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## **Accessory Publication**

# **Synthesis of MUC1 Peptide and Glycopeptide Dendrimers**

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## General materials and methods

Analytical reverse-phase HPLC was performed on a Waters System 2695 separations module with an Alliance series column heater at 30 °C and 2996 photodiode array detector. A Waters Sunfire 5  $\mu\text{m}$ , 2.1  $\times$  150 mm column was used at a flow rate of 0.2 mL min<sup>-1</sup> and results analysed with Waters Empower software. Preparative reverse-phase HPLC was performed using a Waters 600 Multisolvant Delivery System and Waters 500 pump with 2996 photodiode array detector or Waters 490E Programmable wavelength detector operating at 214, 230, 254 or 280 nm. A Waters Sunfire Prep C18 OBD, 19  $\times$  150 mm, 5  $\mu\text{m}$  particle size column was used at a flow rate of 7 mL min<sup>-1</sup> using a mobile phase of 0.1% TFA in water (Solvent A) and 0.1% TFA in acetonitrile (Solvent B). Semi-preparative HPLC was used to purify the final dendrimer products. Purifications were performed on a Waters 600 Multisolvant Delivery System and Waters 500 pump with 2996 photodiode array detector or Waters 490E Programmable wavelength detector operating at 230, 254 or 280 nm. A Waters Sunfire Prep C18, 250  $\times$  10 mm, 5  $\mu\text{m}$  particle size column was used at a flow rate of 3 mL min<sup>-1</sup> using a mobile phase of 0.1% TFA in water (Solvent A) and 0.1% TFA in acetonitrile (Solvent B) for the purification of **13**, **24** and **25**. A Grace Vydac "Protein and Peptide C18", 250  $\times$  10 mm, 10-15  $\mu\text{m}$  particle size column was used at a flow rate of 4 mL min<sup>-1</sup> using a mobile phase of 0.1% TFA in water (Solvent A) and 0.1% TFA in acetonitrile (Solvent B) for the purification of **28**.

LC-MS was performed on a Thermo Separation Products: Spectra System consisting of P400 Pump and a UV6000LP Photodiode array detector on a Phenomenex Luna C18(2) 5  $\mu\text{m}$ , 2.1  $\times$  150 mm column at a flow rate of 0.2 mL min<sup>-1</sup> coupled to a Thermoquest Finnigan LCQ Deca mass spectrometer (ESI) operating in positive mode. Separations involved a mobile phase of 0.1% formic acid in water (Solvent A) and 0.1% formic acid in acetonitrile (Solvent B).

ESI mass spectrometry was performed on a Thermoquest Finnigan LCQ Deca mass spectrometer operating in positive mode. MALDI-Tof mass spectrometry was performed on a Applied Biosystems Voyager DE-STR MALDI-Tof operating in linear mode. A 10 mgmL<sup>-1</sup> solution of  $\alpha$ -cyano-4-hydroxy cinnamic acid containing 1:1 v/v acetonitrile + 0.1% TFA: water + 0.1% TFA was used for generating the

probe-matrix mixture. High resolution mass spectra were recorded on a Bruker 7T Fourier Transform Ion Cyclotron Resonance Mass Spectrometer (FTICR).

Commercial materials were used as received unless otherwise noted. Amino acids, coupling reagents and resins were obtained from Novabiochem. DCM and methanol were distilled from calcium hydride. DMF was obtained as peptide synthesis grade from Auspep or Labscan.

Solid-phase peptide synthesis was carried out in syringes, equipped with teflon filters, purchased from Torviq.

### **Pre-loading Wang resin**

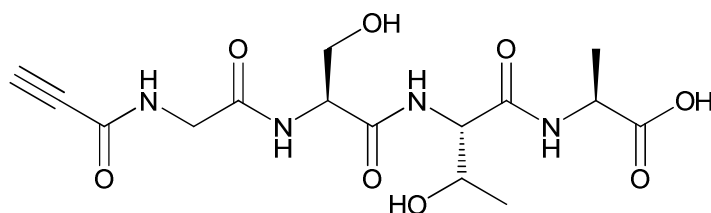
Wang resin was swelled in DMF for 30 min before use. Fmoc-Ala-OH or Fmoc-His(Trt)-OH (8 eq.) was dissolved in anhydrous DCM (final concentration 0.1 M) and cooled to 0 °C. *N,N*-Diisopropylcarbodiimide (4 eq.) was added dropwise and the reaction stirred for 30 min at rt. The reaction mixture was concentrated *in vacuo* and dissolved in DMF (final concentration 0.1 M) containing DMAP (0.1 eq.). This was added immediately to the pre-swelled Wang resin and placed on a shaker for 30 min. The resin was washed with DMF (× 5), DCM (× 5) and DMF (× 5) before capping with acetic anhydride/pyridine solution 1:9 v/v followed by washing with DMF (× 5), DCM (× 5) and DMF (× 5). Resin loading was shown to be quantitative, as determined by deprotecting with 10% piperidine in DMF (2 × 3 min) and measuring the absorbance of piperidine-fulvene adduct at  $\lambda = 301$  nm.

**Iterative peptide assembly (Fmoc-strategy):** *Deprotection:* The resin was treated with 10% piperidine/DMF (2 × 3 min) and washed with DMF (× 5), DCM (× 5) and DMF (× 5). *Amino acid coupling:* A pre-activated solution of protected amino acid (4 eq.), PyBOP (4 eq.) and NMM (8 eq.) in DMF (final concentration 0.1 M) was added to the resin. After shaking for 1 h, the resin was washed with DMF (× 5), DCM (× 5) and DMF (× 5). *Glycosylamino acid coupling:* A pre-activated solution of protected glycosylamino acid **19** or **20** (1.2 eq.), PyBOP (1.2 eq.) and NMM (2.4 eq.) in DMF (final concentration 0.1 M) was added to the resin. After shaking for 16 h, the resin was washed with DMF (× 5), DCM (× 5) and DMF (× 5). *Capping:* Acetic

anhydride/pyridine (1:9 v/v) was added to the resin. After shaking for 3 min the resin was washed with DMF ( $\times 5$ ), DCM ( $\times 5$ ) and DMF ( $\times 5$ ). *Azidoglycine coupling*: A pre-activated solution of azidoglycine **15** (4 eq.), PyBOP (4 eq.) and NMM (8 eq.) in DMF (final concentration 0.1 M) was added to the resin. After shaking for 1 h, the resin was washed with DMF ( $\times 5$ ) and DCM ( $\times 10$ ) and the capping step omitted. *Cleavage*: A mixture of TFA, triisopropylsilane (TIS) and water (90:5:5 v/v/v) was added to the resin. After shaking for 1.5 h, the resin was washed with TFA ( $3 \times 2$  mL) *Work-up*: The combined cleavage solution and TFA washings were concentrated *in vacuo*. The residue was dissolved in water containing 0.1% TFA and purified by preparative HPLC (Gradient 0 to 25% B over 60 min).

