Universidade de Lisboa

Faculdade de Farmácia

Departamento de Química Farmacêutica e Terapêutica

New synthetic methodologies from biorenewable resources

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Abstract

In this thesis will be presented new synthetic methodologies for the production of 5-(hydroxymethyl) furfural (HMF) and its derivatives as one important biorenewable platform molecules available *via* dehydration from sugars. The new approaches aim to overcome the major difficulties related with HMF synthesis and isolation on industrial and laboratory scale and to study the synthesis and possible toxic effects in humans of important HMF derivatives which could be produced on industrial scale in the near future as a replacement of existing fossil based chemical building blocks. Scalable protocol for the isolation of HMF *via* simple precipitation was developed together with chemo-enzymatic, and heterogeneous chromium catalyzed methodologies for its synthesis from glucose.

Biorefinery and biorenewable resources are certainly topics of interest from educational point. Batch and flow student laboratory experiments for the dehydration of fructose to HMF have been optimized to meet this purpose.

The synthesis, physical properties, toxicity and some unconventional applications of specific a class Ionic Liquids namely Magnetic ionic Liquids (MIL) were also studied in collaboration with other laboratories.

The environmental friendly solvents are certainly an important part of developing more "green" and sustainable chemistry methodologies. The stability, basicity and their effect on the organic transformations performed in relatively newly discovered and based mostly on biorenewable resources ionic fluids namely deep eutectic solvents (DES) were studied. It was discovered that one of the most commonly used urea based DES undergo urea decomposition at unexpectedly low temperatures to form ammonia which was assign as responsible for their observed basicity.

The readily available from *Lupinus genus* alkaloid Lupanine was employed as a platform molecule for the synthesis of new sparteine derivatives. Lupanine is a structural analog of sparteine, bearing amide functionality, which was subjected to various transformations resulting in sparteine analogs with potential biological activity and application in asymmetric catalysis.

Resumo

Nesta tese são apresentadas novas metodologias sintéticas para a produção de 5-(hidroximetil)furfural (HMF) e derivados como um dos mais importantes produtos provenientes de fontes bio-renováveis *via* desidratação de açucares. Estas novas abordagens têm como objectivo contornar as extremas dificuldades relacionadas com a síntese do HMF e isolamento a escala industrial e laboratorial. Adicionalmente, efectuou-se a síntese de derivados do HMF, que poderão substituir compostos baseados em combustíveis fosseis existentes, e estudo de possíveis efeitos tóxicos em humanos.

Desenvolveu-se um protocolo com potencial aplicação à escala industrial para o isolamento do HMF através de uma simples precipitação e combinada com metodologias catalíticas quimioenzimáticas e catálise heterogénea com crómio, a partir da glucose.

Do ponto de vista educacional, recursos bio-renovaveis e biorefinaria são certamente tópicos de interesse. Consequentemente, optimizaram-se experiências laboratoriais em lote e em contínuo para a desidratação da frutose para produzir HMF, vocacionada para o ambiente de ensino.

A síntese, propriedades físicas, toxicologia e algumas aplicações não convencionais de uma classe específica de Líquidos Iónicos, nomeadamente Líquidos Iónicos Magnéticos (MIL) também foram estudadas através de colaborações.

Outro tópico de interesse foi o desenvolvimento de solventes mais verdes e amigos do ambiente para o desenvolvimento de metodologias mais sustentáveis do ponto de vista ambiental. Foram ainda estudadas a estabilidade, basicidade e o efeito em transformações orgânicas de fluidos iónicos baseados principalmente em recursos bio-renováveis, nomeadamente misturas eutécticas (DES). Assim, descobriu-se que uma das mais comuns misturas eutécticas baseada em ureia decompõe-se inesperadamente a temperaturas baixas para formar amónia, que consequentemente é responsável pela basicidade observada no meio.

A molécula Lupanina, um alcaloide facilmente retirado do Tremoço (Lupinus genus), é um análogo estrutural da esparteína com um grupo funcional amida. Esta funcionalidade amida foi usada para produzir diferentes derivados da esparteína com potencial atividade biológica e aplicação em catalise assimétrica.

Abbreviations

[Ac]	Acetate
[ASBI][Tf]	3-allyl-1-(4-sulfobutyl) imidazolium trifluoromethanesulfonate
[ASCBI][Tf]	3-allyl-1-(4-sulfurylchloridebutyl) imidazolium trifluoromethanesulfonate
[BDMIM]	1-butyl-2,3-dimethylimidazolium
[BMIM]	1-butyl-3-methyl imidazolium
[BMIM]	1-butyl-3-methyl imidazolium
[BMIM]	1-butyl-3-methylimidazolium
[bmP]	1-butyl-1-methylpyrrolidium
[BMPy]	1-butyl-3-methylpyridinium
[C12MIM]	1-dodecyl-3-methylimidazolium
[C_3SO_3Hmim]	1-methyl-3-(3-sulfopropyl)-imidazolium
[C_8MIM]	1-octyl-3-methylimidazolium
[DIPrim]	1,3-Bis(2,6-diisopropylphenyl) imidazolium
[EMIM]	1-ethyl-3-methylimidazolium
[EMIM]	1-ethyl-3-methylimidazo-lium
[HexMIM]	1-hexyl-3-methylimidazolium
[MBCIm]	1-methyl-3-(butyl-4-chlorosulfonyl) imidazolium
[OMIM]	1-octil-3-methylimidazolim
[OMIM]	1-octil-3-methylimidazolim
[P_{66614}]	trihexyl(tetradecyl)phosphonium
12-MPA	12-molybdophosphoric acid
12-MSA	12-molybdosilicic acid
12-TPA	12-tungstophosphoric acid
12-TSA	12-tungstosilicic acid
2,5-DMF	2,5-Dimethylfuran
4,6-DMDBT	4,6-dimethyldibenzothiophene
5-SMF	5-sulfooxymethylfurfural
ACN	Acetonitrile
AcOH	Acetic acid
AIBN	Azoisobutyronitrile
Aliquat®	*N*-Methyl-*N,N*-dioctyloctan-1-ammonium chloride

AOM	Azoxymethane
APG	N-Ac-Phe-Gly-NH_2 peptide
BHET	bis(hydroxyethyl) terephthalate
BINAM	Bis-naphthylamine
BINOL	Binaphthol
BOC	t*ert*-Butyl carbamate
BT	Benzothiophene
BTPC	Benzyltriphenylphosphonium chloride
BuLi	Butyl Lithium
ChCl	Choline chloride
ChOAc	Choline acetate
CLAB	*Candida antarctica* lipase B
CNF-PSSA	Poly(p-styrenesulfonic acid)-grafted carbon nanofibers
CNT-BSA	Benzenesulfonic acid-grafted carbon nanotubes
CNT-PSSA	Poly(p-styrenesulfonic acid)-grafted carbon nanotubes
Co-gel	Cobalt acetylacetonate encapsulated in sol–gel silica
CSMIL	Supported magnetic ionic liquid nanoparticles
C–SO_3H	Sulfonic acid-functionalized carbon materials
CSZA-3	SO_4^{-2}/ZrO-Al_2O_3
DABCO	1,4-diazabicyclo [2,2,2]octane
DBT	Dibenzothiophene
DBU	1,8-diazabicyclo[5.4.0]undec-7-ene
DCA	Double catalytic activation
DCDMH	1,3-Dichloro-5,5-dimethylhydantoin
DCM	Dichloromethane
DEAD	Diethylazodicarboxylate
DES	Deep eutectic solventes
DFD	Dimethyl furan-2,5-dicarboxylate
DFF	2,5-furandicarbaldehyde
DFT	Density functional theory
DHMF	2,5-dihydroxymethylfurfural
DIC	*N,N′*-Diisopropylcarbodiimide
DMA	N,N-dimethylacetamide
DMAP	4-Dimethylaminopyridine

DMF	N,N-dimethylformamide
DMSO	Dimethyl sulfoxide
DMU	*N,N* dimethyl urea
DTBMP	2,6-Di-*tert*-butyl-4-methylpyridine
ee	Enantiomeric excess
EGDE	Ethylene glycol dimethyl ether
EMF	5-(Ethoxymethyl)furfural
EtOAc	Ethyl Acetate
EtOH	Ethanol
FDA	2,5-furandicarboxylic acid
GC	Gas chromatography
GI	Glucose isomerase
GVL	γ-Valerolactone
HBD	Hydrogen bond donor
HCW	Hot compressed water
HFCS	High fructose corn syrup
HMF	5-hydroxymethylfurfural
HMFCA	5-hydroxymethyl-2-furancarboxylic acid
HMMF	5-hydroxymethyl methylfuroate
HPLC	High performance liquid chromatography
HT	Mg–Al hydrotalcite
IL	Ionic liquids
ILIS	Ionic liquids immobilized on silica gel
LA	Levulinic acid
LDA	Lithium diisopropylamide
MCC	Microcrystalline cellulose
MeOH	Methanol
MIBK	Methyl isobutyl ketone
MIL	Magnetic ionic liquids
MTBE	Methyl *t*-butyl ether
MTPB	Methyltriphenylphosphonium bromide
MW	Microwave irradiation
NBS	N-bromosuccinimide
NHC	N-heterocyclic carbene

NMP	1-methyl-2-pyrrolidinone
PCC	Pyridinium chlorochromate
PEDOT	poly(3,4-ethylenedioxythiophene)
PEG	Polyethylene glycol
PPTS	Pyridinium *p*-toluenesulfonate
PTSA	*p*-Toluenesulfonic acid
PVDF	Polyvinylidene fluoride
PVP	Poly(1-vinyl-2-pyrrolidinone)
ROS	Reactive oxygen species
SCE	Sister chromatin exchange
(-)sp	(-)-Sparteine
TEAB	Tetraethylammonium bromide
TEAC	Tetraethyl ammonium chloride
TEMPO	4-substituted 2,2,6,6-tetramethylpiperidine-1-oxide
Tf_2O	Triflic anhydride
THF	Tetrahydrofuran
TLC	Thin layer chromatography
TMACC	Trimethylammonium chlorochomate
TP	Tantalum phosphate
TPPTS	Trisulfonated triphenylphosphine
VANOL	3,3′-Diphenyl-2,2′-bi-1-naphthalol,-3,3′-Diphenyl-2,2′-bi-1-naphthol
VAPOL	2,2′-Diphenyl-(4-biphenanthrol**)**
VOP	Vanadyl phosphate

Keywords

5-(hydroxymethyl) furfural
Magnetic ionic liquids
Deep eutectic solvents
Sustainable chemistry
Chemo-enzymatic
Carbohydrates
Lupin alkaloids
Sparteine derivatives

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Chapter I
General Introduction

This chapter aims to provide brief overview on the biorefinery concept, as well as the applications of green reaction solvents and in particular ionic liquids and deep eutectic mixtures in biorefinery.

Table of content.

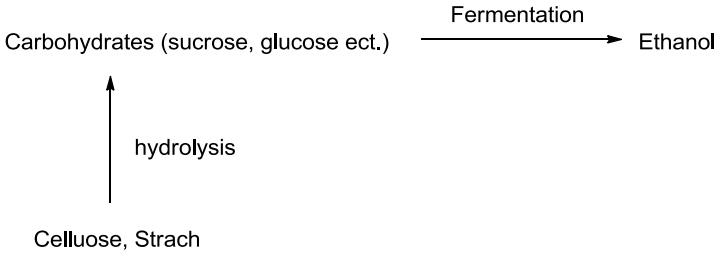
1. Biorefinary and biorenewable feedstock.

Sustainable chemistry becomes an increasingly important topic during the last decades.[1,2] Considerable interest and research seeking new, more sustainable and green methodologies in organic synthesis and processing is ongoing both in industry and academia institutions. The driving force of this research is based on two global reasons:

1. The diminishing fossil resources and their increasing market prices.
2. The need to decrease the greenhouse gas emissions and other environmental impacts related with the production of energy and chemicals.

Certainly the biorefinary and utilization of biomass as a renewable carbon source is a key points in achieving such processes.[3] The International Energy Agency Bioenergy Task 42 defined biorefining is the sustainable processing of biomass into a spectrum of marketable products and energy. Apart of energy issue there are already a considerable range of chemical building blocks derived from renewable resources.[4,5] In 2004 US Department of Energy released a list of "Top10 chemicals'', which could be employed as platform molecules or building blocks for the synthesis of chemicals from biorenewable resources,[6] letter Bozell and Petersen[7] published a revisited version aiming to point out the platform chemicals which received the scientists and industry attention and resulted in significant research and improvements. The new list includes ethanol, furans, glycerol, lactic acid, succinic acid, hydroxypropionic acid/aldehyde, levulinic acid, sorbitol and xylitol. Representative examples of one of the mostly used biorenewable platform chemicals will be further presented.

Commercial production of carbohydrates for ethanol production (Scheme 1) and its use as a biofuel was considered in the beginning of the 20th century.[8] and is one of the first and still major examples of biorefinary process.[9]



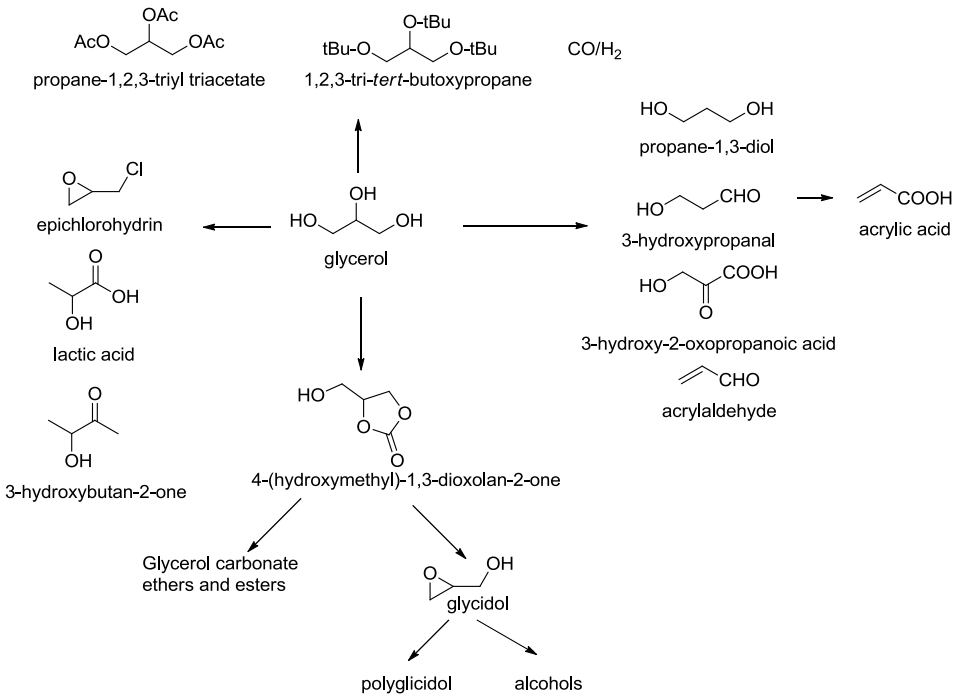
Scheme 1.

Recent technology developments positioned ethanol as an important feedstock for chemical production, improving its potential from only biofuel to also a chemical building blocks precursor and alternative to petrol chemistry.[10] Our days ethanol and related alcohols (propanol, butanol) are of interest as precursors to the corresponding olefins *via* dehydration[11]

and production of H_2.[12] Large scale ethanol production from biomass mostly uses sugar cane or sugar beet juice, corn or wheat and it is also industrially produced in the pulp and paper industry as a by-product. Lignocellulose which is the most abundant polysaccharide in nature and being not competitive with food resources is also widely used as a biomass feedstock. The production of ethanol from lignocellulose, which is composed by cellulose, hemicellulose and lignin, requires initial hydrolysis of the cellulose and hemicellulose to glucose which is further fermented to ethanol. The hydrolysis is achieved via three major processes on industrial scale – acid, enzymatic or thermal.[12]

Glycerol is another platform molecule based on biorenewable resources. The interest on glycerol as a feedstock for biorefinary increased dramatically last decades due to the increased biodiesel production, where it is a by-product in multitone scale.[13]

A numerous applications of crude glycerol to valuable chemical building blocks are already reported in the literature[14] (Scheme 2).

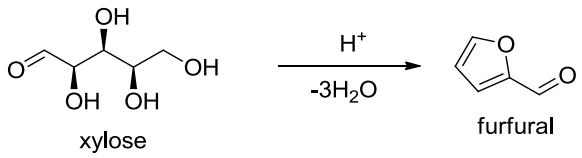


Scheme 2.

The most important biorefinary processes used for the conversion of glycerol are its catalytic dehydration to acrylaldehyde,[15] enzymatic or catalytic dehydration to 1,3-propanediol[14] and enzymatic conversion to lactic acid.[16]

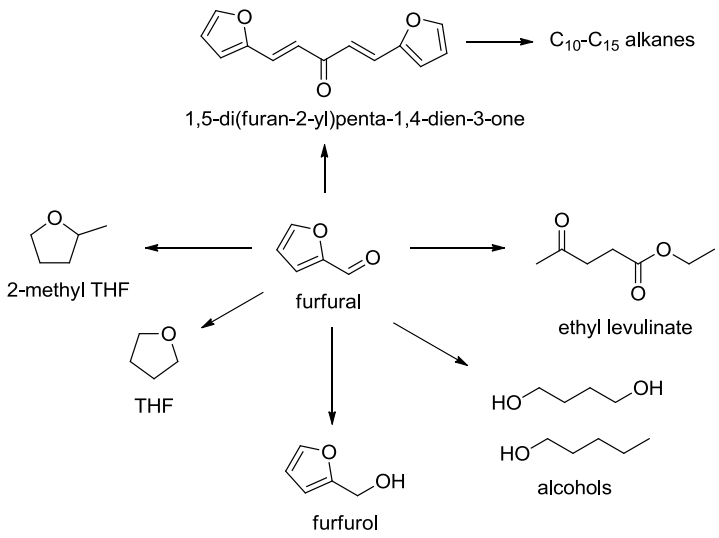
The dehydration of 5- and 6-carbon sugars to give furans is a well-established transformation for the preparation of furfural and 5-hydroxymethylfurfural (HMF) (complete overview of HMF production and applications will be given in chapter II). Hemicellulose

derived xylose is the industrially common feedstock for the production of furfural *via* dehydration using mineral acids as homogeneous catalysts (Scheme 3).[17]



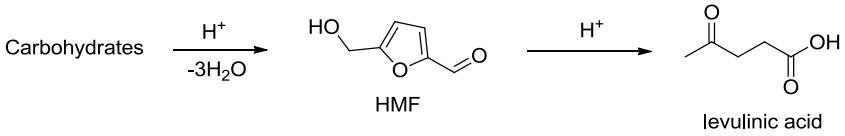
Scheme 3.

The most important further transformation of furfural to valuable chemicals is presented on Scheme 4.[18]



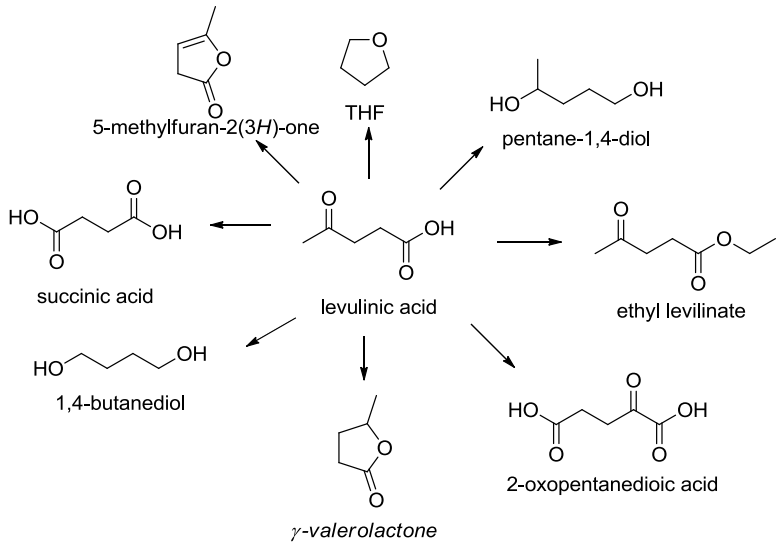
Scheme 4.

Levulinic acid is a gamma-keto acid which can be produced by acid-catalyzed dehydration and hydrolysis of hexose sugars *via* formation of HMF as a major intermediate (Scheme 5).[19]



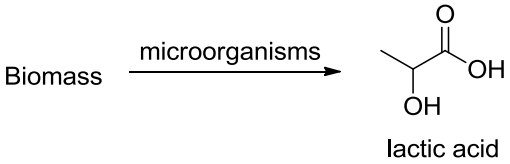
Scheme 5.

Levulinic acid is intensively studied as a precursor for the production of biofuel and fuel additives. The derived 2-methyl-tetrahydrofuran and γ-valerolactone can be readily blended with petroleum products to create cleaner-burning fuels.[19] Compared to ethanol this fuels have the advantage to be water unmixable, thus preventing phase separation when contaminated with water. Ethyl levulinate is already industrially produced as an oxygenated additive for diesel fuel.[20] The brunch of possible levulinic acid transformations is presented on Scheme 6.



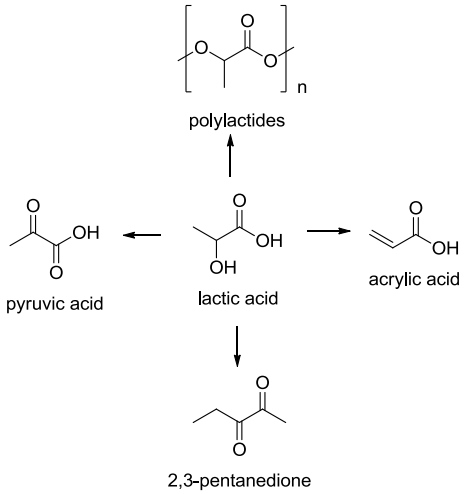
Scheme 6.

Lactic acid is a α-hydroxy carboxylic acid that could be obtained from starch and lignocellulose biomass via biotechnological processes (Scheme 7).[21]



Scheme 7.

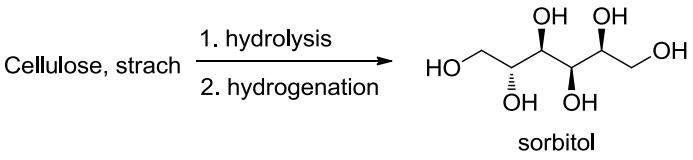
Despite being itself used in food, cosmetic, and pharmaceutical industries[22] lactic acid is also a feedstock for further transformation to chemical building blocks (Scheme 8).[23]



Scheme 8.

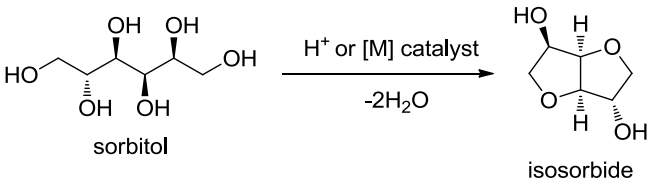
Sorbitol, a sugar derived polyalcohol found in many fruits, is attracting increasing industrial interest as a sweetener, texturizer, and softener.[24] Sorbitol can be produced by hydrogenation from glucose. Cellulose and starch could serve as a biomass feedstock for the

production *via* initial hydrolysis to glucose and subsequent transition metal catalyzed hydrogenation to sorbitol (Scheme 9).[25]



Scheme 9.

Beside the direct use of sorbitol in the industry several further transformations to valuable chemicals are also reported.[26] Sorbitol could be used as a feedstock for the production of biofuels[27] and isosorbide (Scheme 10).[26]

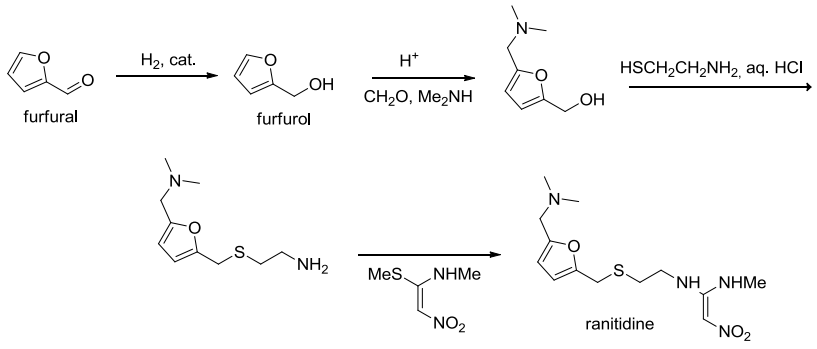


Scheme 10.

2. Application of the biorenewable resources in pharmaceutical chemistry.

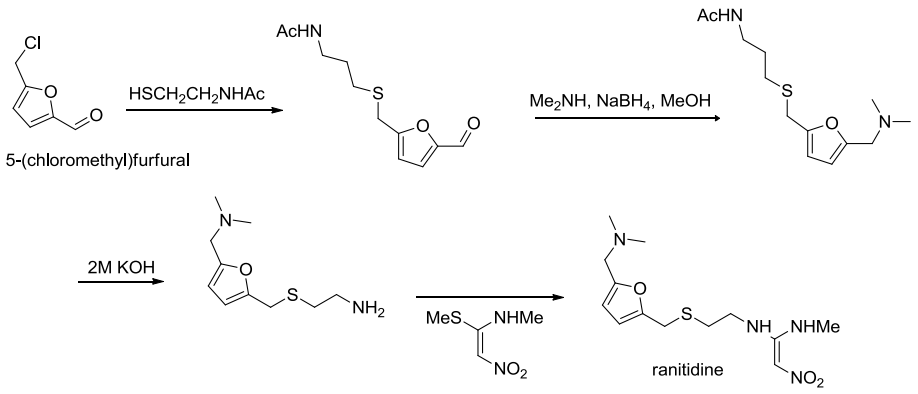
Following the trend for more green and sustainable resources and processes in chemistry, academia and industry institutions working in the field of pharmaceutical chemistry quickly recognized the potential of biorenewable resources as a feedstock in the synthesis of drugs. Some of the platform biorenewable molecules like lactic acid could be directly used in the pharmaceutical industry,[21] while others, mainly furans, serve as precursors for drug synthesis.

Ranitidine, a histamine H2-receptor antagonist, which is used in the management of gastroesophageal reflux disease is an excellent example of a commercial drug synthesized from biorenewable resource. The original patented synthetic route start from furfural and achieve the target compound in 4 steps (Scheme 11).[28]



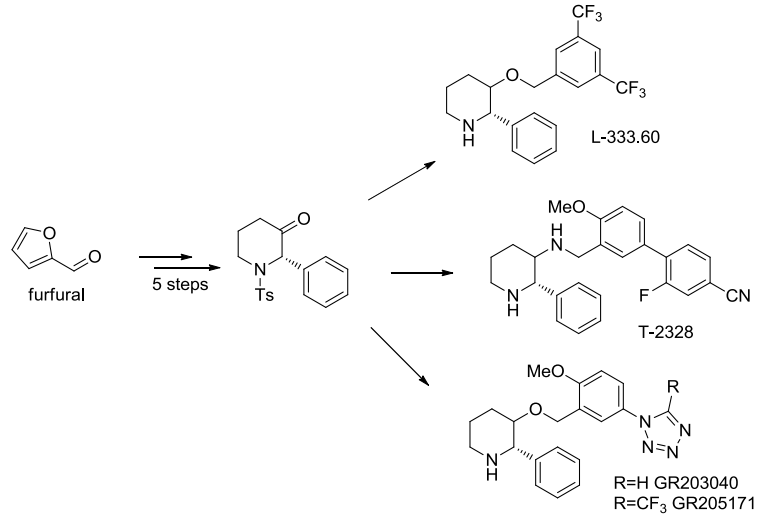
Scheme 11.

More recently and alternative synthesis from cellulose-derived 5-(chloromethyl)furfural was reported by Muscal *et al*. (Scheme 12).[29]



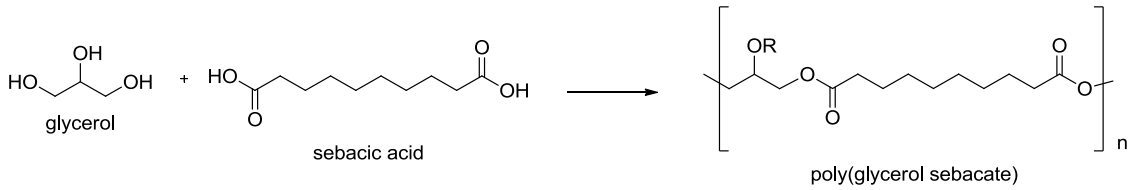
Scheme 12.

Furfural-based facile synthesis of protected (2S)-phenyl-3-piperidone, a common intermediate in drug synthesis was reported very recently by Loh *et al*.(Scheme 13).[30]



Scheme 13.

Another example, although not targeting drug synthesis, is the production of Poly(glycerol sebacate) (PGS) this polyester prepared by polycondensation of glycerol and sebacic acid (Scheme 14) is a biodegradable polymer increasingly used in a variety of biomedical applications since it exhibits biocompatibility and biodegradability both highly relevant properties.[31] Because of the flexible and elastomeric nature it has been widely applied in the soft tissue replacement and the engineering of soft tissues, such as cardiac muscle, blood, nerve, cartilage and retina.



Scheme 14.

These examples along with many others represent the increasing interest of the pharmaceutical industry towards biorenewable feedstock.

3. Application of ionic liquids and deep eutectic solvents in biorefinary.

Ionic liquids (IL) attracted scientist attention during last decades due to their potential as a green alternative for the common organic solvents. IL are considered green due to their extremely low vapor pressure, high thermal stability and recyclability.[32] The application of ionic liquids both as solvents and in some cases catalysts in biorefinary could give an additional value for these processes in terms of sustainability and environmental impact and actually they have always been part of the research in the field. More recently new class of ionic fluids called deep eutectic solvents (DES)[33] which exhibit similar properties as the traditional ILs was discovered but being even more "green" since they are typically produced from biorenewable and environmental friendly resources. Even still quite limited several applications of DES in biorefinary have been already reported.

IL has been applied in all stages of biorefinary processes starting from the pre-treating of biomass. One of their most studied application is in the cellulose processing, where IL have been involved in four major directions - regeneration, chemical modification, enzymatic hydrolysis, and chemical depolymerisation. Along with the cellulose treatment IL were also used in native biomass conversions, like complete and selective dissolution of lignin and hemicellulose.[34] Numerous detailed studies on the use of IL as solvents, catalyst or both together for the further conversion of sugars to furanes were also published in the literature. Xylose and xylan were effectively converted to furfural in presence of IL.[35-37] Certainly the most studied process in this field is the dehydration of carbohydrates (fructose, glucose, cellulose, inulin) into HMF.[37,38] detailed overview of this topic will be provided further on this thesis.

Supported Brönsted acidic IL were used as catalysts for gas phase dehydration of glycerol to acroleine.[39] IL were also found to catalyze the synthesis of glycidol from glycerol and dimethyl carbonate.[40] In the down-stream product separation IL have been applied as an extraction solvents, for example for selective extortion of 1-butanol or ethanol.[34]

As it was already mention the use of DES, being a relatively newly developed ionic fluids, in the biorefinary processes is still quite limited. The reported applications of DES are manly related with the dehydration of carbohydrates to HMF and more detailed information on this topic will be presented in chapters II and III of the thesis.

The great recent scientific and industrial interest on the sustainable chemistry and biorenewable resources provoke us to join this research by developing new approaches for the synthesis of HMF and HMF derived building blocks, aiming to overcome the major problems in their lab and industrial scale production, as well as adapting these approaches for teaching purposes. Additionally, we have performed research on the stability and application in organic synthesis of DES and studied the physical properties and unconventional applications of a specific class of IL namely magnetic ionic liquids.

4. References.

1. Dunn, P. J. *Chem. Soc. Rev.*, **2012**, *41*, 1452.
2. Sheldon, R. A. *Chem. Soc. Rev.*, **2012**, *41*, 1437.
3. Gallezot, P. *Chem. Soc. Rev.*, **2012**, *41*, 1538.
4. Corma, A.; Iborra, S.; Velty, A. *Chem. Rev.*, **2007**, *107*, 2411.
5. Christensen, C. H.; Rass-Hansen, J.; Marsden, C. C.; Taarning, E.; Egeblad, K. *Chemsuschem*, **2008**, *1*, 283.
6. T. Werpy, G. P. *US Department of Energy DOE/GO-102004-1992*, **2004**, http://www.eere.energy.gov/biomass/pdfs/35523.pdf.
7. Bozell, J. J.; Petersen, G. R. *Green Chem.*, **2010**, *12*, 539.
8. Brownlie, D. *Chem. Ind.*, **1940**, *59*, 671.
9. Edgard Gnansounou, A. D. *J. Sci. Ind. Res.*, **2005**, *64*, 809.
10. Rass-Hansen, J.; Falsig, H.; Jørgensen, B.; Christensen, C. H. *J. Chem. Technol. Biotechnol.*, **2007**, *82*, 329.
11. Fan, D.; Dai, D.-J.; Wu, H.-S. *Materials*, **2012**, *6*, 101.
12. Mattos, L. V.; Jacobs, G.; Davis, B. H.; Noronha, F. B. *Chem. Rev.*, **2012**, *112*, 4094.
13. Yang, F.; Hanna, M.; Sun, R. *Biotechnology for Biofuels*, **2012**, *5*, 13.
14. Xiaohu Fan, R. B., Yongchang Zhou *The Open Fuels & Energy Science Journal*, **2010**, *3*, 17.
15. Katryniok, B.; Paul, S.; Dumeignil, F. *ACS Catalysis*, **2013**, *3*, 1819.
16. Li, C.; Lesnik, K.; Liu, H. *Energies*, **2013**, *6*, 4739.
17. Danon, B.; Marcotullio, G.; de Jong, W. *Green Chem.*, **2014**, *16*, 39.
18. Lange, J.-P.; van der Heide, E.; van Buijtenen, J.; Price, R. *ChemSusChem*, **2012**, *5*, 150.
19. Rackemann, D. W.; Doherty, W. O. S. *Biofuels, Bioproducts and Biorefining*, **2011**, *5*, 198.
20. Yadav, G. D.; Yadav, A. R. *Chem. Eng. J. (Lausanne)*, **2014**, *243*, 556.
21. Castillo Martinez, F. A.; Balciunas, E. M.; Salgado, J. M.; Domínguez González, J. M.; Converti, A.; Oliveira, R. P. d. S. *Trends Food Sci. Technol.*, **2013**, *30*, 70.
22. Wee, Y. J.; Kim, J. N.; Ryu, H. W. *Food Technology and Biotechnology*, **2006**, *44*, 163.
23. Mäki-Arvela, P.; Simakova, I. L.; Salmi, T.; Murzin, D. Y. *Chem. Rev.*, **2013**, *114*, 1909.
24. Silveira, M.; Jonas, R. *Appl. Microbiol. Biotechnol.*, **2002**, *59*, 400.
25. Kilpiö, T.; Aho, A.; Murzin, D.; Salmi, T. *Ind. Eng. Chem. Res.*, **2013**, *52*, 7690.

26. Zhang, J.; Li, J.-b.; Wu, S.-B.; Liu, Y. *Ind. Eng. Chem. Res.*, **2013**, *52*, 11799.
27. Zhang, Q.; Jiang, T.; Li, B.; Wang, T.; Zhang, X.; Zhang, Q.; Ma, L. *ChemCatChem*, **2012**, *4*, 1084.
28. B. J. Price, J. W. C., J. Bradshaw *US Patent 4128658*, **1978**.
29. Mascal, M.; Dutta, S. *Green Chem.*, **2011**, *13*, 3101.
30. Koh, P. F.; Wang, P.; Huang, J. M.; Loh, T. P. *Chem. Commun.*, **2014**, *50*, 8324.
31. Rai, R.; Tallawi, M.; Grigore, A.; Boccaccini, A. R. *Prog. Polym. Sci.*, **2012**, *37*, 1051.
32. Mallakpour, S.; Dinari, M. In *Green Solvents II*; Mohammad, A., Inamuddin, D., Eds.; Springer Netherlands: **2012**, p 1.
33. Zhang, Q.; De Oliveira Vigier, K.; Royer, S.; Jerome, F. *Chem. Soc. Rev.*, **2012**, *41*, 7108.
34. Stark, A. *Energy & Environmental Science*, **2011**, *4*, 19.
35. Zhang, L.; Yu, H.; Wang, P.; Dong, H.; Peng, X. *Bioresour. Technol.*, **2013**, *130*, 110.
36. Wu, C.; Chen, W.; Zhong, L.; Peng, X.; Sun, R.; Fang, J.; Zheng, S. *J. Agric. Food Chem.*, **2014**.
37. Binder, J. B.; Blank, J. J.; Cefali, A. V.; Raines, R. T. *ChemSusChem*, **2010**, *3*, 1268.
38. Zakrzewska, M. E.; Bogel-Łukasik, E.; Bogel-Łukasik, R. *Chem. Rev.*, **2010**, *111*, 397.
39. Munshi, M. K.; Lomate, S. T.; Deshpande, R. M.; Rane, V. H.; Kelkar, A. A. *J. Chem. Technol. Biotechnol.*, **2010**, *85*, 1319.
40. Gade, S. M.; Munshi, M. K.; Chherawalla, B. M.; Rane, V. H.; Kelkar, A. A. *Catal. Commun.*, **2012**, *27*, 184.

Chapter II

Synthesis and biological evaluation of 5-(hydroxymethyl) furfural and its derivatives

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In this chapter will be given a complete overview on the toxicity and reported synthetic approaches for the production and transformation of 5-(hydroxymethyl) furfural. Results and discussion on new synthetic methodologies aiming to overcome the difficulties related with its synthesis in lab and industrial scale, as well as the synthesis and toxicity of its derivatives will be presented. A review on the topic and the results from this chapter have been published in 6 peer-reviewed articles.

*1. A. Rosatella, S. Simeonov, R. Frade, C. Afonso, 5-Hydroxymethylfurfural (HMF) a building block platform: Biological properties, Synthesis and synthetic applications, Green Chem., **2011**, 13, 754-793. Listed in Green Chem. top ten most accessed articles in March and April 2011.*

*2. S. Simeonov, J. Coelho, C. Afonso, Integrated Simple Approach for the Production and Isolation of 5-Hydroxymethylfurfural (HMF) from Carbohydrates, ChemSusChem ,**2012**, 5, 1388-1391.*

*3. S. Simeonov, J. Coelho, C. Afonso, C. A. M., Integrated chemo-enzymatic production of 5-hydroxymethylfurfural from glucose, ChemSusChem, **2013**, 6, 997-1000.*

*4. R. Frade, J. Coelho, S. Simeonov, C. Afonso, Emerging Platform from Renewable Resources: Selection Guidelines for Human Exposure of Furan-Based Compounds, Toxicol. Res. **2014,*** DOI: 10.1039/c4tx00019f.

*5. S. Subbiah, S. Simeonov, J. Esperança, L. Rebelo, C. Afonso, Direct transformation of 5-hydroxymethylfurfural to the building blocks 2,5-dihydroxymethylfurfural (DHMF) and 5-hydroxymethyl furanoic acid (HMFA) via Cannizzaro reaction, Green Chem.**2013**, 15, 2849-2853.*

*6. S. Simeonov, C. Afonso, Batch and Flow Synthesis of 5-Hydroxymethylfurfural (HMF) from Fructose as a Bioplatform Intermediate: An Experiment for the Organic or Analytical Laboratory, J. Chem. Edu., **2013**, 90, 1373-1375.*

Table of content.

1. Intruduction

Biorefinary is an important approach for the current need of energy and chemical building blocks for diverse range of applications, that gradually will may replace the actual dependence on fossil resources. During the last years have been reported considerable efforts and achievements on the transformation of carbohydrates into 5-(hydroxymethyl) furfural (HMF). More than 1300 papers could be found in Web of Science database. The interest on this topic increased significantly in the recent years and the number of publication reached above 200 per year (Figure 1).

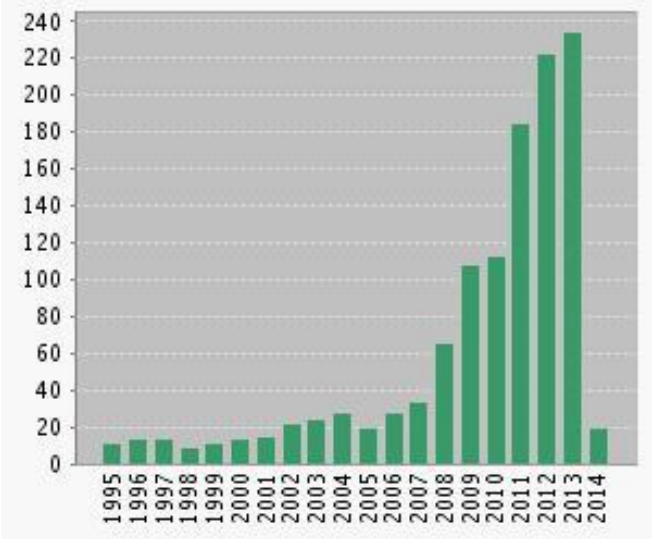


Figure 1. Number of publications per year about 5-hydroxymethylfurfural (Web of Science)

The great scientific and industrial interest and several still not resolved problems, like the difficulties of HMF synthesis from glucose and its difficult isolation and purification provoke us to study the topic. In the beginning of the work full literature search has been performed and it was published a review[1] with authors Dr. Andrea Rosatella (HMF synthesis), Svilen Simeonov (Synthetic applications of HMF) Dr. Raquel Frade (Biological properties) and Prof. Carlos Afonso. An updated version of the review will be provided as an introduction part of this chapter.

Among others primary renewable building blocks, HMF is considered an important intermediate due to its rich chemistry and potential availability from carbohydrates such as fructose, glucose, sucrose, cellulose and inulin. HMF can be most easily obtained from fructose but also more recently from glucose *via* isomerization to fructose, including directly from cellulose.

Since cellulose is formed by anhydro-D-glucopyranose units linked by β-1-4-glycosidic bonds, hydrolytic degradation is necessary to release the sugar monomers. However, it

should not be neither stopped too soon, to avoid formation of oligosaccharides, nor to proceed for too long to prevent monosaccharides to react at high temperatures.[2]

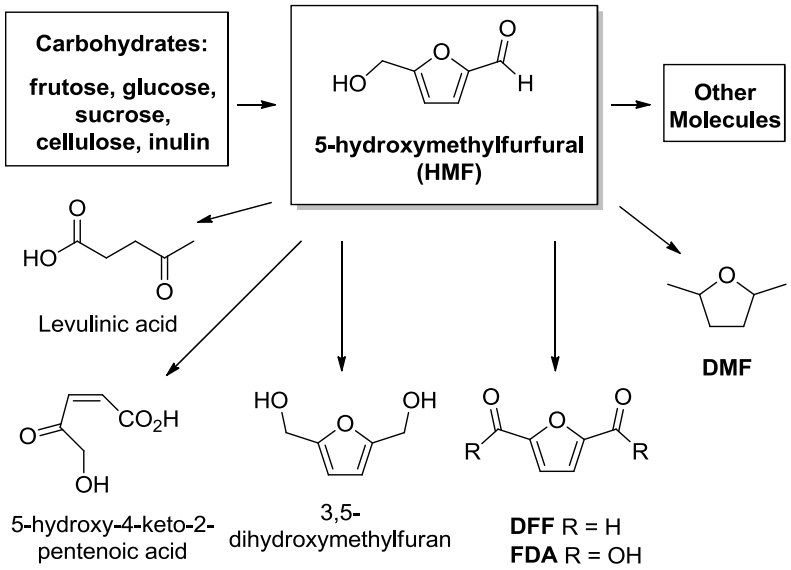
Contrary to cellulose, hemicellulose is a polymer formed by different sugar units as glucose, galactose, mannose, xylose and arabinose, and, it does not form crystalline regions making this polymer more accessible to hydrolysis. Whereas dehydration of hexoses can originate HMF, pentoses can lead to production of furfural. Due to the fact that the rate of dehydration depends on the sugar type and decreases following the order xylose>mannose>glucose, hemicelluloses is hydrolyzed more slowly than cellulose.[2]

HMF is very useful not only as intermediate for the production of the biofuel dimethylfuran (2,5-DMF) and more specific molecules, but also to create other important commodities molecules such as levulinic acid, 2,5-furandicarboxylic acid (FDA), 2,5-furandicarbaldehyde (DFF), dihydroxymethyl-furan and 5-hydroxy-4-keto-2-pentenoic acid (Scheme 1).

HMF have been reported since the final of the 19th century, when Dull *et al.*[3] published the synthesis by heating inulin with oxalic acid solution under pressure. In the same year Kiermayer[4] reported a similar procedure for HMF synthesis, but starting from sugar cane. In the following years several preparation methods along with application of homogeneous and heterogeneous acids, both in aqueous media have been reported.[5-9] This topic was firstly reviewed in 1951 by Newth *et al.*[10] and since then several important reviews have been published: Moye *et al.*[11] reviewed different synthetic methods and industrial applications of HMF. Latter on Harris[12] described the dehydration reactions of carbohydrates in acidic and basic conditions, including their mechanism. In 1981 two reviews where published, one referring the HMF manufacture[13] and other focus on further HMF chemistry[14]. In 1990 and 1991 two important reviews were published by Kuster[15] and Cottier *et al.*[16] respectively, describing the manufacture of HMF. Lewkowski[17] and Moreau *et al.*[18] reviewed the synthesis and further chemistry of HMF. Corma *et al.*[19] dedicated a chapter on HMF synthetic methods in their outstanding biomass transformations review. Woodley *et al.*[20] also summarized some HMF synthetic processes, and Zhang *et al.*[21] connected biomass transformations with imidazolium salts, by including the HMF synthesis with ionic liquids as solvents.[22]

After our team review several following reviews appear in the literature. In 2011 a review on the ionic liquids mediated synthesis of HMF was published by Bogel-Lukasik *et al.*[23] More recently Ebitani *at el.*[24] reviewed the synthesis of HMF as a part of their more general review on the catalytic transformation of biomass into furfurals. The transformation of HMF into polyester building blocks and biofuels was briefly reviewed by Saha *et al.*[25] In 2013 a detailed review on the lab scale and industrial HMF synthesis and its further transformation

into valuable chemicals was published by Vries *et al.*[26] Very recently Abu-Omar *et al.*[27] reviewed the application of biphasic systems in the production of HMF from biomass.



Scheme 1.

1.2 Formation of HMF during baking.

In the bakery industry, the formation of dough consists of a mixture of flour, water, yeast and salt, which after fermentation will be subjected to high temperatures for a certain period of time, for baking. During this process, the dough suffers physical and chemical changes. The temperature leads to water evaporation and formation of new products that contribute to flavor and browning. These products are resultant from Maillard reactions and caramelization. The first consists of a reaction between the carbonyl group of the sugar and the amino group of an aminoacid and is likely to occur at high temperatures (>50ºC) and acidic pH (4-7) and is favored in foods with a high protein and carbohydrate content and intermediate moisture content.[28] Caramelization is the oxidation of sugar and needs more drastic conditions in order to happen as temperatures above 120ºC and more extreme pH (<3 or >9) and low amount of water.[28] These reactions are frequent in bakery products but also in other foods subjected to high temperatures during processing. The reaction of fructose, lactose and maltose with the amino group of lysine to form fructosyl-lysine, lactulosyl-lysine and maltulosyl-lysine (Amadori products) is characteristic of the early stages of Maillard reactions and is responsible for decreasing the available lysine and food nutritional value. Thus, evaluation of these compounds has been suggested to work as control parameters to assess quality of foods.[29] However, other products can be formed and several examples in the literature exist that report the degradation of the sugar in HMF, for instance, during heating of

milk, which has a high concentration of lactose and lysine-rich proteins.[30,31] Under acidic conditions, lactulosyl-lysine can suffers 1,2-enolization through 3-deoxyosulose to form HMF. But, isomerization and degradation of lactose (Lobry de Bruyn-Alberda van Ekenstein transformation) also accounts for the formation of HMF. Quantification of HMF can be used to quantify the extension of the Maillard reaction in foods. Morales, F. J., Romero, C. and Jiménez-Pérez, S. have removed the free lactose from milk samples and quantified HMF released from oxalic acid catalyzed degradation of lactulosyl-lysine compounds, using reversed-phase HPLC. This study demonstrated that this method can be used to determine the extension of the Maillard reaction, however they also showed that this reaction accounts as a minor route for sugar degradation. Other techniques as the 2-thiobarbituric acid (TBA) method, widely applied in dairies, can be used to quantify HMF, as well, but it is less suitable since other aldehydes can take part in the reaction.[32] Many other studies were published but HPLC seems to be the chosen method for HMF determination.[33,34] Previous solubilisation of the sample in water followed by precipitation of proteins with trichloroacetic acid was used to eliminate interferences during HPLC determination of HMF in cookies during the baking process.[34] Thus, HMF determination has also been used as a parameter to evaluate heat effects during manufacture of cereal products.[35-38] Ramírez-Jiménez, A., García-Villanova, B. and Guerra-Hernández, E. have reported formation of HMF during browning of sliced bread and increasing amounts were detected with the increasing of the heating time (14,8 mg/Kg and 2024,8 mg/kg with 5 or 60 minutes of toasting times, respectively).[37] Fallico, B. Arena, E. and Zappalà M. have also reported the effect of the temperature on the HMF formation during roasting of hazelnuts, and they also studied the effect of the oil in this mechanism. Defatted crushed hazelnuts produced less amount of HMF during roasting (2.2 mg/Kg at 150ºC and for 60 minutes) than crushed hazelnuts (8.0 mg/Kg at 150ºC and for 60 minutes) and addition of 10% water to the defatted crushed hazelnuts leaded to an increase of HMF of approximately 32%. Additionally, an increase of the temperature to 175ºC produced an increase of HMF concentrations, as expected (66.5 mg/Kg for crushed hazelnuts and 17.9 mg/Kg for defatted crushed hazelnuts) even using a lower toasting time of 30 minutes.[39] Furthermore, different studies have also demonstrated that formation of HMF is dependent on water and decreases with the increasing of water quantities and that fructose is more efficiently degraded to HMF than glucose.[40] More recently, after our review, Suman *et al.*[41] published a review dedicated to the mitigation strategies of furan and HMF in food. Ozdemir *et al*.[42] studied the relationship between HMF formation and temperature evolution during toasting of bread slices. The authors determined HMF by HPLC in crust and crumb sections of bread slices which were toasted up to 250s in a double-slot toaster. Graduate accumulation

of HMF over the time in up to 392.05 +/-3.72 and 218.20+/-1.56 mg/kg after 250s in crumb and crust part respectively was observed. Study of HMF and furfural formation in cakes during baking in different ovens using validated extraction method was published by Ferreira *et al*.[43] The authors observed that the oven type and baking time strongly influenced HMF formation. Traditional and microwave baking resulted in significant increase of the HMF with the time, up to 41.9 mg kg-^{1} at 60 min and 16.84 mg kg-^{1} at 2.5 min respectively, while steam oven cakes backing exhibit no significant time effect on the HMF formation (28.77, 25.86 and 25.43 mg kg-^{1} at 20, 40 and 60 min respectively were observed). The same group[44] recently studied the presence of HMF and furfural in various commercial bakery products. Cake/pastry samples showed the lowest HMF content (3.0 mg kg^{-1}) while biscuits showed the highest and much more significant content (7.8 mg kg^{-1}). Gökmen *et al*.[45] reported in 2014 baking experiments with biscuits of two different recipes, with and without NaCl, at 180°C, 190°C and 200°C. The authors observed that the presence of NaCl in the biscuit formulation led to higher amounts of HMF at all the tested temperatures and HMF reached highest concentrations at 200°C for both recipes.

1.3 Biological properties

1.3.1 Effects of HMF on the growth of microorganisms

Therefore, the use of lignocelluloses as a carbon source demands an acid hydrolyze before use. And, as already mentioned, this process may release side-products, which are already known to interfere with the microorganisms growth and metabolism. Consequently, some work has been developed with the aim to find microorganisms resistant to these compounds as HMF (**Table 1**)

Several studied strains of *Saccharomyces cerevisiae* were found to be quite tolerant to HMF, however results vary substantially within the studied microorganisms: 1) an addition of 4g/L of HMF to an anaerobic fermentation with *S. cerevisiae* CBS 8066 caused a sudden drop in the CO_2 levels and glucose concentration with the consequent cease of ethanol production after exhaustion of glucose, but biomass concentration did not decrease and reached a step after consumption of all glucose [46]; 2) a lower concentration of 1.5 g/L HMF did not have any effect on ethanol production during the anaerobic fermentation of xylose by *S. cerevisiae* TMB 3001[47]; 3) studies with *S. cerevisiae* ATCC 211239 demonstrated that cell growth was not significantly affected at a HMF concentration below 2.5 g/L but at 3.8 g/L, a long lag phase in the growth curve (approximately 24 hours) was originated [48]; 4) the strain *S. cerevisiae* NRRL Y-12632 was largely affected at a concentration of 2.5 g/L concentration and no growth was detected at a concentration of 3.8 g/L[48]; and 4) bigger amounts of HMF

(7.6 g/L) were also tested in an anaerobic fermentation with *S. cerevisiae* TMB 3400 and were seen to lead to only a 50% decrease of glucose concentration within 24 hours, compared to the control whose glucose had already been used up, with a consequent decrease of the production rate of ethanol.[31]

Table 1. Effect of HMF on the growth and/or ethanol production during fermentation using different strains of microorganisms.

Microorganism	HMF (g/L)	Growth OR/AND Ethanol production
S. cerevisiae CBS 8066[46]	4.0	Growth did not decreased but reached a step after faster consumption of glucose with consequent cease of ethanol production
S. cerevisiae TMB 3001[47]	1.5	No effect on ethanol production
S. cerevisiae ATCC 211239[48]	< 2.5	Growth was not significantly affected.
	3.8	Long lag phase in the growth curve of about 24 h
S. cerevisiae NRRL Y-12632[48]	2.5	Growth largely affected
	3.8	No growth
S. cerevisiae TMB 3400[31]	7.6	Decreased glucose consumption and production rate of ethanol
Pichia stipites NRRL-Y-7124[48]	2.5	Growth not significantly affected
	3.8	No growth
Rhodosporidium toruloids Y4[49]	1.9	Growth was not significantly affected

A different yeast – *Pichia stipitis* NRRL-Y-7124 was also tested and growth was not significantly changed in the presence of 2.5g/L HMF, however it was impaired at a concentration of 3.8 g/L[48]. A different study performed with this last strain revealed that tolerance to HMF improved in stationary phase cultures and was greater in the presence of glucose rather than xylose and, regardless the carbon source, amino acid enrichment of the culture medium enhanced the ability of cells to resist to HMF exposure.[50] *Rhodosporidium toruloids* Y4 was another experimented microorganism and, addition of 1.9 g/L HMF was demonstrated not to change significantly substrate consumption, neither biomass concentration nor lipid content. This yeast strain can accumulate intracellular lipids as high as 60% of its cell dry weight in the presence of glucose, and correspondent fatty acids are similar to those of vegetable oil, constituting an alternative for production of bio-diesel.[49] Two different strains of Escherichia coli (LY01 and KO11) have also been studied and 4.0 g/L HMF was seen to terminate the growth of both strains within 24 hours and consequently, ethanol production as well.[51] Additionally, HMF at a concentration of 0.71 g/L was added to a culture of *Trichosporon cutaneum* 2.1374 but it did not produce an obvious inhibitory effect on cell growth and lipid production.[52]

1.3.2 The effects of HMF in Humans

High concentrations of HMF have been found in some foods as dried fruits[53,54], coffee,[53,54] cereals[38,54] and baking products,[36,38,53-55] but also in medicinal fluids administered intravenously.[56-58] Due to the daily consumption of these foods, the estimated daily intake of HMF is approximately 30-150 mg/per person.[59] Studies with rats and dogs showed that HMF can be toxic if administered at doses of 75 mg/kg body weight.[60] Consequently, several studies have been conducted as an attempt to investigate the effect of HMF in humans.

To assess the effect of HMF in humans several *in vitro* and *in vivo* assays have been conducted. The mutagenic effect have been assessed by the Ames test, which studies the potentiality of the compound to make possible the growth of histidine - deficient bacterial strains plated without histidine supplement, and HMF was seen to be not mutagenic or weakly mutagenic.[61,62] A study performed by Brands, C. M. J. *et al.* have tested different heated mixtures of sugar-casein using the Ames test and have concluded that mutagenicity was related with the extension of Maillard reaction and varied with the type of sugar being fructose more mutagenic than glucose and the reason was based on different reaction mechanisms.[63] Additionally, disaccharides were less mutagenic than monosaccharides because the first induced less mutagenic compounds.[63] However, the compounds responsible for this mutagenicity were not identified, but they were weak compared to 4-nitroquinoline-N-oxide.[63] Additionally, viability of the human hepatocyte cell line – HepG2 in the presence of HMF was not significantly affected (a concentration of 38 mM was necessary to reduce viability in about 50%) and induction of micronuclei formation in this cell line was not detected as well[61]. Furthermore, the presence of HMF protected the human liver cell line – LO2 against the exposure to hydrogen peroxide because it was seen to prevent nitric oxide production, caspase-3 activation and arrestment of the cells in the S phase of the cell cycle.[64] Accordingly with this data, HMF was seen to be present in processed *Fructus Corni* used by the Chinese to invigorate the liver and kidney,[64] and the same compound was also detected in processed steamed *Rehmanniae Radix*, a natural remedy in Chinese medicine used in several diseases as anemia and diabetes.[65] HMF has also been reported to be a promising candidate for therapy of sickle cell disease since it binds efficiently to sickle haemoglobin reverting it to its normal shape.[66]

On the other hand, different studies seem to be in disagreement with the idea that HMF is not harmful since contradictory results have been obtained in different experiments. The human colon cancer cell line – CaCo-2, the human epithelial kidney cell line - HEK 293, the mouse lymphoma cell line – L5178Y and Chinese hamster cell lines – V79 and human sulfotransferase - SULT1A1 expressing V79 were seen to have their DNA damage in the

presence of HMF.[67] Additionally, other derived V79 cell line (V79-hCYP2E1-hSULT1A1) was seen to have higher frequency of sister chromatin exchange (SCE) in the presence of HMF.[68] Furthermore, HMF at the concentration of 23.71 µg/mL induced 50% mortality of nauplii in the brine shrimp bioassay[69] and thermolyzed sucrose, showed to contain 1% HMF, when administered to female rats, treated with the colon carcinogen azoxymethane (AOM) 1 week before, enhanced the growth of the colonic aberrant crypt foci.[70]

One of the hypothesis formulated to try to explain these different effects is the possibility of HMF to be metabolized in a more harmful molecule as 5-sulfooxymethylfurfural (5-SMF)[71], which can be produced through HMF sulfonation by sulfotransferases.[72] The positive result in the Ames test for 5-SMF, towards the strain *Salmonella thyphimurium* TA100,[71] seems to give strength to this idea. 5-SMF was also demonstrated to exhibited a higher skin tumor initiating activity than HMF after its application on mouse skin[71,73] More recently, 5-SMF was quantified *in vivo* after intravenous injection of HMF in mouse.[74] Cytotoxic effect of 5-SMF was also reported in recombinant embryonic kidney cells: 5-SMF was shown to be a substrate for the organic anion transporters OAT1 and OAT3 and to decrease by 80% and 40% respectively, the uptake of the substrates p-aminohippurate and estrone sulphate at a concentration of 1mM, which means that 5-SMF can interfere with the transport of organic anion into renal proximal tubule cells leading to kidney damage.[75] But, other reports fail to attribute to 5-SMF the cause for HMF cytotoxic effects as any correlation was possible to establish between HMF induced DNA damage and sulfotransferase – SULT1A1 activity in some tested cell lines, for instance [67]. Moreover, other studies did not demonstrate the presence of the sulfate metabolite in the urine of male F344 rats and B6C3F1 mice after administration of HMF (5, 10, 100, 500 mg/Kg) being about 60-80% of HMF excreted in the urine.[76] None of this metabolite was also detected in human subjects after consumption of dried plums and/or dried plum juice. In contrast, 4 different metabolites were detected: N-(5-hydroxymethyl-2-furoyl) glycine, 5-hydroxymethyl-2-furoic acid, (5-carboxylic acid-2-furoyl) glycine and (5-carboxylic acid-2-furoyl) aminomethane.[54] Besides, HMF and 5-SMF were both tested in *Min*/+ mouse (heterozygous for a mutation in the tumor suppressor gene *Apc*) and despite increasing of the number of adenomas in the small intestine, they had no effect on its size, compared with the control mice, being classified as weak intestinal carcinogens.[77]

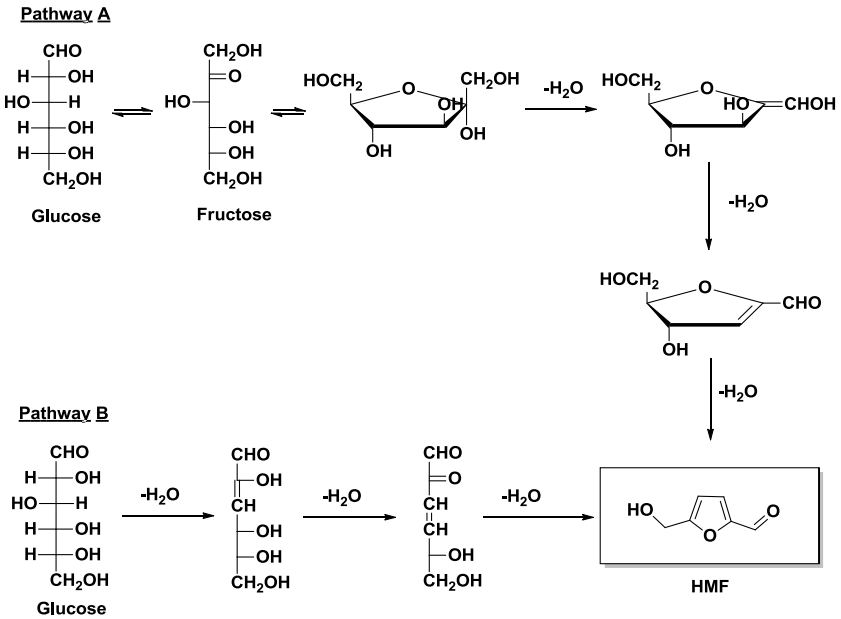
1.4 HMF Synthesis.

Several catalysts have been reported for the dehydration of carbohydrates, and Cottier *et al.*[16] organized them into five groups: organic acids, inorganic acids, salts, Lewis acids, and others. In the last years carbohydrates dehydration catalysts undergo a remarkable evolution

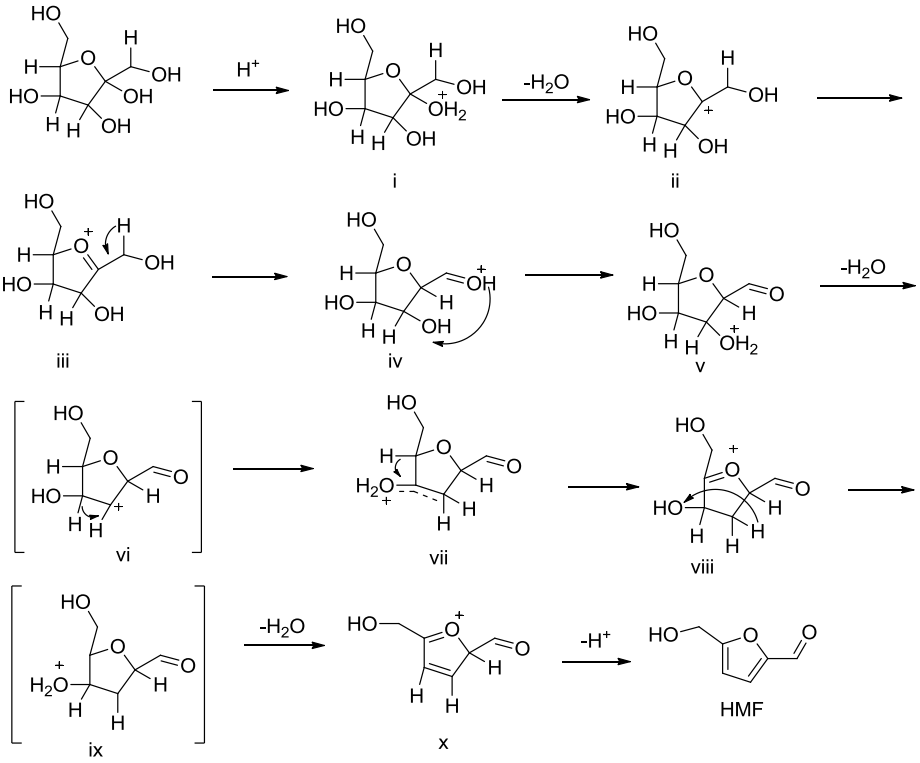
process and several new catalysts have been reported. The carbohydrates dehydration reactions can be arranged by different catalysts, which are divided in four main groups: mineral, organic and solid acids and metal based catalysts.

1.4.1 HMF synthetic problems

HMF is synthesized manly by dehydration of monosaccharides by the loss of three water molecules. Disaccharides or polysaccharides, such as sucrose, cellobiose, inulin or cellulose, can be used as starting material, but a hydrolysis reaction is necessary to depolymerize into their monomers. For example, the main difficulty to transform sucrose into HMF is that the hydrolysis reaction is more efficiently catalyzed by a base, although the dehydration reaction of the fructose monomer is catalyzed by acids. The formation of HMF by dehydration is a very complex process due to the possibility of side reactions. Antal *et al*.[78] reported the possible side products formed by decomposition of fructose in water at high temperatures: isomerization, dehydration, fragmentation and condensation products. The mechanism for fructose dehydration reaction is not clear, and two different pathways are proposed to HMF formation (Scheme 2).[17,78] More recently quantum and molecular mechanics study of the mechanism and energetics of the transformation of fructose to HMF was reported.[79] The proposed mechanism is presented on Scheme 3, where the unstable intermediates are shown in square brackets. The authors found that the reaction proceeds *via* intramolecular hydride transfers, which activation energy is due to the reorganization of the polar solvent environment. The rate determining step was calculated to be the hydride transfer in intermediate **vii**, before the third dehydration, requiring 31.8 kcal/mol of activation free energy.



Scheme 2.



Scheme 3.

1.4.2 Glucose *vs.* Fructose

Glucose (aldose) reactivity is lower than fructose (ketose), this fact have been explained by the much lower relative abundance of acyclic glucose as compared to that of acyclic fructose.[15,80] Glucose can form very stable ring structure, so the enolisation rate in solution is lower than fructose that form less stable ring structures.[15] Since enolisation can be the determining step for HMF formation, fructose will reacts much faster than glucose. On the other hand, fructose forms di-fructose-di-anhydrides in an equilibrium and the most reactive groups are internally blocked, forming less by-products.[15] Glucose forms true oligosaccharides which still contain reactive reducing groups, leaving more risk for cross-polymerization with reactive intermediates and HMF.[15]

1.4.3 HMF isolation methods

In most of the reported studies of HMF synthesis, it was obtained in solution, and the yield determined by HPLC or GC. It is important not only to optimize the synthesis of this compound, but also to develop efficient isolation methods. HMF is not easy to extract from aqueous phase, since the distribution coefficient between the organic and the aqueous phase is not favorable,[15,81] this problem have been partly solved, and several organic solvents have been reported as efficient extraction solvents, such as MIBK (methyl isobutyl ketone)[82, 83-87], DCM[85], ethyl acetate[88-91], THF[92], ether diethylic,[93] or acetone.[94] Organic solvents can improve the HMF synthesis, since they could avoid the formation of by-products, such as

soluble polymers, or humins, among others.[95-97] Polar organic solvents, such as DMSO or DMF,[95,98] have a high boiling point, and due to the reactive nature of HMF at high temperatures,[85,99] distillation is undesirable. Selective absorption of HMF on porous carbons from DMSO has been reported as an alternative approach for it isolation.[100] It was possible to isolate HMF by extraction with a low-boiling point solvent in the presence of ionic liquids.[90,101] In the last few years, several improvements have been achieved in this field, but more efficient separation techniques need to be developed in order to make synthesis economically viable for larger-scale production.

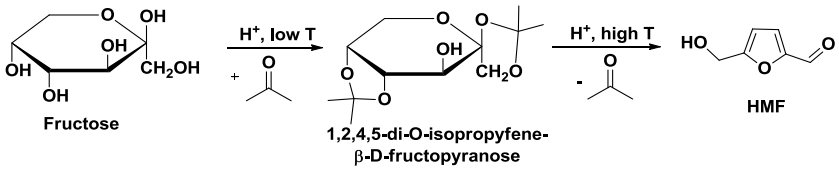
1.4.4 Mineral or organic acid catalysts.

In 1986 Vandam *et al.*[80] studied the dehydration of fructose to HMF in acidic medium. It was observed that the carbohydrate concentration affects the HMF yield, for higher fructose concentrations the HMF yield decreases, probably by reactions of HMF, fructose and their intermediates and formation of larger amount of humins, (**Table 2**, entry 1). The addition of metal chlorides like Cr(III) or Al(III) to the HCl catalyzed dehydration improved the HMF yield (**Table 2**, entry 3). However, the HMF rehydration was also enhanced.[80] The formation of HMF was also affected by the pH, or the nature of the acid, but on the other hand, the rehydration of HMF was not, so an increase of the acid concentration led to an increased HMF yield. The influence of water was also studied, performing the reaction in PEG. This was not the ideal solvent due to the possibility of formation HMF-PEG ethers that induce the formation of levulinic acid, although the HMF yield could be improved to 45%.

HMF synthesis has been already reported before, using PEG 6000 as solvent.[102] A mixture of PEG and fructose (1:1 w/w) became homogeneous after heating and addition of a small amount of acid. Passage of this mixture through a tubular reactor, at high temperatures (120–200°C), led to reasonable HMF yields (**Table 2**, entry 4) in short reaction times, but formation of ethers from the reaction of HMF and PG-600 has been also observed. The isolation of the product from this solvent was a drawback, due to the instability of HMF at high temperatures.

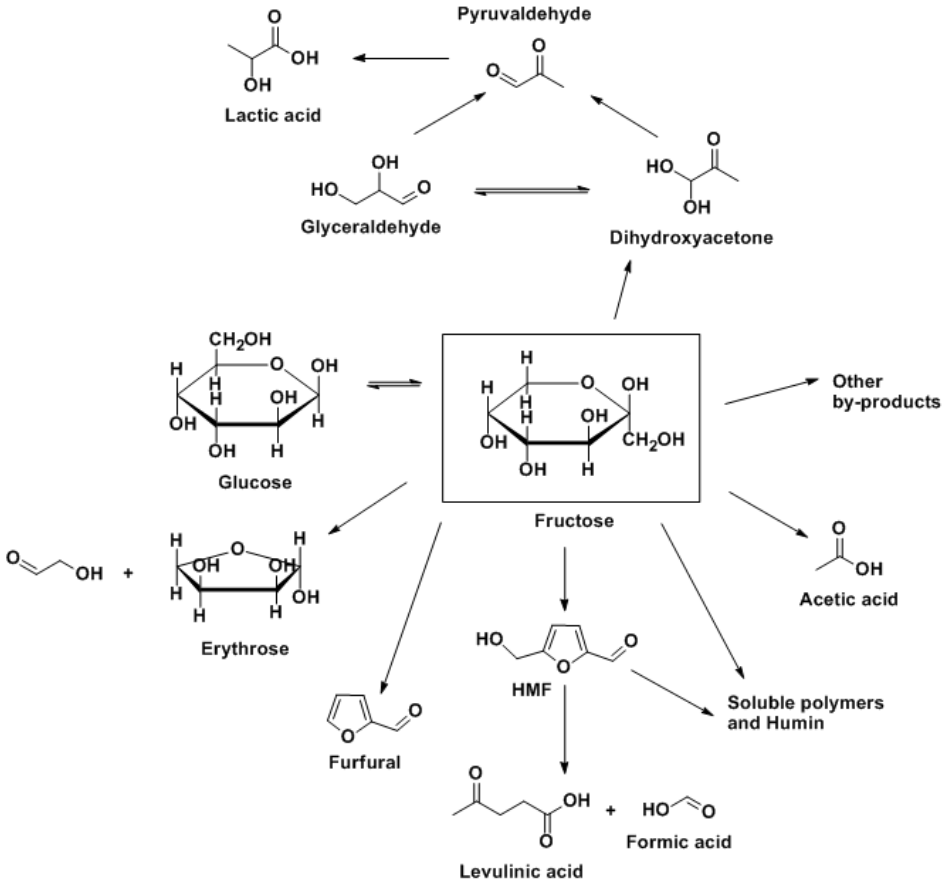
One synthesis of HMF involved dissolving 1,2:4,5-di-o-isopropylidene-β-D-fructopyranose in ethylene glycol dimethyl ether (EGDE) containing water and sulphuric acid as a catalyst.[103] As shown in Scheme 4, the first step was the transformation of fructose into a fructose acetonide derivative followed by rapid dehydration to give HMF. The main advantages of this method were that high reactant concentrations could be achieved using cheap and easily regenerated solvents and the reactive hydroxyl groups of fructose which induce HMF instability are blocked at an earlier stage of the dehydration. However, for

economic reasons, it would be preferable to use a method that directly uses a biomass feedstock rather than another substrate that needs derivatization.



Scheme 4.

Later Asghari *et al.*[104] observed that lower pH led to the rehydration of HMF to levulinic and formic acids, whereas for higher pH the formation of soluble polymers was favored. In this study, hydrochloric, sulphuric, phosphoric, oxalic, citric, maleic, and p-toluenesulfonic acids where tested as catalysts for the dehydration of fructose in sub-critical water (**Table 2**, entry 6). The pH range studied was 1.5-5 using low fructose and several others mono- and di-saccharides concentrations (0.05 M), (**Table 2**, entry 7-12). Different side products where identified and quantified (Scheme 5) using H_3PO_4 as catalyst.

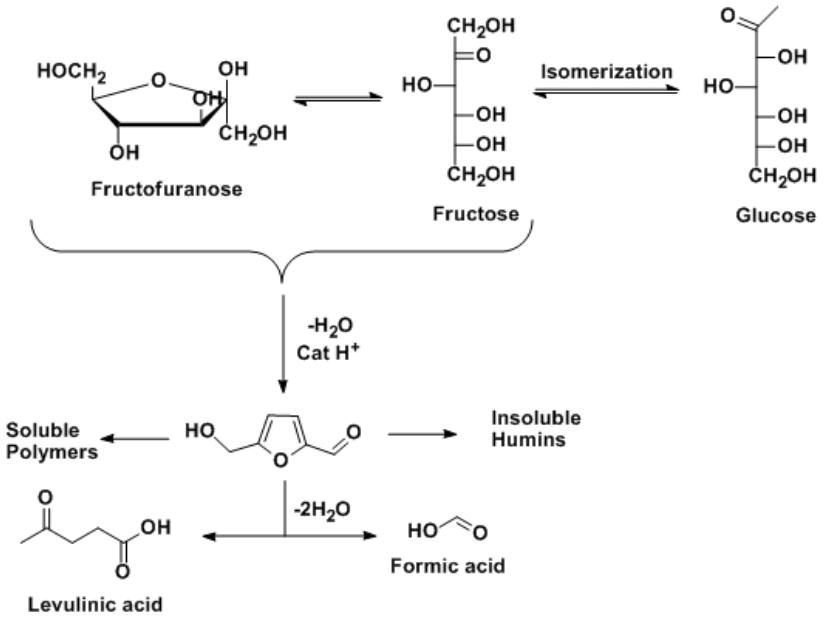


Scheme 5.

Different parameters, such as temperature, pressure and reaction time, of the fructose dehydration reaction in supercritical acetone-water mixture, with sulphuric acid as catalyst

have been studied by Bicker *et al.*[105] Fructose is a carbohydrate with low solubility in acetone, so to enhance the fructose concentration in the reaction mixture water was added to the system. When this system was compared with the same reaction in subcritical water,[104] higher HMF selectivity was observed, especially for glucose and sucrose substrates (**Table 2**, entries 13-16 vs. 6,10,11). No solids (humins) were produced, although other by-products such as furfural, glucose, methylglyoxal, dihydroxyacetone and levulinic acid were formed, although in less than 6% yield. Later the same group reported[106] a study of the same reaction performed in sub- and supercritical methanol and subcritical acetic acid and obtained the resulting furfural-ether, 5-methoxymethylfurfural with 79% selectivity and 99% conversion and –ester, 5-acetoxymethylfurfural with 38% selectivity and 98% conversion.

A microwave assisted dehydration of highly concentrated aqueous fructose to HMF in 100% water and HCl as catalyst was reported by Hansen *et al.*[107] (Scheme 6). In this work a fructose aqueous solution (27 wt %) was microwave irradiated for 1 second (200°C) providing 52% conversion with a HMF selectivity of 63%. For longer irradiation times (60 sec) 95% conversion was achieved, but with lower HMF selectivity, 55%, (**Table 2**, entries 17 and 18). Consequently, a slight improvement was achieved compared to conventional heating (for example **Table 2**, entry 40).



Scheme 6.

Roman-Leshkov *et al.*[86] described an improved method for biphasic acid catalyzed fructose dehydration in high concentrations (30-50 wt.%), by adding modifiers in the both phases of the reaction. As reported before,[80] the high concentrations of fructose increased the amount of side products. To overcame this problem, the authors added to the aqueous phase a polar aprotic solvent (DMSO or 1-methyl-2-pyrrolidinone (NMP)) and/or a hydrophilic

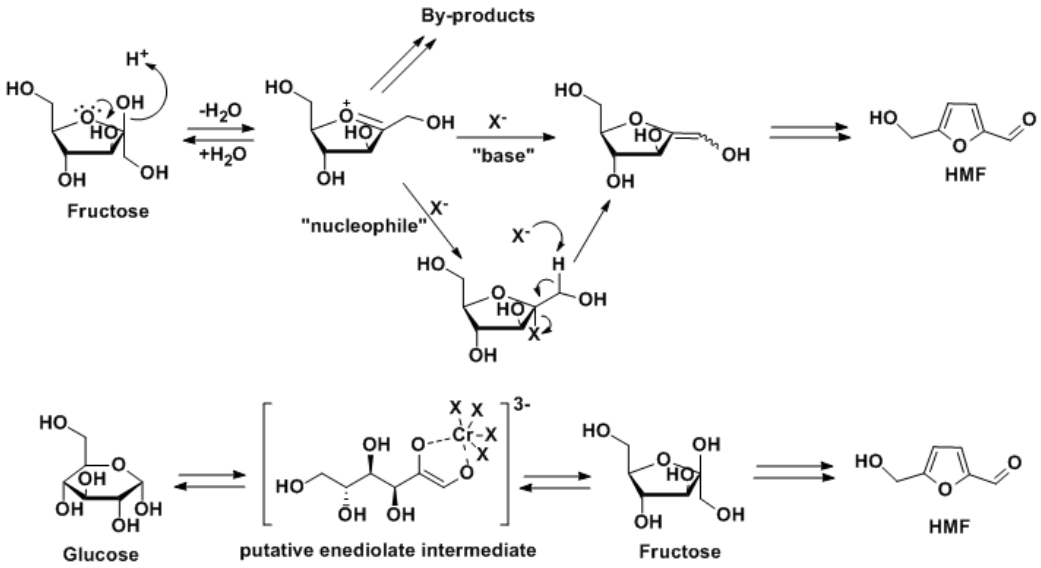
polymer (poly(1-vinyl-2-pyrrolidinone)-PVP), improving the HMF selectivity (**Table 2**, entries 20 and 21). These modifiers in the aqueous phase increased the HMF solubility deteriorating the extraction process with methyl isobutyl ketone (MIBK). By adding 2-butanol into the organic phase, the HMF solubility was increased, improving the extraction process. Several mineral acids were tested and HCl was found to provide best HMF selectivity.

Further studies have been performed[85] by the same authors, where they optimized a glucose dehydration method, achieving up to 53% HMF selectivity (**Table 2**, entries 22 and 23). In this work they employed HCl as catalyst in aqueous phase with DMSO as co-solvent and an extraction phase of MIBK/2-butanol, or DCM. The best conditions for the dehydration of glucose were applied to other saccharides such as inulin, starch, cellobiose and sucrose (**Table 2**, entries 25-34). The main drawback of this method was the HMF separation from the DMSO at the end, which is a complicated process due to reactive nature of HMF at high temperatures.[85,99]

Roman-Leshkov *et al.*[84] reported an acid catalysis dehydration of fructose in a biphasic reactor, where different extraction solvents were studied (**Table 2**, entries 35-40). Since HMF selectivity increased along with the efficiency of the extracting solvent the authors added NaCl to the aqueous phase in order to increase extraction efficiency by a salt-in effect. The advantages of this method are that no DMSO was added to the aqueous phase, and the chosen extracting solvent, 1-butanol, can be obtained by biomass-derived carbohydrates fermentation.[84] The addition of salt also prevents the system to form only one phase, as it was observed when 2-butanol was used as an extraction solvent in absence of NaCl. The fructose concentrations used were higher compared with previous work.[85] However, the achieved conversion and HMF selectivity were in general not that good (**Table 2**, entries 25 *vs.* 35). Different classes of extraction solvent, such as aliphatic alcohols, ketones, and ethers in the C3–C6 range, were tested on the dehydration reaction of fructose in saturated aqueous solution of NaCl.[108] Solvents with four carbon atoms (C-4) provided the highest HMF selectivity within each solvent class. (**Table 2**, entries 47 and 49-51). Higher reaction temperature induced higher HMF selectivity, but on the other hand the temperature has to be sufficiently low to avoid solvent degradation reactions. The application of 1-butanol as an extracting solvent and the effect of different salts on the dehydration reaction were studied. It was shown that KCl and NaCl provide the best combination of extracting efficiency and HMF selectivity (**Table 2**, entries 41-43).[108] The authors described that HMF selectivity could be improved using saturated aq.NaCl but the fructose conversion was lower compared

to pure water (**Table 2**, entries 41 *vs.* 44). No details about the isolation of the final product were provided in this work.

Binder and Raines reported[109] the use of authentic lignocellulosic biomass as feedstok for HMF production in DMA-LiCl (N,N-dimethylacetamide- Lithium chloride) as solvent. Initially they studied fructose as a starting material, with H_2SO_4 as catalyst and DMA-Lithium salt as solvent and tested several additives, such as [EMIM][Cl] (1-ethyl-3-methylimidazolium chloride) and other ionic liquids. When different lithium salts were tested, it was observed that fluoride ions were completely ineffective for this transformation (**Table 2**, entry 56) in contrast with bromide and iodide ions, which tend to be less ion-paired than fluoride and chloride,[109] providing HMF in up to 92% yield (**Table 2**, entry 57). Based on these and others experimental results the authors proposed a reaction mechanism, involving attack of the halide ion toward the formed from fructose oxocarbenium ion (Scheme 7). HMF production starting from glucose was also tested with $CrCl_2$, $CrCl_3$ or $CrBr_3$ as catalyst and DMA-LiCl (or other salts such as LiBr, LiI) as solvent (**Table 2**, entries 58-61). Strong effect of the halide was observed. In presence of chloride anions, $CrCl_2$ as catalyst and DMA-LiCl as solvent, HMF was obtained in up to 60% yield (**Table 2**, entry 58) that was improved to 62% upon the addition of [EMIM][Cl] as additive (**Table 2**, entry 59).The addition of iodide ions together $CrCl_2$ as catalyst did not improved the HMF yields, which was not the case of bromide ions that improved the yield in up to 80% (**Table 2**, entry 61). Cellulose was also tested as a feedstock with chromium chlorides and HCl as catalysts. Dehydration of purified cellulose in solution of DMA-LiCl and [EMIM]Cl and $CrCl_2$ or $CrCl_3$ catalysts provided HMF in up to 54% yield within 2h at 140°C (**Table 2**, entries 62, 63). Due to cellulose insolubility neither lithium iodide nor lithium bromide improved the HMF yields (**Table 2**, entry 64). Finally the synthesis of HMF from lignocellulosic biomass was studied. 48% HMF yield (based on cellulose content of the biomass) was achieved under similar conditions applied for cellulose (**Table 2**, entry 65). The authors propose that the formation of HMF from cellulose in DMA-LiCl proceed *via* saccharification followed by isomerization of the glucose monomers into fructose and further dehydration to give HMF (Scheme 7). In this work it was possible to isolate HMF by an ion-exclusion chromatography in up to75% HMF recovery.



Scheme 7.

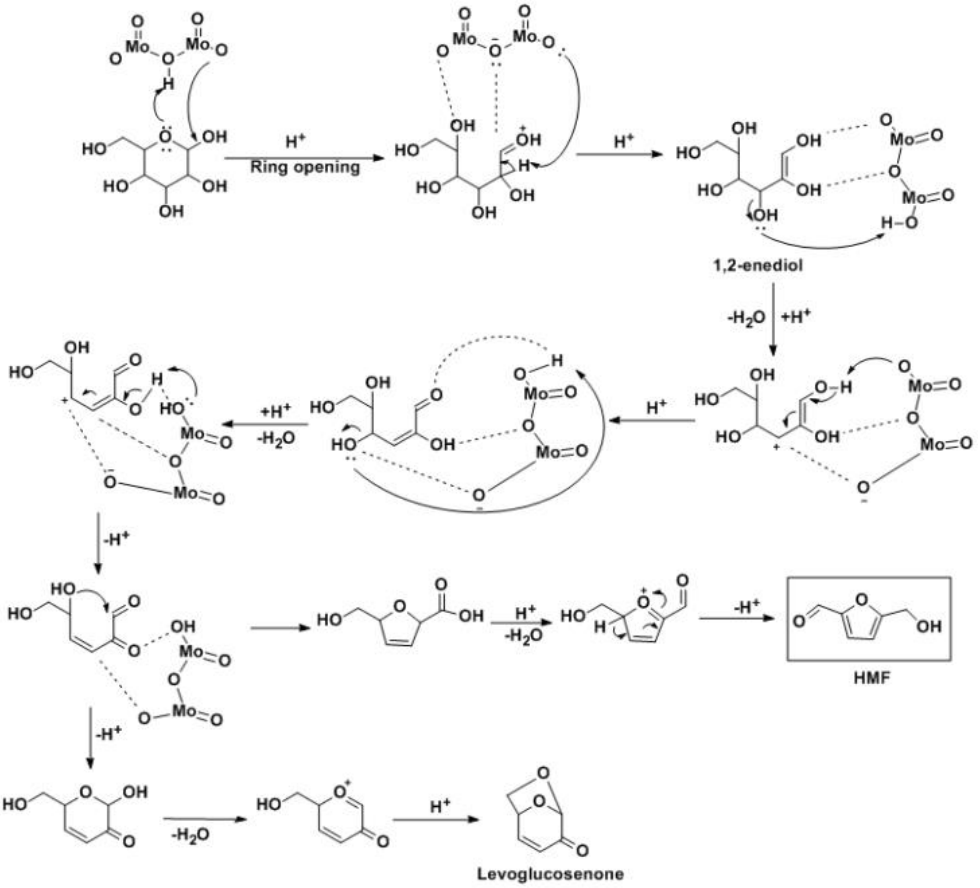
Almost complete conversion of fructose, glucose and mannose was observed in the presence of H_2SO_4 at 120°C within 4 hours, in an ionic liquid [BMIM][Cl] (1-butyl-3-methylimidazolium chloride),[110] in case of fructose HMF was observed to be the main product (**Table 2**, entry 19), in 85% yield. The authors reported that a similar result was obtained when the reaction was performed in absence of H_2SO_4. Although glucose and mannose also undergo almost complete conversion, HMF was not the major product, confirming that dehydration of ketoses is quicker than aldoses.[17] The HMF stability in [BMIM][Cl]/H_2SO_4 was also studied and at the end it was almost completely recovered, only 7% conversion and 1% solid residues were observed. Other studies on HMF stability in [BMIM][Cl] under different reaction conditions[111,112] also resulted in complete HMF recovery confirming that HMF is stable in [BMIM][Cl]. However when HMF and glucose mixtures were tested ([BMIM][Cl]/H_2SO_4 at 120°C after 4 hours), an increase of the solid residues was observed, which indicates that HMF can react with monosaccharides or monosaccharides degradation products under these conditions.

The use of microreactors could provide many advantages compared with the conventional batch reaction conditions, including better control of the reaction conditions (temperature, pressure and residence time), improved safety, and portability.[113] HCl-catalyzed dehydration of fructose in aqueous solutions was performed in a continuous microreactor (**Table 2**, entries 66-70)[114] When the process was compared with an HCl-catalyzed dehydration of fructose under microwave irradiation (**Table 2**, entries 17 and 18), at 200°C the HMF selectivity and fructose conversion were slightly improved (**Table 2**, entry 66). In this work the authors were able to improve the HMF selectivity by decreasing the temperature to 185°C (**Table 2**, entry 67). The conversion of fructose and HMF selectivity were additionally improved by using

DMSO as co-solvent and MIBK/2-butanol as extraction phase (**Table 2**, entry 69). A highly concentrated solution of fructose (50 wt.%) was converted in 98%, with 81% HMF selectivity (**Table 2**, entry 70).

Highly concentrated melt systems formed with choline chloride (ChCl) and up to 50 wt% of carbohydrates were tested in dehydration reactions with different catalysts.[115] For fructose and inulin the best catalyst was PTSA (*p*-toluenesulfonic acid) (**Table 2**, entries 71 and 72), and for glucose and sucrose the best catalyst was $CrCl_2$ providing HMF yields of 45 and 62% respectively (**Table 2**, entries 73 and 74). Although some of the reactions were only analyzed by HPLC it was also reported a method for extraction of HMF with ethyl acetate. A preliminary ecological evaluation was made and the recyclability of the process was studied.

Several liquid (H_2SO_4, CF_3SO_3H, CH_3SO_3H, CF_3COOH, HNO_3, HCl and H_3PO_4) and solid acids, [12-tungstophosphoric acid (12-TPA ($H_3PW_{12}O_{40}$)), 12-molybdophosphoric acid (12-MPA ($H_3PMo_{12}O_{40}$)), 12-tungstosilicic acid (12-TSA ($H_3SiW_{12}O_{40}$)), and 12-molybdosilicic acid (12-MSA ($H_3SiMo_{12}O_{40}$))] were tested as catalysts for the glucose dehydration to HMF in [EMIM][Cl] as a solvent (**Table 2**, entries 75-89).[91] With all of tested catalysts formation of 4 to 20% of humins, and others by-products have been observed. 12-MPA was chosen for further studies due to its best performance and selectivity (**Table 2**, entry 83). Several others ionic liquids, such as [EMIM][Cl], [BDMIM][Cl] (1-butyl-2,3-dimethylimidazolium chloride) and [BMPy][Cl] (1-butyl-3-methylpyridinium chloride) were tested (**Table 2**, entries 85-87). Lower activity of 12-MPA in [BDMIM][Cl] and [BMPy][Cl] was observed compared to the other two. It was suggested that the lower activity in [BDMIM][Cl] is due the acidic proton lost from the imidazolium cation. The addition of acetonitrile as co-solvent to [BMIM][Cl] and [EMIM][Cl] enhances the glucose conversion in up to 99% along with 98% HMF selectivity, moreover no formation of humins was observed (**Table 2**, entries 88 and 89). A glucose dehydration mechanism by 12-MPA was proposed (Scheme 8).

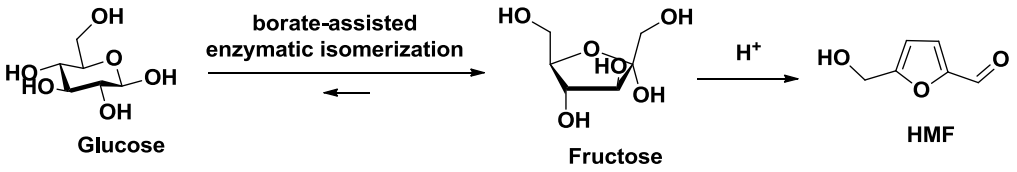


Scheme 8.

The authors suggested that the key intermediate in the reaction pathway was 1,2-enediol. The high selectivity of heteropoly acids was attributed to the stabilization of the reaction intermediates involved in the formation of HMF. In the absence of acetonitrile as a co-solvent, moderate amounts of humins were formed.

CO_2–water system could replace conventional acids such as HCl and H_2SO_4 for the catalysis of some chemical reactions, providing the advantage of simple neutralization *via* depressurization without salt disposal.[116] CO_2 in aqueous solution can generate *in situ* carbonic acid, which acts as catalyst for the dehydration reaction. Due to the correlation of the pH of the solution and CO_2 pressure lower pH values could be achieved under higher CO_2 pressures. Han *et al.*[116] reported an optimum CO_2 pressure of 6 MPa for the dehydration of inulin, resulting in a maximum HMF yield. The authors studied the effects of temperature, time and inulin initial concentration on the HMF yield. Similar behavior was observed when the conditions were varied: HMF yield increased until it reached a maximum value, after which it started decreasing. This was explained by the HMF high reactivity, resulting in by-products formation. The optimal conditions were found to be 6 MPa CO_2, at 200°C, for 0.75 hours, which led to a 53% HMF yield (**Table 2**, entry 90).

One of the main routes for the glucose transformation to HMF involves an isomerization step to fructose, followed by a fast dehydration reaction.[83,93,98,117] Using this approach Huang *et al.*[118] reported a borate-assisted enzymatic isomerization of glucose and sequential dehydration of fructose to HMF in acidic medium (Scheme 9) using 1-butanol as an extracting solvent. The addition of sodium tetraborate during the isomerization step provided 88.2% sugar conversion and after subsequent dehydration 63.3% HMF yield was obtained (**Table 2**, entry 91).



Scheme 9.

A patent published in 2009[119] claims that a biphasic reaction of aqueous solution of fructose, sulphuric acid as catalyst and dioxane as extracting solvent reduce the process time. Yokoyama *et al.*[120] reported the application of acidic ionic liquids as catalysts for the dehydration of fructose. Both 1-methyl-3-(butyl-4-chlorosulfonyl) imidazolium chlorosulfate and 1-methyl-3-(butyl-4-sulfonyl)imidazolium hydrogensulfate were tested and the first one was found to be much more efficient. Acetonitrile was used as a solvent but the authors found that certain amount of water improve the reaction performance. Under the optimized conditions 81% yield of HMF was obtained[120] (**Table 2**, entry 92). Jerome *et al.*(**Table 2**, entry 94) reported that fructose can be conveniently dehydrated to HMF in a ChCl/CO_2 system with up to 72% HMF yield. Moreover HMF was found to be highly stable in the presence of ChCl, presumably through the formation of an eutectic mixture, thus allowing the dehydration to be performed in high content of fructose (up to 100 wt%).

A biphasic system consisting of THF and concentrated $NaHSO_4$–$ZnSO_4$ aqueous solution was developed for efficient degradation of cellulose into HMF by Ma *at al.* (**Table 2**, entry 95). The high concentration of the catalysts in the aqueous solution and the high volume ratio of organic phase to aqueous phase were found to be important for the reaction performance. The depolymerization of cellulose was the rate-determine step, leading to low concentration of glucose in the solution and thus suppressing the side reactions, such as humins and char formation.

Table 2 Conversion of carbohydrates to HMF catalyzed by mineral or organic acids.

Entry	Biomass source	Solvent	Catalyst	T°C	time	Conversion (%)	HMF Selectivity (%)	Isolation/analysis
1[80]	Fructose	H_2O	PTSA	88	3.3h	-	≈20	HPLC
2[80]	Fructose	H_2O/PEG 4000 (1:1)	PTSA	88	3.3h	-	≈45	HPLC
3[80]	Fructose	$H_2O/CrCl_3$	PTSA	88	3.3h	-	≈20	HPLC
4[102]	Fructose	Fructose/PEG 6000 (1:1)	HCl	180	10s	-	65	-
5[103]	Fructose	EG/dimethyl ether[b]	H_2SO_4	200	3.3h	100	70.0	GC
6[104]	Fructose 0.05M	Sub-Critical Water	HCl H_2SO_4 H_3PO_4 citric acid maleic acid PTSA oxalic acid	240	120s	-	44.7 40.3 65.3 49.3 60.0 37.0 17.4	HPLC
7[104]	L-sorbose 0.05M	Sub-Critical Water	H_3PO_4	240	120s	-	50.0	HPLC
8[104]	D-mannose 0.05M	Sub-Critical Water	H_3PO_4	240	120s	-	31.1	HPLC
9[104]	D-galactose 0.05M	Sub-Critical Water	H_3PO_4	240	120s	-	27.3	HPLC
10[104]	D-glucose 0.05M	Sub-Critical Water	H_3PO_4	240	120s	-	30.0	HPLC
11[104]	Sucrose 0.05M	Sub-Critical Water	H_3PO_4	240	120s	-	40.1	HPLC
12[104]	Cellobiose 0.05M	Sub-Critical Water	H_3PO_4	240	120s	-	27.2	HPLC
13[105]	Fructose	Sub-Critical acetone/water (90:10)	H_2SO_4	180, 20MPa	120s	-	77	HPLC
14[105]	Glucose	Sub-Critical acetone/water (90:10)	H_2SO_4	180, 20MPa	120s	-	48	HPLC
15[105]	Sucrose	Sub-Critical acetone/water (90:10)	H_2SO_4	180, 20MPa	120s	-	56	HPLC
16[105]	Inulin	Sub-Critical acetone/water (90:10)	H_2SO_4	180, 20MPa	120s	-	78	HPLC
17[107]	Fructose 27% aq. sol.	H_2O	HCl	200, MW	1s	52	63	HPLC
18[107]	Fructose 27% aq. sol.	H_2O	HCl	200, MW	60s	95	55	HPLC
19[110]	Fructose	[BMIM][Cl]	H_2SO_4	120	4h	100	85	HPLC
20[86]	Fructose 30 wt.%	[7:3(8:2(H2O:DMSO):PVP]/ [7:3 (MIBK:2-butanol)]	HCl	200	3min	89	85	MIBK:2-butanol (7:3) extraction
21[86]	Fructose 50 wt.%	[7:3(8:2(H_2O: DMSO):PVP]/ [7:3 (MIBK:2-butanol)]	HCl	200	3min	92	77	MIBK:2-butanol (7:3) extraction
22[85]	Glucose 10 wt.%	[4:6 (H_2O:DMSO)]/[7:3(MIBK:2-butanol]	HCl	170	10min	43	53	MIBK:2-butanol extraction

23[85]	Glucose 10 wt.%	[5:5 (H_2O:DMSO)]/[7:3(MIBK:2-butanol]	HCl	170	17min	50	47	MIBK:2-butanol extraction
24[85]	Glucose 10 wt.%	[3:7 (H_2O:DMSO)]/[DCM]	-	140	4.5h	62	48	DCM extraction
25[85]	Fructose 10 wt.%	[5:5 (H_2O:DMSO)]/[7:3(MIBK:2-butanol]	HCl	170	4min	95	89	MIBK/2-butanol extraction
26[85]	Fructose 10 wt.%	[3:7 (H_2O:DMSO)]/[DCM]	-	140	2h	100	87	DCM extraction
27[85]	Inulin 10 wt.%	[5:5 (H_2O:DMSO)]/[7:3(MIBK:2-butanol]	HCl	170	5min	98	77	MIBK/2-butanol extraction
28[85]	Inulin 10 wt.%	[3:7 (H_2O:DMSO)]/[DCM]	-	140	2.5h	100	70	DCM extraction
29[85]	Sucrose 10 wt.%	[4:6 (H_2O:DMSO)]/[7:3(MIBK:2-butanol]	HCl	170	5min	65	77	MIBK/2-butanol extraction
30[85]	Sucrose 10 wt.%	[3:7 (H_2O:DMSO)]/[DCM]	-	140	4.5h	82	62	DCM extraction
31[85]	Cellobiose 10 wt.%	[4:6 (H_2O:DMSO)]/[7:3(MIBK:2-butanol]	HCl	170	10min	52	52	MIBK/2-butanol extraction
32[85]	Cellobiose 10 wt.%	[3:7 (H_2O:DMSO)]/[DCM]	-	140	9.5h	85	45	DCM extraction
33[85]	Starch 10 wt.%	[4:6 (H_2O:DMSO)]/[7:3(MIBK:2-butanol]	HCl	170	11min	61	43	MIBK/2-butanol extraction
34[85]	Starch 10 wt.%	[3:7 (H_2O:DMSO)]/[DCM]	-	140	11h	91	40	DCM extraction
35[84]	Fructose 30 wt.%	(H_2O, 35% NaCl)/1-butanol	HCl	180	-	64	84	1-butanol extraction
36[84]	Fructose 30 wt.%	(H_2O, 35% NaCl)/2-butanol	HCl	180	-	71	79	2-butanol extraction
37[84]	Fructose 30 wt.%	(H_2O, 35% NaCl)/1-hexanol	HCl	180	-	78	72	1-hexanol extraction
38[84]	Fructose 30 wt.%	(H_2O, 35% NaCl)/MIBK	HCl	180	-	72	77	MIBK extraction
39[84]	Fructose 30 wt.%	(H_2O, 35% NaCl)/(Toluene/2-butanol)	HCl	180	-	74	88	Toluene/2-butanol extraction
40[84]	Fructose 30 wt.%	(H_2O, 35% NaCl)	HCl	180	-	59	57	HPLC
41[108]	Fructose 30 wt.%	(NaCl sat. solution)/1-butanol	HCl	180	35min	87	82	HPLC
42[108]	Fructose 30 wt.%	(KCl sat. solution)/1-butanol	HCl	180	15min	89	84	HPLC
43[108]	Fructose 30 wt.%	(CsCl sat. solution)/1-butanol	HCl	180	15min	92	80	HPLC
44[108]	Fructose 30 wt.%	1-butanol	HCl	150	35min	93	69	HPLC
45[108]	Fructose 30 wt.%	(NaCl sat. solution)/1-pentanol	HCl	150	35min	75	77	HPLC
46[108]	Fructose 30 wt.%	(NaCl sat. solution)/2-propanol	HCl	150	35min	39	80	HPLC
47[108]	Fructose 30 wt.%	(NaCl sat. solution)/2-butanol	HCl	150	35min	67	85	HPLC
48[108]	Fructose 30 wt.%	(NaCl sat. solution)/2-pentanol	HCl	150	35min	83	82	HPLC
49[108]	Fructose 30 wt.%	(NaCl sat. solution)/2-butanone	HCl	150	35min	84	82	HPLC
50[108]	Fructose 30 wt.%	2-butanone	HCl	150	35min	92	73	HPLC

51[108]	Fructose 30 wt.%	(NaCl sat. solution)/THF	HCl	150	65min	53	83	HPLC
52[108]	Fructose 30 wt.%	THF	HCl	150	35min	95	71	HPLC
53[108]	Fructose 30 wt.%	(NaCl sat. solution)/THF	HCl	160	50min	88	89	HPLC
54[109]	Fructose 10 wt.%	DMA-LiCl	H_2SO_4	100	5h	-	63	HPLC
55[109]	Fructose 10 wt.%	DMA/[EMIM][Cl]	H_2SO_4	100	2h	-	84	HPLC
56[109]	Fructose 10 wt.%	DMA/LiF	H_2SO_4	80	2h	-	0	HPLC
57[109]	Fructose 10 wt.%	DMA/ LiBr NaBr LiI NaI	H_2SO_4	100	4h 2h 6h 5h	-	92 93 89 91	HPLC
58[109]	Glucose 10 wt.%	DMA-LiCl	$CrCl_2$	100	5h	-	60	HPLC
59[109]	Glucose 10 wt.%	DMA-LiCl/[EMIM][Cl]	$CrCl_2$	100	6h	-	62	HPLC
60[109]	Glucose 10 wt.%	DMA/LiI	$CrCl_2$	100	4h	-	54	HPLC
61[109]	Glucose 10 wt.%	DMA/LiBr	$CrBr_2$	100	6h	-	80	HPLC
62[109]	Cellulose	DMA-LiCl/ [EMIM][Cl]	$CrCl_2$ / HCl	140	2h	-	54	HPLC
63[109]	Cellulose	[EMIM][Cl]	$CrCl_2$ / HCl	140	1h	-	53	HPLC
64[109]	Cellulose	DMA/LiI LiBr	$CrCl_2$ / HCl	140	3h	-	<1 <1	HPLC
65[109]	Corn stover	DMA-LiCl/[EMIM][Cl]	$CrCl_2$ / HCl	140	2h	-	48	HPLC
66[114]	Fructose	H_2O	HCl Microreactor	200, 17 bar	1min	97	59	HPLC
67[114]	Fructose	H_2O	HCl Microreactor	185, 17 bar	1min	71	75	HPLC
68[114]	Fructose 10 wt.%	1:2 [(H_2O:DMSO)]/[(MIBK:2-butanol]	HCl Microreactor	185, 17 bar	1min	100	72	HPLC
69[114]	Fructose 30 wt.%	1:5 [(H_2O:DMSO)]/[(MIBK:2-butanol]	HCl Microreactor	185, 20 bar	1min	98	85	HPLC
70[114]	Fructose 50 wt.%	1:5[(H_2O:DMSO)]/[(MIBK:2-butanol]	HCl Microreactor	185, 20 bar	1min	98	81	HPLC
71[115]	Fructose	Fructose/ChCl (4:6)	PTSA	100	30min	-	67	EtOAc extraction/ HPLC
72[115]	Inulin	Fructose/ChCl (5:5)	PTSA	90	1h	-	57	EtOAc extraction/ HPLC
73[115]	Glucose	Fructose/ChCl (4:6)	$CrCl_2$	110	30min	-	45	EtOAc extraction/ HPLC

74[115]	Sucrose	Fructose/ChCl(5:5)	$CrCl_2$	100	1h	-	62	EtOAc extraction/ HPLC
75[91]	Glucose	[EMIM][Cl]	H_2SO_4	120	3h	93	66	EtOAc extraction
76[91]	Glucose	[EMIM][Cl]	CF_3SO_3H	120	3h	87	46	EtOAc extraction
77[91]	Glucose	[EMIM][Cl]	HNO_3	120	3h	56	77	EtOAc extraction
78[91]	Glucose	[EMIM][Cl]	CF_3COOH	120	3h	58	75	EtOAc extraction
79[91]	Glucose	[EMIM][Cl]	HCl	120	3h	53	62	EtOAc extraction
80[91]	Glucose	[EMIM][Cl]	CH_3SO_3H	120	3h	73	58	EtOAc extraction
81[91]	Glucose	[EMIM][Cl]	H_3PO_4	120	3h	17	95	EtOAc extraction
82[91]	Glucose	[EMIM][Cl]	12-TPA	120	3h	82	81	EtOAc extraction
83[91]	Glucose	[EMIM][Cl]	12-MPA	120	3h	71	89	EtOAc extraction
84[91]	Glucose	[EMIM][Cl]	12-TSA	120	3h	69	82	EtOAc extraction
85[91]	Glucose	[BMIM][Cl]	12-MPA	120	3h	71	89	EtOAc extraction
86[91]	Glucose	[BDMIM][Cl]	12-MPA	120	3h	57	88	EtOAc extraction
87[91]	Glucose	[BMPy][Cl]	12-MPA	120	3h	52	87	EtOAc extraction
88[91]	Glucose	[EMIM][Cl]/ACN	12-MPA	120	3h	99	98	EtOAc extraction
89[91]	Glucose	[BMIM][Cl]/ACN	12-MPA	120	3h	99	98	EtOAc extraction
90[116]	Inulin	H_2O	6 MPa CO_2	200	45min	100	53	HPLC
91[118]	Glucose	H_2O	1.GI/ $Na_2B_4O_7$ 2.(HCl/NaCl)/1-butanol	190	45min	88.2	63.3	HPLC
92[120]	Fructose	ACN/water	$[MBCIm]SO_3Cl$[c]	80	3h	-	81[a]	HPLC
93[121]	Glucose	[BMIM]Cl	TPA/boric acid[c]	140	40min		52[a]	HPLC
94[122]	Fructose	ChCl[b]	CO_2, 4MPa	120	1.5h		72[a]	HPLC
95[123]	Cellulose	THF/aq. $NaHSO_4$–$ZnSO_4$		160	1h	96	53	HPLC

[a] HMF yield. [b] Reaction media reused. [c] Catalyst reused.

