SUPPORTING INFORMATION

Design of Multivalent Pharmacological Chaperones against Pompe Disease via Metal-Free Ligation

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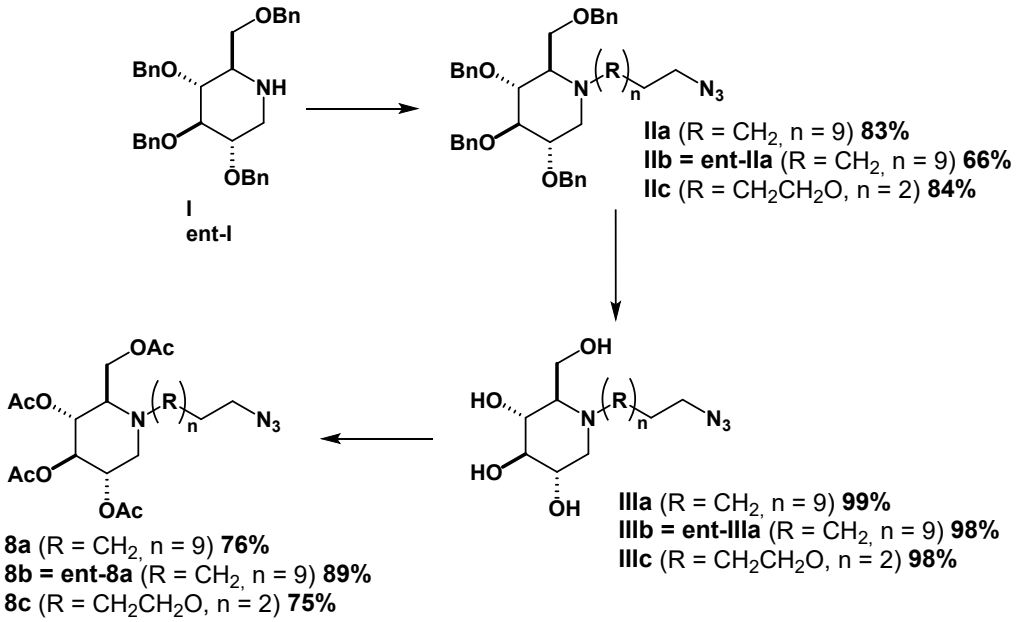
1. Synthesis of new compounds

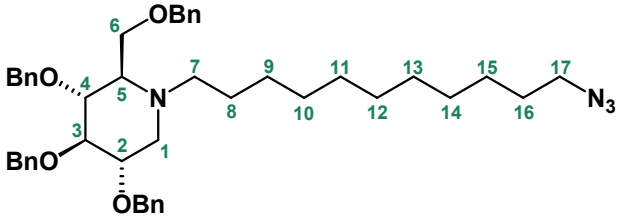
1.1. General synthetic methods

All reagents were obtained from commercial suppliers and used without any further purification. Standard inert atmosphere techniques were used in handling all air and moisture sensitive reagents. CH_2Cl_2 and tetrahydrofuran (THF) were obtained by filtration through a drying column on a filtration system. Dimethylformamide (DMF) and dioxane was purchased anhydrous over molecular sieves. Triethylamine and DIPEA were distilled over KOH under reduced pressure and stored over KOH under nitrogen. Thin-layer chromatography (TLC) analyses were performed on precoated, aluminum-backed silica gel (Merck 60 F254). Visualization of the developed chromatogram was performed by UV light (254 nm) and using 10% phosphomolybdic acid in ethanol, aqueous potassium permanganate ($KMnO_4$). Preparative flash chromatography was performed with SDS silica gel 60 (35-70 µm) from Macherey Nagel. ^{1}H, ^{13}C and ^{31}P NMR spectra were acquired on a Bruker Advance 300, 400 or 500 MHz spectrometer. All spectra were obtained in $CDCl_3$, D_2O or CD_3OD. For $CDCl_3$, spectra were referenced to 7.26 ppm for ^{1}H or 77.16 ppm for ^{13}C, for D_2O to 4.79 for ^{1}H and CD_3OD to 4.87 ppm for ^{1}H or to 49.00 ppm for ^{13}C. NMR data are reported as: chemical shift (δ) in parts per million (ppm), multiplicity (br= broad, s= singlet, d= doublet, t= triplet, q= quartet, p= pentuplet, m= multiplet), coupling constant (J) in Hertz (Hz) and integration. Assignments of ^{1}H and ^{13}C signals were made by DEPT, ^{1}H-^{1}H COSY, HSQC and HMBC experiments. The exponents “1” or “2” will be used for the rotameric protons, and the exponents “A” or “B” will be used for the regioisomeric protons. Dendrimer derivatives synthesis was monitored by mass spectrometry in MALDI-TOF using a Waters Micro MX spectrometer. A 10 mg/mL solution of 2,4,6-trihydroxyacetophenone (THAP) in CH_3OH/H_2O (1:1, v:v) was prepared and used as matrix. A solution of dendrimer derivative was prepared in CH_2Cl_2/CH_3OH (100 µM). Both matrix and compound solution were mixed in 9:1 volume ratio before being deposited onto the plate and dried at rt and atmospheric pressure. High pressure liquid chromatography (HPLC) analyses were performed on a Waters device connected to a PDA (λ= 210 nm or λ= 225 nm or λ= 280 nm) and mass spectrometry for reverse phase analyses. Chiral HPLC analyses were performed to determine enantiomeric excess (ee) with a Lux cellulose-3 column (3 µM, 4.6x150 mm) with a flow of 0.6 $mL.min^{-1}$ or CHIRALPACK® IG column (3 µM; 4.6x100 mm). Mass spectrometry (MS) data were obtained on a ThermoQuest TSQ 7000 spectrometer and high-resolution MS data were obtained with Xevo G2 QTOF spectrometer. Optical rotations were measured at with λ= 589 nm (sodium lamp) a Jasco polarimeter at 20 °C. $[\alpha]_D^{20}$ values are given in $deg.dm^{-1}.cm^3.g^{-1}$. The concentration is indicated in gram per liter.

1.2. Synthesis of alkylated DNJs 8a, 8b and 8c



Compound IIa: (2*R*,3*R*,4*R*,5*S*)-1-(11-azidoundecyl)-3,4,5-tris(benzyloxy)-2-((benzyloxy)methyl)piperidine



Chemical Formula: $C_{45}H_{58}N_4O_4$
Molecular Weight: 718.98 g.mol^{-1}

11-Azidoundecyl 4-methylbenzenesulfonate[1] (365 mg, 0.99 mmol) and K_2CO_3 (183 mg, 1.32 mmol) were added to a solution of compound **I** (215 mg, 0.41 mmol) in dry ACN (2.50 mL) under N_2. The reaction mixture was stirred at 85 °C for 4 days. Then, the mixture was diluted with $CHCl_3$ (5 mL) and concentrated. The residue was diluted with $CHCl_3$ (5 mL) and water (5mL), the aqueous phase was extracted with $CHCl_3$ (3 x 5 mL), the organic phases were collected, dried over Na_2SO_4, and concentrated. The crude product was purified by column chromatography (SiO_2, P.E./E.A. 85:15) to give **IIa** as a yellow oil with a 83% yield (245 mg, 0.34 mmol).

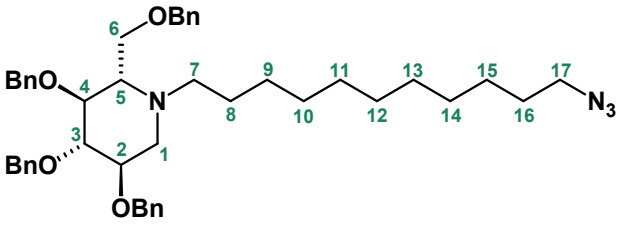
R_f 0.60 (P.E./E.A. 70:30, $KMnO_4$)

^{1}H-NMR ($CDCl_3$, 300 MHz): δ (ppm) 7.40-7.07 (m, 20H, H_{Bn}), 4.96 (d, $J_{Bn\text{-}Bn}$ = 11.2, 1H, H_{Bn}), 4.88 (d, $J_{Bn\text{-}Bn}$ = 11.0, 1H, H_{Bn}), 4.81 (d, *J* = 11.2, 1H, H_{Bn}), 4.67 (dd, *J* = 15.1, 11.6, 2H, H_{Bn}), 4.48 (dd, *J* = 13.6, 12.4, 2H, H_{Bn}), 4.41 (d, *J* = 11.0, 1H, H_{Bn}), 3.71-3.41 (m, 5H, $H_{2+3+4+6}$), 3.26 (t, *J* = 6.9, 1H, H_{17}), 3.09 (dd, *J* = 11.1, 5.0, 1H, H_1), 2.72-2.50 (m, 2H, H_7), 2.34-2.27 (m, 1H, H_5), 2.23 (t, *J* = 11.1, 1H, $H_{1'}$), 1.68-1.49 (m, 4H, H_{8+16}), 1.47-1.07 (m, 14H, $H_{9\text{-}15}$).

^{13}C-NMR ($CDCl_3$, 75 MHz): δ (ppm) 139.2-138.7-138.0 (C_{Bn}), 128.6-128.5-128.5-128.5-128.0-128.0-128.0-127.8-127.7-127.6 (C_{Bn}), 87.5 (C_3), 78.7-78.7 (C_{2+4}), 75.5-75.4-73.6-72.9 (C_{Bn}), 65.4 (C_6), 63.8 (C_5), 54.6 (C_1), 52.5 (C_7), 51.6 (C_{17}), 29.7-29.7-29.7-29.7-29.3-29.0-27.6-26.9-23.7 (C_{8-16}).

HR-MS (ESI) m/z calculated for: $C_{45}H_{59}N_4O_4$ $[M+H]^+$: 719.4536; found: 719.4539

Compound IIb: (2*S*,3*S*,4*S*,5*R*)-1-(11-azidoundecyl)-3,4,5-tris(benzyloxy)-2-((benzyloxy)methyl)piperidine



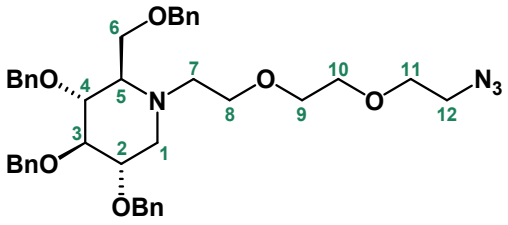
Chemical Formula: $C_{45}H_{58}N_4O_4$
Molecular Weight: 718.98 g.mol^{-1}

11-Azidoundecyl 4-methylbenzenesulfonate[1] (525 mg, 1.430 mmol) and K_2CO_3 (263.70 mg, 1.908 mmol) were added to a solution of compound **ent-I** (240 mg, 0.458 mmol) in ACN dry (2.50 mL) under N_2. The reaction mixture was stirred at 85 °C for 4 days. Then, the mixture was diluted with $CHCl_3$ (5 mL) and concentrated. The residue was diluted with $CHCl_3$ (5 mL) and water (5mL), the aqueous phase was extracted with $CHCl_3$ (3 x 5 mL), the organic phases were collected, dried over Na_2SO_4, and concentrated. The crude product was purified by column chromatography (SiO_2, P.E./E.A. 85:15) to give **IIb** as a yellow oil with a 66% yield (217 mg, 0.30 mmol).

R_f 0.60 (P.E./E.A. 70:30, $KMnO_4$)

Spectral data identical to compound **IIa**.

Compound IIc: (2*R*,3*R*,4*R*,5*S*)-1-(2-(2-(2-azidoethoxy)ethoxy)ethyl)-3,4,5-tris(benzyloxy)-2-((benzyloxy)methyl)piperidine



Chemical Formula: $C_{40}H_{48}N_4O_6$
Molecular Weight: 680.85 g.mol^{-1}

2-(2-(2-Azidoethoxy)ethoxy)ethyl 4-methylbenzenesulfonate[2] (236 mg, 0.72 mmol) and K_2CO_3 (111 mg, 0.80 mmol) were added to a solution of compound **I** (150 mg, 0.29 mmol) in dry ACN (2.00 mL) under N_2. The reaction mixture was stirred at 85 °C for 4 days. Then, the mixture was diluted with $CHCl_3$ (15 mL) and concentrated. The residue was diluted with $CHCl_3$ (15 mL) and water (15mL), the aqueous phase was extracted with $CHCl_3$ (3 x 15 mL), the organic phases were collected, dried over Na_2SO_4, and concentrated.

The crude product was purified by column chromatography (SiO_2, DCM/E.A. 96:4 to 70:30) to give **IIc** as a yellow oil with a 84% yield (164 mg, 0.24 mmol).

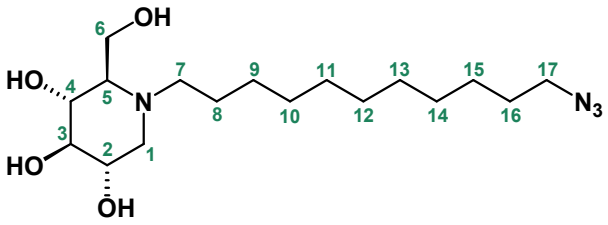
R_f 0.15 (P.E./E.A. 80:20, $KMnO_4$)

^{1}H-NMR ($CDCl_3$, 300 MHz): δ (ppm) 7.40-7.11 (m, 20H, H_{Bn}), 4.99 (d, *J* = 11.7, 1H, H_{Bn}), 4.90 (d, *J* = 10.9, 1H, H_{Bn}), 4.84 (d, *J* = 11.7, 1H, H_{Bn}), 4.75-4.64 (m, 2H, H_{Bn}), 4.53 (d, *J* = 12.0, 1H, H_{Bn}), 4.44 (d, *J* = 12.0, 1H, H_{Bn}), 4.42 (d, *J* = 10.9, 1H, H_{Bn}), 3.82-3.45 (m, 13H, $H_{2\text{-}4+6+peg}$), 3.29 (t, *J* = 5.1 H, 2H, H_{12}), 3,20 (dd, *J* = 11.2, 5.2, 1H, H_1), 2.97 (t, *J* = 5.6, 2H, H_7), 2,53-2,37 (m, 2H, H_5).

^{13}C-NMR ($CDCl_3$, 75 MHz): δ (ppm) 138.6-138.0-136.2 (C_{Bn}), 128.6-128.5-128.5-128.0-128.0-127.9 (C_{Bn}), 87.4 (C_3), 78.8-77.4 (C_{2+4}), 75.3-73.5-72.8-70.8-70.6-70.2-68.1 ($C_{Bn+8\text{-}11}$), 65.9 (C_6), 64.2 (C_5), 57.4 (C_1), 55.4 (C_7), 50.8 (C_{12}).

HR-MS (ESI) m/z calculated for: $C_{40}H_{49}N_4O_6$ $[M+H]^+$: 681.3652; found: 681.3660

Compound IIIa: (2*R*,3*R*,4*R*,5*S*)-1-(11-azidoundecyl)-2-(hydroxymethyl)piperidine-3,4,5-triol



Chemical Formula: $C_{17}H_{34}N_4O_4$
Molecular Weight: 358.48 g.mol^{-1}

BCl_3 (2.15 mL, 1M solution, 2.15 mmol) was added to a solution of **IIa** (230 mg, 0.32 mmol) in DCM (3.80 mL) at -60 °C under N_2. The reaction mixture was allowed to warm to 0 °C and stirred for 4 h. Then, the mixture was quenched with water/MeOH (1:4, 10 mL), concentrated, then washed with the same mixture (3 x 10 mL) to give **IIIa** as a yellow oil with a 99% yield (114 mg, 0.32 mmol).

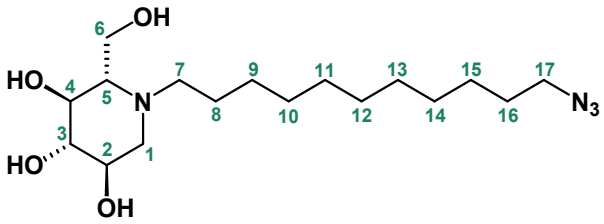
R_f 0.45 (DCM/MeOH 80:20, $KMnO_4$)

^{1}H-NMR (CD_3OD, 300 MHz): δ (ppm) 3.97 (AB part of an ABX system, $\Delta\delta$= 0.13, $J_{A\text{-}B}$ = 12.6, $J_{A\text{-}X}$ = 2.7, $J_{B'X}$ = 1.7, 2H, $H_{6+6'}$), 3.70 (ddd, *J* = 11.0, 9.1, 4.8, 1H, H_2), 3.56 (t, *J* = 9.6, 1H, H_4), 3.41-3.16 (m, 5H, containing at 3.27 (t, *J* = 6.5, 2H, H_{17}) and H_{3+1+7}), 3.14-2.99 (m, 1H, $H_{7'}$), 2.94-2.73 (m, 2H, $H_{1'+5}$), 1.84-1.50 (m, 4H, H_{8+16}), 1.45-1.27 (m, 14H, $H_{9\text{-}15}$).

^{13}C-NMR (CD_3OD, 75 MHz): δ (ppm) 78.4 (C_3), 69.5 (C_4), 68.4 (C_2), 67.3 (C_5), 56.2 (C_6), 55.3 (C_1), 54.0 (C_7), 52.4 (C_{17}), 30.5-30.5-30.5-30.2-30.2-29.8-27.8-27.7-24.4 ($C_{8\text{-}16}$).

HR-MS (DCI-CH_4) m/z calculated for: $C_{17}H_{35}N_4O_4$ $[M+H]^+$: 359.2658; found: 359.2643

Compound IIIb: (2*S*,3*S*,4*S*,5*R*)-1-(11-azidoundecyl)-2-(hydroxymethyl)piperidine-3,4,5-triol

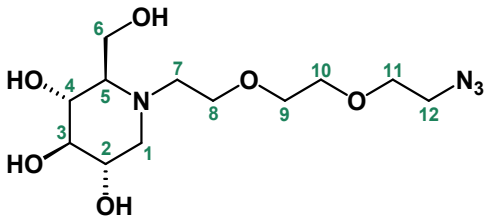


Chemical Formula: $C_{17}H_{34}N_4O_4$
Molecular Weight: 358.48 g.mol^{-1}

BCl_3 (2.00 mL, 1M solution, 2.00 mmol) was added to a solution of **IIb** (215 mg, 0.30 mmol) in DCM (3 mL) at -60 °C under N_2. The reaction mixture was allowed to warm to 0 °C and stirred for 4 h. Then, the mixture was quenched with water/MeOH (1:4, 10 mL), concentrated, then washed with the same solution (3 x 10 mL) to give **IIb** as a yellow oil with a 98% yield (105 mg, 0.29 mmol).

Spectral data identical to compound **IIIa**.

Compound IIIc: (2*R*,3*R*,4*R*,5*S*)-1-(2-(2-(2-azidoethoxy)ethoxy)ethyl)-2-(hydroxymethyl)piperidine-3,4,5-triol



Chemical Formula: $C_{12}H_{24}N_4O_6$
Molecular Weight: 320.35 g.mol^{-1}

BCl_3 (3.00 mL, 1M solution, 3.00 mmol) was added to a solution of **IIc** (305 mg, 0.45 mmol) in DCM (4.50 mL) at -60 °C under N_2. The reaction mixture was allowed to warm to 0 °C and stirred for 4 h. Then, the mixture was quenched with water/MeOH (1:4, 10 mL), concentrated, then washed with the same mixture (3 x 10 mL), concentrated. The crude product was purified by column chromatography (SiO_2, DCM/MeOH 80:20) to give **IIIc** as a yellow oil with a 98% yield (140 mg, 0.44 mmol).

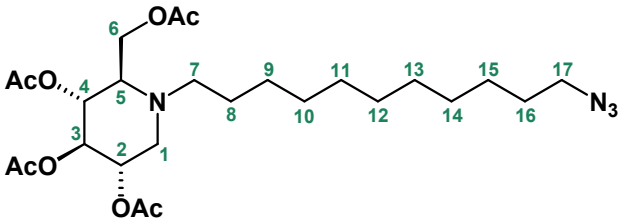
R_f 0.27 (DCM/MeOH 80:20, $KMnO_4$)

^{1}H-NMR (CD_3OD, 300 MHz): δ (ppm) 3.99 (AB part of an ABX system, $\Delta\delta$= 0.04, $J_{A\text{-}B}$ = 12.5, $J_{A\text{-}X}$ = 3.0, $J_{B\text{-}X}$ = 2.5, 2H, $H_{6+6'}$), 3.83 (t, J = 5.0, 2H, H_8), 3.75-3.62 (m, 7H, $H_{2+9\text{-}11}$), 3.57 (t, J = 9.0, 1H, H_4), 3.48-3.15 (m, 6H, $H_{3+1+7+7'}$), 2.96-2.79 (m, 2H, $H_{1'+5}$).

^{13}C-NMR (CD_3OD, 75 MHz): δ (ppm) 78.4 (C_3), 71.3-71.2-70.9-67.0 ($C_{8\text{-}11}$), 69.8 (C_4), 68.7 (C_2), 67.8 (C_5), 56.7 (C_6), 56.1 (C_1), 53.3 (C_7), 51.6 (C_{12}).

HR-MS (DCI-CH_4) m/z calculated for: $C_{12}H_{25}N_4O_6$ $[M+H]^+$: 321.1774; found: 321.1772

Compound 8a: (2*R*,3*R*,4*R*,5*S*)-2-(acetoxymethyl)-1-(11-azidoundecyl)piperidine-3,4,5-triyl triacetate



Chemical Formula: $C_{25}H_{42}N_4O_8$
Molecular Weight: 526.63 g.mol^{-1}

DMAP (6.70 mg, 0.055 mmol) was added to a solution of **IIIa** (55 mg, 0.15 mmol) in Ac_2O (4.50 mL) and pyridine (4.50 mL) under N_2. The reaction mixture was stirred at rt for 4 h. Then, the mixture was cooled at 0 °C, diluted with water (10 mL), extracted with DCM (3 x 10 mL). The collected organic phases were dried over Na_2SO_4, and co-evaporated with toluene (3 x 10 mL). The crude product was purified by column chromatography (SiO_2, P.E./E.A. 80:20) to give **8a** as a yellow oil with a 84% yield (67.3 mg, 0.13 mmol).

