Supplementary information to

Unprecedented selectivity for homologous lectin targets: differential targeting of the viral receptors L-SIGN and DC-SIGN

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Materials and methods

All commercial reagents (Abcr, Carbosynth and Merck) were used without further purification, unless otherwise indicated. When anhydrous conditions were required, the reactions were performed under nitrogen atmosphere. Anhydrous solvents were purchased from Merck with water content ≤ 0.0005%. TEA, CH_2Cl_2, CH_3CN and MeOH were dried over calcium hydride, THF was dried over sodium/benzophenone and freshly distilled. All solvents were of reagent grade or HPLC grade. When required, solvents were deoxygenated by bubbling Ar through the solvent for 30 min. Reactions were monitored by analytical thin-layer chromatography (TLC) performed on Silica Gel 60 F_{254} plates (Merck) or Silica Gel 60 RP-18F_{254}s plates (Merck) with UV detection (254 nm and 366 nm) and/or staining with molybdic reagent or ninhydrin.

- Molybdic reagent: 21 g $(NH_4)_6Mo_7O_{24}{\cdot}4H_2O$ (ammonium heptamolybdate tetrahydrate), 1 g $Ce(SO_4)_2$ and 31 mL H_2SO_4 in 470 mL H_2O;
- Ninhydrin: 300 mg ninhydrin, 3 mL glacial AcOH in 100 mL abs. EtOH;

Flash chromatography was performed according to Still's procedure[1] using Silica gel Macherey-Naegel 60 (40-63 μm, 230-400 mesh, Merck). Automated chromatography was performed on a Biotage Isolera Prime system with double UV detection (254 nm and 280 nm or 366 nm); Biotage SNAP KP-Sil and SFAR cartridges were employed. HPLC purifications were performed on Dionex Ultimate 3000 equipped with Dionex RS Variable Wavelength Detector (column: Atlantis Prep T3 OBDTM 5 μm 19 x 100 mm; flow 10 mL min−1, unless otherwise stated). A JASCO LC-4000 equipped with a C18 cartridge (Phenomenex, Luna 5u) was used for analytical HPLC. ^{1}H- and ^{13}C-NMR experiments were recorded on a Bruker Avance 400 MHz instrument at 298 K (unless otherwise stated). Chemical shifts (δ) are expressed in ppm and are referred to internal standards (TMS). The δ (ppm) axis has been calibrated on the solvent residual signal for which every spectrum was recorded. The signal shapes (^{1}H-NMR) are abbreviated as s (singlet), d (doublet), t (triplet), q (quartet), qui (quintet), sex (sextet), m (multiplet), dd (doublet doublets), td (triplet doublets). COSY and HSQC experiments were used to assist the ^{1}H and ^{13}C resonance assignments. Low resolution mass spectra were recorded on ThermoFisherLCQ apparatus (ESI ionization); high-resolution mass spectra (HRMS) were acquired on a Waters SYNAPT G2 Si ESI QTof instrument available at the MS facility of the Unitech COSPECT at the University of Milan; MALDI spectra were recorded on a Bruker Daltonics Microflex MALDI-TOF apparatus. Specific optical rotation values were measured using a Perkin-Elmer 241 polarimeter, at 589 nm in a 1 dm cell. Microwave-assisted organic synthesis was performed on a Biotage Initiator$^+$ synthesizer.

General procedure for CuAAC reaction

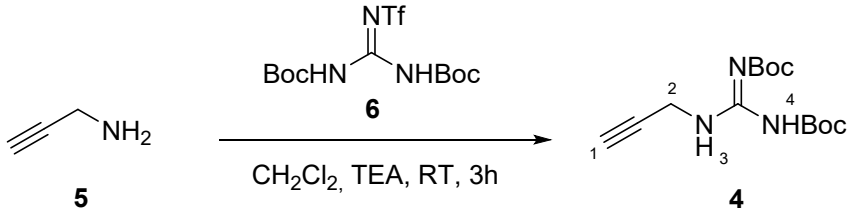
A) A 0.04 M $CuSO_4 \cdot 5H_2O$ and a 0.16 M Na-ascorbate solutions were prepared in deoxygenated water. A 0.4 M alkyne and a 0.4 M azide solutions were prepared in deoxygenated THF. To the alkyne solution (1 mol eq) were added: 0.1 mol eq of $CuSO_4 \cdot 5H_2O$ solution, 0.4 mol eq of Na-ascorbate solution and 1 mol eq of the azide solution. The final concentration of azide being 0.1 M in a 1:1 H_2O:THF mixture. The reaction was stirred at room temperature, under N_2 and protected from light. Upon completion (TLC monitoring), the solvents were evaporated and the crude product purified via flash chromatography.

B) A 0.01 M $CuSO_4 \cdot 5H_2O$ solution and a 0.04 M Na-ascorbate solution were prepared in deoxygenated water. A 0.03 M solution of alkyne, a 0.026 M solution of Tris[(1-benzyl-1H-1,2,3-triazol-4-yl)methyl]amine (**TBTA**) and a 0.2 M solution of azide were prepared in deoxygenated THF. To the alkyne solution were added: 0.2 mol eq of TBTA solution, 0.1 mol eq of $CuSO_4 \cdot 5H_2O$ solution and 0.4 mol eq of Na-ascorbate solution. The mixture was stirred at room temperature for 10 min, then 2.2 mol eq of the azide solution were added. The final concentration of alkyne is therefore 0.016M in a 1:2 H_2O:THF mixture. The reaction was stirred under microwave irradiation at 60 °C until completion (TLC, MALDI or NMR monitoring). Upon total consumption of the alkyne scaffold (4-13 h) the solvents were evaporated, and the crude product purified either via size-exclusion chromatography (SEC) using Sephadex LH-20 resin or directly via reverse phase automated flash chromatography.

General procedure for Zemplén deacetylation

1 mol eq of protected compound was dissolved in dry MeOH and 0.1M, freshly prepared, NaOMe solution (0.3 mol eq NaOMe) was added to 0.18M final concentration of the substrate (0.05M final concentration of NaOMe, unless otherwise stated). The reaction was stirred at room temperature, under N_2. Upon completion (TLC analysis), the reaction was neutralized with Amberlite® IR120 hydrogen form ion-exchange resin, filtered and concentrated *in vacuo* to yield the pure product.

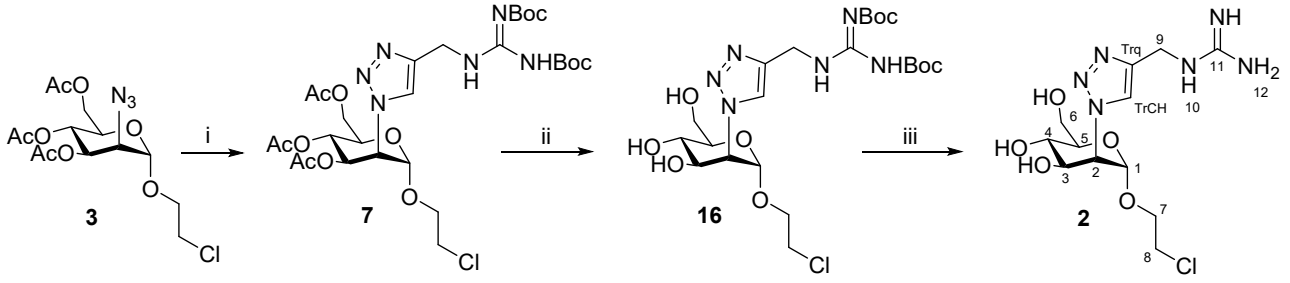
Synthesis of **4**



1,3-Di-Boc-2-(trifluoromethylsulfonyl)guanidine **6**[2,3] (abcr) (1.0 g, 2.5 mmol, 0.9 eq) and triethylamine (385 µL, 2.78 mmol, 1.0 eq) were dissolved in dry CH_2Cl_2 (12.6 mL); then propargylamine **5** (178 µL, 2.78 mmol, 1.0 eq) was added. The reaction was stirred at room temperature, under N_2. Upon completion (3h, TLC monitoring Hex:AcOEt 7:3 R_f(**4**): 0.64) the solvent was removed under reduced pressure. Crude product was purified via automated flash chromatography (Hex with AcOEt gradient from 0% to 30%) to obtain pure product **4** as a white solid (616 mg, 83%).

^{1}H-NMR (400MHz, $CDCl_3$): δ(ppm)= 11.44 (s, 1H, H_4), 8.46 (t, 1H, $J_{3\text{-}2}$ = 5.0 Hz, H_3), 4.24 (dd, 2H, $J_{2\text{-}3}$ = 5.0 Hz, $J_{2\text{-}1}$ = 2.6 Hz, H_2), 2.26 (t, 1H, $J_{1\text{-}2}$ = 2.6 Hz, H_1), 1.45 (s, 18H, H_{tBu}). MS (ESI): m/z calculated for $[C_{14}H_{23}N_3NaO_4]^+$: 320.16 $[M + Na]^+$, found: 320.02; m/z calculated for $[C_{14}H_{22}N_3O_4]^-$: 296.16 $[M - H]^-$, found: 295.99

Synthesis of **2 (Man84)**



i) **4,** H_2O:THF 1:1, RT, ovn; ii) MeONa, MeOH, room temp. 2h;
iii) 20% TFA in CH_2Cl_2 (**2** TFA salt) or 1M aq HCl in CH_2Cl_2, 3 d (**2** chloride salt)

i) CuAAC reaction

Synthesized from **3**[4] (59 mg, 0.15 mmol) and propargyl guanidine **4** according to general procedure A for CuAAC reaction. Purified via flash chromatography (Hex with AcOEt gradient from 0% to 70%) to obtain pure product **7** as a white foam (96.7 mg, 93%).

R_f(**7**): 0.27 in Hex:AcOEt 6:4

^{1}H-NMR (400MHz, $CDCl_3$): δ(ppm)= 11.44 (s, 1H, H_{12}), 8.82 (t, 1H, $J_{10\text{-}9}$ = 4.9 Hz, H_{10}), 7.98 (s, 1H, H_{TrCH}), 5.51 (dd, 1H, $J_{3\text{-}4}$ = 9.9 Hz, $J_{3\text{-}2}$ = 5.2 Hz, H_3), 5.42 (dd, 1H, $J_{2\text{-}3}$ = 5.3 Hz, $J_{2\text{-}1}$ = 0.8 Hz, H_2), 5.26 (t, 1H, $J_{4\text{-}3}$ =

9.9 Hz, H_4), 5.14 (s, 1H, H_1), 4.82 (dd, 1H, $J_{9a\text{-}9b}$ = 15.5 Hz, $J_{9a\text{-}10}$ = 4.9 Hz, H_{9a}), 4.68 (dd, 1H, $J_{9b\text{-}9a}$ = 15.5 Hz, $J_{9b\text{-}10}$ = 4.9 Hz, H_{9b}), 4.33-4.18 (m, 3H, H_6, H_5), 4.02-3.94 (m, 1H, H_{7a}), 3.91-3.83 (m, 1H, H_{7b}), 3.72 (t, 2H, $J_{8\text{-}7}$ = 5.7 Hz, H_8), 2.16 (s, 3H, OAc), 2.05 (s, 3H, OAc), 1.95 (s, 3H, OAc), 1.50 (s, 9H, H_{tBu}), 1.47 (s, 9H, H_{tBu}). ^{13}C-NMR (400MHz, $CDCl_3$): δ(ppm) extrapolated from HSQC = 121.8 (C_{TrCH}), 97.7 (C_1), 68.9 (C_5), 68.7 (C_7), 68.5 (C_3), 64.7 (C_4), 61.9 (C_6), 60.0 (C_2), 42.3 (C_8), 36.2 (C_9), 28.1 (tBu), 20.7 ($\underline{CH_3}CO$), 20.5 ($\underline{CH_3}CO$), 20.4 ($\underline{CH_3}CO$). MS (ESI): m/z calculated for $[C_{28}H_{44}ClN_6O_{12}]^+$: 691.27 $[M + H]^+$, found: 691.58; m/z calculated for $[C_{28}H_{43}ClN_6NaO_{12}]^+$: 713.25 $[M + Na]^+$, found: 713.54; m/z calculated for $[C_{28}H_{42}ClN_6O_{12}]^-$: 689.25 $[M - H]^-$, found: 689.65. HRMS (ESI): m/z calculated for $[C_{28}H_{43}ClN_6NaO_{12}]^+$: 713.2525 $[M + Na]^+$, found: 713.2521.

ii) Zemplén deacetylation

Performed on intermediate **7** (50 mg, 0.07 mmol) according to general procedure for deacetylation (0.02M final concentration of NaOMe).

R_f(**16**): 0.57 in 9:1 CH_2Cl_2:MeOH

^{1}H-NMR (400MHz, CD_3OD): δ(ppm)= 8.18 (s, 1H, H_{TrCH}), 5.14 (s, 1H, H_1), 5.09 (dd, 1H, $J_{2\text{-}3}$ = 5.2 Hz, $J_{2\text{-}1}$ = 0.9 Hz, H_2), 4.68 (s, 2H, H_9), 4.19 (dd, 1H, $J_{3\text{-}4}$ = 9.4 Hz, $J_{3\text{-}2}$ = 5.2 Hz, H_3), 4.04-3.97 (m, 1H, H_{7a}), 3.92-3.78 (m, 4H, H_6, H_{7b}, H_5), 3.77-3.68 (m, 3H, H_8, H_4), 1.52 (s, 9H, H_{tBu}), 1.48 (s, 9H, H_{tBu}). ^{13}C-NMR (400MHz, CD_3OD): δ(ppm) extrapolated from HSQC = 123.4 (C_{TrCH}), 97.9 (C_1), 73.8 (C_5), 68.9 (C_3), 67.7 (C_7), 66.3 (C_4), 60.2 (C_2), 60.7 (C_6), 42.4 (C_8), 35.5 (C_9), 27.1 (H_{tBu}), 26.9 (H_{tBu}). MS (HRMS): m/z calculated for $[C_{22}H_{38}ClN_6O_9]^+$: 565.24 $[M + H]^+$, found: 565.20; m/z calculated for $[C_{22}H_{37}ClN_6NaO_9]^+$: 587.22 $[M + Na]^+$, found: 587.21; m/z calculated for $[C_{22}H_{37}ClN_6O_9]^-$: 563.22 $[M - H]^-$, found: 563.17.

iii) Boc-deprotection (TFA method)

Compound **16** (47.4 mg, 0.083 mmol, 1 eq.) was dissolved in a 4:1 mixture of dry CH_2Cl_2 and TFA (1.66 mL). The reaction was stirred at room temperature, under N_2 for 5h. Upon completion (TLC monitoring, CH_2Cl_2:MeOH 8:2, R_f(**2**): 0.21, staining Molybdic reagent and/or Ninhydrin) the mixture was concentrated *in vacuo* and co-evaporated with toluene three-times to obtain the pure product **2**·**TFAH** as a white foam (39 mg, quantitative).

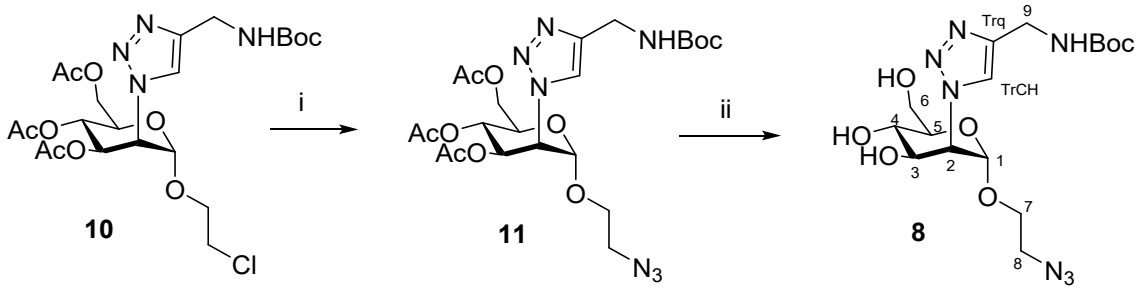
iii) Boc-deprotection (HCl method)

Compound **16** (36.3 mg, 0.064 mmol, 1 eq.) was dissolved in CH_2Cl_2 (1.4 mL), then 190 µL of a 1M aqueous solution of HCl (3.0 eq) were added. The reaction was stirred at room temperature for 3 days. Upon completion (TLC monitoring, CH_2Cl_2:MeOH 8:2, R_f(**2**): 0.21, staining Molybdic reagent and/or Ninhydrin), the mixture was concentrated *in vacuo* and co-evaporated with toluene three-times to obtain pure product **2**·**HCl** as a white foam (22.7 mg, 88%).

^{1}H-NMR (400MHz, CD_3OD): δ(ppm)= 8.24 (s, 1H, H_{TrCH}), 5.16-5.10 (m, 2H, H_1, H_2), 4.51 (s, 2H, H_9), 4.24 (dd, 1H, J_{3-4} = 9.6 Hz, J_{3-2} = 5.3 Hz, H_3), 4.06-3.99 (m, 1H, H_{7a}), 3.91 (d, 2H, J_{6-5} = 3.1 Hz, H_6), 3.87-3.80 (m, 2H, H_{7b}, H_5), 3.79-3.71 (m, 3H, H_8, H_4). ^{13}C-NMR (400 MHz, CD_3OD): δ(ppm)=158.9 (C_{11}), 158.8 (C_{Trq}), 124.9 (C_{TrCH}), 99.4 (C_1), 75.1 (C_5), 70.1 (C_3), 69.3 (C_7), 67.6 (C_4), 65.4 (C_2), 61.8 (C_6), 43.8 (C_8), 37.6 (C_9). MS (HRMS): m/z calculated for $[C_{12}H_{22}ClN_6O_5]^+$: 365.1340 $[M + H]^+$, found: 365.1337; m/z calculated for $[C_{12}H_{21}ClN_6NaO_5]^+$: 387.1159 $[M + Na]^+$, found: 387.1153.

$[\alpha]_D^{27} = +\ 10\ \ (c = 0.5, MeOH)$

Synthesis of **8**



i) NaN_3, DMF, 55 °C, 3d; ii) NaOMe, MeOH, RT, 1h

i) Chlorine-azide exchange

Compound **10**[4] (990 mg, 1.8 mmol, 1 eq) was dissolved in dry DMF (9 mL), then NaN_3 (586 mg, 9 mmol, 5.0 eq) was added, and the reaction stirred at 55 °C for 3 days. Upon completion (^{1}H-NMR monitoring), the mixture was concentrated *in vacuo* and the crude product purified via automated flash chromatography (Hex with AcOEt gradient from 35% to 70%; R_f(**11**): 0.25 in Hex:AcOEt 1:1) to obtain pure product **11** as a white foam (919.4 mg, 92%).

^{1}H-NMR (400 MHz, CD_3OD): δ(ppm)= 8.10 (s, 1H, H_{TrCH}), 5.49 (dd, 1H, J_{3-4} = 10.2 Hz, J_{3-2} = 5.1 Hz, H_3), 5.40 (d, 1H, J_{2-3} = 5.1 Hz, H_2), 5.32 (t, 1H, J_{4-3} = 10.2 Hz, H_4), 5.27 (s, 1H, H_1), 4.41-4.32 (m, 3H, H_{6a}, H_9), 4.30-4.19 (m, 2H, H_{6b}, H_5), 4.01-3.92 (m, 1H, H_{7a}), 3.82-3.75 (m, 1H, H_{7b}), 3.62-3.45 (m, 2H, H_8), 2.16 (s, 3H, OAc), 2.05 (s, 3H, OAc), 1.89 (s, 3H, OAc), 1.45 (s, 9H, H_{tBu}). ^{13}C-NMR (400MHz, CD_3OD): δ(ppm) extrapolated from HSQC = 122.1 (C_{TrCH}), 97.5 (C_1), 68.9 (C_5), 68.7 (C_3), 67.1 (C_7), 64.8 (C_4), 61.6 (C_6), 60.2 (C_2), 50.1 (C_8), 35.6 (C_9), 27.2 (tBu), 19.5 ($\underline{CH_3}CO$), 19.2 ($\underline{CH_3}CO$), 18.9 ($\underline{CH_3}CO$). MS (ESI): m/z calculated for $[C_{22}H_{33}N_7NaO_{10}]^+$: 578.22 $[M + Na]^+$, found: 578.38.

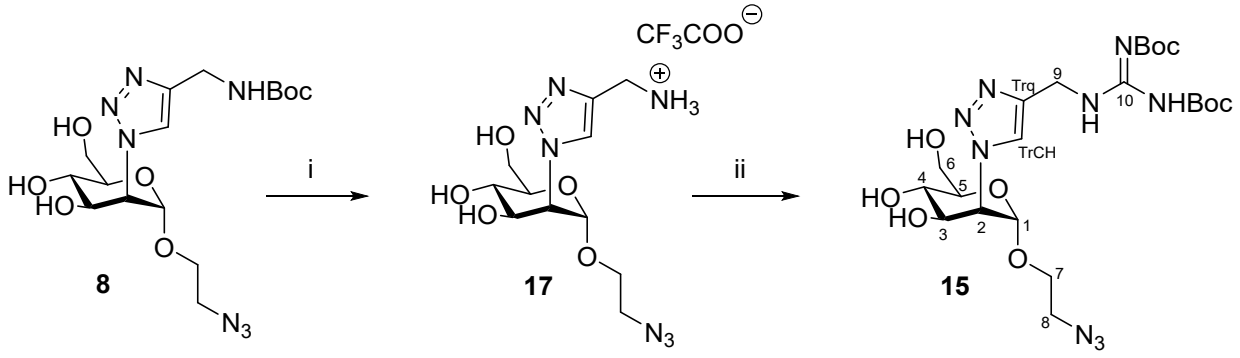
ii) Zemplén deacetylation

Performed on intermediate **11** (50 mg, 0.09 mmol) according to general procedure for deacetylation (37.8 mg, 98%).

R_f(**8**): 0.51 in $CHCl_3$:MeOH 9:1

^{1}H-NMR (400 MHz, CD_3OD): δ(ppm)= 8.06 (s, 1H, H_{TrCH}), 5.12 (s, 1H, H_1), 5.07 (d, 1H, $J_{2\text{-}3}$= 4.7 Hz, H_2), 4.31 (s, 2H, H_9), 4.24-4.17 (m, 1H, H_3), 4.01-3.82 (m, 3H, $H_{7a,}$ H_6), 3.81-3.66 (m, 3H, $H_{7b,}$ H_4, H_5), 3.48 (t, 2H, $J_{8\text{-}7}$ = 4.6 Hz, H_8), 1.45 (s, 9H, H_{tBu}). ^{13}C-NMR (400MHz, CD_3OD): δ(ppm) extrapolated from HSQC = 122.7 (C_{TrCH}), 97.9 (C_1), 73.9 (C_5), 68.9 (C_3), 66.5 (C_7), 66.3 (C_4), 64.2 (C_2), 60.7 (C_6), 50.0 (C_8), 35.4 (C_9), 27.2 (tBu). MS (ESI): m/z calculated for $[C_{16}H_{27}N_7NaO_7]^+$: 452.19 $[M + Na]^+$, found: 452.19; m/z calculated for $[C_{32}H_{54}N_{14}NaO_{14}]^+$: 881.38 $[2M + Na]^+$, found: 881.01; m/z calculated for $[C_{16}H_{26}N_7O_7]^-$: 428.18 $[M - H]^-$, found 428.18. HRMS (ESI): m/z calculated for $[C_{16}H_{27}N_7NaO_7]^+$: 452.1870 $[M + Na]^+$, found: 452.1873

Synthesis of **15**



i) CH_2Cl_2:TFA 9:1, RT, 1h;
ii) 1,3-Di-Boc-2-(trifluoromethylsulfonyl)guanidine **6**, TEA, 1,4-dioxane:H_2O 5:1, RT, ovn.

i) Boc-deprotection

Compound **8** (37.5 mg, 0.087 mmol, 1 eq.) was dissolved in a 9:1 mixture of dry CH_2Cl_2 and TFA (1.77 mL). The reaction was stirred at room temperature, under N_2 for 1h. Upon completion (1h, TLC monitoring, $CHCl_3$:MeOH 8:2, R_f(**17**): 0.21) the mixture was concentrated *in vacuo* and co-evaporated with toluene three-times to obtain the pure TFA salt **17** as a yellowish foam (38.5 mg, quantitative).

^{1}H-NMR (400 MHz, CD_3OD): δ(ppm)= 8.30 (s, 1H, H_{TrCH}), 5.16-5.11 (m, 2H, $H_{1,}$ H_2), 4.28-4.21 (m, 3H, H_9, H_3), 4.01-3.93 (m, 1H, H_{7a}), 3.90 (d, 2H, $J_{6\text{-}5}$ = 3.4 Hz, H_6), 3.82-3.76 (m, 1H, H_5), 3.75-3.66 (m, 2H, H_4, H_{6b}), 3.53-3.46 (t, 2H, $J_{8\text{-}7}$ = 4.7 Hz, H_8). ^{13}C-NMR (400MHz, CD_3OD): δ(ppm) extrapolated from HSQC = 124.3 (C_{TrCH}), 98.1 (C_1), 73.8 (C_5), 68.9 (C_3), 66.7 (C_7), 66.5 (C_4), 64.3 (C_2), 60.7 (C_6), 50.2 (C_8), 34.1 (C_9). MS (ESI): m/z calculated for $[C_{11}H_{29}N_7O_5]^+$: 330.15 $[M + Na]^+$, found: 330.18

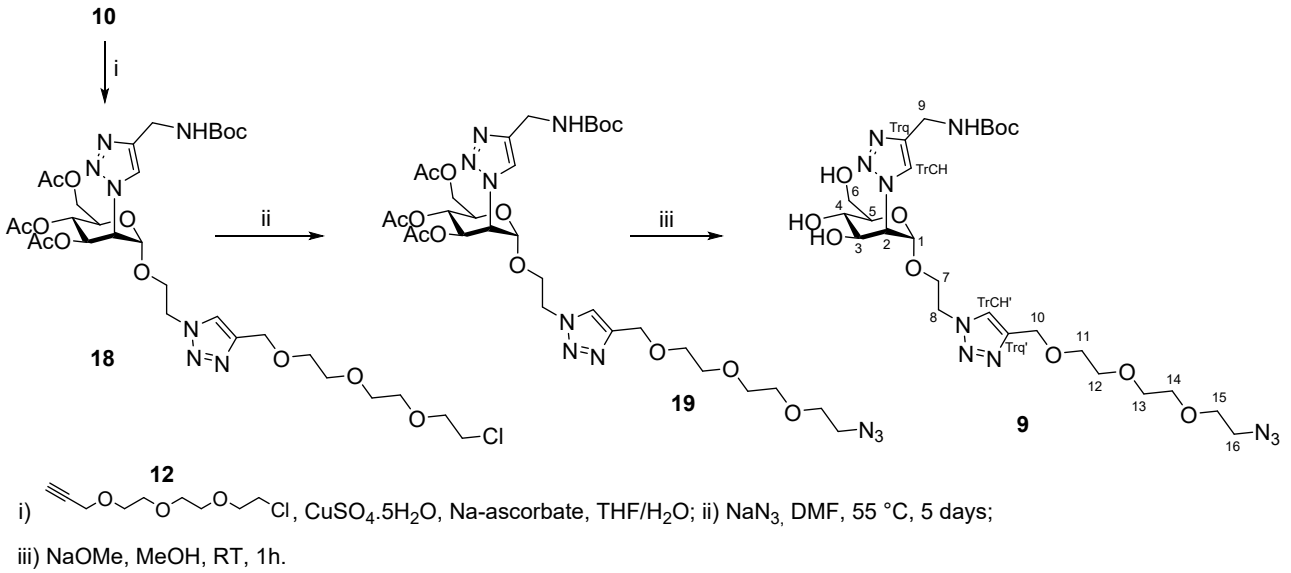
ii) Guanidinylation reaction[5]

The TFA salt **17** (170 mg, 0.38 mmol, 1 eq.) was dissolved in H_2O (316 μL) and diluted with 1,4-dioxane (1.6 mL), then 1,3-Di-Boc-2-(trifluoromethylsulfonyl)guanidine **6** (180.1 mg, 0.46 mmol, 1.2 eq) was

added. After five minutes, triethylamine (158 µL, 1.14 mmol, 3 eq) was slowly added and the reaction stirred at room temperature overnight. Upon completion (TLC monitoring, CH_2Cl_2:MeOH 9:1, R_f(**15**): 0.49) the mixture was diluted with AcOEt and washed with brine three-times. The organic phase was dried over Na_2SO_4, filtered and concentrated *in vacuo*. The crude was purified via automated flash chromatography (CH_2Cl_2 with a MeOH gradient from 0% to 20%) to obtain the pure product **15** as a white foam (177.3 mg, 82%).

^{1}H-NMR (400 MHz, CD_3OD): δ(ppm)= 8.18 (s, 1H, H_{TrCH}), 5.14 (s, 1H, H_1), 5.09 (d, 1H, $J_{2\text{-}3}$ = 5.2 Hz, H_2), 4.65 (s, 2H, H_9), 4.21 (dd, 1H, $J_{3\text{-}4}$ = 9.1 Hz, $J_{3\text{-}2}$ = 5.2 Hz, H_3), 3.99-3.91 (m, 1H, H_{7a}), 3.90-3.83 (m, 2H, H_6), 3.81-3.67 (m, 3H, H_4, H_5, H_{7b}), 3.48 (t, 2H, $J_{8\text{-}7}$ = 5.1 Hz, H_8), 1.51 (s, 9H, H_{tBu}), 1.48 (s, 9H, H_{tBu}). ^{13}C-NMR (400MHz, CD_3OD): δ(ppm) = 164.4 (COO*t*Bu), 157.3 (COO*t*Bu), 153.9 (C_{10}), 144.8 (C_{Trq}), 124.8 (C_{TrCH}), 99.3 (C_1), 84.5 ($C_{quat tBu}$), 80.5 ($C_{quat tBu}$), 75.2 (C_5), 70.2 (C_3), 67.9 (C_7), 67.8 (C_4), 65.3 (C_2), 62.2 (C_6), 51.7 (C_8), 36.9 (C_9), 28.6 (*t*Bu), 28.2 (*t*Bu). MS (ESI): m/z calculated for $[C_{22}H_{37}N_9NaO_9]^+$: 594.26 $[M + Na]^+$, found: 594.40. HRMS (ESI): m/z calculated for $[C_{22}H_{37}N_9NaO_9]^+$: 594.2612 $[M + Na]^+$, found: 594.2618; m/z calculated for $[C_{22}H_{38}N_9O_9]^+$: 572.2792 $[M + Na]^+$, found: 572.2793.

Synthesis of **9**



i) CuAAC reaction

Synthesized from **10** (615 mg, 1.2 mmol) and **12**[6] according to general procedure A for CuAAC reaction. Purified via flash chromatography (CH_2Cl_2 with Acetone gradient from 5% to 100%) to obtain pure product **18** as a white foam (689.4 mg, 82%).

R_f(**18**): 0.66 in CH_2Cl_2:MeOH 9:1

^{1}H-NMR (400MHz, CD_3OD): δ(ppm)= 8.09 (s, 1H, $H_{TrCH'}$), 8.04 (s, 1H, H_{TrCH}), 5.39-5.29 (m, 2H, H_2, H_3), 5.28-5.17 (m, 2H, H_1, H_4), 4.79-4.70 (m, 2H, H_8), 4.68 (s, 2H, H_{10}), 4.33 (s, 2H, H_9), 4.29-4.16 (m, 2H, H_{6a}, H_{7a}), 4.14-3.99 (m, 2H, H_{6b}, H_{7b}), 3.76-3.71 (m, 2H, H_{15}), 3.70-3.62 (m, 11H, H_5, H_{11}-H_{14}, H_{16}), 2.15 (s, 3H, OAc), 2.03 (s, 3H, OAc), 1.88 (s, 3H, OAc), 1.43 (s, 9H, H_{tBu}). ^{13}C-NMR (400MHz, CD_3OD): δ(ppm) extrapolated from HSQC = 124.4 ($C_{TrCH'}$), 122.1 (C_{TrCH}), 97.2 (C_1), 70.9 (C_{15}), 69.8 (C_{11}-C_{14}, C_{16}), 68.7 (C_3), 68.6 (C_5), 65.9 (C_7), 64.4 (C_4), 63.6 (C_{10}), 61.3 (C_6), 60.1 (C_2), 49.5 (C_8), 42.4 (C_{16}), 35.4 (C_9), 27.3 (tBu), 19.5 ($\underline{CH_3}CO$), 19.2 ($\underline{CH_3}CO$), 18.9 ($\underline{CH_3}CO$). MS (ESI): m/z calculated for $[C_{31}H_{48}ClN_7NaO_{13}]^+$: 784.29 [M + Na]$^+$, found: 784.81. HRMS (ESI): m/z calculated for $[C_{31}H_{48}ClN_7NaO_{13}]^+$: 784.2896 [M + Na]$^+$, found: 784.2891.

ii) Chlorine-azide exchange

Compound **18** (500 mg, 0.65 mmol, 1 eq) was dissolved in dry DMF (3.25 mL), then NaN_3 (213.2 mg, 3.28 mmol, 5 eq) was added, and the reaction stirred at 55 °C for 5 days. Upon completion (^{1}H-NMR monitoring), the mixture was concentrated *in vacuo* and the crude product purified via automated flash chromatography (CH_2Cl_2 with MeOH gradient from 3% to 10%) to obtain pure product **19** as a white foam (430 mg, 86%).

