Supplementary Information for:

Pentavalent sialic acid conjugates block coxsackievirus A24 variant and human adenovirus type 37 – viruses that cause highly contagious eye infections

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This PDF file includes:

Material and Methods

Docking and calculations

An in-house crystal structure of CVA24v was prepared by addition and energy minimization of hydrogen atoms, and optimization of the hydrogen bond network in the protein preparation wizard in Maestro, the structure of CVA24v was truncated to include the sialic acid binding site and atoms between these sites including 140 amino acids (Figure S1 C) and 106 water molecules. The pentavalent conjugates **26**, **28**, **40**, **46**, and **48** were built in Maestro[1] by conjugating the five sialic acid residues to core fragments **19** or **20** using spacer fragment **3–5**, **10** or 4-azido-1-butanol. A low-mode conformational search,[2] was performed on the constructed structures using MacroModel[3] the OPLS_2005 force field,[4] a Generalized Born Surface Area water model,[5] and the final conformations were energy minimized using method PR conjugate gradient with maximum 2500 iterations.[6] CVA24v and sialic acid atoms were frozen during the conformational search. The strain on the spacers were investigated by monitoring changes in core conformations, bond angles over carbon atoms in the spacers, and atomic clashes with the CVA24v protein. The optimal angles for C-C-C and C-C-O (e.g. polyethylene glycol) was ~112.5° and ~109.4°, respectively, according to benchmark calculations using the force field OPLS_2005.

Particle stability thermal release assay

CVA24v (1 µg) and compounds (1 mM and 100 µM) were incubated for 30 min at room temperature in a total volume of 20 µl sample buffer (10 mM HEPES pH 8.0, 200 mM NaCl) before adding dyes. PaSTRy was performed as previously described.[7] SYPRO red (stock 5000x, Invitrogen) and SYT09 (stock 50 mM, Thermo Fisher Scientific) were diluted 100x in milli-Q water freshly before each experiment. The dyes were added to the CVA24v plus compound samples to a total volume of 50 µl sample buffer and the final concentrations of the dyes were 3x of SYPRO red and 5 µM of SYT09. Samples and dyes were added in a microamp optical 96-well reaction plate (Applied Biosystems, California, USA) and ran a real-time PCR system (StepOnePlus, Applied Biosystems). The melting curve was set to increase 1 °C every 15 sec (log fluorescence every 1 °C increased), ranging from 25 °C to 99 °C.

Cross-linking and aggregation using negative staining electron microscopy experiment

Compounds **28**, **46**, and **48** were respectively dissolved in PBS to a final concentration of 8 mM, and left on ice for 30 minutes (in the dark). Each compound, or PBS in equal amount, was subsequently added to an eppendorf tube containing purified CVA24v (9.6 mg/mL) or HAdV-37 (0.44 mg/mL) to reach a final concentration of 2 mM in PBS. The compound-virus mixtures were incubated for 30 minutes on ice before adsorbing the mixtures to glow-discharged negative stain electron microscopy grids and staining with 1% (W/V) Uranyl acetate. Images of the grids were recorded on a FEI TF20 microscope fitted with an FEI Ceta detector, at a nominal magnification of 7800 x resulting in a sampling of 13 Å per pixel.

CVA24v binding inhibition assay CVA24v

^{35}S-labeled CVA24v (strain 110390) were produced as previously described.[8] Different concentrations of pentavalent sialic acid conjugates were prepared by serial dilution and incubated with 5000 ^{35}S-Labeled CVA24v/well in a total volume of 50 µl binding buffer 2 (BB2.; Dulbecco's Modified Eagle's Medium (DMEM, Sigma Aldrich) + 0.1% bovine serum albumine (BSA, Roche)) for 1 h on ice. Human cornel cells (HCE) were detached with phosphate-buffered saline containing 0.05% ethylenediaminetetraacetic acid (EDTA, Merck) and recovered in growth medium at 37 °C with agitation. After 1 h, HCE cells (1x10^5 per well) were washed with blocking buffer 2 (BB2) prior to the addition of the virion-compound mixtures and then incubated on ice. After 1 h incubation, cells were washed with PBS to remove non-bound virions before the radioactivity of the cells was measured using a Wallac 1409 scintillation counter (Perkin-Elmer, Waltham, MA). Error bars shown as standard error of mean (SEM) plotted with GraphPad Prism 7 using the function log (inhibitor) vs. response (three parameters). Data are presented as % of control that is the value obtained in the absence of inhibitor. All experiments were performed in duplicates for and a minimum of two times.

CVA24v infection assay

One day prior infection, HCE cells (2 x 10^4 per well) were seeded in a black 96-well plate with transparent bottom. Next day, different concentrations of the pentavalent sialic acid conjugates were prepared by serial dilution and were incubated at 37 °C with 10 CVA24v/cell (approximately 4 x 10^4 cells/well) for 1 h. HCE cells in the black 96-well plate were washed twice with BB2 then incubated with 50 µl of CVA24v (10 CVA24v/cell) + compound mixture per well. After 1 h incubation at 37 °C, cells were washed to remove non–bound virions and incubated in HCE growth medium for 16–18 h. After fixation with 99.5% ice-cold methanol, mouse monoclonal antibodies against enterovirus VP1 (DakoCytomation, Glostrup, Denmark) were diluted 1:200 in PBS and 50 µl was added per well. After incubation for 1 h at room temperature (rt), the cells were washed again and incubated with 50 µl Alexa fluor 488-labeled donkey anti-mouse immunoglobulin G (Thermo fisher scientific) (diluted 1:400 in PBS) per well at rt. One h later, the cells were washed again, and the numbers of infected cells were quantified using a Trophos system. Error bars shown as SEM plotted with GraphPad Prism 7 using the function log (inhibitor) vs. response (three parameters). Data are presented as % of control that is the value obtained in the absence of inhibitor. All experiments were performed in duplicates for and a minimum of two times.

HAdV-37 binding inhibition assay[9]

HCE cells were detached with pre-warmed PBS containing 0.05% EDTA. The cells were counted using automated cell-counter (Countless II, Thermo Fisher, Waltham, USA) and reactivated in suspension with 10% growth medium for 1 h at 37 °C. During reactivation, ^{35}S-labeled HAdV-37 virions (5 × 10^8 per well or 5000 virus particles/cell) were incubated at 4 °C for 1 h on ice in presence of ME0462 and the pentavalent sialic acid conjugates in decreasing concentrations. The compound solutions (50 µL) were prepared in binding buffer (BB: DMEM containing 1% BSA (Roche AB, Stockholm, Sweden), 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES, 20 mM, EuroClone, Milan, Italy, pH 7.5), and

20 U/mL penicillin + 20 µg/mL streptomycin (PEST, Invitrogen, Carlsbad, USA) using successive 10 fold dilution series. After reactivation, the cells were washed once with BB and virus-compound mixtures were added to pre-pelleted cells (1×10^5 per well, in suspension) in a V-bottom 96-well plate. The virus-compound-cell mixtures were incubated for 1h at 4 °C on ice. After 1 h, unbound viruses were washed away with BB and the cell-associated radioactivity was measured by using a Wallac 1409 scintillation counter (Perkin-Elmer, Waltham, USA). Cells incubated with only virions were used as control. Error bars shown as SEM plotted with GraphPad Prism 7 using the function log (inhibitor) vs. response (three parameters). Data are presented as % of control that is the value obtained in the absence of inhibitor. All experiments were performed in duplicates for and a minimum of two times.

X-ray crystallography of pentavalent sialic acid conjugates

Crystals of CVA24v were grown as described previously[10] and soaked with **25** (9 mM), **26** (14 mM), **27** (14 mM), or **28** (9 mM) for 16 h at 4 °C. The crystals were flash-frozen in liquid nitrogen, and data for all four complexes were collected at the Diamond light source (UK) at beamline I03. All data sets were reduced using the XDS package.[11] The structures were solved by applying the phases of the native structure (pdb-code 4Q4W) followed by a simulated annealing approach as implemented in PHENIX[12] to reduce model bias. The following refinement steps were performed with REFMAC5[13] and involved strict NCS parameterization. COOT[14] was used for real-space model corrections. During the final refinement steps the ligand was placed into the simulated annealing omit (F_o-F_c) map and conducted to several steps of reciprocal space refinement. The structures were validated using MOLPROBITY [15] and visualized with PYMOL.[16] A model of the **28** was prepared using PRODRG[17] and COOT taken the sialic acid entities as fixed anchor points. Although the structures of all complexes (**25–28** and **39–46**) were established, we deposited the coordinates for CVA24v-**28** (Table S2) exclusively as all structures showed a similar binding of the starfish-like inhibitors, namely the binding of the sialic acid moiety. None of the linkers connecting the sialic acid entities were visible in the electron density maps, and we conclude that these linkers assume several different conformations.

General chemical procedures

^{1}H NMR and ^{13}C NMR spectra were recorded with a Bruker DRX-400 spectrometer at 400 MHz and 100 MHz respectively, or with a Bruker DRX-600 spectrometer at 600 MHz and 150 MHz respectively. NMR experiments were conducted at 298 K in D_2O (residual solvent peak = 4.79 ppm, δ_H), CD_3OD (residual solvent peak = 3.31 ppm, δ_H) and 49.00 ppm, δ_C) or $CDCl_3$ (residual solvent peak = 7.26 ppm, δ_H and 77.16 ppm, δ_C). Liquid chromatography mass spectrometry (LC-MS) were recorded by detecting positive/negative ion (electrospray ionization, ESI) on Agilent 1,290 infinity II−6,130 Quadrupole using H_2O/CH_3CN (0.1% formic acid) as the eluent system or on Agilent 1,290 infinity−6,150 Quadrupole using YMC Triart C18 (1.9 µm, 20 × 50 mm column) and H_2O/CH_3CN (0.1% formic acid) as the eluent system. High resolution mass spectra (HRMS) data was recorded with Agilent 1290 binary LC System connected to a Agilent 6230 Accurate-Mass Time-of-Flight (TOF) LC/MS (ESI+); calibrated with Agilent G1969-85001 ES-TOF Reference Mix containing ammonium trifluoroacetate, purine and hexakis(1H, 1H, 3H

tetrafluoropropoxy)phosphazine in 90:10 CH_3CN/H_2O. Semi-preparative high performance liquid chromatography (HPLC) was performed on a Gilson system HPLC, using a YMC-Actus Triart C18, 12 nm, S-5 µm, 250 × 20.0 mm with a flow rate 20 $mL.min^{-1}$, detection at 214 nm and eluent system A: aqueous 0.005% formic acid, and B: CH_3CN 0.005% formic acid. Column chromatography was performed on silica gel (Merck, 60 Å, 70–230 mesh ASTM). Thin layer chromatography (TLC) were performed on Silica gel 60 F_{254} (Merck) with detection under ultraviolet (UV) light and/or development with 5% H_2SO_4 in EtOH and heat. Automated flash column chromatography was performed using a Biotage® Isolera One system and purchased pre-packed silica gel cartridges (Biotage® SNAP Cartridge, KP-Sil). Freeze drying was performed by freezing the diluted CH_3CN/water solutions in dry ice-acetone bath and then employing a Scanvac CoolSafe freeze dryer connected to an Edwards 28 rotary vane oil pump. Organic solvents were dried using a Glass Contour Solvent Systems (SG Water USA) except CH_3CN (freshly distilled from CaH_2) and MeOH that were dried over molecular sieves 3 Å. All commercial reagents were used as received. All target compounds were ≥95% pure according to HPLC UV-traces. Statistics were calculated using GraphPad Prism 7 (GraphPad Software, Inc, La Jolla, CA). Microwave reactions were performed using a Biotage® Initiator microwave synthesizer; temperatures were monitored by an internal IR probe; stirring was mediated magnetically and the reaction were carried out in sealed vessels.

General procedure for sialidation – Method A

An oven dried round bottom flask was charged with magnetic stirring bar, activated molecular sieves (4 Å, 9.0 g), thiophenyl donor (1.71 mmol, 1.0 eq), azidoalcohol (4.60 eq) and silver trifluoromethanesulfonate (AgOTf, 2.0 eq). The flask was closed with rubber septa and placed under vaccum in the dark for 16 h. Under dark conditions the flask was transferred to nitrogen atmosphere and at rt was added freshly distilled CH_3CN (45 mL) and anhydrous CH_2Cl_2 (30 mL). The mixture was allowed to stir at rt for 30 min before being cooled to –74 °C degrees. In a separate oven dried v-shaped round bottom flask was added IBr (1.40 eq) and anhydrous CH_2Cl_2 (2.4 mL, final concentration of 1 M) under nitrogen atmosphere. After the IBr was completely dissolved the solution was injected all at once into the stirring solution at –74 °C. The reaction was allowed to perform under dark conditions for 5.5 h at –74 °C. Diisopropylethylamine (DIPEA, 6.0 eq) was then added and the reaction allowed to perform for an additional 30 min before warming to rt. The solution was subsequently filtered through a celite plug and concentrated under reduced pressure. The resulting mixture was pre-purified by automated flash chromatography (ethylacetate (EtOAc)/acetone gradients) before purification on preparative HPLC (CH_3CN/H_2O 20–80% gradient 30 minutes) affording protected sialosides in pure alpha anomeric form. The protected sialosides (0.37 mmol, 1.0 eq) were subsequently dissolved in CH_3OH (44.7 mL) and $NaOCH_3$ (4.5 eq) was added in portion to reach a final concentration of 0.03 M (significantly more concentrated solutions result in breakdown of the sialoside). The reaction was allowed to stir overnight at rt under nitrogen atmosphere before neutralizing (pH 7–8) the mixture with pre-washed Dowex 50x8 H^+-Form. The mixture was concentrated under reduced pressure, re-dissolved in minimal amount of CH_3OH and purified on preparative HPLC (gradient: 5% → 20% CH_3CN/H_2O in 20 min) affording the sialosides **11** and **12**. See chemical synthesis for specific yields and analytical data.

General procedure for sialidation – Method B

An oven dried round bottom flask was charged with magnetic stirring bar, powdered activated molecular sieves (4Å, 3.3 g), xanthate sialoside donor (5.88 mmol, 1.0 eq) and placed under nitrogen atmosphere. To this mixture was added CH_2Cl_2 (50 mL) and under dark conditions a solution of AgOTf (2.0 eq) in freshly distilled CH_3CN (77 mL). The solution was cooled to –74 °C and stirred for 15 minutes, followed by dropwise addition of a solution of IBr (1.0 M in CH_2Cl_2, 1.40 eq). After complete addition of the IBr solution the reaction was stirred for 2h at –74 °C, and DIPEA (6.0 eq) added and another 20 min before warming to rt. The mixture was filtered through a plug of celite and concentrated to dryness. The resulting mixture was purified by flash chromatography (CH_2Cl_2/CH_3OH gradients) affording the protected sialosides as a mixture of alpha and beta anomers. The mixture (3.19 mmol, 1.0 eq) was dissolved in CH_3OH and $NaOCH_3$ (10.0 eq) was added in portion to a final concentration of 0.03 M (significantly more concentrated solutions result in breakdown of the sialoside). The reaction was allowed to perform at rt under nitrogen atmosphere until completion (monitored by LC-MS and TLC). The mixture was neutralized with Amberlyst H^+-Form, filtered and concentrated to dryness. The compound was purified on flash chromatography (CH_2Cl_2/CH_3OH gradients) affording the deprotected sialosides **11–17** as pure alpha anomers. See chemical synthesis for specific yields and analytical data.

General procedure for CuAAC

An oven-dried round bottom flask equipped with magnetic stirring bar was charged with azido-sialoside (0.37 mmol, 11.5 eq). To this was added a solution of pentapropargylated glucoside (1.0 eq, 0.032 mmol) in tetrahydrofuran (THF, 7 mL). To the stirring solution was added $CuSO_4 \cdot 5\ H_2O$ (1.59 eq) and sodium ascorbate (1.55 eq) in H_2O (7 mL). The rbf was equipped with rubber septa and the mixture heated to 50 °C for 5 h and then the reaction was left to perform at rt for 36 h. The THF was removed under reduced pressure and the resulting mixture injected on HPLC (MeCN/H_2O 10% → 25% gradient in 25 minutes) affording the pentavalent methyl ester derivative after freeze-drying. See chemical synthesis for specific yields and analytical data.

General procedure for ester hydrolysis

The pentavalent methyl ester derivate (0.01 mmol, 1.0 eq) was dissolved in CH_3OH (1.35 mL) and to this stirring solution was added a 1 M solution of LiOH (0.156 mL, 15.0 eq). The mixture was stirred for 48 h at rt in the dark. The mixture was neutralized (pH 7–8) with Dowex 50x8 H^+-form, filtered, and concentrated under reduced pressure. The resulting residue was diluted in water and freeze-dried to afford the pentavalent target compound.

General procedure for TBDPS protection

Tertbutyldiphenylsilyl chloride (TBDPSCl, 10 g, 36.38 mmol, 1 eq) was added to a solution of aminoalcohol (43.66 mmol, 1.2 eq) in MeCN (150 mL). The resulting mixture was stirred at rt under nitrogen atmosphere for 16–72 h. The solvent was removed under reduced pressure and the resulting

mixture dissolved in water (50 mL) and titrated with aqueous NaOH (1 M) until pH >12. The resulting solution was extracted with CH_2Cl_2 (four times) and the combined organic layers were washed with NaOH (0.5 M, one time), brine (two times), and water (one time). The organic phase was dried over Na_2SO_4, filtered and concentrated under reduced pressure affording a highly viscous light-yellow oil in quantitative yield.

General procedure for amide coupling and azide formation

To a round-bottom flask equipped with a magnetic stirring bar was added distilled water (36 mL), TBDPS protected aminoalcohol (32.29 mmol, 1.0 eq) and Na_2CO_3 (67.80 mmol, 2.10 eq). The mixture was cooled to 0 °C and under vigorous stirring a solution of acid chloride (38.75 mmol, 1.20 eq) in 1,4-dioxane (36 mL) was dropwise added. After addition was complete, the mixture was allowed to warm to rt and stirred for 5–16 h. At this point the reactions were either: a) continued by addition of NaN_3 (129.16 mmol, 4 eq) and heated to 50 °C, or b) filtered through a plug of celite, concentrated under reduced pressure to remove 1,4-dioxane, extracted with CH_2Cl_2 (three times), washed with water (two times), dried over Na_2SO_4, filtered and concentrated under reduced pressure affording a viscous crude oil. The crude oil (19.72, 1.0 eq) was dissolved in dimethylformamide (DMF, 41.5 mL) under nitrogen atmosphere and NaN_3 (59.16 mmol, 3 eq) added in portion. The reactions were monitored by LC–MS until completion (24–30 h) and solvent removed under reduced pressure. The resulting crude was purified on automated flash chromatography (5% → 50% EtOAc in *n*-heptane) yielding a viscous oil. See chemical synthesis for specific yields.

General procedure for removal of TBDPS protecting group

An oven-dried round bottom flask equipped with a magnetic stirring bar was charged with TBDPS protected azido alcohol (5.46 mmol, 1.0 eq), flushed with N_2, and an. CH_2Cl_2 (11.2 mL) added. To the stirring mixture was added either: a) tetrabutylammoniumfluoride (TBAF, 1 M in THF, 6.0 mL, 1.2 eq), or b) trifluoroacetic acid (TFA, 26.7 eq). The reactions were stirred at rt for 16 h and monitored with TLC. To mixtures indicating incomplete conversion after 16 h was added additional TBAF (1 M in THF, 1.0 eq) and stirring proceeded for 2 h before the reaction mixtures were concentrated under reduced pressure resulting in a crude oil which was purified by automated flash chromatography (CH_3OH/CH_2Cl_2 gradients). See chemical synthesis for specific yields.

Supplementary Text

Docking and Calculations

The length of the spacers connecting the central core fragments **19** (alpha) and **20** (beta) to the five sialic acid residues was investigated computationally. Two different orientations of the chair conformations of **19** and **20** were considered in the investigation. The calculations showed that **26** and **28** containing spacers with 13 main chain atoms were off sufficient length to not cause strain on the spacer atoms; the average bond angles over the carbon atoms was at an optimal of 109 and 113°, which is in agreement with the OPLS_2005 force field for these kinds of angles. Shortening the spacers to contain 11 main chain atoms, as in **40** did not change the C-C-C bond angles. A shorter spacer as in **48** with a total length of eight main chain atoms resulted in significant strain as manifested by an average C-C-C bond angle of 123.3°. In addition, the shorter spacer of **48** also resulted in steric clashes with amino acids of CVA24v.

Supplementary Figures

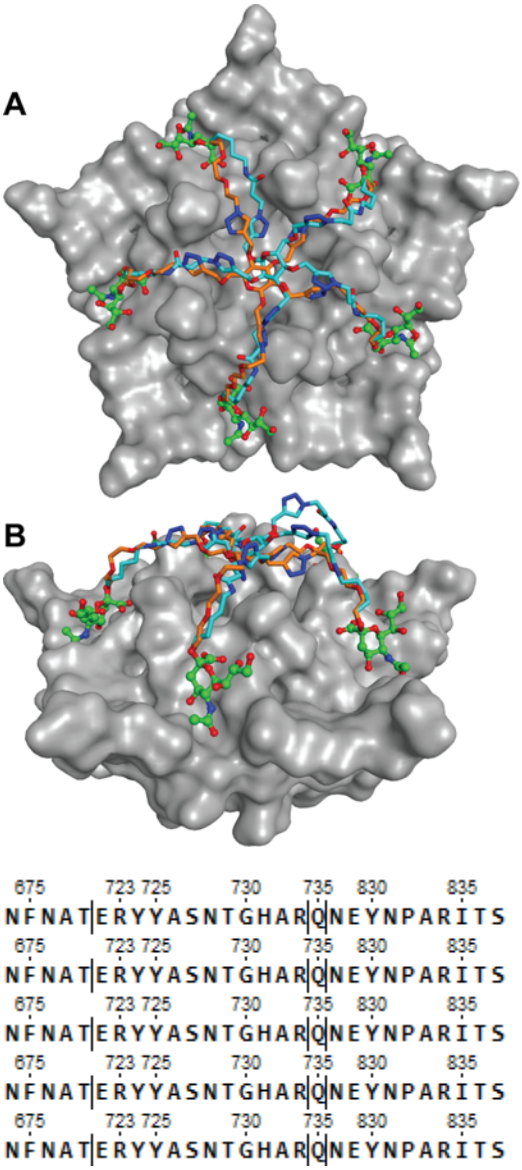


Figure S1. Top view (**A**) and side view (**B**) of CVA24v sialic acid binding region (gray surface) and the design pentavalent inhibitors, **26** (orange), and **28** (cyan) with sialic acid in green. (**C**) Amino acids from the 5 chains of the truncated CVA24v included in spacer design calculations.

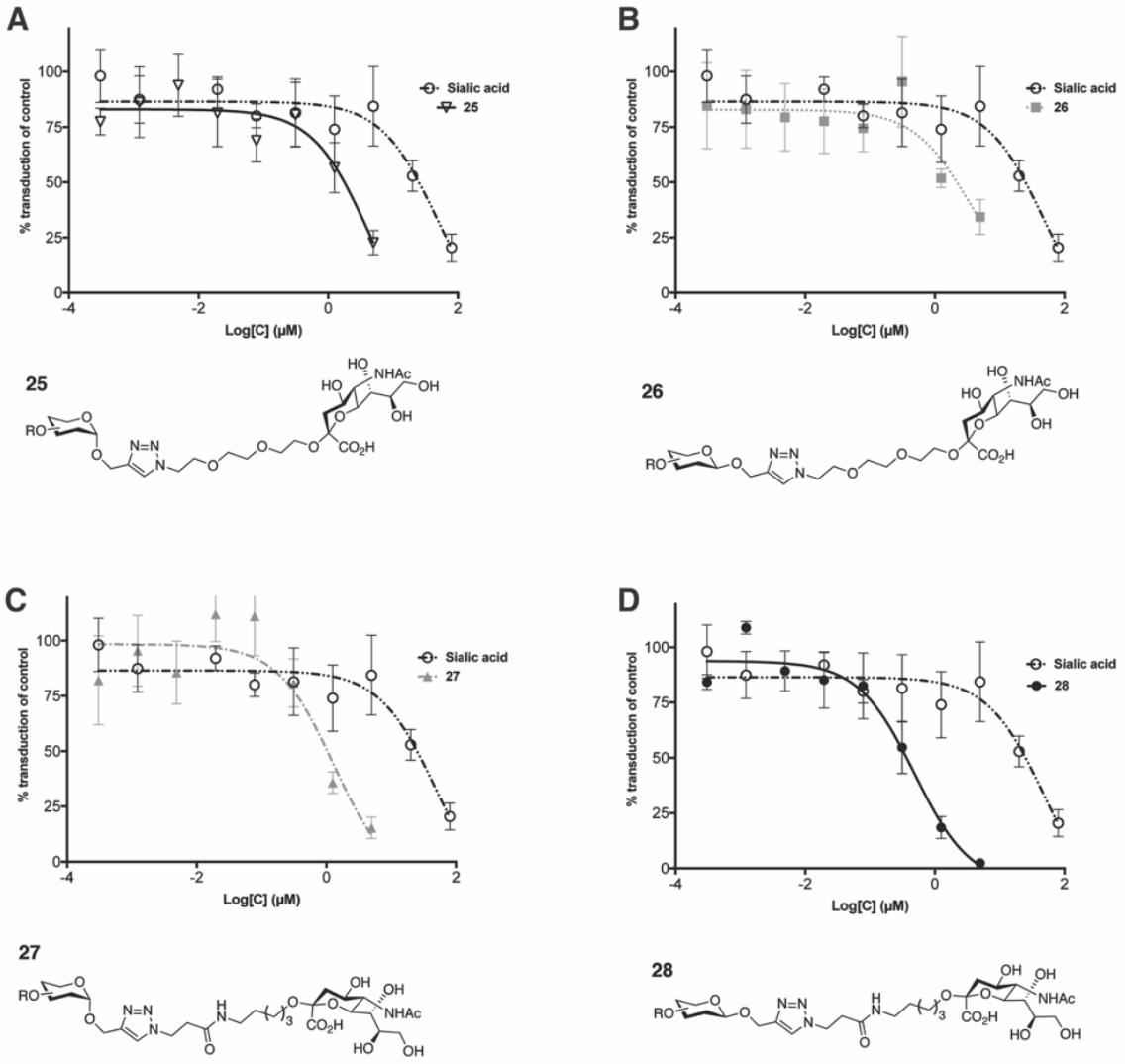


Figure S2. Effect of pentavalent sialic acid conjugates on CVA24v infection of HCE cells at 37 °C. Infection at different concentrations at 37 °C, A) compound **25**, B) compound **26**, C) compound **27**, D) compound **28**. Sialic acid monosaccharide used as a control in all experiments.

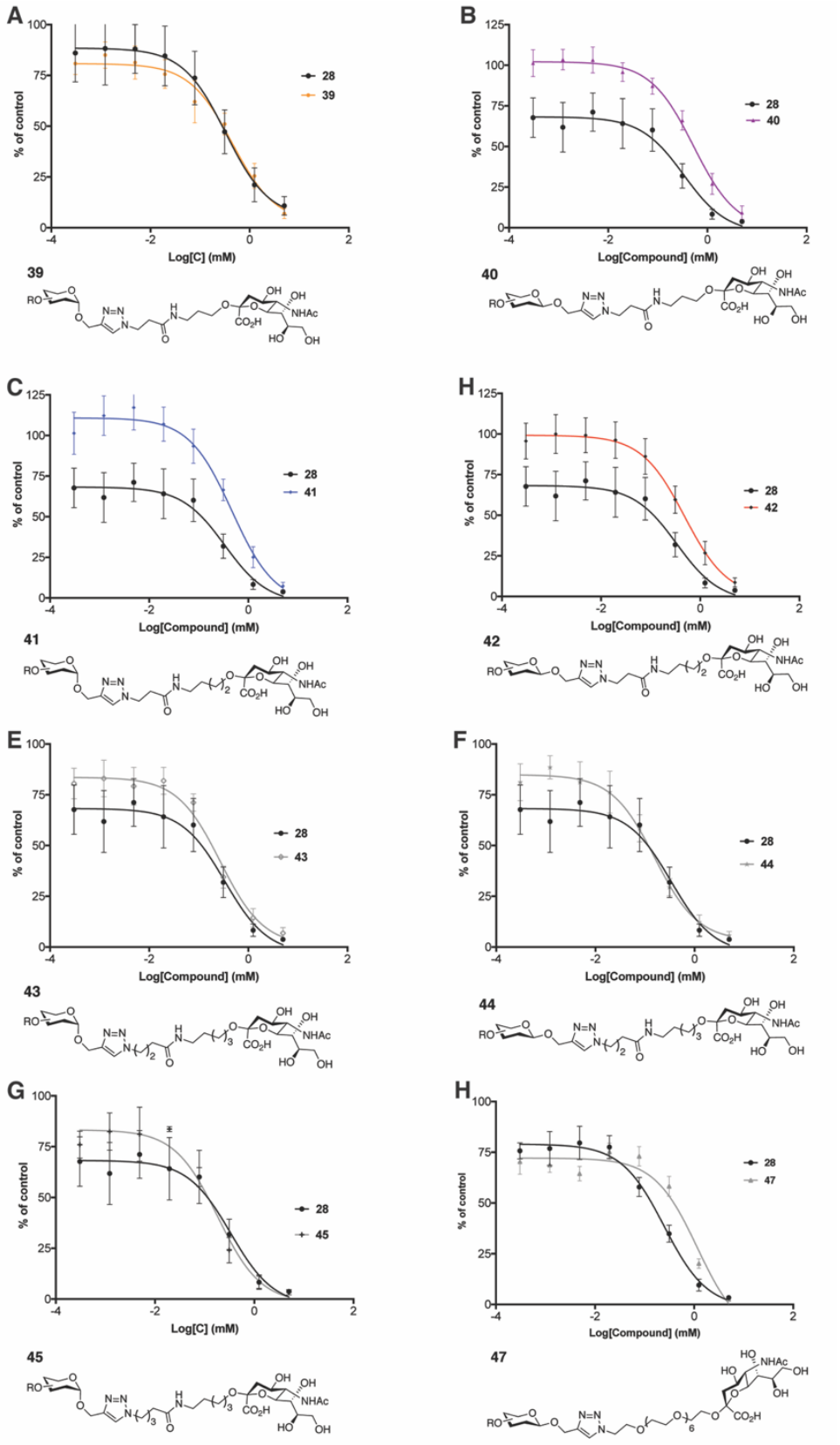


Figure S3. Effect of spacer length on CVA24v binding to HCE cells at 4 °C. Virion binding in presence of inhibitors at different concentrations, A) compound **39**, B) compound **40**, C) compound **41**, D) compound **42**, E) compound **43**, F) compound **44**, G) compound **45**, H) compound **47**.

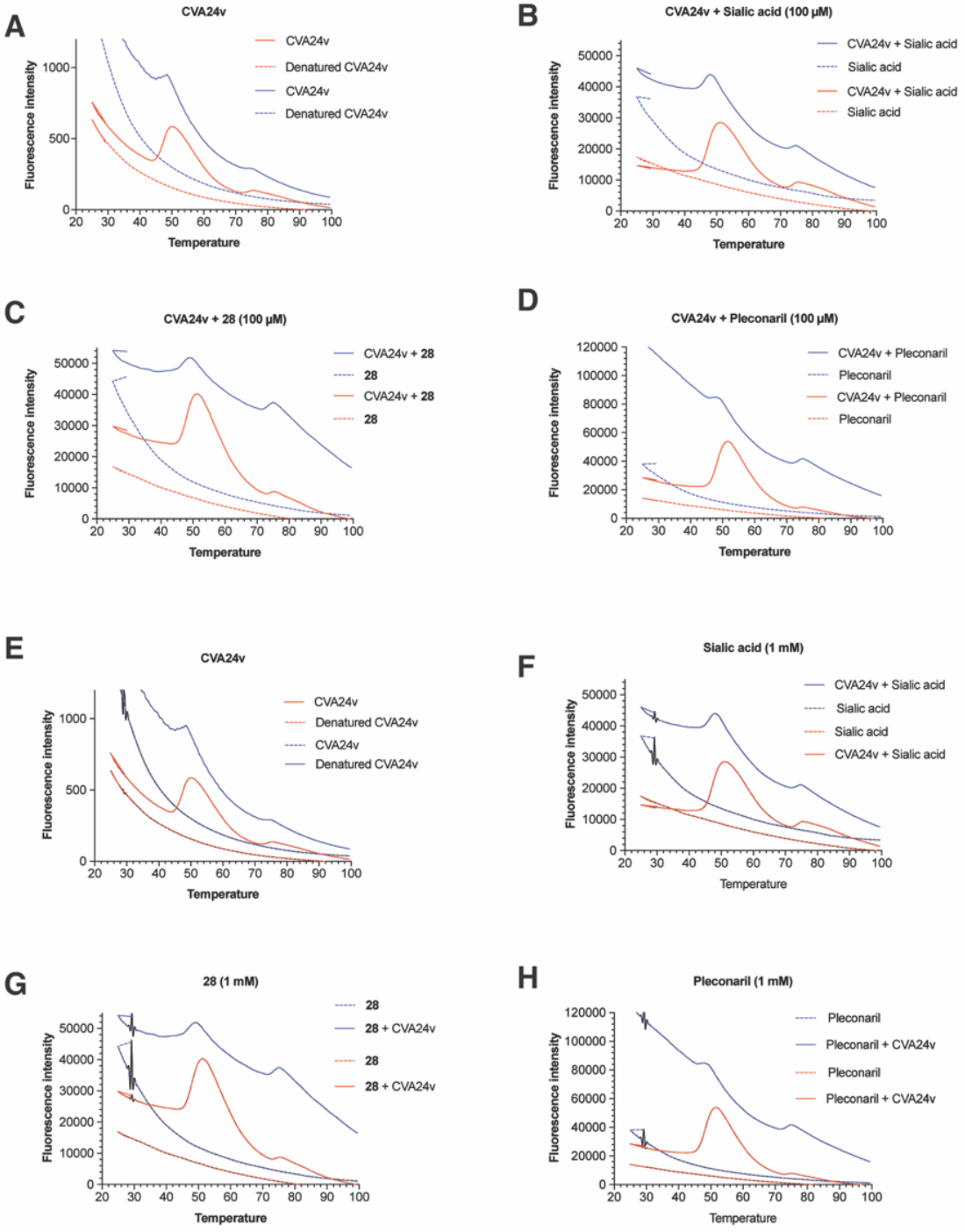


Figure S4. Effect of pleconaril, sialic acid, and 28 on thermal stability of CVA24v. Fluorescence curves of CVA24v with or without treatment with pleconaril, sialic acid, or **28**. Red lines in all curves (A-H) represent detected fluorescence of (SYTO9) upon binding to RNA, while blue lines represent detected fluorescence of (SYPRO red) upon binding to hydrophobic protein patches. A) CVA24v control for B-D. Solid lines represent observed fluorescence of native CVA24v particle upon heat treatment. Dashed lines represent observed fluorescence of denatured CVA24v particle upon heat treatment. B) Effect of sialic acid (100 µM) on CVA24v thermal stability. Dashed lines represent sialic acid in combination with each dye. C) Effect of **28** (100 µM) on CVA24v thermal stability. Dashed lines represent

28 in combination with each dye. D) Effect of pleconaril (100 μM) on CVA24v thermal stability. Dashed lines pleconaril in combination with each dye. E) CVA24v control for F-G. Solid lines represent observed fluorescence of native CVA24v particle upon heat treatment. Dashed lines represent observed fluorescence of denatured CVA24v particle upon heat treatment. F) Effect of sialic acid (1 mM) on CVA24v thermal stability. Dashed lines sialic acid in combination with each dye. G) Effect of **28** (1 mM) on CVA24v thermal stability. Dashed lines sialic acid in combination with each dye. H) Effect of pleconaril (1 mM) on CVA24v thermal stability. Dashed lines sialic acid in combination with each dye.

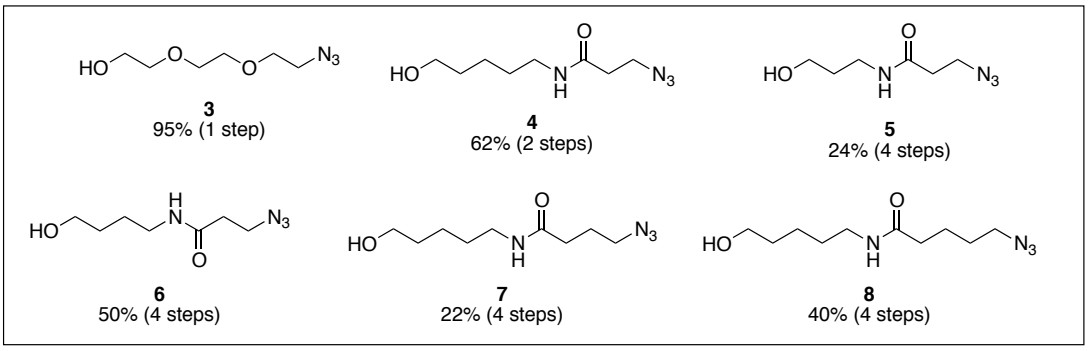
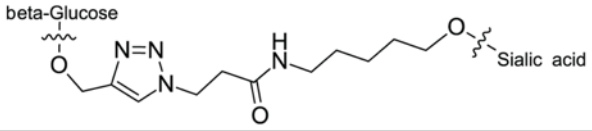
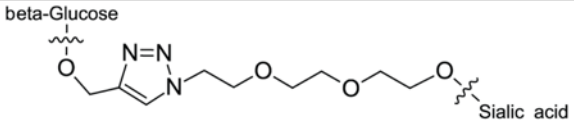
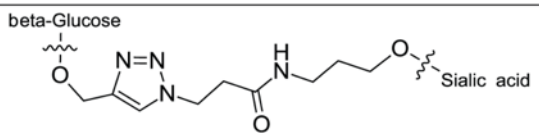
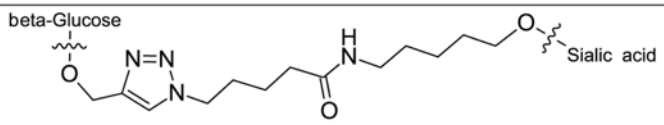
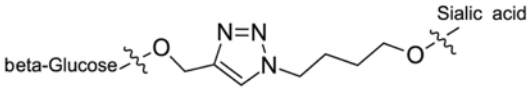


Figure S5. Summary of synthesized spacer fragments. Spacer **3–8** were synthesized in four steps employing the TBDPS protecting group. Spacer **3** was synthesized in one step, and **4** was synthesized in two steps without use of a protecting group.

Supplementary Tables

Table S1. Table of spacer structures and angles over carbons in the designed pentavalent sialic acid conjugates.

ID	Structure	Average C-C-C angle (°)
28		112.7
26		110.8[a]
40		113.0
46		113.0
48		123.3

[a] The angle O-C-C.