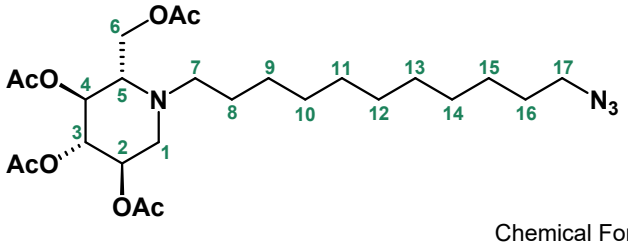
R_f 0.64 (P.E./E.A. 60:40, $KMnO_4$)

^{1}H-NMR ($CDCl_3$, 300 MHz): δ (ppm) 5.08-4.87 (m, 3H, $H_{2\text{-}4}$), 4.11 (d, *J* = 2.8, 2H, H_6), 3.22 (t, *J* = 7.3, 2H, H_{17}), 3.16 (dd, *J* = 11.2, 5.8, 1H, H_1), 2.76-2.45 (m, 3H, $H_{5+7+7'}$), 2.29 (dd, *J* = 11.2, 10.3, 1H, $H_{1'}$), 2.03 (s, 3H, H_{Ac}), 1.98 (s, 6H, H_{Ac}), 1.97 (s, 3H, H_{Ac}), 1.62-1.10 (m, 18H, $H_{8\text{-}16}$).

^{13}C-NMR ($CDCl_3$, 75 MHz): δ (ppm) 170.9-170.4-170.1-169.8 (C_{Ac}), 74.7 (C_3), 69.6, 69.5 (C_{2+4}), 61.9 (C_5), 59.5 (C_6), 52.9 (C_1), 51.8 (C_7), 51.5 (C_{17}), 29.6-29.5-29.5-29.5-29.5-29.2-28.9-27.2-26.7-24.6 ($C_{8\text{-}16}$), 20.9-20.9-20.8-20.7 (C_{Ac}).

HR-MS (ESI) m/z calculated for: $C_{17}H_{35}N_4O_4$ $[M\text{-}4Ac+5H]^+$: 359.2658; found: 359.2665

Compound 8b: (2*S*,3*S*,4*S*,5*R*)-2-(acetoxymethyl)-1-(11-azidoundecyl)piperidine-3,4,5-triyl triacetate



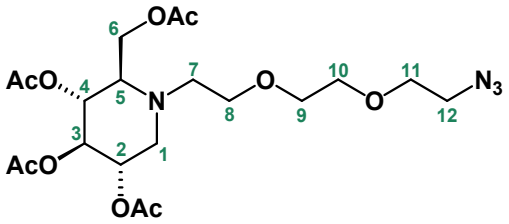
Chemical Formula: $C_{25}H_{42}N_4O_8$
Molecular Weight: 526.63 g.mol^{-1}

IIIb (89.7 mg, 0.248 mmol) was added to a solution of Ac_2O (5.00 mL) and pyridine (5.00 mL) under N_2. The reaction mixture was stirred at rt for 5 h. Then, the mixture was cooled at 0 °C, diluted with water (10 mL), extracted with DCM (3 x 10 mL). The collected organic phases were dried over Na_2SO_4, and co-evaporated with toluene (3 x 10 mL). The crude product does not require any further purification to give **8b** as a colorless oil with 89% yield (116 mg, 0.22 mmol).

R_f 0.64 (P.E./E.A. 60:40, $KMnO_4$)

Spectral data identical to compound **8a**.

Compound 8c: (2*R*,3*R*,4*R*,5*S*)-2-(acetoxymethyl)-1-(2-(2-(2-azidoethoxy)ethoxy)ethyl)piperidine-3,4,5-triyl triacetate



Chemical Formula: $C_{20}H_{32}N_4O_{10}$
Molecular Weight: 488.49 $g.mol^{-1}$

DMAP (5.00 mg, 0.04 mmol) was added to a solution of **IIIc** (130 mg, 0.41 mmol) in Ac_2O (6.00 mL) and pyridine (6.00 mL) under N_2. The reaction mixture was stirred at rt for 4 h. Then, the mixture was cooled at 0 °C, diluted with water (10 mL), extracted with DCM (3 x 10 mL). The collected organic phases were dried over Na_2SO_4, and co-evaporated with toluene (3 x 10 mL). The crude product does not require any further purification to give **8c** as a colorless oil with a 76% yield (116 mg, 0.31 mmol).

R_f 0.62 (P.E./E.A. 50:50, $KMnO_4$)

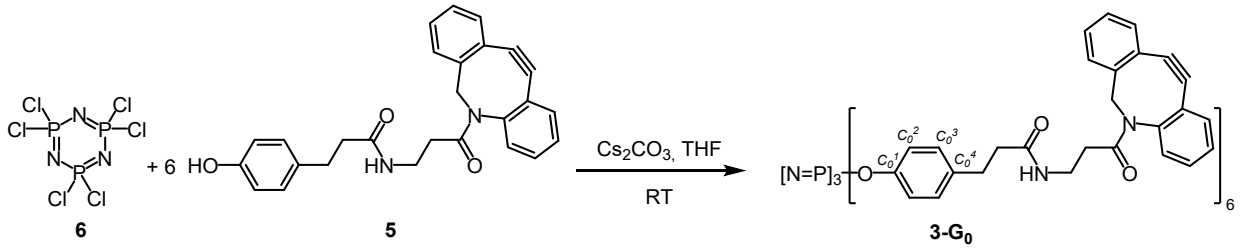
^{1}H-NMR ($CDCl_3$, 300 MHz): δ (ppm) 5.08-4.81 (m, 3H, $H_{2\text{-}4}$), 4.20 (AB part of an ABX system, $\Delta\delta$= 0.10, $J_{A\text{-}B}$ = 12.3, $J_{A\text{-}X}$ = 2.2, $J_{B\text{-}X}$ = 1.6, 2H, $H_{6+6'}$), 3.67-3.44 (m, 8H, $H_{8\text{-}11}$), 3.35 (t, *J* = 4.7, 2H, H_{12}), 3.18 (dd, *J* = 11.8, 4.3, 1H, H_1), 3.02-2.72 (m, 3H, $H_{5+7+7'}$), 2.50 (t, *J* = 10.7, 1H, $H_{1'}$), 2.02 (s, 3H, H_{Ac}), 1.96 (s, 6H, H_{Ac}).

^{13}C-NMR ($CDCl_3$, 75 MHz): δ (ppm) 171.0-170.4-170.0-169.7 (C_{Ac}), 74.7 (C_3),70.7-70.6-70.1-68.4 ($C_{8\text{-}11}$), 69.5 (C_{2+4}), 61.4 (C_5), 59.6 (C_6), 53.5 (C_1), 50.8 (C_7), 50.7 (C_{12}), 20.9-20.9-20.8-20.7 (C_{Ac}).

HR-MS (DCI-CH_4) m/z calculated for: $C_{20}H_{33}N_4O_{10}$ $[M+H]^+$: 489.2197; found: 489.2194

1.3. Synthesis of dendrimer 3-G0 and 4-G1

Compound 3-G0



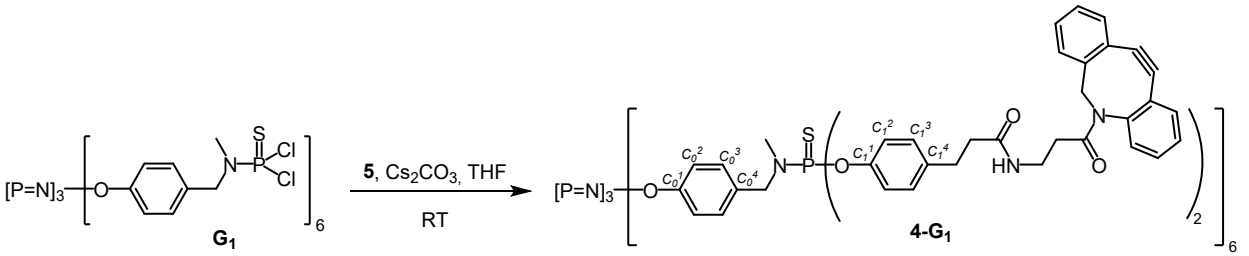
To a suspension of compound **5** (100 mg, 0.23 mmol) and Cs_2CO_3 (153 mg, 0.47 mmol) in THF (15 mL) at rt was added $N_3P_3Cl_6$ (**6**) (12.8 mg, 0.037 mmol). The reaction mixture was stirred overnight at rt. After filtration, the volatiles were removed under reduced pressure. The crude residue was solubilized in THF (2 mL) then precipitated in diethyl ether (100 mL) to give **3-G_0** as a yellow powder in 90% yield (95 mg).

^{31}P-{^{1}H} NMR ($CDCl_3$, 162 MHz): δ (ppm) 8.84 (br s, N=P).

^{1}H NMR ($CDCl_3$, 400 MHz): δ (ppm) 7.71-7.62 (m, 6H, CH_{ADIBO}), 7.45-7.30 (m, 24H, CH_{ADIBO}), 7.28-7.22 (m, 12H, CH_{ADIBO}), 7.22-7.16 (m, 6H, CH_{ADIBO}), 6.94 (d, *J*= 8.4, 12H, $C_0{}^3H$), 6.83 (d, *J*= 8.4, 12H, $C_0{}^2H$), 6.39-6.15 (m, 6H, NH), 5.11 (d, *J*= 13.9, 6H, $ArCH_2NCO$), 4.46 (d, *J*= 12.5, 12H, $C_0{}^4CH_2$), 3.66 (d, *J*= 13.9, 6H, $ArCH_2NCO$), 3.32-3.24 (m, 2H, $\underline{CH_2}NH$), 3.23-3.13 (m, 2H, $\underline{CH_2}NH$), 2.82-2.69 (m, 12H, $\underline{CH_2}CONH$), 2.46-2.37 (m, 6H, $CH_2\underline{CH_2}CON$), 2.30-2.17 (m, 12H, $C_0{}^4CH_2$), 1.96-1.79 (m, 6H, $CH_2\underline{CH_2}CON$).

^{13}C NMR ($CDCl_3$, 101 MHz,): δ (ppm) 172.0 (s, CON), 171.7 (s, CONH), 151.0 (s, C_{ADIBO}), 149.0 (br s, $C_0{}^1$), 148.0 (s, C_{ADIBO}), 137.5 (s, $C_0{}^4$), 132.1 (s, CH_{ADIBO}), 129.2 (s, $C_0{}^3H$), 129.1 (s, CH_{ADIBO}), 128.6 (s, CH_{ADIBO}), 128.3 (s, CH_{ADIBO}), 128.3 (s, CH_{ADIBO}), 127.8 (s, CH_{ADIBO}), 127.2 (s, CH_{ADIBO}), 125.6 (s, CH_{ADIBO}), 122.9 (s, C_{ADIBO}), 122.4 (s, C_{ADIBO}), 120.9 (br s, $C_0{}^2H$), 114.8 (s, C≡C), 107.8 (s, C≡C), 55.5 (s, $Ar\underline{CH_2}NCO$), 38.0 (s, $C_0{}^4\underline{CH_2}$), 35.2 (s, CH_2NH), 34.7 (s, $CH_2\underline{CH_2}CON$), 30.8 (s, $\underline{CH_2}CONH$).

Compound 4-G_1



To a suspension of compound **5** (100 mg, 0.23 mmol) and Cs_2CO_3 (153 mg, 0.47 mmol) in THF (20 mL) maintained at RT was added **G_1** (30.82 mg, 0.017 mmol). The reaction mixture was stirred at RT overnight. After centrifugation to remove the salts, the volatiles were removed under reduced pressure. The crude residue was solubilized in THF (2 mL) and then precipitated with a large volume of diethyl ether (100 mL) to afford **4-G_1** as a yellow powder in 95% yield (103 mg).

^{31}P-{^{1}H} NMR ($CDCl_3$, 121 MHz): δ (ppm) 68.50 (s, P=S), 8.68 (br s, N=P).

^{1}H NMR ($CDCl_3$, 300 MHz): δ (ppm) 7.65 (d, $^{3}J_{HH}$ = 7.5, 12H, CH_{ADIBO}), 7.42-7.31 (m, 42H, CH_{ADIBO}), 7.30-7.25 (m, 30H, CH_{ADIBO}), 7.20-7.14 (br d, $^{3}J_{HH}$ = 8.0, 24H, $C_1{}^3H$), 7.09 (br s, 48H, $C_1{}^2H$, $C_0{}^3H$ and CH_{ADIBO}), 6.93 (br d, $^{3}J_{HH}$ = 8.4, 12H, $C_0{}^2H$), 6.44-6.09 (m, 12H, NH), 5.11 (d, *J* = 13.9, 12H, $ArCH_2NCO$), 4.46 (d, $^{3}J_{HP}$ = 12.5, 12H, $C_0{}^4CH_2$), 3.67 (d, *J*= 13.9, 6H, $ArCH_2NCO$), 3.65 (d, *J*= 13.9, 6H, $ArCH_2NCO$), 3.35-3.12 (m, 24H, CH_2NH), 2.83-2.69 (m, 42H, $\underline{CH_2}CONH$, CH_3NP), 2.48-2.34 (m, 12H, $CH_2\underline{CH_2}CON$), 2.25 (t, $^{3}J_{HH}$ = 8.0, 24H, $C_1{}^4CH_2$), 1.97-1.92 (m, 12H, $CH_2\underline{CH_2}CON$).

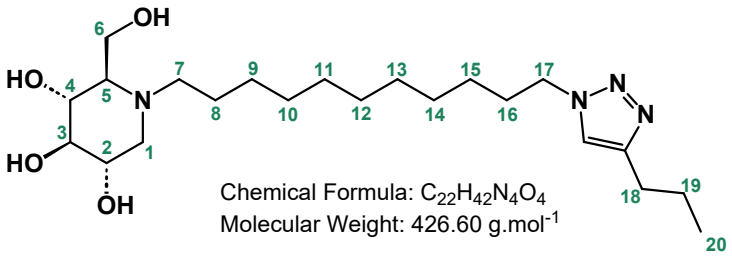
^{13}C NMR ($CDCl_3$, 75 MHz): δ (ppm) 171.86 (s, CON), 171.4 (s, CONH), 151.4 (s, C_{ADIBO}), 150.1 (br s, $C_0{}^1$), 149.4 (d, $^{3}J_{HP}$ = 7.7 Hz, $C_1{}^1$), 148.5 (s, C_{ADIBO}), 138.3 (s, $C_1{}^4$), 134.5 (s, br $C_0{}^4$), 132.4 (s, CH_{ADIBO}), 129.5 (s, $C_1{}^3H$), 129.3 (s, CH_{ADIBO}), 128.8 (s, CH_{ADIBO}), 128.4 (s, CH_{ADIBO}), 128.1 (s, CH_{ADIBO}), 127.9 (s, CH_{ADIBO}), 127.2 (s, CH_{ADIBO}), 125.6 (s, CH_{ADIBO}), 123.2 (s, C_{ADIBO}), 122.5 (s, C_{ADIBO}), 121.1 (s, $C_0{}^2H$), 121.1 (s, $C_1{}^2H$), 114.7 (s, C≡C), 108.1 (s, C≡C), 55.5 (s, $\underline{C}H_2NCO$), 53.6 (br s, CH_2NP), 38.1 (s, $C_1{}^4\underline{CH_2}$), 35.2 (s, CH_2NH), 34.8 (s, $\underline{CH_2}CON$), 33.7 (br s, CH_3NP), 30.9 (s, $\underline{CH_2}CONH$).

1.4. Click reactions

General procedure A for CuAAC:

To a solution of azide (1.1 eq per alkyne) and alkyne (1 eq) in DMF ([alkyne]= 0.01 M) (previously degassed), a pre-mixed solution of $CuSO_4.5H_2O$ (0.3 eq per alkyne) and *L*-ascorbic acid sodium (0.5 eq per alkyne) in water ([$CuSO_4.5H_2O$]= 0.1 M) was added. The mixture was stirred under microwave (20 W) at 80 °C for 30 min. Then, the mixture was extracted with E.A., washed 5 times with a saturated aqueous solution of EDTA. The collected organic phases were dried over $MgSO_4$, and concentrated. The crude mixture was purified by column chromatography (SiO_2, E.A./E.P.). After, the product was diluted in MeOH (0.010 M) and ammonia (32%) (25 eq per alkyne) was added dropwise. The mixture was stirred at rt for 4 at 6 h. Then, the mixture was diluted with MeOH, then co-evaporated with toluene (2 times). MeOH was then added and evaporated to give the desired production without any further purification.

Compound 9a



General procedure **A** starting from **8a** (20.0 mg, 0.038 mmol) and pentyne (37.0 µL, 0.038 mmol) gives compound **9a** as a colorless oil with a **39%** yield (6.40 mg, 0.015 mmol).

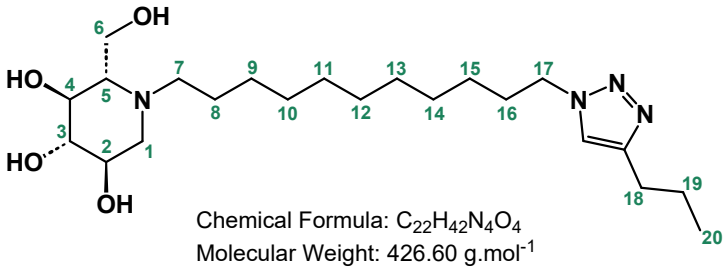
$[\alpha]_D^{20}$ = -6.0 (c 0.2, MeOH)

^{1}H-NMR (MeOD, 300 MHz): δ (ppm) 7.71 (s, 1H, $H_{triazole}$), 4.35 (t, *J* = 7.0, 2H, H_{17}), 3.85 (brs, 2H, H_6), 3.47 (ddd, *J* = 10.5, 9.0, 4.8, 1H, H_2), 3.35 (t, *J* = 9.1, 1H, H_4), 3.13 (t, *J* = 9.0, 1H, H_3), 3.01 (dd, *J* = 11.3, 4.9, 1H, H_1), 2.87-2.75 (m, 1H, H_7), 2.72-2.53 (m, 3H containing at 2.66 (t, *J* = 7.2, 2H, H_{18}) and $H_{7'}$), 2.26-2.10 (m, 2H, $H_{1'+5}$), 1.97-1.83 (m, 2H, H_{16}), 1.69 (h, *J* = 7.5, 2H, H_{19}), 1.57-1.41 (m, 2H, H_8), 1.41-1.19 (m, 14H, H_{9-15}), 0.96 (t, *J* = 7.2, 2H, H_{20}).

^{13}C-NMR (MeOD, 75 MHz): δ (ppm) 149.0 ($C_{triazole}$), 123.1 ($C_{triazole}$), 80.6 (C_3), 72.0 (C_4), 70.7 (C_2), 67.4 (C_5), 59.4 (C_6), 57.7 (C_1), 53.8 (C_7), 51.2 (C_{17}), 31.4 (C_{16}), 30.7-30.7-30.5-30.5-30.0-28.6-28.3-27.5-25.2-23.8-23.7 ($C_{8-15+18-20}$) ppm.

HR-MS (ESI) m/z calculated for: $C_{22}H_{43}N_4O_4$ $[M+H]^+$: 427.3284; found: 427.3277

Compound 9b

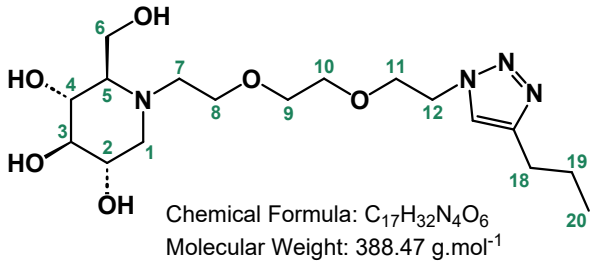


General procedure **A** starting from **8b** (20.0 mg, 0.038 mmol) and pentyne (40 µL, 0.041 mmol) gives compound **9b** as a colorless oil with a **25%** yield (4.1 mg, 0.009 mmol).

$[\alpha]_D^{20}$ = 2.2 (c 0.3, MeOH)

Spectral data identical to compound **9a**.

Compound 9c



General procedure **A** starting from **8c** (16.0 mg, 0.033 mmol) and pentyne (35 µL, 0.035 mmol) gives compound **9c** as a yellow oil with a **48%** yield (6.5 mg, 0.017 mmol).

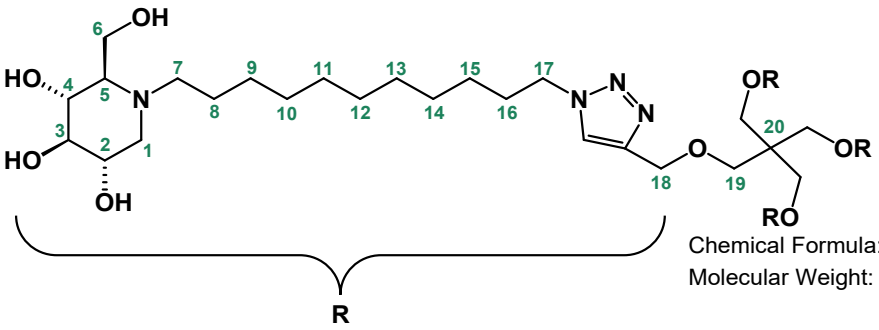
$[\alpha]_D^{20}$ = -1.2 (c 0.3, MeOH)

^{1}H-NMR (D_2O, 300 MHz): δ (ppm) 7.87 (s, 1H, $H_{triazole}$), 4.82 (t, *J* = 5.0, 2H, H_{12}), 3.97 (t, *J* = 5.0, 2H, H_{peg}), (AB part of an ABX system, $\Delta\delta$= 0.13, $J_{A\text{-}B}$ = 12.6, $J_{A\text{-}X}$ = 3.2, $J_{B\text{-}X}$ = 2.7, 2H, $H_{6+6'}$), 3.70-3.50 (m, 7H, $H_{2+4+3+peg}$), 3.43-3.23 (m, 2H, H_{peg}), 3.10 (dd, *J* = 11.9, 5.0, 1H, H_1), 3.05-2.93 (m, 1H, H_7), 2.82-2.69 (m, 1H, $H_{7'}$), 2.69 (t, *J* = 7.2, 2H, H_{18}), 2.43-2.29 (m, 2H, $H_{1'+5}$), 1.67 (h, *J* = 7.5, 2H, H_{19}), 0.96 (t, *J* = 7.5 Hz, 2H, H_{20}).

^{13}C-NMR (D_2O, 300 MHz): δ (ppm) 146.1 ($C_{triazole}$), 125.6 ($C_{triazole}$), 80.5 (C_3), 71.8 (C_{4or2}), 71.4-71.4-70.9-70.4-69.6 ($C_{8\text{-}11+21}$), 70.6 (C_{2or4}), 70.2 (C_{19}), 67.8 (C_5), 65.5 (C_{18}), 59.4 (C_6), 58.7 (C_1), 52.6 (C_7), 51.4 (C_{12}), 46.8 (C_q).

HR-MS (ESI) m/z calculated for: $C_{17}H_{33}N_4O_6$ $[M+H]^+$: 389.2400; found: 389.2399

Compound 12a



Chemical Formula: $C_{85}H_{156}N_{16}O_{20}$
Molecular Weight: 1722.28 g.mol^{-1}

General procedure **A** starting from **8a** (27.1 mg, 0.051 mmol) and tetrakis(2-propynyloxymethyl)methane (3.4 mg, 0.014 mmol) gives compound **12a** as a yellow oil with a **89%** yield (21.0 mg, 0.012 mmol).