1.4.5 Solid acid catalysts.

Vinke *et al.*[124] reported fructose dehydration using a set-up constructed by a column full with ion exchange resin as catalyst and a separate loop for adsorption of HMF on activated carbon. HMF was selectively adsorbed during the reaction, and later extracted with organic solvents. Although the reaction temperature was not that high 77% HMF selectivity could be achieved in 48 hours (**Table 3**, entry 1).

Fructose dehydration in organic solvents instead of in aqueous solutions results in improved HMF selectivity and DMSO was found to be one of the best performing solvents.[95,97,125,126] Halliday *et al.*[126] reported one-pot synthesis of 2,5-diformylfuran (DFF) *via* fructose dehydration to HMF. The author's claimed that they tried to reproduce several reported methods for HMF synthesis and the obtained yields were not reproducible. So they developed a new method for HMF synthesis using an ion-exchange resin as catalyst in DMSO (**Table 3**, entries 2 and 3).

In 2009 Shimizu *et al.*[127] reported fructose dehydration in DMSO and tested several heterogeneous catalysts (heteropoly acid, zeolite and acidic resin). In order to suppress the negative effect of the water formed during the reaction a mild evacuation method was developed by performing the reaction under vacuum (0.97×10^5 Pa). In this way fructose conversion was improved to 100% and HMF yield was increased to 97% (**Table 3**, entry 8). The authors also proved that the evacuation under reduced pressure is more efficient then molecular sieves (**Table 3**, entries 8, 9 and 10). 100% conversion with 100% HMF selectivity was achieved from 50 wt.% fructose solutions in DMSO with powdered (0.15-0.053 mm) amberlyst-15 as catalyst (**Table 3**, entries 6 and 7) even without water evacuation. The authors propose that reducing the particles size of the catalysts enhances the removal of adsorbed water from the surface and near-surface of the catalyst.[127]

In 2007 Dumesic *et al.*[128] reported fructose dehydration in a systems containing NMP as additive in the aqueous phase. MIBK or DCM were used as extracting solvents. Different substrates, such as fructose, inulin and sucrose were studied with ion exchange resin as catalyst. The inulin dehydration was complete with HMF selectivity of 69% (**Table 3**, entry 13). When sucrose was used as a feedstock under the same reaction conditions (**Table 3**, entry 15) only the fructose part of the molecule was observed to react, providing a conversion of 60%, with a HMF selectivity of 74%. The dehydration reaction occurs even without the presence of the resin catalyst, with similar HMF selectivity, but the presence of catalyst allowed a decreased reaction temperature from 120 to 90°C. The authors studied DMSO as a co-solvent in the aqueous phase, and conclude that the increase of DMSO quantity increases

the HMF selectivity. However the use of DMSO was a drawback since it was complicating the HMF separation procedure. (**Table 3**, entries 12, 14 and 16).

As it was already discussed DMSO can improve the fructose dehydration, avoiding the formation of by-products[95,126] such as levulinic acid and humins from HMF, but this solvent has the drawback of difficult separation from the final product. In attempted to overcame this problem, acetone/DMSO (7:3) was reported as a solvent system for the dehydration of fructose in the presence of a strong acid cation-exchange resin catalyst under microwave irradiation.[97] The authors claimed that the use of acetone is beneficial due to the low boiling point so a better separation of the final product could be achieved. Due to the low solubility of fructose in acetone, DMSO was used as a co-solvent. Two different fructose concentrations were tested, and under the same reaction conditions and insignificant decrease of the HMF selectivity in higher fructose concentration was observed (**Table 3**, entries 17 and 18). The catalyst was recycled for at least 5 cycles without the loss of selectivity or efficiency.

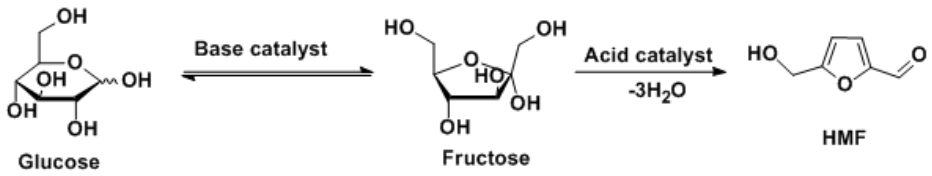
Heating an aqueous fructose solution under microwave irradiation (150°C) with a resin as catalyst provided good conversion (82.6%) but with very low HMF selectivity (**Table 3**, entry 19).[129] In order achieve better yields, acetone was used as a co-solvent since fructose has been shown to rearrange to its furanoid form in acetone–water mixtures, thus favoring the HMF formation.[129] Acetone proved to have positive effect on the reaction outcome and under these reaction conditions the effect of the initial fructose concentration was studied. It was observed that HMF selectivity slowly decreased with increasing the concentrations (**Table 3**, entries 20, 21, 22 and 23). Microwave irradiation was proved to be beneficial compared with conventional heating, providing higher fructose conversion and HMF yield (**Table 3**, entry 28). The catalyst was recycled for 5 cycles without loss of selectivity or efficiency.

The dehydration of fructose using [BMIM][Cl] with amberlyst-15 as catalyst was developed by Qi *et al*.[112] several mineral and Lewis acids, and solid acid anion-exchange resins were tested. However, amberlyst-15 exhibit the best performance. This catalytic system resulted in a 98.6% fructose conversion with a selectivity of 83.3% for HMF, at 80ºC after 10 min (**Table 3**, entries 25 and 26). The water content in [BMIM][Cl] was observed to be important since water in above 5 wt.% resulted in decreased HMF selectivity. Although no HMF self-polymerization products were formed, other by-products were detected by HPLC, such as glucose, levulinic acid and formic acid, even in low yields. The authors tested the HMF stability under the reaction conditions by adding an HMF sample in the reaction, the sample was later recovered in 99.8%. HMF was extracted with ethyl acetate at the end and [BMIM][Cl] and the catalyst were recycled for at least 7 cycles.[112]

The same authors[111] reported the conversion of fructose into HMF using the same catalyst and solvent, but under milder reaction conditions at room temperature (**Table 3**, entry 27). The [BMIM][Cl] viscosity at room temperature was high and it was impossible to stir the reaction mixture without the addition of a co-solvent. Small amounts of different co-solvents were added, such as DMSO, methanol, ethanol, ethyl acetate, supercritic carbon dioxide. Acetone was the most efficient although with all the others organic solvents the conversion and HMF yields were also above 80%. To improve the reaction efficiency fructose has to be pre-dissolved in the ionic liquid in a water bath at 80°C for 20 minutes. The reaction time needs to be longer than previously reported[112] (6 hours *vs.* 10 minutes). However the method has the advantages of being performed at room temperature together with the formation of only 2% of by-products.[111]

A hydrophobic and hydrophilic ionic liquids (1-butyl 3-methyl imidazolium hexafluorophosphate [BMIM][PF_6], and 1-butyl 3-methyl imidazolium tetrafluoroborate [BMIM][BF_4]), were tested as solvents for the fructose dehydration with amberlyst-15 or PTSA as catalysts.[125] The addition of DMSO as co-solvent increased the HMF selectivity, mainly due to the increased fructose solubility. The already reported fructose dehydration in [BMIM][Cl][111] under the same temperature conditions, was much faster, and selective than the one performed in [BMIM][BF_4][125] (**Table 3**, entries 26 *vs.* 32). This observation was explained by the different fructose solubility in [BMIM][BF_4] and [BMIM][Cl]. The addition of DMSO to [BMIM][BF_4] had positive effect on the HMF yield and even better results compared to [BMIM][Cl] alone has been achieved (**Table 3**, entries 28 *vs.* 26).

Takagaki *et al.*[98] reported glucose dehydration to HMF catalyzed by a solid acid/base catalysts *via* one-pot reaction under mild conditions. The base catalyst was required for the isomerization of glucose to fructose, while the acidic catalyst catalyzed the dehydration reaction (Scheme 10). The best base catalyst for the glucose isomerization was Mg–Al hydrotalcite (HT), consisting of layered clays with HCO_3 groups on the surface. The fructose dehydration was carried out with Amberlyst-15. The combination of these two solid catalysts improved the glucose conversion and the selectivity from 0 to 76% (**Table 3**, entries 34 *vs.* 33). Other tested carbohydrates also provided high yields of HMF with the same catalytic system (**Table 3**, entries 36 and 37). The reactions were performed in DMF although DMSO and acetonitrile were also observed to provide good results. However DMF being a highly boiling solvent complicates HMF isolation and is not consistent with industrial production of HMF.



Scheme 10.

Dehydration of fructose to HMF was studied in a batch mode in the presence of dealuminated H-form mordenites as catalysts at 165°C and in a mixture of water and MIBK (1:5 by volume).[82] The HMF selectivity was optimized testing H-mordenite with different Si/Al ratios. It was observed that the selectivity decrease with increasing the Si/A1 ratio, i.e. by increasing the acidity of the catalysts. The optimum Si/A1 ratio was found to be 1:1, (**Table 3**, entry 37) and it high selectivity was correlated with the shape selectivity properties of H-mordenites (bidimensional structure) and particularly with the absence of cavities within the structure leading to further formation of secondary products. High fructose corn syrup (HFCS) was studied by Cho *et al.* (**Table 3**, entry 39) for the production of HMF. Dioxane was found to be the best solvent for the reaction providing 80% HMF yield (by HPLC) at 100ºC after 3h. Moreover the authors described simple EtOAc extraction of HMF after dioxane evaporation to give 72% isolated HMF yield with high purity. Chilukuri *et al.* (**Table 3**, entry 40) studied the synthesis of HMF using mesoporous AlSBA-15 catalysts under biphasic conditions. The effect of Si/Al ratio on the catalytic activity and selectivity was investigated. A good linear correlation between the moderately strong acidity/total acidity ratio and HMF selectively was obtained. A series of sulfonic acid-functionalized carbon materials (C–SO3H), including poly(p-styrenesulfonic acid)-grafted carbon nanotubes (CNT-PSSA), poly(p-styrenesulfonic acid)-grafted carbon nanofibers (CNF-PSSA), benzenesulfonic acid-grafted CMK-5 (CMK-5-BSA), and benzenesulfonic acid-grafted carbon nanotubes (CNT-BSA), have been studied for fructose dehydration to HMF and fructose alcoholysis to alkyl levulinate (**Table 3**, enty 42). Under the optimal conditions HMF and ethyl levulinate yields of up to 89% and 86%, respectively have been obtained.

Table 3 Conversion of carbohydrates to HMF under heterogeneous conditions.

Entry	Biomass source	Reaction conditions				Conversion (%)	HMF Selectivity (%)	Isolation/analysis
		Solvent	Catalyst	T°C	Time			
1[124]	Fructose	H_2O	ion exchange resin/activated carbon	90	48h	-	77	HPLC
2[126]	Fructose	DMSO	Dowex-type ion-exchange resin	110	5h	100	85	GC/MS
3[126]	Fructose	DMSO	Dowex-type ion-exchange resin	80	25h	100	77	GC/MS
4[127]	Fructose	DMSO	Amberlyst-15 Pellets with evacuation (0.97×10^5 Pa)	120	2h	100	92	HPLC
5[127]	Fructose	DMSO	Amberlyst-15 Pellets (0.71-0.5 mm)	120	2h	100	76	HPLC
6[127]	Fructose	DMSO	Amberlyst-15 Powder (0.15-0.053 mm)	120	2h	100	100	HPLC
7[127]	Fructose 50 wt.%	DMSO	Amberlyst-15 Powder (0.15-0.053 mm)	120	2h	100	100	HPLC
8[127]	Fructose	DMSO	$FePW_{12}O_{40}$ with evacuation (0.97×10^5 Pa)	120	2h	100	97	HPLC
9[127]	Fructose	DMSO	$FePW_{12}O_{40}$	120	2h	100	49	HPLC
10[127]	Fructose	DMSO	$FePW_{12}O_{40}$ with evacuation (Sieves 4A)	120	2h	100	69	HPLC
11[128]	Fructose 10 wt.%	[4:6(H_2O:NMP)]/MIBK	Ion exchange resin (DIAION®)	90	18h	98	85	MIBK extraction
12[128]	Fructose 10 wt.%	[5:5 (H_2O:DMSO)]/DCM	-	120	5.5h	92	80	DCM extraction
13[128]	Inulin 10 wt.%	[4:6 (H2O:NMP)]/MIBK	Ion exchange resin (DIAION®)	90	21h	100	69	MIBK extraction
14[128]	Inulin 10 wt.%	[5:5 (H_2O:DMSO)]/DCM	-	120	6.5h	100	61	DCM extraction
15[128]	Sucrose 10 wt.%	[5:5 (H_2O:NMP)]/MIBK	Ion exchange resin (DIAION®)	90	21h	58	74[a]	MIBK extraction
16[128]	Sucrose 10 wt.	[5:5 (H_2O:DMSO)]/DCM	-	120	6.5h	60	69[a]	DCM extraction
17[97]	Fructose 2 wt.%	Acetone/DMSO (7:3)	Dowex-type ion-exchange resin[c]	150 MW	5min 20min	88.2 99.0	89.6 88.3	HPLC
18[97]	Fructose 10 wt.%	Acetone/DMSO (7:3)	Dowex-type ion-exchange resin	150 MW	20min 30min	99.0 99.4	84.1 82.1	HPLC
19[129]	Fructose 2 wt.%	H_2O	Dowex-type ion-exchange resin	150 MW	60min	82.6	34	HPLC
20[129]	Fructose 2 wt.%	Acetone/H_2O (70:30 w/w)	Dowex-type ion-exchange resin	150 MW	10min	91.7	70.3	HPLC
21[129]	Fructose 5 wt.%	Acetone/H_2O (70:30 w/w)	Dowex-type ion-exchange resin	150 MW	10min	98.6	66.6	HPLC
22[129]	Fructose 10 wt.%	Acetone/H_2O (70:30 w/w)	Dowex-type ion-exchange resin	150 MW	10min	99.6	52.7	HPLC
23[129]	Fructose 20 wt.%	Acetone/H_2O (70:30 w/w)	Dowex-type ion-exchange resin	150 MW	10min	98.1	51.5	HPLC
24[129]	Fructose 2 wt.%	Acetone/H_2O (70:30 w/w)	Dowex-type ion-exchange resin	150	10min	22.1	13.7	HPLC
25[112]	Fructose 20 wt.%	[BMIM][Cl][d]	Amberlyst-15[c]	80	10min	98.6	83.3	HPLC

26[112]	Fructose 20 wt.%	[BMIM][Cl]	Amberlyst-15	120	1min	99.3	82.2	HPLC
27[111]	Fructose 20 wt.%	[BMIM][Cl]/Acetone	Amberlyst-15	25	6h	90.3	86.5	HPLC
28[125]	Fructose	[BMIM][BF_4]:DMSO (5:3)	Amberlyst-15	80	32h	-	87	HPLC
29[125]	Fructose	[BMIM][BF_4]:DMSO (5:3)	PTSA	80	32h	-	68	HPLC
30[125]	Fructose	[BMIM][PF_6]:DMSO (5:3)	Amberlyst-15	80	24h	-	80	HPLC
31[125]	Fructose	[BMIM][PF_6]:DMSO (5:3)	PTSA	80	20h	-	75	HPLC
32[125]	Fructose	[BMIM][BF_4]	Amberlyst-15	80	3h	-	52	HPLC
33[98]	Fructose	DMF	HT / Amberlyst-15	100	3h	99	76	HPLC
34[98]	Glucose	DMF	Amberlyst-15	100	3h	69	0	HPLC
35[98]	Glucose	DMF	HT / Amberlyst-15[c]	80	9h	73	58	HPLC
36[98]	Sucrose	DMF	HT / Amberlyst-15	120	3h	58	93	HPLC
37[98]	Cellobiose	DMF	HT / Amberlyst-15	120	3h	52	67	HPLC
38[82]	Fructose	H_2O/MIBK(1:5)	dealuminated H-form mordenites	165	2h	93	73	MIBK extraction/ HPLC
39[130]	HFCS-90	Dioxane	Amberlyst-15	100	3h	-	-	EtOAc extraction[e]
40[131]	Fructose	water: MIBK 1:5 (v/v)	Al-SBA15 (Si/Al = 40)	165	1h	59	88	HPLC
41[132]	Fructose	1,4-dioxane/DMSO	Amberlyst-15	110	3min[b]	98	92	GC
42[133]	Fructose	DMSO	CNT-PSSA	120	30min	99	89	HPLC

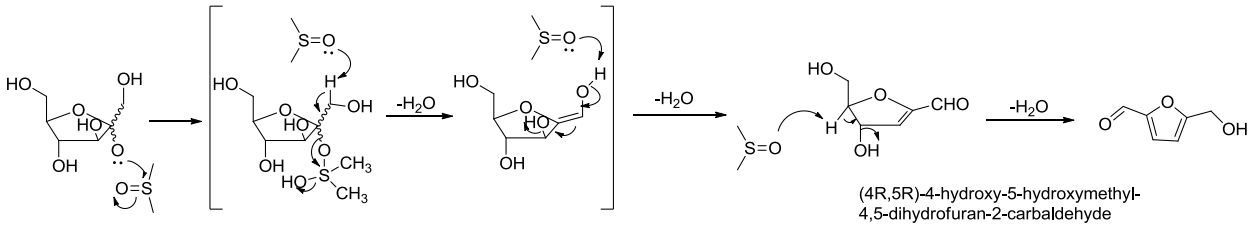
[a] *HMF selectivity is based on fructose content;* [b] *Residence time in flow,* [c] *Catalyst reused,* [d] *Reaction media reused,* [e] *72% isolated yield*

.

1.4.6 Acidic solvents as reaction promoters

In 1987 Musau *et al.*[95] demonstrated that fructose can be converted into HMF in DMSO as solvent at 150°C in absence of catalyst (**Table 4**, entry 1). They have tested different fructose/DMSO molar ratios and found that in case 0.8 ratio optimum conversion was achieved. The authors suggested that DMSO associates initially with only D-fructose at the start of the dehydration reaction, after which the generated water associates with DMSO, reducing the amount of DMSO available to D-fructose. Consequently, DMSO had to be sufficiently in excess to associate with all the water released during the reaction.

Amarasekara *et al.*[134] studied the mechanism of the dehydration of fructose to HMF in DMSO at 150°C by NMR spectroscopy (Scheme 11). It was possible to identify an intermediate (4R,5R)-4-hydroxy-5-hydroxymethyl-4,5-dihydrofuran-2-carbaldehyde using a combination of ^{1}H and ^{13}C NMR spectra.

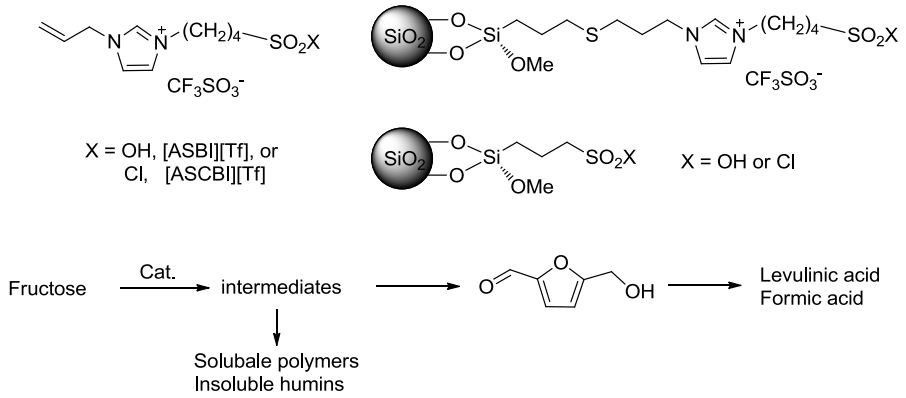


Scheme 11.

In 2006 HMF formation starting from fructose and sucrose, in [HMIM][Cl] (1-H-3-methylimidazolium chloride) as reaction solvent and promoter was reported.[101] [HMIM][Cl] is a protic ionic liquid that can convert completely fructose with 92% HMF selectivity (**Table 4**, entry 2). Kinetic studies showed that the energies of activation for the formation and decomposition of HMF are similar to those reported for the reaction catalyzed by solid catalysts.[101] The dehydration reaction was also tested with sucrose as starting material, and a rapid cleavage of sucrose into glucose and fructose was observed, but as reported before for other conditions,[9] the glucose moiety did not react, although the fructose conversion was complete. After HMF extraction with ethyl ether, the ionic liquid was reused for 5 cycles.

The use of ionic liquids as solvents and reaction promoter was also reported by Yokoyama *et al.*.[135] In this work microwave irradiation was used to heat the fructose dehydration reaction in a Lewis acidic ionic liquid, [ASCBI][Tf] (3-allyl-1-(4-sulfurylchloridebutyl) imidazolium trifluoromethanesulfonate), and a Brønsted acidic ionic liquid [ASBI][Tf] (3-allyl-1-(4-sulfobutyl) imidazolium trifluoromethanesulfonate) and their silica gel immobilized counterparts (Scheme 12).[135] The two ionic liquids with DMSO as co-solvent converted fructose with very good yields, and good selectivity for HMF (**Table 4**, entries 3 and 4). The Lewis acidic ionic liquid was a better reaction medium than the Brønsted one.

These ionic liquids when immobilized on silica converted fructose with 100% yield, but with medium selectivity for HMF (**Table 4**, entries 5 and 6).



Scheme 12.

Several ionic liquids and pyridinium salts were tested, including Brønsted acids, Lewis acids (ChCl/metal chlorides), and bases (ChCl/urea, 1,1,3,3-tetramethylguanidinium trifluoroacetate and lactate) and ChCl-based deep eutectic mixtures for the conversion of fructose to HMF at 80°C for 1 h, without adding any catalyst.[88] The Lewis acids $ZnCl_2$ and $CrCl_3$ in ChCl/metal chloride produced less than 20% of HMF (**Table 4**, entries 8 and 9). The most efficient solvent/catalyst tested was ChCl/citric acid which led to 91.1% conversion with 83.8% HMF selectivity (**Table 4**, entry 7). Ethyl acetate was reported as extraction solvent and showed good efficiency. Due to the immiscibility with the ionic liquid reactive phase the product formed was extracted without any cross-contamination. This ionic liquid system has the advantage being biodegradable and non-toxic.[88]

Recently the same authors reported[90] one pot inulin hydrolysis and fructose dehydration with moderate HMF selectivity (**Table 4**, entrie**s** 10-12) in ChCl based ionic liquids at 80°C. The acidic ChlCl/oxalic acid and ChlCl/citric acid ionic liquid acted as solvent and catalyst and it was possible to recycle ChlCl/oxalic acid system for at least six cycles just by extracting the product with ethyl acetate. A biphasic reaction system with ethyl acetate as extracting solvent provided an improved HMF selectivity (**Table 4**, entry 11).

Fayet *et al.* reported in 1983[136] HMF synthesis from fructose, glucose, sucrose, inulin, and levan (fructose polymer). Different pyridinium salts were tested as promoters. For fructose, inulin and levan, moderated HMF yield was achieved using pyridinium chloride, at 120°C for 30 minutes (**Table 4**, entries 13, 14 and 15) while for glucose or sucrose the HMF yield was very low (**Table 4**, entry 16 and 17).

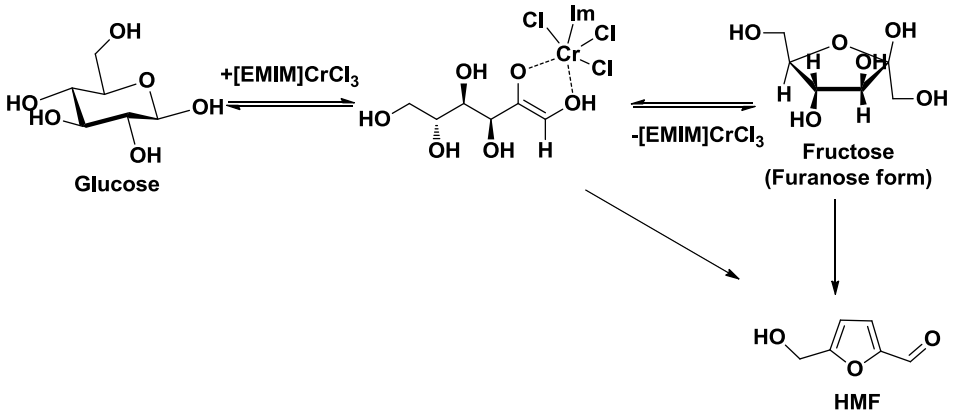
Table 4 Conversion of carbohydrates to HMF using acid solvents promoters

Entry	Biomass source	Reaction medium	T°C	Time	Convertion. %	HMF Selectivity %	Isolation/ analysis
1[95]	Fructose	DMSO	150	2h		92	EtOAc extraction/ Flash chromatography
2[101]	Fructose	1-H-3-methyl imidazolium chloride[b]	90	45min	100	92	Et_2O extraction
3[135]	Fructose	[ASBI][Tf]/DMSO	100 MW	6min	98	80	HPLC
4[135]	Fructose	[ASCBI][Tf]/ DMSO	100 MW	6min	100	84	HPLC
5[135]	Fructose	ILIS-SO_3H/ DMSO	100 MW	4min	100	70.1	HPLC
6[135]	Fructose	ILIS-SO_2Cl/ DMSO	100 MW	4min	100	67.2	HPLC
7[88]	Fructose	ChCl /citric acid[b]	80	1h	91.1	83.8	EtOAc extraction
8[88]	Fructose	ChCl /$CrCl_3$	80	1h	92	<20	EtOAc extraction
9[88]	Fructose	ChCl /$ZnCl_2$	80	1h	25	<7	EtOAc extraction
10[90]	Inulin	ChCl/oxalic acid[b]	80	2h	100	56	EtOAc extraction
11[90]	Inulin	ChCl/oxalic acid EtOAc	80	2h	100	64	EtOAc extraction/ HPLC
12[90]	Inulin	ChCl/citric acid	50+80	2+2h	88	65	EtOAc extraction/ HPLC
13[136]	Fructose	Pyridinium chloride	120	30min	-	70	Flash chromatography
14[136]	Inulin	Pyridinium chloride	120	30min	-	60	
15[136]	Levan	Pyridinium chloride	120	30min	-	60	-
16[136]	Glucose	Pyridinium chloride	120	30min	-	5	
17[136]	Sucrose	Pyridinium chloride	120	30min	-	30	Flash chromatography
18[117]	Fructose 10 wt.%	[EMIM][Cl]	120	3h	100	70	HPLC
19[117]	Glucose 10 wt.%	[EMIM][Cl]	180	3h	40	<5	HPLC

[a] HMF yield. [b]Reused. ***[ASBI][Tf]*** *- 3-allyl-1-(4-sulfobutyl) imidazolium trifluoromethanesulfonate;* ***[ASCBI][Tf]*** *- 3-allyl-1-(4-sulfurylchloridebutyl) imidazolium trifluoromethanesulfonate;* ***ILIS*** *– ionic liquids immobilized on silica gel*

1.4.7 Chromium catalysts

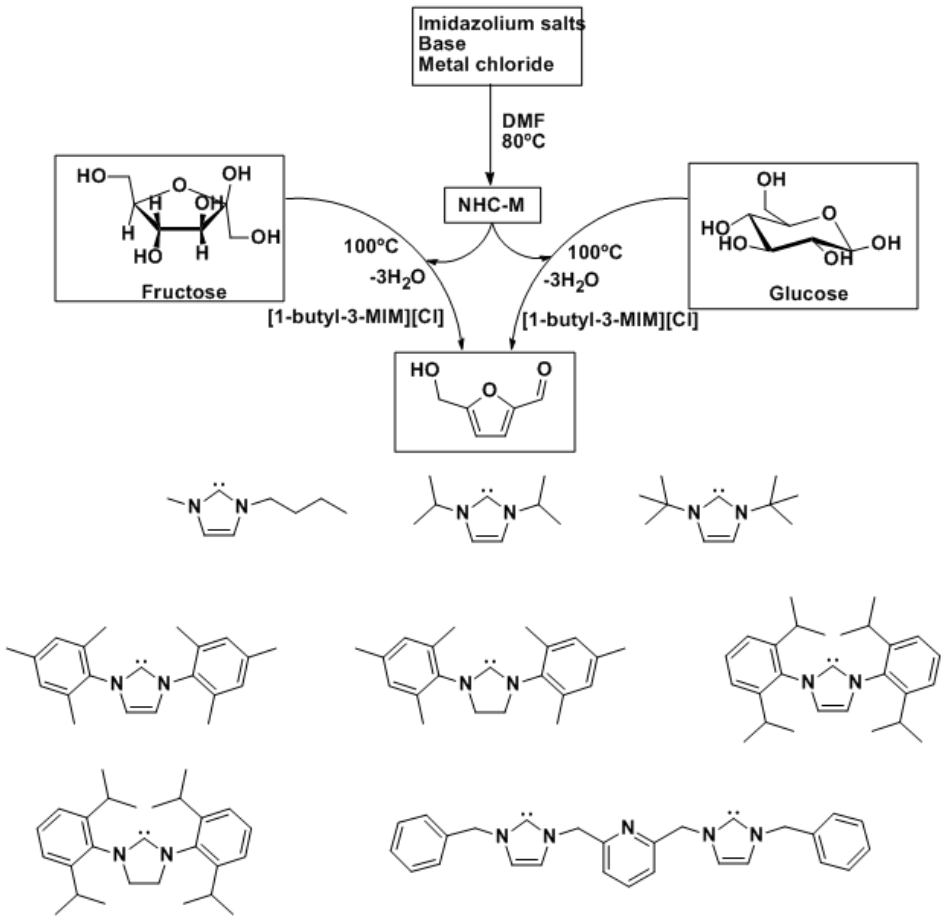
Zhang *et al.* reported[117] the synthesis of HMF starting from fructose or glucose with very good selectivity. They have studied the effect of different ionic liquids on the fructose dehydration. After choosing [EMIM][Cl] as solvent, they tested different catalysts, such as, several metal chlorides, and mineral or Lewis acids. In absence of catalyst they achieved fructose conversion of almost 100%, with approximately 70% selectivity (**Table 5**, entry 1). This system was not so efficient for glucose, since even with an increase of the temperature to 180°C only 40% conversion was obtained, with less than 5% selectivity (**Table 5**, entry 2). However, glucose conversion increased to near 70%, and the selectivity was approximately 90% when a catalytic amount of $CrCl_2$ was added (**Table 5**, entry 3). The authors propose that the catalyst [EMIM][Cl]/$CrCl_2$ was responsible for the isomerization of glucose to fructose and then, fructose was rapidly converted to HMF (Scheme 13).[117] The HMF stability was studied by heating pure HMF at 100°C for 3 h in [EMIM][Cl] in the presence of a catalytic amount of $CrCl_2$ and as a result, 98% of the HMF was recovered, while when no $CrCl_2$ was added to the system the recovery was only 28%. $CuCl_2$, VCl_4, and H_2SO_4 were also studied and provided 85, 86 and 98% recovery, respectively. The results clearly proved that catalytic amount of some metal chlorides can, not only catalyze the dehydration reaction, but also stabilize the final product. This may be one of the main reasons for not observing polymeric by-products, and only a negligible amount of levulinic acid being formed. This method was published in 2008 in a paten.[137]



Scheme 13.

Using [BMIM][Cl] as solvent (100°C, 6 hours), several NHC/metal (N-heterocyclic carbene ligand) complexes were tested as catalysts for the dehydration of fructose and glucose (Scheme 14).[138] The authors concluded that bulky NHC ligands protect the Cr center from reacting [BMIM][Cl] and form a sterically crowded metal center, therefore providing a higher catalytic efficiency. A good HMF selectivity was achieved, using these NHC/Cr

complexes as catalysts, 96% and 81% for fructose and glucose respectively (**Table 5**, entries 7 and 5). The HMF yield was confirmed by GC, but it was also possible to isolate HMF from the reaction medium by a simple diethyl ether extraction. After that, the catalyst and the ionic liquid could be recycled for at least three cycles with some loss of selectivity in the further cycles when glucose was used as substrate. A higher fructose and glucose concentration was tested (20 wt.%) and no decrease of selectivity was observed (**Table 5**, entries 6 and 8).



Scheme 14.

HMF synthesis from different substrates using [EMIM][HSO_4] and [BMIM][Cl], with or without extracting solvents (toluene or MIBK) was studied.[83] [EMIM][HSO_4] with toluene or MIBK as extracting solvents completely converted fructose in 79 and 88% HMF yield, respectively (**Table 5**, entry 9 and 10). This system was not efficient for glucose, and therefore the authors change the ionic liquid to [BMIM][Cl] with $CrCl_3$ as catalyst. This catalyst was chosen instead of $CrCl_2$ since it is more stable and easily handled under air, much cheaper and it is very likely that Cr^{2+} is oxidized to Cr^{3+} in the IL system containing dissolved air and water.[83] The system with [BMIM][Cl]/$CrCl_3$ without a extracting solvent resulted in a 81% HMF yield, which was improved when toluene was added to the reaction system as an extracting solvent (**Table 5**, entry 11 *vs.* 13). This result is comparable with the

reported[139] glucose dehydration in [BMIM][Cl]/$CrCl_3$ with microwave irradiation as a heating source, where the isolated HMF yield was 91% (**Table 5**, entry 18). Others substrates were tested, such as inulin, sucrose, cellobiose and cellulose (**Table 5**, entry 14, 15 and 16, 17 respectively). Although high HMF selectivity was achieved for inulin, and sucrose, the same did not happen with cellobiose or cellulose, even when adding H_2SO_4 into the reaction system for cellulose.

In 2009, Li *et al.*[139] reported the transformation of glucose and cellulose in [BMIM][Cl] with $CrCl_3$ as catalyst affording HMF yields of 91 and 61%, under 400W microwave irradiation for only one and two minutes, respectively (**Table 5**, entries 18 and 19). Different cellulose samples were tested reaching 53-62% HMF yields, indicating that this method is not affected by cellulose type nor the polymerization degree. The high yields obtained from cellulose were explained by the complete cellulose dissolution on the ionic liquids, leaving cellulose chains accessible to chemical transformations, and also because [BMIM][Cl] has excellent dielectric properties for transformation of microwave into heat. Although Zhang *et al.*[117] reported lower HMF yields for glucose transformation with $CrCl_3$ (**Table 5**, entries 4 *vs.* 18) in [EMIM][Cl], the authors believe that the microwave irradiation can improve the catalyst behavior. The mechanism for this transformation still remains unknown, although the authors pursued a pathway to glucose isomerization to fructose.

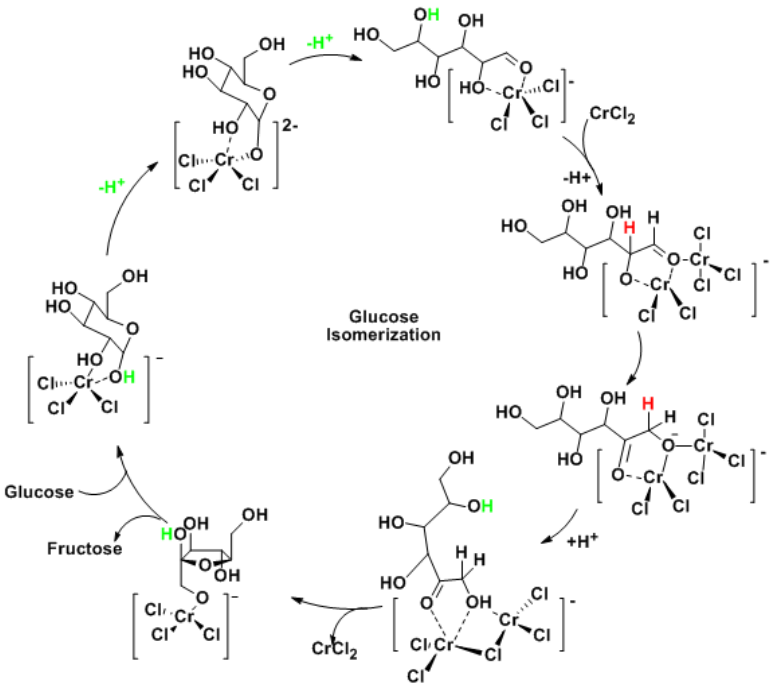
This work was extended by the same authors, where they have tested the microwave-assisted transformation with other biomass resources, such as corn stalk, rice straw and pine wood.[140] In this work $CrCl_3 \bullet 6H_2O$ was used as catalyst in [BMIM][Cl], for 2-3 minutes under 400W microwave irradiation. The improvement of the HMF yield under microwave heating was confirmed again (**Table 5**, entries 23 *vs.* 24). The reaction was also tested with [BMIM][Br] with similar results as with [BMIM][Cl] (**Table 5**, entries 24 *vs.* 25). [BMIM][Br] can also dissolve lignocellulosic biomass, and the authors reported that the reaction medium has little effect on the dehydration efficiency as long as the solvent dissolves it.

Chen *et al.*[94] studied the cellulose conversion into HMF using a ionic-liquid/water mixture with $CrCl_2$ as catalyst. At 120°C, with 10 mol% of $CrCl_2$ in [EMIM][Cl] (no water added) 89% HMF selectivity was observed (**Table 5**, entry 26). This high HMF yield implies that not only glucose was converted to HMF, but also others reducing sugars present after cellulose hydrolysis. To enhance the cellulose hydrolysis and dehydration, water was added to this catalytic system. However, lower HMF yield was obtained, even at higher temperatures (**Table 5**, entries 24 *vs.* 28).

Recently Zhang *et al.*[141] studied cellulose transformation to HMF in [EMIM][Cl] using a pair of two metal catalysts, $CrCl_2$ and $CuCl_2$ and obtained 55.4% HMF yield (**Table 5**, entry 29). In this work optimization was carried out in order to achieve the best molar ratio for the two catalysts $CrCl_2$ and $CuCl_2$, and was observed that as little as 3 mol% of $CrCl_2$ in the paired metal chlorides was sufficient to activate the $CuCl_2$ dominant catalyst in the [EMIM]Cl solvent. The catalyst and the solvent could be recycled for three times using MIBK as extracting solvent.

Studies on sucrose hydrolysis and further dehydration to HMF were performed by Chung,[142] using 1-octil-3-methylimidazolim chloride ([OMIM][Cl]) as solvent and two metal chloride as catalysts ($CrCl_2$ or $ZnCl_2$) in HCl acidic medium. According to the authors, the acidic medium improves the sucrose hydrolysis, and the catalysts $CrCl_2$ or $ZnCl_2$ catalyze further glucose dehydration. Upon the acid addition the hydrolysis was faster, but it was also observed fast fructose disappearance, probably due to the strong chemical reactivity for isomerization, dehydration, fragmentation or condensation reactions. However, the authors didn't quantify the by-products formed. The addition of $CrCl_2$ was beneficial for the system and an improved HMF yield was obtained. (**Table 5**, entry 30).

In 2009 Pidko *et al.*[143] studied the reactivity of $CrCl_2$ towards selective glucose dehydration in an ionic liquid medium, combining different methods, such as kinetic experiments, *in situ* X-ray absorption spectroscopy (XAS), and density functional theory (DFT) calculations. The key reaction of the catalytic system [EMIM][Cl]/$CrCl_2$, as reported before,[117] is the isomerization of glucose to fructose. In this work the authors proposed a mechanism based on the one suggested for the enzymes. The highly concentrated and mobile chloride anions from the ionic liquid promote various (de)protonation reactions important for the glucose isomerization (Scheme 15). In enzymes, such transformations are catalyzed by basic amino acid residues at the active site. The unique transient self-organization of Cr^{2+} dimers to facilitate the rate-controlling H shift in glucose isomerization is possible as a result of the dynamic nature of the Cr complexes and the presence of moderately basic sites in the ionic liquid.



Scheme 15.

Efficient conversion of glucose to HMF was achieved by Jianbing *et al.* (**Table 5**, entry 33). The authors studied the synergic effect of tetraethylammonium bromide (TEAB) and $CrCl_3$ on the reaction rate. Under optimized conditions when 70 mol% TEAB and 12 mol% $CrCl_3 \cdot 6H_2O$ in DMA were used, the yield of HMF was up to 72.7 % after 90 min at 120°C. Similar approach but using tetraethyl ammonium chloride (TEAC) and $CrCl_3 \cdot 6H_2O$ was reported by Lin *et al*. (**Table 5**, entry 34). HMF yield of 71.3% was achieved in 10 min at 130ºC. The TEAC/$CrCl_3$x$6H_2O$ system was found to be tolerant to high water content and high glucose concentration and could be recycled, exhibiting stable activity after five cycles. Good results were achieved also when fructose, sucrose, and cellobiose were used as feedstock.

Efficient catalytic conversion of microcrystalline cellulose (MCC) to HMF, was achieved by Wang *et al*. using acidic ionic liquids (ILs) as catalysts and metal salts as co-catalysts in [EMIM][Ac] as solvent (**Table 5**, entry 35), series of acidic ILs have been tested as well as different metal salts. High yield of HMF in up to 69.7% has been achieved using combination of $[C_4SO_3Hmim][CH_3SO_3]$ and $CuCl_2$.

More recently Chen et al.[144] studied the effect of NHC-$CrCl_x$ complexes on the dehydration of glucose to HMF. These kind of complexes are readily formed from the deprotonating of their precursors – imidazolium salts, which are typically used as solvents for this transformation. Moreover these complexes are believed to be the true catalysts. The authors performed series of experiments with controlled *in situ* formation of NHC-$CrCl_x$ complexes and observed that the NHC ligand serves as a poison to the chromium catalyst

system and a superstoichiometric amount (2 or 3 equiv.) of the NHC ligand can completely shut down the catalysis. It was proposed that strongly σ-donating, largely non-labile NHC ligands render the coordinately saturated Cr center, there by negatively impacting or even completely shutting down the catalyst activity. On the other hand, the free NHC ligand could be responsible for glucose degradation without HMF formation.

Table 5 Conversion of carbohydrates to HMF with heterogeneous chromium based catalysts.

Entry	Biomass source	Reaction conditions				Convertion (%)	HMF Selectivity. (%)	Isolation/ analysis
		Solvent	Catalyst	T(°C)	Time			
1[117]	Fructose 10 wt.%	[EMIM][Cl]	-	120	3h	100	70	HPLC
2[117]	Glucose 10 wt.%	[EMIM][Cl]	-	180	3h	40	<5	HPLC
3[117]	Glucose 10 wt.%	[EMIM][Cl]	$CrCl_2$	100	3h	70	90	HPLC
4[117]	Glucose 10 wt.%	[EMIM][Cl]	$CrCl_2$	100	3h	43	70	HPLC
5[138]	Glucose 10 wt.%	[BMIM][Cl][a]	NHC/$CrCl_2$[b]	100	6h	-	81	Et_2O extraction/ GC
6[138]	Glucose 20 wt.%	[BMIM][Cl]	NHC/$CrCl_2$	100	6h	-	80	GC
7[138]	Fructose 10 wt.%	[BMIM][Cl][a]	NHC/$CrCl_2$[b]	100	6h	-	96	Et_2O extraction/ GC
8[138]	Fructose 20 wt.%	[BMIM][Cl]	NHC/$CrCl_2$	100	6h	-	96	GC
9[83]	Fructose	[EMIM][HSO_4]/Toluene	-	100	30min	100	79	Toluene extraction/HPLC
10[83]	Fructose	[EMIM][HSO_4]/MIBK	-	100	30min	100	88	MIBK extraction/ HPLC
11[83]	Glucose	[BMIM][Cl]/ toluene	$CrCl_3$	100	4h	91	91	HPLC
12[83]	Glucose	[BMIM][Cl]/MIBK	$CrCl_3$	100	4h	79	79	MIBK extraction/ HPLC
13[83]	Glucose	[BMIM][Cl]	$CrCl_3$	100	4h	83	81	HPLC
14[83]	Inulin	[EMIM][HSO_4]/MIBK	-	100	30min	-	73	MIBK extraction/ HPLC
15[83]	Sucrose	[BMIM][Cl]/MIBK	$CrCl_3$	100	4h	-	73	MIBK extraction/ HPLC
16[83]	Cellobiose	[BMIM][Cl]/MIBK	$CrCl_3$	100	4h	-	37	MIBK extraction/ HPLC
17[83]	Cellulose	[BMIM][Cl]/MIBK	$CrCl_3/H_2SO_4$	100	4h	-	9	MIBK extraction/ HPLC
18[139]	Glucose	[BMIM][Cl]	$CrCl_3$	≈200 MW, 400W	1min	-	91	Flash chromatography
19[139]	Cellulose	[BMIM][Cl]	$CrCl_3$	≈200MW, 400W	1min	-	61	Flash chromatography
20[140]	Cellulose	[BMIM][Cl]	$CrCl_3$	≈200 MW, 400W	2.5min	-	62	HPLC
21[140]	Corn stalk	[BMIM][Cl]	$CrCl_3$	≈200 MW, 400W	3min	-	45	HPLC
22[140]	Rice straw	[BMIM][Cl]	$CrCl_3$	≈200 MW, 400W	3min	-	47	HPLC
23[140]	Pine wood	[BMIM][Cl]	$CrCl_3$	≈200 MW, 400W	3min	-	52	HPLC
24[140]	Pine wood	[BMIM][Cl]	$CrCl_3$	100 oil bath	60min	-	6.4	HPLC
25[140]	Pine wood	[BMIM][Br]	$CrCl_3$	≈200 MW 400W	3min	-	44	HPLC

26[94]	Cellulose	[EMIM][Cl]	$CrCl_2$	120	6h	-	89	Acetone extraction/ HPLC
27[94]	Cellulose	[EMIM][Cl]/H_2O	$CrCl_2$	120	12h	-	13	Acetone extraction/ HPLC
28[94]	Cellulose	[EMIM][Cl]/H_2O	$CrCl_2$	140	2h	-	40	Acetone extraction/ HPLC
29[141]	Cellulose 10wt.%	[EMIM][Cl]	$CrCl_2$/$CuCl_2$	120	8h	-	57.5	HPLC
30[142]	Sucrose 20% w/v	[OMIM][Cl]	HCl/$CrCl_2$	120	30min	-	82.0	HPLC
31[142]	Sucrose 30% w/v	[OMIM][Cl]	HCl/$CrCl_2$	120	60min	-	67.7	HPLC
32[142]	Sucrose 50% w/v	[OMIM][Cl]	HCl/$CrCl_2$	120	60min	-	53.2	HPLC
33[145]	Glucose	DMA	TEAB/$CrCl_3$	120	1.5h	-	73	HPLC
34[146]	Glucose	TEAC	$CrCl_3$	130	10min		71	extraction
35[147]	cellulose	[EMIM][Ac]	[C_4SO_3Hmim][CH_3SO_3]/$CuCl_2$	160	3.5h		67	HPLC

1.4.8 Zirconium and Titanium catalyst

Watanabe *et al.* studied glucose and fructose reactivity in hot compressed water with homogeneous or heterogeneous acidic (H_2SO_4 or TiO_2) or alkili additives (NaOH or ZrO_2).[148] They observed that isomerization between glucose and fructose was catalyzed by alkali, and fructose dehydration was promoted by acidic promoters. It seems that the equilibrium favors fructose formation in hot compressed water because the rate of isomerization of fructose into glucose is negligibly compared to that of glucose into fructose. Zirconia (ZrO_2) was a base catalyst that promotes the glucose isomerization. On the other hand Anatase TiO_2 was found to act as an acid catalyst to promote formation HMF.

In 2000 zirconium- and titanium- hydrogenphosphates in α and γ structural arrangements were reported as catalysts for fructose and inulin dehydration to HMF.[87] These reactions were performed in aqueous medium, and even so no appreciable subsequent rehydration to levulinic and formic acids was observed. Among the investigated catalysts, both surface Brønsted and Lewis acid sites were present, and experimental results showed that both acid sites may be involved in the catalytic process. However, as the Lewis acid sites strength is increased a corresponding enhancement of HMF yield was obtained. For example the lower strength of Lewis acid sites present on the external crystal surface of C-TiP_2O_7 with respect to those on C-ZrP_2O_7 decrease the performance of C-TiP_2O_7 catalyst (**Table 6**, entries 4 *vs.* 5 or 6). Inulin showed similar reactivity to that observed for fructose (**Table 6**, entries 3 and 6). Cubic zirconium pyrophosphate and γ -titanium phosphate showed the best performances, in terms of activity and selectivity (**Table 6**, entries 5 and 2).

The same group[149] studied the HMF formation from fructose and glucose in water under microwave irradiation (200°C) as heating source and TiO_2 and ZrO_2 as catalysts The advantage of using these heterogeneous catalysts compared to the homogeneous (such as HCl or H_2SO_4) is the low corrosion and easy separation. With the reported method good fructose conversions were achieved but with low selectivity for HMF (**Table 6**, entries 7-10). The zirconium, or titanium phosphates catalysts used by Benvenuti[87] were observed to provide better performance, than TiO_2 and ZrO_2 described in this work.

Further study on the behavior of solid acid catalyst sulphated zirconia in the fructose dehydration to HMF, performed by the same authors was reported in 2009.[150] In this work they also used microwave heating. The catalyst was characterized, and in aqueous solutions the HMF selectivity was low (37.4%, **Table 6**, entry 11), may be due to the deactivation of the active acid sites of the catalyst by water. As in their previous work the authors[129] changed the solvent to a mixture of acetone-DMSO. The results were more satisfying and 93.6% of

fructose conversion with 72.8% HMF selectivity with SO_4^{-2}/ ZrO_2 as catalyst for 20 minutes at 180°C was achieved (**Table 6**, entry 12).

In the same year other group[93] reported glucose dehydration with SO_4^{-2}/ZrO_2 (CSZ) and $SO_4^{-2}/ZrO\text{-}Al_2O_3$ (CSZA-1 to 5, depending on the Zr–Al mol ratio) catalysts. CSZA 1-5 have acidic and basic active sites, such that increasing the Al ratio increased the number of basic sites. When these catalysts were tested for glucose dehydration, the authors expected that increasing the basic sites on the catalyst will also increase the glucose isomerization, resulting in higher HMF yields. However, this was not observed, and the catalyst with higher acidity and moderate basicity was more favorable for the formation of HMF (**Table 6**, entries 14 *vs.* 15). Another important observation was the fact that the acid sites on the CZA or CSZA catalysts exhibited no catalytic improvement for the conversion of fructose to HMF compared with the same conditions without a catalyst (**Table 6**, entries 17 *vs.*18 or 19). The authors suggested that the production of HMF in this system may not be mainly *via* glucose isomerization–dehydration process. The catalyst CSZA-3 was recycled for at least 5 cycles.

With the objective of coupling in one-pot the hydrolysis and dehydration reactions to produce HMF from lignocellulosic biomasses (i.e. sugarcane bagasse, rice husk and corn cob), heterogeneous catalysts TiO_2, ZrO_2 and mixed-oxide TiO2–ZrO_2 under hot compressed water (HCW) conditions were applied.[151] It was found that the catalyst preparation procedure affected its reactivity, with different Ti/Zr ratios and different calcination temperatures the catalyst acidity/basicity was different. Although these catalysts resulted in good conversions (70-80% for glucose and cellulose, **Table 6**, entries 20 and 21), several other by-products were also formed. The HMF yield was approximately 28% for glucose and 13% for cellulose.[151]

Asghari *et al.*[152] reported fructose and glucose dehydration with zirconium phosphate as catalyst in sub-critical water. It was found that the catalyst is stable under these conditions and induces moderate selectivity from fructose, comparable with that obtained with zirconium pyrophosphate,[87] but affording higher conversions (**Table 6**, entries 22 *vs.* 6).

Table 6 Conversion of carbohydrates to HMF catalyzed by Zr and Ti catalysts.

Entry	Biomass source	Reaction conditions				Conversion (%)	HMF Selectivity (%)	Isolation/ analysis
		Solvent	Catalyst	T°C	Time			
1[87]	Fructose	H_2O	α-Titanium phosphate (α -TiP)	100	0.5h	29.1	98.3	GC
					1h	33.4	83.5	
					2h	34.1	75.4	
2[87]	Fructose	H_2O	γ-Titanium phosphate (γ -TiP)[a]	100	0.5h	36.7	96.1	MIBK extraction/GC
					1h	46.8	88.6	
					2h	56.6	68.7	
3[87]	Inulin	H_2O	γ-Titanium phosphate (γ -TiP)[a]	100	0.5h	31.8	98.1	MIBK extraction/GC
					1h	44.3	94.0	
					2h	91.9	70.7	
4[87]	Fructose	H_2O	Cubic titanium pyrophosphate (C-TiP_2O_7)	100	0.5h	24.8	98.7	GC
					1h	29.3	90.	
					2h	38.7	72.3	
5[87]	Fructose	H_2O	Cubic zirconium-pyrophosphate (C-ZrP_2O_7)[a]	100	0.5h	44.4	99.8	MIBK extraction/GC
					1h	52.2	86.0	
					2h	52.8	81.4	
6[87]	Inulin	H_2O	Cubic zirconium-pyrophosphate (C-ZrP_2O_7)[a]	100	0.5h	26.4	97.8	MIBK extraction/GC
					1h	38.9	89.4	
					2h	50.2	72.3	
7[149]	Fructose2 wt.%	H_2O	ZrO_2	200 MW	5min	65.3	30.6	HPLC
8[149]	Fructose2 wt.%	H_2O	TiO_2	200 MW	5min	83.6	38.1	HPLC
9[149]	Glucose2 wt.%	H_2O	ZrO_2	200 MW	5min	56.7	10.0	HPLC
10[149]	Glucose2 wt.%	H_2O	TiO_2	200 MW	5min	63.8	18.6	HPLC
11[150]	Fructose2 wt.%	H_2O	SO_4^{-2}/ ZrO_2	200 MW	5min	88.7	37.4	HPLC
112[150]	Fructose2 wt.%	Acetone/DMSO 7:3 w/w	SO_4^{-2}/ ZrO_2	180 MW	20min	93.6	72.8	HPLC
13[93]	Glucose7.6 wt.%	DMSO	-	130	4hours	94	4.3	HPLC
14[93]	Glucose7.6 wt.%	DMSO	SO_4^{-2}/ZrO_2	130	4hours	95.2	19.2	Et_2O extraction/ HPLC
15[93]	Glucose3.9 wt.%	DMSO	CSZA-3[a]	130	4hours	99.1	48.0	HPLC
116[93]	Glucose20 wt.%	DMSO	CSZA-3	130	4hours	98.1	39.2	HPLC
17[93]	Fructose20 wt.%	DMSO	-	130	4h	99.6	71.9	HPLC
18[93]	Fructose7.6 wt.%	DMSO	SO_4^{-2}/ZrO_2	130	4h	99.8	67.7	HPLC

19[93]	Fructose7.6 wt.%	DMSO	CSZA-3	130	4h	99.4	56.6	HPLC
20[151]	Cellulose	H_2O (HCW)	ZrO_2 - TiO_2	250	5min	70	13	HPLC
21[151]	Glucose	H_2O (HCW)	ZrO_2 - TiO_2	250	5min	80	28	HPLC
22[152]	Fructose	sub-critical H_2O	ZrP[a]	240 (3.35MPa)	120 sec	80.6	61.3	HPLC
23[152]	Glucose	sub-critical H_2O	ZrP[a]	240 (3.35MPa)	180sec	53.1	39.0	HPLC

[a] *Catalyst reused*

1.4.9 Lanthanides

Lanthanides (III) chlorides are preferred compared to transition metals because they are cheaper and less toxic. Ishida *et al.*[153,154] shown that lanthanide ions could catalyze the glucose dehydration to HMF. No further decomposition in the first 15 minutes was observed, but for longer reaction times the HMF selectivity dropped down. Several lanthanide (III) chlorides ($DyCl_3$, $YbCl_3$, La Cl_3, $NdCl_3$, $EuCl_3$) were tested in water solutions at 140°C and 15 min reaction time. The final product was extracted with benzene. Since lanthanide ions have a high affinity for oxygen, the authors believe that they coordinate with glucose and act as Lewis acid catalysts.

Lanthanides (III) chlorides were tested[155] as catalysts in ionic liquids as solvents for glucose dehydration to HMF. First was studied the HMF stability in different ionic liquids at 100°C and in case of imidazolium ionic liquids with halides as anion was observed the lowest degradation. The strongest Lewis acid $YbCl_3$, exhibit the best performance. However, still moderate HMF yields were obtained (**Table 7**, entries 1-4). The authors suggest that since the yield was favored in hydrophobic ionic liquids the reaction take place *via* different mechanism from the chromium chloride catalytic system, where the yield decreased with the hydrophobicity of the ionic liquids. Glucose dehydration to HMF under mild conditions by using ytterbium triflate and [BMIM]Cl as co-catalyst and solvent was performed by Amin *et al.* (**Table 7**, entry 5). 52% HMF yield has been achieved at 105°C and it was observed that higher temperature and catalyst loading induced formation of insoluble polymers and humins.

Table 7 Conversion of glucose to HMF catalyzed by $YbCl_3$ and $Yb(OTf)_3$.

Entry	Biomass source	Solvent	Catalyst	T°C	time	HMF Selectivity	Isolation/analysis
1[155]	Glucose	[EMIM][Cl]	$YbCl_3$	160	1h	8%	HPLC
2[155]	Glucose	[BMIM][Cl]	$YbCl_3$	160	1h	20%	HPLC
3[155]	Glucose	[HexMIM][Cl]	$YbCl_3$	160	1h	19%	HPLC
4[155]	Glucose	[OMIM][Cl]	$YbCl_3$	160	1h	22.5%	HPLC
5[156]	Glucose	[BMIM]Cl	$Yb(OTf)_3$	105	2.7h	52%	HPLC

1.4.10 Other metal catalysts

Armaroli *et al.*[157,158] reported the use of commercial niobium phosphates, or niobium catalysts prepared by treatment of niobic acid with phosphoric acid as catalysts for sugar dehydration reactions. Different substrates, such as fructose, sucrose and inulin were tested in aqueous medium. It was observed that the HMF selectivity was very high at low reaction times, but along with low sugar conversion (**Table 8**, entries 1-4). For longer reaction times, although the sugar conversions increased up to 65.5%, the HMF selectivity decreased, due to the formation of polymeric by-products.[157,158] In order to overcame this problem the authors reported an extraction process with MIBK as extracting solvent, the conversion was improved and it was possible to recycle both the residual aqueous substrate solution and the

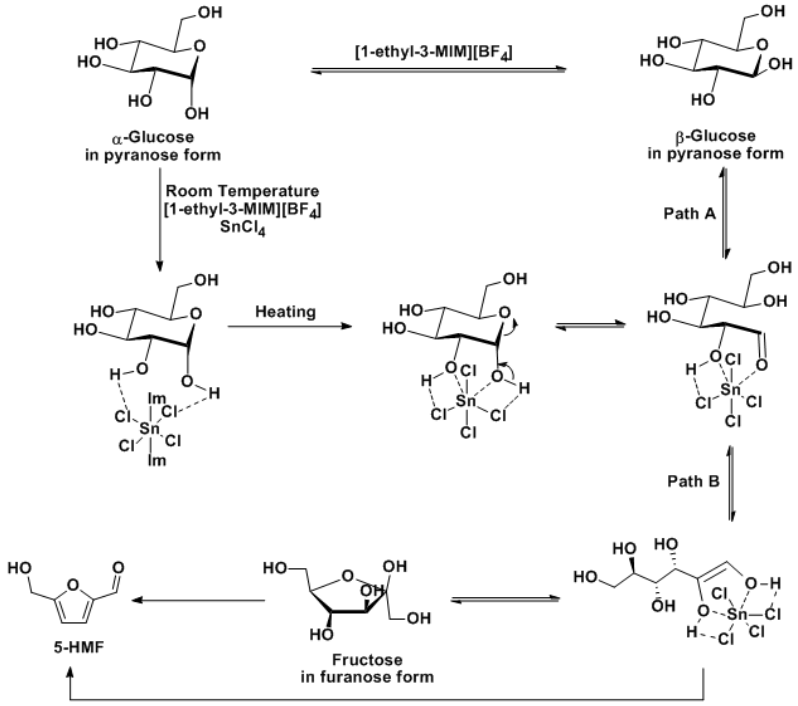
solid catalyst, with fructose and inulin substrates (**Table 8**, entries 3 and 4). A glucose transformation to HMF using a niobium catalyst was published in a patent in 2009.[159] Although the main objective was to synthesize 2,5-furandicarboxilic acid (FDA), Ribeiro *et al*.[96] reported an initial Cobalt acetylacetone ($Co(acac)_3$), SiO_2-gel, or $Co(acac)_3$ encapsulated in sol-gel (Co-gel) catalyzed fructose dehydrated to HMF with moderated conversion and good selectivity. SiO_2-gel provided the best HMF selectivity (**Table 8**, entries 5 *vs.* 6). These results were improved to 100% selectivity despite moderate conversion, when the reaction was carried out in water in an autoclave (**Table 8**, entry 7).

Vanadyl phosphate (VOP) was used as acid catalyst in the dehydration of fructose aqueous solutions to HMF.[160] This catalyst exhibit low selectivity, and moderated fructose conversion (**Table 8**, entry 8). The acidity of the VOP was modified by isomorphous substitution of some VO^{+3} groups with trivalent metals M^{+3} such as Fe^{+3}, Cr^{+3}, Ga^{+3}, Mn^{+3} and Al^{+3}. The catalysts, were tested in aqueous solutions (**Table 8**, entries 10. 11 and 12). FeVOP exhibit the best performance, even at high fructose concentrations (**Table 8**, entries 9-12), without the formation insoluble polymeric by-products or HMF rehydration compounds. Similar activity and selectivity were obtained with inulin as substrate and FeVOP as catalyst (**Table 8**, entry 13).

Several metal chloride were screened in [BMIM][Cl], for the fructose dehydration at room temperature.[92] Tungsten chloride provided the best HMF yield, which was further improved in biphasic reaction with THF as an extracting solvent (**Table 8**, entries 17 *vs.* 18). The authors also developed a continuous batch process for the conversion of fructose to HMF in a THF–[BMIM][Cl] biphasic system, which was tested with a bigger amount of fructose (10 g) as starting material.[92]

Glucose dehydration in DMSO with different metal catalysts was carried out at 100ºC for 3 hours.[89] $SnCl_4$ was the most efficient and was further tested in several ionic liquids. The ionic liquids based on anions having coordination abilities, such as chloride (Cl), bis(trifluoromethane)sulfonimide (NTf_2), trifluoroacetate (TFA), trifluoromethylsulfonate (OTf) or saccharin (SAC), provided lower HMF yields compared with other type of anions (BF_4 - tetrafluoroborate). The authors suggested that these anions could compete with the interaction of glucose and the Sn atom inhibiting the HMF formation. The ionic liquid with best selectivity was [EMIM][BF_4] (**Table 8**, entry 21). Based on these experiments, the authors proposed a mechanism involving a five or six member ring chelate complex of the Sn atom and glucose (Scheme 16). Other saccharides were also tested, such as sucrose, cellobiose, inulin and starch, providing reasonable HMF selectivity (**Table 8**, entries 22-

25).[89] After a product extraction with ethyl acetate, was possible to recycle the $SnCl_4$/[EMIM][BF_4] catalytic system.



Scheme 16.

$SnCl_4$ combined with tetraalkyl ammonium salts as catalysts for the dehydration of various carbohydrates to HMF was reported by Xeu *at al*. The best results were observed using tetrabuthyl ammonium bromide-$SnCl_4$ in 1:1 ratio. Certainly the most interesting results are the ones for the glucose dehydration, where high yield of 69% was obtained using DMSO as a solvent. Zhao *et al.* demonstrated that dehydration of fructose to HMF can be achieved at room temperature using $GeCl_4$ as catalyst in DMSO, the yield was further improved by the presence of [BMIM]Cl (**Table 8**, entry 27). Mesoporous tantalum phosphate (TP) has been successfully used as solid acid catalyst in the dehydration of glucose to HMF by Jiménez-López *et al.* Glucose conversion of 56.3% and 32.8% HMF yield were achieved at 170°C after 1 h in water/MIBK biphasic system. (**Table 8**, entry 28). Conversion of glucose to HMF in DMA was achieved using metal halides as catalysts. The best result was obtained with AlI_3 which provided 52% yield (**Table 8**, entry 30). The combination of ChCl/metal salt catalytic system in biphasic MIBK/water conditions for the conversion of glucose to HMF was studied (**Table 8**, entry 33). $AlCl_3$ exhibit the best performance providing 70% HMF yield which is competitive with the imidazolium ionic liquids and chromium salts. The catalytic system was recycled 6 times.

Table 8 Conversion of carbohydrates to HMF promoted by miscellaneous catalysts.

Entry	Biomass source	Solvent	Catalyst	T (°C)	time	Convertion (%)	HMF Selectivity (%)	Isolation/ analysis
1[157,158]	Fructose 6 wt.%	H_2O	H_3PO_4-treated niobic acid (P/N1)	100	0.5h 1h 2h	31.2 33.3 61.5	93.3 30.3 12.4	GC
2[157,158]	Fructose 6 wt.%	H_2O	Niobium phosphate (NP2)	100	0.5h 1h 2h	28.8 29.3 33.3	100.0 85.2 71.9	GC
3[157]	Fructose 6 wt.%	H_2O	Niobium phosphate (NP2)[a]	100	0.5h 1h	33.6 75.8	98.3 97.8	GC
4[157]	Inulin 6 wt.%	H_2O	Niobium phosphate (NP2)[a]	100	0.5h 1h 1.5h	25.2 48.7 76.3	77.5 74.0 72.0	GC
5 [96]	Fructose	H_2O/MIBK	Co-gel	88	8h	-	46	HPLC/RI
6 [96]	Fructose	H_2O/MIBK	SiO_2-gel	88	8h	-	47	HPLC/RI
7 [96]	Fructose	H_2O	SiO_2-gel	160 20 bar	1.1h	52	100	HPLC/RI
8[160]	Fructose 30 wt.%	H_2O	VOP	80	0.5h 2h	45.1 65.2	32.9 35.8	GC/MS
9[160]	Fructose 6 wt.%	H_2O	FeVOP	80	2h	48.1	42.2	GC/MS
10[160]	Fructose 10 wt.%	H_2O	FeVOP	80	2h	60.9	51.2	GC/MS
11[160]	Fructose 30 wt.%	H_2O	FeVOP	80	1h	70.8	59.6	GC/MS
12[160]	Fructose 40 wt.%	H_2O	FeVOP	80	0.5h	57.7	87.3	GC/MS
13[160]	Inulin 6 wt.%	H_2O	FeVOP	80	2h	41.8	82.7	GC/MS
14[160]	Fructose 6 wt.%	H_2O	CrVOP	80	1h	60.0	48.5	
15[160]	Fructose 6 wt.%	H_2O	AlVOP	80	1h	75.9	57.6	
16[160]	Fructose	H_2O	VOP/TiO_2	80	0.5h 1h	35.5 39.7	93.2 87.4	GC/MS
17[92]	Fructose 20 wt.%	[BMIM][Cl][b]	WCl_6[a]	50	4h	-	63	THF extraction
18[92]	Fructose 20 wt.%	[BMIM][Cl]/ THF[b]	WCl_6[a]	50	4h	-	72	THF extraction
19[92]	Fructose 20 wt.%	[BMIM][Cl]/MIBK[b]	WCl_6[a]	50	4h	-	61	MIBK extraction
20[92]	Fructose 20 wt.%	[BMIM][Cl]/ EtOAc[b]	WCl_6[a]	50	4h	-	59	EtOAc extraction

21[89]	Glucose 17wt.% 20 wt.% 23 wt.% 26 wt.%	[EMim][BF_4][b]	$SnCl_4$[a]	100	3h	98 99 100 99	62 61 61 58	EtOAc extraction
22[89]	Fructose	[EMIM][BF_4]	$SnCl_4$	100	3h	100	62	EtOAc extraction
23[89]	Sucrose 17 wt%	[EMIM][BF_4]	$SnCl_4$	100	3h	100	65	EtOAc extraction
24[89]	Cellobiose	[EMIM][BF_4]	$SnCl_4$	100	3h	100	57	EtOAc extraction
25[89]	Starch	[EMIM][BF_4]	$SnCl_4$	100	3h	100	47	EtOAc extraction
26[161]	Glucose	DMSO	$SnCl_4$-TBAB	100	2h		69[a]	HPLC
27[162]	Fructose	DMSO/[BMIM]Cl	$GeCl_4$	25	12h		70[a]	HPLC
28[163]	Glucose	water/MIBK	mesoporous TP[a]	170	2h	56	33[a]	HPLC
29[164]	Fructose	DMSO	PS-NHC–F^{III}[a]	100	3h	97	75[c]	HPLC
30[165]	Glucose	DMA	AlI_3	120	15min		52	GC
31[166]	Fructose	GVL	$AlCl_3$/HCl	170	20min	94	84	
32[166]	Glucose	GVL	$AlCl_3$/HCl	170	40min	88	70	
33[167]	Glucose	H_2O/MIBK	$AlCl_3$/ChCl[a]	150	15min	90	70	HPLC
34[168]	Cellulose	DMSO	$InCl_3$/[C_3SO_3Hmim][HSO_4][a]	160	5h	84.6	45.3	HPLC

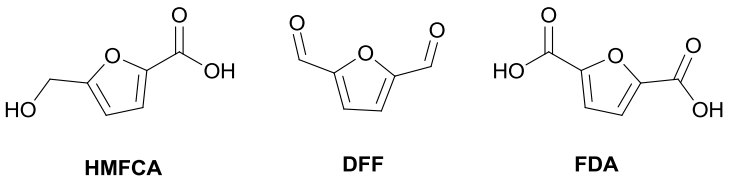
[a] Catalyst was recycled, [b] Solvent reused, [c] 73% isolated HMF yield.

1.5 Synthetic applications of HMF

The structural motifs present in HMF, namely furan, primary hydroxyl and formyl functionalities potentiates further synthetic transformation to other target molecules derived by the following main transformations: selective oxidation and reduction of the formyl, hydroxyl groups and furan ring, carbonyl and hydroxyl homologation and whole skeleton transformations. An overview of the important synthetic transformation of HMF will be provided in this part including the most recent reports in the literature published after our team review.

1.5.1 Oxidation

The oxidation of HMF can be performed selectively to the formyl or hydroxyl groups to form 5-hydroxymethyl-2-furancarboxylic acid (HMFCA) and 2,5-furandicarbaldehyde (DFF) respectively or can involve both groups to give 2,5-furandicarboxylic acid (FDA) which are compounds of a considerable interest as well as starting material for further transformations and chemical building blocks for the industry[17,169] (Scheme 17**)**.

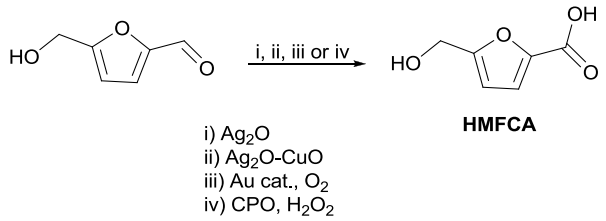


Scheme 17.

1.5.1.1 Selective oxidation of formyl group

There are several examples in the literature for the selective oxidation of the formyl group of HMF to HMFCA by using silver oxide [7,170] or mixture of silver and copper (II) oxides[171] in basic conditions (Scheme 18). Gorbanev *et al.*[172] reported the formation of HMFCA as an intermediate product during the aerobic oxidation of HMF to FDA with Au/TiO_2 catalyst in basic aqueous solution at ambient temperature. They studied the relationship between the formed products and the amount of base or applied O_2 pressure and observed that lower pressures or low concentrations of base afforded relatively more of the intermediate oxidation product HMFCA compared to FDA. Casanova *et al.*[173] also observed the formation of HMFCA as an intermediate product during gold-nanoparticle catalyzed aerobic oxidation of HMF. They described that selective oxidation to HMFCA take place at 25˚C after 4h and reported 100% yield. Davis *et al.*[174] described 92-93% selectivity towards HMFCA with 100% conversion of HMF promoted by Au/C and Au/TiO_2 in basic conditions. Van Deurzen *et al.*[175] oxidized HMF with H_2O_2 and chloroperoxidase (CPO) which is an enzyme known to be effective catalyst for various oxidation reactions with H_2O_2. They observed formation DFF

as a major product and unexpected formation of HMFCA as a minor product in up to 40% yield



Scheme 18.

1.5.1.2 Selective oxidation of the hydroxyl group

The selective oxidation of hydroxyl group of HMF leads to the formation of DFF which is an important monomer for the industry.[176] Numerous examples in the literature described the selective oxidation of hydroxyl group of HMF to DFF using diverse oxidants.

Reijendam *et al.* (**Table 9**, entry 1) obtained DFF in 37% yield by using lead tetraacetate in pyridine. Morikawa oxidized HMF with variety of oxidants and reported higher yields (**Table 9**, entries 2-5). Cottier *et al*. obtained DFF in 58% yield by using DMSO-potassium dichromate oxidative complex at 100˚C. They observed that the application of ultrasonic irradiation afforded DFF in higher yield, 75% (**Table 9**, entry 7). Trimethylammonium chlorochomate (TMACC)-Al_2O_3 oxidative system was also tested in conventional and under sonochemical conditions providing DFF in similar yields of 75% and 72% respectively (**Table 9**, entry 8). The same authors performed oxidation of HMF adsorbed together with pyridinium chlorochromate (PCC) on Al_2O_3 and achieved DFF in 58% yield (**Table 9**, entry 9). McDermott and Stockman also performed oxidation with PCC in CH_2Cl_2 and reported slightly higher yield (**Table 9**, entry 10). Quantitative yield was obtained by Mehdi *et al*. who performed oxidation of HMF with $(NH_4)_2[Ce(NO_3)_6]$ (CAN) in [EMIM][Tf] ionic liquid as a solvent (**Table 9**, entry 11). DFF was obtained *via* CPO catalyzed oxidation of HMF with H_2O_2. Optimum activity for the oxidation of HMF was observed at pH 5 providing 89% conversion and 59% selectivity. The highest selectivity 74% was observed at pH 3 with 25% conversion of HMF (**Table 9**, entry 12). Cotier *et al*.[177] (**Table 9**, entry 13) reported the oxidation of HMF with different 4-substituted 2,2,6,6-tetramethylpiperidine-1-oxide (TEMPO) free radicals and supporting co-oxidants. The best co-oxidant was found to be calcium hypochlorite in the presence of 4-benzoyloxy-TEMPO providing 81% yield. Dess-Martin oxidation of HMF was also found to be effective, providing DFF in 74% yield (**Table 9**, entry 14).

Table 9 Examples of oxidation of HMF to DFF using different oxidants.

Entry	Reaction conditions	Yield (%)
1[178]	$Pb(OAc)_2$, pyridine	37%
2[179, 180]	CrO_3, pyridine	73%, 68%
3[181]	Ac_2O, DMSO	76%
4[181]	HNO_3, DMSO	31-67%
5[181]	N_2O_4, DMSO	76%
6[182]	$BaMnO_4$	93%
7[183]	$K_2Cr_2O_7$, DMSO, ultrasonic irradiation	75%
8[183]	TMACC, Al_2O_3, ultrasonic irradiation	72%
9[17,184]	PCC,Al_2O_3, ultrasonic irradiation	58%
10[185]	PCC, CH_2Cl_2	65%
11[186]	[EMIM][TfO], CAN, 100˚C	100%
12[187]	CPO/ H_2O_2	[a]
13[177]	4-benzoyloxy-TEMPO, $Ca(ClO)_2$	81%
14[188]	Dess-Martin periodinane	74%

[a] *89% conversion of HMF and 59% selectivity*

Several authors investigated the conversion of HMF to DFF with oxygen, air or other more economical and environmental friendly oxidants using different metal based catalysts. Partenheimer and Grushin[189] reported the oxidation of HMF to DFF by using Co/Mn/Zr/Br or Co/Mn/Br catalysts and air as oxidant. They observed that Co/Mn/Zr/Br catalyst was the more active one, providing higher conversion and selectivity. As it was expected under comparable reaction conditions the conversion increased with the temperature. (**Table 10**, entry1). Carlini *et al*. (**Table 10**, entry 2) oxidized HMF to DFF in a biphasic water/MIBK media or in pure organic solvents using various metal-doped unsupported or TiO_2 – supported vanadyl phosphate (VOP) catalysts under pressure of O_2 or Air. The authors reported up to 10% conversion and 60–100% selectivity when water/MIBK mixture was used as a reaction media. Higher conversion rates were obtained in only MIBK as a solvent but with lower selectivity (98% conversion and 50% selectivity). DMF was found to be the best solvent for this transformation providing up to 84% conversion and 97% selectivity. Amarasekara *et al*. reported the conversion of HMF to DFF at room temperature without formation of FDA using NaClO as oxidant and Mn(III)-salen catalysts. Oxygen and H_2O_2 were also tested as more economical oxidants but both failed to give DFF (**Table 10**, entry 3). Cu and V catalysts supported on poly(4-vinylpyridine) crosslinked with 33% of divinylbenzene (PVP) were tested for the heterogeneous catalytic aerobic selective oxidation of HMF. They provided higher activity and better chemoselectivity than the corresponding homogeneous catalysts, when the proper solvent was used for the reaction. The authors also observed that V containing polymeric catalysts were more active than Cu ones (**Table 10**, entry 4). Lilga *et al*. patented a method based on activated MnO_2 oxidation of HMF to DFF in

good yields (**Table 10**, entry 5). Various inorganic vanadium compounds, such as V_2O_5 and $VOHPO_4 \cdot 0.5H_2O$ efficiently catalyzed air oxidation of HMF to DFF. These kinds of V catalysts were found to be active for air-oxidation of not only pure HMF but also crude HMF produced *via* dehydration of fructose. It was observed that the least expensive and most readily available catalyst used, V_2O_5, exhibited one of the highest efficiencies for the process providing DFF in 58% yield calculated on HMF and 43% calculated on fructose. The best result was observed for $VOHPO_4 \cdot 0.5H_2O$, 61% and 45% yield respectively (**Table 10**, entry 6). Very detailed studies on air or O_2 oxidation of HMF promoted by supported platinum catalysts in aqueous solutions were reported by Lilga *et al*. They observed good conversion to DFF by using Pt/SiO_2 catalyst and air as oxidant in neutral solution. Similar conversion and selectivity were achieved with Pt/ZrO_2 and air in acidic solution (**Table 10**, entries 7 and 8).

Table 10 Oxidation of HMF to DFF using metal catalysts.

Entry	Reaction conditions	HMF conversion (%)	DFF Selectivity (%)
1	Co/Mn/Br/Zr or Co/Mn/Br, 70bar Air, 50-75˚C, 2h	60 - 99	38-73
2[190]	VOP or MVOP, O_2 or Air, 80-150˚C	up to 98	up to 99
3[191]	Mn(III)-salen/NaClO, RT	89[a]	100
4[192]	Cu or V based catalysts, Air, DMSO, 130-160˚C.	up to 85	up to >99
5[193]	MnO_2, CH_2Cl_2, reflux, 8h	80	100
6[126]	$VOHPO_4$*$0.5H_2O$, DMSO, 150°C, Air 1atm.	61[a]	-
7[194]	5% Pt/SiO_2, 150psi Air, 60-100°C	60	70
8[194]	5% Pt/ZrO_2, 150psi Air, 100°C, 40% AcOH	50	70
9[195]	CuCl, TEMPO, O_2 2,2-bipyridine	97	92
10[196]	Cu or V based catalysts, acetonitrile, 140ºC, O_2 40atm.	98[a]	
11[197]	Ru supported γ-alumina catalyst, toluene, O_2, 130ºC, 40psi.	99	97
12[195]	V_2O_5/zeolite, DMSO, 125ºC, O_2, 10bar.	84	99
13[198]	Vanadium phosphate oxides, 1bar O_2, 80-110°C	5-99	8-83

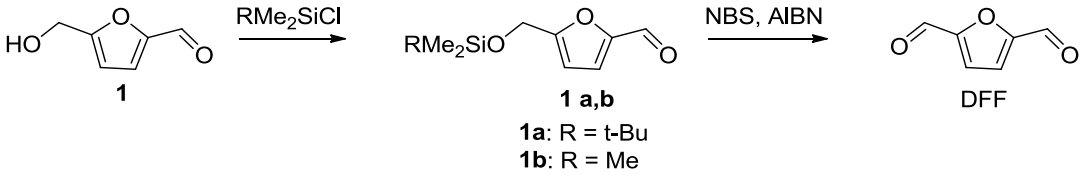
[a] *DFF yield.*

Riisager *et al.* reported CuCl/TEMPO mediated oxygen HMF oxidation to DFF in good yields and selectivity. 2,2-bipyridine was found to improve significantly the HMF conversion. The authors studied also different solvents and observed acetonitrile to be the best one (**Table 10**, entry 9). Immobilization of vanadyl (VO_2^+) and cupric (Cu_2^+) ions on sulfonated carbon and their catalytic activity toward the aerobic oxidation of HMF were performed by Kim *et al.* (**Table 10**, entry 10). VO_2^+-immobilized carbon catalysts showed high stability without any leaching. On the other hand better results were obtained using Cu immobilized catalysts with up to 98% DFF yield, but they have been not so stable and Cu leaching was observed. Selective oxidation of HMF to DFF toward industrial production over Ru supported γ-alumina catalyst using molecular oxygen as oxidant was described by Cho *et al.* (**Table 10**, entry 11). Toluene was found to be the most suitable solvent. The catalyst was successfully reused for 5 cycles. Selective oxidation of HMF to DFF over vanadium phosphate oxide (VPO)-based heterogeneous catalysts in various solvents has been reported

(**Table 10**, entry 13). Maximum yield of 83% has been achieved over $C_{14}VOPO_4$ and $C_{14}VOHPO_4$ after 6 h in toluene at 110ºC. The catalyst has been recycled 3 times.

Electrochemical oxidation of HMF carried out in a divided cell at a platinum anode in a biphasic H_2O/CH_2Cl_2 system was reported by Skowroński *et al.*[199] Various salts were tested as supporting electrolytes. The best result of 68% yield was obtained with Na_2HPO_4 for 7h.

Indirect oxidation of HMF providing DFF in 91% yield has been performed *via* initial protection of the hydroxyl group to 5-*tert*-butyldimethylsylil **1a** and 5-trimethylsilyl **1b** derivatives, followed by oxidation with N-bromosuccinimide (NBS) in the presence of azoisobutyronitrile (AIBN) (Scheme 19).[200]

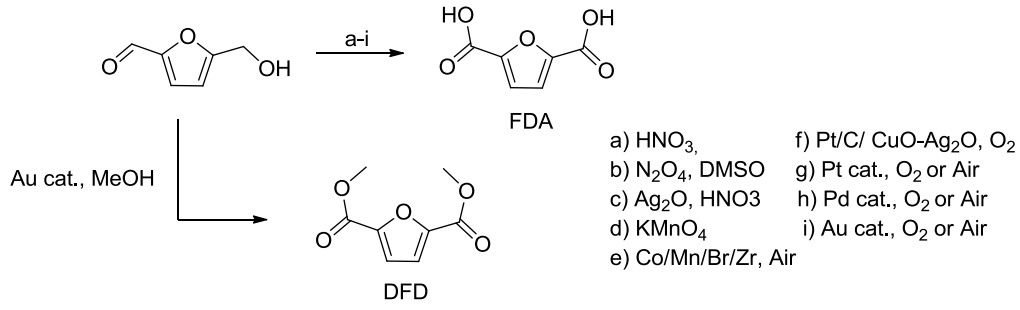


Scheme 19.

1.5.1.3 Oxidation of formyl and hydroxyl group

HMF is a widely exploited precursor for the synthesis of FDA (Scheme 20) which is a potential biorenewable replacement monomer for terephthalic acid in polyethylene terephthalate plastics and has been defined as one of the building blocks of the future.[169]

Morikawa[181] oxidized HMF to FDA using N_2O_4 in DMSO and nitric acid in DMSO. El-Hajj *et al.*[170] use nitric acid for this transformation and obtained FDA in 24% yield. The authors reported higher yields using Ag_2O and HNO_3 or $KMnO_4$ as oxidants 47% and 70% respectively. Cottier *et al.*[17,184] also use nitric acid as oxidant and observed the formation of FDA and 5-formyl-2-furancarboxylic acid, which was resistant for further oxidation under this conditions. The products ratio was found to be dependent on the reaction conditions.



Scheme 20.

Several authors published and patented methods for the oxidation of HMF to FDA using more economical and environmental friendly oxidant and heterogonous metal catalysts. Vinke *et al.*[201] reported an oxidation of HMF to FDA in near-quantitative yield in basic

reaction conditions using Pt/Al_2O_3 as catalyst at 60˚C. Air oxidation of HMF catalyzed by Co/Mn/Br/Zr or Co/Mn/Br resulted in the formation of FDA in up to 61% yield and 2-carboxy-5-formylfuran as a minor product in up to 3% yield.[189] It was observed that the yield increased with the catalyst concentration and temperature but not with the addition of Zr to the Co/Mn/Br. Lew[171] patented an oxidation method using platinum catalyst adsorbed on activated charcoal in basic aqueous solutions and bubbling oxygen and achieved 95% FDA yield. Lilga *et al.*[193] patented a method for the synthesis of FDA from HMF using Pt/ZrO_2 catalyst and air. The authors claimed 100% conversion and 98% selectivity. Gorbanev *et al.*[172] oxidized HMF to FDA in up to 71% yield using commercial heterogeneous Au/TiO_2 nanopracticle catalyst in aq. NaOH under 20 bar of O_2 and ambient temperature. The authors also studied the influence of oxygen pressure and the amount of hydroxide base on the selectivity and yield. Casanova *et al.*[173] carried out this transformation using gold nanoparticle catalysts with different supports in basic aqueous conditions. The oxidative pathway starts with the fast oxidation of HMF into HMFCA, which further oxidation into FDA was the limiting reaction step. $Au\text{-}CeO_2$ and $Au\text{-}TiO_2$ catalysts were found to be the most active providing FDA in >99% yield. Under optimized reaction conditions (10 bar of O_2, 130˚C and NaOH/HMF molar ratio of 4) it was shown that $Au–CeO_2$ provides higher activity and selectivity for FDA. Reduced substrate degradation and increased lifetime of the catalyst was observed by performing the reaction as two-steps procedure, first at 25˚C for 4h followed by 130˚C for 3h. Screening of supported platinum catalysts at different pH in flow reactor was performed by Lilga *et al.*[194] They obtained nearly quantitative yields of FDA using stoichiometric aqueous Na_2CO_3, with air or O_2 over Pt/C or Pt/Al_2O_3 and 98% selectivity with 100% HMF conversion over Pt/ZrO_2 at neutral pH with air. The best result in acidic conditions, 85% selectivity and 100% conversion, was achieved with O_2 and Pt/ZrO_2. Davis *et al.*[174] described O_2 oxidation of HMF to FDA promoted by supported Pt, Pd and Au catalysts in basic aqueous conditions. They observed that Pt/C and Pd/C were more selective towards FDA comparing to Au/C and Au/Ti_2O under identical conditions, providing respectively 79% and 71% selectivity with 100% HMF conversion after 6h. Higher pressures of O_2 and concentrations of base were required for Au catalysts resulting in up to 80% selectivity and 100% conversion after 22h. Riisager *et al.* studied Cu catalyzed HMF oxidation using stoichiometric oxidants. The best results were observed using CuCl/t-BuOOH system in acetonitrile, which provided 45% yield in 48h at room temperature.[195] Thermally stable Fe(III) POP-1 materials containing basic porphyrin subunits and Fe(III) metal were investigated for HMF oxidation with molecular oxygen or air in aqueous medium by Bhaumik *et al.*[202] 80% FDA selectivity and 100% conversion have been

obtained after 10h with 10 bar of air at 100ºC. HMF oxidation to FDA was performed under mild reaction conditions using TiO_2-supported Au and Au–Cu catalysts by Cavani *et al.*[203] The presence of Cu, which by itself was not active, resulted in an increase of the catalytic performance with respect to the Au catalyst alone. After 4 h reaction time at 95ºC, a yield higher than 90% of FDA was achieved in aqueous conditions using 10bar of O_2 as oxidant. Prati et al.[204] showed that the modification of Au/C catalyst with Pt or Pd produces stable and recyclable catalysts for the selective oxidation of HMF to FDA. Although in general Au catalysts present good activity, they suffer rapid deactivation, which was successfully overcome by the authors. Pd modified Au/C catalyst was reused 5 times without loss of activity and 99% conversion and 99% selectivity towards FDA were achieved. Gold nanoclusters of 1 nm in average, encapsulated within the HY zeolite supercage was reported to be highly efficient catalytic system by J. Xu *et al.*[205] who achieved 99% selectivity and conversion. The mechanism of selective oxidation of aqueous HMF at high pH was studied over supported Pt and Au catalysts by Davis *et al.*[206] Results from labeling experiments conducted with $^{18}O_2$ and $H_2{}^{18}O$ indicated that water was the source of oxygen atoms during the oxidation of HMF to HMFCA and FDA. HMF was quantitatively oxidized to FDA at 100°C under 40 bar air in moderately basic aqueous solution in the presence of active carbon supported platinum and bismuth–platinum catalysts by Besson *et al.*[207] It was shown the importance of the nature of the base. The use of 2 equivalents of carbonate with respect to HMF displayed invreased activity compared with the addition of 4 equivalents of bicarbonate, by maintaining the pH at a value favorable for the oxidation to FDA. The catalyst has been recycled 5 times with minor erosion of activity.

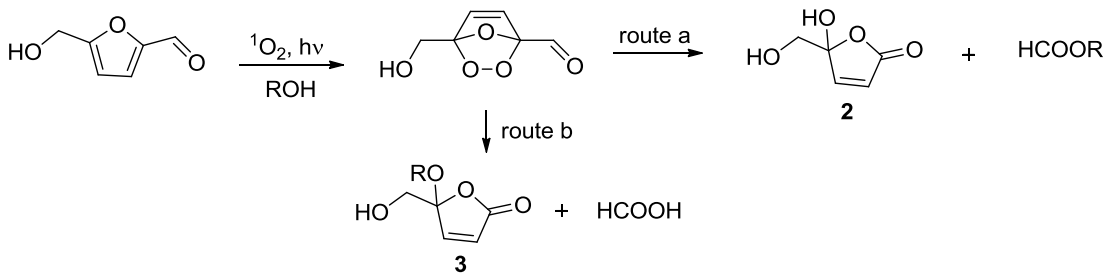
Taarning *et al.*[208] reported the formation of dimethyl furan-2,5-dicarboxylate (DFD) in excellent yield at 130˚C in MeOH in presence of Au/TiO_2 catalyst and basic conditions. When reaction was carried out at room temperature the oxidation takes place only at the formyl group and 5-hydroxymethyl methylfuroate (HMMF) was obtained in excellent yield.

Casanova *et al.*[209] described one pot-base free aerobic oxidative esterification in methanol of HMF into DFD by using $Au\text{-}CeO_2$ catalyst, which could be recovered and reused with minimal loss of activity. It was observed that the temperature and the substrate to catalyst ratio affect the reaction rate. However, quantitative yields of DFD were always obtained.

1.5.1.4 Oxidation of the furan ring

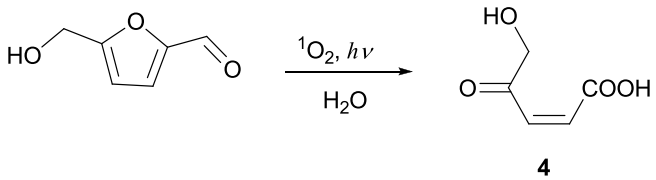
Oxidation of the furan ring of HMF can take place under photo-oxygenation reaction conditions. When alcohol is used as a reaction media the oxidation take place *via* the formation of endoperoxide followed by the attack of an alcohol molecule on the formyl group

or on the carbon atom "5" in the furan ring, leading respectively to the formation of hydroxybutenolide **2** (Scheme 21, route a) as major product and alcoxybutenolide **3** (Scheme 21, route b) as minor product.[210-212]



Scheme 21.

Marisa *et al.*[213] reported the photochemical oxidation of HMF in water providing 5-hydroxy-4-keto-2-pentenoic acid **4** (Scheme 22) which is a possible intermediate or monomer for the chemical industry.

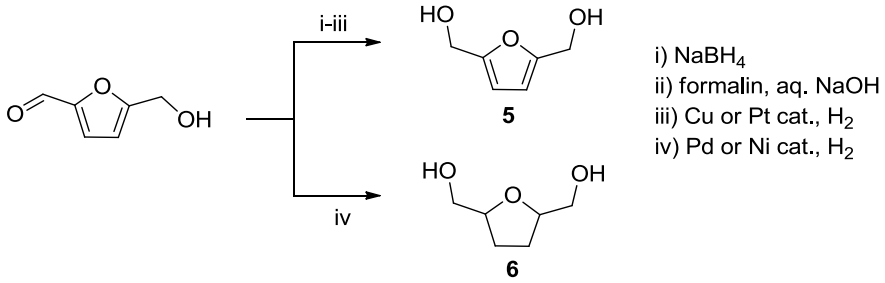


Scheme 22.

1.5.2 Reduction of the furan ring and/or formyl group

Selectively reduction of the formyl group of HMF leads to formation of 2,5-bis-(hydroxymethyl)furan **5** which is an important chemical building block used in the production of polymers and polyurethane foams.[18] Several reports described the reduction of HMF to **5** with sodium borohydride in high yields.[214-217] Turner *et al.*[218] reported the synthesis of **5** in 76.9% yield by using formalin and aq. NaOH. Nickel, copper chromite, platinum oxide, cobalt oxide, molybdenum oxide and sodium amalgam catalysts were also found to be effective for this transformation.[14,17] Hydrogenation of HMF in aqueous media in presence of nickel, copper, platinum, palladium or ruthenium catalysts have been studied by Mentech *et al.*[219] **5** was obtained only as the main product in case of copper or platinum catalysts, while 100% conversion and selectivity towards **5** was observed by using Pt/C, PtO_2 or $2CuO.Cr_2O_3$. The presence of Pd/C[219] or Raney nickel catalysts[218-221] originated the hydrogenation of the furan ring and 2,5-Bis-(hydroxymethyl) tetrahydrofuran **6** was formed as a major product in high yields. Hydrogenation of HMF over supported Ru, Pd, and Pt catalysts in monophasic and biphasic reactor systems in order to determine the effects of the metal, support, solution phase acidity, and the solvent and to elucidate the factors that determine the selectivity of HMF conversion to **6** have been studied by Dumesic *et al.*[222]

High selectivity 91% to **6** and 100% HMF conversion can be achieved when the hydrogenation catalyst is comprised of ruthenium deposited on a support with a high isoelectric point like CeO_2 (Scheme 23).



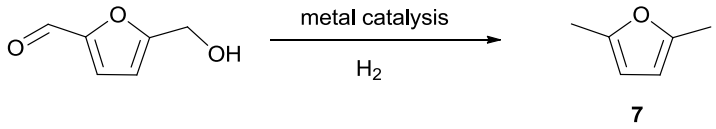
Scheme 23.

1.5.3 Reduction of formyl and hydroxyl group

Reduction of both formyl and hydroxyl group of HMF is one of the synthetic pathways for the synthesis of 2,5-dimethylfuran (2,5-DMF) **7** (Scheme 24) which is a compound of particular interest because of its high energy content and potential use as a biofuel.[84] Roman-Leshkov et al.[84] reported a two-step process for the production of **7**. They subjected HMF obtained from fructose in biphasic reactor to hydrogenation over carbon-supported copper-ruthenium (CuRu/C) catalyst and obtained **7** in very good yields (76-79%).

Two years later Binder *et al.*[109] reported the hydrogenolisys of crude HMF from corn stover in the presence of CuRu/C catalyst providing **7** in 49% yield. Luijkx *et al.*[223] described in the same year the formation of **7** by the hydrogenation of HMF in presence of palladium catalyst.

Chidambaram and Bell[91] reported the hydrogenation of either neat HMF or HMF obtained by dehydration of fructose in a mixture of [EMIM]Cl and acetonitrile promoted by carbon-supported transition metals. They observed the formation of series of products and in particular **7**. Pd/C catalyst was found to be the most active one providing **7** in 16% yield with 47% HMF conversion. Wang *et al.*[224] reported efficient transformation of HMF to **7** using Ru/Co_3O_4 catalyst. Over 93% yield was obtained after 24h in THF at 130°C and 0.7MPa H_2. The authors also discovered that Ru is important for the hydrogenation while, CoO_x spices are important for the hydrogenolysis of the hydroxyl groups. The catalyst displayed good reusability and was recycled for 5 times without loss of activity. Complete conversion of HMF to volatile compounds was achieved in 45 min, without the formation of higher boiling side products by Riisager *et al.*[225] Reduction of HMF at 300 °C for 2 h resulted in 61% total yield of three main products 2,5-DMF, **6** and 2-hexanol. The 2,5-DMF yield was found to be 41% after 3 h at 240°C, and 48%. at 260°C A total yield of 58% of 2,5-DMF and **6** at 260°C was achieved after 3 h.

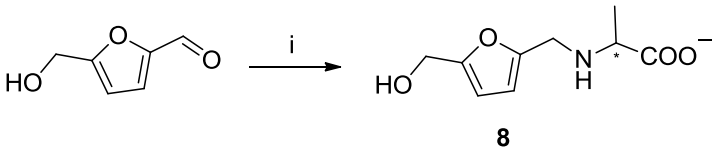


Scheme 24.

1.5.4 Reactions of the formyl group

1.5.4.1 Reductive amination

Villard *at al.*[226] reported a method for the reductive amination of HMF with L-alanine or D-alanine in aq. NaOH in the presence of Raney nickel. The resulting (R) or (S)-N-(1-Carboxyethyl)-2-(hydroxymethyl)-5-(methylamino) furan **8** were isolated in 38% yield as an ammonium salt (Scheme 25).

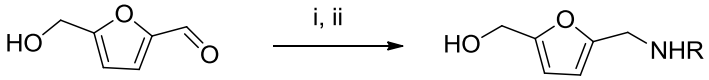


8

i) L or D-alanine, water, aq. NaOH, pH 8.5, Ni/H2, RT, 5bar, 48h

Scheme 25.

The reductive amination of HMF with ammonia allowed the synthesis of 2-(hydroxymethyl)-5-(aminomethyl)-furan **9** in 72% yield as a useful intermediate for further transformations.[227,228] Using the same conditions but switching from liq. NH_3 to aq. $MeNH_2$, 2-(hydroxymethyl)-5-(methylaminomethyl)-furan **10** was obtained in excellent yield of 91% (Scheme 26).[228]

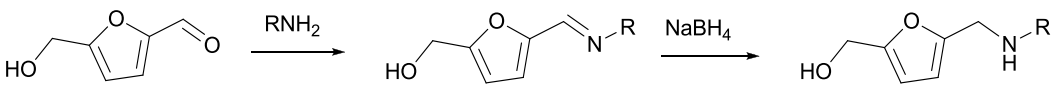


i) liq. NH_3, Raney nickel, H_2
ii) aq. $MeNH_2$, Raney nickel, H_2

9 R= H
10 R= Me

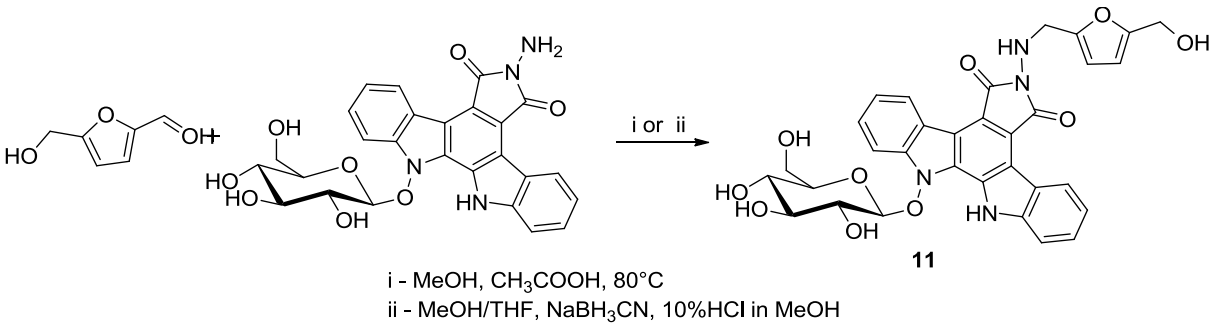
Scheme 26.

Cukalovic and Stevens[81] reported a procedure for the synthesis of several 5-aminomethyl-2-furfuryl alcohols in very good yields starting from HMF and aromatic or aliphatic primary amines. The reaction was performed *via in situ* reduction with $NaBH_4$ of the initially yielded aldimines in water or bio-based solvents, such as methanol and ethanol. Microwave heating was observed to be beneficial and provided higher reaction rates compared to the room temperature reactions (Scheme 27).