Table S2: X-Ray data collection and refinement statistics

	CVA24v-28
Data collection statistics	
Resolution [Å]	50 – 1.81 (1.92 – 1.81)
Space group	I222
Unit cell [Å]	a = 305.38, b = 366.15, c = 365.04
No. of unique reflections	1824197 (292434)
R_{meas} [%]	20.8 (108)
CC(1/2)	99.3 (67.9)
Completeness [%]	99.9 (99.4)
Multiplicity	7.7 (7.7)
I/σ(I)	8.2 (1.8)
Wilson B-factor [$Å^2$]	22.3
Refinement statistics	
R_{factor} [%]	15.7
rmsd bond length	0.005
rmsd bond angle	1.12
Ramachandran angles	
Favoured [%]	96.4
Outliers [%]	0.6

Values for the highest resolution shell are given in parentheses.

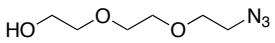
Table S3: pIC50 values for all tested compounds against CVA24v HAdV-37.

	CVA24v			HAdV-37
Compound ID	**Binding (4 °C)**	**Infection (4 °C)**	**Infection (37 °C)**	**Binding (4 °C)**
25	3.37 +/- 0.13	3.14 +/- 0.24	3.62 +/- 0.69	
26	3.17 +/- 0.11	3.01 +/- 0.13	3.53 +/- 0.91	
27	3.80 +/- 0.10	3.91 +/- 0.13	3.11 +/- 0.38	
28	3.64 +/- 0.11*	3.74 +/- 0.16	3.32 +/- 0.20	4.42 +/- 0.12
39	3.35 +/- 0.18			
40	3.28 +/- 0.13			
41	3.33 +/- 0.18			
42	3.32 +/- 0.19			
43	3.57 +/- 0.15			
44	3.80 +/- 0.13			
45	3.74 +/- 0.15			
46	3.75 +/- 0.10			3.68 +/- 0.10
47	3.07 +/- 0.14			
48	3.12 +/- 0.23			4.66 +/- 0.14
ME0462				6.27 +/- 0.11

*Merged data from Figure 2A, 3A and S3.

Chemical synthesis

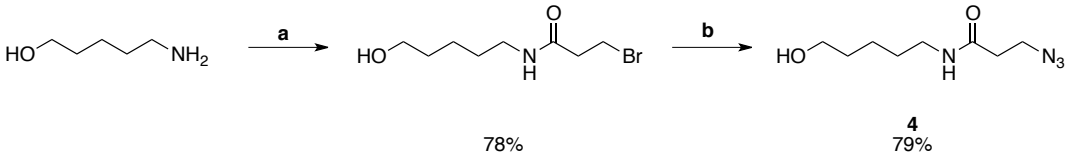
Synthesis of PEG spacer 3



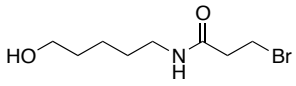
2-(2-(2-azidoethoxy)ethoxy)ethanol (3)

To a round bottom flask was added 2-(2-(2-chloroethoxy)ethoxy)ethanol (18.15 mmol, 1.0 eq) and water (9 mL). To this stirring solution was added NaN_3 (2.0 eq) in portion. The reaction mixture was heated to 75 °C for 70 h. Upon completion of the reaction the mixture was cooled to rt and concentrated under reduced pressure. The resulting residue was suspended in ether (50 mL), filtered and co-evaporated with $CHCl_3$ three times affording 3.017 g of a colorless liquid. Yield 95%. ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.32 (s, 1H), 3.39 (dd, J = 5.8 Hz, 4.7 Hz, 2H), 3.58–3.63 (m, 2H), 3.64–3.70 (m, 6H), 3.70–3.75 (m, 2H). LRMS (ESI) *m/z*: $[M + Na]^+$ Calcd for $C_6H_{13}N_3O_3Na$ 198.08; Found 198.10.

Synthesis of amide spacer 4



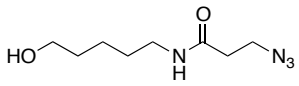
Scheme S1. Synthesis of amide spacer 4. Reagents and conditions: (a): 3-Bromopropionyl chloride, Na_2CO_3, 1,4-dioxane/water (1:1), 0 °C → rt, 5.5 h. (b): NaN_3, DMSO, nitrogen atmosphere, rt, 16 h.



3-bromo-*N*-(5-hydroxypentyl)propanamide

To a round bottom flask was added 5-amino-1-pentanol (12.02 mmol, 1.0 eq), Na2CO3 (1.67 eq) and water (10 mL) and the solution was cooled to 0 °C. To the vigorously stirring solution was added dropwise a solution of 3-Bromopropionyl chloride (1.11 eq) in 1,4-dioxane (10 mL) over 30 minutes and the reaction stirred for 5.5 h while cooling to rt. The reaction was diluted with water (10.0 mL) and extracted with ethyl acetate three times. The organic phases were combined and washed with 0.5 M of HCl solution, brine, dried over Na2SO4, filtered and concentrated to dryness affording 2.226 g of white powder (78% yield). ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.37–1.49 (m, 2H), 1.51–1.67 (m, 5H), 2.73 (t, J =

6.6 Hz, 2H), 3.30 (q, J = 6.6 Hz, 2H), 3.64 (td, J = 6.5 Hz, 2.8 Hz, 4H), 5.46–5.92 (br s, 1H). HRMS (ESI-TOF) *m/z*: $[M + Na]^+$ Calcd for $C_8H_{16}BrNO_2Na$ 260.0256; Found 260.0253.



3-azido-*N*-(5-hydroxypentyl)propanamide (4)

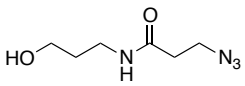
In a round bottom flask was added a magnetic stirrer, 3-bromo-*N*-(5-hydroxypentyl)propanamide (2.10 mmol, 1.0 eq), DMSO (10 mL), and NaN_3 (3.0 eq) in portion. The reaction was allowed to stir for 16 h under nitrogen atmosphere, and the mixture was concentrated by vacuum distillation (not till dryness!). The product was purified on preparative HPLC (10–50% CH3CN in H2O over 25 minutes) affording 0.333 g of a yellow oil in 79% yield after lyophilization. ^1H NMR (400 MHz, $CDCl_3$): δ 1.34 (tdd, J = 9.0 Hz, 6.6 Hz, 5.4 Hz, 2H), 1.5 (m, J = 6.8 Hz, 4H), 2.38 (t, J = 6.5 Hz, 2H), 3.19 (td, J = 6.6 Hz, 5.5 Hz, 3H), 3.55 (q, J = 6.2 Hz, 4H), 6.50–6.78 (br s, 1H). HRMS (ESI-TOF) *m/z*: $[M + Na]^+$ Calcd for $C_8H_{16}N_4O_2Na$ 223.1165; Found 223.1166.

Synthesis of amide spacers 5–8 using TBDPS protecting group

Quant. 58% **6** 85%

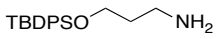
Scheme S2. General synthesis of amide spacer with TBDPS protecting group.

Exemplified with 4-amino-1-butanol and 3-bromopropionyl chloride in synthesis of **6**. Reagents and conditions: (a): TBDPSCl, CH_3CN, rt, 16–72 h. (b): acid chloride, Na_2CO_3, 1,4-dioxane/H_2O (1:1), 0 °C → rt, 5–16 h. (c): NaN_3, DMF, 50 °C, 24–30 h. (d) TBAF, CH_2Cl_2, rt, 16h.



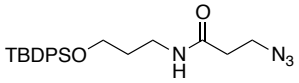
3-azido-N-(3-hydroxypropyl)propanmide (5)

TBDPS cleavage using TFA. Yield 30.0%. ^1H NMR (400 MHz, CD_3OD): δ 1.72 (m, J = 6.7 Hz, 2H), 2.44 (t, J = 6.4 Hz, 2H), 3.22–3.33 (m, overlapped with solvent, 2H), 3.56 (t, J = 6.5 Hz, 2H), 3.59 (t, J = 6.4 Hz, 2H). LRMS (ESI) *m/z*: $[M + Na]^+$ Calcd for $C_6H_{12}N_4O_2Na$ 195.09; Found 195.10.



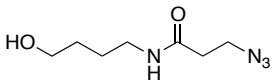
3-((*tert*-butyldiphenylsilyl)oxy)propan-1-amine

Synthesized according to general procedure for TBDPS protection. Quantitative yield. ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.08 (s, 9H), 1.34 (s, 2H), 1.71 (m, J = 6.4 Hz, 2H) 3.76 (t, J = 6.1 Hz, 2H), 7.35–7.46 (m, 6H), 7.66–7.73 (m, 4H). LRMS (ESI) *m/z*: $[M + H]^+$ Calcd for $C_{19}H_{28}NOSi$ 314.19; Found 314.20.



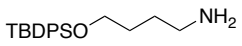
3-azido-*N*-(3-((*tert*-butyldiphenylsilyl)oxy)propyl)propanamide

Synthesized according to general procedure for TBDPS protection. Isolated in 81% over two steps. Azide formation was performed after semi-purification (filtration and extraction). ^{1}H NMR (600 MHz, $CDCl_3$): δ 1.09 (s, 9H), 1.76 (m, J = 5.9 Hz, 2H), 2.25 (t, J = 6.5 Hz, 2H), 3.55 (t, J = 3.55 Hz, 2H), 3.42 (dt, J = 6.7 Hz, 5.6 Hz, 2H), 3.79 (t, J = 5.6 Hz, 2H), 5.99 (br s, 1H), 7.38–7.43 (m, 4H), 7.43–7.48 (m, 2H), 7.62–7.71 (m, 2H). ^{13}C NMR (150 MHz, $CDCl_3$)**:** δ 19.29, 27.04, 31.32, 35.88, 38.08, 47.49, 62.95, 127.94, 129.98, 133.40, 135.62, 169.64. LRMS (ESI) *m/z*: $[M + H]^+$ Calcd for $C_{22}H_{31}N_4O_2Si$ 411.2; found 411.3.



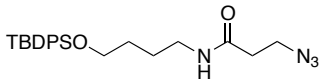
3-azido-*N*-(4-hydroxybutyl)propanamide (6)

TBDPS cleavage using TBAF, 85% yield. ^{1}H NMR (400 MHz, CD_3OD): δ 1.51–1.61 (m, 4H), 2.44 (t, J = 6.4 Hz, 2H), 3.21 (tt, J = 6.9 Hz, 2.3 Hz, 2H), 3.56 (q, J = 6.1 Hz, 4H).^{13}C NMR (150 MHz, CD_3OD): δ 24.79, 28.86, 34.31, 38.36, 46.58, 60.50, 170.80. LRMS (ESI) *m/z*: $[M + Na]^+$ Calcd for $C_7H_{14}N_4O_2Na$ 209.10; Found 209.12.



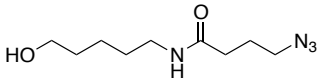
4-((*tert*-butyldiphenylsilyl)oxy)butan-1-amine

Synthesized according to general procedure for TBDPS protection. ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.06 (s, 9H), 0.94–1.22 (br s, NH_2, 2H), 2.67 (t, J = 6.9 Hz, 2H), 3.68 (t, J = 6.3 Hz, 2H), 7.36–7.45 (m, 6H), 7.64–7.72 (m, 4H). LRMS (ESI) *m/z*: $[M + H]^+$ Calcd for $C_{20}H_{30}NOSi$ 328.20; Found 328.3.



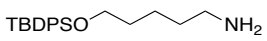
3-azido-*N*-(4-((*tert*-butyldiphenylsilyl)oxy)butyl)propanamide

Synthesized according to general procedure for TBDPS protection, amide coupling and azide formation. Isolated in 58% over two steps. Azide formation was performed after semi-purification (filtration and extraction). ^{1}H NMR (600 MHz, $CDCl_3$): δ 1.06 (s, 9H), 1.56–1.64 (m, 4H), 2.35 (t, J = 6.4 Hz, 2H), 3.28 (q, J = 6.3 Hz, 2H), 3.59 (t, J = 6.4 Hz, 2H), 3.7 (t, J = 5.8 Hz, 2H), 5.67–5.82 (br s, 1H), 7.26–7.44 (m, 6H), 7.63–7.69 (m, 4H^{13}C NMR (150 MHz, CD_3OD): δ 19.33, 26.11, 27.00, 29.93, 36.02, 39.58, 47.58, 63.58, 127.79, 129.77, 133.92, 135.64, 169.77. HRMS (ESI-TOF) *m/z*: $[M + H]^+$ Calcd for $C_{23}H_{33}N_4O2Si$ 425.2368; Found 425.2366.



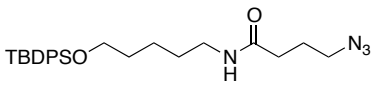
4-azido-(5-hydroxypentyl)butanamide (7)

TBDPS cleavage using TBAF, 87% yield. ^{1}H NMR (400 MHz, CD_3OD): δ 1.36–1.46 (m, 2H), 1.50–1.61 (m, 4H), 1.82–1.93 (m, J = 7.1 Hz, 2H), 2.28 (t, J = 7.4 Hz, 2H), 3.19 (t, J = 7.0 Hz, 2H), 3.35 (t, J = 6.8 Hz, overlapped with CD_3OD 2H), 3.57 (t, J = 6.6 Hz, 2H). HRMS (ESI-TOF) *m/z*: $[M + Na]^+$ Calcd for $C_9H_{18}N_4O_2Na$ 237.1322; Found 237.1322.



5-((*tert*-butyldiphenylsilyl)oxy)pentan-1-amine

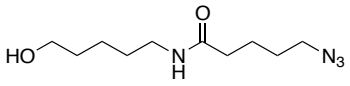
Synthesized according to general procedure for TBDPS protection. ^{1}H NMR (600 MHz, $CDCl_3$): δ 1.06 (s, 3H), 1.34–1.44 (m, 4H), 1.44–1.67 (br s, NH_2, 2H), 1. 1.57 (m, J = 6.9 Hz, 2H), 2.65 (t, J = 6.8 Hz, 2H), 3.67 (t, J = 6.5 Hz, 2H), 7.37–7.40 (m, 4H), 7.40–7.45 (m, 2H), 7.65–7.70 (m, 4H). HRMS (ESI-TOF) *m/z*: $[M + H]^+$ Calcd for $C_{21}H_{32}NOSi$ 342.2248; Found 342.2244.



4-azido-*N*-(5-((*tert*-butyldiphenylsilyl)oxy)pentyl)butanamide

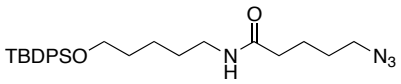
Synthesized according to general procedure for TBDPS protection, amide coupling and azide formation. However, azide formation was performed directly with the addition of NaN_3 without any semi-purification.

Isolated in 25% yield over two steps. ^{1}H NMR (600 MHz, $CDCl_3$): δ 1.05 (s, 9H), 1.36–1.42 (m, 2H), 1.45–1.51 (m, 2H), 1.54–1.61 (m, 2H), 1.92 (m, J = 6.9 Hz 2H), 2.23 (t, J = 7.2 Hz, 2H), 3.23 (q, J = 6.6 Hz, 2H), 3.34 (t, J = 6.6 Hz, 2H), 3.67 (t, J = 6.23 Hz, 2H), 5.50 (br s, 1H), 7.35–7.40 (m, 4H), 7.40–7.44 (m, 2H), 7.66 (dd, J = 8.0, 1.3 Hz, 2H). ^{13}C NMR (150 MHz, $CDCl_3$): δ 19.34, 23.29, 24.97, 26.99, 29.44, 32.23, 33.34, 39.66, 50.92, 63.75, 127.73, 129.67, 134.13, 135.67, 171.62. LRMS (ESI) *m/z*: $[M + H]^+$ Calcd for $C_{25}H_{37}N_4O_2Si$ 453.27; Found 453.3.



5-azido-N-(5-hydroxypentyl)pentamide (8)

Compound was synthesized according to the general procedures described. However, the TBDPS protected azido intermediate was isolated as a mixture and the mixture was used without additional purification. TBAF was used in TBDPS removal and compound subsequently isolated as a clear oil in 40% yield over three steps. ^{1}H NMR (400 MHz, CD_3OD): δ 1.33–1.42 (m, 2H) 1.46–1.73 (m, 8H), 2.2 (t, J = 7.2 Hz, 2H), 3.16 (t, J = 7.0 Hz, 2H), 3.3 (t, J = 6.7 Hz, overlapped with CD_3OD, 2H), 3.54 (t, J = 6.6 Hz, 2H). LRMS (ESI) *m/z*: $[M + Na]^+$ Calcd for $C_{10}H_{20}N_4O_2Na$ 251.15; Found 251.16.

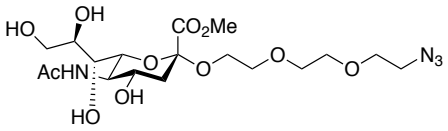


5-azido-*N*-(5-((*tert*-butyldiphenylsilyl)oxy)pentyl)pentanamide

Compound was synthesized according to the general procedure for TBDPS protection, amide coupling and azide formation. This resulted in a mixture which was used directly in the TBDPS deprotection without further purification. LRMS (ESI) *m/z*: $[M + H]^+$ Calcd for $C_{26}H_{39}N_4O_2Si$ 467.28; Found 467.3.

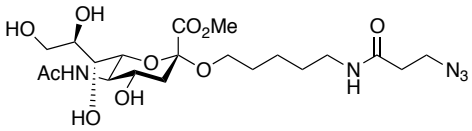
Synthesis and analytical data of azido sialosides 11–18

Azido sialosides were synthesized according to general procedure for sialidation described in the material and methods section, except for azido sialoside **18** (see below). Azido sialoside **11** and **12** were synthesized according to "Method A", while azido sialosides 12–17 were synthesized according to "Method B".



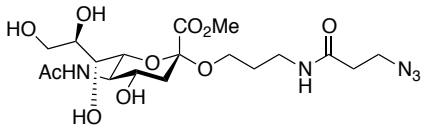
Methyl (2-(2-(2-(2-azidoethoxy)ethoxy)ethoxy)(5-*N*-acetamido-3,5-dideoxy-D-glycero-α-D-galacto-2- nonylopyranosyl))-onate (11)

Synthesized according to "Method A" for sialidation, 28% yield over two steps. ^{1}H NMR (400 MHz, CD_3OD): δ 1.75 (t, J = 12.3 Hz, 1H), 2.0 (s, 3H), 2.7 (dd, J = 12.8 Hz, 4.6 Hz, 1H), 3.38 (t, J = 4.9 Hz, 2H), 3.5 (dd, J = 6.6 Hz, 1.7 Hz, 1H), 3.55–3.70 (m, 12H), 3.73–3.97 (m, 8H). ^{13}C NMR (100 MHz, CD_3OD): δ 22.67, 41.61, 51.77, 53.40, 53.79, 64.69, 64.72, 68.56, 70.20, 71.16, 71.24, 71.49, 71.67, 72.45, 74.90, 100.17, 170.87, 175.15. HRMS (ESI-TOF) *m/z*: $[M + Na]^+$ Calcd for $C_{20}H_{35}N_5O_{10}Na$ 503.1960; Found 503.1944.



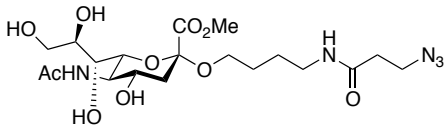
Methyl (2-(5-(3-azidopropanamido)pentyl)oxy)(5-*N*-acetamido-3,5-dideoxy-D-glycero-α-D-galacto-2- nonylopyranosyl))-onate (12)

Synthesis according to "Method A" yielded 12–14% of pure product after two steps. Using "Method B" improved the yield to 35% over two steps. ^{1}H NMR (600 MHz, CD_3OD): δ 1.34–1.43 (m, 2H), 1.49–1.53 (m, 2H), 1.54–1.60 (m, 2H), 1.73 (t, J = 12.4 Hz, 1H), 2.0 (s, 3H), 2.43 (t, J = 6.4 Hz, 2H, 2.67 (dd, J = 12.8 Hz, 4.7 Hz, 1H), 3.19 (t, J = 7.0 Hz, 2H), 3.36 (dt, J = 9.2 Hz, 6.6 Hz, 1H), 3.51 (dd, J = 8.8 Hz, 1.5 Hz, 1H), 3.53–3.58 (m, 3H), 3.60–3.66 (m, 2H), 3.75 (t, J = 10.3 Hz, 1H), 3.78–3.66 (m, overlapped with singlet, 3H), 3.83 (s, 3H). ^{13}C NMR (150 MHz, CD_3OD): δ C-NMR 22.65, 24.34, 29.91, 30.31, 36.35, 40.37, 41.77, 49.57, 53.35, 53.87, 64.70, 64.96, 68.55, 70.23, 72.57, 74.92, 100.19, 171.21, 172.82, 175.26. HRMS (ESI-TOF) *m/z*: $[M + Na]^+$ Calcd for $C_{20}H_{35}N_5O_{10}Na$ 528.2276; Found 528.2279.



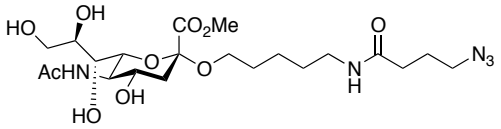
Methyl (2-(3-(3-azidopropanamido)propoxy)(5-*N*-acetamido-3,5-dideoxy-D-glycero-α-D-galacto-2-nonylopyranosyl))-onate (13)

Synthesized according to ”Method B”, 32% after two steps. ^{1}H NMR (400 MHz, CD_3OD): δ 1.69–1.80 (m, 3H), 2.00 (s, 3H), 2.45 (t, J = 6.4 Hz, 2H), 2.67 (dd, J = 12.8 Hz, 4.6 Hz, 1H), 3.27 (td, J = 6.6 Hz, 2.5 Hz, 2H), 3.46 (dt, J = 9.7 Hz, 6.7 Hz, 1H), 3.50–3.60 (m, 4H), 3.61–3.69 (m, 2H), 3.73–3.79 (m, 1H), 3.79–3.87 (m, 6H). ^{13}C NMR (600 MHz, CD_3OD): δ 22.66, 30.29, 36.32, 37.47, 41.55, 49.57, 53.37, 53.86, 62.55, 64.72, 68.56, 70.13, 72.42, 74.84, 100.13, 171.03, 172.96, 175.22. HRMS (ESI-TOF) *m/z*: $[M + Na]^+$ Calcd for $C_{18}H_{31}N_5O_{10}Na$ 500.1963; Found 500.1976.



Methyl (2-2-(4-(3-azidopropanamido)butoxy)(5-*N*-acetamido-3,5-dideoxy-D-glycero-α-D-galacto-2-nonylopyranosyl))-onate (14)

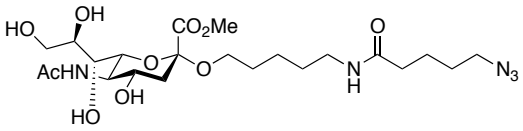
Synthesized according to ”Method B”, 33% after two steps. ^{1}H NMR (600 MHz, CD_3OD): δ 1.50–1.63 (m, 4H), 1.73 (t, J = 12.4 Hz, 1H), 2.0 (s, 3H), 2.43 (t, J = 6.5 Hz, 2H), 2.67 (dd, J = 12.9 Hz, 4.6 Hz, 1H), 3.2 (t, J = 6.2 Hz, 2H), 3.36–3.41 (m, 1H), 3.51 (dd, J = 9.0 Hz, 1.1 Hz, 1H), 3.53–3.59 (m, 3H), 3.60–3.66 (m, 2H), 3.76 (t, J = 10.3 Hz), 3.78–3.88 (m, overlapped with s, 3H), 3.84 (s overlapped with m, 3H). ^{13}C NMR (150 MHz, CD_3OD): δ 22.65, 26.92, 27.99, 36.33, 40.06, 41.75, 49.57, 53.36, 53.86, 64.71, 64.75, 68.55, 70.22, 72.52, 74.92, 100.20, 171.17, 172.84, 175.24. HRMS (ESI-TOF) *m/z*: $[M + Na]^+$ Calcd for $C_{19}H_{33}N_5O_{10}Na$ 514.2119; Found 514.2129.



Methyl (2-(5-(4-azidobutanamido)pentyloxy)(5-*N*-acetamido-3,5-dideoxy-D-glycero-α-D-galacto-2- nonylopyranosyl))-onate (15)

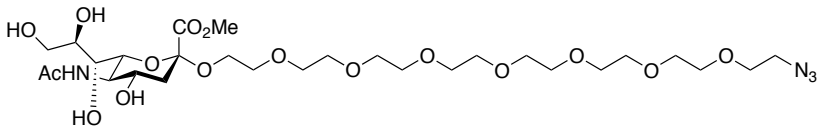
Synthesized according to ”Method B”, 34% after two steps. ^{1}H NMR (400 MHz, CD_3OD): δ 1.28–1.45 (m, 2H), 1.45–1.61 (m, 4H), 1.73 (dd, J = 12.6 Hz, 12.1 Hz, 1H), 1.81–1.91 (m, 2H), 2.0 (s, 3H), 2.27 (t, J = 7.4 Hz, 2H), 2.67 (dd, J = 12.8 Hz, 4.6 Hz, 1H), 3.17 (t, J = 6.9 Hz, 2H), 3.32–3.40 (m, overlapped with CD_3OD, 3H), 3.51 (dd, J = 8.7 Hz, 1.7 Hz, 1H), 3.55 (dd, J = 10.5Hz, 1.7 Hz, 1H), 3.59–3.68 (m, 2H), 3.71–3.88 (m, 7H). ^{13}C NMR (150 MHz, CD_3OD): δ 22.65, 24.24, 24.37, 29.42, 29.98, 30.30, 36.48,

40.27, 41.78, 49.57, 52.13, 53.37, 53.87, 64.70, 64.94, 68.53, 70.23, 72.57, 74.92, 100.18, 171.21, 175.28, 175.61. HRMS (ESI-TOF) *m/z*: $[M + Na]^+$ Calcd for $C_{21}H_{37}N_5O_{10}Na$ 542.2432; Found 542.2437.



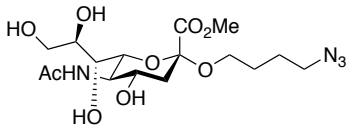
Methyl (2-(5-(5-azidopentanamido)pentyloxy)(5-*N*-acetamido-3,5-dideoxy-D-glycero-α-D-galacto-2- nonylopyranosyl))-onate (16)

Synthesized according to "Method B", 31% after two steps. ^1H NMR (400 MHz, CD_3OD): δ 1.29–1.44 (m, 2H), 1.45–1.79 (m, 9H), 2.00 (s, 3H), 2.21 (t, J = 7.3 Hz, 2H), 2.67 (dd, J = 12.8 Hz, 4.7 Hz, 1H), 3.16 (t, J = 6.9 Hz, 2H), 3.32–3.40 (m, overlapped with CD3OD, 3H), 3.51 (dd, J = 8.6 Hz, 1.9 Hz, 1H), 3.55 (dd, J = 10.4 Hz, 1.8 Hz, 1H), 3.59–3.67 (m, 2H), 3.72–3.87 (m, 7H). ^{13}C NMR (150 MHz, CD_3OD): δ 22.65, 24.24, 24.37, 29.41, 29.98, 30.30, 36.47, 40.27, 41.78, 49.56, 52.12, 53.37, 53.87, 64.70, 64.94, 68.53, 70.22, 72.57, 74.91, 100.18, 171.21, 175.28, 175.60. HRMS (ESI-TOF) *m/z*: $[M + Na]^+$ Calcd for $C_{22}H_{39}N_5O_{10}Na$ 556.2589; Found 556.2600.



Methyl (2-(23-azido-3,6,9,12,15,18,21-heptaoxatricosyloxy)(5-*N*-acetamido-3,5-dideoxy-D-glycero-α-D-galacto-2- nonylopyranosyl))-onate (17)

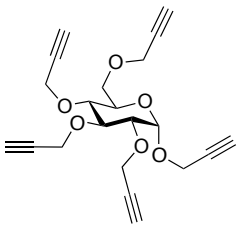
Synthesized according to "Method B", 19% after two steps. 10:1 ratio of alpha and beta anomer. ^1H NMR (600 MHz, CD_3OD): δ 1.75 (t, J = 12.5 Hz), 2.0 (s, 3H), 2.77 (dd, J = 12.8 Hz, 4.7 Hz, 1H), 3.38 (t, J = 4.7 Hz, 2H), 3.44–4.08 (m, 42H). ^{13}C NMR (150 MHz, CD_3OD): δ 22.67, 41.65, 51.80, 53.43, 53.80, 64.70, 64.76, 66.58, 70.22, 71.14, 71.20, 71.55, 71.62, 72.48, 74.93, 100.20, 170.89, 175.15. HRMS (ESI-TOF) *m/z*: $[M + Na]^+$ Calcd for $C_{28}H_{52}N_4O_{16}Na$ 723.3270; Found 723.3285.



Methyl (2-(4-azidobutoxy)(5-*N*-acetamido-3,5-dideoxy-D-glycero-α-D-galacto-2-nonylopyranosyl))-onate (18)

18 was synthesized according to general method B, with minor modifications. In the sialidation step 4-bromo-1-butanol (1.20 eq) was used as the acceptor, yielding an acetyl protected aliphatic bromo sialoside after purification (CH_2Cl_2/CH_3OH 10:0.2 → 10:1) as a mixture of anomers, in addition to elimination product. The mixture (711 mg, 1.135 mmol) was subsequently dissolved in dimethylsulfoxide (DMSO, 32 mL) and treated with NaN_3 (6.0 eq) followed by tetra-n-butylammonium iodide (2.0 eq). The reaction was allowed to stir under nitrogen atmosphere for 22 h. The mixture was diluted in CH_2Cl_2, washed with water, HCl (1 M), and brine. The organic layer was dried with Na_2SO_4, filtered and concentrated under reduced pressure affording crude product, which was used without additional purification. The crude was dissolved in MeOH (68 mL) and $NaOCH_3$ (605.81 mg, 10.0 eq) added portion-wise while stirring. The reaction was allowed to stir at rt under nitrogen atmosphere for 24 h. The solution was neutralized by addition of Dowex 50x8 H^+-form (pre-washed with MeOH), filtered and concentrated to dryness. Mixture was purified using column chromatography (CH_2Cl_2/CH_3OH, from 6 to 10% CH_3OH), affording **18** (247 mg, 0.5875 mmol) in 52.4% yield over the three steps. ^{1}H NMR (600 MHz, CD_3OD): δ 1.52–1.69 (m, 4H), 1.74 (t, J = 12.4 Hz, 1H), 2.00 (s, 3H), 2.68 (dd, J = 12.9 Hz, 4.6 Hz, 1H), 3.26–3.36 (m, overlapped with solvent, 2H), 3.40 (ddd, J = 9.4Hz, 6.3 Hz, 5.5 Hz, 1H), 3.51 (dd, J = 8.8 Hz, 1.4 Hz, 1H), 3.56 (dd, J = 10.5 Hz, 1.5 Hz, 1H), 3.60–3.68 (m, 2H), 3.75 (t, J = 10.2 Hz, 1H), 3.78–3.90 (m, 3H), 3.84 (s, 3H). ^{1}H NMR (150 MHz, CD_3OD): δ 22.66, 26.64, 27.84, 41.70, 52.16, 53.37, 53.84, 64.58, 64.68, 68.51, 70.18, 72.49, 74.93, 100.17, 171.13, 175.24. HRMS (ESI-TOF) *m/z*: $[M + Na]^+$ Calcd for $C_{16}H_{28}N_4O_9Na$ 443.1748; Found 443.1755.

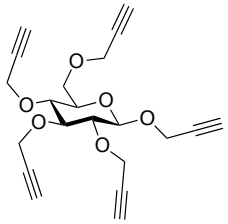
Synthesis of pentapropargylated glucose cores 19 and 20



1,2,3,4,6-penta-*O*-propargyl-α-D-glucopyranoside (19)

An oven-dried MWV was charged with magnetic stirrer and D-(+)-glucose (2.22 mmol, 1.0 eq) was suspended in propargyl alcohol (30.01 eq). Dowex 50x8 (H^+-Form) (100 mg) was then added. The reaction was irradiated in MW for 15 min at 120 °C, and subsequently concentrated to dryness. The resulting residue was filtered through a short silica plug affording a mixture of alpha and beta propargyl

glucosides. The mixture was dissolved in anhydrous DMF (15 mL), cooled to 0 °C, and NaH (8.0 eq) added in-portion. The mixture was stirred at 0 °C for 45 min and propargyl bromide (8.0 eq) dropwise added. The mixture was allowed to warm to rt and allowed to stir at rt for 4 days. The reaction was quenched by water (5 mL), and extracted with Et_2O (20 mL x 3), dried over Na_2SO_4, filtered and concentrated under reduced pressure. The resulting residue was purified by flash chromatography (*n*-heptane/EtOAc 4:1) affording **19** (189 mg, 0.510 mmol) as an amber-colored viscous oil in 38% yield. ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.32–2.58 (m, 5H), 3.53 (dd, J = 9.8 Hz, 8.8 Hz, 1H), 3.68 (dd, J = 9.6 Hz, 3.7 Hz, 1H), 3.72–3.79 (m, 2H), 3.80–3.88 (m, 2H), 4.16–4.35 (m, 6H), 4.39–4.55 (m, 4H), 5.22 (d, J = 3.7 Hz, 1H). ^{13}C NMR (150 MHz, $CDCl_3$): δ 54.80, 58.16, 58.69, 60.20, 60.37, 67.94, 70.12, 74.34, 74.42, 75.06, 75.11, 75.28, 76.34, 78.58, 78.70, 79.43, 79.45, 80.07, 80.15, 81.04, 95.27. HRMS (ESI-TOF) *m/z*: $[M + Na]^+$ Calcd for $C_{21}H_{22}O_6Na$ 393.1308; Found 393.1313.

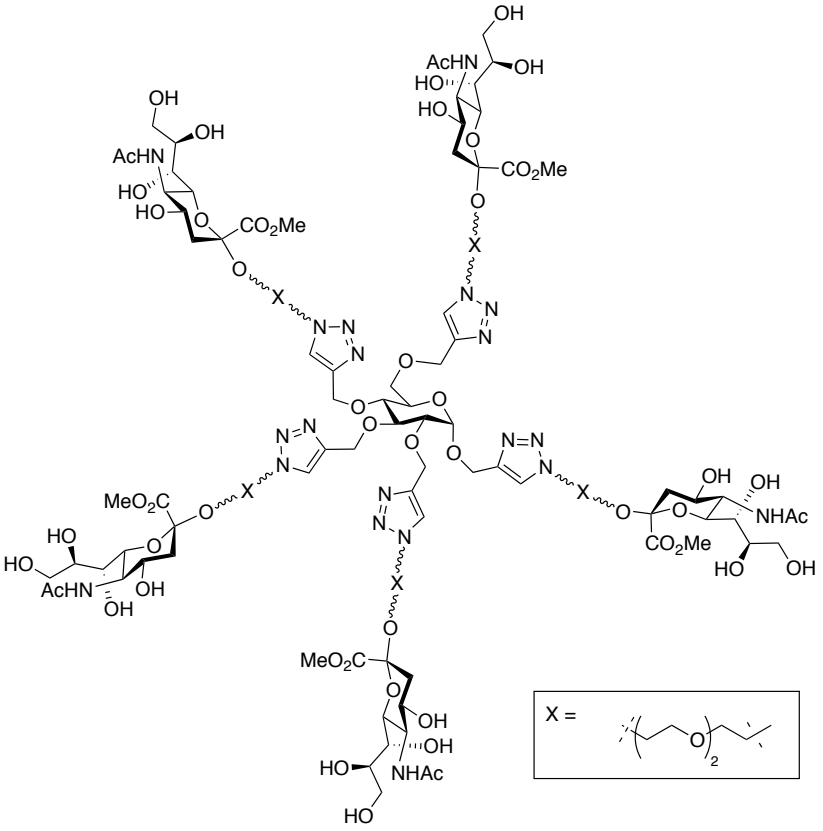


1,2,3,4,6-penta-*O*-propargyl -β-D-glucoryranoside (20)

A round bottom flask was charged with a magnetic stirring bar and commercial 2-propynyl-tetra-*O*-acetyl-β-glucopyranoside (1.29 mmol, 1.0 eq) and CH_3OH (105 mL) was added. To the stirring solution was added NaOCH3 (4.40 eq) in portion. The mixture was stirred for 4 h before neutralization with Amberlite IR 120 (H-Form) to pH ≈7. CH_3OH was removed under reduced pressure to afford a white solid. The white solid was dissolved in anhydrous DMF (19.9 mL) and cooled to 0 °C, to this mixture was added NaH (60% in mineral oil, 7.60 eq) in portion. The mixture was stirred for 45 minutes at 0 °C, and subsequently propargyl bromide (6.0 eq) was added. The mixture was allowed to warm to rt and stirred for 24 h. The reaction was quenched with water (15.0 mL), and extracted with Et_2O (40 mL x 3), dried over Na2SO4, filtered and concentrated under reduced pressure. The resulting mixture was purified by flash chromatography (Heptane/EtOAc 4:1) affording **20** (477.8 mg, 1.29 mmol) as an off-white solid in quantitative yield. ^{1}H NMR (600 MHz, $CDCl_3$): δ 2.21–2.70 (m, 5H), 3.36–3.43 (m, 2H), 3.46 (dd, J = 9.7 Hz, 8.7 Hz, 1H), 3.57 (t, J = 8.9 Hz, 1H), 3.77 (dd, J = 11.0 Hz, 4.7 Hz, 1H), 3.83 (dd, J = 10.9 Hz, 1.8 Hz, 1H), 4.21 (ddd, J = 33.7 Hz, 15.6 Hz, 2.4 Hz, 2H), 4.31–4.58 (m, 10H). ^{13}C NMR (150 MHz, $CDCl_3$): δ 56.05, 58.81, 59.42, 60.17, 60.40, 68.44, 74.27, 74.46, 74.53, 74.56, 74.87, 75.24,

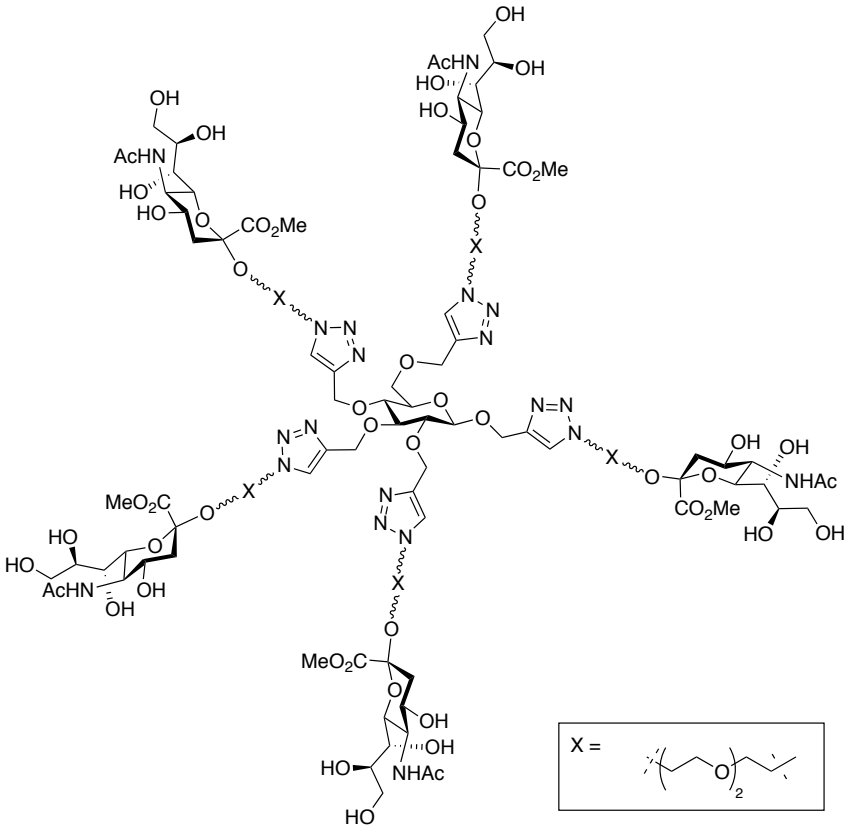
76.03, 78.78, 79.64, 79.89, 80.09, 80.17, 81.06, 83.35, 100.87. HRMS (ESI-TOF) *m/z*: $[M + Na]^+$ Calcd for $C_{21}H_{22}O_6Na$ 393.1308; Found 393.1319.