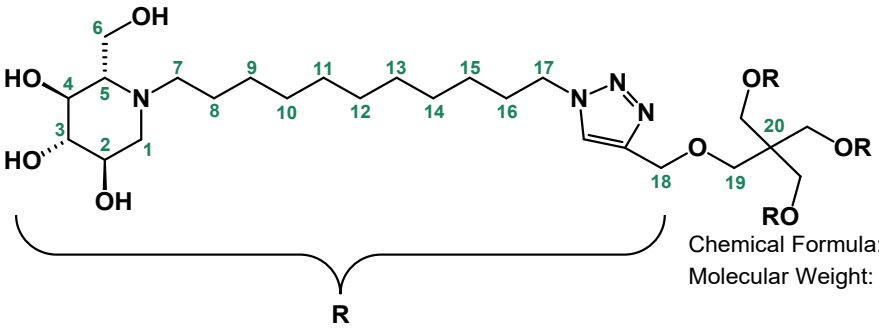
$[\alpha]_D^{20}$ = -6.1 (c 0.9, MeOH)

^{1}H-NMR (MeOD, 300 MHz): δ (ppm) 7.92 (s, 4H, $H_{triazole}$), 4.50 (s, 8H, H_{18}), 4.38 (t, *J* = 7.0, 8H, H_{17}), 3.92-3.82 (m, 8H, H_6), 3.56-3.36 (m, 8H, H_{2+4}), 3.43 (s, 8H, H_{19}), 3.15 (t, *J* = 9.0, 4H, H_3), 3.01 (dd, *J* = 11.2, 4.9, 4H, H_1), 2.93-2.79 (m, 4H, H_7), 2.72-2.57 (m, 4H, $H_{7'}$), 2.35-2.15 (m, 8H, $H_{1'+5}$), 1.94-1.81 (m, 8H, H_{16}), 1.61-1.41 (m, 8H, H_8), 1.42-1.38 (m, 56H, H_{9-15}).

^{13}C-NMR (MeOD, 75 MHz): δ (ppm) 146.2 ($C_{triazole}$), 124.9 ($C_{triazole}$), 80.5 (C_3), 71.9 (C_4), 70.6 (C_2), 69.9 (C_{19}), 67.4 (C_5), 65.4 (C_{18}), 59.3 (C_6), 57.6 (C_1), 53.8 (C_7), 51.4 (C_{17}), 46.4 (C_{20}), 31.3 (C_{16}), 30.7-30.6-30.6-30.6-30.1-28.6-27.5-25.2 (C_{8-15}).

HR-MS (ESI) m/z calculated for: $C_{85}H_{160}N_{16}O_{20}$ $[M+4H]^{4+}$: 431.2999; found: 431.2992

Compound 12b



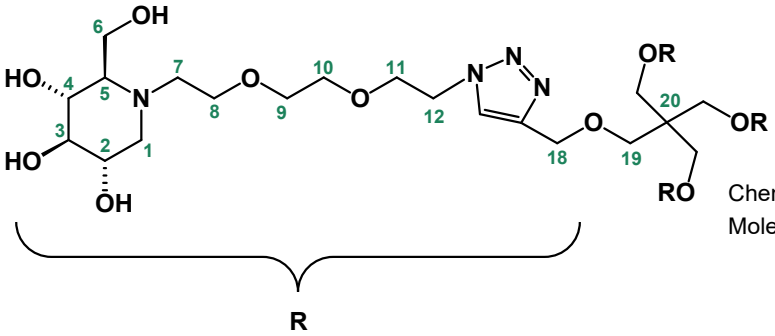
Chemical Formula: $C_{85}H_{156}N_{16}O_{20}$
Molecular Weight: 1722.28 g.mol^{-1}

General procedure **A** starting from **8b** (20.0 mg, 0.038 mmol) and tetrakis(2-propynyloxymethyl)methane (2.6 mg, 0.009 mmol) gives **12b** as a yellow oil with a **81%** yield (12.6 mg, 0.007 mmol).

$[\alpha]_D^{20}$ = 9.1 (c 0.5, MeOH)

Spectral data identical to compound **12a**.

Compound 12c



Chemical Formula: $C_{65}H_{116}N_{16}O_{28}$
Molecular Weight: 1569.73 g.mol^{-1}

General procedure **A** starting from **8c** (36.5 mg, 0.075 mmol) and tetrakis(2-propynyloxymethyl)methane (5.0 mg, 0.017 mmol) gives compound **12c** as a yellow oil with a **72%** yield (19.7 mg, 0.012 mmol).

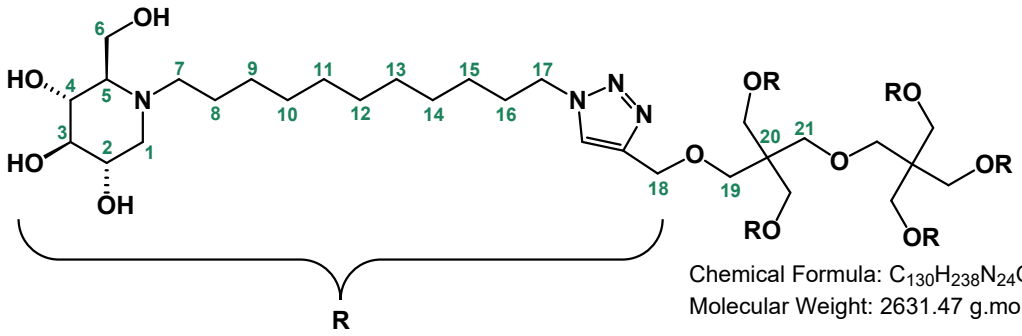
$[\alpha]_D^{20}$ = -2.6 (c 1.0, MeOH)

^{1}H-NMR (CD_3OD, 300 MHz): δ (ppm) 7.98 (s, 4H, $H_{triazole}$), 4.57 (t, *J* = 5.0, 8H, H_{12}), 4.52 (s, 8H, H_{18}), 3.98-3.82 (m, 8H, H_6), 3.88 (m, 8H, H_{peg}), 3.56-3.36 (m, 32H, $H_{2+4+peg}$), 3.46 (s, 8H, H_{19}), 3.16 (t, *J* = 9.1, 4H, H_3), 3.10-2.96 (m, 8H, H_{1+7}), 2.68 (dt, *J* = 14.4, 4.7, 4H, $H_{7'}$), 2.31 (t, *J* = 11.0, 4H, $H_{1'}$), 2.23 (dt, *J* = 9.5, 2.7, 4H, H_5).

^{13}C-NMR (CD_3OD, 75 MHz): δ (ppm) 146.0 ($C_{triazole}$), 125.7 ($C_{triazole}$), 80.4 (C_3), 71.8 (C_{4or2}), 71.4-71.4-70.4-69.6 (C_{peg}), 70.2 (C_{2or4}), 70.0 (C_{19}), 67.7 (C_5), 65.4 (C_{18}), 59.3 (C_6), 58.6 (C_1), 52.6 (C_7), 51.3 (C_{12}), 46.5 (C_q).

HR-MS (ESI) m/z calculated for: $C_{65}H_{120}N_{16}O_{28}$ $[M+4H]^{4+}$: 393.2115; found: 393.2116

Compound 14a



General procedure **A** starting from **8a** (27.0 mg, 0.051 mmol) and hexa(2-propynyloxymethyl) bispentaerythritol (3.9 mg, 0.008 mmol) gives compound **14a** as a yellow oil with a **86%** yield (18.2 mg, 0.007 mmol).

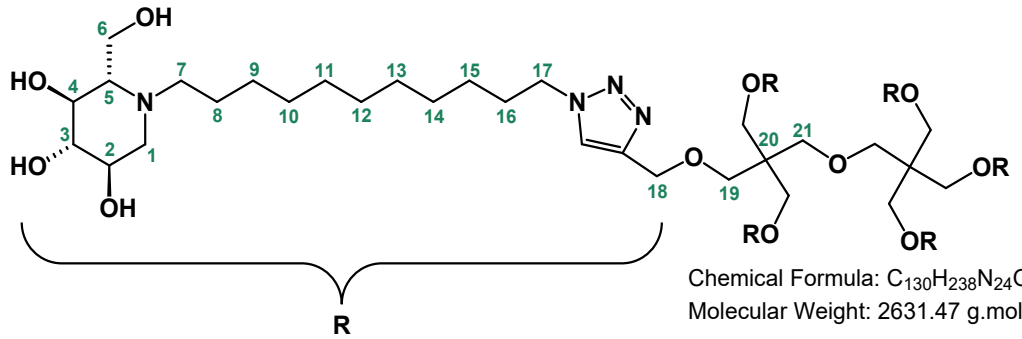
$[\alpha]_D^{20}$ = -7.7 (c 1.0, MeOH)

^{1}H-NMR (CD_3OD, 300 MHz): δ (ppm) 7.93 (s, 6H, $H_{triazole}$), 4.52 (s, 12H, H_{18}), 4.37 (t, *J* = 7.0, 12H, H_{17}), 3.87 (brs, 8H, H_6), 3.56-3.26 (m, 20H, H_{2+4+21}), 3.41 (s, 12H, H_{19}), 3.14 (t, *J* = 9.0, 6H, H_3), 3.01 (dd, *J* = 11.0, 4.5, 6H, H_1), 2.87-2.77 (m, 6H, H_7), 2.66-2.53 (m, 6H, $H_{7'}$), 2.31-2.11 (m, 12H, $H_{1'+5}$), 1.92-1.85 (m, 12H, H_{16}), 1.58-1.40 (m, 12H, H_8), 1.41-1.18 (m, 84H, H_{9-15}).

^{13}C-NMR (CD_3OD, 75 MHz): δ (ppm) 146.3 ($C_{triazole}$), 124.9 ($C_{triazole}$), 80.5 (C_3), 72.0 (C_4), 70.7 (C_2), 70.2 (C_{19}), 67.4 (C_5), 65.5 (C_{18}), 59.4 (C_6), 57.6 (C_1), 53.8 (C_7), 51.4 (C_{17}), 46.7 (C_{20}), 31.4 (C_{16}), 30.7-30.7-30.6-30.6-30.1-28.6-27.5-25.2 (C_{8-15}).

HR-MS (ESI) m/z calculated for: $C_{130}H_{243}N_{24}O_{31}$ $[M+5H]^{5+}$: 527.5641; found: 527.5638

Compound 14b

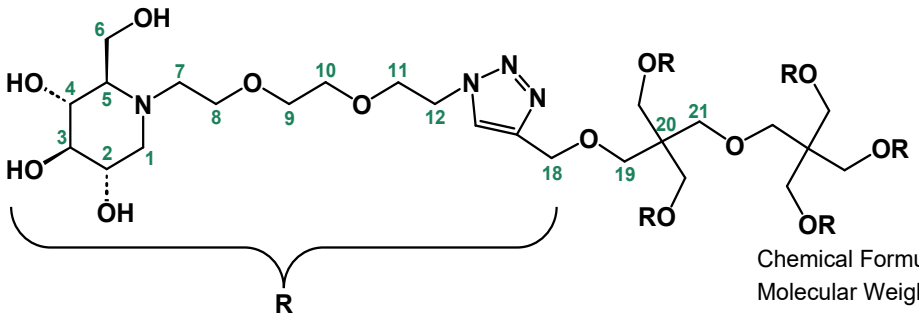


General procedure **A** starting from **8b** (20.5 mg, 0.039 mmol) and hexa(2-propynyloxymethyl) bispentaerythritol (3.0 mg, 0.006 mmol) gives compound **14b** as a yellow oil with a **90%** yield (14.7 mg, 0.006 mmol).

$[\alpha]_D^{20}$ = +2.8 (c 0.3, MeOH)

Spectral data identical to compound **14a**.

Compound 14c



Chemical Formula: $C_{100}H_{178}N_{24}O_{43}$
Molecular Weight: 2404.65 $g.mol^{-1}$

General procedure **A** starting from **8c** (28.5 mg, 0.058 mmol) and hexa(2-propynyloxymethyl) bispentaerythritol (4.4 mg, 0.009 mmol) gives compound **14c** as a yellow oil with a **67%** yield (14.7 mg, 0.005 mmol).

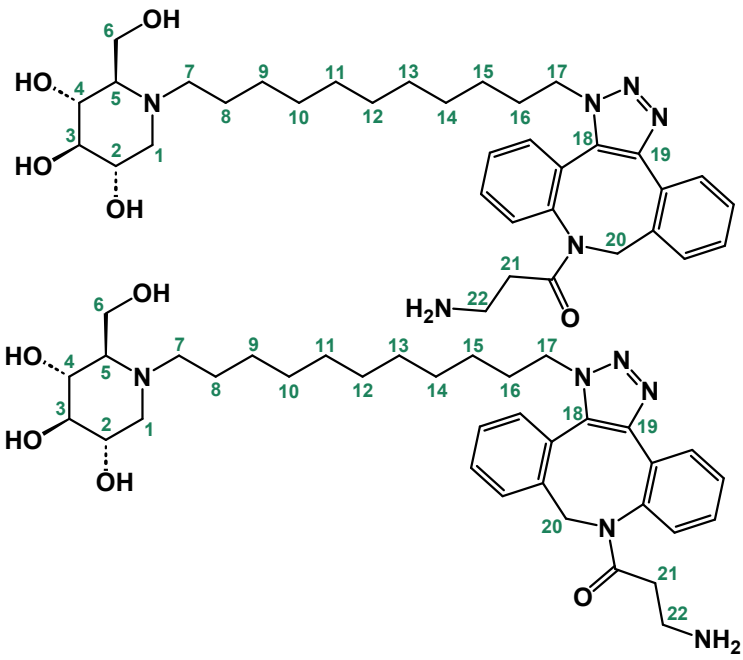
$[\alpha]_D^{20}$ = -3.7 (c 0.8, MeOH)

^{1}H-NMR (CD_3OD, 300 MHz): δ (ppm) 7.97 (s, 6H, $H_{triazole}$), 4.57 (t, *J* = 5.0, 12H, H_{12}), 4.52 (s, 12H, H_{18}), 3.94-3.79 (m, 24H, H_{6+peg}), 3.64-3.32 (m, 52H, $H_{2+4+peg+21}$), 3.35 (s, 12H, H_{19}), 3.16 (t, *J* = 9.1, 6H, H_3), 3.10-2.96 (m, 12H, H_{1+7}), 2.67 (dt, *J* = 14.2, 4.8, 6H, $H_{7'}$), 2.30 (t, *J* = 11.0, 6H, $H_{1'}$), 2.21 (dt, *J* = 9.5, 2.7, 6H, H_5).

^{13}C-NMR (CD_3OD, 75 MHz): δ (ppm) 146.1 ($C_{triazole}$), 125.8 ($C_{triazole}$), 80.5 (C_3), 71.8 (C_{4or2}), 71.4-71.4-70.6-70.4-69.6 (C_{peg+21}), 70.6 (C_{2or4}), 70.3 (C_{19}), 67.7 (C_5), 65.5 (C_{18}), 59.4 (C_6), 58.6 (C_1), 52.6 (C_7), 51.4 (C_{12}), 46.8 (C_q).

HR-MS (ESI) m/z calculated for: $C_{100}H_{183}N_{24}O_{43}$ $[M+5H]^{5+}$: 481.8580; found: 491.8593

Compound 10a



Chemical Formula: $C_{35}H_{50}N_6O_5$
Molecular Weight: 634.82 g.mol-1

Compound **8a** (6.40 mg, 0.012 mmol) was added to a solution of azadibenzocyclooctyne-amine (3.34 mg, 0.012 mmol) in MeOH (previously degassed) (0.36 mL) and THF (previously degassed) (0.15 mL) under Ar. The reaction mixture was stirred at 45°C for 3 h at 1200 rpm. Then, the mixture was concentrated. The crude product was purified by column chromatography (SiO_2, DCM/MeOH/ammonia 80:20:2) to give a yellow oil. After, the product was solubilized in MeOH (1.00 mL), and ammonia (0.19 mL) was added. The reaction mixture was stirred at rt for 4 h. After evaporation of the solvents under reduced pressure, compound **10a** was obtained without further purification as a white solid with a **98%** yield (7.58 mg, 0.012 mmol).

Regioisomer ratio (1/2) 0.72

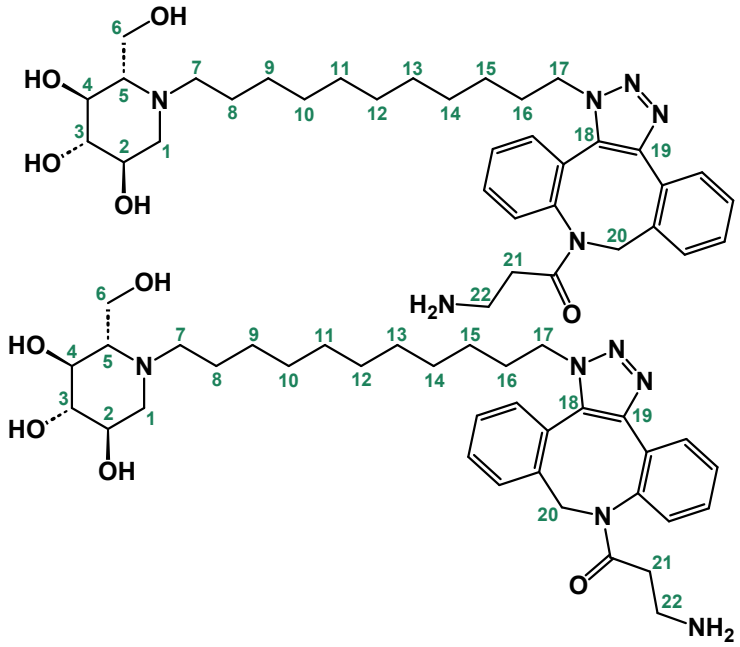
Rotameric ratio (1/2) 0.65

^{1}H-NMR ($CDCl_3$, 300 MHz): δ (ppm) 7.81-7.15 (m, 10H, H_{Ar}), 6.09 (d, *J* = 17.0, 0.42H, H_{20}), 5.91 (d, *J* = 16.0, 0.22H, $H_{20)}$), 5.59 (d, *J* = 18.1 Hz, 0.12H, H_{20}), 5.16 (d, *J* = 14.0, 0.22H, H_{20}), 5.00 (d, *J* = 18.1, 0.12H, $H_{20'}$), 3.73 (d, *J* = 14.0, 0.22H, $H_{20'}$), 4.61-4.29 (m, 2.64H, $H_{17+20'}$), 3.88-3.80 (m, 2H, H_6), 3.49-3.43 (m, 2H, H_2), 3.37-3.25 (m, 2H, H_4), 3.12 (t, *J* = 9.2, 1H, H_3), 2.98 (dd, *J* = 11.3, 5.0, 1H, H_1), 2.83-2.52 (m, 4H, H_{7+22}), 2.24-1.95 (m, 4H, $H_{1'+5+21}$), 1.82-1.05 (m,18H, H_{8-16}).

^{13}C-NMR ($CDCl_3$, 125 MHz): δ (ppm) 173.7-173.4-172.7-172.6 (C_{CO}), 152.5-159.3-146.2-144.9-144.2-143.6-143.5-142.4-141.6-141.4-136.7-136.1-134.9-134.5-134.0-133.5-133.3-133.2-133.0-133.0-132.7-132.4-132.3-132.2-131.2-131.1-131.1-130.8-130.8-130.5-130.4-130.1-130.0-129.9-129.8-129.8-129.7-129.4-129.2-129.2-129.1-128.9-128.9-128.4-128.2-127.0-126.5-125.8-124.2-123.8-115.6-108.7 ($C_{Ar+18-19}$), 72.1 (C_4), 70.7 (C_2), 67.4 (C_5), 59.5 (C_6), 57.8 (C_1), 56.6-56.1-53.6-52.2 (C_{20}), 53.8 (C_7), 50.0-49.7-49.6 (C_{17}), 38.2-38.2-37.9-37.9-37.0-36.8-36.4-31.3-30.9-30.8-30.7-30.6-30.6-30.5-30.3-30.2-29.9-28.6-27.9-27.2-25.2 ($C_{8-16+21-22}$).

HR-MS (ESI) m/z calculated for: $C_{35}H_{51}N_6O_5$ $[M+H]^+$: 635.3921; found: 635.3927

Compound 10b

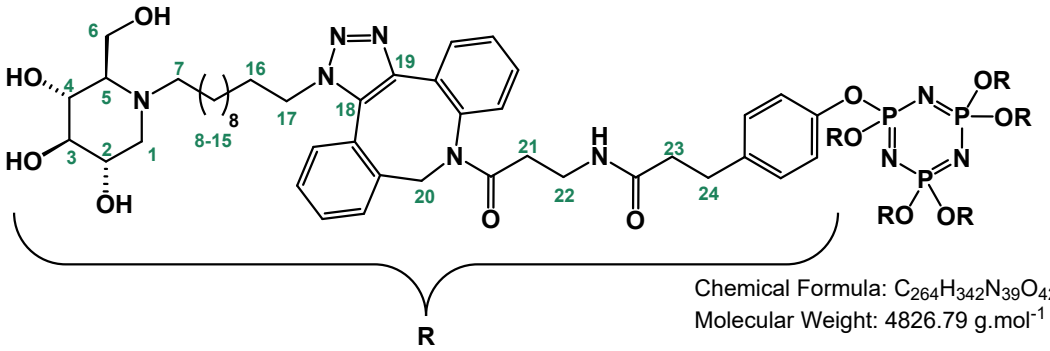


Chemical Formula: $C_{35}H_{50}N_6O_5$
Molecular Weight: 634.82 $g.mol^{-1}$

Compound **10b** was prepared by the same protocol starting from **8b** (10.0 mg, 0.019 mmol). After evaporation of the solvents under reduced pressure, compound **10b** was obtained without further purification as a white solid with a **53%** yield (6.42 mg, 0.010 mmol).

Spectral data identical to compound **10a**.

Compound 15a



Chemical Formula: $C_{264}H_{342}N_{39}O_{42}P_3$
Molecular Weight: 4826.79 g.mol^{-1}

Compound **8a** (12.4 mg, 0.024 mmol) was added to a solution of compound **3** (10.0 mg, 0.004 mmol) in MeOH (previously degassed) (0.30 mL) and THF (previously degassed) (0.70 mL) under Ar. The reaction mixture was stirred at 40 °C for 3 h at 1200 rpm. Then, the mixture was concentrated. The crude product was purified by column chromatography (SiO_2, DCM/MeOH/ammonia 80:16:4) to give a colorless oil. The obtained product was solubilized in MeOH (1.50 mL) and ammonia (0.40 mL) were added. The reaction mixture was stirred at rt for 4 h. After evaporation of the solvents under reduced pressure, compound **15a** was obtained without further purification as a white solid with a **78%** yield (14.0 mg, 0.003 mmol).

