Full Paper

CSIRO PUBLISHING

Aust. J. Chem. **2006**, *59*, 302–306

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Synthesis of NTA_3-DTDA — A Chelator-Lipid that Promotes Stable Binding of His-Tagged Proteins to Membranes

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A six-step reaction sequence is described for the preparation of compound **1** (NTA_3-DTDA), a membrane-penetrating and potent chelator that can be incorporated into liposomes and plasma membrane vesicles containing antigens and thus allowing targeted delivery of such assemblies to a variety of cells for the purposes of eliciting anti-tumour responses. Full spectroscopic characterization of this dendritic-type compound as well as certain of its precursors is reported.

Manuscript received: 3 April 2006.
Final version: 26 April 2006.

Introduction

The delivery of antigen-containing stealth liposomes (SLs) and tumour-derived plasma membrane vesicles (PMVs) to various immune cells can enhance anti-tumour immunity. The approach has potential applications in cancer vaccine development and in establishing tumour immunotherapy regimes.[1–3] Clearly, the cytoselective targeting of such SLs and PMVs would significantly enhance the therapeutic value of the encapsulated or membrane-associated antigens.[1–3] Recent work by one of us (J.G.A.)[2,3] has revealed that the membrane-penetrating and potent chelator 3-(nitrilotriacetic acid)-ditetradecylamine (NTA_3-DTDA or **1**; Fig. 1) is a very useful material in this regard. It can, for example, be used to anchor, via metal ion bridges, oligo-histidine-tagged forms of antibody fragments that target dendritic cells onto either tumour cell-derived PMVs or onto antigen-containing SLs. Such targeting elicits strong anti-tumour responses. However, further developments in this promising area have been restricted by the lack of access to gram quantities of pure samples of compound **1**. Indeed, until now only milligram samples of this material have been available for research purposes and these were contaminated with mono-, di-, and

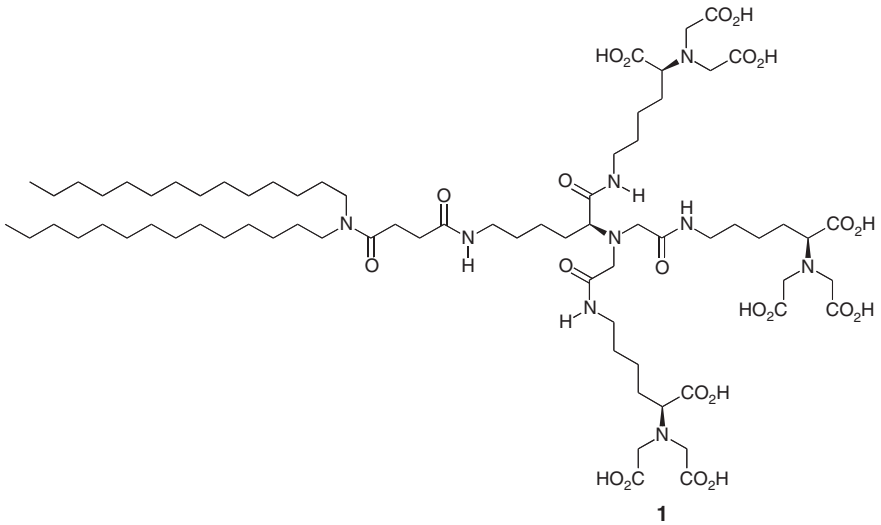

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Fig. 1.

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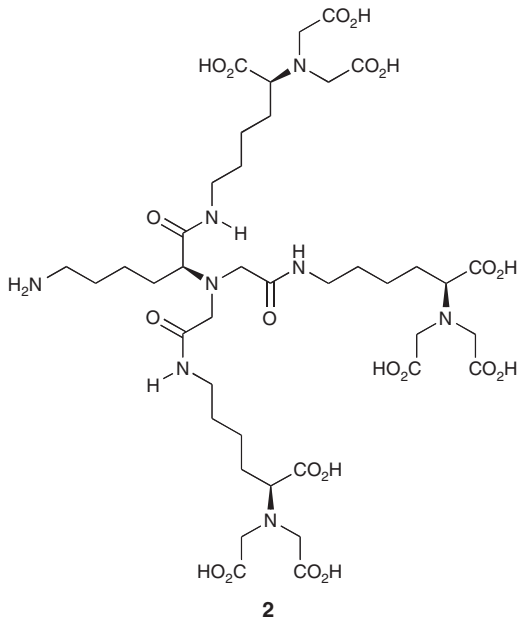

