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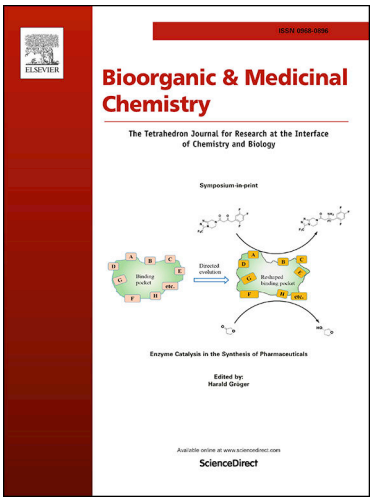

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Bioorganic & Medicinal Chemistry

Inhibition of bacterial growth and galactosyltransferase activity of WbwC by α, ω-bis(3-alkyl-1*H*-imidazolium)alkane salts: effect of varying carbon content

Alexander Kocev[a], Jacob Melamed[a], Shuo Wang[a], Xianqi Kong[b], Jason Z.Vlahakis[b], Yaozu Xu[a,b], Walter A. Szarek[b*], Inka Brockhausen[a*]
[a]*Department of Biomedical and Molecular Sciences, Queen's University, Kingston, Ontario K7L 3N6, Canada*
[b]*Department of Chemistry, Queen's University, Kingston, Ontario K7L 3N6, Canada*

[*]Corresponding authors: Tel: 1 613 533 2643
E-mail addresses: szarekw@chem.queensu.ca (W. A. Szarek), brockhau@queensu.ca (I. Brockhausen).

ABSTRACT

A series of compounds was designed and synthesized having two imidazolium rings separated by a polymethylene spacer and having alkyl substituents on each of the imidazolium rings. The compounds were assayed for their effects on the activity of galactosyltransferase WbwC, and also on the growth of Gram-negative and Gram-positive bacteria, as well as human cells. The inhibition observed on enzyme activities and cell growth was dependent on the total number of carbons in the spacer and the alkyl substituents on the imidazolium rings. These readily synthesized, achiral compounds have potential as antimicrobial and antiseptic agents.

Keywords:
Imidazolium salts
Glycosyltransferase inhibitors
Cell cultures
Bacterial cultures

1. Introduction

The development of new classes of antibacterial drugs is urgent and important as resistance to existing classes of antibiotics emerges. Electron-deficient tetrazolium,[1] triazolium,[2] and imidazolium[3,4] compounds were initially discovered as potent inhibitors of *Plasmodium falciparum* parasite replication.[3] The positively charged imidazolium salts could potentially bind to phosphate groups or other negatively charged groups found in proteins, DNA, membrane lipids, and glycosyltransferase substrates. The latest generation of our compounds consists of bivalent imidazolium salts in which the imidazolium rings are separated by a polymethylene spacer and have alkyl substituents on each of the rings. We previously observed that imidazolium compounds with at least 18 carbons in the aliphatic spacer were effective glycosyltransferase inhibitors and selectively inhibited a number of mammalian and bacterial glycosyltransferases.[4-8] For example, α2,3-sialyltransferase WbwA[6] and β1,3-galactosyltransferase WbwC from *Escherichia coli* O104[7] were strongly inhibited by imidazolium compounds with spacers of 18 or more carbons. However, another galactosyltransferase (WbwB) showed little inhibition.[8] The structures of the bis-imidazolium compounds are not related to structures of glycosyltransferase acceptor or donor substrates and inhibition appears to mainly target the proteins.

We have now synthesized a new series of compounds, varying the spacer length and the size of the alkyl substituents with the aim to improve their potency and possibly specificity. In Table 1 are given details of the structures of the new compounds (**1**–**21**). Here, we studied the inhibition of bacterial β1,3-galactosyltransferase WbwC from highly pathogenic *Escherichia coli* O104[7]. This enzyme binds UDP-Gal as the donor substrate and GalNAc-diphosphate-phenyl-undecyl acceptor substrate. WbwC has been classified by the Carbohydrate-Active Enzymes (CAZy) databank into the GT2 family, having a DDxD motif where Asp residues are critical for activity. The genes encoding WbwC homologs are also found in several other bacteria. An inhibition of these enzymes would reduce the synthesis of O antigens that are virulence factors.

The effects of compounds on the growth of two Gram-negative bacteria (*Escherichia coli* and *Pseudomonas aeruginosa*) and two Gram-positive bacteria (*Bacillus subtilis* and *Staphylococcus epidermidis*), and for comparison, on the proliferation of mammalian cell cultures, were examined. We found that compounds with high carbon content were able to both, inhibit glycosyltransferase WbwC, and block bacterial growth and mammalian cell proliferation. The results suggest the potential of imidazolium compounds as antimicrobials or antiseptic agents.

2. Results and discussion

2.1. Chemical synthesis

The candidate bis-imidazolium salt compounds **1**–**21** were synthesized following a straightforward synthetic approach, as shown in Figure 1. The general strategy involved treating α,ω-alkanediols with methanesulfonyl chloride/triethylamine in THF to afford the α,ω-bis(methanesulfonyloxy)alkanes as described in a previous publication.[4] 1,16-Hexadecanediol was synthesized from 16-hexadecanolide by reduction using $LiAlH_4$ in THF. The 1-alkylimidazoles were obtained by treatment of 1-bromoalkanes with imidazole/tetraethylammonium iodide/sodium hydroxide in toluene following a published procedure.[9] The final bis-imidazolium compounds in the dimesylate form (compounds **1**–**21**) were then obtained by the reaction of the appropriate α,ω-bis(methanesulfonyloxy)alkane with the appropriate 1-alkylimidazole in acetone, as described in our previous publication[4] for the synthesis of 1,22-bis(3-methyl-1*H*-imidazolium-1-yl)docosane dimesylate.