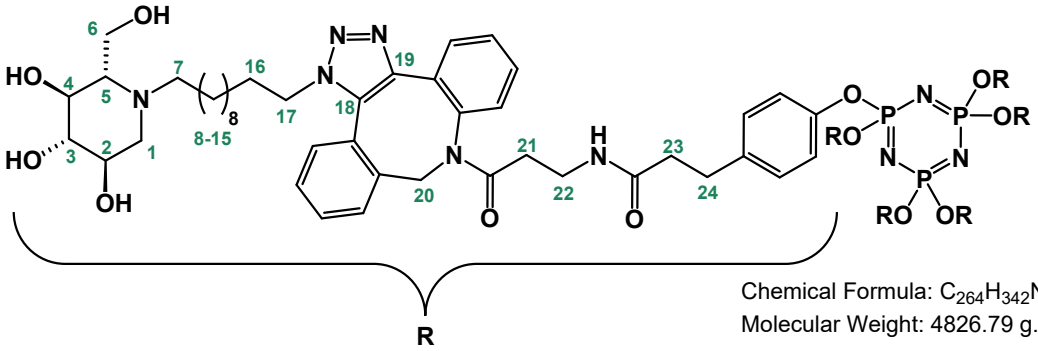
Regioisomer ratio (1/2) 0.54

^{31}P-NMR (CD_3OD/toluene-D8, 300 MHz) δ 9.09 (s, 3P)

MALDI-TOF (Linear positive) m/z calculated for: $C_{264}H_{342}N_{39}O_{42}P_3$ $[M]^+$: 4826.79; found: 4826.97

HR-MS (ESI) m/z calculated for: $C_{264}H_{342}N_{39}O_{42}P_3$ $[M+6H]^{6+}$: 805.4266; found: 805.4274

Compound 15b



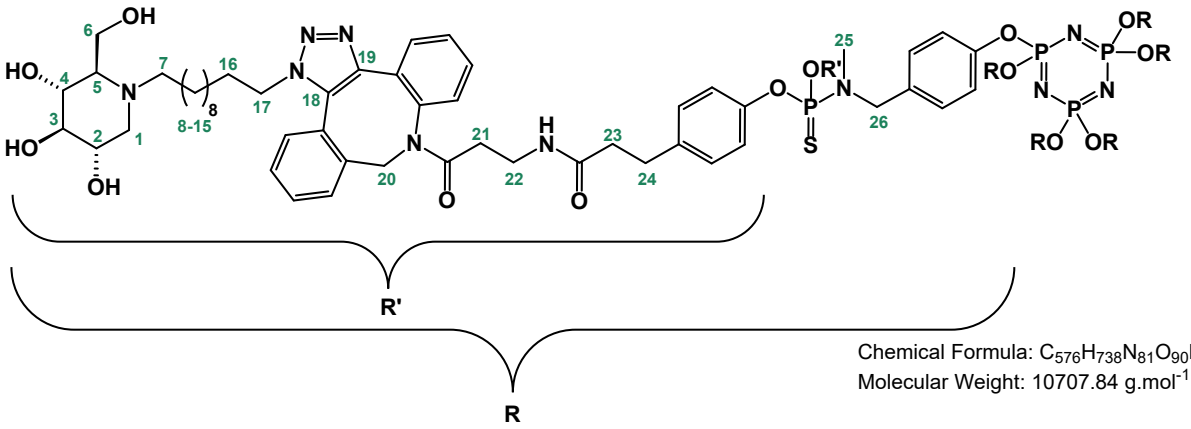
Chemical Formula: $C_{264}H_{342}N_{39}O_{42}P_3$
Molecular Weight: 4826.79 g.mol^{-1}

Compound **15b** was prepared by the same protocol starting from **8b** (12.4 mg, 0.024 mmol). After evaporation of the solvents under reduced pressure, compound **15b** was obtained without further purification give as a colorless oil with a **50%** yield (8.98 mg, 0.002 mmol).

Regioisomer ratio (1/2) 0.54

Spectral data identical to compound **15a**.

Compound 16a MBG262



Chemical Formula: $C_{576}H_{738}N_{81}O_{90}P_9S_6$
Molecular Weight: 10707.84 g.mol^{-1}

Compound **8a** (11.1 mg, 0.021 mmol) was added to a solution of **4** (11.2 mg, 0.002 mmol) in MeOH (previously degassed) (0.30 mL) and THF (previously degassed) (0.70 mL) under Ar. The reaction mixture was stirred at 40 °C for 3 h at 1200 rpm. Then, the mixture was concentrated. The crude product was purified by column chromatography (SiO_2, DCM/MeOH/ammonia 80:16:4) to give a yellow oil. . The obtained product was solubilized in MeOH (0.50 mL), ammonia (0.15 mL) and toluene (0.25 mL) were added. The reaction mixture was stirred at rt for 4 h. After evaporation of the solvents under reduced pressure, compound **16a** was obtained without further purification as a white solid with a **39%** yield (7.30 mg, 0.001 mmol).

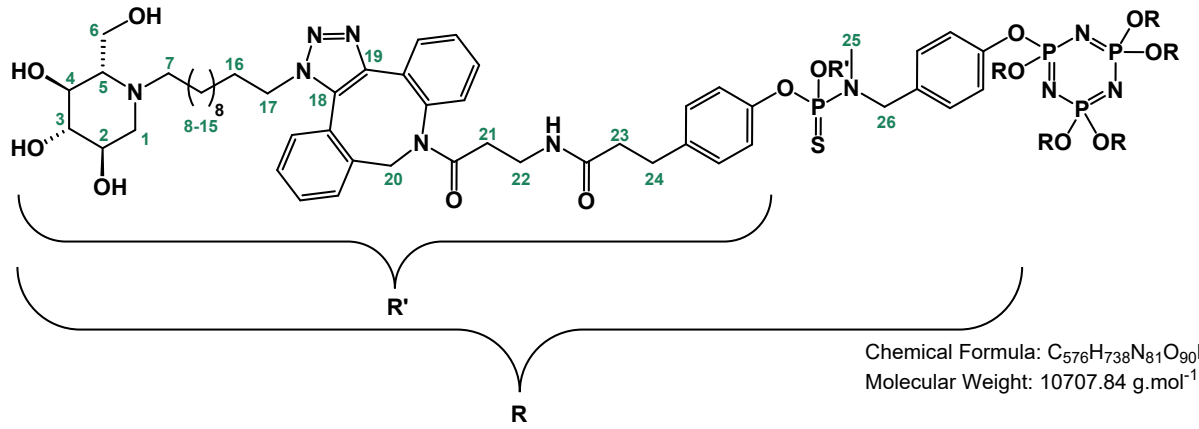
Regioisomer ratio (1/2) 0.54

^{31}P-NMR (CD_3OD, toluene-D8, 300 MHz): δ 68.5 (s, 6P), 9.2 (s, 3P)

MALDI-TOF (Linear positive, 9-aminoacridine Matrix) m/z calculated for: $C_{576}H_{738}N_{81}O_{90}P_9S_6$ $[M]^+$: 10700.2; found: 10720.0

HR-MS (ESI) m/z calculated for: $C_{576}H_{749}N_{81}O_{90}P_9S_6$ $[M+11H]^{11+}$: 974.3874; found: 974.5672

Compound 16b MBG237



Chemical Formula: $C_{576}H_{738}N_{81}O_{90}P_9S_6$
Molecular Weight: 10707.84 g.mol^{-1}

Compound **16b** was prepared by the same protocol starting from **8b** (11.2 mg, 0.021 mmol). After evaporation of the solvents under reduced pressure, compound **16b** was obtained without further purification as a colorless oil with a **67%** yield (12.6 mg, 0.001 mmol).

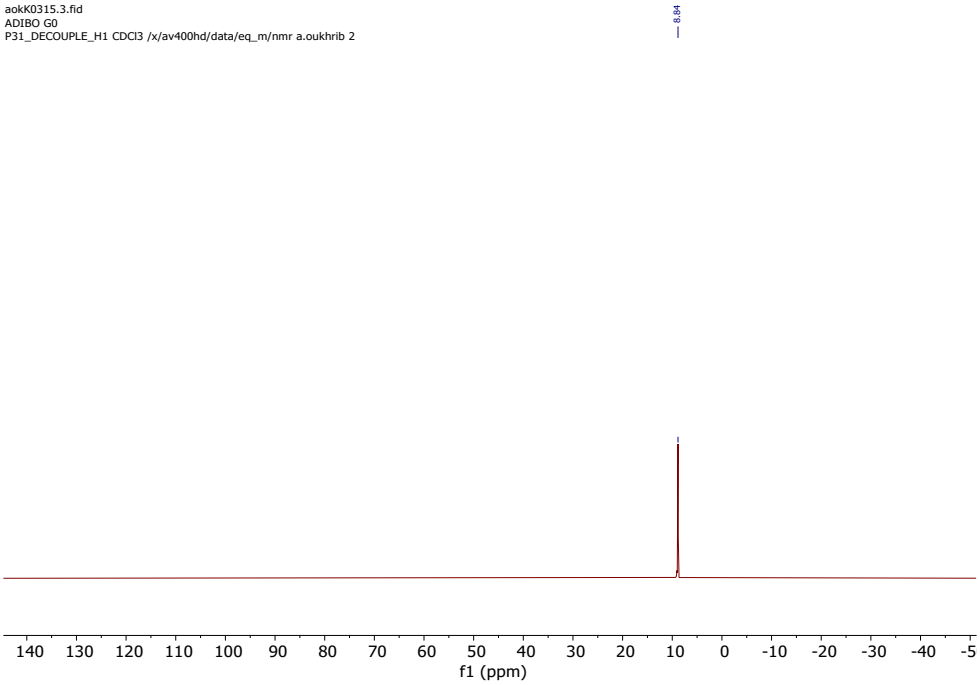
Regioisomer ratio (1/2) 0.54

Spectral data identical to compound **16a**.

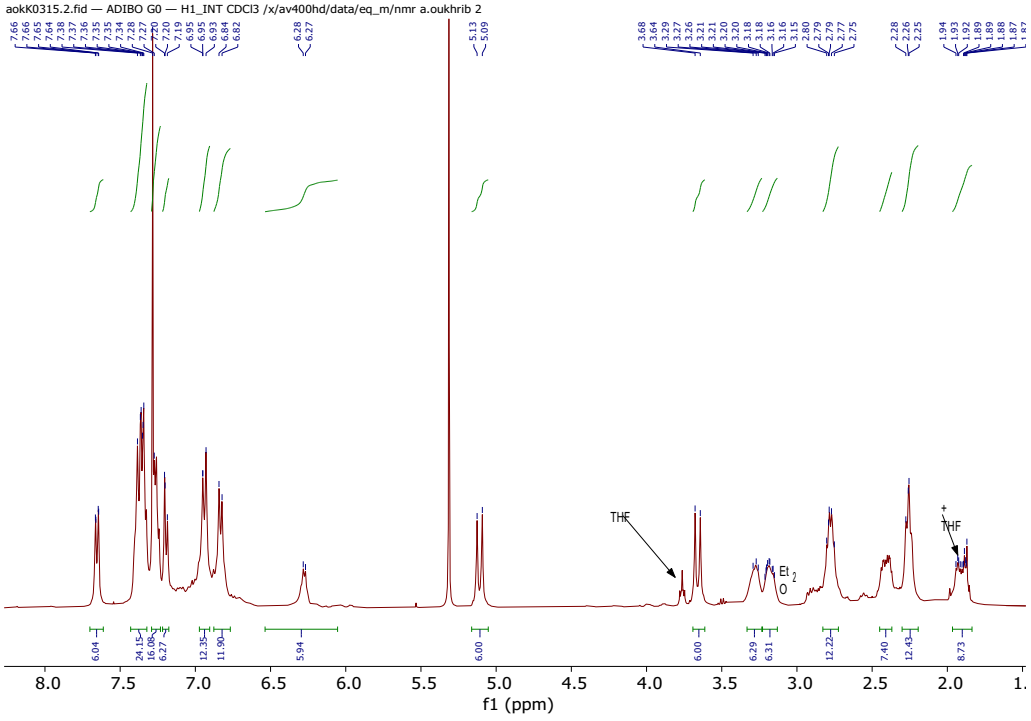
2. NMR spectra for new compounds and mass spectra for 15a and 16a

Compound **3-G_0**

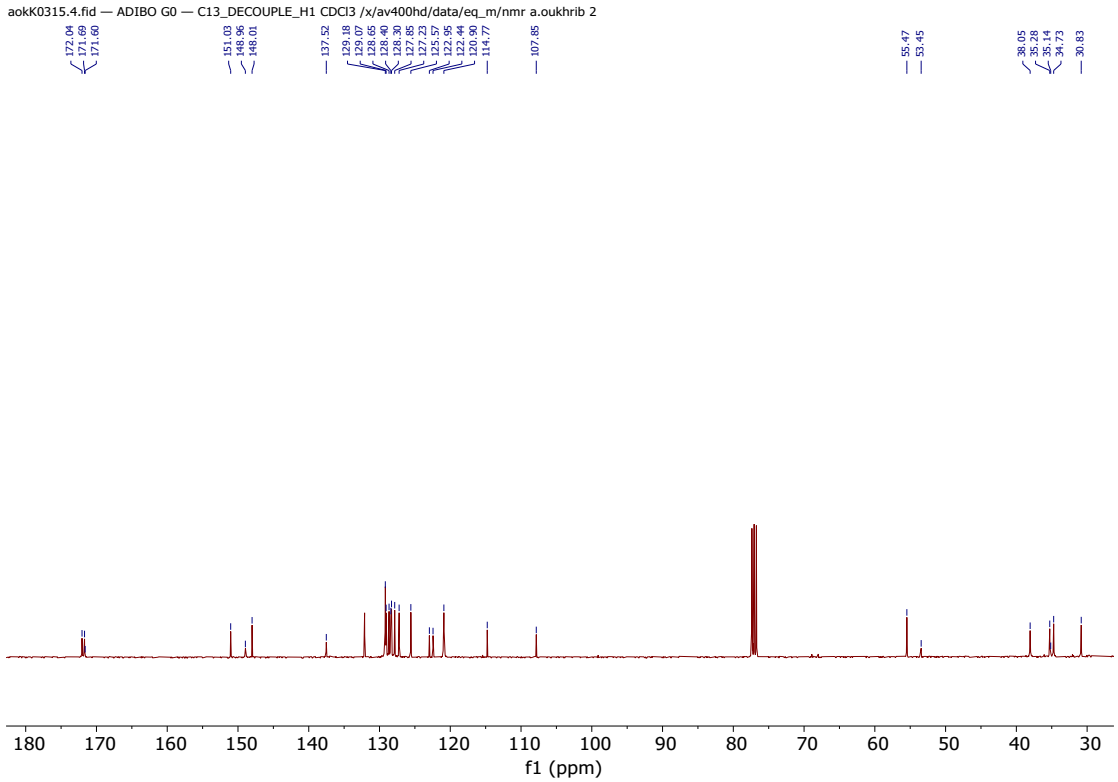
^{31}P-{^{1}H}-NMR ($CDCl_3$, 162 MHz)



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

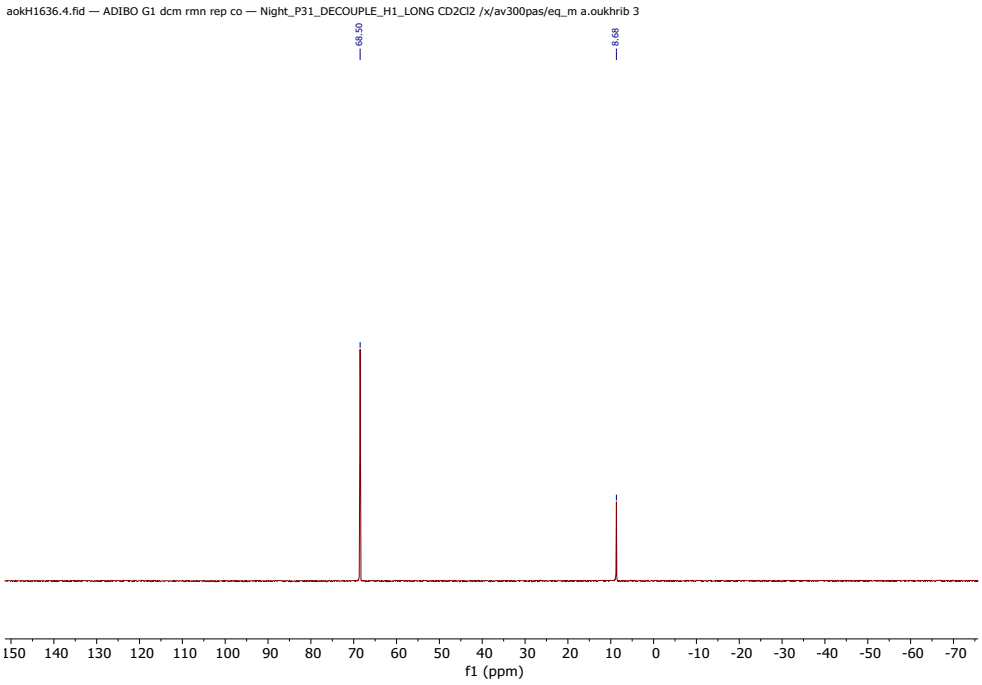


^{13}C-{^{1}H}-NMR ($CDCl_3$, 101 MHz)

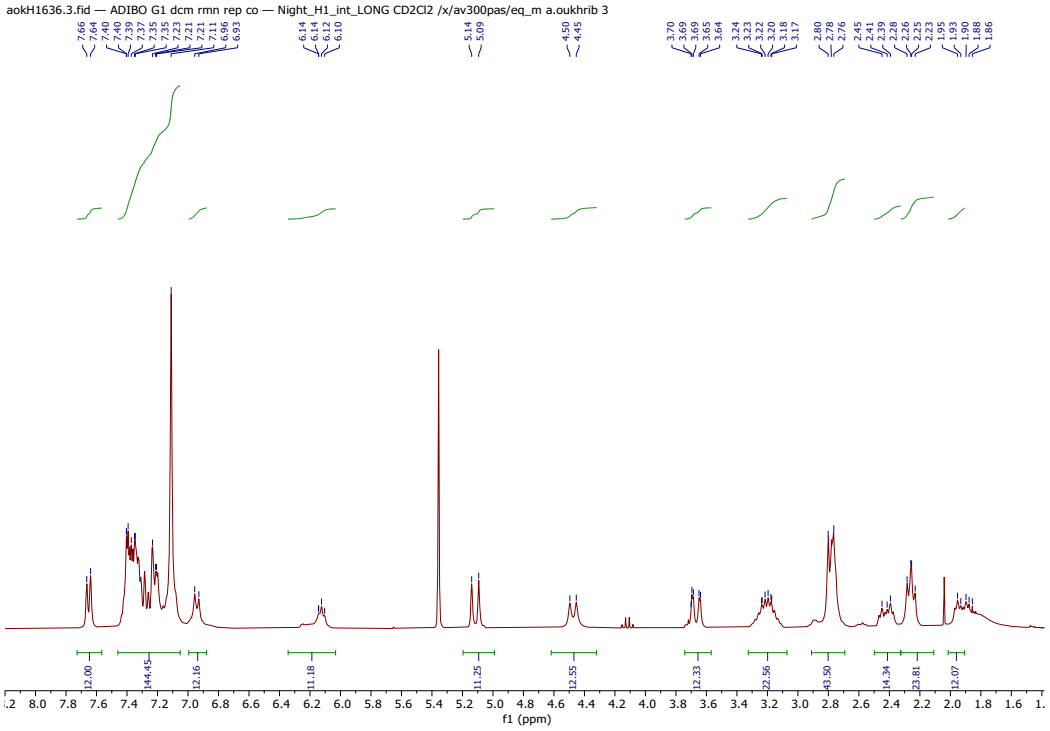


Compound **4-G_1**

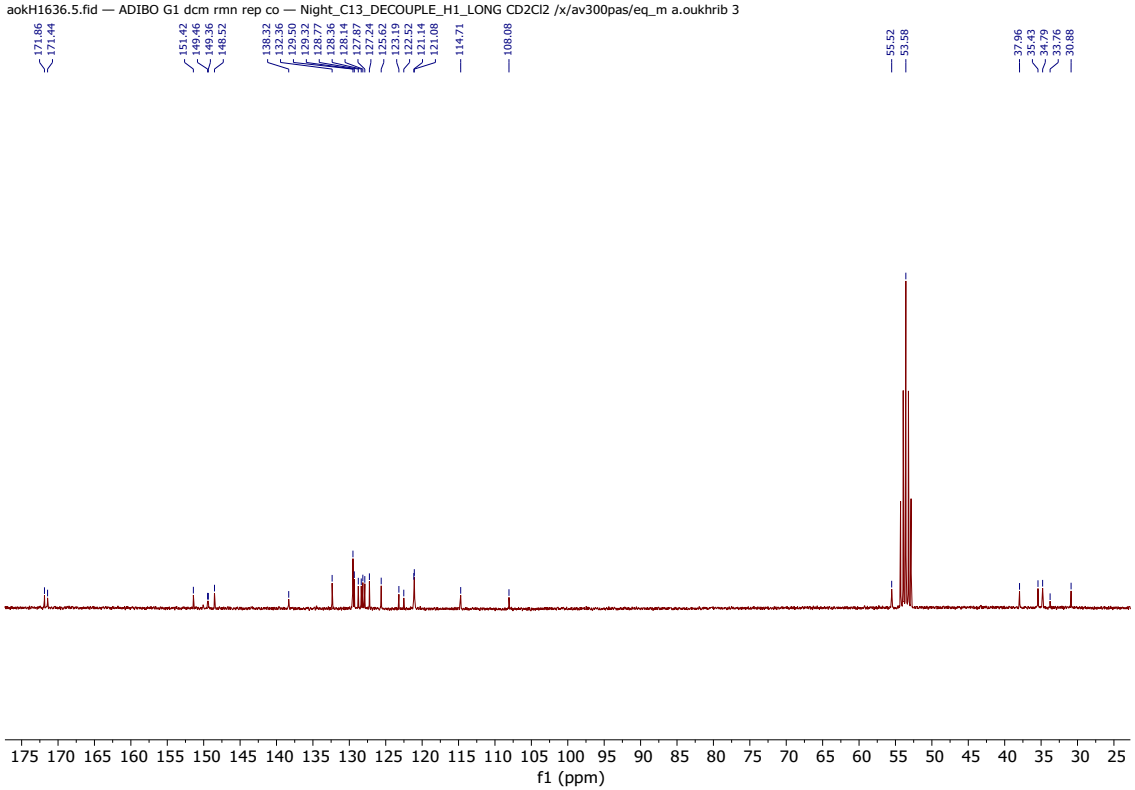
^{31}P-{^{1}H}-NMR ($CDCl_3$, 121 MHz)



