Supporting Information

Incorporation of 'click' chemistry glycomimetics dramatically alters triple-helix stability in an adiponectin model peptide

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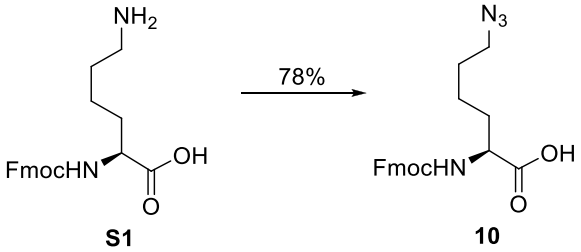
General Information for Organic Synthesis

All reagents were purchased as reagent grade from Sigma Aldrich unless otherwise specified, and were used without further purification. All reactions were done under an atmosphere of nitrogen unless otherwise stated. Analytical thin layer chromatography (TLC) was performed using Millipore TLC silica gel 60 F_{254} plates and compounds were visualised by ultra-violet fluorescence or by staining with 4% sulphuric acid in ethanol (sugar products) or ethanolic ninhydrin solution (amine products) followed by gentle heating of the plate. Flash column chromatography was performed using Grace Davison Discovery Sciences Davisil® chromatographic silica (40-63 µ) with the reported solvents. Molecular sieves were ground, dried and stored in 200 °C oven for at least 24 hours prior to use. Infrared spectra were obtained using a Perkin Elmer Spectrum 100 FT-IR spectrometer, with samples placed on a zinc selenide and diamond crystal. Absorption maxima are reported in wavenumbers (cm^{-1}) with the common abbreviations; s = strong, m = medium, w = weak, br = broad. Optical rotations were determined at the sodium D line (598 nm) at 20 °C, unless otherwise stated, with a Rudolph Research Analytical Autopol IV polarimeter and are given in units deg $dm^{-1}cm^3g^{-1}$. ^1H and ^{13}C nuclear magnetic resonance (NMR) spectra were recorded on a Bruker AVANCE 300 MHz or Bruker ADVANCEIII 400 MHz spectrometer. Chemical shifts are reported in parts per million (ppm) relative to the trimethylsilane signal at δH 0.0 ppm in $CDCl_3/SiMe_4$ solvent. ^1H NMR data values are reported as the chemical shift, relative integral, multiplicity (whereby s = singlet, d = doublet, dd = doublet of doublets, t = triplet, q = quartet, m = multiplet), coupling constant (*J* in Hz) and assignment. ^{13}C NMR values are reported as chemical shift, degree of hybridisation and assignment. High resolution mass spectrometry (HRMS) samples were recorded on a Bruker micrOTOF-Q2 machine using ESI as an ionisation source in a positive mode, unless otherwise specified. Boc-Ser-OH was purchased from Polypeptides. Fmoc-Lys-OH was purchased from GL Biochem.

Synthesis of Pre-'Click' 10, 11, 12 and 13, and 'Clicked' 8 and 9 Building Blocks

(2*S*)-((9*H*-Fluorenylmethoxycarbonyl)amino)-6-azidohexanoic acid[1] 10



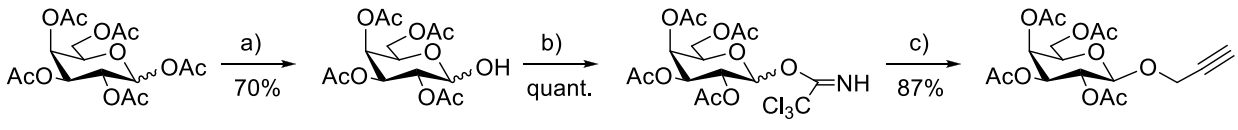
S1 **10**

Reagents and conditions: ISA.HCl (imidazole-1-sulfonyl azide hydrochloride, 1.2 eq.), K_2CO_3 (2.1 eq.), $CuSO_4$ (cat.), MeOH/H_2O (50 mL, 49:1, v/v), rt, 5 h.

Scheme S1 Synthesis of Fmoc-Lys-azide **10**.

Imidazole-1-sulfonyl azide hydrochloride (2.0 g, 9.0 mmol, 1.2 eq.) followed by K_2CO_3 (2.4 g, 17.0 mmol, 2.1 eq.) were added to Fmoc-L-lysine (**S1**, 3.0 g, 8.1 mmol) in MeOH (50 mL, analytical grade). Copper sulphate ($CuSO_4 \cdot 5H_2O$, 20.0 mg, catalytic) and water (3 drops to aid dissolution) were then added to the reaction mixture, which was stirred at rt for 5 h, concentrated *in vacuo* and acidified using HCl (2 M) to reach pH 2. The organic product was extracted into ethyl acetate (3 x 50 mL), washed with brine (50 mL), $NaHCO_3$ (sat. soln., 50 mL), dried using Na_2SO_4 and concentrated *in vacuo*. The crude product was purified by flash column chromatography (ethyl acetate: *n*-hexanes, 3:7 → 1:0) to afford the *title compound* **10** as an off-white solid (2.5 g, 6.3 mmol, 78%). R_f 0.28 (ethyl acetate: *n*-hexanes: acetic acid, 1:1:0.01), the ^1H and ^{13}C NMR data were in agreement with the literature values,[2] $[\alpha]_D^{23}$ -2.3, *c* 0.07, MeOH (lit $[\alpha]_D^{23}$ -1.8, *c* 0.03, MeOH),[1] mp 64.1 - 64.8 °C (no lit. mp), HRMS (ESI) calculated for $C_{21}H_{22}N_4O_4Na^+$ 417.1533, found 417.1536.

Synthetic strategy to 1-O-Propargyl-2,3,4,6-tetra-O-acetyl-β-D-galactopyranose 11

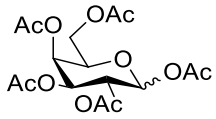


S2 **S3** **S4** **11**

Reagents and conditions: a) Ethylene diamine (1.1 eq.), AcOH (1.4 eq.), THF, 70%; b) Cl_3CCN (4 eq.), K_2CO_3 (3 eq.), CH_2Cl_2, rt, o/n, quant.; c) propargyl alcohol (10 eq.), TMSOTf (0.2 eq.), CH_2Cl_2 (dry), 4Å MS, Ar, -40 °C, 10 min, 87%.

Scheme S2 Synthesis of AcO-Gal-C1-*O*-alkyne **11**.

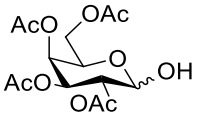
Penta-*O*-acetyl-D-galactopyranose[3,4,5] S2



S2

Perchloric acid (60% solution, 120 µL, 3 µmol, cat.), was added dropwise to an ice-cold solution of D-galactose (0.5 g, 2.8 mmol) in acetic anhydride (20 mL, 180 mmol, excess). Further portions of D-galactose (4.5 g, 25 mmol) were then added and the reaction mixture stirred at rt for 2 h. The organic product was extracted into CH_2Cl_2 (2 x 50 mL), washed with $NaHCO_3$ soln. (sat. soln., 2 x 50 mL), dried using $MgSO_4$ and concentrated *in vacuo* to afford the *title compound* **S2** as a colourless solid (10.9 g, 27 mmol, quant.). R_f 0.38 (ethyl acetate: *n*-hexanes, 1:1), the ^1H and ^{13}C data were in agreement with the literature values [α-anomer[5], β-anomer[4]]. The α/β ratio was calculated from ^1H NMR spectrum to be 5.4/1, mp 84.4 – 86.5 °C (lit. 88 -90 °C),[6] HRMS (ESI) calculated for $C_{16}H_{22}O_{11}Na^+$ 413.1054, found 413.1070.

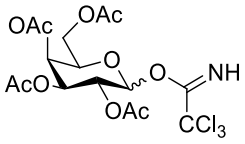
2,3,4,6-Tetra-*O*-acetyl-α,β-D-galactopyranose[7] S3



S3

Glacial acetic acid (0.5 mL, 9.0 mmol, 1.4 eq.) was added dropwise to a solution of ethylenediamine (0.5 mL, 7.6 mmol, 1.1 eq.) in THF (10 mL). A solution of penta-*O*-acetyl-D-galactopyranose (**S2**, 2.5 g, 6.4 mmol) in THF (dry, 10 mL) was then added dropwise to the resulting white suspension. The reaction mixture stirred at rt for 16 h, quenched with deionised water (10 mL) and the organic product was extracted into CH_2Cl_2 (3 x 10 mL), washed with HCl (2 M, 10 mL), $NaHCO_3$ soln.(sat. soln., 10 mL) and deionised water (10 mL), dried using Na_2SO_4 and concentrated *in vacuo*. The crude material was then purified by flash column chromatography (ethyl acetate: *n*-hexanes, 2:3) to afford the *title compound* **S3** as a colourless foam (1.5 g, 4.3 mmol, 70%). R_f 0.26 (ethyl acetate: *n*-hexanes, 1:1), the ^1H and ^{13}C data were in agreement with the literature values.[8] The α/β ratio was calculated from the ^1H NMR spectra to be 1.56/1. HRMS (ESI) calculated for $C_{14}H_{20}O_{10}Na^+$ 371.0949, found 371.0942.

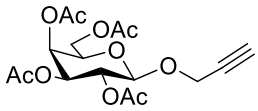
2,3,4,6-Tetra-*O*-acetyl-α,β-D-galactopyranosyl trichloroacetimidate[3] S4



S4

K_2CO_3 (1.20 g, 8.7 mmol, 3 eq.) followed by trichloroacetonitrile (1.2 mL, 10.0 mmol, 4 eq.) were added to 2,3,4,6-tetra-*O*-acetyl-α,β-D-galactopyranose (**S3**, 1.0 g, 2.90 mmol) in CH_2Cl_2 (dry, 10 mL). The reaction mixture was stirred at rt for 16 h, filtered, concentrated *in vacuo* and purified using flash column chromatography (ethyl acetate: *n*-hexanes, 2:3) to afford the *title compound* **S4** as a colourless foam (1.42 g, 2.90 mmol, quant.). R_f 0.46 (ethyl acetate: *n*-hexanes, 1:1), the ^{1}H and ^{13}C data were in agreement with the literature values.[9] The α/β ratio was calculated from ^{1}H NMR spectra to be 0.58/1. HRMS (ESI) calculated for $C_{36}H_{28}Cl_3NO_{10}Na^+$ 762.0671, found 762.0658.

1-*O*-Propargyl-2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranose[10] 11

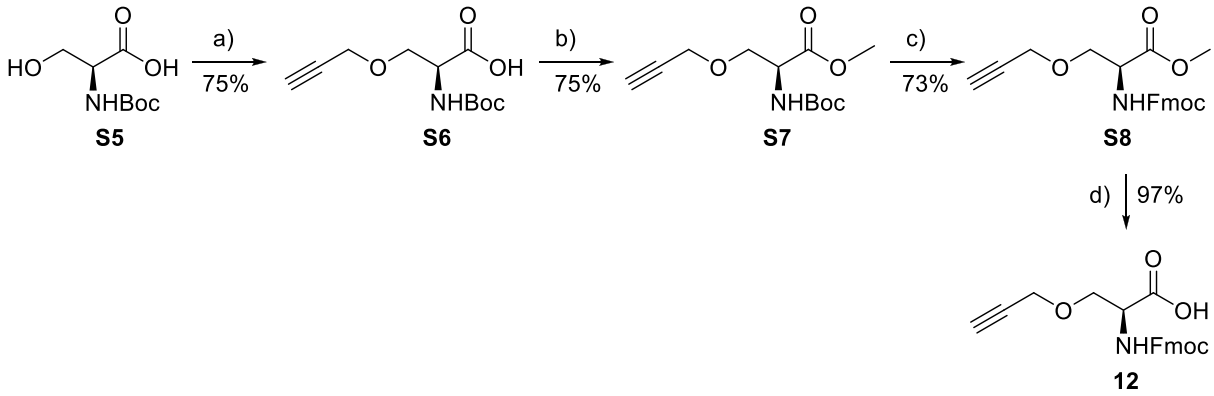


11

2,3,4,6-Tetra-*O*-acetyl-α,β-D-galactopyranosyl trichloroacetimidate (**S4**, 200 mg, 0.4 mmol, 1.7 eq.) in CH_2Cl_2 (dry, 2 mL) was added to a to a flame-dried flask containing molecular sieves (4Å, spatula tip) and the reaction mixture was stirred at rt for 30 min and then cooled to -40 °C. Propargyl alcohol (0.23 mL, 4.2 mmol, 10 eq) followed by trimethylsilyltrifluoromethanesulfonate (15 µL diluted in 0.2 mL dry CH_2Cl_2, 0.08 µmol, 0.2 eq.) were added dropwise to the ice-cold reaction mixture. The reaction mixture was stirred at rt for 30 min, and a further portion of trimethylsilyltrifluoromethanesulfonate (15 µL diluted in 0.2 mL dry CH_2Cl_2, 0.08 µmol, 0.2 eq.) was added and the reaction mixture stirred at rt for a further 15 min. The reaction mixture was then quenched using triethylamine (1 mL), filtered through Celite® and concentrated *in vacuo*. The organic product extracted into ethyl acetate (3 x 5 mL), dried using Na_2SO_4 and concentrated *in vacuo*. The crude product was purified by flash column chromatography (ethyl acetate: *n*-hexanes, 1:2) to afford the *title compound* **11** as a pale yellow oil (0.14 g, 0.35 mmol, 87%). R_f 0.61 (ethyl acetate:

methanol, 1:1), the ^{1}H and ^{13}C NMR data were in agreement with the literature values,[11] $[\alpha]_D^{21}$ -0.8, *c* 1.09, MeOH (lit. $[\alpha]_D^{21}$ -5.3, *c* 1.09, MeOH).[11]

Synthetic strategy to (2*S*)-((9*H*-Fluorenylmethoxycarbonyl)amino)-3-(prop-2-yn-1-yloxy)pro-panoic acid 12

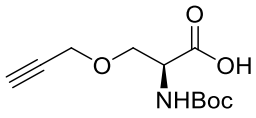


S5 **S6** **S7** **S8** **12**

Reagents and conditions: a) NaH (2.3 eq.), propargyl bromide/ toluene (1.5 eq.), DMF, 1.5 h, rt, 75%; b) K_2CO_3 (1.5 eq.) MeI (1.5 eq.), DMF, rt, o/n, 75%, c)i) TFA (excess), CH_2Cl_2, rt, 2 h, ii) Fmoc-OSu (1 eq.), Na_2CO_3 (1.2 eq.), dioxane/H_2O, rt, o/n, 73% (steps ii and iii); d) 0.8 M $CaCl_2$: 0.1 M LiOH (10 eq.), H_2O, 2 h, rt, 97%.

