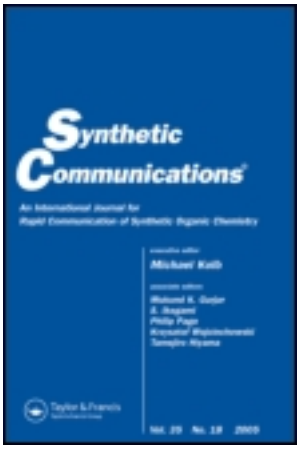

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Efficient Synthesis of Methanesulphonate-Derived Lipid Chains for Attachment of Proteins to Lipid Membranes

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Abstract: We have developed an easy and flexible synthetic methodology to obtain lipid chains containing methanothiosulfonate terminal groups with the aim to attach them to natural proteins as functional groups. There are many proteins found in nature that are modified by lipids, and this is a key part of their function. For example, the prion protein is attached to the plasma membrane via a glycosylphosphatidylinositol (GPI) anchor, and this protein is thought to be the causative agent in diseases such as bovine spongiform encephalopathy (BSE; "mad cow disease") and the human equivalent Creutzfeldt–Jakob disease. However, production of large amounts of protein in bacteria results in proteins that lack these lipid modifications. The lipid chains containing methanothiosulfonate terminal groups that we have synthesized here can be attached to these proteins through the thiol contained in the side chain of the cysteine residue, which can be incorporated into the protein sequence at the desired position.

Keywords: Glycosylphosphatidylinositol, GPI-anchor, prion

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INTRODUCTION

It has been possible for a number of years to engineer bacteria to produce proteins from other organisms. This recombinant technology has enabled structural biologists to produce sufficient quantities of proteins (milligrams) to determine structural features of these biological polymers. However, many proteins in higher organisms are modified by the addition of other functional groups to either amino acid side chains or to the amino or carboxyl termini of the polymer.[1] Proteins produced in bacteria are not modified in this way, which presents a problem when studying the natural modified protein. These additional groups are varied and may either enable the protein to carry out its function or modify the function in some way. Attachment of complex glycosylated phospholipids [glycosylphosphatidylinositol (GPI) modification at the C-terminal, for example] is one of the ways in which proteins can be attached to the cell membrane.[2]

One example of a protein that is attached to the cell membrane in this way is the prion protein (PrP). PrP is of particular interest to biologists and biochemists because it is thought to be the causative agent in an important class of fatal neurodegenerative diseases called transmissible spongiform encephalopathies (TSEs), including scrapie in sheep, bovine spongiform encephalopathy in cattle, and Creutzfeldt–Jakob disease in humans. The GPI group results in the protein being anchored in the outside of the lipid bilayer membrane that surrounds the cell.[3–5] The infectious form of this protein (PrP^{Sc}) is thought to be an alternatively folded form of the normal form (PrP^{C}). To check how the infectious form is produced,[6,7] it is fundamental to anchor the protein in the membrane in a manner similar to that found in nature. It is impractical to use the prion protein with a natural GPI anchor because purification of sufficient amounts for structural analyses is very difficult and the naturally occurring material is highly heterogeneous, consisting of different glycosilated species. For this reason, we have developed a new synthetic route to produce a modified protein GPI mimetic (GPIm). By using methanethiosulfonate chemistry, lipids can be covalently bound to proteins via the thiol group in the side chain of a cysteine residue, which can be engineered at the required position. We have successfully used this approach to study the structure of PrP anchored in lipid membranes using attenuated total internal reflection Fourier transform infrared spectroscopy (FT-IR).[8]

In this article, we present a flexible methodology for the synthesis of glycosylphosphatidylinositol modified (GPIm) compounds. To mimic the properties of the naturally occurring GPI molecule, the following properties are required: two saturated hydrocarbon chains of a length typical of that found in nature, [9] followed by an ethylene glycol linker to mimic the

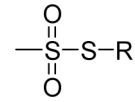


Figure 1. Methanethiosulfonate.

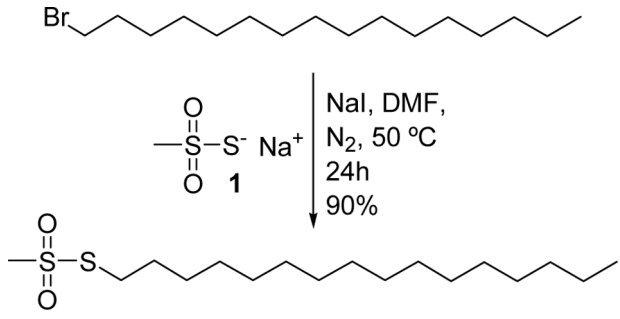
length of carbohydrate moiety in a natural GPI,[10] and finally the coupling methanethisulfonate group to attach GPIm to a cysteine at the C-terminal of the protein. The developed synthetic route is presented next.

RESULTS AND DISCUSSION

With the aim to create a mimic of a lipid anchor-containing protein, we needed to synthesize an anchored lipid to couple with the free thiol group of a genetically engineered cysteine residue at the carboxyl terminus of the prion protein (PrP). Because the coupling will take place via nucleophilic attack by the thiolate anion of the cysteine group, the lipid should contain an activated suitable leaving group. Kenyon and Bruice[11] suggested the use of methanethiosulfonate as reactive leaving group (Fig. 1) because of the specific and quantitative reactivity of thiols toward it to generate a S–S bond.

A simple methanethiosulfonate lipid can be easily prepared by reaction of bromooctadecane and the easily synthesised sodium methanethiosulfonate salt **1** (Scheme 1).[11]

Another factor that must be considered is that the anchored lipid should contain a hydrophilic portion to separate the protein from the membrane surface. Ethyleneglycol chainscan act as suitable flexible spacers.[12]

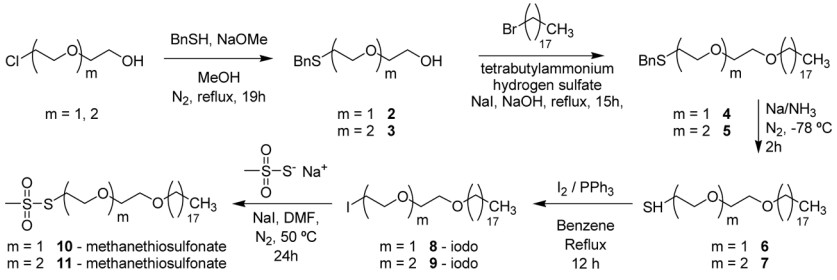


Scheme 1. Preparation of methanethiosulfonate lipid.

Our first synthetic approach to obtain the mimic anchor compound GPIm consisted of the synthesis of two different single methanethiosulfonate lipids with different length chains. The route employed is depicted in Scheme 2. The synthesis began with the reaction of the commercially available alcohols 2-(2-chloroethoxy)ethanol and 2-[2-(2-chloroethoxy)-ethoxy]ethanol with benzyl mercaptan and sodium methoxide in dry methanol, which yielded the benzylthiol protected alcohols **2** and **3** in 88–90% yield. Reaction of these alcohols with 1-bromooctadecane under phase-transfer conditions furnished the ethers **4** and **5** in 72–86% yield, which were successfully reduced with sodium and liquid ammonia to the thiols **6** and **7** in quantitative yield. Reaction of these thiols with triphenylphosphine and iodine gave the iodo-lipids **8** and **9**.[13] Desired methanethiosulfonate lipids **10** and **11** were obtained via reaction of the iodine derivatives **8** and **9** with the initially synthesized sodium methanethiosulfonate **1**[11] (Scheme 2).

Preliminary experiments were performed using compounds **10** and **11** coupled to the prion protein (PrP), via a cysteine residue, engineered at the C-terminus of the protein. Insertion of these lipid-anchored proteins into lipid bilayers was unsuccessful. This was thought to be due to the presence of only one lipid chain compared with the two chains found in the naturally occurring lipid anchor. For this reason, once the synthetic method had been developed with the monoalkyl chain anchors, we turned our attention to the possibility of using double-chain methanethiosulfonate-containing lipids. We have redesigned our synthetic pathway for the preparation of these double-chain compounds, starting from simple and inexpensive starting materials with the objective to target the required molecules.

To mimic as much as possible the length of the naturally occurring GPI anchor,[10] a hexaethyleneglycol linker was chosen as the spacer together with a long C_{16} hydrocarbon chain as hydrophobic anchor.[9]



Scheme 2. Synthesis of two different single methanethiosulfonate chains.

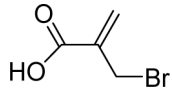


Figure 2. α,α-unsaturated carboxylic acid.

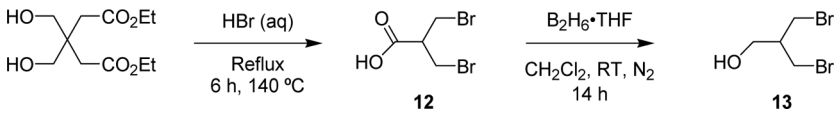
Following the method of Ferris,[14] the inexpensive and widely available diethyl bis(hydroxymethyl)malonate and 48% hydrobromic acid were heated under reflux at 140°C with distillation of ethyl bromide to afford 3-bromo-2-bromomethylpropanoic acid **12** as a crude pale brown solid. It was noteworthy that traces of the α,α-unsaturated carboxylic acid (Fig. 2) were formed during the synthesis of dibromo acid via elimination of HBr.

