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Use of solvents and environmental friendly materials for applications in Green Chemistry

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1. Introduction

In recent years Green Chemistry has become a research area of great interest. Modern lifestyle requires directly or indirectly many chemical substances, such as petrochemicals, polymers, pharmaceuticals, agrochemicals, detergents, cleaning and personal care products, paints and coatings, inorganic chemicals, and, more and more urgently, innovative materials; a large part of those consumer products, during the manufacturing, the use and the waste disposal, will be potentially released in the environment, implicating an inevitable (eco)-toxicological hazard for mankind and the environment. The increasing need to reduce pollution and its effects, and the consequent risk for the human health and the environment, has brought towards a new and safer approach to chemical processes and compounds, described in the "Twelve Principles" of Green Chemistry.

Green Chemistry is defined as "the design, manufacture and application of chemical products and processes to reduce or to eliminate the use and generation of hazardous substances".[1] It means that Green Chemistry principles should be applied to all the aspects of the product life cycle, from its invention to the disposal, including the environmental fate after the use, because there is a direct link between the beginning of a chemical life cycle (synthesis) and the environmental problems due to its release after the use.

The risk related to a process is a crucial factor in Green Chemistry, being an indicator of the potential harm which can be done by the process itself. The risk associated with a toxic chemical is a function of hazard and exposure, according to the general equation:

$$\text{Risk} = \text{Hazard X Exposure}$$

Therefore, there are two ways to reduce the risk: *i*) limiting the exposure or *ii*) limiting the hazard.

The first one, currently followed by legislation, deals with the prevention of the exposure to toxic hazardous chemicals (downstream approach) after

[1] Anastas, P., Warner, J., 1998. Green Chemistry: Theory and Practice, Oxford University Press: New York.

their spreading out. The laws, such as the “Clean Air Act” (1970) or the “Pollution Prevention Act” (1990) in USA, are in general focused on the treatment or removal of pollution and have become known as “command and control” laws.

In many cases these laws fix limits to pollution with little concern to the technological issues useful to reach these goals, and, often, they do not take into account unlucky rare events, such as an accident in a plant, which can result in a significant increase of exposure. Moreover the “end of the pipe” strategies, such as scrubbers on smokestacks and catalytic converters on vehicles, are highly costly.

From these considerations it is clear that environmental issues and pollution prevention have to be faced above all through the second strategy indicated above, by limiting the hazard.

The term “hazard” includes not only physical hazards such as explosiveness, flammability, volatility, but also acute and chronic toxicity, carcinogenicity, ecological toxicity and biodegradability.

In the last years the hazard linked to chemical compounds and processes has become a more and more important social and political issue, requiring a comprehensive regulation. To improve the protection of human health and the environment from the risks that can be posed by chemicals, and to strengthen the former legislative framework on chemicals of the European Union, on 1st June 2007 the regulation REACH (Registration, Evaluation, Authorisation and Restriction of Chemicals) has been approved by the European Parliament. REACH focuses greater responsibility on industries to asses the risks that chemicals may pose to the health and the environment: all manufacturers and importers of chemicals must identify and manage risks linked to manufacturing and marketing, submitting a registration dossier to the European Chemical Agency (ECHA) for substances produced or imported in quantities of 1 tonne or more per year per company. In this way REACH regulation requires that substances of very high concern (new and existing) are ruled out or adequately controlled, progressively substituted by safer substances or technologies, or only used where there is an overall benefit for the society. REACH states also that manufacturers and importers must provide appropriate safety information about the risk to their

downstream users, for example through labelling system and Safety Data Sheets (SDS).

The REACH regulation and the “Twelve Principles” of Green Chemistry can be seen as new important alternative tools for the assessment of hazards of substances and they can act as a guideline to help minimising the hazard of a process, as well as increasing its overall sustainability.

The twelve principles are:

1. **Prevention**. It is better to prevent waste than to treat or clean up waste after it has been created.
2. **Atom Economy**. Synthetic methods should be designed to maximize the incorporation of all materials used in the process into the final product.
3. **Less hazardous chemical syntheses**. Wherever practicable, synthetic methods should be designed to use and generate substances that possess little or no toxicity to human health and the environment.
4. **Designing safer chemicals**. Chemical products should be designed to effect their desired function while minimizing their toxicity.
5. **Safer solvents and auxiliaries**. The use of auxiliary substances (e.g., solvents, separation agents, etc.) should be made unnecessary wherever possible and innocuous when used.
6. **Design for energy efficiency**. Energy requirements of chemical processes should be recognized for their environmental and economic impacts and should be minimized. If possible, synthetic methods should be conducted at ambient temperature and pressure.
7. **Use of renewable feedstock**. A raw material or feedstock should be renewable rather than depleting whenever technically and economically practicable.
8. **Reduce derivatives**. Unnecessary derivatisation (use of blocking groups, protection/ deprotection, temporary modification of physical/chemical processes) should be minimized or avoided if possible, because such steps require additional reagents and can generate waste.

9. **Catalysis**. Catalytic reagents (as selective as possible) are superior to stoichiometric reagents.
10. **Design for degradation**. Chemical products should be designed so that at the end of their function they break down into innocuous degradation products and do not persist in the environment.
11. **Real-time analysis for pollution prevention**. Analytical methodologies need to be further developed to allow for real-time, in-process monitoring and control prior to the formation of hazardous substances.
12. **Inherently safer chemistry for accident prevention**. Substances and the form of a substance used in a chemical process should be chosen to minimize the potential for chemical accidents, including releases, explosions, and fires.

This Thesis focuses mainly on the recommendation of principle number 5, considered from the point of view of the use of safer solvents, with a widening also on principles 4^{th}, 7^{th} and 9^{th}.

In a typical chemical process, solvents are used extensively for dissolving reactants, affecting chemical reactivity, extracting and washing products, separating mixtures, cleaning reaction apparatus. However the major part of the organic solvents currently in the industry, in spite of a large number of self-evident advantages, are characterised by several dangerous effects for the human health and the environment, which are at the base of the need underlined by the 5^{th} principle of Green Chemistry to replace these solvents with safer alternatives.

Many organic solvents are volatile organic compounds (VOCs), and it means that their high volatility, very useful for industrial applications, contributes both to increase the risks of fire and explosion, and to facilitate the release in the atmosphere in which these solvents can act as air pollutants causing ozone deplection, photochemical smog and global warming.

Moreover, many conventional solvents are highly toxic for human beings, animals and plants, and often their chronic toxicological properties are completely unknown. For example, benzene was widely employed as a

solvent, a hand-cleaner and even as an aftershave for decades before its carcinogenicity became appreciated.[2]

Precautions to minimise the effects of these solvents by improved recycling have limited success and can not avoid some losses into the environment. Moreover, the risk connected to potential accidents is still present. For these reasons the replacement of these hazardous compounds with innocuous substitutes, as the 5th principle of Green Chemistry requires, seems to be the only valid alternative for a sustainable use of solvents.

Two main routes towards green solvents have been developed: *i*) the substitution of petro-chemically fabricated solvents with "bio-solvents" from renewable resources, and *ii*) the substitution of hazardous solvents with ones that show better EHS (Environmental, Health and Safety) properties.

The first strategy relies on solvents produced from vegetable biomass such as ethanol by fermentation of sugar-containing feeds, starchy feed materials or lignocellulosic raw materials, leading to the reduction of fossil fuel CO_2 emissions to the atmosphere.

The second strategy is based both on the use of safe and innocuous organic solvents, as acetone and alcohols, and on new generation solvents, as ionic liquids and supercritical fluids.

The choice of the proper alternative should be focused on:[3]

- Workers safety, including carcinogenicity, mutagenicity, reproduction hazards, skin and respiration absorption/sensitisation, and toxicity.
- Process safety, including flammability, explosiveness, potential for emissions through high vapour pressure, static charge, potential for peroxide formation and odour issues.
- Environmental safety and safety for population, including eco-toxicity, persistence, ground water contamination, ozone depletion potential, photo-reactive potential, global warming potential.

[2] Harremoe¨s, P., 2002. The Precautionary Principle in the 20th Century: late lessons from early warnings, James & James/Earthscan, London, 35–48.

[3] Alfonsi, K., Colberg, J., Dunn, P., Fevig, T., Jennings, S., Johnson, T., Kleine, P., Knight, C., Nagy, M., Perry, D., Stefaniak, M., 2008. Green chemistry tools to influence a medicinal chemistry and research chemistry based organisation. Green Chem., 10, 31–36.

In 2008, Pfitzer's scientists elaborated a list in which the solvents are classified according to their compliance to these safety principles (Table 1.1.). The major part of the undesirable solvents have a good alternative in the group of the "green" solvents; for example dichloromethane (which is yet a recommended alternative to other chlorinated solvents) can be replaced by ethyl acetate or *t*-butyl methyl ether (MTBE). The dipolar aprotic solvents group (dimethylformamide, *N*-methylpyrrolidinone) is the only group of unwanted solvents which has not a satisfactory alternative of replacement, because of their unique solvating properties.

Table 1.1. Pfizer solvent selection guide for medicinal chemistry.

Preferred	Usable	Undesirable
Methanol	Cyclohexane	Pentane
Ethanol	Methylcyclohexane	Hexane(s)
1-propanol	Heptane	Diisopropyl ether
2-propanol	Isooctane	Diethyl ether
1-butanol	*t*-butyl methyl ether	Dichloromethane
t-butanol	Acetonitrile	Dichloroethane
Ethyl acetate	2-methyl tetrahydrofuran	Chloroform
Isopropyl acetate	Tetrahydrofuran	Dimethylformamide
Acetone	Dimethylsulfoxide	*N*-methylpyrrolidinone
Methyl ethyl ketone	Acetic acid	Pyridine
	Ethylene glycol	Dimethyl acetate
	Xilene(s)	Dioxane
	Toluene	Dimethoxy ethane
		Benzene
		Carbon tetrachloride

The five main solvent systems which are currently considered as "green" are: *i*) solventless systems, *ii*) water, *iii*) ionic liquids, *iv*) fluorous solvents and *v*) supercritical fluids. Even though the last couple of decades has seen a large development of all of these systems as "clean" alternatives for synthesis and catalysis, it is also becoming increasingly clear that no single system will, in its own right, ever be able to replace completely all conventional reagents and solvents as a truly environmentally friendly alternative. It means that an ideal and universal "green" solvent for all situations does not exist because there are drawbacks associated with all of these systems, both from the point of views of applicability and sustainability.

2. Aim of the Thesis

The main objective of the present Thesis is the study of three alternative solvent groups as sustainable replacement of common organic solvents (5th principle of Green Chemistry). Some aspects of **ionic liquids**, **fluorinated solvents** and **supercritical fluids** have been analysed with a critical approach and their effective "greenness" has been evaluated from the points of view of the synthesis, the properties and the applications. In particular, the attention has been put on the environmental and human health issues, evaluating the eco-toxicity, the toxicity and the persistence, to underline that applicability and sustainability are subjects with equal importance.

Ionic liquids, especially imidazolium salts, have been proposed some years ago as "fully green" alternative solvents; however this epithet does not take into account several "brown" aspects such as their synthesis from petro-chemical starting materials, their considerable eco-toxicity, toxicity and resistance to biodegradation, and the difficulty of clearly outline applications in which ionic liquids are really more advantageous than traditional solvents. For all of these reasons in this Thesis a critical analysis of ionic liquids has been focused on:

- Alternative synthesis by *i)* introducing structural moieties which could reduce the toxicity of the most known liquid salts (4th principle of Green Chemistry), and by *ii*) using starting materials from renewable resources (7th principle of Green Chemistry); this second issue should also lead towards more biodegradable substances (10th principle of Green Chemistry).
- Evaluation of their environmental impact through *i*) eco-toxicological tests (*Daphnia magna* and *Vibrio fischeri* acute toxicity tests, and algal growth inhibition), *ii)* toxicity tests (MTT test, AChE inhibition and LDH release tests) and *iii*) fate and rate of aerobic biodegradation in soil and water.
- Demonstration of their effectiveness as reaction media in organo-catalysis (9th principle of Green Chemistry) and as extractive solvents in the recovery of vegetable oil from terrestrial and aquatic biomass (7th principle of Green Chemistry).

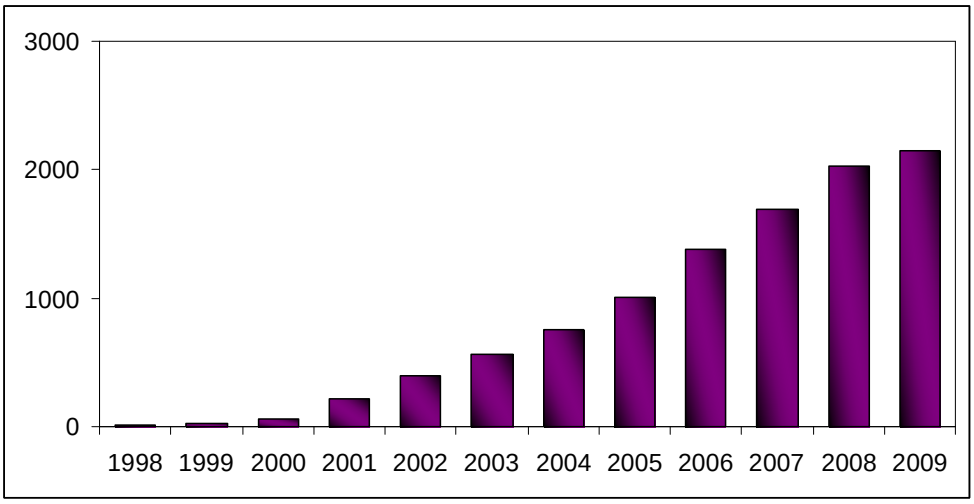
Fluorous solvents and supercritical fluids are less debated alternative solvents than ionic liquids, being their “green” features almost well-established; in particular supercritical carbon dioxide ($scCO_2$) is probably the “greenest” solvent among the alternative solvent systems developed in the last years, enabling to combine numerous advantages both from the point of view of industrial/technological applications and eco-compatibility. Thus in the Thesis the analysis of these two classes of alternative solvents has been mainly focused on their applicability, rather than the evaluation of their environmental impact, as done for ionic liquids.

Specifically the aim has been the evaluation of fluorous solvents and supercritical CO_2 as alternative media for non-aqueous biocatalysis. For this purpose, the hydrophobic ion pairing (HIP), which allows solubilising enzymes in non conventional apolar solvents as fluorous solvents and supercritical CO_2, has been investigated as effective enzymatic derivatisation technique to improve the catalytic activity under homogeneous conditions in non conventional media.

3. Ionic liquids

Ionic liquids (ILs) are the most explored source of alternative solvents, as confirmed by the incredible amount of works in the literature regarding this topic (Figure 3.1.). The great interest for such compounds relies on the fact that they posses several attractive properties such as negligible vapour pressure, chemical and thermal stability, non-flammability, high ionic conductivity, wide electrochemical potential window and ability to act as catalysts. The consequent attention is clearly due to the exploitation of these materials, formerly used for specialised electrochemical applications, as solvents for reactions and material processing, as extraction media or as working fluids in mechanical applications.

Figure 3.1. Publications on ionic liquids: 1998-2009.

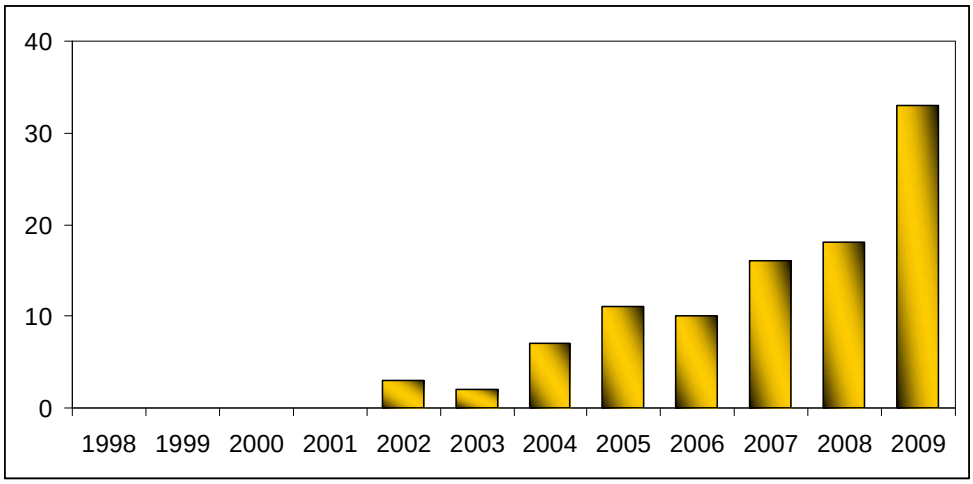


In contrast to conventional solvents that are constituted of molecules, ionic liquids consist of ions and are liquid at room temperature (RTILs) or have a low melting point (generally below 100ºC). A huge amount of different ionic liquids can be envisioned by the simple combination of different anions and cations; by changing the anion species or the cation entity (e.g. varying alkyl chain or the core), it is possible to change the physical properties as hydrophobicity, viscosity, density, and solubility behaviour, and to influence the biological features. For this ability to tune the properties

as function of the chemical structure, ILs can be defined “designer solvents”.[4]

In the light of their recent widespread commercial availability, the synthesis of ionic liquids has been object of further developments. If in the past years the synthesis of ILs was focused on obtaining unique chemico-physical properties (1st ILs generation), such as the absence of volatility and the thermal stability, or a specific targeted behaviour (2nd ILs generation), now one of the main goal is the achieving of specific desirable biological features (3rd ILs generation).[5] Simultaneously the number of studies about the toxicity, biodegradability and environmental fate of ILs has increased, even if this kind of investigation still represents a minimum part of all the literature (Figure 3.2.).

Figure 3.2. Publications on ionic liquids biological properties: 1998-2009.



As indicated by Figure 3.2., in spite of the scientific community is now focusing on ILs sustainability and effective “greenness”, this topics still remains something rather unexplored. The use of non-renewable resources as starting materials, the economic/environmental impact and the cumulative energy demand (which includes the supply of raw materials, the

[4] Earle, M.J., Seddon, K.R., 2000. Ionic liquids. Green solvents for the future. Pure Appl. Chem., 72, 1391-1398.
[5] Hough W.L., Smiglak M., Rodrı´guez H., Swatloski R.P., Spear S.K., Daly D.T., Pernak J., Grisel J.E., Carliss R.D., Soutullo M.D., Davis J.H., Rogers R.D., 2007. The third evolution of ionic liquids: active pharmaceutical ingredients. New J. Chem., 31, 1429–1436.

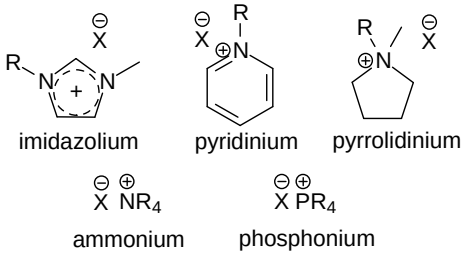
recycling and disposal of chemicals, the energy requires for heating, stirring and cooling), the (eco)-toxicological behaviour and the environmental fate are extremely important parameters to take into account for a complete analysis of ILs sustainability, above all in order to improve the chances of large-scale applications.[6]

Two attempts have been done in this Thesis to address these issues: *i)* the synthesis of imidazolium and pyridinium-based salts bearing functional groups which could reduce their biological activity and increase their biodegradability (Chapter 3.1.1.); *ii)* the use of bio-renewable feedstock for the synthesis of new ammonium salts (Chapter 3.1.5.). Moreover, the efforts to reduce the environmental impact of ILs preparation have been accompanied by investigations of their eco-toxicity (Chapter 3.1.2.), toxicity at cellular and sub-cellular level (Chapter 3.1.3.) and biodegradability (Chapter 3.1.4.).

3.1. Synthesis and properties

The chemical structure of the commonly used cations, e.g. imidazolium, pyridinium, pyrrolidinium, ammonium and phosphonium salts, in the synthesis of ionic liquids is represented in Figure 3.1.1. In particular 1-alkyl-3-methylimidazolium cations have been the most used and deeply investigated cation species over the last 20 years.

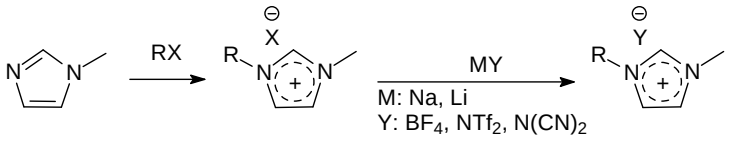
Figure 3.1.1. Examples of cations commonly used for the synthesis of ionic liquids.



[6] a) Kralisch, D., Stark, A., Ko¨rsten, S., Kreisel, G., Ondruschka, B., 2005. Energetic, environmental and economic balances: Spice up your ionic liquid research efficiency. Green Chem., 7, 301–309. b) Zhang, Y., Bakshi, B., Demessie, A, 2008. Life Cycle Assessment of an Ionic Liquid versus Molecular Solvents and Their Applications. Environ. Sci. Technol., 42, 1724–1730.

So far ILs synthesis has been generally based on alkylation reactions of amines, phosphines or sulphides by using alkyl halides or dialkyl sulfates. In principle this reaction is very simple, as indicated in Figure 3.1.2. for the synthesis of imidazolium salts: 1-methylimidazole is stirred with the alkyl halide under nitrogen (to maintain the reaction mixture free of moisture), at a certain temperature and for a specific time according to the reactivity of the alkyl halide. The halide salt, in general immiscible with the starting materials and with many organic solvents (e.g. hexane and ethyl acetate), can be easily separated and purified, and subsequently anion-exchanged with other kinds of anions to afford the suitable ionic liquid (e.g. tetrafluoroborate, hexafluorophosphate, dicyanamide). This process has two main advantages: *i)* a wide range of available cheap alkyl halides and *ii)* high yields of anion-exchange reactions. On the contrary the intrinsic toxicity for human health and for the environment of many alkyl halides (e.g. methyl iodide) represents a disadvantage from the point of view of the sustainability.

Figure 3.1.2. Quaternarization reaction and anion-exchange at the base of the synthesis of common and commercial imidazolium ionic liquids.



The final quality of an ionic liquid is a crucial consideration for its application. For example the transparency in the UV-VIS spectral region is a very important parameter for some spectroscopic applications, but irrelevant when the ionic liquid has to be used as solvent in catalytic reactions, being the coloured impurities usually present only in traces.

In principle the impurities can be summarised in five main categories: *i)* organic compounds, *ii)* water, *iii)* halide salts, *iv*) inorganic salts and *v)* coloured impurities.[7]

[7] a) Scammel, P, Scott, J., Singer, R., 2005. Ionic liquids: the neglected issue. Aust J Chem, 58, 155-158. b) Wagner, M., Hilgers, C., 2008. Quality Aspects and other questions related to commercial ionic liquids. In "Ionic liquids in synthesis, second edition", Wiley-VCH Verlags GmbH & Co. KGaA, Weinheim.

Organic impurities can derived from an incomplete alkylation of the substrate; in general the alkyl halide used as nucleophiles in quaternarization reactions, is easily removable under vacuum, whereas the other non-volatile organic impurity can be eliminated by solvent extraction.

On the contrary, residual water is quite difficult to remove, above all because without a specific drying procedure it is almost impossible to obtain waterless ionic liquids, since their high hygroscopic behaviour. The main strategy to remove water relies in drying the ionic liquid for some days under vacuum at 60-70°C, and then to keep it under nitrogen.

Halides salts are often detected in the final product, as indication of an incomplete anion metathesis, and they can seriously affect the usefulness of the material as solvent for chemical reaction. This kind of impurity can be removed with various strategies, e.g. by titrating with $AgNO_3$, through ion exchange chromatography or by washing the ionic liquid with water. The presence of residual inorganic salt in the ionic liquid is often difficult to remove, above all if the ionic liquid is hydrophilic and can not be washed with water.

Finally it is very difficult to remove coloured impurities because they are often present in very low trace amounts. The strategy to obtain colourless ionic liquids is based on the use of very pure starting materials and mild reaction conditions in their synthesis, followed by purification with activated carbon or filtration through silica/alumina column. Unfortunately, the additional cost of these procedures sometimes prevents a large scale application of ionic liquids.

In comparison with the solvents commonly in use, ionic liquids have several attractive chemico-physical properties which are difficult to obtain with molecular solvents and, above all, which can be tuned by changing the chemical structure of the ions: wide liquid ranges, low melting points, low viscosity, high solubility for many solutes, gas solubility, offer opportunities for controlling the reactivity of chemical reactions as well for obtaining new materials exploitable in many fields.

One of the most interesting properties of ILs is their ability to solubilise both metal salts and organic compounds; in particular they are able to dissolve biological macromolecules that are linked together by

intermolecular hydrogen bonds such as carbohydrates,[8] cellulose,[9] wool keratin,[10] silk fibroin,[11] chitin and chitosan,[12] offering new possibility of exploitation of this kind of renewable bio-materials. The industrial exploitation of cellulose for example is characterised by very harsh extraction and derivatisation conditions, basically because there is a limited number of solvents able to dissolve the fibres. Traditional cellulose dissolution processes, including the cuprammonium and xanthate ones, are often cumbersome or expensive and require the use of harsh conditions and of solvents with high ionic strength which can rather be recycled. In this contest, some ionic liquids represent a very good "green" alternative because they are able to dissolve cellulose and the other bio-polymers in high amount (10% w/w) under mild conditions (room temperature or gently heating), they allow an easy functionalisation of the material and they are simply recoverable by precipitation with water or organic solvents.

3.1.1. Oxygenated ionic liquids

In this Thesis the evaluation of the environmental impact of ILs has been performed by comparing traditional alkyl-substituted cations with oxygenated (or polyethoxylated) ones. The choice of focusing on oxygenated ionic liquids derives both from their large number of applicable chemico-physical properties, but above all from the willing to reduce the

[8] Liu, Q., Janssen, M.H.A., van Rantwijk, F., Sheldon, R.A., 2005. Room-temperature ionic liquids that dissolve carbohydrates in high concentrations. Green Chem., 7, 39–42.

[9] a) Swatloski, R.P., Spear, S.K., Holbrey, J.D., Rogers, R.D., 2002. Dissolution of Cellose with Ionic Liquids. JACS, 124, 4974-4975. b) Kosan, B., Michels, C., Meiste, F., 2008. Dissolution and forming of cellulose with ionic liquids. Cellulose, 15, 59–66. c) Zhu, S., Wu, Y., Chen, Q., Yu, Z., Wang, C., Jin, S., Dinga, Y., Wu, G., 2006. Dissolution of cellulose with ionic liquids and its application: a mini-review. Green Chem., 8, 325–327. d) Mikkola, J., Kirilin, A., Tuuf, J., Pranovich, A., Holmbom, B., Kustov, L., Yu, D., Salmi, T. 2007. Ultrasound enhancement of cellulose processing in ionic liquids: from dissolution towards functionalization. Green Chem., 9, 1229–1237. e) Fukaya, Y., Hayashi, K., Wada, M., Ohno, H., 2008. Cellulose dissolution with polar ionic liquids under mild conditions: required factors for anions. Green Chem., 10, 44–46

[10] Xie, H., Li, S., Zhang, S., 2005. Ionic liquids as novel solvents for the dissolution and blending of wool keratin fibres. Green Chem., 7, 606-608.

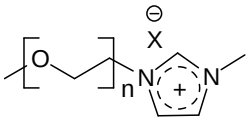
[11] Phillips, D., Drummy, L., Conrady, D., Fox, D., Naik, R., Stone, M., Trulove, P., De Long, H., Mantz, R., 2004. Dissolution and Regeneration of *Bombyx mori* Silk Fibroin Using Ionic Liquids. JACS, 126, 44, 14350–14351.

[12] a) Xie, H., Zhang, S., Li, S., 2006. Chitin and chitosan dissolved in ionic liquids as reversible sorbents of CO2. Green Chem., 8, 630–633. b) Wu, Y., Sasaki, T., Sakurai, K., 2008. A novel biomass-ionic liquid platform for the utilization of native chitin. Polymer, 49, 2321–2327. c) Mine, S., Izawa, H., Kaneko, Y., Kadokawa, J., 2009. Acetylation of a-chitin in ionic liquids. Carbohydrate Research, 344, 2263–2265

hazard and to find a correlation between the chemical structure of the cation and its toxic profile.

Imidazolium-based ILs bearing ether functionalities in the lateral chain (Figure 3.1.1.1.), firstly synthesised in 1996 by Gratzel[13] for photoelectrochemical applications, have very interesting applicative properties, such as high solubility for polar substrates (e.g. carbohydrates and cellulose),[8,14] suitable features as reaction media for biocatalytic processes[15] and for catalytic asymmetric reaction,[16] high CO_2 solubility useful for gas separation processes,[17] good electrochemical behaviour suited to dye-sensitized photoelectrochemical solar cells,[18] and nanoparticles stabilizing properties.[19] Moreover some preliminary (eco)-toxicological data, as it will be described in Chapters 3.1.2. and 3.1.3., indicate that the introduction of one ethoxy moiety in the lateral chain of imidazolium cations can deeply reduce their intrinsic toxicity.

Figure 3.1.1.1. General structure of oxygenated imidazolium-based ionic liquids bearing ethoxy moieties.



[13] Bonhoˆte, P., Dias, A., Papageorgiou, N., Kalyanasundaram, K., Gratzel, M., 1996. Hydrophobic, Highly Conductive Ambient-Temperature Molten Salts. Inorg. Chem., 35, 1168-1178.

[14] Pinkert, A., Marsh, K.N., Pang, S., Staiger, M.P., 2009. Ionic liquids and their interaction with cellulose. Chem. Rev., 109, 6712-6728.

[15] Galletti, P., Moretti, F., Samorì, C., Tagliavini, E., 2007. Enzymatic acylation of levoglucosan in acetonitrile and ionic liquids. Green Chem., 9, 987-991.

[16] Branco, L., Afonso, C.A.M., 2004. Ionic liquids as a convenient new medium for the catalytic asymmetric dihydroxylation of olefins using a recoverable and reusable Osmium/ligand. JOC, 69, 13, 4381- 4389.

[17] Bara, J.E., Gabriel, C.J., Lessmann, S., Carlisle, T.K., Finotello, A., Gin, D.L., Noble, R.D., 2007. Enhanced CO_2 separation selectivity in oligo (ethylene glycol) functionalized room-temperature ionic liquids. Ind. Eng. Chem. Res., 46, 16, 5380-5386.

[18] Stathatos E., Lianos P., Jovanovski V., Orel B., 2005. Dye-sensitized photoelectrochemical solar cells based on nanocomposite organic–inorganic materials. J. Photoch. Photobio. A., 169, 1, 57–61.

[19] Schrekker H.S., Gelesky M.A., Stracke M.P., Schrekker C.M.L., Machado G., Teixeira S.R., Rubim J.C., Jairton Dupont J., 2007. Disclosure of the imidazolium cation coordination and stabilization mode in ionic liquid stabilized gold(0) nanoparticles. J. Colloid Interf. Sci., 316, 1, 189–195.

The synthesis of ether-functionalised imidazolium ionic liquids has been previously described in detail;[20] in this Thesis the procedure reported in the literature has been used to enlarge the class of oxygenated imidazolium salts, including ionic liquids with three and four ethoxy units in the lateral chain, and to synthesise a class of pyridinium analogues (to the best of our knowledge never synthesised before), with one and two ethoxy units (Table 3.1.1.1.).

Table 3.1.1.1. Ionic liquids synthesised in the Thesis.

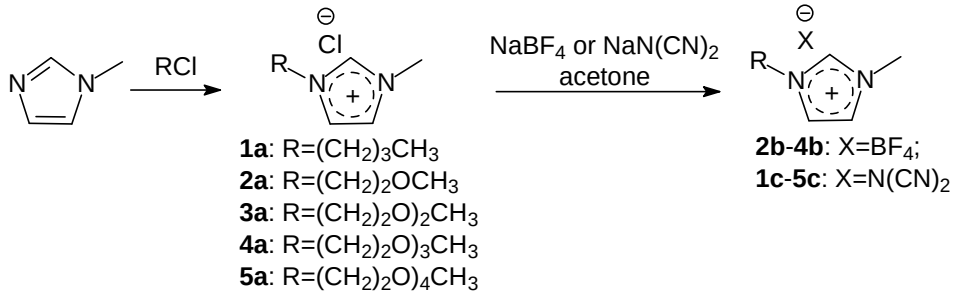
	R	Cation type	Acronym	Anion		
				a	**b**	**c**
1	$CH_3(CH_2)_3$	1-methylimidazolium	BMIM	Cl	BF_4[a]	$N(CN)_2$
2	$CH_3O(CH_2)_2$	1-methylimidazolium	MOEMIM	Cl	BF_4	$N(CN)_2$
3	$CH_3(O(CH_2)_2)_2$	1-methylimidazolium	$M(OE)_2MIM$	Cl	BF_4	$N(CN)_2$
4	$CH_3(O(CH_2)_2)_3$	1-methylimidazolium	$M(OE)_3MIM$	Cl	BF_4	$N(CN)_2$
5	$CH_3(O(CH_2)_2)_4$	1-methylimidazolium	$M(OE)_4MIM$	Cl	-	$N(CN)_2$
6	$CH_3(CH_2)_3$	3-methylpyridinium	BMPy	Cl	-	-
7	$CH_3O(CH_2)_2$	3-methylpyridinium	MOEMPy	Cl	-	-
8	$CH_3(O(CH_2)_2)_2$	3-methylpyridinium	$M(OE)_2MPy$	Cl	-	-

[a]1-Butyl-3-methylimidazolium tetrafluoroborate BMIM BF_4 was purchased from Merck

According to the procedure reported in the literature, the synthesis of ionic liquids was performed in two steps: *i*) a quaternarization reaction to get imidazolium and pyridinium chlorides **1a**-**8a**, followed by *ii*) an anion exchange with $NaBF_4$ (**2b**-**4b**) or $NaN(CN)_2$ (**1c**-**5c**) (Figure 3.1.1.2.).

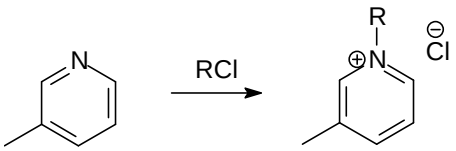
[20] a) Park, S.; Kazlauskas, R.J., 2001. Improved preparation and use of room-temperature ionic liquids in lipase-catalyzed enantio- and regioselective acylations. JOC, 66, 8395-8401. b) Branco, L.C.; Rosa, J.N.; Ramos, J.J.M.; Afonso, C.A.M., 2002. Preparation and characterization of new room temperature ionic liquids. Chem. Eur. J., 8, 3671-3677. c) Fei, Z., Ang, W.H., Zhao, D., Scopelliti, R., Zvereva, E., Katsyuba, S., Dyson, P.J., 2007. Revisiting Ether-Derivatized Imidazolium-Based Ionic Liquids J. Phys. Chem. B., 111, 10095-10108

Figure 3.1.1.2. Synthetic pathway for the synthesis of alkyl and oxygenated imidazolium or pyridinium ionic liquids.



1a: R=$(CH_2)_3CH_3$
2a: R=$(CH_2)_2OCH_3$
3a: R=$(CH_2)_2O)_2CH_3$
4a: R=$(CH_2)_2O)_3CH_3$
5a: R=$(CH_2)_2O)_4CH_3$

2b-**4b**: X=BF_4;
1c-**5c**: X=$N(CN)_2$



6a: R=$(CH_2)_3CH_3$
7a: R=$(CH_2)_2OCH_3$
8a: R=$(CH_2)_2O)_2CH_3$
9a: R=$(CH_2)_2O)_3CH_3$

As mentioned above, the purity and the decolourisation of the ILs are extremely important when biological systems and spectroscopic measurements are involved, as in the case of the present Thesis. To obtain colourless ILs suitable for the envisagement of toxicological properties, a further purification procedure was done, by using activated charcoal according to the literature.[21]

Experimental section

All reagents used were purchased from Aldrich; 1-methylimidazolium, 3-methyl pyridine, 1-chlorobutane and 2-chloroethyl methyl ether were re-distilled before use to limit the formation of coloured impurities.

BMIM BF_4 **1b** (purity 98%) was purchased from Merck (Darmstadt, Germany), and used without any other purification.

[21] a) Gordon, C.M., McLean, A.J., Muldoon, M.J., Dunkin, I.R., 2003. Diffusion-controlled reactions in room temperature ionic liquids. In: Rogers R.D. and Seddon K.R., (Eds.). Ionic liquids as green solvents, progress and prospects. ACS Symposium Series 856, Washington, DC, 357-369. b) Nockemann, P., Binnemans, K., Driesen, K., 2005. Purification of imidazolium ionic liquids for spectroscopic applications. Chem. Phys. Lett., 415, 131–136. c) Earle, M.J., Gordon, C.M., Plechkova, N.V., Seddon, K.R., Welton T., 2007. Decolourization of ionic liquids for spectroscopy. Anal. Chem., 79, 11, 758-764.

The purities of all the ionic liquids were established to be ≥98% through proton and carbon nuclear magnetic resonances (^{1}H NMR and ^{13}C NMR) spectra by integration of proton signals with respect to an internal standard. All spectra were recorded using a 5-mm probe on Varian Inova 300 and Varian Inova 400 spectrometers (Varian, Palo Alto, CA, USA) at 300 or 400 MHz. All spectral data were acquired in acetone-d_6, D_2O or C*D*Cl$_3$ with a known amount of tetrakis(trimethylsilyl)silane as an internal standard and a delay time between successive scans of 20 s to ensure complete proton relaxation and, therefore, quantitative integration.

2-(2-Methoxy-ethoxy)-ethyl chloride ($CH_3(OCH_2)_2)_2Cl$), 2-(2-(2-methoxy-ethoxy)-ethoxy)-ethyl chloride ($CH_3(OCH_2)_2)_3Cl$), and 2-(2-(2-(2-methoxy-ethoxy)-ethoxy)-ethoxy)-ethyl chloride ($CH_3(OCH_2)_2)_4Cl$) were synthesised from the corresponding ethylene glycol monomethyl ether, according to the procedure reported in the literature. [22]

A solution of thionyl chloride (92 mmol) in $CHCl_3$ (15 mL) was added slowly with a dropping funnel to a stirred solution of diethylene glycol monomethyl ether, triethylene glycol monomethyl ether or tetraethylene glycol monomethyl ether (72 mmol), and pyridine (72 mmol) in $CHCl_3$ (60 mL) under N_2. The reaction mixture was stirred for 3 h at reflux and then washed with 300 mL of water, dried with Na_2SO_4, and concentrated under reduced pressure. The crude yellowish product was used without further purification.

$CH_3(OCH_2)_2)_2Cl$, yield >99%. ^{1}H NMR (400 MHz; C*D*Cl$_3$): δ=3.32 (s, 3H, C*H*$_3$O), 3.48-3.50 (m, 2H, $CH_3OCH_2CH_2O(CH_2)_2Cl$), 3.56-3.61 (m, 4H, $CH_3OCH_2CH_2OCH_2CH_2Cl$) and 3.67-3.70 ppm (m, 2H, $CH_3O(CH_2)_2OCH_2CH_2Cl$). ^{13}C NMR (100 MHz; C*D*Cl$_3$): δ_C=42.17, 58.58, 70.10, 70.92, 71.43

$CH_3(OCH_2)_2)_3Cl$ (>99%). ^{1}H NMR (400 MHz; C*D*Cl$_3$): δ=3.38 (s, 3H, C*H*$_3$O), 3.54-3.56 (m, 2H, $CH_3OCH_2CH_2(O(CH_2)_2)_2Cl$), 3.63-3.68 (m, 8H,

[22] Gudipati, V., Curran, D., Wilcox, C., 2002. Solution-Phase Parallel Synthesis with Oligoethylene Glycol Sorting Tags. Preparation of All Four Stereoisomers of the Hydroxybutenolide Fragment of Murisolin and Related Acetogenins. JOC, 71, 9, 3599

$CH_3OCH_2CH_2O(CH_2)_2OCH_2CH_2Cl$) and 3.74-3.77 ppm (m, 2H, $CH_3(O(CH_2)_2)_2OCH_2CH_2Cl$). ^{13}C NMR (100 MHz; $CDCl_3$): δ_C=42.66, 59.01, 70.57, 70.60, 70.64, 71.34, 71.92.

$CH_3(OCH_2)_2)_4Cl$ (90%). ^1H NMR (400 MHz; $CDCl_3$): δ=3.25 (s, 3H, CH_3O), 3.50-3.52 (m, 2H, $CH_3OCH_2CH_2(O(CH_2)_2)_3Cl$), 3.57-3.66 (m, 12H, $CH_3O(CH_2)_2O(CH_2)_2Cl$) and 3.83-3.86 ppm (m, 2H, $CH_3(O(CH_2)_2)_2OCH_2CH_2Cl$). ^{13}C NMR (100 MHz; $CDCl_3$): δ_C=43.02, 58.82, 69.84, 70.42 (×3), 70.62, 71.30, 71.69.

All the chlorides **1a**-**8a** were prepared by adding the corresponding alkyl chloride (72 mmol) to a stirred solution of 1-methylimidazolium or 3-methyl pyridine (1.1 eq, 65.4 mmol); the reaction mixture was stirred for 20 h at 90°C (50°C for BMIM Cl **1a** and BMPy Cl **6a**), and then washed with ethyl acetate and diethyl ether. The solid (or liquid) chloride was dried under vacuum at 70°C over night.

BMIM Cl 1a, yield 98. ^1H NMR (400 MHz; D_2O): δ=0.94-0.97 (m, 3H, $CH_3(CH_2)_3N$), 1.31-1.40 (m, 2H, $CH_3CH_2(CH_2)_2N$), 1.85-1.93 (m, 2H, $CH_3CH_2CH_2CH_2N$), 3.94 (s, 3H, NCH_3), 4.23-4.26 (m, 2H, $CH_3(CH_2)_2CH_2N$), 7.49 (s, 1H, NCH=CHN), 7.54 (s, 1H, NCH=CHN), and 8.78 ppm (s, 1H, N=CHN).

MOEMIM Cl 2a (92%). ^1H NMR (400 MHz; D_2O): δ=3.42 (s, 3H, CH_3O), 3.87-3.90 (m, 2H, $CH_3OCH_2CH_2N$), 3.95 (s, 3H, NCH_3), 4.43-4.46 (m, 2H, $CH_3OCH_2CH_2N$), 7.50 (d, 1H, J=2 Hz, NCH=CHN), 7.56 (d, 1H, J=2 Hz, NCH=CHN), and 8.80 ppm (s, 1H, N=CHN).

M(OE)$_2$MIM Cl 3a (90%). ^1H NMR (400 MHz; D_2O): δ=3.37 (s, 3H, CH_3O), 3.61-3.63 (m, 2H, $CH_3OCH_2CH_2O(CH_2)_2N$), 3.70-3.72 (m, 2H, $CH_3OCH_2CH_2O(CH_2)_2N$), 3.92-3.94 (m, 2H, $CH_3O(CH_2)_2OCH_2CH_2N$), 3.93 (s, 3H, NCH_3), 4.41-4.43 (m, 2H, $CH_3O(CH_2)_2OCH_2CH_2N$), 7.48 (d, 1H, J=2 Hz, NCH=CHN), and 7.54 ppm (d, 1H, J=2 Hz, NCH=CHN).

M(OE)$_3$MIM Cl 4a (90%). ^{1}H NMR (400 MHz; D_2O): δ=3.37 (s, 3H, OCH_3), 3.51-3.64 (m, 8H, $CH_3(O(CH_2)_2)_2OCH_2CH_2N$), 3.82 (t, 2H, J=4.8 Hz, $CH_3(O(CH_2)_2)_2OCH_2CH_2N$), 3.98 (s, 3H, NC$H_3$), 4.48 (t, 2H, J=4.8 Hz, $CH_3(O(CH_2)_2)_2OCH_2CH_2N$), 7.25-7.26 (m, 1H, NC*H*=CHN), and 7.59-7.61 ppm (m, 1H, NCH=C*H*N).

M(OE)$_4$MIM Cl 5a (66%). ^{1}H NMR (400 MHz; D_2O): δ=3.34 (s, 3H, OCH_3), 3.50-3.65 (m, 12H, $CH_3(O(CH_2)_2)_3OCH_2CH_2N$), 3.81 (t, 2H, J=4.8 Hz, $CH_3(OCH_2CH_2)_3OCH_2CH_2N$), 3.96 (s, 3H, NC$H_3$), 4.47 (t, 2H, J=4.8 Hz, $CH_3(OCH_2CH_2)_3OCH_2CH_2N$), 7.31-7.33 (m, 1H, NC*H*=CHN), and 7.61-7.62 ppm (m, 1H, NCH=C*H*N).

BMPy Cl 6a (97%). ^{1}H NMR (400 MHz; D_2O): δ=0.94-0.98 (m, 3H, $CH_3(CH_2)_3N$), 1.35-1.39 (m, 2H, $CH_3CH_2(CH_2)_2N$), 2.00-2.04 (m, 2H, $CH_3CH_2CH_2CH_2N$), 2.58 (s, 3H, CH_3), 4.59-4.62 (m, 2H, $CH_3(CH_2)_2CH_2N$), 7.95-7.99 (m, 1H, *H* meta), 8.39 (d, 1H, J=8 Hz, *H* para), 8.70 (d, 1H, J=6 Hz, *H* orto), and 8.76 ppm (s, 1H, *H* orto)**.** ^{13}C NMR (100 MHz; D_2O): δ_C=12.95, 17.92, 18.94, 32.78, 61.67, 127.62, 140.06, 141.51, 143.91, 146.12.

MOEMPy Cl 7a (98%). ^{1}H NMR (400 MHz; D_2O): δ=2.57 (s, 3H, CH_3), 3.38 (s, 3H, CH_3O), 3.96-3.99 (m, 2H, $CH_3OCH_2CH_2N$), 4.75-4.79 (m, 2H, $CH_3OCH_2CH_2N$), 7.97 (dd, 1H, J=6 and 8 Hz, *H* meta), 8.42 (d, 1H, J=8 Hz, *H* para), 8.66 (d, 1H, J=6, *H* orto), and 8.71 ppm (s, 1H, *H* orto). ^{13}C NMR (100 MHz; D_2O): δ_C=17.80, 58.56, 60.84, 70.38, 127.50, 140.04, 141.91, 144.21, 146.62.

M(OE)$_2$MPy Cl 8a (95%). ^{1}H NMR (400 MHz; D_2O): δ=2.58 (s, 3H, CH_3), 3.32 (s, 3H, CH_3O), 3.55-3.57 (m, 2H, $CH_3OCH_2CH_2O(CH_2)_2N$), 3.67-3.69 (m, 2H, $CH_3OCH_2CH_2O(CH_2)_2N$), 4.05 (t, 2H, J=5.2 Hz, $CH_3O(CH_2)_2OCH_2CH_2N$), 4.76-4.79 (m, 2H, $CH_3O(CH_2)_2OCH_2CH_2N$), 7.97 (dd, 1H, J=6 and 8 Hz, *H* meta), 8.43 (d, 1H, J=8 Hz, *H* para), 8.69 (d, 1H, J=6 Hz, *H* orto), and 8.73 ppm (s, 1H, *H* orto). ^{13}C NMR (100 MHz;

D_2O): δ_C=17.82, 58.20, 60.99, 68.93, 69.78, 71.01, 127.45, 139.94, 141.99, 144.36, 146.62.

Compounds **2b**-**4b** were prepared through an anion exchange with $NaBF_4$. Chloride (**2a**-**4a**, 30 mmol) was added to a suspension of $NaBF_4$ (1.2 eq., 36 mmol) in acetone (75 mL). The mixture was stirred for 2 days at room temperature; the sodium halide precipitate was removed by filtration and the filtrate concentrated to an oil by rotary evaporation. The oil was diluted with CH_2Cl_2 (100 mL) and filtered through silica gel. The solution was washed twice with a small amount of saturated sodium carbonate aqueous solution (CH_2Cl_2: $NaHCO_3$ aq, 10:1) and dried over anhydrous Na_2SO_4. The solvent was removed by rotary evaporation, giving a yellow oil.

MOEMIM BF_4 2b (74%). ^{1}H NMR (300 MHz; acetone-d_6): δ=3.36 (s, 3H, OC*H*$_3$), 3.74 (t, 2H, J=4.5 Hz, OC*H*$_2$CH$_2$N), 3.96 (s, 3H, C*H*$_3$N), 4.38 (t, 2H, J=4.5 Hz, OCH$_2$C*H*$_2$N), 7.27 (m, 1H, NC*H*=CHN), 7.40 (m, 1H, NCH=C*H*N), and 8.81 ppm (s, 1H, N=C*H*N).

M(OE)$_2$MIM BF_4 3b (61%).^{1}H NMR (200 MHz; C*D*Cl$_3$): δ=3.38 (s, 3H, OC*H*$_3$), 3.52-3.57 (m, 2H, CH$_3$OC*H*$_2$CH$_2$O), 3.64-3.68 (m, 2H, CH$_3$OCH$_2$C*H*$_2$O), 3.87 (t, 2H, J=4.6 Hz, OC*H*$_2$CH$_2$N), 3.97 (s, 3H, NC*H*$_3$), 4.40 (t, 2H, J=4.6 Hz, OCH$_2$C*H*$_2$N), 7.28-7.29 (m, 1H, NC*H*=CHN), 7.48-7.50 (m, 1H, NCH=C*H*N), and 8.87 ppm (s, 1H, N=C*H*N).

M(OE)$_3$MIM BF_4 4b (89%). ^{1}H NMR (300 MHz; C*D*Cl$_3$): δ=3.37 (s, 3H, OC*H*$_3$), 3.56-3.57 (m, 2H, CH$_3$OC*H*$_2$CH$_2$), 3.62-3.67 (m, 6H, CH$_3$OCH$_2$C*H*$_2$OC*H*$_2$C*H*$_2$), 3.85 (t, 2H, J=3.8 Hz, OC*H*$_2$CH$_2$N), 3.95 (s, 3H, NC*H*$_3$), 4.38 (t, 2H, J=3.8 Hz, OCH$_2$C*H*$_2$N), 7.32 (d, 1H, J=1.4 Hz, NC*H*=CHN), 7.55 (d, 1H, J=1.4 Hz, NCH=C*H*N), and 8.83 ppm (s, 1H, N=C*H*N).

Compounds **1c**-**5c** were prepared through an anion exchange with $NaN(CN)_2$. Chloride (**1a**-**5a**, 30 mmol) was added to a suspension of $NaN(CN)_2$ (1.2 eq., 36 mmol) in acetone (75 mL). The mixture was stirred

for 2 days at room temperature; the sodium halide precipitate was removed by filtration and the filtrate concentrated to an oil by rotary evaporation. The oil was diluted with acetone and filtered through silica gel. The solvent was removed by rotary evaporation, giving a light yellow oil.

BMIM $N(CN)_2$ 1c (97%)**.** ^{1}H NMR (200 MHz; C*D*Cl_3): δ=0.98 (t, 3H, J=7.4 Hz, C*H*$_3$CH$_2$CH$_2$CH$_2$N), 1.30-1.49 (m, 2H, CH$_3$C*H*$_2$CH$_2$CH$_2$N), 1.82-1.97 (m, 2H, CH$_3$CH$_2$C*H*$_2$CH$_2$N), 4.01 (s, 3H, C*H*$_3$N), 4.23 (t, 2H, J=7.4 Hz, CH$_3$(CH$_2$)$_2$C*H*$_2$N), 7.28-7.45 (m, 2H, NC*H*=C*H*N), and 9.12 ppm (s, 1H, N=C*H*N).

MOEMIM $N(CN)_2$ 2c (93%). ^{1}H NMR (400 MHz; acetone-d_6): δ=3.35 (s, 3H, OC*H*$_3$), 3.81 (t, 2H, J=5.2 Hz, OC*H*$_2$CH$_2$N), 4.07 (s, 3H, C*H*$_3$N), 4.53 (t, 2H, J=5.2 Hz, OCH$_2$C*H*$_2$N), 7.71 (m, 1H, NC*H*=CHN), 7.75 (m, 1H, NCH=C*H*N), and 9.08 ppm (s, 1H, N=C*H*N).

M(OE)$_2$MIM $N(CN)_2$ 3c (65%). ^{1}H NMR (400 MHz; C*D*Cl_3): δ=3.35 (s, 3H, OC*H*$_3$), 3.51-3.53 (m, 2H, CH$_3$OC*H*$_2$CH$_2$O), 3.63-3.65 (m, 2H, CH$_3$OCH$_2$C*H*$_2$O), 3.85 (t, 2H, J=4.6 Hz, OC*H*$_2$CH$_2$N), 3.98 (s, 3H, NC*H*$_3$), 4.41 (t, 2H, J=4.6 Hz, OCH$_2$C*H*$_2$N), 7.37 (d, 1H, J=3.4 Hz, NC*H*=CHN), 7.53 (d, 1H, J=3.4 Hz, NCH=C*H*N), and 9.06 ppm (s, 1H, N=C*H*N).

M(OE)$_3$MIM $N(CN)_2$ 4c (96%). ^{1}H NMR (300 MHz; C*D*Cl_3): δ=3.39 (s, 3H, OC*H*$_3$), 3.56-3.60 (m, 2H, CH$_3$OC*H*$_2$CH$_2$), 3.63-3.73 (m, 6H, CH$_3$OCH$_2$C*H*$_2$OC*H*$_2$C*H*$_2$O), 3.87-3.91 (m, 2H, OC*H*$_2$CH$_2$N), 4.03 (s, 3H, NC*H*$_3$), 4.43-4.47 (m, 2H, OCH$_2$C*H*$_2$N), 7.38-7.40 (m, 1H, NC*H*=CHN), 7.62-7.64 (m, 1H, NCH=C*H*N), and 9.14 ppm (s, 1H, N=C*H*N).

M(OE)$_4$MIM $N(CN)_2$ 5c (69%). ^{1}H NMR (300 MHz; C*D*Cl_3): δ=3.34 (s, 3H, OC*H*$_3$), 3.54-3.56 (m, 2H, CH$_3$OC*H*$_2$), 3.62-3.70 (m, 10H, CH$_3$OCH$_2$C*H*$_2$(OC*H*$_2$C*H*$_2$)$_2$O), 3.91 (t, 2H, J=4.6 Hz, OC*H*$_2$CH$_2$N), 4.02 (s, 3H, NC*H*$_3$), 4.45 (t, 2H, J=4.6 Hz, OCH$_2$C*H*$_2$N), 7.40 (d, 1H, J=3.2 Hz, NC*H*=CHN), 7.64 (d, 1H, J=3.2 Hz, NC*H*=CHN), and 9.15 ppm (s, 1H, N=C*H*N).

3.1.2. Eco-toxicological properties

The environmental impact of ILs and their effective "greenness" have been analyzed in a series of interdisciplinary fundamental studies[23] which have underlined the importance of a preventive evaluation in the ILs design, because of the influence of different structures on pertinent technological, toxicological and eco-toxicological properties. In particular the risk associated to ionic liquids should be analyzed by taking into account several factors, from the release to the biodegradability, from the biological activity to the uncertainty of action respect to traditional solvents. Moreover the need of a full comprehension of the biological effects at different biological organization levels has a fundamental importance, and such knowledge should play a role as a key factor in the modulation and selection of ionic liquid features.

In view of the growing industrial interest towards ionic liquids as alternative solvents, a relevant number of risk scenarios, including those in which they could be present in industrial effluents and then affect aquatic ecosystems, has to be taken into account. For this reason, since now, the biological activity of ionic liquids has been intensely investigated: bioassays using fish,[24] plants,[23b,25] algae, soil invertebrates,[26] animals,[27] mussels,[28]

[23] a) Jastorff, B., Stormann, R., Ranke, J., Molter, K., Stock, F.F., Oberheitmann, B., Hoffmann, W., Nuchter, M., Ondruschka, B., Filser, J.J., 2003. How hazardous are ionic liquids? Structure-activity relationships and biological testing as important elements for sustainability evaluation. Green Chem., 5, 136-142. b) Matzke, M., Stolte, S., Thiele, K., Juffernholz, T., Arning, J., Ranke, J., Welz-Biermann, U., Jastorff, B., 2007. The influence of anion species on the toxicity of 1-alkyl-3-methylimidazolium ionic liquids observed in an (eco)toxicological test battery, Green Chem., 9, 1198-1207. c) Ranke, J., Stolte, S., Sto¨rmann, R., Arning, J., Jastorff, B., 2007. Design of sustainable chemical products - the example of ionic liquids. Chem. Rev., 107, 2183-2206. d) Stolte, S., Matzke, M., Arning, J., Böschen, A., Pitner, W.R., Welz-Biermann, U., Jastorff, B., Ranke, J., 2007. Effects of different head groups and functionalised side chains on the aquatic toxicity of ionic liquids. Green Chem., 9, 1170–1179.

[24] a) Pretti, C., Chiappe, C., Pieraccini, D., Gregori, M., Abramo, F., Monni, G., Intorre, L., 2006. Acute toxicity of ionic liquids to the zebrafish (*Danio rerio*). Green Chem., 8, 238–240. b) Pretti, C., Chiappe, C., Baldetti, I., Brunini, S., Monni G., 2009. Acute toxicity of ionic liquids for three freshwater organisms: *Pseudokirchneriella subcapitata*, *Daphnia magna* and *Danio rerio*. Ecotoxicol. Environ. Safety, 72, 4, 1170-1176.

[25] a) Larson, J.H., Frost, P.C., Lamberti, G.A., 2008. Variable toxicity of ionic liquid-forming chemicals to *Lemnia minor* and the influence of dissolved organic matter. Environ. Toxicol. Chem., 27, 3, 676–681. b) Matzke, M., Stolte, S., Arning, J., Uebers, U., Filser J., 2008. Imidazolium based ionic liquids in soils: effects of the side chain length on wheat (*Triticum aestivum*) and cress (*Lepidium sativum*) as affected by different clays and organic matter. Green Chem., 10, 584–591.

crustaceans and bacteria for detecting the toxic effects in terrestrial and aquatic ecosystems, and toxicity studies at cellular and sub-cellular level have been put forward. The latter ones regard the effects of ILs on cell viability using human and animal cell lines[29] and on enzymatic activity (acetylcholinesterase,[26a,30] AMP deaminase[31] and antioxidant defence enzymes[27b,32]) and will be described in detail in Chapter 3.1.3.

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[26] a) Luo, Y.R., Wang, S.H., Yun, M.X., Li, X.Y., Wange, J.J., Sun, Z.J., 2009. The toxic effects of ionic liquids on the activities of acetylcholinesterase and cellulase in earthworms. Chemosphere, 77 313–318. b) Swatloski, R.P., Holbrey, J.D., Memon, S.B., Caldwell, G.A., Caldwell, K.A., Rogers, R.D., 2004. Using *Caenorhabditis elegans* to probe toxicity of 1-alkyl-3-methylimidazolium chloride based ionic liquids. Chem. Commun., 668-669. c) Bernot, R.J, Kennedy, E.E., Lamberti, G.A., 2005. Effects of ionic liquids on the survival, movement, and feeding behaviour of the freshwater snail, *Physa acuta*. Environ. Toxicol. Chem., 24, 7, 1759-1765.

[27] a) Li, X.Y., Zhou, J., Yu, M., Wang, J.J., Pei, Y.C., 2009. Toxic effects of 1-methyl-3-octylimidazolium bromide on the early embryonic development of the frog *Rana nigromaculata*. Ecotox. Environ. Safety, 72, 2. 552-556. b)Yu, M., Li, S., Li, X., Zhang, B., Wang, J., 2008. Acute effects of 1-octyl-3-methylimidazolium bromide ionic liquid on the antioxidant enzyme system of mouse liver. Ecotoxicol. Environ. Safety, 71, 903– 908.

[28] Costello, D.M., Brown, L.M., Lamberti, G.A., 2009. Acute toxic effects of ionic liquids on zebra mussel (*Dreissena polymorpha*) survival and feeding. Green Chem.., 11, 4, 548-553.

[29] a) Stepnowski, P., Skladanowski, A.C., Ludwiczak, A., Laczyn´ska, E., 2004. Evaluating the cytotoxicity of ionic liquids using human cell line HeLa. Hum. Exp. Toxicol., 23, 11, 513-517. b) Ranke, J., Molter, K., Stock, F., Bottin-Weber, U., Poczobutt, J., Hoffmann, J., Ondruschka, B., Filser, J., Jastorff, B., 2004. Biological effects of imidazolium ionic liquids with varying chain lengths in acute *Vibrio fischeri* and WST-1 cell viability assays. Ecotoxicol. Environ. Safety, 58, 396–404. c) Ranke, J., Mu¨ller, A., Bottin-Weber, U., Stock, F., Stolte, S., Arning, J., Sto¨rmann, R., Jastorff, B., 2007. Lipophilicity parameters for ionic liquid cations and their correlation to in vitro cytotoxicity. Ecotox. Environ. Safety, 67, 430–438. d) Stolte, S., Arning, J., Bottin-Weber, B., Matzke, M., Stock, F., Thiele, K., Uerdingen, M., Welz-Biermann, U., Jastorff, B., Ranke, J., 2006. Anion effects on the cytotoxicity of ionic liquids. Green Chem., 8, 621–629. e) Frade, R.F.M., Matias, A., Branco, L.C., Afonso, C.A.M., Duarte, C.M.M., 2007. Effect of ionic liquids on human colon carcinoma HT-29 and CaCo-2 cell lines. Green Chem., 9, 8, 873–877. f) Wang, X., Ohlin, C.A., Lu, Q., Fei, Z., Hub, J., Dyson, P.J., 2007. Cytotoxicity of ionic liquids and precursor compounds towards human cell line HeLa. Green Chem., 9, 1191–1197. g) Kumar, A., Papaı¨conomou, N., Lee, J., Salminen, J., Clark, D., Prausnitz, J., 2008. In Vitro Cytotoxicities of Ionic Liquids: Effect of Cation Rings, Functional Groups, and Anions. Environ. Toxicol., 388-395. h) Salminen, J., Papaiconomou, N., Kumar, A., Lee, J., Kerr, J., Newman, J., Prausnitz, J., 2007. Physicochemical properties and toxicities of hydrophobic piperidinium and pyrrolidinium ionic liquids. Fluid Phase Equilibria, 261, 421–426. i) Garc´ıa-Lorenzo, A., Tojo, E., Tojo, J., Teijeira, M., Rodr´ıguez-Berrocal, F., P´erez Gonz´alez, M., Mart´ınez-Zorzano, V., 2008. Cytotoxicity of selected imidazolium-derived ionic liquids in the human Caco-2 cell line. Sub-structural toxicological interpretation through a QSAR study. Green Chem., 10, 508-516. l) Frade, R., Rosatella, A., Marques, C., Branco, L., Kulkarni, P., Mateus, N., Afonso, C., Duarte, C., 2009. Toxicological evaluation on human colon carcinoma cell line (CaCo-2) of ionic liquids based on imidazolium, guanidinium, ammonium, phosphonium, pyridinium and pyrrolidinium cations. Green Chem., 11, 1160-1165.

[30] a) Stock, F., Hoffmann, J., Ranke, J., Störmann, R., Ondruschka, B., Jastorff B., 2004. Effects of ionic liquids on the acetylcholinesterase – a structure–activity relationship consideration. Green Chem., 6, 286–290. b) Arning, J., Stolte, S., Bo¨schen, A., Stock, F., Pitner, W.R., Welz-Biermann, U., Jastorff, B., Ranke, J., 2008. Qualitative and quantitative structure activity relationships for the inhibitory effects of cationic head groups,

All the eco-toxicity studies reported in the literature demonstrate that many ILs can deeply affect aquatic and terrestrial organisms and their toxic effect increase by increasing the alkyl chain length of the cation; for the most common classes of ILs, the major contribution to the toxicity is basically due to the cation moiety, even if also the anion contribution could be significant towards some test species. Analogously the cellular and sub-cellular tests indicate the same trend of increasing toxicity by increasing lipophilicity.

The original contribution of this Thesis about ILs biological activity investigation regards how the oxygen atoms number and the chain length in the cation moiety of a specific class of ionic liquids can influence the toxicity, and if these parameters have the same effect at different biological organization levels. To this purpose several oxygenated imidazolium ILs, with a number of oxygen atoms in the lateral chain of the cation from 1 to 4, were synthesised (Chapter 3.1.1.) and tested in eco-toxicity assays, here described, and toxicity tests, described in Chapter 3.1.3., comparing their effect with that of alkyl imidazolium ionic liquids (Figure 3.1.2.1.). The toxicity of alkyl-substituted ionic liquids has been already deeply investigated and for this reason there is a relative plenty of data in the literature; on the other hand, the information about the biological profile of oxygenated imidazolium salts is still preliminary. Thus the aim of this work is to verify if the change in the chemical structure of the cation by introducing ethoxy units can affect in same way both the biological activity and the chemico-physical properties of the ionic liquids.

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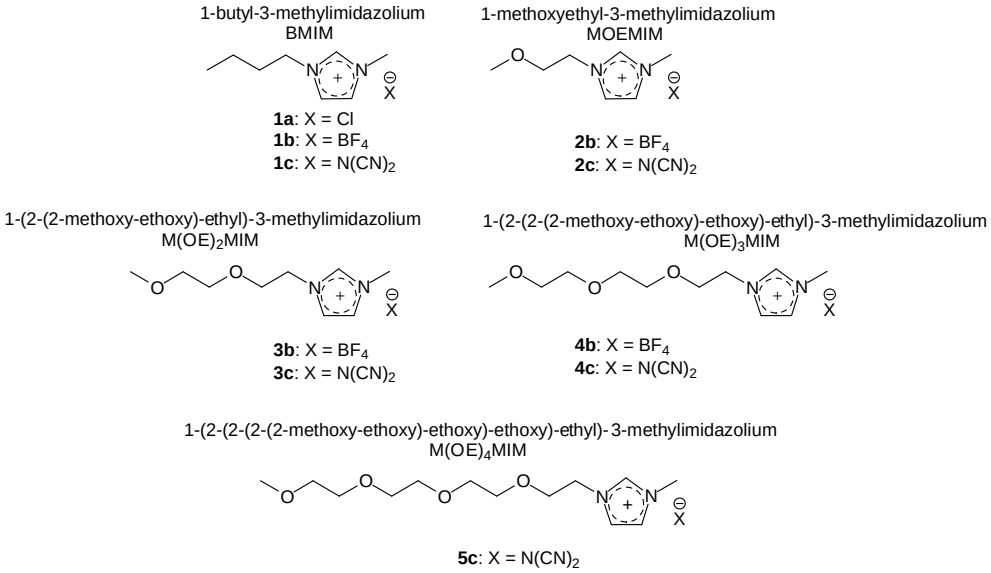
functionalised side chains and anions of ILs on acetylcholinesterase. Green Chem., 10, 47-58.

[31] Składanowski, A.C., Stepnowski, P., Kleszczyński, K., Dmochowska, B., 2005. AMP deaminase in vitro inhibition by xenobiotics: A potential molecular method for risk assessment of synthetic nitro- and polycyclic musks, imidazolium ionic liquids and N-glucopyranosyl ammonium salts. Environ. Toxicol. Phar., 19, 2, 291–296.

[32] a) Yu, M., Wang, S.H., Luo, Y.R., Han, Y.W., Li, X.Y., Zhang, B.J., Wang, J.J., 2009. Effects of the 1-alkyl-3-methylimidazolium bromide ionic liquids on the antioxidant defense system of *Daphnia magna*. Ecotoxicol. Environ. Safety, 72, 1798–1804. b) Lan Tee, K., Roccatano, D., Stolte, S., Arning, J., Jastorff, J., Schwaneberg, U., 2008. Ionic liquid effects on the activity of monooxygenase P450 BM-3. Green Chem., 10, 117–123.

Figure 3.1.2.1. Chemical structure of the tested imidazolium salts.



The eco-toxicological properties of the ILs were evaluated by acute toxicity tests with the crustacean *Daphnia magna* (immobilization), the bacterium *Vibrio fischeri* (inhibition of bioluminescence) and the diatoms *Skeletonema marinoi* and *Phaeodactylum tricornutum* (growth inhibition).

Toxicity towards *Daphnia magna*

Daphnia magna is an indicator species, particularly useful to test the effects of toxins on aquatic ecosystems because of its short lifespan and high reproductive capabilities. The studies reported in the literature about the biological activity of ILs towards *D. magna* regard their acute (Table 3.1.2.1.) and chronic toxicity[24b,33] and their effects on the antioxidant response system.[32a]

[33] a) Wells, A.S., Coombe, V.T., 2006. On the Freshwater Ecotoxicity and Biodegradation Properties of Some Common Ionic Liquids. Org Proc Res, 10, 794. b) Couling, D., Bernot, R., Docherty, K., Dixon, J., Maginn, E., 2006. Assessing the factors responsible for ionic liquid toxicity to aquatic organisms via quantitative structure–property relationship modeling. Green Chem., 8, 82-90. c) Bernot, R.J., Brueseke, M.A., Evans-White, M.A., Lamberti, G., 2005. Acute and chronic toxicity of imidazolium-based ionic liquids towards *Daphnia magna*. Environ Tox Chem., 24, 87-92. d) Luo, Y., Li, X., Chen, X., Zhang, B., Sun, Z., Wang, J., 2008. The Developmental Toxicity of 1-Methyl-3-Octylimidazolium Bromide on *Daphnia magna*. Environ Toxicol., 23, 6, 736-744.

Table 3.1.2.1. Acute toxicity of ILs towards *Daphnia magna* in a 48 h immobilization test (literature data).[24b, 32a, 33]

Entry	Ionic liquid	EC50 μM	Entry	Ionic liquid	EC50 μM
1	BMIM Cl	19-85	18	BPy NTf_2	4
2	BMIM Br	37-50	19	BMPy Br	57
3	BMIM BF_4	48	20	HPy Br	12
4	BMIM PF_6	71-95	21	HMPy Br	4
5	BMIM NTf_2	45	22	OMPy Br	2
6	$Cl(CH_2)_2$MIM Cl	>552	23	TBP	9
7	$Cl(CH_2)_2$MIM NTf_2	>233	24	CY169	29
8	$OH(CH_2)_2$MIM NTf_2	>245	25	CY101	0
9	$OH(CH_2)_3$MIM Cl	340	26	TBA	29
10	HMIM Br	6-11	27	ECOENG500	1
11	OMIM Br	0.05 - 3	28	AMMOENG130	0,9
12	DMIM Br	0.5	29	BMPyr NTf_2	88
13	C12MIM Br	0.2	30	EMMor Br	>476
14	C12MIM Cl	0.01	31	EBMor Br	>397
15	C16MIM Cl	0.01	32	ETHT Br	>507
16	C18MIM Cl	0.005	33	Et_3S Br	>502
17	BPy Cl	116			

The results reported in Table 3.1.2.1. indicate that ionic liquids toxicity towards *D. magna* follows the general trend found in each toxicity and eco-toxicity assay: the more lipophilic is a cation and the higher is its biological activity. For example, an increase of 8 (Entries 13 and 14) and 14 carbon units (Entry 16) in the lateral chain of imidazolium salts, contributes to increase the toxicity respectively of 3 and 4 orders of magnitude respect to 1-butyl-3-methylimidazolium salts (Entries 1 and 2).

All the methylimidazolium cations with short alkyl chain (Entries 6-8) show very low toxicity levels, in spite of different kind of final substituents on the lateral chain (e.g. chloride or hydroxyl group), being their EC50 higher than 100 mg/L, considered to be the limit between practically harmless and moderately toxic compounds.[34]

The general structure of the cation plays a clear role: hetero-aromatic cyclic cations as imidazolium and pyridinium, are more toxic than cyclic and acyclic aliphatic cations as morpholinium (Entries 30-31) and sulfonium

[34] Passino, D.R.M., Smith, S.B., 1987. Acute bioassays and hazard evaluation of representative contaminants detected in Great Lakes fish. Environ. Toxicol. Chem., 6, 901–907.

salts (Entries 32-33), practically harmless. Pyrrolidinium salts ion paired with NTf_2 (Entry 29) are similar to imidazolium analogues (Entry 5) and less toxic than pyridinium ones (Entry 18).
Long-chain quaternary phosphonium and ammonium salts, structurally similar to surfactants, have a similar highly toxic behaviour (Entries 24-25 and 27-28); on the contrary short chain ammonium salts (Entry 26) are less toxic than phosphonium analogues (Entry 23). Among the imidazolium salts, the anion species does not seem to influence the toxicity, as indicated by similar EC50 values of 1-butyl-3-methylimidazolium ILs differently ion-paired (Entries 1-5); also the anion NTf_2, known to be highly toxic for several species, does not influence the toxicity; however the anion PF_6 is slightly less toxic than the other ones. On the contrary, among pyridinium salts the anion species contributes to the toxicity in a stronger way than in the case of imidazolium salts, being the NTf_2 salt (Entry 18) two orders of magnitude more toxic than chloride salt (Entry 17).