R_f(**19**): 0.65 in CH_2Cl_2:MeOH 9:1

^{1}H-NMR (400MHz, CD_3OD): δ(ppm)= 8.09 (s, 1H, $H_{TrCH'}$), 8.04 (s, 1H, H_{TrCH}), 5.39-5.29 (m, 2H, H_2, H_3), 5.28-5.17 (m, 2H, H_1, H_4), 4.79-4.70 (m, 2H, H_8), 4.68 (s, 2H, H_{10}), 4.33 (s, 2H, H_9), 4.29-4.16 (m, 2H, H_{6a}, H_{7a}), 4.14-3.99 (m, 2H, H_{6b}, H_{7b}), 3.72 (m, 11H, H_5, H_{11}-H_{15}), 3.40-3.61 (m, 2H, H_{16}), 2.15 (s, 3H, OAc), 2.03 (s, 3H, OAc), 1.88 (s, 3H, OAc), 1.43 (s, 9H, H_{tBu}). ^{13}C-NMR (400MHz, CD_3OD): δ(ppm) extrapolated from HSQC = 124.4 ($C_{TrCH'}$), 122.1 (C_{TrCH}), 97.2 (C_1), 69.8 (C_{11}-C_{15}), 68.7 (C_3), 68.6 (C_5), 65.9 (C_7), 64.4 (C_4), 63.6 (C_{10}), 61.3 (C_6), 60.1 (C_2), 49.5 (C_8), 42.4 (C_{16}), 35.4 (C_9), 27.3 (tBu), 19.5 ($\underline{CH_3}CO$), 19.2 ($\underline{CH_3}CO$), 18.9 ($\underline{CH_3}CO$). MS (ESI): m/z calculated for $[C_{31}H_{48}N_{10}NaO_{13}]^+$: 791.33 [M + Na]$^+$, found: 791.75

ii) Zemplén deacetylation

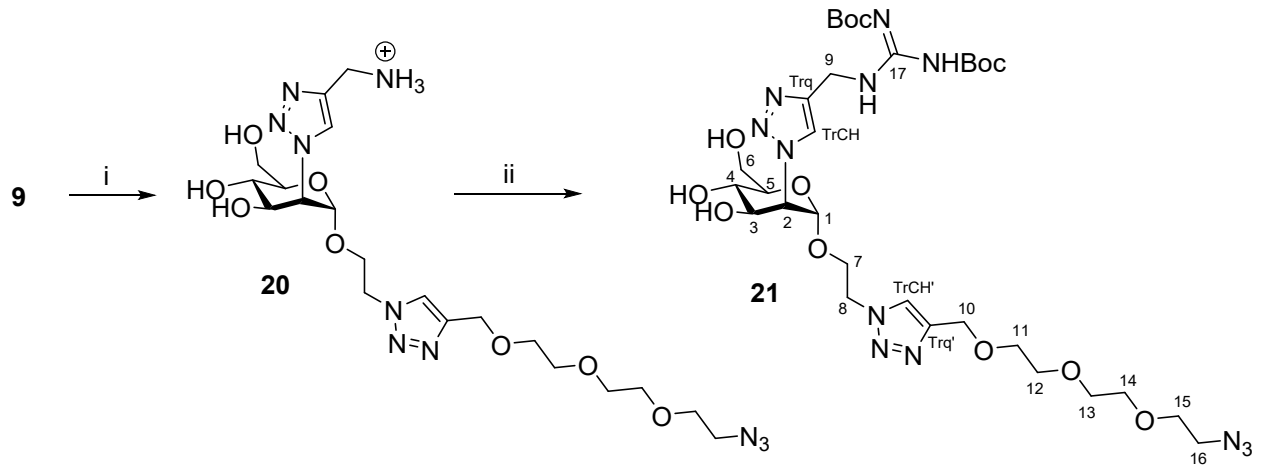
Performed on intermediate **19** (390 mg, 0.51 mmol) according to general procedure for deacetylation (311.3 mg, 95%).

R_f(**9**): 0.50 in CH_2Cl_2:MeOH 9:1

^{1}H-NMR (400MHz, CD_3OD): δ(ppm)= 8.04 (s, 1H, $H_{TrCH'}$), 8.01 (s, 1H, H_{TrCH}), 5.04 (s, 1H, H_1), 4.99 (d, 1H, $J_{2\text{-}3}$ = 5.1 Hz, H_2), 4.71-4.63 (m, 4H, H_8, H_{10}), 4.29 (s, 2H, H_9), 4.21-4.13 (m, 1H, H_{7a}), 4.05 (dd, 1H, $J_{3\text{-}4}$ = 9.4 Hz, $J_{3\text{-}2}$ = 5.1 Hz, H_3), 3.98-3.91 (m, 1H, H_{7b}), 3.85-3.75 (m, 2H, H_6), 3.70-3.62 (m, 11H, H_4, H_{11}-H_{15}), 3.40-3.33 (m, 3H, H_5, H_{16}), 1.44 (s, 9H, H_{tBu}). ^{13}C-NMR (400MHz, CD_3OD): δ(ppm) extrapolated from HSQC = 124.1 ($C_{TrCH'}$), 122.6 (C_{TrCH}), 97.8 (C_1), 73.8 (C_5), 69.8 (C_{11}-C_{15}), 68.9 (C_3), 66.3 (C_4), 65.4 (C_7), 63.5 (C_{10}),

63.3 (C_2), 60.5 (C_6), 50.5 (C_{16}), 49.6 (C_8), 35.3 (C_9), 27.7 (tBu). MS (ESI): m/z calculated for $[C_{25}H_{42}N_{10}NaO_{10}]^+$: 665.30 $[M + Na]^+$, found: 665.58

Synthesis of **21**



i) CH_2Cl_2:TFA 9:1, RT, 1h;
ii) 1,3-Di-Boc-2-(trifluoromethylsulfonyl)guanidine **6**, TEA, 1,4-dioxane:H_2O 5:1, RT, ovn

i) Boc-deprotection

Compound **9** (60 mg, 0.09 mmol, 1 eq.) was dissolved in a 9:1 mixture of dry CH_2Cl_2 and TFA (1.88 mL). The reaction was stirred at room temperature, under N_2 for 2h. Upon completion (TLC monitoring, $CHCl_3$:MeOH 8:2, R_f(**20**): 0.1) the mixture was concentrated *in vacuo* and co-evaporated with toluene three-times to obtain the pure TFA salt **20** as a white foam (54.9 mg, 93%).

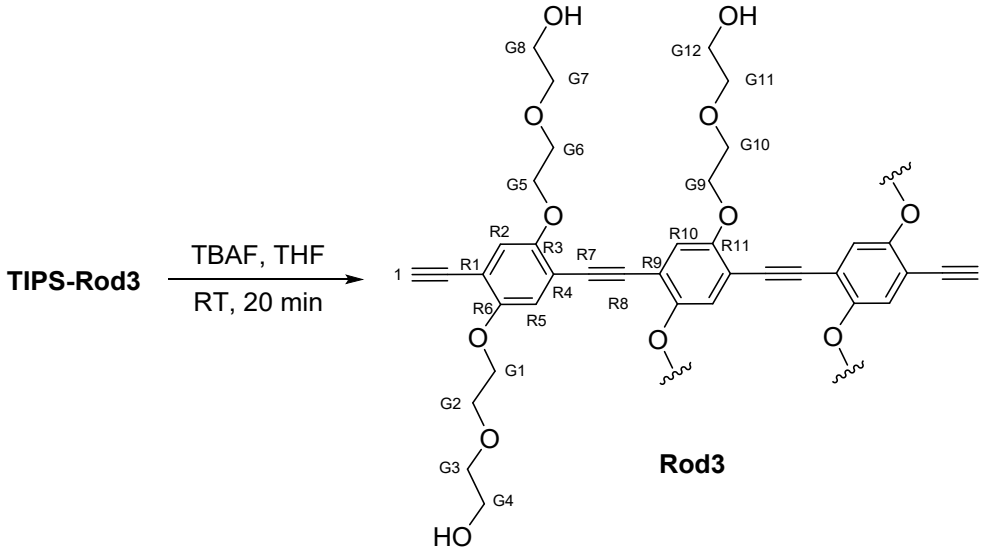
^{1}H-NMR (400MHz, CD_3OD): δ(ppm)= 8.23 (s, 1H, H_{TrCH}), 8.09 (brs, 1H, $H_{TrCH'}$), 5.06-5.01 (m, 2H, H_1, H_2), 4.73-4.62 (m, 4H, H_8, H_{10}), 4.27-4.14 (m, 3H, H_9, H_{7a}), 4.08 (dd, 1H, $J_{3\text{-}4}$ = 9.3 Hz, $J_{3\text{-}2}$ = 5.0 Hz, H_3), 4.01-3.92 (m, 1H, H_{7b}), 3.81 (d, 2H, $J_{6\text{-}5}$ = 3.0 Hz, H_6), 3.72-3.63 (m, 11H, H_4, H_{11}-H_{15}), 3.40-3.34 (m, 3H, H_5, H_{16}). ^{13}C-NMR (400MHz, CD_3OD): δ(ppm) = 125.9 (C_{TrCH}), 99.1 (C_1), 75.2 (C_5), 71.5 (C_{12}, C_{13}), 71.4 (C_{14}), 71.0 (C_{15}), 70.8 (C_{11}), 70.2 (C_3), 67.7 (C_4), 67.1 (C_7), 65.2 (C_2), 64.9 (C_{10}), 61.9 (C_6), 51.7 (C_{16}), 51.1 (C_8), 35.5 (C_9). MS (ESI): m/z calculated for $[C_{20}H_{35}N_{10}O_8]^+$: 543.26 $[M + H]^+$, found: 543.55; m/z calculated for $[C_{20}H_{34}N_{10}NaO_8]^+$: 565.25 $[M + Na]^+$, found: 565.52.

ii) Guanidinylation reaction[5]

The TFA salt **20** (150 mg, 0.23 mmol, 1 eq.) was dissolved in H_2O (1.9 mL) and diluted with 1,4-dioxane (9.6 mL), then 1,3-Di-Boc-2-(trifluoromethylsulfonyl)guanidine **6** (107.3 mg, 0.27 mmol, 1.2 eq) was added. After five minutes, triethylamine (95 μL, 0.69 mmol, 3 eq) was slowly added and the reaction stirred at room temperature overnight. Upon completion (TLC monitoring, CH_2Cl_2:MeOH 9:1, R_f(**21**): 0.55) the mixture was diluted with AcOEt and washed with brine three-times. The organic phase was dried over Na_2SO_4, filtered and concentrated *in vacuo*. The crude was purified via automated flash chromatography (CH_2Cl_2 with a MeOH gradient from 0% to 20%) to obtain the pure product **21** as a white foam (128.1 mg, 71%).

^{1}H-NMR (400MHz, CD_3OD): δ(ppm)= 8.13 (s, 1H, H_{TrCH}), 8.04 (brs, 1H, $H_{TrCH'}$), 5.06 (s, 1H, H_1), 5.01 (dd, 1H, $J_{2\text{-}3}$ = 5.1 Hz, $J_{2\text{-}1}$ = 1.2 Hz, H_2), 4.72-4.60 (m, 6H, H_8, $H_{10,}$ H_9), 4.22-4.13 (m, 1H, H_{7a}), 4.06 (dd, 1H, $J_{3\text{-}4}$ = 9.7 Hz, $J_{3\text{-}2}$ = 5.1 Hz, H_3), 3.98-3.90 (m, 1H, H_{7b}), 3.86-3.76 (m, 2H, H_6), 3.70-3.60 (m, 11H, H_4, H_{11}-H_{15}), 3.41-3.31 (m, 3H, H_5, H_{16}), 1.52 (s, 9H, H_{tBu}), 1.48 (s, 9H, H_{tBu}). ^{13}C-NMR (400MHz, CD_3OD): δ(ppm) = 164.4 (COO*t*Bu), 157.4 (COO*t*Bu), 153.9 (C_{17}), 146.0 ($C_{Trq'}$), 144.8 (C_{Trq}), 125.8 ($C_{TrCH'}$), 124.8 (C_{TrCH}), 99.2 (C_1), 84.5 ($C_{quat\,tBu}$), 80.5 ($C_{quat\,tBu}$), 75.2 (C_5), 71.6, 71.5, 71.4 (C_{12}, C_{13}, C_{14}), 71.0 (C_{15}), 70.8 (C_{11}), 70.3 (C_3), 67.7 (C_4), 67.0 (C_7), 65.2 (C_2), 65.0 (C_{10}), 62.2 (C_6), 51.7 (C_{16}), 51.0 (C_8), 36.9 (C_9), 28.6 (*t*Bu), 28.2 (*t*Bu). MS (ESI): m/z calculated for $[C_{31}H_{53}N_{12}O_{12}]^+$: 785.39 $[M + H]^+$, found: 785.40; m/z calculated for $[C_{31}H_{52}N_{12}NaO_{12}]^+$: 807.37 $[M + Na]^+$, found: 807.44. HRMS (ESI): m/z calculated for $[C_{31}H_{52}N_{12}NaO_{12}]^+$: 807.3725 $[M + Na]^+$, found: 807.3718.

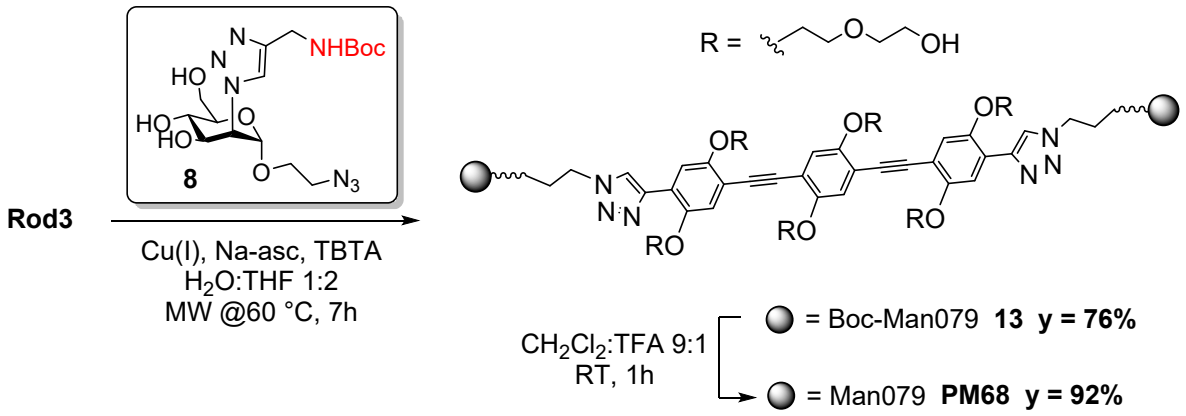
Synthesis of **Rod3**



To a solution of **TIPS-Rod3**[7] (80 mg, 0.063 mmol, 1 eq) in dry THF (1.6 mL), TBAF (1M, 130 μL, 0.13 mmol. 2 eq) was added. The reaction was stirred at room temperature under nitrogen atmosphere for 20 min. Upon completion (TLC monitoring CH_2Cl_2:MeOH 9:1, R_f(**Rod3**): 0.38) the solvent was removed under reduced pressure and crude product purified via automated flash chromatography ($CHCl_3$ with MeOH gradient from 0% to 25%) to obtain pure product as a yellow solid (41.7 mg, 70%)

^{1}H-NMR (400MHz, CD_3OD): δ(ppm)= 7.16 (s, 2H, H_{R2}), 7.14 (s, 2H, H_{R5}), 7.11 (s, 2H, H_{R10}), 4.27-4.18 (m, 12H, H_{G1}, H_{G5}, H_{G9}), 3.93-3.86 (m, 12H, H_{G2}, H_{G6}, H_{G10}), 3.76 (s, 2H, H_1), 3.72-3.65 (m, 24H, H_{G3}, H_{G4}, H_{G7}, H_{G8}, H_{G11}, H_{G12}).

Synthesis of **PM68**



CuAAC reaction

Synthesized from **Rod3** (21 mg, 0.02 mmol) and **8** (**Boc-Man79-N_3**) according to general procedure B for CuAAC reaction. The reaction was stirred under MW irradiation at 60 °C for 7 hours. Crude product was purified via automated reverse phase chromatography (H_2O with a MeOH gradient from 0% to 100%) to obtain product **13** as a yellow foam (30.2 mg, 76%)

R_f(**13**): 0.44 in H_2O:CH_3CN 1:1

^{1}H-NMR (400MHz, CD_3OD): δ(ppm)= 8.66 (s, 2H, H_{TC5}), 8.01 (s, 2H, H_{TrCH}), 7.87 (s, 2H, H_{R2}), 7.24 (s, 2H, H_{R5}), 7.18 (s, 2H, H_{R10}), 5.07 (s, 2H, H_1), 5.02 (d, 2H, $J_{2\text{-}3}$ = 5.1 Hz, H_2), 4.80-4.69 (m, 4H, H_8), 4.35 (t, 4H, $J_{G9\text{-}G10}$ = 4.4 Hz, H_{G9}), 4.32-4.18 (m, 14H, H_9, H_{G1}, H_{G5}, H_{7a}), 4.09 (dd, 2H, $J_{3\text{-}4}$ = 9.6 Hz, $J_{3\text{-}2}$ = 5.1 Hz, H_3), 4.07-4.01 (m, 2H, H_{7b}), 3.99-3.89 (m, 12H, H_{G10}, H_{G2}, H_{G6}), 3.83-3.63 (m, 30H, H_4, H_{G4}, H_{G8}, H_{G12}, H_{G3}, H_{G7}, H_{G11}, H_6), 3.45-3.39 (m, 2H, H_5), 1.43 (s, 18H, H_{tBu}). ^{13}C-NMR (400MHz, CD_3OD): δ(ppm) extrapolated from HSQC = 125.5 (C_{TC5}), 122.8 (C_{TrCH}), 117.6 (C_{R10}), 116.9 (C_{R5}), 111.7 (C_{R2}), 97.6 (C_1), 73.2 (C_5), 72.7 (C_{G3}, C_{G7}, C_{G11}), 72.3 (C_{G4}, C_{G8}, C_{G12}), 69.8 (C_{G5}), 69.5 (C_{G10}, C_{G2}, C_{G6}), 69.4 (C_{G9}), 69.1 (C_3), 67.7 (C_{G1}), 66.2 (C_4), 65.9 (C_7), 63.7 (C_2), 60.8 (C_6), 49.7 (C_8), 35.2 (C_9), 27.1 (*t*Bu).

Boc deprotection

Compound **13** (15 mg, 8 μmol, 1 eq.) was dissolved in a 9:1 mixture of dry CH_2Cl_2 and TFA (800 μL). The reaction was stirred at room temperature, under N_2 for 30 min. Upon completion (^{1}H-NMR monitoring, CD_3OD) the mixture was concentrated *in vacuo* and co-evaporated with toluene three-times. Crude product was purified via semi-preparative HPLC (using the conditions reported below) to obtain the pure product **PM68** as bis-formate salt (12.5 mg, 92%).

HPLC: Flow: 10 mL/min; UV channels: 210 nm; 254 nm; A: H_2O + 0.1% HCO_2H, B: CH_3CN. Gradient: 0-1 min: 20% B, 1-11 min: 20 - 45% B; t_r (**PM68**) = 6.79 min

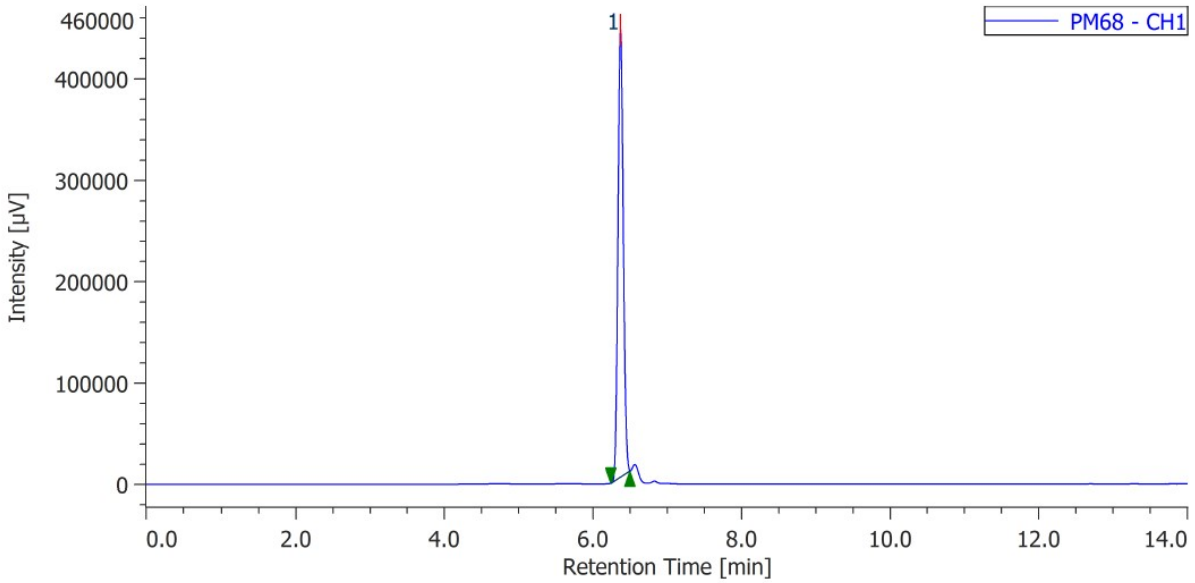
^{1}H-NMR (400MHz, DMSO-d_6): δ(ppm)= 8.56 (s, 2H, H_{TC5}), 8.23 (s, 2H, FA), 8.13 (s, 2H, H_{TrCH}), 7.85 (s, 2H, H_{R2}), 7.22 (s, 2H, H_{R5}), 7.15 (s, 2H, H_{R10}), 5.43 (d, 2H, $J_{OH\text{-}3}$ = 3.3 Hz, OH-3), 5.18 (d, 2H, $J_{OH\text{-}4}$ = 4.5 Hz, OH-4), 4.99 (s, 2H, H_1), 4.88 (d, 2H, $J_{2\text{-}3}$ = 5.2 Hz, H_2), 4.85-4.77 (m, 4H, OH-6, OH-Peg_{int}), 4.74-4.62 (m, 8H, H_8, OH-Peg_{ext}), 4.29-4.18 (m, 12H, H_{G1}, H_{G5}, H_{G9}), 4.09-4.06 (m, 2H, H_{7b}), 4.01 (s, 4H, H_9), 3.98-3.80 (m, 10H, H_{7b}, H_{G2}, H_{G6}).

HRMS (ESI):

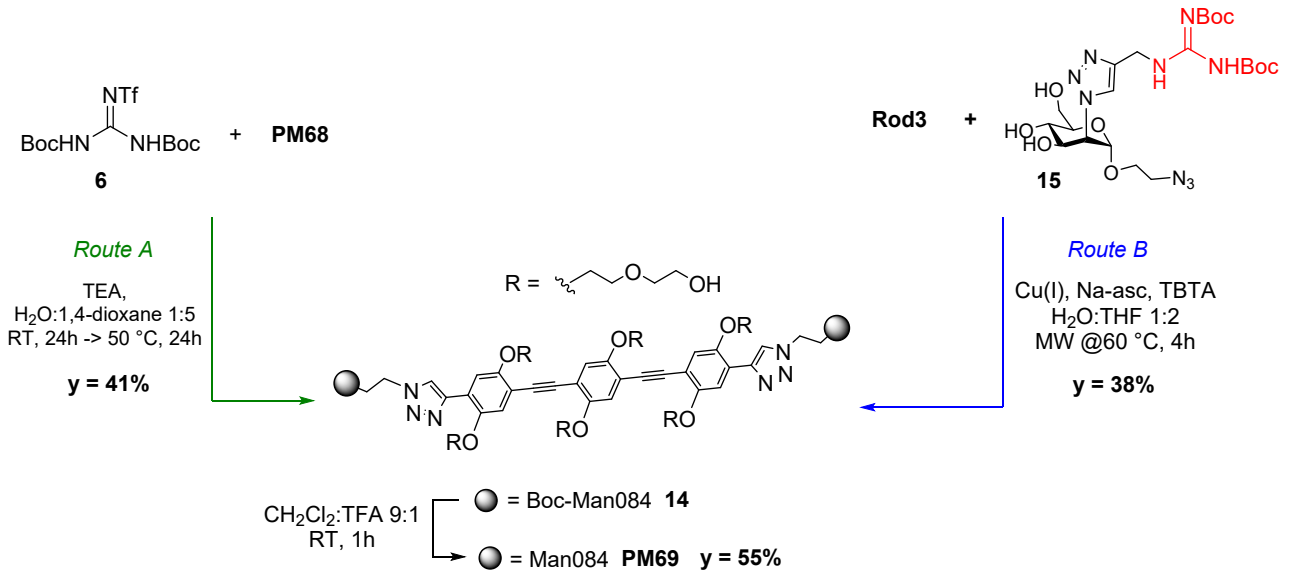
Molecular formula	Ion	Calcd. value (m/z)	Exp. value (m/z)
$[C_{72}H_{101}N_{14}O_{28}]^+$	$[M + H]^+$	1609.6904	1609.6925[i]
$[C_{72}H_{100}N_{14}NaO_{28}]^+$	$[M + Na]^+$	1631.6724	1631.6783[i]
$[C_{72}H_{99}N_{14}Na_2O_{28}]^+$	$[M + 2Na - H]^+$	1653.6543	1653.6533[i]
$[C_{72}H_{102}N_{14}O_{28}]^{2+}$	$[M + 2H]^{2+}$	805.3494	805.3481
$[C_{72}H_{101}N_{14}NaO_{28}]^{2+}$	$[M + Na + H]^{2+}$	816.3404	816.3390
$[C_{72}H_{100}N_{14}Na_2O_{28}]^{2+}$	$[M + 2Na]^{2+}$	827.3313	827.3302

[i] found after deconvolution.

Analytical HPLC: Flow: 1 mL/min; UV channel: 360 nm; A: H_2O + 0.1% HCO_2H, B: CH_3CN + 0.1% HCO_2H. Gradient: 0-2 min: 20% B, 2-11 min: 20 - 65% B; 11-14 min: 65 - 100% B; t_r (**PM68**) = 6.4 min



Synthesis of **PM69**



Route A) Guanidinylation reaction[5]

Dimer **PM68** (13.4 mg, 7 µmol, 1 eq.) was dissolved in H_2O (24 µL) and diluted with 1,4-dioxane (120 µL), then 1,3-Di-Boc-2-(trifluoromethylsulfonyl)guanidine **6** (13.7 mg, 35 µmol, 5 eq) was added. After five minutes, triethylamine (13 µL, 86 µmol, 7 eq) was slowly dropped into the mixture and the reaction was stirred at room temperature. After 24 hours, **6** (13.7 mg, 35 µmol, 5 eq) and triethylamine (13 µL, 86 µmol, 7 eq) were ulteriorly added to the mixture, which was stirred at 50 °C for further 24 hours. Upon completion (TLC and NMR monitoring), the solvents were removed under reduced pressure. Crude product was purified via size-exclusion chromatography on a Sephadex LH-20 column (Ø 3 cm, H 55 cm; eluent: MeOH) to obtain pure product **14** as a yellow foam (6.01 mg, 41%).

Route B) CuAAC reaction

Synthesized from **Rod3** (23 mg, 0.024 mmol) and **15** (**Boc-Man84-N_3**) according to general procedure B for CuAAC reaction. The reaction was stirred under MW irradiation at 60 °C for 4 hours. Crude product was purified via size-exclusion chromatography on a Sephadex LH-20 column (Ø 3 cm, H 55 cm; eluent: MeOH) to obtain pure product **14** as a yellow foam (19 mg, 38%).

R_f(**14**): 0.64 in H_2O:CH_3CN 1:1

^{1}H-NMR (400MHz, CD_3OD): δ(ppm)= 8.61 (s, 2H, H_{TC5}), 8.14 (s, 2H, H_{TrCH}), 7.86 (s, 2H, H_{R2}), 7.20 (s, 2H, H_{R5}), 7.15 (s, 2H, H_{R10}), 5.09 (s, 2H, H_1), 5.04 (d, 2H, $J_{2\text{-}3}$ = 5.1 Hz, H_2), 4.79-4.67 (m, 4H, H_8), 4.67-4.54 (m, 4H, H_9), 4.34 (t, 4H, $J_{G9\text{-}G10}$ = 4.5 Hz, H_{G9}), 4.30-4.18 (m, 10H, H_{G1}, H_{G5}, H_{7a}), 4.11 (dd, 2H, $J_{3\text{-}4}$ = 9.6 Hz, $J_{3\text{-}2}$ = 5.1 Hz, H_3), 4.06-3.99 (m, 2H, H_{7b}), 3.98-3.90 (m, 12H, H_{G10}, H_{G2}, H_{G6}), 3.81-3.65 (m, 30H, H_4, H_{G4}, H_{G8}, H_{G12}, H_{G3}, H_{G7}, H_{G11}, H_6), 3.48-3.40 (m, 2H, H_5), 1.50 (s, 18H, H_{tBu}), 1.47 (s, 18H, H_{tBu}). ^{13}C-NMR (400MHz, CD_3OD): δ(ppm) = 155.3, 154.9 (C_{R6}, C_{R11}), 154.1 (C_{10}), 150.5 (C_{R3}), 126.9 (C_{TC5}), 122.3 (C_{R1}), 119.1 (C_{R10}), 118.4 (C_{R5}), 115.9, 114.5 (C_{R4}, C_{R9}), 113.0 (C_{R2}), 99.2 (C_1), 92.8, 91.7 (C_{R7}, C_{R8}), 84.6 ($C_{quattBu}$), 80.6 ($C_{quattBu}$), 75.3

(C_5), 74.1, 73.7 (C_{G3}, C_{G7}, C_{G11}), 70.9 (C_{G9}, C_{G5} or C_{G1}), 70.8 (C_{G2}, C_{G6}), 70.6 (C_{G10}), 70.3 (C_3), 69.3 (C_{G1} or C_{G5}), 67.7 (C_4), 67.2 (C_7), 65.3 (C_2), 62.4, 62.3, 62.2, 62.1 (C_{G4}, C_{G8}, C_{G12}, C_6), 51.0 (C_8), 37.0 (C_9), 28.6 (*t*Bu), 28.2 (*t*Bu).

MS (MALDI, matrix: DHB, solvent: MeOH):

Molecular formula	Ion	Calcd. value (m/z)	Exp. value (m/z)
$[C_{89}H_{129}N_{18}O_{34}]^+$	$[M - 1Boc + H]^+$	1993.892	1994.935
$[C_{84}H_{121}N_{18}O_{32}]^+$	$[M - 2Boc + H]^+$	1893.839	1894.914
$[C_{79}H_{113}N_{18}O_{30}]^+$	$[M - 3Boc + H]^+$	1793.787	1794.763
$[C_{74}H_{105}N_{18}O_{28}]^+$	$[M - 4Boc + H]^+$	1693.735	1694.096

Boc-deprotection

Compound **14** (19 mg, 9 μmol, 1 eq.) was dissolved in a 9:1 mixture of dry CH_2Cl_2 and TFA (900 μL). The reaction was stirred at room temperature, under N_2 for 1 min. Upon completion (^{1}H-NMR monitoring, CD_3OD) the mixture was concentrated *in vacuo* and co-evaporated with toluene three-times. Crude product was purified via semi-preparative HPLC (using the conditions reported below) to obtain the pure product **PM69** as bis-formate salt (8.9 mg, 55%; yellow foam).

