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PAPER

Facile *N*-alkylation of acridine esters with 1,3-propane sultone in ionic liquids

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Hydrophilic chemiluminescent acridinium esters containing *N*-sulfopropyl groups are extremely useful labels in the clinical diagnostics industry. The synthesis of these labels is normally accomplished by *N*-alkylation of the acridine ester precursors with the carcinogenic reagent 1,3-propane sultone in neat reactions where the alkylating reagent also serves as the solvent. Product yields are often poor, the reactions are not reproducible and are also difficult to scale-up. In our efforts to develop a greener and a more efficient synthesis of *N*-sulfopropyl acridinium esters, we have discovered that commonly used room temperature ionic liquids such as [BMIM][BF_4] and [BMIM][PF_6] are excellent media for the *N*-alkylation of poorly reactive acridine esters with 1,3-propane sultone. Advantages include a significant reduction in the amount of toxic 1,3-propane sultone needed for good conversion to product, and minimal formation of polysulfonated products. The alkylation reaction in ionic liquids is amenable to scale-up for the synthesis of gram quantities of hydrophilic, chemiluminescent acridinium esters.

Introduction

Chemiluminescent acridinium esters have detection limits in the attomole range and are extremely useful labels for both immunoassays and nucleic acid assays in the clinical diagnostics industry.[1] The hydrophilic acridinium ester NSP-DMAE-NHS[2] (Fig. 1, **2b**) and its derivatives containing *N*-sulfopropyl (NSP) groups are used extensively as chemiluminescent labels in automated immunoassays such as in Siemens Healthcare Diagnostics' ADVIA Centaur® systems. A key step in the synthesis of various NSP-DMAE derivatives is the difficult *N*-alkylation of the acridine *N*-hydroxysuccinimide (NHS) ester precursor (Fig. 1, **1b**) with 1,3-propane sultone. The *N*-alkylation of tertiary amines with 1,3-propane sultone can be carried out in organic solvents,[3] but the poor reactivity of the acridine ester **1b** necessitates performing the *N*-alkylation of this substrate with a 50-fold excess of 1,3-propane sultone in a sealed tube where the sultone acts as both alkylating reagent and solvent.[2] Decomposition of the alkylating reagent 1,3-propane sultone[4c] (both thermal and hydrolytic decomposition of propane sultone is reported) competes with the *N*-alkylation of the acridine nitrogen resulting in a tarry reaction mixture from which the acridinium ester is isolated by reverse-phase HPLC. Typically, total yield of product **2b** is only 25% after HPLC purification

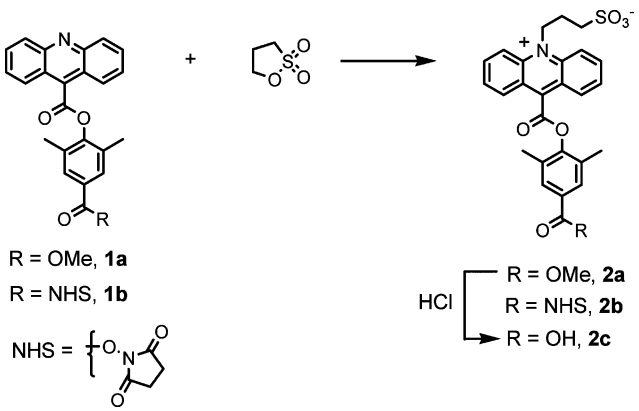

Fig. 1 *N*-Alkylation of acridine esters with 1,3-propane sultone.

because of decomposition and, the formation of polysulfonated material. The reaction is also difficult to scale up and is susceptible to gel formation depending upon the quality of the sultone reagent, which can further lower yields. Polysulfonation also makes HPLC purification of the product **2b** quite challenging.

Besides suffering from low yields and a difficult purification process, another significant drawback is the requirement for conducting the *N*-alkylation reaction in neat 1,3-propane sultone. It has been reported that 1,3-propane sultone is a potent carcinogen[4] that induces cancer in rats even after a single exposure, and that malignant tumors observed in humans exposed to this chemical appear to be consistent with the results seen from these animal studies.[4a] Unfortunately, there

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are few published reports that are preparatively useful for the *N*-alkylation of poorly reactive acridine esters with 1,3-propane sultone under milder conditions without the need for using a large excess of the sultone reagent and extensive chromatography. Adamczyk[5] *et al.* have reported that introduction of the *N*-sulfopropyl group in acridine *N*-sulfonylcarboxamides with poor reactivity can be performed using the reagent neopentyl 3-trifloxypropane sulfonate. However, *N*-alkylation using this reagent required 7 days to afford 34% conversion to product, and moreover the triflate had to be synthesized in three steps from neopentyl alcohol. In addition, acid hydrolysis of the neopentyl group was required to unmask the *N*-sulfopropyl group. In our experience too, with the exception of methyl triflate, other alkyl triflates show poor reactivity towards the acridine nitrogen of acridine esters such as **1a**. Therefore, despite its toxicity, 1,3-propane sultone remains an attractive alkylating reagent for the introduction of the water-soluble, *N*-sulfopropyl group of hydrophilic acridinium esters.

In exploring greener alternatives towards the syntheses of *N*-sulfopropyl acridinium esters by *N*-alkylation of their acridine ester precursors, one of our primary objectives was to reduce the usage of the toxic reagent 1,3-propane sultone in the manufacturing processes of these chemiluminescent labels. Our goal was to both minimize the environmental impact of this carcinogen as well as to alleviate the exposure risk to personnel involved in the manufacture of these compounds. We also wanted to develop a robust *N*-alkylation process that would be reproducible, be of general synthetic utility for a variety of ring-substituted acridine ester precursors and that would be amenable to scale-up for the synthesis of gram quantities of *N*-sulfopropyl acridinium esters without the need for extensive chromatography. Finally, in our attempts to identify a suitable and benign reaction medium for the *N*-alkylation reaction, we wanted to ensure that one carcinogen (1,3-propane sultone) was not merely substituted with another carcinogenic or toxic solvent.

Results and discussion

The *N*-alkylation of acridine esters with 1,3-propane sultone is representative of a substitution-type reaction where uncharged reactants yield a zwitterionic product. In the transition state of the reaction, the acridine nitrogen develops a partial positive charge which is accompanied by the development of a partial negative charge on the (incipient) sulfonate moiety. Based on studies by Hughes, Ingold and co-workers,[6] an increase in polarity of the reaction medium is expected to facilitate this reaction. For organic solvents, the empirically determined Kamlet–Taft parameter π^*, which is a measure of solvent dipolarity/polarizability, is a useful indicator of solvent polarity.[7] In addition, the Kamlet–Taft parameters α and β which denote the hydrogen bond donating and accepting ability respectively of a solvent, are also useful descriptors of a solvent's properties. A comprehensive list of π^*, α and β values of a variety of organic solvents has been compiled by Kamlet *et al.*[7e] For solvents that are traditionally considered non-polar, π^* is low ($\pi^* = 0.43$ for *p*-xylene) and it increases for solvents that are considered polar such as dimethyl sulfoxide ($\pi^* = 1.0$) and water ($\pi^* = 1.09$).

