Electronic Supplementary Material (ESI) for Chemical Communications

Potent divalent inhibitors with rigid glucose click spacers for *Pseudomonas aeruginosa* lectin LecA

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Supporting information

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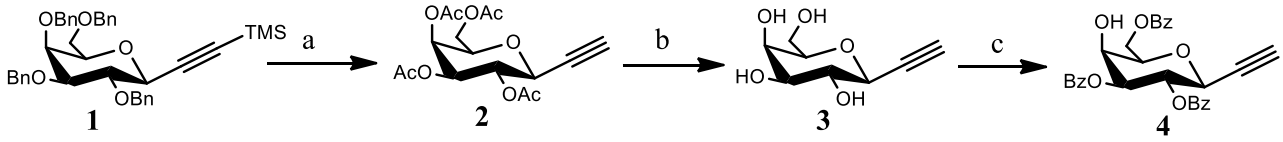
Experimental Section

General: Unless stated otherwise, chemicals were obtain from commercial sources and were used without further purification. Solvents were purchased from Biosolve (Valkenswaard, The Netherlands). All moisture-sensitive reactions were performed under nitrogen atmosphere. Anhydrous THF was dried over Na/benzophenone and freshly distilled prior to use. All the other solvents were dried over molecular sieves 4 Å or 3 Å. TLC was performed on Merck precoated Silica 60 plates. Spots were visualized by UV light, 10% H_2SO_4 in MeOH and triphenylphosphine in THF followed by Ninhydrine. Microwave reactions were carried out in a Biotage microwave Initiator (Uppsala, Sweden). The microwave power was limited by temperature control once the desired temperature was reached. Sealed vessels of 2-5 mL and 10-20 mL were used. Analytical HPLC runs were performed on a Shimadzu automated HPLC system with a reversed-phase column (Alltech, C8, 90 M, 5 mm, 250 L, 4.6 mm, Deerfield, IL, USA) that was equipped with an evoparitive light-scattering detector (PLELS 1000, Polymer Laboratories, Amherst, MA, USA) and a UV/Vis detector operating at 220 nm and 250 nm. Preparative HPLC runs were performed on an Applied Biosystems workstation. Elution was effected by using a linear gradient of 5% MeCN/0.1% TFA in H_2O to 5% H_2O/0.1% TFA in MeCN or by a gradient of 0.1% in H_2O to 30% MeCN/0.1% TFA in H_2O. ^1H NMR (300 MHz) and ^{13}C (75.5 MHz) were performed on a Varian G-300 spectrometer. HSQC, HMBC and TOCSY NMR (500 MHz) were performed with a VARIAN INOVA-500. Electrospray Mass experiments were performed in a Shimadzu LCMS QP-8000. High resolution mass spectrometry (HRMS) analysis was performed using an Applied Biosystems 4700 MALDI TOF/TOF instrument.

LecA inhibiton assay: The lectin LecA was obtained from Sigma-Aldrich and it was FITC labeled according to the procedure of Sigma-Aldrich.[19] Microarray experiments were performed by using PamChip arrays run on a PamStation12 instrument (Pam-Gene B.V., ‘s Hertogenbosch, The Netherlands). Data were obtained by real-time imaging of the fluorescence signal by a CCD camera. Images were analyzed by using BioNavigator software (Pam-Gene). Each array slide contains spots in duplicate. The fluorescence intensities were expressed in arbitrary units and the relative intensities

were the average of the two duplicate spots. Aliquots of a solution of FITC-labeled LecA (20 mg mL-1) in HEPES/PBS buffer (10 mM HEPES, 100 mM NaCl, 0.1% BSA. pH 7.4), containing different concentrations of the inhibitors were incubated for 1 h at r.t. and subsequently added to the glycodendrimer chip. The binding process was monitored for 2 h and the end values of the fluorescence detection were taken for the determination of the IC50 by using Prism 5 (Graphpad Software, Inc.).



Scheme 1. Preparation of the building block **4**. a) i. $BCl_3 \cdot SMe_2$, DCM, microwave, 80°C, 20'; ii. Ac_2O, Py, 70% 2 steps; b) MeONa, MeOH, quant.; c) BzCl, Py, -40 °C, 30%

3,7-Anhydro-4,5,6,8-tetra-*O*-acetyl-1,1,2,2-tetradehydro-1,2-D-glycero-L-mannooctitol, 2. A $BCl_3 \cdot SMe_2$ solution (2M, 6.44 mL, 12.88 mmol) in CH_2Cl_2 was added to a solution of **1** (1 g, 1.61 mmol) in CH_2Cl_2 (12 mL). The mixture was heated under microwave irradiation at 80°C for 20 min. The resulting black solution was neutralized with a saturated $NaHCO_3$ solution (80 mL). The liquid was evaporated, methanol was added and the suspension was filtered. The solvent was evaporated under vacuum and pyridine (15 mL) was added to the residue. The solution was treated with acetic anhydride (2.46 mL, 26 mmol) and the mixture was stirred overnight at r.t.. The mixture was concentrated under vacuum and the residue was dissolved in CH_2Cl_2, washed with 1M $KHSO_4$ solution (15 mL), H_2O (15 mL) and brine (15 mL). The organic layer was dried over sodium sulfate. After evaporation of the solvent the product was purified by column chromatography (ethyl acetate/hexane, 4:6) to give **2** as a white solid (0.430 g, 1.13 mmol, 70%). ^1H NMR (300MHz, $CDCl_3$): δ, 5.39 (d, 1H, $J_{6,5}$=3.34 Hz, H-6), 5.38 (t, 1H, $J_{4,3}$=$J_{4,5}$=9.95 Hz, H-4), 4.97 (dd, 1H, $J_{5,4}$=9.95 Hz, $J_{5,6}$=3.34 Hz, H-5), 4.16 (dd, 1H, $J_{3,1}$=2.19, $J_{3,4}$=9.95 Hz, H-3), 4.09 (d, 2H, $J_{8a,7}$=$J_{8b,7}$=6.40 Hz, H-8ab), 3.88 (t, 1H, $J_{7,8a}$=$J_{7,8b}$=6.40 Hz, H-7), 2.50 (d, $J_{1,3}$=2.19 Hz, H-1), 2.13, 2.05, 2.02 and 1.96 (4s, 4H, $COCH_3$). ^{13}C NMR (75.5 MHz, $CDCl_3$): δ ppm 170.59 (C=O), 170.40 (C=O), 170.26 (C=O), 169.53 (C=O). 78.10 (C-1), 75.45 (C-2), 74.79 (C-7), 71.62 (C-5), 69.07 (C-3), 68.44 (C-4), 67.48

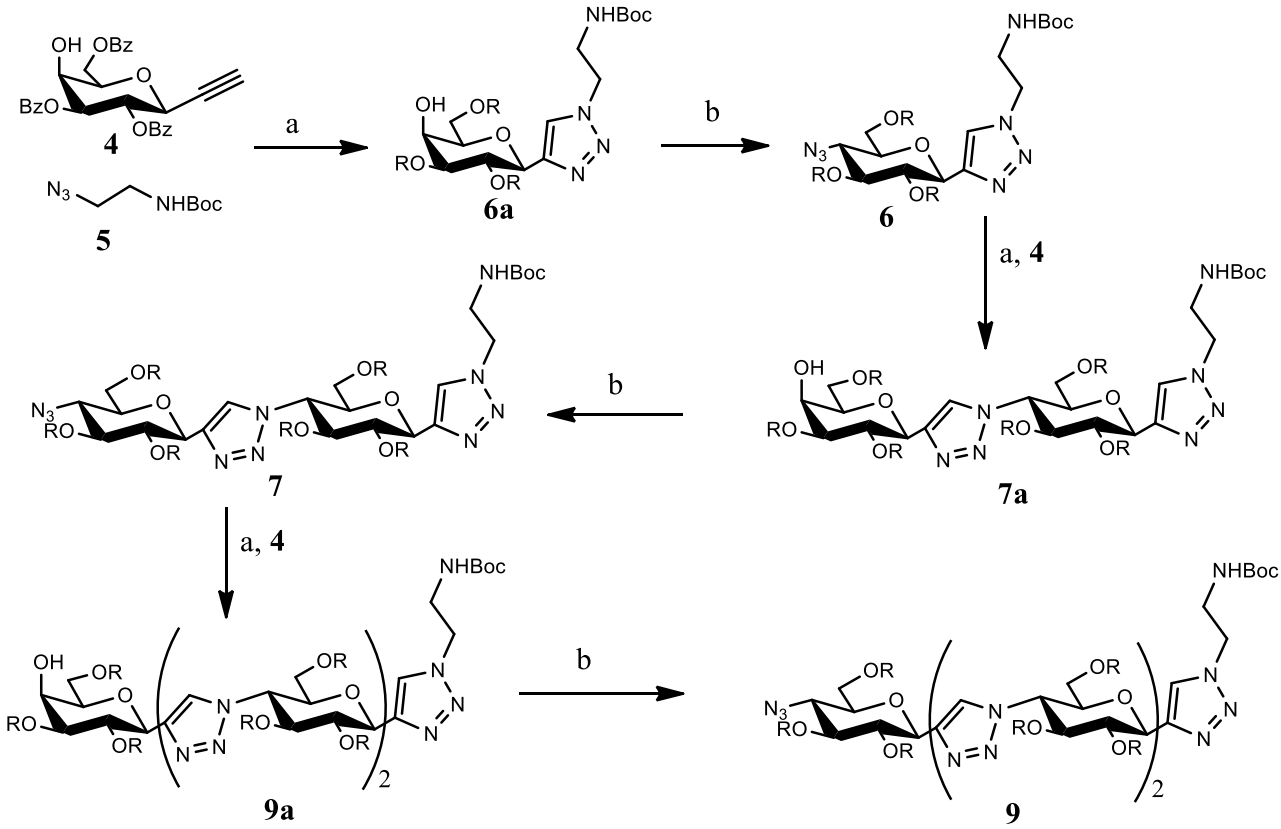
(C-6), 61.78 (C-8), 20.09 ($CO\underline{C}H_3$). MS (ESI) *m/z* calcd for $C_{16}H_{21}O_9$ $(M+H)^+$ 357.12, found 357.32.

3,7-Anhydro-1,1,2,2-tetradehydro-1,2-D-glycero-L-mannooctitol, 3. A solution of **2** (3 g, 8.42 mmol) in methanol (25 mL) was treated with a solution of NaOMe in methanol (30%, 1 mL) and the mixture was stirred for 4 h at r.t. After neutralization with DowexH$^+$, the mixture was filtered and the methanol evaporated in vacuum to give compound **3** as a white foam (1.57 g, 8.35 mmol, quant.) ^{1}H NMR (300MHz, CD_3OD): δ, 3.86 (d, 1H, $J_{6,5}$=3.2 Hz, H-6), 3.86 (dd, 1H, $J_{3,1}$=2.19, $J_{3,4}$=9.55 Hz, H-3), 3.73 (d, 1H, $J_{8a,7}$=6.05 Hz, $J_{8a,8b}$=11.45 Hz, H-8a), 3.66 (d, 1H, $J_{8b,7}$=6.05 Hz, $J_{8b,8a}$=11.45 Hz, H-8b), 3.65 (t, 1H, $J_{4,3}$=$J_{4,5}$=9.55 Hz, H-4), 3.48 (t, 1H, $J_{7,8a}$=$J_{7,8b}$=6.05 Hz, H-7), 3.40 (dd, 1H, $J_{5,4}$=9.55 Hz, $J_{5,6}$=3.30 Hz, H-5), 2.85 (d, $J_{1,3}$=2.19 Hz, H-1). ^{13}C NMR (75.5 MHz, CD_3OD): δ ppm 82.04 (C-1), 80.67 (C-7), 75.70 (C-5), 75.06 (C-2), 72.43 (C-6), 72.25 (C-4), 70.54 (C-3), 62.69 (C-8). MS (ESI) *m/z* calcd for $C_8H_{13}O_5$ $(M+H)^+$ 189.08, found 189.40.

3,7-Anhydro-4,5,8-tri-*O*-acetyl-1,1,2,2-tetradehydro-1,2-D-glycero-L-mannooctitol, 4. Benzoyl chloride (1.97 mL, 17 mmol) in pyridine (10 mL) was added dropwise to a solution of **3** (1 g, 5.3 mmol) in pyridine (25 mL) previously cooled at -40 C. The mixture was kept at -40 C for 30 min and the reaction was quenched with water (50 mL). The product was extracted three times with CH_2Cl_2 (30 mL). After evaporation of the liquid, the residue was dissolved in CH_2Cl_2 and washed with 1M $KHSO_4$, H_2O and brine. The organic layer was dried over sodium sulfate. TLC analysis showed the presence of four different benzoylated compounds, monitored by TLC. The desired compound **4** (toluene/ethylacetate, 4/1; R_f=0.52) was obtained after column chromatography (toluene/ethylacetate, 9:1, 0.796 g, 1.59 mmol, 30%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.05-7.26 (m, 15H, Ar), 6.04 (t, 1H, $J_{4,3}$=$J_{4,5}$=10.18 Hz, H-4), 5.37 (dd, 1H, $J_{5,4}$= 10.18 Hz, $J_{5,6}$=2.95 Hz, H-5), 4.70 (dd, 1H, $J_{8a,7}$= 5.98 Hz, $J_{8a,8b}$=11.60 Hz H-8a), 4.59 (dd, 1H, $J_{8b,7}$= 5.98 Hz, $J_{8b,8a}$=11.60 Hz, H-8b), 4.48 (dd, 1H, $J_{3,1}$=2.05, $J_{3,4}$= 10.18 Hz, H-3), 4.45 (d, 1H, $J_{6,5}$=2.95 Hz, H-6), 4.10 (t, 1H, $J_{7,8a}$=$J_{7,8b}$= 5.98 Hz, H-7), 3.22 (s, 1H, OH), 2.47 (d, 1H, $J_{1,3}$=2.05 Hz H-1). ^{13}C NMR (75.5 MHz, $CDCl_3$): δ ppm 166.62 (C=O), 165.94 (C=O), 165.39 (C=O), 133.47 (C, Ar), 133.32 (C, Ar), 133.30 (C, Ar), 129-129.79 (CH, Ar), 129.50

(CH, Ar), 129.28 (CH, Ar), 128.94 (CH, Ar), 128.50-128.40 (CH, Ar), 78.30 (C-1), 76.43 (C-2), 75.38 (C-7), 74.81 (C-5), 69.16 (C-4), 68.99 (C-3), 67.63 (C-6), 63.53 (C-8). MS (ESI) *m/z* calcd for $C_{29}H_{25}O_8$ $(M+H)^+$ 501.15, found 501.30. The other compounds were collected (1.9 g) and used to recovery compound **2**.

Recovery of compound 2. The crude benzoylated compounds (1.9 g) were dissolved in methanol (20 mL) and treated with 500 μL of 30% MeONa solution in methanol. After 4 h the reaction mixture was neutralized with Dowex and the methanol was removed under vacuum. The residue obtained was dissolved in pyridine 10 mL and 3 mL of acetic anhydride was added. The mixture was stirred at r.t. and it was concentrated under vacuum. The residue was dissolved in CH_2Cl_2 and washed with 1M $KHSO_4$, H_2O and brine. The organic layer was dried over sodium sulfate. After evaporation of the solvent the product was purified by column chromatography (ethyl acetate/hexane, 4:6) to give **2** (1.23 g, 3.45 mmol, 65%).



Scheme 2. Synthesis and elongation of the spacer. a) $CuSO_4$, Na-ascorbate, DMF, 10% H_2O, 30', 80°C, microwave, 85-91%; b) i. Tf_2O, Py, DCM, 0°C, 1h; ii. NaN_3, DMF, r.t., 4h, 80-85% 2 steps.

6a. Compound **5**[20] (0.225 g, 1.2 mmol), $CuSO_4 \cdot 5H_2O$ (0.038 g, 0.15 mmol) and sodium ascorbate (0.060 g, 0.3 mmol) were added to a solution of **4** (0.5 g, 1 mmol) in DMF (15 mL) containing 10% water. The mixture was heated under microwave irradiation at 80°C for 30 min. After evaporation of the solvent the residue was dissolved in CH_2Cl_2. The organic solution was washed three times with water and brine and it was dried over sodium sulfate. The solvent was removed and the white solid compound **5** was purified by column chromatography (EtOAc/Hexane, 2:3) (0.625g, 0.91 mmol, 91%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.02-7.29 (m, 15H, Ar), 7.80 (s, 1H, H-3), 6.07 (t, 1H, $J_{6,5}$=$J_{6,7}$=9.95 Hz, H-6), 5.56 (dd, 1H, $J_{7,6}$=9.95, $J_{3,4}$=3.15 Hz, H-7), 5.04 (d, 1H, $J_{5,6}$=9.95 Hz, H-5), 4.94 (t, 1H, $J_{NH,1ab}$=4.55 Hz, NH), 4.71 (dd, 1H, $J_{10a,9}$=5.94 Hz, $J_{10a,10b}$=11.38 Hz, H-10a), 4.56 (dd, 1H, $J_{10b,9}$=5.94 Hz, $J_{10b,10a}$=11.38 Hz, H-10b), 4.53 (d, 1H, $J_{8,7}$=3.15 Hz, H-8), 4.34 (m, 2H, H-2ab), 4.27 (t, 1H, $J_{9,10a}$=$J_{9,10b}$=5.94 Hz, H-9), 3.49 (m, 2H, H-1ab), 3.38 (s, 1H, OH), 1.42 (s, 9H, $C(CH_3)_3$. ^{13}C NMR (75.5 MHz, $CDCl_3$): δ 166.84 (C=O), 166.02 (C=O), 165.92 (C=O), 156.16 (NH<u>C</u>=O), 145.04 (C-4), 133.80-133.36 (C, Ar), 130.23-128.41 (CH, Ar), 123.52 (C-3), 77.60 (C-9), 75.29 (C-7), 74.15 (C-5), 70.09 (C-6), 68.00 (C-8), 63.51 (C-10), 50.56 (C-2), 40.83 (C-1), 80.17 (<u>C</u>$(CH_3)_3$), 28.53 (C(<u>C</u>$H_3)_3$). MS (ESI) *m/z* calcd for $C_{36}H_{39}N_4O_{10}$ 687.27 ($M+H^+$), found 687.00.

General procedure for the introduction of the azide, step b. Preparation of compounds 6, 7 and 9: Triflic anhydride (10 equiv) was added dropwise to a solution of the carbohydrate (1 equiv) in CH_2Cl_2 (20 mL) containing pyridine 10 %, previously cooled at 0°C. The solution was stirred at 0°C for 1.5 h and the reaction was quenched adding cold 1 M $KHSO_4$ (20 mL). The organic layer was washed two times with cold water and cold brine and dried over sodium sulfate. The solvent was evaporated giving the triflate-carbohydrate compound as a yellow solid which was used without any further purification. The solid was dissolved in DMF (20 mL) and sodium azide (5 equiv) was added. The reaction mixture was stirred at r.t. for 5 h. The solvent was evaporated under vacuum and the residue was dissolved in CH_2Cl_2 and washed three times with water and brine. The organic layer was dried over sodium sulfate. The solvent was evaporated and the residue purified by column chromatography to give a yellowish solid.

General procedure for the "click reaction", step a. Preparation of compounds 7a and 9a: The reactions between compound **4** and the azide–carbohydrate compounds **6** and **7,** respectively, were performed following the experimental procedure reported for the synthesis of the compound **6a**.

6. (0.527 g, 0.74 mmol, 85%) ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.09-7.28 (m, 15H, Ar), 7.66 (s, 1H, H-3), 5.84 (t, 1H, $J_{7,6}$=$J_{7,8}$=9.84 Hz, H-7), 5.65 (t, 1H, $J_{6,5}$=$J_{6,7}$=9.84 Hz, H-6), 5.02 (d, 1H, $J_{5,6}$=9.84 Hz, H-5), 4.85 (t, 1H, $J_{NH,1ab}$=4.55 Hz, NH), 4.74 (dd, 1H, $J_{10a,6}$=2.16 Hz, $J_{10a,10b}$=12.53 Hz, H-10a), 4.62 (dd, 1H, $J_{10b,9}$=4.53 Hz, $J_{10b,10a}$=12.53, Hz, H-10b), 4.39 (m, 2H, H-2ab), 4.03 (t, 1H, $J_{8,7}$=$J_{8,9}$=9.84 Hz, H-8); 3.98 (m, 1H, H-9), 3.52 (m, 2H, H-1ab), 1.40 (s, 9H, $C(CH_3)_3$). ^{13}C NMR (75.5 MHz, $CDCl_3$): δ 166.35 (C=O), 165.82 (C=O), 165.68 (C=O), 156.03 (NH<u>C</u>=O), 144.28 (C-4), 133.97-133.24 (C, Ar), 130.52-128.05 (CH, Ar), 123.34 (C-3), 80.17 ($\underline{C}(CH_3)_3$), 77.33 (C-9), 73.95 (C-5), 75.11 (C-7), 72.36 (C-6), 63.88 (C-10), 61.35 (C-8), 50.52 (C-2), 40.81 (C-1), 28.53 ($C(\underline{C}H_3)_3$). MS (ESI) *m/z* calcd for $C_{36}H_{38}N_7O_9$ $(M+H)^+$ 712,27, found 712.25.

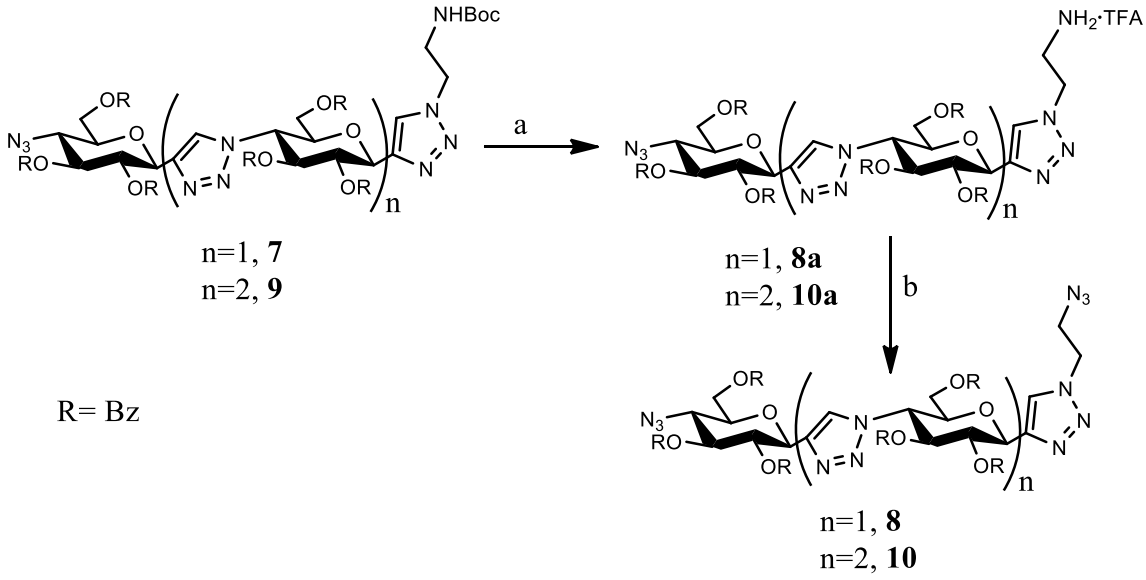
7a. (0.775 g, 0.64 mmol, 86%) ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.1 (1H, H-3'), 7.99-7.11 (m, 30H, Ar), 7.71 (1H, H-3), 6.31 (1H, H-7), 5.93 (1H, H-6'), 5.81 (1H, H-6), 5.52 (1H, H-7'), 5.30 (1H, H-8), 5.28 (1H, H-5), 4.95 (1H, H-5'), 4.89 (1H, NH), 4.71 (1H, H-9), 4.65 (1H, H-10a'), 4.52 (1H, H-10b'), 4.45 (1H, H-10a), 4.40 (1H, H-8'), 4.38 (2H, H-2ab), 4.20 (1H, H-9'), 3.93 (1H, H-10b), 3.50 (2H, H-1ab), 3.50 (1H, OH), 1.38 (9H, $C(CH_3)_3$). ^{13}C NMR (75.5 MHz, $CDCl_3$): δ 166.75 (C=O), 166.02 (C=O), 165.88 (C=O), 165.73 (C=O), 165.63 (C=O), 165.17 (C=O), 165.08 (NH<u>C</u>=O), 156.08 (NH<u>C</u>=O), 145.88 (C-4'), 144.29 (C-4), 134.36-132.83 (Ar), 130.01-127.89 (Ar), 123.90 (C-3), 123.02 (C-3'), 80.13 ($\underline{C}(CH_3)_3$), 77.33 (C-9), 76.87 (C-9'), 75.27 (C-7'), 74.32 (C-5'), 74.20 (C-5), 73.97 (C-7), 72.97 (C-6), 70.32 (C-6'), 68.03 (C-8'), 63.49 (C-10'), 62.68 (C-10), 61.07 (C-8), 50.53 (C-2), 40.77 (C-1), 28.52 ($C(\underline{C}H_3)_3$). MS (ESI) *m/z* calcd for $C_{65}H_{62}N_7O_{17}$ $(M+H)^+$ 1212,42, found 1212.25.

7. (0.593 g, 0.48 mmol, 83%) ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.09-7.14 (m, 15H, Ar), 7.85 (1H, H-3'), 7.69 (1H, H-3), 6.25 (1H, H-7), 5.53 (1H, H-6'), 5.81 (1H, H-6), 5.80 (1H, H-7'), 5.23 (1H, H-8), 5.25 (1H, H-5), 4.95 (1H, H-5'), 4.86 (1H, NH), 4.64 (1H, H-9), 4.66 (1H, H-10a'), 4.61 (1H, H-10b'), 4.40 (1H, H-10a), 3.97 (1H, H-8'), 4.39 (2H, H-2ab), 3.90 (1H, H-9'), 3.98 (1H, H-10b), 3.52 (2H, H-1ab), 1.39 (9H, $C(CH_3)_3$). ^{13}C NMR (75.5 MHz, $CDCl_3$): δ 166.36 (C=O), 165.88 (C=O), 165.80 (C=O), 165.55 (C=O), 165.49 (C=O), 165.07 (C=O), 156.03 (NH<u>C</u>=O), 145.08 (C-4'), 144.06 (C-4), 133.80-133.24 (C, Ar), 130.24-128.29 (CH, Ar), 123.40 (C-3), 122.27 (C-3'), 80.18 (<u>C</u>$(CH_3)_3$), 77.32 (C-9), 77.25 (C-9'), 75.11 (C-7'), 74.25 (C-5), 73.89 (C-5'), 73.82 (C-7), 72.78 (C-6), 72.59 (C-6'), 64.02 (C-10'), 62.73 (C-10), 61.44 (C-8'), 61.13 (C-8), 50.53 (C-2), 40.77 (C-1), 28.52 (C(<u>C</u>$H_3)_3$). MS (ESI) *m/z* calcd for $C_{58}H_{56}N_{10}O_{15}$ $(M+H)^+$ 1237.43, found 1238.00.

9a. (0.618 g, 0.36 mmol, 80%) ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.03-7.10 (m, 45H, Ar), 7.93 (2H, H-3', H-3"), 7.65 (1H, H-3), 6.25 (2H, H-7, H-7'), 5.93 (1H, H-6''), 5.76 (1H, H-6), 5.68 (1H, H-6'), 5.48 (1H, H-7''), 5.23 (1H, H-5), 5.22 (1H, H-8), 5.19 (1H, H-8'), 5.17 (1H, H-5'), 4.91 (1H, H-5''), 4.84 (1H, NH), 4.70 (1H, H-9), 4.68 (1H, H-10a''), 4.65 (1H, H-9'), 4.47 (1H, H-10b''), 4.40 (2H, H-10a, H-8''), 4.38 (3H, H-2ab, H-10a'), 4.15 (1H, H-9''), 4.00 (1H, H-10b'), 3.95 (1H, H-10b), 3.48 (2H, H-1ab), 1.38 (s, 9H, $C(CH_3)_3$). ^{13}C NMR (75.5 MHz, $CDCl_3$): δ 166,74-165,00 (C=O), 156.06 (NH<u>C</u>=O), 145.80 (C-4''), 145.06 (C-4'), 144.16 (C-4), 134.00-132.82 (Ar), 130.30-128.00 (Ar), 123.41 (C-3), 122.51 (C-3'', C-3'), 80.16 (<u>C</u>$(CH_3)_3$), 77.45 (C-9, C-9'), 77.00 (C-9''), 75.28 (C-7''), 74.14 (C-5''), 73.97 (C-5, C-5'), 73.67 (C-7, C-7'), 72.91 (C-6'), 72.72 (C-6), 70.19 (C-6''), 67.88 (C-8''), 63.41 (C-10''), 62.87 (C-10'), 62.64 (C-10), 61.08 (C-8, C-8'), 50.49 (C-2), 40.79 (C-1), 28.53 (C(<u>C</u>$H_3)_3$). MS (ESI) *m/z* calcd for $C_{94}H_{85}N_{10}O_{24}$ $(M+2H)^{2+}$ 869.29, found 869.90.

9. (0.486 g, 0.28 mmol, 80%) ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.10-7.11 (m, 45H, Ar), 7.88 (s, 1H, H-3'), 7.81 (s, 1H, H-3''), 7.69 (s, 1H, H-3), 6.24 (1H, H-7), 6.21 (1H, H-7'), 5.79 (1H, H-6''), 5.78 (1H, H-7''), 5.63 (1H, H-6), 5.50 (1H, H-6'), 5.23 (1H, H-5), 5.21 (1H, H-8), 5.14 (1H, H-8'), 5.09 (1H, H-5'), 4.91 (1H, H-5''), 4.87 (1H, NH),

4.65 (1H, H-10a''), 4.63 (1H, H-9), 4.61 (1H, H-10b''), 4.53 (1H, H-9'), 4.42 (3H, H-10a, H-2ab), 4.32 (1H, H-10a'), 3.95 (3H, H-10b', H-10b, H-8''), 3.86 (1H, H-9''), 3.50 (2H, H-1ab), 1.39 (s, 9H, $C(CH_3)_3$). ^{13}C NMR (75.5 MHz, $CDCl_3$): δ 166,35-165,02 (C=O), 156.02 (NH<u>C</u>=O), 145.04 (C-4''), 144.85 (C-4'), 144.06 (C-4), 133.99-133.05 (C, Ar), 130.43-128.00 (CH, Ar), 123.41 (C-3), 122.39 (C-3''), 122.26 (C-3'), 80.19 ($\underline{C}(CH_3)_3$), 77.50 (C-9, C-9'), 77.30 (C-9''), 74.95 (C-7''), 74.22 (C-5), 74.05 (C-5'), 73.80 (C-5''), 73.70 (C-7), 73.55 (C-7'), 72.66 (C-6, C-6''), 72.36 (C-6'), 63.88 (C-10''), 62.71 (C-10'), 62.61 (C-10), 61.34 (C-8''), 61.19 (C-8), 61.02 (C-8'), 50.54 (C-2), 40.81 (C-1), 28.53 ($C(\underline{C}H_3)_3$). MS (ESI) *m/z* calcd for $C_{87}H_{78}N_{13}O_{21}$ $(M+2H)^{2+}$ 881.80, found 882.50.



Scheme 3. a) TFA, DCM, quant. b) imidazole-1-sulfonyl azide, K_2CO3, $CuSO_4$, MeOH, 82-85%.

General procedure for the removal of the Boc, step a. Preparation of compounds 8a and 10a. Trifluoroacetic acid (5 mL, 50%) was added to a solution of **7** and **9** (0.16 mmol), respectively, in CH_2Cl_2 (10 mL). After 2 h the solvent was evaporated under vacuum to give the compounds **8a** and **10a**, respectively, as yellowish solids that were used without further purification.

8a. MS (ESI) *m/z* calcd for $C_{60}H_{52}N_{10}O_{14}$ $(M+H)^+$ 1137.57, found 1137.37.

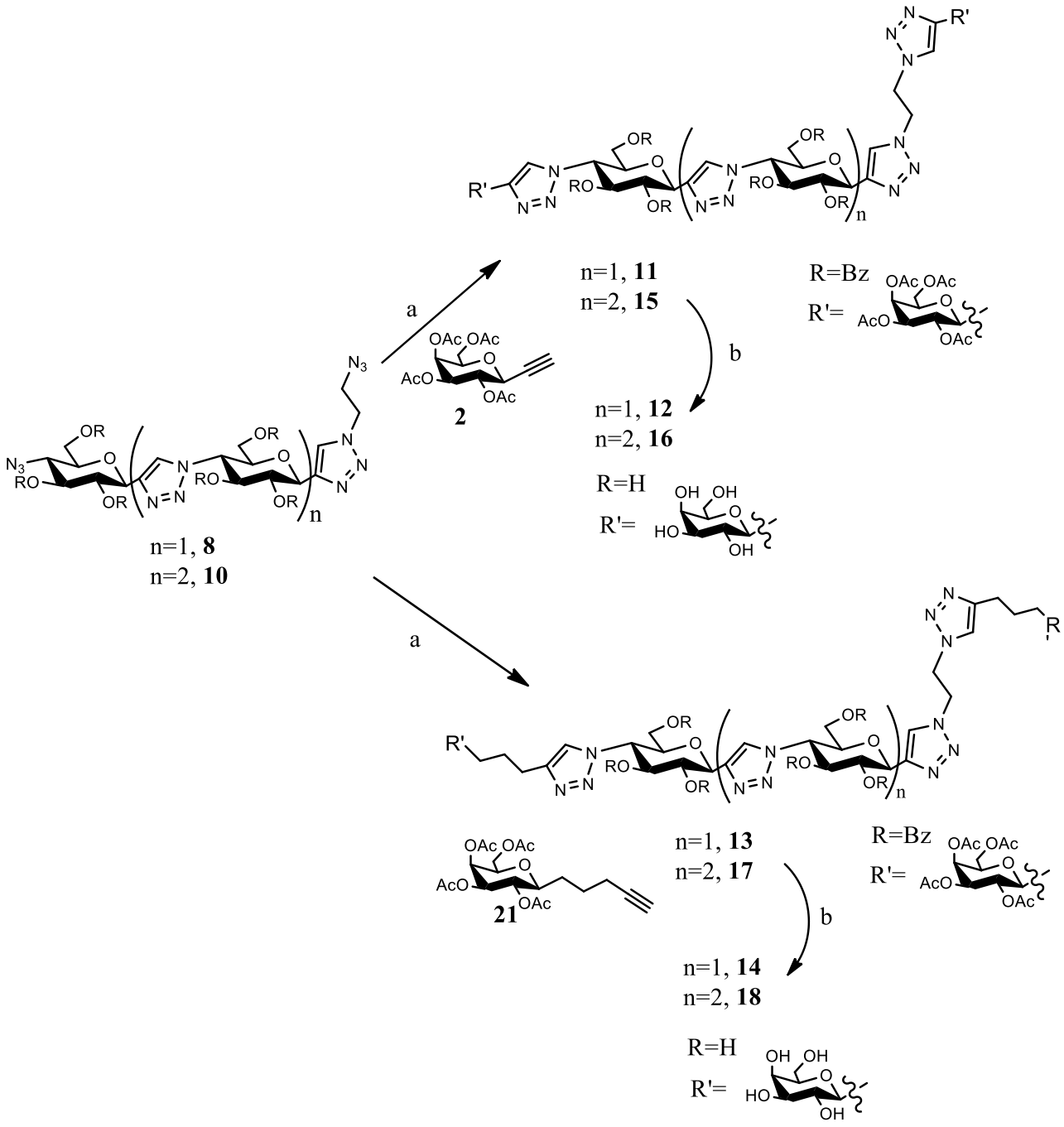
10a. MS (ESI) *m/z* calcd for $C_{89}H_{75}N_{13}O_{21}$ $(M+2H)^{2+}$ 831.77, found 832.30.