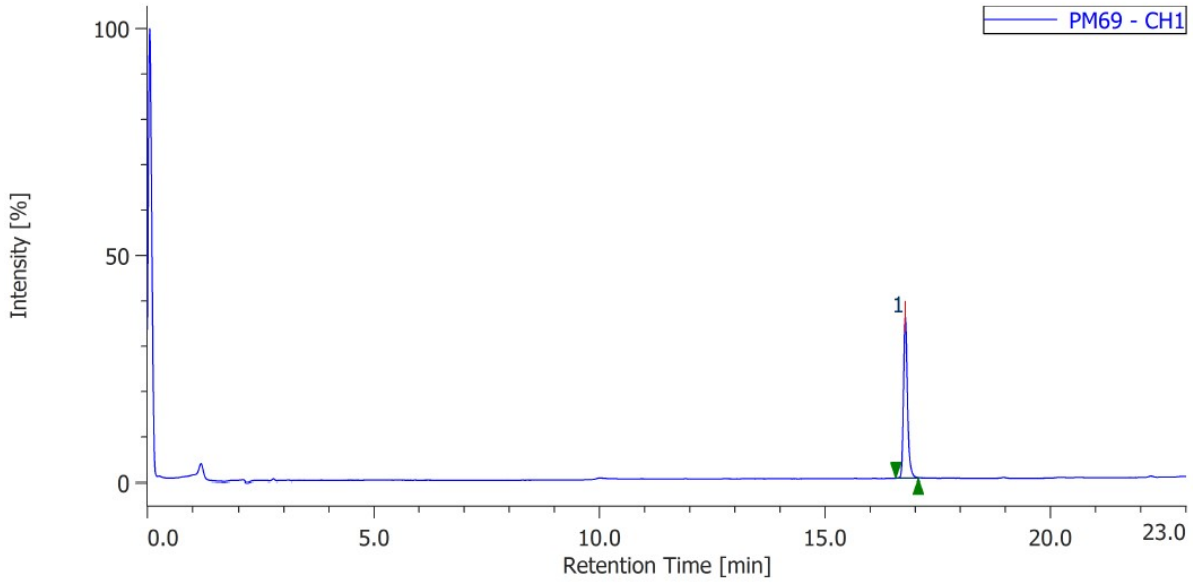
HPLC: Flow: 10 mL/min; UV channels: 210 nm; 254 nm; A: H_2O + 0.1% HCO_2H, B: CH_3CN. Gradient: 0-5 min: 0% B, 5-20 min: 0 - 45% B; t_r (**PM69**) = 16.29 min

^{1}H-NMR (400MHz, DMSO-d_6): δ(ppm)= 8.73 (brs, 2H, NH), 8.57 (s, 2H, H_{TC5}), 8.49 (brs, 2H, FA), 8.16 (s, 2H, H_{TrCH}), 7.88-7.75 (m, 8H, H_{R2}, $NH_, NH_2$), 7.22 (s, 2H, H_{R5}), 7.16 (s, 2H, H_{R10}), 5.49 (brs, 2H, OH-3), 5.31 (brs, 2H, OH-4), 5.05 (s, 2H, H_1), 4.94-4.85 (m, 4H, H_2, OH-6), 4.83-4.58 (m, 10H, H_8, OH-Peg), 4.38 (s, 4H, H_9), 4.31-4.19 (m, 12H, H_{G1}, H_{G5}, H_{G9}), 4.12-4.04 (m, 2H, H_{7a}), 4.00-3.80 (m, 16H, H_{7b}, H_3, H_{G2}, H_{G6}, H_{G10}), 3.71-3.49 (m, 30H, H_6, H_4, H_{G4}, H_{G8}, H_{G12}, H_{G3}, H_{G7}, H_{G11}), 3.44-3.39 (m, 2H, H_5). ^{13}C-NMR (400MHz, CD_3OD): δ(ppm) extrapolated from HSQC = 156.9 (FA), 125.2 (C_{TC5}), 123.3 (C_{TrCH}), 117.8 (C_{R10}), 117.5 (C_{R5}), 111.7 (C_{R2}), 97.5 (C_1), 74.8 (C_5), 73.2 (C_{G4}, C_{G8}, C_{G12}, C_{G3}, C_{G7}, C_{G11}), 69.5 (C_{G10}, $C_{G2.}$ C_{G6}), 68.9 (C_3, C_{G9}, C_{G1}, C_{G5}), 66.5 (C_4), 65.8 (C_7), 64.0 (C_2), 60.6 (C_6), 49.9 (C_8), 36.7 (C_9). HRMS (ESI):

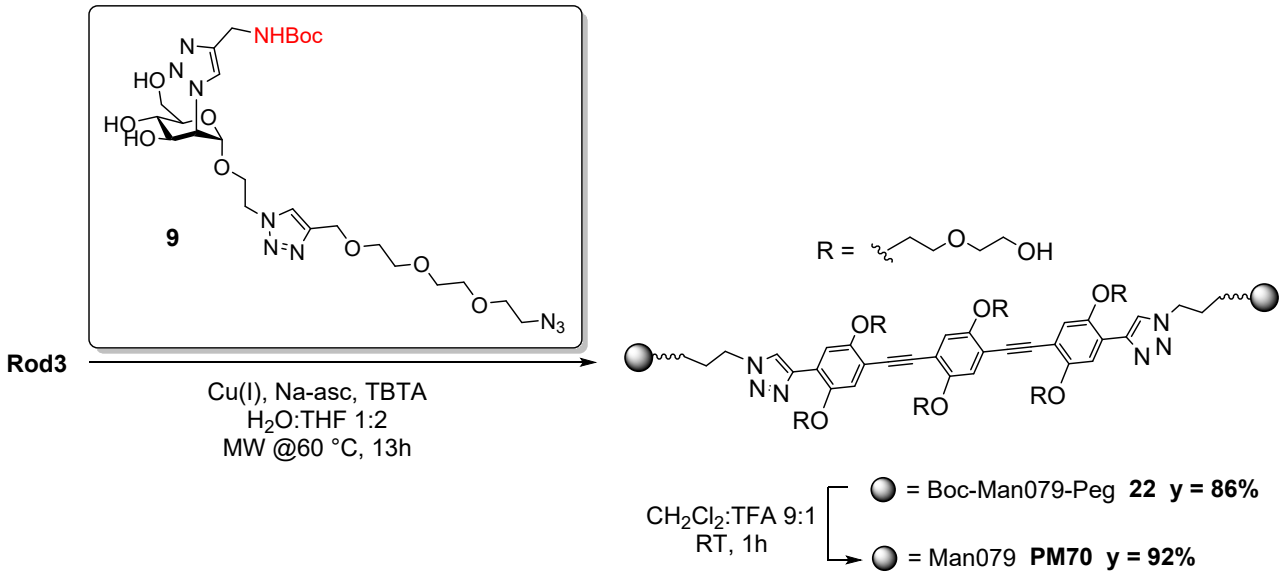
Molecular formula	Ion	Calcd. value (m/z)	Exp. value (m/z)
$[C_{74}H_{105}N_{18}O_{28}]^+$	$[M + H]^+$	1693.7346	1693.7382[i]
$[C_{74}H_{104}N_{18}NaO_{28}]^+$	$[M + Na]^+$	1715.7165	1715.7208[i]
$[C_{74}H_{106}N_{18}O_{28}]^{2+}$	$[M + 2H]^{2+}$	847.3712	847.3713
$[C_{74}H_{105}N_{18}NaO_{28}]^{2+}$	$[M + Na + H]^{2+}$	858.3622	858.3618
$[C_{74}H_{104}N_{18}Na_2O_{28}]^{2+}$	$[M + 2Na]^{2+}$	869.3531	869.3525
$[C_{74}H_{106}N_{18}NaO_{28}]^{3+}$	$[M + 2H + Na]^{3+}$	572.5774	572.5781
$[C_{74}H_{156}N_{18}Na_2O_{28}]^{3+}$	$[M + 2Na + H]^{3+}$	579.9047	579.9049
$[C_{74}H_{104}N_{18}Na_3O_{28}]^{3+}$	$[M + 3Na]^{3+}$	587.2320	587.2321

[i] found after deconvolution.

Analytical HPLC: Flow: 1 mL/min; UV channels: 360 nm; A: H_2O + 0.1% HCO_2H, B: CH_3CN + 0.1% HCO_2H. Gradient: 0-5 min: 0% B, 5-20 min: 0 - 45% B; 20-23 min: 45 - 100% B; t_r (**PM69**) = 16.8 min



Synthesis of **PM70**



CuAAC reaction

Synthesized from **Rod3** (25 mg, 0.026 mmol) and **9** (**Boc-Man79-Peg-N_3**) according to general procedure B for CuAAC reaction. The reaction was stirred under MW irradiation at 60 °C for 13 hours. Crude product was purified via size-exclusion chromatography on a Sephadex LH-20 column (Ø 3 cm, H 55 cm; eluent: MeOH) to obtain pure product **22** as a yellow foam (50 mg, 86%).

R_f(**22**): 0.72 in H_2O:CH_3CN 1:1

^{1}H-NMR (400MHz, CD_3OD): δ(ppm)= 8.59 (s, 2H, H_{TC5}), 8.01 (s, 2H, H_{TrCH}), 7.90 (s, 2H, $H_{TrCH'}$), 7.85 (s, 2H, H_{R2}), 7.22 (s, 2H, H_{R5}), 7.18 (s, 2H, H_{R10}), 5.00 (s, 2H, H_1), 4.97 (d, 2H, $J_{2\text{-}3}$ = 5.2 Hz, H_2), 4.70-4.61 (m, 8H, H_8, H_{16}), 4.53 (s, 4H, H_{10}), 4.34 (t, 4H, $J_{G9\text{-}G10}$ = 4.4 Hz, H_{G9}), 4.30-4.24 (m, 12H, H_{G1}, H_{G5}, H_9), 4.16-4.09 (m, 2H, H_{7a}), 4.03 (dd, 2H, $J_{3\text{-}4}$ = 9.5 Hz, $J_{3\text{-}2}$ = 5.2 Hz, H_3), 3.98-3.87 (m, 18H, H_{G10}, H_{G2}, H_{G6}, H_{7b}, H_{15}), 3.81-3.66 (m, 30H, H_4, H_{G4}, H_{G8}, H_{G12}, H_{G3}, H_{G7}, H_{G11}, H_6), 3.65-3.57 (m, 10H, H_{11}, H_{14}, H_5), 3.54 (s, 8H, H_{12}, H_{13}), 1.42 (s, 18H, H_{tBu}). ^{13}C-NMR (400MHz, CD_3OD): δ(ppm) extrapolated from HSQC = 125.4 ($C_{TrCH'}$), 123.9 (C_{TrCH}), 118.9 (C_{R10}), 118.0 (C_{R5}), 112.7 (C_{R2}), 99.0 (C_1), 73.6 (C_{G3}, C_{G7}, C_{G11}, C_5), 73.3 (C_{G4}, C_{G8}, C_{G12}), 70.7 (C_{G9}, C_{G1} or C_{G5}), 71.0 (C_{11}, C_{12}, C_{13}, C_{14}) 70.4 (C_{G10}, C_{G2}, C_{G6}, C_{15}), 69.9 (C_3), 68.6 (C_{G1} or C_{G5}), 67.1 (C_4), 66.9 (C_7), 64.2 (C_2), 64.6 (C_{10}), 61.9 (C_6), 51.0 (C_8, C_{16}), 36.5 (C_9), 28.5 (*t*Bu).

HRMS (ESI):

Molecular formula	Ion	Calcd. value (m/z)	Exp. value (m/z)
$[C_{100}H_{146}N_{20}NaO_{38}]^+$	$[M + Na]^+$	2258.0005	2258.9993[i]
$[C_{100}H_{146}N_{20}Na_2O_{38}]^{2+}$	$[M + 2Na]^{2+}$	1140.4951	1140.9943
$[C_{100}H_{146}N_{20}Na_3O_{38}]^{3+}$	$[M + 3Na]^{3+}$	767.9933	767.9913

[i] found after deconvolution.

Boc-deprotection

Compound **22** (22 mg, 10 μmol, 1 eq.) was dissolved in a 9:1 mixture of dry CH_2Cl_2 and TFA (980 μL). The reaction was stirred at room temperature, under N_2 for 40 min. Upon completion (^{1}H-NMR monitoring, CD_3OD) the mixture was concentrated *in vacuo* and co-evaporated with toluene three-times. Crude product was purified via semi-preparative HPLC (using the conditions reported below) to obtain the pure product **PM70** as bis-formate salt (19.5 mg, 92%; yellow foam).

HPLC: Flow: 10 mL/min; UV channels: 210 nm; 254 nm; A: H_2O + 0.1% HCO_2H, B: CH_3CN. Gradient: 0-1 min: 0% B, 1-25 min: 0 - 40% B; t_r (**PM70**) = 19.09 min

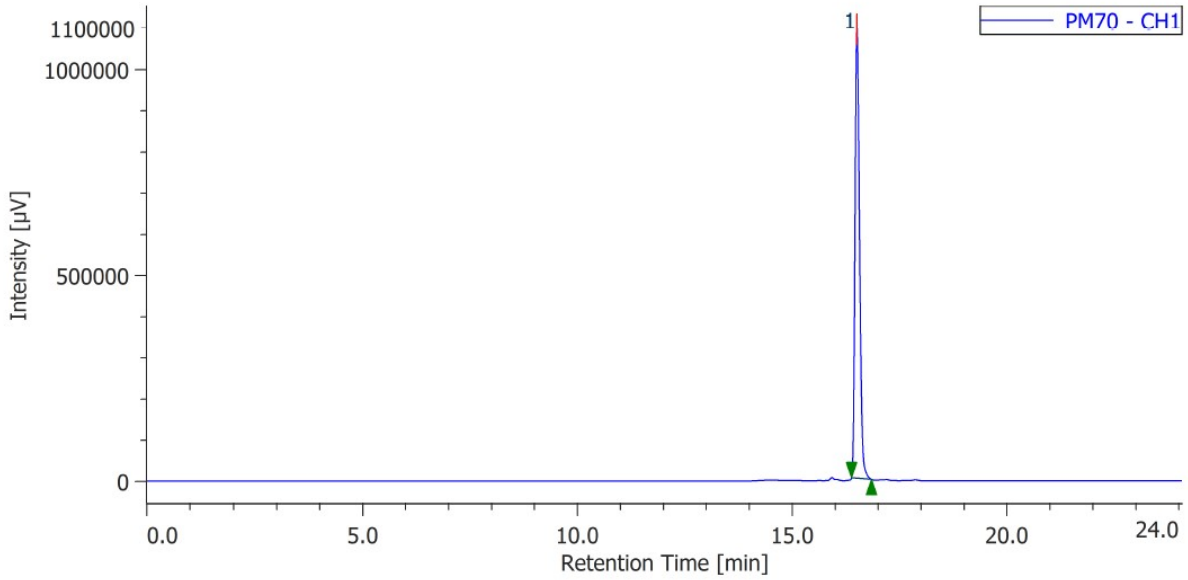
^{1}H-NMR (400MHz, DMSO-d_6): δ(ppm)= 8.51 (s, 2H, H_{TC5}), 8.45 (brs, 2H, FA), 8.07 (s, 2H, H_{TrCH}), 7.97 (s, 2H, $H_{TrCH'}$), 7.86 (s, 2H, H_{R2}), 7.22 (s, 2H, H_{R5}), 7.15 (s, 2H, H_{R10}), 5.35 (brs, 2H, OH-3), 5.18 (brs, 2H, OH-4), 4.96 (s, 2H, H_1), 4.87-4.82 (m, 2H, H_2), 4.77 (brs, 2H, OH-6), 4.68-4.56 (m, 10H, H_8, OH-Peg), 4.51 (s, 4H, H_{10}), 4.23 (m, 12H, H_{G1}, H_{G9}, H_{G5}), 4.05-3.98 (m, 2H, H_{7a}), 3.93-3.80 (m, 16H, H_{7b}, H_3, H_9, H_{16}, H_{G6}), 3.76 (brs, 4H, H_{G10}), 3.69-3.63 (m, 6H, H_4, H_{G2}), 3.60-3.49 (m, 50H, H_6, H_5, H_{11}-H_{15}, H_{G4}, H_{G8}, H_{G12}, H_{G3}, H_{G7}, H_{G11}).

HRMS (ESI):

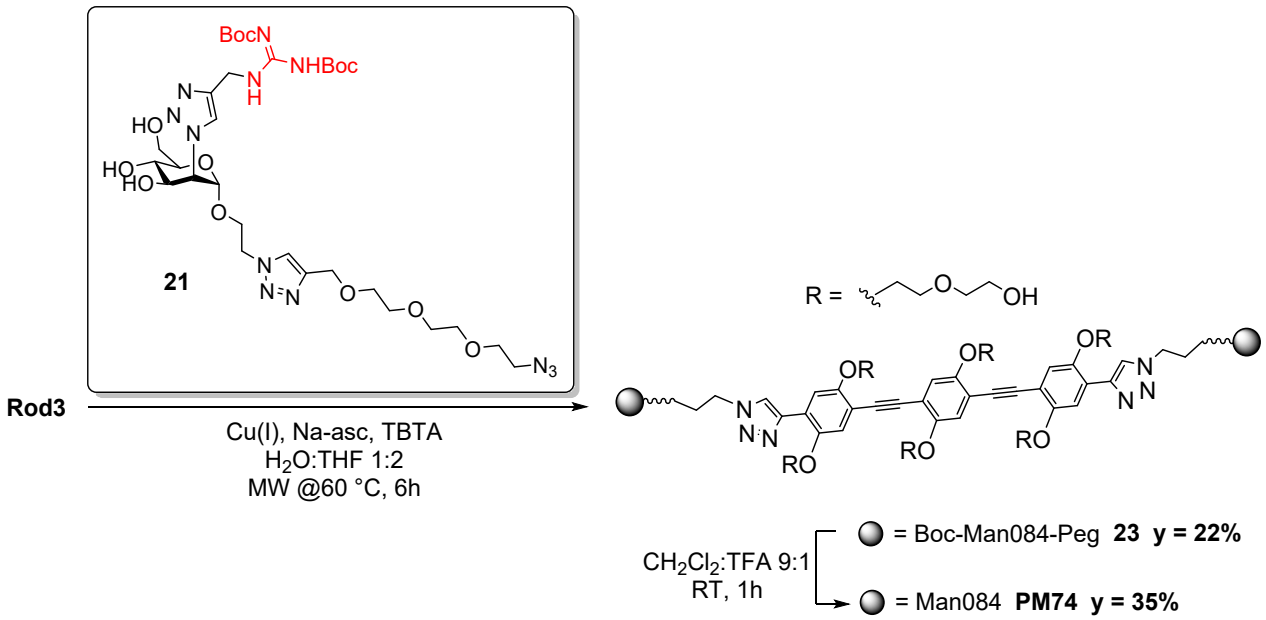
Molecular formula	Ion	Calcd. value (m/z)	Exp. value (m/z)
$[C_{90}H_{131}N_{20}O_{34}]^+$	$[M + H]^+$	2035.9137	2035.9092[i]
$[C_{90}H_{132}N_{20}O_{34}]^{2+}$	$[M + 2H]^{2+}$	1018.4602	1018.4583
$[C_{90}H_{131}N_{20}NaO_{34}]^{2+}$	$[M + Na + H]^{2+}$	1029.4517	1029.4495
$[C_{90}H_{130}N_{20}Na_2O_{34}]^{2+}$	$[M + 2Na]^{2+}$	1040.4427	1040.4409
$[C_{90}H_{133}N_{20}O_{34}]^{3+}$	$[M + 3H]^{3+}$	679.3097	679.3086
$[C_{90}H_{132}N_{20}NaO_3]^{3+}$	$[M + 2H + Na]^{3+}$	686.6371	686.6359
$[C_{90}H_{131}N_{20}Na_2O_{34}]^{3+}$	$[M + 2Na + H]^{3+}$	693.9644	693.9632
$[C_{90}H_{130}N_{20}Na_3O_{34}]^{3+}$	$[M + 3Na]^{3+}$	701.2917	701.2906

[i] found after deconvolution.

Analytical HPLC: Flow: 1 mL/min; UV channel: 360 nm; A: H_2O + 0.1% HCO_2H, B: CH_3CN + 0.1% HCO_2H. Gradient: 0-1 min: 0% B, 1-20 min: 0 - 40% B; 20-23 min: 40 - 100% B; t_r (**PM70**) = 16.5 min



Synthesis of **PM74**



CuAAC reaction

Synthesized from **Rod3** (45 mg, 0.047 mmol) and **21** (**Boc-Man84-Peg-N_3**) according to general procedure D for CuAAC reaction. The reaction was stirred under MW irradiation at 60 °C for 6 hours. Crude product was purified via size-exclusion chromatography on a Sephadex LH-20 column (Ø 3 cm, H 55 cm; eluent: MeOH) to obtain pure product **23** as a yellow foam (25.5 mg, 22%).

R_f(**23**): 0.41 in H_2O:CH_3CN 1:1

^{1}H-NMR (400MHz, CD_3OD): δ(ppm)= 8.59 (s, 2H, H_{TC5}), 8.14 (s, 2H, H_{TrCH}), 7.94 (s, 2H, $H_{TrCH'}$), 7.87 (s, 2H, H_{R2}), 7.24 (s, 2H, H_{R5}), 7.19 (s, 2H, H_{R10}), 5.03 (s, 2H, H_1), 4.98 (d, 2H, $J_{2\text{-}3}$ = 5.2 Hz, H_2), 4.70-4.52 (m, 16H, H_8, H_{16}, H_9, H_{10}), 4.34 (t, 4H, $J_{G9\text{-}G10}$ = 4.4 Hz, H_{G9}), 4.30-4.24 (m, 8H, H_{G1}, H_{G5}), 4.16-4.12 (m, 2H, H_{7a}), 4.04 (dd, 2H, $J_{3\text{-}4}$ = 9.5 Hz, $J_{3\text{-}2}$ = 5.2 Hz, H_3), 3.98-3.87 (m, 18H, H_{G10}, H_{G2}, H_{G6}, H_{7b}, H_{15}), 3.81-3.54 (m, 46H, H_4, H_{G4}, H_{G8}, H_{G12}, H_{G3}, H_{G7}, H_{G11}, H_6, H_{11}, H_{12}, H_{13}, H_{14}), 1.51 (s, 18H, H_{tBu}), 1.48 (s, 18H, H_{tBu}).

HRMS (ESI):

Molecular formula	Ion	Calcd. value (m/z)	Exp. value (m/z)
$[C_{112}H_{167}N_{24}NaO_{42}]^+$	$[M + H]^+$	2520.1670	2520.1633[i]
$[C_{112}H_{166}N_{24}NaO_{42}]^+$	$[M + Na]^+$	2542.1489	2542.1438[i]
$[C_{112}H_{165}N_{24}Na_2O_{42}]^+$	$[M + 2Na - H]^+$	2564.1309	2564.1274[i]
$[C_{112}H_{168}N_{24}O_{42}]^{2+}$	$[M + 2H]^{2+}$	1260.5874	1260.5823
$[C_{112}H_{167}N_{24}NaO_{42}]^{2+}$	$[M + Na + H]^{2+}$	1271.5783	1271.5763
$[C_{112}H_{166}N_{24}Na_2O_{42}]^{2+}$	$[M + 2Na]^{2+}$	1282.5693	1282.5676
$[C_{112}H_{169}N_{24}O_{42}]^{3+}$	$[M + 3H]^{3+}$	840.7275	840.7244
$[C_{112}H_{168}N_{24}NaO_{42}]^{3+}$	$[M + Na + 2H]^{3+}$	848.0548	848.0525

$[C_{112}H_{167}N_{24}Na_2O_{42}]^{3+}$	$[M + 2Na + H]^{3+}$	855.3821	855.3794
$[C_{112}H_{166}N_{24}Na_3O_{42}]^{3+}$	$[M + 3Na]^{3+}$	862.7095	862.7066

[i] found after deconvolution.

Boc-deprotection

Compound **23** (14.5 mg, 5.7 μmol, 1 eq.) was dissolved in a 9:1 mixture of dry CH_2Cl_2 and TFA (570 μL). The reaction was stirred at room temperature, under N_2 for 1h 30 min. Upon completion (^{1}H-NMR monitoring, CD_3OD) the mixture was concentrated *in vacuo* and co-evaporated with toluene three-times. Crude product was purified via semi-preparative HPLC (using the conditions reported below) to obtain the pure product **PM74** as bis-formate salt (4.3 mg, 35%; yellow foam).

HPLC: Flow: 10 mL/min; UV channels: 210 nm; 254 nm; A: H_2O + 0.1% HCO_2H, B: CH_3CN. Gradient: 0-2 min: 0% B, 2-27 min: 0 - 40% B; t_r (**PM74**) = 22.04 min

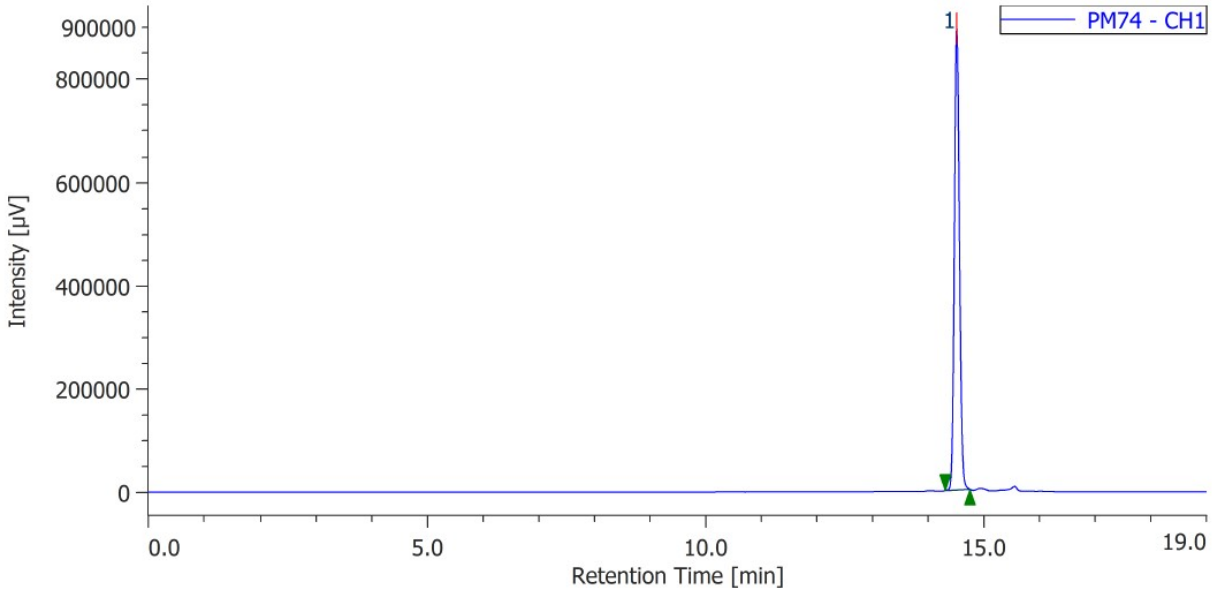
^{1}H-NMR (400MHz, DMSO-d_6): δ(ppm)= 8.57 (brs, 2H, NH), 8.50 (s, 2H, H_{TC5}), 8.44 (brs, 2H, FA), 8.14 (s, 2H, H_{TrCH}), 8.09 (s, 2H, $H_{TrCH'}$), 7.86 (s, 2H, H_{R2}), 7.70 (brs, 6H, NH, NH_2), 7.20 (s, 2H, H_{R5}), 7.16 (s, 2H, H_{R10}), 5.47 (brs, 2H, OH-3), 5.26 (brs, 2H, OH-4), 5.01 (s, 2H, H_1), 4.87 (d, 2H, $J_{2\text{-}3}$ = 4.8 Hz, H_2), 4.73 (brs, 2H, OH-6), 4.68-4.55 (m, 14H, H_8, H_{16}, OH-Peg), 4.51 (s, 4H, H_{10}), 4.39 (s, 4H, H_9), 4.28-4.20 (m, 12H, H_{G1}, H_{G9}, H_{G5}), 4.05-3.98 (m, 2H, H_{7a}), 3.91-3.80 (m, 18H, H_{7b}, H_3, H_5, H_{G6}, H_{G2}, H_{G10}), 3.61-3.48 (m, 50H, H_6, H_4, H_{11}-H_{15}, H_{G4}, H_{G8}, H_{G12}, H_{G3}, H_{G7}, H_{G11}).

HRMS (ESI):

Molecular formula	Ion	Calcd. value (m/z)	Exp. value (m/z)
$[C_{92}H_{135}N_{24}O_{34}]^+$	$[M + H]^+$	2119.9573	2119.9578[i]
$[C_{92}H_{134}N_{24}NaO_{34}]^+$	$[M + Na]^+$	2141.9392	2141.9407[i]
$[C_{92}H_{133}N_{24}Na_2O_{34}]^+$	$[M + 2Na - H]^+$	2163.9211	2163.9216[i]
$[C_{92}H_{136}N_{24}O_{34}]^{2+}$	$[M + 2H]^{2+}$	1060.4825	1060.4799
$[C_{92}H_{135}N_{24}NaO_{34}]^{2+}$	$[M + Na + H]^{2+}$	1071.4735	1071.4708
$[C_{92}H_{134}N_{24}Na_2O_{34}]^{2+}$	$[M + 2Na]^{2+}$	1082.4645	1082.4614
$[C_{92}H_{137}N_{24}O_{34}]^{3+}$	$[M + 3H]^{3+}$	707.3243	707.3232
$[C_{92}H_{136}N_{24}NaO_{34}]^{3+}$	$[M + 2H + Na]^{3+}$	714.6516	714.6503
$[C_{92}H_{135}N_{24}Na_2O_{34}]^{3+}$	$[M + 2Na + H]^{3+}$	721.9789	721.9775
$[C_{90}H_{130}N_{20}Na_3O_{34}]^{3+}$	$[M + 3Na]^{3+}$	729.3062	729.6389

[i] found after deconvolution.

Analitic HPLC: Flow: 1 mL/min; UV channel: 360 nm; A: H_2O + 0.1% HCO_2H, B: CH_3CN + 0.1% HCO_2H.
Gradient: 0-2 min: 0% B, 2-16 min: 0 - 40% B; 16-19 min: 40 - 100% B; t_r (**PM74**) = 14.5 min



Production of L-SIGN and DC-SIGN extracellular domain

L-SIGN and DC-SIGN constructs for SPR, ITC and X-ray crystallographic studies

L-SIGN 3G-ECD (amino acids 78 to 399), L-SIGN CRD (amino acids 266 to 399) and DC-SIGN 3G-ECD (amino acids 66 to 404) were produced as previously described.[4,8,9] Constructs used were supplemented with 3 glycines "3G" residues on the N-terminus side with biomolecular tools, as an anchor for sortagging process, described in the "Surface Plasmon Resonance analysis" supplementary part. This addition does not change the purification protocol and functional properties. The names "lectin 3G-ECD" and "lectin ECD" used hereafter refer to the same entity.

^{15}N-labelled and non-labelled L-SIGN and DC-SIGN for NMR experiments

The DNA fragment coding for the L-SIGN fragment (E262-E400) was inserted into a pET21a plasmid (synthesized by Genscript), amplified in E. coli DH5α cells, and subsequently transformed into E. coli BL21/DE3 cells. One single colony harbouring the expression construct was inoculated into 3 mL Luria Broth (LB) medium containing 100 µg·ml^{-1} ampicillin and then, cultured with shaking for 6 h at 37 °C. For large scale expression of unlabelled protein, 1L of LB

(with antibiotic) was inoculated with the freshly grown culture. For the expression of ^{15}N-labelled protein the cell pellet obtained with the small-scale culture (3ml LB) was transferred to M9 minimal medium containing 1 g/L $^{15}NH4Cl$ with ampicillin. Culture was performed at 37 °C until OD600 was 0.7, induced with 1 mM Isopropyl β-D-1-thio-galactopyranoside (IPTG) and then grown for another 3 h at 20 °C. The culture was harvested by centrifugation at 4500 rpm, and the final pellet was resuspended in lysis buffer (10 mM Tris-HCl pH 8) and sonicated at 4 °C. The protein in the form of inclusion bodies was recovered by ultracentrifugation at 30000 rpm for 1 h. The pellet was then solubilized in Tris-HCl 10 mM (pH 8) with 6 M guanidinium chloride and 0.01% (v/v) b-mercaptoethanol. The residual insoluble fraction was sedimented by ultracentrifugation at 40000 rpm × 2 h. The protein was refolded by dilution then, followed by dialysis. First, the protein sample was diluted 3-fold into buffered solution Tris-HCl 25mM, 1.5M NaCl, 25mM $CaCl_2$, pH 8. Then, subsequent dialysis against no guanidinium chloride (in 25 mM Tris-HCl pH 8, 1.5M NaCl, 25 mM $CaCl_2$) was performed. The soluble fraction containing the folded protein was then purified through size exclusion chromatography in a HiLoad 16/60 Superdex 75 column eluting with 20 mM Tris-HCl, 150 mM NaCl, 5mM $CaCl_2$, 1mM EDTA, pH 8. Finally, fractions containing pure monomeric CRD were washed with 20 mM Tris-HCl, 150 mM NaCl, 5 mM $CaCl_2$, pH 8 to remove the EDTA, and concentrated using 10 MWCO membrane filters. Expression and purification of ^{15}N-DC-SIGN (carbohydrate recognition domain) and the tetrameric extracellular domain of DC-SIGN were performed as previously described [12,13]

Surface Plasmon Resonance (SPR) analysis

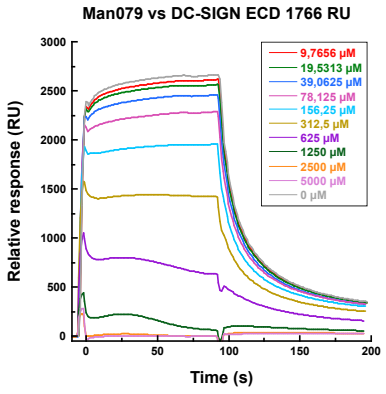
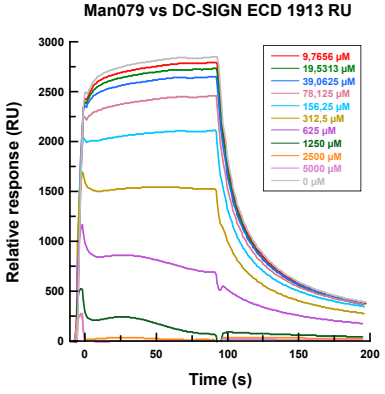
Surface Plasmon Resonance experiments were performed on Biacore T200 instrument at 25°C.

