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Vienna University of Technology

DIPLOMARBEIT

FROM PLANT TO DRUG:

IONIC LIQUIDS FOR THE ISOLATION OF ACTIVE INGREDIENTS

AUSGEFÜHRT AM

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DEDICATION

I want to dedicate my diploma thesis to my grandmother Ilse Hlebowicki who studied chemistry but could not finish her studies because she had to take care of her mother. Unfortunately I never got to know her.

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Liesl, ich möchte mich bei dir und deiner Familie für die schönen Familienfeiern bedanken. Außerdem möchte ich mich bei meinen Taufpaten Evi und Rudi und ihrer Familie bedanken. Opa, Hanni, ihr fehlt mir sehr.

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DEUTSCHE KURZFASSUNG

Im Rahmen dieser Diplomarbeit sollte eine neue Strategie zur Isolation von pharmazeutisch interessanten Wirkstoffen aus Biomasse mittels ionischer Flüssigkeiten entwickelt werden.

Dazu wurden zuerst verschiedene ionische Flüssigkeiten mit einem 1-Alkyl-3-methylimidazol-Ringsystem zum Lösen von Biomasse in quantitativer Ausbeute hergestellt. Zusätzlich wurden funktionalisierte Brønsted-saure ionische Flüssigkeiten in zwei Stufen mit ausgezeichenten Ausbeuten >95% synthetisiert, welche später als Reaktionsmedium und Katalysator angewendet wurden.

Im zweiten Teil der Diplomarbeit wurde Betulin aus Birkenrinde mittels ionischen Flüssigkeiten und konventionellen Lösungsmitteln extrahiert. Es konnte gezeigt werden, dass ionische Flüssigkeiten Betulin mit höherer Ausbeute zugänglich machen. Weiteres wurden die Extraktionsbedingungen optimiert und verschiedenste ionische Flüssigkeiten getestet. Außerdem konnte eine neue Strategie zur Isolation von Betulin aus der Birkenrinde mittels ionischer Flüssigkeiten entwickelt werden, mit der Betulin mit 35 wt% und 87% Reinheit ohne zusätzlichen Reinigungsschritt isoliert werden konnte. Die ionische Flüssigkeit konnte in Ausbeuten bis zu 90% erfolgreich recycliert und zur erneuten Extraktion verwendet werden.

Im letzten Teil der Diplomarbeit wurde der Wirkstoff Shikimisäure, welche der Ausgangsstoff für die Produktion des Grippemittels Tamiflu™ ist, aus der Biomasse Sternanis extrahiert und zugleich zu den Folgeprodukten umgesetzt. Dabei wurde Shikimisäure als auch Sternanis mittels ionischer Flüssigkeiten, die gleichzeitig als Lösungsmittel und Katalysator dienten, *in situ* verestert und ketalisiert. Dadurch konnte die Ausbeute im Vergleich mit herkömmlichen Verfahren deutlich verbessert und die Zahl der Reaktionsschritte reduziert werden. Weiteres konnte der Umgang mit schädlichen, flüchtigen Lösungsmittel bzw. mit dem korrosiven und giftigen Thionylchlorid vermieden werden.

SHORT ABSTRACT

Ionic liquids were successfully synthesized and used for the dissolution of birch bark to obtain the active ingredient Betulin. Furthermore a novel higher yielding and time economic process for the isolation of Betulin from birch bark using ionic liquids was developed. Additionally, an ionic liquid strategy for the reactive dissolution of star anise seeds using different Brønsted acidic ionic liquids as solvent and reaction media towards the isolation of important pharmaceutical intermediates based on Shikimic acid was developed. This procedure provides a single-step, higher yielding and environmentally benign strategy towards the synthesis of the anti-influenza drug Tamiflu™.

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1 Introduction

1.1 Ionic liquids - Definition

Ionic liquids (ILs) are defined as liquids that consist almost exclusively of ions and therefore exhibit ionic conductivity. According to the current definition, ionic liquids have melting points or glass-transition temperatures below 100 °C. In particular, ionic liquids with a melting point around or below room temperature are classified as “room-temperature ionic liquids”. The first ionic liquid was probably discovered in 1888 by Gabriel, who reported the protic ionic liquid ethanolammonium nitrate with a melting point of 52-55 °C.[1] With Walden’s paper about ethylammonium nitrate in 1914 the first room temperature ionic liquid was described.[2] The next significant development was the patent of Hurley and Wier in 1951 who discovered that mixtures of aluminium chloride and ethylpyridinium halides can be used as electrolytes for the electrodeposition of aluminium.[3] However, it was only in the past years that academic and industrial research focused on ionic liquids, which resulted in an almost exponential increase of publications as shown in Figure 1.

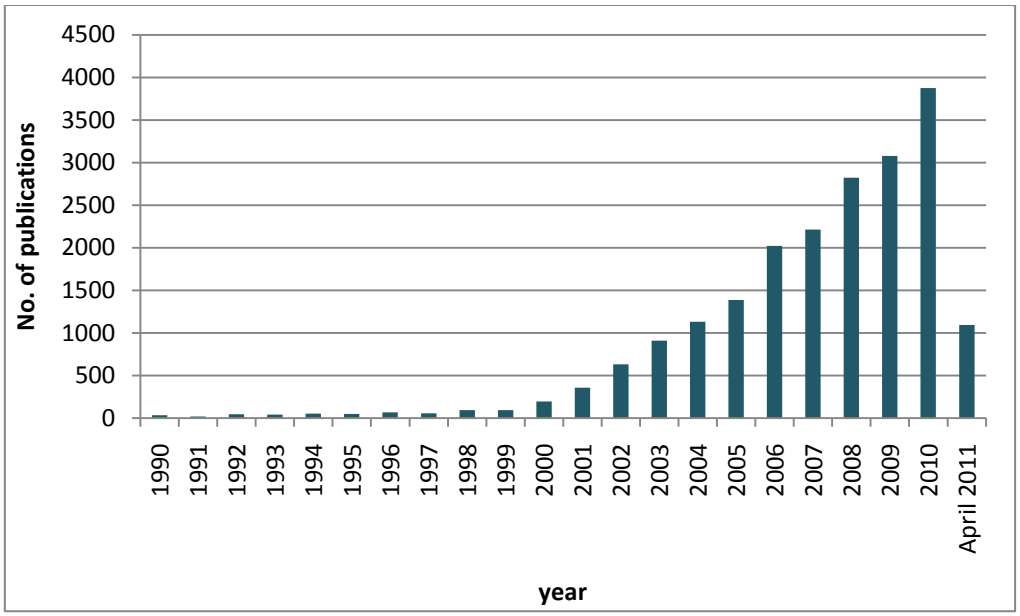

Figure 1: Publications per year dealing with ionic liquids[4]

So far more than 1500 different ionic liquids have been reported, and an almost unlimited number of ionic liquids is theoretically possible by the choice of different ions. Some commonly used cations and anions are shown in Figure 2.

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[1] Gabriel, S. *Ber. Dtsch. Chem. Ges.* **1888**, *21*, 566.
[2] Walden, P. *Bull.Acad. Imper. Sci. (St Petersburg)*, **1914**, 1800.
[3] Hurley, F.H.; Wier, T.P. *J. Electrochem. Soc.* **1951**, *98*, 203.
[4] Sci-Finder™ search performed on 20-04-2011, research term „ionic liquids“; english publications only

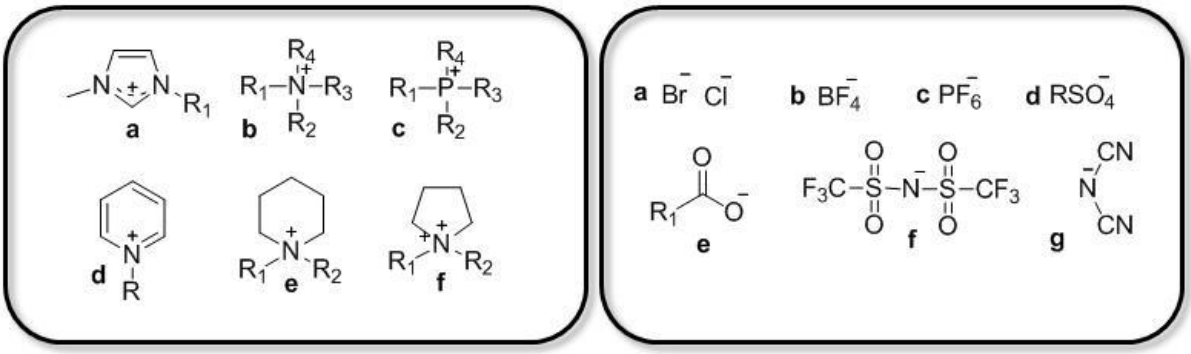

Figure 2: Typical cations and anions of ionic liquids

Typical examples for ionic liquids include 1-alkyl-3-methylimidazolium **a**, tetraalkylammonium **b** and -phosphonium **c**, *N*-alkylpyridinium **d**, *N*,*N*-dialkylpiperidinium **e** and *N*,*N*-dialkylpyrrolidinium cations. In contrast, halides, *e.g.* bromides and chlorides **a**, tetrafluoroborates **b**, hexafluorophosphates **c**, alkyl sulfates **d**, alkylcarboxylates **e**, *e.g.* acetate, bis(trifluoromethylsulfonyl)imides **f** and dicyanamides **g** are the anions most commonly reported in the ionic liquid literature.

With increasing size of anion or cation the melting points of ionic liquids tend to decrease. For instance, if the chloride ion of $[C_2mim]Cl$ is replaced by a larger $AlCl_4^-$ ion, the melting point decreases from 87 °C to 7 °C. The variation of the alkyl chain length also influences the melting point: $[C_2mim]Cl$ (mp 87 °C) has a smaller cation and a higher melting point compared to $[C_4mim]Cl$ (mp 65 °C), since symmetric substituents pack more efficiently. As a consequence, an unsymmetrical design and geometry of the cations results in a lower melting point.

The combination of specific ions as shown in Figure 2 results in some properties that are typical for low-melting salts. In general, ionic liquids have an insignificant vapour pressure and are usually non-flammable. They are also thermally stable at high temperatures up to 400 °C and have a wider liquid range than classical organic solvents. Due to their dual functionality, the variation and functionalization of cations and anions allows the creation of hydrophilic and hydrophobic ionic liquids, which offers a wide range of different applications. Furthermore, ionic liquids possess an unusually wide electrochemical window of up to 6.0 V. Their physical, chemical and biological properties can be fine-tuned by switching anions or cations or designing specific functionalities into the cations and/or anions or by mixing two or more simple ionic liquids.[5]

Because of their tremendous variety of properties ionic liquids can be found in different areas of technology. The potential of ionic liquids in organic synthesis as solvent, reaction media

[5] Freemantle, M. *An Introduction to Ionic Liquids*; The Royal Society of Chemistry:Cambridge, 2010; pp 1-99.

and/or catalyst was early recognized: Biphasic catalysis in or with ionic liquids cannot only improve reactivity, but allows immobilizing, activating and recycling catalysts. Furthermore, ionic liquids can be used for separation and extraction of chemicals from aqueous and molecular organic solvents.[5]

Since 1996 Eastman Chemical Company had been running a process for the isomerisation of 3,4-epoxybut-1-ene to 2,5-dihydrofuran using an ionic liquid. This reaction required a Lewis acid catalyst, and a Lewis basic ionic liquid, $[P_{88818}]I$ that was recovered by liquid-liquid extraction. Unfortunately the market for the product has declined, and thus this process is not running on industrial stage anymore (Figure 3).

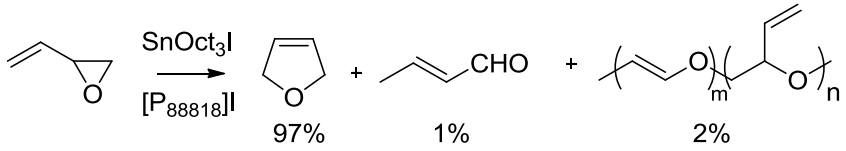

Figure 3: Industrial process for the synthesis of 2,5-dihydrofuran[6]

The BASIL™ (Biphasic Acid Scavenging utilizing Ionic Liquids) process was the first publicly announced room-temperature ionic liquid process which was introduced in the year 2002 in Ludwigshafen, Germany (Figure 4).[6]

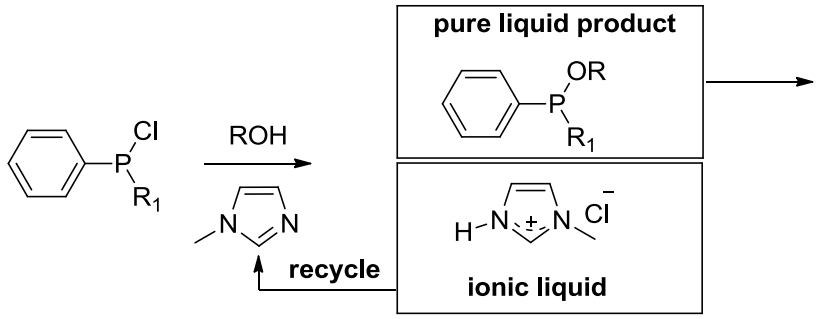

Figure 4: BASIL™ process[6]

In this process alkoxyphenylphosphines are produced, that were originally synthesized using triethylamine as an acid scavenger. The disadvantage of the conventional process was that the by-product triethylammonium chloride formed a dense insoluble paste and made the reaction mixture difficult to handle. Triethylamine was replaced by 1-methylimidazole resulting in the formation of the ionic liquid 1-methylimidazolium chloride. As a consequence, a smaller reactor was used and the space-time yield was tremendously increased, and 1-methylimidazole was recycled.

Central Glass Company investigated ionic liquid technologies for the palladium-catalyzed Sonogashira coupling in the synthesis of alkyl-, aryl-, and diaryl-substituted alkynes that can

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[6] Plechkova, N.V.; Seddon, K.R. *Chem. Soc. Rev*. **2008**, *37*, 123.

be used in optical, electronic and pharmaceutical applications. Typical conventional solvents for this reaction include toluene or hazardous tetrahydrofuran, and a stoichiometric amount of base is required. Central Glass Company introduced tetraalkylphosphonium ionic liquids resulting in a higher efficiency, and thus the reaction has been commercialized in 2007 (Figure 5). Furthermore the remaining ionic liquid-catalyst solution can be recycled several times with little loss of catalytic activity.[6,7]

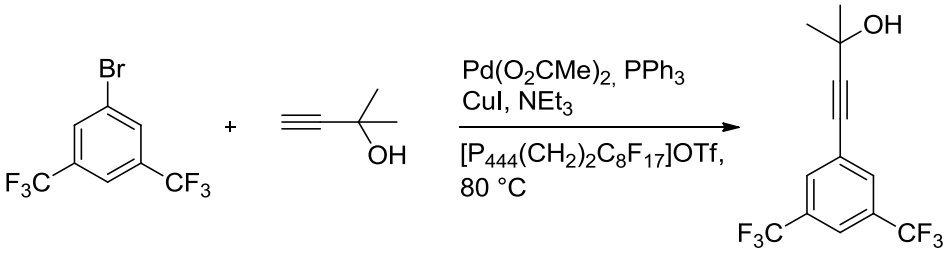

Figure 5: Sonogashira coupling using ionic liquids[6]

Apart from their applications in synthesis and catalysis that have already entered industrial stage, interesting applications of ionic liquids can be found in different areas. To name but a few, ionic liquids can be found in the storage of hazardous gases, as electrolytes, as stationary phases in chromatography, but also in materials applications such as lubricants, thermometers or even active pharmaceutical ingredients and antimicrobial agents.[5]

1.2 Ionic liquids as green solvents

The development of chemical products and processes that reduce or eliminate the use and generation of hazardous substances is the main goal of green chemistry.[8]

As organic solvents are usually volatile and can be flammable, toxic or hazardous, they should be replaced by alternative solvents. Ionic liquids as well as supercritical fluids and perfluorinated fluids can represent an alternative reaction media to avoid hazardous organic solvents.[8] Thus, when chosen carefully, ionic liquids can reduce or prevent chemical wastage and pollution and improve the safety of chemical wastage processes and products.[5] In contrast to conventional organic solvents ionic liquids are generally non-volatile and have a negligible vapour pressure. Additionally, ionic liquids can dissolve a very wide range of organic and inorganic compounds as well as polymeric materials, and thus they can be an attractive alternative as environmentally benign solvents in organic synthesis.[8]

Table 1 gives a brief visual comparison of typical values of organic solvents and ionic liquids.

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[7] http://www.cgc-jp.com/, last accessed 30/4/2011.
[8] Afonso, C.A.M.; Grespo, J.G. *Green Seperation Processes;* Wiley-VCH Verlag GmbH &Co. KGaA: Weinheim, 2005; pp 1-17, 229-230.

Table 1: Comparison of organic solvents with ionic liquids[6]

Property	Organic solvents	Ionic liquids
Number of solvents	>1000	>1 000 000
Applicabiltiy	Single function	Multifunction
Catalytic ability	Rare	Common and tuneable
Chirality	Rare	Common and tuneable
Vapour pressure	Obeys the Clausius-Clapeyron equation	Negligible vapour pressure under normal conditions
Flammability	Usually flammable	Usually nonflammable
Solvation	Weakly solvating	Strongly solvating
Polarity	Conventional polarity concepts apply	Polarity concept questionable
Tuneability	Limited range of solvents available	Virtually unlimited range means "designer solvents"
Cost	Normally cheap	Typically between 2 and 100 times the cost of organic solvents
Recyclability	Green imperative	Economic imperative
Viscosity/cP	0.2-100	22-40 000
Density/g cm^{-3}	0.6-1.7	0.8-3.3
Refractive index	1.3-1.6	1.5-2.2

However, it should be noted that the use of the term “green solvent” has been questioned due to recent toxicity studies about ionic liquids.[9] In addition, imidazolium based cations are synthesized from imidazoles and alkyl halides which are obtained from non-renewable petroleum feedstock, and organic solvents are often used for the synthesis of ILs. However, renewable resources for ionic liquids, *e.g.* nicotinic acid based anions are available to improve this issue. Furthermore, some typically anions such as PF_6^- salts can be quite toxic when in contact with water, and thus a responsible handling of the term “green solvents” is necessary when dealing with ionic liquids.[5]

[9] Pinkert , A.; Marsh, K.N.; Pang, S.; Staiger, M.P. *Chem. Rev.* **2009**, *109, 6715.*

2 State of Art

2.1 Biomass dissolution

2.1.1 Dissolution of biomass with ionic liquids

The term “sustainable development” is based on three interconnected pillars of economic development, social responsibility, and environmental protection. Sustainability means using renewable resources, reducing harm, preventing climate change, the protection of employees, capital growth, *etc*. The feedstock should be used efficiently and its amount should be reduced and recycled as much as possible for a sustainable technological process.

The separation of the major components of lignocellulosic biomass – cellulose, lignin, hemicelluloses – is also known as chemical pulping, which is always connected with high operation costs and the requirement of the recovery of expensive solvents at high temperatures and pressures.[10]

In the early 1980s, Seddon discovered that chloroaluminate ionic liquids could dissolve kerogen under microwave irradiation. Kerogen is a fossilized organic material present in sedimentary rocks and was insoluble in all known solvents except in hydrofluoric acid.[11] The use of ionic liquids can therefore be associated with sustainability due to their potential of directly dissolving and fractionating biomass.

Several years later, Rogers *et al.* found that ionic liquids are capable to dissolve biopolymers due to their polar, ionic character and their hydrogen bonding capacity.[12] Apart from chemical pulping, the dissolution and processing of cellulosic biomass is environmentally problematic and requires the use of a large excess of chemical reagents, *e.g.* NaOH or dimethylsulfoxide/tetrabutylammonium fluoride trihydrate. Cellulose is considered an almost inexhaustible source of raw material for the increasing demand for environmentally friendly and biocompatible products. Novel ionic liquid-based technologies for improved processing of cellulose that take advantage of the direct dissolution of biomass are therefore of considerable interest. Figure 6 shows a tentative mechanism involving both anion and cation of the ionic liquid for the dissolution process of cellulose. Electron donor - electron acceptor complexes are formed from the oxygen and hydrogen atoms of the cellulose with the charged species of the ionic liquid.[9]

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[10] Sun, N.; Rodrıguez, H.; Rahman, M.; Rogers, R.D. *Chem. Commun.* **2011**, *47*, 1405.
[11] Patell, Y.; Seddon, K.R.; Dutta, L. Fleet, A. *Green Industrial Applications of Ionic liquids*, ed. Rogers, R.D.; Seddon, K.R.; Vokov, S. NATO Science Series, Kluwer Academic Publishers, Dordrecht, **2002**, 499.
[12] Swatloski, R.P.; Spear, S.K.; Holbrey, J.D.; Robin D. Rogers, R.D. *J. Am. Chem. Soc.* **2002**, *124*, 4974.

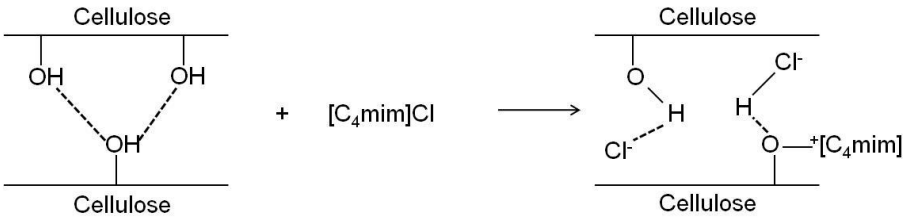


Figure 6: Proposed dissolution mechanism of cellulose in $[C_4mim]Cl$[9]

Another mechanism based on NMR studies suggests that $[C_2mim]OAc$ **27** forms a covalent bond between the C-1 carbon of the glucose unit and the C-2 of the imidazolium core (Figure 7), since the C-1 carbon signal of the glucose unit disappeared after dissolution in the ionic liquid 1-ethyl-3-methylimidazolium acetate.[9]

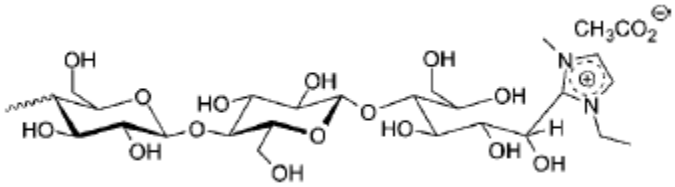


Figure 7: Proposed dissolution mechanism of cellulose in $[C_2mim]OAc$[9]

A number of factors influence the cellulose dissolution, such as viscosity of the ionic liquid, anion and cation of the ionic liquid, dissolution times, *etc*. In general, ionic liquids with low viscosity dissolute biomass more effectively than ionic liquids with high viscosity. Long dissolution times over 12 h at elevated temperatures do not always lead to better results. On the other hand, microwave irradiation instead of conventional heating could improve the solubility of cellulose, meaning that the solubility of cellulose with a degree of polymerization of 1000 could be increased by 150%. Cations based on methylimidazolium and methylpyridinium including allyl-, ethyl-, or butyl-side chains are all suitable for the dissolution of biomass, and the best dissolution results were obtained with the C_4 side chain. Considering the anions, chloride, acetate and formate are all anions with good prospects. The solubility of cellulose was found to increase in following order: $OAc^- < Cl^- < Br^-$.[9]

BASF used $[C_2mim]OAc$ for the dissolution of cellulose, and 5 wt% solutions of cellulose in $[C_2mim]OAc$ are commercially available through Sigma-Aldrich under the trade name CELLIONIC™. Their choice of the ionic liquid $[C_2mim]OAc$ instead of $[C_4mim]Cl$ was based on its lower melting point (-45 °C *vs.* 69 °C), its lower viscosity (10 mPa s *vs.* 147 mPa s, at 80°C) and also its lower corrosive character. Additionally, $[C_2mim]OAc$ was found to be non-toxic (LD_{50} > 2000 mg kg^{-1}) whereas $[C_4mim]Cl$ is moderately toxic (50 mg kg^{-1} < LD_{50} < 300 mg kg^{-1}).[10]

After the initial dissolution process, recovery of the biomass can be easily achieved by precipitating the biopolymers *via* the addition of water, acetone, dichlormethane, acetonitrile

or mixtures of them. Regeneration of cellulose from ionic liquids results in a lower degree of polymerization (50% to 75%) than the initial biopolymer. Furthermore, several cellulose modifications such as acylate, benzoate, and carbamate have been successfully performed using ionic liquids, *e.g.* [C_4mim]Cl, [C_2mim]Cl and [C_2mim]OAc as solvent. An imidazolium ionic liquid consisting of two side chains – a vinyl and a styrene group – can act as both the cellulose solvent and the monomer for the polymerization process for the formation of interwoven networks between the cellulose chains and synthetic polymer strands during the regeneration process.[9] In general, the ionic liquids can be easily recovered after precipitation of the biopolymers, although the evaporation of water is quite energy-consuming and an economical issue that is not yet completely solved.

The processing of biomass is however not limited to the dissolution or functionalization of cellulose: Sun *et al.* investigated the dissolution of shrimp shell with ionic liquids to remove all traces of remaining color from pre-processed langostino shells and to prepare food-grade chitin. Chitin, a linear amino polysaccharide found in the outer skeleton of arthropods was dissolved in chloride- and acetate-based ionic liquids in up to 10% at 110 °C. After dissolution of shrimp shells in [C_2mim]OAc, chitin with high purity and high molecular weight was reconstituted in one step only, whereas a multi-step procedure is currently done in industry.[10]

Another successful example of biomass dissolution in ionic liquids is wood, which usually consists of 40-50% cellulose, 20-40% hemicellulose - a heteropolymer containing of different sugar monomers including glucose, xylose, mannose, *etc.* - and 18-25% (hardwood) or 25-35% (softwood) lignin depending on the wood source. Wood was partially dissolved with a mixture of [C_4mim]Cl and DMSO in a ratio of 84:16 wt%[10], but recent studies showed that [C_2mim]OAc might be more efficient.[13] Softwood (southern yellow pine) as well as hardwood (red oak) were completely dissolved in [C_2mim]OAc up to 5 wt% (0.5 g wood in 10 g ionic liquid) due to the higher basicity of the acetate anion disrupting the inter- and intramolecular hydrogen bonding in biopolymers. It was observed that hardwood is better soluble in ionic liquids than softwood, and this might be explained by the higher lignin-content. In the process presented by Sun *et al.*, cellulose-rich material and lignin were separated and isolated using aqueous acetone solutions, and ionic liquid was recovered after evaporation of the solvents. Figure 8 shows the flowchart for the treatment and processing of wood with ionic liquids.[10]

[13] Sun, N.; Rahman, M.; Qin, Y.; Maxim, M.L.; Rodriguez H.; Rogers, R.D. *Green Chem.* **2009**, *11*, 646–655.