General procedure for the diazotransfer, step b. Preparation of compounds 8 and 10. Imidazole-1-sulfonyl azide hydrochloride (1.2 equiv) was added to the ammonium salt **8a** and **10a** (1 equiv), respectively, K_2CO_3 (2.5 equiv) and $CuSO_4 \cdot 5H_2O$ (0.01 equiv) in MeOH (5 mL) and the mixture was stirred at r.t. for 4 h. The solvent was removed under vacuum and the residue was dissolved in CH_2Cl_2 and washed three times with water and brine. After drying over sodium sulfate the solvent was removed and the compound purified by column chromatography.

8. (0.160 g, 0.14 mmol, 85% from **7**) ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.08-7.12 (m, 30H, Ar), 7.92 (1H, H-3), 7.78 (1H, H-3'), 6.28 (1H, H-7), 5.55 (1H, H-6'), 5.91 (1H, H-6), 5.81 (1H, H-7'), 5.26 (1H, H-8), 5.32 (1H, H-5), 4.96 (1H, H-5'), 4.67 (1H, H-9), 4.63 (1H, H-10a'), 4.59 (1H, H-10b'), 4.39 (1H, H-10a), 3.97 (1H, H-8'), 4.33 (2H, H-2ab), 3.90 (1H, H-9'), 3.98 (1H, H-10b), 3.60 (2H, H-1ab). ^{13}C NMR (75.5 MHz, $CDCl_3$): δ 166.37 (C=O), 165.89 (C=O), 165.86 (C=O), 165.54 (C=O), 165.46 (C=O), 165.14 (C=O), 145.03 (C-4'), 144.14 (C-4), 134.26-133.92 (Ar), 130.46-128.91 (Ar), 123.72 (C-3), 122.51 (C-3'), 77.25 (C-9'), 77.22 (C-9), 75.16 (C-7'), 74.00 (C-5), 73.87 (C-5'), 74.10 (C-7), 72.63 (C-6'), 72.38 (C-6), 64.07 (C-10'), 62.76 (C-10), 61.43 (C-8'), 61.14 (C-8), 50.66 (C-1), 49.59 (C-2). MS (ESI) *m/z* calcd for $C_{60}H_{51}N_{12}O_{14}$ $(M+H)^+$ 1163,36, found 1163.60

10. (0.238 g, 0.14 mmol, 83% from **9**) ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.10-7.13 (m, 45H, Ar), 7.87 (s, 1H, H-3''), 7.80 (s, 1H, H-3'), 7.77 (s, 1H, H-3), 6.22 (1H, H-7), 6.18 (1H, H-7'), 5.86 (1H, H-6''), 5.77 (1H, H-7''), 5.63 (1H, H-6), 5.49 (1H, H-6'), 5.25 (1H, H-5), 5.19 (1H, H-8), 5.11 (2H, H-5', H-8'), 4.90 (1H, H-5''), 4.63 (1H, H-10a''), 4.61 (1H, H-9), 4.58 (1H, H-10b''), 4.52 (1H, H-9'), 4.38 (3H, H-10a, H-2ab), 4.31 (1H, H-10a'), 3.95 (3H, H-10b', H-10b, H-8''), 3.85 (1H, H-9''), 3.68 (2H, H-1ab). ^{13}C NMR (75.5 MHz, $CDCl_3$): δ 166,14-164.83 (C=O), 144.78 (C-4''), 144.53 (C-4'), 143.88 (C-4), 133.49-133.12 (C, Ar), 129.80-128.23 (CH, Ar), 123.47 (C-3), 122.40 (C-3''), 122.15 (C-3'), 76.82 (C-9, C-9', C-9''), 74.73 (C-7''), 73.66 (C-5), 73.62 (C-5'), 73.51 (C-5''), 73.46 (C-7), 73.39 (C-7'), 72.47 (C-6), 72.18 (C-6'), 71.94 (C-6''), 63.68 (C-10''), 62.49 (C-10'), 62.39 (C-10), 61.10 (C-8''), 60.86 (C-8,

C-8'), 50.41 (C-1), 49.35 (C-2). MS (ESI) *m/z* calcd for $C_{89}H_{74}N_{15}O_{21}$ $(M+2H)^{2+}$ 844.77, found 845.40



Scheme 4. Synthesis of the ligands. a) $CuSO_4$, Na-ascorbate, DMF, 10% H_2O, 30', 80°C, microwave, 80-85%. b) MeONa, MeOH, 60-87%.

General procedure for the "click reaction", step a. Preparation of compounds 11, 13, 15, 17: Compound **21** or compound **2** (1.2 equiv), $CuSO_4 \cdot 5H_2O$ (0.15 equiv) and sodium ascorbate (0.3 equiv) were added to a solution of **8** or **10** (1 equiv) in DMF (3 mL) with 10% water. The mixture was heated under microwave irradiation at 80°C for 30 min. After evaporation of the solvent the residue was dissolved in CH_2Cl_2 and washed three times with water and brine. After drying over sodium sulfate the solvent was removed and the compound purified by column chromatography to give a white solid.

11. (0.04 g, 0.02 mmol, 83%) ^{1}H NMR (300 MHz, $CDCl_3$): 8.10-7.10 (30H, Ar), 7.93, 7.73, 7.48, 7.44 (4H, 4×H-3), 6.26, 6.24 (2H, 2×H-7), 5.82, 5.66 (2H, 2×H-6), 5.52-5.41 (3H, 2×H-14, 1×H-12), 5.32-5.06 (7H, 2×H-5, 2×H-13, 2×H-8, 1×H-12), 4.88-4.56 (8H, H-1ab, H-2ab, 2×H-9, 2×H-11), 4.38- 4.26 (2H, 2×H-10a), 4.15-3.95 (6H, 2×H-10b, 2×H-16ab, 2×H-15), 2.11, 2.00, 2.02, 1.99, 1.98, 1.93, 1.87, 1.61 (6×C$\underline{H}_3$C=O). ^{13}C NMR (75.5 MHz, $CDCl_3$): 170.80-170.17, 169.99, 169.73 (C=O, acetyl), 166.04, 165.88, 165.35, 165.08 (C=O, benzoyl), 145.20, 145.13, 144.78, 144.57 (4×C-4), 133.92-133.15 (C, Ar), 130.27-129.45, 128.97-128.18 (CH, Ar), 123.76, 123.74, 122.70, 122.49 (4×C-3), 77.12 (2×C-9), 75.08 (2×C-15), 74.05-73.60 (2×C-5, 2×C-7, 2×C-11), 72.79 (1×C-6), 72.37-71.90 (1×C-6, 2×C-13), 69.00 (2×C-12), 67.80 (2×C-14), 63.04, 62.76 (2×C-10), 61.64-61.10 (2×C-8, 2×C-16), 49.89 (C-2, C-1), 20.93 (C$\underline{H}_3$C=O). MS (ESI) *m/z* calcd for $C_{89}H_{74}N_{15}O_{21}$ $(M+2H)^{2+}$ 938.30, found 939.15.

13. (0.04 g, 0.02 mmol, 85%) ^{1}H NMR (300 MHz, $CDCl_3$): 8.00-7.05 (30H, Ar), 7.91, 7.52, 7.36, 6.89 (4H, 4×H-3), 6.22, 6.19 (2H, 2×H-7), 5.74, 5.67 (2H, 2×H-6), 5.40-5.35 (2H, 2×H-17), 5.23-5.10 (5H, 2×H-5, 2×H-15, 1×H-8), 5.08-4.92 (3H, 2×H-16, 1×H-8), 4.83- 4.60 (6H, 2×H-9, H-1ab, H-2ab), 4.45 (1H, 1×H-14), 4.41-4.10 (3H, 2×H-10a, 1×H-14), 4.23-4.03 (5H, 2×H-19ab, 1×H-10b), 4.00-3.69 (5H, 2×H-13a, 2×H-18, 1×H-10b) 3.50-3.31 (2H, 2×H-13b), 2.59-2.51 (4H, 2×H-11ab), 2.12, 2.09, 2.02, 2.01, 2.00, 1.97, 1.96, 1.95 (6×C$\underline{H}_3$C=O), 2.00-1.58 (4H, 2×H-12ab). ^{13}C NMR (75.5 MHz, $CDCl_3$): 170.70, 170.51, 170.39, 169.75 (C=O, acetyl), 166.08, 165.86, 165.35, 165.06 (C=O, benzoyl), 147.75 (2×C-4), 144.80, 144.45 (2×C-4), 133.85-133.29 (C, Ar), 130.21-129.45 (CH, Ar) 128.94-128.28 (CH, Ar), 122.67, 122.64, 122.34, 122.26 (4×C-3), 101.44 (2×C-14), 77.06, 76.66 (2×C-9), 73.82 (2×C-5, 2×C-

7), 72.50 (2×C-6), 71.9 (2×C-16), 70.80 (2×C-18), 69.20 (2×C-15, 2×C-13), 67.33 (2×C-17), 63.38, 62.64 (2×C-10), 61.50-61.00 (2×C-8, 2×C-19), 49.93 (C-2), 49.45 (C-1), 29.18 (2×C-12), 21.90, 21.78 (2×C-11), 20.89 ($\underline{C}H_3C$=O). MS (ESI) *m/z* calcd for $C_{89}H_{74}N_{15}O_{21}$ $(M+2H)^{2+}$ 996.34, found 996.95.

15. (0.07 g, 0.03 mmol, 80%) ^{1}H NMR (300 MHz, $CDCl_3$): 8.10-7.10 (45H, Ar), 7.92, 7.85, 7.74, 7.48, 7.44 (5H, 4×H-3), 6.326.15 (3H, 3×H-7), 5.85, 5.67, 5.64 (3H, 3×H-6), 5.52-5.42 (3H, 2×H-14, 1×H-12), 5.32-5.06 (7H, 3×H-5, 2×H-13, 3×H-8, 1×H-12), 4.85-4.50 (9H, H-1ab, H-2ab, 3×H-9, 2×H-11), 4.38- 4.25 (3H, 3×H-10a), 4.15-3.90 (7H, 3×H-10b, 2×H-16ab, 2×H-15), 2.11, 2.01, 1.99, 1.97, 1.93, 1.87, 1.85, 1.61 (6×C$\underline{H}_3$C=O). ^{13}C NMR (75.5 MHz, $CDCl_3$): 170.79-170.21, 169.98, 169.71 (C=O, acetyl), 166.03, 165.87, 165.33, 165.06 (C=O, benzoyl), 145.19, 145.12, 144.81, 144.74, 144.58 (5×C-4), 133.97-133.25 (C, Ar), 130.29-129.57, 129.46-128.19 (CH, Ar), 123.77, 123.76, 122.72, 122.51, 122.50 (5×C-3), 77.06 (3×C-9), 75.05 (2×C-15), 74.05-73.60 (3×C-5, 3×C-7, 2×C-11), 72.80-71.90 (3×C-6, 2×C-13), 69.00 (2×C-12), 67.82 (2×C-14), 62.80 (3×C-10), 61.85-61.00 (3×C-8, 2×C-16), 49.86 (C-2, C-1), 20.93 ($\underline{C}H_3C$=O). MS (ESI) *m/z* calcd for $C_{89}H_{74}N_{15}O_{21}$ $(M+2H)^{2+}$ 1201.37, found 1202.05.

17. (0.07 g, 0.03 mmol, 82%) ^{1}H NMR (300 MHz, $CDCl_3$): 8.00-7.10 (45H, Ar), 7.86 (2H, 2×H-3), 7.50, 7.34, 6.89 (3H, 3×H-3), 6.26-6.15 (3H, 3×H-7), 5.78-, 5.58 (3H, 3×H-6), 5.40-5.35 (2H, 2×H-17), 5.23-4.95 (10H, 3×H-5, 2×H-15, 3×H-8, 2×H-16), 4.83- 4.67 (5H, 1×H-9, H-1ab, H-2ab), 4.61, 4.54 (2H, 2×H-9), 4.45 (1H, 1×H-14), 4.40-4.25 (3H, 3×H-10a, 1×H-14), 4.23-4.05 (5H, 2×H-19ab, 1×H-10b), 4.00-3.69 (6H, 2×H-13a, 2×H-18, 2×H-10b), 3.50-3.33 (2H, 2×H-13b), 2.57-2.51 (4H, 2×H-11ab), 2.12, 2.09, 2.02, 2.01, 2.00, 1.98, 1.97, 1.96 (6×C$\underline{H}_3$C=O), 1.67-1.57 (4H, 2×H-12ab). ^{13}C NMR (75.5 MHz, $CDCl_3$): 170.69, 170.51, 170.38, 169.74 (C=O, acetyl), 166.07, 165.83, 165.34, 165.03 (C=O, benzoyl), 147.75 (2×C-4), 144.82, 144.74, 144.45 (3×C-4), 133.92-133.15 (C, Ar), 130.27-129.45 (CH, Ar) 128.97-128.18 (CH, Ar), 123.66 (1×C-3), 122.59 (2×C-3), 122.35, 121.28 (2×C-3), 101.65 (2×C-14), 77.09 (2×C-9), 76.67 (1×C-9), 74.00-73.70 (3×C-5, 3×C-7), 72.60 (3×C-6), 71.21 (2×C-16), 70.83 (2×C-18), 69.24 (2×C-15, 2×C-13), 67.36 (2×C-17), 63.30, 62.70 (3×C-10), 61.55-61.00 (3×C-8, 2×C-19), 49.96 (C-2), 49.46 (C-1), 29.92, 29.15

(2×C-12), 21.93, 21.78 (2×C-11), 20.93 (<u>C</u>H$_3$C=O). MS (ESI) *m/z* calcd for $C_{89}H_{74}N_{15}O_{21}$ $(M+2H)^{2+}$ 1258.92, found 1259.70.

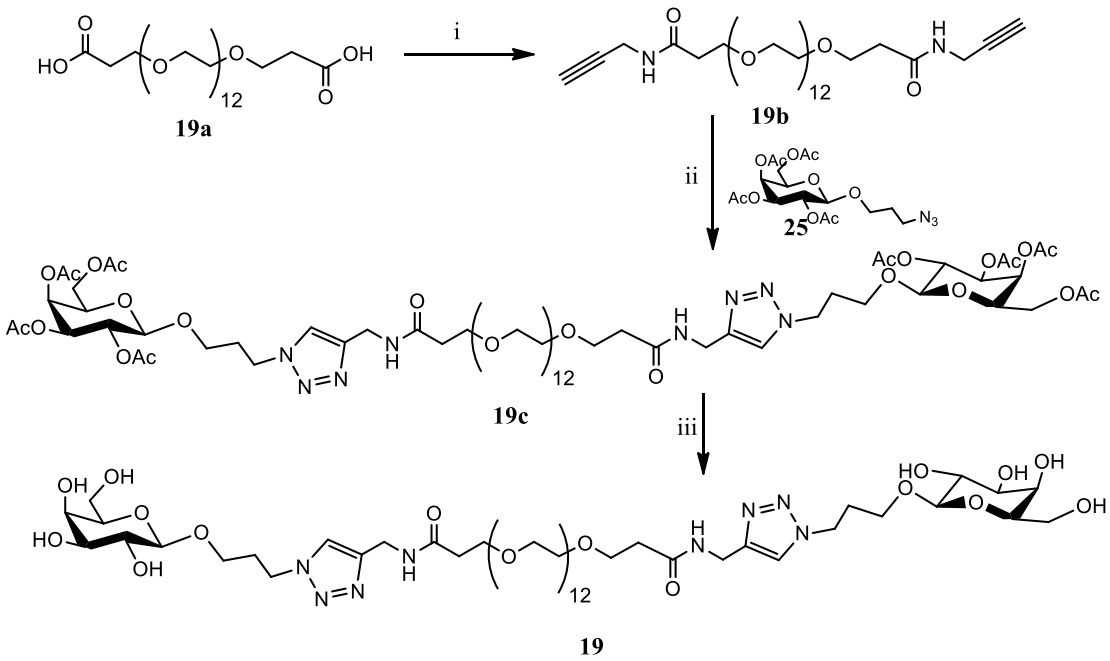
General procedure for the removal of acetyl and benzoyl protecting groups, step b. Preparation of compounds 12, 14, 16, 18. A solution of the protected carbohydrate compounds **11**, **13**, **15**, **17** in methanol (5 mL) was treated, respectively, with a solution of NaOMe in methanol (30%, 200 μL) and the mixture was stirred for 4 h at r.t.. After neutralization with Dowex, the mixture was filtered and the methanol evaporated in vacuum to give the desired compound, which was purified by preparative HPLC.

12. (0.017 g, 0.017 mmol, 87%) ^{1}H NMR (300 MHz, D_2O): 8.34, 8.29, 8.00, 7.96 (4H, 4×H-3), 5.01 (4H, H-1ab, H-2ab), 4.95-4.75 (4H, 2×H-5, 2×H-8), 4.54, 4.45 (2H, 2×H-11), 4.34-4.27 (4H, 2×H-9, 2×H-7), 4.05-3.70 (14H, 2×H-14, 2×H-12, 2×H-6, 2×H-15, 2×H-13, 2×H-16ab), 3.56 (2H, 2×H-10a), 3.34-3.27 (2H, 2×H-10b). ^{13}C NMR (125 MHz, D_2O): 143.40, 143.50, 142.30, 142.20 (4×C-4), 124.22, 124.15, 123.85, 123.83 (4×C-3), 77.98 (2×C-15), 76.98 (2×C-9), 73.41 (3×C-7), 72.80-72.38 (2×C-5, 2×C-11, 2×C-13), 71.80 (2×C-6), 68.98 (2×C-12), 67.85 (2×C-14), 60.99 (2×C-8), 59.98 (2×C-16), 58.75 (2×C-10), 48.90 (C-2, C-1). HRMS (MALDI TOF/TOF) *m/z* calcd for $C_{34}H_{51}N_{12}O_{18}$ $(M+H)^+$ 915.3444 found 915.3411.

14. (0.012 g, 0.012 mmol, 80%) ^{1}H NMR (300 MHz, D_2O): 8.33, 7.99, 7.93, 7.59 (4H, 4×H-3), 4.99-4.92 (4H, H-1ab, H-2ab), 4.85-4.70 (4H, 2×H-5, 2×H-8), 4.39-4.25 (6H, 2×H-9, 2×H-14, 2×H-7), 3.96-3.85 (6H, 2×H-6, 2×H-13a, 2×H-17), 3.79-3.62 (10H, 2×H-13b, 2×H-15, 2×H-16, 2×H-19ab), 3.56-3.49 (4H, 2×H-10a, 2×H-18), 3.31-3.24 (2H, 2×H-10b), 2.95, 2.74 (4H, 2×H-11ab), 1.99, 1.90 (4H, 2×H-12ab). ^{13}C NMR (125 MHz, D_2O): 146.30, 143.30, 143.14 (4×C-4), 123.94, 123.75, 122.12, 122.05 (4×C-3), 101.34 (2×C-14), 76.90 (2×C-9), 73.90-73.00 (2×C-16, 2×C-7), 72.30-71.10 (2×C-15, 2×C-6, 2×C-5), 69.25 (2×C-18), 67.70-67.00 (2×C-17, 2×C-13), 60.90-60.50 (2×C-8), 59.49 (2×C-19), 58.53 (2×C-10), 48.75, 48.52 (C-2, C-1), 27.03, 26.92 (2×C-12), 19.54, 19.35 (2×C-11). HRMS (MALDI TOF/TOF) *m/z* calcd for $C_{40}H_{62}N_{12}NaO_{20}$ $(M+Na)^+$ 1053.4096 found 1053.4061.

16. (0.017 g, 0.015 mmol, 75%) ^{1}H NMR (300 MHz, D_2O): 8.39, 8.38, 8.32, 8.02, 7.99 (5H, 5×H-3), 5.05 (4H, H-1ab, H-2ab), 4.92-4.78 (6H, 3×H-5, 3×H-8), 4.59, 4.49 (2H, 2×H-11), 4.40-4.32 (6H, 3×H-9, 3×H-7), 4.10-3.73 (15H, 2×H-14, 2×H-12, 3×H-6, 2×H-15, 2×H-13, 2×H-16ab), 3.63-3.58 (3H, 3×H-10a), 3.38-3.32 (3H, 3×H-10b). ^{13}C NMR (125 MHz, D_2O): 144.57-143.10 (5×C-4), 124.01-123.40 (5×C-3), 77.83 (2×C-15), 76.81 (3×C-9), 73.24 (3×C-7), 72.53-72.18 (3×C-5, 2×C-11, 2×C-13), 71.70-71.60 (3×C-6), 68.90-68.70 (2×C-12), 67.70 (2×C-14), 60.82 (3×C-8), 59.75 (2×C-16), 58.55 (3×C-10), 48.70 (C-2, C-1). HRMS (MALDI TOF/TOF) *m/z* calcd for $C_{42}H_{62}N_{15}O_{22}$ $(M+H)^+$ 1128.4193 found 1128.4012.

18. (0.016 g, 0.013 mmol, 70%) ^{1}H NMR (300 MHz, D_2O): 8.34 (2H, 2×H-3), 8.02, 7.96, 7.67 (3H, 3×H-3), 4.99-4.94 (4H, H-1ab, H-2ab), 4.87-4.72 (6H, 3×H-5, 3×H-8), 4.37-4.25 (8H, 3×H-9, 2×H-14, 3×H-7), 3.99-3.85 (7H, 3×H-6, 2×H-13a, 2×H-17), 3.78-3.60 (10H, 2×H-13b, 2×H-15, 2×H-16, 2×H-19ab), 3.57-3.47 (5H, 3×H-10a, 2×H-18), 3.32-3.24 (3H, 3×H-10b), 2.95, 2.76 (4H, 2×H-11ab), 1.99, 1.91 (4H, 2×H-12ab). ^{13}C NMR (125 MHz, D_2O): 145.12, 145.09, 143.52, 143.25 (5×C-4), 123.95, 123.82, 122.54, 122.39 (5×C-3), 101.32 (2×C-14), 76.75 (3×C-9), 73.70-73.10 (2×C-16, 3×C-7), 72.50-71.20 (2×C-15, 3×C-6, 3×C-5), 69.27 (2×C-18), 67.60-67.10 (2×C-17, 2×C-13), 60.72 (3×C-8), 59.49 (2×C-19), 58.55 (3×C-10), 48.53 (C-2, C-1), 26.89 (2×C-12), 19.42, 19.15 (2×C-11). HRMS (MALDI TOF/TOF) *m/z* calcd for $C_{48}H_{74}N_{15}O_{24}$ $(M+H)^+$ 1244.5031 found 1244.4996.



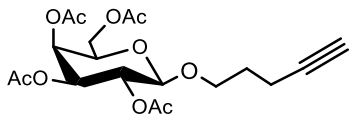
Scheme 5. Synthesis of the PEG based ligand. i) DMAP, propargyl amine, Et_3N, EDCI, DCM, 93%; ii) $CuSO_4$, Na-ascorbate, DMF, 10% H_2O, 30', 80°C, microwave, 65%. b) MeONa, MeOH, 95%.

19b. Propargyl amine (0.05 mL, 0.70 mmol) was added to a solution of **19a** (0.20 g, 0.30 mmol), 4-dimethyl aminopyridine (DMAP, 0.01 g, 0.08 mmol) and triethylamine (0.01 mL, 0.03 mmol) in CH_2Cl_2 (15 mL). The mixture was cooled to 0°C and 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDCI, 0.11 g, 0.60 mmol) was added. The mixture was stirred for 1 h at 0°C and overnight at r.t.. The reaction was quenched with a 1M $KHSO_4$ solution (15 mL) and the organic layer was washed with $NaHCO_3$ saturated solution, water and brine (20 mL). The organic solution was dried over sodium sulfate and after evaporation of the solvent, **19b** was obtained as an oily product (0.21 g, 0.28 mmol, 93%). ^{1}H NMR (300 MHz, $CDCl_3$): 7.00 (2H, NH), 3.95 (m, 4H, H-3), 3.65 (t, 4H, $J_{6,5}$= 5.70 Hz, H-6), 3.60-3.50 (48H, $(OCH_2CH_2)_{12}O$), 2.41 (t, 4H, H-5, $J_{6,5}$= 5.70 Hz), 2.16 (t, 2H, H-1, $J_{1,3}$= 2.50 Hz). ^{13}C NMR (75.5 MHz, $CDCl_3$): 171.28, 80.08, 71.01, 70.53-70.25, 70.21, 70.16, 66.92, 36.56, 28.76 MS (ESI) *m/z* calcd for $C_{36}H_{65}N_2O_{15}$ $(M+H)^+$ 765.44, found 765.75.

19c. The general procedure for the "click reaction" was applied. Starting from compound **19b** (0.19 g, 0.25 mmol) and compound **25**[21] (0.24 g, 0.55 mmol), after column chromatography, **19c** was obtained (0.19 g, 0.16 mmol, 65%). ^{1}H NMR (300 MHz, $CDCl_3$): 7.53 (s, 2H, H-6), 7.29 (2H, NH), 5.31 (d, 2H, $J_{13,12}$= 3.15 Hz, H-13), 5.15 (dd, 2H, $J_{11,10}$= 8.12 Hz, $J_{11,12}$= 10.37 Hz, H-11), 4.95 (dd, 2H, $J_{12,11}$= 10.37, Hz

$J_{12,13}$= 3.15 Hz, H-12), 4.46-4.22 (m, 10H, H-15ab, H-10, H-7), 4.07 (m, 4H, H-4), 3.88-3.76 (m, 4H, H-14, H-9a), 3.68 (t, 4H, $J_{1,2}$= 5.85 Hz), 3.60-3.50 (48H, $(OCH_2CH_2)_{12}O$), 3.38 (m, 2H, H-9b), 2.41 (t, 4H, $J_{2,1}$ = 5.85 Hz, H-2), 2.11 (m, 4H, H-8), 2.11, 2.04, 1.98, 1.93 (24H, C=OC$\underline{H}_3$). ^{13}C NMR (75.5 MHz, $CDCl_3$): 171.28 (NHC=O), 170.32 (C=O), 170.18 (C=O), 170.02 (C=O), 169.56 (C=O), 145.13, 122.60, 101.14, 70.76, 70.65, 70.48-70.35, 70.21, 70.13, 68.76, 67.06, 66.97, 65.85, 61.18, 46.61, 36.71, 34.91, 30.13, 20.81, 20.65, 20.61, 20.5. MS (ESI) *m/z* calcd for $C_{70}H_{116}N_8O_{35}$ $(M+2H)^{2+}$ 814.33, found 814.70.

19. The general procedure for the removal of acetyl protecting groups was applied. Starting from **19c** (0.07 g, 0.04 mmol), compound **19** was obtained (0.05 g, 0.04 mmol, 95%). ^{1}H NMR (300 MHz, D_2O): 7.95 (s, 2H), 4.56 (t, 4H, J=6.85 Hz), 4.49 (s, 4H), 4.37 (d, 2H, J=7.74 Hz), 3.96-3.87 (m, 4H), 3.84-3.50 (m, 64H), 2.58 (t, 4H, J=5.93 Hz), 2.23 (p, 4H, J=6.39 Hz). ^{13}C NMR (125 MHz, D_2O): 171.30 (NHC=O), 143.68 (C, Tr), 122.40 (CH, Tr), 101.14, 73.47, 71.06, 69.07, 68.75-67.20, 66.98, 65.04, 64.65, 59.33, 45.46, 34.28, 32.84, 27.92. HRMS (MALDI TOF/TOF) *m/z* calcd for $C_{54}H_{99}N_8O_{27}$ $(M+H)^+$ 1291.6619 found 1291.6371.

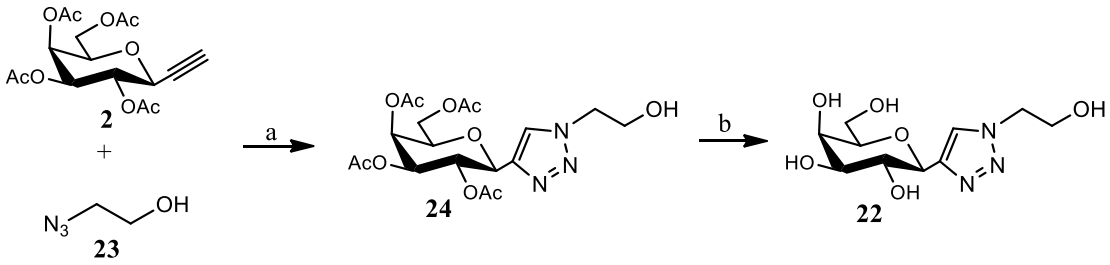


21

21. $BF_3{\cdot}Et_2O$ (2.6 mL, 20.48 mmol) was added dropwise to a solution of β-D-galactose pentaacetate (2 g, 5.12 mmol) and 3-butyn-1-ol (1.55 mL, 20.48 mmol) in CH_2Cl_2 (50 mL) previously cooled at 0°C. The mixture was stirred overnight at room temperature. The solution was neutralized with a saturated $NaHCO_3$ solution and the organic phase was washed with water (30 mL) and once with brine (30 mL). After drying over sodium sulfate, the solvent was removed and the compound purified by column chromatography to give **20** as a white solid (1.48 g, 3.58 mmol, 70%). ^{1}H NMR (300 MHz, $CDCl_3$): δ 5.30 (d, 1H, $J_{9,8}$=2.57 Hz, H-9), 5.10 (t, 1H, $J_{7,6}$=$J_{7,8}$=9.10 Hz, H-7), 4.94 (dd, 1H, $J_{8,7}$= 9.10 Hz, $J_{8,9}$=2.57 Hz, H-8), 4.41 (d, 1H, $J_{6,7}$=9.10 Hz, H-6), 4.07 (m, 2H, H-11ab), 3.88 (m, 2H, H-10, H-5a), 3.56 (m, 1H, H-5b), 2.18 (m, 2H, H-3), 2.18 (1H, H-1), 2.06, 1.99, 1.96, 1.90 (C=OC$\underline{H}_3$), 1.73 (m, 2H, H-4). ^{13}C NMR (75.5 MHz, $CDCl_3$): δ ppm 170.19, 170.10, 169.95, 169.31 (4×C=O), 101.37,

83.29, 70.75, 70.49, 68.83, 68.77, 68.18, 66.96, 61.18, 28.11, 20.61 (C=OC$\underline{H}_3$), 20.53 (2×C=OC$\underline{H}_3$), 20.45 (C=O$\underline{C}H_3$), 14.63. MS (ESI) *m/z* calcd for $C_{19}H_{26}KO_9$ $(M+K)^+$ 437.12, found 437.15.

20. The general procedure for the removal of acetyl protecting groups was applied. Starting from **21** (0.19 g, 0.48 mmol), compound **20**[22] was obtained (0.12 g, 0.47 mmol, 98%). ^{1}H NMR (300 MHz, CD_3OD): δ ppm 4.21 (d, 1H, $J_{6,7}$=6.83 Hz, H-6), 3.96 (dt, 1H, $J_{5a,4}$=6.50 Hz, $J_{5a,5b}$=9.90 Hz, H-5a), 3.84 (s, 1H, H-9), 3.73 (d, 2H, $J_{11ab,10}$=6.36 Hz, H-11ab), 3.65 (dt, 1H, $J_{5b,4}$=6.50 Hz, $J_{5b,5a}$ =9.90 Hz, H-5b), 3.55-3.43 (m, 3H, H-7, H-8, H-10), 2.30 (dt, 2H, $J_{3,4}$=7.00 Hz, $J_{3,1}$ =2.60 Hz, H-3), 2.20 (t, 1H, $J_{1,3}$=2.60 Hz, H-1), 1.80 (p, 2H, $J_{4,3}$=$J_{4,5}$ =6.50 Hz, H-4). ^{13}C NMR (75.5 MHz, CD_3OD): δ ppm 104.94, 84.70, 76.45, 74.86, 72.47, 70.17, 69.63, 69.24, 62.34, 29.99, 15.75. HRMS (MALDI TOF/TOF) *m/z* calcd for $C_{11}H_{18}NaO_6$ $(M+Na)^+$ 269.0996 found 269.0928.



Scheme 6. a) $CuSO_4$, Na-ascorbate, DMF, 10% H_2O, 30', 80°C, microwave, 60%; b) MeONa, MeOH, quant..

24. The general procedure for the "click reaction" was applied. Starting from compound **2** (0.05 g, 0.15 mmol) and compound **23**[23] (0.02 g, 0.22 mmol), after column chromatography, **24** was obtained (0.04 g, 0.09 mmol, 60%). ^{1}H NMR (300 MHz, $CDCl_3$): 7.77 (s, 1H, H-3),5.49 (d, 1H, $J_{8,7}$= 3.15 Hz, H-8), 5.35 (t, 1H, $J_{6,5}$=$J_{6,7}$= 10.10 Hz, H-6), 5.16 (dd, 1H, $J_{7,6}$= 10.10 Hz $J_{7,8}$= 3.15 Hz, H-7), 4.74 (d, 1H, $J_{5,6}$= 10.10 Hz, H-5), 4.48 (m, 2H, H-1ab), 4.17-4.08 (m, 3H, H-9, H-10ab), 3.98 (m, 2H, H-1ab), 2.70 (s, 1H, OH), 2.19, 2.04, 2.00, 1.91 (12H, C=OC$\underline{H}_3$). ^{13}C NMR (75.5 MHz, CD_3Cl): 170.77 (C=O), 170.51 (C=O), 170.45 (C=O), 170.35 (C=O), 144.69 (C-4), 124.04 (C-3), 75.11 (C-9), 74.09 (C-5), 72.00 (C-7) 69.47 (C-6), 67.93 (C-8), 61.96 (C-10), 61.48 (C-1), 53.14 (C-2), 20.99(C=O$\underline{C}$H3). MS (ESI) *m/z* calcd for $C_{18}H_{25}N_3O_{10}$ $(M+H)^+$ 444.16, found 444.70.