The reduction of the dibromo acid **12** was done with diborane according to the method of Anasari et al.[15] to afford 3-bromo-2-bromomethylpropan-1-ol **13** with an overall yield of 44% (Scheme 3).

Using our predesigned route, two types of lipids were synthesized, one containing two sulfide chains, synthesized via the acid **12**, and the other containing two sulfone chains, using the alcohol **13** as starting material (Fig. 3).

Using the method employed by Zhang and Magnusson,[16] dibromo alcohol **13** and hexadecanethiol were treated with caesium carbonate ($CsCO_3$) in dimethylformamide and stirred at room temperature for 24 h to give 3-hexadecylthio-2-(hexadecylthiomethyl) propan-1-ol **14** in good yield (88%) after recrystallization from methanol (Scheme 4).

There are many methods available for the oxidation of alcohols, but we required a reagent that would be able to oxidize both the alcohol and the sulfide in a single step and in good yield. Potassium permanganate was chosen for the oxidation step, as was utilized before by Georges and coworkers[17] for the oxidation of sulfides. A solution of potassium permanganate in water was added to a mixture of dithiolalkyl alcohol **14** in acetic acid at 60°C and stirred for 24 h (Scheme 4). Workup of the reaction afforded the oxidized sulfone **15** as a white solid, which showed the characteristic peak of the carboxyl group in ^{13}C NMR spectra and both the carboxyl and the sulfone in the infrared spectrum.



Scheme 3. Reaction of the dibromo acid.

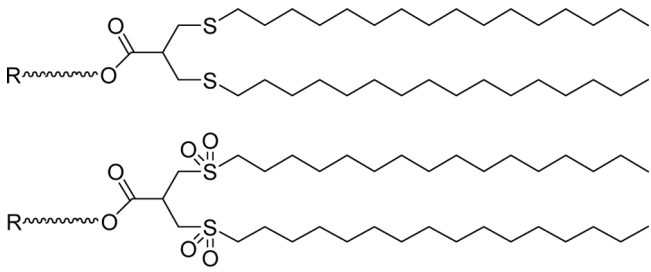
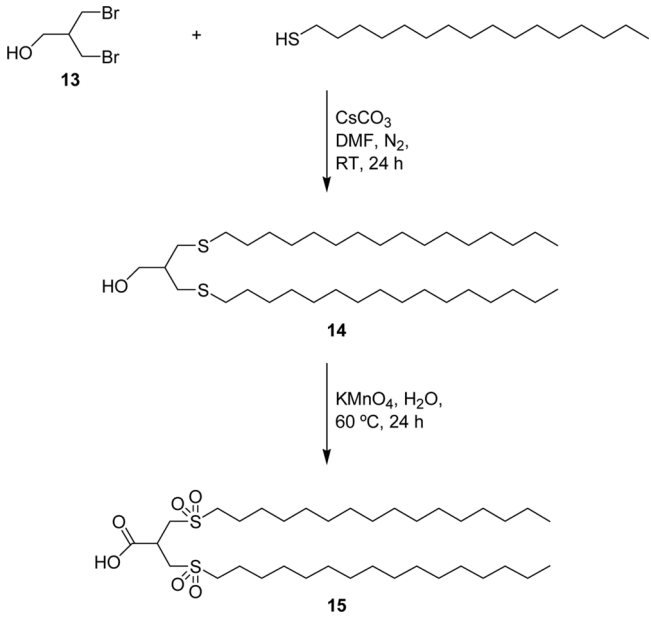


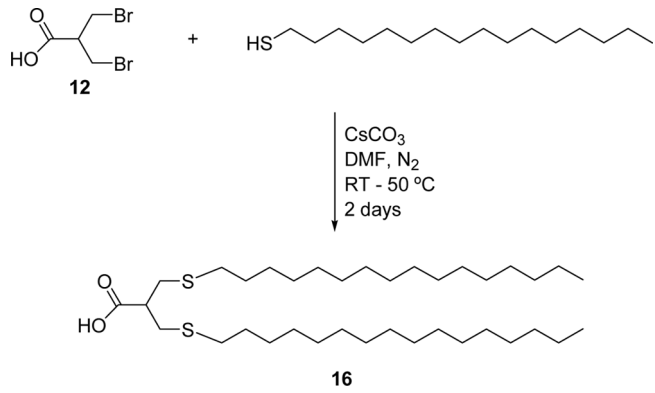
Figure 3. Both sulfide and sulfone lipids were synthesized.

One point of note concerning the oxidized sulfone **15** was its limited solubility in chloroform at room temperature. This decreased solubility had a major effect on the proceeding steps because it limited the reaction conditions that could be used.

With this problem in mind, to improve the solubility of the sulfone derivative acid we decided to attempt in parallel the synthesis of the analogous double-chain sulfide containing acid via direct synthesis from the previously synthesized dibromo acid **12** using the procedure of Zhang



Scheme 4. Synthesis of the sulfone-containing acid.

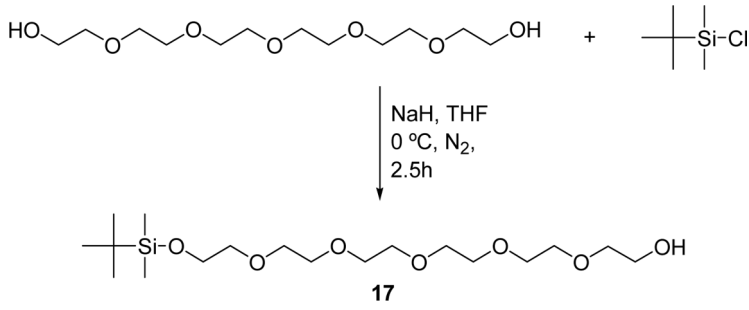


Scheme 5. Synthesis of the sulfide-containing acid.

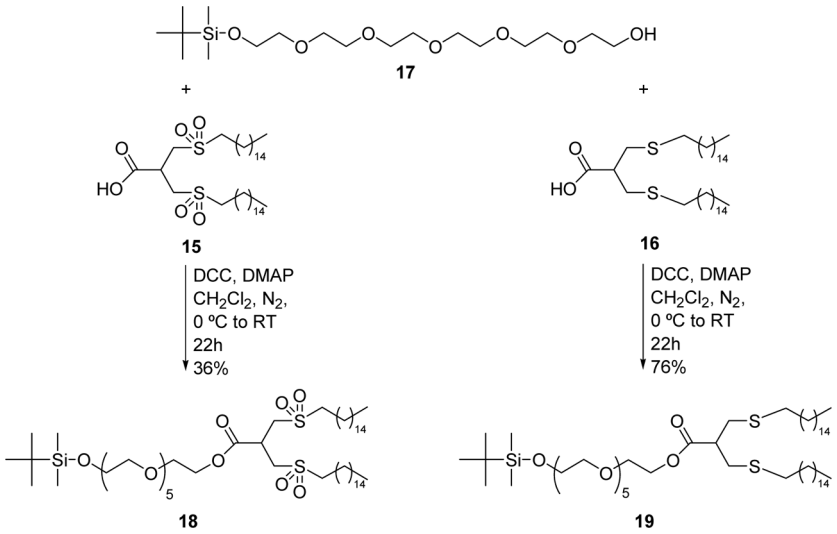
and Magnusson.[16] Reaction of dibromoacid and hexadecanethiol under the previously described conditions[16] yielded the desired dialkylacid **16** (Scheme 5), which was further purified by silica column chromatography.

Once the dithioalkyl and disulfonealkyl acids were prepared, the synthetic sequence to the desired GPIm required the introduction of a spacer unit. This part of the route started with the mono-*tert*-butyldimethylsilyl protection of hexaethylene glycol. Using the method of Bertozzi and Bednarski,[18] reaction of hexaethyleneglycol with terbutyldimethylsilyl chloride (TBDMS-Cl) and sodium hydride at 0°C gave rise a mixture of monosubstituted alcohol **17** and some disubstituted product, which was easily removed by silica chromatography (Scheme 6).

Coupling reactions of the monoprotected alcohol **17** with the sulfone- and sulfide-containing acids **15** and **16**, respectively, were first attempted using [1-(3-dimethylaminopropyl)-3-ethylcarbodiimide



Scheme 6. Synthesis of a monosubstituted alcohol.

