Flexible design of ionic liquids for membrane interactions

Lena Rakers¹ · Frank Glorius¹

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Abstract

In the last years, ionic liquids gained a lot of interest in materials sciences. Especially their interactions with biomolecules like lipids or proteins became more and more fascinating. One substance class of ionic liquids are imidazolium salts, which are usually known as the precursors of the more prominent *N*-heterocyclic carbenes (NHCs). Due to their high stability and easy synthesis, imidazolium salts became more attractive for applications in biological systems. In 2015, imidazolium salts bearing long alkyl chains in their backbone were established as lipid analogs. Interestingly, different effects of the intercalation into a membrane can be observed depending on the characteristics of the structure. Within this tutorial, we would like to highlight the modular synthesis of these compounds leading to a versatile tool box of lipid analogs that can be adjusted depending on the required needs.

Keywords Ionic liquid · Synthesis · Lipid analogs · Imidazolium salts

Introduction

Recently, ionic liquids became increasingly attractive tools in different fields of materials science including their application as for example solvents (Kuchenbuch and Giernoth 2015; Vekariya 2017) or electrolytes (Armand et al. 2009; Watanabe et al. 2017). Moreover, special attention was given to their fascinating interactions with biomolecules like phospholipids or proteins and their potential application as candidates in medicinal chemistry (Sivapragasam et al. 2016; Benedetto and Ballone 2016; Egorova et al. 2017; Benedetto 2017). A prominent class of compounds within this library of ionic liquids is imidazolium salts, which can be easily tuned depending on the required characteristics to lead to a high structural diversity. Furthermore, they show a great stability and can be employed as precursors for the well-known *N*-heterocyclic carbenes (NHCs) for example as ligands in transition metal catalysis (Hopkinson et al. 2014; De Fremont

et al. 2009; Hermann 2002). In addition to their application in organometallic chemistry, interesting interactions with biomolecules were observed (Riduan and Zhang 2013; Benedetto and Ballone 2016; Egorova et al. 2017; Benedetto 2017; Gravel and Schmitzer 2017). In 2015, we designed and synthesized a class of long alkyl chain containing imidazolium salts and analyzed their interactions with 1,2-dipalmitoyl-sn-glycero-3-phosphocholine (DPPC) membranes via biophysical measurements (Wang et al. 2015a, b; Wang et al. 2016; Rühling et al. 2017; Drücker et al. 2017). In contrast to *N*-substituted imidazolium salts, the alkyl chains were attached at the C4- and C5-position of the imidazolium core to resemble the structure of lipids resulting in a small, polar head group and long, flexible alkyl chains. Due to the high structural variability of imidazolium salts, a large number of compounds could be synthesized in a modular fashion. The versatile synthesis of these molecules will be described in here. Some other interesting structures like imidazolium salts with an alkyl chain attached to the *N*-substituent as vesicle forming agents (Blesic et al. 2007; Liu et al. 2016) or imidazolium salt-based artificial anion transporters (Elie et al. 2011; Elie et al. 2013; Gravel and Schmitzer 2015) were also synthesized but their in-depth discussion is beyond the scope of this article. For a description of the membrane interactions of the long-chain imidazolium salts, we would like to refer the reader to a recently published review by Wang et al. (2018).

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✉ Frank Glorius
glorius@uni-muenster.de

¹ Institute of Organic Chemistry, University of Münster, Corrensstrasse 40, 48149 Münster, Germany

Flexible synthesis of C4,C5-alkylated long-chain imidazolium salts

As mentioned above, imidazolium salts became interesting substances for interaction with biomolecules like phospholipids. Due to the facile structural variation, all five positions of the imidazolium core could in principle be modified to adapt the required characteristics individually. In order to create a lipid analog, we attached long alkyl chains in the backbone of the imidazolium core and varied the head group. The overall structural motif can be found in Fig. 1.

Variation of the structure could either occur at the headgroup (nature of the salt (imidazolium, thiazolium, or guanidinium salt), substituent at the nitrogen, C2 substituent, or counter anion) or at the alkyl chains (length) yielding in total six different motifs.

All synthesized compounds have a fatty acid aldehyde as the common starting material (see Scheme 1). The reported molecules were derived from 1-octanal, 1-dodecanal, or 1-hexadecanal resulting in a chain length of C₇, C₁₁, or C₁₅, respectively. Depending on the required needs, the chain length can be adjusted by choosing the appropriate aldehyde, thereby tuning the lipophilicity of the compounds. Nevertheless, choosing aldehydes above a length of C₁₅ resulted in a low solubility of the starting materials and products and therefore diminished yields. Additionally, only substances with symmetrical backbones were synthesized since the benzoin condensation (Scheme 1, step a) would lead to a mixture of products when applying two different aldehydes. With the resulting hydroxy ketone in hand, two different reaction pathways can be chosen: one includes the oxidation to the corresponding diketone (Scheme 1, step b) and the other one includes the synthesis of the α -bromo ketone (Scheme 1, step g), both reactions occurring in good to moderate yields. The imidazole core can be generated via a condensation of the diketone with ammonium acetate and an aldehyde (Scheme 1, step c). If an unsubstituted C2 position is required, paraformaldehyde is chosen, but also other aldehydes yield the imidazole with the corresponding moiety of the aldehyde being integrated as the C2 substituent. It is worth to mention that the purity of ammonium acetate influences the reaction significantly: if contaminated ammonium acetate was used in the

reaction, besides product formation also integration of the corresponding long-chain aldehyde at the C2 position occurred. The aldehyde is probably generated by decomposition of the diketone. Afterwards, the synthesis of the imidazolium salts can be performed by alkylation of the nitrogen atoms with alkyl halides (Scheme 1, step d) to yield a cationic lipid analog. The corresponding halide determines the counter anion of the salt though subsequent salt metathesis is feasible (Dinarès et al. 2009).