Synthesis of pentavalent methyl ester protected sialic acid conjugates



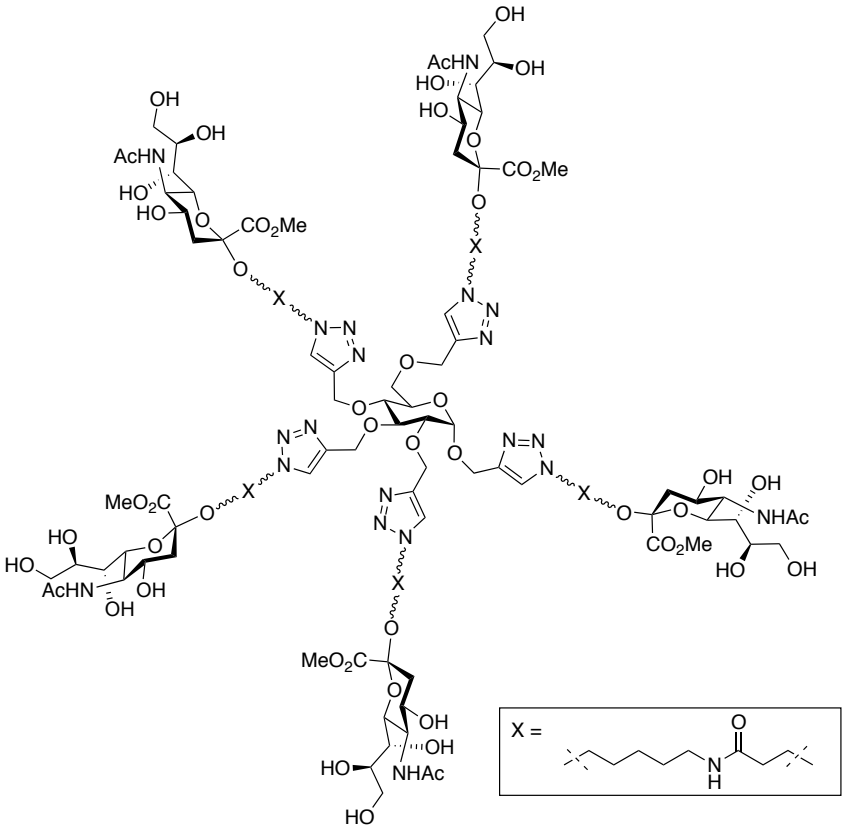
Compound 21

Synthesized according to general procedure for CuAAC, isolated in 49% yield. ^{1}H NMR (600 MHz, CD_3OD): δ 1.69–1.79 (m, 5H), 2.00 (s, 15H), 2.63–2.72 (m, 5H), 3.47–3.94 (m, 105H), 4.49–4.96 (m, overlapped with solvent, 26H), 5.04 (d, J = 3.5 Hz, 1H), 7.99 (s, 1H), 8.05 (s, 1H), 8.07 (s, 1H), 8.07 (s, 2H), 8.09 (s, 1H). ^{13}C NMR (150 MHz, CD_3OD): δ 22.74, 41.66, 51.42, 51.48, 53.58, 53.81, 61.63, 64.69, 64.74, 64.81, 65.29, 66.52, 67.09, 68.56, 69.98, 70.25, 70.41, 71.17, 71.43, 71.55, 71.84, 72.50, 74.96, 78.49, 80.99, 82.54, 97.30, 100.22, 126.00, 126.06, 126.13, 126.18, 126.37, 145.26, 145.71, 145.85, 146.29, 170.90, 175.14. HRMS (ESI-TOF) *m/z*: $[M + 3Na]^+$ Calcd for ($C_{111}H_{182}N_{20}O_{61}Na_3$)/3 946.7144; Found 946.7113.



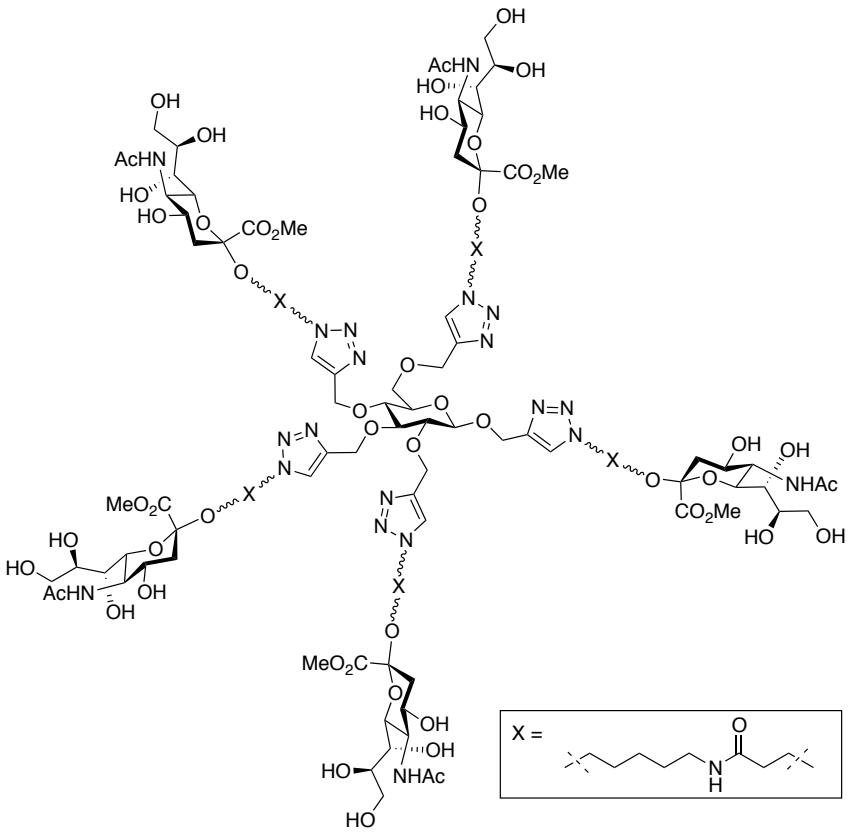
Compound 22

Synthesized according to general procedure for CuAAC, isolated in 42% yield. ^{1}H NMR (600 MHz, CD_3OD): δ 1.74 (t, J = 12.6 Hz, 5H), 2.00 (s, 15H), 2.68 (dd, J = 12.6 Hz, 4.2 Hz, 5H), 3.40–4.05 (m, 110H), 4.50–4.71 (m, 14H), 4.76–4.98 (m, overlapped with solvent, 3H), 7.99 (s, 1H), 8.03 (s, 1H), 8.04 (s, 1H), 8.07 (s, 1H), 8.13 (s, 1H). ^{13}C NMR (150 MHz, CD_3OD): δ 22.76, 41.66, 51.42, 51.48, 53.59, 53.81, 63.27, 64.73, 64.80, 65.32, 66.26, 66.37, 67.12, 68.55, 70.12, 70.25, 70.41, 72.49, 74.96, 75.64, 78.45, 82.88, 85.06, 100.22, 103.54, 126.10, 126.13, 126.44, 145.39, 145.69, 145.75, 146.07, 146.13, 170.89, 175.13. HRMS (ESI-TOF) *m/z*: $[M + 3Na]^+$ Calcd for $(C_{111}H_{182}N_{20}O_{61}Na_3)/3$ 946.7144; Found 946.7125.



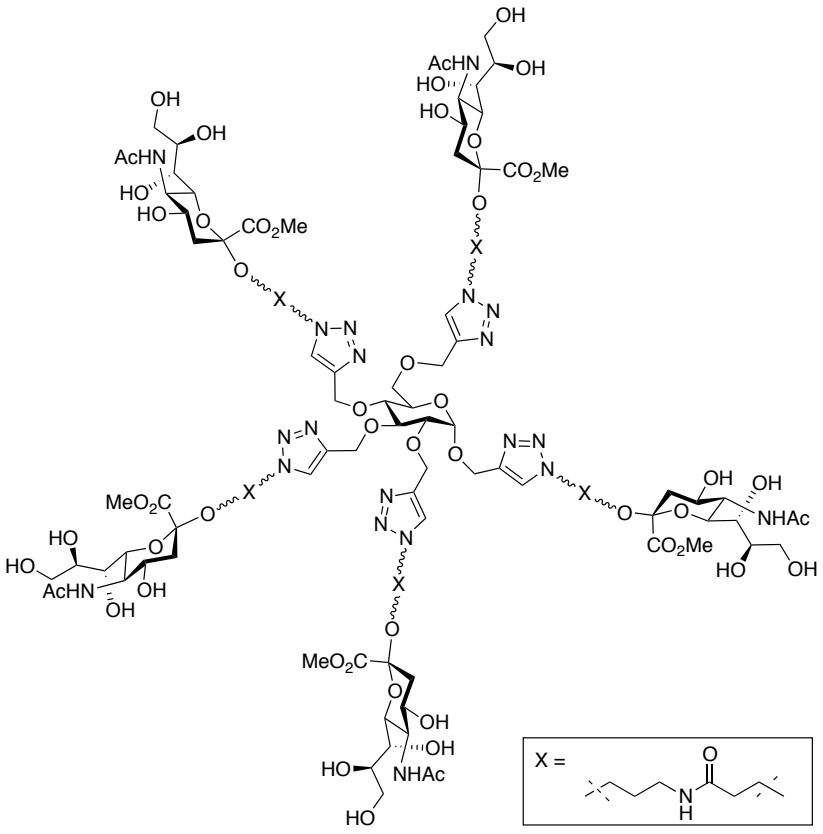
Compound 23

Synthesized according to general procedure for CuAAC, isolated in 41% yield. ^{1}H NMR (400 MHz, CD_3OD): δ 1.23–1.36 (m, 10H), 1.36–1.56 (m, 20H), 1.72 (t, J = 12.3 Hz, 5H), 2.00 (s, 15H), 2.67 (dd, J = 12.8 Hz, 4.7 Hz, 5H), 2.78–2.90 (m, 10H), 3.06–3.16 (m, 10H), 3.34–3.39 (m, overlapped with solvent, 5H), 3.42–3.91 (m, 65H), 4.55–4.91 (m, 16H), 5.01 (d, J = 3.5 Hz, 1H), 7.91 (s, 1H), 7.97 (s, 2H), 7.99 (s, 1H), 8.00 (s, 1H). ^{13}C NMR (150 MHz, CD_3OD): δ 22.73, 24.30, 29.90, 30.28, 37.14, 40.34, 41.80, 53.48, 53.87, 61.51, 64.63, 64.77, 64.98, 65.23, 66.51, 67.08, 68.56, 69.92, 70.27, 71.85, 72.60, 74.93, 78.51, 81.04, 82.53, 97.10, 100.20, 125.40, 125.61, 125.68, 125.70, 125.89, 145.26, 145.70, 145.85, 146.28, 171.21, 175.20. HRMS (ESI-TOF) *m/z*: $[M + 3Na]^+$ Calcd for $(C_{121}H_{197}N_{25}O_{56}Na_3)/3$ 988.4338; Found 988.4337.



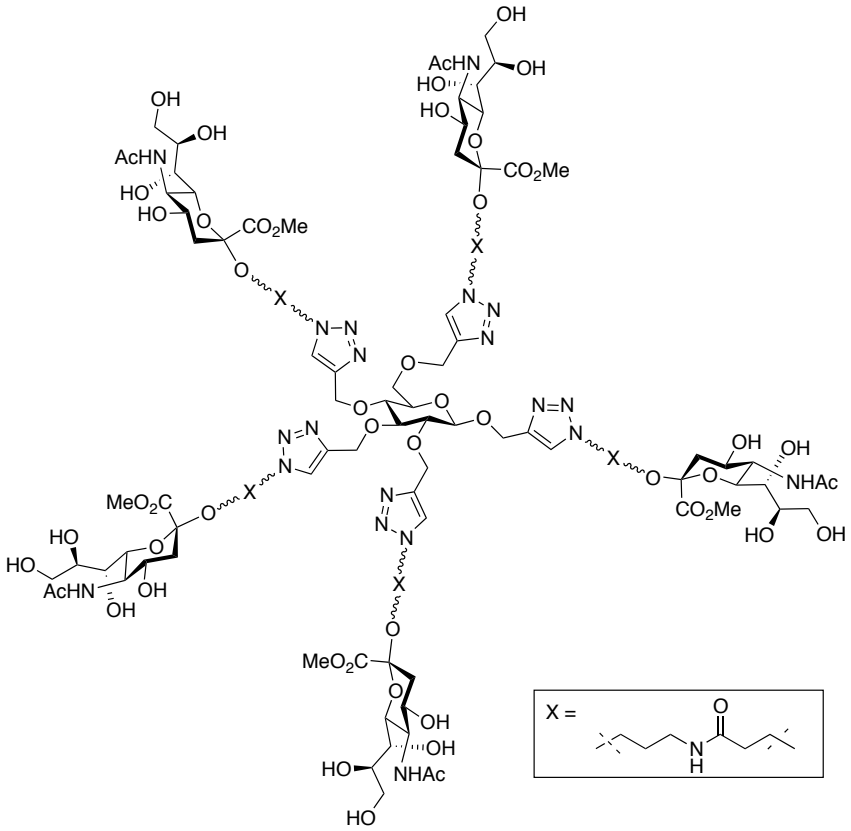
Compound 24

Synthesized according to general procedure for CuAAC, isolated in 73% yield. ^{1}H NMR (400 MHz, CD_3OD): δ 1.21–1.36 (m, 10H), 1.36–1.58 (m, 20H), 1.73 (t, J = 12.2 Hz, 5H), 2.00 (s, 15H), 2.67 (dd, J = 12.7 Hz, 4.6 Hz, 5H), 2.78–2.90 (m, 10H), 3.07–3.18 (m, 10H), 3.26–3.39 (m, overlapped with solvent, 5H), 3.40–3.98 (m, 65H), 4.5 (d, J = 7.9 Hz, 1H), 4.55–4.97 (m, overlapped with solvent, 16H), 7.92 (s, 1H), 7.96 (s, 1H), 7.97 (s, 1H), 7.98 (s, 1H), 8.04 (s, 1H). ^{13}C NMR (150 MHz, CD_3OD): δ 22.73, 24.30, 29.90, 30.28, 37.13, 37.14, 40.34, 41.80, 47.60, 47.68, 53.48, 53.87, 63.18, 64.77, 64.97, 65.26, 66.23, 66.34, 66.90, 67.16, 68.57, 70.00, 70.28, 72.60, 74.93, 75.65, 78.44, 82.92, 85.10, 100.21, 103.52, 125.61, 125.67, 125.97, 145.38, 145.68, 145.76, 146.08, 146.14, 170.29, 175.20. HRMS (ESI-TOF) *m/z*: $[M + 3Na]^+$ Calcd for $(C_{121}H_{197}N_{25}O_{56}Na_3)/3$ 988.4338; Found 988.4332.



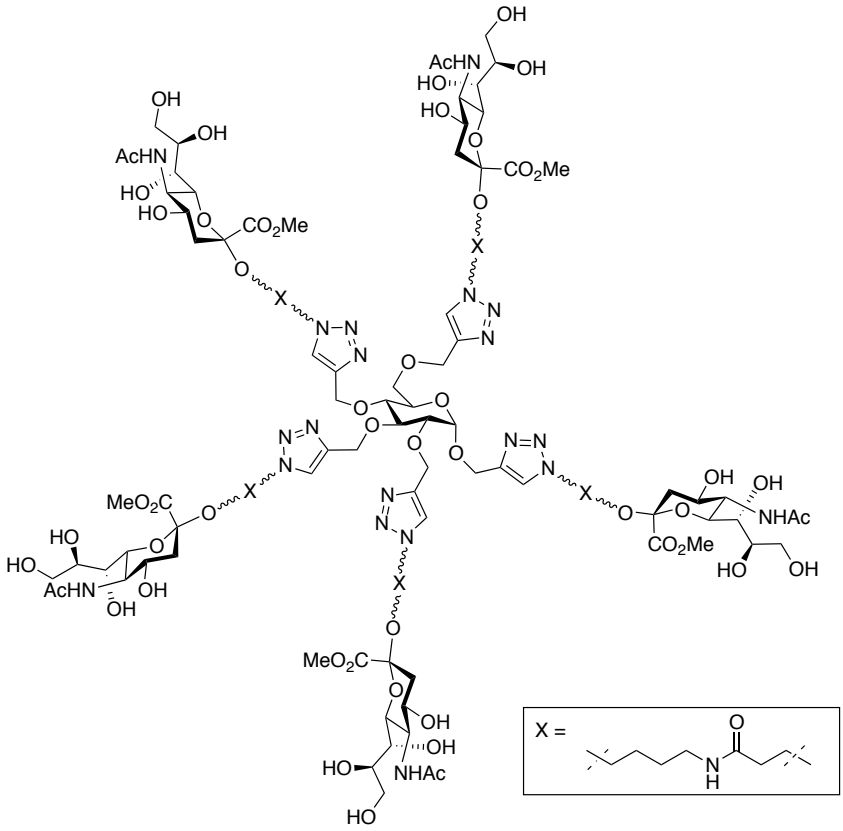
Compound 29

Synthesized according to general procedure for CuAAC, isolated in 27% yield. ^{1}H NMR (600 MHz, CD_3OD): δ 1.59–1.69 (m, 10H), 1.69–1.77 (m, 5H), 2.0 (s, 15H), 2.59–2.68 (m, 5H), 2.77–2.89 (m, 10H), 3.15–3.23 (m, 10H), 3.34–3.40 (m, 5H), 3.43–3.91 (m, 65H), 4.55–4.92 (m, overlapped with solvent, 16h), 5.01 (d, J = 3.3 Hz, 1H), 7.91 (s, 1H), 7.97 (s, 1H), 7.98 (s, 1H), 7.99 (s, 1H), 8.0 (s, 1H). ^{13}C NMR (150 MHz, CD_3OD): δ 21.36, 28.93, 35.76, 35.98, 36.02, 40.09, 46.23, 46.30, 52.11, 52.45, 60.14, 61.07, 61.13, 63.24, 63.43, 63.84, 65.11, 65.68, 67.19, 68.55, 68.79, 70.43, 71.05, 73.42, 77.09, 79.60, 81.15, 95.77, 98.75, 124.23, 124.29, 124.35, 124.39, 124.55, 143.88, 144.32, 144.45, 144.89, 169.63, 170.59, 173.75. HRMS (ESI-TOF) *m/z*: $[M + 3Na]^+$ Calcd for ($C_{111}H_{177}N_{25}O_{56}Na_3$)/3 941.7149; Found 941.7145.



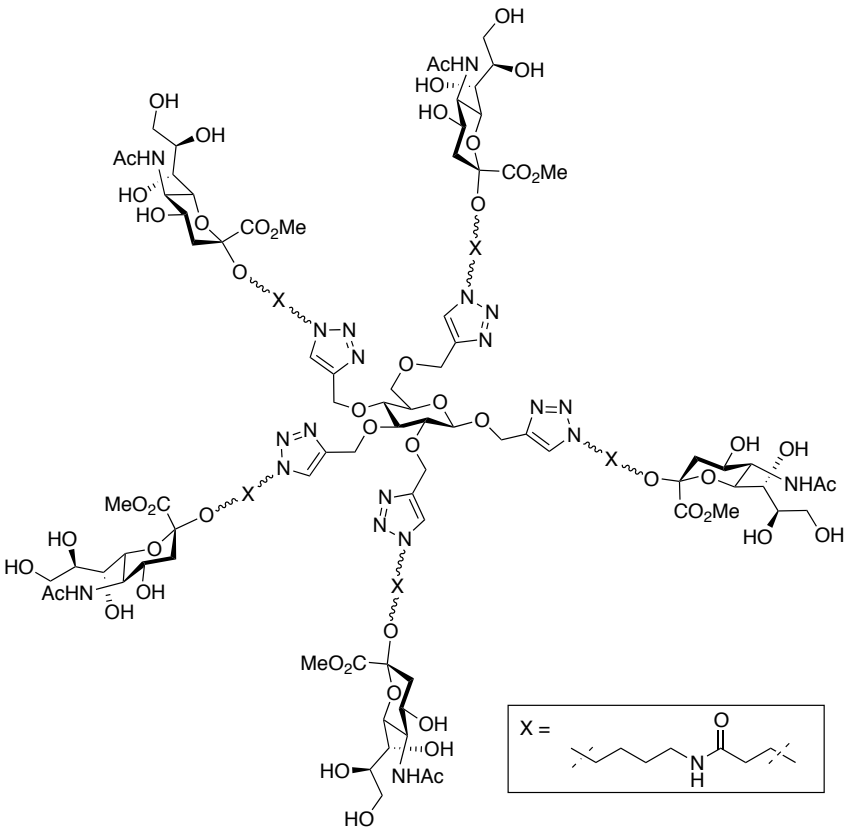
Compound 30

Synthesized according to general procedure for CuAAC, isolated in 36% yield. ^{1}H NMR (600 MHz, CD_3OD): δ 1.58–1.68 (m, 10H), 1.60–1.76 (m, 5H), 2.00 (s, 15H), 2.65 (dd, J = 13.0 Hz, 4.2 Hz, 5H), 2.79–2.89 (m, 10H), 3.17–3.22 (m, 10H), 3.34–3.41 (m, 5H), 3.41–3.91 (m, 65H), 4.54–4.90 (m, overlapped with solvent, 15H), 4.93 (d, J = 12.5 Hz, 1H), 7.92 (s, 1H), 7.95 (s, 1H), 7.97 (s, 1H), 7.98 (s, 1H), 8.04 (s, 1H) ^{13}C NMR (150 MHz, CD_3OD): δ 22.76, 30.34, 37.15, 37.20, 37.42, 41.51, 47.62, 47.71, 53.51, 53.85, 62.47, 62.54, 63.19, 64.83, 65.27, 66.24, 66.35, 67.13, 68.59, 70.01, 70.20, 72.44, 74.83, 75.63, 78.46, 82.93, 85.09, 100.15, 103.52, 125.74, 126.05, 145.41, 145.70, 145.77, 146.08, 146.14, 171.03, 171.98, 172.03, 175.14. HRMS (ESI-TOF) *m/z*: $[M + 3Na]^+$ Calcd for ($C_{111}H_{177}N_{25}O_{56}Na_3$)/3 941.7149; Found 941.7171.



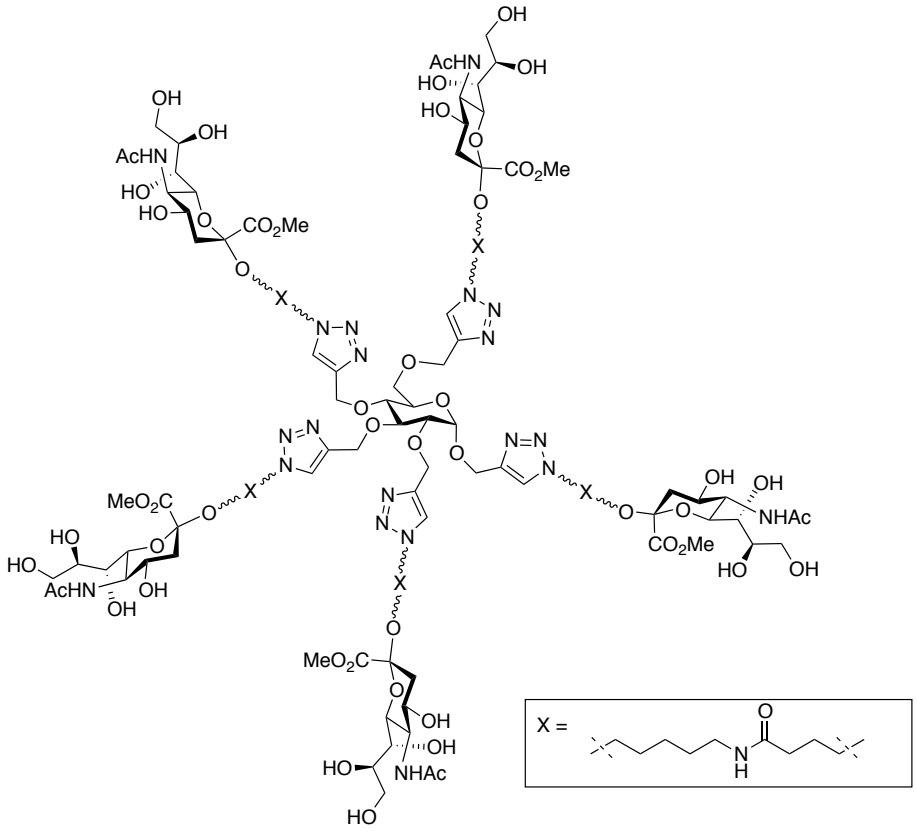
Compound 31

Synthesized according to general procedure for CuAAC, isolated in 48% yield. ^{1}H NMR (600 MHz, CD_3OD): δ 1.41–1.56 (m, 20H), 1.68–1.78 (m, 5H), 2.00 (s, 15H), 2.62–2.70 (m, 5H), 2.78–2.89 (m, 10H), 3.08–3.17 (m, 10H), 3.32–3.37 (m, 5H), 3.44–3.93 (m, 65H), 4.55–4.91 (m, overlapped with solvent, 16H), 5.01 (d, J = 3.3 Hz, 1H), 7.91 (s, 1H), 7.92 (s, 2H), 7.98 (s, 1H), 8.00 (s, 1H). ^{13}C NMR (150 MHz, CD_3OD): δ 22.73, 26.87, 27.88, 27.91, 37.14, 37.17, 39.98, 40.00, 41.78, 47.60, 47.68, 53.50, 53.87, 61.52, 64.63, 64.74, 64.78, 65.23, 65.23, 66.50, 67.08, 68.57, 69.93, 70.26, 71.86, 72.56, 74.93, 78.50, 81.02, 82.50, 97.13, 100.21, 125.55, 125.63, 125.70, 125.71, 125.93, 145.29, 145.72, 145.87, 146.30, 171.17, 171.83, 175.18. HRMS (ESI-TOF) *m/z*: $[M + 3Na]^+$ Calcd for ($C_{116}H_{187}N_{25}O_{56}Na_3$)/3 965.0744; Found 965.0753.



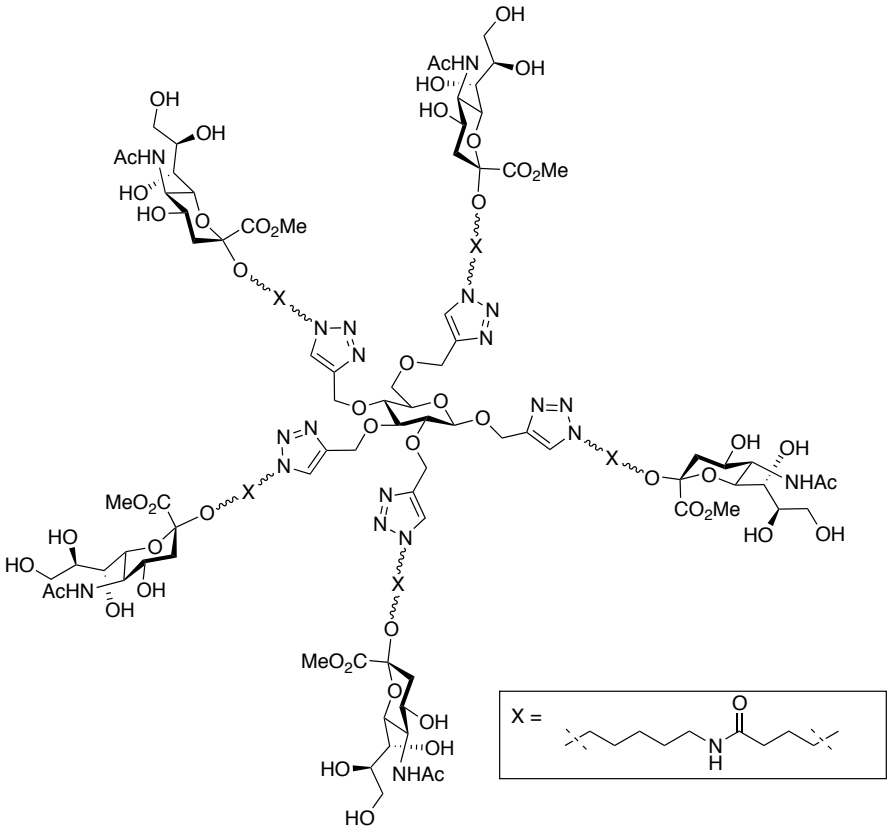
Compound 32

Synthesized according to general procedure for CuAAC, isolated in 34% yield. ^{1}H NMR (600 MHz, CD_3OD): δ 1.42–1.53 (m, 20H), 1.73 (t, J = 12.4 Hz, 5H), 2.00 (s, 15H), 2.66 (dd, J = 12.9 Hz, 4.2 Hz, 5H), 2.79–2.89 (m, 10H), 3.10–3.18 (m, 10H), 3.33–3.40 (m, overlapped with solvent, 5H), 3.41–3.93 (m, 65H), 4.51 (d, J = 7.8 Hz, 1H), 4.56–4.73 (m, 13H), 4.73–4.96 (m, overlapped with solvent, 3H), 7.92 (s, 1H), 7.96 (s, 1H), 7.97 (s, 1H), 7.98 (s, 1H), 8.04 (s, 1H). ^{13}C NMR (150 MHz, CD_3OD): δ 22.74, 26.87, 27.89, 27.92, 37.12, 37.16, 37.18, 39.98, 40.00, 41.78, 47.60, 47.63, 47.68, 53.50, 53.86, 63.20, 64.74, 64.78, 65.25, 66.24, 66.32, 67.16, 68.57, 70.01, 70.27, 72.55, 72.64, 74.93, 75.62, 78.44, 82.90, 85.08, 100.21, 103.53, 125.62, 125.68, 125.71, 125.99, 145.42, 145.70, 145.78, 146.11, 146.16, 171.17, 171.83, 171.86, 175.18. HRMS (ESI-TOF) *m/z*: $[M + 3Na]^+$ Calcd for $(C_{116}H_{187}N_{25}O_{56}Na_3)/3$ 965.0744; Found 965.0757.



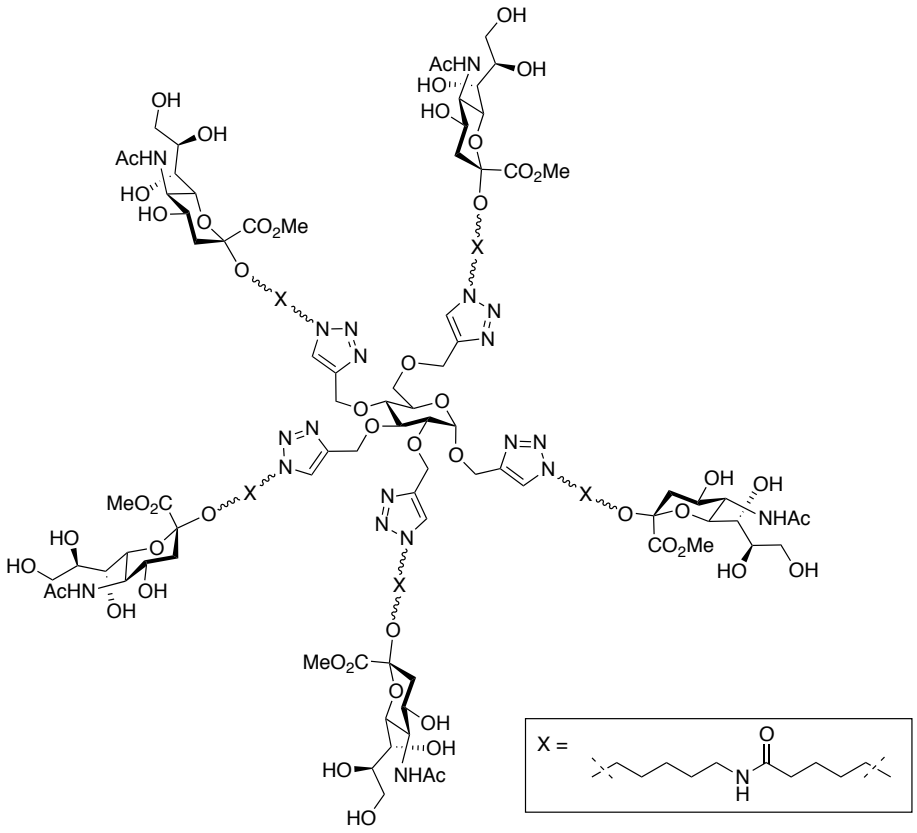
Compound 33

Synthesized according to general procedure for CuAAC, isolated in 41% yield. ^{1}H NMR (600 MHz, D_2O): δ 1.29–1.43 (m, 10H), 1.43–1.58 (m, 20H), 1.72 (t, J= 12.5 Hz, 5H), 2.0 (s, 15H), 2.14–2.26 (m, 20H), 2.66 (dd, J = 12.8 Hz, 4.8 Hz, 5.0H), 3.11–3.20 (m, 10H), 3.32–3.41 (m, overlapped with solvent, 5H), 3.47–3.91 (m, 65H), 4.39–4.51 (m, 10H), 4.56–4.95 (m, overlapped with solvent, 16H), 5.05 (d, J = 3.4 Hz, 1H), 7.99 (s, 1H), 8.04 (s, 1H), 8.05 (s, 1H), 8.06 (s, 1H), 8.06 (s, 1H). ^{13}C NMR (150 MHz, D_2O): δ 22.72, 24.38, 27.49, 29.96, 30.30, 33.57, 40.03, 41.81, 50.74, 50.78, 53.45, 53.88, 61.53, 64.67, 64.76, 64.95, 65.28, 66.55, 67.02, 68.55, 69.95, 70.27, 71.87, 72.61, 74.94, 78.70, 80.97, 82.35, 97.17, 100.19, 125.36, 125.41, 125.50, 125.64, 145.38, 145.87, 145.96, 146.02, 146.39, 171.22, 174.39, 175.22. HRMS (ESI-TOF) *m/z*: $[M + 3Na]^+$ Calcd for $(C_{126}H_{207}N_{25}O_{56}Na_3)/3$ 1011.7932; Found 1011.7931.



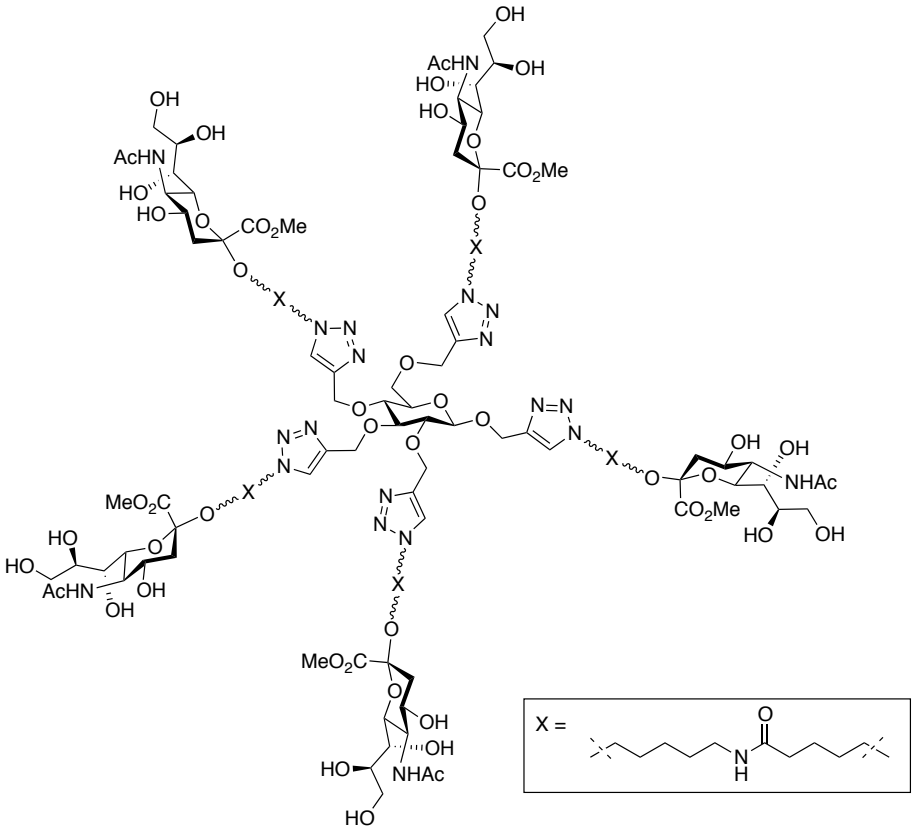
Compound 34

Synthesized according to general procedure for CuAAC, isolated in 62% yield. ^{1}H NMR (600 MHz, D_2O): δ 1.29–1.43 (m, 10H), 1.43–1.59 (m, 20H), 1.72 (t, J = 12.4 Hz, 5H), 2.00 (s, 15H), 2.14–2.26 (m, 20H), 2.66 (dd, J = 12.8 Hz, 4.7 Hz, 5H), 3.11–3.20 (m, 10H), 3.33–3.40 (m, 5H), 3.41–3.93 (m, 65H), 4.39–4.50 (m, 10H), 4.52 (d, J = 7.8 Hz, 1H), 4.56–98 (m, overlapped with solvent, 6H), 7.99 (s, 1H), 8.04 (s, 2H), 8.05 (s, 1H), 8.10 (s, 1H). ^{13}C NMR (150 MHz, D_2O): δ 22.72, 24.38, 27.48, 29.96, 30.30, 33.57, 40.32, 41.82, 50.74, 50.76, 53.44, 53.88, 63.22, 64.76, 64.95, 65.31, 66.21, 66.39, 67.13, 68.55, 70.05, 70.26, 72.61, 74.93, 75.67, 82.81, 84.97, 100.19, 103.54, 125.38, 125.41, 125.45, 125.49, 125.73, 145.55, 145.86, 145.97, 146.21, 146.27, 171.22, 174.29, 175.21. HRMS (ESI-TOF) *m/z*: $[M + 3Na]^+$ Calcd for $(C_{126}H_{207}N_{25}O_{56}Na_3)/3$ 1011.7932; Found 1011.7939.