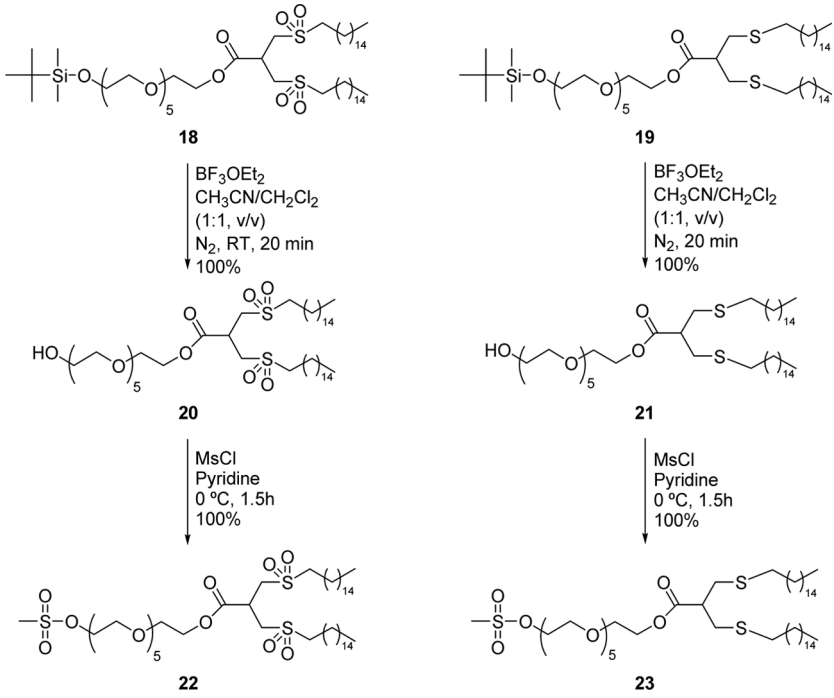


Scheme 7. Coupling the lipid and spacer.

hydrochloride (EDCI) and 4-dimethylaminopyridine (DMAP), using standard peptide coupling conditions. Nevertheless, the reactions with both of the acids gave unsatisfactory yields of the required products. The use of dicyclohexylcarbodiimide (DCC) as suggested by Whitesell and Reynolds[19] provided a more satisfactory yield for coupling of the alcohol **17** with the sulfide-containing acid **16** to yield ester **19** but a low yield for coupling of the alcohol with the sulfone-containing acid **15** to yield **18**. This low but workable yield can be attributed to the low solubility shown by the sulfone acid derivate **15**, as was mentioned before (Scheme 7).

Both TBDMS-protected lipids **18** and **19** were quantitatively deprotected using trifluoroborane etherate in a mixture of dichloromethane and acetonitrile at 0°C according to the procedure employed by King et al.[20] The resulting alcohols **20** and **21** were satisfactorily protected with methanesulfonyl chloride in pyridine at 0°C to give the mesylated derivatives **22** and **23** in quantitative yield (Scheme 8).

The synthesis of methanethiosulfonate derivatives normally proceeds via displacement of a halogen atom from a haloalkane. We have found in the literature different available methods for the conversion of mesylates to halides. Taking into account that iodide as leaving group should provide an easier route to methanesulfonate lipids than bromide,[11] we decided to convert the mesylates **22** and **23** into the related iodide compounds.



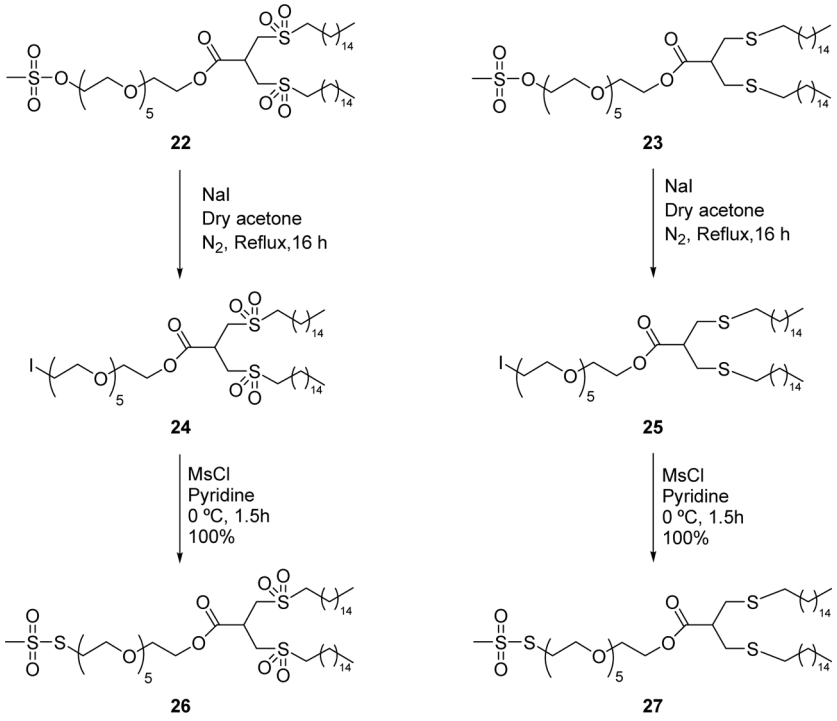
Scheme 8. Synthesis of the mesylated derivatives.

Reaction of mesylates **22** and **23** with iodine and triphenylphosphine[13] provided a reasonable yield of the iodo-lipids, but purification was difficult because of the triphenylphosphine and triphenylphosphine oxides formed during the reaction. However, an alternative reaction with sodium iodide in acetone as described by Poss and Belter[21] performed a clean displacement of the mesylate group and allowed the obtention of the desired iodo-lipids **24** and **25** with excellent yields. Reaction of the iodo compounds with sodium methanethiosulfonate **1** provided the target methanethiosulfonate anchor lipids **26** and **27** (Scheme 9).

The synthetic work presented here provides an easy and straightforward way to obtain synthetically produced glycosylphosphatidylinositol-mimetic lipids to attach as modifying groups in proteins.

EXPERIMENTAL

Melting points were determined using a Stuart Scientific SMP 1 melting-point apparatus and are uncorrected. ^1H and ^{13}C NMR spectra were



Scheme 9. Synthesis of the final products via iodo-lipids.

recorded at either 400 MHz or 500 MHz on Bruker DPX-400 or DRX 500 instruments respectively with coupling constants, *J*, given in hertz (Hz). Fast-atom bombardment (FAB) and EI/CI mass spectra, including high-resolution spectrum, were recorded on a Kratos Autospec spectrometer. Chemicals were purchased from Aldrich, Sigma, or Lancaster at the highest available grade. All general solvents were purchased from Fisons Scientific Equipment at specified laboratory reagent (SLR) grade and purified when required, using the literature procedures. Anhydrous solvents were purchased from Aldrich (Highdry).

Sodium Methanethiosulfonate (1)

Sodium sulfide (72.1 g, 0.3 mol) was dissolved in water (75 cm^3), and the solution was heated at 80°C. Methanesulfonyl chloride (23.3 cm^3, 0.3 mol) was added dropwise to the vigorously stirred solution over a period of 15 min, and the reaction mixture was refluxed overnight. The colorless solution was cooled to room temperature and evaporated under

reduced pressure to give a white solid. The white solid was ground finely, dried in a vacuum oven at 40°C overnight, and then filter-extracted with dry ethanol ($5 \times 50\,cm^3$). The filtrate was cooled in a refrigerator overnight. The resulting crystals were collected by filtration and washed with ice-cold ethanol ($50\,cm^3$). The mother liquors were evaporated under reduced pressure until more crystals started to fall out of solution. The collected crystalline solids were dissolved using the minimum amount of dry ethanol and fine filtered to remove traces of sodium chloride. The filtrate was evaporated to dryness, and the resulting product was recrystallized several times to give the required salt product **1** as a white crystalline solid (5.24 g, 13%). Mp 273–274°C; ^{1}H NMR δ (300 MHz; D_2O) 3.2 (s, 3 H); ^{13}C NMR δ (75 MHz; D_2O) 54.8. Anal. calcd. for $CH_3NaO_2S_2$: C, 8.9; H, 2.3. Found: C, 8.6; H, 2.5.

2-(2-Benzylsulfanylethoxy) Ethanol (2)

Benzyl mercaptan ($31.4\,cm^3$, 267.8 mmol) was added via syringe to a solution of sodium methoxide (24 g, 446.3 mmol) in anhydrous methanol ($500\,cm^3$) under nitrogen atmosphere. Then 2-(2-chloroethoxy)ethanol (50 g, 403 mmol) was added dropwise, and the reaction mixture was heated under reflux overnight. After 19 h, the reaction was poured into a saturated aqueous solution of NaCl ($500\,cm^3$) and the product was extracted with CH_2Cl_2 (3×1 L). The organic phase was dried ($MgSO_4$), filtered, and evaporated under reduced pressure to give a crude oil, which was purified by silica chromatography ($CH_2Cl_2 \rightarrow CH_2Cl_2$-MeOH, 15:1) to give the title compound **4** (75.36 g, 88%) as a clear oil. ^{1}H NMR δ (300 MHz; $CDCl_3$) 2.47 (t, $J = 6.2$, 1 H), 2.64 (t, $J = 6.6$, 2 H), 3.50–3.56 (m, 2 H), 3.60 (t, $J = 6.6$, 2 H), 3.69–3.73 (m, 2 H), 3.77 (s, 2 H), 7.23–7.37 (m, 5 H); ^{13}C NMR δ (75 MHz; $CDCl_3$) 30.7, 36.5, 61.6, 70.1, 71.9, 127.0, 128.4, 128.8, 138.1. MS -CI (m/z) 230.12 ($M + NH_4^+$); $C_{11}H_{20}NO_2S$ requires 230.12.