Ionic liquids[8,9] constitute a relatively new class of non-aqueous, polar compounds that are being explored extensively as reaction media for various synthetic transformations, and their unique properties can confer reactivity not normally observed in common organic solvents. An excellent review of the extensive literature can be found in a two volume book edited by Wasserscheid and Welton.[9] Kamlet–Taft parameters for various ionic liquids have been reported in the literature.[10,17] In general, the reported Kamlet–Taft parameter π^* is high for ionic liquids (typically > 0.8) indicating that these compounds are quite polar. On the other hand, α and β values show considerable variation and depend upon the structure of the cation and anion. For example, the 1-butyl-3-methylimidazolium cation [BMIM] has a higher value of α ($\alpha = 0.625$ for [BMIM][OTf]) than the 1-butyl-1-methylpyrrolidinium cation [BMPY] ($\alpha = 0.396$ for [BMPY][OTf]) indicating that the former cation is a stronger hydrogen bond donor.[17] For anions, β values, which are indicative of their basicity, are higher for anions such as triflate (OTf) and tetrafluoroborate (BF_4) when compared to the bis(trifluoromethylsulfonyl)imide [$N(Tf)_2$] anion,[10,17] Another empirically-derived solvent polarity scale is the Reichardt scale {$E_T(30)$ scale}, and an extensive compilation of $E_T(30)$ values of ionic liquids and other solvents has been published by Reichardt.[11]

In our initial experiments, we wanted to test the hypothesis that increased solvent polarity, as reflected by its π^* value, would facilitate the *N*-alkylation of acridine esters with 1,3-propane sultone. We selected the unsubstituted acridine ester **1a** (Fig. 1) as the substrate for these experiments. The choice of organic solvents was fairly straightforward (solvents listed in Table 1 are commonly used in synthesis) but, the choice of the ionic liquid(s) was not, given that a significant number of ionic liquids are commercially available and, many more have been described in the literature. To guide our selection of ionic liquids for the *N*-alkylation reaction and narrow our choices, we attempted to answer the following questions. Was there relevant literature precedent to support the use of a specific ionic liquid as a reaction medium in analogous reactions such as the *N*-alkylation of heterocycles? What are the toxicological properties of the ionic liquid that would replace 1,3-propane sultone as the solvent in the *N*-alkylation reaction of acridine esters? Would the selected ionic liquid withstand the reaction conditions such as elevated temperature necessary for the *N*-alkylation reaction?

Although the *N*-alkylation of acridines in ionic liquids, to the best of our knowledge, has not been reported in the

Table 1 *N*-Alkylation of acridine ester (**1a**) with 10 equivalents 1,3-propane sultone in various solvents

Solvent	Temperature (°C), reaction time (h)	HPLC conversion to **2a** (%)
p-Xylene	140, 4	5
DMSO	155, 1	<10
DMSO	155, 16	0
Diethylene glycol diethyl ether	155, 1	0
DMF	155, 6	35
Sulfolane	155, 6	60
Sulfolane	155, 24	67
Sulfolane	210, 2	55
[BMIM][BF_4]	155, 16	82
[BMIM][PF_6]	155, 24	87

literature, several preparative *N*-alkylation reactions of nitrogen-containing heterocycles in ionic liquids have been reported, such as the *N*-alkylation of 2,4-thiazolidinones, phthalimides, pyrroles and other nitrogen heterocycles.[12] Specifically, [BMIM][PF_6] was useful for improving yields in the *N*-alkylation of 2,4-thiazolidinones;[12a] both [BMIM][PF_6] and [BMIM][BF_4] were useful reaction media for the *N*-alkylation of phthalimides, indole and benzimidazole with various electrophiles;[12b] both [BMIM][PF_6] and [BMIM][BF_4] were used as solvents for the *N*-alkylation of pyrrole with various electrophiles;[12c] [BMIM][BF_4] was used a co-solvent for the *N*-alkylation of indole and pyrrole[12d] and, [BMIM]BF_4] was used as the solvent for the *N*-alkylation of benzotriazole.[12e] The ionic liquids [BMIM]BF_4] and [BMIM][PF_6] are also readily available from various commercial vendors and seemed to be suitable reaction media for exploring the *N*-alkylation of acridine esters with 1,3-propane sultone.

Until recently, ionic liquids were generally considered to be benign due to their low vapor pressure, but a large number of recent studies have shown that they can be quite toxic to aquatic and other organisms.[13] Many ionic liquids have significant solubility in water and can easily be introduced into the environment by this route. In their screen, Pretti *et al.*[13a] observed that ionic liquids with imidazolium, pyrrolidinium and pyridinium cations were non-toxic to zebrafish but two ionic liquids with quaternary alkylammonium cations and long alkyl chains were more toxic than organic solvents. A comprehensive evaluation of the toxicological properties of both cations and anions of alkyl imidazolium ionic liquids using an (eco)toxicological test battery has also been reported.[13d] Toxicity was related to the lipophilicity of the cation but anion effects were less pronounced. Among anions, BF_4^- or chloride ions were observed to be less toxic than the bis(trifluoromethylsulfonyl)imide anion.[13d] Stolte *et al.* compared the effect of head groups and side chains in the cations of structurally diverse ionic liquids and also related toxicity to the lipophilicity of the cation.[13e] The general consensus that emerges from these studies is that (a) within a class, cations with long alkyl chains should be avoided because they start resembling cationic surfactants which are known to be toxic and, (b) the structure of the cation also contributes to toxicity.[13e,13f] For anions the picture is not as clear except for the bis(trifluoromethylsulfonyl)imide anion. A recent review by Pham *et al.* summarizes the literature on toxicological studies of ionic liquids.[13h] Based on the literature on toxicological studies, both ionic liquids [BMIM]BF_4] and [BMIM][PF_6], with their relatively short alkyl chains do appear to be less toxic than more hydrophobic ionic liquids. A direct comparison of the carcinogenicity/cytotoxicity of 1,3-propane sultone and the two ionic liquids is not available. However, 1,3-propane sultone is a potent alkylating reagent whereas the two ionic liquids can be reasonably expected not to react with proteins and nucleic acids. Ironically, Malhotra and Kumar recently screened a number of imidazolium ionic liquids against 60 human tumor cell lines (National Cancer Institute, USA) and observed anti-tumor activity (unspecified mechanism) only for the more hydrophobic compounds.[14]