22. The general procedure for the removal of acetyl protecting groups was applied. Starting from **24** (0.03 g, 0.07 mmol), compound **22** was obtained (0.02 g, 0.07 mmol, quant). ^{1}H NMR (300 MHz, CD_3OD): δ ppm 8.05 (s, 1H, H-3), 4.49 (t, 2H, $J_{2ab,1ab}$=5.55 Hz, H-2ab), 4.34 (d, 1H, $J_{5,6}$=9.55 Hz, H-5), 3.97-3.83 (m, 4H, H-8, H-9, H-10ab), 3.80-3.63 (m, 3H, H-1ab, H-6), 3.60 (dd, 1H, $J_{7,8}$=3.15 Hz, $J_{7,6}$ =9.55 Hz, H-7). ^{13}C NMR (75.5 MHz, CD_3OD): δ ppm 147.00 (C-4), 125.43 (C-3), 80.93 (C-9), 76.24 (C-5), 76.20 (C-7) 72.23 (C-6), 70.91 (C-8), 62.83 (C-10), 61.58 (C-1), 54.02 (C-2). HRMS (MALDI TOF/TOF) *m/z* calcd for $C_{10}H_{18}N_3O_6$ $(M+H)^+$ 276.1196 found 269.1135.

Calculation of the effective length of the spacer of compound 19

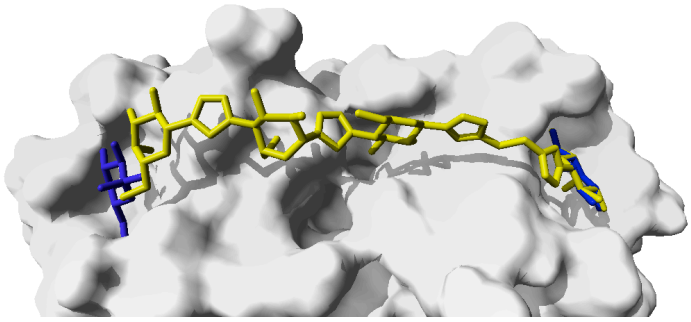
The spacer of compound **19** contains one of the longest commercially available homogeneous PEG molecule. The spacer is of similar length as an all PEG spacer with 20 PEG units (CH_2CH_2O), since they both contain 61 atoms. Using the Flory equation[24] for PEG, $Rf = aN^{\frac{3}{5}}$, where R_f is the length, N is the number of PEG units, and a is the length of one monomer (taken to be 3.5 Å), an effective length of 21.1 Å is calculated. Considering that the spacer of **19** contain two amide bonds and two triazole units, it is likely that its effective length is longer than that. Furthermore flexible PEG and PEG-hybrid spacers are known to give a broad distance distribution, both theoretically[25] and experimentally.[9] Therefore the spacer of **19** is expected to adequately cover the distance between the two binding sites of LecA of 26 Å, measured between the anomeric oxygens of bound galactosides of X-ray structure with pdb code 1OKO.[12b]

Estimating the potential for bivalent binding of 12 and 14

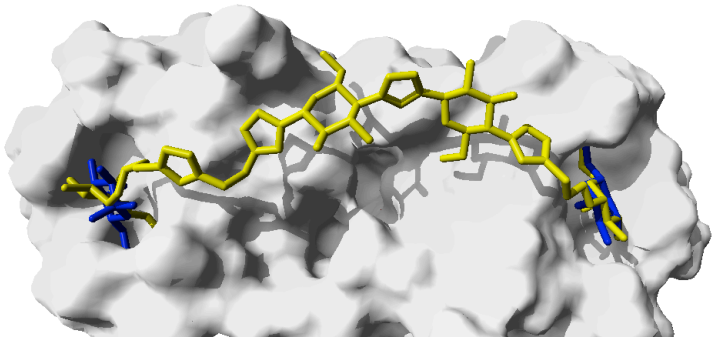
The complex between the rigid and flexible molecule and lectin 1 from *Pseudomonas aeruginosa* was modeled using the X-structure of this lectin complexed to galactose as a template (PDB ID: 1OKO). First, one of the terminal sugar moieties of either the rigid or flexible molecule was superimposed onto the galactose moiety of molecule A in the X-structure with respect to the atoms comprising the sugar-ring. Subsequently, the galactose moieties of molecules A and B were deleted from the structure and the other terminal sugar of either the rigid or the flexible molecule was pulled to the binding site of galactose B using restrained molecular dynamics. The superimposed

sugar and the protein units A and B were kept in a fixed position during the simulation. Restraints were used based on a limited number of hydrogen bonds present between residues of protein unit B and the galactose of B. In the top structure (**A**) our worst ligand, compound **12** was modeled (see yellow structure). Clearly the left galactose of **12** cannot reach the position of galactose B of the X-ray structure (shown in blue). In contrast, as shown in the bottom structure (**B**), both galactosides of our best ligand **14** can bind in both binding sites simultaneously. Modeling was accomplished using the Yasara Structure software (version 11.9.18).

A



B



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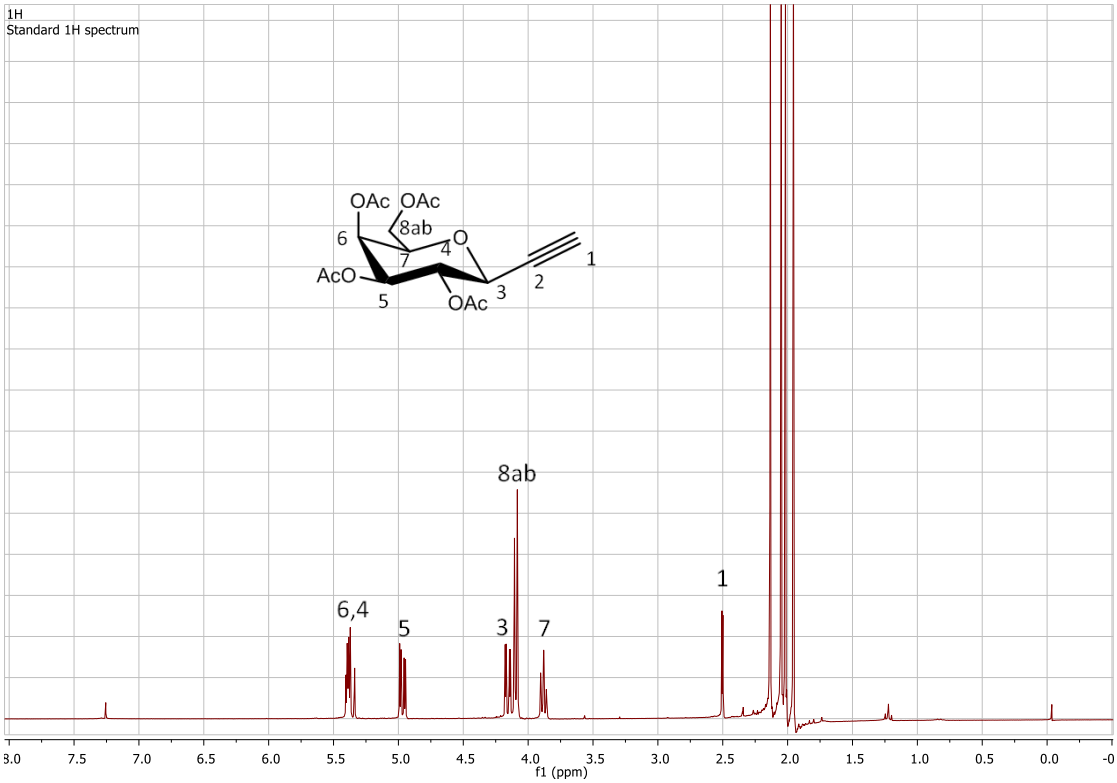
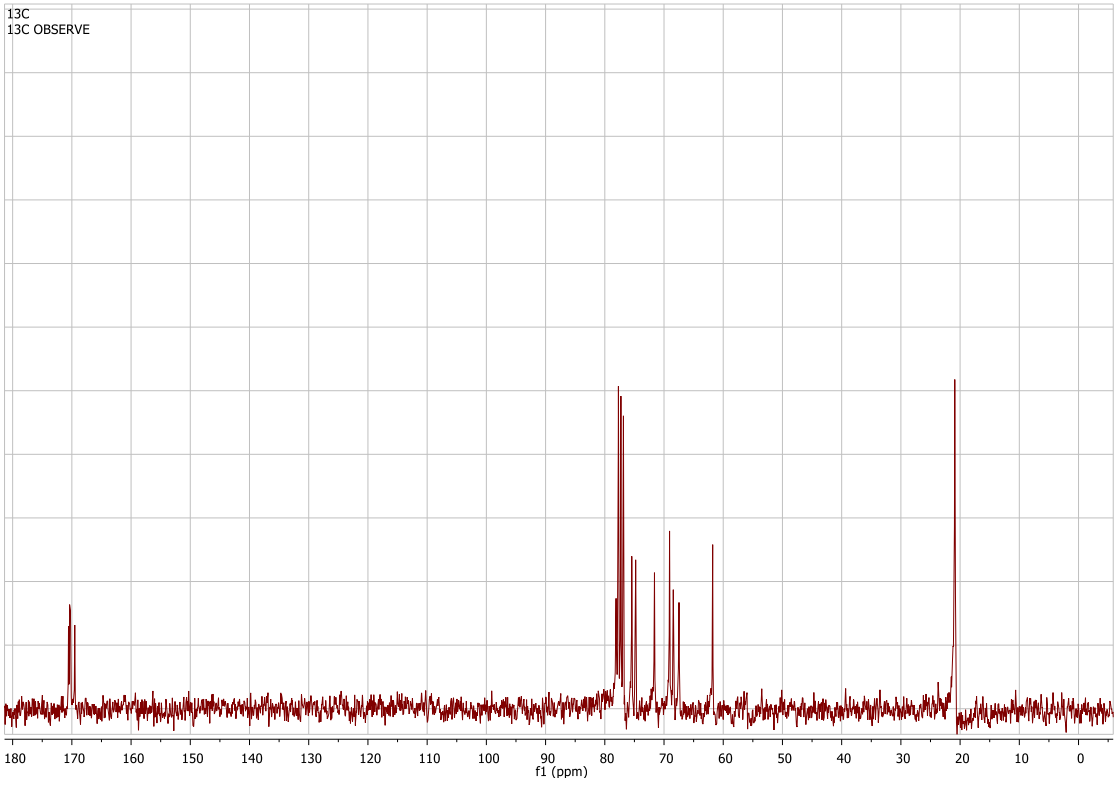
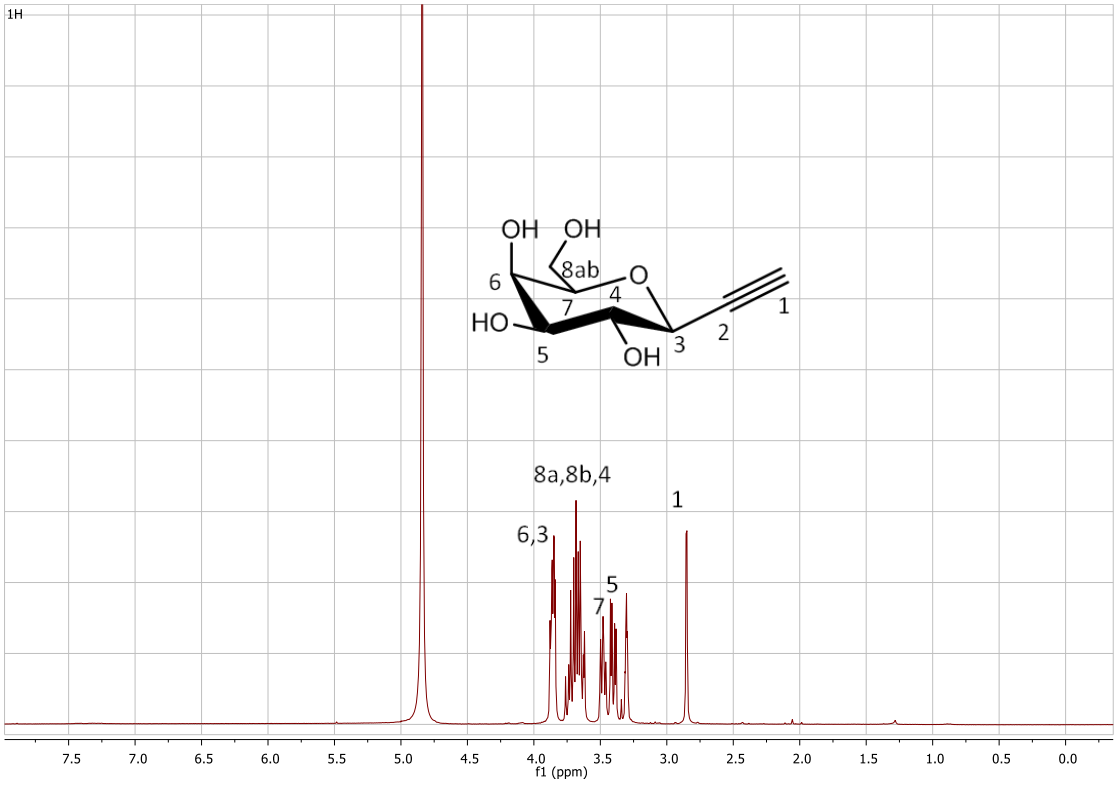
2 in $CDCl_3$





3 in CD_3OD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O
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7
4
1
HO
5
OH
3
2
8a,8b,4
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7
7.5
7.0
6.5
6.0
5.5
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3.5
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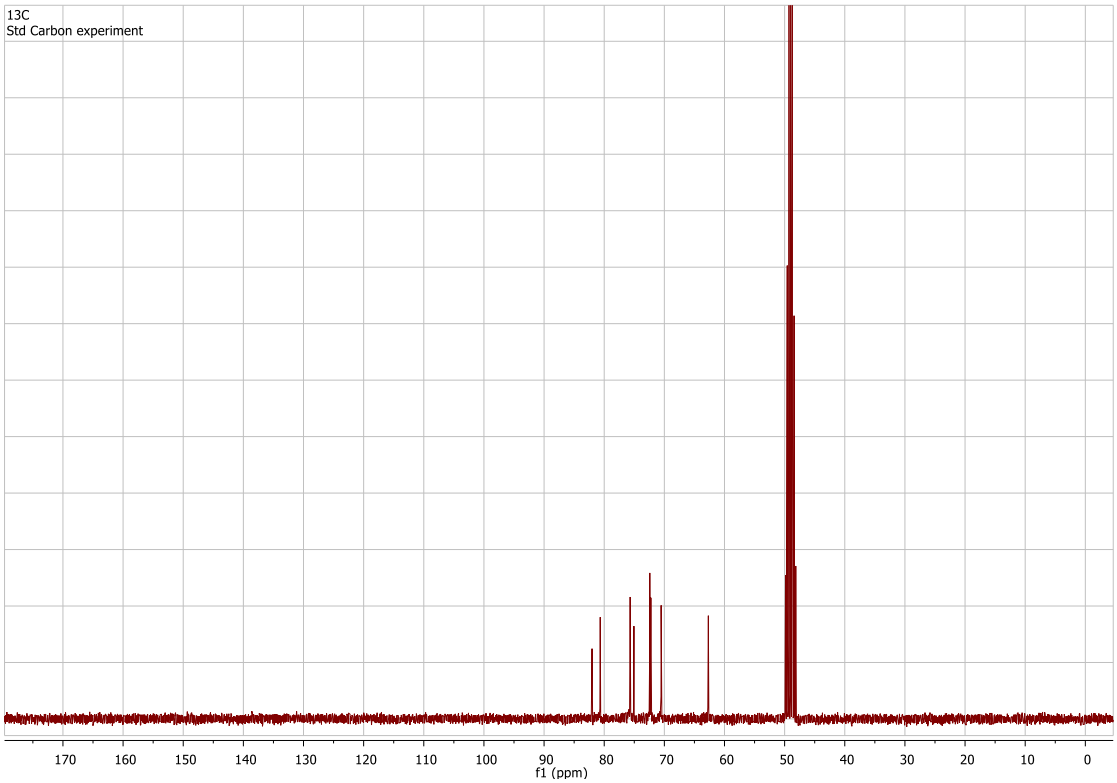
f1 (ppm)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Std Carbon experiment
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130
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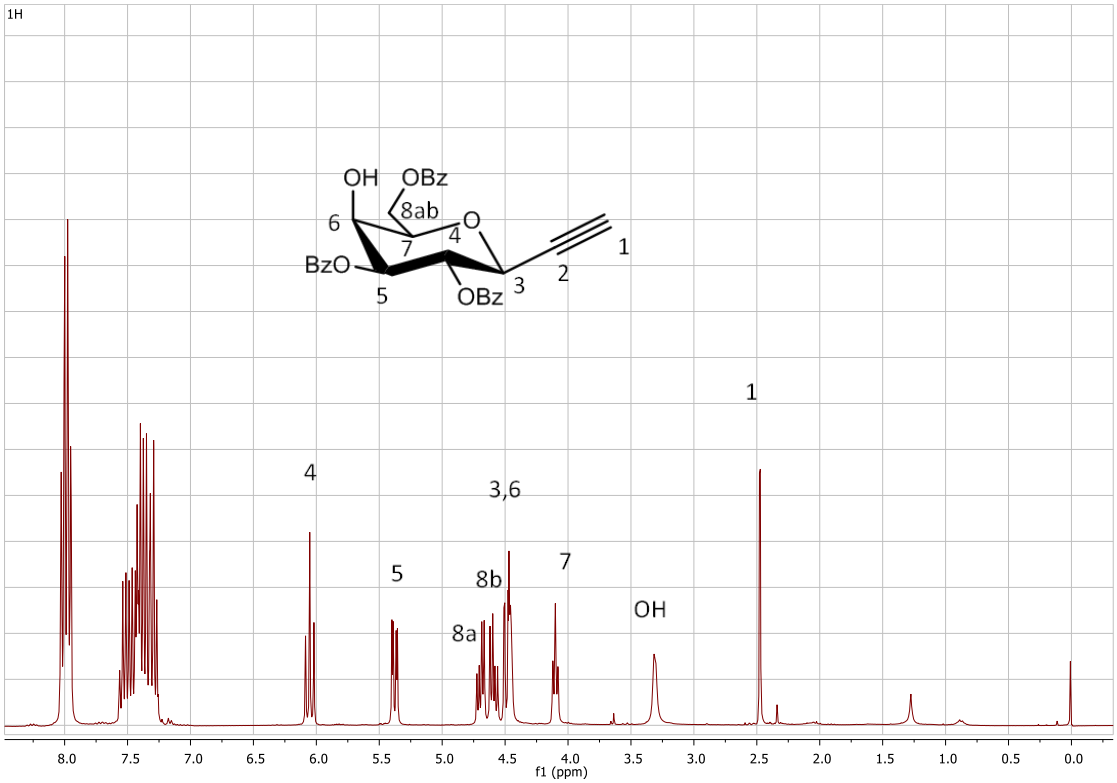
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)

4 in $CDCl_3$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O
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7
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BzO
2
3
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OBz
1
4
3,6
5
8b
7
OH
8a
8.0
7.5
7.0
6.5
6.0
5.5
5.0
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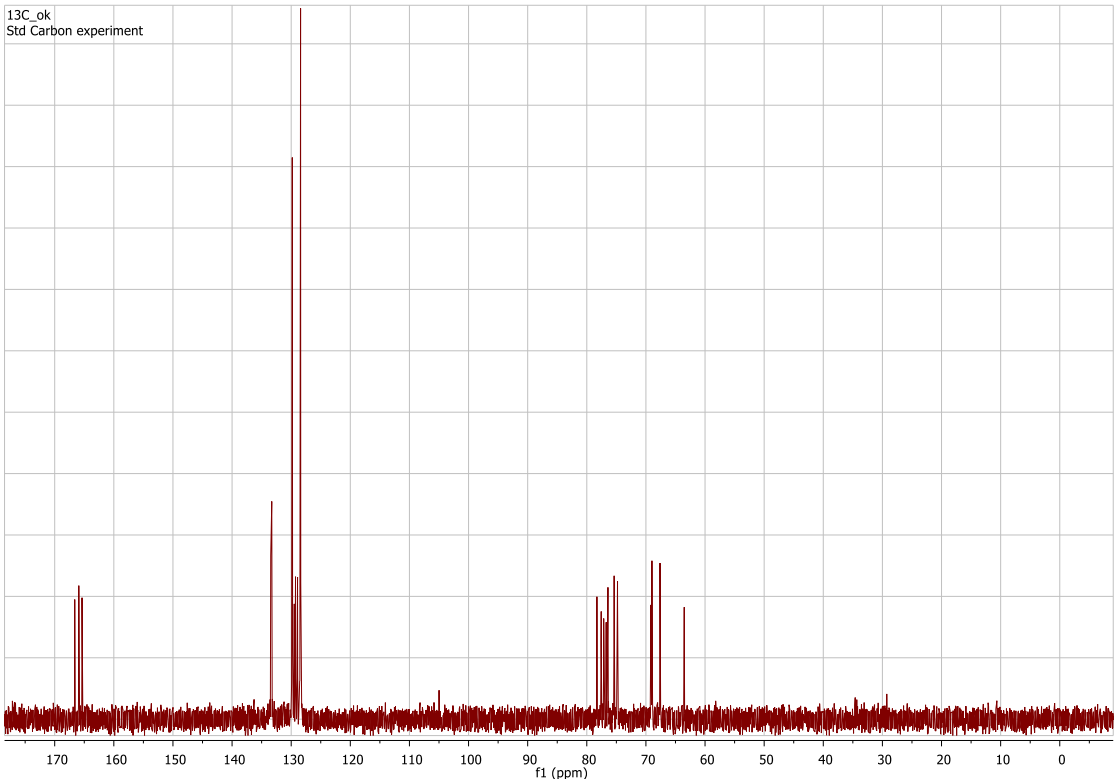
f1 (ppm)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Std Carbon experiment
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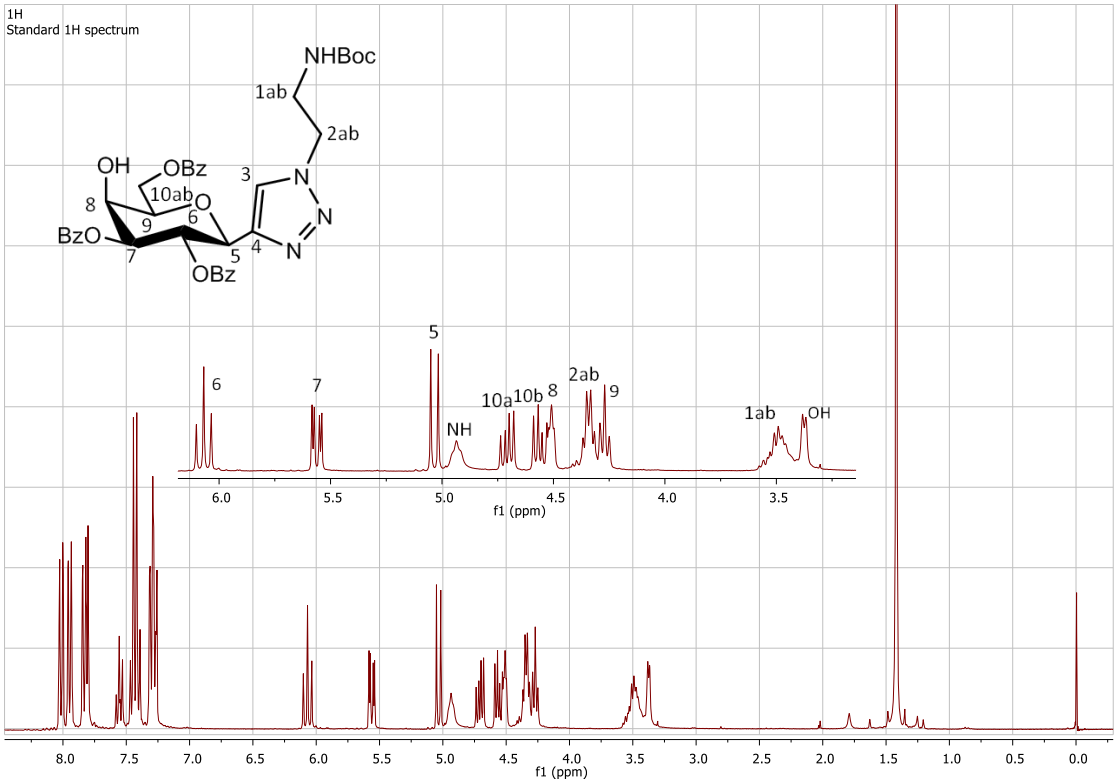
60
50
40
30
20
10
0
f1 (ppm)

6a in $CDCl_3$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N
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BzO
OBz
5
6
7
NH
10a
10b
8
2ab
9
1ab
OH
6.0
5.5
5.0
4.5
4.0
3.5
f1 (ppm)
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7.5
7.0
6.5
6.0
5.5
5.0
4.5
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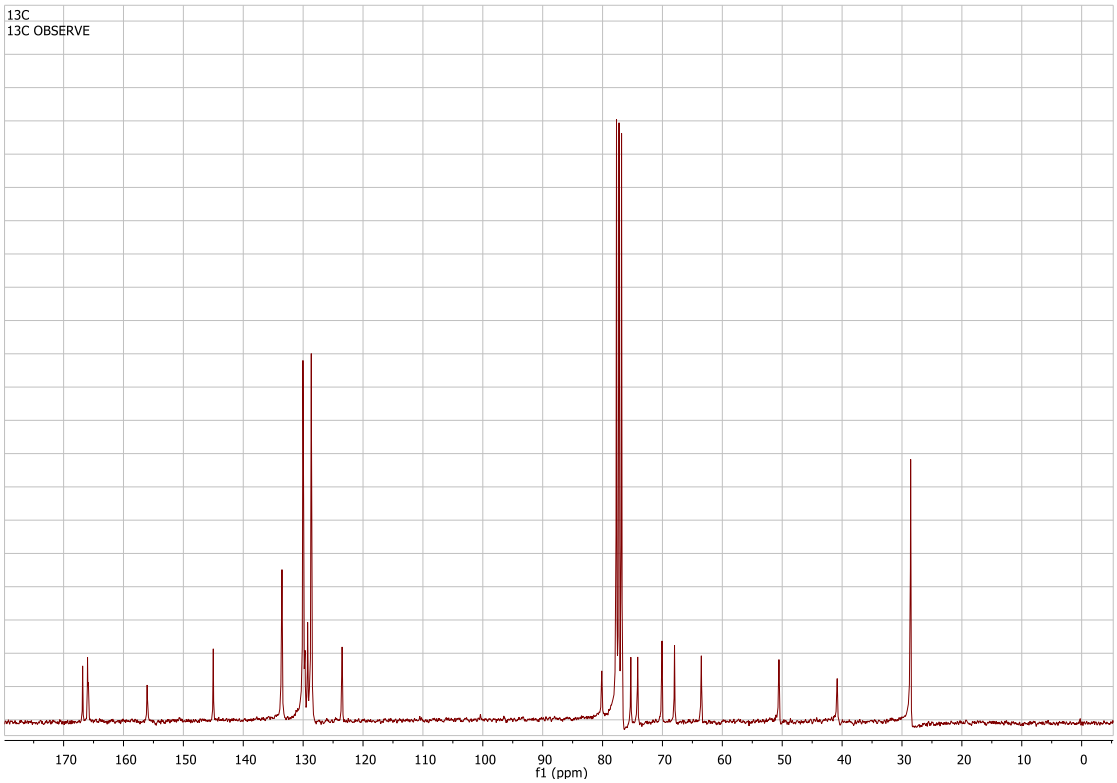
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f1 (ppm)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13C OBSERVE
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10
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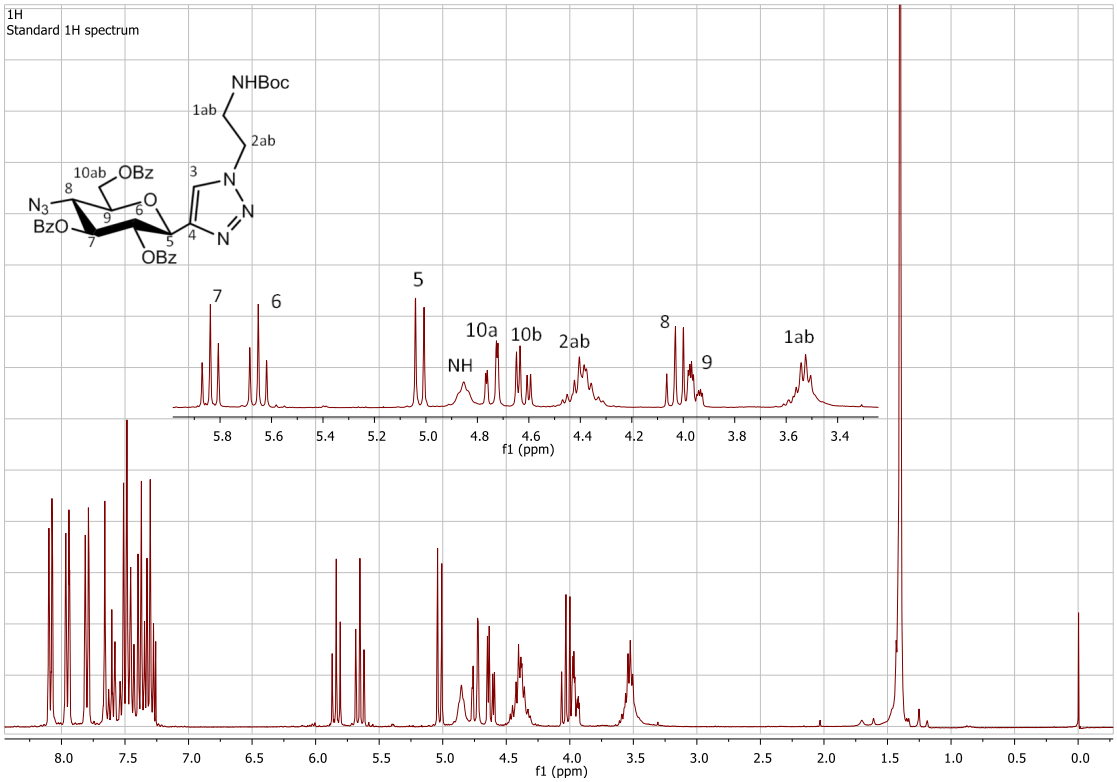
f1 (ppm)

6 in $CDCl_3$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OBz
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2ab
NH
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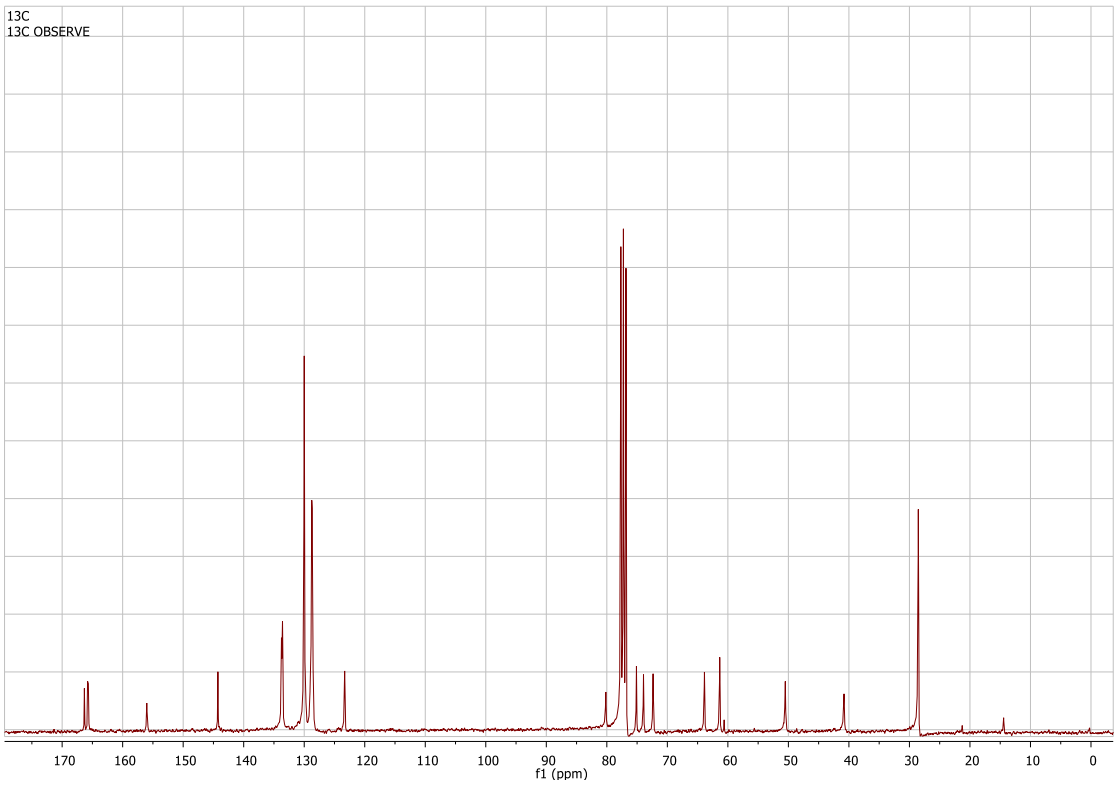
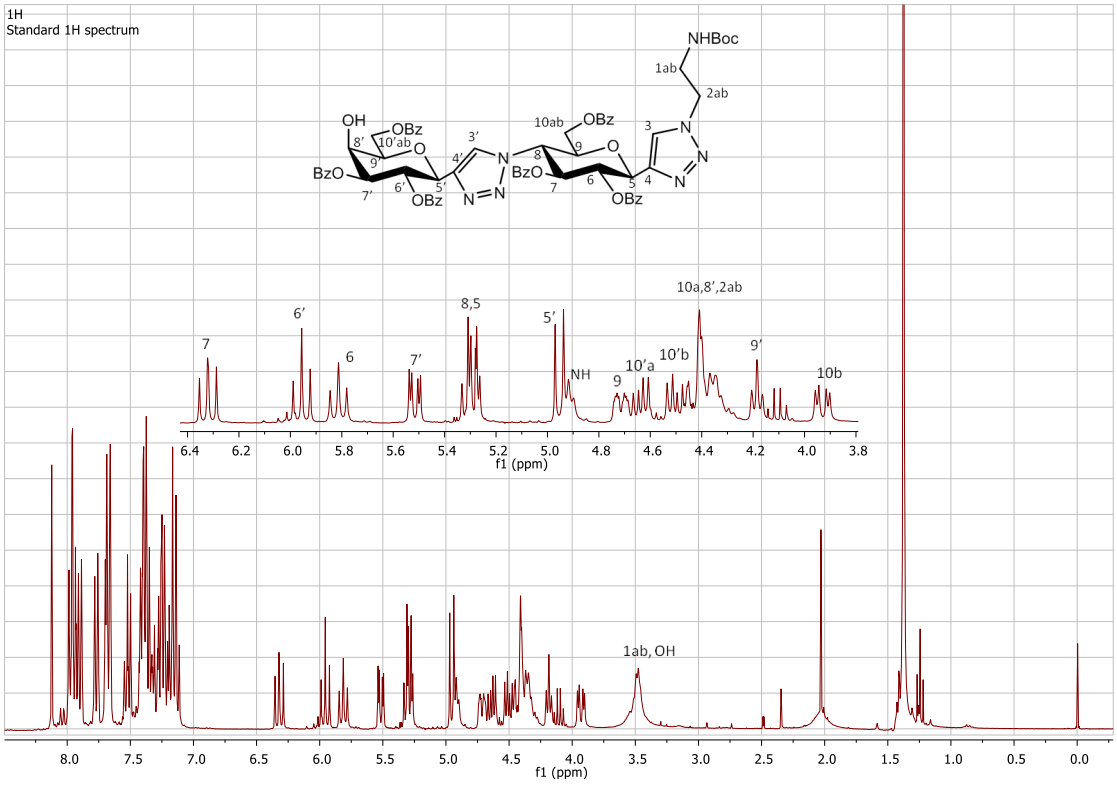
9
1ab
f1 (ppm)