2-[2-(2-Benzylsulfanylethoxy)ethoxy] Ethanol (3)

The compound was prepared according to the procedure described for **2** using the commercially available 2-[2-(2-chloroethoxy)ethoxy]ethanol as starting material. The crude oil obtained was purified by silica chromatography ($CH_2Cl_2 \rightarrow CH_2Cl_2$-MeOH, 15:1) to give the title compound **3** (68.68 g, 90%) as a clear oil. ^{1}H NMR δ (300 MHz; $CDCl_3$) 2.51–2.59 (m, 3 H), 3.50–3.61 (m, 8 H), 3.62–3.68 (m, 2 H), 3.70 (s, 2 H), 7.23–7.37 (m, 5 H); ^{13}C NMR δ (75 MHz; $CDCl_3$) 30.5, 36.5, 61.6, 70.2,

70.2, 70.6, 72.4, 127.0, 128.4, 128.8, 138.2. MS-CI (m/z) 274.1478 ($M + NH_4^+$); $C_{13}H_{24}NO_3S$ requires 274.1477.

[2-(2-Octadecyloxyethoxy)ethylsulfanylmethyl] Benzene (4)

2-(2-Benzylsulfanyl-ethoxy)-ethanol (10 g, 47.15 mmol) and commercially available 1-bromooctadecane (20.9 cm^3, 61.29 mmol) were mixed in a 250-cm^3 round-bottom flask. Aqueous NaOH [37.7 g, 943 mmol, in 38 g of H_2O (50% w/w)], tetrabutylammonium hydrogensulfate (1.28 g, 3.78 mmol), and sodium iodide (0.71 g, 4.72 mmol) were added. The two-phase mixture was stirred vigorously overnight at 90°C under a nitrogen atmosphere. The reaction was followed by thin-layer chromatography (TLC, hexane–EtOAc, 10:1). After stirring overnight, the reaction mixture was poured into water (500 cm^3), and the product was extracted with CH_2Cl_2 ($3 \times 500\ cm^3$). The organic extracts were combined, dried ($MgSO_4$), filtered, and evaporated under reduced pressure to give a crude oil, which was purified by silica chromatography (hexane–EtOAc, 12:1) to give the title compound **4** (18.79 g, 86%) as a clear oil. ^{1}H NMR δ (300 MHz; $CDCl_3$) 0.82 (t, $J = 6.7$, 3 H), 1.15–1.35 (m, 30 H), 1.45–1.54 (m, 2 H), 2.56 (t, $J = 6.8$, 2 H), 3.39 (t, $J = 6.8$, 2 H), 3.47–3.58 (m, 6 H), 3.70 (s, 2 H), 7.13–7.22 (m, 5 H); ^{13}C NMR δ (75 MHz; $CDCl_3$) 14.1, 22.6, 26.0, 29.3, 29.5, 29.6, 29.7, 30.4, 31.8, 36.5, 70.0, 70.3, 70.8, 71.5, 126.8, 128.4, 128.8, 138.4. MS-CI (m/z) 482.4034 ($M + NH_4^+$); $C_{29}H_{56}NO_2S$ requires 482.4032.

{2-[2-(2-Octadecyloxyethoxy)ethoxy]ethylsulfanylmethyl} Benzene (5)

The compound was prepared according with the procedure described for **4.** The obtained crude oil was purified by silica chromatography (hexane–EtOAc, 6:1) to give the pure title compound **5** (14.2 g, 72%) as a white waxy solid. ^{1}H NMR δ (300 MHz; $CDCl_3$) 0.81 (t, $J = 6.8$, 3 H), 1.10–1.32 (m, 30 H), 1.46–1.55 (m, 2 H), 2.56 (t, $J = 6.8$, 2 H), 3.36 (t, $J = 6.8$, 2 H), 3.47–3.58 (m, 10 H), 3.69 (s, 2 H), 7.13–7.27 (m, 5 H); ^{13}C NMR δ (75 MHz; $CDCl_3$) 14.0, 22.6, 26.0, 29.3, 29.4, 29.6, 29.7, 30.5, 31.8, 36.5, 70.0, 70.2, 70.5, 70.6, 70.8, 71.5, 126.9, 128.4, 128.9, 138.3; MS-CI (m/z) 526.4298 ($M + NH4^+$); $C_{25}H_{48}NO_3S$ requires 526.4294.

[2-(2-Octadecyloxy)ethoxy] Ethanethiol (6)

Liquid ammonia (150 cm^3) was condensed into a 250-cm^3, three-necked, round-bottom flask at − 78°C with a dry-ice condenser and a nitrogen

bubbler attached. Small pieces of sodium (1.2 g, 52.2 mmol) were added to obtain a permanent blue coloration. The mixture was stirred at – 78°C for 10 min. After that, a solution of {[2-(2-octadecyloxy)ethoxy]ethylsulfanylmethyl} benzene (3 g, 6.46 mmol) in anhydrous THF (25 cm^3) was added via syringe, and the reaction mixture was stirred at – 78°C for 1 h. The temperature was slowly increased to about – 30°C (~ 1.5 h), and the reaction refluxed for 30 min. The reaction mixture was cooled to – 78°C and quenched with wet THF (75% THF in H_2O, 25 cm^3) until the blue coloration disappeared. The reaction flask was allowed to warm to room temperature with bubbling of nitrogen through the solution. The resulting white suspension was slowly diluted with water (200 cm^3), and the product was extracted with CH_2Cl_2 (3×150 cm^3). The combined organic phases were dried ($MgSO_4$), filtered, and evaporated under reduced pressure to give a crude yellow oil, which was purified by silica chromatography (CH_2Cl_2) to give the title compound **6** (1.80 g, 74%) as a white waxy solid. ^{1}H NMR δ (300 MHz; $CDCl_3$) 0.85 (t, $J = 6.0$, 3 H), 1.15–1.38 (m, 30 H), 1.50–1.61 (m, 3 H), 2.67 (dt, 2 H, $J = 6.4$, 6.6), 3.43 (t, $J = 6.8$, 2 H), 3.52–3.63 (m, 6 H); ^{13}C NMR δ (75 MHz; $CDCl_3$) 14.1, 22.6, 24.2, 26.0, 29.3, 29.5, 29.6, 29.7, 31.8, 69.9, 70.2, 72.8, 71.5. MS-ES + (m/z) 397.3118 (M + Na); $C_{22}H_{46}O_2NaS$ requires 397.3116.

2-[2-(2-Octadecyloxyethoxy)ethoxy] Ethanethiol (7)

Compound **1** was synthesized according the method previously described for **6.** The crude yellow oil obtained was purified by silica chromatography (CH_2Cl_2–EtOAc, 30:1) to give the title compound **7** (1.57 g, 67%) as a white waxy solid. ^{1}H NMR δ (300 MHz; $CDCl_3$) 0.85 (t, $J = 6.4$, 7.0, 3 H), 1.20–1.38 (m, 30 H), 1.50–1.61 (m, 3 H), 2.67 (dt, $J = 6.4$, 6.6, 2 H), 3.43 (t, $J = 6.8$,2 H), 3.50–3.65 (m, 10 H); ^{13}C NMR δ (75 MHz; $CDCl_3$) 14.1, 22.6, 24.2, 26.0, 29.4, 29.5, 29.6, 29.7, 31.8, 70.0, 70.2, 70.5, 70.6, 71.5, 72.8. MS-ES + (m/z) 441.3376 (M + Na); $C_{24}H_{50}O_3NaS$ requires 441.3378.

1-[2-(2-Iodoethoxy)ethoxy] Octadecane 12 (8)

Anhydrous benzene (5 cm^3) was added to a flask containing [2-(2-octadecyloxy)ethoxy] ethanethiol **6** (260 mg, 0.69 mmol) and triphenylphosphine (540 mg, 2.01 mmol) under a nitrogen atmosphere at room temperature. Iodine (263 mg, 1.04 mmol) was added, and the reaction mixture was heated under reflux overnight under a nitrogen atmosphere. After stirring overnight, when the reaction was completed, the mixture was diluted with

dichloromethane (25 cm^3) and washed with water (3 × 15 cm^3). The organic layers were dried over magnesium sulphate ($MgSO_4$), filtered, and evaporated under reduced pressure. The residue was purified by silica chromatography (dichloromethane) to give the title compound 8 (213 mg, 66%) as a white solid. ^{1}H NMR δ (300 MHz; $CDCl_3$) 0.87 (t, $J = 6.7$, 7.0, 3 H),1.27–1.30 (m, 30 H), 1.52–1.59 (m, 2H), 3.25 (t, $J = 6.8$, 2 H), 3.45 (t, $J = 6.8$, 2 H), 3.56–3.66 (m, 4 H), 3.75 (t, $J = 7.0$, 2 H); ^{13}C NMR δ (75 MHz; $CDCl_3$) 2.9, 14.1, 22.7, 26.0, 29.3, 29.5, 29.6, 29.7, 31.9, 70.0, 70.2, 71.6, 72.0. MS-ES + (m/z) 491.2360 (M + Na); $C_{22}H_{45}O_2$ INa requires 491.2362.

