

Polymeric mannosides prevent DC-SIGN-mediated cell-infection by cytomegalovirus

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General Methods

All reagents were purchased from Acros Organics, Alfa Aesar, Carbosynth or Aldrich and were used without further purification. Dichloromethane ethyl acetate and petroleum ether were distilled on a *Buchi rotavapor R-220-SE*. Acetonitril, pyridine, benzene and DMF were freshly distilled from calcium hydride under argon. THF was distilled on sodium, benzophenone and under argon. Reactions requiring anhydrous conditions were performed under argon. Column chromatography was conducted on silica gel Kieselgel SI60 (40–63 μm) from Merck, or on Silica cartridge from Interchim and eluted via a Puriflash 430 with an UV and ELSD detection. Thin layer chromatography (TLC): *Merck Silica gel 60 F254* analytical plates, detection either with UV (254 nm) or dipping in a solution of cerium molybdate, potassium permanganate, ninhydrine and subsequent heating. Microwave experiments were conducted in sealed vials in commercial microwave reactors especially designed for synthetic chemistry (MultiSYNTH, Milestone). The instrument features a special shaking system that ensures high homogeneity of the reaction mixtures. ^1H and ^{13}C NMR spectra were recorded on *Bruker Avance 300* spectrometer fitted with a 5 mm i.d. BBO probe carefully tuned to the recording frequency of 300.13 MHz (for ^1H) and 75.47 MHz (for ^{13}C), the temperature of the probe was set at room temperature (around 293–294 K), on a *Bruker Avance 400* spectrometer fitted with a 5 mm i.d. BBFO+ probe carefully tuned to the recording frequency of 400.13 MHz (for ^1H) and 100.61 MHz (for ^{13}C), the temperature of the probe was set at 303 K, on a *Bruker Avance III 500* spectrometer fitted with a 5mm i.d. $^{13}\text{C}/^1\text{H}$ cryoprobe carefully tuned to the recording frequency of 500.13 MHz (for ^1H) and 125.76 MHz (for ^{13}C), the temperature of the probe was set at 303 K. The spectra are referenced to the solvent in which they were run (7.26 ppm for ^1H CDCl_3 and 77.16 ppm for ^{13}C CDCl_3 , 2.50 ppm for ^1H $\text{DMSO}-d_6$ and 39.52 ppm for ^{13}C $\text{DMSO}-d_6$, 3.31 ppm for ^1H CD_3OD and 49.00 ppm for ^{13}C , 7.16 ppm for ^1H C_6D_6 and 128.06 ppm for ^{13}C C_6D_6 , 5.32ppm for ^1H CD_2Cl_2 and 53.84 ppm for ^{13}C , 4.79 ppm for ^1H D_2O). Chemical shifts (δ) are given in parts per million (ppm) and coupling constants (J) are given in Hz. Multiplicity of signals is indicated as following: s (singlet), d (doublet), t (triplet), q (quadruplet), m (multiplet), brs (broad singlet), dd (doublet of doublet), dt (doublet of triplet)... The numbering used for NMR attribution is different from UPAC numbering, and written on each molecule.

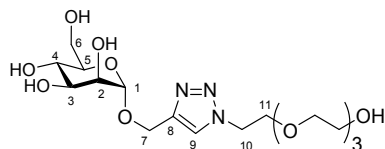
Copper Catalyzed Azide-Alkyne Cycloaddition Method 1.

The azide derivative and the alkyne derivative (1.1 eq./azide function) were dissolved in dioxane (2mL/mmol). A solution of copper sulfate (0.2 eq./azide function) and sodium ascorbate (0.4 eq./azide function) in water (0.5mL/mmol) was added and the mixture was heated at 60°C until completion. The mixture was dissolved in DCM (50mL/mmol), washed with a solution of EDTA (50 mL/mmol) and the aqueous layer was extracted twice with DCM (2 x 50mL/mmol). The organic layer was then dried over MgSO_4 , filtered, concentrated under reduced pressure and purified.

Deprotection of acetyl groups using sodium methanolate method 2.

The protected carbohydrate (1eq.) was dissolved in MeOH/H₂O (1:1, 1 mL/mmol). Amberlite resin IRN 78 OH- 1.25 meq/mL (150 mg/mmol) was added, and the mixture was stirred overnight at rt. The resin was filtered off and washed with methanol and water. The solvent was evaporated under reduced pressure.

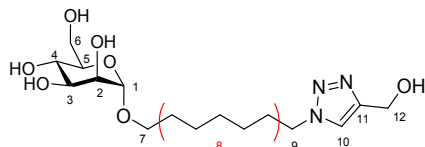
Compound 1



Obtained following the acetate deprotection method 2 to afford **1** (89% yield) as a colorless gum.

$[\alpha]_D^{20} = +29$ (C = 1, MeOH); ¹H NMR (400 MHz, MeOD): δ = 8.07 (1H, s, H-9), 4.85 (1H, d, J = 1.9Hz, H-1), 4.80 (1H, d, J = 12.7Hz, H-7a), 4.65 (1H, d, J = 12.7Hz, H-7b), 4.59 (1H, t, J = 5.2Hz, H-10), 3.90 (2H, t, J = 5.2Hz, H-11), 3.85 (1H, dd, J = 1.9Hz, J = 12.4Hz, H-6a), 3.79 (1H, dd, J = 1.9Hz, J = 3.7Hz, H-2), 3.74-3.83 (16H, m, H-3, H-4, H-5, H-6b, 12xCH₂O); ¹³C NMR (100 MHz, MeOD): δ = 145.1 (C-8), 126.2 (C-9), 100.8 (C-1), 75.0 (C-5), 73.7 (C-3), 72.5 (C-2), 71.5-71.4 (CH₂O), 70.3 (C-10), 68.6 (C-4), 63.0 (C-6), 62.2 (C-7), 51.5 (C-7); HRMS (MALDI) m/z calcd for C₁₇H₃₁N₃O₁₀Na: 460.1907, found 460.1903.

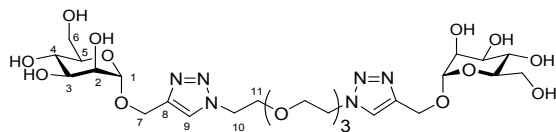
Compound 2



Obtained following the acetate deprotection method 2 to afford **2** (83% yield) as a colorless gum.

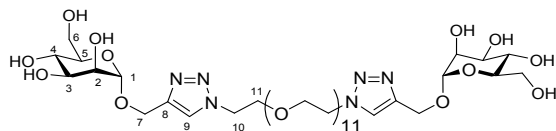
$[\alpha]_D^{20} = +36$ (C = 0.8, MeOH); ¹H NMR (400 MHz, MeOD): δ = 7.90 (1H, s, H-10), 4.72 (1H, d, J = 1.6Hz, H-1), 4.67 (2H, s, H-12), 4.40 (2H, t, J = 7.1Hz, H-9), 3.82 (1H, dd, J = 2.4Hz, J = 11.8Hz, H-6a), 3.77 (1H, dd, J = 1.7Hz, J = 3.3Hz, H-2), 3.74-3.65 (3H, m, H-3, H-6b, H-7a), 3.53-3.57 (1H, m, H-4), 3.55-3.48 (1H, m, H-5), 3.44-3.37 (1H, m, H-7b), 1.97-1.85 (2H, m, 2XH-8), 1.65-1.51 (2H, m, 2XH-8), 1.44-1.27 (6H, m, 6XH-8); ¹³C NMR (100 MHz, MeOD): δ = 149.1 (C-11), 124.0 (C-10), 101.5 (C-1), 74.6 (C-5), 72.7 (C-3), 72.3 (C-2), 68.7 (C-4), 68.4 (C-7), 63.0 (C-6), 56.5 (C-12), 51.3 (C-9), 31.2, 30.4, 29.8, 27.4, 27.1 (C-8); HRMS (ES⁺) m/z calcd for C₁₆H₃₀N₃O₇: 376.2084, found 373.2073.

Compound 3



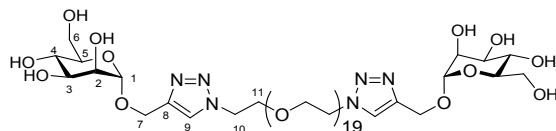
Obtained following the acetate deprotection to afford **3** (97% yield) as a colorless gum. $[\alpha]_D^{20} = +78$ (C = 1, MeOH); ^1H NMR (400 MHz, D_2O): δ = 8.14 (2H, s, H-9), 5.03 (2H, d, J = 1.9Hz, H-1), 4.88 (2H, d, J = 12.7Hz, H-7a), 4.75 (2H, d, J = 12.7Hz, H-7b), 4.69 (4H, t, J = 5.2Hz, H-10), 4.02 (t, J = 5.2Hz, 4 H, H-11), 3.98 (dd, J = 1.9Hz, 2H, J = 12.4Hz, H-6a), 3.91 (dd, J = 1.9Hz, J = 3.7Hz, 2 H, H-2), 3.87-3.78 (4H, m, H-3, H-6b), 3.77-3.70 (4H, m, H-4, H-5), 3.69-3.65 (4H, m, CH_2O), 3.62-3.57 (4H, m, CH_2O); ^{13}C NMR (100 MHz, CDCl_3): δ = 143.5 (C-8), 126.6 (C-9), 99.5 (C-1), 73.0 (C-5), 70.5 (C-3), 70.0 (C-2), 69.7-69.5 (CH_2O), 68.7 (C-10), 66.7 (C-4), 60.9 (C-6), 59.8 (C-7), 50.1 (C-11); HRMS (MALDI) m/z calcd for $\text{C}_{26}\text{H}_{44}\text{N}_6\text{O}_{15}\text{Na}$: 703.2762, found 703.2766.

Compound 4



Obtained following the acetate deprotection to afford **4** (73% yield) as a colorless gum. $[\alpha]_D^{20} = +64$ (C = 1, MeOH); ^1H NMR (400 MHz, D_2O): δ = 8.19 (2H, s, H-9), 5.04 (2H, d, J = 1.9Hz, H-1), 4.91 (2H, d, J = 12.7 Hz, H-7a), 4.79 (2H, d, J = 12.7Hz, H-7b), 4.71 (4H, t, J = 5.2Hz, H-10), 4.05 (4H, t, J = 5.2Hz, H-11), 3.99 (2H, dd, J = 1.9Hz, J = 12.4Hz, H-6a), 3.92 (2H, dd, J = 1.9Hz, J = 3.7Hz, H-2), 3.87-3.78 (4H, m, H-3, H-6b), 3.77-3.73 (28H, m, H-4, H-5, CH_2O), 3.72-3.66 (16H, m, CH_2O); ^{13}C NMR (100 MHz, D_2O): δ = 143.6 (C-8), 125.7 (C-9), 99.5 (C-1), 73.0 (C-5), 70.5 (C-3), 70.0 (C-2), 69.7-69.3 (CH_2O), 68.8 (C-10), 66.7 (C-4), 60.9 (C-6), 59.8 (C-8), 50.1 (C-11); HRMS (ES+) m/z calcd for $\text{C}_{42}\text{H}_{76}\text{N}_6\text{O}_{23}\text{Na}$: 1055.4860, found 1055.4860.

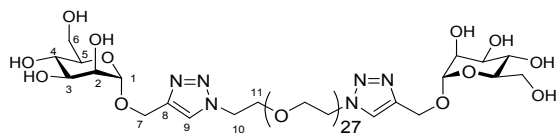
Compound 5



Obtained following the acetate deprotection to afford **5** (71% yield) as a colorless gum. $[\alpha]_D^{20} = +60$ (C = 1, MeOH); ^1H NMR (400 MHz, D_2O): δ = 8.19 (2H, s, H-9), 5.04 (2H, d, J = 1.9Hz, H-1), 4.91 (2H, d, J = 12.7Hz, H-7a), 4.79 (2H, d, J = 12.7 Hz, H-7b), 4.70 (4H, t, J = 5.2Hz, H-10),

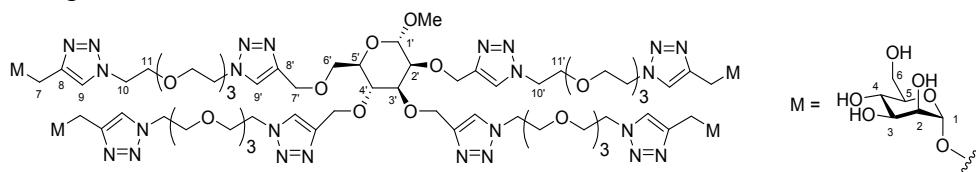
4.04 (4H, t, $J = 5.2\text{Hz}$, H-11), 3.96 (dd, $J = 1.9\text{Hz}$, $J = 12.4\text{Hz}$, H-6a), 3.90 (2H, dd, $J = 1.9\text{Hz}$, $J = 3.7\text{Hz}$, H-2), 3.87-3.78 (4H, m, H-3, H-6b), 3.77-3.69 (4H, m, H-4, H-5), 3.70-3.63 (m, 76 H, CH₂O); ¹³C NMR (100 MHz, D₂O): $\delta = 143.6$ (C-8), 125.6 (C-9), 99.5 (C-1), 73.0 (C-5), 70.5 (C-3), 70.0 (C-2), 69.6 (CH₂O), 68.8 (C-10), 66.7 (C-4), 60.9 (C-6), 59.7 (C-7), 50.1 (C-11); HRMS (MALDI) m/z calcd for C₅₈H₁₀₈N₆O₃₁Na: 1407.6957, found 1407.6953.

Compound 6



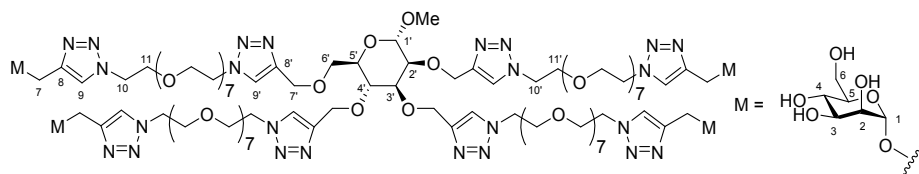
Obtained following the acetate deprotection to afford **6** (74% yield) as a colorless gum. $[\alpha]_D^{20} = +58$ (C = 1, MeOH); ¹H NMR (400 MHz, CD₃OD): $\delta = 8.07$ (2H, s, H-9), 4.86 (2H, d, $J = 1.9\text{Hz}$, H-1), 4.79 (2H, d, $J = 12.7\text{Hz}$, H-7a), 4.65 (2H, d, H-7b), 4.59 (4H, t, $J = 5.2\text{Hz}$, H-10), 3.91 (4H, t, $J = 5.2\text{Hz}$, H-11), 3.96 (2H, dd, $J = 1.9\text{Hz}$, $J = 12.4\text{Hz}$, H-6a), 3.90 (2H, dd, $J = 1.9\text{Hz}$, $J = 3.7\text{Hz}$, H-2), 3.75-3.67 (4H, m, H-3, H-6b), 3.67-3.58 (108H, m, H-4, H-5, 104xCH₂O); ¹³C NMR (100 MHz, CD₃OD): $\delta = 145.1$ (C-8), 126.2 (C-9), 100.8 (C-1), 75.0 (C-5), 72.6 (C-3), 72.0 (C-2), 71.5 (CH₂O), 70.3 (C-10), 68.7 (C-4), 63.0 (C-6), 60.7 (C-7), 51.5 (C-11); HRMS (MALDI) m/z calcd for C₇₄H₁₄₀N₆O₃₉Na₂: 1782.8952, found 1782.8946.