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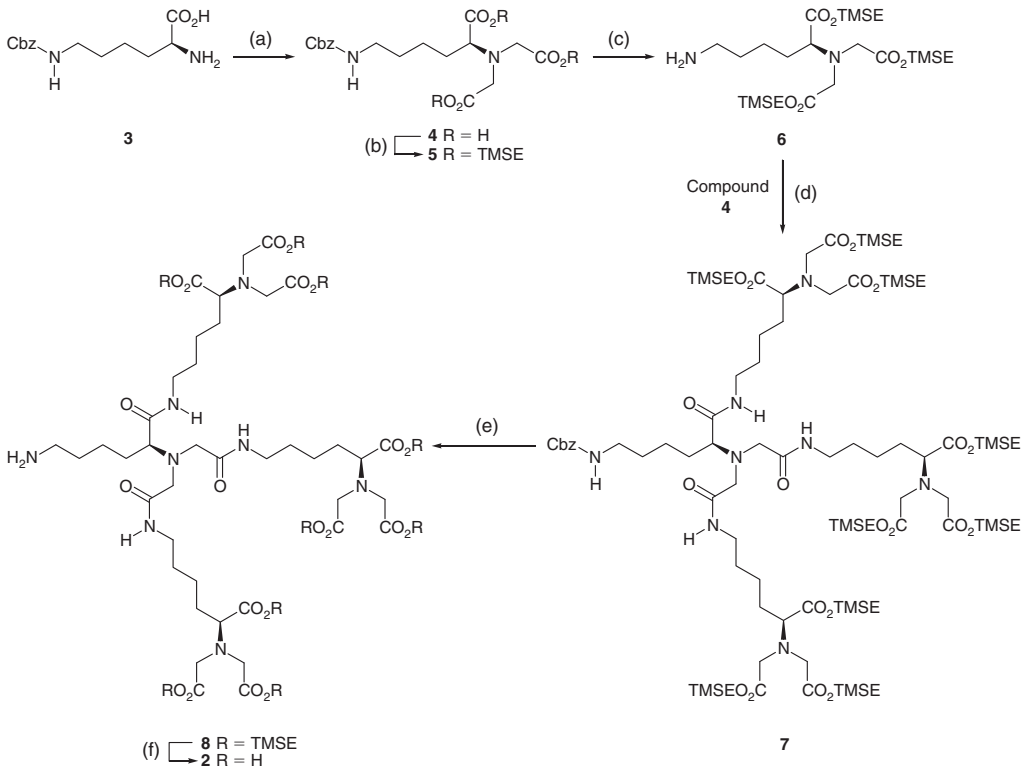
Fig. 2.

tri-methyl ester derivatives arising from the use of methanol as a solvent at a pivotal point in the synthesis.[3,4] Accordingly, we detail below a synthesis of NTA_3-DTDA that allows access to pure material in gram quantities. By related means we have also been able to obtain congener **2** (Fig. 2), incorporating the polar head group of compound **1** and required for undertaking control experiments related to the establishment of the in vivo mode of action of the latter material.