1-{2-[2-(2-Iodoethoxy)ethoxy]ethoxy} Octadecane (9)

Compound **9** was synthesized from **7** according the method previously described for **8**. The crude obtained was purified by silica chromatography (ethyl acetate–dichloromethane, 40:1) to give the title compound **9** (234 mg, 70%) as a white waxy solid; ^{1}H NMR δ (300 MHz; $CDCl_3$) 0.87 (t, $J = 6.7$, 7.0, 3 H), 1.26–1.30 (m, 30 H),1.54–1.62 (m, 2 H), 3.25 (t, $J = 6.8$, 2 H), 3.45 (t, $J = 6.8$, 2 H), 3.56–3.66 (m, 8 H), 3.75 (t, $J = 7.0$, 2 H); ^{13}C NMR δ (75 MHz; $CDCl_3$) 2.9, 14.1, 22.7, 26.1, 29.4, 29.5, 29.6, 29.7, 31.9, 70.1, 70.2, 70.6, 70.7, 71.6, 72.0. MS-ES + (m/z) 535.2623 (M + Na); $C_{24}H_{49}O_3$INa requires 535.2624.

[2-(2-Octadecyloxyethoxy)ethoxy]methanethiosulfonate (10)

Previously synthesized sodium methanethiosulfonate **1** (40 mg, 0.30 mmol) was added to a solution of 1-[2-(2-iodoethoxy)ethoxy] octadecane **8** (100 mg, 0.21 mmol) in anhydrous dimethylformamide (5 cm^3) at room temperature under a nitrogen atmosphere. The reaction mixture was heated at 50°C and was monitored by TLC (dichloromethane). After stirring overnight, the solvent was evaporated under reduced pressure to give a residue that was purified by silica chromatography (dichloromethane) to yield the desired compound **10** (47 mg, 49%) as a waxy solid. Mp 40–41°C; ^{1}H NMR δ (300 MHz; $CDCl_3$) 0.86 (t, $J = 6.7$, 66.7, 3 H), 1.25–1.30 (m, 30 H),1.52–1.59 (m, 2 H), 3.25 (t, $J = 6.8$, 12 H), 3.35–3.44 (m, 7 H), 3.54–3.65 (m, 4 H), 3.79 (t, $J = 5.7$, 2 H); ^{13}C NMR δ (75 MHz; $CDCl_3$) 14.1, 22.7, 26.1, 29.4, 29.5, 29.7, 31.9, 36.6 50.6, 69.9, 70.0. MS-ES + (m/z) 475.2890 (M + Na); $C_{23}H_{48}O_4S_2$Na requires 475.2891.

{2-[2-(2-Octadecyloxyethoxy)ethoxy]ethoxy} Methanethiosulfonate (11)

The compound was synthesized following the procedure described for **10**. The residue obtained was purified by silica chromatography (ethyl

acetate–dichloromethane, 30:1) to the required compound **13** (148 mg, 76%) as a waxy solid; mp 40–41°C; ^{1}H NMR δ (300 MHz; $CDCl_3$) 0.86 (t, $J = 6.6$, 6.6, 3 H), 1.24–1.26 (m, 30 H, 1.51–1.58 (m, 2 H), 3.35–3.44 (m, 7 H), 3.53–3.65 (m, 8 H), 3.79 (t, $J = 5.7$, 2 H); ^{13}C NMR δ (75 MHz; $CDCl_3$) 14.1, 22.7, 26.1, 29.4, 29.5, 29.6, 29.7, 31.9, 36.6, 50.7, 69.9, 70.0. MS-ES + (m/z) 519.2150 (M + Na); $C_{25}H_{52}O_5S_2Na$ requires 519.2154.

3-Bromo-2-bromomethyl Propionic Acid[3] (12)

A mixture of commercial diethyl bis(hydroxymethyl)malonate (25 g, 0.11 mol) and 48% aqueous hydrogen bromide (192 cm^3, 1.70 mol) was heated until the bromoethane liquid began to distil off from the mixture and then distilled vigorously for approximately 1 h. The reaction mixture was refluxed for 5 h at 140°C. The solution was cooled for 1.5 h in an ice bath. The crystals formed were filtered, washed with a little ice-cold water, and dried under vacuum. Around 80 cm^3 of the filtrate was distilled off, and the solution was chilled in ice to yield a further crop of crystals, which were washed and dried as described before to give **12** (12.96 g, 47%) as pale brown crystals. Mp 105–108°C; ν_{max}/cm^{-1} 2881, 1687, 1441, 1302, 1240, 1201, 913; ^{1}H NMR δ (400 MHz; $CDCl_3$) 3.20–3.30 (m, 1 H), 3.69–3.83 (m, 4 H); ^{13}C NMR δ (100 MHz; $CDCl_3$) 29.8, 48.3, 175.4. MS-EI (m/z) 245.8714, (M^+); $C_4H_6Br_2O_2$ requires 245.8716.

3-Bromo-2-bromomethyl Propan-1-ol (13)

3-Bromo-2-bromomethyl propionic acid **12** (2 g, 8.20 mmol) was dissolved in anhydrous dichloromethane (50 cm^3) at room temperature under a nitrogen atmosphere. The solution was cooled to 0°C and then diborane (1 M BH_3 in tetrahydrofuran, 25 cm^3, 24.60 mmol) was added dropwise over a period of 10 min. The reaction mixture was allowed to warm to room temperature and left to stir overnight. The reaction was followed by TLC (CH_2Cl_2–MeOH, 40:1). After 18 h, aqueous 1 M HCl (26 cm^3) was added dropwise at room temperature, and the mixture was allowed to stir for 30 min. The phases were separated, and the aqueous layer was washed with CH_2Cl_2 (3×15 cm^3). The combined organic extracts were dried (Na_2SO_4), filtered, and evaporated under reduced pressure to give an oil, which was purified by silica chromatography (CH_2Cl_2–MeOH, 40:1) to give product **13** (1.75 g, 93%) as a colorless oil. ^{1}H NMR δ (400 MHz; $CDCl_3$) 1.63 (bs,1 H), 2.25 (m, 1 H), 3.56

(m, 4 H), 3.76 (d, $J = 6.0$, 2 H); ^{13}C NMR δ (100 MHz; $CDCl_3$) 32.7, 44.4, 62.5. MS-EI (m/z) 229.8942 (M^+); $C_4H_8Br_2O$ requires 229.8946.

3-Hexadecylsulfanyl-2-hexadecylsulfanylmethyl Propan-1-ol (14)

A solution of 3-bromo-2-bromomethyl propan-1-ol **13** (2 g, 8.70 mmol and hexadecanethiol (8.0 cm^3, 26.01 mmol) in anhydrous DMF (60 cm^3) under a nitrogen atmosphere was cooled to 0°C. Then caesium carbonate (9.2 g, 26.01 mmol) was added, and the reaction mixture was left to stir overnight at room temperature. The reaction was followed by TLC (hexane–EtOAc, 2:1). After 24 h, the reaction was poured into ice/water–CH_2Cl_2, 3:5, 320 cm^3), and the aqueous phase was extracted with CH_2Cl_2 (3×150 cm^3). The combined organic extracts were washed with saturated aqueous NaCl (2×150 cm^3), dried (Na_2SO_4), filtered, and evaporated under reduced pressure. MeOH (80 cm^3) was added to force the crystallization of the desired product **14** (4.49 g, 88%) which was obtained after filtration as a white amorphous solid. Mp 42–44°C; ^{1}H NMR δ (400 MHz; $CDCl_3$) 0.87 (t, $J = 6.6$, 7.3, 6 H), 1.16–1.42 (m, 52 H), 1.54–1.68 (m, 4 H), 1.92–1.99 (m, 1 H), 2.51 (t, $J = 7.3$, 7.5, 4 H), 2.60–2.69 (m, 4 H), 3.75 (d, $J = 5.1$, 2 H); ^{13}C NMR δ (100 MHz; $CDCl_3$) 14.1, 22.7, 28.9, 29.2, 29.4, 29.5, 29.6, 29.7, 31.9, 32.9, 33.7, 40.6, 64.7. MS-FAB 3-NBA (m/z, %) 585.5103 ($M + 1$); $C_{36}H_{73}NOS_2$ requires 585.5102.