Compound 7



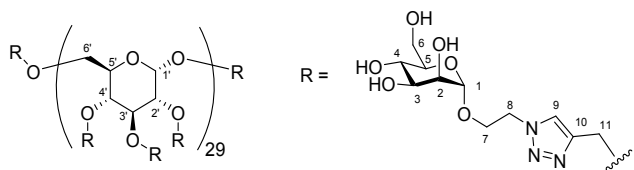
Obtained following the acetate deprotection to afford **7** (78% yield) as a colorless gum. $[\alpha]_D^{20} = +81$ (C = 1, MeOH); ¹H NMR (400 MHz, D₂O): $\delta = 8.13$ -8.01 (8 H, s, H-9, H-9'), 5.02 (4 H, bs, H-1), 4.89-4.67 (16 H, m, H-7, H-7'), 4.67-4.55 (18 H, m, H-11, H-11', H-2', H-11'), 3.97 (21 H, m, H-2, H-10, H-4', H-10'), 3.92-3.80 (12 H, m, H-3, H-6), 3.78-3.68 (8 H, m, H-4, H-5), 3.67-3.52 (36 H, m, H-3', H-5', H-6', CH₂O), 3.41 (3 H, s, OCH₃); ¹³C NMR (100 MHz, D₂O): $\delta = 143.9$, 143.8, 143.5 (C-8, C-8'), 125.7 (C-9), 99.5 (C-1), 98.4 (C-1'), 78.4, 74.5, 73.9 (C-3', C-4', C-5'), 73.0 (C-5), 70.6 (C-3), 70.3 (C-6'), 70.0 (C-2), 69.7-69.5 (CH₂O), 68.7 (C-10), 66.7 (C-4), 63.3 (C-2'), 63.2 (C-7'), 60.9 (C-6), 59.8 (C-7), 55.0 (OCH₃), 50.1 (C-11); HRMS (MALDI) m/z calcd for C₈₇H₁₄₄N₂₄O₄₂: 1098.4935, found 1098.4933.

Compound 8



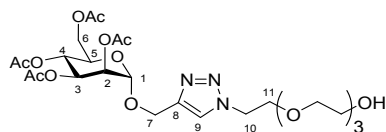
Obtained following the acetate deprotection method 2 to afford **8** (65% yield) as a colorless gum. $[\alpha]_D^{20} = +68$ (C = 1, MeOH); ^1H NMR (400 MHz, D_2O): δ = 8.08-8.01 (8 H, s, H-9, H-9'), 4.94 (4 H, bs, H-1), 4.82-4.65 (16 H, m, H-7, H-7'), 4.61 (18 H, m, H-11, H-11', H-2', H-11'), 3.94 (21 H, m, H-2, H-10, H-4', H-10'), 3.92-3.80 (12 H, m, H-3, H-6), 3.78-3.68 (8 H, m, H-4, H-5), 3.67-3.52 (116 H, m, H-3', H-5', H-6', CH_2O), 3.41 (3 H, s, OCH_3); ^{13}C NMR (100 MHz, D_2O): δ = 143.9, 143.8, 143.6 (C-8, C-8'), 125.6, (C-9), 99.5 (C-1), 73.0 (C-5), 70.5 (C-3), 70.0 (C-2), 69.6 (CH_2O), 68.7 (C-10), 66.7 (C-4), 60.9 (C-6), 59.8 (C-7), 50.1 (C-11); HRMS (MALDI) m/z calcd for $\text{C}_{119}\text{H}_{208}\text{N}_{24}\text{O}_{58}$: 1451.2049, found 1451.2045.

Compound **9**



Obtained following the acetate deprotection method to afford **9** (74% yield) as a yellowish solid. ^1H NMR (400 MHz, D_2O): δ = 8.18 (3 H, m broad, H-9), 5.20 (1 H, s broad, H-1'), 5.00-4.60 (m broad, 9 H, H-1, H-11), 4.20-4.07 (m broad, 3 H, H-6a), 4.04-3.86 (m broad, 9 H, 3xH-2, 3xH-6b, H-2', H-3', H-4'), 3.85-3.49 (21H, H-3, H-4, H-7, H-8, H-5', H-6'), 3.17 (3H, s broad, H-5); ^{13}C NMR (100 MHz, D_2O): δ = 144.4 (C-10), 125.6 (C-9), 99.7 (C-1), 96.7 (C-1'), 79.2 (C-3'), 77.8 (C-2'), 77.1 (C-4'), 73.0 (C-5), 70.6 (C-3'), 70.1 (C-2'), 69.8 (C-5', C-4), 66.6 (C-8), 65.5 (C-6), 63.3 (C-6'), 60.9 (C-7), 50.2 (C-11).

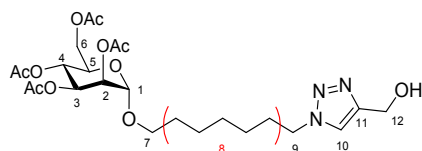
Compound **16**.



Obtained following the CuAAC method 1. The crude product was chromatographed on a silica gel column with 96/4 (DCM/MeOH) as eluent to afford **16** (88% yield) as a yellowish gum. $[\alpha]_D^{20} = +42$ (C = 1, CHCl_3); ^1H NMR (400 MHz, CDCl_3): δ = 7.84 (1H, s, H-9), 5.31-5.24 (2H, m, H-3, H-4), 5.20 (1H, br s, H-2), 4.93 (1H, br s, H-1), 4.81 (1H, d, J = 10.5Hz, H-7a), 4.64 (1H, d, J = 10.5Hz, H-7b), 4.54 (2H, t, J = 4.7Hz, H-11), 4.26 (1H, dd, J = 5.0Hz, J = 12.2Hz, H-6a), 4.12-4.02 (2H, m, H-5, H-6b), 3.86 (2H, t, J = 4.7Hz, H-10), 3.71-3.55 (12 H, m, CH_2O), 2.11, 2.08, 1.99, 1.94 (12H, 4 s, CH_3CO); ^{13}C NMR (100 MHz, CDCl_3): δ = 170.7, 170.1, 169.9, 169.7

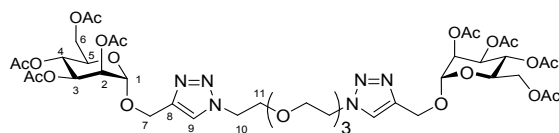
(COCH₃), 143.7 (C-8), 124.8 (C-9), 96.9 (C-1), 72.5, 70.6-70.3 (CH₂O), 69.5 (C-11), 69.5 (C-2), 69.2 (C-3), 68.8 (C-5), 66.2 (C-4), 62.5 (C-6), 61.7 (CH₂OH), 61.0 (C-7), 50.5 (C-10), 20.9-20.6 (COCH₃); HRMS (ES⁺) m/z calcd for C₂₅H₄₀N₃O₁₄: 606.2510 found 606.2500.

Compound **18**.



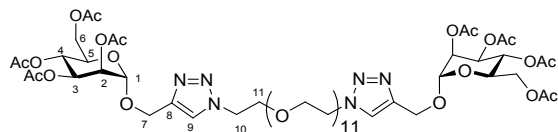
Obtained following the CuAAC method 1. The crude product was chromatographed on a silica gel column with 96/4 (DCM/MeOH) as eluent to afford **18** (73% yield) as a yellowish gum. $[\alpha]_D^{20} = +48$ (C = 1, CHCl₃); ¹H NMR (400 MHz, CDCl₃): δ = 7.52 (1H, s, H-10), 5.30 (1H, dd, *J*₃₋₂ = 3.4 Hz, *J*₃₋₄ = 10.0 Hz, H-3), 5.27-5.20 (1H, m, H-4), 5.18 (1H, dd, *J*₂₋₁ = 1.8 Hz, *J*₂₋₃ = 3.4 Hz, H-2), 4.77-4.72 (3H, m, H-1, H-12), 4.32 (2H, t, *J* = 7.0 Hz, H-9), 4.23 (1H, dd, *J*_{6a-5} = 5.3 Hz, *J*_{6a-6b} = 12.3 Hz, H-6a), 4.07 (1H, dd, *J*_{6b-5} = 2.5 Hz, *J*_{6b-6a} = 12.3 Hz, H-6b), 3.96-3.90 (1H, m, H-5), 3.68-3.58 (1H, m, H-7a), 3.44-3.36 (1H, m, H-7b), 2.12, 2.05, 2.00, 1.96 (12H, 4 s, CH₃CO), 1.92-1.83 (2H, m, H-8), 1.61-1.49 (2H, m, H-8), 1.36-1.27 (6H, m, H-8); ¹³C NMR (100 MHz, CDCl₃): δ = 170.7, 170.2, 170.1, 169.8 (COCH₃), 147.9 (C-11), 121.6 (C-10), 97.6 (C-1), 69.8 (C-2), 69.2 (C-3), 68.5 (C-5), 68.4 (C-7), 66.4 (C-4), 62.6 (C-6), 56.5 (C-12), 50.3 (C-9), 30.2, 29.1, 28.7, 26.4, 26.0 (C-8), 20.9, 20.8, 20.74 (CH₃); HRMS (ES⁺) m/z calcd for C₂₄H₃₈N₃O₁₁: 544.2506 found 544.2504.

Compound **23**.



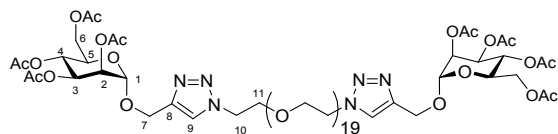
Obtained following the CuAAC method. The crude product was chromatographed on a silica gel column with 95/5 (DCM/MeOH) as eluent to afford **23** (92% yield) as a colorless gum. $[\alpha]_D^{20} = +29$ (C = 1, CHCl₃); ¹H NMR (400 MHz, CDCl₃): δ = 7.76 (2H, s, H-9), 5.32-5.28 (4H, m, H-3, H-4), 5.22 (2H, bs, H-2), 4.96 (2H, d, *J* = 1.6 Hz, H-1), 4.82 (2H, d, *J* = 12.4 Hz, H-7a), 4.66 (2H, d, *J* = 12.4 Hz, H-7b), 4.55 (4H, t, *J* = 4.7 Hz, H-11), 4.29 (2H, dd, *J* = 5.0 Hz, *J* = 12.3 Hz, H-6a), 4.13-4.04 (4H, m, H-5, H-6b), 3.85 (4H, t, *J* = 4.7 Hz, H-10), 3.64-3.57 (8H, m, CH₂O), 2.14, 2.11, 2.02, 1.96 (24H, 4 s, CH₃CO); ¹³C NMR (100 MHz, CDCl₃): δ = 170.8, 170.2, 170.0, 169.7 (COCH₃), 143.4 (C-8), 124.3 (C-9), 96.9 (C-1), 70.6-69.6 (CH₂O), 69.5 (C-11), 69.4 (C-2), 69.2 (C-3), 68.8 (C-5), 66.2 (C-4), 62.5 (C-6), 61.0 (C-7), 52.8 (C-10), 21.0, 20.9, 20.8, 20.7 (COCH₃); HRMS (ESI) m/z calcd for C₄₃H₆₃N₆O₂₃Na: 1039.3608 found 1039.3615.

Compound **24**.



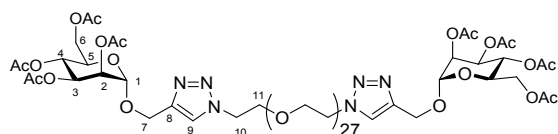
Obtained following the CuAAC method. The crude product was chromatographed on a silica gel column with 95/5 (DCM/MeOH) as eluent to afford **24** (90% yield) as a colorless gum. $[\alpha]_D^{20} = +36$ (C = 1, CHCl₃); ¹H NMR (400 MHz, CDCl₃): δ = 7.76 (2H, s, H-9), 5.29-5.25 (4H, m, H-3, H-4), 5.20 (2H, m, H-2), 4.94 (2H, d, J = 1.6 Hz, H-1), 4.80 (2H, d, J = 12.4Hz, H-7a), 4.65 (2H, d, J = 12.4Hz, H-7b), 4.53 (4H, t, J = 4.7Hz, H-11), 4.27 (2H, dd, J = 5.0 Hz, J = 12.3Hz, H-6a), 4.11–4.02 (4H, m, H-5, H-6b), 3.87 (4H, t, J = 4.7Hz, H-10), 3.64-3.57 (40 H, m, CH₂O), 2.11, 2.09, 2.00, 1.94 (24H, 4 s, CH₃CO); ¹³C NMR (100 MHz, CDCl₃): δ = 170.7, 170.0, 169.9, 169.7 (COCH₃), 143.4 (C-8), 124.3 (C-9), 96.9 (C-1), 70.6 (CH₂O), 69.6 (C-11), 69.5 (C-2), 69.2 (C-3), 68.8 (C-5), 66.2 (C-4), 62.5 (C-6), 61.0 (C-7), 50.4 (C-10), 20.9, 20.8, 20.7 (COCH₃); HRMS (ESI) m/z calcd for C₅₈H₉₂N₆O₃₁Na: 1391.5705 found 1391.5708.

Compound **25**



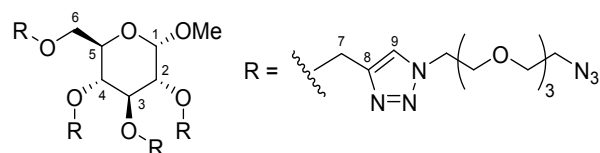
Obtained following the CuAAC method. The crude product was chromatographed on a silica gel column with 9/1 (DCM/MeOH) as eluent to afford **25** (85% yield) as a white solid. $[\alpha]_D^{20} = +54$ (C = 1, CHCl₃); ¹H NMR (400 MHz, CDCl₃): δ = 7.78 (2H, s, H-9), 5.33-5.26 (4H, m, H-3, H-4), 5.22 (2H, bs, H-2), 4.95 (2H, bs, H-1), 4.82 (2H, d, J = 12.4Hz, H-7a), 4.67 (2H, d, J = 12.4Hz, H-7b), 4.55 (4H, t, J = 4.7Hz, H-11), 4.30 (2H, dd, J = 5.0Hz, J = 12.3Hz, H-6a), 4.13–4.05 (4H, m, H-5, H-6b), 3.88 (4H, t, J = 4.7Hz, H-10), 3.66-3.58 (72 H, m, CH₂O), 2.14, 2.11, 2.02, 1.96 (24H, 4 s, CH₃CO); ¹³C NMR (100 MHz, CDCl₃): δ = 170.8, 170.1, 169.9, 169.8 (COCH₃), 143.4 (C-8), 124.4 (C-9), 96.9 (C-1), 70.7 (CH₂O), 69.5 (C-11), 69.5 (C-2), 69.2 (C-3), 68.7 (C-5), 66.2 (C-4), 62.5 (C-6), 61.1 (C-7), 50.4 (C-10), 20.9, 20.8 (COCH₃); HRMS (ESI) m/z calcd for C₇₄H₁₂₄N₆O₃₉Na: 1743.7802 found 1743.7811.

Compound **26**.



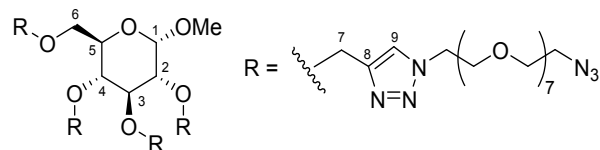
Obtained following the CuAAC method. The crude product was chromatographed on a silica gel column with 9/1 (DCM/MeOH) as eluent to afford **25** (70% yield) as a white solid. $[\alpha]_D^{20} = +43$ (C = 1, CHCl₃); ¹H NMR (400 MHz, CDCl₃): δ = 7.80 (2H, s, H-9), 5.32-5.28 (4H, m, H-3, H-4), 5.23 (2H, m, H-2), 4.97 (2H, d, J = 1.6 Hz, H-1), 4.84 (2H, d, J = 12.4Hz, H-7a), 4.67 (2H, d, J = 12.4Hz, H-7b), 4.56 (4H, t, J = 4.7Hz, H-11), 4.31 (2H, dd, J = 5.0Hz, J = 12.3Hz, H-6a), 4.13–4.05 (4H, m, H-5, H-6b), 3.89 (4H, t, J = 4.7Hz, H-10), 3.64 (104 H, m, CH₂O), 2.14, 2.12, 2.03, 1.97 (24H, 4 s, CH₃CO); ¹³C NMR (100 MHz, CDCl₃): δ = 170.8, 170.1, 169.9, 169.8 (COCH₃), 143.4 (C-8), 124.4 (C-9), 97.0 (C-1), 70.7 (CH₂O), 69.5 (C-11), 69.5, 69.2, 68.7, (C-2, C-3, C-5), 66.2 (C-4), 62.5 (C-6), 61.1 (C-7), 50.4 (C-10), 20.9, 20.8, (COCH₃); HRMS (ESI) m/z calcd for C₉₀H₁₅₆N₆O₄₇Na: 2095.9899 found 2095.9895.

Compound **30**.