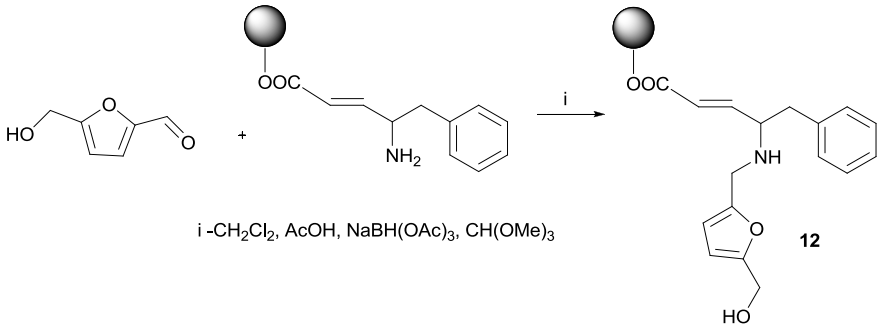
Scheme 27.

Kojiri *at al.*[229] patented a method where they claimed the synthesis of novel indolopyrrolocarbazole derivatives and their studies as antitumor agents. The HMF derivative **11** was achieved *via* two step reductive amination (Scheme 28)



Scheme 28.

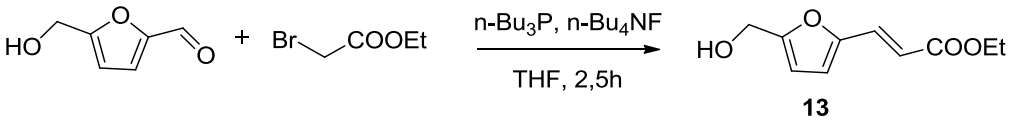
Resin-bound compound **12** was obtained *via* reductive amination of HMF by Sun and Murray[230] and used for further Diels-Alder transformations (Scheme 29).



Scheme 29.

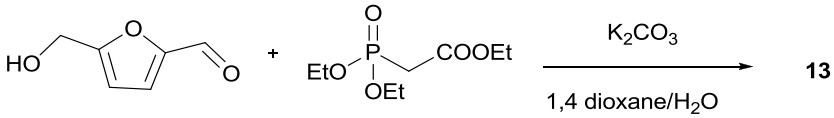
1.5.4.2 Wittig type reactions

One pot fluoride promoted Wittig reaction of HMF was reported from Fumagalli *et al.*[231] The corresponding ethyl 3-(5- (hydroxymethyl) furan-2-yl) acrylate **13** was obtained in 81% yield and 85% diastereoselectivity towards *E* isomer (Scheme 30).



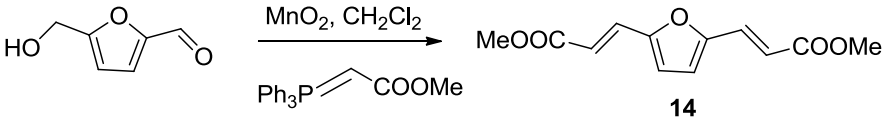
Scheme 30.

The same compound was also synthesized in 90% yield and *E* configuration *via* Wittig-Horner reaction with ethyl 2-(diethoxyphosphoryl)acetate.[232] Later Lasseuguette *et al.*[233] reported the synthesis of **13** in 80% yield using similar reaction conditions (Scheme 31).



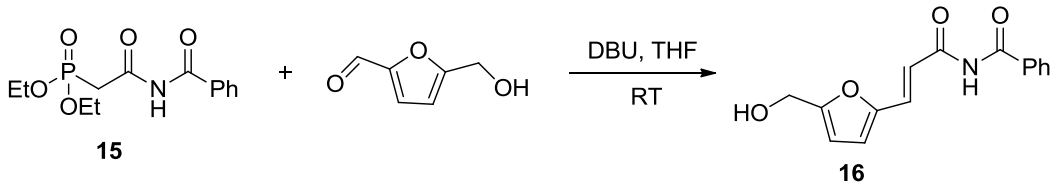
Scheme 31.

HMF has been subjected to Taylor's tandem oxidation/Wittig procedure by McDermott and Stockman[185] providing diester **14** in 87% yield in one step after 4 days as a 6.6:1 mixture of (*E*,*E*)- and (*E*,*Z*)-isomers (Scheme 32).



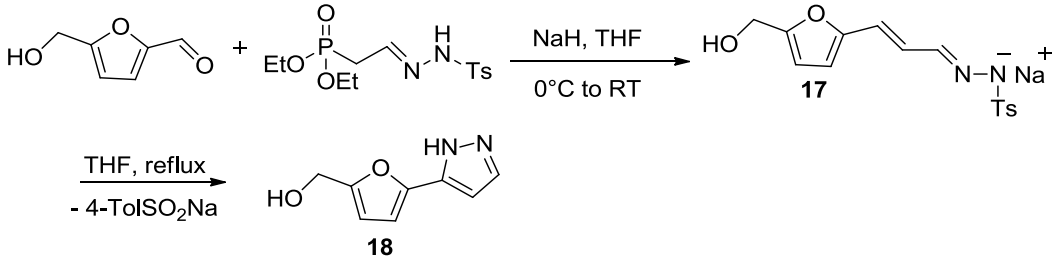
Scheme 32.

Goodman and Jacobsen[234] performed 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU)-mediated Horner-Wadsworth-Emmons reaction of HMF with phosphonate imide **15**. The reaction was carried out in THF providing N-[3-(5-Hydroxymethylfuran-2-yl)-acryloyl]-benzamide **16** in very good yield of 87%. Water was described as another possible solvent obviating any need to run the reaction under inert atmosphere (Scheme 33).



Scheme 33.

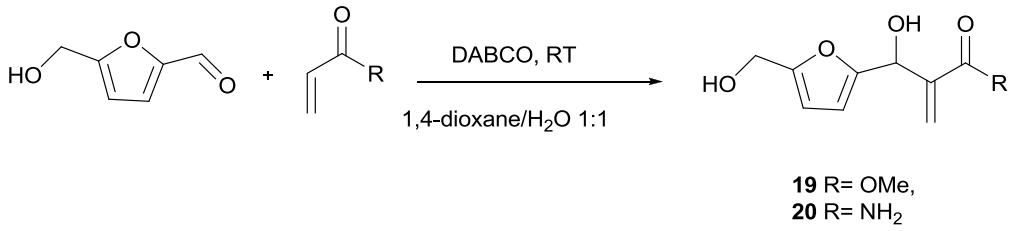
Another Horner-Wadsworth-Emmons reaction of HMF as a part of synthetic procedure for the synthesis of 3(5)-substituted pyrazoles was performed in one step without isolation of the intermediate α,β-unsaturated tosylhydrazone N-sodium salt **17** before the cyclyzation step. The final product **18** was isolated in 60% yield (Scheme 34).[235]



Scheme 34.

1.5.4.3 Baylis-Hillman reaction

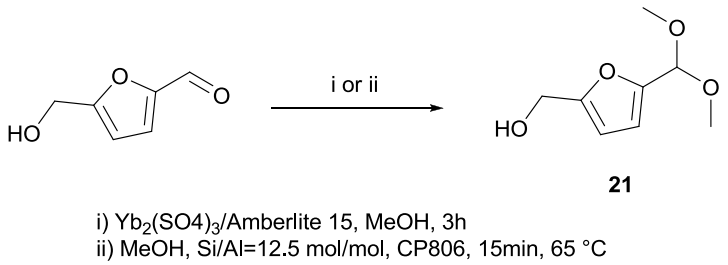
Baylis-Hillman reaction of HMF with methyl acrylate using stoichiometric base catalyst and aqueous medium was reported by Yu *et al.*[236] The corresponding product **19** was obtained in 62% yield after 36h using 1,4-diazabicyclo [2,2,2]octane (DABCO) as catalyst. One year letter Yu and Hu[237] described the formation of compound **20** in 61 % yield after 48h using the same reaction conditions and acryl amide (Scheme 35).



Scheme 35.

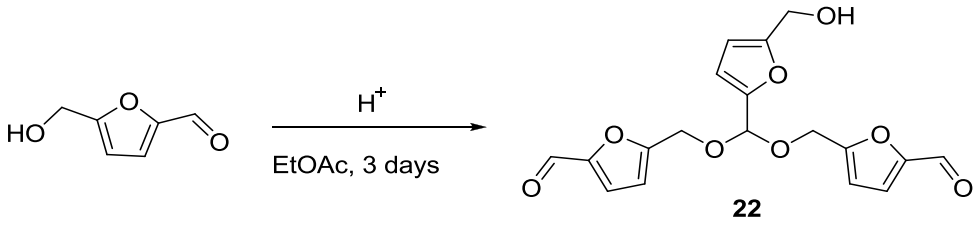
1.5.4.4 Acetal formation

Cottier *et al.*[238] reported reaction of HMF with trimethyl orthoformate in the presence of Ytterbium sulfate supported on Amberlite-15 providing the corresponding [5-(Dimethoxymethyl)-2-furyl] methanol **21** in 80% isolated yield. Higher yield of 96% was achieved by the condensation reaction of HMF and MeOH catalyzed by calcinated and dehydrated Al–beta zeolite (Si/Al = 12.5mol/mol, CP806) (Scheme 36).[209]



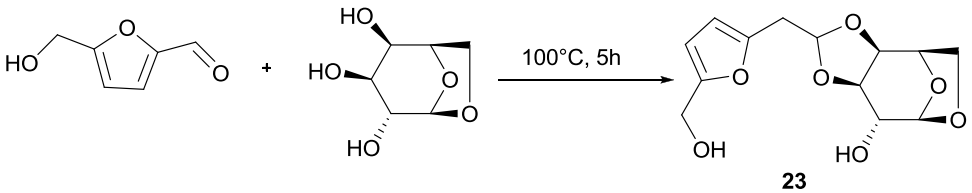
Scheme 36.

The synthesis of 5-hydroxymethyl-2-furaldehyde bis(5-formylfurfuryl) acetal **22** by using strong acid cation exchange resin as catalyst was patented by Terada *et al.*[239] The authors claimed 2.3% yield of **22** and its application for the preparation of flavor improving agents (Scheme 37).



Scheme 37.

The formation of the cyclic acetal **23** was achieved by Urashima *et al.*[240] *via* condensation reaction of levogalactosan and HMF at 100˚C (Scheme 38)



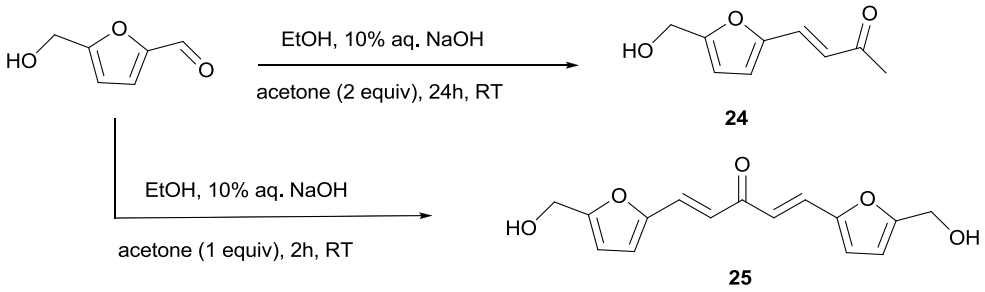
Scheme 38.

1.5.4.5 Aldol condensations

Several authors reported the aldol condensation reactions of HMF as a synthetic strategy for the synthesis of biological active compounds and useful intermediates for the synthesis of biofuels.[241]

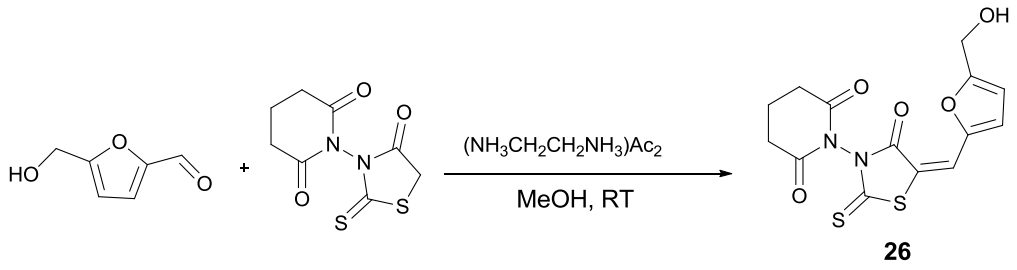
The aldol condensation reaction between HMF and acetophenone was performed in water or methanol in presence of base, providing 5-hydroxymethyl furfurylidene acetophenon in 80 and 82% yield respectively.[242]

The synthesis in 91% yield of naturally occurring furan derivative rehmanone C **24** which have displayed significant biological activity was described by Quiroz-Florentino *et al.*[243] *via* base catalyzed aldol condensation using 0.5 equivalents of acetone and HMF. The same reaction conditions, but using 2 equivalent of acetone provided the bis derivative **25** in 60% yield within 2h. (Scheme 39)



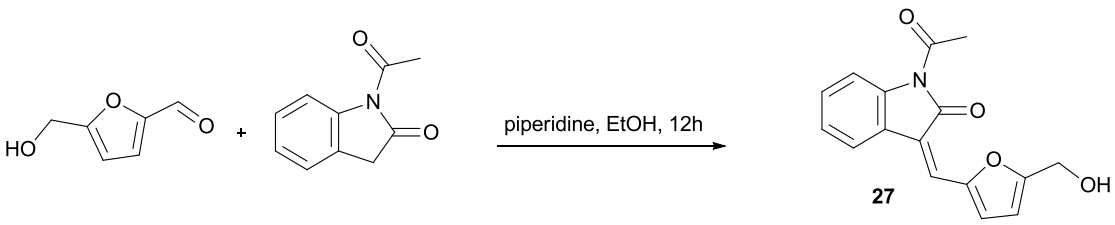
Scheme 39.

Another aldol condensation reaction of HMF towards the synthesis of biological active compound was reported by Hanefeld *et al.*[244] They described a method for the synthesis of rhodanine derivative **26** in 73% yield (Scheme 40).



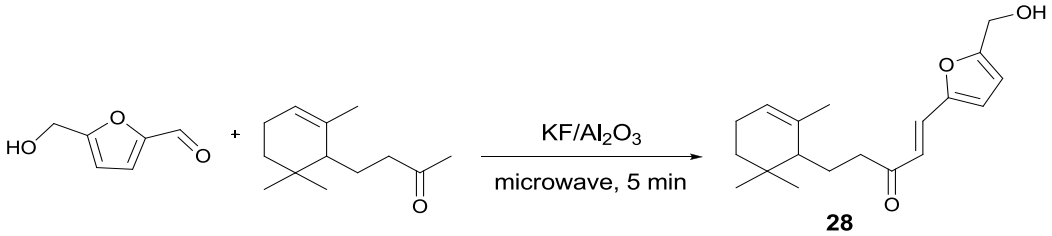
Scheme 40.

Shinobu *et al.*[245] reported the synthesis of 3-((5-(hydroxymethyl)furan-2-yl) methylene)-N-acetyl-2-oxoindoline **27** by using the reaction between HMF and N-acetyloxindole catalyzed by piperidine (Scheme 41).



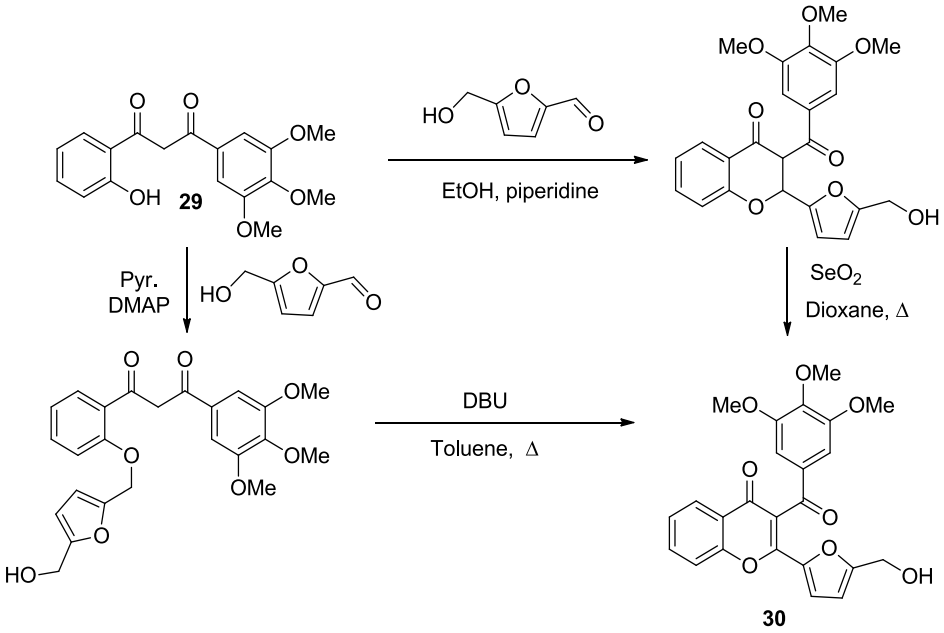
Scheme 41.

The aldol reaction of HMF under microwave irradiation in presence of KF/Al_2O_3 was described by Suryawanshi *et al.*[246] They obtained chalcone **28** in 76% yield and studied it`s antileishmanial activity. (Scheme 42).



Scheme 42.

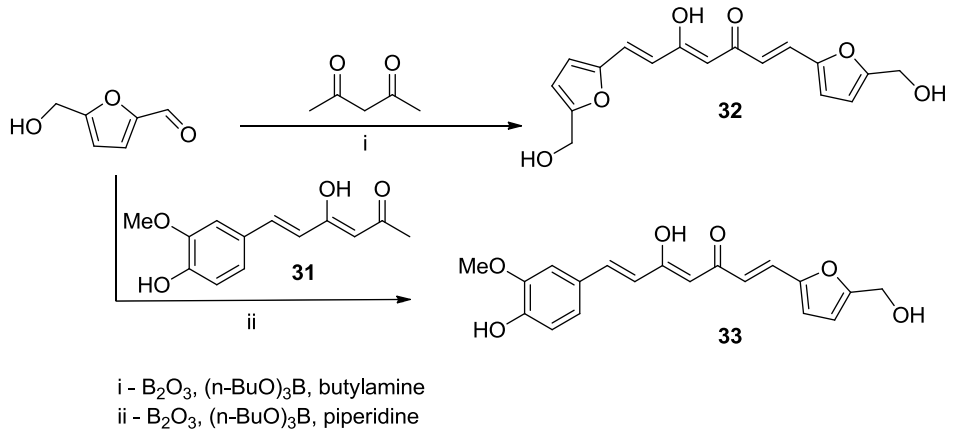
The synthesis and photochemistry of HMF chromone derivative **30** was investigated.[247] Compound **30** was obtained *via* piperidine catalyzed aldol condensation of 1-(2-hydroxyphenyl)-3-(3,4,5-trimethoxyphenyl)propane-1,3-dione **29** with HMF followed by SeO_2 oxidation or *via* DBU induced condensation as a simple and efficient pathway (Scheme 43).



Scheme 43.

The synthesis and citotoxicity studies of two HMF curcumin analogues **32** and **33** were reported.[248] Boric anhydride was first added to the reactions to form a complexes with 2,4-pentanedione or compound **31** in order to protect C-3 position from Knoevenagel

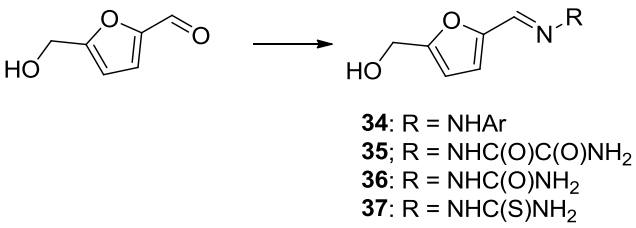
condensation in such way the aldol condensation takes place only at the terminal carbons. Compounds **32** and **33** were obtained in 12% and 32% yield respectively (Scheme 44)



Scheme 44.

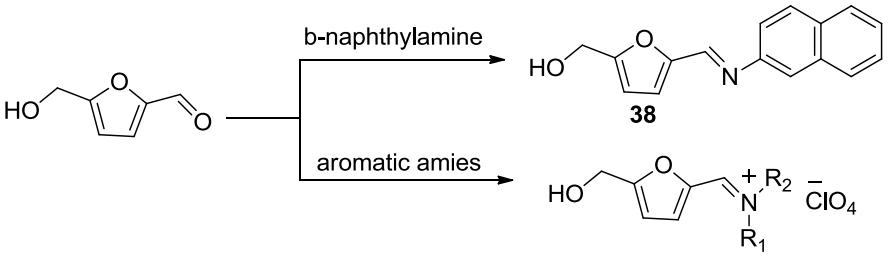
1.5.4.6 Other reactions

There are a considerable number of literature examples for the reactions of the formyl group of HMF with amino compounds resulting in the the formation of a range of products, including arylhydrazones **34**,[249-253] semioxamazone **35**,[254] semicarbazone **36**[255] and thiosemicarbazone **37**[256] (Scheme 45).



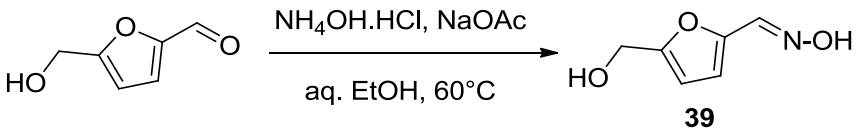
Scheme 45.

The reaction of HMF with aromatic amines led to the formation of Shiff bases such as β-naphthylamine Shiff base **38**[257] and azomethin salts[258] (Scheme 46).



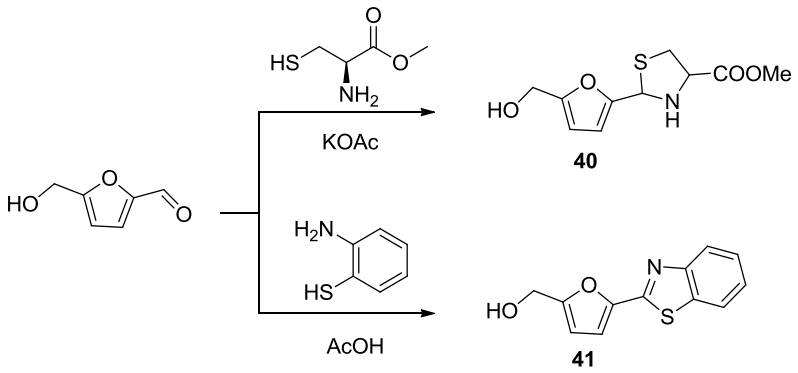
Scheme 46.

The conversion of HMF to its oxime derivative **39**[259] in 95% yield has been described and some biological active HMF proline-oxime containing peptides derivatives were also reported and studied (Scheme 47).[260]



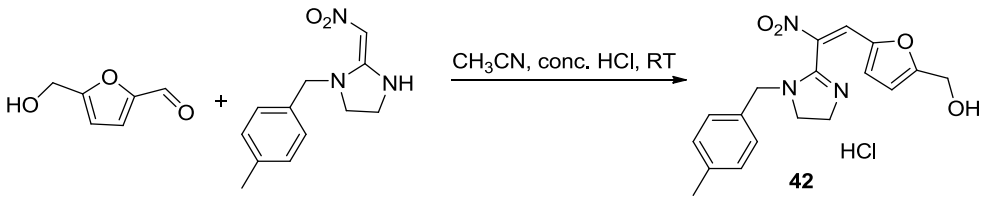
Scheme 47.

The reactions of HMF with 1,2-aminothiols led to the formation of new heterocyclic systems. Undheim. *et al*.[261] reported the synthesis of thiazolidine derivative **40** in 90% yield formed by reaction of L-cysteine methyl ester and HMF in the presence of potassium acetate. Benzothiazole derivative **41** was obtained in quantitative yield from HMF and 2-aminobenzenethiol in presence of acetic acid (Scheme 48)[262]



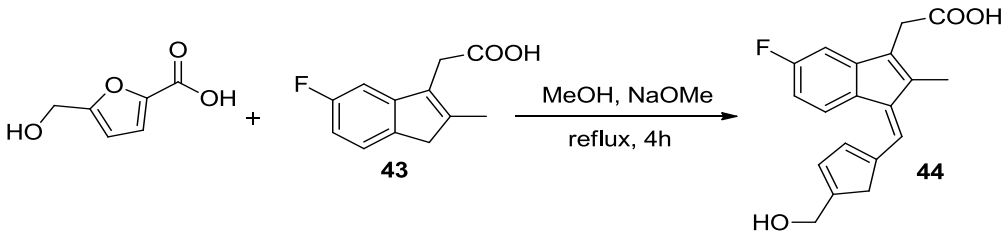
Scheme 48.

The synthesis and studies of insecticidal activities of neonicotinoid **42** was reported by Shao *et al*.[263] The final product was isolated in 72% yield as hydrochloric salt (Scheme 49).



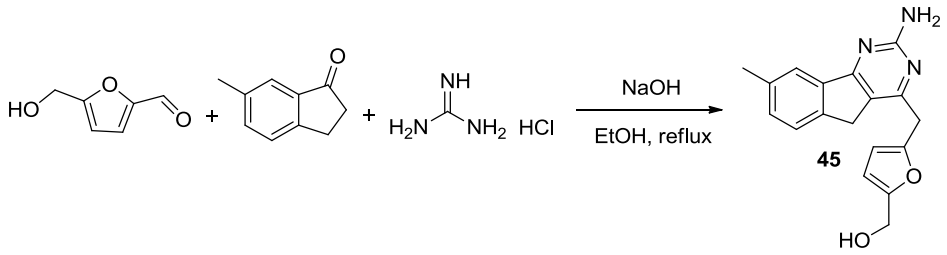
Scheme 49.

Karaguni *et al*.[264] reported a novel HMF inden derivative **44** with anti-proliferative properties obtained in 45% yield *via* one step condensation protocol using 5-fluoro-2-methylindene-3-acetic acid **43** (Scheme 50).



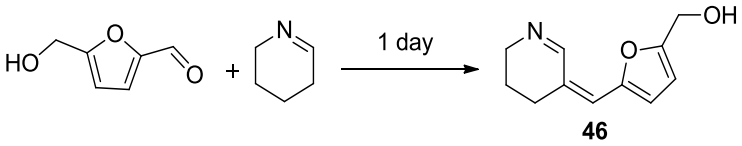
Scheme 50.

The adenosine receptor (A_{2A}) antagonist HMF derivative **45** was obtained *via* three component reaction of HMF[265] (Scheme 51).



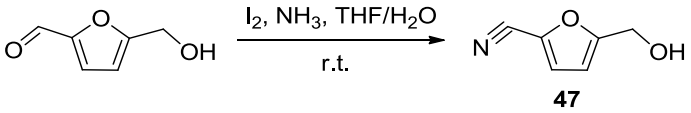
Scheme 51.

Condensation reaction between HMF and 2,3,4,5 tetrahydropyridine was reported by Miller providing the resulting derivative **46** in 64% isolated yield and *E* configuration (Scheme 52).[266]



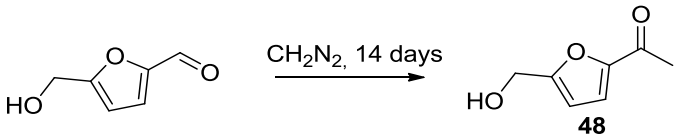
Scheme 52.

Baliani *et al.*[267] described a method for the conversion of aldehyde function of HMF into nitrile **47** in very good yield of 80% by using iodine in aqueous ammonia (Scheme 53).



Scheme 53.

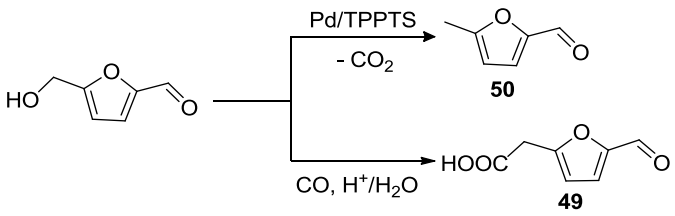
Ramonczai and Vargha[268] performed the reaction of HMF and diazomethane providing 5-hydroxymethyl-2-acetofuran **48** in 40% yield (Scheme 54).



Scheme 54.

HMF was selectively carbonylated to 5-formylfuran-2-acetic acid **49** in acidic aqueous media using a water-soluble palladium complex of trisulfonated triphenylphosphine (TPPTS) as catalyst.[269] The only observed byproduct was 5-methylfurfural **50** formed by the reduction of HMF. The activity and selectivity of the carbonylation was found to be influenced by the Pd/TPPTS molar ratio. The best efficiency was observed for Pd/TPPTS= 6 providing 90% conversion and 71.6% selectivity. The relationship between the selectivity and the nature of the anion of the acid component has also been studied. Acids of weakly or non-coordinating

anions, such as phosphoric, trifluoroacetic, 4-toluenesulfonic and sulphuric acids favors the carbonylation and **49** was the major product, while the acids of strongly coordinated anions such as HBr and HI decrease the selectivity. In fact when HI was used **50** was the only observed product (Scheme 55).

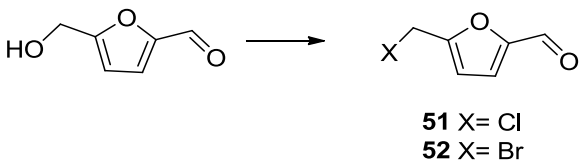


Scheme 55.

1.5.5 Reactions of the hydroxyl group

1.5.5.1 Formation of halides

Halogen substitution of the hydroxyl group of HMF can be easily performed, resulting in the formation of 5-halomethylfurfurals (Scheme 56)



Scheme 56.

Several synthetic protocols for the synthesis of 5-chloromethyfurfural **51** were described in the literature. Treatment of HMF with gaseous or 36% aq. HCl in various organic solvents led to the formation of **51** in moderate to very good yields (**Table 11**, entry 1). Sanda *et al*. reported a method for the synthesis of **51** using chlorotrimethylsilane and $CHCl_3$ or Me_2SO-Et_2O as solvents in similar yields. However, $CHCl_3$ was found to be the best solvent. (**Table 11** entry 2). Very detailed studies on the Vilsmeier reaction as a synthetic pathway for the synthesis of **51** was reported from Sanda *et al.* Different reaction conditions and Vilsmeier activating reagents were tested (**Table 11**, entry 3-5). DMF was found to be the best solvent for the reaction. The authors also reported preparative scale experiments and studied the influence of different co-solvents, HMF concentrations and the rate of the $POCl_3$ addition.

Table 11. Conversion of HMF into **51**.

Entry	Reaction conditions	Yield (%)
1[8,270]	Gaseous or 36% aq. HCl	64-87
2[270]	Me_3SiCl, $CHCl_3$, 6h	92
3[271]	SO_2Cl_2, DMF, 50°C, 8h	86
4[271]	$POCl_3$, DMF, 5°C, 5h	92
5[271]	$MeSO_2Cl$, DMF, 65°C, 8h	84
6[270]	$SOCl_2$	53
7[270]	$SOCl_2$ + pyridine	71

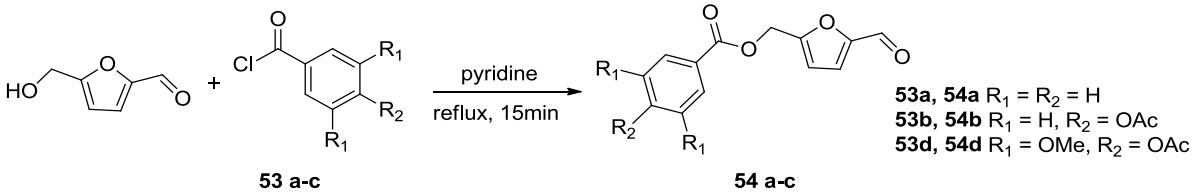
Screening of $SOBr_2$, PBr_3 and PBr_5 reagents for the synthesis of 5-bromomethyfurfural **52** was performed by Sanda *et al*. and it was obtained in moderate yields (**Table 12**, entries 1-4). Excellent yields were achieved by the reaction of HMF with Me_3SiBr, using $CHCl_3$ or 1,1,2-trichloroethane as solvents (**Table 12**, entries 5 and 6). The treatment of HMF with solution of HBr in Et_2O or aq. HBr in CCl_4 resulted in the formation of **52** in moderate yields (**Table 12**, entries 7 and 8).

Table 12. Conversion of HMF into 52.

Entry	Reaction conditions	Yield (%)
1[270]	$SOBr_2$	63
2[270]	$SOBr_2$ + pyridine	75
3[270]	PBr_3 + Et_3N	29
4[270]	PBr_5 + $CaCO_3$	62
5[270,272]	Me_3SiBr, $CHCl_3$	98[270], 88
6[270]	Me_3SiBr, $CHCl_2CH_2Cl$	99
7[270,273]	HBr, Et_2O	64[270], 40
8[270]	47% HBr, CCl_4	70

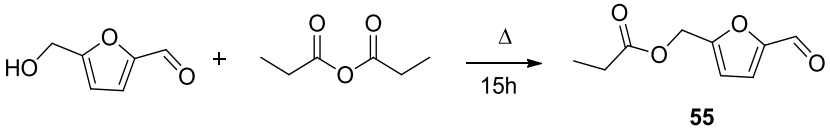
1.5.5.2 Esterification

There are several reported examples describing the formation of various aromatic HMF esters using reaction of HMF with corresponding aromatic acid chlorides under base conditions. Jogia *et al*.[274] reported the formation of HMF ester derivatives **54a-c** *via* a reaction of HMF with aromatic acid chlorides **53a-c** in pyridine in moderate yields 30-66% (Scheme 57).



Scheme 57.

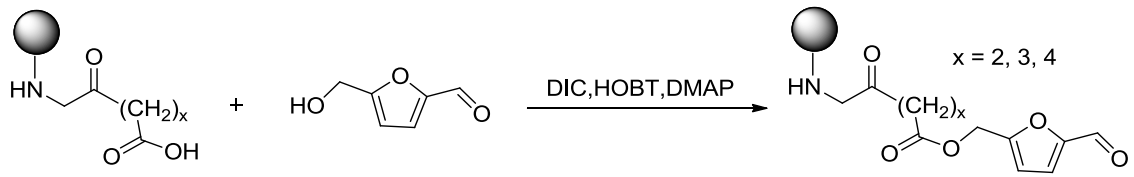
Compound **54a** was obtained in 84% yield also by Bognar *et al*.[275] using the same reaction at room temperature. The reaction of acetic anhydride with HMF in the presence of NaOAc leading to formation of 5-acetoxymethylfurfural in 81% yield was reported by Cottier *at al*.[212] The synthesis of 5-propionoxymethylfurfural **55** in 66% yield resulted from the reaction of propionic anhydride and HMF. Compound **55** is an important fungicide for the industry[17] and its synthesis was patented by Cope (Scheme 58).[276]



Scheme 58.

The authors also claimed that the conversion can be modified by reacting HMF with propionic acid catalyzed by small amount of strong acid.

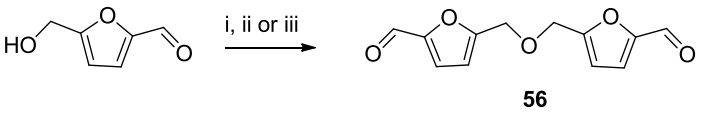
The widely exploited DIC/DMAP procedure was used by Gupta *et al.*[277] for the esterification of HMF and Sieber amide resin loaded with aliphatic dicarboxylic acids (Scheme 59). The resulting solid phase supported HMF esters were used for the combinatorial synthesis of furan-based libraries of compounds.



Scheme 59.

1.5.5.3 Formation of ethers

The condensation reaction of HMF with different alcohols is a synthetic pathway, which provides various HMF ether derivatives. Timko and Cram[216] described the synthesis of 5,5′-diformylfurfuryl ether **56** from HMF in 44% yield using azeotropicall water distillation and toluene in the presence of 4-toluensulfonic acid. Chundury and Szmant[278] carried out several experiments in order to obtain **56** in high yields. Different solvents and acidic catalysts were tested, using a Dean-Stark trap. The highest reported yield was 76% with 4-toluensulfonic acid as catalyst in presence of P_2O_5. Formation of **56** in 38% yield was reported from Cottier *et al.*[212] by refluxing HMF in benzene using Dean-Stark trap in the presence of ion exchange resin IR 120 (H^+) (Scheme 60)

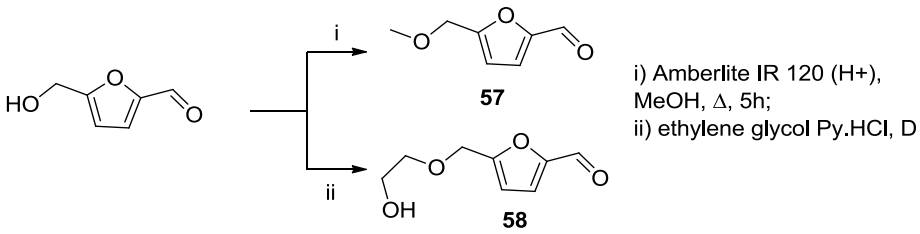


56

i) Toluene, reflux, 0.2% 4-toluenesulfonic acid, 4h
ii) Toluene, reflux, 0.2% 4-toluenesulfonic acid, P_2O_5, 12h
iii) Benzene, reflux, IR 120 (H^+), 5h

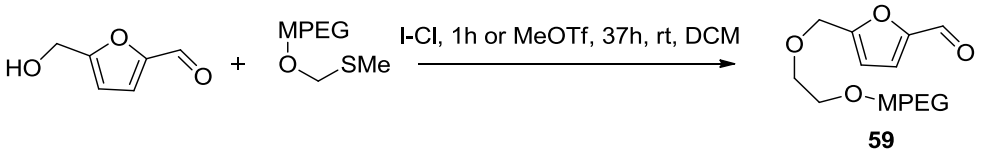
Scheme 60.

The 5-(methoxymethyl)furan-2-carbaldehyde **57** and derivative **58** were obtained in 50% and 24% yield respectively[210] by using the reactions of HMF with MeOH in presence of Amerlite IR 120 H^+ for **57** and ethylene glycol with Py.HCl catalyst for **58** (Scheme 61).



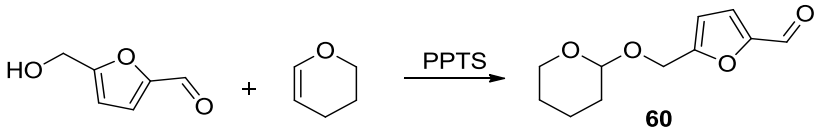
Scheme 61.

Oikawa *et al*.[279] reported the synthesis of MPEG derivative of HMF **59** which was used for further transformations in tandem Ugi/Diels-Alder reactions. The reaction was carried out with iodine monochloride or MeOTf in presence of molecular sieves providing 88% or 75% yield respectively (Scheme 62).



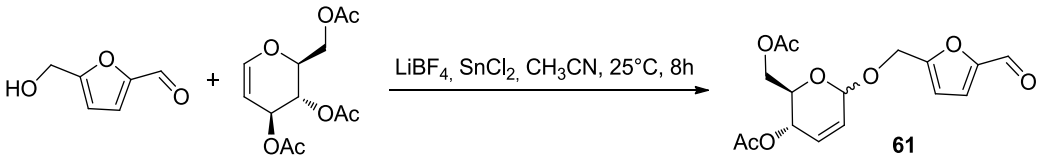
Scheme 62.

El-Hajj et al.[280] reported the reaction between HMF and dihydropyran catalyzed by pyridinium p-toluenesulfonate (PPTS) providing 5-(2-tetrahydropyranyl) oxymethyl furfural 60 in 72% yield (Scheme 63).



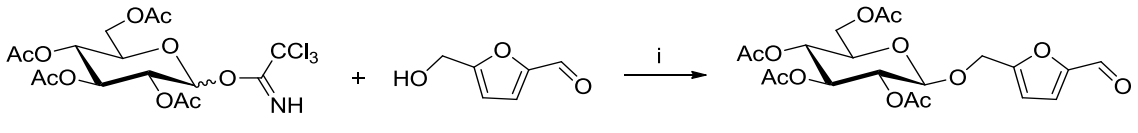
Scheme 63.

The Lewis acid-catalysed rearrangement of glycals in the presence of alcohol known as the Ferrier reaction was used by Filho *et al*.[281] to obtain a 2,3-unsaturated glycoside ether derivative of HMF **61**. Different catalytic systems were tested and the best yield of 93% was achieved using a mixture of lithium tetrafluoroborate and tin(II)chloride. The formation of both α and β-annomers was observed in a ratio of 85/15 respectively (Scheme 64).



Scheme 64.

The synthesis of β-glucopyranoside ether **63β** derivative of HMF in 32% yield after column chromatography was reported by using the reaction between **62α/β** and HMF in the presence of $BF_3.Et_2O$ (Scheme 65). [238]



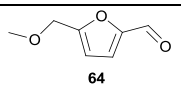
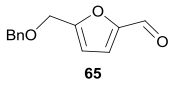
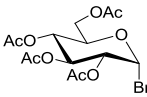
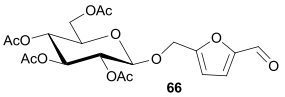
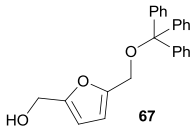
62α/β **63**β

i) CH_3CN, CH_2Cl_2, $BF_3.Et_2O$, MS4A, RT, 2h, column chromatography

Scheme 65.

Several examples were reported on the formation of ether derivatives of HMF using widely exploited Williamson synthesis, as presented in **Table 13**. Other examples for the protection of hydroxyl group of HMF such as *tert*-butyldimethylsylil ether[200,238,282] or trimethylsilyl ether[200] using imidazole as catalyst in DMF were also described.

Table 13 Williamson synthesis of HMF ester derivatives.

Entry	Reaction conditions	Product	Yield (%)
1[243]	MeI, NaH, THF, RT, 16 h.	 **64**	94
2[212]	Benzyl bromide, Ag_2O, DMF, RT, 53h.	 **65**	72
3[238]	 , CH_2Cl_2, Ag_2O, RT, 5h	 **66**	11
4[283]	Triphenylmethyl chloride, pyridine, 40min.	 **67**	4.5g[a]

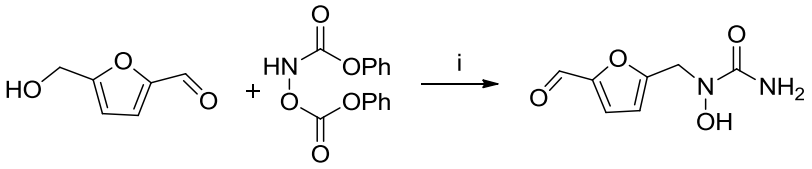
[a] *Starting from 7.8g of HMF*

After our review, etherification and reductive etherification of both HMF or its sugar precursor D-(–)-fructose in alcoholic solutions and presence of solid acid catalysts was reported by Bell *et al.*[284] They employed this approach for the production of potential bio-diesel candidates, 5-(alkoxymethyl)furfural, 5-(alkoxymethyl) furfural dialkylacetal, and alkyl levulinate. Novel magnetic Fe_3O_4-SiO_2 MNP supported heteropolyacid $H_3PW_{12}O_{40}$ catalyst for the synthesis of 5-(Ethoxymethyl)furfural (EMF) from HMF and fructose was developed by Li *et al.*[285] EMF was obtained in high yield of 83.6% by direct etherification of HMF, and in moderate 54.8% yield from fructose *via* one-pot reaction. The catalyst was easily recovered with magnet and reused 6 times without significant loss of activity. One-pot synthesis of EMF from glucose using Sn-BEA and Amberlyst catalysts was reported by Tsapatsis *et al.*[286] The reaction proceeds at 90ºC *via* initial isomerization of the glucose to fructose catalyzed by zeolite Sn-Beta, followed by dehydration of the fructose to HMF and its subsequent etherification catalyzed by amberlyst 131. An EMF yield of 31% was achieved. Direct conversion of fructose into EMF catalyzed by an organic−inorganic hybrid solid catalyst $[MIMBS]_3PW_{12}O_{40}$ was reported.[287] Initially direct etherification of HMF was tested

in ethanol solution at 70°C and provided 90.7% yield of EMF. However, 70ºC was not sufficient temperature for the one-pot conversion of fructose to EMF. After the temperature was raised to 90°C high EMF yield of 90.5% was obtained from fructose with 5 mol% catalyst for 24h. The recycling experiments demonstrated that the catalyst could be reused several times without losing catalytic activity, with an average EMF yield of approximate 90%.

1.5.5.4 Other reactions

The reaction of HMF with N,O-(bisphenoxycarbonyl) hydroxylamine **68** under Mitsunobu conditions providing hydroxyurea derivative **69** was reported by Lewis *et al.*[288] (Scheme 66).

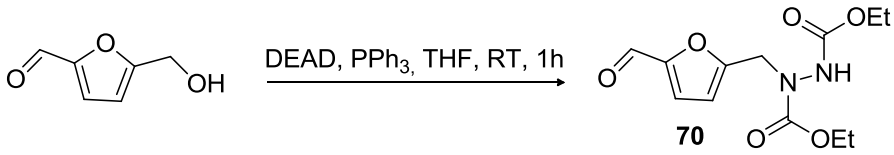


68 **69**

i) PPh_3, DIAD/THF, 2. NH_3/water/THF

Scheme 66.

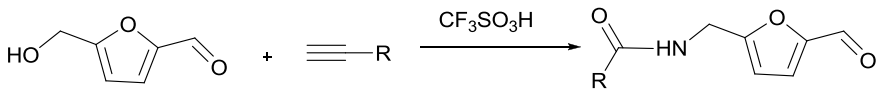
Dow *et al.*[289] described the reaction of HMF and diethylazodicarboxylate (DEAD) using Mitsunobu type reaction conditions in the absence of other nucleophile. Hydrazine derivative **70** was obtained in 12% yield (Scheme 67).



70

Scheme 67.

The reported by Cotier *et al.*[212] reaction of HMF and bezonitrile or acetonitrile catalyzed by trifluoromethanesulfonic acid resulted 5-benzamidomethyl-2-furfural **71** and 5-acetamidomethyl-2-furfural **72** in 48% and 50% yield respectively (Scheme 68)

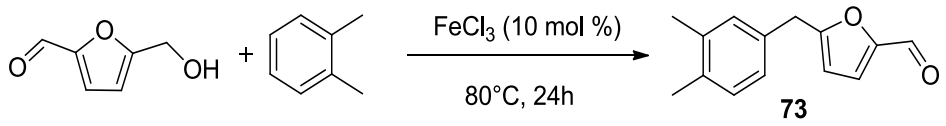


71 R = Ph
72 R = Me

Scheme 68.

$FeCl_3$ catalyzed Friedel-Crafts reaction of HMF with *o*-xylene providing 37% yield and 62% regioselectivity towards 4-alkylated product **73** was reported by Iovel *et. al.* [290] They

explained that the moderate yield (compared to the much higher yields when other benzyl alcohols were used) was due to the "self-arylation" of HMF during the reaction (Scheme 69).

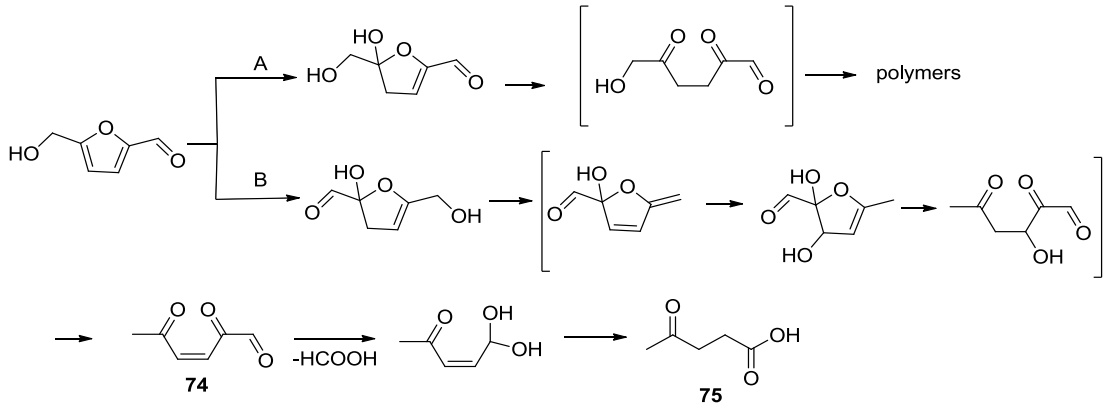


Scheme 69.

1.5.6 Furan ring reactions

1.5.6.1 Hydrolysis

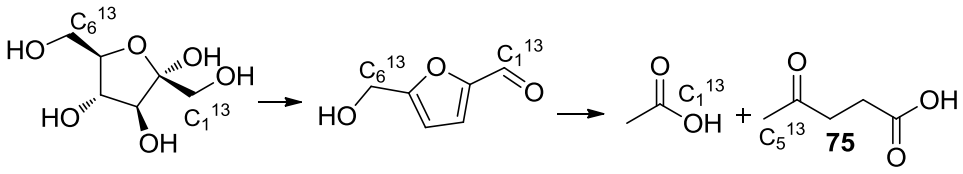
It's known that cleavage of the furan ring of HMF take place in acidic conditions.[291] This process was found to be very important, especially when starting directly from biomass due to the formation of levulinic acid (LA) as final product. LA, together with its derivatives, are important chemical building blocks with various applications such as production of fuels, fuel additives, and polymers.[292,293] Two possible pathways were proposed by Horvat *et al*.[294] for this transformation. Pathway **A** goes via 2,3 water addition on HMF and leads to polymerization, while pathway **B** proceeds *via* 4,5 addition of water, resulting in the formation of 2,5-dioxo-3-hexenal **74**, which fragments to levulinic **75** and formic acids (Scheme 70).



Scheme 70.

After our review, Weitz et al.[295] investigated the pathways for the formation of HMF by dehydration of D-fructose and for the formation of LA and formic acid from HMF by rehydration using *in situ* ^{13}C and ^{1}H NMR with both unlabeled and ^{13}C-labeled fructose. The authors reported that the fructose dehydration to HMF follows a similar mechanism in different solvents and with different catalysts, in which the C-1 or C-6 carbon of fructose maps onto the corresponding carbons of HMF. ^{13}C-labeled HMF produced *via* the reaction of ^{13}C-labeled fructose was used to probe the pathway of the HMF rehydration in different solvents and for different catalysts. The results demonstrated that the C-1 and C-6 carbon of

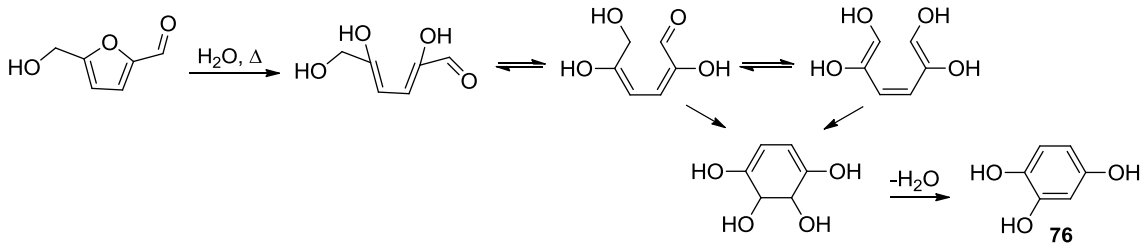
HMF are mapped onto the carbon of formic acid and C-5 carbon of levulinic acid, respectively (Scheme 71). This mapping of the ^{13}C-labeled HMF into LA and formic acid was also consistent with the already published proposed generalized mechanism (Scheme 70).



Scheme 71.

A number of studies on the kinetics of the acid catalyzed HMF degradation to LA have been reported in the literature using different acid catalysts, acid concentrations and temperature ranges.[296-299] Very detailed kinetic studies on this process were reported by Heeres *et al.*[300] The experiments were carried out with various acid catalysts and acid concentrations between 0.05-0.1M in a temperature window of 98-181˚C. The effect of the initial concentrations of HMF has been also studied in the ranges of 0.1-1M. The LA was obtained in up to 94% yield using sulfuric acid as catalyst.

The hydrothermolisys reaction of HMF at 27.5Mpa and 290 to 400°C was performed by Luijkx *et al.*[301] and resulted in the formation of 1,2,4-benezenetriol **76** as the major product in up to 46% yields and 50% HMF conversion. The authors also described a possible pathway for this transformation (Scheme 72).



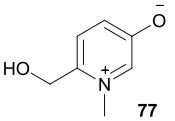
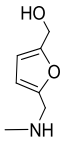
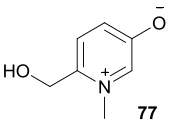
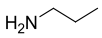
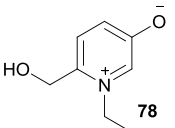
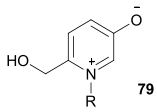
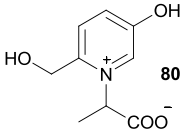
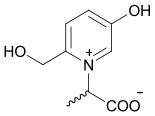
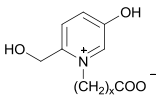
Scheme 72.

1.5.6.2 Synthesis of betaine salts

The synthesis of different betaine salts from HMF with primary amines or amino acids is important, because they seemed to be promising targets for further research due to their taste-modulatory activity.[302] N-methyl-3-oxidopyridinium betaine **77** was obtained *via* the one-step reaction of HMF and $MeNH_2$ in low yield (**Table 14**, entry 1). Much higher yield was achieved by Müller *et al.*,[228] who reported a two steps protocol for the synthesis of **77.** Firstl, they performed reductive amination to obtain 2-(hydroxymethyl)-5-(aminomethyl)-furan **10** which was in turn exposed to bromine in water to give **77** in good yield (**Table 14**, entry 2). The synthesis of **78** in moderate yields was performed in one step in basic conditions (**Table**

14, entry 3). The betaine salt **79** was obtained by the reaction of HMF and N-acetyllysine from Pachmayr, and later by Koch but the yields were not provided (**Table 14**, entry 4). The synthesis and taste-enhancing activity of compound **80** were reported by Ottinger *et al.*[303] (**Table 14**, entry 5). (+)-(*S*)-**80** enantiomer was found to be the physiologically active one, whereas (-)-(*R*)-**80** did not affect sweetness perception at all. Racemization was observed during the synthesis of betaine (+)-(*S*)-**38** by reaction between HMF and L-alanine under alkaline conditions, resulting in lower taste-enhancing activity. Villard *et al.*[226] (**Table 14**, entry 6) reported an alternative two step synthetic protocol for the preparation of enantiopure final products although in lower yields. Soldo and Hofmann extended these investigations by the synthesis and screening of the Bitter-Suppressing properties of pyridinium betaines **81a-c**[304] (**Table 14**, entry 7).

Table 14 Transformation of HMF to 2-hydroxymethyl-pyridinium derivatives

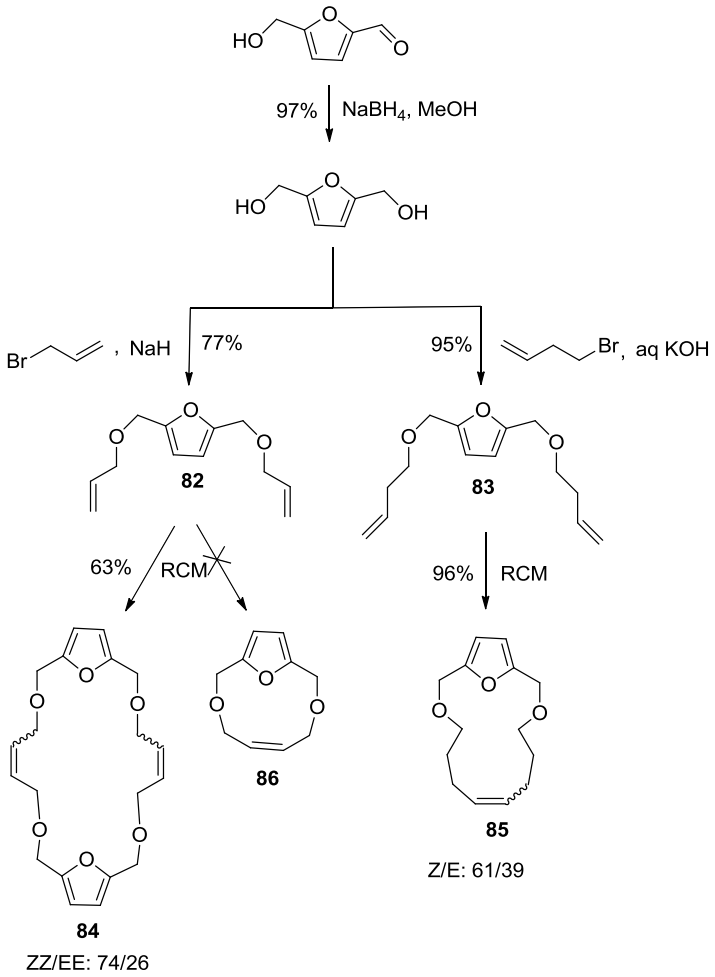
Entry	Starting compound	Reaction conditions	Product	Yield (%)
1[305,306]	HMF	$MeNH_2$, $EtOH/H_2O$	 **77**	10[a]
2[228]		Br_2, H_2O, 0˚C	 **77**	78[b]
3[305,307]	HMF	 ,H_2O/EtOH, NaOH, pH= 9.4, reflux, 3 days.	 **78**	43-45
4[305,306]	HMF	N-Acetyllysin, EtOH, NaOH	 **79** R = $(CH_2)_4CH(NHCOCH_3)COOH$	-
5[303]	HMF	Alanine, NaOH, H_2O/EtOH, pH= 9.4, reflux, 48h	 **80**	51
6[226]	HMF	1- L or D-alanine, water, aq. NaOH (32%) pH 8.5, Ni/H_2, RT, 5bar, 48h; 2-water, 0°C, Br_2/MeOH (0.5h), RT (1h)	 **(+)-(S)-80** or **(-)-(R)-80**	13[b]
7[304]	HMF	glycine, β-alanine, or γ-aminobutyric acid, H_2O/EtOH, NaOH, pH= 9.4, RT (1.5h), reflux (24h)	 **81** a) x = 1; b) x = 2; c) x = 3	**81a** - 22 **81b** - 12 **81c** - 5

[a] *Pachmayr et al.[306] reported 10% in presence of AcOH while Koch et al.[305] treated HMF with aq. $MeNH_2$ for 3 days under reflux and used the crude product directly for further transformation.* [b] *Yield from the second step.*

1.5.7 Synthesis of heteromacrocycles

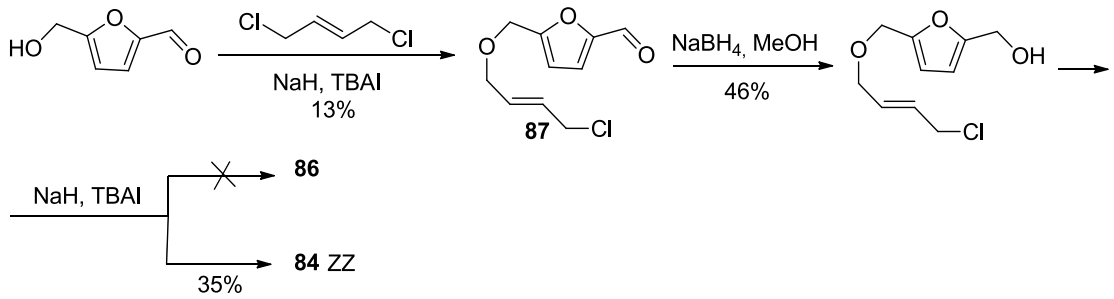
The hydroxyl and aldehyde functional groups present in the HMF molecule are appealing structural motifs for the synthesis of heteromacrocycles which are compounds of considerable interest due to their biological activity and complexation properties.

Heteromacricyclic compounds **84** and **85** were prepared from HMF 2,5-disubstituted furans **82** and **83** *via* a ring closing metathesis (RCM) catalyzed by commercially available benzylidene-bis (tricyclohexylphosphine) ruthenium dichloride Grubs catalyst.[214] The formation of heteromacrocycle **84** instead of **86** was explained by the authors as due to the possible conformational constraints in the original substrate (Scheme 73).



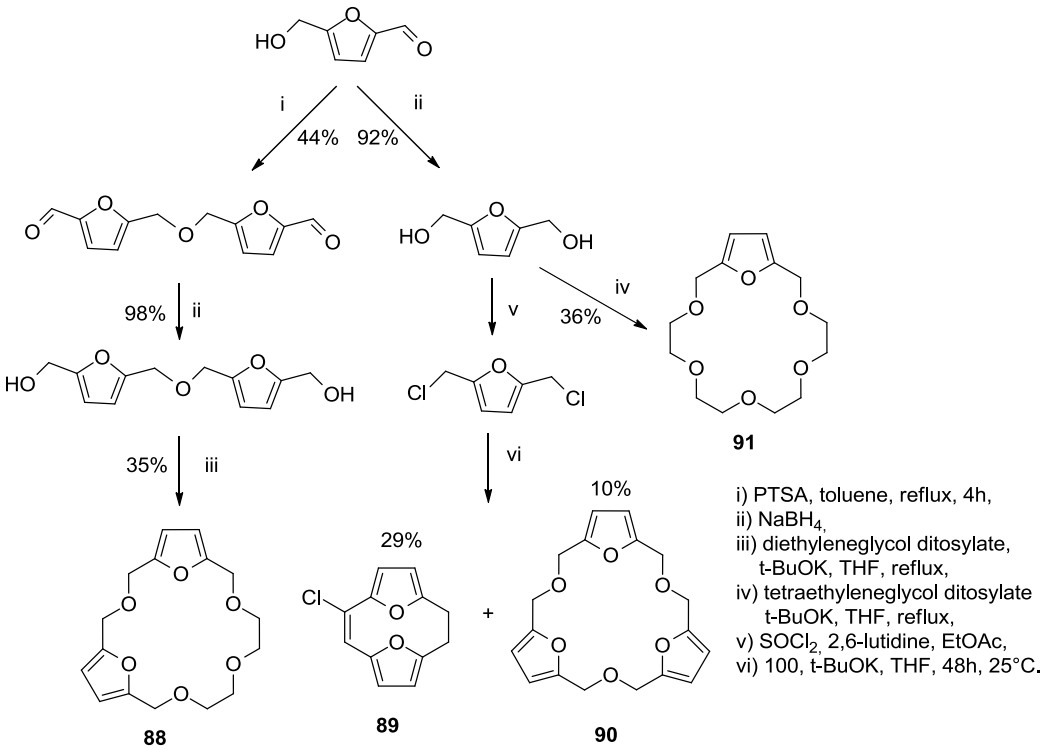
Scheme 73.

Another attempt to obtain **86** in two steps from HMF chloro alcohol **87** was also unsuccessful, the macrocycle **84** was again the only formed product in 35% yield (Scheme 74).



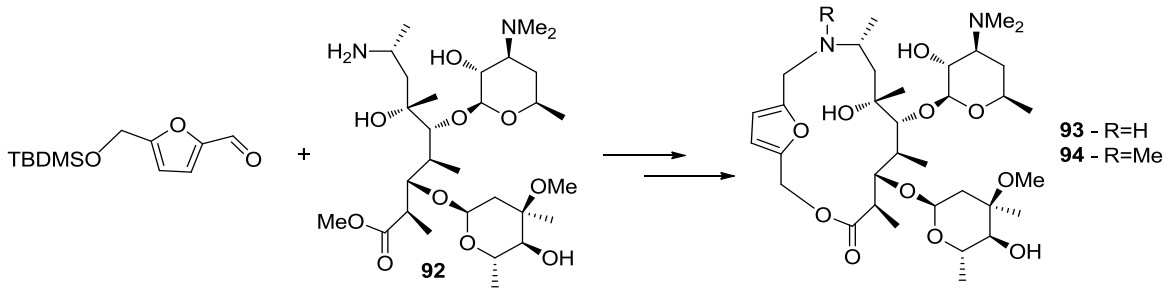
Scheme 74.

Heteromacrocycles **88-91** were obtained starting from HMF in low to moderate yields.[308] These kind of compounds are themselves hosts for binding organic and inorganic cations. More importantly, they can serve as starting materials for preparing host compounds whose periphery is lined with a variety of binding and shaping units (Scheme 75).



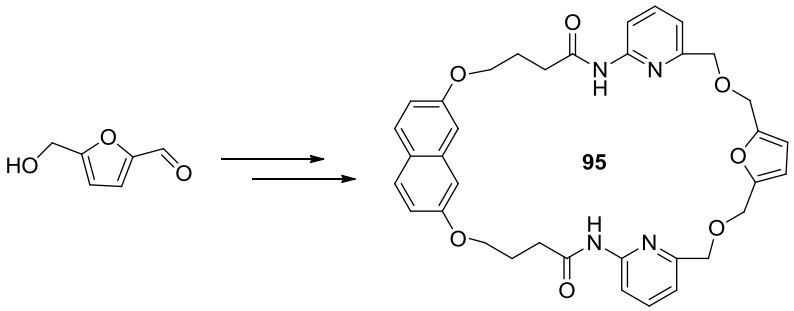
Scheme 75.

Waddell *et al.*[309] developed a method for the synthesis of heteromacrocyclic derivatives of HMF **93** and **94**, which have the same eastern positions as the erythromycin derived azalide antibiotics 9-deoxo-9a-aza-9a-methyl-8a-homoerythromycin A and 9-deoxo-8a-aza-8a-methyl-8a-homoerythromycin A, but more functionalized western positions, due to the a introduction of tetrahydrofuran ring derived from the HMF. **93** and **94** were prepared in several steps from erythromycin-derived acyclic fragment **92** and protected HMF as 5-*tert*-butyldimethylsylil ether **4a** (Scheme 76).



Scheme 76.

A macrocyclic fluorescent receptor **95** was synthesized from HMF, and binding studies with three different types of dicarboxylic acids were performed[217] (Scheme 77).



Scheme 77.

1.5.8 Conclusions

Recently, considerable efforts have been made in order to achieve more efficient integrated processes for the transformation of carbohydrates into HMF. Considerable improvement has been reported for the conversion of fructose to HMF, whereas the transformation of glucose, sucrose and cellulose remains difficult. The main drawback for the transformation of glucose-based carbohydrates to HMF is the isomerization to fructose, which requires different conditions from the fructose dehydration step. As a result, overall process based on two independent steps is more desirable, and has been already explored by combining basic/acid[98] or enzymatic/acid[118] catalytic systems. More efficient reaction conditions (lower temperature, and higher carbohydrate initial concentration), higher conversions and HMF selectivity are desirable, and these processes have to be environmentally friendly.

The presence of two functional groups in the molecule of HMF, combined with the furan ring, makes it an appealing starting material for various chemical transformations. Several transformations of HMF as a substrate involving formyl or hydroxyl group (or both) have been reported in the literature. Serious attention was paid to the oxidation and reduction because they provide convenient synthetic pathways for the production of chemical building blocks for the polymer industry and biofuels starting from renewable materials. Heterogeneous metal catalysts and air as oxidant is the modern approach for performing

selective oxidation of HMF and synthesis of FDA and DFF. A lot of research in this direction was already done and some really good results have been achieved for the oxidation of HMF to FDA in water - the most economical and environmental friendly solvent. Nevertheless, still considerable amonths of base and high temperatures are required for this transformation, and future investigations will focus on the resolution of these issues.

Synthesis of 2,5-bis-(hydroxymethyl)furan which is already in use for the production of polyurethane foams and 2,5-DMF (a compound of considerable interest because of its potential as biofuel and fuel additive) has been carried out by hydrogenolysis. Very good results were already reported for the synthesis of 2,5-bis-(hydroxymethyl)furan by hydrogenolisys promoted by Pt catalyst, while the reduction of HMF to 2,5-DMF resulted in low to moderate yields and selectivity. Screening of more effective catalysts for this transformation needs to be considered.

Some catalysts were found to be effective for the transformations of not only neat HMF, but also crude HMF resulting from the dehydration of carbohydrates. This approach leads to reduced reaction costs, and it is important for industry that the search for new catalysts leading to high yields and selectivity continues.

In view of the reported data, many questions about HMF and its derivative product remain to be answered. There are a number of reports that do not agree about the toxicology of these compounds to humans and therefore more experimental work needs to be developed - for instance, in the study of the toxicology of HMF derivatives and their effect on the wider environment.

2. Results and discussion.

2.2 Integrated approach for the production and isolation of HMF from carbohydrates.[310]

2.2.1 HMF synthesis in batch conditions.

As it was already discussed one of the major difficulties regarding the synthesis of HMF in laboratory and industrial scale is its difficult separation and purification from the reaction media. Since the crystallization is one of the best separation processes to use industrially, we explored the possibility of using readily available, easily crystallized, and low-volatile tetraalkyl ammonium salts as reaction media, promoting the production of HMF under acid catalyzed conditions by melting of the reaction media and solubilization of carbohydrates at the temperature required for the reaction. Furthermore, after cooling, the reaction media could be precipitated at room temperature by using biorenewable EtOH and EtOAc solvents,

allowing isolation of the HMF in the mother liquor just by evaporation of the organic solvent followed by the reaction media, catalyst and solvents reuse (Figure 2).

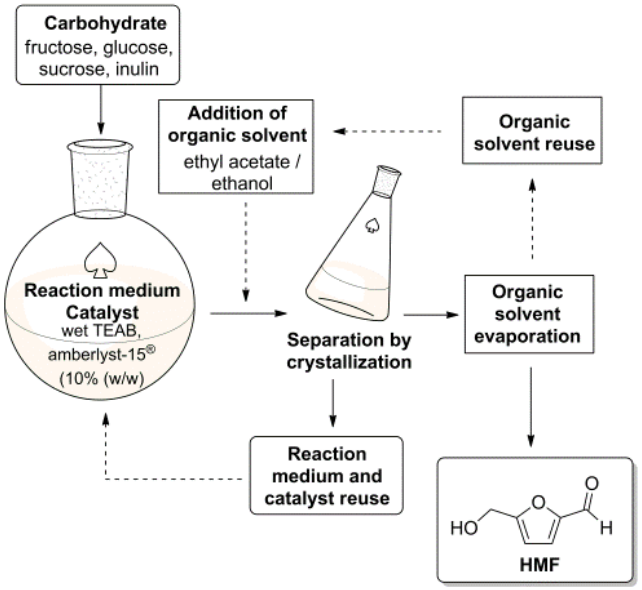


Figure 2. Integrated approach for the production and isolation of HMF from carbohydrates.

Several tetraalkyl ammonium salts in presence or absence of amberlyst-15 catalyst were screened for the conversion of fructose to HMF at 100°C. The total reaction time was 15 min for the major experiments. In some of the cases the reaction time was extended up to 1.5h. The results are presented in **Table 15**.

Table 15. Screening of tetraalkyl ammonium salts as a reaction media for the dehydration of fructose to HMF in presence of amberlyst-15®.[a]

Entry	Reaction media	Reaction time	Catalyst %	Yield %[b]
1	CholineCl	1.5h	-	50
2	CholineCl	15min	10%	59
3	$[Me]_4NCl$	1.5h	-	0
4	[Me]4NCl	15min	10%	0
5	$[Me]_4NBr$	1.5h	-	0
6	$[Me]_4NBr$	15min	10%	0
7	$Me_4N^+Br^-$ + 0.6ml H_2O	20min	10%	26
8	$[Et]_4NCl.H_2O$	1.5h	-	46
9	$[Et]_4NCl.H_2O$	15min	10%	78
10	$[But]_4NBr$	15min	10%	80
11	NH_4Br	15min	10%	0
12	Aliquat®	15min	10%	65[d]
13	Et_4NBr(old)[c]	15min	10%	91
14	Pr_4NBr	15min	10%	91
15	Pr_4NCl	1.5h	10%	28

[a]All experiments were performed in a 1 g scale of fructose (commercial grade from supermarket) and fructose/ammonium salt ratio (w/w) of 1:5. [b] Isolated yield obtained by dissolution of the reaction mixture in ethanol followed by precipitation with ethyl acetate, filtration and removal of traces of ammonium salt by filtration with silica. [c] Old (>15 years) and wet (average water content of 14 % w/w) TEAB. [d] After column chromatography.

Tetramethyl ammonium salts (**Table 15**, entry 3-6) and ammonium bromide (**Table 15**, entry 10) were found to be not suitable for this transformation, no HMF formation was

observed in all the cases. ChCl which is one of the most promising green reaction media, since it is biorenewable and available in big quantities was observed to provide only moderate yields of HMF (**Table 15**, entry 1-2). Moderate yields were also observed when $[Et]_4NCl.H_2O$ (**Table 15**, entry 7-8) and Aliquat (**Table 15**, entry 11) were used. The best results were obtained with tetrabutyl (**Table 15**, entry 9), tetrapropyl (**Table 15**, entry 13) and tetraethyl (**Table 15**, entry 12) ammonium bromides. Tetrabutyl ammonium bromide was difficult to crystalize using EtOH and EtOAc, the precipitation resulted in formation of slurry, which was difficult to handle and resulted in lower isolated yield of 80% *vs* 91%, compared to TEAB. It was also observed that in general, bromide salts provided better yields compared to chloride ones. Tetrapropyl and TEAB provided similar results and both were successfully precipitated and HMF was quantitatively isolated. The lower price of TEAB (15€/kg, quotation from scienTEST, Germany) was the reason to be chosen as the best reaction media for this transformation and for further studies. The effectiveness of the crystallization procedure was proofed by NMR experiment, where no HMF signals have been observed in the precipitated TEAB.

In order to be justified the need of amberlyst-15 as catalyst, series of experiments under catalyst free conditions have been performed and the results are presented in **Table 16**.

Table 16. Experiments for the transformation of fructose to HMF using ammonium salts as reaction media without catalyst.[a]

Entry	Reaction media (rm)	initial water content % (w/w)	Pre-heated TºC	Time Pre-heated T (min)	Final heated T ºC	Time of final heated temp (min/h)	Yield (%)[b]	Purity (%)
1	$Et_4N^+Br^-$	14[d]	80	10	100	15min	41	[g]
2	$Et_4N^+Br^-$	14[d]	80	12	110	15min	61	[g]
3	$Et_4N^+Br^-$	14[d]	80	15	120	15min	43	[g]
4	$Et_4N^+Br^-$	14[d]	80	12	110	30min	79	[g]
5	$Et_4N^+Br^-$	14[d]	80	15	110	35min	75	[g]
6	$Et_4N^+Br^-$	e,c	80	10	100	30min	71	88[h]
7[i]	$Et_4N^+Br^-$[i]	10[f]	80	10	100	1.5h	50	96[h]
8	$Pr_4N^+Br^-$	c	80	10	100	1.5h	63	99[h]
9	$Pr_4N^+Br^-$	c	80	10	100	2.5h	61	99[h]
10	$Pr_4N^+Br^-$	c	80	12	110	1.5h	77	99[h]

[a]All experiments were performed in a 1 g scale of fructose (commercial grade from supermarket) and fructose/ammonium salt ratio (w/w) of 1:5. [b] Isolated yield obtained by dissolution of the reaction mixture in ethanol followed by precipitation with ethyl acetate, filtration and removal of traces of ammonium salt by filtration with silica. [c] Used commercial sample of ammonium salt. [d] Old (>15 years) and wet (average water content of 14 % w/w) TEAB. [e] Old sample of $Et_4N^+Br^-$ (average water content of 14 % w/w w) dried under vacuum (< 1 mmHg, rt, 4-5 h). [f] Determined by Karl Fisher on the commercial sample followed by addition of water. [g] Isolated HMF pure by TLC. [h] Purity of HMF determined by HPLC. [i] 2 g scale of fructose was used.

It was observed that the reaction proceeds under the same conditions in absence of amberlyst-15, but in lower yield 91% *vs* 41% (**Table 15**, entry 12 *vs* **Table 16**, entry 1). Raising the reaction temperature to 110ºC provided HMF in moderate yield of 61% (**Table 16**, entry 2). Further increase of the reaction temperature to 120ºC was not beneficial and

only 43% yield has been achieved, possibly due to side reactions of the fructose and instability of the final product (**Table 16**, entry 3). The best yield of 79% was observed when the reaction was performed for 30 min at 110ºC, longer than 30 min reaction time had negative effect and provided lower yields (**Table 16**, entry 4 *vs* **Table 16**, entry 5). Furthermore Pr_4NBr has been tested as reaction media under catalyst free conditions. Lower reaction rates compared to TEAB have been observed in all the cases and the best yield of 77% has been achieved after 1.5h at 110ºC (**Table 16**, entry 10). HMF exhibit higher stability in Pr_4NBr due to its lower catalytic activity compared to TEAB. Under the same conditions in TEAB only 50% yield of HMF was isolated due to its decomposition (**Table 16**, entry 7).

Since amberlyst-15 was found to be effective catalyst for the transformation and provided significant improvement on the reaction outcome, further optimization of the catalyst loading was carried out. The yield was gradually increasing up to 10% catalyst loading, further increase to 15% didn't provide any benefit and 10% catalyst loading was accepted as the optimum. (**Table 17**).

Table 17. Transformation of fructose to HMF in TEAB with different amounts of amberlyst-15.

Fructose /TEAB[a] ratio	Catalyst %	T (ºC)	Time	Yield (%)
1:5	1	100	15min	64
1:5	2	100	15min	71
1:5	5	100	15min	79
1:5	10	100	15min	91
1:5	15	100	15min	91

[a] Old (>15 years) and wet (average water content of 14 % w/w) TEAB.

The initial experiments in this work (**Table 15**, entry 12) were performed using old >15years TEAB which was available in our lab at that time. When the experiment was repeated with new TEAB obtained from Sigma-Aldrich, reduced yield and purity have been observed. Since it is known that TEAB is hygroscopic, we determined the water amount by Karl-Fisher in the old and new TEAB and it was found to be 14% *vs* 1% respectively. Further optimization (**Table 18**) showed that the presence of small amounts of water were important to achieve clean transformation, 10% water was found to provide the best result (**Table 18**, entry 2).

Table 18. Dehydration of fructose to HMF in TEAB in different conditions.

fructose /TEAB ratio	water %	catalyst %	Pre-heated temp (ºC)	Time Pre-heated (min)	Final heated T (ºC)	Time of final heated temp	Yield[a] (%)	Purity[b] (%)
1:5	5	10	80	10	100	15min	80	77
1:5	10	10	80	10	100	15min	91	97
1:5	15	10	80	10	100	15min	71	98

[a] Isolated yield, [b] determined by HPLC

In addition, an initial preheating (10 min, from 80 to 100°C) proved to be desirable in order to achieve high HMF purity without need of further purification (**Table 19**). Studies on

the fructose/(TEAB, 10%w/w H_2O) ratio have been also performed. 1:4 ratio provided high isolated yield of HMF but with moderate purity (88%) (**Table 19**, entry 1), high yields and excellent purity have been achieved by using 1:5 ratio (**Table 19**, entry 7), presumably due to the suppressed formation of polymer side products and humins caused by the dilution and higher fructose/water ratio. Even better results in terms of yield and purity were achieved using 1:10 ratio (**Table 19**, entry 6). Further dilution up to 1:20 resulted in significantly lower yields, even when 20%ww of catalyst was used (**Table 19**, entry 7-8). The lower isolated yield is probably caused by the higher fructose/water ratio in the system and possible problems with the mass and heat transfer. The transformation was successfully scaled up to 20g fructose remaining high yields and purity of the isolated HMF (**Table 19**, entry 5).

Table 19. Transformation of fructose to HMF in 1 to 20 g scale and different fructose/TEAB ratios catalysed by Amberlyst-15® using TEAB as reaction media.[a]

Entry	Fructose (g)	Fructose / Et_4NBr ratio (w/w)	water content % (w/w)	catalyst (%w/w)	Pre-heated T (ºC)	Time Pre-heating	Final heated T (ºC)	Time of final heating	Yield (%)[b]	Purity (%)
1	1	1:4	10	15	80	10min	100	15 min	96	88[e]
2	5	1:5	14[c]	5			100	15min	88	[d]
3	10	1:5	14[c]	10			100	15min	85	[d]
4	5	1:5	10	10	80	10min	100	15min	86	96[e]
5	20	1:5	10	10	80	10min	100	15min	92	98[e]
6	10	1:10	10	10	80	10min	100	15min	97	99 [e]
7	1	1:20	10	10	80	10min	100	15min	29	99[e]
8	1	1:20	10	20	80	10min	100	15min	57	99[e]

[a]All experiments were performed in a 5-20 g scale of fructose (commercial grade from supermarket) and TEAB containing water . [b] Isolated yield obtained by dissolution of the reaction mixture in ethanol followed by precipitation with ethyl acetate, filtration and removal of traces of ammonium salt by filtration with silica. [c] Old (>15 years) and wet (average water content of 14 % w/w w) TEAB. [d] Isolated HMF pure by NMR and TLC. [e] Purity of HMF determined by HPLC.

Recycling experiments have been performed using 1:5 fructose/TEAB 10% water (w/w) although it provided slightly lower yield and purity compared to 1:10, we were satisfied with the results and the possibility to develop more green and sustainable process using less TEAB. Unfortunately under optimized conditions significant erosion of the yield has been observed only after four cycles (**Table 20**).

Table 20. Recycling experiments 1:5 fructose:TEAB ratio (w/w).[a]

Cycle	Yield %	Purity %
1	92	98
2	86	97
3	93	93
4	64	91

[a]All experiments were performed in a 20 g scale of fructose (commercial grade from supermarket) and TEAB containing 10%w of water using a preaheatinhg step 80-100ºC for 10 min followed by 15 min at 100ºC.

The most reasonable explanation for this result was the downstream contamination of the reaction media from the formed during the reactions side products, which had negative effect in the following cycles. In order to overcome this issue the fructose/TEAB ratio was

increased to 1:10 (w/w) and under the same reaction condition excellent recycling results were obtained. The system was successfully recycled 6 times with a minor yield erosion and outstanding purity. When reduced yield has been observed after 7th cycle fresh catalyst has been added and the system was fully recovered (**Table 21**).

Table 21. Recycling experiments 1:10 fructose to TEAB ratio (w/w)[a, b]

Cycle	Yield %	Purity %
1	98	99
2	95	99
3	94	99
4	91	99
5	89	99
6	97	96
7	63	95
8[c]	123(93)[e]	94

[a]The experiments have been performed by Jaime Coelho, [b] combined yield from 7th and 8th cycles [b]All experiments were performed in a 2 g scale of fructose (commercial grade from supermarket) and TEAB containing 10%w of water using a preaheatinhg step 80-100ºC for 10 min followed by 15 min at 100ºC.c The recovered TEAB was purified and fresh Amberlyst-15 (10 %) was added. [e] Combined yield of the 7th and 8th cycles.

The integrated process was also explored for the direct transformation of glucose, inulin and sucrose to HMF using already reported catalysts for this transformation. All catalysts tested so far under non-optimized conditions shown that the transformation occurs in moderate isolated yields, although in high purity (**Table 22**).

Table 22. Preparation of HMF from other carbohydrates in TEAB.[a]

Entry	Carbohydrate	Catalyst	Catalyst [w/w%]	Yield%	Purity %
1	Sucrose	Amberlyst-15®	10	32	90
2	Inulin	Amberlyst-15®	10	55	98
3	Glucose	PMA	10	15	87
4	Glucose	Boric acid	34	26	85
5	Glucose	$CrCl_3.6H_2O$	3	35	82

***PMA**- Phospho-molibdic acid, [a] All experiments were performed in a 2.0 g scale of carbohydrate and TEAB containing 10 % of water (w/w) and carbohydrate/TEAB ratio (w/w) of 1:5 and catalyst. For PMA and Boric acid reactions, 100ºC for 90 min. were applied instead of 15 min reaction.*

2.2.2 HMF synthesis in flow conditions

The continuous transformation of fructose to HMF was also explored by passing fructose dissolved at 90°C in TEAB containing 25% of water (w/w) through amberlyst-15 (3.5 g) supported in an in-house-made glass tube reactor at 100ºC (Figure 3 and Figure 4).

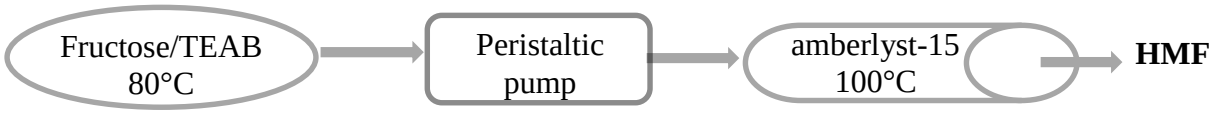


Figure 3. Scheme of the system for continuous dehydration of fructose to HMF.

In-house glass reactor full with amberlyst-15®

Glass reactor placed inside the domestic oven

Operating system outside the oven -pump (top middle) and on the bottom the feed flask (right) and the collected reaction mixture (left).

Figure 4. Photographs of the continuous apparatus.

In order to become possible to pump the reaction mixture through the reactor, it was required to be homogeneous liquid. To achieve that we screened the minimum amount of water and preheating temperature needed. We set 80ºC as optimal temperature in order to eliminate the risk of fructose decomposition before the mixture reaches the reactor. It was observed that even 15% w/w of water was sufficient to form homogeneous liquid at 80ºC. However, when the mixture was pumped, fast cooling down caused blocking of the supplying tubes. Further studies showed that the minimum amount of water, which could provide safe pumping of the preheated reaction mixture was 25% w/w.

The reaction media/fructose ratio and the flow have been also studied (**Table 23**). We observed that, in contrast to the batch process, the ratio has minor effect on the yield and purity of the final product (**Table 23**, entry 2, 5 and 6), presumably due to the very high catalyst loading, since only small amount of the reaction mixture is in contact with the catalyst at a time. The flow rate effect was insignificant up to 0.9ml/min. Similar results were obtained when the flow was speeded up 3 times from 0.3ml/min (**Table 23**, entry 1) to 0.9ml/min (**Table 23**, entry 2). Only after it was increased up to 1.2ml/min, 10% erosion of the yield was observed (**Table 23**, entry 3). The best obtained result was 90% yield and 97% (**Table 23**, entry 2).

Table 23. Continuous preparation of HMF from fructose.[a]

Entry	(Fructose+water)/TEAB ratio [w/w]	Flow mL/min	Yield %	Purity %
1	1:20	0.3	90	91
2	1:20	0.9	90	97
3	1:15	1.2	80	97
4	1:15	0.9	91	93
5	1:10	0.9	85	92

[a]All experiments were performed by passing continuously a 1 g of fructose TEAB containing 25 % (w/w) of water, through a glass reactor containing Amberlyst-15® (3.5 g) heated at 100ºC.

In conclusion, we were able to develop an integrated, simple, efficient, reusable and scalable method for the transformation of carbohydrates (mainly fructose) into HMF that overcome the major problem in big scale HMF production, its isolation and purification. Simple crystallization (precipitation) of the reaction medium (TEAB) using renewable solvents ethanol and ethyl acetate followed by solvent evaporation provided HMF in excellent yields and purity without any further purification. This method also opens the opportunity to discover more efficient catalytic system that may allow the direct conversion of glucose, or ultimately cellulose to HMF under conditions that can be more easily transferred to large scale production.

2.3 Integrated chemo-enzymatic production of HMF from glucose.[311]

Having in hands the optimized condition for HMF isolation after fructose dehydration, we decided to extend and optimize the scope of the process toward glucose dehydration.

Fructose dehydration to HMF is much easier to achieve compared to glucose. However, glucose is much more desirable biorenewable starting material since it is available from cellulose.[15] We focused our attention on the possibility to develop an integrated process based on catalytic isomerization of glucose to fructose, which could be further dehydrated to HMF in high yields.

The isomerization of glucose to fructose in basic aqueous conditions *via* aldose-ketose isomerization using $Ca(OH)_2$, NaOH, KOH is known for more than a century and is called Lobry de Bruyn–van Ekenstein transformation.[312,313] Despite the use of inexpensive catalysts, this approach suffer serious drawbacks, like low glucose concentration, long reaction times and random degradation of the glucose resulting in low yields. Moreover the acid catalysis required for the dehydration of the formed fructose to HMF will need additional neutralization step in the overall process resulting in the formation of wastes and lower *E*-factor. From that point of view the use of heterogeneous catalysis for this isomerization would be much more desirable.

Zeolites have been studied recently as heterogeneous catalyst for glucose-fructose isomerization.[314-316] Although some good results have been achieved, this approach still provides relatively low selectivity and yields. Moreover some of the zeolites are difficult to be accessed.

Industrially glucose to fructose isomerization is carried out enzymatically, using immobilized glucose isomerize enzyme (GI). The enzymatic isomerization provides equilibrium of 50%/50% glucose to fructose ratio and high selectivity towards fructose, and despite it exhibits some serious drawbacks, typical for enzymatically catalyzed processes, like inactivation of GI at high temperatures, narrow pH operational window, gradually loss of activity and high prices of the GI it is performed in industrial scales for the production of high fructose corn syrup (HFCS).[317-319] Considering the fact that the enzymatic fructose isomerization is a well-established big scale process and can be operated under flow conditions, we decided to employ it as an initial step for the HMF synthesis for glucose. Before our work one paper described[326] chemo-enzymatic concept for the conversion of glucose into HMF in seawater. Glucose was isomerized to the equilibrium by GI and in a second step the formed fructose was dehydrate to HMF by oxalic acid catalysis in a biphasic 2-methyltetrahydrofuran/seawater system. The HMF was *in situ* extracted in the organic phase and up to 57% yields have been achieved. This work provides an interesting alternative for the HMF synthesis from glucose since it describes the direct application of seawater, avoiding the use of drinkable water sources. Although this approach exhibit some environmental advantages it provides only moderate HMF yields, and although it was discussed by the authors as a possibility, no recycling studies of the enzyme, catalyst or the remaining after the isomerization glucose have been performed. Another work from He *et al.* [327] described the integration of enzymatic and acid catalysis for the selective conversion of glucose into HMF. The authors performed borate-assisted GI isomerization of glucose into fructose in high fructose yield (87.8%). The resulting sugar mixture was dehydrated in water–

1-butanol media using HCl as catalyst to produce HMF in up to 63.3% yield. Although this method provides higher yield of HMF it has the drawback of using sodium tetraborate in borate-to-glucose molar ratio of 0.5 in the isomerization step. Moreover again no recycling experiments have been performed by the authors. Considering the serious drawbacks of these two pioneer papers, the development of a complete chemo-enzymatic process for the conversion of glucose to HMF, providing high isolated yields and system recyclability was still an open topic.

By taking our previous experience, we explored an integrated approach for glucose-fructose-HMF conversion after enzymatic isomerization of glucose to fructose using commercially available enzyme (sweetzyme®) and the already developed by us TEAB/H_2O reaction media as presented on Figure 5.

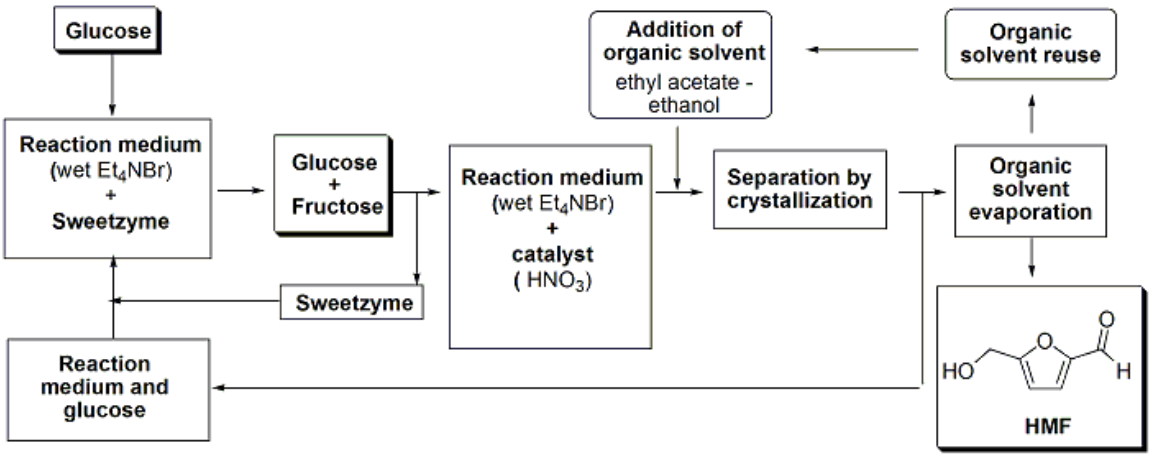


Figure 5. Integrated approach for the production and isolation of HMF from glucose.

Initially the enzymatic activity in TEAB/H_2O has been studied. Unfortunately under the previously optimized for the fructose dehydration TEAB/H_2O 9/1 ratio, sweetzyme was completely inactive. Further experiments established that the minimum amount of water required for the isomerization is 50%, allowing almost maximum conversion of 50% glucose (Figure 6).

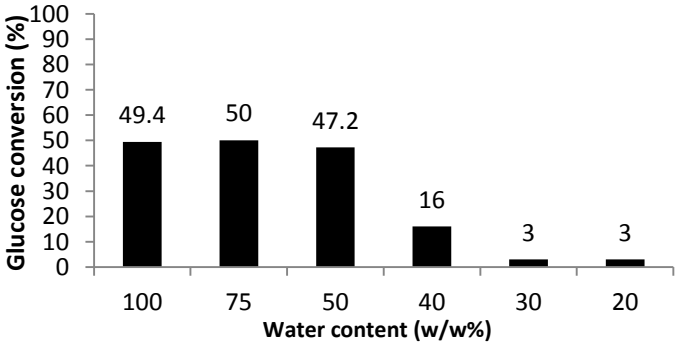


Figure 6. Effect of the water content on the glucose-fructose isomerization by sweetzyme in TEAB (70 ^oC, 14 h).

The rate of the isomerization using 1:1 ratio of TEAB/H_2O was observed to be slower compared to pure water. However, the equilibrium has been achieved in reasonable time scale (Figure 7, **Table 24**, entry 1 vs 3)

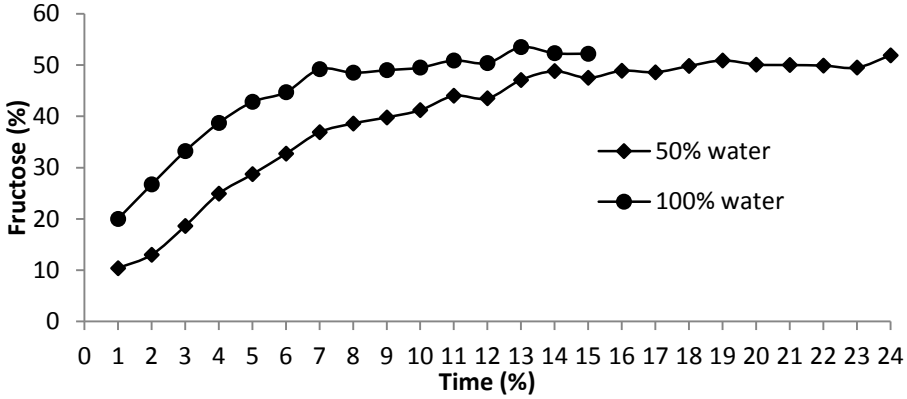


Figure 7. Glucose conversion with 3% w/w sweetzyme in water or in 50:50 water/TEAB at 70ºC.

$MgSO_4$ which is known to work as a promoter for this isomerization under 100% aqueous conditions (Figure 8, **Table 24**, entry 1 vs 2) was not observed to be beneficial in 1/1 TEAB/H_2O (Figure 9 and **Table 24**, entry 3 *vs* 4).

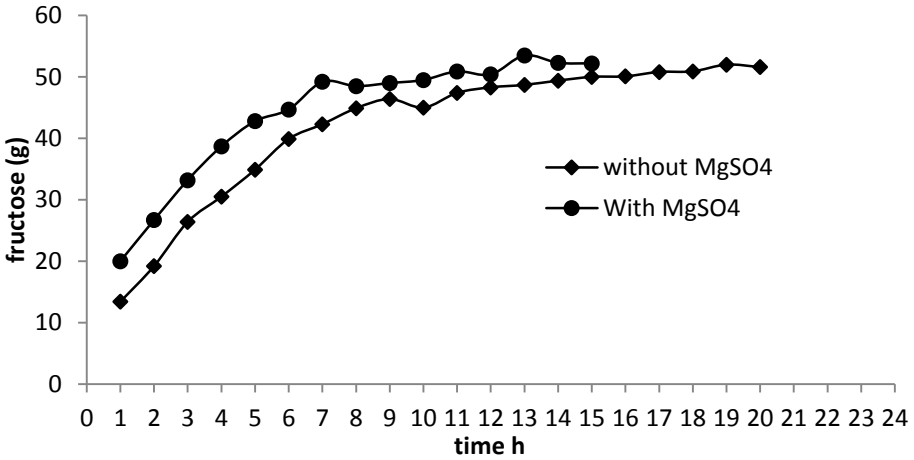


Figure 8. Glucose conversion with 3% w/w sweetzyme in water 70ºC in the presence or absence of 20 mg $MgSO_4$.

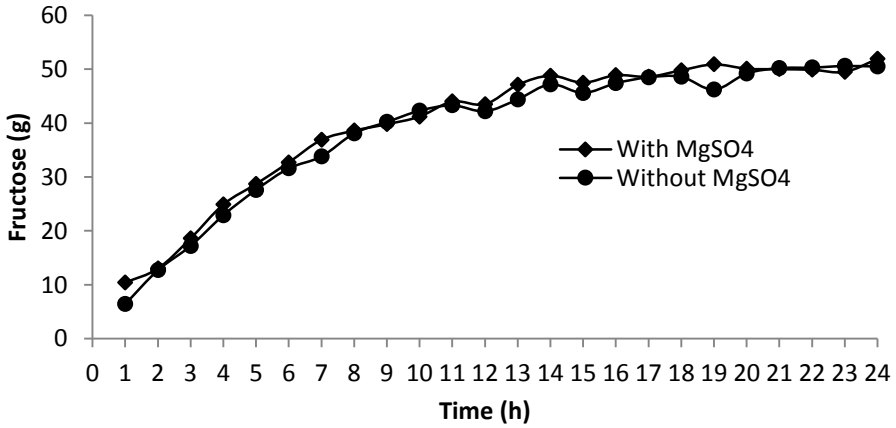


Figure 9. Glucose conversion with 3% w/w sweetzyme in 50% TEAB and 50% water mixture in the presence or absence of 20 mg of $MgSO_4$ at 70ºC.

As it was expected the increased amount of the enzyme resulted in significant increase of the reaction rate (Figure 10, **Table 24**, entry 3,5 and 7).

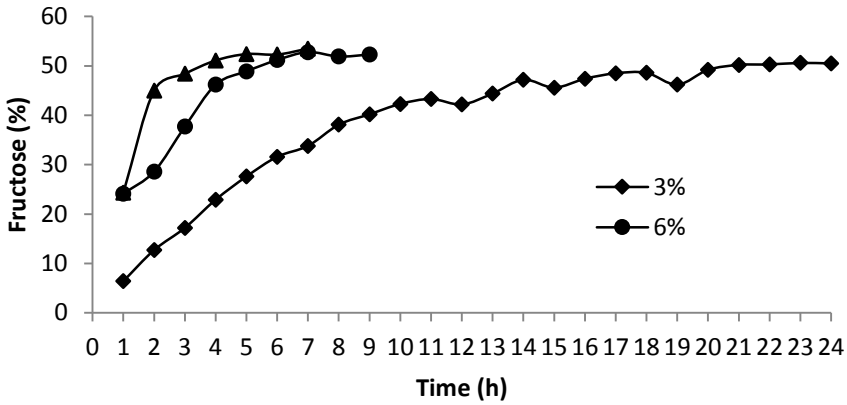


Figure 10. Glucose conversion with 3, 6 or 10% w/w sweetzyme in 50% TEAB and 50% water mixture at 70°C.

Since it is known for GI to be unstable for long time at high temperatures, we studied also the isomerization at lower temperature of 60°C, as it was expected significant decrease of the reaction rate has been observed (Figure 11, **Table 24**, entry 5 vs 6).

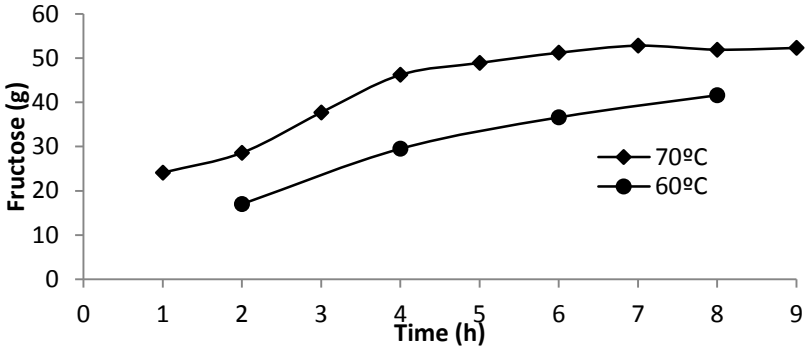


Figure 11. Glucose conversion with 6% w/w sweetzyme in 50% TEAB and 50% water mixture at 60°C and 70ºC**.**

We performed kinetics studies on the isomerization reaction using a single step fructose-glucose conversion model. The two associated equilibrium rate constants have been calculated using Runge-Kutta fourth order algorithm and the least square method for minimization of errors. The kinetic equations are presented in Figure 12.

$$G \underset{K_2}{\overset{K_1}{\rightleftharpoons}} F$$

$$-\frac{d[G]}{dt} = k_1[G] - k_2[F]$$

Figure 12. Kinetic equations used for isomerization rates studies

The times required to achieve 25% (50% of the maximum conversion) and 42% (the maximum conversion used for industrial production) of fructose have been also calculated. The results are presented in **Table 24**.

Table 24. Isomerization reaction rates with sweetzyme in wet TEAB.[a]

Entry	Enzyme %(w/w)	T(ºC)	k_1 (h^{-1}) ($x10^{-3}$)[b]	k_2 (h^{-1}) ($x10^{-3}$)[b]	t_{25}(h)[c]	t_{42}(h)[c]
1	3	70	112	105	2.8	6.9
2	3[d]	70	162	150	1.8	4.8
3	3	70	75	69	4.4	9.8
4	3[d]	70	80	73	4.0	10.3
5	6	70	203	172	1.2	3.5
6	6	60	90	85	3.2	8.2
7	10	70	297	254	1.1	1.9

[a] Glucose (1.2 g) in 10 mL of water (entries 1 and 2) or 10 g of 1:1 TEAB-water (entries 3-7). [b] Determined rate constants ($-d[G]/dt = K_1[G]-K_2[F]$). [c] t_{25}=Time required to achieve 25% of fructose; t_{42}=time required to achieve 42% of fructose, determined by HPLC. [d] In the presence of 20 mg of $MgSO_4$.

1:1 ratio of TEAB/water (w/w) and 6% w/w of enzyme at 70°C were chosen as the best conditions for further experiments because the equilibrium was reached within 6-7h (Figure 10) thus allowing us to save time by performing overnight experiments.

The optimization of the catalytic conversion of fructose to HMF was performed using 1:1 mixture of fructose and glucose with a special attention at the stability of the glucose under the reaction conditions. In an ideal scenario fructose has to be completely converted to HMF and the glucose should remain intact and reused for further enzymatic isomerization to fructose. A range of readily available acid catalysts have been tested for their ability to selectively convert fructose to HMF in presence of glucose and under conditions, which could be integrated with the glucose isomerization step just by prior enzyme filtration and partial water evaporation. The fructose conversion and glucose degradation was analyzed by HPLC analysis. Selected experiments are presented in **Table 25**.

Table 25. Production HMF from fructose in the presence of glucose.

Entry	Catalyst	Water (%)	Temp. (ºC)	Time(min)	Fructose convertion (%)	Remaining glucose (%)
1	Amberlyst-15, 10%[a]	10	100	15	95	90
2	Anberlyst-15, 10%[a]	13.3	100	15	91	90
3	H_3PO_4, 20%	10	100	60	93	100
4	H_3PO_4, 30%	10	100	15	100	100
5	HNO_3, 10%[a]	10	100	10	100	88
6	HNO_3, 10%[a,b]	10	80	15	100	100

[a] Experiments have been performed by Jaime Coelho, [b] reaction performed in closed vessel.

The previously used by us catalyst, amberlyst 15, for the conversion of fructose to HMF exhibit high reactivity, but low selectivity resulting in 10% glucose decomposition after 15 min. Since the presence of water was known to provide better HMF purity and induce higher stability of the carbohydrates, we performed an experiment using higher water amount 10 *vs* 13.3%. However, the rate of glucose decomposition remained the same (**Table 25**, entry 1 vs 2). Furthermore H_3PO_4 and HNO_3 have been tested as catalysts. Since H_3PO_4 is a weaker acid, higher catalyst loading and longer reaction times have been required, but excellent selectivity was observed. No glucose degradation in presence of 20% H_3PO_4 and 93% fructose conversion were achieved after 60 min at 100°C. The fructose conversion increased

to 100% along with no glucose decomposition in presence of 30% H_3PO_4. At the end of the process H_3PO_4 was neutralized with NaOH and we were able to isolate the formed $Na_3PO_4x12H_2O$ in high yield of 95%. Sodium phosphates are commercial salts with high market value and industrially are synthesized *via* the same process of H_3PO_4 neutralization. In this way the two processes of HMF and sodium phosphates production can be applied together and diminished the high catalyst loading drawback of the process.