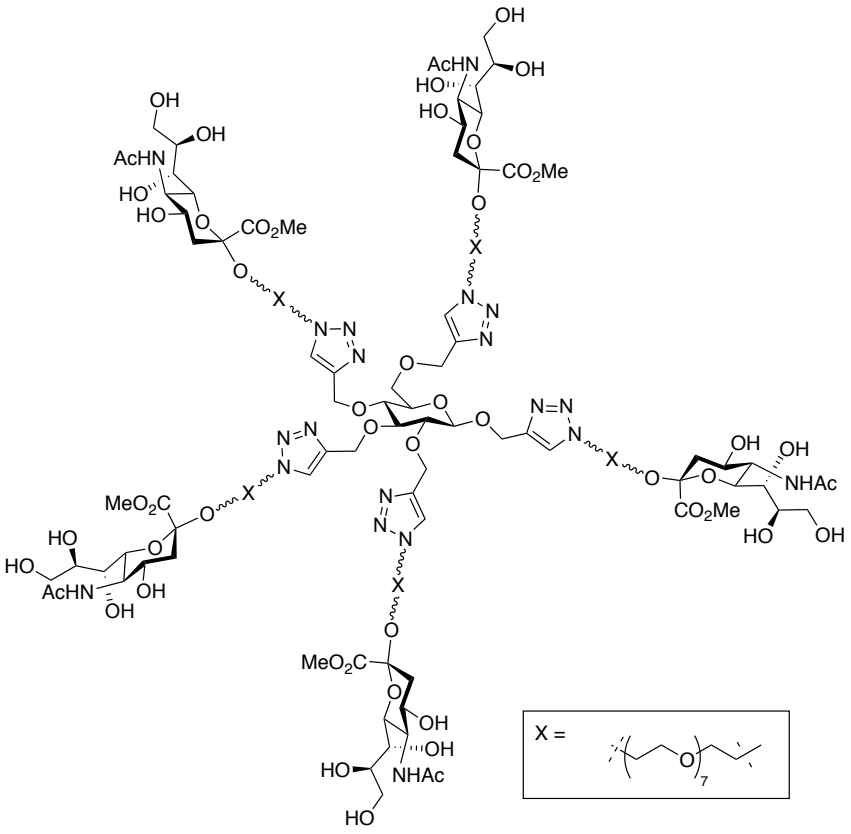
Compound 35

Synthesized according to general procedure for CuAAC, isolated in 46% yield. ^{1}H NMR (600 MHz, D_2O): δ 1.27–1.42 (m, 10H), 1.42–1.67 (m, 30H), 1.72 (t, J = 12.3 Hz, 5H), 1.84–1.95 (m, 10H), 2.0 (s, 15H), 2.16–2.30 (m, 10H), 2.67 (dd, J = 12.9 Hz, 4.7 Hz, 5H), 3.07–3.20 (m, 10H), 3.39–3.32 (m, overlapped with solvent, 5H), 3.44–3.93 (m, 55H), 4.29–4.48 (m, 10H), 4.55–4.94 (m, overlapped with solvent, 16H), 5.04 (d, J = 3.5 Hz, 1H), 7.98 (s, 1H), 8.03 (s, 1H), 8.04 (s, 1H), 8.05 (s, 1H), 8.05 (s, 1H). ^{13}C NMR (150 MHz, D_2O): δ 22.72, 23.93, 24.38, 29.99, 30.32, 30.81, 36.28, 40.28, 41.83, 50.97, 51.00, 51.03, 53.44, 53.90, 61.57, 64.66, 64.76, 64.93, 65.31, 66.56, 67.06, 68.54, 69.72, 70.27, 71.88, 72.61, 74.94, 78.50, 81.02, 82.40, 97.15, 100.19, 125.29, 125.38, 125.53, 145.36, 145.85, 145.97, 146.01, 146.40, 171.22, 175.22, 175.28. HRMS (ESI-TOF) *m/z*: $[M + 3Na]^+$ Calcd for $(C_{131}H_{217}N_{25}O_{56}Na_3)/3$ 1035.1526; Found 1035.1534.



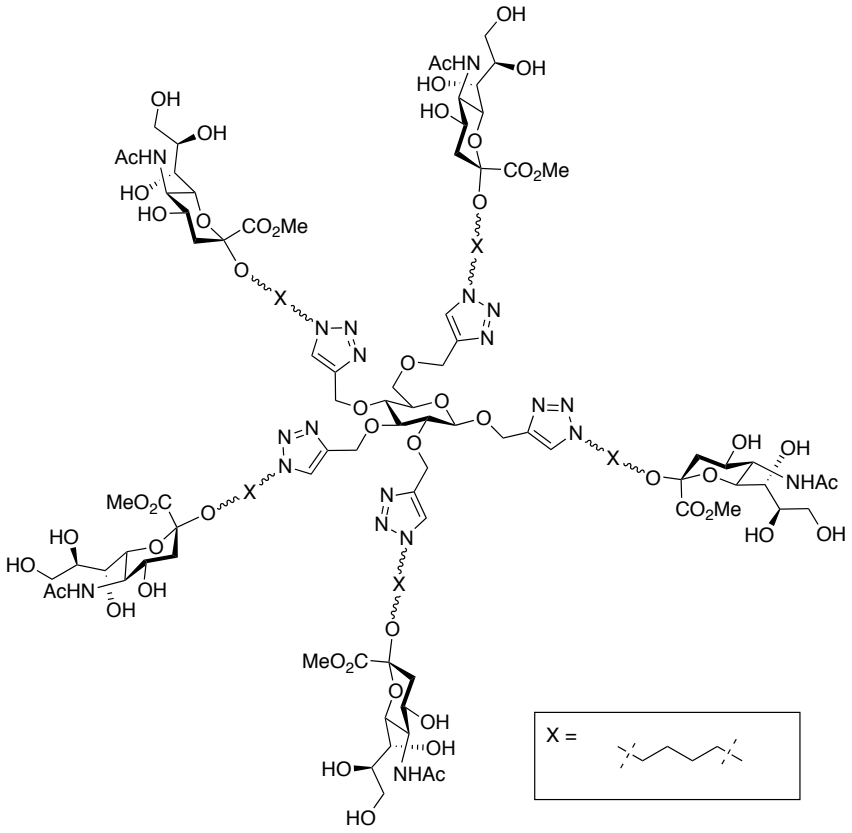
Compound 36

Synthesized according to general procedure for CuAAC. 45% yield. ^{1}H NMR (600 MHz, D_2O): δ 1.27–1.41 (m, 10H), 1.43–1.56 (m, 20H), 1.56–1.65 (m, 10H), 1.72 (t, J = 12.4 Hz, 5H), 1.86–1.95 (m, 10H), 2.00 (s, 15H), 2.17–2.27 (m, 10H), 2.67 (dd, J = 12.9 Hz, 4.6 Hz, 5H), 3.10–3.18 (m, 10H), 3.33–3.38 (m, overlapped with solvent, 5H), 3.41–3.90 (m, 65H), 4.36–4.46 (m, 10H), 4.51 (d, J = 7.7 Hz, 1H), 4.56–4.96 (m, overlapped with solvent, 5H), 7.99 (s, 1H), 8.03 (s, 2H), 8.04 (s, 1H), 8.10 (s, 1H). ^{13}C NMR (150 MHz, D_2O): δ 22.72, 23.91, 24.38, 29.99, 30.30, 30.80, 36.27, 40.28, 41.83, 50.98, 51.03, 54.80, 63.23, 64.76, 64.93, 65.33, 67.14, 68.54, 70.07, 70.26, 72.60, 74.93, 75.67, 78.46, 82.84, 84.95, 100.18, 103.53, 125.29, 125.37, 125.40, 125.64, 145.53, 145.84, 145.94, 146.21, 146.26, 171.22, 175.23, 175.27. HRMS (ESI-TOF) *m/z*: $[M + 3Na]^+$ Calcd for ($C_{131}H_{217}N_{25}O_{56}Na_3$)/3 1035.1526; Found 1035.1539.



Compound 37

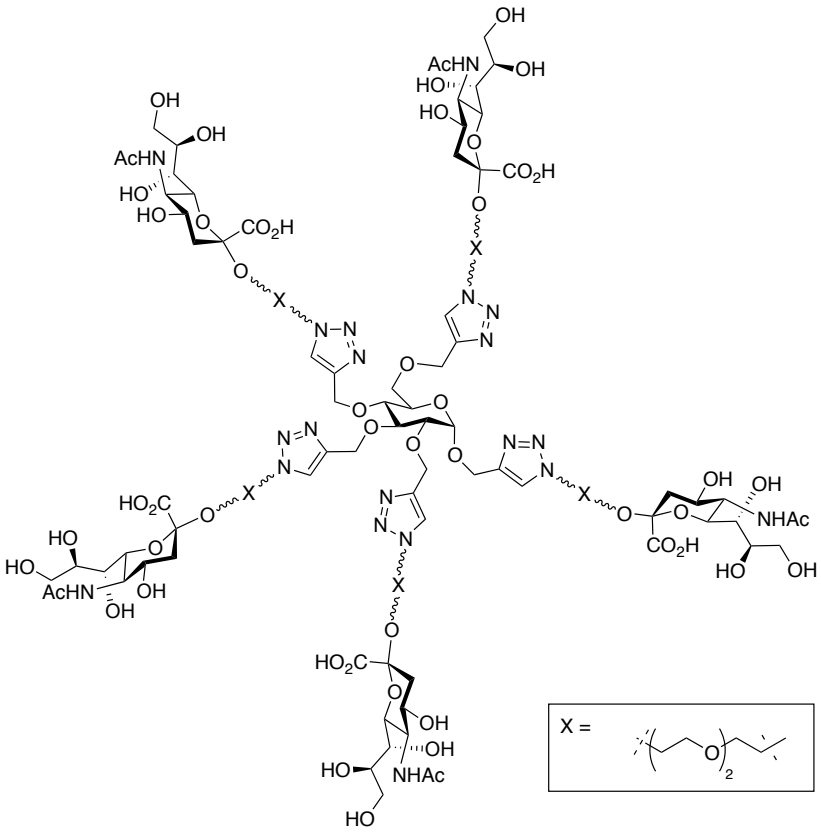
Synthesized according to general procedure for CuAAC, isolated in 43% yield. ^{1}H NMR (600 MHz, D_2O): δ 1.75 (t, J = 12.3 Hz, 5H), 2.00 (s, 15H), 2.69 (dd, J = 12.8 Hz, 4.6 Hz, 5H), 3.25–4.08 (m, overlapped with solvent, 210 H) 4.50–5.00 (m, overlapped with solvent, 17H), 8.02 (s, 1H), 8.06 (s, 1H), 8.06 (s, 1H), 8.08 (s, 1H), 8.14 (s, 1H). ^{13}C NMR (150 MHz, D_2O): δ 22.74, 41.68, 51.40, 51.45, 51.47, 53.23, 53.52, 53.82, 63.25, 63.92, 64.71, 64.78, 65.28, 65.40, 66.28, 66.41, 67.17, 67.64, 68.56, 70.16, 70.23, 71.20, 71.27, 71.46, 71.56, 72.49, 74.95, 75.74, 78.46, 82.89, 85.14, 99.97, 100.21, 103.55, 126.11, 126.44, 145.33, 145.71, 145.74, 146.03, 146.10, 170.90, 175.14. HRMS (ESI-TOF) *m/z*: $[M + 3Na]^+$ Calcd for $(C_{161}H_{282}N_{20}O_{86}Na_3)/3$ 1313.5995; Found 1313.5990.



Compound 38

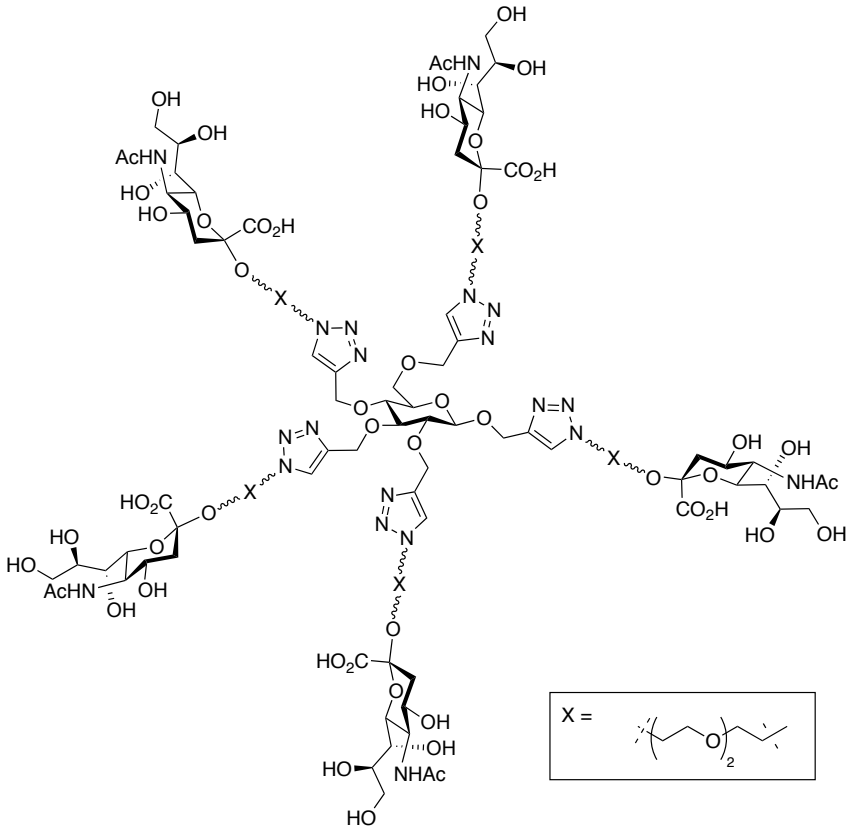
Synthesized according to general procedure for CuAAC, isolated in 58% yield. ^{1}H NMR (600 MHz, D_2O): δ 1.41–1.57 (m, 10H), 1.73 (t, J = 12.3 Hz, 5H), 1.90–2.02 (m, 10H), 2.00 (s, 15H), 2.66 (dd, J = 12.8 Hz, 4.4 Hz, 5H), 3.37–3.88 (m, 70H), 4.36–4.46 (m, 10H), 4.51 (d, J = 7.8 Hz, 1H), 4.60–4.70 (m, 3H), 4.73–4.97 (m, overlapped with solvent, 3H), 7.98 (s, 1H), 8.03 (s, 1H), 8.03 (s, 1H), 8.04 (s, 1H), 8.09 (s, 1H). ^{13}C NMR (150 MHz, D_2O): δ 22.72, 27.50, 28.16, 41.73, 50.99, 51.03, 53.51, 53.85, 63.24, 64.42, 64.77, 65.33, 66.22, 66.37, 67.11, 68.54, 70.07, 70.23, 72.50, 74.96, 75.66, 78.41, 82.79, 84.91, 100.19, 103.54, 125.32, 125.40, 125.66, 145.54, 145.82, 145.91, 146.20, 146.23, 171.10, 175.19. HRMS (ESI-TOF) *m/z*: $[M + H + Na]^+$ Calcd for $(C_{101}H_{163}N_{20}O_{51}Na)/2$ 1247.5331; Found 1247.5355.

Synthesis of final pentavalent sialic acid conjugates



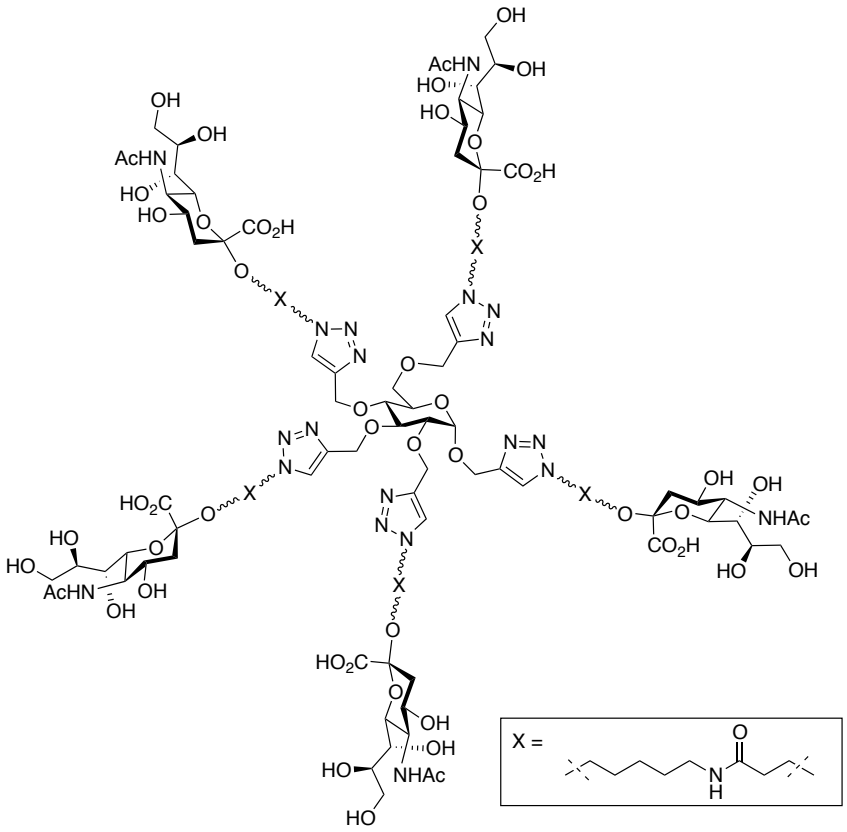
Compound 25 (ME0753)

Synthesized according to general procedure for ester hydrolysis, isolated in 86% yield. ^{1}H NMR (400 MHz, D_2O): δ 1.57 (t, J = 12.2 Hz, 5H), 1.94 (s, 15H), 2.64 (dd, J = 12.4 Hz, 4.6 Hz, 5H), 3.38–3.97 (m, 90H), 4.38–4.83 (m, overlapped with solvent, 16H), 4.95 (d, J = 3.8 Hz, 1H), 7.88 (s, 1H), 7.94 (s, 1H), 7.96 (s, 1H), 8.00 (s, 1H), 8.01 (s, 1H). ^{13}C NMR (600 MHz, D_2O): δ 22.02, 40.25, 49.93, 50.00, 51.89, 60.10, 62.60, 63.08, 63.13, 63.19, 64.78, 65.27, 67.35, 68.21, 68.25, 69.39, 69.54, 69.57, 71.70, 72.56, 76.33, 78.92, 80.41, 95.43, 100.49, 125.21, 125.35, 125.48, 125.67, 125.69, 143.45, 143.48, 143.70, 143.82, 144.04, 173.40, 175.02. HRMS (ESI-TOF) *m/z*: $[M + H + Na]^+$ Calcd for $(C_{106}H_{173}N_{20}O_{61}Na)/2$ 1362.5468; Found 1362.5458.



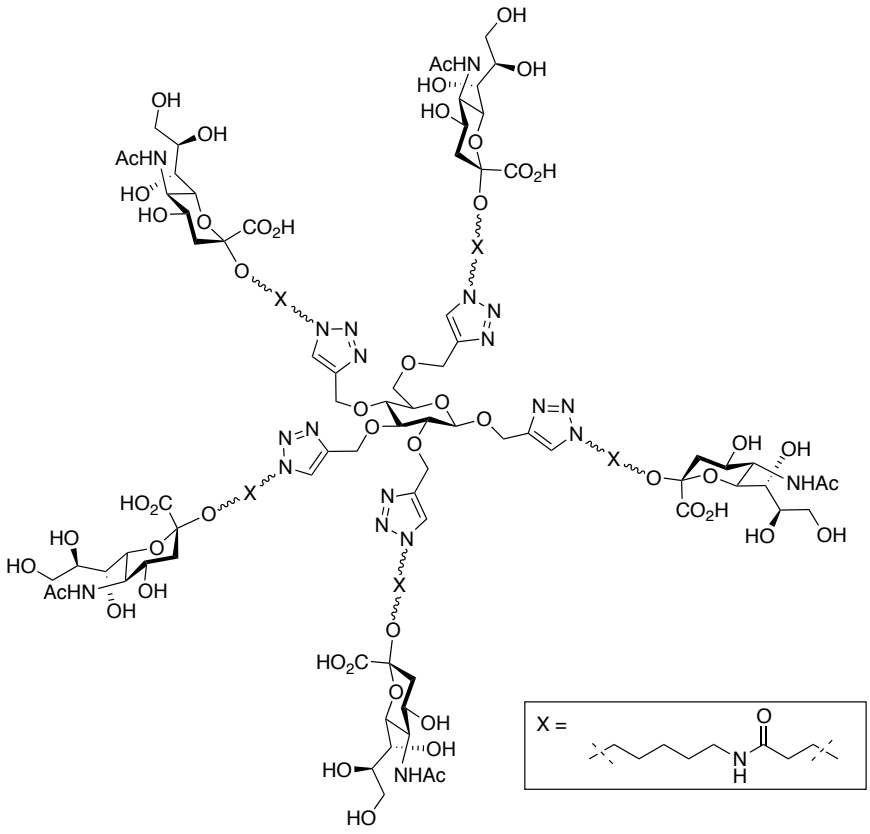
Compound 26 (ME0742)

Synthesized according to general procedure for ester hydrolysis, isolated in quantitative yield. ^{1}H NMR (600 MHz, D_2O): δ 1.67 (t, J = 12.2 Hz, 5H), 2.04 (s, 15H), 2.73 (dd, J = 12.3 Hz, 4.6 Hz, 5H), 3.28–4.04 (m, 90H), 4.44–5.06 (m, 17H), 7.98 (s, 1H), 8.00 (s, 1H), 8.02 (s, 1H), 8.10 (s, 1H), 8.11 (s, 1H). ^{13}C NMR (150 MHz, D_2O): δ 22.02, 40.25, 49.91, 49.95, 51.89, 62.23, 62.60, 63.19, 64.65, 64.71, 65.33, 67.71, 68.21, 68.25, 68.79, 69.39, 69.56, 71.70, 72.56, 73.34, 76.60, 80.76, 82.80, 100.49, 101.61, 125.22, 125.33, 125.37, 125.64, 125.68, 143.57, 143.63, 143.68, 143.96, 173.40, 175.02**.** HRMS (ESI-TOF) *m/z*: $[M + H + Na]^+$ Calcd for $(C_{106}H_{173}N_{20}O_{61}Na)/2$ 1362.5468; Found 1362.5475.



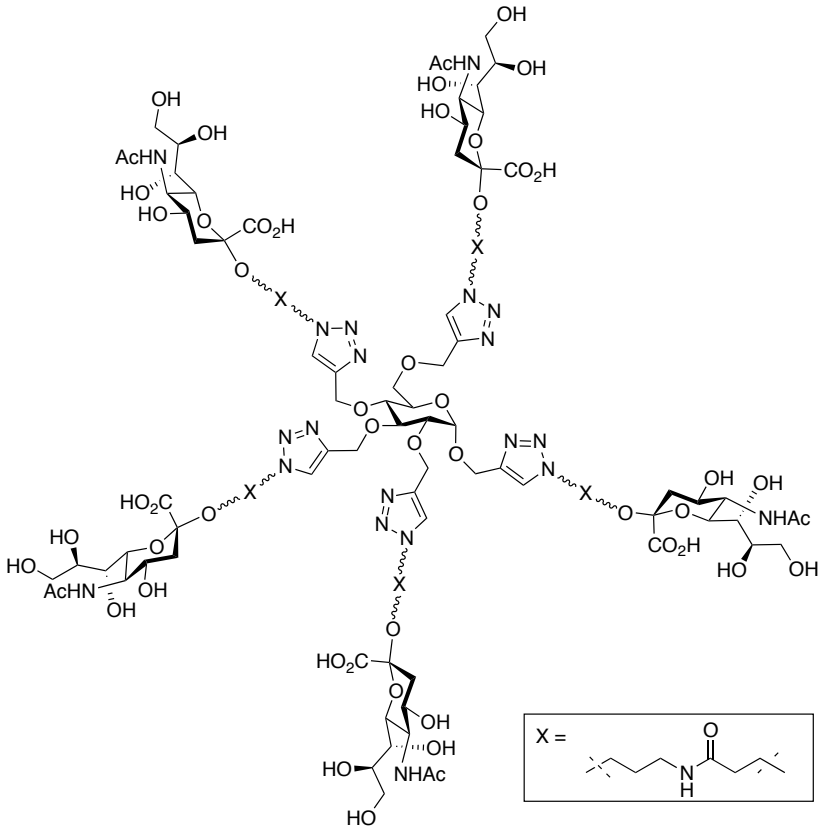
Compound 27 (ME0741)

Synthesized according to general procedure for ester hydrolysis, isolated in quantitative yield. ^{1}H NMR (600 MHz, D_2O): δ 1.13–1.27 (m, 10H), 1.30–1.43 (m, 10H), 1.43–1.54 (m, 10H), 1.63 (t, J = 12.2 Hz, 5H), 2.04 (s, 15H), 2.73 (dd, J = 12.3 Hz, 4.7 Hz, 5H), 2.79–2.91 (m, 10H), 2.97–3.12 (m, 10H), 3.35–3.45 (m, 5H), 3.46–3.93 (m, 50H), 4.45–4.89 (m, 16H), 5.02 (d, J = 3.4 Hz, 1H), 7.89 (s, 1H), 7.95 (s, 1H), 7.97 (s, 1H), 8.01 (s, 1H), 8.02 (s, 1H). ^{13}C NMR (150 MHz, D_2O): δ 22.02, 24.41, 22.45, 27.82, 28.52, 36.03, 36.07, 36.12, 39.18, 40.48, 46.62, 46.74, 51.91, 60.00, 62.57, 63.05, 63.10, 64.58, 64.76, 65.28, 67.43, 68.21, 68.31, 69.55, 71.77, 72.55, 76.34, 78.84, 80.48, 95.34, 100.63, 124.94, 125.02, 125.17, 125.30, 125.37, 143.36, 143.54, 143.61, 143.72, 143.96, 171.74, 173.59, 175.03. HRMS (ESI-TOF) *m/z*: $[M + H + Na]^+$ Calcd for $(C_{116}H_{188}N_{25}O_{56}Na)/2$ 1425.1259; Found 1425.1257.



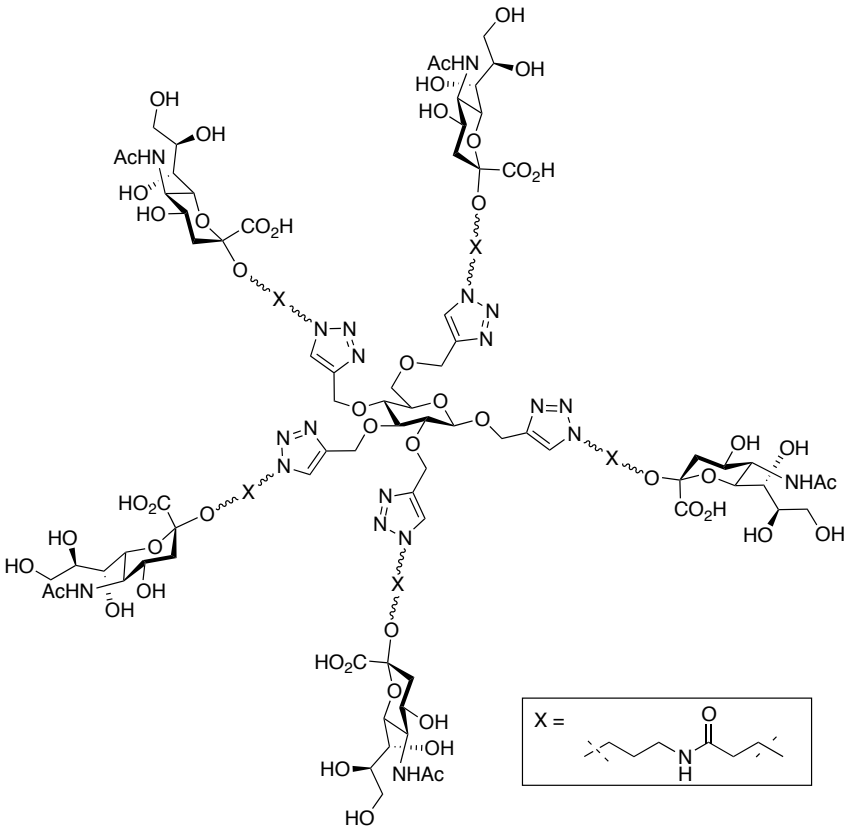
Compound 28 (ME0752)

Synthesized according to general procedure for ester hydrolysis, isolated in quantitative yield.^{1}H NMR (600 MHz, D_2O): δ 1.13–1.26 (m, 10H), 1.30–1.41 (m, 10H), 1.43–1.53 (m, 10H), 1.63 (t, J = 12.2 Hz, 5H), 2.04 (s, 15H), 2.73 (dd, J = 12.4 Hz, 4.3 Hz, 5H), 2.77–2.90 (m, 10H), 2.97–3.11 (m, 10H), 3.31–3.93 (m, 55H), 4.49–4.99 (m, overlapped with solvent, 17H), 7.90 (s, 1H), 7.92 (s, 1H), 7.93 (s, 1H), 8.02 (s, 1H), 8.03 (s, 1H). ^{13}C NMR (150 MHz, D_2O): δ 22.02, 22.40, 22.43, 27.62, 28.52, 36.04, 36.10, 36.16, 39.18, 40.48, 46.63, 46.65, 46.75, 51.92, 62.13, 62.57, 63.19, 64.57, 64.66, 65.33, 67.78, 68.21, 68.31, 71.77, 72.55, 73.35, 76.54, 80.73, 82.82, 100.63, 101.58, 124.96, 125.01, 125.09, 125.28, 125.36, 143.48, 143.58, 143.61, 143.85, 171.75, 171.81, 173.59, 175.03. HRMS (ESI-TOF) *m/z*: $[M + H + Na]^+$ Calcd for $(C_{116}H_{188}N_{25}O_{56}Na)/2$ 1425.1229; Found 1425.1257.



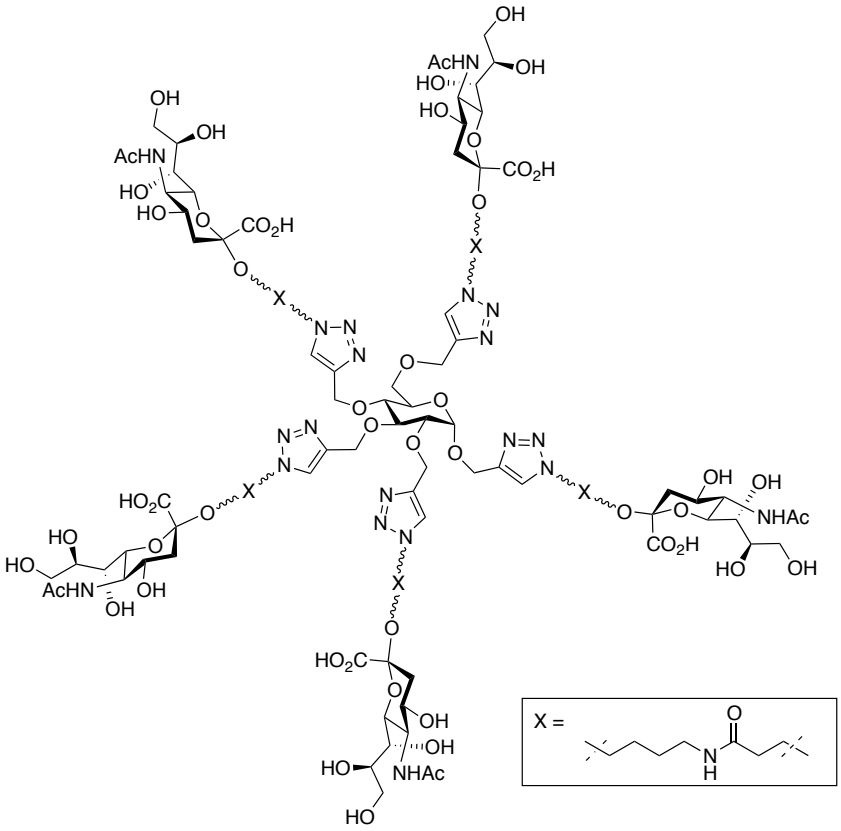
Compound 39 (ME0969)

Synthesized according to general procedure for ester hydrolysis, isolated in quantitative yield. ^{1}H NMR (600 MHz, D_2O): δ 1.54–1.71 (m, 15H), 2.00 (s, 15H), 2.67–2.75 (m, 5H), 2.81–2.91 (m, 10H), 3.06–3.23 (m, 10H), 3.32–3.93 (m, 55H), 4.51 (d, J = 12.4 Hz, 1H), 4.57 (d, J = 12.7 Hz, 1H), 4.60–4.86 (m, overlapped with solvent, 14H), 5.01 (d, J = 3.4 Hz, 1H), 7.90 (s, 1H), 7.95 (s, 1H), 7.97 (s, 1H), 8.01 (s, 1H), 8.02 (s, 1H). ^{13}C NMR (150 MHz, D_2O): δ 22.02, 28.48, 36.00, 36.03, 36.09, 36.43, 36.50, 40.30, 46.55, 46.67, 51.90, 60.00, 62.02, 62.08, 62.59, 62.68, 63.08, 64.76, 65.27, 67.32, 68.20, 68.28, 69.51, 71.75, 72.55, 76.30, 78.87, 80.44, 95.36, 100.56, 124.94, 125.06, 125.18, 125.33, 125.40, 143.35, 143.52, 143.57, 143.62, 143.70, 143.90, 166.86, 171.89, 171.91, 171.95, 173.62, 175.02. HRMS (ESI-TOF) *m/z*: $[M + H + Na]^+$ Calcd for $(C_{106}H_{168}N_{25}O_{56}Na)/2$ 1355.0477; Found 1355.0482.



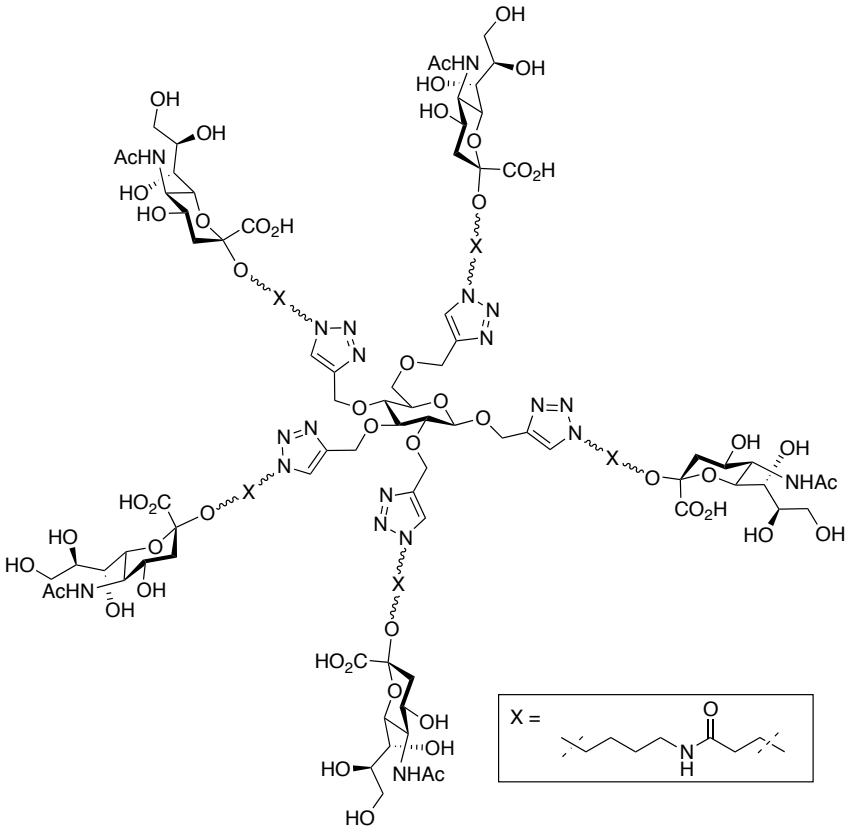
Compound 40 (ME0970)

Synthesized according to general procedure for ester hydrolysis, isolated in 97% yield. ^{1}H NMR (600 MHz, D_2O): δ 1.57–1.70 (m, 15H), 2.04 (s, 15H), 2.67–2.75 (m, 5H), 2.80–2.91 (m, 10H), 3.09–3.23 (m, 10H), 3.32–3.91 (m, 55H), 4.53 (d, J = 12.2 Hz, 1H), 4.58–4.84 (m, 15H), 4.96 (d, J = 12.7 Hz, 1H), 7.90 (s, 1H), 7.92 (s, 1H), 7.94 (s, 1H), 8.02 (s, 1H), 8.03 (s, 1H). ^{13}C NMR (150 MHz, D_2O): δ 22.02, 28.48, 36.02, 36.07, 36.12, 36.42, 36.45, 40.30, 46.54, 46.57, 46.67, 51.90, 62.02, 62.05, 62.17, 62.59, 63.15, 64.60, 64.70, 65.32, 67.72, 68.20, 68.29, 71.75, 72.55, 73.30, 76.58, 80.72, 82.79, 100.56, 101.57, 124.94, 125.05, 125.10, 125.32, 125.39, 143.46, 143.57, 143.82, 171.88, 171.91, 171.95, 173.62, 175.01. HRMS (ESI-TOF) *m/z*: $[M + H + Na]^+$ Calcd for ($C_{106}H_{168}N_{25}O_{56}Na$)/2 1355.0477; Found 1355.0448.