3-(Hexadecane-1-sulfonyl)-2-(hexadecane-1-sulfonylmethyl) Propionic Acid (15)

Acetic acid (150 cm^3) was added to a flask containing 3-hexadecylsulfanyl-2-hexadecylsulfanylmethyl propan-1-ol **14** (2 g, 3.41 mmol). The mixture was heated in an oil bath at 60°C until all of the starting material was dissolved. A saturated solution of potassium permanganate (21.6 g, 136.4 mmol) in water (200 cm^3) was then added, and the mixture was stirred overnight at 60°C. The reaction was followed by TLC (CH_2Cl_2–MeOH, 5:1). After 24 h, the reaction was allowed to cool to room temperature. Then sodium metabisulfate (33.7 g, 177.3 mmol) was added, which resulted in an instantaneous color change of the solution from a purple to white. The reaction was acidified with 5 M HCl (aq), and the product was extracted with $CHCl_3$ (3×300 cm^3). The combined extracts were combined and evaporated under reduced pressure, and the crude product was purified by silica chromatography (CH_2Cl_2–MeOH, 5:1 v/v) to give product **15** (1.41 g, 63%) as a white amorphous solid. Mp 62–64°C; ν_{max}/cm^{-1}

3109, 2956, 2916, 2849, 1726, 1464, 1317, 1276, 1189, 1126, 1109; ^{1}H NMR δ (400 MHz; pyridine-d^6) 0.88 (t, $J = 6.7$, 7.0, 6 H), 1.15–1.45 (m, 52 H), 1.95–2.05 (m, 4 H), 3.45 (t, $J = 7.8$, 8.0, 4 H), 4.15–4.40 (m, 5 H); ^{13}C NMR δ (100 MHz; pyridine-d^6) 14.3, 22.4, 23.0, 28.7, 29.4, 29.6, 29.7, 29.8, 29.9, 30.0; 29.5, 29.6, 29.7, 32.1, 32.7, 36.1, 53.4, 54.2, 178.4. MS-FAB 3-NBA (m/z, %) 687.4655 ($M + Na^+$); $C_{36}H_{72}O_6S_2Na$ requires 687.4668.

3-Hexadecylsulfanyl-2-hexadecylsulfanylmethyl Propionic Acid (16)

3-Bromo-2-bromomethyl propionic acid **15** (2 g, 8.201 mmol) and hexadecanethiol (7.6 cm^3, 24.60 mmol) were dissolved in anhydrous DMF (60 cm^3) at room temperature under a nitrogen atmosphere. Caesium carbonate (9.2 g, 26.01 mmol) was added, and the reaction mixture was stirred overnight, at 50°C, under a nitrogen atmosphere. The reaction was followed by TLC. (For TLC, an aliquot was removed to vial containing 1 M HCl (aq), $CHCl_3$ was added, the vial was shaken, and the $CHCl_3$ was phase spotted using as eluent hexane–EtOAc, 4:1 v/v + 0.5% acetic acid.) After 24 h, the reaction was allowed to cool to room temperature and poured into ice/water–$CHCl_3$ (3:5, 320 cm^3), and the organic phase was separated. The aqueous phase was acidified with 5 M HCl (aq), and product was extracted with $CHCl_3$ (150 cm^3 × 3). The extracts were washed with saturated aqueous NaCl (2 × 200 cm^3), dried (Na_2SO_4), filtered, and evaporated under reduced pressure. MeOH (40 cm^3) was added to favor the crystallization as a white solid, which was filtered and purified by silica chromatography (hexane–EtOAc, 4:1, + 0.5% acetic acid) to give product **16** (2.15 g, 44%) as a white amorphous solid; mp 52–53°C; ν_{max}/cm^{-1} 3110, 2955, 2917, 2850, 1694, 1470, 1431, 716; ^{1}H NMR δ (400 MHz; $CDCl_3$) 0.87 (t, $J = 6.7$, 7.0, 6 H), 1.15–1.38 (m, 52 H), 1.50–1.60 (m, 4 H), 2.52 (t, $J = 7.3$, 7.5, 4 H), 2.75–2.90 (m, 5 H); ^{13}C NMR δ (75 MHz; $CDCl_3$) 14.1, 22.7, 28.9, 29.3, 29.4, 29.5, 29.6, 29.7, 31.9, 32.7, 46.1, 178.4. MS-FAB 3-NBA (m/z, %) 645.4676 ($M + 2Na^+$); $C_{36}H_{72}O_2S_2Na_2$ requires 645.4691.

2-{2-[2-(2-{2-[2-(*tert*-Butyldimethylsilanyloxy)ethoxy]ethoxy} ethoxy)ethoxy]ethoxy} Ethanol (17)

A solution of hexaethyleneglycol (9.2 g, 32.58 mmol) in anhydrous THF (20 cm^3) under a nitrogen atmosphere was cooled to 0°C and stirred for 10 min. Sodium hydride (1.3 g, 32.58 mmol, 60% dispersion in mineral oil) was added. Stirring was continued until effervescence had ceased.

Then a solution of *tert*-butyldimethylsilyl chloride (4.9 g, 32.58 mmol) in anhydrous THF (20 cm^3) was added dropwise over a period of 2.5 h, at 0°C, keeping the atmosphere of nitrogen. After the addition was complete, the ice bath was removed, and the reaction mixture was allowed to warm to room temperature with stirring over 30 min. The reaction was monitored by TLC using CH_2Cl_2–MeOH, (15:1) solvent mixture. The reaction was cooled to 0°C, and methanol (10 cm^3) was added dropwise to destroy the excess of sodium hydride. The solvent was evaporated under reduced pressure to give a yellow oil. The oil was dissolved in cyclohexane (100 cm^3) and washed twice with water (30 cm^3). The organic layer was dried ($MgSO_4$), filtered, and evaporated under reduced pressure to give a yellow oil, which was purified by silica chromatography to give product **17** (4.9 g, 36%) as a colorless oil; ^{1}H NMR δ (400 MHz; $CDCl_3$) 0.04 (s, 6 H), 0.87 (s, 9 H), 2.58 (bs, 1 H), 3.53 (dd, $J = 5.3, 5.5$, 11H), 3.59 (dd, $J = 4.1, 4.7$, 2 H), 3.60–3.65 (m, 16 H), 3.70 (dd, $J = 4.3, 4.8$, 2 H), 3.74 (dd, $J = 4.7, 5.5$, 2 H); ^{13}C NMR δ (100 MHz; $CDCl_3$) – 5.3, 25.9, 61.7, 62.7, 70.3, 70.5, 70.6, 70.7, 72.5, 72.6. MS-EI (m/z, %) 397.2625 (100.%, M^+.); $C_{18}H_{41}O_7Si$ requires 397.2622).

3-(Hexadecane-1-sulfonyl)-2-(hexadecane-1-sulfonylmethyl)-propionic Acid 2-{2-[2-(2-{2-[2-(*tert*-Butyl-dimethyl-silanyloxy)ethoxy]-ethoxy}ethoxy)ethoxy]ethoxy} Ethyl Ester (18)

2-{2-[2-(2-{2-[2-(*tert*-Butyldimethylsilanyloxy)ethoxy]ethoxy}ethoxy)-ethoxy]-ethoxy}ethanol **15** (0.26 g, 0.640 mmol) was dissolved in anhydrous toluene (8 cm^3) under an atmosphere of nitrogen. A Dean–Stark trap was attached, and the solution was heated until about 4 cm^3 of toluene were distilled. The remaining solvent was evaporated under reduced pressure and dried under vacuum. Anhydrous CH_2Cl_2 (30 cm^3) was added to a flask containing 3-(hexadecane-1-sulfonyl)-2-(hexadecane-1-sulfonylmethyl) propionic acid (0.42 g, 0.640 mmol), dried alcohol **15** (0.26 g, 0.640 mmol), and DMAP (8 mg, 0.064 mmol) at room temperature under a nitrogen atmosphere. The mixture was gently warmed to dissolve the acid. The mixture was cooled to 0°C, and then a solution of dicyclohexylcarbodiimide (DCC) (0.26 g, 1.280 mmol) in anhydrous CH_2Cl_2 (3 cm^3) was added dropwise over a period of 10 min. Dicyclohexylurea (DCU) precipitates before addition of DCC is complete. The ice bath was removed, and the reaction mixture was left to stir overnight. The reaction was followed by TLC ($CHCl_3$–MeOH, 30:1). After 22 h, the reaction was filtered to remove DCU and evaporated under reduced pressure. The crude product was purified by silica chromatography ($CHCl_3$–EtOAc, 1:1), yielding **18** (0.24 g, 36%) as a

waxy solid; ^{1}H NMR δ (500 MHz; $CDCl_3$) 0.04 (s, 6 H), 0.87 (t, $J = 7.0$, 7.4, 6 H), 0.89 (s, 9 H), 1.20–1.40 (m, 48 H), 1.37–1.47 (m, 4 H), 1.79–1.85 (m, 4 H), 3.02 (app t, $J = 8.1$, 8.1, 4 H), 3.53 (t, $J = 5.3$, 6.6, 2 H), 3.60–3.68 (m, 16 H), 3.70 (app t, $J = 4.7$, 5.0, 2 H), 3.73 (t, $J = 5.3$, 5.6, 2 H), 4.33 (app t, $J = 4.7$, 5.0, 2 H); ^{13}C NMR δ(125 MHz; $CDCl_3$) −5.30, 14.1, 21.8, 22.7, 25.9, 28.4, 29.0, 29.2, 29.3, 29.5, 29.6, 31.9, 34.7, 51.7, 54.4, 62.7, 65.2, 68.6, 70.4, 70.5, 70.6, 70.7, 72.6, 170.0. MS-FAB 3-NBA (m/z) 1087.6876 ($M + 2Na^+$); $C_{54}H_{109}O_{12}S_2SiNa_2$ requires 1087.6925.