^{1}H-NMR ($CDCl_3$, 300 MHz)

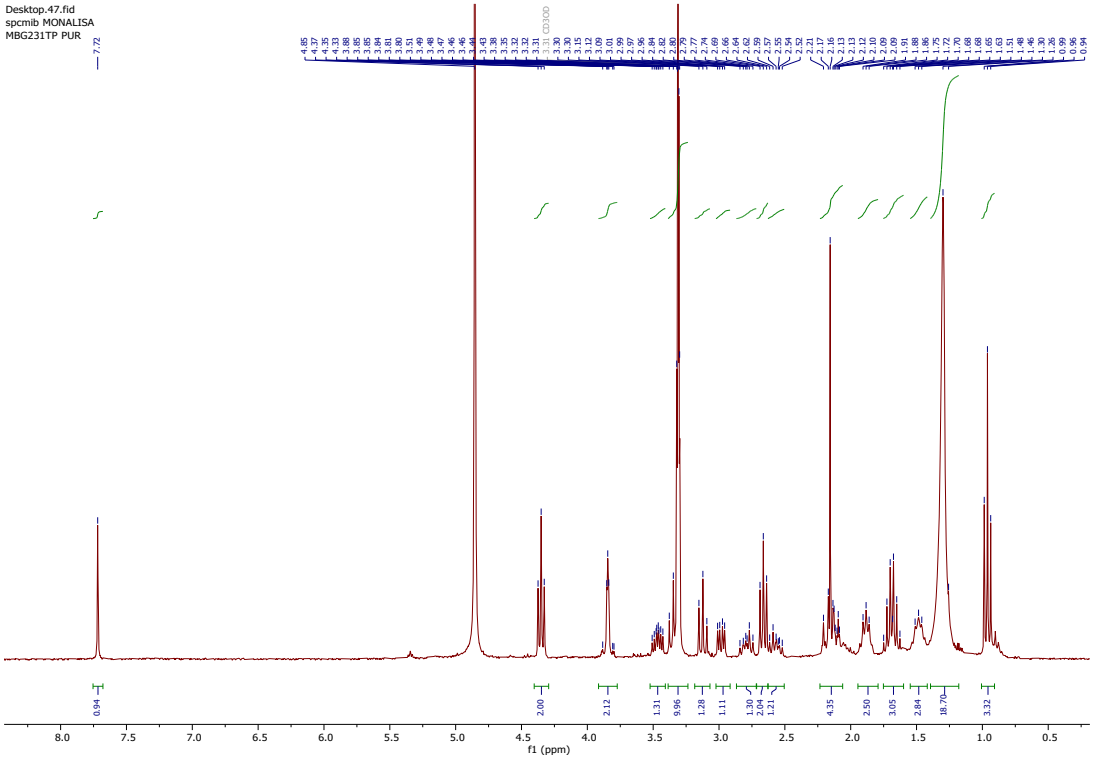


^{13}C-{^{1}H}-NMR ($CDCl_3$, 75 MHz)

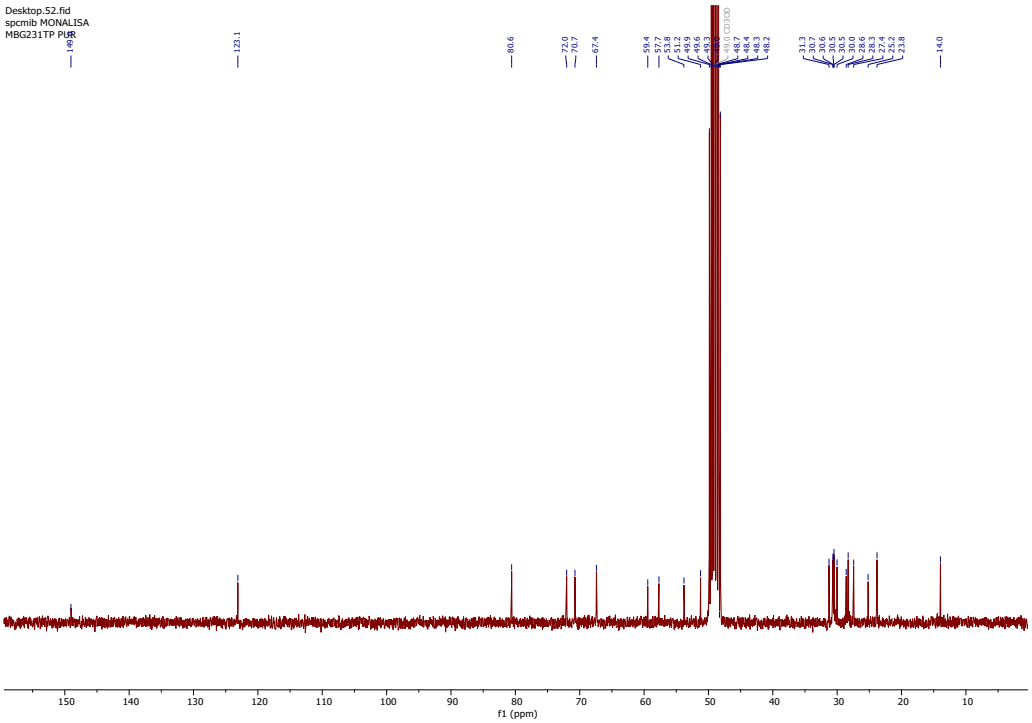


Compound **9b**

^{1}H-NMR (MeOD, 300 MHz)

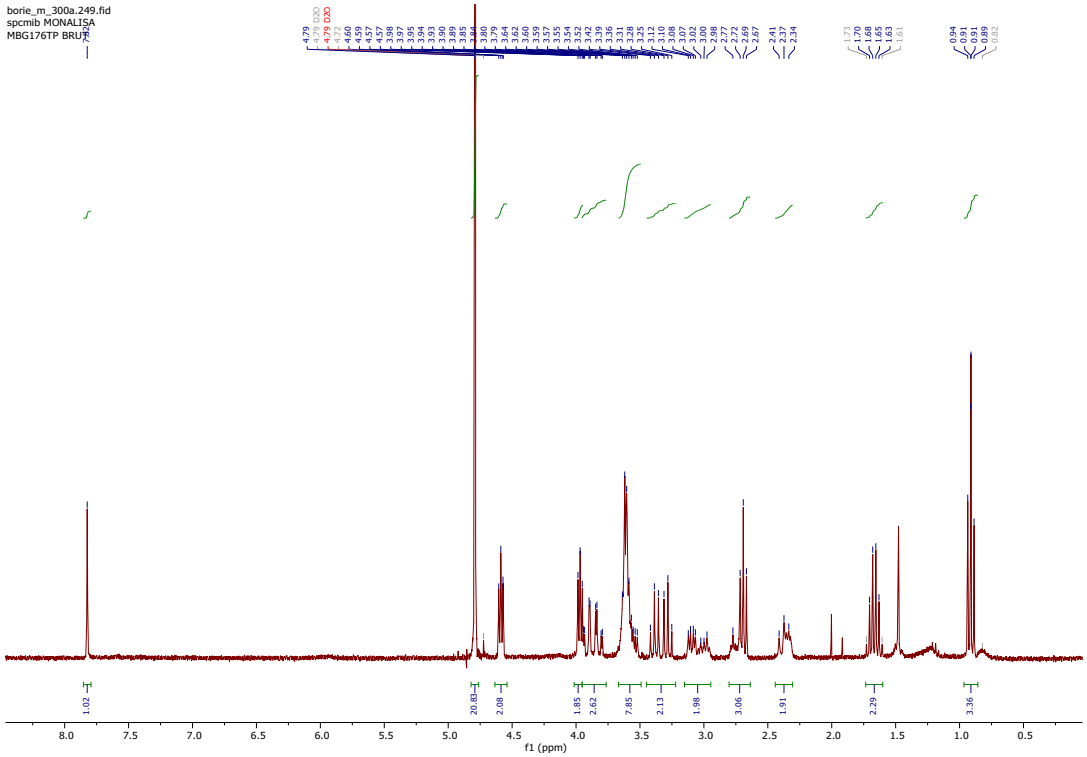


^{13}C-NMR (MeOD, 75 MHz)



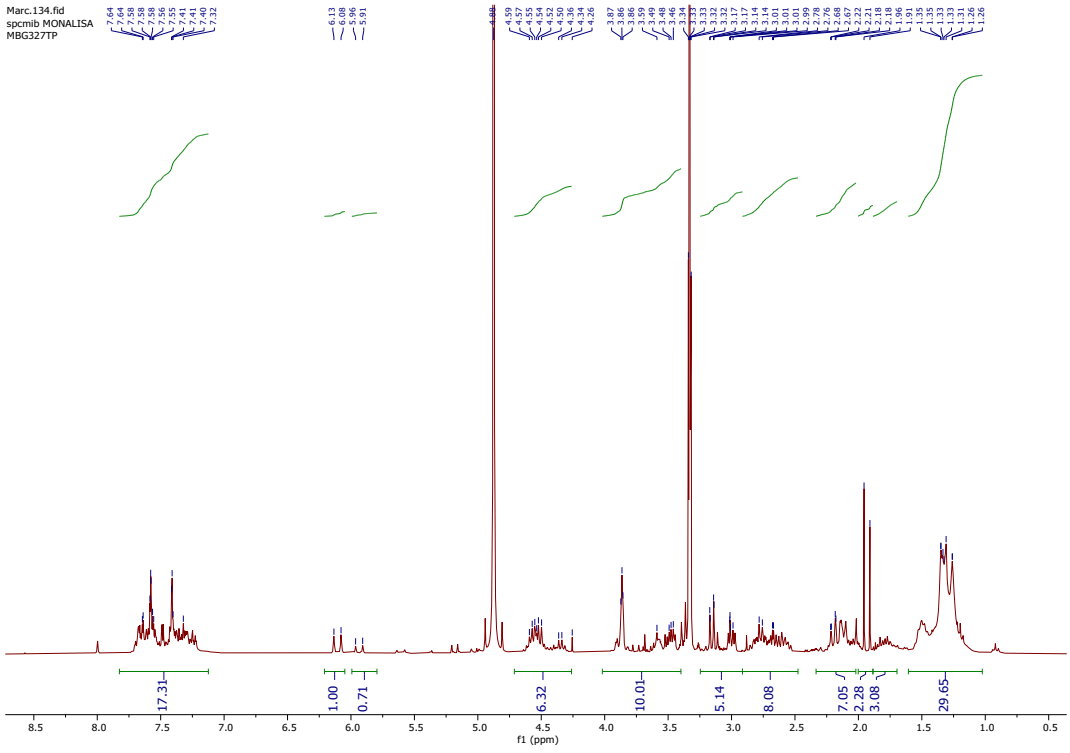
Compound **9c**

^{1}H-NMR (MeOD, 300 MHz)

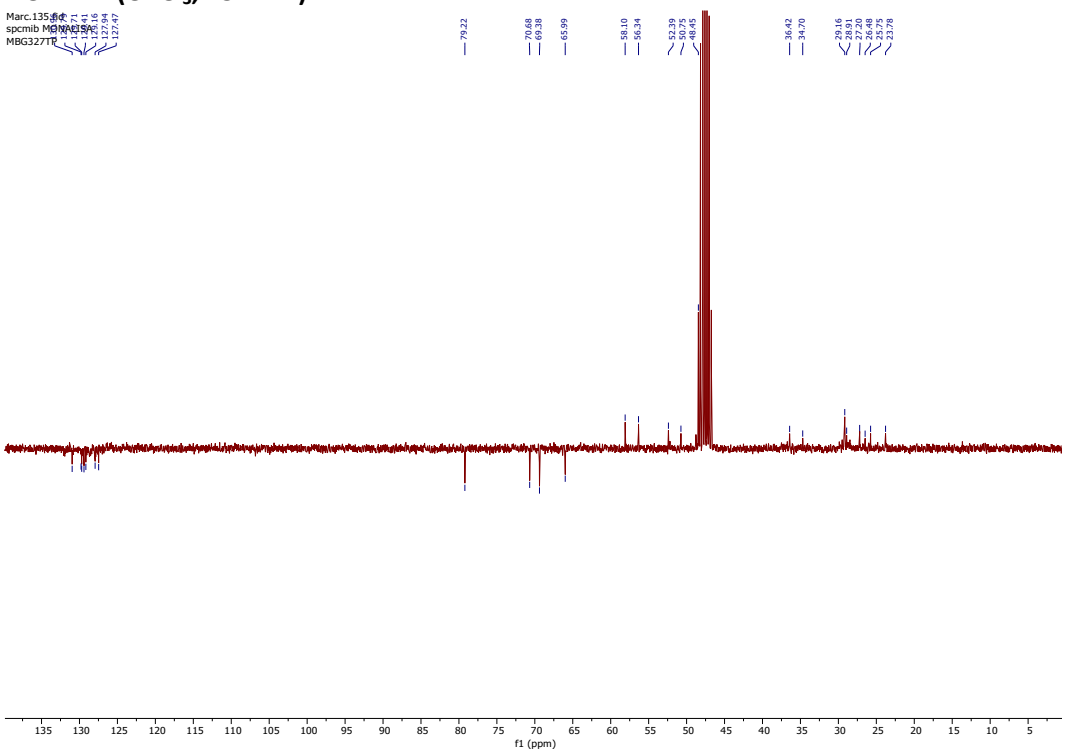


Compound **10a**

^{1}H-NMR ($CDCl_3$, 300 MHz)

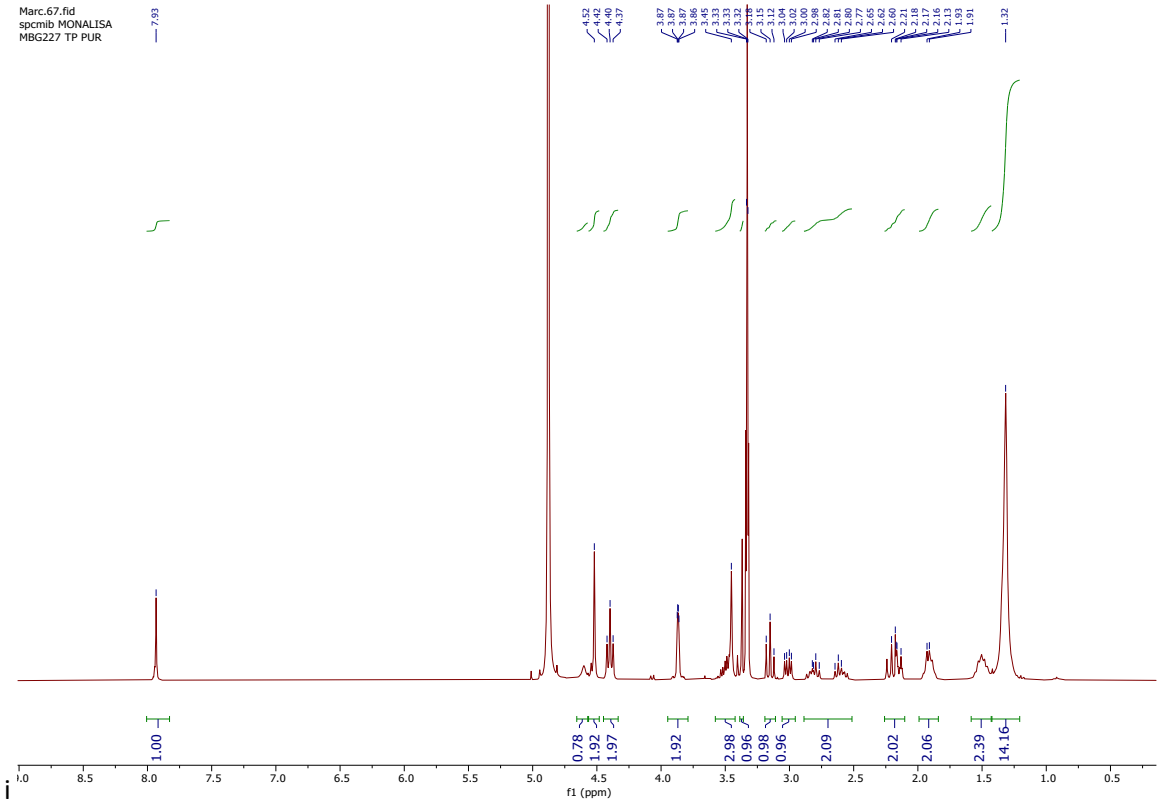


^{13}C-NMR ($CDCl_3$, 75 MHz)



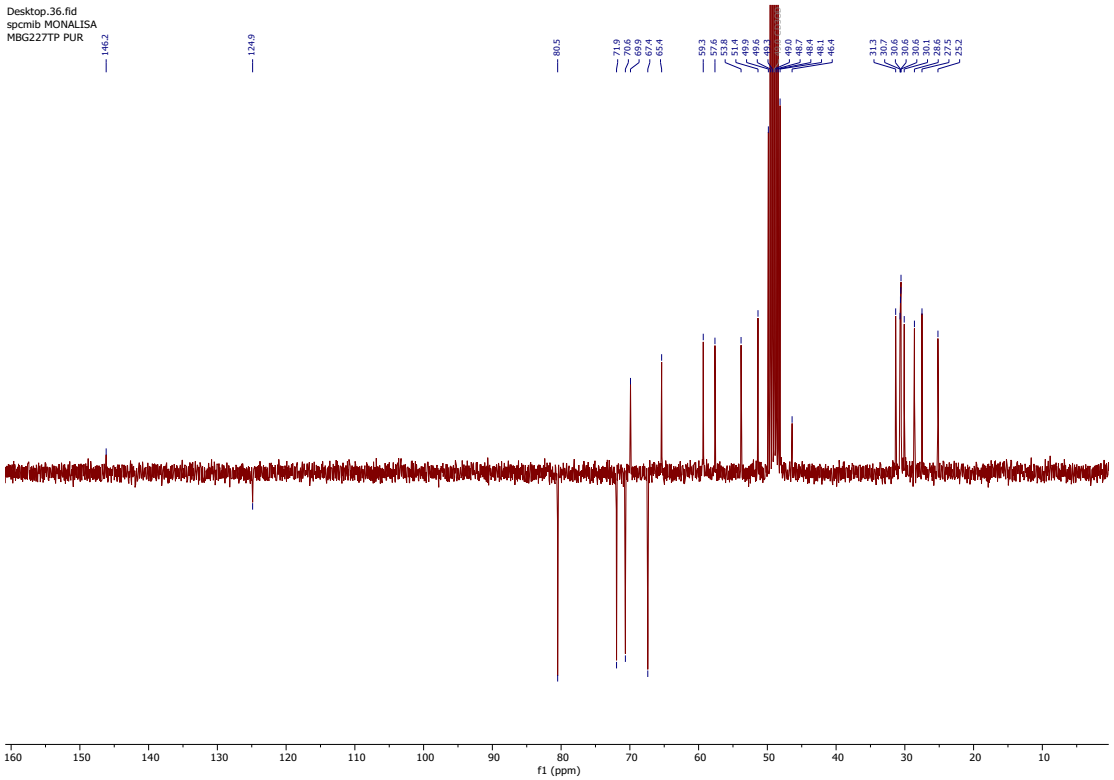
Compound **12a**

^{1}H-NMR (MeOD, 300 MHz)



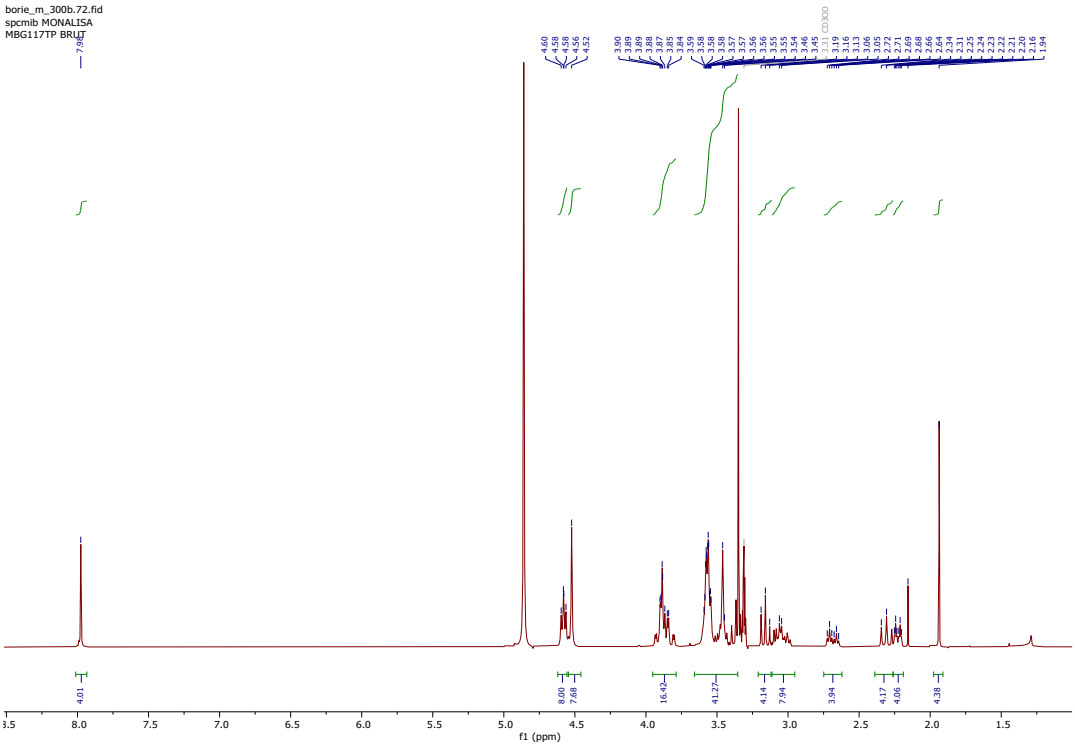
i

^{13}C-NMR (MeOD, 75 MHz)