Scheme S3 Synthesis of Fmoc-Ser-alkyne **12**.

(2*S*)-(*tert*-Butoxycarbonyloxycarbonyl)amino)-3-(prop-2-yn-1-yloxy)propanoic acid[1] S6

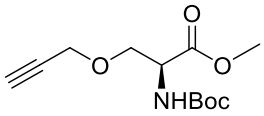


S6

Boc-L-serine (**S5**, 6.0 g, 29.0 mmol) was added to an ice-cold solution of NaH (2.7 g, 0.1 mmol, 2.3 eq.) in DMF (30 mL) and the reaction mixture stirred for 30 min. Propargyl bromide (80 wt.% in toluene, 4.7 mL, 0.05 mmol, 1.5 eq.) was added dropwise and the reaction mixture stirred at rt for 1.5 h. The reaction mixture was the quenched with ice-cold deionised water (30 mL), concentrated in *vacuo* and acidified using HCl (1 M) to pH 2. The organic product was extracted into ethyl acetate (3 x 50 mL), dried using Na_2SO_4 and concentrated *in vacuo.* The crude product was purified using flash column chromatography (*n*-hexanes: ethyl acetate: acetic acid, 5: 1: 0.1) to afford the *title compound* **S6** as an orange oil (6.9 g, 28.5 mmol, 98%). R_f 0.34 (ethyl acetate: acetic acid, 1:0.01), the ^{1}H and ^{13}C NMR

data were in agreement with the literature values,[1] $[\alpha]_D^{24}$ +5.5, *c* 0.52, MeOH (lit. $[\alpha]_D^{23}$ +0.1, *c* 1.0, MeOH).[1]

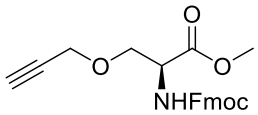
(2*S*)-Methyl-(*tert*-butoxycarbonyloxycarbonyl)amino)-3-(prop-2-yn-1-yloxy)propanoate[12,13] S7



S7

K_2CO_3 (5.9 g, 42.6 mmol, 1.5 eq.) followed by MeI (2.2 mL, 42.6 mmol, 1.5 eq.) were added to a solution of (2*S*)-(*tert*-butoxycarbonyloxycarbonyl)amino)-3-(prop-2-yn-1-yloxy)propanoic acid (**S6**, 6.9 g, 28.4 mmol) in DMF (40 mL). The reaction mixture was stirred overnight at room temperature and quenched with brine (50 mL). The organic product extracted with ethyl acetate (2 x 100 mL), washed with water (4 x 50 mL), dried using Na_2SO_4 and concentrated *in vacuo*. The crude product was purified using flash column chromatography (ethyl acetate: *n*-hexanes, 1:4 → 1:1) to afford the *title compound* **S7** as a yellow oil (5.5 g, 21.4 mmol, 75%). R_f 0.65 (ethyl acetate: *n*-hexanes, 4: 1), the ^1H and ^{13}C NMR data were in agreement with the literature values,[12] $[\alpha]_D^{21}$ +14.7, *c* 1.20, $CHCl_3$ (lit. $[\alpha]_D^{21}$ +17.8, *c* 2.7, $CHCl_3$).[14]

(2*S*)-Methyl-((9*H*-fluorenylmethoxycarbonyl)amino)-3-(prop-2-yn-1-yloxy)propanoate S8

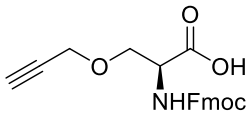


S8

TFA (40 mL) was added to an ice-cold solution of (2*S*) Methyl (*tert* butoxycarbonyloxycarbonyl)amino)- 3-(prop-2-yn-1-yloxy)propanoate (**S7**, 7.7 g, 30.0 mmol) in CH_2Cl_2 (20 mL) and the reaction mixture stirred at rt for 2 h then concentrated *in vacuo*. The crude product was then dissolved in a mixture of dioxane: 10 % Na_2CO_3 solution (3:5, 80 mL) and cooled to 0 °C. A solution of *N*-(9*H*-fluorenylmethoxycarbonyl)succinimide (10.2 g, 30.0 mmol, 1 eq.) in dioxane (20 mL) was added dropwise to the ice-cold reaction mixture, which was then stirred for at rt for 16 h. The organic product was extracted into ethyl acetate (3 x 20 mL), washed with brine (sat. soln. 20 mL), dried using Na_2SO_4 and concentrated *in vacuo.* The crude product was

purified by flash column chromatography (ethyl acetate: *n*-hexanes, 1:4 → 1:1), followed by recrystallisation from ethyl acetate: *n*-hexanes (1:2) to yield the *title compound* **S8** as a colourless solid (8.3 g, 21.2 mmol, 73%). R_f 0.28 (ethyl acetate: *n*-hexanes, 1: 4), δ_H (400 MHz; $CDCl_3$; Me_4Si) 2.44 (1 H, t, $^4J_{3,1}$ 2.2, ≡C*H*), 3.72-3.77-4.01 (2 H, m, β-CH_2), 3.78 (3 H, s, OCH_3), 4.16 (2 H, d, $^4J_{1,3}$ 2.2, CH_2C≡), 4.24 (1 H, t, $J_{CH,CH2}$ 7.3, C*H* Fmoc), 4.35-4.46 (2 H, m, CH_2 Fmoc), 4.53-4.57 (1 H, m, α-C*H*), 5.66 (1 H, d, $J_{NH,a-H}$ 8.3, N*H*), 7.32 and 7.39 (2 H, t, *J* 7.4 C*H* Fmoc Ar), 7.61 (2 H, m, C*H* Fmoc Ar), 7.76 (2 H, d, *J* 7.6, C*H* Fmoc Ar). δ_C (100 MHz, $CDCl_3$) 47.1 (CH, Fmoc-*C*H), 52.7 (CH_3, O*C*H_3), 54.2 (CH, α-*C*), 58.6, (CH_2, *C*H_2C≡), 67.2 (CH_2, *C*H_2 Fmoc), 69.6 (CH_2, β-*C*), 76.7 (CH, ≡*C*H), 78.8 (quat., CH_2*C*≡), 120.0, 125.1 and 127.1 (CH, Fmoc Ar), 127.7, 141.3 and 143.9 (quat., Fmoc Ar), 156.0 (C=O), 170.6 (C=O); $[\alpha]_D^{23}$ −1.7, *c* 0.70, MeOH (no lit $[\alpha]_D$), mp 83 – 85 °C (from ethyl acetate/*n*-hexanes) (no lit. mp). ν_{max}/cm^{-1} 3402m (N-H), 3263m (C-H, alkyne), 2115w (C≡C), 1756s and 1709s (C=O), 1528s (N-H), 1442m (C-C, aromatic), 1340s (C-H, aliphatic), 1203s, 1107s and 1075s (C-O), 1035s (C-N); HRMS (ESI) calculated for $C_{22}H_{21}NO_5Na^+$ 402.1312, found 402.1308.

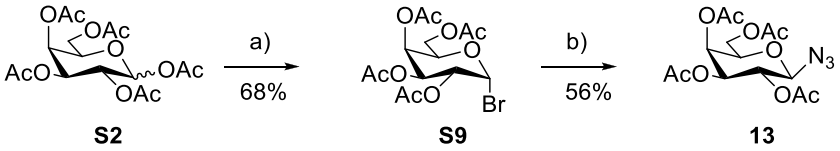
(2*S*)-((9*H*-Fluorenylmethoxycarbonyl)amino)-3-(prop-2-yn-1-yloxy)propanoic acid[15] 12



12

A solution of LiOH (0.1 M, 9.4 mL) was added dropwise to a solution of (2*S*)-methyl-((9*H*-fluorenylmethoxycarbonyl)amino)- 3-(prop-2-yn-1-yloxy)propanoate (**S8**, 1.77 g, 4.8 mmol) in a mixture of 0.8 M $CaCl_2$: 0.1 M LiOH (70 mL: 9.36 mL, 10 eq.). The reaction mixture was stirred at rt for 2 h, quenched with ice-cold water (30 mL) then acidified using 5% citric acid solution to reach pH 4. The organic product was extracted into ethyl acetate (3 x 10 mL), washed with brine (10 mL), dried using Na_2SO_4 and concentrated *in vacuo*. The crude product was purified by flash column chromatography (ethyl acetate: *n*-hexanes 1:1 → 100% ethyl acetate + 2% acetic acid) to afford the *title compound* **12** as a colourless powder (1.6 g, 4.37 mmol, 97%). R_f 0.14 (ethyl acetate: *n*-hexanes: acetic acid, 1: 0.02), the ^{1}H and ^{13}C NMR data were in agreement with the literature values,[1] $[\alpha]_D^{24}$ +6.5, *c* 0.62, MeOH (lit. $[\alpha]_D^{23}$ +6.2, *c* 1.00, MeOH),[1] mp 144.6 - 145.2 °C (lit. mp not reported).

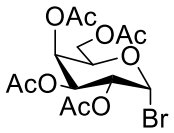
Synthetic strategy to 2,3,4,6-Tetra-O-acetyl-β-D-galactopyranosyl azide 13



Conditions and reagents: a) HBr/AcOH (33%, 10 eq.), CH_2Cl_2, rt, 4 h, 68%; b) NaN_3 (5 eq.), $NaHCO_3$ (excess), Bu_4NHSO_4 (1 eq.), H_2O/CH_2Cl_2 (1:1, v/v), 56%.

Scheme S4 Synthesis of AcO-Gal-N_3 **13**.

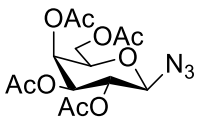
2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranosyl bromide[16] S9



S9

Hydrogen bromide (33% in acetic acid, 16 mL, 10 eq.) was added dropwise to an ice-cold solution of 1,2,3,4,6-penta-*O*-acetyl-α,β-D-galactopyranose (**S2**, 10.8 g, 27.0 mmol) in CH_2Cl_2 (50 mL). The reaction mixture was stirred at rt for 4 h, quenched with ice-cold water (150 mL) and $NaHCO_3$ (sat. aq.) was added to reach pH 7. The organic product was extracted into CH_2Cl_2 (3 x 50 mL), washed with $NaHCO_3$ (sat. aq., 50 mL), dried using Na_2SO_4 and concentrated *in vacuo*. The crude product was purified by flash column chromatography (ethyl acetate: *n*-hexanes, 2:1) to afford the *title compound* **S9** as a colourless foam (7.8 g, 19.0 mmol, 68%). R_f 0.39 (ethyl acetate: *n*-hexanes, 1:2) , the ^1H and ^{13}C NMR data were in agreement with the literature values,[16] $[\alpha]_D^{21}$ +237.8, *c* 0.96 , $CHCl_3$ (lit. $[\alpha]_D^{21}$ +225.0, *c* 0.60, CH_3Cl)[17], mp 76.7- 79.5 °C (lit. 86 - 89 °C).[17]

2,3,4,6-Tetra-*O*-acetyl-β-D-galactopyranosyl azide[16] 13

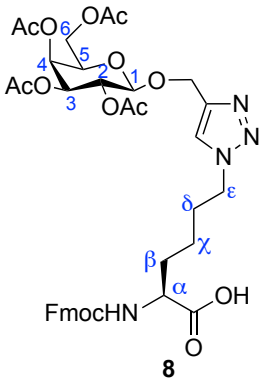


13

Tetrabutylammonium hydrogen sulfate (6.5 g, 19.0 mmol, 1 eq.) followed by $NaHCO_3$ (sat. aq., 70 mL) were added to a solution of 2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranosyl bromide (**S9**, 7.8 g, 19.0 mmol) in CH_2Cl_2 (dry, 70 mL). Sodium azide (6.2 g, 95.0 mmol, 5 eq.) was then added, the reaction mixture stirred at rt for 2 d then concentrated *in vacuo*. The organic

product was extracted into ethyl acetate (3 x 50 mL), washed with $NaHCO_3$ (sat. aq., 2 x 10 mL), dried using Na_2SO_4 and concentrated *in vacuo*. The crude product was then recrystallised from hot ethanol (10 mL) to afford the *title compound* **13** as a colourless foam (4.0 g, 10.7 mmol, 56%). R_f 0.30 (ethyl acetate: *n*-hexanes, 1:2), the ^1H and ^{13}C NMR data were in agreement with the literature values,[18] $[\alpha]_D^{21}$ -14.7, *c* 2.35, $CHCl_3$ (lit. $[\alpha]_D^{21}$ -10.3, *c* 1.00, $CHCl_3$).[18]

(2*S*)-2-[((9*H*-Fluorenyl-9-yl-)methoxy)carbonyl]amino-6-(((2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl(oxy)methyl)-1*H*-1,2,3-triazol-1-yl)hexanoic acid, 8

