Monodisperse polyethylene glycols “brushes” with enhanced lipophilicity, thermo and plasma stability

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1. General information

Unless otherwise indicated, all reagents were obtained from commercial supplier and used without prior purification. DCM, DMF and THF were dried and freshly distilled prior to use. Flash chromatography was performed on silica gel (200–300 mesh) with MeOH/DCM as eluents. NMR analysis of the prepared compounds were recorded using a Bruker NMR spectrometer, operated at 400 MHz for \(^1\)H and 100 MHz for \(^{13}\)C, using the solvent signal as the internal reference. Chemical shifts are expressed in ppm and coupling constants (\(J\)) are in Hertz (Hz). The splitting patterns for \(^1\)H NMR spectra are denoted as follows: s (singlet), d (doublet), dd (double doublet), t (triplet), q (quartet), and m (multiplet). ESI mass spectra were recorded on a Thermo Scientific Q Exactive Focus mass spectrometer. MALDI-TOF mass spectra were recorded on a Bruker Ultraflex III TOF/TOF spectrometer using the reflection mode for positive ions with \(\alpha\)-cyano-4-hydroxycinnamic acid or 2,5-dihydroxybenzoic acid as matrix.

2. Synthesis of compounds

2,4,8,10-tetraoxa-3,9-dithiaspiro[5.5]undecane 3,3,9,9-tetraoxide 2.

SOCl\(_2\) (26.2 g, 16.0 mL, 220.4 mmol) was slowly added to a stirring suspension of pentaerythritol (10.0 g, 73.5 mmol) in dry THF/DCM (150 mL/150 mL) containing 4 Å MS (10.0 g). After the addition, the reaction mixture was refluxed overnight. The reaction was concentrated under vacuum and the residue was dissolved in EA (250 mL), washed with NaHCO\(_3\) solution (200 mL) and water (200 mL, 2 times). The organic layer was separated and concentrated, the residue was recrystallized from EA/PE to give the spirobi[bicyclic sulfite ester] intermediate. The intermediate was dissolved in a mixture of CH\(_3\)CN (100 mL), CCl\(_4\) (100 mL) and water (150 mL) at 0 °C. NaIO\(_4\) (31.4 g, 146.9 mmol) and RuCl\(_3\)-3H\(_2\)O (0.96 g, 3.7 mmol) were sequentially added to the solution and the resulting mixture was stirred for 2 h. The mixture was filtrated through a pad of celite, and the organic layer was concentrated, purified by flash chromatography on silica gel with EA as eluents to give spirobi[cyclic sulfate ester] 2 (11.5 g, 60% yield) as white solid. \(^1\)H NMR (400 MHz, d\(_{6}\)-acetone) \(\delta\) 5.04 (s, 8H).
Diol 3a. Under an atmosphere of Ar, to a suspension of NaH (5.9 g, 60% in mineral oil, 148.5 mmol) in dry DMF (100 mL) was added a solution of 12 (20.8 g, 99.9 mmol) in dry DMF (100 mL) at 0 ºC. Then a solution of 2 (7.2 g, 27.0 mmol) in dry DMF (50 mL) was added to the mixture after stirring for 2 h. The resulting mixture was stirred overnight at rt. The reaction was neutralized with 2 N HCl and DMF was removed under reduced pressure. The residue was dissolved in THF (300 mL), and H₂O (2.4 mL, 135.0 mmol) was added, the mixture was acidified to pH 3 with concentrated sulfuric acid and stirred at 60 ºC. After the consumption of the sodium sulfate intermediate, the reaction was quenched with saturated NaHCO₃ solution and concentrated under vacuum, the residue was purified by flash chromatography on silica gel with MeOH/DCM (1/20) as eluents to give 3a (12.1 g, 85% yield) as light yellow oil. ¹H NMR (400 MHz, CDCl₃) δ 3.69-3.58 (m, 30H), 3.55 (dd, J = 5.7, 3.5 Hz, 4H), 3.51 (s, 4H), 3.38 (s, 6H). ¹³C NMR (100 MHz, CDCl₃) δ 71.7, 71.5, 70.6, 70.42, 70.39, 70.3, 70.1, 63.8, 58.9, 45.2. HRMS (ESI) calcd for C₂₃H₄₈NaO₁₂+ [M+Na]⁺ 539.3038, found 539.3032.

Diol 3b was prepared from 2 and 13 by following the same procedure for 3a as light yellow oil (13.7 g, 80% yield). ¹H NMR (400 MHz, CDCl₃) δ 3.71-3.61 (m, 64H), 3.60-3.52 (m, 8H), 3.40 (s, 6H). ¹³C NMR (100 MHz, CDCl₃) δ 71.8, 70.6, 70.4, 70.0, 64.1, 58.9, 45.1. HRMS (ESI) calcd for C₃₉H₈₀KO₂₀⁺ [M+K]⁺ 907.4875, found 907.4854.