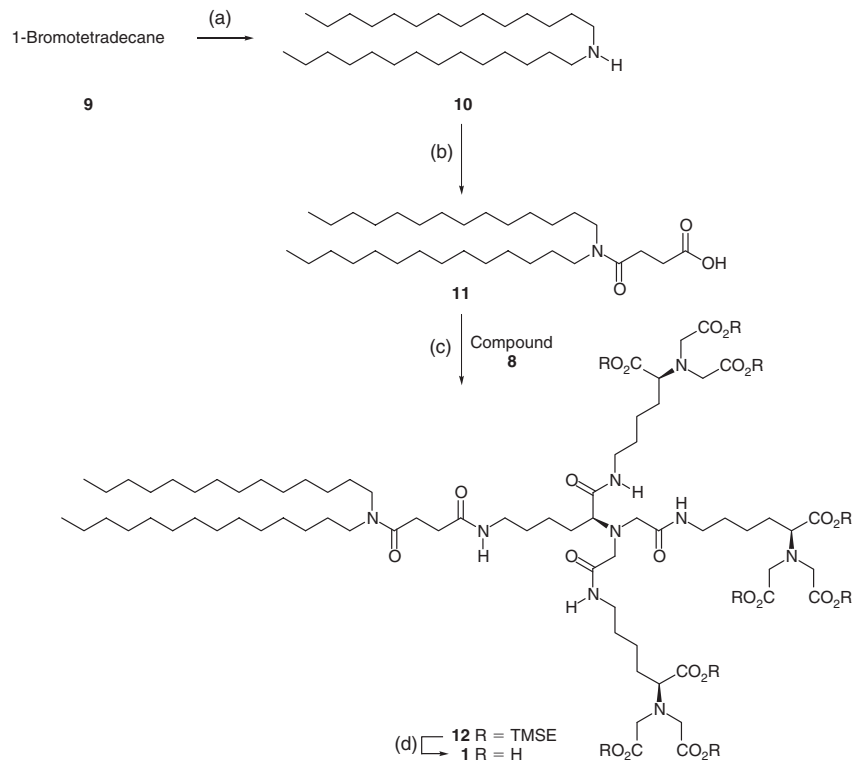
Results and Discussion

The reaction sequence leading to the 2-(trimethysilyl)ethyl (TMSE) ester protected form, **8**, of the head group compound **2** is shown in Scheme 1. Thus, following previously described protocols[5] that were inspired by the work of Tampé and co-workers,[6] the commercially available and carbobenzyloxy (Cbz) protected form, **3**, of lysine was treated with an excess of α-bromoacetic acid in aqueous alkali. As a result, and after work-up with mineral acid, the previously reported[7] triacid **4** was obtained in 93% yield. Esterification of compound **4** with 2-trimethylsilylethanol using *N*-(3-dimethylaminopropyl)-*N′*-ethylcarbodiimide hydrochloride (EDAC·HCl)[8] as coupling agent then afforded triester **5** in 90% yield. This product was characterized by ^{1}H NMR, ^{13}C NMR, and IR spectroscopy, as well as by low- and high-resolution ESI mass spectrometry (MS). All such data were in full accord with the assigned structure. Hydrogenolytic cleavage of the Cbz-group within compound **5** was accomplished under standard conditions using an atmosphere of dihydrogen in the presence of 10% palladium on carbon, and so giving the primary amine **6** (98%) which was coupled, using EDAC·HCl, with one-third of a molar equivalent of the precursor triacid **4**. In this manner the dendritic-type compound **7** was obtained in 81% yield. Removal of Cbz-group within dendrimer **7** was achieved hydrogenolytically under the same conditions as employed in the analogous conversion **5** → **6** and with methanol as reaction solvent. The product primary amine **8** was then subjected to treatment with trifluoroacetic acid (TFA) in order to effect removal of the TMSE groups and thus generating the target nona-acid **2**. However, ESI mass spectral analysis of this product inevitably revealed that it was contaminated with significant quantities of the corresponding mono-, di-, and tri-methyl ester derivatives. These by-products presumably arise through carry over of methanol from the preceding hydrogenolysis step and the participation of this alcohol in acid-catalyzed trans-esterification and/or esterification reactions when compound **8** is treated with TFA. Various attempts, including those involving the application of freeze drying techniques, were made to remove residual methanol from product **8** but methyl ester by-products continued to contaminate the free acid **2** derived from it. These difficulties were ultimately addressed by using the much more weakly nucleophilic solvent 2,2,2-trifluoroethanol in the hydrogenolysis step. This led to amine **8**, the pivotal precursor to the final target **1**, in 98% yield. Cleavage of the ester residues within compound **8** using TFA then proceeded to give the polyacid **2** in quantitative yield and, therefore, uncontaminated with ester by-products. The compound could be purified by simple washing with toluene, acetonitrile, then diethyl ether. This process avoided the need to use HPLC techniques for purification which, as established during earlier studies,[4] necessarily involved methanol as an eluting solvent and so resulting in further contamination of the target material by mono-, di-, and tri-methyl ester derivatives. The ESI mass spectrum of compound **2** revealed a protonated molecular ion at *m/z* 995.5 and an accurate mass measurement on this species established that it was of the expected composition. The ^{13}C NMR spectrum displayed only five of the expected twelve carbonyl carbon resonances but this is undoubtedly due to the high degree of similarity of the relevant atoms within this pseudo-symmetrical system and the resulting overlap of signals. Indeed, this sort of situation was encountered throughout the entire series of compounds described herein.

The completion of the synthesis of compound **1** is shown in Scheme 2 and involved, in the initial stages, the preparation of a suitably functionalized synthon corresponding to the lipophilic tail of this target. Thus, commercially available 1-bromotetradecane was treated with ammonia according to the procedure of Altin, White, and Easton[5] and in this way the secondary amine **10** was obtained. Reaction of this last compound with succinic anhydride in the presence of triethylamine then afforded succinate **11** (42%). This then engaged in an EDAC·HCl-promoted coupling reaction with compound **8** so as to generate, in 72% yield, the TMSE-protected form, **12**, of the final target. Exposure of compound **12** to neat TFA at 0–18°C for 4 h then gave compound **1** in 92% yield and as a white solid with a broad melting range. The negative ion ESI mass spectrum of this material revealed an $(M-H)^-$ species at *m/z* 1485 while a doubly charged ion was observed at *m/z* 742 and represented the base peak. The ^{13}C NMR spectrum of this C_{72}-containing and pseudo-symmetrical compound displayed thirty-two resonances as