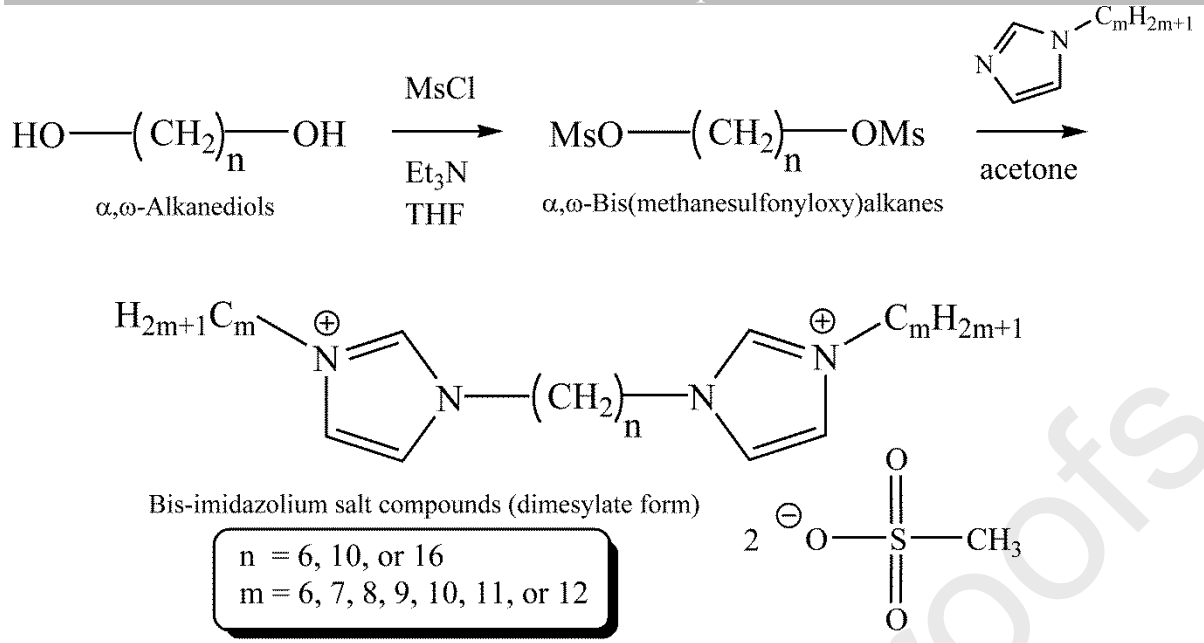


Figure 1. The synthesis of bis-imidazolium salt compounds (dimesylate form).

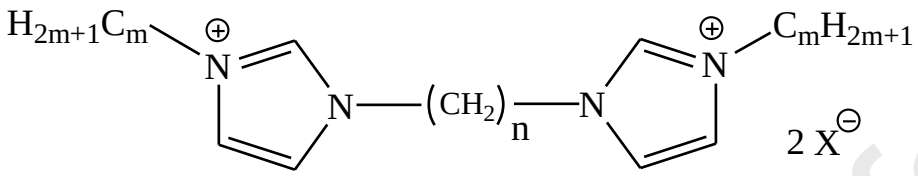
Reaction of β1,3-galactosyltransferase WbwC from *E. coli* O104:

UDP-[^{3}H]Gal + GalNAcα-PO_3-PO_3-$(CH_2)_{11}$-O-phenyl →

UDP + [^{3}H]Galβ1-3GalNAcα-PO_3-PO_3-$(CH_2)_{11}$-O-phenyl

Figure 2.
In vitro β1,3-**galactosyltransferase reaction by WbwC utilizing a synthetic analog of the natural diphosphate-undecaprenol-based acceptor substrate** GalNAcα-PO_3-PO_3-$(CH_2)_{11}$-O-phenyl (GalNAc-PP-PhU). The reaction product can be isolated by C18 chromatography and quantified by scintillation counting.

Table 1. Inhibition of β1,3- galactosyltransferase activity of WbwC by α,ω-bis(3-alkyl-1*H*-imidazolium)alkane salts (at 0.5 mM concentration). The acceptor concentration was 0.1 mM and inhibitory compounds were at 0.5 mM. All assay mixtures contained 10% methanol. Compounds were tested in at least duplicate determinations. The IC_{50} values were determined of highly active inhibitors at increasing inhibitor concentrations.



Compound	Number of carbon atoms in spacer (n)	Number of carbon atoms in each alkyl substituent (m)	Total number of carbons (spacer + alkyl substituents, n+2m)	% Inhibition at 0.5mM	IC_{50} (μM)
1	6	6	18	6	
2	6	7	20	16	
3	6	8	22	13	
4	6	9	24	<10	
5	6	10	26	92	263
6	6	11	28	99	117
7	6	12	30	90	43
8	10	6	22	<1	
9	10	7	24	<1	
10	10	8	26	64	123
11	10	9	28	95	23
12	10	10	30	98	152
13	10	11	32	98	62
14	10	12	34	91	59
15	16	6	28	88	48
16	16	7	30	95	11
17	16	8	32	93	18
18	16	9	34	93	35
19	16	10	28	92	147
20	16	11	38	92	123
21	16	12	40	92	121
22	4	1	6	9	
23	6	1	8	7	
24	8	1	10	21	
25	14	1	16	10	
26	15	1	17	11	
27	16	1	18	<1	
28	18	1	20	<1	
29	20	1	22	91	230
30	22	1	24	91	159

The syntheses of compounds **22**–**30** were reported in ref. 4. Compounds **1**–**21**, **29**, and **30** were prepared as dimesylate salts, and compounds **22**–**28** were prepared as dichloride salts.

2.2. Inhibition of β1,3-galactosyltransferase WbwC

The compounds **1**–**21** were soluble at 5 mM concentration in MeOH, EtOH and DMSO. Assays containing candidate compounds were carried out at 37°C with 10 % MeOH present. However, strong inhibition was also observed in control assays lacking MeOH, suggesting that there is significant solubility of compounds in the aqueous assay solution.

In bacterial cells glycosyltransferases are thought to be located at the cytoplasmic face of the inner membrane where they access the nucleotide sugar-donor substrates, as well as the diphosphate–lipid acceptor substrates that are anchored in the membrane. Bis-imidazolium compounds having spacers of 18 to 22 carbons and a methyl group on each of the imidazolium rings were previously found[4] to be strong inhibitors of selected glycosyltransferases while compounds having shorter spacers and unsusbstituted imidazole rings were ineffective. In the present study, the new compounds **1**–**21** were examined with β1,3-Gal-transferase WbwC from *E. coli* O104[7]. Included also are results with compounds **22**–**30** that only have 1-carbon substituents on the imidazolium rings. The donor substrate was UDP-[^{3}H]Gal and the acceptor substrate was 0.1 mM GalNAc-PP-PhU (Figure 2). The results of the inhibition studies are summarized in Table 1. The comparison of inhibition of purified enzyme with enzyme in supernatants revealed remarkable similarity (data not shown). The length of the polymethylene spacer as well as that of the alkyl substituents had a significant effect on the inhibitory properties of the compounds. High inhibition (>90 %) of WbwC activity was achieved with compounds having spacers of 20 and 22 carbons, or with compounds having a total number of carbons (spacer + alkyl substituents) of 26 to 40.

When comparing compounds having a 6-carbon spacer, the alkyl substituent had to have a length of at least 10 carbons to yield effective inhibition. The IC_{50} values were 263 and 117 μM for compounds **5** and **6** having 10 and 11 carbons in each alkyl substituent. The IC_{50} value was lower (43 μM) for compound **7** having longer (12-carbon) substituents. Similarly, in the series of compounds having 10-carbon spacers, compounds **8** and **9** having shorter substituents were not inhibiting WbwC, while compounds having substituents of 8 to12 carbons were inhibitors with IC_{50} values between 23 and 152 μM. The entire series of compounds having 16-carbon spacers and 6- to 12-carbon substituents (compounds **15** to **21**) were successful inhibitors of WbwC with IC_{50} values of 11 to 147 μM (Table 1).

Compounds having only 1-carbon substitutents (**22** to **30**) and 4- to 18-carbon spacers showed minimal inhibition while compounds **29** and **30**, having 20- and 22-carbon spacers, strongly inhibited with IC_{50} values of 230 and 159 μM, respectively.