Finally, *N*-alkylation of the poorly reactive acridine nitrogen with 1,3-propane sultone requires elevated temperature (~150 °C) and ionic liquids selected for this reaction should be able to withstand these temperatures without decomposition. It has been shown that [BMIM][PF_6] can be distilled at 300 °C and high vacuum with minimal decomposition,[15a] but lower decomposition temperatures (150 °C) were reported for [BMIM][$N(Tf)_2$][15b] measured by gravimetry. Measurement of free imidazoles by potentiometic titration has been reported to be a more accurate method for measuring ionic liquid decomposition,[15c] and [BMIM][BF_4] was observed to be more stable than [BMIM][PF_6], although the total amount of imidazoles formed at 140 °C was quite small for both ionic liquids even after heating for several days. Based on these studies, both [BMIM][PF_6] and [BMIM][BF_4] appear to be quite stable so as to withstand the reaction conditions required for the *N*-alkylation of acridine esters with 1,3-propane sultone.

To assess the impact of the polarity of the solvent on the *N*-alkylation of acridine esters with propane sultone, we investigated this *N*-alkylation reaction in various solvents (Table 1) using **1a** as the substrate and measured the formation of the product NSP-DMAE methyl ester (Fig. 1, **2a**), by HPLC analysis. The solvents that we investigated ranged from non-polar *p*-xylene to polar aprotic solvents such as dimethyl formamide (DMF), dimethyl sulfoxide (DMSO), diethylene glycol diethyl ether, sulfolane and, the two ionic liquids [BMIM][BF_4] and [BMIM][PF_6]. The Kamlet–Taft parameters of these solvents are listed in Table 3, and although they are from different studies the same set of dyes (4-nitroaniline and *N*,*N*-diethyl-4-nitroaniline) were used to calculate these parameters.[10] Kamlet–Taft parameters for diethylene glycol diethyl ether was not listed by Kamlet *et al.*[7e] but the structurally similar methyl ether was reported to have $\pi^* = 0.64$. We conducted the *N*-alkylation of acridine methyl ester **1a** (25–50 mg) with ten equivalents of 1,3-propane sultone in these various solvents using the same concentrations of reagents and measured conversion to the acridinium ester **2a** by HPLC analysis. The results of these measurements are summarized in Table 1. The concentration of the acridine ester substrate was kept as high as possible (~0.25 M) for efficient reaction, and all reactions gave homogeneous solutions at the temperatures indicated in Table 1. The *N*-alkylation reaction in the non-polar solvent *p*-xylene, as expected, led to very poor conversion. In the polar aprotic solvent DMSO, product formation was minimal at short reaction times and extended heating led to substantial decomposition with no trace of product. The reaction in DMF led to only modest conversion of 35% after 6 h of heating and, extended reaction times only led to decomposition. Hardly any reaction was observed at a short reaction time in diethylene glycol diethyl ether, another polar aprotic solvent whose Kamlet–Taft parameter π^* indicates that it is less polar than both DMF and DMSO. Among the polar aprotic solvents that were investigated, the solvent sulfolane proved to be superior to DMF, and afforded modest conversion (60%) to product after 6 h at 155 °C. A longer reaction time of 24 h improved conversion somewhat to 67% without apparent decomposition of product or starting material by HPLC analysis but produced a foul-smelling reaction mixture indicating that the solvent may have decomposed. When the reaction was conducted at a higher temperature of 210 °C for 2 h in sulfolane, further improvement was not observed. Inclusion of five equivalents of the organic bases 2,6-di-*tert*-butylpyridine or 2,6-di-*tert*-butyl-4-methylpyridine in sulfolane

Table 2 *N*-Alkylation of acridine ester (**1a**) with 1,3-propane sultone in various ionic salts

Ionic liquid	1,3-Propane sultone (equivalents)	Reaction time (h)	HPLC conversion to **2a** (%)
[BMPY][N(Tf)$_2$]	2.5	4	25
[BMPY][N(Tf)$_2$]	5.0	4	35
[BMPY][N(Tf)$_2$]	5.0	24	40
[BMIM][BF$_4$]	5.0	24	45
[BMPY][BF$_4$]	2.5	24	39
[BMPY][BF$_4$]	5.0	24	43
[THTDP][N(Tf)$_2$]	2.5	24	<10
[THTDP][N(Tf)$_2$]	5.0	24	<10

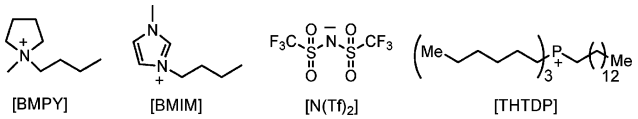


Table 3 Kamlet–Taft parameters[a] of the solvents used in the *N*-alkylation of acridine ester (**1a**) with 1,3-propane sultone

Solvent	π^*	α	β
p-Xylene	0.43	0	—
DMF	0.88	0	0.69
DMSO	1.00	0	0.76
Sulfolane	0.98	0	—
[BMPY][N(Tf)$_2$]	0.954	0.427	0.252
[BMIM][PF$_6$]	1.032	0.634	0.207
[BMIM][BF$_4$]	1.047	0.627	0.376
[THTDP][N(Tf)$_2$]	0.83	0.37	0.27

[a] From refs. 7e and 10.

at 155 °C for 16 h also did not improve conversion from 60%. In contrast to the other solvents, as indicated by the results in Table 1, both ionic liquids [BMIM][PF$_6$] and [BMIM][BF$_4$] proved to be excellent media for the *N*-alkylation reaction, affording >80% conversion to the *N*-sulfopropyl acridinium ester. These alkylation reactions proceeded cleanly with little evidence of decomposition by HPLC analysis, and were run for 16–24 h for optimal conversion.