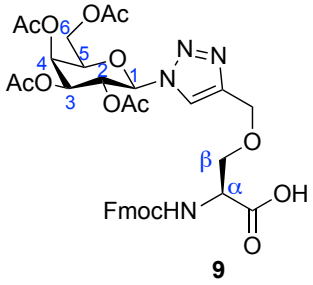


8

$CuSO_4.5H_2O$ (83 mg, 0.33 mmol, 0.3 eq.) and sodium ascorbate (131 mg, 0.66 mmol, 0.6 eq.) in degassed, deionised water (5 mL) were added to 1-*O*-propargyl-2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranose (**11**, 433 mg, 1.09 mmol, 1.1 eq.) in degassed ethanol (2 mL) and the reaction mixture heated at 80 °C for 15 min. (2*S*)-((9*H*-Fluorenylmethoxycarbonyl)amino)-6-azidohexanoic acid (**10**, 411 mg, 1.09 mmol, 1.0 eq.) was added to the reaction mixture, which was then heated at 80 °C for 1 h. The reaction mixture was concentrated *in vacuo* and the crude product was extracted into ethyl acetate (3 x 10 mL), washed with saturated ammonium chloride solution (10 mL), dried using Na_2SO_4 and concentrated *in vacuo*. The crude product was purified by flash column chromatography (ethyl acetate: *n*-hexanes: methanol, 1: 1: 0 → 1: 0: 0 → 1: 0: 0.05) to afford the *title compound* **8**, as a colourless foam (803 mg, 1.09 mmol, quant.). R_f 0.35 (ethyl acetate: methanol, 9:1), ν_{max}/cm^{-1} 2922m and 2853 (C-H, aliphatic), 1744s (C=O), 1531w, 1451w (C=C, aromatic), 1369m, 1217s (C-O), 1170m, 1132m, 1044s (C-O); δ_H (400 MHz; $CDCl_3$; Me_4Si) 1.24 - 1.41 (2 H, m, γ-CH_2), 1.75 - 2.12 (16 H, m, δ-CH_2, β -CH_2, 3 x OAc), 3.91 - 3.95 (1 H, t, *J* 6.4, H-5), 4.07 - 4.23 (3 H, m, H_2-6, C*H* Fmoc), 4.33 - 4.39 (5 H, m, ε -CH_2, CH_2 Fmoc, α-C*H*), 4.60 - 4.63 (1 H, d, *J* 7.8, H-1), 4.76 - 4.96 (2 H, dd, *J* 62.0, 11.8, β-CH_2OCH_2), 5.03 - 5.07 (1 H, dd, *J* 10.4, 3.3, H-3), 5.17 - 5.23 (1 H,

m, H-2), 5.38 - 5.40 (1 H, d, *J* 3.1, H-4), 5.71 (1 H, brs, N-*H*), 7.26 - 7.31 (2 H, t, *J* 8.6, C*H* Fmoc Ar), 7.35 - 7.40 (2 H, t, *J* 7.4, C*H* Fmoc Ar), 7.53 - 7.60 (3H, m, C*H* Fmoc Ar, C=C*H*N), 7.73 - 7.75 (2 H, d, *J* 7.5, C*H* Fmoc Ar). δ_C (400 MHz; $CDCl_3$; Me_4Si) 20.5, 20.6, 20.7 20.8 (CH_3, 4 x OAc), 21.9 (CH_2, γ-*C*), 29.6 (CH_2, δ-*C*), 31.6 (CH_2, β-*C*), 47.1 (CH, Fmoc-*C*H), 50.1 (CH_2, ε-*C*), 52.0 (CH, α-*C*), 61.3 (CH_2, H_2-6), 62.7 (CH_2, β-CH_2OCH_2), 67.0 (CH, C-4), 67.1 (CH_2, Fmoc-CH_2), 68.0 (CH, C-2), 69.0 (CH, C-3),70.7 (CH, C-5) 100.4 (C CH, C-1), 120.0 (CH, Fmoc, Ar), 122.9 (CH, C=*C*HN), 125.1, 127.1, 127.7 (*C*H, Fmoc Ar), 143.7 (quat., *C*=CHN), 143.8, 156.1 (quat., *C*=O Fmoc Ar), 170.1, 170.2, 170.3, 170.6 (quat., *C*=O, 4 x OAc –one peak overlapping), $[\alpha]_D^{24}$ -4.9, *c* 0.30, MeOH, mp 64.1 – 64.8 °C (from ethyl acetate/ *n*-hexanes); HRMS (ESI+) for $C_{38}H_{44}N_4O_{14}Na$ 803.2746, found 803.2757.

(2*S*)-2-[((9*H*-Fluorenyl-9-yl-)methoxy)carbonyl)amino]-3-(((2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-1*H*-1,2,3-triazol-4-yl)methyl)oxy)propanoic acid, 9



9

$CuSO_4 \cdot 5H_2O$ (410 mg, 1.64 mmol, 0.3 eq.) and sodium ascorbate (651 mg, 3.28 mmol, 0.6 eq.) in degassed, deionised water (5 mL) were added to (2*S*)-((9*H*-fluorenylmethoxycarbonyl)amino)-3-(prop-2-yn-1-yloxy)propanoic acid (**12**, 2.00 g, 5.47 mmol) in degassed ethanol (2 mL) and the reaction mixture was heated at 80 °C for 10 min. 2,3,4,6-Tetra-*O*-acetyl-α-D-galactopyranosyl azide (**13**, 2.04 mg, 5.47 mmol, 1.0 eq.) was added to the reaction mixture, which was then heated at 50 °C for 1 h. The reaction mixture was concentrated *in vacuo* and the crude product extracted into ethyl acetate (3 x 10 mL), washed with saturated ammonium chloride solution (10 mL), dried using Na_2SO_4 and concentrated *in vacuo*. The crude product was purified by flash column chromatography (ethyl acetate: *n*-hexanes: methanol, 1: 1: 0 → 1: 0: 0 → 1: 0: 0.05) to afford the *title compound* **9** as a colourless foam (4.03 g, 5.47 mmol, quant.). R_f 0.32 (ethyl acetate: methanol, 9:1), ν_{max}/cm^{-1} 3407w (N-H), 2928w (C-H, aliphatic), 1737s (C=O), 1515m and 1450m (C=C, aromatic), 1368m, 1206s (C-O), 1154m, 1107m, 1043s (C-O)). δ_H (400 MHz;

$CDCl_3$; Me_4Si) 1.88, 1.99, 2.03, 2.21 (each 3 H, s, OAc), 3.82 - 4.00 (2 H, dd, $J_{\alpha,\beta}$ 64.2, $J_{\beta a \beta b}$ 6.9, β-C*H*$_2$), 4.13 - 4.25 (4 H, m, CH Fmoc, H-5, CH_2-6), 4.30 - 4.45 (2 H, m, C*H*$_2$ Fmoc), 4.56 (1 H, brs, α-C*H*), 4.63 - 4.78 (2 H, dd, *J* 42.8, *J* 12.8, β-CH_2OC*H*$_2$), 5.23 - 528 (1 H, dd, *J* 10.2, *J* 3.4, H-3), 5.49 - 5.55 (2H, m, H-2 and H-4), 5.80 - 5.83 (1 H, d, *J* 9.2, Gal-C1-*H*), 5.89-5.92 (1 H, d, *J* 8.1, N-*H*), 7.28 - 7.32 (2H, m, C*H* Fmoc Ar), 7.37 - 7.40 (2 H, t, *J* 7.4, C*H* Fmoc Ar), 7.60-7.64 (2 H, br t, *J* 7.5, C*H* Fmoc Ar), 7.75 - 7.76 (2 H, d, *J* 7.5, C*H* Fmoc Ar), 7.82 (1 H, s, C=C*H*N). δ_C (400 MHz; $CDCl_3$; Me_4Si) 20.2, 20.5, 20.6, 21.0 (CH_3, 4 x OAc), 47.1 (CH, Fmoc-*C*H), 54.2 (CH, α-*C*H), 61.3 (CH_2, C-6), 64.2 (CH_2, β-CH_2O*C*H_2), 66.9 (CH, C-2 or C-4), 67.3 (CH_2, Fmoc-*C*H_2) 68.0 (CH, C-2 or C-4), 69.8 (CH_2, β-*C*H_2), 70.6 (CH, C-3), 74.2 (CH, C-5), 86.3 (CH, Gal-*C*1H), 119.9 (CH, Fmoc, Ar), 121.5 (CH, C=*C*HN), 125.3, 127.1, 127.7 (CH, Fmoc, Ar), 141.2 (quat., *C*=CHN), 143.7, 143.9 (quat., Fmoc Ar), 156.5 (quat. *C*=O, Fmoc), 169.5, 169.8, 170.0, 170.7, 171.2 (quat. *C*=O, OAc and CO_2H). mp 80.2 – 81.8 °C (from ethyl acetate/ n-hexanes); $[\alpha]_D^{24}$ -3.3, *c* 1.30, MeOH, HRMS (ESI+) for $C_{35}H_{38}N_4O_{14}Na$ calcd 761.2277, found 761.2268.

General Information for Peptide Synthesis

All solvents and reagents were used as supplied. 1-[Bis(dimethylamino)methylene]-1H-1,2,3-triazolo[4,5-b]pyridinium 3-oxid hexafluorophosphate (HATU), 1H-Benzotriazolium 1-[bis(dimethylamino)methylene]-5chloro-hexafluorophosphate (1-),3-oxide (HCTU) and Fmoc-Rink amide linker were purchased from GL Biochem (Shanghai, China). Dimethylformamide (DMF) (AR grade) and acetonitrile (HPLC grade) were purchased from Scharlau (Barcelona, Spain). *N,N'*-Diisopropylethylamine (DIPEA) and diisopropylcarbodiimide (DIC) were purchased from Aldrich (St Louis, MO). Tris(2-carboxyethyl)phosphine hydrochloride (TCEP.HCl) and triisopropylsilane (TIPS) were purchased from Alfa Aesar (Wardhill, MA). Guanidine hydrochloride (Gn.HCl) was purchased from MP Biomedicals (Auckland, New Zealand). 4-[(*R*,*S*)-α-[1-(9H-fluoren-9-yl]methoxycarbonylamino]-2,4-dimethoxy]phenoxyacetic acid (Rink amide) linker was purchased from GL Biochem. Trifluoroacetic acid (TFA) was purchased from Oakwood Chemicals (River Edge, NJ). Hydroxymethylphenoxy propionic acid (HMPP) linker was purchased from Novabiochem(R) (Billerica, MA). Aminomethyl polystyrene (AM-PS) resin was synthesised "in house" according to the method previously published by Brimble *et al*.[19] Fmoc-amino acids were purchased from GL Biochem with the following side chain protections: Fmoc-Pro-OH, Fmoc-Gly-OH, Fmoc-Lys(Boc)-OH, Fmoc-Ser(^{t}Bu)-OH, Fmoc-Glu(O^{t}Bu). Fmoc-Hyp(4*R*)-OH was purchased from Sigma-Aldrich. All peptides were synthesised by Fmoc-solid phase peptide synthesis (Fmoc-SPPS) using an aminomethyl polystyrene resin (AM-PS, 0.98 g/mmol loading) functionalised with a Rink amide linker. Unless otherwise noted, purified peptides were >95% pure by HPLC analysis.

General Method A (attachment of Rink amide linker to AM-PS resin)

AM-PS resin was swollen in CH_2Cl_2 (2 mL). In a separate vial, DIC (2 eq.) was added to a slurried mixture of Rink amide linker (2 eq.) and 6-Cl-HOBt (2 eq.) in DMF (1 mL) and the slurry was vortex-mixed until a clear solution was formed. This clear solution was added to the swollen resin, stirred for 3 h, drained and washed (2 x CH_2Cl_2, 1 x DMF). The resulting peptidyl-resin was allowed to sit at rt for 1.5 h, at which point the Kaiser test gave a negative result. The resin was then dried by washing with methanol and a stream of N_2.

General Method B (single coupling cycle, CEM™ microwave)

The dried peptidyl-resin from General Method A was transferred to the CEM™ microwave synthesiser (CEM Corporation, Mathews, NC) and coupling cycles performed utilising the following conditions: *Fmoc deprotection*: 20% piperidine/ DMF (75 °C, 50 W, 1.5 mL, 2 x 3 min), *Resin wash*: DMF (2 mL, 5 x), *Coupling*: Fmoc-amino acid (5 eq.) with HATU (4.6 eq.), DIPEA (10 eq.) in DMF (1.5 mL) (75 °C, 25 W, 5 min), *Resin wash*: DMF (2 mL, 5 x).

General Method C (single coupling cycle, Tribute™ synthesiser)

The dried peptidyl-resin from General Method A was transferred to the Tribute™ synthesiser (Protein Technologies, Tuscon, Az) and the following couple cycle performed utilising the following coupling conditions: *Single coupling cycle: Fmoc deprotection*: 20% piperidine/ DMF (3 mL, 2 x 5 min), *Resin wash*: DMF (2 mL, 5 x 30 s), *Coupling*: Either HCTU or HATU (2 mL, 0.23 M in DMF, 4.6 eq.) and Fmoc-amino acid (0.5 mmol, 5 eq., N_2, 2 min), DIPEA (0.5 mL, 2 M in NMP, 10 eq, N_2, 45 min); *Resin wash:* DMF (2mL, 2 x 30 s).

General Method D (double coupling cycle, Tribute™ synthesiser)

The dried peptidyl-resin from General Method A was transferred to the Tribute™ synthesiser (Protein Technologies, Tuscon, Az) and coupling cycles performed utilising the following conditions: *Double coupling cycle:* As per General method B with coupling cycles performed twice to improve yields.

General Method E (Fmoc SPPS, Liberty™ microwave synthesiser and piperidine for Fmoc deprotection step)

The dried peptidyl-resin from General Method C was transferred to the Liberty 12 Microwave Peptide Synthesiser (CEM Corporation, Mathews, NC) and coupling cycles performed utilising the following conditions for all amino acids: *Single coupling cycle: Fmoc deprotection*: 40% piperidine/ DMF (3 mL, 2 x 3 min, 75 °C, 25 W), *Resin wash*: DMF (7 mL, 3 x), *Coupling*: HCTU (0.45 M, 4.5 eq.), DIPEA (2 M in NMP, 10 eq.) and Fmoc-amino acid (2.5 mL, 0.2 M in DMF, 5 eq., 5 min, 75 °C, 25 W), *Resin wash:* DMF (3 x 7 mL).

General Method F (double coupling cycle, Tribute™ synthesiser, increased coupling agent eq.)

The dried peptidyl-resin from General method A was transferred to Tribute™ synthesiser (Protein Technologies, Tuscon, Az) and coupling cycles performed utilising the following conditions:

Double coupling cycle: *Deprotection*: 20% piperidine/ DMF (3 mL, 2 x 5 min), *Resin wash*: DMF (2 mL, 5 x 30 s), *Coupling*: HCTU (2 mL, 0.23 M in DMF, 7 eq., 2 min, performed bubbling under N_2), *Base*: *N*-methylmorpholine (NMM, 5.1 M, 10 eq.) in *N*-methyl-2-pyrrolidinone (NMP, 0.5 mL), and amino acid (10 eq.) (20 min, performed bubbling under N_2); *Resin wash:* DMF (2mL, 2 x 30 s).

Second coupling: *Base*: in *N*-methylmorpholine (NMM, 5.1 M, 10 eq.) in *N*-methyl-2-pyrrolidinone (NMP, 0.5 mL), *Coupling*: HCTU (1 mL, 0.23 M in DMF, 7 eq., 2 min, performed bubbling under N_2), and amino acid (10 eq.) (20 min, performed bubbling under N_2); *Resin wash:* DMF (2 mL, 2 x 30 s).