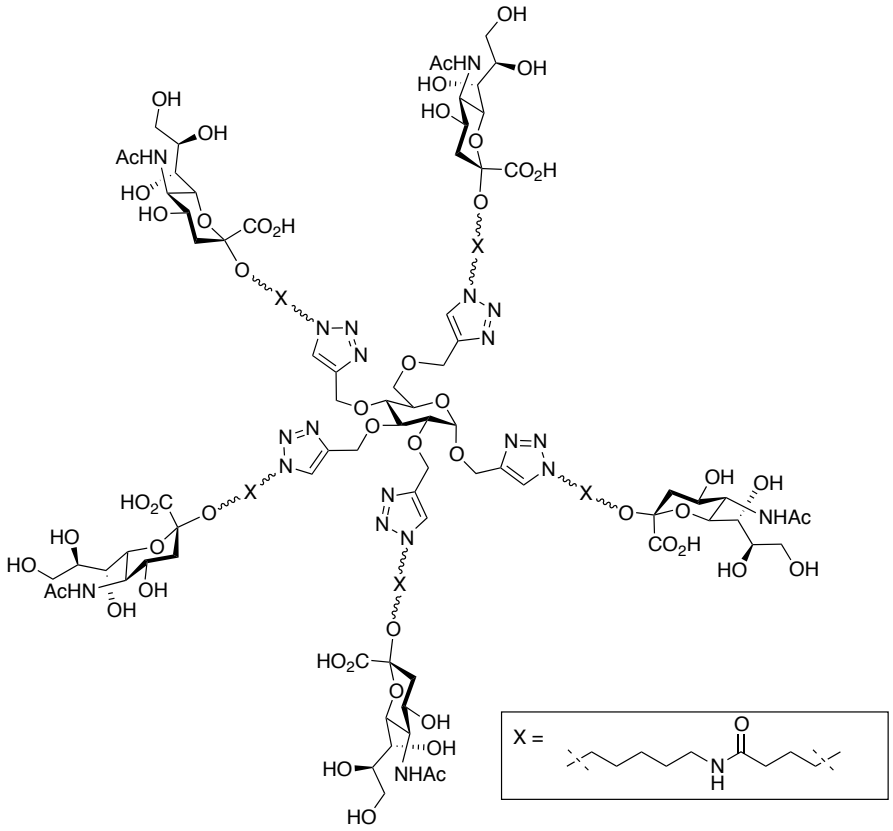
Compound 41 (ME0971)

Synthesized according to general procedure for ester hydrolysis, isolated in quantitative yield. ^{1}H NMR (600 MHz, D_2O): δ 1.28–1.49 (m, 20H), 1.63 (t, J = 12.1 Hz, 5H), 2.03 (s, 15H), 2.68–2.76 (m, 5H), 2.79–2.90 (m, 10H), 3.03–3.15 (m, 10H), 3.37–3.93 (m, 55H), 4.46–4.91 (m, overlapped with solvent, 16H), 5.01 (d, J = 3.4 Hz, 1H), 7.90 (s, 1H), 7.95 (s, 1H), 7.97 (s, 1H), 8.01 (s, 1H), 8.02 (s, 1H). ^{13}C NMR (150 MHz, D_2O): δ 22.02, 24.83, 26.24, 35.99, 36.02, 36.08, 40.43, 46.58, 46.70, 51.91, 60.01, 62.57, 63.07, 64.20, 64.75, 65.26, 67.38, 68.20, 68.30, 69.54, 71.74, 72.55, 76.30, 78.86, 80.43, 95.34, 100.60, 124.92, 125.03, 125.17, 125.33, 125.39, 143.36, 143.53, 143.61, 143.71, 143.94, 171.84, 171.89, 173.59, 175.03. HRMS (ESI-TOF) *m/z*: $[M + 2H]^+$ Calcd for $(C_{111}H_{179}N_{25}O_{56})/2$ 1379.0958; Found 1379.0923.



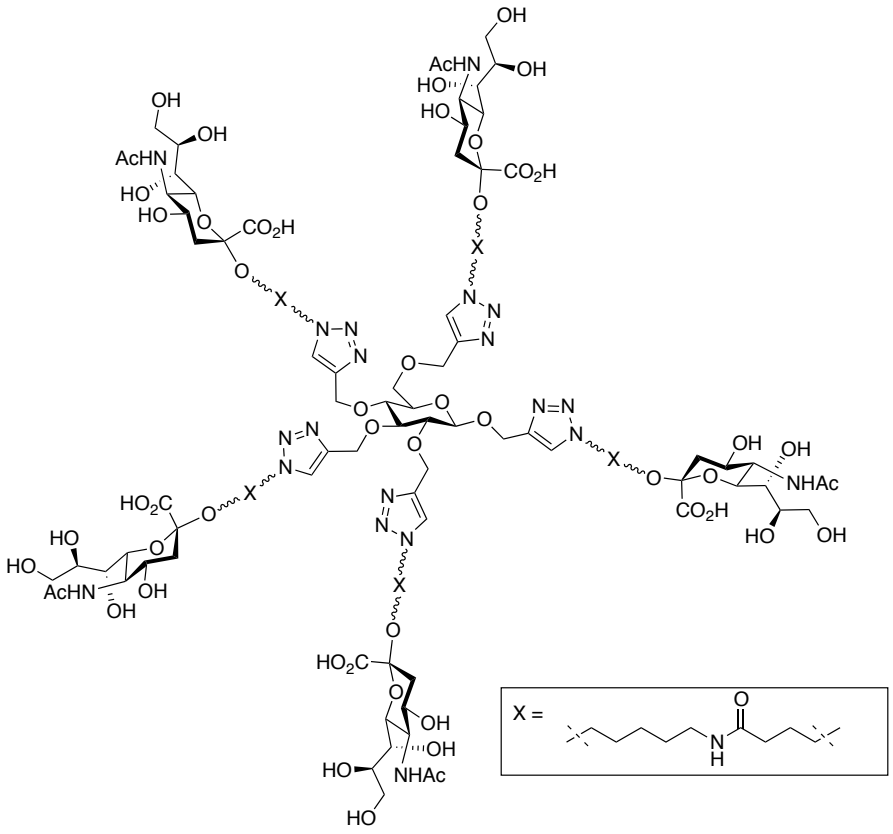
Compound 42 (ME0972)

Synthesized according to general procedure for ester hydrolysis, isolated in 91% yield. ^{1}H NMR (600 MHz, D_2O): δ 1.36–1.49 (m, 20H), 1.63 (t, J = 11.9 Hz, 5H), 2.04 (s, 15H), 2.70–2.76 (m, 5H), 2.80–2.89 (m, 10H), 3.03–3.14 (m, 10H), 3.31–3.93 (m, 55H), 4.53 (d, J = 12.2 Hz, 1H), 4.56–4.87 (m, overlapped with solvent, 15H), 4.96 (d, J = 12.5 Hz, 1H), 7.09 (s, 1H), 7.92 (s, 1H), 7.94 (s, 1H), 8.02 (s, 1H), 8.03 (s, 1H). ^{13}C NMR (150 MHz, D_2O): δ 22.02, 24.83, 26.24, 26.27, 36.01, 36.06, 36.12, 38.96, 38.98, 40.44, 46.59, 46.71, 51.91, 62.17, 62.57, 63.15, 64.20, 64.59, 64.66, 65.33, 67.73, 68.20, 68.31, 71.74, 72.55, 73.32, 76.55, 80.71, 82.80, 100.60, 101.58, 124.93, 125.01, 125.09, 125.31, 125.37, 143.49, 143.59, 143.85, 171.83, 171.85, 171.89, 173.59, 175.02. HRMS (ESI-TOF) *m/z*: $[M + H + Na]^+$ Calcd for $(C_{111}H_{178}N_{25}O_{56}Na)/2$ 1390.0868; Found 1390.0858.



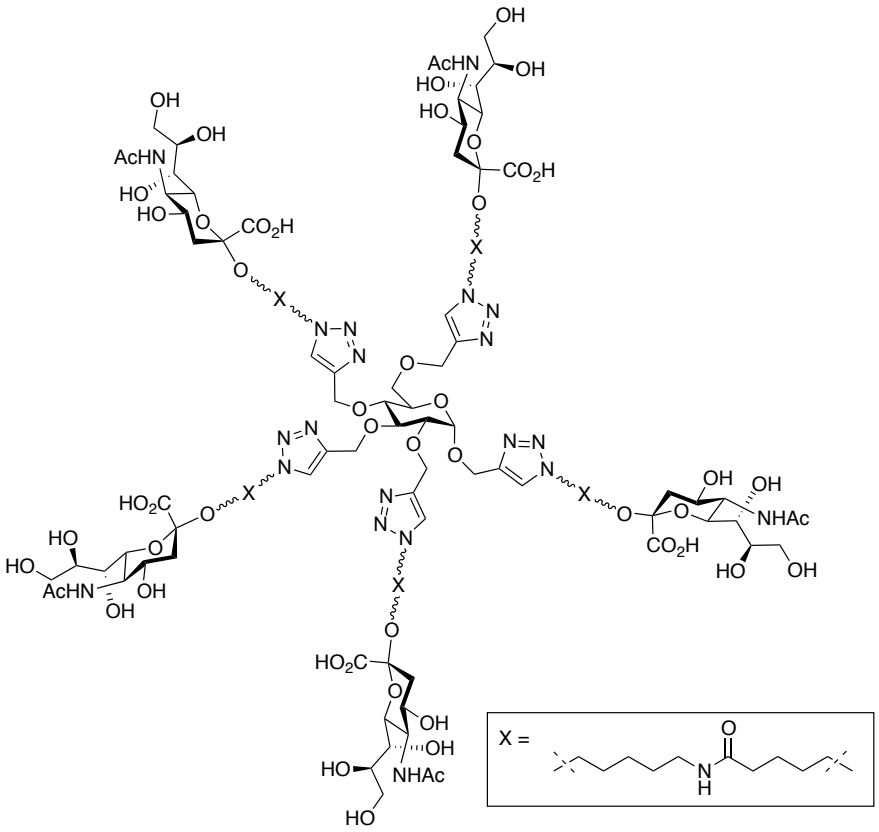
Compound 43 (ME0973)

Synthesized according to general procedure for ester hydrolysis, isolated in 97% yield. ^{1}H NMR (600 MHz, D_2O): δ 1.34–1.37 (m, 10H), 1.38–1.49 (m, 10H), 1.50–1.58 (m, 10H), 1.62 (t, J = 12.2 Hz, 5H), 2.03 (s, 15H), 2.09–2.26 (m, 20H), 2.73 (dd, J = 12.5 Hz, 4.7 Hz, 5H), 3.04–3.14 (m, 10H), 3.37–3.93 (m, 55H), 4.33–4.52 (m, 11H), 4.57 (d, J = 12.8 Hz, 1H), 4.61–4.89 (m, overlapped with solvent, 4H), 5.07 (d J = 3.5 Hz, 1H), 7.91 (s, 1H), 7.97 (s, 1H), 7.99 (s, 1H), 8.04 (s, 1H), 8.05 (s, 1H). ^{13}C NMR (150 MHz, D_2O): δ 22.01, 22.55, 25.67, 25.71, 25.78, 27.91, 28.58, 32.41, 32.47, 39.26, 40.49, 49.59, 49.63, 49.67, 49.70, 51.92, 60.12, 62.56, 63.08, 63.16, 64.63, 64.84, 65.26, 67.33, 68.20, 68.29, 69.57, 71.77, 72.56, 76.34, 78.88, 80.22, 95.45, 100.63, 124.73, 124.97, 125.03, 125.25, 143.46, 143.59, 143.68, 143.81, 143.97, 173.61, 174.49, 174.52, 174.54, 175.04. HRMS (ESI-TOF) *m/z*: $[M + 2H]^+$ Calcd for $(C_{121}H_{199}N_{25}O_{56})/2$ 1449.1741; Found 1449.1720.



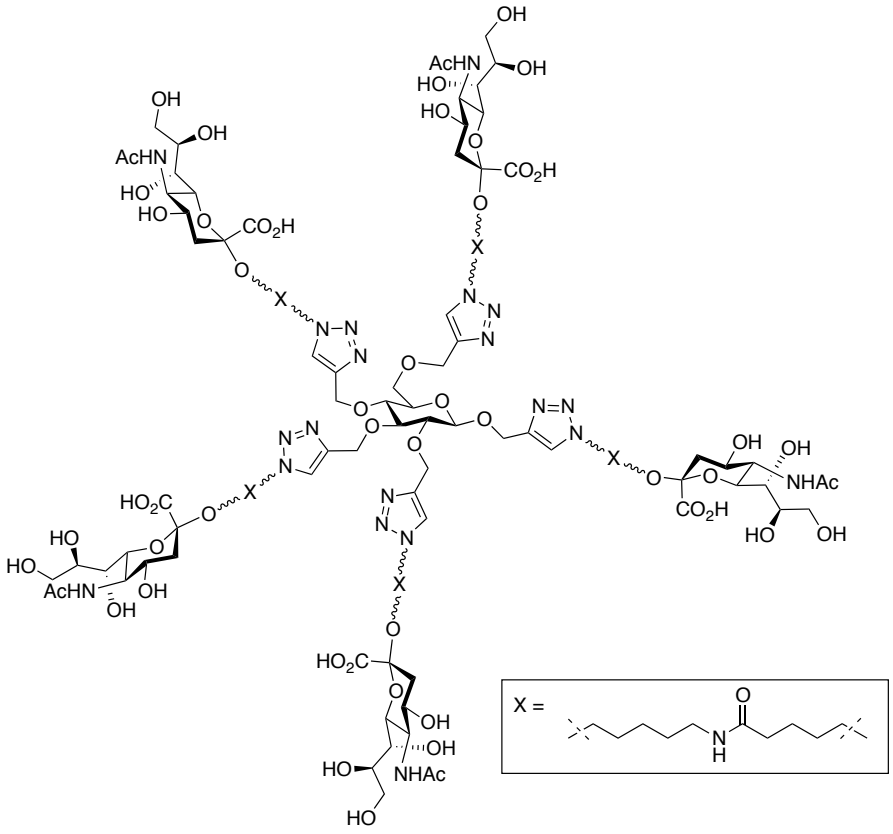
Compound 44 (ME0974)

Synthesized according to general procedure for ester hydrolysis, isolated in quantitative yield. ^{1}H NMR (600 MHz, D_2O): δ 1.25–1.36 (m, 10H), 1.38–1.49 (m, 10H), 1.49–1.58 (m, 10H), 1.63 (t, J = 12.1 Hz, 5H), 2.03 (s, 15H), 2.10–2.26 (m, 20H), 2.73 (dd, J = 12.4 Hz, 4.7 Hz, 5H), 3.03–3.14 (m, 10H), 3.28–3.94 (m, 55H), 4.32–4.49 (m, 10H), 4.52 (d, J = 12.3 Hz, 1H), 4.58–4.88 (m, overlapped with solvent, 5H), 4.96 (d, J = 12.6 Hz, 1H), 7.92 (s, 1H), 7.95 (s, 1H), 7.96 (s, 1H), 8.05 (s, 1H), 8.06 (s, 1H). ^{13}C NMR (150 MHz, D_2O): δ 22.01, 22.55, 25.71, 25.79, 27.90, 27.93, 28.58, 32.47, 39.27, 40.48, 49.59, 49.63, 49.67, 51.91, 62.29, 62.56, 63.16, 65.36, 67.68, 68.20, 68.29, 71.77, 72.56, 73.35, 76.59, 80.69, 82.71, 100.63, 101.63, 124.73, 124.90, 124.96, 125.22, 125.27, 143.58, 143.66, 143.67, 143.92, 173.61, 174.49, 174.52, 175.04. HRMS (ESI-TOF) *m/z*: $[M + 2H]^+$ Calcd for $(C_{121}H_{199}N_{25}O_{56})/2$ 1449.1741; Found 1449.1727.



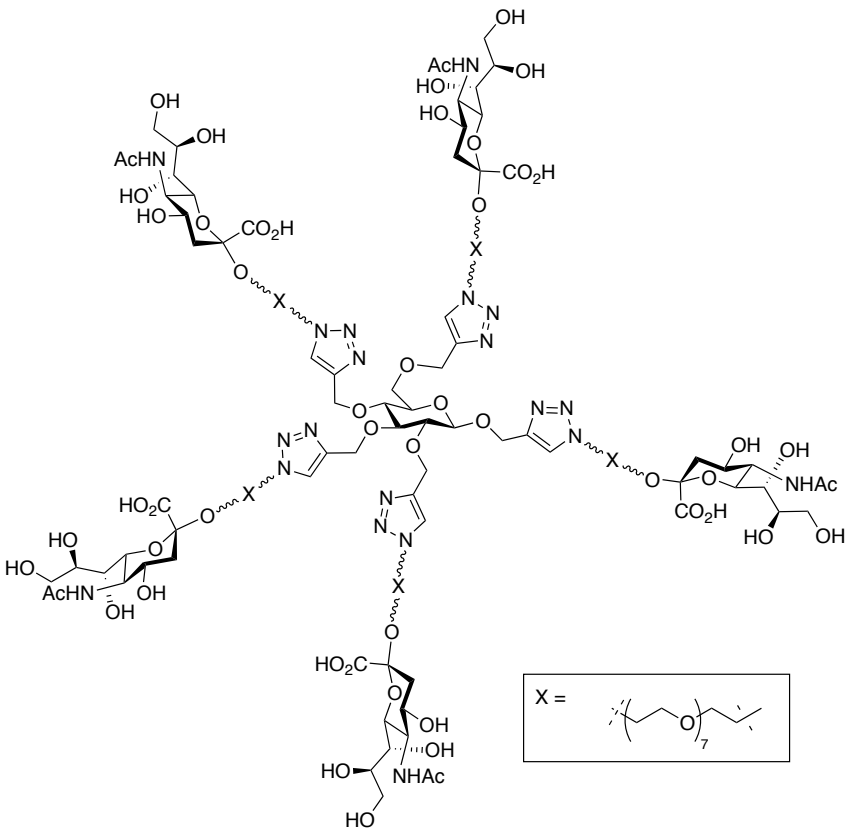
Compound 45 (ME0975)

Synthesized according to general procedure for ester hydrolysis, isolated in quantitative yield. ^{1}H NMR (600 MHz, D_2O): δ 1.26–1.35 (m, 10H), 1.40–1.58 (m, 30H), 1.63 (t, J = 12.1 Hz, 5H), 1.76–1.94 (m, 10H), 2.03 (s, 15H), 2.16–2.28 (m, 10H), 2.73 (dd, J = 12.3 Hz, 4.7 Hz, 5H), 3.03–3.17 (m, 10H), 3.36–3.94 (m, 55H), 4.32–4.48 (m, 11H), 4.56 (d, J = 13.1 Hz, 1H), 4.63 (d, J = 12.4 Hz, 1H), 4.65–4.86 (m, overlapped with solvent, 3H), 5.05 (d, J = 3.3 Hz, 1H), 7.89 (s, 1H), 7.94 (s, 1H), 7.98 (s, 1H), 8.03 (s, 1H), 8.05 (s, 1H). ^{13}C NMR (150 MHz, D_2O): δ 22.01, 22.28, 22.54, 27.95, 28.57, 28.81, 28.84, 28.91, 34.95, 34.98, 39.18, 40.95, 50.01, 51.93, 60.16, 62.56, 64.61, 64.80, 65.30, 67.28, 68.20, 68.29, 69.58, 71.77, 72.56, 76.28, 78.91, 80.25, 95.39, 100.63, 124.62, 124.80, 124.90, 125.13, 125.15, 143.47, 143.57, 143.68, 143.83, 144.03, 173.60, 175.04, 175.67, 175.71, 175.72. HRMS (ESI-TOF) *m/z*: [M + H + Na]$^{+}$ Calcd for ($C_{126}H_{208}N_{25}O_{56}Na$)/2 1495.2042; Found 1495.2037.



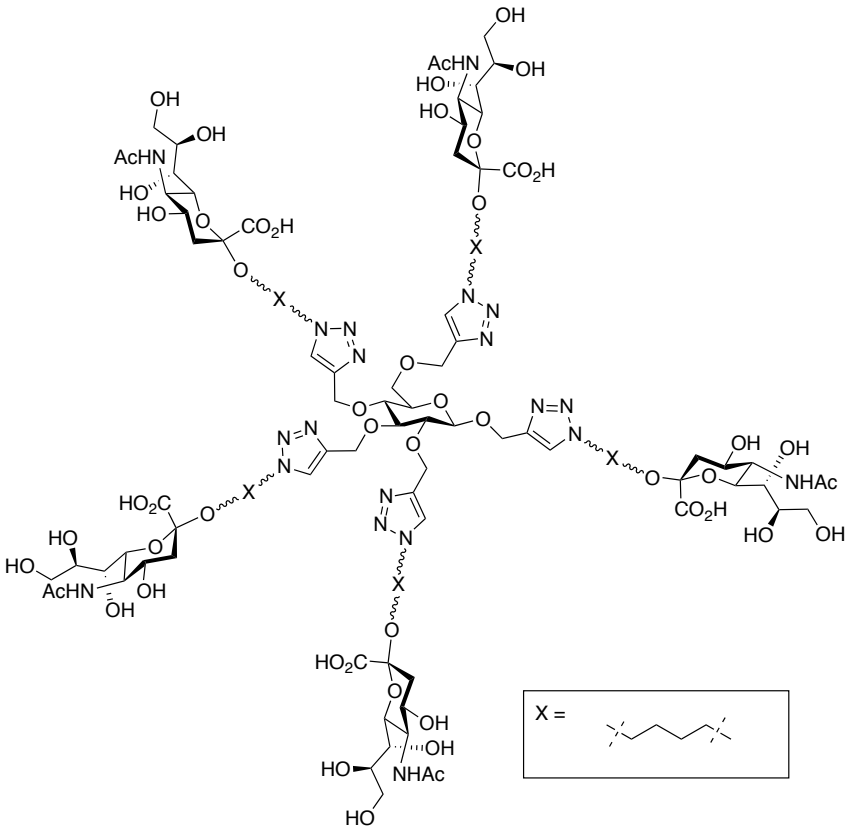
Compound 46 (ME0976)

Synthesized according to general procedure for ester hydrolysis, isolated in 91% yield. ^{1}H NMR (600 MHz, D_2O): δ 1.26–1.37 (m, 10H), 1.39–1.59 (m, 30H), 1.63 (t, J = 12.1 Hz, 5H), 1.78–1.93 (m, 10H), 2.04 (s, 15H), 2.16–2.28 (m, 10H), 2.74 (dd, J = 12.4 Hz, 4.7 Hz, 5H), 3.07–3.17 (m, 10H), 3.36–3.92 (m, 55H), 4.31–4.46 (m, 10H), 4.5 (d, J = 12.5 Hz, 1H), 4.56–4.85 (m, overlapped with solvent, 5H), 4.96 (d, J = 12.8 Hz, 1H), 7.91 (s, 1H), 7.94 (s, 1H), 7.94 (s, 1H), 8.05 (s, 1H), 8.05 (s, 1H). ^{13}C NMR (150 MHz, D_2O): δ 22.01, 22.28, 22.55, 27.95, 28.58, 28.83, 28.91, 34.97, 39.19, 40.50, 49.90, 49.95, 49.99, 51.92, 62.31, 62.56, 63.14, 64.61, 64.71, 65.38, 67.63, 68.20, 68.30, 71.77, 72.60, 73.35, 76.54, 80.74, 82.71, 100.63, 101.65, 124.65, 124.76, 124.82, 125.11, 125.16, 143.60, 143.65, 143.66, 143.97, 173.60, 175.04, 175.70, 175.71, 175.73. HRMS (ESI-TOF) *m/z*: $[M + H + Na]^+$ Calcd for $(C_{126}H_{208}N_{25}O_{56}Na)/2$ 1495.2042; Found 1495.2048.



Compound 47 (ME1058)

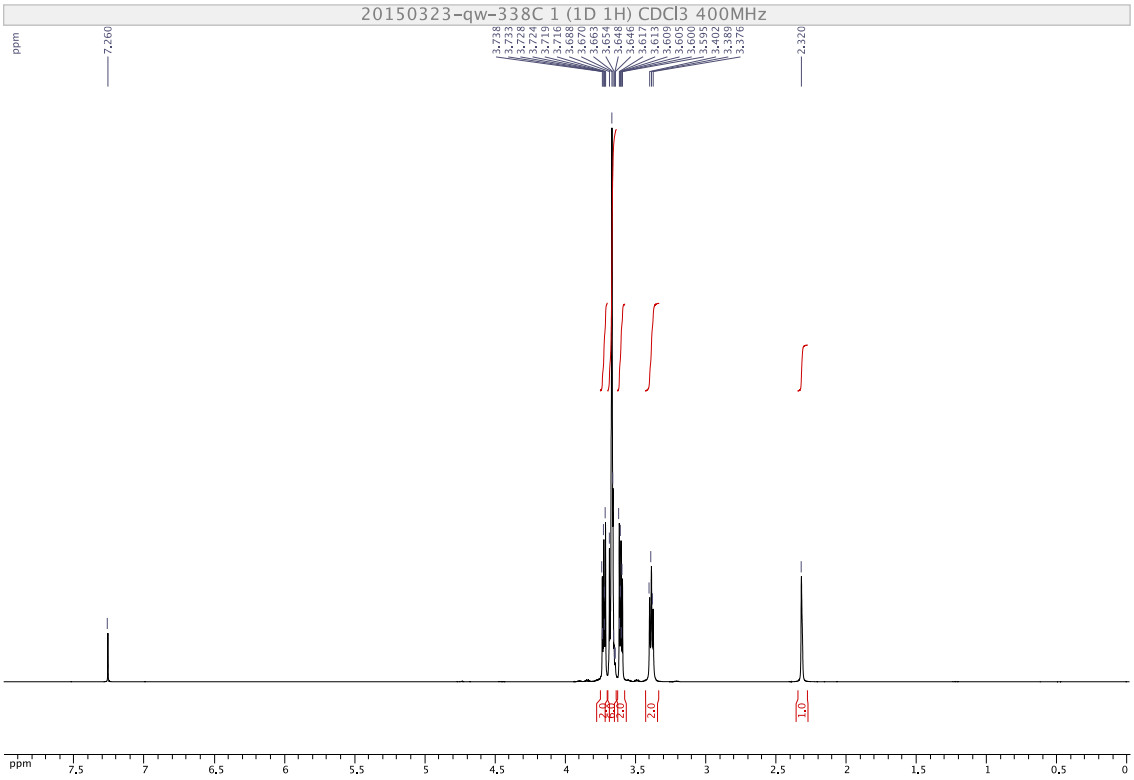
Synthesized according to general procedure for ester hydrolysis, isolated in quantitative yield. 10:1 ratio of alpha/beta anomer. ^{1}H NMR (600 MHz, D_2O): δ 1.68 (t, J = 12.2 Hz, 5H), 2.04 (s, 15H), 2.74 (dd, J = 12.5 Hz, 4.8 Hz, 5H), 3.31–4.14 (m, 195H), 4.47–5.03 (m, overlapped with solvent, 17H), 8.00 (s, 1H), 8.03 (s, 1H), 8.04 (s, 1H), 8.11 (s, 1H), 8.13 (s, 1H). ^{13}C NMR (150 MHz, D_2O): δ 22.04, 40.30, 50.00, 50.04, 61.66, 62.18, 62.62, 63.23, 63.52, 64.64, 64.74, 65.38, 66.94, 67.85, 68.24, 66.33, 68.75, 69.49, 69.56, 69.63, 69.77, 69.98, 70.20, 71.72, 72.58, 73.44, 76.66, 80.20, 82.90, 100.10, 100.52, 101.65, 125.27, 125.35, 125.41, 125.61, 125.70, 143.58, 143.70, 143.98, 144.01, 168.35, 171.06, 173.44, 175.04. HRMS (ESI-TOF) *m/z*: $[M + 2H + Na]^+$ Calcd for $(C_{156}H_{274}N_{20}O_{86}Na)/3$ 1275.5870; Found 1275.5886.



Compound 48 (ME1057)

Synthesized according to general procedure for ester hydrolysis, isolated in 62% yield. ^{1}H NMR (600 MHz, D_2O): δ 1.38–1.57 (m, 10H), 1.66–1.78 (m, 5H), 1.81–2.09 (m, 10H), 2.04 (s, 15H), 2.58–2.74 (m, 5H), 3.30–3.93 (m, 55H), 4.27–5.01 (m, 17H), 7.90 (s, 1H), 7.95 (s, 2H), 8.06 (s, 2H). ^{13}C NMR (150 MHz, D_2O): δ 22.04, 25.78, 26.01, 26.03, 26.16, 26.20, 26.26, 28.20, 28.24, 38.65, 39.59, 50.10, 51.81, 52.06, 60.81, 63.03, 63.16, 63.82, 64.61, 65.25, 66.73, 57.66, 67.76, 68.10, 68.23, 70.14, 70.39, 70.78, 71.16, 72.67, 72.77, 73.34, 76.48, 80.58, 80.66, 82.59, 95.34, 99.44, 101.60, 101.64, 124.68, 124.89, 125.22, 143.48, 143.77, 172.01, 172.04, 173.48, 174.82, 175.00. HRMS (ESI-TOF) *m/z*: $[M + H + Na]^+$ Calcd for $(C_{96}H_{153}N_{20}O_{51}Na)/2$ 1212.4940; Found 1212.4931.

Spacer 3



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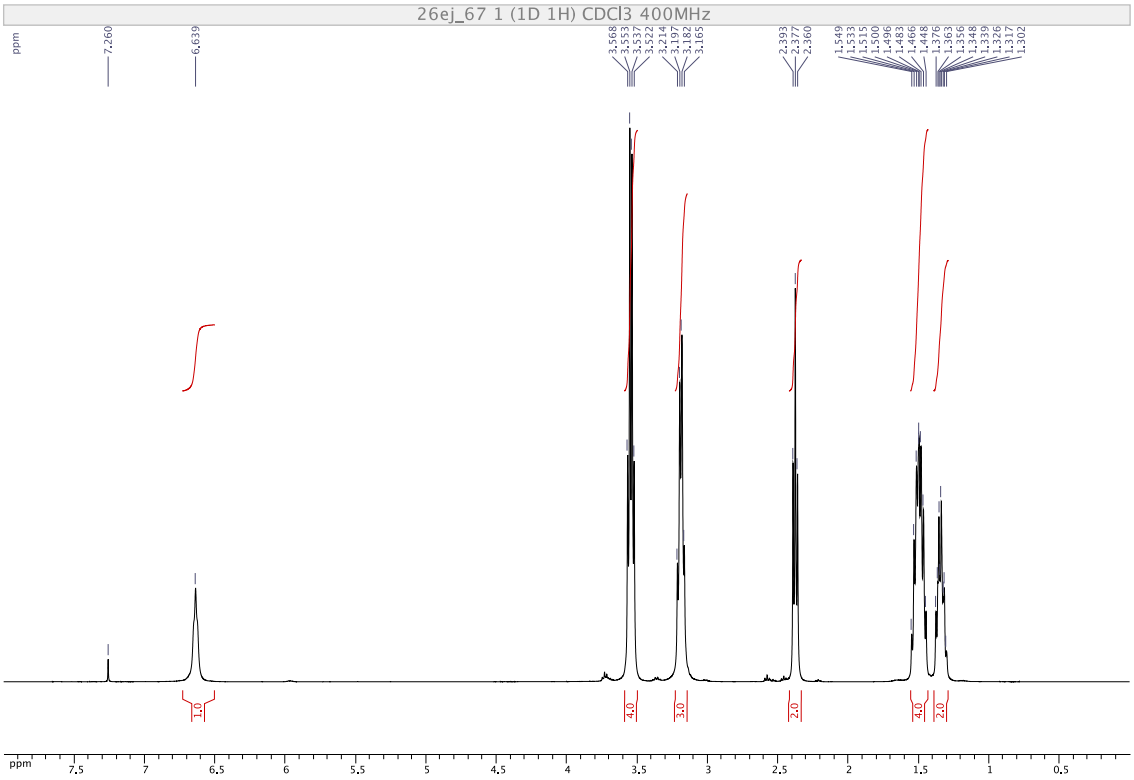

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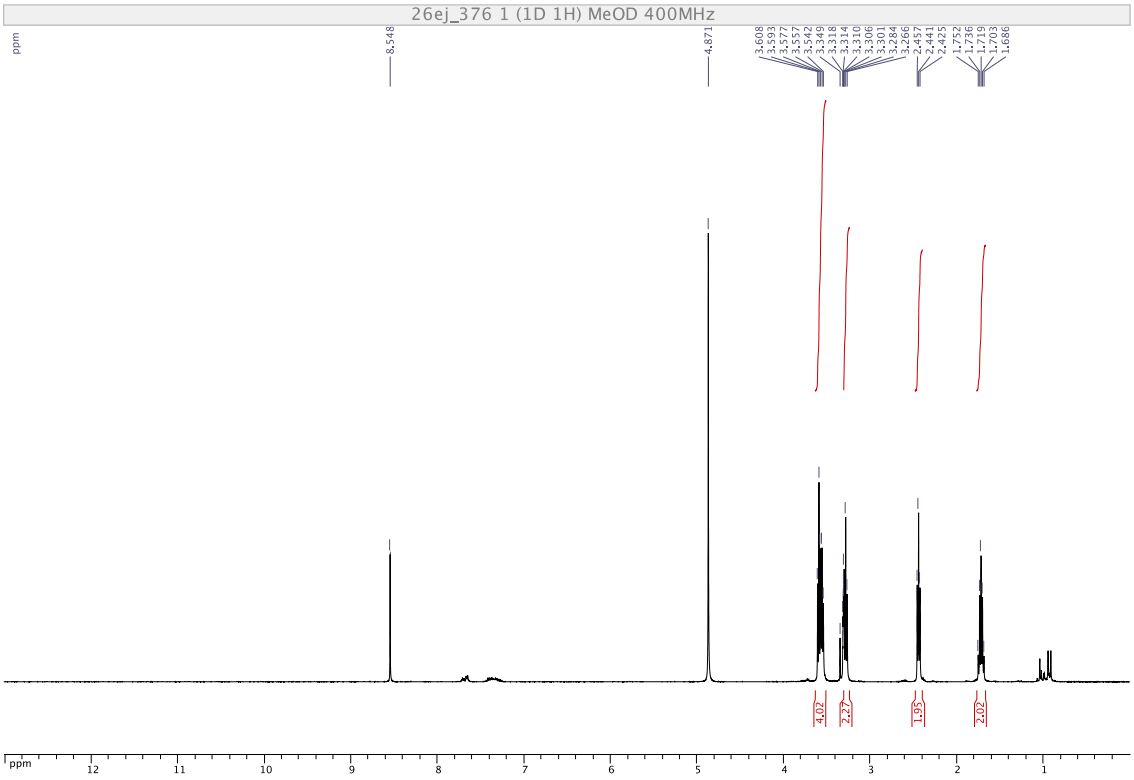

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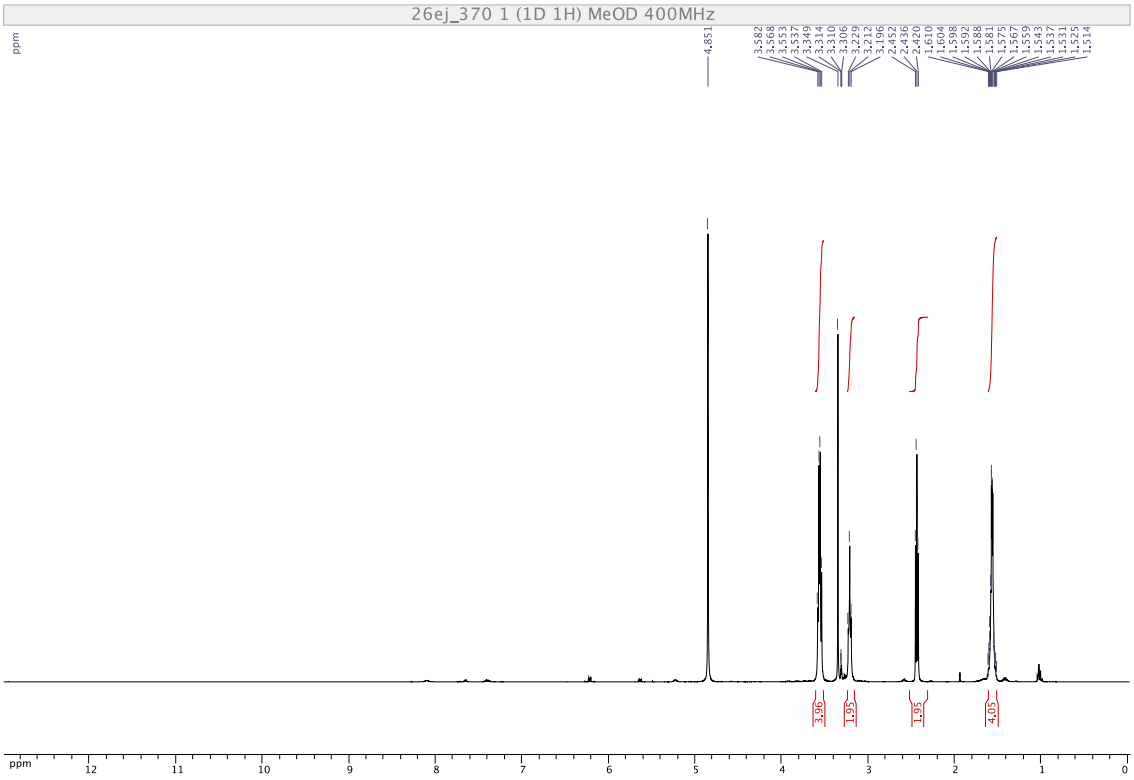