The investigation of the chronic toxicity of ionic liquids through multigenerational studies reveals that these substances are able to exert a toxic effect on the development of both individual animals and population, and in particular the number of first brood, the brood size and the total number of offspring significantly decline at high concentrations of ionic liquids.[33c,d] Also the antioxidant response in *D. magna* is related to high exposure concentrations and above all to the length of the alkyl chain in the cation, demonstrating that oxidative stresses play an important role in ionic liquids induced toxicity.
In spite of acute and chronic results, the action mechanism of ILs towards *Daphnia magna* is still unknown; some hypothesis, including enzyme inhibition, disruption of membrane permeability and structural DNA damage, have been formulated, but they still need to be confirmed

In this Thesis the acute biological effects of alkyl and oxygenated imidazolium-based ILs towards *Daphnia magna*, were checked in two sets of experiments. The chemical structures of the tested ionic liquids, ion

paired with tetrafluoroborate and dicyanamide anions, are shown in Figure 3.1.2.1.

In a first preliminary experiment, the acute toxicity of 1-butyl-3-methylimidazolium tetrafluoroborate (BMIM BF_4, **1b**) was compared to that of 1-methoxyethyl-3-methylimidazolium tetrafluoroborate (MOEMIM BF_4, **2b**) and 1-methoxyethyl-3-methylimidazolium dicyanamide (MOEMIM $N(CN)_2$, **2c**), to investigate how the introduction of one oxygen in the lateral chain of imidazolium cations could affect the toxicity.

Subsequently, a further experiment was done to deepen the effect of increasing the chain length and the oxygen units in the cation, through a comparison between BMIM salts and oxygenated imidazolium ionic liquids bearing a number of oxygen atoms in the lateral chain from 1 to 4 (MOEMIM BF_4 **2b** and $N(CN)_2$ **2c**, $M(OE)_2MIM$ BF_4 **3b** and $N(CN)_2$ **3c**, $M(OE)_3MIM$ BF_4 **4b** and $N(CN)_2$ **4c**, and $M(OE)_4MIM$ $N(CN)_2$ **5c**).

The toxicity to *D. magna* was assessed using a 48 h static acute immobilization test according to the procedures established by the Organization for Economic Co-operation and Development Guideline 202.[35] The 50% effect concentrations (EC50) were calculated by fitting the experimental concentration–response curves to a logistic model using the nonlinear regression procedures implemented in Statistica software.

In the first experiment the toxicity was assessed in two trials, one in which BMIM BF_4 and MOEMIM BF_4 were comparatively tested, and one in which the two MOEMIMs salts were compared; in both the trials a reference toxicant ($K_2Cr_2O_7$) was checked along. Three replicates for each treatment concentrations were done.

Also in the second experiment the toxicity was assessed in two trials. In the first trial, BMIM BF_4 and $N(CN)_2$ were tested together with MOEMIMs, $M(OE)_2MIMs$ and $M(OE)_3MIMs$, while in the second trial, the $N(CN)_2$ series was compared (oxygen atoms in the lateral chain from 0 to 4). Two replicates for each treatment concentrations were done. In this experiment the differences in the EC50 values were tested by ANOVA. An orthogonal two-factor design (the cation and the anion were the two factors) was used

[35] Organization for Economic Cooperation and Development. 2004. *Daphnia* sp. Acute Immobilization Test. OECD Guideline 202. Paris, France.

for the first trial, instead a one-factor design was used in the second trial, since only one anion was tested. *Post-hoc* pair wise comparisons were performed by Student-Newman-Keuls (SNK) test.

The results of the experiments are shown in Table 3.1.2.2. and Table 3.1.2.3.

Table 3.1.2.2. First experiment: the 50% effect concentrations (EC50, μM) of different ionic liquids to *Daphnia magna* in a 48 h immobilization test (values in parentheses are the 95% confidence intervals).

	EC50 48 h μM	
	1st trial	2nd trial
BMIM BF_4	23 (22-24)	-
MOEMIM BF_4	917 (807-1026)	974 (855-1088)
MOEMIM $N(CN)_2$	-	1008 (980-1042)

Table 3.1.2.3. Second experiment: the 50% effect concentrations (EC50, μM) of different ionic liquids to *Daphnia magna* in a 48 h immobilization test (values in parentheses are the 95% confidence intervals).

	EC50 48 h μM	
	1st trial	2nd trial
BMIM BF_4	54 (28–107)	—
BMIM $N(CN)_2$	72 (29–179)	87 (49–155)
MOEMIM BF_4	774 (589–1018)	—
MOEMIM $N(CN)_2$	862 (687–1083)	1010 (816–1250)
$M(OE)_2MIM$ BF_4	930 (669–1291)	—
$M(OE)_2MIM$ $N(CN)_2$	1140 (910–1428)	1137 (923–1402)
$M(OE)_3MIM$ BF_4	650 (440–960)	—
$M(OE)_3MIM$ $N(CN)_2$	808 (413–1583)	1185 (869–1616)
$M(OE)_4MIM$ $N(CN)_2$	—	926 (784–1095)

Published EC50 value of BMIM BF_4 (Table 3.1.2.1., Entry 3) is in the same range of the values found in both the experiments, even if the slight differences could be explained by test water quality and experimental procedures.

The most important point which emerges from both the experiments with *D. magna* is that the introduction of one oxygen in the lateral chain of imidazolium cations reduces the toxicity of one order of magnitude, both with tetrafluoroborate and dicyanamide anions.

These results are confirmed by the ANOVA analysis and the *post-hoc* SNK test of the second experiment: the cation results in a significant contribute to the toxicity ($P < 0.001$) and the differences among all the oxygenated cations and BMIMs are statistically significant, indicating the alkyl cation as more toxic.

Analyzing the EC50 values of all the oxygenated compounds, it is clear that the major contribution to the toxicity reduction is given by the introduction of the first oxygen in the lateral chain of the cation: a further increasing of the oxygen atoms number does not further reduce the toxicity, as it is indicated by similar EC50 values of all the oxygenated ILs, and in fact the statistical analysis can not define clear-cut groups among them.

The crustacean seems being also sensitive to the anion, which results in a significant contribute to the toxicity by ANOVA analysis ($P = 0.032$), being the BF_4 salts slightly, but consistently, more toxic than the $N(CN)_2$ salts; we can exclude that this effect can be due to the formation of hydrofluoric acid as a consequence of the hydrolysis of BF_4 anion because all the stock solutions were prepared within 24 hours before the beginning of the tests. The anion dicyanamide confirms to be one of the less toxic anion for *D. magna*, in the same range of hexafluorophosphate salts (Table 3.1.2.1., Entry 4).

Toxicity towards *Vibrio fischeri*

The *Vibrio fischeri* acute bioluminescence inhibition assay is a standard eco-toxicological bioassay, widely used in environmental toxicity studies to screen aquatic ecosystems and to investigate the toxic behaviour of chemical compounds. Several different luminescence inhibition tests of *V.*

fischeri have been developed so far, most of them are designed for analysis of aqueous samples, such as Microtox®, which can be used into a wide range of applications, as the analysis of industrial effluent and discharges, waters, soils and sediments, and new products.

The database about the acute effect of chemical compounds towards *Vibrio fischeri* is very wide, and also the biological activity of ILs towards this species (Table 3.1.2.4.) is well documented in the literature.[23b,23d,29b,33b,36]

Table 3.1.2.4. Acute toxicity of ILs towards *Vibrio fischeri* in a 15 min inhibition of bioluminescence test (literature data). [23b,23d,29b,33b,36]

Entry	Ionic liquid	EC50 µM	Entry	Ionic liquid	EC50 µM
1	EMIM X	21380	27	OMIM X	10
2	OH-EMIM X	7762	28	NMIM BF_4	5
3	OH-PMIM X	>19953	29	DMIM BF_4	1
4	PMIM BF_4	8710	30	DMIM Cl	3
5	BMIM OTs	3311	31	DMIM X	1
6	BMIM BF_4	3500	32	C14MIM X	1
7	BMIM $OctSO_3$	70	33	C16MIM X	2
8	BMIM $(CF_3)_2N$	3000	34	C18MIM X	28
9	BMIM $N(CN)_2$	966-4677	35	BPy X	1514
10	BMIM Cl	897-5129	36	BPy $N(CN)_2$	410-2042
11	BMIM Br	1175-10232	37	BPy Cl	440
12	BMIM NTf_2	300-2454	38	BPy Br	538-2512
13	BMIM X	2951	39	BMPy $N(CN)_2$	98-457
14	BEIM BF_4	631	40	BMPy Br	130-562
15	EOMMIM X	10233	41	HMPy Br	30-115
16	MOEMIM X	15136	42	OMPy Br	2-6
17	EOEMIM X	19055	43	TMA Br	>100000
18	MOPMIM X	>10000	44	TEA Br	>100000
19	PentMIM BF_4	1380	45	TBA Br	1862
20	HMIM BF_4	1514	46	TBP Br	513
21	HMIM X	813	47	CY169	1175
22	HEIM BF_4	141	48	CY101	2570
23	HeptMIM BF_4	275	49	BMPyr X	>19953
24	HMIM Br	6-26	50	BMMor X	18621
25	OMIM Br	1-4			
26	OMIM BF_4	25			

[36] Docherty, K., Kulpa, C., 2005. Toxicity and antimicrobial activity of imidazolium and pyridinium ionic liquids. Green Chem., 7, 185–189.

The results reported in Table 3.1.2.4. indicate that also for *Vibrio fischeri***,** ILs toxicity increases by increasing the lipophilicity of the cation, according to a baseline toxicity which causes a non-specific disturbance of the structure functioning of biological membranes, being the longer alkyl chains able to be incorporated into the polar headgroups of the phospholipidic bilayer of the membranes, causing narcotic effects.

Specifically in the case of *V. fischeri*, by increasing of two carbon units the lateral chain of 1-ethyl-3-methylimidazolium cation (Entry 1), the toxicity increases of two orders of magnitude (Entries 13, 21, 27), with a further constant increase of one order of magnitude up to 1-decyl-3-methylimidazolium cation (Entry 31), 10000 times more toxic than 1-ethyl-3-methylimidazolium salts. However there is also an evidence of a cut-off for very lipophilic compounds with long alkyl side chains (> C_{10}) which causes a divergence from the correlation toxicity-lipophilicity, probably due to an insufficient water solubility of these cations (Entries 32, 33 and 34).

Interestingly, the introduction of one oxygen atom in the lateral chain of imidazolium-based cation, contributes to reduce the toxicity of the ionic liquids of one order of magnitude (Entries 15-18).

Pyridinium salts are more toxic than the imidazolium analogues (Entries 35-38); moreover the introduction of a methyl on the ring enhances the biological effects (Entries 39-40). The non-aromatic ILs, as short chain quaternary ammonium salts (Entries 43-44) and morpholinium cations (Entries 49-50), are among the least toxic compounds. Comparing the EC50 values of tetrabutyl ammonium salts (Entry 45) with the phosphonium analogues (Entry 46), it is clear that phosphonium ILs are more toxic, even if by increasing the chains length (Entries 47 and 48), the toxicity decreases.

The anion species seems to contribute to the toxicity in spite of the large disagreement among the literature data; tetrafluoroborate seems to be the least toxic species (Entry 6, 20 and 26), followed by chloride and dicyanamide (Entries 9 and 10), by bromide, tosylate and bis(trifluoromethyl)imide (Entries 24, 26, 5 and 6), by bis(trifluoromethylsulfonyl)imide (Entry 12), and by octylsulfate (Entry 7) which is the most toxic among the anion probably because of its high lipophilicity.

In this Thesis the biological effects of alkyl and oxygenated imidazolium-based ILs towards *Vibrio fischeri*, were checked in two sets of experiments, and arranged analogously to the experiments for *Daphnia magna*. The chemical structures of the tested ionic liquids, ion paired with tetrafluoroborate and dicyanamide anions, are shown in Figure 3.1.2.1.

In the first preliminary experiment, the acute toxicity of BMIM BF_4, **1b**, was compared to that of MOEMIM BF_4 **2b** and $N(CN)_2$ **2c**.

In the second experiment BMIM BF_4 **1b** and $N(CN)_2$ **1c** were analysed together with MOEMIM BF_4 **2b** and $N(CN)_2$ **2c**, $M(OE)_2MIM$ BF_4 **3b** and $N(CN)_2$ **3c**, $M(OE)_3MIM$ BF_4 **4b** and $N(CN)_2$ **4c**, and $M(OE)_4MIM$ $N(CN)_2$ **5c**.

The toxicity to *V. fischeri* was measured as inhibition of bioluminescence after 15 min using Microtox® equipment and consumables. The assay was carried out in accordance with the 90% basic test for pure compounds protocol as described in the Microtox user's manual.[37] The 50% effect concentrations (EC50) were calculated by fitting the experimental concentration–response curves to a logistic model using the nonlinear regression procedures implemented in Statistica.

In the first experiment the toxicity was assessed in three trials, in each one using three vials of Microtox® "reagent" (lyophilized *V. fischeri*) from the same lot. In each test, a reference toxicant ($ZnSO_4 \cdot 7H_2O$), BMIM BF_4, MOEMIM BF_4, and MOEMIM $N(CN)_2$ were tested simultaneously.

As for *Daphnia magna* the second experiment was divided in two sets: in the first the toxicity of BMIM BF_4 and $N(CN)_2$ were tested together with MOEMIMs, $M(OE)_2MIMs$ and $M(OE)_3MIMs$, while in the second, the $N(CN)_2$ series was compared (oxygen atoms in the lateral chain from 0 to 4). The experiment was performed in three trials, which were carried out using three vials of Microtox® "reagent". Due to constraints imposed by the protocol, it was not possible to test the substances simultaneously or to test all the substances using a single vial of bacteria. To approximate simultaneous testing, substances were tested according to a rotation scheme: the first trials for all the substances were carried out sequentially; when all

[37] Azur Environmental. 1998. 90% Basic Test for Pure Compounds. Carlsbad, CA, USA.

the substances were tested once, the second trials were carried out and then the third.

In the second experiment the differences in the EC50 values were tested by ANOVA. An orthogonal two-factor design (the cation and the anion were the two factors) was used for the first part of the experiment, in which alkyl and oxygenated (from 1 to 3 oxygen units) ILs were tested, instead a one-factor design was used in the second part, since only the anion dicyanamide was tested. *Post-hoc* pair wise comparisons were performed by Student-Newman-Keuls (SNK) test.

The results of the experiments are shown in Table 3.1.2.5. and Table 3.1.2.6.

Table 3.1.2.5. First experiment: the 50% effect concentrations (EC50s, μM) of different ionic liquids to *Vibrio fischeri* in a 15 min Microtox® inhibition of bioluminescence test (values in parentheses are the 95% confidence intervals. The confidence interval of the mean of the three trials is computed as $\bar{X} \pm t(2, 0.975)s/\sqrt{3}$).

	EC50 15 min μM			
	1st trial	2nd trial	3rd trial	Mean
BMIM BF_4	1115 (602-1624)	1743 (739-2748)	1128 (889-1363)	1327 (438-2221)
MOEMIM BF_4	14645 (7167-22123)	15864 (4868-26904)	11548 (7377-15719)	14018 (8491-19548)
MOEMIM $N(CN)_2$	9105 (5197-13014)	11571 (9376-13767)	14157 (5665-22650)	11610 (5337-17887)

Table 3.1.2.6. Second experiment: the 50% effect concentrations (EC50s μM) of different ionic liquids to *Vibrio fischeri* in a 15 min Microtox® inhibition of bioluminescence test (values in parentheses are the 95% confidence intervals. The confidence interval of the mean of the three trials is computed as $\bar{X} \pm t(2, 0.975)s/\sqrt{3}$).

	EC50 15 min μM	
	1st set	2nd set
BMIM BF_4	1635 (1442-1854)	—
BMIM $N(CN)_2$	1353 (723-2530)	—
MOEMIM BF_4	8093 (904-72493)	—
MOEMIM $N(CN)_2$	12078 (5660-25770)	8431 (6160–11540)
$M(OE)_2MIM$ BF_4	6151 (549-68959)	—
$M(OE)_2MIM$ $N(CN)_2$	8253 (3266-20856)	7011 (4726–10400)
$M(OE)_3MIM$ BF_4	2823 (284-28046)	—
$M(OE)_3MIM$ $N(CN)_2$	5311 (1229-22964)	4588 (2918–7215)
$M(OE)_4MIM$ $N(CN)_2$	—	448 (319–631)

Published EC50 values of BMIM $N(CN)_2$ and MOEMIM cation (Table 3.1.2.4., Entries 9 and 16) are in the same range of the values found in both the experiments. However the EC50 for BMIM BF_4 found here is about three times lower than the literature value (Table 3.1.2.4., Entry 6); the differences could be explained by different test water quality and experimental procedures.

A previous work by Stolte et al.[23d] has already demonstrated a reduction in the toxic effect of ILs towards *Vibrio fischeri* by increasing the polarity of the cation. The difference between the EC50 value of 1-butyl-3-methylimidazolium cation (Table 3.1.2.4., Entry 13) and 1-methoxyethyl-3-methylimidazolium cation (Table 3.1.2.4., Entry 16) found by Stolte et al., corresponds to the difference of one order of magnitude found in this work between BMIM BF_4 and MOEMIM BF_4, and between BMIM $N(CN)_2$ and MOEMIM $N(CN)_2$. The values reported in this Thesis are lower than those

reported in the literature with the same cations ion-paired with halides, probably because of a presuming toxic effect of BF_4 and $N(CN)_2$ anions.

As found for *Daphnia magna*, also for *Vibrio fischeri* the major contribute to reduce the toxicity is given by the introduction of one oxygen in the lateral chain of imidazolium cation. However the acute toxic effect of oxygenated ILs by increasing the length of the side chain is different from what observed with *Daphnia magna*: the further addition of ethoxy moieties causes an increase in the toxicity, and MOE_4MIM $N(CN)_2$ is the most toxic IL among the oxygenated ones, even showing a lower EC50 value than the alkyl BMIM $N(CN)_2$. It emerges that the bacterium is more sensitive to an elongation of the length chain than the crustacean, in spite of the increasing number of oxygen units which should increase the overall polarity of the cation.

The statistical analysis indicates that in the first set of the second experiment the cation is the only significant factor ($P < 0.001$), thus it is not possible to detect any anion effect. Again the *post-hoc* SNK test detects significant differences between BMIM and all the oxygenated cations, but no significant differences among the latter ones, in spite they clearly show a trend of increasing toxicity with increasing the length of the oxygenated side chain. However this trend is confirmed by the statistical analysis of the second set of the experiment in which the EC50 differences among cations are highly significant ($P < 0.001$). The toxicity clearly increases with the length of the oxygenated side chain and all the differences are significant, according to the SNK test, with the exception of difference between $M(OE)_2MIMs$ and MOEMIMs.

Toxicity towards algae

Algal dose–response bioassays have become widely used as biological tools in environmental impact studies to predict the effects of chemical compounds in aquatic environments. Growth inhibition tests with freshwater algae and seawater algae are included in the set of eco-toxicological tests recommended by the European Committee for Standardization (CEN), the International Organization for Standardization (ISO), and the Organization for Economic Cooperation and Development (OECD). Moreover they were

demanded by EU regulations (Dir. 67/548, 88/379, and 76/769; Reg. 793/93), and they are required by the new EU regulatory framework REACH.

So far, the toxicity of ILs towards algae has only been tested to a preliminary extent in few studies. The major part of them used green algae species, such as *Selenastrum capricornutum, Scenedesmus vacuolatus* and *quadricauda, Chlorella vulgaris, Chlamydomonas reinhardtii* and *Oocystis submarina* (Table 3.1.2.7.).[24b,38]

Table 3.1.2.7. Toxicity of ionic liquids towards green algae (literature data).[24b,38]

S. capricornutum					
EC50 μM 48h		**EC50 μM 72h**		**EC50 μM 96h**	
PMIM Br	2884	BMIM NTf_2	63	PMIM Br	1380
BMIM Br	2884	ClC2MIM Cl	421	BMIM Br	1047
BMIM PF_6	158	ClC2MIM NTf_2	167	BMIM Br	2138
BMIM Cl	220	C2OHMIM NTf_2	139	BMIM BF_4	3467
HMIM Br	371	HOC3MIM Cl	169	BMIM BF_4 OLD	148
OMIM Br	45	HMIM Cl	693	BMIM SbF_6	135
C12MIM Cl	0.004	BPy NTf_2	17	BMIM PF_6	1318
C16MIM Cl	0.01	BMPyr NTf_2	>237	BMIM BF_4	2512
C18MIM Cl	0.03	AMMOENG130	1.4	BMIM OTf	2188
BPy Cl	367	Chol PF_6	152	BMIM $OctSO_3$	2239
BPy Br	2884	EMMor Br	>476	BMIM Br	2137
BPyr Br	4677	EBMor Br	>397	BMIM Cl	2884
TBA Br	933			HMIM Br	288
ECOENG500	0.07			OMIM Br	38
TBP Br	79			BPy Br	4898
CY169	16			BPyr Br	12303
CY101	0.08			TBA Br	2239

[38] a) Cho, C., Pham, T., Jeon, Y., Vijayaraghavan, K., Choe, W., Yun, Y., 2007. Toxicity of imidazolium salt with anion bromide to a phytoplankton Selenastrum capricornutum: Effect of alkyl-chain length. Chemosphere, 69, 1003-1007. b) Pham, T., Cho, C., Min, J., Yun, Y., 2008. Alkyl-Chain Length Effects of Imidazolium and Pyridinium Ionic Liquids on Photosynthetic Response of Pseudokirchneriella subcapitata. J Biosci and Bioeng., 105, 425-428. c) Pham, T., Cho, C., Jeon, Y., Vijayaraghavan, Min, J., K., Yun, Y., 2008. Effect of imidazolium based ionic liquids on the photosynthetic activity and growth rate of Selenastrum capricornutum. Environ Tox Chem., 27, 7, 1583-1589. d) Cho, C., Pham, T., Jeon, Y., Yun, Y., 2008. Influence of anions on the toxic effects of ionic liquids to a phytoplankton Selenastrum capricornutum. Green Chem., 10, 67-72. e) Cho, C., Jeon, Y., Pham, T., Vijayaraghavan, K., Yun, Y., 2008. The ecotoxicity of ionic liquids and traditional organic solvents on microalga Selenastrum capricornutum. Ecotox Environ Safety, 71, 1, 166-171. f) Latała, A., Stepnowski, P., N˛edzi, M., Mrozik, W., 2005. Marine toxicity assessment of imidazolium ionic liquids: Acute effects on the Baltic algae *Oocystis submarina* and *Cyclotella meneghiniana.* Aquatic toxicology, 73, 91-98. g) Kulacki, K., Lamberti, G., 2008. Toxicity of imidazolium ionic liquids to freshwater algae. Green Chem., 10, 104.

Scenedesmus vacuolatus and *quadricauda*					
EC50 µM 24h				**EC50 µM 96h**	
EMIM X	603	C18MIM X	>0.01	BMIM Br	22
BMIM BF_4	130	C18MIM BF_4	0.005	HMIM Br	0.3
BMIM Cl	140	C2OHMIM X	>1000	OMIM Br	0.02
BMIM $OctSO_3$	60	CCNMIM X	>1000	BMIM Br[a]	60
BMIM NTf_2	50	C3OHMIM X	>1000	HMIM Br[a]	0.02
BMIM $(CF_3)N$	840	EOMMIM X	891	OMIM Br[a]	0.02
BMIM X	178	MOEMIM X	1820		
HMIM X	1	EOEMIM X	1778		
OMIM X	0.002	BPy X	389		
DMIM X	0.0003	BMPyr X	2344		
C14MIM X	0.003	MBMor X	>1000		
C16MIM X	>0.01				

C. reinhardtii		*C. vulgaris*		*O. submarina*	
EC50 µM 96h		**EC50 µM 72h**		**EC50 µM 72h**	
BMIM Br	4883	EMIM Cl	6331	EMIM Cl	13573
HMIM Br	1052	BMIM Cl	1026	BMIM Cl	2224
OMIM Br	145	HMIM Cl	64	HMIM Cl	753
BMIM Br[a]	9766	OMIM Cl	15	OMIM Cl	79
HMIM Br[a]	3443	DMIM Cl	4	DMIM Cl	8
OMIM Br[a]	184	BMPy Cl	2110	BMPy Cl	1924

[a] enriched medium

Recently Stepnowski et al.[39] studied also the biological effects of ionic liquids towards seawater diatoms, *Cyclotella meneghiniana*, *Skeletonema marinoi* and *Bacillaria paxillifer*, and towards the cyanobacterium *Geitlerinema amphibium* (Table 3.1.2.8.).

[39] a) Latała, A., N˛edzi, M., Stepnowski, P., 2009. Toxicity of imidazolium and pyridinium based ionic liquids towards algae. *Chlorella vulgaris*, *Oocystis submarina* (green algae) and *Cyclotella meneghiniana*, *Skeletonema marinoi* (diatoms). Green Chem., 11, 580-588. b) Latała, A., N˛edzi, M., Stepnowski, P., 2009. Toxicity of imidazolium and pyridinium based ionic liquids towards algae. *Bacillaria paxillifer* (a microphytobenthic diatom) and *Geitlerinema amphibium* (a microphytobenthic blue green alga). Green Chem., 11, 1371-1376. c) Latała, A., N˛edzi, M., Stepnowski, P., 2010. Toxicity of imidazolium ionic liquids towards algae. Influence of salinity Variations. Green Chem., 12, 60-64.

Table 3.1.2.8. Toxicity of imidazolium-based ionic liquids towards diatoms and cyanobacteria (literature data) in a 72 h growth inhibition test.[39]

	EC50 μM 72h			
	Skeletonema marinoi	*Cyclotella meneghiniana*	*Bacillaria paxillifer*	*Geilerinema amphibium*
EMIM Cl	112	59	34	31
BMIM Cl	3	7	6	4
HMIM Cl	1	2	2	0.9
OMIM Cl	0.4	0.7	1	0.1
DMIM Cl	0.08	0.3	0.9	0.02

All of these studies reached very interesting results, indicating a specific mechanism of toxic activity complexly correlated with several aspects, including the ionic liquid structure, the physiology of the algal species and the environmental conditions.

Influence of the ionic liquids structure

The ILs toxicity increases by increasing the lipophilicity of the cation, according to a baseline toxicity described above;[23d,33a,38a,b] however, as for *Vibrio fischeri*, there is also an evidence of a cut-off for very lipophilic compounds with long alkyl side chains (> C_{10}) which causes a divergence from the correlation toxicity-lipophilicity.

The effect of different cation species on the toxicity follows a clear trend: the long chain quaternary ammonium (AMMOENG) and phosphonium salts are the most toxic compounds, instead pyrrolidinium and morpholinium ILs are the least ones.[24b] Imidazolium and pyridinium-based ionic liquids, with various substituents on the lateral chain, are moderately toxic, and their effect increases by increasing the incubation time (on the contrary for ammonium and phosphonium salts the toxicity decreases by increasing the exposure time).[38a,38e] The introduction of one oxygen in the lateral chain of imidazolium salts can reduce the toxicity of the cation.[23d]

For many species (e.g. *Scenedesmus vacuolatus*) both the cation and the anion affect the toxicity. The most toxic anion is bis(trifluoromethylsulfonyl)imide (NTf_2), as confirmed by other bioassays,

for example with terrestrial organisms,[23b] followed by tetrafluoroborate (BF_4) which can generate fluoride upon hydrolysis,[38c,d] and trifluoromethanesulfonate (OTf) which is a very lipophilic anion strongly associated with the cation, enhancing the cell wall penetration ability of ILs.[39a]

Physiology of the algal species

Ionic liquids exhibit adsorption behaviour not only through hydrophobic interactions but also through ionic interactions: namely they have a great ability to accumulate at the interface between algal cells and water, causing a disruption of the integral membranes. Moreover the cell wall of algae is known to play a critical role in the transport of materials, including toxins, in and out of the cell, and the availability within the membrane structure depends on membranes surface chemistry. Stepnowski et al.[39] and Kulacki and Lamberti[38g] demonstrated that the sensitivity of different algae depends on different cell walls: diatoms, with silanol functional groups in the cell wall, are very good biosorbents for charged chemicals (e.g. heavy metals and ionic liquids), corresponding to a larger sensitivity than green algae; among green algae, those with a cellulose-made wall (e.g. *Scenedesmus quadricauda*) are less resistant than those with a glycoprotein cell wall (e.g. *Chlamydomonas reinhardtii*). Stepnowski[39a] suggested also that a further explanation for the higher sensitivity of diatoms relies in the presence of many proteins (frustulins, silaffins and pleuralins) on the cell surface, enriched in acidic amino-acids residues which can increase the ion-pairing with ionic liquids, causing a stronger interaction and a consequent stronger effect.

The chemical composition of the cell wall is not the only one parameter which influences the toxic process; another important physiological feature is the size of the algal cell, because smaller cells have an overall larger external surface area, which correspond to a more efficient transport of toxins into the cell. For example a 10-fold difference in the size should produce a 100-fold difference in sensitivity.[39a]

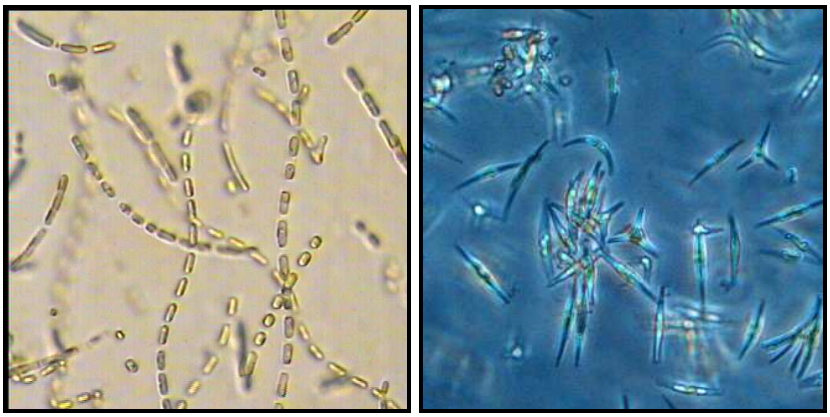
Being more resistant, some planktonic green algae species (e.g. *Ooocystis submarina*) appear to acclimatise to low concentrations of short chain imidazolium-based ILs, indicated by a restoring in the growth ability after few days of exposition.[38f] On the contrary, for benthic organisms (e.g. *Bacillaria paxillifer* and *Geitlerinema amphibium*) imidazolium salts with short lateral chain, as propyl or butyl, are more harmful than long chain ILs,[39b] which undergo a rapid and very strong adsorption to sedimentary matter before becoming bio-available for benthic species.

Environmental conditions

The physico-chemical features of the environment strictly influence the toxicity. For example, it has been observed that an increasing in the salinity of the algal water medium, causes a decreasing in the biological effect of ILs, probably due to a high chloride ions concentration which can reduce the interaction with algal cell wall by a stronger ion-pairing with ILs cations.[38f,39c] Moreover high concentrations of inorganic cations can compete with ILs cations for the anionic sites available on the algal surfaces. Both these effects can contribute to prevent the uptake of toxicant from the medium by the tested algal cells.

In this Thesis a preliminary experiment has been done to investigate the biological effects of 1-butyl-3-methylimidazolium chloride BMIM Cl **1a**, towards the diatoms *Skeletonema marinoi* and *Phaeodactylum tricornutum* (Figure 3.1.2.2.).

Figure 3.1.2.2. *Skeletonema marinoi* (left) and *Phaeodactylum tricornutum* (right) from Adriatic Sea.



Phaeodactylum tricornutum can be considered an "atypical" diatom:[40] its cells are of two characteristic types, oval and fusiform, the first possesses one only silica valve on each cell (the remainder of the cell wall being unsilicified), typical of a pennate diatom type, the second devoid of any organized siliceous structure; oval and fusiform cells both contain approximately the same amount of silica (0.4-0.5% dry weight).

For this peculiar conformation and because it has never been used before in toxicity assays with ionic liquids, *Phaeodactylum tricornutum* was chosen for the comparison with a "typical"diatom, *Skeletonema marinoi*.

There is only one work in the literature about *Skeletonema marinoi*, reported by Stepnowski et al.[39a] It describes the effect of the alkyl chain elongation on the growth inhibition, indicating that by increasing the lateral chain of imidazolium-based cation from 2 carbon units (1-ethyl-3-methylimidazolium) to 10 carbon units (1-decyl-3-methylinidazolium), the growth inhibition increases of 4 order of magnitude.

The two aims at the basis of the present study are:

i) checking the hypothesis of toxic action proposed by Stepnowski who suggests the interaction of the ILs with the proteins on the cell surface and with the silanol groups of the frustul; on the basis of what previously exposed, it is expected the *Phaeodactylum tricornutum*, an "atypical" diatom, will be less sensitive than *Skeletonema marinoi* to ILs;

ii) verifying the effect of the salinity on the growth inhibition of *Skeletonema marinoi*.

About the second point Stepnowski has already reported that by increasing the salinity of the water from 8 psu to 32 psu, the effect of ILs on the growth inhibition of green algae (*Oocystis submarina* and *Chlorella vulgaris*) and diatoms (*Cyclotella meneghiniana*) is reduced by 7 times.[39c] The strains of *Skeletonema marinoi* used by Stepnowski[39a] comes from the Baltic Sea and is adapted to a salinity of 8 psu; on the contrary, the strain of *Skeletonema marinoi* used in this Thesis comes from Adriatic Sea, which has a typical salinity value of 35 psu.

[40] Lewin, J., Lewin, R., Philpott, D., 1958. Observations on *Phaeodactylum tricornutum* J. *gen.* Microbiol., 18, 418-426.

The toxicity towards algae was assessed using a 72 h growth inhibition test, according to the procedures established by the Organization for Economic Co-operation and Development Guideline 201.[41] The 50% effect concentrations (EC50) were calculated by fitting the experimental concentration–response curves to a logistic model using the nonlinear regression procedures implemented in Statistica software (Statsoft, Tulsa, OK, USA).

The acute toxicity of 1-butyl-3-methylimidazolium chloride (BMIM Cl) was investigated towards both the species. In each experiment two replicates for each treatment concentrations were done.

The results of the experiments are shown in Table 3.1.2.9.

Table 3.1.2.9. Influence of ionic liquids on the growth of *Skeletonema marinoi* and *Phaeodactylum tricornutum* in a 72 h growth inhibition test.

	EC50 ± SE (μM)	
	Skeletonema marinoi	*Phaeodactylum tricornutum*
BMIM Cl	161.7 ± 13.2	2875 ± 9.7

As shown in Table 3.1.2.9., the EC50 values for the two diatoms are largely different: *Skeletonema marinoi* is more sensitive than *Phaeodactylum tricornutum*, being the EC50 of the first diatom (162 μM) lower of one order of magnitude than the EC50 of the other one (2875 μM).

The EC50 value here obtained with BMIM Cl for *S. marinoi* adapted to a salinity of 35 psu (162 μM), is higher of two order of magnitude than the EC50 reported in the literature with the same diatom adapted to a salinity of 8 psu (3 μM, Table 3.1.3.8.),[39a] and it is easy to ascribe this large difference to the different salinity conditions.

In spite of the preliminary character of this study, two important considerations can be done:

i) the salinity exerts a significant influence on ionic liquid toxicity. The results of this work indicate that at higher salinity algal growth is inhibited with a significantly lesser extent, specifically almost 50

[41] Organization for Economic Cooperation and Development. 2006. Freshwater Alga and Cyanobacteria, Growth Inhibition Test OECD Guideline 201. Paris, France.

times respect to low salinity. The salinity effect on ionic liquids toxicity towards diatoms and green algae reported in the literature by Stepnowski[39c] is less intense than what observed in this study, being the growth inhibition reduced by just eight–ten times. However this difference can be correlated to a higher sensitivity of *Skeletonema marinoi* than other kind of tested diatoms with bigger cell sizes, as *Cyclotella meneghiniana*;[39a]

ii) the difference of one order of magnitude in the EC50 values of *S. marinoi* and *P. tricornutum* probably indicates a mechanism of action which involves the silica cell wall or the proteins which synthesise the frustul. In fact the diatom (*P. tricornutum*) with less proteins/silica content is less sensitive to BMIM Cl of about 20 times.

Further experiments are in progress to deepen and understand the mechanism at the base of the difference in sensitivity of *S. marinoi* and *P. tricornutum*, and to extend the investigation also towards oxygenated ionic liquids to compare the results with what obtained for *D. magna* and *V. fischeri*.

A final comparison among the results obtained in the three eco-toxicological assays has been also done, by evaluating the hazard ranking of the different cations according to Pretti et al.[24b] and Passino et al.[34] (Table 3.1.2.10.).
The cations are classified as harmless (0, EC50 > 1000 mg/L), practically harmless (+, EC50 100–1000 mg/L), moderately toxic (++, EC50 10–100 mg/L), slightly toxic (+++, EC50 1–10 mg/L) and highly toxic (++++, EC50 0.1–1 mg/L).

Table 3.1.2.10. Hazard ranking for the imidazolium cations tested in the eco-toxicity assays.

	Daphnia magna	*Vibrio fischeri*	*Skeletonema marinoi*	*Phaeodactylum tricornutum*
BMIMs	++	+	++	+
MOEMIMs	+	0	nt[c]	nt[c]
$M(OE)_2MIMs$	+	0	nt[c]	nt[c]
$M(OE)_3MIMs$	+	0[a]/+[b]	nt[c]	nt[c]
$M(OE)_4MIMs$	+	+	nt[c]	nt[c]

[a] $N(CN)_2$ anion; [b] BF_4 anion; [c] nt: not tested

As indicated by Table 3.1.2.10. for *Daphnia magna* and *Skeletonema marinoi* the alkyl cation BMIM results moderately toxic independently by the anion (BF_4, $N(CN)_2$ and Cl), whereas for *Vibrio fischeri* and *Phaeodactylum tricornutum* it is pratically harmless.

All the oxygenated cations are pratically harmless for *Daphnia magna*, without any difference among them related to the anion.

On the contrary for *Vibrio fischeri* just the cations with 1 or 2 ethoxy units in the lateral chain are harmless, being their EC50 higher than 1500 mg/L; $M(OE)_4MIM$ is ranked in the category of pratically harmless compounds, being its EC50 (152 mg/L) in the range 100-1000 mg/l, whereas the cation $M(OE)_3MIM$ is in the category of harmless compound when ion-paired with $N(CN)_2$ anion, but classificable as pratically harmless if ion-paired with BF_4 (even if its EC50 is definitely higher than that of $M(OE)_4MIM$, being 890 mg/L).

Conclusions

The results here presented about tests with *Daphnia magna*, *Vibrio fischeri* and algae clearly indicate that the difference in toxicity between alkyl and oxygenated cations relies in differences of polarity, according to the general trend of decreasing toxicity by decreasing the lipophilicity.

Daphnia magna shows the higher sensitivity to ILs, followed by *Skeletonema marinoi* and by *Vibrio fischeri* and *Phaeodactylum tricornutum*. The EC50 values for the crustacean are in the micromolar range (50-80 μM) for BMIM cation and in the low millimolar range (0.8-1.2

mM) for compounds with a prolonged oxygenated lateral chain. For *V. Fisheri* and *Phaodactylum tricornutum* the values definitely move to the millimolar range.

A special remark should be done for the diatom *Skeletonema marinoi*: the data indicate a significant influence of salinity variations on algal toxicity, decreasing the EC50 by almost 50 times by increasing the salinity from 8 psu to 35 psu; thus the results show that the toxic effect of ILs on algae can be moderated by environmental conditions such as salinity. This is a clear indication that the composition of the abiotic environment has to be taken into account when the toxicity of ILs in various biological test systems is analysed.

It is worth to stress that both the patent of "fully green" than the epithet of "terrible toxicant" attributed to ionic liquids by the scientific community is unjustified. Indeed, ionic liquids generally are useful substances, whose (eco)-toxicological features are to be assessed in each individual case and for each possible application; here it has been shown that harmless variants of the more commonly used ionic liquids, such as polyoxygenated methyl imidazolium ones, are very attractive. In any case, ionic liquid solvents still maintain a large potential for helping to improve green chemistry.

Finally it is important to underline that a so large difference in EC50 values found with different organisms probably suggests difference mechanism of action species-dependent; the understanding of these detailed mechanisms is still unknown and represents one of the main goal in the evaluation of ionic liquids environmental impact and "greenness". Probably only by combining enzymatic and cellular *in vivo* tests with eco-toxicity assays involving several target organisms it will be possible to disclose the biological activity and identify ILs mode of action.

Experimental section

Ionic liquids

1-Butyl-3-methylimidazolium tetrafluoroborate (BMIM BF_4) was purchased from Merck (Darmstadt, Germany); the other ionic liquids were synthesised according the experimental procedures described in the Chapter 3.1.1. of the Thesis.

Daphnia magna acute toxicity assay

Daphnia magna was cultured at CIRSA, Interdepartmental Centre of Research for the Environmental Science, University of Bologna, (Ravenna, Italy), and maintained at 20 ± 1 °C under a 16:8 (light:dark) photoperiod.

The toxicity of ILs to *D. magna* was assessed using a 48-h static acute immobilization test according to the procedures set out in the Organization for Economic Co-operation and Development Guideline 202 (OECD, 2004). Five neonates (age, <24 h; born from parthenogenic females) were placed in each 100-mL beaker. Two replicate beakers for each of eight treatment concentrations (control plus seven toxicant concentrations) were prepared and maintained at 20 ± 1 °C under a 16:8 (light:dark) photoperiod. Each test vessel was checked for immobilized individuals at 24 and 48 h after the beginning of the test. Animals not able to swim within 15 s after gentle agitation of the test vessel were considered to be immobilized, even though they could still move their antennae.

In the first set of experiments, two trials were conducted. In the first trial, BMIM BF_4 and MOEMIM BF_4 were tested, while in the second trial, the two MOEMIMs salts were compared; in both the tests a reference toxicant ($K_2Cr_2O_7$) was checked. Test concentrations, identified through a preliminary range-finding test, were arranged in a geometric series ranging from 3 to 30 mg/L for BMIM BF_4 and from 100 to 400 mg/L for MOEMIM BF_4 and MOEMIM $N(CN)_2$.

In the second set of experiments, two experiments, each one including three independent trials, were conducted. In the first experiment, ILs BMIM BF_4 and $N(CN)_2$, MOEMIM BF_4 and $N(CN)_2$, $M(OE)_2MIM$ BF_4 and $N(CN)_2$ and $M(OE)_3MIM$ BF_4 and $N(CN)_2$ were tested; in the second one, the $N(CN)_2$ series (BMIM, MOEMIM, $M(OE)_2MIM$, $M(OE)_3MIM$ and $M(OE)_4MIM$). Within each trial, all the substances were tested simultaneously. Test concentrations of each ionic liquid, identified through a preliminary range-finding test, were arranged in geometric series.

Vibrio fischeri acute toxicity test

The freeze-dried luminescent bacteria, *Vibrio fischeri* (NRRL B-11177), and the reconstitution solution were supplied by Azur Environmental

(Carlsbad, CA, USA). The reagent was stored at -20 °C and rehydrated prior to testing. Toxicity to *V. fischeri* was measured as inhibition of bioluminescence using Microtox® (Strategic Diagnostics, Newark, DE, USA) equipment and consumables. The assay was carried out in accordance with the 90% basic test for pure compounds protocol as described in the Microtox® user's manual.

In the first set of experiments three trials were carried out using three vials of Microtox reagent from the same lot. In each test, a reference toxicant ($ZnSO_4 \cdot 7H_2O$), BMIM BF_4, MOEMIM BF_4 and MOEMIM $N(CN)_2$ were tested. Nine concentrations of each substance were tested in a 1:2 dilution series including a control. The highest concentration was 3600 mg/L for BMIM BF_4 and 72000 mg/L for MOEMIM BF_4 and MOEMIM $N(CN)_2$.

Also in the second set of experiments with all the oxygenated ionic liquids three trials were carried out for each substance using three vials of Microtox® "reagent" (lyophilized *V. fischeri*) from the same lot. Nine concentrations of each substance in a 1:2 dilution series and a control were tested. Due to constraints imposed by the protocol, it was not possible to test the substances simultaneously or to test all the substance using a single vial of bacteria. To approximate simultaneous testing, substances were tested according to a rotation scheme: the first trials for all the substances were carried out sequentially; when all the substances were tested once, the second trials were carried out and then the third.

The endpoint used to establish the concentration-response relationship was the bioluminescence of the bacteria, measured at each concentration as the ratio between the light emission after 15 min of exposure and the emission at time 0, expressed as a percentage of the same ratio in the control:

$$100 \frac{I_{15}/I_0}{I_{15}^c/I_0^c}$$

Where: I_0: light emission at time 0; I_{15}: emission after 15 min, I^c: emission of the control treatment. Light emission of the bacteria was measured using a Microtox® Model 500 Toxicity Analizer.

Skeletonema marinoi and *Phaeodactylum tricornutum* growth inhibition test

The diatoms *Skeletonema marinoi* and *Phaeodactylum tricornutum* were isolated from coastal waters of the Adriatic Sea.

The test algae were batch-cultured in f/2 medium prepared in distilled water. The culture salinity of 35 PSU was similar to that in the Adriatic Sea, the original environment of the test organisms.

The stock cultures of test organisms were acclimatised for 10 days at 20°C and illuminated with 25 mmol photons m^{-2} s^{-1} from daylight type fluorescent lamps (photoperiod 16 : 8).

The acute toxicity tests of ILs towards algae were carried out using modified versions of the methods recommended in the European Committee for Standardization's guidelines.[41] The main modifications were the use of f/2medium, the photoperiod and the choice of the test strains.

The final batch cultures used in the experiments were obtained by mixing a known amount of cells in the log growth phase with sterile medium. The initial cell number was constant and was measured as optical density (OD) at two wavelengths (665 and 750 nm). The optical densities of *S. marinoi* at 665 nm and 750 nm were 0.022 and 0.020 respectively; the optical densities of *P. tricornutum* at 665 nm and 750 nm were 0.043 and 0.040 respectively

Algal suspension aliquots of 5 mL were transferred to glass conical flasks (75 mL), and to each of these, different volumes of an aqueous IL stock solution were added. The final concentrations of BMIM Cl experiments ranged from 21 μM to 350 μM for in *S. marinoi*, and from 404 μM to 6197 μM for *Phaeodactylum tricornutum*. All experiments were run in duplicate.

After 72 h incubation the number of cells in the cultures was determined by OD measurement. The variability of the results did not exceed 5% on the inhibition scale. The ILs were tested on a wide range of concentrations, which enabled the EC50 values to be calculated.

Data analysis.

The 50% effect concentration (EC50) of each substance for *D. magna, V. fischeri* and algae was estimated by fitting the experimental concentration-response curves to a logistic model:

$$y = \frac{a}{1 + \left(\frac{x}{EC50}\right)^{b}}$$

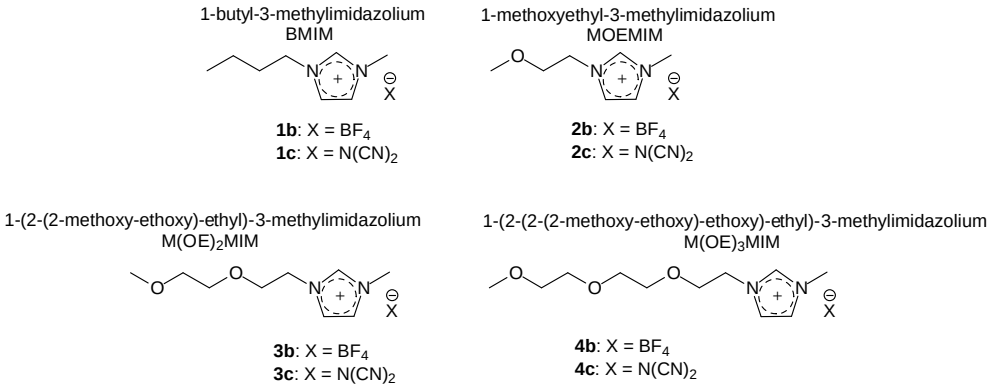
Where: y = endpoint value; x = substance concentration; a = expected endpoint value in absence of toxic effect; b = slope parameter. The parameters of the equation, including the EC50, were estimated using the non-linear regression procedures implemented in Statistica (Statsoft, Tulsa, OK, USA). The statistical significance of the differences among EC50s was tested by one-way analysis of variance (ANOVA). Once ANOVA resulted significant, the *post-hoc* Student-Newman-Keuls (SNK) test was carried out to identify which substances were significantly different from each other. The statistical tests were performed on log transformed data to achieve homogeneity of variances.

3.1.3. Toxicological properties

In the latest years the toxic effect of ionic liquids at cellular and sub-cellular levels have been deeply explored, by investigating the effects on cell viability and on enzymatic activity. The main result which emerges from this kind of studies is the confirmation of the trend “increasing lipophilicity -increasing toxicity”, related to the incorporation of long alkyl chains into the polar headgroups of the phospholipidic membranes bilayer.

In this Thesis a combined toxicological study was performed through the application of different testing protocols to alkyl and oxygenated imidazolium ILs (Figure 3.1.3.1.), previously tested in the eco-toxicity assays towards *Daphnia magna*, *Vibrio fischeri* and algae (Chapter 3.1.2.). A set of tests was performed by using rat pheochromocytoma (PC12) cell lines: the spectrophotometric MTT-test was employed to evaluate cell viability; the acetylcholinesterase (AChE) inhibition was measured since neurotoxicity has been indicated as a possible effect of ionic liquids, being the enzyme AChE a possible target of these compounds; the leakage of lactate dehydrogenase (LDH), frequently used as an end-point for cytotoxicity studies, was assessed as an end-point of membrane integrity.

Figure 3.1.3.1. Imidazolium-based ionic liquids, ion paired with tetrafluoroborate and dicyanamide anions, tested at cellular and sub-cellular levels.



Cell viability

The use of mammalian cells cultures (human and animal) for a screening of the hazard assessment of chemical compounds is an innovative and very important approach in environmental sciences. Generally, cell cultures

provide a quick and convenient mean to gather information about biological activities of chemical substances, avoiding *in vivo* testing and the ethical and practical associated problems.

The effects of several ILs on cell viability have been previously investigated in different human and animal cell lines, including some cells which derive from tissues that undergo the first contact between the organism and a toxin, such as human breast cancer cells (MCF7),[29g,h] prototypical human epithelial cells (HeLa, HT-29 and Caco-2)[29a,e,f,i,l] and promyelotic rat cells (the leukemia cells IPC-81 and the glioma cells C6).[29b-d] These studies reveal that both the cation and anion species contribute to the reduction of cell viability, and once again, a correlation between biological effect, inducing the cell death, and lipophilicity of the IL is found, allowing to presume a mechanism of action that involves the interaction of long alkyl chain with plasmatic membrane.

Specifically, the lipophilicity of the cation does not play a significant inhibition growth factor up to six carbon units in the lateral chain, but a further increase of the length restores the lipophilicity-toxicity linear correlation.

In general cations with short chain alkyl groups have a critical concentration window over which they “jump” from nearly non toxic to highly toxic, instead cations with long alkyl chains show a gradual increase in the toxicity when increasing the concentrations.

Interestingly the presence of ethoxy units, as in the cation 1-(2-(2-methoxy-ethoxy)-ethyl)-3-methylimidazolium $M(OE)_2MIM$, does not affect the cell viability, confirming the results obtained with the eco-toxicity assays reported in the Chapter 3.1.2. of the Thesis and in the literature.

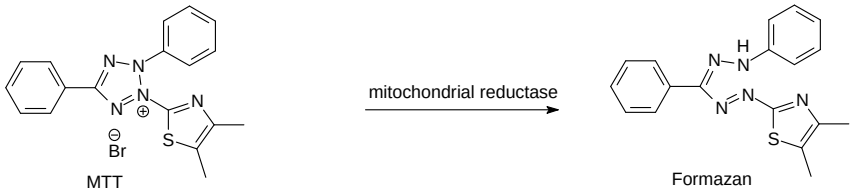
As reported also for *Daphnia magna*, ILs with aromatic cyclic cations as imidazolium and pyridinium, are more toxic than those containing cyclic and acyclic cations like short chain ammonium and phosphonium; however long chain ammonium salts, as Aliquat, are the most toxic compounds, inducing high toxicity and killing most of the cells even at low doses.

Regarding the anion, the lipophilicity and/or the vulnerability to hydrolytic cleavage seem to be the key features leading to anion cytotoxicity: highly

fluorinated anions as SbF_6, NTf_2, $N(CF_3)_2$ or $(C_2F_5)_3PF_3$, are very toxic, whereas halides are the least toxic ones. For example bis(trifluoromethylsulfonyl)imide (NTf_2) ion paired with short chain cation is one order of magnitude more toxic than bromide, but by increasing the chain length in the cation, the anion effect disappears and the predominant factor which leads the toxic action is just the lipophilicity. Tetrafluoroborate and dicyanamide anions show a three times higher effect than halides.

In this Thesis the cellular responses of the pheocromocytoma rat cell line PC12 exposed to ionic liquids were investigated through the spectrophotometric MTT-test, a sensitive and quantitative colorimetric assay that measures cell viability based on the ability of mitochondrial succinyl dehydrogenase in living cells to convert the yellow substrate (MTT) into a dark blue formazan product (Figure 3.1.3.2).

Figure 3.1.3.2. MTT reduction to formazan in living cells.



MTT
3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
(yellow)

Formazan
1,3,5-Triphenyltetrazolium
(purple)

1-Butyl-3-methylimidazolium tetrafluoroborate, BMIM BF_4, was tested as prototypical ionic liquid; its EC50 value was calculated from seven independent experiments run in quadruplicate, and compared with the literature values (Table 3.1.3.1.).

Then the cytotoxic effects related to the elongation of the lateral chain and the increasing of the oxygen atoms number were evaluated in a second set of experiments where cells were exposed to the other ILs represented in Figure 3.1.3.1. The screening was performed comparing the effects of all the ionic liquids used at 1 mM concentration, chosen on the basis of the EC50 values obtained for BMIM BF_4 (Figure 3.1.3.3.).

Table 3.1.3.1. EC50 values (mM) of BMIM BF_4 and the standard error associated, SE (n=7, number of independent experiments) determined using the MTT test, and comparison with the literature data with other cell lines.

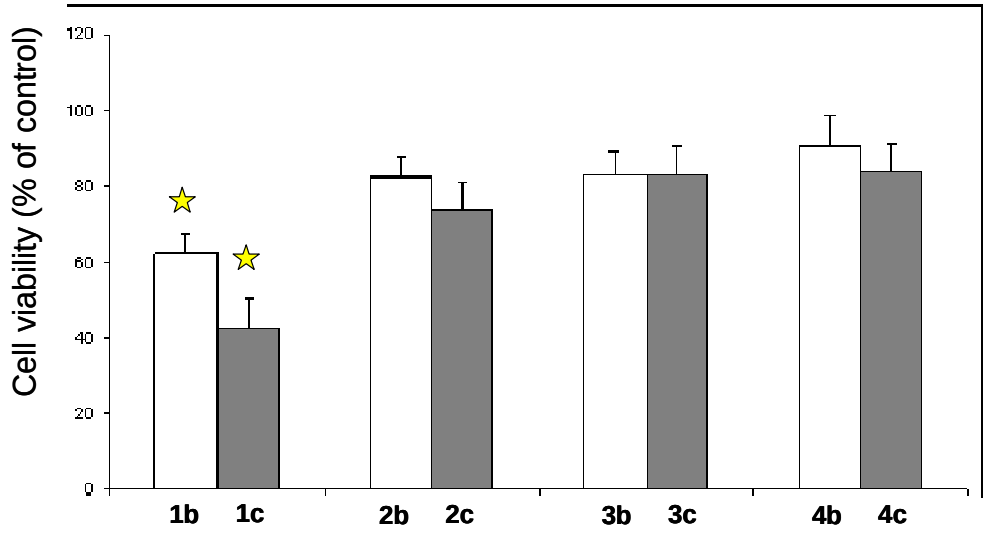
Cell line	EC50 ± SE (mM)
pheocromocytoma rat cell PC12	1.06 ± 0.07 (present work)
rat leukemia cells IPC-81	1.32[29b]-1.70[29d]
rat glioma cells C6	>1.00[29b]
human epithelial cells Caco-2	>3.02[29e]
human epithelial cells HeLa	4.55[29f]-5.30[29a]
human epithelial cells HT-29	>6.03[29e]

The EC50 value for BMIM BF_4 here obtained by the MTT test using pheocromocytoma rat cell line PC12 is in agreement with that obtained with the other rat cell lines tested in the literature (IPC-81 and C6). As indicated by the EC50 values reported in Table 3.1.3.1., human cell lines seem to be slightly less sensitive than animal ones.

It is interesting to note that Ranke et al.[29b] found a correlation between the cytotoxic effect of short alkyl chain ILs towards cell lines and acute eco-toxicity effect towards the bacterium *Vibrio fischeri* (in spite of the different incubation time of the two tests, e.g. 48 h *vs* 30 min), explainable by the fact that both the cell viability and the inhibition of bioluminescence indicate the metabolic state of the cells. This correlation deposes also in favour of the hypothesis that ILs interact with a very basic physiological mechanism, common to both prokaryotic and eukaryotic cells.

The data presented in this Chapter and in Chapter 3.1.2., confirm the proposed correlation, ranging the EC50 value of BMIM BF_4 for *Vibrio fischeri* from 1.32 mM to 1.63 mM.

Figure 3.1.3.3. Effects of different ILs on cell viability assessed on a rat PC12 cell line. Data are expressed as the mean ± SE of seven independent experiments, each run in quadruplicate, ☆ = P<0.001 versus unexposed cells (control).



The results presented in Figure 3.1.3.3. indicate the higher cytotoxicity of the BMIM cation, paired with both BF_4 **1b** or $N(CN)_2$ **1c** anion, respect to the oxygenated cations, independently by the anion and the number of ethoxy units in the lateral chain. This trend is in agreement with the data reported by Frade et al.[29e], who stated that the presence of a polar group in the lateral chain of imidazolium cations enhances the cell viability, being for example $M(OE)_2MIM\ BF_4$ and $M(OE)_2MIM\ PF_6$ practically harmless if compared with the alkyl analogues.

BMIM cations result to be the only ILs which cause a significant effect on cell viability respect to the control.

Acetylcholinesterase (AChE) inhibition assay

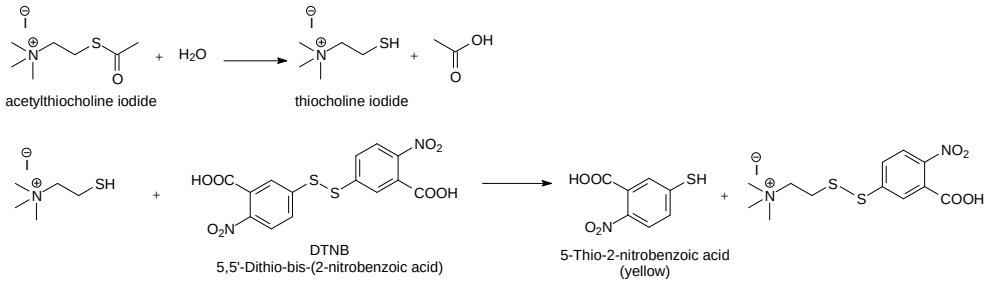
The influence of ionic liquids on the activity of the enzyme acetylcholinesterase has been widely studied in these last years by the group of Prof. Bernard Jastorff, University of Bremen (Germany).[23c,30] They were the first to suggest this enzyme as a target for ILs biological activity, mainly through a structure-activity analysis. Imidazolium and pyridinium ILs for example, have the core structure of the cation characterised by quaternary nitrogen atoms combined with certain lipophilicity, which seem to be key

factors in the drug design of new pharmaceutical drugs for the treatment of diseases of the nervous system.[42] The cationic head groups of ILs can be bounded through the positively charged aromatic core to the negatively charged residues at the entrance of acetylcholinesterase active site, and the lipophilic side chains can enforce this interaction by a strong affinity with the narrow gorge at the entrance of the active site, rich in lipophilic aromatic amino residues.

Interestingly Arning et al.[30b] found that polar functionalised side chains (ether or hydroxyl) exhibit a lower inhibitory potential than their lipophilic alkyl analogues.

In this Thesis the sub-cellular responses of the pheocromocytoma rat cell line PC12 exposed to ionic liquids were investigated through the inhibition of the intracellular AChE, using a colorimetric assay based on the reduction of the dye 5,5'-dithio-bis-(2-nitrobenzoic acid) (DTNB) to the yellow coloured 5-thio-2-nitrobenzoic acid by the thiocholine moiety obtained after AChE hydrolysis of the substrate acetylthiocholine iodide (Figure 3.1.3.4.).

Figure 3.1.3.4. DTNB reduction to 5-thio-2-nitrobenzoic acid in living cells.



1-Butyl-3-methylimidazolium tetrafluoroborate, BMIM BF_4, was tested as prototypical ionic liquid; its EC50 value was calculated from four independent experiments run in quadruplicate, and compared with the literature value (Table 3.1.3.2.).

Then the inhibitory effects related to the elongation of the lateral chain and the increasing of the oxygen atoms number were evaluated in a second set of experiments where cells were exposed to the other ILs represented in

[42] Kaur, J., Zhang, M., Curr. 2000. Med. Chem., 7, 273–294.

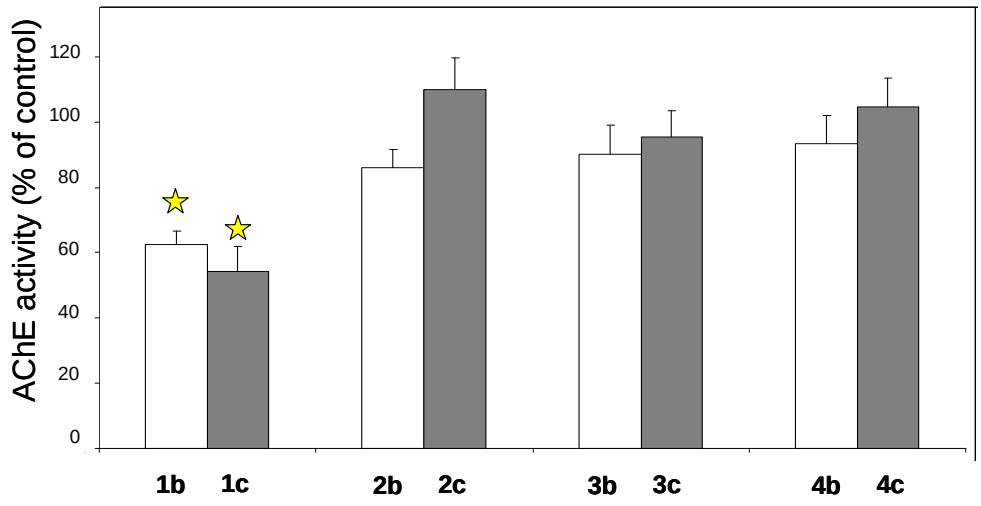
Figure 3.1.3.1. All substances were used at the concentration of 1 mM, chosen considering the EC50 values obtained for BMIM BF_4 (Figure 3.1.3.5.).

Table 3.1.3.2. EC50 values (mM) of BMIM BF_4 and the standard error associated SE (n=4, number of independent experiments), determined using AChE inhibition assay, and comparison with the literature data.

AChE	**EC50 ± SE (mM)**
AChE from pheocromocytoma rat cell PC12	0.54 ± 0.13 (present work)
AChE from electric eel (*Electrophorus electricus*)	0.15[30a]
AChE from electric eel (*Electrophorus electricus*)	0.08[30b] (*)

(*) BMIM halide

Figure 3.1.3.5. Effects of different ILs on AChE inhibition assessed on a rat PC12 cell line. Data are expressed as the mean ± SE of four independent experiments, each run in quadruplicate, ☆ = P<0.001 versus unexposed cells (control value = 6.20 ± 0.58 nmol min^{-1} mg $protein^{-1}$).



The EC50 value of BMIM BF_4 is in the same range of the values reported in the literature, obtained with AChE from electric eel (*Electrophorus electricus*).

From Figure 3.1.3.5. it emerges that, as for the cell viability assay, the oxygenated cation salts do not inhibit the activity of AChE, independently by the anion and the number of ethoxy units in the lateral chain, the

measured activity of AChE being very similar to that of the control test. These result are in line with the data of Arning et al.[30b] who have reported an increase of one and two orders of magnitude in the EC50 value of BMIM halide (0.082 mM) when one oxygen is introduced in the alkyl chain as ether (MOEMIM, EC50 = 0.38 mM) or as hydroxyl group ($HO(CH_2)_3MIM$, EC50 = 1 mM) respectively.

Lactate dehydrogenase (LDH) release

The present work represents the first study concerning the evaluation of the lactate dehydrogenase (LDH) release after exposition of cell culture to ILs, even if this assay is a frequently used test for cell viability, being the leakage of lactate dehydrogenase a clear end-point of membrane integrity.

In this Thesis the cellular responses of the pheocromocytoma rat cell line PC12 exposed to ionic liquids were investigated through the LDH release, a spectrophotometric assay used as an end-point for cytotoxicity studies, based on the measurement of LDH released from the cytosol of damaged cells into the culture medium. An increase in the amount of membrane-damaged cells results in the increase of LDH enzyme activity in the culture medium, spectroscopically measured through the reduction of pyruvate by the enzyme with NADH.

1-Butyl-3-methylimidazolium tetrafluoroborate, BMIM BF_4, was tested as prototypical ionic liquid; its EC50 value was calculated from three independent experiments run in triplicate, and confronted with the literature value of cationic surfactants (Table 3.1.3.3.). In the literature the LDH release associated with the exposition to surfactants has been already measured[43] using cultured human cells (keratinocytes and fibroblasts); among all the surfactants, cetyl trimethylammonium bromide (CTAB) and benzetonium chloride are the ones with the major structural similarities to ILs, both of them bearing a cationic head group with longer alkyl chains (Figure 3.1.3.6.).

[43] a) Van Ruissen F., Le M., Carroll J.M., van der Valk P.G.M., Schalkwijk J., 1998. Differential effects of detergents on keratinocyte gene expression. J. Invest. Dermatol., 110, 358–363. b) Arechabala, B., Coiffard, C., Rivalland, P., Coiffard, L., de Roeck-Holtzhauer, Y., 1999. Comparison of Cytotoxicity of Various Surfactants Tested on Normal Human Fibroblast Cultures using the Neutral Red Test, MTT Assay and LDH Release. J. Appl. Toxicol., 19, 163–165.

Then the inhibitory effects related to the elongation of the lateral chain and the increasing of the oxygen atoms number were evaluated in a second set of experiments where cells were exposed to the other ILs represented in Figure 3.1.3.1. All substances were used at the concentration of 1 mM, chosen considering the EC50 values obtained for BMIM BF_4 (Figure 3.1.3.7.).

Figure 3.1.3.6. Chemical structure of the cationic surfactants investigated in the literature for the LDH release.

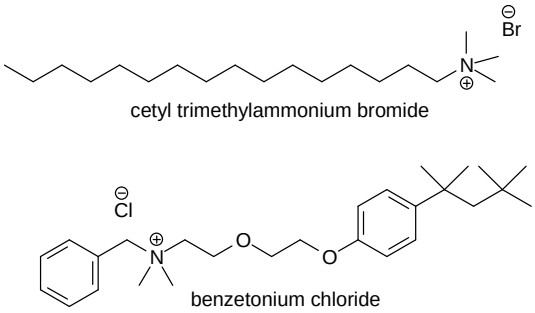


Table 3.1.3.3. EC50 values (mM) of BMIM BF_4 and the standard error associated SE (n=3, number of independent experiments), determined using LDH release assay, and comparison with the literature data on ammonium salts.

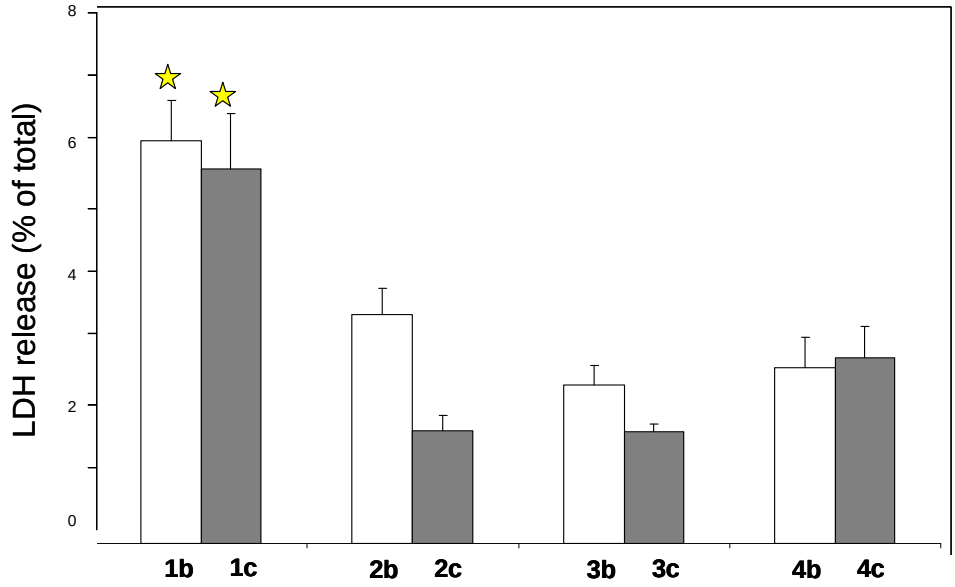
Compound	**EC50 ± SE (mM)**
BMIM BF_4	1.66 ± 0.22 (present work)
Cetyl trimethylammonium bromide	0.008[43a]
Benzetonium chloride	0.01[43b]

As shown in Table 3.1.3.3. both the EC50 values reported for long chain ammonium salts are two orders of magnitude lower than the EC50 value found in the present study for BMIM BF_4; these data support the typical trend at the base of the biological effects of ILs and cationic surfactants, confirming again that an increasing in the length of alkyl chains in cationic head group determines a higher toxicity.

The EC50 value for BMIM BF_4 in lactate dehydrogenase release test, end-point for cytotoxic effects, is well in agreement with the other value

obtained in this Thesis as measure of cell viability, the MTT test (Table 3.1.3.1.), being the EC50 1.66 mM and 1.06 mM respectively.

Figure 3.1.3.7. Effects of different ILs on LDH release assessed on a rat PC12 cell line. Data are expressed as the mean ± SE of three independent experiments, each run in triplicate, ☆ = P<0.001 versus unexposed cells (control value = 2.60 ± 0.31%).



From Figure 3.1.3.7. it emerges that, as for the cell viability assay, BMIM BF_4 and BMIM $N(CN)_2$ are the only two ILs which give a toxic effect significantly different from the control. MOEMIM $N(CN)_2$ and $M(OE)_2MIM\ N(CN)_2$ are the least toxic substances, but a no defined trend can be related to the elongation of the chain in the cation.

Conclusions

The effects of oxygenated imidazolium-based ILs have been assessed at cellular and sub-cellular levels, by MTT test, AChE inhibition assay and LDH release assay using cultured cell lines. Independently by the biological approach, all results are in agreement, showing a lower toxicity for compounds with oxygenated lateral chains than for those having purely alkyl lateral chains (BMIMs). As for eco-toxicity tests, these results indicate that an appropriate choice of cation and anion structures is important not

only to design the IL with improved and suitable chemico-physical properties but also to obtain safer and eco-friendly ILs.

The EC50 values for BMIM BF_4, here used as prototypical ionic liquid, are very similar in both the cell viability tests (MTT and LDH leakage).

Comparing the cellular responses with the eco-toxicity data reported in Chapter 3.1.2., it emerges that either in bacterial (*Vibrio fischeri*) or in mammalian cell cultures, the sensitivity to the ILs is lower than in crustacean or diatoms, being the EC50 values of BMIM BF_4 in the range of millimolar for the cellular responses and for the bacterium, and in the range of micromolar for the toxicity towards *Daphnia magna* and *Skeletonema marinoi*.

Therefore, in *vivo* and in *vitro* experiments are in progress to deepen the physiological responses affected by different ILs and to study the alteration of the biological membranes physical properties, possibly related to ILs mechanisms of action.

Experimental section

Ionic liquids

1-Butyl-3-methylimidazolium tetrafluoroborate (BMIM BF_4) was purchased from Merck (Darmstadt, Germany); the other ionic liquids were synthesised according the experimental procedures described in the Chapter 3.1.1. of the Thesis.

