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Synthesis of nitrilotriacetic acid terminated tethers for the binding of His-tagged proteins to lipid bilayers and to gold

Ammathnadu S. Achalkumar [a,*], Richard J. Bushby [a], Stephen D. Evans [b]

[a] Centre for Molecular NanoScience (CMNS), University of Leeds, Leeds, LS2 9JT, UK
[b] School of Physics and Astronomy, University of Leeds, Leeds, LS2 9JT, UK

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ABSTRACT

Nitrilotriacetic acid terminated tethers for trapping of His-tagged proteins have been synthesized and characterized. Compound **1**, containing a lipophilic cholesterol anchor, hydrophilic oligoethoxy chain linker, and nitrilotriacetic acid terminus, can be used for attaching His-tagged proteins to phospholipid bilayers. Compound **2**, containing a gold binding thioacetate anchor, hydrophilic oligoethoxy chain, and nitrilotriacetic acid terminus, can be used to tether His-tagged proteins to a gold surface.

An efficient synthesis is reported for nitrilotriacetic acid terminated tethers for binding of His-tagged proteins to lipid bilayers and to gold surfaces.

1. Introduction

Biological membranes are essential components of living cells as they enclose the cell contents, compartmentalize the cell, and form the boundary of cell organelles. They are the seat of signal recognition, transduction, and amplification and the sites at which both metabolism and photosynthesis occur. Furthermore, an understanding of biomembranes is fundamental to medicine, since drugs often target membrane proteins. Biomembranes are made up of a complex mixture of phospholipids, proteins, sterols and other components (Fig. 1) but the biological functionality of the membrane depends on the presence of integral proteins (present inside the bilayer) and peripheral proteins (on its surface).

Supported bilayer phospholipid membranes (sBLMs) and phospholipid vesicles are the two most commonly used models of natural biomembranes.[1–5] sBLMs are stable, they can be reproducibly prepared,[4,6] and they permit a wide range of surface analytical techniques to be employed ranging from atomic force microscopy (AFM) to impedance spectroscopy. Reviews by Boxer illustrate the mechanism of sBLMs formation, motion within and on sBLMs, their interaction with cells, methods used to pattern sBLMs, and recent trends in the field.[1,2] Phospholipid vesicles are simple to prepare and can be made with some control of both size and dispersity. As with natural biomembranes, it is incorporation or attachment of proteins that provides sBLMs and phospholipid vesicles with biological functionality.

Attachment of proteins can be achieved simply by physisorption or by binding through a natural ligand. Physisorption is often non-specific and depends on several factors, such as pH, ion strength or temperature. Protein binding using a natural ligand is better but cannot be applied to cases where the protein/ligand binding site also needs to be studied and hence other methods are required.

We have reported protein tethers, where the reactive head-group is an *N*-hydroxy succinimido ester.[7] These can be attacked by proteins that contain free $-NH_2$ residues. Thus at pH 8–9, lipid vesicles functionalized with an *N*-hydroxy succinimido ester react with human IgG, binding the antibody to the outside of the vesicle.[8] Using tethers bearing a maleimide head-group[9–11] it is possible to bind proteins that contain free –SH residues. Maleimide containing tethers have been used to create 'immunoliposomes' using an antibody that targets the epidermal growth factor receptor that is over expressed in brain tumor cells. The –SH residues were introduced into the antibody by thiolation using Traut's reagent.[9]

These methods involving tethering of proteins through $-NH_2$ or –SH residues are fairly non-specific. More specific protein binding can be achieved using biotin or nitrilotriacetic acid (NTA)–

* Corresponding author. E-mail address: achalkumar78@gmail.com (A.S. Achalkumar).

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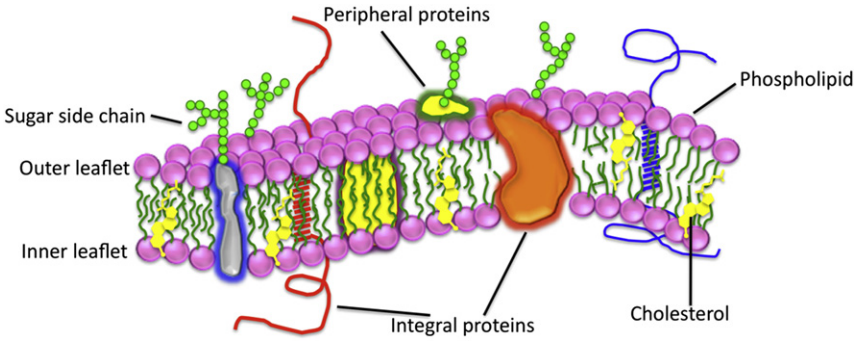


Fig. 1. Schematic of biomembrane.

nickel functionalized tethers. A biotin functionalized tether allows specific binding of avidin or streptavidin.[12] (NTA)–nickel complexes (or the very similar iminodiacetic acid (IDA)–copper complexes)[13] allow specific binding of histidine-tagged (His-tagged) proteins (Fig. 2). These reactions have the advantage that His-tagged proteins of all types are relatively easy to obtain and that the binding can be reversed by lowering the pH or by the addition of imidazole or EDTA. Furthermore, this complexation does not affect the protein activity. There is a commercially available NTA tether known as DOGS–NTA.[14] The development of these protein-specific binding tethers has been driven by the need to attach different types of complex protein to lipid vesicles and to sBLMs and from the potential use of such systems in drug delivery, medical imaging systems, biosensors, and drug screening arrays.[15]