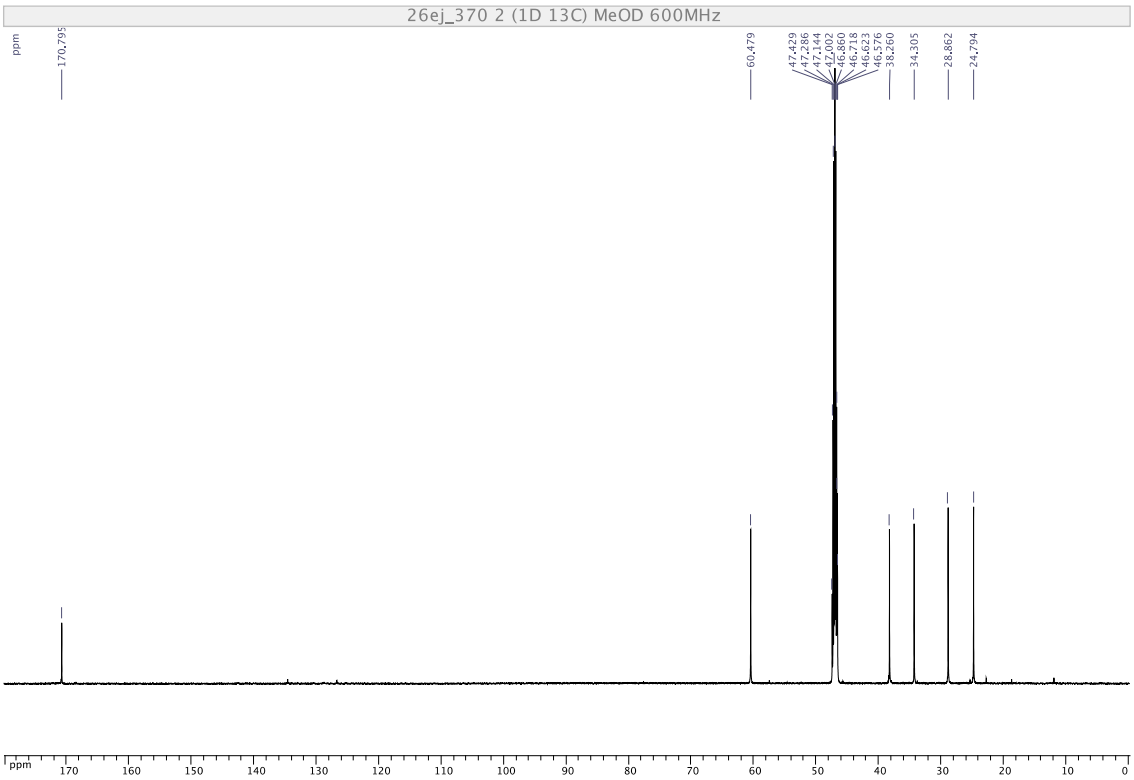
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70
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50
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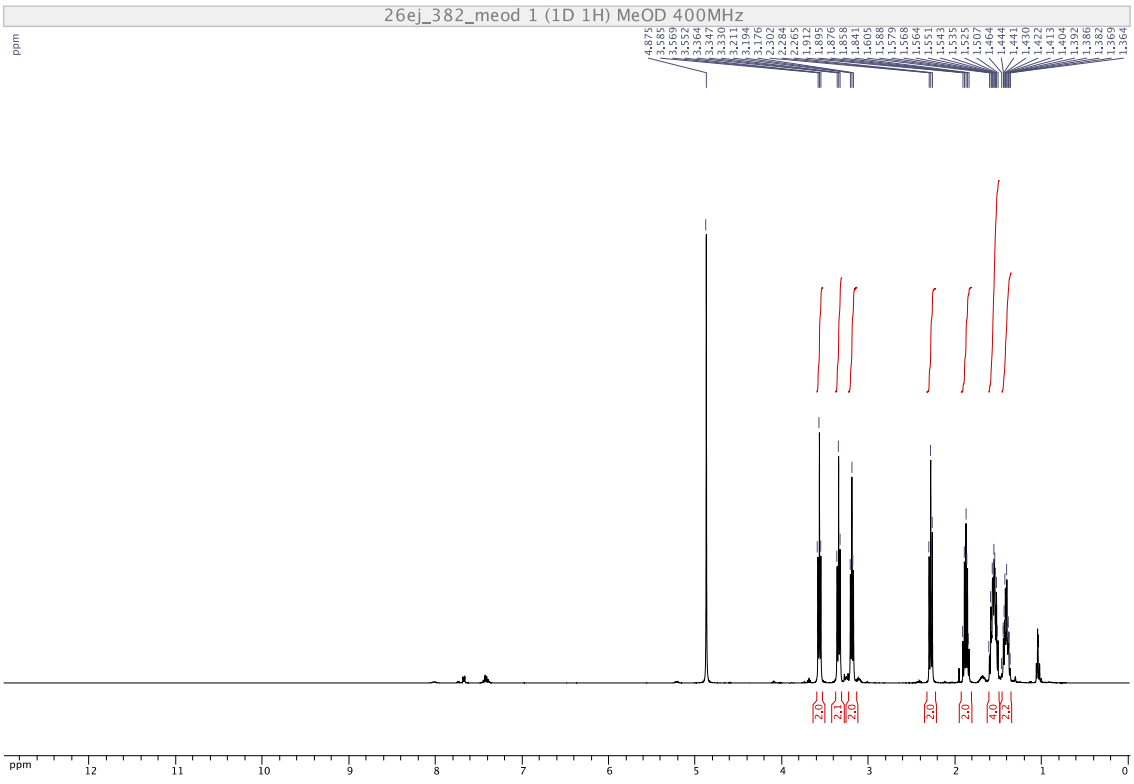
30
20
10
0

Spacer 7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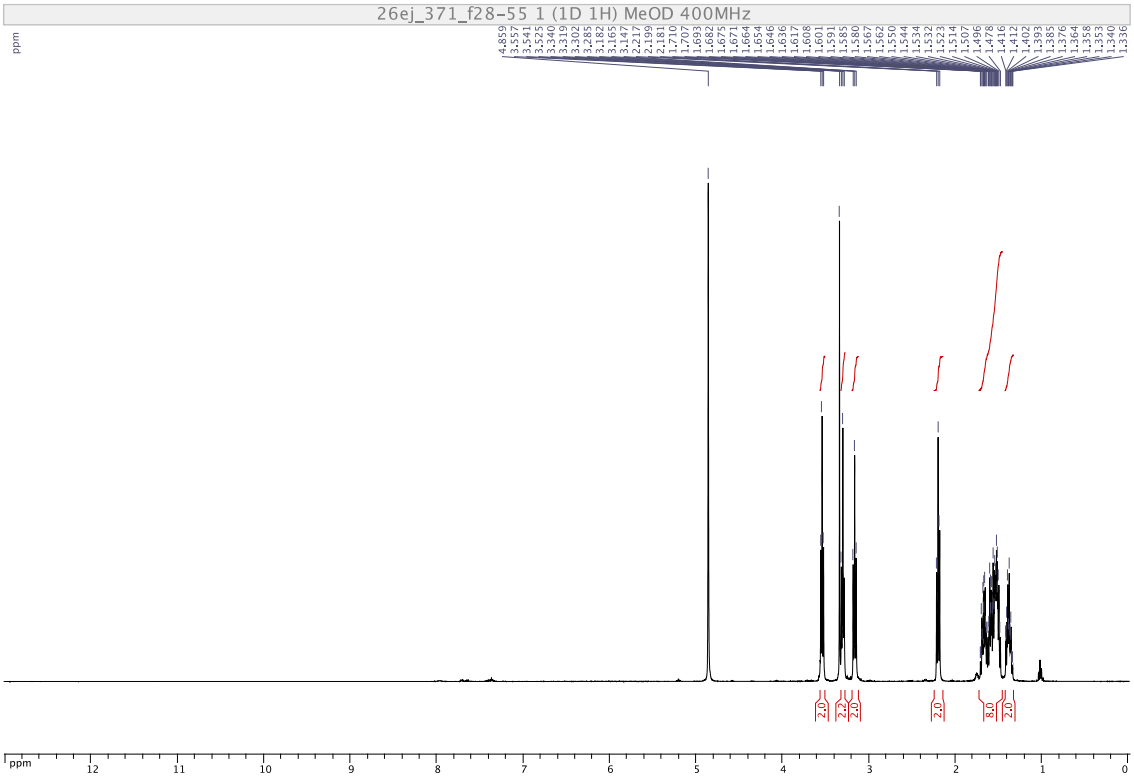
Spacer 8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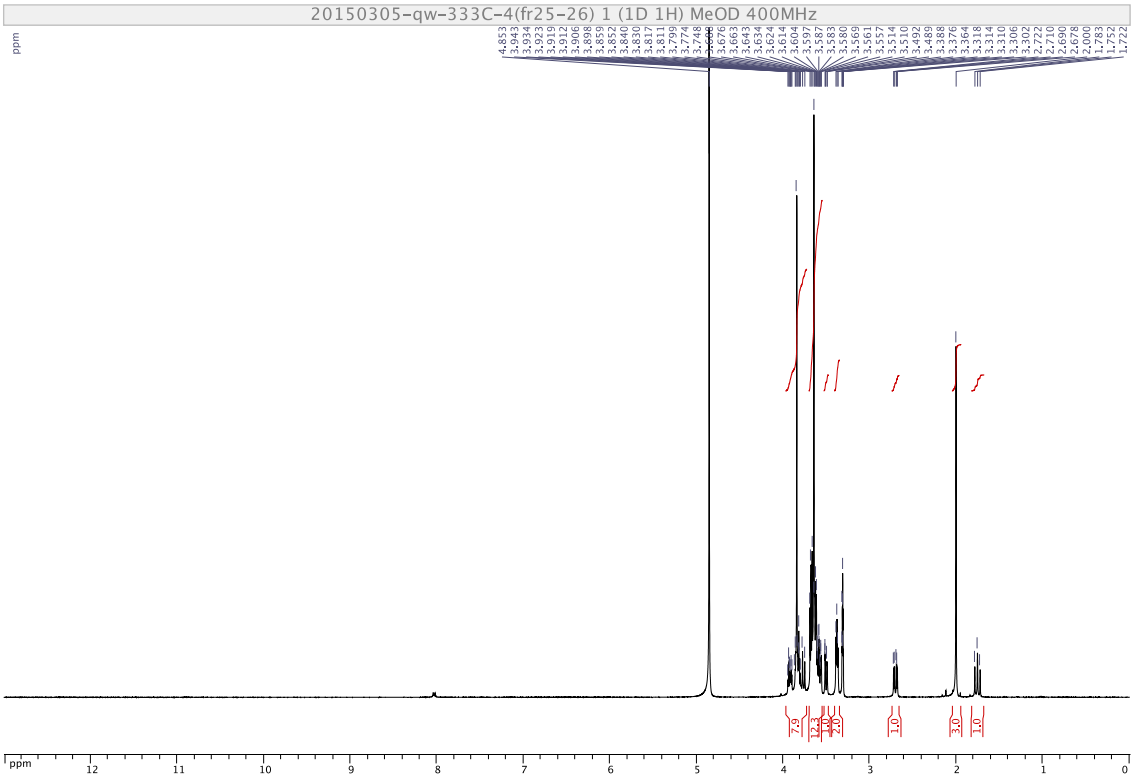
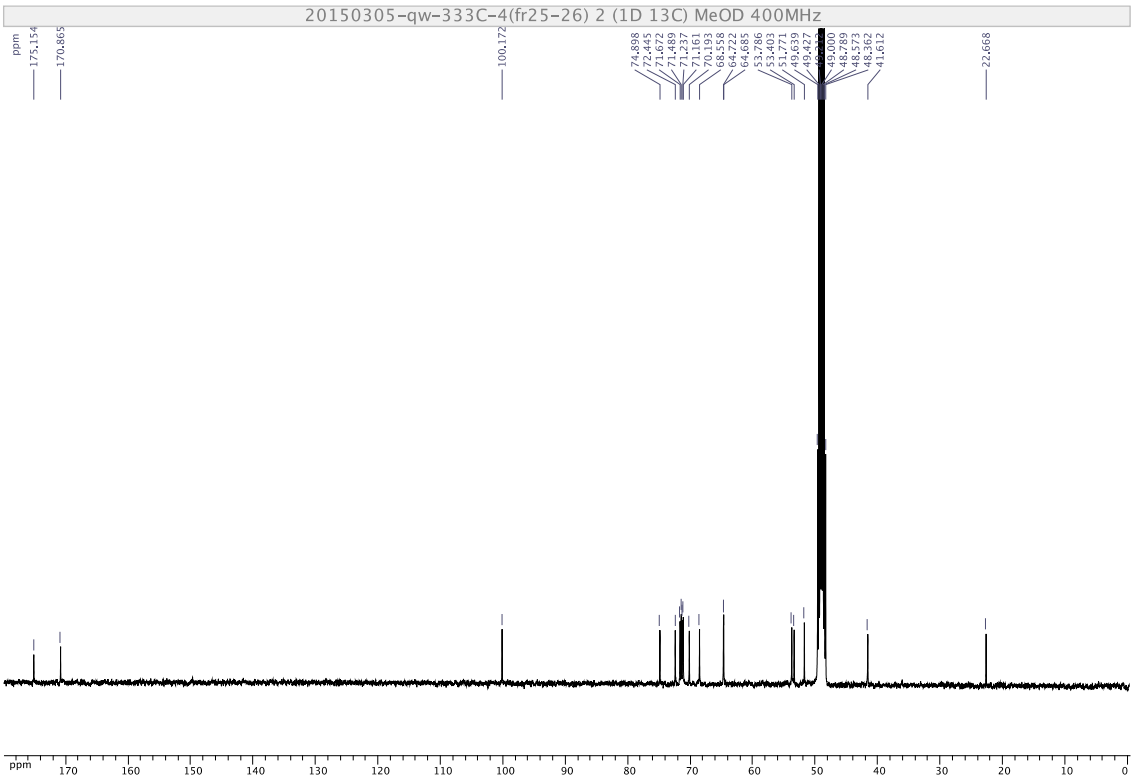
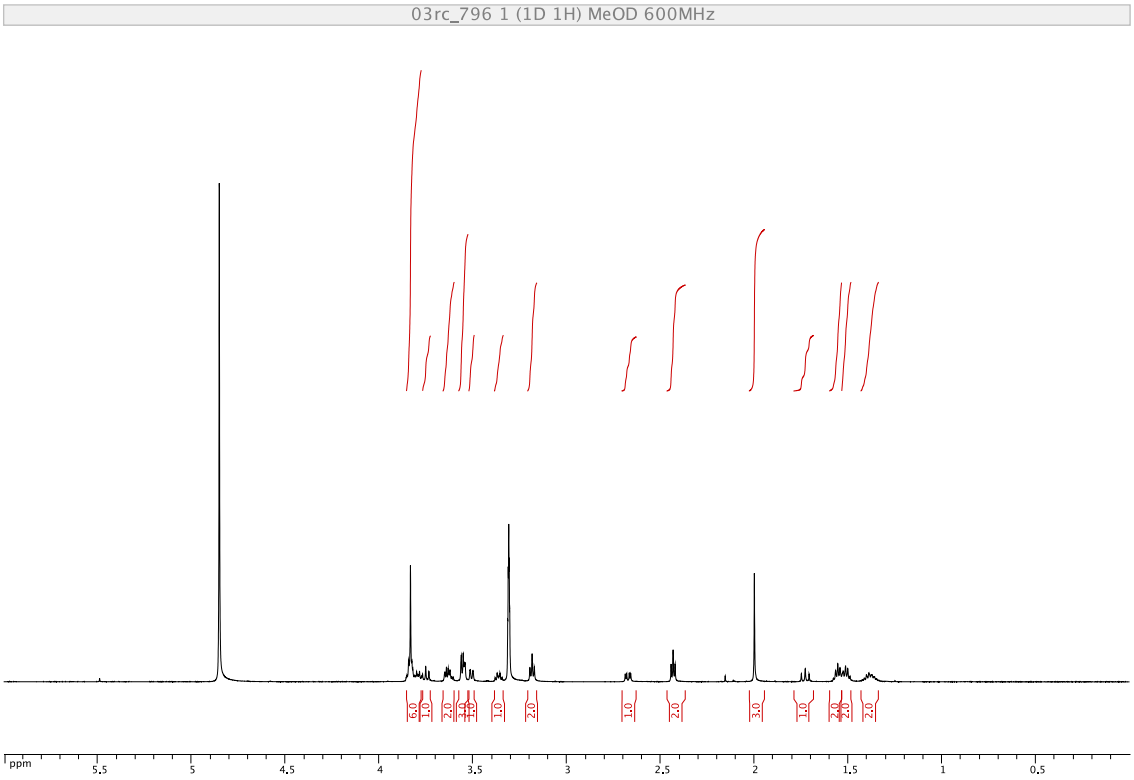
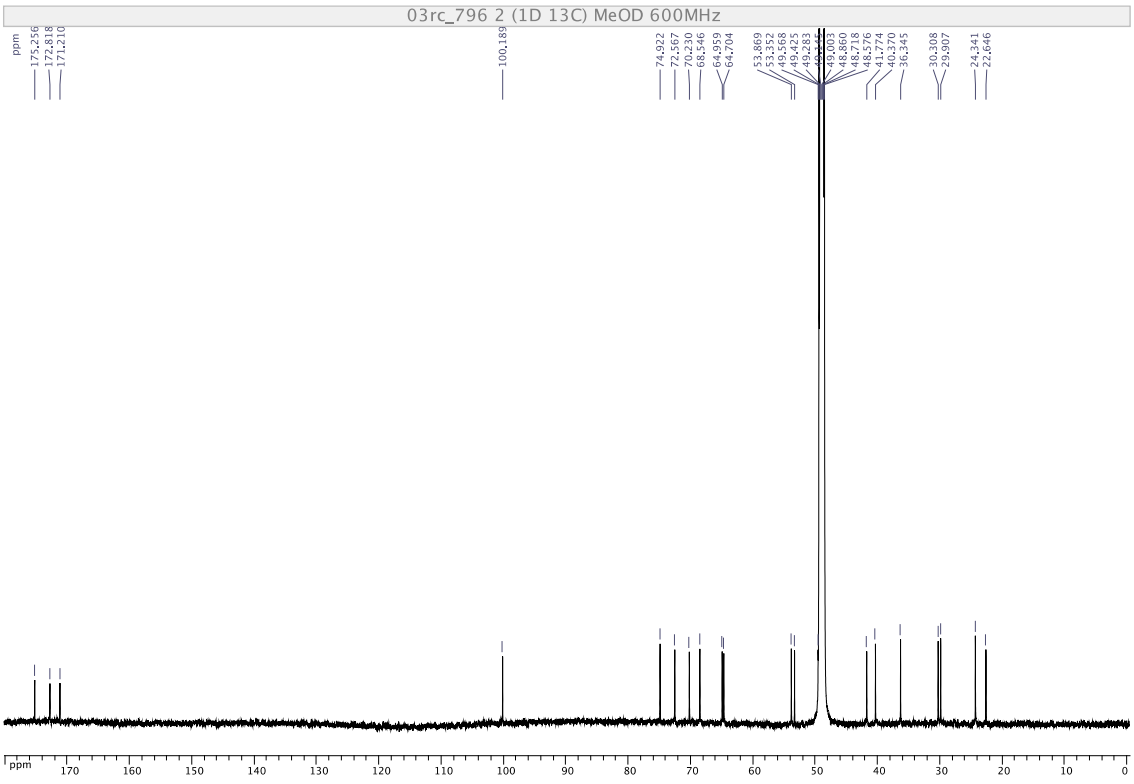
Azidosialoside 11