Novel compounds are denoted in *italics*.

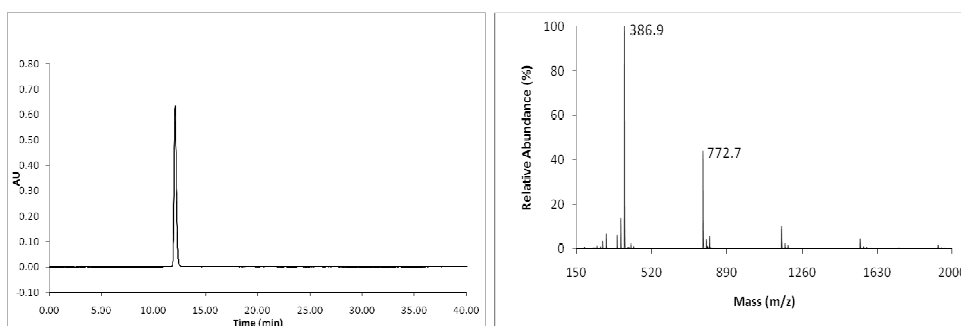
#### Synthesis of *alkynyl-tetrapeptide 9*.



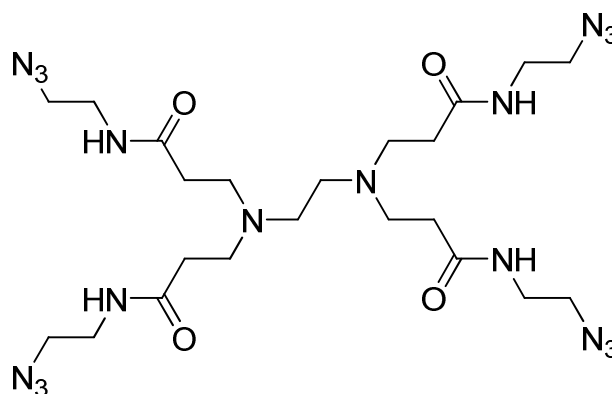
Wang resin was pre-loaded with Fmoc-Ala-OH and the tetrapeptide assembled using the iterative peptide assembly protocol described above. *Propiolic acid coupling*: A pre-activated solution of propiolic acid (8 eq.), EEDQ (8 eq.) in DMF (final concentration 0.1 M) was added to the resin. After shaking for 1 h, the resin was washed with DMF ( $\times 5$ ) and DCM ( $\times 10$ ) and the capping step omitted. *Cleavage*: A mixture of TFA, triisopropylsilane (TIS) and water (90:5:5 v/v/v) was added to the resin. After shaking for 1.5 h, the resin was washed with TFA ( $3 \times 2$  mL) *Work-up*: The combined cleavage solution and TFA washings were concentrated *in vacuo*. The residue was dissolved in water containing 0.1% TFA and purified by preparative HPLC (Gradient 0 to 25% B over 60 min) to afford the desired *alkynyl-tetrapeptide 12* as a white solid.

25  $\mu$ M scale, Yield = 7.1 mg, 73%, yield based on the Fmoc loading of the penultimate glycine residue.

$\nu_{\max}/\text{cm}^{-1}$  3284, 2111, 1671, 1632, 1556; ESI ( $m/z$ ):  $[M+H]^+$ : 386.9,  $[2M+H]^+$ : 772.7; HPLC:  $t_R$ : 12.0 min (Gradient 0 to 25% B over 40 min);  $^1\text{H}$  NMR (MeOD, 400 MHz): 4.50 (1H, app. t,  $J$  5.7 Hz, CH), 4.43-4.35 (2H, m,  $2 \times$  CH), 4.25 (1H, m, CH), 3.97 (2H, s, CH<sub>2</sub>), 3.89 (1H, dd,  $J$  5.4, 10.9 Hz, CHH), 3.78 (1H, dd,  $J$  6.1, 10.9 Hz, CHH), 3.66 (1H, s,  $\equiv$ -H), 1.41 (3H, d,  $J$  7.3 Hz, CH<sub>3</sub>), 1.21 (3H, d,  $J$  6.4 Hz, CH<sub>3</sub>);  $^{13}\text{C}$  NMR (MeOD, 100 MHz): 172.7, 172.2, 170.0, 169.9, 155.1, 77.8, 76.6, 68.1, 62.9, 60.1, 56.8, 43.5, 20.3, 17.7, one undetected double up; HRMS calcd for C<sub>15</sub>H<sub>22</sub>N<sub>4</sub>O<sub>8</sub>Na:  $[M+Na]^+$ , 409.13299. Found:  $[M+Na]^+$ , 409.13290.



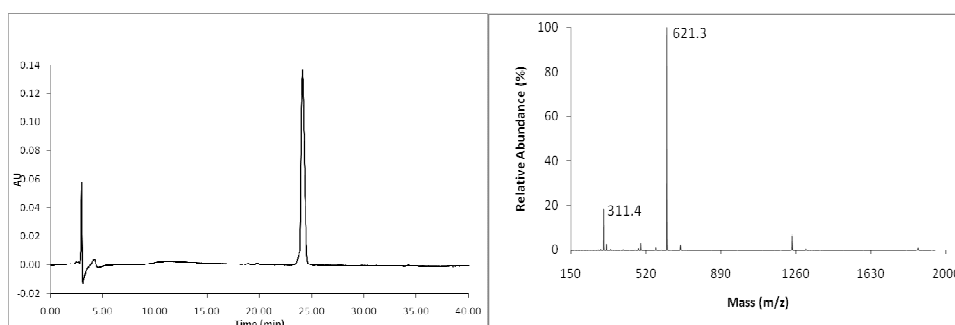
### Synthesis of azido-PAMAM dendrimer core 12