Diol 3c was prepared from 2 and 14 by following the same procedure for 3a as light yellow oil (12.4 g, 71% yield). ¹H NMR (400 MHz, CDCl₃) δ 3.68-3.63 (m, 84H), 3.62-3.59 (m, 12H), 3.57-3.54 (m, 4H), 3.49 (s, 4H), 3.38 (s, 6H). ¹³C NMR (100 MHz, CDCl₃) δ 71.8, 71.4, 70.6, 70.45, 70.42, 70.38, 70.2, 63.6, 58.9, 45.3. HRMS (ESI) calcd for (C₅₅H₁₁₄O₂₈)₂⁺/2 [(M+2H⁺)/2] 611.3743, found 611.3761.

Cyclic sulfate 4a. A solution of SOCl₂ (6.9 g, 58.4 mmol) in dry DCM (200 mL) was added dropwise into a solution of 3a (12.1 g, 7.4 mL, 23.4 mmol) and Et₃N (9.5 g, 13.0 mL, 93.6 mmol) in dry DCM (350 mL) at 0 ºC, then the resulting mixture was allowed to warm to rt and stirred for another 4 h. The reaction was quenched with water. The organic layer was collected and the aqueous layer was extracted with DCM. The combined organic layer was concentrated. Then the residue was dissolved in a mixture of CH₃CN (300 mL), CCl₄ (300 mL) and water (450
mL) at 0 ºC. NaIO₄ (10.0 g, 46.8 mmol) and RuCl₃·3H₂O (0.31 g, 1.17 mmol) were sequentially added to the solution and the resulting mixture was stirred at 0 ºC overnight. The mixture was filtrated through a pad of celite, and the organic layer was concentrated, purified by flash chromatography on silica gel with MeOH/DCM (1/20) as eluents to give 4a (11.2 g, 83% yield) as light yellow oil. ¹H NMR (400 MHz, CDCl₃) δ 4.68 (s, 4H), 3.72-3.61 (m, 28H), 3.57 (dd, J = 5.5, 3.6 Hz, 8H), 3.40 (s, 6H). ¹³C NMR (100 MHz, CDCl₃) δ 75.3, 71.8, 71.0, 70.54, 70.51, 70.48, 70.4, 70.2, 68.0, 59.0, 39.4. HRMS (ESI) calcd for C₂₃H₄₆NaO₁₄S⁺ [M+Na]⁺ 601.2500, found 601.2491.

Cyclic sulfate 4b was prepared from 3b by following the same procedure for 4a as light yellow oil (11.2 g, 80% yield). ¹H NMR (400 MHz, CDCl₃) δ 4.68 (s, 4H), 3.86-3.48 (m, 68H), 3.41 (s, 6H). ¹³C NMR (100 MHz, CDCl₃) δ 75.3, 71.8, 71.0, 70.6, 70.5, 70.4, 70.2, 68.1, 59.0, 39.5. HRMS (ESI) calcd for C₃₉H₇₈NaO₂₂S⁺ [M+Na]⁺ 953.4598, found 953.4580.

Cyclic sulfate 4c was prepared from 3c by following the same procedure for 4a as light yellow oil (10.2 g, 80% yield). ¹H NMR (400 MHz, CDCl₃) δ 4.66 (s, 4H), 3.69-3.63 (m, 84H), 3.63-3.60 (m, 8H), 3.58-3.53 (m, 8H), 3.39 (s, 6H). ¹³C NMR (100 MHz, CDCl₃) δ 75.2, 71.6, 70.7, 70.3, 70.2, 70.1, 70.0, 67.7, 58.6, 39.2. HRMS (ESI) calcd for (C₅₅H₁₁₂O₃₀S)²⁺ [(M+2H⁺)/2] 642.3474, found 642.3454.

Azide 5a. NaN₃ (1.5 g, 22.4 mmol) was added to a solution of 4a (8.6 g, 14.9 mmol) in DMF (150 mL), and the mixture was stirred overnight at 80 ºC. Excess NaN₃ was removed by filtration through a pad of celite, and DMF was evaporated. The residue was then dissolved in THF (150 mL), then H₂O (1.3 mL, 74.5 mmol) was added. The solution was acidified with concentrated sulfuric acid and stirred at rt for 3 h. The reaction was quenched with saturated NaHCO₃ solution and concentrated under vacuum, the residue was purified by flash chromatography on silica gel with MeOH/DCM (1/20) as eluents to give 5a (7.7 g, 95% yield) as light yellow oil. ¹H NMR (400 MHz, CDCl₃) δ 3.68-3.57 (m, 30H), 3.55 (dd, J = 5.7, 3.6 Hz, 4H), 3.42 (t, J = 6.1 Hz, 6H), 3.37 (s, 6H). ¹³C NMR (100 MHz, CDCl₃) δ 71.8, 70.7, 70.6, 70.43, 70.41, 70.3, 70.2, 62.8, 58.9, 51.4, 45.4. HRMS (ESI) calcd for C₂₃H₄₇N₃NaO₁₁⁺ [M+Na]⁺ 564.3103, found 564.3091.
Azide 5b was prepared from 4b by following the same procedure for 5a as light yellow oil (9.6 g, 90% yield). 1H NMR (400 MHz, CDCl₃) δ 3.71-3.53 (m, 66H), 3.46-3.41 (m, 6H), 3.39 (s, 6H). 13C NMR (100 MHz, CDCl₃) δ 71.8, 70.9, 70.6, 70.44, 70.38, 70.1, 63.7, 58.9, 51.4, 45.2. HRMS (ESI) calcd for C₉₉H₇₉KN₃O₁₉⁺ [M+K]⁺ 932.4939, found 932.4921.

