Supplemental data

Structure-Activity Relationships of Lipopolysaccharide Sequstration in *N*-Alkylpolyamines

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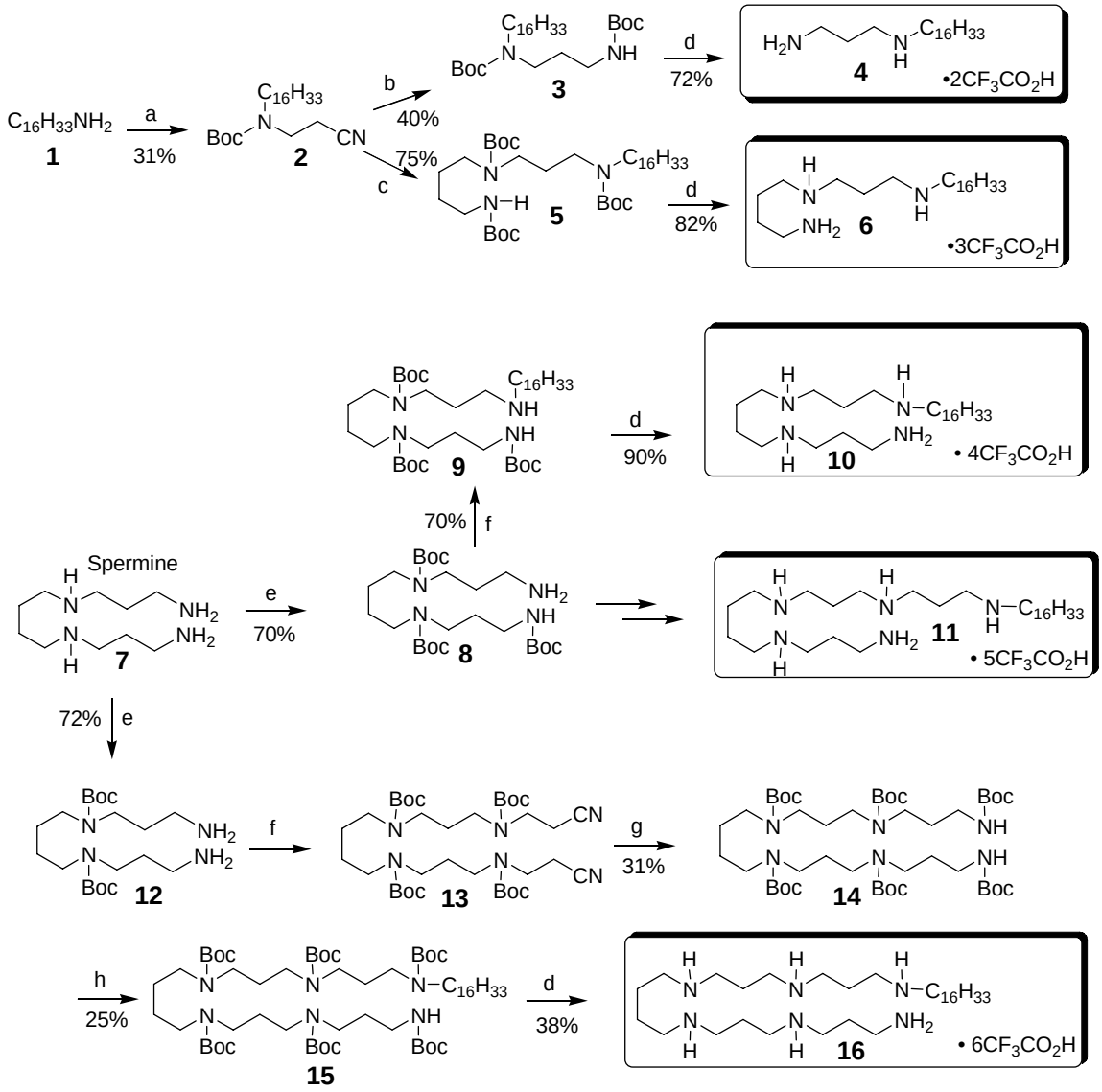
Scheme 1



Reagents:

a. (i)CH_2=CHCN, MeOH, r.t, 15h; (ii) Boc_2O (excess), MeOH, r.t, 12h.

b. AcOH, $Pd(OH)_2$/C, H_2, 50 psi; Boc_2O (excess), MeOH, r.t, 12h.

c. (i) 1,4-Diaminobutane (excess), $Pd(OH)_2$/C, H_2, 60 psi; (ii) Boc_2O (excess), MeOH, r.t, 12 h.

d. CF_3CO_2H (excess), r.t.