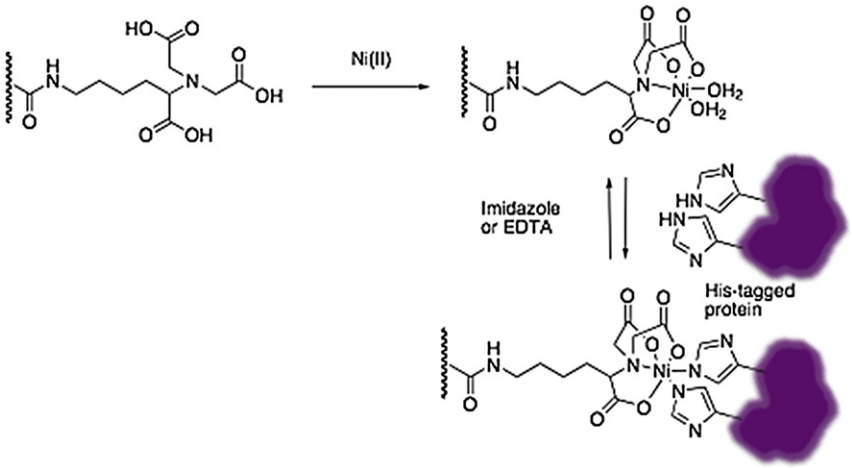


Fig. 2. Schematic of the trapping of His-tagged proteins by NTA–Ni(II) complex.

The main goal of the present work is to demonstrate a general strategy for constructing NTA terminated tethers, which can anchor His-tagged proteins to sBLMs on silica. As part of the same work we have developed an NTA terminated tether, which can anchor His-tagged proteins to a gold surface; a variant on existing gold binding tethers.[16]

2. Results and discussion

The functional end of these tethers is a nitrilotriacetic acid fragment, which on complexation with Ni(II) can bind His-tagged proteins. This NTA fragment is connected to a hydrophilic oligoethoxy chain so as to make it water compatible and provide a space between the protein and the surface. In the case of tether **1** this hydrophilic molecular segment is connected to a cholesterol segment (Fig. 3). Cholesterol is an ideal choice as a hydrophobic segment, because naturally in a biomembrane cholesterol adopts a conformation where its hydrophobic part inserts into the bilayer, while the hydrophilic 3-hydroxy group points to the aqueous phase. In the case of the present cholesterol tether **1**, the hydrophilic region comprising oligoethoxy chain with NTA fragment replaces this 3-hydroxy group. Thus the structure should hardly perturb the natural structure of the membrane. In the case of tether **2** we incorporated a thioacetate group at the anchor end of the hydrophobic undecyl chain, which can bind to the gold surface. The syntheses of these tethers are outlined in Schemes 1 and 2. Synthesis of the carbamate **3**,[7] the monoprotected oligoethoxy amine **9**[7] and the NTA fragment **4**[17] were carried out as previously reported. In the process we have investigated several routes for introducing the nitrilotriacetic acid residue but that shown, involving *tert*-butyl ester deprotection was the most successful.

For the synthesis of tether **1** (Scheme 1) carbamate **3** was treated with the amine-terminated triprotected NTA moiety **4** under mild basic conditions to obtain compound **5**. This compound was deprotected using trifluoroacetic acid to obtain the NTA tether in quantitative yield. Synthesis of thioacetate based tether **2** was carried as follows (Scheme 2). 12-Bromododecanoic acid was treated with potassium thioacetate to obtain the thioacetate derivative **7**. The acid **7** was activated as its *N*-hydroxy succinimido ester **8** and this ester was treated with monoprotected amine **9** to obtain compound **10**. Amine **11** was obtained by the acid catalyzed deprotection of compound **10** in quantitative yield. Compound **11** was then treated with disuccinimidyl carbonate under mild basic conditions to obtain carbamate **12** in moderate yield. This

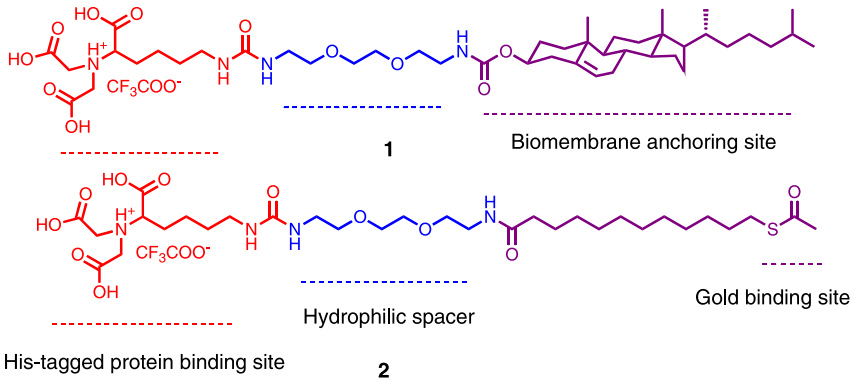
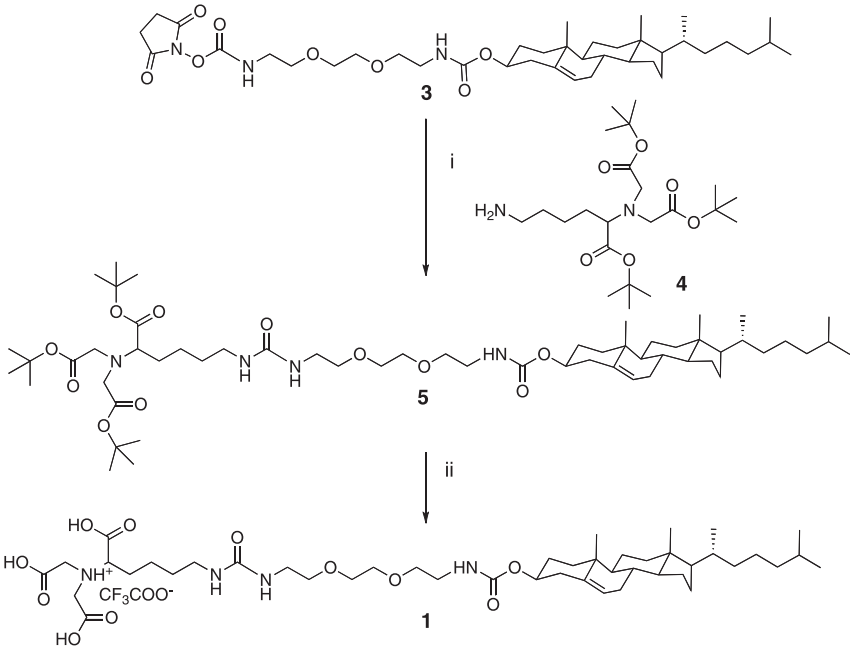