Azide 5c was prepared from 4c by following the same procedure for 5a as light yellow oil (9.5 g, 96% yield). 1H NMR (400 MHz, CDCl₃) δ 3.73-3.52 (m, 98H), 3.45-3.40 (m, 6H), 3.38 (s, 6H). 13C NMR (100 MHz, CDCl₃) δ 71.7, 70.6, 70.5, 70.34, 70.26, 70.0, 62.8, 58.8, 51.4, 45.3. HRMS (ESI) calcd for C₅₅H₁₁₂N₃O₂₇⁺ [M+H⁺] 1246.7478, found 1246.7482.

Ester 6a. Under an atmosphere of Ar, to a suspension of NaH (1.4 g, 60% in mineral oil, 35.5 mmol) in dry THF (100 mL) was added a solution of 5a (7.7 g, 14.2 mmol) in dry THF (100 mL) at 0 ºC. After stirred for 1 h, tert-butyl bromoacetate (6.9 g, 5.2 mL, 35.5 mmol) was added to the reaction, and the resulting mixture was stirred overnight at rt. The reaction was neutralized with 2 N HCl and concentrated, the residue was purified with flash chromatography on silica gel with MeOH/DCM (1/20) as eluents to give 6a (7.2 g, 77% yield) as light yellow oil. 1H NMR (400 MHz, CDCl₃) δ 3.95 (s, 2H), 3.71-3.54 (m, 32H), 3.50 (s, 2H), 3.44 (d, J = 8.4 Hz, 6H), 3.40 (s, 6H), 1.49 (s, 9H). 13C NMR (100 MHz, CDCl₃) δ 169.5, 81.3, 71.8, 70.8, 70.54, 70.5, 70.47, 70.4, 70.2, 69.8, 69.2, 58.9, 51.8, 45.3, 28.0. HRMS (ESI) calcd for C₂₉H₅₇N₃NaO₁₃⁺ [M+Na]⁺ 678.3784, found 678.3774.

Ester 6b was prepared from 5b by following the same procedure for 6a as light yellow oil (7.5 g, 80% yield). 1H NMR (400 MHz, CDCl₃) δ 3.94 (s, 2H), 3.71-3.57 (m, 64H), 3.50 (s, 2H), 3.44 (d, J = 8.2 Hz, 6H), 3.40 (s, 6H), 1.49 (s, 9H). 13C NMR (100 MHz, CDCl₃) δ 169.5, 81.3, 71.8, 70.8, 70.54, 70.48, 70.4, 70.2, 69.8, 69.2, 58.9, 51.8, 45.3, 28.1. HRMS (ESI) calcd for C₄₉H₈₉KN₃O₂₁⁺ [M+K]⁺ 1046.5620, found 1046.5608.

Ester 6c was prepared from 5c by following the same procedure for 6a as light yellow oil (8.3 g, 80% yield). 1H NMR (400 MHz, CDCl₃) δ 3.93 (s, 2H), 3.69-3.54 (m, 96H), 3.48 (s, 2H), 3.43 (s, 4H), 3.41 (s, 2H), 3.38 (s, 6H), 1.47 (s, 9H). 13C NMR (100 MHz, CDCl₃) δ 169.3, 81.0, 71.7, 70.7, 70.3,
70.2, 70.1, 69.6, 69.0, 58.7, 51.6, 45.2, 27.9. HRMS (ESI) calcd for (C_{61}H_{123}N_{3}O_{29})^{2+}/2 [(M+2H^{+})/2] 680.9116, found 680.9117.

Amide 7a. To a solution of 6a (7.1 g, 10.8 mmol) in THF (100 mL) was added triphenylphosphine (8.5 g, 32.3 mmol), and the resulting mixture was stirred for 1 h at 40 °C. Then H_{2}O (1.9 mL, 108.3 mmol) was added and the reaction mixture was stirred overnight at this temperature. The solution was concentrated, the residue was then added H_{2}O (200 mL) and filtrated, the filtrate was washed with Et_{2}O and extracted with H_{2}O. The combined aqueous phase was concentrated and used without further purification. The crude product was dissolved in THF (100 mL) and added a saturated solution of NaHCO_{3} (1.8 g, 21.6 mmol). Then a solution of 9-fluorenylmethyl chloroformate (4.2 g, 16.2 mmol) in THF (30 mL) was slowly added to the mixture at 0 °C. The resulting mixture was allowed to warm to rt and stirred overnight. After the consumption of the amine intermediate, the reaction was concentrated and the residue was purified with flash chromatography on silica gel with MeOH/DCM (1/20) as eluents to give 7a (8.7 g, 95% yield) as light yellow oil. ^{1}H NMR (400 MHz, CDCl_{3}) δ 7.78 (d, J = 7.5 Hz, 2H), 7.70 (d, J = 7.5 Hz, 2H), 7.41 (t, J = 7.4 Hz, 2H), 7.32 (t, J = 7.1 Hz, 2H), 4.36 (d, J = 7.4 Hz, 2H), 4.26 (s, 1H), 3.97 (s, 2H), 3.71-3.50 (m, 34H), 3.47 (t, J = 6.2 Hz, 4H), 3.40 (d, J = 6.5 Hz, 8H), 1.51 (d, J = 9.6 Hz, 9H). ^{13}C NMR (100 MHz, CDCl_{3}) δ 170.4, 157.0, 144.2, 141.2, 127.6, 127.0, 125.3, 119.9, 81.9, 72.0, 71.9, 71.2, 71.0, 70.59, 70.55, 70.51, 70.46, 70.3, 68.6, 66.5, 59.0, 47.3, 44.5, 42.8, 28.2. HRMS (ESI) calcd for C_{44}H_{69}NNaO_{15}^{+} [M+Na]^{+} 874.4559, found 874.4544.