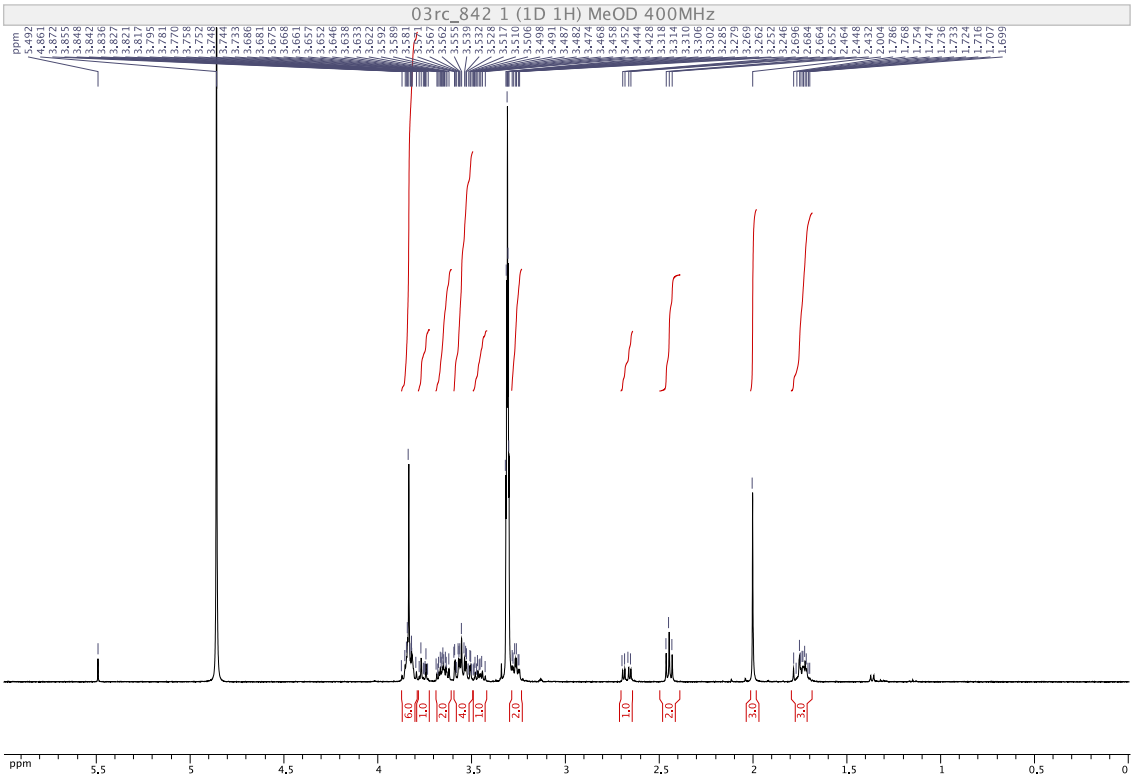


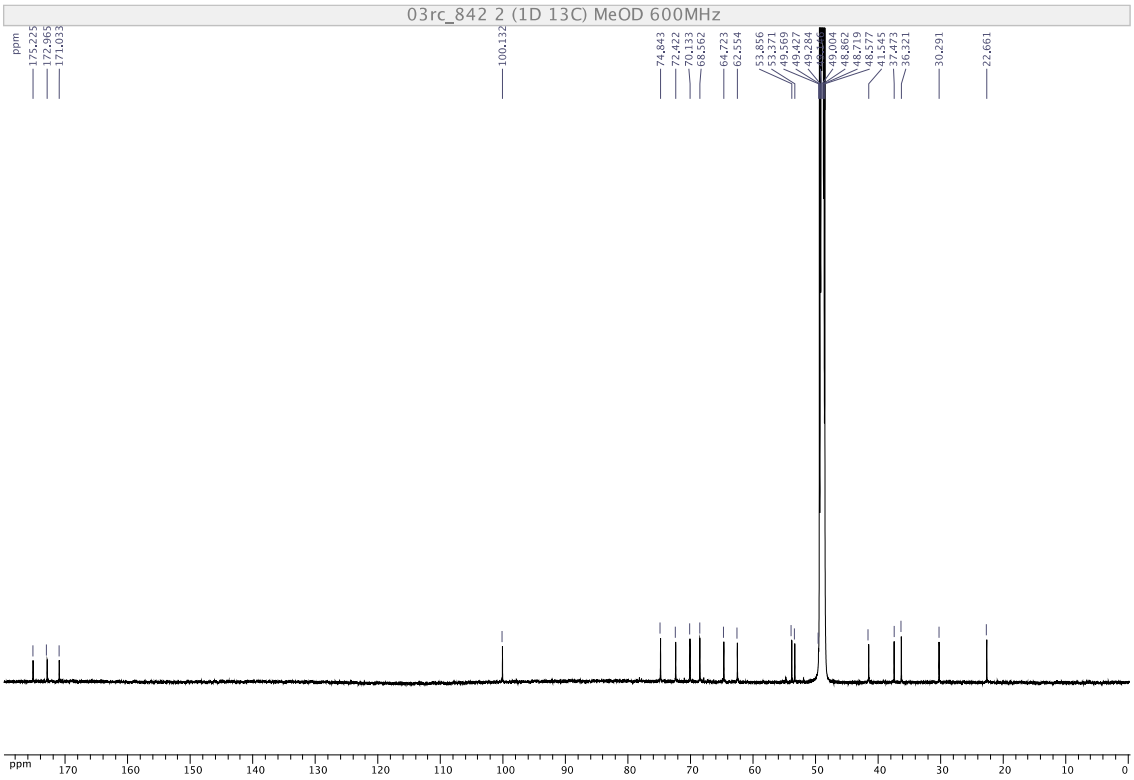
Azidosialoside 12



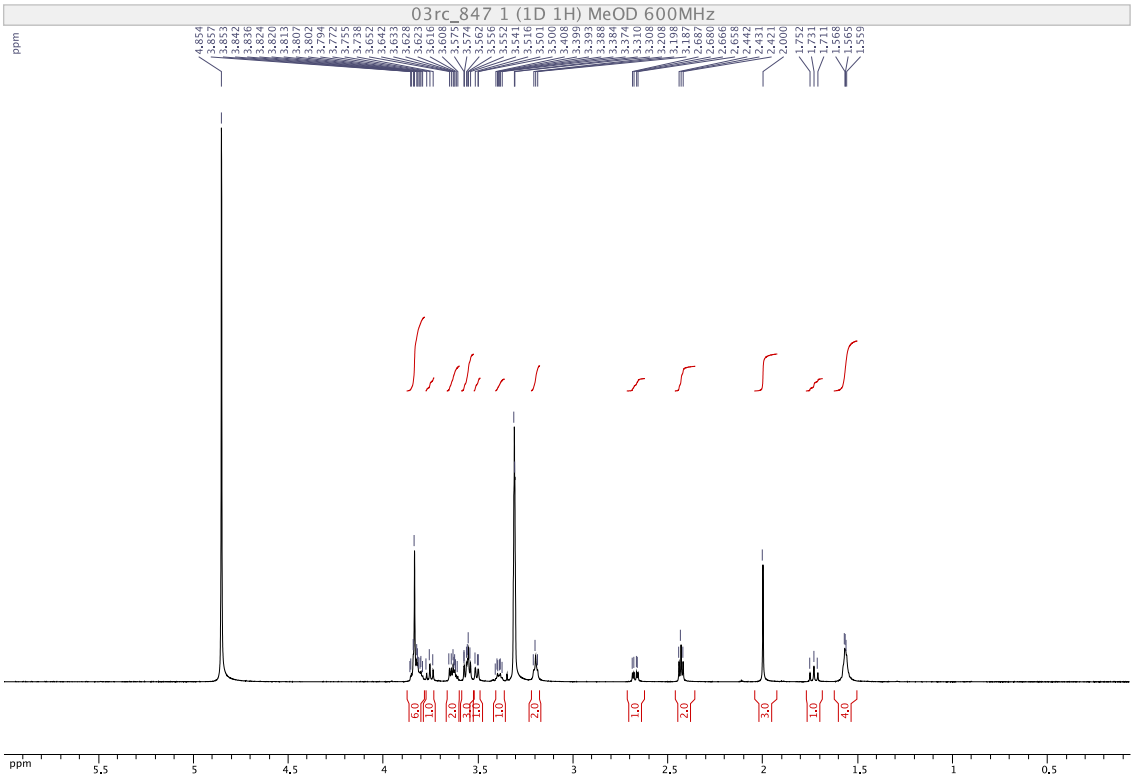


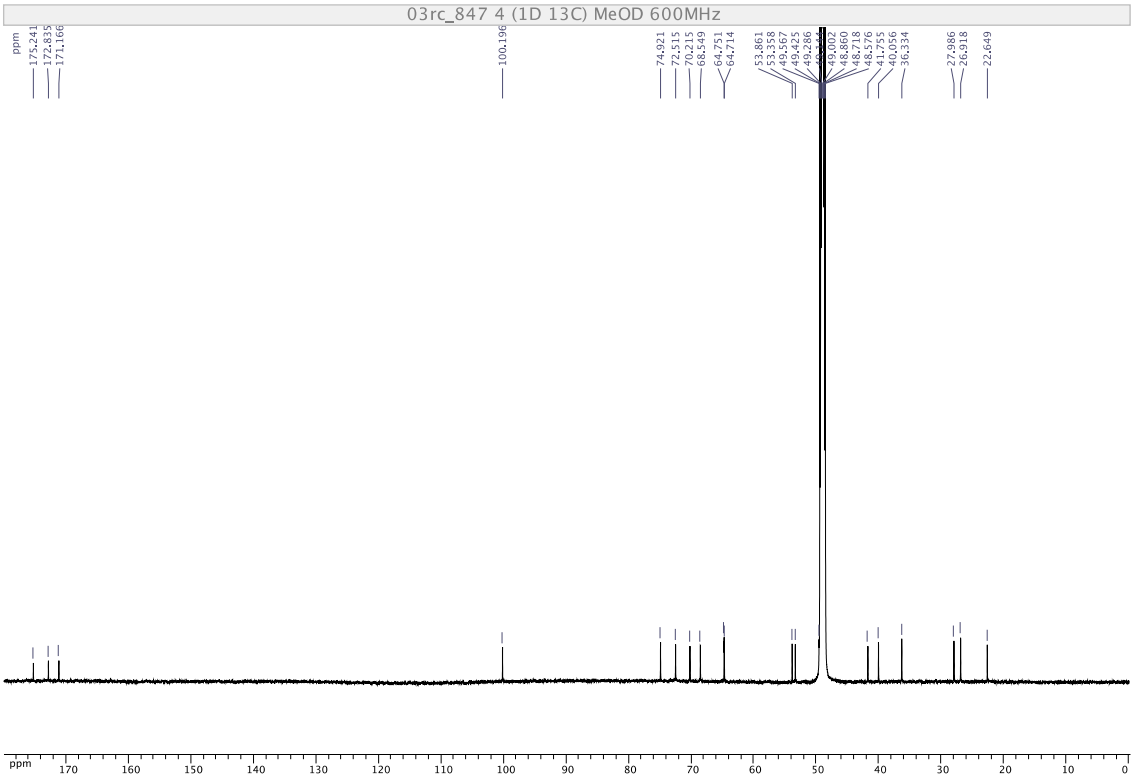
Azidosialoside 13



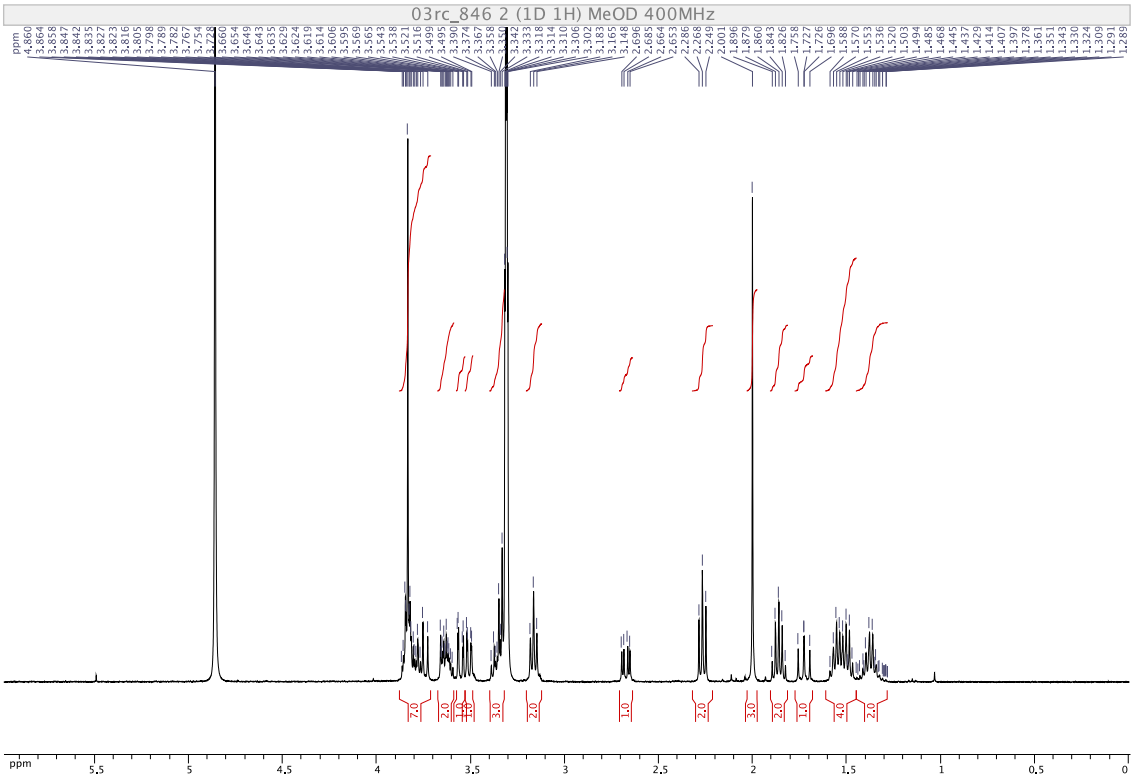


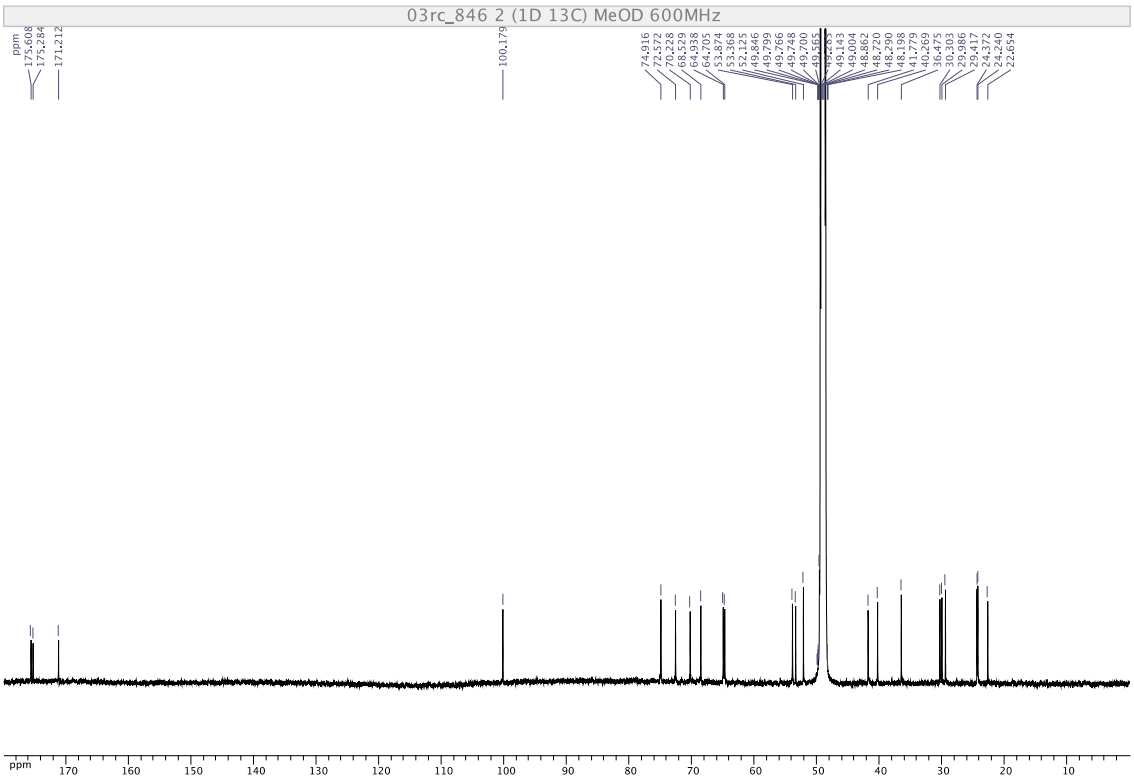
Azidosialoside 14



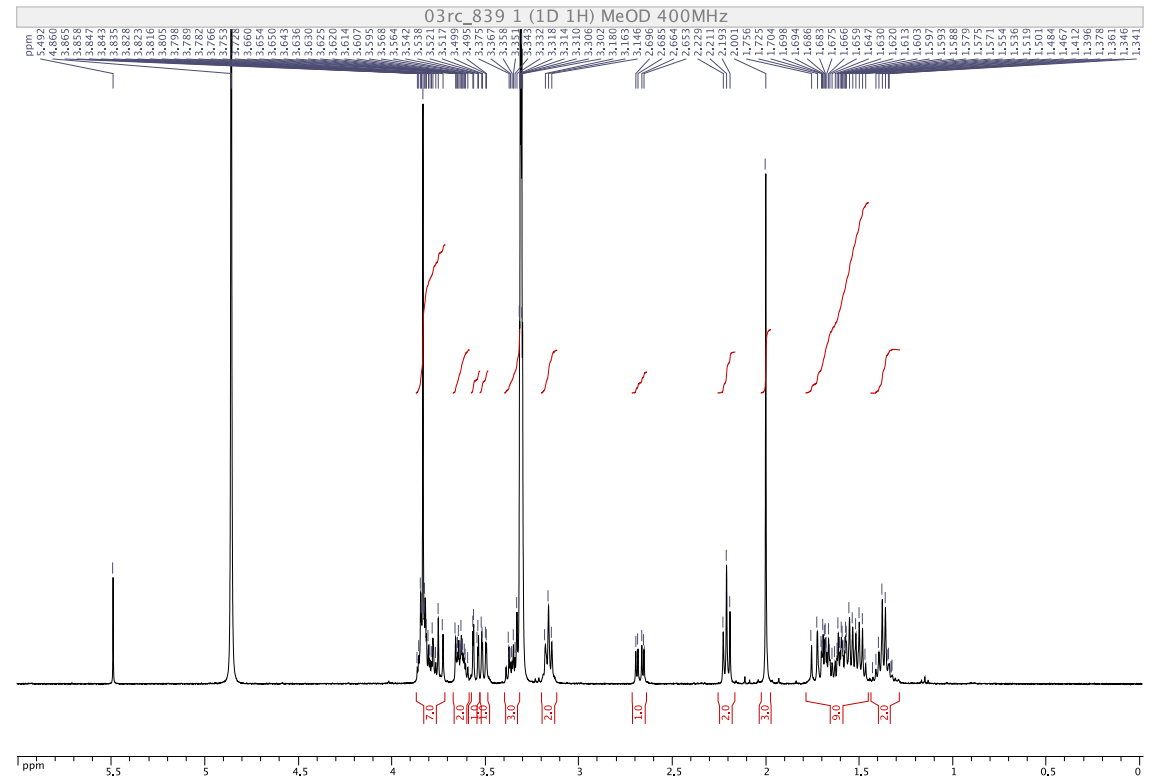


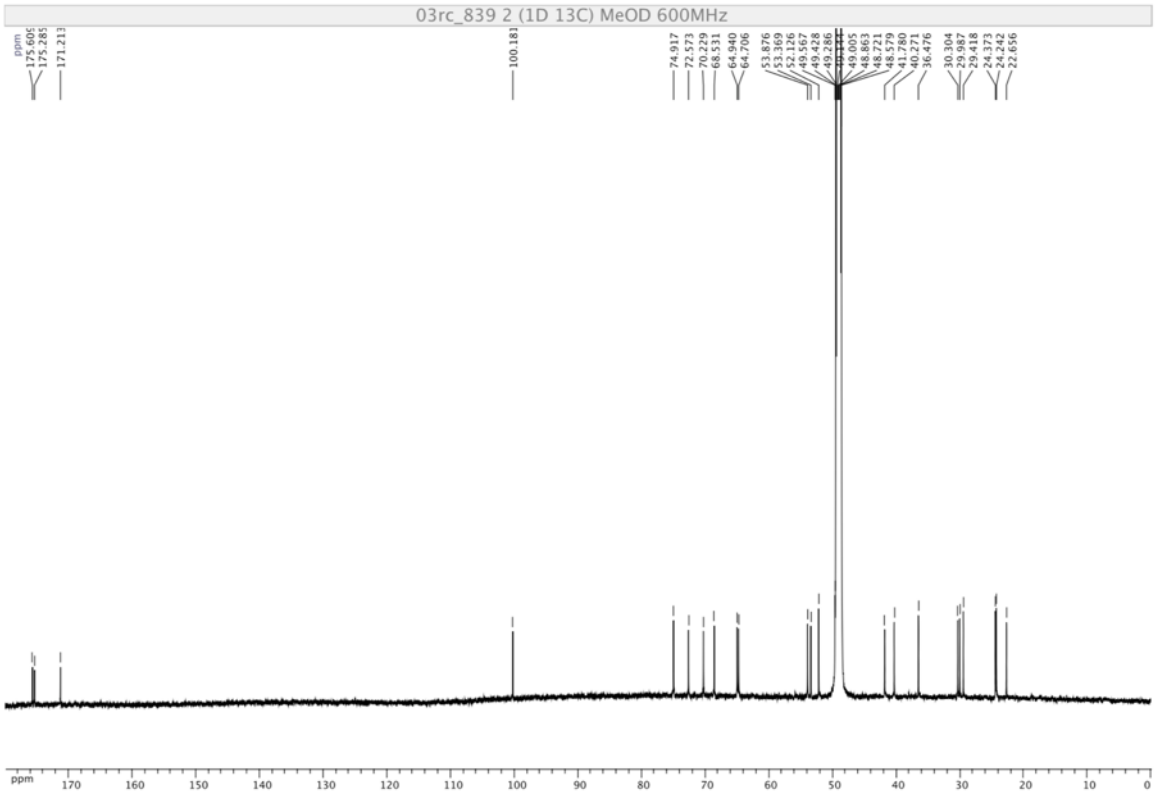
Azidosialoside 15



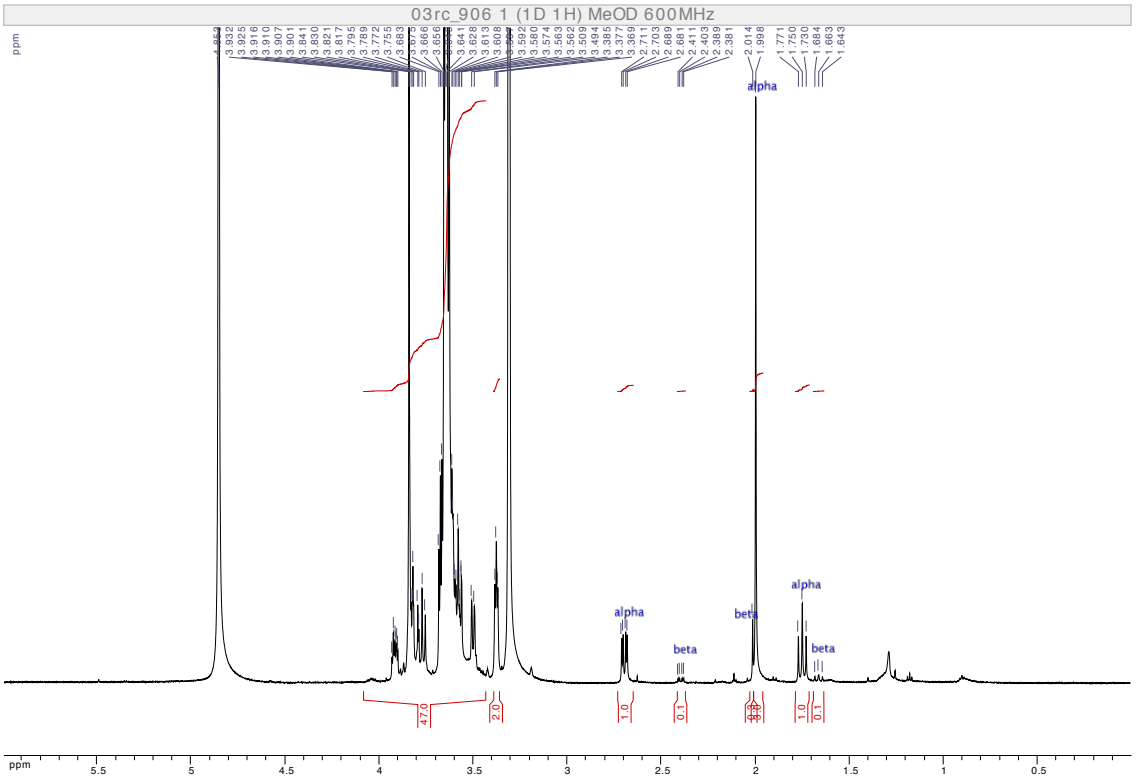


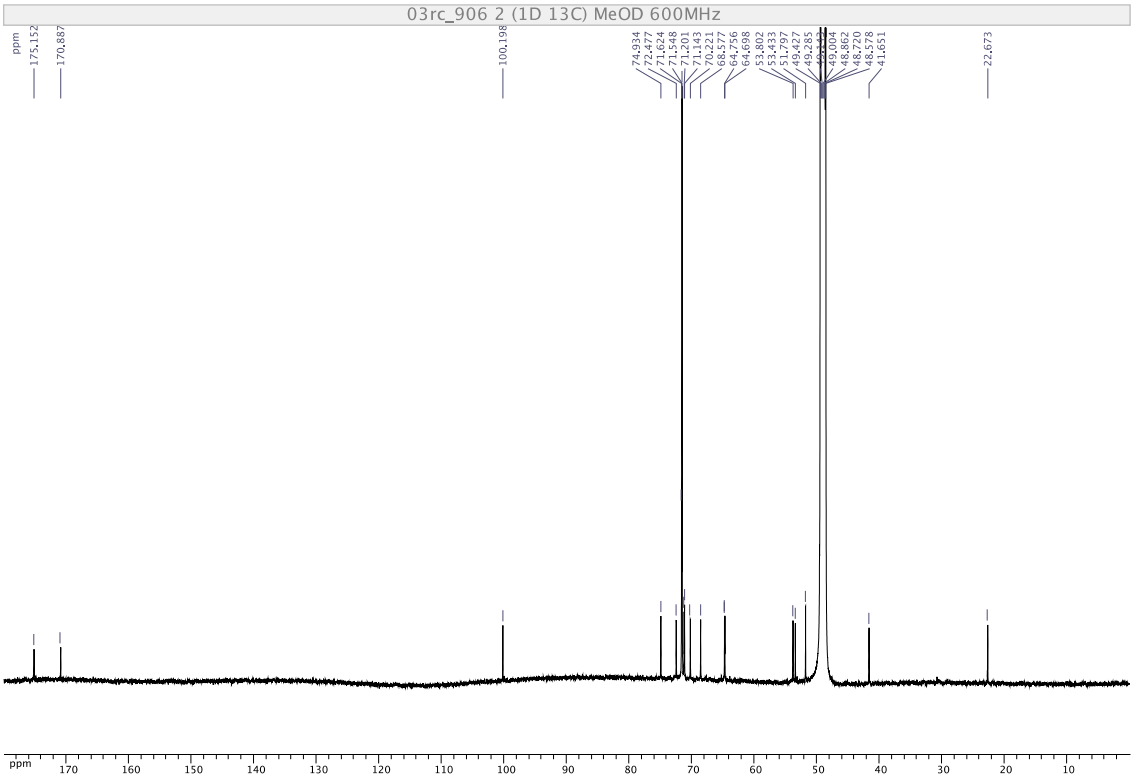
Azidosialoside 16



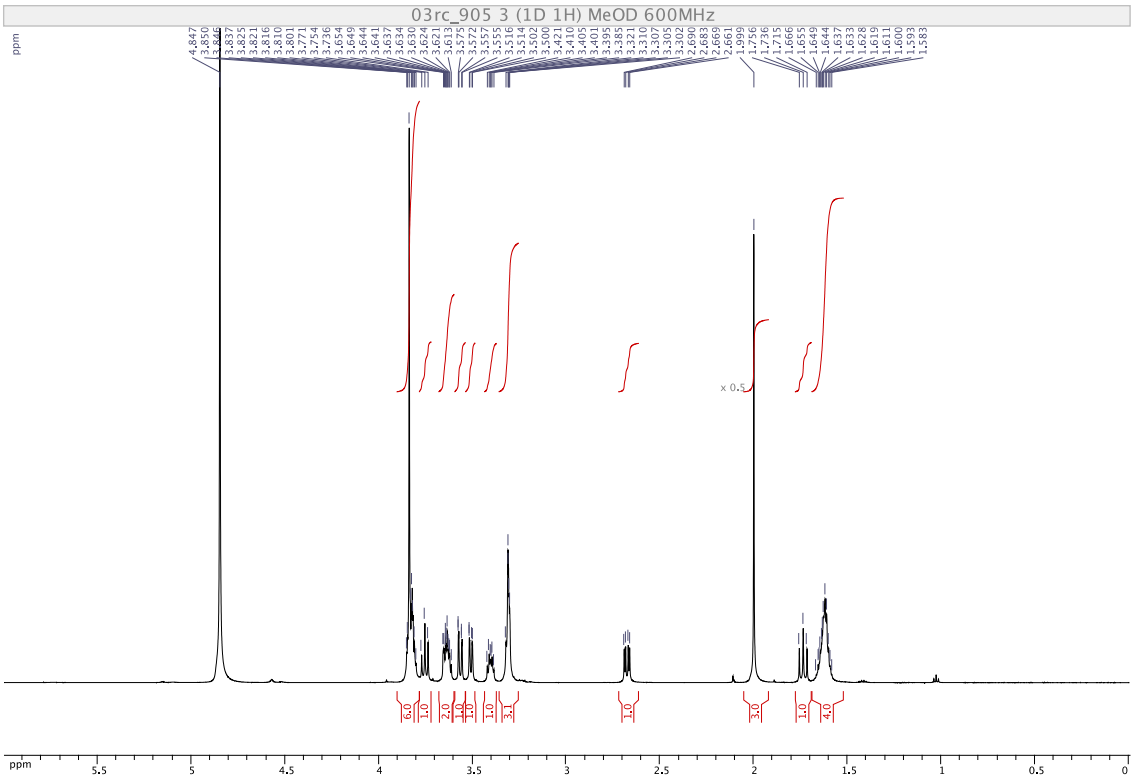


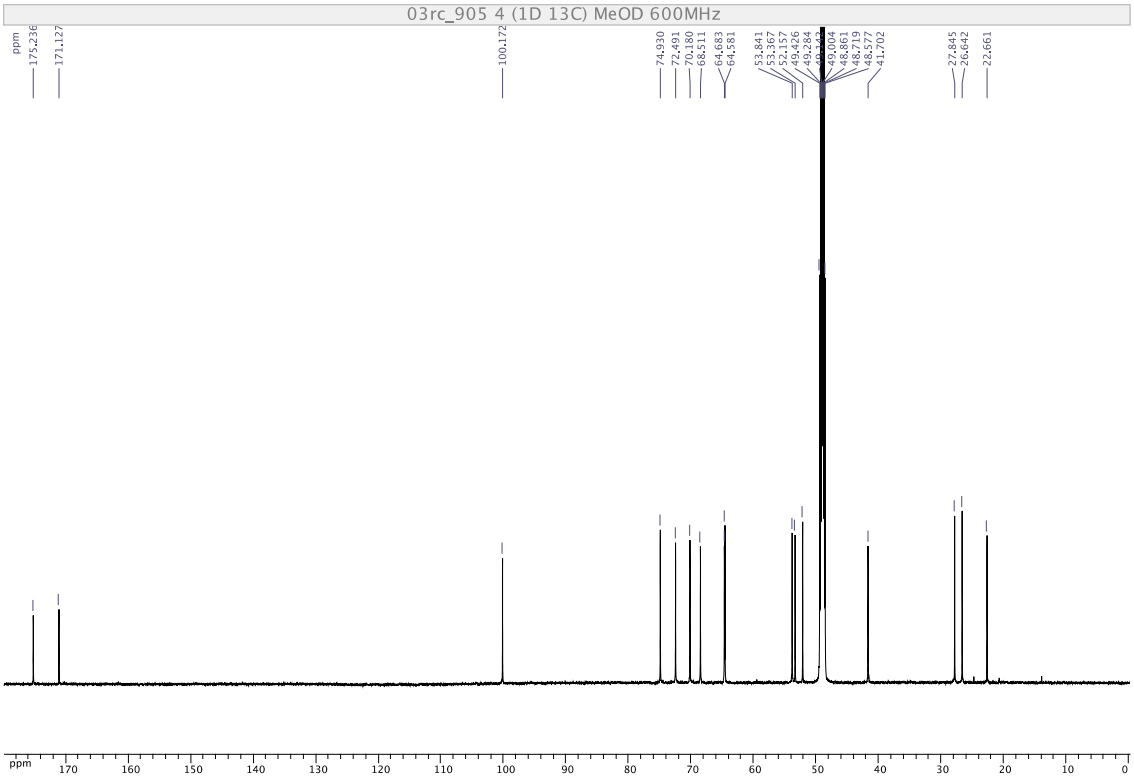
Azidosialoside 17



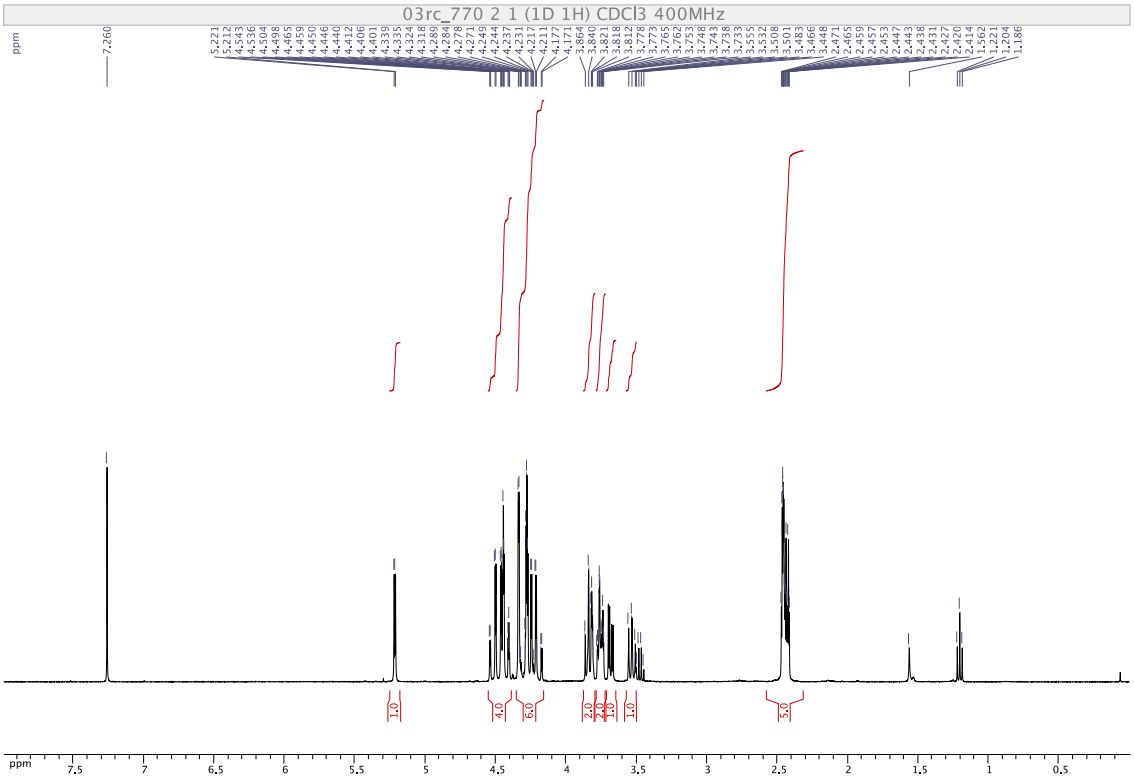


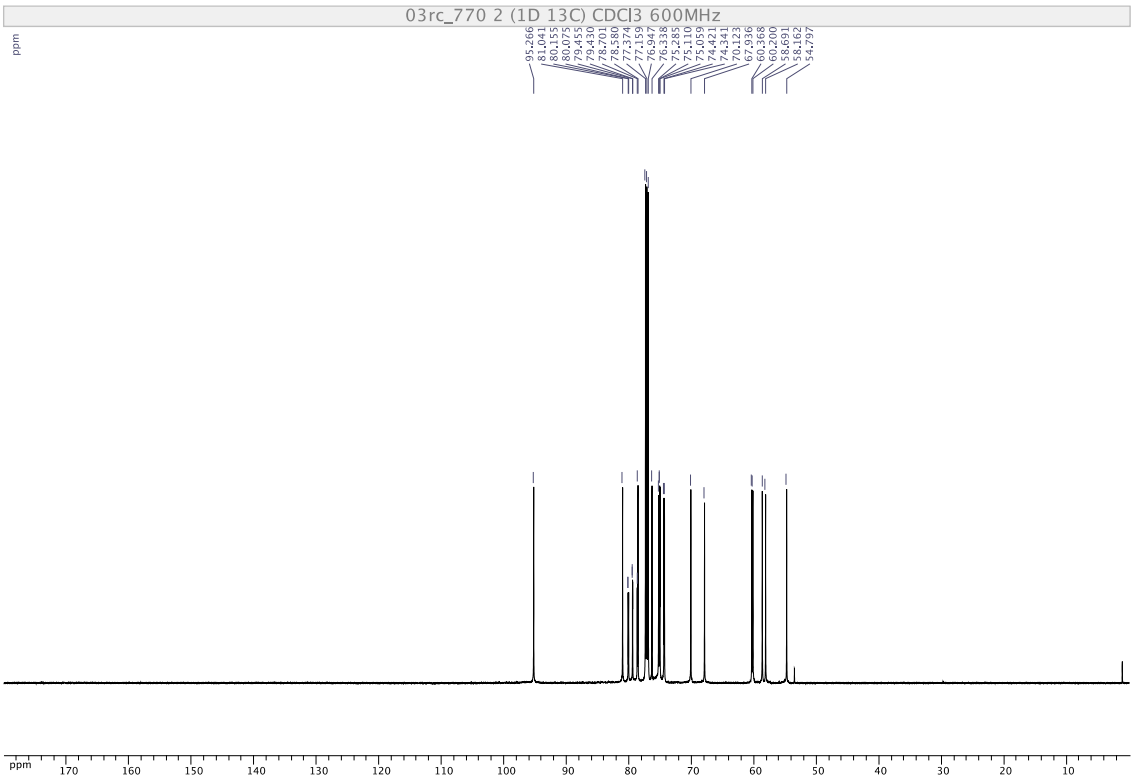
Azidosialoside 18



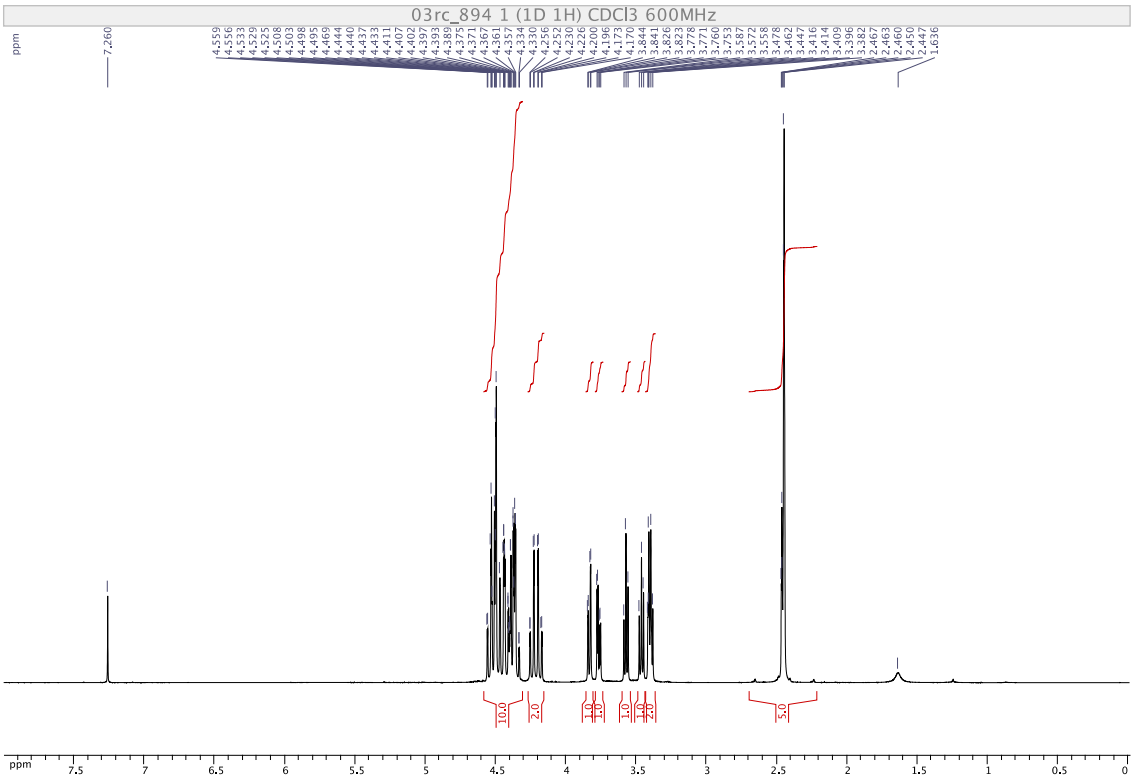


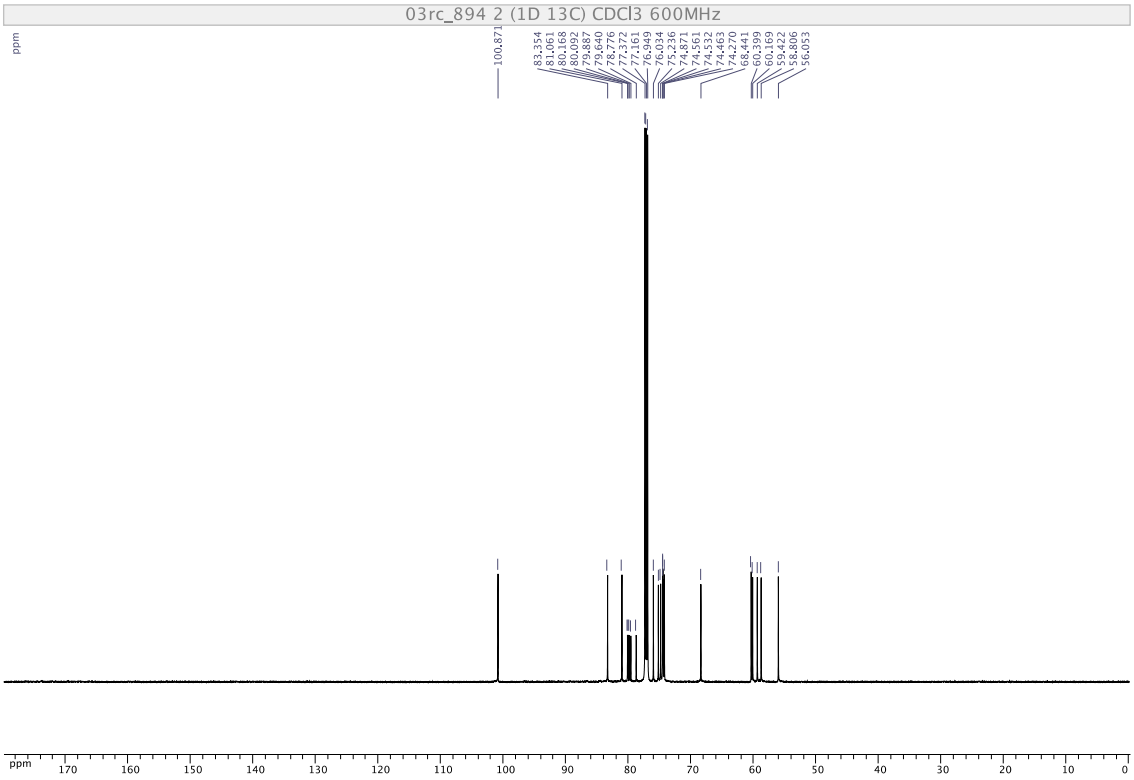
Glucose core 19



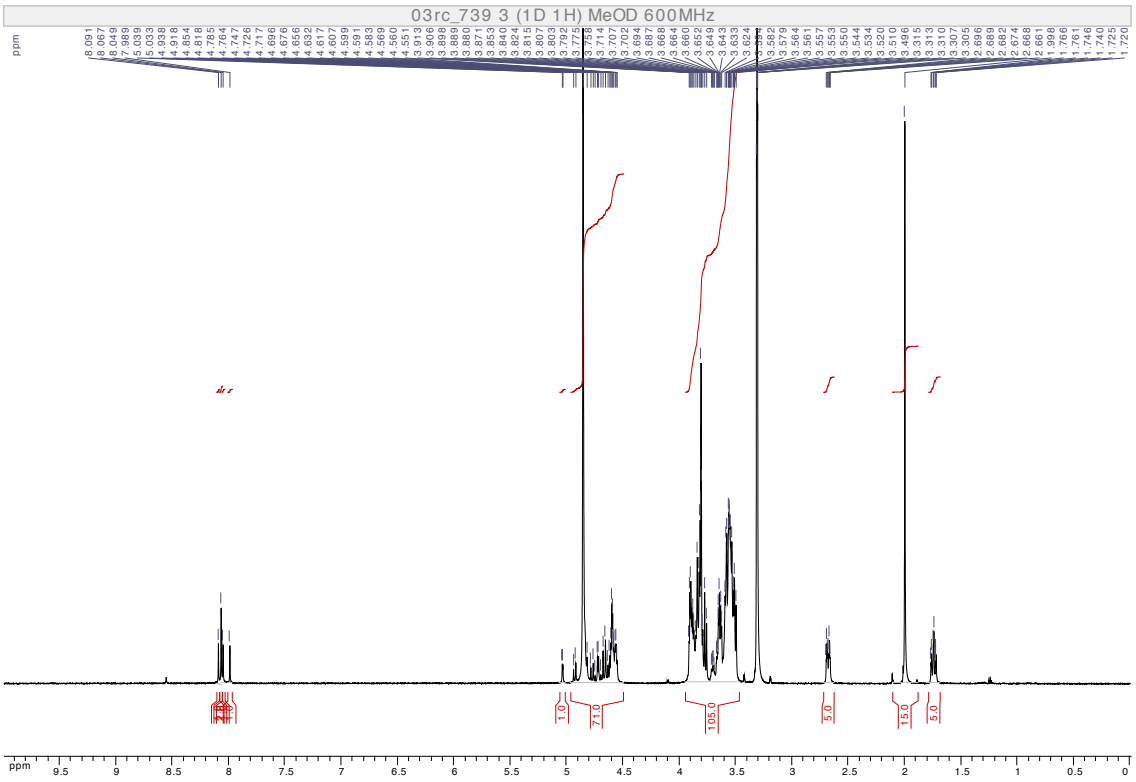


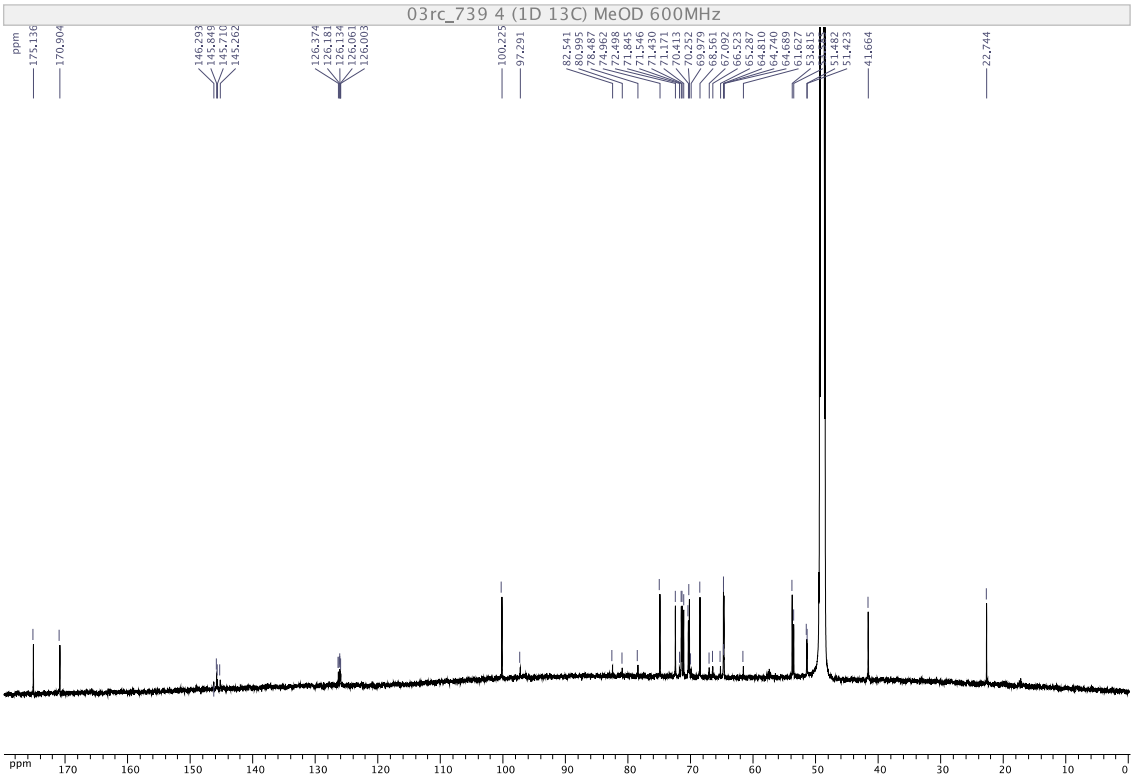
Glucose core 20



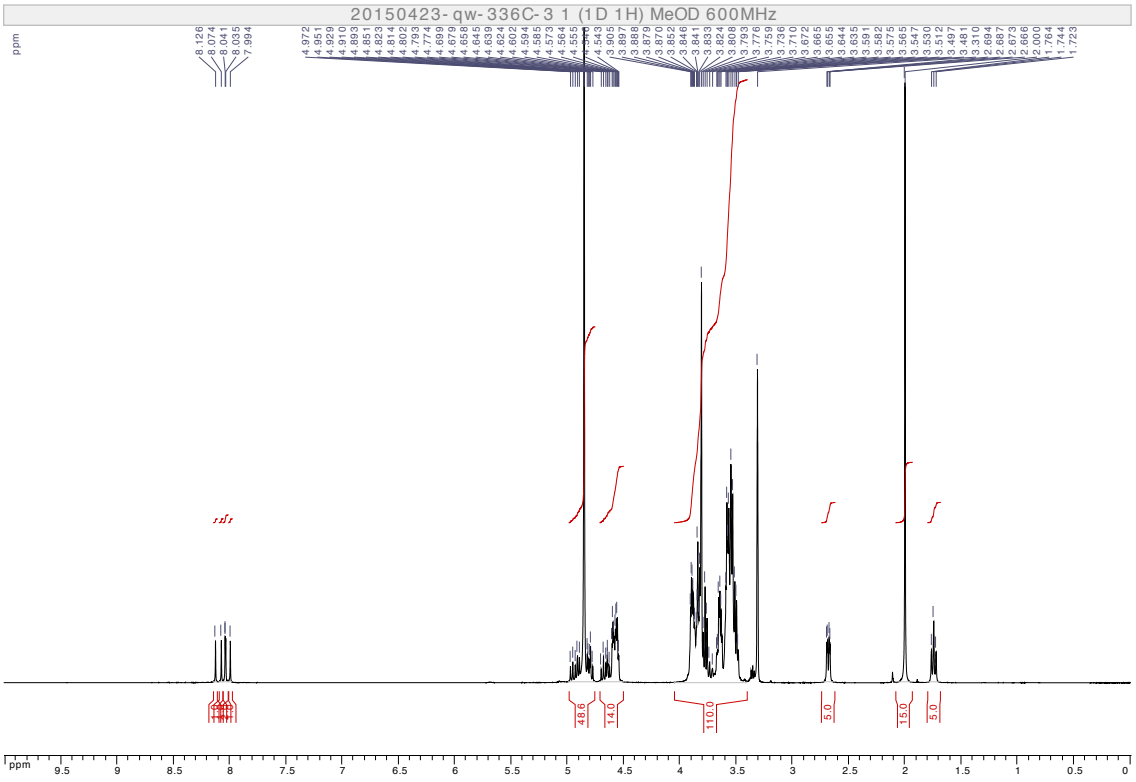


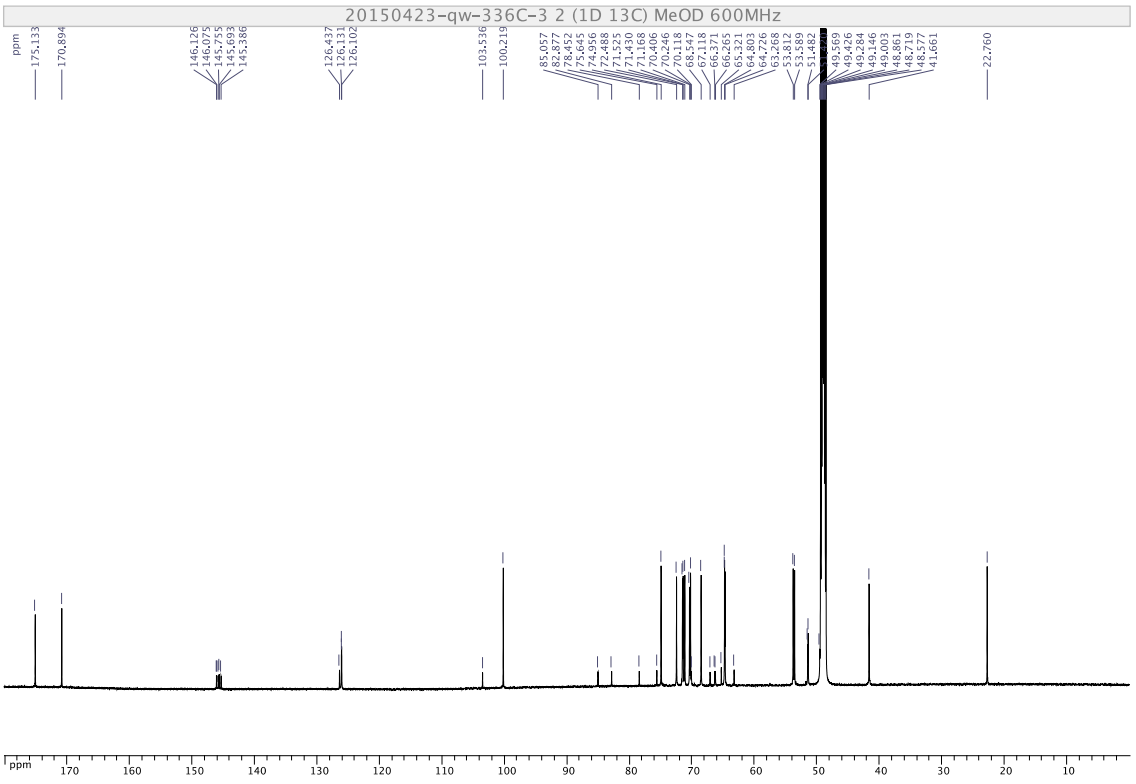
Compound 21


03rc_739 3 (1D 1H) MeOD 600MHz
ppm
1.0
7.10
105.0
5.0
15.0
5.0

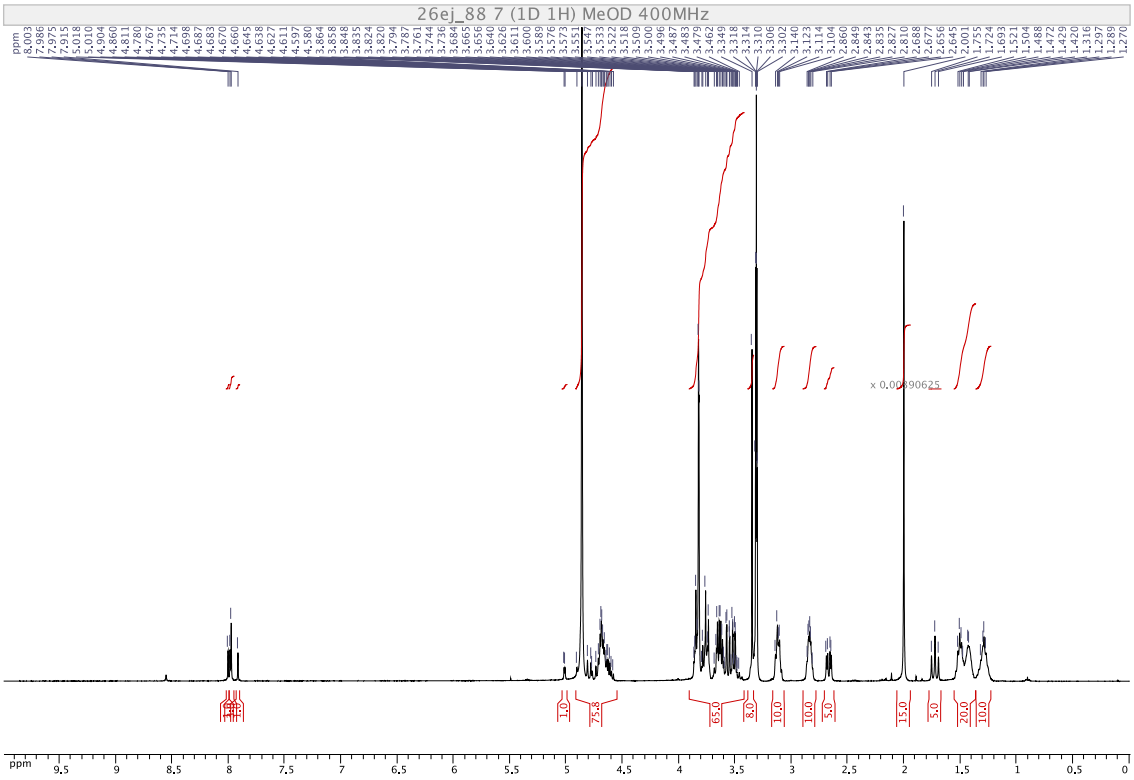

03rc_739 4 (1D 13C) MeOD 600MHz
ppm
175.136
170.904
146.293
145.849
145.710
145.262
126.374
126.181
126.134
126.061
126.003
100.225
97.291
82.541
80.995
78.487
74.962
72.498
71.845
71.546
71.430
71.171
70.413
70.252
69.979
68.561
67.092
66.523
65.287
64.810
64.740
64.689
61.627
53.815
51.482
51.423
41.664
22.744