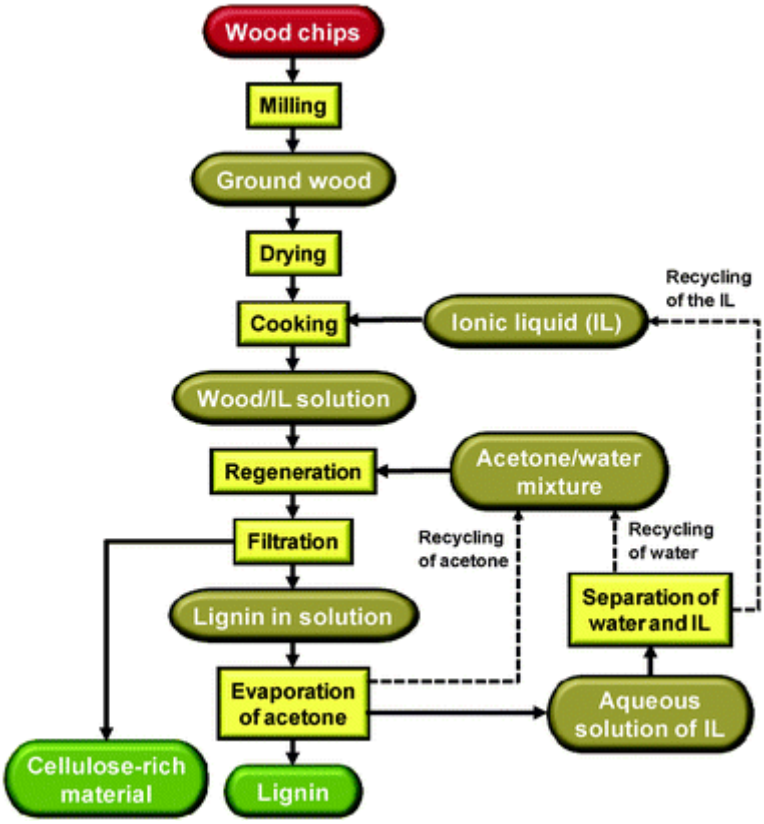


Figure 8: Flowchart for the process of dissolution and regeneration of wood in the ionic liquid[13]

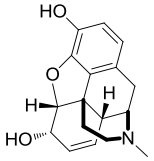
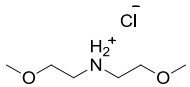
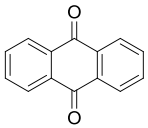
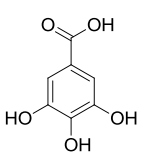
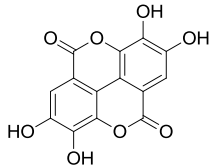
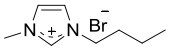
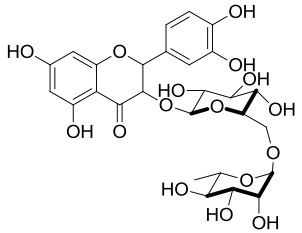
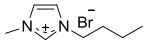
According to Zavrel *et al.* the ionic liquid 1-allyl-3-methylimidazolium chloride ([Amim]Cl) **16** was more effective for the dissolution of wood chips than [C_2mim]OAc.[14] On the other hand, the research group of Welton reported that the dissolution and swelling of wood chips was best performed using [C_4mim]OAc.[15] In any case, swelling and dissolution of the biomass was affected by moisture, and drying of the ionic liquids before use was required.[10]

2.1.1.1 Active ingredient isolation with ionic liquids

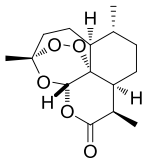
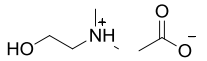
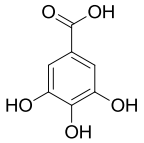
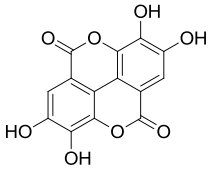
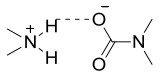
Apart from the advantages in biomass processing or functionalization, the dissolution of biomass in ionic liquids also allows the isolation of active ingredients from biomass using ionic liquids. However, it was only recently that research in this area started, and some examples are represented in this chapter (Table 2).

[14] Zavrel, M.; Bross, D.; Funke, M.; Buchs J.; Spiess, A.C. *Bioresour. Technol*. **2009**, *100*, 2580.
[15] Brandt, A.; Hallett, J.P.; Leak, D.J.; Murphy, R.J.; Welton, T. *Green Chem,* **2010**, *12*, 672.

Table 2: Active ingredient isolation using ionic liquids

Entry	Active Ingredient	Biomass	Ionic Liquid	Reference
1	Different alkaloids, *e.g.*  Morphin	Various biomass of plant or fungal origin	 bis(2-methoxyethyl)ammonium chloride	Walker[16]
2	Anthraquinone 	*Rheum officinale*	$[C_{1\text{-}10}mim]Y$, Y= BF_4^-, PF_6^-, OAc^- imidazolium based ionic liquids	Wen[17]
3	Polyphenols, *e.g.*  Gallic acid  Ellagic acid	*Psidium Guajava* leaves, *Smilax china* tubers	 1-butyl-3-methylimidazolium based ionic liquids	Du[18]
4	Rutin 	*S. chinensis*, *Flos Sophorae*	 1-butyl-3-methylimidazolium bromide	Zheng[19]

[16] Walker, A. WO 2007/110637 A1.
[17] Wen, P.; Dengxiang, J.; Jianbing, J.; Xiaoyang, S.; *Faming Zhuanli Shenqing Gongkai Shuomingshu* **2008**, CN 101219942 A.
[18] Du, F-Y.; Xiao, X-H.; Luo, X-J.; Li, G-K. *Talanta* **2009**, *78*, 1177.
[19] Zheng, H.; Wang, Y.; Kong, J.; Nie, C.; Yuan, Y. *Talanta* **2010**, *83*, 582.

5	Artemisinin		*Artemisia annua*	 bis(2-methoxyethyl)ammonium acetate	Bioniqs Ltd..[20]
6	Tannins, *e.g.*	 Gallic acid  Ellagic acid	*Acacia Catechu, Terminalia Chebula*	 DIMCARB	McFarlane[21]

[20] Extraction of Artemisinin using Ionic Liquids **2008**, Project Report 003-003/3, Bioniqs Ltd., York, UK.
[21] Chowdhury, S.A.; Vijayaraghavan, R.; MacFarlane, D.R. *Green Chem.* **2010**, *12*, 1023.

Figure 9 shows the flowchart for the extraction/isolation of active ingredients. After initial dissolution of biomass in the ionic liquid, the biopolymers can be precipitated and separated from the active ingredient. Optionally, the biopolymers can be further used in biorefinery, and the recovered ionic liquid can be reused in another extraction/isolation process.

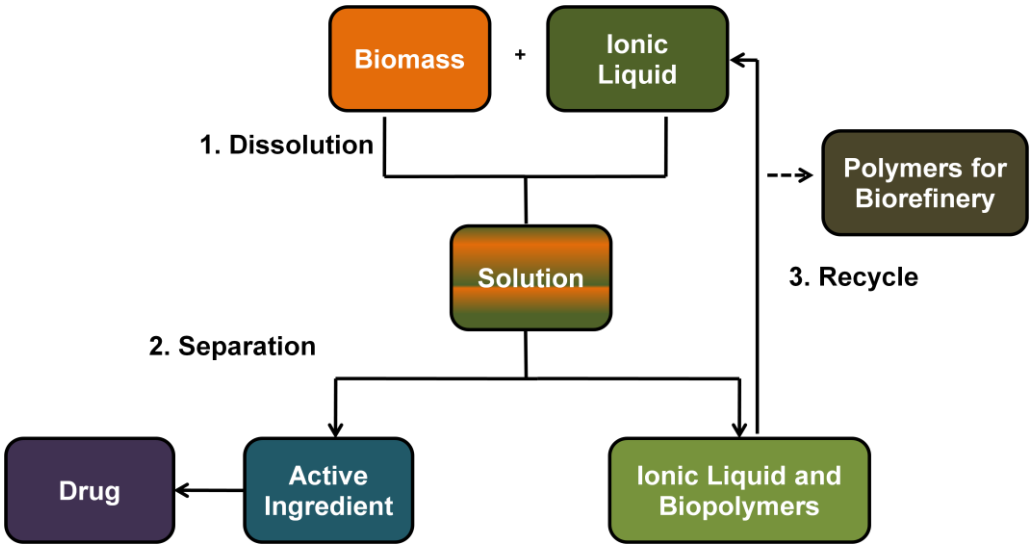


Figure 9: Flowchart for the extraction/isolation process of active ingredients with ionic liquids

Essential oils, naturally occurring alkaloids and other materials of pharmaceutical or nutritional value are typically extracted from plant material using hazardous and often toxic volatile organic solvent. In the example of alkaloids, conventional extraction methods use water-immiscible organic solvents such as chloroform at high temperature to extract organic compounds such as alkaloids, but also terpenes, oils, *etc.* from the biomass. The following extraction of the organic extract with aqueous acid pushes the alkaloid as a salt into the water phase. After removing water the alkaloid can be purified by conventional chromatography and recrystallisation. However, this process is far from flawless: Due to multiple extractions steps substantial amounts of the alkaloid are lost. Furthermore not only the target molecule but also impurities are extracted, and the process tends to use large volumes of hazardous and environmentally unfriendly solvents.[16]

In 2005 Li and co-workers used [C_4mim]Cl and K_2HPO_4 to extract codeine, papaverine and morphine from plant material.[22] The following patent of 2007 disclosed a strategy to isolate any alkaloid from plant or fungal origin, and caffeine was extracted from green tea leaves and ground coffee beans using bis(2-methoxyethyl)ammonium chloride as solvent. In another example, the ionic liquids ethanolammonium formate and *N*-methyl-bis(2-methoxyethyl)ammonium chloride were used for the extraction of the alkaloids morphine (53%), codeine (23%) and thebaine (3%) from dried poppy heads. The plants (1.5 g) were

[22] Li, S.; He, C.; Liu, H.; Li, K.; Liu, F. *J. Chromatogr. B* **2005**, *826,* 58.

mixed with 10 ml of ionic liquid on a tilt table for 72 hours. A sample was taken and centrifuged to remove debris. A KH_2PO_4 buffer solution was added, and the alkaloids were detected *via* HPLC analysis after filtration.[16]

In 2008 Wen *et al*. isolated anthraquinone from *Rheum officinale* using various imidazolium-based ionic liquids of the type $[C_n mim]Y$ with different alkyl side chains ranging from C_1-C_{10}. Different anion including BF_4^-, PF_6^-, OAc^-, $CF_3SO_3^-$ or $N(SO_2CF_3)_2^-$ were used, and the ionic liquids could be recycled.[17]

In another example for active ingredient isolation with ionic liquids, Du *et al.* presented microwave-assisted extraction (MAE) as attractive technique for polyphenols, particularly since ionic liquids absorb microwave energy efficiently. Polyphenolic compounds such as gallic acid, ellagic acid, quercetin and *trans*-resveratrol are used for pharmaceuticals and dietary supplements; however their extraction from *Psidium Guajava* (*P. guajava*) leaves and *Smilax china* (*S*. *china*) tubers suffers again from the excessive use of volatile organic solvents. In a typical procedure, 1.0 g of *P. guajava* leaves or *china* tubers and 20 ml of different ionic liquids as well as their aqueous solution were extracted for 10 min at 60-70 °C. After filtration and dilution the mass content of the polyphenolic compounds was determined, and improved extraction yields of polyphenolic compounds were observed with ionic liquids compared to organic solvents. Furthermore, the polyphenolic active compounds were found to be stable under microwave-assisted extraction at temperatures even up to 100 °C for 20 min in the ionic liquids, and no degradation was observed. The best results were obtained with the ionic liquid $[C_4mim]Br$ when different ionic liquids were tested, suggesting that the hydrogen bond acidity as well as the hydrophobicity plays an important role for solvation and interactions with polyphenolic compounds. Furthermore different cations such as tetramethylammonium $(CH_3)_4N^+$), 1-butyl-3-methylimidazolium ($[C_4mim]^+$) and butylpyridinium (C_4Py^+) with chloride as counter ion were tested, indicating that the aromatic character of the *N*-butylpyridinium cation leads to stronger solvation interactions and to higher extraction yields compared to the other ionic liquids.[18]

In 2010 the research group of Wang used microwave-assisted extraction for the extraction of the pharmaceutically active Rutin from *S. chinensis* and *Flos Sophorae*. In this case, improved yield was obtained with aqueous solutions of ionic liquids rather than with pure water and multiple interactions of the ionic liquid were held responsible in the higher solubility of Rutin compared to conventional solvents. The highest extraction yields with 5 mg active per gram of the herb samples were obtained with the ionic liquids $[C_4mim]Br$ and $[C_4mim]OTs$.[19]

Since the extraction of Artemisinin – an anti-malaria drug – from *Artemisia annua* with hexane suffers from co-extraction of many impurities, a promising isolation process of

Artemisinin using the ionic liquid bis(2-methoxyethyl)ammonium acetate had been developed by Bioniqs Ltd resulting in a better extraction (60% extraction efficiency by hexane and 79% by ionic liquid) and in a decrease of co-extracted impurities. Unfortunately, Bioniqs Ltd was closed and this process never made it to production scale.[20]

Another interesting strategy for the extraction of biomass was presented by McFarlane *et al.* who used a distillable ionic liquid, *N*,*N*-dimethylammonium *N*'*N*'-dimethylcarbamate (DIMCARB) at room temperature to extract hydrolysable tannin materials from plant sources such as catechu (*Acacia Catechu*) and myrobolan (*Terminalia Chebula*). Tannins are generally defined as water soluble organic substances present in plant extracts that effect the transformation of animal hide into leather.[21] Typically, vegetable tannins are phenol-rich compounds that show antitumor, anticarcinogenic, antimicrobial and antiviral effects.[23] In the leather industry tannins replace „chrome tanning" and can herefore avoid the handling with Cr(VI), which is considered being highly toxic, mutagenic and carcinogenic. Conventional extraction methods for tannins require harsh conditions and a high solvent/solid ratio resulting in poor extraction yields. DIMCARB is a distillable, protic ionic liquid and is formed by combining CO_2 and dimethylamine in an approximately 1:2 ratio. Figure 10 represents the dynamic equilibria in the DIMCARB system showing a two step proton transfer for the formation of the dimethyl ammonium ion and the dimethylcarbamate ion. In contrast to conventional ionic liquids, the formation of DIMCARB is reversible, and distillation at 45 °C reforms CO_2 and dimethylamine.[21] Greaves and Drummond categorized DIMCARB rather as an ionic media as an ionic liquid because it consists of non-stoichiometric ratios of ions and contains a number of neutral and ionic species in equilibrium.[24]

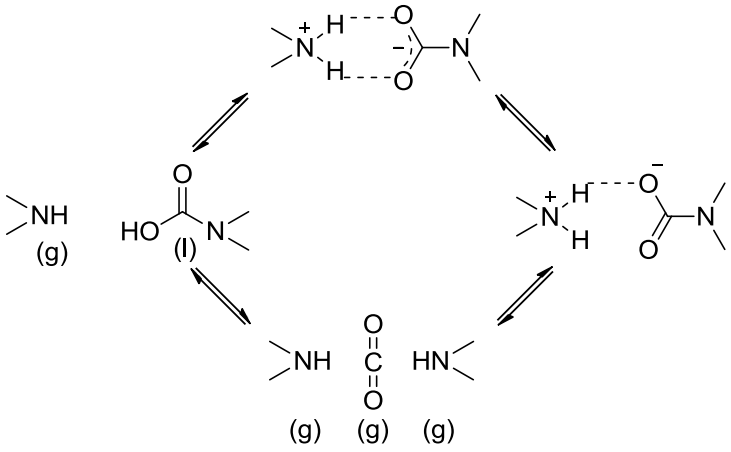


Figure 10: Dynamic equilibria in the DIMCARB system.[21]

[23] Chang, C.W.; Hsu F.L.; Lin, J.Y. *J. Biomed. Sci.* **1994**, *1*, 163.
[24] Greaves, T.M.; Drummond, C.J. *Chem. Rev.* **2008**, *108*, 206.

A mixture of 5.0 g of either myrobalan nut or catecheu was treated with 25.0 g of DIMCARB ionic liquid and stirred at room temperature for varying times. After filtration of undissolved plant material and evaporation of DIMCARB, water was added, and the aqueous solution was filtered to remove the so-called “condensed tannins”. The filtrate consisting of so-called “water-soluble tannins” could be directly used for the leather tanning process or evaporated to yield the pure hydrolysable tannins such as ellagic aicd. In contrast to conventional solvents ellagic acid was obtained in higher yields and the products are more stable against bacterial moulds as evidenced by microbial analysis. Furthermore only a third of water was necessary compared to the conventional process.[21]

2.1.2 Protic Ionic Liquids

Protic ionic liquid (PILs) can be seen as a sub-class of ionic liquids that differ in the strucure of the cation: Aprotic ionic liquids are salts consisting of alkylated cations and anions, whereas protic ionic liquids (PILs) are composed of a protonated cation. Some typical examples of aprotic ionic liquids and PILs are represented in Figure 11.[5]

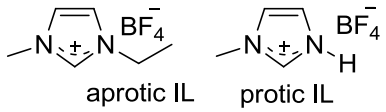


Figure 11: Example for a aprotic and a protic IL

In contrast to alkylated ionic liquids, PILs have a proton available for hydrogen bonding but do to some extent posses some typical ionic liquid properties, such as conductivity or low vapour pressure. Figure 12 shows common cations and anions of PILs.

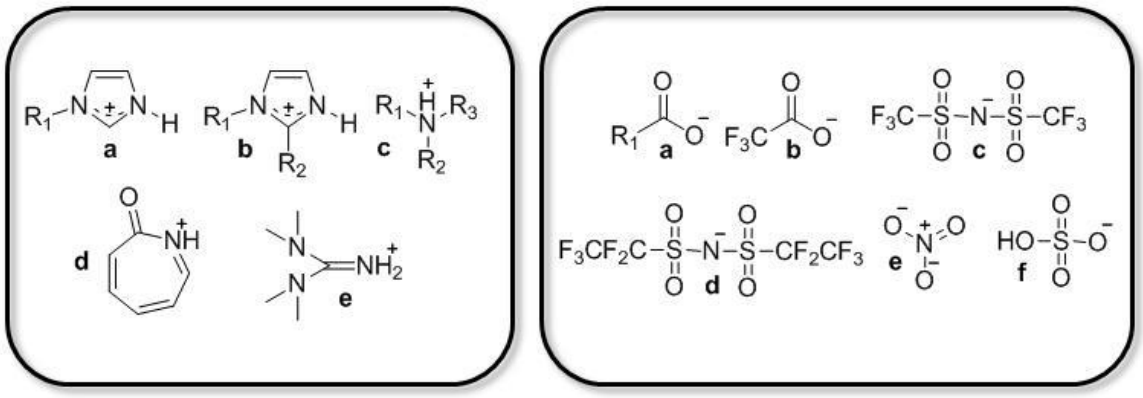


Figure 12: Typical cations and anions of PILs

The composition of protic ionic liquids is very similar to conventional ionic liquids: 1-Alkylimidazolium cations **a**, 1,2-dialkylimidazolium cations **b**, primary, secondary, or tertiary ammonium cations **c**, protonated caprolactam **d** and 1,1,3,3-tetramethylguanidinium cations **e** are most frequently found in PILs. Common anions include carboxylates **a**, trifluoroacetates

b, bis(trifluoromethanesulfonyl)imides **c**, bis(perfluoroethylsulfonyl)imides **d**, nitrates **e** and hydrogen sulphates **f**.

Protic ionic liquids are easily prepared via neutralization of the cation precursor, *e.g.* the amine with a stoichiometric amount of a Brønsted acid. However, it should be noted that a certain difference in pK_a of acid and base about ΔpK_a~10 is required. Otherwise, an incomplete degree of protonation results that has a tremendous influence on the physical properties of the salt.[24]

2.1.2.1 Protic ionic liquids as catalyst and/or reaction media

An interesting application of protic ionic liquids is their use as catalyst and/or reaction media in organic synthesis, and some examples are listed below.

In 1989 Jaeger and Tucker reported the use of protic ionic liquids as a catalyst and reaction media in a Diels-Alder reaction; however the *endo*/*exo* ratio and the conversion rate did not significantly differ from pure water.[25]

In 2002 Hangarge *et al.* used ethylammonium nitrate as solvent and catalyst in a Knoevennagel reaction (Figure 13) resulting in high yields up to 97% compared to only 47% in conventional solvents, shorter reaction times and very mild reaction conditions. In contrast, the yields of aprotic ionic liquids with BF_4^- or PF_6^- anions (54-77%) were significant lower than the yields of PILs, but higher than those of conventional solvents.[26]

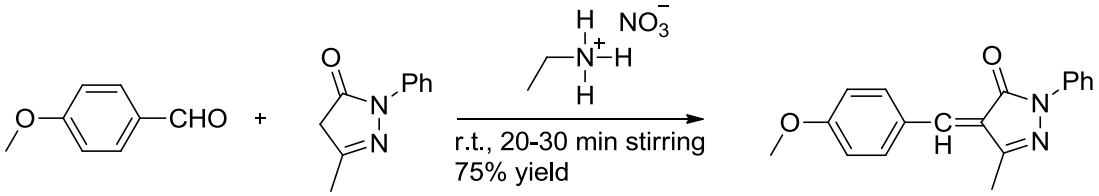


Figure 13: Example for the Knoevennagel reaction catalyzed by protic ionic liquid

Another example for the successful use of protic imidazolium- and pyridinium ionic liquids as solvent and catalyst is the Fischer esterification. The protic ionic liquid $[HC_1im]BF_4$ was used in a variety of esterification reactions of different alcohols and acids with excellent results. Furthermore, the PIL was reused 8 times maintaining 100% selectivity with conversion decreasing only slightly from 97% to 94%.[27]

The use of pyridinium-based protic ionic liquids for the esterification of cyclic olefins with acetic acid showed that the anion had a strong influence: Ionic liquids composed of the bis(trifluoromethanesulfonyl)imide (TFSI) anion gave excellent conversion and selectivity,

---

[25] Jaeger, D. A.; Tucker, C. E. *Tetrahedron Lett.* **1989**, *30*, 1785.
[26] Hangarge, R. V.; Jarikote, D. V.; Shingare, M. S. *Green Chem.* **2002**, *4*, 266.
[27] Evans, D. F. *Langmuir* **1988**, *4*, 3.

whereas the trifluoroacetic acid (TFA) promoted excellent selectivity but only poor conversion. In contrast, the HSO_4^- anion did not catalyze the reaction at all.[28]

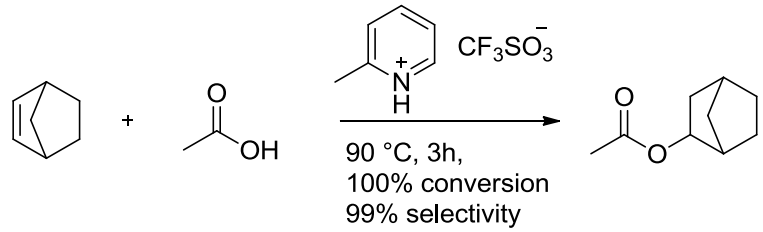


Figure 14: Esterification of norbornene and acetic acid using a pyridinium-based protic ionic liquid

Apart from esterification, protic ionic liquids can be successfully used in acetalization reactions, *e.g*. for the protection of aldehydes and ketones. (Figure 15) Again, the anion of the [HC_1im] based ionic liquid was found to have a strong influence on the reaction: When using protic ionic liquids composed of BF_4^-, $CF_3SO_3^-$, $PhSO_3^-$ and $CF_3CO_2^-$, the conversion was decreasing from 96, 89, 87 to 0%. On the other hand, the use of the protic ionic liquids [HC_1im]PF_6 and [HC_1im]Br resulted in a selective formation of the undesired Aldol product, whereas non-protic ionic liquids achieved poor to moderate selectivities of 8-60%.

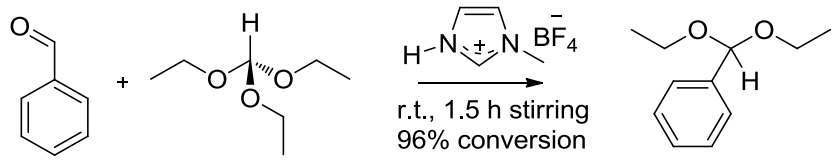


Figure 15: Acetalization of benzaldehyde using a protic ionic liquid[29]

2.1.3 Brønsted acidic Ionic Liquids

Brønsted acidic ionic liquids can be considered as a special class of ionic liquids that are closely related to protic ionic liquids. They are either protic ionic liquids or aprotic ionic liquids depending on whether the available proton is on the cation or the anion (Figure 16).[24]

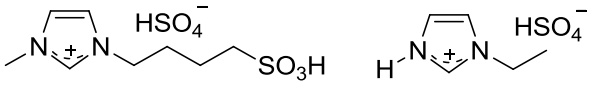


Figure 16: Aprotic Brønsted acidic ionic liquid (left) and protic acidic ionic liquid (right)

They research group of Davis was the first that reported the synthesis and application of Brønsted acidic ionic liquids.[30] An alkanesulfonic acid was covalently bound to the ionic liquid cation resulting in a strong Brønsted acidic ionic liquid. Due to the properties of these SO_3H-functionalized ionic liquids – they are flexible, non-volatile, non corrosive and

[28] Duan, Z. Y.; Gu, Y. L.; Zhang, J.; Zhu, L. Y.; Deng, Y. Q. *J. Mol. Catal., A: Chem.* **2006**, *250*, 163.
[29] Du, Y. Y.; Tian, F. L. *Synth. Commun.* **2005**, *35*, 2703.
[30] Cole, A.C.; Jensen, J.L.; Ntai, I.; Kim, L.T.; Weaver, K.J.; Forbes, D.C.; Davis, J.H.Jr. *JACS.* **2002**, *124*, 5962.

immiscible with many classical organic solvents – they have great potential for the replacement of conventional homogenous and heterogeneous acidic catalysts.[31]

2.1.3.1 Brønsted acidic ionic liquids as catalyst and/or reaction media

For example, Brønsted acidic ionic liquids have been successfully used as solvents and catalysts for several acid-promoted organic reactions such as Fischer esterification and the pinacole/benzopinacole rearrangement.

Davis' paper focused on phosphonium-based Brønsted acidic ionic liquids, since tetraorganophosphonium tosylates had already been established for several organic reactions. 1-Octanol was selectively converted to dioctyl ether in 16-56% isolated yield in the presence of Brønsted acidic ionic liquid within 60 minutes, and only minimal by-product formation was observed. Although the use of *p*-toluenesulfonic acid hydrate resulted in a better yield of dioctyl ether, more by-products were formed and the separation was more difficult. Furthermore, the Brønsted acidic phosphonium-based ionic liquid could be easily separated as a solid after cooling, whereas the use of volatile organic solvents complicated the isolation strategy.[30]

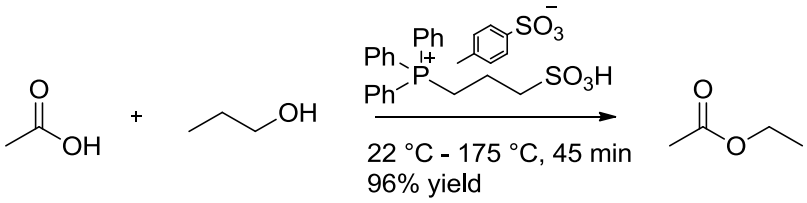


Figure 17: Esterification of acetic acid using a Brønsted acidic phosphonium-based ionic liquid

Another research group used this phosphonium-based ionic liquid as solvent and catalyst in a Fischer esterification for the formation of ethyl acetate from ethanol and acetic acid. The ionic liquid was successfully recycled, and the yield of the ester increased from cycle 1 to 3 and decreased in cycle 4. This accumulation of water that is formed as by-product in the ionic liquid was held responsible for this variation of the yield.[30]

Xing *et al*. synthesized pyridinium-based ionic liquids with an alkanesulfonic acid group and used them for the esterification of benzoic acid (Figure 18). The catalytic activity was strongly dependent on the anion, and with increasing Brønsted acidity the catalytic activity of the ionic liquids also increased. Best results were obtained with the HSO_4^- anion, and the obtained esters could be easily separated *via* decantation. After removal of water the ionic liquids were successfully reused.

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[31] Gui, J.; Ban, H.; Cong, X.; Zhang, X.; Hu, Z.; Sun, Z. *J. Mol. Catal. A: Chem*. **2005**, *225*, 28.

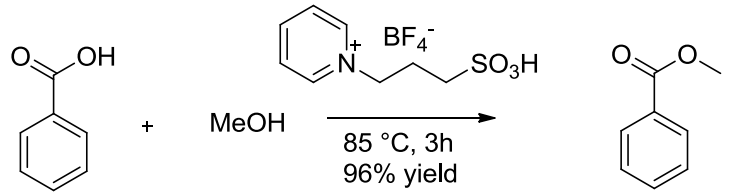

Figure 18: Brønsted acidic pyridinium based ionic liquid for the esterification of benzoic acid[32]

Shen *et al.* used imidazolium-based Brønsted acidic ionic liquids with an alkanesulfonic acid side chain as catalyst and reaction media for a Claisen-Schmidt condensation. (Figure 19) In contrast to conventional homogenous acidic catalysts these ionic liquids were immiscible with many organic solvents. Anions such as BF_4^-, HSO_4^- or *p*-toluenesulfonate showed high catalytic activities whereas the catalytic activity of the $H_2PO_4^-$ – an anion of average acid strength – was much lower.