Fig. 3. Molecular design of tethers **1** and **2**.



Scheme 1. Synthesis of tether **1**. Reagents and conditions. (i) Diisopropyl ethylamine (DIPEA), THF, rt, 17 h, 97%; (ii) Trifluoroacetic acid (TFA), CH_2Cl_2, rt, 17 h, 100%.

carbamate was later treated with amine-terminated triprotected NTA moiety **4** to obtain compound **13** in good yield. Finally compound **13** was deprotected under acid catalyzed condition to obtain the target molecule **2** in quantitative yield.

3. Conclusions

We have developed short and efficient routes to cholesterol and thioacetate terminated NTA tethers (**1** and **2**), which on complexation with Ni(II) can be used to bind His-tagged proteins to biomembranes (particularly sBLMs but also to vesicles or microbubbles) or to gold surfaces. The results of these experiments will be reported separately.

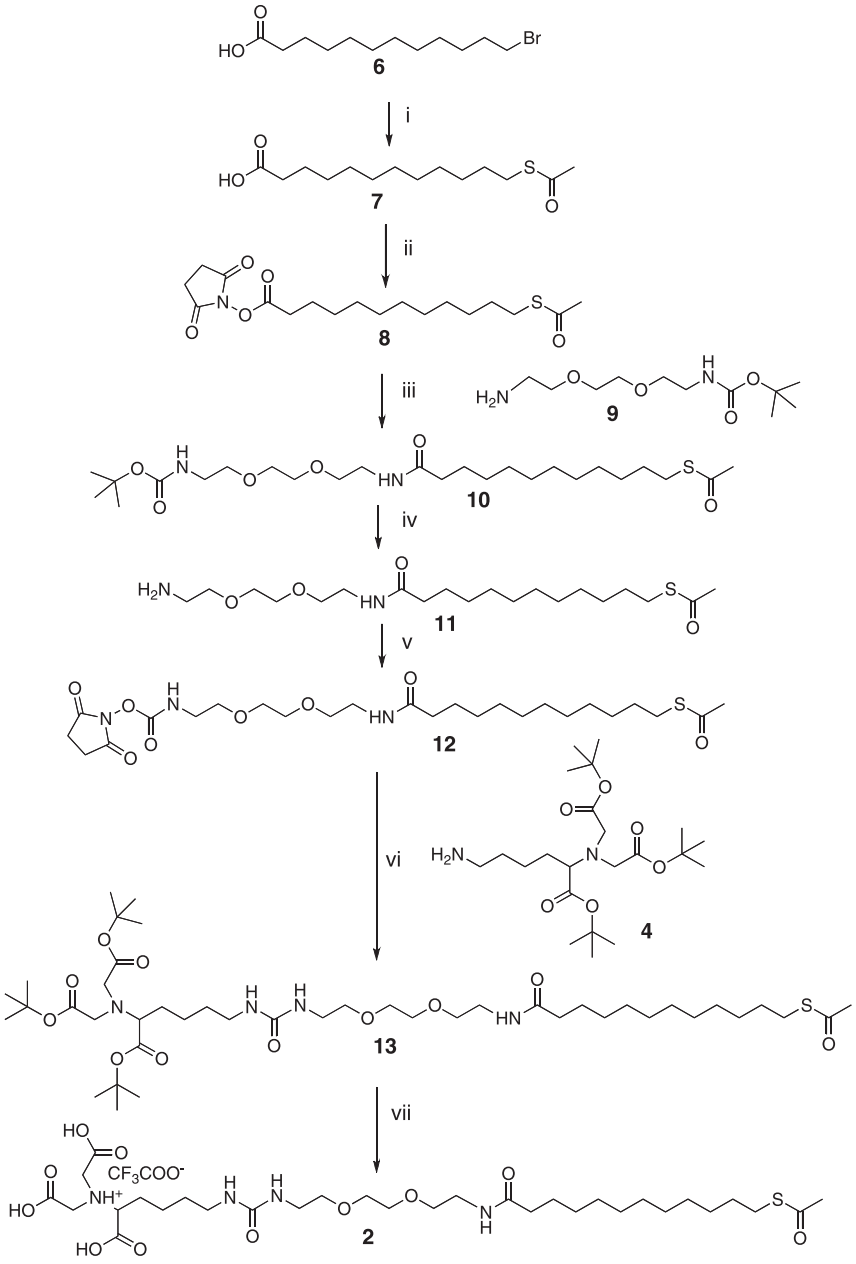
4. Experimental

4.1. General information

The starting materials were purchased from Sigma–Aldrich and used without further purification. All the solvents were purified and dried by standard methods prior to use. The crude samples were purified by column chromatography on silica gel (400 mesh). Thin layer chromatography (TLC) was performed on aluminum sheets pre-coated with silica gel (Merck, Kieselgel 60, F_{254}). IR spectra were recorded using Perkin–Elmer 1760X FT-IR spectrometer. ^{1}H NMR spectra were recorded using a Bruker AMX-500 (500 MHz) spectrometer and the chemical shifts are reported in parts per million (ppm) relative to tetramethylsilane (TMS) as an internal standard. Mass spectra were recorded on a VG Autospec spectrometer. Elemental analysis was carried out using Eurovector model EA 3000 CHNS analyzer. The determination of the melting points of the compounds was carried using a polarizing microscope (Leitz DMRXP) in conjunction with a programmable hot stage (Mettler FP90).

4.2. Synthetic procedures