13C
13C OBSERVE
f1 (ppm)

7a in $CDCl_3$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Standard 1H spectrum
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2ab
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OBz
10'ab
8'
9'
3'
4'
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BzO
7'
6'
5'
OBz
N=N
10ab
OBz
9
8
7
6
5
4
3
N
N
BzO
OBz
10a,8',2ab
8,5
6'
5'
7
6
7'
NH
9
10'a
10'b
9'
10b
6.4 6.2 6.0 5.8 5.6 5.4 5.2 5.0 4.8 4.6 4.4 4.2 4.0 3.8
f1 (ppm)
1ab, OH
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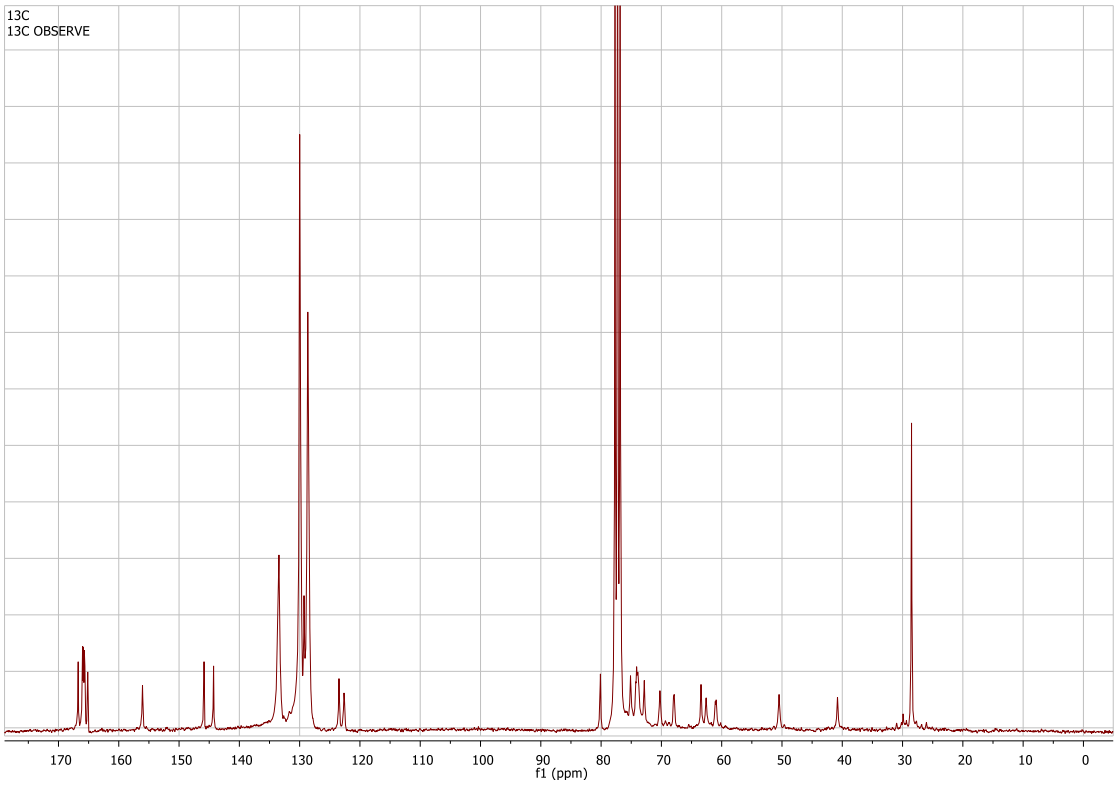
f1 (ppm)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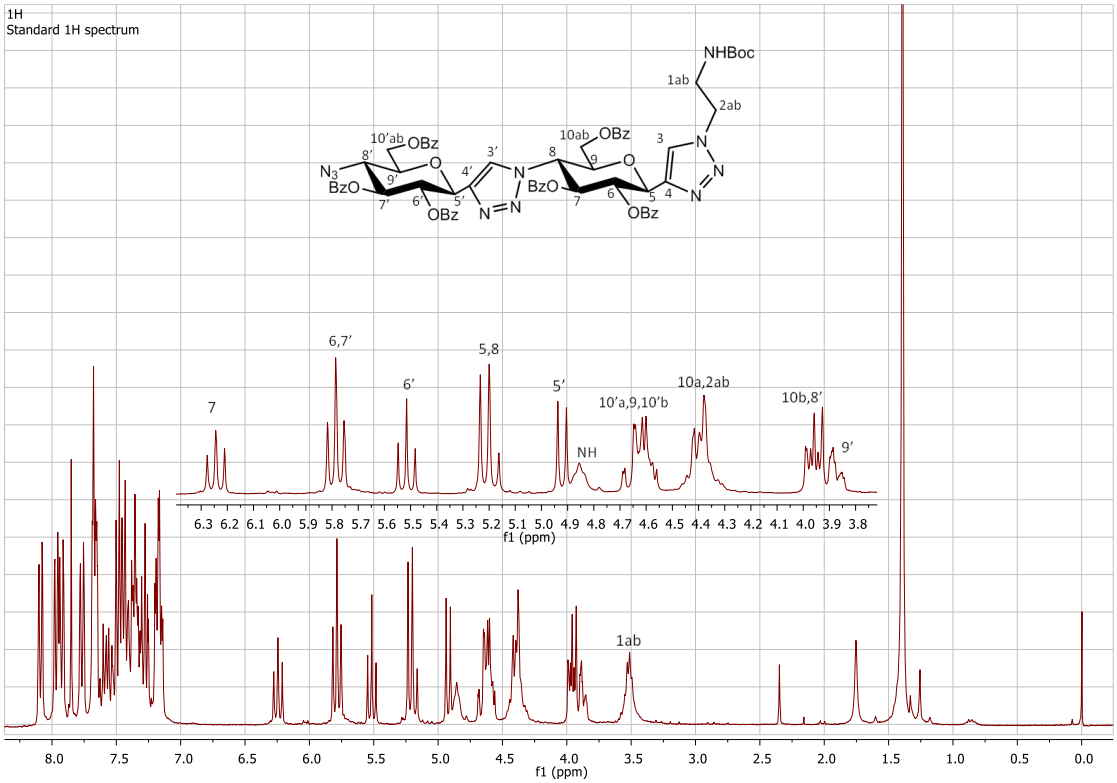
f1 (ppm)

7 in $CDCl_3$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2ab
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OBz
OBz
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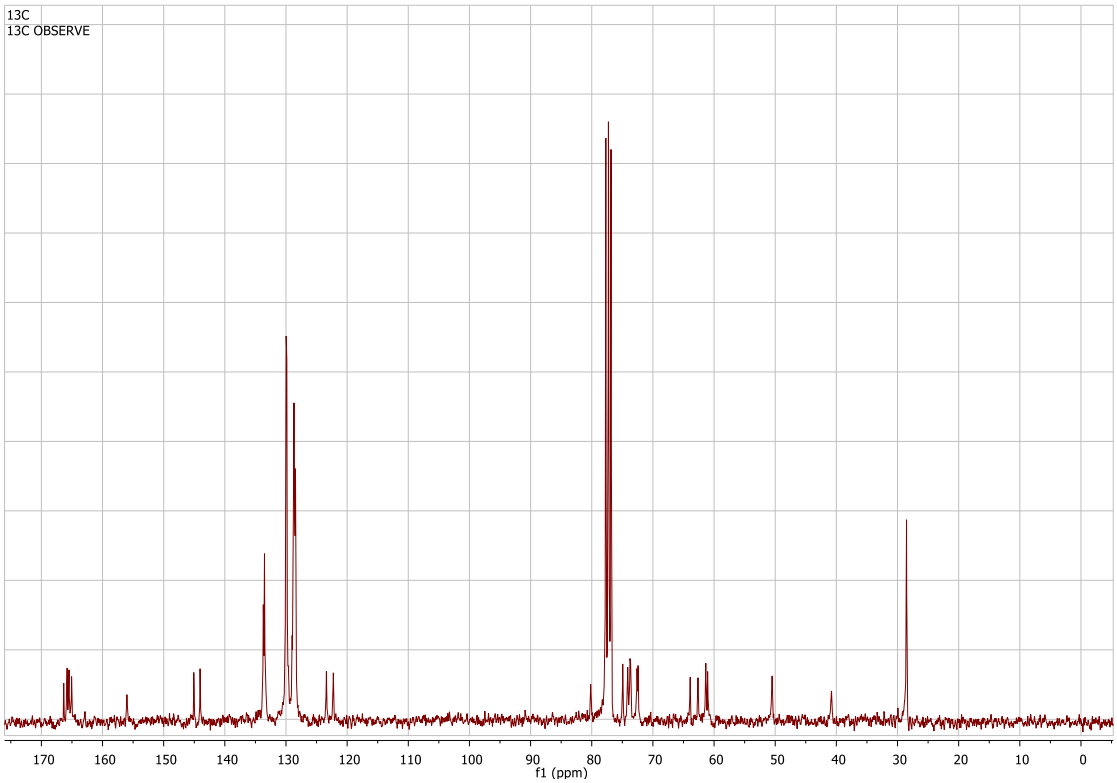
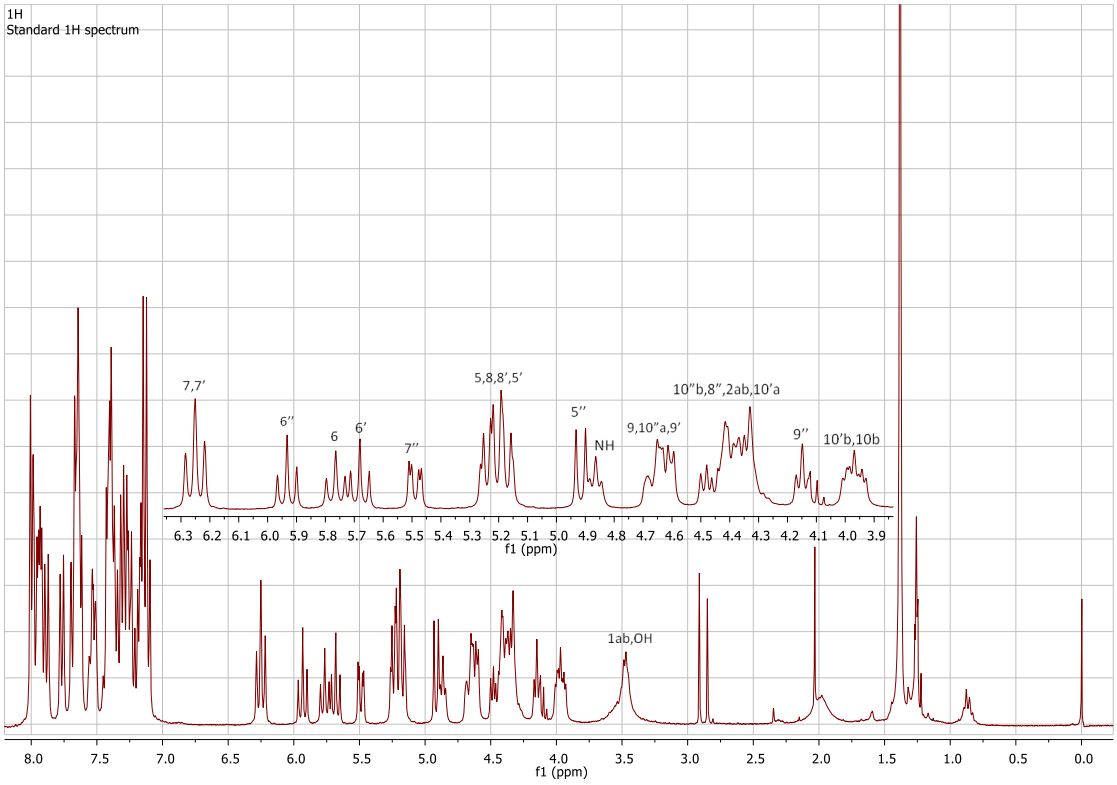
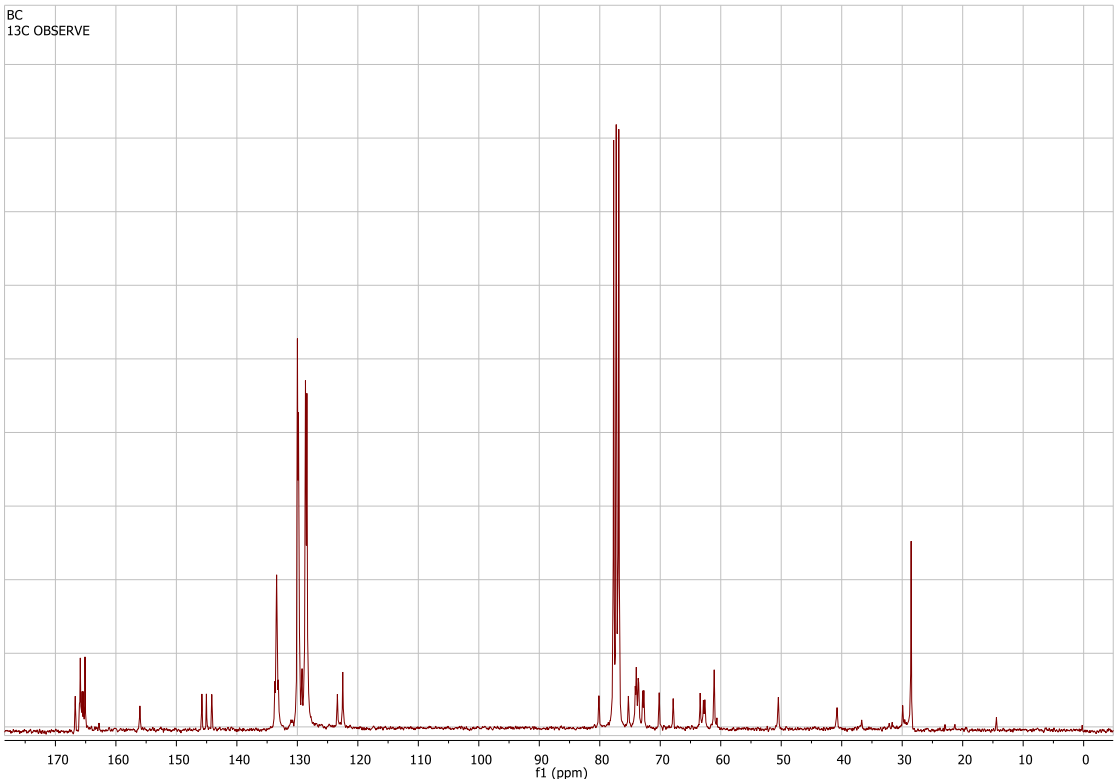
10b,8'
NH
9'
1ab
f1 (ppm)

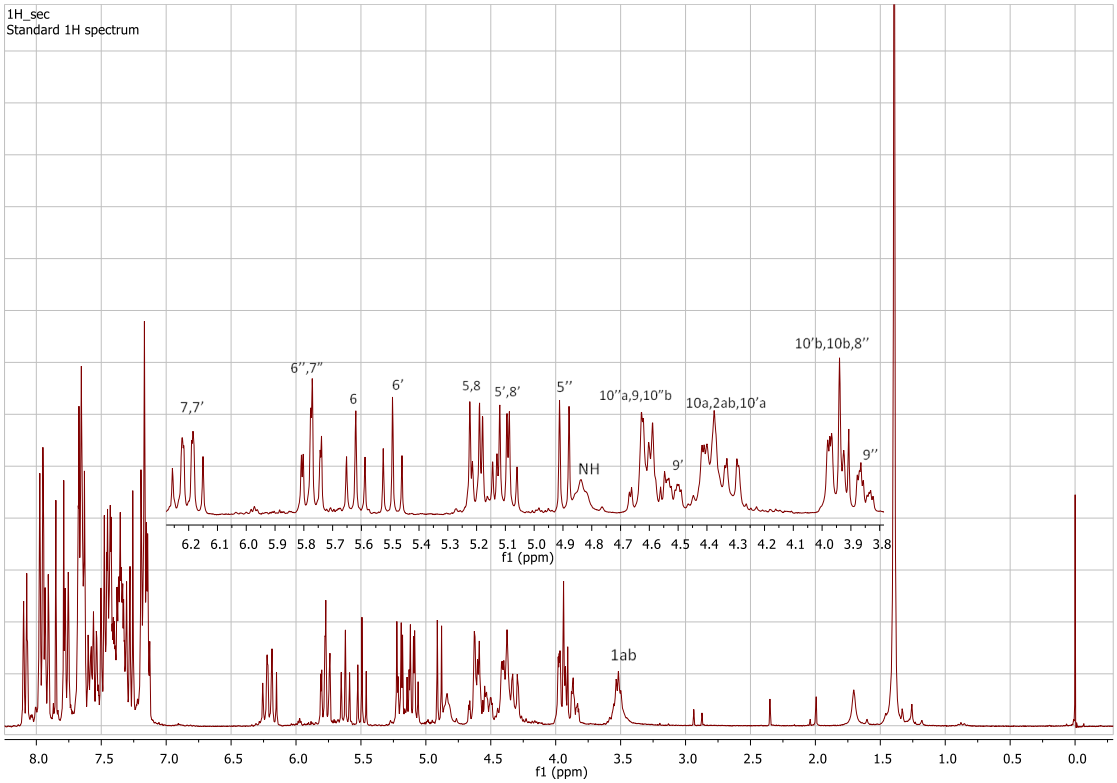

13C
13C OBSERVE
f1 (ppm)

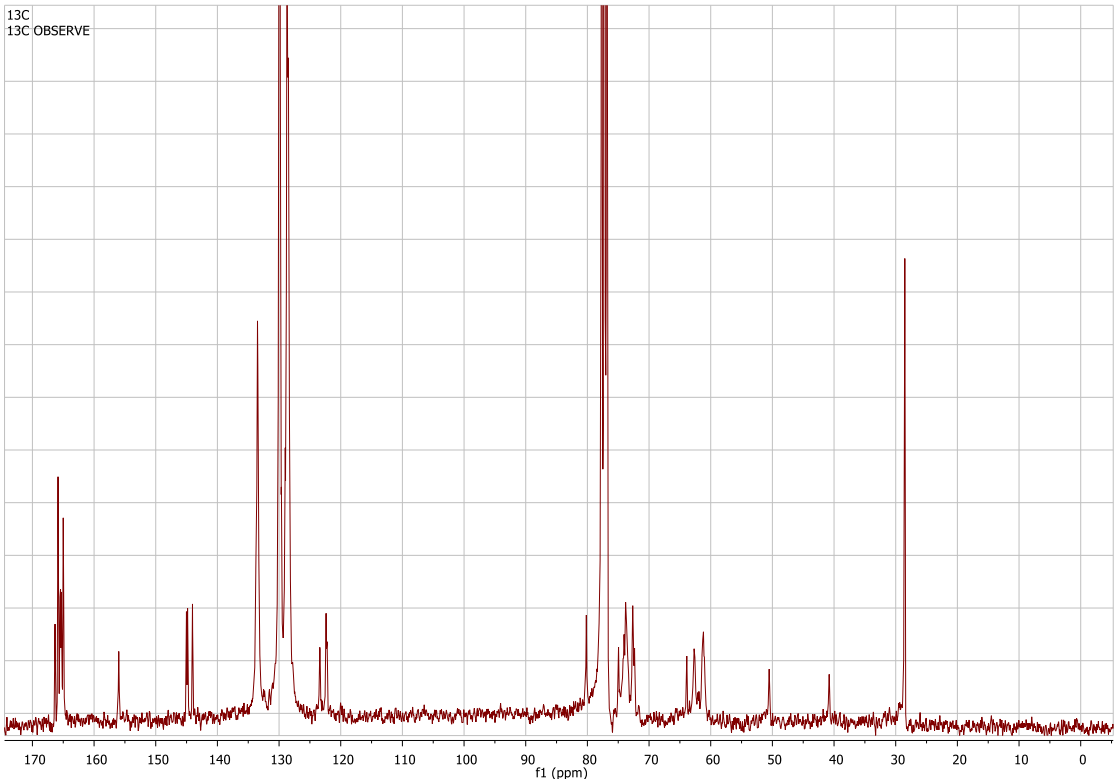
9a in $CDCl_3$


1H
Standard 1H spectrum
7,7'
6''
6
6'
7''
5,8,8',5'
5''
NH
9,10''a,9'
10''b,8'',2ab,10'a
9''
10'b,10b
1ab,OH
f1 (ppm)

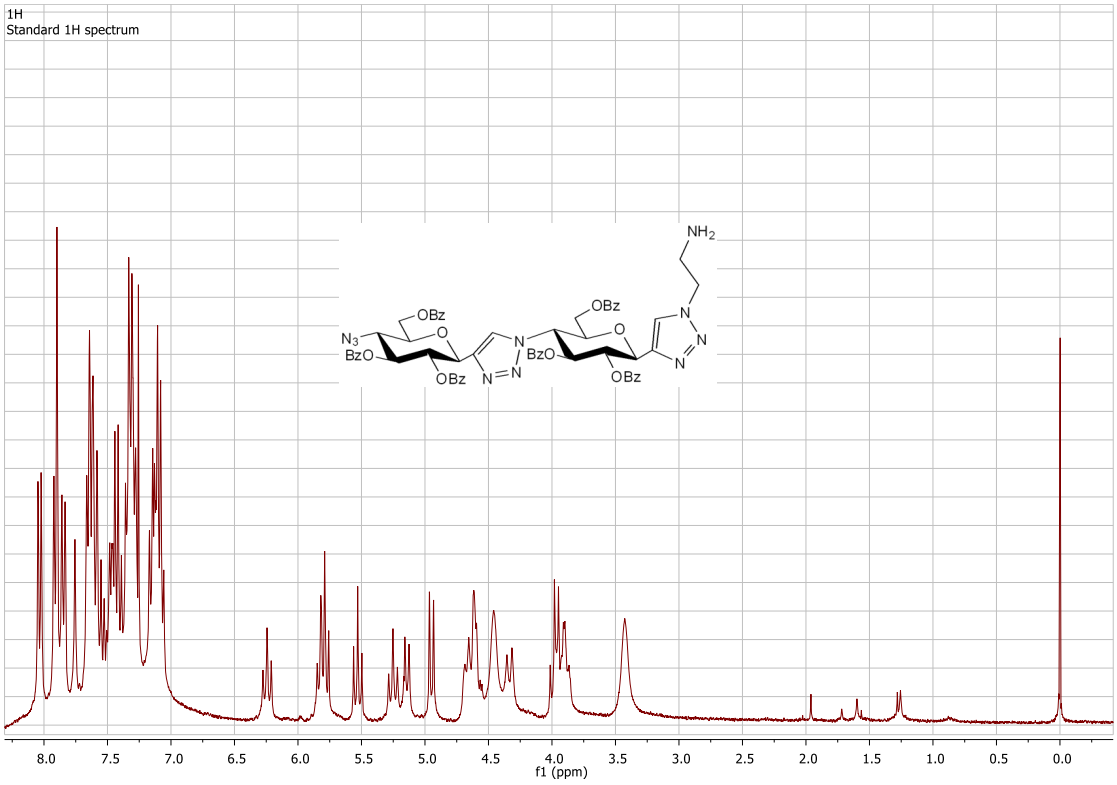

BC
13C OBSERVE
f1 (ppm)

9 in $CDCl_3$

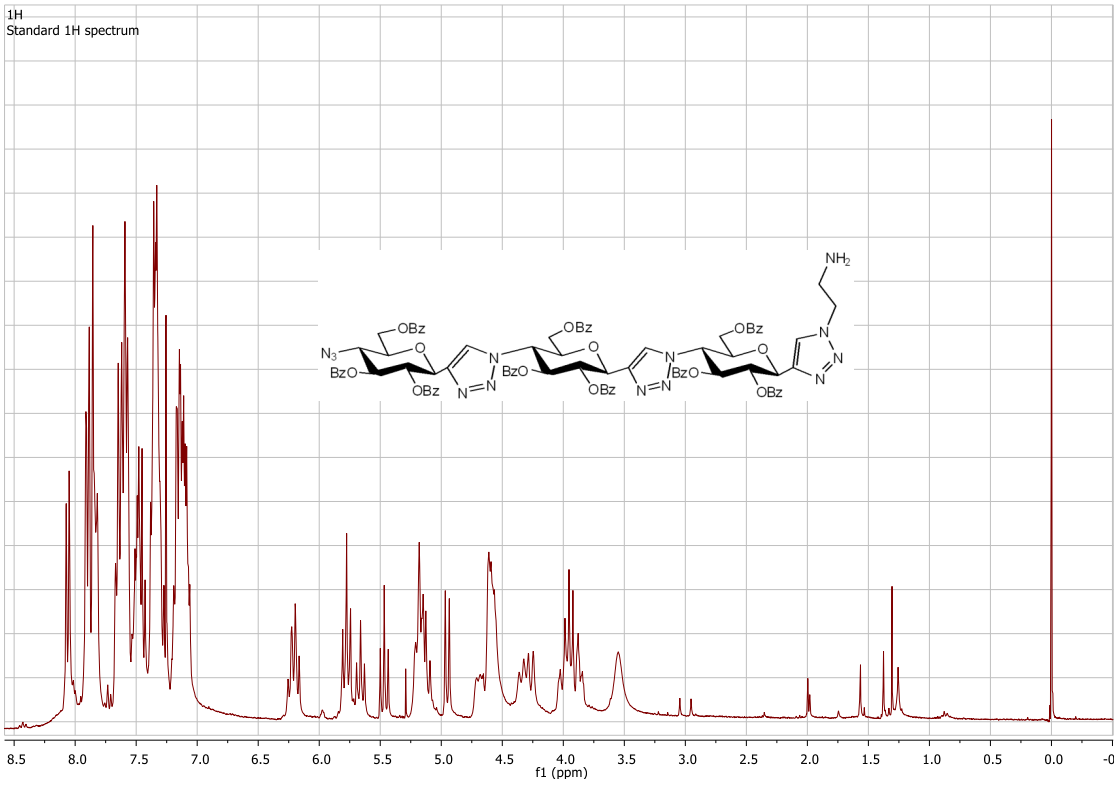

1H_sec
Standard 1H spectrum
7,7'
6'',7''
6
6'
5,8
5',8'
5''
NH
10''a,9,10''b
9'
10a,2ab,10'a
10'b,10b,8''
9''
1ab
f1 (ppm)


13C
13C OBSERVE
f1 (ppm)