General Method G (acetyl capping of *N*-terminus using acetic anhydride, Tribute™ synthesiser)

The peptidyl resin was dried by washing with methanol and a stream of N_2. The dried peptidyl resin was then transferred to the Tribute™ synthesiser (Protein Technologies, Tuscon, Az) and the following acetyl capping cycle performed: *Fmoc deprotection*: 20% piperidine/ DMF (2 mL, 2 x 7 min), *Resin wash:* DMF (2 mL, 6 x 30 s); *Acetylation*: acetic anhydride (2 mmol, 40 eq.), DIPEA in NMP (0.5 mL, 2 M, 10 eq., N_2, 2 x 20 min); *Resin wash:* DMF (2mL, 2 x 30 s).

General Method H (acetyl capping of *N*-terminus using acetic anhydride/ DIPEA, rotary mixing)

The peptidyl resin was dried by washing with methanol and a stream of N_2. The dried peptidyl resin was then transferred to a round bottomed flask and rotary mixed with a solution of acetic anhydride/ DIPEA/ DMF (20% v/, 20 eq.) for 30 min at rt. The acetylated peptidyl resin was then washed with DMF (5mL, 3 x).

General Method I (resin cleavage method 1 using agitation)

The cleavage cocktail (2 mL, 95% v/v TFA, 2.5% TIPS, 2.5% DI H_2O, v/v/v) was added to the peptidyl resin and agitated for 90 min at rt. The resin was then drained, washed with TFA (2 mL x 2) and concentrated under a stream of nitrogen. The peptide was precipitated from the reaction slurry using cold diethyl ether (20 mL, 4 °C, 20 min) and recovered by centrifugation (5 minutes at 3,000 rpm). The peptide precipitate was then washed with diethyl ether (cold, 10 mL), centrifuged (5 min at 3,000 rpm) dried under a stream of stream of N_2, dissolved in CH_3CN: H_2O (1:1 containing 0.1% TFA) and lyophilised to yield the crude peptide.

General Method J (resin cleavage method 2 using agitation)

Following General Method I but using the following cleavage cocktail (2 mL, 93% v/v TFA, 3.5% TIPS, 3.5% DI H_2O).

General Method K (resin cleavage method 3 using agitation)

Following General Method I but using the following cleavage cocktail (2 mL, 90% v/v TFA, 5% TIPS, 5% DI H_2O).

General method L (deacetylation)

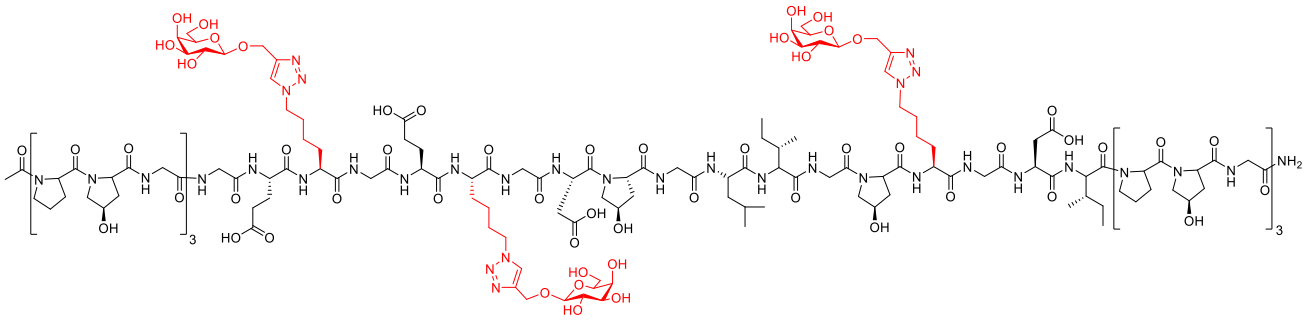
Freshly prepared 1 M NaOMe solution in methanol was added to a solution of the crude peptide in methanol (AR grade) until the pH of the reaction mixture was pH 11. The reaction mixture was stirred at rt for 4 h after which the pH was measured to be 7. Therefore, a further portion of NaOMe solution (1 M) was added to obtain pH 11, and the reaction mixture was stirred at rt for 16 h, neutralised using Dowex H^+ beads, filtered and concentrated *in vacuo*. The pH of the crude peptide solution was adjusted to pH 2 using HCl (2 M) and the product recovered by centrifugation (10 min at 4,000 rpm).

RP-HPLC

Unless otherwise stated, semi-preparative RP-HPLC was performed on a Dionex Ultimate 3000 equipped with a 4 channel UV detector, using a Phenomenex Luna C18 column (5 µm,10 mm x 250 mm) at a flow rate of 5 mL/min.

Synthesis of CMP-Adpn Peptides 4, 5, 6 and 7

CMP-Adpn-Lys-3*neo*Gal-NH_2 4



Peptide **4** was synthesised on a 0.033 mmol scale. The derivatised Fmoc-Lys residue **8** was incorporated into the peptide sequence using the following manual coupling conditions in a CEM™ microwave (CEM Corporation, Mathews, NC) according to **General Method B**. The other non-functionalised amino acids were synthesised using The Tribute™ synthesiser (Protein Technologies, Tuscon, Az). Resin bound Fmoc-**K"**GDI$(GPO)_3NH_2$-Rink-φ was synthesised using **General method C** but using a larger volume of 20% piperidine/ DMF deprotection solution (2 mL instead of 1.5 mL). The peptidyl resin was then elongated using **General Method D** to afford Fmoc-GDOGLIGE**K"**GDI$(GPO)_3NH_2$-Rink-φ. Further elongation of the peptide was then carried out using **General Method E** to afford Fmoc-E**K"**GE**K"**GDOGLIGE**K"**GDI$(GPO)_3NH_2$-Rink-φ but with larger volume of 40% piperidine/ DMF deprotection solution (2 mL instead of 1.5 mL). The final amino acids were then added using **General Method C** using HCTU as the coupling agent and shorter coupling time for each amino acid (10 min instead of 20 min) to afford Fmoc-$(GPO)_3$GE**K"**GE**K"**GDOGLIGO**K"**GDI$(GPO)_3NH_2$-Rink-φ. *N*-Terminal acetylation was conducted using **General Method G** followed by cleavage from resin using **General Method J** to afford the crude *O*-acetylated peptide CMP-Adpn-Lys-3Gal-OAc, which was identified by FI-MS as having the $[M+3H]^{3+}$ (calculated 1560.6, observed 1561.1.1) and $[M+4H]^{4+}$ (calculated 1170.7, observed 1170.9) charged states.

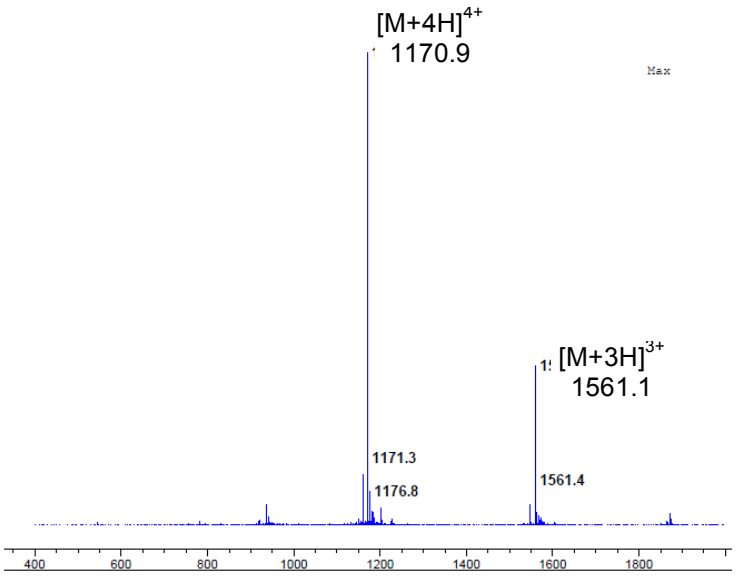


Figure S1 Flow-injection mass spectrometry trace for CMP-Adpn-Lys-3Gal-OAc.

The crude *O*-acetylated peptide CMP-Adpn-Lys-3Gal-OAc (19.2 mg) was then deacetylated according to **General Method K** to afford the crude peptide (3.7 mg, 0.88 µmol) which was purified using RP-HPLC using a gradient of 5 - 40% B, 0.5% B per min to afford the *title compound* **4** as a colourless powder (2.6 mg, 1.9% overall yield including synthesis of acetylated peptide). R_t 12.2 min, linear gradient 5 – 40 % B over 35 min; [where buffer A = 0.1% TFA in DI H_2O, Buffer B = 0.1% TFA in CH_3CN]; LRMS (ESI+) $[M+3H]^{3+}$ 1392.8, calc. 1392.6; $[M+4H]^{4+}$ 1044.9, calc. 1044.7.

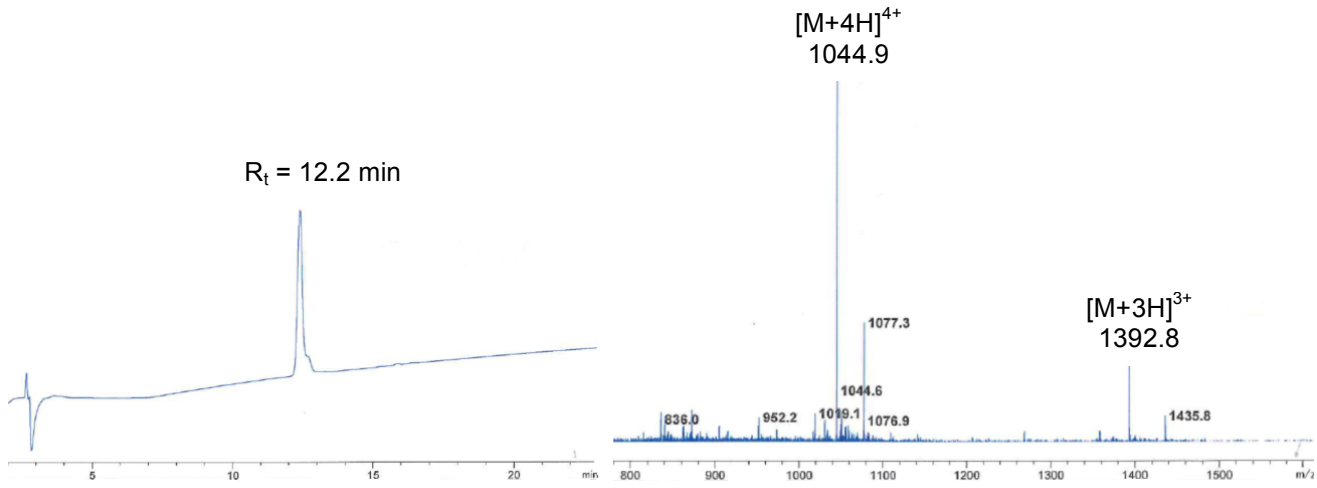
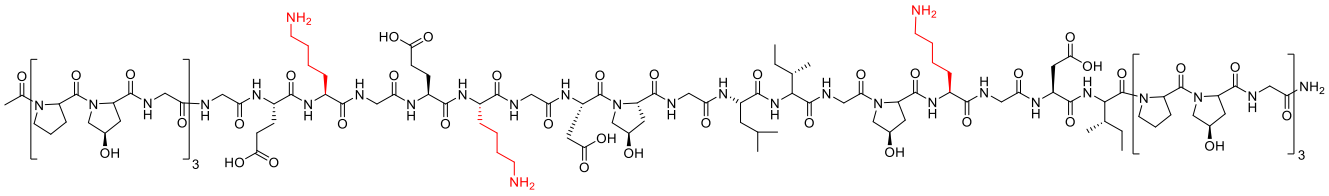


Figure S2 RP-HPLC trace and low resolution mass spectrometry trace for CMP-Adpn-Lys-3*neo*Gal-NH_2 **4**. The analytical RP-HPLC was performed using an analytical column (Phenomenex Gemini C_{18} column (5 µm, 150 mm x 4.6 mm) at a flow rate of 1 mL/min, linear gradient 5 - 40% B over 35 min, where buffer A = 0.1% TFA in DI H_2O, Buffer B = 0.1% TFA in CH_3CN.

CMP-Adpn-Lys 5

Ac-$(GPO)_3(GEK)_2GDOGLIGEKGDI(GPO)_3$-$NH_2$



This peptide was synthesised on a 0.05 mmol scale using 0.85 g/mmol loading. Fmoc-$(GPO)_3(GEK)_2GDOGLIGOKGDI(GPO)_3$-Rink-ϕ was synthesised according to **General Method C** using HCTU/NMM in NMP and piperidine as the coupling and Fmoc-deprotection agents respectively. The peptidyl-resin was then *N*-terminal acetylated using **General Method G** followed by cleavage from resin using **General Method L** to afford the crude peptide (10.7 mg, 31.3 µmol, 31.3% crude yield) which was purified using RP-HPLC using a gradient of 5 – 20% B, 0.2 %B per min to afford the *title compound* **5** as a colourless powder (2.8 mg, 1.6% yield). R_t 12.5 min, linear gradient 5 – 40 % B over 35 min; [where buffer A = 0.1% TFA in DI H_2O, Buffer B = 0.1% TFA in CH_3CN]; LRMS (ESI+) $[M+3H]^{3+}$ 1148.6, calc. 1148.6; $[M+4H]^{4+}$ 861.8, calc. 861.7.