The results indicate that the length of the spacers of the compounds control the inhibition, and in addition, the alkyl substituents played a critical role in creating highly effective inhibitors. In contrast, inhibitors of mammalian Gal-transferases (e.g., 1-thio-*N*-butyrylGlcN*β*-(2-naphthyl)[10] did not inhibit WbwC activity. Interestingly, strong inhibitors (compounds **5, 7, 11, 14, 15, 21**) had only minor effects on the activity of bovine milk β1,4-galactosyltransferase using 0.5 mM GlcNAcβ-Bn as the acceptor and 0.5 mM candidate compound.

In order to examine if the inhibition of the hydrophobic and positively charged compounds was due to their interactions with glycosyltransferase substrates, the compounds were mixed with the donor substrate UDP-[^{3}H]Gal and buffers at different pH values and ratios, followed by chromatography using a C_{18} Sep-Pak cartridge Plus (Waters). In the absence of buffer, compounds **6** and **12** caused a 50 % retention of UDP-[^{3}H]Gal by the C_{18} Sep-Pak column at a molar ratio of 2.5:1 (compound **6** or **12**:UDP-[^{3}H]Gal). This result was likely due to electrostatic interaction between the phosphates of UDP and imidazolium groups, thereby increasing the hydrophobic property of UDP-Gal when complexed with the compounds. However, in the presence of buffer at pH 7, no retention of UDP-[^{3}H]Gal was observed under these conditions.

Both UDP-[^{3}H]Gal and [^{3}H]Galβ1-4GlcNAc-PP-PhU[11], quantitatively bound to AG-1x8 anion-exchange columns due to the presence of phosphate groups, and inhibitor **7** had no effect on this binding. It was concluded that binding of negatively charged enzyme substrates to positively

charged compounds was not the cause of glycosyltransferase inhibition. Therefore, the inhibition appeared to be glycosyltransferase-protein specific and likely based on spatially defined charge and hydrophobic interactions. This conclusion is corroborated by previous findings that only a selected number of glycosyltransferases were inhibited although they shared donor and acceptor substrates.[4]

2.3. Effect of compounds on bacterial growth

To assess the effect of bis-imidazolium compounds on live bacteria in culture, the compounds were added to bacterial culture media and overnight growth was estimated by counting colony numbers. At 50 μM concentration in DMSO, uncharged compounds, namely, 1,16-bis(1*H*-imidazol-1-yl)hexadecane, 1,18-bis(1*H*-imidazol-1-yl)octadecane , and 1,20-bis(1*H*-imidazol-1-yl)eicosane[4], and positively charged compounds **22** and **23** with short spacers did not show any effect on bacterial growth of *P. aeruginosa* PAO1 cells. A minor growth effect was seen with compound **28** (having an 18-carbon spacer) at 50 μM and with compound **25** (having a 14-carbon spacer) at 5 to 25 μM. In contrast, significant growth inhibition was seen by compound **29** at 50 μM having a 20-carbon spacer. Compound **30** (having a 22-carbon spacer) at 50 μM concentration showed no colonies, and at 25 μM only 5% of the colonies were visible (Table 2).

In contrast, compounds **10** and **14** at 5 to 25 μM strongly inhibited PAO1 bacterial growth while compound **20** had a less potent effect but eliminated colonies at 25 μM (Table 2). The mammalian galactosyltransferase inhibitor 1-thio-*N*-butyrylGlcN*β*-(2-naphthyl)[5] at 50 μM had no effect on PAO1 bacterial growth.

In comparison, Gram-negative *Escherichia coli* BL21 showed strong inhibition by compounds **10**, **14** and **20** with 26, 34 and 38 aliphatic carbons, respectively, at 5, 10, and 25 μM concentrations with no apparent colonies. Compound **25** (with a 14-carbon spacer) was much less effective at the same concentrations. Thus, the inhibitory effects were slightly different between the two Gram-negative bacteria. The effects of candidate compounds on the growth of Gram-positive bacteria *Staphylococcus epidermidis* and *Bacillus subtilis* closely resembled those of *E. coli*. No or few colonies were seen after treatment with compounds **10**, **14**, and **20** at 5 to 25 μM concentrations, and compound **25** did not appear to affect *S. epidermidis.* In addition, compound **30** at 50 μM eliminated colonies of *B. subtilis* and *S. epidermidis*.

These four strains of bacteria have different glycosyltransferases and produce different polysaccharides. Our results suggest that the candidate compounds having a total number of carbons (spacer + alkyl substituents) of 26 to 38 may have a general effect on disrupting the functions of bacterial membranes. This effect might be attributable to the possible detergent-like properties of candidate compounds by making the membranes leaky, or affecting the structure of the membrane that is a prerequisite for the functions of membrane-bound or membrane-associated enzymes and proteins. It is unlikely that this growth inhibition is mediated through inhibition of the cytoplasmic enzymes that assemble polysaccharides. However, of the previously synthesized compounds[4] and the newly synthesized compounds (**1** to **21**) tested here, the inhibition of Gal-transferases paralleled the inhibition of bacterial growth, suggesting a common underlying mechanism. These possibilities should be explored in future experiments.

Table 2. Effect of α,ω-bis-(3-alkyl-1*H*-imidazolium)alkanes on growth of bacteria.[a]
P.a., *Pseudomonas aeruginosa* PAO1; E.c., *Escherichia coli* BL21; B.s., *Bacillus subtilis*; S.e., *Staphylococcus epidermidis.* ND, not determined. Bacteria were pre-incubated for 4 hours at 37 °C with and without candidate compound in 1% MeOH and then incubated overnight at 37°C. [a]In the absence of candidate compound, but with 1% MeOH in the culture medium, the number of colonies was set to 100%. All experiments were carried out in at least duplicate determinations. The average percentages of colony numbers counted on each plate are shown as percentage of controls lacking inhibitory compounds.

Compound	Total number of carbons (spacer + alkyl substituents)	Concentration µM	Inhibition of colony number (%)			
			P.a.	E.c.	B.s.	S.e
22	6	50	0	ND	ND	ND
23	8	50	0	ND	ND	ND
25	16	5	0	0	8	0
		10	4	32	20	0
		25	22	51	51	0
29	22	50	>90	ND	ND	ND
30	24	25	>95	ND	ND	ND
		50	100	ND	ND	ND
10	26	5	96	100	100	100
		10	100	100	100	100
		25	100	100	100	100
14	34	5	>98	100	100	100
		10	100	100	100	100
		25	>98	100	100	100
20	38	5	28	100	100	100
		10	77	100	100	100
		25	100	100	100	100