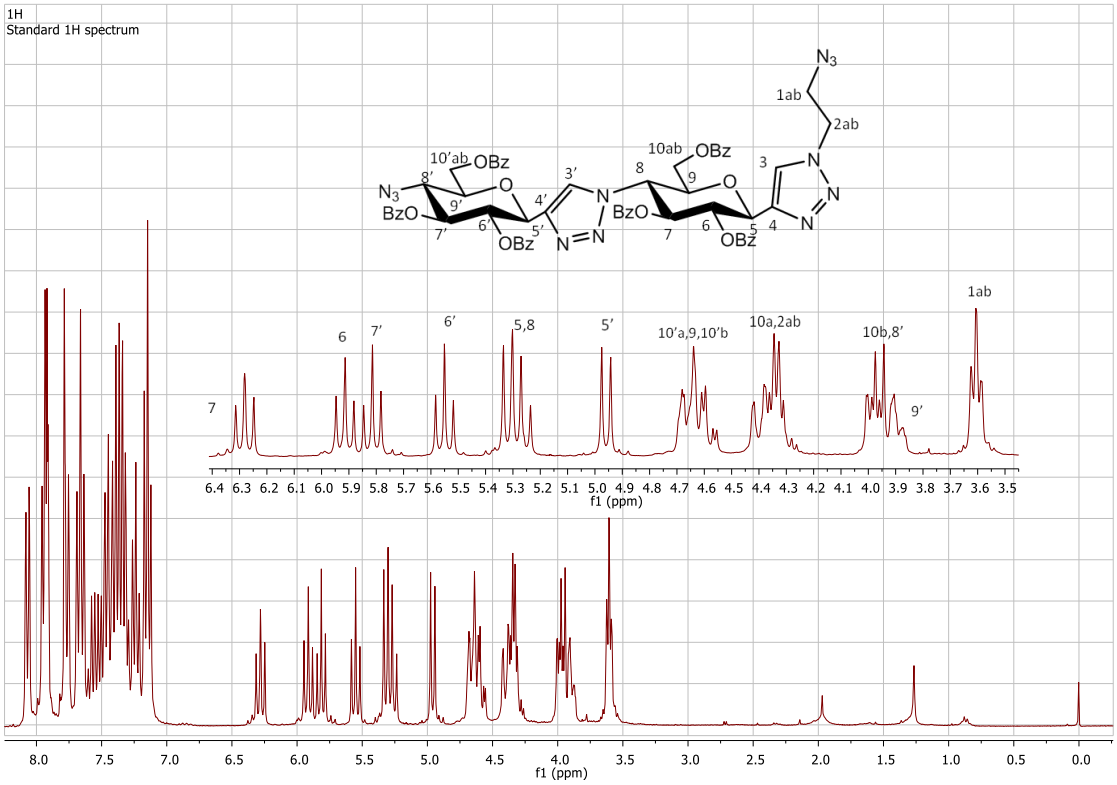
8a in $CDCl_3$

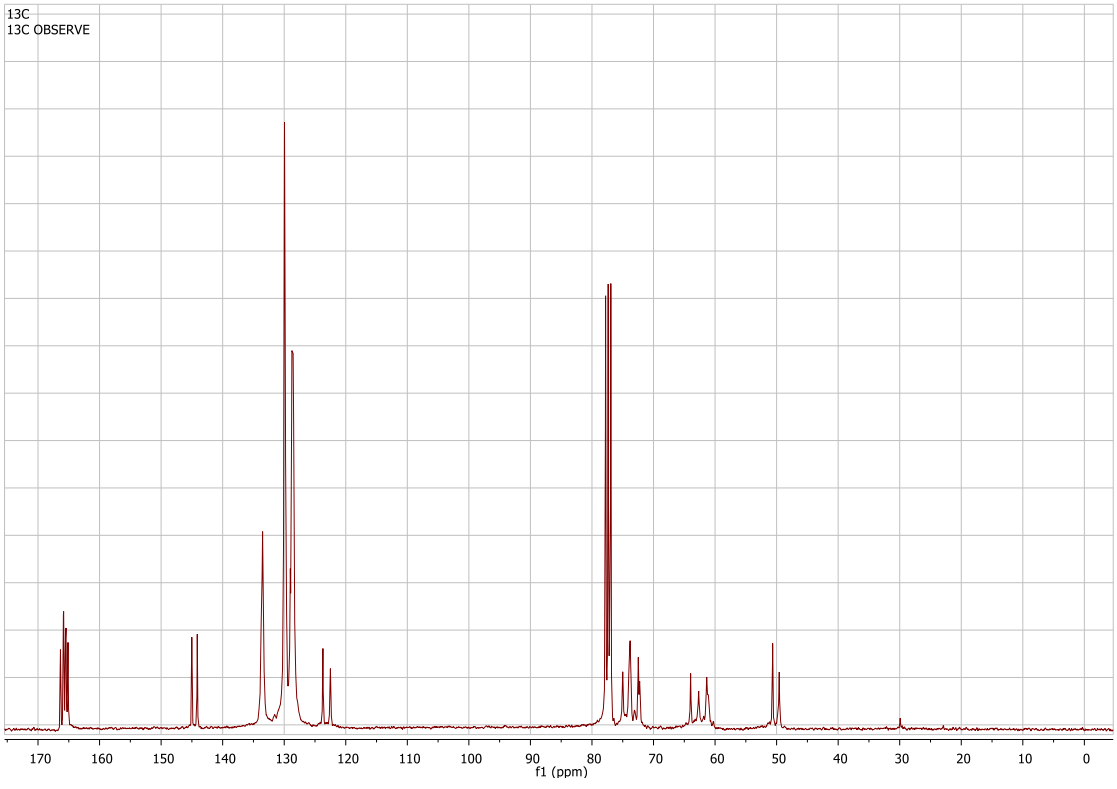


10a in $CDCl_3$

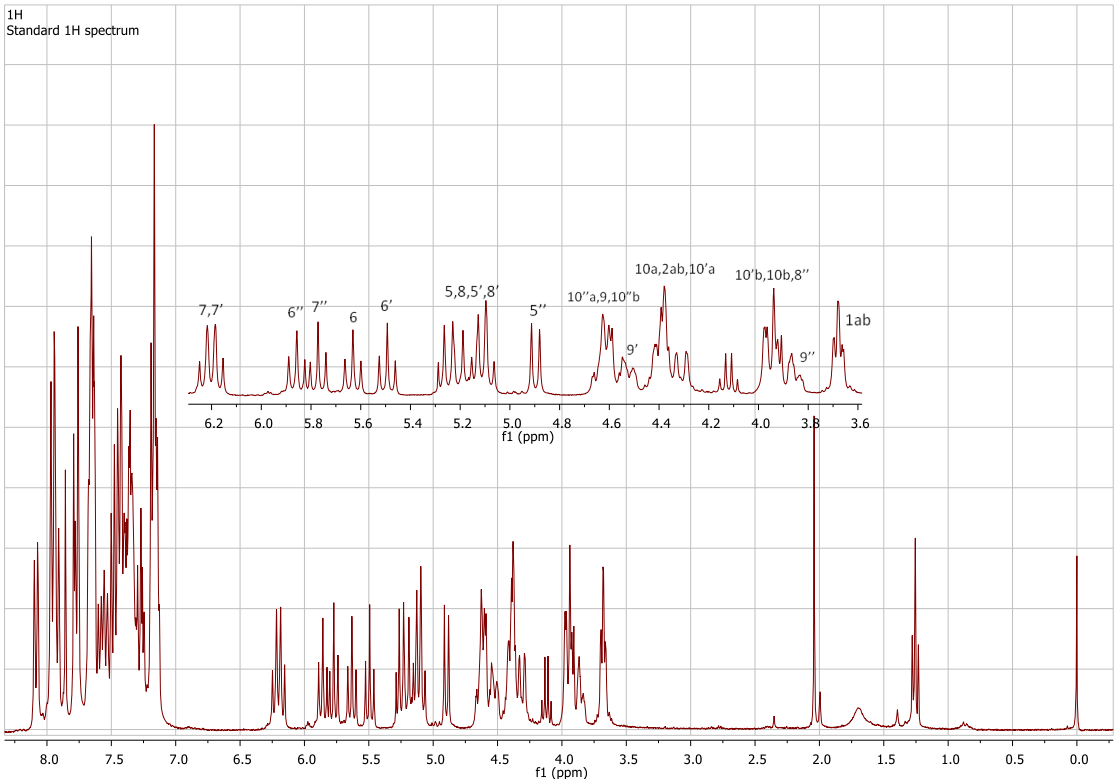


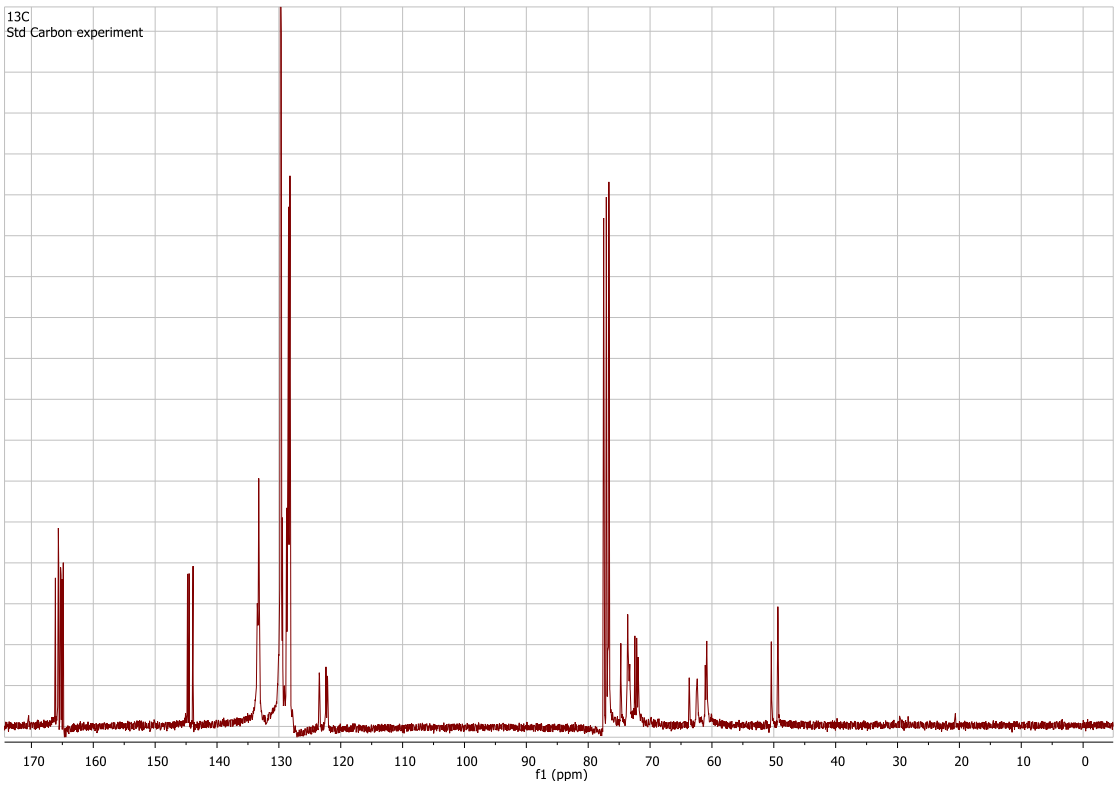
8 in $CDCl_3$


1H
Standard 1H spectrum
N3
1ab
2ab
10'ab
OBz
10ab
OBz
BzO
BzO
OBz
OBz
6
7'
6'
5,8
5'
10'a,9,10'b
10a,2ab
10b,8'
1ab
7
9'
f1 (ppm)

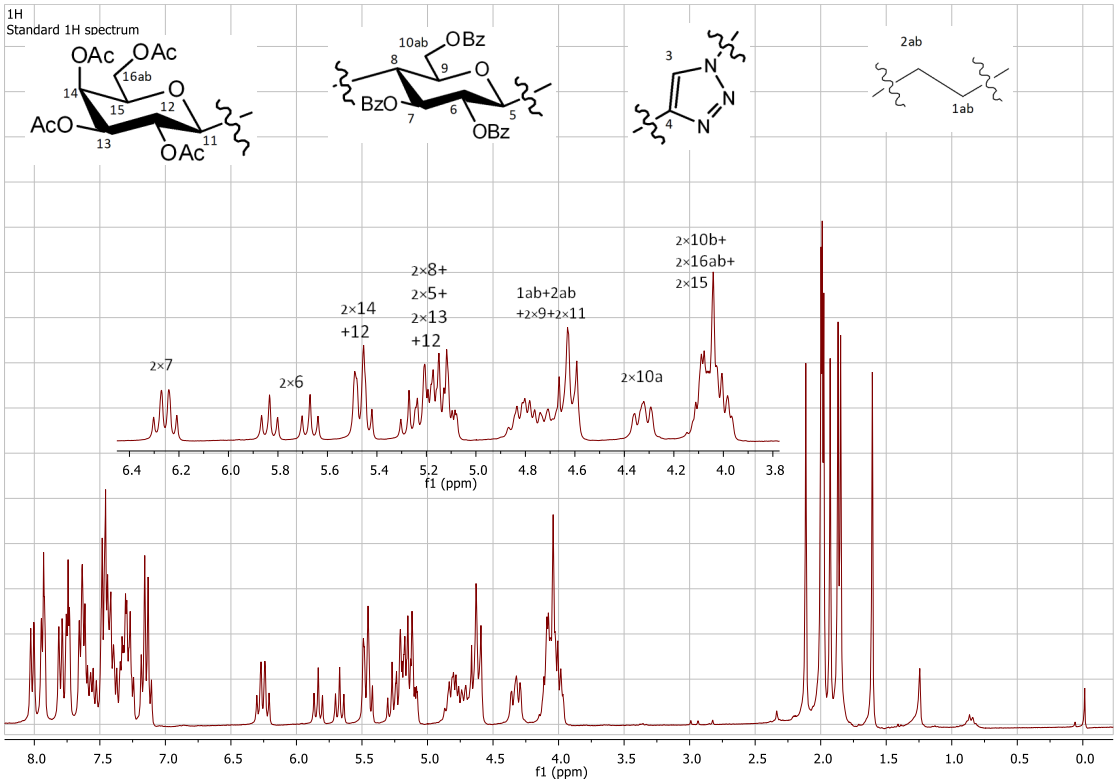

13C
13C OBSERVE
f1 (ppm)

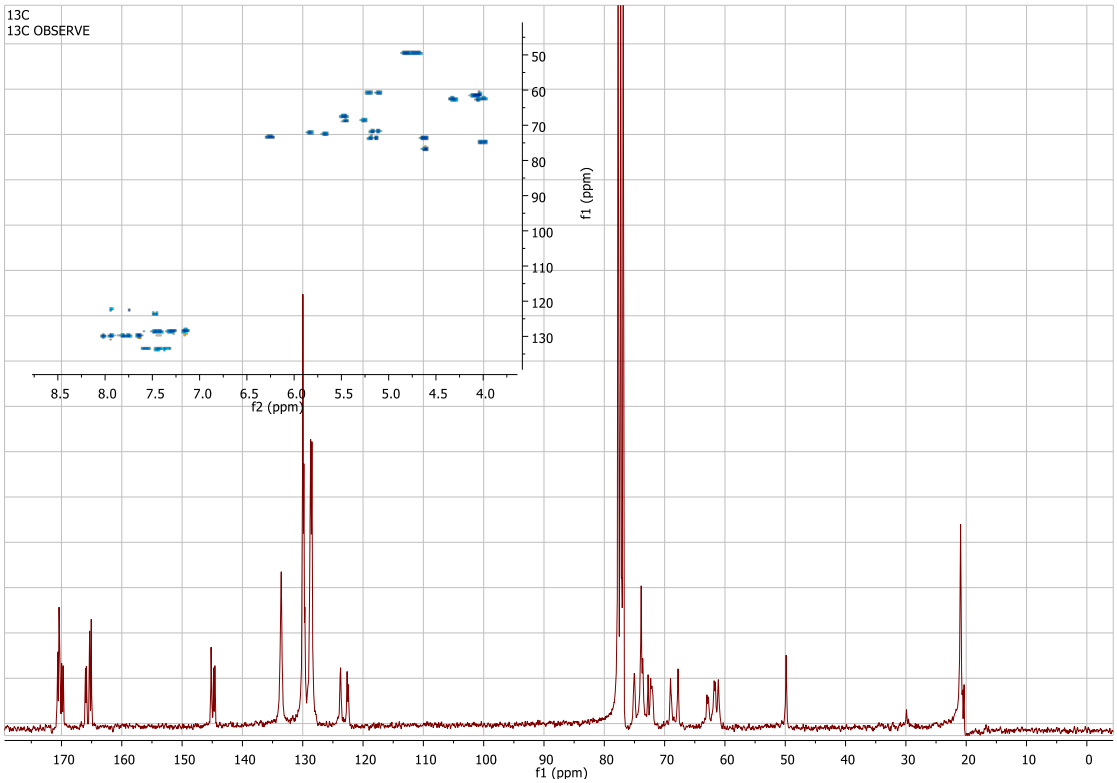
10 in $CDCl_3$


1H
Standard 1H spectrum
7,7′
6″
7″
6
6′
5,8,5′,8′
5″
10″a,9,10″b
10a,2ab,10′a
9′
10′b,10b,8″
9″
1ab
f1 (ppm)

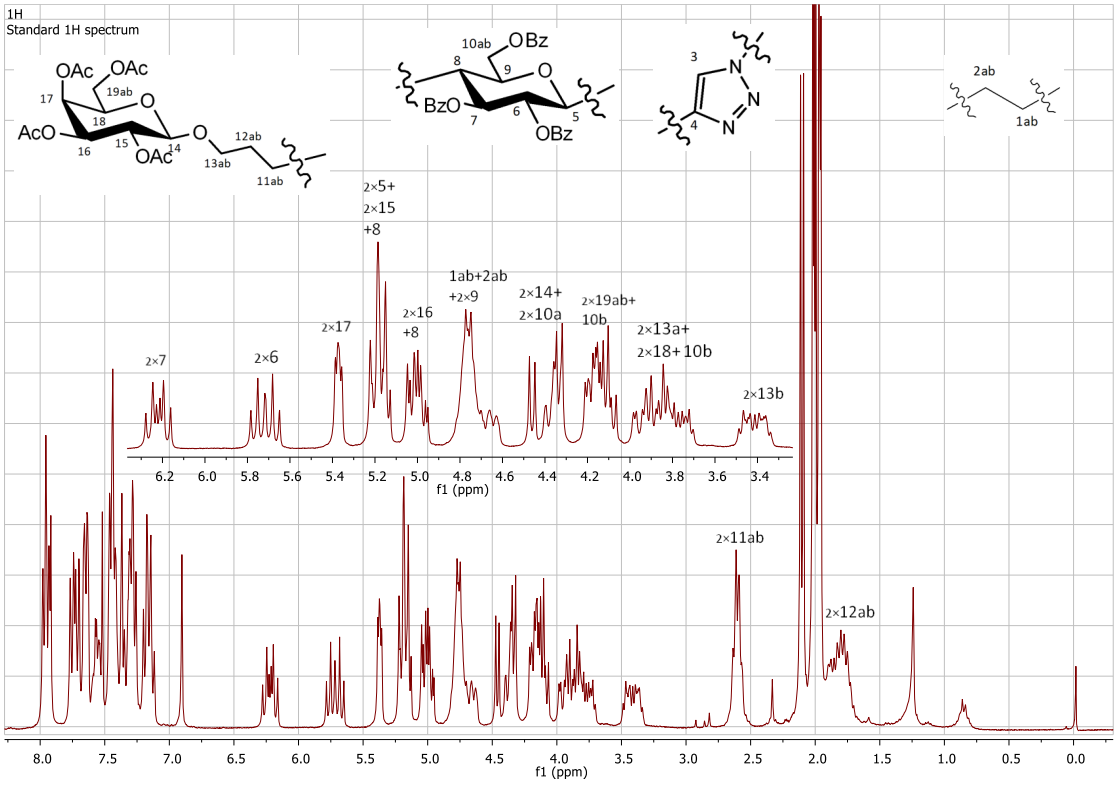

13C
Std Carbon experiment
f1 (ppm)

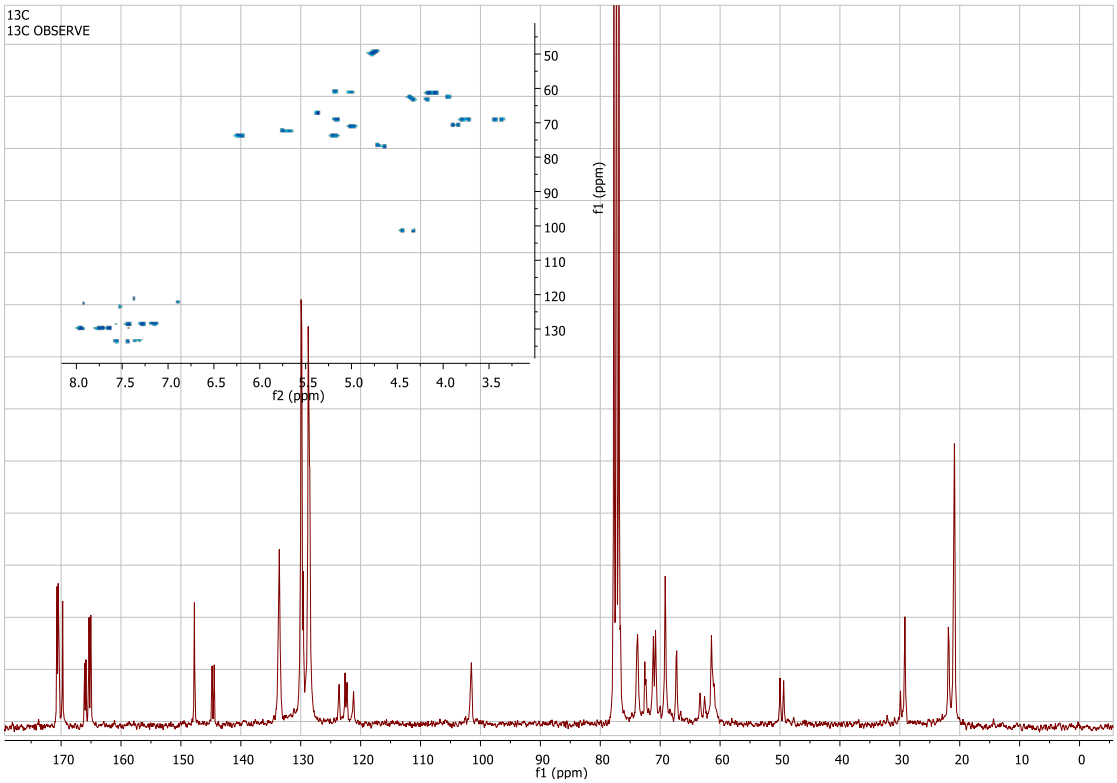
11 in $CDCl_3$


1H
Standard 1H spectrum
OAc
OAc
16ab
14
15
12
O
AcO
13
OAc
11
10ab
OBz
8
9
O
BzO
7
6
5
OBz
3
N
N
4
N
2ab
1ab
2×7
2×6
2×14
+12
2×8+
2×5+
2×13
+12
1ab+2ab
+2×9+2×11
2×10a
2×10b+
2×16ab+
2×15
f1 (ppm)

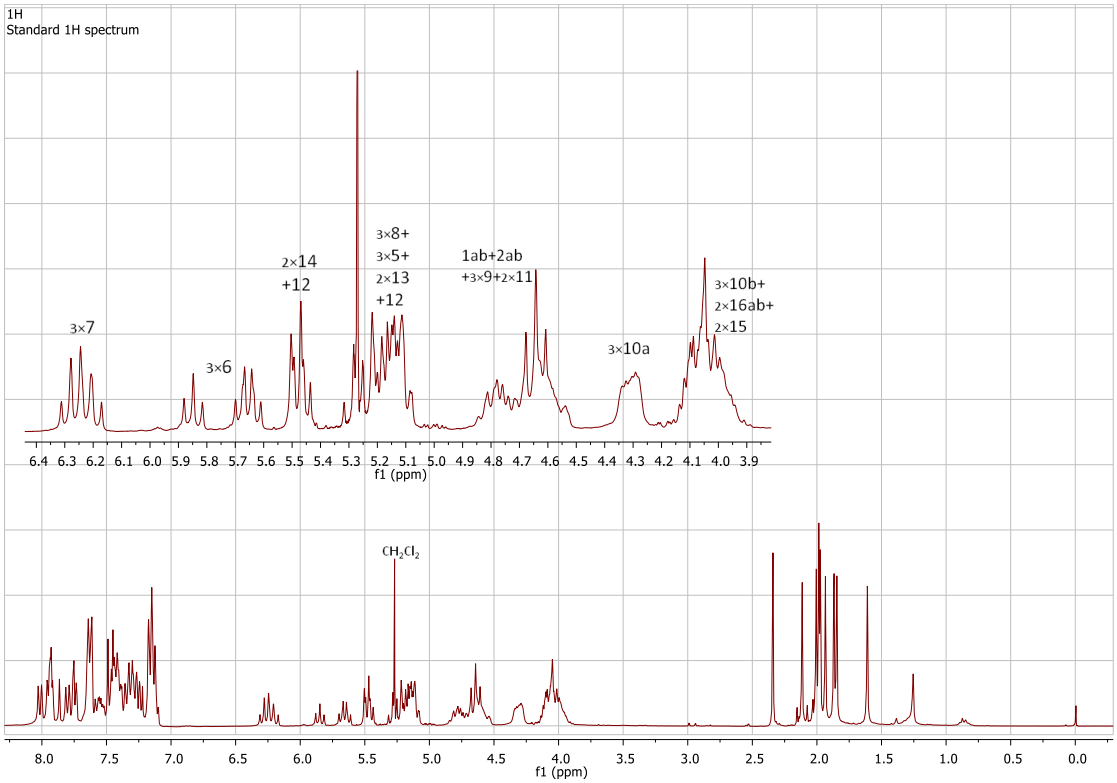

13C
13C OBSERVE
f1 (ppm)
f2 (ppm)

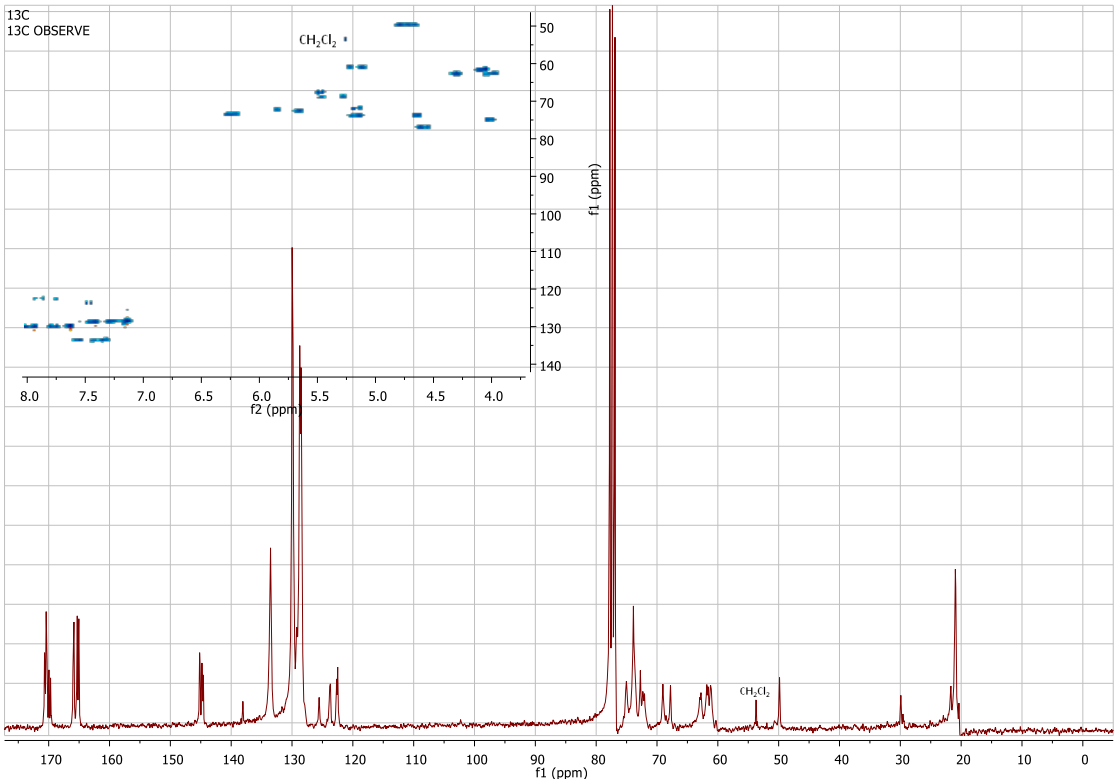
13 in $CDCl_3$


1H
Standard 1H spectrum
OAc
OAc
19ab
17
O
18
AcO
16
15
OAc
14
O
12ab
13ab
11ab
10ab
OBz
8
9
O
BzO
7
6
5
OBz
3
N
N
4
N
2ab
1ab
2×5+
2×15
+8
1ab+2ab
+2×9
2×14+
2×10a
2×19ab+
10b
2×17
2×16
+8
2×13a+
2×18+10b
2×7
2×6
2×13b
2×11ab
2×12ab
f1 (ppm)

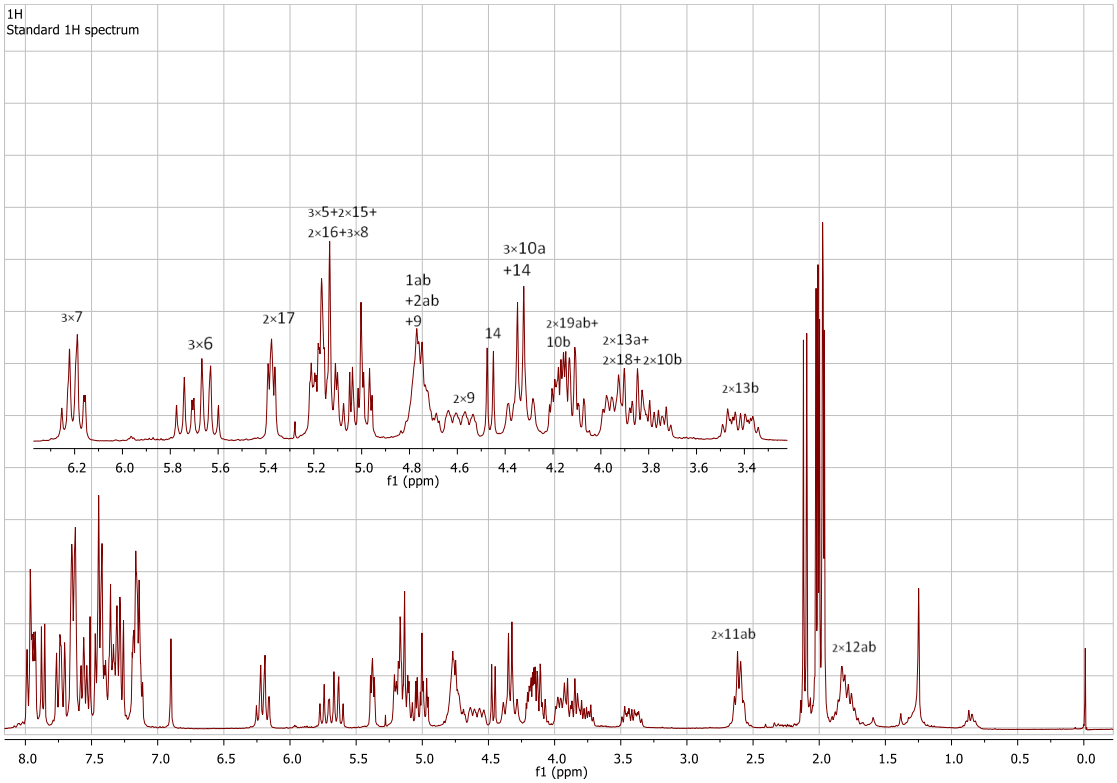

13C
13C OBSERVE
f1 (ppm)
f2 (ppm)

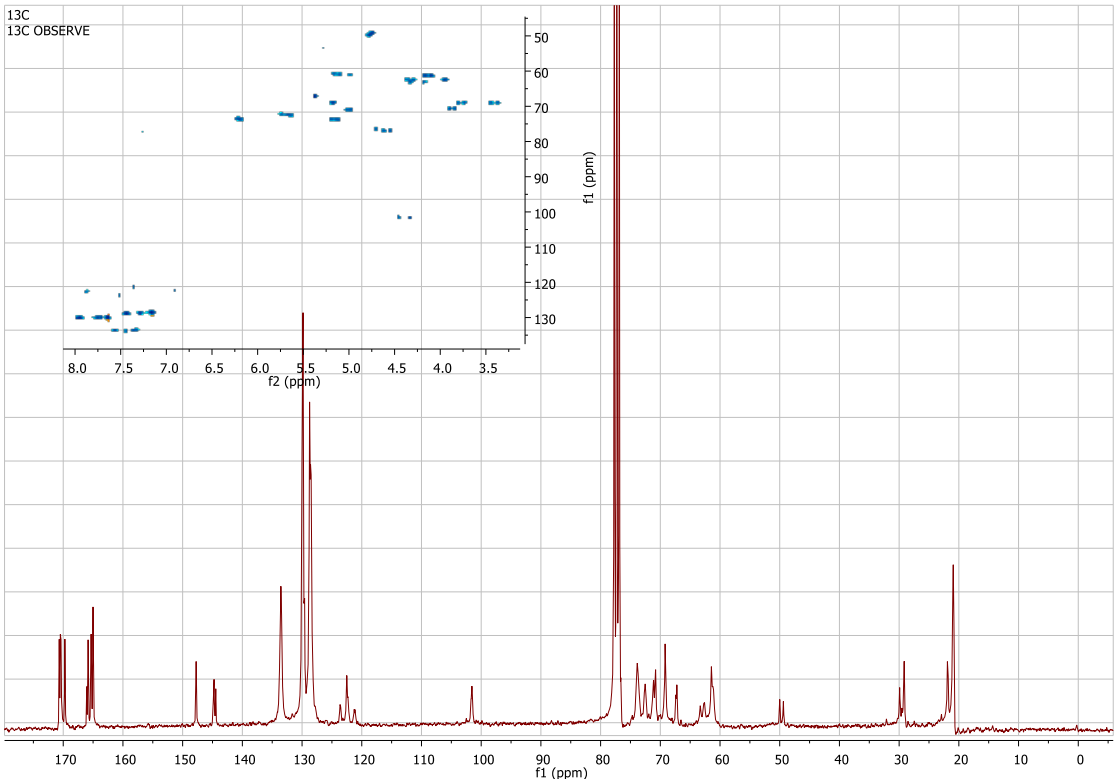
15 in $CDCl_3$


1H
Standard 1H spectrum
3×7
3×6
2×14
+12
3×8+
3×5+
2×13
+12
1ab+2ab
+3×9+2×11
3×10a
3×10b+
2×16ab+
2×15
f1 (ppm)
CH2Cl2

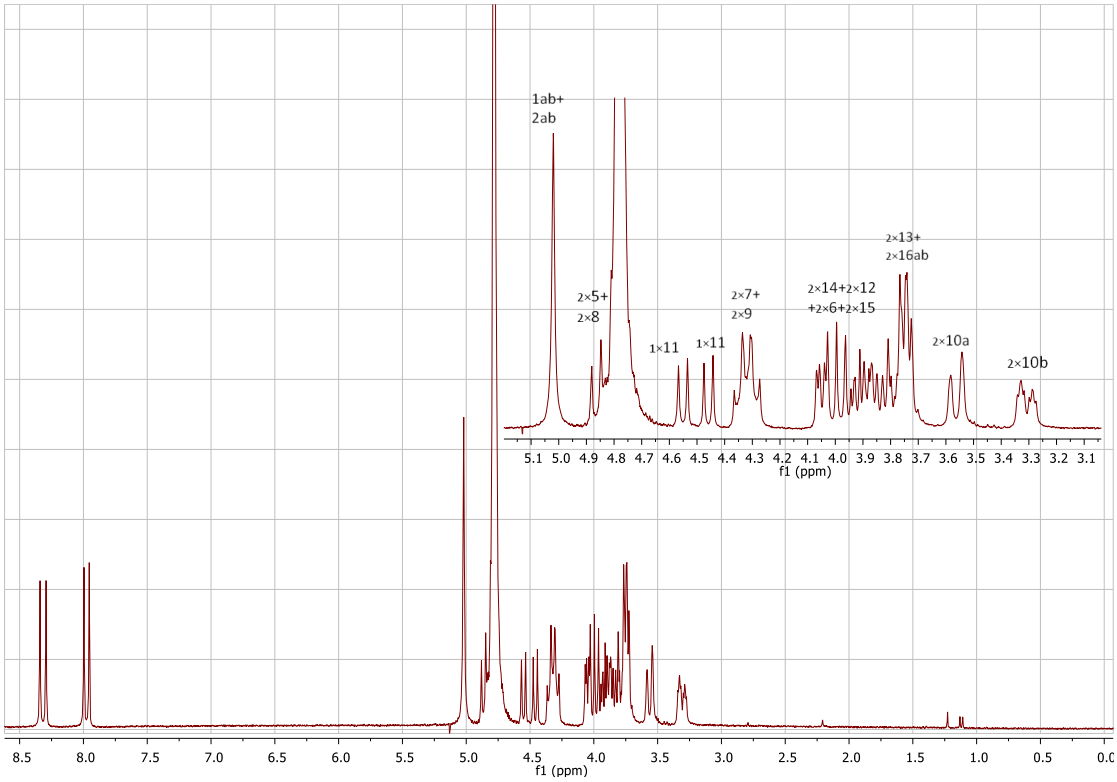

13C
13C OBSERVE
CH2Cl2
f2 (ppm)
f1 (ppm)
CH2Cl2

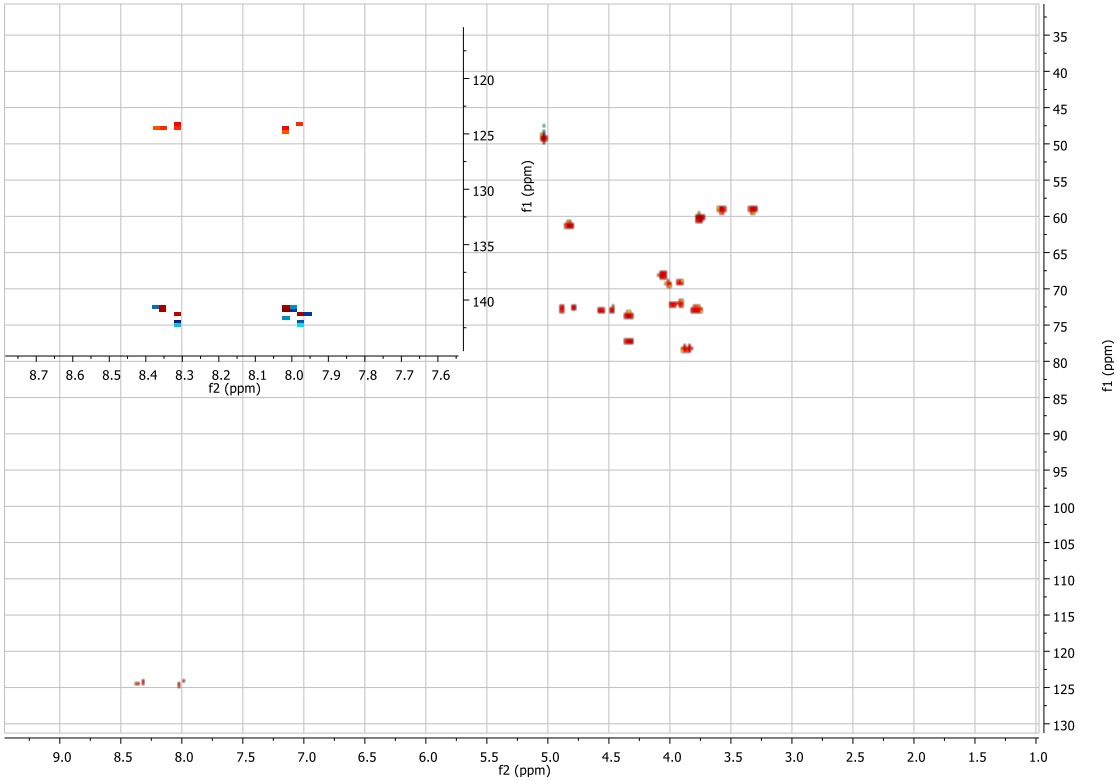
17 in $CDCl_3$


1H
Standard 1H spectrum
3×7
3×6
2×17
3×5+2×15+
2×16+3×8
1ab
+2ab
+9
2×9
14
3×10a
+14
2×19ab+
10b
2×13a+
2×18+2×10b
2×13b
2×11ab
2×12ab
f1 (ppm)