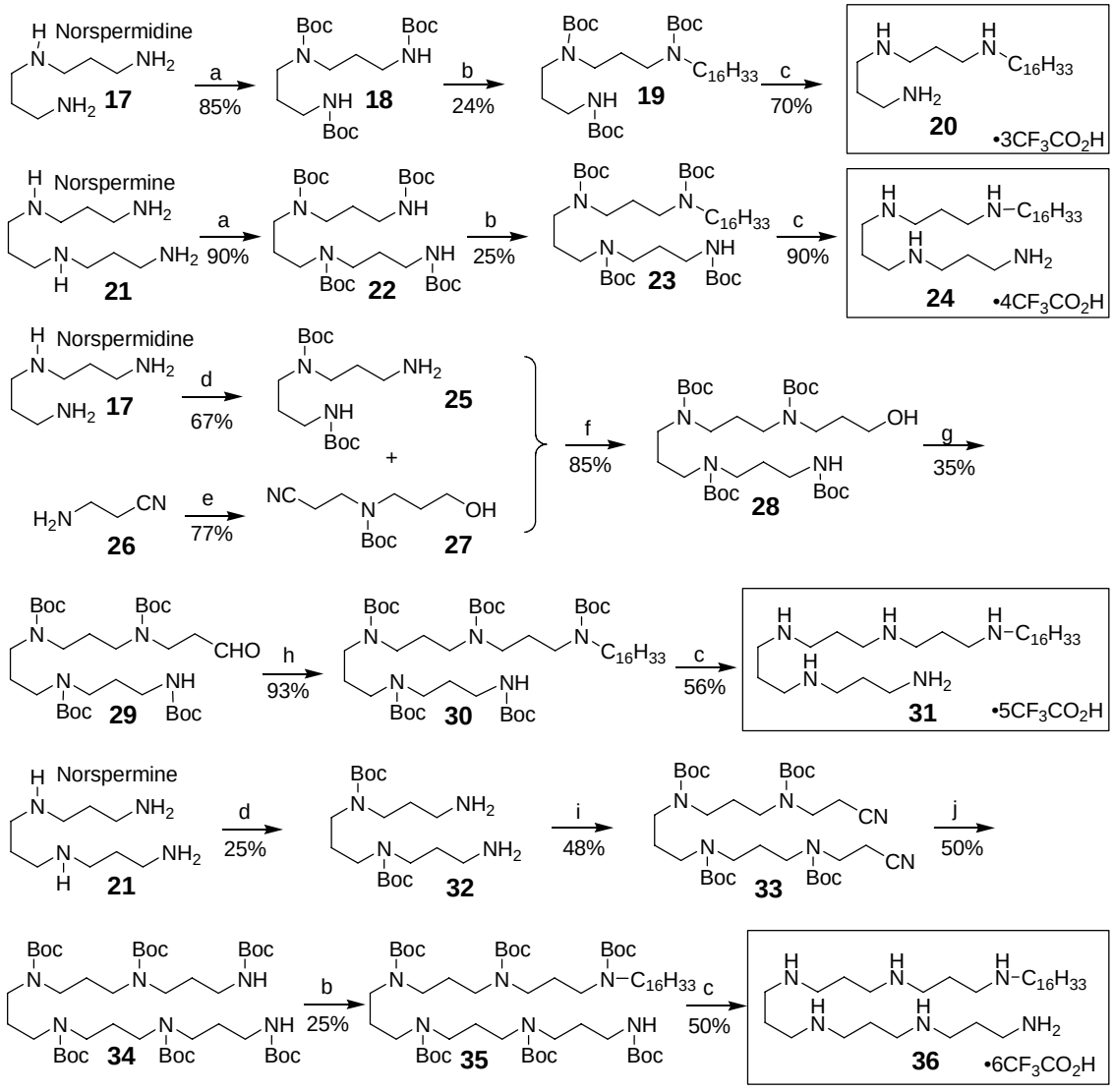
e. (i) CF_3COOEt (1or 2 Eq.), MeOH. -78^{o}C to 0^o C, 1h; (ii) Boc_2O (excess); (iii) LiOH, THF, r.t.

f. (i) CH_2=CHCN (2 Eq.), MeOH, r.t, 15 h; (ii) Boc_2O (excess), MeOH, r.t, 12h.

g. AcOH, $Pd(OH)_2$/C, H_2, 50 psi; Boc_2O (excess), MeOH, r.t, 12 h.

h. NaH, $C_{16}H_{33}I$, DMF, -15^{o}C-r.t, 24h.

Scheme 2



Reagents: a. Boc_2O (excess), MeOH, rt, 12 h. **b.** NaH (excess), $C_{16}H_{33}I$, DMF, - 15^oC-r.t., 24 h. **c.** TFA, r.t, 45 min **d.** (i) CF_3COOEt, MeOH, -78 ^oC to 0 ^oC, 1h; (ii) Boc_2O (excess), MeOH, r.t, 12 h; (iii) LiOH,THF, r.t. **e.** 3-bromopropan-1-ol, K_2CO_3, DMF, 60^oC (ii) Boc_2O (excess), MeOH, r.t, 12h. **f.** (i) MeOH, $Pd(OH)_2/C$, H_2, 60 psi; (ii) Boc_2O (excess), MeOH, r.t, 12h. **g.** PCC, DCM, r.t. **h.** (i)$C_{16}H_{33}NH_2$, $NaCNBH_3$, glacial AcOH (5 drops), anhyd. MeOH, r.t, 24h (ii) Boc_2O (excess), MeOH, r.t, 12h. **i.** i) CH_2CHCN, MeOH, r.t, 6 h; ii) Boc_2O (excess), MeOH, rt, 12 h. **j.** i) $Pd(OH)_2/C$, Glacial acetic acid, H_2, 60 psi; ii) Boc_2O (excess), MeOH, rt, 12 h.

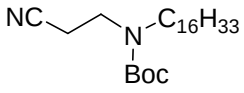
Experimental Data

Materials and Methods

Proton and carbon nuclear magnetic resonance spectra were recorded using a Bruker DRX 400 MHz or Bruker DRX 500 MHz spectrometer. All chemical shifts were recorded (δ) as parts per million (ppm), and all the samples were dissolved in $CDCl_3$ using residual solvent as internal standard unless otherwise noted. Mass spectra were obtained from Agilent ESI-TOF mass spectrometer at a mass accuracy of 20 ppm. All moisture-sensitive reactions were performed using either oven or flame dried glassware (120^oC) under a positive pressure of argon unless otherwise noted. Solvents and reagents that are commercially available were used without further purification unless otherwise noted. All silica gel 635 (60-100 mesh) used for column chromatography was purchased from Sigma-Aldrich, Inc. while thin layer chromatography were performed using silica gel CCM pre-coated aluminum sheets, purchased from Sorbent Technologies, Inc. All compounds were concentrated and dried using standard rotary evaporator and high vacuum techniques.

Synthesis of Spermine and Spermidine Series

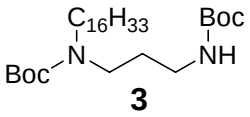
Conversion of Hexadecylamine 1 to 2. To a solution of hexadecylamine (10 g, 41.3 mmol) in anhydrous methanol (50 mL) at room temperature was added acrylonitrile (2.72 mL, 41.3 mmol) and the mixture stirred at room temperature for 15 h. After removal of solvent under high vacuum, the crude *bis*-nitrile derivative was dissolved in 30 mL of DCM followed by addition of a solution of di-*tert*-butyl dicarbonate (42.95 g, 206 mmol) in DCM (15 mL). The resulting solution was stirred for 12 h at room temperature, concentrated in vacuum, and purified by flash column chromatography (EtOAc: Hexane = 8 : 92 to 10 : 90) to give a colorless oil **2** (14g, 89%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.96 (t, 3H), 1.29 (s, 26H), 1.40-1.55 (s, 8H), 2.8 (br d, 2H), 3.0 (br m, 2H), 3.3 (t, 2H); ^{13}C NMR (100.6 MHz, $CDCl_3$) δ 14.09, 20.90, 24.62, 25.88, 27.41, 28.35, 29.6, 31.9, 46.58, 46.77, 78.71, 79.19, 79.50, 120, 155.32, 155.95; MS (ESI) calculated for $C_{24}H_{46}N_2O_2$ *m/z* 394.35 found 417.30 $(MNa)^+$.