3-Hexadecylsulfanyl-2-hexadecylsulfanylmethyl-propionic Acid 2-{2-[2-(2-{2-[2-(*tert*-Butyldimethylsilanyloxy)ethoxy]ethoxy}ethoxy)ethoxy]ethoxy} Ethyl Ester (19)

The compound was prepared according with the procedure described for **18** using product **16** as starting material. The crude obtained was purified by silica chromatography ($CHCl_3$–MeOH, 80:1 v/v) to give product **19** (1.24 g, 76%) as a waxy solid; ^{1}H NMR δ (500 MHz; $CDCl_3$) 0.05 (s, 6 H), 0.87 (t, $J = 7.2$, 7.6, 6 H), 0.89 (s, 9 H), 1.20–1.40 (m, 52 H), 1.50–1.60 (m, 4 H), 2.50 (t, $J = 7.2$, 7.5, 4 H), 2.73–2.88 (m, 5 H), 3.54 (t, $J = 5.3$, 5.6, 2 H), 3.60–3.68 (m, 16 H), 3.70 (t, $J = 5.0$, 5.0, 2H), 3.75 (t, $J = 5.6$, 6.3, 2 H), 4.28 (t, $J = 4.7$, 5.0, 2 H); ^{13}C NMR δ (125 MHz; $CDCl_3$) − 5.26, 14.1, 22.7, 25.9, 28.9, 29.3, 29.4, 29.5, 29.6, 29.7, 31.9, 32.6, 33.1, 46.3, 62.7, 63.9, 69.1, 70.5, 70.6, 70.7, 72.7, 173.2. MS-FAB 3-NBA (m/z) 1001.7315 ($M + Na^+$); $C_{54}H_{110}O_8S_2SiNa_2$ requires 1001.7309.

3-(Hexadecane-1-sulfonyl)-2-(hexadecane-1-sulfonylmethyl) Propionic Acid 2-[2-(2-{2-[2-(2-Hydroxy-ethoxy)ethoxy]ethoxy}ethoxy)ethoxy] Ethyl Ester (20)

3-(Hexadecane-1-sulfonyl)-2-(hexadecane-1-sulfonylmethyl)-propionic acid 2-{2-[2-(2-{2-[2-(*tert*-butyl-dimethylsilanyloxy)ethoxy]ethoxy}ethoxy)-ethoxy-ethoxy-ethyl ester **18** (0.18 g, 0.175 mmol) was dissolved in mixture of anhydrous CH_2Cl_2–acetonitrile, 1:1 (2 cm^3) at room temperature under a nitrogen atmosphere. The solution was cooled to 0°C, and then $BF_3{\cdot}OEt_2$ (44 µl, 0.349 mmol) was added dropwise over a period of 10 min. TLC after 20 min ($CHCl_3$–EtOAc–MeOH, 2:20:0.7) indicated completion of the reaction. Saturated aqueous $NaHCO_3$ (5 cm^3) was then added, and the product was extracted with $CHCl_3$ (3×10 cm^3). The organic phase was washed with water (5 cm^3), dried (Na_2SO_4), filtered, and evaporated under reduced pressure to give product **20** (0.160 g,

99%) as a white solid. ^{1}H NMR δ (500 MHz; $CDCl_3$) 0.86 (t, $J = 6.6$, 7.2, 6 H), 1.20–1.34 (m, 48 H), 1.37–1.50 (m, 4 H), 1.79–1.88 (m, 4 H), 3.03 (appt t, $J = 6.0$, 6.0, 4 H), 3.59–3.70 (m, 23 H), 3.71–9.75 (m, 4 H), 4.28 (app t, $J = 4.7$, 4.7, 2 H); ^{13}C NMR δ (125 MHz; $CDCl_3$) 14.1, 21.8, 22.7, 28.4, 29.1, 29.3, 29.4, 29.5, 29.6, 29.7, 31.9, 34.8, 51.7, 54.5, 61.6, 65.2, 68.7, 70.2, 70.4, 70.5, 70.6, 72.6, 170.1; MS-FAB 3-NBA (m/z) 951.6241 ($M + Na^+$). $C_{48}H_{96}O_{12}S_2Na$ requires 951.6232).

3-Hexadecylsulfanyl-2-hexadecylsulfanylmethyl Propionic Acid 2-[2-(2-{2-[2-(2-Hydroxyethoxy)ethoxy]ethoxy}ethoxy)ethoxy] Ethyl Ester (21)

The compound was prepared according with the procedure described for **20** using **19** as starting material. The reaction yielded **21** (0.78 g, 98%) as a waxy solid. ^{1}H NMR δ (500 MHz; $CDCl_3$) 0.84 (t, $J = 6.5$, 7.0, 6 H), 1.15–1.44 (m, 52 H), 1.49–1.59 (m, 4 H), 2.49 (t, $J = 7.3$, 7.5, 4 H), 2.73–2.85 (m, 5 H), 3.55–3.75 (m, 22 H), 4.28 (t, $J = 4.8$, 4.8, 2 H); ^{13}C NMR δ (125 MHz; $CDCl_3$) 14.1, 22.7, 28.9, 29.2, 29.3, 29.5, 29.6, 29.7, 31.9, 32.6, 33.0, 46.1, 63.9, 69.1, 70.2, 70.4, 70.5, 72.4, 173.2. MS-FAB 3-NBA (m/z) 887.6433 ($M + Na^+$); $C_{48}H_{96}O_8S_2Na$ requires 887.6435).

3-(Hexadecane-1-sulfonyl)-2-(hexadecane-1-sulfonylmethyl)-propionic Acid 2-[2-(2-{2-[2-(2-Methanesulfonyloxyethoxy)ethoxy]ethoxy}ethoxy) ethoxy] Ethyl Ester (22)

A solution of 3-(hexadecane-1-sulfonyl)-2-(hexadecane-1-sulfonylmethyl) propionic acid 2-[2-(2-{2-[2-(2-hydroxyethoxy)ethoxy]ethoxy}ethoxy)-ethoxy] ethyl ester **20** (0.3 g, 0.323 mmol) in anhydrous pyridine (8 cm^3) under a nitrogen atmosphere was cooled to 0°C. Then a solution of methanesulfonyl chloride (33 μl, 0.426 mmol) in anhydrous pyridine (2 cm^3) was added dropwise over a period of 10 min. The ice bath was removed, and the reaction mixture was left to stir at room temperature. The reaction was followed by TLC ($CHCl_3$–EtOAc–MeOH, 2:20:0.7). After 1 h, the reaction was filtered through cotton and evaporated under reduced pressure. The residue was purified by silica chromatography to remove residual pyridine ($CHCl_3$–EtOAc–MeOH, 2:20:0.7) to give product **22** (0.31 g, 96%) as a pale brown waxy solid. ^{1}H NMR δ (400 MHz; $CDCl_3$) 0.87 (t, $J = 6.5$, 7.0, 6 H), 1.20–1.34 (m, 48 H), 1.37–1.47 (m, 4 H), 1.78–1.88 (m, 4 H), 3.03 (appt t, $J = 6.0$, 6.0, 4 H), 3.08 (s, 3 H), 3.55–3.70 (m, 21 H), 3.71 (app t, $J = 4.5$, 5.0, 2 H), 3.73–3.77 (m, 2 H), 4.20–4.40 (m, 4 H); ^{13}C NMR δ (100 MHz; $CDCl_3$)

14.1, 21.8, 22.7, 28.4, 29.0, 29.3, 29.5, 29.6, 29.7, 31.9, 34.7, 37.7, 51.7, 54.5, 65.2, 68.6, 69.0, 69.3, 70.4, 70.5, 70.6, 70.7, 170.2; MS-FAB 3-NBA (m/z) 1029.6016 ($M + Na^+$); $C_{49}H_{98}O_{14}S_3Na$ requires 1029.6013.

3-Hexadecylsulfanyl-2-hexadecylsulfanylmethyl Propionic Acid 2-[2-(2-{2-[2-(2-Methanesulfonyloxyethoxy)ethoxy]ethoxy}ethoxy)ethoxy] Ethyl Ester (23)

The compound was prepared according to the procedure described for **22** using **21** as starting material. The residue obtained was purified by silica chromatography to remove residual pyridine ($CHCl_3$–EtOAc, 1:1) to yield product **23** (0.44 g, 100%) as a brown solid. ^{1}H NMR δ (500 MHz; $CDCl_3$) 0.86 (t, $J = 6.6$, 7.0, 6 H), 1.20–1.40 (m, 52 H), 1.50–1.57 (m, 4 H), 2.49 (t, $J = 7.3$, 7.5, 4 H), 2.76–2.85 (m, 5 H), 3.06 (s, 3 H), 3.59–3.67 (m, 16 H), 3.69 (t, $J = 4.8$, 5.3, 2 H), 3.73–3.76 (m, 2 H), 4.27 (app t, $J = 4.8$, 5.3, 2 H), 4.36 (m, 2 H); ^{13}C NMR δ (125 MHz; $CDCl_3$) 14.1, 22.7, 28.8, 29.2, 29.3, 29.5, 29.7, 31.9, 32.6, 33.0, 37.7, 46.2, 63.9, 69.0, 69.1, 69.3, 70.4, 70.5, 70.7, 173.2; MS-FAB 3-NBA (m/z) 965.6220 ($M + Na^+$), $C_{49}H_{98}O_{10}S_3Na$ requires 965.6254.