Amide 7b was prepared from 6b by following the same procedure for 7a as light yellow oil (7.2 g, 82% yield). ^{1}H NMR (400 MHz, CDCl_{3}) δ 7.78 (d, J = 7.5 Hz, 2H), 7.69 (d, J = 7.4 Hz, 2H), 7.41 (t, J = 7.4 Hz, 2H), 7.31 (dd, J = 7.4, 6.7 Hz, 2H), 4.35 (d, J = 7.4 Hz, 2H), 4.25 (t, J = 7.4 Hz, 1H), 3.97 (s, 2H), 3.71-3.53 (m, 66H), 3.51 (d, J = 5.8 Hz, 2H), 3.46 (t, J = 6.3 Hz, 4H), 3.39 (s, 6H), 1.50 (s, 9H). ^{13}C NMR (100 MHz, CDCl_{3}) δ 170.4, 157.0, 144.2, 141.2, 127.6, 127.0, 125.3, 119.8, 81.8, 71.92, 71.87, 71.2, 70.9, 70.54, 70.50, 70.49, 70.44, 70.2, 68.6, 66.5, 59.0, 47.3, 44.5, 42.8, 28.1. HRMS (ESI) calcd for C_{60}H_{101}NNaO_{23}^{+} [M+Na]^{+} 1226.6657, found 1226.6646.
Amide 7c was prepared from 6c by following the same procedure for 7a as light yellow oil (7.5 g, 81% yield). \(^1\)H NMR (400 MHz, CDCl\(_3\)) \(\delta\) 7.78 (d, \(J = 7.5\) Hz, 2H), 7.70 (d, \(J = 7.4\) Hz, 2H), 7.41 (t, \(J = 7.4\) Hz, 2H), 7.32 (dd, \(J = 7.4, 6.6\) Hz, 2H), 6.53 (s, 1H), 4.35 (d, \(J = 7.4\) Hz, 2H), 4.26 (d, \(J = 7.2\) Hz, 1H), 3.97 (s, 2H), 3.71-3.54 (m, 9H), 3.51 (d, \(J = 5.8\) Hz, 2H), 3.47 (t, \(J = 6.3\) Hz, 4H), 3.40 (s, 6H), 1.52 (s, 9H).

\(^{13}\)C NMR (100 MHz, CDCl\(_3\)) \(\delta\) 170.4, 157.0, 144.1, 141.2, 127.5, 127.0, 125.2, 119.8, 81.8, 71.8, 71.0, 70.9, 70.44, 70.38, 70.2, 68.6, 66.4, 58.9, 47.3, 44.5, 42.7, 28.1. HRMS (ESI) calcd for (C\(_{76}\)H\(_{135}\)NO\(_3\))\(^{2+}\)/2 [(M+2H\(^{+}\))/2] 778.9504, found 778.9492.

Branched M-PEGs amino acid 8a. A solution of 7a (4.8 g, 5.6 mmol), anisole (0.91 g, 0.92 mL, 8.5 mmol) and TFA (19.3 g, 12.5 mL, 169.0 mmol) in dry DCM (100 mL) was stirred overnight at 30 °C. The reaction was then concentrated, the residue was dissolved in water and washed with Et\(_2\)O, then the water phase was extracted with DCM (200 mL, 3 times). The combined organic layer was dried over anhydrous Na\(_2\)SO\(_4\), concentrated under vacuum to give 8a (4.5 g, 99% yield) as light yellow oil. \(^1\)H NMR (400 MHz, CDCl\(_3\)) \(\delta\) 7.77 (d, \(J = 7.5\) Hz, 2H), 7.63 (d, \(J = 7.4\) Hz, 2H), 7.40 (t, \(J = 7.3\) Hz, 2H), 7.32 (t, \(J = 7.3\) Hz, 2H), 4.42 (d, \(J = 6.8\) Hz, 2H), 4.22 (s, 1H), 4.04 (s, 2H), 3.70-3.50 (m, 32H), 3.46 (s, 2H), 3.42-3.34 (m, 10H), 3.30 (d, \(J = 6.3\) Hz, 2H). \(^{13}\)C NMR (100 MHz, CDCl\(_3\)) \(\delta\) 173.0, 157.2, 144.0, 141.2, 127.6, 127.0, 125.2, 119.9, 71.8, 71.6, 71.1, 70.8, 70.49, 70.47, 70.4, 70.3, 70.1, 66.4, 58.9, 47.3, 44.5, 42.6. HRMS (ESI) calcd for C\(_{40}\)H\(_{61}\)NNaO\(_{15}\)\(^{+}\) [M+Na\(^+\)] 818.3933, found 818.3921.