Cell culture

Rat pheochromocytoma PC12 cells were cultured in the Interdepartmental Centre of Research for the Environmental Science (CIRSA), University of Bologna, (Ravenna, Italy). Cells were grown in high glucose D-MEM (Dulbecco's Modified Eagle Medium), supplemented with 10% heat-inactivated horse serum, 5% heat-inactivated foetal calf serum, 1% penicillin-streptomycin. The cells were cultured at 37 °C in an incubator with humidified air and 5% CO_2. Approximately 2.5 x 10^5 cells were plated 2-3 days before each experiment into 6-well cell culture plates for AChE assay, and 1.25 x 10^5 cells into 12-well cell culture plates for MTT and LDH determinations. Cell exposures to ILs were always performed for 16h

at 37 °C in 5% CO_2. The experiments were organized in two sets. In a first set (concentrations ranging from 0.5 to 25 mM) the EC50 value for BMIM BF_4 was calculated; in a second set cells were exposed to a single concentration of different ILs. EC50 corresponds to the concentration causing the 50% of the maximal effect.

MTT assay

The MTT assay is a sensitive and quantitative colorimetric assay that measures cell viability based on the ability of mitochondrial succinyl dehydrogenase in living cells to convert the yellow substrate (MTT) into a dark blue formazan product. The assay was performed as described by Mosmann et al., 1983. After cell exposure to ILs the culture medium was replaced with 1 mL of MTT solution in DMEM and incubated at 37 °C in 5% CO_2 for 1.5 h. Then the medium was carefully removed and formazan crystals were dissolved in 1 mL of isopropanol and centrifugated for 2 min at 15,000 x *g*. The absorbance was measured using a Multi Sample DU800 Beckman spectrophotometer at a wavelength of 570 nm (test) and 650 nm (background) respectively. Results are given as percentage of the control absorbance.

AChE inhibition

The inhibition of the intracellular AChE was measured using a colorimetric assay based on the reduction of the dye 5,5'-dithio-bis-(2-nitrobenzoic acid) (DTNB) by the thiocholine moiety obtained after AChE action on the substrate acetylthiocholine iodide. After the experimental treatments, cells were washed with ice-cold phosphate-buffered saline solution (PBS), detached by scraping and centrifuged for 10 min at 800 × g at 4 °C. The pellet was resuspended in ice-cold 100 mM Na-phosphate buffer, pH 7.4, containing 1% Nonidet-P40. After 1 h on ice, homogenate was centrifuged at 3000 × g at 4 °C for 10 min and the resulting supernatant was used for the intracellular enzyme determination. The assay was performed using the Ellman procedure[44] with acetylthiocholine iodide as substrate, and sample

[44] Ellman G.L., Courtney K.D., Andres V., Featherstone R.M., 1961. A new and rapid colorimetric determination of acetylcholinesterase activity. Biochem. Pharmacol., 7, 88 – 95.

protein concentration was estimated according to Lowry et al.[45]using bovine serum albumin as standard. In a typical assay, 0.06 mg of protein was incubated at 30 °C in a final volume of 1.2 mL containing: 100 mM phosphate buffer, pH 7.4 and 0.3 mM DTNB. The enzymatic activity was quantified spectrophotometrically at 412 nm, monitoring the incubation at 1 min intervals for 5 min. The AChE activity was expressed as $nmol \cdot min^{-1} \cdot mg$ of $protein^{-1}$. In each experiment a blank without substrate was measured to evaluate the reaction of thiols with DTNB. Treatment with BW284c51, a selective inhibitor of AChE, indicated that at least 95% of the enzymatic activity we observed was due to specific AChE.

LDH release

The LDH release is a spectrophotometric assay used as an end-point for cytotoxicity studies, based on the measurement of lactate dehydrogenase (LDH) released from the cytosol of damaged cells into the culture medium. An increase in the amount of membrane-damaged cells results in the increase of LDH enzyme activity in the culture medium. The test procedure was according to Kendig and Tarloff[46] with slight modifications. After cell exposure to ILs the culture medium was recovered and 1 mL of a lysis solution containing 0.1% Triton X-100 in 100 mM sodium phosphate (pH 7.4) was added to each culture well. Cells were incubated for 5 min at room temperature and lysis was confirmed at the microscope. Culture medium and cell lysate were centrifuged for 2 min at 15,000 x *g*. LDH activity was then assayed in the supernatants by addition of 2.85 mL of 0.3 mM pyruvate in 50 mM phosphate (pH 7.4), 50 μL of 8 mM NADH to 100 μL of sample. Absorbance was immediately recorded every min for 5 min at 340 nm. The % of LDH release was calculated as the ratio between the LDH release in the medium and the total LDH release (medium plus cell lysate) and referred to as percent of total.

[45] Lowry O.H., Rosenbrough N.J., Farr A.L., Randall R.J., 1951. Protein measurement with the Folin phenol reagent. J. Biol. Chem., 193, 265 –275.
[46] Kendig D.M. and Tarloff J.B, 2007. Inactivation of lactate dehydrogenase by several chemicals: implications for in vitro toxicology studies. Toxicol. In Vitro, 21,125-132.

3.1.4. Biodegradation properties

Jastorff and co-workers in 2003[23a] were the first who started to think how hazardous ionic liquids could be, by taking into account the risk associated with their toxicity, in spite of their initial fame of "green solvents". They underlined the importance of a multidimensional risk analysis, based on five eco-toxicological indicators (release, spatiotemporal range, bioaccumulation, biological activity and uncertainty), which allows a direct comparison with common organic solvents.[23c]

The need to study the toxicity and biodegradation of ionic liquids is of paramount importance; in fact, when an ionic liquid has completed its operational use, or even during its use for unexpected spill, it can be released to surface water and soils and its disposal becomes an issue, above all because it represents a waste with potential eco-toxicity or biological activity. According to the 10th principle of the Green Chemistry, "chemical products should be designed so that at the end of their function they break down into innocuous degradation products and do not persist in the environment": for this reason, the knowledge of the destiny of a compound in the various environments and of its abiotic and biotic degradation pathways and kinetics is worthy.

In these years the investigations about the environmental fate and the biodegradation of ionic liquids have been focused mainly on three topics:

- the sorption to solid matters and abiotic degradation
- the biotic degradation in water and soil compartments
- the design of more biodegradable compounds

<u>Sorption to solid matters and abiotic degradation</u>

Recent studies[47] have demonstrated that 1-alkyl-3-methylimidazolium cations can be strongly absorbed on different types of soil and marine sediments, and that the sorption is ruled by several factors: *i)* hydrophobic interactions with the organic matter, ruled by the lipophilicity of the alkyl

[47] a) Stepnowski, P., 2005.. Preliminary assessment of the sorption of some alkyl imidazolium cations as used in ionic liquids to soils and sediments. Aust. J. Chem., 58, 170-173. b) Beaulieu, J.J, Tank, J.L., Kopacz, M., 2008. Sorption of imidazolium-based ionic liquids to aquatic sediments. Chemosphere, 70, 1320–1328. c) Matzke, M., Thiele, K., Müller, A., Filser, J., 2009. Sorption and desorption of imidazolium based ionic liquids in different soil types. Chemosphere, 74, 568–574.

side chains; *ii)* non-hydrophobic interactions (e.g. electrostatic interactions) between charged sites on the sorbent surfaces and polar ionisable groups (e.g. ammonium); *iii)* the strength of the ion pairing within the ionic liquid itself (e.g. BMIM BF_4, thanks to the strong dissociation of the ion pair, results in high sorption to soil matrix); *iv)* the presence of clays which enhances the sorption.

Stepnowski et al.[48] have also checked in a series of in-depth studies, the abiotic degradation of imidazolium ionic liquids through different photochemical methods (UV, UV/H_2O_2 and UV/TiO_2): they have demonstrated that the stability towards direct photolysis is strictly correlated with the length of the alkyl chain, being octyl- or hexyl-methylimidazolium ILs more stable than butyl- or ethyl- ones.

Biotic degradation

Several studies have been accomplished to determine the aerobic biodegradation of ionic liquids in water:[33a,49] all of them describe, unexpectedly, a significant rate of primary biodegradation for (eco)-toxicologically unfavourable compounds carrying long alkyl side chains, such as octyl or hexyl ionic liquids, in contrast with short chain ILs, which are less biologically active, but more refractory. This trend is typical for all the most common cationic head groups (imidazolium, pyridinium, ammonium and phosphonium), indicating that a certain lipophilicity of the test compounds is an essential criterion for selecting biodegradable ionic liquids. The presence of linear alkyl chains, particularly containing four or more carbons, is an important factor in designing biodegradable compounds because carbon units can act as potential sites for attack by oxygenase

[48] a) Stepnowski, P., Zaleska, A., 2005. Comparison of different advanced oxidation processes for the degradation of room temperature ionic liquids. J. Photochem. Photobio. A: Chem., 170, 45–50. b) Siedlecka, E.M., Mrozik, W., Kaczy´nski, Z., Stepnowski, P., 2008. Degradation of 1-butyl-3-methylimidazolium chloride ionic liquid in a Fenton-like system. J. Haz Mat., 154, 893–900. c) Czerwicka, M., Stolte, S., Muller, A., Siedlecka, E.M., Gołębiowski, M., 2009. Identification of ionic liquid breakdown products in an advanced oxidation system. J. Haz. Mat., 171, 478–483.

[49] a) Docherty, K.M., Dixon, J.K., Kulpa C.F., 2007. Biodegradability of imidazolium and pyridinium ionic liquids by an activated sludge microbial community. Biodegradation, 18, 481–493. b) Stolte, S., Abdulkarim, S., Arning, J., Blomeyer-Nienstedt, A.K., Bottin-Weber, U., Matzke, M., Ranke, J., Jastorff, B., Thoming, J., 2008. Primary biodegradation of ionic liquid cations, identification of degradation products of 1-methyl-3-octylimidazolium chloride and electrochemical wastewater treatment of poorly biodegradable compounds. Green Chem., 10, 214–224.

enzymes. However, a very high lipophilicity (e.g. phosphonium and imidazolium cations with C_{12}, C_{16} and C_{18} longer chains) corresponds to an increased toxicity and to a stronger inhibitory potential towards the microbial community.[33a] A possible explanation for these experimental data is that middle-long chain ILs, being more toxic, can act as selecting agents for microorganisms capable of utilising them as a carbon source, allowing a more rapid growth of these biodegrading microorganisms which do not compete with any other microorganisms for carbon in the medium.[49a]

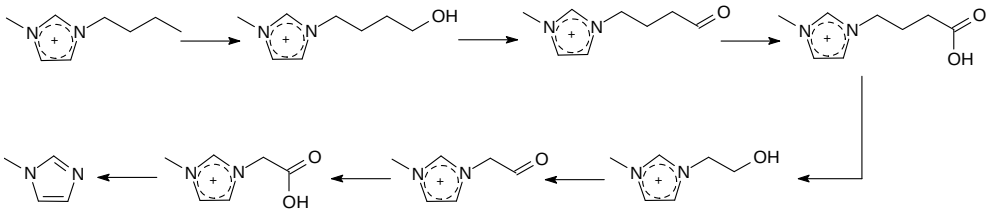
Docherty and co-workers[49a] also demonstrated that imidazolium ILs without any modification of alkyl lateral group are never readily biodegradable, being in the best case partially mineralised after the test period as for 1-octyl-3-methylimidazolium and 1-hexyl-3-methylimidazoilum cations. On the contrary pyridinium ILs are generally more readily biodegradable than imidazolium ILs; 1-octyl-3-methylpyridinium bromide and 1-hexyl-3-methylpyridinium bromide for example are classifiable as readily biodegradable and fully mineralised respectively.

Docherty et al.,[49a] Stolte et al.[49b] and Yun et al.[50] gave a clear picture of biodegradation pathways (Figure 3.1.4.1.), indicating that primary biodegradation mainly occurs through an initial ω-oxidation, followed by a β-oxidative cleavage of the lateral chain, as confirmed by different biological transformation products carrying hydroxyl, carbonyl and carboxyl groups detected after the test period. As suggested by Jastorff et al.[51] in an initial theoretical study, the uncharged imidazole ring, in spite of it is less toxic than imidazolium ring, remains intact and not utilised as a carbon source by the microbial community.

[50] Pham, T., Cho, C., Jeon, C., Chung, Y., Lee, M., Yun, Y. 2009. Identification of metabolites involved in the biodegradation of the ionic liquid 1-butyl-3-methylpyridinium bromide by activated sludge microorganisms. Environ. Sci. Technol., 43, 516–521.

[51] Jastorff, B., Mo¨ lter, K., Behrend, P., Bottin-Weber, U., Filser, J., Heimers, A., Ondruschka, B., Ranke, J., Schaefer, M., Schro¨der, H., Stark, A., Stepnowski, P., Stock, F., Sto¨rmann, R., Stolte, S., Welz-Biermann, U., Ziegert, S., Tho¨ming, J. 2005. Progress in evaluation of risk potential of ionic liquids—basis for an eco-design of sustainable products. Green Chem., 7, 362–372.

Figure 3.1.4.1. Biodegradation pathway of 1-butyl-3-methylimidazolium cation.



Design of more biodegradable compounds

In the design of more biodegradable compounds the most important parameters are: *i)* the presence of potential sites of enzymatic hydrolysis (e.g. esters and amides), *ii)* the introduction of oxygen in the form of hydroxyl, aldehyde or carboxylic acid groups, *iii)* the presence of unsubstituted linear alkyl chains (more than four carbons) and phenyl rings, possible sites of attack by oxygenases.[52]

However, *i)* the incorporation of oxygen containing functional groups such as alcohols, aldehyde and carboxylic acids can deeply change the features of ionic liquids, including their chemical reactivity, influencing their use as reaction media, and *iii)* the introduction of phenyl groups is known to produce compounds with elevated melting points, so no usable as solvents at room temperature.

Ionic liquids with ester or amide functionality, with no reactive oxygen atoms (e.g. ethers) or with long linear lateral chains, seem to fit both the needs of usability and promptness to biodegradation.

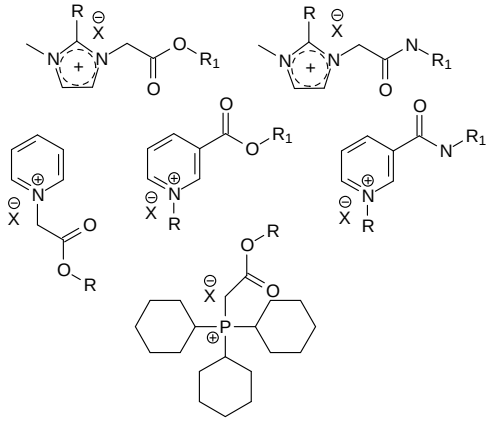
The first attempt to synthesise more biodegradable ionic liquids in according to these principles was done by Scammels and co-workers[53] (Figure 3.1.4.2.), who introduced ester and amide groups in the alkyl side

[52] Boethling, R.S., 1996. Designing Safer Chemicals, ACS Symp. Ser. 640, 156.

[53] a) Gathergood, N., Garcia, M.T., Scammells, P.J., 2004. Biodegradable ionic liquids: Part I. Concept, preliminary targets and evaluation. Green Chem., 6, 166-175. b) Gathergood, N., Scammells, P.J., Garcia, M.T.,2006. Biodegradable ionic liquids Part III. The first readily biodegradable ionic liquids. Green Chem., 8, 156–160. c) Harjani, J.R., Singer, R.D., Garcia, M.T., Scammells, P.J., 2008. The design and synthesis of biodegradable pyridinium ionic liquids Green Chem., 10, 436–438. d) Harjani, J.R., Singer, R.D., Garcia, M.T., Scammells, P.J., 2009. Biodegradable pyridinium ionic liquids: design, synthesis and evaluation. Green Chem., 11, 83–90; e) Morrissey, S., Pegot, B., Coleman, D., Garcia, M.T., Ferguson, D., Quilty, B., Gathergood, N., 2009. Biodegradable, non-bactericidal oxygen-functionalised imidazolium esters: A step towards 'greener' ionic liquids. Green Chem., 11, 475–483.

chain of imidazolium or pyridinium cation and analysed the effect of different anions. The results indicates that ILs with an amide in the side chain are poor biodegradable in water, whereas the incorporation of an ester results in a significant increase in biodegradation; moreover the octyl sulfate anion is considerably the most biodegradable anion.[54] Further investigations on the biodegradability of ester-ionic liquids were done on phosphonium cations,[55] but in this case the incorporation of ester functionality into a side chain and the selection of octyl sulfate anion, fail in improving the biodegradation.

Figure 3.1.4.2. Examples of ester-based ionic liquids.



Stepnowski et al.[56] kept on the design of biodegradable ionic liquids by synthesising 1-alkoxymethyl-3-hydroxypyridinium (potential biocides) ion paired with acesulphamate and saccharinate anions, carrying several sites of enzymatic hydrolysis; in spite of they can not be considered as rapidly biodegradable, the biodegradation rate of most of these compounds tends to increase with time, up to a complete mineralisation.

[54] Harjani, J.R., Farrell, J., Garcia, M.T., Singer, R.D., Scammells, P.J., 2009. Further investigation of the biodegradability of imidazolium ionic liquids. Green Chem.., 11, 821–829.
[55] Atefi, F., Garcia, M.T., Singer, R.D., Scammells, P.J., 2009. Phosphonium ionic liquids: design, synthesis and evaluation of biodegradability. Green Chem., 11, 1595–1604.
[56] Stasiewicz, M., Mulkiewicz, E., Tomczak-Wandzel, R., Kumirsk, J., Siedleck, E.M., Go"ebiowski, M., Gajdus, J., Czerwick, M., Stepnowski, P., 2008. Assessing toxicity and biodegradation of novel, environmentally benign ionic liquids (1-alkoxymethyl-3-hydroxypyridinium chloride, saccharinate and acesulfamates) on cellular and molecular level. Ecotoxicol Environ. Safety, 71, 157–165.

In this Thesis two different studies were performed in order to deepen ILs biodegradability in other environmental compartments than water and to find a correlation between the chemical structure of the cations and their biodegradability, as done for the investigation of (eco)-toxicological properties in Chapters 3.1.2. and 3.1.3.. Thus the aerobic biodegradation of four imidazolium ionic liquids was monitored for the first time in soil, and the biodegradability of a class of pyridinium ionic liquids, bearing an increasing number of ethoxy units in the lateral chain, was analysed in water.

Biodegradation of oxygenated and non-oxygenated imidazolium based ionic liquids in soil

Kumar et al.[57] have previously analysed by GC-MS the presence of breakdown products when 1-butyl-3-methylimidazolium tetrafluoroborate (BMIM BF_4) was added to an aerobic aqueous solution inoculated with soil-bacteria.

In the present study of Thesis, the degradation of ionic liquids in soil was monitored over six months, using the ASTM D 5988-96 method,[58] devised for determining the degree of aerobic biodegradation of materials under laboratory conditions by the action of microorganisms present in soil. The test method ASTM D 5988-96, previously found to be suitable for evaluating the degree and rate of aerobic biodegradation of polymeric materials,[59] measures the amount of CO_2 produced by microbiological activity and adsorbed by a KOH solution.

The tests were carried out on the four ionic liquids 1-butyl-3-methylimidazolium tetrafluoroborate (BMIM BF_4, **1b**), 1-butyl-3-methylimidazolium dicyanamide (BMIM $N(CN)_2$, **1c**), 1-methoxyethyl-3-methylimidazolium tetrafluoroborate (MOEMIM BF_4, **2b**) and 1-

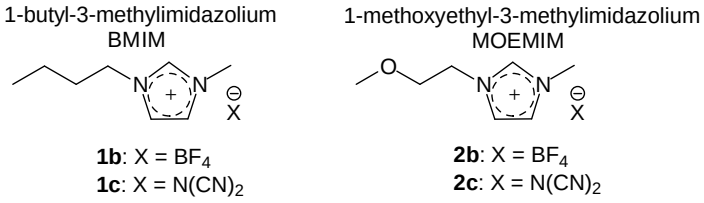
[57] Kumar, S., Ruth, W., Sprenger, B., Kragl, U., 2006. On the biodegradation of ionic liquid 1-Butyl-3-methylimidazolium terafluoroborate. Chem. Today, 24, 24-26.

[58] Annual Book of ASTM Standards, 1997. Vol. 08.03.A.

[59] a) Modelli, A., Calcagno, B., Scandola, M., 1999. Kinetics of aerobic polymer degradation in soil by means of the ASTM D 5988–96 standard method. J. Environ. Polym. Degrad., 7, 109–116. b) Modelli, A., Rondinelli, G., Scandola, M., Mergaert, J., Cnockaert, M.C., 2004. Biodegradation of chemically-modified flax fibres in soil and 'in vitro' with selected bacteria. Biomacromolecules, 5, 596–602.

methoxyethyl-3-methylimidazolium dicyanamide (MOEMIM $N(CN)_2$, **2c**) (Figure 3.1.4.3).

Figure 3.1.4.3. Alkyl and oxygenated imidazolium ionic liquids.



1b: X = BF_4
1c: X = $N(CN)_2$

2b: X = BF_4
2c: X = $N(CN)_2$

Since several recent studies correlate the toxicity or inhibitory properties of ionic liquids with atomic charges, theoretical calculations at the B3LYP/6-31G(d) level were carried out to obtain insight into the atomic charge distributions of BMIM and MOEMIM cations.

In a typical experiment 0.5 g of BMIM BF_4, BMIM $N(CN)_2$, MOEMIM BF_4 and MOEMIM $N(CN)_2$ were analysed, each in triplicate, in a single batch. A blank without ILs was analysed along (Figure 3.1.4.4.). Titrations with KOH solution were carried out every 2-3 days during the first two months, and then weekly over the last months.

Figure 3.1.4.4. Desiccators used for the biodegradation of imidazolium-based ionic liquids in soils.

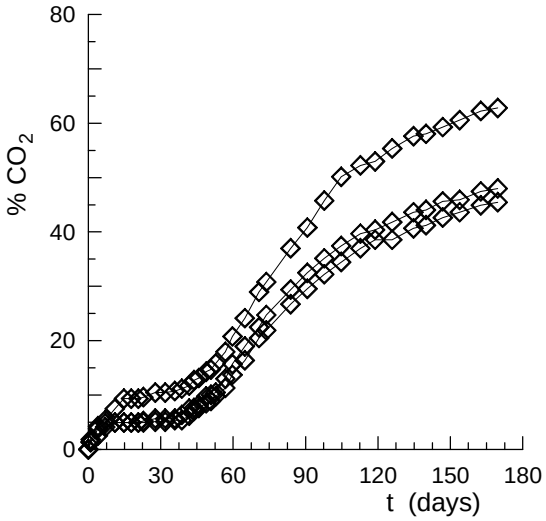
The carbon content of each compound expressed as percentage by weight and as amount corresponding to 0.5 g of ILs, and the quantity of expected CO_2 from a complete degradation are reported in Table 3.1.4.1.

Table 3.1.4.1. Carbon content of each compound, expressed as percentage by weight and as amount corresponding to 0.5 g of ILs, and expected CO_2 (mmol) derives from a fully biodegradation.

	Carbon content in 0.5 g of ILs (mg)	**Carbon content by weight (%)**	**Expected CO_2 (mmol)**
BMIM BF_4	212.5	42.5	17.7
BMIM $N(CN)_2$	292.7	58.5	23.4
MOEMIM BF_4	184.0	36.8	15.3
MOEMIM $N(CN)_2$	260.9	52.1	21.7

The total percent CO_2 production of BMIM BF_4, BMIM $N(CN)_2$, MOEMIM BF_4 and MOEMIM $N(CN)_2$ as a function of time, measured in the three tests is reported in Figure 3.1.4.5., Figure 3.1.4.6., Figure 3.1.4.7. and Figure 3.1.4.8. The rate of degradation (percent of carbon per day) is given by the slope of the tangent to each curve.

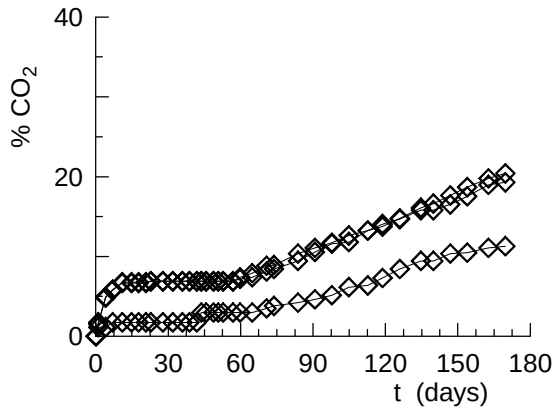
Figure 3.1.4.5. Production of carbon dioxide, as a function of time, measured in the tests with 0.5 g of BMIM BF_4.



It can be noted that, in spite of the complexity of the heterogeneous systems analysed, the results of the triplicate experiments are quite reproducible. The

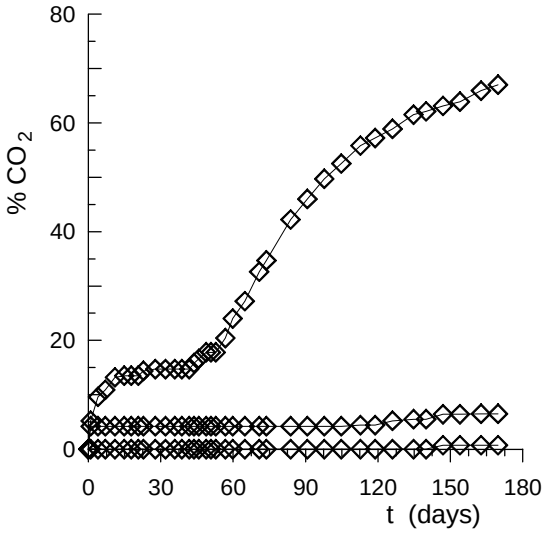
induction time (if any) to start the degradation process is less than one day, indicating that the microorganisms contained in the soil can promptly produce suitable enzymes to degrade the ionic liquid. However, after about one week the degradation rate drops suddenly and essentially no further evolution of CO_2 is measured for more than one month. The degradation process then re-starts after 40-45 days, with a rate of 0.7% day $^{-1}$. Production of 50% (averaged over the three tests) of the expected total CO_2 is measured after 151 days. On the suspension of the tests after six months, a residual average degradation rate > 0.1% day $^{-1}$ is still observed, and the total percentage degradation is 52.1 ± 6.6 % (standard error calculated from the standard deviations of the tests and blanks).

Figure 3.1.4.6. Production of carbon dioxide, as a function of time, measured in the tests with 0.5 g of BMIM $N(CN)_2$.



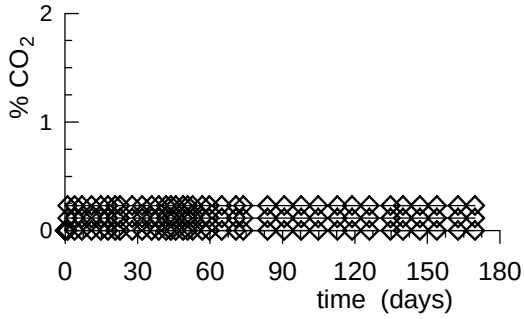
Also in this case the reproducibility over the three tests is good. As noted for the tetrafluoroborate analogue, a prompt production of CO_2 is measured over the first 5-7 days. Then no further evolution of CO_2 is observed for about two months, after which the degradation process re-starts at a rate of about 0.2% day $^{-1}$. The trend of CO_2 production, as a function of time, is thus similar to that observed for BMIM BF_4, although the induction period is even longer and the degradation rate significantly lower. After six months the average percentage degradation is 17.0 ± 4.2%.

Figure 3.1.4.7. Production of carbon dioxide, as a function of time, measured in the tests with 0.5 g of MOEMIM BF_4.



The occurrence of a large discrepancy between two replicates, where the production of CO_2 is very small, if any, and the third one, is immediately evident. In principle, this can be due to an accidental presence of additional organic carbon in the soil of the latter test. However, whereas for readily degradable materials (such as starch[59a] and cellulose[59b]) the degradation rate is maximum during the first few days and then gradually decreases, here the trend (in addition to the extent of degradation after six months) is similar to that displayed by the BMIM analogue. For the two reproducible tests, the average extent of degradation (3.6%) after six months is smaller than the evaluated standard error (± 6.0%).

Figure 3.1.4.8. Production of carbon dioxide, as a function of time, measured in the tests with 0.5 g of MOEMIM $N(CN)_2$.



In all three tests (except for a very small amount after the first day) no production of CO_2 relative to the blanks can be monitored. After six months

the average percentage degradation is 0.1 ± 4.0%. Moreover, inspection of the data shows that the amount of HCl required for titration of KOH in the three MOEMIM $N(CN)_2$ tests is not equal, but significantly smaller than that required for the blanks. This finding could be consistent with an inhibitory effect on the activity of the microorganisms (or their enzymes), which would cause a reduction of the degradation rate of the organic carbon present in the soil relative to the blank tests.

Interestingly, it can be noted that the observed effect of the oxygen atom in the alkyl side-chain on biodegradability in soil is unexpected on the basis of the smaller toxicity of MOEMIM cation towards aquatic organisms, including bacteria, than BMIM cation, as reported previously in Chapter 3.1.2. of the Thesis. On the other hand, the present data also indicate significantly different behaviours between the two cations.

In addition to the different toxicity of the oxygen derivatives towards aquatic organisms, although the discrepancy of one of the tests with MOEMIM BF_4 is hardly explainable, the present study of biodegradability in soil has indicated that replacement of a CH_2 group with an oxygen atom in the alkyl chain is of crucial importance for the degradability of imidazolium ionic liquids in soils.

Therefore the electronic structures of 1-R-3-methylimidazolium cations, their dependence on the length of the alkyl chain (R) and the presence of a heteroatom (oxygen or nitrogen) were investigated.

In Table 3.1.4.2. the atomic charges of the ring nitrogen atoms for the derivatives with R = $CH_3(CH_2)_n$ (n = 0, 1, 3, 4 and 5), $CH_3O(CH_2)_2$ and $CH_3NH(CH_2)_2$, obtained with B3LYP/6-31G(d) calculations using the Mulliken approximation[60] are reported.

[60] Mulliken, R.S., 1949. The theory of molecular orbitals. J. Chim. Phys. 46, 497–542

Table 3.1.4.2. B3LYP/6-31G(d) Mulliken atomic charges (δ) of the ring nitrogen atoms in 1-R-3-methylimidazolium cations

R	δ N(3)	δ N(1)
CH_3	-0.387	-0.387
CH_3CH_2	-0.390	-0.383
$CH_3(CH_2)_3$	-0.390	-0.388
$CH_3O(CH_2)_2$	-0.390	-0.391
$CH_3NH(CH_2)_2$	-0.390	-0.388
$CH_3(CH_2)_4$	-0.390	-0.387
$CH_3(CH_2)_5$	-0.390	-0.387

According to the B3LYP results, both nitrogen atoms in imidazolium cations are largely negative. Moreover, their charges are only very slightly affected by the length of the alkyl chain (from one to six carbon atoms) or replacement of a CH_2 group with an oxygen atom or a NH group. In all derivatives the three ring carbon atoms are predicted to be positive, as well as all hydrogen atoms in the ring and in the lateral alkyl chain. The alkyl-chain carbon atoms are predicted to be negatively charged (mainly that of the terminal CH_3 group), but, due to the positive charges of the hydrogen atoms, the net charge of the CH_2 and terminal CH_3 groups is always positive. These features of the charge distributions are common to all the derivatives considered, but those with R = $CH_3O(CH_2)_2$ and $CH_3NH(CH_2)_2$ present a peculiarity. The charges of the oxygen atom and NH group are calculated to be -0.463 and -0.233, respectively, so that the saturated side-chains possess a largely negative heteroatomic centre. On this point, as suggested by Kaur and Zang, the presence of an ionisable (basic) heteroatom in compounds which act as acetylcholinesterase inhibitors is found to be an important parameter.[61] That calculation supports the hypothesis that oxygen-containing lateral chains of ionic liquids are presumable centres of basic reactivity. However the evidence that such chemical feature could be the reason of their refractivity to biodegradation is still lacking.

[61] Kaur, J., Zhang, M.-Q., 2000. Molecular modelling and QSAR of reversible acetylcholinesterase inhibitors. Curr. Med. Chem. 7, 273–294.

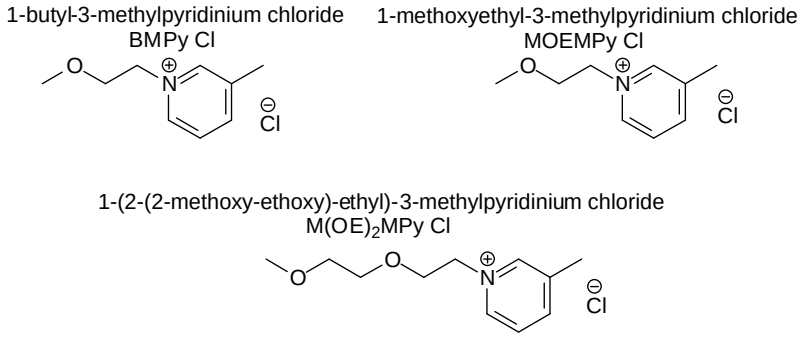
Biodegradation of oxygenated and non-oxygenated pyridinium based ionic liquids in water

As described above, the literature data suggest that pyridinium based ILs are more biodegradable than imidazolium ones; in particular pyridinium cations bearing an ester substituent at positions 1 or 3 show excellent biodegradability, attesting the tendency of the pyridine ring to mineralise, in contrast with imidazole ring.

The introduction of an oxygen atom, specifically in the form of ester group, is a key factor in increasing the biodegradability; however in the literature the effect of other oxygen containing functional groups has not still investigated.

Thus in this Thesis the biodegradability in water of the class of pyridinium ILs synthesised as described in Chapter 3.1.1. (Figure 3.1.4.9.), with a number of oxygen atoms in the lateral chain of the cation from 0 to 2, was checked by using the Closed Bottle Test (OECD 301D)[62], to investigate the effect of the oxygen atoms number on the mineralisation.

Figure 3.1.4.9. Alkyl and oxygenated pyridinium ionic liquids.



In this test each IL was added to an aerobic aqueous mineral medium inoculated with a small number of microorganisms derived from the secondary effluent of the municipal waste-water treatment plant of Rimini city (Italy), and the depletion of dissolved molecular oxygen was measured over a 28-d period. The amount of oxygen taken up by the microbial population during the biodegradation of the test substance, corrected for

[62] OECD Chemical Group, Ready Biodegradability: Closed Bottle Test. Method 301 D, OECD Revised Guidelines for Tests for Ready Biodegradability, Paris, France, 1993.

uptake by the blank inoculum run in parallel, was expressed as a percentage of the theoretical oxygen demand (ThOD).

In a typical run, for each ionic liquid, 10 bottles containing test substance and inoculum (test suspension), 10 containing only inoculum (inoculum blank), 10 containing reference compound (sodium acetate) and inoculum (procedure control), and 6 bottles containing test substance, reference compound and inoculum (toxicity control), were prepared. The toxicity control was evaluated in order to verify any potential toxic/inhibitor effect of each IL, measuring if the microorganisms, in spite of the presence of an ionic liquid, could degrade or not the reference compound (sodium acetate).

Duplicate bottles of each series were analysed at the start of the test for dissolved oxygen and the remaining bottles were incubated at 20 °C ± 1°C in the dark. Compounds which reached a biodegradation level higher than 60% in 10 days are referred to be “readily biodegradable”.

The experiment was carried out on sodium acetate (NaOAc), 1-butyl-3-methylpyridinium chloride (BMPy Cl, **6a**), 1-methoxyethyl-3-methylpyridinium chloride (MOEMPy Cl, **7a**) and 1-(2-(2-methoxy-ethoxy)-ethyl)-3-methylpyridinium chloride (M$(OE)_2$MPy Cl, **8a**) (Figure 3.1.4.9.). The experimental concentrations for each compound and the relative ThOD are reported in Table 3.1.4.3.

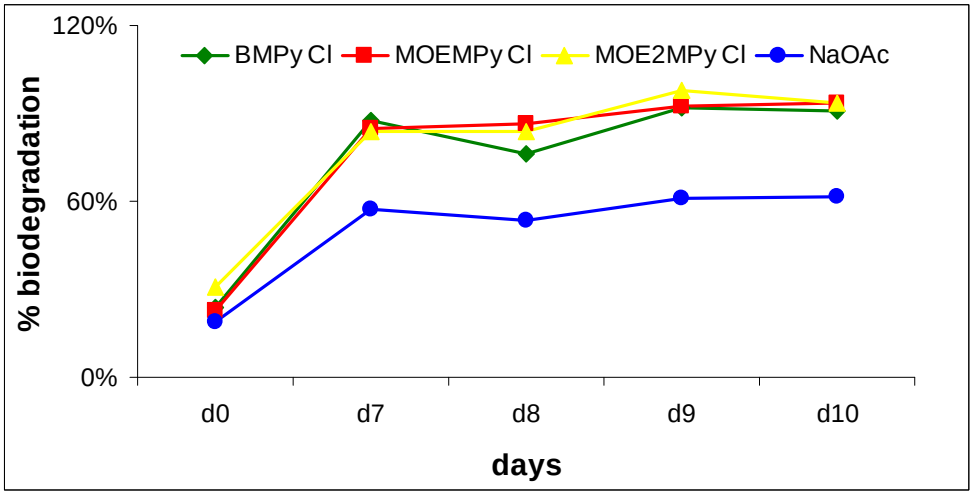
Table 3.1.4.3. Experimental concentrations for the tested compounds and the relative theoretical oxygen demand ThOD.

X	**Concentration$_x$ (mg/L)**	**ThOD$_x$ (mgO_2/L)**
BMPy Cl	2.46	5.5
MOEMPy Cl	2.84	5.3
M$(OE)_2$MPy Cl	2.88	5.4
NaOAc	10.3	8.0

For all the substances, the concentrations and the ThOD were in the range of acceptability for the test conditions of the OECD guideline (concentrations 2-10 mg/L, ThOD 5-10 mg/L). The inoculum derived from the secondary effluents of the municipal wastewater treatment plant of Rimini city (Italy), was added as 2.5 mL of filtrate per litre of medium.

The results of the biodegradation are summarised in Figure 3.1.4.10.

Figure 3.1.4.10. Biodegradation curves of alkyl and oxygenated pyridinium ionic liquids.



As shown in Figure 3.1.4.10., the experiment was interrupted after 10 days because the biodegradation of the ionic liquids was (surprisingly) almost complete. Specifically it was 91% for BMPy Cl, 94% for MOEMPy Cl and 93% for $M(OE)_2MPy$ Cl, indicating all the compounds as readily biodegradable (according to OECD standards, a readily biodegradable compound shows 60–70% or greater biodegradation by activated sludge microbial inoculate, within a 10-day window in a 28-day test period).

No influence of the oxygen atoms number, including their absence, on the biodegradation rate is detected.

The surprising percentage here found for BMPy Cl seems in disagreement with previous literature data[33a,49a,d] which indicate 1-butyl-3-methylpyridinium bromide (BMPy Br) and 1-butyl-3-methylpyridinium chloride (BMPy Cl) as very recalcitrant compounds.

However recently Yun and co-workers[50] have suggested that the amount of the ionic liquid used in the biodegradability test is a crucial factor for the inhibition of the microbial community biodegrading activity since ILs have a certain dose-dependant biological activity also towards these mineralising microorganisms. The authors have reported that the biodegradation of BMPy Br starts after 21 days and it is almost complete after 28 days, contrarily to what observed in other studies, because of, as they explain, the

differences among BMPy Br concentrations and relative different adaptation times of the microorganisms. In fact the amount of BMPy Br used by Yun et al. (10.7 mg/L) is almost 7 times lower than that used in other works (77 mg/L).[49a] The BMPy Cl amount used in the present study was 2.46 mg/L, about 4 times and 31 times lower than the concentration reported in the literature, thus a possible explanation of the high level of biodegradation reached in only 10 days here reported could reside in this difference.

The experiments to determine the potential toxic/inhibitor effect of each IL indicated that the microorganisms, in spite of the presence of the ionic liquids, degrade sodium acetate at the same rate of the samples without ILs. The biodegradation rate for sodium acetate is 61% after 10 days, as required for the validity of the biodegradability test.

Conclusions

Aerobic biodegradation of four imidazolium ionic liquids in soil was evaluated for the first time. The tests were carried out on BMIM BF_4, BMIM $N(CN)_2$, MOEMIM BF_4 and MOEMIM $N(CN)_2$, by measuring the total production of CO_2 over six months according to ASTM D 5988-96. Although the two cations differ only in the replacement of a CH_2 group of the *n*-butyl chain with an oxygen atom, their degradability tests gave quite different results. The two ionic liquids with BMIM cation were shown to be biodegradable, although the rate of degradation of BF_4 derivative was larger than that of $N(CN)_2$ analogue. Interestingly, in both cases the production of CO_2, as a function of time, was characterized by a peculiar trend. After a prompt start (over the first few days), no CO_2 evolution was observed over nearly two months, after which the degradation process re-started. In contrast (except for a large and unexplained discrepancy of one of the six tests) no significant evolution of CO_2 was observed over six months with MOEMIM derivatives, with either BF_4 and $N(CN)_2$ anions.

Density functional theory calculations at the B3LYP/6-31G(d) level gave insight into the atomic charges and electronic structures of the substituted imidazolium cations, and highlighted significant differences caused by replacement of a CH_2 group with an oxygen atom in the alkyl side-chain. The oxygen atom of MOEMIM cation was predicted to bear a large

negative charge, while the net charges of the CH_2 groups in its 1-butyl analogue were positive. Maybe, these electrons are involved in the peculiar behaviour of MOEMIM containing ionic liquids towards biodegradation.

Aerobic biodegradation of three pyridinium ionic liquids in water was evaluated to investigate the effect of an increasing oxygen atoms number in the cation on the biodegradability. The tests were carried out on a series of chlorides, bearing 0 (BMPy), 1 (MOEMPy), and 2 ($M(OE)_2MPy$) ethoxy units in the lateral chain of the cation, by measuring the amount of oxygen taken up by the inoculated microbial population during biodegradation according to OECD guidelines.

All the ionic liquids were almost completely degraded after 10 days, independently by the number of oxygen in the lateral chain of the cation, showing an opposed behaviour to what previously found by other authors. Our results are probably due to the lower concentration of ionic liquids employed in the present study, not high enough to inhibit the microorganisms, allowing mineralisation to occur. This findings allows to underline the importance of hypothesising a scenario of application when eco-toxicological and biodegradation studies are performed on substances for which the industrial use is still uncertain, as for ionic liquids, and from this scenario extrapolating the reasonable amount of the substance which can be eventually reversed in the environment.

Experimental section

Ionic liquids

1-Butyl-3-methylimidazolium tetrafluoroborate (BMIM BF_4) was purchased from Merck (Darmstadt, Germany); the other ionic liquids were synthesised according the experimental procedures described in the Chapter 3.1.1. of the Thesis.

Biodegradation in soil

Desiccators of approximately 2 L internal volume, sealed air-tight, were used for biodegradation experiments according to ASTM D 5988-96. In each test 0.5 g of ionic liquid was mixed with about 300 g of soil, sieved to 2-mm particle size. The soil was collected from the coastal area called

Piallassa Baiona, close to Ravenna, Italy, and taken only from the surface (maximum depth 10 cm). A pH = 7.9 and a 5% content of volatile solids (at 550 °C) of the soil were measured. The C:N ratio was adjusted to a value of 15:1 with ammonium phosphate solution. Distilled water was also added to bring the moisture content of the soil to about 90% of the moisture holding capacity (29% of dry soil), determined using ASTM D 425 (Standard Methods for the Examination of Water and Wastewater, 1989). The amount of CO_2 produced was determined by titrating 0.4 M KOH solutions placed in the test and the blank desiccators (same conditions, but without sample) with 0.25 M HCl to a phenolphthalein end-point. The frequency of titrations ranged from daily to weekly, depending on the degradation rate. The O_2 content of the vessel (about 19 mmol) never fell by more than 10% in the interval between successive titrations. In addition to the ionic liquids, 0.5 g of powdered starch was degraded to verify the activity of the microorganisms. The method requires that at least 70% of the starch be degraded after six months. We achieved this percentage of degradation after two months. Blank experiments were carried out under the same conditions, using soil without any additional carbon source. Each test (and blank) was run in triplicate. The temperature was 25 ± 2 °C over the six-month period of observation.

Optimization of the geometrical structures and charge distributions (Mulliken populations) were obtained with the DFT/B3LYP hybrid functional,[63] by using the standard 6-31G(d) basis set and the Gaussian-03 package.[64]

Biodegradation in water

Solutions of sodium acetate (reference substance), BMPy Cl, MOEMPy Cl, and $M(OE)_2MPy$ Cl (as sole sources of organic carbon) were prepared separately in previously aerated mineral medium.[62] In the preliminary experiment, followed for 10 days, sodium acetate, BMPy Cl, MOEMPy Cl and $M(OE)_2MPy$ Cl were checked; the concentrations of the solutions were

[63] Becke, A.D., 1993. Density-functional thermochemistry III. The role of exact exchange. J. Chem. Phys. 98, 5648–5652.
[64] Frisch, M.J. et al., 2004. Gaussian 03, Revision D.01. Gaussian Inc., Wallingford, CT.

reported in Table 3.1.4.4., in which also the theoretical oxygen demand (calculated according to the Formula 3.1.4.1.)[62] for each compound have been indicated.

Formula 3.1.4.1. ThOD calculation (without nitrification), for a generic compound X (elemental composition $C_cH_hCl_{cl}N_nNa_{na}O_o$), in concentration [X] (mg/L) and with molecular weight MW_x (g/mol).

$$ThOD_X\ (mg\ O_2/L) = 16*[2c + 1/2*(h - cl - 3n) + 1/2na - o]*[X]*MW_x^{-1}$$

Table 3.1.4.4. ThOD and concentration used in the test.

X	[X]	MW_X	c	h	cl	n	o	na	$ThOD_X$
BMPy Cl	2.46	185.7	10	16	1	1	0	0	5.5
MOEMPy Cl	2.84	187.67	9	14	1	1	1	0	5.3
$M(OE)_2MPy$ Cl	2.88	231.72	11	18	1	1	2	0	5.4
NaOAc	10.3	82.03	2	3	0	0	2	1	8.0

The solutions were then inoculated with microorganisms collected from a secondary effluent of a municipal wastewater treatment plant (Rimini, Italy), and each well-mixed solution was carefully dispensed into a series of BOD bottles so that all the bottles were completely full. A control with inoculum, but without test chemicals was run parallel for the determination of oxygen blanks. Duplicate bottles of each series were analysed immediately for dissolved oxygen and the remaining bottles were incubated at 20 °C±1 °C in the dark. Bottles of all series were withdrawn in duplicate for dissolved oxygen analysis over the 28-day incubation period. The biodegradation after *n* days was expressed as the ratio of the biochemical oxygen demand (BOD) to the theoretical oxygen demand both of them expressed as mg O_2/L for each compound. The measurements of dissolved oxygen were recorded with an oxygen electrode and meter (electrode method).

3.1.5. New ionic liquids from renewable resources

The global energetic consumption are constantly increasing and at the present they are mainly satisfied by oil, methane and coal; moreover about the 98% of the synthesis of organic chemical compounds commonly used in the industry is based on non-renewable fossil material. Thus, obtaining chemicals from renewable material is of growing importance in facing environmental concerns over fossil fuels consumption. Biomass is the most important renewable resource available both for energy and chemical compounds production, convertible through chemico-physical processes (e.g. extraction and transesterification), bio-chemical processes (e.g. digestion and fermentation), or thermo-chemical processes (e.g. combustion, gasification and pyrolysis). The thermo-chemical treatment of the biomass usually affords a large number of oxygenated compounds, such as anhydrosugars and furans, which can be used in different chemical applications.[15,65]

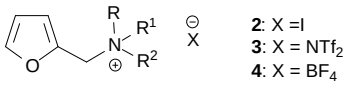
Furfural **1** (2-furaldehyde) is a cheap chemical, industrially obtained in a large amount from the treatment of lignocellulosic materials, derived from agricultural or forestry wastes, with acidic water at high temperatures. It is currently produced in more than 25000 tons/year globally, with a 10% yield of the original biomass. It can be also recovered in the liquid fraction obtained from the pyrolysis of wood and cellulose.[66]

In this Thesis furfural has been used as building block for the synthesis of a class of furan-containing quaternary ammonium iodides **2a**–**i**, bis(trifluoromethylsulfonyl)imide (NTf_2 salts) **3a**–**h**, and tetrafluoroborates (BF_4 salts) **4a**–**h**, whose application can be envisioned in the fields of ionic liquid solvents, surfactants or biocides in analogy with benzyl quaternary ammonium salts (Table 3.1.5.1).

[65] Lichtenthaler, F.W., 2007, in Methods and Reagents for Green Chemistry, ed. P. Tundo, A. Perosa and F. Zecchini, John Wiley & Sons, Hoboken, Chapter 2, 23–64.

[66] a) Lichtenthaler, F.W., Peters, S., 2004. Carbohydrates as green raw materials for the chemical industry. C. R. Chim., 7, 65–90. b) Hoydonckx, H.E., Van Rhijn, W., Van Rhijn, W., De Vos, D.E., Jacobs, P.A., 2007. Furfural and Derivatives, in Ullmann's Encyclopedia of Industrial Chemistry, Wiley-VCH Verlag GmbH & Co. KGaA, DOI: 10.1002/14356007.a12_119.pub2.

Table 3.1.5.1. Furan-derived quaternary ammonium salts **2a-i** (X = I), **3a-h** (X = NTf_2), **4a-h** (X = BF_4).



Compound	R	R^1	R^2
a	butyl	butyl	methyl
b	butyl	dodecyl	methyl
c	octyl	butyl	methyl
d	octyl	dodecyl	methyl
e	butyl	methyl	methyl
f	butyl	butyl	butyl
g	octyl	methyl	methyl
h	octyl	butyl	butyl
i	hexyl	methyl	methyl

Long-chain ammonium salts, such as benzalkonium chloride (BAC), show very good antimicrobic and tensioactive features, for which they are largely and commonly employed as cationic surfactants in detergent and pharmaceutical formulations, as phase transfer agents in chemical industry and as biocides.[67] The use of quaternary ammonium salts with low melting points is of growing importance also in the field of room-temperature ionic liquids (ILs).[68]

Nowadays, either commonly used ionic liquids or quaternary ammonium surfactants are synthesised from petroleum derivatives with polluting and expensive methodologies; therefore, the synthesis of those chemical

[67] a) Pernak, J., Sobaszkiewicz, K., Mirska, I., 2003. Anti-microbial activities of ionic liquids. Green Chem., 5, 52–56. b) Pernak, J., Smiglak, M., Griffin, S., Hough, W., Wilson, T., Pernak, A., Zabielska-Matejuk, J., Fojutowski, A., Kita, K., Rogers, R., 2006. Long alkyl chain quaternary ammonium-based ionic liquids and potential applications**.** Green Chem., 8, 798–806. c) Kuca, K., Marek, J., Stodulka, P., Musilek, K., Hanusova, P., Hrabinova, M., Jun, D., 2007. Preparation of Benzalkonium Salts Differing in the Length of a Side Alkyl Chain. Molecules, 12, 2341–2347. d) Kucˇ a, K., Vivala, M., Dohnal, V., 2004. J. Appl. Biomed., 2, 195–198.

[68] a) Ionic liquids in synthesis, ed. P. Wasserscheid and T. Welton, Wiley-VCH, Weinheim, 2008. b) Meindersma, G., Maase, M., De Haan, A., 2007. Ionic liquids, in Ullmann's Encyclopedia of Industrial Chemistry, Wiley-VCH Verlag GmbH & Co. KGaA, DOI: 10.1002/14356007.114_101.

commodities from renewable raw materials, such as furfural, can be of great potential impact.[69]

The synthesis of ionic liquids from natural compounds has been faced in the last years by exploiting natural molecules both as anions and as cations.

The most common organic anions found in nature are chiral carboxylate salts, such as acetate, mandelate, lactate, and tartrate, [70] but the major part of these ion-pairing with traditional ILs cations (e.g. imidazolium) gives salts with higher melting points than the corresponding tetrafluoroborate and hexafluorophosphate salts. Other sources of natural anions are the aminoacids pool, indeed exploited both as anion and cation,[71] (S)-10-camphorsulfonate[72] and the borate anions based on chiral natural acids.[73]

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[69] Poletti, L., Chiappe, C., Lay, L., Pieraccini, D., Polito, L., Russo, G., 2007. Glucose-derived ionic liquids: exploring low-cost sources for novel chiral solvents Green Chem., 9, 337–341.

[70] a) Jodry, J., Mikami, K., 2004. New chiral imidazolium ionic liquids: 3D-network of hydrogen bonding Tetrahedron Letters, 45, 4429–4431. b) Wang, Z., Wang, Q., Zhanga, Y., Bao, W., 2005. Synthesis of new chiral ionic liquids from natural acids and their applications in enantioselective Michael addition. Tetrahedron Letters, 46, 4657–4660. c) Allen, C., Richard, P., Ward, A., van de Water, L., Masters, A., Maschmeyer, T., 2006. Facile synthesis of ionic liquids possessing chiral carboxylates. Tetrahedron Letters, 47, 7367–7370. d) Earle, M., McCormac, P., Seddon, K., 1999, Diels–Alder reactions in ionic liquids . A safe recyclable alternative to lithium perchlorate–diethyl ether mixtures**.** Green Chem, 1, 23.

[71] a) Bao, W., Wang, Z., Li, Y., 2003. Synthesis of Chiral Ionic Liquids from Natural Amino Acids J. Org. Chem., 68, 591-593. b) Ni, B., Headley, A., Li, G., 2005. Design and Synthesis of C-2 Substituted Chiral Imidazolium Ionic Liquids from Amino Acid Derivatives J. Org. Chem., 70, 10600-10602. c) Chen, X., Li, X., Hua, A., Wang, F., 2008. Advances in chiral ionic liquids derived from natural amino acids. Tetrahedron: Asymmetry, 19, 1–14. d) Guillen, F., Bre´geon, D., Plaquevent, J., 2006. (*S*)-Histidine: the ideal precursor for a novel family of chiral aminoacid and peptidic ionic liquids Tetrahedron Letters, 47, 1245–1248. e) Gao, H., Hu, Z., Wang, J., Qiu, Z., Fan, F., 2008. Synthesis and Properties of Novel Chiral Ionic Liquids from L-Proline. Aust. J. Chem., 61, 521–525. f) Fukumoto, K., Yoshizawa, M., Ohno, H., 2005. Room Temperature Ionic Liquids from 20 Natural Amino Acids. J. Am. Chem. Soc., 127, 8, 2398–2399.

[72] a) Schulz, P., Muller, N., Bosmann, A., Wasserscheid, P., 2007. Effective Chirality Transfer in Ionic Liquids through Ion-Pairing Effects Angew. Chem. Int. Ed., 46, 1293 – 1295. b) Zhou, W., Xu, L., Qiu, H., Lai, G., Xia, C., Jiang, J., 2008. Synthesis of a Novel Chiral Ionic Liquid and Its Application in Enantioselective Aldol Reactions Helvetica Chimica Acta , 91, 53-59. c) Nobuoka, K., Kitaoka, S., Kunimitsu, K., Iio, M., Harran, T., Wakisaka, A., Ishikawa, Y., 2005. Camphor Ionic Liquid: Correlation between Stereoselectivity and Cation-Anion Interaction. J. Org. Chem., 70, 10106-10108. d) Machado, M., Dorta, R., 2005. Synthesis and Characterization of Chiral Imidazolium Salts Synthesis, 15, 2473–2475.

[73] a) Yu, S., Lindeman, S., Tran, C., 2008. Chiral Ionic Liquids: Synthesis, Properties, and Enantiomeric Recognition. J. Org. Chem., 73, 2576-2591. b) Xu, W., Wang, L., Nieman, R., Angell, C., 2003. Ionic Liquids of Chelated Orthoborates as Model Ionic Glassformers. J. Phys. Chem. B, 107, 11749-11756. c) Herzig, T., Schreiner, C., Gerhard, D., Wasserscheid, P., Gores, H., 2007. Characterisation and properties of new ionic liquids with the difluoromono[1,2-oxalato(2-)-O,O']borate anion. Journal of Fluorine Chemistry, 128, 612–618. d) Gausepohl, R., Buskens, P., Kleinen, J., Bruckmann, A., Lehmann, C., Klankermayer, J., Leitner, W., 2006. Highly Enantioselective Aza-Baylis–Hillman Reaction in a Chiral Reaction Medium Angew. Chem. Int. Ed., 45, 3689 –3692.

Also the field of ILs based on natural cations has been widely explored by using natural nitrogen-containing compounds as amines (e.g. ephedrine),[74] amino alcohols[75] and ammonium salts (e.g. choline chloride).[76] Recently other compounds as nicotine,[77] menthol,[78] and carbohydrates,[69,79] have been explored as starting materials in the synthesis of new chiral ionic liquids. Also furfural has been already investigated as building block for the synthesis of ammonium salts. The first example was proposed in 1940 by Nabenhauer[80] who prepared some short chain dimethylalkyl furan ammonium salts, called "furtrethoniums", showing cholinergic or muscarinic properties and enabling to stimulate the neuromuscular mechanism through the parasympathetic nervous system.[81] Furmethide (2-*N*,*N*,*N*-trimethylfurfurylammonium iodide), in particular, is the most studied furan-containing ammonium salts among the short chain dimethylalkyl furans synthesised since now,[82] because of its high similarity with the natural alkaloid muscarine. In 1945 Weilmuenster and co-workers[83]

[74] Wasserscheid, P., Bösmann, A., Bolm, C., 2002. Synthesis and properties of ionic liquids derived from the 'chiral pool'. Chem Commun, 200–201. b) Vo Thanh, G., Pegot, B., Loupy, A., 2004. Solvent-Free Microwave-Assisted Preparation of Chiral Ionic Liquids from (-)-*N*-Methylephedrine. Eur. J. Org. Chem., 1112-1116

[75] a) Levillain, J., Dubant, G., Abrunhosa, I., Gulea, M., Gaumont, A., 2003. Synthesis and properties of thiazoline based ionic liquids derived from the chiral pool**.** Chem. Commun., 2914–2915. b) Ni, B., Headley, A., Li, G., 2005. Design and Synthesis of C-2 Substituted Chiral Imidazolium Ionic Liquids from Amino Acid Derivatives. J. Org. Chem., 70, 10600–10602.

[76] a) Abbott, A., Capper, G., Davis, D., Rasheed, R., Tambyrajah, V., 2002. Quaternary ammonium zinc- or tin-containing ionic liquids: water insensitive, recyclable catalysts for Diels-Alder reactions Green Chem., 4, 24–26. b) Fukaya, Y., Iizuka, Y., Sekikawa, K., Ohno, H., 2007. Bio ionic liquids: room temperature ionic liquids composed wholly of biomaterials. Green Chem., 9, 1155–1157. c) Abbott, A., Boothby, B., Capper, G., Davis, D., 2004. Deep Eutectic Solvents Formed between Choline Chloride and Carboxylic Acids: Versatile Alternatives to Ionic Liquids. J. Am. Chem. Soc., 126, 9142– 9147.

[77] Shibagaki, M., Matsushita, H. Kaneko, H., 1983. The preparation of N'-alkylnicotinium salts. Heterocycles, 20, 497–500

[78] J. Pernak, J. Feder-Kubis, 2005. Synthesis and Properties of Chiral Ammonium-Based Ionic Liquids**.** Chem. Eur. J., 11, 4441–4449.

[79] a) Kumar, V., Pei, C., Olsen, C., Scha¨ffer, S., Parmarb, V., Malhotra, S., 2008. Novel carbohydrate-based chiral ammonium ionic liquids derived from isomannide. Tetrahedron: Asymmetry, 19, 664–671. b) Handy, S., Okello, M., Dickenson, G., 2003. Solvents from Biorenewable Sources: Ionic Liquids Based on Fructose. Org. Lett., 2513– 2515.

[80] Nabenhauer, F.P., 1940. US Pat., 2,185,220.

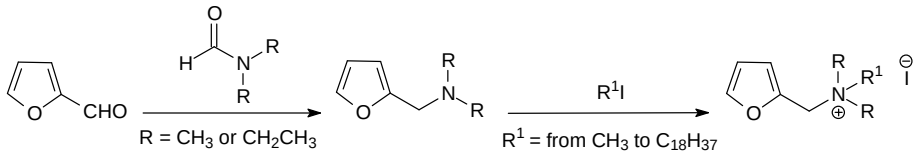
[81] a) Kordik, H., Williams, D., 1952. Studies on the structure-action relationships of the choline group. Br. J. Pharmacol, 7, 103–116. b) Manfredini, S., Guarneri, M., Simoni, D., Grana, E., Borselli, C., Zonta, F., Feriani, A., Gaviraghi, G., Toson, G., 1994. Cholinergic agents structurally related to furtrethonium. Eur. J. Med. Chem., 29, 153–161.

[82] Michal, P., El-Fakahany, E., Dolezal, V., 2007. Muscarinic M_2 Receptors Directly Activate $G_{q/11}$ and G_s G-Proteins. J. Pharmacol. Exp. Ther., 320, 607–614.

[83] Weilmuenster, E.A., Jordan, C.N., 1945. The preparation and properties of some furfuryl quaternary ammounium compounds. J. Am. Chem. Soc., 67, 415–416.

proposed a synthetic method for the synthesis of dimethylalkylammonium furans (Figure 3.1.5.1.) whose key step was the reaction of a large excess of *N,N*-dialkylformamide with furfural at 170°C, followed by quenching with very strong alkaline solutions.

Figure 3.1.5.1. Synthesis of furfuryl quaternary ammonium compounds proposed by Weilmuenster.



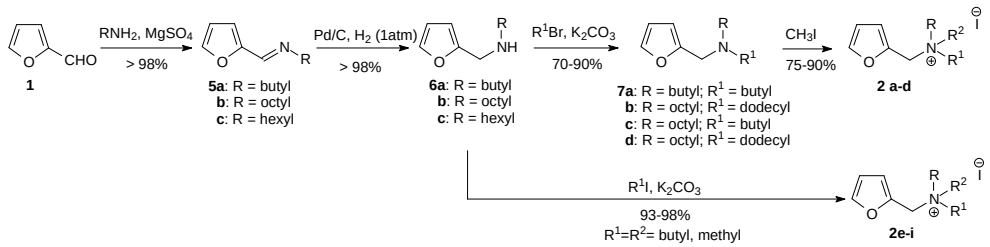
The approach reported in this Thesis to the synthesis of quaternary furan-based ammonium salts combines the use of a starting material obtainable from renewable resources with mild operation conditions. In view of practical applications, the evaluation of the thermal properties of the new ion pairs together with preliminary eco-toxicological evaluation of some representative products has been also accomplished.

Synthesis of furan-based ammonium iodides 2a–i

Ammonium iodides **2a**–**i** are synthesised through a solventless procedure at room temperature in three or four steps according to the general route described in Figure 3.1.5.2.: furfural imines **5a**, **5b** and **5c** are quantitatively obtained from furfural **1** and butylamine, octylamine or hexylamine, respectively, and then reduced by using hydrogen (1 atm on Pd/C, 10%) to give secondary amines **6a**, **6b** and **6c**. Alkylation with bromobutane or bromododecane gives the tertiary amines **7a**–**d** which are finally converted into the ammonium salts **2a**–**d** by treatment with iodomethane. Products **2e**–**i** can be obtained directly from secondary amines **6a**, **6b** and **6c** and 2.2 equivalents of iodomethane or iodobutane. Although the synthesis is multistep, the overall yields from furfural **1** to the ammonium salts **2a**–**i** are very high and range from 75 to 90%. Purity of compounds, assessed by means of quantitative ^{1}H NMR with internal standard, is generally very good; when necessary, especially for toxicity tests, ion pairs can be further

purified by flash chromatography on a short silica gel column, or, for solid compounds **2a**, **2e**, **2f** and **2i** by grinding in ethyl acetate.

Figure 3.1.5.2. Synthesis of furan-based ammonium salts.



Ion pairs **2a**, **2e**, **2f** and **2i** with short lateral chains or high degree of symmetry are solid with melting points around 100°C, whereas iodide salts with four different substituents on ammonium (**2c**) or long lateral chains (**2b** and **2d**, **2g** and **2h**) are viscous liquids at room temperature (Table 3.1.5.3.).

Preparation of 3a–h and 4a–h

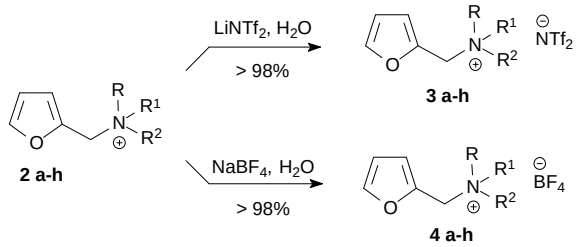
The iodide anion is further exchanged with two inorganic salts (lithium bis(trifluoromethylsulfonyl)imide $LiNTf_2$ and sodium tetrafluoroborate $NaBF_4$) by an anion metathesis in water, to give the ammonium salts **3a-h** and **4a-h** respectively.

According to the general procedure described in Figure 3.1.5.3., the ammonium iodides **2a**–**h** are converted in to **3a**–**h** upon treatment with $LiNTf_2$ in water.[84] Ion pairs **3a**–**h** are liquid at room temperature, except for compound **3f**, and insoluble in water; the presence of NTf_2 anion, in fact strongly lowers the melting point with respect to the corresponding iodides and increases the fluidity of viscous ones as a general feature.

The exchange with $NaBF_4$ gives the ionic salts **4a**–**h**; except for compound **4e** which is solid, all the tetrafluoroborate ammonium salts are liquid at room temperature. The yields of the exchange reactions are always close to 100%.

[84] Gupta, O.D., Armstrong, P.D., Shreeve, J.M., 2003. Low melting and slightly viscous ionic liquids via protonation of trialkylamines by perfluoroalkyl β-diketones. Tetrahedron Lett., 44, 9367–9370.

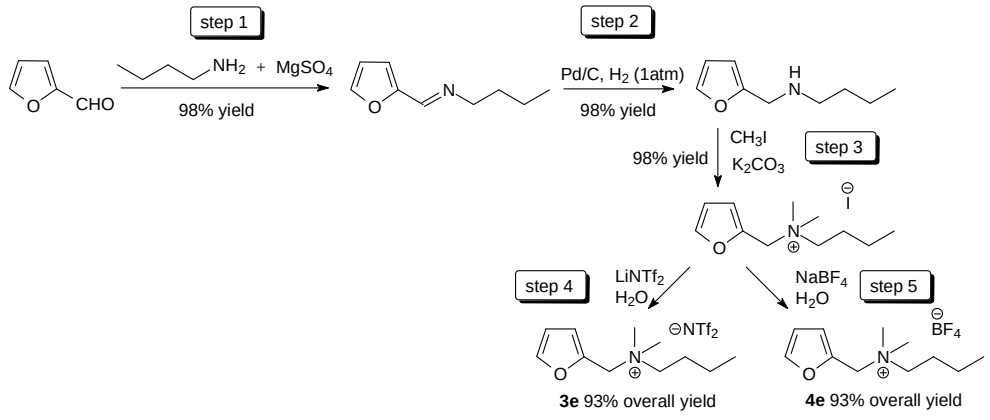
Figure 3.1.5.3. Anion exchange with $LiNTf_2$ and $NaBF_4$.



Considering the whole process it can be pointed out that *i*) all the reactions depicted in Figure 3.1.5.2. are solvent free, or performed in water, as in the case of the anion metathesis; *ii*) the temperature conditions do not exceed the room temperature; *iii*) the overall yields are very high. To deepen the analysis of the sustainability and efficiency of the process and to quantify its "greenness", five green metrics have been used and compared: *i*) atom economy which calculates how much of the reactants remains in the final product; *ii*) carbon efficiency that indicates the percentage of carbon in the reactants that remain in the final product; *iii*) mass intensity which includes everything (reactants, reagents, solvents, catalysts) used in a process or process step with the exception of water; *iv*) E-factor which considers the quantity of waste produced for a given mass of product; *v*) reaction mass efficiency that describes the percentage of reactants' mass that remains in the product.

The synthesis of the ammonium salts **3e** and **4e** (*N*-butyl-*N*,*N*-dimethyl-2-furfurylammonium NTf_2 and BF_4) (Figure 3.1.5.4.) has been chosen as representative of all the other salts.

Figure 3.1.5.4. Synthesis of *N*-butyl-*N*,*N*-dimethyl-2-furfurylammonium NTf_2 **3e** and BF_4 **4e** analysed from the sustainability point of view through five green metrics.



The carbon efficiency CE, the mass intensity MI, the E-factor and the reaction mass efficiency RME are calculated for each single step described in Figure 3.1.5.4. (Table 3.1.5.2.), whereas the atom economy AE is calculated for the global synthetic pathway, according to the formulas reported below.

For the generic reaction:

$$A + B \longrightarrow C$$

- the carbon efficiency (CE) is defined as:[85]

$$CE = \frac{(n° \text{ moles C} \times n° \text{ carbons C})}{(n° \text{ moles A} \times n° \text{ carbons A}) + (n° \text{ moles B} \times n° \text{ carbons B})} \times 100$$

- the mass intensity (MI) is defined as:

$$MI = \frac{\text{total mass used in a process or in a process step (kg)}}{\text{mass of products (kg)}}$$

- the E-factor, proposed by Sheldon,[86] is defined as:

$$\text{E-factor} = \frac{\text{total waste (kg)}}{\text{mass of product (kg)}}$$

and it can be also calculated from mass intensity MI as:

$$\text{E-factor} = MI - 1$$

- the reaction mass efficiency (RME) is defined as:[85]

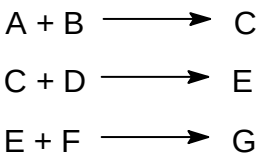
$$RME = \frac{(\text{mass of product C})}{(\text{mass of A} + \text{mass of B})} \times 100$$

- the atom economy (AE), proposed by Trost,[87] in the case of the general synthetic process:

[85] a) Curzons, A., Constable, D., Mortimer D., Cunningham, V., 2001. So you think your process is green, how do you know?—Using principles of sustainability to determine what is green–a corporate perspective Green Chem., 3, 1–6. b) Constable, D., Curzons, A., Cunningham, V., 2002. Metrics to 'green' chemistry—which are the best? Green Chem, 4, 521–527.

[86] a) Sheldon, R., 1992. Chem. Ind. (London), 903–906. Sheldon, R., 1997. Chem. Ind. (London), 12–15.

[87] a) Trost, B., 1991. The atom economy--a search for synthetic efficiency Science, 254, 1471-1477. b) Trost, B., 1994. Atom Economy - A Challenge for Organic Synthesis: Homogeneous Catalysis Leads the Way. Angew Chem Int Ed, 34, 3, 259-281.



is defined as:

$$AE = \frac{\text{(mw of product G)}}{\text{(mw of A + mw of B+ mw of D+ mw of F)}} \times 100$$

Table 3.1.5.2. Green metrics calculation for the synthesis of ammonium salts **3e** and **4e**: carbon efficiency (CE), mass intensity (MI), E-factor, reaction mass efficiency (RME) and atom economy (AE).

step	**CE**	**MI**	**E-factor**	**RME**	**AE**
	%	**kg/kg**	**kg/kg**	**%**	**%**
1	96	1.5	0.5	87	
2	98	1.1	0.1	99	
3	96	2.4	1.4	67	
4	98	1.2	0.2	79	
5	98	1.5	0.5	64	
Average for 3e	97	1.5	0.5	83	77
Average for 4e	97	1.6	0.6	79	63

By using the five green metrics to evaluate the synthesis of ammonium salts **3e** and **4e**, it emerges that all of them are in good agreement, being the step 2, the reduction of the imine, the "greenest" one, and the step 3, the alkylation with iodomethane, the worst one. In general the global procedure can be considered "green", from all the green metrics points of view.

Carbon efficiency CE, which includes yield and stoichiometry of reactants and products, is very high for all the steps, but less than 100% because of the excess stoichiometry in almost each step. However in terms of carbon efficiency it is rather difficult improving the process more than this.

Mass intensity MI takes into account the yield, stoichiometry, the solvent, and the reagent used in the reaction mixture. In the ideal situation, MI would be 1, indicating that all the mass used in the process is incorporated into the product. In the case of the synthetic pathway described in Figure 3.1.5.4. all the steps which involve inorganic salts (e.g. $MgSO_4$ in step 1, K_2CO_3 in step 3, $LINTf_2$ in step 4 and $NaBF_4$ in step 5) show quite high values of MI,

instead the reduction of butyl-imine to butyl-amine (step 2) shows the lowest value, very close to 1. By expressing mass intensity as its reciprocal and making it a percentage, mass productivity MP is obtained. MP values for the global synthesis of **3e** and **4e** are respectively 66% and 62%, indicating that in both the processes about 40% of the total mass used to make **3e** and **4e** is wasted.

The E-factor indicates the quantity of waste that is produced for a given mass of product, and in the ideal situation, it would be 0, meaning that no wastes are produced during the synthesis. The mean values reported in Table 3.1.5.2. are quite low and, as for the mass intensity, the least "green" steps are those in which inorganic salts are used and not recovered.