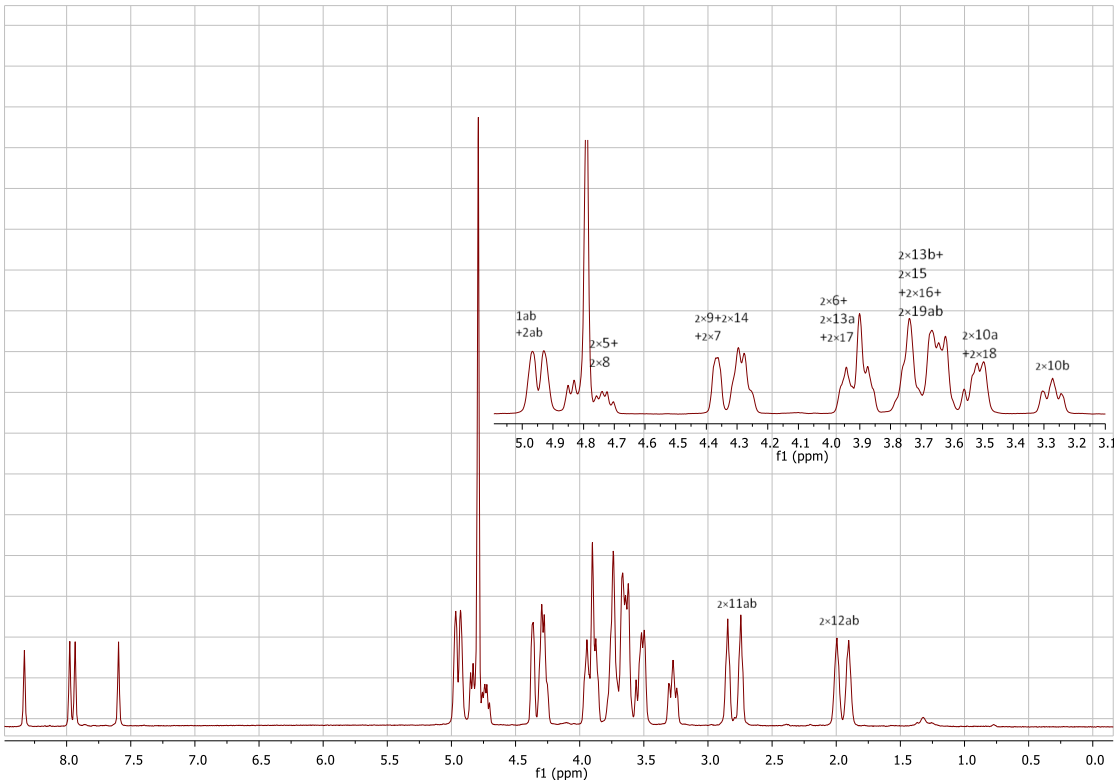

13C
13C OBSERVE
f1 (ppm)
f2 (ppm)

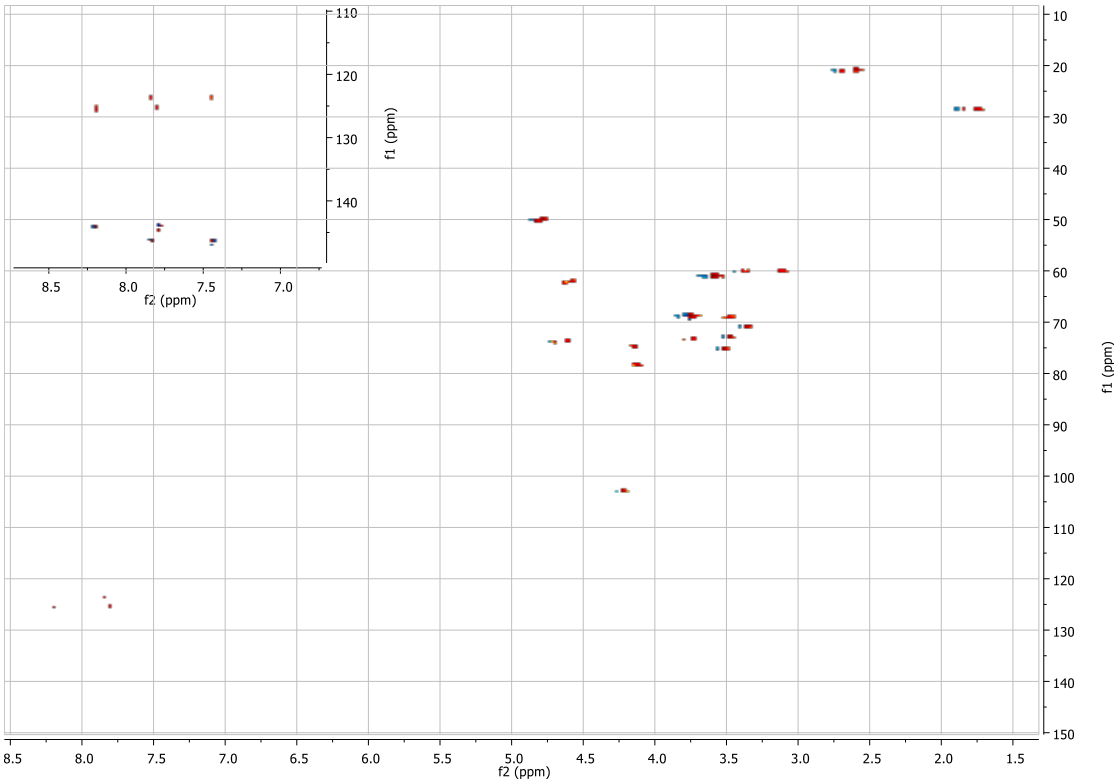
12 in D_2O


1ab+
2ab
2×5+
2×8
1×11
1×11
2×7+
2×9
2×14+2×12
+2×6+2×15
2×13+
2×16ab
2×10a
2×10b
f1 (ppm)

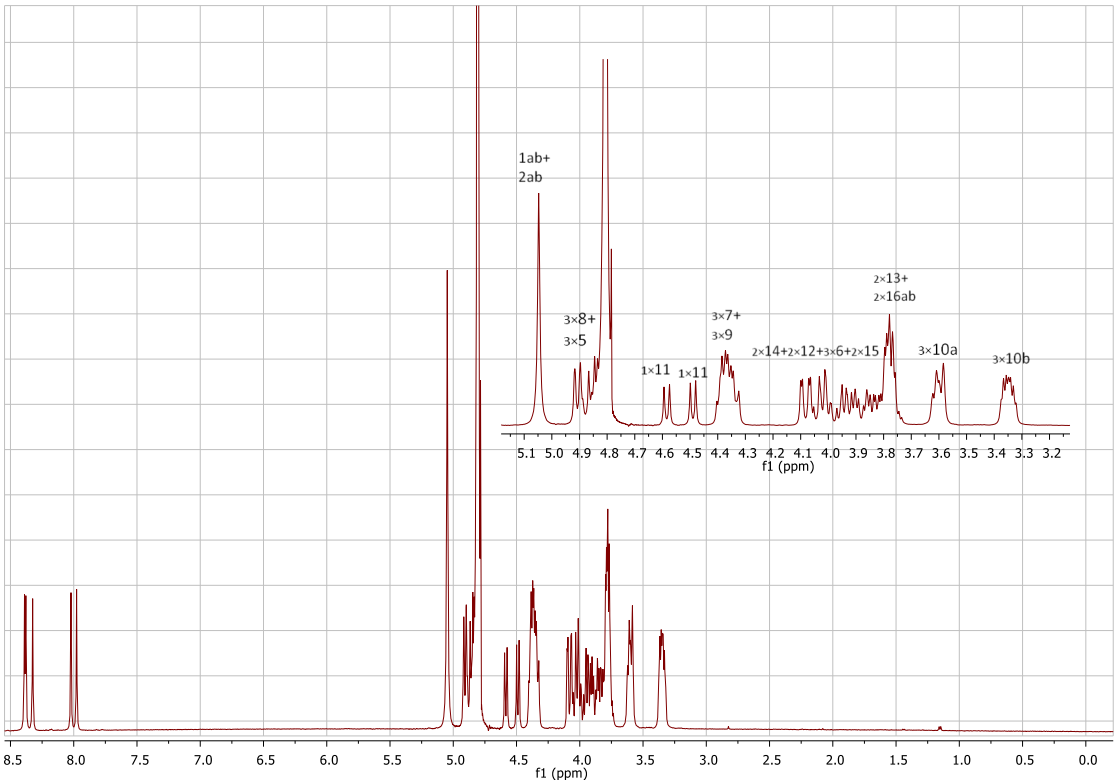

f2 (ppm)
f1 (ppm)
f2 (ppm)
f1 (ppm)

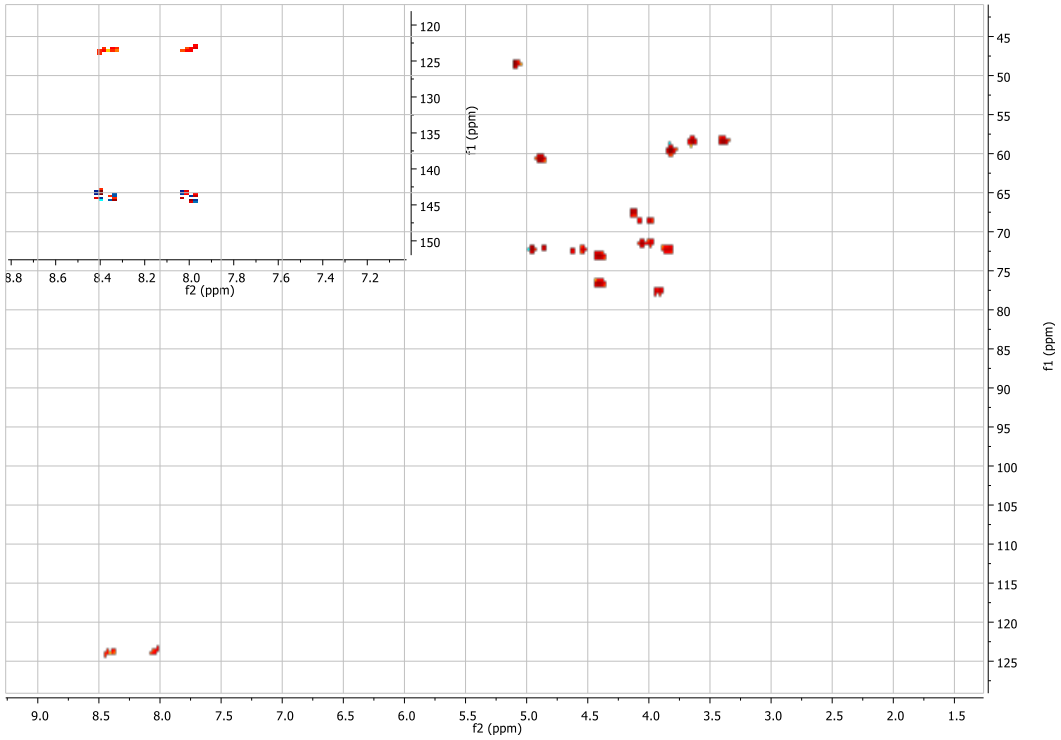
14 in D_2O


1ab
+2ab
2×5+
2×8
2×9+2×14
+2×7
2×6+
2×13a
+2×17
2×13b+
2×15
+2×16+
2×19ab
2×10a
+2×18
2×10b
2×11ab
2×12ab
f1 (ppm)

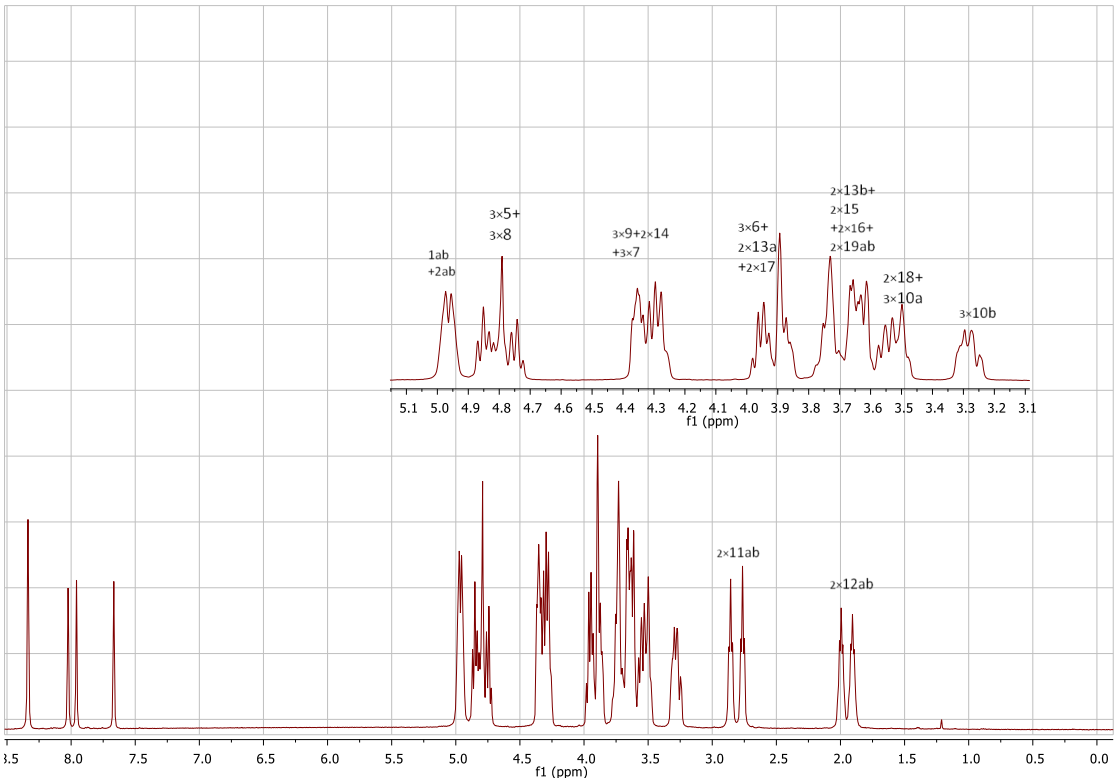

f1 (ppm)
f2 (ppm)

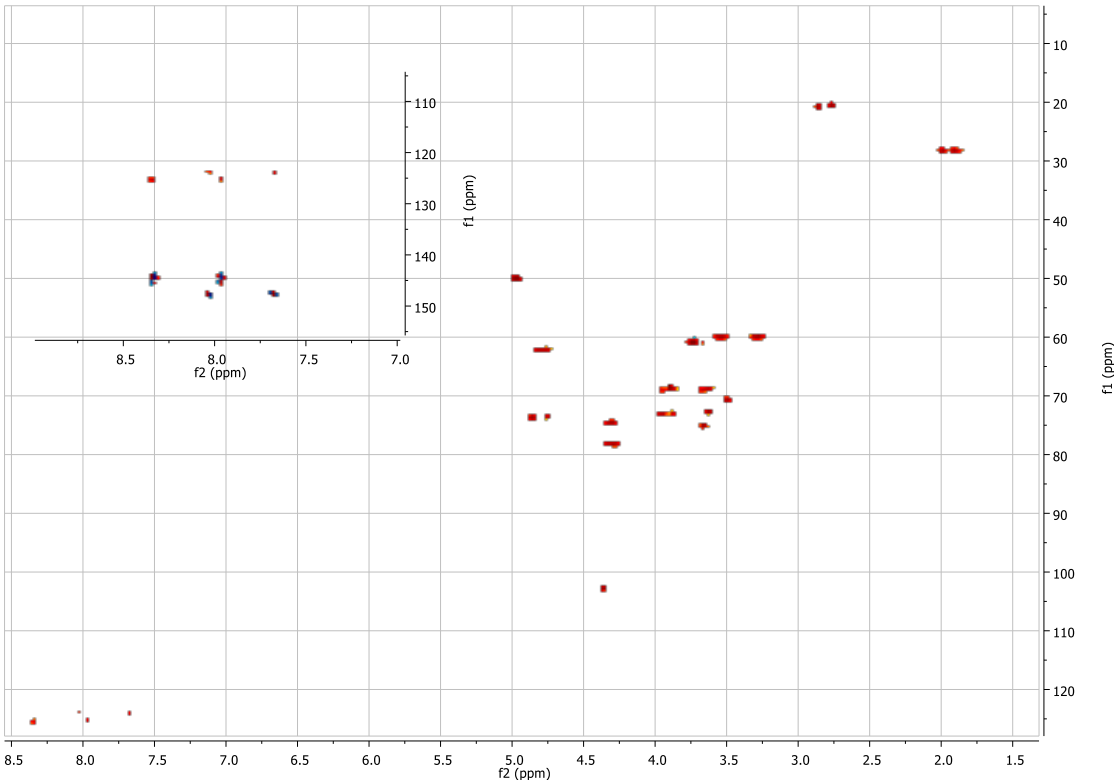
16 in D_2O


1ab+
2ab
3×8+
3×5
1×11
1×11
3×7+
3×9
2×14+2×12+3×6+2×15
2×13+
2×16ab
3×10a
3×10b
f1 (ppm)

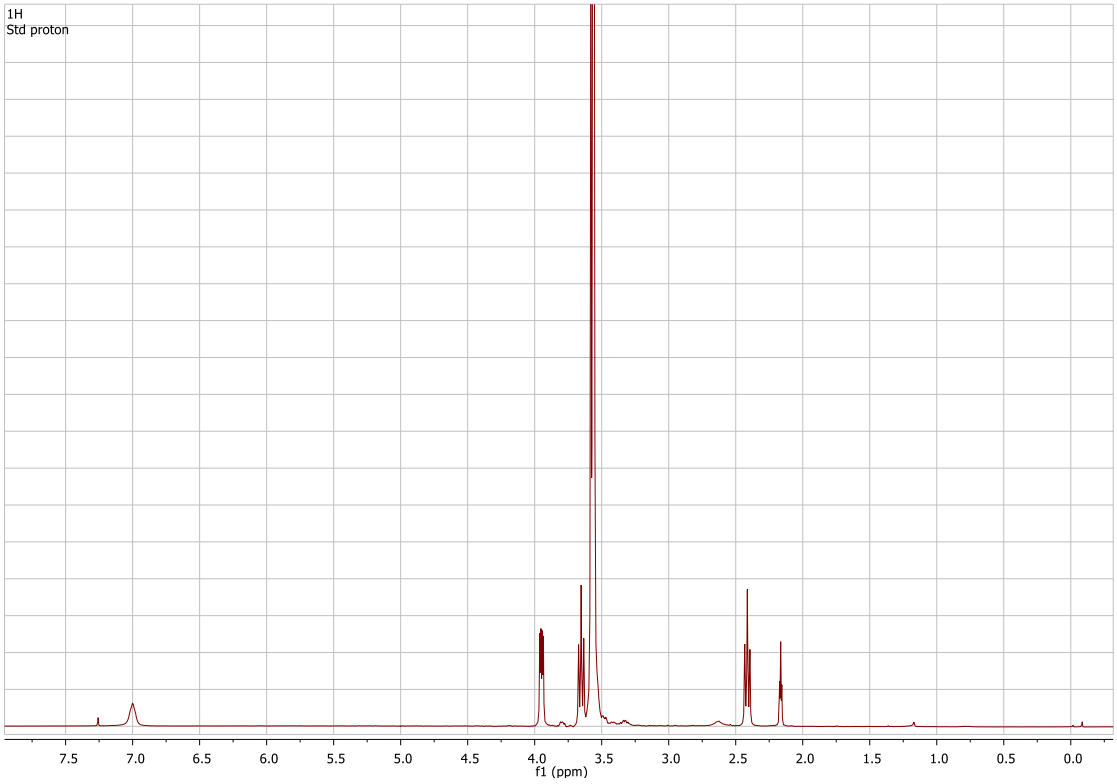

f2 (ppm)
f1 (ppm)
f2 (ppm)
f1 (ppm)

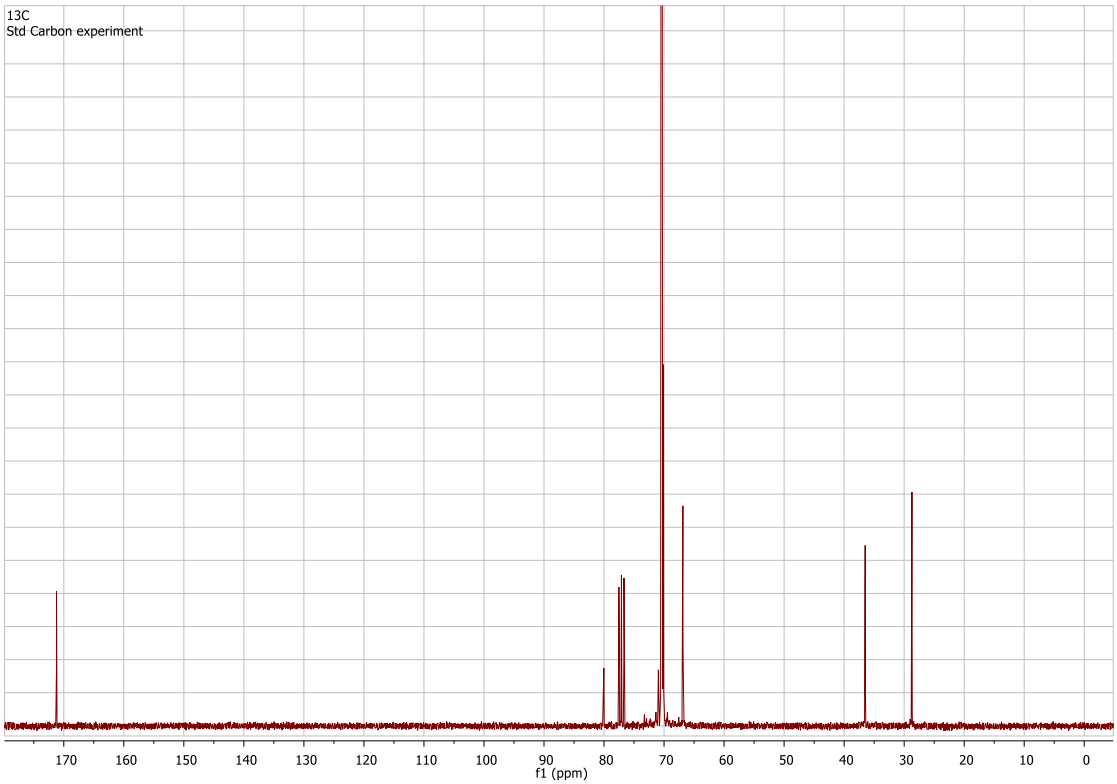
18 in D_2O


1ab
+2ab
3×5+
3×8
3×9+2×14
+3×7
3×6+
2×13a
+2×17
2×13b+
2×15
+2×16+
2×19ab
2×18+
3×10a
3×10b
2×11ab
2×12ab
f1 (ppm)

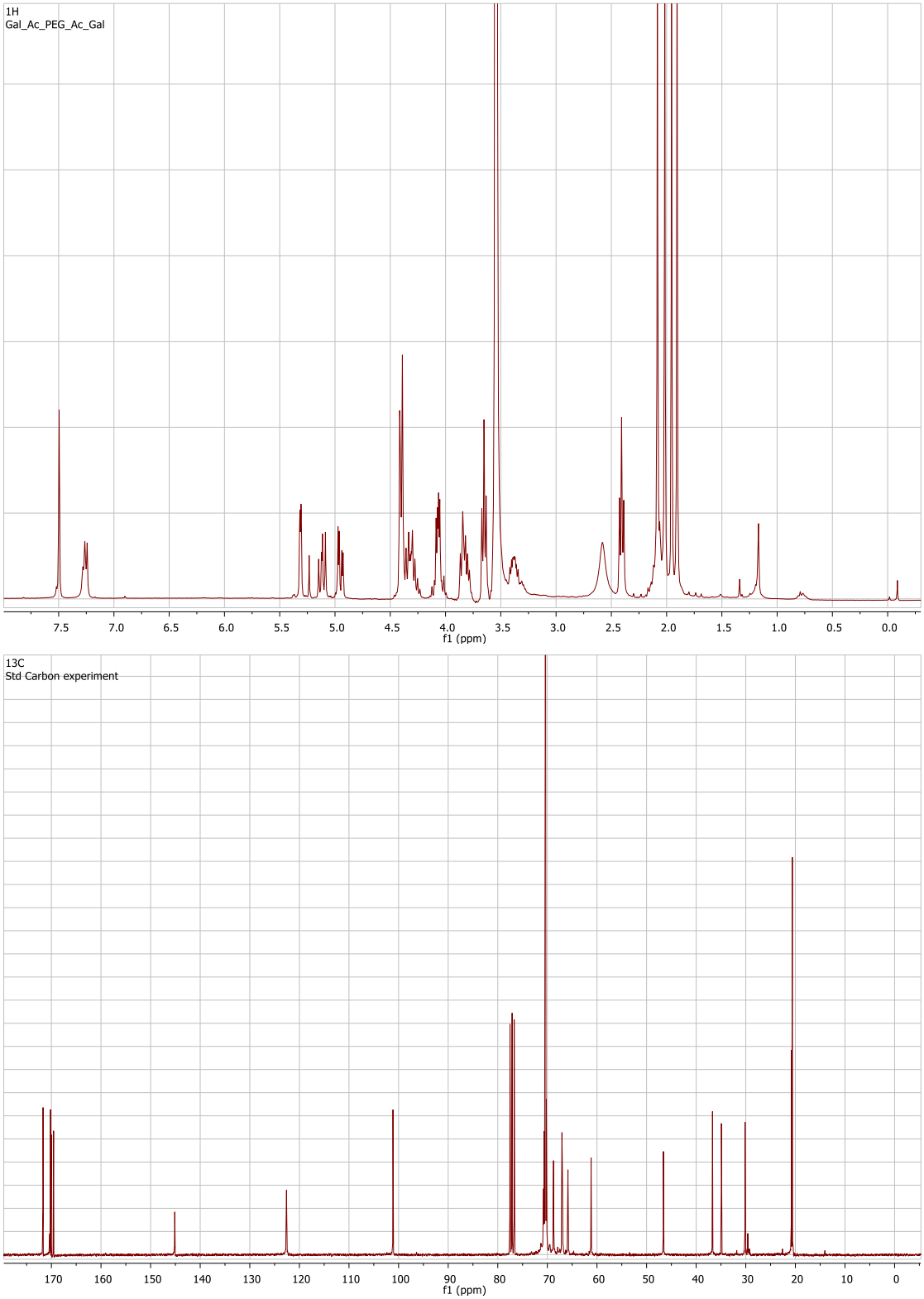

f1 (ppm)
f2 (ppm)

19b in $CDCl_3$

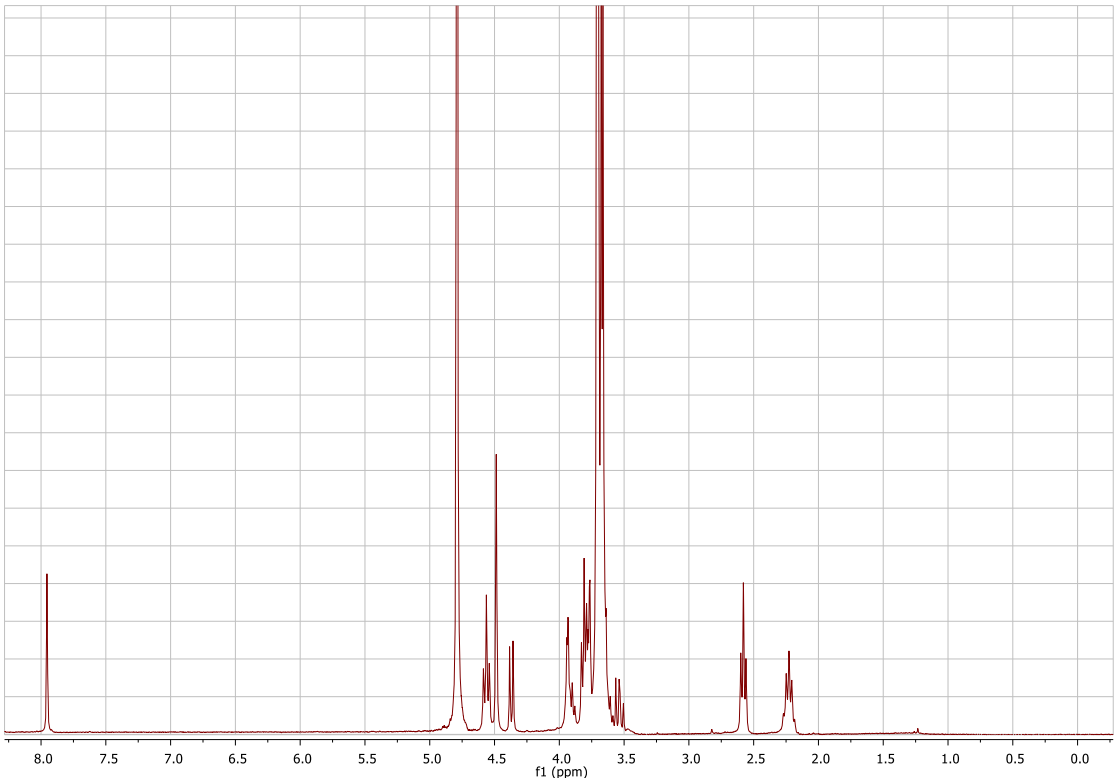


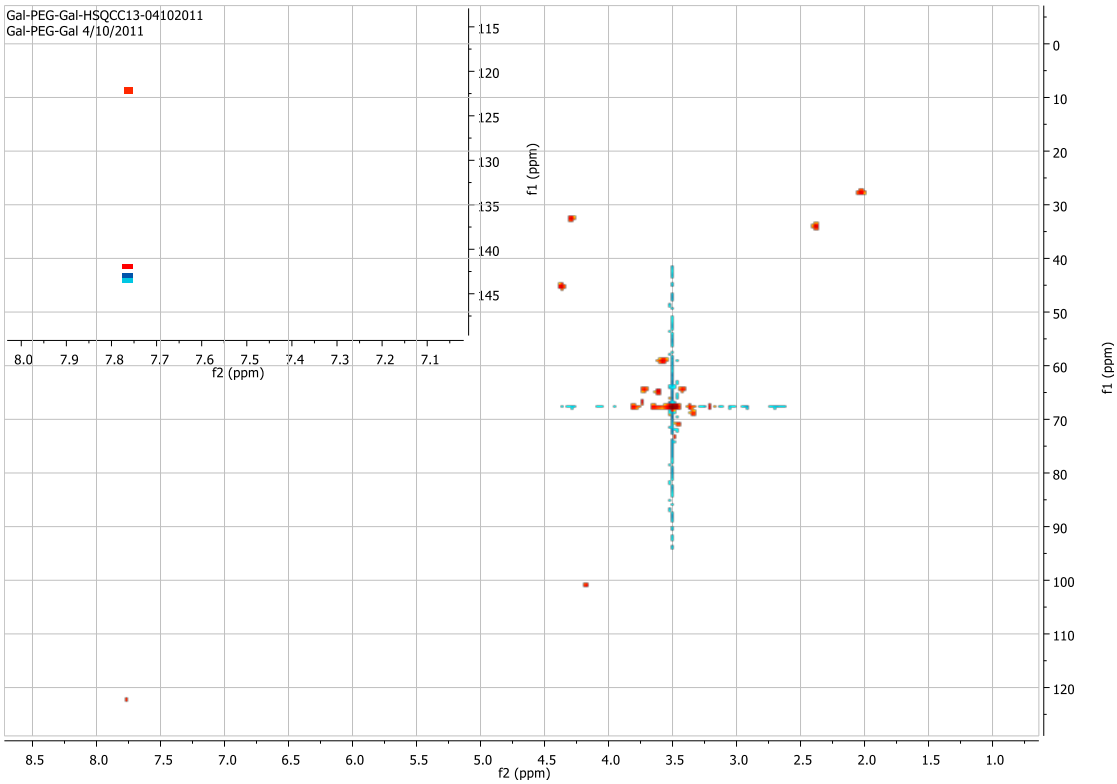


19c in $CDCl_3$

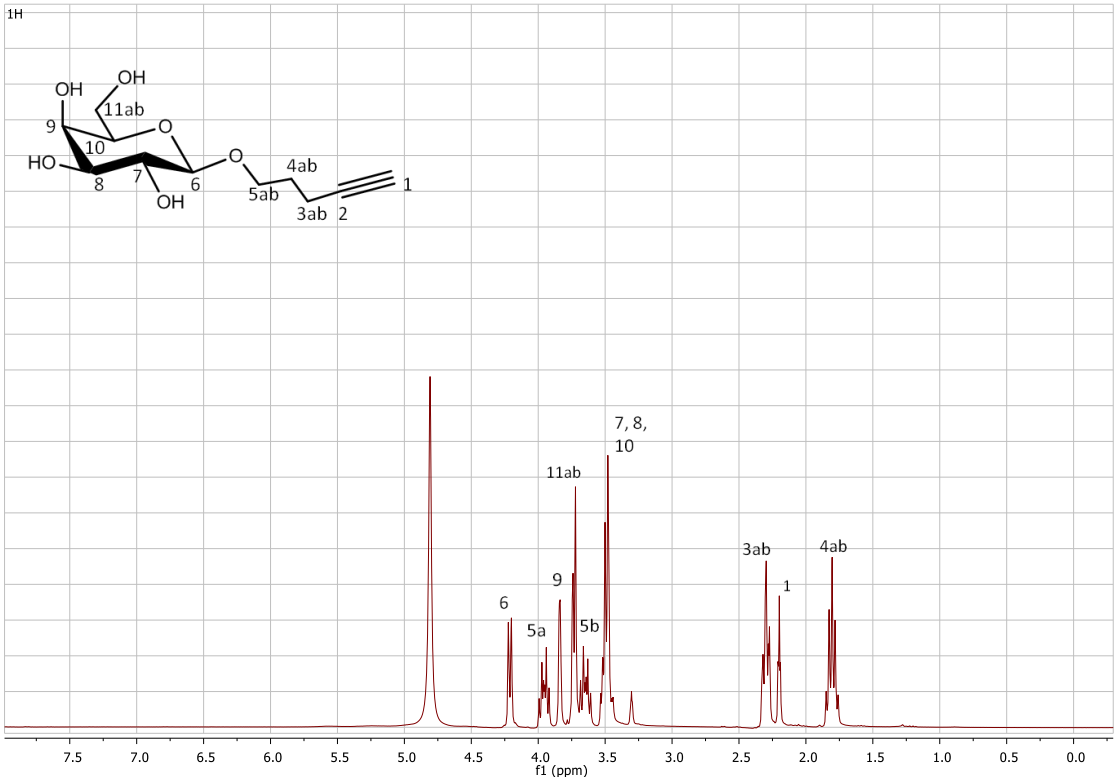


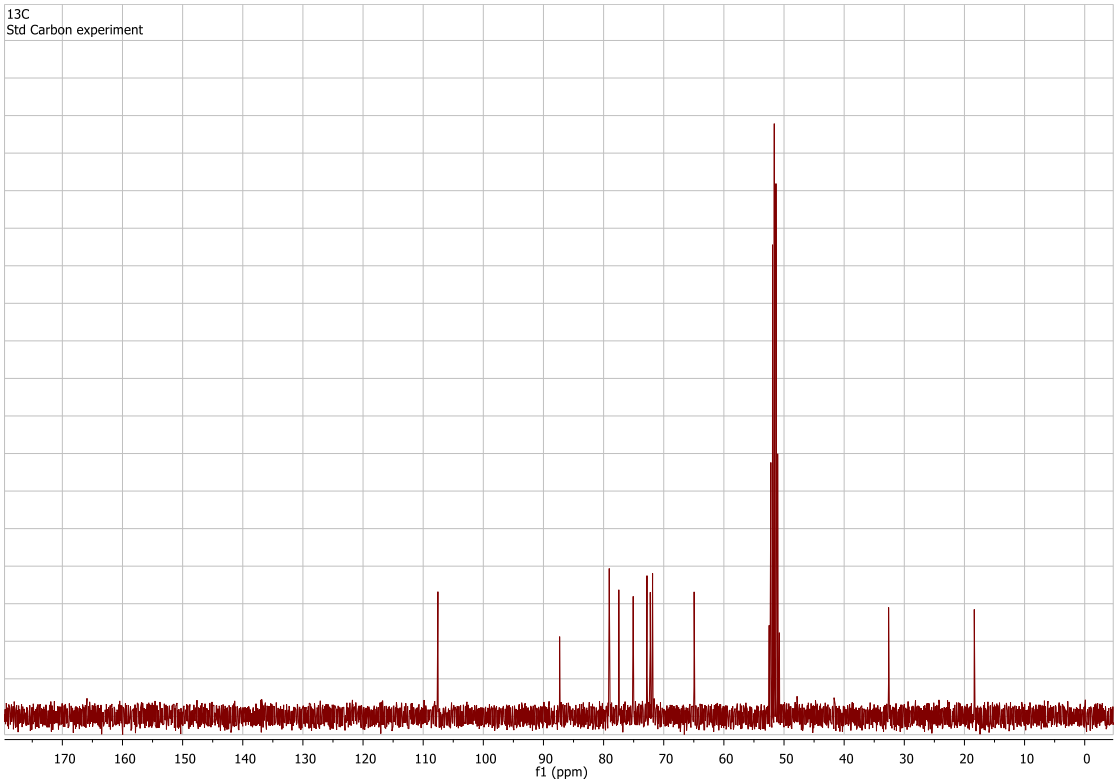
19 in D_2O


8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f1 (ppm)