Competition experiments were performed on surfaces functionalized with the Spike protein, using a CM4 sensorchip. The functionalization process of the sensorchip and compounds titrations used are described in Pollastri *et al*[4] : Flow cell 1 (Fc1) to 3 were activated with 60 µL of a mixture of EDC-NHS (according to manufacturer). Fc1 was functionalized with 16.7 µL of BSA at 20 µg/mL in 10 mM NaOAc pH 4, at 5 µL/min. Spike HexaPro was immobilized at the same flow rate on Fc2 and Fc3 at 20 µg/mL in 100 mM NaOAc pH 5. Remaining activated groups were neutralized with 60 µL of 1 M Ethanolamine pH 8.5. After functionalization, all surfaces were washed with 100 µL of 10 mM HCl followed by 100 µL of 50 mM NaOH/1M NaCl at 100 µL/min. Finally, Fc1, 2 and 3 were functionalized respectively at 904, 1766, and 1913 RU.

Direct interaction assays were carried out on a CM3 sensorchip. DC-SIGN and L-SIGN surfaces were processed through a high-affinity biotin/streptavidin interaction. Lectins were biotinylated in N-terminus through a “sortagging process” : a depsi-biotin peptide with LPXTG end (X as any amino acid)

was linked to the N-terminus extremity of lectins complemented with 3 glycine residues. The sortase enzyme links these two ends and binds the biotin to each lectin protomer. Biotinylated lectins were then captured on surfaces previously functionalized with streptavidin. The position of the biotin on the CLR thus enables all the active sites to be oriented in the same direction at the surface, for optimal presentation to glycomimetic compounds. Streptavidin was functionalized of Fc1, Fc2 and Fc3, which were first activated with 60 µL of a mixture of ECD-NHS (according to the manufacturer). Streptavidin was diluted at 100 µg/mL in 10 mM NaOAc pH 4 and immobilized using 78 µL at 5 µL/min. Fc1 was used as a reference surface. Remaining activated groups were neutralized with 60 µL of 1 M Ethanolamine pH 8.5. After functionalization, all surfaces were washed with 128 µL of 10 mM HCl followed by 128 µL of 50 mM NaOH/1M NaCl at 100 µL/min. Finally, Fc1, 2 and 3 were functionalized respectively at 1322, 1393, and 1492 RU. Biotinylated DC-SIGN and L-SIGN were respectively diluted at 1.2 µg/mL and 4 µg/mL in HBS-P+ buffer and captured on Fc2 (for DC-SIGN) and Fc3 (for L-SIGN) surfaces using 100 µL at 5 µL/min. Non-specific binding was washed by injecting 48 µL of 50 mM NaOH/1M NaCl NaOH at 100 µL/min. Fc2 and Fc3 were functionalized at 1747 and 1774 RU. Surfaces functionnality was tested by injections of BSA α1-3, α1-6 Mannotriose (Dextra, 14 atom spacer, product n°NGP1336) at 500 nM, with an association time of 250 sec and a dissociation time of 150 sec at a 30 µL/min flow rate. The dimers were injected on surfaces with decreasing concentrations from 125 µM to 1.9 nM, using a 1:2 dilution. Concentrations injected were adapted depending on the prediction of the apparent K_D obtained (see sensorgrams below). Flow rate was set at 20 µL/min in 25 mM Tris pH 8, 150 mM NaCl, 4 mM CaCl2 supplemented with 0,05% Tween, with an association and dissociation time set to 200 and 150 seconds respectively. Surface regeneration was done between each injection for 10 seconds at 100 µL with a 100-second stabilization period, using 50 mM glycine NaOH pH 12 0.15% Triton 25 mM EDTA.

Figue S1: Sensorgrams of competition experiment

		Spike surface 1766 RU	Spike surface 1913 RU
DC-SIGN competition assays	Man079		
		IC_{50} = 316.8 ± 24 µM	IC_{50} = 319.2 ± 22.4 µM

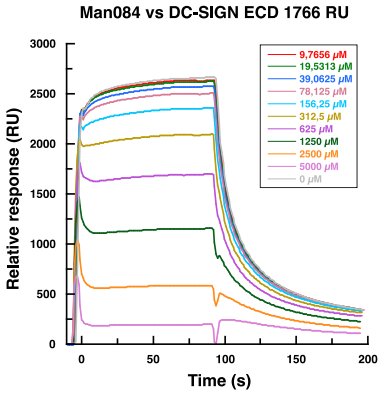
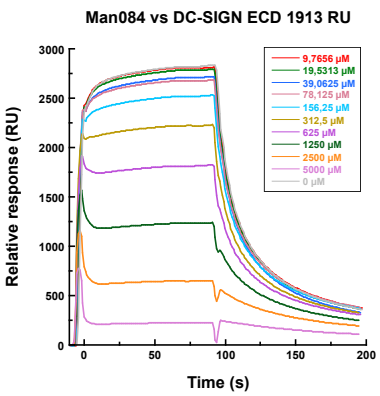
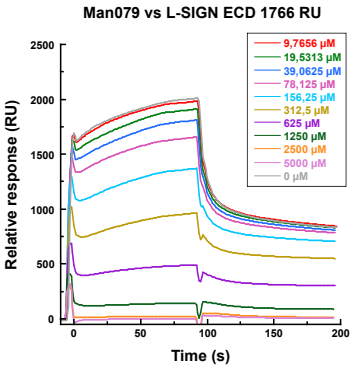
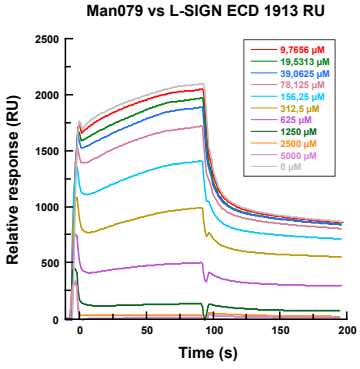
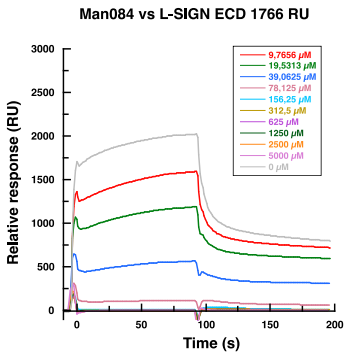
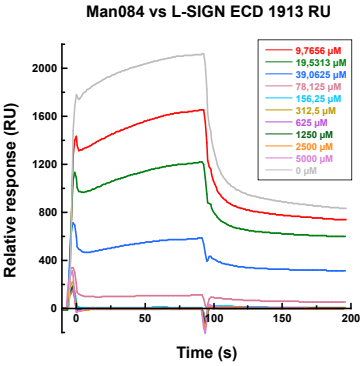
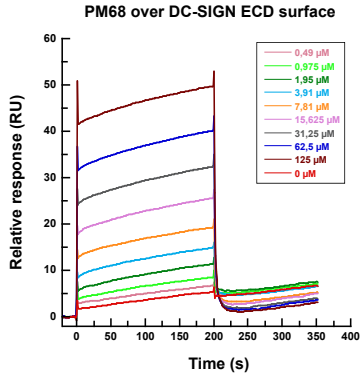
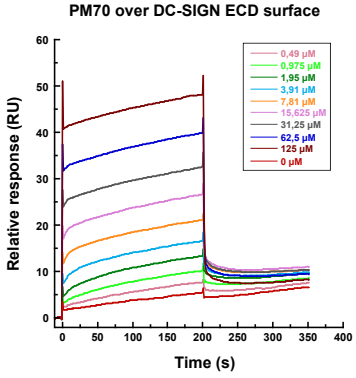
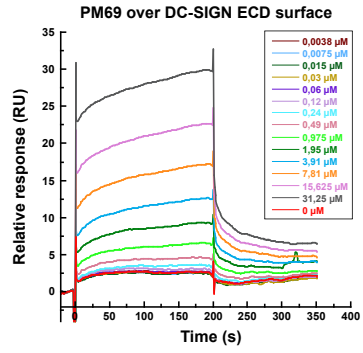
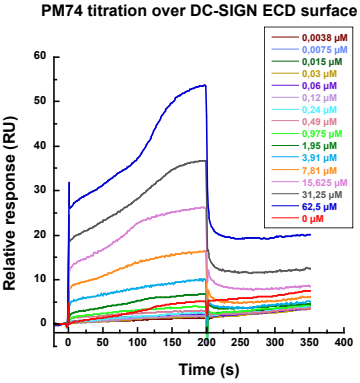
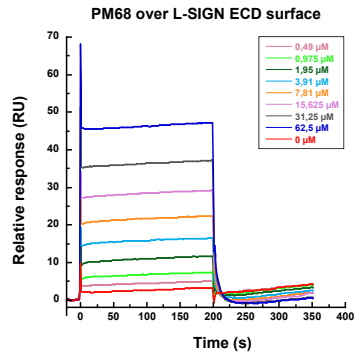
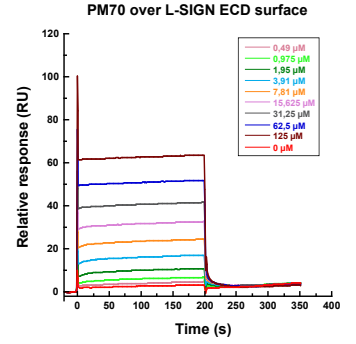
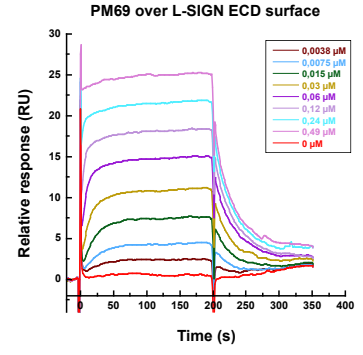
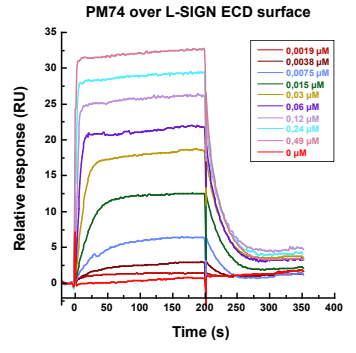
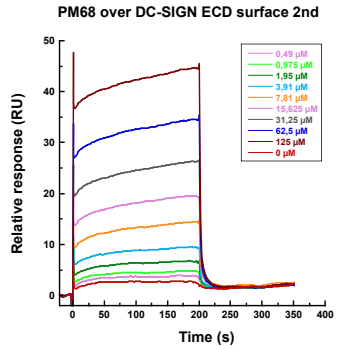
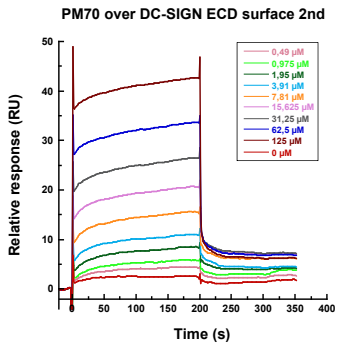
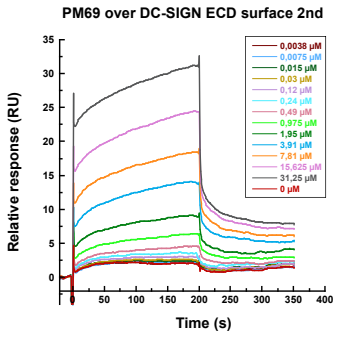
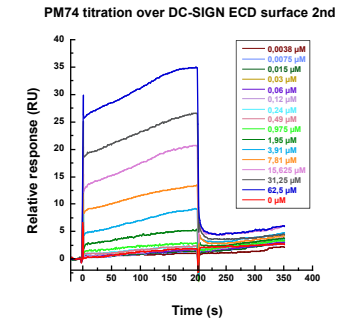
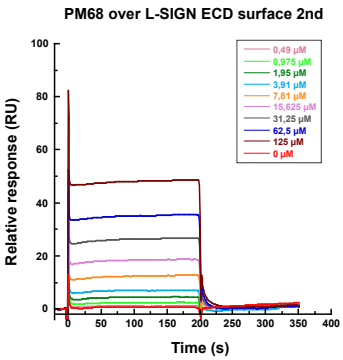
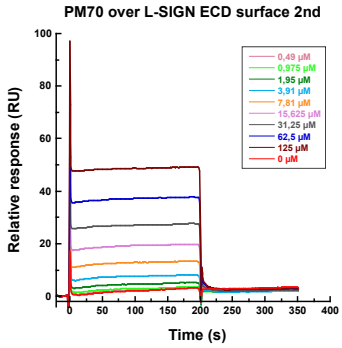
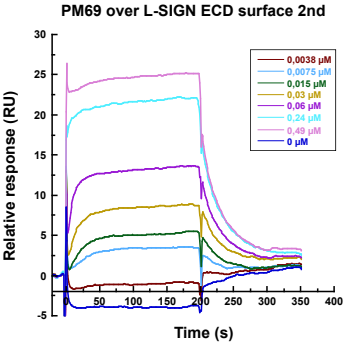
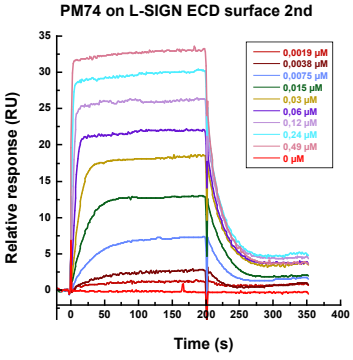
	Man084		
		IC_{50} = 1051 ± 436 µM	IC_{50} = 1062 ± 474 µM
L-SIGN competition assays	Man079		
		IC_{50} = 284.9 ± 21.2 µM	IC_{50} = 271.1 ± 21.1 µM
	Man084		
		IC_{50} = 24.11 ± 0.75 µM	IC_{50} = 23.68 ± 0.96 µM

Figure S2 : Sensorgrams of direct interaction experiments (duplicate)

First serie of measurements

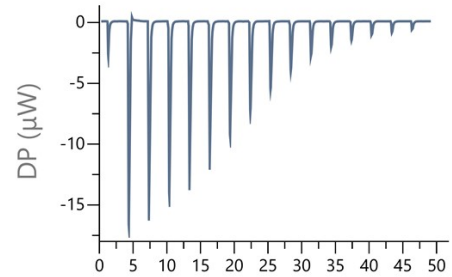
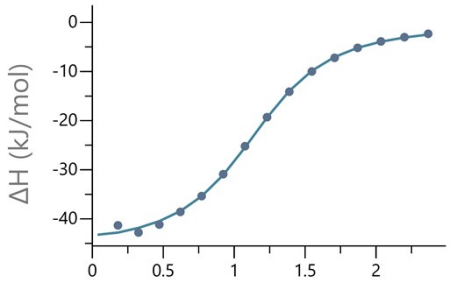
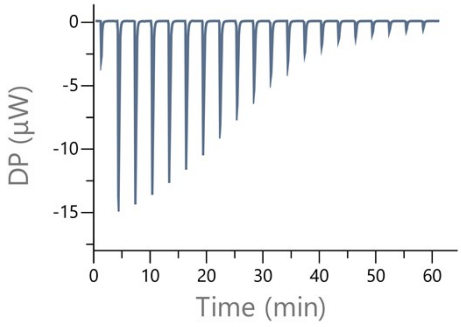
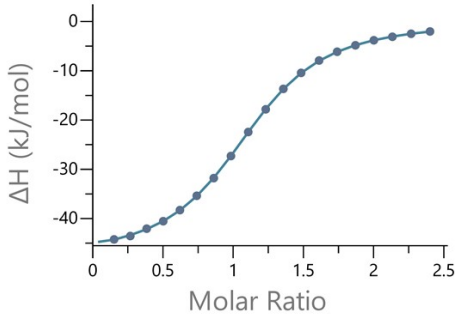
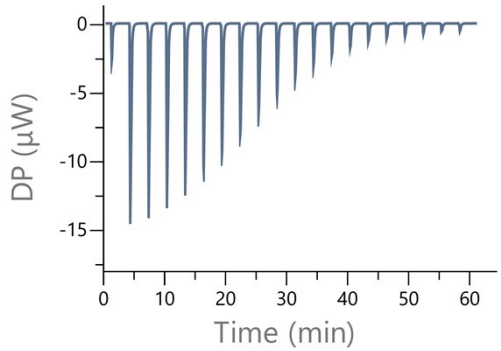
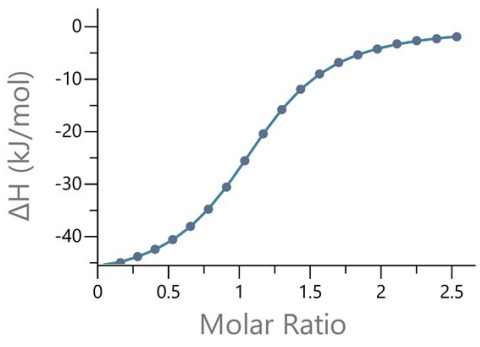
DC-SIGN surface	*Man79-dimers*		
		Apparent K_D = 28.8 ± 5.1 µM	**Apparent K_D = 24.5 ± 5.1 µM**
	Man84-dimers		
		Apparent K_D = 10.3 ± 1.1 µM	**Apparent K_D = 38.8 ± 3.1 µM**
L-SIGN surface	*Man79-dimers*		
		Apparent K_D = 16.1 ± 3.3 µM	**Apparent K_D = 21.7 ± 3.8 µM**
	Man84-dimers		
		Apparent K_D = 0.0501 ± 0.007 µM	**Apparent K_D = 0.0249 ± 0.0036 µM**
Second serie of measurements			

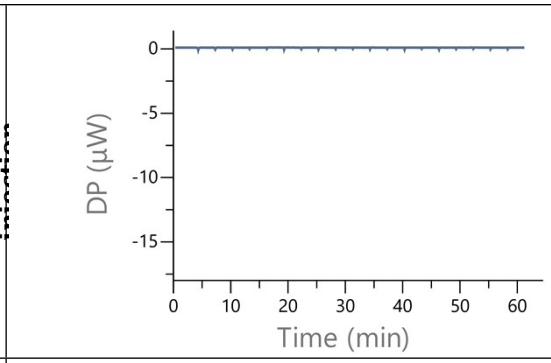
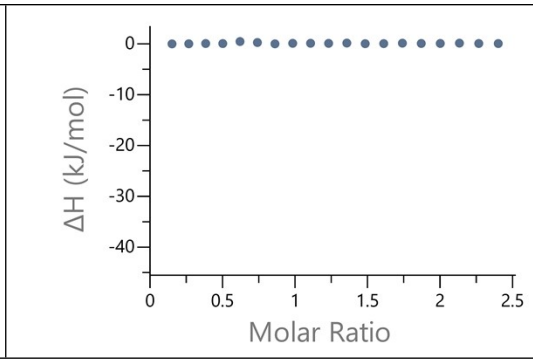
DC-SIGN surface	*Man79-dimers*		
		Apparent K_D = 38.5 ± 6 µM	**Apparent K_D = 31.7 ± 6 µM**
	Man84-dimers		
		Apparent K_D = 9.31 ± 0.77 µM	**Apparent K_D = 20.9 ± 1.5 µM**
L-SIGN surface	*Man79-dimers*		
		Apparent K_D = 44.4 ± 7.6 µM	**Apparent K_D = 44.1 ± 4.8 µM**
	Man84-dimers		
		Apparent K_D = 0.0537 ± 0.012 µM	**Apparent K_D = 0.0247 ± 0.0038 µM**

Isothermal titration calorimetry

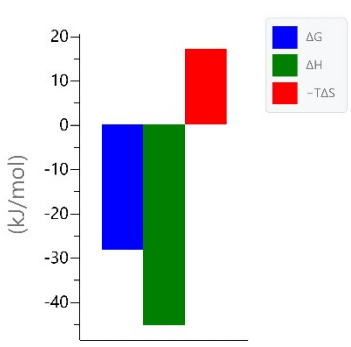
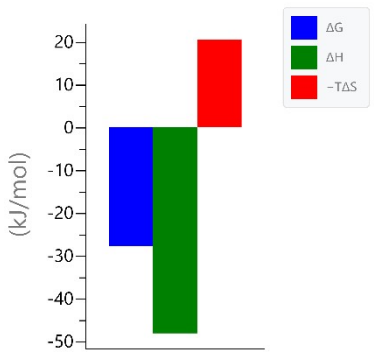
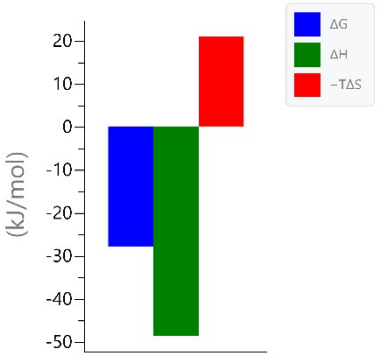
Characterization assays by ITC were performed on MicroCal PEAQ-ITC (Malvern Panalytical) at 25°C. L-SIGN 3G-ECD and Man84 were respectively diluted at 172 µM and 2 mM with 25 mM Tris pH 8, 150 mM NaCl, 4 mM CaCl2 buffer. L-SIGN was injected on the Sample Cell, equilibrated 3 times with buffer 25 mM Tris pH 8, 150 mM NaCl, 4 mM $CaCl_2$. 40 µL of Man84 sample were loaded into the syringe. 20 injections of 2 µL were performed into the Sample cell (initial injection of 0.5 µL, deleted for analysis), each one at 0,5 µL/s with 180 seconds between two injections. All the parameters were determined via the MicroCal PEAQ-ITC Analysis Software.

Figure S3

Run 1		
	K_D = 11.3 µM ; ΔH = - 45.4 kJ/mol ; Stoichiometry N = 1.13 ;	
Run 2		
	K_D = 13.6 µM ; ΔH = -48.2 kJ/mol ; Stoichiometry N = 1.10 ;	
Run 3		
	K_D = 13.3 µM ; ΔH = -48.8 kJ/mol ; Stoichiometry N = 1.09 ;	

Control : buffer injection		
	K_D = 0 µM ; ΔH = 0 kJ/mol ; Stoichiometry N = 0 ;	

Thermodynamic parameters:

Run 1	**Run 2**	**Run 3**
		
ΔG = -28.3 kJ/mol ΔH = -45.4 kJ/mol -TΔS = 17.1 kJ/mol	ΔG = -27.8 kJ/mol ΔH = -42.8 kJ/mol -TΔS = 20.4 kJ/mol	ΔG = -27.9 kJ/mol ΔH = -48.8 kJ/mol -TΔS = 20.9 kJ/mol

NMR Interaction Experiments

General information. 500 µL samples were prepared and precision NMR tubes with 5 mm outer diameter (New Era Enterprises, Vineland, USA) were employed. 1H-NMR resonances of **Man84** were assigned through standard 2D-TOCSY (60 and 90 ms mixing times), 2D-NOESY (400 ms mixing time), and 1H,13C-HSQC experiments at 298K on a 1 mM sample in D2O. NMR experiments were acquired on a Bruker 800 MHz spectrometer.

Chemical Shift Perturbation (CSP) Analysis. For DC-SIGN, experiments were acquired at 308K on a sample containing 40 µM ^{15}N-labelled protein in buffered (25 mM Tris-d11, 150 mM NaCl, 4 mM $CaCl_2$, 2 mM DTT-d10, pH 8) H_2O/D_2O 9:1. In the case of L-SIGN, experiments were acquired at 301K on a sample of 29 µM of ^{15}N-labelled protein in buffered (20 mM Tris-HCl, 150 mM NaCl, 5 mM $CaCl_2$, pH 8) H_2O/D_2O 9:1. ^{1}H,^{15}N-HSQC experiments with 128 (T1) x 1536 (T2) complex data points in ^{15}N and ^{1}H dimensions, respectively were acquired either in the absence or in the presence of the ligand. 10 and 100 equivalents of **Man84** were titrated into protein samples of L-SIGN and DC-SIGN, respectively. Backbone resonance assignments deposited in the Biological Magnetic Resonance Data Bank (BMRB

27854 and 25046, for DC-SIGN and L-SIGN, respectively) were employed for data analysis. Average chemical shift perturbation (CSP) of the protein-backbone NH groups was analysed using CcpNmr Analysis software and calculated using the formula: $\Delta\delta$ (ppm) = $[(\Delta\delta H^2 + (0.14 \cdot \Delta\delta N)^2)/2]^{½}$).

Transferred NOESY experiments. Samples contained: for DC-SIGN, 11µM of the tetrameric extracellular domain and 750 µM of **Man84** (17 equivalents with respect to the number of carbohydrate binding sites), and for L-SIGN, 40 uM of the monomeric CRD (non-labelled) and 1.6mM of **Man84** (protein:ligand ratio 1:10), in buffered D_2O (L-SIGN: 20 mM Tris-d11, 150 mM NaCl, 5 mM $CaCl_2$, pH 8; DC-SIGN: 25 mM Tris-d11,150 mM NaCl, 4 mM $CaCl_2$, 2 mM DTT-d10). 2D-NOESY experiments were recorded on a Bruker AVANCE 2 800 MHz spectrometer equipped with cryoprobe at 298K. The experiments were acquired with 2048x128 data points with mixing times of 50ms for the sample with DC-SIGN and 100ms for the sample with L-SIGN.

Crystallogenesis and crystal structure determination

Crystals were obtained for reservoir conditions with 0.18M tri-ammonium citrate and 20% (w/v) PEG 3350, with a 2:1 ratio of protein/reservoir. The processing of these data was carried out with the A chain of the PDB 1SL6 architecture (without ligand) as a model and the Man84 molecule added later. Data collection was performed at id30A-1 beamline (MASSIF-1), ESRF Grenoble, 900 images were collected at 100°K, with an oscillation range of 0.2°, an exposure time of 0.05s per image, and a wavelength of 0.965459 Å.

Data sets were processed and using the programs XDS by playing on “Strong_Pixel” parameter to filter the weaker diffraction spots. This parameter was increased by a factor of 2 at each assay of indexation, until a good quality of feet (<10) was obtained for a space group different from space group 1 (P1). Such results were obtained with STRONG_PIXEL=24 (default was 6) resulting in CC(1/2)>50% until resolution of 1.8 Å. Space group was confirmed using POINTLESS and turns out to be space group 4 ($P2_1$). Finally, reflections were scaled using XSCALE. Statistics of data processing are summarized in Table 1. Matthew’s coefficient was calculated to 1 using the program MATTHEWS_COEF from the CCP4 suite. Phasing was performed by molecular replacement with a known structure of DC-SIGN CRD (a chain of pdb code: 1SL6) depleted of the ligand and water molecules. The best solution resulting from molecular replacement was used as the starting model for structure refinement. The structure refinement was performed by cycling between manual building using the program COOT and energy minimization with the program REFMAC from the CCP4 package. Geometry of the structure was verified using Procheck program with check module of the CCP4 program suite. Finally, the structure was verified using Procheck program with CHECK module of the CCP4 program suite. Data processing and refinement statistics are described in Table 2. The crystal structure of DC-SIGN CRD/Man84 has been deposited in Protein Data Bank under PDB code 8RCY.

Data collection statistics	
Wavelength (Å)	0.965459
Space group	$P12_11(4)$
Unit cell parameters (Å)	a=33.43, b=48.89, c=35.67, α=90.0, β=103.53, γ=90.0
Resolution (Å)	48.88-1.8(1.91-1.8)
Unique reflections	10206 (1441)
Completeness (%)	97.6 (87.1)
I/σ	9.09 (1.52)
CC (1/2) (%)	99.6 (74.4)
Structure refinement statistics	
Resolution (Å)	34.68-1.8
Refinement factors	
Used reflections/free (%)	9695/485
R_{cryst}[c]	0.1626
R_{free}[c]	0.2125
rmsd from ideality	
Bond angles (deg)	1.5080
Bond lengths (Å)	0.0076
Ramachandran plot (%)	
Most favoured regions	95.87
Additional allowed regions	3.3
Disallowed regions	0.83
Average B-factors (Å^2)	28.0

Table S1: L-SIGN CRD/Man084 complex data collection and structure refinement statistics.

Production of SARS-CoV-2 and EBOV-pseudotyped rVSV-luc and trans-infection assays

Trans-infection assays with **PM74, PM66, PM71** and mannan were performed by using pseudoviruses (PSV) based in a recombinant vesicular stomatitis virus-luciferase (rVSV-luc) system and a reference strain of SARS-CoV-2 and EBOV-pseudotyped. rVSV-luc was produced following previously published protocols.[10,11] First, BHK-21 cells were transfected using Lipofectamine 3000 (Fisher Scientific) to express Ebola virus Makona Glycoprotein (EBOV-GP, GenBank KM233102.1) or Spike protein of SARS-CoV-2 (Wu-hu-1 D614G). On the next day, transfected cells were inoculated with a replication-deficient rVSV-luc pseudotype (MOI: 3) (Kerafast, Boston, MA). After 1 h incubation at 37°C, cells were washed three times with PBS and then DMEM supplemented with 5% heat-inactivated FBS, 25 µg/mL gentamycin and 2 mM L-glutamine were added. PSV were collected 24 h post-inoculation, clarified from cellular debris by centrifugation and stored at -80°C. Luciferase activity was determined by luciferase assay (Steady-Glo Luciferase Assay System, Promega).

PSV were normalized for infectivity to a multiplicity of infection of 0.5 and the inhibitory effect of the glycomimetic compounds: **PM74, PM66** and **PM71** were evaluated in trans-infection experiments using Jurkat cells expressing DC-SIGN and L-SIGN and Vero E6 cells. Jurkat DC-SIGN and Jurkat L-SIGN cells were first pre-incubated 20 min with the corresponding concentration of the compounds before being challenged with SARS-CoV-2 and EBOV-rVSV-luc. **PM74, PM66** and **PM71** were tested at 2 different concentrations: 5 µM and 500 nM, and mannan was tested at 25µg/mL. Cells were then incubated with the SARS-CoV-2 and EBOV-rVSV-luc during 2 h at room temperature with rotation. Cells were then centrifuged at 1200 rpm for 5 minutes and washed three times with PBS supplemented with 0.5 % BSA and 1 mM CaCl2. Cells were then resuspended in RPMI medium and co-cultivate with adherent VeroE6 cells. After 24 h, the supernatant was removed and monolayer of VeroE6 was washed with PBS three times. Cells were then lysed and assayed for luciferase expression (Glomax Navigator, Promega).

To calculate PM-74 50% inhibitory concentration (IC50) for SARS-CoV-2 trans-infection DC-SIGN or L-SIGN Jurkat cells were tested by triplicates in a range of concentrations from 100 µM to 0.01 nM. IC50 and 95% confident interval was calculated by Graph Pad Prism V8. See Figure S4 below.