2

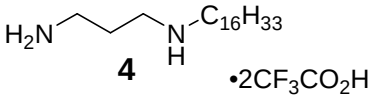
Conversion of 2 to 3. Compound **2** (0.8 g, 2.02 mmol) was hydrogenated over $Pd(OH)_2$/C (0.08g) at 50 psi pressure in glacial AcOH (20 mL) for 12 h. AcOH was removed under reduced pressure followed by addition of excess of di-*tert*-butyl dicarbonate (2.82 g, 12.9 mmol) in methanol (15 mL). After removal of solvent under vacuum, the residue was purified by flash column chromatography (EtOAc: Hexane = 2 : 98 to 4 : 96) to yield **3** as a colorless oil (0.4 g, 40%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.90 (m, 3H), 1.25 (br s, 26H), 1.45-1.47 (m, 20H), 1.66-1.81 (m, 3H), 4.75 (br s, 4H), 3.29

(br s, 2H); ^{13}C NMR (100.6 MHz, $CDCl_3$) δ 14.13, 22.70, 26.89, 28.46, 29.37, 29.60, 29.63, 29.66, 29.70, 31.93; MS(ESI) calculated for $C_{29}H_{58}N_2O_4$ *m/z* 498.44 found 521.50 $(MNa)^+$



3

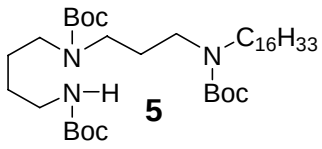
Conversion of 3 to 4. Compound **3** (0.4 g, 0.8 mmol) was dissolved in excess of dry trifluoroacetic acid (25 mL) and stirred at room temperature for 30 min. Excess solvent was removed by purging nitrogen and the residue was thoroughly washed with diethyl ether to obtain white flaky solid **4** (0.3 g, 71.4%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 0.87 (m, 3H), 1.24 (br s, 26H), 1.58 (br s, 2H), 1.89 (m, 2H), 2.89 (m, 4H), 2.99 (m, 2H); ^{13}C NMR (100.6 MHz, DMSO-d_6) δ 14.41, 22.55, 24.13, 25.80, 26.32, 28.96, 29.16, 29.24, 29.38, 29.50, 31.75, 36.50, 44.20, 47.15, 116.17, 119.15, 158.65, 158.96; MS(ESI) calculated for $C_{19}H_{42}N_2$ (free amine) *m/z* 298.33 found 299.38 $(MH)^+$.



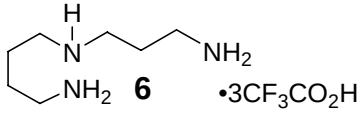
4

Conversion of 2 to 5. A solution of mono-nitrile **2** (0.57 g, 1.45 mmol) and 1, 4-diaminobutane (1.02 g, 11.6mmol) in methanol (25 mL) was hydrogenated over $Pd(OH)_2/C$ (0.05g) at 60 psi pressure for 12 h. The catalyst was removed by filtration and the residue was washed thoroughly with methanol. After removal of solvent under high vacuum, the crude amine alkylated compound (0.33 g, 0.70 mmol, 49.3%) was dissolved in methanol (20 mL) to which a solution of di-*tert*-butyl dicarbonate (1.53 g, 7.0 mmol)

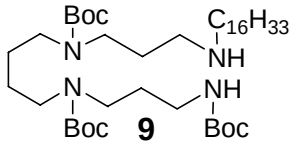
in methanol (5 mL) was added. The resulting solution was stirred for 12 h at ambient temperature, concentrated in vacuum and purified by flash column chromatography (EtOAc: Hexane = 8 : 92 to 10 : 90) to give compound **5** (0.35 g, 74.5%) as a viscous oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.82 (m, 3H), 1.19 (br s, 26H), 1.39 (m, 33H), 1.66 (br s, 2H), 3.10 (br s, 10H); ^{13}C NMR (100.6 MHz, $CDCl_3$) δ 14.03, 22.59, 25.54, 26.77, 27.32, 28.33, 28.39, 29.27, 29.32, 29.50, 29.57, 29.60, 31.84, 40.13, 44.33, 44.66, 46.60, 47.04, 78.78, 78.97, 79.15, 155.39, 155.93; MS(ESI) calculated for $C_{38}H_{75}N_3O_6$ *m/z* 669.57 found 692.61 $(MNa)^+$.



Conversion of 5 to 6. The resulting Boc-protected polyamine **5** (0.35 g, 0.70 mmol) was dissolved in excess of dry trifluoroacetic acid (25 mL) and stirred at room temperature for 30 min. Excess solvent was removed by purging nitrogen and the residue was thoroughly washed with diethyl ether to obtain white flaky solid **6** (0.3 g, 81%). ^{1}H NMR (400 MHz, DMSO-d_6) 0.86 (m, 3H), 1.24 (br s, 26H), 1.56-1.62 (m, 6H), 1.96 (m, 2H), 2.80-2.99 (m, 10H), 8.79-8.90 (m, 4H); ^{13}C NMR (100.6 MHz, DMSO-d_6) 14.40, 22.55, 22.79, 23.00, 24.59, 25.84, 26.33, 28.95, 29.16, 29.23, 29.37, 29.46, 29.50, 31.75, 38.66, 44.30, 44.34, 46.55, 47.20, 116.09, 119.07, 158.58, 158.89, 159.21; MS(ESI) calculated for $C_{23}H_{51}N_3$ (free amine) *m/z* 369.41 found 370.45 $(MH)^+$.



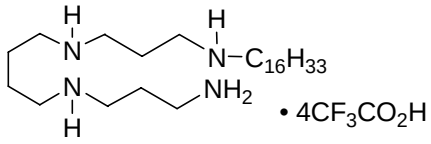
C**onversion of 8 to 9** To a solution of the Compound **8** (0.989 g, 1.97 mmol) [which was synthesized from spermine **7** using a reported procedure (*J. Med. Chem*. **2107**; *50*(4); 877-888)] was added acetic acid (0.7 mL) in dry methanol (30 mL) followed by the addition of hexadecylaldehyde (0.315 g, 1.31 mmol), and also the addition of sodium cyanoborohydride (0.124 g, 0.1.97 mmol) in one portion. The colorless solution was stirred at room temperature for 24 h. The reaction was quenched by the addition of concentrated HCl to pH 2, followed by basification of the resulting solution to pH 12 by addition of solid NaOH. The solution was concentrated under reduced pressure, and the residue was extracted with ether (3 x 50 mL). The combined organic layers were washed with saturated aqueous sodium chloride and dried over anhydrous Na_2SO_4. Removal of solvent and purification of the crude product by column chromatography (CH_2Cl_2/MeOH/aq.ammonia = 9 : 1 : 0.05) provided the compound **9** as a colorless oil (0.655g, 69%). ^{1}H NMR ($CDCl_3$, 400 MHz) δ 3.27-3.12 (m, 10H), 2.62 (br s, 4H), 1.74-1.66 (m, 4H), 1.50-1.32 (br s, 34H), 1.32-1.23 (m, 27H), 0.89 (t, J = 8.0 Hz, 3H); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 156.1(3C), 79.5, 50.1, 46.8, 46.5, 44.2, 37.4, 31.9, 30.1, 29.69, 29.65, 29.62, 29.60, 29.56, 29.36, 28.5, 27.4, 26.1, 25.6, 22.7, 14.1; MS (ESI, *m/z*) calculated for $C_{41}H_{83}N_4O_6$ (MH^+) 727.6312, found 727.6307.