2.4. Effect of candidate compounds on mammalian cells in culture

Human embryonic kidney (AD293), lung cancer (A549) and prostate cancer (PC-3)[12] cells, as well as human ovarian cancer cells, OC-3-VGH, showed inhibition of cell proliferation by candidate compounds measured by the MTT assay. DMSO (0.1%) was used as the solvent for compounds in cell cultures that were not compatible with MeOH. Uncharged compounds, namely, 1,16-bis-(1*H*-imidazol-1-yl)hexadecane, 1,18-bis-(1*H*-imidazol-1-yl)octadecane, and 1,20-bis-(1*H*-imidazol-1-yl)eicosane[4] at 50 μM, as well as solvent controls did not affect cell growth. However, compounds with longer spacers, namely, compound **28** (18 spacer carbons) and compound **29** (20 spacer carbons) showed up to 80% inhibition of cell proliferation with IC_{50} values varying between 5 and 12 μM for OC-3-VGH cells. The effects on PC-3, A549 and AD293 cells were similar. The mammalian galactosyltransferase inhibitor 1-thio-*N*-butyrylGlcN*β*-(2-naphthyl)[5] had a comparatively minor effect on OC-3-VGH cells and at 1 mM reduced growth by 50 %. To further examine the effect of **29**, the scratch wound healing assay of ovarian cancer cells was employed. The migration of OC-3-VGH cells was significantly inhibited after 18 h incubation with 500 μM **29**, compared to vehicle-treated control cells containing the same amount of DMSO solvent (data not shown). The effects of the candidate compounds were not cancer-cell specific or selective. As with bacteria, it is possible that the inhibitory effects on whole cells are mediated through interactions with components of cell membranes. It remains to be shown if compounds penetrate membranes and act on internal structures such as phosphorylated lipids or DNA in order to block cell growth.

3. Conclusions

The work described showed that, in the case of the newly synthesized bis-imidazolium compounds **1**–**21**, those having a total number of non-imidazolium carbons (spacer + alkyl substituents) of at least 26, afforded potent inhibitors of two bacterial glycosyltransferases (see Table 1). Targeted delivery of these inhibitors into bacterial cells has the potential of blocking the biosynthesis of outer polysaccharides which are virulence factors. It remains to be shown if the bis-imidazolium salts induce inactive, glycosyltransferase-protein conformations. The structural features determine the inhibitory potential of the compounds. It is noteworthy that compounds that exhibited strong inhibition of glycosyltransferase activity (e.g. compounds **14** and **20**, Table 1), also inhibited the growth of bacteria (Table 2). and could act as adjuvant antibiotics but the relationship between glycosyltransferase inhibition and growth inhibition has not yet been clarified. Several mechanisms for inhibition are possible, for example, the compounds may disturb the structures and functions of membrane components, thereby exhibiting cell-growth inhibition of both Gram-negative and Gram-positive bacteria as well as human cells. It remains to be shown if compounds penetrate through membranes and inactivate compounds critical for growth. Future work will be directed towards the differential toxic effects in cells and animal models, and the effective concentrations necessary for the design of bacteria-specific inhibitors.

4. Experimental

4.1. General

Melting points were measured on a Mel-Temp II apparatus and are uncorrected. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker Avance 400 spectrometer in DMSO-d_6 or CD_3OD. The chemical shifts are reported in δ (ppm) relative to tetramethylsilane.[13] The compounds synthesized were deemed to be >95% pure by ^{1}H NMR analysis. High-resolution ESI mass spectra were performed by the AIMS Mass Spectrometry Laboratory (Department of Chemistry) at The University of Toronto on an Agilent 6538 UHD instrument.

Compounds **1**–**21** were synthesized for the present study, and the experimental details are reported herein; syntheses for compounds **22**–**30** are described in a previous publication.[4] All other chemical reagents were obtained from Sigma–Aldrich and used without prior purification.

4.2. General procedure for the formation of α,ω-bis(methanesulfonyloxy)alkanes

Under an atmosphere of nitrogen, the α,ω-alkanediol (8.5 mmol, 1 equiv) was dissolved in warm tetrahydrofuran (20 mL) and the solution was then cooled to 0 ºC. To this solution was added triethylamine (2.5 equiv) and then methanesulfonyl chloride (2.2 equiv). The mixture was stirred at room temperature overnight, concentrated, and then dried under high vacuum. Water was added, and the resulting solid was collected by filtration and washed with water (250 mL). The solid material was dried under high vacuum to give the product.

4.3. General procedure for the formation of 1-alkylimidazoles

A mixture of imidazole (22 mmol, 1 equiv) and the alkyl bromide (1 equiv) in toluene (30 mL) in the presence of tetraethylammonium iodide (0.2 equiv) and sodium hydroxide (3 equiv) was heated at reflux temperature for 10 h. The resulting mixture was cooled to room temperature, water was added, and the mixture extracted twice with ethyl acetate. The combined organic extracts were washed with brine, dried over sodium sulfate, and concentrated. Purification by flash-column chromatography on silica gel using ethyl acetate as an eluent, followed by drying under high vacuum gave the product.

4.4. General procedure for the formation of bis-imidazolium compounds (dimesylate form)

Under an atmosphere of nitrogen, the α,ω-bis(methanesulfonyloxy)alkane (0.36 mmol, 1 equiv) was mixed with the appropriate 1-alkylimidazole (5 equiv) and the mixture was heated at 80–90 ºC for 1h; acetone (20 mL) was then added and the mixture was stirred at reflux temperature overnight, concentrated, and dried under high vacuum. The resulting golden oil was dissolved in methanol, the solution was concentrated, and the residual oil was washed with diethyl ether (10 mL). This dissolution/concentration/washing procedure was repeated two more times. The solid material was dried under high vacuum to give the product.

4.5. Characterization of bis-imidazolium compounds (dimesylate form) synthesized following the general procedure outlined in Section 4.4

4.5.1. 1,6-Bis(3-hexyl-1*H*-imidazolium-1-yl)hexane dimesylate (1)

Obtained in 82% yield from reaction of 1,6-bis(methanesulfonyloxy)hexane with 1-hexylimidazole affording a yellow oil: ^{1}H NMR (400.13 MHz, DMSO-d_6): δ 0.86 (t, J = 7.0 Hz, 6H), 1.16–1.33 (m, 16H), 1.71–1.85 (m, 8H), 2.32 (s, 6H), 4.17 (t, J = 7.2 Hz, 8H), 7.82 (s, 4H), 9.26 (s, 2H); ^{13}C NMR (100.61 MHz, DMSO-d_6): δ 13.8 (2C), 21.8 (2C), 24.8 (2C), 25.1 (2C), 29.0 (2C), 29.2 (2C), 30.4 (2C), 39.8 (2C), 48.6 (2C), 48.8 (2C), 122.5 (4C), 136.0 (2C); HRMS (ESI) $[M\text{-}C_2H_6S_2O_6]^{2+}$ calcd for $C_{24}H_{44}N_4$: 194.1778. Found: 194.1778.

4.5.2. 1,6-Bis(3-heptyl-1*H*-imidazolium-1-yl)hexane dimesylate (2)

Obtained in 85% yield from reaction of 1,6-bis(methanesulfonyloxy)hexane with 1-heptylimidazole affording a golden oil: ^{1}H NMR (400.13 MHz, DMSO-d_6): δ 0.85 (t, J = 6.8 Hz, 6H), 1.15–1.33 (m, 20H), 1.71–1.85 (m, 8H), 2.31 (s, 6H), 4.16 (t, J = 7.0 Hz, 8H), 7.81 (s, 4H), 9.26 (s, 2H); ^{13}C NMR (100.61 MHz, DMSO-d_6): δ 13.9 (2C), 21.9 (2C), 24.8 (2C), 25.4 (2C), 28.0 (2C), 29.0 (2C), 29.3 (2C), 31.0 (2C), 39.8 (2C), 48.7 (2C), 48.8 (2C), 122.5 (4C), 136.0 (2C); HRMS (ESI) $[M\text{-}C_2H_6S_2O_6]^{2+}$ calcd for $C_{26}H_{48}N_4$: 208.1934. Found: 208.1939.