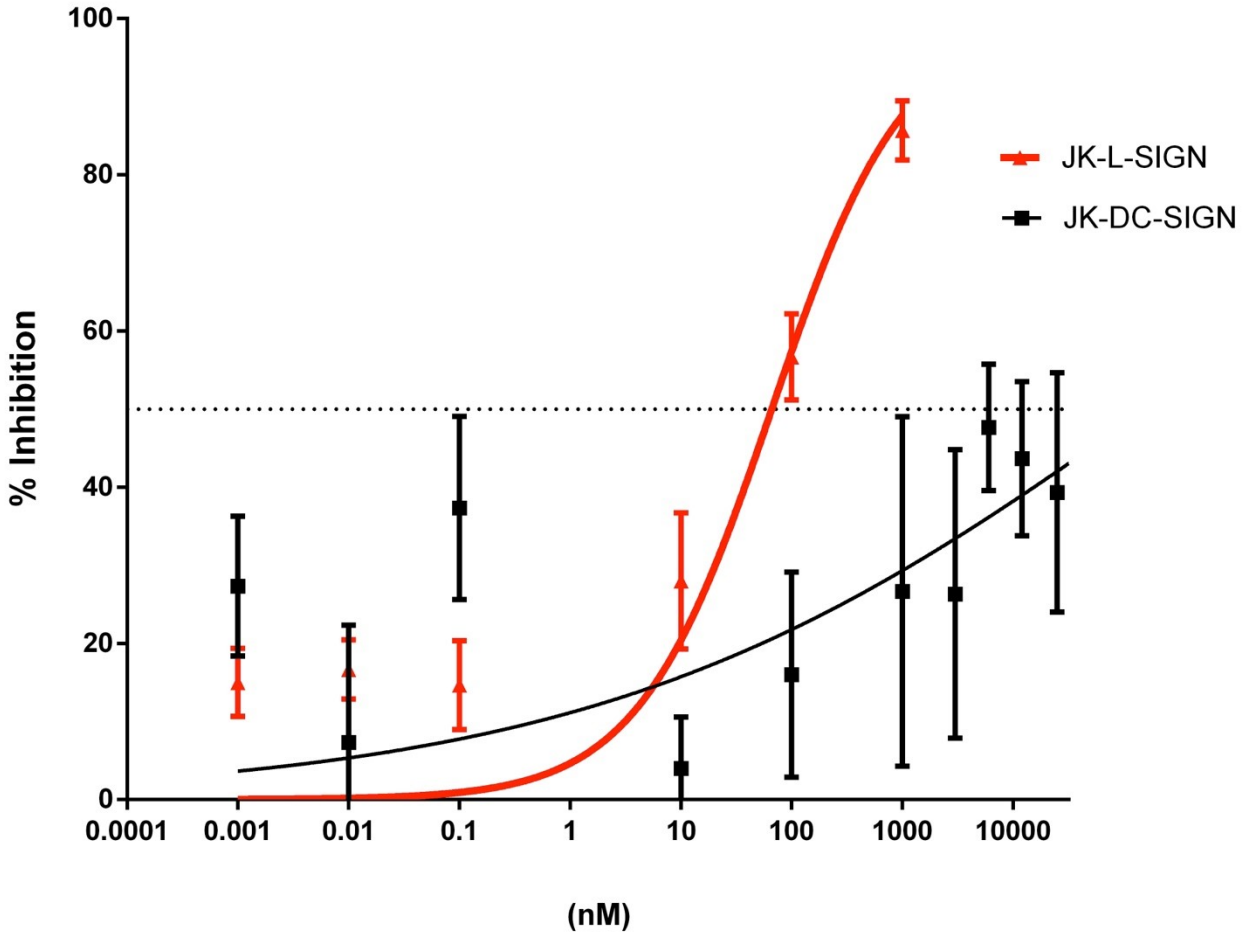
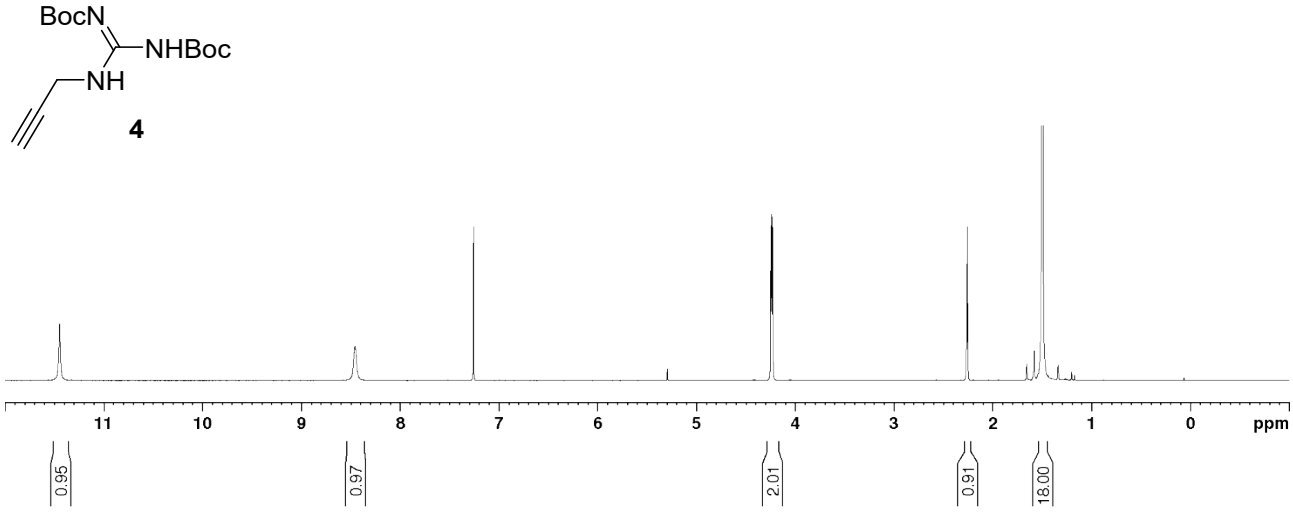


Figure S4: PM74 inhibition of DC-SIGN and L-SIGN-mediated trans-infection of SARS-CoV-2. DC-SIGN or L-SIGN expressing Jurkat cells were incubated with SARS-CoV-2 pseudoviruses (SARS-CoV-2_S-VSV) in the presence of PM74 and after extensive washing cells were incubated onto susceptible Vero E6 cells. Mean values ± SEM are represented.

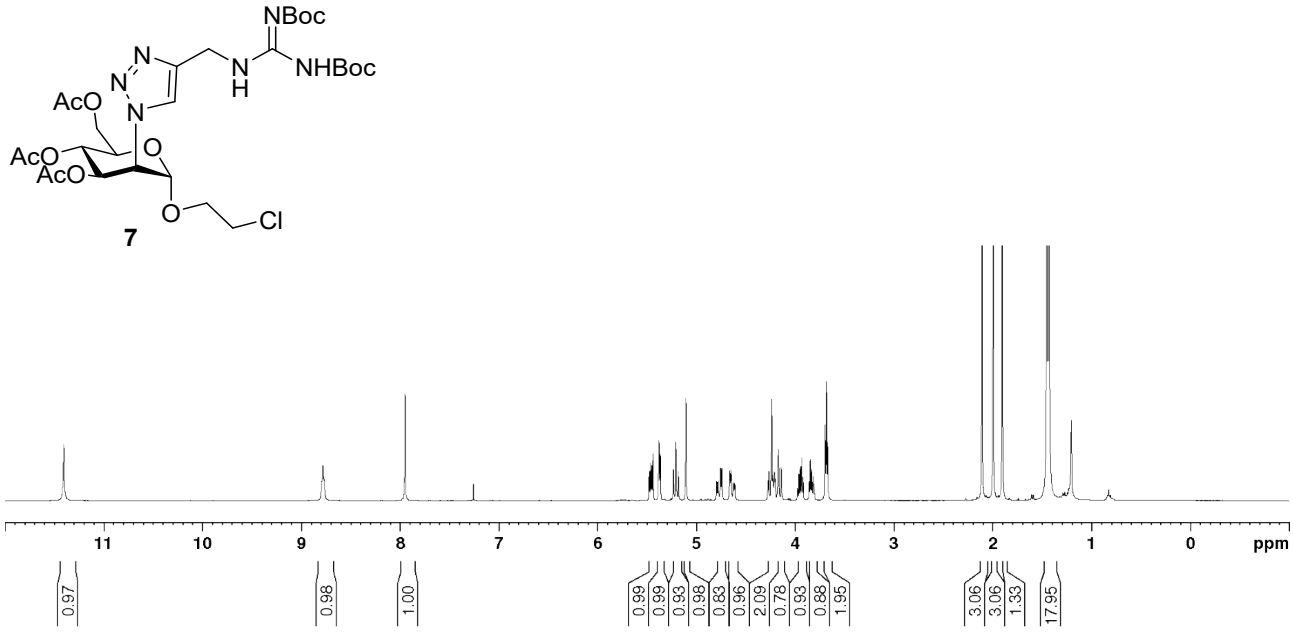
References

[1] W. C. Still, M. Kahn, A. Mitra, *Journal of Organic Chemistry* **1978**, *43*, 2923–2925.
[2] K. Feichtinger, C. Zapf, H. L. Sings, M. Goodman, *J Org Chem* **1998**, *63*, 3804–3805.
[3] K. Feichtinger, H. L. Sings, T. J. Baker, K. Matthews, M. Goodman, *J Org Chem* **1998**, *63*, 8432–8439.
[4] S. Pollastri, C. Delaunay, M. Thépaut, F. Fieschi, A. Bernardi, *Chemical Communications* **2022**, *58*, 5136–5139.
[5] C. S. Eubanks, J. E. Forte, G. J. Kapral, A. E. Hargrove, *J Am Chem Soc* **2017**, *139*, 409–416.
[6] F. Piron, C. Oprea, C. Cismaş, A. Terec, J. Roncali, I. Grosu, *Synthesis (Stuttg)* **2010**, *2010*, 1639–1644.
[7] F. Pertici, N. Varga, A. van Duijn, M. Rey-Carrizo, A. Bernardi, R. J. Pieters, *Beilstein Journal of Organic Chemistry* **2013**, *9*, 215–222.
[8] S. Achilli, J. T. Monteiro, S. Serna, S. Mayer-Lambertz, M. Thépaut, A. Le Roy, C. Ebel, N.-C. Reichardt, B. Lepenies, F. Fieschi, C. Vivès, *Int J Mol Sci* **2020**, *21*, 5290.
[9] G. Tabarani, M. Thépaut, D. Stroebel, C. Ebel, C. Vivès, P. Vachette, D. Durand, F. Fieschi, *Journal of Biological Chemistry* **2009**, *284*, 21229–21240.
[10] M. A. Whitt, *J Virol Methods* **2010**, *169*, 365–374.
[11] M. Thépaut, J. Luczkowiak, C. Vivès, N. Labiod, I. Bally, F. Lasala, Y. Grimoire, D. Fenel, S. Sattin, N. Thielens, G. Schoehn, A. Bernardi, R. Delgado, F. Fieschi, *PLoS Pathog* **2021**, *17*, DOI 10.1371/journal.ppat.1009576.
[12] P. Valverde, S. Delgado, J. D. Martínez, J.-B. Vendeville, J. Malassis, B. Linclau, N.-C. Reichardt, F. J. Cañada, J. Jiménez-Barbero and A. Ardá, *ACS Chem. Biol.*, 2019, **14**, 1660–1671.
[13] J. D. Martínez, P. Valverde, S. Delgado, C. Romanò, B. Linclau, N. C. Reichardt, S. Oscarson, A. Ardá, J. Jiménez-Barbero and F. J. Cañada, *Molecules*, 2019, **24**, 2337.

NMR spectra of ligands



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



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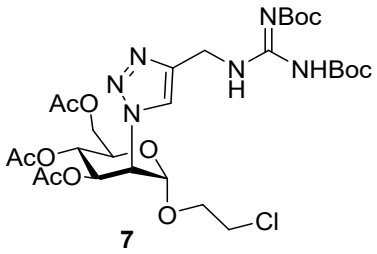

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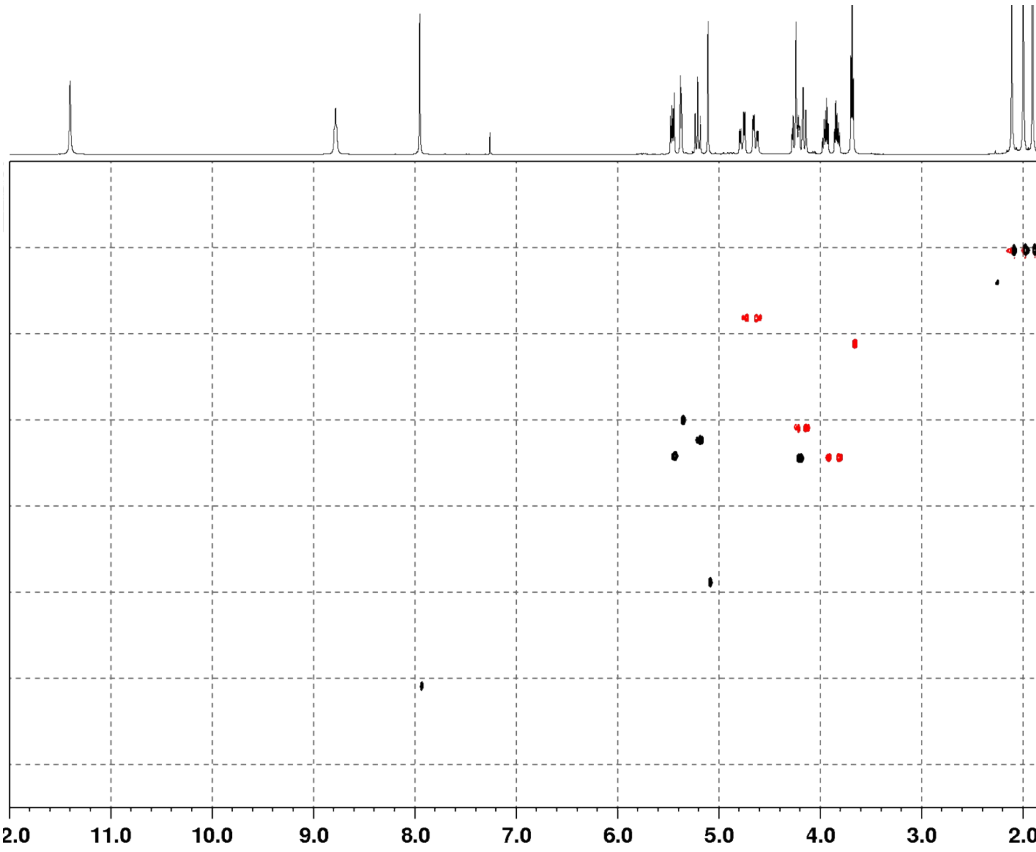

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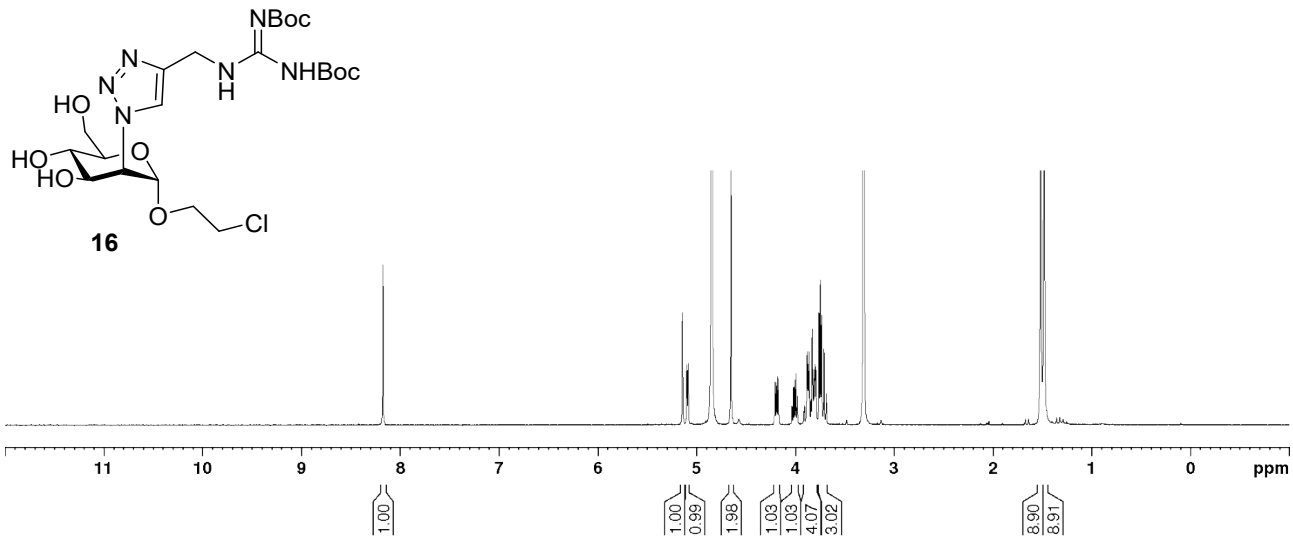

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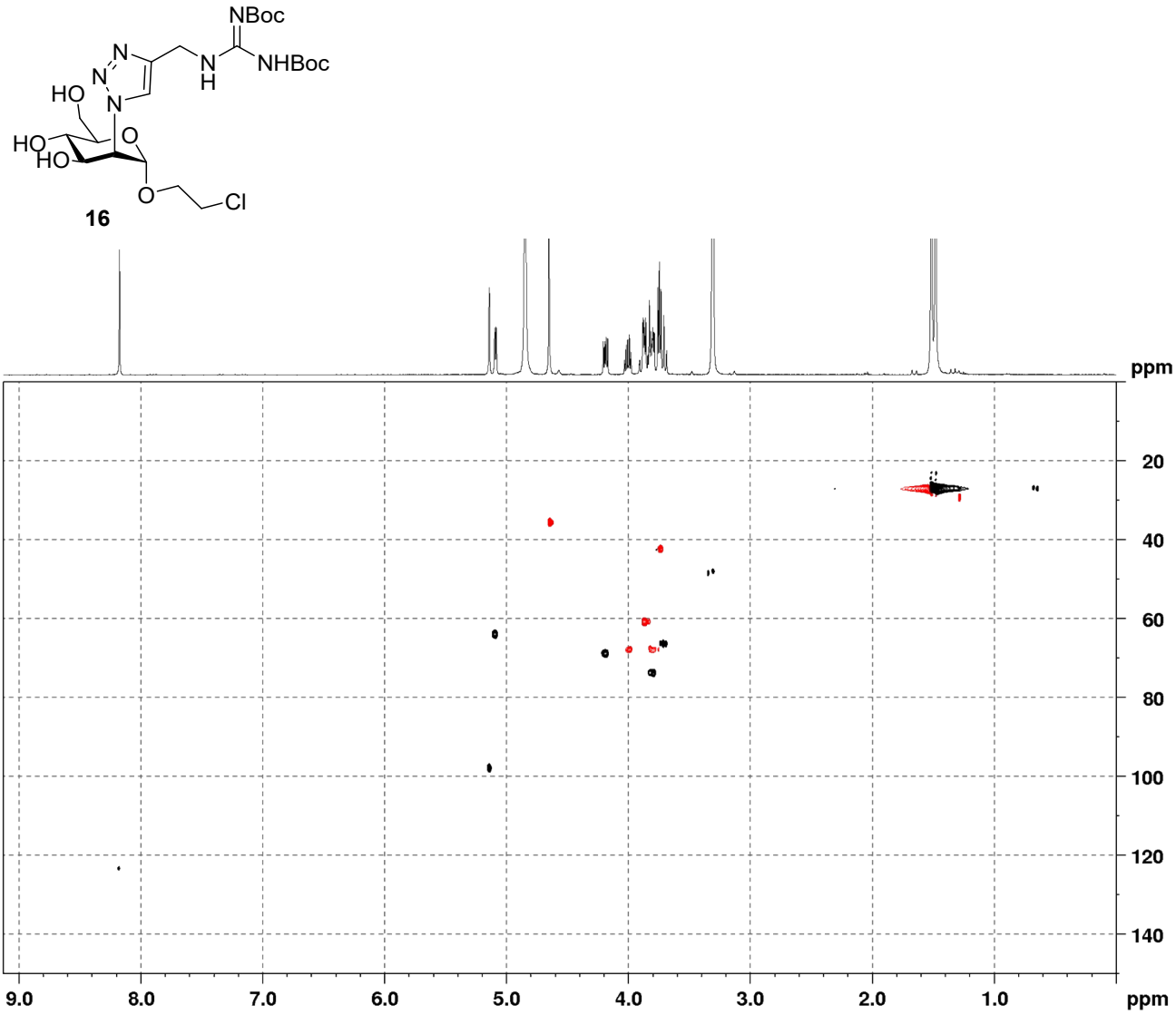

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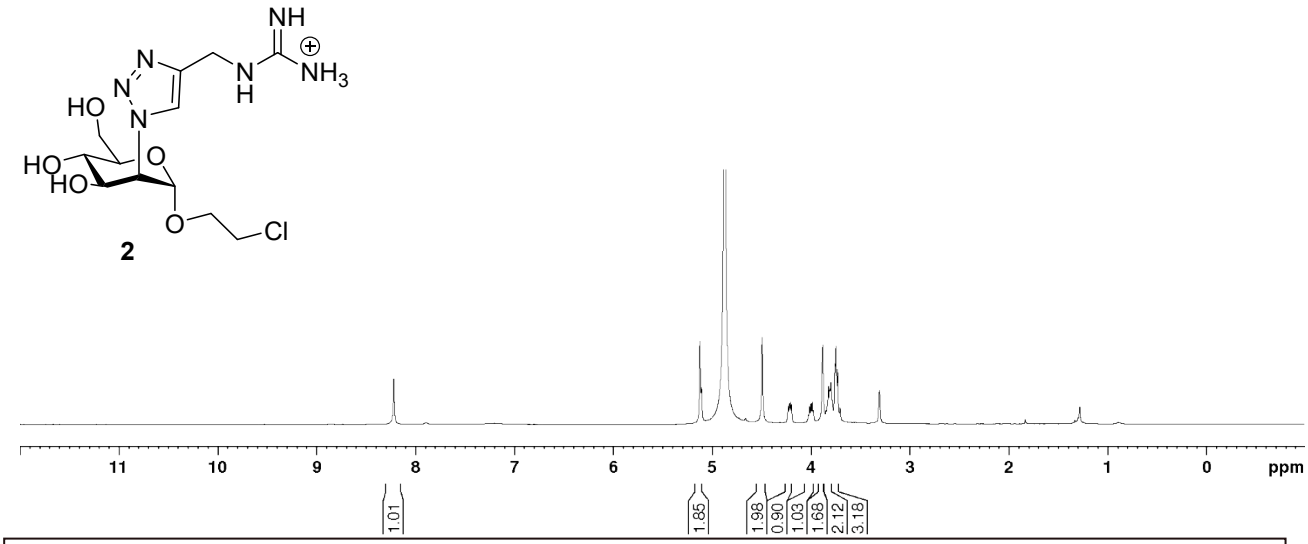

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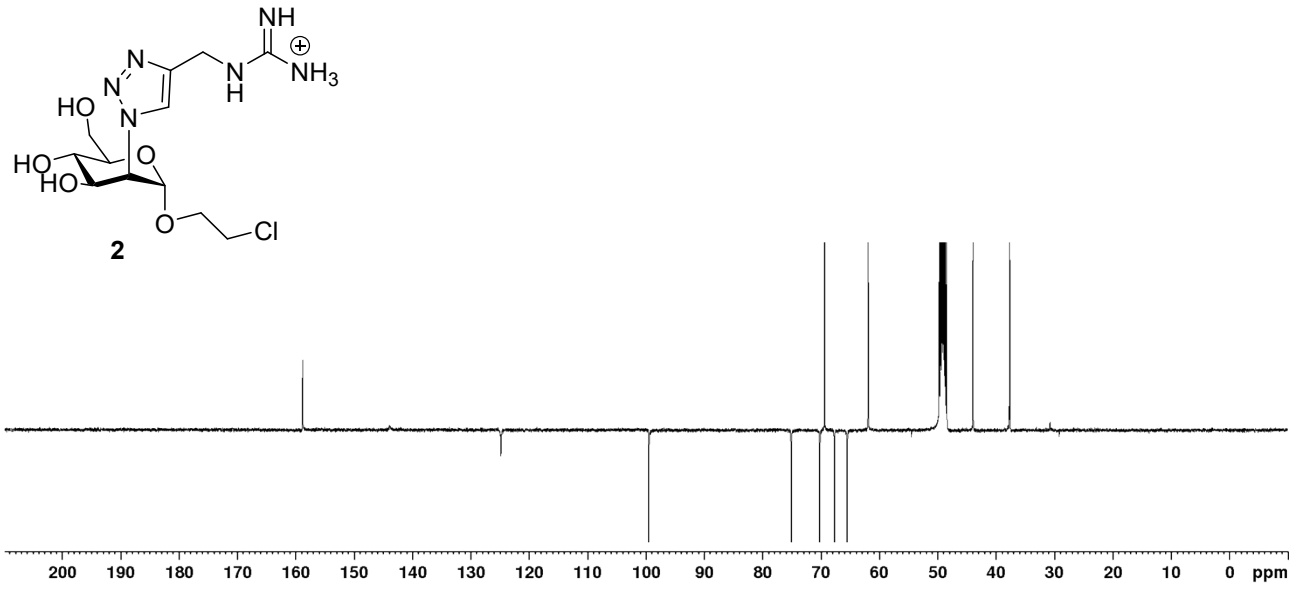

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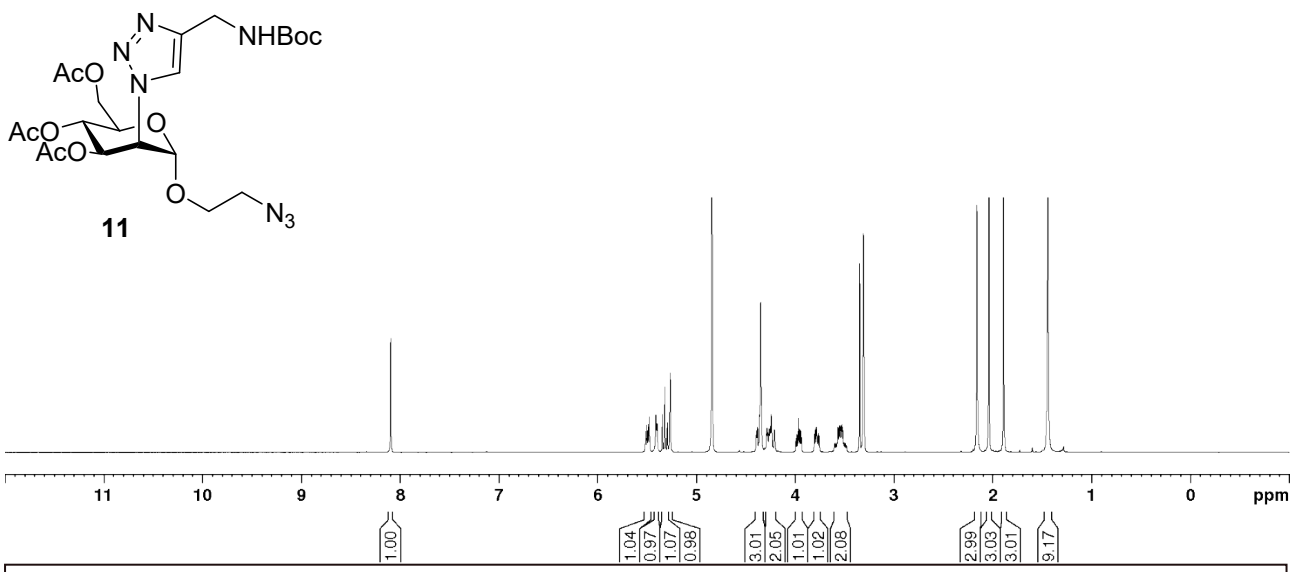

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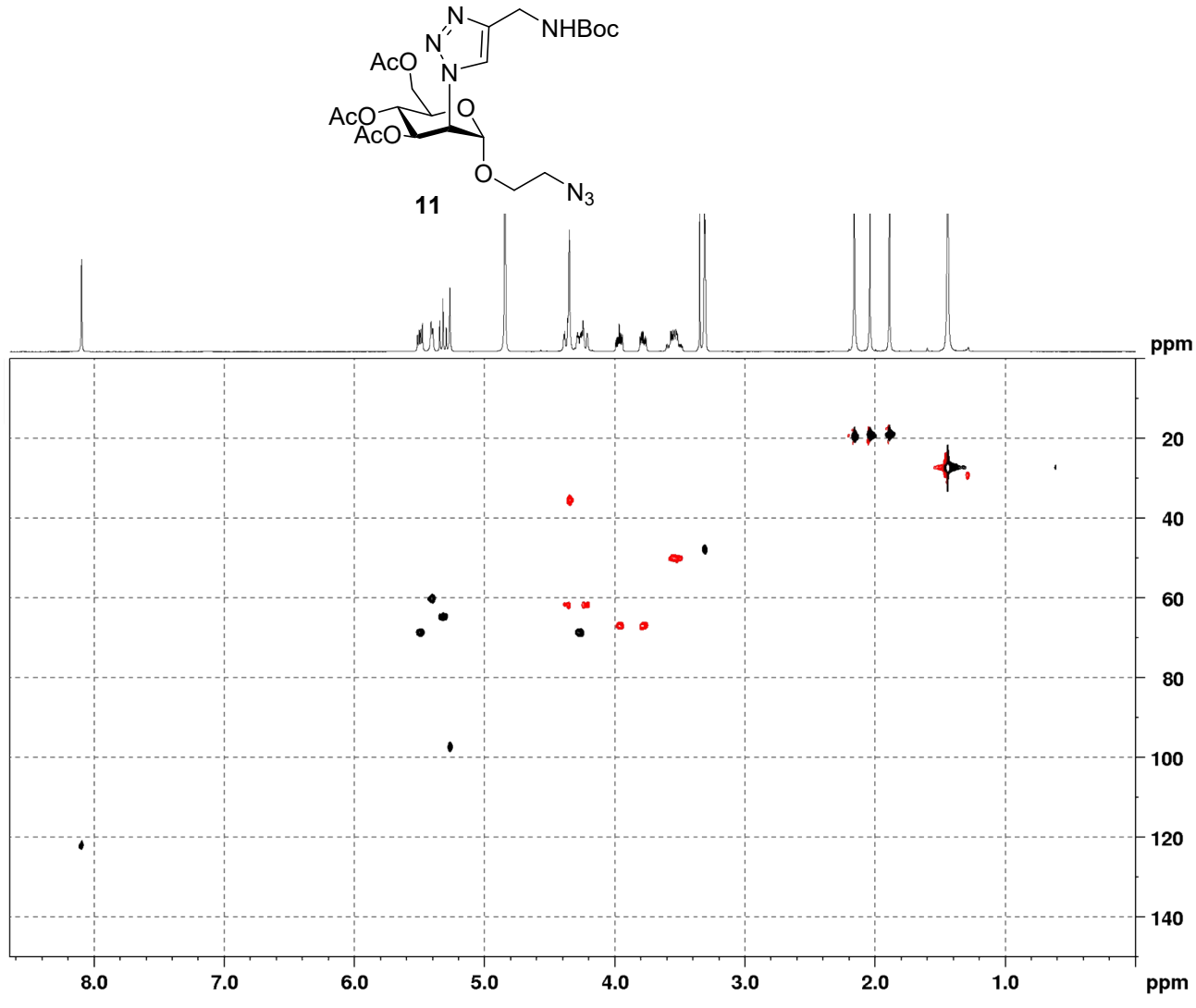

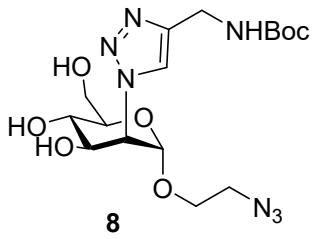

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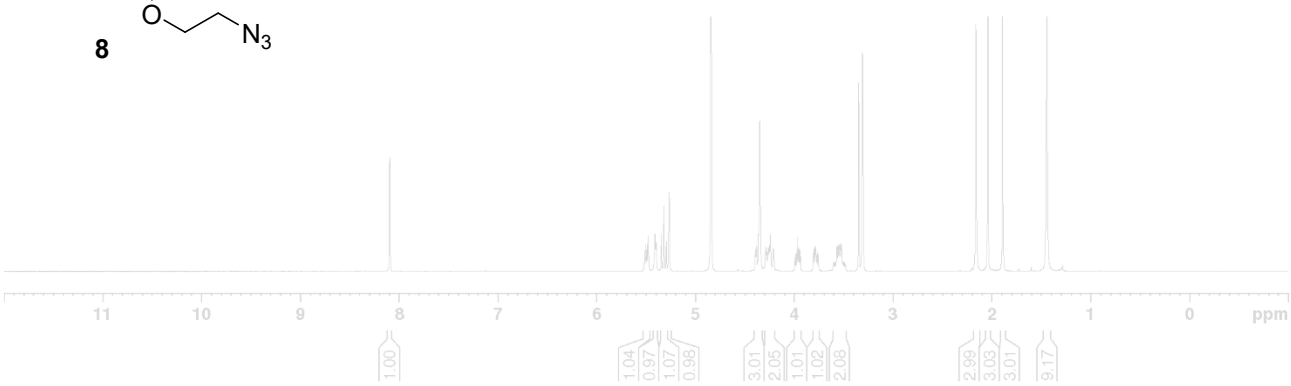

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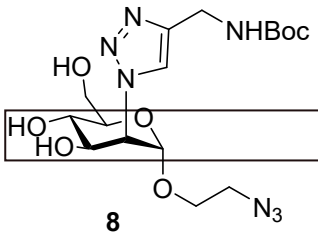