Reaction mass efficiency (RME) includes atom economy (AE), yield and the stoichiometry of reactants, by combining both process and chemistry features, and it is probably the best green metric to calculate the whole sustainability of a chemical process. By comparing the values of RME in steps 1 (87%), 2 (99%) and 3 (67%) with those reported in the literature[43a] about general aminations (54%), hydrogenations (74%), and ammonium alkylations (60%), it emerges that in each step the values here obtained are higher, indicating that the proposed pathway is greener than the major part of common chemical synthesis.

Atom economy is a calculation of how much of the reactants remain in the final product. In spite of the very high atom economies for single steps 1, 2 and 3 (Figure 3.1.5.4), the overall AE is reduced by the final anion metathesis from iodide. However it is important to note that the use of alkyl halides in the quaternarization step has the advantages of producing ammonium salts with anions easily exchangeable in water.

Properties of ion pairs 2a–i, 3a–h, 4a–h

The solubility of furfural-based quaternary ammonium salts has been checked in some common solvents: all salts are highly soluble in acetone, ethanol, methanol, dichloromethane and chloroform, but insoluble in diethyl ether and cyclohexane.

The solubility in water and in ethyl acetate, however, heavily depends on the anion and other structural features (Table 3.1.5.3.). For example, iodides

and tetrafluoroborates with short lateral chains such as **2a**, **2e**, **2f** and **4a**, **4e**, **4f**, do not dissolve in ethyl acetate whereas those bearing longer chains, such as **2b** and **4b**, are soluble. An opposite behaviour is found in water: **2a**, **2e**, **2f**, and **4a**, **4e**, **4f** are water soluble while compounds with longer chains are not. The partial solubility of compounds **2g** and **4g** in water has been quantitatively measured by ^{1}H NMR in D_2O, being 40 and 28 mg/L, respectively. All the ionic liquids **3a**–**h** with hydrophobic NTf_2 anion are totally miscible with ethyl acetate and water insoluble.

Table 3.1.5.3. Phase state and selected solubility properties of furan-based ionic pairs **2a**–**h**, **3a**–**h**, **4a**–**h.**

Salt	Physical state.	Solubility		Salt	Physical state.	Solubility	
		H_2O	AcOEt			H_2O	AcOEt
2a	s	+	-	**3d**	l	-	+
2b	vl	-	+	**3e**	l	-	+
2c	l	-	+	**3f**	s	-	+
2d	vl	-	+	**3g**	l	-	+
2e	s	+	-	**3h**	l	-	+
2f	s	+	-	**4a**	l	+	-
2g	l	40 mg/L	+	**4b**	vl	-	+
2h	l	-	+	**4c**	l	-	+
2i	s	+	-	**4d**	vl	-	+
TBAI[a]	s	+	-	**4e**	s	+	-
BTBAI[b]	s	+	-	**4f**	l	+	-
3a	l	-	+	**4g**	l	28 mg/L	+
3b	l	-	+	**4h**	l	-	+
3c	l	-	+				

s = solid; l = liquid; vl = viscous liquid
+ = soluble; - = not soluble
[a] tetrabutyl ammonium bromide
[b] benzyltributyl ammonium iodide

Thermal analysis data, essential to establish the temperature range of use of the prepared organic salts, are collected in Table 3.1.5.4. Melting temperature and glass transition temperature have been established by differential scanning calorimetry (DSC), while decomposition temperatures have been obtained through thermogravimetric analysis (TGA).

Table 3.1.5.4. Thermal analysis data of furan-based ionic pairs **2a–h**, **3a–h**, **4a–h.**

Salt	T_{dec}			T_g	T_m	Salt	T_{dec}			T_g	T_m
	T_1	T_2	T_3				T_1	T_2	T_3		
2a	187	264	303	-46	102	**3d**	252	378	433	-47	
2b	190	266	306	-47		**3e**	-	353	400	-46	
2c	187	229	288	-47		**3f**	-	345	403	-47	66
2d	187	261	298	-47		**3g**	291	360	393	-46	
2e	196	264	-	-47	110	**3h**	290	366	391	-46	
2f	173	228	264	-47	107	**4a**	192	267	-	-47	
2g	192	262	296	-47		**4b**	184	260	-	-47	
2h	162	225	-	-47		**4c**	191	275	-	-47	
2i	195	251	308	-47	78	**4d**	182	271	-	-47	
TBAI[a]	223	-	-	-47	141[c]	**4e**	198	264	-	-47	57
BTBAI[b]	187	-	-	-46	143[c]	**4f**	187	263	-	-46	
3a	250	377	-	-46		**4g**	189	260	-	-47	
3b	246	287	415	-47		**4h**	165	254	-	-47	
3c	318	367	387	-47							

T_{dec}: temperatures of decomposition (°C)
T_g: glass transition temperature (°C)
T_m: melting temperature (°C)
[a] tetrabutyl ammonium bromide
[b] benzyltributyl ammonium iodide
[c] Source: Individual Material Safety Data Sheets

Furan iodides **2a–i** are stable from room temperature to at least 170°C, moreover no significant material loss due to water or other volatile impurities has been observed in this region; at higher temperatures they show three thermal decomposition steps: the first (T_1) ranges from 162°C (**2h**) to 196°C (**2e**), the second (T_2) from 225°C (**2h**) to 266°C (**2b**), and the third (T_3) from 264°C (**2f**) to 308°C (**2i**); all of them are in the same range of decomposition temperatures of other quaternary ammonium compounds reported in the literature.[88]

To understand the thermogravimetric behaviour of furan-based iodides, also two commercially available ammonium salts, tetrabutylammonium iodide (TBAI) and benzyltributylammonium iodide (BTBAI) were analysed: they have only one decomposition temperature at 223°C and at 187°C,

[88] a) MacFarlane, D., Forsyth, S., Golding, J., Deacon, G., 2002. Ionic liquids based on imidazolium, ammonium and pyrrolidinium salts of the dicyanamide anion. Green Chem., 4, 444–448. b) Steinert, S., Voigt, W., Glausch, R., Neuschutz, M., 2005. Thermal characteristics of solid–solid phase transitions in long-chain dialkyl ammonium salts Thermochim. Acta, 435, 28–33. c) Zhang, Q., Li, Z., Zhang, J., Zhang, S., Zhu, L., Yang, J., Zhang, X., Deng, Y., 2007. Physicochemical Properties of Nitrile-Functionalized Ionic Liquids. J. Phys. Chem. B, 111, 2864–2872. d) Kourai, H., Yabuhara, T., Shirai, A., Maeda, T., Nagamune, H., 2006. Syntheses and antimicrobial activities of a series of new bis-quaternary ammonium compounds Eur. J. Med. Chem., 41, 437–444.

respectively, in the same range of the first and second decomposition temperatures T_1 and T_2 of iodides **2a–i**. Therefore it is possible to suppose that the higher T_3 temperatures of furan-containing iodides **2a–i** could be correlated with the decomposition of the aromatic furan ring.

The bis(trifluoromethylsulfonyl)imides **3a–h** are more stable than the corresponding iodides, in fact they show a first irreversible loss of mass at higher temperature: T_1, ranging from 246°C (**3b**) to 318°C (**3c**), a second decomposition temperature T_2 from 287°C (**3b**) to 378°C (**3d**), and a third, T_3, between 387°C (**3c**) and 433°C (**3d**). It is reasonable to think that the poorly basic NTf_2 anion is less prone to promote elimination reactions.

The tetrafluoroborate ammonium salts have only two decomposition temperatures: the first, T_1, ranges from 165°C (**4h**) to 198°C (**4e**), and the second, T_2, from 254°C (**4e**) to 275°C (**4c**), displaying thermogravimetric behaviour similar to the corresponding iodides.

Other thermal properties of the ion pairs have been evaluated by DSC, cooling the samples at –90°C and then heating them to 130°C at a rate of 10 °C/min, in two cycles.

All the salts show a glass transition temperature (T_g) at around -47°C, as the conventional ammonium based ion pairs[89] such as TBAI, independently by the anion type. The melting points (T_m) of the four solid iodides range from 78°C (**2i**) to 110°C (**2e**), with a reduction of the temperatures of melting when increasing the length of the alkyl chain.

By exchanging the anion from iodide to tetrafluoroborate, ammonium salts with lower melting temperatures are obtained. For example, compounds **2a** and **2f** afford liquids **4a** and **4f** at room temperature, and compound **4e** has a substantially lower melting point than **2e**, respectively 57°C and 110°C. Comparing TBAI and BTBAI with compound **2f**, it is possible to note that the furan moiety causes a decrease in the melting point, relative to an alkyl or benzyl substituent, with a T_m reduction of about 40°C (the temperature of melting of **2f** is 107°C). The melting point of the only solid bis(trifluoromethylsulfonyl)imide salt (**3f**) is 66°C, lower than those of the

[89] a) Pernak, J., Feder-Kubis, J., 2005. Synthesis and Properties of Chiral Ammonium-Based Ionic Liquids. Chem.–Eur. J., 11, 4441–4444. b) Yuan, X., Zhang, S., Lu, X., 2007. Hydroxyl Ammonium Ionic Liquids: Synthesis, Properties, and Solubility of SO_2. J. Chem. Eng. Data, 52, 596–599.

other two classes (**2f** and **4f**), according to the general behaviour of the NTf_2 anion.

As most of the obtained furan-ammonium salts are liquid at room temperature, the application as alternative reaction media can be envisaged; in particular NTf_2 salts possess the best physical properties for a use as solvents, since they combine a low melting point with a high decomposition temperature and a reduced viscosity.

In order to investigate also the chemical stability of the prepared ammonium salts to different conditions, the behaviour of **2e**, chosen as a model compound, was checked in four solutions at different pH (1, 2, 7 and 11) recording quantitative ^{1}H NMR spectra in acetone-d_6 with a known amount of tetrakis(trimethylsilyl)silane (TTMS) as internal standard.

2e is stable under basic and weakly acidic conditions for at least 24 h, but a fast decomposition occurs at very low pH.

Evaluation of acute eco-toxicity

Benzalkonium chlorides (BAC) are among the most used and efficient antimicrobic agents in the biocides field; however, the acute toxicity towards non-target living organisms is quite high (for example the EC50 values towards both the crustacean *Daphnia magna* and the bacterium *Vibrio fischeri* range from 0.02 to 0.22 mg/L,[90] and from 0.06 to 0.15 mg/L,[91] respectively) and recent studies have demonstrated that they cause genotoxic effects in mammalian and plant cells at environmentally relevant concentrations.[92]

The toxicological studies developed in recent years have demonstrated that short chain furan-based ammonium salts, such as furmethide, have a deep

[90] Garcia, M.T., Ribosa, I., Guindulain, T., Sanchez-Leal, J., Vives-Rego, J., 2001. Fate and effect of monoalkyl quaternary ammonium surfactants in the aquatic environment**.** Environ. Pollut., 111, 169–175.

[91] Jawecki, G., Grabinska-Sota, E., Narkiewicz, P., 2003. The toxicity of cationic surfactants in four bioassays. Ecotoxicol. Environ. Saf., 54, 87–91.

[92] Ferk, F., Misˇıkl, M., Hoelzl, C., Uhl, M. Fuerhacker, M., Grillitsch, B., Parzefall, W., Nersesyan, A., Micˇieta1, K., Grummt, T., Ehrlich, V., Knasmuller, S., 2007. Benzalkonium chloride (BAC) and dimethyldioctadecyl-ammonium bromide (DDAB), two common quaternary ammonium compounds, cause genotoxic effects in mammalian and plant cells at environmentally relevant concentrations. Mutagenesis, 22, 6, 363–370.

interaction with biological systems, so the toxicity and the environmental fate of the synthesised ammonium ion pairs needs to be evaluated.

Accordingly, the acute eco-toxicity towards *Daphnia magna* and *Vibrio fischeri* of the water-soluble iodides **2a**, **2e**, **2f**, **2g** and **2i** was evaluated. In addition, the toxicity of TBAI and BTBAI was assessed for comparison. *D. magna* and *V. fischeri* were selected because they are among the most widely used species in aquatic toxicity studies, allowing comparison with a large number of substances. Toxicity to the marine bacterium *V. fischeri* can also be considered a preliminary, aspecific measure of biocide activity both toward pathogens and non-target bacteria. The freshwater crustacean *D. magna* is representative of eukaryotic non-target species. The toxicity to *D. magna* was assessed using a 48 h static acute immobilisation test, according to the procedures set out in the Organisation for Economic Co-operation and Development (OECD) guideline 202.[93]

Toxicity to *V. fischeri* was measured as inhibition of bioluminescence using Microtoxs ® equipment and consumables. The assay was carried out in accordance with the 90% basic test for pure compounds protocol, as described in the Microtox user's manual.[94]

For each substance three trials of both the toxicity tests were carried out; details are described in the Experimental section.

The main results are presented in Table 3.1.5.5.

[93] Organization for Economic Cooperation and Development. Daphnia sp. Acute Immobilization Test. OECD Guideline 202, Paris, France, 2004.

[94] Azur Environmental, 90% Basic Test for Pure Compounds, 1998.

Table 3.1.5.5. The 50% effect concentrations (EC50, μM) of the ammonium salts TBAI, BTBAI, **2a**, **2e-i** to *Daphnia magna* in a 48 h immobilization test and to *Vibrio fischeri* in a 15 min inhibition of bioluminescence test.

	Daphnia magna		*Vibrio fischeri*	
	EC50	**SNK[a] test**	**EC50**	**SNK[b] test**
TBAI	7.6 (6.6–8.7)	a	769 (652–908)	a
BTBAI	6.3 (1.6-24.3)	ab	158 (115–218)	b
2f	6.0 (3.6–9.8)	ab	240 (167–345)	c
2a	6.7 (6.4–7.0)	a	105 (82–135)	d
2e	6.3 (5.1–7.8)	ab	2189 (1530–3129)	e
2i	6.0 (4.0–9.1)	ab	34 (26–45)	f
2g	4.6 (3.3–6.5)	b	3.5 (1.7–6.9)	g

[a]The reported values are means of three independent trials (in parenthesis: lower and upper limits of the confidence interval).[b]Results of the Student-Newman-Keuls post-hoc statistical test for pairwise comparison of EC50 (μmol/L). Substances sharing the same letter are not significantly different.

The EC50 values of the tested ammonium salts for *D. magna* range from 4.6 to 6.7 μM. The differences tested by ANOVA are significant (P = 0.016), however the post-hoc SNK test only discriminates **2g**, the most toxic compound, from **2a** and TBAI, the least toxic; other substances are not significantly different from each other or from **2g**, **2a** and TBAI.

The EC50 values of the tested ammonium salts for *V. fischeri* range from 3.5 to 2189 μM. The differences tested by ANOVA are highly significant (P < 0.001); moreover, the post-hoc test indicates that any compound is significantly different from all the others.

Thus, while *D. magna* is in general more sensitive to the tested ammonium salts, toxicity to *V. fischeri* is more influenced by the characteristics of the lateral chains of the cation. In particular, **2e** has the lowest toxicity to *V.*

fischeri. EC50 dramatically decreases (i.e. toxicity increases) as a single lateral chain is lengthened from butyl (**2e**) to hexyl (**2i**) to octyl (**2g**). This trend is shown also by *D. magna*, however the differences are much less marked and not statistically significant.

Substitution of a methyl with a second butyl (from **2e** to **2a**) also greatly increases toxicity to *V. fischeri*; yet, the substitution of the last methyl with a third butyl (from **2a** to **2f**) slightly reduces toxicity. The substitution of the benzyl ring with a furan ring (from BTBAI to **2a**) slightly decreases toxicity. On the other hand, substitution of an aromatic ring with a butyl (from **2f** or BTBAI to TBAI) produces a larger reduction of toxicity.

Even if preliminary, these results indicate that toxicity of furan-based quaternary ammonium salts is comparable to the analogous benzyl-based salts, in particular the toxicity profile towards *V. fischeri* indicates potentially the same applications as biocides.

Conclusions

The synthesis of furan-based ion pairs from furfural has been accomplished in very good yields, good purity of the products and wide versatility. The main goal of preparing ILs suitable to be used as reaction and general-purpose solvents has been also attained, especially for NTf_2 salts **3a**–**h** that combine low melting points with high decomposition temperatures and reduced viscosities. Regarding the possible applications as surfactants and biocides, furan-based salts could be a valuable alternative to benzyltributylammonium salts and benzalkonium chloride that are produced from non-renewable resources. Further studies are in progress, in particular to characterise biocide efficiency toward pathogens and other non-target organisms and to eliminate the use of alkyl halide in the alkylation step.

Experimental section

General

Most chemicals are purchased from Sigma-Aldrich and used without further purification. Reactions are monitored by means of TLC using silica gel sheets (Merck 60 F_{254}); the products are separated by flash chromatography on silica gel (Aldrich, 230-400 mesh). NMR spectra are

recorded using a 5 mm probe on a Varian Inova 200, Varian Inova 300 or Varian Mercury 400 spectrometer, all chemical shifts are quoted relative to deuterated solvent signals with chemical shifts (δ) given in ppm and coupling costants (*J*) values given in Hz. GC-MS spectra are obtained using a AGILENT 6850 gas chromatograph on a SUPELCO SPB-5 capillary column (temperature programme: 50°C for 5 min then 10°C/min till 300°C) coupled with a mass spectrometer (quadrupole) AGILENT 5975. Thermogravimetric measurements (TGA) are carried out using a TA-SDT Q600. The analysis are performed at 10°C/min from room temperature to 500°C under N_2 flow. Differential scanning calorimetry (DSC) is performed using TA-DSC Q100 in the temperature range from -90°C to 130°C under N_2 flow, at an heating rate of 10°C/min.

Purity of new compounds was established to be at least 98% through proton nuclear magnetic resonance (^{1}H NMR) spectra by integration of proton signals with respect to an internal standard. To this purpose, spectral data have been acquired in deuterated solvent with a known amount of tetrakis(trimethylsilyl)silane (TTMS) as an internal standard and a delay time between successive scans of 20 s to ensure complete proton relaxation and therefore quantitative integration.[95]

Synthesis and characterization of products **5a-c**, **6a-c**, and **7a-d**.

***N*-Butylfurfurylimine 5a.** Freshly distilled furfural **1** (4.30 mL, 52 mmol) is charged in a two necked round bottom flask under N_2; then anhydrous $MgSO_4$ (3.13 g, 26 mmol) is added, followed by butylamine (5.14 mL, 52 mmol). The exothermic reaction is monitored by means of TLC and GC-MS; after 2 hours the conversion is complete. Imine **5a** is obtained by distillation under reduced pressure (T_{eb} =60°C, p = 10 mbar) as a colourless liquid (7.7 g, 98%). ^{1}H NMR (400 MHz, C*D*Cl$_3$): δ = 0.94 (t, ^3J (H, H) = 7.2 Hz, 3H, N(CH$_2$)$_3$C*H*$_3$), 1.33-1.42 (m, 2H, N(CH$_2$)$_2$C*H*$_2$CH$_3$), 1.66-1.71 (m, 2H, NCH$_2$C*H*$_2$CH$_2$CH$_3$), 3.58 (t, ^3J (H, H) = 6.8 Hz, 2H, NC*H*$_2$(CH$_2$)$_2$CH$_3$), 6.47 (dd, ^3J (H, H) = 1.8 Hz, ^3J (H, H) = 3.2 Hz, 1H, 4-H furan), 6.72 (d, ^3J

[95] a) Malz, F., Jancke, H., 2005. Validation of quantitative NMR J. Pharm. Biomed. Anal., 38, 813–823. b) Rizzo, V., Pinciroli, V., 2005. Quantitative NMR in synthetic and combinatorial chemistry J. Pharm. Biomed. Anal., 38, 851–857.

(H, H) = 3.2 Hz, 1H, 3-H furan), 7.50 (d, ^3J (H, H) = 1.8 Hz, 1H, 5-H furan), 8.08 ppm (s, 1H, N=C*H*); GC rt 17.4 min; MS (70 eV, EI): m/z 151 (20) [M^+]; 122 (75) [M^+- CH_3CH_2]; 108 (100) [M^+- $CH_3(CH_2)_2$]; 94 (25) [M^+- $CH_3(CH_2)_3$]; 81 (100) [M^+- $CH_3(CH_2)_3N$]; 67 (6) [M^+- $CH_3(CH_2)_3$ NCH]; 53 (20).

***N*-Octylfurfurylimine 5b.** The imine is prepared from **1a** and octylamine in 98% yield after distillation under reduced pressure (T_{eb} = 95°C, p = 9 10^{-2} mbar) with the same procedure of compound **5a**. ^{1}H NMR (200 MHz, C*D*Cl_3): δ = 0.88 (t, ^3J (H, H) = 6.2 Hz, 3H, $N(CH_2)_7CH_3$), 1.28-1.31 (m, 10H, $N(CH_2)_2(CH_2)_5CH_3$), 1.68-1.75 (m, 2H, $NCH_2CH_2(CH_2)_5CH_3$), 3.58 (t, ^3J (H, H) = 7.4 Hz, 2H, $NCH_2(CH_2)_6CH_3$), 6.47 (dd, ^3J (H, H) = 1.6 Hz, ^3J (H, H) = 3.2 Hz, 1H, 4-H furan), 6.72 (d, ^3J (H, H) = 3.2 Hz, 1H, 3-H furan), 7.51 (d, ^3J (H, H) = 1.6 Hz, 1H, 5-H furan), 8.08 (s, 1H, N=C*H*); GC rt 18.2 min; MS (70 eV, EI): m/z 207 (5) [M^+]; 192 (3) [M^+- CH_3]; 178 (15) [M^+- CH_3CH_2]; 164 (30) [M^+- $CH_3(CH_2)_2$]; 150 (100) [M^+- $CH_3(CH_2)_3$]; 136 (10) [M^+- $CH_3(CH_2)_4$]; 122 (45) [M^+- $CH_3(CH_2)_5$]; 108 (60) [M^+- $CH_3(CH_2)_6$]; 94 (25) [M^+- $CH_3(CH_2)_7$]; 80 (50) [M^+- $CH_3(CH_2)_7N$]; 67 (5) [M^+- $CH_3(CH_2)_7$ NCH]; 53 (15).

***N*-Hexylfurfurylimine 5c.** The imine is prepared from **1a** hexylamine in 97% yield after distillation under reduced pressure (T_{eb} = 90°C, p = 1 10^{-1} mbar) with the same procedure of compound **5a**. ^{1}H NMR (200 MHz, C*D*Cl_3): δ = 0.88 (t, ^3J (H, H) = 3.2 Hz, 3H, $N(CH_2)_5CH_3$), 1.23-1.38 (m, 6H, $N(CH_2)_2(CH_2)_3CH_3$), 1.65-1.75 (m, 2H, $NCH_2CH_2(CH_2)_3CH_3$), 3.56 (t, ^3J (H, H) = 4.6 Hz, 2H, $NCH_2(CH_2)_4CH_3$), 6.46 (dd, ^3J (H, H) = 1.2 Hz, ^3J (H, H) = 2.2 Hz, 1H, 4-H furan), 6.71(d, ^3J (H, H) = 2.2 Hz, 1H, 3-H furan), 7.50 (d, ^3J (H, H) = 1.2 Hz, 1H, 5-H furan), 8.07 (s, 1H, N=C*H*); GC rt 15.6 min; MS (70 eV, EI): m/z 179 (5) [M^+]; 164 (10) [M^+- CH_3]; 150 (100) [M^+- CH_3CH_2]; 136 (10) [M^+- $CH_3(CH_2)_2$]; 122 (60) [M^+- $CH_3(CH_2)_3$]; 108 (70) [M^+- $CH_3(CH_2)_4$]; 94 (35) [M^+- $CH_3(CH_2)_5$]; 80 (75) [M^+- $CH_3(CH_2)_5N$]; 67 (5) [M^+- $CH_3(CH_2)_5$ NCH]; 53 (15).

***N*-Butylfurfurylamine 6a**. Freshly distilled imine **5a** (7.55 g, 50 mmol) is charged in a two necked round bottom flask with Pd/C 10 wt % (750 mg) and connected to a rubber balloon filled with H_2 (1 atm); the reaction is stirred at room temperature and checked by means of TLC and GC-MS. After 24 hours Pd/C is filtered off on Celite and the amine is obtained by distillation under reduced pressure (T_{eb} =40°C, p = 8.10^{-2} mbar) as a colourless liquid (7.5 g, 98%). ^{1}H NMR (200 MHz, C*D*Cl$_3$): δ = 0.90 (t, ^3J (H, H) = 7.0 Hz, 3H, N(CH$_2$)$_3$C*H*$_3$), 1.25-1.52 (m, 4H, NCH$_2$(C*H*$_2$)$_2$CH$_3$), 2.60 (t, ^3J (H, H) = 6.6 Hz, 2H, NC*H*$_2$(CH$_2$)$_2$CH$_3$), 3.78 (s, 2H, NC*H*$_2$-furan), 6.17 (d, ^3J (H, H) = 3.2 Hz, 1H, 3-H furan), 6.31 (dd, ^3J (H, H) = 1.8 Hz, ^3J (H, H) = 3.2 Hz, 1H, 4-H furan), 7.33 (d, ^3J (H, H) = 1.8 Hz, 1H, 5-H furan); GC rt 17.1 min; MS (70 eV, EI): m/z 153 (15) [M$^+$]); 110 (30) [M$^+$- CH$_3$(CH$_2$)$_2$]; 96 (15) [M$^+$- CH$_3$(CH$_2$)$_3$]; 81 (100) [M$^+$- CH$_3$(CH$_2$)$_3$ NH]; 53 (15).

***N*-Octylfurfurylamine 6b**. The amine is prepared in 98% yield as a pale yellow oil after distillation under reduced pressure (T_{eb} =90°C, p = 7.10^{-2} mbar) starting from imine **5b** as described for compound **6a**. ^{1}H NMR (400 MHz, C*D*Cl$_3$): δ = 0.87 (t, ^3J (H, H) = 6.4 Hz, 3H, N(CH$_2$)$_7$C*H*$_3$), 1.26-1.27 (m, 10H, N(CH$_2$)$_2$(C*H*$_2$)$_5$CH$_3$), 1.44-1.50 (m, 2H, NCH$_2$C*H*$_2$(CH$_2$)$_5$CH$_3$), 2.59 (t, ^3J (H, H) = 6.8 Hz, 2H, NC*H*$_2$(CH$_2$)$_6$CH$_3$), 3.76 (s, 2H, NC*H*$_2$-furan), 6.15 (d, ^3J (H, H) = 3.2 Hz, 1H, 3-H furan), 6.30 (dd, ^3J (H, H) = 2.2 Hz, ^3J (H, H) = 3.2 Hz, 1H, 4-H furan), 7.35 (d, ^3J (H, H) = 2.2 Hz, 1H, 5-H furan); GC rt 17.1 min; MS (70 eV, EI): m/z 209 (25) [M$^+$]; 180 (5) [M$^+$- CH$_3$CH$_2$]; 166 (5) [M$^+$- CH$_3$(CH$_2$)$_2$]; 152 (5) [M$^+$- CH$_3$(CH$_2$)$_3$]; 110 (100) [M$^+$- CH$_3$(CH$_2$)$_6$]; 96 (20) [M$^+$- CH$_3$(CH$_2$)$_7$]; 81 (100) [M$^+$- CH$_3$(CH$_2$)$_7$ NH]; 53 (15).

***N*-Hexylfurfurylamine 6c**. The amine is prepared in 95% yield as a pale yellow oil after distillation under reduced pressure (T_{eb} = 70°C, p = 7.10^{-2} mbar) starting from imine **5c** as described for compound **6a**. ^{1}H NMR (400 MHz, C*D*Cl$_3$): δ = 0.85 (t, ^3J (H, H) = 6.2 Hz, 3H, N(CH$_2$)$_5$C*H*$_3$), 1.23-1.27 (m, 6H, N(CH$_2$)$_2$(C*H*$_2$)$_3$CH$_3$), 1.47-1.52 (m, 2H, NCH$_2$C*H*$_2$(CH$_2$)$_3$CH$_3$), 2.61 (t, ^3J (H, H) = 7.2 Hz, 2H, NC*H*$_2$(CH$_2$)$_4$CH$_3$), 3.77 (s, 2H, NC*H*$_2$-furan),

6.19 (d, ^3J (H, H) = 3.0 Hz, 1H, 3-H furan), 6.32 (dd, ^3J (H, H) = 2.2 Hz, ^3J (H, H) = 3.0 Hz, 1H, 4-H furan), 7.34 (d, ^3J (H, H) = 2.2 Hz, 1H, 5-H furan); GC rt 15.4 min; MS (70 eV, EI): m/z 181 (5) [M^+]; 110 (40) [M^+- $CH_3(CH_2)_4$]; 96 (10) [M^+- $CH_3(CH_2)_5$]; 81 (100) [M^+- $CH_3(CH_2)_5$ NH]; 53 (10).

***N,N*-dibutylfurfurylamine 7a.** Freshly distilled amine **6a** (1.53 g, 10 mmol), K_2CO_3 (1.38 g, 10 mmol) and bromobutane (1.07 mL, 10 mmol) are charged in a two necked round bottom flask and stirred at room temperature for 24 hours. The reaction is monitored by GC-MS, when the conversion is completed, K_2CO_3 is filtered off and the product is purified by distillation under reduced pressure (T_{eb} = 70°C, p = 8.10^{-2} mbar) giving a pale yellow oil (1.24 g, 80%). ^{1}H NMR (400 MHz, C*D*Cl_3): δ = 0.90 (m, 6H, 2 × C*H*$_3(CH_2)_3$N), 1.27-1.33 (m, 4H, 2 × CH_3C*H*$_2(CH_2)_2$N), 1.43-1.49 (m, 4H, 2 × CH_3CH_2C*H*$_2CH_2$N), 2.42 (m, 4H, 2 × $CH_3(CH_2)_2$C*H*$_2$N), 3.64 (s, 2H, NC*H*$_2$-furan), 6.15 (d, ^3J (H, H) = 3.0 Hz, 1H, 3-H furan), 6.31 (dd, ^3J (H, H) = 1.8 Hz, ^3J (H, H) = 3.0 Hz, 1H, 4-H furan), 7.36 (d, ^3J (H, H) = 1.8 Hz, 1H, 5-H furan); GC rt 20.6 min; MS (70 eV, EI): m/z 209 (10) [M^+]; 166 (45) [M^+- $CH_3(CH_2)_2$]; 81 (100) [M^+- $CH_3(CH_2)_3$N $CH_3(CH_2)_3$]; 53 (10).

***N*-butyl-*N*-dodecylfurfurylamine 7b** is obtained with the same procedure of **7a** starting from **6a** and bromododecane,70% yield after flash chromatography (cyclohexane/AcOEt 8/2). ^{1}H NMR (200 MHz, C*D*Cl_3): δ = 0.86-0.94 (m, 6H, C*H*$_3(CH_2)_{11}$N, C*H*$_3(CH_2)_3$N), 1.26-1.35 (m, 20H, CH_3(C*H*$_2)_9(CH_2)_2$N, CH_3C*H*$_2(CH_2)_2$N), 1.50-1.52 (m, 4H, $CH_3(CH_2)_9$C*H*$_2CH_2$N, CH_3CH_2C*H*$_2CH_2$N), 2.44-2.49 (m, 4H, $CH_3(CH_2)_{10}$C*H*$_2$N, $CH_3(CH_2)_3$C*H*$_2$N), 3.72 (s, 2H, NC*H*$_2$-furan), 6.22 (d, ^3J (H, H) = 3.2 Hz, 1H, 3-H furan), 6.33 (dd, ^3J (H, H) = 2.2 Hz, ^3J (H, H) = 3.2 Hz, 1H, 4-H furan), 7.38 (d, ^3J (H, H) = 2.2 Hz, 1H, 5-H furan); GC rt 26.5 min; MS (70 eV, EI): m/z 321 (10) [M^+]; 278 (40) [M^+- $CH_3(CH_2)_2$]; 166 (65) [M^+- $CH_3(CH_2)_{10}$]; 81 (100) [M^+- $CH_3(CH_2)_{11}$ N $CH_3(CH_2)_3$].

***N*-Butyl-*N*-octylfurfurylamine 7c.** The amine **7c** is prepared from amine **6b** with the same procedure described for compound **7a**, obtained as a pale

yellow oil in 90% yield after distillation under reduced pressure (T_{eb} =100°C, p = 9 10^{-2} mbar). ^{1}H NMR (400 MHz, C*D*Cl$_3$): δ = 0.86 (t, ^3J (H, H) = 6.4 Hz, 3H, C*H*$_3$(CH$_2$)$_7$N), 0.93 (t, ^3J (H, H) = 7.2 Hz, 3H, N(CH$_2$)$_3$C*H*$_3$), 1.24-1.36 (m, 12H, CH$_3$(C*H*$_2$)$_5$(CH$_2$)$_2$N, CH$_3$C*H*$_2$(CH$_2$)$_2$N), 1.75-1.79 (m, 4H, CH$_3$(CH$_2$)$_5$C*H*$_2$CH$_2$N, CH$_3$CH$_2$C*H*$_2$CH$_2$N), 2.72-2.77 (m, 4H, CH$_3$(CH$_2$)$_6$C*H*$_2$N, CH$_3$(CH$_2$)$_2$C*H*$_2$N), 4.08 (s, 2H, NC*H*$_2$-furan), 6.40 (dd, ^3J (H, H) = 1.8 Hz, ^3J (H, H) = 3.2 Hz, 1H, 3-H furan), 6.52 (d, ^3J (H, H) = 3.2 Hz, 1H, 4-H furan), 7.45 (d, ^3J (H, H) = 1.8 Hz, 1H, 5-H furan); GC rt 20.3 min; MS (70 eV, EI): m/z 265 (10) [M$^+$]; 222 (30) [M$^+$- CH$_3$(CH$_2$)$_2$]; 166 (50) [M$^+$- CH$_3$(CH$_2$)$_6$]; 81 (100) [M$^+$- CH$_3$(CH$_2$)$_3$ N CH$_3$(CH$_2$)$_7$], 53 (5).

N-**Dodecyl-*N*-octyl-furfuryl amine 7d.** The amine is prepared from octyl-furfuryl amine **6b** as described for compound **7b,** in a 70% yield. ^{1}H NMR (400 MHz, C*D*Cl$_3$): δ = 0.85-0.88 (m, 6H, C*H*$_3$(CH$_2$)$_{11}$N, C*H*$_3$(CH$_2$)$_7$N), 1.24-1.28 (m, 28H, CH$_3$(C*H*$_2$)$_9$(CH$_2$)$_2$N, CH$_3$(C*H*$_2$)$_5$(CH$_2$)$_2$N), 1.71-1.73 (m, 4H, CH$_3$(CH$_2$)$_9$C*H*$_2$CH$_2$N, CH$_3$ (CH$_2$)$_5$C*H*$_2$CH$_2$N), 2.67 (m, 4H, CH$_3$(CH$_2$)$_{10}$C*H*$_2$N, CH$_3$(CH$_2$)$_6$C*H*$_2$N), 4.00 (s, 2H, NC*H*$_2$-furan), 6.38 (dd, ^3J (H, H) = 1.6 Hz, ^3J (H, H) = 3.2 Hz, 1H, 4-H furan), 6.38 (d, ^3J (H, H) = 3.2 Hz, 1H, 3-H furan), 7.43 (d, ^3J (H, H) = 1.6 Hz, 1H, 5-H furan); GC rt 27.3; MS (70 eV, EI): m/z 377 (10) [M$^+$]; 278 (75) [M$^+$- CH$_3$(CH$_2$)$_6$]; 264 (10) [M$^+$- CH$_3$(CH$_2$)$_7$]; 222 (80) [M$^+$- CH$_3$(CH$_2$)$_{10}$]; 208 (10) [M$^+$- CH$_3$(CH$_2$)$_{11}$]; 81 (100) [M$^+$- CH$_3$(CH$_2$)$_{11}$ N CH$_3$(CH$_2$)$_7$].

***N,N*-Dibutyl-*N*-methyl-2-furfurylammonium iodide 2a**. Tertiary amine **7a** (2.0 g, 10 mmol) and iodomethane (6.9 mL, 11.1 mmol) were stirred at room temperature for 24 hours. The excess of iodomethane was evaporated under vacuum and the solid obtained was crystallized from ethyl acetate and ethanol (3.0 g, 85% yield). ^{1}H NMR (400 MHz, C*D*Cl$_3$): δ = 0.99-1.03 (m, 6H, 2 × N(CH$_2$)$_3$C*H*$_3$), 1.40-1.49 (m, 4H, 2 × N(CH$_2$)$_2$C*H*$_2$CH$_3$), 1.75-1.84 (m, 4H, 2 × NCH$_2$C*H*$_2$(CH$_2$)$_2$CH$_3$), 3.29 (s, 3H, NC*H*$_3$), 3.33-3.51 (m, 4H, 2 × NC*H*$_2$(CH$_2$)$_2$CH$_3$), 4.99 (s, 2H, NC*H*$_2$-furan), 6.49 (dd, ^3J (H, H) = 2.0 Hz, ^3J (H, H) = 3.2 Hz, 1H, 4-H furan), 7.06 (d, ^3J (H, H) = 3.2 Hz, 1H, 3-H furan), 7.59 ppm (d, ^3J (H, H) = 2.0

Hz, 1H, 5-H furan); ^{13}C NMR (100MHz, C*D*Cl$_3$): δ = 13.6 (×2C), 19.7 (×2C), 24.5 (×2C), 48.5, 57.9, 61.2 (×2C), 111.7, 117.9, 142.0, 145.4 ppm.

***N*-Butyl-*N*-dodecyl-*N*-methyl-2-furfurylammonium iodide 2b**. Prepared starting from tertiary amine **7b** with the same procedure reported for **2a.** The viscous liquid obtained can be purified by a short flash chromatography eluting with CH_2Cl_2/acetone (1/1) (3.5 g, 75% yield). ^{1}H NMR (400 MHz, C*D*Cl$_3$): δ =0.86 (t, ^3J (H, H) = 6.4 Hz, 3H, C*H*$_3$(CH$_2$)$_{11}$N), 0.99 (t, ^3J (H, H) = 7.2 Hz, 3H, C*H*$_3$(CH$_2$)$_3$N), 1.24-1.30 (m, 16H, CH$_3$(C*H*$_2$)$_8$(CH$_2$)$_3$N), 1.34-1.38 (m, 4H, CH$_3$(CH$_2$)$_8$C*H*$_2$(CH$_2$)$_2$N, CH$_3$C*H*$_2$(CH$_2$)$_2$N), 1.76-1.83 (m, 4H, CH$_3$(CH$_2$)$_9$C*H*$_2$CH$_2$N, CH$_3$CH$_2$C*H*$_2$CH$_2$N), 3.27 (s, 3H, NC*H*$_3$), 3.32-3.47 (m, 4H, CH$_3$(CH$_2$)$_{10}$C*H*$_2$N, CH$_3$(CH$_2$)$_2$C*H*$_2$N), 4.96 (s, 2H, NC*H*$_2$-furan), 6.46 (dd, ^3J (H, H) = 2 Hz, ^3J (H, H) = 3.6 Hz, 1H, 4-H furan), 7.03 (d, ^3J (H, H) = 3.6 Hz, 1H, 3-H furan), 7.51 ppm (d, ^3J (H, H) = 2 Hz, 1H, 5-H furan). ^{13}C NMR (100MHz, C*D*Cl$_3$): δ =13.6, 14.0, 19.6, 22.5, 22.6, 24.5, 26.2, 29.0, 29.2, 29.3, 29.3, 29.5 (×2C), 31.8, 48.5, 57.7, 61.2, 61.4, 111.5, 117.7, 142.0, 145.2 ppm.

***N*-Butyl-*N*-methyl-*N*-octyl-2-furfurylammonium iodide 2c**. Prepared starting from tertiary amine **7c** with the same procedure reported for **2a.** The viscous liquid obtained can be purified by flash chromatography eluting with CH_2Cl_2/acetone (1/1) (3.2 g, 78% yield). ^{1}H NMR (400 MHz, C*D*Cl$_3$): δ = 0.88 (t, ^3J (H, H) = 6.8 Hz, 3H, N(CH$_2$)$_7$C*H*$_3$), 1.01 (t, ^3J (H, H) = 7.6 Hz, 3H, N(CH$_2$)$_3$C*H*$_3$), 1.23-1.47 (m, 12H, CH$_3$C*H*$_2$(CH$_2$)$_2$N, CH$_3$(C*H*$_2$)$_5$(CH$_2$)$_2$N), 1.77-1.81 (m, 4H, CH$_3$CH$_2$C*H*$_2$CH$_2$N, CH$_3$(CH$_2$)$_5$C*H*$_2$CH$_2$N), 3.29 (s, 3H, NC*H*$_3$), 3.31-3.48 (m, 4H, CH$_3$(CH$_2$)$_2$C*H*$_2$N, CH$_3$(CH$_2$)$_6$C*H*$_2$N), 5.00 (s, 2H, NC*H*$_2$-furan), 6.49 (dd, ^3J (H, H) = 1.6 Hz, ^3J (H, H) = 3.4 Hz, 1H, 4-H furan), 7.07 (d, ^3J (H, H) = 3.4 Hz, 1H, 3-H furan), 7.52 ppm (d, ^3J (H, H) = 1.6 Hz, 1H, 5-H furan). ^{13}C NMR (100MHz, C*D*Cl$_3$): δ = 13.6, 14.0, 19.6, 22.5, 22.6, 24.5, 26.2, 28.9, 29.0, 31.5, 48.4, 57.8, 61.2, 61.3, 111.6, 117.9, 142.0, 145.3 ppm.

***N*-Dodecyl-*N*-methyl-*N*-octyl-2-furfurylammonium iodide 2d**. Prepared starting from tertiary amine **7d** with the same procedure reported for **2a.** The viscous liquid obtained can be purified by flash chromatography eluting with CH_2Cl_2/acetone (1/1) (4.6 g, 90% yield). ^{1}H NMR (400 MHz, C*D*Cl_3): δ = 0.83 (t, ^3J (H, H) = 6.4 Hz, 6H, C*H*$_3$(CH$_2$)$_{11}$N, C*H*$_3$(CH$_2$)$_7$N), 1.24-1.35 (m, 28H, CH$_3$(C*H*$_2$)$_9$(CH$_2$)$_2$N, CH$_3$(C*H*$_2$)$_5$(CH$_2$)$_2$N), 1.75-1.77 (m, 4H, CH$_3$(CH$_2$)$_9$C*H*$_2$CH$_2$N, CH$_3$(CH$_2$)$_4$C*H*$_2$CH$_2$N), 3.23 (s, 3H, NC*H*$_3$), 3.30-3.40 (m, 4H, CH$_3$(CH$_2$)$_{10}$C*H*$_2$N, CH$_3$(CH$_2$)$_6$C*H*$_2$N), 4.92 (s, 2H, NC*H*$_2$-furan), 6.43 (dd, ^3J (H, H) = 1.8 Hz, ^3J (H, H) = 3.4 Hz, 1H, 4-H furan), 7.00 (d, ^3J (H, H) = 3.4 Hz, 1H, 3-H furan), 7.47 ppm (d, ^3J (H, H) = 1.8 Hz, 1H, 5-H furan). ^{13}C NMR (100MHz, C*D*Cl_3): δ =13.9, 13.9, 22.4, 22.5, 22.5, 26.1, 28.8, 28.8, 28.9, 29.1, 29.2, 29.2, 29.4 (×4C), 31.4, 31.7, 48.5, 57.7, 61.2, 61.4 , 111.4, 117.5, 141.9, 145.1 ppm.

***N*-Butyl-*N*,*N*-dimethyl-2-furfuryl ammonium iodide 2e**. *N*-butylfurfuryl amine **6a** (1.5 g, 10 mmol) and K_2CO_3 (3.0 g, 22 mmol) were charged in a two necked round bottom flask, then iodomethane (1.3 mL, 22 mmol) was added and the reaction was stirred at room temperature for 24 hours. K_2CO_3 was filtered off and the excess of iodomethane evaporated under vacuum. The solid obtained was crystallized from ethanol and ethyl acetate (3.1 g, 98% yield). ^{1}H NMR (400 MHz, C*D*Cl_3): δ =1.02 (t, ^3J (H, H) = 7.2 Hz, 3H, N(CH$_2$)$_3$C*H*$_3$), 1.43-1.48 (m, 2H, N(CH$_2$)$_2$C*H*$_2$CH$_3$), 1.82-1.86 (m, 2H, NCH$_2$C*H*$_2$CH$_2$CH$_3$), 3.36 (s, 6H, 2 × NC*H*$_3$), 3.50-3.54 (m, 2H, NC*H*$_2$(CH$_2$)$_2$CH$_3$), 5.04 (s, 2H, NC*H*$_2$-furan), 6.05 (dd, ^3J (H, H) = 3.4 Hz, ^3J (H, H) = 2 Hz, 1H, 4-H furan), 7.05 (d, ^3J (H, H) = 3.4 Hz, 1H, 3-H furan), 7.54 ppm (d, ^3J (H, H) = 2 Hz, 1H, 5-H furan). ^{13}C NMR (100MHz, C*D*Cl_3): δ = 13.3, 19.1, 24.3, 50.3 (×2C), 59.4, 63.8, 111.1, 117.2, 141.9, 145.1 ppm.

***N*,*N*,*N*-Tributyl-2-furfuryl ammonium iodide 2f**. *N*-butylfurfuryl amine **6a** (1.5 g, 10 mmol) and K_2CO_3 (3.0 g, 22 mmol); were charged in

a two necked round botton flask, then iodobutane (2.5 mL, 22 mmol) was added and the reaction was stirred at 50°C for 24 hours. K_2CO_3 was filtered off and the oil extracted with H_2O/diethyl ether; the water phase was concentrated and the solid crystallized from ethyl acetate and ethanol (3.6 g, 93% yield). ^{1}H NMR (400 MHz, C*D*Cl$_3$): δ = 1.00-1.04 (m, 9H, 3 × N(CH$_2$)$_3$C*H*$_3$), 1.42-1.48 (m, 6H, 3 × N(CH$_2$)$_2$C*H*$_2$CH$_3$), 1.77-1.85 (m, 6H, 3 × NCH$_2$C*H*$_2$CH$_2$CH$_3$), 3.29-3.34 (m, 6H, 3 × NC*H*$_2$CH$_2$)$_2$CH$_3$), 4.90 (s, 2H, NC*H*$_2$-furan), 6.49 (dd, ^{3}*J* (H, H) = 2 Hz, ^{3}*J* (H, H) = 3.2 Hz, 1H, 4-H furan), 6.96 (d, ^{3}*J* (H, H) = 3.2 Hz, 1H, 3-H furan), 7.52 ppm (d, ^{3}*J* (H, H) = 2 Hz, 1H, 5-H furan). ^{13}C NMR (100MHz, C*D*Cl$_3$): δ = 13.6 (×3C), 19.7 (×3C), 24.4 (×3C), 55.6, 59.2 (×3C), 111.6, 117.3, 141.9, 145.2 ppm.

***N*-Octyl-*N*,*N*-dimethyl-2-furfurylammonium iodide 2g**. Prepared starting from amine **6b** with the same procedure reported for **2e.** The liquid obtained was purified by flash chromatography eluting with CH_2Cl_2/acetone (1/1) (3.5 g, 96% yield). ^{1}H NMR (400 MHz, C*D*Cl$_3$): δ = 0.88 (t, ^{3}*J* (H, H) = 6.4 Hz, 3H, N(CH$_2$)$_7$C*H*$_3$), 1.27-1.37 (m, 10H, N(CH$_2$)$_2$(C*H*$_2$)$_5$CH$_3$), 1.83-1.85 (m, 2H, NCH$_2$C*H*$_2$(CH$_2$)$_5$CH$_3$), 3.36 (s, 6H, 2 × NC*H*$_3$), 3.46-3.51 (m, 2H, NC*H*$_2$(CH$_2$)$_6$CH$_3$), 5.05 (s, 2H, NC*H*$_2$-furan), 6.49 (dd, ^{3}*J* (H, H) = 3.4 Hz, ^{3}*J* (H, H) = 2 Hz, 1H, 4-H furan), 7.05 (d, ^{3}*J* (H, H) = 3.4 Hz, 1H, 3-H furan), 7.54 ppm (d, ^{3}*J* (H, H) = 2 Hz, 1H, 5-H furan). ^{13}C NMR (100MHz, C*D*Cl$_3$): δ = 13.5, 13.9, 19.6, 22.4, 24.3, 26.3, 28.9, 31.5, 55.5, 59.1, 59.3, 111.5, 117.2, 141.9, 145.1 ppm.

***N*,*N*-Dibutyl-*N*-octyl-2-furfurylammonium iodide 2h**. Prepared starting from amine **6b** with the same procedure reported for **2f.** The liquid obtained can be purified by flash chromatography eluting with CH_2Cl_2/acetone (1/1) (4.2 g, 95% yield). ^{1}H NMR (400 MHz, C*D*Cl$_3$): δ = 0.88 (t, ^{3}*J* (H, H) = 6.8 Hz, 3H, N(CH$_2$)$_7$C*H*$_3$), 1.02 (t, ^{3}*J* (H, H) = 7.2 Hz, 6H, 2 × N(CH$_2$)$_3$C*H*$_3$), 1.24-1.38 (m, 14H, 2 × CH$_3$C*H*$_2$(CH$_2$)$_2$N, CH$_3$(C*H*$_2$)$_5$(CH$_2$)$_2$N), 1.78-1.82 (m, 6H, 2 × CH$_3$CH$_2$C*H*$_2$CH$_2$N, CH$_3$(CH$_2$)$_5$C*H*$_2$CH$_2$N), 3.27-3.34 (m, 6H, 2 × CH$_3$(CH$_2$)$_2$C*H*$_2$N,

$CH_3(CH_2)_6CH_2N$), 4.92 (s, 2H, NCH_2-furan), 6.50 (dd, ^3J (H, H) = 1.6 Hz, ^3J (H, H) = 3.2 Hz, 1H, 4-H furan), 6.97 (d, ^3J (H, H) = 3.2 Hz, 1H, 3-H furan), 7.52 ppm (d, ^3J (H, H) = 1.6 Hz, 1H, 5-H furan). ^{13}C NMR (100MHz, $CDCl_3$): δ =13.5 (×2C), 13.9, 19.6 (×2C), 22.4 (×2C), 24.3 (×2C), 26.2, 28.8, 28.8, 31.4, 55.4, 59.1, 59.3 (×2C), 111.5, 117.1, 141.9, 145.1 ppm.

***N*-Hexyl-*N*,*N*-dimethyl-2-furfurylammonium iodide 2i**. Prepared starting from amine **6c** with the same procedure reported for **2e.** The solid obtained was crystallized from ethanol and ethyl acetate (2.8 g, 97% yield). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 0.89 (t, ^3J (H, H) = 6.8 Hz, 3H, $N(CH_2)_5CH_3$), 1.29-1.35 (m, 6H, $N(CH_2)_2(CH_2)_3CH_3$), 1.82-1.86 (m, 2H, $NCH_2CH_2(CH_2)_3CH_3$), 3.36 (s, 6H, 2 × NCH_3), 3.47-3.56 (m, 2H, $NCH_2(CH_2)_4CH_3$), 5.04 (s, 2H, NCH_2-furan), 6.48 (dd, ^3J (H, H) = 3.4 Hz, ^3J (H, H) = 1.8 Hz, 1H, 4-H furan), 7.04 (d, ^3J (H, H) = 3.4 Hz, 1H, 3-H furan), 7.54 ppm (d, ^3J (H, H) = 1.8 Hz, 1H, 5-H furan). ^{13}C NMR (100MHz, $CDCl_3$): δ =13.7 (×2C), 22.2, 22.7, 25.7 31.0, 50.6, 59.6, 64.4, 111.4, 117.6, 142.1, 145.3 ppm.

***N*,*N*-Dibutyl-*N*-methyl-2-furfurylammonium bis(trifluoromethylsulfonyl)imide 3a**. According to the general procedure reported in the literature ammonium salt **2a** (0.35 g, 1mmol) was dissolved in deionized water (1 mL) and then an aqueous solution of $LiN(SO_2CF_3)_2$ (0.28 g, 1 mmol) was added. The reaction was stirred at room temperature for 24 hours, the product was extracted with ethyl acetate and the solvent was evaporated under vacuum (0.50 g, 99% yield). ^{1}H NMR (400 MHz, $CDCl_3$): δ =1.00 (t, ^3J (H, H) = 7.2 Hz, 6H, 2 × $N(CH_2)_3CH_3$), 1.39-1.44 (m, 4H, 2 × $N(CH_2)_2CH_2CH_3$), 1.73-1.78 (m, 4H, 2 × $NCH_2CH_2CH_2CH_3$), 3.00 (s, 3H, NCH_3), 3.14-3.18 (m, 4H, 2 × $NCH_2CH_2)_2CH_3$), 4.46 (s, 2H, NCH_2-furan), 6.51 (dd, ^3J (H, H) = 2 Hz, ^3J (H, H) = 3.4 Hz, 1H, 4-H furan), 6.80 (d, ^3J (H, H) = 3.4 Hz, 1H, 3-H furan), 7.56 ppm (d, ^3J (H, H) = 2 Hz, 1H, 5-H furan). ^{13}C NMR (100MHz, $CDCl_3$): δ =13.3 (×2C), 19.4 (×2C), 24.1 (×2C), 48.0, 57.7, 61.5 (×2C), 111.6, 117.3, 119.7 (q, *J* 318, CF_3), 141.5, 145.6 ppm.

***N*-Butyl-*N*-dodecyl-*N*-methyl-2-furfurylammonium bis(trifluoromethylsulfonyl)imide 3b**. Prepared starting from ammonium iodide **2b** (0.46 g, 1 mmol) with the same procedure reported for **3a** (0.61 g, 99% yield). ^{1}H NMR (400 MHz, C*D*Cl$_3$): δ = 0.88 (t, ^{3}J (H, H) = 6.4 Hz, 3H, C*H*$_3$(CH$_2$)$_{11}$N), 1.00 (t, ^{3}J (H, H) = 7.2 Hz, 3H, C*H*$_3$(CH$_2$)$_3$N), 1.24-1.28 (m, 16H, CH$_3$(C*H*$_2$)$_8$(CH$_2$)$_3$N), 1.32-1.35 (m, 4H, CH$_3$(CH$_2$)$_8$C*H*$_2$(CH$_2$)$_2$N, CH$_3$C*H*$_2$(CH$_2$)$_2$N), 1.75-1.77 (m, 4H, CH$_3$(CH$_2$)$_9$C*H*$_2$CH$_2$N, CH$_3$CH$_2$C*H*$_2$CH$_2$), 3.00 (s, 3H, NC*H*$_3$), 3.13-3.20 (m, 4H, CH$_3$(CH$_2$)$_{10}$C*H*$_2$N, CH$_3$(CH$_2$)$_2$C*H*$_2$N), 4.48 (s, 2H, NC*H*$_2$-furan), 6.51 (dd, ^{3}J (H, H) = 2 Hz, ^{3}J (H, H) = 3.4 Hz, 1H, 4-H furan), 6.81 (d, ^{3}J (H, H) = 3.4 Hz, 1H, 3-H furan), 7.56 ppm (d, ^{3}J (H, H) = 2 Hz, 1H, 5-H furan). ^{13}C NMR (100MHz, C*D*Cl$_3$): δ = 13.4, 14.1, 19.5, 22.3, 22.6, 24.2, 26.1, 28.9, 29.2 (×2C), 29.3, 29.5 (×2C),, 31.8, 48.1, 57.8, 61.4, 61.6, 111.8, 117.5, 119.8 (q, *J* 319, CF$_3$), 141.5, 145.6 ppm.

***N*-Butyl-*N*-methyl-*N*-octyl-2-furfurylammonium bis(trifluoromethylsulfonyl)imide 3c**. Prepared starting from ammonium iodide **2c** (0.40 g, 1 mmol) with the same procedure reported for **3a** (0.56 g, 99% yield). ^{1}H NMR (400 MHz, C*D*Cl$_3$): δ = 0.88 (t, ^{3}J (H, H) = 6.4 Hz, 3H, N(CH$_2$)$_7$C*H*$_3$), 1.00 (t, ^{3}J (H, H) = 7.2 Hz, 3H, N(CH$_2$)$_3$C*H*$_3$), 1.28-1.44 (m, 12H, CH$_3$C*H*$_2$(CH$_2$)$_2$N, CH$_3$(C*H*$_2$)$_5$(CH$_2$)$_2$N), 1.73-1.77 (m, 4H, CH$_3$CH$_2$C*H*$_2$CH$_2$N, CH$_3$(CH$_2$)$_5$C*H*$_2$CH$_2$N), 2.99 (s, 3H, NC*H*$_3$), 3.15-3.19 (m, 4H, CH$_3$(CH$_2$)$_2$C*H*$_2$N, CH$_3$(CH$_2$)$_6$C*H*$_2$N), 4.47 (s, 2H, NC*H*$_2$-furan), 6.50 (dd, ^{3}J (H, H) = 1.6 Hz, ^{3}J (H, H) = 3.2 Hz, 1H, 4-H furan), 6.80 (d, ^{3}J (H, H) = 3.2 Hz, 1H, 3-H furan), 7.55 ppm (d, ^{3}J (H, H) = 1.6 Hz, 1H, 5-H furan). ^{13}C NMR (100MHz, C*D*Cl$_3$): δ = 13.3, 14.9, 19.5, 22.3, 22.5, 24.2, 26.1, 28.8, 28.9, 31.5, 48.0, 57.7, 61.5, 61.6, 111.7, 117.4, 119.8 (q, *J* 319, CF$_3$), 141.5, 145.6 ppm.

***N*-Dodecyl-*N*-methyl-*N*-octyl-2-furfurylammonium bis(trifluoromethylsulfonyl)imide 3d**. Prepared starting from ammonium iodide **2d** (0.51 g, 1 mmol) with the same procedure reported

for **3a** (0.67g, 99% yield). ^{1}H NMR (400 MHz, C*D*Cl$_3$): δ = 0.89 (t, ^{3}J (H, H) = 6.8 Hz, 3H, C*H*$_3$(CH$_2$)$_{11}$N), 1.01 (t, ^{3}J (H, H) = 7.2 Hz, 3H, N(CH$_2$)$_7$C*H*$_3$), 1.24-1.36 (m, 28H, CH$_3$(C*H*$_2$)$_9$(CH$_2$)$_2$N, CH$_3$(C*H*$_2$)$_5$(CH$_2$)$_2$N), 1.74-1.78 (m, 4H, CH$_3$(CH$_2$)$_9$C*H*$_2$CH$_2$N, CH$_3$CH$_2$C*H*$_2$CH$_2$N), 3.01 (s, 3H, NC*H*$_3$), 3.14-3.20 (m, 4H, CH$_3$(CH$_2$)$_{10}$C*H*$_2$N, CH$_3$(CH$_2$)$_2$C*H*$_2$N), 4.48 (s, 2H, NC*H*$_2$-furan), 6.52 (dd, ^{3}J (H, H) = 1.6 Hz, ^{3}J (H, H) = 3.2 Hz, 1H, 4-H furan), 6.82 (d, ^{3}J (H, H) = 3.2 Hz, 1H, 3-H furan), 7.56 ppm (d, ^{3}J (H, H) = 1.6 Hz, 1H, 5-H furan). ^{13}C NMR (100MHz, C*D*Cl$_3$): δ = 13.4, 14.0, 19.5, 22.3, 22.5, 24.2, 26.1, 28.8 (×10C), 31.5, 48.1, 57.9, 61.4, 61.6, 111.8, 117.5, 119.6 (q, *J* 318, CF$_3$), 141.4, 145.7 ppm.

***N*-Butyl-*N*,*N*-dimethyl-2-furfurylammonium bis(trifluoromethylsulfonyl)imide 3e**. Prepared starting from ammonium iodide **2e** (0.30 g, 1 mmol) with the same procedure reported for **3a** (0.46 g, 99% yield). ^{1}H NMR (400 MHz, C*D*Cl$_3$): δ = 1.00 (t, ^{3}J (H, H) = 7.2 Hz, 3H, N(CH$_2$)$_3$C*H*$_3$), 1.39-1.44 (m, 2H, N(CH$_2$)$_2$C*H*$_2$CH$_3$), 1.78-1.82 (m, 2H, NCH$_2$C*H*$_2$CH$_2$CH$_3$), 3.07 (s, 6H, 2 × NC*H*$_3$), 3.21-3.24 (m, 2H, NC*H*$_2$(CH$_2$)$_2$CH$_3$), 4.48 (s, 2H, NC*H*$_2$-furan), 6.51 (dd, ^{3}J (H, H) = 3.6, ^{3}J (H, H) = 1.8 Hz, 1H, 4-H furan), 6.82 (d, ^{3}J (H, H) = 3.6 Hz, 1H, 3-H furan), 7.57 ppm (d, ^{3}J (H, H) = 1.8 Hz, 1H, 5-H furan). ^{13}C NMR (100MHz, C*D*Cl$_3$): δ = 13.3, 19.3, 24.5, 50.4 (×2C), 60.0, 64.4, 111.7, 117.4, 119.7 (q, *J* 319, CF$_3$), 141.6, 145.8 ppm.

***N*,*N*,*N*-Tributyl-2-furfurylammonium bis(trifluoromethylsulfonyl)imide 3f**. Prepared starting from ammonium iodide **2f** (0.39 g, 1 mmol) with the same procedure reported for **3a** (0.54 g, 99% yield). ^{1}H NMR (400 MHz, C*D*Cl$_3$): δ = 1.00-1.04 (m, 9H, 3 × N(CH$_2$)$_3$C*H*$_3$), 1.40-1.45 (m, 6H,3 × N(CH$_2$)$_2$C*H*$_2$CH$_3$), 1.70-1.76 (m, 6H, 3 × NCH$_2$C*H*$_2$CH$_2$CH$_3$), 3.09-3.13 (m, 6H, 3 × NC*H*$_2$(CH$_2$)$_2$CH$_3$), 4.48 (s, 2H, NC*H*$_2$-furan), 6.52 (dd, ^{3}J (H, H) = 2 Hz, ^{3}J (H, H) = 3.4 Hz, 1H, 4-H furan), 6.75 (d, ^{3}J (H, H) = 3.4 Hz, 1H, 3-H furan), 7.56 ppm (d, ^{3}J (H, H) = 2 Hz, 1H, 5-H furan). ^{13}C NMR (100MHz, C*D*Cl$_3$): δ = 13.3 (×3C), 19.4 (×3C), 23.7 (×3C), 54.5, 58.5

(×3C), 111.5, 116.7, 119.8 (q, *J* 319, CF_3), 141.5, 145.5 ppm.

***N*-Octyl-*N*,*N*-dimethyl-2-furfurylammonium bis(trifluoromethylsulfonyl)imide 3g**. Prepared starting from ammonium salt **2g** (0.36 g, 1 mmol) with the same procedure reported for **3a** (0.51 g, 99% yield). ^{1}H NMR (400 MHz, C*D*Cl_3): δ = 0.88 (t, ^3J (H, H) = 6.8 Hz, 3H, $N(CH_2)_7CH_3$), 1.23-1.34 (m, 10H, $N(CH_2)_2(CH_2)_5CH_3$), 1.78-1.82 (m, 2H, $NCH_2CH_2(CH_2)_5CH_3$), 3.05 (s, 6H, 2 × NCH_3), 3.16-3.21 (m, 2H, $NCH_2(CH_2)_6CH_3$), 4.46 (s, 2H, NCH_2-furan), 6.50 (dd, ^3J (H, H) = 3.4 z, ^3J (H, H) = 1.4 Hz, 1H, 4-H furan), 6.81 (d, ^3J (H, H) = 3.4 Hz, 1H, 3-H furan), 7.56 ppm (d, ^3J (H, H) = 1.4 Hz, 1H, 5-H furan). ^{13}C NMR (100MHz, C*D*Cl_3): δ =13.9 (×2C), 22.4, 22.6, 25.9, 28.8, 28.8, 31.5, 50.4, 60.0, 64.3, 111.7, 117.4, 119.6 (q, *J* 319, CF_3), 141.5, 145.8 ppm.

***N*,*N*-Dibutyl-*N*-octyl-2-furfurylammonium bis(trifluoromethylsulfonyl)imide 3h**. Prepared starting from ammonium salt **2h** (0.44 g, 1 mmol) with the same procedure reported for **3a** (0.60 g, 99% yield). ^{1}H NMR (400 MHz, C*D*Cl_3): δ = 0.89 (t, ^3J (H, H) = 6.8 Hz, 3H, $N(CH_2)_7CH_3$), 1.01 (t, ^3J (H, H) = 7.6 Hz, 6H, 2 × $N(CH_2)_3CH_3$), 1.28-1.45 (m, 14H, 2 × $CH_3CH_2(CH_2)_2N$, $CH_3(CH_2)_5(CH_2)_2N$), 1.71-1.77 (m, 6H, 2 × $CH_3CH_2CH_2CH_2N$, $CH_3(CH_2)_5CH_2CH_2N$), 3.08-3.13 (m, 6H, $CH_3(CH_2)_2CH_2N$, $CH_3(CH_2)_6CH_2N$, 4.50 (s, 2H, NCH_2-furan), 6.51 (dd, ^3J (H, H) = 1.6 Hz, ^3J (H, H) = 3.2 Hz, 1H, 4-H furan), 6.75 (d, ^3J (H, H) = 3.2 Hz, 1H, 3-H furan), 7.54 ppm (d, ^3J (H, H) = 1.6 Hz, 1H, 5-H furan). ^{13}C NMR (100MHz, C*D*Cl_3): δ =13.4 (×2C), 14.0, 19.5 (×2C), 21.9, 22.5, 23.9 (×2C), 26.2, 28.8, 28.9, 31.5, 54.8, 58.7 (×2C), 59.0, 111.7, 117.0, 119.3 (q, *J* 318, CF_3), 141.5, 145.5 ppm.

***N*,*N*-Dibutyl-*N*-methylfurfuryl ammonium tetrafluoroborate 4a**. Ammonium salt **2a** (0.35 g, 1 mmol) was dissolved in deionized water (1 mL) and then $NaBF_4$ (0.1 g, 1 mmol) is added. The reaction is stirred at room temperature for 24 h. The product is extracted with ethyl acetate,

the solvent is evaporated under vacuum (0.31 g, 99% yield). ^{1}H NMR (400 MHz, C*D*Cl$_3$): δ = 0.99 (t, ^{3}J (H, H) = 7.6 Hz, 2 ×6H, N(CH$_2$)$_3$C*H*$_3$), 1.37-1.46 (m, 4H, 2 × N(CH$_2$)$_2$C*H*$_2$CH$_3$), 1.72-1.81 (m, 4H, 2 × NCH$_2$C*H*$_2$CH$_2$CH$_3$), 3.11 (s, 3H, NC*H*$_3$), 3.20-3.33 (m, 4H, 2 × NC*H*$_2$(CH$_2$)$_2$CH$_3$), 4.68 (s, 2H, NC*H*$_2$-furan), 6.47 (dd, ^{3}J (H, H) = 1.8 Hz, ^{3}J (H, H) = 3.6 Hz, 1H, 4-H furan), 6.93 (d, ^{3}J (H, H) = 3.6 Hz, 1H, 3-H furan), 7.52 ppm (d, ^{3}J (H, H) = 1.8 Hz, 1H, 5-H furan). ^{13}C NMR (100MHz, C*D*Cl$_3$): δ = 13.5 (×2C), 19.6 (×2C), 24.3 (×2C), 48.1, 57.7, 61.2 (×2C), 111.6, 117.6, 142.0, 145.3 ppm.

***N*-Butyl-*N*-dodecyl-*N*-methylfurfurylammonium tetrafluoroborate 4b**. Prepared starting from ammonium salt **2b** (0.46 g, 1 mmol) with the same procedure reported for **4a** (0.42 g, 99% yield). ^{1}H NMR (400 MHz, C*D*Cl$_3$): δ = 0.87 (t, ^{3}J (H, H) = 6.8 Hz, 3H, C*H*$_3$(CH$_2$)$_{11}$N), 1.00 (t, ^{3}J (H, H) = 7.2 Hz, 3H, C*H*$_3$(CH$_2$)$_3$N), 1.25-1.36 (m, 16H, CH$_3$(C*H*$_2$)$_8$(CH$_2$)$_3$N), 1.40-1.45 (m, 4H, CH$_3$(CH$_2$)$_8$C*H*$_2$(CH$_2$)$_2$N, CH$_3$C*H*$_2$(CH$_2$)$_2$N), 1.74-1.78 (m, 4H, CH$_3$(CH$_2$)$_9$C*H*$_2$CH$_2$N, CH$_3$CH$_2$C*H*$_2$CH$_2$N), 3.10 (s, 3H, NC*H*$_3$), 3.20-3.30 (m, 4H, CH$_3$(CH$_2$)$_{10}$C*H*$_2$N, CH$_3$(CH$_2$)$_2$C*H*$_2$N), 4.67 (s, 2H, NC*H*$_2$-furan), 6.48 (dd, ^{3}J (H, H) = 2.2 Hz, ^{3}J (H, H) = 3.4 Hz, 1H, 4-H furan), 6.94 (d, ^{3}J (H, H) = 3.4 Hz, 1H, 3-H furan), 7.52 ppm (d, ^{3}J (H, H) = 2.2 Hz, 1H, 5-H furan). ^{13}C NMR (100MHz, C*D*Cl$_3$): δ = 13.5, 14.0, 19.6, 22.4, 22.6, 24.3, 26.2, 29.0, 29.3, 29.3, 29.4, 29.5 (×2C), 31.8, 48.1, 57.8, 61.2, 61.3, 111.7, 117.8, 141.9, 145.4 ppm.

***N*-Butyl-*N*-methyl-*N*-octyl-2-furfurylammonium tetrafluoroborate 4c**. Prepared starting from ammonium salt **2c** (0.40 g, 1 mmol) with the same procedure reported for **4a** (0.36 g, 99% yield). ^{1}H NMR (400 MHz, C*D*Cl$_3$): δ = 0.88 (t, ^{3}J (H, H) = 6.4 Hz, 3H, N(CH$_2$)$_7$C*H*$_3$), 1.00 (t, ^{3}J (H, H) = 7.2 Hz, 3H, N(CH$_2$)$_3$C*H*$_3$), 1.25-1.47 (m, 12H, CH$_3$C*H*$_2$(CH$_2$)$_2$N, CH$_3$(C*H*$_2$)$_5$(CH$_2$)$_2$N), 1.72-1.81 (m, 4H, CH$_3$CH$_2$C*H*$_2$CH$_2$N, CH$_3$(CH$_2$)$_5$C*H*$_2$CH$_2$N), 3.10 (s, 3H, NC*H*$_3$), 3.20-3.30 (m, 4H, CH$_3$(CH$_2$)$_2$C*H*$_2$N, CH$_3$(CH$_2$)$_6$C*H*$_2$N), 4.68 (s, 2H, NC*H*$_2$-furan), 6.48 (dd, ^{3}J (H, H) = 2 Hz, ^{3}J (H, H) = 3.2 Hz, 1H, 4-H furan), 6.95 (d, ^{3}J (H, H) = 3.2 Hz, 1H, 3-H furan), 7.52 ppm (d, ^{3}J (H, H) = 2 Hz, 1H, 5-H furan).

^{13}C NMR (100MHz, C*D*Cl$_3$): δ =13.5, 14.0, 19.6, 22.4, 22.5, 24.3, 26.2, 28.9 (×2C), 31.5, 48.1, 57.8, 61.1, 61.3, 111.7, 117.7, 142.1, 145.3 ppm.

***N*-Dodecyl-*N*-methyl-*N*-octyl-2-furfurylammonium tetrafluoroborate 4d**. Prepared starting from ammonium salt **2d** (0.51 g, 1 mmol) with the same procedure reported for **4a** (0.47 g**,** 99% yield). ^{1}H NMR (400 MHz, C*D*Cl$_3$): δ = 0.88 (t, ^3J (H, H) = 6.8 Hz, 3H, C*H*$_3$(CH$_2$)$_{11}$N), 1.00 (t, ^3J (H, H) = 7.2 Hz, 3H, N(CH$_2$)$_7$C*H*$_3$), 1.24-1.36 (m, 28H, CH$_3$(C*H*$_2$)$_9$(CH$_2$)$_2$N, CH$_3$(C*H*$_2$)$_5$(CH$_2$)$_2$N), 1.75-1.79 (m, 4H, CH$_3$(CH$_2$)$_9$C*H*$_2$CH$_2$N, CH$_3$ (CH$_2$)$_4$C*H*$_2$CH$_2$N), 3.09 (s, 3H, NC*H*$_3$), 3.21-3.27 (m, 4H, CH$_3$(CH$_2$)$_{10}$C*H*$_2$N, CH$_3$(CH$_2$)$_6$C*H*$_2$N), 4.64 (s, 2H, NC*H*$_2$-furan), 6.48 (dd, ^3J (H, H) = 2 Hz, ^3J (H, H) = 3.4 Hz, 1H, 4-H furan), 6.92 (d, ^3J (H, H) = 3.4 Hz, 1H, 3-H furan), 7.53 ppm (d, ^3J (H, H) = 2 Hz, 1H, 5-H furan). ^{13}C NMR (100MHz, C*D*Cl$_3$): δ =13.5, 14.0, 19.6, 22.4, 22.5, 24.2, 26.2, 28.9 (×10C), 31.5, 48.0, 57.7, 61.2, 61.3, 111.7, 117.6, 142.0, 145.3 ppm.