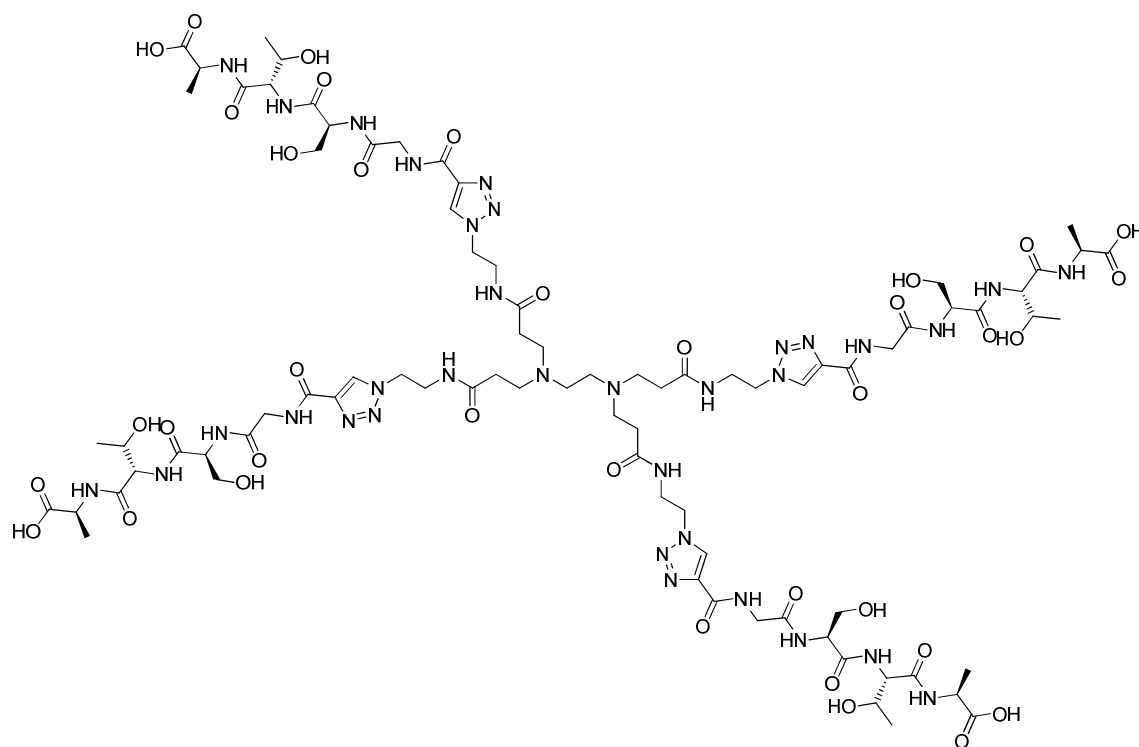
PAMAM dendrimer, ethylenediamine core, generation 0.0 (1.0 mL of a 20 wt. % solution in methanol, 0.4 mmol) was added to a stirred suspension of potassium carbonate (0.40 g, 3.1 mmol) and copper sulfate pentahydrate (40 mg, 0.2 mmol) in methanol (1 mL). Imidazole-1-sulfonyl azide hydrochloride salt (**11**)<sup>[2]</sup> (0.4 g, 1.9 mmol) was then added and the reaction stirred at rt for 1 h. The insoluble potassium salts were filtered and the filter washed with methanol. The filtrate was concentrated

*in vacuo* and the residue purified by preparative HPLC (Gradient 0 to 50% B over 60 min) to afford the desired *dendrimer core* **12** as a yellow oil. (50 mg, 25%).

$\nu_{\max}/\text{cm}^{-1}$  3280, 3048, 2941, 2098, 1651, 1556; ESI (m/z):  $[\text{M}+\text{H}]^+$ : 621.3,  $[\text{M}+2\text{H}]^{2+}$ : 311.4; HPLC:  $t_{\text{R}}$ : 24.1 min (Gradient 0 to 50% B over 40 min);  $^1\text{H}$  NMR (MeOD, 400 MHz): 3.31 (16H, m,  $8 \times \text{CH}_2$ ), 3.18 (4H, br. s,  $2 \times \text{CH}_2$ ), 3.14 (8H, t,  $J$  6.6 Hz,  $4 \times \text{CH}_2$ ), 2.57 (8H, t,  $J$  6.5 Hz,  $4 \times \text{CH}_2$ );  $^{13}\text{C}$  NMR (MeOD, 100 MHz): 173.3, 51.5, 50.3, 50.0, 40.0, 31.3; HRMS calcd for  $\text{C}_{22}\text{H}_{41}\text{N}_{18}\text{O}_4$ :  $[\text{M}+\text{H}]^+$ , 621.3553. Found:  $[\text{M}+\text{H}]^+$ , 621.3554.



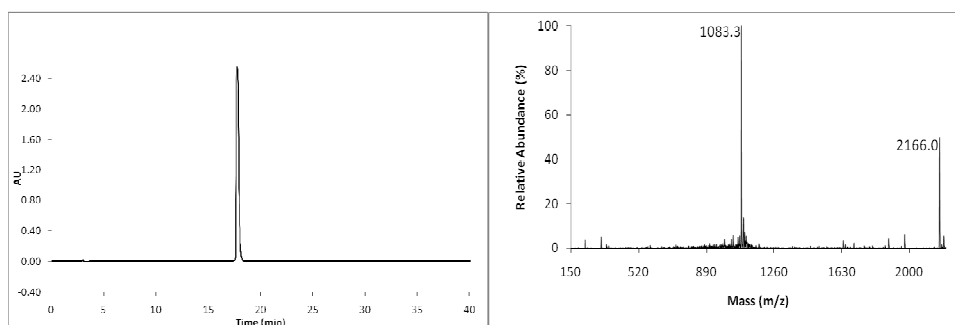
### Synthesis of *peptide dendrimer* **13**



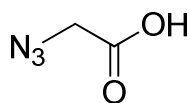
Azido-dendrimer core **12** (1 mg, 1.6  $\mu\text{mol}$ , 1 eq.) and alkynyl-tetrapeptide **9** (3.7 mg, 9.6  $\mu\text{mol}$ , 6 eq.) were dissolved in *t*-butanol/water 1:1 (v/v) (100  $\mu\text{L}$ ). Aqueous copper sulfate pentahydrate (1.6  $\mu\text{L}$  of 0.1 M solution, 0.1  $\mu\text{mol}$ , 0.1 eq.) and aqueous

sodium ascorbate (1.6  $\mu\text{L}$  of 1 M solution, 0.1  $\mu\text{mol}$ , 1 eq.) were added sequentially and stirred at rt for 20 h. The product was purified by semi-preparative HPLC (Gradient 0 to 25% B over 60 min) to afford the desired *peptide dendrimer 13* as a white solid.