As it was expected HNO_3 was observed to be much more active catalyst compared to amberlyst-15 and H_3PO_4, 100% conversion of fructose has been achieved even only after 10 min but unfortunately with low selectivity (**Table 25**, entry 4). However, we decided to take advantage of the high catalytic activity of HNO_3 and optimize the reaction conditions. Excellent results have been observed when the reaction was performed at lower temperature in closed vessel, thus avoiding water evaporation (**Table 25**, entry 5). Comparison between the reactions performances using open or closed vessels are presented on Figure 13.

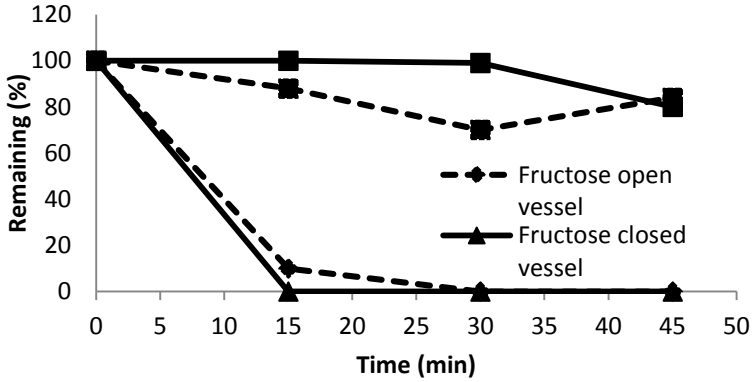


Figure 13. 10% HNO_3, 10% H_2O, 80ºC, open vessel vs closed vessel.

A full screening of the reaction conditions for H_3PO_4 and HNO_3 catalyzed fructose dehydration in presence of glucose is presented in **Table 26**.

Table 26. Production and isolation of HMF from fructose in the presence of glucose.[a]

Entry	sugar (g): rm (g)[b]; ov/cv[c]	Water %(w/w)	Catalyst %(w/w)	T ºC	Time (min)	Isolated yield of HMF (%)[d]	Purity of HMF (%)[e]	Remaining glucose (%)[e]
1	3:15; ov	10	H_3PO_4 20%	80 to 100 100	15 60	85	99	>99
2	4:30; ov	10	H_3PO_4 30%	80 to 100 100	10 30	87	97	>99
3	2:10; ov	10	H_3PO_4 30%	100	40	91	99	99
4	2:10; cv	10	H_3PO_4 30%	110	20	90	97	94
5	2:10; cv	10	H_3PO_4 30%	100	30	89	99	94
6	2:15; cv	10	HNO_3 10%	90	15	94	70	95
7	2:15; cv	10	HNO_3 10%	80	15	91	90	99
8	6:45; cv	10	HNO_3 10%	80	15	70	95	95

[a] *1:1 Mixture of glucose and fructose in TEAB-water.* [b] *Amount (g) of fructose and glucose, reaction medium (rm, in g).* [c] *Reaction performed in open vessel (ov) or closed vessel (cv).* [d] *Isolated yield based on fructose.* [e] *Determined by HPLC.*

Temperature, catalyst loading, carbohydrates loading, reaction time and open or closed vessel experiments have been varied. H_3PO_4 catalyzed reaction showed much higher resistance towards different reaction condition, excellent results have been observed in all the cases. On the other hand for HNO_3, as it was already mentioned, lower reaction temperature combined with closed reaction vessel were required for high yields and purity of the isolated HMF.

Furthermore we studied the reaction under flow conditions. Since higher volumes of water are required for the flow operation leading to decreased reaction rates, we chose strong acids as catalysts for this transformation. Both H_2SO_4 and HNO_3 were tested under homogeneous catalytic conditions. The same domestic oven and peristaltic pump setup presented on Figure 4 have been used, but since the reaction was performed under homogeneous conditions the reactor was replaced with the one presented on Figure 14.

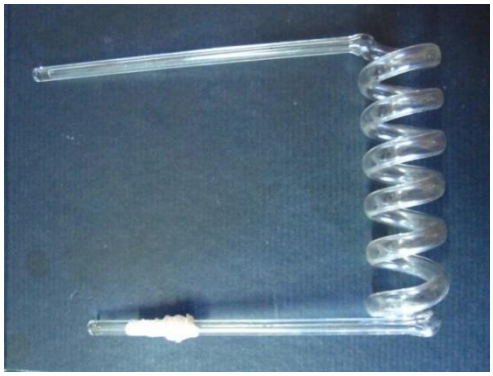


Figure 14. Homemade glass reactor: internal diameter (5 mm), internal volume (12 mL).

Very good yields of HMF with high purity but with low glucose selectivity have been achieved in all the cases (**Table 27**).

Table 27. Production and isolation of HMF from fructose in the presence of glucose.

Entry	sugar (g); rm (g)	Water %(w/w)	Catalyst %(w/w)	T °C	Time (min)	Isolated yield of HMF (%)[a]	Purity of HMF (%)[b]	Remaining glucose (%)[b]
1	4;35	28	H_2SO_4 25%	95	0.5 ml/ min	80	97	89
2	5; 32	24	H_2SO_4 16%	95	0.8 ml/ min	75	97	89
3	4; 24	25	HNO_3 15%	95	0.5 ml/ min	87[b]	99	nd[c]

[a] Isolated yield based on fructose. [b] Determined by HPLC; [c]nd = not determined.

Having the optimized batch conditions in hands, we decided to perform the recycling experiments using 10% HNO_3 as catalyst since it requires shorter reaction times and lower catalyst loading. After each dehydration reaction, before the HMF isolation, HNO_3 was neutralized with equimolar amount of sodium carbonate till pH 7, thus avoiding sweetzyme deactivation at the following cycles, which is known to be unstable under acidic conditions and rapidly and irreversibly, loses its activity.

The enzymatic isomerization was initially performed as an overnight reaction at 70°C, using 6w% of sweetzyme® and 10g of glucose dissolved in 1/1, TEAB/water. Under these

conditions significant loss of enzyme activity was observed only after 5 cycles, although very high overall yield of 83% of HMF was isolated (**Table 28**).

Table 28. Integrated recycling experiments with glucose isomerization at 70°C[a]

Cycles	Reaction	Fructose loading (g)	Glucose loading (g)	Isolated HMF (g)	HMF purity (%)[b]	Glucose Conv. (%)[b]	Fructose Conv. (%)[b]	Fructose/ Glucose ratio after enzymatic reaction
1	Dehydration	5	5	2.7 (77%)	98	0	94	
	Isomerization		5					1.0
2	Dehydration			1.8	100	0	55[c]	
	Isomerization		2.5[d]					1.1
3	Dehydration			2.8	99	3	95	
	Isomerization		5					0.9
4	Dehydration			2.5	100	0	94	
	Isomerization		5					0.6
5	Dehydration			2.1	98	3	98	
	Isomerization		5					0.3 (1.0)[e]
	Total	5	27.5	11.9 (83%)[f]				

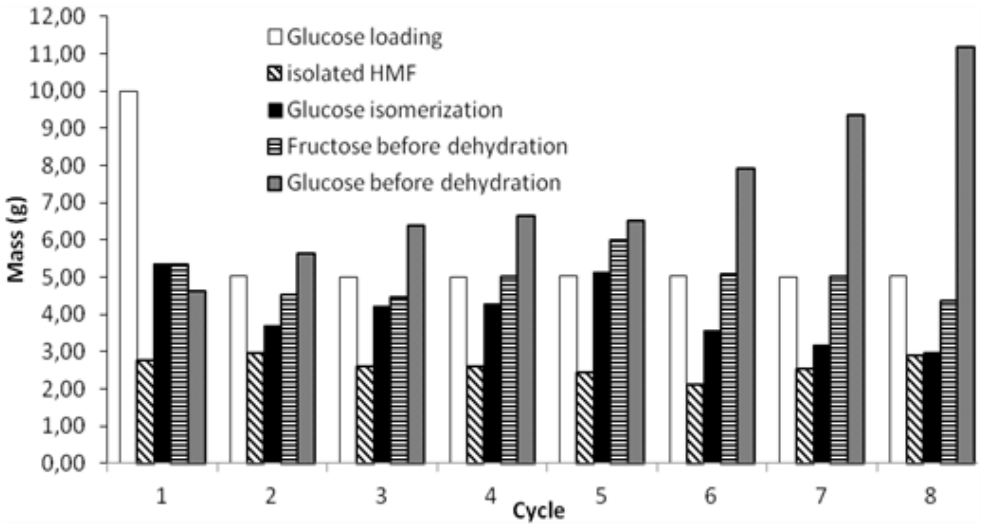
[a] Experiments have been performed by Jaime Coelho. [b] Determined by HPLC. [c]Low yield of fructose conversion due to the use of > 10% w/w H_2O. [d] Only 2.5 g of glucose loading since 55% yield was obtained in the previous reaction. [e] Due to low glucose conversion, freshly enzyme was replaced and 1.0 ratio was obtained after 6 hours of enzymatic reaction. [f] After 5 cycles of dehydration reaction and 4 cycles of isomerization reaction, 7.3 g of glucose was presented in the final reaction mixture. This value was obtained by HPLC analysis. Thus 27.5-7.3= 20.2 g of glucose was consumed which gives a theoretical HMF yield of 14.4 g. Therefore, 11.9 g of isolated HMF correspond to 83% yield based of consumed glucose and fructose.

Taking into account the possible deactivation of the enzyme caused by the temperature, we repeated the recycling isomerization experiments at 60 instead of 70°C thus extending the high sweetzyme activity over 8 cycles (**Table 29**).

Table 29. Integrated recycling experiments with glucose isomerization at 60°C[a]

Cycle	Reaction	Glucose loading (g)	Isolated HMF (g)	HMF purity[b] (%)	Glucose Conv.[b] (%)	Fructose Conv.[b] (%)	Fructose/Glucose ratio after enzymatic reaction
1	Isomerization	10					1.0
	Dehydration		2,8	100	7	84	
2	Isomerization	5					0.9
	Dehydration		3.0	100	1	94	
3	Isomerization	5					0.8
	Dehydration		2,6	100	7	83	
4	Isomerization	5					0.9
	Dehydration		2,6	100	1	83	
5	Isomerization	5					1.0
	Dehydration		2,5	100	1	74	
6	Isomerization	5					0.8
	Dehydration		2,1	100	5	64	
7	Isomerization	5					0.7
	Dehydration		2,6	100	2	72	
8	Isomerization	5					0.6
	Dehydration		2,9	100	6	96	
	Total	45	21.1 (87%)[c]				

[a] Experiments have been performed by Jaime Coelho. [b] Determined by HPLC. [c] After 8 cycles, 10.5 g of glucose was presented in the final reaction mixture. This value was obtained by HPLC analysis. Thus 45-10.5= 34.5 g of glucose was consumed which gives a theoretical HMF yield of 24.2 g. Therefore, 21.1g of isolated HMF correspond to 87% yield based of consumed glucose.



In conclusion, it was successfully developed integrated process for glucose transformation to HMF. The process combines an industrially established enzymatic isomerization of glucose to fructose, with a following selective dehydration of the fructose to HMF under acid catalysis. The remaining glucose and sweetzyme were recycled for at least 8 cycles and 87% overall isolated yield of HMF, based on the glucose, has been achieved, which is one of the best results ever reported in the literature.

2.4 Dehydration of glucose to HMF over supported chromium catalysts.

Despite the enzymatic conversion of glucose to fructose provides many advantages and is an industrial method, it also suffers many drawbacks due to the specific properties and sensitivity of the enzymes. Moreover in case of HMF synthesis the dehydration of the obtained fructose should be performed as a separate step. Certainly metal catalysis, which could provide direct conversion of glucose to HMF in high yields and selectivity together with long catalyst life time and recyclability will be an attractive alternative for the industry. Among many tested transition and lanthanide metal catalysts, chromium was proven to be the best for this transformation. $CrCl_2$ and $CrCl_3$ provided up to 91% HMF yield in [EMIM][Cl] and [BMIM][Cl] ionic liquids[117] *via* initial chromium catalyzed isomerization of glucose to fructose and subsequent dehydration to HMF (Scheme 13). Despite these methods provided one of the best reported yields, they exhibit serious drawbacks in terms of difficult HMF isolation and high prices of the ionic liquids, which are not consistent with industrial applications.

Although our previous results in applying $CrCl_3$ catalysis for glucose dehydration in TEAB were not very encouraging, since only 35% yield and 82% purity were obtained (**Table 22**, entry 5), we decided to explore more the topic aiming to construct a recyclable catalytic system based on chromium, which will provide higher yields and purity. In terms of

recyclability heterogeneous catalysis is much more desirable, compared to homogeneous, which provoke us to look for possible supported chromium catalysts. Studding the literature, we observed that strong acid ion-exchange resins are capable of adsorbing Cr(III) salts[320,321] thus providing access to possible heterogeneous catalysts for glucose dehydration to HMF. Several strongly acidic and one chelating resin/$CrCl_3$ catalysts have been prepared by heating the resins at 80ºC in ethanol solution of $CrCl_3$ for 4h. The resins were then filtered, dried and applied as catalysts. Initially amberlyst-15/$CrCl_3$ was prepared and used in 20%w/w catalyst loading under our previously optimized conditions for the batch fructose dehydration (TEAB containing 10%w/w H_2O at 100ºC), but using glucose as feedstock, 50% HMF yield and excellent purity have been achieved (**Table 30**, entry 1). An improved yield of 60% (**Table 30**, entry 2) was achieved when 100% w/w of the catalyst was used, further increase was found to be undesirable since only 43% yield has been obtained using 200w% of the catalyst (**Table 30**, entry 3). Less amount of water in the reaction mixture provided better yield but low purity (**Table 30**, entry 5). Significant improvement of the yield in up to 73%, maintaining high purity, was achieved when the reaction temperature was raised to 120ºC and combined with longer reaction time (60min) (**Table 30**, entry 4). Reaction times longer than 60min resulted in both lower yield and purity presumably due to the unstable nature of HMF (**Table 30**, entry 7).

Table 30. Dehydration of glucose with Amberlyst-15/$CrCl_3$ catalyst under different conditions.[a]

Entry	Catalyst (%w/w)[b]	Reaction media	TºC	Time (min)	Yield %[d]	Purity % (HPLC)
1	20	TEAB+10w%H_2O	100	45	50	>95
2	100	TEAB+10w%H_2O	100	45	60	>95
3	200	TEAB+10w%H_2O	100	45	43	-
4	100	TEAB+10w%H_2O	120	60	73	>95
5	100	TEAB+4w%H_2O	100	60	65	90
6	50	TEAB+10w%H_2O	120	60	59	>95
7	100	TEAB+10w%H_2O	120	90	68	92

[a]All the experiments have been performed in closed vessel using reaction media/glucose 10/1 w/w ratio. [b] Weight % calculated /for glucose. [c] RM = reaction media.[d] Isolated yields.

Furthermore we tested different commercially available resins. The sorption of $CrCl_3$ was carried in the same manner as for amberlyst-15. The results are presented in **Table 31**. Although maintaining high purity, all the tested resins provided lower yields compared to amberlyst-15, only in the case of amberlyst IRC86 the result was competitive (**Table 31**, entry 2). We also started a screening of different organic solvents as reaction media. Dioxane a common organic solvent, which could provide cheaper alternative to TEAB was tested. Unfortunately due the low solubility of glucose even at high temperatures, we observed aggregation of the catalyst caused by the adsorption of the glucose over it and as a result only traces of HMF has been obtained.

Table 31. Screening of different resins for dehydration of glucose to HMF[a]

Entry	Resin	Yield %[d]	Purity % HPLC
1	Amberlyst 36[b]	62	>95
2	Amberlyst IRC86[b]	68	>95
3	Amberlite IRC748[c]	27	>95
4	Amberlyst IRC86[b]	33	>95
5	Amberlyst 120[b]	10	>95

[a]All the experiments have been performed in closed vessel at 120º for 1h using 100%w/w of the catalyst and 10/1 w/w reaction media/glucose ratio. [b]Strong acid ion exchange resin. [c]Chelating ion exchange resin, [d]Isolated yields.

In conclusion, it was developed a catalytic system for glucose dehydration to HMF based on Cr(III)/resin supported catalysts, which could provide an attractive alternative for this transformation on large scale. The application of heterogeneous catalysis could also provide an improved recyclability. Amberlyst-15/$CrCl_3$ was observed to exhibit the best performance providing 73% yield of HMF in high purity. The work is still ongoing in our laboratory and more experiments towards screening of other organic solvents as reaction media and catalyst recycling will be performed in due course.

2.5 Synthesis of HMF as a student laboratory experiment.[322]

Since the protocol for the batch synthesis of HMF from fructose was repeated by different researchers in our lab and was found to be robust and easy to perform and it doesn't require special and expensive equipment, as well as dangerous and hazardous materials, we decided to develop a student laboratory experiment based on it. Two protocols have been developed using batch or flow conditions, which will provide the students the possibility to compare the two processes in terms of efficiency and green chemistry credits. Moreover the students are introduced to a challenging and innovative reaction in the biorefinery applying an innovative and recyclable approach and using homogeneous or heterogeneous acid catalysis and crystallization as a very simple and industrially useful separation technology.

The experiments were reproduced in the teaching laboratory environment by the students from the 2nd year of pharmaceutical science course (5 years course). The batch experiments were performed in 1g fructose scale and since the reaction was performed at 100ºC it was simply used boiling water bath without temperature control in order to be minimized the equipment requirements. The results presented in **Table 32**, entries 1-4, were performed using new TEAB and amberlyst-15, while experiment presented in entry 5, was performed with recovered TEAB and catalyst from the previous ones. Similar results were observed in both cases showing that the reaction media and catalyst can be successfully reused for the next students class.

Table 32. Results from the students experiments in batch conditions.

Entry	HMF Yield %	HMF Purity %
1	94	98
2	93	97
3	97	97
4	91	96
5[a]	90	95

[a] *The reaction was performed using recovered TEAB and catalyst from the previous experiments.*

In addition, a flow process protocol for the synthesis of HMF from fructose using 5% H_2SO_4 as catalyst under homogeneous conditions was developed. Standard glass column for flash chromatography connected with an in-house made glass reactor (internal diameter: 4 mm, internal volume: 13 mL, length: 1.02 meters), placed in a boiling water bath were used for this experiment (Figure 15).

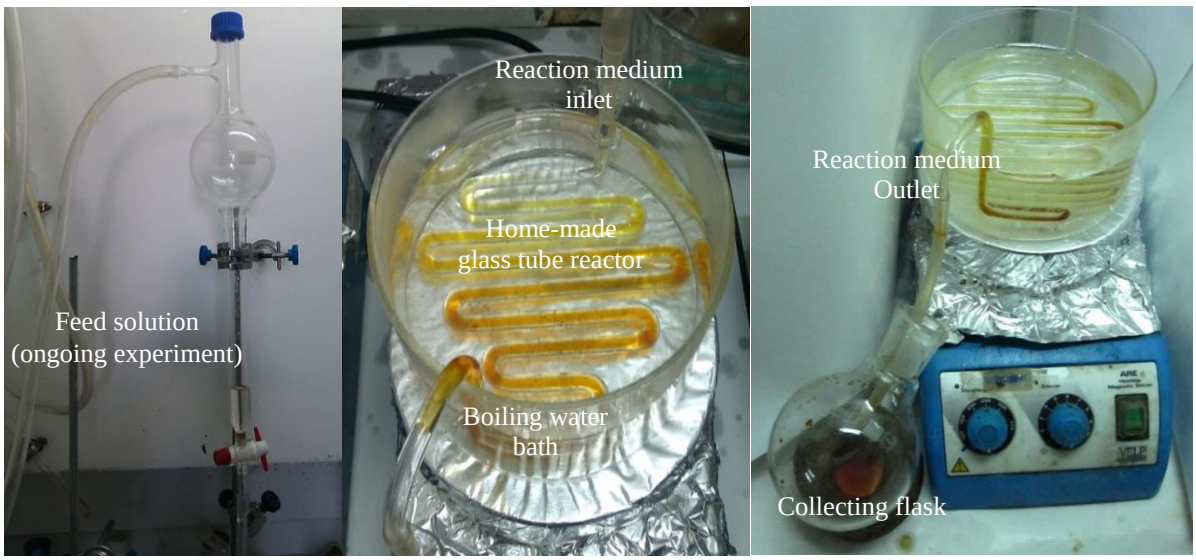


Figure 15. Photographs of the equipment used for the flow conversion of fructose to HMF.

The flow conversion was also successfully repeated by the students, providing HMF in 77% yield and 92% purity. In one experiment the students added by mistake 10 ml of 5%H_2SO_4 instead of 6 ml and in this case 65% yield and 91% purity has been observed. The batch and flow experiments could be performed during the same lab class providing the students a possibility to compare the two approaches.

2.6 Synthesis and biological evaluation of HMF derivatives.

This work has been performed together with Dr. Raquel Frade who did the biological evaluations. My contribution was the synthesis of some of the derivatives presented in **Table 33**.

Since HMF and its derivatives are one of the most studied and developed intermediates for the production of chemical building blocks for the industry based on biorenewable resources that can replace the existing ones mainly based on fossil resources, it is very important to

study their toxic effects in humans. In this context we studied the toxic impact of HMF and a range of HMF derivatives and other furan-based molecules (Figure 16 and **Table 33**) in the CRL-1502 cell line with the aim to provide key guidelines about the most human-friendly building blocks. CRL-1502 cells are human normal skin fibroblasts thus allowing the study of the impact of such compounds in healthy tissue. In addition, was included in this study the already in use and established bioplatform furan-based molecules furfural and furfuryl alcohol and non-aromatic levulinic acid that allows a comparison with the new potential emerging molecules containing the furan ring namely HMF and analogues. HMF was synthesized *via* already developed by us procedure from fructose in TEAB/water 9:1 and amberlyst-15 as catalysts in batch conditions. Dimer **56** (Figure 16) was formed as a side product during the fructose dehydration to HMF in longer reaction time and it was possible to isolate in 10% yield after column chromatography. 5-(ethoxymethyl) furfural (**Table 33**, entry 4) was prepared directly from HMF *via* acid catalyzed etherification with absolute ethanol. The final product was obtained in 57% yield after 5h reflux and column chromatography. 2,5-Dihydroxymethylfurfural (DHMF) was obtained after $NaBH_4$ reduction of HMF. Since DHMF is water soluble it was not possible the isolation *via* extraction work up of the reaction mixture. A modified procedure was applied using filtration of the dissolved in DCM/MeOH 9/1 reaction mixture followed by column chromatography thus providing 80% of the final product. Further the obtained DHMF was subjected to Williamson etherification using NaH as base and bromoethane to give 39% yield of 2,5-bis(ethoxymethyl)furan. DFF was obtained from HMF subjected to a Swern oxidation. The product was isolated in 31% yield after column chromatography and recrystallization. Dimer **95** was obtained in 86% yield from acid catalyzed reaction of HMF with hydrazine hydrate in EtOH. The final product was not soluble in EtOH and precipitates from the reaction mixture and can be simply isolated by filtration. The synthesized compounds together with the ones synthesized by Jaime Coelho were used for biological evaluation performed by Dr. Raquel Frade.

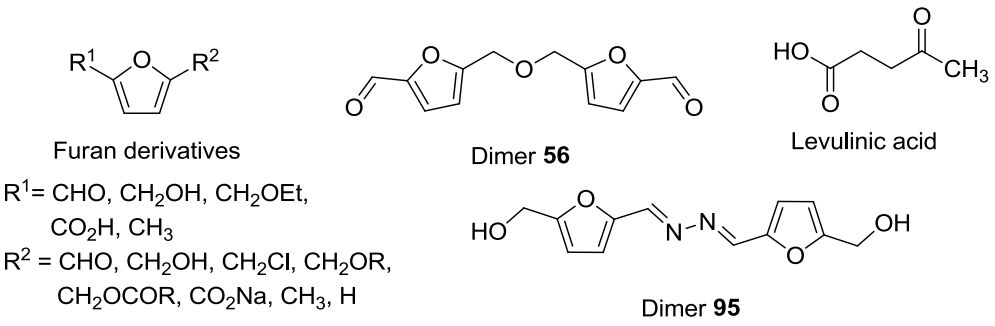


Figure 16. Tested HMF derivatives.

Table 33. Cell viability and ROS measured for the tested compounds.[a]

Entry	R_1	R_2	%V±SD (500µM)	%ROS ±SD	Toxicity[c]
1	CHO	CH_2OH	94±3	106±7	NT
2[b]	CHO	CH_2OSO_3Na	68±5[d]	120±11	MT
3[b]	CHO	CH_2Cl	70±10[b]	**379±60**[d]	MT
4	CHO	CH_2OEt	90±15	107±22	NT
5[b]	CHO	CH_2OCH_2Ph	72±13[d]	**177±14**[d]	MT
6[b]	CHO	$CH_2OSi(CH_3)_2{}^tBu$	75±24[d]	**158±36**[d]	MT
7[b]	CHO	CH_2OCOCH_3	86±11	108±34	NT
8[b]	CHO	CH_2OCO*n*-C_5H_{11}	22±3[d]	**370±44**[d]	**T**
9[b]	CHO	CH_2OCOPh	62±7[d]	**153±17**[d]	MT
10	CHO	CHO	32±2[d]	113±12	**T**
11	CHO	H	95±8	101±30	NT
12	CH_2OH	CH_2OH	96±10	**254±32**[d]	MT
13	CH_2OH	CH_2OEt	95±10	116±7	NT
14	CH_2OH	COONa	95±17	89±13	NT
15	CH_2OH	H	99±6	101±28	NT
16	CH_2OEt	CH_2OEt	114±15	97±21	NT
17[b]	COOH	COOH	93±11	86±7	NT
18	CH_3	CH_3	65±8[d]	**172±20**[d]	MT
19	CH_3	H	93±6	84±20	NT
20	Dimer **56**		51±6[d]	134±19	**T**
21	Dimer **95**		109±5[d]	109±19	NT
22	Levulinic acid		98±5	122±52	NT

[a] *The biological evaluations have been performed by Dr. Raquel Frade.* [b] *The compound has been synthesized by Jaime Coelho.* [c] *T, MT and NT stands for highly cytotoxic, moderately cytotoxic and not cytotoxic, respectively;* [d] $p<0.05$

When CRL-1502 cells were incubated with several doses of 5-SMF for 72 hours, cell viability suffered a decline of about 30%, however HMF was seen not to lead to visible variations. One-way ANOVA analysis demonstrated that these differences are statistically significant ($p<0.05$) and we may assume that 5-SMF constitutes a compound of a slightly higher probability to generate cytotoxicity (**Table 33**). Some derivatives of HMF are suggested as promising candidates for polymer production (**Table 33**, entry 10, 14, and 17), biofuel replacement dimethyl furan (**Table 33**, entry 18) or biofuel additives levulinic acid (**Table 33**, entry 22) which were also included in this study. The HMF derivatives (**Table 33**, entry 14, 17) and levulinic acid did not affect significantly cell viability whereas dimethyl furan induced a viability decrease at a similar extent as 5-SMF; and results were statistically significant ($p<0.05$) when compared to our control molecule HMF (**Table 33**, entry 1). For the HMF derivatives containing R_2 groups CH_2Cl or CH_2OCOPh the CRL-1502 cells experienced a weak decrease in viability, which did not go below 60% (**Table 33**, entry 3 and 9), however for CH_2OCOCH_3 group the effect was even more insignificant of 86% (**Table 33**, entry 7).

Other studied compounds such as HMF benzyl and TBS ether derivatives were also demonstrated to be insignificantly cytotoxic (**Table 33**, entry 5 and 6). However, in the

presence of the more lipophilic ester side chain (**Table 33**, entry 8)**,** induced cytotoxicity was undoubtedly enhanced and the DFF (**Table 33**, entry 10), was the second most toxic compound. Within the remaining furan-based compounds, the dimer **56** (Figure 16), was considered moderately cytotoxic since it reduced approximately 50% viability at the maximum tested dose (**Table 33**, entry 20)

Data showed that either the dimer **56** or FDA (**Table 33**, entry 17) are safer choices than the DFF. Surprisingly, in several examples we could conclude that the cytotoxicity increased from the mono to the di-substituted furan ring (**Table 33**, DFF entry 10 *vs* furfuraldehyde entry 11, 2,5-dihydroxymethylfurfural (DHMF) entry 12 *vs* furfuryl alcohol entry 15 and 2,5-DMF entry 18 *vs* 2-methylfuran entry 19), which indicates that a furan derivative is more prone to generate cytotoxicity in case it is di-substituted. HMF derivatives (**Table 33**, entry 4, 13, 14, 16) and the dimer **95** (**Table 33**, entry 21) were not seen to decrease viability of this cell line. Ether substituents were then seen not to cause cytotoxicity (**Table 33**, entry 4, 13 and 16) in this model and, comparing, DHMF **(Table 33,** entry 1**2)** and FDA (**Table 33**, entry 17), we may conclude that the change of CH_2OH group by COOH group or vice-versa does not seem to affect cell viability.

The compounds presented in **Table 33**, entry 2, 3, 5, 6, 9, 18, and 20, did induce to some extent decrease in cell viability and were more toxic than HMF. Additionally, and with exception of compounds 5-SMF entry 2, DFF entry 10 and dimer **56** entry 20, data showed that cytotoxic compounds lead to reactive oxygen species (ROS) generation and, the decreasing trend of oxidant potential is: **Table 33**, entry $3 \approx 8 \gg 5 \approx 18 > 6 \approx 9$, where the most toxic is a 5-(chloromethyl) furfural (**Table 33**, entry 3). This effect was not detected in cells exposed to HMF (**Table 33**, entry 1). Therefore, the general predisposition for cytotoxicity observed in the viability assay is here confirmed by their ability to cause redox state alterations. But, we may assume that the mode of action of compound presented in **Table 33**, entry 2, 10 and 20 is likely very different from compounds entry 3, 5, 6, 8, 9, and 18 since their mediated total intracellular ROS increasing is not substantial to justify cytotoxicity.

From the group of compounds demonstrated not to induce viability alterations, only DHMF **(Table 33,** entry 12) was seen to play a role as ROS generator and consequently, there is a possibility for inducing cell alterations leading to cell death or cell transformation in a long-term incubation. Interestingly, if we compare DHMF, sodium salt HMFA and FDA (**Table 33**, entry 12, 14 and 17), it is clear that the functionalization of the furan ring with hydroxylmethyl group increases the potentiality for the compound to cause harmful effects on a long term basis. Information gather suggests that derivatization with COOH is indeed more

preferential than derivation with CH_2OH which could not be concluded from the viability assay discussed previously. Compounds from **Table 33** entries 1, 4, 5, 7, 13-17, 19, and 22 were not seen to affect oxidative status in addition to their lack of cytotoxicity in the neutral red assay meaning that these are very likely the safest studied compounds.

In conclusion, and within the studied furan derivatives, we can organize them into two different groups: one group constituted by compounds that did not decrease viability and did not enhance ROS levels (entries 1, 4, 7, 11, 13-17, 19, 21, 22) in which furfuraldehyde (entry 11), furfuryl alcohol (entry 15) and levulinic acid (entry 22) were also included for comparison purpose and a second group formed by the remaining compounds. In this last group, we can distinguish three different types of compounds:

a) Compounds not involved in ROS generation but weakly cytotoxic SMF (**Table 33**, entry 2) or extremely cytotoxic DFF (**Table 33**, entry 10);

b) Others that induced both ROS and viability decrease (**Table 33**, entry 3, 5, 6, 8, 18 and 20);

c) DHMF that was not seen to decrease viability but it was seen to form ROS significantly.

From all the data, just compounds entries 1, 4, 7, 11, 13-17, 19, 21 and 22 can be considered as the safest compounds within the studied array of compounds, since compounds that induce ROS may induce later cell damage. In this line, the potential useful HMF derivatives such as the 5-(chloromethyl) furfural, DFF (potential polymer monomer) and 2,5-DMF (potential biofuel) maybe considered their use with limit human exposure.

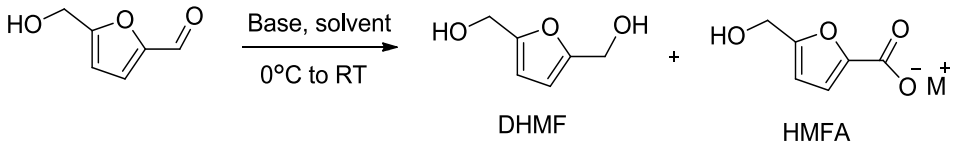
Regarding the controversial data in the literature for HMF toxicological impact, we have not seen any significant cellular impact in our work. In addition, S5MF was shown to be just a moderated cytotoxic compound in the tested cell line.

2.7 Transformation of HMF into 2,5-dihydroxymethylfurfural (DHMF) and 5-hydroxymethyl-2-furancarboxylic acid (HMCFA) via Cannizzaro reaction.

This work was performed together with my colleague Sowmiah Subbiah. My participation was on the synthesis of HMF under solvent free conditions, developing the NMR analysis of the reaction mixtures and crystallization and purification process.

Cannizzaro reaction is the base-induced disproportionation reaction of an aldehyde lacking a hydrogen atom at an α-position to the carbonyl group. One molecule of the aldehyde acts as a hydride donor while the other functions as an acceptor, resulting in a carboxylic acid salt and an alcohol product, respectively. The Cannizzaro reaction is of limited use as it produces an equimolar mixture of both products only one of which is not the target molecule. However, when applied to HMF, the Cannizzaro reaction would be one of the most efficient routes for the simultaneous production of DHMF and HMCFA

both of which are equally important potential derivatives of HMF and circumvents the need of oxidant (for HMFCA) or reductor (for DHMF). Another advantage to the Cannizzaro reaction over the standard reduction or oxidation conditions to HMF is the relative affordability and lack of toxicity of hydroxide ions. Very little research, only two studies, on the Cannizzaro reaction of HMF are available (1910,1919), both of which employed an aqueous alkali solution.[323,324] To the best of our knowledge, only very recently has the formation of DHMF and HMFCA from HMF in an ionic liquid been reported.[325] Our aim was to optimize the synthesis and purification of DHMF and HMFCA *via* the Cannizzaro reaction with HMF (Scheme 78).



Scheme 78.

Initially we performed a screening of different bases. NaH (**Table 34**, entry 1) show the best performance but it is expensive and requires dry solvents. NaOH (**Table 34**, entry 2) provided reasonable yields although in longer reaction times and it can be used in aqueous conditions. The other tested bases did not provide good results.

Table 34. Screening with various bases (performed by Sowmiah Subbiah) [a]

Entry	Base	Solvent	Time (h)	Yield[b] (%)	
				DHMF	HMFA
1	NaH	Dry THF	4	90	89
2	NaOH	Water	36	82	81
3	Li_2CO_3	Water	12	7	7
4	$Ba(OH)_2$	Water	12	NR	NR
5	$Ca(OH)_2$	Water	12	NR	NR
6	K_2CO_3	Water	12	NR	NR
7	KO^{t}Bu	CH_3CN	12	31	34

[a] General conditions: 100mg of HMF (0.8mmol) was dissolved in the corresponding solvent (4 ml) at 0 °C, the base (0.88mmol) was added in a closed vial and stirred at room temperature and the reaction was monitored by TLC. [b]Yield determined by proton NMR using 1 equivalent of sodium acetate (NaOAc, 0.8mmol) as internal standard. NR represents 'No reaction' was observed by NMR.

The amount of NaOH has been studied further on. Theoretically the reaction required the use of 0.5eq. of base but under this condition significant drop of the yield has been observed (Table 35 entry 2). The use of 1.1eq of NaOH showed the best performance, very good yields in shorter time has been achieved (**Table 36**, entry 1). In order to be minimized the amount of required NaOH we tried to perform the reaction in solvent free conditions using 0.55eq.of NaOH. The NaOH solubility in neat HMF was observed to be very poor. To overcome this problem and also to avoid the difficult water evaporation during the reaction work up, minimum amount of EtOH was added. Incomplete conversion was observed after 38h

reaction time, by TLC. After addition of more 0.06eq NaOH the reaction time was extended for additional 24h. No significant improvement under these conditions was observed and after work up 61% HMFCA and 51% DHMF were isolated ,which are similar results obtained when 0.6eq of NaOH were used under standard conditions (**Table 36**, entry 4).

Table 36. Screening of NaOH quantity (performed by Sowmiah Subbiah)[a]

Entry	NaOH (equiv)	Time (h)	Yield[b] (%)	
			DHMF	HMFA
1	1.1	18	86	87
2	0.5	38	28	35
3	0.55	48	46	40
4	0.6	48	53	51
5	0.9	48	80	81

[a] General conditions: To 50mg of HMF (0.4mmol) in water (2 ml) at 0 °C, NaOH (varying equivalent) was added in a closed vial and after 1 h was stirred at room temperature; the reaction was monitored by TLC. [b]Yield determined by proton NMR using 0.15 equivalent of NaOAc (0.04mmol) as an internal standard. After reaction, the water was evaporated and the mixture was washed with diethylether and dried before preparing NMR sample to remove any unreacted HMF.

With best conditions in hand, we explored the simple purification methods to obtain both HMFA and DHMF individually without the need for column chromatography or acid-base separation techniques, since they led to significant losses of Cannizzaro products. Our simpler process is based on the recrystallization technique. After the evaporation of water and simple washing of the crude solid with EtOAc 60% of DHMF were obtained in the solution. The recrystallization of the resulting solid from EtOH/EtOAc provided 98% pure recrystallized product HMFCA (83%) and around 27% diol was easily isolated from the mother liquor by removing non-polar impurities by washing with ether/hexane (total yield of DHMF = 87%).

In conclusion, we have developed an efficient and eco-friendly Cannizzaro reaction of HMF for the simultaneous synthesis of both DHMF and HMFA. The Cannizzaro reaction of HMF ensures an economical process to effectively isolate both DHMF and HMFA by crystallization rather than an acid base extraction. A scalable, simply purified, high yield reaction would make the present process even more useful and attractive.

3. Experimental part.

3.2 Experimental results for the integrated approach for the production and isolation of HMF from carbohydrates.

General: All reagents were purchased from Sigma-Aldrich, Alfa Aesar and Merck and were used without further purification. Old (>15 years) and wet (14 % w/w) TEAB from Laboratorios Azevedos Sociedade Industrial Farmaceutica Lisboa, HPLC analysis have been performed on Dionex P680 pump, Dionex UVD 340S diode array detector, detection at 275nm, manual injector with 20µl loop, column HICHROM C18, 250x4.6mm, R_t (HMF) =

8.7 min or Kromasil 100, C18, 250x4.6mm. R_t (HMF) = 10.7 min. Mobile phase gradient from 1:99 to 50:50 for 40 min acetonitrile:water, flow 1 mL/min, The purity of HMF was determined by comparing the obtained integration area of HMF with other observed minor peaks. The determination of water content in the ammonium salt was performed on Metrohm Karl Fisher coulometer (831KF coulometer Metrohm, 768KF oven Metrohm, 703TI stend Metrohm) equipped with oven: temperature for the analysis was 220˚C.

3.2.1 General procedure for the transformation of fructose (1 g scale) to HMF and isolation using ammonium salts as reaction media:

Without catalyst: To 5g of corresponding ammonium salt was added 1g of fructose. The mixture was placed in a heated silicon bath for the time mentioned in **Table 37**. The resulting solid was washed with EtOAc (50 ml). The solvent was decanted and the solid was dissolved in hot EtOH (2 ml) then under vigorous stirring was added EtOAc (200ml). The resulting precipitated was filtered out and the combined solutions were filtered through a pad of silica gel (10 g) and evaporated to give brown liquid of crude HMF.

Table 37. Experiments for the transformation of fructose to HMF using ammonium salts as reaction media without catalyst.

Entry	Reaction media (rm)	initial water content % (w/w)	Pre-heated temp (ºC)	Time Pre-heated temp (min)	Final heated temp (ºC)	Time of final heated temp (min/h)	Yield (%)[b]	Purity (%)
1	$Pr_4N^+Cl^-$	c			100	1.5 h	0	
2	$Me_4N^+Br^-$	c			100	1.5 h	0	
3	H_4N^+Br + 1mL H_2O	c			100	1.5 h	0	
4	$Et_4N^+Cl^-.H_2O$	c			100	1.5 h	46	g
5	Choline chloride	c			100	1.5 h	51	g
6	$Et_4N^+Br^-$	14[d]	80	10	100	15 min	41	g
7	$Et_4N^+Br^-$	14[d]	80	12	110	15 min	61	g
8	$Et_4N^+Br^-$	14[d]	80	15	120	15 min	43	g
9	$Et_4N^+Br^-$	14[d]	80	12	110	30 min	79	g
10	$Et_4N^+Br^-$	14[d]	80	15	110	35 min	75	g
11	$Et_4N^+Br^-$	e,c	80	10	100	30 min	71	88[h]
12[i]	$Et_4N^+Br^-$[i]	10[f]	80	10	100	1.5 h	50	96[h]
13	$Pr_4N^+Br^-$	c	80	10	100	1.5 h	63	99[h]
14	$Pr_4N^+Br^-$	c	80	10	100	2.5 h	61	99[h]
15	$Pr_4N^+Br^-$	c	80	12	110	1.5 h	77	99[h]

In the presence of catalyst: To 5g of corresponding ammonium salt was added 1g of fructose and amberlyst-15 as catalyst. The mixture was placed in a heated silicon bath for mentioned in (Table 38) time. The resulting solid was washed with EtOAc (50 ml). The solvent was decanted and the solid was dissolved in hot EtOH (2 ml) then under vigorous stirring was added EtOAc (200ml). The resulting precipitated was filtered out and the combined solutions were filtered through a pad of silica gel (10 g) and evaporated to give brown liquid of crude HMF.

Table 38. Experiments for the transformation of fructose to HMF catalyzed by amberlyst-15, using ammonium salts as reaction media.

Entry	Reaction media (rm)	fructose / rm ratio (w/w)	water content % (w/w)	Amount catalyst (% w/w)	Pre-heated temp (ºC)	Time Pre-heated temp (min)	Final heated temp (ºC)	Time of final heated temp (min/h)	Yield (%)[b]	Purity (%)
1	$Me_4N^+Cl^-$	1:5	c	10			100	15 min	0	
2	$Me_4N^+Br^-$	1:5	c	10			100	15 min	0	
3	$Me_4N^+Br^-$ + 0.6ml H_2O	1:5	10[c]	10			100	20 min	26	d
4	$Et_4N^+Cl^-$ .H_2O	1:5	c	10			100	1.5 h	78	d
5	$Bu_4N^+Cl^-$[a]	1:5	c	10			100	15 min	80[h]	d
6	$Pr_4N^+Cl^-$	1:5	c	10			100	1.5h	28	d
7	Choline	1:5 [i]	c	5			100	15 min	59	d
8	$Et_4N^+Br^-$	1:5	14[e]	1			100	15 min	64	d
9	Et_4N^+Br	1:5	14[e]	2			100	15 min	71	d
10	$Et_4N^+Br^-$	1:5	14[e]	5			100	15 min	79	d
11	$Et_4N^+Br^-$	1:5	14[e]	5			100	15 min	80	d
12	$Et_4N^+Br^-$	1:5	14[e]	15			100	15 min	91	d
13	Et_4NBr	1:5 [g]	10[f]	5	80	10	100	15 min	71	96 [l]
14	Et_4NBr	1:5 [g]	10[f]	10	80	10	100	15 min	91	97 [l]
15	Et_4NBr	1:5 [g]	10[f]	15	80	10	100	15 min	90	95 [l]
16	Et_4NBr	1:4 [g]	10[f]	15	80	10	100	15 min	96	88 [l]
17	Et_4NBr	1:5 [g]	5[f]	10	80	10	100	15 min	80	77 [l]
18	Et_4NBr	1:5 [g]	15[f]	10	80	10	100	15 min	71	98 [l]
19	$Pr_4N^+Br^-$	1:5	c	10	80	10	100	15 min	91	70 [l]
20	$Pr_4N^+Br^-$	1:5 [k]	c	10	80	10	100	15 min	87	88 [l]
21	Et_4NBr	1:10 [g]	10[f]	10	80	10	100	15 min	100	99.3 [l]
22	Et_4NBr	1:20 [j]	10[f]	10	80	10	100	15 min	29	99 [l]
23	Et_4NBr	1:20 [j]	10[f]	20	80	10	100	15 min	57	99 [l]

[a] *HMF was isolated by short silica gel column chromatography using EtOAc as a mobile phase.*

3.2.2 Optimized procedure for the batch dehydration of 2g fructose to HMF in 1:5 fructose/TEAB ratio (w/w).

To 9.1g of TEAB (1% water content w/w) was added 0.9ml of water. The resulting mixture (10 g, 10% water content w/w) was mixed with 2g of fructose and 0.2g of amberlyst-15 (10% w/w). The mixture was placed at 80˚C and heated up to 100˚C in 10 min. and stirred at 100 ˚C for 15 min. The mixture was cooled down to r.t. and the water was evaporated. The resulting solid was washed with EtOAc (50 ml). The solvent was decanted and the solid was dissolved in hot EtOH (2 ml) then under vigorous stirring was added EtOAc (200ml). The resulting precipitated was filtered out and the combined solutions were filtered through a pad of silica gel (10g) and evaporated to give brown liquid of HMF (1.25g, 91%) in 97% purity by HPLC.

3.2.3 Procedure for the transformation of fructose (20 g scale) to HMF and isolation using TEAB as reaction media and reaction media reuse:

1st Cycle: To 91 g of TEAB (1% water content w/w) was added 9.0 ml of water. The resulting mixture (100 g, 10% water content (w/w)) was mixed with 20g of Fructose and 2g

of amberlyst-15® (10w%). The mixture was placed at 80˚C and heat up to 100˚C in 10 min. and stirred at 100 ˚C for 15 min. The mixture was cooled down to r.t. and the water was evaporated. The resulting solid was washed with EtOAc (200 ml). The solvent was decanted and the solid was dissolved in hot EtOH (10 ml) then under vigorous stirring was added EtOAc (1000ml). The resulting precipitated was filtered out and the combined solutions were filtered through a pad of silica gel (20 g) and evaporated to give brown liquid of crude HMF (12.9g, 92%) with 99% purity by HPLC.

2nd Cycle: The recovered TEAB was recrystallized additionally from EtOH and ethyl acetate and dried for 5h under vacuum (rotatory pump, <1 mmHg) resulting in 86g recovery. The amount of water was determined by Karl Fisher – 2%, then 7.6 ml of water was added to achieve 10% water amount and 20g of fructose was added. The mixture was placed at 80˚C and heat up to 100˚C in 10 min. and stirred at 100 ˚C for 15 min. The mixture was cooled down to r.t. and the water was evaporated. The resulting solid was washed with EtOAc (200 ml). The solvent was decanted and the solid was dissolved in hot EtOH (10 ml) then under vigorous stirring was added EtOAc (1000ml). The resulting precipitated was filtered out and the combined solutions were filtered through a pad of silica gel (20g) and evaporated to give brown liquid of crude HMF (12 g, 86%) with 97% purity by HPLC and NMR.

3rd Cycle: The recovered TEAB was recrystallized additionally from EtOH and ethyl acetate and dried for 5h under vacuum (rotatory pump, <1 mmHg) resulting in 68g recovery. The amount of water was determined by Karl Fisher – 3%. 5.3 ml of water was added to achieve 10% water amount and 14g of fructose was added. The mixture was placed at 80˚C and heat up to 100˚C for 10 min and at 100 ˚C for 15 min. The mixture was cooled down to r.t. and the water was evaporated. The resulting solid was washed with EtOAc (200 ml). The solvent was decanted and the solid was dissolved in hot EtOH (10 ml) then under vigorous stirring was added EtOAc (1000ml). The resulting precipitated was filtered out and the combined solutions were filtered through a pad of silica gel (20g) and evaporated to give brown liquid of crude HMF (9.1 g, 93%) with 93% purity by HPLC.

4th Cycle: The recovered TEAB was recrystallized additionally from EtOH and ethyl acetate and dried for 5h under vacuum (rotatory pump, <1 mmHg) resulting in 60g recovery. The amount of water was determined by Karl Fisher – 2%, then 31g of new TEAB was added together with 8.4 ml of water to achieve 10% water amount then 20g of fructose was added. The mixture was placed at 80˚C and heat up to 100˚C for 10 min. and stirred at 100 ˚C for 15 min. The mixture was cooled down to r.t. and the water was evaporated. The resulting solid was washed with EtOAc (200 ml). The solvent was decanted and the solid was dissolved in hot EtOH (10 ml) then under vigorous stirring was added EtOAc (1000ml). The precipitation

was not good forming gum type brown solid. It was filtered out and the combined solutions were filtered through a pad of silica gel (20g) and evaporated to give brown liquid of crude HMF (9g, 64%) with 91% purity by HPLC.

3.2.4 Procedure for the transformation of fructose (10 g scale) to HMF in 1:10 fructose/TEAB ratio (w/w).

To 91 g of TEAB (1% water content w/w) was added 9 ml of water. The resulting mixture (100 g, 10% water content w/w) was mixed with 10g of fructose and 1g of amberlyst-15 (10% w/w). The mixture was placed at 80˚C and heat up to 100˚C in 10 min and then stirred at 100˚C for 15 min. The reaction was cooled down to r.t. and the water from the resulting solid mixture was evaporated. Then, the mixture was dissolved in hot EtOH (30 ml) and under vigorous stirring was added EtOAc (1500ml). The resulting precipitated was filtered out and the solution were filtered through a pad of silica gel (20g) and evaporated to give HMF as orange oil (6.8g, 97%) with 99% purity by HPLC.

3.2.5 Procedure for the transformation of fructose (2 g scale) to HMF in 1:10 fructose/TEAB ratio (w/w) and reaction media reuse.

1st to 7th Cycles: To 18.2 g of TEAB (1% water content w/w) was added 1.8 ml of water. The resulting mixture (10 g, 10% water content w/w) was mixed with 2g of fructose and 0.2g of amberlyst-15®. The mixture was placed at 80 ˚C and heat up to 100 ˚C for 10 min. and then stirred at 100˚C for 15 min. The reaction was cooled down to r.t. and the water was evaporated. The water from the resulting solid mixture was evaporated. Then it was dissolved in hot EtOH (10 ml) and under vigorous stirring was added EtOAc (500ml). The resulting precipitated was filtered out and the solution was filtered through a pad of silica gel (10g) and evaporated to give HMF as orange oil.

The collected TEAB mixed with amberlyst-15® was dried under vacuum (4-5 h, rotator pump, <1 mmHg) and recycled using the same conditions for 7 times.

8th Cycle: The recovered TEAB and amberlyst-15® from the seventh cycle was dissolved in 300ml MeOH, and 4g of charcoal was added. The mixture was heated with stirring until it started to boil and was immediately filtered through celite. The solvent was evaporated to give 14g of purified TEAB.

To the purified TEAB (14g) was added 1.6ml of water. The resulting mixture (15.6 g, 10% water content w/w) was mixed with 1.6g of fructose and 0.16g of smashed Amberlyst-15®. The mixture was placed at 80˚C and heat up to 100˚C for 10 min and then stirred at 100˚C for 15 min. The reaction was cooled down to r.t. and the water from the resulting solid mixture was evaporated. Then it was dissolved in hot EtOH (10 ml) and under vigorous stirring was

added EtOAc (500ml). The resulting precipitated was filtered out and solution was filtered through a pad of silica gel (10g) and evaporated to give HMF as orange oil (1.38g, 123%) with 94% purity by HPLC. The observed 123% yield is due to the transformation of accumulated non-reacted fructose from the previous cycle, which corresponds to combined yields of 7^{th} and 8^{th} cycles of 93%.

3.2.6 Procedures for the transformation of glucose to HMF using TEAB as reaction media:

To 9.1g of TEAB was added 0.9ml of water. The resulting 10g mixture with 10% water amount was mixed with 2g of glucose and 60mg of $CrCl_3.6H_2O$. The mixture was placed at 80˚C and heat up to 100˚C for 10 min and then was stirred at 100˚C for 15 min. The mixture was cooled down to r.t. and the water was evaporated. The resulting solid was washed with EtOAc (50 ml). The solvent was decanted and the solid was dissolved in hot EtOH (2 ml) then under vigorous stirring was added EtOAc (200ml). The resulting precipitate was filtered out and the combined solutions were filtered through a pad of silica gel (10g) and evaporated to give brown liquid of crude HMF (500mg, 35%) with 82% purity by HPLC.

To 9.1g of TEAB was added 0.9ml of water. The resulting 10g mixture with 10% water amount was mixed with 2g of glucose and 200mg phosphomolibdic acid. The mixture was placed at 80˚C and heat up to 100˚C for 10 min and then was stirred at 100 ˚C for 15 min. The mixture was cooled down to r.t. and the water was evaporated. The resulting solid was washed with EtOAc (50 ml). The solvent was decanted and the solid was dissolved in hot EtOH (2 ml) then under vigorous stirring was added EtOAc (200ml). The resulting precipitated was filtered out and the combined solutions were filtered through a pad of silica gel (10g) and evaporated to give brown liquid of crude HMF (200mg, 14%) with 87% purity by HPLC.

To 9.1g of TEAB was added 0.9ml of water. The resulting 10g mixture with 10% water amount was mixed with 2g of glucose and 670 mg boric acid. The mixture was placed at 80˚C and heat up to 100˚C for 10 min and then was stirred at 100˚C for 15 min. The mixture was cooled down to r.t. and the water was evaporated. The resulting solid was washed with EtOAc (50 ml). The solvent was decanted and the solid was dissolved in hot EtOH (2 ml) then under vigorous stirring was added EtOAc (200ml). The resulting precipitated was filtered out and the combined solutions were filtered through a pad of silica gel (10g) and evaporated to give brown liquid of crude HMF (350mg, 25%) with 85% purity by HPLC.

3.2.7 Procedure for the transformation of sucrose to HMF using TEAB as reaction media:

To 9.1g of TEAB (1% water content w/w) was added 0.9ml of water. The resulting mixture (10 g, 10% water content w/w) was mixed with 2g of sucrose and 0.2g of amberlyst-15 (10% w/w). The mixture was placed at 80˚C and heat up to 100˚C for 10 min and then was stirred at 100˚C for 15 min. The mixture was cooled down to r.t. and the water was evaporated. The resulting solid was washed with EtOAc (50 ml). The solvent was decanted and the solid was dissolved in hot EtOH (2 ml) then under vigorous stirring was added EtOAc (200ml). The resulting precipitated was filtered out and the combined solutions were filtered through a pad of silica gel (10g) and evaporated to give brown liquid of HMF (0.4g, 32%) with 90% purity by HPLC.

3.2.8 Procedure for the transformation of inulin to HMF using TEAB as reaction media:

To 9.1g of TEAB (1% water content w/w) was added 0.9ml of water. The resulting mixture (10 g, 10% water content w/w) was mixed with 2g of inulin and 0.2g of amberlyst-15 (10% w/w). The mixture was placed at 80˚C and heat up to 100˚C for 10 min and then was stirred at 100˚C for 15 min. The mixture was cooled down to r.t. and the water was evaporated. The resulting solid was washed with EtOAc (50 ml). The solvent was decanted and the solid was dissolved in hot EtOH (2 ml) then under vigorous stirring was added EtOAc (200ml). The resulting precipitated was filtered out and the combined solutions were filtered through a pad of silica gel (10g) and evaporated to give brown liquid of HMF (0.75g, 55%) with 98% purity by HPLC.

3.2.9 General procedure for the continuous transformation of fructose (1-5 g scale) to HMF using TEAB as reaction media.

A mixture of TEAB containing 25% water (w/w) and fructose was passed continuously through a glass reactor of 100 mm pathway and 10 mm diameter filled with amberlyst-15 (3.5 g) heated at 100˚C inside a domestic oven (Solac) and using a peristaltic pump (Ismatec Reglo) equipped with 0.8 mm silicon tube (see details of each experiment in **Table 39**). The water from the collected reaction mixture was evaporated using rotavapor and then rotatory vacuum pump (<1 mmHg). Then the solid was dissolved in a minimum amount of hot EtOH and the TEAB was precipitated with the addition of EtOAc. The mixture was filtered and the filtrate was evaporated.

Table 39 Continuous preparation of HMF from fructose using TEAB as reaction media.

Entry	Fructose (g)	Fructose / solvent ratio (w/w)	Flow (mL/min)	Yield[b] (%)	Purity (%)[c]

1	2g	1:5	1.8	80	90
2[d]	2g	1:15	1.2	80	99
3[e]	2g	1:15	1.2	70	99
4	1g	1:20	0.3	90	91
5	1g	1:20	0.9	90	97
6	1g	1:15	0.9	91	93
7	1g	1:10	0.9	85	92
8	5g	1:10	0.9	74	94

3.2.10 NMR spectras and HPLC chromatograms

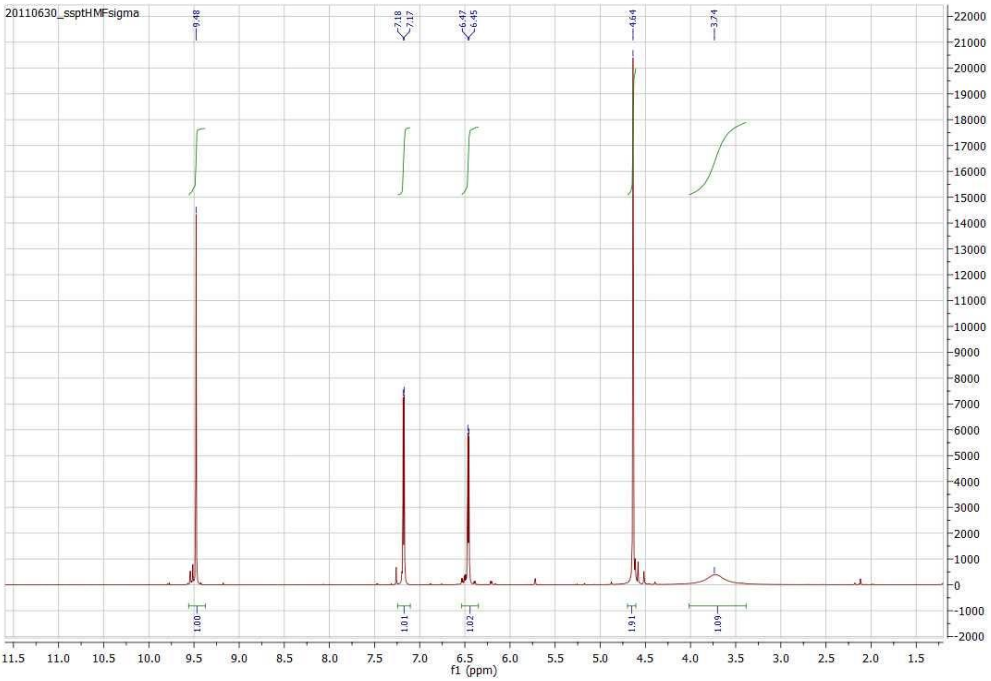


Figure 17 [1]H NMR spectra of commercial HMF (Aldrich Ref . H40807) (96% purity by HPLC).

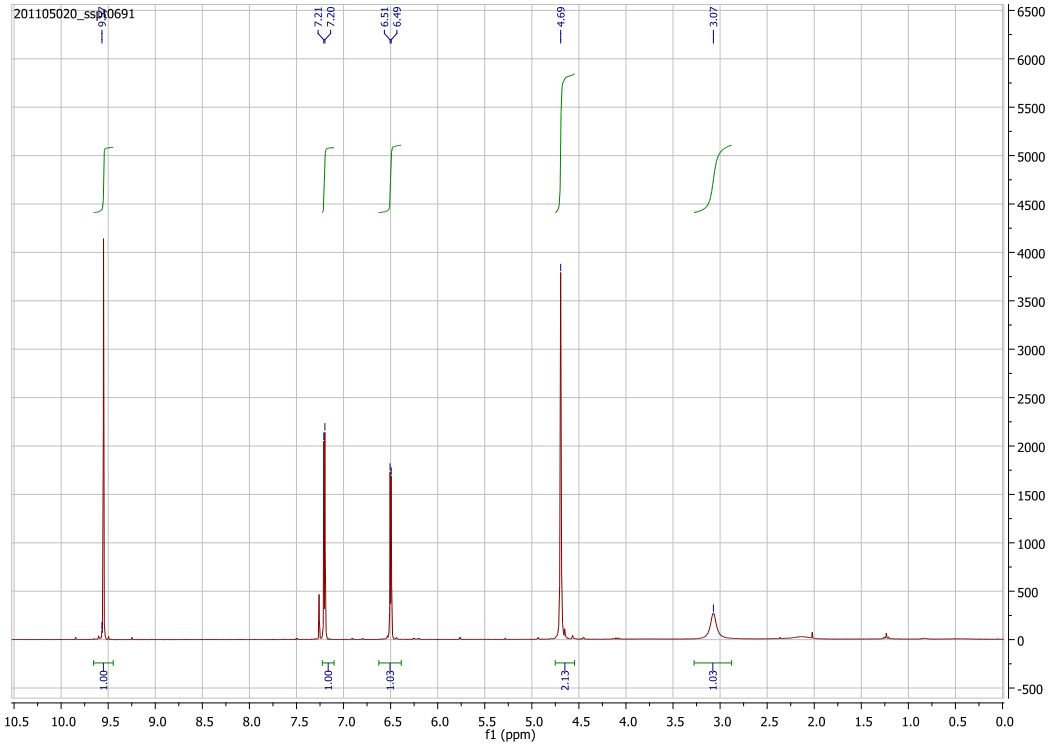


Figure 18 Example of ^{1}H NMR spectra of HMF obtained from fructose in 2 g scale (98% purity by HPLC).

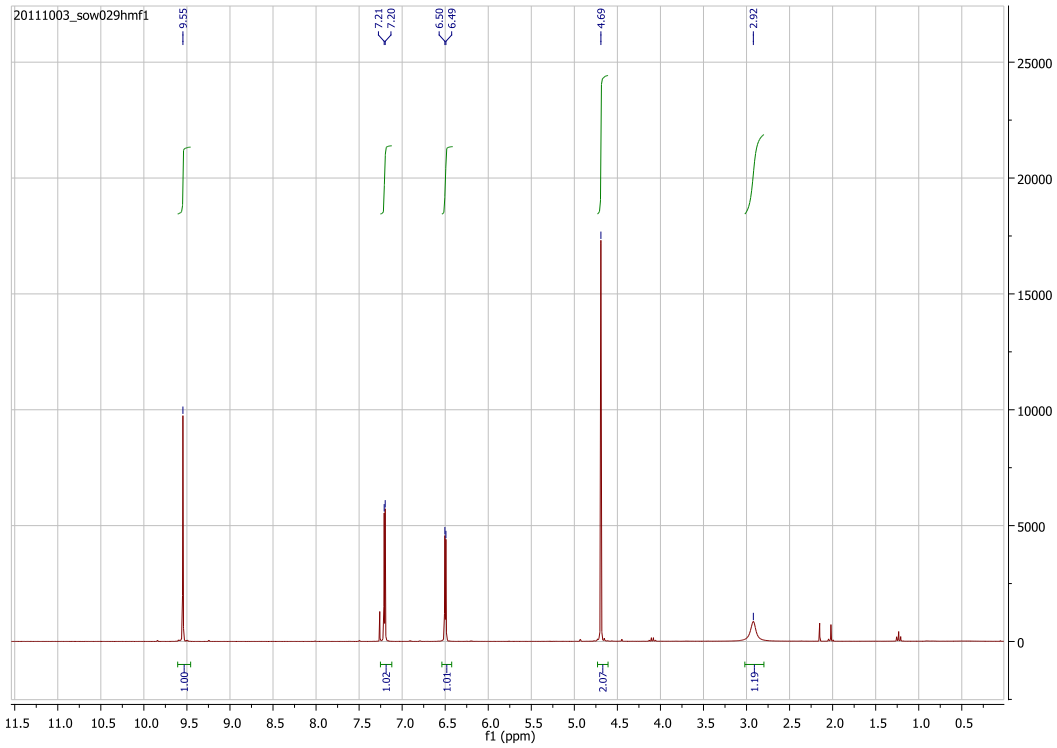


Figure 19 ^{1}H NMR spectra of HMF obtained from fructose in 20 g scale (1st cycle, 99% purity by HPLC).

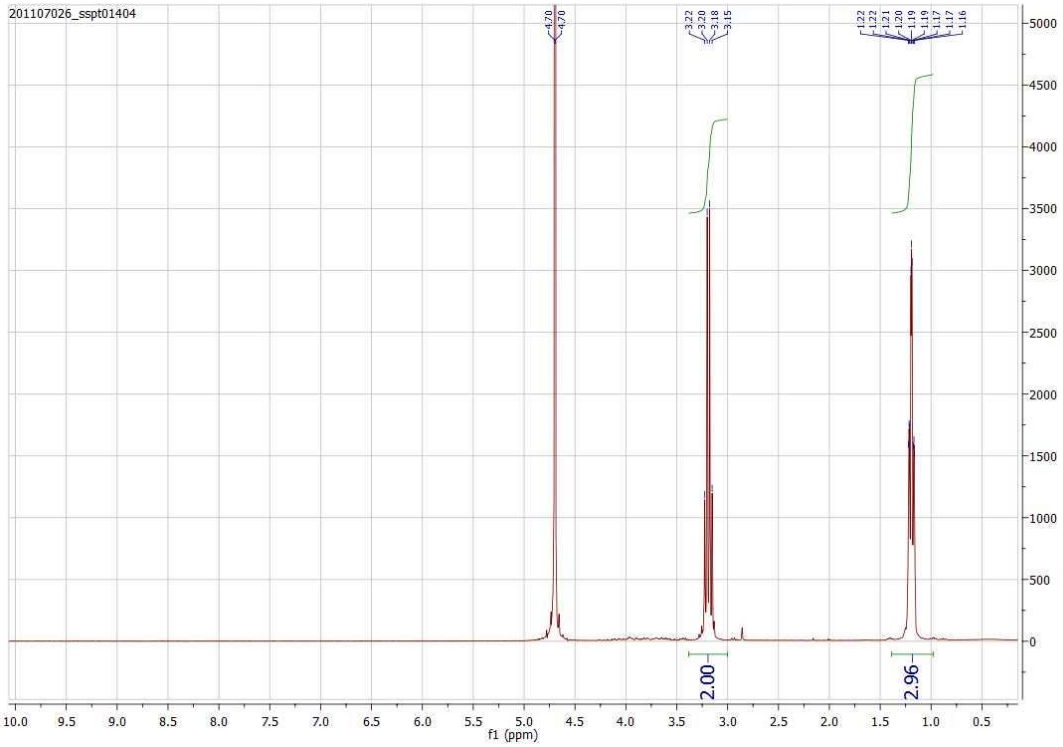


Figure 20 Example of ^{1}H NMR spectra of the TEAB after precipitation. No remaining HMF detected.

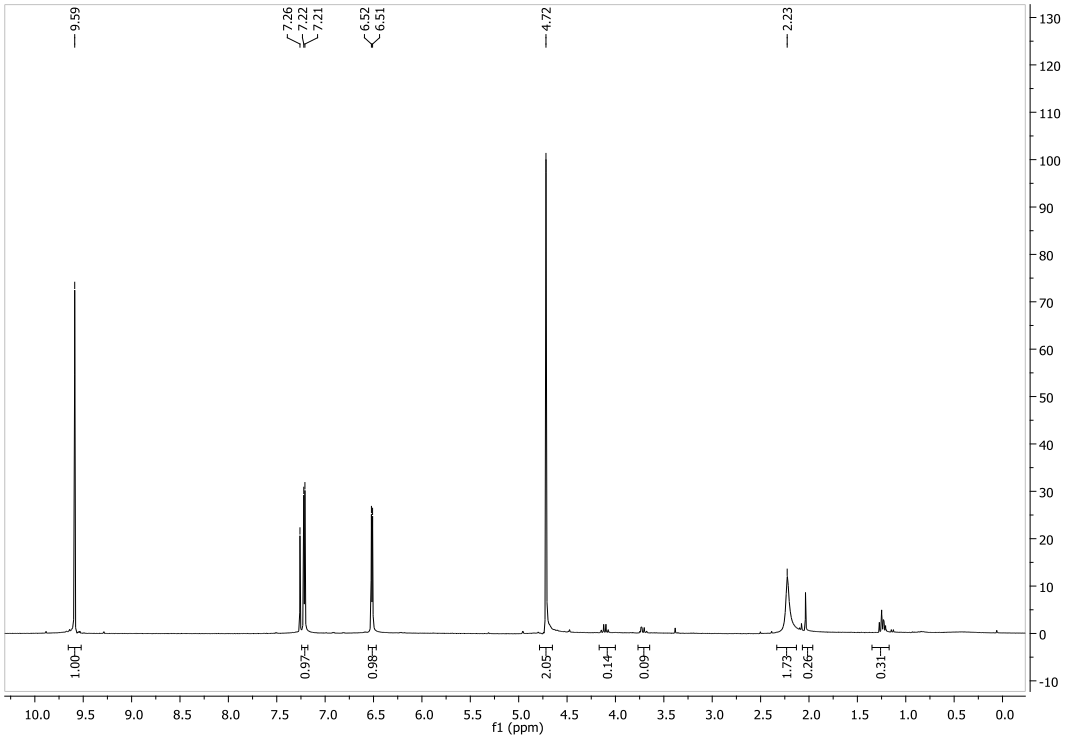


Figure 21 ^{1}H NMR spectra of HMF obtained from fructose in 2 g scale ratio 1:10 (1st cycle, 100% purity by HPLC).

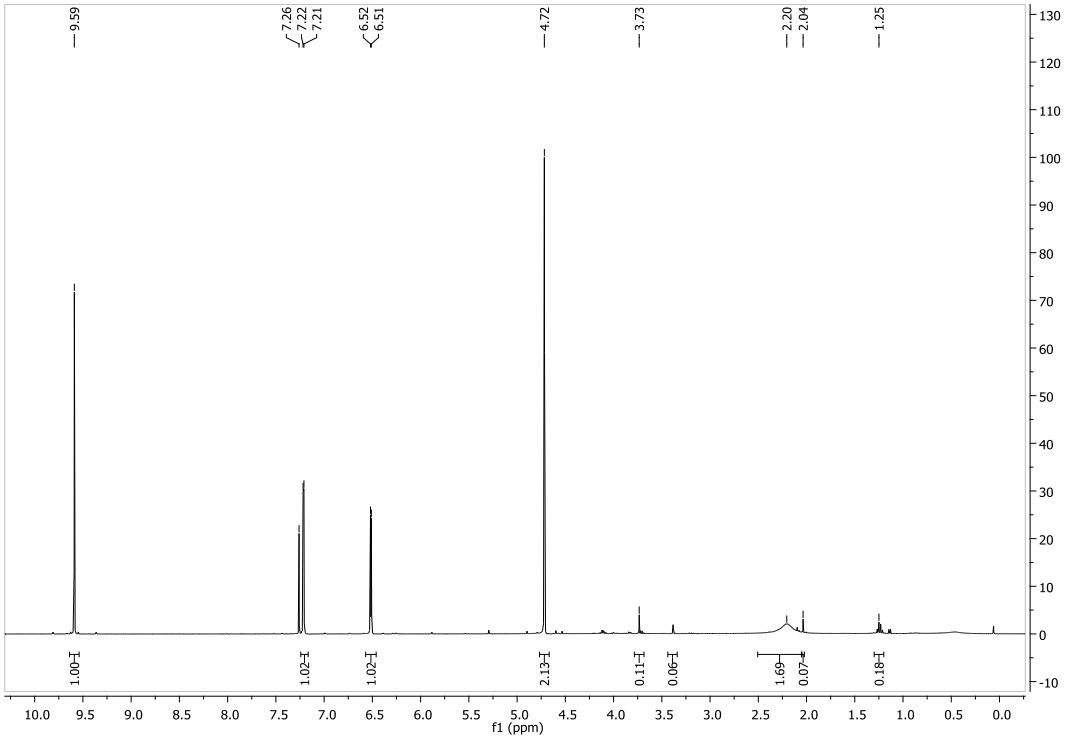


Figure 22. ^{1}H NMR spectra of HMF obtained from fructose in 2 g scale ratio 1:10 (6th cycle, 96.3% purity by HPLC).

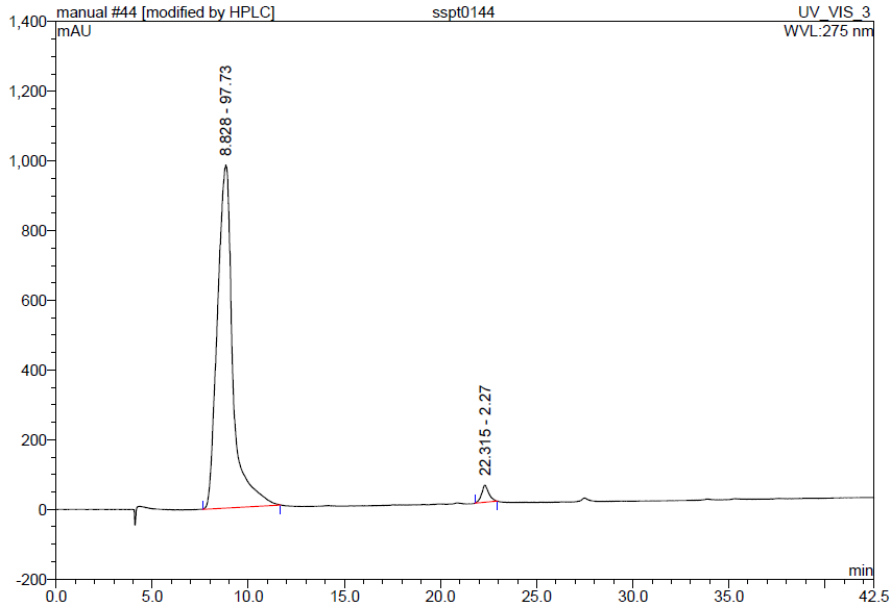


Figure 23 HPLC chromatogram of HMF obtained from fructose in 2 g scale using HICHROM C18, 250x4.6mm column (97.7% purity by HPLC).

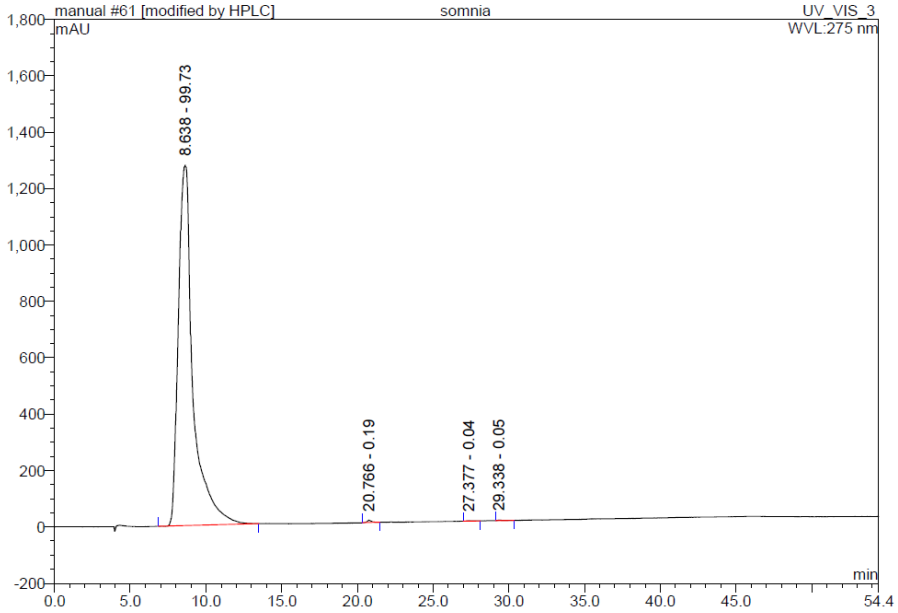


Figure 24 HPLC chromatogram of HMF obtained from fructose in 20 g scale using HICHROM C18, 250x4.6mm column (1st cycle, 99.7% purity by HPLC).

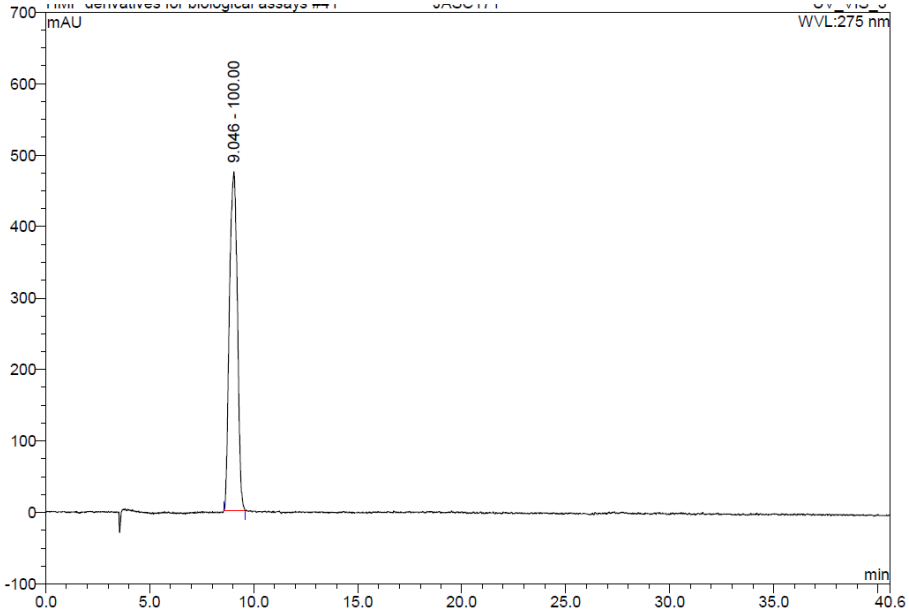


Figure 25 HPLC chromatogram of HMF obtained from fructose in 2 g scale ratio 1:10 (1st cycle, 100% purity by HPLC).

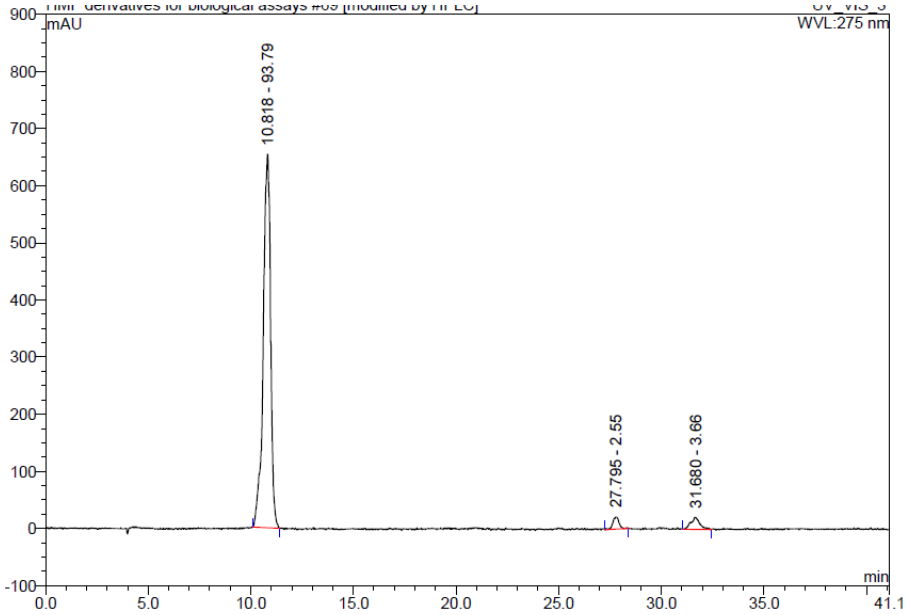


Figure 26 HPLC chromatogram of HMF obtained from fructose in 2 g scale ratio 1:10 (8th cycle, 93.8% purity by HPLC).

3.3 Integrated chemo-enzymatic production of HMF from glucose.

General: All reagents were purchased from Sigma-Aldrich, Alfa Aesar and Merck and have been used without further purification. TEAB – Sigma cat. Nº 14023-1kg (water content 1 % w/w), TEAB 98% Alfa Aesar A13835, Amberlyst-15® (wet) cat. Nº 216399- 500G, HNO_3 65% solution Merck, H_3PO_4 85% solution Merck pro analysis, HCl 36% solution HCl Scharlau, Fructose Merck extra pure and commercial grade from supermarket, D(+)-Glucose anhydrous Merck Art. 8337.

The HMF purity was determined using HPLC analysis performed with Dionex P680 pump, Dionex UVD 340S diode array detector, detection at 275nm, manual injector with 20µl loop, column HICHROM C18, 250x4.6mm, Rt (HMF) = 9.5 min or Kromasil 100, C18, 250x4.6mm. Rt (HMF) = 11.4 min. Mobile phase gradient from 1:99 to 50:50 for 40 min acetonitrile:water, flow 1 mL/min, The purity of HMF was determined by comparing the obtained integration area of HMF with other observed minor peaks.

The Glucose and Fructose analysis was determined using HPLC analysis performed with Shimadzu LC-20AT pump, Merck Differential Refractometer RI-71, Manual injector with 20µL loop. The analysis has been performed using Phenomenex Luna-NH2 250x4.6mm 5µm column, flow 2ml/min and mobile phase acetonitrile and water 87:13. Rt (Fructose) = 5.4 min and Rt (Glucose) = 7.2 min.

3.3.1 Enzymatic glucose/fructose isomerization

Influence of the water content on the enzymatic glucose/fructose isomerization with in TEAB.

General procedure: TEAB was dissolved in H_2O then 0.5 g of Glucose and 15 mg of sweetzyme (3% w/w) have been added and the mixture was placed in Kugelrohr at 70°C and 50 rpm overnight (14 h). Conversion was determined by HPLC analysis.

Table 40. Amount of TEAB and water used

Water content	Et_4NBr (g)	H_2O (mL)
75	0.75	2.25
50	1.5	1.5
40	1.8	1.2
30	2.1	0.9
20	2.4	0.6

3.3.2 Kinetic studies

Glucose conversion into fructose with 3% w/w sweetzyme in 50% TEAB and 50% water mixture: 5 g TEAB was dissolved in 5 ml H_2O then 1.2 g of glucose and 36 mg of sweetzyme have been added and the mixture was placed in Kugelrohr at 70°C and 50 rpm. Samples on every 1 hour have been analyzed by HPLC.

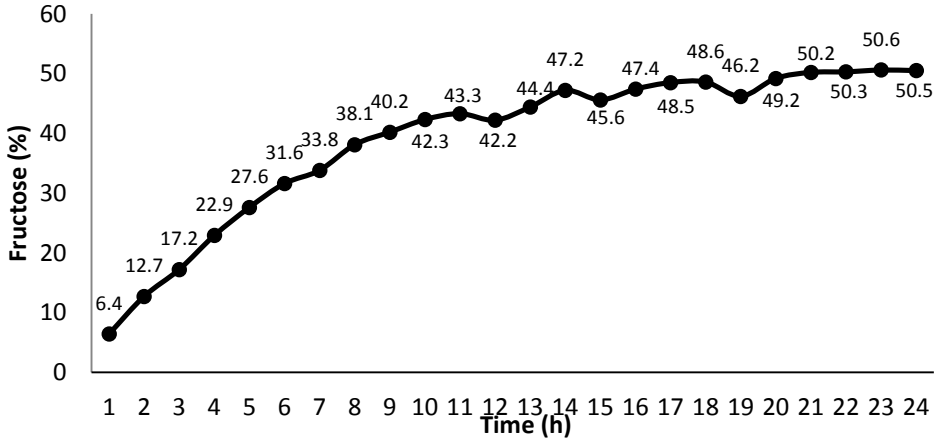


Figure 27 Glucose conversion with 3% w/w sweetzyme in 50% TEAB and 50% water mixture at 70°C.

Glucose conversion with 3% w/w sweetzyme and 20 mg $MgS0_4$ in 50% TEAB and 50% water mixture: 5 g TEAB was dissolved in 5 ml H_2O then 1.2 g of glucose, 20 mg $MgSO_4$ and 36mg of sweetzyme have been added and the mixture was placed in Kugelrohr at 70°C and 50 rpm. Samples on every 1 hour have been analyzed by HPLC.

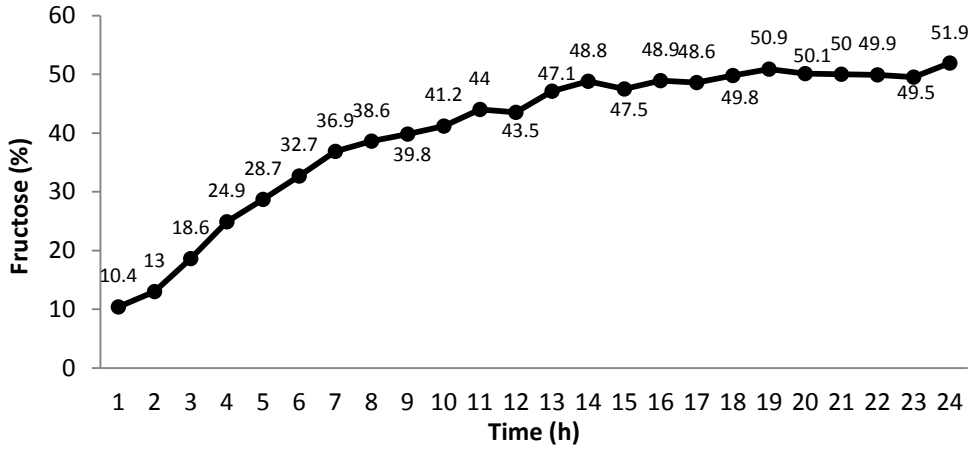


Figure 28 Glucose conversion with 3% w/w sweetzyme and 20 mg $MgSO_4$ in 50% TEAB and 50% water mixture.

Glucose conversion with 3% w/w sweetzyme in water: 1.2 g of glucose was dissolved in 10 ml of H_2O and 36 mg of sweetzyme has been added. The mixture was placed in Kugelrohr at 70°C and 50 rpm. Samples on every 1 hour have been analyzed by HPLC.

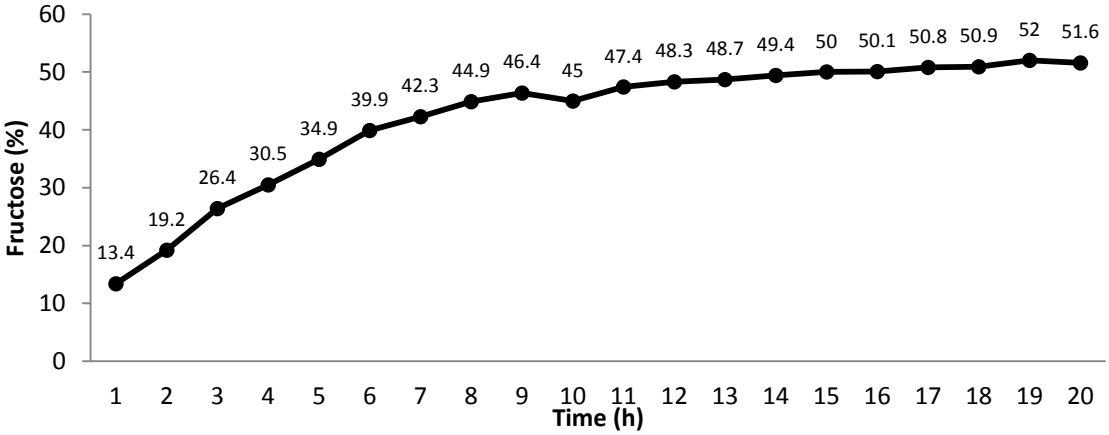


Figure 29 Glucose conversion with 3% w/w sweetzyme in water at 70ºC

Glucose conversion with 3% w/w sweetzyme and 20 mg of $MgSO_4$ in water: 1.2 g of glucose and 20 mg of $MgSO_4$ were dissolved in 10 ml of H_2O and 36mg of sweetzyme has been added. The mixture was placed in Kugelrohr at 70°C and 50 rpm. Samples on every 1 hour have been analyzed by HPLC.

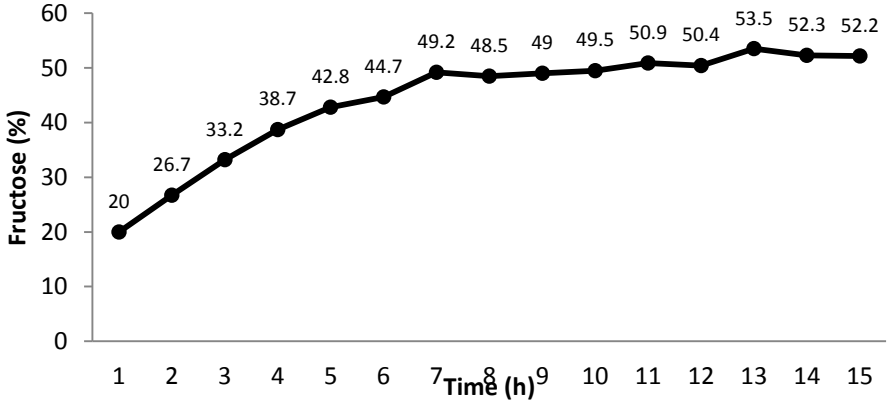


Figure 30 Glucose conversion with 3% w/w sweetzyme and 20mg $MgSO_4$ in water

Glucose conversion with 6% w/w sweetzyme in 50% TEAB and 50% water mixture: 5 g TEAB was dissolved in 5 ml H_2O then 1.2 g of glucose and 72 mg of sweetzyme have been added and the mixture was placed in Kugelrohr at 70°C and 50 rpm. Samples on every 1 hour have been analyzed by HPLC.

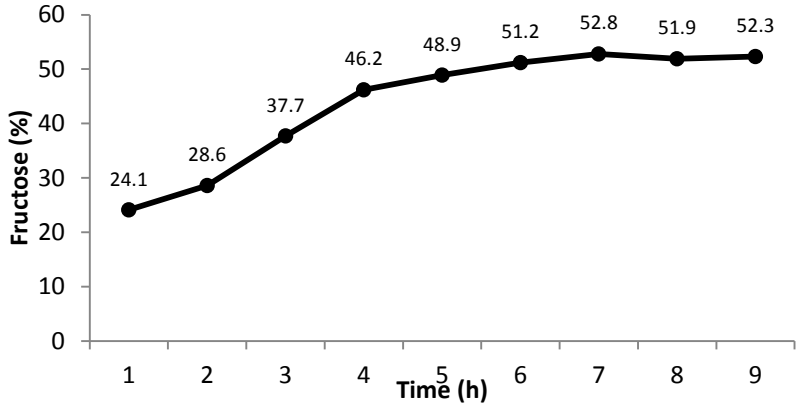


Figure 31 Glucose conversion with 6% w/w sweetzyme in 50% TEAB and 50% water mixture at 70ºC.

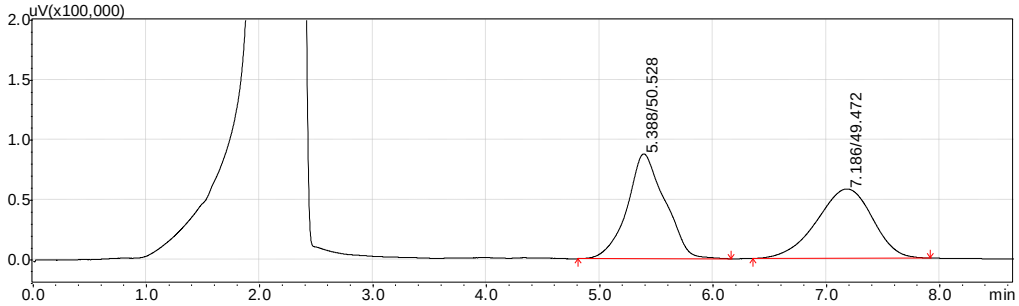


Figure 32 HPLC Chromatogram after 9h of enzymatic reaction. Fructose Rt-5.3min, Glucose Rt-7.18min.

Glucose conversion with 10% w/w sweetzyme in 50% TEAB and 50% water mixture: 5 g TEAB was dissolved in 5 ml H_2O then 1.2 g of glucose and 120 mg of sweetzyme have been added and the mixture was placed in Kugelrohr at 70°C and 50 rpm. Samples on every 1 hour have been analyzed by HPLC.

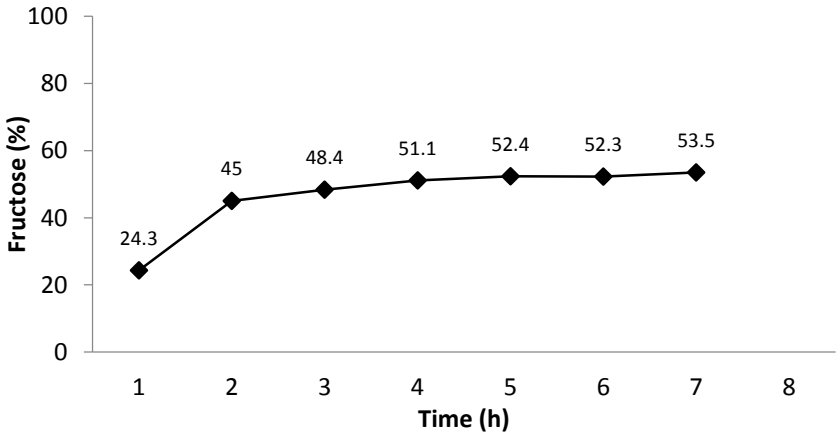


Figure 33 Glucose conversion with 10% w/w sweetzyme in 50% TEAB and 50% water mixture at 70ºC.

Glucose conversion with 6% w/w sweetzyme in 50% TEAB and 50% water mixture at 60°C: 5 g TEAB was dissolved in 5 ml H_2O then 1.2 g of glucose and 72 mg of sweetzyme have been added and the mixture was placed in Kugelrohr at 60°C and 50 rpm. Samples on every 2 hours have been analyzed by HPLC.

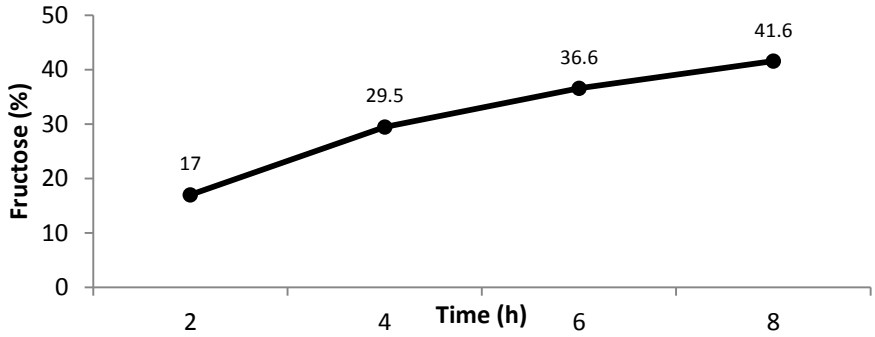
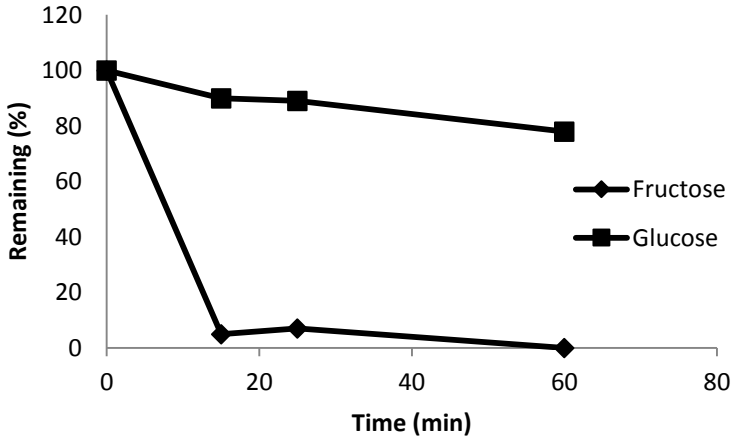


Figure 34 Glucose conversion with 6% w/w sweetzyme in 50% TEAB and 50% water mixture at 60°C.

3.3.3 Dehydration reactions.

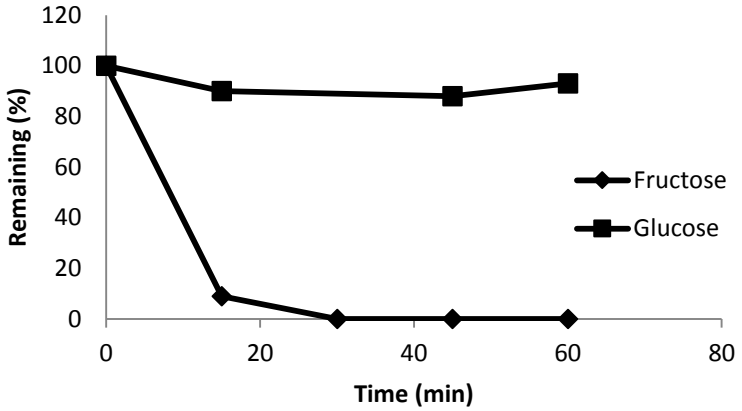
10% Amberlyst-15, 10% H_2O, 80 to 100ºC, acid added at RT, open vessel, 2g sugar/15g rm: 13.5 g of TEAB was mixed with 1 g Fructose and 1 g Glucose then 1.5 mL H_2O and 100 mg amberlyst-15 (10% w/w) were added to a 250 mL round bottom flask with cap. The mixture was placed at 80°C and a sample was collected when a homogeneous solution was obtained. The reaction mixture was heated up to 100°C for 10 min. Samples have been taken.



Time (min)	Glucose (%)	Fructose (%)
0	100	100
15	90	5
25	89	7
60	78	0

10% Amberlyst-15, 13.3 % H_2O, 80 to 100ºC, acid added at RT, open vessel, 2g sugar/15 g rm: 13 g of TEAB was mixed with 1 g Fructose and 1 g Glucose then 2 mL H_2O

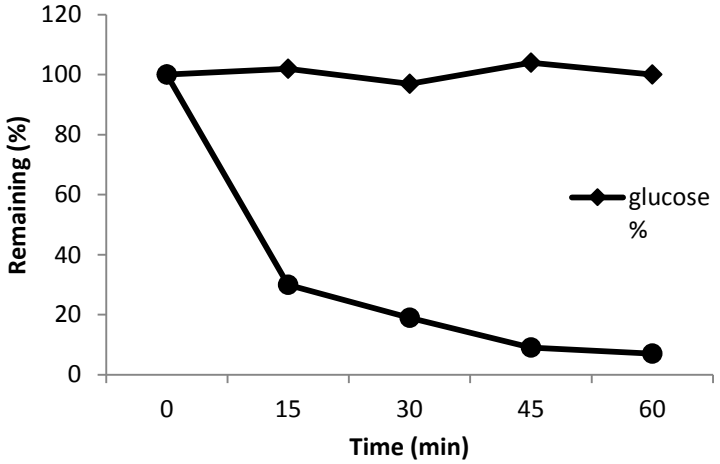
and 100 mg amberlyst-15 (10% w/w) were added to a 250 mL round bottom flask with cap. The mixture was placed at 80°C and a sample was collected when a homogeneous solution was obtained. The reaction mixture was heated up to 100°C for 10 min. Samples have been taken on every 15 min.



Time (min)	Glucose (%)	Fructose (%)
0	100	100
15	90	9
30	nd	0
45	88	0
60	93	0

nd – not determined

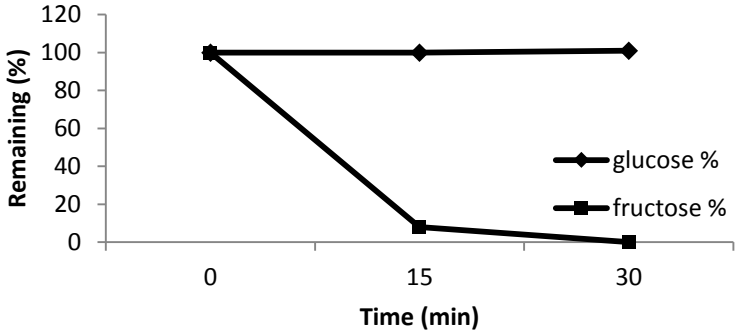
20% H_3PO_4, 10% H_2O, 80 to 100ºC, acid added at RT, open vessel, 3g sugar/15g rm: 13.5 g of TEAB was mixed with 1.5 g Fructose and 1.5 g Glucose then 1.5 ml H_2O and 350 mg 85% H_3PO_4 (20% w/w) were added. The mixture was placed at 80°C and heated up to 100°C for 10 min. Samples have been taken on every 15 min.



Time (min)	Glucose (%)	Fructose (%)
0	100	100
15	102	30
30	97	19
45	104	9
60	100	7

The reaction mixture has been neutralized directly with 0.25 g $NaHCO_3$ in order to be formed NaH_2PO_4. The water was evaporated and the mixture dissolved in 5ml hot absolute EtOH. The white precipitate of NaH_2PO_4 was filtered and the TEAB and remaining glucose was crystalized with the addition of 200ml of EtOAc. The precipitate was filtered out and dried to give 15 g (13.5 g TEAB+1.5 g Glucose). The solution was filtered through silica gel and evaporated to give 0.9 g (85%) HMF as orange oil with 99% purity by HPLC.

30% H_3PO_4, 10% H_2O, 80 to 100ºC, acid added at RT, open vessel, 3g sugar/15g rm: 13.5 g of TEAB was mixed with 1.5 g Fructose and 1.5 g Glucose then 1.5 ml H_2O and 525mg 85% H_3PO_4 (30% w/w) were added. The mixture was placed at 80°C and heated up to 100°C for 10 min. Samples have been taken on every 15 min.



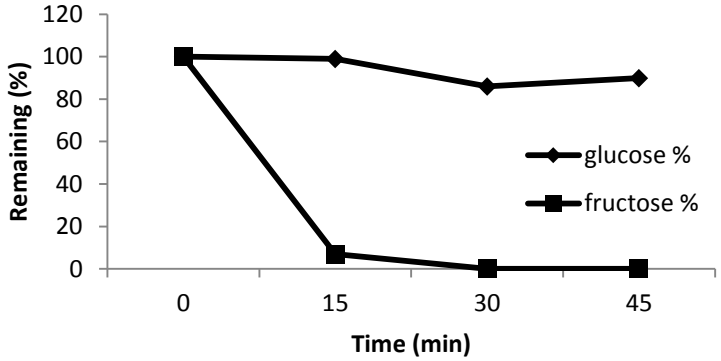
Time (min)	Fructose (%)	Glucose (%)
0	100	100
15	8	100
30	0	100

30% H_3PO_4, 10% H_2O, 80 to 100ºC, acid added at RT, open vessel, 4g sugar/30g rm, isolation: 27g of TEAB was mixed with 2 g Fructose and 2 g Glucose then 3 ml H_2O and 700mg 85% H_3PO_4 (30% w/w) were added. The mixture was placed at 80°C and heated up to 100°C for 10 min and then stirred at 100°C for 30 min. The acid was neutralized with equimolar amount of $NaHCO_3$ (1.5 g) and the water was evaporated. The mixture was dissolved in absolute EtOH and the Na_3PO_4 was filtered. The solvent was evaporated and the mixture dissolved in 10ml of absolute EtOH and the and the TEAB and remaining glucose was crystalized with the addition of 400 ml of EtOAc. The precipitate was filtered and the solution was passed through pad of silica gel. The solvent was evaporated to give 1.22g of HMF as orange oil in 87% yield and 97% purity by HPLC.

30% H_3PO_4, 10% H_2O, 100ºC, acid added at RT, open vessel, 2g sugar/10g rm, isolation: 9 g of TEAB was mixed with 1 g Fructose and 1 g Glucose then 1 ml H_2O and 350mg 85% H_3PO_4 (30% w/w) were added. Then the mixture was stirred at 100°C for 40 min. The acid was neutralized with equimolar amount of $NaHCO_3$ (0.77g) and the water was

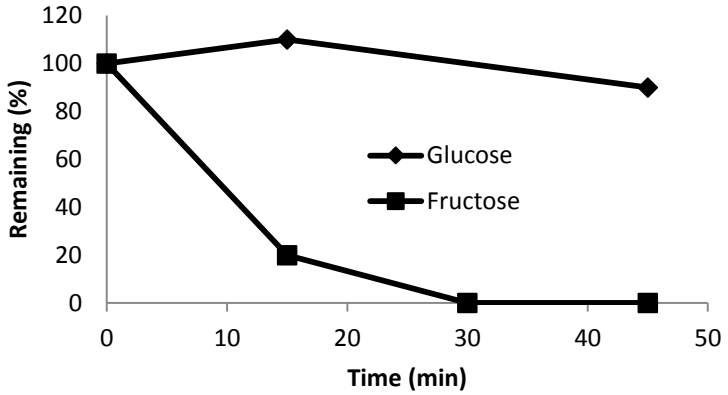
evaporated. The mixture was dissolved in absolute EtOH and the Na_3PO_4 was filtered. The solvent was evaporated and the mixture dissolved in 5ml of absolute EtOH and the and the TEAB and remaining glucose was crystalized with the addition of 200 ml of EtOAc. The precipitate was filtered and the solution was passed through pad of silica gel. The solvent was evaporated to give 0.64 g HMF as orange oil in 91% yield and 99% purity by HPLC.