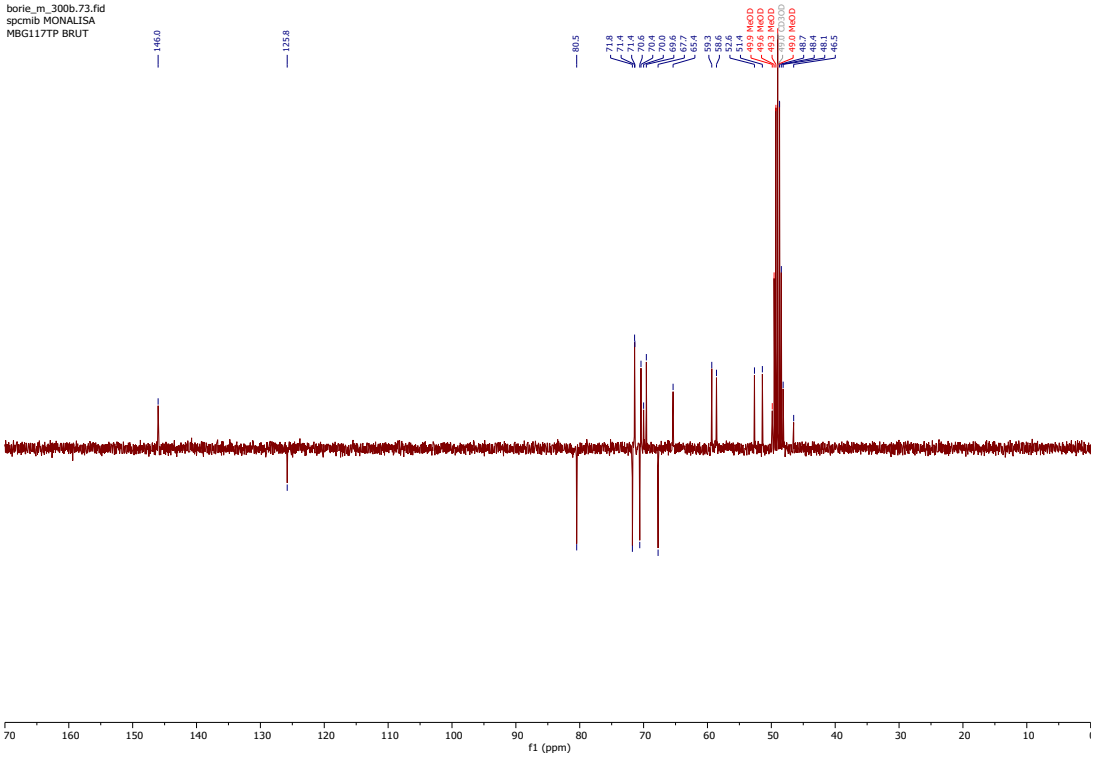


Compound **12c**

^{1}H-NMR (MeOD, 300 MHz)

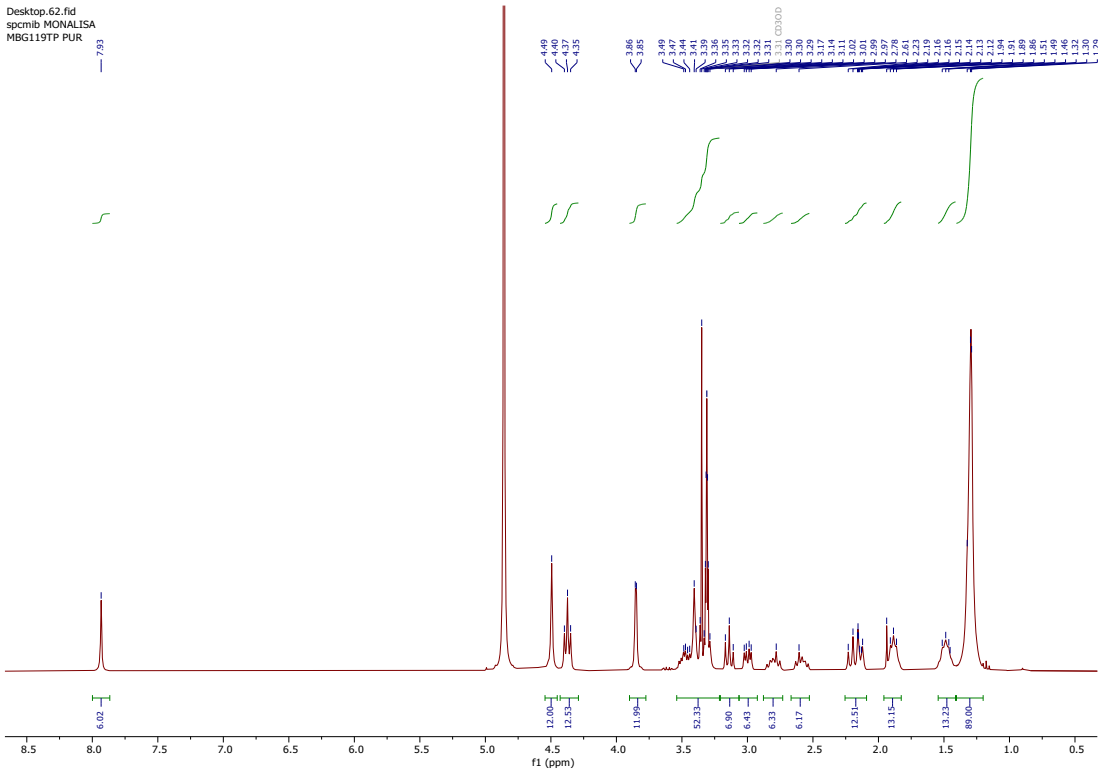


^{13}C-NMR (MeOD, 75 MHz)

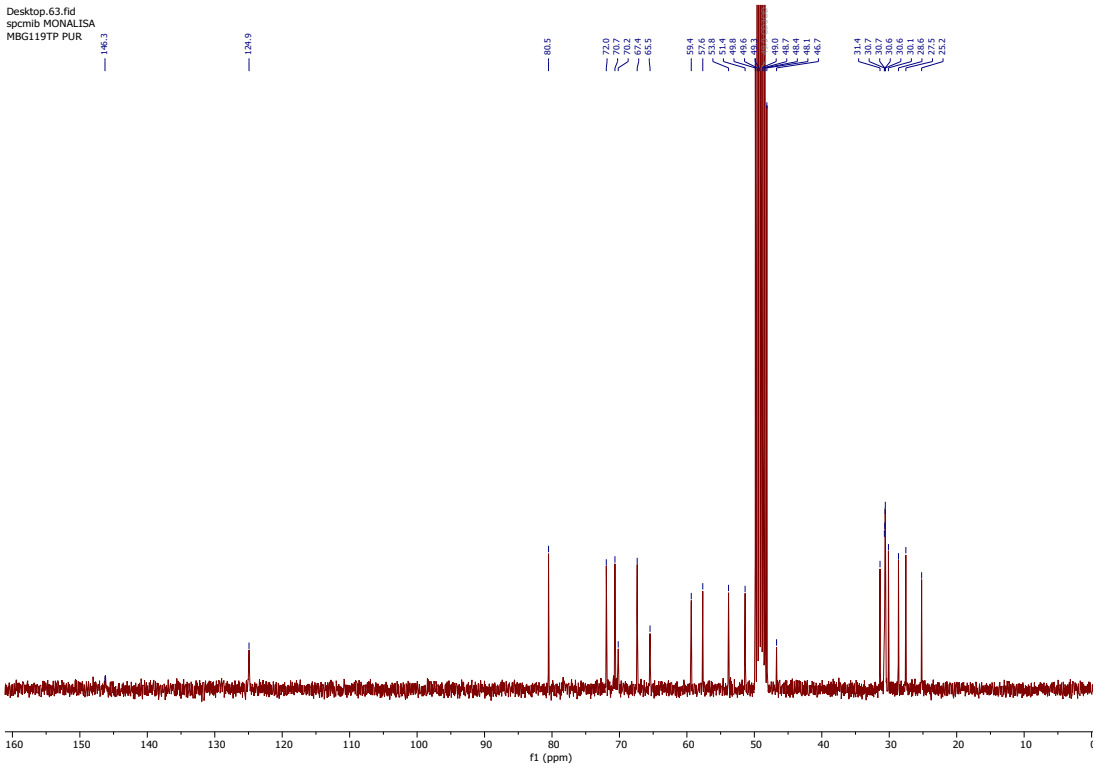


Compound **14a**

^{1}H-NMR (MeOD, 300 MHz)

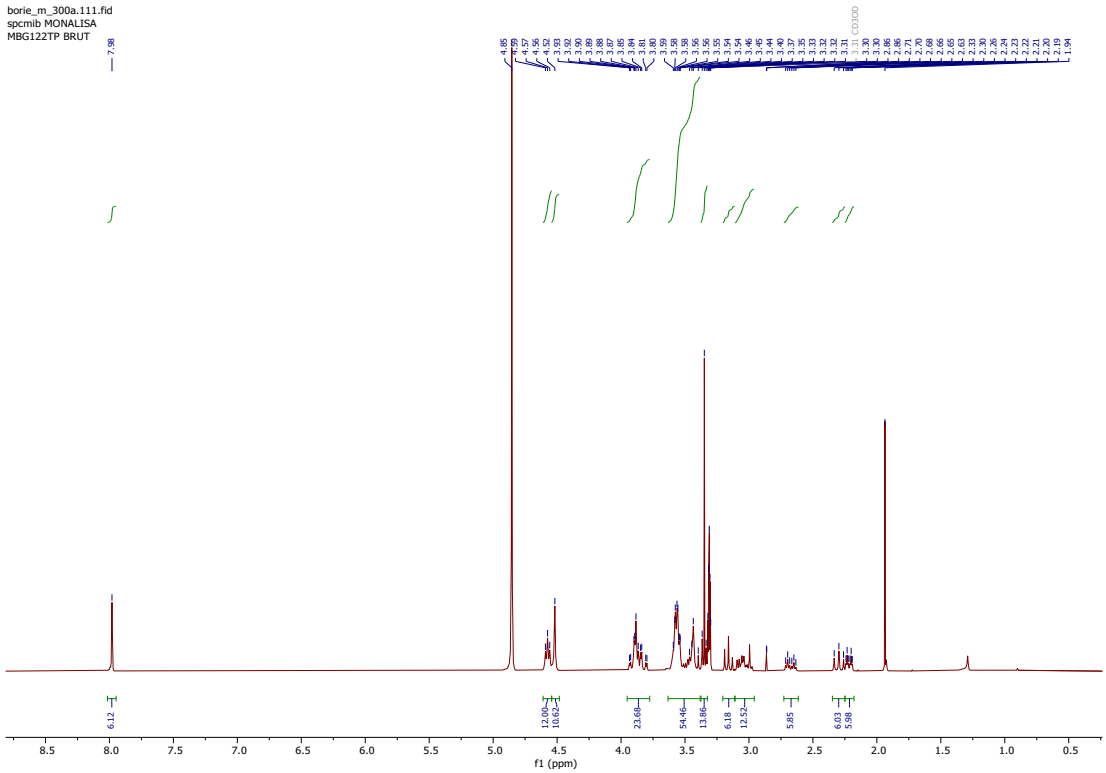


^{13}C-NMR (MeOD, 75 MHz)

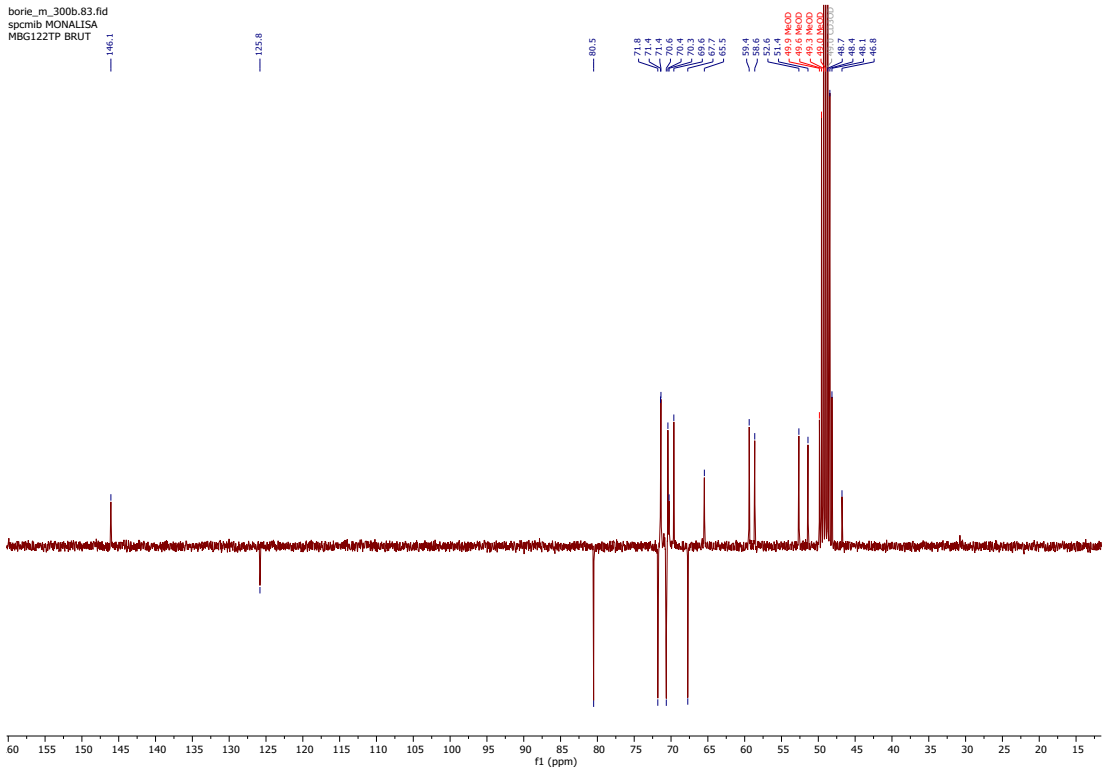


Compound **14c**

^{1}H-NMR (MeOD, 300 MHz)

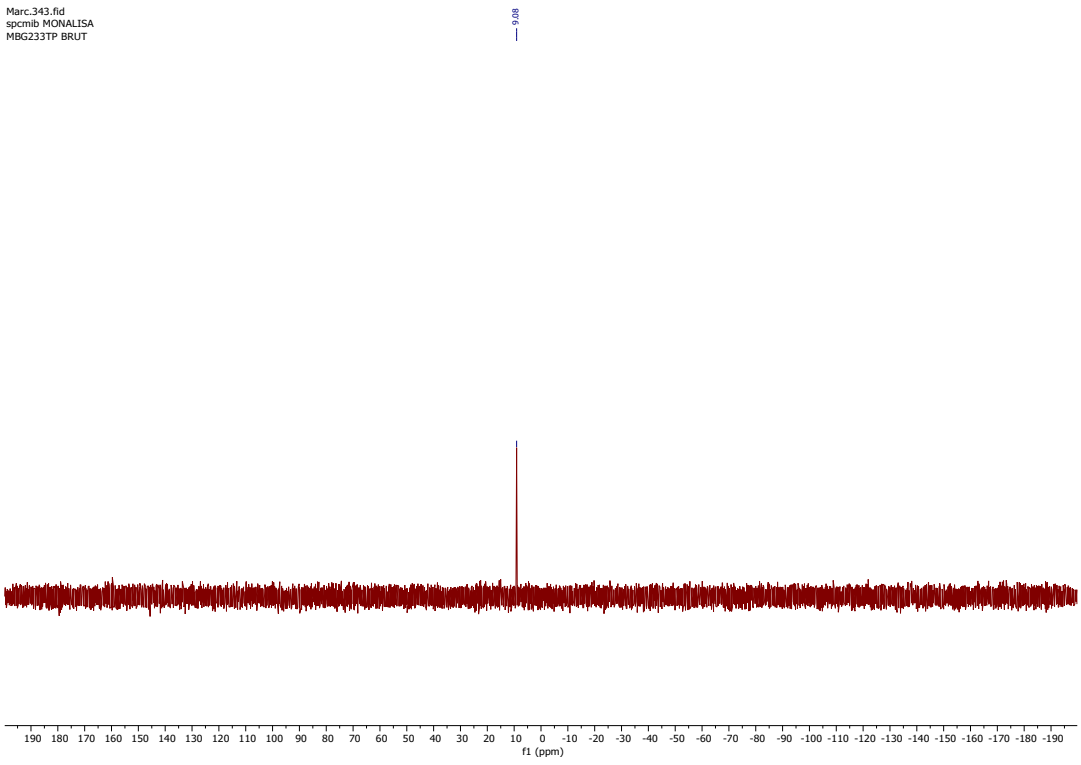


^{13}C-NMR (MeOD, 75 MHz)

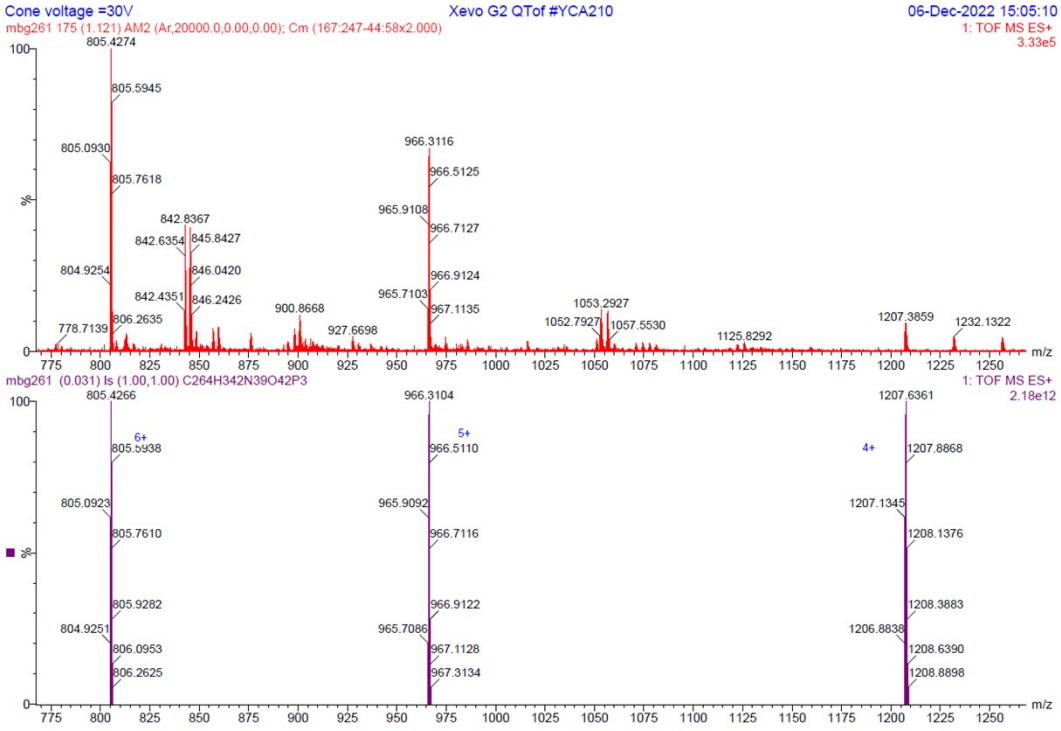


Compound **15a**

^{31}P-NMR (CD_3OD/toluene-D8, 300 MHz)



HR-MS (ESI)


Cone voltage =30V
Xevo G2 QTof #YCA210
06-Dec-2022 15:05:10
mbg261 175 (1.121) AM2 (Ar,20000.0,0.00,0.00); Cm (167:247-44:58x2.000)
1: TOF MS ES+
3.33e5
805.4274
805.5945
805.0930
805.7618
966.3116
966.5125
965.9108
966.7127
842.8367
842.6354
845.8427
804.9254
846.0420
966.9124
842.4351
846.2426
965.7103
967.1135
900.8668
1053.2927
1052.7927
1057.5530
806.2635
778.7139
927.6698
1125.8292
1207.3859
1232.1322
m/z
mbg261 (0.031) Is (1.00,1.00) C264H342N39O42P3
1: TOF MS ES+
2.18e12
805.4266
966.3104
1207.6361
6+
5+
4+
805.5938
966.5110
1207.8868
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966.9122
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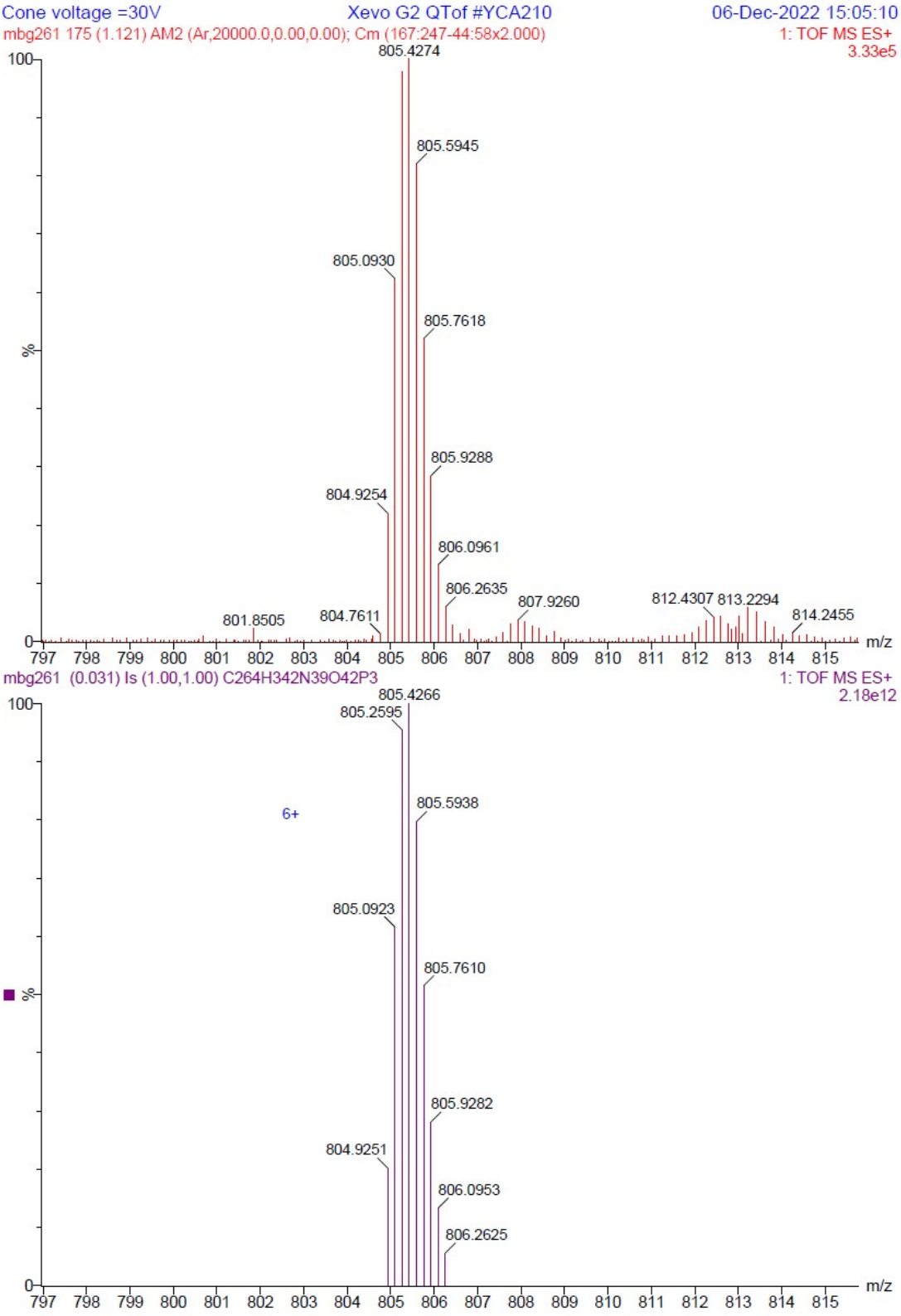
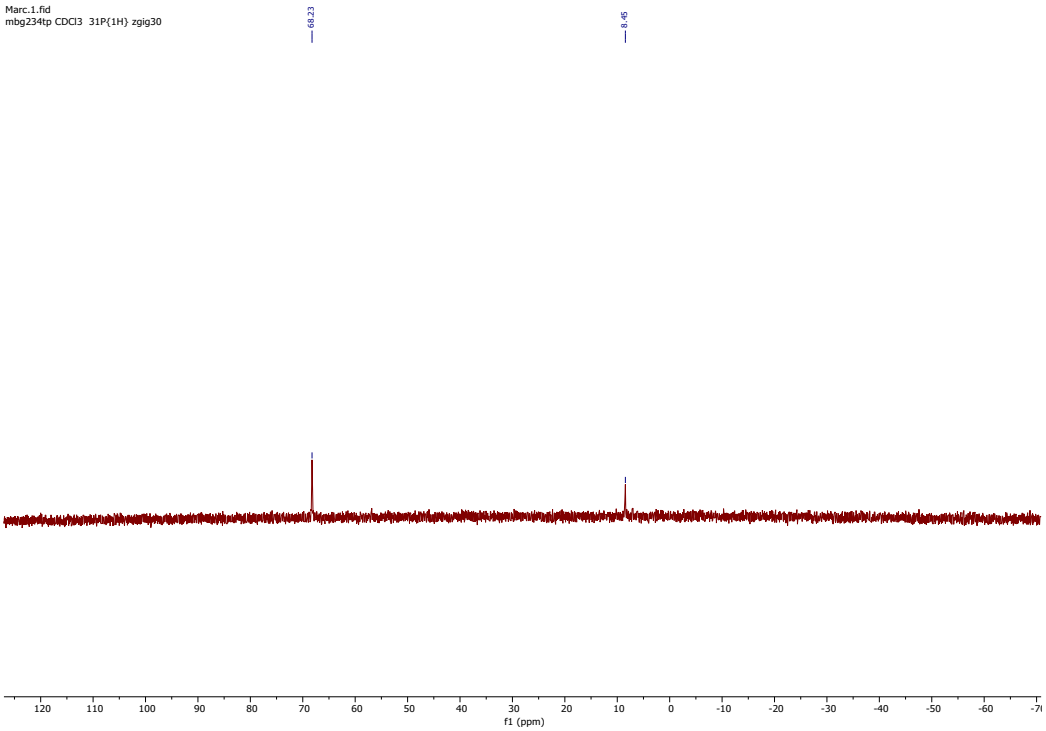
804.9251
965.7086
1206.8838
806.0953
967.1128
1208.6390
806.2625
967.3134
1208.8898
m/z