*4.2.1. Compound **5**.* To a stirred solution of compound **3** (0.15 g, 0.21 mmol) and compound **4** (0.092 g, 0.21 mmol) in dry THF (6 ml) was added dry diisopropyl ethylamine (0.035 ml, 0.21 mmol). The reaction mixture was stirred for 17 h at room temperature under an argon atmosphere. The solvent was removed in vacuo and the residue was dissolved in chloroform and washed with water (3×30 ml), brine (20 ml) and dried over anhydrous Na_2SO_4. Evaporation under reduced pressure yielded a viscous liquid, which was purified by flash column chromatography on silica gel with 5%



Scheme 2. Synthesis of tether **2**. Reagents and conditions. (i) CH_3COSK, DMF, rt, 12 h, 65%; (ii) *N*-hydroxy succinimide, DCC, DMAP, THF, 17 h, 84%; (iii) Triethylamine (TEA), THF, 17 h, 84%; (iv) TFA, CH_2Cl_2, rt, 17 h; (v) Disuccinimidyl carbonate (DSC), TEA, THF, 17 h, 39%; (vi) DIPEA, THF, rt, 17 h, 95%; (vii) TFA, CH_2Cl_2, rt, 17 h, 100%.

methanol–chloroform eluent mixture to obtain a colorless viscous liquid (0.211 g, 97%). R_f=0.5 (5% MeOH–CH_3Cl_3); IR (KBr Pellet) ν_{max} in cm^{-1}: 3355, 2960, 2403, 1738, 1562, 1454, 1370, 1216, 960, 843, 801, 778, 596; ^1H NMR (500 MHz, $CDCl_3$): 5.37 (s, 2H, 2× NH), 5.2 (s, 1H, 1× olefinic H), 5.1 (s, 1H, 1× NH) 4.48 (m, 1H, O–CH– of Cholesteryl), 3.61 (s, 4H, 2× OCH_2), 3.56 (m, 4H, 2× OCH_2), 3.55 (m, 4H, 2× $COCH_2$), 3.48 (m, 2H, CH_2NH), 3.44 (m, 2H, CH_2NH), 3.37 (m, 2H, CH_2NH), 3.3 (m, 1H, *CH*N), 2.35 (m, 2H, CH_2), 2.28 (m, 2H, CH_2), 0.87–2 (m, 30H, 6× CH, 9× CH_2, Cholesteryl, 3× CH_2, Lysine),1.4 (s, 27H, 3× $C(CH_3)_3$), 1 (s, 3H, 1× CH_3, Cholesteryl), 0.92 (d, 3H, J=6.5 Hz, 1× CH_3, Cholesteryl), 0.91 (d, 3H, J=6 Hz, 1× CH_3), 0.86 (d, 3H, J=2.1 Hz, 1× CH_3), 0.68 (s, 3H, 1× CH_3, Cholesteryl); ^{13}C NMR (125 MHz, $CDCl_3$): δ 182.9, 158.4, 155.7, 150, 135.3, 133.8, 130.8, 70.2, 64.5, 60.9, 56.6, 56.1, 55.4, 50, 43.2, 42.3, 39.5, 38.5, 36.5, 35.8, 32.2, 28.2, 24.3, 23.8, 22.8, 22.6, 21, 19.3, 18.7, 11.8. ESI-HRMS: *m*/*z*: calcd for $C_{57}H_{100}N_4O_{11}$: 1017.74. Found: 1017.75 $[M]^+$; elemental analysis calcd (%) for $C_{57}H_{100}N_4O_{11}$: C, 67.29; H, 9.91; N, 5.51. Found: C, 67.60; H, 9.7; N, 5.2.