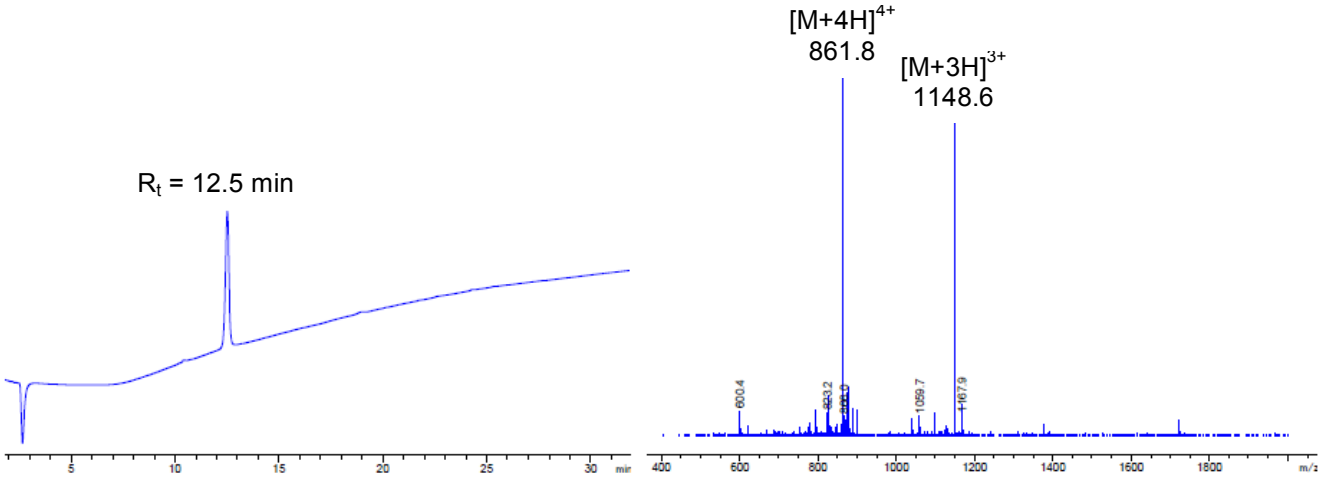
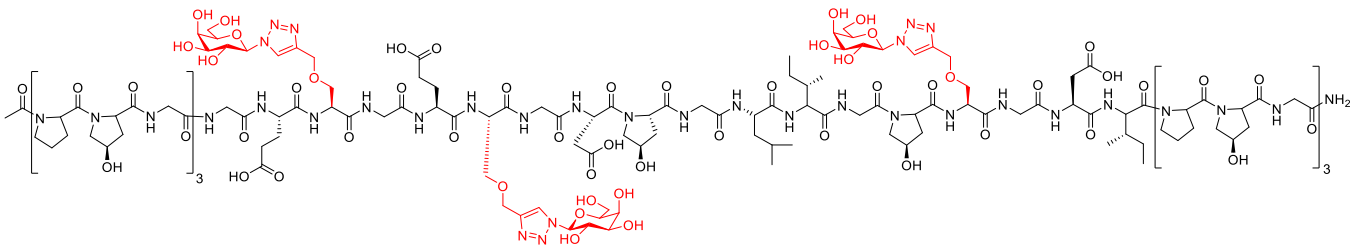


Figure S3 RP-HPLC trace and low resolution mass spectrometry trace for CMP-Adpn-Lys **5**. The analytical RP-HPLC was performed using an analytical column (Phenomenex Gemini C_{18} column (5 µm, 150 mm x 4.6 mm) at a flow rate of 1 mL/min, linear gradient 5 - 40% B over 35 min, where buffer A = 0.1% TFA in DI H_2O, Buffer B = 0.1% TFA in CH_3CN.

CMP-Adpn-Ser-3*neo*Gal-NH_2 6



This peptide synthesis was conducted on a 0.025 mmol scale using 0.85 g/mmol loading. Resin bound Fmoc-GDI$(GPO)_3$Rink-φ was synthesised using the Tribute™ Peptide Synthesiser (Protein Technologies Inc.) according to **General method C**, using HCTU/ NMM in NMP and piperidine as the coupling and Fmoc-deprotection agents respectively. Elongation of the peptidyl resin was conducted using the CEM™ microwave (CEM Corporation, Mathews, NC) according to **General Method B** with HCTU/ DIPEA and piperidine as the as the coupling and Fmoc-deprotection agents respectively. The derivatised Fmoc-Ser residue **14** was coupled into the peptide sequence manually using the CEM™ microwave and **General Method B**. These conditions afforded the peptidy-resin Fmoc-GPOGE**S"**GE**S"**GDOGLIGO**S"**GDI$(GPO)_3$Rink-φ. The final amino acids were incorporated using the Tribute™ Peptide Synthesiser and **General Method F** with HATU/NMM in NMP and piperidine to afford Fmoc-$(GPO)_3$GE**S"**GE**S"**GDOGLIGO**S"**GDI$(GPO)_3$Rink-φ. The peptidyl-resin was then *N*-terminal acetylated using **General Method G** followed by cleavage from resin using **General Method L** to afford the crude *O*-acetylated peptide CMP-Adpn-Ser-3Gal-OAc which was identified by FI-MS as having the $[M+3H]^{3+}$ (calculated 1504.6, observed 1505.1) and $[M+4H]^{4+}$ (calculated 1128.7, observed 1128.7) charged states.

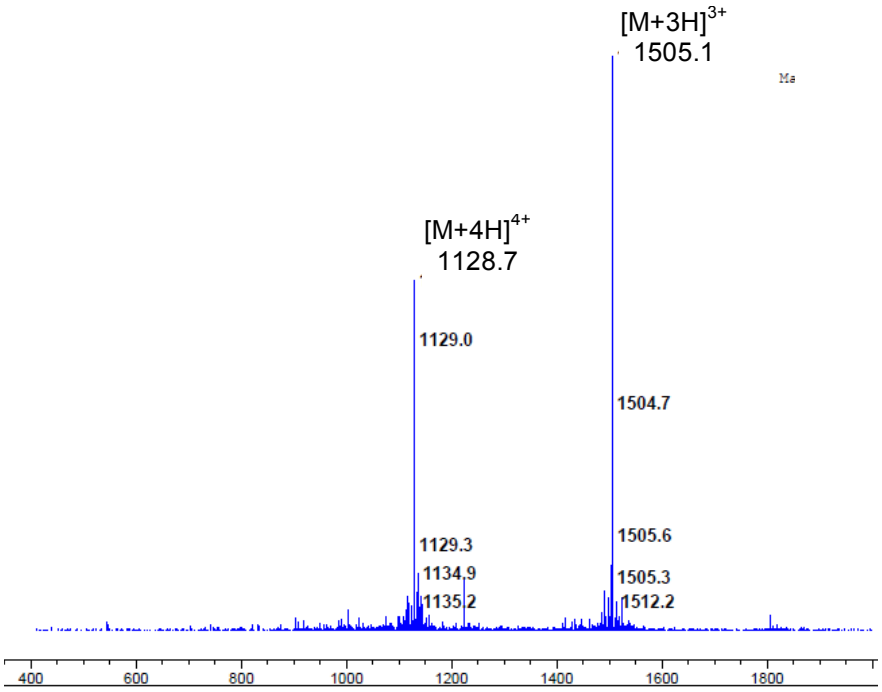


Figure S4 Flow-injection mass spectrometry trace for CMP-Adpn-Ser-3*neo*Gal-OAc

The crude *O*-acetylated peptide (16.7 mg) was then deacetylated according to **General Method K** to afford the crude peptide (4 mg, 0.98 µmol), which was purified using RP-HPLC using a gradient of 5 - 40% B, 0.5% B per min to afford the *title compound* **6** as a colourless powder (0.9 mg, 0.9% overall yield including synthesis of acetylated peptide). R_t 13.6 min, linear gradient 5 – 40 % B over 35 min; [where buffer A = 0.1% TFA in DI H_2O, Buffer B = 0.1% TFA in CH_3CN]; LRMS (ESI+) $[M+3H]^{+3}$ 1350.9, calc. 1350.7; $[M+4H]^{4+}$ 1013.5, calc. 1013.3.

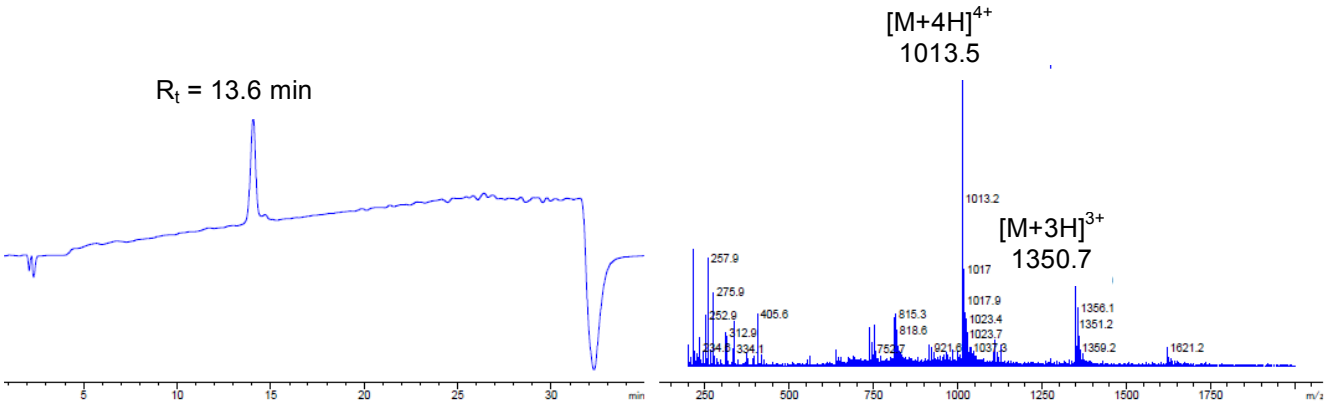
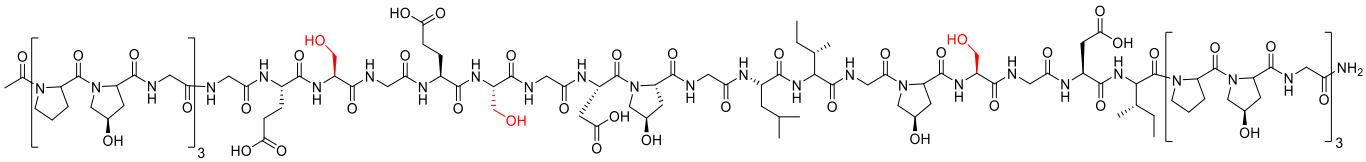


Figure S5 RP-HPLC trace and low resolution mass spectrometry trace for CMP-Adpn-Ser-3*neo*Gal-NH_2 **6**. The analytical RP-HPLC was performed using an analytical column (Phenomenex Gemini C_{18} column (5 µm, 150 mm x 4.6 mm) at a flow rate of 1 mL/min, linear gradient 5 - 40% B over 35 min, where buffer A = 0.1% TFA in DI H_2O, Buffer B = 0.1% TFA in CH_3CN.

CMP-Adpn-Ser 7

Ac-$(GPO)_3$-$(GES)_2$GDOGLIGESGDI$(GPO)_3$-NH_2



This peptide was synthesised on a 0.033 mmol scale using 0.85 g/mmol loading. Fmoc-O$(GES)_2$GDOGLIGOSGDI$(GPO)_3$-Rink-ϕ was synthesised according to **General Method C** (single coupling cycles) using HCTU/NMM in NMP and piperidine as the coupling and Fmoc-deprotection agents respectively. The final amino acids were then incorporated using **General Method D** (double coupling cycles) and using HCTU/NMM in NMP and piperidine as the coupling and Fmoc-deprotection agents respectively to afford Fmoc-$(GPO)_3$-$(GES)_2$GDOGLIGOSGDI$(GPO)_3$-Rink-ϕ**.** The peptidyl-resin was then *N*-terminal acetylated using **General Method H** and then cleaved from resin using **General Method L** to afford the crude peptide (13.5 mg, 4.06 µmol), which was purified batch wise using RP-HPLC using a gradient of 5 – 20 % B, 0.5 % B per min to afford the *title compound* **7** as a colourless powder (4.8 mg, 4.4% overall yield including synthesis of acetylated peptide). R_t 15.8 min, linear gradient 5 – 40 % B over 35 min; [where buffer A = 0.1% TFA in DI H_2O, Buffer B = 0.1% TFA in CH_3CN]; LRMS (ESI+) $[M+2H]^{2+}$ 1660.7, calc. 1660.7; $[M+3H]^{3+}$ 1107.5, calc. 1107.5, $[M+4H]^{4+}$ 831.0, calc. 830.9.

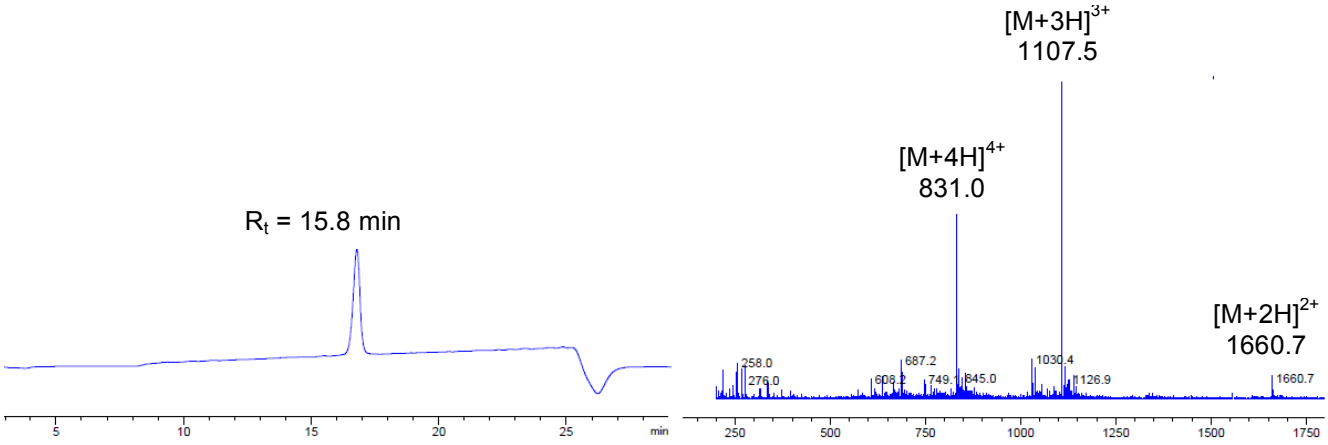


Figure S6 RP-HPLC trace and low resolution mass spectrometry trace for CMP-Adpn-Ser **7**. The analytical RP-HPLC was performed using an analytical column (Phenomenex Gemini C_{18} column (5 µm, 150 mm x 4.6 mm) at a flow rate of 1 mL/min, linear gradient 5 - 40% B over 35 min, where buffer A = 0.1% TFA in DI H_2O, Buffer B = 0.1% TFA in CH_3CN.

General Information for Circular Dichroism Studies and Bond Length Calculations

Circular dichroism spectra were recorded using Applied Photophysics PiStar Spectrometer v.4.2.12 using a cuvette of path length 10 mm (106-QS, Hellma Analytics, Müllheim, Germany). The peptide solutions used were 0.2 mM (by weight using a 4.d.p. balance, Sartorius Analytic, A2005) in 10 mM potassium phosphate buffer at pH 7.4, which had been incubated at 6 °C for a minimum of 15 hours. The spectra were obtained at 6 °C between wavelengths of 180 and 310 nm with a bandwidth of 2 nm, steps of 1 nm, with a 3 s averaging time and averaged from 5 scans. The baseline scans were recorded and averaged using 10 mM potassium phosphate buffer and were subtracted from sample scans and mean molar ellipticity was calculated using Pro-Data Viewer (version 4.1.1, Applied Photophysics Ltd., UK). The thermal transition experiments recorded were run from 15 to 93 °C in 0.5 °C steps with a smooth ramp rate and set at a wavelength of 215 nM.