The results in Table 1 are consistent with the prediction[6] that an increase in the polarity of the reaction medium will facilitate the *N*-alkylation reaction of acridine ester **1a** with 1,3-propane sultone. This reaction has a charged transition state, and the extent of conversion to product increases with an increase in the polarity of the solvent as reflected by its Kamlet–Taft parameter π^*. The only exception is DMSO where the alkylating reagent may have reacted with the solvent.[16]

In the next set of experiments (Table 2), we attempted to determine whether the acidity and basicity of the ions comprising the ionic liquid would also affect the *N*-alkylation reaction of the acridine ester substrate **1a** with 1,3-propane sultone. Besides solvating the transition state of the reaction, the cations and anions of an ionic liquid can also interact with the two reacting species. For example, Crowhurst *et al.*[17] have reported that in the reaction of tertiary tri-*n*-butylamine with methyl-*p*-nitrobenzenesulfonate, with the same triflate anion, the less acidic [BMPY] cation (lower α) afforded faster reaction rates than the [BMIM] cation which was attributed, in part to reduction in the nucleophilicity of the amine by its interaction with the more acidic [BMIM] cation. In the case of primary *n*-butylamine, faster reaction rate was also observed with the more basic triflate anion (higher β) than the bis(trifluoromethylsulfonyl)imide anion when paired with the [BMPY] cation. In this case the triflate anion was thought to stabilize the transition state of the reaction more effectively by being a stronger hydrogen bond acceptor for the incipient ammonium ion.

For the *N*-alkylation of acridine ester **1a** with 1,3-propane sultone, the ionic solvents selected for this second study (Table 2) were 1-butyl-1-methylpyrrolidinium tetrafluoroborate [BMPY][BF$_4$], 1-butyl-1-methylpyrrolidinium bis(trifluoromethylsulfonyl)imide [BMPY][N(Tf)$_2$] and the phosphonium ionic liquid trihexyltetradecylphosphonium bis(trifluoromethylsulfonyl)imide [THTDP] [N(Tf)$_2$]. The [BMPY] cation has a lower value of α and was not expected to interact as well as the [BMIM] cation with the acridine nitrogen. Consequently a faster reaction with 1,3-propane sultone and better conversion to product was expected in ionic liquids containing this cation. We also compared the impact of anion basicity by pairing the [BMPY] cation with both the more basic tetrafluoroborate anion (higher β) as well as the less basic bis(trifluoromethylsulfonyl)imide anion. The phosphonium ionic liquid represented a unique case, and was selected because while it has the lowest π^* among the various ionic liquids in this study and therefore was expected to be less effective in transition state stabilization in the *N*-alkylation reaction, it also has the lowest value of α and clearly would be the least effective in deactivating the acridine nitrogen.

All the reactions listed in Table 2 were conducted in a similar manner using the same concentrations of reagents (~0.1 M acridine ester **1a** in the ionic salt limited by solubility) and only 2.5–5 equivalents of distilled 1,3-propane sultone. We reasoned that by using only a limited quantity of the alkylating reagent, differences in reactivity in the various ionic salts would be more easily discerned. The results in Table 2 indicate that both sets of cations ([BMPY] and [BMIM]) are equally effective in solvating the transition state of the *N*-alkylation reaction when paired with tetrafluoroborate anion. All three ionic salts [BMPY][BF$_4$], [BMIM][BF$_4$] and [BMPY][N(Tf)$_2$] also afforded similar conversion of 40–45% to product after 24 h using 5 equivalents of 1,3-propane sultone. Enhanced reactivity and improved conversion to product was not observed with the [BMPY] cation even with the more basic tetrafluoroborate anion. Anion basicity (β) also did not have any impact on the *N*-alkylation reaction as indicated by the similar levels of conversion observed with the [BF$_4$] anion (β = 0.376) and the [PF$_6$] anion (β = 0.207) when paired with the [BMIM] cation (Table 1) or, when the [BMPY] cation was paired with [BF$_4$] anion and less basic [N(Tf)$_2$] anion (Table 2). Our results suggest that for the *N*-alkylation of acridine esters, the polarity of the ionic liquid as reflected by the Kamlet–Taft parameter π^* and, its ability to solvate the transition state of the reaction is the most important parameter dictating reactivity and conversion. Consistent with this conclusion are the results observed in [THTDP][N(Tf)$_2$] which showed the poorest conversion because it is the least polar ionic liquid investigated in this study (lowest π^*) despite containing a cation which should interact only weakly (lowest α) with the acridine nitrogen of **1a**. The acridine

nitrogen of **1a** and similar acridine esters is very hindered and large organic cations may be sterically excluded from interacting with it.

Having identified suitable ionic liquids and reaction conditions for the *N*-alkylation reaction of acridine ester **1a** with reduced quantities of 1,3-propane sultone, we next turned our attention to determine whether the reaction in ionic liquids was practical *i.e.* would these reactions be amenable to scale-up for the synthesis of gram quantities of *N*-sulfopropyl acridinium esters and would product isolation be straightforward without the need for extensive chromatography? While the reactions in Table 1 were carried out on a small scale (25–50 mg acridine ester), using distilled 1,3-propane sultone, unsurprisingly, scale-up of the reaction to 1–2 g of the acridine ester **1a** led to even better conversion to product (>85%). Moreover, the larger scale reactions did not require distillation of the toxic sultone reagent and were more tolerant to the quality of the sultone reagent with no evidence of polymerization or gel formation in the reactions. For example, the reaction in [BMIM][PF_6] was carried with 2 grams of the acridine methyl ester **1a** and was conveniently worked up by diluting the reaction mixture in ethyl acetate and collecting the crude product **2a** by filtration. Hydrolysis of the methyl ester with hydrochloric acid followed by simple precipitation afforded good recovery (1.68 g, 66%) of the *N*-sulfopropyl acridinium carboxylic acid **2c** in excellent purity (94%). A similar reaction carried out in [BMIM][BF_4] using 1 g of the acridine ester **1a** gave, after hydrolysis of the methyl ester **2a**, 0.79 g (62%) of the *N*-sulfopropyl acridinium carboxylic acid **2c**. The alkylation reaction in the two ionic liquids also showed minimal polysulfonation. This is illustrated in Fig. 2, where HPLC analyses of the *N*-alkylation reactions of acridine methyl ester **1a** with 1,3-propane sultone in either neat 1,3-propane sultone or in [BMIM][PF_6], are shown. As is evident from Fig. 2, the reaction in ionic liquid shows mostly monosulfonated product **2a** eluting at 6.7 min, whereas the reaction conducted in neat 1,3-propane sultone shows a significant amount of polysulfonation that is manifested as a series of peaks of diminishing intensity eluting after the main product peak. In Fig. 2, the starting material **1a** elutes at 10.3 min. Polysulfonated acridinium ester byproducts can be converted to the desired monosulfonate by acid hydrolysis but clearly, acid-sensitive functional groups in the acridinium ester are also expected to be cleaved by this procedure.