***N*-Butyl-*N*,*N*-dimethyl-2-furfurylammonium tetrafluoroborate 4e**. Prepared starting from ammonium salt **2e** (0.30 g, 1 mmol) with the same procedure reported for **4a** (0.26 g, 99% yield). ^{1}H NMR (400 MHz, C*D*Cl$_3$): δ = 0.99 (t, ^3J (H, H) = 7.2 Hz, 3H, N(CH$_2$)$_3$C*H*$_3$), 1.37-1.47 (m, 2H, N(CH$_2$)$_2$C*H*$_2$CH$_3$), 1.76-1.84 (m, 2H, NCH$_2$C*H*$_2$CH$_2$CH$_3$), 3.18 (s, 6H, 2 × NC*H*$_3$), 3.31-3.35 (m, 2H,NC*H*$_2$(CH$_2$)$_2$CH$_3$), 4.70 (s, 2H, NC*H*$_2$-furan), 6.48 (dd, ^3J (H, H) = 3.2 Hz, ^3J (H, H) = 2 Hz, 1H, 4-H furan), 6.93 (d, ^3J (H, H) = 3.2 Hz, 1H, 3-H furan), 7.53 ppm (d, ^3J (H, H) = 2 Hz, 1H, 5-H furan). ^{13}C NMR (100MHz, C*D*Cl$_3$): δ =13.5, 19.5, 24.6, 50.4 (×2C), 59.7, 64.3, 111.6, 117.6, 141.9, 145.5 ppm.

***N*,*N*,*N*-Tributyl-2-furfurylammonium tetrafluoroborate 4f**. Prepared starting from ammonium salt **2f** (0.39 g, 1 mmol) with the same procedure reported for **4a** (0.35g, 99% yield). ^{1}H NMR (400 MHz, C*D*Cl$_3$): δ =1.00 (t, ^3J (H, H) = 7.2 Hz, 9H, 3 ×N(CH$_2$)$_3$C*H*$_3$), 1.39-1.45 (m, 6H, 3 × N(CH$_2$)$_2$C*H*$_2$CH$_3$), 1.73-1.77 (m, 6H, 3 × NCH$_2$C*H*$_2$CH$_2$CH$_3$), 3.18 (t, ^3J (H, H) = 8.8 Hz, 6H, 3 ×

$NCH_2(CH_2)_2CH_3$), 4.62 (s, 2H, NCH_2-furan), 6.49 (dd, ^3J (H, H) = 2 Hz, ^3J (H, H) = 3.2 Hz, 1H, 4-H furan), 6.83 (d, ^3J (H, H) = 3.2 Hz, 1H, 3-H furan), 7.52 ppm (d, ^3J (H, H) = 2 Hz, 1H, 5-H furan). ^{13}C NMR (100MHz, $CDCl_3$): δ =13.5 (×3C), 19.6 (×3C), 23.9 (×3C), 54.9, 58.9 (×2C), 111.7, 117.1, 141.9, 145.3 ppm.

***N*-Octyl-*N*,*N*-dimethyl-2-furfurylammonium tetrafluoroborate 4g**. Prepared starting from ammonium salt **2g** (0.36 g, 1 mmol) with the same procedure reported for **4a** (0.32 g, 99% yield). ^{1}H NMR (400 MHz, $CDCl_3$): δ =0.86-0.90 (m, 3H, $N(CH_2)_7CH_3$), 1.27-1.35 (m, 10H, $N(CH_2)_2(CH_2)_5CH_3$), 1.79-1.82 (m, 2H, $NCH_2CH_2(CH_2)_5CH_3$), 3.19 (s, 6H, 2 × NCH_3), 3.30-3.34 (m, 2H, $NCH_2(CH_2)_6CH_3$), 4.73 (s, 2H, NCH_2-furan), 6.49 (dd, ^3J (H, H) = 3.6 Hz, ^3J (H, H) = 1.8 Hz, 1H, 4-H furan), 6.96 (d, ^3J (H, H) = 3.6 Hz, 1H, 3-H furan), 7.53 ppm (d, ^3J (H, H) = 1.8 Hz, 1H, 5-H furan). ^{13}C NMR (100MHz, $CDCl_3$): δ =13.5, 13.6, 19.7, 22.5, 22.8, 26.2, 29.0, 31.5, 50.5, 59.8, 64.2, 111.7, 117.8, 141.9, 145.5 ppm.

***N*,*N*-Dibutyl-*N*-octyl-2-furfurylammonium tetrafluoroborate 4h**. Prepared starting from ammonium salt **2h** (0.44 g, 1 mmol) with the same procedure reported for **4a** (0.40 g, 99% yield). ^{1}H NMR (400 MHz, $CDCl_3$): δ =0.88 (t, ^3J (H, H) = 6.6 Hz, 3H, $N(CH_2)_7CH_3$), 1.01 (t, ^3J (H, H) = 7.2 Hz, 6H, 2 × $N(CH_2)_3CH_3$), 1.26-1.44 (m, 14H, 2 × $CH_3CH_2(CH_2)_2N$, $CH_3(CH_2)_5(CH_2)_2N$), 1.74-1.76 (m, 6H, 2 × $CH_3CH_2CH_2CH_2N$, $CH_3(CH_2)_5CH_2CH_2N$), 3.13-3.19 (m, 6H, $CH_3(CH_2)_2CH_2N$, $CH_3(CH_2)_6CH_2N$), 4.59 (s, 2H, NCH_2-furan), 6.49 (dd, ^3J (H, H) = 1.6 Hz, ^3J (H, H) = 3.2 Hz, 1H, 4-H furan), 6.81 (d, ^3J (H, H) = 3.2 Hz, 1H, 3-H furan), 7.53 ppm (d, ^3J (H, H) = 1.6 Hz, 1H, 5-H furan). ^{13}C NMR (100MHz, $CDCl_3$): δ =13.5 (×2C), 14.0, 19.6 (×2C), 22.0, 22.5, 23.9 (×2C), 26.3, 28.8, 28.9, 31.5, 54.8, 58.8 (×2C), 59.0, 111.7, 117.0, 142.0, 145.2 ppm.

Solubility in water

To verify the miscibility in water of the compounds **2g** and **4g** we

recorded two ^{1}H NMR spectra in D_2O (5 mm probe on a Varian Mercury 600 spectrometer) with a known amount of 3-(trimethylsilyl)propionic acid-d_4 sodium salt (TSP) (28.9 μmol for each proton in 1 mL of D_2O) as internal standard and a delay time between successive scans of 20 s to ensure complete proton relaxation and, therefore, quantitative integration.

The samples has been prepared by sonication of the NMR tube, charged with D_2O, TSP and ammonium salts **2g** or **4g** with three ultrasound cycles of 30 min. Then the spectra have been recorded and the integrals have been calculated respect to TSP signal.

Test of **2e** stability to different pH conditions

PH stability has been established recording quantitative ^{1}H NMR spectra in acetone-d_6 (5 mm probe on a Varian Mercury 400 spectrometer) with a known amount of tetrakis(trimethylsilyl)silane (TTMS) as internal standard and a delay time between successive scans of 20 s to ensure complete proton relaxation and, therefore, quantitative integration.

Four solutions of the compound **2e** have been prepared in acetone-d_6: neutral pH, basic pH with triethylamine, weakly acidic pH with acetic acid and strongly acidic pH with hydrochloridic acid.

After 24 hours the spectra have been recorded and the integrals have been calculated respect to TTMS signal.

Daphnia magna acute toxicity tests

The essay was carried out according to the procedures set out in the Organization for Economic Co-operation and Development (OECD) guideline 202. Five neonates (age < 24 h; born from parthenogenic females grown in a batch culture) have been placed in each 100 mL beaker, containg 40 mL of test solution; two replicate beakers for each of eight treatment concentrations (control plus seven toxicant concentrations) have been used. Test concentrations, identified through a preliminary range finding test, were arranged in a geometric series. Three consecutive trials were carried out for each substance, for a total of six replicates at each concentration. Substances **2a**, **2e**, **2f**, **2g** and **2i**, were

tested simultaneously; this was not possible for TBAI and BTBAI that were tested some weeks later.

The temperature was 20 ± 1°C, with a 16:8 h light:dark photoperiod. Each test vessel has been checked for immobilized individuals at 24 and 48 h after the beginning of the test. Animals not able to swim within 15 s after gentle agitation of the test vessel have been considered immobilized, even though they could still move their antennae. The endpoint used to establish the concentration-response relationship was the number of individuals still active after 48 h of exposure.

Vibrio fischeri acute toxicity test

The assay was carried out in accordance with the 90% basic test for pure compounds protocol. Three trials were carried out for each substance using three vials of Microtox® "reagent" (lyophilized *V. fischeri*) from the same lot. Nine concentrations of each substance were tested in a 1:2 dilution series and a control. Due to constraints imposed by the protocol, it was not possible to test the substances simultaneously or to test all the substance using a single vial of bacteria. To approximate simultaneous testing, substances were tested according to a rotation scheme: the first trials for all the substances were carried out sequentially; when all the substances were tested once, the second trials were carried out and then the third. Light emission of the bacteria was measured after 0, 5 and 15 min of exposure using a Microtox® Model 500 Toxicity Analizer.

Data analysis.

The 50% effect concentration (EC50) of each substance for *D. magna* and *V. fischeri* was estimated by fitting the experimental concentration-response curves to a logistic model:

$$y = \frac{a}{1 + \left(\frac{x}{EC50}\right)^{b}}$$

Where: y = endpoint value; x = substance concentration; a = expected endpoint value in absence of toxic effect; b = slope parameter. The parameters of the equation, including the EC50, were estimated using the non-linear regression procedures implemented in Statistica (Statsoft,

Tulsa, OK, USA). An independent estimate of EC50 was obtained for each of the three experimental trials. The statistical significance of the differences among EC50s was tested by one-way analysis of variance (ANOVA). Once ANOVA resulted significant, the *post-hoc* Student-Newman-Keuls (SNK) test was carried out to identify which substances were significantly different from each other. The statistical tests were performed on log transformed data to achieve homogeneity of variances.

Green metrics calculation

Carbon efficiency is calculated according to the literature procedures, by dividing the carbon content of the product for the carbon content of the reactants.

In the calculation of mass intensity water is not included; it is obtained as the ratio among the mass of the reagents (including inorganic salts) and the mass of the product. The E factor is calculated as the value of mass intensity minus 1.

Reaction mass efficiency is obtained as the ratio among the mass of the product and the mass of the reactants (excluding inorganic salts and water).

Atom economy for the overall process is calculated by diving the molecular weight of the product by the molecular weight of all the reactants used for its synthesis (except the inorganic salts).

3.2. Applications

In this Thesis two novel applications of ionic liquids as solvents for catalytic reactions (Chapter 3.2.1.) and for the extraction of vegetable oil from biomass (Chapter 3.2.2.) have been investigated.

The first application regards the use of ionic liquids as reaction media for the chemoselective allylation and the asymmetric alkylation of carbonyl compounds, by derivatising a traditional ligand as 2,2'-binaphthol (BINOL) with ionic-tags.

The second application regards the use of a new class of ionic liquids, called switchable polarity solvents (SPS), as extraction media for aquatic (algae) and terrestrial (oleaginous seed plants) biomass.

3.2.1. Catalysis in ionic liquids as reaction media

Chemoselective allylation of ketones in ionic liquids containing sulfonate anions

Allylation of carbonyl compounds is one of the most powerful tools for the formation of C-C bonds when complex organic molecules have to be assembled. Two classes of allylating reagents can be used: strong reagents, such as allylmagnesium halides, which give fast addition but require the use of strictly anhydrous solvents and an inert atmosphere; and mild reagents, such as allylsilanes or stannanes, which are stable to common reaction conditions, but react only in the presence of an activator or catalyst (usually a Lewis acid). Among the reagents of the second class, tetraallyltin is a good choice for the allylation of poorly reactive ketones because it is nonreactive when used alone, but much more reactive than allysilanes or trialkylallyl stannanes when polar protic solvents, such as acidic water[96] or methanol,[97] or a Lewis acid activator are employed. Moreover, all four allyl groups of tetraallyltin can be transferred to the ketone, exploiting 1:4 molar ratio between reagents, which significantly lowers the amount of tin at stake in the process and therefore enhances the overall atom economy. As a benefit,

[96] Yanagisawa, A., Inoue, H, Morodome, M., Yamamoto, H., 1993. Highly chemoselective allylation of carbonyl compounds with tetraallyltin in acidic aqueous media. J. Am. Chem. Soc., 15, 10356– 10357

[97] Cokley, T.M., Harvey,P.J., Marshall, R.L., McCluskey, A., Young, D.J., 1997. Solvent-Mediated Allylation of Carbonyl Compounds with Allylic Stannanes. J. Org. Chem., 62, 1961– 1964

two of the "Twelve Principles" of Green Chemistry are followed: the development of catalytic (or promoted) processes using mild reagents (9th Principle) and the maximum incorporation of reagents' mass into the products (2nd Principle). Exploiting the above described features, the most successful application of tetraallyltin, discovered by Tagliavini[98] and improved by Walsh,[99] is the enantioselective allylation of ketones catalyzed by chiral binapthol (BINOL)-Ti complexes, usually carried out in dichloromethane. An interesting version of this asymmetric catalytic methodology in highly concentrated isopropanol solutions has been recently developed by Walsh;[100] however, also in this case, dichloromethane is required for the preparation of the catalyst.

A variety of reaction conditions have been used for the addition of tetraallyltin to different C=O or C=N groups: aldehydes, imines, and ketones. However, only recently the increasing attention towards environmental concerns has brought to the willing of replacing toxic or hazardous organic solvents, like dichloromethane, with more environmentally friendly solvents, like ionic liquids. The first examples of allylation of aldehydes and ketones in ionic liquids have been reported by Gordon et al.[101] in the presence of Lewis acids or without any catalyst (Figure 3.2.1.1.).

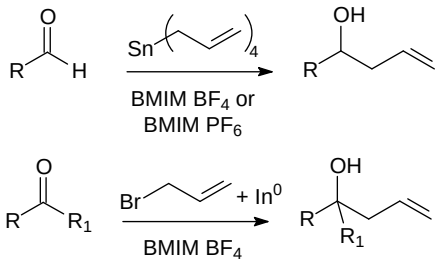
[98] Casolari, S., D'Addario, D., Tagliavini, E., 1999. BINOL-Ti-Catalyzed Synthesis of Tertiary Homoallylic Alcohols: The First Catalytic Asymmetric Allylation of Ketones. Org. Lett., 1, 1061 –1063

[99] a) Waltz, K.M., Gavenonis, J., Walsh, P.J., 2002. A Simple, Reliable, Catalytic Asymmetric Allylation of Ketones Angew. Chem., 114, 3849; b) Waltz, K.M., Gavenonis, J., Walsh, P.J., 2002. A Simple, Reliable, Catalytic Asymmetric Allylation of Ketones. Angew. Chem. Int. Ed., 41, 3697-3699; c) Gon. Kim, J., Waltz, K.M., Garcia, I.F., Kwiatkowski, D., Walsh, P.J., 2004. Catalytic Asymmetric Allylation of Ketones and a Tandem Asymmetric Allylation/Diastereoselective Epoxidation of Cyclic Enones. J. Am. Chem. Soc., 126, 12580 – 12585; d) Gon Kim, J., Camp, E.H., Walsh, P.J., 2006. Catalytic Asymmetric Methallylation of Ketones with an (H_8-BINOLate)Ti-Based Catalyst. Org. Lett., 8, 4413 – 4416

[100] Wooten, A.J., Gon Kim, J., Walsh, P.J., 2007. Highly Concentrated Catalytic Asymmetric Allylation of Ketones. Org. Lett., 9, 381–384.

[101] a) Gordon, C.M., McCluskey, A., 1999. Ionic liquids: a convenient solvent for environmentally friendly allylation reactions with tetraallylstannane. Chem. Commun., 1431–1432; b) Gordon, C.M., Ritchie, C., 2002. Indium and tin-mediated allylation in ionic liquids. Green Chem, 4, 124–128;

Figure 3.2.1.1. Synthesis of homoallylic alcohols in ionic liquids.

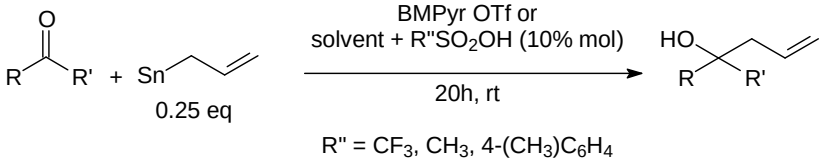


The synthesis of homoallylic alcohols from aldehydes and tetraallyltin in 1-butyl-3-methylimidazolium tetrafluoroborate (BMIM BF_4) and 1-butyl-3-methylimidazolium hexafluorophosphate (BMIM PF_6) proceeds very well with simple aromatic aldehydes, like benzaldehyde, or with both electron-withdrawing substituents (e.g. *p*-Cl) and weakly electron-donating substituents (e.g. *p*-OMe), but no reaction occurs in presence of strongly electron-donating substituents (e.g. Me_2N). The worst results are obtained with aliphatic and conjugated aldehydes.

The reaction has been also accomplished by generating the allylating agent *in situ*, from allyl bromide and indium metal (Barbier reaction), in BMIM BF_4. This kind of system works very well on aldehydes, aromatic and aliphatic, but it is less reactive towards unactivated ketones

In the present Thesis the chemoselective allylation of ketones by tetraallyltin in ionic liquids was exploited for the first time (Figure 3.2.1.2.). The solvent systems checked in the present study were: *N*-methyl-*N*-butylpyrrolidinium trifluoromethansulfonate (BMPyr OTf); BMIM BF_4 containing 10% mole of trifluoromethanesulfonic acid (TfOH), methanesulfonic acid (MsOH), or 4-methylbenzensulfonic acid (TsOH); molecular organic solvents (chloroform, dichloromethane, acetonitrile) with 10% mole of TfOH or BMPyr OTf.

Figure 3.2.1.2. Allylation of ketones in sulfonate-containing solvent systems.



The initial set of experiments was performed on 2-octanone as a model substrate (Table 3.2.1.1.).

Table 3.2.1.1. Allylation of 2-octanone under different solvent systems (20h, rt).

Entry	Allylating agent	Solvent system	Isolated yield (%)
1	tetraallyltin	BMPyr OTf	92
2	tetraallylsilane	BMPyr OTf	0
3	allyltributyltin[a]	BMPyr OTf	0
4	tetraallyltin	BMIM BF_4	0
5	tetraallyltin	BMIM BF_4/TfOH	94[b]
6	tetraallyltin	BMIM BF_4/MsOH	84
7	tetraallyltin	BMIM BF_4/TsOH	91
8	tetraallyltin	MOEMIM BF_4/TfOH	78
9	tetraallyltin	$CHCl_3$/TfOH	59
10	tetraallyltin	CH_2Cl_2/TfOH	49
11	tetraallyltin	CH_3CN/TfOH	60
12	tetraallyltin	$CHCl_3$/BMPyr OTf	66

All reactions were performed on 2-octanone (0.33 mmol) and tetraallyltin (0.08 mmol) in solvent (0.3 mL); when necessary, 0.03 mmol of sulfonic acid was used. [a] 0.33 mmol of allylating agent. [b] 24h reaction time.

As shown in Table 3.2.1.1., tetraallyltin is a suitable reagent, but tetraallylsilane or allyltributyltin are completely inert.

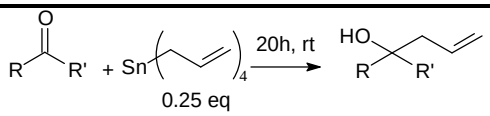
BMPyr OTf is the best solvent, giving the corresponding homoallylic alcohol in very good yield (92%, Entry 1) under mild reaction conditions, even if about 20 hours are required to achieve a good conversion because of a long induction period.

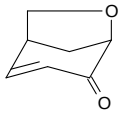
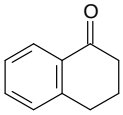
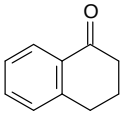
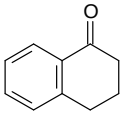
For the solvents lacking sulfonate, a small amount of sulfonic acid (TfOH, MsOH, or TsOH) is required, because without this addition, no reaction occurs. The combination BMIM BF_4/TfOH is as successful as BMPyr OTf, with a very high yield of product (94 % in 24h, Entry 5), instead the use of an oxygenated ionic liquid (1-methoxyethyl-3-methylimidazolium tetrafluoroborate MOEMIM BF_4), is less efficient (78%, Entry 8). The

combination BMIM BF_4/sulfonic acid gives the best yields when trifluoromethanesulfonic acid (TfOH) and 4-methylbenzensulfonic acid (TsOH) are used (94% and 91% respectively, Entries 5 and 7), but it is slightly less active with methanesulfonic acid (84% yield, Entry 6).
The traditional organic solvents are not as suitable as ionic liquids for this kind of reaction: the yields obtained in combination with TfOH range from 60% in acetonitrile (Entry 11) to 49% in dichloromethane (Entry 10). Notably, the presence of 10% BMPyr OTf as a source of triflate anion in $CHCl_3$ gives the best results among organic solvents (66%, Entry 12).

Further experiments were performed on different substrates: open-chain and cyclic dialkyl ketones, α,β-unsaturated ketones, aryl alkyl ketones, and aldehydes (Table 3.2.1.2.).

Table 3.2.1.2. Allylation of various substrates under different solvent systems (20h, rt).



Entry	R	R'	Solvent system	Isolated yield (%)
1	CH_3	C_6H_{13}	BMPyr OTf	92
2	i-C_3H_7	C_4H_9	BMPyr OTf	53[a]
3	s-C_4H_9	CH_3	BMPyr OTf	35
4	$(CH_2)_5$		BMPyr OTf	95
5	$(CH_2)_4$		BMPyr OTf	65
6			BMPyr OTf	100[b]
7	CH_3	Ph-CH=CH	BMPyr OTf	80[c]
8	CH_3	Ph-CH=CH	CH_3CN/TfOH	41
9	CH_3	Ph-C≡C	BMPyr OTf	87[c]
10	CH_3	α-naphtyl	BMPyr OTf	39
11	CH_3	α-naphtyl	BMPyr NTf_2	5
12			BMPyr NTf_2	16
13			BMPyr OTf	21[d]
14			CH_3CN/TfOH	32
15	CH_3	4-(HO)-C_6H_4	BMPyr OTf	10
16	CH_3	4-(MeO)-C_6H_4	BMPyr OTf	17
17	CH_3	4-(Br)-C_6H_4	BMPyr OTf	60
18	CH_3	4-(NO_2)-C_6H_4	BMPyr OTf	88
19	H	Ph	BMPyr OTf	98[e]
20	H	Ph-CH=CH	BMPyr OTf	90[e]

All reactions were performed on substrates (0.33 mmol) and tetraallyltin (0.08 mmol) in solvent (0.3 mL); when necessary, 0.03 mmol of TfOH was used. [a] 144h reaction time. [b] The isomer S was predominant (only traces of the R isomer). [c] 48h reaction time. [d] 1 eq of tetraallyltin. [e] 30 min reaction time.

Linear (Entry 1) and cyclic (Entries 4-5) dialkyl ketones generally give good yields, but an increase in the sterical hindrance, such as in Entries 2-3, makes the reaction sluggish and longer times are required to achieve only fair yields (53% and 35% respectively).

The yields of homoallylic alcohol from α,β-unsaturated ketones are higher in ionic liquid (80% and 87% with the alkenyl and the alkynyl chains respectively, Entries 7 and 9) than in acetonitrile (41%, Entry 8).

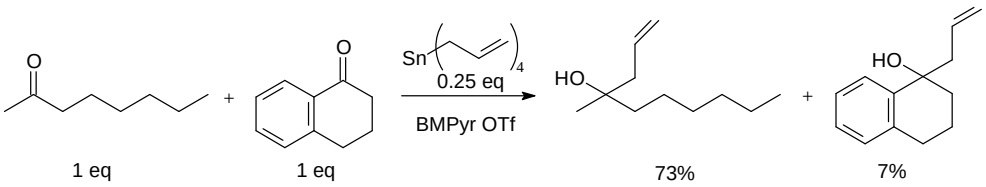
The allylation of levoglucosenone (Entry 6) is complete in 20h (100% yield) and highly stereoselective (practically, only one stereoisomer is formed), and this result is particularly remarkable, allowing further exploitations of this interesting compound obtainable from renewable feedstock.

The reactivity of aryl alkyl ketones strongly depends on the electronic properties of the aromatic ring: electron-neutral or electron-rich substrates (Entries 10-16) are much less reactive than dialkyl ketones in BMPyr OTf (yields from 39% to 10%), probably because of a reduced electrophilicity of the carbonyl carbon. On the contrary, a substrate bearing an electron-withdrawing substituent like 4-nitroacetophenone (Entry 18) shows high reactivity (88% yield) and 4-bromoacetophenone (Entry 17) behaves intermediately (60%). In the case of α-tetralone, the poor result achieves in BMPyr OTf (21% yield) can be slightly improved in CH_3CN/TfOH (32%, Entry 14). The use of BMIM NTf_2 instead of BMIM OTf gives worse results (Entries 11-12), particularly in the case of 2-acetonaphthone with a decrease in the yield of 8-fold (5% against 39%).

The scarce reactivity of some aromatic ketones appears to be a peculiar feature of the present reaction and it is opposite to the trend of the asymmetric catalytic reaction by Tagliavini[98] and Walsh.[99a]

To check the chemoselectivity of the allylation reaction in ionic liquids, 2-octanone, as model for dialkyl ketones, and α-tetralone, as model for aryl alkyl ketones, were reacted in a competitive experiment, with tetraallyltin in a 1:1:0.25 molar ratio (Figure 3.2.1.3.).

Figure 3.2.1.3. Chemoselective allylation of aliphatic ketones.



The corresponding homoallylic alcohols of 2-octanone and α-tetralone are obtained in a ratio greater than 10:1.

As expected, aldehydes are more reactive than ketones under the same reaction conditions: both benzaldehyde (Table 3.2.1.2, Entry 19) and cinnamaldehyde (Entry 20) afford the corresponding secondary homoallylic alcohols in shorter times (30min) and in very high yields (98% and 90% respectively). In this case, the aromatic counter effect is not observed. Comparing these results with those obtained by Gordon et al.,[101a] the reaction of allylation with tetraallyltin or allylbromide and indium in BMIM BF_4 gives lower yields, respectively 79% and 69% with benzaldehyde and cinnamaldehyde, in a longer time (16h).

In addition to their low volatility, a major advantage of ionic liquids is the easy recovery of the product from the reaction mixture, due to the scarce miscibility of the reaction solvent with hydrocarbons and ethers. In this Thesis, the synthesised homoallylic alcohols were usually recovered by extraction with diethyl ether and ethyl acetate, without any other workup.

By this procedure, the ionic liquid solvent was directly recovered and reused without further purification, as demonstrated by the recycle experiments performed with levoglucosenone as substrate (Table 3.2.1.3.).

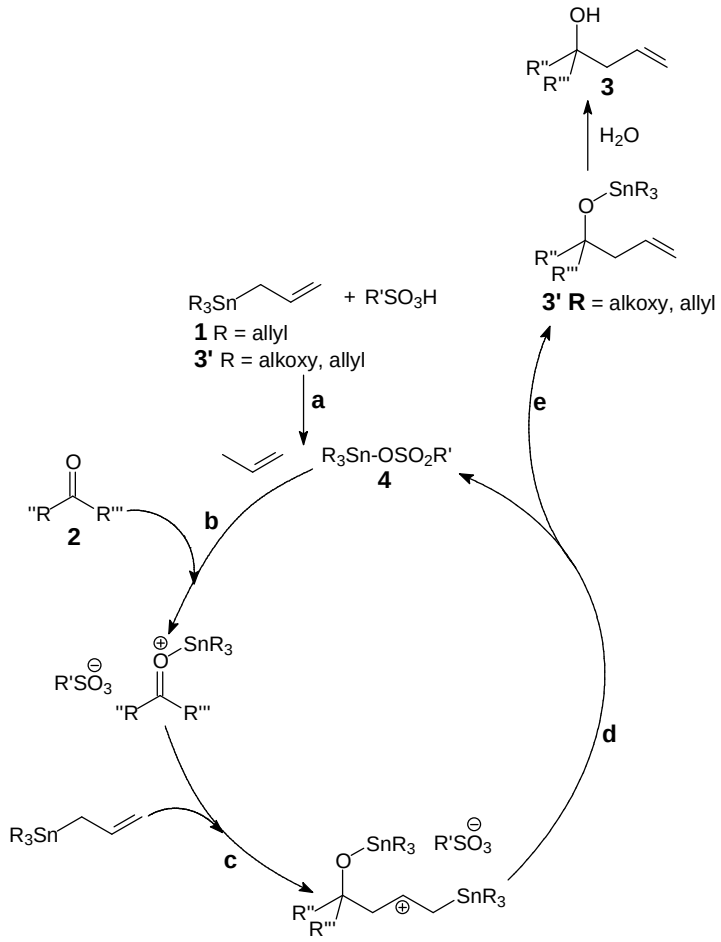
Table 3.2.1.3. Recycling of solvent system.

Solvent system	**Isolated yield (%)**			
	1st cycle	2nd cycle	3rd cycle	4th cycle
BMPyr OTf	100	99	96	100
BMIM BF_4/TfOH	94	99	97	99

During the first four cycles, solvent extraction and addition of fresh reagents, no loss of activity is recorded, both in BMPyr OTf and BMIM BF_4/TfOH systems. In the case of BMIM BF_4/TfOH system, TfOH is added again to achieve optimal performance (if TfOH is not added in the second cycle, the yield of product is lowered to 56%).

The unique role of sulfonate anions in promoting the allylation of ketones in both molecular organic solvents and ionic liquid solvents was first hypothesised (Figure 3.2.1.4.) and then investigated with NMR spectroscopy (Figure 3.2.1.5.).

Figure 3.2.1.4. Mechanism of the initiation step in the systems with sulfonic acid.

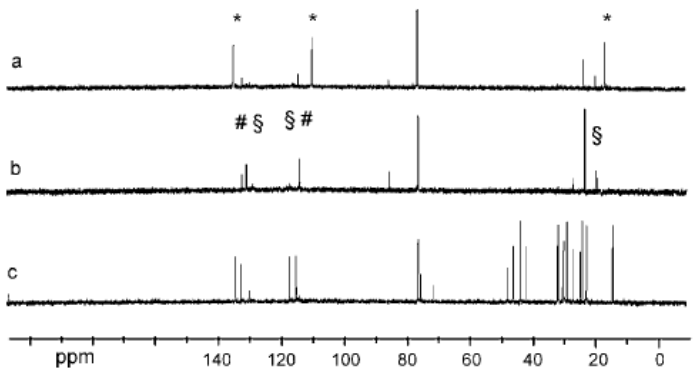


The first case taken into account is a system without sulfonate, in which the reaction has to be promoted by the addition of a sulfonic acid.

When a catalytic amount of sulfonic acid is added to a solution of tetraallyltin, probably a protonolysis reaction occurs, giving rise to propene and to a tin sulfonate derivative, indicated as **4** in Figure 3.2.1.4. (R=allyl, step **a**). This intermediate is prone to dissociate into a sulfonate anion and trialkyltin cation; the latter is a strong Lewis acid that is able to coordinate the carbonylic oxygen (step **b**), hence activating the ketonic carbon towards the addition of the allyl stannane through an intramolecular or, most likely, an intermolecular pathway (step **c**). In the final step **d**, a sulfonate ion eventually assists the cleavage of a carbon–tin bond, giving rise to the homoallylic product **3'** (as a tin alkoxide) and restoring the tri-organotin sulfonate catalyst **4**. To account for the stoichiometry of the reaction (molar ratio tetraallyltin **1**:ketone **2** = 1:4), an allyl transfer from an allyltin alkoxide **3'** (R=allyl) presumably occurs (namely, **3'** plays the role of the allylating reagent) producing tin compounds progressively containing more alkoxy and less allyl substituents as previously found. The tertiary alcohol **3** eventually forms during the workup or from some residual water in the solvent.

To test this mechanistic hypothesis, ^{13}C NMR spectra (^{1}H NMR spectra were also obtained, but they are more complex and difficult to interpret) of a mixture of tetraallyltin and trifluoromethanesulfonic acid in C*D*Cl_3 in 10:1 (Figure 3.2.1.5.a) and 1:1 (Figure 3.2.1.5.b) molar ratios were recorded.

Figure 3.2.1.5. ^{13}C NMR spectra of: a) tetraallyltin + TfOH (1 : 0.1); b) tetraallyltin + TfOH (1 : 1); c) 2-octanone + tetraallyltin + TfOH (1 : 0.25 : 0.1). Signals attributed to tetraallyltin (*), propene (§) and **4** (#).



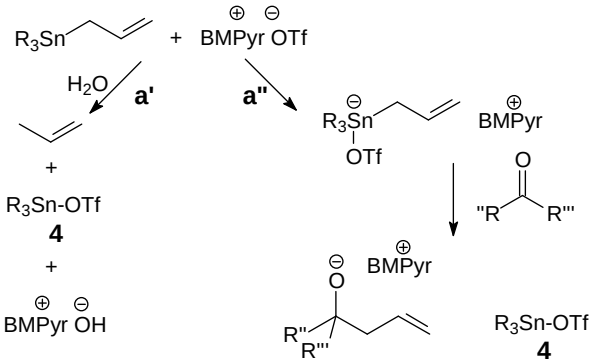
The former spectrum, besides the peaks of tetraallyltin (16.07, 111.01, and 136.53 ppm), shows smaller peaks at 19.35 ppm, 115.71 ppm, and 133.73 ppm that correspond to propene. In the spectrum of the 1:1 mixture, tetraallylytin can not be detected, while signals of propene are accompanied by strong signals at 23.03 and 86.33, attributed to isopropyl triflate coming from the addition of TfOH to propene. A small amount of the same compound is also seen in the previous spectrum.

Finally, a third set of peaks is detected in the 1:1 spectrum, characterised by chemical shifts: 26.75, 115.42, and 132.34 that are tentatively attributed to triallyltin triflate **4** (R=allyl, R'=CF_3).

The ^{13}C NMR spectrum (Figure 3.2.1.5.c) of a mixture of tetraallyltin and 2-octanone, alongside trifluoromethanesulfonic acid in a $CDCl_3$ solution, reveals that a fast reaction occurs. After a short time, the signals of tetraallyltin are no longer visible in the spectrum. Three main sets of vinylic carbons can be observed: the first one (118.46 ppm, 134.15 ppm) is attributed to the homoallylic alcohol product by comparison with the reported values.[98] The other two sets (116.40, 136.18 and 116.12, 131.42 ppm), can be due to homoallylic tin alkoxides **3'** (R"= C_6H_{13}, R'''=CH_3) having a different number of alkoxy substituents bonded to the tin atom.

The second case taken into account is a system sulfonate anion such as BMPyr OTf (Figure 3.2.1.6.).

Figure 3.2.1.6. Mechanism of the initiation step in BMPyr OTf.



The pathway of the allylation reaction is presumably similar, but a different initiation (step **a'**) is required. In this case, the source of proton to produce

propene from tetraallyltin could be from some residual water in the solvent. An alternative pathway (step **a''**) takes into account the initial base-promoted addition of tetraallyltin to the ketone with triflate acting as the base. Furthermore, a tin triflate species **4** is formed, which is able to initiate the same catalytic cycle described in Figure 3.2.1.4.

To test this mechanistic hypothesis, ^{13}C NMR spectra were carried out. When tetraallyltin and BMPyr OTf are mixed in C*D*Cl_3 solution, no change is observed in the ^{13}C NMR spectrum respect to the signals of the components. Moreover, by adding tetraallyltin to pure BMPyr OTf in the NMR tube, a biphasic mixture is obtained, and the ^{13}C NMR spectrum reveals only signals of the two compounds. Therefore, we tentatively suppose that a reversible addition of triflate anion to tetraallyltin occurs (according to step **a"**), but the amount of stannate anion formed is very low and can not be detected by NMR spectroscopy.

Only in the presence of the ketone, the successive allylation reaction gives rise to allyltin triflate **4**, initiating the catalytic cycle. This assumption is in agreement with the long induction period for the reactions carry out in the BMPyr OTf systems.

Asymmetric catalysis in ionic liquids with modified BINOLs

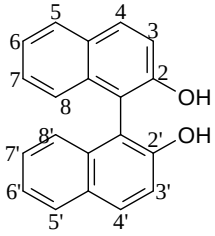
Asymmetric catalysis is one of the most efficient and environmentally sustainable route to obtain enantiomerically enriched products and for this reason it has a central importance in the modern synthetic and pharmaceutical chemistry.

In particular, enantioselective catalysis using chiral metal complexes, formed from the interaction of a metal ion or atom and a chiral ligand, provides one of the most general and flexible methods for asymmetric synthesis.[102] Metals, either in high or low oxidation state, are known to possess a range of specific activities that can be influenced by ligand properties. The ligand must has suitable functionalities, lack of appropriate elements of symmetry, and substituents capable of differentiating the available space in the vicinity of the metal centre. Although many successful chiral ligands are completely asymmetric (C1-symmetry), most

[102] Noyori, R. 1989. Chemical multiplication of chirality: science and application. Chem. Soc. Rev.,18, 187-208.

of the ligands which have found a large utility in the field of enantioselective reactions posses axial chirality and a C2-symmetry. One of the most famous example of this kind of ligands is 2,2′-binaphthol (BINOL) (Figure 3.2.1.7.), synthesised for the first time in 1926[103] and successfully exploited by Noyori in 1979 for the enatioselective reduction of aromatic ketones and aldehydes using a modified lithium aluminium hydride.[104]

Figure 3.2.1.7. 2,2′-binaphthol(BINOL).



After this first application, a large number of BINOL derivatives has been synthesised, modifying the backbone with substituents which affected the steric environment around the metal centre and the electronic properties of the oxygen atoms involved in the formation of the Lewis acidic metal complexes.

The most common substitutions of BINOL have been accomplished in positions 3,3', 6,6' and 7,7'.[105]

The modifications in 3,3' are easily obtained by treating BINOL with an organolithium compound (e.g. butyl lithium), followed by a reaction of the resulting aryl carbanion with an electrophile; the degree of substitution is strictly determined by the number of organolithium equivalents, having for example a mono-substitution with 2.2 equivalents or a di-substitution with 3 equivalents.[106]

[103] Pummerer R.; Prell, E.; Rieche, A. , 1926. Preparation of binaphthylene dioxide.Chem. Ber., 59, 2159.

[104] Noyori, R., Tomino, I.; Tanimoto, Y. 1979. Virtually complete enantioface differentiation in carbonyl group reduction by a complex aluminum hydride reagent. JACS. 101, 3129-3131.

[105] Chen, Y., Yekta, S., Yudin, A.K., 2003. Modified BINOL Ligands in Asymmetric Catalysis. Chem. Rev., 103, 3155-3211.

[106] Cox, P. J., Wang, W., Snieckus, V. 1992. Expedient route to 3- and 3,3′-substituted 1,1′-bi-2-naphthols by directed ortho metalation and suzuki cross coupling methods. Tetrahedron Lett., 33,2253-2256

The modifications in 6,6' start from 6,6'-dibromo BINOL, easily obtained through an aromatic bromination of BINOL. For example, 6,6'-dilithio BINOL, precursor for a wide range of derivatives, can be achieved by lithium-halogen exchange. In this case the degree of substitution is not easy to control and the mono-substituted BINOL is often obtainable in very low yields.

The modifications in 7,7' are less common but they can be very useful to increase the enantioselectivity by increasing the steric bulk around the metal centre.

The applications of BINOL and its derivatives in asymmetric catalysis are enormous,[107] from asymmetric oxidations (e.g. epoxidation of olefins or oxidation of sulfides) to asymmetric reductions (e.g. ketones and imines reduction), and above all in C-C bond forming reactions (e.g. ene reactions, allylation, aldol and Diels-Alder reactions). Chiral metal BINOL complexes can be synthesised with a variety of Lewis acidic metal entities, such as aluminium, boron, titanium, lanthanum, and ytterbium.

In this Thesis in particular, a complex of BINOL with titanium (IV) was used to investigate the possibility of realizing catalytic asymmetric transformations in ionic liquids and in other environmental friendly solvent systems. The tested reactions were the allylation of ketones with tetraallyltin and the alkylation of aldehydes with diethylzinc. This work has been done during the PhD under the supervision of Prof. Paul Dyson at the Ecole Polytechnique Fédérale de Lausanne (Swiss), thanks to a fellowship granted by INCA, the Interuniversity Consortium "Chemistry for the Environment" (Venice, Italy).

The asymmetric allylation of carbonyl compounds with titanium (IV)-BINOL complexes has been deeply investigated.[108] The first example was reported by Mikami[109] in the addition of allylic silanes and stannanes to

[107] Brunel, J.M., 2005. BINOL: A Versatile Chiral Reagent. Chem. Rev., 105, 857-897

[108] Denmark, S.E., Fu, J., 2003. Catalytic Enantioselective Addition of Allylic Organometallic Reagents to Aldehydes and Ketones. Chem. Rev., 103, 2763-2793.

[109] Aoki, S.; Mikami, K.; Terada, M.; Nakai, T., 1993. Enantio- and diastereoselective catalysis of addition reaction of allylic silanes and stannanes to glyoxylates by binaphthol-derived titanium complex. Tetrahedron, 49, 1783.

glyoxylates, and then the exploitation of allylstannanes was further developed by independent works of Tagliavini,[110] and Keck.[111] Both of them obtained very high yields and enantioselectivity on aromatic and aliphatic aldehydes, and discovered that the addition of molecular sieves is an important parameter for high reactivity and selectivity. Tagliavini prepared the catalyst by mixing $TiCl_2(O\text{-}i\text{-}Pr)_2$ and BINOL (ratio 1:1), and used this complex (20 mol %) to catalysed the asymmetric allylation; in the method developed by Keck, the catalyst was prepared by mixing $Ti(O\text{-}i\text{-}Pr)_4$ and BINOL (ratio 1:1) or by adding triflic acid (ratio 2:1), and used in lower amount (10 mol %) to obtain the same good results of Tagliavini.
In 1999 Tagliavini et al.[98] deepened their work reporting the first catalytic asymmetric allylation of ketones, using tetraallyltin as allylating reactant and BINOL-titanium as chiral catalyst, in good yields and moderate enantioselectivity. Successively Walsh improved the catalytic system getting very high enantioselectivity, by adding 2-propanol as additive,[99b,99c] using H8-BINOL as chiral ligand[99d] and developing the reaction under high concentrated conditions.[100]

The asymmetric addition of diethylzinc to aldehydes is a widely studied reaction,[112] and it works very well with a large number of chiral ligands (e.g. dimethylaminoisobornenol discovered by Noyori).[113] The first use of BINOL-titanium complex to catalyse this reaction was reported by Nakai[114] and Chan,[115] and since then, the number of BINOL derivatives with substitutions in different positions, increased rapidly with very good results in terms of yields and enantioselectivity.

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[110] Costa, A.L., Piazza, G., Tagliavini, E., Trombini, C., Umani-Ronchi, A., 1993. Catalytic Asymmetric Synthesis of Homoallytic Alcohols. JACS.,115, 7001-7002.
[111] Keck, G.E., Tarbet, K.H., Geraci, L.S., 1993. Catalytic Asymmetric Allylation of Aldehydes. JACS, 115, 8467-8468
[112] a) Lin Pu, L., Yu. H.B., 2001. Catalytic Asymmetric Organozinc Additions to Carbonyl Compounds. Chem. Rev., 101, 757-824. b) Knochel, P., Singer, R.D., 1993. Preparation and Reactions of Polyfunctional Organozinc Reagents in Organic Synthesis. Chem. Rev., 2117-2188
[113] Kitamura, M., Suga, S., Kawai, K., Noyori, R., 1986. Catalytic asymmetric induction. Highly enantioselective addition of dialkylzincs to aldehydes. JACS, 108, 6071.
[114] Mori, M.; Nakai, T. Tetrahedron Lett. 1997, 38, 6233-6236.
[115] a) Zhang, F.Y., Yip, C.W., Cao, R., Chan, A.S.C., 1997. Enantioselective addition of diethylzinc to aromatic aldehydes catalyzed by Ti(BINOL) complex. Tetrahedron: Asymmetry, 8, 585-589. b) Zhang, F.Y., Chan, A.S.C., 1997. Enantioselective addition of diethylzinc to aromatic aldehydes catalyzed by titanium-5,5′,6,6′,7,7′,8,8′-octahydro-1,1′-bi-2-naphthol complex. Tetrahedron: Asymmetry, 8, 3651-3655.

Chiral metal BINOL complexes are commonly used in homogeneous asymmetric catalysis in organic solvents such as dichloromethane. However there are some difficulties associated with the recovery and the re-use of this expensive catalytic systems. To work out this type of problem in this Thesis two different solutions have been proposed.

The first one is the development of a reaction system which allows the extraction of the final products, leaving in the reaction media the chiral metal complex. Specifically ionic liquids were used as reaction media for both the asymmetric allylation and alkylation of carbonyl compounds, exploiting their immiscibility with many organic solvents to recover the products; moreover, in order to increase the affinity of the catalyst with ionic liquids, and guarantee its retaining in the reaction media, a class of new ionic BINOL derivatives were synthesised and checked.

The second strategy is represented by the heterogenisation of homogeneous chiral catalysts, as by anchoring the catalyst on solid matrices by covalent or ionic interactions,[116] to create functional solid materials (e.g. "Functional Solid Ionic Material", FIM).[117] In this contest a BINOL derivative supported on a polystyrene matrix through ionic bonds have been designed and realised.

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[116] a) Ding, K., Wang, Z., Wang, X., Liang, Y., Wang, X., 2006. Self-Supported Chiral Catalysts for Heterogeneous Enantioselective Reactions. Chem. Eur. J., 12, 5188 – 5197; b) Bautista, F.M., Caballero, V., Campelo, J.M., Luna, D., Marinas, J.M., Romero, A.A., Romero, I., Serrano, I., Llobet, A., 2006. Heterogeneization of a new Ru(II) homogeneous asymmetric hydrogenation catalyst containing BINAP and the N-tridentate bpea ligand, through covalent attachment on amorphous $AlPO_4$ support. Top. Catal., 40, 193-205. c) Stephenson, P., Kondor, B., Licence, P., Scovell, K., Ross, S.K., Poliakoff, M., 2006. Continuous Asymmetric Hydrogenation in Supercritical Carbon Dioxide using an Immobilised Homogeneous Catalyst. Adv. Synt. Cat., 348, 1605-1610. d) Kucherenko, A., Struchkova, M.I., Zlotin, S.G. 2006. The (*S*)-Proline/Polyelectrolyte System: An Efficient, Heterogeneous, Reusable Catalyst for Direct Asymmetric Aldol Reactions. Eur. J. Org. Chem., 2000-2004. e) Chiral Catalyst Immobilization and Recycling, (Eds.: D. E. de Vos, I. F. Vankelecom, P. A. Jacobs), Wiley-VCH, Weinheim, 2000; f) Song, C.E., Lee, S., 2002. Supported Chiral Catalysts on Inorganic Materials. Chem. Rev., 102, 3495 –3524; g) Fan, Q., Li, Y-M., Chan, A.C.S., 2002. Recoverable Catalysts for Asymmetric Organic Synthesis. Chem. Rev., 102, 3385 – 3466; h) de Vos, D.E., Dams, M., Sels, B.F., Jacobs, P.A., 2002. Ordered Mesoporous and Microporous Molecular Sieves Functionalized with Transition Metal Complexes as Catalysts for Selective Organic Transformations. Chem. Rev., 102, 3615 –3640.

[117] a) Geldbach, T.G., Zhao, D., Castello, N.C., Laurenczy, G., Weyershausen, B., Dyson, P.J., 2006. Biphasic Hydrosilylation in Ionic Liquids: A Process Set for Industrial Implementation. J. Am. Chem. Soc., 128, 9773. b) Gu, Y., Ogawa, C., Kobayashi, J., Mori, Y., Kobayashi, S., 2006. A Heterogeneous Silica-Supported Scandium/Ionic Liquid Catalyst System for Organic Reactions in Water Angew. Chem. Int. Ed. Eng., 45, 7217.

Transition metal catalysis in ionic liquids is a very promising field for applications in organic synthesis and asymmetric catalysis.[118] A variety of catalytic reactions was successfully performed *i)* exploiting the immiscibility of ionic liquids with many organic solvents, leading to a heterogenous phase reaction media useful for catalyst separation and recovery, or *ii)* utilising "task specific ionic liquids" as metal ligands,[119] guarantying the recycling of the ionic auxiliary. This last strategy, particularly interesting for precious chiral auxiliaries, leads also to enhance the catalyst stability and to avoid its leaching.

In the literature there are few examples of chiral ligands functionalisation with ionic tag, and just only one regards BINOL. The group of Moreau developed first a camphorsulfonamides derivate[120] and then a modified BINOL[121] (Figure 3.2.1.8.), both of them functionalised with an ionic imidazolium, successfully used for the enantioselective addition of diethylzinc to aldehydes in dichloromethane.

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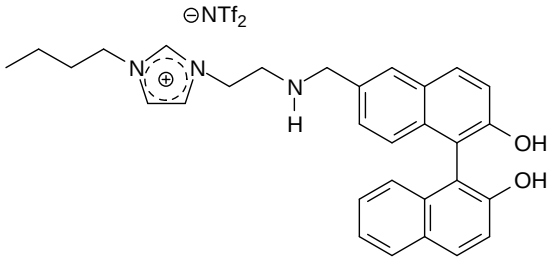
[118] Wasserscheid, P.; Welton, T. Ionic Liquids in Synthesis; Wiley-VCH: Weinheim, 2002. Recent review articles: a) Welton, T., 1999. Room-Temperature Ionic Liquids. Solvents for Synthesis and Catalysis. Chem. Rev., 99, 2071–2083; b) Wasserscheid, P.; Keim, W., 2000. Ionic Liquids - New Solutions for Transition Metal Catalysis. Angew. Chem., Int. Ed., 39, 3772–3789; c) Sheldon, R., 2001. Catalytic reactions in ionic liquids Chem. Commun., 2399–2407; d) Gordon, C. M., 2001. New developments in catalysis using ionic liquids. Appl. Catal. A: General 2001, 222, 101–117

[119] a) Audric, N.; Clavier, H.; Mauduit, M.; Guillemin, J.-C.,2003. An Ionic Liquid-Supported Ruthenium Carbene Complex: A Robust and Recyclable Catalyst for Ring-Closing Olefin Metathesis in Ionic Liquids. JACS, 125, 9248; b) Yao, Q.; Zhang, Y., 2003**.** Olefin Metathesis in the Ionic Liquid 1-Butyl-3-methylimidazolium Hexafluorophosphate Using a Recyclable Ru Catalyst: Remarkable Effect of a Designer Ionic Tag**.** Angew. Chem., Int. Ed., 42, 3395; c) Bronger, R. P. J.; Silva, S. M.; Kamer, P. C. J.; van Leeuwen, P. W. N. M., 2002. A novel dicationic phenoxaphosphino-modified Xantphos-type ligand—a unique ligand specifically designed for a high activity, selectivity and recyclability**.** Chem. Commun., 3044; d) Wasserscheid, P.; Waffenschmidt, H.; Machnitzki, P.; Kottsieper, K. W.; Stelzer, O., 2001. Cationic phosphine ligands with phenylguanidinium modified xanthene moieties—a successful concept for highly regioselective, biphasic hydroformylation of oct-1-ene in hexafluorophosphate ionic liquids Chem. Commun, 451; e) Favre, F.; Olivier-Bourbigou, H.; Commereuc, D.; Saussine, L., 2001, Hydroformylation of 1-hexene with rhodium in non-aqueous ionic liquids : how to design the solvent and the ligand to the reaction **.** Chem. Commun., 1360; f) Brasse, C. C.; Englert, U.; Salzer, A., Wasserscheid, P., 2000. Ionic Phosphine Ligands with Cobaltocenium Backbone: Novel Ligands for the Highly Selective, Biphasic, Rhodium-Catalyzed Hydroformylation of 1-Octene in Ionic Liquids. Organometallics, 19, 3818.

[120] Gadenne, B., Hesemann P., Moreau, J., 2004. Ionic liquids incorporating camphorsulfonamide units for the Ti-promoted asymmetric diethylzinc addition to benzaldehyde. Tet Let, 45, 8157-8160.

[121] Gadenne, B., Hesemann P., Moreau, J., 2005. Easily recoverable BINOL ligand with ionic tag for asymmetric catalysis. Tet. Asym., 15, 2001-2006

Figure 3.2.1.8. BINOL with an imidazolium tag synthesised by Moreau et al.[121]

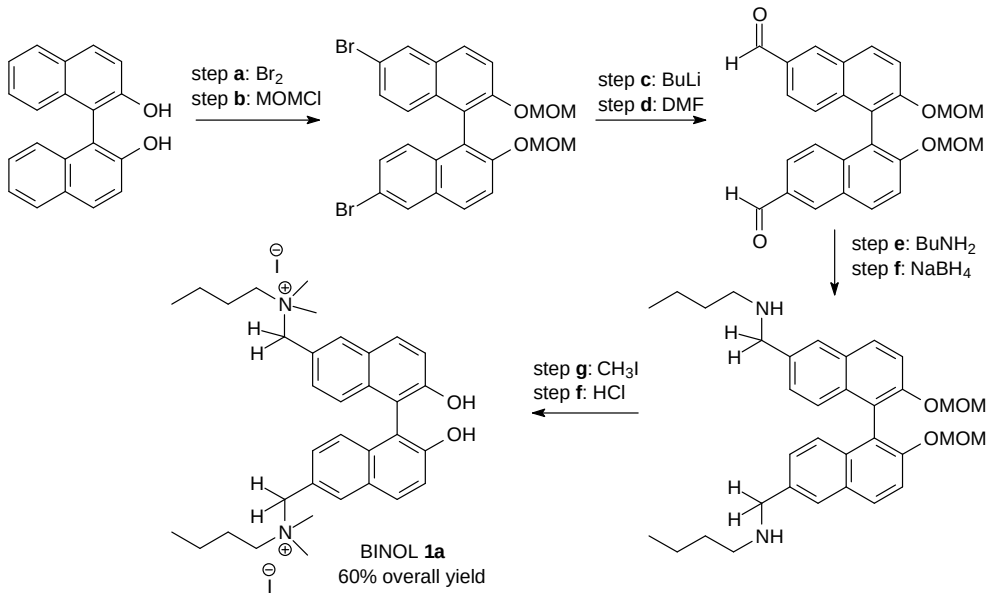


The approach followed in this Thesis is based on a different modification of BINOL backbone, introducing ammonium instead of imidazolium moieties. The first class of derivatives (ionic BINOLs **1**) shows two ammonium moieties on the positions 6,6'.

The synthetic pathway, according to the literature procedure,[122] was based on the two typical initial steps of aromatic bromination in 6,6' (Figure 3.2.1.9., step **a**) and protection of the OH groups (step **b**), followed by a di-metallation with butyl lithium (step **c**) and by formylation with N,N-dimethylformamide DMF to get protected 6,6'-diformyl BINOL (step **d**). This compound was treated with butylamine (step **e**), synthesising a di-imine in position 6,6', which was then reduced to amine (step **f**); the final steps were the dimethylation of nitrogens with methyl iodide (step **g**) and the deprotection of the OH groups (step **h**), to get bis-quaternary ammonium derivative of BINOL **1a**, precursor for the following transformation, in a 60% overall yield.

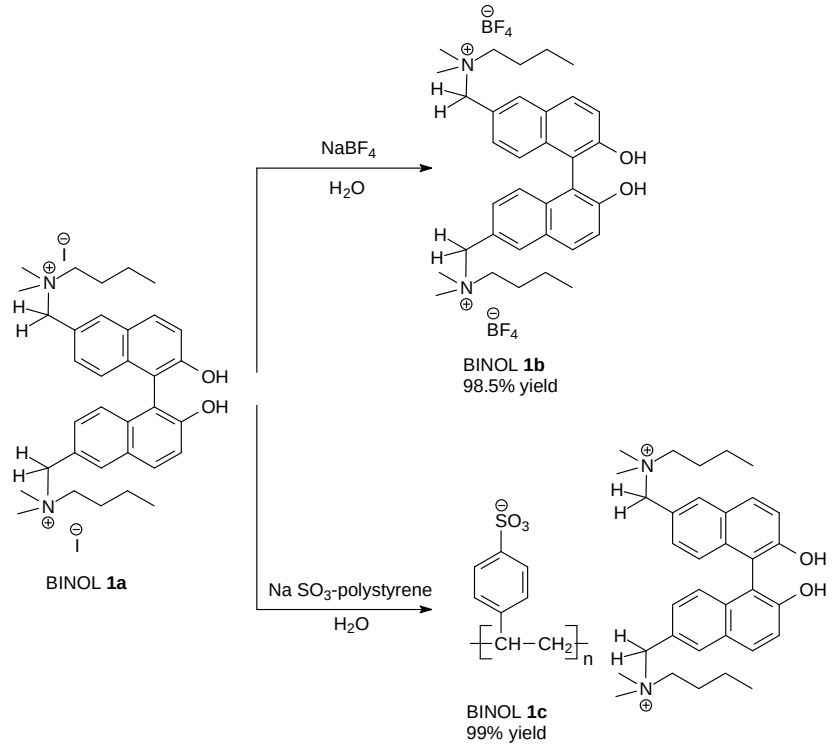
[122] a) Dotsevi, G., Sogah, Y., Gram., D.,1979. Host-guest complexation. 14. Host covalently bound to polystyrene resin for chromatographic resolution of enantiomers of amino acid and ester salts. JACS., 101, 3035-3042; b) Ishitani, H., Veno, M., Kobayashi, S., 2000. Enantioselective Mannich-Type Reactions Using a Novel Chiral Zirconium Catalyst for the Synthesis of Optically Active β-Amino Acid Derivatives. JACS, 122, 8180-8186; c) Dong, C., Zhang, J., Zheng, W., Zhang, L., Yu, Z., Choi, M., Chan, A., 2000, Heterogeneous asymmetric addition of diethylzinc to aromatic aldehydes catalyzed by Ti(IV)/imine bridged poly(*R*)-binaphthol. Tet. Asym., 11, 2449-2454.

Figure 3.2.1.9. Synthesis of BINOL **1a**.



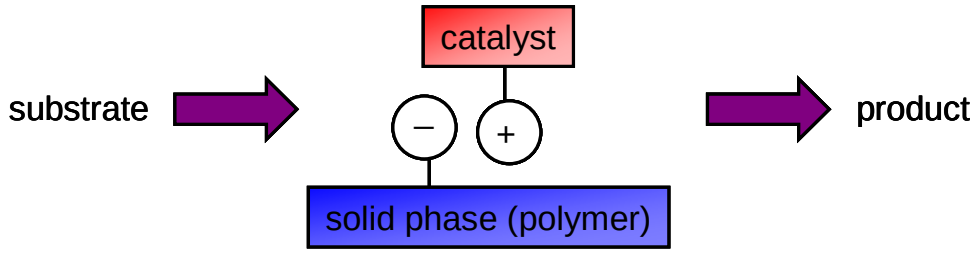
It is known that iodide is not a good anion for the catalysis and for this reason two new ionic derivatives have been prepared (**1b** and **1c**). Both of them are obtained in quantitative yields by simple anion exchanges in water, with sodium tetrafluoroborate (**1b**) and not-linked sodium polystyrene sulfonated (**1c**) (Figure 3.2.1.10.).

Figure 3.2.1.10. Synthesis of BINOLs **1b** and **1c**.



Polymer **1c** in particular, has been designed in order to develop supported ionic liquid catalysts as "Functional Solid Ionic Materials" (FIM) for heterogeneous catalysis (Figure 3.2.1.11.) as mentioned above.

Figure 3.2.1.11. Supported ionic liquid catalysts as functional solid ionic materials.



The anchoring of a catalysts on solid matrices through covalently immobilization of the catalytic species on inorganic materials, organic polymers or dendrimers has been widely used. For example BINOL has been supported on ordered mesoporous silica[123] (e.g. MCM-41 or SBA.15), on styrene through a free radical polimerisation[124] or on dendrimers.[125]

In the present Thesis the heterogenisation is based on the use of polymer-supported ionic species, obtained with ionic interaction instead of covalently techniques: the polymer carries the negative charge with the group SO_3^-, and BINOL moiety carries the positive charge with the quaternary ammonium groups in position 6 and 6'.

[123] a) Pathak, K., Bhatt, A., Abdi, S., Kureshy, R., Khan, N., Ahmad, I., Jasra, R., 2006. Enantioselective addition of diethylzinc to aldehydes using immobilized chiral BINOL–Ti complex on ordered mesoporous silicas. Tet. Asym., 17, 1506–1513; b) Liu, G., Gao, Y., Lu, X., Liu, M., Zhang, F., Li, H., 2008. Microwave-assisted catalytic allylation of aldehydes promoted by a mesoporous silica-supported BINOL ligand in solid media. Chem. Commun., 3184–3186; c) Bhatt, A., Pathak, K., Jasra, R., Kureshy, R., Khan, N., Abdi, S., 2006. Chiral lanthanum–lithium–binaphthol complex covalently bonded to silica and MCM-41 for enantioselective nitroaldol (Henry) reaction . J. Mol. Cat. A: Chem., 244, 110–117; d) Pathak, K., Ahmad, I., Abdi, S., Kureshy, R., Khan, N., Jasra, R., 2008. The synthesis of silica-supported chiral BINOL: Application in Ti-catalyzed asymmetric addition of diethylzinc to aldehydes. J. Mol. Cat. A: Chem., 280, 106–114.

[124] Jayaprakash, D., Sasai, H., 2001. Synthesis and catalytic applications of soluble polymer-supported BINOL. Tet. Asym., 12, 2589–2595.

[125] Yin, L., Li, R., Wang, F., Wang, H., Zheng, Y., Wang, C., Ma, J., 2007. The synthesis of dendritic BINOL ligands and their applications in the asymmetric addition of diethylzinc to benzaldehyde. Tet. Asym., 18, 1383–1389.

BINOL **1b** is a reddish solid, highly soluble in all the common slightly polar solvents such as acetone, acetonitrile, ethylacetate and alcohols, but totally insoluble in halogenated solvents like chloroform.
On the contrary BINOL **1c** is insoluble in all the organic solvents and in water.

In the last few years different papers have evaluated the activity of un modified BINOL in catalysing Diels-Alder reactions,[126] Friedel-Crafts reactions,[127] asymmetric reduction of ketones[128] and allylations of aldehydes[129] in ionic liquids. Lewis acid catalysts based on BINOL-titanium were never tested before.
In this Thesis asymmetric reactions in ionic liquids using BINOL-Ti(O-*i*-Pr)$_4$ as catalyst were carried out for the first time. Indeed, the only previous example of exploitation of an ionic modified-BINOL-titanium complex for asymmetric catalysis reported in the literature by Moreau et al.[121] was performed in organic solvents and not in ionic liquids.
Two different types of asymmetric reactions have been studied: the addition of tetraallyltin to ketones and the addition of diethylzinc to aldehydes.

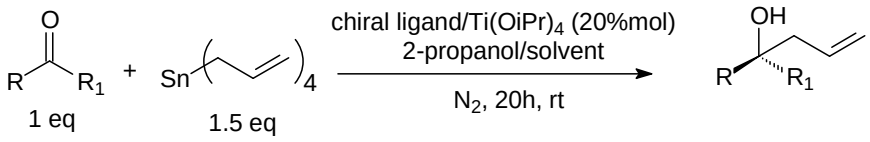
Addition of tetraallyltin to ketones

As mentioned above, the allylations of ketones with tetraallyltin were developed with very good results by Tagliavini and co-workers and by Walsh and co-workers; the yields and the enantiomeric excesses obtained especially by Walsh, were very high, so there are few possibilities to improve the system from this point of view. Therefore, the aim of the present study is not to improve enantioselectivity of the process, but to verify the possibility of recovering and recycling the catalytic complex and of performing the allylation reactions in "green" solvents such as ionic liquids.

[126] Fu, F., Teo, Y., Loh, T., 2006. Catalytic Enantioselective Diels−Alder Reaction in Ionic Liquid via a Recyclable Chiral In(III) Complex. Organic Letters, 8, 26, 5999-6001.
[127] Malhotra, S., Xiao, Y., 2006. Enantioselective Friedel–Crafts Reactions of Aromatic Amines with Ethyl Glyoxylate in Pyridinium-Based Ionic Liquids. Aust. J. Chem., 2006, 59, 7, 468-472
[128] Xiao, Y., Malhotra, S., 2006. Asymmetric reduction of aromatic ketones in pyridinium-based ionic liquids. Tet. Asym., 17, 7, 1062-1065.
[129] Teo, Y., Goh, E., Loh, T., 2005. Catalytic enantioselective allylation of aldehydes via a chiral indium(III) complex in ionic liquids. Tet. Lett., 46, 27, 4573-4575.

The reactions were initially performed following a slight modification of the experimental conditions reported by Walsh et al[100] (Figure 3.2.1.12.).

Figure 3.2.1.12. Typical procedure of asymmetric allylation of ketones (chiral ligand = BINOL, **1b**, **1c**).



Reactions were carried out in anhydrous acetonitrile, a good solvent for this transformation,[99d] able to dissolve ligand **1b**, and in the ionic liquid 1-butyl-4-methyl-pyridinium tetrafluoroborate (BMPy BF_4). It is known that hydrophilic ionic liquids, such as those having BF_4 anion, retain a lot of water, which can cause the hydrolytic formation of TiO_2 from Ti(O-*i*-Pr)$_4$, and for this reason BMPy BF_4 was carefully dried by keeping 10 days at 70°C under vacuum.

Walsh and co-workers demonstrated that 2-propanol plays a positive role in enantioselectivity by increasing the initiation times of the reaction,[99b] so an amount of this alcohol was used as an additive to both the solvent systems.

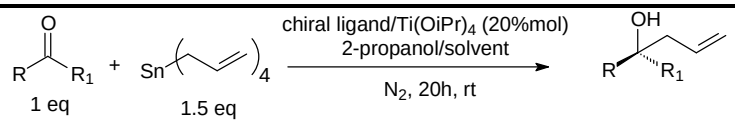
In the first experiments also two imidazolium based ionic liquids were investigated: 1-butyl-3-methyl-imidazolium tetrafluoroborate (BMIM BF_4) and 1-methoxyethyl-3-methyl imidazolium tetrafluoroborate (MOEMIM BF_4). Both of them were submitted to the same drying procedure as BMPy BF_4 but in spite of this, after the addition of Ti(O-*i*-Pr)$_4$, white precipitates appeared, probably indicating the formation of TiO_2, and the reaction did not afford the product. For this reason the reaction in imidazolium ionic liquids was not further investigated, and the study was pursued only in BMPy BF_4 and CH_3CN.

BINOL, **1b** and **1c** were used as chiral ligands; BINOL and **1b** were totally soluble in acetonitrile and in BMPy BF_4, instead **1c** was unsoluble. When Ti(O-*i*-Pr)$_4$ was added, BINOL solution turned yellow in both the solvents, **1b** turned reddish, and **1c** turned yellowish; this change in the colour of the ligand solution suggested the formation of the complexes BINOLs-titanium.

As substrates, two electron-rich ketones (acetonaphthone and *p*-methoxyacetophenone) and one unsaturated ketone (*E*-4-phenyl-3-buten-2-

one) were used. All the homoallylic alcohol products have been purified by chromatographic column to quantify the yields and analysed by chiral HPLC to determine the enantiomeric excesses (Table 3.2.1.4.).

Table 3.2.1.4. Asymmetric allylation of various substrates under different solvent systems (20h, rt).



Entry	R	R_1	ligand	solvent	yield %	ee %
1	CH_3	Ph-CH=CH	BINOL	CH_3CN	>99	35
2	CH_3	Ph-CH=CH	BINOL	BMPy BF_4	>99	11.5
3	CH_3	Ph-CH=CH	**1b**	CH_3CN	>99	10
4	CH_3	Ph-CH=CH	**1b**	BMPy BF_4	>99	2.6
5	CH_3	Ph-CH=CH	**1c**	CH_3CN	61.3	0
6	CH_3	Ph-CH=CH	**1c**	BMPy BF_4	45	0
7	CH_3	α-naphtyl	BINOL	CH_3CN	94	40
8	CH_3	α-naphtyl	BINOL	BMPy BF_4	68.6	7.3
9	CH_3	α-naphtyl	**1b**	CH_3CN	50.3	0
10	CH_3	α-naphtyl	**1b**	BMPy BF_4	42.8	3.2
11	CH_3	α-naphtyl	**1c**	CH_3CN	6.8	1.7
12	CH_3	α-naphtyl	**1c**	BMPy BF_4	13	3
13	CH_3	4-(MeO)-C_6H_4	BINOL	CH_3CN	97	10
14	CH_3	4-(MeO)-C_6H_4	BINOL	BMPy BF_4	70	5.7
15	CH_3	4-(MeO)-C_6H_4	**1b**	CH_3CN	93	9.5
16	CH_3	4-(MeO)-C_6H_4	**1b**	BMPy BF_4	91	3.2
17	CH_3	4-(MeO)-C_6H_4	**1c**	CH_3CN	37	2.7
18	CH_3	4-(MeO)-C_6H_4	**1c**	BMPy BF_4	42.3	1.9

The results reported in Table 3.2.1.4. indicate that the complex BINOL-titanium in acetonitrile gives very high yields with all the substrates (Entries 1, 7, 13). The yields are better or comparable with those obtained by Tagliavini et al.[98] and Walsh et al.[99b] on the same substrates in

dichloromethane. However the enantiomeric excesses are definitely worse (best ee of 40% with acetonahpthone).

BINOL-titanium complex shows to be also able of catalysing the allylation reaction in 1-butyl-4-methyl-pyridinium tetrafluoroborate, and to the best of our knowledge this is the first time that a titanium-complex is active in an ionic liquid. However, the enantioselectivities achieved in the ionic liquid are constantly lower than those achieved in CH_3CN (Entries 2, 8, 14),.

Ligand **2b** affords comparable yields to those obtained with BINOL, both in CH_3CN (Entries 3, 9, 15) and in BMPy BF_4 (Entries 4, 10, 16), but lower enantiomeric excesses.

Ligand **2c** on the contrary is the least reactive catalyst, above all in the reactions with acetonahpthone, and it gives almost racemic mixtures with all the substrates. The low conversions could be related to mass transfer limitations, typically of catalysts immobilized on solid supports, which could be increased by longer reaction times; however the very discouraging enantiomeric excesses leave few possibilities for further improvements.

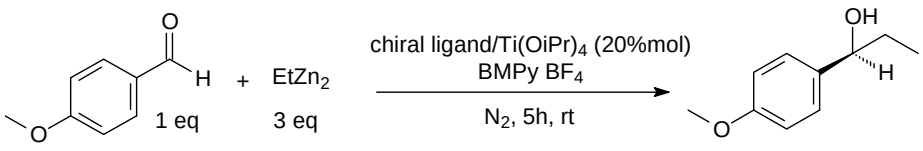
Addition of diethylzinc to aldehydes

The BINOL-titanium catalysed addition of diethylzinc to aldehydes is a well known reaction, generally performed in dichloromethane at low temperature (e.g. 0°C or -20°C) with very high yields and enantiomeric excesses. However in the literature, there are no examples about this kind of asymmetric catalysis with a BINOL-titanium complex in ionic liquids; it is reported that the alkylation of aldehydes could occur also with neat diethylzinc in a series of imidazolium and pyridinium ionic liquids[130] without titanium species and chiral ligands. The authors of this work reported that 1-butyl-3-methylimidazolium ionic liquids could react with diethylzinc to give carbene complexes because of relatively acidic hydrogen at the C_2 position of the imidazole ring. Pyridinium ionic liquids instead, did not show this problem and were the solvents of choice, giving the best yields, easily recovered and reused.

[130] Law, M., Wong, K., Chan T., 2004. Organometallic reactions in ionic liquids. Alkylation of aldehydes with diethylzinc. Green Chem., 6, 241–244

In this Thesis the diethylzinc addition to aldehydes was performed following the experimental conditions reported by Ding et al[131] (Figure 3.2.1.13.).

Figure 3.2.1.13. Typical procedure of asymmetric addition of diethylzinc to aldehydes. (chiral ligand = BINOL, **1b**, **1c**).