4.2.2. Tether ***1***. To a stirred solution of compound **5** (0.211 g, 0.207 mmol) in dichloromethane (5 ml), was added a solution of trifluoroacetic acid (0.6 ml, 8.3 mmol) in dichloromethane (5 ml) dropwise. The reaction mixture was vigorously stirred for 17 h at room temperature. The solvent was removed in vacuo and the residue was triturated in diethyl ether (10 ml) to remove the nonpolar impurities and traces of acid to give a colorless sticky solid. The yield was quantitative. R_f=0.3 (5% MeOH–CH_3Cl_3); IR (KBr Pellet) ν_{max} in cm^{-1}: 3453, 2945, 2714, 2511, 1728, 1366, 1204, 1135, 1032, 960, 914, 837, 801, 780, 722, 600, 517; ^1H NMR (500 MHz, $CDCl_3$): 5.37 (s, 2H, 2× NH), 5.21 (s, 1H, 1× olefinic H), 5.1 (s, 1H, 1× NH) 4.5 (m, 1H, O–CH– of Cholesteryl), 3.68 (s, 1H, 1× $CHNH^+$), 3.61 (s, 4H, 2× OCH_2), 3.56 (m, 4H, 2× OCH_2), 3.55 (m, 4H,

2× $COCH_2$), 3.49 (m, 5H, 2× CH_2NH, 1× CHN), 3.38 (m, 2H, CH_2NH), 2.35 (m, 2H, CH_2), 2.28 (m, 2H, CH_2), 0.87–2 (m, 30H, 6× CH, 9× CH_2, Cholesteryl, 3× CH_2, Lysine), 1 (s, 3H, 1× CH_3, Cholesteryl), 0.92 (d, 3H, J=6.5 Hz, 1× CH_3, Cholesteryl), 0.91 (d, 3H, J=6 Hz, 1× CH_3), 0.87 (d, 3H, J=2.1 Hz, 1× CH_3), 0.68 (s, 3H, 1× CH_3, Cholesteryl); ^{13}C NMR (125 MHz, $CDCl_3$): δ 196.4, 175.1, 174.3, 171.1, 160, 158.2, 155.1, 117.6, 102, 90.1, 70.4, 70.1, 67.9, 56.6, 56, 50, 45.5, 40.3, 39.4, 36.6, 36.4, 28.8, 24.3, 23.8, 22.8, 22.6, 21, 19.3, 18.7, 11.8. ESI-HRMS: m/z: calcd for $C_{45}H_{74}N_4O_{11}$: 847.54. Found: 847.54 $[M]^+$.

4.2.3. Compound ***7***. To a mixture of 12-bromododecanoic acid (**6**) (3.00 g, 10.7 mmol) and potassium thioacetate (1.84 g, 16.1 mmol) was added 25 ml anhydrous DMF and the mixture was stirred under N_2 atmosphere for 17 h. The solvent was removed in vacuo and the residue dissolved in EtOAc (50 ml), washed with water (5×50 ml) and brine (2×50 ml). The combined organic extracts were dried over anhydrous Na_2SO_4 and concentrated to yield a brown solid. This was adsorbed on silica gel and subjected to column chromatography using 25% EtOAc–hexanes eluent mixture to yield an off-white powder (1.9 g, 65%). R_f=0.5 (40% EtOAc–hexanes); mp 58–60 °C; IR (KBr Pellet) ν_{max} in cm^{-1}: 3366, 3045, 2917, 2851, 1697, 1472, 1429, 1409, 1353, 1324, 1302, 1278, 1252, 1226, 1203, 1184, 1138, 1107, 955, 916, 714, 682, 632, 533; ^{1}H NMR (500 MHz, $CDCl_3$): δ 2.86 (t, J=5.5 Hz, 2H, 1× SCH_2), 2.35 (t, J=5.5 Hz, 2H, 1× CH_2COOH), 2.32 (s, 3H, 1× $COCH_3$), 1.62 (m, 2H, 1× CH_2CH_2S), 1.04 (m, 2H, 1× CH_2CH_2CO), 1.26–1.33 (m, 14H, 7× CH_2); ^{13}C NMR (125 MHz, $CDCl_3$): δ 196.2, 178.5, 33.8, 30.7, 29.5, 29.4, 29.1, 28.8, 24.7, 12.7. ESI-HRMS: m/z: calcd for $C_{14}H_{26}O_3S$+Na: 297.15. Found: 297.15 $[M+Na]^+$; elemental analysis calcd (%) for $C_{14}H_{26}O_3S$: C, 61.27; H, 9.55; S, 11.68. Found: C, 61.2; H, 9.65; S, 11.75.

4.2.4. Compound ***8***. To a stirred solution of compound **7** (1.5 g, 5.5 mmol), *N*-hydroxy succinimide (0.63 g, 5.5 mmol), and DMAP (0.033g, 0.27 mmol) in dry THF (15 ml) was added a solution of DCC (1.24 g, 6.01 mmol) in THF (20 ml). This was stirred under a N_2 atmosphere for 17 h at room temperature. The reaction mixture was filtered to remove the dicyclohexyl urea and concentrated. The residue obtained was purified by column chromatography using 40% EtOAc–hexanes to yield a brown solid (1.7 g, 84%). R_f=0.3 (30% EtOAc–hexanes); mp 54–56 °C; IR (KBr Pellet) ν_{max} in cm^{-1}: 3706, 3511, 3352, 2911, 2849, 2403, 2287, 2255, 2117, 1816, 1789, 1730, 1683, 1549, 1470, 1414, 1365, 1203, 1068, 877, 812, 764, 719, 635, 555, 513; ^{1}H NMR (500 MHz, $CDCl_3$): δ 2.85 (m, 6H, 1× SCH_2, 2× $COCH_2CH_2CO$), 2.6 (t, J=6.5 Hz, 2H, 1× CH_2COO), 2.32 (s, 3H, 1× $COCH_3$), 1.75 (m, 2H, 1× CH_2CH_2S), 1.27–1.58 (m, 16H, 7× CH_2, 1× CH_2CH_2CO); ^{13}C NMR (125 MHz, $CDCl_3$): δ 196.4, 169.6, 169.1, 146.4, 137, 107, 93.9, 69.9, 60.3, 31.3, 31, 29.9, 29.8, 29.7, 29.6, 29.5, 29.2, 25. ESI-HRMS: m/z: calcd for $C_{18}H_{29}NO_5S$+Na^+: 394.17. Found: 394.17 $[M+Na]^+$; elemental analysis calcd (%) for $C_{18}H_{29}NO_5S$: C, 58.2; H, 7.87; N, 3.77; S, 8.63. Found: C, 58.4; H, 7.95; N, 3.9; S, 8.3.