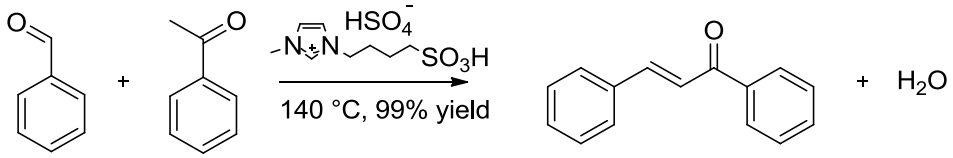

Figure 19: Claisen Schmidt reaction and Brønsted acidic ionic liquids[33]

Another application of Brønsted acidic ionic liquids was reported by Li *et al.* who published the ionic liquid mediated acetalisation of aldehydes. (Figure 20)

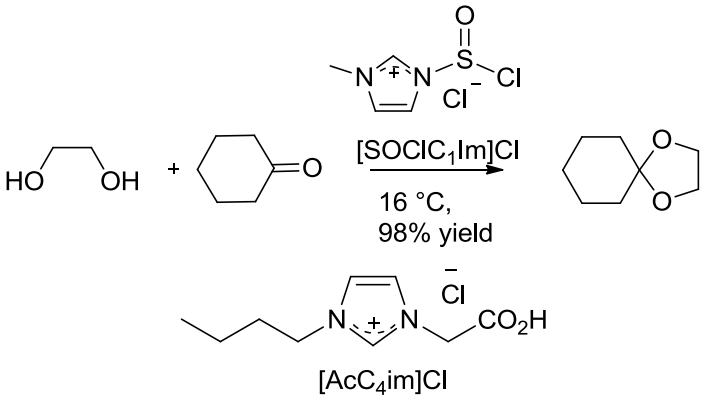

Figure 20: Ionic liquids as catalyst and reaction media for actalization[34]

The acetalization of butyl aldehyde and isoamyl alcohol was performed in excellent yield using the ionic liquid 1-butyl-3-(carboxymethyl)imidazolium chloride [AcC_4im]Cl. These results indicated that the synergism between the N^+ in the imidazolium cation and the acidic side chain was responsible for the improved catalytic activity. Furthermore side reactions such as polymerization and oxidation could be suppressed in the presence of the ionic liquid.

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[32] Xing, H.; Wang, T.; Zhou, Z.; Dai, Y. *Ind. Eng. Chem. Res.* **2005,** *44,* 4147.
[33] Shen, J.; Wang, H.; Liu, H.; Sun, Y.; Liu, Z. *J. Mol. Catal. A: Chem*. 2008, *280,* 24.
[34] Li, D.; Shi, F.; Peng, J.; Guo, S.; Deng, Y. *J. Org. Chem.* **2004**, *69*, 3582.

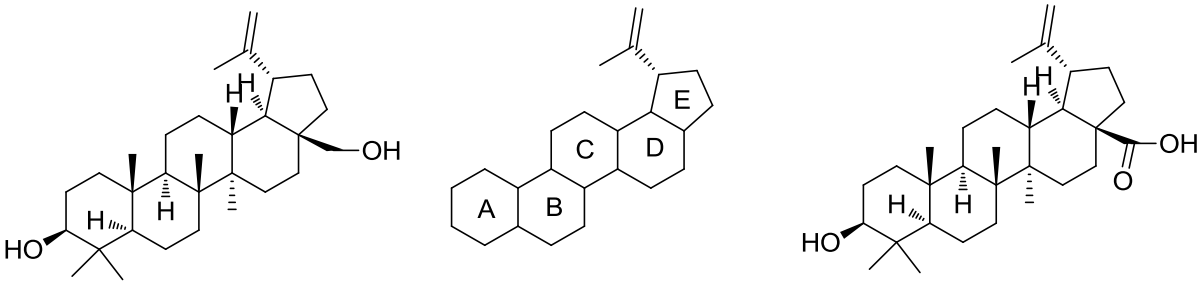
In contrast, Brønsted acidic ionic liquids with a -SO_3H group in the side chain gave different results. No catalytic activity was observed using the zwitterion 4-(1-methylimidazolium-3-yl)butane-1-sulfonate, whereas conversion increased after protonation of the zwitterion with different acids. However, side reactions also increased with stronger acids. The best results were obtained with the ionic liquid [$SOClC_1Im$]Cl, and the ketalization of different aldehydes and ketones such as cyclohexanone, benzaldehyde, propionaldehyde and butyraldehyde with ethanediol and butanediol could be performed in 80-99% yield and excellent selectivity >99%, and the products could be easily isolated via decantation.[34]

2.2 Active ingredients

2.2.1 Betulin and its derivates

2.2.1.1 Natural occurance

Betulin (**1**, lup-20(29)-ene-3β,28-diol is a naturally occurring pentacyclic triterpene alcohol (~30 wt% in birch bark) with a lupane skeleton (Figure 21). Already several hundred years ago people used Betulin and its derivatives obtained from birch bark as remedies against skin diseases. Pliny the elder, a roman naturalist first mentioned birches as "*gallica arbor*" which means "tree of the gauls", and produced a sap of the birch bark that was used as plaster. Later on during the 12th,13th and 14th century scientists were using birch bark against wounds and renal calculi. In 1788, Lowitz described Betulin for the first time as white flakes which occur by putting the white cortex of a birch next to a fire. However, it was only in 1950 when the structure of Betulin was first determined and the lupane skeleton identified.[35] To date, there is no total synthesis reported in literature.



1 Betulin | lupane skeleton | **2** Betulinic acid

Figure 21: Betulin **1**, lupane skeleton and Betulinic acid **2**

[35] www.betulin.de, last accessed 18/4/2011.

2.2.1.2 Biological activity

Betulin and its derivates show antitumor, anti-HIV, antiviral, antibacterial, anti-inflammatory and antimalarial activities. In particular, Betulinic acid **2** (Figure 21) could be used in treatment against tumours and can be obtained from Betulin **1** in a two step synthesis in 75% overall yield (Figure 22).[36]

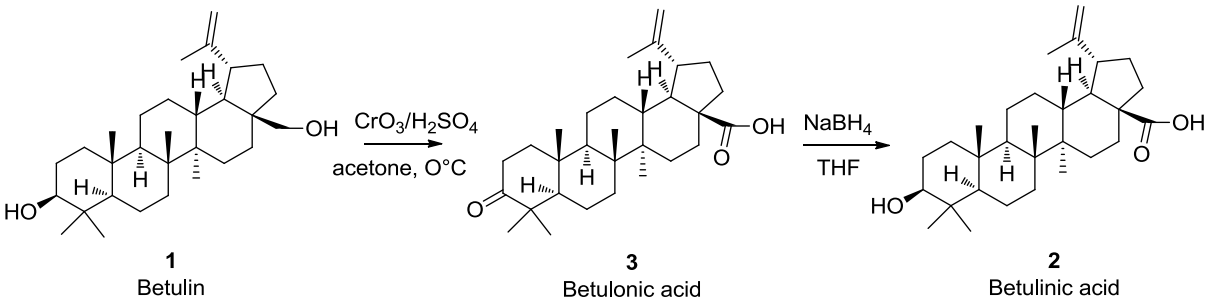


Figure 22: Synthesis of Betulinic acid **2**

Betulin **1**, Betulinic acid **2** and Betulonic acid **3** show antiviral activities, *e.g.* against herpes simplex type 1. In contrast, their antibacterial activities are rather poor, and they were found to be inactive against *Escherichia coli* and other bacteria. Anti-inflammatory activitiy has been reported for Betulin and Betulinic acid, and they were effective against skin inflammation and ear edema. Betulin is inactive against malaria whereas Betulinic acid shows moderate activity against *Plasmodium falciparum*.[36]

Song *et al.* discovered that Betulin could be used as a leading compound for development of drugs for hyperlipidermia. Betulin inhibits the pathway of SREBPs (sterol regulatory element-binding proteins) that offers a treatment against metabolic diseases, for instance type II diabetes and atherosclerosis. SREBPs are major transcription factors and responsible for the expression of genes involved in biosynthesis of cholesterol, fatty acid and triglyceride.[37]

Apart from its pharmaceutical properties, Betulin in the form of birch bark extract is commonly used as additive to shampoo, skincare, dental-care and hare-care products. Furthermore, the remedy Betual® consisting of Betulin and birch bark extract is used as a dietary supplement, for liver protection, prevention and treatment of acute alcoholic intoxication and against hangover intensity.[38]

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[36] Sami, A.; Taru, M.; Salme, K.; Jari Y.K.; *Eur. J. of Pharm. Sci.* **2006**, *29*, 1.
[37] Tang, J.J.; Li, J.G.; Qi, W.; Qiu, W.W.; Li, P.S.; Li, B.L.; Song, B.L. *Cell Metabolism* **2010**, *13*, 44.
[38] Krasutsky, P.A. *Nat. Prod. Rep.* **2006**, *23*, 921.

2.2.1.3 Conventional isolation of Betulin

Betulin is typically isolated from birch bark using a conventional solvent extraction process, and several organic solvents have been reported in literature (Table 3).

Table 3: Comparison of different extraction and isolation methods using conventional solvents

Entry	Solvent	Yield [wt%]	Purity	Purification	Reference
1	EtOH	26[a]	n.a.	chromatography	Drag[39]
2	Chloroform	17	>95%	chormatography	Pichette[40]
3	Toluene	0.3	96%	recrystallization	Sauter[41]

[a] Crude material before purification

However, all processes require large amounts of volatile solvents to obtain moderate yields, and the obtained crude actives need to be further purified *via* expensive column chromatography or repeated crystallization.

In 2009, Drag *et al.* reported an extraction yield of 26 wt% using anhydrous EtOH as solvent. Starting from 250 g birch bark and 1.5 l of EtOH (17 wt% solution) they obtained 66 g of crude Betulin, which had to be further purified by column chromatography.[39]

The isolation of Betulin from birch bark with chloroform was described by Pichette *et al*. They refluxed 100 g of birch bark in 1000 ml of chloroform (6 wt% solution) for several hours. After column chromatography on silica with petrolether/ethyl acetat as eluent, 17 g of Betulin (17 wt%, >95% purity according to GC) were obtained.[40]

According to a patent from 2003, a 41 wt% solution of birch bark in toluene (2.5 kg birch bark, 4.5 l toluene) was stirred at 70 °C for 2 h. Toluene was decanted and the residue was again refluxed with another 4.0 l of toluene for 2 h. After filtration and evaporation of the solvent, the crude Betulin was washed with 500 ml of petrolether and yielded in 1.8 wt%. (70.2% purity according to GC). For further purification, Betulin was redissolved in 8000 ml of toluene and after extraction with NaOH and water 29 g of Betulin were obtained (74% yield). It was further purified by recrystallization from EtOH to give 37% of Betulin in purity 90% according to GC. A second recrystallization step from EtOH with addition of charcoal can further improve the purity (96%) but again a loss of Betulin was observed. (56% yield). The over all yield was only 0.3 wt% of Betulin with a purity of 96% purity according to GC.[41]

[39] Drag, M.; Surowiak, P.; Drag-Zalesinska, M.; Dietel, M.; Lage, H.; Oleksyszyn, J. *Molecules* **2009**, *14*, 1639.
[40] Pichette, A.; Liu, H.; Roy, C.; Tanuay, S.; Simard, F.; Lavoie, S. *Synth. Commun.* **2004**, *34*, 3932.
[41] Sauter, M.; Bender, C. WO 03/066658 A2

2.2.1.4 Bevirimat

Bevirimat (**4**, 3-*O*-(3',3'-dimethylsuccinyl)Betulinic acid) that is probably the most promising derivative of Betulin was found to be active against Human Immunodeficiency Virus (HIV) infection and is currently under clinical trials. Although the treatment of HIV infection progressed by the availability of distinct classes of antiretrovirals that inhibit HIV replication, the current therapy is limited by toxicity and the eventual development of resistance. Due to the fact that Bevirimat is well absorbed and tolerated, it passed clinical phase I and II and is at the moment in clinical phase III.[42]

Bevirimat 4 can be easily synthesized from Betulinic Acid **2** and 2,2-dimethylsuccinic anhydride *via* esterification in refluxing pyridine. (Figure 23) For further purification, recrystallization from MeOH is required, and Bevirimat is typically obtained in 70% yield.

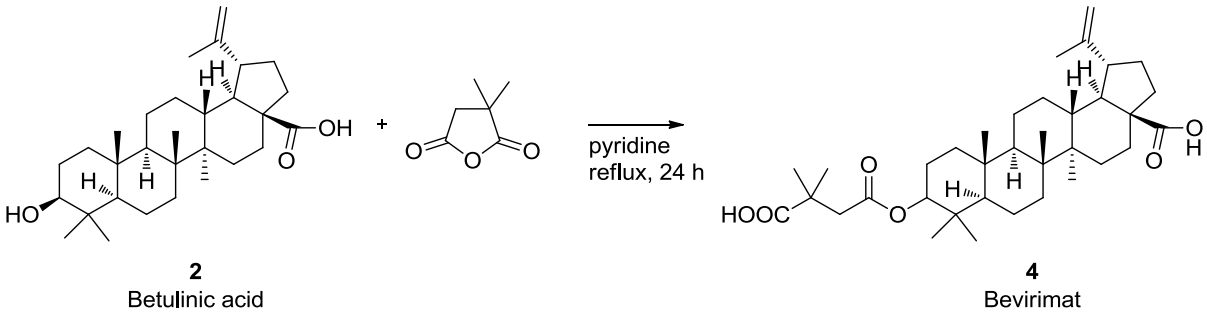

Figure 23: Synthesis of Bevirimat[43]

2.2.2 Shikimic Acid

2.2.2.1 Natural occurrence and isolation

Shikimic acid ((3*R*,4*S*,5*R*)-3,4,5-trihydroxycyclohex-1-ene-1-carboxylic acid, **5**, Figure 24) obtained its name from the fruit *Illicium religiosum* – named *shikimi-no-ki in* Japanese – from which it was first isolated in 1885 by Eykman.[44] Although the stereochemistry of Shikimic acid could not be determined at that time, Eykman described the structure as a cyclic trihydroxycyclohexenecarboxylic acid. It was only in the 1930s that the stereochemistry could be finally determined.

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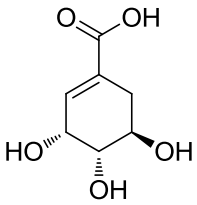
[42] Smith, P.F.; Ogundele, A.; Forrest, A.;Wilton J.; Salzwedel, K.; Doto J.; Allaway, G.P.; Martin, D.E. *Antimicrob. Agents Chemother*. **2007**, *51*, 3574.
[43] Hashimoto, F.; Kashiwada, Y.; Cosentino, L.M.; Chen, C-H.; Garrett, P.E.; Lee, K-H. *Biorg. Med. Chem.* **1997**, *5*, 2133.
[44] *Eykman, J. F. Ber. Dtsch. Chem. Ges.,* **1891**, *24*, 1278.



5
Shikimic acid

Figure 24: Structure of Shikimic acid

Shikimic acid is typically isolated from Chinese star anise seeds (*Illicium verum*) but the isolation yields are low (3-7%).[45,46] In the past years, novel methods for the biochemical prepapration of Shikimic acid by genetically modified *Escherichia coli* strains have been reported.[47] Furthermore, Shikimic acid can be also obtained by fermentation from ginkgo leaves.[48] There are also several total synthetic approaches for Shikimic acid available that are *e.g*. either a Diels-Alder reaction and chiral synthesis, the partial hydration of benzene and its derivatives or a chiral synthesis starting from (-)-quinic acid and other carbohydrates (Figure 25).[49]

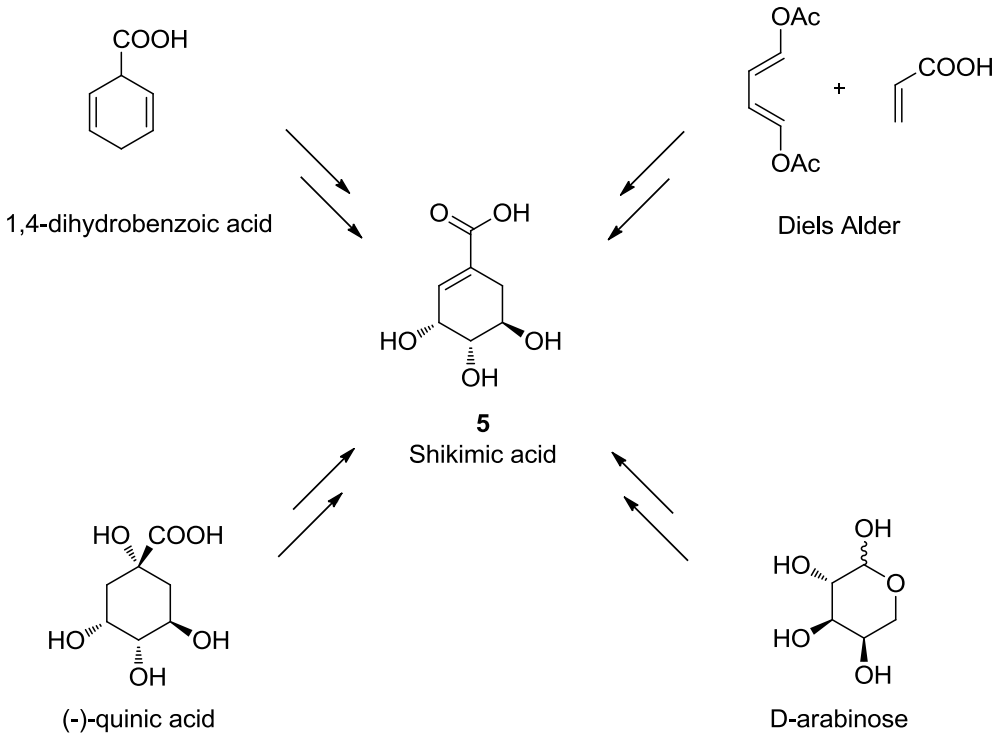


Figure 25: Starting materials for the synthesis of Shikimic acid

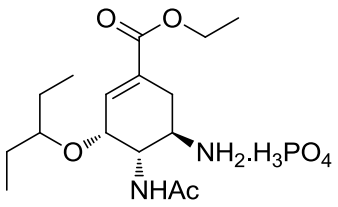
[45] Ressmann, A.K.; Gaertner, P.; Bica, K. *Green Chem.* **2011**, DOI:10.1039/C1GC15058H.
[46] Draths, K. M.; Knop, D.R.; Frost, W. *J. Am. Chem. Soc.* 1999, **121**, 1603.
[47] Johansson, L.; Lindskog, A.; Silfversparre, G.; Cimander, C.; Nielsen, K.F.; Liden, G. *Biotechnol. Bioeng.* **2005**, *92*, 541.
[48] Federspiel, M.; Fischer, R.; Hennig, M.; Mair, H-J.; Oberhauser, T.; Rimmler, G.; Albiez, T.; Bruhin, J.; Estermann, H.; Gandert, C.; Göckel, V.; Götzö, S.; Hoffmann, U.; Huber, G.; Janatsch, G.; Lauper, S.; Röckel-Stäbler, O.; Trussardi, R.; Zwahlen, A.G. *Org. Process Res. Dev.* **1999**, *3*, 266.
[49] Jiang, S.; Singh, G. *Tetrahedron*, 1998, *54*, 4697.

2.2.2.2 Biological activity of Shikimic acid and its derivatives

Shikimic acid is one of the primary intermediates in the shikimate pathway, a biochemical pathway for the synthesis of aromatic amino acids which can be found in most organisms except for mammals. Shikimic acid is also an industrially interesting chiral starting material for production of many different chemical compounds, such as the anti-influenza drug Tamiflu™. Furthermore, Shikimic acid derivates can be used as herbicides and have antibacterial activities due to their potential of blocking the shikimate pathway without negative effects in mammals.[50]

2.2.2.3 Tamiflu™

Tamiflu™ (Oseltamivir phosphate **6**, Figure 26) is currently used for treatment and prevention of influenza. It became famous for the treatment of the swine flu virus (H1N1 human flu) which has become a global influenza pandemic.Tamiflu™ is taken orally and hydrolyzed by an esterase enzyme to the corresponding carboxylic acid, which is a potent inhibitor of neuraminidases A and B.[50] It was discovered by Kim *et al.* from Gilead Sciences.[51]



6

Tamiflu™ (Oseltamivir phosphate)

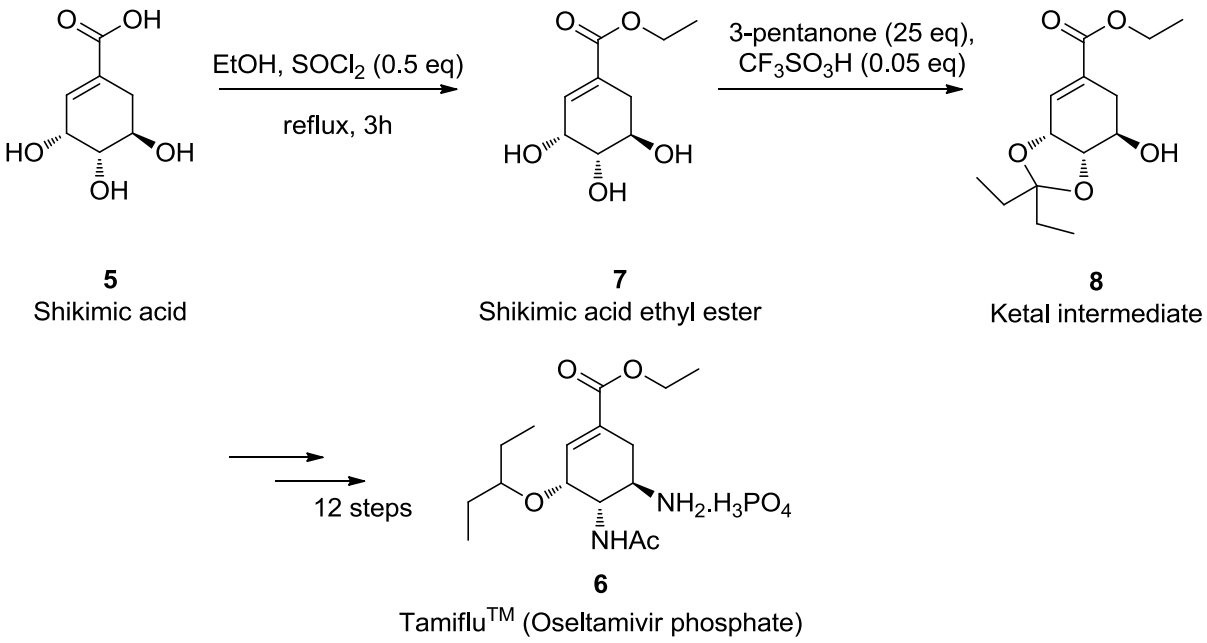
Figure 26: Structure of Tamiflu™

The production of Tamiflu™ is still dependent on the isolation of Shikimic acid for Chinese star anise seeds, although alternative fermentation processes have been developed. Due to the fact that isolation yields are very low, there has been a world-wide shortage in the production of Tamiflu™ in 2005.[45]

In the current manufacturing process, the synthesis of Oseltamivir phosphate **6** involves the formation of Shikimic acid ethyl ester (**7**, (*3R,4S,5R*)-3,4,5-trihydroxycyclohex-1-ene-1-carboxylic acid, ethyl ester) in 97% yield using 0.5 equivalents of toxic and corrosive thionyl chloride. The second intermediate is the ketal (**8**, (*3aR,7R,7aS*)-2,2-diethyl-*3a,6,7,7a*-tetrahydro-7-hydroxy-1,3-benzodioxole-5-carboxylic acid, ethyl ester) that is formed with 3-pentanone and CF_3SO_3H in 97% yield (Figure 27). Recently, Roche reported an alternative

[50] Wichienukul, P.; Akkarasamiyo, S.; Kongkathip, N.; Kongkathip, B. *Tetrahedron Lett*., **2010**, *51*, 3208.
[51] (a) Kim, C. U.; Lew, W.; Williams, M. A.; Wu, H.; Zhang, L.; N.; Chen, X.; Escarpe, P. A.; Mendel, D. B.; Laver, W. G.; Stevens, R. C. *J. Med. Chem*. **1998**, *41*, 2451. (b) Kim, C. U.; Lew, W.; Williams, M. A.; Liu, H.; Zhang, L.; Swaminathan, S.; Bischofberger, N.; Chen, M. S.; Mendel, D.B.; Tai, C. Y.; Laver, W. G.; Stevens, R. C. *J. Am. Chem. Soc*. **1997**, *119*, 681.

streamlined one-step procedure that could be performed without thionyl chloride by using 3,3-diethoxypentane as active reagent.[48]



5
Shikimic acid

7
Shikimic acid ethyl ester

8
Ketal intermediate

6
Tamiflu™ (Oseltamivir phosphate)

Figure 27: Roches' process for the industrial synthesis of Tamiflu™[48]

3 Task

In this diploma thesis, ionic liquids should be established as novel and environmentally benign solvent for the isolation of pharmaceutically active ingredients from plant material.

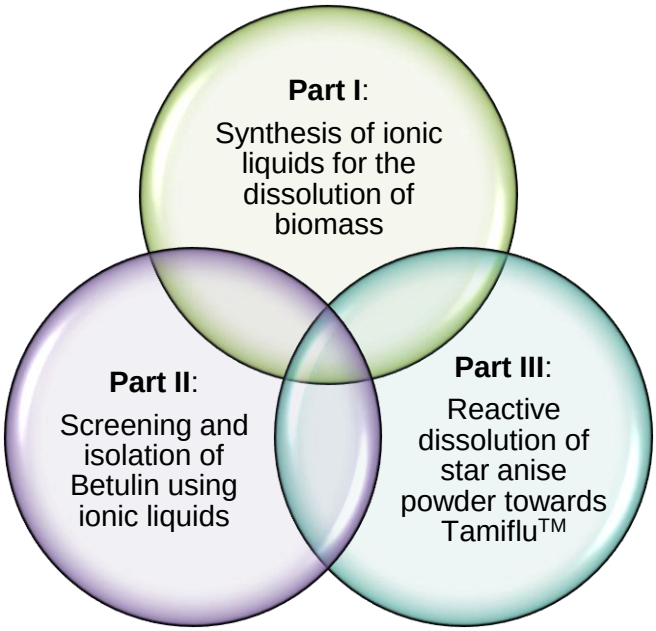


Figure 28: Task

The aim of the first part of this diploma thesis was to synthesize several ionic liquids for the dissolution of biomass *via* alkylation of 1-methylimidazole or 1-ethylimidazole. Additionally, a set of Brønsted acidic ionic liquids should be synthesized for the reactive dissolution of biomass.

In the second part these ionic liquids should be used for the dissolution of birch bark to extract the active ingredient Betulin. Extraction yields should be compared with conventional solvents and extraction process parameters such as temperature, concentration, time and ionic liquid type should be investigated. Based on the optimized results, a novel isolation process for Betulin using ionic liquids should be developed, and the ionic liquid should be recovered.

In the third part a reactive dissolution strategy for the improved synthesis of Tamiflu™ starting from Shikimic acid should be developed. In this process, the Brønsted acidic ionic liquid should be used as solvent and as catalyst, and the *in situ* esterification and ketalization of pure Shikimic acid but also of star anise biomass should be investigated.