As for allylation reactions, the ionic liquid investigated for this reaction was 1-butyl-4-methyl-pyridinium tetrafluoroborate (BMPy BF_4). No comparison with a traditional organic solvent was done; anhydrous dichloromethane, in which the reaction is usually performed, does not solubilise both the ligands **1b** and **1c**.

Diethylzinc used in the present study was a 1 M solution in hexane and, in spite of it has been reported that BMPy BF_4 is immiscible with hexane, the reactions were performed in homogeneous solution. However some experiments were also done to understand if the reaction could eventually occur in heterogeneous conditions.

BINOL, **1b** and **1c** were used as chiral ligands; 4-methoxy-benzaldehyde (*p*-anisaldehyde) has been chosen as model substrate. After the stated reaction time, the alcohol products were purified by chromatographic column to evaluate the yields and analysed by chiral HPLC to determine the enantiomeric excess (Table 3.2.1.5.).

[131] Shen, X., Guo, H., Ding, K., 2000. The synthesis of a novel non-*C*2 symmetric H4-BINOL ligand and its application to titanium-catalyzed enantioselective addition of diethylzinc to aldehydes. Tet. Asym., 11, 4321-4327.

Table 3.2.1.5. Asymmetric addition of diethylzinc to *p*-anisaldehyde in with different chiral ligands (5 h, rt).

Entry	Chiral catalyst	Solvent system	T °C	Isolated yield%	ee%
1	BINOL/Ti(O-*i*-Pr)$_4$	BMPy BF_4	rt	90	64
2	**1b**/Ti(O-*i*-Pr)$_4$	BMPy BF_4	rt	75	45
3	**1c**/Ti(O-*i*-Pr)$_4$	BMPy BF_4	rt	51	8
4	BINOL[a]	BMPy BF_4	rt	-	-
5	BINOL/Ti(O-*i*-Pr)$_4$ [b]	BMPy BF_4	rt	-	-
6	BINOL/Ti(O-*i*-Pr)$_4$ [c]	BMPy BF_4	rt	-	-
7	BINOL/Ti(O-*i*-Pr)$_4$	hexane	rt	100	74
8	BINOL/Ti(O-*i*-Pr)$_4$	-[d]	-20°C	100	78
9	BINOL/Ti(O-*i*-Pr)$_4$ [e]	-[d]	-20°C	100	60
10	**1b**/Ti(O-*i*-Pr)$_4$	-[d]	-20°C	100	-
11	**1b**/Ti(O-*i*-Pr)$_4$ [e]	-[d]	-20°C	50	-
12	-	-[d]	-20°C	100	-

[a] without Ti(O-*i*-Pr)$_4$
[b] hexane was evaporated from Et_2Zn solution before addition to a mixture containing BMPy BF_4 and the catalyst
[c] hexane was evaporated from Et_2Zn solution after it was added to a mixture containing BMPy BF_4 and the catalyst
[d] no additional solvent was employed, except 1M Et_2Zn hexane solution
[e] 0.05 equiv of ligand instead of 0.2

The results reported in Table 3.2.1.5. show that all the ligands are suitable for working out the reaction in ionic liquid: BINOL-titanium is the best catalyst both in terms of yields and enantioselectivities (90% and 64% respectively, Entry 1), even if the difference with the complex **1b**-titanium is not highly relevant (75% yield and 45% ee, Entry 2). Compound **1c** is an inferior ligand (51% yield and 8% ee, Entry 3), showing a parallel trend with what observed for allylation reactions.

The lower enantiomeric excess obtained with BINOL-Ti catalyst in BMPy BF_4 respect to traditional solvents could be due to the higher reaction temperature (room temperature instead of 0°C or -20°C), required to prevent a large increase in the viscosity of the solvent system.

The reaction performed with BINOL but without the addition of Ti(O-*i*-Pr)$_4$ does not work (Entry 4).

The results obtained with **1b** are inferior to those obtained by Moreau et al. with imidazolium-BINOL (>99% yield, 80% ee) but those authors used dichloromethane at low temperature.

The results obtained with ligand **1c** are not satisfactory, in comparison with other examples of supported BINOLs. BINOL supported on ordered mesoporous silica,[123a] for example, catalysed the alkylation of aldehydes with diethylzinc with good yields and enantiomeric excesses (e.g. 86% yield and 70% ee with *p*-anisaldehyde), analogously to BINOL covalently supported on polystyrene[124] (e.g. 61% yield and 83% ee with benzaldehyde).

The effect of the hexane contained in the solution of diethylzinc (1 M in hexane) was also investigated.

The asymmetric addition of diethylzinc to *p*-anisaldehyde gives good results in hexane (100% yield and 74% ee, Entry 7), but when it is tried to remove the hexane under vacuum from Et_2Zn solution and perform the reaction in the ionic liquid, no reaction occurs, both by evaporating the hexane and then adding the IL mixture containing the catalytic complex (a white precipitate appeared, Entry 5), and by evaporating the hexane after the addition of Et_2Zn solution to the IL mixture (Entry 6).

A set of experiments was also done in highly concentrated conditions, without any additional solvent except the hexane contained in Et_2Zn solution (Entries 8-11).

In these condition, at -20°C, BINOL-Ti gives the expected results: 100% yield, 78% ee, with 20% catalyst and 60% ee with 5% catalyst (Entries 8, 9). On the contrary **1b**-Ti affords good yield but no selectivity (Entry 10). This result is probably due to the catalytic activity of $Ti(O\text{-}i\text{-}Pr)_4$ alone, as demonstrated by entry 12 where no ligand is added.

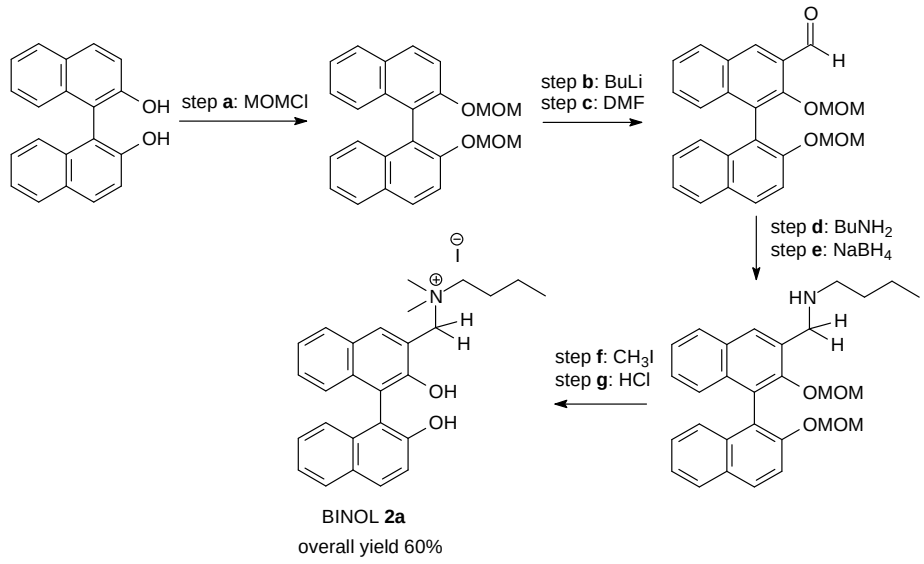
Summarising the results obtained in the allylations and alkylations of carbonyl compounds with the first class of derivatives (ionic BINOLs **1b** and **1c**) synthesised in the Thesis, it is clear that the introduction of two ammonium moieties on the positions 6,6' of BINOL gives good results in terms of conversion, but scarce enantioselectivity. **1c** in particular, designed to develop supported ionic liquid catalysts as "Functional Solid Ionic

Materials" (FIM) for the heterogeneous catalysis, is poorly active in all the checked conditions.

For this reason, a second class of derivatives (ionic BINOLs **2a,b**) was synthesised, introducing a single ammonium moiety in position 3 of BINOL.

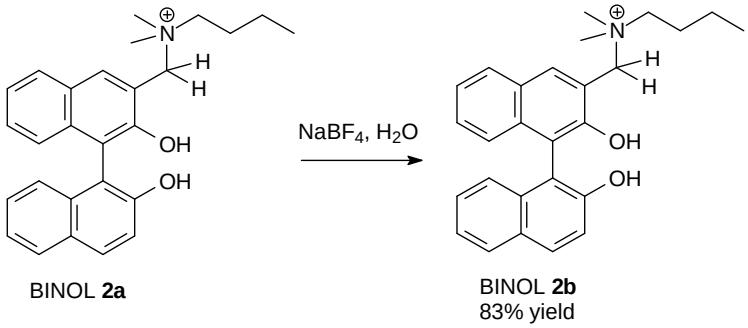
The synthetic pathway (Figure 3.2.1.14.) was based on an initial protection of the OH groups (step **a**), followed by a mono-metallation with butyl lithium (step **b**) and by a formylation with DMF to get 3-formyl-protected BINOL (step **c**). This compound was treated with butylamine (step **d**), synthesising a mono-imine in position 3, and then reduced to amine (step **e**); the final steps were the dialkylation with methyl iodide (step **f**) and the deprotection of the OH groups (step **g**), to get **2a** in a 60% overall yield (all the steps gave very high yields >98%, except steps **b** and **c** which had only 62% of conversion).

Figure 3.2.1.14. Synthesis of BINOL **2a**.



BINOL **2a** was then treated with sodium tetrafluoroborate to get BINOL **2b** (Figure 3.2.1.15.).

Figure 3.2.1.15. Synthesis of BINOL **2b**.

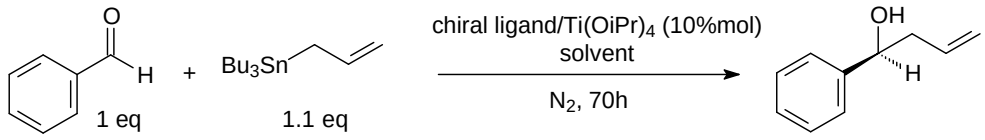


BINOL **2b** was a reddish solid, highly soluble in many common polar solvents such as acetone, acetonitrile, ethyl acetate and alcohols, and also soluble in halogenated solvents, differently from BINOL **1b**.

BINOL **2b** was used to study the asymmetric allylation of aldehydes with allyltributyltin in dichloromethane and in 1-butyl-4-methylpyridinium tetrafluoroborate, in comparison with unmodified BINOL.

The reactions were performed following the experimental conditions reported in the literature[111] (Figure 3.2.1.16.).

Figure 3.2.1.16. Typical procedure of asymmetric allylation of aldehydes (chiral ligand = BINOL and **2b**).



Anhydrous dichloromethane and 1-butyl-4-methyl-pyridinium tetrafluoroborate (BMPy BF_4) were used as solvent systems, and BINOL and **2b** as chiral ligands. Benzaldehyde has been chosen as model substrate. The enantiomeric excesses were established by chiral GC of the silyl ether obtained by silylation of the homoallylic alcohol product, according to Tagliavini et al.[110] (Table 3.2.1.6.).

Table 3.2.1.6. Asymmetric allylation of benzaldehyde with allyltributyltin.

Entry	Chiral ligand	Solvent system	Ligand: Ti(O-*i*-Pr)$_4$	T (°C)	Isolated yield %	ee %
1	BINOL	CH_2Cl_2	1:1	-20°C	21	77
2	BINOL	CH_2Cl_2	2:1	-20°C	90	98
3	BINOL	CH_2Cl_2	2:2	-20°C	45	74
4	BINOL	BMPy BF_4	1:1	-20°C	30	45
5	**2b**	CH_2Cl_2	1:1	-20°C	10	12
6	**2b**	CH_2Cl_2	2:1	-20°C	10	65
7	**2b**	BMPy BF_4	2:1	-20°C	8	20
8	**2b**	CH_2Cl_2	2:1	rt	20	0
9	**2b**	BMPy BF_4	2:1	rt	22	0

The finding of optimal experimental condition for the BINOL-Ti catalyzed allylation of aldehydes in CH_2Cl_2 has been extensity debated.[111,132]. In our hands, the ratio ligand:titanium markedly influences the efficiency of the catalyst: ratio BINOL:titanium 1:1 (10% mol, Entry 1 and 20% mol, Entry 3) gives fair enantioselectivity (77% and 74%) but low yields (21% and 45%); instead ratio 2:1 (Entry 2) gives both excellent enantiomeric excess and conversion (98% and 90% respectively). With **2b**, a ratio ligand:titanium 1:1 (Entry 5) gives low yield (10%) and low ee (12%), while a ratio 2:1 (Entry 6) significantly increases the enantioselectivity to 65%.

In the literature, few findings have been reported about the asymmetric allylation of aldehydes in ionic liquids catalysed by chiral In(III) complexes,[133] and only one in which In(III)-BINOL catalyst was involved.[129] The best result was obtained in 1-hexyl-3-methylimidazolium hexafluorophosphate (HMIM PF_6) in which the diethylzinc addition to benzaldehyde afforded 60% conversion and 72% enantiomeric excess, while

[132] Weigand, S., Reinhard Bruckner, R., 1996. Ti^{iv}- BINOLate-Catalyzed Highly Enantioselective Additions of b-Substituted Allylstannanes to Aldehydes. Chem Europ J., 2, 9, 1077-1084.

[133] a) Lu, J., Ji, S., Qian, R., Chen, J., Liu, Y., Loh, T., 2004. InCl3-Promoted Allylation of Aldehydes in Ionic Liquid: Scope and Enantioselectivity Studies. Synlett, 3, 534–536; b) Lu, J., Ji, S., Teo, Y., Loh, T., 2005. Asymmetric allyltributylstannane addition to ketones catalyzed by chiral PYBOX–In(III) complex immobilized in ionic liquid. Tet Let., 46, 7435–7437

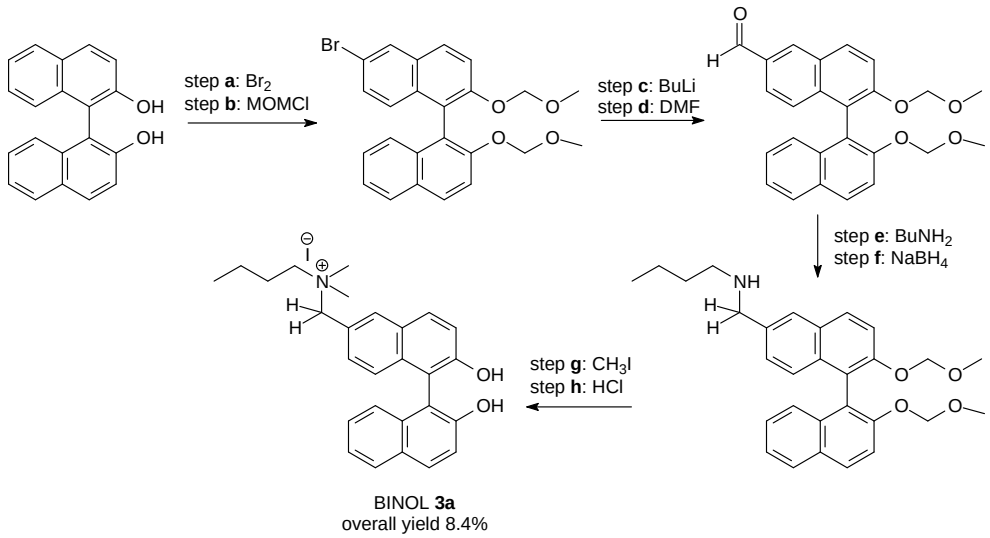
in HMIM BF_4 only 20% ee is reported, lower to what obtained here in Entry 4.

Summarising the results obtained in the allylations of aldehydes with the second class of derivatives (ionic BINOLs **2**) it is possible to say that also the introduction of a single ammonium moiety on the positions 3 of BINOL is not successful. Moreover, also in this case, the use of an ionic liquid solvent decreases both the yield and the selectivity respect to organic solvents. The poor activity of **2b** can be attributed to the increase of the steric hindrance in region of space where the crucial complexation of the aldehyde substrate should occur. For this reason, a last different derivatisation (ionic BINOL **3a**) was attempted, introducing one single ammonium moiety in position 6 of the backbone.

The synthetic pathway (Figure 3.2.1.17.), according to the literature procedure,[124] was based on an initial mono-bromination in position 6 (step **a**) followed by protection of the OH groups (step **b**). In both the previous derivatisation (BINOLs **1** and BINOLs **2**), the initial steps were very successful and gave the desired products in very high yields; in this case step **a** was much less efficient, with an initial yield of just 35% (comparable to the yield reported in the literature, 47%). The next steps were a metallation with butyl lithium (step **c**) and a formylation with DMF (step **d**). These steps gave 23% yield of 6-formyl-protected BINOL, about the half than in the literature (67%).

This compound was reductively aminated and methylated as usually (**e**, **f**, **g**). Final deprotection afforded BINOL **3a** in a 8.0% overall yield.

Figure 3.2.1.17. Synthesis of BINOL **3a**.



The low amount of product obtained has not yet made possible to check the catalytic performance of the new ligand. This task will be pursed in the next future.

Conclusions

A new procedure for the allylation of ketones and aldehydes with tetraallyltin in ionic liquids was developed. The reaction afforded high yields both in sulfonate-containing ILs (*N*-methyl-*N*-butylpyrrolidinium trifluoromethansulfonate) and in ILs without sulfonate (1-butyl-3-methylimidazolium tetrafluoroborate) upon addition of a small amount of sulfonic acid. The checked reaction resulted in peculiar chemoselectivity favouring aliphatic substrates towards aromatic ketones and good stereoselectivity in the allylation of levoglucosenone. Finally ILs-based systems could be easily and successfully recycled, making the described procedure environmentally benign.

Extension of the present methodology to other substrates and efforts to control stereochemistry will be the object of future studies.

In collaboration with Prof. Paul Dyson (Ecole Polytechnique Fédérale de Lausanne, Swiss) asymmetric allylations and alkylation of ketones and aldehydes were performed in ionic liquids and organic solvents for the first time with modified BINOL-titanium complexes.

A series of ionic BINOL derivatives was synthesized, introducing quaternary ammonium moieties in position 6,6' (BINOLs **1**), 3 (BINOLs **2**) or 6 (BINOLs **3**), ion paired with tetrafluoroborate and polystyrene SO_3 anions.

BINOL and the new ionic derivatives have been used to catalyse asymmetric additions of tetraallyltin and diethylzinc to carbonyl compounds in organic solvents (acetonitrile and dichloromethane) and in ionic liquid (1-butyl-3-methylpyridinium tetrafluoroborate); however, in spite of the high conversions, enantioselectivities were unsatisfactory.

Supporting chiral ligands on solid materials as polystyrene by exploiting the ion pairing as for BINOL **3**, revealed to be an intriguing but unsuccessful goal.

The other ionic BINOLs derivatives, ion paired with tetrafluoroborate anion, could catalyse the alkylation of carbonyl compounds under homogeneous conditions, but affording low enantioselectivity (BINOLs **1,** modified in positions 6,6') or low conversions (BINOLs **2**, modified in position 3).

Experimental section

Chemicals were purchased from Sigma–Aldrich with a purity grade of 98% or better, and used without further purification. The ionic liquids (purity >98%) were purchased from Merck (Darmstadt, Germany).

Reactions were monitored by means of TLC using silica gel sheets (Merck 60F254); the products were purified by flash chromatography on silica gel (Aldrich, 230–400 mesh). NMR spectra were recorded on Varian spectrometers: Inova 300, Mercury 400, and Inova 600; chemical shifts are quoted relative to the deuterated solvent signals with chemical shifts (d) given in ppm and coupling constants (J) given in Hz. GC-MS spectra were obtained using an AGILENT 6850 gas chromatograph on a SUPELCO SPB-5 capillary column (temperature programmed: 50°C for 5 min then 10°C min^{-1} until 300°C) coupled with a quadrupole mass spectrometer AGILENT 5975. Elemental analyses were obtained with a Perkin–Elmer 2400 Series II CHNS/O Analyzer equipped with a Perkin AD-4 autobalance.

Enantiomeric excesses obtained in the allylation of ketones with tetraallyltin were determined by HPLC on CHIRALCEL OD column (λ 254 nm, 0.5 mL/min flux, hexane/iPrOH).

Enantiomeric excesses obtained in the alkylation of *p*-methoxybenzaldehyde with diethylzinc were determined by HPLC on CHIRALCEL OD column (λ 210 nm, 0.5 mL/min flux, hexane/iPrOH from 100/0 to 80/20 in 20 min).

Enantiomeric excesses obtained in the allylation of benzaldehyde with allyltributyltin were determined by GC over capillary pentyldimethyl-β-cyclodextrin column (BETACDX-PS 086, 25 m length, 0.25 mm i.d., 0.25 μm film thickness; H_2 carrier, 1 mL/min flux). Temperature programmed: isotherm at 55°C for 100 min.

Levoglucosenone was obtained through pyrolysis of cellulose by following the procedure reported in the literature.[134]

General procedure for the allylation of carbonyl compounds with tetraallyltin in BMPyr OTf

Carbonyl compound (0.33 mmol) was dissolved in BMPyr OTf (0.3 mL) and stirred for a few minutes at room temperature. Tetraallyltin (0.019 mL, 0.08 mmol) was added and the reaction was stirred at room temperature for 20 h. At the end of the reaction, the products were extracted with diethyl ether (2×3 mL) and 1:1 ethyl acetate/diethyl ether mixture (2×3 mL); the extracts were combined and the solvent was removed in vacuo. The homoallylic alcohol products were purified by column chromatography.

General procedure for the allylation of carbonyl compounds with tetraallyltin in BMIM BF_4.

The carbonyl compound (0.33 mmol) was dissolved in 1-butyl-3-methylimidazolium tetrafluoroborate (0.3 mL) and stirred for a few minutes at room temperature. Tetraallyltin (0.019 mL, 0.08 mmol) was added,

[134] Z. J. Witczak, in Levoglucosenone and Levoglucosans: Chemistry and Applications (Ed.: Z. J. Witczak), ATL Press, Mount Prospect, 1994.

followed by TfOH (2.6 mL, 0.03 mmol), and the reaction was stirred at room temperature for 20 h. At the end of the reaction, the products were extracted with diethyl ether (2×3 mL) and ethyl acetate (2×3 mL). The extracts were combined and the solvent was removed in vacuo. The homoallylic alcohol products were purified by column chromatography.
In some cases MsOH (1.9 mL, 0.03 mmol) or TsOH (5.7 mg, 0.03 mmol) were used in the place of TfOH.

Allylation of carbonyl compounds with tetraallyltin in organic solvents

The carbonyl compound (0.33 mmol) was dissolved in an organic solvent (0.3 mL; $CHCl_3$, CH_2Cl_2, or CH_3CN). Tetraallyltin (0.019 mL, 0.08 mmol) was added, followed by TfOH (2.6 mL, 0.03 mmol), and the reaction was stirred at room temperature for 20 h. After removal of the organic solvent, the products were purified by column chromatography.

Competitive allylation of 2-octanone and α-tetralone

2-octanone (0.052 mL, 0.33 mmol) and 2j (0.045 mL, 0.33 mmol) were dissolved in BMPyr OTf (0.3 mL) and stirred for a few minutes at room temperature. Tetraallyltin (0.019 mL, 0.08 mmol) was added and the reaction was stirred at room temperature for 20 h. Following the usual workup, the products were purified by column chromatography; the corresponding homoallylic alcohol of 2-octanone was obtained in 73% yield (124 mg) and the corresponding homoallylic alcohol of α-tetralone in 7% (13 mg).

Allylation of levoglucosenone with tetraallyltin in BMPyr OTf with recycling of solvent.

Levoglucosenone (42 mg, 0.33 mmol) was dissolved in BMPyr OTf (0.3 mL) and stirred for a few minutes at room temperature. Tetraallyltin (0.019 mL, 0.08 mmol) was added and the reaction was stirred at room temperature for 20 h. At the end of the reaction, the reaction mixture was extracted with diethyl ether (2×3 mL) and 1:1 ethyl acetate/diethyl ether (2×3 mL); the extracts were combined and the solvent was removed in vacuo. The homoallylic alcohol product was purified by column chromatography. From

the residual BMPyr OTf, any trace of the extraction solvent was removed by rotary evaporation. Afterwards, levoglucosenone and tetraallyltin were added in the same amounts as the previous cycle and the reaction was carried out for 20 h. The same workup was applied for each cycle.

Allylation of levoglucosenone with tetraallyltin in BMIM BF_4 with recycling of solvent

Levoglucosenone (42 mg, 0.33 mmol) was dissolved in BMIM BF_4 (0.3 mL) and stirred for few minutes at room temperature. Tetraallyltin (0.019 ml, 0.08 mmol) was added followed by TfOH (2.6 mL, 0.03 mmol). After 20 h at room temperature, the reaction mixture was extracted with diethyl ether (2×3 mL) and ethyl acetate (2×3 mL). The product was then purified by column chromatography. From the residual BMIM BF_4, any trace of the extraction solvent was removed by rotary evaporation. Afterwards, levoglucosenone, tetraallyltin, and TfOH were added in the same amounts as the previous cycle and the reaction was carried out for 20 h. The same workup was applied for each cycle.

The corresponding homoallylic allylic alcohols of 2-octanone, cyclohexanone, cyclopentanone, *trans*-4-phenyl-3-buten-2-one, acetonaphthone, α-tetralone, 4-methoxyacetophenone, 4-bromoacetophenone, 4-nitroacetophenone, benzaldehyde, cinnamaldehyde are known compounds. Spectroscopic features of the products obtained in the present work exactly corresponded to those reported in the literature.

4-(2-methylethyl)oct-1-en-4-ol: ^{1}H NMR (400 MHz, C*D*Cl_3): δ=0.89–0.91 (m, 9H, C*H*$_3$), 1.28–1.43 (m, 6H, C*H*$_2$), 1.72–1.79 (m, 1H, C*H*$(CH_3)_2$), 2.16 (dd, 1H, J=7.4 Hz, J=13.8 Hz, CH_2=CHC*H*$_2$), 2.26 (dd, 1H, J=7.4 Hz, J=13.8 Hz, CH_2=C*H*CH_2), 5.06–5.11 (m, 2H, C*H*$_2$=CH), 5.79–5.88 ppm (m, 1H, CH_2=C*H*). ^{13}C NMR (100 MHz, C*D*Cl_3): δ=14.0, 16.8, 23.3, 25.2, 25.3, 34.4, 35.9, 40.6, 75.4, 118.1, 134.3 ppm.

GC–MS: room temperature, 12.6 min, m/z (%) 129 (80) $[M\text{-}CH_2{=}CHCH_2]^+$, 85 (100), 71 (60), 57 (50). Elemental analysis (%) calcd for $C_{11}H_{22}O$ (170.3): C 77.58, H 13.02, O 9.40; found: C 77.66, H 12.98, O 9.42.

4,5-Dimethylhept-1-en-4-ol: ^{1}H NMR (400 MHz, C*D*Cl$_3$): δ= 0.84–0.90 (m, 6H, C7-H and C4-Me), 1.05 (d, 3H, J=7.4 Hz, C5-Me), 1.18–1.39 (m, 2H, C6-H), 1.54–1.71 (m, 1H, C5-H), 2.17–2.21 (m, 2H, C3-H), 5.06–5.11 (m, 2H, C1-H), 5.79–5.88 ppm (m, 1H, C2-H). ^{13}C NMR (100 MHz, C*D*Cl$_3$): δ=12.7, 13.7, 23.4, 24.0, 44.0, 44.4, 74.5, 118.5, 134.0 ppm.
GC–MS: room temperature, 9.38 min, m/z (%) 101 (100) [M-CH_2=$CHCH_2$]$^+$, 85 (80), 57 (60). Elemental analysis (%) calcd. for $C_9H_{18}O$ (142.2): C 76.00, H 12.76, O 11.25; found: C 75.85, H 12.78, O 11.22.

(4S)-4-Allyl-6,8-dioxa-bicyclo[3.2.1]oct-2-en-4-ol: ^{1}H NMR (300 MHz, C*D*Cl$_3$): δ=1.67 (bs, 1H, O*H*), 2.37–2.41 (m, 2H, CH_2=CHCH_2), 3.68–3.73 (m, 1H, C6-H), 3.79 (d, 1H, J=6.6 Hz, C6-H), 4.67 (t, 1H, J=2.7 Hz, C5-H), 5.08–5.12 (m, 1H, CH_2=CH), 5.15 (s, 1H, C1-H), 5.26 (d, 1H, J=2.1 Hz, CH_2=CH), 5.60 (dd, 1H, J=2.1 and 9.9 Hz, C4-H), 5.86–5.94 (m, 1H, CH_2=C*H*), 6.02 ppm (dd, 1H, J= 4.5 and 9.9 Hz, C3-H). ^{13}C NMR (75 MHz, C*D*Cl$_3$): δ=41.4, 69.6, 71.5, 72.4, 103.8, 118.2, 128.5, 132.1, 132.5 ppm.
GC–MS: room temperature, 14.0 min; m/z (%) 168 [M$^+$]; 127 (10) [M-CH_2CH=CH_2]$^+$; 97(50); 81 (100); 53 (25). Elemental analysis (%) calcd. for $C_9H_{12}O_3$ (168.2): C 64.27, H 7.19, O 28.54; found: C 64.14, H 7.21, O 28.45.

3-Methyl-1-phenylhex-5-en-1-yn-3-ol: ^{1}H NMR (300 MHz, C*D*Cl$_3$): δ=1.61 (s, 3H, CH_3), 2.29 (bs, 1H, O*H*), 2.49 (dd, 1H, J= 7.6 Hz, J=13.6 Hz, C4-H), 2.62 (dd, 1H, J=6.6 Hz, J=13.6 Hz, C4-H), 5.27 (m, 2H, C6-H), 6.12 (m, 1H, C5-H), 7.28–7.48 ppm (m, 5H, Ph). ^{13}C NMR (50 MHz, C*D*Cl$_3$): 29.3, 48.2, 67.4, 83.5, 83.6, 119.6, 122.6, 128.2, 128.3, 131.7, 133.3.
GC–MS: room temperature, 17.0 min, m/z (%) 145 (100) [M-CH_2=$CHCH_2$]$^+$, 129 (10). Elemental analysis (%) calcd. for $C_{13}H1_4O$ (185.25): C 83.83, H 7.58, O 8.59; found: C 83.56, H 7.59, O 8.61.

4-(4-hydroxyphenyl)pent-1-en-4-ol: ^{1}H NMR (400 MHz, C*D*Cl$_3$): δ=1.25 (t, 3H, CH_3), 2.53–2.55 (m, 2H, CH_2=CHCH_2), 5.08–5.13 (m, 2H, CH_2=CH), 5.26–5.28 (m, 1H, CH_2=C*H*), 6.81–6.83 (m, 2H, Ar-*H* ortho),

7.25–7.33 ppm (m, 2H, Ar-*H* meta).^{13}C NMR (100 MHz, C*D*Cl_3): δ=29.6, 48.4, 73.8, 115.1, 119.3, 126.8, 133.6, 154.6, 161.3 ppm.

GC–MS: room temperature, 17.9 min, m/z (%) 160 (50) $[M\text{-}H_2O]^+$, 137 (100) $[M\text{-}CH_2{=}CHCH_2]^+$, 115 (20). Elemental analysis (%) calcd. for $C_{11}H_{14}O_2$ (170.2): C 74.13, H 7.92, O 17.95; found: C 74.28, H 7.90, O 17.91.

Synthesis of BINOL **1a**

Synthesis of (*R*)-6,6'-dibromo-2,2'-dihydroxy-1,1'-binaphalene

(*R*)-BINOL (1000 mg, 3.5 mmol) was dissolved in CH_2Cl_2 (20 mL) at room temperature. The solution was cooled at -78°C and bromine (0.48 mL, 9.3 mmol, 2.67 equiv.) was added dropwise over a period of 30 min with constant stirring. After the addition was complete the solution was allowed to stir for 3 h rising the temperature to room temperature. The excess of Br_2 was destroyed by the addition of aqueous sodium bisulfite. The layers were separated and the organic layer was washed with saturated NaCl solution and dried over anhydrous Na_2SO_4. Evaporation of the solvent gave the crude product as a pasty solid, which was recrystallised from cyclohexane afforded the product as a colourless foamy solid (3.38 mmol, 96.7% yield).

(*R*)-6,6'-dibromo-2,2'-bis(methoxymethyloxy)-1,1'-binaphthalene

6,6'-dibromo-BINOL (1503 mg, 3.38 mmol) in anhydrous THF (10 mL) was added dropwise to a solution of NaH (676 mg, 16.9 mmol, 5 equiv. as 60% dispersion in oil) in THF (7 mL). The mixture was stirred for 1 h and chloromethyl methyl ether (0.64 mL, 8.45 mmol, 2.5 equiv.) in THF (2 mL) was added dropwise. The mixture was stirred for further 1.5 h then poured into water and the product extracted with ethyl acetate. The extract was washed with saturated NaCl solution and saturated $NaHCO_3$ solution, then dried over anhydrous Na_2SO_4 and concentrated to afford the product (3.38 mmol, >99% yield)

(*R*)-6,6'-diformyl-2,2'-bis(methoxymethyloxy)-1,1'-binaphthalene

To a solution of 6,6'-dibromo-BINOL-MOM (1798 mg, 3.38 mmol) in dry THF was added TMEDA (2.2 mL, 14.8 mmol, 4.4 equiv.) and then *n*-BuLi

(5.9 mL, 14.8 mmol, 4.4 equiv. 2.5 M solution in hexane) dropwise at −78°C. The solution was stirred for 5 h and dry DMF (1.7 mL, 22.3 mmol, 6.6 equiv.) was slowly added. The temperature was raised to -50°C and the solution was stirred for 45 min. It was then poured into saturated NH_4Cl, extracted with ethylacetate and dried over anhydrous Na_2SO_4. The crude product was obtained upon evaporation of the solvent as a pale yellow oil which was washed with cyclohexane to afforded the product as a pale yellow solid (2.46 mmol, 73.3% yield).

^{1}H NMR (400 MHz, C*D*Cl_3): δ=3.20 (s, 6H, C*H*$_3$), 5.06 (d, 2H, J = 6.7 Hz, OC*H*$_2$OCH$_3$), 5.17 (d, 2H, J = 7.2 Hz, OC*H*$_2$OCH$_3$), 7.21 (d, 2H, J = 8.8 Hz, Ar-*H*), 7.71 (d, 4H, J = 9.6 Hz, Ar-*H*), 8.16 (d, 2H, J = 9.2 Hz, Ar-*H*), 8.40 (s, 2H, Ar-*H*), 10.12 ppm (s, 1H, OC*H*).

(*R*)-6,6'-dibutylimine-2,2'-bis(methoxymethyloxy)-1,1'-binaphthalene

6,6'-Diformyl-BINOL-MOM (1062 mg, 2.46 mmol) was dissolved in anhydrous diethyl ether (7 mL) then anhydrous $MgSO_4$ (592 mg, 4.9 mmol, 2 equiv.) was added, followed by butylamine (0.5 mL, 5.4 mmol, 2.2 equiv.). The solution was stirred for 8 h, then filtered over celite and concentrated to give the product (2.26 mmol, 92% yield).

^{1}H NMR (200 MHz, C*D*Cl_3): δ=0.96 (t, 6H, J = 7.2 Hz, N(CH$_2$)$_3$C*H*$_3$), 1.28-1.46 (m, 4H, N(CH$_2$)$_2$C*H*$_2$CH$_3$), 1.64-1.75 (m, 4H, NCH$_2$C*H*$_2$CH$_2$CH$_3$), 3.18 (s, 6H, OC*H*$_3$), 3.66 (t, 4H, J = 7 Hz, NC*H*$_2$(CH$_2$)$_2$CH$_3$), 5.01 (d, 2H, J = 6.8 Hz, OC*H*$_2$OCH$_3$), 5.13 (d, 2H, J = 6.8 Hz, OC*H*$_2$OCH$_3$), 7.15-7.30 (m, 2H, Ar-*H*), 7.60-7.74 (m, 4H, Ar-*H*), 8.01-8.12 (m, 2H, Ar-*H*), 8.40 (s, 2H, Ar-*H*), 9.81 ppm (s, 1H, NC*H*).

(*R*)-6,6'-dibutylamine-2,2'-bis(methoxymethyloxy)-1,1'-binaphthalene

6,6'-Dibutylimine-BINOL-MOM (1223 mg, 2.26 mmol) was dissolved in MeOH (7 mL) at 0°C, $NaBH_4$ (171 mg, 4.52 mmol, 2 equiv.) was slowly added. The solution was stirred for 8 h, then quenched with saturated aq NH_4Cl, extracted with ethyl acetate, dried over anhydrous Na_2SO_4 and concentrated to afford the product (2.24 mmol, 99.5% yield).

^{1}H NMR (400 MHz, C*D*Cl_3): δ=0.85 (t, 6H, J = 7.2 Hz, N(CH$_2$)$_3$C*H*$_3$), 1.26-1.32 (m, 4H, N(CH$_2$)$_2$C*H*$_2$CH$_3$), 1.82-1.84 (m, 4H, NCH$_2$C*H*$_2$CH$_2$CH$_3$),

2.87-2.95 (m, 4H, NC*H*$_2$(CH$_2$)$_2$CH$_3$), 3.14 (s, 6H, OC*H*$_3$), 3.83 (m, 2H, N*H*$_2$-Ar), 4.01 (m, 2H, N*H*$_2$-Ar), 4.95 (d, 1H, J = 6.8 Hz, OC*H*$_2$OCH$_3$), 5.02 (d, 1H, J = 6.8 Hz, OC*H*$_2$OCH$_3$), 7.08 (d, 4H, J = 8.8 Hz, Ar-*H*), 7.54 (d, 2H, J = 9.2 Hz, Ar-*H*), 7.89 ppm (d, 4H, J = 2.8 Hz, Ar-*H*).

(*R*)-6,6'-dibutyl-dimethylammonium iodide-2,2'-bis(methoxymethyloxy)-1,1'-binaphthalene

6,6'-Dibutylamine-BINOL-MOM (1223 mg, 2.24 mmol) and K_2CO_3 (1362 mg, 9.8 mmol, 4.4 equiv.) were dissolved in CH_3CN (7 mL), then iodomethane (0.61 mL, 9.8 mmol, 4.4 equiv.) was added and the reaction was stirred at room temperature for 24 hours. K_2CO_3 was filtered off and the excess of iodomethane evaporated under vacuum (2.24 mmol,> 99% yield).

^{1}H NMR (200 MHz, DMSO): δ=0.93 (t, 6H, J = 7.0 Hz, N(CH$_2$)$_3$C*H*$_3$), 1.25-1.35 (m, 4H, N(CH$_2$)$_2$C*H*$_2$CH$_3$), 1.75-1.78 (m, 4H, NCH$_2$C*H*$_2$CH$_2$CH$_3$), 2.98 (s, 6H, OC*H*$_3$), 3.08 (s, 6H, OC*H*$_3$), 3.23-3.25 (m, 4H, NC*H*$_2$(CH$_2$)$_2$CH$_3$), 3.32 (s, 12H, N(C*H*$_3$)$_2$), 4.64 (s, 4H, NC*H*$_2$-Ar), 5.12 (d, 1H, J = 7.4 Hz, OC*H*$_2$OCH$_3$), 5.17 (d, 1H, J = 7.4 Hz, OC*H*$_2$OCH$_3$), 7.05 (d, 2H, J = 8.8 Hz, Ar-*H*), 7.40 (d, 2H, J = 9.0 Hz, Ar-*H*), 7.74 (d, 2H, J = 9.2 Hz, Ar-*H*), 8.13-8.16 (m, 4H, Ar-*H*), 8.20 ppm (s, 2H, Ar-*H*).

(*R*)-6,6'-dibutyl-dimethylammonium iodide-2,2'-dihydroxy-1,1'-binaphthalene 1a

An ice cooled solution of 6,6'-dibutyl-dimethylammonium iodide-BINOL-MOM (1917 mg, 2.24 mmol) in MeOH/HCl (20 mL) was stirred for 8 h, then poured into water (50 mL), and the product was extracted with ethyl acetate, dried over anhydrous Na_2SO_4 and concentrated to give BINOL **1a** in >99% yield (2.24 mmol).

^{1}H NMR (400 MHz, acetone): δ=0.95 (t, 6H, J = 7.2 Hz, N(CH$_2$)$_3$C*H*$_3$), 1.25-1.44 (m, 4H, N(CH$_2$)$_2$C*H*$_2$CH$_3$), 2.02-2.06 (m, 4H, NCH$_2$C*H*$_2$CH$_2$CH$_3$), 2.27 (s, 12H, N(C*H*$_3$)$_2$), 3.53-3.57 (m, 4H, NC*H*$_2$(CH$_2$)$_2$CH$_3$), 4.84 (s, 4H, NC*H*$_2$-Ar), 7.17 (d, 2H, J = 8.8 Hz, Ar-*H*), 7.45 (m, 4H, Ar-*H*), 8.02 (d, 2H, J = 8.8 Hz, Ar-*H*), 8.23 ppm (s, 2H, Ar-*H*).

Synthesis of BINOL **1b**

BINOL **1a** (100 mg, 0.13 mmol) was added to a solution of $NaBF_4$ (28.5 mg, 0.26 mmol, 2 equiv.) in water (3 mL), and the mixture was stirred for 24 h at 60°C. The water phase was extracted with ethyl acetate, and BINOL **1b** was obtained after removing the solvent by rotary evaporation (0.12 mmol, 98.5% yield).

^{1}H NMR (400 MHz, acetone): δ=0.99 (t, 6H, J = 7.2 Hz, $N(CH_2)_3CH_3$), 1.40-1.45 (m, 4H, $N(CH_2)_2CH_2CH_3$), 2.06-2.07 (m, 4H, $NCH_2CH_2CH_2CH_3$), 3.22 (s, 12H, $N(CH_3)_2$), 4.03-4.09 (m, 4H, $NCH_2(CH_2)_2CH_3$), 4.80 (s, 4H, NCH_2-Ar), 7.19 (d, 2H, J = 8.4 Hz, Ar-*H*), 7.48 (d, 2H, J = 8.8 Hz, Ar-*H*), 7.74 (d, 2H, J = 9.2 Hz, Ar-*H*), 8.01-8.51 ppm (m, 4H, Ar-*H*).

Synthesis of BINOL **1c**

BINOL **1a** (232 mg, 0.30 mmol) was added to a solution polystyrene 30% wt solution in water (402 mg, 0.00060 mmol, 0.002 equiv.) in water (3 mL), and the mixture was stirred for 18 h at 60°C. BINOL **1c** was precipitated by adding acetone to the water phase, and then washed several time with water and acetone (0.00059 mmol, 99% yield). BINOL **1c** is insoluble in all the organic solvents and in water so an ^{1}H-NMR characterization was not possible; the compound has been analyzed by IR, and the typical absorbance of the sulfonic group and OH stretching have been detected, indicating the ion pairing between ammonium-BINOL and sulfonated polystyrene.

Synthesis of BINOL **2a**

Synthesis of (*R*)-2,2'-bis(methoxymethyloxy)-1,1'-binaphthalene

(*R*)-BINOL (1000 mg, 3.5 mmol) in anhydrous THF (5 mL) was added dropwise to a solution of NaH (698.2 mg, 17.5 mmol, 5 equiv. as 60% dispersion in oil) in THF (7 mL). The mixture was stirred for 1 h and chloromethyl methyl ether (0.66 mL, 8.7 mmol, 2.5 equiv.) in THF (2 mL) was added dropwise. The mixture was stirred for further 1.5 h then poured into water and the product extracted with ethyl acetate. The extract was washed with saturated NaCl solution and saturated $NaHCO_3$ solution, then

dried over anhydrous Na_2SO_4 and concentrated to afford the product (3.5 mmol, >99% yield)

(*R*)-3-formyl-2,2'-bis(methoxymethyloxy)-1,1'-binaphthalene

To a stirred solution of BINOL-MOM (2168 mg, 5.8 mmol) in THF (7 mL) at -78°C was added TMEDA (1.0 mL, 6.9 mmol, 1.2 equiv.) and then *n*-BuLi (2.6 mL, 6.5 mmol, 1.13 equiv. 2.5 M solution in hexane) over 15 min. The m ixture was wormed to 0°C and stirred for 30 min. After cooling to -78°C, DMF (0.5 mL, 6.9 mmol, 1.2 equiv.) in THF (5 mL) was added dropwise for 30 min and then warmed to 0°C and stirred for further 40 min. the resultant yellow solution was quenched with saturated aq NH_4Cl. After addition of 1 N aq HCl. The solution was extracted with diethyl ether and washed with saturated aq. $NaHCO_3$ and brine. It was then dried over anhydrous Na_2SO_4 and concentrated to afford a residue which was purified by flash column chromatography (SiO_2, hexane/ethyl acetate 10/1) (3.59 mmol, 62% yield).

^{1}H NMR (400 MHz, C*D*Cl_3): δ=3.01 (s, 3H, C*H*$_3$), 3.17 (s, 3H, C*H*$_3$), 4.64 (d, 1H, J = 6 Hz, OC*H*HOCH$_3$), 4.75 (d, 1H, J = 6 Hz, OCH*H*OCH$_3$), 5.05 (d, 1H, J = 7.2 Hz, OC*H*HOCH$_3$), 5.16 (d, 1H, J = 7.2 Hz, OCH*H*OCH$_3$), 7.15-7.49 (m, 6H, Ar-*H*), 7.62 (d, 1H, J = 8.8 Hz, Ar-*H*), 7.90 (d, 1H, J = 8.8 Hz, Ar-*H*), 8.03 (dd, 2H, J = 9.2 Hz, J = 15.2, Ar-*H*), 8.58 (s, 1H, Ar-*H*), 10.60 ppm (s, 1H, OC*H*).

(*R*)-3-butylimine-2,2'-bis(methoxymethyloxy)-1,1'-binaphthalene

3-Formyl-BINOL-MOM (1444 mg, 3.59 mmol) was dissolved in anhydrous diethyl ether (7 mL) then anhydrous $MgSO_4$ (430 mg, 3.59 mmol, 1 equiv.) was added, followed by butylamine (0.4 mL, 3.9 mmol, 1.1 equiv.). The solution was stirred for 8 h, then filtered over celite and concentrated to give the product (3.54 mmol, 98.6% yield).

^{1}H NMR (200 MHz, C*D*Cl_3): δ=1.01 (t, 3H, J = 7.4 Hz, N(CH$_2$)$_3$C*H*$_3$), 1.27-1.90 (m, 4H, NCH$_2$(C*H*$_2$)$_2$CH$_3$), 2.94 (s, 3H, OC*H*$_3$), 3.15 (s, 3H, OC*H*$_3$), 3.79 (t, 2H, J = 5.8 Hz, NC*H*$_2$(CH$_2$)$_2$CH$_3$), 4.62 (d, 1H, J = 5.6 Hz, OCH*H*OCH$_3$), 4.68 (d, 1H, J = 5.6 Hz, OC*H*HOCH$_3$), 5.01-5.19 (m, 2H,

OC*H*$_2$OCH$_3$), 7.15-7.64 (m, 6H, Ar-*H*), 7.88-8.06 (m, 4H, Ar-*H*), 8.89 (s, 1H, Ar-*H*), 10.60 ppm (s, 1H, NC*H*).

(*R*)-3 -butylamine-2,2'-bis(methoxymethyloxy)-1,1'-binaphthalene

3-Butylimine-BINOL-MOM (1619 mg, 3.54 mmol) was dissolved in MeOH (7 mL) at 0°C, $NaBH_4$ (134 mg, 3.54 mmol, 1 equiv.) was slowly added. The solution was stirred for 8 h, then quenched with saturated aq NH_4Cl, extracted with ethyl acetate, dried over anhydrous Na_2SO_4 and concentrated to afford the product (3.5 mmol, 98.8% yield).

^{1}H NMR (200 MHz, C*D*Cl$_3$): δ=0.93 (t, 3H, J = 7.2 Hz, N(CH$_2$)$_3$C*H*$_3$), 1.24-1.49 (m, 2H, N(CH$_2$)$_2$C*H*$_2$CH$_3$), 1.77-1.93 (m, 2H, NCH$_2$C*H*$_2$CH$_2$CH$_3$), 3.01 (t, 2H, J = 8.4 Hz, NC*H*$_2$(CH$_2$)$_2$CH$_3$), 3.17 (s, 3H, OC*H*$_3$), 3.19 (s, 3H, OC*H*$_3$), 4.44 (d, 1H, J = 5.6 Hz, OCH*H*OCH$_3$), 4.47 (s, 2H, N*H*$_2$-Ar), 4.65 (d, 1H, J = 5.6 Hz, OC*H*HOCH$_3$), 5.06 (d, 1H, J = 7 Hz, OCH*H*OCH$_3$), 5.15 (d, 1H, J = 7 Hz, OCH*H*OCH$_3$), 7.28-7.48 (m, 4H, Ar-*H*), 7.62 (d, 2H, J = 9.2 Hz, Ar-*H*), 7.89-8.04 (m, 4H, Ar-*H*), 8.30 ppm (s, 1H, Ar-*H*).

(*R*)-3-butyl-dimethylammonium iodide-2,2'-bis(methoxymethyloxy)-1,1'-binaphthalene

3-Butylamine-BINOL-MOM (1638 mg, 3.5 mmol) and K_2CO_3 (1604 mg, 7.7 mmol, 2.2 equiv.) were dissolved in CH_3CN (7 mL), then iodomethane (0.48 mL, 7.7 mmol, 2.2 equiv.) was added and the reaction was stirred at room temperature for 24 hours. K_2CO_3 was filtered off and the excess of iodomethane evaporated under vacuum (3.5 mmol,> 99% yield).

^{1}H NMR (200 MHz, C*D*Cl$_3$): δ=1.03 (t, 3H, J = 7.0 Hz, N(CH$_2$)$_3$C*H*$_3$), 1.44-1.62 (m, 2H, N(CH$_2$)$_2$C*H*$_2$CH$_3$), 1.80-1.94 (m, 2H, NCH$_2$C*H*$_2$CH$_2$CH$_3$), 3.05 (s, 3H, OC*H*$_3$), 3.17 (s, 3H, OC*H*$_3$), 3.40 (s, 6H, N(C*H*$_3$)$_2$), 3.73-3.81 (m, 2H, NC*H*$_2$(CH$_2$)$_2$CH$_3$), 4.42 (d, 1H, J = 5.4 Hz, OCH*H*OCH$_3$), 4.56 (s, 4H, NC*H*$_2$-Ar), 4.58 (d, 1H, J = 5.4 Hz, OC*H*HOCH$_3$), 5.02-5.13 (m, 1H, OCH*H*OCH$_3$), 5.19-5.36 (m, 1H, OCH*H*OCH$_3$), 7.03 (d, 1H, J = 8.4 Hz, Ar-*H*), 7.07 (d, 1H, J = 8.4 Hz, Ar-*H*), 7.23-7.64 (m, 6H, Ar-*H*), 7.90-8.12 (m, 2H, Ar-*H*), 8.63 ppm (s, 1H, Ar-*H*).

(*R*)-3-butyl-dimethylammonium iodide-2,2'-dihydroxy-1,1'-binaphthalene 2a

An ice cooled solution of 3-butyl-dimethylammonium iodide-BINOL-MOM (2100 mg, 3.5 mmol) in MeOH/HCl (20 mL) was stirred for 8 h, then poured into water (50 mL), and the product was extracted with ethyl acetate, dried over anhydrous Na_2SO_4 and concentrated to give BINOL **2a** in >99% yield (3.5 mmol).

^{1}H NMR (400 MHz, DMSO): δ=0.98 (t, 3H, J = 7.2 Hz, $N(CH_2)_3CH_3$), 1.33-1.39 (m, 2H, $N(CH_2)_2CH_2CH_3$), 1.82-1.86 (m, 2H, $NCH_2CH_2CH_2CH_3$), 3.15 (s, 6H, $N(CH_3)_2$), 3.41-3.45 (m, 2H, $NCH_2(CH_2)_2CH_3$), 4.84 (s, 4H, NCH_2-Ar), 6.92 (d, 1H, J = 8.4 Hz, Ar-*H*), 6.98 (d, 1H, J = 8.4 Hz, Ar-*H*), 7.19-7.45 (m, 4H, Ar-*H*), 7.91-7.99 (m, 4H, Ar-*H*), 8.22 ppm (s, 1H, Ar-*H*).

Synthesis of BINOL **2b**

BINOL **2a** (883 mg, 1.72 mmol,) was added to a solution of $NaBF_4$ (227.2 mg, 2.06 mmol, 1.2 equiv.) in water (3 mL), and the mixture was stirred for 24 h at 60°C. The water phase was extracted with ethyl acetate, and BINOL **2b** was obtained after removing the solvent by rotary evaporation (1.43 mmol, 83% yield).

^{1}H NMR (200 MHz, DMSO): δ=0.95 (t, 3H, J = 7.6 Hz, $N(CH_2)_3CH_3$), 1.21-1.35 (m, 2H, $N(CH_2)_2CH_2CH_3$), 1.79-1.94 (m, 2H, $NCH_2CH_2CH_2CH_3$), 3.08 (s, 6H, $N(CH_3)_2$), 3.34-3.49 (m, 2H, $NCH_2(CH_2)_2CH_3$), 4.74 (s, 4H, NCH_2-Ar), 6.87 (d, 1H, J = 8.4 Hz, Ar-*H*), 6.94 (d, 1H, J = 8.4 Hz, Ar-*H*), 7.15-7.36 (m, 4H, Ar-*H*), 7.88-7.96 (m, 4H, Ar-*H*), 8.17 ppm (s, 1H, Ar-*H*).

Synthesis of BINOL **3a**

Synthesis of (*R*)-6-bromo-2,2'-dihydroxy-1,1'-binaphalene

(*R*)-BINOL (1000 mg, 3.5 mmol) was dissolved in CH_2Cl_2 (7 mL) and cooled to−78°C. Bromine (0.2 mL, 4.5 mmol, 1.3 equiv.) was added dropwise over a period of 30 min with constant stirring. After the addition was completed, the solution was allowed to warm to room temperature and stirred for 1.5 h. The excess of Br_2 was destroyed by the addition of sodium

bisulfite. The layers were separated and the organic layer was washed with saturated NaCl solution and dried over anhydrous Na_2SO_4. Evaporation of the solvent gave the crude product as a pasty solid. Flash column chromatography (SiO_2, hexane/ether 4/1) afforded the product as a colourless foamy solid (1.2 mmol, 35% yield).

(*R*)-6-Bromo-2,2'-bis(methoxymethyloxy)-1,1'-binaphthalene

6-Bromo-BINOL (350 mg, 0.95 mmol) in anhydrous THF (3 mL) was added dropwise to a solution of NaH (191 mg, 4.8 mmol, 5 equiv. as 60% dispersion in oil) in THF (2 mL). The mixture was stirred for 1 h and chloromethyl methyl ether (0.18 mL, 2.4 mmol, 2.5 equiv.) in THF (1 mL) was added dropwise. The mixture was stirred for further 1.5 h then poured into water and the product extracted with ethyl acetate. The extract was washed with saturated NaCl solution and saturated $NaHCO_3$ solution, then dried over anhydrous Na_2SO_4 and concentrated to afford the product (0.93 mmol, 98% yield)

(*R*)-6-Formyl-2,2'-bis(methoxymethyloxy)-1,1'-binaphthalene

To a solution of 6-bromo-BINOL-MOM (250 mg, 0.93 mmol) in dry THF was added TMEDA (0.16 mL, 1.1 mmol, 1.2 equiv.) and *n*-BuLi (0.44 mL, 1.1 mmol, 1.2 equiv., 2.5 M solution in hexane) dropwise at −78°C. The solution was stirred for 5 h and then dry DMF (0.12 mL, 1.6 mmol, 1.8 equiv.) was slowly added. After being stirred for 45 min the solution was allowed to warm to −50°C, poured into saturated NH_4Cl solution, extracted with ethyl acetate, and dried over anhydrous Na_2SO_4. The crude product was obtained upon evaporation of the solvent as a pale yellow oil.

Flash column chromatography (SiO_2, hexane/ethyl acetate 10/1) afforded the product as a pale yellow solid (0.27 mmol, 23% yield):

^{1}H NMR (600 MHz, C*D*Cl$_3$): δ=3.13 (s, 3H, C*H*$_3$), 3.17 (s, 3H, C*H*$_3$), 4.97 (d, 1H, J = 7.2 Hz, OC*H*HOCH$_3$), 5.03 (d, 1H, J = 7.2 Hz, OCH*H*OCH$_3$), 5.08 (d, 1H, J = 7.0 Hz, OC*H*HOCH$_3$), 5.13 (d, 1H, J = 7.0 Hz, OCH*H*OCH$_3$), 7.08 (d, 1H, J = 8.4, Ar-*H*), 7.21-7.23 (m, 2H, Ar-*H*), 7.34 (t, 1H, J = 7.8 Hz, Ar-*H*), 7.57 (d, 1H, J = 9 Hz, Ar-*H*), 7.67 (d, 2H, J = 9 Hz,

Ar-*H*), 7.87 (d, 1H, J = 8.4 Hz, Ar-*H*), 7.96 (d, 1H, J = 9 Hz, Ar-*H*), 8.10 (d, 1H, J = 9.0 Hz, Ar-*H*), 8.36 (s, 1H, Ar-*H*), 10.09 ppm (s, 1H, OC*H*).

(*R*)-6-butylimine-2,2'-bis(methoxymethyloxy)-1,1'-binaphthalene

6-Formyl-BINOL-MOM (108 mg, 0.27 mmol) was dissolved in anhydrous diethyl ether (7 mL) then anhydrous $MgSO_4$ (32.4 mg, 0.27 mmol, 1 equiv.) was added, followed by butylamine (0.03 mL, 0.3 mmol, 1.1 equiv.). The solution was stirred for 8 h, then filtered over celite and concentrated to give the product (0.26 mmol, >99% yield).

^{1}H NMR (200 MHz, C*D*Cl_3): δ=1.0 (t, 3H, J = 7.4 Hz, N$(CH_2)_3$C*H*$_3$), 1.28-1.34 (m, 2H, N$(CH_2)_2$C*H*$_2$CH$_3$), 1.52-1.63 (m, 2H, NCH$_2$C*H*$_2$CH$_2$CH$_3$), 3.13 (s, 3H, OC*H*$_3$), 3.19 (s, 3H, OC*H*$_3$), 3.68 (t, 2H, J = 5.8 Hz, NC*H*$_2$$(CH_2)_2CH_3$), 4.60 (d, 1H, J = 5.6 Hz, OCH*H*OCH$_3$), 4.70 (d, 1H, J = 5.6 Hz, OC*H*HOCH$_3$), 5.08-5.14 (m, 2H, OC*H*$_2$OCH$_3$), 7.15-7.30 (m, 6H, Ar-*H*), 7.88-8.06 (m, 4H, Ar-*H*), 8.89 (s, 1H, Ar-*H*), 9.70 ppm (s, 1H, NC*H*).

(*R*)-6-butylamine-2,2'-bis(methoxymethyloxy)-1,1'-binaphthalene

6-Butylimine-BINOL-MOM (121 mg, 0.26 mmol) was dissolved in MeOH (7 mL) at 0°C, $NaBH_4$ (10 mg, 0.26 mmol, 1 equiv.) was slowly added. The solution was stirred for 8 h, then quenched with saturated aq NH_4Cl, extracted with ethyl acetate, dried over anhydrous Na_2SO_4 and concentrated to afford the product (0.25 mmol, 98.6% yield).

^{1}H NMR (200 MHz, C*D*Cl_3): δ=0.93 (t, 3H, J = 7.2 Hz, N$(CH_2)_3$C*H*$_3$), 1.24-1.49 (m, 2H, N$(CH_2)_2$C*H*$_2$CH$_3$), 1.77-1.93 (m, 2H, NCH$_2$C*H*$_2$CH$_2$CH$_3$), 3.01 (t, 2H, J = 8.4 Hz, NC*H*$_2$$(CH_2)_2CH_3$), 3.17 (s, 3H, OC*H*$_3$), 3.19 (s, 3H, OC*H*$_3$), 3.90 (s, 2H, N*H*$_2$-Ar), 4.05 (d, 1H, J = 5.6 Hz, OC*H*HOCH$_3$), 4.44 (d, 1H, J = 5.6 Hz, OCH*H*OCH$_3$), 5.06 (d, 1H, J = 7 Hz, OCH*H*OCH$_3$), 5.15 (d, 1H, J = 7 Hz, OCH*H*OCH$_3$), 7.34-7.52 (m, 4H, Ar-*H*), 7.70 (d, 2H, J = 9.2 Hz, Ar-*H*), 7.89-8.04 (m, 4H, Ar-*H*), 8.80 ppm (s, 1H, Ar-*H*).

General procedure for the allylation of carbonyl compounds with tetraallyltin and BINOLs/titanium complex in anhydrous CH_3CN or BMPy BF_4.

$Ti(O\text{-}i\text{-}Pr)_4$ (0.009 mL, 0.03 mmol, 20% mol) was added to a solution of BINOL (8.6 mg, 0.03 mmol, 20% mol), modified BINOL **1b** (20.6 mg, 0.03 mmol, 20% mol) or **1c** (as monomer, 20.9 mg, 0.03 mmol, 20% mol), in 1 mL of anhydrous CH_3CN or BMPy BF_4 (strongly dried at 70°C under vacuum for 10 days). The mixture was stirred for 10 min under N_2, and then 2-propanol (0.034 mL, 0.45 mmol, 3 equiv.), ketone (0.15 mmol) and tetraallyltin (0.054 mL, 0,225 mmol, 1.5 equiv.) were added. After 20 h the reaction was quenched with saturated NH_4Cl and extracted with CH_2Cl_2, filtered over celite and concentrated. The crude product was purified by column chromatography and analysed by chiral HPLC.

2-(2'-naphtyl)-4-penten-2-ol. HPLC analysis on CHIRALCEL OD column (λ 254 nm, 0.5 mL/min flux, hexane/iPrOH from 100/0 to 80/20 in 20 min, then 80/20 until 30 min). rt_1 = 19.0 min; rt_2 = 20.9 min.

1-phenyl-3-hydroxy-3-methyl-1,5-hexandiene. HPLC analysis on CHIRALCEL OD column (λ 254 nm, 0.5 mL/min flux, hexane/iPrOH from 100/0 to 95/5 in 20 min, then 95/5 until 30 min, then from 95/5 to 10/90 in 45 min). rt_1 = 29.3 min; rt_2 = 30.0 min.

2-(4-metoxyphenyl)-4-penten-2-ol. HPLC analysis on CHIRALCEL OD column (λ 254 nm, 0.5 mL/min flux, hexane/iPrOH from 100/0 to 95/5 in 20 min). rt_1 = 22.6 min; rt_2 = 23.3 min.

General procedure for the alkylation of *p*-methoxybenzaldehyde with diethylzinc and BINOLs/titanium complex in BMPy BF_4.

$Ti(O\text{-}i\text{-}Pr)_4$ (0.052 mL, 0.175 mmol, 1.4 equiv.) was added to a solution of BINOL (7.16 mg, 0.025 mmol), modified BINOL **1b** (17.21 mg, 0.025 mmol) or **1c** (as monomer, 17.45 mg, 0.025 mmol), in 1.5 mL of BMPy BF_4 (strongly dried at 70°C under vacuum for 10 days). The mixture was stirred for 15 min under N_2, and then diethylzinc was added (0.375 mL, 0.375

mmol, 3 equiv., 1 M solution in hexane); after 15 min *p*-anisaldehyde (0.015 mL, 0.125 mmol) was added. After 5 h the reaction was quenched with saturated NH_4Cl and extracted with ethyl acetate and concentrated. The crude product was purified by column chromatography and analysed by chiral HPLC.

1-Phenyl-butan-1-ol. GC-MS analysis (temperature programmed: 50°C for 5 min then 10°C min^{-1} until 300°C): rt 16 min. HPLC analysis: rt_1 = 18.9 min (minor product); rt_2 = 19.9 min (major product).

General procedure for the allylation of benzaldehyde with allyltributyltin and BINOLs/titanium (1/1) complex in anhydrous CH_2Cl_2 and BMPy BF_4.
$Ti(O\text{-}i\text{-}Pr)_4$ (0.02 mL, 0.066 mmol, 10% mol) was added under N_2 to a solution of BINOL (19 mg, 0.066 mmol, 10% mol), or modified BINOL **2b** (32.3 mg, 0.066 mmol, 10% mol) in 2 mL of anhydrous CH_2Cl_2 or BMPy BF_4 (strongly dried at 70°C under vacuum for 10 days). 4Å activated molecular sieves (264 mg) were added and the solution was stirred for 1 h under reflux (in the case of BMPy BF_4 at 60°C). Benzaldehyde (0.067 mL, 0.656 mmol) was added; the solution was stirred at room temperature for 10 minutes and then cooled at -78°C. Allyltributyltin (0.226 mL, 0.73 mmol, 1.1 equiv.) was added, the reaction was stirred for 10 min at -78°C and then placed at -20°C for 70 h. The reaction was quenched with saturated $NaHCO_3$ and extracted with CH_2Cl_2, filtered over celite and concentrated. The crude product was purified by column chromatography and silylated for chiral GC-FID analysis.

General procedure for the allylation of benzaldehyde with allyltributyl tin and BINOLs/titanium (2/1) complex in anhydrous CH_2Cl_2 and BMPy BF_4.
The procedure was the same as described above but with BINOL (38 mg, 0.13 mmol, 20% mol), or modified BINOL **2b** (64.6 mg, 0.13 mmol, 20% mol).
1-Phenyl-3-buten-1-ol was previously silylated with 200 µl of bis-trimethylsilyl-trifluoro-acetamide containing 1 % trimethyl-chlorosilane and 10 µl of pyridine, and then the enantiomeric excesses were determined by GC-FID. rt_1 = 80.80 min; rt_2 = 85.95 min.

3.3.2. Switchable polarity solvents (SPS)

The second application of ionic liquids described in this Thesis is their use as switchable polarity solvents for the extraction of vegetable oils from biomass.

Switchable polarity solvents (SPS) are a "new" class of fascinating solvents which can be considered as reversible ionic liquids.

The concept of "switchable compounds" and in particular of "switchable solvents" was proposed for the first time by Jessop et al.;[135] this smart idea is based on the possibility to switch some properties of a substance on and off when a "trigger" is applied, in a reversible way from a version, with one set of properties, to another that has very different properties. For solvents such properties can be polar/apolar, volatile/non-volatile, protic/aprotic.

Chemical processes often involve many steps which can require many specific solvents; after each step the solvent has to be removed and replaced with a new one, more suitable for the following step, increasing the economic cost and environmental impact of the process. Switchable solvents represent a valid answer to these cumbersome procedures, because their properties can be adjusted for the following step while still in the reaction vessel, enabling the same solvent to be used for several consecutive reaction or separation steps.

The first SPS proposed by Jessop et al.[135,136] were based on a non-ionic solvent mixture, generally composed by an alcohol (or water) and an amine, which could be converted into an ionic liquid upon exposure to CO_2 and easily reconverted into the non-ionic form by removing CO_2 through bubbling N_2 into the system.

After these first examples, the number of switchable compounds increased rapidly (Figure 3.3.2.1.). It includes switchable surfactants based on water or aliphatic primary amines and acetamidine,[137] switchable solvents

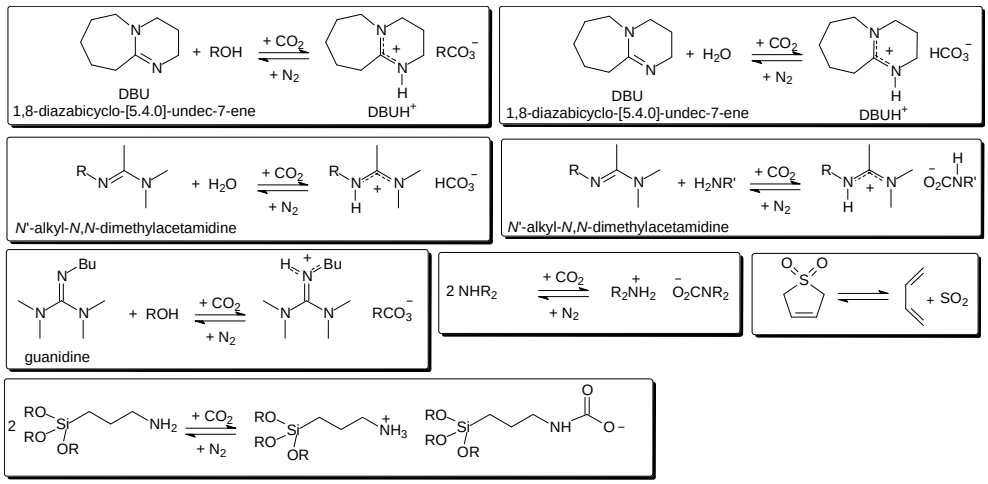
[135] Jessop, P. G., Heldebrant, D.J., Li, X., Eckert, C. A., Liotta, C. L., 2005. Reversible non polar-to-polar solvent. Nature. 436, 1102.

[136] Jessop, P. G., Heldebrant, D. J., Thomas, C. A., Eckert, C. A. Liotta, C. L., 2005. The Reaction of 1,8-Diazabicyclo-[5.4.0]-undec-7-ene (DBU) with Carbon Dioxide. JOC, 70, 5335-5338.

[137] a) Jessop, P. G., Liu, Y., Cunningham, M., Eckert, C.A., Liotta, C.L., 2006. Switchable surfactants. Science. 313, 958-960. b) Weiss, R. G., Yamada, T., Lukac, P. J., George, M., 2007. Reversible, Room Temperature Ionic Liquids. Amidinium Carbamates Derived from Amidines and Aliphatic Primary Amines with Carbon Dioxide. Chem. Mater. 19 (5), 967-969.

consisting of amidine/alcohol or guanidine/alcohol mixtures, successfully used in the polymerisation of styrene,[138] single liquid component SPS[139] based on primary or secondary amines easily converted into liquid carbamates, used for example in the successful polymerisation of cyclohexene. The most recent example of SPS has been proposed by Eckert et al.[140] who used butadiene sulfone and piperylene sulfone as switchable solvents which could act simultaneously as solvent and as acid catalyst in the hydrolysis of β-pinene

Figure 3.3.2.1. Examples of switchable polarity solvents.



One of the most interesting properties for the practical application of switchable polarity solvents is their switching solubility behaviour, correlated with their reversible polarity; Jessop et al.[135] reported that an equimolar mixture of DBU (1,8-diazabicyclo-[5.4.0]-undec-7-ene) and 1-hexanol behaves as a slightly polar solvent, similar to chloroform, enabling to dissolve apolar compounds such as hydrocarbons, whereas the liquid

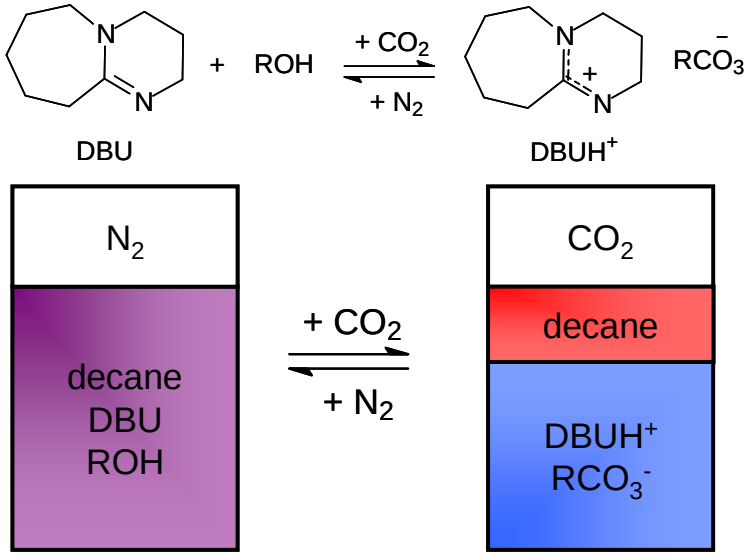
[138] Jessop, P. G., Phan, L., Andreatta, J. R., Horvey, L. K., Edie, C. F., Luco, A., Mirchandani, A., Darensbourg, D. J., 2008. Switchable Polarity Solvents Prepared with a Single Liquid Component. JOC, 73 (1), 127-132.

[139] a) Jessop, P. G., Phan, L., Chiu, D., Heldebrant, D. J., Huttenhower, H., John, E., Li, X., Pollet, P., Wang, R., Eckert, C. A., Liotta, C. L., 2008. Switchable Solvents Consisting of Amidine/Alcohol or Guanidine/Alcohol Mixtures. Ind. Eng. Chem. Res., 47 (3), 539-545. b) Eckert, C. A., Blasucci, V., Dilek, C., Huttenhower, H., John, E., Llopis-Mestre, V., Pollet, P., Liotta, C. L., 2009. One-component, switchable ionic liquids derived from siloxylated amines. Chem. Commun., 116–118.

[140] Eckert, C. A., Donaldson, M. E., Llopis Mestre, V., Vinci, D., Liotta, C. L., 2009. Switchable Solvents for in-Situ Acid-Catalysed Hydrolysis of β-Pinene. Ind. Eng. Chem. Res., 48 (5), 2542-2547.