4.5.3. 1,6-Bis(3-octyl-1*H*-imidazolium-1-yl)hexane dimesylate (3)

Obtained in 82% yield from reaction of 1,6-bis(methanesulfonyloxy)hexane with 1-octylimidazole affording a golden oil: ^{1}H NMR (400.13 MHz, DMSO-d_6): δ 0.85 (t, J = 6.6 Hz, 6H), 1.15–1.33 (m, 24H), 1.72–1.84 (m, 8H), 2.31 (s, 6H), 4.16 (t, J = 7.0 Hz, 8H), 7.81 (s, 4H), 9.25 (s, 2H); ^{13}C NMR (100.61 MHz, DMSO-d_6): δ 13.9 (2C), 22.0 (2C), 24.8 (2C), 25.5 (2C), 28.3

(2C), 28.4 (2C), 29.0 (2C), 29.3 (2C), 31.1 (2C), 39.8 (2C), 48.7 (2C), 48.8 (2C), 122.5 (4C), 136.0 (2C); HRMS (ESI) $[M\text{-}C_2H_6S_2O_6]^{2+}$ calcd for $C_{28}H_{52}N_4$: 222.2091. Found: 222.2097.

4.5.4. 1,6-Bis(3-nonyl-1*H*-imidazolium-1-yl)hexane dimesylate (4)

Obtained in 78% yield from reaction of 1,6-bis(methanesulfonyloxy)hexane with 1-nonylimidazole affording a golden oil: ^{1}H NMR (400.13 MHz, DMSO-d_6): δ 0.85 (t, J = 6.6 Hz, 6H), 1.14–1.34 (m, 28H), 1.72–1.85 (m, 8H), 2.31 (s, 6H), 4.16 (t, J = 6.8 Hz, 8H), 7.81 (s, 4H), 9.26 (s, 2H); ^{13}C NMR (100.61 MHz, DMSO-d_6): δ 13.9 (2C), 22.1 (2C), 24.8 (2C), 25.5 (2C), 28.3 (2C), 28.5 (2C), 28.8 (2C), 29.0 (2C), 29.3 (2C), 31.2 (2C), 39.8 (2C), 48.6 (2C), 48.8 (2C), 122.4 (4C), 136.0 (2C); HRMS (ESI) $[M\text{-}C_2H_6S_2O_6]^{2+}$ calcd for $C_{30}H_{56}N_4$: 236.2247. Found: 236.2252.

4.5.5. 1,6-Bis(3-decyl-1*H*-imidazolium-1-yl)hexane dimesylate (5)

Obtained in 73% yield from reaction of 1,6-bis(methanesulfonyloxy)hexane with 1-decylimidazole affording a golden oil: ^{1}H NMR (400.13 MHz, DMSO-d_6): δ 0.85 (t, J = 6.6 Hz, 6H), 1.15–1.34 (m, 32H), 1.72–1.84 (m, 8H), 2.31 (s, 6H), 4.16 (t, J = 7.0 Hz, 4H), 4.16 (t, J = 7.0 Hz, 4H), 7.80 (s, 2H), 7.81 (s, 2H), 9.25 (s, 2H); ^{13}C NMR (100.61 MHz, DMSO-d_6): δ 13.9 (2C), 22.1 (2C), 24.8 (2C), 25.5 (2C), 28.3 (2C), 28.6 (2C), 28.8 (2C), 28.9 (2C), 29.0 (2C), 29.3 (2C), 31.3 (2C), 39.8 (2C), 48.6 (2C), 48.8 (2C), 122.4 (4C), 136.0 (2C); HRMS (ESI) $[M\text{-}C_2H_6S_2O_6]^{2+}$ calcd for $C_{32}H_{60}N_4$: 250.2404. Found: 250.2409.

4.5.6. 1,6-Bis(3-undecyl-1*H*-imidazolium-1-yl)hexane dimesylate (6)

Obtained in 68% yield from reaction of 1,6-bis(methanesulfonyloxy)hexane with 1-undecylimidazole affording a golden oil: ^{1}H NMR (400.13 MHz, DMSO-d_6): δ 0.85 (t, J = 6.6 Hz, 6H), 1.15–1.33 (m, 36H), 1.72–1.84 (m, 8H), 2.32 (s, 6H), 4.16 (t, J = 7.2 Hz, 4H), 4.17 (t, J = 7.0 Hz, 4H), 7.80 (s, 2H), 7.81 (s, 2H), 9.26 (s, 2H); ^{13}C NMR (100.61 MHz, DMSO-d_6): δ 13.9 (2C), 22.1 (2C), 24.8 (2C), 25.5 (2C), 28.3 (2C), 28.7 (2C), 28.8 (2C), 28.91 (2C), 28.94 (2C), 29.0 (2C), 29.3 (2C), 31.3 (2C), 39.8 (2C), 48.6 (2C), 48.8 (2C), 122.5 (4C), 136.0 (2C); HRMS (ESI) $[M\text{-}C_2H_6S_2O_6]^{2+}$ calcd for $C_{34}H_{64}N_4$: 264.2560. Found: 264.2568.

4.5.7. 1,6-Bis(3-dodecyl-1*H*-imidazolium-1-yl)hexane dimesylate (7)

Obtained in 62% yield from reaction of 1,6-bis(methanesulfonyloxy)hexane with 1-dodecylimidazole affording a beige waxy solid: mp 44–59 °C; ^{1}H NMR (400.13 MHz, DMSO-d_6): δ 0.85 (t, J = 6.2 Hz, 6H), 1.13–1.34 (m, 40H), 1.72–1.84 (m, 8H), 2.32 (s, 6H), 4.16 (t, J = 7.2 Hz, 4H), 4.17 (t, J = 7.0 Hz, 4H), 7.81 (s, 4H), 9.26 (s, 2H); ^{13}C NMR (100.61 MHz, DMSO-d_6): δ 13.9 (2C), 22.1 (2C), 24.8 (2C), 25.5 (2C), 28.3 (2C), 28.7 (2C), 28.8 (2C), 28.90 (2C), 28.99 (4C), 29.03 (2C), 29.3 (2C), 31.3 (2C), 39.8 (2C), 48.6 (2C), 48.8 (2C), 122.4 (4C), 136.0 (2C); HRMS (ESI) $[M\text{-}C_2H_6S_2O_6]^{2+}$ calcd for $C_{36}H_{68}N_4$: 278.2717. Found: 278.2720.