4 Results and discussion

4.1 Synthesis of ionic liquids

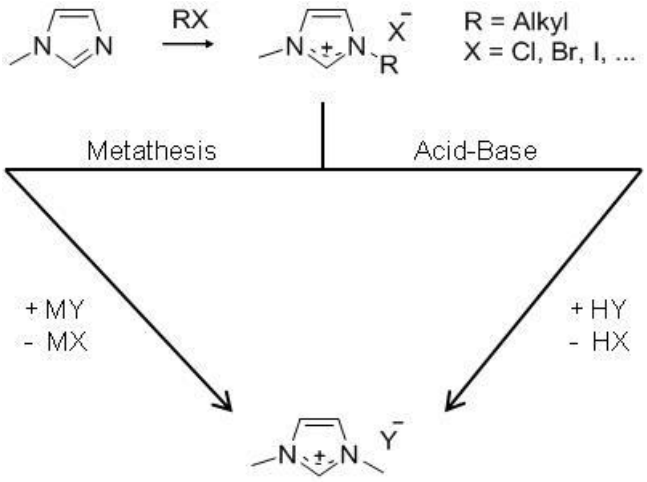


Figure 29: General scheme for the synthesis of ionic liquids[9]

In general, the first step in the synthesis of ionic liquids is an alkylation of amines or phosphines. (Figure 29) The preparation of alkyl cations of ammonium-, imdiazolium-, pyridinium- and phosphonium-based ionic liquids is performed with a nucleophile such as alkyl halide, alkyl sulphonate or dialkyl sulphate. Figure 30 shows the alkylation of 1-methylimidazole with *n*-butylbromide.

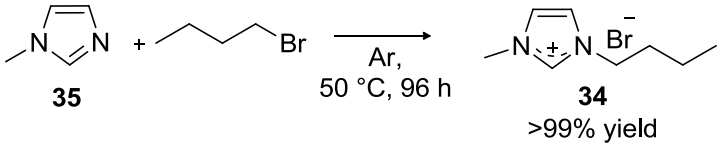


Figure 30: Synthesis of [C_4mim]Br *via* alkylation with *n*-butylbromide

Reactions were generally performed with small excess of alkylating reagent for a complete conversion of 1-methylimidazole as its removal is difficult due to its high boiling point of 198 °C, whereas the alkylation reagent, *e.g.* butylbromide can be easily removed *in vacuo*. The reaction was typically performed with moderate heating to facilitate complete alkylation, however the temperature should be kept <100 °C since otherwise dark materials were obtained. In addition the reactions were generally performed under inert atmosphere to avoid discoloration. Furthermore, ionic liquids are generally hygroscopic and moisture was avoided by working under inert atmosphere. The conversion of the reaction was controlled *via* NMR until 1-methylimidazole was completely reacted, and remaining volatiles were removed *in vacuo*. In any case, the yield for the alkylation step was quantitative.

Alkylated ionic liquids are also precursors for the synthesis of other ionic liquids by anion metathesis. This allows not only preparing ionic liquids with anions that cannot be introduced *via* direct alkylation, but also modifies the physical properties and often leads to a reduction of the melting point. The anion exchange is usually performed between the organic ionic liquid precursor and an inorganic anion source, *e.g*. the alkali salt of the corresponding acid.

In general, there are several different methods to perform the anion methathesis:

- Anion exchange with the silver salt of the anion: The precipitation of the by-product such as silver halides can be easily done, although this method is quite expensive Drake *et al.* reported in 2005 a preparation strategy using silver nitrate for 1-alkyl-4-amino-1,2,4-triazolium nitrate ionic liquids. The reaction was performed in hot methanol and the silver halides could be removed by filtration. (Figure 31).

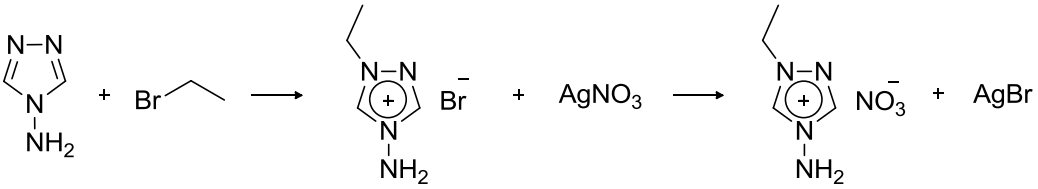


Figure 31: Alkylation and anion metathesis

- Anion exchange with alkali or earth alkali salts of the anion is a cheap and simple preparation method for hydrophobic ionic liquids, since the by-product can be easily removed *via* aequous extraction. However the production of hydrophilic ionic liquids with this method is problematic, since the by-product *e.g.* NaCl needs to be precipitated in an organic solvent and the isolated ionic liquid often suffers from halide and metal impurities.
- Anion exchange with the acid: If the corresponding acid of the desired anion is stronger, the anion exchange can be directly performed from the acid. An example is the preparation of the PF_6^- anion with an aqueous solution of the acid HPF_6 or neutral salts such as $NaPF_6$ or NH_4PF_6 (Figure 32). As the product is immiscible with water and forms a separate phase that can easily be separated from the aqueous layer.[52]

$$[C_4mim]Cl + HPF_6 \longrightarrow [C_4mim]PF_6 + HCl$$

Figure 32: Preparation of $[C_4mim]PF_6$ *via* acid-base reaction[5]

[52] Wasserscheid, P.; Welton, T. *Ionic Liquids in Synthesis, 2nd ed.*; Wiley-VCH: Weinheim, 2008; Chapter 8.

- Ion exchange resin is used for anions that cannot be introduced *via* acid-base reaction. A typical example is the preparation of [C_2mim]OAc. The chloride anion of [C_2mim]Cl is first replaced by a hydroxide ion *via* ion exchange resin. Reaction with stoichiometric amounts of acetic acid results in the formation [C_2mim]OAc (Figure 33).[53]

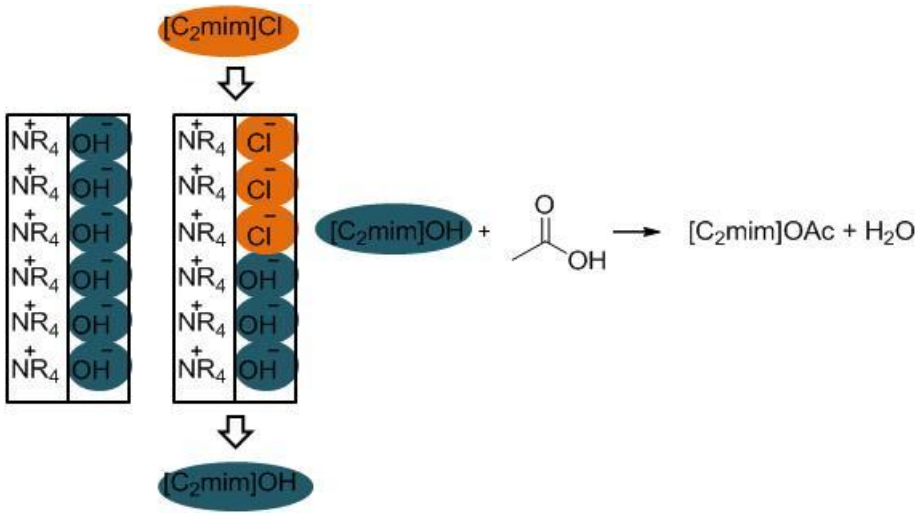
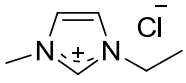
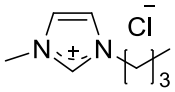
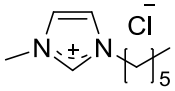
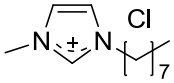
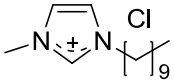
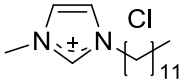
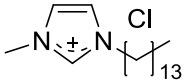
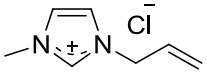
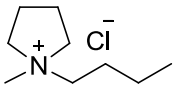
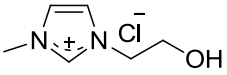
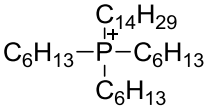
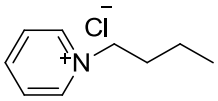
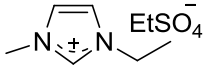
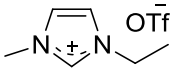
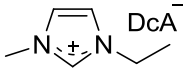
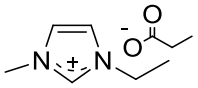
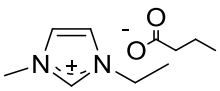
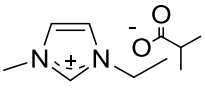
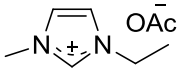
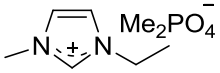
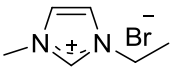
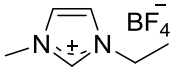
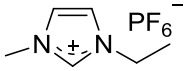
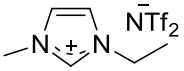
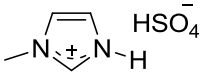
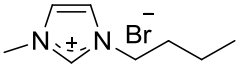


Figure 33: Synthesis of [C_2mim]OAc *via* anion exchange resin

In the course of this thesis several ionic liquids have been synthesized according to the strategies described above, whereas many others that were used for the subsequent biomass dissolution were already available from previous projects or obtained from commercial suppliers. In Table 4, all ionic liquids used for the extraction of Betulin are shown; however, yields are only given for those prepared during this thesis.

[53] Szarvas, L.; Maase, M.; Massonne, K. WO 2005/085207A2.

Table 4: Ionic liquids used in extraction process

1-Alkyl-3-methylimidazolium chloride ionic liquids			
 **9** [C_2mim]Cl	 **10** [C_4mim]Cl	 **11** [C_6mim]Cl	
 **12** [C_8mim]Cl	 **13** [C_{10}mim]Cl	 **14** [C_{12}mim]Cl	 **15** [C_{14}mim]Cl
Other chloride ionic liquids			
 **16** [amim]Cl	 **17** [C_4mPyrl]Cl	 **18** [OHC_2mim]Cl >99% yield	 **19** [P_{66614}]Cl
 **20** [C_4Py]Cl			
Hydrophilic [C_2mim]Y ionic liquids			
 **21** [C_2mim]$EtSO_4$ >99% yield	 **22** [C_2mim]OTf	 **23** [C_2mim]DCA	 **24** [C_2mim]propionate
 **25** [C_2mim]*n*-butyrate	 **26** [C_2mim]*iso*-butyrate	 **27** [C_2mim]OAc	 **28** [C_2mim]Me_2PO_4 >99% yield
 **29** [C_2mim]Br			
Hydrophobic [C_2mim]Y ionic liquids			
 **30** [C_2mim]BF_4	 **31** [C_2mim]PF_6	 **32** [C_2mim]NTf_2	
Protic ionic liquid		**[C_4mim]Y**	
 **33** [C_2mim]HSO_4		 **34** [C_4mim]Br >99% yield	

4.1.1 Synthesis of protic ionic liquids

In comparison to permant and alkylated ionic liquids, the synthesis of protic ionic liquids requires a proton transfer from a Brønsted acid to a Brønsted base.

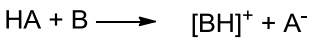


Figure 34: Synthesis of PILs

Ideally the proton transfer from acid to base is complete but this is often unlikely. Depending on the difference of pK_a between acid and base, an incomplete proton transfer occurs, resulting in the presence of complex mixtures of the salt, a neutral acid and base, but also in the aggregation and association of either ions or neutral species. A look at the aqueous pK_a values of the precursor acids and bases may estimate how complete a proton transfer is. A large ΔpK_a ((pK_a(base) - pK_a(acid)) indicates a better proton transfer, and if ΔpK_a >10 the proton transfer is typically complete, as shown for the protic ionic liquid in Figure 35.[24]

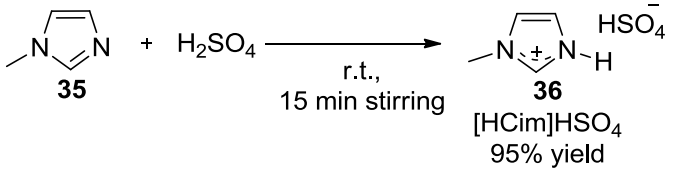


Figure 35: Example for a protic ionic liquid synthesized in this thesis

4.1.2 Synthesis of Brønsted acidic ionic liquids

The synthesis of Brønsted acidic ionic liquids was first described by Forbes *et al*. In the first step, a zwitterionic precursor is prepared *via* reaction of the nucleophiles *N*-butylimidazole or triphenylphosphine with 1,4-butane- or 1,3-propanesultone. In case of ammonium-based Brønsted acidic ionic liquids this can be a nitrogen-containing nucleophil, *e.g.* amines, whereas the reaction with alkylphosphines results in the formation of phosphonium-based ionic liquids.

In the second step, the obtained zwitterion is protonated. A stoichiometric amount of the zwitterions is reacted with an acid possessing a pK_a sufficiently low to convert the sulfonate group into an alkanesulfonic acid in the side chain. The resulting ionic liquid consists of a sulfonic acid-functionalized cation and the conjugate base of the acid as anion. For both zwitterion formation and acidification step quantitative yields were reported.[30]

This procedure by Forbes was adapted to synthesize a small set of Brønsted acidic ionic liquids for biomass dissolution. In the first step 1-methylimidazole and 1,4-butanesultone were reacted in anhydrous THF at 70 °C for 48 h to form the zwitterionic intermediate. The zwitterions precipitated from the solution, and the yield was quantitative. In the second step,

the zwitterion was protonated by adding several Brønsted acids, and after short reaction times the ionic liquids were obtained with overall yields of >95% (Figure 36).

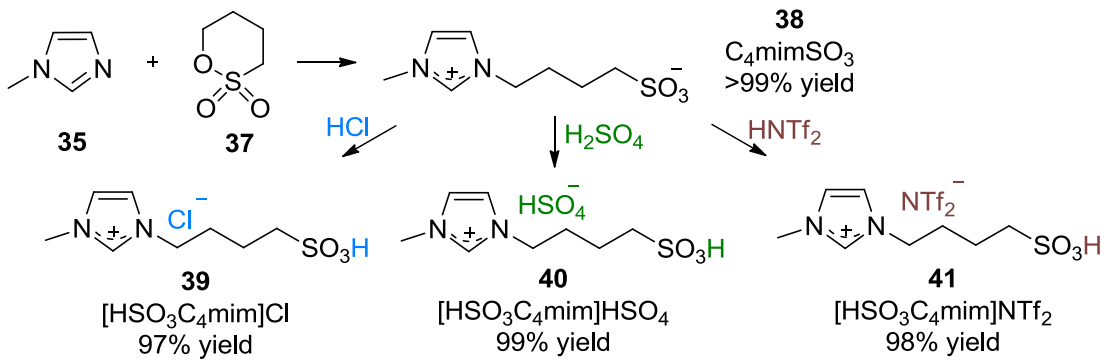


Figure 36: Synthesized Brønsted ionic liquids

4.2 Dissolution of biomass: Betulin

4.2.1 General strategy for the extraction of Betulin

As conventional extraction of birch bark is currently performed with waste amounts of volatile solvents (chapter 2.2.1.3) giving moderate to poor yields of Betulin, a new strategy should be developed. As ionic liquids are able of dissolving biomass, they were chosen as alternative solvents. Initially, it should be investigated if extraction process using ionic liquid can increase the extraction yield compared to conventional solvents. Therefore a working process including an HPLC strategy for the detection of Betulin in the presence of ionic liquid was developed to allow a comparison of ionic liquids with conventional solvents, but also a quick screening of different isolation conditions or ionic liquid types.

Figure 37: Working process for the extraction of Betulin from birch bark

Figure 37 shows the working process, which consists of following parts:

- Dissolution of birch bark in ionic liquids under different conditions: Birch bark was harvested from local birch trees in July 2010 and the wooden parts were peeled of manually. The birch bark was cut in a conventional kitchen blender being frozen in liquid nitrogen first and sieved to obtain particles with < 1 mm particle size. The powder was dried in a vacuum drying oven at 60 °C/20 mbar for 48 h. The extraction

yield of the ionic liquid was compared with yields obtained with conventional solvents. Furthermore different parameters should be varied to find out the optimal conditions:

- Comparison with conventional solvents
- Concentration
- Temperature
- Reaction time
- Microwave heating
- Different ionic liquids

- Precipitation of biopolymers: EtOH or MeOH was added to the dark slurry which allowed the precipitate of undesired biopolymers, whereas Betulin stayed in solution. It should be mentioned that this precipitation step could be also performed with other organic solvents; however both MeOH and EtOH are generally classified as environmentally benign solvents. While both alcohols were suitable here, EtOH has the advantage of being pharmaceutically compatible compared to the toxic but cheaper MeOH.
- Centrifugation: For the removal of the precipitated biopolymers centrifugation was used instead of filtration as it is cheap, fast and easy to handle.
- HPLC analysis was used for the determination of the extraction yield and is precisely described in the next chapter. The yield of the active component is calculated according to the formula

$$\text{yield [wt\%]} = \frac{\text{Active ingredient from HPLC [mg]}}{\text{Crude biomass [mg]}} \cdot 100$$

and reported in mass percent compared to the crude biomass.

4.2.1.1 HPLC analysis for the detection of Betulin

In order to efficiently screen the exact amount of Betulin in the presence of ionic liquids, a HPLC strategy was developed. Due to the high polarity of ionic liquids, a reversed phase column was chosen with acetonitrile and water as eluent. This allowed a clean separation of the ionic liquid that was eluted in the beginning, whereas the rather apolar active ingredient Betulin was eluated later on. For the exact determination of Betulin, an internal standard was required that was added to the sample. After screening several components including alcohols (*e.g.* phenol, 2,6-diphenylphenol, 2-cyclohexen-1-ol), olefins (*e.g.* 1-methyl-1-cyclohexen, sqalen) and aromatics (*e.g.* naphtalin) as internal standard, 1-methyl-1-cyclohexen was chosen since the retention time was between the ionic liquid and Betulin and the peaks did not overlap. Furthermore, UV detection could be easily done at 210 nm for the analyte and the internal standard.

After the chromatographic conditions were established, calibration curves were prepared by adding an exact amount of internal standard (0.1 mg) to a dilution series of Betulin ranging from 2 mg/ml - 0.01 mg/ml.

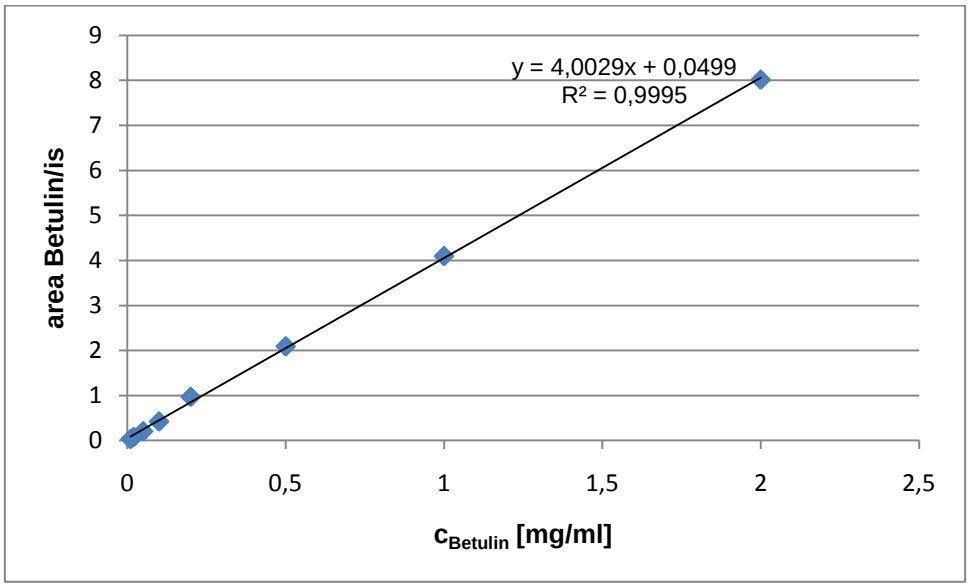


Figure 38: Standard calibration

Figure 38 shows the calibration curve for Betulin using 1-methyl-1-cyclohexene as internal standard. It is linear in a range from 2 mg/ml - 0.01 mg/ml with an excellent correlation coefficient R^2 > 0.999. A good separation within 20 min and retention times of 8.7 min (internal standard) and 17.6 min (Betulin) were obtained using acetonitrile:water 80:20 as eluent on a C-18 reversed phase column.

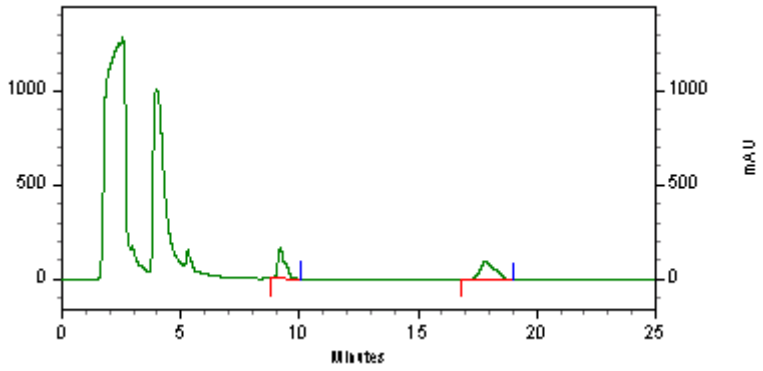


Figure 39: Chromatogram of an extraction sample

Figure 39 shows a chromatogram of an extraction sample after dilution with MeOH. Besides the active Betulin and the internal standard, the ionic liquid and some unidentified substances, *e.g.* biopolymers are eluted in the beginning.

4.2.1.2 Comparison with conventional organic solvents

After establishing an HPLC strategy, the extraction efficiency of conventional solvents and ionic liquids was compared. Chloroform, toluene and ethanol were chosen as organic solvent since they have already been reported for the extraction of Betulin from birch bark (chapter 2.2.1.3). Additionally, water and ethanol were investigated since they are not only traditional solvents in extraction processes, but also generally considered to be environmentally benign solvents. Since the ionic liquid [C_2mim]OAc has already been established for biomass processing (chapter 2.1.1), this ionic liquid was chosen for the comparison with conventional solvents. The extraction experiments were performed with a 5 wt% solution of birch bark in organic solvent or [C_2mim]OAc (corresponding to 50 mg birch bark in 950 mg solvent) and the solution was stirred at either reflux (MeOH, EtOH, chloroform) or at 100 °C in case of toluene and ionic liquid overnight.

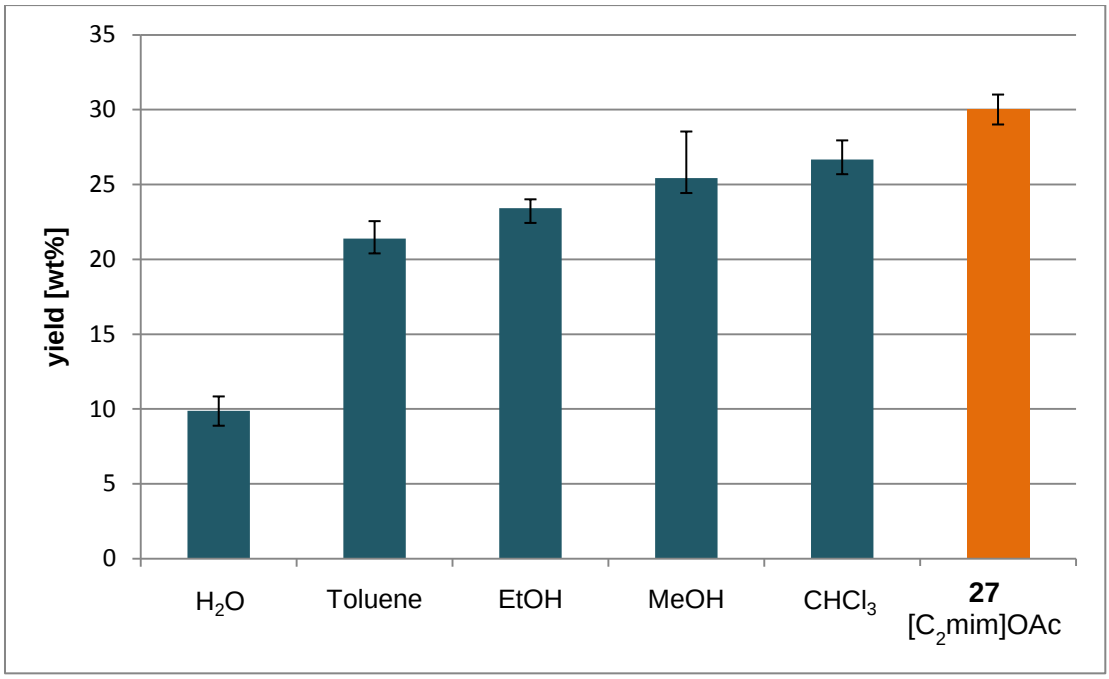


Figure 40: Conventional extraction, 5 wt% birch bark in solvent

The results showed that extraction with ionic liquid can significantly improve the extraction yield of Betulin compared to conventional solvents. While water showed only poor extraction yields of 10 wt%, toluene, chloroform, ethanol and methanol gave moderate to good yields between 22 wt% and 27 wt%. The best yield of 30 wt% was obtained using [C_2mim]OAc as solvent, which might be explained by the ability of ionic liquids of dissolving biomass: In the dissolution process, the cell structure of the lignocellulosic biomass is destroyed giving a better access to the active component. As a result more of the active ingredients located inside are available for in the extraction with ionic liquids (Figure 41).

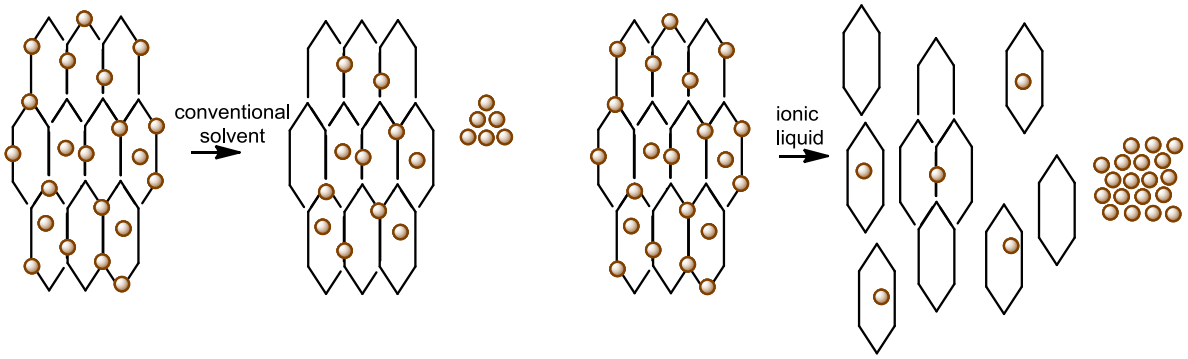


Figure 41: Extraction of Betulin with conventional solvent and ionic liquid

Subsequently, the volume of the conventional organic solvent was increased to see if the extraction yield also increases and can reach the result of the ionic liquid extraction. Figure 42 represents the results of 0.5 wt% solution of birch bark in conventional solvents compared to a 5 wt% solution of birch bark in ionic liquid. It could be shown that the amount of the volatile organic solvents has to be increased for 10 times to reach the same extraction efficiency as the ionic liquid. Even with this excess of solvent toluene and chloroform gave lower yields than the ionic liquid, whereas EtOH and MeOH gave comparable results.