DBU hexylcarbonate, after CO_2 treatment, is a polar liquid, very similar to dimethylformamide and immiscible with hydrocarbons (Figure 3.3.2.2.).

Figure 3.3.2.2. SPS based on an equimolar mixture of 1,8-diazabicyclo-[5.4.0]-undec-7-ene (DBU) and an alcohol, and its polarity switching after the addition of CO_2.



The miscibility/immiscibility with apolar compounds has been exploited in two innovative applications.[139b,141] The first example concerns the recovery of alkanes from contaminated heavy crude oil, exploiting the immiscibility of alkanes with polar ionic liquids in which the impurities are retained.

The second application is an alternative to the industrial use of *n*-hexane in the extraction of soy oil from crushed soybeans. In the extraction of soybean oil, a low polarity organic solvents is necessary in the first step, but in the following recovery phase the high affinity of the solvent for the extracted matrix represents a drawback; in this contest, the use of a solvent which can be switched from low polar, useful in the extraction phase, to high polar, suitable in the recovery phase, represents the key to reduce the energetic and economic costs of the extractive process. This brilliant idea has inspired the work described below in the Thesis about the extraction of biofuels from biomass with SPS.

[141] Jessop. P. G., Phan, L., Brown, H., White, J., Hodgson, A., 2009. Soybean oil extraction and separation using switchable or expanded solvents. Green Chem. 11, 53-59.

Extraction of biofuels from terrestrial and aquatic biomass with SPS

The need to replace fossil fuels with fuels derived from renewable biomass is currently focused on biodiesel from oleaginous plant seeds and ethanol from sugarcane/corn; however this first generation of biofuels, primarily produced from food crops and mostly oil seeds, are limited in their ability to achieve targets for biofuel production, climate change mitigation and economic growth; moreover the recent dramatic increase of the price of food stocks has become a worldwide emergency. All these environmental and social concerns are now shifting the attention towards the development of next generation biofuels,[142] mainly produced from non-food feedstocks.

In the case of terrestrial plant sources, the production of second generation biofuels relies on the conversion of the highly abundant and widespread non-edible lignocellulosic fraction of plants; a third generation relies on the aquatic environment and it is represented by biofuels from micro and macroalgae. Lipids, which include acylglycerols and hydrocarbons, represent the most valuable fraction of microalgal biomass as their high energy content per mass unit is similar to conventional fuels. Several oleaginous microalgae (with lipid content exceeding 20% of their dry weight) have been exploited to this purpose,[143] and the biodiesel obtained has been claimed to be more convenient than conventional biodiesel from plant seeds.[144] Benefits rising from the utilisation of aquatic over terrestrial biomass include: *i*) higher sunlight use efficiency (about 5% vs. 1.5%),[145] *ii*) utilisation of marginal areas (e.g. desert and coastal regions), *iii*) possible coupling with other activities (e.g. wastewater treatment, CO_2 sequestration), *iv*) minor dependence on climatic conditions, *v*) availability of a larger number of species and *vi*) easier genetic manipulation to modify chemical composition (e.g. lipid content).[144,146] However, the industrial

[142] Williams, P. J. L., 2007. Biofuel: microalgae cut the social and ecological costs. Nature. 450, 478.

[143] Hu, Q., Sommerfeld, M., Jarvis, E., 2008. Microalgal triacylglycerols as feedstocks for biofuel production: perspectives and advances. Plant J. 54 (4), 621-639.

[144] a) Chisti, Y., 2007. Biodiesel from microalgae. Biotechnol. Adv. 25 (3), 294-306. b) Chisti, Y., 2008. Biodiesel from microalgae beats bioethanol. Trends. Biotechnol. 26 (3), 126-131.

[145] Posten, C., Schaub, G., 2009. Microalgae and terrestrial biomass as source for fuels—A process view. J. Biotechnol. 142 (1), 64-69.

[146] a) An, J.Y., Sim, S. J., Lee, J. S., Kim, B. W., 2003. Hydrocarbon production from secondarily treated piggery wastewater by the green alga *Botryococcus braunii*. J. Appl. Phycol. 15 (2-3), 185–191. b) Rosenberg, J. N., Oyler, G. A., Wilkinson L., Betenbaugh M.

development of fuels from microalgae is still hampered by higher overall costs with respect to both fossil fuel and first generation of biofuels counterparts: operating open ponds and bioreactors are expensive and the harvesting of algal biomass is energy costly.[147] For this reason the net energy balance from microalgae cultivation is still debated.[148] Besides the cost of growing and collecting microalgae, also downstream processes are to be taken into account to evaluate the overall productivity.

The current methods for vegetable oils extraction from biomass involve physical or chemical processes; the first is typically used to produce more traditional food oils (e.g., olive oil) by squeezing under high pressure the raw materials without using any solvent. The second method, mainly used for industrial oils such as soybean and corn oils, is based on an extraction with solvents (e.g. *n*-hexane), to obtain vegetable oil in higher yields and with a quicker and less expensive process.[149] However the existing solvent approach is characterised by several problematic aspects, such as the high solvent/biomass ratio and solvent losses (e.g. *n*-hexane losses are 1 kg per ton of beans processed).[150] In the last years different efforts have been done to reduce the use of toxic and polluting organic solvents to improve the sustainability of the extraction procedures from biomass, for example by using supercritical fluids.[151] As suggested by Jessop et al.,[141] switchable

J., 2008. A green light for engineered algae: redirecting metabolism to fuel a biotechnology revolution. Curr. Opin. Biotech. 19 (5), 430–436. c) Shen Y., Yuan W., Pei Z., Mao E., 2008. Culture of microalga *Botryococcus* in livestock wastewater. T. ASABE. 51 (4), 1395-1400.

[147] a) Benemann, J. R., 1997. CO_2 mitigation with microalgae systems. Energ. Convers. Manage. 38, S475-S479. b) Molina Grima, E., Belarbi, E. H., Acie´n Ferna´ndez, F. G., Robles Medina, A., Chisti, Y., 2003. Recovery of microalgal biomass and metabolites: process options and economics. Biotechnol. Adv. 20, 491–515. c) Wang, B., Li, Y. Q., Wu, N., Lan, C. Q., 2008. CO_2 bio-mitigation using microalgae. Appl. Microbiol. Biot. 79 (5), 707-718.

[148] a) Chisti Y., 2008. Response to Reijnders: Do biofuels from microalgae beat biofuels from terrestrial plants? Trends Biotechnol. 26 (7), 351-352. b) Reijnders, L., 2008. Do biofuels from microalgae beat biofuels from terrestrial plants? Trends Biotechnol. 26 (7), 349-350.

[149] Gouveia, L., Oliveira, A. C., 2009. Microalgae as a raw material for biofuels production. J. Ind. Microbiol. Biot. 36 (2), 269-274.

[150] Erikson, D.R., 1995. Practical Handbook of Soybean Processing and Utilisation, AOCS Press and United Soybean Board, St. Louis.

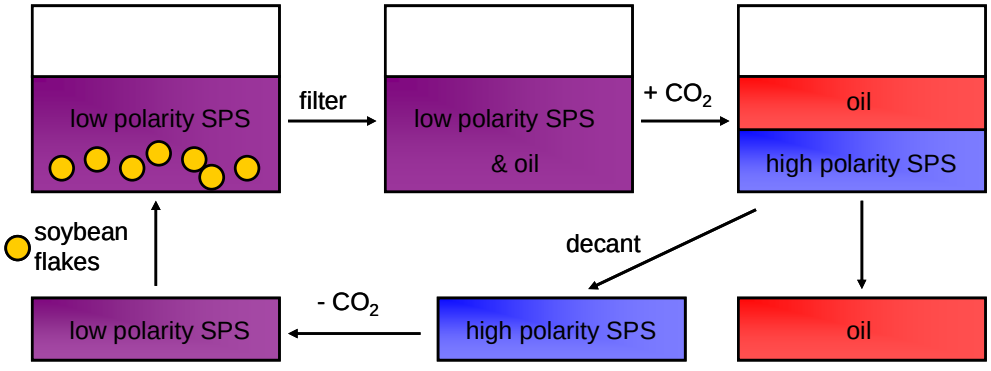
[151] a) Mendes, R. L., Nobre, B. P., Cardoso, M. T., Pereira, A. P., Palavra, A. F., 2003. Supercritical carbon dioxide extraction of compounds with pharmaceutical importance from microalgae. Inorg. Chim. Acta. 356, 328-334. b) McHugh, M.A., Krukoni, V.J., 1994. Biotechnology and food process engineering, Chapter 6 Supercritical fluid extraction. International Union of Food Science and Technology

polarity solvents, thanks to their peculiar polarity properties, could represent a new sustainable alternative to traditional organic solvents in vegetable oils extraction, and for this reason in this Thesis the potential of SPS as new solvents for the extraction of biofuels from terrestrial and aquatic natural matters has been investigated. In particular SPS were used in the extraction of two kinds of terrestrial biomass, soybean flakes and sunflower seeds, rich in triacylglycerols easily convertible in biodiesel of 1st generation, and one aquatic biomass, the microalga *Botryococcus braunii*, a freshwater colonial green microalga capable to produce high levels of liquid hydrocarbons which can be processed by cracking, similarly to hydrocarbons from fossil oil.

Extraction of oil from soybean flakes

The strategy proposed by Jessop et al.[141] to extract soybean oil from soybean flakes is based on the use of a SPS in its low polarity form to extract the soybean oil from the flakes, and then switching the solvent to its high polarity form to induce immiscibility with the oil, so that the solvent can be decanted from the oil (Figure 3.3.2.3.).

Figure 3.3.2.3. Schematic for soy oil extraction and separation with SPS.



In this Thesis the extraction system of soybean flakes was developed by investigating the effect of different alcohols in the extraction performance of the SPS, by setting the best extraction conditions, and by comparing SPS efficiency with that of traditional organic solvents (*n*-hexane, chloroform, methanol).

In a typical experiment, samples of grinded soybean flakes were extracted at room temperature without stirring, with each of the following solvent

systems: *i*) *n*-hexane, *ii*) equimolar mixtures of DBU/2-propanol, *iii*) equimolar mixtures of DBU/ethanol *iv*) equimolar mixtures of DBU/octanol, *v*) equimolar mixtures of DBU/diethylene glycol monomethyl ether. After 24 h the residual biomass was filtered off, an aliquot of each extract was submitted to transesterification and then analysed by GC-MS (Table 3.3.2.1.). Soybean flakes samples were also extracted with *n*-hexane and a chloroform/methanol mixture at higher temperature, and the results were compared with the extraction efficiencies obtained at room temperature with SPS and *n*-hexane (Figure 3.3.2.4.).

Table 3.3.2.1. Fatty acid methyl esters yields obtained from soybean flakes samples, calculated on biomass weight basis (24 h, rt).

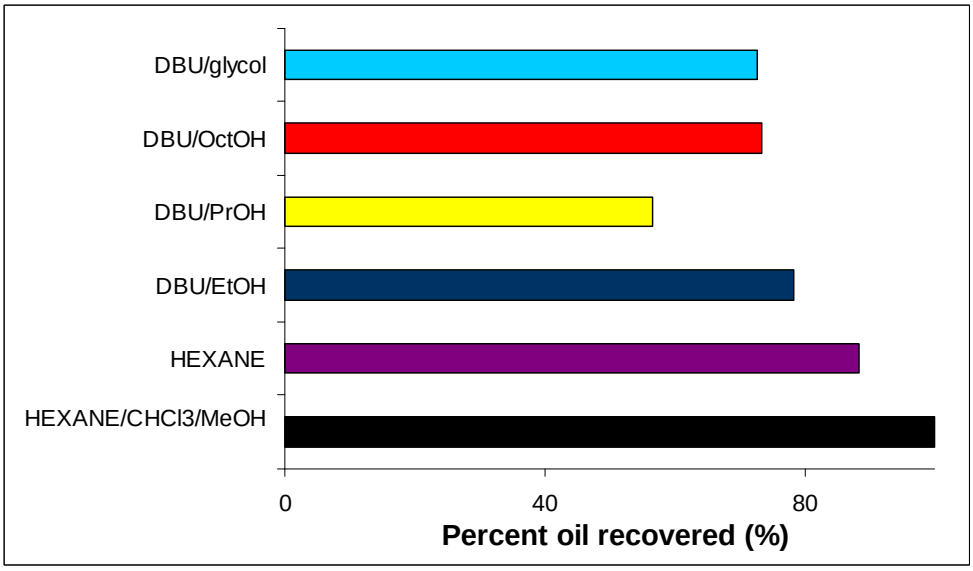
Methyl esters	***n*-hexane**	**DBU/ propanol**	**DBU/ ethanol**	**DBU/ octanol**	**DBU/ glycol**
Palmitic	2.0 ± 0.01%	1.2 ± 0.04%	1.7 ± 0.05%	1.8 ± 0.02%	1.7 ± 0.06%
Linoleic	6.5 ± 0.03%	4.1 ± 0.05%	5.5 ± 0.01%	5.1 ± 0.09%	5.0 ± 0.06%
Oleic	4.0 ± 0.06%	2.7 ± 0.01%	3.8 ± 0.09%	3.3 ± 0.02%	3.4 ± 0.05%
Stearic	0.7 ± 0.07%	0.45 ± 0.08%	0.7 ± 0.02%	0.7 ± 0.05%	0.6 ± 0.01%
Total	13.2 ± 0.7%	8.4 ± 0.2%	11.6 ± 0.4%	10.9 ± 0.5%	10.8 ± 0.4%

As shown in Table 3.3.2.1., the best results in the extraction of fatty acids are obtained with *n*-hexane (13 ± 0.7% total fatty acids yield), followed by DBU/ethanol (12 ± 0.4% yield), by DBU/octanol (11 ± 0.5% yield) and DBU/diethylene glycol monomethyl ether (11 ± 0.4% yield). The results indicate that the length of the alcohol lateral chain does not affect the extraction efficiency, being the SPS with a C-2 alcohol as efficient as the SPS with a C-8 alcohol; moreover also the polarity of the alcohol in the SPS does not affect the efficiency, because there are no differences among the most polar SPS (DBU/diethylene glycol monomethyl ether) and the least polar one (DBU/octanol).

The equimolar mixture DBU/2-propanol is the least efficient extraction system, affords the lowest yields (8 ± 0.2%).

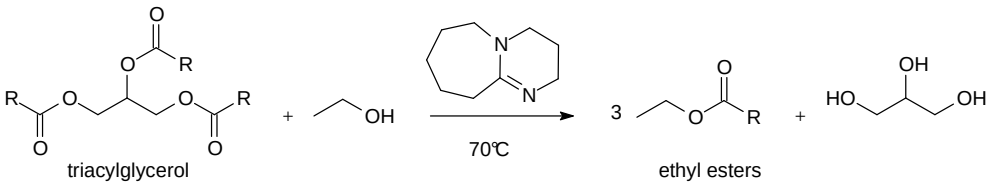
As expected, all the tested solvents show no selectivity among different fatty acids.

Figure 3.3.2.4. Extraction yields of soybean oil from flakes (24 h, rt) with the SPS, DBU/ethanol, DBU/2-propanol, DBU/octanol, DBU/, diethylene glycol monomethyl ether, and *n*-hexane. Percent oil recovery based upon 15% total oil in the flakes extracted with *n*-hexane/chloroform/methanol at 80°C for 4 h.



In his work Jessop[141] extracted the soybean flakes at room and higher temperatures, comparing the extraction efficiency of an equimolar mixture DBU/ethanol with that of *n*-hexane, DBU and ethanol alone. He reported that at room temperature the SPS DBU/ethanol extracted oil more slowly than ethanol or DBU alone, and much more slowly than *n*-hexane control at 60°C, with a recovery of only 45%. By increasing the temperature to 70°C a collateral transesterification of the soy oil extracted by the SPS DBU/ethanol occurred, resulting in the formation of ethyl esters catalysed by DBU (Figure 3.3.2.5.).

Figure 3.3.2.5. Transesterification of soy oil triacylglycerols catalysed by DBU.[141]



In this Thesis, the experiments of soybean flakes extraction were performed at room temperature but for longer time (24 h). The results reported in Figure 3.3.2.4. show that the oil recovery with SPS ranges from 57% (DBU/2-propanol) to 78% (DBU/ethanol), the latter being only slightly lower than *n*-hexane (88%); so an increasing of the extraction time and a decreasing of the extraction temperature, improve the oil recovery and avoid undesirable side transesterification at high temperature.

Extraction of oil from sunflower seeds

The extraction of high valuable vegetable oil from terrestrial biomass was deepened with sunflower seeds. In this set of experiments the SPS DBU/octanol was chosen, and compared with *n*-hexane and octanol alone. Jessop[141] suggested the possibility that water in natural matrixes may interfere with SPS causing a loss in the recovery of the oil probably due to the high water solubility of alcohols as ethanol. For this reasons the SPS DBU/octanol, with water immiscible alcohol was used; moreover it showed very similar efficiency to DBU/ethanol in the extraction of soybean flakes.

Octanol alone was also tested as extractive solvent in order to verify if the addition of DBU to achieve the SPS system, before or after the extraction process, could affect in some way the efficiency of the process itself.

In a typical experiment, samples of grinded sunflower seeds were extracted at room temperature without stirring, with each of the following solvent systems: *i*) *n*-hexane, *ii*) equimolar mixtures of DBU/octanol, *iii*) octanol. After 24 h the residual biomass was filtered off, an aliquot of each extract was submitted to transesterification and then analysed by GC-MS (Table 3.3.2.2.).

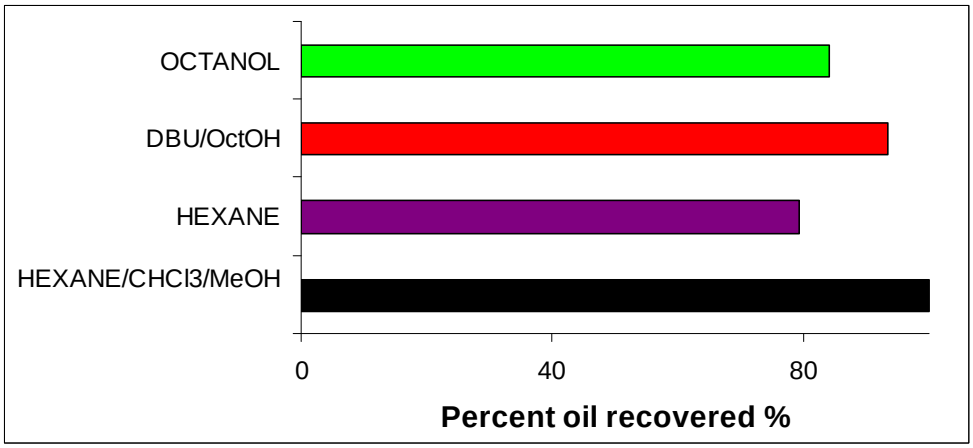
Sunflower seeds samples were also extracted with *n*-hexane and a chloroform/methanol mixture at higher temperature, and the results were compared with the extraction efficiencies obtained at room temperature with SPS, *n*-hexane and octanol (Figure 3.3.2.6.).

Table 3.3.2.2. Fatty acid methyl esters yields obtained with *n*-hexane, octanol, DBU/octanol, from sunflower seeds (24 h, rt), calculated on biomass weight basis.

Methyl esters	*n*-hexane	octanol	DBU/octanol
Palmitic	1.8 ± 0.09%	2.2 ± 0.3%	2.2 ± 0.4%
Linoleic	11.4 ± 0.3%	12.6 ± 1.9%	13.7 ± 3.1%
Oleic	8.6 ± 1%	8.3 ± 0.07%	9.8 ± 2%
Stearic	1.2 ± 0.1%	1.4 ± 0.04%	1.4 ± 0.1%
Total	23 ± 1.6%	24.4 ± 2.2%	27.1 ± 2.4%

As shown in Table 3.3.2.2., the efficiencies of the tested extractive systems are very similar; the best yields of fatty acids are obtained with DBU/octanol (27 ± 2.4% total fatty acids yield), followed by octanol (24 ± 2.2% yield), and by *n*-hexane (23 ± 1.6% yield). The use of octanol as the sole extraction solvent, adding DBU only after the finishing of extraction, does not change the extractive performances of the SPS DBU/octanol.

Figure 3.3.2.6. Extraction yields of sunflower oil from seeds (24 h, rt) with DBU/octanol, octanol and *n*-hexane. Percent oil recovery based upon 29% total oil in the flakes extracted with *n*-hexane/chloroform/methanol at 80°C for 4 h.



The results reported in Figure 3.3.2.6. show that the sunflower oil recovery with DBU/octanol is almost complete (93%), followed by octanol (84% oil recovery) and by *n*-hexane (79%).

Comparing the results with those obtained with soybean flakes, the efficiency of the SPS DBU/octanol is better with sunflower seeds than with

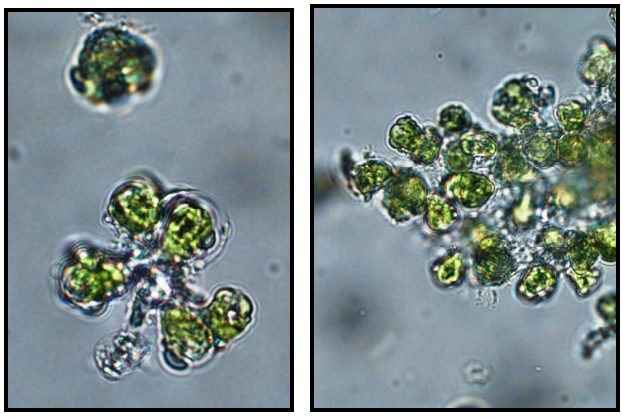
soybean flakes (73%), on the contrary the oil recovery with *n*-hexane is better with soybean flakes (88%) than with sunflower seeds (79%).

These first results obtained with two terrestrial biomasses rich in triacylglycerols have shown that switchable polarity solvents can be a valid alternative to the traditional solvents current in use for the industrial extraction of vegetable oils, being the extraction efficiency very similar.

Extraction of lipids from *Botryococcus braunii*

To deepen the potential of SPS as biomass-oil extractive systems, they have also been used with the microalga *Botryococcus braunii*, to recover an algal oil rich in long unsaturated hydrocarbons. *Botryococcus braunii* in fact is a freshwater colonial green microalga proposed as a future renewable source of fuels because capable to produce high levels of liquid hydrocarbons (Figure 3.3.2.7.).

Figure 3.3.2.7. *Botryococcus braunii*, A strain.



There are three main *B. braunii* races, each one synthesising different types of olefinic hydrocarbons: the A race accumulates linear olefins, odd numbered from C_{23} to C_{31}, chiefly C_{27}, C_{29}, and C_{31} dienes or trienes; the B race produces polyunsaturated triterpenes (botryococcenes), while the L race synthesises one single tetraterpenoid hydrocarbon named lycopadiene.[152]

[152] a) Metzger, P., Casadevall, E., Pouet, M. J., Pouet, Y., 1985. Structures of some botryococcenes: branched hydrocarbons from the b-race of the green alga *Botryococcus braunii*. Phytochemistry. 24, 2995-3002. b) Metzger, P., Berkaloff, C., Casadevall, E.,

Both A and B strains contain similar amounts of lipids (approximately 30% on a dry weight basis) but with a very different composition: in the A strain hydrocarbons, non-polar lipids and polar lipids are respectively 25%, 60% and 15% of the total lipids, whereas in the B strain the percentage are 71%, 9% and 20%. It means that a quarter of the dried biomass of the B strain is composed by hydrocarbons, specifically triterpenoid hydrocarbons, C_{30}–C_{37} botryococcenes and C_{31}–C_{34} methylated squalenes.[158a]

Some studies on the biosynthesis of *B. braunii* hydrocarbons have shown that oleic acid is the direct precursor of dienes and trienes[153] and that decarboxylation of very long chain fatty acid derivatives, activated by a β-substituent, is the final step leading to the formation of the terminal unsaturation.[154]

Specifically for *B. braunii* the bulk of hydrocarbons is located in an external cellular pool and it can be recovered from algal biomass by means of physical (cold press) and chemical processes (extraction with solvents) or both,[155] the latter being more effective.

In this Thesis the lipid extraction efficiency of two SPS systems based on DBU was compared with those of traditional solvents (*n*-hexane, chloroform/methanol) in the extraction of freeze-dried and liquid samples of *B. braunii* A race, kindly donated by the group of algal physiology of CIRSA (Interdepartmental Centre of Research for Environmental Science), Ravenna.

Coute, A., 1985. Alkadiene- and botryococcene-producing races of wild strains of *Botryococcus braunii*. Phytochem., 24, 2305-2312.

[153] Templier, J., Largeau, C., and Casadevall, E., 1987. Effect of Various Inhibitors on Biosynthesis of Non-Isoprenoid Hydrocarbons in Botryococcus braunii. Phytochem., 1987, 26, 377–383.

[154] ChanYong, T.P., Largeau, C., Casadevall, E., 1986. Biosynthesis of non-isoprenoid hydrocarbons by the microalga *Botryococcus braunii*: evidence for an elongation decarboxylation mechanism; activation of decarboxylation. Nouv J Chim., 10, 701–707.

[155] Jae-Yon, L., Chan, Y., So-Young, J., Chi-Yong, A., Hee-Mock, O., 2009. Comparison of several methods for effective lipid extraction from microalgae. Bioresource Technol, in press

Characterisation of the lipid composition of *Botryococcus braunii*

The characterisation of the lipid content of the microalga *B. braunii* was performed by extracting 100 mg of freeze-dried alga with *n*-hexane,[156] and with a chloroform/methanol mixture.[157] The extracted oil was fractionated by column chromatography to obtain hydrocarbons, non-polar and polar lipids (Table 3.3.2.3.); each fraction was submitted to direct silylation (to quantify the amount of free fatty acids) and transesterification to methyl esters (to quantify the amount of triacylglycerols), and finally analysed by GC-MS (Table 3.3.2.4.).

Table 3.3.2.3. Content and Composition of Lipids in A Strains of *B. braunii*.

Lipid		
Content [a]		28.8%
Composition [b]		
	Hydrocarbons	29.5%
	Non-polar lipids	61.8%
	Polar lipids	8.7%

[a] grams per 100 g of dry matter
[b] percentage of total lipids

Table 3.3.2.4. Composition of fatty acids in the non-polar lipids fraction, considered as the sum of free fatty acids, and bounded fatty acids.

Fatty acids	**Free fatty acids**	**Bounded fatty acids**
Palmitic	0.23 ± 0.1%	0.69 ± 0.09%
Linoleic	-	0.17 ± 0.06%
Oleic	0.16 ± 0.2%	1.8 ± 0.1%
Stearic	0.24 ± 0.04%	0.07 ± 0.05%
Total	0.64 ± 0.3%	2.7 ± 0.3%

The results presented in Table 3.3.2.3. are in the same range of the lipids content and composition reported in the literature for this strain.[158] The total

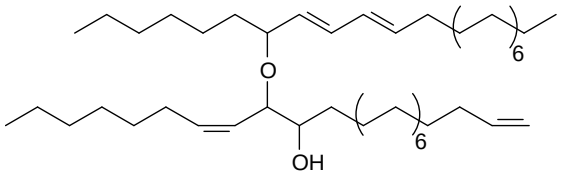
[156]Largeau, C., E. Casadevall, E., Berkaloff, C., Dhmelincourt, P., 1980. Sites of accumulation and composition of hydrocarbons in *Botryococcus braunii*. Phytochem., 19, 1043-1051.
[157] Bligh, E. G., Dyer, W. J., 1959. A rapid method of total lipid extraction and purification. Can. J. Biochem. Physiol., 37, 911-917.
[158]a) Yamaguchi, K., Nakano, H., Murakami, M., Konosu, S., Nakayama, O., Kanda, M., Nakamura, A., Iwamoto, H., 1987. Lipid Composition of a Green Alga, *Botryococcus*

amount of lipids is approximately 30% on a dry weight basis; the main part of the total lipids is represented by a non-polar lipids fraction (61.8%), followed by hydrocarbons (29.5%) and polar lipids (8.7%) fractions.

In addition to hydrocarbons, *B. braunii* also synthesises classic non-polar lipids such as fatty acids, triacylglycerols and sterols, and polar lipids such as non-polysaccharide biopolymers of very high molecular weight (104 Da to 4×106 Da), polyaldehydes and polyacetals.

The results shown in Table 3.3.2.4., in agreement with the literature data, indicate that bounded fatty acids and free fatty acids represent only a very small fraction of non-polar lipids, 2.7% and 0.6% respectively. The main part of the non-polar fraction of A strain lipids[159] is composed by a class of ether lipids closely related to hydrocarbons and not to glycerol; in particular the predominant compound is an alkadienyl-O-alkatrienyl ether with an oxygen bridge between two C_{27} hydrocarbon chains (Figure 3.3.2.8.).[159a]

Figure 3.3.2.8. Characteristic ether lipid of *Botryococcus braunii* A strain.



alkadienyl-O-alkatrienyl ether

In this Thesis the extraction of this kind of ether compounds, in spite their represent the higher amount of non-polar lipid produced by *Botryococcus braunii*, has not been evaluated because of difficult analytical determination by GC-MS. The procedure and the results described below have been focused on GC detectable hydrocarbons and fatty acids.

Extraction of lipids from freeze-dried algae

The choice of the alcohol that forms the carbonate anion in the SPS is a critical point to determine the properties of the obtainable ionic liquid.

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braunii. Agric. Biol. Chem., 51 (2), 493-498. b) Metzger, P., Largeau, C., 2005. *Botryococcus braunii*: a rich source for hydrocarbons and related ether lipids. Appl Microb Biotechnol, 66, 5, 486-496.

[159] a) Metzger, P. and Casadevall, E., 1991. Botryococcoid ethers, ether lipids from *Botryococcus braunii*. Phytochem., 30, 1439. b) Metzger, P., Casadevall, E., 1992. Ether lipids from *Botryococcus braunii* and their biosynthesis. Phytochem., 31, 2341.

Previous works[136,160] have demonstrated that bicarbonate and methyl carbonate DBU salts have melting points lower than room temperature, but DBU salts with longer alkyl chains are liquid at room temperature,[139a] therefore suitable for the extraction process. For this reason, the SPS DBU/ethanol and DBU/octanol were selected for the extraction of algal samples, to obtain, after CO_2 treatment, liquid carbonate (DBU ethyl carbonate and DBU-octyl carbonate have melting points of 35°C and 30°C respectively).[139a]

DBU alone was also tested as extractive solvent in order to verify if the addition of octanol to achieve the SPS system, before or after the extraction process could affect in some way the efficiency of the process itself.

Samples of freeze-dried alga were extracted at 60°C for 4 h with each of the following solvent systems: *i*) *n*-hexane followed by a 2/1 chloroform/methanol mixture, *ii*) DBU, *iii*) equimolar mixtures of DBU/ethanol, or *iv*) equimolar mixtures of DBU/octanol.

After cooling, an aliquot of each extract was spiked with internal standards (hexatriacontane and tridecanoic acid), submitted to silylation and transesterification, and analysed by GC-MS to determine the quantity of extracted hydrocarbons and fatty acids. The yields of total hydrocarbons and of the five major linear olefins ($C_{27}H_{52}$, $C_{29}H_{56}$, $C_{29}H_{54}$ and $C_{31}H_{60}$) and the yields relative to fatty acids (sum of free fatty acids and triacylglycerols) obtained from freeze-dried samples using different solvents are shown in Table 3.3.2.5. and in Table 3.3.2.6. In the case of DBU/ethanol and DBU/octanol the extraction time was also evaluated by GC-MS analysis at increasing time intervals (Figure 3.3.2.9.).

[160] a) Jessop, P. G., Munshi, P., Main, A. D., Linehan, J., Tai, C. C., 2002. Hydrogenation of Carbon Dioxide Catalysed by Ruthenium Trimethylphosphine Complexes: The Accelerating Effect of Certain Alcohols and Amines. JACS, 124 (27), 7963-7971. b) Prez, E. R., Santos, R. H. A., Gambardella, M. T. P., de Macedo, L. G. M., Rodrigues-Filho, U. P., Launay, J., Franco, D. W., 2004. Activation of Carbon Dioxide by Bicyclic Amidines. JOC, 69, 8005–8011.

Table 3.3.2.5. Hydrocarbon percentage yields on a dry weight basis (mean of three replicates ± standard deviation) from freeze-dried samples (60°C, 4 h), obtained with *n*-hexane, DBU, equimolar mixtures of DBU/octanol, and DBU/ethanol.

hydrocarbons	**hexane**	**DBU**	**DBU/octanol**	**DBU/ethanol**
$C_{27}H_{52}$	0.65 ± 0.2%	1.4 ± 0.6%	1.5 ± 0.5%	1.2 ± 0.3%
$C_{29}H_{56}$	2.8 ± 1%	5.3 ± 1%	5.6 ± 2%	4.8 ± 1%
$C_{29}H_{54}$	0.57 ± 0.3%	0.52 ± 0.70%	1.5 ± 0.8%	0.10 ± 0.2%
$C_{29}H_{54}$	1.4 ± 0.7%	3.59 ± 1.86%	3.1 ± 0.3%	2.8 ± 0.1%
$C_{31}H_{60}$	2.5 ± 1%	4.3 ± 1%	4.4 ± 0.5%	3.6 ± 0.7%
Total	7.8 ± 3%	15 ± 6%	16 ± 2%	12 ± 2%

As shown in Table 3.3.2.5., the best results in the extraction of hydrocarbons are obtained with the equimolar mixture DBU/octanol (16 ± 2% total hydrocarbons yield), followed by DBU alone (15 ± 6% yield), and by DBU/ethanol (12 ± 2% yield). As described for the extraction experiments of vegetable oils rich in triacylglycerols, the SPS DBU/octanol and DBU/ethanol show almost similar efficiencies; however in the case of an algal oil rich in hydrocarbons, the higher yields obtained with DBU/octanol than with DBU/ethanol can be ascribed to a major similarity of the apolar matrix with an apolar alcohol as octanol.

The two SPS are both better than *n*-hexane, that affords the lowest yields (7.8 ± 3%), after an extraction process performed at the same temperatures and after the same time interval (60°C and 4 h).

As expected, all the tested solvents show no selectivity among different olefins.

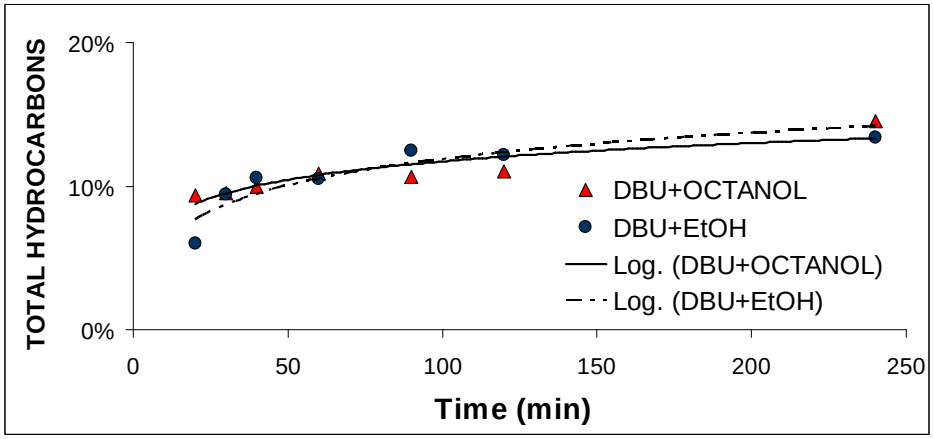
Table 3.3.2.6. Fatty acid methyl esters percentage yields on a dry weight basis (mean of three replicates ± standard deviation) from freeze-dried samples (60°C, 4 h), obtained with *n*-hexane/chloroform/methanol, DBU, equimolar mixtures of DBU/octanol, and DBU/ethanol.

Methyl esters	**hexane/ $CHCl_3$/MeOH**	**DBU**	**DBU/octanol**	**DBU/ethanol**
Palmitic	0.69 ± 0.09%	0.15 ± 0.03%	0.17 ± 0.01%	0.11 ± 0.08%
Linoleic	0.17 ± 0.06%	0.02 ± 0.01%	-	0.10 ± 0.04%
Oleic	1.8 ± 0.1%	0.36 ± 0.08%	0.39 ± 0.03%	0.34 ± 0.2%
Stearic	0.07 ± 0.05%	0.05 ± 0.04%	0.11 ± 0.08%	0.04 ± 0.02%
Total	2.7 ± 0.3%	0.56 ± 0.2%	0.67 ± 0.1%	0.59 ± 0.2%

The yields relative to fatty acids, presented in Table 3.3.2.6. are rather low with DBU (0.6 ± 0.2%) as well as with the two switchable mixtures (0.7 ± 0.1% DBU/octanol and 0.6 ± 0.2% DBU/ethanol). These extracting media are less efficient than a *n*-hexane/chloroform/methanol mixture (2.7 ± 0.3% yield). No selectivity towards a specific fatty acid is observed in any case.

The possibility that the presence of free fatty acids in the algal oil could affect the separation process was also taken into account. Free fatty acids in fact could protonate DBU and be preferentially retained in the extraction mixture as carboxylates forming an ion-pairing with protonated DBU, causing a poor separation after CO_2 addition. In the case of the A strain of *Botryococcus braunii*, the percentage of free fatty acids is 0.6% on a dry weight basis (according to data of Yamaguchi et al.[158a]), irrelevant if compared with the amount of DBU used for the extraction, eventually reactable with the free acids (about 0.18 mg of free fatty acids respect to 1 g of DBU).

Figure 3.3.2.9. Hydrocarbons extraction efficiency of freeze-dried algal samples with time using equimolar mixtures of DBU/octanol (▲), and DBU/ethanol (●). Dots and dashes curve, and continuous line represent the logarithmic regression of DBU/octanol and DBU/ethanol data respectively.



The effect of alcohol chain length on the rate of extraction was also investigated by determining the extraction yields at selected time intervals (Figure 3.3.2.9.). DBU/octanol and DBU/ethanol exhibit approximately the same behaviour, with similar hydrocarbons amount extracted after 240 min (14% and 13% yields respectively). The hydrocarbons extraction with DBU/octanol after 20 minutes is 65% of the yield at 240 min, indicating that the process is quite efficient from the beginning.

Extraction of lipids from algal cultures

The direct extraction from algal cultures was performed with *n*-hexane, octanol and equimolar DBU/octanol mixture; in each extraction system, the solvent layer was stratified over the water phase, avoiding the direct contact of the extraction agent with the algal cells. The extraction with DBU/octanol from cultures required a previous adjustment of the pH to alkaline conditions to achieve phase separation.

The extractions were performed at room temperature, centrifuging the samples at different speeds, simulating a high (3000 rpm) and a low energy (300 rpm) liquid/liquid extraction process from the growth medium.

An aliquot was withdrawn from *B. braunii* resuspended cultures and added to the extraction medium. Then the biphasic system was centrifuged (no stirring) at 300 rpm for 2 or 24 h, or at 3000 rpm for 4 h. The following extracting media were tested: *i*) *n*-hexane, *ii*) octanol, *iii*) equimolar mixture

of DBU/octanol. In the latter case, the pH of the aqueous phase was previously brought to 13 with KOH, to ensure the complete separation of DBU from water.

Octanol alone was tested as extractive solvent in order to verify if the addition of DBU to achieve the SPS system, before or after the extraction process, could affect in some way the efficiency of the process itself.

Centrifuging was chosen as the best procedure to obtain a clearer separation (useful for small samples operation) of the aqueous and organic phases. This method allows to avoid vigorous stirring that should end up into an untreatable foaming, still maintaining a good extraction efficiency.

The water content in the upper organic phase, after the adjusting of the pH of the aqueous phase with KOH, was 7% (g H_2O/100 mL SPS, Karl Fischer titration). To avoid the formation of DBU bicarbonate, a further step to remove the residual water was necessary before treating the SPS with CO_2. Water was removed from DBU/octanol by bubbling N_2 for 30 min at room temperature; after this time the residual H_2O in the system was less than 0.1%, without any loss of the organic components.

An aliquot of the upper organic phase was spiked with hexatriacontane as internal standard, and analysed by GC-MS to determine the quantity of extracted hydrocarbons. The extraction yields after 2 h and 24 h of centrifugation at 300 rpm, and after 4 h at 3000 rpm, are shown in Table 3.3.2.7., Table 3.3.2.8. and Table 3.3.2.9. respectively.

Table 3.3.2.7. Hydrocarbon extraction percentage efficiency calculated on a dry weight basis (mean of three replicates ± standard deviation) from liquid culture samples (300 rpm, rt, 2 h), with octanol, *n*-hexane, and equimolar mixture of DBU/octanol.

Hydrocarbons	**octanol**	**hexane**	**DBU/octanol**
$C_{27}H_{52}$	0.08 ± 0.02%	-	0.12 ± 0.05%
$C_{29}H_{56}$	0.19 ± 0.04%	0.16 ± 0.06%	0.91 ± 0.3%
$C_{29}H_{54}$	-	-	-
$C_{29}H_{54}$	0.37 ± 0.03%	0.17 ± 0.09%	0.72 ± 0.3%
$C_{31}H_{60}$	0.58 ± 0.1%	0.3 ± 0.2%	0.75 ± 0.6%
Total	1.2 ± 0.4%	0.6 ± 0.02%	2.5 ± 0.6%

Table 3.3.2.8. Hydrocarbon extraction percentage efficiency calculated on a dry weight basis (mean of three replicates ± standard deviation) from liquid culture samples (300 rpm, rt, 24 h), with octanol, *n*-hexane, and equimolar mixture of DBU/octanol.

Hydrocarbons	octanol	hexane	DBU/octanol
$C_{27}H_{52}$	0.24 ± 0.2%	0.11 ± 0.1%	0.60 ± 0.05%
$C_{29}H_{56}$	1.4 ± 0.5%	1.3 ± 0.6%	2.9 ± 1%
$C_{29}H_{54}$	-	0.55 ± 0.4%	0.25 ± 0.1%
$C_{29}H_{54}$	1.2 ± 0.4%	1.8 ± 0.8%	2.3 ± 0.9%
$C_{31}H_{60}$	1.6 ± 0.1%	1.9 ± 0.6%	2.3 ± 0.8%
Total	4.4 ± 0.4%	5.6 ± 1%	8.2 ± 1%

Table 3.3.2.9. Hydrocarbon extraction percentage efficiency calculated on a dry weight basis (mean of three replicates ± standard deviation) from liquid culture samples (3000 rpm, rt, 4 h), with octanol, *n*-hexane, and equimolar mixture of DBU/octanol.

Hydrocarbons	octanol	hexane	DBU/octanol
$C_{27}H_{52}$	-	0.33 ± 0.2%	0.51 ± 0.3%
$C_{29}H_{56}$	0.98 ± 0.4%	1.3 ± 0.3%	2.2 ± 0.1%
$C_{29}H_{54}$	0.25 ± 0.1%	0.37 ± 0.07%	0.25 ± 0.1%
$C_{29}H_{54}$	0.65 ± 0.4%	1.4 ± 0.3%	2.0 ± 0.4%
$C_{31}H_{60}$	2.6 ± 0.2%	1.7 ± 0.05%	2.3 ± 0.1%
Total	4.4 ± 0.5%	5.1 ± 0.7%	7.0 ± 0.8%

Although the extraction process is somewhat sluggish, the equimolar mixture DBU/octanol after 24 h at 300 rpm gives 8.2% hydrocarbon yield (Table 3.3.2.8.), slightly higher than what obtainable from freeze-dried sample with *n*-hexane under reflux (7.8% yield) but inferior to the yield achieved with DBU/octanol on freeze-dried samples (16%).

Similarly to what observed with dried samples, the best extractive solvent for liquid samples is the switchable mixture DBU/octanol (8.2 ± 1% yield), followed by *n*-hexane and octanol with 5.6 ± 1% and 4.4 ± 0.4% yield respectively, confirming a positive role of DBU in the extraction process when mixed with octanol.

The effect of the centrifugation speed was also evaluated: for all the tested solvents, at higher rate (3000 rpm, Table 3.3.2.9.) the extraction results

faster, obtaining in 4 h approximately the same yields (DBU/octanol 7.0 ± 0.8% yield, *n*-hexane 5.1 ± 0.7% and with 4.4 ± 0.5% octanol) allowed to obtain at 300 rpm for 24 h (Table 3.3.2.8.). This can be explained by the fact that by raising the centrifuge rate, less dense algae (with higher hydrocarbon content) will move quickly to the top of the water phase and release the hydrocarbons in the upper organic layer by contact.

Recovery of hydrocarbons with DBU/octanol system

A complete non-ionic/ionic cycle to recover the extracted hydrocarbons was performed.

After the extraction of freeze-dried algae with DBU/octanol was completed and analysed by GC-MS, the algal biomass was filtered off and CO_2 was bubbled into the stirred solution at 40°C for 1 h. Upon formation of DBU-octyl carbonate salt, and separation of a hydrocarbon layer at the top of the ionic liquid phase, *n*-hexane was added to the surface layer without mixing, and the resulting *n*-hexane solution was decanted and subjected to GC-MS analysis, to verify the degree of contaminations with DBU and octanol of the algal oil. Then, the DBU-octyl carbonate mixture was heated at 60°C, while N_2 bubbled through the solution, to convert back the system into the non-ionic form, which was subjected to GC-MS analysis, to verify the degree of hydrocarbon recovery.

The efficiency of a non-ionic/ionic cycle in recovering pure hydrocarbons is about 81% of the total amount of hydrocarbons extracted, with 8.1% of hydrocarbons retained in the ionic SPS phase in the second half of the cycle, and about a 10% mechanical loss probably due to the small size of the sample. The results indicate that the ionic form retains a rather small amount of the hydrocarbons extracted with the equimolar mixture DBU/octanol and this amount can be still reduced by increasing the size of the extracted sample.

The GC-MS analysis of the hydrocarbons obtained after the procedure described above indicates that the oil still contained small amounts of octanol (0.3%) and DBU (0.4%); bubbling extra CO_2 for 1 hour at 40°C decreases the levels of contamination to undetectable values because of the precipitation of all the ionic liquid from the oil.

Conclusions

The potential role of switchable polarity solvents as a green technology for the extraction of vegetable oil from terrestrial and aquatic biomass has been investigated.

The extraction efficiency of terrestrial biomass rich in triacylglycerols, as soy bean flakes and sunflower seeds, was comparable to those of traditional organic solvents, being the yield of vegetable oils recovery very similar.

Switchable polarity solvents as been also exploited for the first time in the extraction of hydrocarbons from the microalga *Botryococcus braunii*, demonstrating the efficiency of the process for the extraction of both dried microalgal biomass and directly of the aqueous growth medium. The DBU/octanol system exhibited better extraction efficiency than conventional solvents, both with dried and liquid samples. This is an important issue considering that the harvest and the dewatering of algal biomass have a large impact on overall costs and energy balance.

Besides the efficiency in the hydrocarbons extraction, SPS have the advantage to be recyclable non-volatile/non-inflammable systems, therefore suited for-non-hazardous small plants for biofuel production located nearby algal cultivation sites.

Experimental section

Chemicals

DBU (Aldrich, 98 % grade) was distilled (b.p. = 82°C, 0.8 mbar) and stored under N_2. All other solvents and chemicals were used as received from Aldrich. Supercritical grade CO_2 (99.95 %, H_2O < 20 ppm), nitrogen (99.998 %, H_2O < 3 ppm) were used as received from Air Liquide.

Soybean flakes and sunflower seeds were finely gridded before use.

Botryococcus braunii A race (SAG 807-1) was obtained from the Algal Culture Centre, University of Göttingen, Germany. SAG 807-1 was originally isolated from a water sample collected in Madingly Brick Pits, Cambridge, by M. R. Droop in 1950.

The alga was grown in a modified Chu13 medium (Largeau et al., 1980) containing the following components (g/l): KNO_3 (0.2), K_2HOP_4 (0.04),

$MgSO_4 \cdot 7H_2O$ (0.1), $CaCl_2 \cdot 6H_2O$ (0.08), Fe citrate (0.01), citric acid (0.1); micro elements: B, Mn (both at 0.5 ppm), Zn (0.05 ppm), Cu, Co, Mo (0.02 ppm). The pH was adjusted to 7.5 with KOH before autoclaving. Culture vessel (2l Erlenmeyer flask) was aerated and thermostatically controlled at 20°C in a shaking incubator under 120 $\mu Em^{-2}s^{-1}$ light intensity with 16-h light : 8-h dark cycle. The aliquot of the culture used for the extractions was routinely harvested, by centrifugation, at the beginning of the stationary phase. The dry weight of algal biomass was determined gravimetrically; aliquots of 10 mL algal suspension were filtered through a pre-weighted, pre-combusted (60°C, 24 h) glass fibre filter (Whatman GF/F, 47 mm, nominal pore size 0.7 µm). The filters were then dried at 60°C to a constant weight.

Extraction of oil from soybean flakes

Samples of soybean flakes (40 mg) were extracted at room temperature for 24 h with each of the following solvent systems: *i*) *n*-hexane (0.6 mL), *ii*) equimolar mixtures of DBU/2-propanol (2 mmol, 0.299 mL/0.153 mL), *iii*) equimolar mixtures of DBU/ethanol (2 mmol, 0.299 mL/0.116 mL), *iv*) equimolar mixtures of DBU/octanol (2 mmol, 0.299 mL/0.315 mL), *v*) equimolar mixtures of DBU/ diethylene glycol monomethyl ether (2 mmol, 0.299 mL/0.235 mL).

As control, samples were also extracted with *n*-hexane (4 mL) followed by a mixture 2/1 of chloroform/methanol (4 mL), at 80°C for 4 h.

After cooling, the residual biomass was filtered off, an aliquot of each extract was spiked with internal standard diluted with *n*-hexane and analysed by GC-MS. Tridecanoic acid was utilised as internal standard to quantify free fatty acids and total fatty acids, sum of free fatty acids and triacylglycerols, assuming an unitary response factor relative to tridecanoic acid.

Extraction of oil from sunflower seeds

Samples of sunflower seeds (40 mg) were extracted at room temperature for 24 h with each of the following solvent systems: *i*) *n*-hexane (0.6 mL), *ii)* octanol (0.6 mL), *iii*) equimolar mixtures of DBU/octanol (2 mmol, 0.299

mL/0.315 mL), *v*) equimolar mixtures of DBU/diethylene glycol monomethyl ether (2 mmol, 0.299 mL/0.235 mL).

As control, samples were also extracted with *n*-hexane (4 mL) followed by a mixture 2/1 of chloroform/methanol (4 mL), at 80°C for 4 h.

After cooling, the residual biomass was filtered off, an aliquot of each extract was spiked with internal standard diluted with *n*-hexane and analysed by GC-MS. Tridecanoic acid was utilised as internal standard to quantify free fatty acids and total fatty acids, sum of free fatty acids and triacylglycerols, assuming an unitary response factor relative to tridecanoic acid.

Extraction, fractionation and analysis of *Botryococcus braunii* lipids.

In a typical procedure (with slight modifications)[158a], 100 mg of freeze-dried alga was extracted twice with *n*-hexane at 60°C (2 mL and 2 h each time), and with a chloroform/methanol mixture at 80°C (2/1, 2 mL and 2 h each time), filtered, collected and evaporated under vacuum. The total lipids (l00 mg) were fractionated on a chromatographic column packed with silica gel using 250 mL of hexane, 150 mL of chloroform, and 150 mL of methanol in this order to isolate hydrocarbons, nonpolar lipids except hydrocarbons and polar lipids, respectively. The isolated lipids in each eluate were measured gravimetrically after evaporation of the solvent.

The *n*-hexane fraction, composed by the four main long chain alkenes $C_{27}H_{52}$, $C_{29}H_{56}$, $C_{29}H_{54}$ and $C_{31}H_{60}$ which represented the calibration solution for GC-MS analysis, was used to determine the GC-MS response factor relative to hexatriacontane (Sigma-Aldrich).

The chloroform fraction, spiked with tridecanoic acid as internal standard, was submitted to silylation and transesterification to quantify free fatty acids and total fatty acids, sum of free fatty acids and triacylglycerols, assuming an unitary response factor relative to tridecanoic acid.

Extraction of lipids from freeze-dried *Botryococcus braunii* samples

Samples of freeze-dried alga (30 mg) were extracted at 60°C for 4 h with each of the following solvent systems: *i*) *n*-hexane (4 mL) followed by a mixture 2/1 of chloroform/methanol (4 mL), *ii*) DBU (3 mL), *iii*) equimolar

mixtures of DBU/ethanol (6.6 mmol, 1 mL/0.388 mL), *iv*) or DBU/octanol (6.6 mmol, 1 mL/1 mL).

After cooling, an aliquot of each extract was spiked with internal standards (hexatriacontane and tridecanoic acid), diluted with *n*-hexane and analysed by GC-MS.

Extraction of lipids from *Botryococcus braunii* cultures

An aliquot (3 mL) was withdrawn from cultures at concentration of 0.8 g algal dry weight/l and added to the extraction medium in a centrifuge tube, without stirring and at room temperature. The biphasic system was then centrifuged at 300 rpm for 2 or 24 h, or at 3000 rpm for 4 h. The following extracting media were tested: *i*) *n*-hexane (3 mL), *ii*) octanol (3 mL), *iii*) equimolar mixture of DBU/octanol (9.9 mmol, 1.5 mL/1.5 mL). In the latter case, the pH of the aqueous phase was brought to 13 with KOH, to allow the complete separation of DBU from water.

The upper organic phase was collected and dried at room temperature under N_2 (1 l min^{-1}) for about 30 min, until the water content was less than 0.1% by Karl Fischer titration.

An aliquot of the upper organic phase was spiked with hexatriacontane as internal standard, diluted with *n*-hexane and analysed by GC-MS.

GC-MS analysis

B. braunii hydrocarbons were analysed without any pre-treatment. The amount of free fatty acids in soybean flakes, sunflower seeds and algal samples was obtained by silylation and GC-MS analysis. 5 mg of the extract were spiked with 100 µl of internal standard solution, 200 µl of bis-trimethylsilyl-trifluoro-acetamide containing 1 % trimethyl-chlorosilane and 10 µl of pyridine.

The amount of total fatty acids (sum of free and triacylglycerols) in soybean flakes, sunflower seeds and algal samples was obtained by transesterification into the corresponding methyl esters (FAMEs). The extract was refluxed for 10 min with 4 mL methanolic NaOH 0.5 M; then 5 ml of $MeOH/BF_3$ were added and the mixture refluxed for 2 min followed by the addition of 10 mL of *n*-hexane. After cooling, a saturated NaCl

aqueous solution was added under stirring, then the hexane layer containing FAMEs was separated, dried over anhydrous sodium sulfate and diluted prior to GC–MS analysis.[161]

Analyses were performed by a 6850 Agilent HP gas chromatograph connected to a 5975 Agilent HP quadrupole mass spectrometer. Analytes were separated by a HP-5 fused-silica capillary column (stationary phase poly[5 % diphenyl/95 % dimethyl]siloxane, 30 m, 0.25 mm i.d., 0.25 mm film thickness) using helium as carrier gas (at constant pressure, 33 cm s^{-1} linear velocity at 200°C). Measurement solutions were injected under splitless condition with injector temperature set at 280°C. Mass spectra were recorded under electron ionisation (70 eV) at a frequency of 1 scan s^{-1} within the 12–450 *m/z* range. The following thermal program was used: 130°C for three minutes, then 10°C/min up to 300°C and hold for 10 min.

The quantity of extracted compounds was reported as percentage yields of the extracted biomass. This latter figure was calculated on the weight of soybean flakes and sunflower seeds samples, on a dry weight basis in the case of freeze-dried algal samples, and on the algal biomass content of the liquid samples following filtration in the case of cultures. Reported yields were averaged from three replicate extractions.

[161] Fabbri, D., Baravelli, V., Chiavari, G., Prati, S., 2005. Profiling fatty acids in vegetable oils by reactive pyrolysis–gas chromatography with dimethyl carbonate and titanium silicate. J. Chromatogr. A. 1100, 218–222.

4. Fluorous solvents and supercritical fluids

In this Thesis the analysis of alternative solvent systems as "greener" replacement for traditional organic solvents has been focused on ionic liquids (described above in Chapter 3), fluorous solvents and supercritical fluids, described below, mainly by taking into account their applicability as reaction media in biocatalysis.

4.1. Fluorous solvents

Properties and applications

'Fluorous' was the term coined for highly fluorinated (or perfluorinated) solvents by Horvath,[162] in an analogous way to "aqueous" for water-based systems. Perfluorinated compounds (PFCs) have been produced since the 1950s and used for many industrial applications as cosmetics, fire fighting foams, food packaging, water and grease repellents, coatings for fabrics. This widespread use is due to PFCs unique physical-chemical properties, and in particular to a better effectiveness and a higher chemical and thermal stability than non-fluorinated chemicals, correlated to the strength, the chemical inertness and the low polarizability of C-F bonds.

Nowadays fluorine chemistry has also an important role in clean technology, both in catalysis and in solvents replacement fields. Several applications in organic chemistry involve perfluoroalkanes like FC-72 (a mixture of perfluorohexanes) or PFMC (perfluoromethylcyclohexane), which are extraordinarily non-polar and form biphasic mixtures with many organic solvents or with water. However fluorous solvents commonly exhibit temperature-dependent miscibility (thermomorphism) with organic solvents (Table 4.1.1.), and it means that they can be used in fluorous/organic biphasic systems (FBS) in which catalysts, with high affinity for fluorous solvents, and organic reagents, soluble in the organic solvents, can react in one-phase at higher temperature and separated in a biphasic system at lower temperature. In this sense fluorous solvents can be considered as switchable, as the switchable polarity solvents described in Chapter 3.2.2. of the Thesis, being their solubility simply switchable by

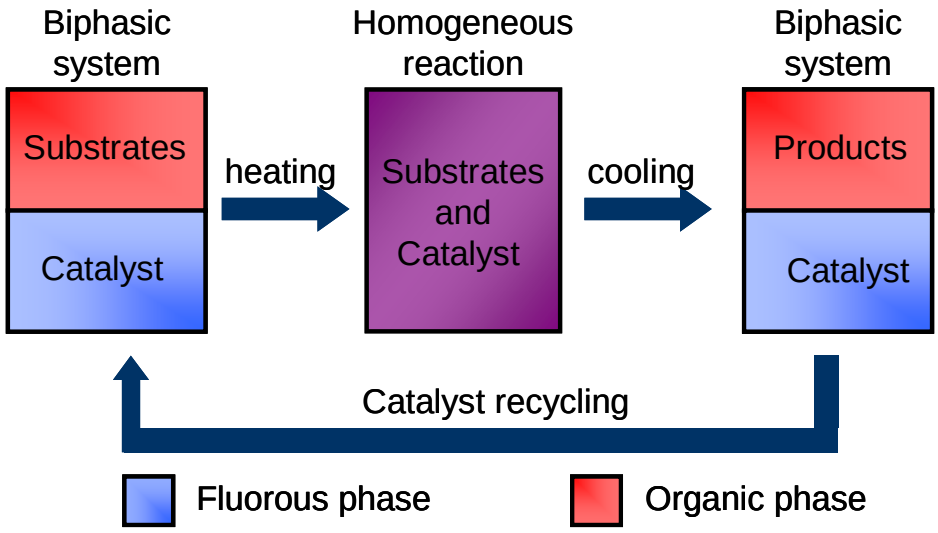
[162] Horvàth, I., Ràbai, J., 1994. Facile Catalyst Separation Without Water: Fluorous Biphase Hydroformylation of Olefins. Science, 266, 72 – 75.

changing the temperature. This peculiar feature has led to the development of fluorous biphasic chemistry, in which the efficiency and the recycling of the system are improved by a better and easier separation of products from catalysts (Figure 4.1.1.).

Table 4.1.1. Temperature-dependent miscibility of some perfluorinated solvents in a 1:1 ratio with organic solvents.[163]

	Two phases at (°C)	One phase at (°C)
PFMC/CCl_4	rt	> 26.7
PFMC/$CHCl_3$	rt	> 50.1
PFMC/hexane	0	rt
PFMC/Et_2O	0	rt
FC-72/hexane	0	> 24

Figure 4.1.1. Fluorous biphasic system (FBS) and their "green" potential in catalytic reactions.

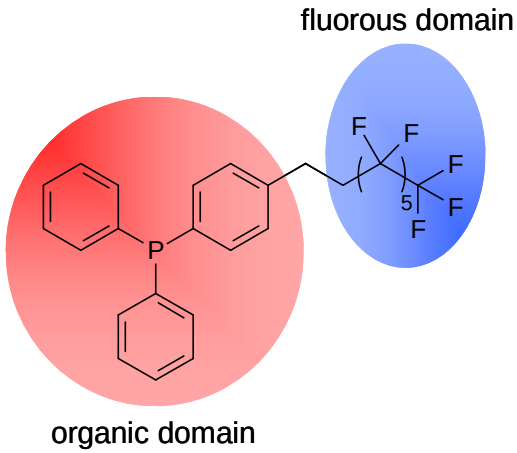


The main strategy for the best exploiting of the FBS potentiality in catalysis is based on the principle "like dissolves like", improving the catalysts affinity for fluorous solvents thanks to the introduction of fluorous moiety

[163] a) Hildebrand, J.H., Cochran, D.R.F. 1949. Liquid-Liquid Solubility of Perfluoromethylcyclohexane with Benzene, Carbon Tetrachloride, Chlorobenzene, Chloroform and Toluene. JACS, 71, 22-25.; b) Gladysz, J. A., Curran, D. P., Horva´th, I., 2004. Handbook of Fluorous Chemistry. Wiley-VCH Verlag GmbH & Co. KGaA: Weinheim, Germany; c) Bedford, R. G., Dunlap, R. D., 1958. Solubilities and Volume Changes Attending Mixing for the System: Perfluoro-n-hexane-n-Hexane JACS, 1958, 80, 282-285.

(e.g. fluorous ponytails). In this way the catalyst can incorporate an "organic" domain which controls the reactivity of the molecule, and a "fluorous" domain useful for the separation. Triphenylphosphines for example (Figure 4.1.2.) have been modified by introducing fluorinated ponytails and used to catalyse hydroboration[164] or hydroformilation[165] of alkenes in FBS.

Figure 4.1.2. Fluorous triphenylphosphine as catalyst in FBS.



Also with the enzymes it is possible to introduce a fluourous domain in the catalyst, for example through the technique of the hydrophobic ion-pairing (HIP) which creates an ionic couple between an anionic fluorous surfactant and the basic amino acids residues of enzymes (as it will be described in Chapter 4.3.1.).

The use of fluorous catalysts also finds applications in supercritical CO_2 systems because of the high solubility of fluorinated compounds in supercritical phase. The reason of this compatibility is not well understood, although there is some evidence that specific interactions between the fluid and the fluorous group may exist.[166]

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[164] Juliette, J., Gladysz, J., Horváth, I., 1997. Transition Metal Catalysis in Fluorous Media: Practical Application of a New Immobilization Principle to Rhodium-Catalyzed Hydroboration. Angew Chem Int Ed., 36, 15, 1610-1612.

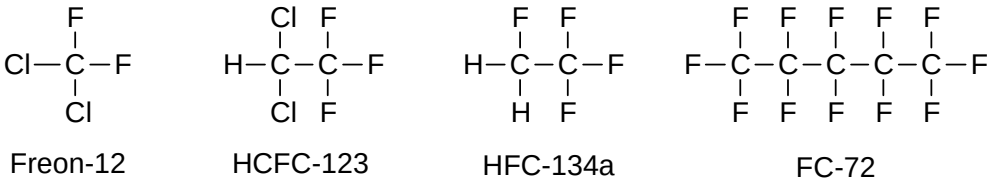
[165] Horváth, I., Kiss, G., Cook, R., Bond, J., Stevens, P., Rábai, J., Mozeleski, E., 1998. Molecular Engineering in Homogeneous Catalysis: One-Phase Catalysis Coupled with Biphase Catalyst Separation. The Fluorous-Soluble $HRh(CO)\{P[CH_2CH_2(CF_2)_5CF_3]_3\}_3$ Hydroformylation System. JACS, 120, 13, 3133-3143

[166] McHugh, M., Park, I., Reisinger, J., Ren, Y., Lodge, T., Hillmyer, M., 2002. Solubility of CF_2-Modified Polybutadiene and Polyisoprene in Supercritical Carbon Dioxide. Macromolecules, 35, 4653

Environmental and toxicological aspects

In spite of the large number of applications in the field of the clean technologies, fluoro-chemicals have also been involved in some of the most negative aspects of the past chemical industry. One of the first uses of fluorinated compounds was as alternative of chlorofluorocarbons (CFCs), phased out by the Montreal Protocol in 1987 because of their contribution to stratospheric ozone depletion. CFCs were firstly replaced by hydrochlorofluorocarbons (HCFCs) in refrigeration and blowing agent applications, and then by hydrofluorocarbons (HFCs) (Figure 4.1.3.).

Figure 4.1.3. Examples of the most known CFCs, HCFCs, HFC and PFCs.



HFCs are a less harmful alternative to CFCs because they are chlorine-free and have no significant contribution to ozone destruction. However, HFCs and PFCs can not be considered as totally environmental friendly because of some negative aspects:

- they may act as greenhouse gases because of their large global warming potential (GWP); HFCs and PFCs absorb IR energy in the window 8-12 μm which is largely transparent in the natural atmosphere. Moreover their persistence in the environment is very high because of C-F bond strength, and the consequent stability, which is especially appreciated when perfluorinated compounds are used as solvents, assumes a negative role in terms of degradation. PFCs are largely immune to the oxidative chemical processes in the lower atmosphere that break down most atmospheric pollutants, and the removal mechanism by UV radiation in the mesosphere is extremely slow. As result PFCs accumulate in the atmosphere and remain there for several thousand years;
- their synthesis involves large quantities of fluorine and hydrogen fluoride, very corrosive and harsh. There are two main processes for the industrial synthesis of HFCs and PFCs, and in both of them the amount

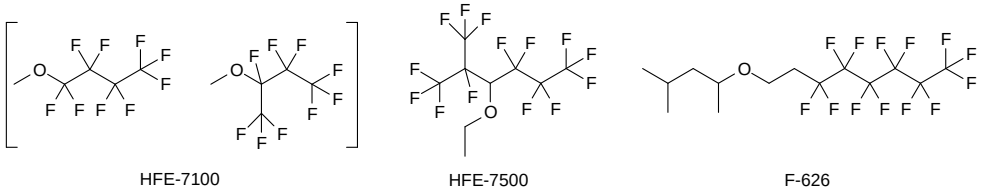
of fluorinated agents is very high. The first is the electrochemical fluorination, developed for the first time by Simons,[167] in which a constant electric current is passed through an electrolyte solution containing a mixture of liquid anhydrous hydrogen fluoride and an organic compound which has to be fluorinated (substrate), causing the replacement of the hydrogen atoms of the substrate by fluorine; the second is the telomerisation, developed for the first time by Du Pont,[168] and involves the radical polymerisation of the monomer tetrafluoroethylene with perfluoroalkyl iodides (e.g. pentafluoroethyl iodide);

- their resistance to biodegradation and the potential to accumulate in organisms and to biomagnificate. All of the known biologically produced fluorinated molecules contain only one fluorine atom, in contrasts with many man-made PFCs which are fully fluorinated. Natural fluorous compounds, such as monofluoroacetate and fluoro-organic acids (e.g. fluorooleic and fluoropalmitic acids), produced by different plants species, can rarely undergo to the direct breaking of the carbon-fluorine bond and, more often, the functional groups or bonds attached to the fluorinated moiety are involved in the biodegradation. Analogously PFCs can undergo in the environment to the same abiotic and biotic transformations, becoming precursors of more persistent perfluorinated compounds, such as perfluorooctane sulfonate (PFOS) and perfluorooctanoate (PFOA), which do not breakdown further. In addition both PFOS and PFOA are very bioaccumulative and toxic, and that is why they are classified as POP[169] (persistent organic pollutants) by the Stockholm Convention;[170]
- the high costs of fluorous solvents limit their industrial interest.

[167] Simons, J.H., 1949. Production of fluorocarbons. J. Electrochem Soc., 95, 47-64.
[168] a) Brace, N.O., (DuPont), 1961. US 3 145 222; b) Blanchard, W.A., Rhode, J.C., (Du Pont), 1965. US 3 226 449.
[169] European Union, 2006, Directive 2006/122/ECOF of the European Parliament and of the council of 12 December 2006. Official Journal of the European Union, L/372/32-34, 27.12.2006
[170] Council Decision 2006/507/EC of 14 October 2004 concerning the conclusion, on behalf of the European Community, of the Stockholm Convention on Persistent Organic Pollutants (OJ L 209, 31.7.2006, p. 1).

One “greener” alternative to perfluorinated compounds is represented by hydrofluoroethers (HFEs), which have been recently introduced as the fluorous phase of a biphasic system with different miscibility properties.[171] The commercially available HFEs,[172] such as HFE-7100, HFE-7500 and F-626 (Figure 4.1.4.), show a higher polarity than PFC because of the introduction of one oxygen atoms between a fluoroalkyl group and alkyl group.

Figure 4.1.4. Examples of the most known HFEs.



HFEs are often less expensive than their fluorocarbon cousins and they are also more environmentally friendly because of considerably lower persistency (shorter atmospheric lifetimes) and global warming potential.

As observed with many hydrocarbons, the insertion of ether oxygen makes the hydrogen atoms more labile and increases the rate of reaction with OH radicals. The same reactivity can be observed with HFEs and PFCs; in particular in HFE-7100 or HFE-7500, in which the oxygen atoms are located between the fluoroalkyl group and the alkyl group, all the hydrogen atoms are on carbons with no fluorine substitutions and this structure reduces the atmospheric lifetimes (Table 4.1.2.).

[171] a) Matsubara, H., Yasuda, S., Sugiyama, H., Ryu, I., Fujii, Y., Kita, K., 2002. A new fluorous/organic amphiphilic ether solvent, F-626: execution of fluorous and high temperature classical reactions with convenient biphase workup to separate product from high boiling solvent. Tetrahedron, 58, 4071–4076; b) Fukuyama, T., Arai, M., Matsubara, H., Ryu, I., 2004. Mizoroki−Heck Arylation of α,β-Unsaturated Acids with a Hybrid Fluorous Ether, F-626: Facile Filtrative Separation of Products and Efficient Recycling of a Reaction Medium Containing a Catalyst. JOC, 69, 8105–8107; c) Yu, M. S., Curran, D. P., Nagashima, T., 2005. Increasing Fluorous Partition Coefficients by Solvent Tuning. Org. Lett., 7, 3677–3680; d) Curran, D. P., Bajpai, R., Sanger, E., 2006. Purification of Fluorous Mitsunobu Reactions by Liquid-Liquid Extraction. Adv. Synth. Catal., 1621–1624.

[172] HFE solvents are produced by 3M under the trade-name: Novec; http://www.3m.com/Product/information/Novec-Engineered- Fluid.html.

Table 4.1.2. Atmospheric lifetime τ (based on reaction with OH radicals) and global warming potentials GWP (calculated at time horizon ITH of 100 years) for some commercial chlorofluorocarbons (CFCs), perfluorocarbons (PFCs), hydrofluorocarbons (HFCs), and hydrofluoroethers (HFEs).[173]

Trade Name	Formula	τ (years)	GWP (100 years ITH)
CFC-13	CF_3Cl	640	14000
CFC-11	$CFCl_3$	45	4600
Freon 14	CF_4	>50000	5700
Freon 116	C_2F_6	10000	11900
HFC-23	CHF_3	260	12000
HFC-152a	$C_2H_4F_2$	1.4	120
HFE-7100	$C_4F_9OCH_3$	4.1	320
HFE-7500	$C_3F_7CF(OC_2H_5)CF(CF_3)_2$	2.2	100

In addiction HFEs are non-flammable, have low toxicity and possess peculiar physical-chemical properties to replace PFCs and HFCs in a large number of applications.

4.2. Supercritical fluids

Properties and applications

In 1822, Baron Charles Cagniard de la Tour discovered for the first time that there is a temperature, called critical temperature T_c, above which a single substance can exist as a fluid and not as either liquid or gas.

A supercritical fluid (SCF) is a highly compressed gas that displays liquid like properties. The term "supercritical" describes any substance, the temperature and pressure of which are higher than their critical values (T_c and p_c), and which has a density close to or higher than its critical density (d_c).[174] T_c and p_c describe the critical point, which is located at the "end" of the vapour pressure equilibrium curve (Figure 4.2.1.)). In these conditions a fluid exists as single phase and its pressure can be raised indefinitely without condensing into a liquid. If the temperature is not much higher than

[173] IPCC 3rd Assessment Report on Climate Change: The Scientific Basis, Chapter 6, 2001.
[174] Darr J., Poliakoff, M., 1999. New Directions in Inorganic and Metal-Organic Coordination Chemistry in Supercritical Fluids. Chem. Rev., 99, 495-541

T_c, supercritical fluids are obtained, having gas-like diffusivities and low viscosity coefficients (which increase the permeability through porous solids and the mass transfer), and liquid-like densities and dissolving powers.