Obtained following the CuAAC method. This reaction afforded the compound **30** (84% yield) as a colorless gum. $[\alpha]_D^{20} = +16$ (C = 1, CHCl₃); ¹H NMR (400 MHz, CDCl₃): δ = 7.93, 7.87, 7.78, 7.75 (4H, s, H-9), 4.92 (1 H, d, J = 11.3Hz, H-7), 4.81 (2 H, bs, H-7), 4.73 (1H, d, J = 11.3Hz, H-7), 4.72 (1H, bs, H-2), 4.71 (1H, d, J = 3.2Hz, H-1), 4.67 (1H, d, J = 11.3Hz, H7), 4.58 (1H, d, J = 11.3Hz, H-7), 4.56 (2 H, m, H-7), 4.53-4.48 (6H, m, H-4, CH₂O), 3.89-3.75 (m, 13 H, H-3, H-5, H-6, CH₂O), 3.67-3.56 (42 H, m, CH₂O), 3.36 (m, 8 H, CH₂N₃), 3.30 (3 H, bs, OCH₃); ¹³C NMR (100 MHz, CDCl₃): δ = 145.2-145.0 (C-8), 124.4-124.0 (C-9), 99.0 (C-1), 79.8, 74.8, 74.5, (C-3, C-4, C-5), 70.8-69.4 (CH₂O), 69.2, 66.2, 64.9, 64.3 (C-2, C-6, C-7), 54.9 (OCH₃), 50.8 (CH₂N₃), 50.3-50.2 (CH₂N); HRMS (ESI) m/z calcd for C₅₁H₈₇N₂₄O₁₈: 1323.6630 found 1323.6626.

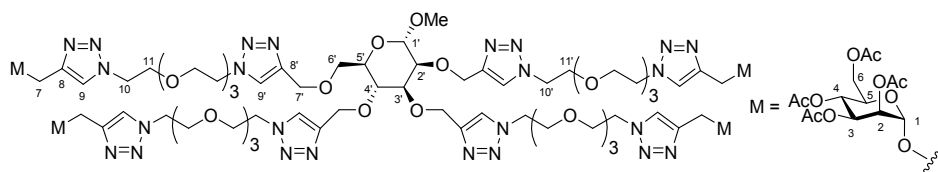
Compound **31**.



Obtained following the CuAAC method. This reaction afforded the compound **31** (75% yield) as a colorless gum. $[\alpha]_D^{20} = +14$ (C = 1, CHCl₃); ¹H NMR (400 MHz, CDCl₃): δ = 7.92, 7.86, 7.78, 7.75 (4H, s, H-9), 4.94 (1 H, d, J = 11.3Hz, H-7), 4.82 (2 H, bs, H-7), 4.73 (1H, d, J = 11.3Hz, H-7), 4.72 (1H, bs, H-2), 4.67 (1H, d, H-1), 4.64 (1H, d, H7), 4.58 (1H, d, H-7), 4.56 (2H, m, H-7), 4.53-4.50 (6H, m, H-4, CH₂O), 3.89-3.75 (m, 13 H, H-3, H-5, H-6, CH₂O), 3.68-3.55 (106 H, m, CH₂O), 3.36 (m, 8 H, CH₂N₃), 3.31 (3 H, bs, OCH₃); ¹³C NMR (100 MHz, CDCl₃): δ = 144.9 (C-8), 124.2-124.0 (C-9), 98.9 (C-1), 79.7, 74.8, 74.5, (C-3, C-4, C-5), 70.8-69.4 (CH₂O), 69.3, 66.1, 64.7, 64.1

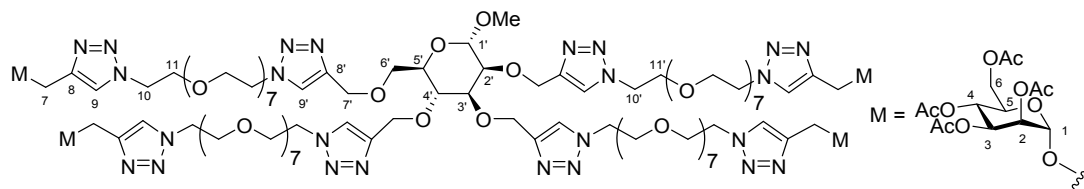
(C-2, C-6, C-7), 54.8 (OCH₃), 50.8 (CH₂N₃), 50.7, 50.1 (CH₂N); HRMS (ESI) [m+2H]/2z calcd for C₈₃H₁₅₂N₂₄O₃₄: 1014.5452 found 1014.5455.

Compound 32.



Obtained following the CuAAC method. The crude product was chromatographed on a silica gel column with 9/1 (DCM/MeOH) as eluent to afford **32** (78% yield) as a colorless gum. $[\alpha]_D^{20} = +36$ (C = 1, CHCl₃); ¹H NMR (400 MHz, CDCl₃): δ = 7.93, 7.85, 7.78, 7.75, 7.74 (8H, s, H-9, H-9'), 5.26 (8H, m, H-3, H-4), 5.20 (4H, bs, H-2), 4.94 (4 H, *J* = 2.0, H-1), 4.91-4.60 (16 H, m, H-7, H-1', H-2', H7'), 4.54-4.45 (18 H, m, H-7, H-11, H-11', H-7') 4.28 (4 H, dd, *J* = 12.3, 5.2Hz, H-6a), 4.12-4.01 (9 H, m, H-5, H-6b, H-4'), 3.85 (20 H, m, H-10, H-3', H-5', H-6', H-10'), 3.56 (32 H, m, CH₂O), 3.27 (3 H, s, OCH₃), 2.12, 2.09, 2.00, 1.94 (48 H, 4 s, CH₃CO); ¹³C NMR (100 MHz, CDCl₃): δ = 170.7, 170.1, 169.9, 169.8 (COCH₃), 145.0, 144.9, 144.8, 143.3 (C-8, C-8'), 124.3, 124.1, 124.0 (C-9, C-9'), 99.0, 96.9 (C-1'), 96.9 (C-1), 79.8, 74.7, 74.7, (C-3', C-4', C-5'), 71.4 (C-6'), 70.6, 69.5, 69.4, 69.2, 68.8 (C-11, C-11', CH₂O), 69.3, 69.2, 68.8, 66.2, 64.8 (C-2, C-3, C-4, C-5, C-7, C-7'), 64.2 (C-2'), 63.6 (C-7'), 62.5 (C-6), 61.0 (C-7'), 55.0 (OCH₃), 53.5, 50.4, 50.2 (C10, C-10'), 20.9, 20.8, 20.7, (COCH₃).

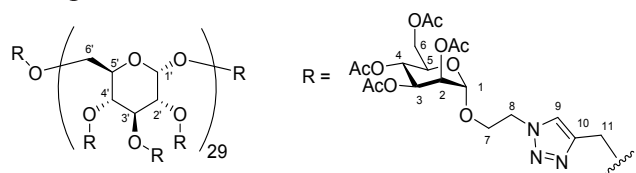
Compound 33.



Obtained following the CuAAC method. The crude product was chromatographed on a silica gel column with 9/1 (DCM/MeOH) as eluent to afford **32** (90% yield) as a colorless gum. $[\alpha]_D^{20} = +18$ (C = 1, CHCl₃); ¹H NMR (400 MHz, CDCl₃): δ = 7.95, 7.88, 7.79, 7.72 (8H, s, H-9, H-9'), 5.39-5.29 (8H, m, H-3, H-4), 5.29-5.26 (4H, m, H-2), 4.87 (4 H, *J* = 2.0, H-1), 4.92-4.68 (18 H, m, H-7, H-1', H-2', H7'), 4.67-4.49 (18 H, m, H-7, H-11, H-11', H-7') 4.31 (4 H, dd, *J* = 5.2Hz, *J* =

12.3, H-6a), 4.16–4.04 (9 H, m, H-5, H-6b, H-4'), 3.93–3.80 (20 H, m, H-10, H-3', H-5', H-6', H-10'), 3.60 (106 H, m, CH₂O), 3.30 (3 H, s, OCH₃), 2.14, 2.09, 2.03, 1.98 (48 H, s, CH₃CO); ¹³C NMR (100 MHz, CDCl₃): δ = 170.8, 170.1, 170.0, 169.9 (COCH₃), 145.1–143.4 (C-8, C-8'), 124.4, 124.3, 124.2, 124.1 (C-9, C-9'), 99.1 (C-1'), 96.8 (C-1), 79.6, 74.7, 74.3 (C-3', C-4', C-5'), 71.3 (C-6'), 70.6–69.1 (C-11, C-11', CH₂O), 69.2, 69.1, 68.8, 66.3, 64.3 (C-2, C-3, C-4, C-5, C-7, C-7'), 64.2 (C-2'), 63.6 (C-7'), 62.3 (C-6), 61.2 (C-7'), 55.3 (OCH₃), 52.9, 52.6 (C10, C-10'), 21.0, 20.8, 20.7, (COCH₃).

Compound **35**



Copper sulfate (40 mg, 0.16 mmol) and sodium ascorbate (65 mg, 0.32 mmol) were added to a solution of propargyl dextran (20mg, 72.4 μmol of monosaccharide unit) and **15** (125 mg, 0.30 mmol) in dioxane-H₂O (5 mL, 4–1). The mixture was irradiated at 80 °C for 2 x 45 min in a sealed vessel, with addition of copper sulfate sulfate (13.3 mg, 53.3 μmol) and sodium ascorbate sulfate (21.7 mg, 0.11 mmol) for the second run. Residual copper was removed with EDTA (94 mg, 0.32 mmol) for 2 h, and the mixture was poured into a NH₄Cl satd. solution (20 mL) and extracted with ethyl acetate (20 mL). The organic layer was dried (MgSO₄), filtered and the solvent removed under reduced pressure. The crude product was dissolved in a small amount of CH₂Cl₂ and the product was precipitated with Et₂O (30mL). The precipitate was collected by filtration, washed with Et₂O (50mL) and precipitate twice from Et₂O, to afford **35** (64 mg, 54%) as a yellow solid. ¹H NMR (500 MHz, CDCl₃): δ = 8.17–7.70 (3H, br s, H-9), 5.18 (6H, br, H-3, H-4) 5.00–4.41 (13H, m broad, H-1, H-2, H-11, H-1'), 4.37–3.20 (27H, br, H-6a, H-8, H-7, H-2',3',4',5',6'), 2.27–2.77 (36H, m, CH₃CO); ¹³C NMR (100 MHz, CDCl₃): δ = 171.2, 170.7, 170.0, 169.8 (CH₃CO), 145.4 (C-10), 124.4 (C-9), 97.7 (C-1, C-1'), 77.3 (C-3'), 69.3, 69.2, 68.9 (C-2, C-5', C-3, C-5), 66.4 (C-4), 65.9 (C-11), 62.4 (C-6), 60.5 (C-7), 60.1 (C-6'), 49.7 (C-8), 21.1, 20.9, 20.8 (CH₃CO).

Materials and Methods

Cells

U937 cells (monocytic leukemia; ATCC® CRL-1593.2™, LGC Standards, UK) stably expressing full length DC-SIGN were generated by lentiviral transduction and propagated as already described elsewhere (Halary, Immunity, 2002). DC-SIGN expression was validated by flow cytometry with an APC-conjugated anti-human DC-SIGN mAb (clone DCN46) on a LSR II apparatus (BD Biosciences, Franklin Lakes, NJ). MRC-5 fibroblasts (RD Biotech, France) were propagated in DMEM, 2mM glutamine, 10% FCS (HyClone/GE Healthcare, Wauwatosa, WI). MRC-5 cells were used at 80-90% confluency for *trans*-infection assays.

Viral envelope glycoprotein binding assay

Parental or DC-SIGN-expressing U937 cells were resuspended in TBS, 1 mM CaCl₂, 2mM MgCl₂, 0.1% bovine serum albumin (BSA) and then seeded in 96-well plates at 1.10⁵ cells per well. Biotin-labelled recombinant HCMV gB (2 µg/ml) was then added to cells for 20 min at 4°C. Cells were washed three times with cold TBS, 1 mM CaCl₂, 2 mM MgCl₂, 0.1% BSA and further stained with APC- or PE-conjugated streptavidin (BD Biosciences, Franklin Lakes, NJ) before being analyzed on a LSR II flow cytometer (BD Biosciences, Franklin Lakes, NJ) and the FlowJo software (Tree Star, Ashland, OR).

***Trans*-infection experiments**

To assess the blocking properties of HM-based compounds described above in this section, a DC-SIGN-expressing U937 cell line and the parental U937 cells as a negative control were used in a *trans*-infection assay described elsewhere (Halary, Immunity, 2002). MRC-5 fibroblasts were seeded in flat-bottom 96-well plates the day before starting the *trans*-infection assay in DMEM, 2mM glut, 10% FCS. Briefly, DC-SIGN-expressing U937 cells were incubated with inhibitors at indicated concentrations for 30 min at 4°C before adding a constant dose of AD-GFP (MOI=2), a AD169 genetically-modified CMV strain that allowed for the GFP expression in infected cells (REF

princeps). After being washed thoroughly, CMV-loaded cells were applied on a MRC-5 cell monolayer. A short centrifugation step (5', x300g) was used to favour cell-to-cell contact and viral transmission which was assessed after 48h by counting the total number of GFP positive MRC-5 cells in each cell culture well through an automated processing described hereafter.

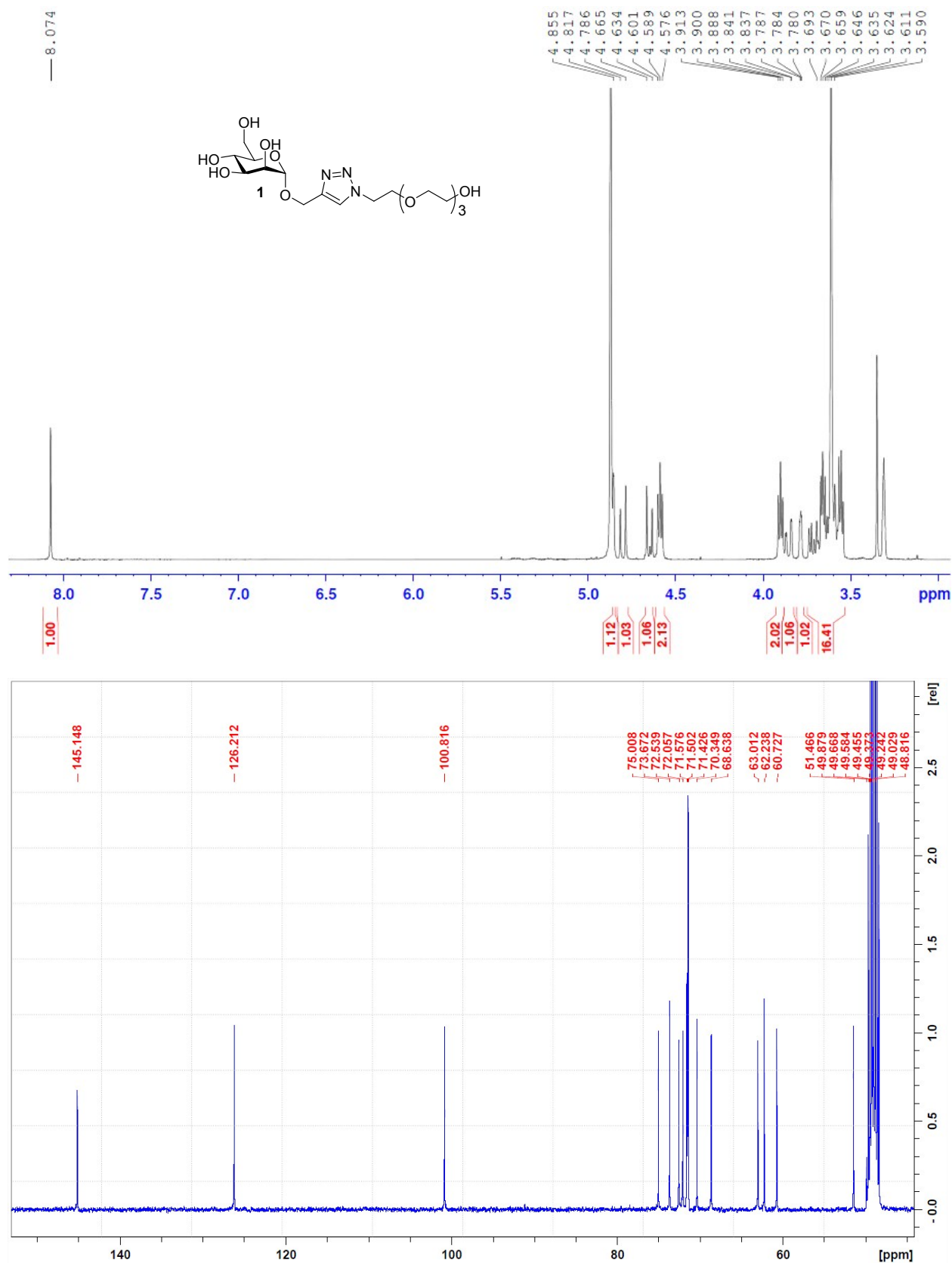
Automated infected cell counting

Absolute numbers of infected cells were obtained by analysing the total well area with a Cellomics® ArrayScan® VTI HCS Reader (Thermo Scientific) equipped with a x10 objective. For each field, phase-contrast and fluorescence (band-path filter 485nm±20nm) channels were recorded simultaneously. Image stitching was performed with the HCS Studio Cell Analysis Software (Thermo Scientific) and allowed to reconstruct an image of the whole well for all conditions. Digitalized images were processed with the “particles analysis” plugin from the Fiji software. Results were plotted as absolute numbers of infected cells (GFP positive cells) per well against the base 10 logarithm of the inhibitor concentrations. IC50 values were determined on the GraphPad Prism 5.0 software using a nonlinear regression analysis (GraphPad Software Inc., La Jolla, CA, USA).