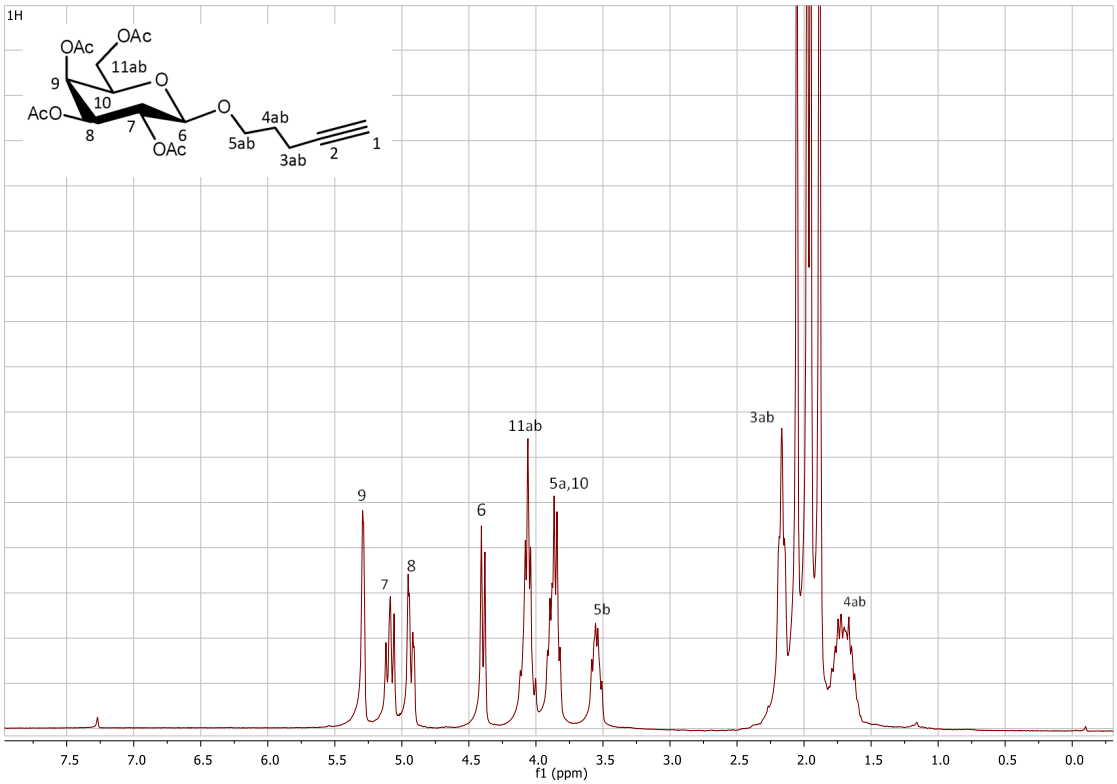

Gal-PEG-Gal-HSQCC13-04102011
Gal-PEG-Gal 4/10/2011
115
120
125
130
135
140
145
f1 (ppm)
8.0
7.9
7.8
7.7
7.6
7.5
7.4
7.3
7.2
7.1
f2 (ppm)
0
10
20
30
40
50
60
70
80
90
100
110
120
f1 (ppm)
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
f2 (ppm)

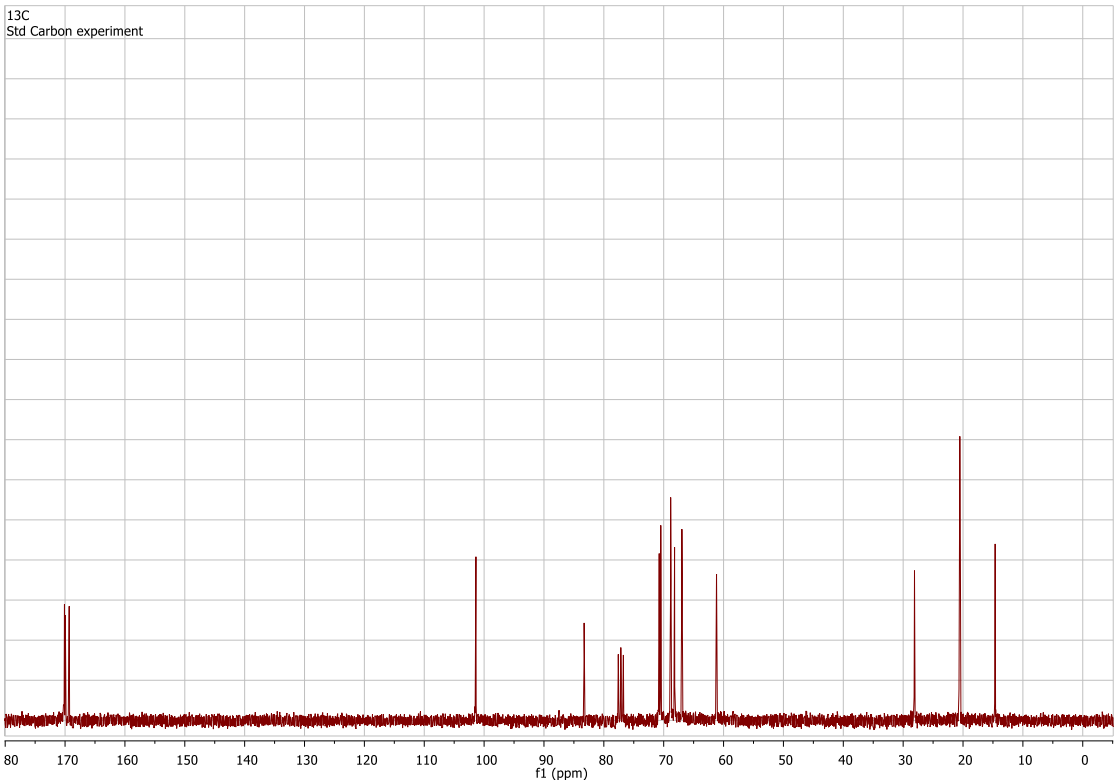
20 in CD_3OD


1H
OH
OH
11ab
9
10
O
HO
8
7
6
OH
O
4ab
5ab
3ab
2
1
7, 8,
10
11ab
9
6
5a
5b
3ab
1
4ab
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f1 (ppm)

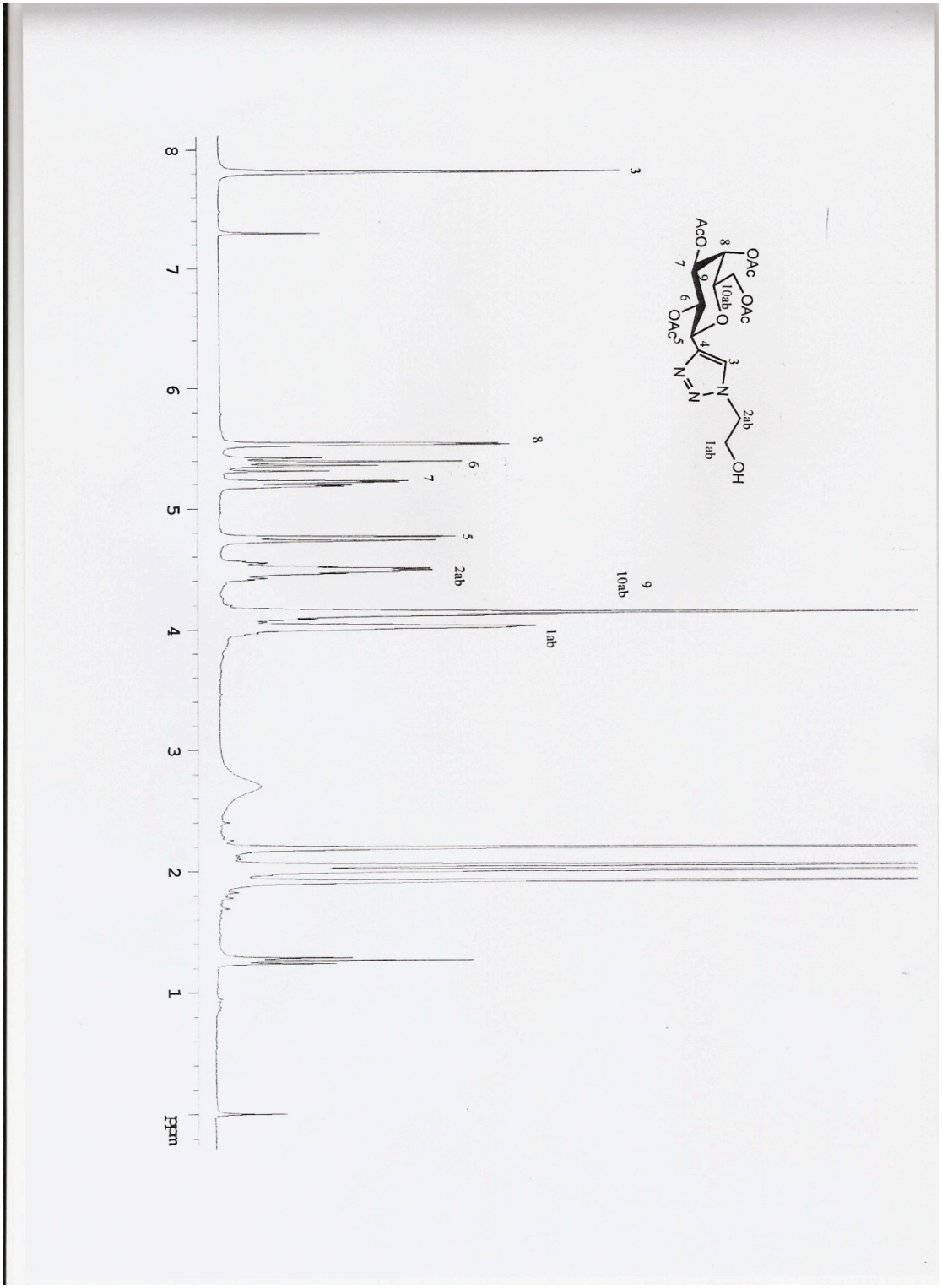

13C
Std Carbon experiment
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)

21 in $CDCl_3$

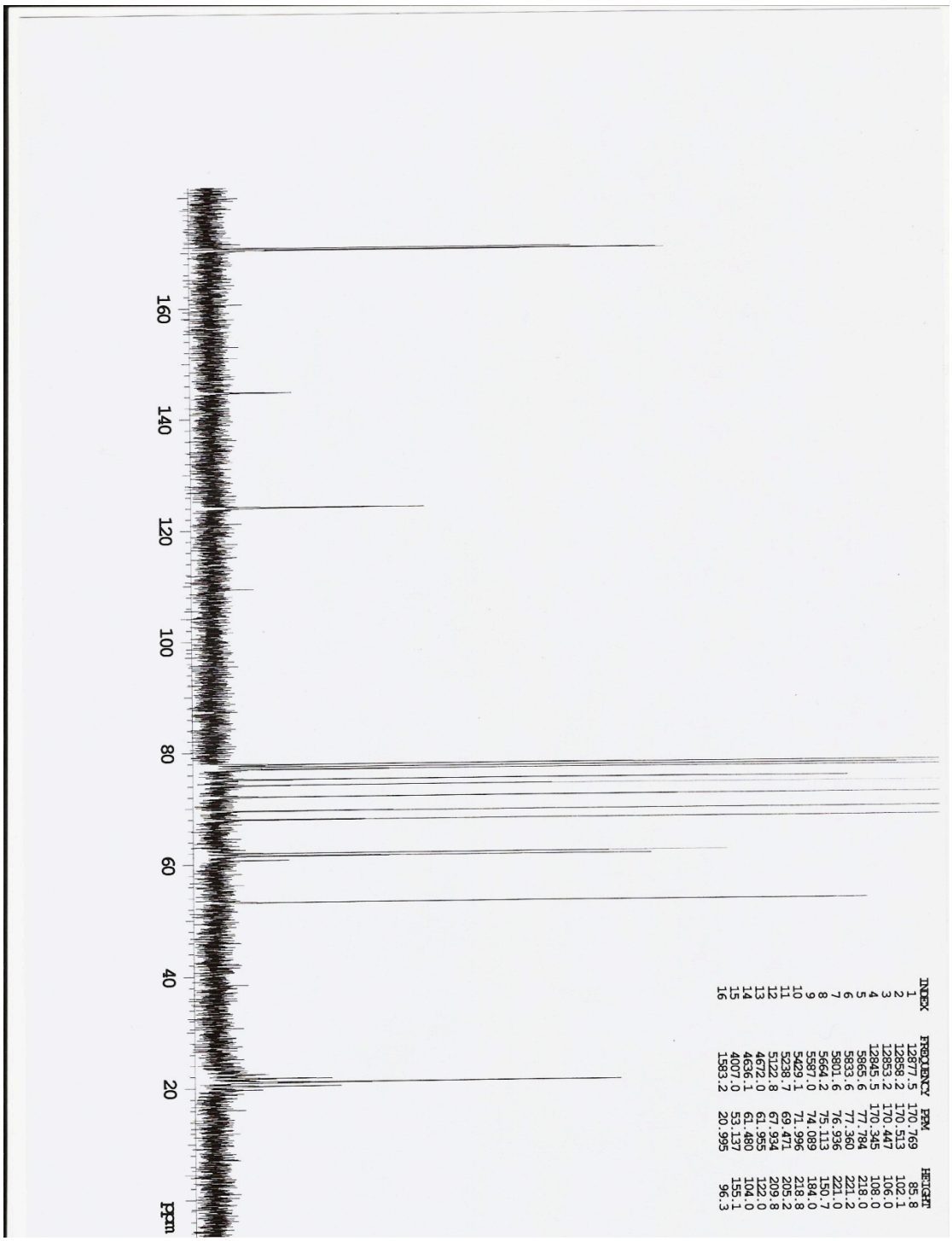

1H
OAc
OAc
11ab
9
O
10
AcO
8
7
6
OAc
O
5ab
4ab
3ab
2
1
9
7
8
6
11ab
5a,10
5b
3ab
4ab
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f1 (ppm)


13C
Std Carbon experiment
80
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)

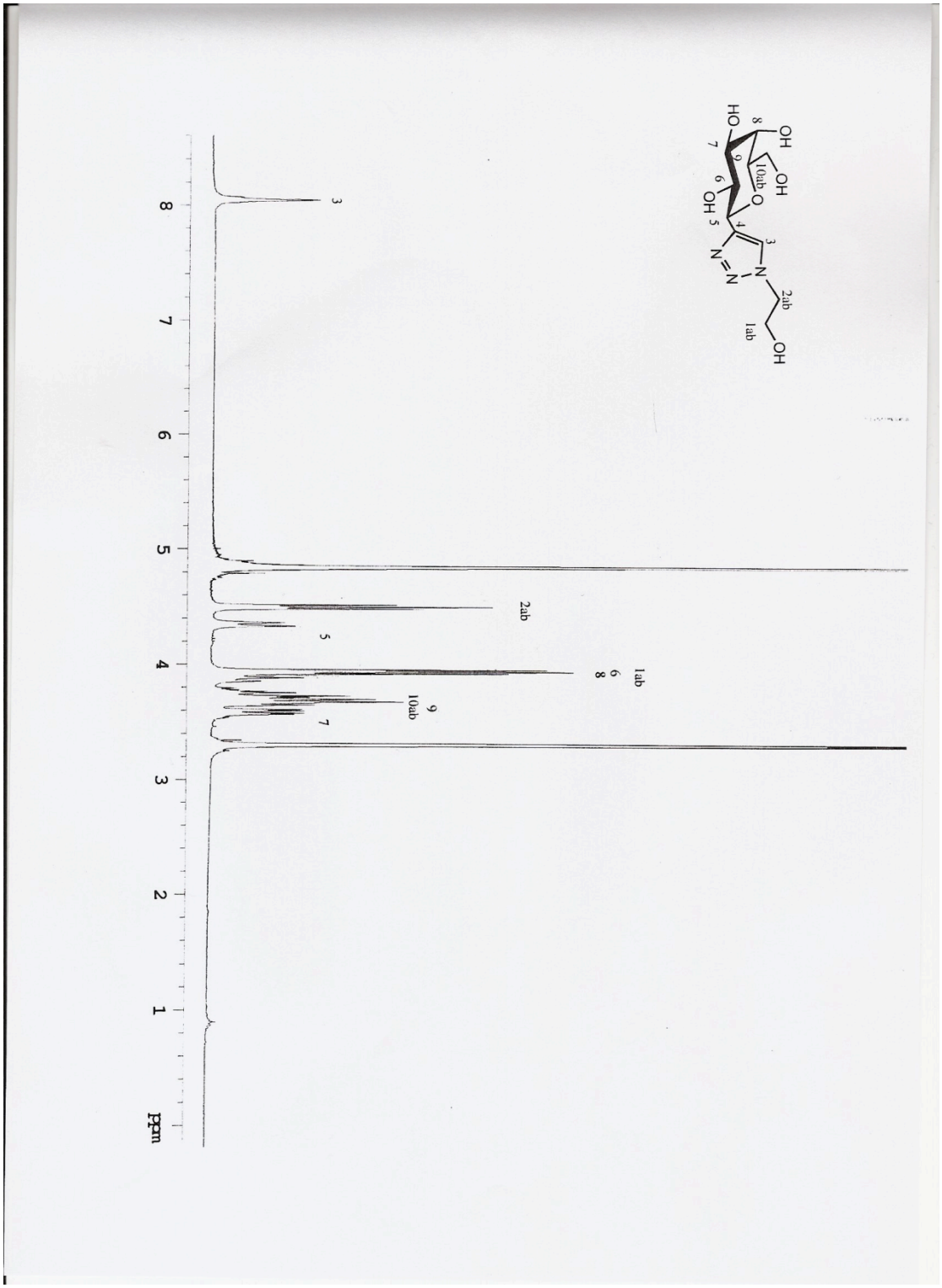
24 in $CDCl_3$



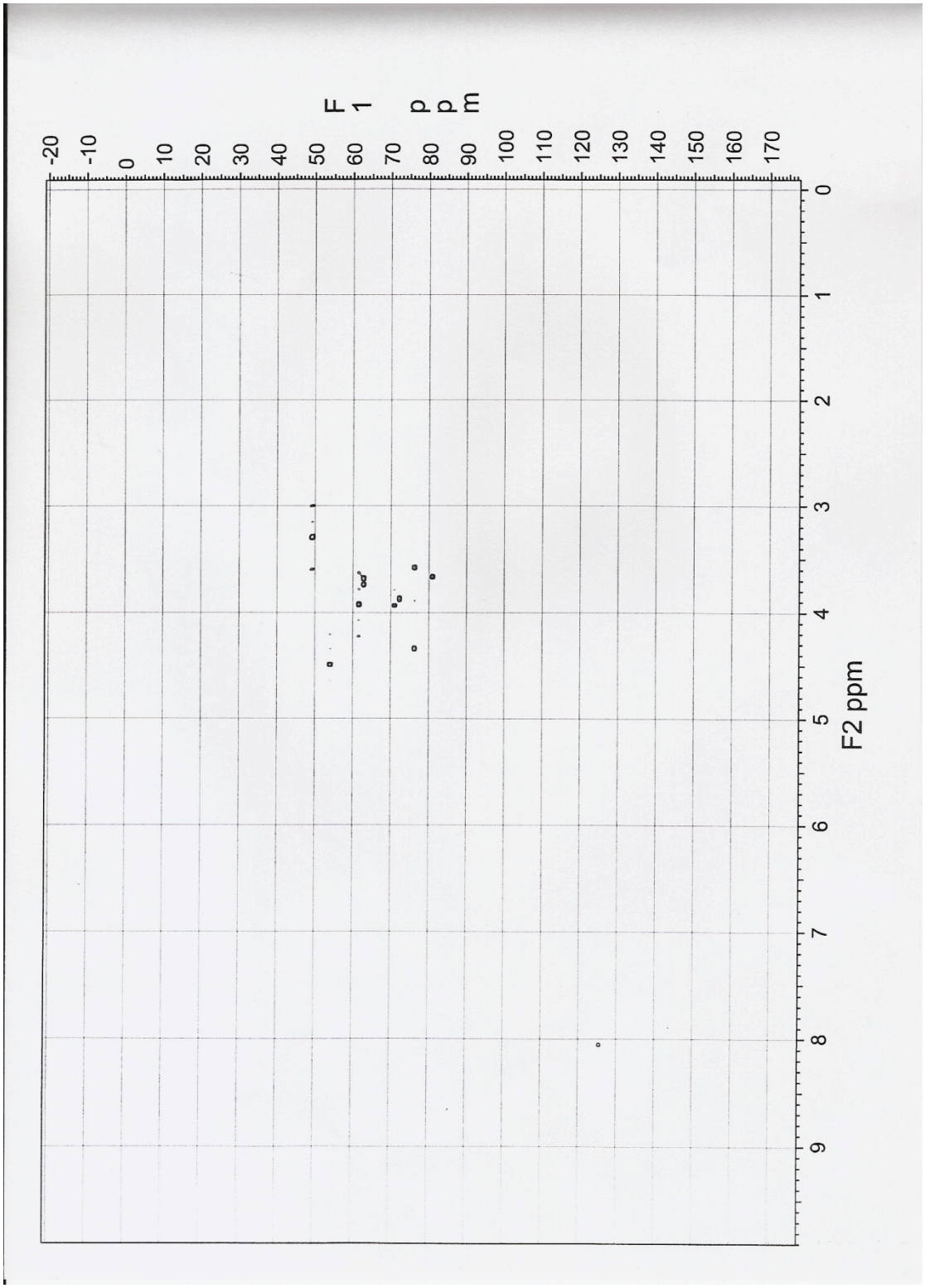
24 in $CDCl_3$



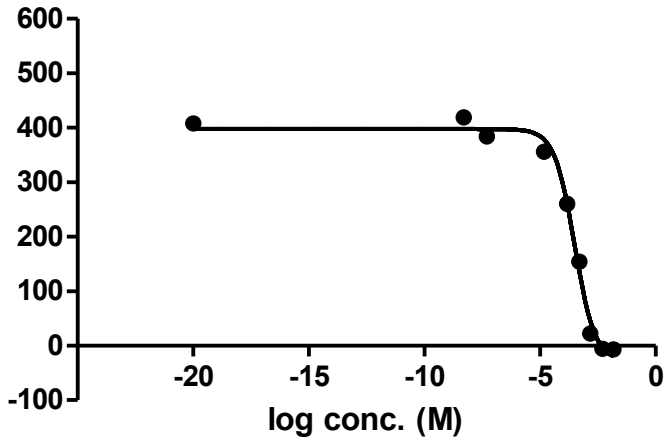
22 in CD_3OD



22 in CD_3OD

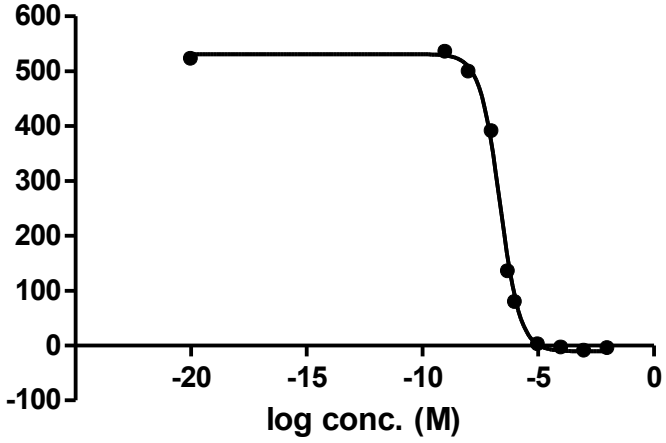


12 IC_{50}=314 ± 62 μM



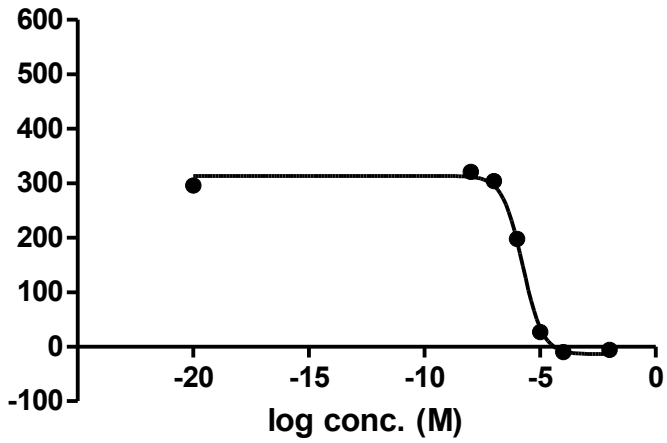
LOGIC50	-3,503
IC50 (M)	0,0003141
LOGIC50 (error)	0,07849
R²	0,9915

14 IC_{50}=223 ±23 nM



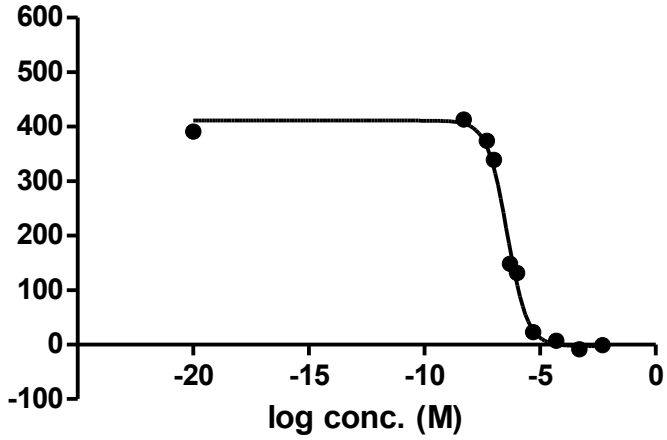
LOGIC50	-6,655
IC50 (M)	2,213e-007
LOGIC50 (error)	0,04342
R²	0,9971

16 IC_{50}=1.76 ± 0.34 μM



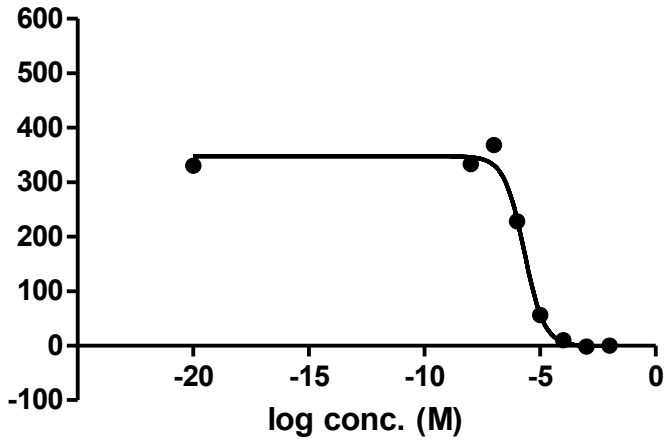
LOGIC50	-5,753
IC50 (M)	1,766e-006
LOGIC50 (error)	0,07599
R²	0,9957

18 IC_{50}=383 ± 61 nM



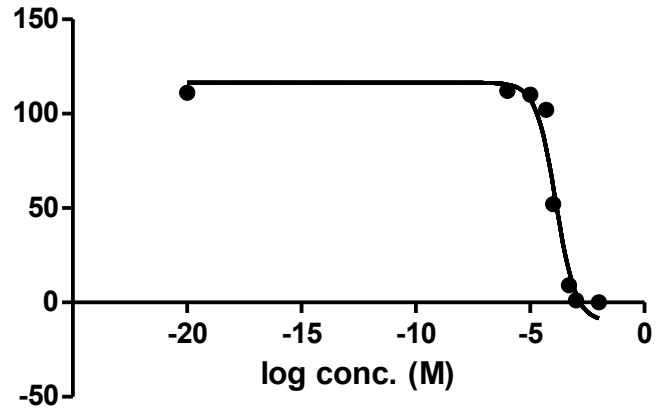
LOGIC50	-6,417
IC50 (M)	3,830e-007
LOGIC50 (error)	0,06460
R²	0,9929

19 IC_{50}=2.06 ± 0.59 μM



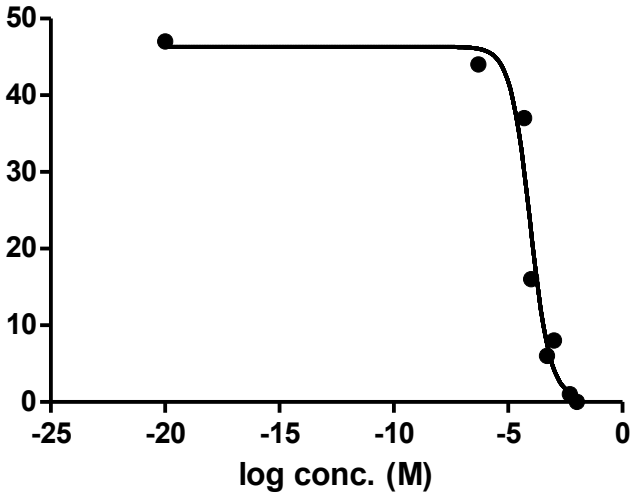
LOGIC50	-5,687
IC50 (M)	2,056e-006
LOGIC50 (error)	0,1108
R²	0,9902