4.5.8. 1,10-Bis(3-hexyl-1*H*-imidazolium-1-yl)decane dimesylate (8)

Obtained in 75% yield from reaction of 1,10-bis(methanesulfonyloxy)decane with 1-hexylimidazole affording a yellow oil: ^{1}H NMR (400.13 MHz, DMSO-d_6): δ 0.85 (t, J = 6.6 Hz, 6H), 1.13–1.33 (m, 24H), 1.71–1.84 (m, 8H), 2.31 (s, 6H), 4.16 (t, J = 7.0 Hz, 4H), 4.17 (t, J = 6.8 Hz, 4H), 7.81 (s, 4H), 9.25 (s, 2H); ^{13}C NMR (100.61 MHz, DMSO-d_6): δ 13.8 (2C), 21.8 (2C), 25.1 (2C), 25.5 (2C), 28.3 (2C), 28.7 (2C), 29.2 (2C), 29.3 (2C), 30.5 (2C), 39.8 (2C), 48.8 (4C), 122.5 (4C), 136.0 (2C); HRMS (ESI) $[M\text{-}C_2H_6S_2O_6]^{2+}$ calcd for $C_{28}H_{52}N_4$: 222.2091. Found: 222.2097.

4.5.9. 1,10-Bis(3-heptyl-1*H*-imidazolium-1-yl)decane dimesylate (9)

Obtained in 77% yield from reaction of 1,10-bis(methanesulfonyloxy)decane with 1-heptylimidazole affording a beige waxy solid: mp 58–62 °C; ^{1}H NMR (400.13 MHz, DMSO-d_6): δ 0.86 (t, J = 6.6 Hz, 6H), 1.13–1.34 (m, 28H), 1.72–1.84 (m, 8H), 2.31 (s, 6H), 4.17 (t, J = 6.4 Hz, 8H), 7.81 (s, 4H), 9.24 (s, 2H); ^{13}C NMR (100.61 MHz, DMSO-d_6): δ 13.9 (2C), 21.9 (2C), 25.4

(2C), 25.5 (2C), 28.0 (2C), 28.3 (2C), 28.8 (2C), 29.25 (2C), 29.28 (2C), 31.0 (2C), 39.8 (2C), 48.8 (4C), 122.5 (4C), 136.0 (2C); HRMS (ESI) $[M\text{-}C_2H_6S_2O_6]^{2+}$ calcd for $C_{30}H_{56}N_4$: 236.2247. Found: 236.2252.

4.5.10. 1,10-Bis(3-octyl-1*H*-imidazolium-1-yl)decane dimesylate (10)

Obtained in 72% yield from reaction of 1,10-bis(methanesulfonyloxy)decane with 1-octylimidazole affording a golden oil: ^{1}H NMR (400.13 MHz, CD_3OD): δ 0.90 (t, J = 6.8 Hz, 6H), 1.22–1.43 (m, 32H), 1.82–1.95 (m, 8H), 2.70 (s, 6H), 4.23 (t, J = 7.4 Hz, 8H), 7.66 (s, 2H), 7.67 (s, 2H), 9.05 (s, 2H); ^{13}C NMR (100.61 MHz, CD_3OD): δ 14.4 (2C), 23.7 (2C), 27.27 (2C), 27.29 (2C), 30.0 (2C), 30.1 (2C), 30.2 (2C), 30.5 (2C), 31.09 (2C), 31.13 (2C), 32.9 (2C), 39.6 (2C), 50.87 (2C), 50.89 (2C), 123.8 (4C), 137.2 (2C); HRMS (ESI) $[M\text{-}C_2H_6S_2O_6]^{2+}$ calcd for $C_{32}H_{60}N_4$: 250.2404. Found: 250.2412.

4.5.11. 1,10-Bis(3-nonyl-1*H*-imidazolium-1-yl)decane dimesylate (11)

Obtained in 71% yield from reaction of 1,10-bis(methanesulfonyloxy)decane with 1-nonylimidazole affording a beige waxy solid: mp 65–71 °C; ^{1}H NMR (400.13 MHz, DMSO-d_6): δ 0.85 (t, J = 6.8 Hz, 6H), 1.13–1.32 (m, 36H), 1.71–1.83 (m, 8H), 2.31 (s, 6H), 4.16 (t, J = 6.8 Hz, 8H), 7.81 (s, 4H), 9.24 (s, 2H); ^{13}C NMR (100.61 MHz, DMSO-d_6): δ 13.9 (2C), 22.0 (2C), 25.4 (2C), 25.5 (2C), 28.31 (2C), 28.35 (2C), 28.5 (2C), 28.8 (4C), 29.2 (2C), 29.3 (2C), 31.2 (2C), 39.8 (2C), 48.8 (4C), 122.5 (4C), 136.0 (2C); HRMS (ESI) $[M\text{-}C_2H_6S_2O_6]^{2+}$ calcd for $C_{34}H_{64}N_4$: 264.2560. Found: 264.2570.

4.5.12. 1,10-Bis(3-decyl-1*H*-imidazolium-1-yl)decane dimesylate (12)

Obtained in 75% yield from reaction of 1,10-bis(methanesulfonyloxy)decane with 1-decylimidazole affording a golden oil: ^{1}H NMR (400.13 MHz, CD_3OD): δ 0.90 (t, J = 6.8 Hz, 6H), 1.20–1.45 (m, 40H), 1.81–1.98 (m, 8H), 2.70 (s, 6H), 4.22 (t, J = 7.2 Hz, 8H), 7.66 (s, 4H), 9.04 (s, 2H); ^{13}C NMR (100.61 MHz, CD_3OD): δ 14.4 (2C), 23.7 (2C), 27.27 (2C), 27.31 (2C), 30.06 (2C), 30.08 (2C), 30.4 (2C), 30.5 (2C), 30.55 (2C), 30.59 (2C), 31.10 (2C), 31.14 (2C), 33.0 (2C), 39.6 (2C), 50.87 (2C), 50.90 (2C), 123.8 (4C), 137.2 (2C); HRMS (ESI) $[M\text{-}C_2H_6S_2O_6]^{2+}$ calcd for $C_{36}H_{68}N_4$: 278.2717. Found: 278.2722.

4.5.13. 1,10-Bis(3-undecyl-1*H*-imidazolium-1-yl)decane dimesylate (13)

Obtained in 56% yield from reaction of 1,10-bis(methanesulfonyloxy)decane with 1-undecylimidazole affording a brown oily residue; ^{1}H NMR (400.13 MHz, DMSO-d_6): δ 0.85 (t, J = 6.8 Hz, 6H), 1.13–1.32 (m, 44H), 1.71–1.83 (m, 8H), 2.30 (s, 6H), 4.15 (t, J = 7.2 Hz, 8H), 7.80 (s, 4H), 9.21 (s, 2H); ^{13}C NMR (100.61 MHz, CD_3OD): δ 14.4 (2C), 23.7 (2C), 27.26 (2C), 27.31 (2C), 30.1 (4C), 30.4 (2C), 30.48 (2C), 30.54 (2C), 30.6 (2C), 30.7 (2C), 31.09 (2C), 31.12 (2C), 33.1 (2C), 39.6 (2C), 50.85 (2C), 50.87 (2C), 123.77 (2C), 123.81 (2C), 137.2 (2C); HRMS (ESI) $[M\text{-}C_2H_6S_2O_6]^{2+}$ calcd for $C_{38}H_{72}N_4$: 292.2873. Found: 292.2878.