30% H_3PO_4, 10% H_2O, 110ºC, acid added at RT, open vessel, 2g sugar/10g rm: 9 g of TEAB was mixed with 1 g Fructose and 1 g Glucose then 1 ml H_2O and 235mg 85% H_3PO_4 (20% w/w) were added. The mixture was placed at 110°C. Samples have been taken on every 15 min.



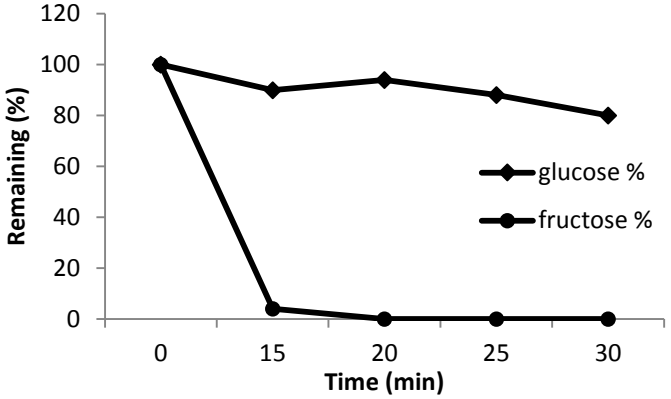
Time (min)	Glucose (%)	Fructose (%)
0	100	100
15	99	7
30	86	0
45	90	0

30% H_3PO_4, 10% H_2O, 110ºC, acid added at RT, closed vessel, 2g sugar/10 g rm: 9 g of TEAB was mixed with 1 g Fructose and 1 g Glucose then 1 ml H_2O and 0.23ml 85% H_3PO_4 (30% w/w) were added. The mixture was placed at 110°C in a closed reactor. Samples have been taken on every 15 min.



Time (min)	Glucose (%)	Fructose (%)
0	100	100
15	110	20
30	90	0
45	90	0

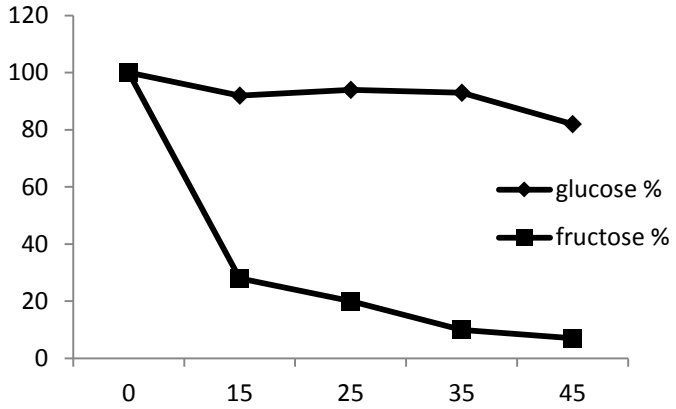
30% H_3PO_4, 10% H_2O, 110ºC, acid added at 110ºC, closed vessel, 2g sugar/10 g rm: 9 g of TEAB was mixed with 1 g Fructose and 1 g Glucose then 1 ml H_2O was added. The mixture was placed at 110°C in closed reactor. When the mixture riches 110°C, giving clear solution, 0.23ml 85% H_3PO_4 (30% w/w) was added and the time counting was started. Samples have been taken on every 5 min.



Time (min)	Glucose (%)	Fructose (%)
0	100	100
15	90	4
20	94	0
25	88	0
30	80	0

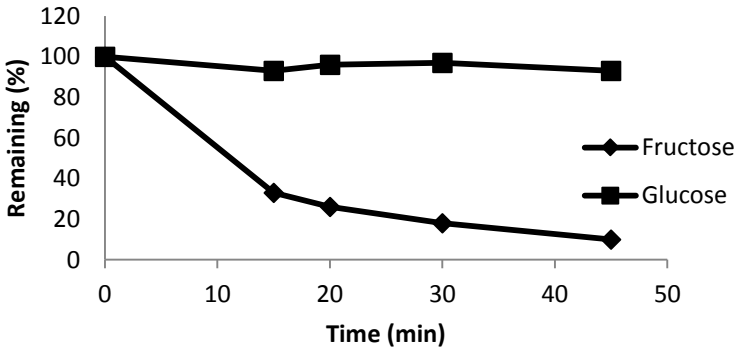
30% H_3PO_4, 10% H_2O, 110ºC, acid added at 110ºC, closed vessel, 2g sugar/10 g rm, isolation: 9 g of TEAB was mixed with 1 g Fructose and 1 g Glucose then 1 ml H_2O was added. The mixture was placed at 110°C in a closed reactor. When the mixture riches 110°C, giving clear solution, 0.23ml 85% H_3PO_4 (30% w/w) was added and the time counting was started. The acid was neutralized with equimolar amount of $NaHCO_3$ (0.77g) and the water was evaporated. The mixture was dissolved in absolute EtOH and the Na_3PO_4 was filtered. The solvent was evaporated and the mixture dissolved in 5ml of absolute EtOH and the TEAB and remaining glucose was crystalized with the addition of 200 ml of EtOAc. The precipitate was filtered and the solution was passed through pad of silica gel. The solvent was evaporated to give 0.66 g of HMF as orange oil in 94% yield and 97% purity by HPLC.

30% H_3PO_4, 10% H_2O, 110ºC, acid added at 110ºC, closed vessel, 2g sugar/10 g rm: 18 g of TEAB was mixed with 2 g Fructose and 2 g Glucose then 2 ml H2O was added. The mixture was placed at 110°C in closed reactor. When the mixture riches 110°C giving clear solution 0.41ml 85% H_3PO_4 (30% w/w) was added and the time counting was started.



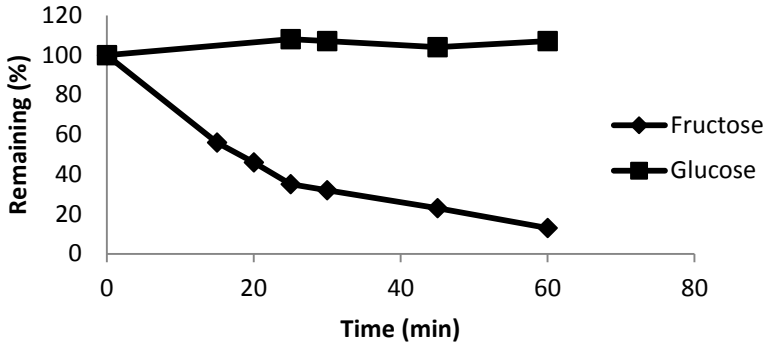
Time (min)	Glucose (%)	Fructose (%)
0	100	100
15	92	72
25	94	80
35	93	90
45	85	93

10% H_3PO_4, 10% H_2O, 120ºC, acid added at 120ºC, closed vessel, 2g sugar/15 g rm: 13.5 g of TEAB was mixed with 1 g Fructose and 1 g Glucose then 1.5 mL H_2O was added. The mixture was placed at 120°C in a closed reactor. When the mixture riches 120°C, giving clear solution, 70 µL 85% H_3PO_4 (10% w/w) was added and the time counting was started. Samples have been taken for HPLC analysis.



Time (min)	Fructose (%)	Glucose (%)
0	100	100
15	33	93
20	26	96
30	18	97
45	10	93

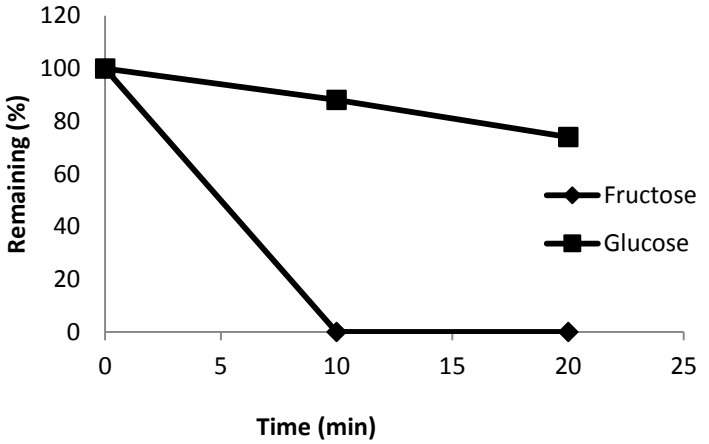
20% H_3PO_4, 10% H_2O, 80 to 110ºC, acid added at 80ºC, closed vessel, 2g sugar/15 g rm: 13.5 g of TEAB was mixed with 1 g Fructose and 1 g Glucose then 1.5 mL H_2O was added. The mixture was placed at 80°C in a closed reactor. When the mixture riches 80°C, giving homogeneous solution, 140 µL 85% H_3PO_4 (10% w/w) was added and the reaction mixture heated up to 110ºC for 20 min. When the mixture riches 110ºC the time counting was started. Samples have been taken for HPLC analysis.



Time (min)	Fructose (%)	Glucose (%)
0	100	100
15	56	nd
20	46	nd
25	35	108
30	32	107
45	23	104
60	13	107

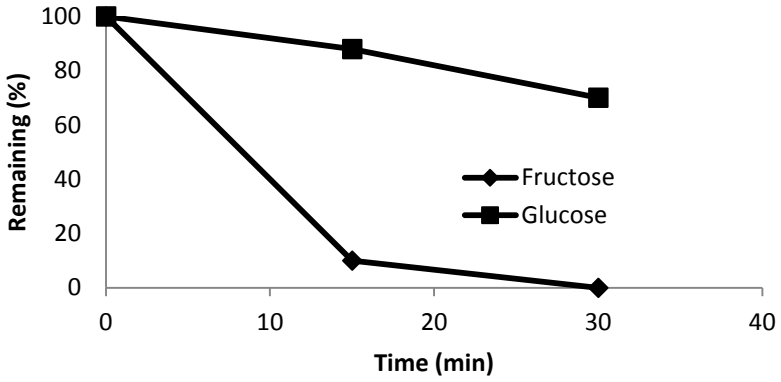
nd – not determined

10% HNO_3, 10% H_2O, 80 to 100ºC, acid added at RT, open vessel, 2g sugar/15 g rm : 13.5 g of TEAB was mixed with 1 g Fructose and 1 g Glucose then 1.5 mL H_2O and 0.11mL 65% HNO_3 (10% w/w) were added to a 250 mL round bottom flask with a cap. The mixture was placed at 80°C and a sample was collected. The reaction mixture was heated up to 100°C for 10 min and second sample was collected. After 10 min reaction at 100ºC was collected the last sample.



Time (min)	Glucose (%)	Fructose (%)
0 (80ºC)	100	100
10 (100ºC)	88	0
20	74	0

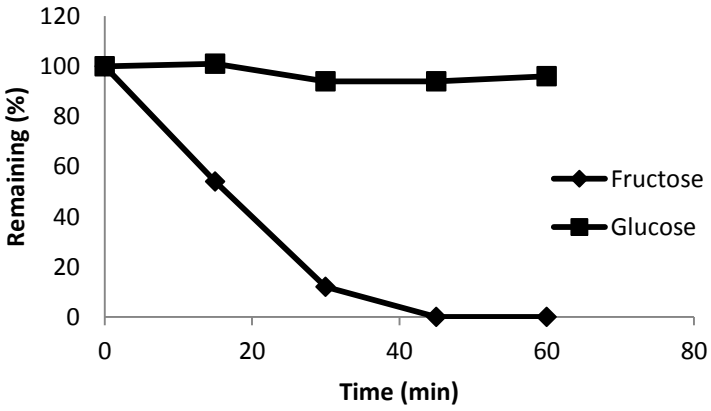
10% HNO_3, 10% H_2O, 80ºC , acid added at RT, open vessel, 2g sugar/15 g rm: 13.5 g of TEAB was mixed with 1 g Fructose and 1 g Glucose then 1.5 mL H_2O and 0.11mL 65% HNO_3 (10% w/w) were added to a 250 mL round bottom flask with cap. The mixture was placed at 80°C and samples have been taken on every 15 min.



Time (min)	Glucose (%)	Fructose (%)
0	100	100
15	88	10
30	70	0

10% HNO_3, 50% H_2O, 80 to 100ºC, acid added at RT, open vessel, 2g sugar/15 g rm : 7.5 g of TEAB was mixed with 1 g Fructose and 1 g Glucose then 7.5 mL H_2O and 0.11 mL 65% HNO_3 (10% w/w) were added to a 250 mL round bottom flask with a cap. The mixture was placed at 80°C and a sample was collected. The reaction mixture was heated up to 100°C for 10 min. Further samples have been taken during the reaction. No reaction was observed in 60 minutes.

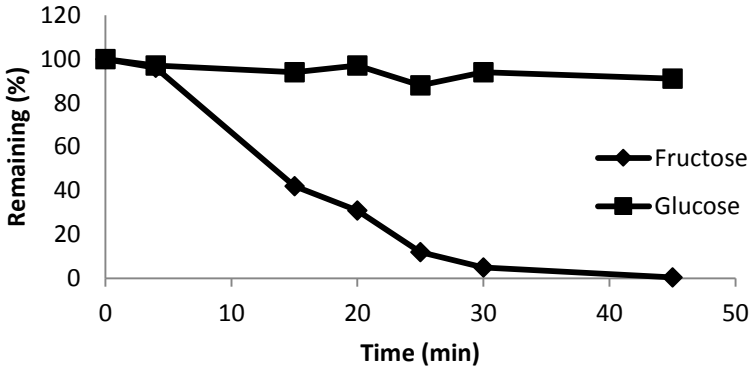
10% HNO_3, 15% H_2O, 80ºC, acid added at RT, open vessel, 2g sugar/15 g rm : 12.8g of TEAB was mixed with 1 g Fructose and 1 g Glucose then 2.2 mL H_2O and 0.11 mL 65% HNO_3 (10% w/w) were added to a 250 mL round bottom flask with a cap. The mixture was placed at 80°C and samples have been taken on every 15 min.



Time (min)	Glucose (%)	Fructose (%)
0	100	100
15	101	54
30	94	12
45	94	0
60	96	0

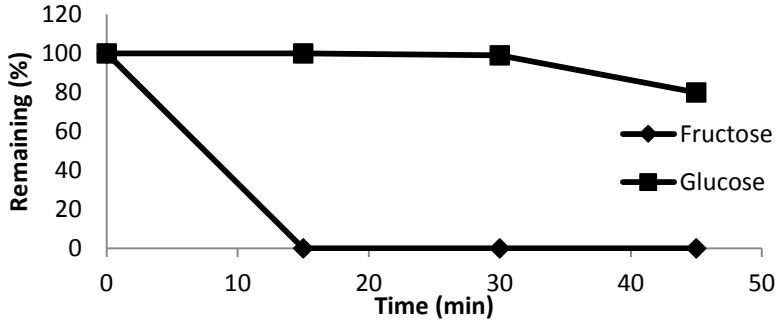
15% HNO_3, 15% H_2O, 80ºC, acid added at RT, open vessel, 2g sugar/15 g rm: 12.8g of TEAB was mixed with 1 g Fructose and 1 g Glucose then 2.2 mL H_2O and 0.17 mL 65%

HNO_3 (10% w/w) were added to a 250 mL round bottom flask with a cap. The mixture was placed at 80°C and samples have been taken.



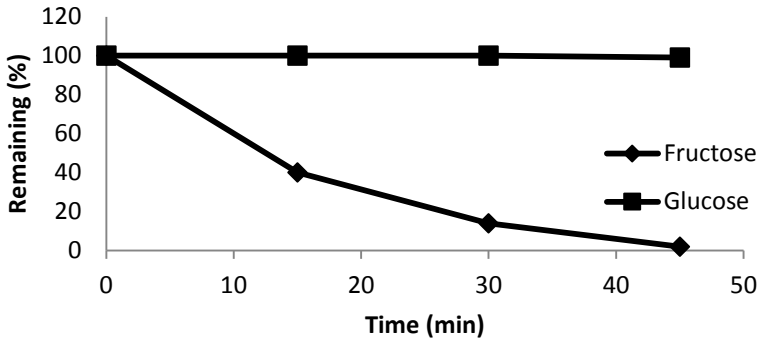
Time (min)	Glucose (%)	Fructose (%)
0	100	100
4	97	96
15	94	42
20	97	31
25	88	12
30	94	5
45	91	0.5

10% HNO_3, 10% H_2O, 80ºC, acid added at RT, closed vessel, 2g sugar/15 g rm: 13.5g of TEAB was mixed with 1 g Fructose and 1 g Glucose then 1.5 mL H_2O and 0.11mL 65% HNO_3 (10% w/w) were added to closed vessel reactor. The mixture was placed at 80°C and samples have been taken on every 15 min.



Time (min)	Glucose (%)	Fructose (%)
0	100	100
15	100	0
30	99	0
45	80	0

10% HNO_3, 15% H_2O, 80ºC, acid added at RT, closed vessel, 2g sugar/15 g rm: 12.8g of TEAB was mixed with 1 g Fructose and 1 g Glucose then 2.2 mL H_2O and 0.11mL 65% HNO_3 (10% w/w) were added to closed vessel reactor. The mixture was placed at 80°C and samples have been taken on every 15 min.



Time (min)	Glucose (%)	Fructose (%)
0	100	100
15	100	40
30	100	14
45	99	2

10% HNO_3, 10% H_2O, 90ºC, acid added at 90ºC, closed vessel, 2g sugar/15 g rm, isolation: 13.5 g of TEAB was mixed with 1 g Fructose and 1 g Glucose then 1.5 mL H_2O was added. The mixture was placed at 90°C in closed reactor. When the mixture riches 90°C, giving clear solution, 0.11 mL 65% HNO_3 (10% w/w) was added and the time counting was started. After 15 min reaction at 90°C, the reaction mixture was dissolved in absolute EtOH and transferred to a round bottom flask. Then the acid was neutralized with equimolar amount of $NaHCO_3$ (133.5 mg). The solvent was evaporated and the mixture dissolved in 5 mL of absolute EtOH and the TEAB, $NaNO_3$ and remaining glucose was crystalized with the addition of 200 mL of EtOAc. The precipitate was filtered and the solution was passed through pad of silica gel. The solvent was evaporated to give 0.66 g of HMF as orange oil in 94% isolated yield and 70% purity by HPLC. The isolated precipitate (14.1 g) was dissolved in 14 mL of H_2O and passed through pad of activated carbon and celite. 1 g of Glucose and 123mg of sweetzyme were added and the mixture was placed in Kugelrohr at 70°C and 50 rpm overnight. 50% glucose conversion into fructose was observed by HPLC analysis.

Time (min)	Glucose (%)	Fructose (%)
0	100	100
15	95	0

10% HNO_3, 10% H_2O, 80ºC, acid added a**t 80ºC, closed vessel, 2g sugar/15 g rm, isolation:** 13.5 g of TEAB was mixed with 1 g Fructose and 1 g Glucose then 1.5 mL H_2O was added. The mixture was placed at 80°C in closed reactor. When the mixture riches 80°C, giving clear solution, 0.11 mL 65% HNO_3 (10% w/w) was added and the time counting was started. After 15 min reaction at 80°C, the reaction mixture was dissolved in absolute EtOH and transferred to a round bottom flask. Then the acid was neutralized with equimolar amount of $NaHCO_3$ (133.5 mg). The solvent was evaporated and the mixture dissolved in 5 mL of absolute EtOH and the TEAB, $NaNO_3$ and remaining glucose was crystalized with the

addition of 200 mL of EtOAc. The precipitate was filtered and the solution was passed through pad of silica gel. The solvent was evaporated to give 0.64 g of HMF as orange oil in 91% isolated yield and 90% purity by HPLC.

Time (min)	Glucose (%)	Fructose (%)
0	100	100
15	99	0

10% HNO_3, 10% H_2O, 80ºC, acid added at 80ºC, closed vessel, 6 g sugar/45 g rm, isolation: 40.5 g of TEAB was mixed with 3 g Fructose and 3 g Glucose then 4.5 mL H_2O was added. The mixture was placed at 80°C in closed reactor. When the mixture riches 80°C, giving clear solution, 0.33 mL 65% HNO_3 (10% w/w) was added and the time counting was started. After 15 min reaction at 80°C, the reaction mixture was dissolved in absolute EtOH and transferred to a round bottom flask. Then the acid was neutralized with equimolar amount of $NaHCO_3$ (400.5 mg) and 20% of $NaNO_3$ was filtered. The solvent was evaporated and the mixture dissolved in 5 mL of absolute EtOH and the TEAB, remaining $NaNO_3$ and glucose was crystalized with the addition of 200 mL of EtOAc. The precipitate was filtered and the solution was passed through pad of silica gel. The solvent was evaporated to give 1.47 g of HMF as orange oil in 70% isolated yield and 95% purity by HPLC.

10% HCl, 15% H_2O, 90ºC, acid added at RT, open vessel, 3g sugar/15 g rm, isolation: 12.7 g of TEAB was mixed with 1.5 g Fructose and 1.5 g Glucose then 2.3 ml water and 10% w/w HCl (0.42ml) were added. The reaction was stirred at 90°C for 15min. The acid was neutralized with equimolar amount of $NaHCO_3$ and the water was evaporated. The mixture was dissolved in absolute EtOH and the NaCl was filtered. The solvent was evaporated and the mixture dissolved in 5ml of absolute EtOH and the TEAB and remaining glucose was crystalized with the addition of 200 ml of EtOAc. The precipitate was filtered and the solution was passed through pad of silica gel. The solvent was evaporated to give 0.89 g of HMF as orange oil in 85% yield and 87% purity by HPLC. 27% glucose has been lost.

3.3.4 Reaction media and sweetzyme reutilization

General procedure for Fructose dehydration: Reaction mixture containing 67.5g TEAB, 7.5mL water (10% w/w/), 5g Fructose and 5g Glucose was placed in a closed vessel reactor. The mixture was stirred at 80ºC until a homogeneous solutions was obtained. Then 0.55 mL of 65% HNO_3 (10% w/w) were added and the mixture was stirred at 80°C for 25 min. 100 mg of the reaction mixture were collected at 0 and 25 min for HPLC analysis. The reaction mixture was then dissolved in absolute EtOH (50 mL) and transferred to a round bottom flask. The acid was neutralized with equimolar amount of $NaHCO_3$ (667.5 mg) and

the solvent evaporated. The obtained solid mixture was extracted with AcOEt (2x100mL) and then dissolved in hot absolute EtOH (25mL) and the TEAB, $NaNO_3$ and glucose was crystalized with the addition of EtOAc (800mL). The precipitate was filtered and all the collected organic phases were passed through pad of 10g silica gel. The solvent was evaporated to give HMF as orange oil.

General procedure for Glucose isomerization: The recovered reaction medium and glucose were dissolved in 50% w/w of H_2O and passed through pad of activated carbon and celite. To the mixture, 5 g of Glucose and sweetzyme (600 mg used from the 1st cycle, which was reuse in the next cycles) were added and the mixture was placed in rotavap at 70°C and 50 rpm overnight. The glucose/fructose ratio was determined by HPLC analysis. Sweetzyme was decanted and stored in the fridge for the next cycle. Water was evaporated and the obtained solid was used for the fructose dehydration reaction.

10% HNO_3, 10% H_2O, 80ºC, acid added at 80ºC, closed vessel, 10g sugar/75 g rm, isolation, enzymatic isomerization performed at 60ºC.

General procedure for Glucose isomerization: A mixture of 10 g of Glucose, 67.5 g TEAB, 67.5 mL H_2O and 600 mg sweetzyme was placed on a rotavap at 60°C and 50 rpm overnight. The glucose/fructose ratio was determined by HPLC analysis. Sweetzyme was decanted and stored in the fridge for the next cycle. Water was evaporated and the obtained solid was used for the fructose dehydration reaction.

General procedure for Fructose dehydration: The reaction mixture from the glucose isomerization was placed in closed vessel reactor and 7.5 mL water was added. The mixture was stirred at 80ºC until a homogeneous solutions was obtained. Then 0.55 mL of 65% HNO_3 (10% w/w) was added and the mixture was stirred at 80 °C for 25 min. 100 mg of the reaction mixture were collected at 0 and 25 min for HPLC analysis. The reaction mixture was then dissolved in absolute EtOH (50 mL) and transferred to a round bottom flask. The acid was neutralized with equimolar amount of $NaHCO_3$ (667.5 mg) and the solvent evaporated. The obtained solid mixture was extracted with AcOEt (2 × 100 mL) and then dissolved in hot absolute EtOH (25mL) and the TEAB, $NaNO_3$ and glucose were crystalized with the addition of EtOAc (800 mL). The precipitate was filtered and all the collected organic phases were passed through pad of 10g silica gel. The solvent was evaporated to give HMF as orange oil.

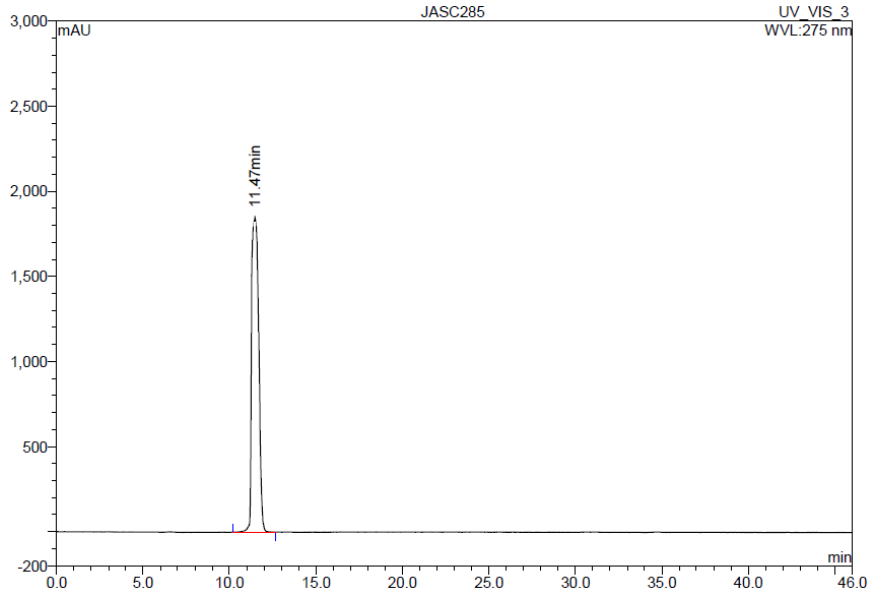


Figure 35. Representative HPLC chromatogram of the isolated HMF

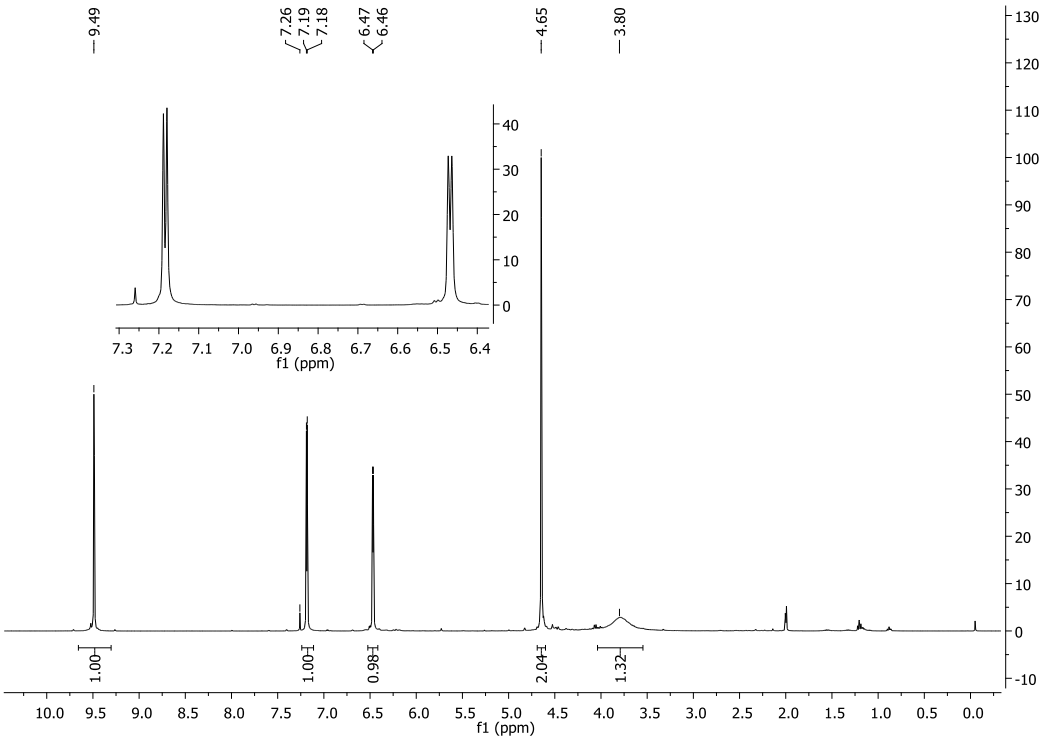


Figure 36 ^{1}H NMR of the isolated HMF.

Continuous transformations: The experiments have been performed using an in-house made serpentine glass reactor with 5 mm internal diameter and 12ml internal volume. The reactor was heated in domestic oven (Solac) having originally built in temperature control. The reaction mixture was passed through the reactor using Watson Marlon 120S peristaltic pump.

General procedure for Sulfuric acid catalyzed continuous dehydration: 25 g of TEAB was mixed with 2 g Fructose and 2 g Glucose and 10ml 5% H_2SO_4. The mixture was passed

with 0.5ml/min flow through a glass reactor heated at 95°C. The acid was neutralized with equimolar amount of $NaHCO_3$ (0.8 g) and the water was evaporated. The mixture was dissolved in absolute EtOH and the Na_2SO_4 was filtered. The solvent was evaporated and the mixture dissolved in 10ml of absolute EtOH and the TEAB and remaining glucose was crystalized with the addition of 400 ml of EtOAc. The precipitate was filtered and the solution was passed through pad of silica gel. The solvent was evaporated to give 1.12 g of HMF as orange oil in 80% yield and 97% purity by HPLC; 89 % of glucose content.

3.4 Dehydration of glucose to HMF using supported chromium catalyst.

General: All reagents were purchased from Sigma-Aldrich, Alfa Aesar and Merck and have been used without further purification. Fructose was commercial grade from supermarket. HPLC analysis have been performed on Dionex P680 pump, Dionex UVD 340S diode array detector, detection at 275nm, manual injector with 20µl loop, column HICHROM C18, 250x4.6mm, R_t (HMF) = 8.7 min or Kromasil 100, C18, 250x4.6mm. R_t (HMF) = 10.7 min. Mobile phase gradient from 1:99 to 50:50 for 40 min acetonitrile:water, flow 1 mL/min, The purity of HMF was determined by comparing the obtained integration area of HMF with other observed minor peaks.

Preparation of chromium supported catalysts: 3g of the corresponding resin were heated for 4h at 80ºC in a solution of 1g $CrCl_3$*$6H_2O$ in 10ml MeOH in a closed vessel. Then the resin was filtered out and washed with 50ml of MeOH and 100ml and dried on air overnight.

Optimization of the reaction conditions

20%w/w Amberlyst-15/$CrCl_3$, 100ºC: To 9 g of TEAB was added 1ml of water. The resulting mixture (10 g, 10% water content w/w) was mixed with 1g of glucose. The mixture was heated up to 100 ˚C. Then 200mg Amberlyst-15/$CrCl_3$ was added and stirred at 100 ˚C for 45 min. The mixture was cooled down to r.t. and the water was evaporated. The resulting solid was washed with EtOAc (50 ml). The solvent was decanted and the solid was dissolved in hot EtOH (2 ml) then under vigorous stirring was added EtOAc (200ml). The resulting precipitated was filtered out and the combined solutions were filtered through a pad of silica gel (10g) and evaporated to give HMF as brown liquid (350mg, 50%) and >95% purity by HPLC.

100%w/w Amberlyst-15/$CrCl_3$, 100ºC: To 9 g of TEAB was added 1ml of water. The resulting mixture (10 g, 10% water content w/w) was mixed with 1g of glucose. The mixture was heated up to 100ºC. Then 1g Amberlyst-15/$CrCl_3$ was added and stirred at 100˚C for 45 min. The mixture was cooled down to r.t. and the water was evaporated. The resulting solid was washed with EtOAc (50 ml). The solvent was decanted and the solid was dissolved in

hot EtOH (2 ml) then under vigorous stirring was added EtOAc (200ml). The resulting precipitated was filtered out and the combined solutions were filtered through a pad of silica gel (10g) and evaporated to give HMF as brown liquid (420mg, 60%) and >95% purity by HPLC.

100%w/w Amberlyst-15/$CrCl_3$, 100ºC 4%w/w water: To 9.6 g of TEAB was added 0.4ml of water. The resulting mixture (10 g, 4% water content w/w) was mixed with 1g of glucose. The mixture was heated up to 100ºC. Then 1g Amberlyst-15/$CrCl_3$ was added and stirred at 100˚C for 60min. The mixture was cooled down to r.t. and the water was evaporated. The resulting solid was washed with EtOAc (50 ml). The solvent was decanted and the solid was dissolved in hot EtOH (2 ml) then under vigorous stirring was added EtOAc (200ml). The resulting precipitated was filtered out and the combined solutions were filtered through a pad of silica gel (10g) and evaporated to give HMF as brown liquid (455mg, 65%) and 90% purity by HPLC.

200%w/w Amberlyst-15/$CrCl_3$, 100ºC: To 9 g of TEAB was added 1ml of water. The resulting mixture (10 g, 10% water content w/w) was mixed with 1g of glucose. The mixture was heated up to 100˚C. Then 1g Amberlyst-15/$CrCl_3$ was added and stirred at 100˚C for 45 min. The mixture was cooled down to r.t. and the water was evaporated. The resulting solid was washed with EtOAc (50 ml). The solvent was decanted and the solid was dissolved in hot EtOH (2 ml) then under vigorous stirring was added EtOAc (200ml). The resulting precipitated was filtered out and the combined solutions were filtered through a pad of silica gel (10g) and evaporated to give HMF as brown liquid (300mg, 43%).

100%w/w Amberlyst-15/$CrCl_3$, 120ºC, 60min: To 9 g of TEAB was added 1ml of water. The resulting mixture (10 g, 10% water content w/w) was mixed with 1g of glucose. The mixture was heated up to 120˚C. Then 1g Amberlyst-15/$CrCl_3$ was added and stirred at 120˚C for 60min. The mixture was cooled down to r.t. and the water was evaporated. The resulting solid was washed with EtOAc (50 ml). The solvent was decanted and the solid was dissolved in hot EtOH (2 ml) then under vigorous stirring was added EtOAc (200ml). The resulting precipitated was filtered out and the combined solutions were filtered through a pad of silica gel (10g) and evaporated to give HMF as brown liquid (510mg, 73%) and >95% purity by HPLC.

50%w/w Amberlyst-15/$CrCl_3$, 120ºC, 60min: To 9 g of TEAB was added 1ml of water. The resulting mixture (10 g, 10% water content w/w) was mixed with 1g of glucose. The mixture was heated up to 120˚C. Then 0.5g Amberlyst-15/$CrCl_3$ was added and stirred at 120˚C for 60min. The mixture was cooled down to r.t. and the water was evaporated. The resulting solid was washed with EtOAc (50 ml). The solvent was decanted and the solid was

dissolved in hot EtOH (2 ml) then under vigorous stirring was added EtOAc (200ml). The resulting precipitated was filtered out and the combined solutions were filtered through a pad of silica gel (10g) and evaporated to give HMF as brown liquid (410mg, 59%) and >95% purity by HPLC.

100%w/w Amberlyst-15/$CrCl_3$, 120ºC, 90min: To 9 g of TEAB was added 1ml of water. The resulting mixture (10 g, 10% water content w/w) was mixed with 1g of glucose. The mixture was heated up to 120˚C. Then 1g Amberlyst-15/$CrCl_3$ was added and stirred at 120˚C for 90min. The mixture was cooled down to r.t. and the water was evaporated. The resulting solid was washed with EtOAc (50 ml). The solvent was decanted and the solid was dissolved in hot EtOH (2 ml) then under vigorous stirring was added EtOAc (200ml). The resulting precipitated was filtered out and the combined solutions were filtered through a pad of silica gel (10g) and evaporated to give HMF as brown liquid (476mg, 68%) and >92% purity by HPLC.

General procedure for the ion exchange resins screening: 7g TEAB was mixed with 0.7ml H_2O and 0.7g glucose then 0.7g of the corresponding resin-$CrCl_3$ was added and the mixture was stirred at 120ºC for 1h. After cooling down the resulting solid was washed with EtOAc (50 ml). The solvent was decanted and the solid was dissolved in hot EtOH (2 ml) then under vigorous stirring EtOAc (200ml) was added. The resulting precipitated was filtered out and the combined solutions were passed through a pad of silica gel (10g) and evaporated to give HMF as brown liquid.

3.5 Experimental data for the synthesis of HMF as a student laboratory experiment.

3.5.1 General procedure for batch conversion of fructose to HMF.

Laboratory session (2 hours)

1. In a fume hood heat a water bath on a magnetic hot plate until it start to boil.
2. Prepare a single-necked round bottom flask (250 ml) equipped with a magnetic stir bar and charge it with 9.1g of TEAB (1% water content w/w), 1g of Fructose, 0.1g amberlyst-15 and 0.9ml of water.
3. Place the reaction mixture in the boiling water bath and stir it for 15 min.
4. Remove the flask and cool down the reaction mixture to room temperature and evaporate the water using rotary evaporator.
5. Wash the solid with 50 ml of EtOAc, decant and collect the solvent then add 5 ml of EtOH to the solid and heat it until it is fully dissolved.
6. Add 300 ml of EtOAc to the hot EtOH solution under vigorous stirring and filter out the resulting precipitate and the catalyst.

7. Prepare a filter with a path of silica gel (10 g) and filter through it the combined solutions from step 5 and 6.
8. Evaporate the solvent on a rotary evaporator and determined the HMF yield.

Students results: The experiment was reproduced in the teaching laboratory environment by the students from the 2nd year of pharmaceutical science course (5 years course). The experiments that have been performed using new TEAB and Amberlyst-15 provided 91-94% yield of HMF and 96-98% purity by HPLC, while the experiment performed with recovered TEAB and catalyst from the previous experiment resulted in 90% HMF yield and 95% purity by HPLC.

3.5.2 General procedure for continuous conversion of fructose to HMF.

Laboratory session (2 hours)

1. Charge an Erlenmeyer flask (250ml) with 18g of TEAB, 2g of fructose and 6ml. 5% H_2SO_4 and stirrer the mixture with magnetic stir bar until it formed a homogeneous solution.
2. Connect a glass column supplied with compressed air to the flow reactor and submerge the reactor in a water bath. Heat the water bath on a magnetic hot plate till the water start to boil.
3. Transfer the solution prepared in step 1 to the column and apply slight positive pressure of compressed air. Adjust the flow with the column drain to around 1 drop per 3-4 sec. (0.5-0.6ml/min). Collect the reaction mixture in a round bottom flask (500 ml).
4. Remove the flask and cool down the reaction mixture to room temperature then neutralize it with 0.514g $NaHCO_3$. Add 100 ml of ethanol and remove the formed Na_2SO_4 by filtration through filter paper and evaporate the mixture using rotary evaporator.
5. Wash the resulting solid with 50 ml of EtOAc, decant and collect the solvent then add 6ml of EtOH to the solid and heat it until it is fully dissolved.
6. Add 300 ml of EtOAc to the hot EtOH solution under vigorous stirring and filter out the resulting precipitate.
7. Prepare a filter with a path of silica gel (10g) and filter through it the combined solutions from step 5 and 6.
8. Evaporate the solvent on a rotary evaporator and determine the HMF yield.

Students results: The flow conversion was repeated by the students providing HMF in 77% yield and 92% purity by HPLC In one experiment the students added by mistake 10ml of 5%H_2SO_4 instead of 6 ml and in this case 65% yield and 91% purity has been observed.

3.6 Toxicological evaluation of HMF derivatives

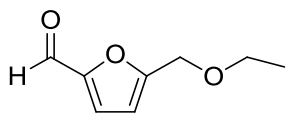
General: All solvents were freshly distilled from commercial grade sources. Commercially available reagents were used as received without further purification otherwise notice. Preparative thin-layer chromatography plate was prepared with silica gel 60 GF254 MercK (ref 1.07730.1000), whereas flash chromatography was carried out on silica gel 60M purchased from MN (Ref. 815381). Reaction mixtures were analyzed by TLC using ALUGRAM SIL G/UV254 from MN (Ref. 818133, silica gel 60), and visualization by UV and phosphomolybdic acid stain.

HPLC analysis was performed on Dionex P680 pump, Dionex UVD 340S diode array detector, detection at 275 and 225 nm, manual injector with 20 μL loop, column HICHROM C18, 250x4.6mm, or Kromasil 100, C18, 250x4.6mm. Mobile phase gradient from 1:99 to 50:50 for 40 min acetonitrile:water, and then 50:50 for the time indicated in the chromatogram, flow 1 mL/min, The HPLC purity was determined by comparing the integration area of the main signal with other observed minor peaks and are represented in the chromatograms by relative area. Retention times were determined by using the mobile phase gradient from 1:99 to 90:10 in 50 min.

GC–MS analyses were performed on Gas Chromatograph Mass Spectrometer-QP2010S, Shimadzu by using the column TRB-5MS-Teknokroma (30 m × 0.25 mm × 0.25 μm). GC program: column oven Tinitial=: 50.0 °C, Tfinal=: 250.0 °C, slope = 5ºC/min ; injection temperature: 250 °C; pressure: 77.9 kPa, total flow: 17.7 mL/min; column flow: 1.34 mL/min; linear velocity: 42.0 cm/sec; purge flow: 3.0 mL/min split ratio: 10.0, high press. inj. pressure: 100.0 kPa, high press. inj. time: 1.00 min. MS program: start time: 3.00 min; end time: 50.00 min; event time: 0.50 s; scan speed: 666; start: m/z = 40.00; end: m/z = 350.00.

NMR spectra were recorded at room temperature in a Bruker AMX 300 or Bruker AMX 400 using $CDCl_3$, D_2O or DMSO-d6 as solvents and $(CH3)_4Si$ (1H) as internal standard.

5-(Ethoxymethyl)furan-2-carbaldehyde.



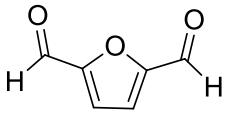
HMF (1.0 g, 8.0 mmol) was dissolved in absolute EtOH (15 mL) then 3 drops of concentrated H_2SO_4 were added and mixture was stirred for 5 h at reflux temperature. The reaction mixture was neutralized with aqueous saturated solution of $NaHCO_3$ and extracted with EtOAc. The organic phase was dried with $MgSO_4$ and evaporated. The product was

purified by silica flash chromatography using EtOAc/Hexane to give 703 mg (57% yield) of the desired product.

^{1}H NMR (300 MHz, $CDCl_3$) δ 1.20 (t, J = 7.0 Hz, 3H), 3.55 (q, J = 7 Hz, 2H), 4.49 (s, 2H), 6.49 (d, J = 3.52 Hz, 1H), 7.18 (d, J = 3.52 Hz, 1H), 9.56 (s, 1H);

^{13}C NMR (300 MHz, $CDCl_3$) δ 15.1, 64.8, 66.7, 111.1, 152.6, 158.8, 177.9.

Furan-2,5-dicarbaldehyde.

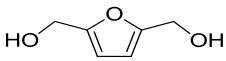


To a flame dried schlenk with CH_2Cl_2 (100 mL) at -78ºC under argon atmosphere oxalyl chloride (0.85 mL, 1.25 equiv.) was added followed by dropwise addition of DMSO (1.13 mL, 2 equiv.). After stirring for 15 minutes, a solution of HMF (1.0 g, 8.0 mmol) in CH_2Cl_2 (10 mL) was added dropwise and the resulting mixture was stirred at -78ºC for an additional 30 minutes. Et_3N was then added dropwise and the mixture was allowed to warm up to RT. After 1 hour of stirring at RT, water (50 mL) was added and the resulting mixture was washed with HCl 1M, extracted with CH_2Cl_2 and finally dried over Na_2SO_4. The resulting crude mixture was purified by flash chromatography with Et_2O/Hexane. The collected solid was further recrystallized from hexane/EtOAc to give 305 mg (31% yield) of the product as white needles.

^{1}H NMR (300 MHz, $CDCl_3$) δ 7.3 (s, 2H), 9.86 (s, 2H);

^{13}C NMR (300 MHz, $CDCl_3$) δ 119.4, 154.4, 179.4.

2,5-dihydroxymethylfuran (DHMF)

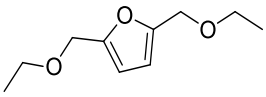


To a solution of HMF (2g, 16mmol) in dry THF (10 mL), $NaBH_4$ (2.4g, 64 mmol) was added portionwise. The reaction mixture was stirred at RT overnight. MeOH (7 mL) and acetic acid (9 mL) were added and stirred at RT for 15 min then 50 mL of MeOH was added and the solvent removed under vacuum. Then twice 50 ml MeOH were added and evaporated. The mixture was dissolved in DCM/MeOH 9:1 and passed through a pad of silica gel. The solvent was evaporated and the product was purified by silica flash chromatography using EtOAc/Hexane to give 1.6g (80% yield) of the product.

^{1}H NMR (300 MHz, D_2O) δ 4.51 (s, 4H), 6.31 (s, 2H);

^{13}C NMR (300 MHz, D_2O) δ 55.8, 109.0, 153.6.

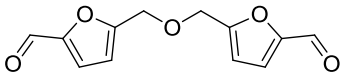
2,5-Bis(ethoxymethyl)furan.



(5-(ethoxymethyl)furan-2-yl)methanol (390mg, 2.5mmol) was dissolved in acetonitrile (5 mL) then NaH (0.2g, 8.3mmol) was added followed by dropwise addition of bromoethane (0.6 mL, 8 mmol). The reaction mixture was stirred at RT overnight. The reaction was quenched with H_2O and the acetonitrile was evaporated and the water phase extracted with Et_2O. The organic phase was dried with $MgSO_4$ and evaporated. The product was purified by silica flash chromatography using EtOAc/Hexane to give 180 mg (39%) of the desired product. The purity was determind by HPLC.

^{1}H NMR (300 MHz, $CDCl_3$) δ 1.20 (t, J = 7.0 Hz, 6H), 3.52 (q, J = 7 Hz, 4H), 4.41 (s, 4H), 6.24 (s, 2H).

(5,5'-(Oxybis(methylene))bis(furan-5,2-diyl))dimethanol.

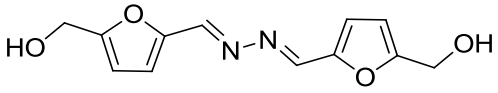


The title compound was isolated by silica gel column chromatography EtOAc/Hexane as a 10% side product from the following reaction: To 9.1g of TEAB (1% water content w/w) was mixed with 2g of fructose and 0.2g of smashed Amberlyst-15 (10% w/w). The mixture was placed at 80 ˚C and heated up to 100 ˚C for 10 min. Then the mixture was stirred at 100 ˚C for 30 min. The mixture was cooled down to RT and the resulting solid was washed with EtOAc (50 mL). The solvent was decanted and the solid was dissolved in hot EtOH (2 mL) then under vigorous stirring EtOAc (200 mL) was added. The resulting precipitated was filtered out and the combined solutions were filtered through a pad of silica gel (10g) and evaporated to give brown liquid of HMF (1.25g, 71%) in 88% purity by HPLC. The purity of the final product was determined by HPLC.

^{1}H NMR (300 MHz, $CDCl_3$) δ 4.63 (s, 4H), 6.57 (d, J = 3.55 Hz, 2H), 7.21 (d, J = 3.55 Hz, 2H), 9.63 (s, 2H);

^{13}C NMR (300 MHz, $CDCl_3$) δ 64.8, 112.0, 122.0, 152.9, 157.4, 177.9.

(5,5'-((1E,1'E)-hydrazine-1,2-diylidenebis(methanylylidene))bis(furan-5,2-diyl))dimethanol.



HMF (1.0 g, 8.0 mmol) and hydrazine monohydrate (0.4 mL, 8.0 mmol) was dissolved in absolute EtOH (15 mL) then 3 drops of concentrated H_2SO_4. The mixture was stirred at RT

for 1h. The resulting solid was filtered and washed with absolute EtOH to give the desired product 1.7 g (86% yield).

^{1}H NMR (300 MHz, DMSO-d_6) δ 4.45 (s, 2H), 4.47 (s, 2H), 5.43 (t, 2H), 6.50 (s, 2H), 7.02 (s, 2H), 8.44 (s, 2H);

^{13}C NMR (300 MHz, DMSO-d_6) δ 55.8, 109.6, 118.4, 148.4, 150.2, 159.4.

3.7 Cannizaro reaction of HMF experimenyal results.

3.7.1 Representative procedure for the Cannizzaro reaction of HMF and product isolation

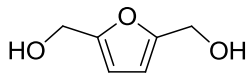
3g of HMF (24 mmol) were dissolved in water (30ml) and then cooled to 0ºC. After 0.87g (22 mmol) of NaOH at 0 ºC were added, the resulting mixture was stirred at room temperature in a closed vessel for about 18h; TLC confirmed the completion of the reaction. After evaporating the water, ethyl acetate (2x50ml) was added to the solid residue to separate the DHMF diol (0.85g, 60%). From the remaining solid, carboxylate salt HMFCA was isolated by recrystallization with ethanol (approx 2ml)/ethylacetate (100ml), yielding HMFCA salt (1.4g, 83% yield) hygroscopic solid which was stored in a refrigerator. More DHMF (0.4g, 27% yield) was isolated after evaporation of the mother liquor.

Isolated DHMF: 87% yield, 98% purity

Isolated HMFA salt: 83% yield, 95% purity

The diol DHMF can be further purified by washing with ether/hexane and hexane to remove any non-polar impurities retained after the recrystallization process.

2,5-dihydroxymethylfuran



Off-white solid isolated by initial purification by recrystallization and further cleansing by washing with diethylether;

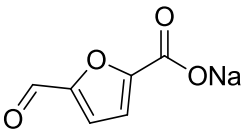
Mp 76 ºC (lit. mp 76– 78 ºC)

^{1}H NMR(400MHz, D_2O): δ 6.22 (s, 2H), 4.42 (s, 4H);

^{13}C NMR(400MHz, D_2O): δ 153.6, 108.9, 55.8.

ESI(+) MS: m/z 127.89; 111.04 ($C_6H_7O_2$).

5-hydroxymethyl-2-furancarboxylic acid sodium salt.



Brown hygroscopic solid purified by recrystallization in ethanol/ethyl acetate

^{1}H NMR(400MHz, D_2O): δ 6.81(d, J = 3.3Hz, 1H), 6.33 (d, J = 3.3Hz,1H), 4.45 (s, 2H);

^{13}C NMR(400MHz, D_2O): δ 166.3, 155.8, 149.0, 115.4, 109.9, 55.7;

ESI (-) MS: m/z 140.89.

3.7.2 Procedure for the Cannizaro reaction under sovent free conditions.

HMF 6.5g, NaOH(0.55eq) 1.13g and 1ml EtOH were stirred for 3 days at RT. The reaction was not completed by TLC. Then additional 0.13g NaOH were added and stirred for one more day. Although full conversion of HMF was not acheievd by TLC the reaction was worked up by precipitation of the sodium acid salt from EtOH and EtOAc. 2g of HMFA sodium salt were isolated (61% yield). The diol was isolate in a mixture with not converted HMF. After it has been performed NMR it was calculated yield of 1.7g DHMF (51% yield) and 2.3g unreacted HMF (65% conversion).

4. References.

1. Rosatella, A. A.; Simeonov, S. P.; Frade, R. F. M.; Afonso, C. A. M. *Green Chem.*, **2011**, *13*, 754.
2. Saeman, J. F. *Ind. Eng. Chem.*, **1945**, *37*, 43.
3. Dull, G. *Chemiker-Zeitung*, **1895**, 216.
4. Kiermayer, J. *Chemiker-Zeitung*, **1895**, *19*, 1003.
5. Fenton, H. J. H.; Gostling, M. *J. Chem. Soc.*, **1901**, *79*, 807.
6. Fenton, H. J. H.; Robinson, F. *J. Chem. Soc.*, **1909**, *95*, 1334.
7. Reichstein, T. *Helv. Chim. Acta*, **1926**, *9*, 1066.
8. Reichstein, T.; Zschokke, H. *Helv. Chim. Acta*, **1932**, *15*, 249.
9. Haworth, W. N.; Jones, W. G. M. *J. Chem. Soc.*, **1944**, 667.
10. Newth, F. H. *Adv. Carbohydr. Chem.*, **1951**, *6*, 83.
11. Moye, C. J.; Krzeminski, Z. S. *Aust. J. Chem.*, **1963**, *16*, 258.
12. Feather, M. S.; Harris, J. F. In *Adv. Carbohydr. Chem. Biochem.*; Academic Press: **1973**; 28, 161.
13. Gaset, A.; Gorrichon, J. P.; Truchot, E. *Inf. Chim.* , **1981**, *212*, 179.
14. Faury, A.; Gaset, A.; Gorrichon, J. P. *Inf. Chim.*, **1981**, *214*, 203.
15. Kuster, B. F. M. *Starch-Starke*, **1990**, *42*, 314.
16. Cottier, L.; Descotes, G. *Trend. Heterocycl. Chem.*, **1991**, *2*, 233.
17. Lewkowski, J. *Arkivoc*, **2001**, *2*, 17.
18. Moreau, C.; Belgacem, M. N.; Gandini, A. *Top. Catal.*, **2004**, *27*, 11.
19. Corma, A.; Iborra, S.; Velty, A. *Chem. Rev.*, **2007**, *107*, 2411.
20. Boisen, A.; Christensen, T. B.; Fu, W.; Gorbanev, Y. Y.; Hansen, T. S.; Jensen, J. S.; Klitgaard, S. K.; Pedersen, S.; Riisager, A.; Stahlberg, T.; Woodley, J. M. *Chem. Eng. Res. Des.*, **2009**, *87*, 1318.
21. Zhang, Y. G.; Chan, J. Y. G. *Energy Environ. Sci.*, **2010**, *3*, 408.
22. Search made in ISI-Web of Knowledge by topic with 5-hydroxymethylfurfural
23. Zakrzewska, M. E.; Bogel-Łukasik, E.; Bogel-Łukasik, R. *Chem. Rev.*, **2010**, *111*, 397.
24. Takagaki, A.; Nishimura, S.; Ebitani, K. *Catal. Surv. Asia*, **2012**, *16*, 164.

25. Dutta, S.; De, S.; Saha, B. *ChemPlusChem*, **2012**, *77*, 259.
26. van Putten, R.-J.; van der Waal, J. C.; de Jong, E.; Rasrendra, C. B.; Heeres, H. J.; de Vries, J. G. *Chem. Rev.*, **2013**, *113*, 1499.
27. Saha, B.; Abu-Omar, M. M. *Green Chem.*, **2014**, *16*, 24.
28. Kroh, L. W. *Food Chem.*, **1994**, *51*, 341.
29. Erbersdobler, H. F.; Hupe, A. *Z Ernahrungswiss*, **1991**, *30*, 46.
30. Berg, H. E.; Van Boekel, M. A. J. S. *Neth Milk Dairy Journal*, **1994**, *48*, 157.
31. Martin, C.; Jonsson, L. J. *Enzyme Microb. Technol.*, **2003**, *32*, 386.
32. Morales, F. J.; Romero, C.; Jiménez-Pérez, S. *J. Agric. Food Chem.*, **1997**, *45*, 1570.
33. Ramirez-Jimenez, A.; Guerra-Hernandez, E.; Garcia-Villanova, B. *J. Agric. Food Chem.*, **2000**, *48*, 4176.
34. Ameur, L. A.; Trystram, G.; Birlouez-aragon, I. *Food Chem.*, **2005**, *98*, 790.
35. Fernandez-Artigas, P.; Guerra-Hernandez, E.; Garcia-Villanova, B. *J. Agric. Food Chem.*, **1999**, *47*, 2872.
36. Ramírez-Jiménez, A.; García-Villanova, B.; Guerra-Hernandez, E. *Food Res. Int.*, **2000**, *33*, 833.
37. Ramirez-Jimenez, A.; García-Villanova, B.; Guerra-Hernandez, E. *J. Sci. Food Agric.*, **2001**, *81*, 513.
38. Rufián-Henares, J. A.; Delgado-Andrade, C.; Morales, F. J. *J. Cereal Sci.*, **2006**, *43*, 63.
39. Fallico, B.; Arena, E.; Zappalà, M. *Food Chem.*, **2003**, *81*, 569.
40. Ait Ameur, L.; Mathieu, O.; Lalanne, V.; Trystram, G.; Birlouez-Aragon, I. *Food Chem.*, **2007**, *101*, 1407.
41. Anese, M.; Suman, M. *Food Res. Int.*, **2013**, *51*, 257.
42. Kirit, A. B.; Erdogdu, F.; Ozdemir, Y. *J. Food Process. Eng.*, **2013**, *36*, 241.
43. Petisca, C.; Henriques, A. R.; Pérez-Palacios, T.; Pinho, O.; Ferreira, I. M. P. L. V. O. *Food Chem.*, **2013**, *141*, 3349.
44. Petisca, C.; Henriques, A. R.; Pérez-Palacios, T.; Pinho, O.; Ferreira, I. M. P. L. V. O. *J. Food Comp. Anal.*, **2014**, *33*, 20.
45. Van Der Fels-Klerx, H. J.; Capuano, E.; Nguyen, H. T.; Ataç Mogol, B.; Kocadağlı, T.; Göncüoğlu Taş, N.; Hamzalıoğlu, A.; Van Boekel, M. A. J. S.; Gökmen, V. *Food Res. Int.*, **2014**, *57*, 210.
46. Taherzadeh, M. J.; Gustafsson, L.; Niklasson, C.; Liden, G. *Appl. Microbiol. Biotechnol.*, **2000**, *53*, 701.
47. Wahlbom, C. F.; Hahn-Hagerdal, B. *Biotechnol. Bioeng.*, **2001**, *78*, 172.
48. Liu, Z. L.; Slininger, P. J.; Dien, B. S.; Berhow, M. A.; Kurtzman, C. P.; Gorsich, S. W. *J. Ind. Microbiol. Biotechnol.*, **2004**, *31*, 345.
49. Hu, C.; Zhao, X.; Zhao, J.; Wu, S.; Zhao, Z. K. *Bioresour. Technol.*, **2009**, *100*, 4843.
50. Slininger, P. J.; Gorsich, S. W.; Liu, Z. L. *Biotechnol. Bioeng.*, **2009**, *102*, 778.
51. Zaldivar, J.; Martinez, A.; Ingram, L. O. *Biotechnol. Bioeng.*, **1999**, *65*, 24.
52. Chen, X.; Li, Z. H.; Zhang, X. X.; Hu, F. X.; Ryu, D. D. Y.; Bao, J. *Appl. Biochem. Biotechnol.*, **2009**, *159*, 591.
53. Murkovic, M.; Pichler, N. *Mol. Nutr. Food Res.*, **2006**, *50*, 842.
54. Prior, R. L.; Wu, X.; Gu, L. *J. Agric. Food Chem.*, **2006**, *54*, 3744.
55. Husoy, T.; Haugen, M.; Murkovic, M.; Jobstl, D.; Stolen, L. H.; Bjellaas, T.; Ronningborg, C.; Glatt, H.; Alexander, J. *Food Chem. Toxicol.*, **2008**, *46*, 3697.
56. Hryncewicz, C. L.; Koberda, M.; Konkowski, M. S. *J. Pharm. Biomed. Anal.*, **1996**, *14*, 429.
57. Jellum, E.; Borresen, H. C.; Eldjarn, L. *Clin. Chim. Acta*, **1973**, *47*, 191.
58. Wieslander, A. P.; Andren, A. H.; Nilsson-Thorell, C.; Muscalu, N.; Kjellstrand, P. T.; Rippe, B. *Perit. Dial. Int.*, **1995**, *15*, 348.
59. Janzowski, C.; Glaab, V.; Samimi, E.; Schlatter, J.; Eisenbrand, G. *Food Chem. Toxicol.*, **2000**, *38*, 801.
60. Ulbricht, R. J.; Northup, S. J.; Thomas, J. A. *Fundam. Appl. Toxicol.*, **1984**, *4*, 843.
61. Severin, I.; Dumont, C.; Jondeau-Cabaton, A.; Graillot, V.; Chagnon, M. C. *Toxicol. Lett.*, **2010**, *192*, 189.
62. Wagner, K. H.; Reichhold, S.; Koschutnig, K.; Cheriot, S.; Billaud, C. *Mol. Nutr. Food Res.*, **2007**, *51*, 496.
63. Brands, C. M.; Alink, G. M.; van Boekel, M. A.; Jongen, W. M. *J. Agric. Food Chem.*, **2000**, *48*, 2271.
64. Ding, X.; Wang, M. Y.; Yao, Y. X.; Li, G. Y.; Cai, B. C. *J. Ethnopharmacol.*, **2010**, *128*, 373.
65. Lin, A. S.; Qian, K.; Usami, Y.; Lin, L.; Itokawa, H.; Hsu, C.; Morris-Natschke, S. L.; Lee, K. H. *J. Nat. Med.*, **2008**, *62*, 164.
66. Abdulmalik, O.; Safo, M. K.; Chen, Q.; Yang, J.; Brugnara, C.; Ohene-Frempong, K.; Abraham, D. J.; Asakura, T. *Br. J. Haematol.*, **2005**, *128*, 552.

67. Durling, L. J.; Busk, L.; Hellman, B. E. *Food Chem. Toxicol.*, **2009**, *47*, 880.
68. Glatt, H.; Schneider, H.; Liu, Y. *Mutat. Res.*, **2005**, *580*, 41.
69. Khondkar, P.; Rahman, M. M.; Islam, A. *Phytother. Res.*, **2005**, *19*, 816.
70. Zhang, X. M.; Chan, C. C.; Stamp, D.; Minkin, S.; Archer, M. C.; Bruce, W. R. *Carcinogenesis*, **1993**, *14*, 773.
71. Surh, Y. J.; Liem, A.; Miller, J. A.; Tannenbaum, S. R. *Carcinogenesis*, **1994**, *15*, 2375.
72. Teubner, W.; Meinl, W.; Florian, S.; Kretzschmar, M.; Glatt, H. *Biochem. J.*, **2007**, *404*, 207.
73. Lee, Y. C.; Shlyankevich, M.; Jeong, H. K.; Douglas, J. S.; Surh, Y. J. *Biochem. Biophys. Res. Commun.*, **1995**, *209*, 996.
74. Monien, B. H.; Frank, H.; Seidel, A.; Glattt, H. *Chem. Res. Toxicol.*, **2009**, *22*, 1123.
75. Bakhiya, N.; Monien, B.; Frank, H.; Seidel, A.; Glatt, H. *Biochem. Pharmacol.*, **2009**, *78*, 414.
76. Godfrey, V. B.; Chen, L. J.; Griffin, R. J.; Lebetkin, E. H.; Burka, L. T. *J Toxicol Environ Health A*, **1999**, *57*, 199.
77. Svendsen, C.; Husoy, T.; Glatt, H.; Paulsen, J. E.; Alexander, J. *Anticancer Res.*, **2009**, *29*, 1921.
78. Antal, M. J.; Mok, W. S. L.; Richards, G. N. *Carbohydr. Res.*, **1990**, *199*, 91.
79. Caratzoulas, S.; Vlachos, D. G. *Carbohydr. Res.*, **2011**, *346*, 664.
80. Vandam, H. E.; Kieboom, A. P. G.; Vanbekkum, H. *Starch-Starke*, **1986**, *38*, 95.
81. Cukalovic, A.; Stevens, C. V. *Green Chem.*, **2010**, *12*, 1201.
82. Moreau, C.; Durand, R.; Razigade, S.; Duhamet, J.; Faugeras, P.; Rivalier, P.; Ros, P.; Avignon, G. *Appl. Catal., A*, **1996**, *145*, 211.
83. Lima, S.; Neves, P.; Antunes, M. M.; Pillinger, M.; Ignatyev, N.; Valente, A. A. *Appl. Catal., A*, **2009**, *363*, 93.
84. Roman-Leshkov, Y.; Barrett, C. J.; Liu, Z. Y.; Dumesic, J. A. *Nature*, **2007**, *447*, 982.
85. Chheda, J. N.; Roman-Leshkov, Y.; Dumesic, J. A. *Green Chem.*, **2007**, *9*, 342.
86. Roman-Leshkov, Y.; Chheda, J. N.; Dumesic, J. A. *Science*, **2006**, *312*, 1933.
87. Benvenuti, F.; Carlini, C.; Patrono, P.; Galletti, A. M. R.; Sbrana, G.; Massucci, M. A.; Galli, P. *Appl. Catal., A*, **2000**, *193*, 147.
88. Hu, S. Q.; Zhang, Z. F.; Zhou, Y. X.; Han, B. X.; Fan, H. L.; Li, W. J.; Song, J. L.; Xie, Y. *Green Chem.*, **2008**, *10*, 1280.
89. Hu, S. Q.; Zhang, Z. F.; Song, J. L.; Zhou, Y. X.; Han, B. X. *Green Chem.*, **2009**, *11*, 1746.
90. Hu, S. Q.; Zhang, Z. F.; Zhou, Y. X.; Song, J. L.; Fan, H. L.; Han, B. X. *Green Chem.*, **2009**, *11*, 873.
91. Chidambaram, M.; Bell, A. *Green Chem.*, **2010**, *12*, 1253.
92. Chan, J. Y. G.; Zhang, Y. G. *Chemsuschem*, **2009**, *2*, 731.
93. Yan, H. P.; Yang, Y.; Tong, D. M.; Xiang, X.; Hu, C. W. *Catal. Commun.*, **2009**, *10*, 1558.
94. Zhang, Y. T.; Du, H. B.; Qian, X. H.; Chen, E. Y. X. *Energy Fuels*, **2010**, *24*, 2410.
95. Musau, R. M.; Munavu, R. M. *Biomass*, **1987**, *13*, 67.
96. Ribeiro, M. L.; Schuchardt, U. *Catal. Commun.*, **2003**, *4*, 83.
97. Qi, X. H.; Watanabe, M.; Aida, T. M.; Smith, R. L. *Ind. Eng. Chem. Res.*, **2008**, *47*, 9234.
98. Takagaki, A.; Ohara, M.; Nishimura, S.; Ebitani, K. *Chem. Commun.*, **2009**, 6276.
99. Brown, D. W.; Floyd, A. J.; Kinsman, R. G.; Roshanali, Y. *J. Chem. Technol. Biotechnol.*, **1982**, *32*, 920.
100. Rajabbeigi, N.; Ranjan, R.; Tsapatsis, M. *Microporous Mesoporous Mater.*, **2012**, *158*, 253.
101. Moreau, C.; Finiels, A.; Vanoye, L. *J. Mol. Catal. A: Chem.*, **2006**, *253*, 165.
102. Kuster, B. F. M.; Laurens, J. *Starke*, **1977**, *29*, 172.
103. Chen, J. D.; Kuster, B. F. M.; Vanderwiele, K. *Biomass & Bioenergy*, **1991**, *1*, 217.
104. Asghari, F. S.; Yoshida, H. *Ind. Eng. Chem. Res.*, **2006**, *45*, 2163.
105. Bicker, M.; Hirth, J.; Vogel, H. *Green Chem.*, **2003**, *5*, 280.
106. Bicker, M.; Kaiser, D.; Ott, L.; Vogel, H. *J. Supercrit. Fluids*, **2005**, *36*, 118.
107. Hansen, T. S.; Woodley, J. M.; Riisager, A. *Carbohydr. Res.*, **2009**, *344*, 2568.
108. Roman-Leshkov, Y.; Dumesic, J. A. *Top. Catal.*, **2009**, *52*, 297.
109. Binder, J. B.; Raines, R. T. *J. Am. Chem. Soc.*, **2009**, *131*, 1979.
110. Sievers, C.; Musin, I.; Marzialetti, T.; Olarte, M. B. V.; Agrawal, P. K.; Jones, C. W. *Chemsuschem*, **2009**, *2*, 665.

111. Qi, X. H.; Watanabe, M.; Aida, T. M.; Smith, R. L. *Chemsuschem*, **2009**, *2*, 944.
112. Qi, X. H.; Watanabe, M.; Aida, T. M.; Smith, R. L. *Green Chem.*, **2009**, *11*, 1327.
113. Roberge, D. M.; Ducry, L.; Bieler, N.; Cretton, P.; Zimmermann, B. *Chem. Eng. Technol.*, **2005**, *28*, 318.
114. Tuercke, T.; Panic, S.; Loebbecke, S. *Chem. Eng. Technol.*, **2009**, *32*, 1815.
115. Ilgen, F.; Ott, D.; Kralisch, D.; Reil, C.; Palmberger, A.; Konig, B. *Green Chem.*, **2009**, *11*, 1948.
116. Wu, S.; Fan, H.; Xie, Y.; Cheng, Y.; Wang, Q.; Zhang, Z.; Han, B. *Green Chem.*, **2010**, *12*, 1215.
117. Zhao, H. B.; Holladay, J. E.; Brown, H.; Zhang, Z. C. *Science*, **2007**, *316*, 1597.
118. Huang, R. L.; Qi, W.; Su, R. X.; He, Z. M. *Chem. Commun.*, **2010**, *46*, 1115.
119. Chernyak, M. Y.; Smirnova, M. A.; Tarabanko, V. E.; RU2363698-C1, 2009: 2009.
120. Okano, T.; Qiao, K.; Bao, Q.; Tomida, D.; Hagiwara, H.; Yokoyama, C. *Appl. Catal., A*, **2013**, *451*, 1.
121. Hu, L.; Sun, Y.; Lin, L.; Liu, S. *Biomass and Bioenergy*, **2012**, *47*, 289.
122. Liu, F.; Barrault, J.; De Oliveira Vigier, K.; Jérôme, F. *ChemSusChem*, **2012**, *5*, 1223.
123. Shi, N.; Liu, Q.; Zhang, Q.; Wang, T.; Ma, L. *Green Chem.*, **2013**, *15*, 1967.
124. Vinke, P.; Vanbekkum, H. *Starch-Starke*, **1992**, *44*, 90.
125. Lansalot-Matras, C.; Moreau, C. *Catal. Commun.*, **2003**, *4*, 517.
126. Halliday, G. A.; Young, R. J.; Grushin, V. V. *Org. Lett.*, **2003**, *5*, 2003.
127. Shimizu, K.; Uozumi, R.; Satsuma, A. *Catal. Commun.*, **2009**, *10*, 1849.
128. Chheda, J. N.; Dumesic, J. A. *Catal. Today*, **2007**, *123*, 59.
129. Qi, X. H.; Watanabe, M.; Aida, T. M.; Smith, R. L. *Green Chem.*, **2008**, *10*, 799.
130. Jeong, J.; Antonyraj, C. A.; Shin, S.; Kim, S.; Kim, B.; Lee, K.-Y.; Cho, J. K. *J. Ind. Eng. Chem.*, **2013**, *19*, 1106.
131. Lucas, N.; Kokate, G.; Nagpure, A.; Chilukuri, S. *Microporous Mesoporous Mater.*, **2013**, *181*, 38.
132. Aellig, C.; Hermans, I. *ChemSusChem*, **2012**, *5*, 1737.
133. Liu, R.; Chen, J.; Huang, X.; Chen, L.; Ma, L.; Li, X. *Green Chem.*, **2013**, *15*, 2895.
134. Amarasekara, A. S.; Williams, L. D.; Ebede, C. C. *Carbohydr. Res.*, **2008**, *343*, 3021.
135. Bao, Q. X.; Qiao, K.; Tomida, D.; Yokoyama, C. *Catal. Commun.*, **2008**, *9*, 1383.
136. Fayet, C.; Gelas, J. *Carbohydr. Res.*, **1983**, *122*, 59.
137. Zhao, H.; Holladay, J. E.; Zhang, Z. C.; WO2008019219-A1: 2008.
138. Yong, G.; Zhang, Y. G.; Ying, J. Y. *Angew. Chem., Int. Ed.*, **2008**, *47*, 9345.
139. Li, C. Z.; Zhang, Z. H.; Zhao, Z. B. K. *Tetrahedron Lett.*, **2009**, *50*, 5403.
140. Zhang, Z. H.; Zhao, Z. B. K. *Bioresour. Technol.*, **2010**, *101*, 1111.
141. Yu, S.; Brown, H. M.; Huang, X. W.; Zhou, X. D.; Amonette, J. E.; Zhang, Z. C. *Appl. Catal., A*, **2009**, *361*, 117.
142. Chun, J. A.; Lee, J. W.; Yi, Y. B.; Hong, S. S.; Chung, C. H. *Korean J. Chem. Eng.*, **2010**, *27*, 930.
143. Pidko, E. A.; Degirmenci, V.; van Santen, R. A.; Hensen, E. J. M. *Angew. Chem., Int. Ed.*, **2010**, *49*, 2530.
144. Dunn, E. F.; Liu, D.; Chen, E. Y. X. *Appl. Catal., A*, **2013**, *460–461*, 1.
145. Hai Yan Zhang, X. J. L., Ning Ai, Yong Nie, Jian Bing Ji *Appl. Mech. Mater.*, **2013**, *316-317*, 157.
146. Hu, L.; Sun, Y.; Lin, L. *Ind. Eng. Chem. Res.*, **2011**, *51*, 1099.
147. Ding, Z.-D.; Shi, J.-C.; Xiao, J.-J.; Gu, W.-X.; Zheng, C.-G.; Wang, H.-J. *Carbohydr. Polym.*, **2012**, *90*, 792.
148. Watanabe, M.; Aizawa, Y.; Iida, T.; Aida, T. M.; Levy, C.; Sue, K.; Inomata, H. *Carbohydr. Res.*, **2005**, *340*, 1925.
149. Qi, X. H.; Watanabe, M.; Aida, T. M.; Smith, R. L. *Catal. Commun.*, **2008**, *9*, 2244.
150. Qi, X. H.; Watanabe, M.; Aida, T. M.; Smith, R. L. *Catal. Commun.*, **2009**, *10*, 1771.
151. Chareonlimkun, A.; Champreda, V.; Shotipruk, A.; Laosiripojana, N. *Bioresour. Technol.*, **2010**, *101*, 4179.
152. Asghari, F. S.; Yoshida, H. *Carbohydr. Res.*, **2006**, *341*, 2379.
153. Ishida, H.; Seri, K.-i. *J. Mol. Catal. A: Chem.*, **1996**, *112*, L163.
154. Seri, K.; Inoue, Y.; Ishida, H. *Bull. Chem. Soc. Jpn.*, **2001**, *74*, 1145.
155. Stahlberg, T.; Sorensen, M. G.; Riisager, A. *Green Chem.*, **2010**, *12*, 321.
156. Utami, S. P.; Amin, N. S. *Ind. Crop. Prod.*, **2013**, *41*, 64.
157. Carlini, C.; Giuttari, M.; Galletti, A. M. R.; Sbrana, G.; Armaroli, T.; Busca, G. *Appl. Catal., A*, **1999**, *183*, 295.

158. Armaroli, T.; Busca, G.; Carlini, C.; Giuttari, M.; Galletti, A. M. R.; Sbrana, G. *J. Mol. Catal. A: Chem.*, **2000**, *151*, 233.
159. Hara, M.; Nakajima, K.; Yamashita, S.; JP2009215172-A, 2009: 2009.
160. Carlini, C.; Patrono, P.; Galletti, A. M. R.; Sbrana, G. *Appl. Catal., A*, **2004**, *275*, 111.
161. Tian, G.; Tong, X.; Cheng, Y.; Xue, S. *Carbohydr. Res.*, **2013**, *370*, 33.
162. Zhang, Z.; Liu, B.; Zhao, Z. *Carbohydr. Polym.*, **2012**, *88*, 891.
163. Jiménez-Morales, I.; Teckchandani-Ortiz, A.; Santamaría-González, J.; Maireles-Torres, P.; Jiménez-López, A. *Appl. Catal., B*, **2014**, *144*, 22.
164. Kim, Y.-H.; Shin, S.; Yoon, H.-J.; Kim, J. W.; Cho, J. K.; Lee, Y.-S. *Catal. Commun.*, **2013**, *40*, 18.
165. Ren, Q., Huang, Y., Ma, H., Wang, F., Gao, J., and Xu, J. *BioRes.*, **2013**, *8*, 1563.
166. Gallo, J. M. R.; Alonso, D. M.; Mellmer, M. A.; Dumesic, J. A. *Green Chem.*, **2013**, *15*, 85.
167. Liu, F.; Audemar, M.; De Oliveira Vigier, K.; Cartigny, D.; Clacens, J.-M.; Costa Gomes, M. F.; Padua, A. A. H.; De Campo, F.; Jerome, F. *Green Chem.*, **2013**, *15*, 3205.
168. Li, H.; Zhang, Q.; Liu, X.; Chang, F.; Hu, D.; Zhang, Y.; Xue, W.; Yang, S. *RSC Adv.*, **2013**, *3*, 3648.
169. Werpy, T.; Petersen, G. *Top Value Added Chemicals from Biomass*, **2004**, *1*, 26.
170. El-Hajj, T.; Masroua, A.; Martin, J. C.; Descotes, G. *Bull. Soc. Chim. Fr.*, **1987**, 855.
171. Lew, B. W. In *US Pat., 3326944, Chem. Abstr. 1968, 68, P49434n.*
172. Gorbanev, Y. Y.; Klitgaard, S. K.; Woodley, J. M.; Christensen, C. H.; Riisager, A. *Chemsuschem*, **2009**, *2*, 672.
173. Casanova, O.; Iborra, S.; Corma, A. *Chemsuschem*, **2009**, *2*, 1138.
174. S. E.Davis; L. R.Houkb; E. C.Tamargoa; A. K.Datyeb; J.Davis, R. *Catal. Today*, **2011**, 55.
175. van Deurzen, M. P. J.; van Rantwijk, F.; Sheldon, R. A. *J. Carbohydr. Chem.*, **1997**, *16*, 299.
176. Gandini, A.; Belgacem, M. N. *Prog. Polym. Sci.*, **1997**, *22*, 1203.
177. Cottier, L.; Descotes, G.; Lewkowski, J.; Skowronski, R.; Viollet, E. *J. Heterocyclic. Chem.*, **1995**, *32*, 927.
178. Van Reijendam, J. W.; Heeres, G. J.; Janssen, M. J. *Tetrahedron*, **1970**, *26*, 1291.
179. Morikawa, S. *Noguchi Kenkyusho Jiho, 1978, **21**, 25, Chem. Abstr. 1979, 90, 103740d.*
180. Clennan, E. L.; Mehrsheikh-Mohammadi, M. E. *J. Am. Chem. Soc.*, **1984**, *106*, 7112.
181. Morikawa, S. *Noguchi Kenkyusho Jiho, 1979, **22**, 20, Chem. Abstr. 1980, 92, 198181a, .*
182. Elhajj, T.; Masroua, A.; Martin, J. C.; Descotes, G. *Bull. Soc. Chim. Fr.*, **1987**, 855.
183. Cottier, L.; Descotes, G.; Lewkowski, J.; Skowronski, R. *Org. Prep. Proced. Int.*, **1995**, *27*, 564.
184. Cottier, L.; Descotes, G.; Lewkowski, J.; Skowronski, R. *Pol. J. Chem.*, **1994**, *68*, 693.
185. McDermott, P. J.; Stockman, R. A. *Org. Lett.*, **2005**, *7*, 27.
186. Mehdi, H.; Bodor, A.; Lantos, D.; Horvath, I. T.; De Vos, D. E.; Binnemans, K. *J. Org. Chem.*, **2007**, *72*, 517.
187. vanDeurzen, M. P. J.; vanRantwijk, F.; Sheldon, R. A. *J. Carbohydr. Chem.*, **1997**, *16*, 299.
188. Davies, R.; Hedebrant, U.; Athanassiadis, I.; Rydberg, P.; Tornqvist, M. *Food Chem. Toxicol.*, **2009**, *47*, 1950.
189. Partenheimer, W.; Grushin, V. V. *Adv. Synth. Catal.*, **2001**, *343*, 102.
190. Carlini, C.; Patrono, P.; Galletti, A. M. R.; Sbrana, G.; Zima, V. *Appl. Catal. a-Gen.*, **2005**, *289*, 197.
191. Amarasekara, A. S.; Green, D.; McMillan, E. *Catal. Commun.*, **2008**, *9*, 286.
192. Navarro, O. C.; Canos, A. C.; Chornet, S. I. *Top. Catal.*, **2009**, *52*, 304.
193. Lilga, M. A.; Hallen, R. T.; Hu, J.; White, J. F.; Gray, M. J. In *US patent, US2008103318-A1* 2007; Vol. US2008103318-A1.
194. Lilga, M. A.; Hallen, R. T.; Gray, M. *Top. Catal.*, **2010**, *53*, 1264.
195. Hansen, T. S.; Sádaba, I.; García-Suárez, E. J.; Riisager, A. *Appl. Catal., A*, **2013**, *456*, 44.
196. Le, N.-T.; Lakshmanan, P.; Cho, K.; Han, Y.; Kim, H. *Appl. Catal., A*, **2013**, *464–465*, 305.
197. Antonyraj, C. A.; Jeong, J.; Kim, B.; Shin, S.; Kim, S.; Lee, K.-Y.; Cho, J. K. *J. Ind. Eng. Chem.*, **2013**, *19*, 1056.
198. Grasset, F. L.; Katryniok, B.; Paul, S.; Nardello-Rataj, V.; Pera-Titus, M.; Clacens, J.-M.; De Campo, F.; Dumeignil, F. *RSC Adv.*, **2013**, *3*, 9942.
199. Skowronski, R.; Cottier, L.; Descotes, G.; Lewkowski, J. *Synthesis*, **1996**, 1291.
200. Cottier, L.; Descotes, G.; Lewkowski, J. *Synthetic. Commun.*, **1994**, *24*, 939.

201. P.Vinke; W. van der Poel; Bekkum, H. v. *Stud. Surf. Sci. Catal*, **1991**, *59*, 385.
202. Saha, B.; Gupta, D.; Abu-Omar, M. M.; Modak, A.; Bhaumik, A. *J. Catal.*, **2013**, *299*, 316.
203. Albonetti, S.; Pasini, T.; Lolli, A.; Blosi, M.; Piccinini, M.; Dimitratos, N.; Lopez-Sanchez, J. A.; Morgan, D. J.; Carley, A. F.; Hutchings, G. J.; Cavani, F. *Catal. Today*, **2012**, *195*, 120.
204. Villa, A.; Schiavoni, M.; Campisi, S.; Veith, G. M.; Prati, L. *ChemSusChem*, **2013**, *6*, 609.
205. Cai, J.; Ma, H.; Zhang, J.; Song, Q.; Du, Z.; Huang, Y.; Xu, J. *Chem. Eur. J.*, **2013**, *19*, 14215.
206. Davis, S. E.; Zope, B. N.; Davis, R. J. *Green Chem.*, **2012**, *14*, 143.
207. Ait Rass, H.; Essayem, N.; Besson, M. *Green Chem.*, **2013**, *15*, 2240.
208. Taarning, E.; Nielsen, I. S.; Egeblad, K.; Madsen, R.; Christensen, C. H. *Chemsuschem*, **2008**, *1*, 75.
209. Casanova, O.; Iborra, S.; Corma, A. *J. Catal.*, **2009**, *265*, 109.
210. Cottier, L.; Descotes, G.; Nigay, H.; Parron, J. C.; Gregoire, V. *Bull. Soc. Chim. Fr.*, **1986**, 844.
211. Alibes, R.; Font, J.; Mula, A.; Ortuno, R. M. *Synthetic. Commun.*, **1990**, *20*, 2607.
212. Cottier, L.; Descotes, G.; Eymard, L.; Rapp, K. *Synthesis*, **1995**, 303.
213. Marisa, C.; Ilaria, D.; Marotta, R.; Roberto, A.; Vincenzo, C. *J. Photoch. Photobio. A*, **2010**, *210*, 69.
214. Cottier, L.; Descotes, G. R.; Soro, Y. *Synthetic. Commun.*, **2003**, *33*, 4285.
215. Moye, C. J. *Rev. Pure. Appl. Chem.*, **1964**, *14*, 161.
216. Timko, J. M.; Cram, D. J. *J. Am. Chem. Soc.*, **1974**, *96*, 7159.
217. Goswami, S.; Dey, S.; Jana, S. *Tetrahedron*, **2008**, *64*, 6358.
218. Turner, J. H.; Rebers, P. A.; Barrick, P. L.; Cotton, R. H. *Anal. Chem.*, **1954**, *26*, 898.
219. Schiavo, V.; Descotes, G.; Mentech, J. *Bull. Soc. Chim. Fr.*, **1991**, 704.
220. Haworth, W. N.; Jones, W. G. M.; Wiggins, L. F. *J. Chem. Soc.*, **1945**, 1.
221. Cope, A. C.; Baxter, W. N. *J. Am. Chem. Soc.*, **1955**, *77*, 393.
222. Alamillo, R.; Tucker, M.; Chia, M.; Pagan-Torres, Y.; Dumesic, J. *Green Chem.*, **2012**, *14*, 1413.
223. Gerard C. A. Luijkx, N. P. M. H., Gred van Rantwijk, Leendert Maat, Herman van Bekkum *Heterocycles*, **2009**, *77*, 1037.
224. Zu, Y.; Yang, P.; Wang, J.; Liu, X.; Ren, J.; Lu, G.; Wang, Y. *Appl. Catal., B*, **2014**, *146*, 244.
225. Hansen, T. S.; Barta, K.; Anastas, P. T.; Ford, P. C.; Riisager, A. *Green Chem.*, **2012**, *14*, 2457.
226. Villard, R.; Robert, F.; Blank, I.; Bernardinelli, G.; Soldo, T.; Hofmann, T. *J. Agr. Food. Chem.*, **2003**, *51*, 4040.
227. Elming, N.; Clausonkaas, N. *Acta Chem. Scand.*, **1956**, *10*, 1603.
228. Muller, C.; Diehl, V.; Lichtenthaler, F. W. *Tetrahedron*, **1998**, *54*, 10703.
229. Kojiri, K.; Kondo, H.; Arakawa, H.; Ohkubo, M.; Suda, H. *US pat.,US6703373-B1*.
230. Sun, S. G.; Murray, W. V. *J. Org. Chem.*, **1999**, *64*, 5941.
231. Fumagalli, T.; Sello, G.; Orsini, F. *Synthetic. Commun.*, **2009**, *39*, 2178.
232. Mouloungui, Z.; Delmas, M.; Gaset, A. *Synthetic. Commun.*, **1984**, *14*, 701.
233. Lasseuguette, E.; Gandini, A.; Belgacem, M. N.; Timpe, H.-J. *Polymer*, **2005**, *46*, 5476.
234. Goodman, S. N.; Jacobsen, E. N. *Adv. Synth. Catal.*, **2002**, *344*, 953.
235. Almirante, N.; Cerri, A.; Fedrizzi, G.; Marazzi, G.; Santagostino, M. *Tetrahedron Lett.*, **1998**, *39*, 3287.
236. Yu, C. Z.; Liu, B.; Hu, L. Q. *J. Org. Chem.*, **2001**, *66*, 5413.
237. Yu, C.; Hu, L. *J. Org. Chem.*, **2001**, *67*, 219.
238. Cottier, L.; Descotes, G.; Soro, Y. *J. Carbohyd. Chem.*, **2005**, *24*, 55.
239. Terada, I.; Takeda, T.; Kobayashi, T.; Hiramoto, T.; Tsuyoshi, K.; WO2008044784-A1: 2008.
240. Urashima, T.; Suyama, K.; Adachi, S. *Carbohydr. Res.*, **1985**, *135*, 324.
241. Dumesis, J. A.; Huber, G. W.; Chheda, J. N.; Barrett, C. J.; Dumesic, J. A.; WO2007103858-A2, p 60.
242. Skowronski, R.; Grabowski, G.; Lewkowski, J.; Descotes, G.; Cottier, L.; Neyret, C. *Org. Prep. Proced. Int.*, **1993**, *25*, 353.
243. Quiroz-Florentino, H.; Aguilar, R.; Santoyo, B. M.; Diaz, F.; Tamariz, J. *Synthesis*, **2008**, 1023.
244. Hanefeld, W.; Schlitzer, M.; Debski, N.; Euler, H. *J. Heterocyclic Chem.*, **1996**, *33*, 1143.
245. Shinobu, N.; Shao, J.; Kobayashi, M.; Mori, T.; Masataka, K.; Noriaki, S.; Takao, M.; WO2008056634-A1, p 2130829.
246. Suryawanshi, S. N.; Chandra, N.; Kumar, P.; Porwal, J.; Gupta, S. *Eur. J. Med. Chem.*, **2008**, *43*, 2473.
247. Cummings, R. T.; Dizio, J. P.; Krafft, G. A. *Tetrahedron Lett.*, **1988**, *29*, 69.

248. Lin, L.; Shi, Q.; Nyarko, A. K.; Bastow, K. F.; Wu, C. C.; Su, C. Y.; Shih, C. C. Y.; Lee, K. H. *J. Med. Chem.*, **2006**, *49*, 3963.
249. Muther, A.; Tollens, B. *Ber. Dtsch. Chem. Ges.*, **1904**, *37*, 306.
250. Wahhab, A. *J. Am. Chem. Soc.*, **1948**, *70*, 3580.
251. Volksen, W. *Arch. Pharm. Ber. Dtsch. Pharm. Ges.*, **1954**, *287*, 459.
252. Kato, H. *B. Agr. Chem. Soc. Japan*, **1959**, *23*, 551.
253. Michail, K.; Matzi, V.; Maier, A.; Herwig, R.; Greilberger, J.; Juan, H.; Kunert, O.; Wintersteiger, R. *Anal. Bioanal. Chem.*, **2007**, *387*, 2801.
254. Erdmann, E. *Ber. Dtsch. Chem. Ges.*, **1910**, *43*, 2391.
255. Erdmann, E.; Schaefer, C. *Ber. Dtsch. Chem. Ges*, **1910**, *43*, 2398.
256. Gardner, T. S.; Smith, F. A.; Wenis, E.; Lee, J. *J. Org. Chem.*, **1951**, *16*, 1121.
257. Cooper, W. F.; Nuttall, W. H. *J. Chem. Soc.*, **1912**, *101*, 1074.
258. Kallinich, G. *Arch. Pharm. Ber. Dtsch. Pharm. Ges.*, **1958**, *291/63*, 274.
259. Amarasekara, A. S.; Edigin, O.; Hernandez, W. *Lett. Org. Chem.*, **2007**, *4*, 306.
260. Liu, F.; Stephen, A. G.; Fisher, R. J.; Burke, T. R., Jr. *Bioorg. Med. Chem. Lett.*, **2008**, *18*, 1096.
261. Undheim, K.; Roe, J.; Greibrok.T *Acta Chem. Scand.*, **1969**, *23*, 2501.
262. Sattler, L.; Zerban, F. W.; Clark, G. L.; Chu, C. C. *J. Am. Chem. Soc.*, **1951**, *73*, 5908.
263. Shao, X.; Li, Z.; Qian, X.; Xu, X. *J. Agric. Food. Chem.*, **2009**, *57*, 951.
264. Karaguni, I. M.; Glusenkamp, K. H.; Langerak, A.; Geisen, C.; Ullrich, V.; Winde, G.; Moroy, T.; Muller, O. *Bioorg. Med Chem. Lett.*, **.2002**, *12*, 709.
265. Matasi, J. J.; Caldwell, J. P.; Hao, J.; Neustadt, B.; Arik, L.; Foster, C. J.; Lachowicz, J.; Tulshian, D. B. *Bioorg. Med. Chem. Lett.*, **2005**, *15*, 1333.
266. Miller, R. *Acta Chem. Scand. B*, **1987**, *41*, 208.
267. Baliani, A.; Bueno, G. J.; Stewart, M. L.; Yardley, V.; Brun, R.; Barrett, M. P.; Gilbert, I. H. *J. Med. Chem.*, **2005**, *48*, 5570.
268. Ramonczai, J.; Vargha, L. *J. Am. Chem. Soc.*, **1950**, *72*, 2737.
269. Papadogianakis, G.; Maat, L.; Sheldon, R. A. *J. Chem. Soc., Chem. Commun.*, **1994**, 2659.
270. Sanda, K.; Rigal, L.; Gaset, A. *Carbohydr. Res.*, **1989**, *187*, 15.
271. Sanda, K.; Rigal, L.; Delmas, M.; Gaset, A. *Synthesis*, **1992**, 541.
272. Villain-Guillot, P.; Gualtieri, M.; Bastide, L.; Roquet, F.; Martinez, J.; Amblard, M.; Pugniere, M.; Leonetti, J. P. *J. Med. Chem.*, **2007**, *50*, 4195.
273. Newth, F. H.; Wiggins, L. F. *J. Chem. Soc.*, **1947**, *169*, 396.
274. Jogia, M. K.; Vakamoce, V.; Weavers, R. T. *Aust. J. Chem.*, **1985**, *38*, 1009.
275. Bognar, R.; Herczegh, P.; Zsely, M.; Batta, G. *Carbohydr. Res.*, **1987**, *164*, 465.
276. A. C. Cope Cope, A. C. US Patent, 3079449 1963.
277. Gupta, P.; Singh, S. K.; Pathak, A.; Kundu, B. *Tetrahedron*, **2002**, *58*, 10469.
278. Chundury, D.; Szmant, H. H. *Ind. Eng. Chem. Prod. Res. Dev.*, **1981**, *20*, 158.
279. Oikawa, M.; Ikoma, M.; Sasaki, M. *Tetrahedron Lett.*, **2005**, *46*, 415.
280. Elhajj, T.; Martin, J. C.; Descotes, G. *J. Heterocycl. Chem.*, **1983**, *20*, 233.
281. de Freitas Filho, J. R.; Srivastava, R. M.; Soro, Y.; Cottier, L.; Descotes, G. *J. Carbohydr. Chem.*, **2001**, *20*, 561.
282. Schinzer, D.; Bourguet, E.; Ducki, S. *Chem. Eur. J.*, **2004**, *10*, 3217.
283. Bredereck, H. *Ber. Dtsch. Chem. Ges.*, **1932**, *65*, 1833.
284. Balakrishnan, M.; Sacia, E. R.; Bell, A. T. *Green Chem.*, **2012**, *14*, 1626.
285. Wang, S.; Zhang, Z.; Liu, B.; Li, J. *Catal. Sci. Technol.*, **2013**, *3*, 2104.
286. Lew, C. M.; Rajabbeigi, N.; Tsapatsis, M. *Ind. Eng. Chem. Res.*, **2012**, *51*, 5364.
287. Bing, L.; Zhang, Z.; Deng, K. *Ind. Eng. Chem. Res.*, **2012**, *51*, 15331.
288. Lewis, T. A.; Bayless, L.; Eckman, J. B.; Ellis, J. L.; Grewal, G.; Libertine, L.; Marie Nicolas, J.; Scannell, R. T.; Wels, B. F.; Wenberg, K.; Wypij, D. M. *Bioorg. Med. Chem. Lett.*, **2004**, *14*, 2265.
289. Dow, R. L.; Kelly, R. C.; Schletter, I.; Wierenga, W. *Synth. Commun.*, **1981**, *11*, 43.
290. Iovel, I.; Mertins, K.; Kischel, J.; Zapf, A.; Beller, M. *Angew. Chem., Int. Ed.*, **2005**, *44*, 3913.
291. Cunningham, M.; Doree, C. *Biochem. J.*, **1914**, *8*, 438.

292. Bozell, J. J.; Moens, L.; Elliott, D. C.; Wang, Y.; Neuenscwander, G. G.; Fitzpatrick, S. W.; Bilski, R. J.; Jarnefeld, J. L. *Resources, Conservation and Recycling*, **2000**, *28*, 227.
293. Alonso, D. M.; Bond, J. Q.; Dumesic, J. A. *Green Chem.*, **2010**, *12*, 1493.
294. Horvat, J.; Klaic, B.; Metelko, B.; Sunjic, V. *Tetrahedron Lett.*, **1985**, *26*, 2111.
295. Zhang, J.; Weitz, E. *ACS Catalysis*, **2012**, *2*, 1211.
296. Teunissen, H. P. *Recl. Trav. Chim. Pays-Bas*, **1930**, *49*, 784.
297. Heimlich, K. R.; Martin, A. N. *J. Am. Pharm. Assoc.*, **1960**, *49*, 592.
298. Baugh, K. D.; McCarty, P. L. *Biotechnol. Bioeng.*, **1988**, *31*, 50.
299. Kuster, B. F. M.; S. van der Baan, H. *Carbohydr. Res.*, **1977**, *54*, 165.
300. Girisuta, B.; Janssen, L. P. B. M.; Heeres, H. J. *Green Chem.*, **2006**, *8*, 701.
301. Luijkx, G. C. A.; Vanrantwijk, F.; Vanbekkum, H. *Carbohydr. Res.*, **1993**, *242*, 131.
302. Soldo, T.; Blank, I.; Hofmann, T. *Chem. Senses*, **2003**, *28*, 371.
303. Ottinger, H.; Soldo, T.; Hofmann, T. *J. Agric. Food. Chem.*, **2003**, *51*, 1035.
304. Soldo, T.; Hofmann, T. *J. Agric. Food. Chem.*, **2005**, *53*, 9165.
305. Koch, J.; Pischetsrieder, M.; Polborn, K.; Severin, T. *Carbohydr. Res.*, **1998**, *313*, 117.
306. Pachmayr, O.; Ledl, F.; Severin, T. *Z. Lebensm.-Unters.-Forsch.*, **1986**, *182*, 294.
307. Frank, O.; Ottinger, H.; Hofmann, T. *J. Agric. Food. Chem.*, **2001**, *49*, 231.
308. Timko, J. M.; Cram, D. J. *J Am Chem Soc*, **1974**, *96*, 7159.
309. Waddell, S. T.; Eckert, J. M.; Blizzard, T. A. *Heterocycles*, **1996**, *43*, 2325.
310. Simeonov, S. P.; Coelho, J. A.; Afonso, C. A. *ChemSusChem*, **2012**, *5*, 1388.
311. Simeonov, S. P.; Coelho, J. A.; Afonso, C. A. *ChemSusChem*, **2013**, *6*, 997.
312. Lobry De Bruyn, C. A.; Alberda Van Ekenstein, W. *Recl. Trav. Chim. Pays-Bas*, **1895**, *14*, 203.
313. Angyal, S. In *Glycoscience*; Stütz, A., Ed.; Springer Berlin Heidelberg: **2001**; Vol. 215, p 1.
314. Lima, S.; Dias, A. S.; Lin, Z.; Brandão, P.; Ferreira, P.; Pillinger, M.; Rocha, J.; Calvino-Casilda, V.; Valente, A. A. *Appl. Catal., A*, **2008**, *339*, 21.
315. Moliner, M.; Román-Leshkov, Y.; Davis, M. E. *Proc. Natl. Acad. Sci. U.S.A.*, **2010**, *107*, 6164.
316. Saravanamurugan, S.; Paniagua, M.; Melero, J. A.; Riisager, A. *J. Am. Chem. Soc.*, **2013**, *135*, 5246.
317. Ge, Y.; Zhou, H.; Kong, W.; Tong, Y.; Wang, S.; Li, W. *Appl. Biochem. Biotechnol.*, **1998**, *69*, 17.
318. Fournier, R. L.; Varanasi, S.; Byers, J. P.; Chen, G. *Biotechnol. Bioeng.*, **1996**, *52*, 718.
319. Havewala, N. B.; Pitcher, W. H. In *Enzyme Engineering Volume 2*; Springer US: **1974**, p 315.
320. Mustafa, S.; Shah, K. H.; Naeem, A.; Ahmad, T.; Waseem, M. *Desalination*, **2010**, *264*, 108.
321. Mustafa, S.; Shah, K. H.; Naeem, A.; Waseem, M.; Ahmad, T.; Sarfraz, S.; Irshad, M. *Chin. J. Chem.*, **2010**, *28*, 27.
322. Simeonov, S. P.; Afonso, C. A. M. *J. Chem. Educ.*, **2013**, *90*, 1373.
323. Blanksma, J. J. *Recl. Trav. Chim. Pays-Bas*, **1910**, *29*, 403.
324. Middendorp, J. A. *Recl. Trav. Chim. Pays-Bas*, **1919**, *38*, 1.
325. Kang, E.-S.; Chae, D. W.; Kim, B.; Kim, Y. G. *J. Ind. Eng. Chem.*, **2012**, *18*, 174.

Chapter III

Stability and basicity of urea based deep eutectic mixtures.

In this chapter will be presented a brief overview of the synthesis and applications of deep eutectic mixtures in organic synthesis. Investigation on the stability and the origin of the basicity of the most used urea based deep eutectic mixtures will be discussed.

Table of content.

1. Introduction

During the last decades has been observed immerge scientific interest on more green and sustainable approaches in organic synthesis.[1,2] ILs were intensively studied as promising green solvents and catalysts for diverse organic transformations[3-5] and are considered green mainly because of their low vapor pressure, high thermal stability and recyclability. Despite ILs advantages there are some serious doubts about their real greenness when consider their all life cycle, and the toxic and environmental effects during their synthesis, application and desposal.[6,7] More recently new class of ionic fluids called deep eutectic solvents (DES), which exhibit similar properties as the traditional ILs but being much more ecofriendly and cheap are of growing interest.[8-10]

By definition DES are typically formed by two or three components, which interact each other *via* hydrogen bond interactions to form a eutectic mixture, which has melting point lower than each of the individual ingredients. Most of them are liquid at temperatures below 70°C and even at RT, and can be used as safe and inexpensive solvents for diverse applications. Usually DES are obtained by mixing quaternary ammonium salts with metal salts or a hydrogen bond donor (HBD)[11-13] which has the ability to form a complex with the halide anion of the quaternary ammonium salt. A general formula, which defines DES, $R_1R_2R_3R_4N^+X^-Y^-$ was described by Abbott *et al.*[13] in 2007.

Type 1 DES Y = MClx, M = Zn, Sn, Fe, Al, Ga

Type 2 DES Y = MClx_yH2O, M = Cr, Co, Cu, Ni, Fe

Type 3 DES Y =R_5Z with Z =$^-CONH_2$, ^-COOH, ^-OH

1.2 Formation and Freezing points (T_f) of some common DES.

As it was already mention DES are formed by mixing two solids, which *via* hydrogen bonds self-organization, forms a new phase, which is characterized by a lower freezing point than the individual ingredients. DES, which exhibit freezing points less than 50ºC are considered the most interesting and applicable as cheap and green solvents.

Abbott *et al.*[14] reported in 2003 various DES using cheap and environmental friendly ammonium salt choline chloride (ChCl) combined with several urea and amide HBD in 1:2 molar ratio (**Table 1**). The mixtures were formed by stirring at 80ºC until homogeneous mixture was formed.

Table 1. Freezing points (T_f) of mixtures of ChCl with amides in a 1:2 molar ratio.

Entry	Amide	T_f °C
1	urea	12
2	1-methylurea	29
3	1,3-dimethylurea	70
4	1,1-dimethylurea	149
5	thiourea	69
6	acetamide	51
7	bemzamide	92
8	tetramethylurea	[a]

[a] *No homogeneous liquid formed.*

The DES that exhibit the lowest T_f was ChCl-urea mixture (**Table 1,** entry 1). Because of the low T_f and the both ChCl and urea availability, low toxicity and low price so reported DES is one of the most used as a solvent in organic synthesis. Further on the authors prepared several urea based DES with variety of quaternary ammonium salt and were able to achieve much lower T_f in some cases down to -38°C (**Table 2**).

Table 2. Freezing points of urea with quaternary ammonium salts of the form $R_1R_2R_3R_4N^+$ X^- in 2:1 molar ratio.