Scheme 1. Reagents and conditions: (a) α-Bromoacetic acid (4.3 mol equiv.), NaOH, water, 18–50°C, 18 h. (b) EDAC·HCl (4.6 mol equiv.), DMAP (3.3 mol equiv.), 2-(trimethylsilyl)ethanol (4.4 mol equiv.), CH_2Cl_2, 0–18°C, 72 h. (c) H_2 (1 atm), 10% Pd on C, MeOH, 18°C, 16 h. (d) Compound **4** (0.27 mol equiv.), EDAC·HCl (1.3 mol equiv.), DMAP (1.0 mol equiv.), CH_2Cl_2, 0–18°C, 72 h. (e) H_2 (1 atm), 10% Pd on C, TFE, 18°C, 16 h. (f) TFA, 0–18°C, 4 h.



Scheme 2. Reagents and conditions: (a) See ref. [5]. (b) See ref. [5]. (c) Compound **8** (0.5 mol equiv.), EDAC·HCl (1.5 mol equiv.), DMAP (1.5 mol equiv.), CH_2Cl_2, 0–18°C, 16 h. (d) TFA, 0–18°C, 4 h.

a result of the overlap of various signals arising from the presence of many near equivalent carbons within the structure. Reversed-phase HPLC analysis of this material revealed it was an essentially homogeneous material while biological evaluations established that the product had the expected capacity to anchor histidine-tagged proteins onto SLs and PMVs.

Conclusions

A practical method for the preparation of the important SL- and PMV-targeting compound NTA_3-DTDA (**1**) that delivers gram quantities of pure forms of this material has been established. Such methodology should be capable of ready adaptation to the construction of related assemblies and so allowing the fine-tuning of the biological properties of this fascinating class of compound.

Experimental

General Procedures

Proton (^{1}H) and carbon (^{13}C) NMR spectra were recorded on either a Varian Inova 500 spectrometer operating at 500 MHz for proton and 126 MHz for carbon or a Gemini 300 NMR spectrometer, operating at 300 MHz (for proton) and 75 MHz (for carbon). Unless otherwise specified, spectra were acquired at 20°C in deuterochloroform ($CDCl_3$) that had been filtered through basic alumina immediately before use. Chemical shifts are recorded as δ values in parts per million (ppm). Infrared spectra (ν_{max}) were recorded on a Perkin–Elmer 1800 Series FTIR Spectrometer and samples were analyzed as thin films on KBr plates. Low resolution mass spectra were recorded on a Micromass–Waters LC-ZMD single quadrupole liquid chromatograph-MS or VG Quattro II triple quadrupole MS instrument using electrospray techniques. High resolution mass spectra were recorded on an AUTOSPEC spectrometer. Dichloromethane was distilled from calcium hydride and THF was distilled, under nitrogen, from sodium benzophenone ketyl. Where necessary, reactions were performed under a nitrogen atmosphere.

*Compound **4***

A magnetically stirred solution of α-bromoacetic acid (25.0 g, 180 mmol) in NaOH (90 mL of a 1.5 M aqueous solution) was cooled to 0°C then a solution of compound **3** (12.0 g, 42 mmol) in NaOH (150 mL of a 1.5 M aqueous solution) was added dropwise over 2 h. The ensuing mixture was allowed to warm to 18°C, stirred at this temperature for 16 h, and then at 50°C for 2 h. The cooled reaction mixture was treated, dropwise, with HCl (240 mL of a 1 M aqueous solution), and the ensuing white precipitate filtered off and washed with HCl (120 mL of a 0.1 M, aqueous solution) then distilled water (2 × 120 mL). The resulting solid was dried under high vacuum to afford the title compound **4**[7] (15.75 g, 93%) as a white solid, mp 162.4–167.4°C (lit.[7] 170°C). (HRMS (+ve ESI): Found $[M+H]^+$ 397.1610, $[M+Na]^+$ 419.1428. $C_{18}H_{24}N_2O_8$ requires $[M+H]^+$ 397.1611, $[M+Na]^+$ 419.1430.) δ_H (300 MHz, CD_3OD) 7.38–7.28 (5H, complex m, $-C_6H_5$), 5.06 (2H, s, $-CH_2Ph$), 3.62 (4H, ABq, J 18, 2 × $-NCH_2CO_2H$), 3.46 (1H, dd, J 8.1 and 6.3, –NC*H*–), 3.12 (2H, t, J 6.6, $-NHCH_2-$), 1.87–1.37 (6H, complex m, $-NCH_2(CH_2)_3-$) (signals due to exchangeable protons not observed). δ_C (75 MHz, CD_3OD) 175.8(1), 175.7(8), 158.9, 138.4, 129.4, 128.9, 128.8, 67.3, 66.7, 55.3, 41.4, 30.5(4), 30.4(8), 24.6. ν_{max} (nujol mull)/cm^{-1} 3374, 2924, 2855, 1698, 1535, 1456, 1377, 1265, 1144, 1022, 975, 892, 776, 739, 698. *m/z* (+ve ESI) 793 ($[2M+H]^+$, 6%), 419 (9), 397 ($[M+Na]^+$, 63), 353 (100).