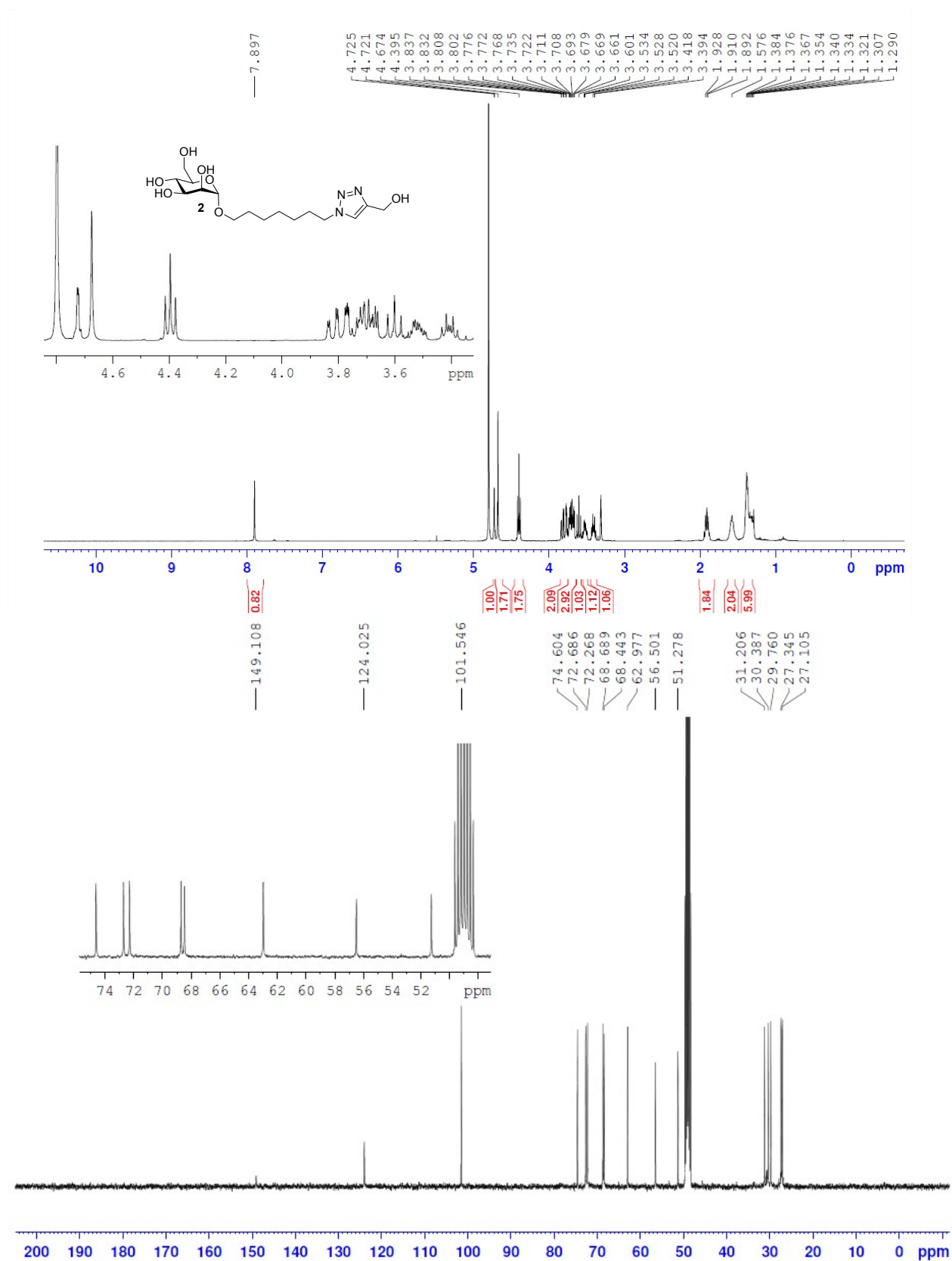
Cell viability assay

Cell viability was determined by DAPI (4',6-Diamidino-2-Phenylindole, Dihydrochloride) exclusion after incubation of U937 cells with all tested compounds for two hours at 37°C. Cells were then washed and resuspended into 300µL of PBS per condition. Ten microliters of a 1µM DAPI solution were extemporaneously to cells and immediately analysed on a LSR II flow cytometer.

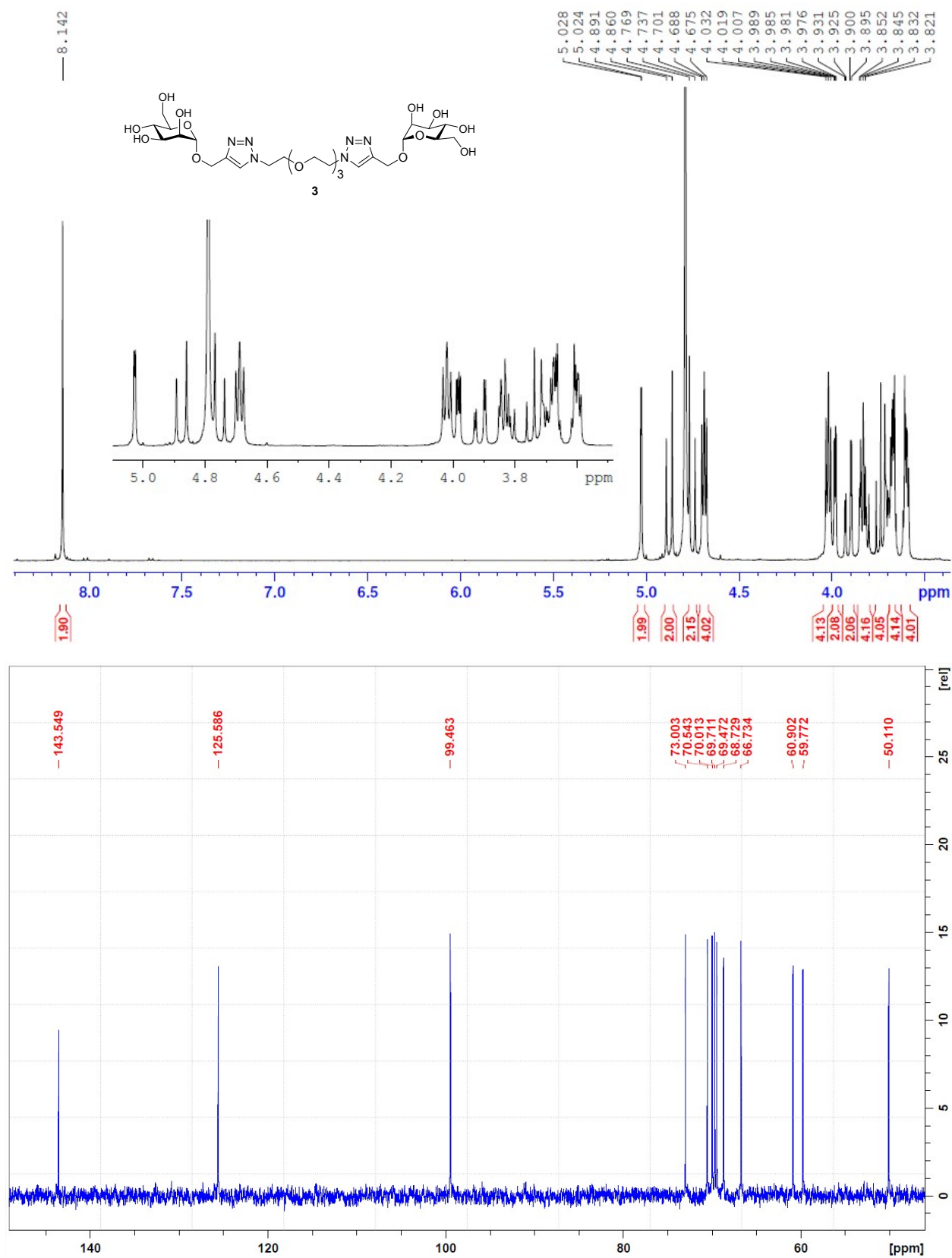
Compound 1



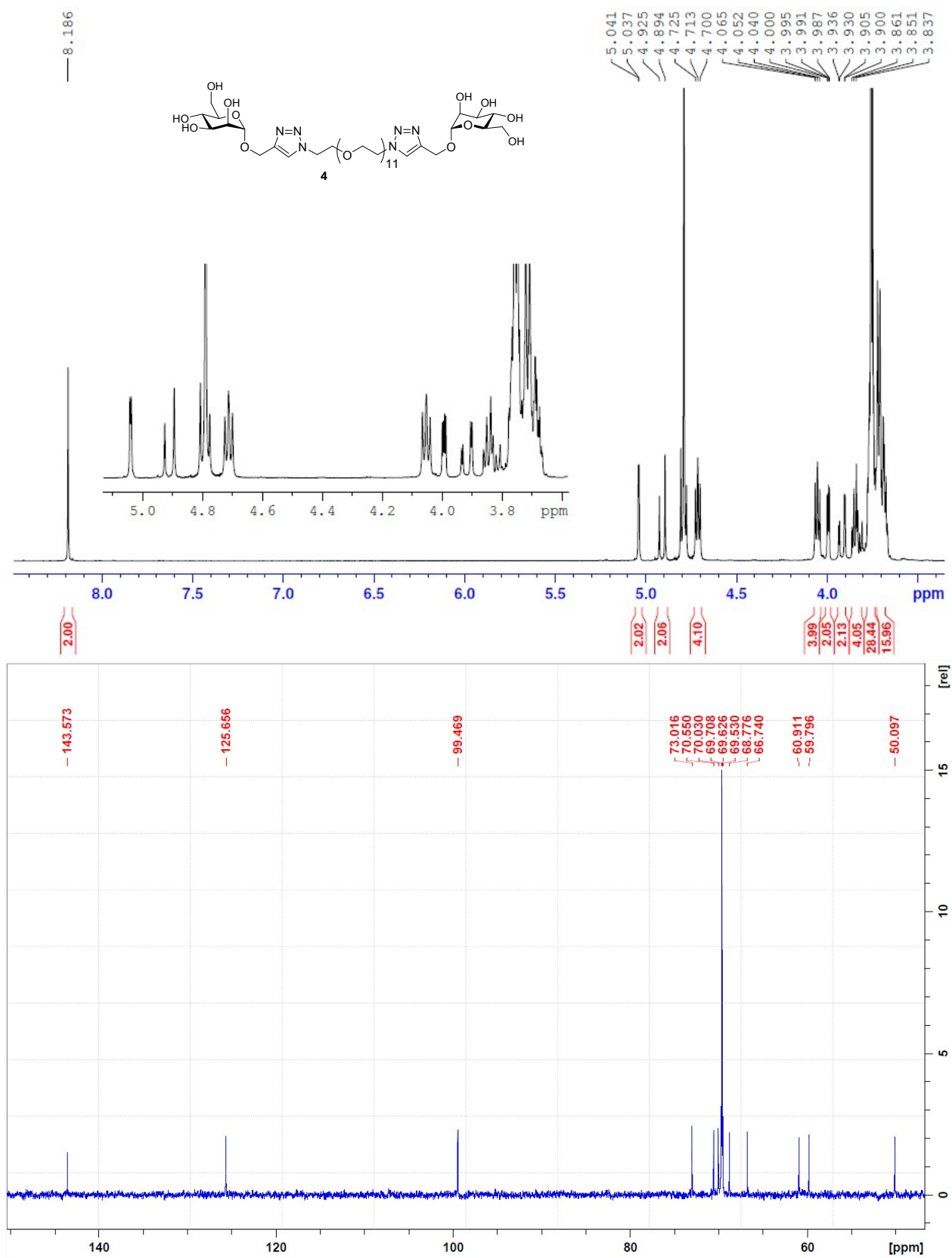
Compound 2



Compound 3



Compound 4

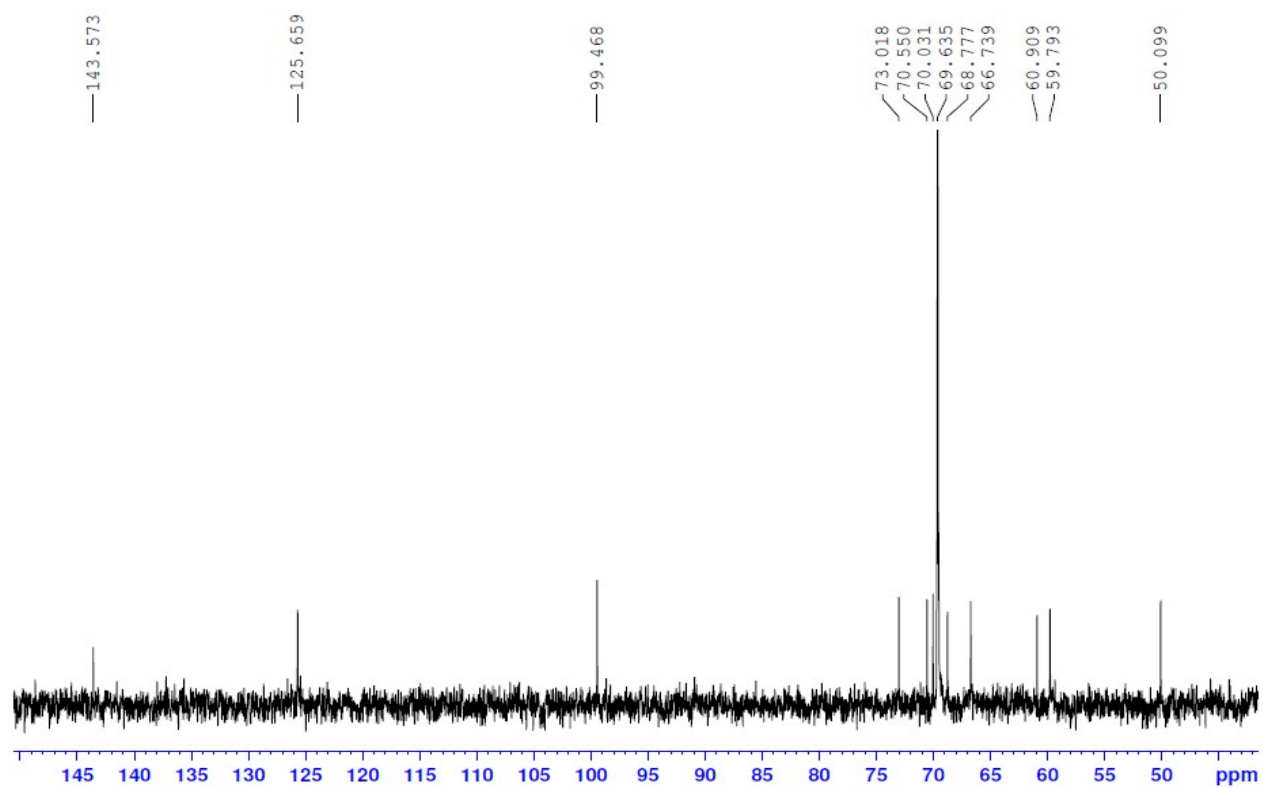


Chemical structure of compound **5** is shown above the spectrum. The structure consists of two α -D-glucopyranosyl units linked via azo groups to a poly(ethylene glycol) chain.