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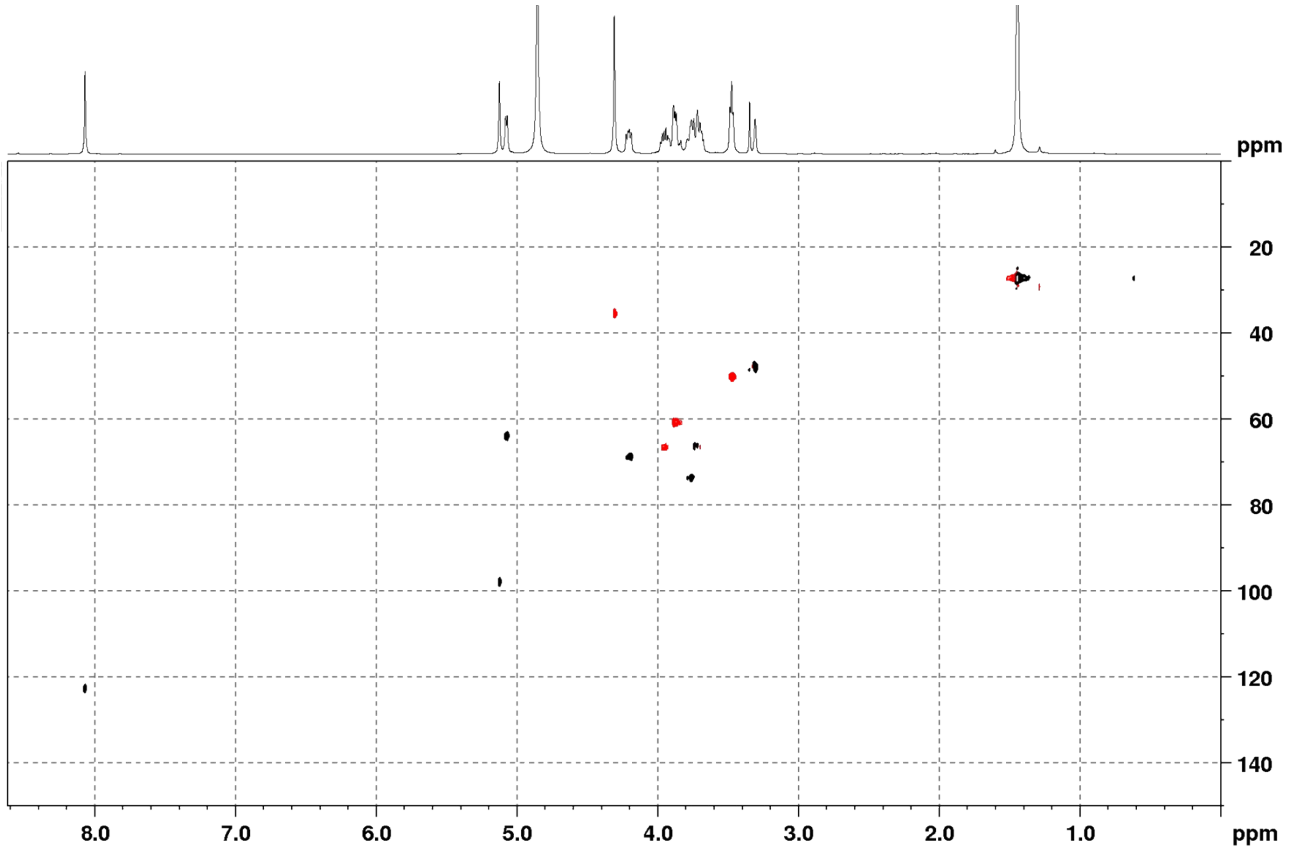

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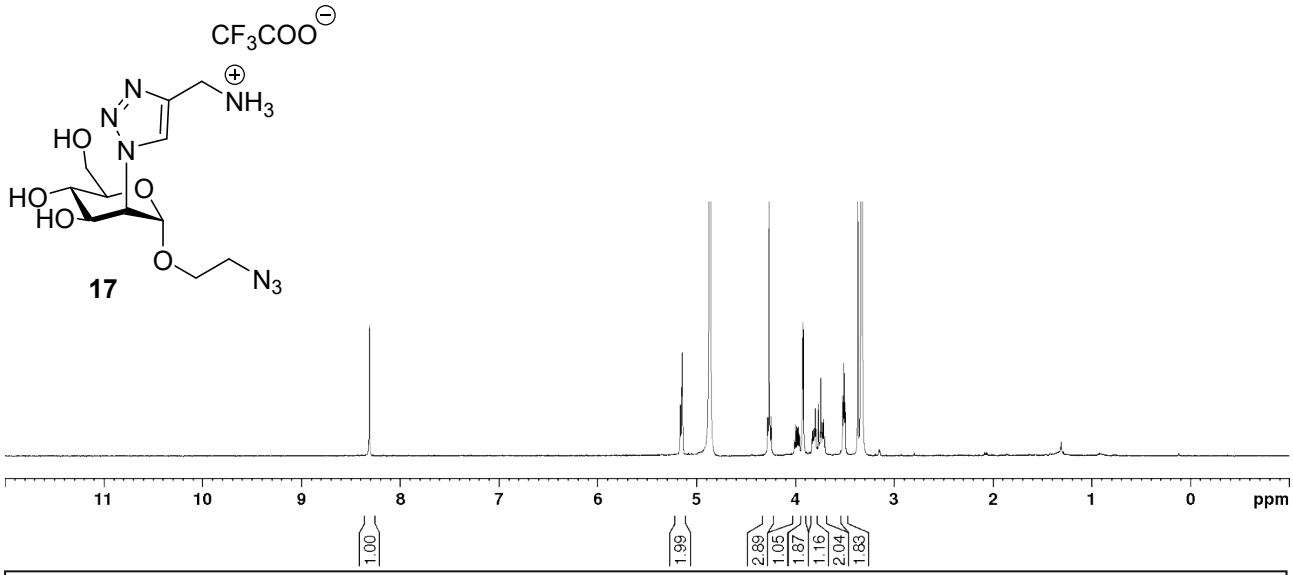

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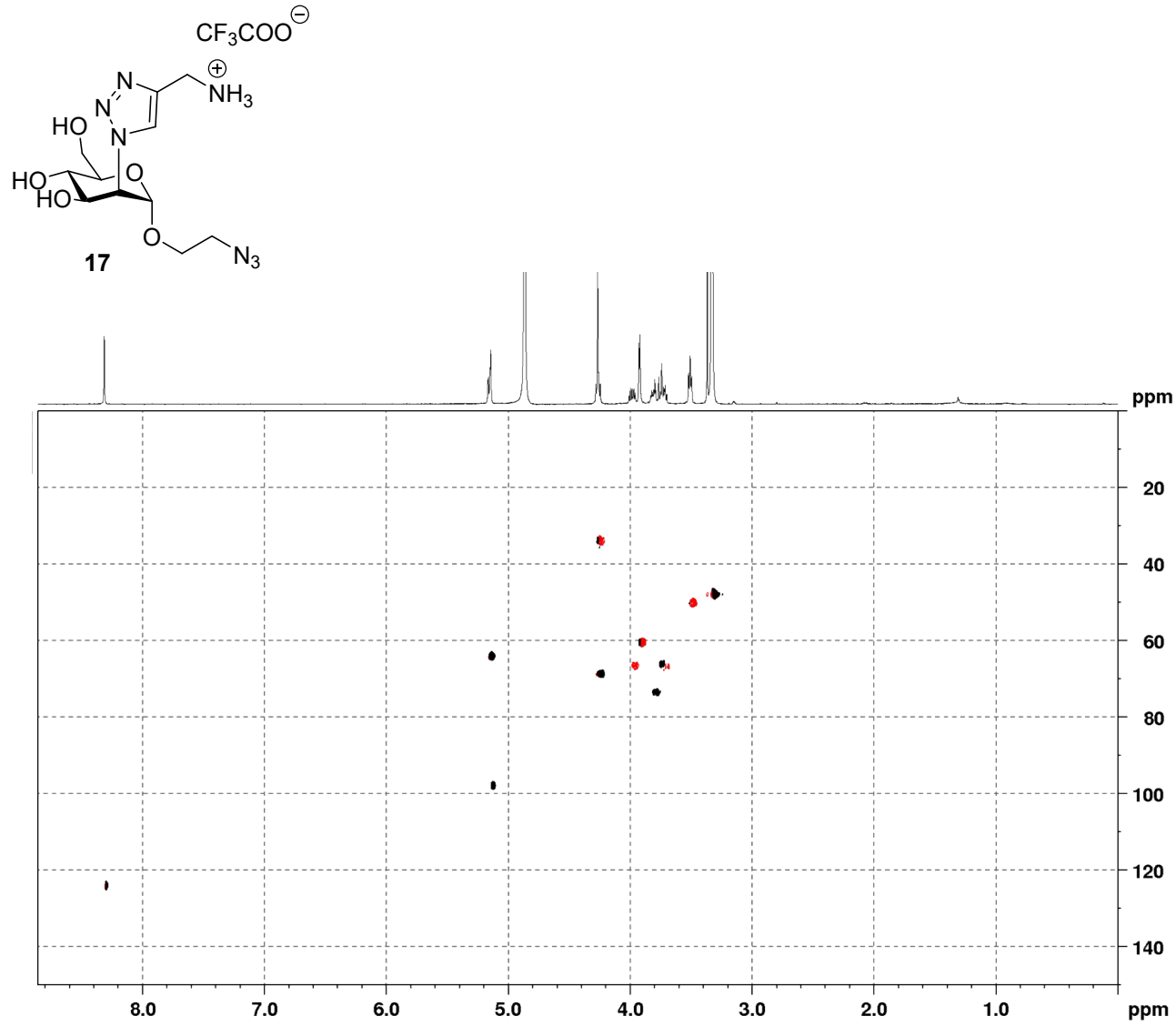

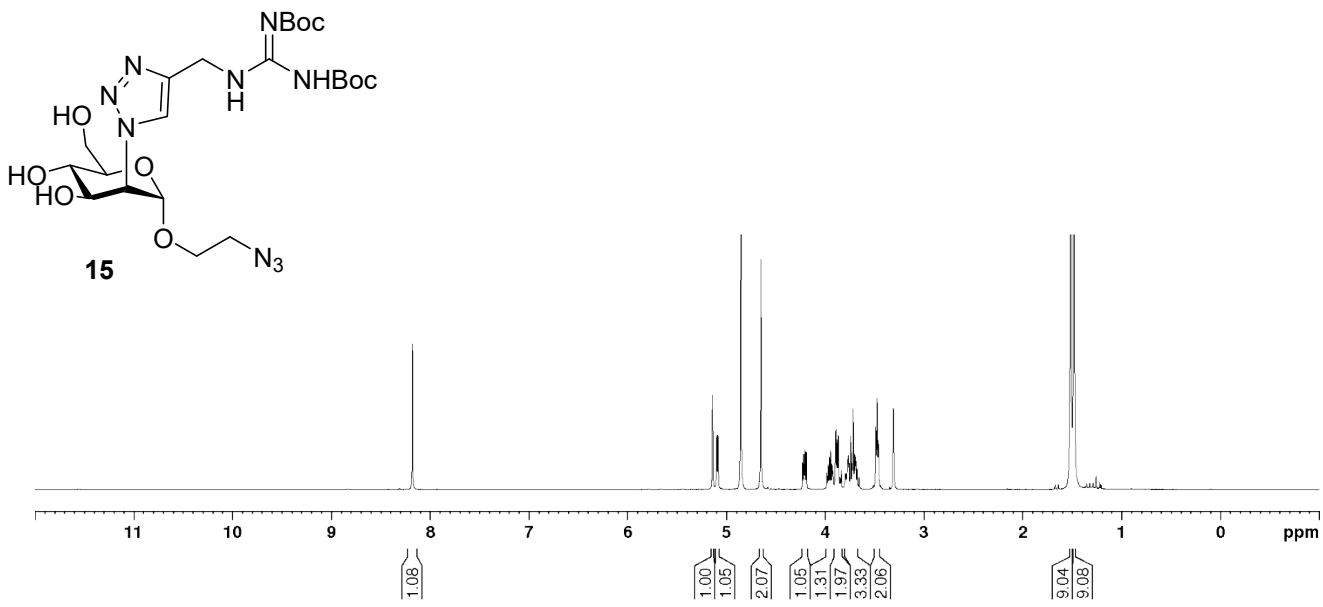

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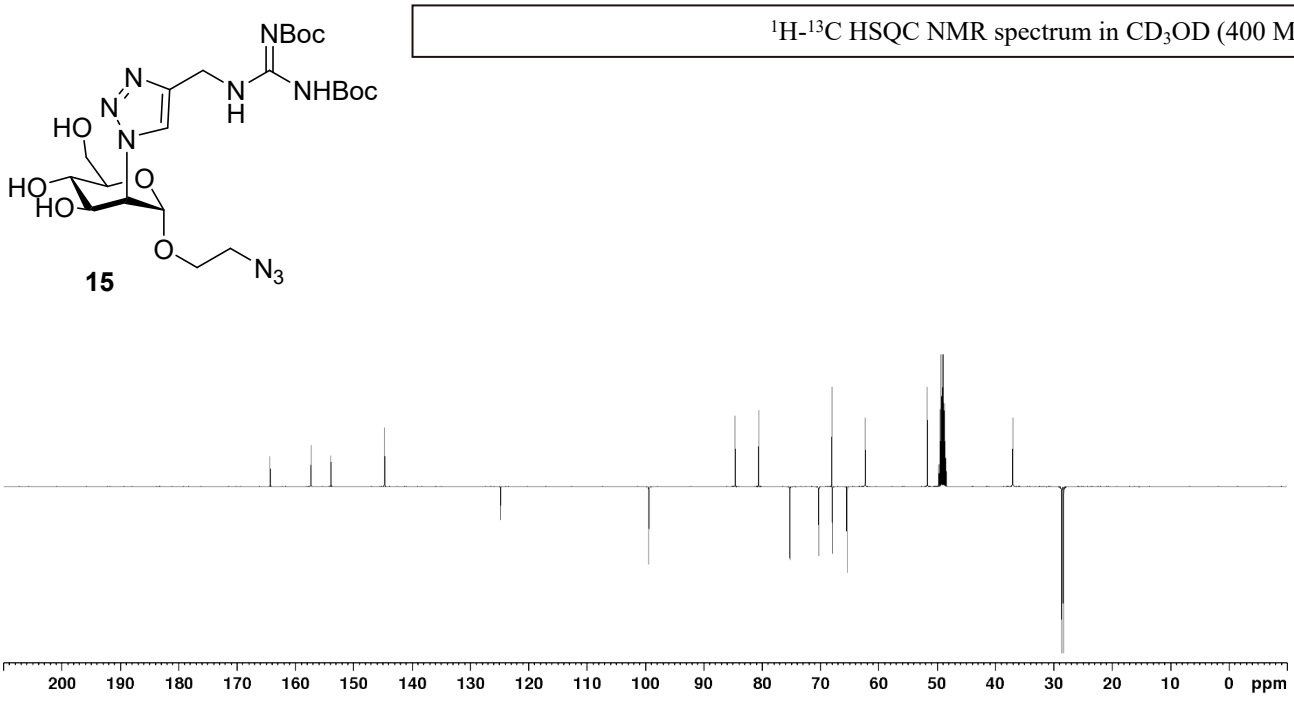

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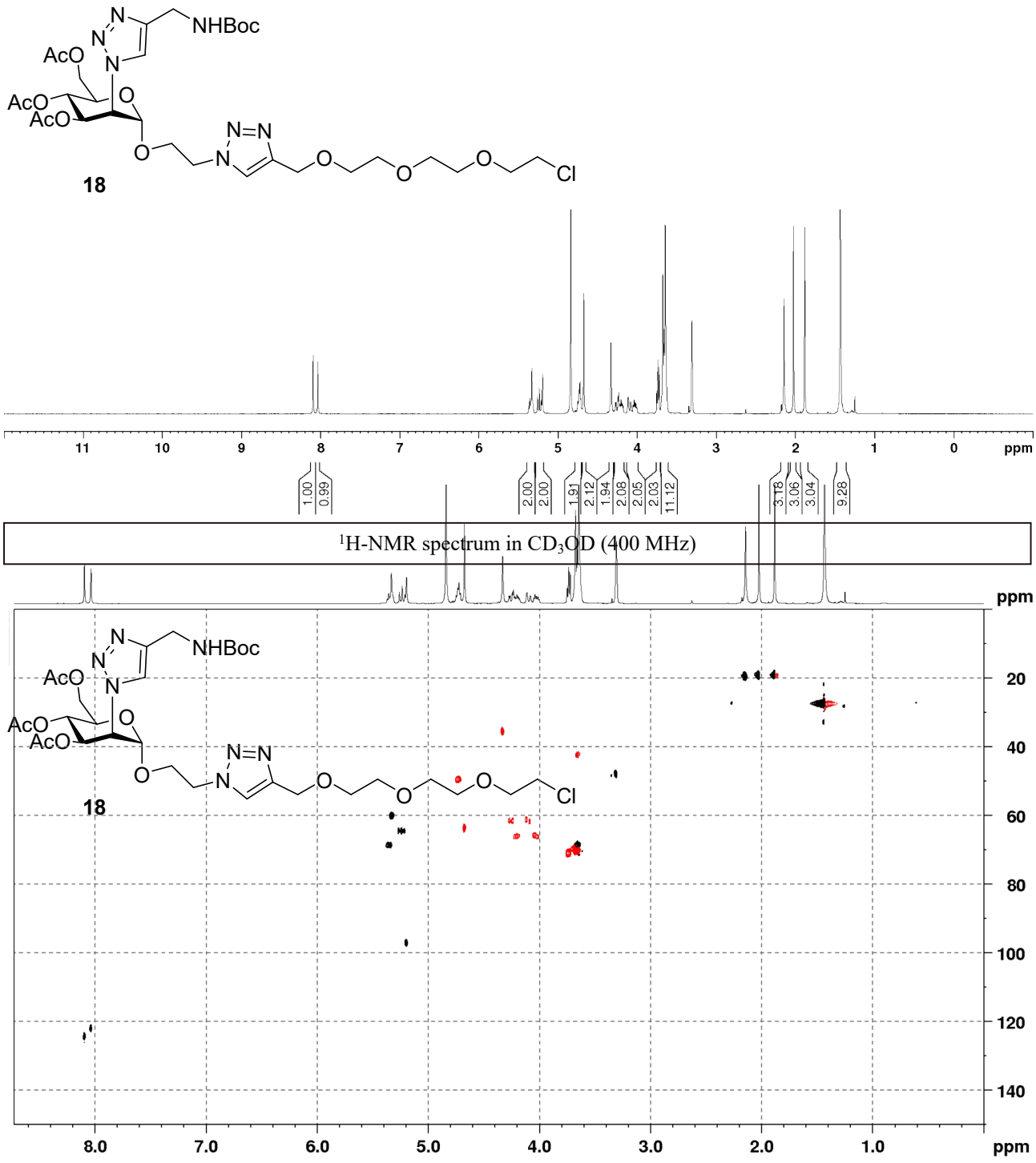

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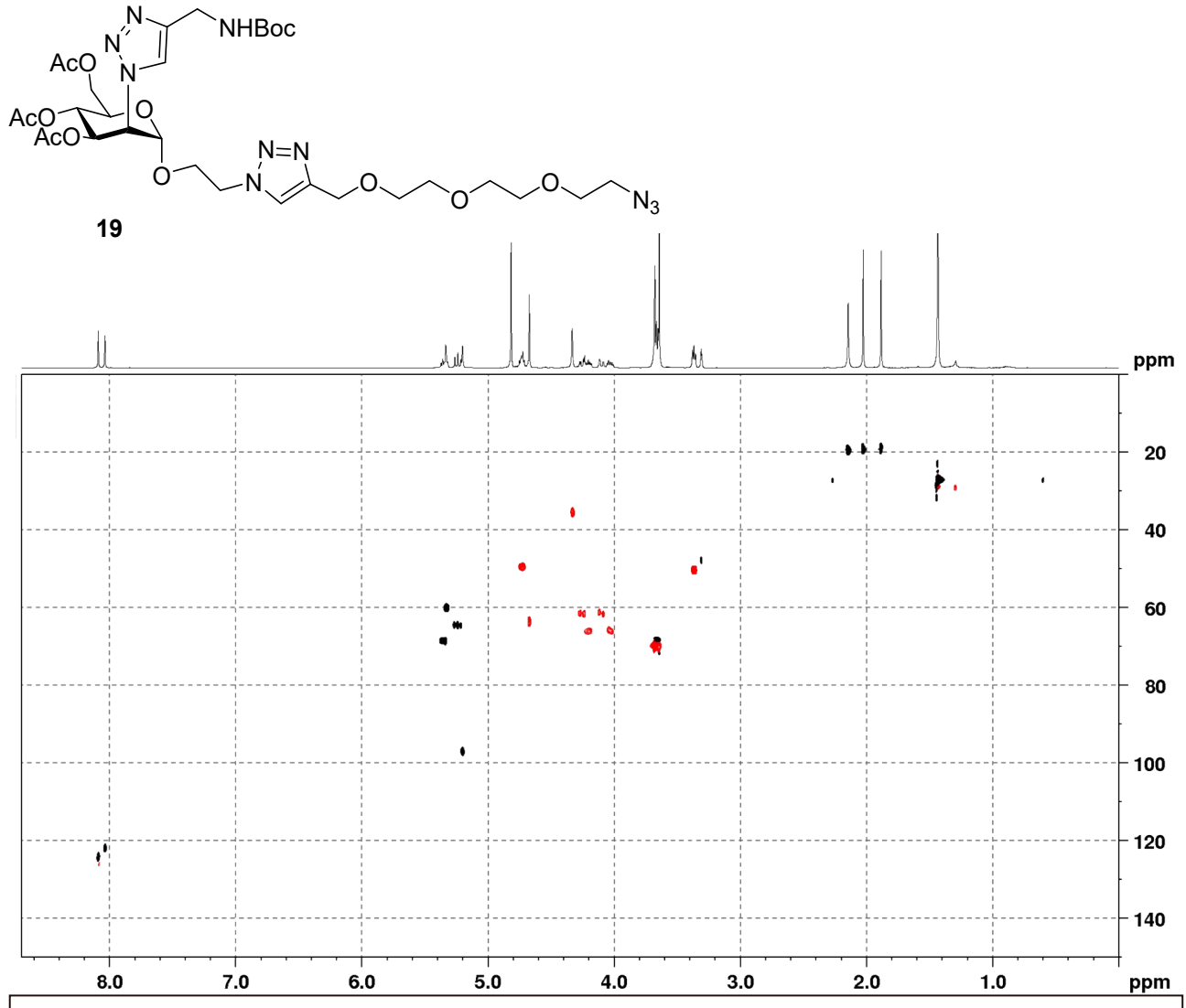

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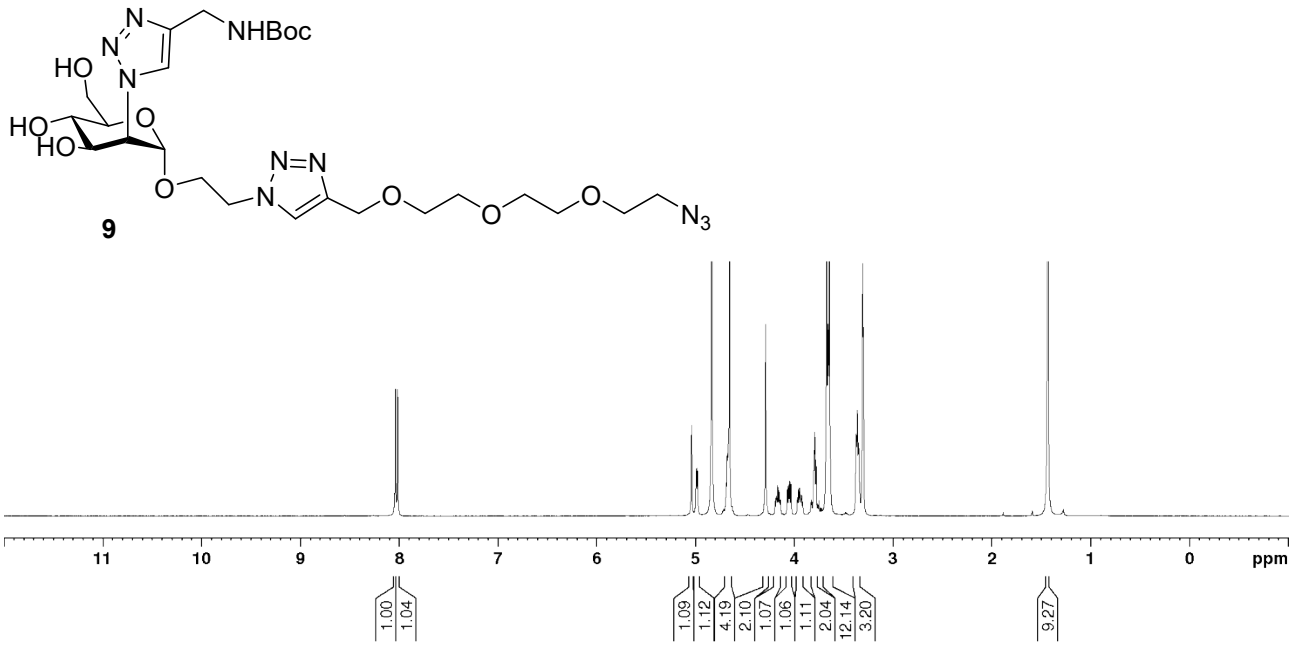

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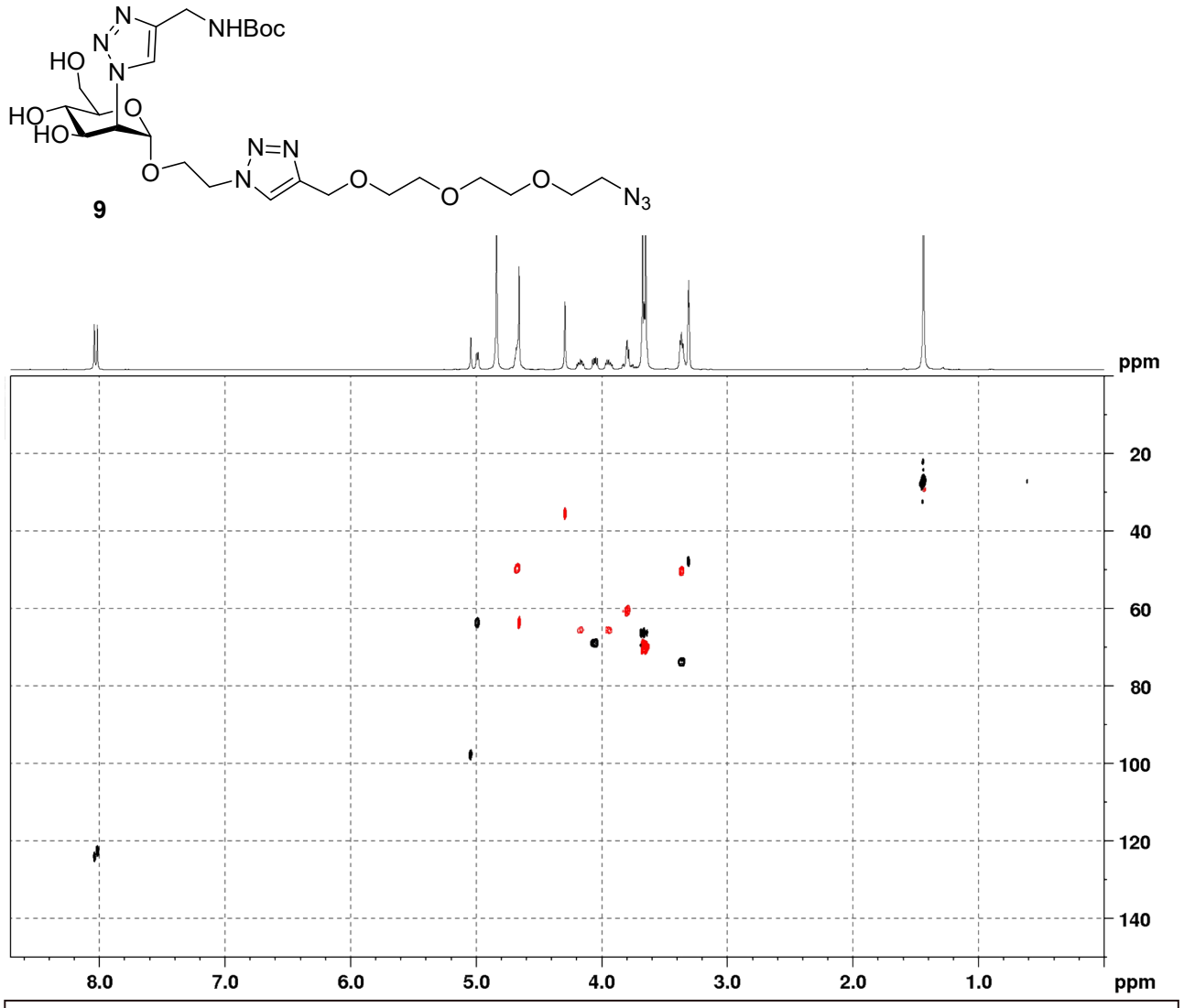

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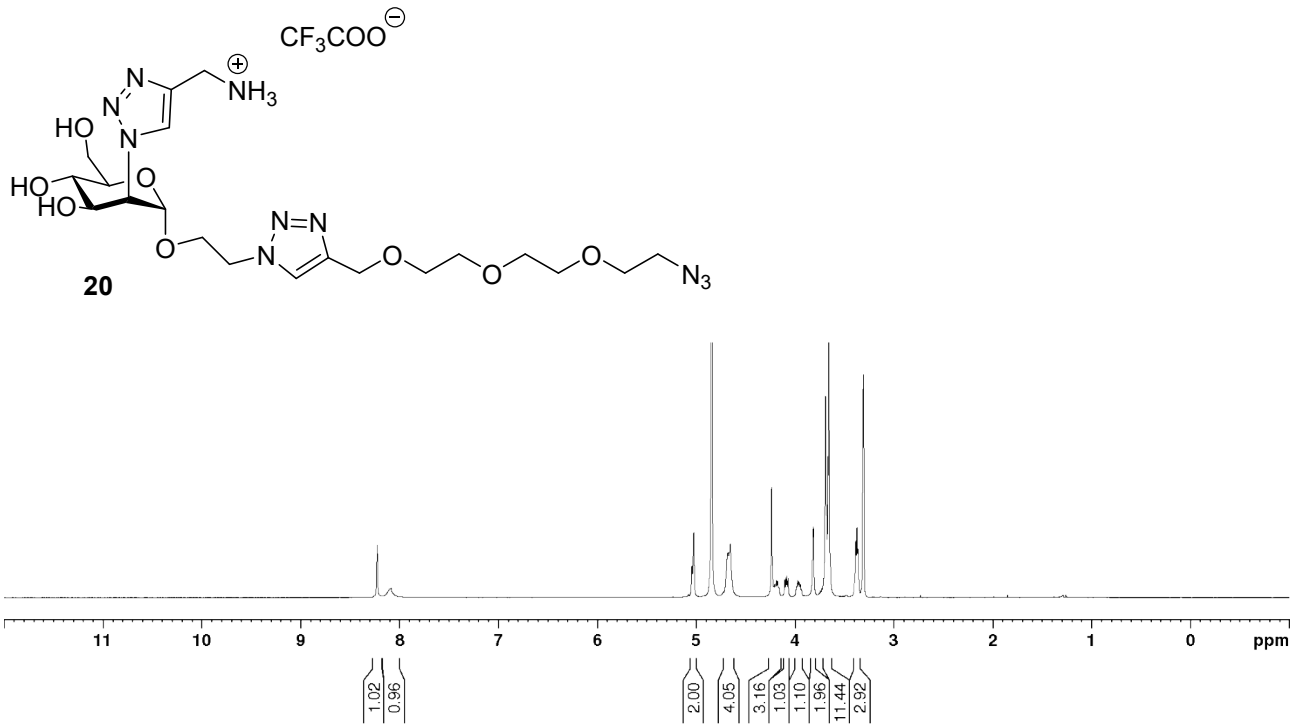

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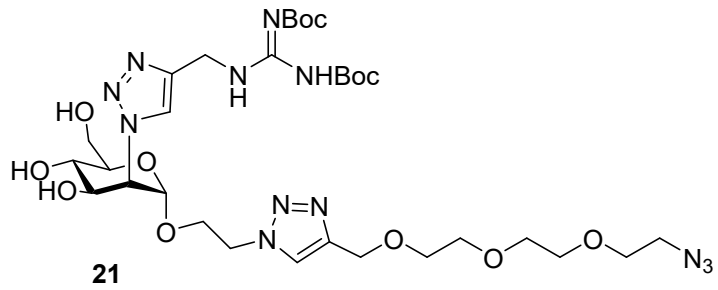