Branched M-PEGs amino acid 8b was prepared from 7b by following the same procedure for 8a as light yellow oil (6.8 g, 99% yield). \(^1\)H NMR (400 MHz, CDCl\(_3\)) \(\delta\) 7.77 (d, \(J = 7.5\) Hz, 2H), 7.63 (d, \(J = 7.4\) Hz, 2H), 7.40 (t, \(J = 7.4\) Hz, 2H), 7.32 (t, \(J = 7.3\) Hz, 2H), 4.42 (d, \(J = 6.8\) Hz, 2H), 4.22 (s, 1H), 4.04 (s, 2H), 3.70-3.50 (m, 32H), 3.46 (s, 2H), 3.42-3.34 (m, 10H), 3.30 (d, \(J = 6.3\) Hz, 2H). \(^{13}\)C NMR (100 MHz, CDCl\(_3\)) \(\delta\) 171.9, 157.2, 144.0, 141.2, 127.6, 127.0, 125.2, 119.9, 71.8, 71.6, 71.1, 70.8, 70.49, 70.47, 70.4, 70.3, 70.1, 66.4, 58.9, 47.3, 44.5, 42.6. HRMS (ESI) calcd for C\(_{56}\)H\(_{93}\)NNaO\(_{23}\)\(^{+}\) [M+Na\(^+\)] 1170.6031, found 1170.5995.

Branched M-PEGs amino acid 8c was prepared from 7c by following the same procedure for 8a as light yellow oil (6.5 g, 99% yield). \(^1\)H NMR (400 MHz, CDCl\(_3\)) \(\delta\) 7.76 (d, \(J = 7.5\) Hz, 2H), 7.63 (d, \(J = 7.4\) Hz, 2H), 7.40 (t, \(J = 7.4\) Hz, 2H), 7.32 (t, \(J = 7.4\) Hz, 2H), 4.42 (d, \(J = 6.8\) Hz, 2H), 4.22 (t, \(J = 6.7\) Hz, 1H), 4.06 (s, 2H), 3.71–3.57 (m, 60H), 3.56 (dd, \(J = 5.7, 3.5\) Hz, 4H), 3.48 (s, 2H), 3.41 (s, 4H), 3.39 (s, 6H), 3.31 (d, \(J = 6.3\) Hz, 2H). \(^{13}\)C NMR (100 MHz, CDCl\(_3\)) \(\delta\) 171.9, 157.2, 144.0, 141.3, 127.6, 127.0, 125.1, 119.9, 71.9, 71.6, 71.2, 70.8, 70.6, 70.51, 70.48, 70.46, 70.4, 70.1, 68.6, 66.3, 59.0, 47.4, 44.5, 42.6. HRMS (ESI) calcd for C\(_{56}\)H\(_{93}\)NNaO\(_{23}\)\(^{+}\) [M+Na\(^+\)] 1170.6031, found 1170.5995.
= 7.4 Hz, 2H), 7.39 (t, J = 7.4 Hz, 2H), 7.32 (dd, J = 10.7, 4.1 Hz, 2H), 6.21 (s, 1H), 4.41 (d, J = 6.9 Hz, 2H), 4.22 (t, J = 6.8 Hz, 1H), 4.04 (s, 2H), 3.75-3.51 (m, 96H), 3.47 (s, 2H), 3.40 (s, 4H), 3.38 (s, 6H), 3.30 (d, J = 6.3 Hz, 2H).

$^{13}$C NMR (100 MHz, CDCl$_3$) $\delta$ 172.0, 157.1, 144.0, 141.2, 127.6, 127.0, 125.2, 119.9, 71.8, 71.6, 71.1, 70.8, 70.5, 70.1, 68.4, 66.3, 59.0, 47.3, 44.5, 42.6. HRMS (ESI) caled for (C$_{72}$H$_{127}$NO$_3$)$_{2+}$/2 [(M+2H$^+$)/2] 750.9190, found 750.9190.

Linear M-PEGs amino acid 8d was prepared as reported in the literature$^{[1]}$. $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 7.78 (d, J = 7.5 Hz, 2H), 7.64 (d, J = 7.4 Hz, 2H), 7.42 (t, J = 7.5 Hz, 2H), 7.34 (dd, J = 7.9, 7.0 Hz, 2H), 4.42 (d, J = 7.0 Hz, 2H), 4.25 (t, J = 6.8 Hz, 1H), 4.17 (s, 2H), 3.78-3.57 (m, 28H), 3.56-3.31 (m, 4H).