Yield = 2.6 mg, 75%;  $\nu_{\text{max}}/\text{cm}^{-1}$  3273, 2981, 1651, 1446; ESI (m/z): Calculated Mass  $[\text{M}+\text{H}]^+$ : 2165.9, Mass Found 2166.0, Calculated Mass  $[\text{M}+2\text{H}]^{2+}$ : 1083.3, Mass Found 1083.3; HPLC:  $t_{\text{R}}$ : 17.8 min (Gradient 0 to 50% B over 40 min);  $^1\text{H}$  NMR (1:1 v/v MeOD/ $\text{CDCl}_3$ , 400 MHz): 8.37 (4H, s, 4  $\times$  CH), 4.55 (4H, app. t,  $J$  5.5 Hz, 4  $\times$  CH), 4.36-4.29 (8H, m, 8  $\times$  CH), 4.24-4.22 (4H, m, 4  $\times$  CH), 3.92 (8H, s, 4  $\times$   $\text{CH}_2$ ), 3.84-3.75 (8H, m, 4  $\times$   $\text{CH}_2$ ), 3.70 (16H, m, 8  $\times$   $\text{CH}_2$ ), 3.18-3.08 (12H, m, 6  $\times$   $\text{CH}_2$ ), 2.58 (8H, m, 4  $\times$   $\text{CH}_2$ ), 1.37 (12H, d,  $J$  7.3 Hz, 4  $\times$   $\text{CH}_3$ ), 1.17 (12H, d,  $J$  6.4 Hz, 4  $\times$   $\text{CH}_3$ ); HRMS calcd for  $\text{C}_{82}\text{H}_{130}\text{N}_{34}\text{O}_{36}$ :  $[\text{M}+2\text{H}]^{2+}$ , 1083.46880. Found:  $[\text{M}+2\text{H}]^{2+}$ , 1083.46890.



### Synthesis of azidoglycine 15



A solution of methyl bromoacetate (0.80 mL, 8.5 mmol) and sodium azide (0.60 g, 9.0 mmol, 1.1 eq.) in DMF (5 mL) was stirred at room temperature for 2.5 h. The reaction mixture was diluted with water (50 mL) and extracted with diethylether (4  $\times$  10 mL). The combined organic fractions were washed with water (4  $\times$  10 mL), dried ( $\text{Na}_2\text{SO}_4$ ) and concentrated *in vacuo* to obtain azidoglycine methyl ester as a yellow oil (235 mg, 24%) which was used without further purification. Potassium hydroxide (0.3 g, 5.0 mmol) was dissolved in a solution of THF/ $\text{H}_2\text{O}$  (1:1 v/v, 8 mL) and added to azidoglycine methyl ester. The reaction was stirred at 40  $^\circ\text{C}$  for 2.5 h before washing with ethyl acetate (10 mL). The aqueous fraction was acidified to pH 1 with



3 M HCl, then extracted with ethyl acetate (4 x 20 mL) and the combined organic fractions dried over Na<sub>2</sub>SO<sub>4</sub> and concentrated *in vacuo* to obtain azidoglycine **15** as a yellow oil (154 mg, 75%).

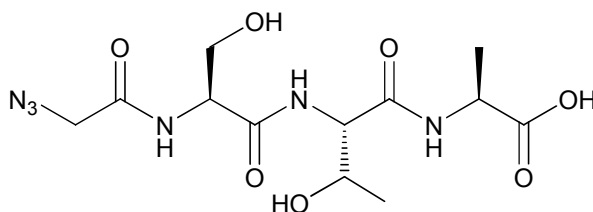
ESI (m/z): [M-H]: 99.8; <sup>1</sup>H NMR (CDCl<sub>3</sub>, 300 MHz): 9.36 (1H, br s, OH), 3.97 (2H, s, CH<sub>2</sub>); <sup>13</sup>C NMR (CDCl<sub>3</sub>, 50 MHz): 173.6, 50.0

These data are in agreement with that previously reported by Kim *et al.*<sup>[1]</sup>

## Synthesis of azidopeptides **14** and **26** and azido-glycopeptide **18**

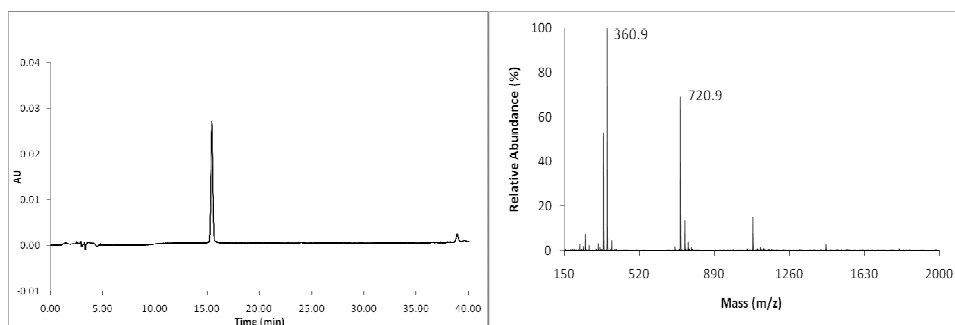
### *Tetrapeptide (14)*

*Tetrapeptide 14* was synthesised using the iterative peptide assembly described above.



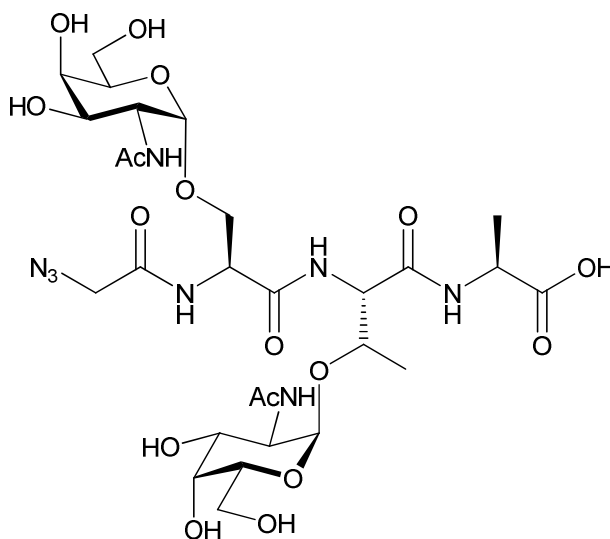
26 μM scale, Yield = 9.6 mg, quant., yield based on the Fmoc loading of the penultimate serine residue.