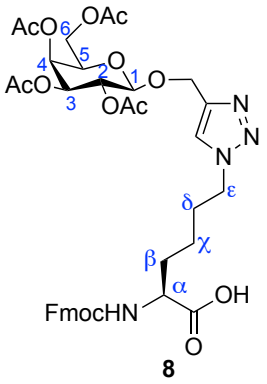
Using the MenD tuberculosis crystal structure provided by Dr Jodie Johnson, School of Biological Sciences, The University of Auckland, the through bond and through space distances between the Cα atom and glycan attachment sites were calculated using ChemDraw 3D (Table 1).

Table 1. Side-chain bond lengths calculated for lysine and serine residues.

Amino acid	Through bond length (Å)	Through space length (Å)
Lysine	7.29	5.63
Serine	2.94	2.45

NMR Spectra For Novel Organic Compounds

(2*S*)-2-[((9*H*-Fluorenyl-9-yl-)methoxy)carbonyl]amino-6-(((2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl(oxy)methyl)-1*H*-1,2,3-triazol-1-yl)hexanoic acid, 8



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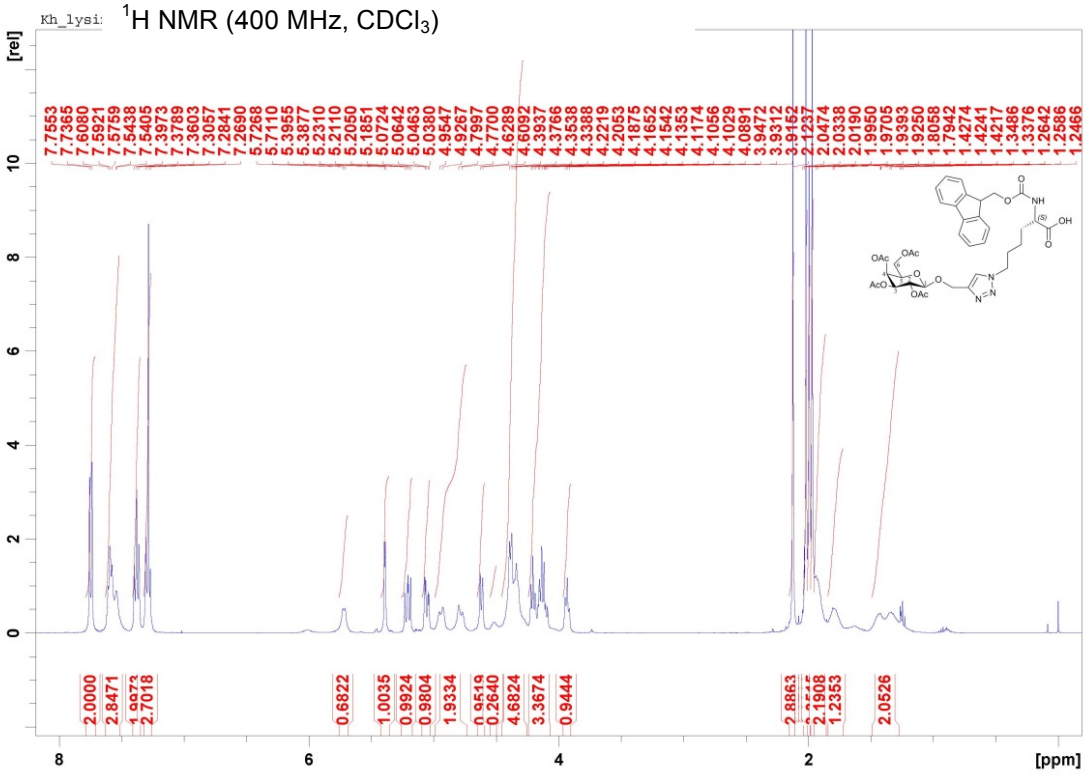
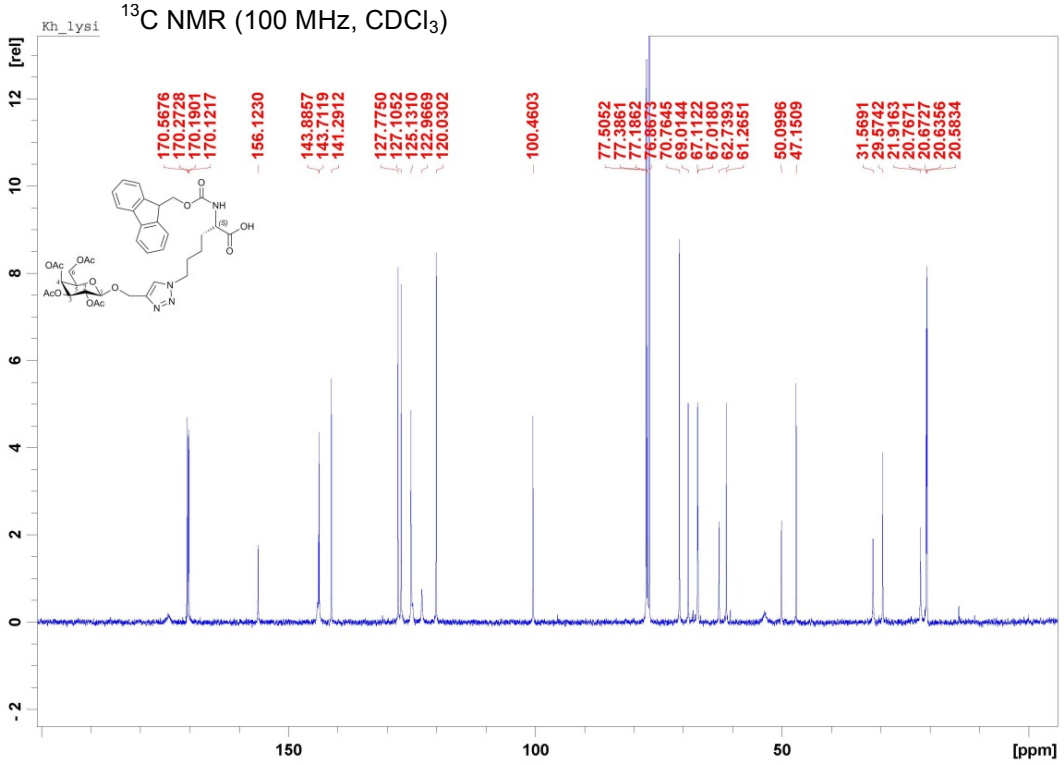
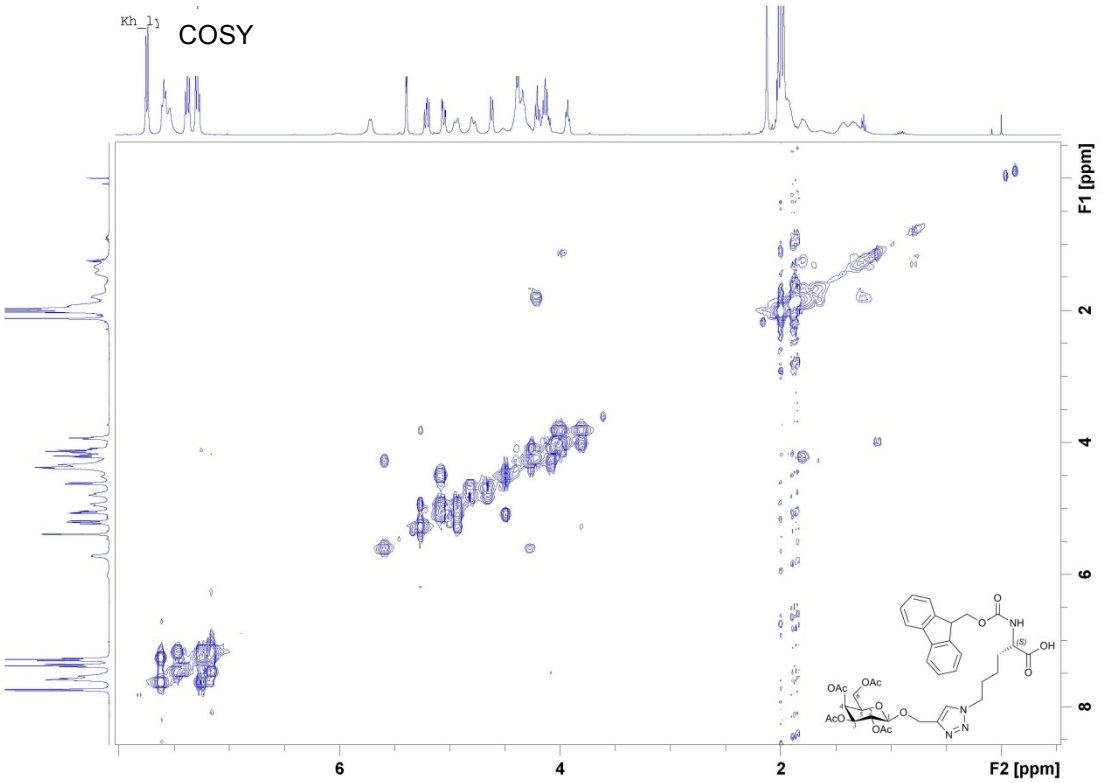


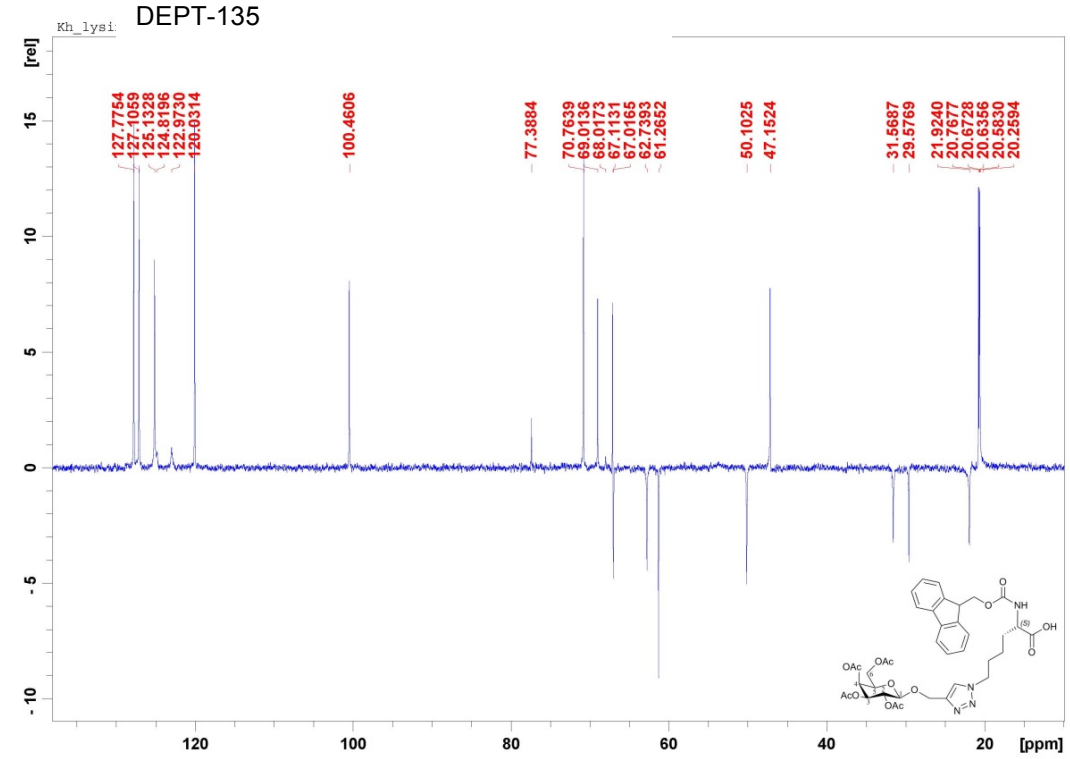
^{13}C NMR (100 MHz, $CDCl_3$)