9

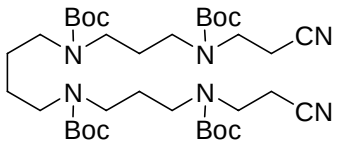
Conversion of 9 to 10 The Boc-protected monoalkyl polyamine **9** (0.64 g, 0.88 mmol) was dissolved in dry trifluoroacetic acid (10 mL) and the solution stirred at room temperature for 20 h. Excess solvent was removed under reduced pressure, and the

residue was thoroughly triturated with dichloromethane and diethyl ether to provide the product **10** as a white flaky solid (0.609 g, 90%). ^{1}H NMR (DMSO-d_6, 400 MHz) δ 8.86-8.73 (m, 3H), 7.93 (br s, 2H), 2.99-2.92 (m, 14H), 1.93 (br s, 4H), 1.64-1.58 (m, 6H), 1.26 (br s, 26H), 0.86 (t, J = 8.0 Hz, 3H); ^{13}C NMR (DMSO-d_6, 125 MHz) δ 158.7, 158.4, 158.2, 64.9, 46.7, 46.1, 43.9, 36.2, 31.3, 29.0, 28.9, 28.7, 28.6, 28.5, 25.8, 25.4, 23.8, 22.6, 22.3, 22.1, 13.1; HRMS (ESI, *m/z*) calculated for $C_{26}H_{59}N_4$ (MH^+) 427.4740, found 427.4738.



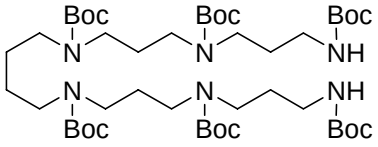
10

Conversion of 12 to 13. To a solution of compound **12** also synthesized from spermine **7** using a reported procedure (*J. Med. Chem*. **2107**; *50*(4); 877-888)] (1.0 g, 2.48 mmol) in methanol (50 mL) was added acrylonitrile (0.26 g, 5 mmol) and stirred at room temperature for 15 h. After removal of solvent under high vacuum, the crude *bis*-nitrile derivative (1.2 g, 95 %) was dissolved in DCM (30 mL) followed by the addition of a solution of di-*tert*-butyl dicarbonate (1.05 g, 4.8 mmol) in DCM (10 mL). The resulting solution was stirred for 90 min at ambient temperature, concentrated *in vacuo* and purified by flash column chromatography (EtOAc: Hexane = 40 : 60) to give compound **13** (1.12 g, 64 %) as a viscous oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.45 and 1.47 (2s, 42H), 1.72-1.79 (m, 4H), 2.55-2.68 (m, 4H), 3.08-3.20 (br s, 6H), 3.21-3.30 (m, 4H), 3.46-3.51 (m, 4H); ^{13}C NMR (100.6 MHz, $CDCl_3$) δ 17.0, 17.6, 25.6, 26.0, 28.4, 28.5, 43.5, 44.0, 44.6, 45.5, 46.6, 47.0, 79.5, 80.6, 154.7, 155.2, 155.5; MS (ESI) calculated for $C_{36}H_{64}N_6O_8$ *m/z* 708.4, found 709.5 $(MH)^+$.



13

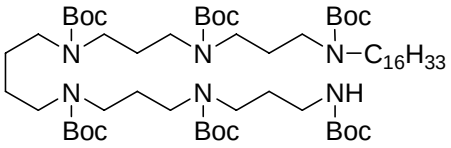
Conversion of 13 to 14. 1.5 g of compound **13** was hydrogenated over 0.15g of $Pd(OH)_2/C$ at 60 psi pressure in glacial AcOH (20 mL) for 12 h. AcOH was removed under reduced pressure followed by addition of excess of di-*tert*-butyl dicarbonate (2.3 g, 10.5 mmol) in methanol (15 mL) to afford **14** as colorless oil (0.6 g). Yield 31%; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.34 (br s, 54H), 1.56 (br s, 4H), 1.63 (br s, 8H), 2.90-3.06 (m, 15H), 3.06-3.15 (m, 5H); ^{13}C NMR (100.6 MHz, $CDCl_3$) δ 14.09, 20.90, 24.62, 25.88, 27.41, 28.35, 37.31, 43.69, 44.70, 46.58, 46.77, 60.22, 78.71, 79.19, 79.50, 155.32, 155.95; MS(ESI) calculated for $C_{46}H_{88}N_6O_{12}$ *m/z* 916.65 found 939.64 $(MNa)^+$.



14

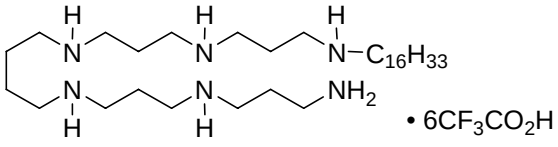
Conversion of 14 to 15. 0.34 g (0.4 mmol) of compound **14** was added to the suspension of 60% NaH (0.2 g, 8.3 mmol) in DMF and was stirred for 10 min at 0^{o}C after which 0.14 g of iodohexadecane (0.4 mmol) was added and thereafter was stirred for 24h. The reaction mixture was quenched with 10% HCl and extracted with EtOAc. After removal of the solvent under reduced pressure, the desired Boc-protected, mono-alkylated compound (0.11 g, 26.2%) was isolated at (EtOAc: Hexane = 20 : 80). ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.70-0.73 (br s, 3H), 1.25 (br s, 26H), 1.44-1.46 (m, 54H), 1.54-1.73 (m, 14H), 3.15-3.47 (m, 22H); ^{13}C NMR (100.6 MHz, $CDCl_3$) δ 14.11, 22.67, 25.82, 26.86, 28.44, 28.47, 29.34, 29.41, 29.59, 29.64, 29.67, 31.90, 37.32, 44.79, 47.07, 79.08, 79.31,

155.42, 156.05; MS(ESI) calculated for $C_{62}H_{120}N_6O_{12}$ *m/z* 1140.89 found 1163.77 $(MNa)^+$.