Entry	R_1	R_2	R_3	R_4	X^-	T_f ºC
1	C_2H_5	C_2H_5	C_2H_5	C_2H_5	Br	113
2	CH_3	CH_3	CH_3	C_2H_4OH	Cl	12
3	CH_3	CH_3	CH_3	C_2H_4OH	BF_4	67
4	CH_3	CH_3	CH_3	C_2H_4OH	NO_3	4
5	CH_3	CH_3	CH_3	C_2H_4OH	F	1
6	CH_3	CH_3	$PhCH_2$	C_2H_4OH	Cl	-33
7	CH_3	CH_3	C_2H_5	C_2H_4OH	Cl	-38
8	CH_3	CH_3	CH_3	$PhCH_2$	Cl	26
9	CH_3	CH_3	CH_3	C_2H_4OAc	Cl	-14
10	CH_3	CH_3	CH_3	C_2H_4Cl	Cl	15
11	CH_3	$PhCH_2$	C_2H_4OH	C_2H_4OH	Cl	-6
12	CH_3	CH_3	CH_3	C_2H_4OF	Br	55

Again Abbott and co-workers[15] reported in 2004, ChCl based DES but using organic acids HBD in 1:1 molar ratio instead of amides (**Table 3**).The authors tested the viscosity, surface tension and conductivity of the formed DES and concluded that their physical properties and phase behavior are similar to ILs and are dependent on the number of acid functionalities, aryl/alky substituents and the composition of the mixture.

Table 3. Freezing points (T_f) of mixtures of ChCl with organic acids in 1:1 molar ratio.

Entry	Organic acid	T_f °C
1	adipic	85
2	benzoic	95
3	citric	69
4	malonic	10
5	oxalic	34
6	phenylacetic	25
7	phenylpropionic	20
8	succinic	71
9	tricarballylic	90

In 2011 Maugeri *et al.*[12] reported the formation of DES based on ChCl and various organic acids and sugars as biorenewable HBD, providing in some cases chiral DES (**Table 4**).

Table 4. DES based on ChCl and biorenewable HBD.

Entry	HBD	ChCl:HBD (molar ratio)	Mp ºC
1	levulinic acid	1:2	Liquid at RT
2	itaconic acid	1:1	57
3	xylitol	1:1	Liquid at RT
4	D-sorbitol	1:1	Liquid at RT
5	L-tartaric acid	1:0.5	47
6	D-isosorbide	1:2	Liquid at RT
7	4-hydroxybenzoic acid	1:0.5	87
8	caffeic acid	1:0.5	67
9	*p*-coumaric acid	1:0.5	67
10	*trans*-cinnamic acid	1:1	93
11	suberic acid	1:1	93
12	gallic acid	1:0.5	77

The authors studied also the Mp temperature depression upon the addition of glycerol to the DES, which were not liquid at RT. Significantly lower Mp were achieved and moreover in some cases the addition of glycerol resulted in DES formation which was not possible without it (**Table 5**).

Table 5. The effect of glycerol addition on the Mp of DES

Entry	HBD	ChCl:HBD:Glycerol (molar ratio)	Mp ºC, without glycerol	Mp ºC, with glycerol
1	itaconic acid	1:0.5:0.5	57	Liquid at RT
2	L-tartaric acid	1:0.5:0.25	47	Liquid at RT
3	4-hydroxybenzoic acid	1:0.5:0.25	87	63
4	caffeic acid	1:0.5:0.5	67	Liquid at RT
5	*p*-coumaric acid	1:0.5:0.25	76	63
6	*trans*-cinnamic acid	1:1:0.5	93	87
7	suberic acid	1:0.5:0.5	No DES formed	73
8	gallic acid	1:0.25:0.25	No DES formed	53

AlNashef *et al.*[16] prepared and studied the physical properties of DES based on phosphonium salts, methyltriphenylphosphonium bromide (MTPB) or benzyltriphenyl phosphonium chloride (BTPC) and various HBD (**Table 6**).

Table 6. Phosphonium salts based DES.

Entry	DES composition	Mole ratio (phosphonium salt/HBD)	T_f ºC
1	MTPB/glycerol	1:1.75	-4.03
2	MTPB/2,2,2-trifluoro acetamide	1:8	-69.29
3	BTPC/glycerol	1:5	50.36
4	BTPC/ethylene glycol	1:3	47.91
5	BTPC/2,2,2-trifluoro acetamide	3:1	99.72

The same group[17] reported in 2011 the preparation of MTPB based DES and their application for the removal of glycerol from palm oil biodiesel. The DES were formed by

mixing MTPB and glycerol, triethylene glycol and ethylene glycol as HBD in different molar ratios (**Table 7**).

Table 7. Composition and T_f of MTPB based DES.

Entry	HBD	Molar ratio (MTPB:HBD)	T_f ºC
1	glycerol	1:2	3-4
2	glycerol	1:3	-5.5
3	glycerol	1:4	15.6
4	ethylene glycol	1:3	-46
5	ethylene glycol	1:4	-50
6	ethylene glycol	1:5	-48
7	triethylene glycol	1:3	-8
8	triethylene glycol	1:4	-19
9	triethylene glycol	1:5	-21

Again AlNashef *et al.*[18] synthesized and measured the densities of various DES based on ChCl and *N,N* diethylenethanol ammoinium chloride combined with glycerol or ethylene glycol as HBD (**Table 8**). In this work the density of the previously reported by them MTPB based DES were also measured.

Table 8. Composition and T_f of ChCl and *N,N* diethylenethanol ammoinium chloride DES.

Entry	salt	HBD	Molar ratio (MTPB:HBD)	T_f ºC
1	ChCl	glycerol	0.5:0.5	8
2		glycerol	0.33:0.67	-36
4		glycerol	0.25:0.75	-33
3		ethylene glycol	0.36:0.64	-33
5		ethylene glycol	0.33:0.67	-66
6		ethylene glycol	0.28:0.72	4
7	N,N diethylenethanol ammoinium chloride	glycerol	0.33:0.67	-1
8		glycerol	0.25:0.75	2
9		glycerol	0.2:0.8	2
10		ethylene glycol	0.33:0.67	-31
11		ethylene glycol	0.25:0.75	-22
12		ethylene glycol	0.2:0.8	-22

Hashim *et al.*[19] reported the synthesis of DES based on ChCl and D-glucose as HBD in different ratios (**Table 9**). The authors observed that the T_f decreased with raising the amount of ChCl up to 2:1 molar ratio, further increase up to 2.5:1 resulted in higher T_f. When D-glucose above 1:1 molar ratio was used no clear DES were obtained and formation of white semisolid mixtures have been observed.

Table 9. ChCl:D-glucose DES and their T_f.

Entry	ChCl:D-glucose molar ratio	T_f ºC
1	1:1	31
2	1.5:1	24
3	2:1	15
4	2.5:1	44

Several DES based on carbohydrates, ureas and inorganic salts (**Table** 10) were prepared by Imperato *et al.*[20] and used as solvents for Diels-Alder reactions.

Table 10. DES of cyrbohydrates, urea and inorganic salts.

Entry	Carbohydrate (% w)	Urea (% w)	Salt (% w)	Mp.ºC
1	Fructose (60%)	Urea (40%)	-	65
2	Sorbitol (70%)	Urea (20%)	NH_4Cl (10%)	67
3	Maltose (50%)	DMU (40%)	NH_4Cl (10%)	73
4	Glucose (50%)	Urea (40%)	$CaCl_2$ (10%)	75
5	Mannose (30%)	DMU (70%)	-	75
6	Sorbitol (40%)	DMU (60%)	-	77
7	α-Cyclodextrin (30%)	DMU (70%)	-	77
8	Citric acid (40%)	DMU (60%)	-	65

1.3 Acidity and basicity of DES.

Zhou et al.[21] applied Hammett function to evaluate the basicity of ChCl/urea (1:2 molar ratio) DES. For a basic solution Hammett function measures the tendency of the solution to capture protons and in case of weak acid indicators it is defined by the following equation:

$H\text{-} = pK(HI) + \log([I^-]/[HI])$

where pK(HI) is the thermodynamic ionization constant of the indicator in water, $[I^-]$ and [HI] represent the molar concentrations of anionic and neutral forms of the indicator, respectively. Medium to high H- refer to strong basicity. The authors used 4-nitrobenzylcyanide as indicator and calculated H- value to be 10.86 suggesting the ChCl/urea DES is slightly basic. The authors also observed that H- values decreased when 1-3% water have been added. Due to its basicity this DES was capable to absorb small amounts of CO_2. H- of the DES was observed to decrease to 6.25 after 1 atm. of CO_2 was applied. The acidity and basicity were found to be reversible. When after the CO_2, N_2 was bubbled together with heating the initial H- was restored.

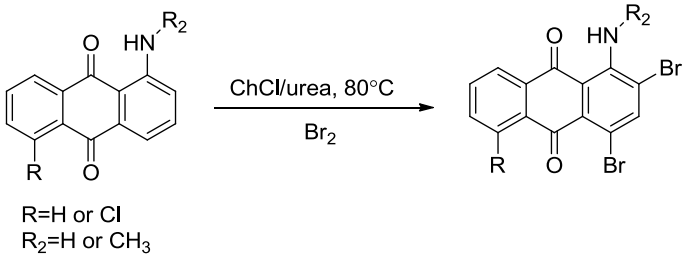
AlNashef *et al.*[16] measured the pH of several phosphonium salt based DES as a function of the temperature. MTPB/glycerol (1:1.75), MTPB/ethylene glycol (1:4), BTPC/glycerol (1:5) and BTPC/ethylene glycol (1:3) DES exhibit neutral or close to neutral pH, which is not influenced by the temperature (5-95ºC range). While MTPB/2,2,2-trifluoro acetamide (1:8) has a low pH of 2.5 at 20ºC which increased when the temperature was raised.

ChCl based DES with polyols HBD was observed by Maria *et al.*[12] to exhibit neutral pH. Gano *et al.*[22] measured the relation between the temperature and pH of 3 DES formed from K_2CO_3 and glycerol in 1:4, 1:5 and 1:6 molar ratios respectively. As it was expected the DES with higher equivalents of K_2CO_3 (1:4 ratio) exhibit the highest pH of 13.5 which decreased to 12.5 with raising the temperature (20-80ºC range). The same decrease of the pH values was also observed for the other two studied DES.

2. Application of DES in organic synthesis.

2.2 Base catalyzed reactions.

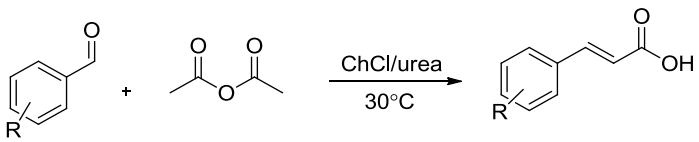
The observed basicity of some DES was used from the researchers to promote base catalyzed reactions. Shankarling *et al*.[23] reported electrophilic substitution of 1-aminoanthra-9,10-quinone derivatives in ChCl/urea (1:2) DES (Scheme 1).



Scheme 1.

The products were obtained in high yields of up to 95% and the reaction was observed to be significantly accelerated in DES compared to the conventional solvents, such as MeOH and $CHCl_3$. This effect can be attributed to the basic nature of the ChCl/urea, although no explanation was provided by the authors. The brominated 1-aminoanthra-9,10-quinones were isolated after precipitation with water. Then water was evaporated and DES was reused by the authors over 5 cycles without significant effect on the reaction outcome.

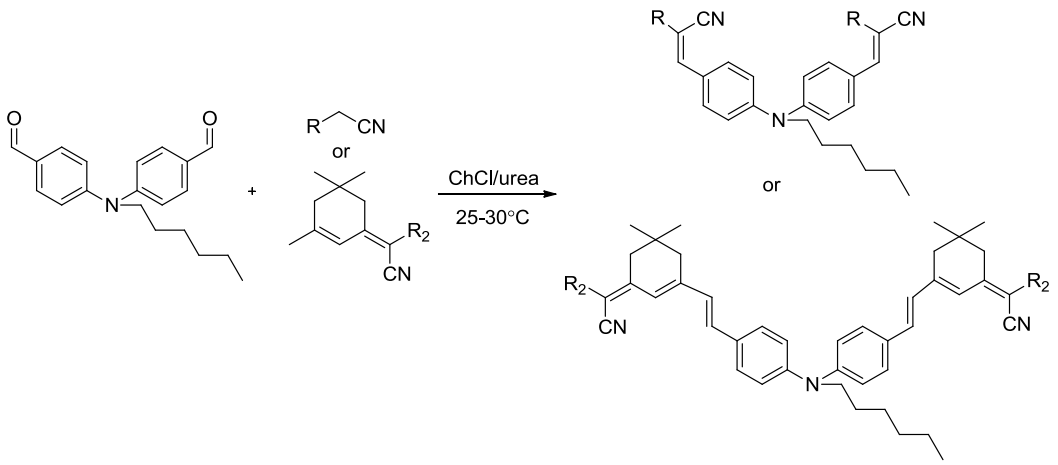
Perkin reaction of benzaldehyde derivatives in ChCl/urea DES was reported by the same group (Scheme 2).[24]



Scheme 2.

Cinnamic acid derivatives were obtained in very good to excellent yields (62-92%) and ChCl/urea was recycled by the authors 4 times with minor yields erosion. The reaction using benzaldehyde as starting material was compared with the conventional reaction conditions (benzaldehyde/acetic anhydride/sodium acetate trihydrate in a molar ratio of 1:2:3). The reaction performed in DES was observed to proceed at much lower temperature 30 *vs* 140ºC, thus allowing energy saving of 62%.

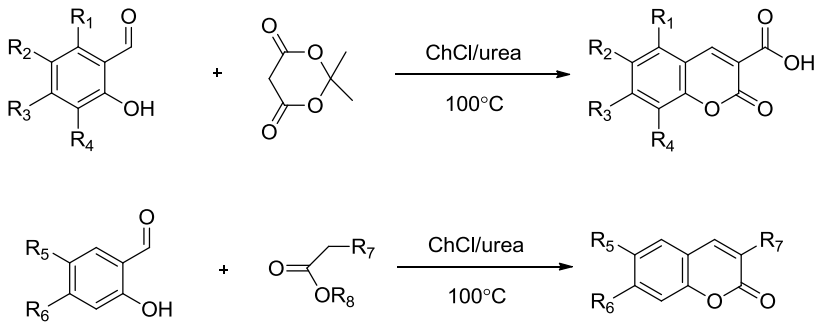
Shankarling *et al*.[25] reported the synthesis of novel Y-shaped acceptor-π-donor-π-acceptor-type compounds from 4,4′-hexyliminobisbenzaldehyde *via* Knovenagel condensation (**Scheme 3**). The authors performed the synthesis using conventional conditions (reflux in absolute EtOH and piperidine as catalyst), lipase biocatalyst or DES and compared the yields and recyclability among the three methods.



Scheme 3.

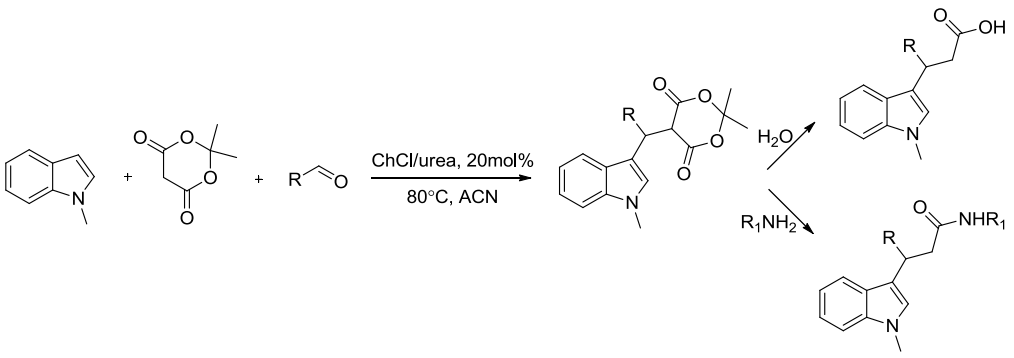
It was shown that ChCl/urea DES may provide an attractive alternative for the synthesis of valuable chromophores in high yields (75-95%). During the study, lipase was recycled by the authors for up to four cycles, there was no significant decrease in product yield after completion of the first cycle, but the yield declined to 50% after the completion of the fourth one, while DES was recycled up to 5 times without significant decrease of the reaction yield.

Coumarine derivatives were synthesized by Satyanarayan *et al.* from active methylene compounds such as meldrum's acid, diethylmalonate, ethyl cyanoacetate, dimethylmalonate, *via* Knovenagel condensation with various salicylaldehydes in presence of ChCl/urea DES without using any solvents or additional catalyst (Scheme 4). The target coumarines were isolated in excellent yields (92-98%).



Scheme 4.

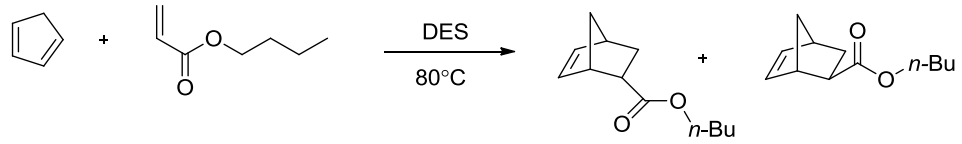
Kumar *et al.*[26] reported ChCl/urea catalyzed one-pot synthesis of indole-3-propanamide derivatives. The reactions have been performed in acetonitrile and ChCl/urea DES was applied as a catalyst in 20 mol% (Scheme 5).



Scheme 5.

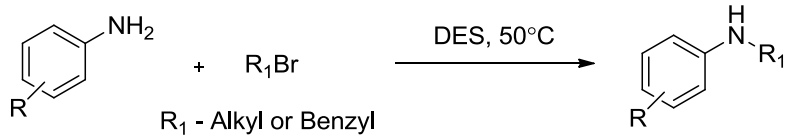
Under optimized conditions various indole-3-propanamide derivatives were obtained in high yields (74-92%).

König *et al.*[27] performed 5mol% L-proline catalyzed Diels-Alder reaction of cyclopentadiene and *n*-butyl acrylate in L-carnitine/urea melt (2:3) and obtained 93% yield after 4h at 80ºC (Scheme 6).



Scheme 6.

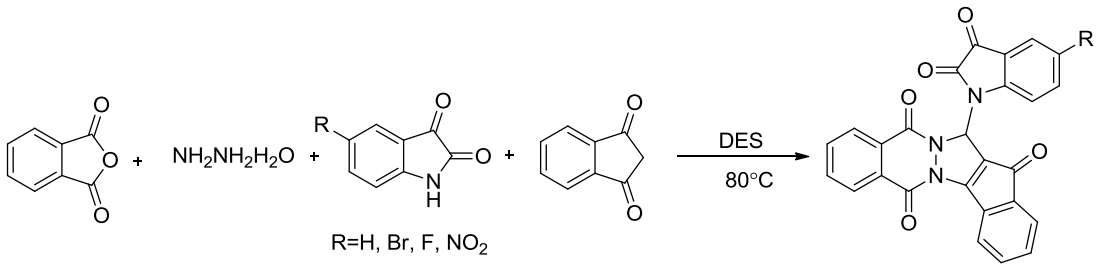
Mono *N*-alkylation of aromatic amines was reported by Shankarling *et al.*[28] using ChCl/glycerol or ChCl/urea DES as catalysts and reaction media (Scheme 7). Due to its higher basicity ChCl/urea DES was much more efficient allowing the synthesis of a range of mono-*N*-alkylated aromatic amines in high yields (70-89%).



Scheme 7.

After selective extraction of the products ChCl/urea was recycled by the authors 5 times without significant erosion of the yields, while in case of alternative lipase-catalyzed reaction graduate enzyme deactivation was observed over 4 cycles.

Kumar *et al.*[29] recently reported the synthesis of spirooxindoles in ChCl/urea (1:2) DES (Scheme 8). Compared to the conventional reaction conditions, in EtOH using Et_3N or piperidine as catalyst, the reaction rate was significantly higher in ChCl/urea and very good yields (83-93%) have been achieved. ChCl/urea was reused by the authors over 4 cycles with insignificant loss of activity.

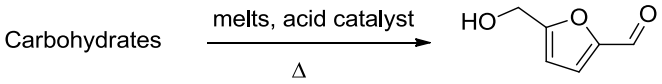


Scheme 8.

2.3 Acid catalyzed reactions.

Han *et al.*[30] reported dehydration of fructose to HMF in ChCl based DES. The authors observed that ChCl/urea (1:2) DES and ChCl/metal chlorides ($ZnCl_2$, $CrCl_3$) based DES were poorly efficient in the dehydration of fructose to HMF, while the application of Brönsted acid DES composed of ChCl and organic acids provided good yields of up to 76% in case of ChCl/citric acid. Due to the low solubility of HMF in ChCl/citric acid the reaction could be performed as a biphasic system with EtOAc, as an extraction solvent, affording HMF in 91% yield. In a following work the same group extend the application of ChCl/citric acid and ChCl/oxalic acid DES for the tandem depolymerization/dehydration reaction of inulin to HMF, which was obtained in 51 and 56% yield respectively.[31]

Dehydration of fructose to HMF in carbohydrates/urea or ChCl melts was reported by König *et al.*[32] (Scheme 9).



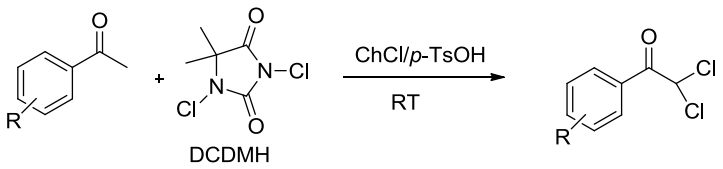
Scheme 9.

In this work high concentration of carbohydrates fructose, glucose inulin and sucrose were used in the presence of an acid catalyst (**Table 11**). As it was expected among other carbohydrates the highest yields were obtained from fructose in up to 67% in case of PTSA (Table 11, entry 5). The authors also studied the dehydration in carbohydrates/urea melts. When urea or DMU were used poor results were observed in all the cases and the highest obtained yield was 27%, when amberlyst-15 was used as a catalyst. Furthermore it was systematically studied the effect of different ureas on the reaction outcome and was found that in case of $FeCl_3$ catalyzed dehydration of fructose/urea melts, simple urea didn't provide any yield of HMF, only 8% was achieved for DMU and significant increase of the yield up to 89% was observed for *N,N*-tetramethyl urea (TMU).

Table 11. Acid-catalyzed dehydration of carbohydrates melts to HMF.

Entry	Catalyst	HMF yield.			
		Fructose/ChCl 2:3(w/w)	**Inulin/ChCl 2:3(w/w)**	**Glucose/ChCl 1:1(w/w)**	**Sucrose/ChCl 1:1(w/w)**
1	$FeCl_3$	59%	55%	15%	27%
2	$ZnCl_2$	8%	3%	6%	6%
3	$CrCl_2$	40%	36%	45%	62%
4	$CrCl_3$	60%	46%	31%	43%
5	PTSA	67%	57%	15%	25%
6	$Sc(OTf)_2$	55%	44%	9%	28%
7	Amberlyst 15	40%	54%	9%	27%
8	Montmorillonite	49%	7%	7%	35%

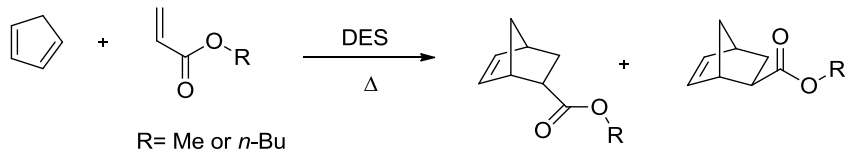
Zou *et al.*[33] used ChCl/PTSA (1:1) DES for a selective dichlorination of acetophenone derivatives using 1,3-Dichloro-5,5-dimethylhydantoin (DCDMH) (Scheme 10).



Scheme 10.

At the end of the reaction, the products were easily and selectively extracted from the ChCl/PTSA DES using MTBE as an extraction solvent, thereby allowing the authors to successfully recycle such DES at least 5 times.

Diels‑Alder reaction in various DES based on carbohydrate or derived polyols (such as fructose, maltose,lactose, mannitol, glucose and sorbitol) in combination with either urea or DMU was studied by Imperato *et al.*[20] All the DES were found to be efficient for the reaction of cyclopentadiene and methyl or *n*-buthyl acrylate (Scheme 11).

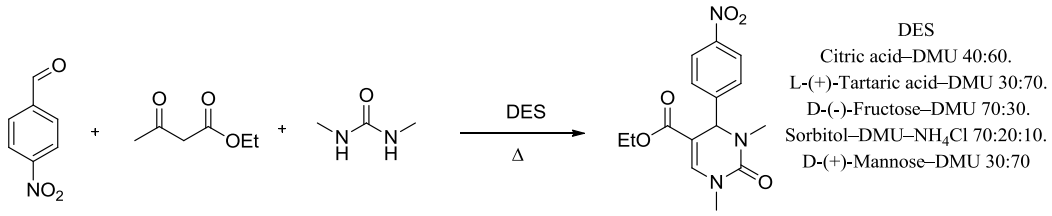


Scheme 11.

In all cases high, 72‑100% yields were obtained, while the endo/exo selectivity was observed to vary from 2.7:1 to 5:1.

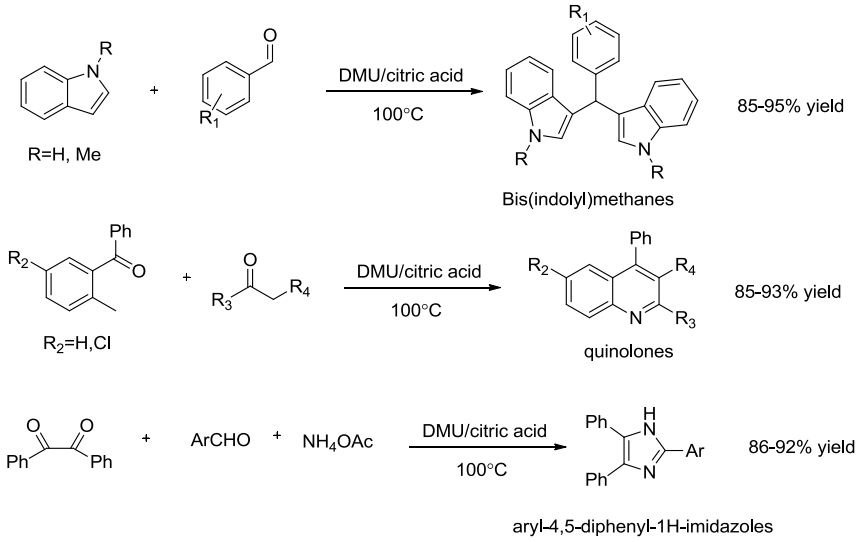
Koenig *et al.*[34] performed the synthesis 3,4-dihydropyrimidin-2-ones *via* Biginelli reaction in low melting organic acid/urea mixtures. The authors used a model reaction of 4-nitrobenzaldehyde, ethylacetoacetate and DMU for DES screening (Scheme 12) L-(+)-Tartaric acid–DMU 30:70 DES was found to be the best providing the final product in 96% yield in

12h. The methodology exhibit broad substrate scope and the authors applied L-(+)-Tartaric acid DES for the synthesis of number of different 4-dihydropyrimidin-2-ones derivatives.



Scheme 12.

Dimethylurea/citric acid DES (6:4) was used by Khabazzadeh *et al.*[35] as a solvent and catalyst for multicomponent reactions. Bis(indolyl)methanes, quinolones and aryl-4,5-diphenyl-1H-imidazole were prepared in excellent yields (Scheme 13).

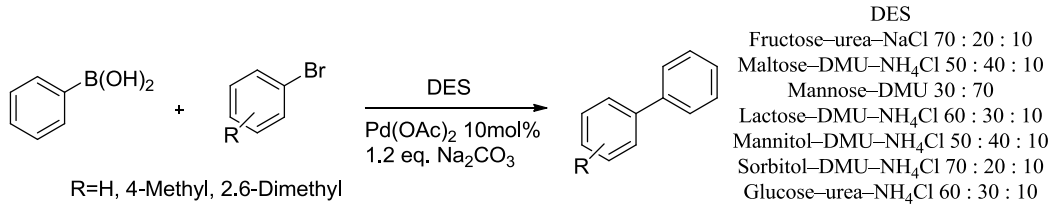


Scheme 13.

The authors observed that reaction rates were accelerated in DES without additional catalyst, compared to solvent free reaction conditions or conventional organic solvents in presence of acid catalyst. DES was recycled 3 times without any effect on the reaction outcome.

2.4 Metal-catalyzed reactions.

Pd catalyzed Suzuki coupling of phenyl boronic acid with aryl bromides in different carbohydrates/urea/inorganic salts eutectic mixtures was reported by König et al.[11] (Scheme 14). Quantitative conversions in all tested DES was observed and the target products were isolated in 78‑98% yields.



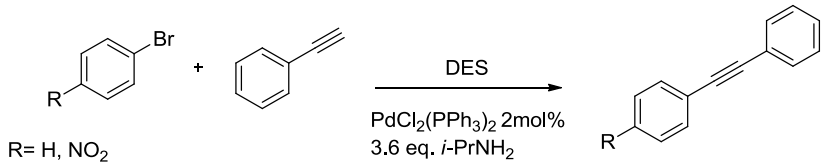
Scheme 14.

Homogeneous Pd catalyzed Heck reaction was reported by the same group[27] using DES composed of D-mannose and DMU in 3:7 ratio(Scheme 15). Several Pd catalysts were tested and among, $PdCl_2(PPh_3)_2$ provided the best yields in up to 91%.

Scheme 15.

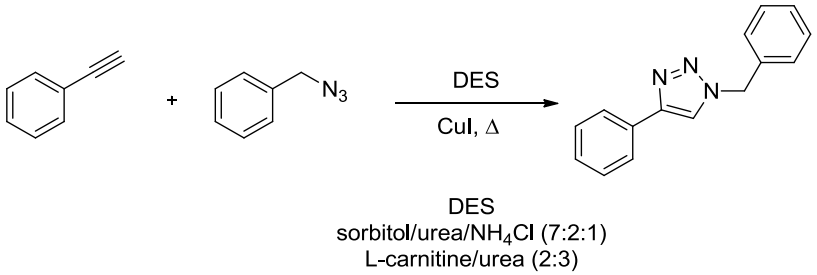
Catalytic Heck coupling can also be performed in L-carnitine/urea DES in 2:3 ratio. Because of the slightly higher viscosity of such a melt lower reaction rates were observed.

In the same work the authors also tested D-mannose/DMU (3:7) DES as a solvent for Sonogashira cross-coupling reactions. Interestingly it was observed that under these conditions the reaction can be performed without assistance of copper providing the final products in 61‑79% yield (Scheme 16).



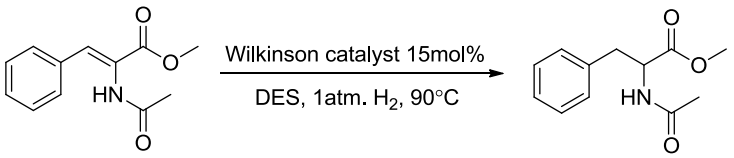
Scheme 16.

CuI catalyzed Click reaction between benzylazide and phenyl acetylene was successfully performed in DES (Scheme 17).[11]93% yield in sorbitol/urea/NH_4Cl has been achieved. In case of L-carnitine/urea the reaction rate was slightly enhanced providing 96% yield.



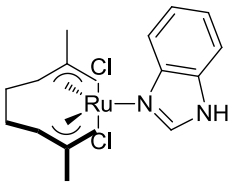
Scheme 17.

Hydrogenation reaction of methyl α-cinnamate in various carbohydrate DES using Wilkinson`s catalyst was reported in 2006.[11] Among the tested melts, citric acid/DMU (2:3) was the most efficient providing the final product in quantitative yield (Scheme 18). Unfortunately no asymmetric induction was observed when chiral DES (sorbitol/DMU/NH4Cl (7:2:1) or mannitol/DMU/NH_4Cl (5:4:1) were used as a reaction media.

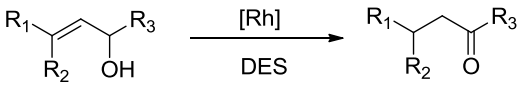


Scheme 18.

Alvarez et al.[36] reported in 2013 the application of DES as reaction media for Rh-catalyzed redox isomerization of allylic alcohols into carbonyl compounds (Scheme 19).



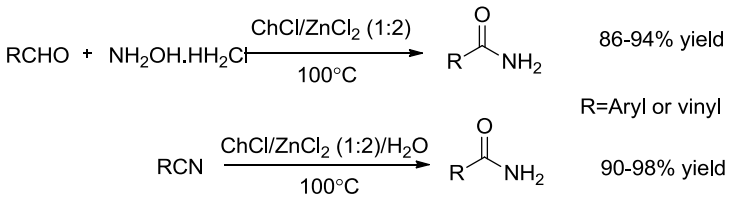
[Ru(η³:η³-C10H16)Cl₂(benzimidazole)]



Scheme 19.

High activities, selectivity and nearly quantitative yields in short reaction times and with low catalyst loadings (0.2 mol% in Ru) were achieved for monosubstituted allylic alcohols in ChCl/glycerol (1:2) DES, while for their disubstituted counterparts, high catalyst loading and longer reaction times were always required.

ChCl/$ZnCl_2$ (1:2) was used as an efficient and reusable solvent system for the synthesis of primary amides from aldehydes and nitriles.



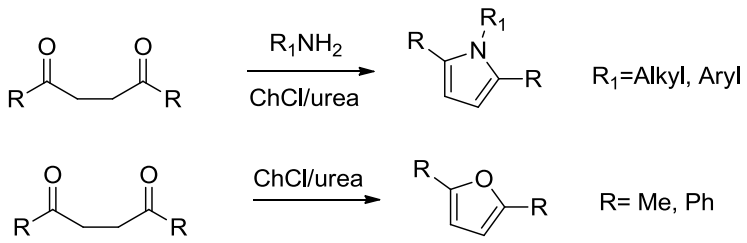
Scheme 20.

Compared to the already reported in the literature catalysts for these transformations, ChCl/$ZnCl_2$ proved to be more efficient besides being also more environmental friendly and

cheap. Moreover it was recycled by the authors for 5 cycles, although with some graduate loss of activity.

2.5 Other reactions.

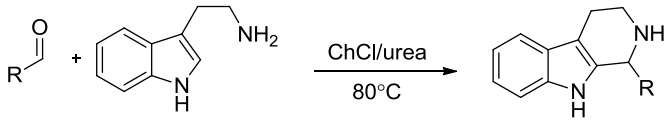
Lavender et al.[37] recently reported that Paal‑Knorr reactions are effectively catalyzed by ChCl/urea DES (Scheme 21). The reaction conditions were quite mild and do not require the addition of an additional Bronsted or Lewis acid catalyst. The authors explained the catalytic activity with the weak hydrogen-bonding ability of urea that serves as an organocatalyst.



Scheme 21.

The corresponding furan or pyrrole derivatives were obtained in high yields (77-97%) in 12h at 80ºC. After product isolation, DES was successfully recycled by the authors over 4 cycles with minor erosion of the reaction yield. ChCl/glycerol DES was also applied for the reaction of 2,5-hexanedione with benzylamine but was found to be less effective providing the corresponding pyrrole derivative in 67% yield.

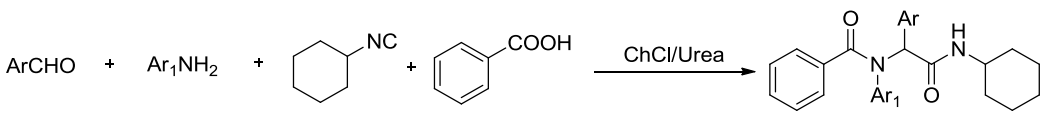
The urea organocatalitic activity in ChCl/urea DES was also used by Wright *et al.*[38] who performed acid-free Pictet‑Spengler reaction (Scheme 22).



Scheme 22.

The final products were obtained in high yields in all cases (79-99%) and the reaction media was recycled 5 times by the authors.

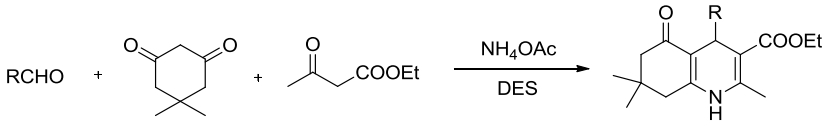
Multicomponent Ugi reaction in ChCl/urea melt was reported recently by Azizi *et al.*[39] The reaction exhibit broad substrate scope and various Ugi adducts were obtained in good to excellent yields (60–92%) (Scheme 23).



Scheme 23.

The reaction rate was found to be significantly improved in ChCl/Cl as a solvent compared to neat reaction conditions or common organic solvents. Moreover DES was recycled at least 4 times by the authors.

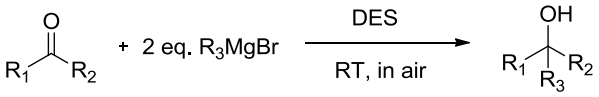
Ghadge et al.[40] used ChCl based DES with urea, organic acids or glycerol HBD as a reaction media for one-pot multicomponent synthesis of 1,4-duhidropyridine derivatives (Scheme 24).



Scheme 24.

All the DES provided very good results but ChCl/urea was found to be the most efficient, excellent yields have been achieved in all the cases (77-95%). In terms of recyclability, excellent results were obtained by the authors over 5 cycles.

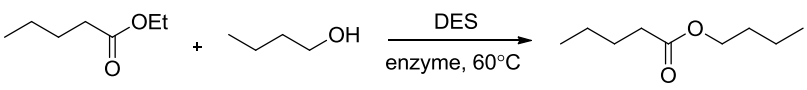
Interesting application of ChCl based DES in organometallic chemistry was recently reported.[41] Grignard and organolithium reagents were found to be stable and undergo addition to carbonyls in ChCl/glycerol, ChCl/urea and even in ChCl/water mixtures in air (Scheme 25).



Scheme 25.

Although the attempts to generate Grignard reagent in ChCl/glycerol (1:1) failed, when commercial ethereal solutions were used in 2 eq. moderate to good yields of the corresponding alcohols were obtained. It was observed that although in lower yield (78 *vs* 60%) vinylmagnesium bromide reacts also in pure DES (without ethereal co-solvent) with 2-methoxy-acetophenone. The authors extended the scope of the reaction using organolithium reagents. Very good yields of 60-90% were obtained in ChCl/glycerol and ChCl/water mixtures in very short reaction times of 2-3s at room temperature and no need of reaction cooling as it is required for the conventional conditions using ethereal solvents.

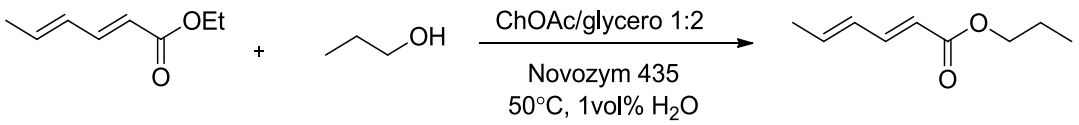
DES were found to be also suitable reaction media for various enzyme-catalyzed reactions. In 2008 Kazlauskas et al.[42] published a pioneer work on the biocatalysis in various DES. The authors investigated the activity of enzymes in the transesterification of ethyl valerate with butanol (Scheme 26).



Scheme 26.

In contrast with their poor stability in aqueous solutions of ChCl or urea, the enzymes exhibit good stability in ChCl/urea DES. In the presence of lyophilized *Candida antarctica* lipase B (CLAB) or its immobilized form on acrylic resin (iCLAB) more than 90% conversion of ethyl valerate to butyl valerate was achieved in ChCl/urea or ChCl/glycrol DES. Interestingly, in ChCl/glycerol DES, side transesterification reaction between ethyl valerate and glycerol occurred in a very low rate (<0.5%). Furthermore the application of ChCl/urea and ChCl/glycerol DES was extended to iCLAB catalyzed aminolysis of ethyl valerate with butylamine. The reaction rates and final conversion (>90%) were similar in ChCl:glycerol, ChCl:urea or toluene. Finally the authors reported that DESs were also suitable as co-solvents for reactions in aqueous solutions, where they enhanced hydrolase-catalyzed reactions. The rates of esterase-catalyzed hydrolysis of p-nitrophenyl acetate were found to increase moderately upon the addition of 10vol% of ChCl/glycerol DES. The rate of epoxide-hydrolase catalyzed hydrolysis of styrene oxide was significantly increased up to 20-fold in presence of 25vol% of ChCl/glycerol.

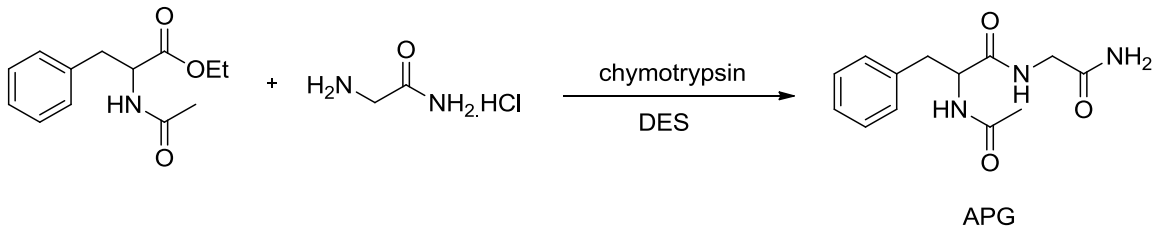
Zhao and co-workers[43] reported in 2011 the synthesis of novel choline acetate (ChOAc)/glycerol DES, which were considerably less viscous than those derived from ChCl and urea, and are capable of maintaining high biocatalytic activity of CALB. The authors investigated the transesterification of ethyl sorbitate with 1-propanol catalyzed by *Candida antarctica* lipase B immobilized on acrylic resin, Novozym® 435 (Scheme 27).



Scheme 27.

The highest initial rates were observed in ChOAc/glycerol (1:2) and ChCl/urea (1:2) DES, the first one being slightly better 1.02 *vs* 1.00 μmol min^{-1} g^{-1}, over 99% selectivity was obtained in both cases. Moreover Novozym 435 was highly stable in ChOAc/glycerol (1:1.5) maintaining 92% and 50% of its activity after 48h and 168h of pre-incubation, respectively.

More recently Maria *et al.*[44] reported chymotrypsin catalyzed peptide synthesis in ChCl DES. The synthesis of the protected *N*-Ac-Phe-Gly-NH_2 peptide (APG) was used as a prototypical reaction in different DES, starting from *N*-acetylphenylalanine ethyl ester and glycine-amide hydrochloride (Scheme 28).



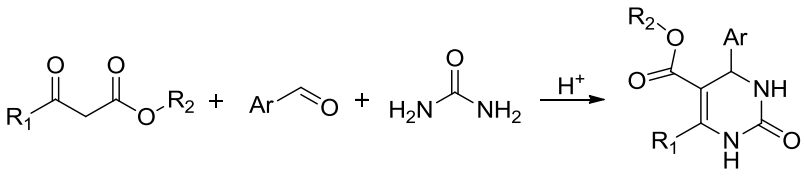
Scheme 28.

When glycerol or isosorbide were used as HBD in DES (ChCl:HBD, 1:2) full conversion and complete selectivity was observed. ChCl/urea (2:1) also provided high conversions despite the denaturing effect of urea on the enzyme. Interestingly, the use of ChCl/xylitol (1:1) DES provided much lower conversion. Furthermore the non-immobilized enzyme can be reused over several cycles although gradient inactivation has been observed by the authors.

3. Results and discussion.

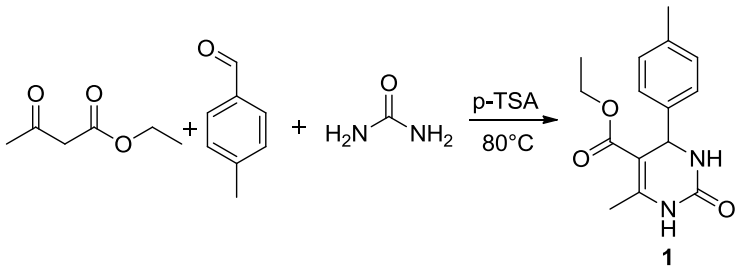
In our research we were interested on studding the possible application of chiral DES in asymmetric synthesis. Such an alternative would be highly attractive because such a DES could be easily accessed from cheap and environmental friendly natural compounds like carbohydrates or derived polyols. We decided to study previously obtained in our lab Sorbitol/Urea (47mol%/53mol%) DES as a reaction media for asymmetric Biginelli reaction.

The Beginelli reaction is an acid-catalyzed, three-component reaction between an aldehyde, a ß-ketoester and urea resulting in the formation of dihydropyrimidones, compounds with interesting pharmacological properties associated with their heterocyclic scaffold (Scheme 29).[45,46]



Scheme 29.

Initially Beginelli reaction was performed using ethyl acetoacetate and p-tolyl aldehyde as substrates under acid catalysis with PTSA at 90°C in Sorbitol/Urea (47mol%/53mol%) DES, the resulting dihydropyrimidone derivative **1** was obtained in 95% yield after 12h (Scheme 30). Unfortunately after been analyzed by HPLC no enantioselectivity was observed.



Scheme 30.

Further, we decided to perform the same reaction without PTSA or any other acid catalyst in order to study the DES catalytic activity itself, which would be much more likely to result in asymmetric induction. The same reaction was performed at 90°C in absence of PTSA. At the end no Beginelli adduct was observed but the reaction resulted in the formation of a new product, which was determined by NMR to be diethyl 2,4,6-trimethyl-1,4-dihydropyridine-3,5-dicarboxylate 2.

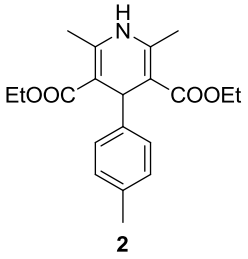
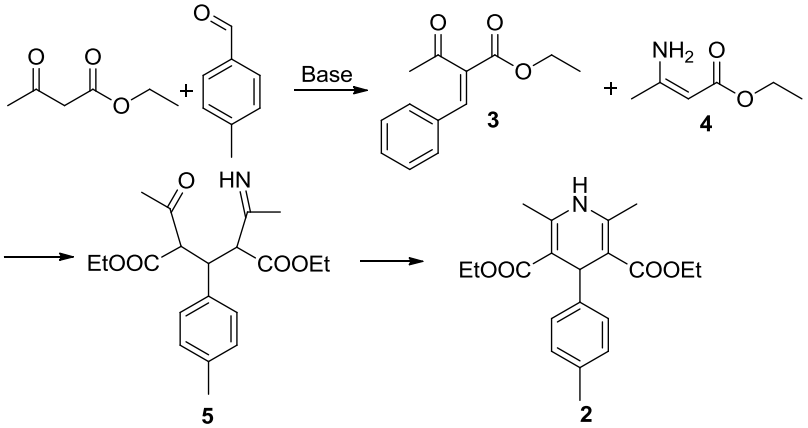


Figure 1.

Obviously the formation of **2** proceeds *via* Hantzsch dihydropyridine synthesis[47] which include initial Knovenagel condensation between ethyl acetoacetate and *p*-tolyl aldehyde and the formation of intermediate **3** which further reacts with ester enamine **4**, produced by condensation of the second equivalent of ethyl acetoacetate with ammonia. The formed intermediate **5** undergo cyclization to give 1,4-dihydropyridine derivative **2** (Scheme 31).



Scheme 31.

The unexpected formation of **2** rise the question about the origin of ammonia in the reaction mixture. We used standard Nesstler reagent test, which gives yellow color in presence of ammonia (Scheme 32) and it was confirmed in an aqueous solution of sorbitol/urea DES, while the blank tests of pure urea and sorbitol aqueous solutions failed to give yellow color (Table 12).

$$NH_4^+ + 2[HgI_4]^{2-} + 4OH^- \longrightarrow \underset{\text{yellow color}}{HgO{\cdot}Hg(NH_3)I} + 7I^- + 3H_2O$$

Scheme 32.

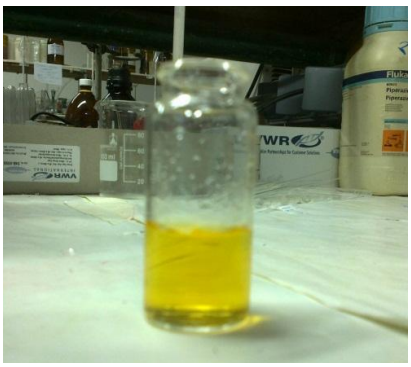


Figure 2. Positive Nesstler reagent reaction.

Table 12. Nessler reagent test results.

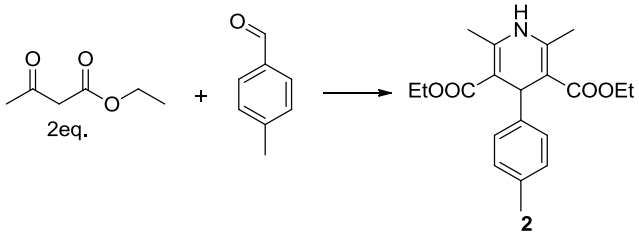
Entry	Tested mixture	Result
1	Sorbitol/Urea (47mol%/53mol%)[a]	positive
2	Urea aqueous solution[b]	negative
3	Sorbitol aqueous solution[b]	negative

[a] Prepared by heating sorbitol and urea mixture for 3h at 80ºC. [b] 0.1g solution in 4 ml of distilled water.

The observed negative results for urea and sorbitol proved that ammonia is not originated as an impurity from their production and is probably a result of some decomposition of urea during the formation and use of DES.

These results are also providing a clue that partial decomposition of urea to ammonia during the heating and formation of urea based DES is responsible for their observed basicity and is the real catalyst for some reported base catalyzed reactions in this type of DES, such as Perkin reactions and Knovenagel condensations. The results are somehow unexpected since the significant thermal urea decomposition requires higher temperatures, above 150°C.[48] Further on the synthesis of **2** was used as a clock reaction for the formation of ammonia in different alcohols combined with urea as reaction media. The reactions were monitored by TLC (Table 13).

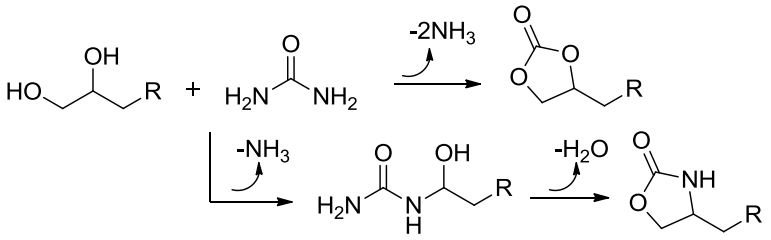
Table 13. Screening of different alcohols for the synthesis of **2**[a]



Entry	Alcohol	Result[b]
1	Methanol	No reaction
2	Ethanol	No reaction
3	ChCl	Traces
4	Sorbitol	Product
5	Glycerol	Product

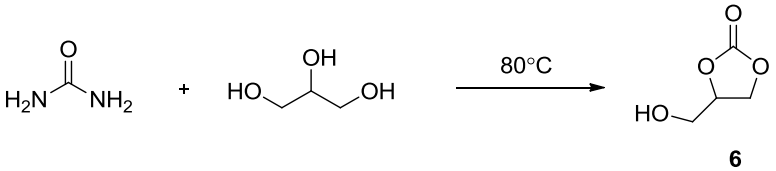
[a] *The reaction were performed overnight at 80°C using 1:1 w/w ratio of the alcohol and urea.* [b] *Product detected by TLC*

Obviously the formation of ammonia is not a result of simple thermal decomposition of urea since the formation of **2** was observed only in case of glycerol and sorbitol (Table 13, entry 4 and 5) and not in MeOH and EtOH (Table 13, entry 1 and 2) at the same temperature. The most rationalized explanation of the origin of ammonia is a possible formation of cyclic carbonates and their related intermediates[49] (Scheme 33) from the reaction of urea with polyalcohols that can proceed at moderate temperatures, even below 100°C.



Scheme 33.

In order to prove the formation of cyclic carbonates a mixture of glycerol and urea (10:4 molar ratio) was heated at 80ºC with steering for 3 weeks to form 4-(Hydroxymethyl)-1,3-dioxolan-2-one 6 (Scheme 34).



Scheme 34.

The reaction mixture was analyzed by ^{13}C NMR and the formation of **6** together with other side products, which were not assigned, was confirmed.

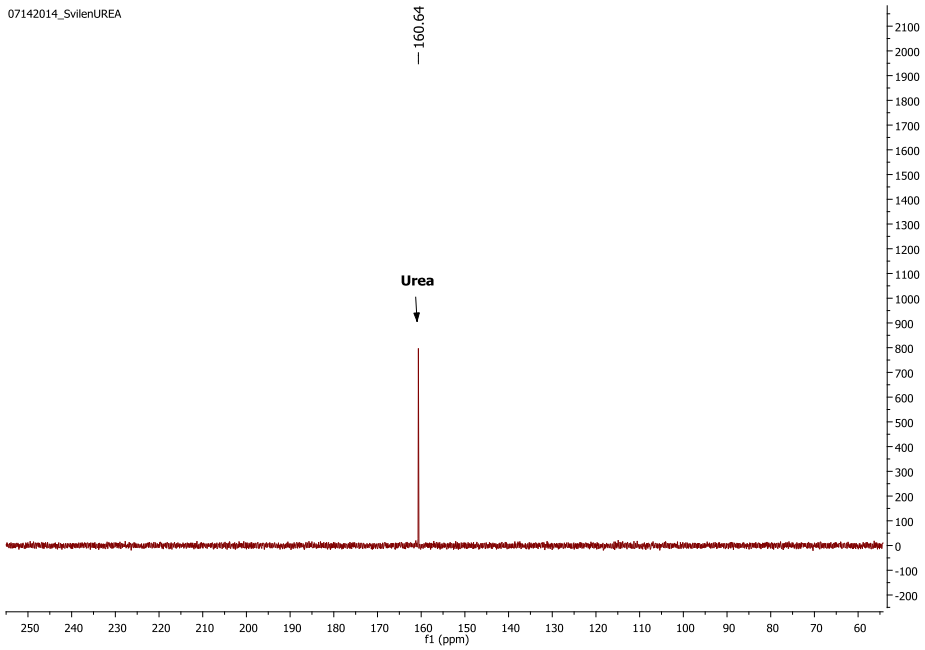
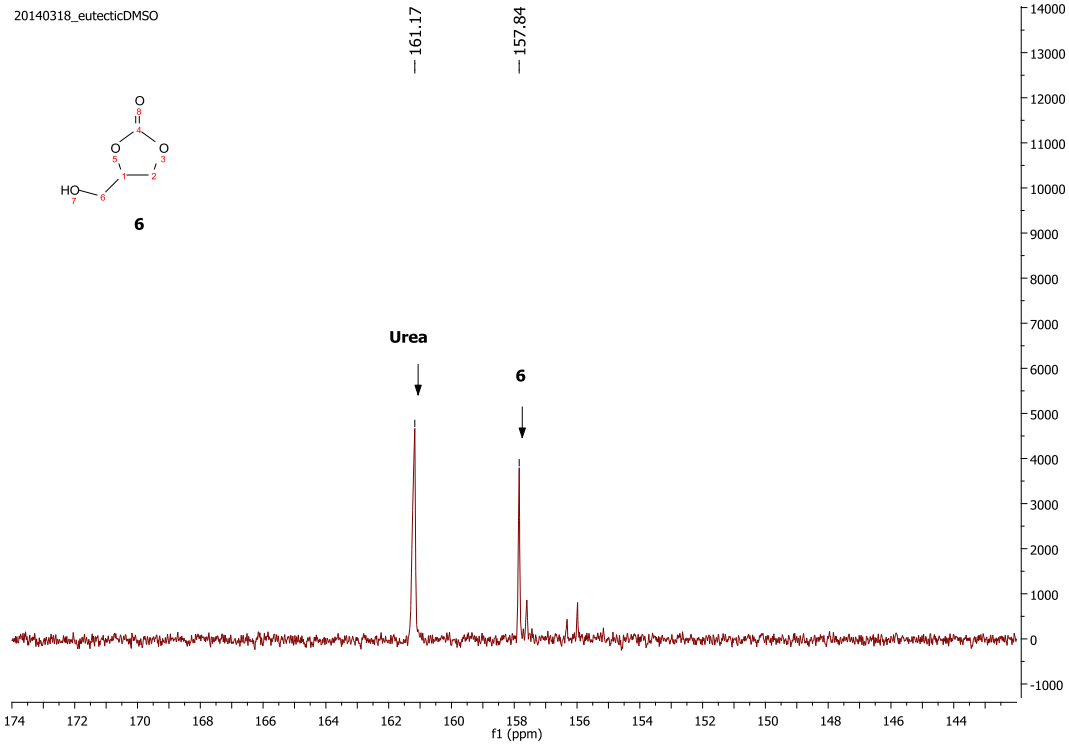


Figure 3. Reference ^{13}C NMR spectra of pure urea in DMSO-d6.



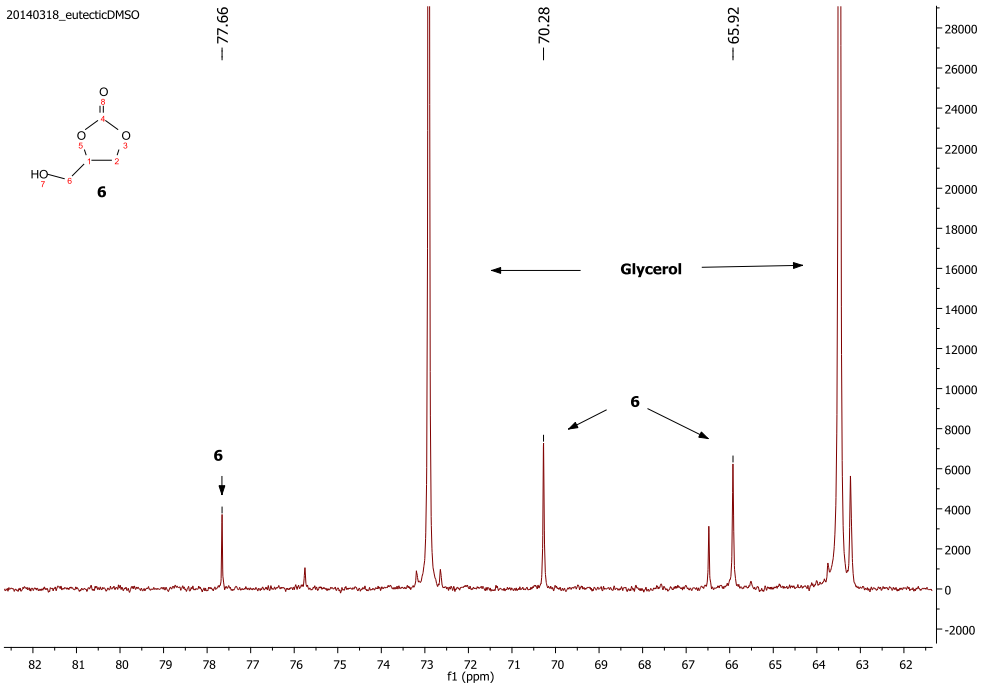
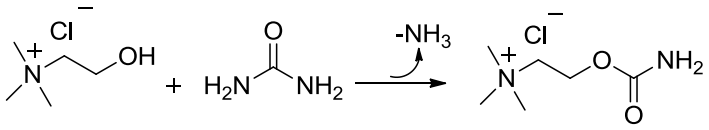


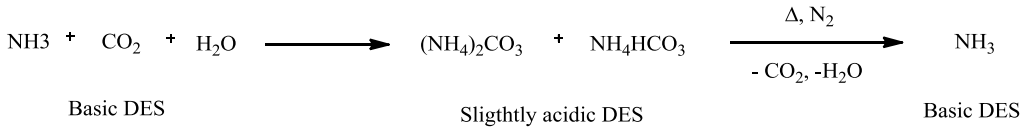
Figure 4. ^{13}C NMR spectra of glycerol urea mixture (80ºC, 3 weeks) in DMSO-d6.

Our results could also explain the basicity of one of the most commonly used urea based DES ChCl/urea (1:2), although the rate of urea decomposition seems to be lower compared to polyalcohol based DES, since only traces of product **2** were observed (Table 13, entry 3). Probably the lower amount of formed ammonia is due to the fact that ChCl, being mono alcohol, cannot form cyclic carbonates (Scheme 35) *via* favorable intermolecular cyclization (Scheme 33).



Scheme 35.

Going back to the literature and the studies on the basicity and acidity of DES, it is obvious that the only DES that exhibit unexpected basicity are the urea based ones. The other DES are either neutral or the origin of the basicity or acidity is due to the nature of the ingredients. We believe that our observation provide simple and rational explanation of this phenomena. Zhou *et al*.[21] observed the absorption of CO_2 by ChCl/urea DES that could also be due to the presence of ammonia, which reacts to form ammonium carbonate or bicarbonate and switches the pH of the DES when all the ammonia is reacted. Moreover when the saturated with CO_2 DES is heated up to 60ºC and flashed with N_2, the known to be thermally unstable ammonium salts transforms back to ammonia and CO_2 restoring the initial basicity of the ChCl/urea DES, as reported by the authors (Scheme 36).



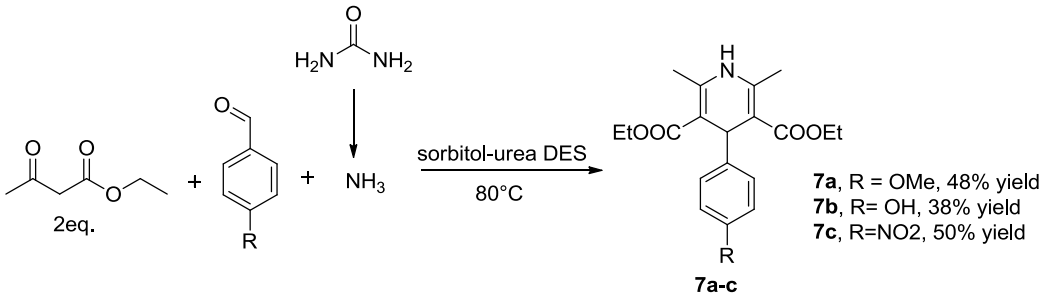
Scheme 36.

The reported by König *et al*.[32] of poor results when D-fructose/urea melt was subjected to $FeCl_3$ catalyzed dehydration of fructose to HMF could also be due to ammonia formation. The authors did not observed any HMF yield when D-fructose/urea melt was used (Table 14, entry 1), since HMF is known to be unstable under basic conditions ammonia could be a reason for its decomposition. Moreover the reaction was performed at 100ºC which is higher than the used by us 80ºC, thus resulting in even higher rate of urea decomposition. Switching the D-fructose melts to DMU and tetramethyl urea (TMU), which are much less prompt to form carbonates and liberate methylammonia or dimethylammonia, the authors observed HMF formation in 8 and 89% respectively (**Table 14**).

Table 14. HMF formation from D-fructose in low melting mixtures catalyzed by 10mol% $FeCl_3$

Entry	Melt composition	HMF yield.
1	D-fructose:urea, 2:3w:w ratio	No product
2	D-fructose:DMU, 2:3w:w ratio	8%
3	D-fructose:TMU, 9:1w:w ratio	89%

We also screened different aryl aldehydes under the same conditions used for the synthesis of **2**, moderate yields up to 50% were obtained for 1,4 dihydropyridine derivatives **7a-c**. Proving that the formation of ammonia should be taken into account, when these type of DES are used, not only as a catalyst, but also as a reactive nucleophile (Scheme 37).



Scheme 37.

Having these results in hands, we investigated the stability of already reported DES in a systematic way. We trapped the liberated during the formation of DES ammonia in an aqueous acid solution and after titration with a base in presence of indicator, we calculate the amount of ammonia, which could be directly related to the rate of urea decomposition. 80°C was chosen for our study because it can mimic commonly used by many researchers conditions for the

formation of DES. In a typical experiment DES was heated at 80°C for 7h and flashed with N_2, which was then bubbled trough 10ml 0.05M water solution of H_2SO_4, at the end the solution was titrated with 0.05M solution of KOH using phenolphthalein as indicator, the results are summarized in **Table 15**. For the synthesis of DES we used one and the same amount of urea or DMU (2.4g) and recalculated the other ingredients as they are reported in the literature.

Table 15. Results from the titration of the captured ammonia in 10ml 0.05M water solution of H_2SO_4 after 7h at 80ºC.

Entry	DES/molar ratio	0.05M KOH, ml.[a]	NH_3 or CH_3NH_2 mmol	NH_3 yield %[c,d]
1[14]	ChCl+Urea/1:2	18.5	0.075	0.09
2[b]	Sorbitol+Urea/5.2:4	9.3	0.535	0.66
3	Pure Urea	20	0.000	0
4[20]	Glucose+urea+$CaCl_2$/1.6:4:0.5	19	0.005	0.006
5	Glycerol+urea/10:4	4.9	0.755	0.94
6[14]	ChCl+DMU/1:2	19.5	0.025	0.03
7	Glycerol+DMU/10:4	19.5	0.025	0.03
8	Sorbitol+DMU/5.2:4	19.3	0.035	0.04
9[20]	Fructose+urea/3:2	18	0.100	0.12

[a] Theoretical volume of 0.05M KOH needed for complete neutralization of H_2SO_4 solution (10ml, 005M) is 20ml. [b]ureas/sorbitol DES was previously prepared in this molar ratio in our laboratory. [c] Calculated as a percent from the theoretical yield of ammonia from the full urea decomposition. [d] The ammonia, which remain dissolved in the DES is not taken into account and the actual yields are expected to be higher.

The obtained results were with agreement with our previous observation that the urea decomposition is accelerated in presence of polyalcohols compared to ChCl. As it was expected pure urea was complete stable at 80ºC and no ammonia was trapped (Table 15, entry 3). The highest rate of ammonia formation was observed in case of glycerol (Table 15, entry 5) and sorbitol (Table 15, entry 2). In case of ChCl only small amount of ammonia was detected, which explained why compound **2** was detected only as traces in our previous experiments (Table 13, entry 3). When carbohydrates like glucose and fructose were employed for the formation of DES with urea, only minor amounts of ammonia were detected. However, after 7 hours at 80°C the color of these DES turns brown, probably due to carbohydrates decomposition and formation of humins, which also should be taken into account when this type of DES are used as a reaction media with heating. Switching from urea to dimethylurea DMU more stable DES were obtained since DMU is less reactive and almost no formation of methylamine was observed, even when combined with glycerol and sorbitol.

4. Conclusion.

In summary, we have investigated the stability of various urea based DES. A simple and rational explanation of the previous observed by other researches unusual basicity of these DES was provided by urea decomposition and formation of ammonia, even at lower than expected temperatures. The presence of ammonia was detected by an unexpected formation of

dihydropyridines *via* Hantzsch synthesis in sorbitol/urea DES and further on confirmed with Nesstler reagent test. We also found out that the origin of ammonia is the urea decomposition during the formation of DES and it is not an impurity in the ingredients. The stability of several reported urea and DMU based DES was tested by ammonia trapping in acid solution and titration. The results shown that urea decomposition is accelerated in mixtures with polyalcohols presumably due to the formation of cyclic carbonates, while in mixture with monoalcohols it was observed to be stable. ChCl was found to be an exception, although being monoalcohol, some urea decomposition was detected. However, in much lower rate compared to glycerol and sorbitol. As it was expected DMU based DES were more stable since DMU is less prompt to react with alcohols to form carbonates. Our observations could be important for all the researchers using urea based DES in organic synthesis since, as we shown, ammonia could be a reactive nucleophile in organic reactions. The formation of ammonia and DES basicity should also be considered when enzymatic reactions are performed in such solvents because it can affect the reactivity and the stability of the enzymes.

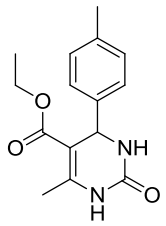
5. Experimental

General: All the reagents used were purchased from Sigma-Aldrich or Merck and were used without further purification. The reaction evolution was followed by TLC using silica Merck Kieselgel 60 F254 plates, and revealed by ultraviolet light at 254 nm and 325 nm. NMR spectra were recorded at room temperature in a Bruker AMX 300 or Bruker AMX 400 using $CDCl_3$ or DMSO-d_6 as solvents.

Preparation of Sorbitol-Urea DES: 13,96 g of urea and 49,8 g of sorbitol were mixed and stirred at 80°C for 3h till homogeneous liquid mixture was formed.

Begineli reaction.

Ethyl 6-methyl-2-oxo-4-(p-tolyl)-1,2,3,4-tetrahydropyrimidine-5-carboxylate (1).[50]



5g of sorbitol/urea DES were placed in a round bottom flack then *p*-tolyl aldehyde (1mmol, 120mg), ethyl acetoacetate (1mmol, 130mg) and *p*-TsOH (0.2mmol, 34mg) were added and stirred overnight at 90°C. The reaction was dissolved in 10 ml of water. After complete dissolution, the product was extracted with ethyl acetate 2x25ml. The organic phase was dried

over Na_2SO_4 and evaporated and the crude was purified with automatic flash chromatography machine -CombiFlash using gradient mixing of hexane and ethylacetate to give 260.4 mg (95%) of the target compound with no enantioselectivity by HPLC.

Reported M.p 170-172 °C; found: 171-172ºC.

The spectral data (^{1}H and ^{13}C NMR) is identical with the reported one.[51]

^{1}H NMR (300 MHz, DMSO-d6) δ (ppm) 9.16 (s, 1H, NH), 7.68 (br s, 1H, NH), 7.12 (s,4H), 5.10 (d, 1H, J=3.3 Hz), 3.36 (q, 2H, J=7.1 Hz), 2.49 (s, 3H), 2.24 (s, 3H), d 1.10 (t, 3H, J=7.1 Hz)

^{13}C NMR (100 MHz, DMSO-d6) δ (ppm), 166.3, 152.9, 148.9, 142.8, 137.3, 129.7, 126.8, 100.2, 59.9, 54.5, 21.4, 18.5, 14.8.

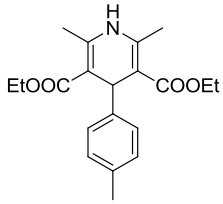
The HPLC analysis was performed using Shimadzu LC-20AT HPLC pump, SPD-M20A PDA detector, manual injector coupled with 20µl loop and Chiralpak OD 250x4mm column, mobile phase was Hexane:*i*-PrOH 95:5, flow 1ml/min, detection at 254nm. Enantiomers retention times: 10.4 and 11.8 min.

Synthesis of Nessler reagent: A saturated solution of $HgCl_2$ (~2.2g in 35ml of distilled water) was added to a solution of 5g of KI in 5ml of distilled water until the excess is indicated by the formation of a precipitate. Then 20ml of 5N NaOH were added and the mixture diluted to 100ml with a distilled water. The solution was left to settle and the clear liquid was draw off.

Nessler test: To a solution of 0.1g sorbitol/urea DES, urea or sorbitol in 4ml of distilled water were added several drops of the Nessler reagent. The appearance of yellow color was followed as a mark for a presence of ammonia.

General procedure for the synthesis of 1,4 dihydropyridine derivatives: 5g of sorbitol/urea DES were placed in a round bottom flack then 1mmol of the corresponding aromatic aldehyde and 2 mmol of ethyl acetoacetate were added and stirred overnight at 90°C. The reaction was dissolved in 10 ml of water. After complete dissolution, the product was extracted with ethyl acetate 2x25ml. The organic phase was dried over Na_2SO_4 and evaporated and the crude was purified with automatic flash chromatography machine (CombiFlash) using gradient mixing of hexane and ethylacetate.

Diethyl 2,6-dimethyl-4-(p-tolyl)-1,4-dihydropyridine-3,5-dicarboxylate (2).[52]



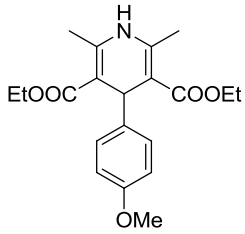
Light yellow solid, yield: 140mg, 41%. Reported M.p: 136–137°C;[52] found: 140-142ºC.

^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm) δ 7.09 (d, *J* = 7.6 Hz, 2H), 6.93 (d, *J* = 7.6 Hz, 2H), 5.67 (s, 1H), 4.88 (s, 1H), 4.01 (q, *J* = 7.0 Hz, 4H), 2.24 (s, 6H), 2.20 (s, 3H), 1.15 (t, *J* = 7.1 Hz, 6H). **^{13}C NMR (75 MHz, $CDCl_3$) δ (ppm)** δ 167.82, 143.93, 135.62, 128.68, 127.95, 104.36, 59.82, 39.22, 21.17, 19.68, 14.38.

ESI-MS: calculated for $[C_{20}H_{25}NO_4 + H]^+$/z: 344,24; found: (M+H)/z: 344,15

IR (KBr, cm^{-1}): 3342.64 (NH), 2982.11 (Ar-H), 2936.77 (CH), 1693.65 (C=O).

Diethyl 4-(4-methoxyphenyl)-2,6-dimethyl-1,4-dihydropyridine-3,5-dicarboxylate (7a).[53]



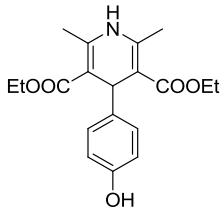
Light yellow solid, yield: 160mg, 48%. Reported M.p: 148–153°C;[53] found: 156-157ºC.

^{1}H NMR (400 MHz, CDCl3) δ (ppm) 7.19 (d, *J* = 8.7 Hz, 2H), 6.74 (d, *J* = 8.7 Hz, 2H), 5.62 (s, 1H,), 4.92 (s, 1H), 4.21 – 3.99 (m, 4H,), 3.75 (s, 3H,), 2.32 (s, 6H), 1.22 (t, *J* = 7.1 Hz, 6H). **^{13}C NMR (101 MHz, CDCl3) δ (ppm)** 167.83, 158.00, 143.66, 140.46, 129.10, 113.31, 104.54, 59.84, 55.27, 38.86, 19.74, 14.41.

ESI-MS: calculated for $[C_{20}H_{25}NO_5 + H]^+$/z: 360,42; found: (M+H)/z: 360,08

IR (KBr, cm^{-1}): 3342.64 (NH), 2983.88 (Ar-H), 2956.87 (CH), 1689.64 (C=O).

Diethyl 4-(4-hydroxyphenyl)-2,6-dimethyl-1,4-dihydropyridine-3,5-dicarboxylate (7b).[54]



Yellow solid, yield: 131mg, 38%. Reported M.p: 230-231ºC;[54] found: 219-220ºC.

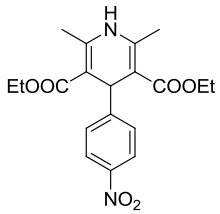
1H NMR (400 MHz, DMSO) δ (ppm) 9.07 (s, 1H), 8.70 (s, 1H), 6.92 (d, *J* = 8.5 Hz, 2H), 6.57 (d, *J* = 8.5 Hz, 2H), 4.74 (s, 1H), 4.03 – 3.93 (m, 4H), 2.23 (s, 6H), 1.13 (t, *J* = 7.1 Hz, 6H).

13C NMR (101 MHz, DMSO) δ (ppm) 167.45, 155.78, 145.11, 139.25, 128.63, 114.86, 102.63, 59.23, 38.20, 18.55, 14.55

ESI-MS: calculated for $[C_{19}H_{23}NO_5 + H]+/z$: 346,40; found: (M+H)/z: 346,02

IR (KBr, cm-1): 3346.50 (N-H), 2985.81 (Ar-H), 2937.59 (CH), 1662.64 (C=O), 1442.75 (C-OH).

Diethyl 2,6-dimethyl-4-(4-nitrophenyl)-1,4-dihydropyridine-3,5-dicarboxylate (7c).[53]



Yellow solid, yield: 189mg, 50%. Reported M.p: 118–127°C;[53] found: 129-130ºC.

^{1}H NMR (400 MHz, CDCl3) δ (ppm) 8.07 (d, *J* = 8.8 Hz, 2H), 7.44 (d, *J* = 8.8 Hz, 2H), 5.84 (s, 1H), 5.08 (s, 1H), 4.11 – 4.05 (m, *J* = 7.1, 3.1 Hz), 2.34 (s, 6H), 1.21 (t, *J* = 7.1 Hz, 6H).

^{13}C NMR (101 MHz, CDCl3) δ (ppm) 167.10, 155.14, 146.34, 144.70, 128.92, 123.30, 103.18, 60.02, 40.14, 19.65, 14.27.

ESI-MS: calculated for $[C_{19}H_{22}N_2O_6 + H]+/z$: 375,40, found: (M+H)/z: 375,06

IR (KBr, cm 1): 3319.49 (NH), 2926.01 (Ar-H), 2852.72 (CH), 1701.22 (C=O).

6. References.

1. Anastas, P. T. *Chem. Rev.*, 2007, *107*, 2167.
2. Horváth, I. T.; Anastas, P. T. *Chem. Rev.*, 2007, *107*, 2169.
3. Hallett, J. P.; Welton, T. *Chem. Rev.*, 2011, *111*, 3508.
4. Park, S.; Kazlauskas, R. J. *Curr. Opin. Biotechnol.*, 2003, *14*, 432.
5. van Rantwijk, F.; Sheldon, R. A. *Chem. Rev.*, 2007, *107*, 2757.
6. Renner, R. *Environ. Sci. Technol.*, 2001, *35*, 410A.
7. Thuy Pham, T. P.; Cho, C.-W.; Yun, Y.-S. *Water Res.*, 2010, *44*, 352.
8. Zhang, Q.; De Oliveira Vigier, K.; Royer, S.; Jerome, F. *Chem. Soc. Rev.*, 2012, *41*, 7108.
9. Dai, Y.; van Spronsen, J.; Witkamp, G.-J.; Verpoorte, R.; Choi, Y. H. *J. Nat. Prod.*, 2013, *76*, 2162.
10. Ru; Konig, B. *Green Chem.*, 2012, *14*, 2969.
11. Imperato, G.; Hoger, S.; Lenoir, D.; Konig, B. *Green Chem.*, 2006, *8*, 1051.
12. Maugeri, Z.; Dominguez de Maria, P. *RSC Adv.*, 2012, *2*, 421.
13. Abbott, A. P.; Barron, J. C.; Ryder, K. S.; Wilson, D. *Chem. Eur. J.*, 2007, *13*, 6495.
14. Abbott, A. P.; Capper, G.; Davies, D. L.; Rasheed, R. K.; Tambyrajah, V. *Chem. Commun.*, 2003, 70.
15. Abbott, A. P.; Boothby, D.; Capper, G.; Davies, D. L.; Rasheed, R. K. *J. Am. Chem. Soc.*, 2004, *126*, 9142.

16. Kareem, M. A.; Mjalli, F. S.; Hashim, M. A.; AlNashef, I. M. *J. Chem. Eng. Data*, 2010, *55*, 4632.
17. Shahbaz, K.; Mjalli, F. S.; Hashim, M. A.; AlNashef, I. M. *Energy Fuels*, 2011, *25*, 2671.
18. Shahbaz, K.; Baroutian, S.; Mjalli, F. S.; Hashim, M. A.; AlNashef, I. M. *Thermochim. Acta*, 2012, *527*, 59.
19. Hayyan, A.; Mjalli, F. S.; AlNashef, I. M.; Al-Wahaibi, Y. M.; Al-Wahaibi, T.; Hashim, M. A. *J. Mol. Liq.*, 2013, *178*, 137.
20. Imperato, G.; Eibler, E.; Niedermaier, J.; Konig, B. *Chem. Commun.*, 2005, 1170.
21. Li, W.; Zhang, Z.; Han, B.; Hu, S.; Song, J.; Xie, Y.; Zhou, X. *Green Chem.*, 2008, *10*, 1142.
22. J. Naser, F. M., B. Jibril, S. Al-Hatmi, Z. Gano *Int. J. Chem. Eng. Appl.*, 2013, *4*, 114.
23. Phadtare, S. B.; Shankarling, G. S. *Green Chem.*, 2010, *12*, 458.
24. Pawar, P. M.; Jarag, K. J.; Shankarling, G. S. *Green Chem.*, 2011, *13*, 2130.
25. Sonawane, Y. A.; Phadtare, S. B.; Borse, B. N.; Jagtap, A. R.; Shankarling, G. S. *Org. Lett.*, 2010, *12*, 1456.
26. Siddalingamurthy, E.; Mahadevan, K. M.; Shrungesh Kumar, T. O. *Synth. Commun.*, 2013, *43*, 3153.
27. Ilgen, F.; Konig, B. *Green Chem.*, 2009, *11*, 848.
28. Singh, B.; Lobo, H.; Shankarling, G. *Catal. Lett.*, 2011, *141*, 178.
29. Rajawat, A.; Khandelwal, S.; Kumar, M. *RSC Adv.*, 2014, *4*, 5105.
30. Hu, S.; Zhang, Z.; Zhou, Y.; Han, B.; Fan, H.; Li, W.; Song, J.; Xie, Y. *Green Chem.*, 2008, *10*, 1280.
31. Hu, S.; Zhang, Z.; Zhou, Y.; Song, J.; Fan, H.; Han, B. *Green Chem.*, 2009, *11*, 873.
32. Ilgen, F.; Ott, D.; Kralisch, D.; Reil, C.; Palmberger, A.; Konig, B. *Green Chem.*, 2009, *11*, 1948.
33. Chen, Z.; Zhou, B.; Cai, H.; Zhu, W.; Zou, X. *Green Chem.*, 2009, *11*, 275.
34. Gore, S.; Baskaran, S.; Koenig, B. *Green Chem.*, 2011, *13*, 1009.
35. Bafti, B.; Khabazzadeh, H. *J. Chem. Sci.*, 2014, *126*, 881.
36. Vidal, C.; Suárez, F. J.; García-Álvarez, J. *Catal. Commun.*, 2014, *44*, 76.
37. Handy, S.; Lavender, K. *Tetrahedron Lett.*, 2013, *54*, 4377.
38. Handy, S.; Wright, M. *Tetrahedron Lett.*, 2014, *55*, 3440.
39. Azizi, N.; Dezfooli, S.; Hashemi, M. M. *C. R. Chim.*, 2013, *16*, 1098.
40. Pednekar, S.; Bhalerao, R.; Ghadge, N. *J. Chem. Sci.*, 2013, *125*, 615.
41. Vidal, C.; García-Álvarez, J.; Hernán-Gómez, A.; Kennedy, A. R.; Hevia, E. *Angew. Chem., Int. Ed.*, 2014, *53*, 5969.
42. Gorke, J. T.; Srienc, F.; Kazlauskas, R. J. *Chem. Commun.*, 2008, 1235.
43. Zhao, H.; Baker, G. A.; Holmes, S. *Org. Biomol. Chem.*, 2011, *9*, 1908.
44. Maugeri, Z.; Leitner, W.; Domínguez de María, P. *Eur. J. Org. Chem.*, 2013, *2013*, 4223.
45. Suresh; Sandhu, J. S. *Arkivoc*, 2012, 66.
46. Kappe, C. O. *Eur. J. Med. Chem.*, 2000, *35*, 1043.
47. Saini, A. K., Sanjay Sandhu, Jagir S *J.Sci. Ind. Res.*, 2008, *67*, 95.
48. Schaber, P. M.; Colson, J.; Higgins, S.; Thielen, D.; Anspach, B.; Brauer, J. *Thermochim. Acta*, 2004, *424*, 131.
49. Li, Q.; Zhang, W.; Zhao, N.; Wei, W.; Sun, Y. *Catal. Today*, 2006, *115*, 111.
50. Jing, X.; Li, Z.; Pan, X.; Shi, Y.; Yan, C. *J. Iran. Chem. Soc.*, 2009, *6*, 514.
51. Liberto, N. A.; de Paiva Silva, S.; de Fátima, Â.; Fernandes, S. A. *Tetrahedron*, 2013, *69*, 8245.
52. Bandyopadhyay, D.; Maldonado, S.; Banik, B. K. *Molecules*, 2012, *17*, 2643.
53. Affeldt, R. F.; Benvenutti, E. V.; Russowsky, D. *New J. Chem.*, 2012, *36*, 1502.
54. Koukabi, N.; Kolvari, E.; Khazaei, A.; Zolfigol, M. A.; Shirmardi-Shaghasemi, B.; Khavasi, H. R. *Chem. Commun.*, 2011, *47*, 9230.

Chapter IV

Physical properties, toxicity and unconventional applications of Magnetic Ionic Liquids

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This chapter aims to provide brief overview on the reported in the literature synthesis, physical properties and applications of a specific class Ionic Liquids namely Magnetic Ionic Liquids (MILs). Some original research and discussion on the effect of magnetic field on the transport of various compounds trough bulky MILs membranes and on the organic reactions performed in MILs will be presented. The results have been published in 3 peer-reviewed publications:

1. R. Frade, S. Simeonov, A. Rosatella, F. Siopa, C. Afonso, Toxicological evaluation of magnetic ionic liquids in human cell lines, CHEMOSPHERE, ***2013****, 92, 100-105.*

2. I. de Pedro, A. Garcia-Saiz, J. Gonzalez, I. de Larramendi, T. Rojo, C. Afonso, S. Simeonov, J. Waerenborgh, J. Blanco, B. Ramajo, J. Fernandez, Magnetic ionic plastic crystal: choline[$FeCl_4$], Phys. Chem. Chem. Phys., ***2013****, 15, 12724-12733.*

3. J. Albo, E. Santos, L. Neves, S. Simeonov, C. Afonso, J. Crespo, A. Irabien, Separation performance of CO_2 through Supported Magnetic Ionic Liquid Membranes (SMILMs), Sep. Purif. Technol., ***2012****, 97, 26-33.*

Table of content.

1. Introduction.

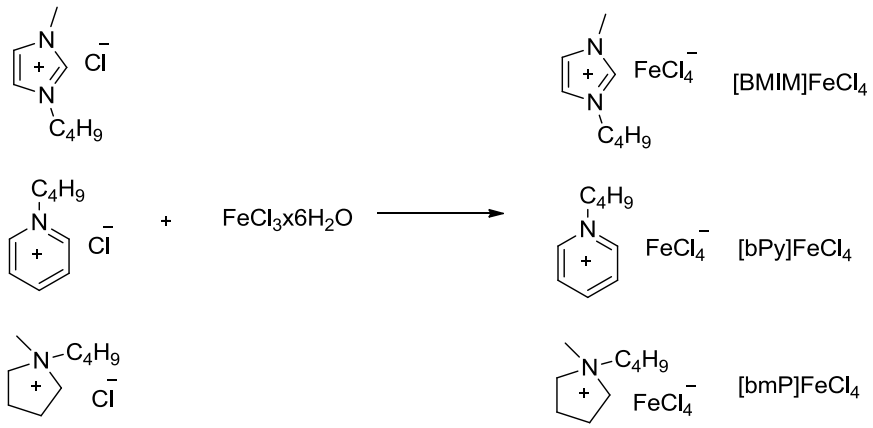
Ionic Liquids (IL) are ionic compounds (salts) which are liquid below 100 ℃ and more commonly, IL have melting points below room temperature. Typically the ions in IL are poorly coordinated and at least one ion has a delocalized charge and one component is organic, which prevents the formation of a stable crystal lattice. IL are considered as a "green" replacement of the commonly used organic solvents because of some unique properties, like their extremely low vapor pressure and high thermal stability, which offers advantages such as ease of containment, product recovery, and recycling ability.[1] Numerous articles in the literature describe their application as solvents and catalysts for diverse organic transformations,[2-4] as well as industrial applications.[5] Another advantage of IL is the possibility their properties to be tuned for specific applications by carefully choosing the cataion and anion combinations.[6-9]

An unique class of IL namely magnetic ionic liquids MIL was discovered in 2004 by Hayashi and Hamaguchi.[10] The authors observed that an ionic liquid synthesized by mixing 1-butyl-3-methylimidazolium chloride [BMIM]Cl and $FeCl_3$ exhibit high response towards external magnetic field. The structure of the MIL was proven to be [BMIM]$FeCl_4$ and the formation of high spin $FeCl_4^-$ anion responsible for the magnetic properties was observed by visible absorption spectroscopy.

Figure 1. Response of [BMIM]$FeCl_4$ towards external magnetic field.

1.1. Synthesis and physical properties of MILs.

The pioneer paper of Hayashi and Hamaguchi open a new research field of the magnetism and specific properties of this class of IL. Li et al.[11] reported the synthesis and characterization of three species of room temperature MILs, 1-butyl-3-methylimidazolium tetrachloroferrate ([BMIM]$FeCl_4$), N-butylpyridium tetrachloroferrate ([bPy]$FeCl_4$) and 1-butyl-1-methylpyrrolidium tetrachloroferrate ([bmP]$FeCl_4$) (Scheme 1).



Scheme 1.

The magnetic susceptibilities of the three MILs were measured at a certain temperatures. The results showed that the magnetic susceptibility of [bmP]$FeCl_4$ is the lowest, while the magnetic susceptibility of [bPy]$FeCl_4$ is a little higher than that of [BMIM]FeCl4 at the same order of magnitude. In addition, the magnetic properties of [BMIM]FeCl4, [bPy]FeCl4 and [bmP]FeCl4 were observed to be paramagnetic from 5 K to 300 K.

The synthesis, magnetic properties of [CMMIM]$FeCl_4$ MIL (Figure 2) and cellulose solubility in it was reported by Ito *et al.*[12]

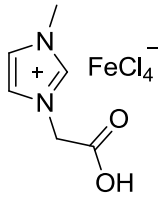
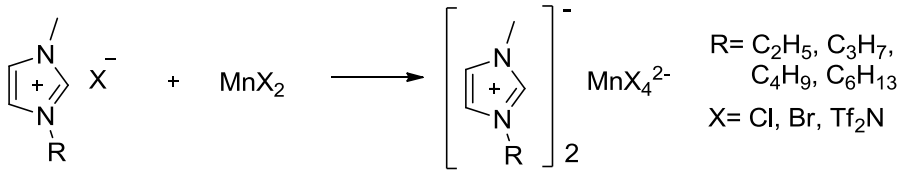


Figure 2. Chemical structures of [CMMIM]$FeCl_4$.

Magnet behavior of [CMMIM]FeCl4 was examined with magnetic field range from −10,000 to 10,000 Oe at 273 K and showed a linear response, the magnetic susceptibility of [CMMIM]$FeCl_4$ was calculated to be 16.3×10^{-5} emu g^{-1}.

MILs based on the phosphonium cation $[P_{66614}]^+$ and magnetic anions: $[GdCl6]^{3-}$, $[MnCl4]^{2-}$, $[FeCl4]^-$ and $[CoCl4]^{2-}$ were synthesized and the influence of the temperature on their viscosity was studied by modeling estimation and experimentally.[13] Good agreement of the theory and experimental results was observed, presenting a mean percentage deviation of 7.64%.

Mudring *et al.*[14] reported the synthesis and study of the physico-optical properties of several Mn^{2+} based MILs with chloro, bromo, bis(trifluoromethanesulfonyl)amido (Tf_2N) ligands and *n*-alkyl-methylimidazolium cations as counter ions (Scheme 2).



Scheme 2.

Broad range of MILs based on Mn^{2+}, Gd^{3+}, Ho^{3+}, Dy^{3+}, Mn^{2+} and Fe^{+} chlorides or bromides and 1-butyl-3-methyl imidazolium [BMIM], 1-butyl-2,3-dimethyl imidazolium [BDMIM], methyltrioctylammonium [Aliq] or alanine methyl ester [AlaC1] were synthesized and their physico-chemical, magnetic, and thermal properties were studied by Whitesides *et al*.[15] The main focus of the work was the application of the MILs for measurements of density using magnetic levitation (Figure 3).

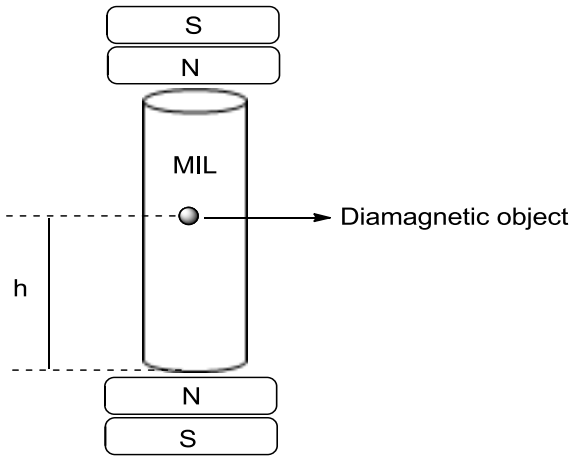


Figure 3. Schematic set-up for measuring the density of diamagnetic objects in MILs by magnetic levitation.

The experiments have been performed using NdFeB magnets faced with the same poles to each other. The levitation height (h) of the diamagnetic object can be directly correlated to its density. Compared to the conventional methods using solutions of paramagnetic salts, MILs have the advantages to be nonvolatile, highly thermally stable and possess low melting points.

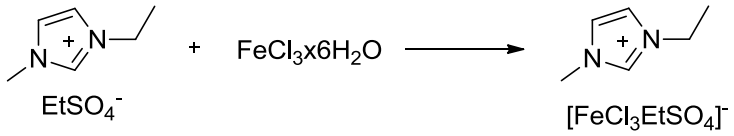
Williams *et al*.[16] synthesized number of MILs from Co, Mn, Fe or Gd halides and imidazolium or phoosphonium ionic liquids. Most of the MILs were obtained mainly by direct mixing of the transition metal halides and corresponding ionic liquid, although in some cases heating or $CHCl_3$-H_2O 1:1 v/v mixture as a reaction media were required. Additionally the authors studied some physical, electrochemical, and magnetic properties of selected MILs.

Koo *et al*.[17] reported the synthesis and recovery of [BMIM]$FeCl_4$ from its 50%v/v biphasic systems with water, using electromagnet. Although the separation of [BMIM]$FeCl_4$ from

20%v/v homogenous water solution was unsuccessful it was observed variation of the concentration as a function of the magnetic field strength. Later the problem of recovering [BMIM]$FeCl_4$ from homogeneous water solutions was solved by Chen *et al.*[18] *via* a simple two-step method of phase-division by adding inorganic salt, plus chemical extraction, or alternatively, ultracentrifugation or ultra-strong magnetic field. This method was successfully applied for the separation of low contents of 1%v solutions of [BMIM]$FeCl_4$.

The thermal stability of various imidazolium tetrachloroferrate ionic liquids was studied by Voronchikhina *et al.*[19] The authors observed that all the studied MILs were stable up to 380–400°C, in air and the cation nature was a key factor in their thermal stability. The decomposition was carried out in several stages with formation of undecomposed residue.

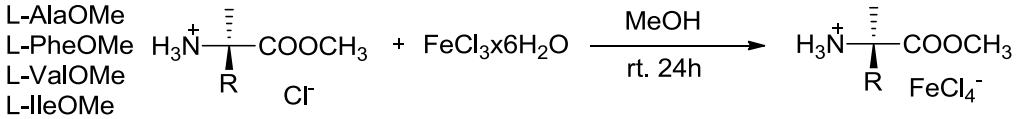
MIL bearing hybrid-type anion was synthesized by Oshiki *et al.*[20] by mixing 1-Ethyl-3-methylimidazolium ethylsulfate ([EMIM][$EtSO_4$]) and $FeCl_3x6H_2O$ at room temperature without solvent (Scheme 3). The obtained MIL was observed to be unstable and gradually decomposed to form [EMIM][FeCl4] and an unidentified precipitate, by a disproportionate reaction.



Scheme 3.

The attempt to be obtained $CoCl_4(EtSO_4)_2^{2+}$ MIL failed since direct decomposition to $CoCl_6^{2+}$ anion was observed.

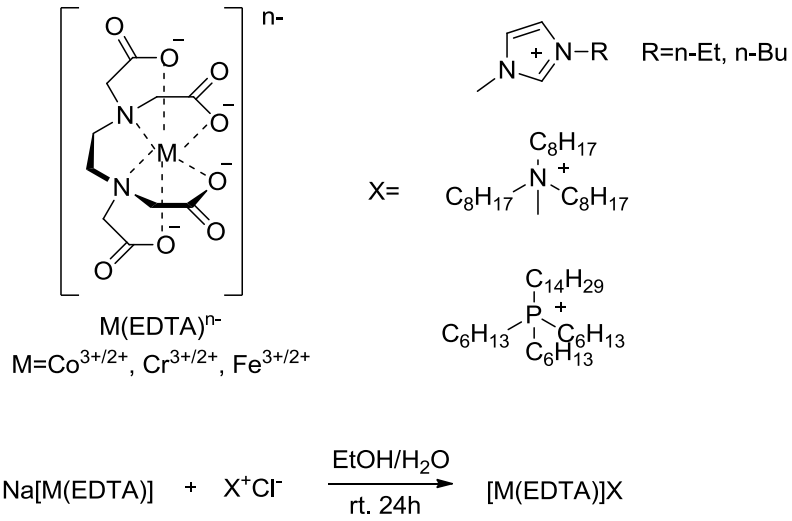
In 2009 Warner *et al.*[21] reported room temperature chiral MILs derived from amino acids (Scheme 4).



Scheme 4.

All the obtained MILs exhibit paramagnetic properties and can be potentially used in asymmetric synthesize and catalysis.

New type of MILs based on Co, Cr, or Fe ethylenediaminetetraacetic complexes were reported by Pina *et al.*[22] (Scheme 5).



Scheme 5.

The obtained MILs possess electrochromic and paramagnetic properties and can switch reversibly from diamagnetic to paramagnetic states upon electrochromic reduction/oxidation. The authors also pointed out their possible application in redox flow batteries.

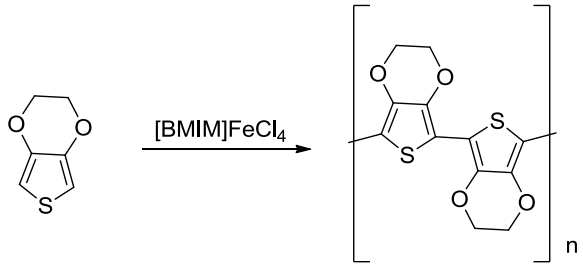
$[C_{12}MIM]_3[DyBr_6]$ (C_{12}MIM=1-dodecyl-3-methylimidazolium) MIL with interesting luminescent behavior, as well as mesomorphic and magnetic properties was reported by Mudring *et al.*[23] The compound exhibit thermotropic liquid crystalline behavior and forms smectic mesophases. Its emission color can be tuned from white to orange-yellow by the choice of the excitation wavelength. Sample excitation with λ=366nm led to the blue-whitish luminescence from the imidazolium cation itself. With λ=254 nm the common Dy(III) emission was observed and the sample appeared orange. The compound showed superparamagnetism and can be manipulated by an external magnetic field, the effective magnetic moment was determined to be μ_{eff} =9.6μB at room temperature.

Irabien *at al.*[24] reported the influence of external magnetic field on the viscosity and gas permeability of $[P_{66614}][CoCl_4]$, $[P_{66614}][FeCl_4]$, $[P_{66614}][MnCl_4]$ and$[P_{66614}][GdCl_6]$ MILs supported on commercial hydrophobic PVDF porous support. The MILs were synthesized in our group by mixing $[P_{66614}]$Cl and corresponding metal chlorides in DCM at room temperature. It was observed that an external magnetic field between 0 and 2T increases the gas permeability for CO_2, N_2 and air without changing the permeability ratio and decreases MILs viscosity, depending on the MILs magnetic susceptibility. $[P_{66614}][GdCl_6]$ showed the maximum CO_2 permeability increase (21.64%) in comparison with the result when no magnetic field was applied.

1.2. Application of MILs in organic synthesis.

Although significant attention to the physical properties of MILs have been paid by many researchers, the examples for their application in organic reactions remains quite limited.

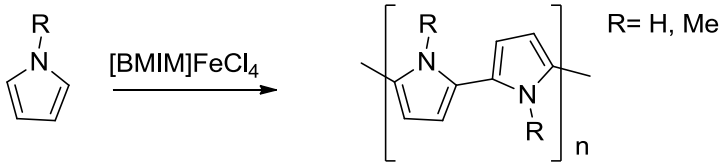
The synthesis of poly(3,4-ethylenedioxythiophene) PEDOT nanospheres with their size ranging around 60 nm has been reported by Ma *et al*.,[25] adding the monomer into [BMIM]$FeCl_4$ without the use of any additional dopant or oxidant (Scheme 6). The MIL led to the formation of uniform nanospheres with a relatively narrow size distribution confined to submicrometer-sized domains. The authors compared the polymers produced in [BMIM]$FeCl_4$ to those synthesized in conventional solution and emulsion polymerizations. The yield and conductivity of the formed in MIL nanospheres were observed to be better.



Scheme 6.

[BMIM]$FeCl_4$ assisted synthesis of polypyrrole/AgCl nanocomposites and their application as a H_2O_2 biosensor was described by Yan *et al*.[26] The MIL served as an oxidant in the interface polymerization system. The polymerization reactions were carried out in water-MIL biphasic for 24h at room temperature.

Nanostructured conducting polypyrrole and poly(*N*-methylpyrrole) were synthesized by adding monomers into [BMIM]$FeCl_4$ (Scheme 7).[27]



Scheme 7.

In this process, self-organized conducting polymer nanostructures, such as particles and tubes, were formed without and with magnetic field. The self-assembled local structures in the solvent ionic liquid are likely to serve as templates of highly organized nanostructured polymers. More attractive nanostructured polymers were obtained from the polymerization of *N*-methylpyrrole resulting in the formation of tubes with nanoscaled inner holes and walls. The polymer morphologies were observed to be differently assembled according to the monomer

structure and the reaction conditions with and without magnetic field. From the polymerization without applying magnetic field, spherical nanoparticles were mainly obtained, while under magnetic field, highly organized shapes, such as rods and tubes, were observed.

Depolymerization of poly(ethylene terephthalate) (PET) in ethylene glycol catalyzed by [BMIM]$FeCl_4$ was reported by Zhang *et al*.[28] Since the use of PET plastics is rising every year their degradation and recycling *via* glycolysis is an important process from economical and environmental point of view. The authors showed that [BMIM]$FeCl_4$ can effectively catalyze the depolymerization process. PET glycolysis reactions were carried out under atmospheric pressure, reaction temperatures ranging from 140ºC to 178°C and times of 3–7h. The highest selectivity of 76.4% for bis(hydroxyethyl) terephthalate (BHET) monomer was observed at 150ºC, along with only 16.5% PET conversion. Full depolymerization was observed at 178°C, but with only moderate BHET selectivity (59.2%).

[BMIM]$FeCl_4$ was also applied from Mat *et al*.[29] as catalyst for esterification of oleic acid to biodiesel. 83.4% conversion has been achieved under optimized condition using ethanol-oleic acid ratio 22-1 at 65°C. Kinetic studies showed that the transformation followed a pseudo-first order reaction, with activation energy and pre-activation energy of 17.97 kJ/mol and 181.62 min^{-1}, respectively. The obtained values were relatively lower compared to other homogeneous or heterogeneous catalysts for esterification of oleic acid, thus indicated that MILs could be promising new type of catalysts for conversion of high free fatty acids feeds to biodiesel.

[BMIM]Fe_2Cl_7 was synthesized by Misuk *et al*.[30] by mixing [BMIM]Cl with excess of $FeCl_3$ resulting in equilibrium mixture of both [BMIM]Fe_2Cl_7, and [BMIM]$FeCl_4$. The authors studied the catalytic behavior of the MIL in a so-called liquid fixed-bed (LFB) in a micro-/meso-structured reactor. The used set-up is presented on Figure 4.

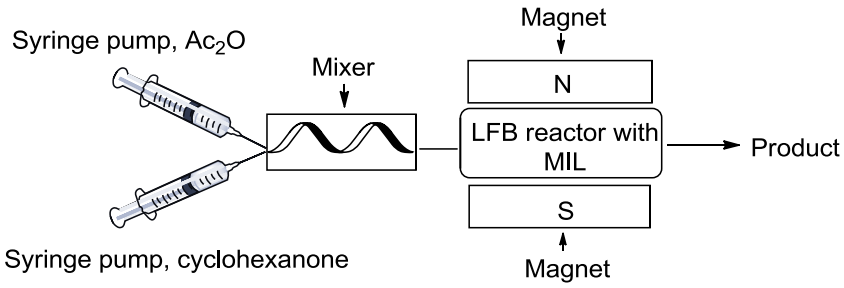
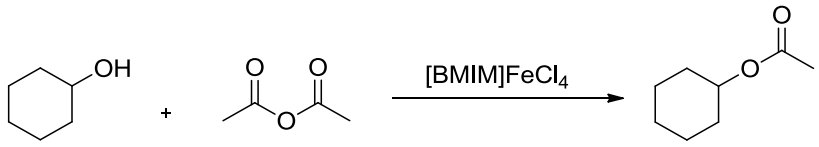


Figure 4. Sketch of the experimental set-up.