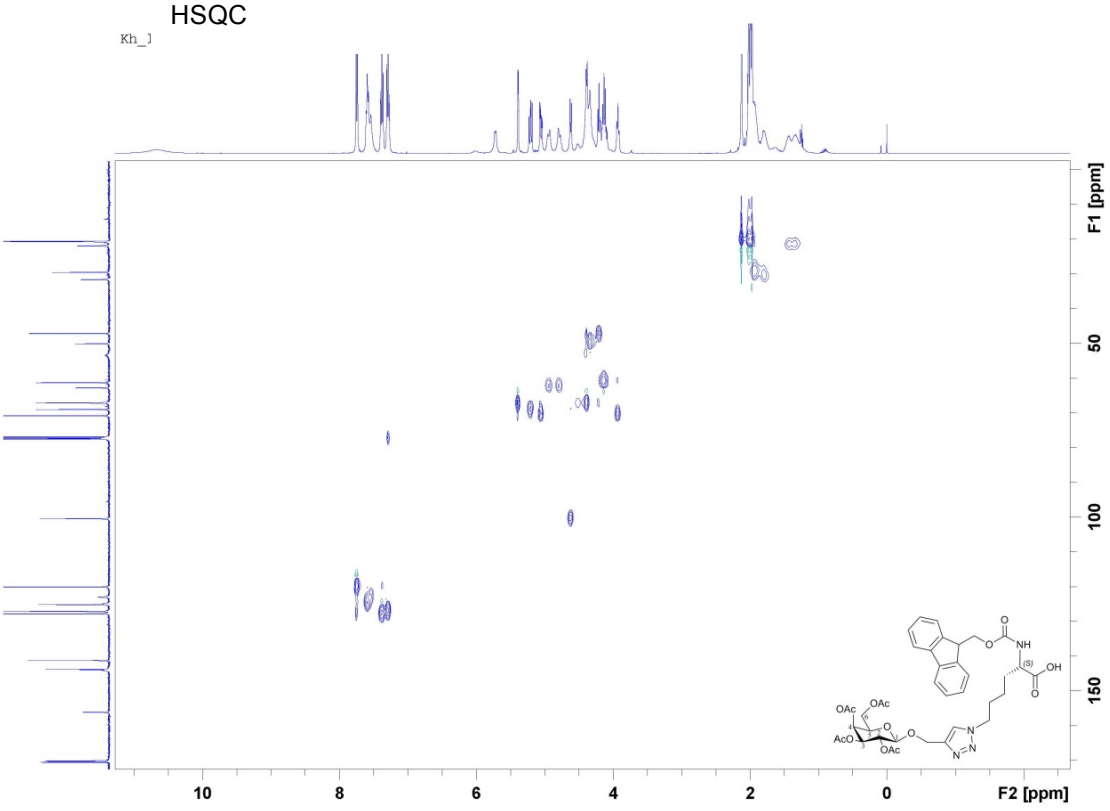
COSY



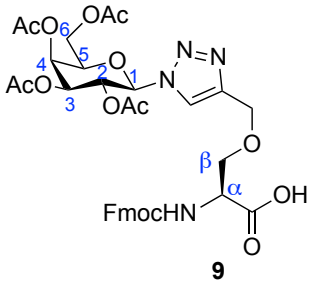
DEPT-135



HSQC

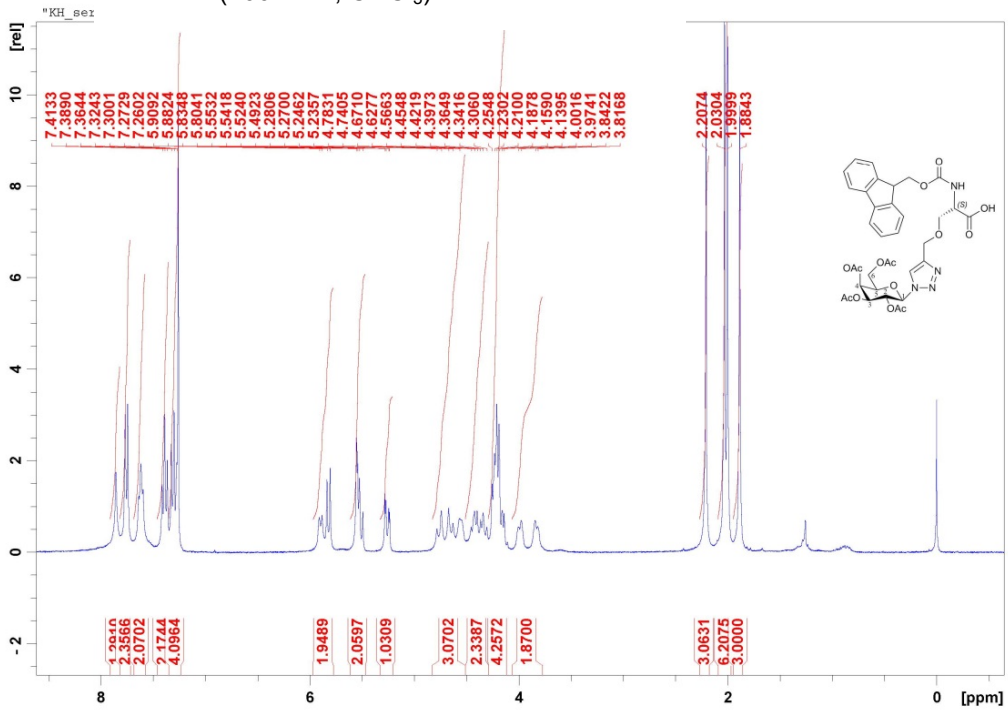


(2*S*)-2-[((9*H*-Fluorenyl-9-yl-)methoxy)carbonyl)amino]-3-(((2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-1*H*-1,2,3-triazol-4-yl)methyl)oxy)propanoic acid, 9

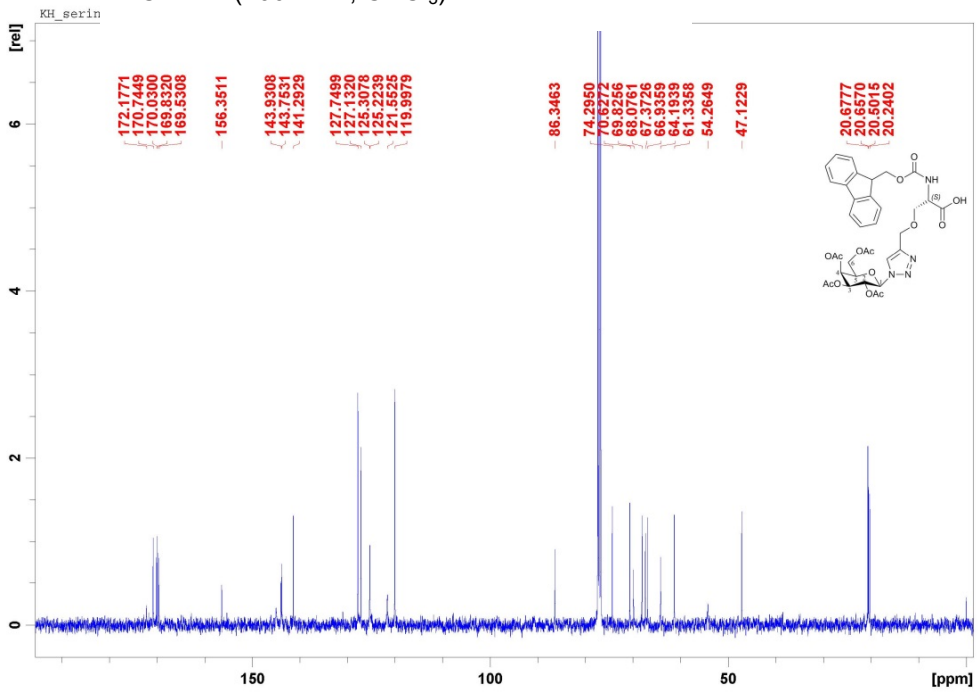


9

^{1}H NMR (400 MHz, $CDCl_3$)



^{13}C NMR (100 MHz, $CDCl_3$)



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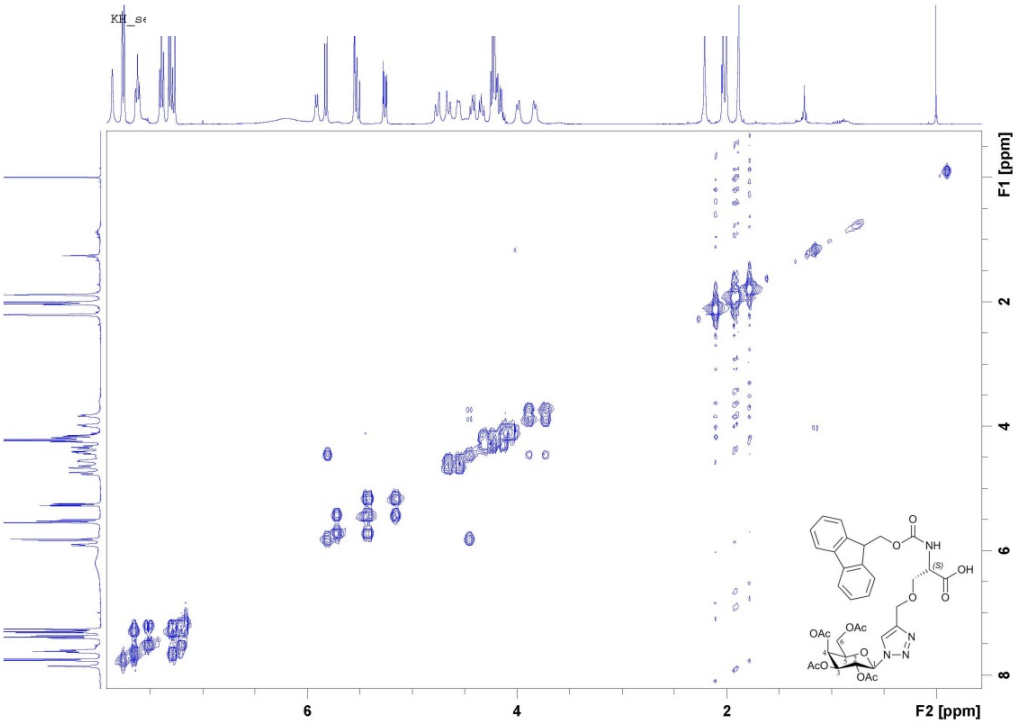
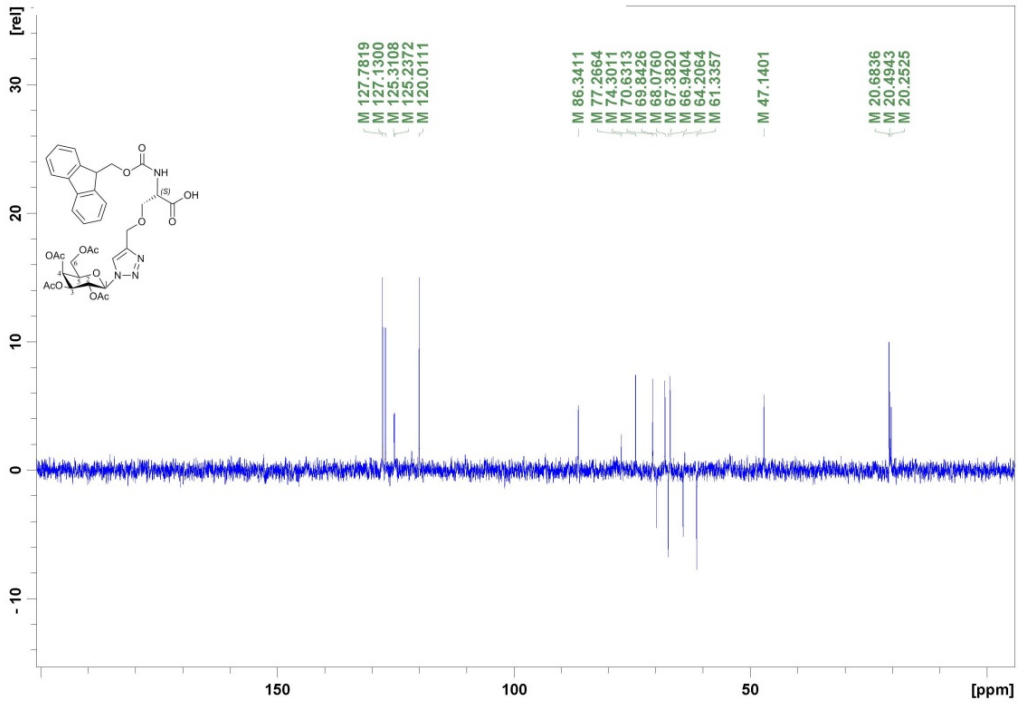
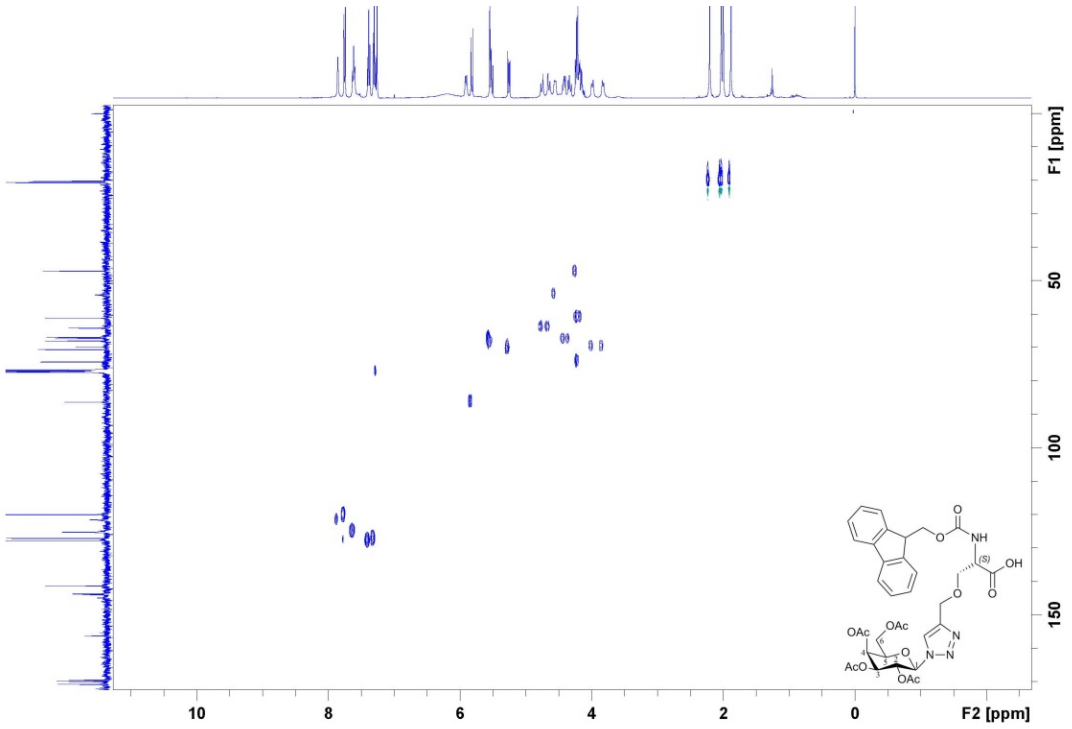