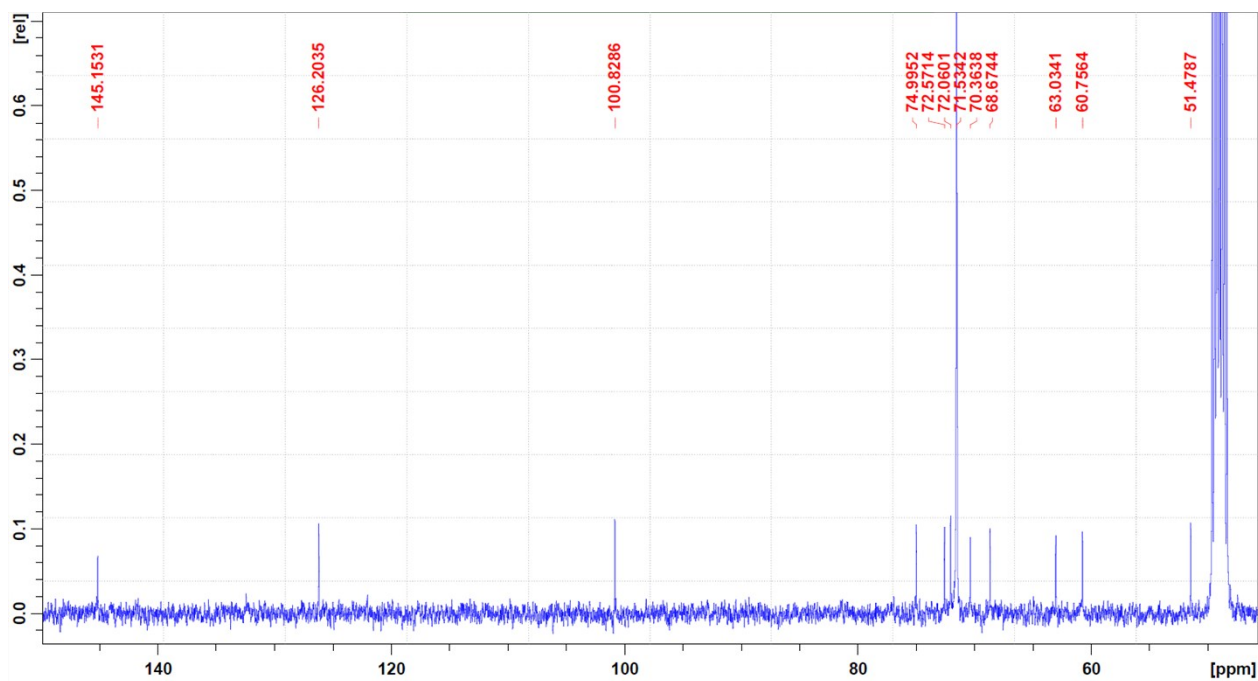
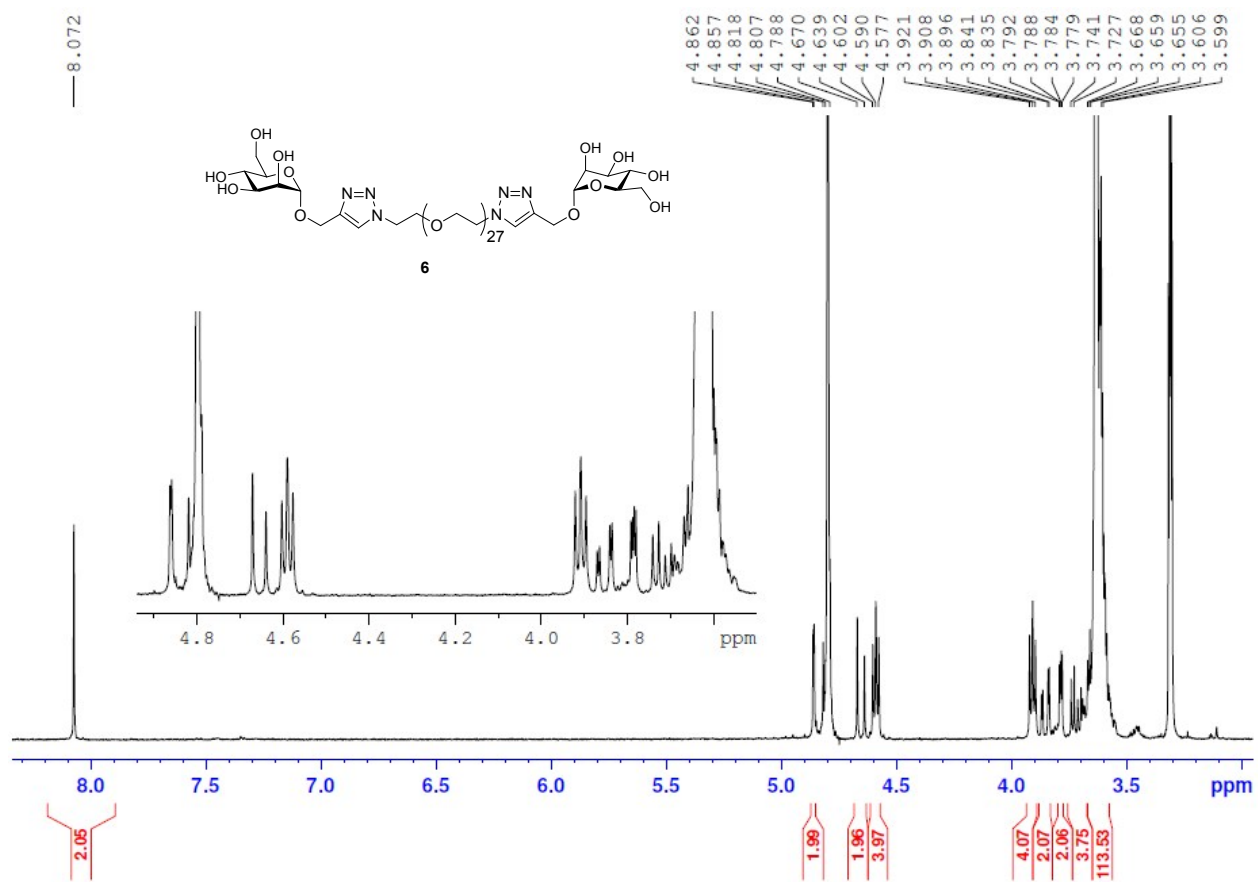
¹H NMR spectrum (DMSO-d₆) of compound **5** is displayed below the structure. The x-axis represents chemical shift in ppm, ranging from 3.5 to 8.2. The spectrum shows several peaks corresponding to the protons in the molecule. Integration values are provided below the baseline.

Integration values (from left to right): 1.96, 2.04, 2.08, 4.05, 4.00, 2.00, 2.19, 4.24, 95.55.

Chemical shift values (from left to right): 8.189, 5.042, 4.927, 4.702, 4.000, 3.995, 3.987, 3.905, 3.851 ppm.



Compound 6



¹H NMR (400 MHz, CDCl₃)

Chemical structure of **7**: M = COCC1OC(CO)C(O)C(O)C1O

¹³C NMR (100 MHz, CDCl₃)

¹H NMR (400 MHz, CDCl₃)

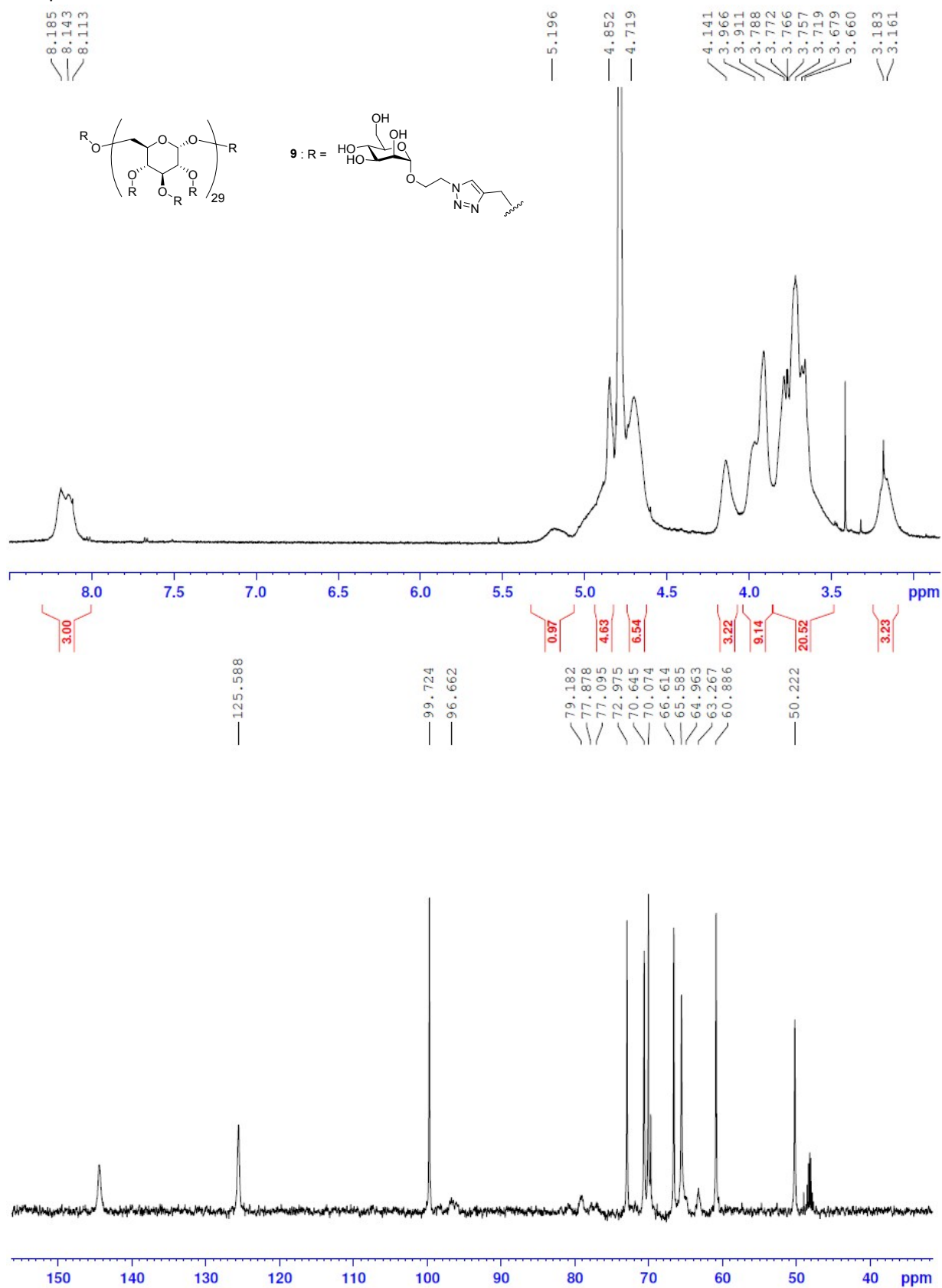
Chemical Shift (ppm)	Integration
8.086, 8.053, 8.042, 7.957	5.26, 1.93, 0.85
4.943, 4.939, 4.823, 4.791, 4.753, 4.607	4.00, 8.31, 20.26
3.945, 3.742, 3.632, 3.336	22.00, 16.87, 116.67, 3.17

¹³C NMR (100 MHz, CDCl₃)

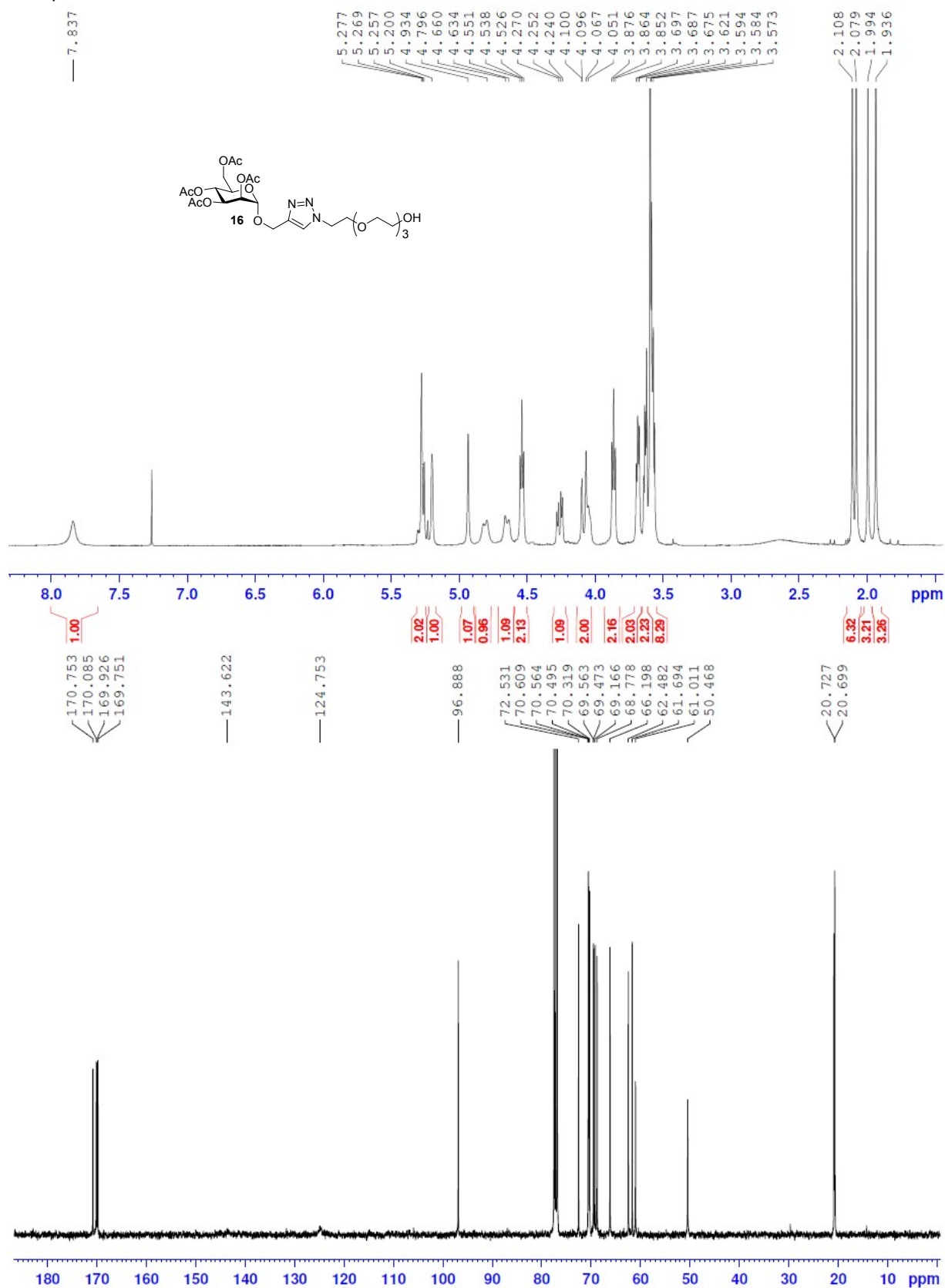
Chemical Shift (ppm)
143.970, 143.833, 143.573
125.634
99.465
73.022, 70.560, 70.036, 69.703, 69.628, 69.531, 68.772, 66.742
60.913, 59.782
50.086

Chemical Structure of 8: A central sugar moiety (likely a derivative of D-glucose) with four azide-terminated side chains (M groups) attached via ether linkages. The structure is shown with stereochemistry and a repeating unit of 7.