$\nu_{\max}/\text{cm}^{-1}$  3278, 3078, 2944, 2108, 1629, 1549; ESI (m/z): [M+H]<sup>+</sup>: 360.9, [2M+H]<sup>+</sup>: 720.9; HPLC:  $t_R$ : 15.5 min (Gradient 0 to 25% B over 40 min); <sup>1</sup>H NMR (MeOD, 400 MHz): 4.88 (1H, app t, *J* 5.9 Hz, CH), 4.55-4.38 (2H, m, 2 × CH), 4.27-4.25 (1H, m, CH), 3.98 (2H, s, CH<sub>2</sub>), 3.83 (1H, dd, *J* 5.5, 10.9 Hz, CHH), 3.77 (1H, dd, *J* 6.2, 10.9 Hz, CHH), 1.41 (3H, d, *J* 7.3 Hz, CH<sub>3</sub>), 1.23 (3H, d, *J* 6.4 Hz, CH<sub>3</sub>); <sup>13</sup>C NMR (MeOD, 100 MHz): 175.8, 172.5, 172.2, 170.4, 68.2, 62.9, 60.0, 56.6, 52.7, 49.5, 20.0, 17.6; HRMS calcd for C<sub>12</sub>H<sub>20</sub>N<sub>6</sub>O<sub>7</sub>Na: [M+Na]<sup>+</sup>, 383.1286. Found: [M+Na]<sup>+</sup>, 383.1286.



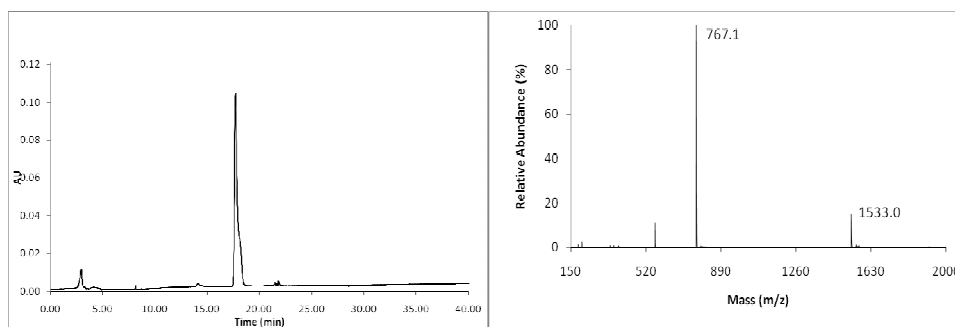
### ***Glycosylated tetrapeptide (18)***

Acetylated glycopeptide **22** was synthesised using the iterative peptide assembly described above. After cleavage from the resin, a 5% aqueous solution of hydrazine hydrate (2 mL) was added to **22** to remove the acetate groups on the carbohydrates. After standing at rt for 40 min, the reaction mixture was diluted with water containing 0.1% TFA (1.5 mL) and purified by preparative HPLC (Gradient 0 to 25% B over 60 min) to afford *glycosylated tetrapeptide 18* as a white solid.



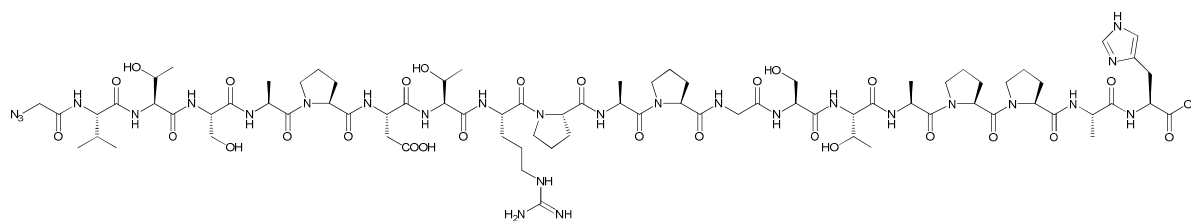
30  $\mu\text{M}$  scale, Yield = 5.9 mg, 26%, yield based on the Fmoc loading of the penultimate serine residue.

$\nu_{\text{max}}/\text{cm}^{-1}$  3366, 2119, 1676, 1438; ESI (m/z):  $[\text{M}+\text{H}]^+$ : 767.1,  $[\text{2M}+\text{H}]^+$ : 1533.0; HPLC:  $t_{\text{R}}$ : 17.7 min (Gradient 0 to 25% B over 40 min).



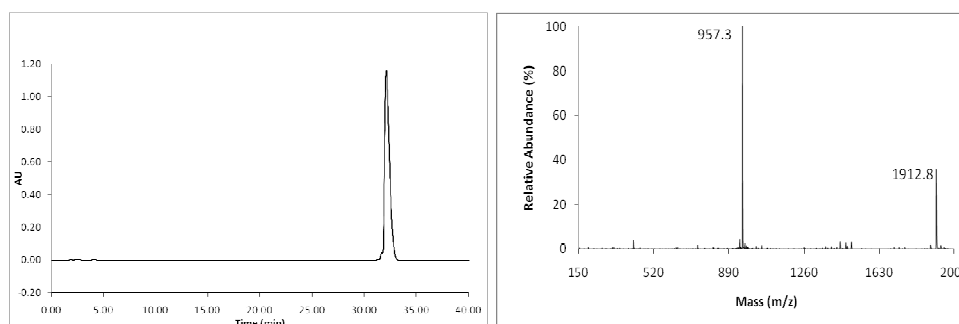
### ***Eicosapeptide (26)***

*Eicosapeptide 26* was synthesised using the iterative peptide assembly described above.