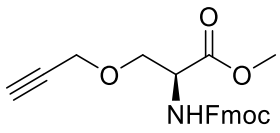
DEPT-135



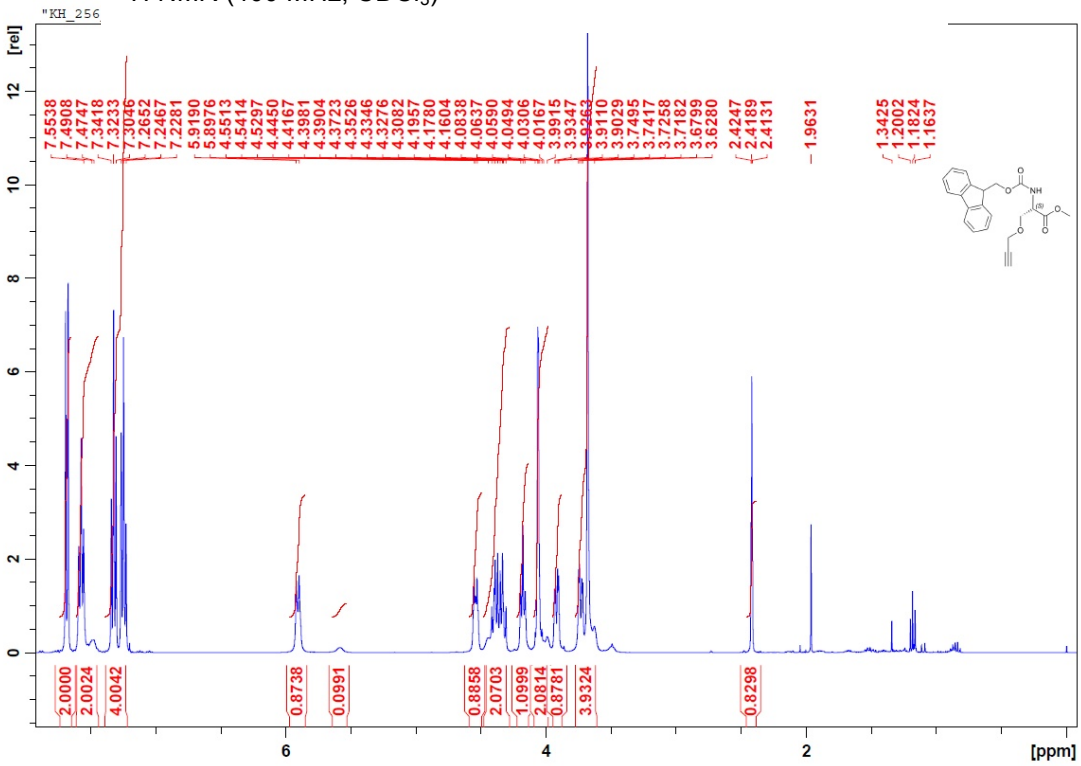
HSQC



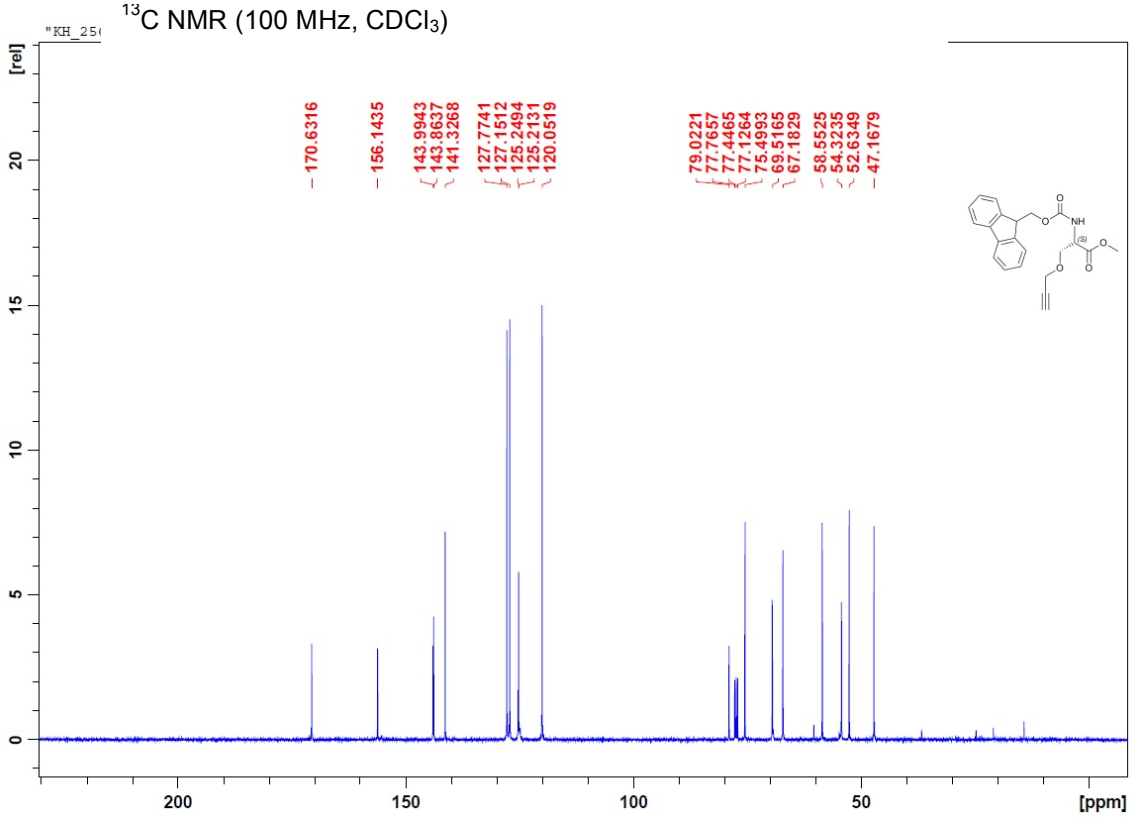
(2*S*)-Methyl-((9*H*-fluorenylmethoxycarbonyl)amino)-3-(prop-2-yn-1-yloxy)propanoate, S8



^{1}H NMR (400 MHz, $CDCl_3$)



^{13}C NMR (100 MHz, $CDCl_3$)



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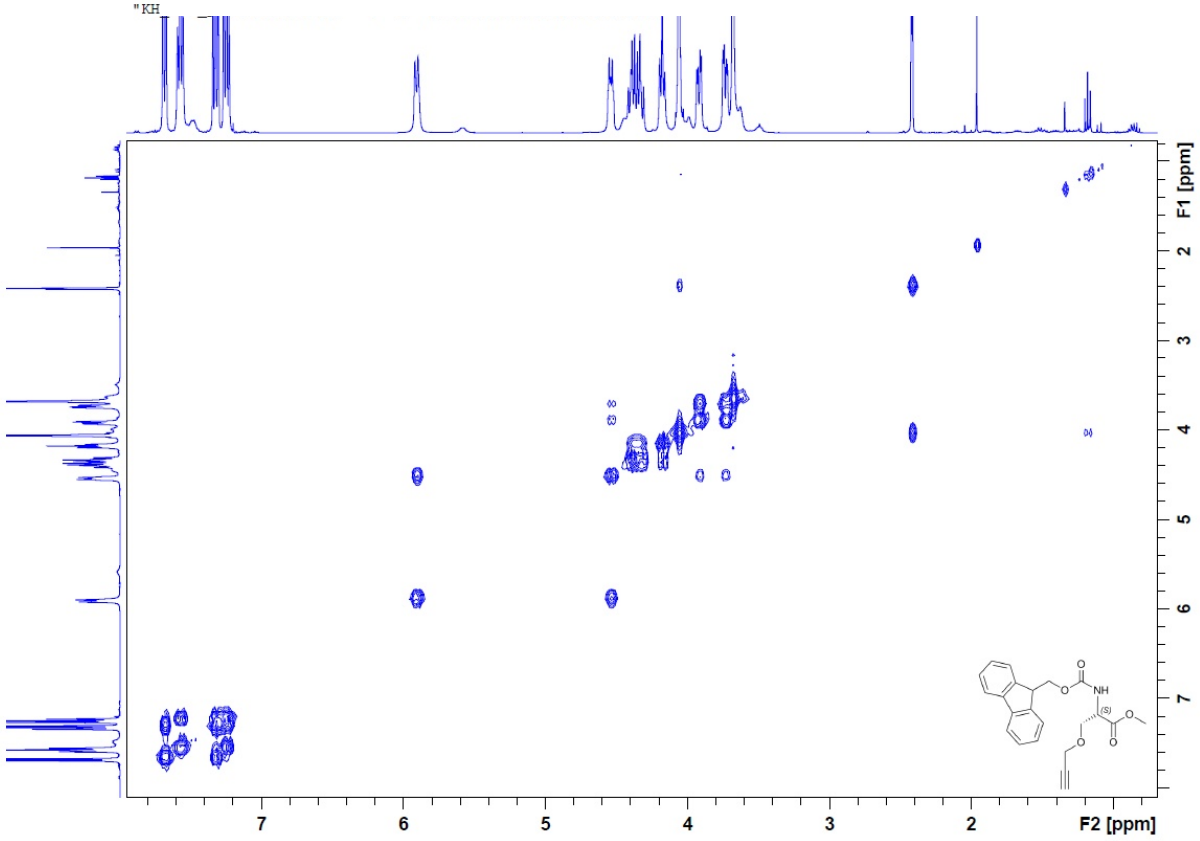

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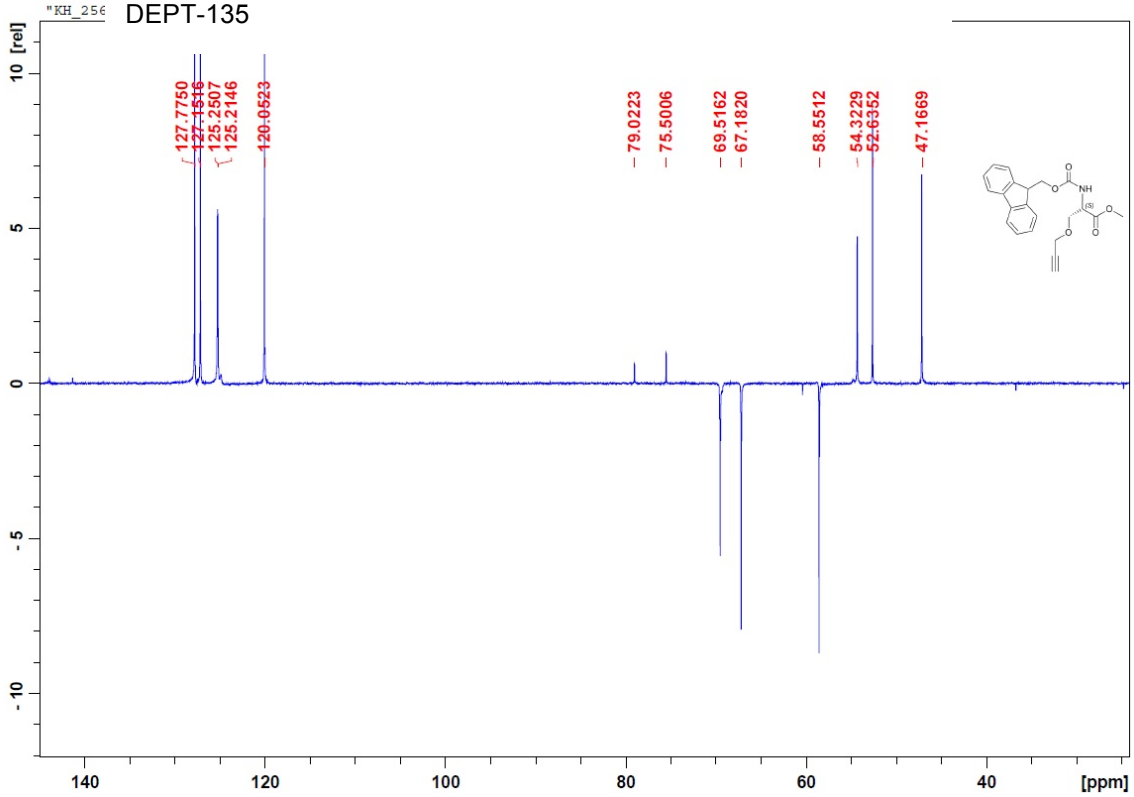

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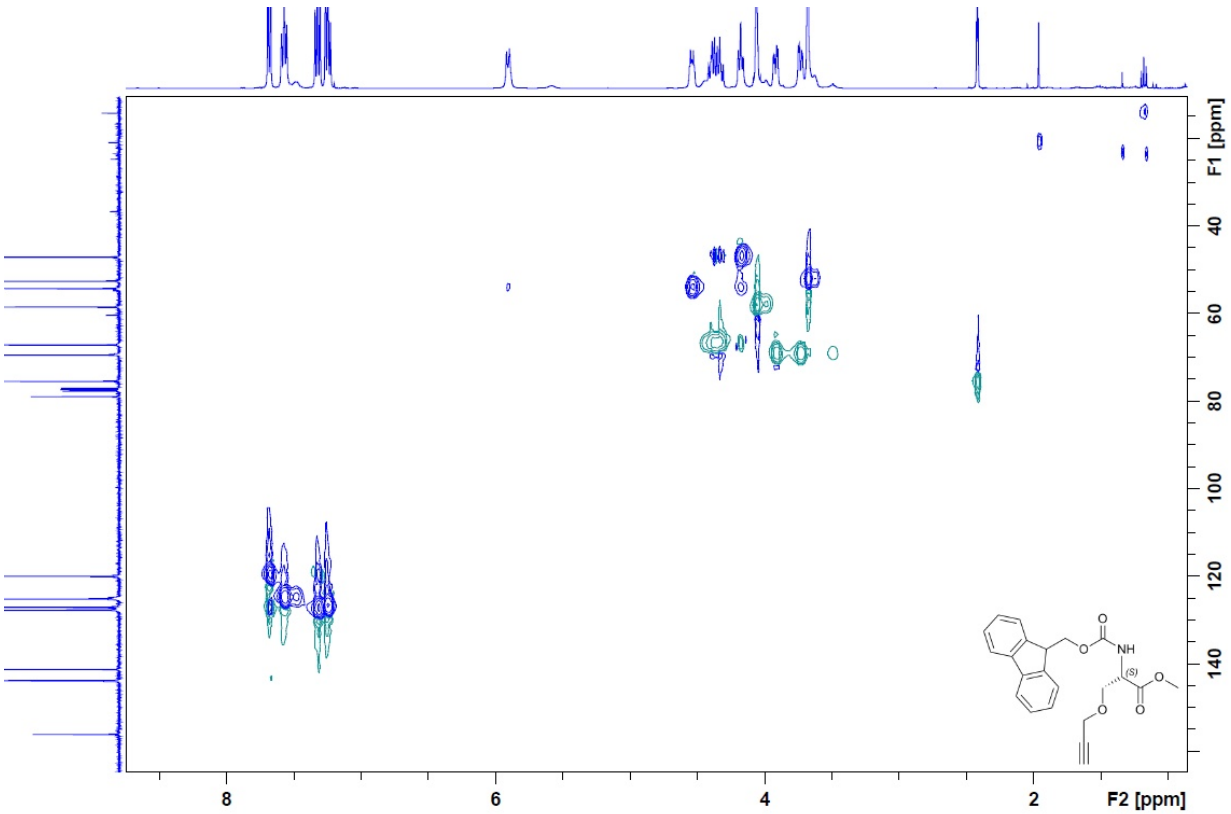

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