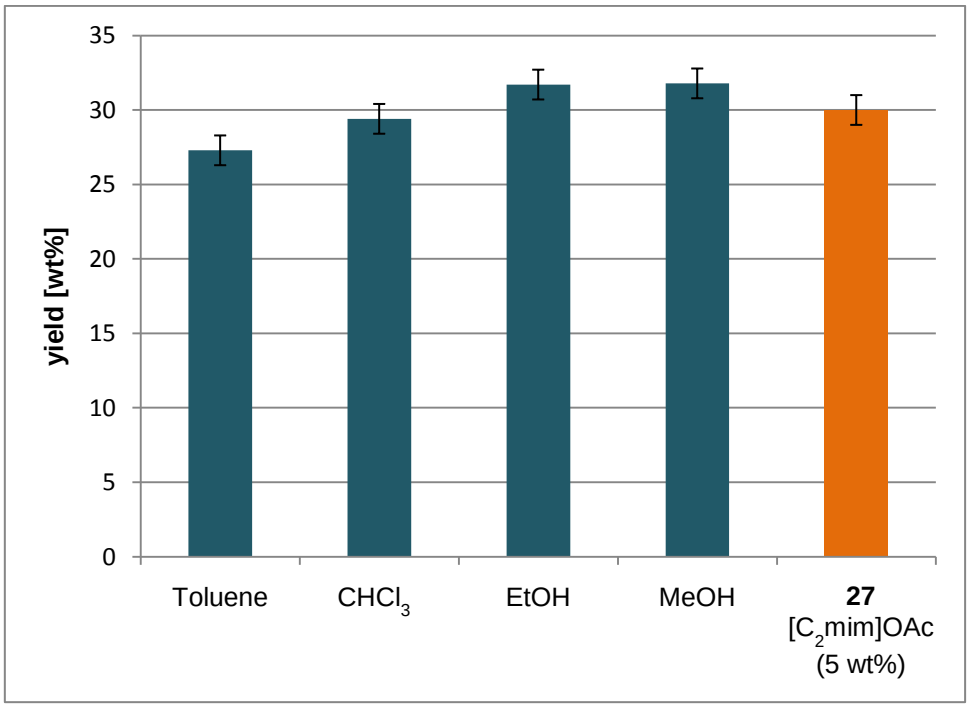


Figure 42: Conventional extraction, 0.5 wt% birch bark in solvent

4.2.1.3 Microscopy of biomass in ionic liquids

In order to get a closer look on biomass dissolution in ionic liquids, several samples were investigated under a light microscope. Different concentration of birch bark in [C_2mim]OAc ranging from 0.1 to 10 wt% were examined under the microscope to estimate the solubility of birch bark in ionic liquid. It was observed that the structure of birch bark was changing and seemed to become transparent under ionic liquid treatment. It seemed that biomass was swelling and mostly dissolved, particularly when low concentrations of birch bark of 1 wt% were used. However, it was not possible to quantify the exact solubility of biomass, which is probably caused by the inhomogeneous consistency of birch bark biomass.

Furthermore, a time screening showed changes in the biomass consistence over longer stirring, as can be seen in Figure 43.

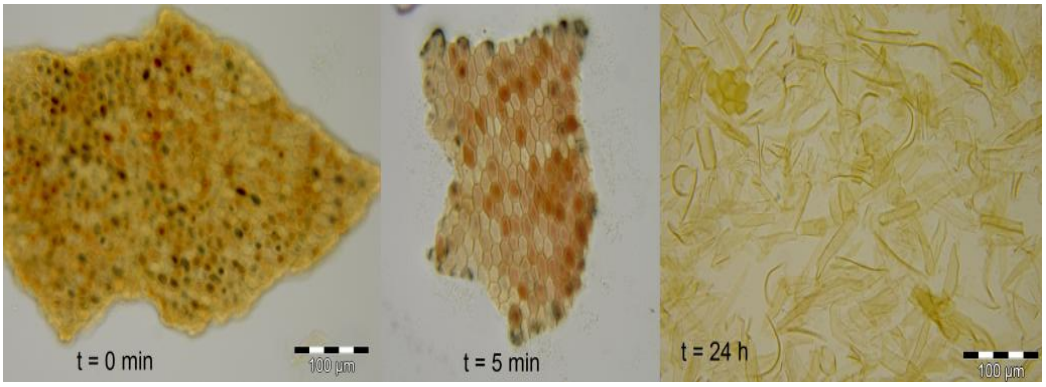


Figure 43: Microscopy of birch bark

4.2.1.4 Optimisation of extraction conditions

Since the initial extraction experiments and the comparison with conventional solvents gave promising results, different conditions such as time, concentrations of biomass in [C_2mim]OAc and temperatures were screened to identify the best extraction conditions. Variation of the extraction time showed that longer extraction times did not lead to better results. The yield increased from 28 wt% after 5 min to 30 wt% after 2 h and levels off to 30 wt% after 24 h. The same pattern was observed by Chowdhury and co-workers by extracting tannins using DIMCARB.[21] Due to the results presented above it was decided to work with 2 h as optimum extraction time.

Figure 44 shows that the yield increased with increasing temperature which might be explained by the lower viscosity of the biomass-ionic liquid mixtures at higher temperatures. Furthermore with increasing concentration of birch bark the yield also increased. The only exception is the value of 10 wt% at 25 °C. This again might be explained by the viscosity, since the sample with 10 wt% biomass in ionic liquid became almost unstirrable at room temperature.

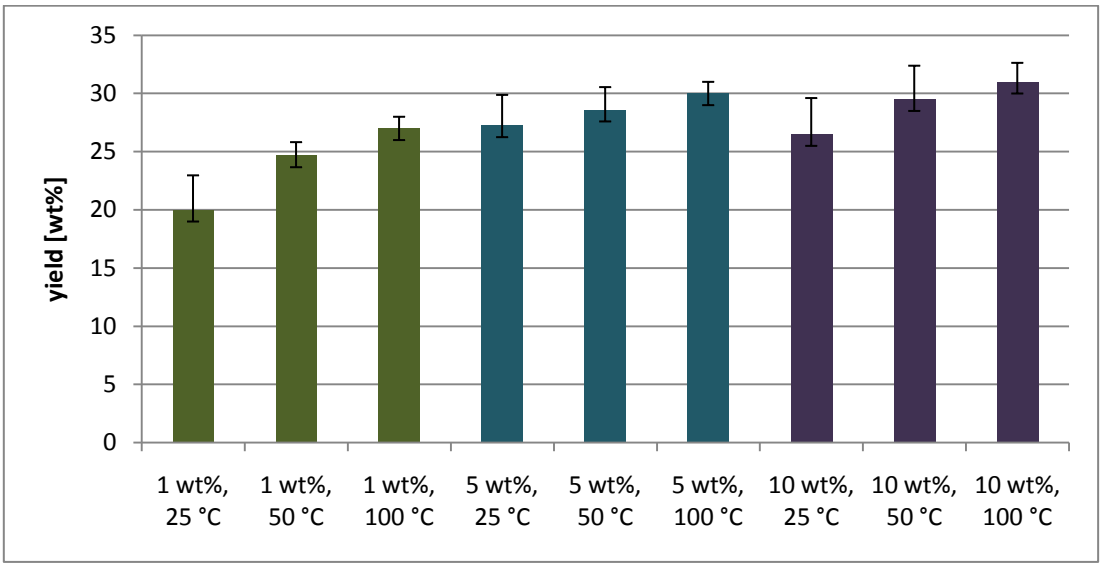


Figure 44: Extraction yield with different ratios of birch bark and [C_2mim]OAc

Microwave assisted extraction

According to literature extraction time of active ingredients from plant material can be shortened using microwave irradiation.[18] This can be a special advantage for the ionic liquid-based extraction of actives from biomass: Microwave energy is transferred *via* dipole rotation or ionic conduction, and thus ionic liquids absorb microwave irradiation very well due to their ionic character[54], suggesting that microwave irradiation might provide a clean and efficient alternative to conventional heating. As described in chapter 2.1.1.1 microwave assisted extraction is a suitable extraction process *e.g.* for the extraction of polyphenolic compounds from *Psidium Guajava* leaves and *Smilax china* tubers.

Figure 45 shows that the reaction time could indeed be shortened from 2 h to 15 min using microwaving irradiation at 100 °C with comparable yields. A similar pattern as described above was observed, and with increasing concentration of birch bark the yield increased as well.

[54] Hayes, B.L. *Microwave Synthesis*; CEM Publishing: Matthews, 2002; p 67.

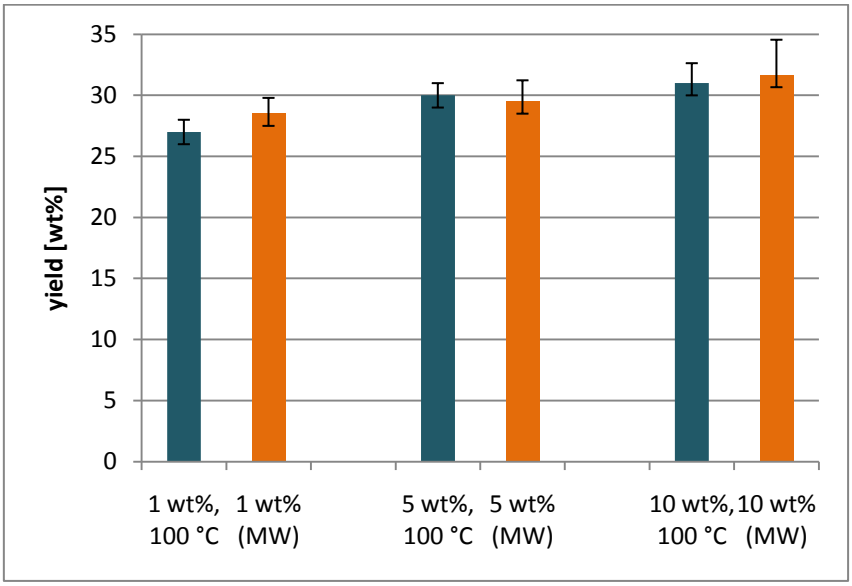


Figure 45: Microwave assisted extraction (orange) compared to conventional heating (blue) of birch bark using [C_2mim]OAc at 100 °C

4.2.1.5 Investigation of different ionic liquids

It was only recently that Sun *et al.* reported that the solubility of lignin in ILs is controlled by the anions and is increasing in the order PF_6^- <<< Br^- ≈ Cl^- < CH_3SO_4.[10] Since the initial extraction experiments of birch bark using [C_2mim]OAc gave promising results, it was decided to screen different anions in a 5 wt% solution of birch bark with different ionic liquids comprising the 1-ethyl-3-methylimidazolium cation but various anions. Although the 10 wt% solution of birch bark in [C_2mim]OAc gave slightly better yields, the 5 wt% solution was chosen due to the lower viscosity. Microwave irradiation for 15 minutes was chosen on the basis of its time efficiency.

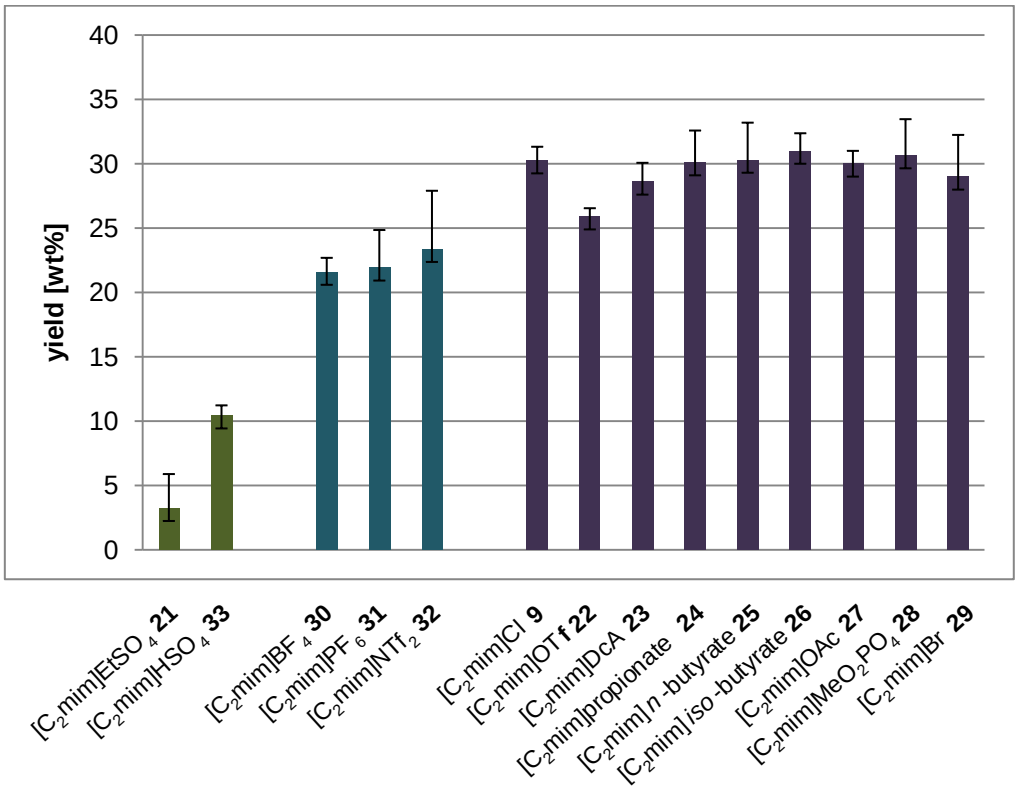


Figure 46: Ionic liquids based on [C_2mim]-cation with different anions

The results of the screening with different [C_2mim]-based ionic liquids showed that the Brønsted acidic and protic ionic liquid **33** and [C_2mim]$EtSO_4$ as an exception in the tested hydrophilic ionic liquids failed in extraction experiments (Figure 46). Moderate yields were obtained using hydrophobic ionic liquids including BF_4^- anion, PF_6^- anion and NTf_2^- anion which are comparable with those of conventional solvents. On the contrast, good to excellent yields were obtained with hydrophilic ionic liquids such as the halides [C_2mim]Br and [C_2mim]Cl, but also with [C_2mim]DCA, [C_2mim]MeO_2PO_4 and [C_2mim]OTf. Similarly, all carboxylate based ionic liquids such as [C_2mim]OAc, [C_2mim]propionate, [C_2mim]*n*-butyrate, [C_2mim]*iso*-butyrate performed well, and little influence of the chain length in the carboxylate anion was observed.

A similar pattern was observed by Zheng *et al*., who observed the highest yields for the extraction of rutin from herb samples using [C_4mim]Br and [C_4mim]TsO, whereas moderate yields were obtained with [C_4mim]BF_4.[19] The results with different anions shown in Figure 46 are also consistent with Sun's solubility study of lignin in ionic liquids that increased in the order PF_6^- <<< Br^- ≈ Cl^- < $CH_3SO_4^-$.[10] Furthermore it should be mentioned that the extraction was also performed with [C_4mim]Br and gave an excellent yield of 32 wt%.

Variation of cations

Since good extraction yields were obtained with the ionic liquid [C_2mim]Cl it was decided to further investigate the influence of the cation based on the chloride anion. (Figure 47)

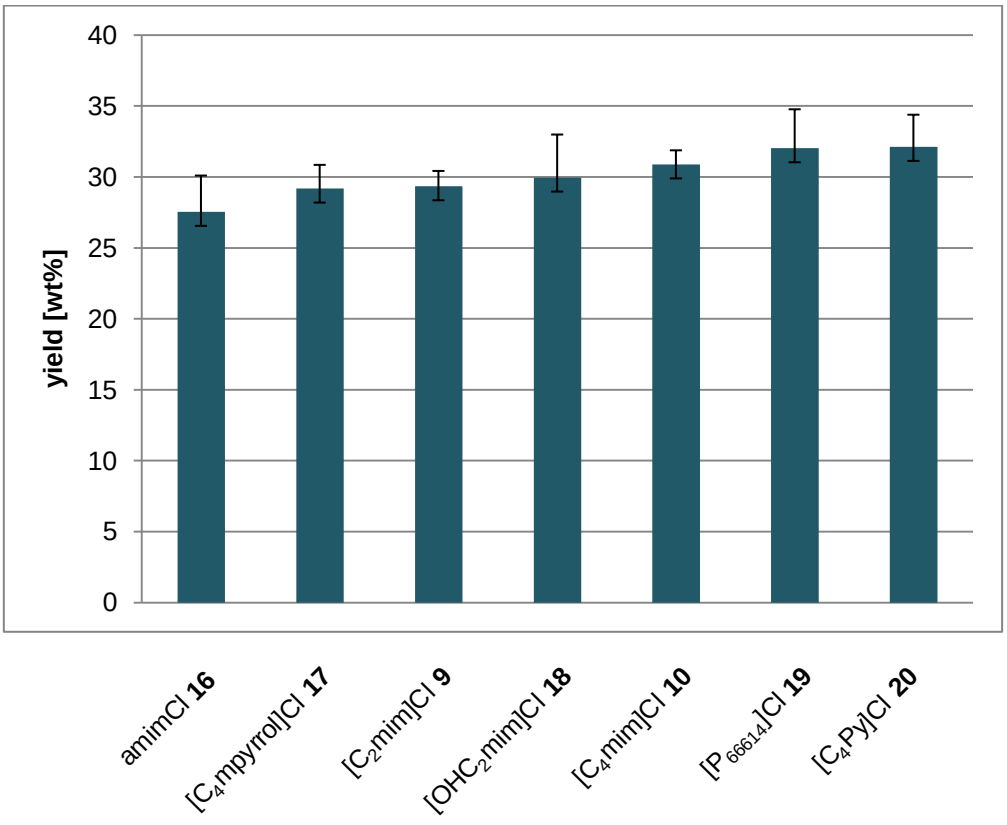


Figure 47: Variation of the cation

As already mentioned, the solubility of lignin was reported to depend on the anion rather than on the cation. When several chloride-based ionic liquids were screened in the isolation of Betulin from birch bark, little difference was observed, and all tested cations gave good to excellent yields. The best results were obtaining using the pyridinium-based ionic liquid, which is probably related to the aromatic character of the *N*-butylpyridinium cation that might lead to stronger solvation interactions and thus to a slightly better extraction compared to the other ionic liquids.[18]

Variation of chain length of cation

In the next step, the length of the alkyl side chain of the 3-alkyl-1-methylimidazolium cation was modified to further investigate the influence of the cation in the extraction process. Therefore ionic liquids [$C_n$mim]Cl with an alkyl side chain varying from n=2 to n=14 were used in extraction experiments and the results are shown in Figure 48.

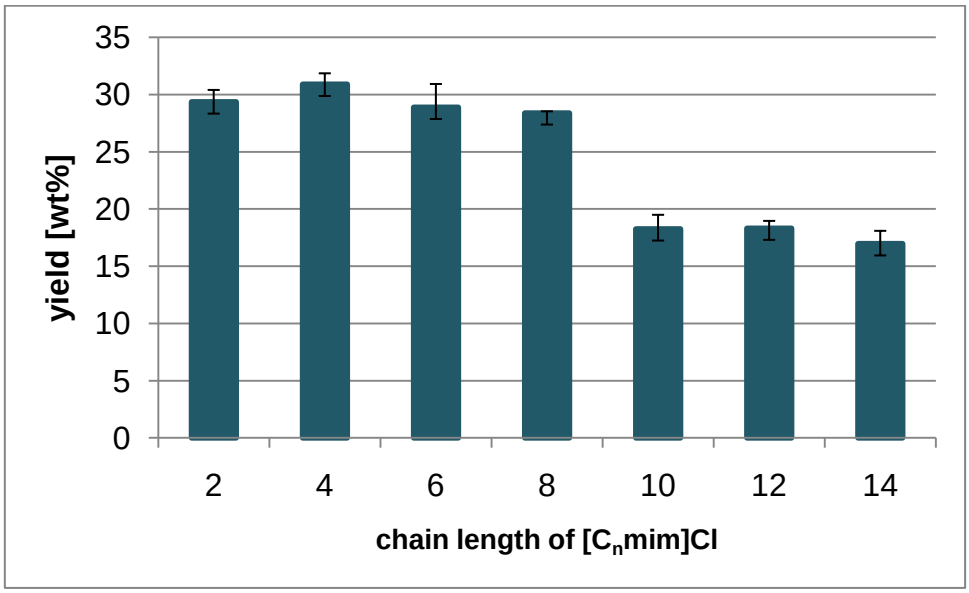


Figure 48: Variation of chain length of the cation of [$C_n$mim]Cl

It was found that a longer side chain results in a constant drop of yield, and thus ionic liquids containing a short side chain gave better extraction yields. This again shows the importance of hydrophilicity for the extraction of biomass, and is comparable with the pattern that was observed when testing different anions. With increasing chain length, the hydrophobicity of 1-alkyl-3-methylimidazolium chlorides is gradually increasing, hence a lower yield is obtained. The best results of 31 wt% extraction yield were obtained with the butyl side chain (n=4). This is again in accordance with results from Pinkert *et al.* who observed a similar influence using ionic liquids with a C_2 to C_{20} side chain of methylimidazolium cations for cellulose dissolution, and reported the best results for the butyl side chain.[9] Likewise, Du and co-workers observed similar results using [$C_n$mim]Br (n=2,4,6) for the extraction of polyphenolic compounds such as gallic acid, ellagic acid and quercetin from *Psidium Guajava* leaves and *Smilax china* tubers, and the best results were obtained with [C_4mim]Br.[18]

4.2.2 Isolation of Betulin

After the extraction conditions were optimized with excellent yields, it was decided to scale-up this ionic liquid-based strategy and to develop a process for the preparative isolation of Betulin from birch bark (Figure 49). The scale-up was done starting with 1 g of birch bark, and a 10 wt% solution of biomass in the ionic liquid [C_2mim]OAc was chosen to keep the amount of the expensive ionic liquid as low as possible. Furthermore, microwave irradiation was used to develop a rapid and efficient process.

Figure 49: Work procedure of the isolation of Betulin

In the initial dissolution process, a solution of birch bark (1 g) in [C_2mim]OAc (9 g, corresponding to a 10 wt% solution) was heated for 15 min at 100 °C using microwave irradiation. In the next step, EtOH or MeOH was added to the dark slurry. This allowed precipitating the undesired biopolymers, whereas Betulin stayed in solution. While both alcohols were suitable here, EtOH has the advantage of being pharmaceutically compatible compared to the toxic but cheaper MeOH. The biomass was removed *via* filtration, and Betulin was precipitated by adding a specific amount of water (Table 5).

After filtration Betulin was dried under reduced pressure and its purity was analyzed *via* HPLC and NMR. Furthermore, the remaining filtrate was evaporated to recover the ionic liquid.

Initially, a good isolation yield of 32 wt% and 39 wt% was obtained, but only the moderate purity of 59% and 67% was observed (Table 5, entry 1 and 2). However, we found that the addition of charcoal before the separation of the biomass greatly increased the purity of the isolated Betulin, and up to 90% purity according to HPLC could be obtained (entry 3 and 4). It should be noted that this is a remarkably high purity, particularly since only a simple precipitation step is involved in here.

Table 5: Isolation of Betulin and recovery of the ionic liquid

Entry[a]	Water [%]	Yield [mg]	Yield [wt%]	Purity [%][b]	Recovery of IL[%]
1[c]	50	324	32	59	n.a.
2[c]	30	386	39	67	n.a.
3	20	348	35	87	92
4	10	284	28	90	80
5	20	319	32	68	82[d]

[a] Conditions: 10 wt% solution (1 g birch bark in 9 g [C_2mim]OAc), MW, 100 °C, 15 min. [b] Purity determined by HPLC. [c] Performed without charcoal. [d] Recycled ionic liquid used.

Furthermore, it was found that the amount of water for the precipitation could be reduced from 50% to 10% (entries 1-4). This did not only lead to a higher purity of the isolated Betulin, but did also improve the recovery of the ionic liquid, since this allowed a faster and energy-saving azeotropic distillation of EtOH/water from the filtrate.

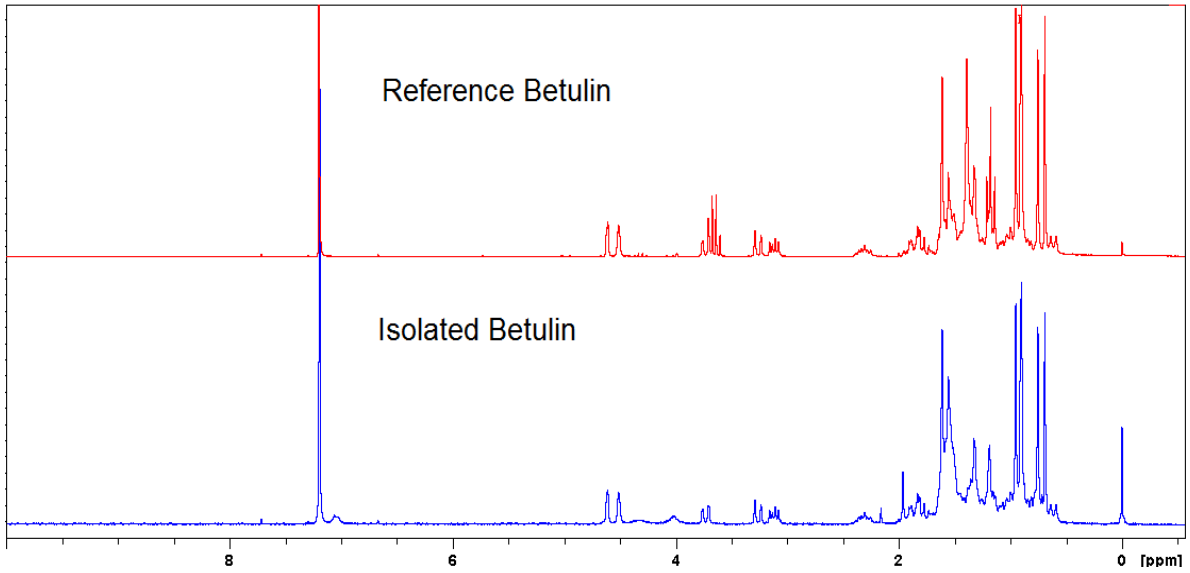


Figure 50: ^{1}H-NMR of the reference Betulin (top) and the isolated Betulin (bottom)

When comparing the ^{1}H NMR spectra of pure Betulin with the material obtained *via* precipitation from the ionic liquid solution, the structure of Betulin was confirmed, thus proving that no undesired reaction between ionic liquid and active ingredient took place (Figure 50). Furthermore, good purity was found for the crude Betulin in accordance with previous HPLC measurements that gave an average purity of 90%. It is also important to recognize that no traces of ionic liquid were detected in the spectra of the isolated Betulin, indicating a good separation and purity of the active ingredient. However, if necessary the obtained material could be easily purified *via* simple crystallization from ethanol.

The ionic liquid was recovered in good yields from 80-92% and could be used for a second isolation run with excellent yield but lower purity of the precipitated Betulin. The recovery of the ionic liquid after the second recycling circle was also successful, and 82% of [C_2mim]OAc could be recovered. When investigating the purity of the recovered ionic liquid *via* ^{1}H no impurities or remaining Betulin were detected, indicating that the recovered ionic liquid can indeed be reused without further purification (Figure 51).

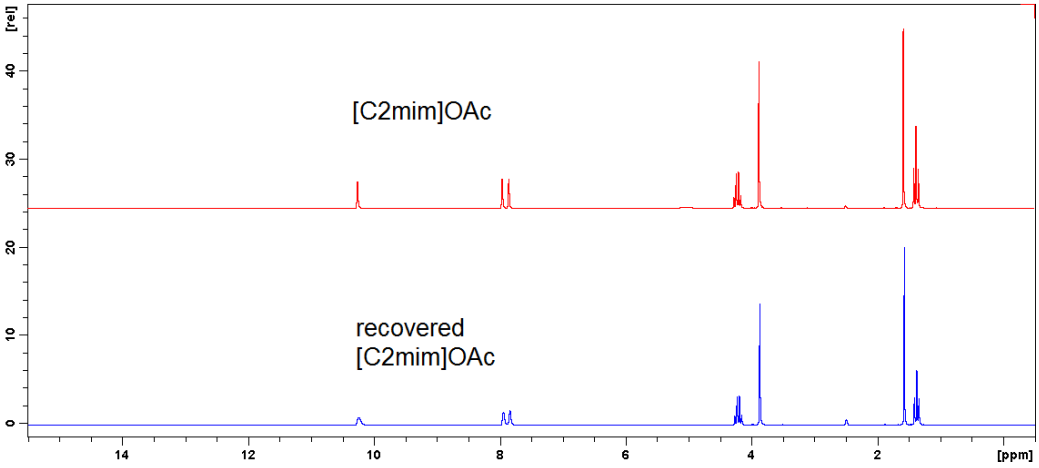


Figure 51: ^{1}H-NMR of commercial [C_2mim]OAc (top) and the recovered ionic liquid (bottom)

4.3 Reactive dissolution of Shikimic acid

Since the extraction of Betulin from birch bark with ionic liquids was very successful, it was decided to further investigate the reactive dissolution combining *in situ* extraction and reaction of an active ingredient from biomass towards a drug. Shikimic acid, which can be isolated form star anise, was chosen as active ingredient due to the fact that it is the major precursor for the production of the anti-influenza drug Tamiflu™. The first reaction step in the total synthesis of Tamiflu™ is the acid catalyzed formation of the ethyl ester followed by a ketalization step. As they are both acid catalyzed processes, it might be possible to perform both reactions *in situ* from biomass. Figure 52 shows the strategy for reactive dissolution of star anise using EtOH and an acidic ionic liquid as catalyst for the formation of Shikimic acid ethyl ester as well as the reactive dissolution towards the ketal intermediate using EtOH, 3-pentanone and a Brønsted acidic ionic liquid as reaction media and catalyst.