*Compound **5***

A magnetically stirred solution of compound **4** (8.00 g, 20.2 mmol) and 4-(*N*,*N*-dimethylamino)pyridine (DMAP) (8.10 g, 66.3 mmol) in dichloromethane (500 mL) maintained under argon was cooled to 0°C then *N*-(3-dimethylaminopropyl)-*N*′-ethylcarbodiimide hydrochloride (EDAC·HCl) (18.00 g, 93.9 mmol) was added and the resulting mixture was left to stir at 0°C for 0.5 h. 2-(Trimethylsilyl)ethanol (10.50 g, 88.8 mmol) was then added to the reaction mixture and the resulting solution was stirred at 0°C for a further 1 h then warmed to 18°C and allowed to stir at this temperature for 3 days. The by now light-yellow reaction mixture was treated with distilled water (500 mL) and the separated aqueous layer was extracted with dichloromethane (3 × 100 mL). The combined organic fractions were dried (Na_2SO_4), filtered and concentrated under reduced pressure to afford a clear, colourless oil. Subjection of this material to flash chromatography (silica, 1:9 → 1:1 v/v ethyl acetate/hexane gradient elution) and concentration of the appropriate fractions (R_f 0.4 in 1:3 v/v ethyl acetate/hexane) afforded *compound **5*** (12.60 g, 90%) as a clear, colourless oil. (HRMS (+ve ESI): Found $[M+H]^+$ 697.3707, $[M+Na]^+$ 719.3545. $C_{33}H_{60}N_2O_8Si_3$ requires $[M+H]^+$ 697.3736, $[M+Na]^+$ 719.3555.) δ_H (300 MHz, $CDCl_3$) 7.38–7.29 (5H, complex m, $-C_6H_5$), 5.09 (2H, s, $-CH_2Ph$), 4.91 (1H, m, –N*H*), 4.21–4.10 (6H, complex m, 3 × $-OCH_2-$), 3.61 (4H, s, 2 × $-NCH_2CO_2-$), 3.40 (1H, t, J 7.7, –NC*H*–), 3.18 (2H, m, $-NHCH_2-$), 1.76–1.32 (6H, complex m, $-NCH_2(CH_2)_3-$), 1.04–0.93 (6H, complex m, 3 × $-CH_2Si-$), 0.04 (9H, s, 3 × $-CH_3$), 0.02 (18H, s, 6 × $-CH_3$). δ_C (75 MHz, $CDCl_3$) 172.9, 171.6, 156.5, 136.8, 128.5, 128.1, 128.0, 66.5, 64.7, 62.9, 62.8, 52.8, 40.8, 30.1, 29.4, 23.1, 17.6, 17.4, –1.5. ν_{max} (neat)/cm^{-1} 3381, 2953, 2899, 1728, 1526, 1455, 1413, 1250, 1217, 1159, 1061, 1042, 988, 937, 860, 838, 756, 696, 664, 608. *m/z* (+ve ESI) 719 ($[M+Na]^+$, 1%), 698 ($[M+H]^+$, 100).

*Compound **6***

A magnetically stirred solution of compound **5** (11.75 g, 16.9 mmol) in methanol (1000 mL) was treated with 10% palladium on charcoal (700 mg). The ensuing mixture was degassed, flushed with dihydrogen, stirred for 16 h at 18°C under an atmosphere of dihydrogen then filtered and the filtrate concentrated under reduced pressure. In this manner *compound **6*** (9.28 g, 98%) was obtained as a clear, colourless oil. (HRMS (+ve ESI): Found $[M+H]^+$ 563.3382. $C_{25}H_{54}N_2O_6Si_3$ requires $[M+H]^+$ 563.3368.) δ_H (300 MHz, $CDCl_3$) 4.22–4.12 (6H, complex m, 3 × $-OCH_2-$), 3.59 (4H, s, 2 × $-NCH_2CO_2-$), 3.41 (1H, t, J 7.7, –NC*H*–), 2.86–2.71 (2H, complex m, NH_2CH_2-), 1.78–1.34 (6H, complex m, $-NCH_2(CH_2)_3-$), 1.04–0.93 (6H, complex m, 3 × $-CH_2Si-$), 0.04 (9H, s, 3 × $-CH_3$), 0.03 (18H, s, 6 × $-CH_3$) (signal due to amine protons not observed). δ_C (75 MHz, $CDCl_3$) 173.0, 171.6, 64.9, 62.8, 62.7, 52.8, 41.8, 33.1, 30.4, 23.2, 17.6, 17.4, −1.5. ν_{max} (neat)/cm^{-1} 2953, 2899, 1745, 1592, 1454, 1416, 1388, 1346, 1250, 1159, 1061, 1042, 974, 937, 860, 837, 764, 695, 664, 608. *m/z* (+ve ESI) 564 (47%), 563 ($[M+H]^+$, 100).