The absence of polysulfonation in ionic liquids allowed for a significant improvement in product yield of NSP-DMAE-NHS ester **2b** as exemplified by the *N*-alkylation of acridine NHS ester precursor **1b** with ten equivalents of 1,3-propane sultone in [BMIM][PF_6]. *N*-Alkylation of 0.5 g of the acridine ester precursor (Fig. 1, **1b**) led to, after HPLC purification, 0.356 g (56%) of the product NSP-DMAE-NHS ester **2b** which, represents a greater than doubling of the yield using five-fold less toxic 1,3-propane sultone over the procedure described by Law[2] using the same reaction scale. Although feasible, we did not attempt to recycle the ionic liquid from these *N*-alkylation reactions to minimize exposure to the toxic sultone reagent. The formation of hydrogen fluoride in [BMIM][PF_6] in the presence of traces of water has been reported to be a concern,[18] which we avoided by drying the ionic liquid at high vacuum over phosphorus pentoxide prior to reaction.

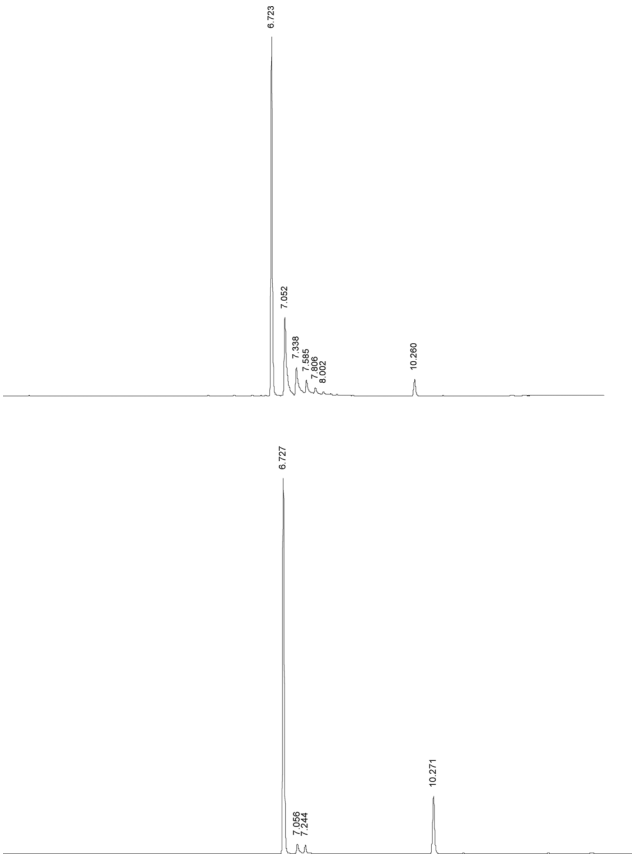
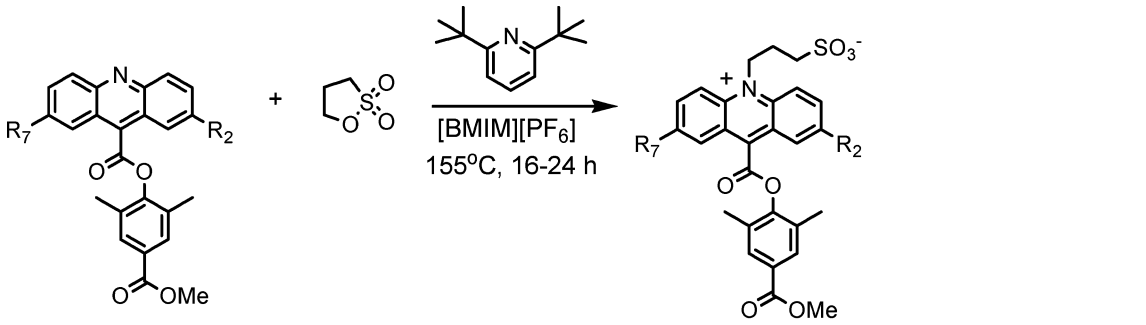


Fig. 2 HPLC chromatograms of *N*-alkylation reactions of **1a** with 1,3-propane sultone, (a) neat reaction (upper panel) and, (b) in [BMIM][PF_6] respectively.

The *N*-alkylation reaction in ionic liquids could be extended to other acridine ester substrates as well as illustrated in Fig. 3. Acridinium ester labels with alkoxy groups at C-2 and/or C-7 of the acridinium ring exhibit enhanced light output and improved sensitivity in immunoassays when compared to unsubstituted acridinium esters.[19] The synthetic precursors of these acridinium esters with alkoxy groups in the acridine ring were also *N*-alkylated cleanly with 1,3-propane sultone in ionic liquids. The presence of the alkoxy groups necessitated using a slightly greater excess of the sultone reagent and inclusion of the base 2,6-di-*tert*-butylpyridine to obtain efficient conversion. Even though these reactions were conducted under anhydrous conditions, we suspect that acidic impurities in the propane sultone reagent may protonate the more basic acridine nitrogen of these alkoxy-substituted substrates thereby requiring base for neutralization. As illustrated in Fig. 3, *N*-alkylation reactions of both monoalkoxy-substituted and dialkoxy-substituted acridine ester precursors with 15 equivalents of 1,3-propane sultone in [BMIM][PF_6] in the presence of 2,6-di-*tert*-butylpyridine (7.5 equivalents) afford excellent conversion to the *N*-sulfopropyl acridinium esters. These reactions too proceeded very cleanly with minimal polysulfonation and the products could be conveniently separated from the base and ionic liquid by chromatography on silica gel. The compound **6b** is a critical synthetic intermediate in the synthesis of a hydrophilic, high-light-output, acridinium ester label that is currently used in Siemens Healthcare Diagnostics' ADVIA Centaur,



3a, R_7 = H, R_2 = -OCH$(CH_3)_2$

3b, R_7 = H, R_2 = -OCH$(CH_3)_2$, 90%

4a, R_2 = R_7 = -OCH$(CH_3)_2$

4b, R_2 = R_7 = -OCH$(CH_3)_2$, 93%

5a, R_7 = H, R_2 = -OCH$(CH_2OCH_3)_2$

5b, R_7 = H, R_2 = -OCH$(CH_2OCH_3)_2$, 81%

6a, R_2 = R_7 = -O$(CH_2CH_2O)_5CH_2CH_2$OMe

6b, R_2 = R_7 = -O$(CH_2CH_2O)_5CH_2CH_2$OMe, 90%

Fig. 3 *N*-Alkylation of alkoxy-substituted acridine esters with 1,3-propane sultone.

high-sensitivity troponin assay (TnI-Ultra® assay). The presence of the bulky methoxyhexa(ethylene) glycol moieties in the acridine ring further reduces the reactivity of the acridine nitrogen of **6a**, requiring a vast excess (typically > 100-fold) of 1,3-propane sultone to obtain good conversion in the neat reaction. In [BMIM][PF_6], the *N*-alkylation of **6a** with 15 equivalents of 1,3-propane sultone and in the presence of 7.5 equivalents of 2,6-di-*tert*-butylpyridine led to 90% conversion to the acridinium ester **6b**, thereby greatly curtailing the amount of the toxic alkylating reagent. Isolation of the acridinium ester **6b** was equally straightforward using chromatography on silica gel.