15

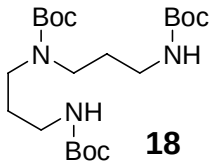
Conversion of 15 to 16. The resulting Boc-protected mono-alkylated polyamine (0.19g, 0.19 mmol) was dissolved in excess (25 mL) of dry trifluoroacetic acid and stirred at room temperature for 1 h. Excess solvent was removed by purging nitrogen and the residue was thoroughly washed with diethyl ether to obtain white flaky solid **17** (0.170 g, 90%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 0.85 (t, *J* = 6.6Hz, 3H), 1.25 (br s, 26H), 1.41 (br s, 6H) 1.55-1.65 (br s, 6H), 2.88 (br m, 18H); ^{13}C NMR (100.6 MHz, DMSO-d_6) 22.8, 23.0, 24.2, 25.8, 26.3, 28.9, 29.2, 29.3, 29.4, 31.7 , 36.6, 39.5, 39.7, 40.1, 40.4, 40.6, 44.3, 44.5, 46.5, 47.2; MS(ESI) calculated for $C_{29}H_{65}N_5$ (free amine) *m/z* 483.4 found 484.4 $(MH)^+$.



16

Synthesis of Nor-Spermidine and Nor-Spermine series

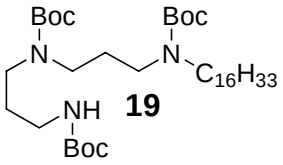
Conversion of 17 to 18. To a solution of norspermidine **17** (0.5 g, 3.81 mmol) in methanol (20 mL), di-*tert*-butyl dicarbonate (8.4 g, 38.1 mmol) dissolved in methanol (5 mL) was added. The resulting solution was stirred for 12 h at ambient temperature, concentrated in vacuum and purified by flash column chromatography (EtOAc: hexane = 20 : 80 to 25 : 75) to afford the Boc protected product **18** **(**1.4 g, 85 %) as a viscous oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.38 (s, 27H), 1.86 (m, 4H), 3.0 (t, 4H) 3.2 (t, 4H); ^{13}C NMR (100.6 MHz, $CDCl_3$) δ 28.4, 37.5, 44.1, 79.8, 156; MS(ESI) calculated for $C_{21}H_{41}N_3O_6$ *m/z* 431.3 found 454.2 $(MNa)^+$.



18

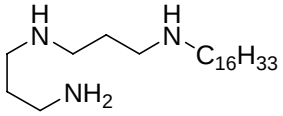
Conversion of 18 to 19. A solution of **18** (1.4 g, 3.24 mmol) and 60% of sodium hydride suspension (6.25 g, 259.8 mmol) in DMF (20 mL) was cooled to -15 ^{o}C and hexadecyl iodide (1.14 g, 3.24 mmol) was then added to it dropwise under nitrogen atmosphere. The resulting mixture was stirred for another 1 h at -15 ^{o}C and was allowed to stir overnight at room temperature. The reaction mixture was quenched with 10% HCl solution in water, extracted with EtOAc, dried with Na_2SO_4 and concentrated. The residue was purified by flash column chromatography (EtOAc: hexane = 8 : 92 to 10 : 90) to afford **19** (0.5 g, 24%) as a viscous oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.9 (t, 3H), 1.27 (br s, 25H), 1.38 (s, 27H), 1.86 (m, 4H), 3.0 (t, 6H) 3.2 (t, 2H); ^{13}C NMR (100.6 MHz, $CDCl_3$) δ 14.1, 22.7,

24.1, 26.3, 27, 28.4, 29.3, 29.6, 31.8, 39.9, 45.7, 46, 48.6, 79.8, 156; MS(ESI) calculated for $C_{37}H_{73}N_3O_6$ *m/z* 655.5 found 678.5 $(MNa)^+$.



19

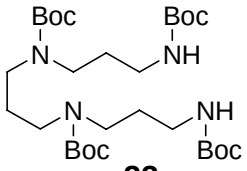
Conversion of 19 to 20. The resulting product **19** was dissolved in excess (25 mL) of dry trifluoroacetic acid and stirred at room temperature for 45 minutes. Excess solvent was removed by purging nitrogen and the residue was thoroughly washed with diethyl ether to obtain white flaky solid **20** (0.35 g, 70%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 0.88 (t, *J* = 6.6Hz, 3H), 1.29-1.38 (s, 25H), 1.55 (br m, 2H) 1.75 (br m, 4 H), 2.55 (t, 4 H), 2.65 (t, 6 H); ^{13}C NMR (100.6 MHz, DMSO-d_6) δ 14.1, 22.7, 24.1, 26.3, 27, 27.3, 29.3, 29.6, 30.5, 31.8, 39.4, 45.7, 46, 46.3, 49.9; MS(ESI) calculated for $C_{22}H_{49}N_3$ *m/z* 355.3 found 356.4 $(MH)^+$.



20 •$3CF_3CO_2H$

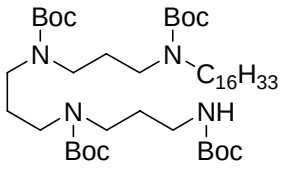
Conversion of 21 to 22. To a solution of norspermine **21** (0.5 g, 2.65 mmol) in methanol (20 mL) di-*tert*-butyl dicarbonate (5.8 g, 26.5 mmol) dissolved in methanol (7 mL) was added. The resulting solution was stirred for 12 h at ambient temperature, concentrated in vacuum and purified by flash column chromatography (EtOAc: hexane = 15 : 85) to afford the Boc protected product **22** (1.3 g, 83 %) as a viscous oil. ^{1}H NMR (400 MHz, MeOD) δ 1.5 (s, 36H), 1.86 (m, 4H), 3.0 (t, 4H) 3.2 (t, 8H); ^{13}C NMR (100.6 MHz,

$CDCl_3$) δ 26, 28.4, 37.5, 44.1, 47.6, 79.6, 156; MS(ESI) calculated for $C_{29}H_{56}N_4O_8$ *m/z* 588.41 found 611.39 $(MNa)^+$.