Monomethylated PEG$_4$ 12. Under an atmosphere of Ar, a solution of macrocyclic sulfate 10 (45.0 g, 175.6 mmol) in dry DMF (350 mL) was added to the suspension of sodium methanolate (14.2 g, 263.4 mmol) in dry DMF (100 mL), the resulting mixture was stirred at rt overnight. DMF was evaporated under vacuum, the residue was then dissolved in THF (400 mL), then H$_2$O (9.5 mL, 526.8 mmol) was added. The solution was acidified with concentrated sulfuric acid and stirred at rt for 3 h. The reaction was quenched with saturated NaHCO$_3$ solution and concentrated under vacuum, the residue was purified by flash chromatography on silica gel with MeOH/DCM (1/20) as eluents to give 12 (34.2 g, 94% yield) as light yellow oil. $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 3.77-3.72 (m, 2H), 3.72-3.60 (m, 12H), 3.60-3.55 (m, 2H), 3.40 (s, 3H).

Monomethylated PEG$_8$ 13. Under an atmosphere of Ar, to a suspension of NaH (11.1 g, 60% in mineral oil, 278.5 mmol) in dry THF (100 mL) was added a solution of 12 (29.0 g, 139.3 mmol) in dry THF (150 mL) at 0 ºC. After stirred for 30 min, a solution of macrocyclic sulfate 10 (53.5 g, 208.9 mmol) in dry THF (200 mL) was added to the reaction, and the resulting mixture was stirred overnight at rt. Then, H$_2$O (7.5 mL, 417.8 mmol) was added. The solution was acidified with concentrated sulfuric acid and stirred at rt for 3 h. The reaction was quenched with saturated NaHCO$_3$ solution and concentrated under vacuum, the residue was purified with flash chromatography on silica gel with MeOH/DCM (1/20) as eluents to give 13 (49.6 g, 93% yield) as light yellow oil. $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 3.78-3.60 (m, 30H), 3.59-3.53 (m, 2H), 3.39 (s,
Monomethylated PEG₁₂ 14 was prepared from 13 by following the same procedure for 12 as light yellow oil (30.0 g, 93% yield) as light yellow oil. ¹H NMR (400 MHz, CDCl₃) δ 3.76-3.58 (m, 46H), 3.57-3.51 (m, 2H), 3.38 (s, 3H).

General procedure for peptide synthesis
All peptides were synthesized using the Fmoc-strategy manually in a sintered glass reaction funnel fitted with a three-way stopcock under a positive pressure of nitrogen gas using dry solvents. Rink amide-AM resin was used. Fmoc-protected amino acids (2.5 eq, relative to the resin loading) and HATU (2.4 eq) (or TBTU (2.4 eq) and HOBt (2.5 eq)) were dissolved in a minimum amount of DMF, DIPEA (5.0 eq) was then added and mixed thoroughly. The solution was added immediately to the resin, and agitated for 2-4 h at rt. For each coupling step, a double coupling was carried out. A negative TNBS test was used to confirm reaction completion. Once the coupling reaction was completed, the resin was drained under vacuum and washed with DCM/DMF several times. Then the resin was subjected to Fmoc removal. The N-Fmoc protecting group was removed by treating the resin with DBU/piperidine/DMF (1/1/48 v/v) (8 min, 4 times). Once the desired peptide was generated, the final Fmoc protecting group was removed and the activated 1-naphthoic acid was coupled to the peptide following the coupling procedure. The resin was then washed thoroughly, and the desired peptide was cleaved using TFA/TES/DCM (10/1/10 v/v) (40 min, 5 times). All peptides were purified using semi-preparative HPLC with UV detection at 254 nm using a RP C18 column (10 μm; 30 × 250 mm), the mobile phase consist of water (Mobile phase A) and acetonitrile (Mobile phase B) at a flow rate of 10 mL/min, starting at 80% mobile phase A and 20% mobile phase B.

Peptide 15. ¹H NMR (400 MHz, D₂O) δ 8.25 (s, 2H), 7.98-7.85 (m, 5H), 7.75-7.70 (m, 2H), 7.58-7.51 (m, 3H), 3.93 (s, 3H), 3.85-3.77 (m, 8H), 3.73-2.92 (m, 230H). MS (MALDI) m/z calcd for C₁₃₆H₂₅₄N₆NaO₆₁+ [(M+Na)]⁺ 2970.7, found 2970.8.
Peptide 16.  $^1$H NMR (400 MHz, D$_2$O) $\delta$ 8.27 (s, 2H), 8.03-7.91 (m, 3H), 7.90-7.84 (m, 2H), 7.74 (d, $J = 7.7$ Hz, 2H), 7.61-7.50 (m, 3H), 3.92 (s, 2H), 3.87-3.76 (m, 8H), 3.74-2.98 (m, 3H). MS (MALDI) m/z calcd for $C_{216}H_{414}N_6O_{101}^+[(M+Na)]^+$ 4731.7, found 4730.0.

Peptide 17.  $^1$H NMR (400 MHz, D$_2$O) $\delta$ 8.27 (s, 2H), 7.99-7.91 (m, 3H), 7.90-7.85 (m, 2H), 7.78-7.71 (m, 2H), 7.59-7.51 (m, 3H), 3.92 (s, 3H), 3.89-3.02 (m, 5H). MS (MALDI) m/z calcd for $C_{296}H_{574}N_6O_{141}^+[(M+Na)]^+$ 6492.8, found 6492.0.