Compound 7

A magnetically stirred solution of compound **4** (300 mg, 0.76 mmol) and DMAP (350 mg, 2.86 mmol) in dichloromethane (150 mL) maintained under argon was cooled to 0°C then EDAC·HCl (710 mg, 3.70 mmol) was added and the ensuing mixture left to stir at 0°C for 0.5 h. A solution of compound **6** (1.60 g, 2.84 mmol) in dichloromethane (100 mL) was then added and the reaction mixture stirred at 0°C for 1 h, allowed to warm to 18°C, left at this temperature for 3 days, then quenched with water (250 mL). The separated aqueous layer was extracted with dichloromethane (3 × 50 mL) and the combined organic fractions then dried (Na_2SO_4), filtered, and concentrated under reduced pressure to afford a light-yellow oil. Subjection of this material to flash chromatography (silica, 1:3 → 1:1 v/v ethyl acetate/hexane gradient elution) and concentration of the appropriate fractions (R_f 0.7 in 3:1 v/v ethyl acetate/hexane) afforded compound **7** (1.24 g, 81%) as a clear, colourless oil. (HRMS (+ve ESI): Found $[M+H]^+$ 2030.1138, $[M+Na]^+$ 2052.1040. $C_{93}H_{180}N_8O_{23}Si_9$ requires $[M+H]^+$ 203.1163, $[M+Na]^+$ 2052.0983.) δ_H (300 MHz, $CDCl_3$) 7.42 (2H, broad s, –N*H*), 7.36–7.27 (6H, complex m, $-C_6H_5$ and –N*H*), 5.15 (1H, t, J 5.7, –N*H*), 5.07 (2H, s, $-CH_2Ph$), 4.20–4.10 (18H, complex m, 9 × $-OCH_2-$), 3.58 (12H, s, 6 × $-NCH_2CO_2-$), 3.36 (5H, t, J 7.5, Cbz–$NHCH_2-$ and 3 × $-NCHCO_2-$), 3.28–3.12 (10H,

complex m, 2 × –NCH_2C(O)NH– and 3 × –C(O)NHCH_2–), 3.12–3.02 (1H, broad s, –NC*H*C(O)N–), 1.74–1.28 (24H, complex m, 4 × –NCH$_2$(CH_2)$_3$–), 1.04–0.92 (18H, complex m, 9 × –CH_2Si–), 0.04 (27H, s, 9 × –CH_3), 0.02 (54H, s, 18 × –CH_3). δ_C (75 MHz, $CDCl_3$) 172.9(2), 172.8(8), 172.4, 171.6, 156.5, 136.8, 128.5, 128.1, 128.0, 66.5, 65.7, 64.8, 64.7, 62.9, 62.8, 56.2, 50.7, 40.6, 39.2, 30.1, 29.9, 29.5, 29.3, 28.8, 28.2, 24.0, 23.1, 23.2, 17.6, 17.3, −1.4. ν_{max} (neat)/cm^{-1} 2953, 2899, 1729, 1667, 1535, 1453, 1345, 1250, 1217, 1157, 1061, 1042, 976, 936, 860, 837, 760, 695. *m/z* (+ve ESI) 2032 ([M + H]$^+$, 5%), 1016 ([M + 2H]$^{2+}$, 100).