20 IC_{50}=133 ± 60 μM

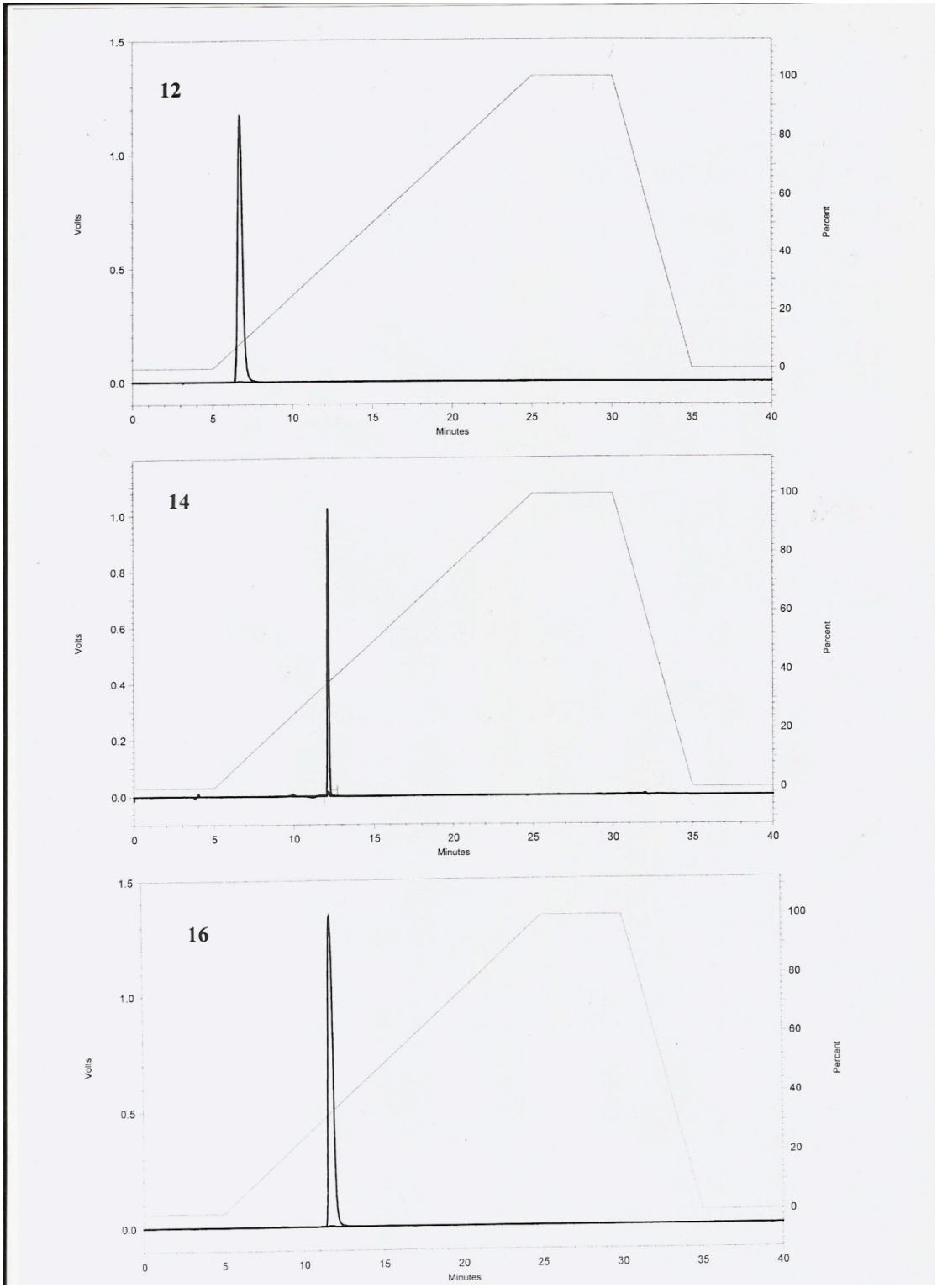


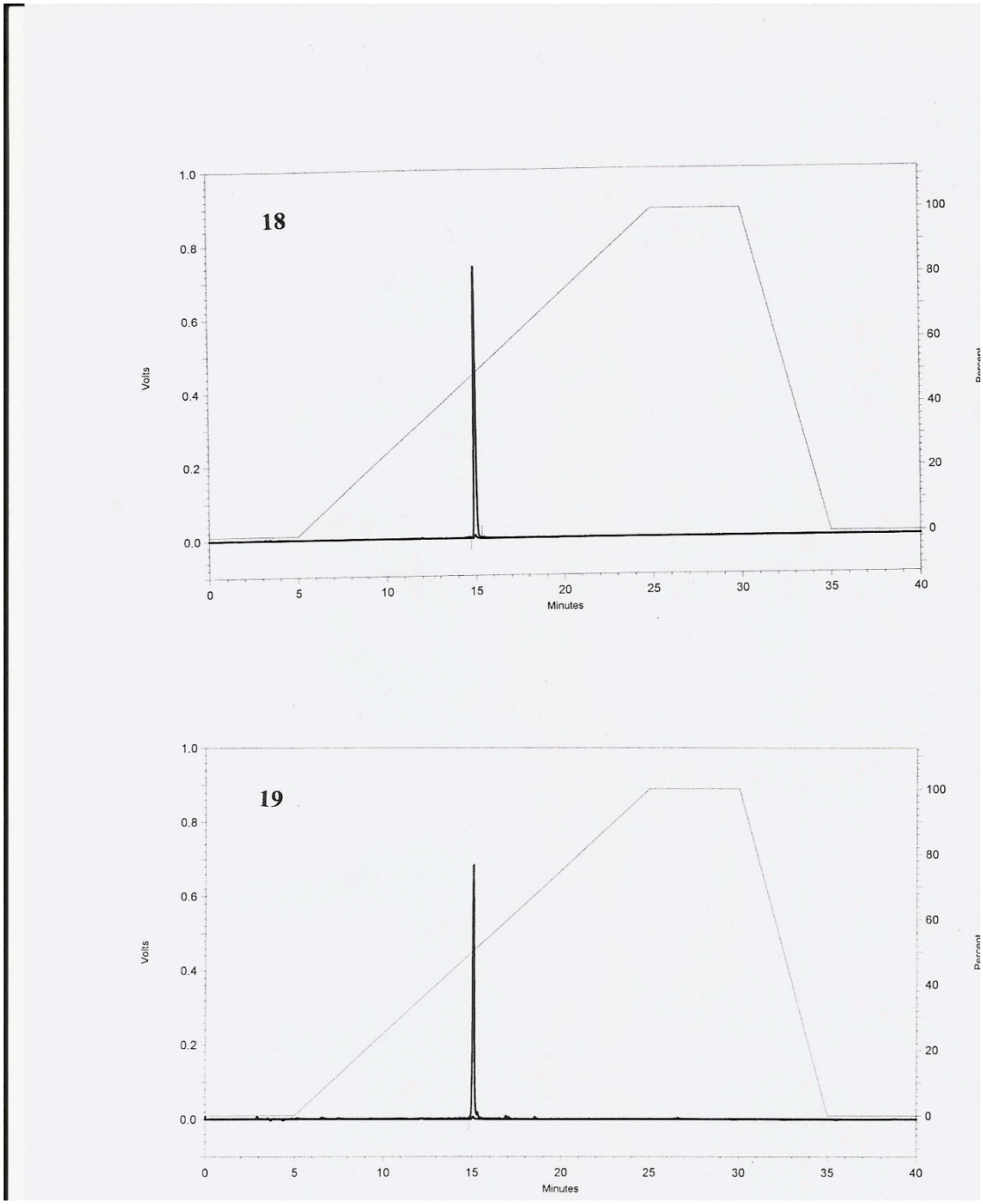
LOGIC50	-3,877
IC50 (M)	0,0001327
LOGIC50 (error)	0,1646
R²	0,9644

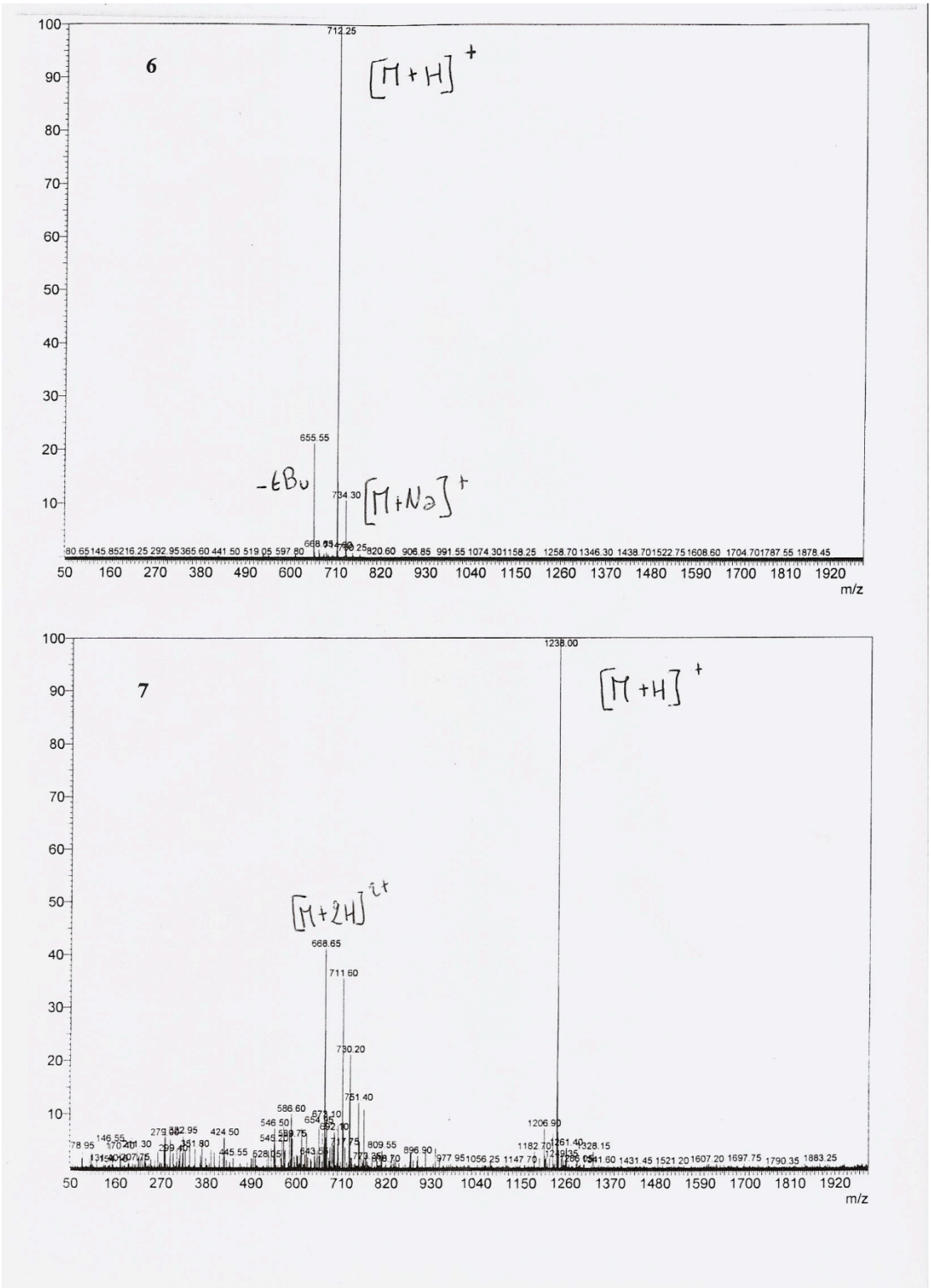
22 IC_{50}=92 ± 37 μM

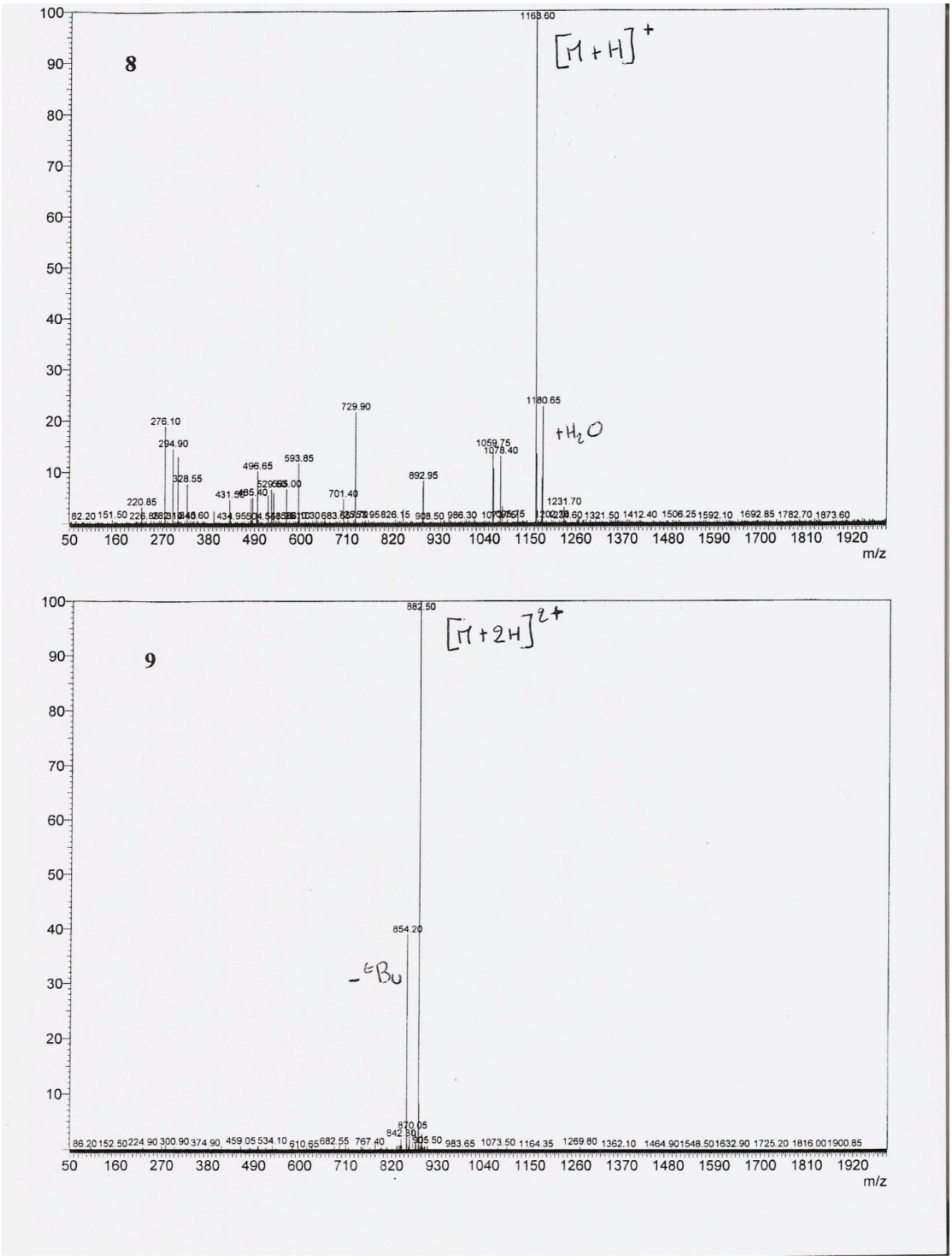


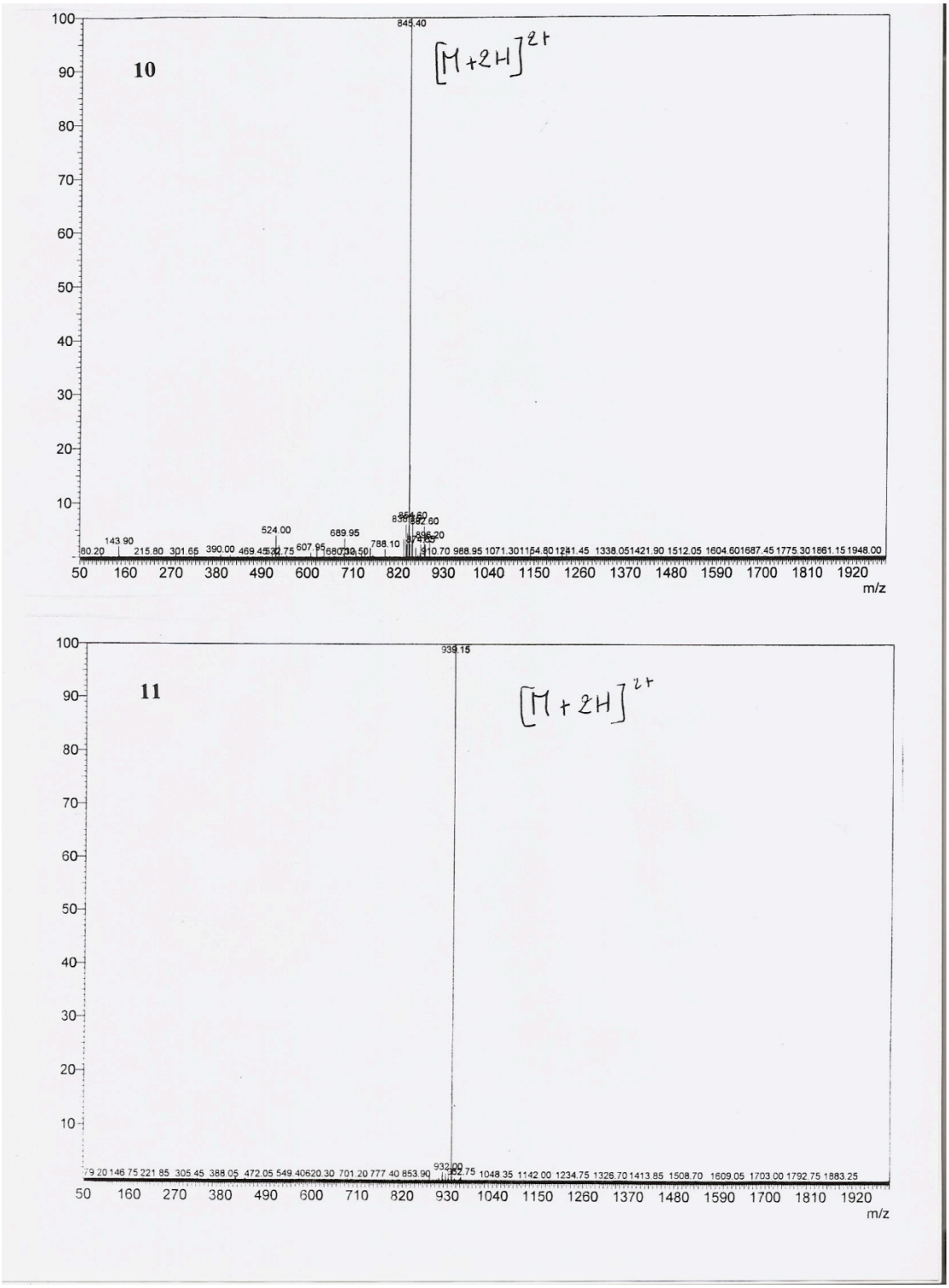
LOGIC50	-4.035
IC50 (M)	9,224e-005
LOGIC50 (error)	0,1636
R^2	0,9595

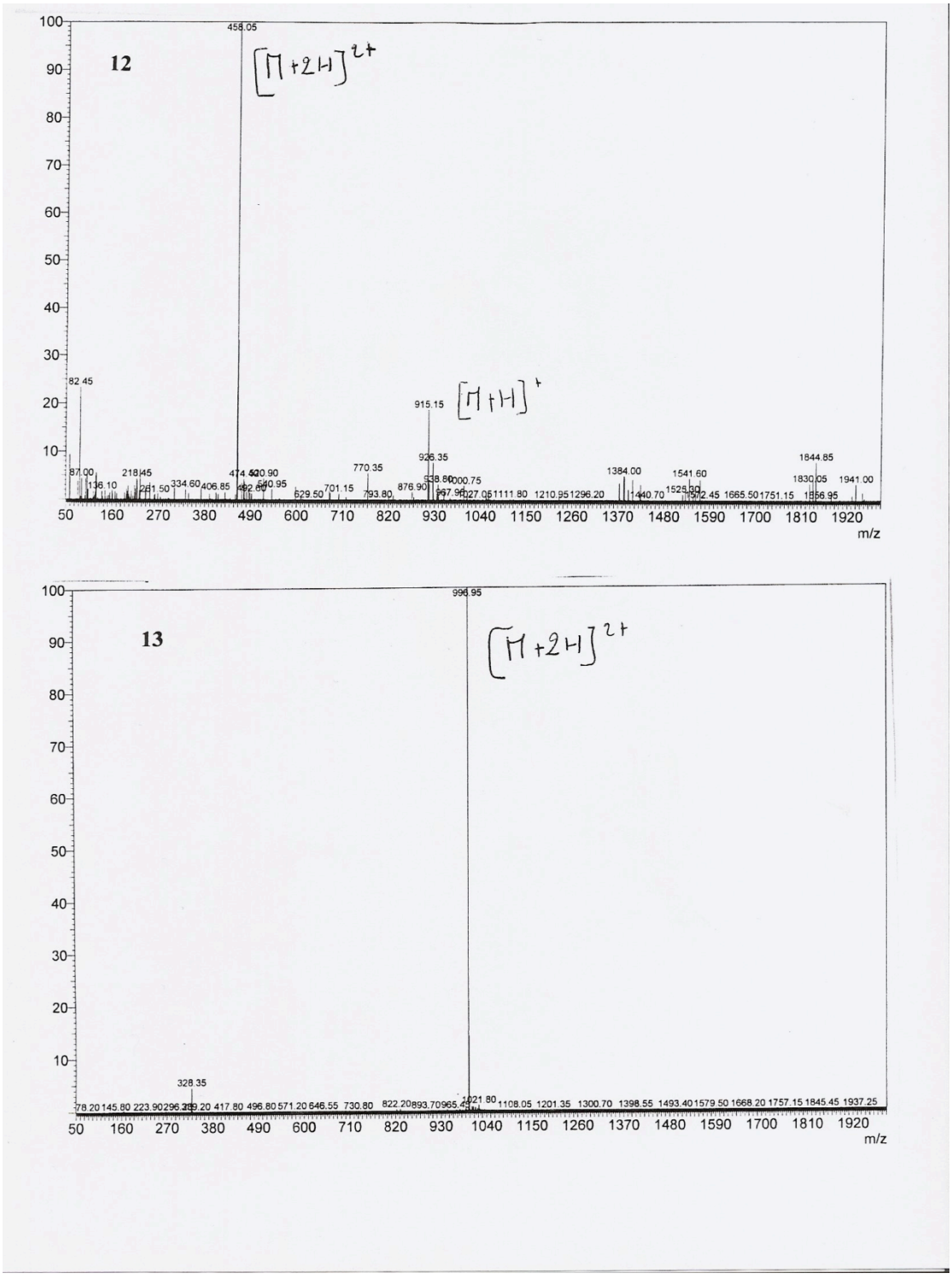

12
Volts
Percent
Minutes
14
Volts
Percent
Minutes
16
Volts
Percent
Minutes


18
Volts
Minutes
Percent
19
Volts
Minutes
Percent








12
458.05
[M+2H]2+
915.15
[M+H]+
926.35
82.45
87.00
136.10
218.45
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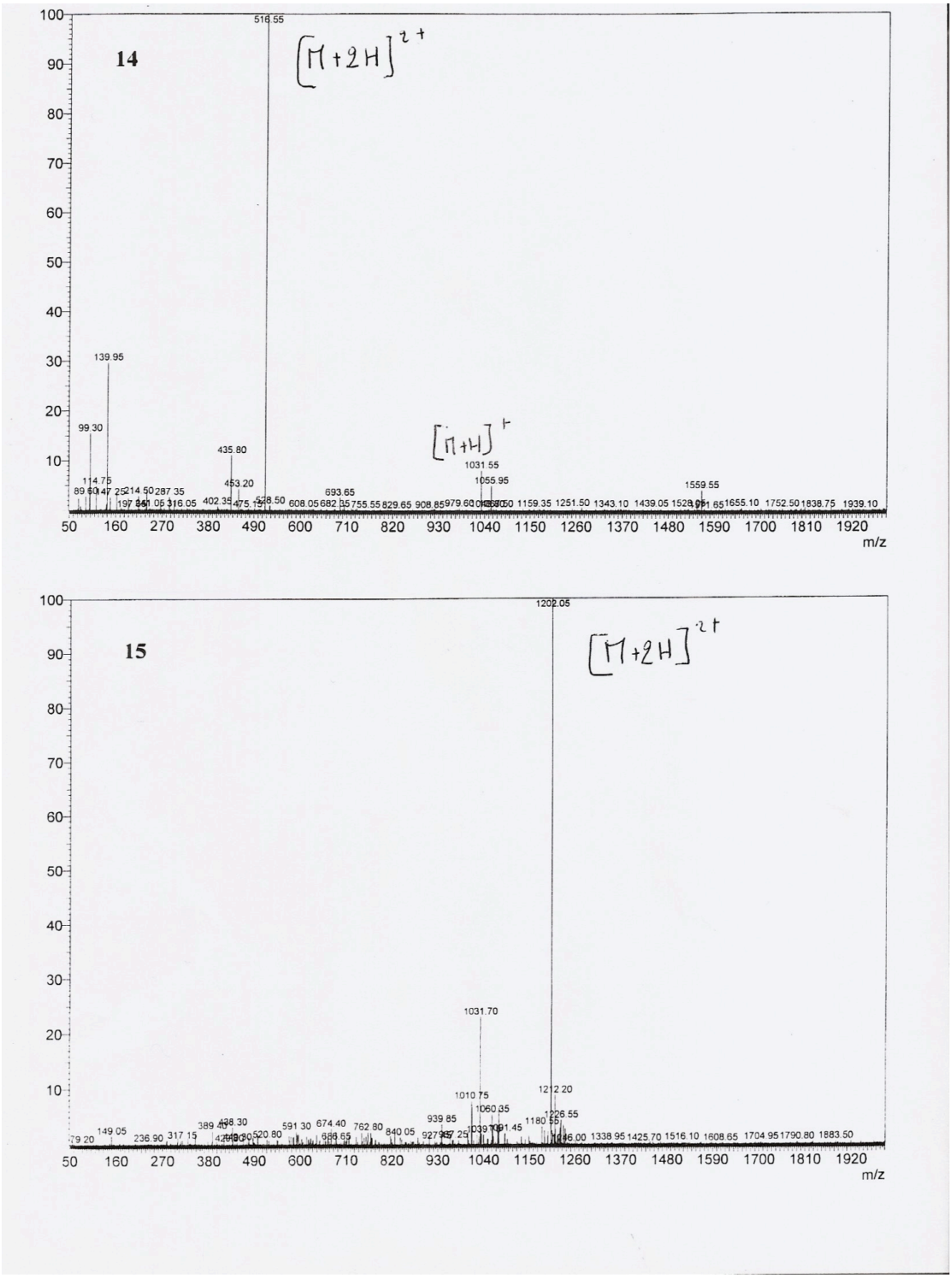
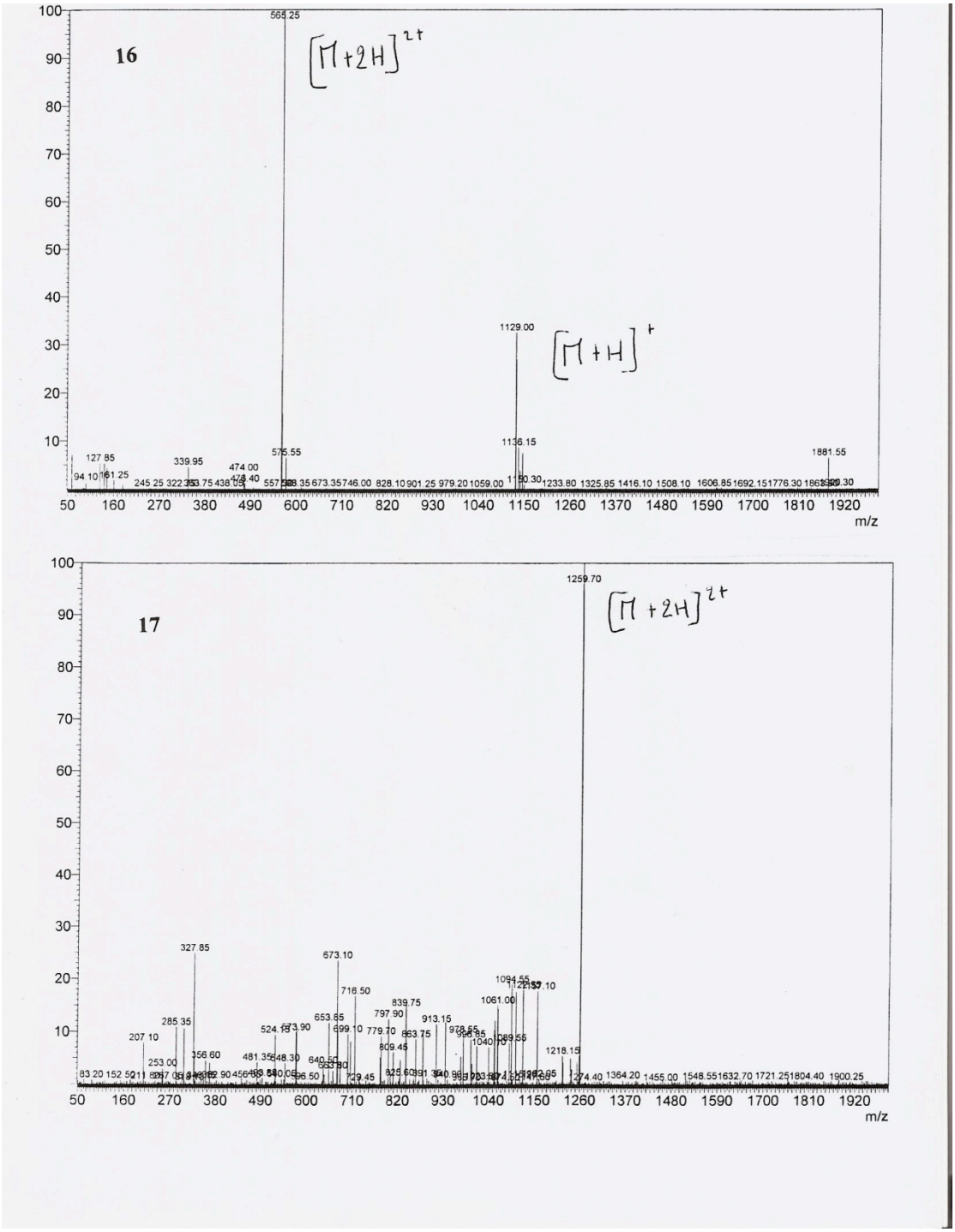
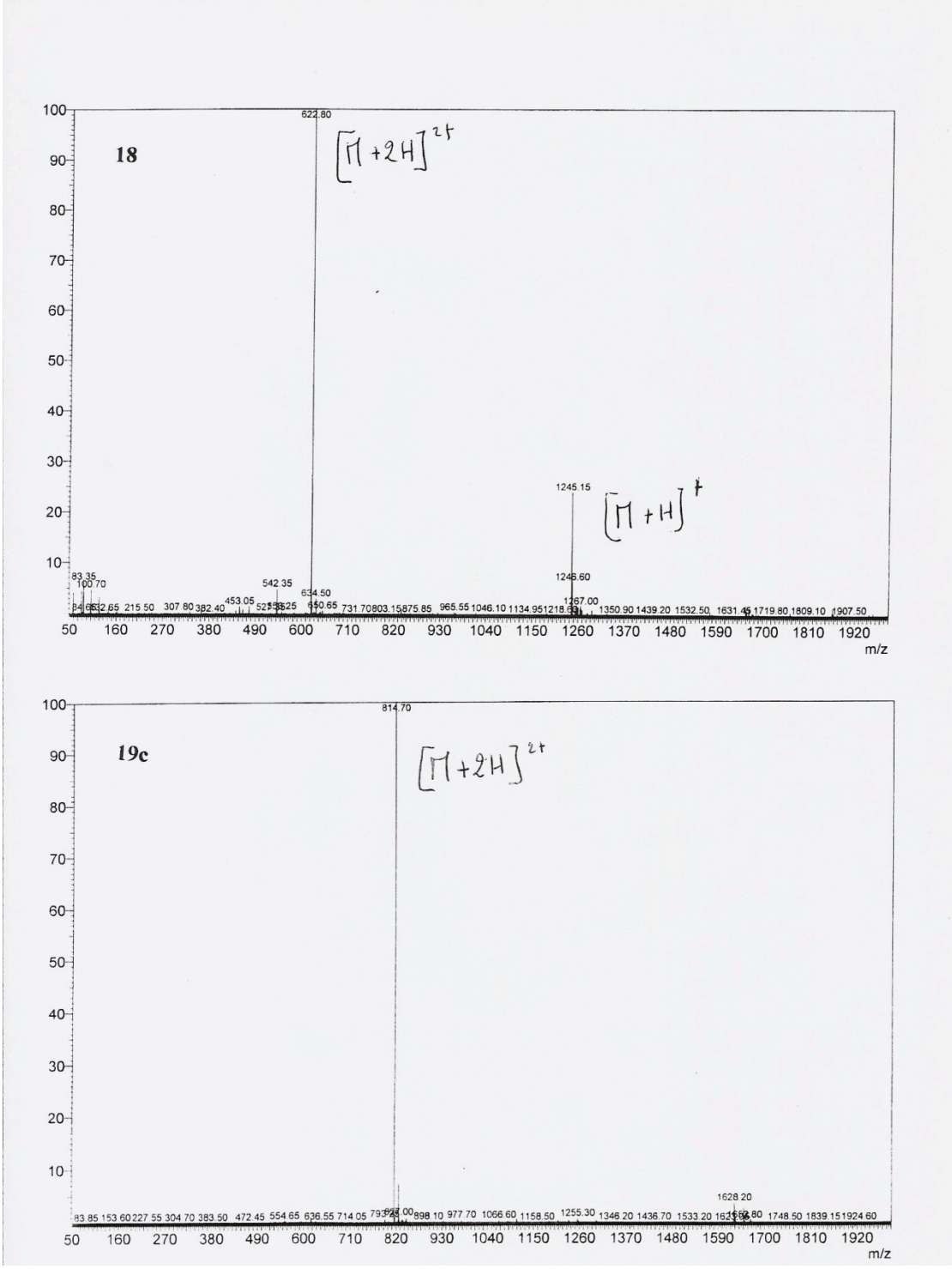
406.85
474.50
520.90
540.95
629.50
701.15
770.35
793.80
876.90
1000.75
1111.80
1210.95
1296.20
1384.00
1440.70
1541.60
1528.80
1665.50
1751.15
1844.85
1830.05
1941.00
50 160 270 380 490 600 710 820 930 1040 1150 1260 1370 1480 1590 1700 1810 1920
m/z
13
996.95
[M+2H]2+
328.35
78.20 145.80 223.90 296.20 417.80 496.80 571.20 646.55 730.80 822.20 893.70 965.45 1021.80 1108.05 1201.35 1300.70 1398.55 1493.40 1579.50 1668.20 1757.15 1845.45 1937.25
50 160 270 380 490 600 710 820 930 1040 1150 1260 1370 1480 1590 1700 1810 1920
m/z




16
565.25
[M+2H]2+
1129.00
[M+H]+
1136.15
575.55
1881.55
339.95
127.85
474.00
m/z
17
1259.70
[M+2H]2+
327.85
673.10
716.50
839.75
797.90
913.15
1061.00
1218.15
285.35
207.10
253.00
356.60
653.85
699.10
779.70
863.75
809.45
m/z


18
622.80
[M+2H]2+
1245.15
[M+H]+
1246.60
1267.00
542.35
634.50
83.35
100.70
453.05
m/z
19c
814.70
[M+2H]2+
1628.20
m/z