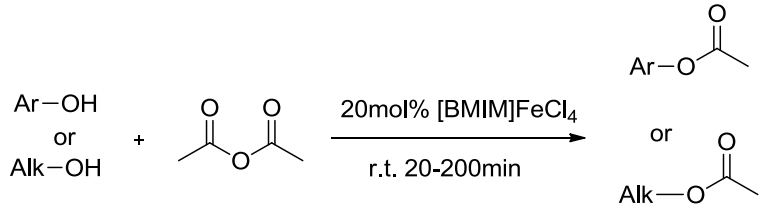
The acetylation of cyclohexanonol with Ac_2O was used as a model reaction (Scheme 8). Droplets of the mixed reactants were passed through the MIL, which was magnetically fixed

by NdFeB magnets inside the reactor. 78.5% yield of the product was achieved with approx. 14s residence time of the reactants. No MIL leaching was observed in the collected reaction mixture. For comparison, the reaction was performed under batch conditions, similar yield of 79% was achived, when 65%mol $[BMIM]Fe_2Cl_7$ was used as catalyst for 180 min. However, additional separation step was required to recover the MIL at the end.



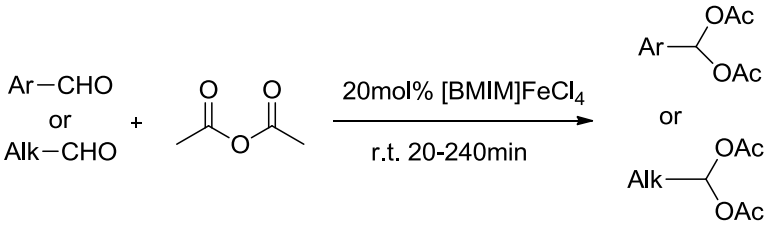
Scheme 8.

Peng *et al*.[31] also reported acetylation reactions catalyzed by $[BMIM]FeCl_4$. Alcohols and phenols were converted to the corresponding acetyl esters at room temperature in good yields, up to 94%, without using additional solvents (Scheme 9).



Scheme 9.

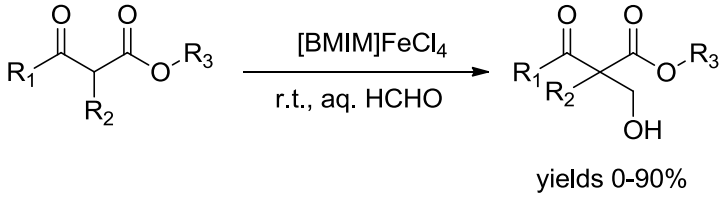
In the same work $[BMIM]FeCl_4$ was employed as a catalyst for the transformation of alkyl and aryl aldehydes to the corresponding 1,1-diacetaes (Scheme 10), while ketones were observed to be unreactive under these conditions. The catalyst was recovered and reused by the authors over 6 cycles without loss of activity.



Scheme 10.

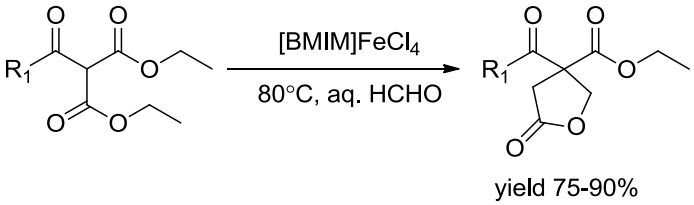
$[BMIM]FeCl_4$ was reported to be effectively catalyzing hydroxylmethylation reactions in aqueous media.[32] Various β-ketoesters and cyclic β-ketoesters were successfully hydroxylmethylated with aq. formaldehyde (Scheme 11). The authors tested also other MILs based on Co, Ni, Cu and Ti, but $[BMIM]FeCl_4$ proved to be the best. The reactions have been

performed at room temperature using 10%mol of [BMIM]$FeCl_4$, similar yields have been observed when the catalyst was applied in only 0.1% mol, although in longer reaction times.



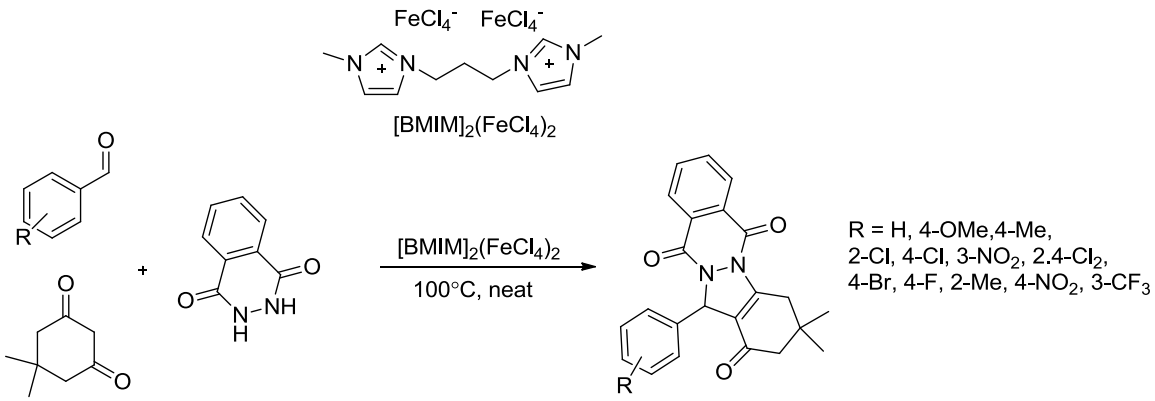
Scheme 11.

The catalyst was successfully recycled over 5 cycles. However, longer reaction times where required in the following cycles. When β-ketoethylmalonates were used as substrates at 80°C, the authors observed *in situ* lactonization and formation of 3-disubstituted butyrolactones in very good yields (Scheme 12).



Scheme 12.

Dicationic MIL, $[BMIM]_2(FeCl_4)_2$ was used as an environmentally benign catalyst under solvent-free conditions for the synthesis of 2*H*-indazolo[2,1-*b*]phthalazinetriones (Scheme 13).[33]

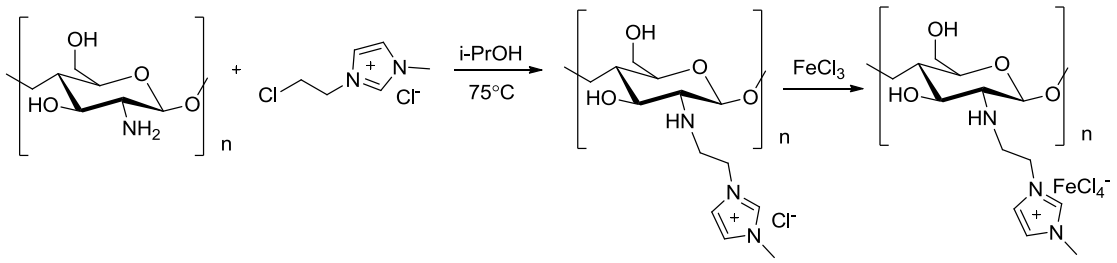


Scheme 13.

The reactions were carried out at 100ºC in presence of 20mol% of the catalysts for 10-15 min and resulted in 86-91% yields. The solid products were isolated by filtration and washed with water, after concentration of the water phase the MIL was separated by using 1.5T

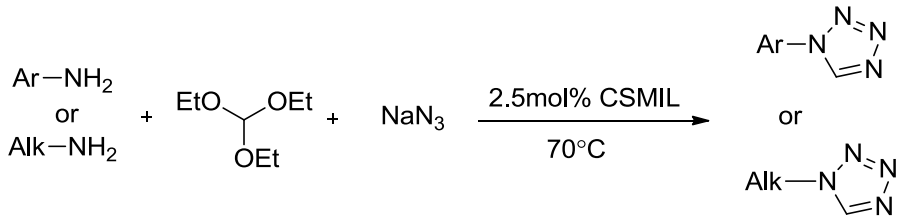
magnetic field and reused. Gradient loss of catalytic activity from 89% to 76% has been observed over 4 cycles.

Efficient synthesis of 1- and 5-substituted 1H-tetrazoles from nitriles and amines has been described, using chitosan supported magnetic ionic liquid nanoparticles (CSMIL) as heterogeneous catalyst.[34] The catalyst was synthesized from the most abundant biopolymer in the nature and also cheap industrial waste chitosan and 3-(2-chloroethyl)-1-methyl imidazolium chloride and was further transformed to CSMILFeCl$_4$ by reaction with FeCl$_3$ (Scheme 14).



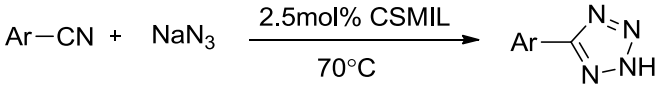
Scheme 14.

The obtained CSMIL was applied in 2.5mol% as catalyst for the synthesis of 1-substituted 1H-tetrazoles (Scheme 15). High yields of 73-91% were obtained in all the cases. The required reaction temperature of 70°C was lower compared to the reported for other catalytic systems.



Scheme 15.

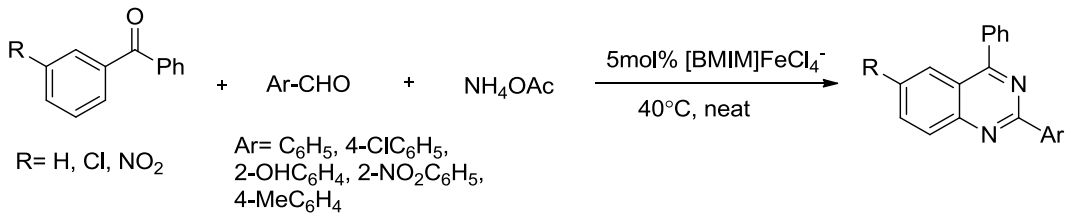
CSMIL was also tested as catalyst for the synthesis of 5-substituted-1H-tetrazoles from the reaction of NaN$_3$ and the corresponding cyanide (Scheme 16).



Scheme 16.

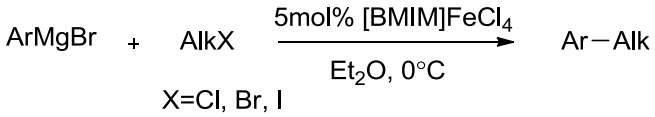
Again high yields of up to 90% have been achieved, under solvent free conditions. The catalyst was successfully recovered by using magnetic field and recycled for 5 times accompanied by the loss of its catalytic activity, due to, the observed by CEM, gelification of chitosan that can easily cover the surface of the MIL particles.

Multicomponent, solvent-free, green synthesis of quinazolines, catalyzed by [BMIM]$FeCl_4$ has been reported by Saha *et al*.[35] (Scheme 17). Employing 5% mol of the catalyst at 40ºC under solvent free condition the corresponding products were obtained in very good yields, above 90%. The [BMIM]$FeCl_4$ was recovered and reused with negligible loss of activity over 4 cycles.



Scheme 17.

Gaertner *et al*.[36] reported in 2006 a cross-coupling reaction of aryl grignard reagents and alkylhalides catalyzed by [BMIM]$FeCl_4$ (Scheme 18). Moderate to high yields, 20-89% have been achieved. The reactions were performed at 0°C using 5 mol% of the catalyst in biphasic system with Et_2O and proved to be completely air and moisture stable, thus could be carried out without inert atmosphere. The catalyst was reused over 5 cycles by the authors.



Scheme 18.

Latter Qi *et al*.[37] reported that 1,3-Bis(2,6-diisopropylphenyl) imidazolium iron tetracholridechloride, [DIPrim]$FeCl_4$ (Figure 5) can promote the same cross-coupling in similar yields.

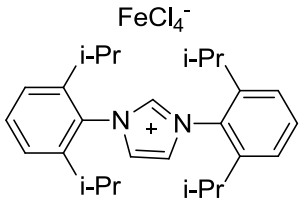
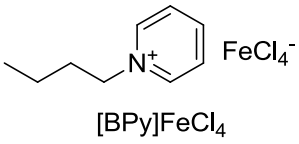


Figure 5.

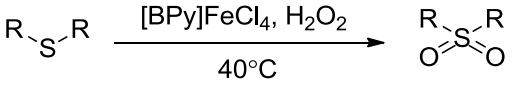
Room temperature MILs were successfully applied for extraction and desulfurization processes. Warner *et al*.[38] reported efficient extraction of phenolic compounds from aqueous media by [P_{66614}]$FeCl_4$. Neodemium magnet (1.4T) was moved circularly by use of an oribital shaker, thus insuring good mixing by forcing the suspended MIL to move synchronously in an aqueous phenolic solution. The authors investigated the conditions for the extraction, including extraction time, volume ratio between MIL and aqueous phase, pH of aqueous solution, and

the structures of the phenolic compounds. Compounds bearing chlorine or nitro substituents exhibited better distribution. The magnetic extraction achieved equilibrium in 20 min and the phenols were found to have higher distribution ratios under acidic conditions. Compared to the extraction with non-magnetic ionic liquids, $[P_{66614}]FeCl_4$ provided higher efficiency.

N-butylpyridinium tetrachloroferrate ($[BPy]FeCl_4$) was applied as catalyst for oxidative desulfurization of fuels.[39] The authors investigated the desulfurization of model oil containing dibenzothiophene (DBT), benzothiophene (BT) and 4,6-dimethyldibenzothiophene (4,6-DMDBT) (Scheme 3).



$[BPy]FeCl_4$



Scheme 19.

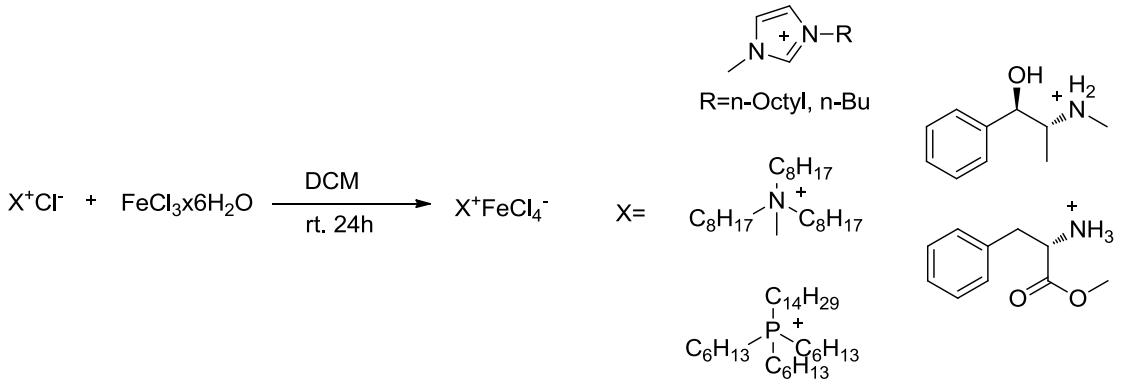
95.3%, 75.0% and 54.8% sulfur removal for DBT, BT and 4,6-DMDBT respectively have been achieved in $[BPy]FeCl_4$ for 10 min. $[BPy]FeCl_4$ is solid at room temperature and was melted at 40ºC, after completion of the process and cooling down the solidified MIL was recovered by external magnetic field and reused five times without significant loss of catalytic efficiency.

The same concept was applied by Zhao *at al*.[40] The authors synthesized 1-*n*-butyric acid-3-methylimidazolium chloride/*x*$FeCl_3$ $[C_3H_6COOHMIM]Cl/xFeCl_3$ by using different ratios of $[C_3H_6COOHMIM]Cl$ and $FeCl_3$. The MIL resulting from 1:2 ratio exhibit the highest efficiency and 100%, 100% and 93.7% sulfur removal for DBT, BT and 4,6-DMDBT respectively have been achieved. In contrast with $[BPy]FeCl_4$, $[C_3H_6COOHMIM]Cl/2FeCl_3$ was liquid at room temperature and the desulfurization process was carried out at lower temperature of 30°C. The MIL was reused 3 times after magnetic field separation. Slight decrease of the efficiency was observed during the 2nd and 3rd cycle, 95.2% and 90.1% sulfur removal, respectively. This was explained by the enriched concentrations of oxidative products in the MIL with increasing the recycling times.

2. Results and discussion.

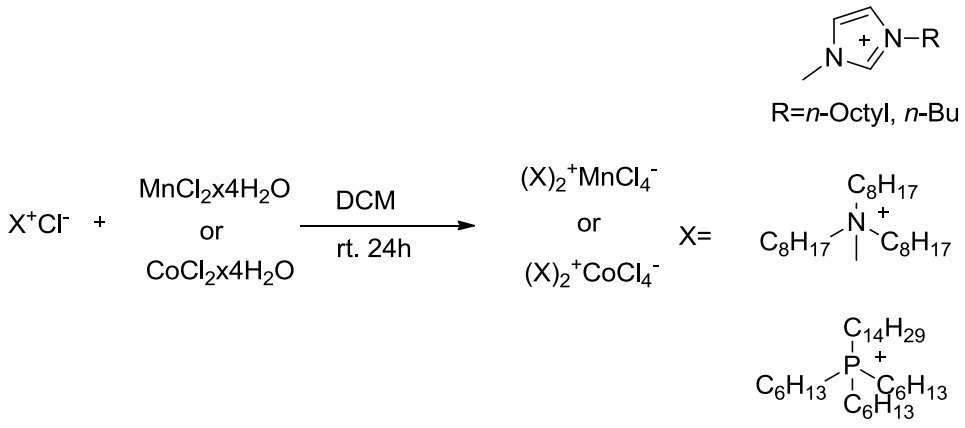
2.1. Synthesis and characterization of MILs

Various MILs based on Fe, Co, Gd and Mn were obtained *via* reported procedures, mixing metal chloride hydrate salts with quaternary amonium, phosphonium or imidazolium chlorides in suitable solvent, typically MeOH or DCM, and overnight stirring at room temperature. Fe containing MILs were obtained as brown liquids and only [(+)ephedrine]$FeCl_4$ was obtained as a brown solid at room temperature (Scheme 20).



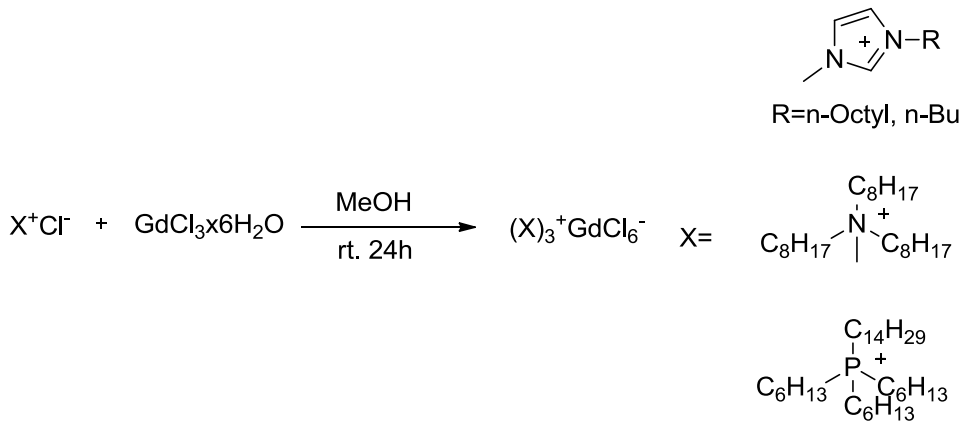
Scheme 20.

Mn and Co containing MILs were isolated respectively as viscous green or blue liquids from the reaction of $MnCl_2 \cdot 4H_2O$ or $CoCl_2 \cdot 6H_2O$ with corresponding quaternary amonium, phosphonium or imidazolium chlorides under the same conditions used for the iron MILs (Scheme 21).



Scheme 21.

Gadolinium MILs were obtained as colorless viscous liquids from the reaction of $GdCl_3x6H_2O$. The synthesis were carried out for 24h using MeOH as solvent (Scheme 22).



Scheme 22.

After the evaporation of the solvent using rotary evaporator. The traces of solvents and water have been removed under vacuum for 48 h at $1–4\times10^{-2}$ mbar (rotatory pump) and 4 h to 6×10^{-5} mbar (difusion pump) with stirring at 60ºC.

2.2. Toxicological evaluation of magnetic ionic liquids in human cell lines.

The toxicity of $[C_8MIM]FeCl_4$, $[C_8MIM]_2MnCl_4$, $[C_8MIM]_2CoCl_4$ and $[C_8MIM]_3GdCl_6$ together with several MILs containing different choline type cataions (synthesized by Dr. Andrea Rosatella and Dr. Filipa Siopa) have been studied.[41] The toxicology tests have been performed by Dr. Raquel Frade with CaCo-2 cells and in normal skin fibroblasts (CRL-1502).

As expected, $[C_8MIM]$ based MILs reduced the viability of CaCo-2 cells, and all the tested MILs behaved similarly with exception of $[C_8MIM]FeCl_4$ that induced a different toxicity curve (Figure 6) and additionally its toxicity was similar to the reported non-magnetic $[C_8MIM]$ ionic liquids.[42] At lower $[C_8MIM]FeCl_4$ concentrations, mitochondrial metabolism was enhanced and decreasing afterwards, in the presence of higher concentrations. Viability went down to 40% at 1.2 $x10^{-3}$ M concentration. For the other magnetic anions, a more rapidly decrease of viability was attained and the observed toxicity induced by $[C_8MIM]_3GdCl_6$, $[C_8MIM]_2CoCl_4$ and $[C_8MIM]_2MnCl_4$ was in the same range (Figure 6).

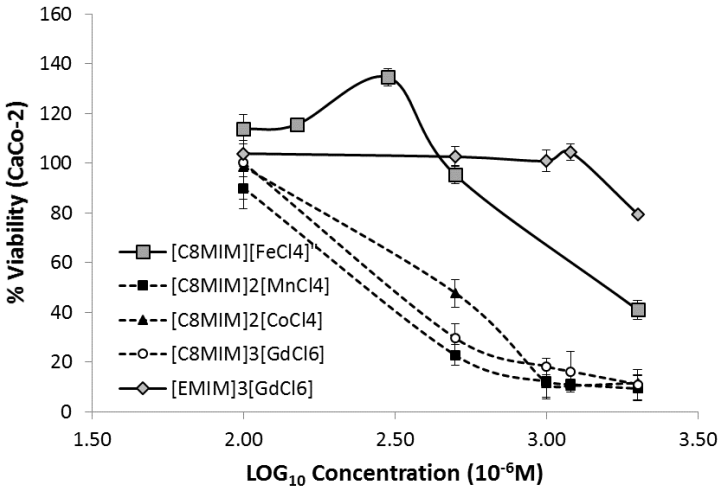


Figure 6. CaCo-2 monolayer was incubated with [C_8MIM/EMIM] – based MILs for 24 h before viability assessment. Experimental points are the average of six replicates and the error bars are ±SD.

Accordingly with the results obtained for the CaCo-2 model, [C_8MIM]$FeCl_4$ was also the least toxic for the human skin fibroblast cells (entry 1, Table 1**).** All the other magnetic anions induced similar responses as presented before. It is reasonable to consider $FeCl_4$ as the most suitable magnetic anion since in solution originates chloride, that has no intrinsic toxicity, and iron that also participates in cell metabolism. But, with data obtained in this work it is also feasible to consider $GdCl_6$ as the second best studied magnetic anion (entry 4, Table 1). It was also observed that the major effect on the toxicity of MILs is caused by the cataion.

Table 1. Determined IC50 (lM) in human skin fibroblast (CRL-1502) cells after 24h incubation. R is the goodness of fit of the adjusted equation to the experimental data.

Entry	MIL	IC_{50}(µM)
1	[C_8MIM]$FeCl_4$	1217(R=0.9081)
2	[C_8MIM]$_3GdCl_6$	678.9(R=0.8865)
3	[C_8MIM]$_2CoCl_4$	541.8(R=0.8827)
4	[C_8MIM]$_2$MnCl4	422.8(R=0.9950)

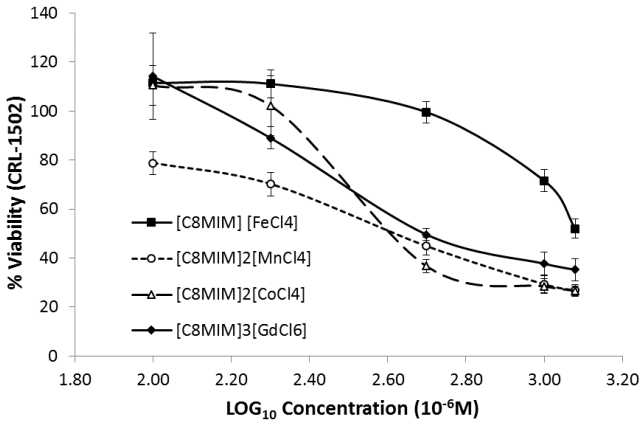


Figure 7. CRL-1502 cells were incubated with [C_8MIM] – based MILs for 24 h before viability assessment. Experimental points are the average of six replicates and the error bars are ±SD.

2.3. Physical properties of MILs.

The magnetic properties and the performance of the synthesized phoshonium based MILs for the separation of CO_2 were studied in Departamento Ingeniería Química y Química Inorgánica, E.T.S. de Ingenieros Industriales y Telecomunicación, Universidad de Cantabria.[43] The magnetic moment measurments of $[P_{66614}]FeCl_4$, $[P_{66614}]_2MnCl_4$, $[P_{66614}]CoCl_4$ and $[P_{66614}]_3GdCl_6$ are presented in (Table 2).

Table 2. Magnetic properties of the MILs.

Entry	MIL	χ_m^T (emu K/mol)	μ_{eff} (μ_B/ion)	Θ_p (K)
1	$[P_{66614}]FeCl_4$	4.29	5.89	-0.5
2	$[P_{66614}]_2MnCl_4$	4.23	5.40	-1.6
3	$[P_{66614}]_2CoCl_4$	2.10	4.00	3
4	$[P_{66614}]_3GdCl_6$	6.51	6.32	1.29

$[P_{66614}]FeCl_4$ and $[P_{66614}]_2MnCl_4$ exhibit similar magnetic behavior (entry 1 and 2, Table 2), while lower values were observed for $[P_{66614}]CoCl_4$ (entry 3, Table 2). The highest response to magnetic field was measured in case of $[P_{66614}]_3GdCl_6$ (entry 4, Table 2). The results were in good agreement with the reported in the literature magnetic moments. $[CoCl4]^{2-}$ (2.01–2.48 emu K/mol), $[FeCl4]^{2-}$ (3.74–4.46 emu K/mol), and $[MnCl4]^{2-}$ (4.14–4.76 emu K/mol) but it does not agree well with the expected value for the gadolinium anion (7.72 emu K/ mol).[16]

Furthermore the MILs were supported on either hydrophilic or hydrophobic polyvinylidene fluoride (PVDF) membranes by soaking the MILs into the membrane using vacuum. The gas permeability of the supported membranes was tested for CO_2, N_2 and Air. The experiment were performed by placing the membranes between two compartments. Pure

gas was introduced in both the compartments and 0.45bar pressure was applied in one of them (feeding compartment). This pressure difference leads to a flux across the membrane into the other compartment (permeate compartment). The pressure in the both compartments was measured at 25ºC. The permeability of the pure gas through the membrane was calculated from the pressure data for the feed and permeate compartments, according to the following equation:

$$\ln\left(\frac{[P_{feed}] - [P_{perm}]_0}{[P_{feed}] - [P_{perm}]}\right) = \ln\left(\frac{\Delta P_0}{\Delta P}\right) = \frac{D.H.\beta}{\delta}\ t$$

Where P_{feed} and P_{perm} are the pressures in the feed and permeate compartments respectively. D (m^2s^{-1}) is the diffusivity, H is the partition coefficient, t is the time (s) and d is the membrane thick ness (m). The geometric parameter β (m^{-1}) is:

$$\beta = A_m\left(\frac{1}{V_{feed}} + \frac{1}{V_{perm}}\right)$$

Where A_m is the membrane area (m^2) and V_{feed} and V_{perm} are the volume of the feed and permeate compartments (m^3), respectively.

The ideal selectivity ($S_{A/B}$) can be determined by dividing the permeabilities of two different gases (A and B).

$$S_{a/b} = \frac{P_a}{P_b}$$

The results from the measurements and calculations are presented in (Table 3). It can be observed that $[P_{66614}]FeCl_4$ exhibit the highest permeability in both membranes, being higher when combining with a PVDF hydrophobic support (entry 2, Table 3). However, $[P_{66614}]_2MnCl_4$ displays the best CO_2/N_2 separation performance in combination with hydrophobic PVDF (entry 3, Table 3). The pure gas permeation results have also indicated that these supported MILs are more selective for CO_2 compared to N_2 and Air and could be potentially applied for selective removal of CO_2.

Table 3. Permeability and selectivity for the supported MILs.

Entry	MIL	Support	Gas	Permeability (barrer)	CO_2/N_2 selectivity	CO_2/air selectivity
1	$[P_{66614}]_2CoCl_4$	Hydrophobic PVDF	CO_2	147.06	23.24	14.24
			N_2	6.33		
			Air	10.33		
		Hydrophilic PVDF	CO_2	149.95	22.70	15.22
			N_2	6.61		
			Air	9.85		
2	$[P_{66614}]FeCl_4$	Hydrophobic PVDF	CO_2	259.04	24.17	20.98
			N_2	10.72		
			Air	12.34		
		Hydrophilic PVDF	CO_2	206.36	29.51	18.41
			N_2	6.99		
			Air	11.21		
3	$[P_{66614}]_2MnCl_4$	Hydrophobic PVDF	CO_2	202.63	41.20	26.90
			N_2	4.92		
			Air	7.53		
		Hydrophilic PVDF	CO_2	155.01	21.08	18.42
			N_2	7.35		
			Air	8.41		
4	$[P_{66614}]_3GdCl_6$	Hydrophobic PVDF	CO_2	176.35	30.80	19.40
			N_2	5.73		
			Air	9.09		
		Hydrophilic PVDF	CO_2	159.96	23.91	17.37
			N_2	6.69		
			Air	9.21		

2.4. Studies of the influence of external magnetic field on the compounds transport trough MILs.

The aim of this research, performed in our laboratory, was to be studied the transport rates of compounds trough bulky MIL membranes and the effect of the external magnetic field on these rates. In a typical experiment two U-tubes were charged with MIL and unmixable solvent was placed on each of its sides (hexane or *i*-Pr_2O), one with dissolved compounds of interest (feeding solution) and pure solvent on the other side (guest solution). One of the U-tubes was placed between magnets with 0.4T magnetic field (Figure 8).

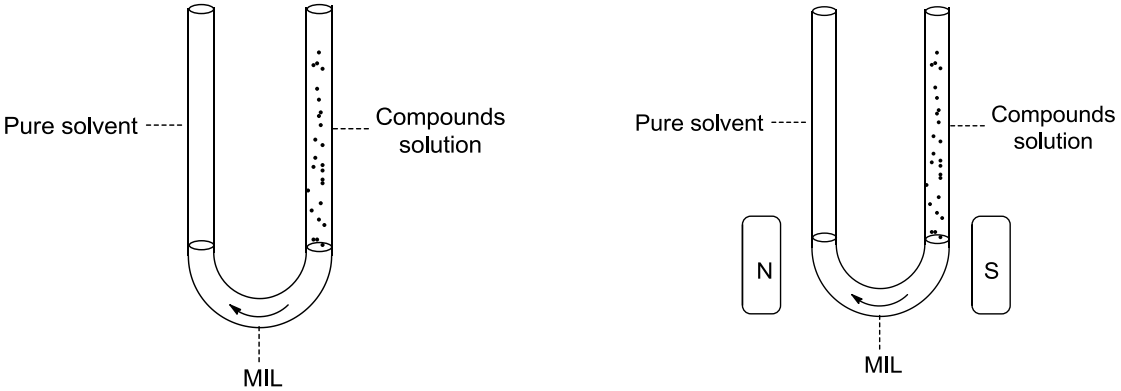


Figure 8. Set-up used for transportation studies.

Samples from the guest solution were taken every 24h and analyzed by GC. Initially it was studied the transport of 0.01M (for each compound) solution of 2-butanol, cyclohexanone and 2-octanol in hexane through [Aliquat]$FeCl_4$, dodecane was used as an internal standard for the GC analysis. The experiment was performed for 10 days and it was observed that the transport in present of magnetic field is slightly accelerated. However, the observed differences were very small (Figure 9 and Figure 10).

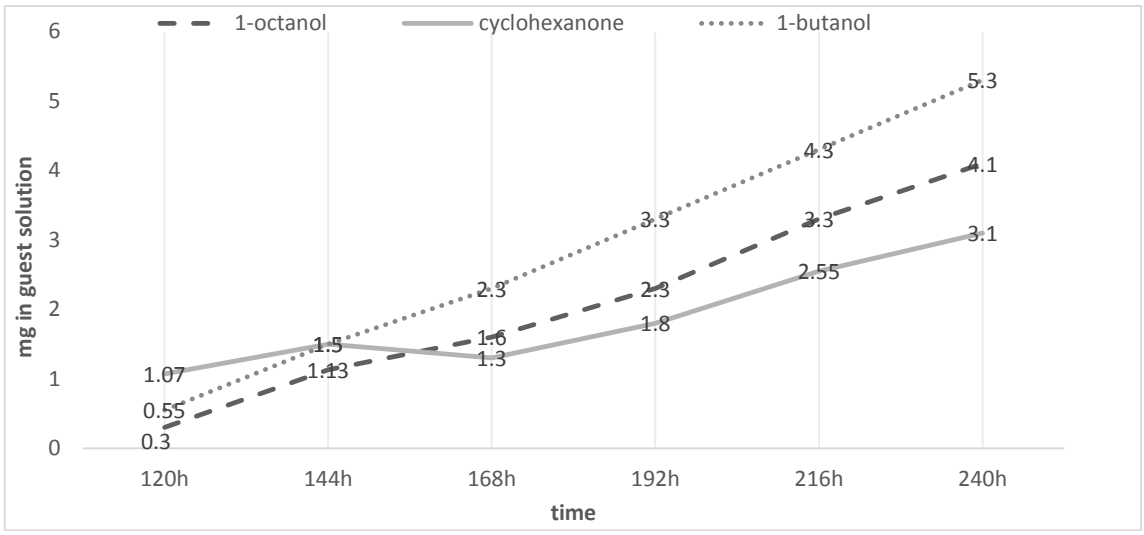


Figure 9. Transport rates in presence of magnetic field.

Table 4. Compounds distribution after 10 days in the presence of magnetic field.

Entry	Compound	Amount in feeding solution	Amount in [aliquat]$FeCl_4$	Amount in guest solution
1	1-butanol	117mg/63%	63.4mg/34%	5.6mg/3%
2	cyclohexanone	85.3mg/34.7%	157.3mg/63.4%	3.4mg/1.4%
3	1-octanol	226.5/69.5%	94.9mg/29.1%	4.6mg/1.4%

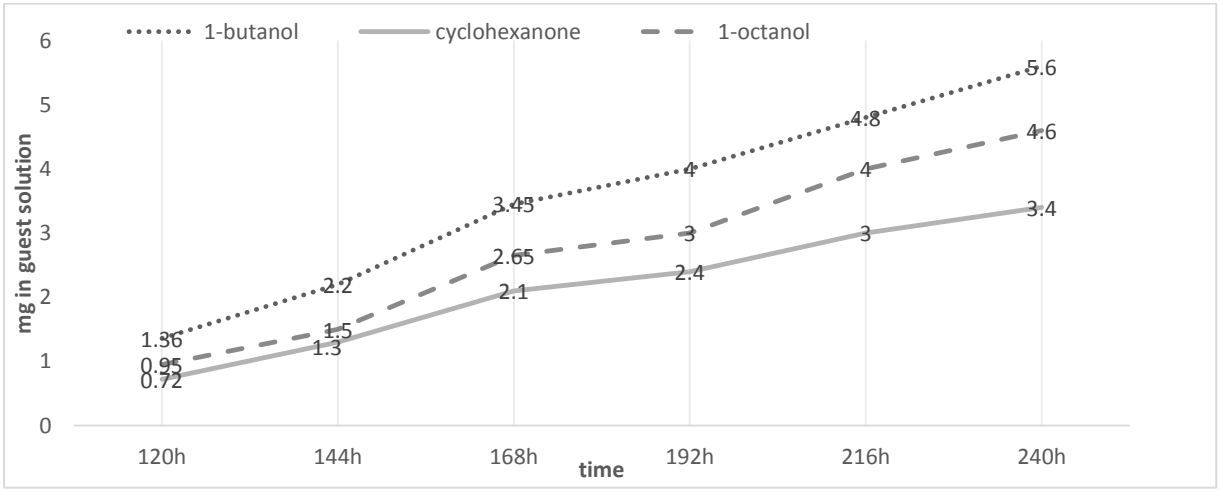


Figure 10. Transport in absence of magnetic field.

Table 5. Compounds distribution after 10 days in absence of magnetic field.

Entry	Compound	Amount in feeding solution	Amount in [aliquat]$FeCl_4$	Amount in guest solution
1	1-butanol	135mg/73%	45.7mg/24.6%	5.3mg/2.8%
2	cyclohexanone	113mg/45.9%	130mg/52.8%	3.1mg/1.3%
3	1-octanol	253/77.6%	68.9mg/21.1%	4.1mg/1.25%

Only 0.2% higher transport was observed for 1-octanol (entry 1, Table 4 vs entry 1,Table 5), 0.1%% for cyclohexanone (entry 2, Table 4 vs entry 2, Table 5) and 0.15% for 1-butanol (entry 3, Table 4 vs entry 3, Table 5). The rate of the transport was also observed to be very slow, less than 3% mass transfer after 10 days was found in the guest solutions.

We tried to repeat and confirm the results by constructing the same experiment. However, the attempts were unsuccessful. The transport rates were even slower, causing problems with the analysis at such low concentrations, non-explainable variations and unrepresentative results. The sensitive of the FID detector for cyclohexanone was low and it was not detected in the guest solution even after a week, in the following experiments (Figure 11).

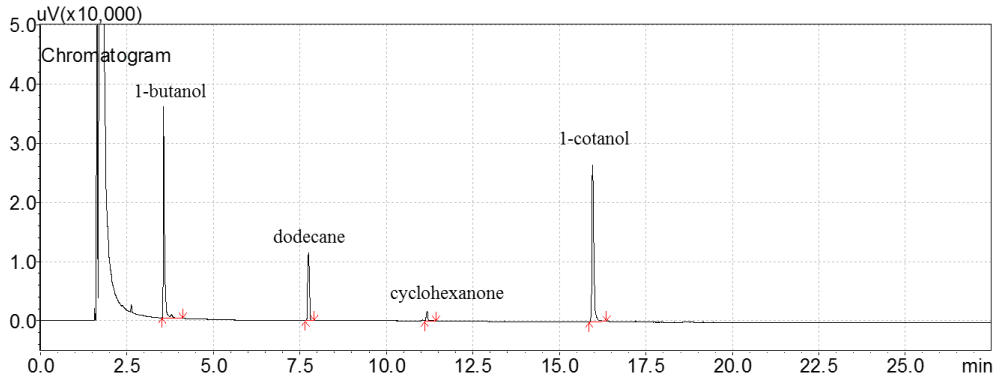


Figure 11. GC chromatogram of the 0.0005M calibration solution.

Taking into account the distribution of the tested compounds in the system we observed that in case of magnetic field their distribution in [aliquat]$FeCl_4$ was significantly higher in up to 10.6% for cyclohexanone (entry 2, Table 4 vs entry 2, Table 5). This phenomena was studied and in a typical experiment one and the same amount of MIL was placed in 2 test tubes, a solution of 2-butanol, cyclohexanone or 2-octanol 0.01M in hexane was carefully added above the MIL. One of the tubes was placed inside 0.4T magnetic field (Figure 12). Samples were taken every 24h and analyzed by GC. Three different experiments have been performed using [Aliquat]$FeCl_4$, [Aliquat]$_2^-$ $MnCl_4^{2-}$ and $[P_{66614}]_2^-$ $CoCl_4^{2-}$ MILs. However, no difference in the distribution ratio, caused by the magnetic field, was observed after 10 days.

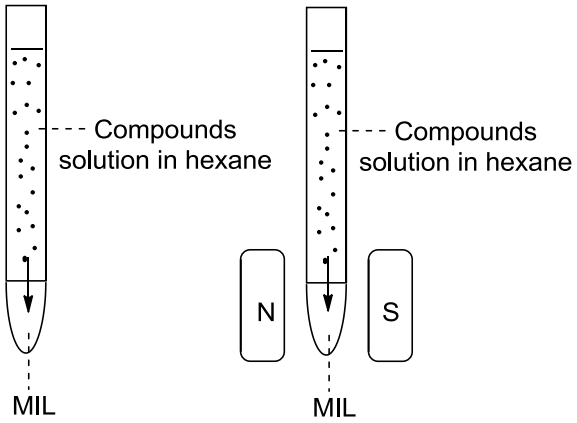


Figure 12. Experimental set-up for studying the extraction in MIL.

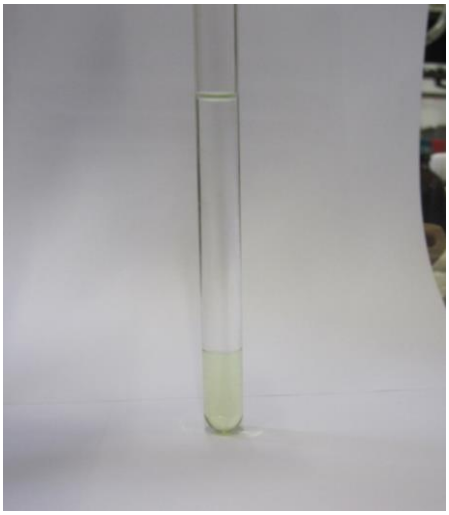


Figure 13. MIL – $[aliquat]_2MnCl_4$ 0.01M solution of 1-pentanol, cyclehexanone, 2-octanol in hexane.

For the further studies we decided to optimize the experiment by varying the MIL, compounds, their concentration and solvents, as well as running the experiments for longer times. First we performed an experiment using feeding solution with higher concentration, which should increase the transport rate caused by the higher concentration difference. Unfortunately when the concentration was increased to 0.1M the MIL became partly soluble in the feeding solution and the experiments failed. As a next step we changed the tested compounds. 1-butanol was replaced with 1-pentanol, the second was easier to analyze with GC since it has higher boiling point and didn't interfere with the solvent peak tailing. Cyclohexanone was replaced with 1-phenylethanol because the FID detector is more sensitive for it, thus allowing to be analyzed correctly in lower concentrations. Moreover we kept the feeding solution to be hexane but replaced the guest one with *i*-Pr_2O, in which theoretically the tested compounds should exhibit higher solubility and higher transportation rates. The experiment time was dramatically increased and samples were taken on weekly bases and

analyzed by GC. The extended experiment time was observed to be problematic. In general, it was difficult to keep volatile solvents for such a long time. Numerous experiments failed because of the solvent evaporation, before we found a suitable experimental set up. Since no influence of the magnetic field on the distribution ratio of the compounds in MILs was confirmed, in the following experiments only the concentration of the compounds in the guest solution was analyzed. The experiment has been performed in two U shaped tubes. Both the tubes were charged with 3ml of [Aliquat]$FeCl_4$ and simultaneously were introduced 8ml of the feeding (0.01M concentration for each compound in hexane) on one side, and 8ml of the guest solvent *i*-Pr_2O on the other (Figure 14).

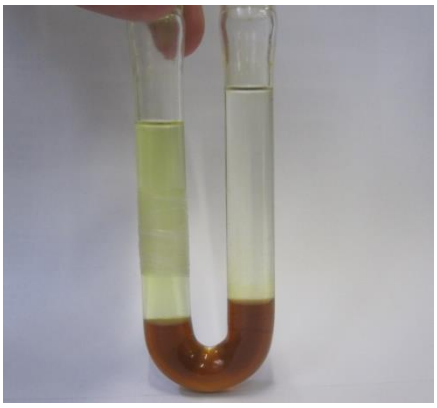


Figure 14. Experimental U tube for the transport studies. MIL – [aliquat]$FeCl_4$. Feeding solution 0.01M solution of 1-pentanol, 1-octanol and 1-phenylethanol in hexane. Guest solution *i*-Pr_2O.

The tubes were well closed with plastic caps and secured with parafilm. One of the tubes was placed in 0.4T magnetic field. As in the previous experiment faster transport rate for all the compounds was observed in presence of magnetic field, presumably due to a certain degree of molecular organization in the MIL, thus allowing faster transport of the compounds (Figure 15,Figure 16 and Figure 17).

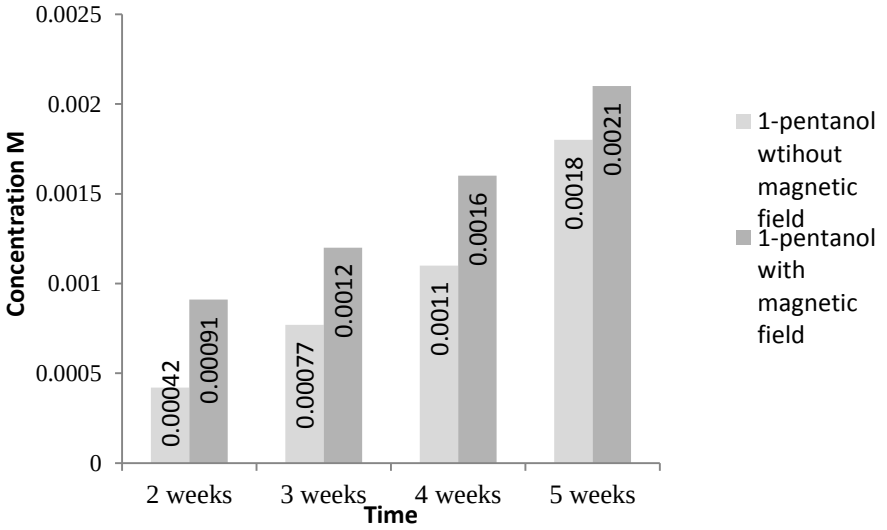


Figure 15. Transport rate of 1-pentanol trough [aliquat]$FeCl_4$

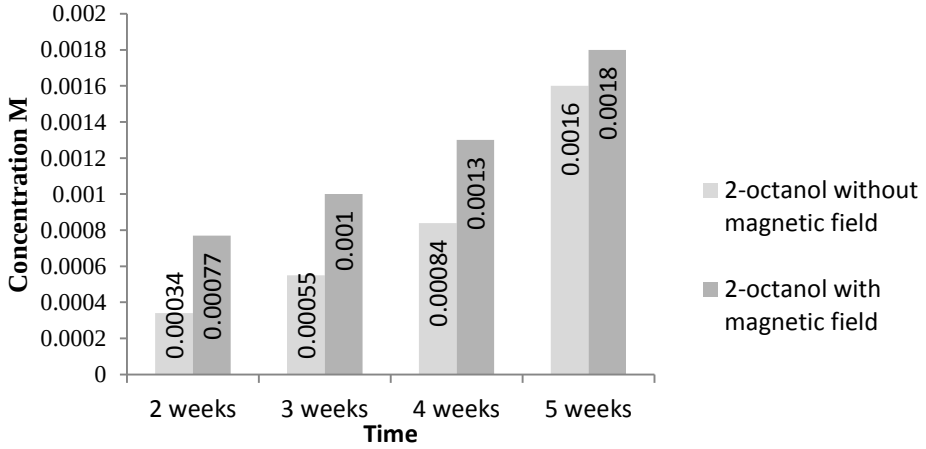


Figure 16. Transport rate of 1-octanol trough [aliquat]$FeCl_4$

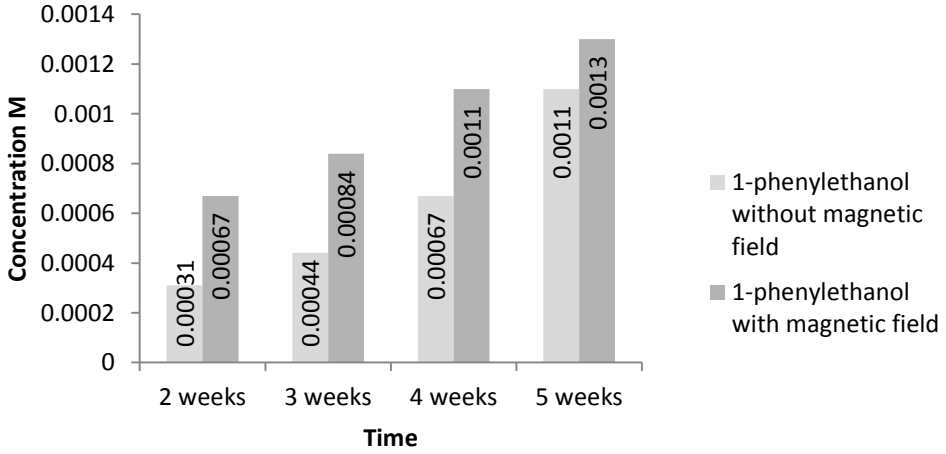


Figure 17. Transport rate of 1-phenylethanol trough [aliquat]$FeCl_4$

It was also observed that the difference in the transport rate was much higher in the beginning of the experiment. Approximately 2 times faster transport in presence of magnetic field was observed for all the compounds after 2 weeks (2 weeks values, Table 6). At the end

of the experiment after 5 weeks the difference was much more insignificant and gradually dropped down to around 1.12-1.18 higher concentration in presence of magnetic field (5 week values, Table 6).

Table 6. Transport rates in absence and in presence of magnetic field.

Entry	compound	2 weeks[a]		3 weeks[a]		4 weeks[a]		5 weeks[a]	
		MF[b]	No MF[b]	MF[b]	No MF[b]	MF[b]	No MF[b]	MF[b]	No MF[b]
1	1-pentanol	9.1%	4.2%	12%	7.7%	16%	11%	21%	18%
2	1-octanol	7.7%	3.4%	10%	5.5%	13%	8.4%	18%	16%
3	1-phenyletahnol	6.7%	3.1%	8.4%	4.4%	11%	6.7%	13%	11%

[a] *The values are presenting the concentration of each compound found in the guest solution as a percent use of the initial concentration of the feeding solution.*[b] *MF=Magnetic field.*

Further the effect on the transport caused by different MIL cataions was tested. [Aliquat]$FeCl_4$ was replaced with [C_8MIM]$FeCl_4$ and the experiment performed with the same experimental set up. Since we were not sure what will be the transport rate in this case the samples have been taken more frequently 48h, 72h and after 1 week (Figure 18, Figure 19 and Figure 20).

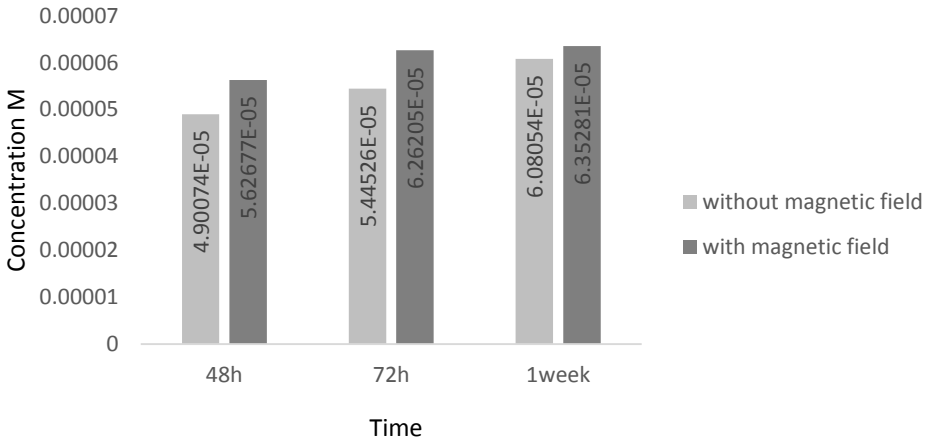


Figure 18. Transport rate of 1-pentanol trough [C_8MIM]$FeCl_4$.

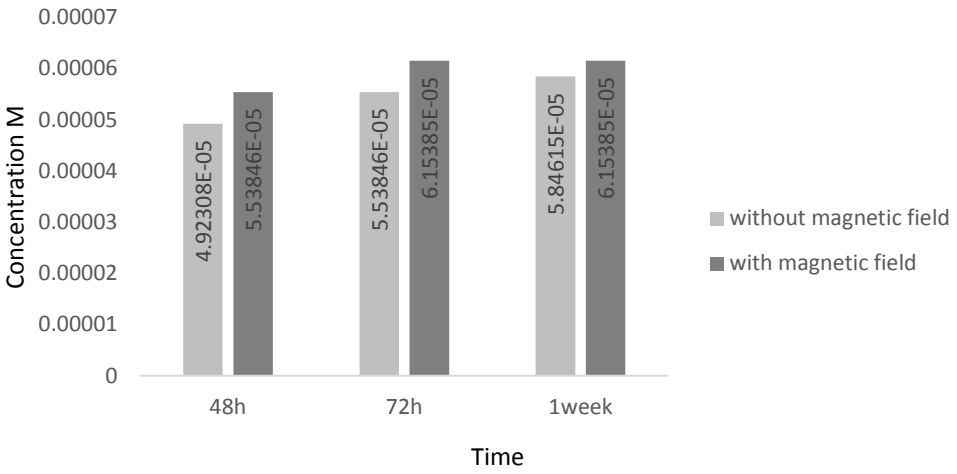


Figure 19. Transport rate of 1-octanol trough $[C_8MIM]FeCl_4$.

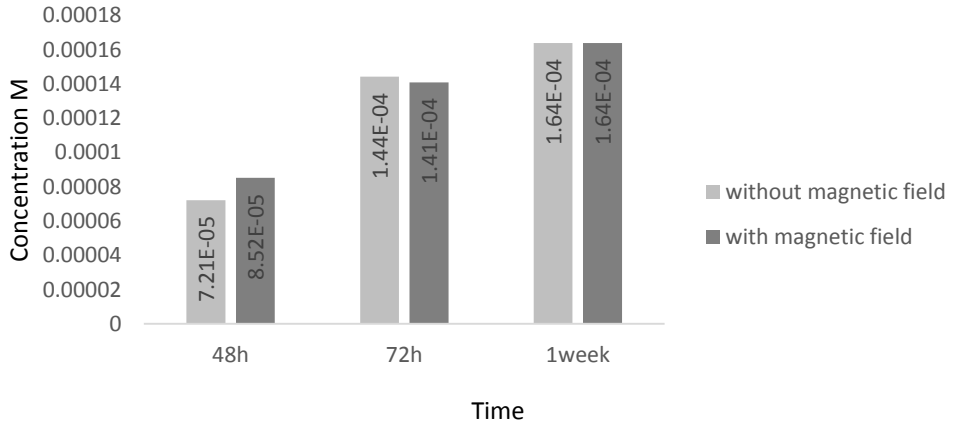


Figure 20. Transport rate of 1-phenyl ethanol trough $[C_8MIM]FeCl_4$.

As it was expected in case of $[C_8MIM]FeCl_4$ 1-phenyl ethanol exhibited the fastest transport rate, due to its higher solubility in $[C_8MIM]FeCl_4$ caused by the aromatic nature of the imidazolium cataion, while the opposite was observed for the transport trough $[Aliquat]FeCl_4$. We observed that in general all the transport rates were slower for $[C_8MIM]FeCl_4$ compared to $[Aliquat]FeCl_4$. The differences in mass transfer were higher for all the compounds in presence of magnetic field at the beginning and dropped down with the time. Only after 1 week they become insignificant, at which point the experiment was stopped. The results clearly showed that $[Aliquat]FeCl_4$ is a better choice for the transport studies.

2.5. Studies on the effect of the magnetic field on the organic reactions performed with MIL.

We explored the idea that the magnetic field may could have an effect on the reaction outcome in presence of MILs in terms of the reaction rates and stereochemistry. Initially we investigated the already reported acetylation of alcohols using $[C_8MIM]FeCl_4$ as catalyst.[31] 1-

phenylethanol was acetylated into 1-phenylethyl acetate using acetic anhydrate. The reaction catalyzed by $[C_8MIM]FeCl_4$ was observed to be much slower than the reported in the literature. 88% yield after 90min were obtained by Peng *at al.* using $[BMIM]FeCl_4$, while in our case the reaction reached 80% yield only after 6 days, presumably due to the lower catalytic activity of $[C_8MIM]FeCl_4$ and the absence of stirring. Initially the reaction has been performed using $[C_8MIM]FeCl_4$ as catalyst at room temperature. Two vials were taken and each one was charged with 10 mmol 1-phenylathanol, 15 mmol acetic anhydride and 2 mmol $[C_8MIM]FeCl_4$. One of the vials was placed in 0.4T magnetic field. Samples have been taken on each 24h and analyzed by GC. The concentration of the product was used for direct measurement of the reaction rate (Figure 21).

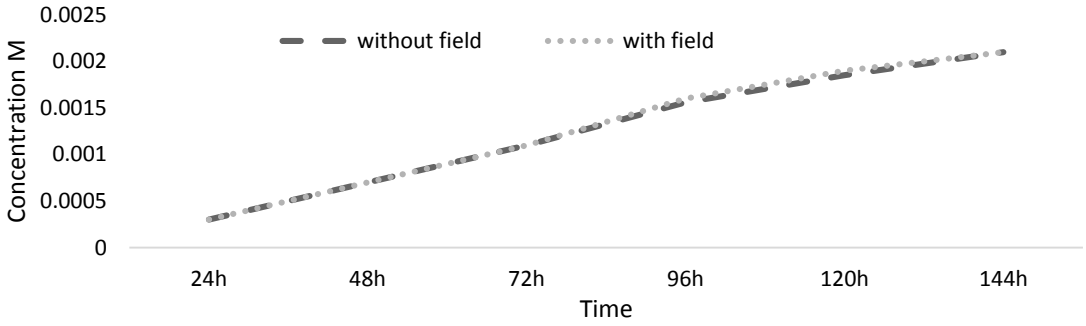


Figure 21. 1-phenylethanol acetylation reaction rate with catalytic amount $[C_8MIM]FeCl_4$.

Unfortunately no difference of the reaction rate caused by the magnetic field was observed. Both the reactions exhibit the same rate and the experiment was stopped after 144h.

Furthermore we decided to increase significantly the amount of $[C_8MIM]FeCl_4$ and use it as a reaction solvent, thus inducing higher magnetic field effect. The experiment has been repeated but using 4ml $[C_8MIM]FeCl_4$ as solvent. Again no effect on the reaction rate was observed both of the reaction proceed with the same rate for 144h (Figure 22).

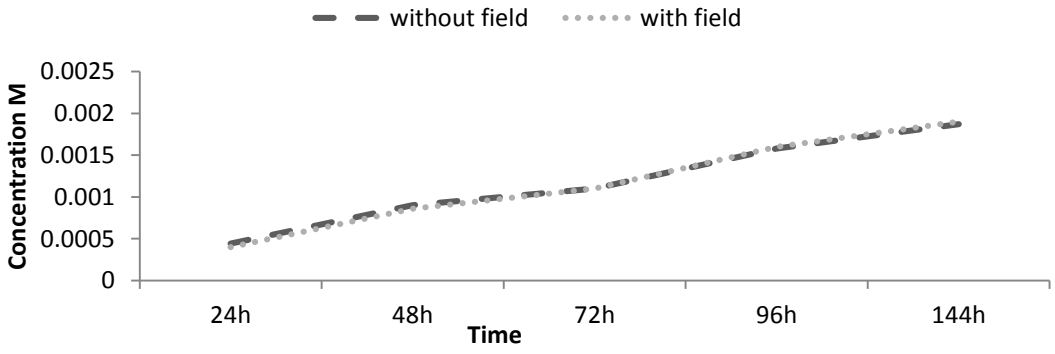
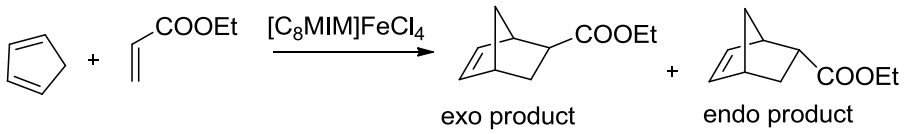


Figure 22. 1-phenylethanol acetylation reaction rate with $[C_8MIM]FeCl_4$ as solvent.

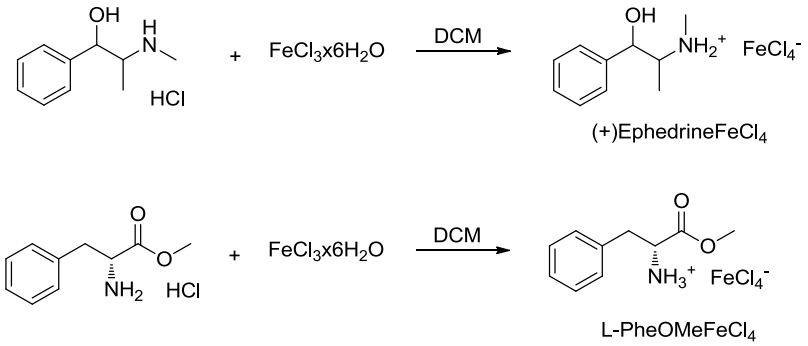
Next we studied the effect of the magnetic field on the stereoselectivity of reactions performed in MILs. We chose Diels-Alder reaction between cyclopentadiene and ethyl acrylate as a model, because it is easy to perform at room temperature and the endo/exo product stereoselectivity is known to be highly dependent on the properties of the solvent (Scheme 23). We were expecting by changing the properties of the MIL using magnetic field to affect the reaction stereoselectivity.



Scheme 23.

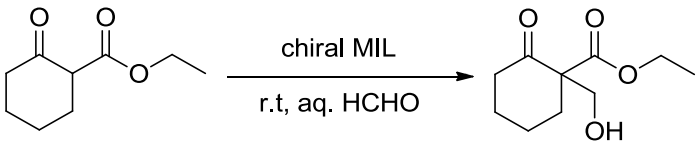
The experiments were carried out in separate vials, one of them placed in 0.4T magnetic field, using [C_8MIM]FeCl4 as reaction media. The reactions were analyzed after 8h by GC analysis. No effect of the magnetic field has been observed. 84/16 endo/exo ratio was found in both cases. Even when the experiment was repeated under more powerful magnetic field of 1.5T, produced by an electromagnet, the endo/exo ratio remain the same.

We also explore some possibilities for chiral MILs to be applied in asymmetric catalysis. Using the already reported by Warner *et al.*[21] procedure two chiral MILs [L-PheOMe]$FeCl_4$ and [(+)ephedrine]$FeCl_4$ were obtained *via* reactions of L-phenylalanine methyl ester hydrochloride or (+)-ephedrine hydrochloride with $FeCl_3x6H_2O$. [(+)ephedrine]$FeCl_4$ was isolated as brown solid while [L-PheOMe]$FeCl_4$ was a brown liquid (Scheme 24).



Scheme 24.

The chiral MILs were tested for an asymmetric version of the reported by Gaertner *et al.*[32] hydroxymethylation of β-keto esters catalyzed by [BMIM]$FeCl_4$. The reaction was performed using [(+)ephedrine]$FeCl_4$ or [L-PheOMe]$FeCl_4$ in catalytic amounts. The model substrate was cyclohexenone-2-ethylcarboxylate (Scheme 25). No enantioselectivity was observed for the both catalysts, with or without magnetic field being applied.



Scheme 25.

3. Conclusions

Various MIL based on Fe, Co, Mn and Gd were synthesized and the physical and toxicological properties for selected samples were evaluated. Experiments aiming to observe the difference on the transport rates of several compounds trough MIL have been performed. Although the results provided some clues that the transport was accelerated in presence of magnetic field, we have faced a lot of problems, due to the long experimental times that caused numerous experiments to fail because of solvent evaporation. Moreover the slow transport led to problematic analysis, due to the low concentrations in the guest solutions, causing non-explainable variations and unrepresentative results for many experiments. Optimization of the experimental set up and the tested compound-MIL systems are required for the future in order the results to be clearly confirmed. Additionally the possible effects induced by magnetic field on organic reactions carried out in presence of MIL, have been studied. However no clear effects caused by the magnetic field were observed.

4. Experimental.

General: All reagents were purchased from Sigma-Aldrich, Alfa Aesar, Merck, Carlo Erba and Panreac and have been used without further purification. The 0.4T magnetic field experiments have been performed using in-house made set up using constant magnets while 1.5T experiments were performed using GMW dipole electromagnet. NMR spectra were recorded at room temperature in a Bruker AMX 300 or Bruker AMX 400 using $CDCl_3$. HPLC analysis were performed on Dionex P680 pump, Dionex UVD 340S diode array detector. GC analysis were performed using Shimadzu GC-2014.

4.1. MIL synthesis.

General procedure: To a stirred solution of the corresponding aliquat, imidazolium or $P_{[66614]}$ chlorides in CH_2Cl_2 or MeOH was added the corresponding metal chloride hydrated salt MCln.$_x$H2O (1 equiv. for $FeCl_3x6H_2O$; 0.5 equiv. for $CoCl_2x6H_2O$ and $MnCl_2x4H_2O$, and 0.3 equiv. for $GdCl_3x6H_2O$). The reaction mixture was stirred overnight at room temperature. The solvent was evaporated on a rotary evaporator at 50°C, and then kept under vacuum for 48h at 1–4x10^{-2} mbar (rotatory pump) and 4h to 6x10^{-5} mbar (diffusion pump) under stirring at 60°C.

$[C_8MIM]FeCl_4$ was obtained as brown liquid, yield 64g (99%) from (C8MIM)Cl, 38g (1eq.) and $FeCl_3x6H_2O$, 44g (1eq.) in 200ml CH_2Cl_2 following the general procedure.
$[C_8MIM]_2MnCl_4$ was obtained as a viscous oil, yield 11.1 g (82%) from (C8MIM)Cl, 10.8g (1eq.) and $MnCl_2x4H_2O$, 4.6g (0.5 eq.) in 50 ml CH_2Cl_2 following the general procedure.
$[C_8MIM]_2CoCl_4$ was obtained as a blue viscous oil, yield 16 g (89%) from (C8MIM)Cl, 14g (1eq.) and $CoCl_2x4H_2O$, 7.2g (0.5 eq.) in 50 ml CH_2Cl_2 following the general procedure.
$[C_8MIM]_3GdCl_6$ was obtained as a viscous oil, yield 1.3 g (27%) from (C8MIM)Cl, 3.5g (3 eq.) and $GdCl_3x6H_2O$, 1.86g (0.3eq.) in 50 ml MeOH following the general procedure.
[Aliquat]$FeCl_4$ was obtained as brown liquid, yield 87.6g (95%) from AliquatCl, 66g (1eq.) and $FeCl_3x6H_2O$, 44g (1eq.) in 200ml CH_2Cl_2 following the general procedure.
[Aliquat]$_2MnCl_4$ was obtained as a viscous oil, yield 19.5g (83%) from AliquatCl, 20.4g (1eq.) and $MnCl_2x4H_2O$, 5g (0.5 eq.) in 50 ml CH_2Cl_2 following the general procedure.
[Aliquat]$_2CoCl_4$ was obtained as a blue viscous oil, yield 25.2g (80%) from AliquatCl, 27g (1eq.) and $CoCl_2x4H_2O$, 8g (0.5 eq.) in 50 ml CH_2Cl_2 following the general procedure.
[Aliquat]$_3GdCl_6$ was obtained as a viscous oil, yield 3.2 g (40%) from AliquatCl, 6.5g (3 eq.) and $GdCl_3x6H_2O$, 2g (0.3eq.) in 50 ml MeOH following the general procedure.
$[P_{[66614]}]FeCl_4$ was obtained as brown liquid, yield 101g (93%) from $P_{[66614]}Cl$, 82g (1eq.) and FeCl3x6H2O, 44g (1eq.) in 200ml CH_2Cl_2 following the general procedure.
$[P_{[66614]}]_2MnCl_4$ was obtained as a viscous oil, yield 23g (80%) from $P_{[66614]}Cl$, 25.5g (1eq.) and $MnCl_2x4H_2O$, 5g (0.5 eq.) in 50 ml CH_2Cl_2 following the general procedure.
$[P_{[66614]}]_2CoCl_4$ was obtained as a blue viscous oil, yield 31g (81%) from $P_{[66614]}Cl$, 34g (1eq.) and $CoCl_2x4H_2O$, 8g (0.5 eq.) in 50 ml CH_2Cl_2 following the general procedure.
$[P_{[66614]}]_3GdCl_6$ was obtained as a viscous oil, yield 3.4g (35%) from $P_{[66614]}Cl$, 8.2g (1eq.) and $GdCl_3x6H_2O$, 2g (0.3eq.) in 50 ml MeOH following the general procedure.
[BMIM]$FeCl_4$ was obtained as brown liquid, yield 53.8g (98%) from (BMIM)Cl, 28.5g (1eq.) and $FeCl_3x6H_2O$, 44g (1eq.) in 200ml CH_2Cl_2 following the general procedure.
[BMIM]$_2MnCl_4$ was obtained as a viscous oil, yield 10g (85%) from (BMIM)Cl, 8.8g (1eq.) and $MnCl_2x4H_2O$, 5g (0.5 eq.) in 50 ml CH_2Cl_2 following the general procedure.
[BMIM]$_2CoCl_4$ was obtained as a blue viscous oil, yield 13.5g (84%) from (BMIM)Cl, 11.7g (1eq.) and $CoCl_2x4H_2O$, 8g (0.5 eq.) in 50 ml CH_2Cl_2 following the general procedure.
[BMIM]$_3GdCl_6$ was obtained as a viscous oil, yield 1.5 g (35%) from (BMIM)Cl, 2.8g (3 eq.) and $GdCl_3x6H_2O$, 2g (0.3eq.) in 50 ml MeOH following the general procedure.

4.2. Chiral MIL synthesis.

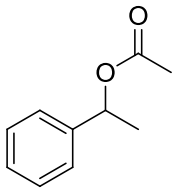
General procedure: To a stirred solution of 1eq. (+)-ephedrine hydrochloride or L-phenylalanine methyl ester hydrochloride in MeOH was added 1eq. $FeCl_3x6H_2O$. The reaction mixture was stirred overnight at room temperature. The solvent was evaporated on a rotary evaporator at 50°C, and then kept under vacuum for 48h at 1–$4x10^{-2}$ mbar (rotatory pump) and 4h to $6x10^{-5}$ mbar (difusion pump) under stirring at 60°C.

[L-PheOMe]$FeCl_4$ was obtained as a brown liquid, yield 13.3g (96%) from L-phenylalanine methyl ester hydrochloride 8g (1eq.) and $FeCl_3x6H_2O$, 10g (1eq.) in 50ml MeOH following the general procedure.

[(+)ephedrine]$FeCl_4$ was obtained as a brown solid, yield 8.9g (94%) from (+)-ephedrine hydrochloride 7.5g (1eq.) and $FeCl_3x6H_2O$, 10g (1eq.) in 50ml MeOH following the general procedure.

4.3. Organic reaction using MILs.

1-phenylethyl acetate.[44]



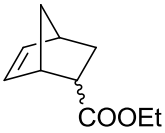
a) [C_8MIM]$FeCl_4$ as catalyst: Two equal vials were charged with acetic anhydride (1ml, 10.6mmol) and 1-phenyl ethanol (1ml, 8.3mmol) then [C_8MIM]$FeCl_4$ (0.78g, 2mmol) was added. One of the vials was placed in 0.4T magnetic field. Samples for GC analysis where taken every 24h over 6 days.

b) [C_8MIM]$FeCl_4$ as solvent: Two equal vials were charged with 4ml [C_8MIM]$FeCl_4$ then acetic anhydride (0.1ml, 1.6mmol) and 1-phenylethanol (0.1ml, 0.83mmol) were added. One of the vials was placed in 0.4T magnetic field. Samples for GC analysis where taken every 24h over 6 days.

GC analysis: 0.01g samples were taken from the reaction mixtures, diluted with 1ml CH_2Cl_2 and 1ml 0.01M solution of dodecane in CH_2Cl_2 as an internal standard and directly analyzed. GC conditions – 80-200ºC/ 8°C/min, Injector – 280ºC, FID 280°C, Column 30m/0.25mm RTX-5, carrier He.

^{1}H NMR ($CDCl_3$) δ 7.25-7.38 (m, 5H), 5.91 (q, 1H, J = 6.4 Hz), 2.07 (s, 3H), 1.52 (d, 3H, J = 5.9 Hz).
^{13}C NMR (CDCl3) δ 170.5, 127.8, 141.6, 128.6, 126.1, 72.3, 22.2, 21.34.

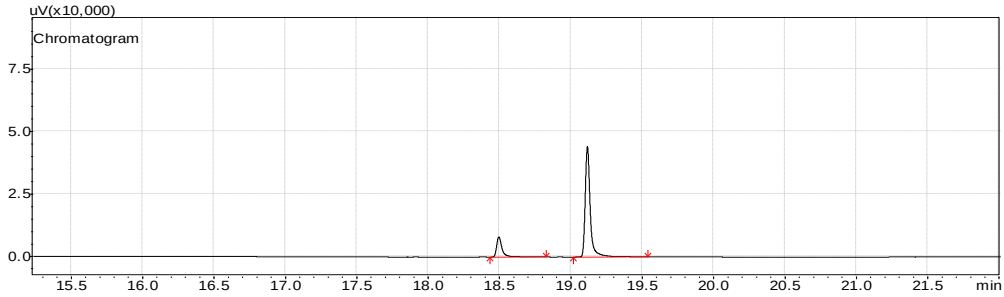
Ethyl bicyclo[2.2.1]hept-5-ene-2-carboxylate.[45]



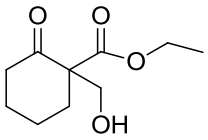
In two equal vials (0.2ml, 2.4mmol) freshly distilled cyclopentadiene was added to a solution of ethyl acrylate (0.25ml, 2.4mmol) in 4ml $[C_8MIM]FeCl_4$. One of the vials was placed in magnetic field (0.4T normal magnet or 1.5T electromagnet) the reaction mixtures was kept overnight and endo/exo ratio monitored on the next day by GC using Shimadzu GC-2014 machine. Injector temperature – 250ºC, FID temperature - 250ºC, Capillary column 30x25 HP-5, Oven: 60ºC for 10min, then up to 200ºC with 10ºC/min gradient, carrier gas: He.

^{1}H NMR (300 MHz, $CDCl_3$) (mixture of endo/exo 84/16) δ 6.11 (dd, *J* = 5.6, 3.0 Hz, 0.84H), 6.04 (dd, *J* = 8.2, 4.6 Hz, 0.14H), 5.85 (dd, *J* = 5.6, 2.8 Hz, 0.84H) 4.11 – 3.93 (m, 2H), 3.13 (br s, 1H), 2.91 – 2.74 (m, 2H), 1.90 – 1.71 (m, 1H), 1.44 – 1.29 (m, 2H), 1.10 – 0.74 (m, 4H).

GC Chromatogram (mixture of endo/exo 84/16):



Ethyl 1-(hydroxymethyl)-2-oxocyclohexanecarboxylate.[32]



Two equal vials were charged with ethyl 2-oxocyclohexanecarboxylate (0.8ml, 5mmol) and 37% aq, formaldehyde (0.7ml, 8.6mmol HCHO) then [(+)Ephedrine]$FeCl_4$ (182mg,0.5mmol) or L-PheOMe$FeCl_4$ (182mg, 0.5mmol) was added. One of the vials was placed in 0.4T magnetic field and the reactions kept overnight. 20ml of water has been added and extracted with Et_2O (2x50ml). The organic phase was dried over $MgSO_4$ and solvent evaporated on rotary evaporator. The crude product was directly analyzed using Chiral HPLC. Column Chiralpack AD, i-PrOH:Hexane 3:97 and detection at 240nm.

^{1}H NMR (300 MHz, $CDCl_3$) δ 4.24 (q, *J* = 7.2 Hz, 2H), 3.75 (dd, *J* = 37.1, 11.8 Hz, 2H), 2.86 (s, 1H), 2.11 – 1.44 (m, 6H), 1.27 (t, *J* = 7.1 Hz, 3H).

^{13}C NMR (75 MHz, $CDCl_3$) δ 210.77, 171.28, 77.58, 77.16, 76.74, 66.40, 62.63, 61.71, 41.02, 32.84, 26.99, 22.01, 14.15.

4.4. GC analysis of the transport studies:

Samples of 0.1ml from the guest solution were taken, diluted with 1ml CH_2Cl_2 and 1ml 0.01M solution of dodecane in CH_2Cl_2 as an internal standard and directly analyzed. GC conditions: Oven temperature: 60ºC for 10min, then 60-200ºC/ 8°C/min, Injector – 280ºC, FID 280°C, Column 30m/0.25mm RTX-5, carrier gas He.

5. References.

1. Mallakpour, S.; Dinari, M. In *Green Solvents II*; Springer Netherlands: 2012, p 1.
2. Hallett, J. P.; Welton, T. *Chem. Rev.*, **2011**, *111*, 3508.
3. Martins, M. A. P.; Frizzo, C. P.; Moreira, D. N.; Zanatta, N.; Bonacorso, H. G. *Chem. Rev.*, **2008**, *108*, 2015.
4. Zhang, S.; Sun, J.; Zhang, X.; Xin, J.; Miao, Q.; Wang, J. *Chem. Soc. Rev.*, **2014**.
5. Plechkova, N. V.; Seddon, K. R. *Chem. Soc. Rev.*, **2008**, *37*, 123.
6. McCrary, P. D.; Beasley, P. A.; Gurau, G.; Narita, A.; Barber, P. S.; Cojocaru, O. A.; Rogers, R. D. *New J. Chem.*, **2013**, *37*, 2196.
7. Yue, C.; Fang, D.; Liu, L.; Yi, T.-F. *J. Mol. Liq.*, **2011**, *163*, 99.
8. Visser, A. E.; Swatloski, R. P.; Reichert, W. M.; Mayton, R.; Sheff, S.; Wierzbicki, A.; Davis, J. J. H.; Rogers, R. D. *Chem. Commun.*, **2001**, 135.
9. Sawant, A. D.; Raut, D. G.; Darvatkar, N. B.; Salunkhe, M. M. *Green Chem. Lett. Rev.*, **2011**, *4*, 41.
10. Hayashi, S.; Hamaguchi, H.-o. *Chem. Lett.*, **2004**, *33*, 1590.
11. Wang, J.; Yao, H.; Nie, Y.; Zhang, X.; Li, J. *J. Mol. Liq.*, **2012**, *169*, 152.
12. Muraoka, J.; Kamiya, N.; Ito, Y. *J. Mol. Liq.*, **2013**, *182*, 76.
13. Daniel, C. I.; Albo, J.; Santos, E.; Portugal, C. A. M.; Crespo, J. G.; Irabien, A. *Fluid Phase Equilib.*, **2013**, *360*, 29.
14. Pitula, S.; Mudring, A.-V. *Chem.--Eur. J.*, **2010**, *16*, 3355.
15. Bwambok, D. K.; Thuo, M. M.; Atkinson, M. B. J.; Mirica, K. A.; Shapiro, N. D.; Whitesides, G. M. *Anal. Chem.*, **2013**, *85*, 8442.
16. Del Sesto, R. E.; McCleskey, T. M.; Burrell, A. K.; Baker, G. A.; Thompson, J. D.; Scott, B. L.; Wilkes, J. S.; Williams, P. *Chem. Commun.*, **2008**, 447.
17. Lee, S.; Ha, S.; You, C.-Y.; Koo, Y.-M. *Korean J. Chem. Eng.*, **2007**, *24*, 436.
18. Wang, M.; Li, B.; Zhao, C.; Qian, X.; Xu, Y.; Chen, G. *Korean J. Chem. Eng.*, **2010**, *27*, 1275.
19. Zhuravlev, O. E.; Verolainen, N. V.; Voronchikhina, L. I. *Russ. J. Appl. Chem.*, **2011**, *84*, 1158.
20. Takagi, Y.; Kusunoki, Y.; Yoshida, Y.; Tanaka, H.; Saito, G.; Katagiri, K.; Oshiki, T. *Aust. J. Chem.*, **2012**, *65*, 1557.
21. Li, M.; De Rooy, S. L.; Bwambok, D. K.; El-Zahab, B.; DiTusa, J. F.; Warner, I. M. *Chem. Commun.*, **2009**, 6922.
22. Branco, A.; Branco, L. C.; Pina, F. *Chem. Commun.*, **2011**, *47*, 2300.
23. Getsis, A.; Balke, B.; Felser, C.; Mudring, A.-V. *Crystal Growth & Design*, **2009**, *9*, 4429.
24. Santos, E.; Albo, J.; Daniel, C. I.; Portugal, C. A. M.; Crespo, J. G.; Irabien, A. *J. Membr. Sci.*, **2013**, *430*, 56.
25. Li, L.; Huang, Y.; Yan, G.; Liu, F.; Huang, Z.; Ma, Z. *Mater. Lett.*, **2009**, *63*, 8.
26. Wei, Y.; Zhao, Y.; Li, L.; Yang, X.; Yu, X.; Yan, G. *Polymer. Adv. Tech.*, **2010**, *21*, 742.
27. Kim, J.-Y.; Kim, J.-T.; Song, E.-A.; Min, Y.-K.; Hamaguchi, H.-o. *Macromolecules*, **2008**, *41*, 2886.

28. Wang, H.; Yan, R.; Li, Z.; Zhang, X.; Zhang, S. *Catal. Commun.*, **2010**, *11*, 763.
29. Mohammad Fauzi, A. H.; Amin, N. A. S.; Mat, R. *Appl. Energy*, **2014**, *114*, 809.
30. Misuk, V.; Breuch, D.; Löwe, H. *Chem. Eng. J.*, **2011**, *173*, 536.
31. Wang, D.-S.; Li, G.-Y.; Peng, Y.-Q. *J. Chin. Chem. Soc.*, **2009**, *56*, 834.
32. Bica, K.; Gaertner, P. *Eur. J. Org. Chem.*, **2008**, *2008*, 3453.
33. Bijan Mombani Godajdar, A. R. K., Mohammad Mahmoodi; Hashemi *Heterocycles*, **2013**, *87*, 559.
34. Khalafi-Nezhad, A.; Mohammadi, S. *RSC Adv.*, **2013**, *3*, 4362.
35. Panja, S. K.; Saha, S. *RSC Adv.*, **2013**, *3*, 14495.
36. Bica, K.; Gaertner, P. *Org. Lett.*, **2006**, *8*, 733.
37. Yan, C.; Wang, L.; Gao, H.; Sun, H.; Shen, Q. *Chin. Sci. Bull.*, **2012**, *57*, 1953.
38. Deng, N.; Li, M.; Zhao, L.; Lu, C.; de Rooy, S. L.; Warner, I. M. *J. Hazard. Mater.*, **2011**, *192*, 1350.
39. Zhu, W.; Wu, P.; Yang, L.; Chang, Y.; Chao, Y.; Li, H.; Jiang, Y.; Jiang, W.; Xun, S. *Chem. Eng. J.*, **2013**, *229*, 250.
40. Jiang, W.; Zhu, W.; Li, H.; Xue, J.; Xiong, J.; Chang, Y.; Liu, H.; Zhao, Z. *Chem. Eng. Technol.*, **2014**, *37*, 36.
41. Frade, R. F. M.; Simeonov, S.; Rosatella, A. A.; Siopa, F.; Afonso, C. A. M. *Chemosphere*, **2013**, *92*, 100.
42. Frade, R. F. M.; Rosatella, A. A.; Marques, C. S.; Branco, L. C.; Kulkarni, P. S.; Mateus, N. M. M.; Afonso, C. A. M.; Duarte, C. M. M. *Green Chem.*, **2009**, *11*, 1660.
43. Albo, J.; Santos, E.; Neves, L. A.; Simeonov, S. P.; Afonso, C. A. M.; Crespo, J. G.; Irabien, A. *Sep. Purif. Technol.*, **2012**, *97*, 26.
44. Mäki-Arvela, P.; Sahin, S.; Kumar, N.; Heikkilä, T.; Lehto, V.-P.; Salmi, T.; Murzin, D. Y. *Appl. Catal., A*, **2008**, *350*, 24.
45. Clapham, G.; Shipman, M. *Tetrahedron*, **2000**, *56*, 1127.

Chapter V

Synthesis of sparteine like derivatives from lupanine

In this chapter will be provided an overview of the lupin alkaloids and in particular of sparteine as an important ligand for asymmetric catalysis. Preliminary results of our research on the application of readily available alkaloid lupanine as a platform molecule for the synthesis of sparteine derivatives will be described.

Table of content.

1. Inroduction.

1.1. Lupin alkaloids.

Lupinus, commonly known as lupin or lupine (North America), is a genus of flowering plants in the legume family, Fabaceae. The genus includes over 200 species, with centers of diversity in North and South America. Smaller centers occur in North Africa and the Mediterranean. Seeds of various species of lupins have been used as a food for over 3000 years around the Mediterranean. The *Lupinus* plants are known to produce a family of alkaloids namely lupin alkaloids,[1,2] used as a defensive mechanism and causes bitter taste and toxicity of the seeds, which has to be soaked in water prior to use. This class of alkaloids shares a quinolizidine core structure and is well studied and found to exhibit a broad spectra of biological activities such as antibacterial,[3] antufungal,[4] and neurological effects.[4] Some examples are presented on Figure 1.

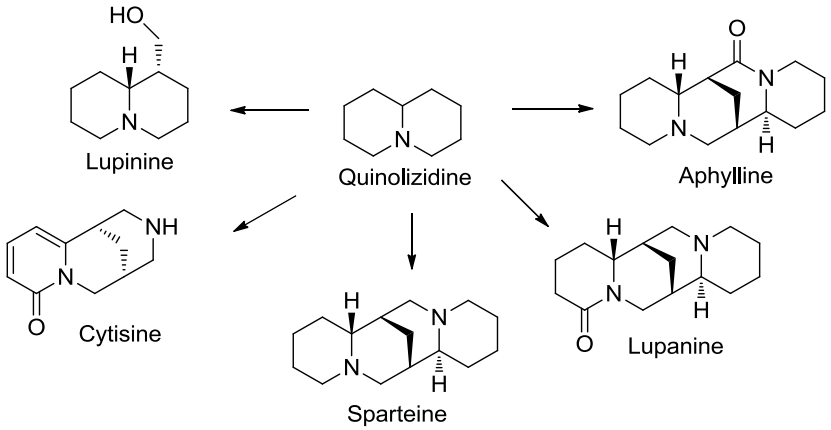


Figure 1.

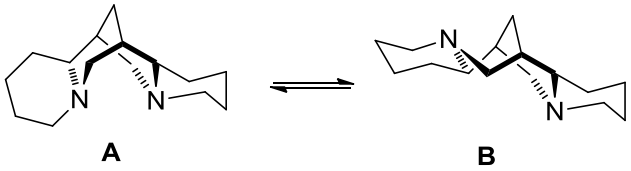
Among the different lupin alkaloids, (-)sparteine (Figure 1), an alkaloid that can be extracted from Scotch broom and is also a predominant alkaloid in *Lupinus mutabilis***,** is the mostly isolated and investigated due to its properties as an antiarrhythmic agent[5] and more importantly as a ligand for asymmetric catalysis and in particular enantioselective lithiation, copper and palladium catalysis.[6]

1.2. Sparteine as a chiral ligand for asymmetric catalysis.

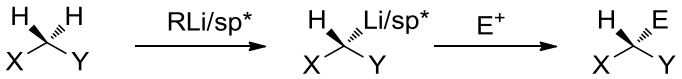
1.2.1. Application of sparteine in asymmetric lithiations.

Lithium deprotonation-substitution sequence is a powerful and widely used synthetic tool for carbon-carbon and carbon-silicon bond formation. In non-polar solvents lithium carbanions exhibit low reactivity caused by the formation of oligomeric structures such as dimers,

tetramers or higher aggregates. The level of the oligomerisation could be decreased, thus increasing the reactivity, by complexation of lithium with a coordinating ligand. Enantioslective control of the reaction can be achieved *via* chelation with chiral ligands such as (-)sparteine, which exist in one of its tautomeric forms as a cage-like ligand (Scheme 1, form A) and is observed to provide good enantiocontrol in lithium deprotonation/substitution reactions (Scheme 2).[7]

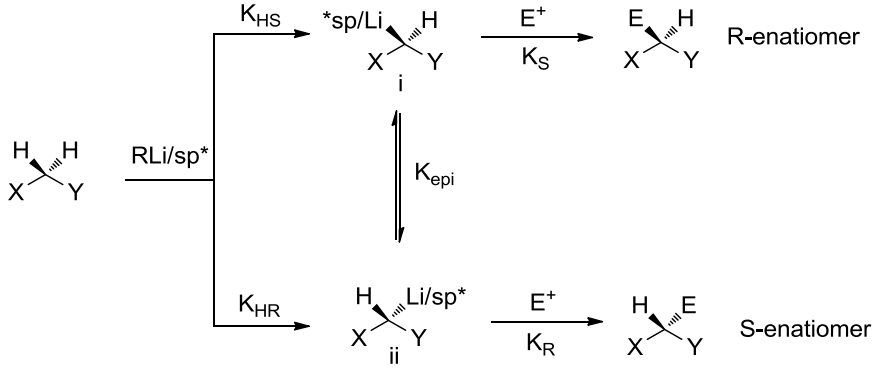


Scheme 1.



Scheme 2.

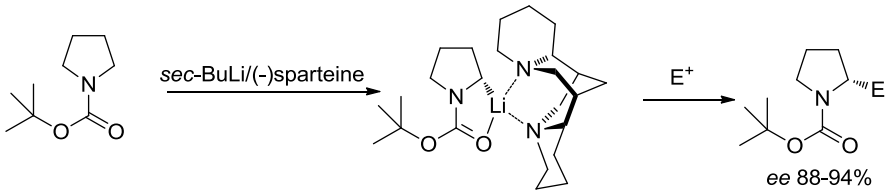
The asymmetric induction in such type of reactions occurs *via* kinetic resolution (Scheme 3). In case of $K_R \sim K_s > K_{epi}$ the ratio between the R and S enantiomer correspond to the position of the equilibrium between intermediates **i** and **ii**.



Scheme 3.

In another scenario the rate constants K_R and K_S could differ greatly and K_{epi} to be much larger than them ($K_{epi} >> K_S > K_R$) in this case the enantiomeric excess of the reaction will be determined by the ratio K_R/K_S.[8] Hoppe and co-workers demonstrated another mechanism, in which the equilibrium between two diastereomeric complexes **i** and **ii** in solution is disturbed by a selective crystallization of one of them, subsequent electrophilic substitution of the residual organolithium substrate provided enantioenriched products.[9]

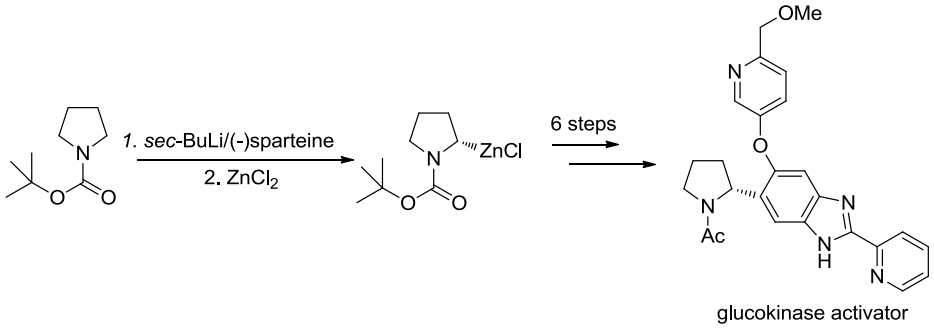
In a pioneer work Beak *et al.*[10] reported that asymmetric deprotonation of *N*-Boc pyrrolidine with *sec*-BuLi/(-)sparteine, which after subsequent substitution with various electrophiles provided enantioenriched 2-substituted *N*-Boc pyrrolidines in high *ee* (Scheme 4).



Scheme 4.

The reactions were carried out in Et_2O at -78 ºC and favored the abstraction of pro-(*S)* hydrogen.

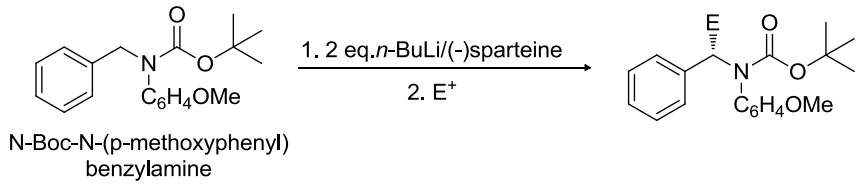
Later on this transformation was applied on kg scale in drug research, where researchers at Merck use it as a key step for the synthesis of a glucokinase activator (Scheme 5).[11]



Scheme 5.

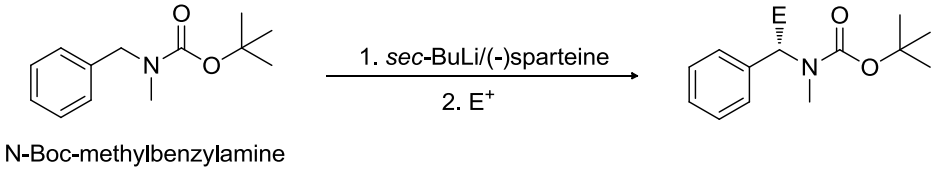
Taking into account the possible multikilogram application of this reaction Blakemore *et al.*[12] performed screening of the reaction *ee* at different temperatures and observed that the transformation could be carried out at temperatures well above -78ºC with satisfactory results. Using shorter lithiation times (2-30s) and temperature of -50 to -20ºC the corresponding 2-substituted *N*-Boc pyrrolidines could be obtained in up to 92% yield and 86% *ee*.

The benzylic protons α to a carbamate nitrogen could also be selectively deprotonated *via* sparteine controlled lithiation. *N*-Boc-*N*-(p-methoxyphenyl) benzylamine was reacted with *n*-BuLi/(-)sparteine and the formed lithiocarbanions, further trapped with electrophiles to give the corresponding derivatives in 93-96% *ee* (Scheme 6).[13]



Scheme 6.

Limat *et al*.[14] discovered that the *ee* from reactions of *N*-Boc-methylbenzylamine with *s*-BuLi/(-)sparteine depend not only on the solvent and electrophile but also on the time the reaction was kept before quenching (Scheme 7).

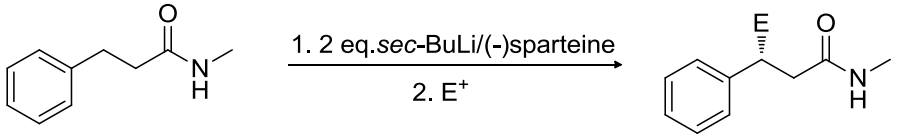


N-Boc-methylbenzylamine

Scheme 7.

Only after about 2h at -75ºC maximum *ee* was obtained and it was observed that switching from hexane to THF resulted in an inversed configuration of the products.

Other benzylic systems has been also studied as substrates for asymmetric deprotonations. Beak *et al*.[15] performed a reaction of *N*-Methyl-3-phenylpropionamide with 2 equivalents of *sec*-BuLi and (-)sparteine (Scheme 8).



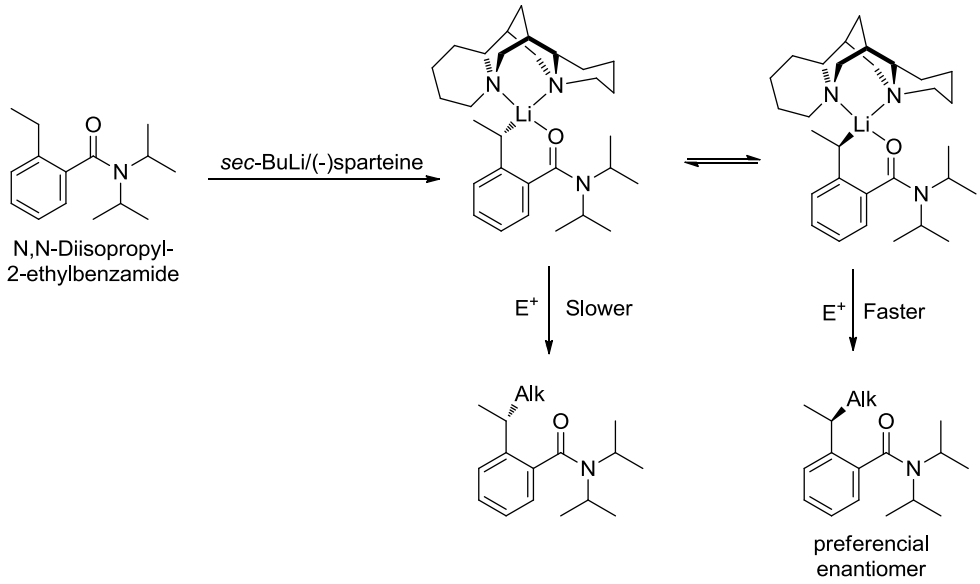
N-Methyl-3-phenylpropionamide

65-94% *ee*

Scheme 8.

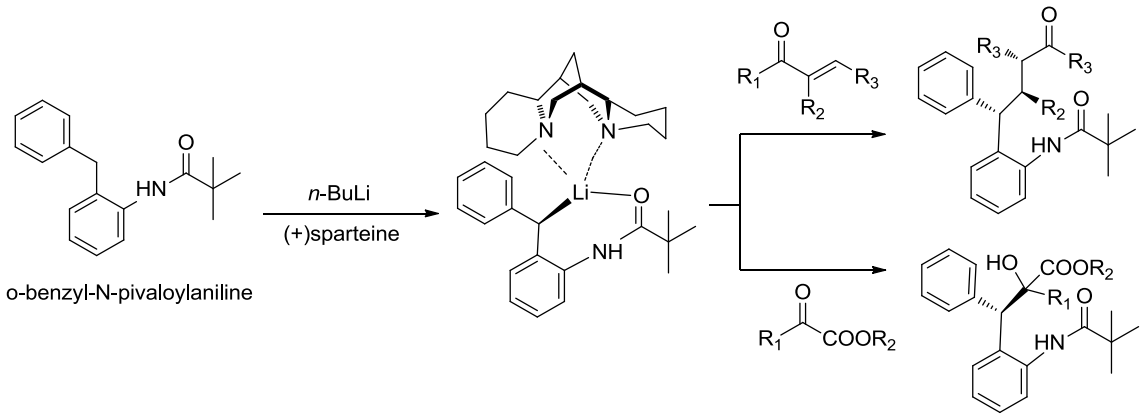
Similar results were obtained when *N*-Methyl-3-phenylpropionamide was pretreated with *sec*-BuLi and (-)sparteine was only added subsequently, thus providing a clue, which was later confirmed,[16] that the enantioselectivity is a result of favored equilibrium between the two organolithium carbanion epimers in the postdeprotonation step.

The benzylic position of *N*,*N*-Diisopropyl-2-ethylbenzamide was alkylated by Beak *et al*.[17] *via* deprotonation with *sec*-BuLi/(-)sparteine and substitution with alkyl halides and alkyl tosylates. The experimental data indicates that the origin of the enantioselectivity is a rare case of dynamic kinetic resolution, where the two organolithium carbanion epimers are in equilibrium and one reacts preferentially.



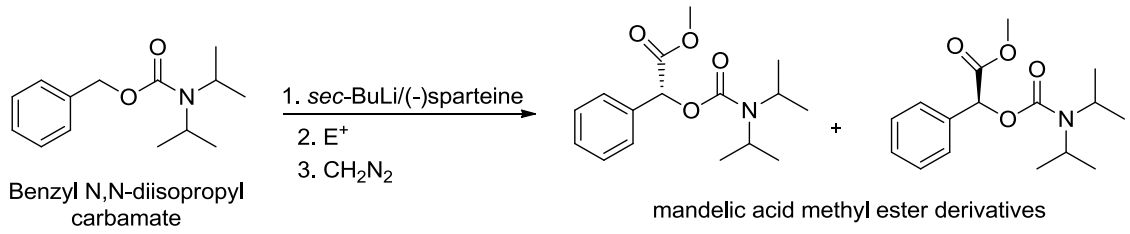
Scheme 9.

Park *et al.*[18] recently reported substitution of *o*-benzyl-*N*-pivaloylaniline with α-keto esters and α,β-unsaturated ketones. The highly diastereoenriched organolithium intermediate generated from (+)sparteine and *n*-BuLi undergoes a reaction with ketone electrophiles to afford the corresponding tertiary alcohols and 1,4-adducts in good yields of up to 84% and with high ee of up to 98% (Scheme 10).



Scheme 10.

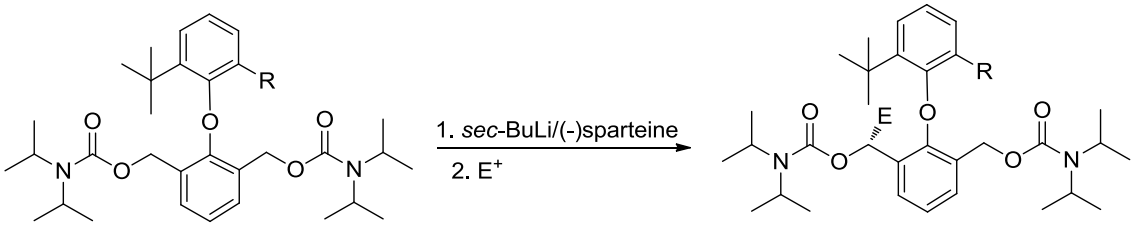
Asymmetric lithiations α to an oxygen atom have been also investigated. Benzyl *N*,*N*-diisopropylcarbamate was deprotonated with *sec*-BuLi and (-)sparteine in Et_2O at -78ºC, after 4h the epimeric mixture was trapped with CO_2 and subsequently esterificated with diazomethane to give mandelic acid methyl ester derivatives.



Scheme 11.

The reaction outcome was found to be dependent on the solvent. In Et_2O only 14% ee was obtained, while when the reaction was performed in hexane 84% enantioselectivity was achieved. The strong influence of the solvent and an experiment where the authors filtered the formed after the deprotonation precipitate from the solution and after treated them separately observed 38% and 90% *ee* respectively, confirmed that crystallization of one of the epimers leads to a dynamic resolution.

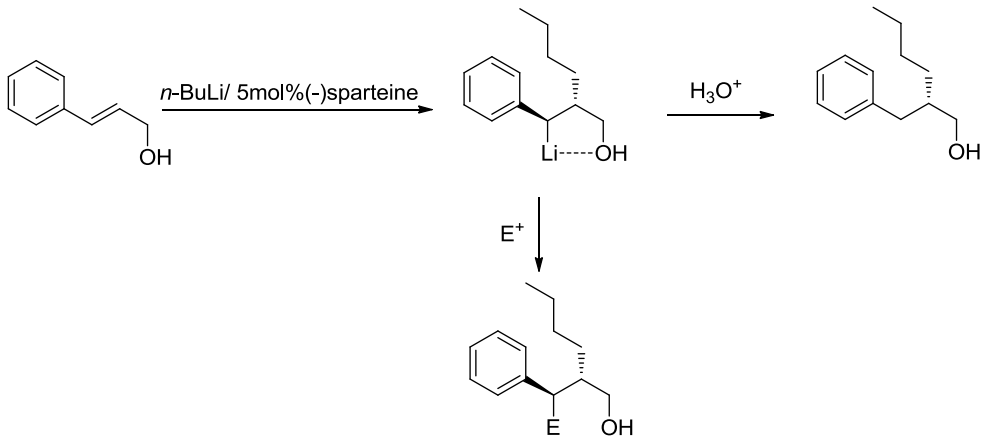
More recently a carbamate-directed benzylic lithiation for the diastereo- and enantioselective synthesis of diaryl ether atropisomers has been reported.[19]



Scheme 12.

Enantioselective deprotonation of one of the two benzylic positions led to atropisomeric products with 80:20 e.r.; an electrophilic quench provided the functionalized atropisomeric diastereoisomers in up to 97:3 d.r ratio.

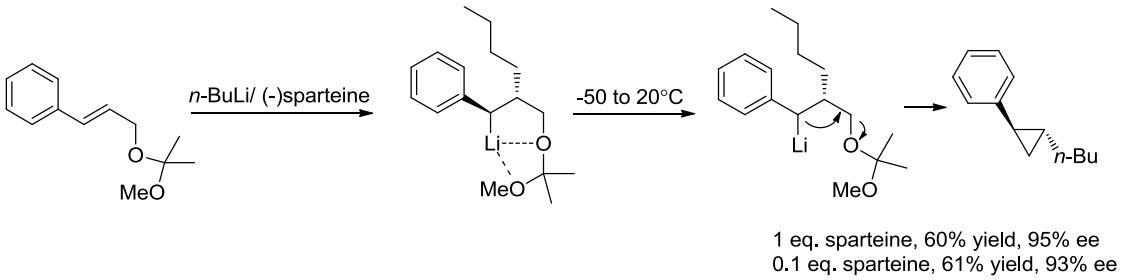
The discussed up to now stoichiometric sparteine catalyzed asymmetric lithiation reactions are well investigated, but the reports of catalytic applications are rare. The described by Normant *et al.*[20] enantioselective carbolithiation of cinnamyl derivatives *via* initial asymmetric addition of *n*-BuLi to cinnamyl alcohol (Scheme 13) is one of the first reports on the use of sparteine as a catalytic activator of organolithium carbanions.