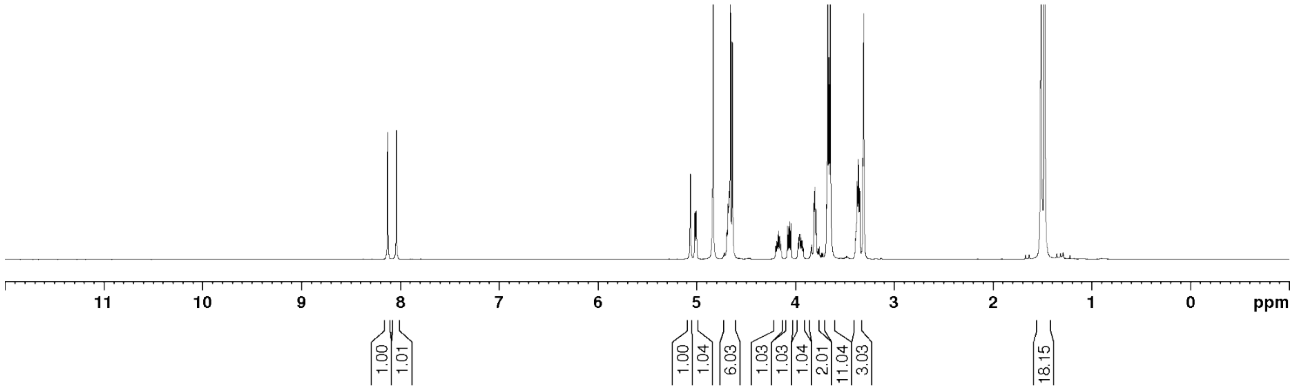

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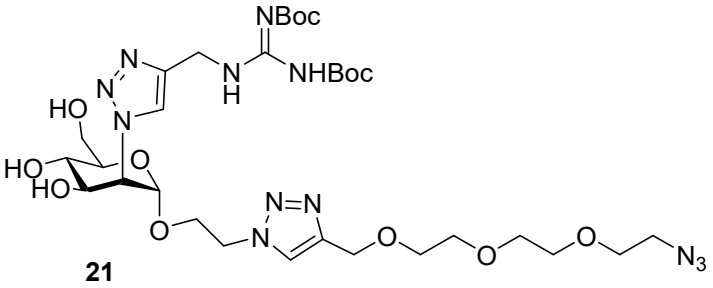

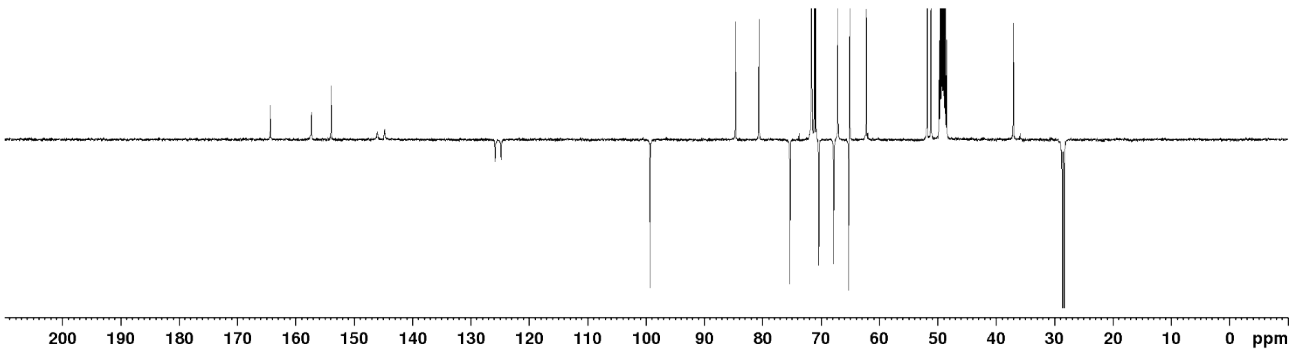

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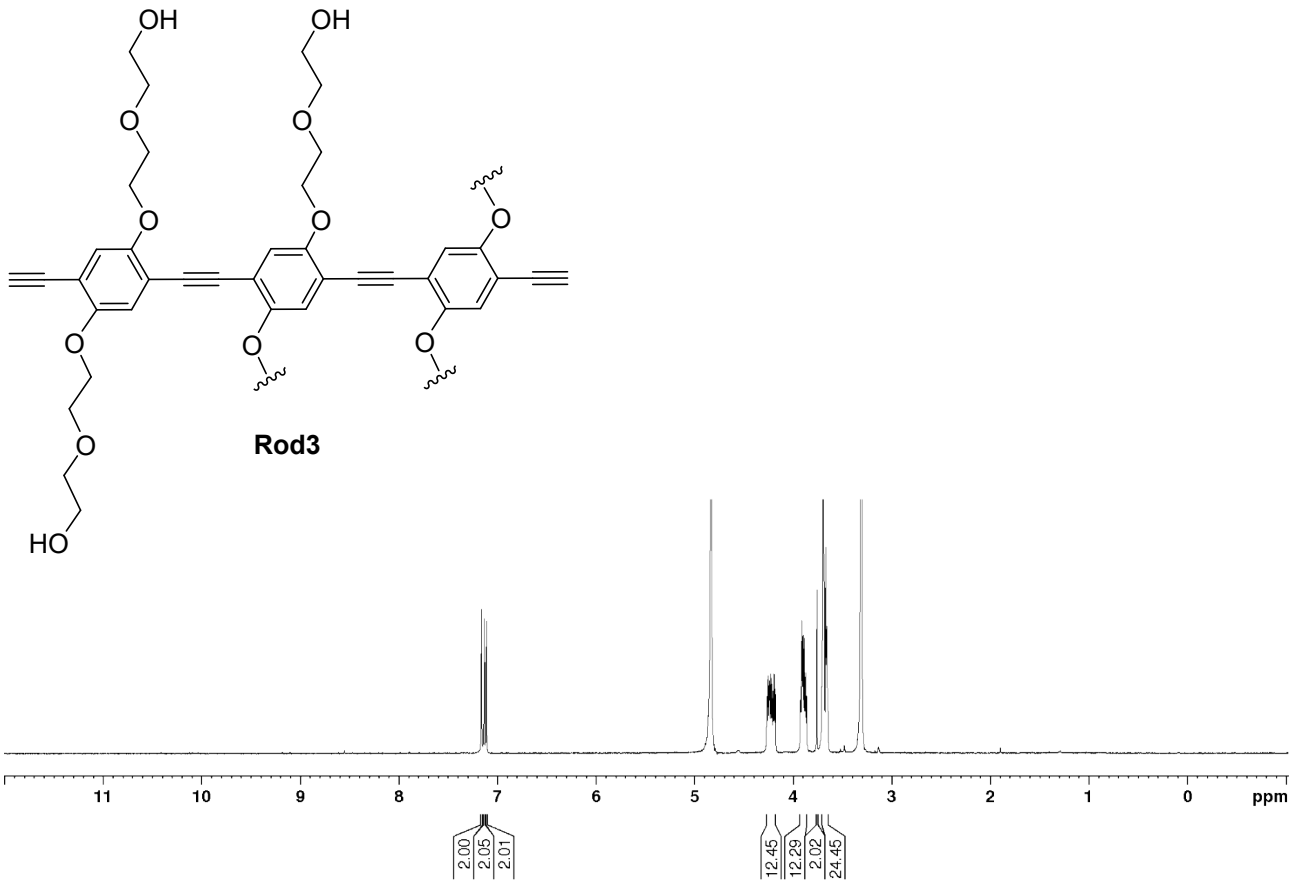

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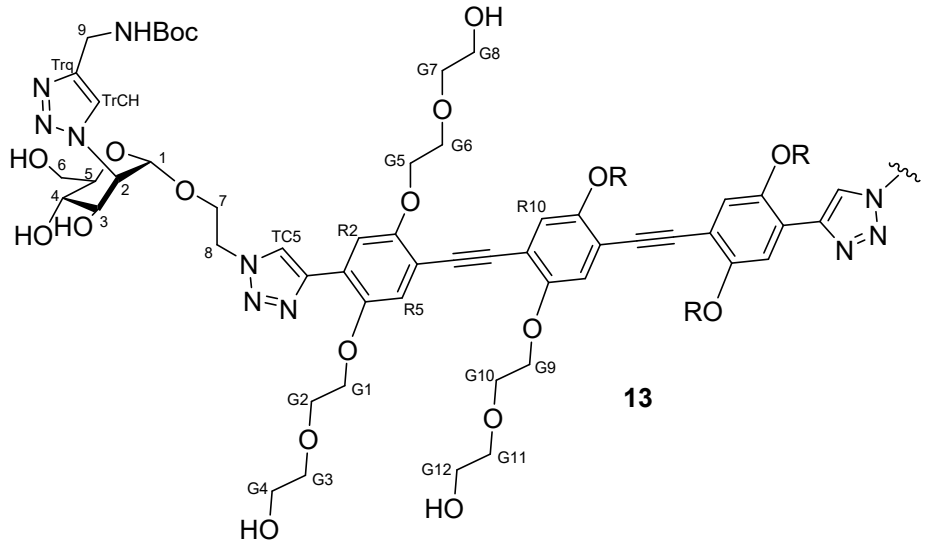

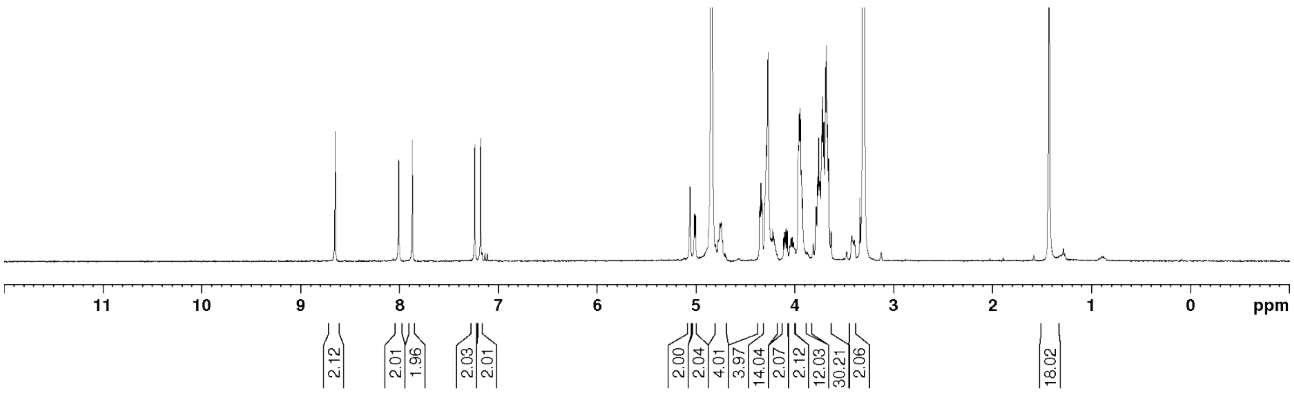

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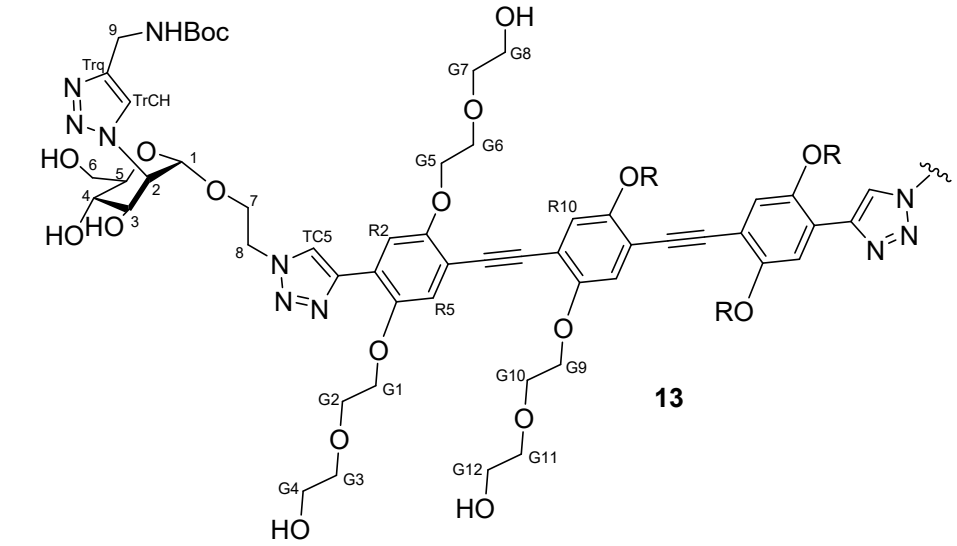

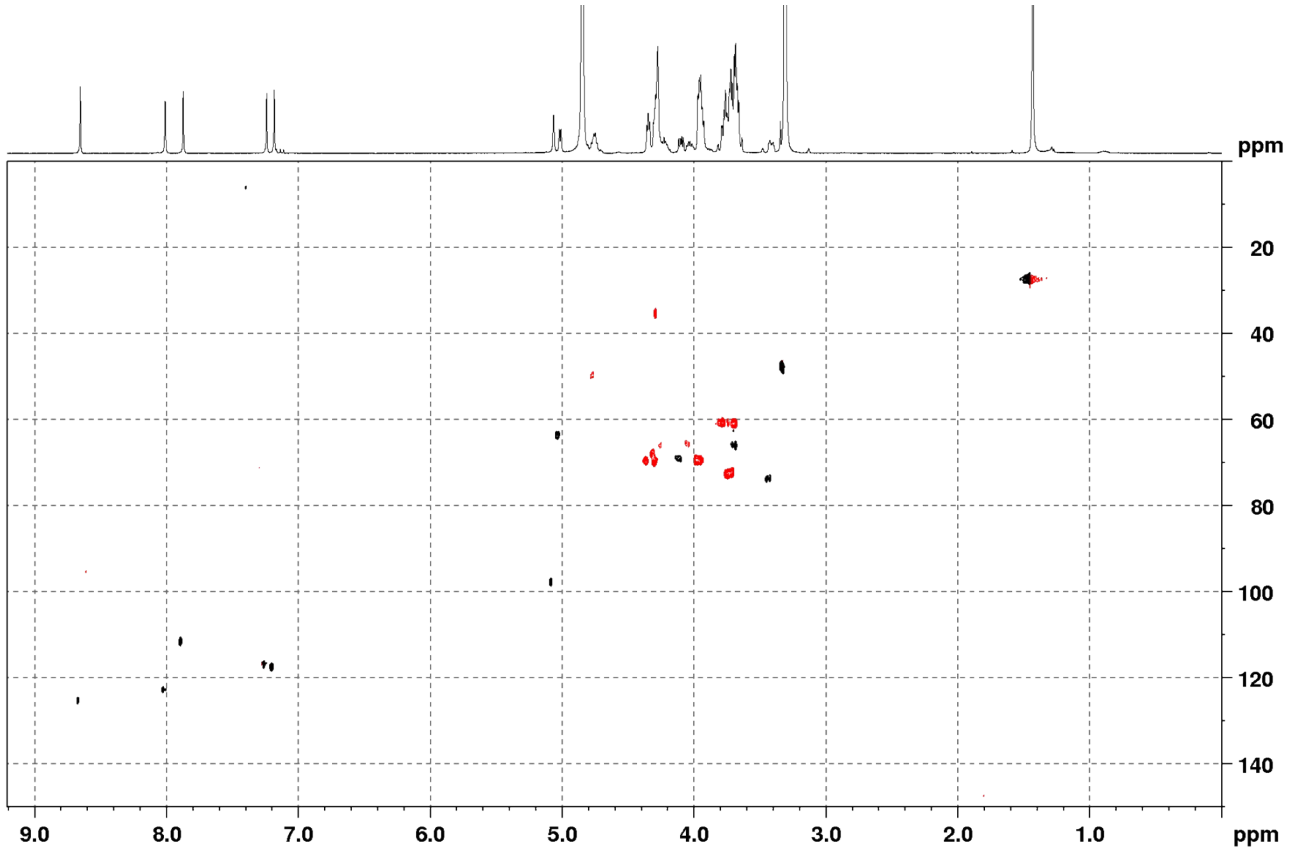

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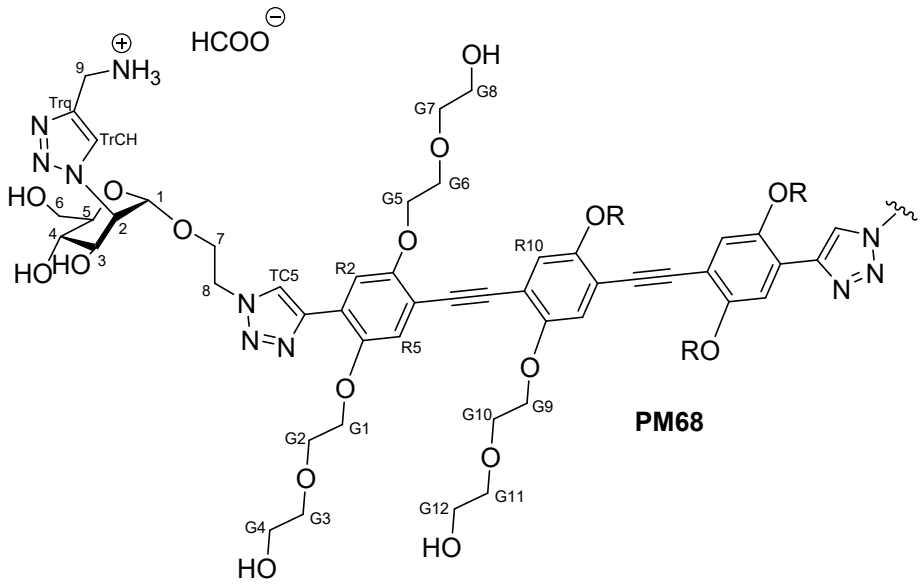
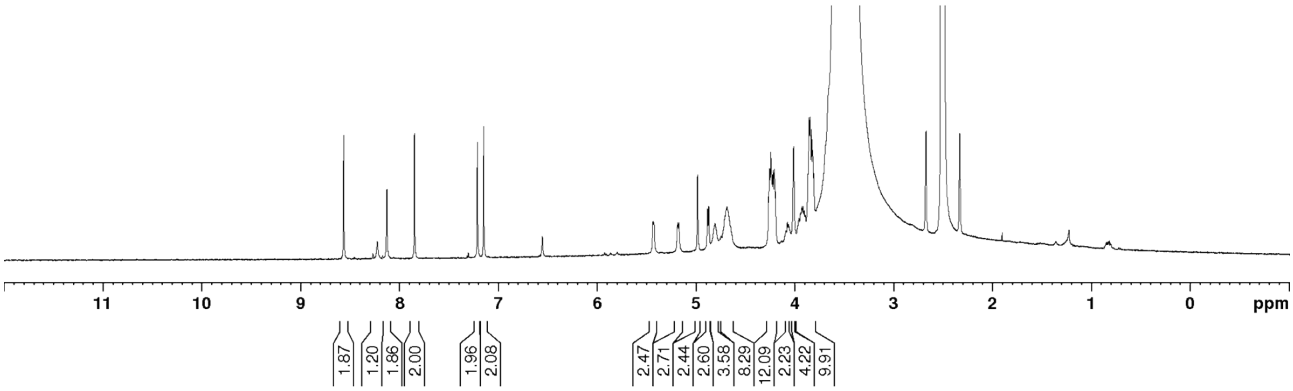
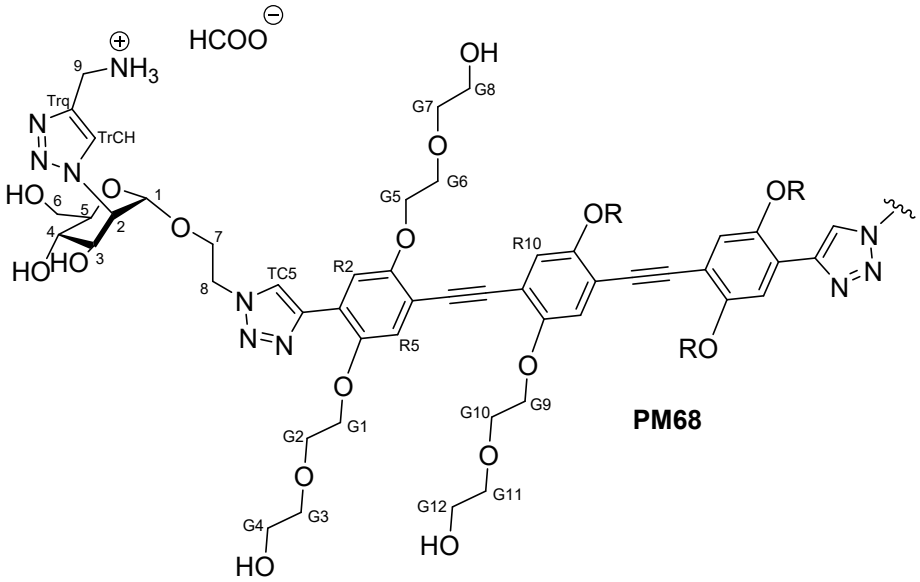




^{1}H-NMR spectrum in DMSO-d_6 (400 MHz)



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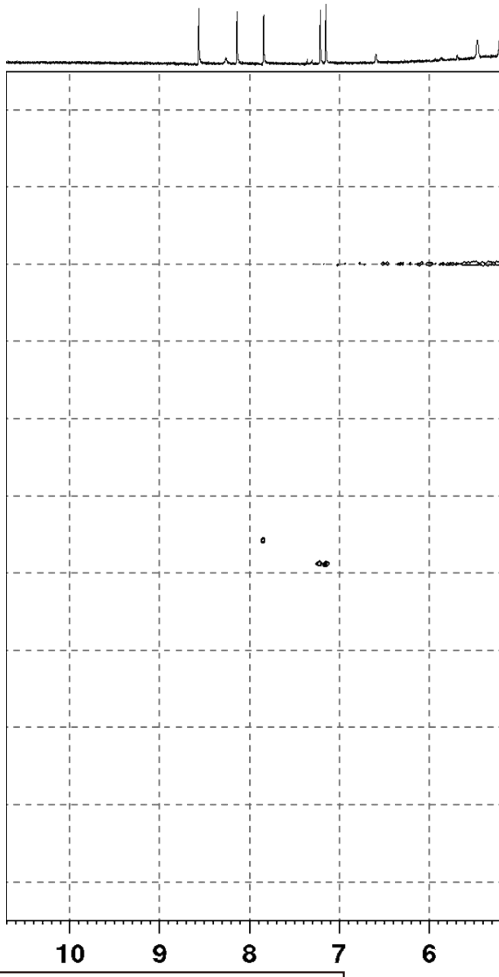

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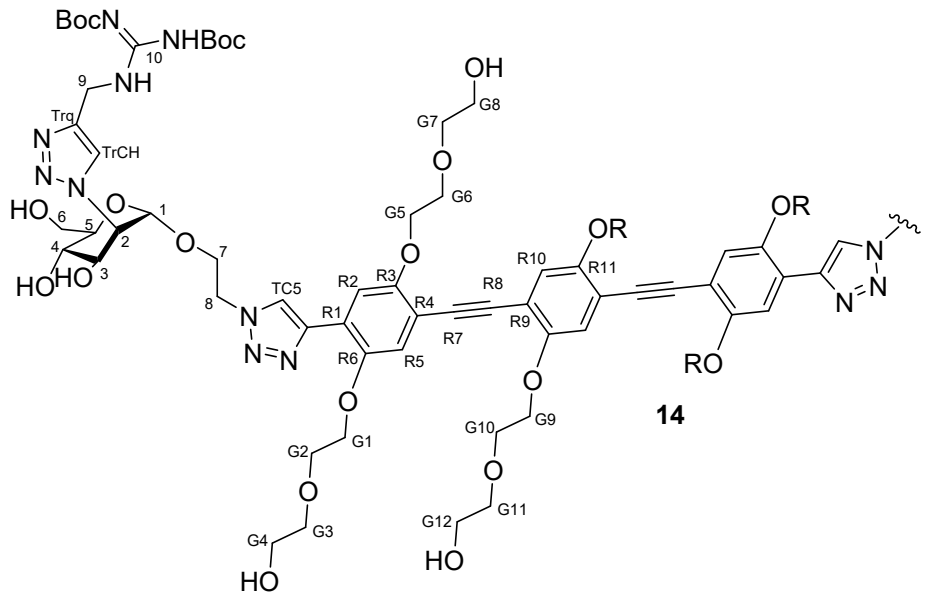

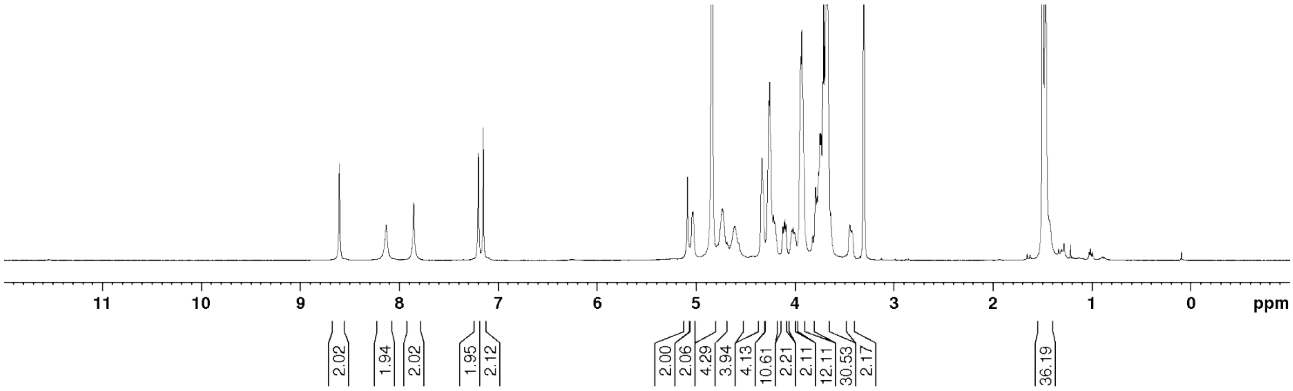

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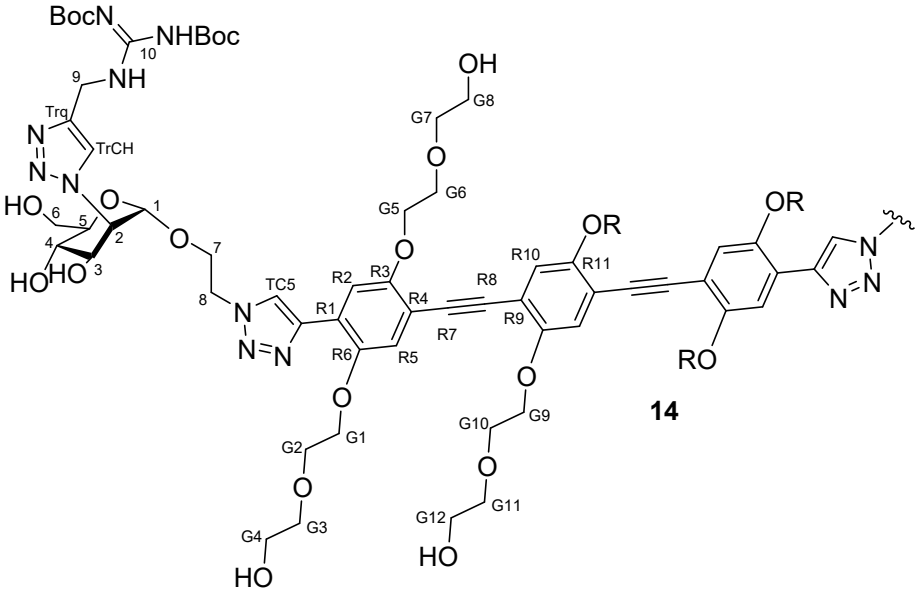