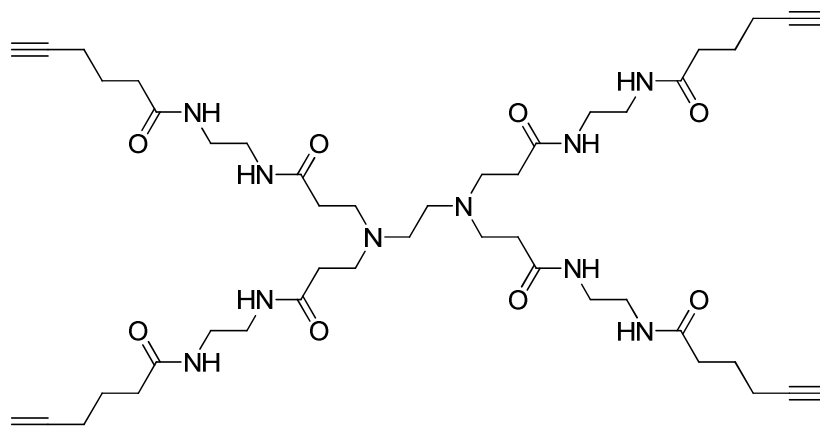


17  $\mu$ M scale, Yield = 31.9 mg, 98%, yield based on the Fmoc loading of the penultimate valine residue.

$\nu_{\max}/\text{cm}^{-1}$  3299, 2984, 2109, 1633, 1537, 1454; ESI (m/z):  $[\text{M}+\text{H}]^+$ : 1912.8,  $[\text{M}+2\text{H}]^{2+}$  957.3; HPLC:  $t_R$ : 32.2 min (Gradient 0 to 25% B over 40 min); HRMS calcd for  $\text{C}_{80}\text{H}_{126}\text{N}_{27}\text{O}_{28}$ :  $[\text{M}+\text{H}]^+$ , 1912.9260. Found:  $[\text{M}+\text{H}]^+$ , 1912.9202.

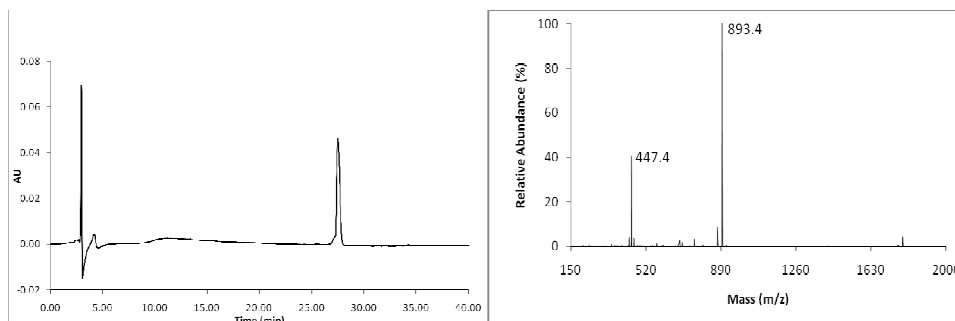


## Synthesis of *alkynyl-PAMAM dendrimer 23*

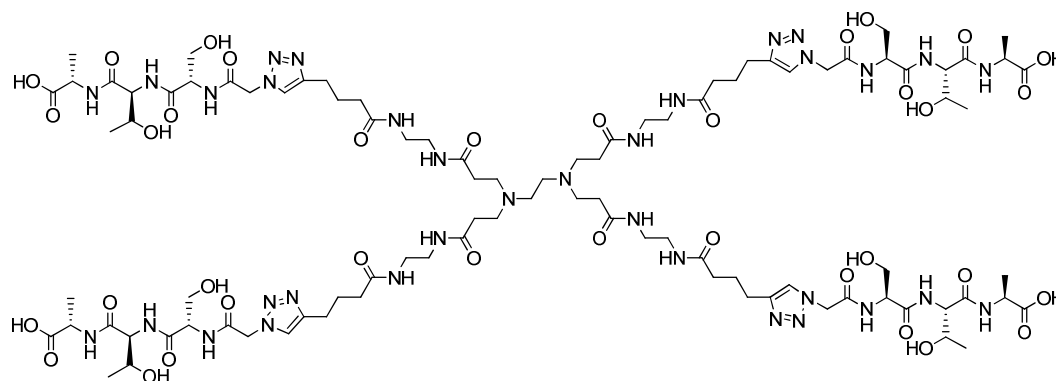


PAMAM dendrimer, ethylenediamine core, generation 0.0 (0.25 mL of a 20 wt. % solution in methanol, 0.1 mmol) and EEDQ (0.50 g, 2.0 mmol) were dissolved in methanol (1 mL). 5-Hexynoic acid (0.22 mL, 2.0 mmol) was added dropwise and the reaction stirred at rt for 16 h. The reaction mixture was concentrated *in vacuo* and the resulting residue purified by preparative HPLC (Gradient 0 to 50% B over 60 min) to afford the desired *dendrimer core 23* as a yellow oil. (61 mg, 68%).

$\nu_{\max}/\text{cm}^{-1}$  3293, 3085, 2941, 2117, 1639, 1543, 1432; ESI (m/z):  $[\text{M}+\text{H}]^+$ : 893.4,  $[\text{M}+2\text{H}]^{2+}$ : 447.4; HPLC:  $t_{\text{R}}$ : 27.5 min (Gradient 0 to 50% B over 40 min);  $^1\text{H}$  NMR (1:1 v/v MeOD/ $\text{CDCl}_3$ , 400 MHz): 3.30 (16H, br. s,  $8 \times \text{CH}_2$ ), 3.10 (12H, m,  $6 \times \text{CH}_2$ ), 2.57 (8H, t,  $J$  6.6 Hz,  $4 \times \text{CH}_2$ ), 2.33 (8H, dd,  $J$  7.4, 7.4 Hz,  $4 \times \text{CH}_2$ ), 2.24 (8H, td,  $J$  2.6, 7.0 Hz,  $4 \times \text{CH}_2$ ), 2.11 (4H, t,  $J$  2.6 Hz,  $4 \times \equiv\text{-H}$ ), 1.82 (8H, m,  $4 \times \text{CH}_2$ );  $^{13}\text{C}$  NMR (1:1 v/v MeOD/ $\text{CDCl}_3$ , 100 MHz): 173.4, 171.2, 84.1, 70.3, 50.2, 40.2, 39.6, 35.8, 31.5, 25.7, 18.6, one undetected double up; HRMS calcd for  $\text{C}_{46}\text{H}_{73}\text{N}_{10}\text{O}_8$ :  $[\text{M}+\text{H}]^+$ , 893.5607. Found:  $[\text{M}+\text{H}]^+$ , 893.5613.