Compound ***8***

A magnetically stirred solution of compound **7** (224 mg, 0.11 mmol) in 2,2,2-trifluoroethanol (20 mL) was treated with 10% palladium on charcoal (15 mg), the ensuing mixture degassed, flushed with dihydrogen, and then stirred under an atmosphere of dihydrogen at 18°C for 16 h. The reaction mixture was then filtered through a pad of Celite and the filtrate concentrated under reduced pressure to afford *compound* ***8*** (204 mg, 97%) as a clear, colourless oil. (HRMS (+ve ESI): Found [M + H]$^+$ 1897.0812. $^{12}C_{84}{}^{13}C_1H_{174}N_8O_{21}Si_9$ requires [M + H]$^+$ 1897.0829.) δ_H (300 MHz, $CDCl_3$) 7.97 (2H, m, –NH_2), 7.74 (1H, m, –N*H*), 7.51 (1H, m, –N*H*), 6.53 (1H, m, –N*H*), 4.20–4.04 (18H, complex m, 9 × –OCH_2–), 3.55 (12H, s, 6 × –NCH_2CO$_2$–), 3.48–2.88 (16H, complex m, H_2NCH_2–, 4 × –NC*H*C(O)–, 2 × –NCH_2C(O)NH–, and 3 × –C(O)NHCH_2–), 1.82–1.22 (24H, complex m, 4 × –NCH$_2$(CH_2)$_3$–), 1.02–0.88 (18H, complex m, 9 × –CH_2Si–), 0.01 (27H, s, 9 × –CH_3), −0.01 (54H, s, 18 × –CH_3). δ_C (75 MHz, $CDCl_3$) 173.0, 171.8, 171.7, 171.6, 65.0, 64.7, 63.0(4), 62.9(7), 62.9(2), 62.8(6), 52.8, 39.3, 30.1, 29.4, 29.0, 23.8, 23.3, 17.6, 17.4, −1.4. ν_{max} (neat)/cm^{-1} 3281, 3063, 2953, 2899, 1745, 1668, 1542, 1421, 1384, 1345, 1250, 1158, 1061, 1042, 976, 937, 860, 837, 763, 694, 663, 608. *m/z* (+ve ESI) 1899 (64%), 1898 (96), 1897 ([M + H]$^+$, 100), 961 (65), 950 (27), 614 (36), 564 (40).

Compound ***2***

A sample of compound **8** (200 mg, 0.10 mmol) was cooled to 0°C, treated with trifluoroacetic acid (5 mL), and the resulting mixture stirred at 0°C for 2 h then at 18°C for a further 2 h. The reaction mixture was concentrated at reduced pressure to a pale-brown resin that was washed successively with toluene (2 × 10 mL), acetonitrile (2 × 10 mL), and diethyl ether (10 mL), and so affording *compound* ***2*** (98 mg, 100%) as a white solid, mp 79.0–98.5°C (dec.). (HRMS (+ve ESI): Found [M + H]$^+$ 995.4449. $C_{40}H_{66}N_8O_{21}$ requires [M + H]$^+$ 995.4421.) δ_H (300 MHz, D_2O) 3.97 (15H, m, 6 × –CH_2CO$_2$– and 3 × –NC*H*CO$_2$–), 3.65 (4H, ABq, *J* 17.0, 2 × –NCH_2C(O)NH–), 3.53 (1H, m, –NC*H*C(O)NH–), 3.20 (6H, broad s, 3 × –NHCH_2–), 2.92 (2H, t, *J* 7.4, H_2NCH_2–), 2.00–1.44 (24H, complex m, 4 × –NCH$_2$(CH_2)$_3$–) (signals due to exchangeable protons not observed). δ_C (75 MHz, [D_6]DMSO) 174.4, 174.2, 174.1, 171.7, 171.2, 64.9, 64.6, 55.8, 54.6, 54.4, 38.4, 29.5, 28.9, 27.0, 23.3, 22.9. ν_{max} (nujol mull)/cm^{-1} 3267, 2924, 2855, 1923, 1724, 1644, 1458, 1377, 1240, 1024, 981, 897. *m/z* (+ve ESI) 996 (49%), 995 ([M + H]$^+$, 100), 498 (11).