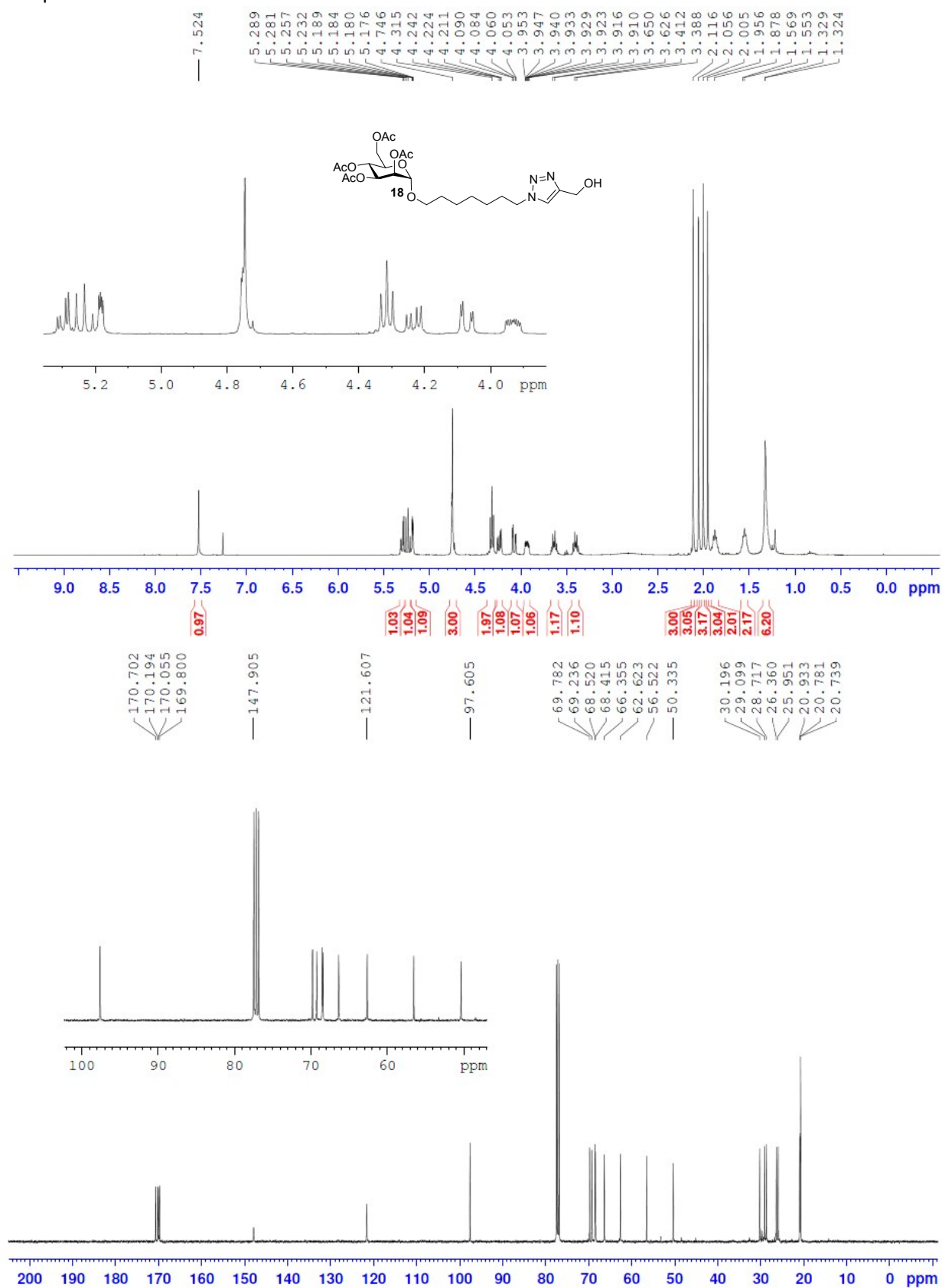
Compound 9



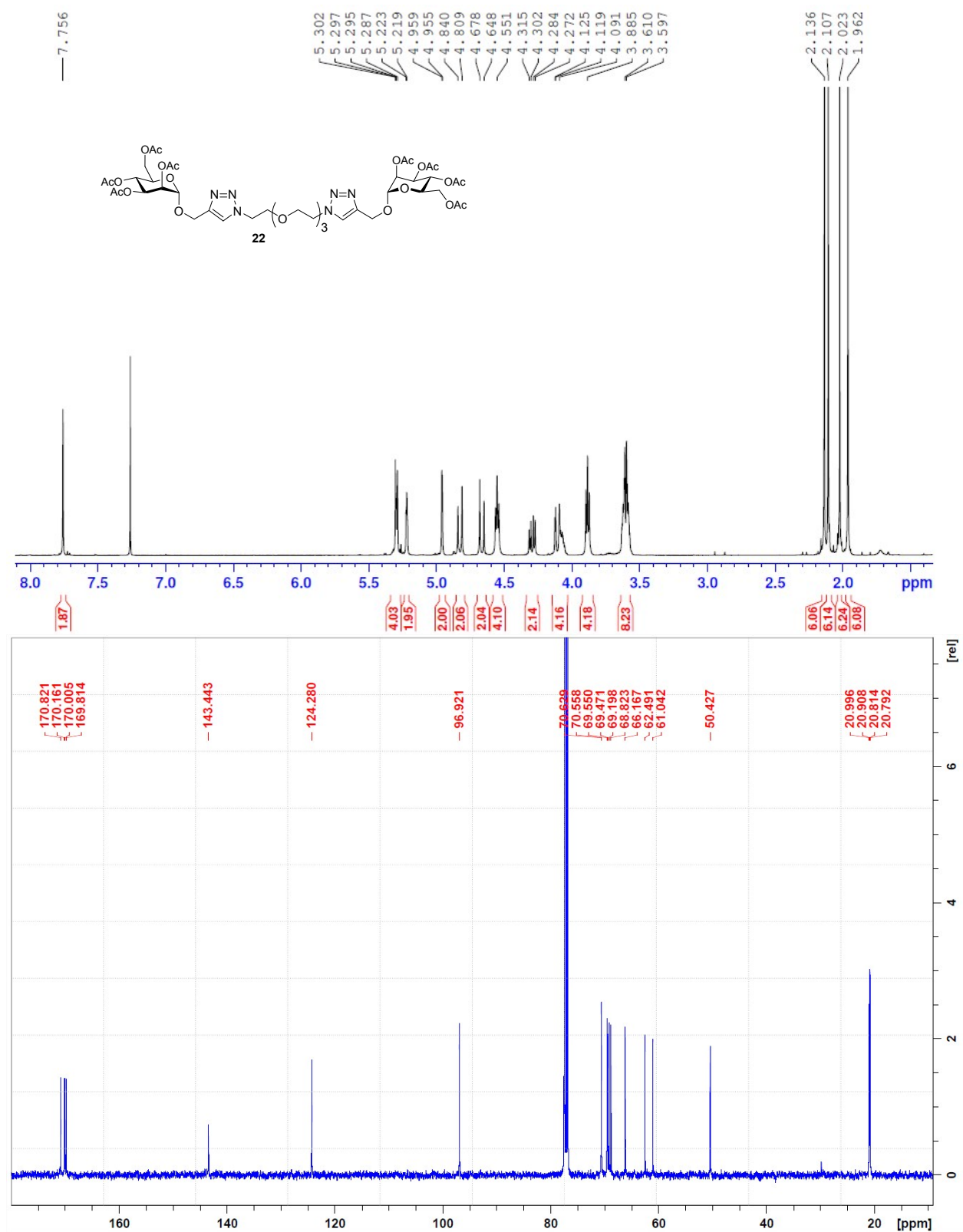
Compound 16



Compound 18



Compound 22

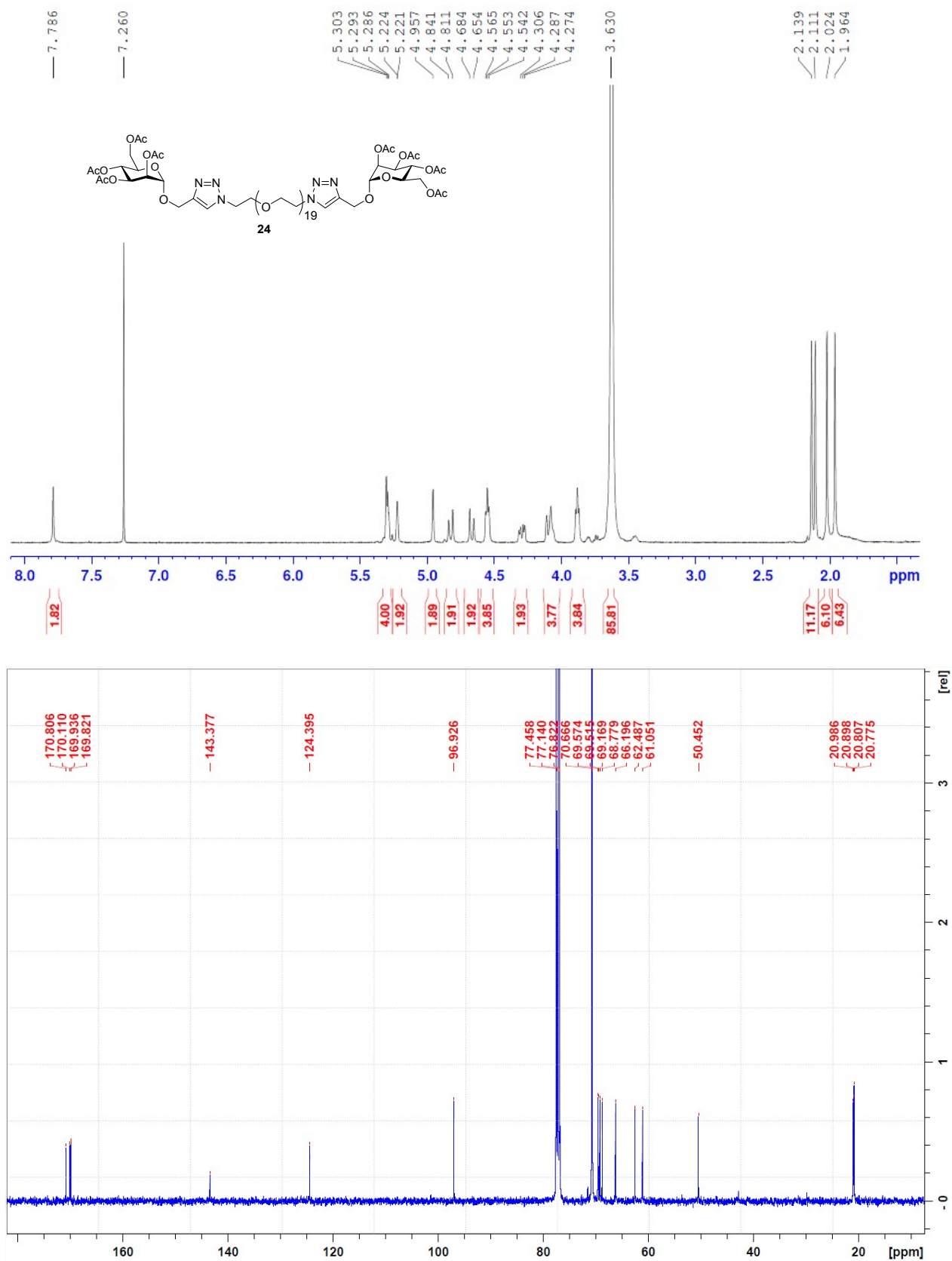


Chemical structure of 23: A bis-azobenzene derivative with two 2,3,4,6-tetra-O-acetyl-β-D-glucopyranosyl groups attached via methylene spacers to the azobenzene moieties. The central azobenzene core is substituted with a 11-membered ring containing two azobenzene units.

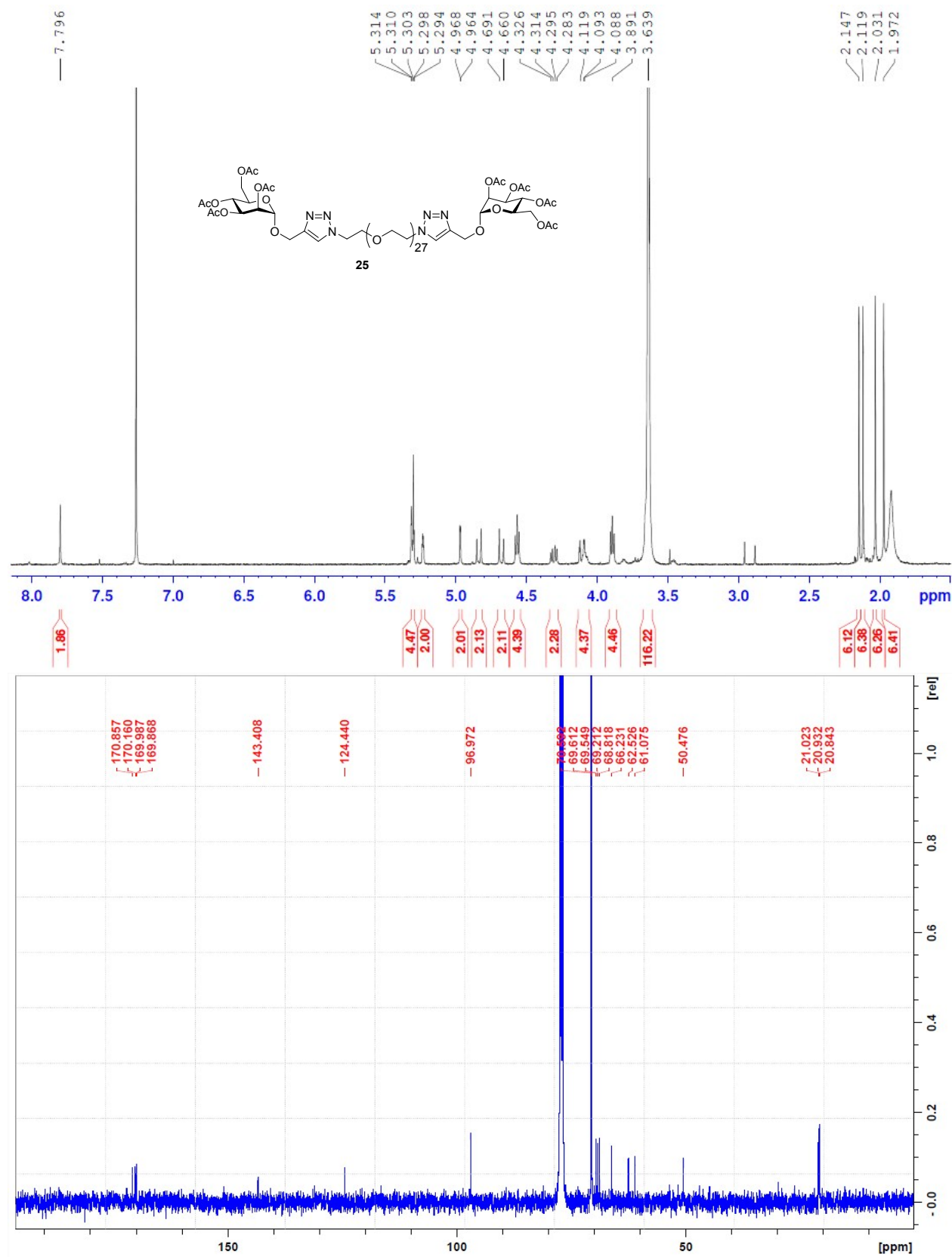
¹H NMR (400 MHz, CDCl₃) peaks (ppm): 7.763, 5.282, 5.276, 5.262, 5.207, 5.202, 5.200, 5.195, 4.939, 4.935, 4.821, 4.791, 4.670, 4.639, 4.530, 4.290, 4.277, 4.260, 4.247, 4.100, 4.094, 4.068, 3.866, 3.607, 3.600, 2.115, 2.086, 2.001, 1.942.

¹³C NMR (100 MHz, CDCl₃) peaks (ppm): 170.717, 170.026, 169.857, 169.751, 143.361, 124.316, 96.906, 70.624, 70.580, 69.564, 69.474, 69.152, 68.777, 66.214, 62.473, 61.037, 50.410, 20.911, 20.826, 20.740, 20.705.