HR-MS (ESI) m/z calculated for: $C_{264}H_{342}N_{39}O_{42}P_3$ $[M+6H]^{6+}$

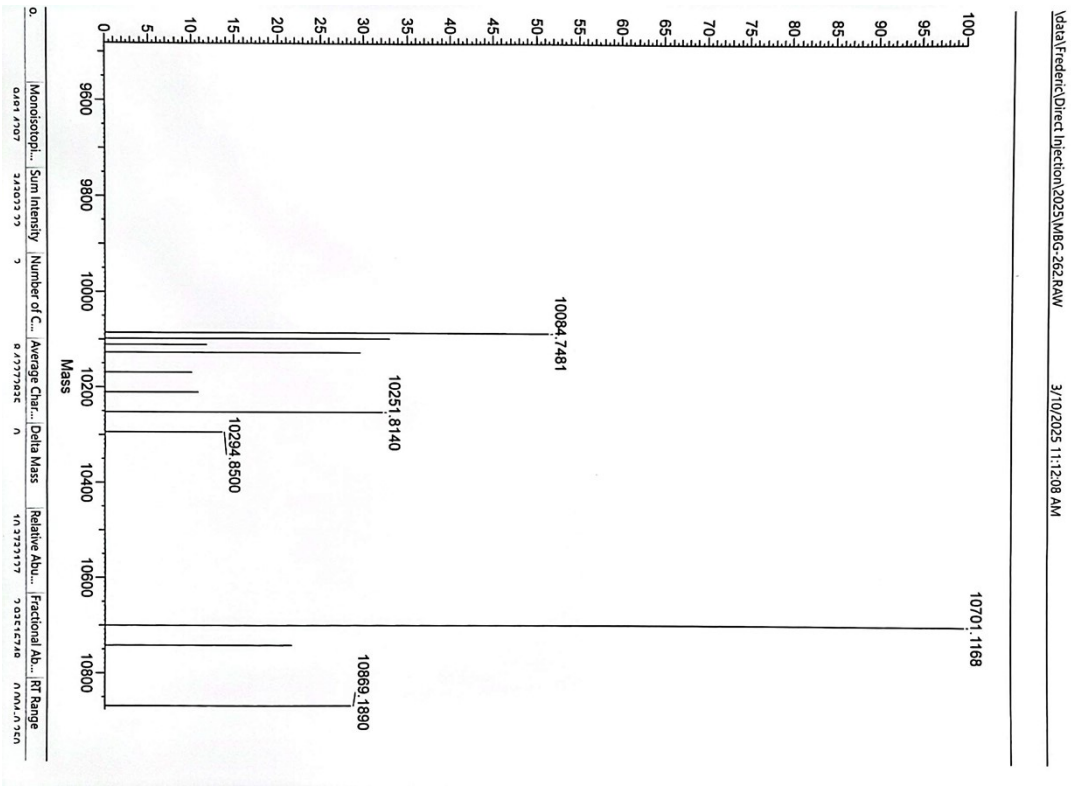


Compound **16a**

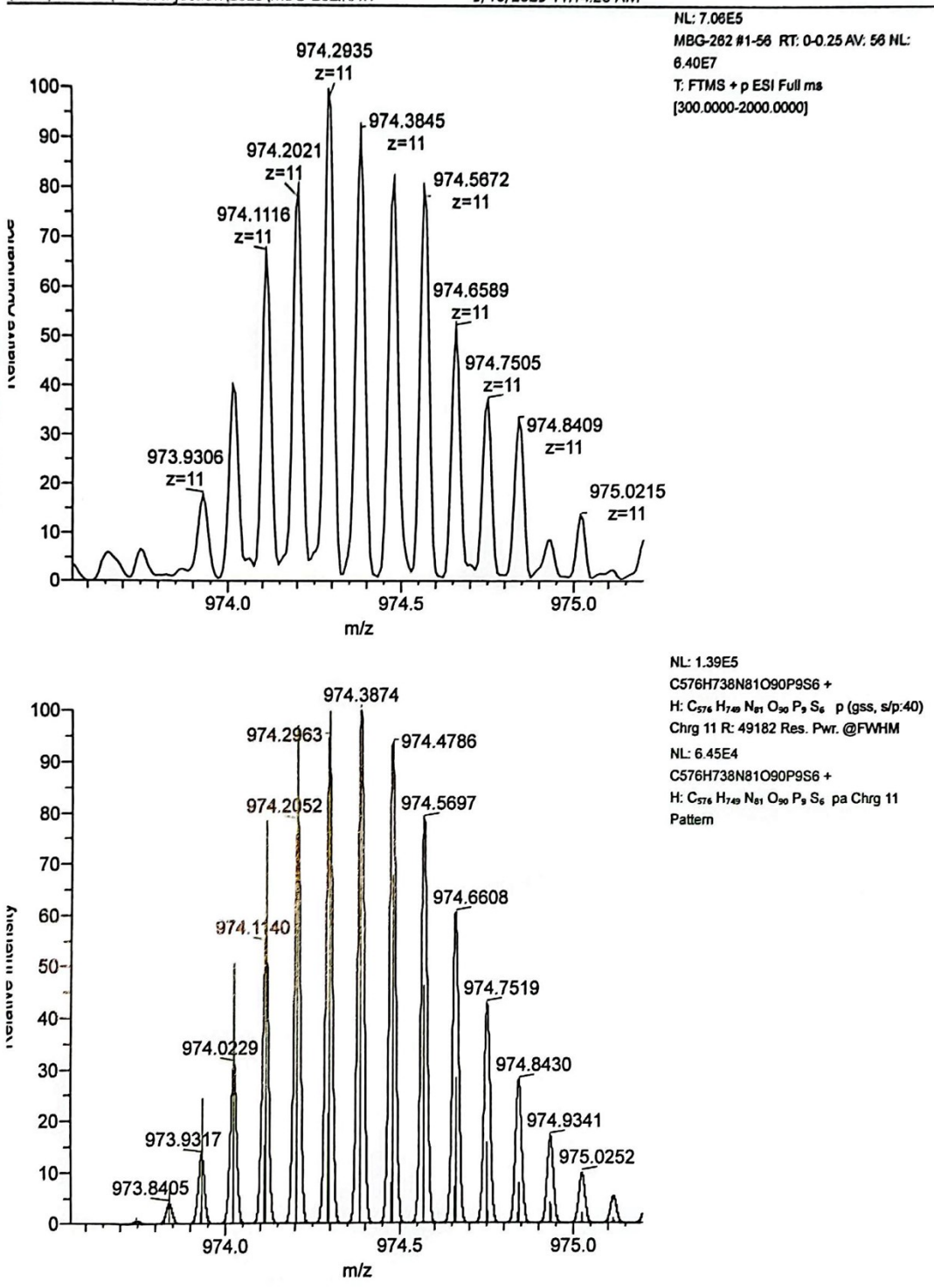
^{31}P-NMR (CD_3OD, toluene-D8, 300 MHz)



HR-MS (ESI)