Peptide 18.  $^1$H NMR (400 MHz, D$_2$O) $\delta$ 8.11 (s, 1H), 7.78-7.67 (m, 3H), 7.62 (d, $J = 8.2$ Hz, 1H), 7.49-7.37 (m, 2H), 7.30-6.97 (m, 25H), 3.90-3.29 (m, 17H), 3.29-2.68 (m, 8H). MS (MALDI) m/z calcd for $C_{181}H_{303}N_{12}O_{66}^+[(M+NH_4)]^+$ 3701.1, found 3699.0.

Peptide 19.  $^1$H NMR (400 MHz, D$_2$O) $\delta$ 8.16 (s, 1H), 7.89-7.78 (m, 3H), 7.66 (d, $J = 8.9$ Hz, 1H), 7.58-7.47 (m, 2H), 7.32-7.03 (m, 25H), 3.87-3.31 (m, 4H), 3.29-2.81 (m, 8H). MS (MALDI) m/z calcd for $C_{341}H_{619}N_{11}O_{146}^+[(M+Na)]^+$ 7228.1, found 7227.8.

Peptide 20.  $^1$H NMR (400 MHz, D$_2$O) $\delta$ 8.33 (s, 1H), 7.98-7.91 (m, 3H), 7.78 (d, $J = 8.1$ Hz, 1H), 7.62-7.55 (m, 2H), 4.65-2.30 (m, 56H), 1.82-1.11 (m, 40H). MS (MALDI) m/z calcd for $C_{326}H_{634}N_{16}O_{146}^+[(M+Na)]^+$ 7133.3, found 7136.0.

Peptide 21.  $^1$H NMR (400 MHz, d$_6$-DMSO) $\delta$ 8.18 (t, $J = 5.2$ Hz, 2H), 7.72-7.54 (m, 3H), 7.38-7.09 (m, 2H), 4.35-4.25 (m, 2H), 3.90 (s, 5H), 3.81 (s, 2H), 3.73-3.11 (m, 18H), 2.86-2.66 (m, 6H), 2.15-1.00 (m, 20H). MS (MALDI) m/z calcd for $C_{131}H_{244}N_{16}O_{51}^+[(M+Na)]^+$ 2880.7, found 2880.7.

Peptide 22.  $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 8.09-7.72 (m, 7H), 7.69-7.43 (m, 10H), 4.68-4.48 (m, 5H), 4.31-3.25 (m, 17H), 2.60-2.30 (m, 10H), 2.29-2.06 (m, 6H), 1.98 (dd, $J = 14.5$, 6.3 Hz, 4H). MS (MALDI) m/z calcd for $C_{126}H_{219}N_{11}O_{61}^+[(M+Na)]^+$ 2885.3, found 2885.4.

Peptide 23.  $^1$H NMR (400 MHz, CDCl$_3$) $\delta$ 9.70-9.38 (m, 5H), 7.94-7.78 (m, 4H), 7.73-7.31 (m, 16H), 7.22-6.98 (m, 13H), 6.81 (s, 4H), 4.98-4.60 (m, 5H), 4.11-3.85 (m, 10H), 3.83-2.98 (m, 17H). MS
(MALDI) m/z calcd for C_{156}H_{234}N_{16}NaO_{51}^{+} [(M+Na)]^{+} 3170.6, found 3169.5.

Peptide 24. $^1$H NMR (400 MHz, CDCl$_3$) δ 8.49-8.03 (m, 9H), 8.02-7.68 (m, 8H), 7.66-7.08 (m, 25H), 5.16-4.53 (m, 5H), 4.34-2.56 (m, 180H). MS (MALDI) m/z calcd for C_{146}H_{229}N_{11}NaO_{51}^{+} [(M+Na)]^{+} 2975.6, found 2975.8.


All chromatographic separations were performed on RP C$_{18}$ column at 37 °C, using either a
linear gradient (20–100%) of solvent B in solvent A over 15 min (solvent A = water, solvent B = acetonitrile) at a flow rate of 1.0 mLmin\(^{-1}\) for peptides 15-19, 23-24, or a linear gradient (20–100%) of solvent B in solvent A over 20 min (solvent A = 0.1% TFA in water, solvent B = 0.1% TFA in acetonitrile) at a flow rate of 1.0 mLmin\(^{-1}\) for peptides 20-22, with detection by UV absorption at 254 nm.

Figure S1 HPLC of M-PEGs peptides 15-24

4. Biocompatibility study

The biocompatibility studies of peptides were investigated in L929 cell lines in vitro by
MTT assay. Briefly, L929 cells were seeded into a 96-well plate at a density of 8.0 x 10^4 cells/well in 100 μL of alpha-MEM containing 10% FBS and 1% streptomycin double antibody. The cells were cultured for 1 day at 37 °C in 5% CO₂ atmosphere. Afterwards, the cells were incubated with peptides 13-22 respectively for 24 h at 37 °C. The concentration of peptides ranged from 3.1 μM to 400 μM. Cells treated with only media were used as control. After incubation, MTT stock solution (1 mg/mL in PBS, 20 μL) was added to each well and incubated for another 4 h. The media were replaced by 100 μL DMSO to dissolve the formazan blue crystal. The relative cell viability (%) was determined spectrophotometrically by comparing the absorbance of each well at 490 nm with control well using a microplate reader (Bio Tek Instruments, USA).