In case neutral compounds are required, the C2 unsubstituted imidazolium salts can be further functionalized. Deprotonation of the imidazolium salt in the presence of elemental sulfur leads to the formation of the corresponding thiourea (Scheme 1, step e). A neutral gold complex can be synthesized by forming a silver complex of the imidazolium salt first. Then, transmetalation to a gold precursor yields the corresponding gold complexes (Scheme 1, step f). Despite the application for membrane interactions, NHC-gold complexes serve as catalysts (Marion and Nolan 2008) in for example the hydration of alkynes (Rühling et al. 2015) or as potential anticancer candidates (Oehninger et al. 2013; Patil et al. 2015), which would render long-chain NHC gold complexes as interesting compounds for various applications. The synthesis of other headgroups or bigger *N*-substituents begins with the corresponding α -bromo ketone. Guanidinium salts can be generated by cyclization of the α -bromo ketone with *N*-Boc-guanidine followed by removal of the protecting group and protonation (Scheme 1, step h). Since functionalization of imidazoles with aromatic halides is difficult, aromatic *N*-substituents can be installed by reacting the α -bromo ketone with formamides (Scheme 1, step i). This approach allows the introduction of unsymmetrical *N*-substituents depending on the applied formamides (Hirano et al. 2008). Similarly to the alkylated imidazolium salts, the arylated imidazolium salts can be further functionalized to form the neutral gold complexes (Scheme 1, step j). However, the arylated imidazolium salts require no transmetalation step, so that the direct complex synthesis with chloro(tetrahydrothiophene)gold as precursor can be performed. Finally, another class of compounds that can be generated from fatty acid-derived aldehydes is thiazolium salts. First, the thiazole core is built up by cyclization of the α -bromo ketone with formamide and phosphorus pentasulfide (Scheme 1, step k). The alkylation of the *N*-substituent occurs similarly to the

Fig. 1 Variation of the headgroup or tail enables the modulation of the characteristics of the lipid analogs

Scheme 1 Synthesis of the long-chain compounds starting from the fatty acid aldehyde. The variation of the structure is highlighted in different colors (chain length: bold black; nature of the salt: red; N-substituent: blue; C2-substituent: orange). **Conditions:** **a:** 3-benzyl-5-(2-hydroxyethyl)-4-methylthiazol-3-ium, NEt_3 , EtOH, reflux; **b:** VOCl_3 , MeCN, 80 °C, O_2 atm.; **c:** Y = H: paraformaldehyde, Y = Me: acetaldehyde, NH_4OAc , HOAc, EtOH, 110 °C; **d:** R' = Me: methyl iodide, R' = Bn: benzyl bromide, K_2CO_3 , MeCN, 90 °C; **e:** S_8 , KOtBu , THF, RT; **f:**

Ag_2O , CH_2Cl_2 , RT; dimethyl sulfide gold chloride complex, CH_2Cl_2 , 50 °C; **g:** DDQ, $(n\text{Bu})_4\text{NBr}$, PPh_3 , CH_2Cl_2 , RT; **h:** N-Boc-guanidine, THF, 55 °C; CH_2Cl_2 , TFA, RT; **i:** HCl in 1,4-dioxane, RT; **j:** R = Me: MeI, 60 °C, R = Bn: CS_2 , NaOH, benzyl amine, DMSO, RT; **k:** Ar = Mes: dimesitylformamidine, Ar = Dipp: bis(2,6-diisopropylphenyl)formamidine, $i\text{Pr}_2\text{EtN}$, MeCN, 120 °C; Ac_2O , HBr, toluene, 90 °C; **l:** chloro(tetrahydrothiophene)gold complex, K_2CO_3 , acetone, 60 °C

alkylation of imidazoles via the reaction with alkyl halides (Scheme 1, step l) with the corresponding halide becoming the counter anion of the salt.

Due to the high level of structural flexibility of the synthesis, a diverse library of compounds could be synthesized and their effects on DPPC bilayers could be analyzed (Wang et al. 2015a, b; Wang et al. 2016; Rühling et al. 2017; Drücker et al. 2017). In general, different trends of the integrational behavior could be observed. Short alkyl chains (C_7) possess a more detergent-like

character, while greater chain lengths (C_{15}) enhance the level of intercalation and therefore reveal a lipid analogy. Studies of the cytotoxicity and antitumor and antimicrobial activity demonstrate a negative correlation with the chain length. Additionally, the head group has a great effect on the integrational behavior. Rigidification of the bilayers occurs for small and hydrophilic headgroups like methylated imidazolium or guanidinium salts. Contrary to this, bulky or hydrophobic headgroups like benzylated imidazolium salts promote the fluidization of the bilayer.

Conclusion

With this tutorial, we provide an overview on the synthesis plan of lipid analogues cationic or neutral compounds. Due to the high modularity of the syntheses, different characteristics can be adjusted easily by choosing the correct building blocks. Tuning of the headgroups or the chain length could influence the integrational behavior tremendously and could lead to different applications. Apart from the application as lipid analogs in lipid bilayers, the synthesized compounds could serve as catalysts, detergents, or anticancer agents rendering them as a highly versatile compound class. Moreover, the given overview could help to identify pathways for the synthesis of new compounds.

Compliance with ethical standards

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Conflict of interest Lena Rakers declares that she has no conflict of interest. Frank Glorius declares that he has no conflict of interest.

Ethical approval This article does not contain any studies with human participants or animals performed by any of the authors.

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