22

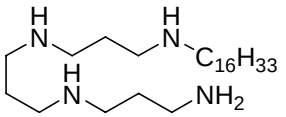
Conversion of 22 to 23. A solution of **22** (1 g, 1.7 mmol) and 60% sodium hydride (3.26 g, 136 mmol) in DMF (30 mL) was cooled to -15 ^oC and hexadecyl iodide (0.6 g, 1.7 mmol) was then added to it dropwise under nitrogen atmosphere. The resulting mixture was stirred for another 1 h at -15 ^oC and was allowed to stir overnight at room temperature. The reaction mixture was quenched with 10% HCl solution in water, extracted with EtOAc, dried with Na_2SO_4 and concentrated. The residue was purified by flash column chromatography (EtOAc: hexane = 10 : 90) to afford **23** (0.35 g, 25%) as a viscous oil. ^1H NMR (400 MHz, $CDCl_3$) δ 0.9 (t, 3H), 1.27 (br s, 25H), 1.38 (s, 27H), 1.86 (m, 4H), 3.0 (t, 6H) 3.2 (t, 2H); ^{13}C NMR (100.6 MHz, $CDCl_3$) δ 14.1, 22.7, 24.1, 26.3, 27, 28.4, 29.3, 29.6, 31.8, 39.9, 45.7, 46, 48.6, 79.8, 156; MS(ESI) calculated for $C_{45}H_{88}N_4O_8$ *m/z* 812.6 found 835.64 $(MNa)^+$.



23

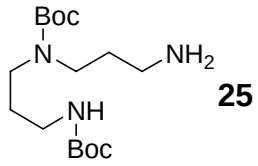
Conversion of 23 to 24. The resulting product **23** was dissolved in excess (25 mL) of dry trifluoroacetic acid and stirred at room temperature for 8 h. Excess solvent was removed by purging nitrogen and the residue was thoroughly washed with diethyl ether to obtain

white flaky solid **24** (0.21 g, 90%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 0.88 (t, *J* = 6.6Hz, 3H), 1.29-1.38 (s, 25H), 1.55 (br m, 2H) 1.75 (br m, 4 H), 3.0 (t, 12 H); ^{13}C NMR (100.6 MHz, DMSO-d_6) δ 14.1, 22.7, 24.1, 26.3, 27, 27.3, 29.3, 29.6, 30.5, 31.8, 39.4, 45.7, 46, 46.3, 49.9; MS(ESI) calculated for $C_{25}H_{56}N_4$ *m/z* 412.4 found 413.49 $(MH)^+$.



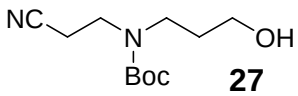
24 •4CF_3CO_2H

Conversion of 17 to 25. To a solution of amine **17** (3 g, 22.9 mmol) in methanol (50 mL) at -78 ^{o}C was added dropwise ethyl trifluoroacetate (2.72 mL, 22.9 mmol) over 30 min and the solution was stirred for another 30 min. The temperature was raised to 0 ^{o}C and an excess of di-*tert*-butyl dicarbonate (40.0 g, 183.2 mmol) in methanol (20 mL) was added over 10 min. The reaction was then warmed to 25 ^{o}C for 15 h. After removal of solvent under vacuum, the crude product was dissolved in THF (20 mL) followed by addition of a solution of lithium hydroxide (5.5 g, 229 mmol) in water (40 mL). The resulting solution was stirred at ambient temperature for 24 h. The reaction mixture was extracted with chloroform. The combined extracts were washed with water and brine and dried over sodium sulfate. After removal of solvent under vacuum, the residue was purified by flash column chromatography with use of (MeOH : DCM = 4 : 96 to 6 : 94) to afford the product **25** as colorless viscous oil (5 g, 15.1 mmol) in 67 % yield.^{1}H NMR (400 MHz, $CDCl_3$) δ 1.44-1.47 (m, 18H), 2.74-2.79 (m, 4H), 3.10-3.11 (br s, 2H), 3.24-3.47 (m, 6H); ^{13}C NMR (100.6 MHz, $CDCl_3$); 28.34, 28.40, 80.49, 156.12; MS(ESI) calculated for $C_{16}H_{33}N_3O_4$ *m/z* 331.24 found 332.23 $(MH)^+$.



25

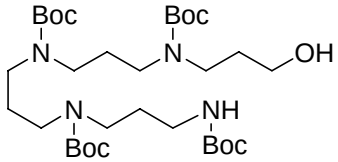
Conversion of 26 to 27. To a solution of 3-aminopropanenitrile **26** (2.08 mL, 28.6 mmol) in dry DMF and K_2CO_3 (6 g, 43.5 mmol) was added 3-bromopropan-1-ol (2.49 mL, 28.6 mmol) and the reaction mixture was refluxed for 12 h. After removal of DMF under reduced pressure, an excess of di-*tert*-butyl dicarbonate (24.9 g, 114.2 mmol) in methanol (20 ml) was added and the resulting solution was stirred at ambient temperature for 24 h. After removal of solvent under vacuum, the residue was purified by flash column chromatography at (EtOAc: Hexane = 20 : 80) to afford the product **27** as colorless viscous oil (5 g, 76.9%). ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.5 (br s, 9H), 1.66-1.79 (m, 2H), 2.61-2.68 (m, 2H), 3.31-3.49 (m, 4H), 3.59-3.68 (m, 2H); ^{13}C NMR (100.6 MHz, $CDCl_3$); 17.44, 28.30, 30.66, 43.53; MS (ESI) calculated for $C_{11}H_{20}N_2O_3$ *m/z* 228.14 found 251.14 $(MNa)^+$.



27

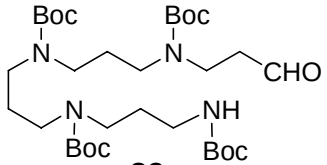
Conversion of 25 and 27 to 28. A solution of *mono*-nitrile **27** (2.19 g, 9.6 mmol) and *tris*-amine **25** (1.31 g, 3.9 mmol) in methanol (20 mL) was hydrogenated over $Pd(OH)_2/C$ (0.3 g) at 60 psi pressure for 12 h. The catalyst was removed by filtration and the residue was washed thoroughly with methanol. After removal of solvent under high vacuum, the crude secondary amine compound (2.0 g, 3.7 mmol, 92%) was dissolved in methanol (30 mL) followed by the addition of a solution of di-*tert*-butyl dicarbonate (16.4 g, 75.14 mmol) in methanol (7 mL). The resulting solution was stirred for 12 h at ambient temperature, concentrated in vacuum and purified by flash column chromatography at

(EtOAc: Hexane = 35 : 65 to 38 : 62) to give the product **28** (2.0 g, 84.7%) as a viscous oil . ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.25-1.27 (m, 36H), 1.43-1.57 (m, 8H), 2.91-3.16 (m, 14H), 3.37 (br s, 2H); ^{13}C NMR (100.6 MHz, $CDCl_3$); 20.79, 28.25, 28.28, 29.45, 30.58, 31.69, 36.11, 36.51, 37.55, 42.82, 44.88, 50.39, 53.44, 58.31, 59.51, 60.12, 62.01, 78.61, 79.29, 79.44, 79.75, 81.73, 86.66, 87.97, 89.65, 91.87, 92.70, 96.85, 98.04, 114.45, 117.31, 155.18, 155.74, 155.95, 156.42, 156.95, 170.83; MS(ESI) calculated for $C_{32}H_{62}N_4O_9$ *m/z* 646.45 found 669.46 $(MNa)^+$.