5. Determination of n-Octanol/Water partition coefficients (logP).

The logP values of peptides 15-24 (except peptide 23 which was too hydrophobic to be detected from the aqueous phase) were measured following shake-flask method. Briefly, the peptide was dissolved in distilled water saturated with n-octanol. Then 1 mL of this solution was mixed with an equal volume of n-octanol saturated with distilled water and mixed on a vortex device. After shaking the mixture overnight, n-octanol phase was separated by centrifugation. Equal-volume samples of the shaken water phase and the starting solution were subsequently taken and analyzed by HPLC. The peak area was measured at λ = 254 nm, and compared with calibration curve to obtain the concentration of the peptide. LogP values were determined from: Lg[(C_s-C_w)/C_w], where C_s and C_w are the concentrations of the starting water solution and the water phase of the compound, respectively.

6. DLS data.

The size distribution and zeta potential of peptides 18 and 24 in water (0.3 mg/mL) were
determined by Dynamic Light Scattering (DLS) using Zetasizer Nano 3690 at 25 °C. The scattering angle was 90°.

Figure S2 DLS data of peptide 18 (0.3 mg/mL) in water with average particle size of 208.4 nm.

Figure S3 DLS data of peptide 24 (0.3 mg/mL) in water with average particle size of 212.3 nm.

7. Transmission Electron Microscopy
M-PEGs 18 and 24 were dissolved in water (0.3 mg/mL) respectively, and prepared on carbon-
coated copper grids for TEM. 5 μL of the sample solution was drop cast on the grid and blotted after 60 seconds. Then the grids were stained with 1% (wt/vol) uranyl acetate solution, and were air-dried overnight. TEM images were taken on a JEM-2100 at an acceleration voltage of 200 kV.

Figure S4 TEM images of M-PEGs (a) 18 and (b) 24.

8. LCST determination.

The turbidity measurements were recorded using a UV-visible Lambda 35 spectrometer (Perkin Elmer, USA) at 700 nm. Peptides 18 and 24 were dissolved in water (3.0 mM and 1.2 mM). The transmittance was measured between 29 °C and 64 °C through temperature-controlled heating and cooling cycles, and the sample was equilibrated for 10 min at each temperature before measurement.

<table>
<thead>
<tr>
<th>peptides</th>
<th>LCST (°C) (3.0 mM)</th>
<th>LCST (°C) (1.2 mM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>18</td>
<td>56</td>
<td>60</td>
</tr>
<tr>
<td>24</td>
<td>34</td>
<td>44</td>
</tr>
</tbody>
</table>


Adult male SD rat (180 g - 230 g) was anesthetized with amobarbital sodium and whole blood was collected by orbital bleeding. Rat plasma was obtained by centrifugation of rat whole blood. All animal procedures were approved by Wuhan University Animal Ethics Committee. The in vitro stability studies of peptides 15, 17, 18 and 24 were conducted in SD rat plasma. In brief, 2.0 mg of peptide was incubated with 600 μL of rat plasma at 37 °C for 72 h with gentle stirring. After 1, 3, 6, 12, 24, 36, 48, 60 and 72 h, a sample (20 μL) was collected and mixed with 1.0 mL methanol. The
mixture was filtered and analyzed by HPLC. The integrated values of these peaks were compared to those of $t = 0$ min in plasma for each peptide and expressed as a fraction of the initial compound that was remaining at the given time point.

Reference

$\text{H} - \text{OCH}_2\text{CH}_2\text{OMe}$

$\text{H} - \text{OCH}_2\text{CH}_2\text{OMe}$

$^1\text{H NMR (CDCl}_3, 400\text{ MHz)}$
$^1\text{H NMR (CDCl}_3, 400 \text{ MHz)}$
$^{1}H$ NMR (CDCl$_3$, 400 MHz)
$^1$H NMR (CDCl$_3$, 400 MHz)
Peptide 15

$^1$H NMR (D$_2$O, 400 MHz)
Peptide 16
$^1$H NMR (D$_2$O, 400 MHz)
Peptide 17

$^1$H NMR (D$_2$O, 400 MHz)

MALDI-TOF, CCA, 2, 20180510
Peptide 18

$^1$H NMR (D$_2$O, 400 MHz)

MALDI-TOF, CCA, 3, 20180510
Peptide 19

$^1$H NMR (D$_2$O, 400 MHz)

MALDI-TOF, CCA, 2, 20180718
Peptide 20

$^1$H NMR (D$_2$O, 400 MHz)

MALDI-TOF, CCA, 4, 20180510
Peptide 21

$^1$H NMR (d$_6$-DMSO, 400 MHz)
Peptide 22

$^1$H NMR (CDCl$_3$, 400 MHz)
Peptide 23

$^1$H NMR (CDCl$_3$, 400 MHz)
Peptide 24

$^1$H NMR (CDCl$_3$, 400 MHz)