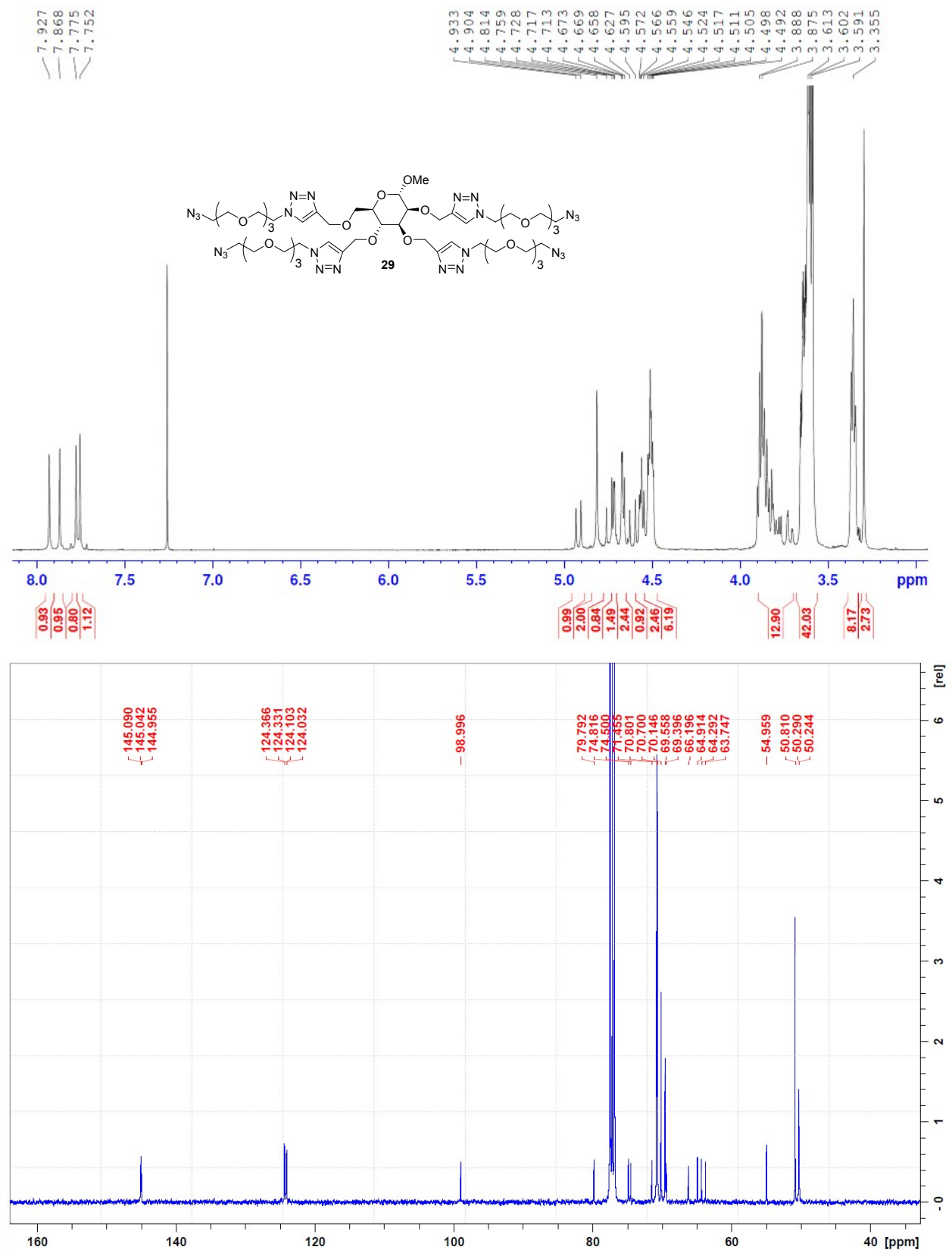
Compound 24



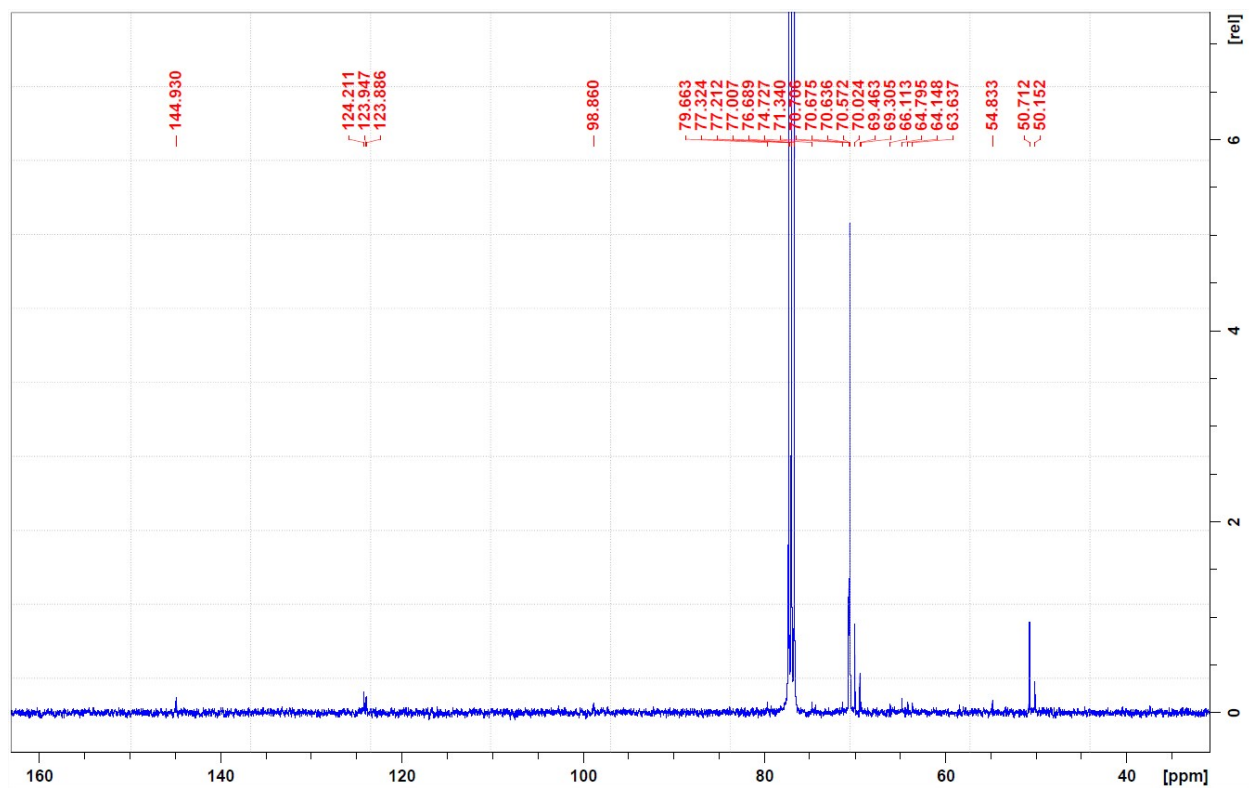
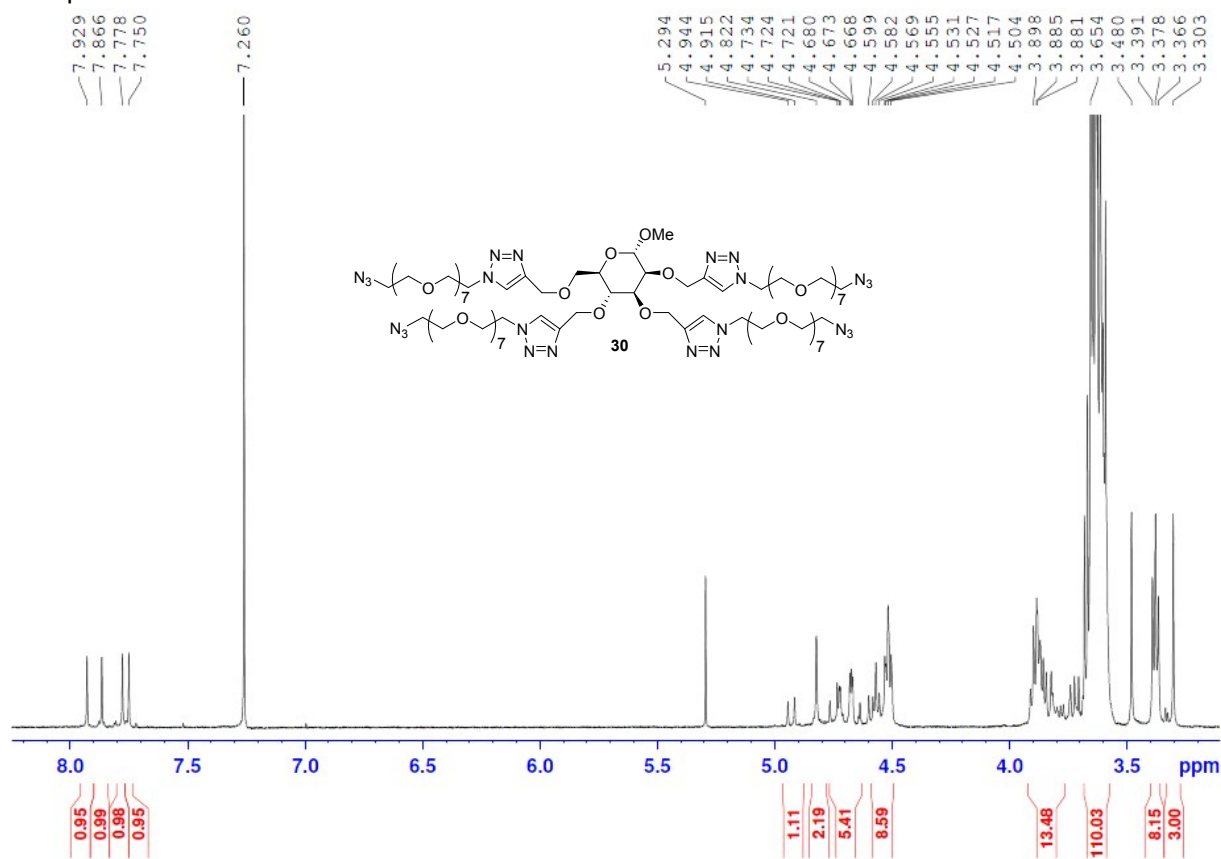
Compound 25



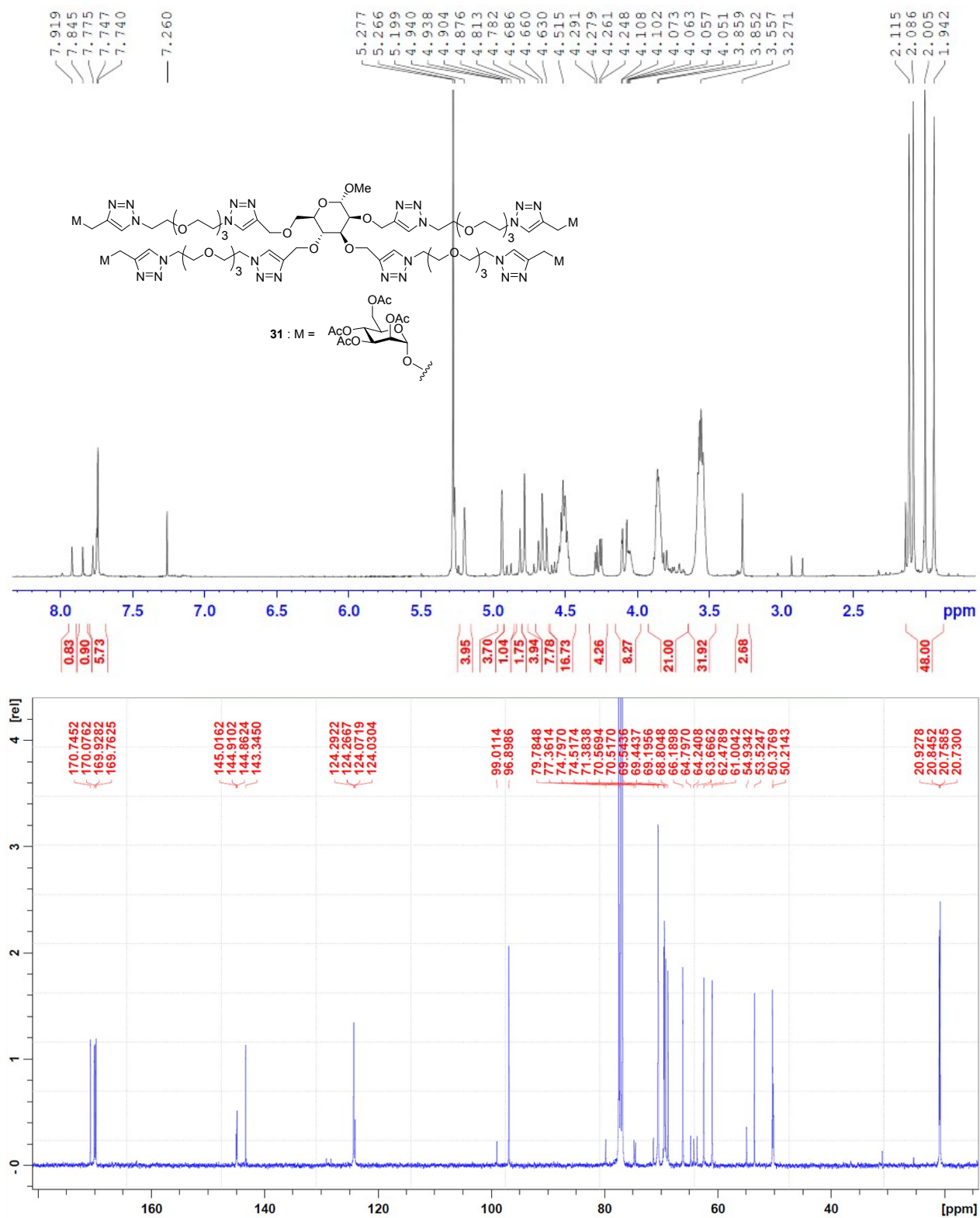
Compound 29



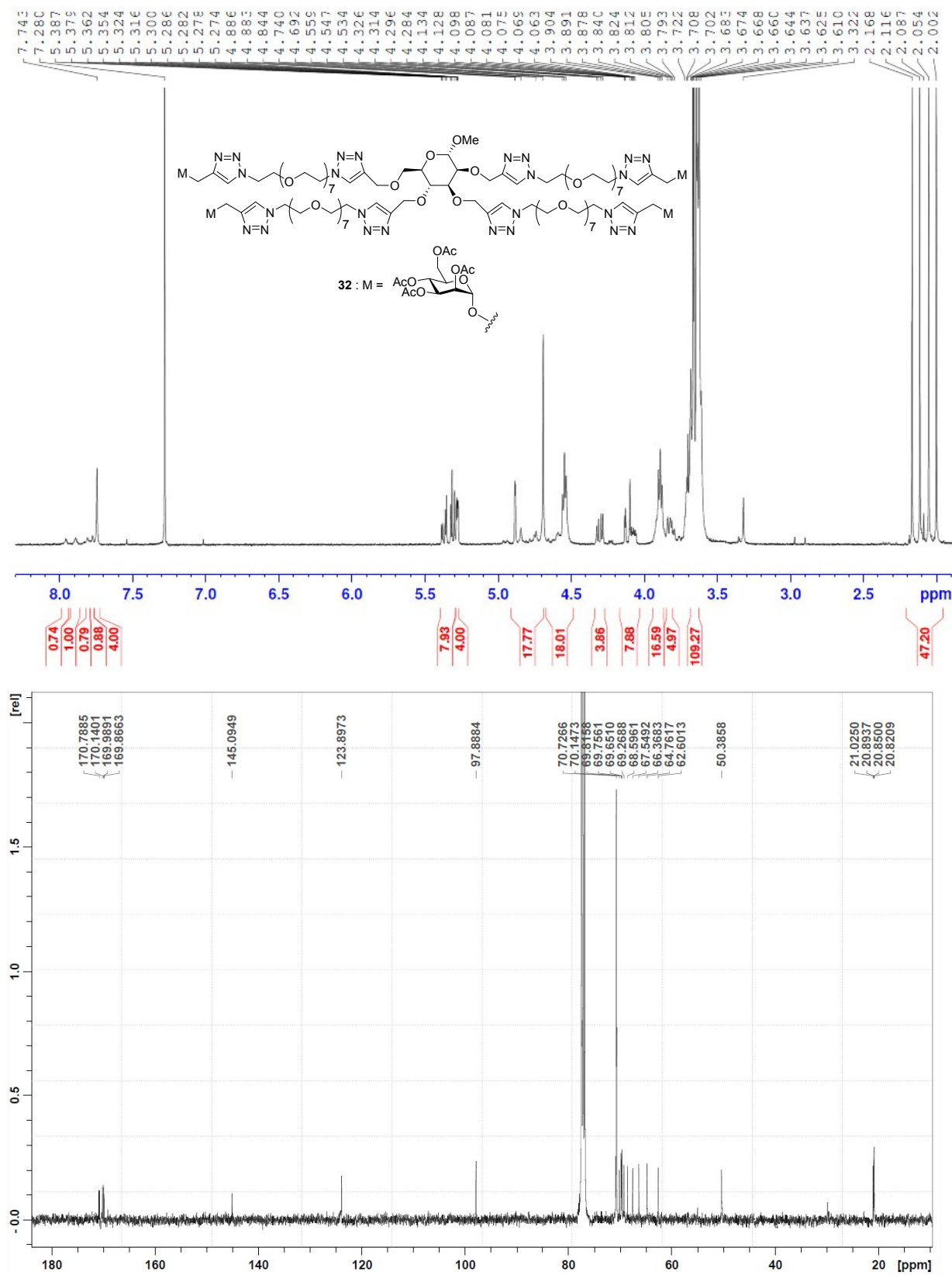
Compound 30



Compound 31



Compound 32



Chemical structures:

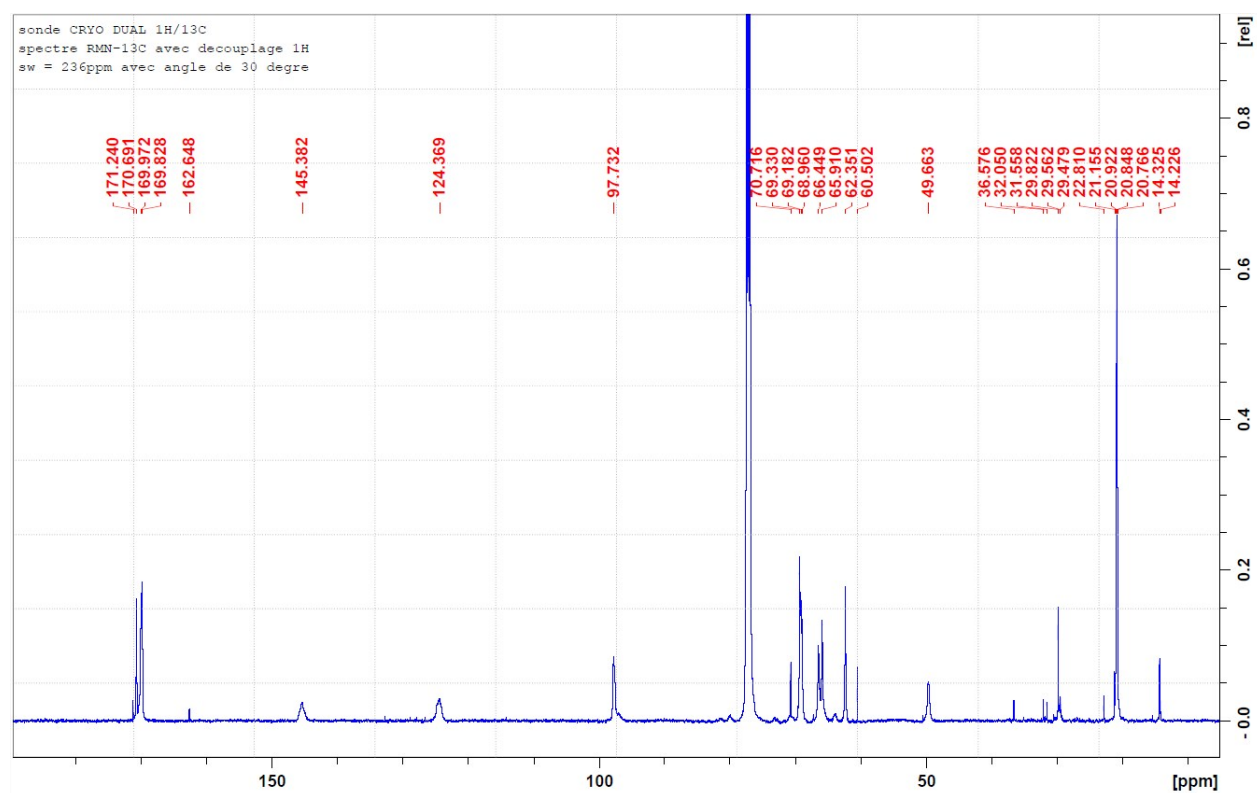
Repeating unit: R-O-(C6H6O5O)29-R

Substituent **34**: R = AcO[C@H]1[C@@H](O[C@@H]2[C@H](CO[C@@H]2COC3=CN=CN=C3CC)O[C@H](CO[C@@H]4[C@H](OC(=O)C)[C@@H](OC(=O)C)[C@H](OC(=O)C)O4)O[C@H](CO[C@@H]5[C@H](OC(=O)C)[C@@H](OC(=O)C)[C@H](OC(=O)C)O5)O[C@H]1

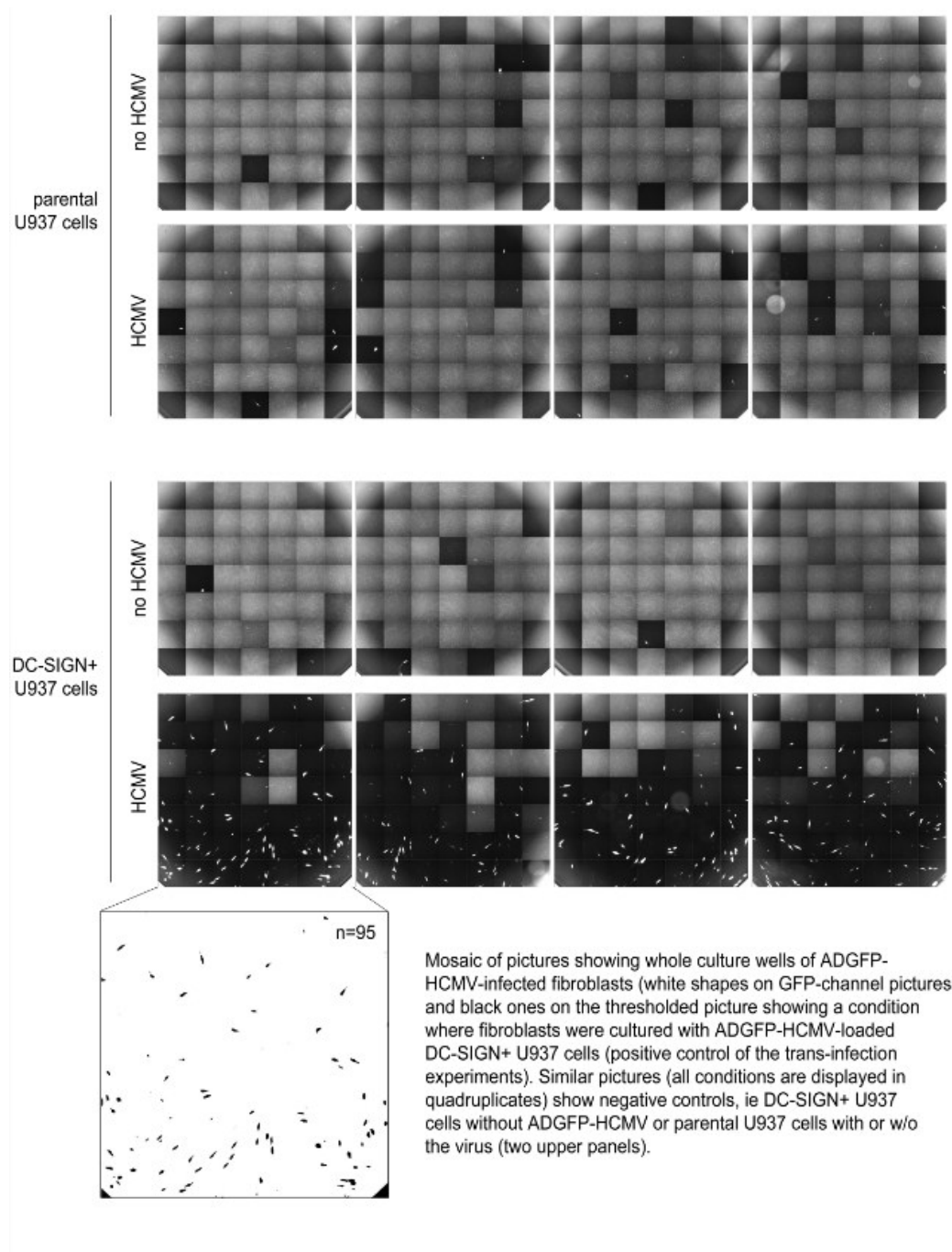
¹H NMR spectrum (CDCl₃):

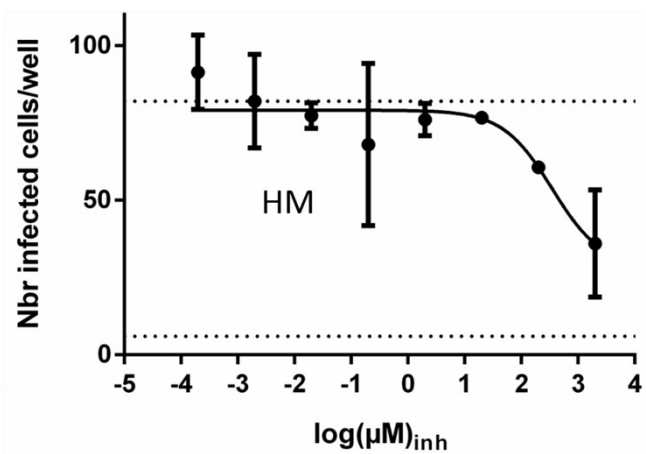
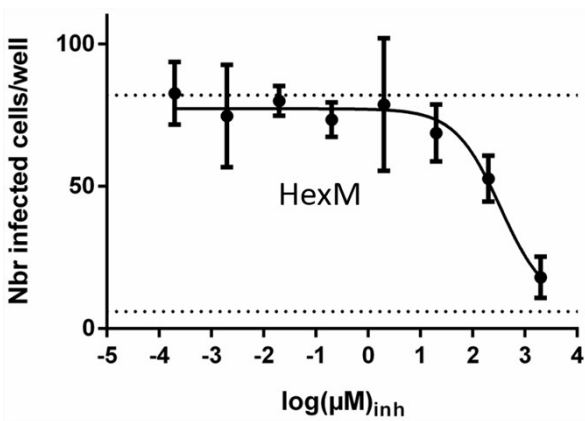
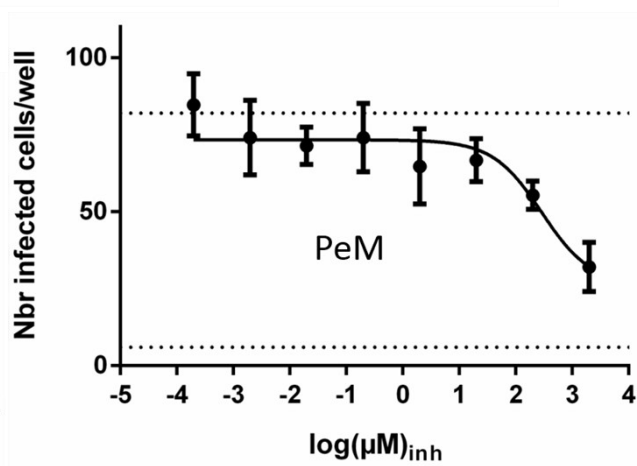
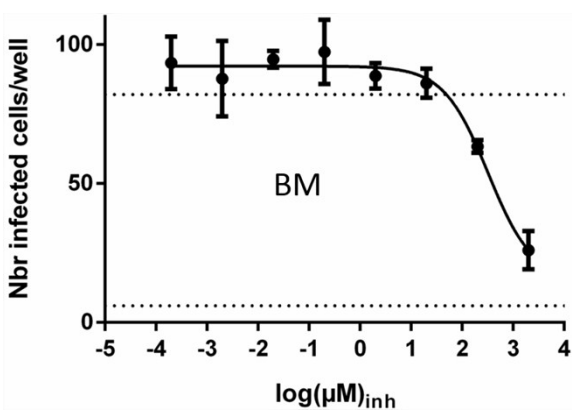
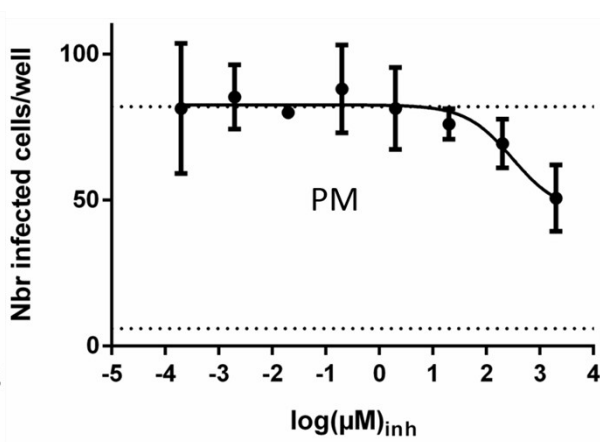
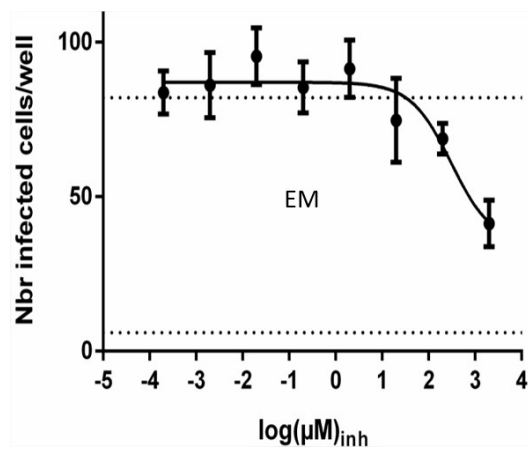
Chemical shift (ppm): 8.009, 7.946, 7.862, 5.186, 4.840, 4.829, 4.805, 4.609, 4.198, 4.041, 3.923, 3.897, 3.735, 3.632, 3.622, 2.103, 2.060, 2.033, 2.023, 2.014.

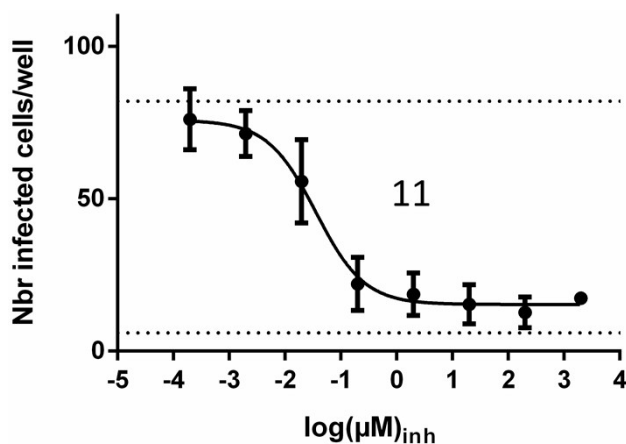
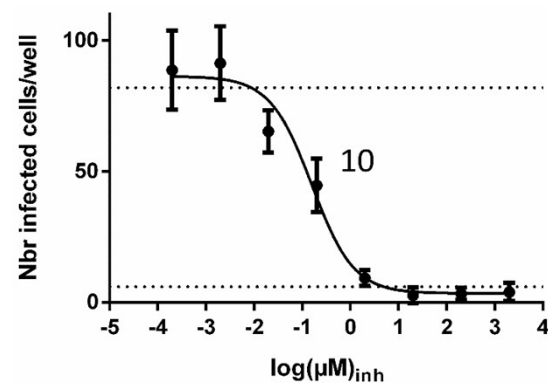
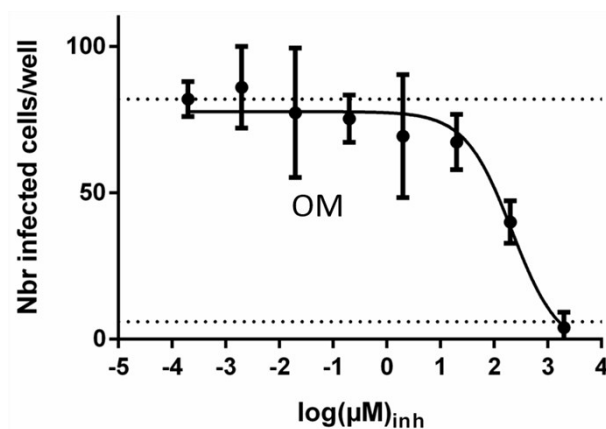
Integration values: 3.00, 7.89, 13.75, 26.60, 36.19.



Trans-infection experiments







Cpds	Val	IC ₅₀ ^{Mol} (μM)	IC ₅₀ ^{Lig} (μM)	IC ₅₀ ^{Mon*o} (μM, 95%CI)
MM	1	220	=	= (120-402)
EM	1	307	=	= (33-2900)
PM	1	307	=	= (35-2901)
BM	1	322	=	= (155-669)
PeM	1	289	=	= (65-1269)
HxM	1	337	=	= (97-1166)
HM	1	348	=	= (48-2497)
OM	1	213	=	= (77-594)
2	1	59	=	= (26-130)
10	23	0.021	0.474	0.159 (0.09-0.29)
11	89	0.0012	0.107	0.035 (0.018-0.071)
12	902	0.00002	0.018	0.006 (0.002-0.015)

* IC₅₀^{Mon*} = expressed in mol of monomer; 95% confidence intervals from triplicates