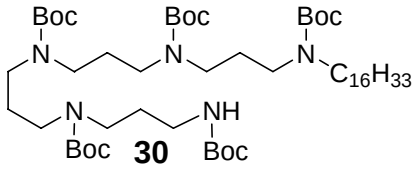
28

Conversion of 28 to 29. A solution of the alcohol **28** (1.35 g, 2.08 mmol) and PCC (1 g, 4.32 mmol) in dry DCM was stirred for 3 h. After removal of the solvent under reduced pressure the crude product was purified by column chromatography (EtOAC: Hexane = 30 : 70 to 35 : 65) to give the product **29** (0.46 g, 34.3%) as a viscous oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.38 (br s, 36H), 1.46-1.82 (m, 8H), 3.04-4.08 (m, 14H), 9.73 (s, 1H); ^{13}C NMR (100.6 MHz, $CDCl_3$); 24.21, 24.55, 24.63, 27.51, 28.37, 28.90, 36.60, 37.00, 37.32, 43.67, 44.77, 78.78, 79.44, 79.60, 91.62, 155.31, 156.07, 197; MS(ESI) calculated for $C_{32}H_{60}N_4O_9$ *m/z* 644.44 found 667.45 $(MNa)^+$.



29

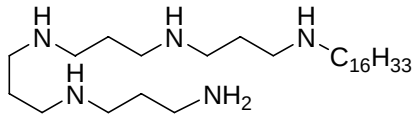
Conversion of 29 to 30. To a solution of 0.46 g of the aldehyde **29** and hexadecylamine (0.26 g, 1.08 mmol) in absolute methanol (20 mL), sodium cyanoborohydride (.09 g, 1.43 mmol) and 5-6 drops of glacial acetic acid was added. The reaction mixture was allowed to stir at room temperature for 24 h. After removal of the solvent under reduced pressure the crude product was purified by column chromatography (MeOH: DCM = 1 : 99) to give the alkylated secondary amine (0.51 g, 0.58 mmol, 82.3%) was dissolved in a solution of methanol (20 mL) to which a solution of di-*tert*-butyl dicarbonate (1.3 g, 5.9 mmol) in methanol (10 mL) was added. The resulting solution was stirred for 12 h at ambient temperature, concentrated in vacuum and purified by flash column chromatography (EtOAc: Hexane = 20 : 80 to 25 : 75) to give the product **30** (0.52 g, 92.8%) as a viscous oil . ^{1}H NMR (400 MHz, MeOD) δ 0.91-0.94 (m, 3H), 1.32 (br s, 28H), 1.46-1.55 (m, 45H), 1.71-1.78 (m, 8H), 3.04-3.09 (m, 2H), 3.22-3.33 (m, 16H); ^{13}C NMR (100.6 MHz, MeOD); 19.61, 22.43, 26.55, 27.58, 27.91, 28.29, 29.17, 29.37, 29.40, 29.48, 31.76, 37.64, 44.50, 44.82, 60.09, 78.22, 78.42, 79.31, 79.52, 79.55, 79.72, 155.67, 155.81, 156.96; MS(ESI) calculated for $C_{53}H_{103}N_5O_{10}$ *m/z* 969.77 found 992.80 $(MNa)^+$.



30

Conversion of 30 to 31. The polyamine **30** (0.52 g, 0.54 mmol) was dissolved in excess of dry trifluoroacetic acid (20 mL) and stirred at room temperature for 30 min. Excess solvent was removed by purging nitrogen and the residue was thoroughly washed with diethyl ether to obtain white flaky solid **31** (0.31 g, 56.3%). ^{1}H NMR (400 MHz, DMSO-

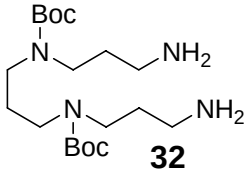
d_6) δ 0.84-0.86 (m, 3H), 1.24 (br s, 26H), 1.56-1.73 (br s, 2H), 1.89-2.13 (br s, 8H), 2.67-2.88 (m, 18H), 8.98-9.09 (m, 6H); ^{13}C NMR (100.6 MHz, DMSO-d_6); 22.55, 28.95, 29.50, 31.75, 44.52 MS(ESI) calculated for $C_{28}H_{63}N_5$ *m/z* 469.51 (free amine) found 470.51 $(MH)^+$.



31

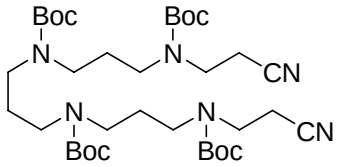
Conversion of 21 to 32. To a solution of norspermine **21** (1 g, 5.31 mmol) in methanol (30 mL) at -78 ^{o}C was added dropwise ethyl trifluoroacetate (1.26 mL, 10.62 mmol) over 30 min and the solution was stirred for another 1 h. The temperature was then increased to 0 ^{o}C and an excess of di-*tert*-butyl dicarbonate (11.6 g, 53.1 mmol) in methanol (10 mL) was added over 10 min. The reaction was then warmed to room temperature and stirred for another 20 h. After removal of solvent under high vacuum, the crude product was dissolved in THF (30 mL) followed by addition of a solution of lithium hydroxide (.38 g, 15.93 mmol) in water (10 mL). The resulting solution was stirred at ambient temperature for 24h. The reaction mixture was extracted with chloroform (4 x). The combined extracts were washed with water (1 x) and brine (1 x), dried over Na_2SO_4. After removal of solvent under vacuum, the residue was purified by flash column chromatography (MeOH: DCM: aq NH_4OH = 1 : 9 : 0.1) to afford the title compound **32** as a viscous oil (.41 g, 20.4%).^{1}H NMR (400 MHz, $CDCl_3$) δ 1.25 (s, 18H), 1.47 (t, 4H), 1.55 (br t, 2H), 2. 47 (t, 4H), 2.9 (br s, 4H), 3.0 (br s, 4H); ^{13}C NMR (100.6 MHz, $CDCl_3$)

δ 28.45, 31.2, 32.1, 38.7, 39, 43.7, 44.1, 49.2, 77.6, 79. 18, 156.0; MS(ESI) calculated for $C_{19}H_{40}N_4O_4$ *m/z* 388.30 found 389.30 $(MH)^+$.