4.2.5. Compound ***10***. Triethylamine (0.6 ml, 8.4 mmol) was added to a stirred solution of compound **8** (1.57g, 4.23 mmol) and compound **9** (1.00 g, 4.23 mmol) in dry THF (20 ml) and stirred for 17 h under a N_2 atmosphere. The solvent was removed in vacuo and the residue was redissolved in EtOAc (50 ml). This solution was washed with water, brine (50 ml each) and finally dried over anhydrous Na_2SO_4. Evaporation under reduced pressure yielded a brown residue, which was purified by flash column chromatography on silica gel with 5% methanol–chloroform eluent mixture to obtain a brown solid (1.78 g, 84%). R_f=0.4 (5% MeOH–$CHCl_3$); mp 60–62 °C; IR (KBr Pellet) ν_{max} in cm^{-1}: 3321, 2917, 2851, 1677, 1469, 1349, 1250, 1128, 1043, 972, 874, 780, 746, 715, 628; ^{1}H NMR (500 MHz, $CDCl_3$): 5.9 (s, 1H, 1× NH), 4.98 (s, 1H, 1× NH), 3.61 (s, 4H, 2× OCH_2), 3.55 (m, 4H, 2× NCH_2CH_2O), 3.47 (m, 2H, NCH_2), 3.34 (m, 2H, NCH_2), 2.85 (t, 2H, J=8 Hz, SCH_2), 2.32 (s, 3H, 1× $COCH_3$), 2.17 (m, 2H, 1× CH_2CH_2CO), 1.45 (s, 9H, $C(CH_3)_3$),1.26–1.6 (m, 18H, 7× CH_2, 1× CH_2CH_2S, 1× CH_2CH_2CO); ^{13}C NMR (100 MHz, $CDCl_3$): δ 196.1, 172.9, 156, 121.3, 79.4, 70.3, 40.4, 39.2, 36.7, 30.6, 29.5, 29.1, 28.4, 25.8, 24.9; ESI-HRMS: m/z: calcd for $C_{25}H_{48}N_2O_6S$+Na: 527.31. Found: 527.32 $[M+Na]^+$; elemental analysis calcd (%) for $C_{25}H_{48}N_2O_6S$: C, 59.49; H, 9.59; N, 5.55; S, 6.35. Found: C, 59.2; H, 9.6; N, 5.5; S, 6.05.

4.2.6. Compound ***11***. To a stirred solution of compound **10** (1.00 g, 1.98 mmol) in dichloromethane (20 ml), was added a solution of trifluoroacetic acid (3.00 ml, 39.6 mmol) in dichloromethane (10 ml) dropwise. The reaction mixture was vigorously stirred for 17 h at room temperature. The solvent was removed in vacuo and the residue was triturated in diethyl ether (10 ml). This brown viscous liquid was used for the next step without any further purification. The yield was quantitative. R_f=0.3 (5% MeOH–$CHCl_3$); brown viscous liquid; IR (KBr Pellet) ν_{max} in cm^{-1}: 3093, 2929, 2857, 1778, 1682, 1458, 1431, 1355, 1203, 1175, 1143, 958, 837, 799, 722, 707, 630, 598; ^{1}H NMR (400 MHz, $CDCl_3$): 7.6 (s, 3H, 1× NH_3^+), 3.77 (s, 4H, 2× OCH_2), 3.68 (m, 4H, 2× NCH_2CH_2O), 3.62 (m, 2H, NCH_2), 3.5 (m, 2H, NCH_2), 3.25 (s, 1H, 1× NH), 2.85 (t, 2H, J=7.5 Hz, SCH_2), 2.33 (s, 3H, 1× $COCH_3$), 2.27 (t, 2H, J=8.5 Hz, 1× CH_2CH_2CO), 1.57 (m, 4H, 1× CH_2CH_2S, 1× CH_2CH_2CO), 1.25–1.34 (m, 14H, 7× CH_2); ^{13}C NMR (100 MHz, $CDCl_3$): δ 196.4, 176.9, 160.7, 160.1, 159.8, 117.1, 113.3, 70.2, 70, 69.8, 66.4, 40.1, 40.1, 39.8, 36.2, 30.6, 29.3, 29, 28.7, 25.8, 14.9; ESI-HRMS: m/z: calcd for $C_{20}H_{41}N_2O_4S$: 405.28. Found: 405.3 $[M+H]^+$.