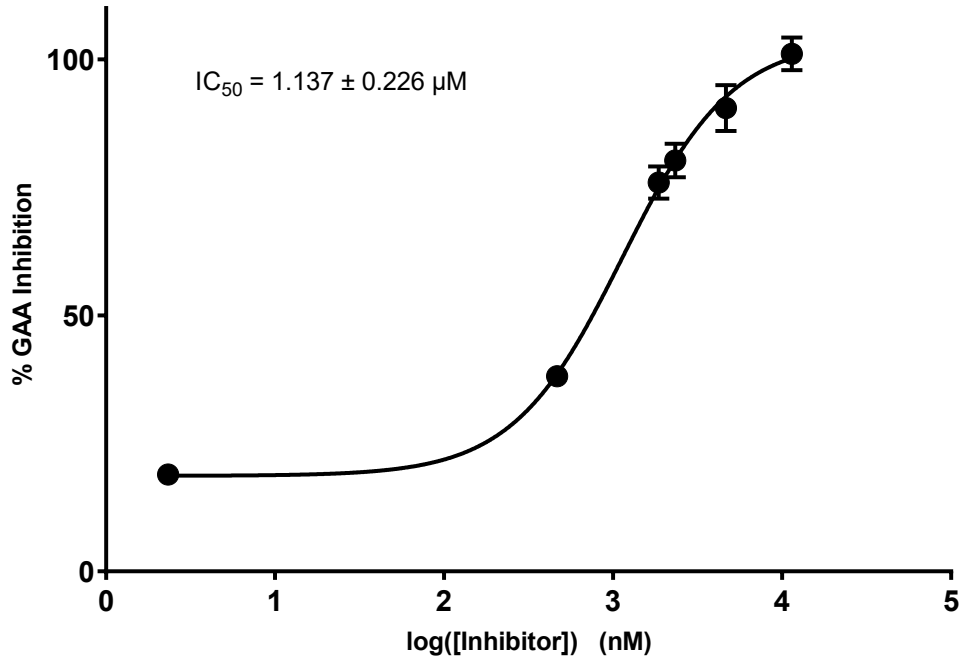


HR-MS (ESI) m/z cal

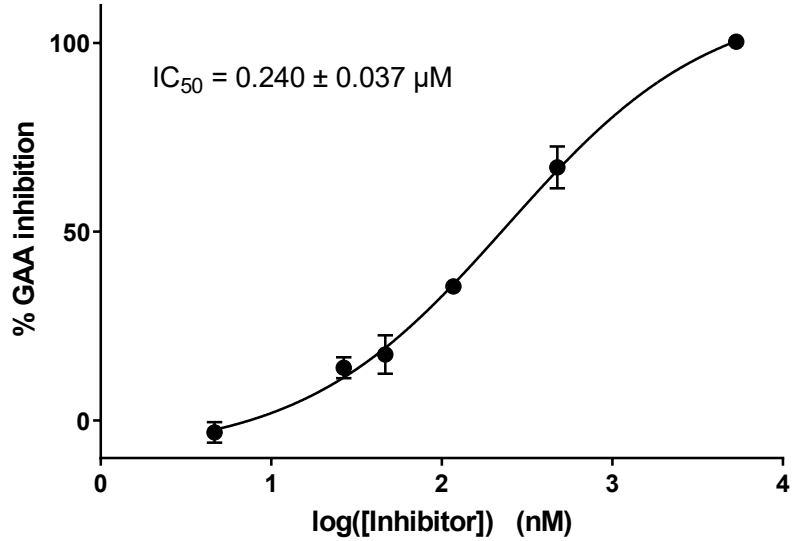


3. IC_{50} curves for *rh*GAA inhibition

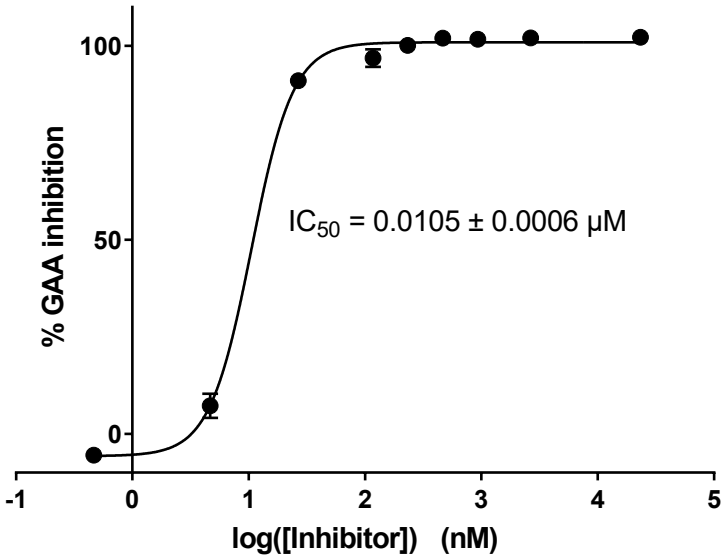
Miglitol



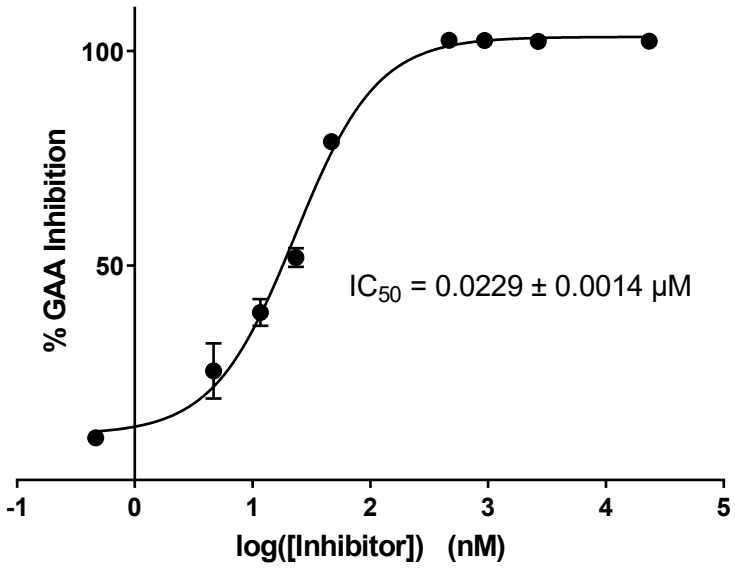
Compound **9a**



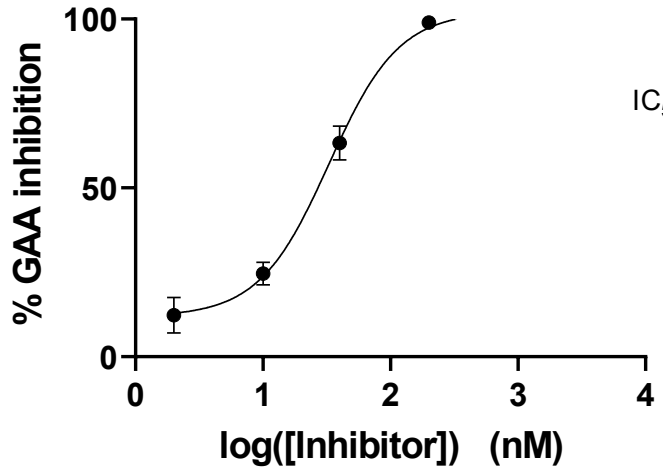
Compound **12a**



Compound **14a**

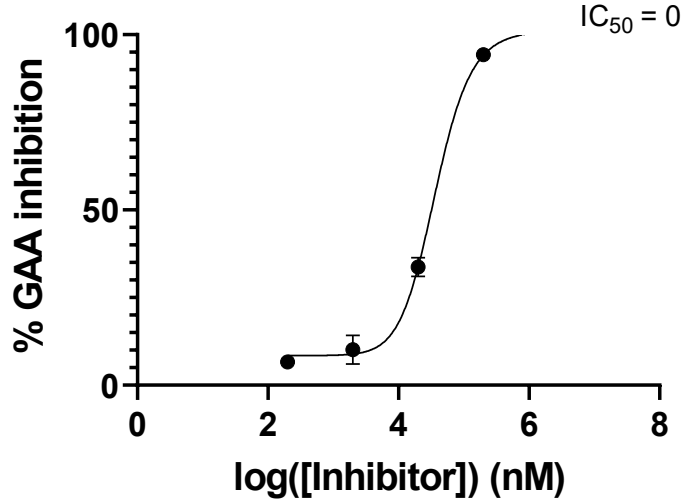


Compound **10a**



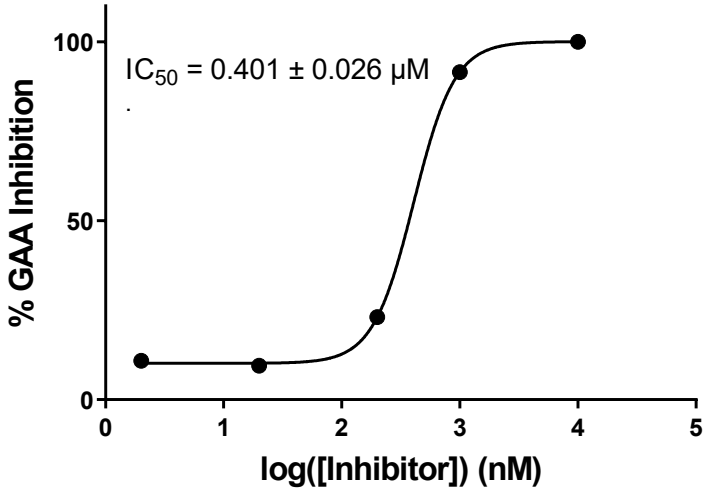
IC_{50} = 0.0329 ± 0.0046 μM

Compound **15a**

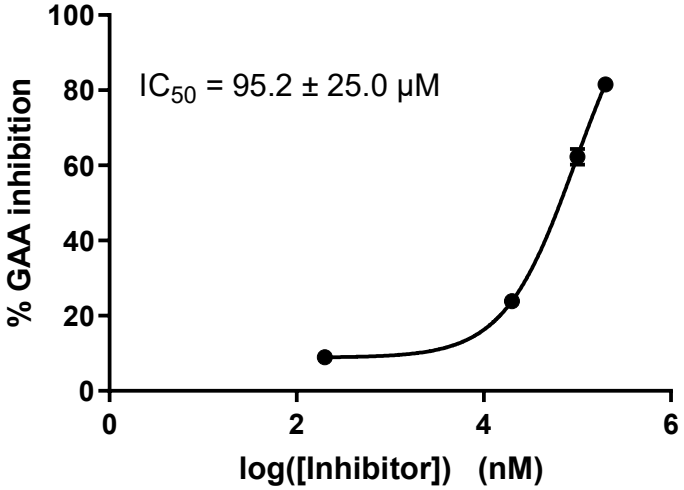


IC_{50} = 0.0379± 0.0048 μM

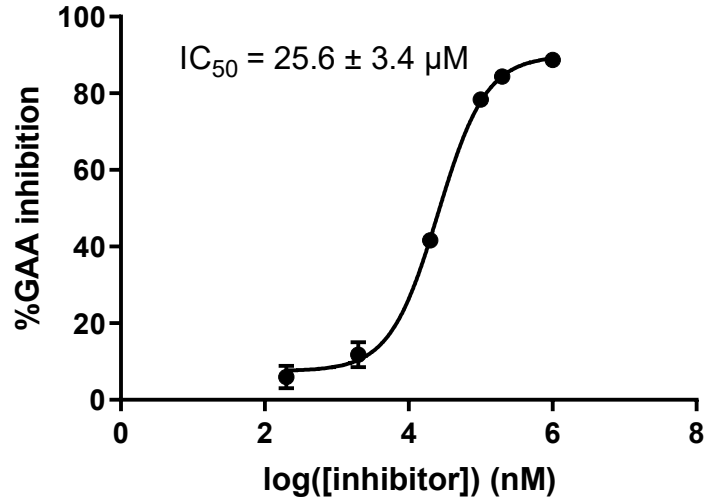
Compound **16a**



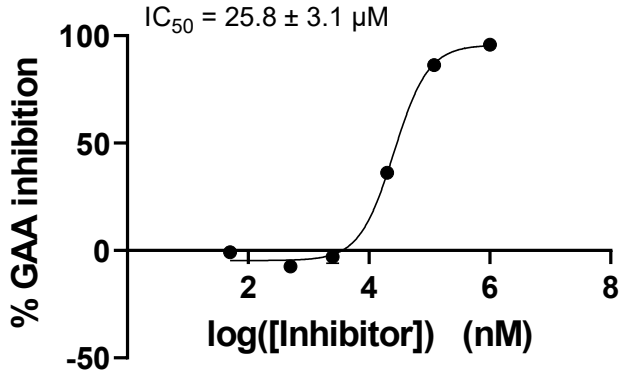
Compound **12b**



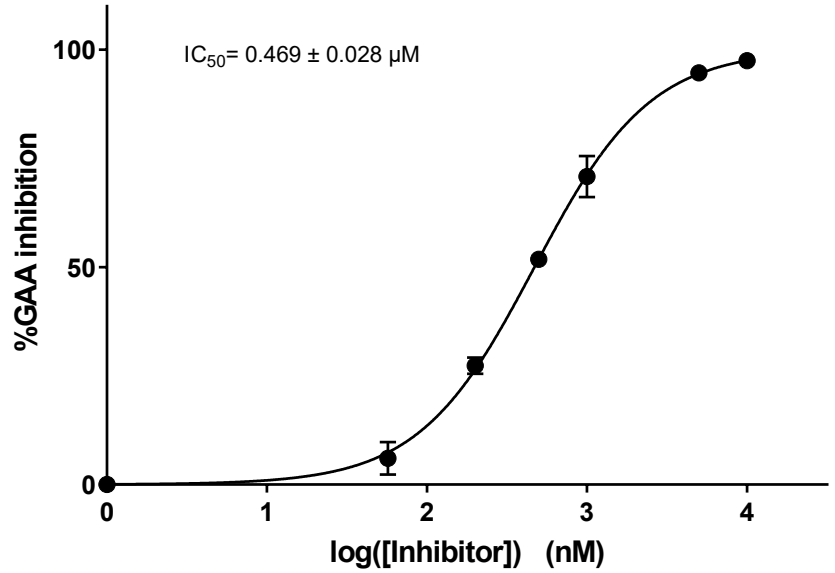
Compound **14b**



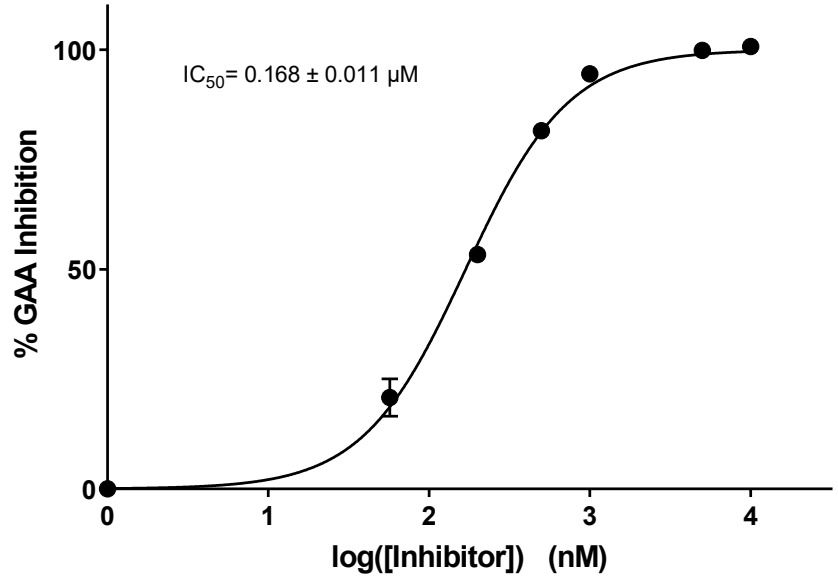
Compound **10b**



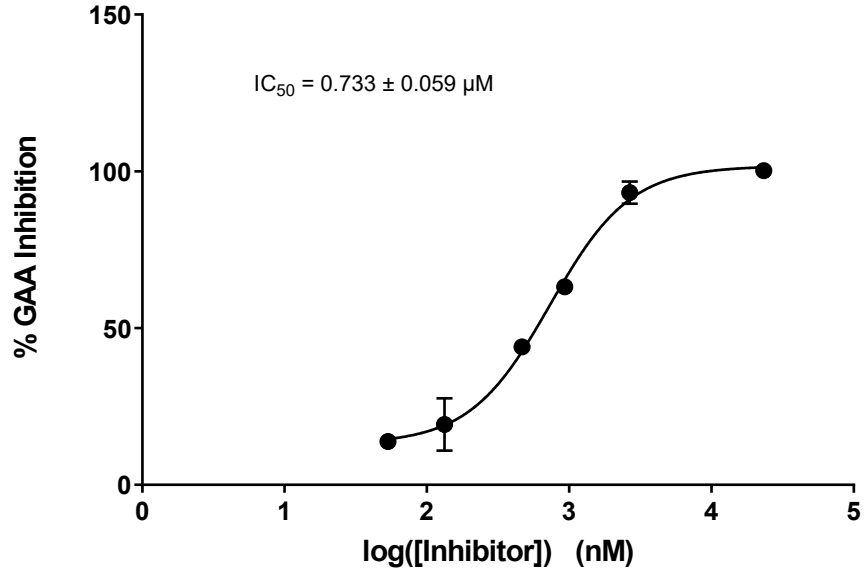
Compound **9c**



Compound **12c**

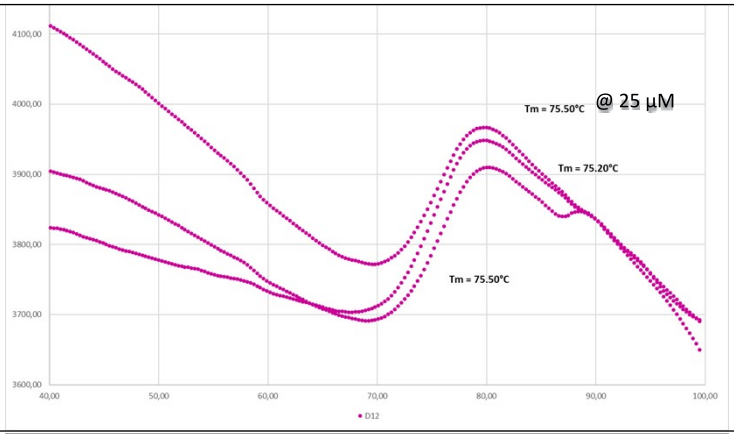


Compound **14c**

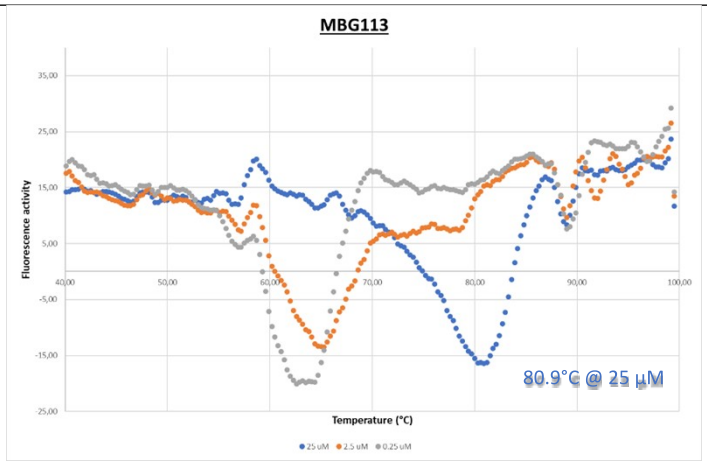


4. Differential scanning fluorimetry curves of compounds for stabilization of *rh*GAA at pH 7.4

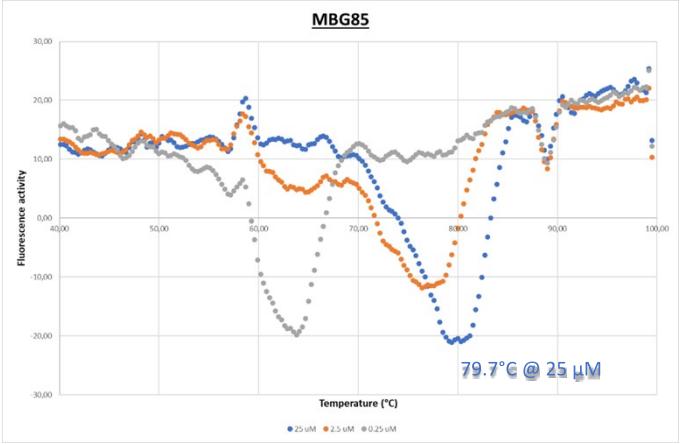
NN-DNJ



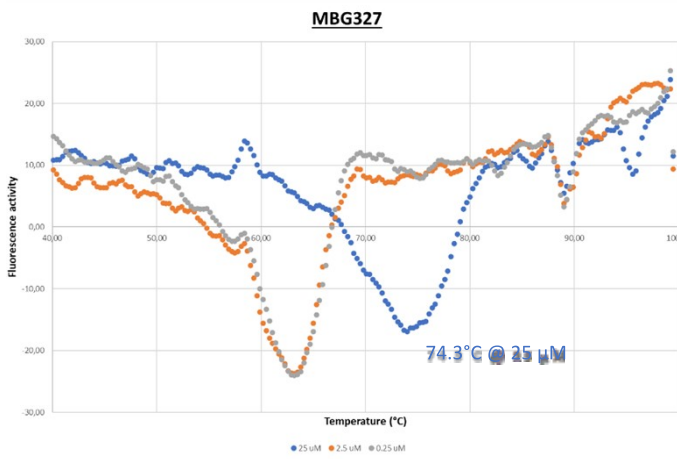
12a



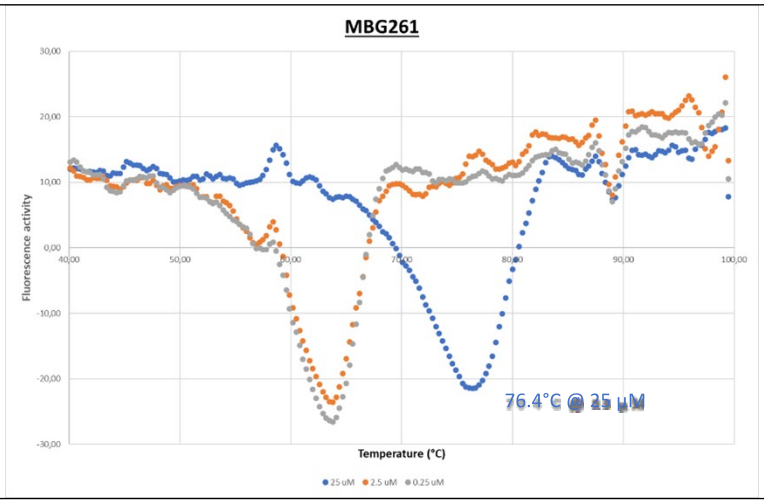
14a



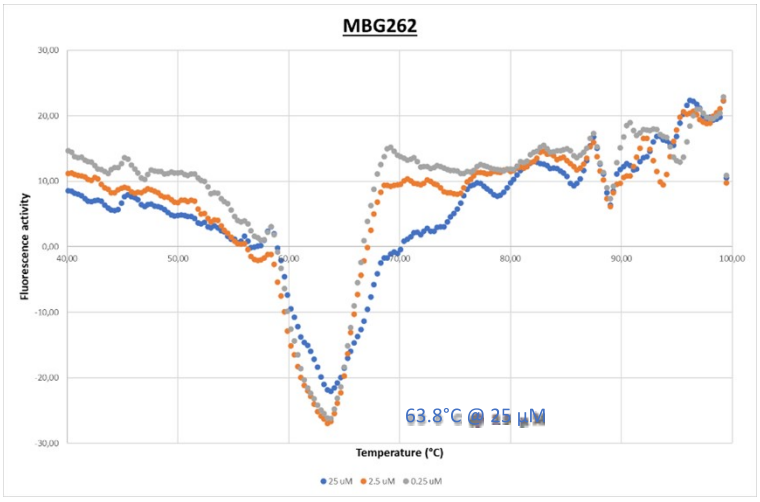
10a


MBG327
Fluorescence activity
Temperature (°C)
74.3°C @ 25 µM
25 uM
2.5 uM
0.25 uM

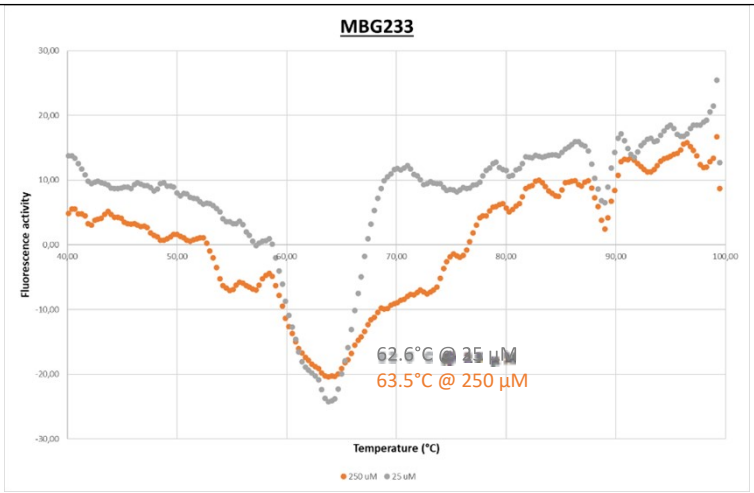
15a


MBG261
Fluorescence activity
Temperature (°C)
76.4°C @ 25 µM
25 uM
2.5 uM
0.25 uM

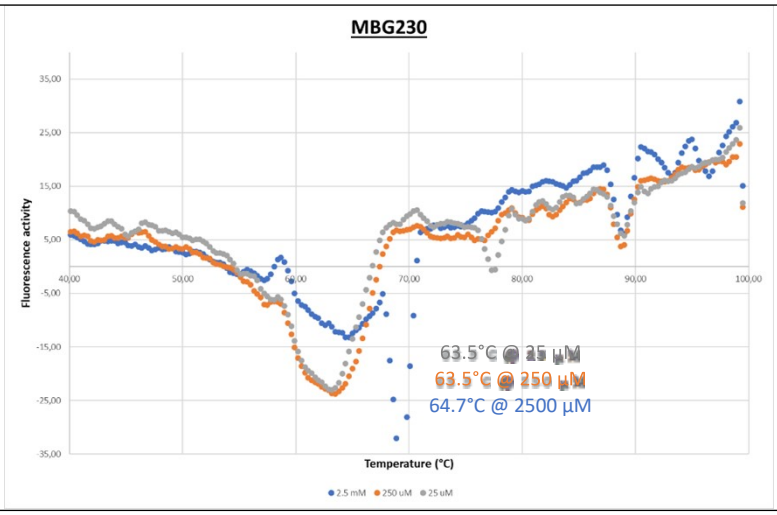
16a


MBG262
Fluorescence activity
Temperature (°C)
63.8°C @ 25 µM
25 uM
2.5 uM
0.25 uM

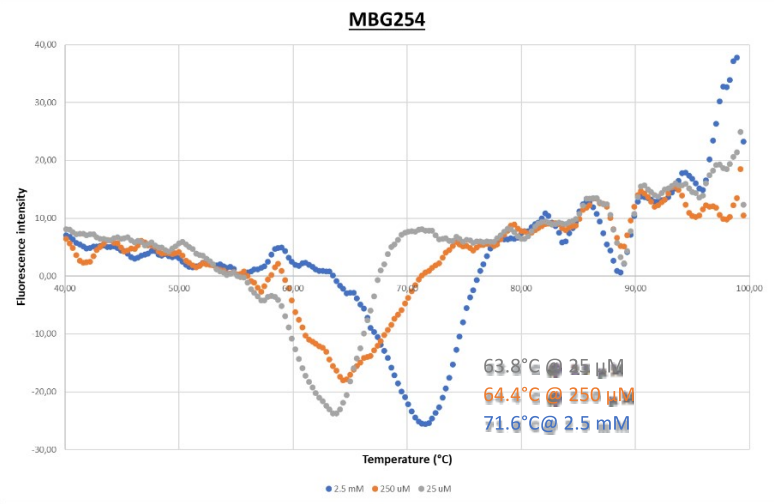
12b


MBG233
Fluorescence activity
Temperature (°C)
62.6°C @ 25 µM
63.5°C @ 250 µM
250 uM
25 uM

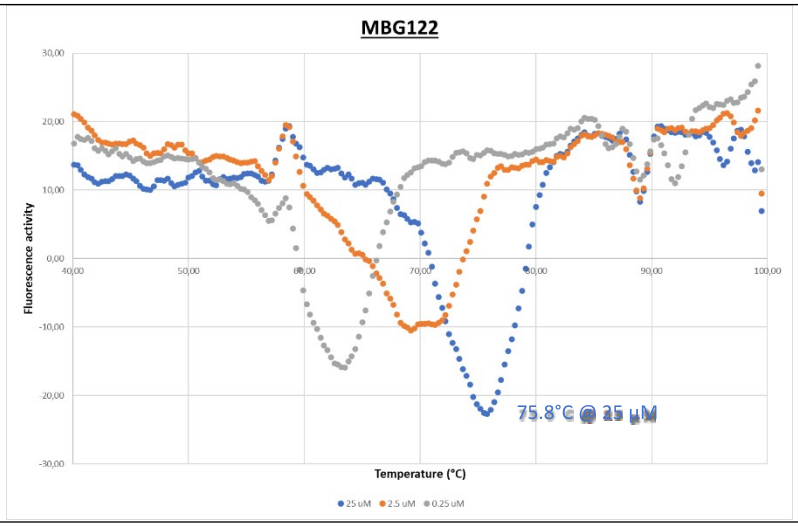
14b


MBG230
Fluorescence activity
Temperature (°C)
63.5°C @ 25 µM
63.5°C @ 250 µM
64.7°C @ 2500 µM
2.5 mM
250 uM
25 uM

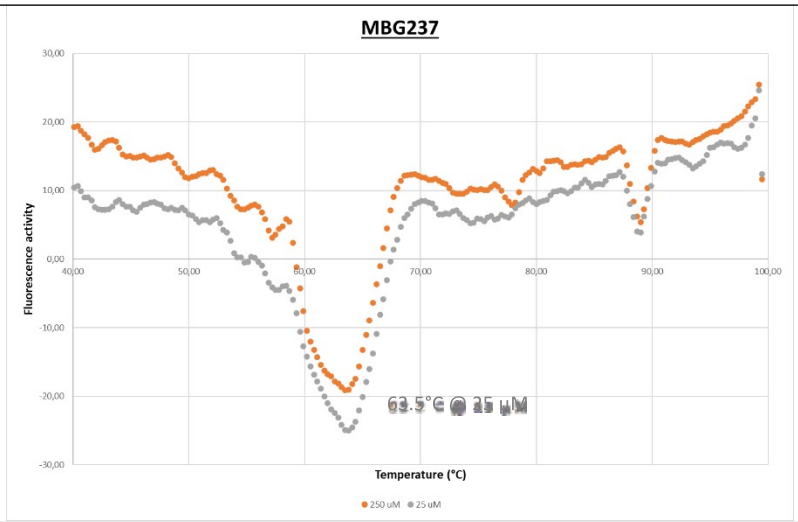
10b


MBG254
Fluorescence intensity
Temperature (°C)
63.8°C @ 25 µM
64.4°C @ 250 µM
71.6°C@ 2.5 mM
2.5 mM
250 uM
25 uM

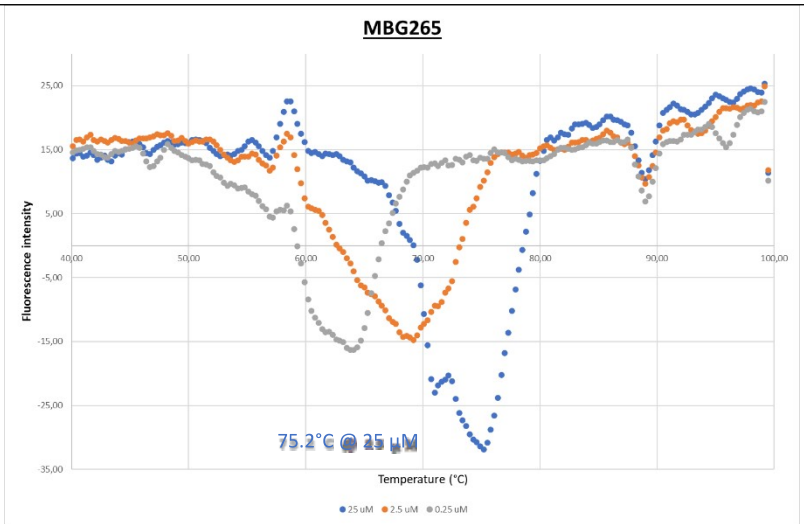
15b


MBG122
Fluorescence activity
Temperature (°C)
75.8°C @ 25 µM
25 uM
2.5 uM
0.25 uM

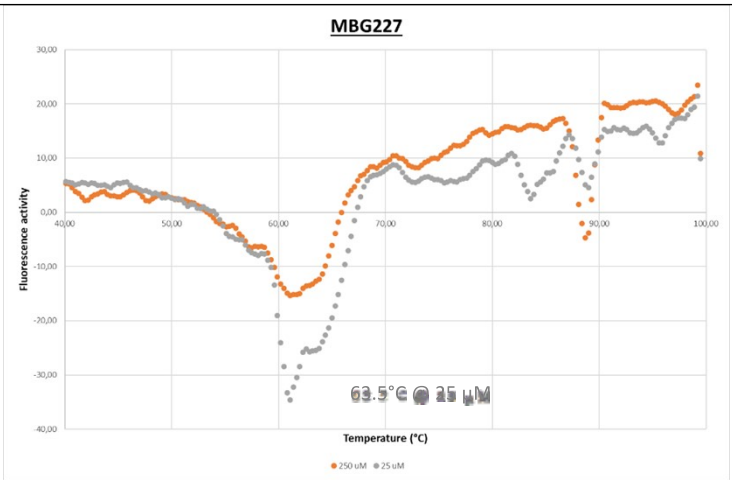
16b


MBG237
Fluorescence activity
Temperature (°C)
63.5°C @ 25 µM
250 uM
25 uM

12c


MBG265
Fluorescence intensity
Temperature (°C)
75.2°C @ 25 µM
25 uM
2.5 uM
0.25 uM

14c


MBG227
30,00
20,00
10,00
0,00
-10,00
-20,00
-30,00
-40,00
40,00
50,00
60,00
70,00
80,00
90,00
100,00
Fluorescence activity
Temperature (°C)
250 uM
25 uM

5. References

(1) Chen, D.; Zhan, J.; Zhang, M.; Zhang, J.; Tao, J.; Tang, D.; Shen, A.; Qiu, H.; Yin, S. A Fluorescent Supramolecular Polymer with Aggregation Induced Emission (AIE) Properties Formed by Crown Ether-Based Host-Guest Interactions. *Polym. Chem.* **2015**, *6* (1), 25–29. https://doi.org/10.1039/c4py01206b.

(2) Lundt, I.; Steiner, A. J.; Stütz, A. E.; Tarling, C. A.; Ully, S.; Withers, S. G.; Wrodnigg, T. M. Fluorescently Tagged Iminoalditol Glycosidase Inhibitors as Novel Biological Probes and Diagnostics. *Bioorg. Med. Chem.* **2006**, *14* (6), 1737–1742. https://doi.org/10.1016/j.bmc.2005.10.021.