32

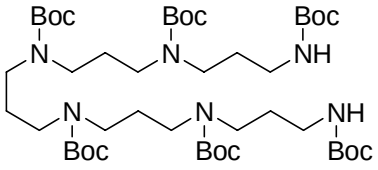
Conversion of 32 to 33. To a solution of the amine **32** (0.4 g, 1.05 mmol) in anhydrous methanol (15 mL) at room temperature was added acrylonitrile (0.14 mL, 2.11 mmol) and the mixture stirred at room temperature for 15 h. After removal of solvent under high vacuum, the crude *bis*-nitrile derivative was dissolved in 15 mL of DCM to which a solution of di-*tert*-butyl dicarbonate (2.28 g, 10.5 mmol) in DCM (7 mL) was added. The resulting solution was stirred for 12 h at room temperature, concentrated in vacuum, and purified by flash column chromatography (EtOAc: Hexane = 17: 83 to 20 : 80) to give a white flaky solid **33** (0.35 g, 48 %) ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.33 (s, 36H), 1.64 (br m, 6H), 2.5 (br d, 4H), 3.06 (br s, 8H), 3.1 (t, 4H), 3.3 (t, 4H); ^{13}C NMR (100.6 MHz, $CDCl_3$) δ 16.80, 20.9, 44.61, 45.2, 46.34, 80.3, 118.2, 155.21 MS(ESI) calculated for $C_{35}H_{62}N_6O_8$ *m/z* 694.46 found 717.39 $(MNa)^+$.



33

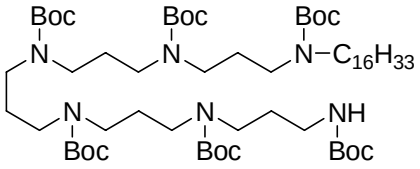
Conversion of 33 to 34. A solution of *bis*-nitrile **33** (0.35 g, 0.50 mmol) in glacial AcOH (10 mL) was hydrogenated over $Pd(OH)_2$/C (0.1 g) at 60 psi pressure for 12 h. The catalyst was removed by filtration and the residue was washed thoroughly with methanol.

After removal of solvent under high vacuum, the amine was reacted with excess di-*tert*-butyl dicarbonate (1.1 g, 5 mmol). The resulting solution was stirred for 12 h at ambient temperature, concentrated in vacuum and purified by flash column chromatography (EtOAc: Hexane = 15: 75 to 20 : 80) to give compound **34** (0.225 g, 50%) as a viscous oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.44 (s, 54H), 1.64 -1.72 (br m, 10H), 3.0 (t, 18H), 3.23 (t, 4H); ^{13}C NMR (100.6 MHz, $CDCl_3$); 28.4, 37.27, 44.73, 77.37, 79.45, 155.34; MS(ESI) calculated for $C_{45}H_{86}N_6O_{12}$ *m/z* 902.63 found 925.51 $(MNa)^+$.



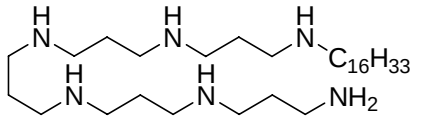
34

Conversion of 34 to 35. A solution of **34** (0.2 g, 0.22 mmol) and 60% sodium hydride (0.42 g, 17.73 mmol) in DMF (10 mL) was cooled to -15 ^{o}C and hexadecyl iodide (0.6 g, 0.22 mmol) was then added to it dropwise under nitrogen atmosphere. The resulting mixture was stirred for another 1 h at -15 ^{o}C and was allowed to stir overnight at room temperature. The reaction mixture was quenched with 10% HCl solution in water, extracted with EtOAc, dried with Na_2SO_4 and concentrated. The residue was purified by flash column chromatography (EtOAc: Hexane = 30 : 70 to 35 : 65) to afford **35** (62 mg, 25%) as a viscous oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.9 (t, 3H), 1.27 (br s, 28H), 1.38 (s, 54H), 1.86 (m, 12H), 3.0 (t, 22H); ^{13}C NMR (100.6 MHz, $CDCl_3$) δ 14.1, 22.7, 24.1, 26.3, 27, 28.4, 29.3, 29.6, 31.9, 44.80, 77.33, 155.38; MS(ESI) calculated for $C_{61}H_{118}N_6O_{12}$ *m/z* 1126.88 found 1149.7 $(MNa)^+$.



35

Conversion of 35 to 36. The resulting product **35** (60 mg, .05 mmol) was dissolved in excess (10 mL) of dry trifluoroacetic acid and stirred at room temperature for 45 min. Excess solvent was removed by purging nitrogen and the residue was thoroughly washed with diethyl ether to obtain white flaky solid **36** (30 mg, 50%). ^{1}H NMR (400 MHz, DMSO-d_6) δ 0.88 (t, J = 6.6Hz, 3H), 1.29-1.38 (s, 25H), 1.55 (br m, 2H) 1.75 (br m, 6 H), 3.0 (t, 12 H); ^{13}C NMR (100.6 MHz, DMSO-d_6) δ 14.1, 22.7, 24.1, 26.3, 27, 27.3, 29.3, 29.6, 30.5, 31.8, 39.4, 45.7, 46, 46.3, 49.9; MS(ESI) calculated for $C_{31}H_{70}N_6$ *m/z* 526.57 found 527.56 $(MH)^+$.



36 $.6CF_3CO_2H$

NF-κB induction Assay: The induction of NF-κB (a key transcriptional activator of the innate immune system) was quantified using HEK-Blue-4™ cells. Stable expression of secreted alkaline phosphatase (sAP) under control of NF-κB/AP-1 promoters is inducible by LPS, and extracellular sAP in the supernatant is proportional to NF-κB induction. HEK- Blue-4™ cells were incubated at a density of ~10^5 cells/ml in a volume of 80 µl/well, in 384-well, flat-bottomed, cell culture-treated microtiter plates until confluency was achieved, and subsequently graded concentrations of stimuli. sAP was assayed spectrophotometrically using an alkaline phosphatase-specific chromogen (present in HEK-detection medium as supplied by the vendor) at 620 nm.