In light of the encouraging results observed in the *N*-alkylation of acridine esters in ionic liquids, we attempted to replace the sultone reagent with non-toxic 3-halopropylsulfonate salts as alkylating reagents. However, these reagents afforded minimal conversion even after extensive heating in ionic liquids. The current study also prompts the question whether there is scope for further improvement in the synthetic process of *N*-sulfopropyl acridinium esters. Our results suggest that an increase in the polarity (π^*) of ionic liquids may afford faster reactions and may permit further reduction of the alkylating reagent 1,3-propane sultone in these reactions. For example, ethylammonium nitrate (π^* = 1.24) and *sec*-butylammonium thiocyanate (π^* = 1.28) are more polar[20] than [BMIM][BF_4] and [BMIM][PF_6] but for obvious reasons cannot be used for *N*-alkylation reactions. Several of the quaternary tetraethylammonium salts reported in this study are fairly low melting (π^* values were not reported), and similar salts with inert anions could be useful in the *N*-alkylation reaction of acridine esters, and moreover they may also be less toxic than imidazolium-based ionic liquids.[13e,f]

Conclusions

In conclusion, we have discovered that the *N*-alkylation of poorly reactive acridine phenolic esters with 1,3-propane sultone in the ionic liquids [BMIM][BF_4] and [BMIM][PF_6] afford excellent conversion to the commercially useful, chemiluminescent, *N*-sulfopropyl acridinium esters using only a limited quantity of the carcinogenic sultone reagent. In contrast to *N*-alkylation reactions in neat 1,3-propane sultone, the reactions in ionic liquids are (a) amenable to scale-up to gram quantities, (b) are reproducible, (c) proceed cleanly with minimal polysulfonation or polymerization and, (d) permit product isolation in a straightforward manner. Ionic liquids may offer a unique reaction medium for similar and difficult *N*-alkylation reactions of heterocycles.

Experimental

General

Chemicals and ionic liquids were purchased from Sigma–Fluka–Aldrich (Milwaukee, WI, USA). The ionic liquids were dried under high vacuum over phosphorus pentoxide prior to use. HPLC analyses and purification were performed using either a Beckman–Coulter HPLC system or a Waters HPLC system. For HRMS (High Resolution Mass Spectra), samples were dissolved in HPLC-grade methanol and analyzed by direct-flow injection (injection volume = 5 μL) ElectroSpray Ionization (ESI) on a Waters Qtof API US instrument in the positive ion mode. NMR spectra of the *N*-sulfopropyl acridinium esters were recorded in CF_3COOD on a Varian 500 MHz spectrometer.

General procedure for the small scale *N*-alkylation of 2′,6′-dimethyl-4′-methoxycarbonylphenyl acridine-9-carboxylate (1a) with 1,3-propane sultone.

The following procedure illustrated in DMF solvent was typical. A mixture of 2′,6′-dimethyl-4′-methoxycarbonylphenyl acridine-9-carboxylate **1a** (50 mg, 0.13 mmol) and distilled 1,3-propane sultone (160 mg, 10 equivalents) in anhydrous DMF (0.5 mL) was heated in an oil bath at 155 °C. After 6 h, HPLC analysis was performed using a 3.9 × 300 mm, 10 micron,

C_{18} column from Phenomenex and a 30-minute gradient of 10% → 100% MeCN/water (each with 0.05% TFA) at a flow rate of 1 mL min^{-1} and UV detection at 260 nm. Product **2a** eluted at 17.5 min. Comparison with the amount of unreacted starting material eluting at 26 min indicated approximately 35% conversion. When HPLC analysis was performed using a 4.0 × 50 mm, YMC, 3 micron C_{18} column and 10-minute gradient of 10% → 90% MeCN/water (each with 0.05% TFA) at a flow rate of 1 mL min^{-1} and UV detection at 260 nm, product eluted at 6.7 min and starting material at 10.3 min as illustrated in Fig. 2.

Small-scale reactions in other solvents were performed similarly. Reaction with neat 1,3-propane sultone was carried out in a sealed tube with 2′,6′-dimethyl-4′-methoxycarbonylphenyl acridine-9-carboxylate **1a** (25 mg, 0.065 mmol) and 1,3-propane sultone (396 mg, 50 equivalents).

N-Alkylation on a gram scale of 2′,6′-dimethyl-4′-methoxycarbonylphenyl acridine-9-carboxylate (1a) with 1,3-propane sultone in [BMIM][PF_6] followed by hydrolysis

To an 8 dram vial was added 2.0 g (5.19 mmol) of the acridine ester **1a**, 15 g (52.8 mmol) of 1-butyl-3-methylimidazolium hexafluorophosphate [BMIM][PF_6] (Fluka) and 6.34 g (51.9 mmol) of 1,3-propane sultone (Aldrich) under nitrogen. This sealed vial was heated to 155 °C for 24 h. Then the reaction mixture was cooled to ~40 °C and added dropwise into 500 mL of ethyl acetate with stirring which gave a yellow precipitate. The mixture was stirred at room temperature for 2 h and filtered. The filter cake was washed with ethyl acetate (3 × 50 mL) to give 6.19 g of a yellow solid (**2a** containing ethyl acetate) which was used directly in the next step as follows. A small portion of **2a** was purified by HPLC for spectral analysis. δ_H (500 MHz, CF_3COOD) 2.64 (s, 6 H), 3.05 (br s, 2 H), 3.94 (br s, 2 H), 4.17 (d, 3 H, J = 1.47), 5.96 (dd, 2 H, J = 17.12, 8.31), 8.10 (s, 2 H), 8.23 (t, 2 H, J = 7.70), 8.65 (t, 2 H, J = 8.07), 8.88 (d, 2 H, J = 8.56), 9.02 (d, 2 H, J = 9.05); HRMS m/z $(M + H)^+$ 508.1438 (508.1430 calculated).