4.2.7. Compound ***12***. Dry triethylamine (0.4 ml, 2.8 mmol) was added to a stirred solution of compound **11** (0.42g, 0.82 mmol) and *N*,*N*′-disuccinimidyl carbonate (0.25 g, 0.98 mmol) in dry THF (5 ml). The reaction mixture was stirred for 17 h at room temperature under an argon atmosphere. The solvent was removed in vacuo and the residue was subjected to flash column purification on silica gel with 5% methanol–chloroform as eluent to get product as a dull brown solid (0.17 g, 39%). R_f=0.8 (5% MeOH–$CHCl_3$); mp 96–98 °C; IR (KBr Pellet) ν_{max} in cm^{-1}: 3303, 2918, 2851, 1770, 1742, 1694, 1642, 1548, 1470, 1423, 1378, 1206, 1135, 990, 955, 919, 811, 714, 630; ^{1}H NMR (500 MHz, $CDCl_3$): 6.2 (s, 1H, 1× NH), 6.1 (s, 1H, 1× NH), 3.58–3.63 (m, 8H, 2× OCH_2, 2× NCH_2CH_2O), 3.47 (m, 4H, 2× NCH_2), 2.86 (t, 2H, J=8 Hz, SCH_2), 2.83 (s, 4H, $COCH_2CH_2CO$), 2.32 (s, 3H, 1× $COCH_3$), 2.17 (t, 2H, J=7.5 Hz, 1× CH_2CH_2CO), 1.59 (m, 4H, 1× CH_2CH_2S, 1× CH_2CH_2CO), 1.25 (m, 14H, 7× CH_2); ^{13}C NMR (100 MHz, $CDCl_3$): δ 196.2, 169.8, 161.7, 70.4, 70.3, 70.1, 69.2, 41.9, 39.2, 36.7, 30.6, 29.5, 29.1, 28.8, 25.7, 25.5; ESI-HRMS: m/z: calcd for $C_{25}H_{43}N_3O_8S$+Na: 568.27. Found: 568.27 $[M+Na]^+$; elemental analysis calcd (%) for $C_{25}H_{43}N_3O_8S$: C, 55.03; H, 7.94; N, 7.7; S, 5.88. Found: C, 55.2; H, 7.84; N, 7.7; S, 5.8.

4.2.8. Compound ***13***. To a stirred solution of compound **12** (0.1 g, 0.18 mmol) and compound **4** (0.079 g, 0.18 mmol) in dry THF (5 ml) was added dry diisopropyl ethylamine (0.03 ml, 0.18 mmol). The reaction mixture was stirred for 17 h at room temperature under an argon atmosphere. The solvent was removed in vacuo and the residue was dissolved in chloroform and washed with water (3×30 ml), brine (20 ml) and dried over anhydrous Na_2SO_4. Evaporation under reduced pressure yielded a viscous liquid, which was purified by flash column chromatography on silica gel with 5% methanol–chloroform eluent mixture to obtain a yellowish viscous liquid (0.15 g, 95%). R_f=0.7 (5% MeOH–CH_3Cl_3); IR (KBr Pellet) ν_{max} in cm^{-1}: 3297, 2976, 2926, 2855, 1742, 1726, 1692, 1643, 1554, 1458, 1392, 1368, 1251, 1223, 1143, 989, 954, 847, 752, 627; ^{1}H NMR (500 MHz, $CDCl_3$): 6.3 (s, 2H, 2× NH), 6.1 (s, 1H, 1× NH), 3.61 (s, 4H, 2× OCH_2), 3.55–3.57 (m, 8H, 2× $COCH_2$, 2× NCH_2CH_2O), 3.43–3.46 (m, 4H, 2× NCH_2), 3.4 (m, 1H, CHN), 3.38 (m, 2H, CH_2NH), 2.86 (t, 2H, J=8 Hz, SCH_2), 2.32 (s, 3H, 1× $COCH_3$), 2.17 (t, 2H, J=7.5 Hz, 1×

CH_2CH_2CO), 1.56 (m, 4H, 1× CH_2CH_2S, 1× CH_2CH_2CO), 1.45 (s, 27H, 3× $C(CH_3)_3$), 1.25–1.34 (m, 20H, 7× CH_2, 3× CH_2, Lysine); ^{13}C NMR (125 MHz, $CDCl_3$): δ 196.2, 181.8, 173.7, 172.5, 170.4, 158.7, 155.9, 90.9, 80.9, 81.1, 70.8, 69.9, 67.9, 64.9, 53.9, 53.6, 46.7, 39.3, 36.7, 30.7, 28.8, 25.6, 22.7, 21.4, 13.6; ESI-HRMS: m/z: calcd for $C_{43}H_{81}N_4O_{11}S$: 861.55. Found: 861.56 $[M]^+$; elemental analysis calcd (%) for $C_{43}H_{80}N_4O_{11}S$: C, 59.97; H, 9.36; N, 6.51; S, 3.72. Found: C, 59.75; H, 9.3; N, 6.35, S, 3.85.