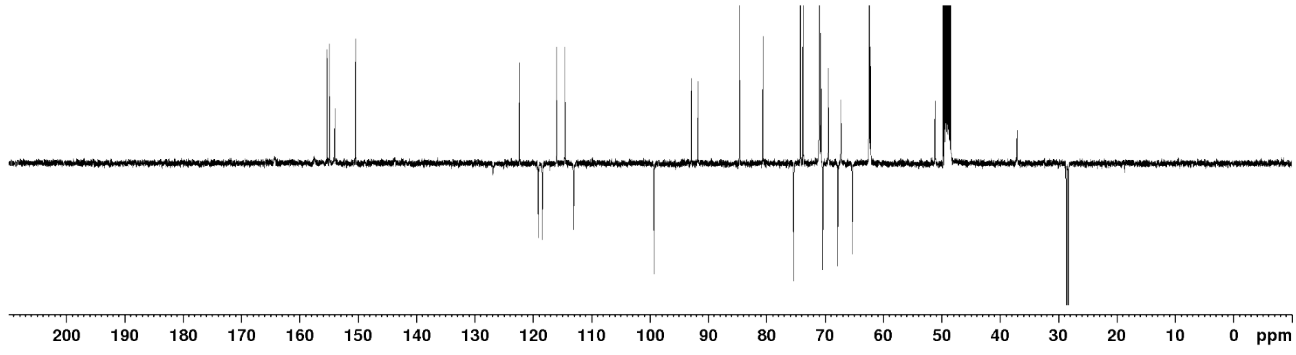

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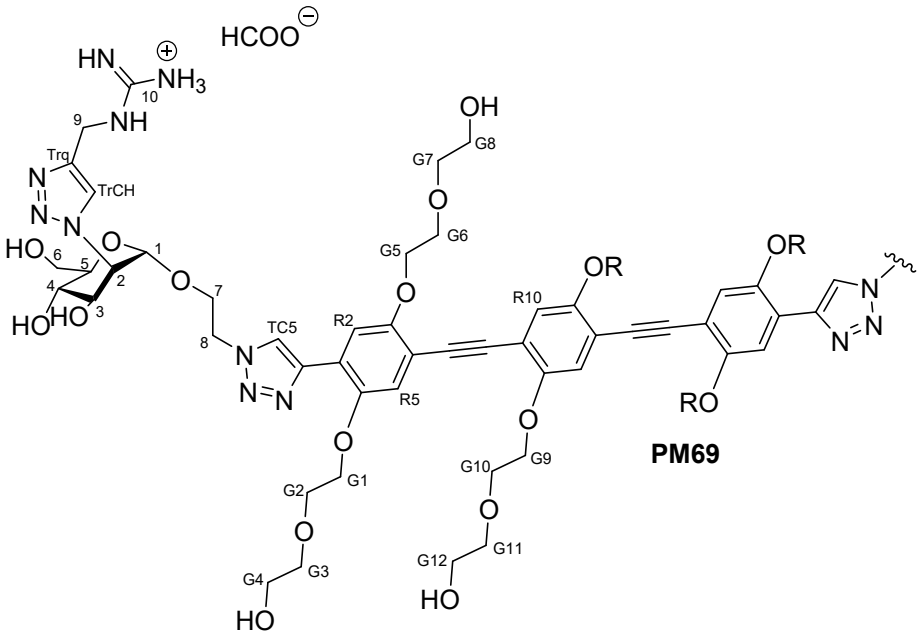
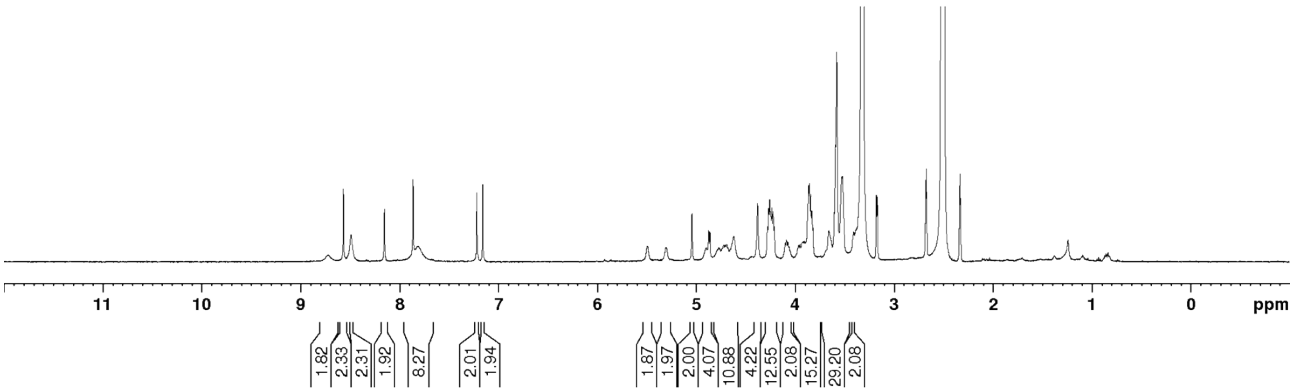
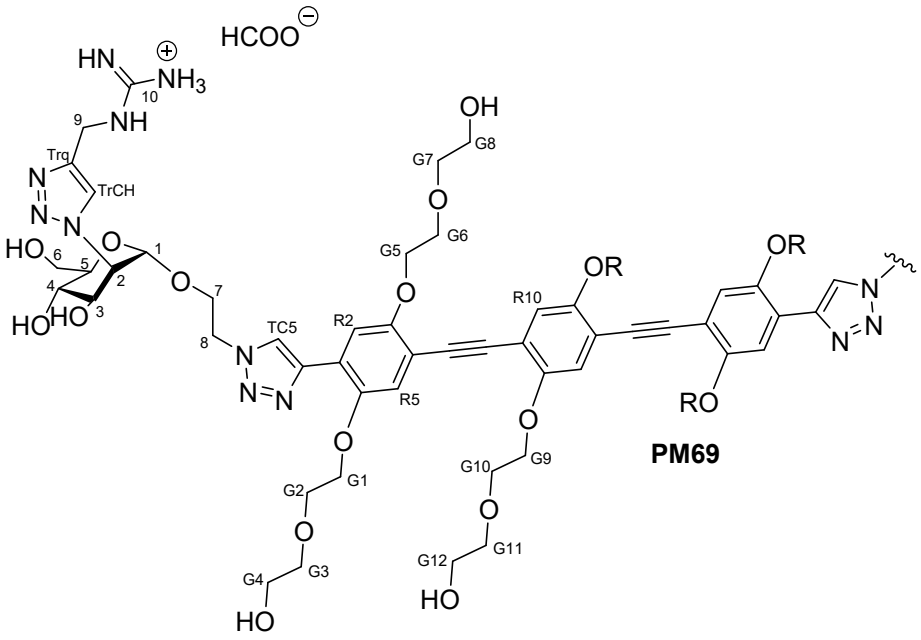
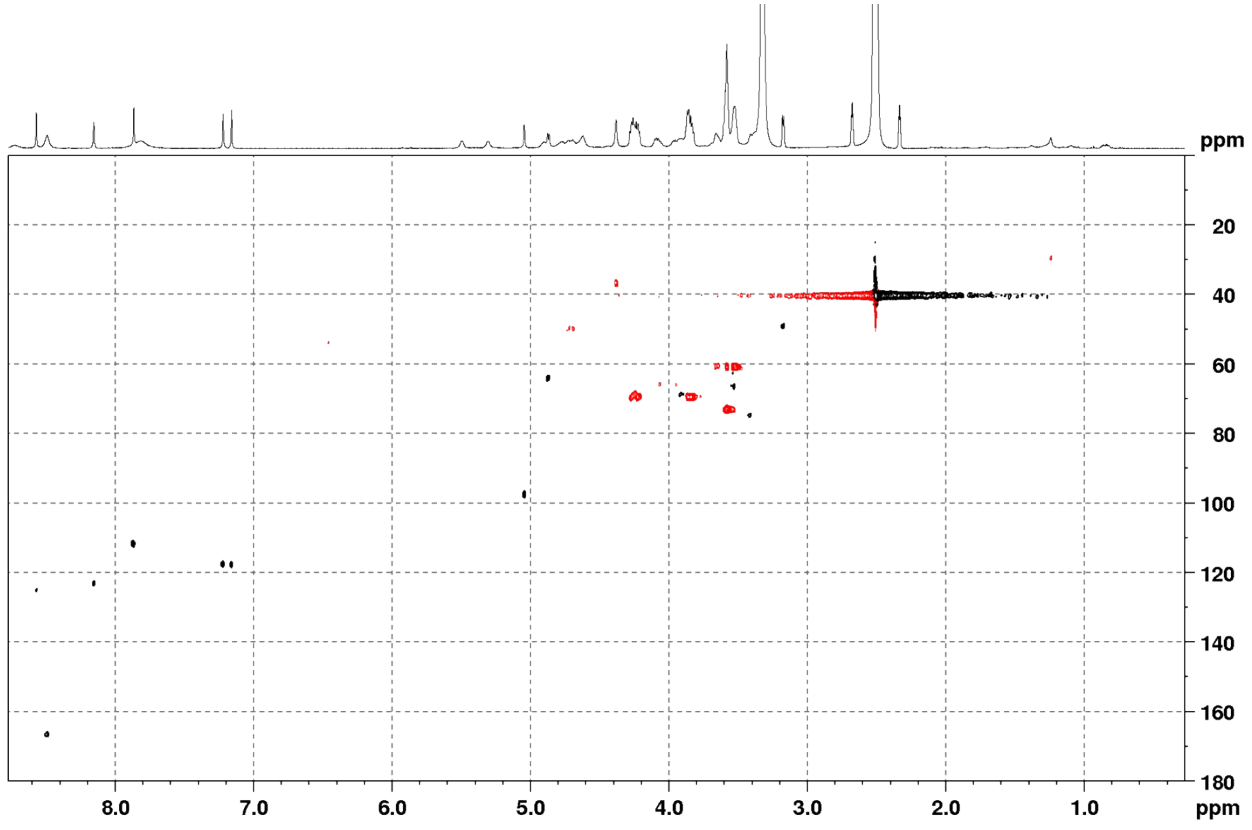


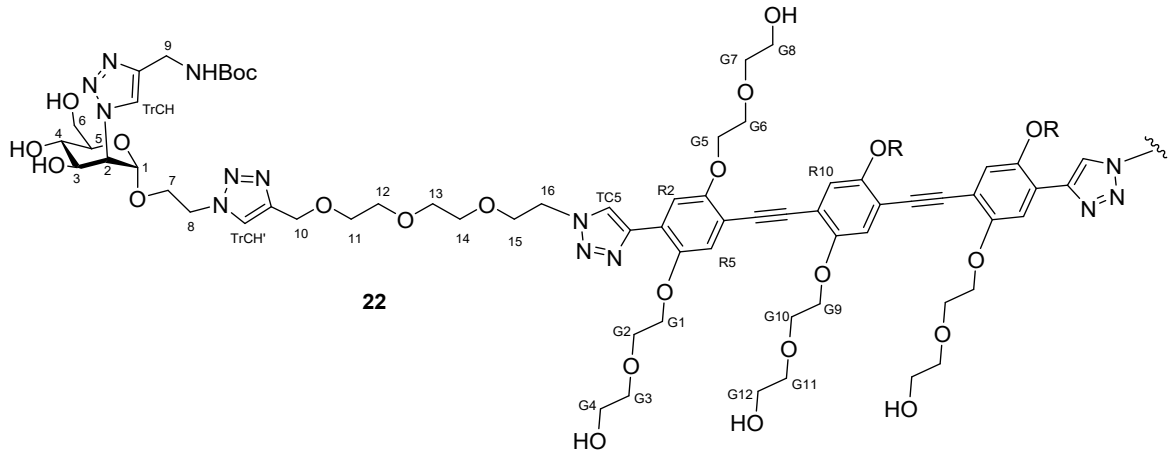


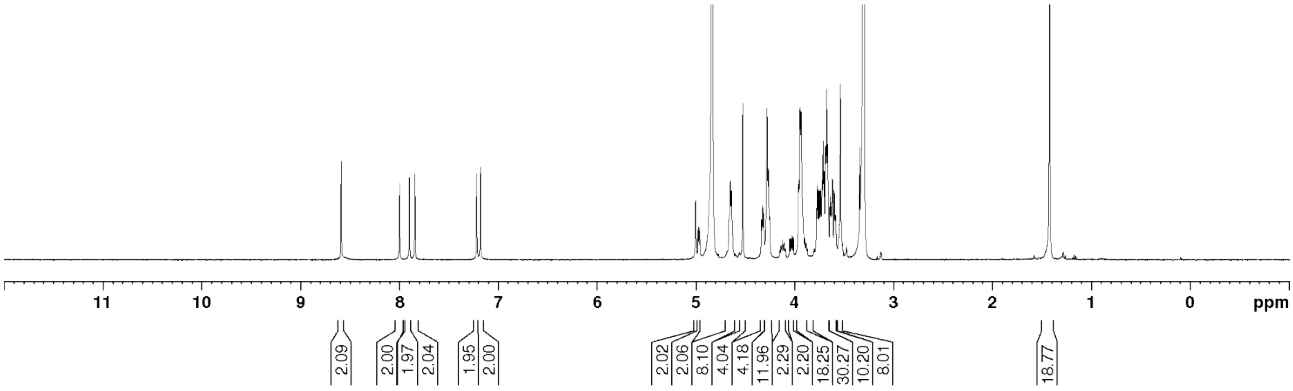
^{1}H-NMR spectrum in DMSO-d_6 (400 MHz)



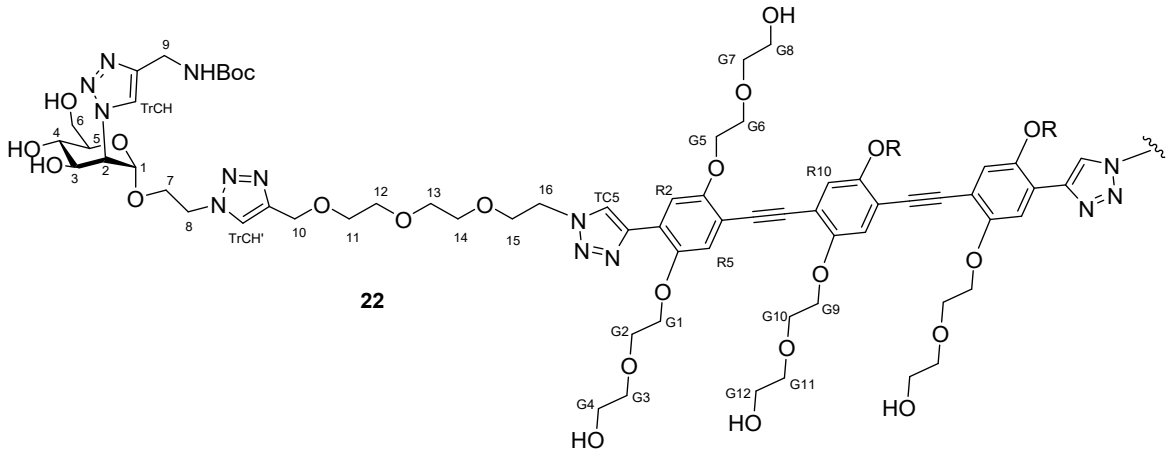


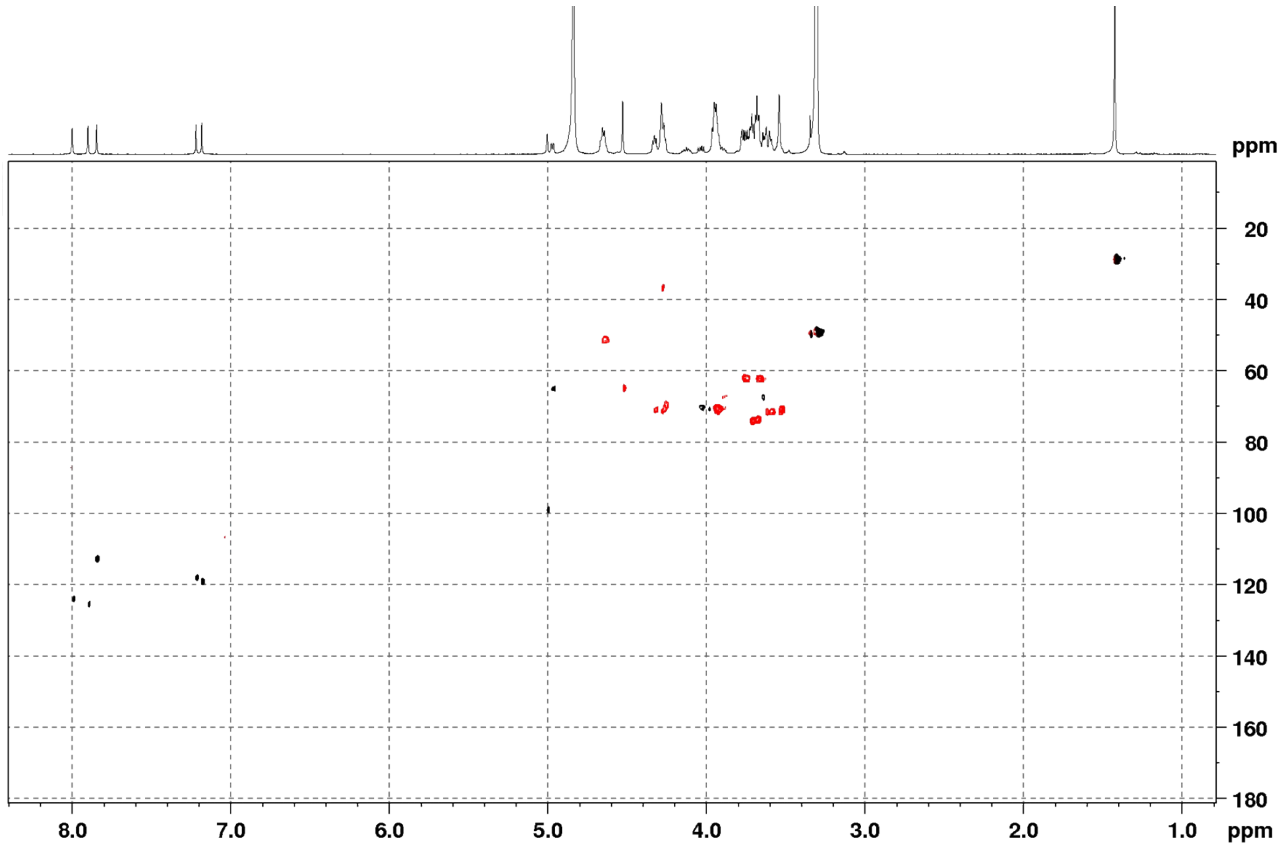
^{1}H-^{13}C HSQC NMR spectrum in DMSO-d_6 (400 MHz)



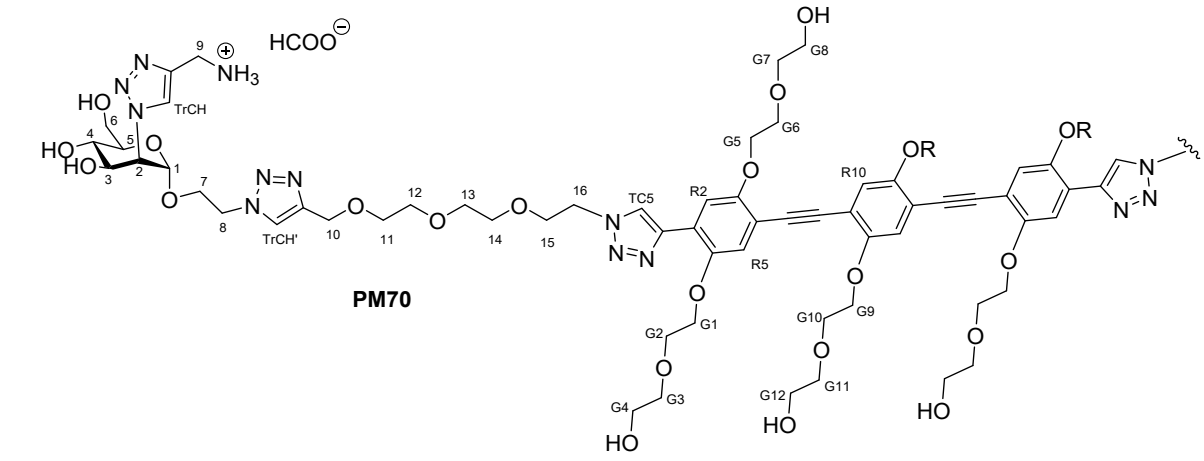


^{1}H-NMR spectrum in CD_3OD (400 MHz)

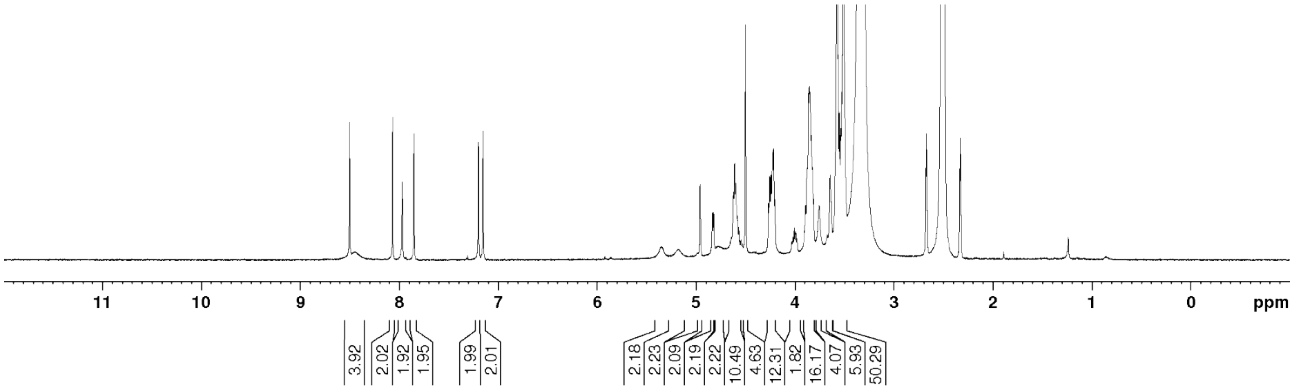




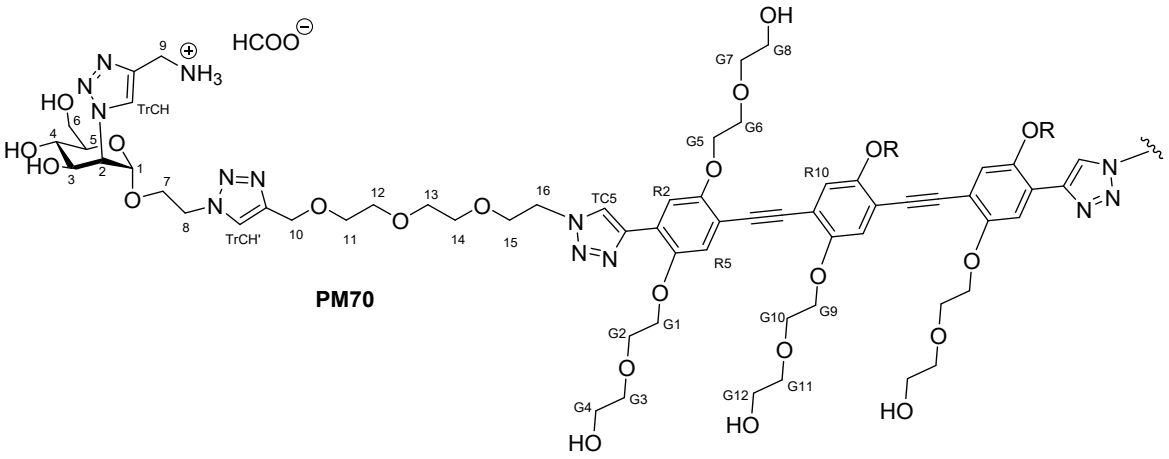
^{1}H-^{13}C HSQC NMR spectrum in CD_3OD (400 MHz)



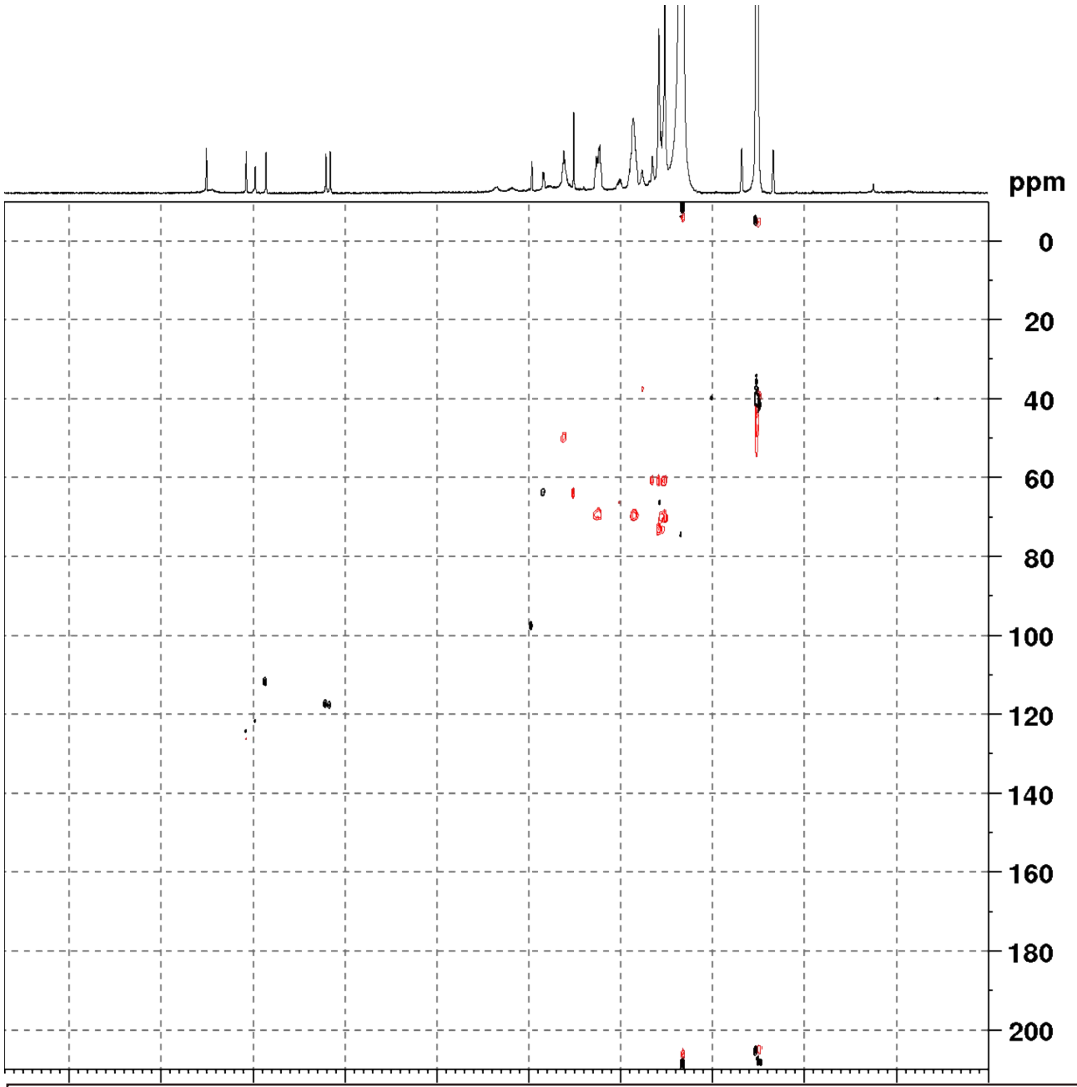
PM70



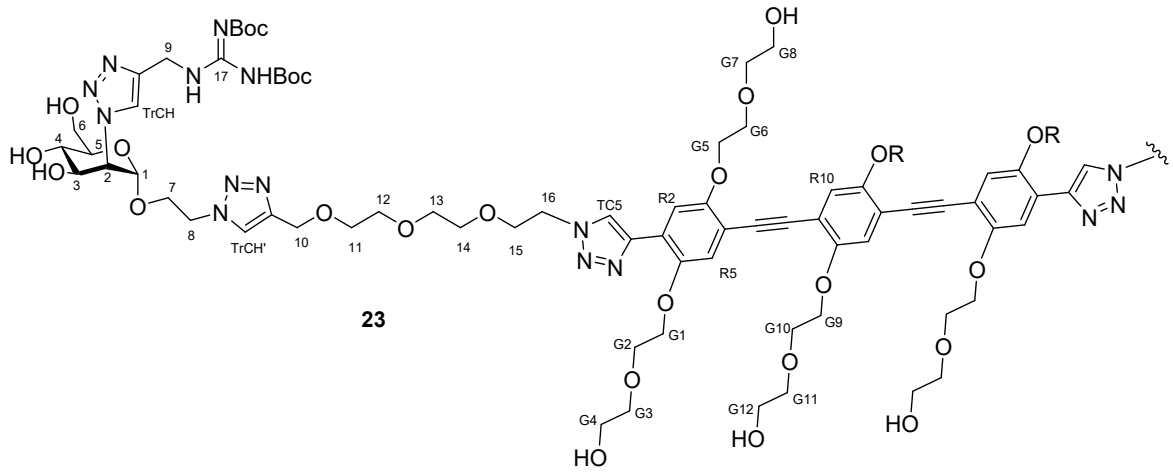
^{1}H-NMR spectrum in DMSO-d_6 (400 MHz)

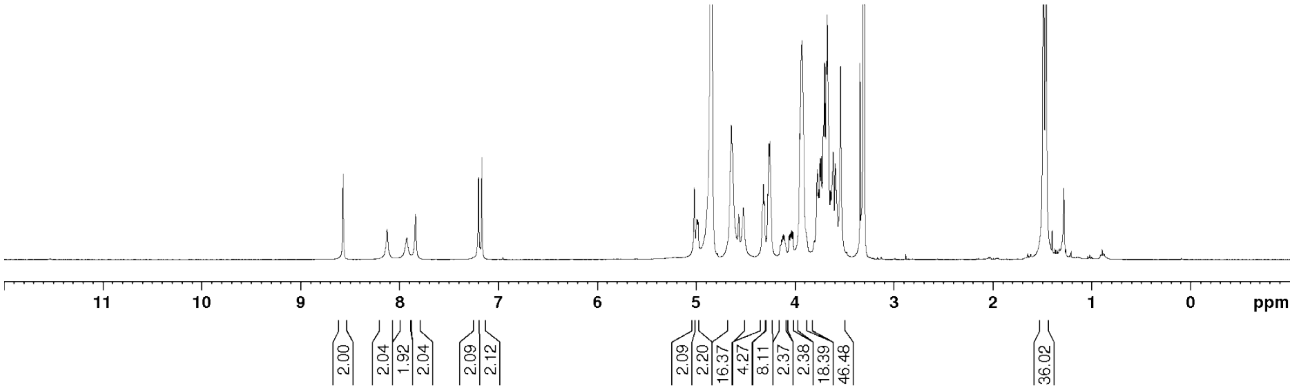


SP2
86

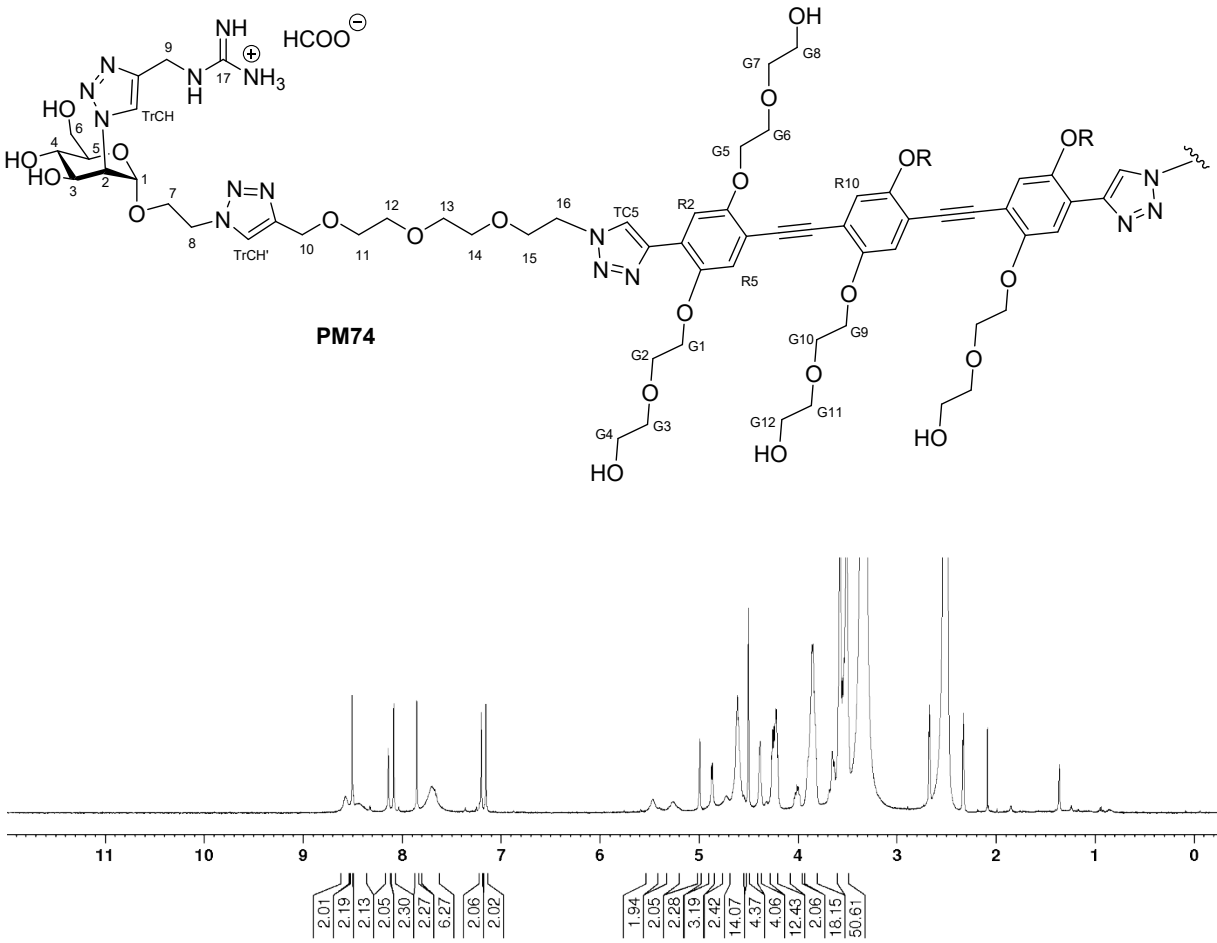


^{1}H-^{13}C HSQC NMR spectrum in DMSO-d_6 (400 MHz)

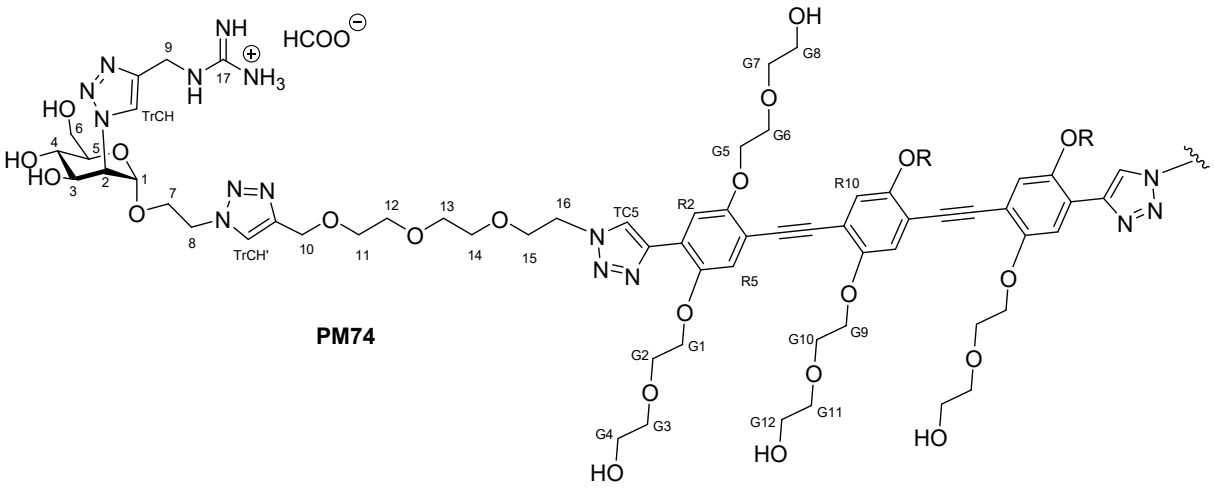




^{1}H-NMR spectrum in CD_3OD (400 MHz)

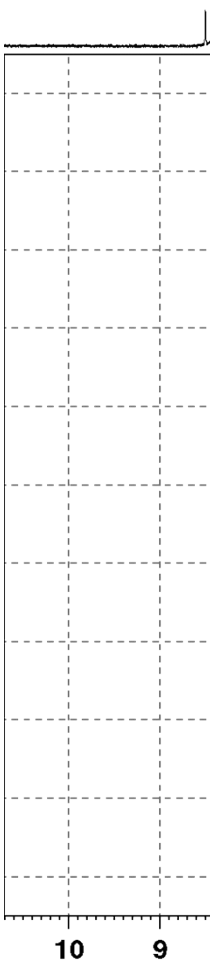


^{1}H-NMR spectrum in DMSO-d_6 (400 MHz)



PM74

SP3
07



^{1}H-^{13}C HSQC NMR spectrum in DMSO-d_6 (400 MHz)