The above 6.19 g of crude acridinium ester **2a** was added to 80 mL of 1 N HCl. The reaction mixture was heated to 110 °C for 4 h and then cooled to 2–8 °C overnight. A yellow precipitate was formed which was recovered by filtration. The filter cake was washed with de-ionized water (40 mL × 3) and diethyl ether (20 mL × 2) and dried under high vacuum over P_2O_5 which gave the acridinium carboxylic acid **2c** as a yellow powder (1.68 g, 66% yield from **1a**, 94% purity by HPLC). δ_H (500 MHz, CF_3COOD) 2.65 (s, 6 H), 3.04 (m, 2 H), 3.93 (t, 2 H, J = 6.11), 5.96 (m, 2 H), 8.16 (s, 2 H), 8.23 (dd, 2 H, J = 8.68, 6.97), 8.64 (t, 2 H, J = 8.07), 8.87 (d, 2 H, J = 8.07), 9.02 (d, 2 H, J = 9.54); HRMS m/z $(M + H)^+$ 494.1273 (494.1273 calculated).

N-Alkylation on a gram scale of 2′,6-dimethyl-4′-methoxycarbonylphenyl acridine-9-carboxylate (1a) with 1,3-propane sultone in [BMIM][BF_4] followed by hydrolysis

To an 8 dram vial was added 1.0 g (2.6 mmol) of the acridine ester **1a**, 10 g (44.2 mmol) of 1-butyl-3-methylimidazolium tetrafluoroborate [BMIM][BF_4] (Fluka) and 3.18 g (26.0 mmol) of 1,3-propane sultone (Aldrich) under nitrogen. This sealed vial was heated to 155 °C for 24 h. Then the reaction mixture was cooled to room temperature which gave a clear, red liquid (>85% conversion to product by HPLC). Product isolation and conversion to the acridinium carboxylic acid **2c** was performed as described previously (0.793 g, 62%).

N-Alkylation on a half-gram scale of 2′,6-dimethyl-4′-*N*-succinimidyloxycarbonylphenyl acridine-9-carboxylate (1b) with 1,3-propane sultone in [BMIM][PF_6]

To an 8 dram vial was added 0.50 g (1.07 mmol) of 2′,6-dimethyl-4′-*N*-succinimidyloxycarbonylphenyl acridine-9-carboxylate **1b**, 5 g (17.6 mmol) of 1-butyl-3-methylimidazolium hexafluorophosphate [BMIM][PF_6] (Fluka) and 1.31 g (10.7 mmol) of 1,3-propane sultone (Aldrich) under nitrogen. This sealed vial was heated to 155 °C for 16 h. Then the reaction mixture was cooled to ~40 °C and added dropwise to 100 mL of ethyl acetate with stirring which gave a yellow precipitate. The mixture was stirred at room temperature for 2 h and filtered. The filter cake was washed with ethyl acetate (30 mL × 2) and dried under high vacuum which gave 0.753 g of a yellow solid. The crude product was purified by preparative HPLC on a Waters HPLC system using an YMC, C_{18} 50 × 500 mm column, with a solvent flow rate of 60 mL min^{-1}, UV detection at 260 nm, elution being performed with 35% MeCN/water (with 0.05% TFA). After lyophilization of the HPLC fractions, the product NSP-DMAE-NHS ester **2b**, (0.356 g, 56%), was obtained as a yellow powder. δ_H (500 MHz, CF_3COOD) 2.67 (s, 6 H), 3.05 (br s, 2 H), 3.25 (br s, 4 H), 3.94 (t, 2 H, J = 6.11), 5.96 (d, 2 H, J = 8.56), 8.17 (s, 2 H), 8.23 (dd, 2 H, J = 8.56, 6.85), 8.64 (t, 2 H, J = 8.07), 8.86 (d, 2 H, J = 8.31), 9.03 (d, 2 H, J = 9.29); HRMS m/z $(M + H)^+$ 591.1432 (591.1437 calculated).

N-Alkylation of 2′,6′-dimethyl-4′-methoxycarbonylphenyl 2-isopropyloxyacridine-9-carboxylate (3a) with 1,3-propane sultone in [BMIM][PF_6]

A mixture of **3a** (0.213 g, 0.48 mmol), 1,3-propane sultone (0.88 g, 7.21 mmol, 15 equivalents) and 2,6-di-*tert*-butylpyridine (0.79 mL, 3.6 mmol, 7.5 equivalents) in [BMIM][PF_6] (3 mL) was heated and stirred at 155 °C in a tightly capped, round bottom flask under an inert atmosphere. After 16 h, the reaction was cooled to room temperature. A small portion (2 uL) was withdrawn, diluted with MeCN (0.2 mL)and analyzed by HPLC using a 3.9 × 300 mm, 10 micron, C_{18} column from Phenomenex and a 30-minute gradient of 10% → 100% MeCN/water (each with 0.05% TFA) at a flow rate of 1 mL min^{-1} and UV detection at 260 nm. Product **3b** eluted at 19.5 min. Comparison with the amount of unreacted starting material **3a** eluting at 30 min indicated 90% conversion. The reaction mixture was diluted with ethyl acetate (5 mL) and loaded onto a column of silica gel. The column with eluted with 500 mL each of ethyl acetate to remove base and starting material followed by 40% methanol/ethyl acetate to elute product. Product fractions were combined and concentrated under reduced pressure to give a dark yellow solid (0.25 g, 93%) which could be used as such further elaboration. For spectral analysis, a small portion was purified by preparative HPLC as described previously to remove trace (ionic liquid) impurities. δ_H (500 MHz, CF_3COOD) 1.50 (d, 6 H, J = 6.11), 2.53 (s, 6 H), 2.91 (m, 2 H), 3.32 (br s, 2 H), 4.06 (s, 3 H), 4.90 (dt, 1 H, J = 12.17, 6.02), 5.80 (m, 2 H), 7.84 (d, 1 H, J = 2.69), 7.98 (s, 2 H), 8.05 (dd, 1 H, J = 8.80, 6.85), 8.18 (dd, J = 10.03,

2.69), 8.40 (m, 1 H), 8.68 (dd, 1 H, J = 8.68, 0.86), 8.81 (d, 1 H, J = 3.18), 8.82 (d, 1 H, J = 3.91); HRMS m/z (M + H)$^+$ 588.1678 (588.1668 calculated).