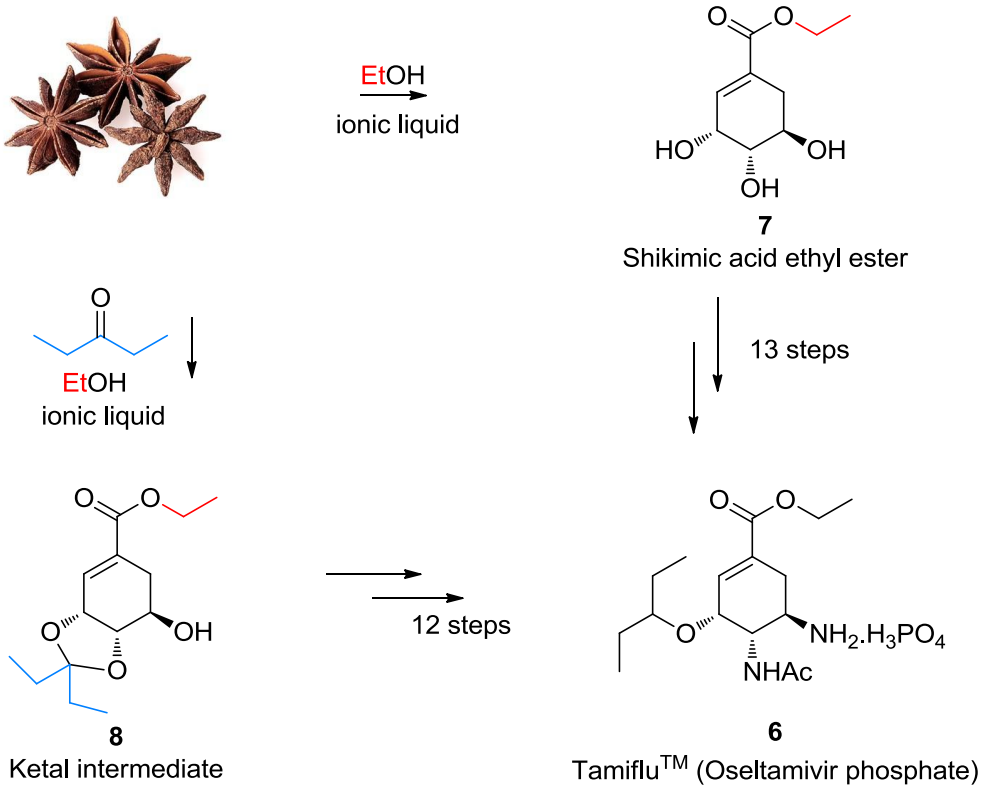


Figure 52: Strategy for the reactive dissolution using star anise powder

4.3.1 Esterification

The first step in the synthesis of Tamiflu™ is the esterification of the Shikimic acid **5** to obtain the Shikimic acid ethyl ester (**7**, (*3R,4S,5R*)-3,4,5-trihydroxycyclohex-1-ene-1-carboxylic acid, ethyl ester). Since this esterifaction is currently done with toxic and corrosive thionyl chloride, the reactive dissolution of star anise could avoid the use of toxic reagents and shorten the process. To develop an ionic liquid process, the esterification was initially performed with pure Shikimic acid using ionic liquid as catalyst only, and if the reaction is successful as well with star anise powder (Figure 53).

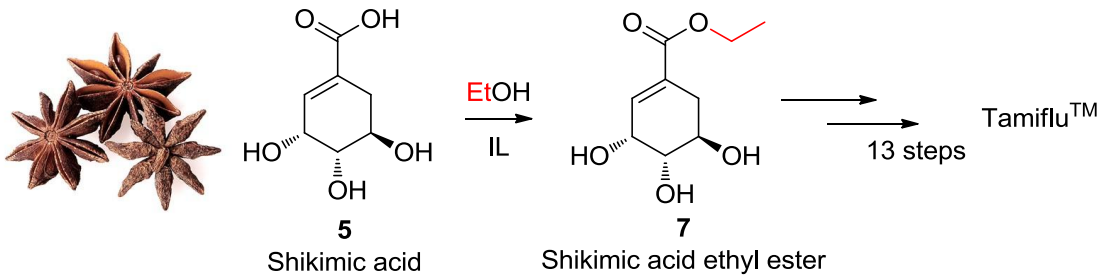


Figure 53: Esterification of Shikimic acid and star anise powder

4.3.2 Method for the detection of Shikimic acid and its derivatives

In order to quickly screen the extraction of the active ingredient Shikimic acid in the presence of ionic liquid, but also the conversion of Shikimic acid **5** towards Shikimic acid ethyl ester **7** or to ketal **8** in the reactive dissolution of biomass with catalytically active ionic liquids, an HPLC strategy was developed. In a similar manner as described in 4.2.1.1, calibration curves for Shikimic acid and Shikimic acid ethyl ester were prepared with a linearity range from 0.01 mg to 2 mg and excellent correlation coefficients R^2 > 0.999 using phenol as internal standard. Due to the high polarity of the analytes, an RHM-monosaccharide H^+ column was chosen and H_2O/5% trifluoroacetic acid was used as eluent.

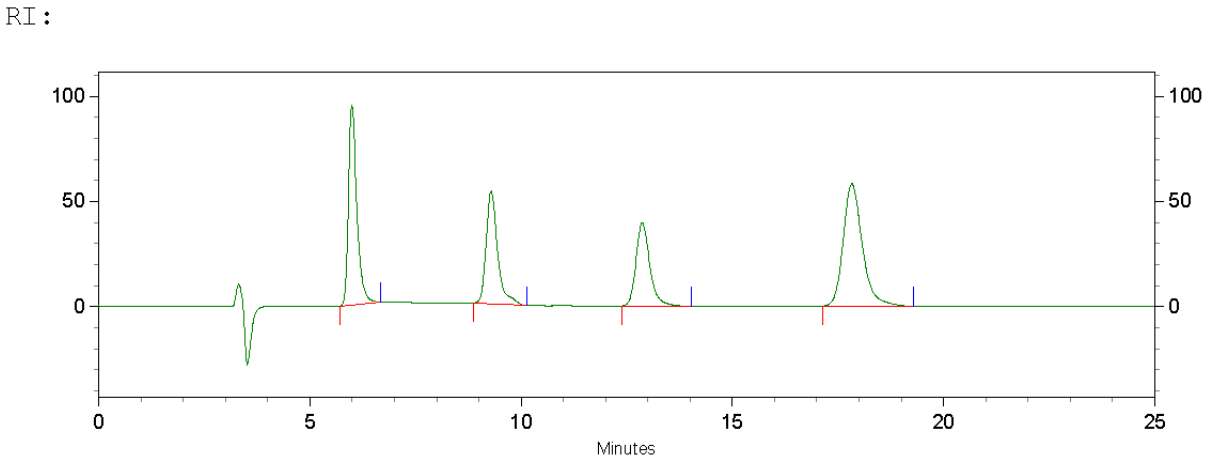


Figure 54: Chromatogramm of Shikimic acid, Shikimic acid ethyl ester, ionic liquids and phenol

With this chromatographic set-up, all compounds could be separated. In a typical chromatogram Shikimic acid **5** and Shikimic acid ethyl ester **7** were eluted after 6.0 min and 9.3 min, the ionic liquid $[HSO_3C_4mim]NTf_2$ **41** after 12.9 min and phenol as internal standard after 17.8 min. The compounds did not interfere with any by-products from lignocelluloses degradation allowing the direct determination of yield and conversion from the crude biomass extract.

Similarly, an HPLC strategy for the simultaneous detection of ketal intermediate, Shikimic acid, ionic liquid and biomass had to be developed. In contrast to Shikimic acid and Shikimic acid ethyl ester, the acid-labile ketal intermediate could not be analysed using this chromatographic set-up. For the determination of ketal intermediate **8**, a reversed phase column was used with CH_3CN and H_2O (50:50) as eluent leading to retention times of 6.0 min for the internal standard phenol and 14.3 min for the ketal.

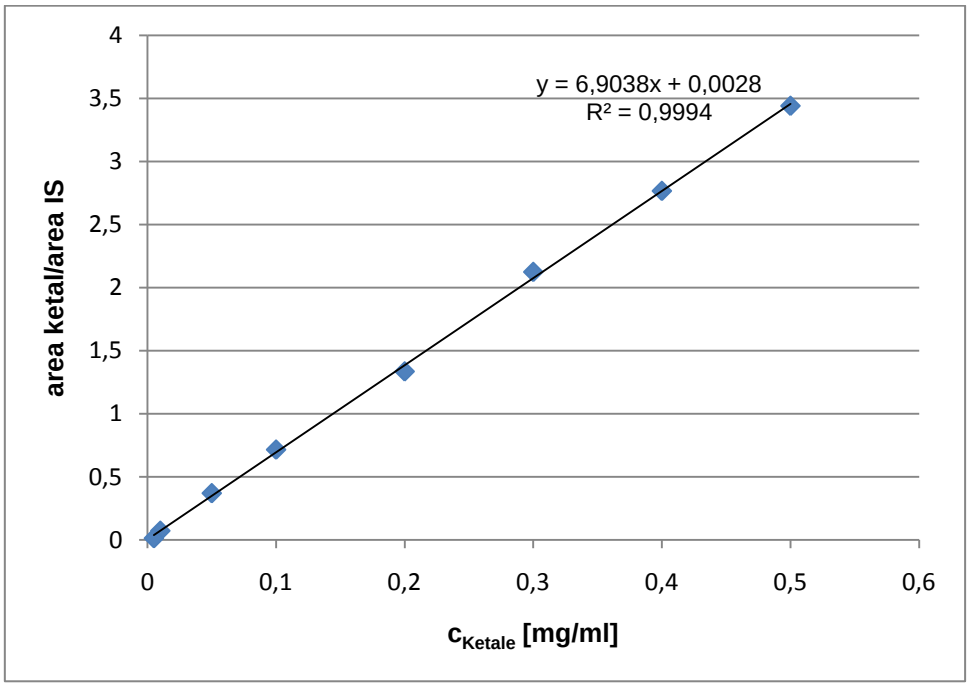


Figure 55: Standard calibration of the ketal intermediate

Figure 55 shows the calibration curve of the ketal intermediate using phenol as internal standard. It is linear in a range from 0.6 mg/ml - 0.01 mg/ml with an excellent correlation coefficient $R^2 > 0.999$.

4.3.2.1 Esterification of a carboxylic acid with an alcohol

Emil Fischer was the first to discover that treating a carboxylic acid with an excess of alcohol in the presence of an acidic catalyst results in an ester. Figure 56 shows the mechanism of the acid-catalyzed esterificaton of a carboxylic acid. A proton from an acid is added to the oxygen atom of the carboxyl group resulting in a by mesomerism stabilised cation. The nucleophilic oxygen of the alcohol attacks the positively charged carbon atom. Water is cleaved off and the ester is formed. The equilibrium can be shifted towards the ester by an excess of alcohol or by removing the produced water from the reaction.

In the production of Tamiflu™ (-)-Shikimic acid was first transferred into the ester under acidic conditions using H_2SO_4 (0.05 equiv) or TsOH (0.1 equiv) in boiling EtOH. As the reaction proceeded slowly and even after 20 h 5% starting material remained unreacted EtOH/$SOCl_2$ were used instead of sulfuric acid or *p*-toluenesulfonic acid. After 3 h only <2% of Shikimic acid was observed.[48]

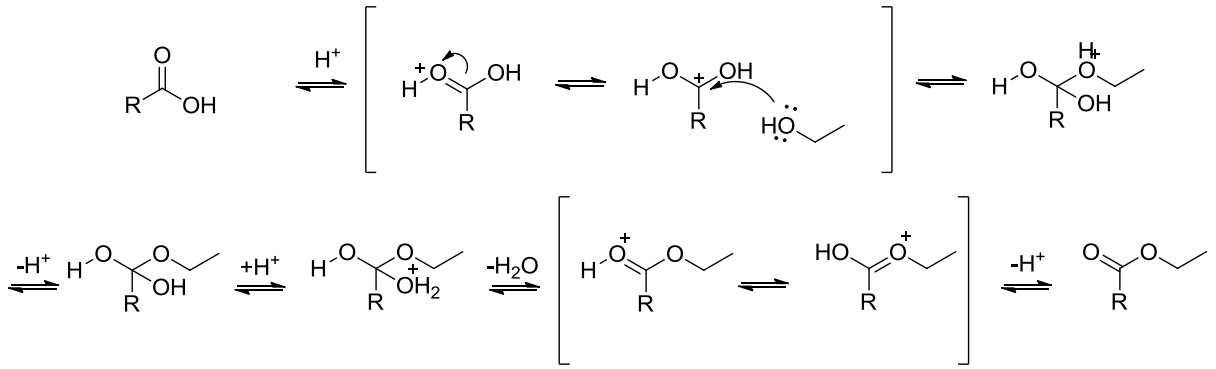


Figure 56: Mechanism of the esterification of a carboxylic acid[55]

This acid catalyzed esterification can be also done using acidic ionic liquids as catalysts, and Arfan *et al*. suggested a mechanism for the use of Brønsted acidic ionic liquids. The hydrogen sulphate counteranion of the ionic liquid initiates the esterification by donating a proton to the carboxylic acid. The carboxyl acid is further attacked by the hydroxyl group of the alcohol and water is eliminated. The hydrogen sulphate counterion is regenerated by the transfer of the proton and thus the ester is formed. In many cases, the ester can be separated from the ionic liquid by decantation.[56] Furthermore, Joseph *et al*. used the aprotic butylpyridinium chloride–aluminium(III) chloride for the esterification of alcohols and carboxylic acids with good to excellent conversion and selectivity, indicating that the proton is not necessary for this reaction (Figure 57).[57]

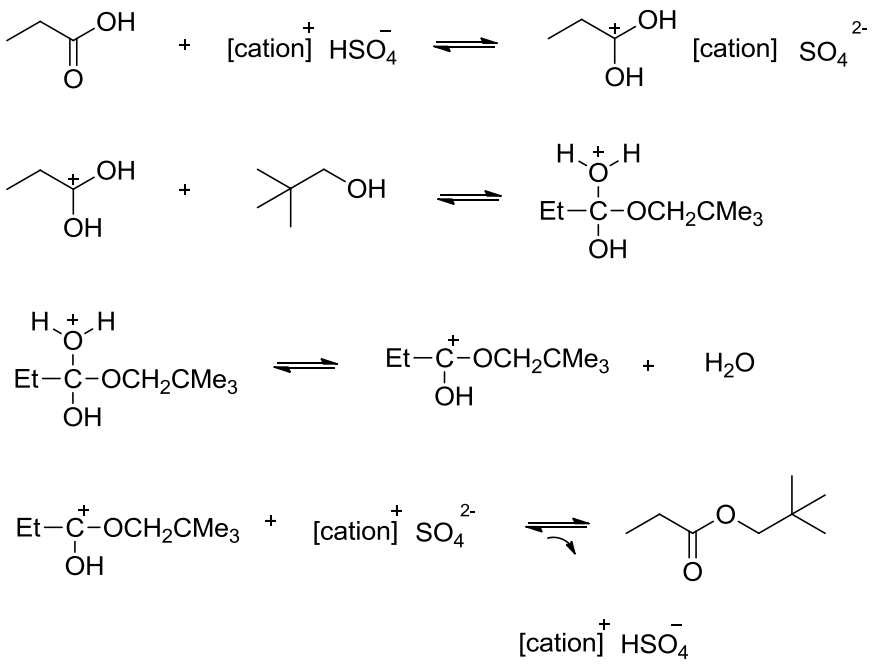


Figure 57: Esterification catalyzed by an ionic liquid

[55] Beyer, H.; Walter, W. *Lehrbuch der Organischen Chemie, 22nd ed.*;. S. Hirzelverlag: Stuttgart, 1991; p 259.
[56] Arfan, A.; Bazureau, J.P. *Org. Process Res. Dev*. **2005**, *9*,743.
[57] Joseph, T.; Sahoo, S.; Halligudi, S. B. *J. Mol. Catal., A: Chem.* **2005**, *234*, 107.

4.3.2.2 Esterification of Shikimic acid with ionic liquids

The esterification of Shikimic acid was performed using stoichiometric amounts of Broensted acidic ionic liquid as catalyst in an excess of anhydrous EtOH for 24 h at 80 °C. For comparison, the reaction was also performed using sulfuric acid or without a catalyst.

Table 6: Esterification of Shikimic acid

Entry	Catalyst	Conditions[a]	yield [%][b]
1	$[HSO_3C_4mim]NTf_2$ **41**	1 eq.	**99**
2	$[HSO_3C_4mim]HSO_4$ **40**	1 eq.	96
3	$[HSO_3C_4mim]HSO_4$ **40**	0.1 eq.	95
4	$[HSO_3C_4mim]HSO_4$ **40**	1 eq./MW[c]	88
5	$[HSO_3C_4mim]Cl$ **39**	1 eq.	81
6	$[HCim]HSO_4$ **36**	1 eq.	14
7	$[C_2mim]HSO_4$ **33**	1 eq.	3
8	H_2SO_4	1 eq.	83
9	without catalyst	-	0

[a] Performed with 1 mmol of Shikimic acid and 1 ml of anhydrous EtOH for 24 h at 80 °C.

Table 6 shows that sulfonic acid functionalized ionic liquids are suitable catalysts for the esterification of Shikimic acid. The dual acidic hydrogensulfate ionic liquid as well as the chloride salt and the bromide salt (84% yield)[45] showed good to excellent conversion to the ethyl ester, which can be explained by the acidity of these functionalized ionic liquids.[45] Recent UV-vis studies of Xu *et al*. showed that the acidity of $[HSO_3C_4mim]Y$ decreases in the order $Y = HSO_4^- > Br^- > Cl^-$.[58] Complete conversion was shown with the bistriflimide ionic liquid $[HSO_3C_4mim]NTf_2$ **41**. $[C_2mim]HSO_4$ **33** and the protic ionic liquid $[HCim]HSO_4$ **36** failed as a catalyst and only low conversion was observed. These results indicate that the sulfonic acid group in the side chain of the cation is responsible for the catalytic activity. A similar result was observed by Shen *et al.*, who investigated the Claisen-Schmidt condensations using Brønsted acidic ionic liquids as catalyst and reaction media and found that $-SO_3H$ functionalized ionic liquids gave higher yields than ionic liquids without the sulfonic acid group. Furthermore, they showed that anions of higher acid strength, such as BF_4^-, HSO_4^- or *p*-toluenesulfonate showed high catalytic activities with the same $–SO_3H$ functionalized cation. Changing the anion into $H_2PO_4^-$ – an anion of middle acid strength – catalytic activity was much lower.[33]

[58] Xu, D-Q.; Wu, J.; Luo, S-P.; Zhang, J-X.; Wu, J-Y.; Du, X-H.; Xu, Z. *Green Chem.* **2009**, *11*, 1239.

The reaction time could be tremendously shortened by microwave irradiation. Only 30 minutes of irradiation time were needed to obtain 88% conversion. Using stoichiometric amounts of concentrated sulfuric acid under conventional heating lower conversion (83%) was observed, and no conversion was observed in the absence of a catalyst.[45]

4.3.2.3 Esterification of star anise powder with ionic liquids

Once the esterification of pure Shikimic acid was succesfully performed, Shikimic acid was replaced by star anise powder. Initially, different ratios of biomass, EtOH and ionic liquid were tested to find the best conditions for the esterification of star anise powder. A 10 wt% solution of star anise powder was used in different ratios of ionic liquid and EtOH. (Figure 58)

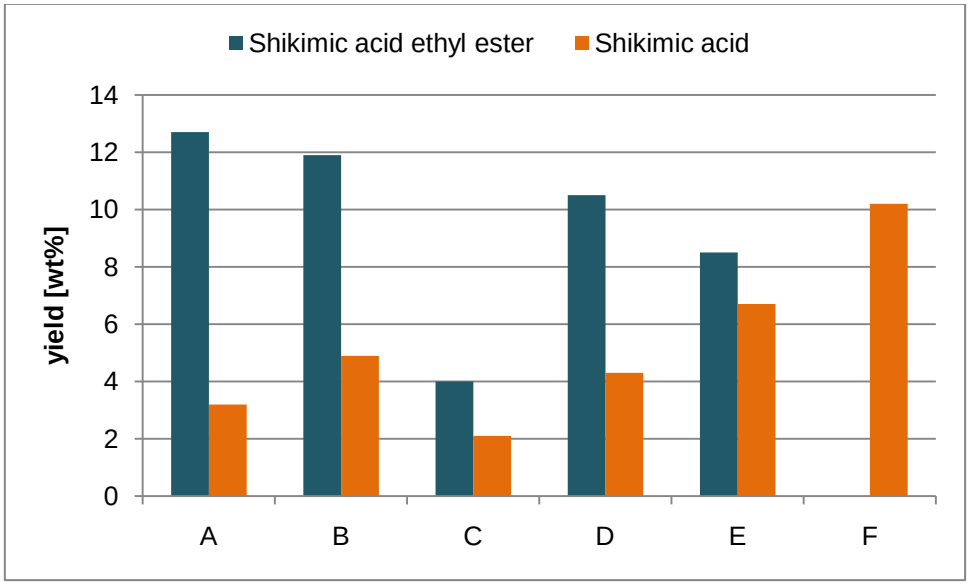


Figure 58: Esterification of star anise powder – screening

A: 100 mg ionic liquid **40**, 800 mg EtOH, 24 h, 80°C

B: 450 mg ionic liquid **40**, 450 mg EtOH, 24 h, 80 °C

C: 800 mg ionic liquid **40**, 100 mg EtOH, 24 h, 80 °C

D: 100 mg ionic liquid **40** 800 mg EtOH, MW, 30 min, 100 °C

E: 100 mg H_2SO_4, 800 mg EtOH, 24, 80 °C

F: 900 mg EtOH, 24 h, 80 °C

The best results were obtained with one mass-equivalent of ionic liquid and star anise powder (approximately 100 mg) and 800 mg of EtOH (corresponding to a 10 wt% solution of biomass in the ionic liquid/EtOH mixture), and Shikimic acid ethyl ester was obtained in 12.7% yield. The amount of ionic liquid was further increased to 450 mg ionic liquid and the mass of EtOH was reduced to 450 mg. This resulted in a higher total isolation yield of ethyl ester and Shikimic acid although the conversion to the ester was reduced, indicating that an

excess of ionic liquid leads to a better processing of biomass. When the amount of ionic liquid was further increased to 800 mg ionic liquid/100 mg EtOH, this resulted in a lower isolation and conversion yield. This might be explained by the increase of viscosity which made the sample almost unstirrable. Furthermore, EtOH acts as a co-solvent and thus better results were achieved when more EtOH was present. Again the reaction time could be shortened using microwave irradiation to 30 minutes and 100 °C, although a slight decrease in conversion and isolation yield was observed. In contrast to the ionic liquids sulfuric acid as a catalyst gave only moderate yields. When the reaction was performed without catalyst, no conversion to the ester was observed and only Shikimic acid was extracted.

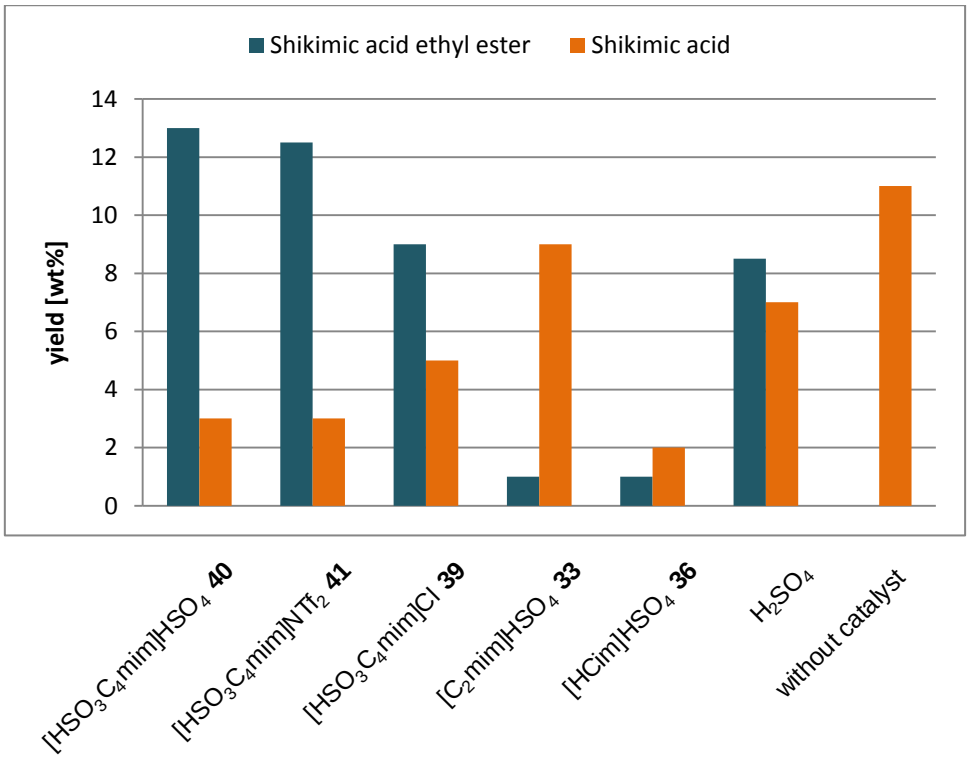


Figure 59: Esterification of star anise powder using differents catalysts

Figure 59 shows the results of different ionic liquids used as a catalyst for the esterification. The results were comparable with those obtained for pure Shikimic acid. Again, the ionic liquids $[HSO_3C_4mim]NTf_2$ and $[HSO_3C_4mim]HSO_4$ performed very well and gave yields over 12 wt%. Similarly, the hydrogen sulfate based ionic liquid **34** and the protic ionic liquid **36** did not catalyse the esterification and only poor yields of the ester were obtained.