4.5.14. 1,10-Bis(3-dodecyl-1*H*-imidazolium-1-yl)decane dimesylate (14)

Obtained in 51% yield from reaction of 1,10-bis(methanesulfonyloxy)decane with 1-dodecylimidazole affording a light brown waxy solid: mp 46–47 °C; ^{1}H NMR (400.13 MHz, DMSO-d_6): δ 0.85 (t, J = 6.8 Hz, 6H), 1.12–1.32 (m, 48H), 1.72–1.83 (m, 8H), 2.30 (s, 6H), 4.16 (t, J = 7.0 Hz, 8H), 7.81 (s, 4H), 9.24 (s, 2H); ^{13}C NMR (100.61 MHz, DMSO-d_6): δ 13.9 (2C), 22.1 (2C), 25.4 (2C), 25.5 (2C), 28.3 (2C), 28.4 (2C), 28.7 (2C), 28.79 (2C), 28.82 (2C), 28.9 (2C), 28.98 (2C), 29.00 (2C), 29.2 (2C), 29.3 (2C), 31.3 (2C), 39.8 (2C), 48.8 (4C), 122.5 (4C), 136.0 (2C); HRMS (ESI) $[M\text{-}C_2H_6S_2O_6]^{2+}$ calcd for $C_{40}H_{76}N_4$: 306.3030. Found: 306.3038.

4.5.15. 1,16-Bis(3-hexyl-1*H*-imidazolium-1-yl)hexadecane dimesylate (15)

Obtained in 71% yield from reaction of 1,16-bis(methanesulfonyloxy)hexadecane with 1-hexylimidazole affording a golden oil: ^{1}H NMR (400.13 MHz, CD_3OD): δ 0.91 (t, J = 6.2 Hz, 6H),

1.20–1.42 (m, 36H), 1.81–1.96 (m, 8H), 2.70 (s, 6H), 4.23 (t, *J* = 7.0 Hz, 8H), 7.66 (s, 4H), 9.04 (s, 2H); ^{13}C NMR (100.61 MHz, CD_3OD): δ 14.3 (2C), 23.5 (2C), 26.9 (2C), 27.3 (2C), 30.1 (2C), 30.6 (2C), 30.7 (2C), 30.78 (2C), 30.81 (2C), 31.0 (2C), 31.1 (2C), 32.2 (2C), 39.5 (2C), 50.9 (4C), 123.8 (4C), 137.2 (2C); HRMS (ESI) $[M\text{-}C_2H_6S_2O_6]^{2+}$ calcd for $C_{34}H_{64}N_4$: 264.2560. Found: 264.2569.

4.5.16. 1,16-Bis(3-heptyl-1*H*-imidazolium-1-yl)hexadecane dimesylate (16)

Obtained in 75% yield from reaction of 1,16-bis(methanesulfonyloxy)hexadecane with 1-heptylimidazole affording a golden oil: ^{1}H NMR (400.13 MHz, CD_3OD): *δ* 0.90 (t, *J* = 6.8 Hz, 6H), 1.22–1.43 (m, 40H), 1.83–1.95 (m, 8H), 2.70 (s, 6H), 4.23 (t, *J* = 7.2 Hz, 8H), 7.66 (s, 4H), 9.04 (s, 2H); ^{13}C NMR (100.61 MHz, CD_3OD): δ 14.4 (2C), 23.6 (2C), 21.2 (2C), 27.3 (2C), 29.7 (2C), 30.1 (2C), 30.6 (2C), 30.7 (2C), 30.78 (2C), 30.81 (2C), 31.1 (4C), 32.8 (2C), 39.5 (2C), 50.9 (4C), 123.8 (4C), 137.2 (2C); HRMS (ESI) $[M\text{-}C_2H_6S_2O_6]^{2+}$ calcd for $C_{36}H_{68}N_4$: 278.2717. Found: 278.2723.

4.5.17. 1,16-Bis(3-octyl-1*H*-imidazolium-1-yl)hexadecane dimesylate (17)

Obtained in 68% yield from reaction of 1,16-bis(methanesulfonyloxy)hexadecane with 1-octylimidazole affording a golden oil: ^{1}H NMR (400.13 MHz, CD_3OD): *δ* 0.90 (t, *J* = 6.8 Hz, 6H), 1.22–1.42 (m, 44H), 1.82–1.95 (m, 8H), 2.70 (s, 6H), 4.23 (t, *J* = 7.2 Hz, 8H), 7.66 (s, 4H), 9.05 (s, 2H); ^{13}C NMR (100.61 MHz, CD_3OD): δ 14.4 (2C), 23.7 (2C), 27.26 (2C), 27.28 (2C), 30.0 (2C), 30.1 (2C), 30.2 (2C), 30.6 (2C), 30.7 (2C), 30.78 (2C), 30.82 (2C), 31.1 (4C), 32.9 (2C), 39.5 (2C), 50.9 (4C), 123.8 (4C), 137.2 (2C); HRMS (ESI) $[M\text{-}C_2H_6S_2O_6]^{2+}$ calcd for $C_{38}H_{72}N_4$: 292.2873. Found: 292.2881.

4.5.18. 1,16-Bis(3-nonyl-1*H*-imidazolium-1-yl)hexadecane dimesylate (18)

Obtained in 62% yield from reaction of 1,16-bis(methanesulfonyloxy)hexadecane with 1-nonylimidazole affording a beige waxy solid: mp 66–69 °C; ^{1}H NMR (400.13 MHz, CD_3OD): *δ* 0.90 (t, *J* = 6.8 Hz, 6H), 1.20–1.42 (m, 48H), 1.82–1.95 (m, 8H), 2.70 (s, 6H), 4.23 (t, *J* = 7.2 Hz, 8H), 7.66 (s, 4H), 9.04 (s, 2H); ^{13}C NMR (100.61 MHz, CD_3OD): δ 14.4 (2C), 23.7 (2C), 27.26 (2C), 27.29 (2C), 30.08 (2C), 30.11 (2C), 30.3 (2C), 30.5 (2C), 30.6 (2C), 30.7 (2C), 30.80 (2C), 30.83 (2C), 31.1 (4C), 33.0 (2C), 39.5 (2C), 50.9 (4C), 123.8 (4C), 137.2 (2C); HRMS (ESI) $[M\text{-}C_2H_6S_2O_6]^{2+}$ calcd for $C_{40}H_{76}N_4$: 306.3030. Found: 306.3035.