N-Alkylation of 2′,6′-dimethyl-4′-methoxycarbonylphenyl 2,7-di-isopropyloxyacridine-9-carboxylate (4a) with 1,3-propane sultone in [BMIM][PF$_6$]

A mixture of **4a** (0.220 g, 0.44 mmol), 1,3-propane sultone (0.80 g, 6.6 mmol, 15 equivalents) and 2,6-di-*tert*-butylpyridine (0.724 mL, 3.3 mmol, 7.5 equivalents) in [BMIM][PF$_6$] (3 mL) was heated and stirred at 155 °C in a tightly capped, round bottom flask under an inert atmosphere. After 16 h, the reaction was cooled to room temperature. A small portion (2 uL) was withdrawn, diluted with MeCN (0.2 mL) and analyzed by HPLC using a 3.9 × 300 mm, 10 micron, C$_{18}$ column from Phenomenex and a 30-minute gradient of 10% → 100% MeCN/water (each with 0.05% TFA) at a flow rate of 1 mL min^{-1} and UV detection at 260 nm. Product **4b** eluted at 22.3 min. Comparison with the amount of unreacted starting material **4a** eluting at 31 min indicated 93% conversion. The reaction mixture was diluted with ethyl acetate (5 mL) and loaded onto a column of silica gel. The column was eluted with 500 mL each of ethyl acetate to remove base and starting material followed by 40% methanol/ethyl acetate to elute product. Product fractions were combined and concentrated under reduced pressure to give a dark yellow solid (0.286 g, quantitative) which could be used as such further elaboration. For spectral analysis, a small portion was purified by preparative HPLC as described previously to remove trace (ionic liquid) impurities. δ_H (500 MHz, CF_3COOD) 1.59 (d, 12 H, J = 6.11), 2.64 (s, 6 H), 2.98 (br s, 2 H), 3.89 (br s, 2 H), 4.17 (s, 3 H), 4.99 (hep, 2 H, J = 6.08), 5.86 (br s, 2 H), 7.89 (d, 2 H, J = 1.96), 8.09 (s, 2 H), 8.17 (dd, 2 H, J = 9.90, 1.83), 8.83 (d, 2 H, J = 10.03); HRMS m/z (M + H)$^+$ 624.2271 (624.2267 calculated).

N-Alkylation of 2′,6′-dimethyl-4′-methoxycarbonylphenyl 2-[(1,3-dimethoxypropyl)oxy]acridine-9-carboxylate (5a) with 1,3-propane sultone in [BMIM][PF$_6$]

A mixture of **5a** (0.095 g, 0.188 mmol), 1,3-propane sultone (0.345 g, 2.83 mmol, 15 equivalents) and 2,6-di-*tert*-butylpyridine (0.310 mL, 1.41 mmol,7.5 equivalents) in [BMIM][PF$_6$] (1.3 mL) was heated and stirred at 155 °C in a tightly capped, round bottom flask under an inert atmosphere. After 16 h, the reaction was cooled to room temperature. A small portion (2 uL) was withdrawn, diluted with MeCN (0.2 mL) and analyzed by HPLC using a 3.9 × 300 mm, 10 micron, C$_{18}$ column from Phenomenex and a 30-minute gradient of 10%→70% MeCN/water (each with 0.05% TFA) at a flow rate of 1 mL min^{-1} and UV detection at 260 nm. Product **5b** eluted at 23 min. Comparison with the amount of unreacted starting material **5a** eluting at 32 min indicated 81% conversion. The reaction mixture was diluted with ethyl acetate (5 mL) and loaded onto a column of silica gel. The column was eluted with 250 mL each of ethyl acetate to remove base and starting material followed by 40% methanol/ethyl acetate to elute product. Product fractions were combined and concentrated under reduced pressure to give a dark yellow oil (0.200 g, quantitative) which could be used as such further elaboration. For spectral analysis, a small portion was purified by preparative HPLC as described previously to remove trace (ionic liquid) impurities. δ_H (500 MHz, CF_3COOD) 2.54 (s, 6 H), 2.92 (m, 2 H), 3.62 (s, 6 H), 3.82 (t, 2 H, J = 6.24), 4.05 (br s, 4 H), 4.08 (s, 3 H), 5.19 (m, 1 H), 5.84 (m, 2 H), 8.00 (s, 2 H), 8.03 (d, 1 H, J = 1.71), 8.11 (t, 1 H, J = 7.83), 8.25, dd, 1 H, J = 9.78, 1.71), 8.46 (t, 1 H, J = 8.07), 8.78 (d, 1 H, J = 8.80), 8.88 (d, 1 H, J = 9.54), 8.91 (d, 1 H, J = 10.03); HRMS m/z (M + Na)$^+$ 648.1874 (648.1879 calculated).

N-Alkylation of 2′,6′-dimethyl-4′-methoxycarbonylphenyl-2,7-bis(3,6,9,12,15,18-hexaoxanonadec-1-yloxy)acridine-9-carboxylate (6a) with 1,3-propane sultone in [BMIM][PF$_6$]

A mixture of **6a** (0.158 g, 0.162 mmol), 1,3-propane sultone (0.297 g, 2.433 mmol 15 equivalents) and 2,6-di-*tert*-butylpyridine (0.269 mL, 1.217 mmol, 7.5 equivalents) in [BMIM][PF$_6$] (0.9 mL) was heated and stirred at 155 °C in a 2 dram vial, under an inert atmosphere. After 16 h, the reaction was cooled to room temperature. A small portion (2 uL) was withdrawn, diluted with MeCN (0.2 mL) and analyzed by HPLC using a 4.0 × 50 mm, YMC, 3 micron C$_{18}$ column and 10-minute gradient of 10% → 90% MeCN/water (each with 0.05% TFA) at a flow rate of 1 mL min^{-1} and UV detection at 260 nm, Product **6b** eluted at 7.0 min. Comparison with the amount of unreacted starting material **6a** eluting at 9.0 min indicated 90% conversion. The reaction mixture was diluted with ethyl acetate (5 mL) and loaded onto a column of silica gel. The column was eluted with 500 mL each of ethyl acetate to remove base and starting material followed by 40% methanol/ethyl acetate to elute product. Product fractions were combined and concentrated under reduced pressure to give a dark yellow solid (0.110 g, 62%, 92% purity by HPLC) which could be used as such further elaboration. For spectral analysis, a small portion was purified by preparative HPLC as described previously to remove trace (ionic liquid) impurities. δ_H (500 MHz, CF_3COOD) 2.65 (s, 6 H), 3.01 (br s, 2 H), 3.68 (s, 6 H), 3.90 (t, 2 H, J = 6.11), 3.97 (m, 4 H), 3.99–4.05 (m, 28 H), 4.09 (m, 4 H), 4.19 (s, 7 H), 4.33 (br s, 4 H), 4.60 (m, 4 H), 5.92 (br s, 2 H), 7.93 (d, 2 H, J = 2.45), 8.11 (s, 2 H), 8.22 (dd, J = 9.78, 2.20), 8.92 (d, 2 H, J = 10.03); HRMS m/z (M + H)$^+$ 1096.4746 (1096.4787 calculated).

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