4.2.9. Tether ***2****.* To a stirred solution of compound **13** (0.15 g, 0.17 mmol) in dichloromethane (5 ml), was added a solution of trifluoroacetic acid (0.52 ml, 7 mmol) in dichloromethane (5 ml) dropwise. The reaction mixture was vigorously stirred for 17 h at room temperature. The solvent was removed in vacuo and the residue was triturated in diethyl ether (10 ml) to remove the nonpolar impurities and traces of acid to get a sticky brown solid. The yield was quantitative. R_f=0.3 (5% MeOH–CH_3Cl_3); sticky brown solid; IR (KBr Pellet) ν_{max} in cm^{-1}: 3329, 2925, 2854, 1736, 1692, 1642, 1562, 1458, 1352, 1203, 1138, 957, 899, 720, 630; ^1H NMR (500 MHz, $CDCl_3$): 4.6 (s, 1H, 1× CHNH^+), 3.9 (s, 2H, 2× NH), 3.61 (s, 4H, 2× OCH_2), 3.56 (m, 4H, 2× OCH_2), 3.49 (s, 4H, 2× $COCH_2$), 3.39 (m, 5H, 2× CH_2NH, 1× CHN), 3.38 (m, 2H, CH_2NH), 3.15 (s, 1H, 1× NH), 2.84 (t, 2H, J=8 Hz, SCH_2), 2.32 (s, 3H, 1× $COCH_3$), 2.22 (m, 2H, 1× CH_2CH_2CO), 1.55 (m, 4H, 1× CH_2CH_2S, 1× CH_2CH_2CO), 1.25–1.34 (m, 20H, 7× CH_2, 3× CH_2, Lysine); ^{13}C NMR (125 MHz, $CDCl_3$): δ 196.4, 175.2, 174.8, 174.3, 173.8, 168.8, 160.9, 160.4, 158.2, 155.1, 121.4, 117.6, 70.5, 40.3, 39.4, 38.7, 36.6, 36.4, 30.3, 29.5, 28.8, 28.1, 25.9, 23.5, 23.1, 13.2. ESI-HRMS: m/z: calcd for $C_{31}H_{56}N_4O_{11}S+Na^+$: 715.36. Found: 715.35 $[M+Na]^+$.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.tet.2011.06.049.

References and notes

1. Boxer, S. G. *Curr. Opin. Chem. Biol.* **2000**, *4*, 704–709.
2. Groves, J. T.; Boxer, S. G. *Acc. Chem. Res.* **2002**, *35*, 149–157.
3. Jenkins, A. T. A.; Boden, N.; Bushby, R. J.; Evans, S. D.; Knowles, P. F.; Miles, R. E.; Ogier, S. D.; Schonherr, H.; Vancso, G. J. *J. Am. Chem. Soc.* **1999**, *121*, 5274–5280.
4. Plant, A. L.; Gueguetchkeri, M.; Yap, W. *Biophys. J.* **1994**, *67*, 1126–1133.
5. Sackmann, E. *Science* **1996**, *271*, 43–48.
6. Sheikh, K. H.; Christenson, H. K.; Bushby, R. J.; Evans, S. D. *J. Phys. Chem. B* **2007**, *111*, 379–386.
7. Han, X.; Achalkumar, A. S.; Bushby, R. J.; Evans, S. D. *Chem.—Eur. J.* **2009**, *15*, 6363–6370.
8. Carroll, T. R.; Davison, A.; Jones, A. G. *J. Med. Chem.* **1986**, *29*, 1821–1826.
9. Pan, X.; Wu, G.; Yang, W.; Barth, R. F.; Tjarks, W.; Lee, R. J. *Bioconjugate Chem.* **2007**, *18*, 101–108.
10. Kley, J. T.; Fischert, T.; Massing, U. *Monatsh. Chem.* **1998**, *129*, 319–327.
11. Menger, F. M.; Bian, J.; Seredyuk, V. A. *Angew. Chem., Int. Ed.* **2004**, *43*, 1265–1267.
12. (a) Lentacker, I.; De Smedt, S. C.; Demeester, J.; Van Marck, V.; Bracke, M.; Sanders, N. N. *Adv. Funct. Mater.* **2007**, *17*, 1910–1916; (b) Weiss, S.; Milner, P.; Nelson, A. *Electrochim. Acta* **2005**, *50*, 4248–4256; (c) Booth, C.; Bushby, R. J.; Cheng, Y.; Evans, S. D.; Liu, Q.; Zhang, H. *Tetrahedron* **2001**, *57*, 9859–9866.
13. Hayden, C. C.; Hwang, J. S.; Abate, E. A.; Kent, M. S.; Sasaki, D. Y. *J. Am. Chem. Soc.* **2009**, *131*, 8728–8729.
14. (a) http://avantilipids.com/. (b) Chikh, G. G.; Li, W. M.; Schutze- Redelmeier, M.-P.; Meunier, J.-C.; Bally, M. B. *Biochim. Biophys. Acta* **2002**, *1567*, 204–212; (c) Gizeli, E.; Glad, J. *Anal. Chem.* **2004**, *76*, 3995–4001; (d) Lim, Y. T.; Lee, K. Y.; Lee, K.; Chung, B. H. *Biochem. Biophys. Res. Commun.* **2006**, *344*, 926–930; (e) Bilek, G.; Kremser, L.; Wruss, J.; Blaas, D.; Kenndler, E. *Anal. Chem.* **2007**, *79*, 1620–1625; (f) Tanaka, M.; Hermann, J.; Haase, I.; Fischer, M.; Boxer, S. G. *Langmuir* **2007**, *23*, 5638–5644.
15. Achalkumar, A. S.; Bushby, R. J.; Evans, S. D. *Soft Matter* **2010**, *6*, 6036–6051.
16. (a) Tinazli, A.; Tang, J.; Valiokas, R.; Picuric, S.; Lata, S.; Piehler, J.; Liedberg, B.; Tamp, R. *Chem.—Eur. J.* **2005**, *11*, 5249–5259; (b) Lata, S.; Piehler, J. *Anal. Chem.* **2005**, *77*, 1096–1105; (c) Lauer, S. A.; Nolan, J. P. *Cytometry* **2002**, *48*, 136–145; (d) Khan, F.; He, M.; Taussig, M. J. *Anal. Chem.* **2006**, *78*, 3072–3079.
17. Dorn, I. T.; Neumaier, K. R.; Tampe, R. *J. Am. Chem. Soc.* **1998**, *120*, 2753–2763.