3-(Hexadecane-1-sulfonyl)-2-(hexadecane-1-sulfonylmethyl) Propionic Acid 2-[2-(2-{2-[2-(2-Iodoethoxy)ethoxy]ethoxy}ethoxy)ethoxy] Ethyl Ester (24)

A solution of 3-(hexadecane-1-sulfonyl)-2-(hexadecane-1-sulfonylmethyl)propionic acid 2-[2-(2-{2-[2-(2-methanesulfonyloxyethoxy)ethoxy]ethoxy}ethoxy)ethoxy]ethyl ester **22** (0.28 g, 0.28 mmol) and sodium iodide (58 mg, 0.39 mmol) in anhydrous acetone (10 cm^3) was refluxed overnight under a nitrogen atmosphere. The reaction was followed by TLC ($CHCl_3$–EtOAc–MeOH, 2:20:0.2). After 22 h, the solvent was evaporated under reduced pressure. The residue was dissolved in $CHCl_3$ (50 cm^3), washed with water (25 $cm^3 \times 2$), dried (Na_2SO_4), filtered, and evaporated under reduced pressure to give product (0.19 g, 66%) as a brown waxy solid. ^{1}H NMR δ (500 MHz; $CDCl_3$) 0.87 (t, $J = 6.9$, 6.9, 6 H), 1.20–1.37 (m, 48 H), 1.38–1.45 (m, 4 H), 1.79–1.87 (m, 4 H), 3.03 (app t, $J = 6.9$, 8.0, 4 H), 3.25 (t, $J = 7.2$, 7.6, 2 H), 3.54–3.66 (m, 21 H), 3.71 (app t, $J = 4.7$, 5.0, 2 H), 3.74 (t, $J = 7.2$, 7.9, 2 H), 4.34 (app t, $J = 4.7$, 4.7, 2 H); ^{13}C NMR δ (125 MHz; $CDCl_3$) 2.9, 14.1, 21.8, 22.7, 28.4, 29.0, 29.3, 29.5, 29.6, 29.7), 31.9, 34.7, 51.7, 54.5, 65.2, 68.6,

70.2, 70.4, 70.6, 70.7, 72.0, 170.0; MS-FAB 3-NBA (m/z, %) 1045.5788 ($M + Na^+$); $C_{49}H_{98}IO_{13}S_4Na$ requires 1045.5747.

3-Hexadecylsulfanyl-2-hexadecylsulfanylmethyl Propionic Acid 2-[2-(2-{2-[2-(2-iodoethoxy)ethoxy]ethoxy}ethoxy)ethoxy] Ethyl Ester (25)

The compound was prepared according with the procedure described for **24** using **23** as starting material. The reaction yielded pure product **25** (0.41 g, 90%) as a brown waxy solid. ^{1}H NMR δ (400 MHz; $CDCl_3$) 0.87 (t, $J = 6.5$, 7.1, 6 H), 1.19–1.40 (m, 52 H), 1.50–1.59 (m, 4 H), 2.49 (t, $J = 7.5$, 7.5, 4 H), 2.75–2.84 (m, 5 H), 3.25 (t, $J = 6.8$, 7.0, 2 H), 3.60–3.65 (m, 16 H), 3.70 (br t, $J = 5.0$, 5.0, 2 H), 3.74 (t, $J = 6.8$, 7.3, 2 H), 4.27 (br t, $J = 4.8$, 5.0, 2 H); ^{13}C NMR δ (75 MHz; $CDCl_3$) 14.1, 22.7, 28.9, 29.2, 29.4, 29.5, 29.6, 29.7, 31.9, 32.6, 33.1, 46.3, 63.9, 69.0, 70.2, 70.5, 70.6, 70.7, 70.8, 72.0, 173.2. MS-FAB 3-NBA (m/z) 997.5462 ($M + Na^+$); $C_{48}H_{95}IO_7S_2Na$ requires 997.5484.

3-(Hexadecane-1-sulfonyl)-2-(hexadecane-1-sulfonylmethyl) Propionic Acid 2-[2-(2-{2-[2-(2-methanesulfonylsulfanylethoxy)ethoxy]ethoxy}ethoxy)ethoxy] Ethyl Ester (26)

3-(Hexadecane-1-sulfonyl)-2-(hexadecane-1-sulfonylmethyl) propionic acid 2-[2-(2-{2-[2-(2-iodoethoxy)ethoxy]ethoxy}ethoxy)ethoxy] ethyl ester **24** (0.18 g, 0.17 mmol) was dissolved in anhydrous DMF (8 cm^3) at room temperature under a nitrogen atmosphere. Sodium methanethiosulfonate (30 mg, 0.22 mmol) was added, and the mixture was heated at 50°C overnight. The reaction was followed by TLC ($CHCl_3$–EtOAc–MeOH, 2:20:0.2). After 17 hours, the reaction was allowed to cool to room temperature, and the solvent was evaporated under reduced pressure. The crude residue was purified by silica chromatography ($CHCl_3$–EtOAc–MeOH, 2:20:0.2) to give the required lipid **26** (0.13 g, 74%) as a brown orange waxy solid. ^{1}H NMR δ (500 MHz; $CDCl_3$) 0.87 (t, $J = 6.6$, 7.0, 6 H), 1.20–1.36 (m, 48 H), 1.37–1.47 (m, 4 H), 1.77–1.87 (m, 4 H), 3.03 (appt t, $J = 8.0$, 8.3, 4 H), 3.37 (t, $J = 5.8$, 5.8, 2 H), 3.41 (s, 3 H), 3.56–3.68 (m, 21 H), 3.72 (app t, $J = 4.8$, 4.8, 2 H), 3.78 (t, $J = 5.8$, 5.9, 2 H), 4.34 (app t, $J = 4.8$, 4.8, 2 H); ^{13}C NMR δ (125 MHz; $CDCl_3$) 14.1, 21.8, 22.6, 28.4, 29.0, 29.2, 29.3, 29.5, 29.6, 29.7, 31.9, 34.8, 36.6, 50.7, 51.7, 54.5, 65.2, 68.6, 69.9, 70.4, 70.5, 70.6, 170.1. MS-FAB 3-NBA (m/z) 1045.5788 ($M + Na^+$), $C_{49}H_{98}O_{13}S_4Na$ requires 1045.5747.

3-Hexadecylsulfanyl-2-hexadecylsulfanylmethyl Propionic Acid 2-[2-(2-{2-[2-(2-Methanesulfonylsulfanylethoxy)ethoxy]ethoxy}ethoxy)ethoxy] Ethyl Ester (27)

The compound was prepared according to the procedure described for **26** using **25** as precursor. The crude residue obtained was purified by silica chromatography ($CHCl_3$–EtOAc, 1:4) to give the target anchor lipid **27** (0.31 g, 90%) as a brown orange solid. ^{1}H NMR δ (400 MHz; $CDCl_3$) 0.87 (t, $J = 6.0$, 6.8, 6 H), 1.18–1.42 (m, 52 H), 1.50–1.57 (m, 4 H), 2.50 (app t, $J = 7.0$, 7.8, 4 H), 2.75–2.86 (m, 5 H), 3.37 (t, $J = 5.8$, 5.8, 2 H), 3.40 (s, 3 H), 3.60–3.65 (m, 16 H), 3.70 (app t, $J = 5.0$, 5.0, 2 H), 3.79 (t, $J = 5.8$, 5.8, 2 H), 4.27 (t, $J = 5.0$, 5.0, 2 H); ^{13}C NMR δ (75 MHz; $CDCl_3$) 14.1, 22.7, 28.9, 29.2, 29.3, 29.5, 29.6, 29.7, 31.9, 32.6, 33.1, 36.6, 46.3, 50.7, 63.9, 69.1, 69.9, 70.4, 70.5, 70.6, 173.2. MS-FAB 3-NBA (m/z) 981.5952 ($M + Na^+$); $C_{49}H_{98}O_9S_4Na$ requires 981.5991.

CONCLUSION

In summary, we have described an easy and efficient route to synthesize mimetic lipid anchors containing a coupling methanethiosulfonate group suitable to attach to a cysteine residue of a protein. These mimetic lipid–coupled proteins can be used as models for many proteins naturally tethered to membranes via lipid tails, and this strategy has been applied to the study of fatal neurodegenerative diseases in cattle and humans.[8]

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