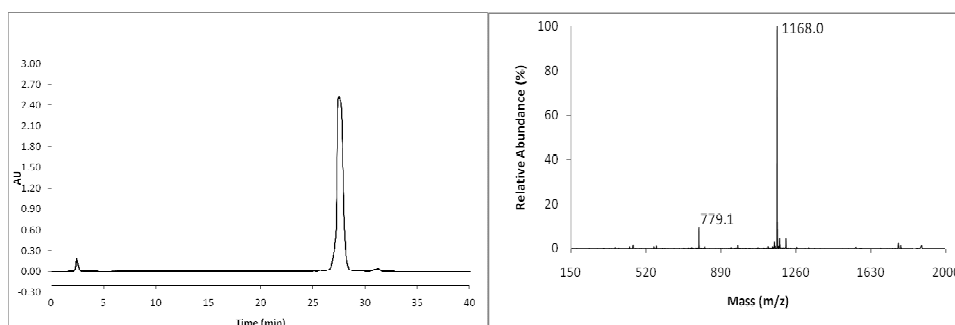


## Synthesis of peptide dendrimer **24**

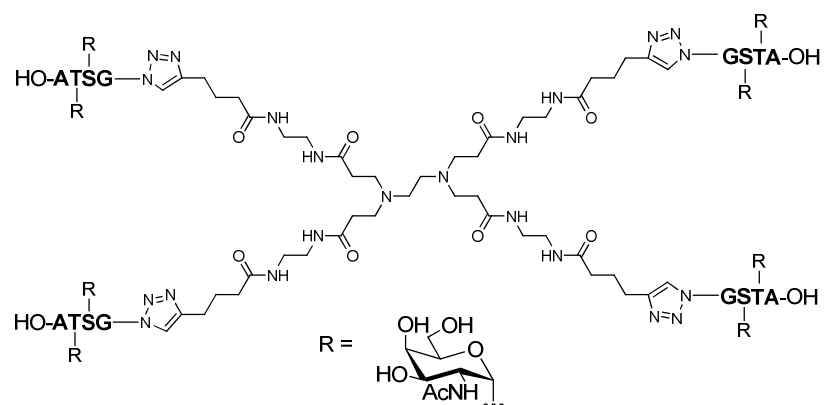


Alkynyl-dendrimer core **23** (0.9 mg, 1.0  $\mu\text{mol}$ , 1 eq.) and azidopeptide **14** (4.3 mg, 12  $\mu\text{mol}$ , 6 eq.) were dissolved in *t*-butanol/water 1:1 (v/v) (100  $\mu\text{L}$ ). Aqueous copper sulfate pentahydrate (4  $\mu\text{L}$  of 0.1 M solution, 0.4  $\mu\text{mol}$ , 0.4 eq.) and aqueous sodium ascorbate (4  $\mu\text{L}$  of 1 M solution, 4.0  $\mu\text{mol}$ , 4.0 eq.) were added sequentially and the reaction placed on a shaker for 48 h. The product was purified by semi-preparative HPLC (Gradient 0 to 25% B over 60 min) to afford the desired peptide dendrimer **24** as a white solid.

Yield = 2.6 mg, 75%;  $\nu_{\text{max}}/\text{cm}^{-1}$  3385, 2940, 1648, 1449; ESI (m/z):  $[\text{M}+2\text{H}]^{2+}$ : 1168.0,  $[\text{M}+3\text{H}]^{3+}$ : 779.1, HPLC:  $t_{\text{R}}$ : 27.5 min (Gradient 0 to 25% B over 40 min);  $^1\text{H}$  NMR (MeOD, 400 MHz): 7.82 (4H, s, CH), 5.25 (8H, s,  $4 \times \text{CH}_2$ ), 4.57 (4H, app t,  $J$  5.5 Hz,  $4 \times \text{CH}$ ), 4.40-4.35 (8H, m,  $8 \times \text{CH}$ ), 4.27-4.21 (4H, m,  $4 \times \text{CH}$ ), 3.89 (4H, dd,  $J$  5.5, 10.8 Hz,  $4 \times \text{CHH}$ ), 3.81 (4H, dd,  $J$  5.9, 10.9 Hz,  $4 \times \text{CHH}$ ), 3.31-3.22 (28H, m,  $14 \times \text{CH}_2$ ), 2.72 (8H, t,  $J$  7.3 Hz,  $4 \times \text{CH}_2$ ), 2.63 (8H, m,  $4 \times \text{CH}_2$ ), 2.22 (8H, t,  $J$  7.2 Hz,  $4 \times \text{CH}_2$ ), 1.99-1.92 (8H, m,  $4 \times \text{CH}_2$ ), 1.37 (12H, d,  $J$  7.3 Hz,  $4 \times \text{CH}_3$ ), 1.20 (12H, d,  $J$  6.3 Hz,  $4 \times \text{CH}_3$ ); HRMS calcd for  $\text{C}_{94}\text{H}_{154}\text{N}_{34}\text{O}_{36}$ :  $[\text{M}+2\text{H}]^{2+}$ , 1167.5627. Found:  $[\text{M}+2\text{H}]^{2+}$ , 1167.5615.