4.5.19. 1,16-Bis(3-decyl-1*H*-imidazolium-1-yl)hexadecane dimesylate (19)

Obtained in 65% yield from reaction of 1,16-bis(methanesulfonyloxy)hexadecane with 1-decylimidazole affording a beige waxy solid: mp 83–87 °C; ^{1}H NMR (400.13 MHz, CD_3OD): *δ* 0.90 (t, *J* = 6.8 Hz, 6H), 1.20–1.42 (m, 52H), 1.83–1.95 (m, 8H), 2.70 (s, 6H), 4.23 (t, *J* = 7.2 Hz, 8H), 7.66 (s, 4H), 9.04 (s, 2H); ^{13}C NMR (100.61 MHz, CD_3OD): δ 14.4 (2C), 23.7 (2C), 27.25 (2C), 27.28 (2C), 30.07 (2C), 30.11 (2C), 30.4 (2C), 30.5 (2C), 30.6 (4C), 30.7 (2C), 30.80 (2C), 30.83 (2C), 31.08 (2C), 31.09 (2C), 33.0 (2C), 39.5 (2C), 50.9 (4C), 123.8 (4C), 137.2 (2C); HRMS (ESI) $[M\text{-}C_2H_6S_2O_6]^{2+}$ calcd for $C_{42}H_{80}N_4$: 320.3186. Found: 320.3192.

4.5.20. 1,16-Bis(3-undecyl-1*H*-imidazolium-1-yl)hexadecane dimesylate (20)

Obtained in 58% yield from reaction of 1,16-bis(methanesulfonyloxy)hexadecane with 1-undecylimidazole affording a beige waxy solid: mp 82–85 °C; ^{1}H NMR (400.13 MHz, DMSO-d_6): *δ* 0.85 (t, *J* = 6.4 Hz, 6H), 1.11–1.32 (m, 56H), 1.71–1.83 (m, 8H), 2.30 (s, 6H), 4.16 (t, *J* = 7.0 Hz, 8H), 7.80 (s, 4H), 9.23 (s, 2H); ^{13}C NMR (100.61 MHz, CD_3OD): δ 14.4 (2C), 23.7 (2C), 27.26 (2C), 27.29 (2C), 30.07 (2C), 30.12 (2C), 30.45 (2C), 30.56 (2C), 30.62 (4C), 30.64 (2C), 30.7 (2C), 30.8 (2C), 30.9 (2C), 31.07 (2C), 31.09 (2C), 33.1 (2C), 39.5 (2C), 50.9 (4C), 123.8 (4C), 137.2 (2C); HRMS (ESI) $[M\text{-}C_2H_6S_2O_6]^{2+}$ calcd for $C_{44}H_{84}N_4$: 334.3343. Found: 334.3341.

4.5.21. 1,16-Bis(3-dodecyl-1*H*-imidazolium-1-yl)hexadecane dimesylate (21)

Obtained in 49% yield from reaction of 1,16-bis(methanesulfonyloxy)hexadecane with 1-dodecylimidazole affording a beige waxy solid: mp 91–95 °C; ^{1}H NMR (400.13 MHz, CD_3OD): δ 0.90 (t, J = 6.2 Hz, 6H), 1.21–1.43 (m, 60H), 1.83–1.95 (m, 8H), 2.70 (s, 6H), 4.23 (t, J = 7.0 Hz, 8H), 7.66 (s, 4H), 9.05 (s, 2H); ^{13}C NMR (100.61 MHz, CD_3OD): δ 14.5 (2C), 23.7 (2C), 27.25 (2C), 27.28 (2C), 30.07 (2C), 30.11 (2C), 30.46 (2C), 30.56 (2C), 30.61 (2C), 30.63 (2C), 30.70 (2C), 30.74 (4C), 30.81 (2C), 30.84 (2C), 31.06 (2C), 31.09 (2C), 33.1 (2C), 39.5 (2C), 50.9 (4C), 123.8 (4C), 137.2 (2C); HRMS (ESI) $[M\text{-}C_2H_6S_2O_6]^{2+}$ calcd for $C_{46}H_{88}N_4$: 348.3499. Found: 348.3506.

4.6. Glycosyltransferase assays

The β1,3-Gal-transferase WbwC from *Escherichia coli* serotype O104: H4[7] was expressed in BL21 bacteria and the gene in plasmids was re-sequenced. Bacterial suspensions in 10% glycerol and 90 % PBS were stored at -20ºC. For enzyme activity assays, bacterial suspensions were sonicated 3 times for 15 sec and then centrifuged. The supernatant was an excellent source of active and stable enzyme and was used to determine the IC_{50} values. The expressed WbwC protein was also purified using a Ni-NTA column as described.[7] Gal-transferase activities were assayed in standard assays in a total volume of 40 µL in the presence of 5 mM $MnCl_2$, 0.125M Tris-HCl buffer pH 7.5, with donor substrate 0.8 mM UDP-[^{3}H]Gal and 0.1 mM acceptor substrate GalNAc-PO_3-PO_3-$(CH_2)_{11}$-O-phenyl. 10% MeOH was present in control assays. Mixtures were incubated for 1 h at 37ºC. Reaction products were isolated using a C18 Sep-Pak cartridge (short), washed first with 4 mL water and then eluted with 3 mL MeOH, and evaluated by scintillation counting. Candidate compounds (Table 1) were added from 5 mM stock solutions in MeOH, and were present at 0.5 mM in the assay mixtures, and from 0.01 to 0.5 mM for inhibition assays. A 10 min pre-incubation of the enzymes did not increase the inhibition. The inhibitor of bovine β1,4-Gal-transferase, 1-thio-*N*-butyrylGlcN*β*-(2-naphthyl)[5] was tested with WbwC under the same conditions. Bovine milk β1,4-galactosyltransferase (Sigma) was assayed similarly, but with UDP-[^{3}H]Gal, acceptor substrate GlcNAcβ-Bn and candidate compounds at 1 mM and 0.05% bovine serum albumin in the assay, as described before.[4]

4.7. Growth of bacteria

Antimicrobial assays were performed using the colony-forming unit (CFU) assay. Gram-negative bacteria *Escherichia coli* (BL21) or *Pseudomonas aeruginosa* PAO1, and Gram-positive bacteria *Bacillus subtilis* and *Staphylococcus epidermidis* were grown in LB medium from a single colony with agitation at 37 °C. The bacterial number was then evaluated by measuring the optical density (OD) at 550 nm corresponding to approximately 10^8 CFU/mL). The bacteria were pelleted, washed with phosphate-buffered saline (PBS) and then concentrated to 5×10^8 CFU/mL in PBS. The bacterial suspension (100 µL) was incubated with candidate compound and a final concentration of 1% MeOH in PBS for 4 h and poured onto solid LB agar. Bacterial colonies were counted after incubation overnight at 37 °C.

4.8. Mammalian cell cultures

Human embryonal kidney cells (AD293), lung cancer (A549) and prostate cancer (PC-3, LNCaP) cells[12] were obtained from ATCC and grown as recommended. Human ovarian cancer cells (OC-3-VGH) were donated by G. Lee, University of British Columbia. Candidate compounds (0.5 mM final concentration in the growth medium) were dissolved in DMSO (final concentration 0.1 %) and added to the cell medium; cells were then grown on 96-well plates and incubated for 24 h at 37°C followed by analyses of cell proliferation by MTT assays in at least three identical experiments. MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium bromide, Thermo-Fisher) was used to assess cell proliferation accroding to the manufacturer's instructions.

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Declaration of interests

☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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