Compound ***12***

A magnetically stirred solution of acid **11** (242 mg, 0.48 mmol) and 4-(*N*,*N*-dimethyl)aminopyridine (87 mg, 0.71 mmol) in dichloromethane (25 mL) maintained under argon was cooled to 0°C then EDAC·HCl (136 mg, 0.71 mmol) was added and the ensuing mixture left to stir at 0°C for 0.5 h. A solution of amine **8** (440 mg, 0.23 mmol) in dichloromethane (25 mL) was added and the ensuing mixture stirred at 0°C for 1 h, allowed to warm to 18°C, stirred at this temperature for 16 h, then quenched with water (50 mL). The separated aqueous layer was extracted with dichloromethane (3 × 25 mL) and the combined organic fractions then dried (Na_2SO_4), filtered, and concentrated under reduced pressure to afford a clear, colourless oil. Subjection of this material to flash chromatography (silica, 1:1 → 3:1 v/v ethyl acetate/hexane gradient elution) and concentration of the appropriate fractions (R_f 0.8 in ethyl acetate) afforded compound **12** (399 mg, 72%) as a clear, colourless oil. (HRMS (+ve ESI): Found [M + H]$^+$ 2387.5506. $C_{117}H_{235}N_9O_{23}Si_9$ requires [M + H]$^+$ 2387.5498.) δ_H (300 MHz, $CDCl_3$) 7.60 (4H, broad s, 3 × N*H*), 4.21–4.10 (18H, complex m, 9 × –OCH_2), 3.60 (12H, s, 6 × –NCH_2CO$_2$–), 3.37 (4H, t, *J* 7.5, 4 × –NC*H*CO$_2$–), 3.31–3.14 (16H, complex m, 2 × –NCH_2C(O)NH–, 4 × –C(O)NHCH_2–, and –C(O)N(CH_2–)$_2$), 2.68–2.60 (2H, complex m, –C(O)CH_2CH$_2$C(O)–), 2.58–2.48 (2H, complex m, –C(O)CH$_2$CH_2C(O)–), 1.90–1.18 (72H, complex m, 4 × –NCH$_2$(CH_2)$_3$– and 2 × –CH$_2$(CH_2)$_{12}$CH$_3$), 1.04–0.93 (18H, complex m, 9 × –CH_2Si–), 0.90 (6H, t, *J* 6.2, 2 × –CH_3), 0.04 (27H, s, 9 × –CH_3), 0.03 (54H, s, 18 × –CH_3). δ_C (75 MHz, $CDCl_3$) 172.8(4), 172.8(2), 172.7, 171.5, 171.4, 171.1, 65.5, 64.8, 64.7, 62.8(0), 62.7(9), 62.7(0), 62.6(6), 60.4, 56.1, 52.7, 48.1, 46.3, 39.3, 38.9, 31.9, 29.7, 29.7, 29.6(4), 29.6(3), 29.6(1), 29.5(8), 29.4(6), 29.4(0), 29.0, 27.8, 27.1, 27.0, 23.4, 23.2, 22.7, 21.0, 17.6, 17.3, 14.1, −1.5. ν_{max} (neat)/cm^{-1} 3301, 3073, 2953, 2926, 2855, 1746, 1651, 1546, 1461, 1427, 1379, 1250, 1158, 1061, 1042, 976, 937, 860, 837, 762, 695, 663, 608. *m/z* (+ve ESI) 2389 ([M + H]$^+$, <<1%), 1195 (28%), 537 (100).

Compound ***1***

A chilled sample of compound **12** (395 mg, 0.165 mmol) was cooled to 0°C, treated with trifluoroacetic acid (5 mL) and the ensuing mixture allowed to stand for 2 h at 0°C and then for an additional 2 h at 18°C. All volatiles were then removed under reduced pressure to afford a pale-brown resin that was washed with toluene (2 × 10 mL) and acetonitrile (2 × 10 mL) to afford compound **1** (225 mg, 92%) as a white solid, mp 169.0–193.9°C (dec.). (HRMS (–ve ESI): Found [M − H]$^-$ 1484.9028. $C_{72}H_{127}N_9O_{23}$ requires [M − H]$^-$ 1484.8967.) δ_H (300 MHz, [D_6]DMSO) 8.27 (2H, m, 2 × –N*H*), 8.01 (1H, m, –N*H*), 7.74 (1H, m, –N*H*), 3.47 (12H, ABq, *J* 17.9, –NCH_2CO$_2$–), 3.38–2.90 (14H, complex m, 2 × –NCH_2C(O)NH–, 3 × –C(O)NHCH_2–, and –C(O)N(CH_2–)$_2$), 2.48–2.40 (2H, complex m, –C(O)CH_2CH$_2$C(O)–), 2.27 (2H, m, –C(O)CH$_2$CH_2C(O)–), 1.68–1.10 (78H, complex m, 2 × –CH_2(CH_2)$_{12}$CH$_3$), 0.84 (6H, t, *J* 6.8, 2 × –CH_3) (signals due to nine carboxylic acid protons not observed). δ_C (75 MHz, [D_6]DMSO) 174.0, 173.3, 171.7, 171.4, 171.1, 170.7, 64.8, 64.4, 55.8, 53.4, 47.2, 45.3, 31.4, 30.7, 29.4, 29.1(8), 29.1(5), 29.0(7), 28.9, 28.6, 27.8, 27.4, 26.5, 26.3, 23.5, 23.2, 22.2, 14.0. ν_{max} (nujol mull)/cm^{-1} 3305, 2924, 2854, 1922, 1730, 1631, 1460, 1377, 1249, 977, 896, 722. *m/z* (–ve ESI) 1485 ([M − H]$^-$, 5%), 769 (6), 742 ([M − 2H]$^{2-}$, 100), 495 (60), 371 (16). HPLC R_t 12.7 min (Alltima (Alltech) C-18, 5 μm, 240 × 4.6 mm column, 959:40:1 v/v/v methanol/water/TFA (as isocratic solvent), flow rate 1 mL min^{-1}).

Acknowledgments

We thank Lipotek Pty Ltd for financial support including the provision of a post-doctoral stipend to D.A.O.

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