4.3.3 *In situ* esterification and ketalization

Since the second step in the synthesis of Tamiflu™ after esterifaction is also an acid-catalyzed process, the intention was to perform both steps in a simple one-pot reaction. The *in situ* esterification and ketalization was initially performed on pure Shikimic acid and then transferred on star anise powder. (Figure 60)

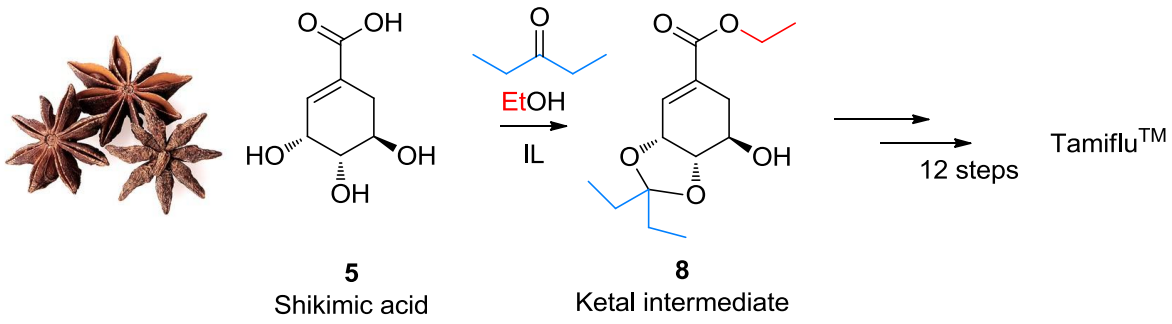


Figure 60: *In situ* esterification and ketalization of Shikimic acid/star anise

4.3.3.1 Mechanism of ketalization

Ketones and aldehydes react with 1,2-diols to form cyclic five-membered ketals or acetals, whereas six-membered rings are obtained with 1,3-diols. Ketals and acetals are used as protecting groups against bases, reducing and oxidizing agents and can be removed by acid-catalyzed hydrolysis. The ketalization of Shikimic acid ethyl ester is shown in Figure 61. The acid protonates the carbonyl oxygen of the ketone, resulting in higher susceptibility for nucleophilic attack. A hemiketal is formed after the loss of a proton. The oxygen atoms of the hemiketal are equally basic meaning that both can be protonated. In the next step water is eliminated, and the second alcohol group attacks the nucleophilic carbon atom. In the last step, the ketal is formed after a proton is lost.[59]

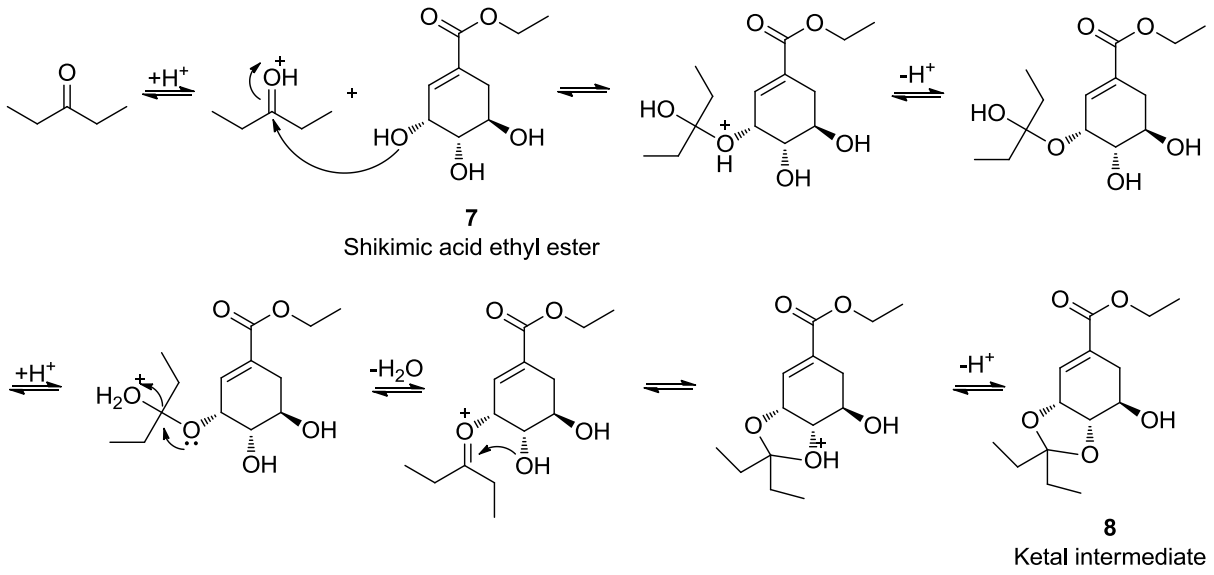


Figure 61: Mechanism of the ketalization of Shikimic acid

Although three hydroxy groups are available in Shikimic acid, the ketal is selectively formed between position 3 and 4. This can be explained by the *cis*-configuration of both hydroxy

[59] Bruice, P.Y. *Organic chemistry*, 5th ed.;,Pearson Education, Inc.: Upper Saddle River, 2007; pp 817-820.

groups that are oriented on the same side of the ring. Therefore ketalization can only occur between position 3 and 4, and a five-membered ring is formed.

In 2004, Li *et al.* presented the ionic liquid-catalyzed ketalization of cyclohexanone, benzaldehyde, propionaldehyde, and butyraldehyde with glycol using imidazolium-based ionic liquids with up to 99% conversions and >99% selectivity. They proposed a mechanism for the acetalization catalyzed by imidazolium-based ionic liquids. (Figure 62) The key factor in this reaction is the synergistic combination of the acidic group and the N^+ in the imidazolium cation.

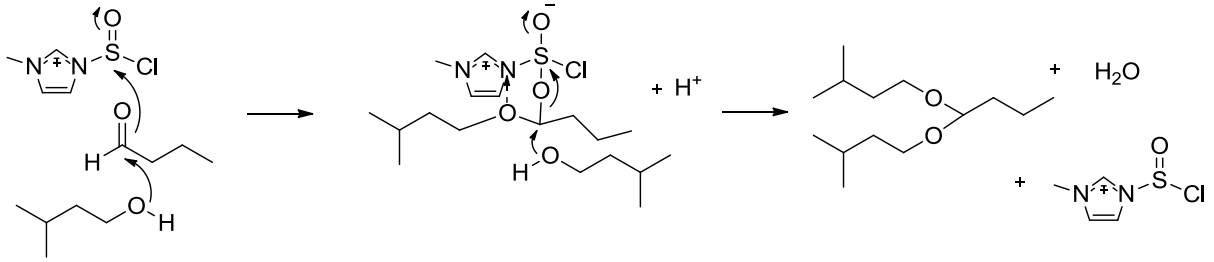


Figure 62: Mechanism of acetalization catalyzed by ILs[34]

4.3.3.2 *In situ* esterification and ketalization of Shikimic acid

The *in situ* esterification and ketalization was initially performed with 0.1 mmol of Shikimic acid and an equimolar amount of catalyst, 0.5 ml of anhydrous ethanol and 0.5 ml of 3-pentanone at 80 °C for 24 h. (Table 7) A similar pattern was found compared to the esterification of Shikimic acid with ionic liquids, and the best conversion was obtained with the ionic liquid $[HSO_3C_4mim]NTf_2^-$.**41**. The ketalization could be also performed when the amount of $[HSO_3C_4mim]HSO_4$ **40** was reduced to 0.1 equivalent, and the conversion was only slightly decreasing from 64% to 62%. Surprisingly, the reaction with concentrated sulfuric acid gave a better yield than in previous experiments. Again microwave irradiation shortened the reaction time from 24 h to 30 minutes, although a slightly lower conversion was observed.

Table 7: *In situ* esterification and ketalization of Shikimic acid

Entry	Catalyst	Eq.[a]	Ketal [mmol][b]	Yield [%]
1	$[HSO_3C_4mim]HSO_4$ **40**	1	0.064	64
2	$[HSO_3C_4mim]HSO_4$ **40**	0.1	0.062	62
3	$[HSO_3C_4mim]HSO_4$ **40**	1, MW	0.048	48
4	$[HSO_3C_4mim]$ NTf_2 **41**	1	0.079	79
5	H_2SO_4	1	0.071	71
6	without catalyst	-	0	0

[a] Performed with 1 mmol of Shikimic acid, 0.5 ml of anhydrous EtOH and 0.5 ml 3-pentanone for 24 h at 80 °C. [b] Determined *via* HPLC analysis from the crude product.

4.3.3.3 *In situ* esterification and ketalization of star anise powder

Due to the successful *in situ* esterification and the previous ketalization of the pure Shikimic acid, it was decided to investigate the *in situ* ketalization of star anise powder. The reaction was scaled to 1 g star anise powder in a 10 wt% solution of catalyst, anhydrous EtOH and 3-pentanone for 24 h at 80 °C. (Table 8)

Table 8: *In situ* esterification and ketalization of star anise powder

Entry	Catalyst	IL [g]/EtOH [g]/3-pentanone[g][a]	Yield [wt%][b]	Isolated Yield [wt%][c]
1	$[HSO_3C_4mim]$ NTf_2 **41**	1/4/4	9.6	8.8
2	$[HSO_3C_4mim]HSO_4$ **40**	1/4/4	6.9	6.1
3	$[HSO_3C_4mim]HSO_4$ **40**	4.5/2.25/2.25	10.4	10.3
4	$[HSO_3C_4mim]HSO_4$ **40**	1/4/4 MW[d]	6.1	5.9
5	$[HCim]HSO_4$ **36**	1/4/4	0.5	n.d.
6	$[C_2mim]HSO_4$ **33**	1/4/4	<0.1	n.d.
7	H_2SO_4	1/4/4[e]	6.2	n.d.
8	without catalyst	0/4.5/4.5	<0.1	n.d.

[a] Performed using 1.00 ± 0.05 g star anise powder for 24 h at 80 °C. [b] Determined *via* HPLC analysis from the crude product. [c] Isolated yield after preparative HPLC. [d] Performed under microwave irradiation for 30 min at 100 °C. [e] Performed using 1.0 g of H_2SO_4 for 24 h at 80 °C.

In contrast to previous experiments the product was isolated after work-up and the yield was determined by HPLC (Table 8, yield) from the crude product. After the given reaction time, the reaction mixture was hydrolyzed with aqueous $NaHCO_3$ solution and remaining biomass was removed *via* filtration. The crude mixture was extracted with ethyl acetate and further treated with charcoal. This extraction process allowed not only to separate any unreacted

Shikimic acid, but could also remove water soluble degradation products such as sugars that are formed during the acid-catalyzed hydrolysis of lignocellulosic biomass.

Again, excellent catalytic activity of the sulfonic acid functionalized ionic liquids $[HSO_3C_4mim]HSO_4$ and $[HSO_3C_4mim]NTf_2$ was observed and up to 10.4% of the ketal could be isolated. In contrast to the esterification of star anise, an increase of ionic liquid led to a better yield of ketal **8**, and a maximum yield of 9.7% of ketal could be obtained using 4.5 g of ionic liquid $[HSO_3C_4mim]NTf_2$ as solvent and catalyst. Microwave irradiation could also shorten the reaction time to 30 minutes and yielded 6.1% of the ketal. Again, the ionic liquids $[HCim]HSO_4$ and $[C_2mim]HSO_4$ did not catalyse this reaction, and no conversion was observed without a catalyst.

In order to verify the results and to obtain a pure product, the ketal intermediate was further purified *via* preparative HPLC and the compound was isolated in up to 10.3 wt%. However it should be mentioned that this additional purification step is not necessary for further reaction, since the crude material was already isolated in excellent purity. Figure 63 shows a chromatogram of the crude ketal after isolation from the biomass *via* simple extraction, and an excellent purity of >90% was found in HPLC and GC-MS chromatography.

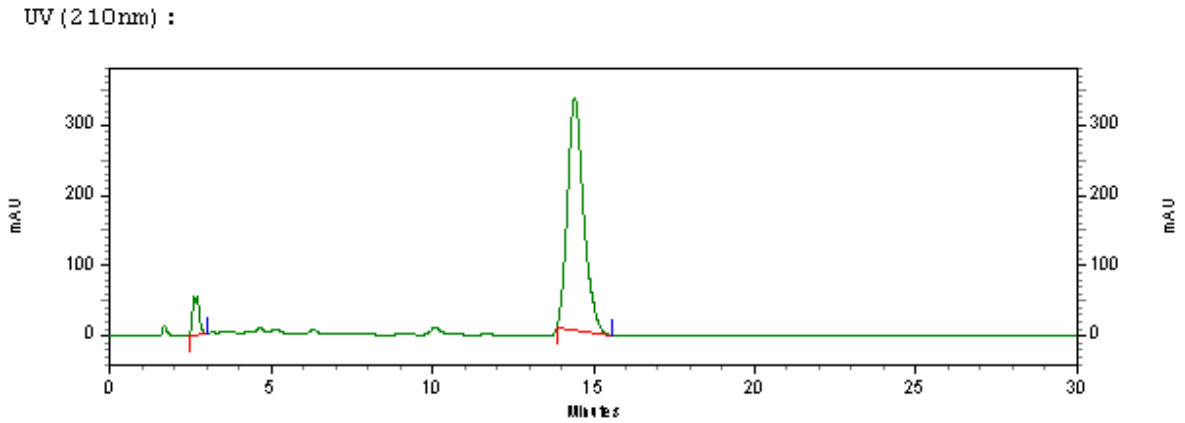


Figure 63: Chromatogram of the crude ketal intermediate after extraction

5 Summary

In the first part of the thesis, several ionic liquids based on 1-methylimidazole and 1-ethylimidazole **18**, **21**, **29**, **30** were synthesized *via* alkylation in quantitative yield. Additionally, Brønsted acidic ionic liquids **39**, **40**, **41** were synthesized in a two-step synthesis in excellent overall yield of >95%.

Once a set of different ionic liquids was prepared, the ionic liquid-assisted extraction of the active ingredient Betulin **1** from birch bark was investigated. An HPLC strategy was developed to screen different reaction conditions, and it was found that ionic liquids extract Betulin better from birch bark than conventional solvents. Consequently, a new isolation strategy of Betulin using the ionic liquid 1-ethyl-3-methylimidazolium acetate **27** was developed on large scale. Betulin could be isolated in up to 30 wt% yield with excellent purities of 90%, which is a considerable improvement to the current isolation strategies that involve volatile, hazardous organic solvents and long extraction times. Furthermore the ionic liquid could be easily recovered after the process and reused for another extraction.

In the third part of this project the reactive dissolution of star anise biomass towards the anti-influenza drug Tamiflu^{TM} **6** using Brønsted acidic ionic liquids as solvent and catalyst was developed. The ionic liquid was not only used as solvent and reaction media, but also as catalyst for the esterifaction and ketalization of the active ingredient Shikimic acid **5**. After reactive dissolution, the ketal intermediate **8** in the synthesis of Tamiflu^{TM} could be isolated from star anise powder in 10.4% yield and excellent purity of >95%. This process does not only eliminate the use of toxic and corrosive thionyl chloride, but simplifies the current industrial three-step process into a simple one-step procedure with improved yield.

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6 Experimental part

6.1 Materials and methods

Chemicals: Commercially available reagents and solvents were used as received from Sigma Aldrich unless otherwise specified. [C_2mim]OAc **27** was purchased from Iolitec (Heilbronn, Germany). Diethyl ether, light petrol (60-80 °C fraction), ethyl acetate and dichloromethane were distilled prior to use. Anhydrous THF and was predried over KOH and distilled from Na/benzophenone. *N*-Methylimidazole was distilled from KOH prior to use.

Ionic liquids **10**-**15**, **20**, **24**-**26** were prepared as previously reported and analytical data were in accordance with literature.

Biomass: Birch bark was harvested from a local birch tree in July 2010. The wooden parts were peeled of manually. The birch bark was cut in a conventional kitchen blender being frozen in liquid nitrogen first and sieved to obtain particles with < 1 mm particle size. The powder was dried in a vacuum drying oven at 60 °C/ 20 mbar for 48 h. Star anise powder was bought at a local market and used as received.

^{1}H and ^{13}C NMR spectra were recorded on a Bruker AC 200 at 200 and 50 MHz or on a Bruker AC 400 at 200 and 50 MHz, resp., using the solvent peak or TMS as reference. ^{13}C NMR spectra were run in proton-decoupled mode and multiplicities from DEPT were referred as s (singlet), d(doublet), t (triplet), q (quartet), quin (quintet), sext (sextet), sept (septet) and m (multiplet). d_3-Chloroform was filtered over basic Al_2O_3 for acid-sensitive compounds.

HPLC: HPLC analysis was performed on a Thermo Finnigan Surveyor chromatograph equipped with a PDA plus (190-360 nm) and refractive index (RI) detector.

For the determination of Betulin a Phenomenex Luna 10 µm C18 100A column (250 × 4.60 mm) was used with CH_3CN/H_2O 80/20 as solvent and a flow of 1 ml/min; detection was done at 210 nm. (*Method A*)

For analysis of Shikimic acid **5** and Shikimic acid ethyl ester **7**, a Phenomenex Resex RHM-monosaccharide H^+ column (150 × 7.80 mm) was used as stationary phase with H_2O/5% trifluoroacetic acid as solvent and a flow of 0.6 ml/min; detection was done *via* refractive index (*Method B*).

For the determination of ketal **8**, a Phenomenex Luna 10 µm C18(2) 100A column (250 × 4.60 mm) was used with CH_3CN/H_2O 50/50 as solvent and a flow of 1 ml/min; detection was done at 210 nm (*Method C*).

Preparative HPLC: Preparative HPLC was performed on a Shimadzu LC-8A 80 device with a SIL-10AP autosampler, SPD-20A dectector and FRC-10A fraction collector. For separation, a Phenomenex Luna 10 µm RP18(2) 100A (250 × 21.20 mm) was used with CH_3CN/H_2O 40/60 as solvent and a flow of 20 ml/min. The injection volume was 3 ml and the detection wavelength was 210 nm (*Method D*).

GC–MS: GC-MS analyses were conducted on a VOYAGER Quadrupol (Thermo Finnigan) directly interfaced to a GC 8000 TOP gas chromatograph using a BGB-5 (30 m × 0.32 mm i.d., 1.0 µm 5 film thickness) cross-bonded dimethyl polysiloxane capillary column. The oven program temperature was 80 °C (2 min)//10 °C/min//280 °C (3 min). Source and transfer line temperatures were set at 200 and 280 °C, resp.

Microwave assisted reactions: Microwave reactions were performed on a BIOTAGE Initiator^{TM} sixty microwave unit. The reported times are hold times.

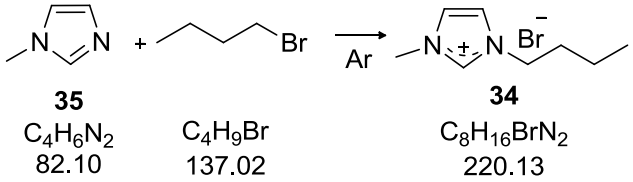
Microscopy: Microscopy was accomplished using an Orthoplan-Pol bright field transmission light microscope with a NPL Fluotar 16/0.45 objective and a Märzhäuser microscope stage. The pictures were taken and analysed with analySIS (Soft Imagine System) pro 5.0.

TLC: TLC analysis was done with precoated aluminium-backed plates (Silica gel 60 F254, Merck). Compounds were visualized by spraying with 5% phosphomolybdic acid hydrate in ethanol and heating.

6.2 Synthesis of ionic liquids

6.2.1 1-Methylimidazolium-based ionic liquids

6.2.1.1 1-Butyl-3-methylimidazolium bromide



35 $C_4H_6N_2$ 82.10 | C_4H_9Br 137.02 | **34** $C_8H_{16}BrN_2$ 220.13

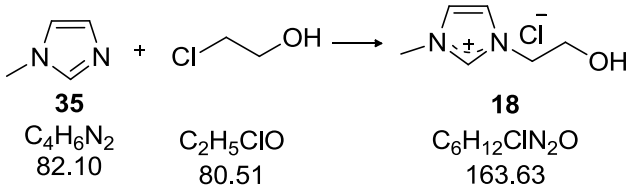
41.01	g	0.499	mol	1-Methylimidazole
87.04	g	0.635	mol	1-Brombutane

To 41 g of freshly distilled 1-methylimidazole 87 g of 1-brombutane were added. The mixture was refluxed at 50 °C for 96 h under argon until NMR control indicated complete conversion. After cooling to RT the solution was washed with ethyl acetate (3 × 10 ml) and dried *in vacuo* ($1 \cdot 10^{-2}$ mbar) at 80 °C for 24 h. A dark yellow oil was obtained in 99.7% yield.[60]

^{1}H-NMR (200 MHz, $CDCl_3$): δ_H = 0.88 (3H, t, J=0.59, N-CH_2-$(CH_2)_3$-$\underline{CH_3}$), 1.33 (2H, sext, J=6.06, N-CH_2-CH_2-$\underline{CH_2}$-CH_3), 1.84 (2H, quin, J=3.86, N-CH_2-$\underline{CH_2}$-CH_2-CH_3), 4.06 (3H, s, N$\underline{CH_3}$), 4.27 (2H, t, J=0.32 Hz, N-$\underline{CH_2}$-$(CH_2)_2$-CH_3), 7.48 (1H, t, J=1.76 Hz, H-4), 7.64 (1H, t, J=1.76 Hz, H-5), 10.29 (1H, s, H-2).

Analytical data were in accordance with literature values.[61]

6.2.1.2 1-(2-Hydroxyethyl)-3-methylimidazolium chloride



35 $C_4H_6N_2$ 82.10 | C_2H_5ClO 80.51 | **18** $C_6H_{12}ClN_2O$ 163.63

10.02	g	0.122	mol	1-Methylimidazole
10.80	g	0.134	mol	2-Chloroethanol

Chloroethanol was added dropwise under stirring to freshly distilled 1-methylimidazole. The mixture was stirred at 80 °C for 96 h to obtain a white solid. The solid was washed with ethyl

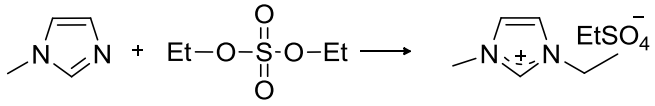
[60] Changzhi L; Qian W; Zongbao K.Z, *Green Chem.* **2007**, *10*, 180.
[61] Obermayer, D.; Kappe, C.O. *Org. Biomol. Chem.* **2010**, *8*, 120.

acetate (2 × 30 ml) and with 30 ml of Et_2O. Drying *in vacuo* ($1 \cdot 10^{-2}$ mbar) at 80 °C for 24 h yielded compound **18** as white solid in 99%.[62]

^{1}H-NMR (200 MHz, d_6-DMSO): δ_H = 2.83 (2H, q, J=5.09 Hz, -CH_2-$\underline{CH_2}$-OH), 2.98 (3H, s, -CH_3), 3.34 (2H, t, J=5.08, -$\underline{CH_2}$-CH_2-OH), 4.47 (1H, s, H-2), 6.85 (2H, m, H-4, H-5), 8.31 (1H, s, OH).

Analytical data were in accordance with literature values.[63]

6.2.1.3 1-Ethyl-3-methyl imidazolium ethylsulfate



35 $C_4H_6N_2$ 82.10 | $C_4H_{10}O_4S$ 154.18 | **21** $C_8H_{17}N_2O_4S$ 237.30

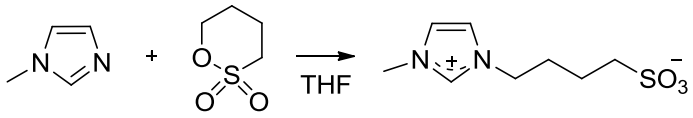
7.51	g	0.0915	mol	1-Methylimidazole
13	ml	0.0995	mol	Diethylsulfate

Freshly distilled diethylsulfate (15 mbar, 98 °C) was slowly added to freshly distilled 1-methylimidazole under cooling in an ice/water bath. The mixture was stirred for 96 h at 50 °C. After drying *in vacuo* ($1 \cdot 10^{-2}$ mbar) at 80 °C for 24 h compound **21** was obtained as colourless liquid in 99% yield.[64]

^{1}H-NMR (200 MHz, D_2O): δ_H = 1.28 (3H, t, J=7.14, $\underline{CH_3}$-CH_2-OSO_3), 1.48 (3H, t, J=7.43, -CH_2-$\underline{CH_3}$), 3.87 (3H, s, -CH_3), 4.08 (2H, q, J=7.17, CH_3-$\underline{CH_2}$-OSO_3), 4.21 (2H, q, J=7.37, -$\underline{CH_2}$-CH_3), 7.40 (1H, s, H-5), 7.47 (1H, s, H-4), 8.68 (1H, s, H-2).

Analytical data were in accordance with literature values.[65]

6.2.1.4 4-(1-Methylimidazolium-3-yl)butane-1-sulfonate



35 $C_4H_6N_2$ 82.10 | **37** $C_4H_8O_3S$ 136.17 | **38** $C_8H_{14}N_2O_3S$ 218.27

[62] Fraga-Dubreuil, J.; Bazureau, J.P. *Tetrahedron Lett*. **2001**, *42,* 6098.
[63] Juarez, R.; Martın, R.; Alvaro, M.; Garcia, H. *Appl. Catal, A*, **2009**, *369*, 134.
[64] Blesic M.; Swadzba-Kwasny,M.; Belhocine T.; Gunaratne, H.Q.N.; Canongia Lopes J.N.; Gomes, M.F.; Padua A.A.H.; Seddon K.R.; Rebelo L.P.N., *Phys. Chem. Chem. Phys*. **2009**, *11*, 8939
[65] Gomez, E.; Gonzales, B.; Calvar, N.; Tojo, E.; Dominguez, A.; *J. Chem. Eng. Data* **2006,** *51,* 2096.

20.01	g	0.244	mol	1-Methylimidazole
33.55	g	0.246	mol	1,4-Butansulton
100	ml			anhydrous THF

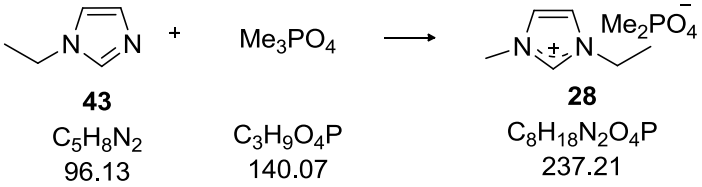
Freshly distilled 1-methylimidazole was dissolved in anhydrous THF and 1,4-butansulton was slowly added. The solution was refluxed at 70 °C for 48 h. The white precipitate was collected via filtration and washed with diethylether. The white solid was dried under reduced pressure at 30 °C/20 mbar for 24 h and obtained in >99% yield.[30]

^{1}H-NMR (200 MHz, D_2O): δ_H = 1.73 (2H, m, -CH_2-$\underline{CH_2}$-CH_2-CH_2-SO_3), 2.01 (2H, m, -CH_2-CH_2-$\underline{CH_2}$-CH_2-SO_3), 2.94 (2H, t, J=7.63 Hz, -$\underline{CH_2}$-CH_2-CH_2-CH_2-SO_3), 3.89 (3H, s, -CH_3), 4.24 (2H, t, J=6.94, -CH_2-CH_2-CH_2-$\underline{CH_2}$-SO_3), 7.43 (1H, m, H-4), 7.49 (1H, m, H-5), 8.74 (1H, s, H-2).

Analytical data were in accordance with literature values.[66]

6.2.2 1-Ethylimidazolium based ionic liquids

6.2.2.1 1-Ethyl-3-methylimidazolium dimethylphosphate



30.01	g	0.312	mol	1-Ethylimidazole
38.9	ml	0.332	mol	Trimethylphosphat

Trimethylphosphate (38 ml) was added dropwise to 1-ethylimidazol under argon atmosphere. The mixture was heated to 80 °C and stirred for 24 h. As NMR did not indicate complete conversion, another 0.9 ml of trimethylphosphate were added and stirring was continued for 72 hours. The mixture was washed with ethyl acetate (3 × 50 ml). The solvent was evaporated to dryness and the residue was dried *in vacuo* ($1 \cdot 10^{-2}$ mbar) at 80 °C for 24 h. An orange oil was obtained in 99%).[67]

^{1}H-NMR (200 MHz, $CDCl_3$): δ_H = 1.52 (3H, t, J=4.21, N-CH_2-$\underline{CH_3}$), 3.53 (6H, d, J=10.56 Hz, P(O$\underline{CH_3}$)$_2$), 4.00 (3H, s, N$\underline{CH_3}$), 4.30 (2H, q, J=2.48 Hz, N-$\underline{CH_2}$-CH_3), 7.38 (2H, m, H-4, H-5), 10.50 (1H, s, H-2).

Analytical data were in accordance with literature values.[67]

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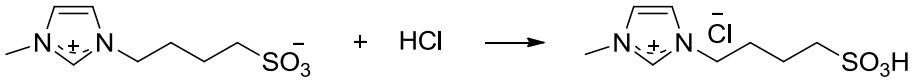
[66] Harjani, J.R.; Farrell, J.; Garcia, M.T.; Singer R.D.; Scammells, P.J. Green Chem. **2009**, *11*, 821.
[67] Kuhlmann, E.; Himmler, S.; Giebelhaus, H.; Wasserscheid, P. *Green Chem*. **2007**, *9*, 233.

6.2.3 Brønsted acidic ionic liquids

6.2.3.1 General procedure

To 1 eq. of zwitterion **38** 0.99 eq. of the protonation reagent which was dissolved in water or ethanol were added. The clear solution was stirred for 15 min and the solution was diluted with EtOH. and the solvent was evaporated. Remaining volatile material was removed under reduced pressure ($1 \cdot 10^{-2}$ mbar) with stirring at 80 °C for 24 h.

6.2.3.2 3-Methyl-1-(4-sulfobutyl)imidazolium chloride



38
$C_8H_{15}N_2O_3S$
219.28

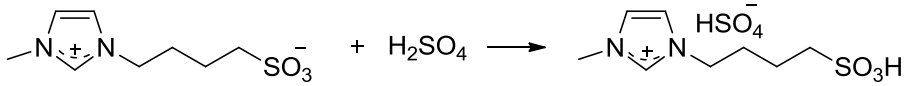
39
$C_8H_{15}ClN_2O_3S$
254.73

3.51	g	16.1	mmol	4-(1-Methylimidazolium-3-yl)butane-1-sulfonate **38**
1.60	g	15.9	mmol	Hydrochloric acid (36%)
15	ml			Water dest.