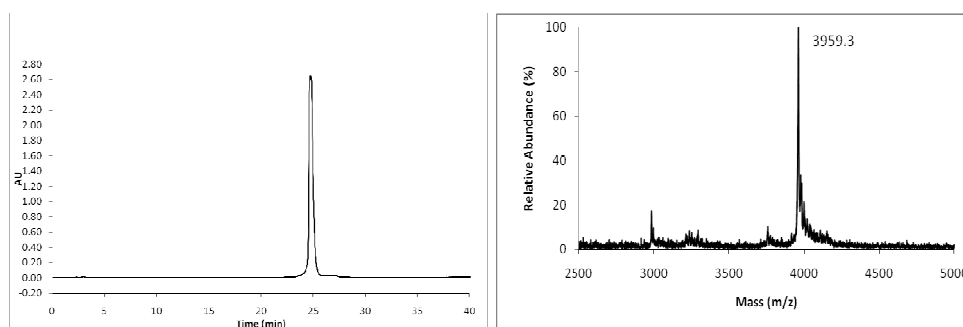


## Synthesis of glycopeptide dendrimer **25**

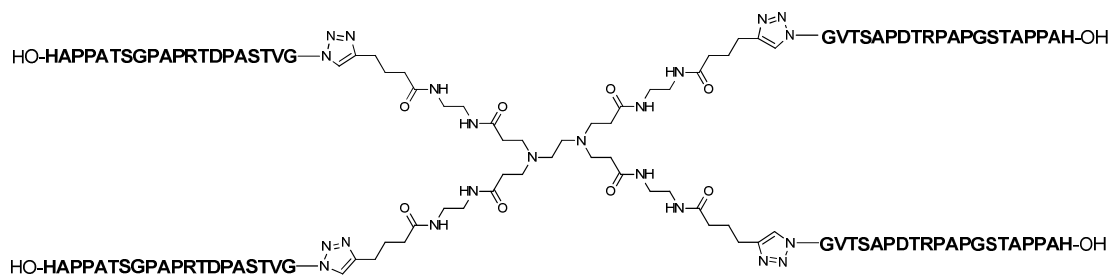


Alkynyl-dendrimer core **23** (0.9 mg, 1.0  $\mu\text{mol}$ , 1 eq.) and azido-glycopeptide **18** (3.1 mg, 4.0  $\mu\text{mol}$ , 4 eq.) were dissolved in *t*-butanol/water 1:1 (v/v) (50  $\mu\text{L}$ ). Aqueous copper sulfate pentahydrate (4  $\mu\text{L}$  of 0.1 M solution, 0.4  $\mu\text{mol}$ , 0.4 eq.) and aqueous sodium ascorbate (4  $\mu\text{L}$  of 1 M solution, 4.0  $\mu\text{mol}$ , 4 eq.) were added sequentially and the reaction placed on a shaker for 48 h. The reaction mixture was purified by semi-preparative HPLC (Gradient 0 to 25% B over 60 min) to afford the desired *glycopeptide dendrimer 25* as a white solid.

Yield = 0.8 mg, 20%;  $\nu_{\text{max}}/\text{cm}^{-1}$  3436, 1639, 1472; MALDI-Tof ( $m/z$ ):  $[\text{M}+\text{H}]^+$ : 3959.3; HPLC:  $t_{\text{R}}$ : 24.8 min (Gradient 0 to 25% B over 40 min); HRMS calcd for  $\text{C}_{158}\text{H}_{260}\text{N}_{42}\text{O}_{76}$ :  $[\text{M}+4\text{H}]^{4+}$ , 990.4437. Found:  $[\text{M}+4\text{H}]^{4+}$ , 990.4425.

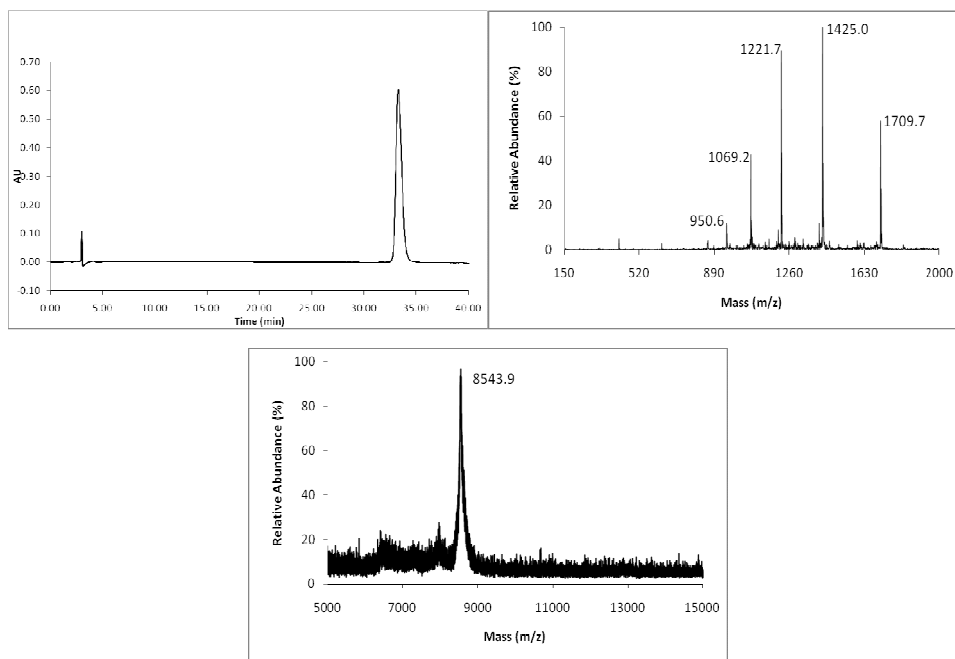


## Synthesis of peptide dendrimer 28



Alkynyl-dendrimer core **23** (0.9 mg, 1.0  $\mu\text{mol}$ , 1 eq.) and azidopeptide **26** (7.7 mg, 4.0  $\mu\text{mol}$ , 4 eq.) were dissolved in *t*-butanol/water 1:1 (v/v) (100  $\mu\text{L}$ ). Aqueous copper sulfate pentahydrate (4  $\mu\text{L}$  of 0.1 M solution, 0.4  $\mu\text{mol}$ , 0.4 eq.) and aqueous sodium ascorbate (4  $\mu\text{L}$  of 1 M solution, 4.0  $\mu\text{mol}$ , 4 eq.) were added sequentially and the reaction placed on a shaker for 48 h. The reaction mixture was purified using semi-preparative HPLC (Gradient 0 to 25% B over 60 min) to afford the desired *peptide dendrimer 28* as a white solid.

Yield = 4.8 mg, 56%;  $\nu_{\text{max}}/\text{cm}^{-1}$  3284, 2968, 1651, 1538, 1443; ESI ( $m/z$ ):  $[\text{M}+5\text{H}]^{5+}$ : 1709.7,  $[\text{M}+6\text{H}]^{6+}$  1425.0,  $[\text{M}+7\text{H}]^{7+}$ : 1221.7,  $[\text{M}+8\text{H}]^{8+}$  1069.2,  $[\text{M}+9\text{H}]^{9+}$ : 950.6; MALDI-ToF: Calculated Mass  $[\text{M}+\text{H}]^{+}$ : 8543.7, Mass Found 8543.9; HPLC:  $t_{\text{R}}$ : 33.3 min (Gradient 0 to 25% B over 40 min).



## References

- [1] H. Kim, J. K. Cho, S. Aimoto, Y-S. Lee. *Org. Lett.* **2006**, 8, 1149-1151.
- [2] E.D. Goddard-Borger, and R.V. Stick, *Org. Lett.*, **2007**, 9, 3797-3800.