Scheme 13.

The carbanion was further either hydrolyzed or trapped with various electrophiles to give the corresponding adducts with complete diastereoselectivity and high enantioselectivity (80–83%). Initially the authors performed the reaction using stoichiometric amount of sparteine but since *n*-BuLi alone was observed to be unreactive toward the cynamyl alcohol a chelating activation was required, thus allowing the reaction to be enantiocontroled even with catalytic amounts of (-)sparteine. The same *ee* has been achieved with 5mol% of the ligand, although in lower yield.

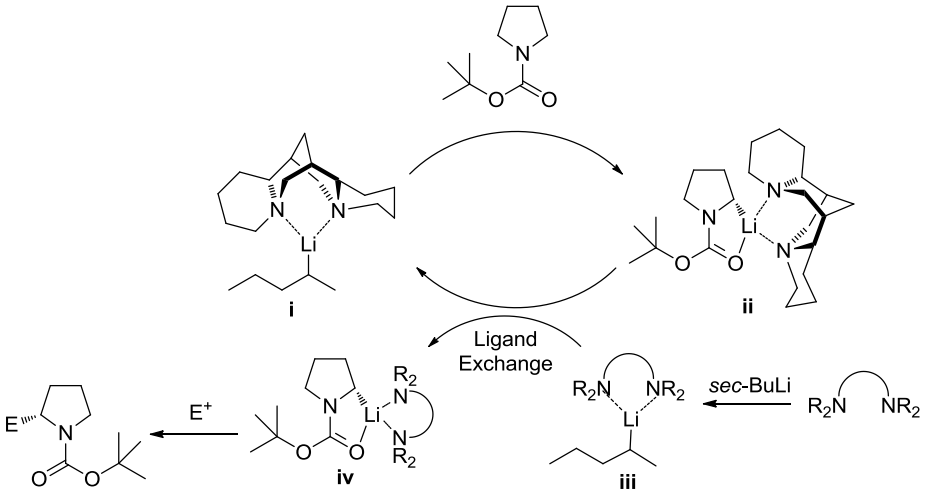
Later the methodology was extended to the preparation of chiral disubstituted cyclopropanes.[21] Cinnamyl acetals were used for carbolithiation with various organolithiums at -50ºC. After generation of the chiral benzylic organolithium carbanion, intramolecular elimination of the acetal occurred when the reaction mixture was allowed to warm up to room temperature. In that case, thermodynamic equilibration causes epimerization and promotes the formation of the more thermodynamically stable *trans*-cyclopropane. The reaction was performed with 1 and 0.1 eq. of sparteine and insignificant differences of the yield and *ee* were observed (Scheme 14).



Scheme 14.

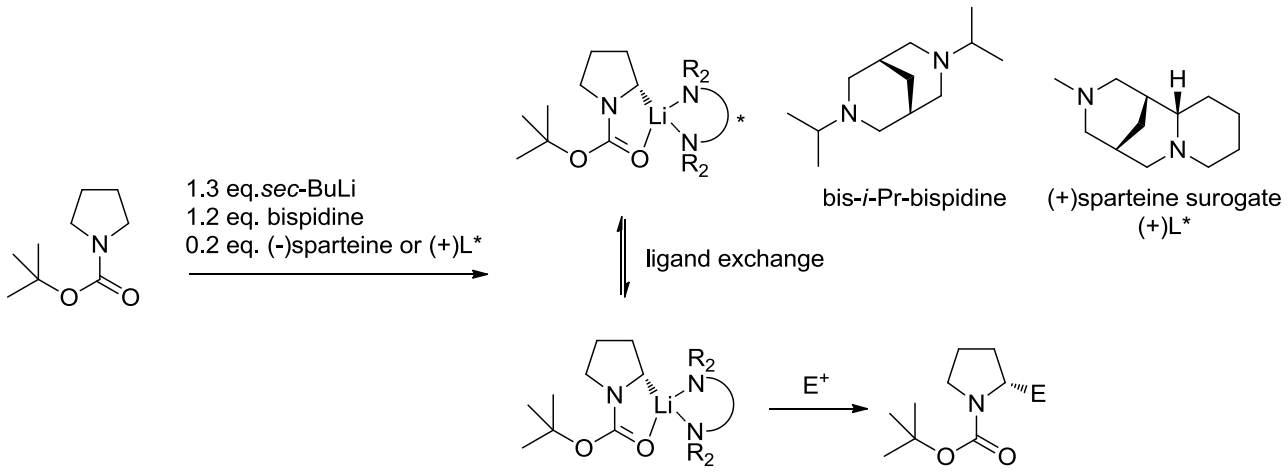
More recently the researchers focus their attention on developing ligand exchange approaches applying sparteine in a combination with non-chiral ligands thus decreasing the amount of the

required chiral promoter. For such an approach to work, several criteria must be met: (1) ligand exchange must occur (2) organolithiums **ii** and **iv** must be configurationally stable during the ligand exchange; and (3) deprotonation of *N*-Boc pyrrolidine using s-BuLi/(-)-sparteine complex **i** must be faster than that using the achiral s-BuLi/diamine complex **iii** (Scheme 15).



Scheme 15.

In 2005 O`Brien *et al*.[22] studied different diamines as non-chiral ligands for catalytic asymmetric lithiations catalyzed by (-)sparteine or previously developed by the same group (+)sparteine surrogate.[23] Bis-*i*-Pr-bispidine was observed to provide the best performance and the authors successfully carried out different asymmetric lithiation reactions using substoichiometric amount of 0.2 eq. of the chiral ligands (Scheme 16).



Scheme 16.

In a following paper[24] the authors studied other series of different stoichiometric non-chiral ligands for catalytic asymmetric deprotonation of *N*-Boc pyrrolidine. With three of them, TMEDA-like diamine ligands (Figure 2), the authors achieved satisfactory results.

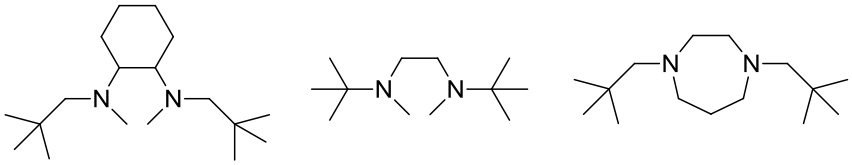
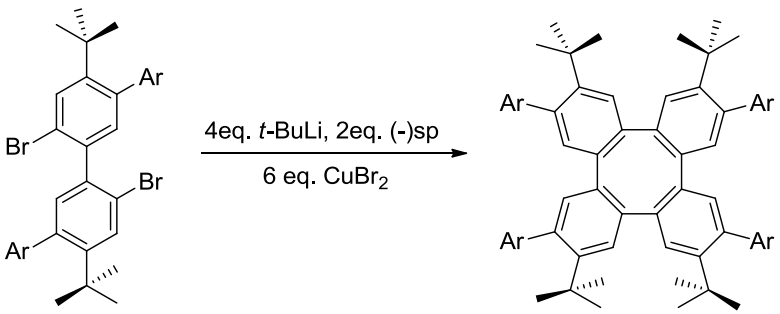


Figure 2.

More recently again O`Brien group together with Campos, a researcher from Merck Research Laboratories, studied various bispidines as stoichiometric ligands in the two-ligand catalytic asymmetric deprotonation of *N*-Boc pyrrolidine. However bis-*i*-Pr-bispidine remained the best stoichiometric recycling diamine for such catalytic asymmetric deprotonation reactions.[25]

1.2.2. Sparteine as a chiral ligand for copper catalysis.

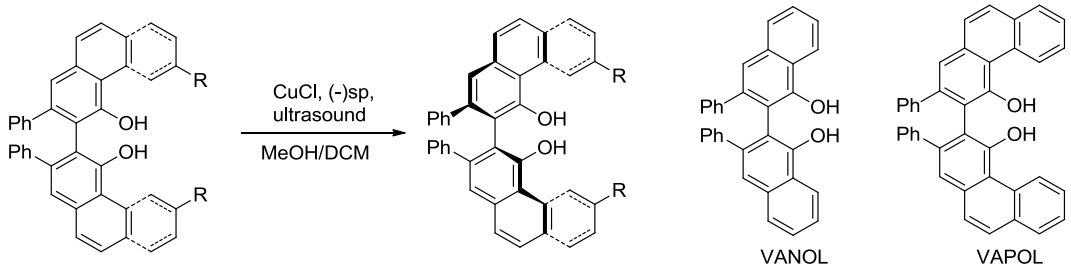
Rajca *et al*.[26] described an efficient asymmetric synthesis of chiral tetra-o-phenylenes, based on (–)-sparteine\CuBr2-mediated coupling of 2,2-dilithiobiaryls (Scheme 17).



Scheme 17.

The tetra-o-phenylene adducts were isolated in approximately 80% yields, with 50% *ee*. The authors reported that 2–3 fold increase in the number of equivalents of *t*-BuLi and/or (–)sparteine and/or $CuBr_2$ had no effect on ee's or yields.

Wulff *et al*.[27] demonstrated a copper-mediated deracemezation of vaulted biaryl ligands VANOL and VAPOL in presence of (-)sparteine (Scheme 18).

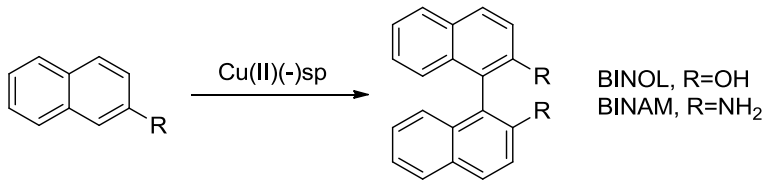


Scheme 18.

The optimal conditions involved *in situ* generation of copper(II) species using 1.4 equiv. of copper chloride and 2.8 equiv. of (–)-sparteine under sonification in the presence of air. The

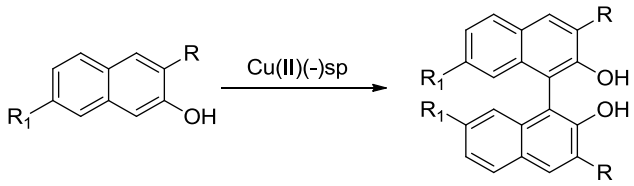
deracemization occurs in 1.75h for VANOL and 6h for VAPOL in 79 and 84% yield, respectively and 99% *ee* in both cases.

Kocovsky *et al*.[28] reported enantioselective oxidative coupling using (-)sparteine as a chiral ligand and $CuCl_2$ as oxidant under stoichiometric conditions (Scheme 19). The Cu(II)/(-)sparteine complexes were generated *in situ* and applied for 2-naphthol and 2-naphtylamine coupling. Formation of precipitate in the mother liquor was observed in case of 2-napthol. Working up the precipitate led to the isolation of (-)binaphthol ((-)BINOL) in 14% yield. The authors proposed that the isolation of (-)BINOL is a result of deracemization of the formed racemic BINOL, rather than truly asymmetric oxidative coupling. The theory was confirmed after racemic BINOL was treated with Cu(II)/(-)sparteine complex under the same conditions as for the coupling. The formation of a precipitate was observed again, and both the precipitate and the mother liquor were worked up separately. The precipitate provided 36% yield of enantiomerically pure (–)BINOL, while the mother liquor gave the same enantiomer with 59% ee and 60% yield. Work-up of the whole mixture (without the separation) gave a crude product in 80% ee and 94% yield. In contrast to BINOL the bis-naphthylamine (BINAM) was obtained in much higher *ee* from asymmetric coupling rather than deracemization. The coupling reaction provided BINAM in 84% *ee* and 19% yield, while racemic BINAM in 95% yield was recovered from the deracemization experiment.



Scheme 19.

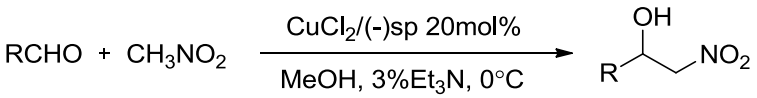
Catalytic oxidative coupling of different naphtols was reported by Nakajima *et al*.[29] using *in situ* prepared CuCl/(-)sparteine complex in 10 mol% as catalyst and O_2 as oxidant**.** The corresponding binaphtols were obtained in low to moderate yields and *ee*, 31-54% and 10-47% respectively (Scheme 20).



Scheme 20.

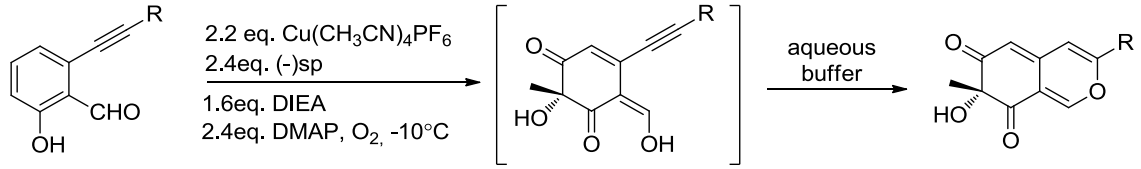
Kantam *et al*.[30] reported in 2006 enantioselective nitroaldol (Henry) reaction catalyzed by $CuCl_2$/(-)sparteine or $Cu(OAc)_2$/(-)sparteine complexes in presence of Et_3N. The authors

reported that the reaction outcome is dependent on the temperature, solvent and amount of base, under optimum conditions the corresponding nitroaldol adducts were obtained in high yields 60-95% and very good *ee* 73-99% (Scheme 21).



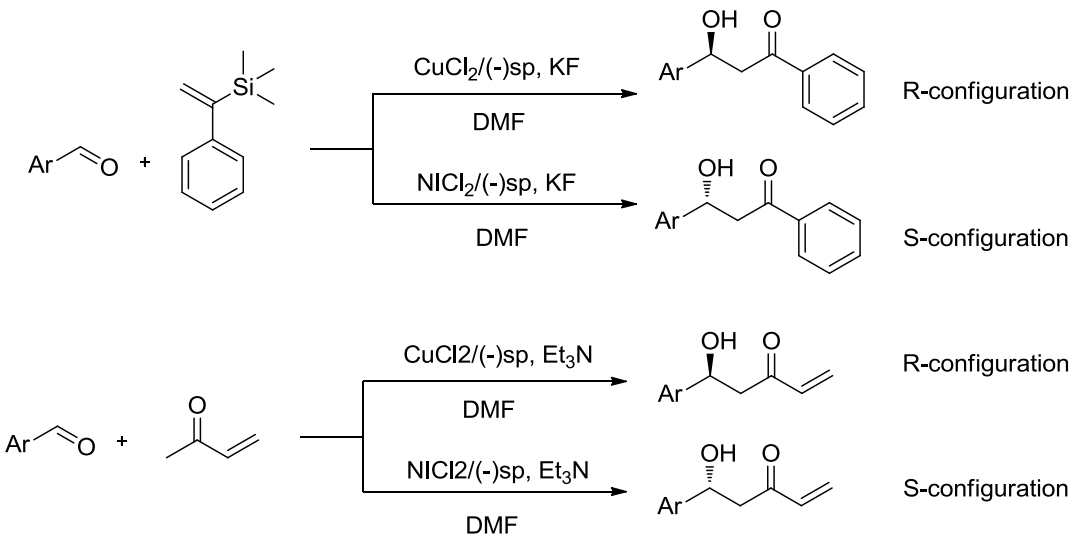
Scheme 21.

Enantioselective synthesis of diverse azaphilones was achieved by Porco et al.[31] *via* copper-mediated oxidative dearomatization. Cu_2[(-)-sparteine]$_2O_2$ complex was generated from $Cu(CH_3CN)_4PF_6$ and (-)-sparteine and applied as catalyst in 2.2 eq. The authors reported that the addition of diisopropylethylamine (DIEA) provided cleaner oxidation reactions. The reaction proceed *via* the formation of vinylogous acids as intermediates and 4-(dimethylamino)pyridine (DMAP) was identified as an efficient additive to promote full conversions. Subsequent the intermediates were subjected to buffer mediated cycloisomerization to give the corresponding azaphilones in 44-72% yield and 95-97% *ee* (Scheme 22).



Scheme 22.

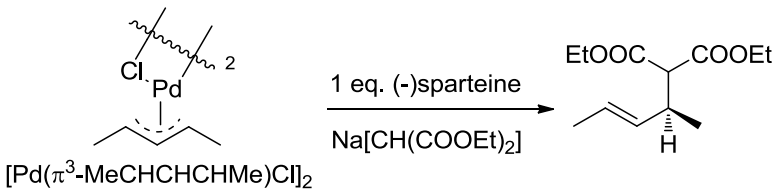
$CuCl_2$/(-)sparteine complex was applied as catalysts under fluoride anion-promoted double catalytic activation (DCA) for asymmetric Mukaiyama aldol reaction of 1-phenyl-1-trimethylsiloxyethylene The corresponding Mukaiyama adducts were obtained in moderate to good yields 25-86% and low to moderate *ee* 8-63%. The authors reported enantioreversal effect on the reaction when $NiCl_2$/(-)sparteine complex was used as a catalyst under the same conditions. The same effect was observed also for the direct aldol reaction of methyl vinyl ketone under Et_3N promoted DCA conditions (Scheme 23).[32]



Scheme 23.

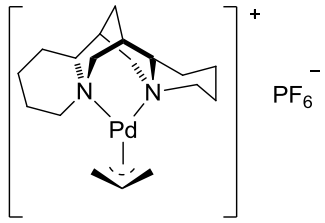
1.2.3. Sparteine as a chiral ligand for palladium catalysis.

Several examples of Pd/(-)sparteine complexes as catalysts for asymmetric allylic alkylations were reported from different researchers. A pioneer work in this area was published by Trost *et al.*,[33] where they performed a stoichiometric allylic alkylation of $[Pd(\pi^3\text{-MeCHCHCHMe})Cl]_2$ complex with $Na[CH(COOEt)_2]$ and in presence of 1eq. (-)sparteine obtained the corresponding product in 20% *ee* (Scheme 24).

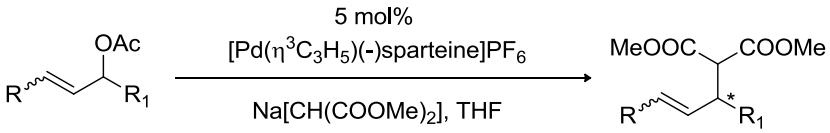


Scheme 24.

Latter Togni[34] employed $[Pd(\eta^3C_3H_5)(\text{-})\text{sparteine}]PF_6$ complex as a chiral precursor for asymmetric alkylation of allylic acetates with $Na[CH(COOMe)_2]$. Using 5 mol% of the catalyst and starting from either racemic or achiral allylic acetates the corresponding adducts were obtained in up to 90% yield and 85% *ee* (Scheme 25). However, the substrate scope of the system seems to be restricted to either cyclic substrates or those bearing aryl substituents.



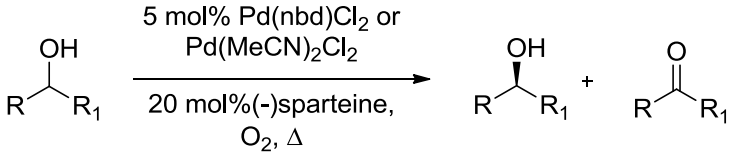
$[Pd(\eta^3C_3H_5)(-)sparteine]PF_6$



Scheme 25.

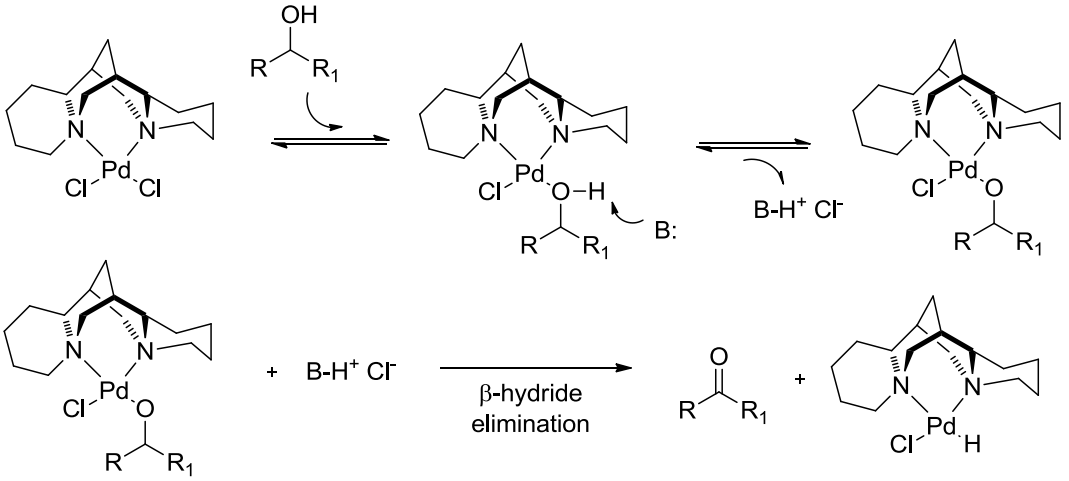
In a following work Cho *et al.*[35] compared $[Pd(\eta^3C_3H_5)(-)sparteine]PF_6$ and $[Pd(\eta^3C_3H_5)(-)isosparteine]PF_6$ complexes for the same transformation employing several solvents in parallel reactions. The results showed that both (-)sparteine and (-)isosparteine can indeed behave as a chiral bidentate ligands. However, the second was preferred overall presumably because its pocket depth is deeper than that of (-)sparteine thus affecting the stability of the Pd complex.

Detailed studies on (-)sparteine as a suitable bidentate ligand for Pd(II) oxidative kinetic resolution of secondary alcohols were carried out mainly by the Stoltz and Sigman groups. In early papers Stoltz *et al.*[36] and Sigman *et al.*[37] established that generated *in situ* $Pd[(-)sparteine]Cl_2$ complex, from $Pd(nbd)Cl_2$ (Stoltz) or $Pd(MeCN)_2Cl_2$ (Sigman), and (-)sparteine is capable to catalyze enantioselective oxidation of a variety of benzylic and allylic alcohols with O_2, providing excellent levels of asymmetric induction (Scheme 26).



Scheme 26.

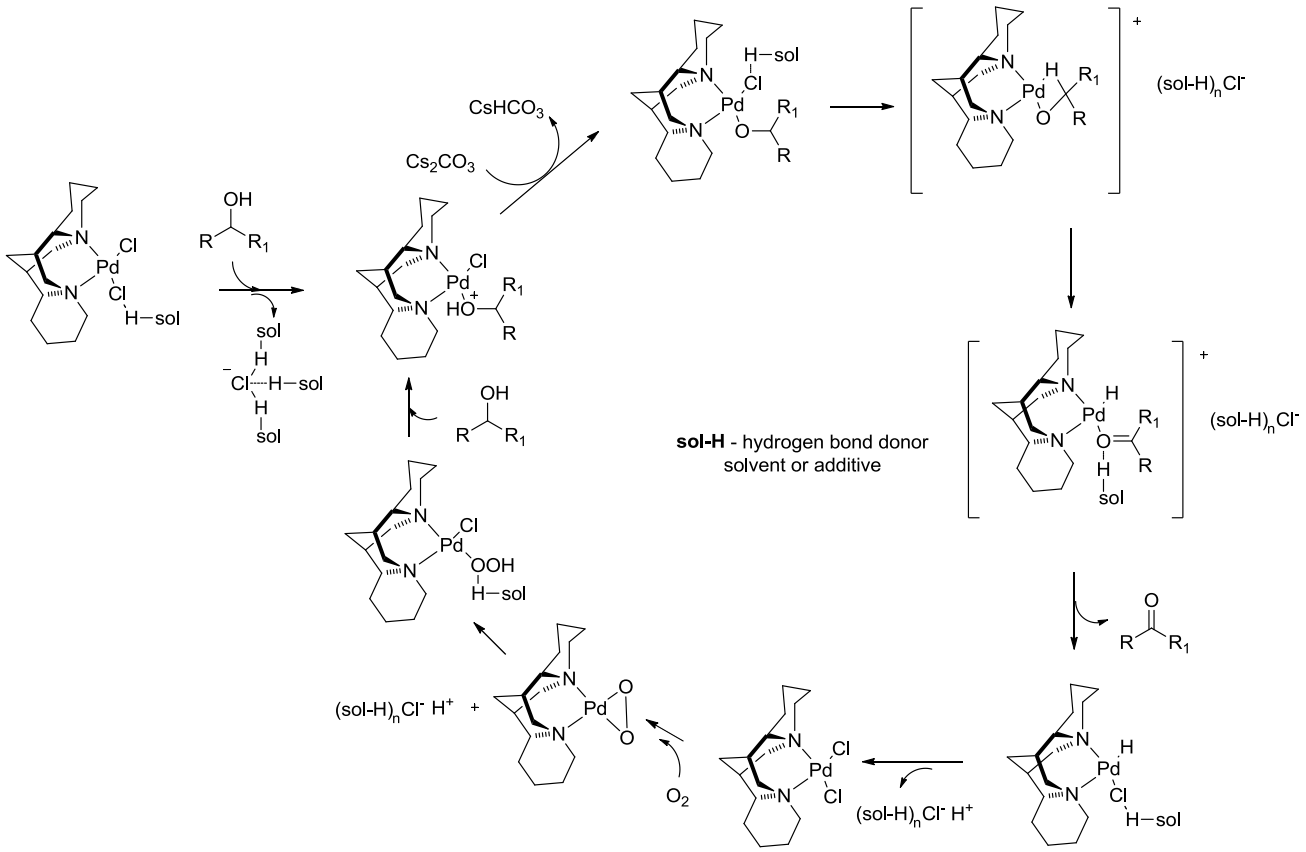
Both research groups observed that the use of excess of (-)sparteine in regards to $Pd(nbd)Cl_2$ or $Pd(MeCN)_2Cl_2$ is essential for the transformation and $Pd[(-)sparteine]Cl_2$ complex itself is incompetent as a catalyst without additional (-)-sparteine. In following paper Sigman *et al.*[38] explained that observation by proposing a reaction mechanism, which involves a base-promoted pathway where (–)sparteine works as a base (Scheme 27).



Scheme 27.

Following these hypotheses, the authors[39] replaced the exogenous (–)sparteine with weakly coordinating achiral bases (pk_a of conjugate acids from 3 to 11 in H_2O). Carbonates were found to provide effective oxidative resolution, although higher loadings were necessary. Na_2CO_3 in 50 mol% with 5% Pd[(-)sparteine]Cl_2 exhibit the same performance like the previously reported system with exogenous (–)-sparteine.

Stoltz group also carried out an optimization of their system. They discovered that the presence of Cs_2CO_3 and *t*-BuOH significantly accelerates the transformation,[40] which in its original version, using toluene as solvent at 80ºC under O_2, required 1 week reaction time. Upon the addition of Cs_2CO_3 and *t*-BuOH in 1.2eq. and 4eq. respectively, the same performance was achieved in only 16h. In a following paper the authors hypothesized that the exogenous alcohol was affecting reaction rates by forming hydrogen bonds when aiding the solvation of chloride anions (Scheme 28).[41]



Scheme 28.

Following these suggestions the authors performed solvent screening aiming to explore the effect of hydrogen bond donation. $CHCl_3$ was identified as the most effective solvent, providing high reaction rates even at room temperature and ambient air as oxidant. *t*-BuOH was discovered not to be beneficial for the reaction anymore. However, Cs_2CO_3 still enhanced the reaction rate. After the ratio of the different additives in chloroform was optimized, the best system was found to be: 5 mol % $Pd(nbd)Cl_2$, 12 mol % (–)sparteine, molecular sieves (3˚A), ambient air (1 atm.), Cs_2CO_3 (0.4 equiv.), 23˚C and these are the most mild and selective conditions for the Pd catalyzed oxidative kinetic resolution of secondary alcohols, reported up to now. In the same year Stoltz *et al.*[42] proved the importance of this transformation by applying it as a tool to access important enantiopure pharmaceutical building blocks for the synthesis of various drugs, including the antidepressants fluoxetine hydrochloride (Prozac®), norfluoxetine, tomoxetine and nisoxetine, the orally active leukotriene receptor antagonist monteleukast sodium (Singulair®) and Merck`s human neurokinin-1 (hNK-1) receptor antagonist (Figure 3).

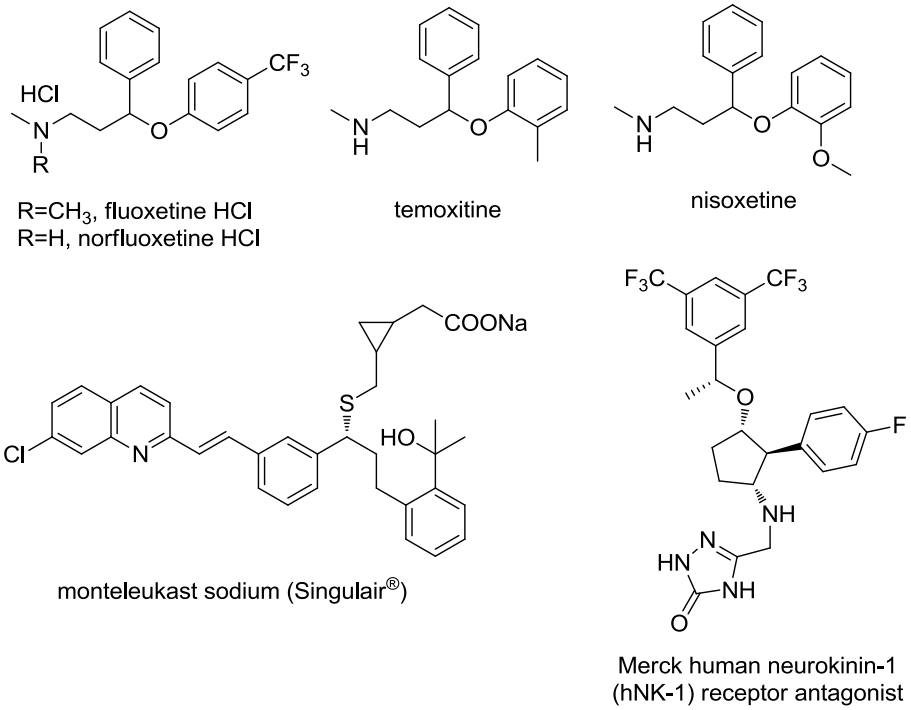
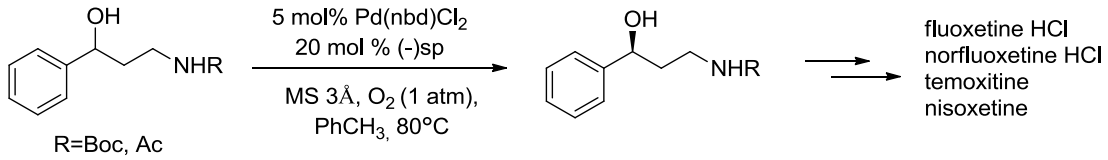


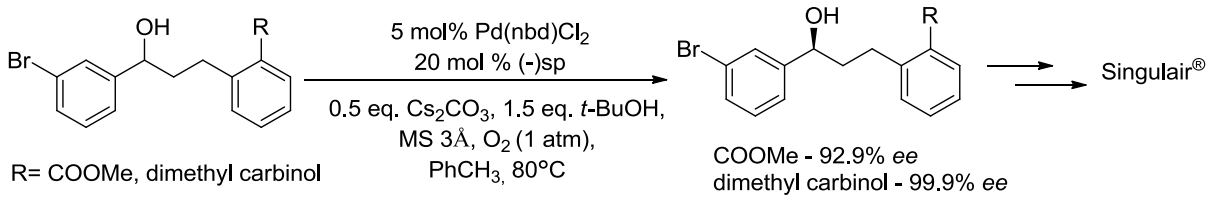
Figure 3.

Two different precursors for the synthesis of fluoxetine, norfluoxetine, tomoxetine and nisoxetine were successfully resolved in up to 97% *ee* (Scheme 29). The required reaction times for the Boc and Ac precursors were 24 and 14.5h, respectively.



Scheme 29.

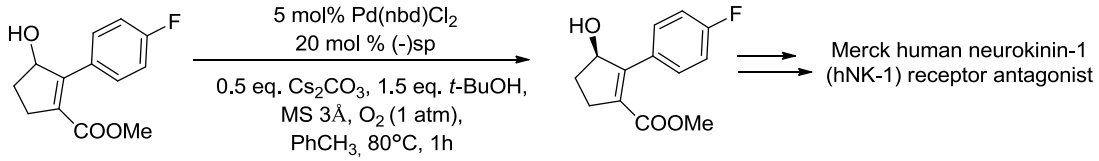
In order to minimize the experimental time for the resolution of the Singulair® precursors (Scheme 30) the authors employed their recently developed, at the time, procedure involving Cs_2CO_3 and *t*-BuOH as additives. The reaction rate was significantly accelerated and both precursors were resolved in high *ee* within 4.5h.



Scheme 30.

The oxidative kinetic resolution of the precursor for Merck`s human neurokinin-1 (hNK-1) receptor antagonist was carried out under various conditions. The same yield (55.5%) and *ee*

(99.5%) were achieved in presence or absence of Cs_2CO_3 and *t*-BuOH. However the reaction time in their presence was shorter 4 *vs* 1h (Scheme 31). The authors also tested $CHCl_3$ as a solvent at room temperature. 50.8% yield and 94% *ee* were achieved within 9h using 5 mol % $Pd(nbd)Cl_2$, 12 mol% (-)sparteine, 0.4 equiv Cs_2CO_3, MS 3Å and O_2 (1 atm).



Scheme 31.

In 2009 Stoltz group[43] published a review on the topic, where they summarize their own results and discussed the scope, applications and limitations of this technology. The authors pointed out, that although the method is applicable for a broad substrate scope, some limitations still exist. A number of alcohols display limited rates of oxidation, preventing their resolution. Benzylic alcohols with ortho-substituents and sterically hindered alcohols exhibit decreased rates of oxidation. The presence of vicinal heteroatoms blocks the oxidations, presumably through catalyst coordination and deactivation. In addition to unreactive alcohols, some types of alcohols are resolved with poor selectivity. Certain class of substrates are poorly resolved due to the steric difference between the two alcohol substituents, which is too small for the catalyst to adequately distinguish between the enantiomers. Secondary alcohols bearing electron-poor aromatic substituents are much less selectively resolved than their electron-rich counterparts. However no significant improvements were achieved in that area after the Stoltz review.

2. Results and discussion.

2.1. Synthesis and applications of lupanine derived sparteine analogs.

In our research we were interested to apply lupanine (Figure 4) as a platform molecule for the synthesis of sparteine derivatives with different functionalities. Lupanine is a lupin alkaloid available in considerable high quantities from *Lupinus genus* and it is actually presented as contaminant in the waste water from the production of lupine beans for food.[44]

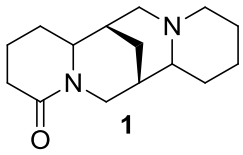


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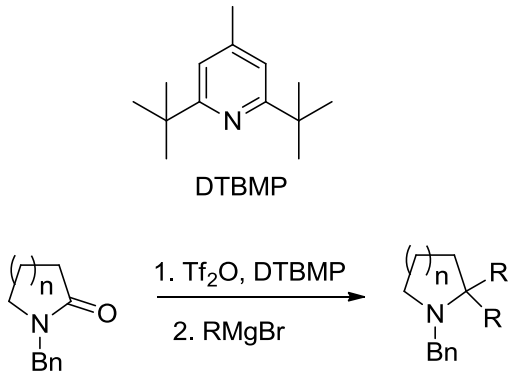
Figure 4.

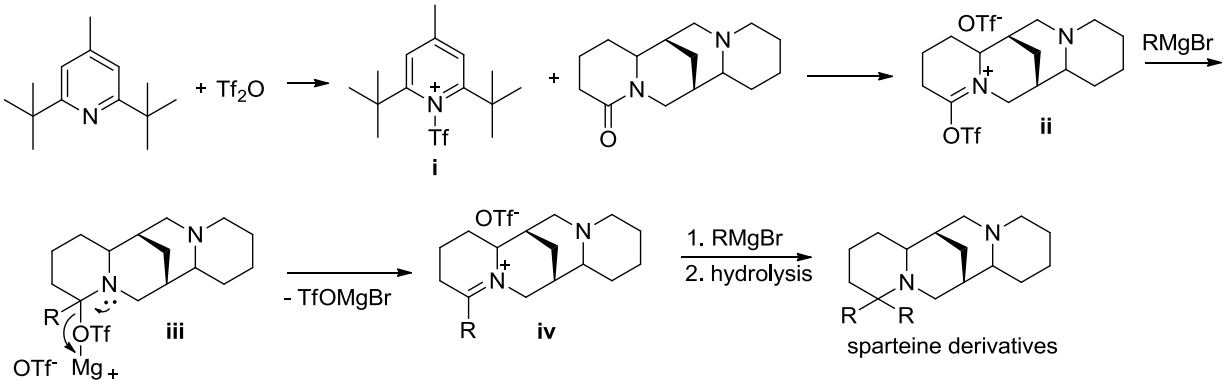
Moreover being a structural analog of sparteine, but bearing amide functionality, is a convenient staring material for the synthesis of sparteine analogs *via* functionalization of the amide group. It is also available in racemic or close to racemic natural form and could be resolved *via* crystallization with (−)-Camphor-10-sulfonic acid[45] to its individual enantiomers, thus providing access to both enatiomeric series of derivatives.

We anticipated that the reported by Huang *et al*.[46] (Scheme 32) direct sequential reductive alkylation of lactams with Grignard reagents could be applied for lupanine and provide access to sparteine analogs bearing two substituents α to one of the nitrogens.



Scheme 32.

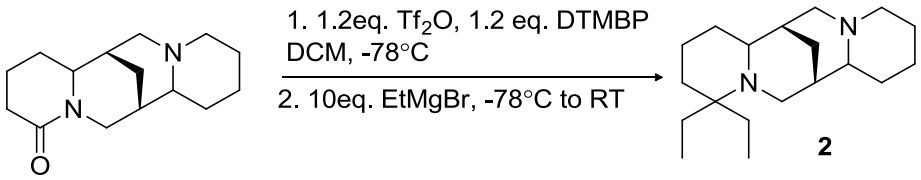
According to the described proposed mechanism the 2,6-Di-*tert*-butyl-4-methylpyridine (DTBMP) first reacts with triflic anhydride to generate the reactive pyridinium intermediate **i**, which reacts with lupanine to form highly electrophilic iminium triflate intermediate i**i**, the last after a reaction with 1 equivalent of Grignard reagent forms N,O-acetal **iii**. Then, elimination of OTf^- assisted by the lone pair of electrons on the nitrogen atom and metal cation leads to the formation of iminium ion **iv**, which after being trapped by a second equivalent of Grignard and hydrolyzed provides the target disubstituted sparteine derivatives (Scheme 33).



Scheme 33.

We were expecting from this kind of sparteine analogs to exhibit an improved performance as ligands in asymmetric lithiations or Pd catalyzed alcohol kinetic resolutions since they possess higher steric hindrance caused by the two substituents on one side of the molecule, thus possibly allowing better stereocontrol on the reactions compared to sparteine itself.

Initially we performed an addition of EtMgBr to lupanine under the already reported conditions.[46]



Scheme 34.

The reaction was performed *via* initial activation of racemic lupanine with 1.2eq. Tf_2O and DTMBP at -78ºC for 1h in DCM, followed by an addition of 5eq. excess of freshly prepared EtMgBr in Et_2O. The final product **2** was isolated as white solid in 31% yield after column chromatography. The yield of the reaction, which was lower compared to the reported yields for other lactams (70-90%), led us to look for a possible reason. One possible explanation could be a reaction of Tf_2O with the amine functionality of the lupanine forming a salt (Figure 5), thus decreasing the equivalents of T_2O available for the amide activation.

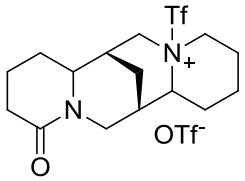


Figure 5.

The reaction was repeated using 2eq. of Tf_2O and DTMBP and much higher, and competitive to the reported in the literature, yield of 61% of **2** was achieved. Further increase of the equivalents didn't provide any improvement and the same yield, using 3eq.of Tf_2O and DTMBP was obtained.

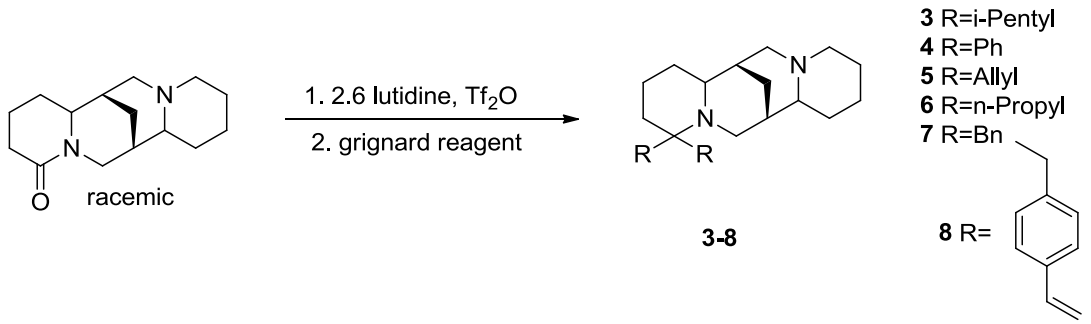
Having in hands the optimized conditions we decided to study other pyridinium bases as activation promoters. The reason was the high price of DTMBP and the observed negative effect on the reaction related with its purity (lower reaction yields have been achieved with one of the obtained from Alfa Aesar DTMBP). 2,6 lutidine and 2,4,6 collidine have been tested and both of them provided similar yield of **2** (Table 1).

Table 1. Screening of different pyridinium bases for the synthesis of derivative **2**[a]

Entry	Pyridinium base	Yield of 2, %
1	2,6 lutidine	60
2	2,4,6 collidine	63
3	DTMPB	61

[a] *All the reaction were performed with racemic Lupanine in DCM with 2eq. of Tf_2O and pyridinium base at -78ºC for 1h, followed by an addition of 10eq. excess of freshly prepared EtMgBr in Et_2O.*

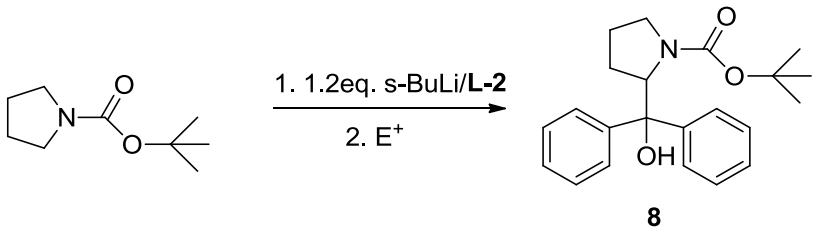
Further on we carried out the synthesis of other sparteine derivatives **3-8** under the optimized conditions and choose 2,6 lutidine as a base because provides similar yields as the other pyridinium bases and it was more easy to be purify the products at the end and it (Scheme 35).



Scheme 35.

It was not possible to obtain compounds **3** and **4** presumably due to the increased steric hindrance when two *i*-pentyl or phenyl groups have to be inserted to one and the same carbon. Compounds **5-8** were obtained in 40-53% yield as white or slightly colorful solids and along with derivative **2** were submitted for biological evaluations, which are still ongoing.

Compound **2** was synthesized also from enantiopure L-lupanine in order to be tested as a ligand (**L-2**) for asymmetric lithiation of *N*-Boc-pyrrolidine and subsequent substitution using benzophenone as electrophile to give chiral **8** (Scheme 36).

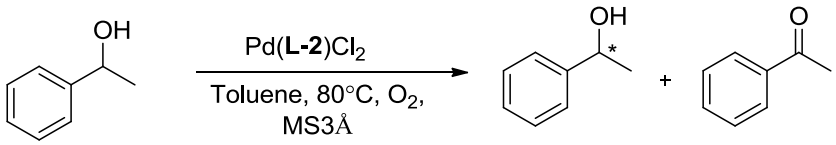


Scheme 36.

Unfortunately **L-2**, was observed to be completely inactive for this transformation and no conversion to **8** was obtained under the reported in the literature conditions, which were

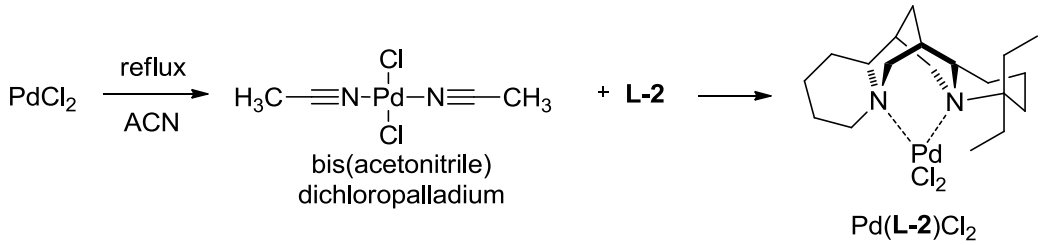
pretreatment of *N*-Boc-pyrrolidine with 1.2 eq. *sec*-BuLi/**L-2** in dry Et_2O at -78ºC, followed by the addition of 1.2 eq. of benzophenone and subsequent slow warm up of the reaction mixture to room temperature. Looking for a possible reasons for this negative result, we decided to test the reaction with higher temperature, thus increasing the reactivity of the *sec*-BuLi/**L-2** complex. The reaction has been repeated twice, performing the pretreatment of the *N*-Boc-pyrrolidine with *sec*-BuLi/**L-2** at 0ºC and room temperature. However both experiments failed to give **8.** Since it is known for the asymmetric lithiation reactions to be very dependent at the solvent and also in order to increase the solubility of **L-2**, which was observed to not be fully soluble in Et_2O, we change the reaction solvent to dry THF, unfortunately again no product was obtained.

L-2 was also tested as a ligand for Pd catalyzed 1-phenyl ethanol kinetic resolution using the reported by Stoltz *et al*.[36] procedure (Scheme 37).



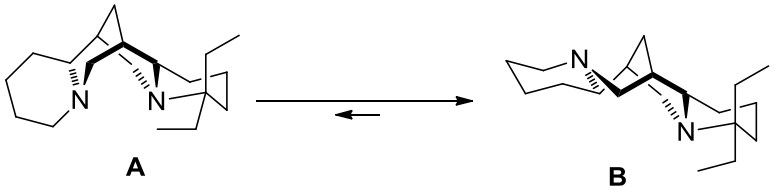
Scheme 37.

The reaction has been performed *via in situ* ligand exchange of $Pd(nbd)Cl_2$ complex with **L-2** in toluene followed by stirring the reaction mixture at 80ºC under oxygen in presence of molecular sieves. No *ee* of the 1-phenyl ethanol was achieved, along with no acetophenone formation, which means that **L-2** is an unreactive ligand for this oxidative kinetic resolution. We also tried to isolate the $Pd(\mathbf{L\text{-}2})Cl_2$, using already reported procedure for the synthesis of $Pd((-)sparteine)Cl_2$ complex *via* formation of bis(acetonitrile)dichloropalladium by refluxing of $PdCl_2$ in acetonitrile and *in situ* exchange of the two acetonitrile ligands with **L-2** (Scheme 38). Unfortunately all the attempts to obtain $Pd(\mathbf{L\text{-}2})Cl_2$ were unsuccessful.



Scheme 38.

A possible explanation for the completely different behavior of **L-2** as a ligand for asymmetric catalysis compared to (-)sparteine could be the effect of the two ethyl substituents on the conformational equilibrium of the molecule (Scheme 39).



Scheme 39.

We speculate that the presence of the substituents causes higher energy barrier between the thermodynamically favored conformer **B** and thermodynamically unfavored **A**, resulting in a predominant abundance of **B**, which is unavailable for complexation with Li and Pd due to the bigger distance between the nitrogen atoms. The more stable conformational structure of the molecule caused by the shifting of the equilibrium, could also be a reason for the unexpected fact that **L-2** is a solid at room temperature (Mp. 101-103ºC), while (-)sparteine is a liquid (Mp. 30ºC).

Our observations were also supported by the obtained x-ray structure of **2** (Figure 6), which confirmed that the compound exist as its conformer **B** in the crystal form. Additional DFT calculations, which will provide the correct value of the energy barrier will be performed.

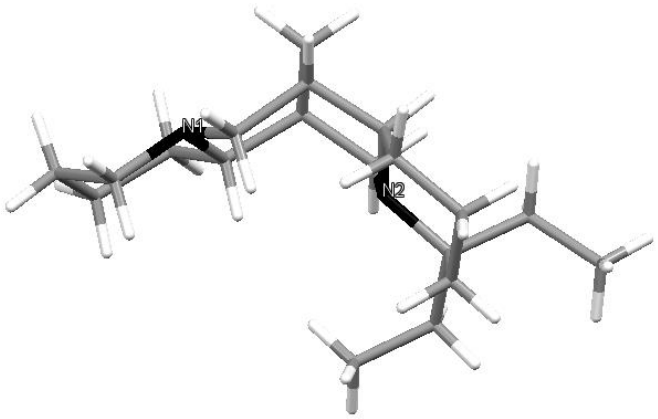
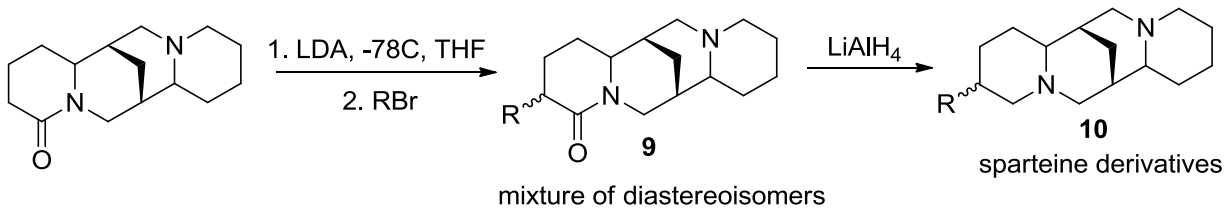


Figure 6.

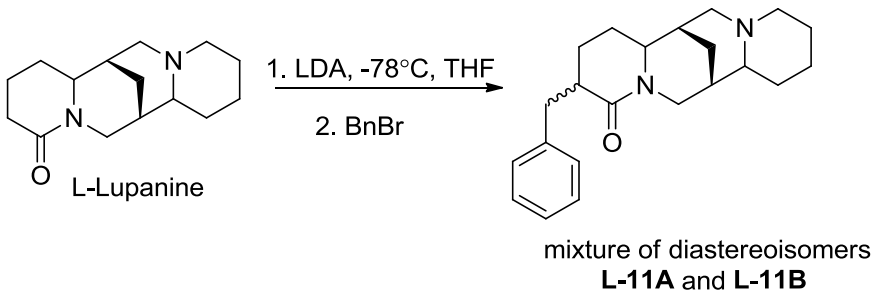
Another synthetic pathway to access sparteine derivatives, tacking advantage from the amide moiety of lupanine, is presented on Scheme 40.



Scheme 40.

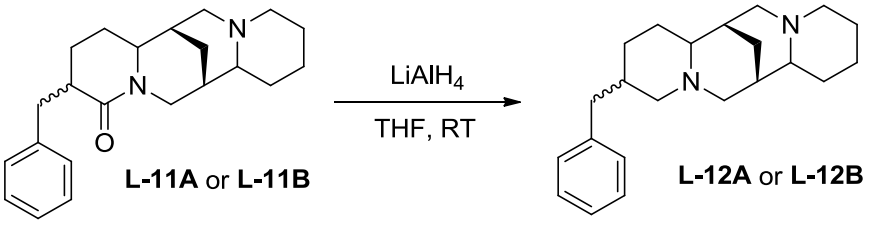
After initial deprotonation of enantiopure lupanine with lithium diisopropylamide (LDA) the formed lithium enolate can be trapped with alkyl or benzyl bromides as electrophiles to give lupanine derivatives **9** as mixture of diastereoisomers, which after separation and reduction with $LiAlH_4$ will provide sparteine derivatives **10**, bearing a substituent β to one of the nitrogens.

Derivatives **L-11A,B** were successfully synthesized using this strategy in 59% yield as mixture of diastereoisomers. The reaction was performed *via* pretreatment of the L-lupanine with 2.5eq. LDA in THF for 4h at -78ºC, followed by an addition of 3eq. of benzyl bromide. (Scheme 41).



Scheme 41.

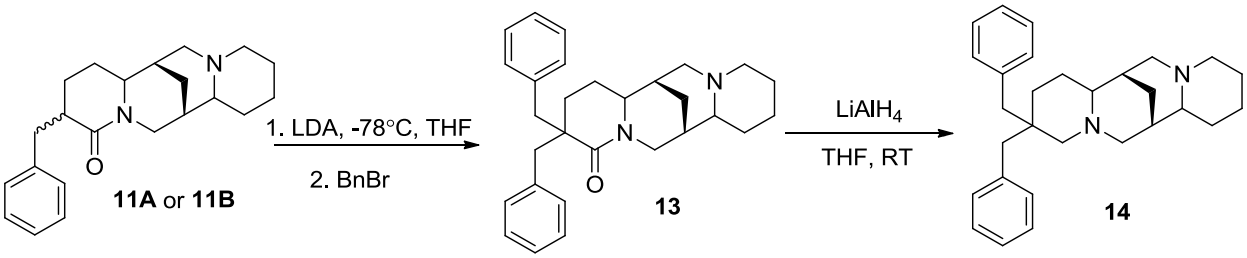
n-BuLi and *sec*-BuLi have been also tested as bases for the reaction but both provided poor results, low lupanine conversion and a lot of side products were observed by TLC. Full conversion of the lupanine (by TLC) was achieved after slow warming up of the reaction mixture to room temperature before the addition of the benzyl bromide (at -78ºC after cooling down) but lower yield of 48% of **L-11A,B** was obtained**,** probably due to L-lupanine decomposition under these conditions. The mixture of diastereoisomers was partly separated by silica gel column chromatography and the obtained pure diastereoisomers **L-11A** and **L-11B** were further handled individually and reduced with $LiAlH_4$ to the corresponding sparteine derivatives **L-12A** and **L-12B** (Scheme 42).



Scheme 42.

Initially the reduction was performed under reflux in THF in presence of 3eq. of $LiAlH_4$, which are common conditions for this kind of amide reductions. But in the particular case partial decomposition of compound **L-11A** was observed. Surprisingly we found out that the reaction could be performed under much milder conditions and 81% yield of derivatives **L-12A** and **L-12B** was obtained after overnight stirring at room temperature.

The diastereomeric mixture **L-11A,B** was also subjected to a second deprotonation with LDA followed by an addition of benzyl bromide, compound **13** was obtained in 40% yield and subsequently reduced in 80% yield with $LiAlH_4$ to give sparteine derivative **14** (Scheme 43).

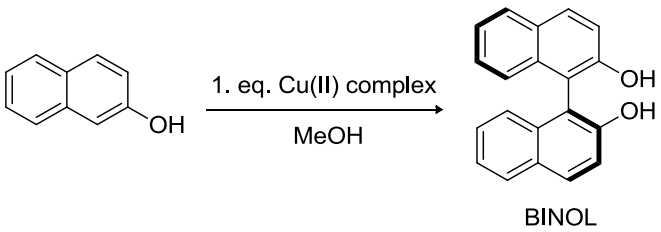


Scheme 43.

Synthesis of other derivatives is ongoing and the compounds will be provide for biological evaluations and tested as ligands for asymmetric catalysis in a due course.

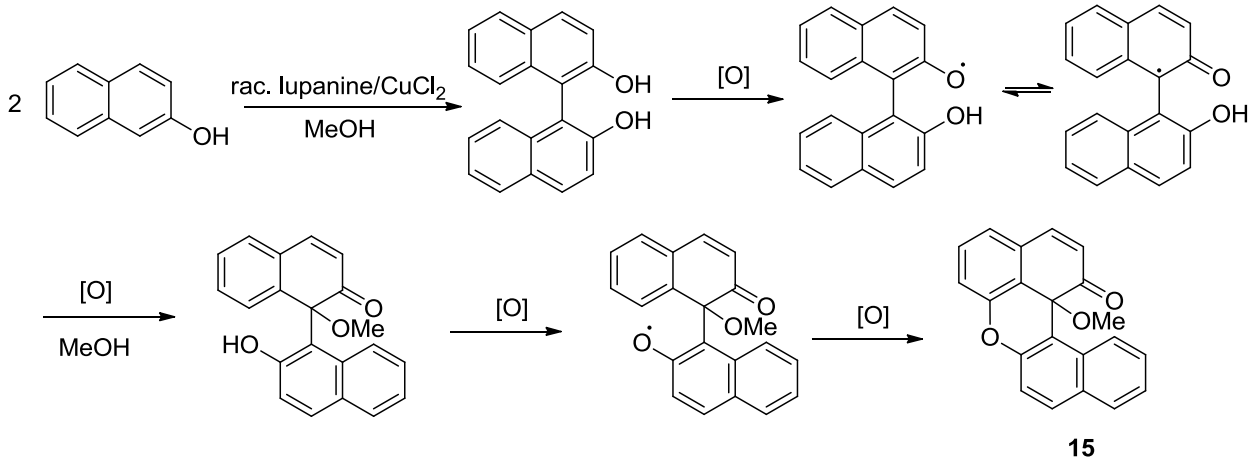
2.2. Complexes of lupanine and $CuCl_2$ and their catalytic behavior.

In our research we were also interested on the direct application of enantiopure lupanine as ligand for asymmetric metal catalysis and in particular asymmetric Cu(II) catalyzed oxidative coupling of 2-naphtol to BINOL.[28] This coupling is typically carried out in MeOH by *in situ* formation of Cu(II)-ligand complex in stoichiometric quantity, followed by addition of 2-napthol (Scheme 44).



Scheme 44.

Initially we decided to test the catalytic activity of the racemic lupanine as ligand, before performing the asymmetric version of the reaction. After mixing 1:1 equivalents of racemic lupanine and $CuCl_2$ in MeOH we observed immediate formation of a complex, which precipitates from the solution, at that point we added 1 eq. of 2-naphthol and stirred the reaction mixture under Ar overnight. After work up of the reaction no BINOL was observed, instead it was isolated in 10% yield an already reported product **15,** which is a result from further radical oxidations involving the addition of one molecule of the solvent (MeOH) (Scheme 45).[47]



Scheme 45.

Since the mechanism for the formation of **15** required O_2 for the further oxidations we repeated the reaction but under ambient air instead of Ar, and under these conditions **15** was isolated in 40% yield.

Take advantage of the exhibited high catalytic activity of the $CuCl_2$/rac-lupanine complex, we decided to check if product **15** will be available in its enantioenriched form under asymmetric catalysis. The reaction was performed using the same conditions and L-lupanine as ligand surprisingly, instead of formation of **15** we observed racemic BINOL, although in low yield (10%), to be the major reaction product along with minor amount of side products. This unexpected result showed that more than one lupanine ligand complex with Cu, thus allowing the formation of different type of complexes from racemic and enantiopure lupanine

respectively. Triggered by the big difference in the catalytic activity of these complexes we performed a screening of various conditions and solvents, which would allow as to obtain single crystals for x-ray analysis. Unfortunately $CuCl_2$/rac-lupanine complex was observed to be completely insoluble in the suitable organic solvents, while $CuCl_2$/L-lupanine was possible to crystalize. The crystals are submitted for x-ray analysis but upon the completion of this theses the results were still not available.

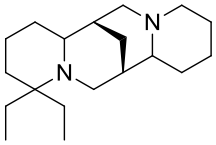
3. Conclusions.

In conclusion we were able to successfully synthesized various sparteine like derivatives taking advantage from the amide functionality of readily available lupain alkaloid – Lupanine. The compounds were fully characterized and are under biological evaluations. Up to now we were not able to apply them as ligands for asymmetric organic transformation. However, this work is still ongoing and the ligands will be tested for other asymmetric reactions.

4. Experimental.

General: All the reagents used were purchased from Sigma-Aldrich or Merck and were used without further purification. The reaction evolution was followed by TLC using silica Merck Kieselgel 60 F254 plates, and revealed by ultraviolet light at 254 nm and 325 nm. NMR spectra were recorded at room temperature in a Bruker AMX 300 or Bruker AMX 400 using $CDCl_3$ as solvent.

*Synthesis of diethyl sparteine derivative **2.***



A flame dried schlenk was charged with racemic lupanine, 248 mg (1mmol), 2.6-ditertbutyl-4-methyl pyridine, 410 mg (2 mmol) and 5 ml of dry DCM. The solution was cooled down to -78ºC and Tf_2O 0.33 ml (2 mmol) was added dropwise. The resulting mixture was stirred at -78ºC for 45 min, when EtMgBr, 10 ml (10 mmol) 1M solution in Et_2O was added dropwise. The reaction mixture was allowed to slowly warm up to r.t. over 5h. The reaction was quenched with 5 ml 1M aq. HCl and concentrated under vacuum, dissolved in 20 ml 1M aq. HCl and washed with Et_2O 2x25 ml. The aqueous phase was basified with Na_2CO_3 and extracted with EtOAc 3x25 ml. The organic phase was dried over Na_2SO_4 and evaporated. The crude product was purified with silica flash chromatography using gradient mixing of hexane and EtOAc containing 5% Et_3N. The final product was obtained as a white solid in 61% yield (176mg)

For the biological studies the product was additionally recrystallized from water/EtOH to give 120 mg (41% yield) of snow white crystals, which were also submitted for x-ray analysis.

Grignard reagent preparation: A flame dried 2 neck round bottom flask, equipped with condenser, was charged with Mg turnings, 240 mg (10 mmol) and a small crystal of I_2, then 10 ml dry Et_2O was added, then ethyl bromide, 0.75 ml (10 mmol) was added dropwise in order to keep gentle reflux, the reaction was stirred for additional 30 min after the addition was completed.

^{1}H NMR (400 MHz, $CDCl_3$) δ 2.80 (d, *J* = 11.3 Hz, 1H), 2.64 (t, *J* = 10.8 Hz, 1H), 2.51 (d, *J* = 10.8 Hz, 1H), 2.39 (m, 2H), 2.22 – 2.10 (m, 1H), 1.96 (m, 3H), 1.88 – 1.64 (m, 3H), 1.63 – 1.13 (m, 15H), 1.06 – 0.95 (m, 1H), 0.91 (t, *J* = 7.4 Hz, 3H), 0.76 (t, *J* = 7.6 Hz, 3H).

^{13}C NMR (101 MHz, $CDCl_3$) δ 64.53, 58.98, 57.11, 55.95, 53.78, 49.94, 36.64, 34.84, 34.35, 30.65, 30.16, 28.81, 28.10, 26.16, 25.22, 21.53, 19.63, 9.28, 7.18.

IR (neat) - 2933, 1737, 1456, 1373, 1229 cm^{-1}

HRMS Calculated for $[M+H]^+$ $C_{19}H_{35}N_2$, 291.2800. Found 291.2790.

Mp. 101-103ºC

*Synthesis of **2** with 2.6 lutidine.*

A flame dried schlenk was charged with racemic lupanine, 248 mg (1 mmol), 2.6 lutidine, 0.23 ml (2 mmol) and 5ml of dry DCM. The solution was cooled down to -78ºC and Tf_2O 0.33 ml (2 mmol) was added dropwise. The resulting mixture was stirred at -78ºC for 45 min when EtMgBr, 10ml (10mmol) 1M solution in Et_2O was added dropwise. The reaction mixture was allowed to slowly warm up to r.t. over 5h. The reaction was quenched with 5 ml 1M aq. HCl and concentrated under vacuum, dissolved in 20 ml 1M aq. HCl and washed with Et_2O 2x25 ml. The aqueous phase was basified with Na_2CO_3 and extracted with EtOAc 3x25 ml. The organic phase was dried over Na_2SO_4 and evaporated. The crude product was purified with silica flash chromatography using gradient mixing of hexane and EtOAc containing 5% Et_3N. The final product was obtained as a white solid in 60% yield (172 mg).

*Synthesis of **2** with 2.4.6 collidine.*

A flame dried schlenk was charged with racemic lupanine, 248 mg (1 mmol), 2.4.6 collidine, 0.26 ml (2 mmol) and 5 ml of dry DCM. The solution was cooled down to -78ºC and Tf_2O 0.33 ml (2 mmol) was added dropwise. The resulting mixture was stirred at -78ºC for 45 min when EtMgBr, 10 ml (10 mmol) 1M solution in Et_2O was added dropwise. The reaction mixture was allowed to slowly warm up to r.t. over 5h. The reaction was quenched with 5 ml 1M aq. HCl and concentrated under vacuum, dissolved in 20 ml 1M aq. HCl and washed with

Et_2O 2x25 ml. The aqueous phase was basified with Na_2CO_3 and extracted with EtOAc 3x25 ml. The organic phase was dried over Na_2SO_4 and evaporated. The crude product was purified with silica flash chromatography using gradient mixing of hexane and EtOAc containing 5% Et_3N. The final product was obtained as a white solid in 63% yield (183 mg).

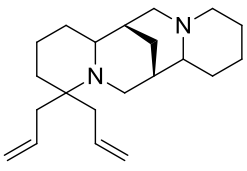
*Synthesis of racemic **2** in 2.4 g scale:*

A flame dried schlenk was charged with L-lupanine, 2.48 g (10 mmol), 2.6 lutidine, 2.3 ml (20 mmol) and 50 ml of dry DCM. The solution was cooled down to -78ºC and Tf_2O 3.3ml (20 mmol) was added dropwise. The resulting mixture was stirred at -78ºC for 45min when EtMgBr, 80 ml (80 mmol) 1M solution in Et_2O was added dropwise. The reaction mixture was allowed to slowly warm up to r.t. over 5h. The reaction was quenched with 15 ml 1M aq. HCl and concentrated under vacuum, dissolved in 100 ml 1M aq. HCl and washed with Et_2O 2x100 ml. The aqueous phase was basified with Na_2CO_3 and extracted with EtOAc 3x100 ml. The organic phase was dried over Na_2SO_4 and evaporated. The crude product was purified with silica flash chromatography using gradient mixing of hexane and EtOAc containing 5% Et_3N. The final product was obtained as a white solid in 58% yield (1.8 g). The product was recrystallized from water/EtOH to give 50% (1.56 mg) of highly pure product.

*Synthesis of **L-2**:*

A flame dried schlenk was charged with L-lupanine, 1 g (4 mmol), 2.6 lutidine, 0.93 ml (8 mmol) and 20 ml of dry DCM. The solution was cooled down to -78ºC and Tf_2O 1.35 ml (8 mmol) was added dropwise. The resulting mixture was stirred at -78ºC for 45 min when EtMgBr, 40 ml (40 mmol) 1M solution in Et_2O was added dropwise. The reaction mixture was allowed to slowly warm up to r.t. over 5h. The reaction was quenched with 15 ml 1M aq. HCl and concentrated under vacuum, dissolved in 100 ml 1M aq. HCl and washed with Et_2O 2x100 ml. The aqueous phase was basified with Na_2CO_3 and extracted with EtOAc 3x100 ml. The organic phase was dried over Na_2SO_4 and evaporated. The crude product was purified with silica flash chromatography using gradient mixing of hexane and EtOAc containing 5% Et_3N. The final product was obtained as a white solid in 54% yield (618 mg). The product was recrystallized from water/EtOH to give 45% (525 mg) of highly pure final product.

*Synthesis of diallyl sparteine derivative **5.***



A flame dried schlenk was charged with racemic lupanine, 248 mg (1 mmol), 2.6-lutidine, 0.23ml (2 mmol) and 5 ml of dry DCM. The solution was cooled down to -78ºC and Tf_2O 0.33ml (2 mmol) was added dropwise. The resulting mixture was stirred at -78ºC for 45 min when 10ml (10 mmol) 1M solution in Et_2O of AllylMgBr was added dropwise. The reaction mixture was allowed to slowly warm up to r.t. over 5h. The reaction was quenched with 5 ml 1M aq. HCl and concentrated under vacuum, dissolved in 20 ml 1M aq. HCl and washed with Et_2O 2x25 ml. The aqueous phase was basified with Na_2CO_3 and extracted with EtOAc 3x25 ml. The organic phase was dried over Na_2SO_4 and evaporated. The crude product was purified with silica flash chromatography using gradient mixing of hexane and EtOAc containing 5% Et_3N. The final product was obtained as a white solid in 40% yield (126 mg**).**

Grignard reagent preparation: A flame dried 2 neck round bottom flask, equipped with condenser, was charged with Mg turnings, 240 mg (10mmol) and a small crystal of I_2, then 10 ml dry Et_2O was added, then allyl bromide, 0.87ml (10mmol) was added dropwise in order to keep gentle reflux, the reaction was stirred for additional 30 min after the addition was completed.

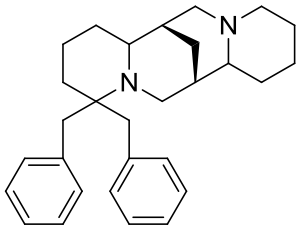
^{1}H NMR (400 MHz, $CDCl_3$) δ 6.15 – 5.90 (m, 1H), 5.79 – 5.55 (m, 1H), 5.29 (m, 2H), 5.10 (m, 2H), 3.66 (d, *J* = 12.9 Hz, 1H), 3.50 (t, *J* = 14.9 Hz, 2H), 3.34 (d, *J* = 12.6 Hz, 1H), 3.22 (d, *J* = 11.7 Hz, 1H), 3.08 – 2.94 (m, 2H), 2.75 (m, 1H), 2.57 – 2.39 (m, 2H), 2.29 – 2.17 (m, 1H), 2.15 – 2.00 (m, 3H), 1.91 (m, 3H), 1.85 – 1.36 (m, 11H).

^{13}C NMR (101 MHz, $CDCl_3$) δ 133.94, 133.00, 119.10, 118.95, 63.41, 60.17, 59.66, 52.53, 51.21, 47.88, 42.23, 33.63, 33.34, 32.87, 31.71, 29.93, 27.29, 22.89, 22.72, 18.40, 17.95.

HRMS Calculated for $[M+H]^+$ $C_{29}H_{39}N_2$, 315.2800. Found 315.2786.

IR (neat) 2941, 1738, 1365, 1260, 1224, 1150, 1030 cm^{-1}

*Synthesis of dibenzyl sparteine derivative **7**:*



A flame dried schlenk was charged with racemic lupanine, 248 mg (1 mmol), 2.6-lutidine, 0.23ml (2 mmol) and 5 ml of dry DCM. The solution was cooled down to -78ºC and Tf_2O 0.33 ml (2 mmol) was added dropwise. The resulting mixture was stirred at -78ºC for 45min when 10 ml (10 mmol) 1M solution in Et_2O of BnMgBr was added dropwise. The reaction mixture was allowed to slowly warm up to r.t. over 5h. The reaction was quenched with 5ml

1M aq. HCl and concentrated under vacuum, dissolved in 20ml 1M aq. HCl and washed with Et_2O 2x25 ml. The aqueous phase was basified with Na_2CO_3 and extracted with EtOAc 3x25 ml. The organic phase was dried over Na_2SO_4 and evaporated. The crude product was purified with silica flash chromatography using gradient mixing of hexane and EtOAc containing 5% Et_3N. The final product was obtained as a white solid in 43% yield (178 mg**).**

Grignard reagent preparation: A flame dried 2 neck round bottom flask, equipped with condenser, was charged with Mg turnings, 240 mg (10 mmol) and a small crystal of I_2, then 10 ml dry Et_2O was added, then benzyl bromide, 1.2 ml (10 mmol) was added dropwise in order to keep gentle reflux, the reaction was stirred for additional 30 min after the addition was completed.

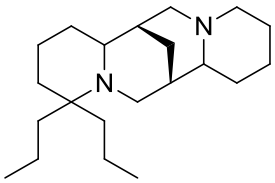
^{1}H NMR (400 MHz, CDCl3) δ 7.38 – 6.92 (m, 10H), 3.06 (m, Hz, 3H), 2.70 (d, *J* = 13.0 Hz, 1H), 2.61 (d, *J* = 11.3 Hz, 1H), 2.55 – 2.33 (m, 4H), 2.13 (dd, *J* = 11.1, 3.4 Hz, 1H), 1.97 (m, 1H), 1.85 – 1.54 (m, 5H), 1.53 – 1.06 (m, 11H), 1.04 – 0.88 (m, 2H).

^{13}C NMR (101 MHz, $CDCl_3$) δ 140.10, 138.83, 131.44, 130.85, 128.06, 127.55, 125.88, 125.78, 62.70, 60.86, 59.83, 55.54, 53.31, 50.44, 46.40, 42.14, 36.79, 35.24, 34.50, 31.72, 30.49, 28.05, 26.01, 24.89, 20.57.

HRMS Calculated for $[M+H]^+$ $C_{29}H_{39}N_2$, 415.3313. Found 415.3089.

IR (neat) 2938, 1665, 1631, 1029 cm^{-1}

*Synthesis of di n-propyl sparteine derivative **6.***



A flame dried schlenk was charged with racemic lupanine, 248 mg (1 mmol), 2.6-lutidine, 0.23 ml (2 mmol) and 5 ml of dry DCM. The solution was cooled down to -78ºC and Tf_2O 0.33 ml (2 mmol) was added dropwise. The resulting mixture was stirred at -78ºC for 45 min when 10 ml (10 mmol) 1M solution in Et_2O of *n*-PrMgBr was added dropwise. The reaction mixture was allowed to slowly warm up to r.t. over 5h. The reaction was quenched with 5 ml 1M aq. HCl and concentrated under vacuum, dissolved in 20 ml 1M aq. HCl and washed with Et_2O 2x25 ml. The aqueous phase was basified with Na_2CO_3 and extracted with EtOAc 3x25 ml. The organic phase was dried over Na_2SO_4 and evaporated. The crude product was purified with silica flash chromatography using gradient mixing of hexane and EtOAc containing 5% Et_3N. The final product was obtained as a white solid in 50% yield (160 mg**).**

Grignard reagent preparation: A flame dried 2 neck round bottom flask, equipped with condenser, was charged with Mg turnings, 240 mg (10 mmol) and a small crystal of I_2, then 10 ml dry Et_2O was added, then *n*-propyl bromide, 0.9 ml (10 mmol) was added dropwise in order to keep gentle reflux, the reaction was stirred for additional 30 min after the addition was completed.

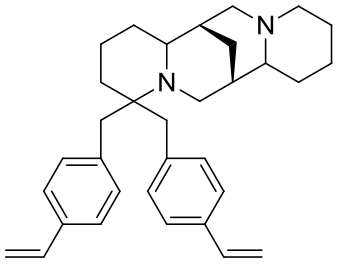
^{1}H NMR (400 MHz, CDCl3) δ 3.48 (d, *J* = 12.4 Hz, 2H), 3.34 (d, *J* = 12.1 Hz, 1H), 3.24 (d, *J* = 12.0 Hz, 1H), 3.13 (dd, *J* = 27.0, 12.5 Hz, 3H), 2.90 (td, *J* = 13.4, 3.1 Hz, 1H), 2.01 (m 2H), 1.98 – 1.31 (m, 19H), 1.29 – 1.15 (m, 2H), 0.99 (m, 6H).

^{13}C NMR (101 MHz, $CDCl_3$) δ 64.56, 62.65, 61.76, 53.41, 52.90, 47.26, 38.59, 33.57, 32.43, 31.73, 31.04, 29.50, 27.02, 24.06, 22.12, 18.08, 17.81, 17.06, 15.56, 14.60, 14.57.

HRMS Calculated for $[M+H]^+$ $C_{29}H_{39}N_2$, 319.3113. Found 319.3111.

IR (neat) 2938, 2873, 1738, 1261, 1222, 1150 cm^{-1}

*Synthesis of distyril sparteine derivative **8**:*



A flame dried schlenk was charged with racemic lupanine, 248 mg (1 mmol), 2.6-ditertbutyl-4-methyl pyridine, 410 mg (2 mmol) and 5 ml of dry DCM. The solution was cooled down to -78ºC and Tf_2O 0.33 ml (2 mmol) was added dropwise. The resulting mixture was stirred at -78ºC for 45min when 10 ml (10 mmol) 1M solution in Et_2O of the corresponding Grignard reagent was added dropwise. The reaction mixture was allowed to slowly warm up to r.t. over 5h. The reaction was quenched with 5 ml 1M aq. HCl and concentrated under vacuum, dissolved in 20 ml 1M aq. HCl and washed with Et_2O 2x25 ml. The aqueous phase was basified with Na_2CO_3 and extracted with EtOAc 3x25ml. The organic phase was dried over Na_2SO_4 and evaporated. The crude product was purified with silica flash chromatography using gradient mixing of hexane and EtOAc containing 5% Et_3N. The final product was obtained as a white solid in 53% yield (247 mg**).**

Grignard reagent preparation: A flame dried 2 neck round bottom flask, equipped with condenser, was charged with Mg turnings, 240 mg (10 mmol) and a small crystal of I_2, then 10 ml dry Et_2O was added, then 4-vinylbenzyl chloride, 1.4 ml (10 mmol) was added dropwise in

order to keep gentle reflux the reaction was stirred for additional 30 min after the addition was completed.

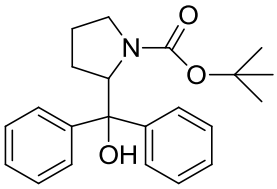
^{1}H NMR (400 MHz, CDCl3) δ 7.25 (d, *J* = 8.3 Hz, 4H), 7.23 – 7.16 (m, 2H), 7.12 (d, *J* = 8.2 Hz, 2H), 6.75 – 6.46 (m, 2H), 5.63 (ddd, *J* = 17.6, 13.1, 0.8 Hz, 2H), 5.12 (ddd, *J* = 14.0, 10.9, 0.8 Hz, 2H), 3.06 (t, *J* = 14.5 Hz, 2H), 2.65 (dd, *J* = 33.6, 12.1 Hz, 2H), 2.56 – 2.35 (m, 4H), 2.13 (dd, *J* = 11.1, 3.6 Hz, 1H), 1.96 (d, *J* = 11.2 Hz, 1H), 1.89 – 1.75 (m, 1H), 1.75 – 1.53 (m, 4H), 1.46 (m, 5H), 1.38 – 1.28 (m, 2H), 1.21 (m, 4H), 1.04 – 0.96 (m, 1H), 0.80 (m, 1H).

^{13}C NMR (101 MHz, CDCl3) δ 139.91, 138.76, 136.95, 136.74, 135.35, 135.25, 131.55, 130.99, 125.98, 125.52, 113.21, 112.87, 62.75, 61.13, 59.90, 55.59, 53.29, 50.53, 42.20, 36.79, 35.17, 34.52, 31.98, 30.51, 28.05, 25.98, 24.77, 20.62.

HRMS Calculated for $[M+H]^+$ $C_{33}H_{43}N_2$, 467.3426. Found 467.3440.

IR (neat) 2943, 1738, 1261, 1224, 1152, 1030 cm^{-1}

Racemic tert-butyl 2-(hydroxydiphenylmethyl)pyrrolidine-1-carboxylate ***8.***[12]



A flame dried schlenk was charged with TMEDA, 0.37 ml (2.5 mmol) and 5 ml dry Et_2O. The solution was cooled down to -78°C and 1.4M solution of sec-BuLi, 1.8 ml, (2.5 mmol) was added. The mixture was stirred 15 min at -78°C when Boc-pyrrolidine 340 mg (2 mmol), dissolved in 2 ml Et_2O, was added. The reaction mixture was stirred at -78°C for 1h and benzophenone, 455 mg (2.5 mmol) solution in 5 ml Et_2O, was added and the reaction was allowed to warm up to r.t. overnight. The reaction was poured in water and extracted with EtOAc 3x20ml. The organic phase was dried over Na_2SO_4 and evaporated. The product was isolated in 68% yield (600 mg) as white solid after flash chromatography using hexane/EtOAc gradient mixing and additional recrystallization from water/EtOH.

^{1}H NMR (400 MHz, CDCl3) δ 7.41 (dd, *J* = 9.7, 4.4 Hz, 4H), 7.38 – 7.18 (m, 6H), 6.46 (s, 1H), 4.92 (dd, *J* = 8.9, 3.6 Hz, 2H), 3.38 (d, *J* = 8.1 Hz, 2H), 2.89 (s, 2H), 2.11 (m, 1H), 2.03 – 1.83 (m, 1H), 1.73 (s, 1H), 1.46 (s, 9H), 0.83 (s, 1H).

Synthesis of ***8*** *using derivative* ***L-2*** *as catalyst.*

1. A flame dried schlenk was charged with **L-2**, 145mg (0.5 mmol) and 5 ml dry Et_2O. The solution was cooled down to -78°C and 1.4M solution of sec-BuLi, 0.36 ml, (0.5 mmol) was added. The mixture was stirred 15 min at -78°C when Boc-pyrrolidine 68 mg (0.4 mmol),

dissolved in 2 ml Et_2O, was added. The reaction mixture was stirred at -78°C for 1h and benzophenone, 91 mg (0.5 mmol) solution in 2 ml Et_2O, was added and the reaction was allowed to warm up to r.t. overnight. No product was detected by TLC.

2. A flame dried schlenk was charged with racemic **2**, 145mg (0.5 mmol) and 5 ml dry Et_2O. The solution was cooled down to -78°C and 1.4M solution of sec-BuLi, 0.36 ml, (0.5 mmol) was added. The mixture was stirred 15 min at -78°C when Boc-pyrrolidine 68 mg (0.4 mmol), dissolved in 2 ml Et_2O, was added. The reaction mixture was transferred to ice bath stirred at 0°C for 1h then benzophenone, 91 mg (0.5 mmol) solution in 2 ml Et_2O, was added and the reaction was allowed to warm up to r.t. overnight. No product was detected by TLC.

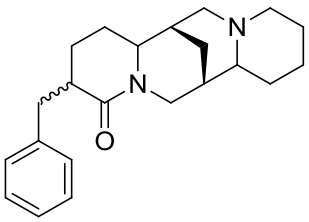
3. A flame dried schlenk was charged with racemic **2**, 145mg (0.5 mmol) and 5 ml dry Et_2O. The solution was cooled down to -78°C and 1.4M solution of sec-BuLi, 0.36 ml, (0.5 mmol) was added. The mixture was stirred 15 min at -78°C when Boc-pyrrolidine 68 mg (0.4 mmol), dissolved in 2 ml Et_2O, was added. The acetone bath was removed and the reaction stirred at RT for 1h, then benzophenone, 91 mg (0.5 mmol) solution in 2 ml Et_2O, was added and the reaction was allowed to warm up to r.t. overnight. No product was detected by TLC.

4. A flame dried schlenk was charged with racemic **2**, 145mg (0.5 mmol) and 5 ml dry THF. The solution was cooled down to -78°C and 1.4M solution of sec-BuLi, 0.36 ml, (0.5 mmol) was added. The mixture was stirred 15 min at -78°C when Boc-pyrrolidine 68 mg (0.4 mmol), dissolved in 2 ml THF, was added. The acetone bath was removed and the reaction stirred at RT for 1h, then benzophenone, 91 mg (0.5 mmol) solution in 2 ml THF, was added and the reaction was allowed to warm up to r.t. overnight. No product was detected by TLC.

*Complex of **2** with $PdCl_2$.*

$PdCl_2$ (35 mg, 0.2 mmol, 1.0 equiv) was suspended in CH_3CN (5 mL) and refluxed under Ar until formation of $(CH_3CN)_2PdCl_2$ was complete, as indicated by the switch in the color to yellow-orange. The mixture was allowed to cool to RT at which time **L-1** (1) (58 mg, 0.2 mmol, 1.0 equiv) was added immediate change in the color to deep red-brown was observed. The reaction was stirred at RT for 1 h under Ar.

*Synthesis of derivative **L-11A,B** under optimized conditions.*



A flame dried schlenk was charged with diisopropylamine, 2.8 ml (20 mmol) and 30 ml dry THF. The mixture was cooled down to -78ºC and n-BuLi (1.6M), 12.5 ml (20 mmol) was added drop wise. The mixture was stirred at -78ºC for 15 min and then at RT for 30 min. After cooling down again to -78ºC L or L-lupanine 2 g (8 mmol) was added as a solution in 10 ml dry THF. The reaction mixture was stirred for 4h at -78ºC when benzyl bromide 3 ml (25 mmol) was added dropwise, the reaction was stirred for additional 2h and quenched with 5 ml 1M aq.HCl. After warming up the reaction mixture was poured in 100 ml 1M aq. HCl and washed with MTBE 2x100ml. The aqueous phase was basified with Na_2CO_3 and extracted with EtOAc 3x100 ml. The organic phase was dried over Na_2SO_4 and evaporated. The crude product was purified with silica flash chromatography using gradient mixing of hexane and EtOAc containing 5% Et_3N. The final product was obtained as two diastereomers in 59% yield (1.6 g). The less polar diastereoisomers **L-11A** was isolated as colorless oil, 900 mg. The more polar isomer **L-11B** was isolated as colorless oil, 700 mg.

Less polar diastereoisomer L-11A- ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.22 – 7.13 (m, 2H), 7.08 (m, 3H), 4.47 (dt, *J* = 13.1, 2.3 Hz, 1H), 3.58 – 3.41 (m, 1H), 3.19 (ddd, *J* = 19.1, 15.2, 6.4 Hz, 1H), 2.77 – 2.57 (m, 2H), 2.49 – 2.32 (m, 4H), 2.17 – 1.98 (m, 1H), 1.99 – 1.72 (m, 3H), 1.66 – 1.06 (m, 14H), 0.94 (t, *J* = 7.2 Hz, 1H).

^{13}C NMR (101 MHz, $CDCl_3$) δ 172.82, 140.34, 129.05, 128.05, 125.74, 63.73, 60.93, 55.19, 52.78, 46.93, 43.79, 37.37, 34.81, 33.47, 32.29, 26.69, 26.46, 25.20, 24.58, 24.36.

HRMS Calculated for $[M+H]^+$ $C_{33}H_{43}N_2$, 339.2436. Found 339.2423.

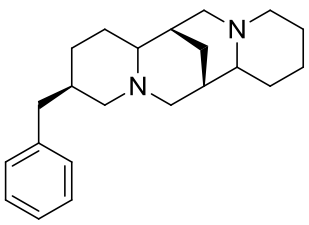
More polar diastereoisomer L-11B- ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.25 – 7.17 (m, 2H), 7.17 – 7.08 (m, 3H), 4.44 (dt, *J* = 13.2, 2.3 Hz, 1H), 3.25 – 3.11 (m, 2H), 2.81 (dd, *J* = 12.6, 11.0 Hz, 1H), 2.75 – 2.55 (m, 3H), 2.51 – 2.39 (m, 2H), 2.08 (m, 1H), 2.00 – 1.80 (m, 3H), 1.75 – 1.57 (m, 3H), 1.57 – 1.10 (m, 10H), 0.94 (t, *J* = 7.2 Hz, 1H).

^{13}C NMR (101 MHz, $CDCl_3$) δ 173.39, 139.56, 129.06, 128.35, 126.15, 63.79, 60.68, 55.35, 52.68, 47.00, 43.15, 37.65, 34.72, 33.03, 32.53, 26.72, 24.92, 24.48, 22.28, 21.35.

HRMS Calculated for $[M+H]^+$ $C_{33}H_{43}N_2$, 339.2436. Found 339.2433.

IR (neat) 2933, 1738, 1633, 1440, 1364, 1229 cm^{-1}

*Synthesis of derivative **L-12A**.*



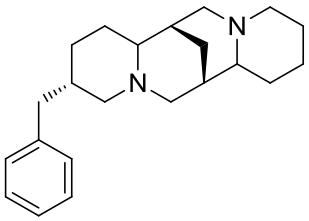
A flame dried schlenk was charged with **L-11A,** 340 mg (1 mmol) and 5 ml 1M $LiAlH_4$ in THF was added and the mixture was stirred at room temperature overnight. The reaction was quenched with 5 ml MeOH poured into water and extracted with EtOAc 3x25 ml. The organic phase was dried over Na_2SO_4 and evaporated. The crude product was purified with silica flash chromatography using gradient mixing of Hexane and EtOAc containing 5% Et_3N to give 70 mg, 22% yield of **L-12A.**

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.33 – 7.25 (m, 2H), 7.24 – 7.12 (m, 3H), 2.80 (d, *J* = 11.5 Hz, 1H), 2.70 (m, 2H), 2.52 (m, 2H), 2.43 (m, 1H), 2.31 (dd, *J* = 11.1, 3.1 Hz, 1H), 2.14 – 2.05 (m, 1H), 2.05 – 1.79 (m, 5H), 1.78 – 1.66 (m, 4H), 1.59 (m, 2H), 1.54 – 1.23 (m, 6H), 1.08 (d, *J* = 11.9 Hz, 1H), 0.96 (m, 1H).

^{13}C NMR (101 MHz, $CDCl_3$) δ 140.69, 129.26, 128.25, 125.88, 66.39, 64.53, 62.31, 61.96, 55.53, 53.66, 41.52, 38.20, 36.14, 34.75, 32.87, 31.24, 29.23, 27.76, 26.02, 24.93.

HRMS Calculated for $[M+H]^+$ $C_{33}H_{43}N_2$, 325.2644. Found 325.2632.

*Synthesis of derivative **L-12B.***



A flame dried schlenk was charged with **L-11B,** 340 mg (1 mmol) and 5 ml 1M $LiAlH_4$ in THF was added and the mixture was stirred at room temperature overnight. The reaction was quenched with 5ml MeOH poured into water and extracted with EtOAc 3x25 ml. The organic phase was dried over Na_2SO_4 and evaporated. The crude product was purified with silica flash chromatography using gradient mixing of Hexane and EtOAc containing 5% Et_3N to give 70 mg, 22% yield of **L-12B.**

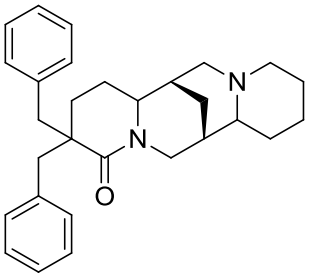
^{1}H NMR (400 MHz, $CDCl_3$) δ 7.19 (m, 2H), 7.15 – 7.06 (m, 3H), 2.95 (dd, *J* = 13.2, 9.1 Hz, 1H), 2.83 – 2.58 (m, 3H), 2.43 – 2.24 (m, 3H), 2.04 – 1.74 (m, 7H), 1.74 – 1.36 (m, 9H), 1.32 – 1.16 (m, 2H), 1.08 – 0.93 (m, 2H).

^{13}C NMR (101 MHz, $CDCl_3$) δ 142.31, 129.33, 128.23, 125.65, 77.48, 77.16, 76.84, 66.57, 65.21, 62.03, 58.21, 56.08, 54.29, 37.59, 36.34, 36.11, 34.86, 33.19, 28.27, 27.74, 26.11, 25.16, 24.14.

HRMS Calculated for $[M+H]^+$ $C_{33}H_{43}N_2$, 325.2644. Found 325.2629.

IR (neat) 2925, 2758, 1738, 1441, 1366, 1228 cm^{-1}

*Synthesis of derivative **13.***



A flame dried schlenk was charged with diisopropylamine, 0.42 ml (3 mmol) and 10 ml dry THF. The mixture was cooled down to -78ºC and n-BuLi 1.6M, 1.9 ml (3 mmol) was added drop wise. The mixture was stirred at -78ºC for 15 min and then at RT for 30 min. After cooling down again to -78ºC diastereomeric mixture of **11A,B** 260 mg (0.8 mmol) was added as a solution in 1 ml dry THF. The reaction mixture was stirred for 4h at -78ºC when benzyl bromide 0.47 ml (4 mmol) was added dropwise, the reaction was stirred for additional 2h and quenched with 1ml 1M aq.HCl. After warming up the reaction mixture was poured in 50 ml 1M aq. HCl and washed with MTBE 2x25 ml. The aqueous phase was basified with Na_2CO_3 and extracted with EtOAc 3x25 ml. The organic phase was dried over Na_2SO_4 and evaporated. The crude product was purified with silica flash chromatography using gradient mixing of Hexane and EtOAc containing 5% Et_3N to give **13** in 40% yield, 171 mg.

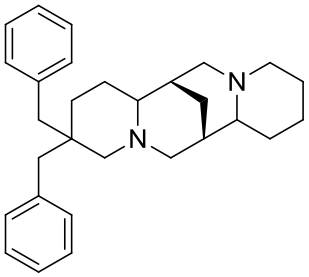
^{1}H NMR (400 MHz, $CDCl_3$) δ 7.26 – 7.00 (m, 10H), 4.44 (dt, *J* = 13.3, 2.1 Hz, 1H), 3.43 (d, *J* = 12.9 Hz, 1H), 3.29 (d, *J* = 13.2 Hz, 1H), 2.76 – 2.43 (m, 4H), 2.34 – 2.20 (m, 2H), 1.93 (m, 1H), 1.89 – 1.75 (m, 1H), 1.75 – 1.34 (m, 10H), 1.33 – 1.06 (m, 5H), 0.99 – 0.89 (m, 2H).

^{13}C NMR (101 MHz, $CDCl_3$) δ 175.30, 138.38, 138.11, 130.95, 130.84, 128.43, 127.86, 126.57, 126.31, 64.36, 60.40, 55.38, 52.09, 48.43, 47.49, 45.69, 45.53, 34.75, 33.37, 32.45, 26.14, 26.09, 25.22, 24.74, 23.53.

HRMS Calculated for $[M+H]^+$ $C_{33}H_{43}N_2$, 429.2906. Found 429.2911.

IR (neat) 2924, 2852, 1629, 1453 cm^{-1}

*Synthesis of derivative **14**.*



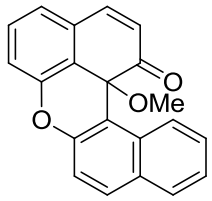
A flame dried schlenk was charged with **13**, 60 mg (0.14 mmol), 3ml 1M solution of $LiAlH_4$ in THF was added and the mixture was refluxed overnight. Then 10 ml MTBE were added and the reaction was quenched with 0.2 ml H_2O. The formed white solid was filtered out and the cake washed with 20 ml MTBE. The filtrate was dried over Na_2SO_4 and evaporated to give 80% yield (46 mg) of **14**.

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.31 (d, *J* = 7.3 Hz, 2H), 7.24 (t, *J* = 7.4 Hz, 2H), 7.14 (ddd, *J* = 13.3, 6.9, 4.1 Hz, 4H), 7.00 – 6.93 (m, 2H), 3.21 (d, *J* = 13.1 Hz, 1H), 2.73 (dd, *J* = 23.0, 11.8 Hz, 2H), 2.52 (d, *J* = 13.1 Hz, 1H), 2.47 – 2.32 (m, 4H), 2.20 (dd, *J* = 11.4, 2.0 Hz, 1H), 1.99 (d, *J* = 10.9 Hz, 2H), 1.94 – 1.86 (m, 1H), 1.85 – 1.64 (m, 4H), 1.60 (d, *J* = 11.4 Hz, 1H), 1.51 (m, 4H), 1.40 (m, 1H), 1.37 – 1.22 (m, 4H), 1.07 (m, 1H), 0.93 (d, *J* = 11.9 Hz, 1H).

^{13}C NMR (101 MHz, $CDCl_3$) δ 139.64, 138.26, 131.18, 131.15, 127.90, 127.69, 125.98, 125.95, 65.90, 65.32, 61.75, 61.51, 56.06, 54.26, 44.97, 41.68, 38.09, 36.20, 34.87, 32.77, 32.46, 27.70, 26.06, 25.25, 25.07.

HRMS Calculated for $[M+H]^+$ $C_{33}H_{43}N_2$, 415.3131. Found 415.3105.

*Synthesis of **15**.*[47]



To a solution of $CuCl_2.2H_2O$, 140 mg (1 mmol) in 10 ml MeOH (HPLC grade) was added rac. Lupanine, 240 mg (1 mmol). Imidate formation of green precipitate was observed, then a solution on of 2-naphthol, 144 mg (1 mmol) in 5 ml MeOH was added and the reaction was stirred under overnight. The reaction mixture was poured in to water and extracted with EtOAc (2x50 ml). The organic phase was dried over $MgSO_4$ and evaporated. The crude product was purified with Combiflash flash purification system using gradient mixing from 100% hexane to 50% EtOAc. The final product was obtained in 40% yield (63 mg).

^{1}H NMR (300 MHz, $CDCl_3$) δ 8.08 – 7.99 (m, 1H), 7.80 (d, *J* = 8.9 Hz, 1H), 7.76 – 7.68 (m, 1H), 7.37 – 7.19 (m, 4H), 7.10 (d, *J* = 10.0 Hz, 1H), 7.03 – 6.94 (m, 2H), 6.18 (d, *J* = 10.0 Hz, 1H), 2.75 (s, 3H).

^{13}C NMR (75 MHz, $CDCl_3$) δ 197.89, 152.41, 151.78, 139.45, 133.49, 132.95, 132.12, 131.15, 130.87, 128.35, 127.87, 126.14, 126.01, 124.84, 124.21, 117.37, 116.63, 116.18, 107.53, 75.93, 51.77.

5. References.

1. Boschin, G.; Resta, D. In *Natural Products*; Springer Berlin Heidelberg: 2013, p 381.
2. Veen, G.; Schmidt, C.; Witte, L.; Wray, V.; Czygan, F.-C. *Phytochemistry*, **1992**, *31*, 4343.
3. Tyski, S.; Markiewicz, M.; Gulewicz, K.; Twardowski, T. *J. Plant Physiol.*, **1988**, *133*, 240.
4. Zamora-Natera, F.; Garcia-Lopez, P.; Ruiz-Lopez, M.; Salcedo-Perez, E. *Agrociencia*, **2008**, *42*, 185.
5. Senges, J.; Ehe, L. *Naunyn-Schmiedeberg's Arch. Pharmacol.*, **1973**, *280*, 265.
6. Chuzel, O.; Riant, O. In *Chiral Diazaligands for Asymmetric Synthesis*; Lemaire, M., Mangeney, P., Eds.; Springer Berlin Heidelberg: 2005; Vol. 15, p 59.
7. Hoppe, D.; Hense, T. *Angew. Chem., Int. Ed.*, **1997**, *36*, 2282.
8. Caddick, S.; Jenkins, K. *Chem. Soc. Rev.*, **1996**, *25*, 447.
9. Zschage, O.; Hoppe, D. *Tetrahedron*, **1992**, *48*, 5657.
10. Kerrick, S. T.; Beak, P. *J. Am. Chem. Soc.*, **1991**, *113*, 9708.
11. Klapars, A.; Campos, K. R.; Waldman, J. H.; Zewge, D.; Dormer, P. G.; Chen, C.-y. *J. Org. Chem.*, **2008**, *73*, 4986.
12. Gelardi, G.; Barker, G.; O'Brien, P.; Blakemore, D. C. *Org. Lett.*, **2013**, *15*, 5424.
13. Park, Y. S.; Boys, M. L.; Beak, P. *J. Am. Chem. Soc.*, **1996**, *118*, 3757.
14. Schlosser, M.; Limat, D. *J. Am. Chem. Soc.*, **1995**, *117*, 12342.
15. Beak, P.; Du, H. *J. Am. Chem. Soc.*, **1993**, *115*, 2516.
16. Gallagher, D. J.; Du, H.; Long, S. A.; Beak, P. *J. Am. Chem. Soc.*, **1996**, *118*, 11391.
17. Thayumanavan, S.; Lee, S.; Liu, C.; Beak, P. *J. Am. Chem. Soc.*, **1994**, *116*, 9755.
18. Ko, Y. K.; Im, C.; Do, J.; Park, Y. S. *Eur. J. Org. Chem.*, **2014**, *2014*, 3460.
19. Page, A.; Clayden, J. *Beilstein J. Org. Chem.*, **2011**, *7*, 1327.
20. Klein, S.; Marek, I.; Poisson, J.-F.; Normant, J.-F. *J. Am. Chem. Soc.*, **1995**, *117*, 8853.
21. Norsikian, S.; Marek, I.; Poisson, J.-F.; Normant, J. F. *J. Org. Chem.*, **1997**, *62*, 4898.
22. McGrath, M. J.; O'Brien, P. *J. Am. Chem. Soc.*, **2005**, *127*, 16378.
23. Dearden, M. J.; Firkin, C. R.; Hermet, J.-P. R.; O'Brien, P. *J. Am. Chem. Soc.*, **2002**, *124*, 11870.
24. Bilke, J. L.; O'Brien, P. *J. Org. Chem.*, **2008**, *73*, 6452.
25. Barker, G.; O'Brien, P.; Campos, K. R. *Arkivoc*, **2011**, 217.
26. Rajca, A.; Wang, H.; Bolshov, P.; Rajca, S. *Tetrahedron*, **2001**, *57*, 3725.
27. Zhang, Y.; Yeung, S.-M.; Wu, H.; Heller, D. P.; Wu, C.; Wulff, W. D. *Org. Lett.*, **2003**, *5*, 1813.
28. Smrcina, M.; Lorenc, M.; Hanus, V.; Sedmera, P.; Kocovsky, P. *J. Org. Chem.*, **1992**, *57*, 1917.
29. Noji, M.; Nakajima, M.; Koga, K. *Tetrahedron Lett.*, **1994**, *35*, 7983.
30. Maheswaran, H.; Prasanth, K. L.; Krishna, G. G.; Ravikumar, K.; Sridhar, B.; Kantam, M. L. *Chem. Commun.*, **2006**, 4066.
31. Zhu, J.; Grigoriadis, N. P.; Lee, J. P.; Porco, J. A. *J. Am. Chem. Soc.*, **2005**, *127*, 9342.
32. Maheswaran, H.; Joseph, P. J. A.; Prasanth, K. L.; Priyadarshini, S.; Satyanarayana, P.; Likhar, P. R.; Kantam, M. L. *Tetrahedron: Asymmetry*, **2010**, *21*, 2158.
33. Trost, B. M.; Dietsch, T. J. *J. Am. Chem. Soc.*, **1973**, *95*, 8200.
34. Togni, A. *Tetrahedron Asymmetry*, **1991**, *2*, 683.
35. Kang, J.; Oh Cho, W.; Cho, H.; Geun *Tetrahedron: Asymmetry*, **1994**, *5*, 1347.
36. Ferreira, E. M.; Stoltz, B. M. *J. Am. Chem. Soc.*, **2001**, *123*, 7725.
37. Jensen, D. R.; Pugsley, J. S.; Sigman, M. S. *J. Am. Chem. Soc.*, **2001**, *123*, 7475.
38. Mueller, J. A.; Jensen, D. R.; Sigman, M. S. *J. Am. Chem. Soc.*, **2002**, *124*, 8202.
39. Mandal, S. K.; Sigman, M. S. *J. Org. Chem.*, **2003**, *68*, 7535.
40. Bagdanoff, J. T.; Ferreira, E. M.; Stoltz, B. M. *Org. Lett.*, **2003**, *5*, 835.
41. Bagdanoff, J. T.; Stoltz, B. M. *Angew. Chem., Int. Ed.*, **2004**, *43*, 353.

42. Caspi, D. D.; Ebner, D. C.; Bagdanoff, J. T.; Stoltz, B. M. *Adv. Synth. Catal.*, **2004**, *346*, 185.
43. Ebner, D. C.; Bagdanoff, J. T.; Ferreira, E. M.; McFadden, R. M.; Caspi, D. D.; Trend, R. M.; Stoltz, B. M. *Chem. Eur. J.*, **2009**, *15*, 12978.
44. Carmali, S.; Alves, V. D.; Coelhoso, I. M.; Ferreira, L. M.; Lourenço, A. M. *Sep. Purif. Technol.*, **2010**, *74*, 38.
45. Ebner, T.; Eichelbaum, M.; Fischer, P.; Meese, C. O. *Arch. Pharm. (Weinheim, Ger.)*, **1989**, *322*, 399.
46. Xiao, K.-J.; Luo, J.-M.; Ye, K.-Y.; Wang, Y.; Huang, P.-Q. *Angew. Chem., Int. Ed.*, **2010**, *49*, 3037.
47. Tan, D.-M.; Li, H.-H.; Wang, B.; Liu, H.-B.; Xu, Z.-L. *Chin. J. Chem.*, **2001**, *19*, 91.