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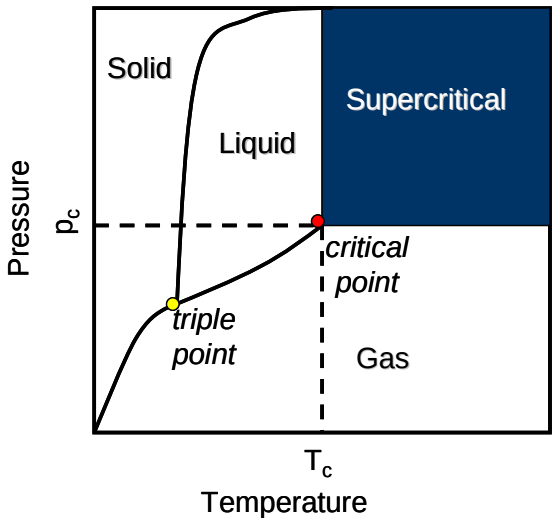
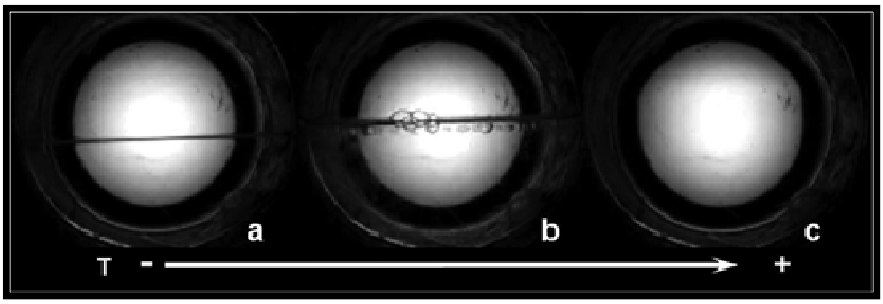
Figure 4.2.1. Phase diagram for a pure substance.



The passage from a biphasic liquid-gas phase to a supercritical phase is shown in Figure 4.2.2. If a biphasic mixture of a gas and a liquid is heated through the critical temperature T_c in a sealed vessel (Figure 4.2.2.a), molecules in the liquid phase evaporate into the gas phase (Figure 4.2.2.b); in these conditions, the gas phase becomes denser as the liquid phase becomes less dense. When the temperature exceeds T_c, the system is in one phase and under supercritical conditions (Figure 4.2.2.c).[175]

Figure 4.2.2. View cell showing the phase behaviour of a biphasic mixture of a gas and a liquid which becomes supercritical by heating.



[175] Licence, P., Litchfield, D., Dellar, M. P., Poliakoff, M. 2004. "Supercriticality"; a dramatic but safe demonstration of the critical point. Green Chem., 6, 352-354.

The process described above is reversible but the cooling of the SCF is not the reverse of heating up a liquid. The reason is that as the temperature of the fluid drops below T_c, droplets of liquid form uniformly in the fluid and fall because of the gravity and gas rises until a complete separation of the two distinct phases.

Supercritical fluids have the property of being tuneable solvents, meaning that the properties of the solvents can be changed by changing the pressure, besides the temperature as in normal liquids, above all near the critical point; for example the dissolving power is pressure-dependent as it was discovered for the first time in 1879 by Hannay and Hogarth. [176]

Supercritical carbon dioxide ($scCO_2$) is the most studied and employed supercritical fluid, currently used in a wide range of industrial applications and chemical reactions. The attention towards $scCO_2$ as a suitable solvents for organic chemistry was born as a consequence of the need to reduce the energy consumption in industrial processes; it is easy to understand that from an energy saving point of view it is better to remove a solvent by depressurising the system at room temperature than by distillation, which is the industrial process which requires the higher energetic costs. For this reason the use of $scCO_2$ and supercritical fluids as substitutes for organic solvents represents an important tool for waste and energy consumption reductions in solvent-based industrial productions. Coffee decaffeination, hops extraction, essential oil production, waste extraction/recycling, analytical instrumentations, homogeneous and heterogeneous catalytic reactions, biocatalysis, are only some examples of the applicative fields of $scCO_2$, which represents a really promising approach to pollution prevention.

Supercritical carbon dioxide has a critical temperature of 31 °C and a critical pressure of 74 bar (very easily obtainable), it is non-toxic and non-flammable, it is easy to remove (it is a gas at room temperature and pressure) and potentially recyclable, it has a good mass transfer and high diffusion rates, and it is cheap and readily available. One of the best features

[176] Hannay, J.B., Hogarth, J., 1879. On the Solubility of Solids in Gases, Proc. Roy. Soc., (London). 29, 324.

of $scCO_2$ is its high gas solubility, which has lead to its successful exploitation as solvent in hydrogenations and hydrogen transfer reactions.[177] Moreover $scCO_2$ is a non-polar solvent with similar properties to *n*-hexane, so it is a very good solvent for apolar compounds, but it can be also suitable to solubilise low polar compounds as caffeine, thanks to the quadripole moment of CO_2 molecules.[178] However one of the main disadvantages relies in its relatively poor solubility towards polar compounds and many polymers (except for fluoropolymers which are extremely apolar because of the high content of fluorine), and for this reason in many applications the use of a co-solvent is required. However, it is often enough to add a few percent of substances like chloroform or methanol to achieve a dramatic modification of the dissolving properties of $scCO_2$. One another disadvantage is correlated to the need to operate at relative high pressure with appropriate equipments, which can be capital intensive.

Environmental and toxicological aspects

Supercritical CO_2 can be described as a "green" solvent due to its non toxicity and non flammability, and in spite of the negative pressure around carbon dioxide as a greenhouse gas, the supercritical CO_2 used nowadays in the industry, does not contribute to the global warming because it is a recyclable by-product of other processes, such as ammonia synthesis or fossil fuel combustion. Moreover, after each use, $scCO_2$ can be recovered and recycled with the result of no net increase of CO_2 in the environment.

Finally the use of $scCO_2$ as replacement for traditional organic solvents, contributes to eliminate or reduce the use of compounds which are synthesised from non-renewable resources and which have high hazardous potentials as environmental pollutants and for human health.

This allows supercritical fluids to be considered a "greener" valid alternative for hazardous traditional solvents; in fact when an environmentally friendly gas, e.g. a non-toxic substance like CO_2, or liquid

[177] Hitzler, M.G., Smail, F.R., Ross, S.K., Poliakoff, M., 1998. The Selective Catalytic Hydrogenation of Organic Compounds in Supercritical Fluids as a Continuous Process. Organic Process Research & Development, 2, 137-146. b) Hitzler, M.G., Poliakoff, M., 1997. Continuous Hydrogenation of Organic Compounds in Supercritical Fluids. Chem. Commun., 1667-1668.

[178] Beckman, E., 2004. Supercritical and near-critical CO_2 in green chemical synthesis and processing. J. of Supercritical Fluids, 28, 121–191

becomes supercritical, and it can be used as solvents, several environmental benefits can be obtained.

4.3. Applications of fluorous biphasic systems (FBS) and supercritical carbon dioxide ($scCO_2$) in biocatalysis

This Chapter of the Thesis describes the use of fluorous biphasic systems and supercritical CO_2 as reaction media for homogeneous biocatalytic transesterifications. The aim of the present work is to optimize a new proteins derivatisation technique, called hydrophobic ion-pairing (HIP), for the solubilisation of enzymes both in fluorous solvents and in supercritical fluids, and to find the best reaction conditions for homogeneous biocatalytic transesterifications in these two solvent systems. Specifically the experiments have been developed during this PhD work at the University of Nottingham (UK) in collaboration with Prof. Martyn Poliakoff and Neil Thomas, thanks to a fellowship of the University of Bologna (Marco Polo Project).

Biocatalysis in non-aqueous solvents is a field which has been deeply explored in the last decades. One of the most important parameters in the successful exploitation of enzymes in non-polar media is the amount of water associated to the protein and necessary to its activity, specificity and stability. The water necessary to the enzyme, essential for its catalytic activity because taking part to all the interactions (e.g. hydrogen bonds) which maintain the proteic structure, is restricted to a monolayer around the molecule; thus the replacement of water phase with non-aqueous solvents which do not interfere with the essential water monolayer, has represented the key for the development of new frontiers in biocatalysis of the last decades.

There has been an intense amount of interest in the ability of enzymes to function in apolar media, not only traditional organic apolar solvents (e.g. *n*-hexane), but also alternative solvents, such as supercritical carbon dioxide and fluorous solvents. Apolar and hydrophobic media in fact are the best solvents for biocatalytic reaction in non-conventional media, because their interaction with the enzyme essential water is minimum. Consequently the

ability to produce active enzymes, soluble in $scCO_2$ and fluorous solvents, opens up a range of new interesting technologies.

The use of supercritical fluids as non aqueous solvents for enzyme-catalysed reactions, was first investigated in 1985 by Randolph et al., Hammond et al., and Nakamura et al.,[179] who used enzymes as simple suspensions in SCFs. $ScCO_2$ in particular is considered the best supercritical solvents for biocatalysis because of its advantageous properties described above and because its critical parameters are compatible with the conditions required for enzymatic reactions. However, CO_2 is involved in two chemical processes that have the potential to cause enzyme inactivation:[180] the formation of carbamates between CO_2 and lysine residues on the surface of the enzyme,[181] and the formation of carbonic acid by reaction between CO_2 and any water present in the system, lowering the pH of the aqueous microenvironment around the enzyme.[182] For these reasons several attempts have been done to stabilise enzymes in supercritical fluids; some examples include the use of immobilised enzymes (e.g. the lipase *Candida antarctica* B immobilised on a macroporous acrylic resin by Novozym 435), lipid-coated enzymes,[183] sol gels,[184] cross-linked enzyme crystals (CLECs),[185]

[179] a) Randolph, T. W., Blanch, H. W., Prausnitz, J. M., Wilke, C. R., 1985. Enzymatic catalysis in a supercritical fluid. Biotechnol. Lett., 7, 325. b) Hammond, D. A., Karel, M., Klibanov, A. M., Krukonis, V. J., 1985. Enzymatic reactions in supercritical gases. Appl. Biochem. Biotechnol., 11, 393. c) Nakamura, K.; Chi, Y. M.; Yamada, Y.; Yano, T., 1985. Lipase activity and stability in supercritical carbon dioxide. Chem. Eng. Commun., 45, 207.

[180] Hobbs, H., Thomas, T., 2007. Biocatalysis in Supercritical Fluids, in Fluorous Solvents, and under Solvent-Free Conditions. Chem. Rev., 107, 2786-2820.

[181] a) Kamat, S., Critchley, G., Beckman, E. J., Russell, A. J., 1995. Biocatalytic synthesis of acrylates in organic solvents and supercritical fluids: III. Does carbon dioxide covalently modify enzymes? Biotechnol. Bioeng., 46, 610-620. b) Habulin, M.; Knez, Z. 2001. Pressure stability of lipase and their use in different systems. Acta Chim. Slov., 48, 521-532. c) Habulin, M.; Knez, Z., 2001. Activity and stability of lipases from different sources in supercritical carbon dioxide and near-critical propane. J. Chem. Technol. Biotechnol., 76, 1260-1266.

[182] Kamat, S. V., Beckman, E. J., Russell, A. J., 1995. Enzyme Activity in Supercritical Fluids. Crit. Rev. Biotechnol., 15, 41-71.

[183] Okahata, Y., Hatano, A., Ijiro, K., 1995. Enhancing enantioselectivity of a lipid-coated lipase *via* imprinting methods for esterification in organic solvents. Tet Asym, 6, 1311-1322.

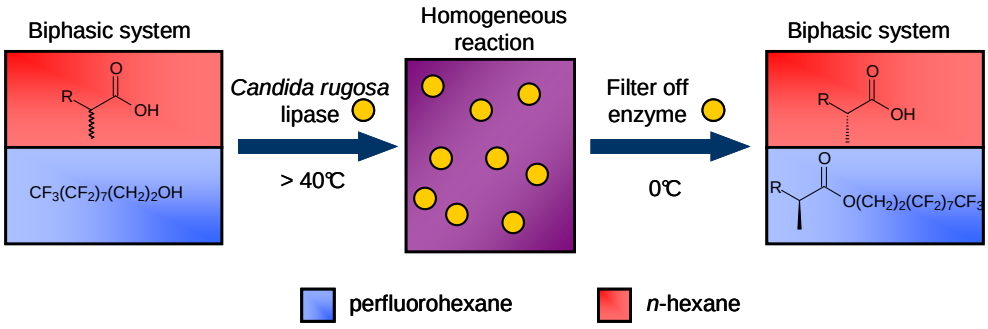
[184] van Unen, D., Engbersen, J., Reinhoudt, D., 2001. Sol-gel immobilization of serine proteases for application in organic solvents. Biotechnol Bioeng., 75, 154-158.

[185] Lalonde, J., Govardhan, C., Khalaf, N., Martinez, A., Visuri, K., Margolin, A., 1995. Cross-Linked Crystals of Candida rugosa Lipase: Highly Efficient Catalysts for the Resolution of Chiral Esters. JACS, 117, 6845-6852

cross-linked enzyme aggregates (CLEAs),[186] or enzymes combined with suitable surfactants to form reverse micelles/microemulsions.[187]

The use of fluorous solvents as non aqueous solvents for enzyme-catalysed reactions is not a deeply explored field, mainly because enzymes are not soluble in fluorous phases and there are few enzymatic substrates compatible with these media. In particular the use of fluorous solvents in fluorous biphasic system (FBS), by exploiting their temperature-dependent miscibility with organic solvents, has been reported only in three studies. The first example reports the resolution of *rac*-α-methylpentanoic acid by *Candida rugosa* lipase via fluorinated tagging with a perfluorinated alcohol in a *n*-hexane/perfluorohexane FBS, where the restore of the biphasic condition allows a straightforward separation of the *(S)*-perfluoroalkyl ester from the *(R)* unreacted acid (Figure 4.3.1.).[188]

Figure 4.3.1. Kinetic resolution of *rac*-α-methylpentanoic acid catalysed by *Candida rugosa* lipase in fluorous biphasic systems.[188]



The other two examples have recently been proposed by the group of Martyn Poliakoff and Neil Thomas in their innovative works on the

[186] a) Sheldon, R.A., Schoevaart, R., Van Langen, L.M., 2005. Continuous kinetic resolution catalysed by cross-linked enzyme aggregates, 'CLEAs', in supercritical CO_2. Biocatal. Biotransform., 23, 141–147. b) Hobbs, H., Kondor, B., Stephenson, P., Sheldon, R., Thomas, N., Poliakoff, M., 2006. Continuous kinetic resolution catalysed by cross-linked enzyme aggregates, CLEAs, in supercritical CO_2. Green Chem., 8, 816–821.

[187] Gupte, A., Nagarajan, R., Kilara, A., 1995. Enzymic oxidation of cholesterol in reverse micelles. Ind. Eng. Chem. Res., 34, 2910-2922.

[188] Beier, P., O' Hagan, D., 2002. Enantiomeric partitioning using fluorous biphase methodology for lipase-mediated (trans)esterifications**.** Chem Commun., 1680-1681.

solubilisation of two enzymes, cytochrome c[189] and α-chymotrypsin,[190] in fluorous solvents and in supercritical carbon dioxide by the technique of the hydrophobic ion-pairing (HIP), as described in the next Chapters 4.3.1. and 4.3.2. of the Thesis.

4.3.1. Hydrophobic Ion-Pairing

The technique of the hydrophobic ion-pairing (HIP) employs ionic surfactants at very low concentrations (below critical micellar concentration CMC), to replace counter ions on the surface of the enzyme with ionic surfactants, whose non-polar tails interact with non-aqueous solvents, increasing the solubility of the enzyme in these solvents.

Typically, HIP occurs between an anionic surfactant and the cationic residues (protonated lysine, arginine and histidine) on the surface of a protein (positively charged) dissolved in an aqueous phase with a pH below its isoelectrical point (pI).

While applications of an ion-pairing with a surfactant as sodium dodecyl sulfate (SDS) enable to prepare ion-pairing enzymes complex that works in water miscible solvents (e.g. octanol[191] or DMF[192]), the use of surfactants as sodium bis(2-ethylhexyl)sulfoccinate (AOT), reduces the water solubility of the surfactant-protein complex, guarantees the extraction of the protein in water-immiscible solvents, such as alkanes and chlorocarbons,[193] and enhances the catalytic activity, the stability and the temperature resistance of the enzyme itself.

The same idea at the base of HIP between an organic surfactant and a protein to extract the protein in organic solvents can be also exploited for

[189] Hobbs, H., Kirke, H., Poliakoff, M., Thomas, N., 2007. Homogeneous biocatalysis in both fluorous biphasic and supercritical carbon dioxide system. Angew. Chem. Int. Ed., 46, 7860-7863.

[190] Benaissi, K., Thomas, N., Poliakoff, M. 2010. Solubilisation of α-chymotrypsin by HIP in fluorous systems and supercritical carbon dioxide and demonstration of efficient enzyme recycling. Green Chem., 12, 54-59.

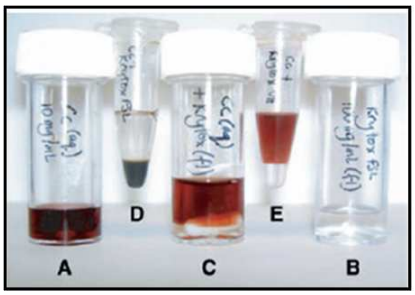
[191] Matsuura, J., Powers, M., Manning, M., Shefter, E., 1993. Structure and stability of insulin dissolved in 1-octanol. JACS, 115, 1261-1264.

[192] Meyer, J., Kendrick, B., Matsuura, J., Ruth, J., Bryan, P., Manning, M., 1996. Generation of soluble and active subtilisin and a-chymotrypsin in organic solvents via HIP. Int J Peptide Protein Res, 47, 177-181.

[193] a) Meyer, J., Matsuura, J., Kendrick, B., Evans, E., Evans, G., Manning, M., 1995. Solution behaviour of a-chymotrypsin dissolved in non polar organic solvents via HIP. Biopolymers, 35, 451-456. b) Paradkar, V., Dordick, J., 1994. Mechanism of extraction of chymotrypsin into isooctane at very low concentrations of aerosol OT in the absence of reversed micelles. Biotechnol Bioeng., 43, 529-540.

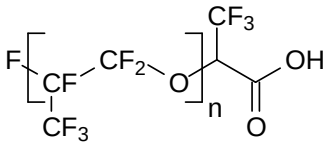
the solubilisation of proteins in more apolar solvents, as fluorous solvents and $scCO_2$, by using a fluorophilic surfactant. Until now, the only one example about the solubilisation of proteins in fluorous solvents or supercritical fluids by HIP was published by Poliakoff and Thomas' group: they have successfully extracted some proteins such as cytochrome c and α-chymotrypsin[189] in fluorous solvents (Figure 4.3.1.1.), by means of HIP with two fluorous carboxylate surfactants, Krytox FSL 157 and KDP 4606 respectively (Figure 4.3.1.2.), and these enzymatic complexes were active both in FBS and in $scCO_2$.

Figure 4.3.1.1. Hydrophobic ion-pairing of cytochrome c (Cc) with the surfactant Krytox.

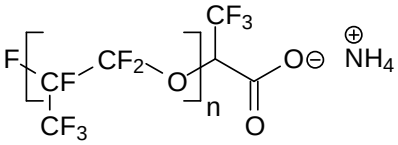


A: Cytochrome c in water phase
B: Krytox surfactant in fluorous phase
C and **D:** Biphasic mixture before HIP (top: aqueous phase with the enzyme; bottom: fluorous phase with the surfactant)
E: Biphasic mixture after HIP (top: aqueous phase; bottom: fluorous phase with the enzyme-surfactant complex)

Figure 4.3.1.2. Structure of Krytox FSL 157 and KDP 4606 (DuPont®).



Krytox FSL 157 (mw = 2500; n = 17)



KDP 4606 (mw = 1400; n = 9)

In this Thesis several enzymes, lipases and proteases, were evaluated as candidates for the HIP with the fluorous surfactant KDP 4606. For each enzyme the molecular weight, the number of basic amino acid residues (lysine, arginine and histidine), the isoelectric point, and the extinction coefficient at 280 nm (ε_{280}, for determination of protein concentration) were determined; the protein purity was assessed by using the bicinchoninic acid

(BCA) assay (Figure 4.3.1.3.). All the parameters are reported in Table 4.3.1.1.

Figure 4.3.1.3. Determination of proteins purity through the spectrophotometric bicinchoninic acid (BCA) assay.

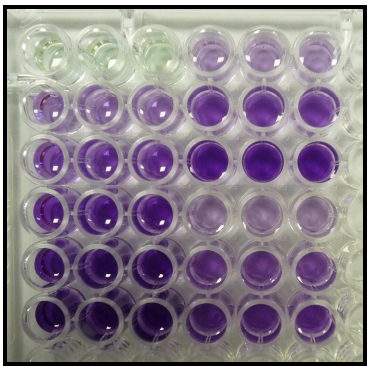


Table 4.3.1.1. Proteins molecular weight (MW), isoelectric point (pI), number of basic amino acid residues (lysine, arginine, and histidine), extinction coefficient at 280 nm (ε_{280}),[a] and purity (BCA assay).

Protein	**MW**	**pI**	**basic residues**	**ε 280 (M^{-1} cm^{-1})**	**purity %**
α-chymotrypsin	25653	8.3	19	20400	99
Aspergillus niger lipase	64654	5.5	60	106453	47
Candida rugosa lipase	58550	4.6	40	57550	13
Candida Antarctica lipase	35517	8.1	22	41285	38
Candida cylindracea lipase	59218	4.8	42	57550	10
Rhizopus arrhizus lipase	42138	7.0	39	43360	28
Rhizopus niveus lipase	42138	7.0	39	43360	28

[a]: source ExPASy (Expert Protein Analysis System) tool 'Protparam'

The ideal ratio between a protein and a surfactant for their hydrophobic ion-pairing is given by the formula:

$$(bs + 1) * m_p / MW_p = m_s / MW_s$$

Where:

bs= basic amino acid residues (lysine, arginine and histidine)

m_p= protein mass

MW_p= protein molecular weight

m_s= surfactant mass

MW_p=surfactant molecular weight

According to the formula, the ideal ratios between the tested proteins and the surfactant KDP 4606 are reported in Table 4.3.1.2.

Table 4.3.1.2. Ideal mass ratio between enzymes and the surfactant KDP.

Enzyme	**Enzyme:KDP ideal mass ratio**
α-chymotrypsin	1:1
Aspergillus niger lipase	1:1.5
Candida rugosa lipase	1:1.5
Candida Antarctica lipase	1:1.5
Candida cylindracea lipase	1:1
Rhizopus arrhizus lipase	1:2
Rhizopus niveus lipase	1:2

According to the formula and to the hydrophobic ion-pairing parameters for example, the ideal chymotrypsin-surfactant mass ratio should be 1:1. However, previous works[189,190] have demonstrated that the best results in the complexation could be obtained with a higher amount of surfactant, up to a ratio of 1:3. For this reason the following formation of each protein-KDP complex was also investigated at ratios different from the ideal ones.

In a typical experiment the protein was dissolved in aqueous buffer with a pH lower than its pI, to guarantee a positively charge on the surface of the protein, and containing $CaCl_2$, a "salting in" salt, which has been demonstrated to have a positive effect in the complexation, may due to its ability in aiding phase separation following the extraction.[194] KDP was dissolved in fluorous solvents (perfluoromethylcyclohexane PFMC or 3-ethoxy-1,1,1,2,3,4,4,5,5,6,6,6-dodecafluoro-2-trifluoromethyl-hexane HFE 7500), and the two solutions were mixed together for a specific time. After that the mixture was centrifuged and the upper fluorous phase was removed

[194] Bindhu, L.V., Abraham, E., 2003. Preparation and kinetic studies of surfactant–horseradish peroxidise ion paired complex in organic media. Biochem. Eng. J., 15, 47–57.

and analysed by UV spectrophotometer at 280 nm to determine the mass of protein extracted from the aqueous phase.

In Figure 4.3.1.4 it is reported an example of the hydrophobic ion-pairing between the protease α-chymotrypsin (CMT) and the fluorinated surfactant KDP. In Table 4.3.1.3. the complexation efficiencies (ce %) obtained with different proteins and KDP are summarised.

Figure 4.3.1.4. Hydrophobic ion-pairing between α-chymotrypsin (CMT) and the fluorinated surfactant KDP.

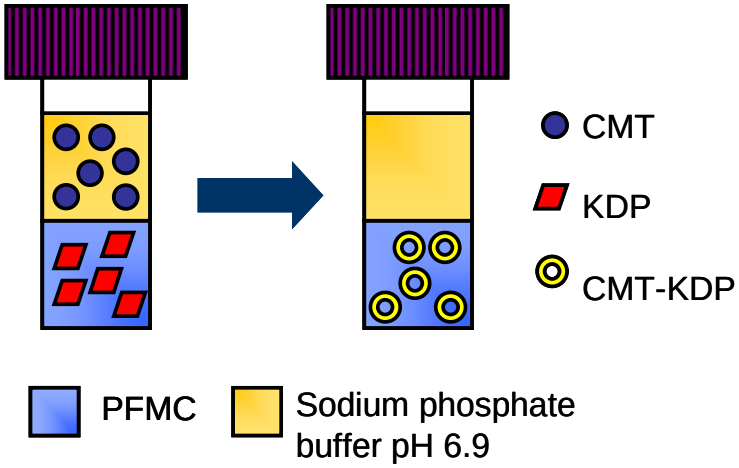


Table 4.3.1.3. Effect of different times of reaction, fluorous phases (perfluoromethylcyclohexane PFMC and hydrofluoroether HFE-7500), enzyme-surfactant ratio (mass ratio), and aqueous phase-fluorous phase ratio (volume ratio) on the hydrophobic ion-pairing complexation efficiency (ce) of proteins with the surfactant KDP.

Entry	**Protein**	**time (min)**	**fluorous phase**	**enzyme: KDP**	**buffer: fluorous phase**	**ce %**
1	α-chymotrypsin	10	PFMC	1:3	1:1	82
2	*A. niger* lipase	30	PFMC	1:1	1:1	11
3	*A. niger* lipase	30	PFMC	1:1.5	1:1	18
4	*A. niger* lipase	30	PFMC	1:2	1:1	8
5	*A. niger* lipase	30	PFMC	1:2.5	1:1	7
6	*C. rugosa* lipase	60	HFE-7500	1:1	1:1	51
7	*C. rugosa* lipase	120	HFE-7500	1:1	1:1	46
8	*C. rugosa* lipase	120	PFMC	1:3	2:1	98
9	*C. antarctica* lipase	120	HFE-7500	1:1.5	1:1	26
10	*C. antarctica* lipase	60	HFE-7500	1:1	1:1	65
11	*C. antarctica* lipase	60	PFMC	1:1	1:1	11
12	*C. cylindracea* lipase	60	HFE-7500	1:1	1:1	29
13	*C. cylindracea* lipase	60	HFE-7500	1:2	2:1	28
14	*C. cylindracea* lipase	60	HFE-7500	2:1	2:1	48
15	*C. cylindracea* lipase	60	HFE	1:1	1.3:1	31
16	*C. cylindracea* lipase	60	PFMC	2:1	1.3:1	70
17	*R. arrhizus* lipase	20	HFE	1:2	1:1	9
18	*R. arrhizus* lipase	20	HFE	2:2	2:1	17
19	*R. arrhizus* lipase	20	PFMC	2:2	2:1	20
20	*R. niveus* lipase	45	HFE	1:50	1:1	58
21	*R. niveus* lipase	45	HFE	1:2	1:1	39
22	*R. niveus* lipase	45	HFE	2:2	2:1	14
23	*R. niveus* lipase	45	PFMC	1:2	1:1	31

The fluorous phase HFE 7500 has been tested in comparison with PFMC, because it represents a "greener" alternative to common fluorinated solvents, as previous reported in Chapter 4.1. The effect of the two solvents in the complexation is not always the same, and for example with some enzymes, as *Candida antarctica* lipase, HFE 7500 results a better solvent

than PFMC (Entries 10 and 11), but with other enzymes such as *Candida cylindracea* lipase, the reverse is true (Entries 14 and 16). Moreover the maximum complexation efficiency obtained with HFE 7500 is 65% (*Candida antarctica* lipase), lower than the maximum complexation efficiency obtained with PFMC (98%, *Candida rugosa* lipase, Entry 18). For these reasons, in spite of a higher environmental compatibility and the lower price of hydrofluoroethers, the fluorous phase used for the following experiments in the Thesis was the perfluorinated PFMC.

As shown in Table 4.3.1.3., the best results with PFMC are obtained with α-chymotrypsin (ce 82%, Entry 1) and with *Candida rugosa* lipase (ce 98%, Entry 8). However the time required for the complexation is 2 hours in the case of the lipase and only 10 minutes in the case of the protease; moreover, as reported in Table 4.3.1.1., the purity of chymotrypsin is much higher (99%) than that of *Candida antarctica* (13%). Therefore, α-chymotrypsin, solubilised in PFMC according to the experimental conditions reported in Table 4.3.1.3. (Entry 1), is the enzyme of choice for the transesterification reaction performed in fluorous biphasic system and in $scCO_2$.

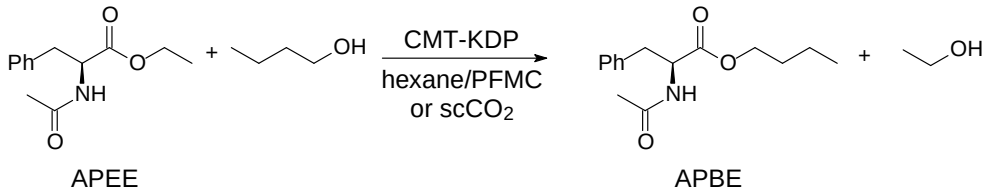
The complexation efficiency obtained here (82 ± 12%) is slightly higher than the values reported previously by Thomas et al. (70%)[189] and by Benaissi et al. (51 ± 4%).[190]

4.3.2. Transesterification of *N*-acetyl-L-phenylalanine ethyl ester in Fluorous Biphasic Systems (FBS) and in supercritical carbon dioxide ($scCO_2$).

In a previous initial work by Benaissi et al.,[190] the authors investigated the catalytic activity of two chymotrypsin(CMT)-surfactant complexes with the fluorinated surfactant KDP and the organic surfactant AOT, soluble respectively in perfluoromethylcyclohexane (PFMC) and in *n*-hexane. The transesterification of a model compound was performed in the fluorous biphasic system PFMC/hexane (biphasic at 0°C and homogeneous at 40°C), and in supercritical CO_2, indicating a higher activity of the solubilised protease-surfactant complex than that of the suspended protease in both the solvent systems.

In this Thesis the activity of the enzymatic complex chymotrypsin-surfactant KDP (CMT-KDP) has been deepened on a model reaction, the transesterification of *N*-acetyl-L-phenylalanine ethyl ester (APEE) with *n*-butanol (Figure 4.3.2.1.), with particular focus on the optimisation of the reaction conditions and on the recycling of the catalyst.

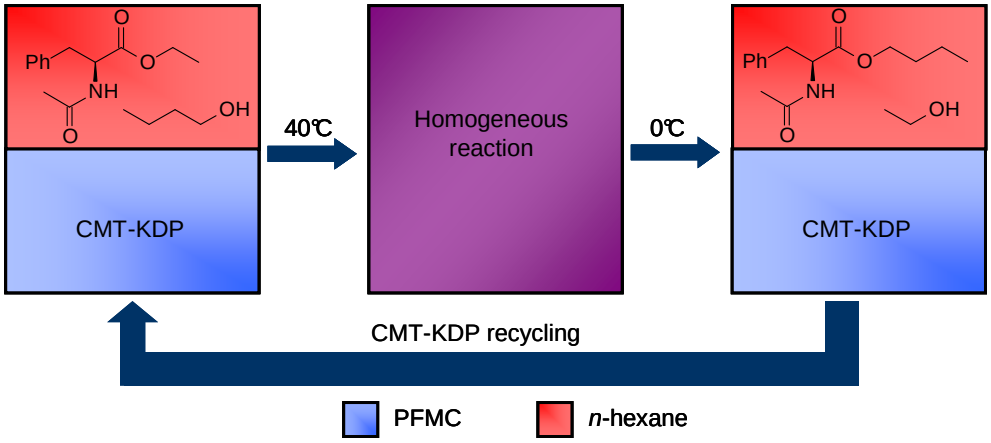
Figure 4.3.2.1. Transesterification of *N*-acetyl-L-phenylalanine ethyl ester (APEE) with *n*-butanol, to give *N*-acetyl-L-phenylalanine butyl ester (APBE).



APEE APBE

Biocatalytic reactions in fluorous biphasic system have been developed by solubilising at room temperature the protease α-chymotrypsin in perfluoromethylcyclohexane, and the reagents (*N*-acetyl-L-phenylalanine ethyl ester and *n*-butanol) in *n*-hexane, being the fluorous and the organic solvents immiscible under this temperature conditions. On heating at 40°C, the two phases became miscible, allowing homogeneous reaction to occur. After cooling at 0°C, the two phases were separated, leaving the products (*N*-acetyl-L-phenylalanine butyl ester and ethanol) in *n*-hexane, and the enzyme-surfactant complex in perfluoromethylcyclohexane, permitting the recycling of the catalyst (Figure 4.3.2.2.). The organic phase was then recovered and analyzed to determine APBE yields by gas chromatography.

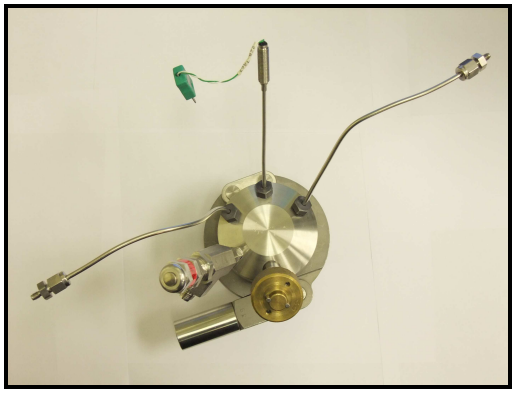
Figure 4.3.2.2. Transesterification of *N*-acetyl-L-phenylalanine ethyl ester with *n*-butanol under fluorous biphasic system (perfluoromethylcyclohexane(PFMC)/*n*-hexane) with the enzymatic complex chymotrypsin-surfactant KDP (CMT-KDP).



The complex chymotrypsin-KDP was also used to catalyse the same reaction in supercritical carbon dioxide. The reactions were carried out in a purpose built autoclave for high pressure (Figure 4.3.2.9.), by solubilising the enzyme in perfluoromethylcyclohexane, adding the reagents (*N*-acetyl-L-phenylalanine ethyl ester and *n*-butanol), and heating at 40°C. After 2 h, the system was depressurized by placing the autoclave in a dry-acetone bath, and the residual mixture in the autoclave was recovered and analyzed to determine APBE yields by gas chromatography.

3

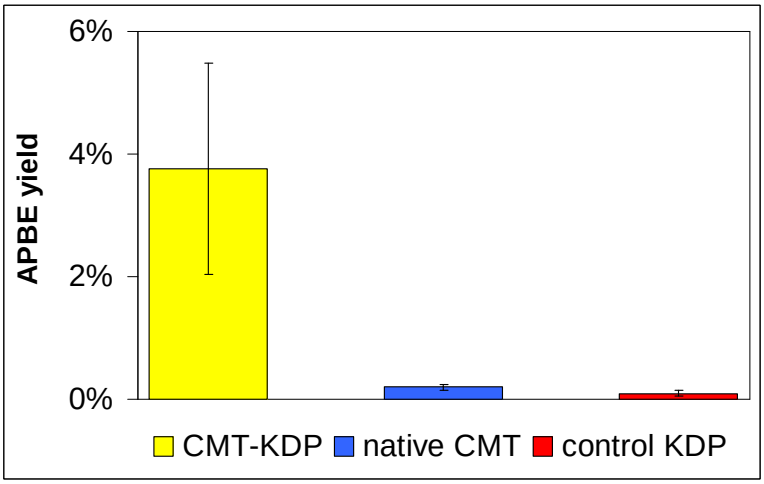
Figure 4.3.2.9. Small volume autoclave (8.5 mL) used for the biocatalytic transesterification of *N*-acetyl-L-phenylalanine ethyl ester in $scCO_2$.



Enzyme activity

The activity of the enzyme-surfactant complex in FBS (Figure 4.3.2.3.) and in $scCO_2$ (Figure 4.3.2.4.) was initially compared with the activity of the native chymotrypsin under the same conditions; as a control reaction, the transesterification of *N*-acetyl-L-phenylalanine ethyl ester was also performed with just the surfactant KDP

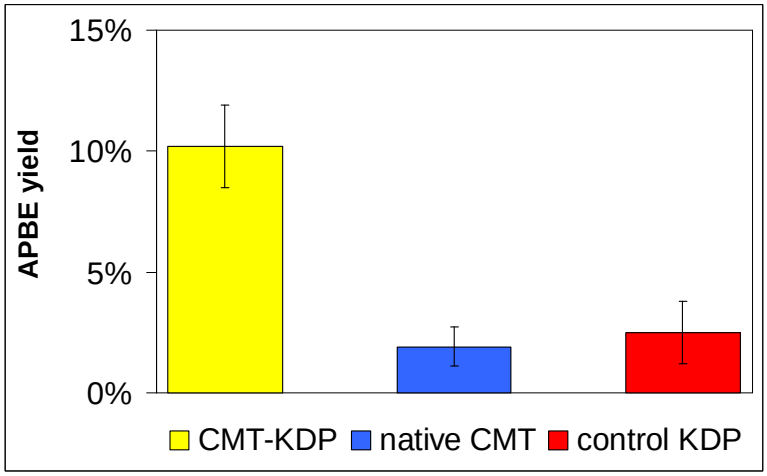
Figure 4.3.2.3. Activity of various catalysts (chymotrypsin-surfactant complex, native chymotrypsin and surfactant KDP) for the transesterification of *N*-acetyl-L-phenylalanine ethyl ester in FBS.



Native chymotrypsin and the surfactant KDP show negligible catalytic activity, with transesterification yields of 0.2 ± 0.05% and 0.1 ± 0.08% respectively; the transesterification occurs in higher yields only with α-chymotrypsin-KDP complex (3.8 ± 1.7% yield), thanks to the solubility of

the enzyme in the fluorous biphasic system, with the consequent homogeneous conditions of reaction.

Figure 4.3.2.4. Activity of various catalysts (chymotrypsin-surfactant KDP complex, native chymotrypsin and surfactant KDP) for the transesterification of *N*-acetyl-L-phenylalanine ethyl ester in $scCO_2$.



As for the reaction under fluorous biphasic system condition, α-chymotrypsin is largely less active in its native form (1.9 ± 0.8% APBE yield) than in its complex with KDP which catalysed the reaction in 10.2 ± 1.7% yield, in good agreement with the literature values.[189,190]

Since previous works[189,190] have already deepened the activity of various enzymes ion-paired with KDP in $scCO_2$ and demonstrated their reusability over several cycles, indicating that pressurisations and depressurisations procedures do not affect the enzymatic activity, the optimisation of the reaction conditions and the evaluation of the recyclability of the enzymatic complex have been analysed just for the fluorous biphasic system PFMC/*n*-hexane.

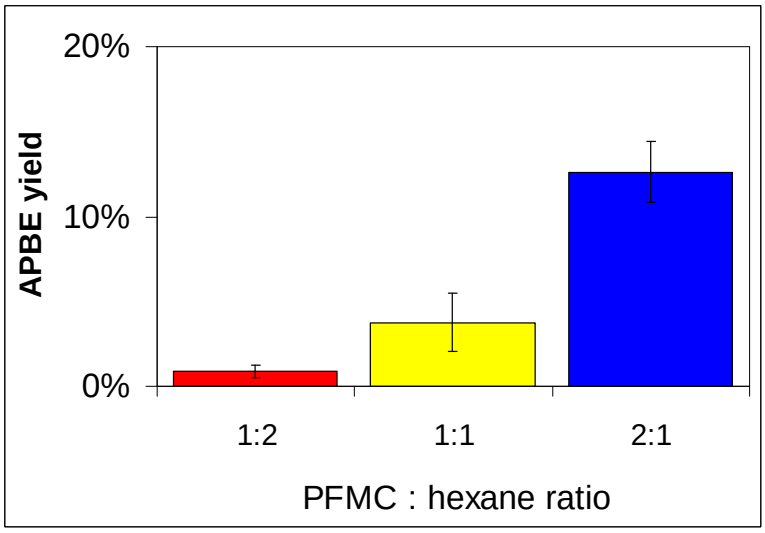
Optimisation of reaction conditions in FBS

The influence of the perfluoromethylcyclohexane:*n*-hexane ratio, of the total volume of the fluorous biphasic system, of the substrate concentrations and of the reaction time was examined.

- Perfluoromethylcyclohexane : *n*-hexane ratio and total fluorous biphasic system volume.

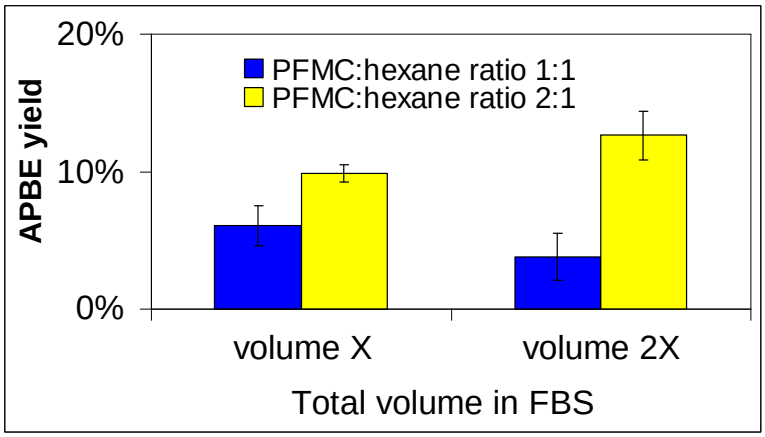
  The effect of three different PFMC:hexane ratios (1:2, 1:1, 2:1) in the fluorous biphasic system was investigated (Figure 4.3.2.5.). The effect of the amount of the reaction medium was also checked, halving the total volume of the FBS (from 2 mL to 1 mL with PFMC:hexane ratio 1:1, and from 3 mL to 1.5 mL with PFMC:hexane ratio 2:1) (Figure 4.3.2.6.).

  Figure 4.3.2.5.Effect of PFMC:hexane ratio on the yields (*N*-acetyl-L-phenylalanine ethyl ester 4.25 mM, 2h, 40°C).



  The amount of the fluorous phase in the FBS deeply influences the efficiency in the transesterification, increasing the yield of 14-fold by increasing the amount of PFMC respect to the amount of hexane in the FBS. In particular the yield increases from 0.9 ± 0.4% with PFMC:hexane ratio 1:2, to 12.6 ± 0.4% with a ratio 2:1.

Figure 4.3.2.6. Effect of the total FBS volume on the yields (*N*-acetyl-L-phenylalanine ethyl ester 4.25 mM, 2h, 40°C).

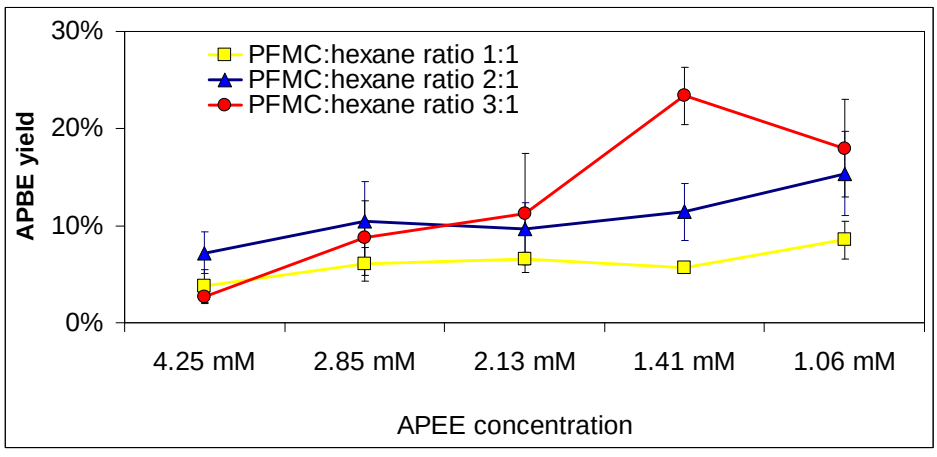


Doubling the amount of the volume of the reaction media has marginal effects on the efficiency of the reaction, as can be seen in Figure 4.3.2.6. The following experiments were performed with a total FBS volume of 2 mL, 3 mL and 4 mL for the PFMC:hexane ratio 1:1, 2:1 and 3:1 respectively.

- Substrate concentrations.

  The effect of *N*-acetyl-L-phenylalanine ethyl ester concentrations was investigated in the systems with PFMC:hexane ratio 1:1, 2:1 and 3:1 by changing the initial substrate molarity from 4.25 mM to 1.06 mM (Figure 4.3.2.7.).

Figure 4.3.2.7. Effect of *N*-acetyl-L-phenylalanine ethyl ester (APEE) concentrations on the yields (2h, 40°C).



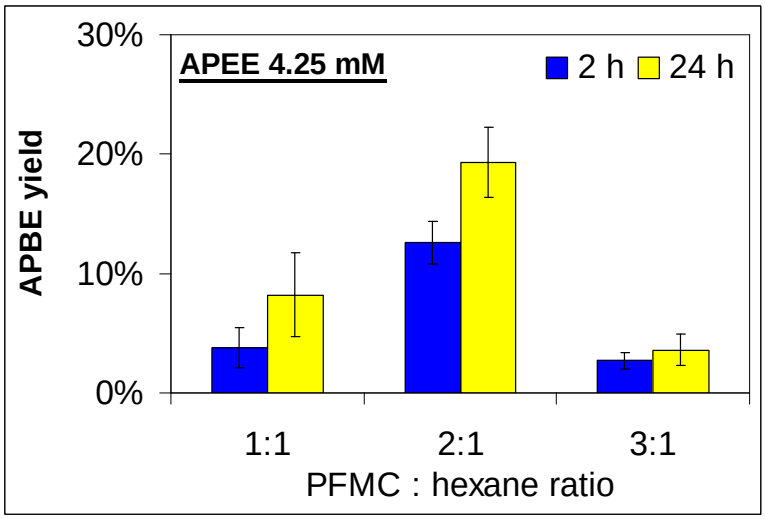
As expected, the yields increase by decreasing the substrate molarities in each system and in particular of 2 times with both PFMC:hexane ratios 1:1 and 2:1, and of 9 times with the ratio 3:1.

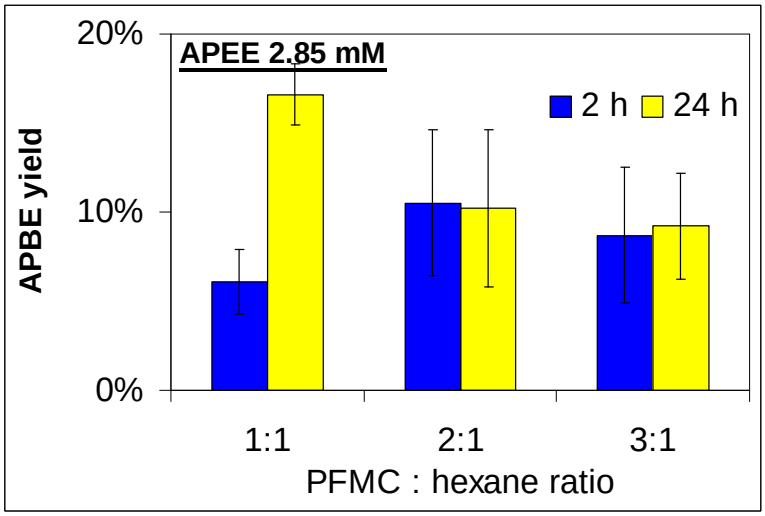
The higher yield is obtained at 1.41 mM APEE concentration with the PFMC:hexane ratio 3:1 (23.4 ± 3.0%).

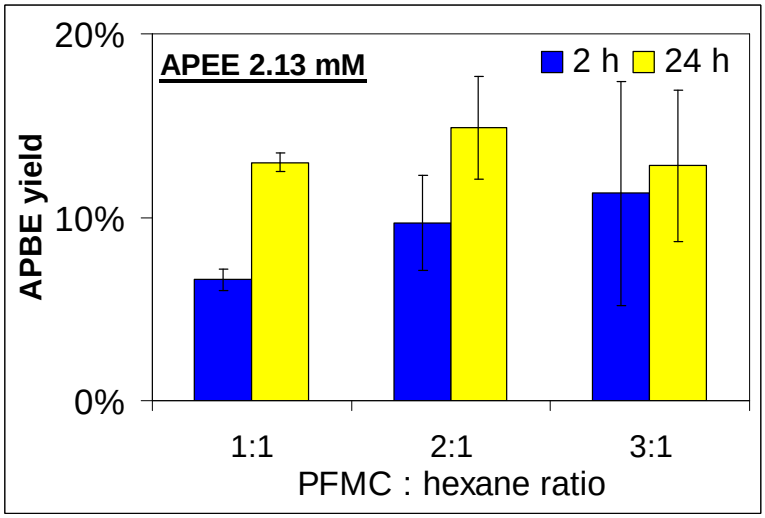
- Reaction time

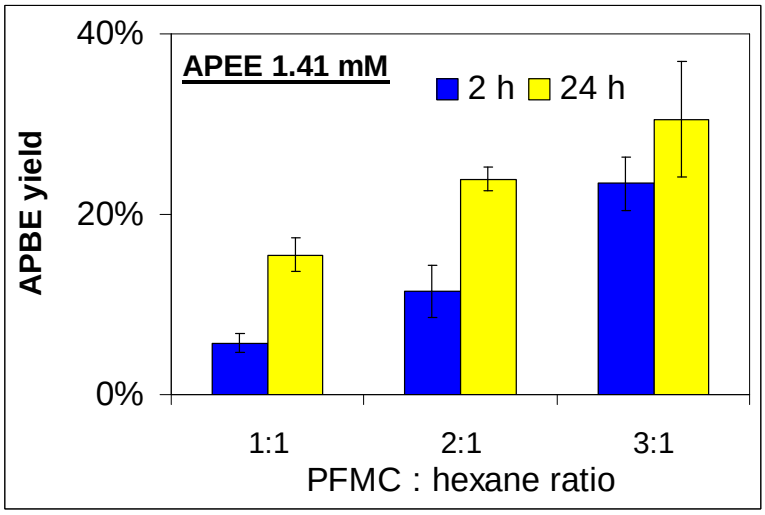
  The effect of the time on the transesterification of *N*-acetyl-L-phenylalanine ethyl ester has been investigated in three systems with different PFMC:hexane ratio (1:1, 2:1 and 3:1) and different *N*-acetyl-L-phenylalanine ethyl ester concentrations (4.25 mM, 2.85 mM, 2.13 mM, 1.41 mM and 1.06 mM), analysing the yields after 2 and 24 hours (Figure 4.3.2.8.).

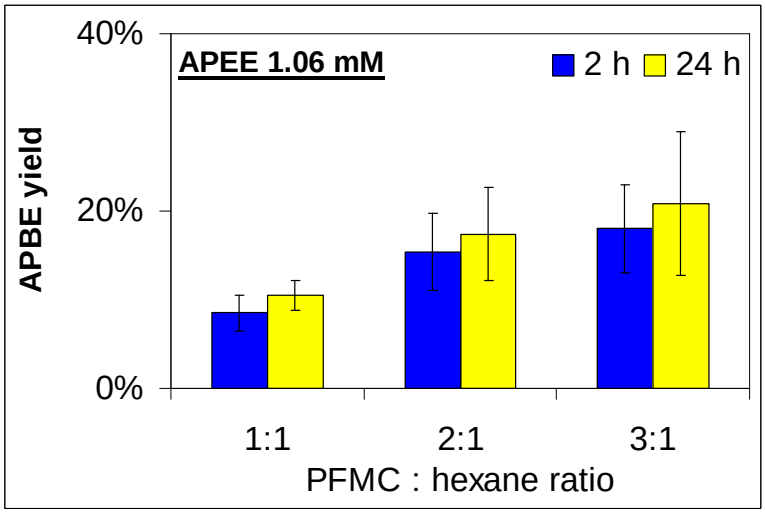
Figure 4.3.2.8. Effect of the time on the yields of *N*-acetyl-L-phenylalanine butyl ester at different substrate molarities.




APEE 2.85 mM
2 h
24 h
APBE yield
20%
10%
0%
1:1
2:1
3:1
PFMC : hexane ratio


APEE 2.13 mM
2 h
24 h
APBE yield
20%
10%
0%
1:1
2:1
3:1
PFMC : hexane ratio


APEE 1.41 mM
2 h
24 h
APBE yield
40%
20%
0%
1:1
2:1
3:1
PFMC : hexane ratio



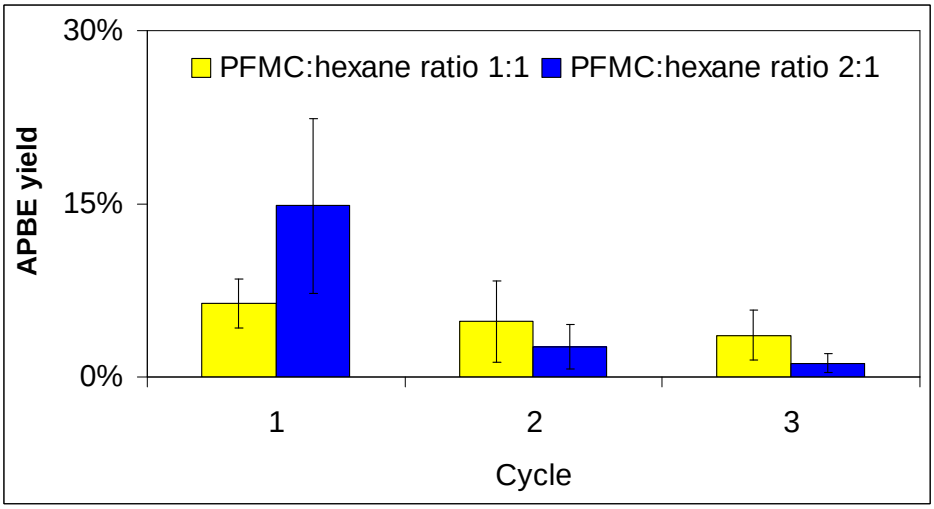
The effect of the time is not so relevant because the yields increase not more than 3 times between 2 and 24 hours in each system. In particular with an initial *N*-acetyl-L-phenylalanine ethyl ester concentration of 1.41 mM a larger effect of the time is detected: by increasing the time of reaction from 2 to 24 hours, the yields increase 3, 2 and 1.5 times, respectively in the systems with PFMC:hexane ratio 1:1, 2:1 and 3:1.

The best yield (30.5 ± 6.4%) is obtained after 24 hours with PFMC:hexane ratio 3:1 and an initial *N*-acetyl-L-phenylalanine ethyl ester concentration of 1.41 mM.

Recycling of the catalyst

The reusability of the complex chymotrypsin-surfactant KDP was investigated over three cycles with the systems PFMC:hexane 1:1 and 2:1, replacing after 2 hours of reaction the hexane phase with a fresh batch of the substrates (Figure 4.3.2.9.).

Figure 4.3.2.9. Recycling of the enzymatic complex chymotrypsin-surfactant KDP (*N*-acetyl-L-phenylalanine ethyl ester 4.25 mM, 2h, 40°C).



Unlike the results reported by Benaissi et al.,[190] which indicate that α-chymotrypsin exhibits a good stability in the fluorous biphasic system over 4 cycles (*N*-acetyl-L-phenylalanine butyl ester yields of about 8%), in this case the recyclability of the enzyme is unsuccessful. In fact a dramatic decrease in the catalytic complex activity is observed over three enzyme recycles in both systems, respectively of 12 times and 1.5 times with the PFMC:hexane ratio 2:1 and 1:1.

Conclusions

The technique of hydrophobic ion pairing (HIP) has been used to solubilise several enzymes in fluorous solvents by using the fluorinated surfactant KDP, checking the effect of different HIP parameters (fluorous phase, fluorous phase:aqueous phase ratio, enzyme:surfactant ratio, time) on the complexation efficiency. Among the checked conditions, the best results were obtained with the protease α-chymotrypsin (CMT) in perfluoromethylcyclohexane (PFMC) (complexation efficiency 82 ± 12%).

The activity of CMT-KDP complex was investigated on a model reaction in fluorous biphasic systems (FBS) and under supercritical CO_2 ($scCO_2$) conditions, and compared with the native form of the enzyme.

α-Chymotrypsin solubilised in PFMC through the hydrophobic ion-pairing with the fluorous surfactant KDP gave higher product yields than the

suspended enzyme, which showed negligible catalytic activity, both in FBS and in $scCO_2$.

By analysing the effect of several parameters on the enzymatic activity in FBS, it emerged that the amount of fluorous phase influenced the yield of product in a remarkable way; the yield increased of 12 times by increasing the ratio PFMC:hexane from 1:2 to 2:1. As expected, also by lowering the substrate concentration and by increasing the time of reaction the yields of product increased.

Unluckily the evaluation of the reusability of the enzyme-surfactant complex CMT-KDP in FBS indicated that the enzyme lost its activity over three cycles, in disagreement with the literature data which suggested a good recyclability. Further investigations are necessary to deepen this last issue which represents the main attractive feature of FBS as alternative "green" solvents in biocatalytic applications.

Experimental section

α-Chymotrypsin (CMT, type II from bovine pancreas, 51 U mg-1) and all the chemicals used in the protein purity assay (bicinchoninic acid BCA $CuSO_4$ solution 4% and bovine serum albumin BSA standard) and transesterification reactions (*n*-hexane, *N*-acetyl-L-phenyl alanine ethyl ester APEE and *n*-butanol) were obtained from Aldrich, and used without any further purification.

The fluorinated surfactant KDP 4606 was donated by DuPont®. Perfluoromethylcyclohexane (PFMC) and 3-ethoxy-1,1,1,2,3,4,4,5,5,6,6,6-dodecafluoro-2-trifluoromethyl-hexane (HFE-7500) were purchased from Fluorochem.

High purity CO_2 was purchased from CryoService. Gases hydrogen, helium and air were purchased from BOC Gases.

Protein purity

The BCA assay was used to determine the purity of proteins before their use. BCA reagent was prepared from bicinchoninic acid (BCA, 15 mL) and $CuSO_4$ solution 4% (300 µL). A bovine serum albumin (BSA) standard curve was prepared for a range of concentrations (0 – 0.1 mg/mL, 200 µL)

by dilution with the experimental buffer. The protein sample (200 µL) was prepared so that the concentration would fall within the range of the standard curve. To each BSA standard and protein sample (200 µL) BCA reagent was added (800 µL) and incubated at 40 °C for 30 min. A Tecan 'Sunrise' 96-well plate reader was used to measure the samples in triplicate at 595 nm.

Solubilisation of CMT in PFMC

The protein (1.1 mg/mL) was dissolved in sodium phosphate buffer (1 mL, 10 mM, pH 7) containing 5 mM $CaCl_2$ and it was stirred for 10 min at room temperature with a solution of KDP (3 mg) in PFMC (1 mL). The resulting solution was centrifuged at 8000 rpm for 3 min to allow phase separation to occur, and the UV absorbance of the protein complex in the fluorous phase was measured at 280 nm (ε = 20400 M^{-1} cm^{-1}). UV measurements were performed with an Agilent 8453 UV spectrophotometer. Complexation efficiency was calculated by dividing the mass of protein extracted by the original mass of protein.

Transesterification of APEE with n-butanol under FBS conditions

CMT-KDP in PFMC (1 mg/mL) was added to APEE (1 eq) and *n*-butanol (100 eq) in hexane; the reaction was stirred at 40ºC for 2 or 24 hours, and aliquots were collected and analyzed by GC, after the phases separation by cooling of the system at 0ºC. Each experiment was performed in triplicate.

In the CMT-KDP recycling experiments, after a reaction time of 2 h, the system was cooled to about 0°C, the hexane phase was removed, and the products were analyzed by gas chromatography. Afresh batch of substrate solution was added to the remaining enzyme–surfactant complex in PFMC, and the reaction was carried out as before. Three cycles were completed in total.

Transesterification of APEE with n-butanol in $scCO_2$

Reactions in $scCO_2$ were performed in a stainless-steel high pressure batch reactor (autoclave) specially designed at the University of Nottingham (internal volume of 8.5 mL). The chymotrypsin-KDP complex, APEE and

n-butanol were added into the reactor prior to sealing. A band heater, thermocouple, input and output pressure pipes were then connected to the reactor to enable temperature and pressure control of the system. The system was then heated to the desired temperature and liquid CO_2 was pumped into the reactor using a high pressure NWA PM-101 Pickel pump until the desired pressure was achieved. The reaction mixture was stirred for 2 h and then the system was depressurised by placing the autoclave in a dry ice–acetone bath. The residual mixture in the autoclave was dissolved in acetone and analysed by GC.

GC-FID analysis

The yields were determined by gas liquid chromatography (GLC) using a Shimadzu GC 2010 chromatograph fitted with a RTX5-FAST column (fused silica, crossbond 5% diphenyl / 95% dimethylpolisiloxane, 10 m × 0.1 mm × 0.1 μm). Measurement solutions were injected under a split ratio 75:1, with injector temperature set at 250°C. The following thermal program was used: 100°C, 10°C/min up to 250°C and held for 2 min. The FID temperature was 350°C.

5. Conclusions

This Thesis has the main goal of studying and developing new “green” solvents. However, as it clearly appears from the initial directory, the concept of “green” solvents is rather smoky: many different classes of substances have been classified as “green” on the basis of completely different, sometimes mutually contradicting, properties. Some solvents, for example, can be considered as “green” because harmless to humans, but at the same time they contribute to spread pollutants in the environment; others can be suitable for catalyst recycle, but maybe synthesised from “brown” starting materials. Environmentally concerned chemists should approach the problem of “green” solvents in a flexible and pragmatic way, where each improvement, also the least important, could be a benefit for the scientific community and for the entire society.

The choice for one specific green solvent in which carrying out a process depends on economical and technical aspects as well as on the intrinsic characteristics of a given reaction and/or solvent. It is important to emphasize that the ideal “green” solvent, suitable for each application, does not exist, but it is possible to find from time to time a suitable option for a specific need that will reduce the impact of that application on the health of humans and environment.

Moreover, as shown in this Thesis, a fundamental parameter which can not be forgotten in the choice and evaluation of a proper solvent, is its (eco)-toxicological hazard potential. Ionic liquids for example, to the study of which the most part of this Thesis has tried to give a contribution, are considerable “green” solvents thanks to their non-volatility and non-flammability, but they are often powerfully toxic substances.

In order to reduce the risk for human health and the environment, according to the principle of Green Chemistry, the design of inherently safer chemicals with suitable properties for technical application is a fundamental goal. In this contest a multidisciplinary and interdisciplinary analysis is strictly necessary, for evaluating both the application skills and the biological activity of the chemicals themselves.

The analysis of the three “green” solvents categories, ionic liquids, supercritical fluids and fluorous solvents, accomplished in this Thesis, has been done following this approach.

Supercritical fluids have been considered as the “greenest” solvents among the alternatives proposed for the replacement of traditional organic solvents; supercritical CO_2 in particular is non toxic, non flammable, easily to separate from the products of a reaction and obtainable as byproduct of other industrial processes. Also fluorous solvents can be defined as “green” solvents, mainly because of the facility of recycling when used in combination with organic solvents and of very low levels of toxicity; however it is important to underline that their effective “greenness” is still a matter of debate because of their high persistence in the environment.

In this Thesis the evaluation on these two kind of alternative solvents has been mainly focused on their exploitation as non conventional media for homogeneous biocatalytic reactions. Several enzymes were successfully dissolved in supercritical carbon dioxide and in fluorous solvents by means of hydrophobic ion pairing with a highly fluorinated surfactant. These apolar enzyme-surfactant complexes were used to catalyse transesterification reactions under homogeneous conditions, affording very good results in comparison with native enzymes, indicating that the use of the hydrophobic ion pairing can introduce many new possibilities of exploitations for biocatalysis in non-conventional media. In spite of these relevant findings, it has been also emerged that catalyst recycle is not a trivial problem in such reaction media and a further investigation is mandatory; moreover, the range of applicability of hydrophobic ion pairing technique must be widely deepened.

Ionic liquids, which represent the most controversial and discussed alternative solvents of the last decades, deserve a deepen analysis: the possibility of tuning both the chemico-physical properties and the (eco)-toxicological behaviour by changing the chemical structure of the solvents, opens to a wide range of opportunities for the combination of sustainability and industrial applicability.

In the present Thesis an analysis of ILs environmental fate have been accomplished in a series of biological studies and biodegradability tests,

aiming to reduce the environmental impact and the biological activity by introducing specific moieties in the cationic structure; short polyethoxylated chains have clearly demonstrated a great potential for this purpose.

The biological studies, focused on the eco-toxicity towards crustacean, bacteria and algae and on toxicity assays at cellular and sub-cellular levels, have revealed that the introduction of one or more oxygen atoms in the lateral chain of imidazolium cations can deeply reduce the biological activity respect to alkyl derivative ILs, achieving the goal of tuning the chemical structure to modify the toxicity. The biodegradability assays in soil have demonstrated that the increase in the polarity of the cations does not increase the biodegradability, being the oxygenated imidazolium salts completely not biodegradable. Alkyl imidazolium salts are more biodegradable, even if the after 6 months the level of biodegradation does not exceed the 50%. On the contrary pyridinium ILs in water appear to be readily biodegradable under aerobic conditions; in this case the introduction of oxygen atoms in the lateral chain of the cation does not influence the biodegradability rate, being alkyl and oxygenated pyridinium salts degradable at the same extent. Soil degradation studies are indeed very long lasting and this time drawback strongly hampers the feasibility of deep assessment of a wide category of substances like ionic liquids. The development of different approaches to the aim of biodegradation assessment will be pursued in the next future.

New sustainable syntheses and the applicability of ILs have been also evaluated by using renewable feedstock as starting material for new synthesis and by investigating ILs uses as solvents for both catalytic reactions and extraction processes.

To address the issue of improving the sustainability of ILs synthesis in this Thesis a chemical derived from renewable feedstock, furfural, has been used as building block; the new furan-based ammonium salts thus prepared have shown both good thermal stability and low viscosity, suitable for their exploitation as solvents, and interesting biological activity towards target organisms, indicating a potential use as biocides.

Finally two successful applications of ILs have been proposed.

Sulfonate anion-containing ILs have been used as reaction media for allylation reactions of carbonyl compounds, affording very high yields, good selectivity and easy recyclability of the solvent; the important goal of stereocontrol of the synthetic process will be addressed fatherly in the next future.

Switchable polarity solvents, considered as a new generation of ionic liquids thanks to their ability to switch from one ionic form to a non-ionic state by bubbling CO_2, have been exploited in the extraction of vegetable oils of potential interest as biofuels, from terrestrial and aquatic biomass. The extraction of algal biomass in particular has been accomplished very successfully, developing a new technology which gives the opportunity to bypass the steps of biomass treatment and extracts the oil directly from the algal culture.

Abbreviations

Ionic liquids

IL ionic liquid

RTIL room temperature ionic liquid

EMIM 1-ethyl-3-methylimidazolium

PMIM 1-propyl-3-methylimidazolium

BMIM 1-butyl-3-methylimidazolium

BEIM 1-butyl-3-ethylimidazolium

EOMMIM 1-ethoxymethyl-3-methylimidazolium

EOEMIM 1-ethoxyethyl-3-methylimidazolium

MOPMIM 1-methoxypropyl-3-methylimidazolium

PentMIM 1-penthyl-3-methylimidazolium

HEIM 1-hexyl-3-ethylimidazolium

HMIM 1-hexyl-3-methylimidazolium

HeptMIM 1-hepthyl-3-methylimidazolium

OMIM 1-octyl-3-methylimidazolium

NMIM 1-nonyl-3-methylimidazolium

DMIM 1-decyl-3-methylimidazolium

C12MIM 1-dodecyl-3-methylimidazolium

C14MIM 1-tetradecyl-3-methylimidazolium

C16MIM 1-hexadecyl-3-methylimidazolium

C18MIM 1-octadecyl-3-methylimidazolium

MOEMIM 1-methoxyethyl-3-methylimidazolium

M(OE)$_2$MIM 2-(2-methoxy-ethoxy)-ethyl-3-methylimidazolium

M(OE)$_3$MIM 2-(2-(2-methoxy-ethoxy)-ethoxy)-ethyl-3-methylimidazolium

M(OE)$_4$MIM 2-(2-(2-(2-methoxy-ethoxy)-ethoxy)-ethoxy)-ethyl-3-methylimidazolium

BMPy 1-butyl-3-methylpyridinium

BPy 1-butylpyridinium

HMPy 1-hexyl-3-methylpyridinium

HPy 1-hexylpyridinium

OPy 1-octylpyridinium

OMPy 1-octyl-3-methylpyridinium

MOEMPy 1-methoxyethyl-3-methylpyridinium

$M(OE)_2MPy$ 2-(2-methoxy-ethoxy)-ethyl-3-methylpyridinium

TMA tetramethylammonium

TEA tetraethylammonium

TBA tetrabutylammonium

BTBA benzyltributylammonium

TBP tetrabutylphosphonium

CY169 tributylethylphosphonium diethylphosphate

CY101 trihexyl(tetradecyl)posphonium bromide

ECOENG500 Peg-5 cocomonium methosulfate

AMMOENG130 ethylmethyl(dioctadecyl)ammonium chloride

Aliquat trioctylmethylammonium

BMPyr 1-butyl-1-methylpyrrolidinium

EMMor ethyl-methylmorpholinium

EBMor ethyl-butylmorpholinium

BMMor butyl-methylmorpholinium

ETHT ethyltetrahydrothiophenium

Et_3S triethylsulfonium

Cl chloride

BF_4 tetrafluoroborate

$N(CN)_2$ dicyanamide

PF_6 hexafluorophosphate

NTf_2 bis(trifluoromethylsulfonyl)imide

Br bromide

X halide

$OctSO_3$ octylsulfate

$(CF_3)_2N$ bis(trifluoromethyl)imide

SbF_6 hexafluoroantimonate

I iodide

OTf triflate

(Eco)-toxicity and biodegradation properties of ionic liquids

EC50 50% effect concentration

SE standard error

SNK Student-Newman-Keuls test

OECD Organization for Economic Co-operation and Development

MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide

AChE acetylcholinesterase

LDH lactate dehydrogenase

ASTM D 5988-96 standard method of ASTM

B3LYP/6-31G(d) Becke, three-parameter, Lee-Yang-Parr

ThOD theoretical oxygen demand

Applications of ionic liquids

BINOL 2,2'-binaphthol

$Ti(O\text{-}i\text{-}Pr)_4$ titanium tetraisopropoxide

Et_2Zn diethylzinc

TfOH trifluoromethansulfonic acid

MsOH methansulfonic acid

TsOH 4-methylbenzensulfonic acid

FIM functional solid ionic materials

SPS switchable polarity solvents

DBU 1,8-diazabicyclo-[5.4.0]-undec-7-ene

GC gas chromatography

FID flame ionisable detector

MS mass spectroscopy

ee enantiomeric excess

HPLC high performance liquid chromatography

NMR nuclear magnetic resonance

Tg glass transition temperature

Tm melting temperature

Tdec decomposition temperature

Fluorous solvents and supercritical fluids

FBS fluorous biphasic system

$scCO_2$ supercritical carbon dioxide

SCF supercritical fluids

T_c	critical temperature
p_c	critical pressure
PFCs	perfluorinated compounds
CFCs	chlorofluorocarbons
HCFCs	hydrochlorofluorocarbons
HFCs	hydrofluorocarbons
HFEs	hydrofluoroethers
PFMC	perfluoromethylcyclohexane
HIP	hydrophobic ion pairing
GWP	global warming potential
POPs	persistant organic pollutants
AOT	aerosol OT
SDS	sodium dodecyl sulfate
KDP	surfactant KDP 4606
pI	isoelectric point
CMC	critical micelle concentration
BCA	bicinchoninic acid
BSA	bovine serum albumin
CMT	α-chymotrypsin
Cc	Cytochrome c
APEE	*n*-acetyl-L-phenylalanine ethyl ester
APBE	*n*-acetyl-L-phenylalanine butyl ester
ExPASy	Expert Protein Analysis System
ε_{280}	extinction coefficient at 280 nm
mw	molecular weight
bs	basic residues

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