Preparation according to 6.2.3.1 from compound **38** and HCl gave compound **39** as a colourless oil in 97% yield.[58]

^{1}H-NMR (200 MHz, D_2O): δ_H = 1.71 (2H, m, -CH_2-$\underline{CH_2}$-CH_2-CH_2-SO_3), 1.99 (2H, m, -CH_2-CH_2-$\underline{CH_2}$-CH_2-SO_3), 2.91 (2H, t, J=7.63 Hz, -$\underline{CH_2}$-CH_2-CH_2-CH_2-SO_3), 3.86 (3H, s, -CH_3), 4.22 (2H, t, J=6.94, -CH_2-CH_2-CH_2-$\underline{CH_2}$-SO_3), 7.41 (1H, m, H-4), 7.47 (1H, m, H-5), 8.71 (1H, s, H-2).

6.2.3.3 3-Methyl-1-(4-sulfobutyl)imidazolium hydrogensulfate



38
$C_8H_{15}N_2O_3S$
219.28

40
$C_8H_{16}N_2O_7S_2$
316.35

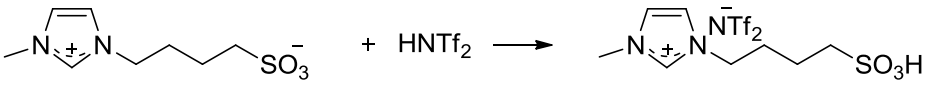
10.01	g	45.8	mmol	4-(1-Methylimidazolium-3-yl)butane-1-sulfonate **38**
4.95	g	45.5	mmol	Sulfuric acid (96%)
15	ml			Water dest.

Preparation according to 6.2.3.1 from compound **38** with H_2SO_4 gave compound **40** as a brown oil in 99% yield.[68]

^{1}H-NMR (200 MHz, D_2O): δ_H = 1.62 (2H, m, -CH_2-$\underline{CH_2}$-CH_2-CH_2-SO_3), 1.90 (2H, m, -CH_2-CH_2-$\underline{CH_2}$-CH_2-SO_3), 2.82 (2H, t, J=7.63 Hz, -$\underline{CH_2}$-CH_2-CH_2-CH_2-SO_3), 3.77 (3H, s, -CH_3), 4.12 (2H, t, J=7.04, -CH_2-CH_2-CH_2-$\underline{CH_2}$-SO_3), 7.31 (1H, m, H-4), 7.37 (1H, m, H-5), 8.61 (1H, s, H-2).

Analytical data were in accordance with literature values.[68]

6.2.3.4 3-Methyl-1-(4-sulfobutyl)imidazolium bis(trifluoromethansulfonyl)imide



38
$C_8H_{15}N_2O_3S$
219.28

41
$C_{10}H_{15}F_6N_3O_7S_3$
499.43

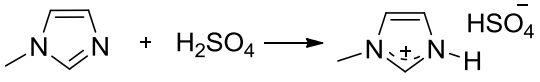
0.98	g	4.51	mmol	4-(1-Methylimidazolium-3-yl)butane-1-sulfonate **38**
1.25	g	4.45	mmol	Bis(trifluormethane)sulfonimide
10	ml			Ethanol

Preparation according to 6.2.3.1 from compound **38** and $HN(Tf)_2$ gave compound **41** in 10 ml Ethanol as a orange oil in 98% yield.[69]

^{1}H-NMR (200 MHz, D_2O): δ_H = 1.71 (2H, m, -CH_2-$\underline{CH_2}$-CH_2-CH_2-SO_3), 1.99 (2H, m, -CH_2-CH_2-$\underline{CH_2}$-CH_2-SO_3), 2.89 (2H, t, J=4.01 Hz, -$\underline{CH_2}$-CH_2-CH_2-CH_2-SO_3), 3.85 (3H, s, -CH_3), 4.21 (2H, t, J=0.68 Hz, -CH_2-CH_2-CH_2-$\underline{CH_2}$-SO_3), 7.40 (1H, s, H-4), 7.46 (1H, s, H-5), 8.71 (1H, s, H-2).

Analytical data were in accordance with literature values.[69]

6.2.3.5 1-H, 3-Methylimidazolium hydrogensulfate



35
$C_4H_6N_2$
82.10

36
$C_4H_9N_2O_4S$
181.19

[68] Gui, J.; Ban, H.; Cong, X.; Zhang, X.; Hu, Z.; Sun, Z. *J. Mol. Catal. A: Chem*. **2005**, *225*, 28.
[69] Ning Yan, N.; Yuan, Y.; Dykeman, R.; Kou, Y.; Dyson, P.J. *Angew. Chem. Int. Ed.* **2010**, *49*, 5551.

10.02	g	0.122	mol	1-Methylimidazole
12.35	g	0.121	mol	Sulfuric acid (96%)
15	ml			Water dest.

Freshly distilled 1-methylimidazole was added drop wise to a solution of sulfuric acid (12.3 g) in 15 ml of distilled water. The clear solution was stirred for 15 min at room temperature, and the solvent was evaporated. Remaining volatile material was removed under reduced pressure ($1 \cdot 10^{-2}$ mbar) with stirring at 80 °C for 24 h to yield ionic liquid **36** as coluorless oil in 95% yield.

^{1}H-NMR (200 MHz, D_2O): δ_H = 3.83 (3H, s, $-CH_3$), 7.34 (2H, s, H-4, H-5), 8.56 (1H, s, H-2).

6.3 Dissolution of biomass for the extraction of Betulin

6.3.1 Preparation of the standard calibration of Betulin

A stock solution of 20 mg Betulin in 10 ml methanol (solution A) was prepared. Solution B - H were prepared by diluting solution A. (see Table 9)

Table 9: Solutions for standard calibration

Solution	[ml]	solution	diluted to [ml]	Betulin [mg/ml]
A	-	-	-	2
B	1	A	2	1
C	0.5	A	2	0.5
D	1	A	10	0.2
E	1	B	10	0.1
F	1	C	10	0.05
G	1	D	10	0.02
H	1	E	10	0.01

A sample of 1 ml was taken from the solution and 0.2 ml of a 1-methyl-1-cyclohexene stock solution (50 mg in 100 ml methanol) were added. The samples were directly analysed *via* HPLC according to *Method A*.

6.3.2 Extraction of Betulin from birch bark

6.3.2.1 General extraction procedure using conventional solvents

Preparation of a 5 wt% solution:

A 5 ml screw-cap vial was charged with a 5 wt% solution of birch bark (0.0500 ± 0.0090 g) in solvent (0.950 g) and stirred at 100 °C for 2 h. The solution was diluted to 10 ml with the solvent used in extraction process.

A sample of 0.2 ml was taken from the solution, 0.8 ml acetonitrile and 0.2 ml of a 1-methyl-1-cyclohexene stock solution (50.0 mg in 100 ml methanol) were added. The samples were centrifuged for 10 min at 13000 min^{-1} and the supernatant was directly analysed *via* HPLC according to *Method A*.

Preparation of a 0.5 wt% solution:

A 5 ml screw-cap vial was charged with a 0.5 wt% solution of birch bark (0.0500 ± 0.0090 g) in solvent (9.950 g) and stirred at 100 °C for 2 h. The solution was diluted to 50 ml with the solvent used in extraction process.

A sample of 1 ml was taken from the solution and 0.2 ml of a 1-methyl-1-cyclohexene stock solution (50.0 mg in 100 ml methanol) was added. The samples were centrifuged for 10 min at 13000 min^{-1} and the supernatant was directly analysed *via* HPLC according to *Method A*.

6.3.2.2 General extraction procedure using ionic liquids under conventional heating

Variation of different parameters: Variation of extraction time:

A 5 ml screw-cap vial was charged with a 5 wt% solution of birch bark (0.0500 ± 0.0090 g) in ionic liquid (0.9500 ± 0.0150 g) and stirred at 100 °C for 5 min, 10 min, 15 min, 30 min, 1h, 2 h, 6 h, 12 h and 24 h. The solution was diluted to 50 ml with EtOH or MeOH.

A sample of 1 ml was taken from the solution and 0.2 ml of a 1-methyl-1-cyclohexene stock solution (50.0 mg in 100 ml methanol) were added. The samples were centrifuged for 10 min at 13000 min^{-1} and the supernatant was directly analysed *via* HPLC according to *Method A*.

Variation of different parameters: Variation of concentration of birch bark in ionic liquid and temperature:

A 5 ml screw-cap vial was charged with a 1,5 or 10 wt% solution of birch bark (0.0100 ± 0.0050 g, 0.0500 ± 0.0090 g, 0.1000 ± 0.0090 g) in ionic liquid (0.9900 ± 0.0150 g, 0.9500 ± 0.0150 g, 0.9000 ± 0150 g) and stirred at 25 °C, 50 °C or 100 °C for 2 h. The solution was diluted to 50 ml with EtOH or MeOH.

A sample of 1 ml was taken from the solution and 0.2 ml of a 1-methyl-1-cyclohexene stock solution (50.0 mg in 100 ml methanol) were added. The samples were centrifuged for 10 min at 13000 min^{-1} and the supernatant was directly analysed *via* HPLC according to *Method A*.

6.3.2.3 General extraction procedure using ionic liquids under Microwave irradiation

A 5 ml microwave vial was charged with a 1.5 or 10 wt% solution of birch bark (0.0100 ± 0.0050 g, 0.0500 ± 0.0090 g, 0.1000 ± 0.0090 g) in ionic liquid (0.9900 ± 0.0150 g, 0.9500 ± 0.0150 g, 0.9000 ± 0.0150 g) and sealed with a Teflon septum and heated for 15 min at 100

°C under microwave irradiation (high absorption level). The solution was diluted to 50 ml with EtOH or MeOH.

HPLC analysis was accomplished according to 6.3.2.2.

Screening of different ionic liquids: Variation of anions

A 5 ml microwave vial was charged with a 5 wt% solution of birch bark (0.0500 ± 0.0090 g) in ionic liquid (0.9500 ± 0.0150 g) and sealed with a Teflon septum and heated for 15 min at 100 °C under microwave irradiation (high absorption level). The solution was diluted to 50 ml with EtOH or MeOH.

HPLC analysis was accomplished according to 6.3.2.2.

Table 10: Different anions used for extraction

- **9** $[C_2mim]Cl$
- **10** $[C_4mim]Cl$
- **22** $[C_2mim]OTf$
- **23** $[C_2mim]DCA$
- **24** $[C_2mim]$propionate
- **25** $[C_2mim]$*n*-butyrate
- **26** $[C_2mim]$*iso*-butyrate
- **27** $[C_2mim]OAc$
- **28** $[C_2mim]MeO_2PO_4$
- **29** $[C_2mim]Br$
- **30** $[C_2mim]BF_4$
- **31** $[C_2mim]PF_6$
- **32** $[C_2mim]NTf_2$
- **33** $[C_2mim]HSO_4$
- **34** $[C_4mim]Br$

Screening of different ionic liquids: Variation of cations

Preparation was accomplished with **9** $[C_2mim]Cl$, **10** $[C_4mim]Cl$, **17** $[C_4mpyrrol]Cl$, **18** $[OHC_2mim]Cl$, **19** $[P_{6\,6\,6\,14}]Cl$ and **20** $[C_4Py]Cl$, according to 6.3.2.3.

HPLC analysis was accomplished according to 6.3.2.2.

Screening of different ionic liquids: Variation of chain length of cation

Screening was accomplished with **9** $[C_2mim]Cl$, **10** $[C_4mim]Cl$, **11** $[C_6mim]Cl$, **12** $[C_8mim]Cl$, **13** $[C_{10}mim]Cl$, **14** $[C_{12}mim]Cl$ and **15** $[C_{14}mim]Cl$ according to 6.3.2.2.

HPLC analysis was accomplished according to 6.3.2.2.

6.3.2.4 Microscopy

Different concentration of birch bark in ionic liquid: variation of the amount of birch bark and ionic liquid:

A 5 ml screw-cap vial was charged with birch bark and IL, stirred at 100 °C for 24 h (Table 11).

Table 11: Samples for microscopy (1)

Birch bark [mg]	[C_2mim]OAc [g]	Solution [wt%]
100.1	0.918	10
50.2	0.960	5
20.2	0.982	2
10.2	0.989	1
5.1	0.993	0.5
2.2	0.999	0.2
1.0	1.001	0.1

Samples were taken with a Pasteur pipette and examined under the microscope.

Different concentrations of birch bark in ionic liquid: variation of the ionic liquid

A 5 ml screw-cap vial was charged with birch bark and IL, stirred at 100 °C for 24 h (Table 12).

Table 12: Samples for the microscopy (2)

Birch bark [mg]	[C_2mim]OAc [g]	Solution [wt%]
10.5	0.090	10
10.2	0.186	5
9.7	0.486	2
9.8	0.989	1
9.9	1.996	0.5

Samples were taken with a Pasteur pipette and examined under the microscope.

Variation of different parameters: Time screening

A 5 ml screw-cap vial was charged with 0.0498 g birch bark and 0.9516 g [C_2mim]OAc and stirred at 100 °C. Samples were taken with a Pasteur pipette after 0 min, 1 min, 5 min,10 min, 15 min, 30 min and after 1 h, 2 h, 6 h, and 24 h and examined under the microscope.

6.3.3 Isolation of Betulin

6.3.3.1 General procedure

A 20 ml microwave vial was charged with 1.0000 ± 0.010 g birch bark and 9.0000 ± 0.0150 g [C_2mim]OAc, sealed with a Teflon septum and heated for 15 min at 100 °C under microwave irradiation (high absorption level). The dark slurry was diluted to 50 ml with EtOH and treated with charcoal. The suspension was stirred for 30 min and filtered over a batch of silica. The

solvent was evaporated of to a volume of 30 ml. Water was added and the solution was stored in a fridge for >6 h. The solution was filtered off and the solid material was dried *in vacuo* at 30 °C/20 mbar for 24 h to isolate crude Betulin as off-white solid in 32 wt% yield.

HPLC analysis:

An exact amount of dry Betulin <10 mg was dissolved in 10 ml acetonitrile. A sample of 1 ml was taken and 0.2 ml of a 1-methyl-1-cyclohexene stock solution (50 mg in 100 ml methanol) was added. The samples were directly analysed *via* HPLC according to *Method A*.

^{1}H-NMR (200 MHz, $CDCl_3$): δ_H = 0.65-2.05 (21H, m), 2.39 (1H, m),3.19 (1H, dd, J = 5.08 Hz), 3.34 (1H, s, J = 10.8 Hz), 3.80 (1H, d, J = 10.9 Hz), 4.59 (1H, s), 4.69 (1H, s).

Analytical data were in accordance with literature values.[70]

6.3.3.2 Recovery of ionic liquid

The clear filtrate obtained after isolation of Betulin in 7.3.3.1 was filtered and the solvent was evaporated to dryness. The remaining ionic liquid was dried *in vacuo* ($1 \cdot 10^{-2}$ mbar) at 80 °C for 24 h.

^{1}H-NMR (200 MHz, d_6-DMSO): δ_H = 1.37 (3H, t, J = 7.24 Hz, $-CH_2-\underline{CH_3}$), 1.57 (3H, s, $CO\underline{CH_3}$), 3.87 (3H, s, $-CH_3$), 4.21 (2H, q, J = 7.24 Hz, $-\underline{CH_2}-CH_3$), 7.84 (1H, s, H-4), 7.94 (1H, s, H-5), 10.24 (1H, s, H-2).

Analytical data were in accordance with the commercially available ionic liquid.

6.4 Reactive dissolution: Shikimic acid

6.4.1 Preparation of standard calibration for Shikimic acid derivates

6.4.1.1 Standard calibration – Shikimic acid

The standard calibration of Shikimic acid was accomplished according to 6.3.1.

6.4.1.2 Standard calibration – Shikimic acid ethyl ester

The standard calibration of Shikimic acid ethyl ester was accomplished according to 6.3.1.

6.4.1.3 Standard calibration – Ketal intermediate

The standard calibration of Shikimic acid ethyl ester was accomplished according to 6.3.1.

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6.4.2 Esterification of Shikimic acid

6.4.2.1 General procedure for the formation of (*3R,4S,5R*)-3,4,5-trihydroxycyclohex-1-ene-1-carboxylic acid, ethyl ester using ionic liquids

A 5 ml screw-cap vial was charged with Shikimic acid (17.4 mg, 0.1 mmol), 0.1-1 equivalents of ionic liquid **34**, **36**, **39** - **41** and 1 ml of anhydrous EtOH. The clear solution was heated with stirring for 24 h at 80 °C.

HPLC analysis:

The sample was diluted with water to 10.0 ml. A sample of 1.0 ml was taken and 0.2 ml of a phenol stock solution (250.0 mg in 100 ml H_2O) were added. The samples were centrifuged for 10 min at 13000 min^{-1} and the supernatant was directly analysed *via* HPLC according to *Method B*.

6.4.2.2 General procedure using sulfuric acid

A 5 ml screw-cap vial was charged with Shikimic acid (17.4 mg, 0.1 mmol), 1 equivalent (29.0 mg) of sulfuric acid (96%) and 1 ml of anhydrous EtOH. The clear solution was heated with stirring for 24 h at 80 °C.

HPLC analysis was accomplished according to 6.4.2.1.

6.4.3 Esterification of star anise powder

6.4.3.1 General procedure for the formation of (*3R,4S,5R*)-3,4,5-trihydroxycyclohex-1-ene-1-carboxylic acid, ethyl ester, different concentrations of ionic liquids

A 5 ml screw-cap vial was charged with 100.0 ± 9.0 mg star anise powder and a different concentration of ionic liquid **40** and anhydrous EtOH (see Table 13) and heated with stirring for 24 h at 80 °C.

Table 13: Different compositions for the formation of Shikimic acid ethyl ester

Entry	Star anise powder [mg]	IL [mg]	Anhydrous EtOH [mg]	Total [g]
1	100	100	800	1
2	100	450	450	1
3	100	800	100	1

HPLC analysis:

The sample was diluted with water to 50.0 ml. A sample of 1.0 ml was taken and 0.2 ml of a phenol stock solution (250.0 mg in 100 ml H_2O) was added. The samples were centrifuged for 10 min at 13000 min^{-1} and the supernatent was directly analysed *via* HPLC according to *Method B*.

6.4.3.2 General procedure of the esterification of star anise using ionic liquids

A 5 ml screw-cap vial was charged with 100.0 ± 9.0 mg star anise powder and 100.0 ± 9.0 mg ionic liquid **34**, **36**, **39** - **41** and 800 mg of anhydrous EtOH. The resulting suspension was heated with stirring for 24 h at 80 °C.

HPLC analysis was accomplished according to 6.4.3.1.

6.4.3.3 General procedure for the esterification of star anise using sulfuric acid

A 5 ml screw-cap vial was charged with 100.0 ± 9.0 mg star anise powder and 100.0 ± 9.0 mg of sulfuric acid (96%) and 800 mg of anhydrous EtOH. The resulting suspension as heated with stirring for 24 h at 80 °C.

HPLC analysis was accomplished according to 6.4.3.1.

6.4.3.4 General procedure for the microwave assisted esterification of star anise

A microwave vial (5 ml) was charged with 100.0 ± 9.0 mg of star anise powder, 100.0 ± 9.0 mg of 3-methyl-1-(4-sulfobutyl)imidazolium hydrogensulfate and 800 mg of anhydrous EtOH, sealed with a Teflon septum and heated for 30 min at 80 °C under microwave irradiation (high absorption level).

HPLC analysis was accomplished according to 6.4.3.1.

6.4.4 *In situ* esterification and ketalization of Shikimic acid

6.4.4.1 General procedure for the formation of (*3aR,7R,7aS*)-2,2-diethyl-*3a,6,7,7a*-tetrahydro-7-hydroxy-1,3-benzodioxole-5-carboxylic acid, ethyl ester

A 5 ml screw-cap vial was charged with Shikimic acid (17.4 mg, 0.1 mmol), 1 eq. of catalyst, 0.5 ml of anhydrous EtOH and 0.5 ml of 3-pentanone and heated with stirring for 24 h at 80 °C.

HPLC analysis: The sample was diluted with CH_3CN to 10.0 ml. A sample of 0.2 ml was taken, diluted with 0.8 ml of CH_3CN and 0.2 ml of a phenol stock solution (50.0 mg in 100 ml CH_3CN) was added. The samples were directly analysed *via* HPLC according to *Method C*.

6.4.4.2 General procedure for the microwave assisted esterification and ketalization of Shikimic acid

A 5 ml microwave vial was charged with Shikimic acid (17.4 mg, 0.1 mmol), 0.0342 g of $[HSO_3C_4mim]HSO_4$, 0.5 ml of anhydrous EtOH and 0.5 ml of 3-pentanone and heated for 30 min at 80 °C under microwave irradiation (high absorption level).

HPLC analysis was accomplished according to 6.4.4.1.

6.4.5 *In situ* esterification and ketalization of star anise powder

6.4.5.1 General procedure for the formation of (*3aR,7R,7aS*)-2,2-diethyl-*3a,6,7,7a*-tetrahydro-7-hydroxy-1,3-benzodioxole-5-carboxylic acid, ethyl ester

A large microwave vial (20 ml) with magnetic stirrer flee was charged with 1.000 ± 0.0015 g of star anise powder and different concentration of catalyst (ionic liquid **34**, **36**, **39** - **41** or sulfuric acid), EtOH and 3-pentanone. The flask was sealed with a Teflon septum and heated at 80 °C (oil bath temperature) with stirring overnight. The mixture was poured into saturated $NaHCO_3$ solution (30 ml), filtered and extracted with EtOAc. The combined organic layers were treated with charcoal, dried over Na_2SO_4, filtered over a batch of silica and evaporated to dryness. The light yellow oil was dried *in vacuo* ($1 \cdot 10^{-2}$ mbar) for 1 h.

HPLC analysis: The sample was diluted with CH_3CN to 50.0 ml. A sample of 0.2 ml was taken, diluted with 0.8 ml of CH_3CN and 0.2 ml of a phenol stock solution (50.0 mg in 100 ml CH_3CN) was added. The samples were directly analysed *via* HPLC according to *Method C.*

6.4.5.2 General procedure for the microwave assisted esterification and ketalization of star anise

A large microwave vial (20 ml) with magnetic stirrer flee was charged with 1.0000 ± 0.0100 g of star anise powder, 1.0 g of IL **40**, 4.0 g of EtOH and 4.0 g of 3-pentanone. The flask was sealed with a Teflon septum and heated for 30 min at 80 °C under microwave irradiation (high absorption level). The mixture was poured into saturated $NaHCO_3$ solution (30 ml), filtered and extracted with EtOAc. The combined organic layers were treated with charcoal, dried over Na_2SO_4, filtered over a batch of silica and evaporated to dryness. The light yellow oil was dried *in vacuo* ($1 \cdot 10^{-2}$ mbar) for 1 h.

HPLC analysis was accomplished according to 6.4.5.1.

The crude product was further purified *via* preparative HPLC according to *Method D* to give pure **8** as colourless oil.

^{1}H-NMR (200 MHz, $CDCl_3$): δ_H = 0.88 (6 H, q, J = 7.43 Hz), 1.28 (3H, t, J = 7.14 Hz), 1.65 (4H, 2q, J = 7.21 Hz), 2.22 (1H, m), 2.76 (1H, dd, J_1 = 17.61 Hz, J_2 = 4.11 Hz), 3.89 (1H, m), 4.09 (1H, t, J = 6.94 Hz), 4.20 (2H, q, J = 7.10 Hz), 4.75 (1H, m), 6.92 (1H, m).

^{13}C-NMR (100 MHz, $CDCl_3$): δ_H = 7.9 (q), 8.5 (q), 14.1 (q), 29.1 105 (t), 29.3 (t), 29.7 (t), 61.1 (t), 68.9 (d), 72.2 (d), 77.8 (d), 113.6 (s), 130.4 (s), 134.0 (s), 166.2 (s).

Analytical data were in accordance with literature.[50]

7 References

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8 Appendix

8.1 Abbreviations

[AcC_4im]Cl	1-butyl -3(carboxymethyl)-imidazolium chloride
[amim]Cl	1-allyl-3-methylimidazolium chloride
[C_{10}mim]Cl	1-decyl-3-methylimidazolium chloride
[C_{12}mim]Cl	1-dodecyl-3-methylimidazolium chloride
[C_{14}mim]Cl	1-methyl-3-tetradecylimidazolium chloride
[C_2mim]BF_4	1-ethyl-3-methylimidazolium tetrafluoroborate
[C_2mim]Br	1-ethyl-3-methylimidazolium bromide
[C_2mim]Cl	1-ethyl-3-methylimidazolium chloride
[C_2mim]DcA	1-ethyl-3-methylimidazolium dicyanamide
[C_2mim]$EtSO_4$	1-ethyl-3-methyl imidazolium ethylsulfate
[C_2mim]HSO_4	1-ethyl-3-methylimidazolium hydrogensulfate
[C_2mim]*iso*-butyrate	1-ethyl-3-methylimidazolium *iso*-butyrate
[C_2mim]*n*-butyrate	1-ethyl-3-methylimidazolium *n*-butyrate
[C_2mim]NTf_2	1-ethyl-3-methylimidazolium bis(trifluoromethansulfonyl)imide
[C_2mim]OAc	1-ethyl-3-methylimidazolium acetate
[C_2mim]OTf	1-ethyl-3-methylimidazolium triflate
[C_2mim]PF_6	1-ethyl-3-methylimidazolium hexafluorophosphate
[C_2mim]propionate	1-ethyl-3-methylimidazolium propionate
[C_4mim]Br	1-butyl-3-methylimidazolium bromide
[C_4mim]Cl	1-butyl-3-methylimidazolium chloride
[C_4mim]TsO	1-butyl-3-methylimidazolium tosylate
[C_4mpyrrol]Cl	1-butyl-1-methylpyrrolidinium chloride
[C_4Py]Cl	1-butylpyridinium chloride
[C_6mim]Cl	1-hexyl-3-methylimidazolium chloride
[C_8mim]Cl	1-methyl-3-octylimidazolium chloride

$[HCim]HSO_4$	1-H, 3-methylimidazolium hydrogensulfate
$[HSO_3C_4mim]Cl$	3-methyl-1-(4-sulfobutyl)imidazolium chloride
$[HSO_3C_4mim]HSO_4$	3-methyl-1-(4-sulfobutyl)imidazolium hydrogensulfate
$[HSO_3C_4mim]NTf_2$	3-methyl-1-(4-sulfobutyl)imidazolium bis(trifluoromethansulfonyl)imide
$[OHC_2mim]Cl$	1-(2-hydroxyethyl)-3-methylimidazolium chloride
$[P_{666\ 14}]Cl$	tetradecyltrihexylphosphonium chloride
$[SOClC_1Im]Cl$	1H-imidazolium, 1-(chlorosulfinyl)-3-methyl-, chloride
AIL	aprotic ionic liquid
BASIL™	Biphasic Acid Scavenging utilizing Ionic Liquids
C_4mimSO_3	4-(1-methylimidazolium-3-yl)butane-1-sulfonate
d	doublet
DIMCARB	*N*,*N*-dimethylammonium *N*'*N*'-dimethylcarbamate
DMSO	dimethylsulfoxide
Et_2O	diethylether
EtOH	ethanol
HIV	Human Immunodeficiency Virus
HPLC	high performance liquid chromatography
IL	ionic liquid
m	multiplet
MAE	Microwave-assisted extraction
MeOH	methanol
NMR	nuclear magnetic resonance
PIL	protic ionic liquid
q	quartet
quant	quantitative
quin	quintet
s	singlet
S. china	*Smilax china*

sept	septet
sext	sextet
SREBPs	Sterol regulatory element-binding proteins
t	triplet
TFA	trifluoroacetic acid
TFSI	bis(trifluoromethanesulfonyl)imide
THF	tetrahydrofurane
TLC	thin layer chormatography