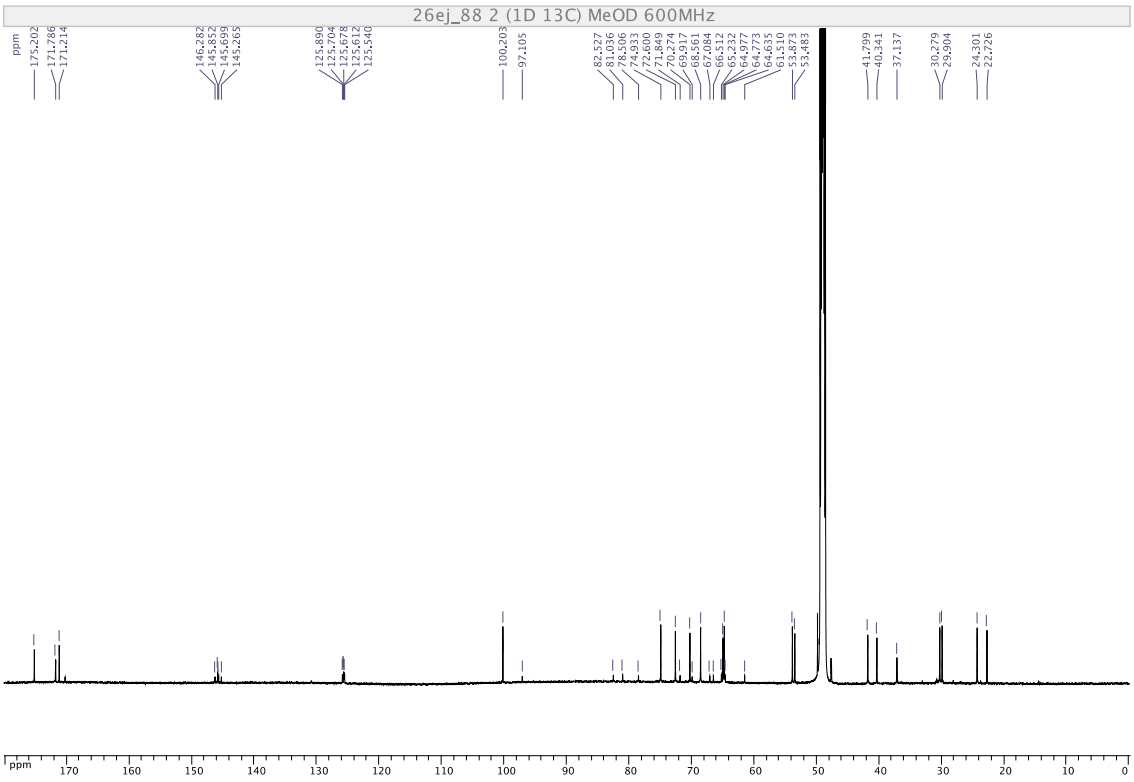
Compound 22


20150423- qw- 336C- 3 1 (1D 1H) MeOD 600MHz
ppm
48.6
14.0
110.0
5.0
15.0
5.0

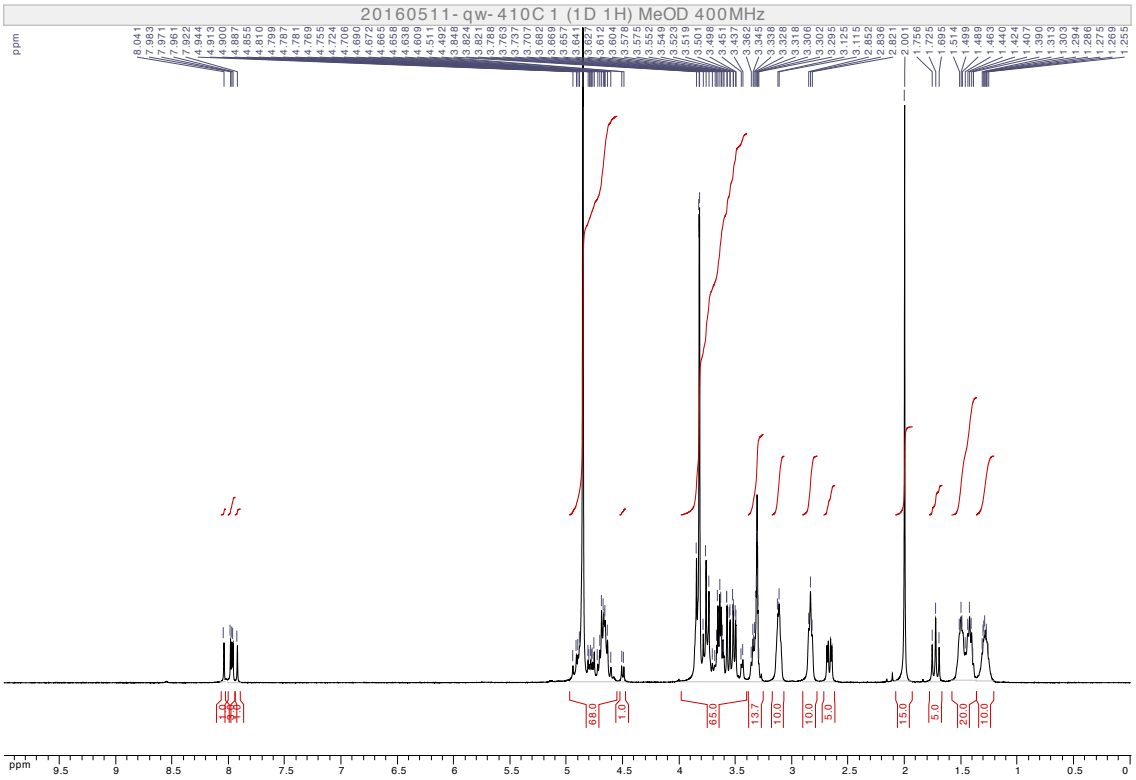

20150423-qw-336C-3 2 (1D 13C) MeOD 600MHz
ppm

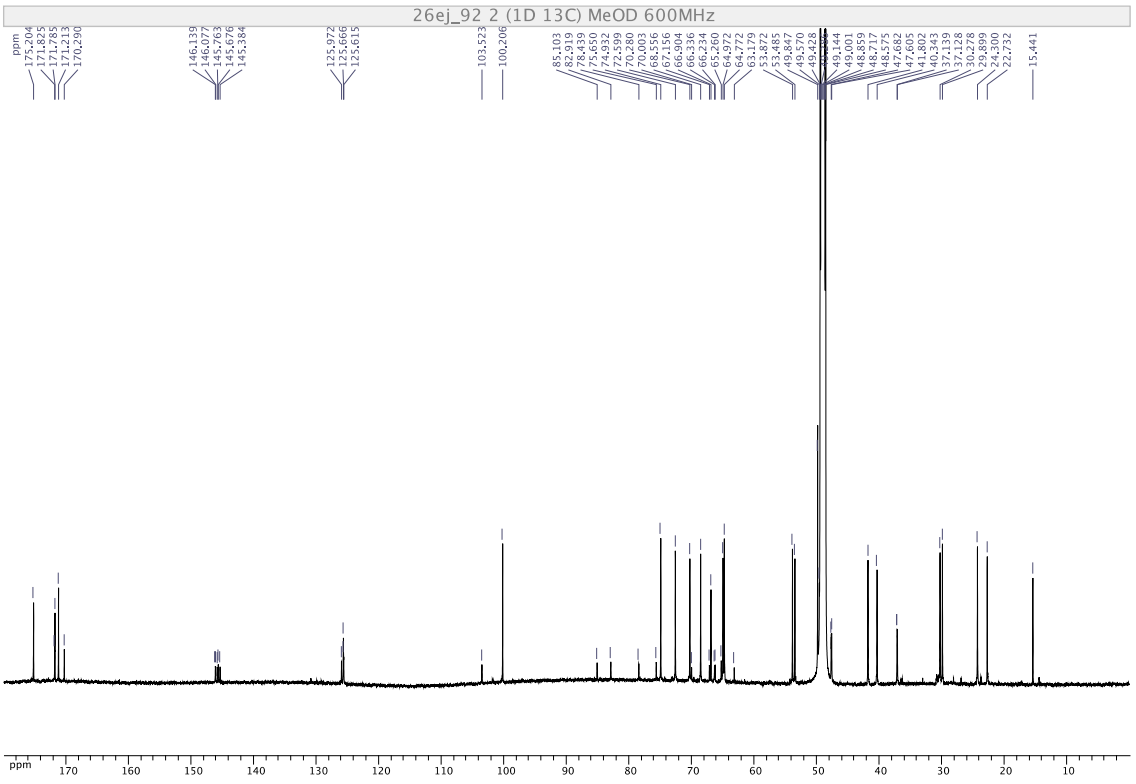
Compound 23



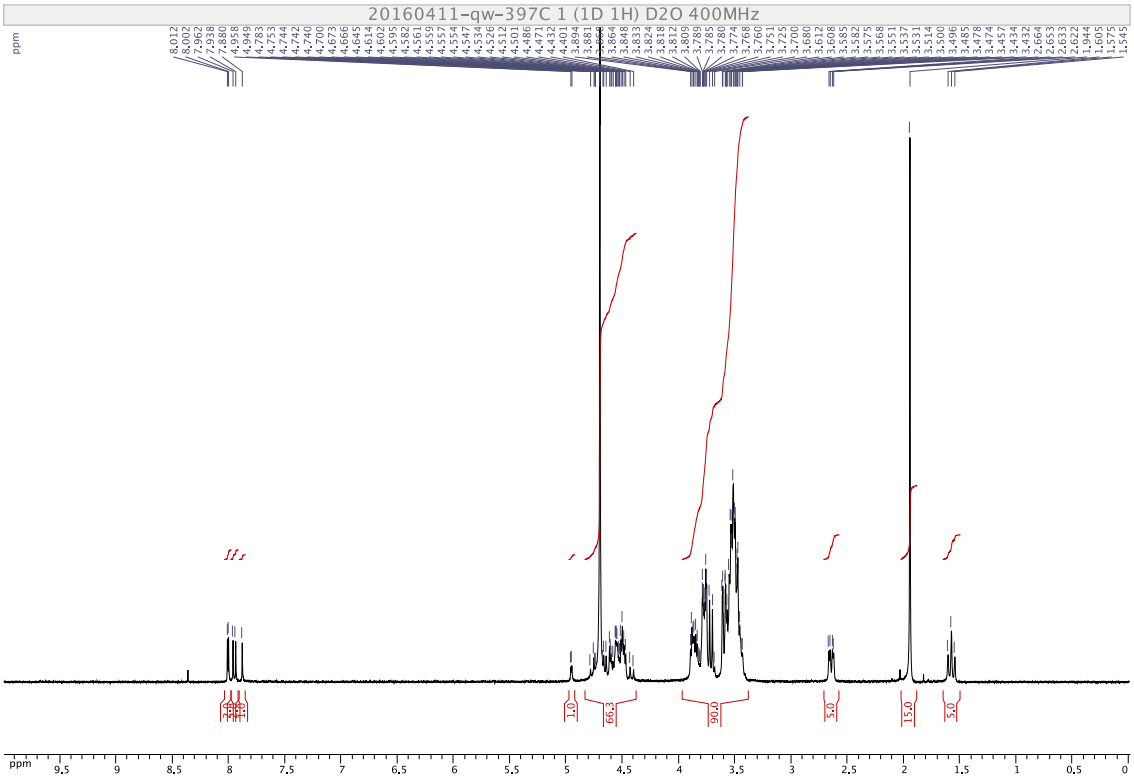


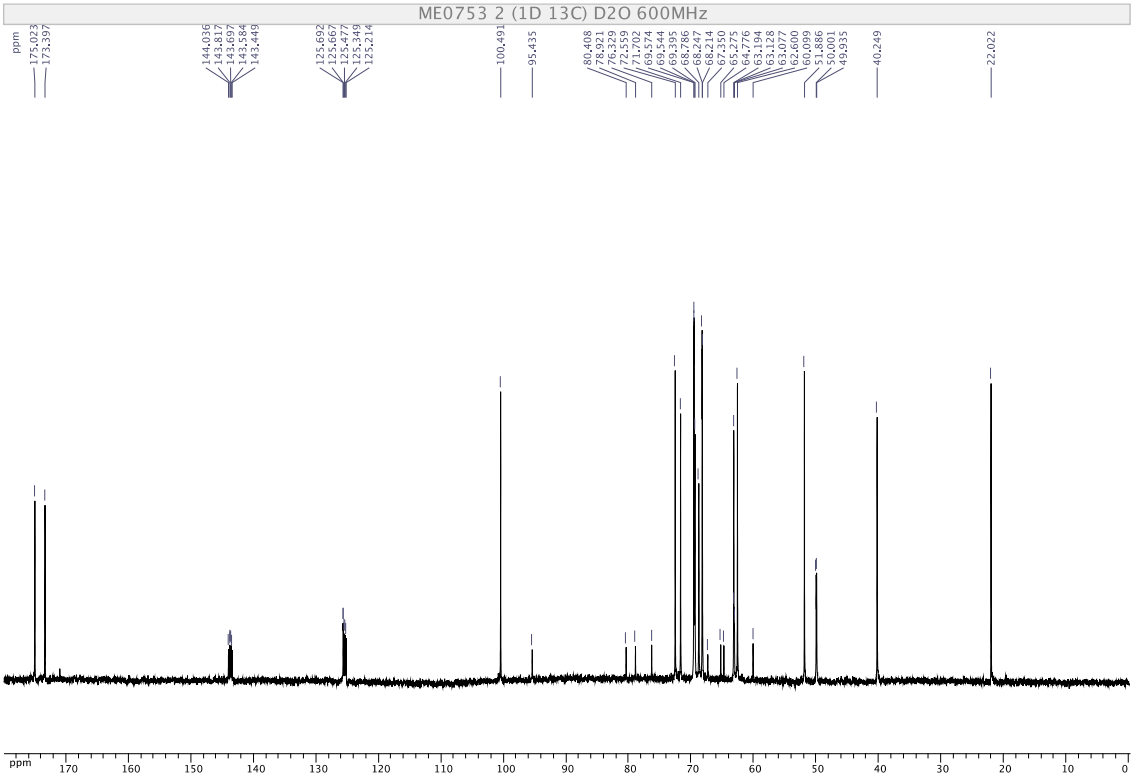
Compound 24


20160511-qw-410C 1 (1D 1H) MeOD 400MHz

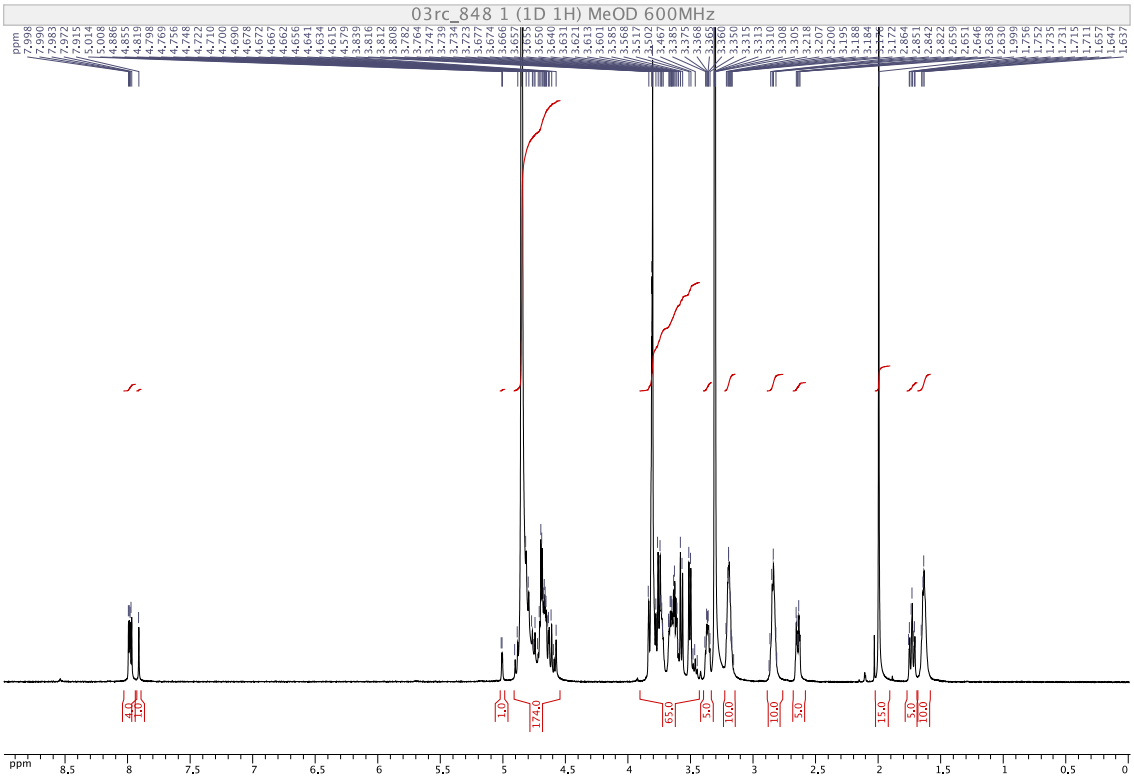

26ej_92 2 (1D 13C) MeOD 600MHz

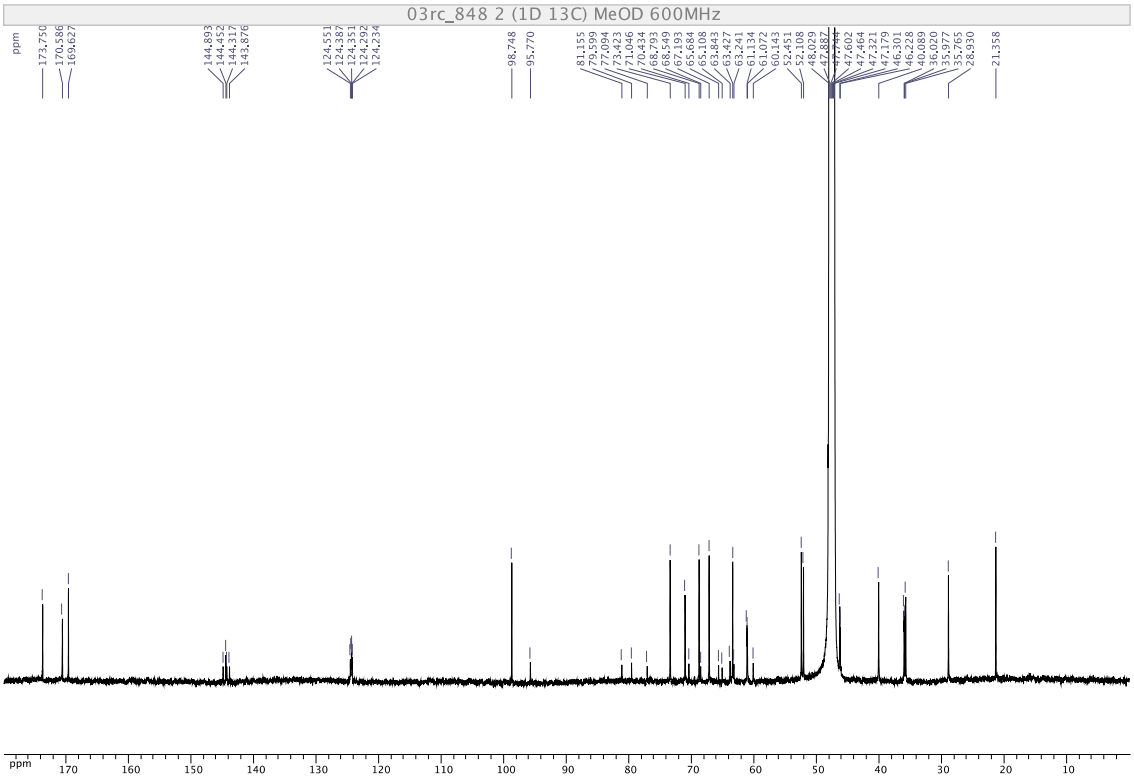
Compound 25


20160411-qw-397C 1 (1D 1H) D2O 400MHz
ppm
1.0
66.3
90.0
5.0
15.0
5.0

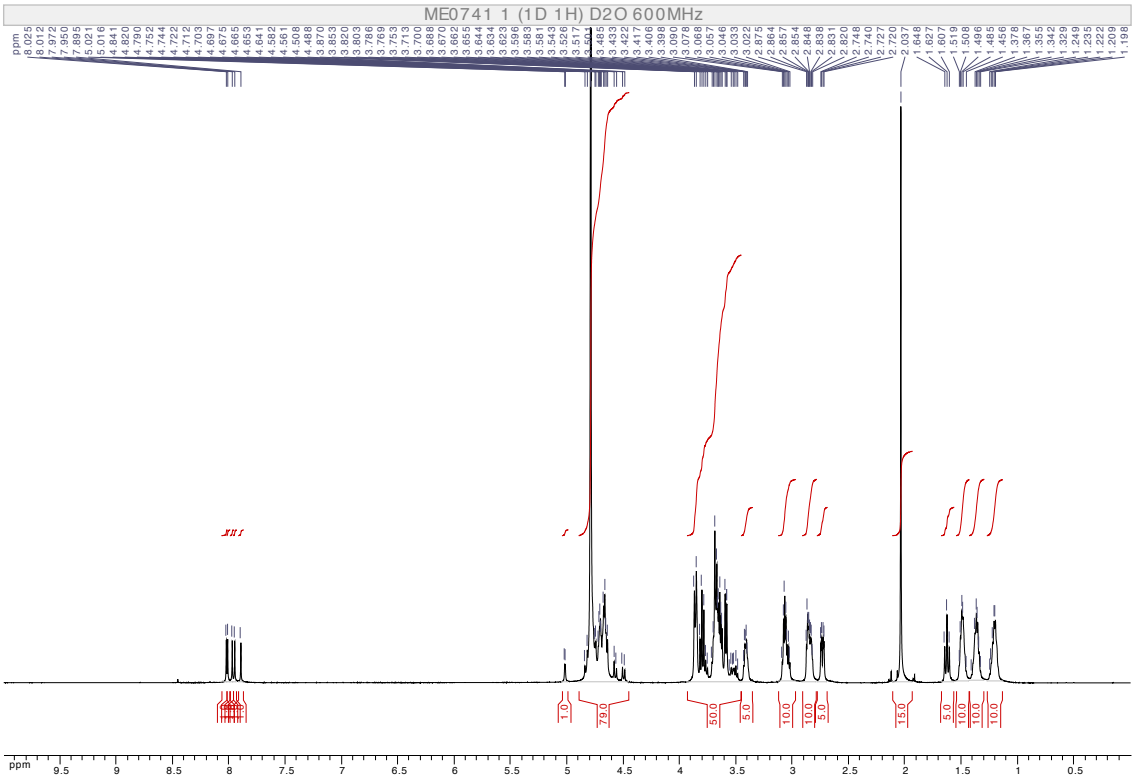

ME0753 2 (1D 13C) D2O 600MHz
ppm

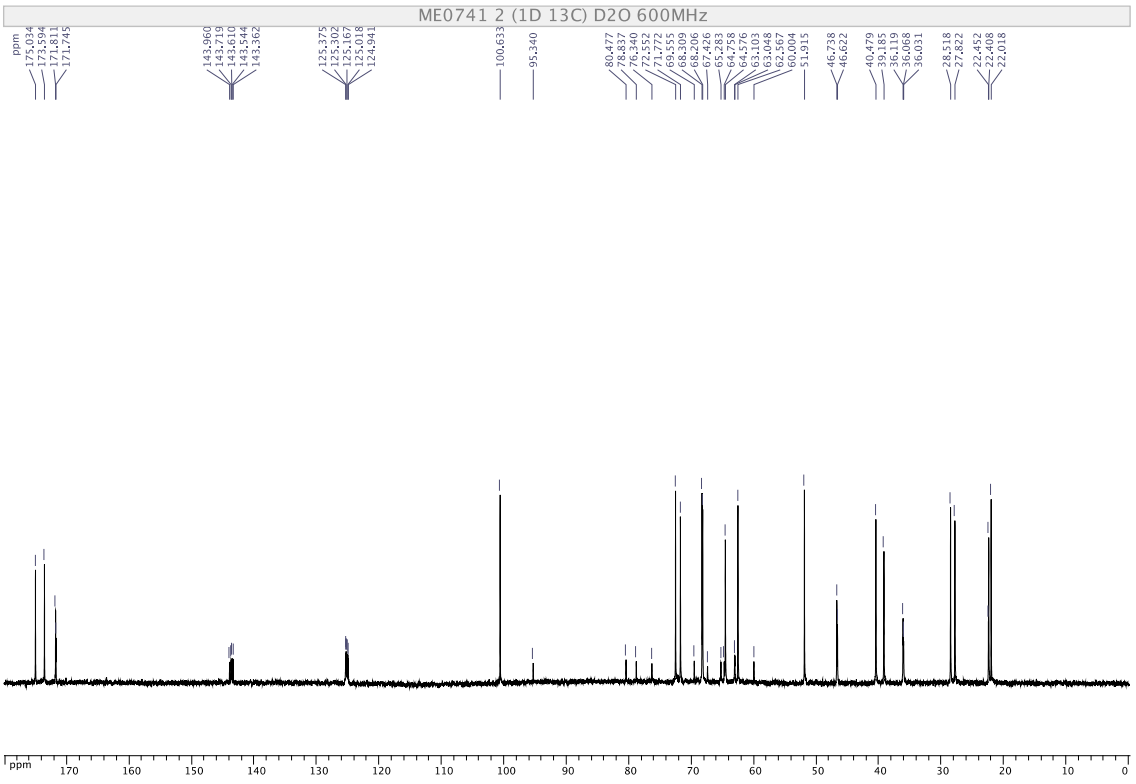
Compound 26


03rc_848 1 (1D 1H) MeOD 600MHz
ppm

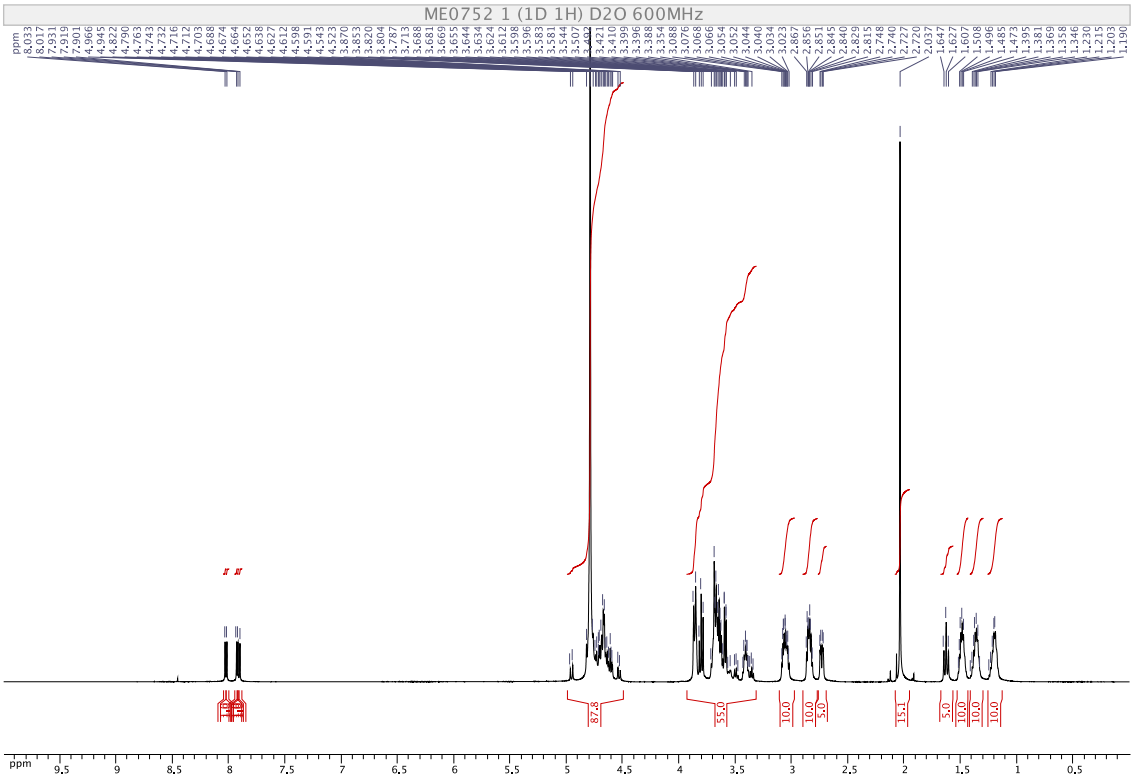

03rc_848 2 (1D 13C) MeOD 600MHz
ppm

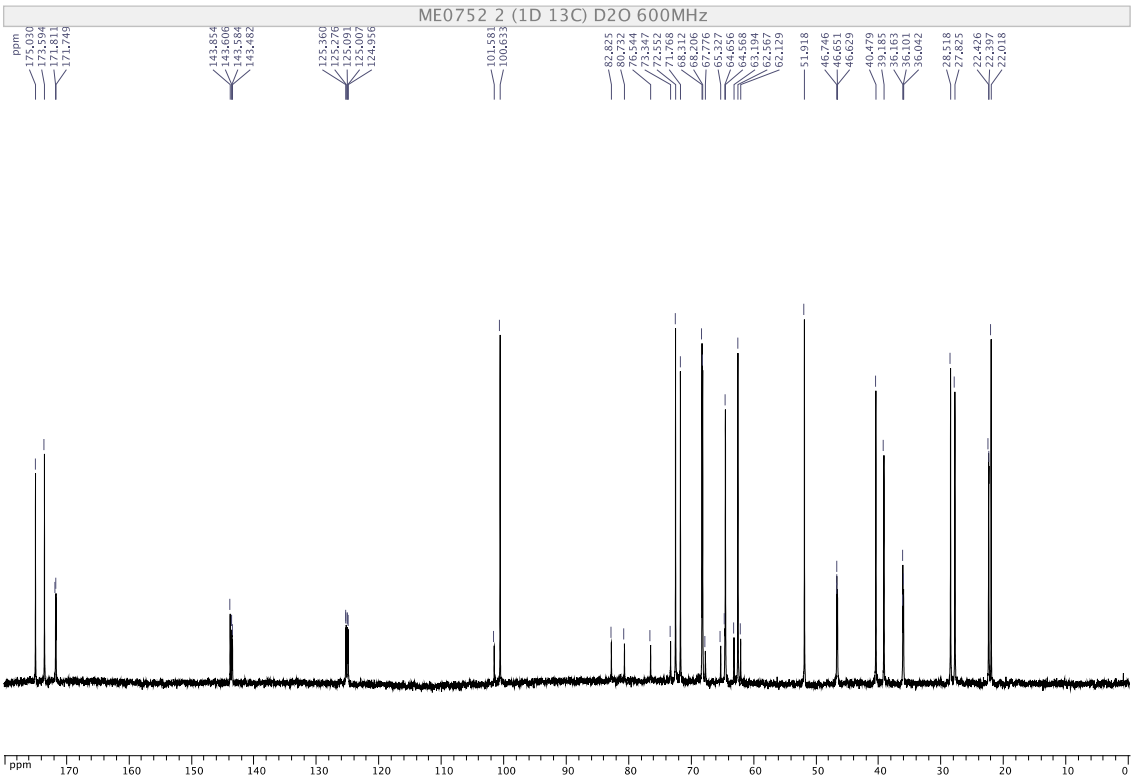
Compound 27



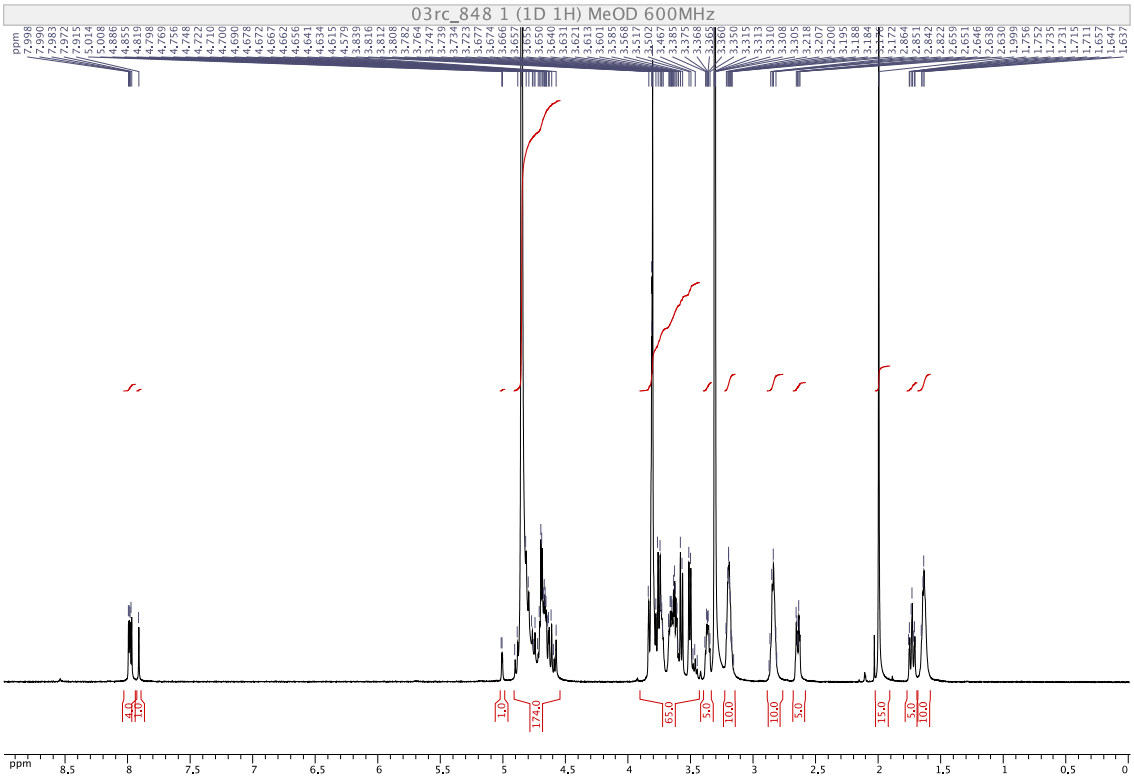


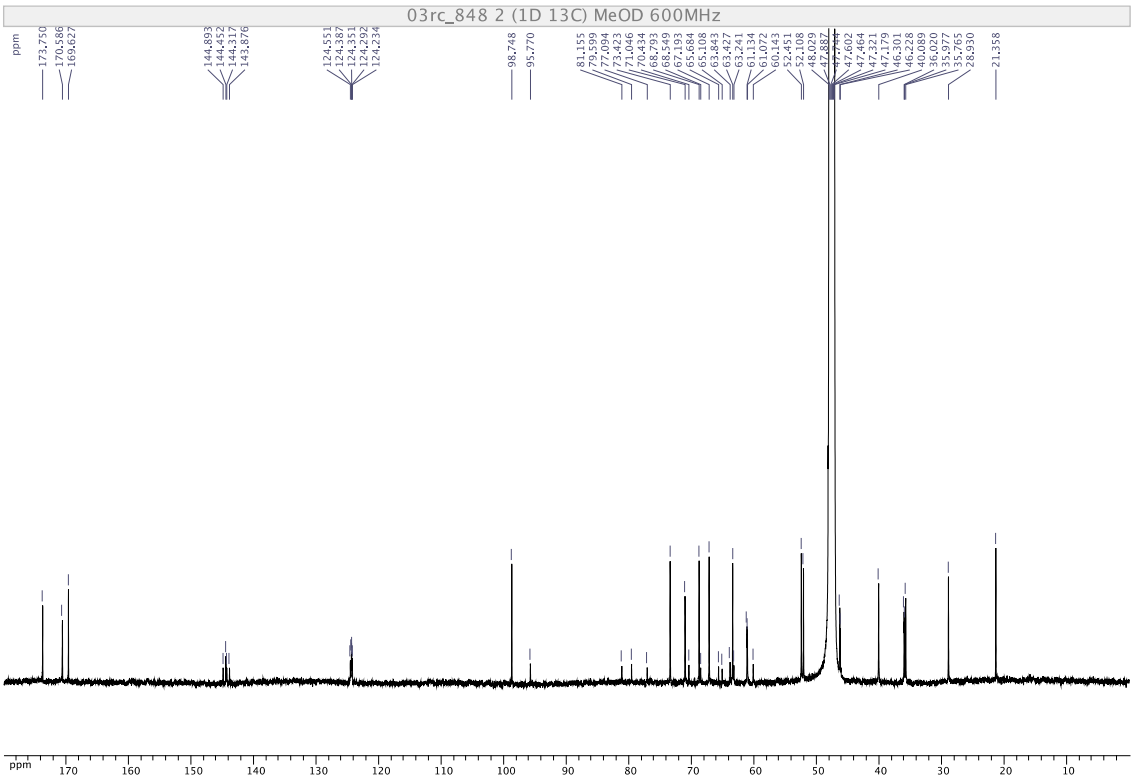
Compound 28


ME0752 1 (1D 1H) D2O 600MHz
ppm
87.6
55.0
10.0
10.0
5.0
15.1
5.0
10.0
10.0
10.0

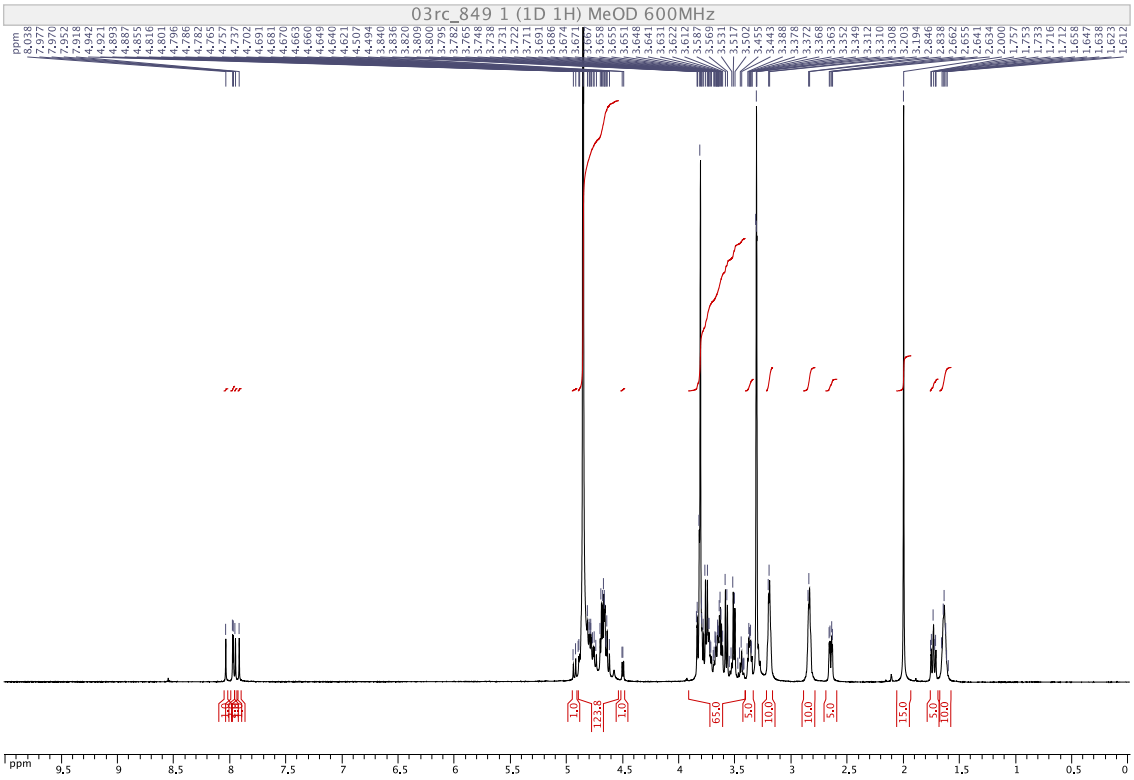

ME0752 2 (1D 13C) D2O 600MHz
ppm

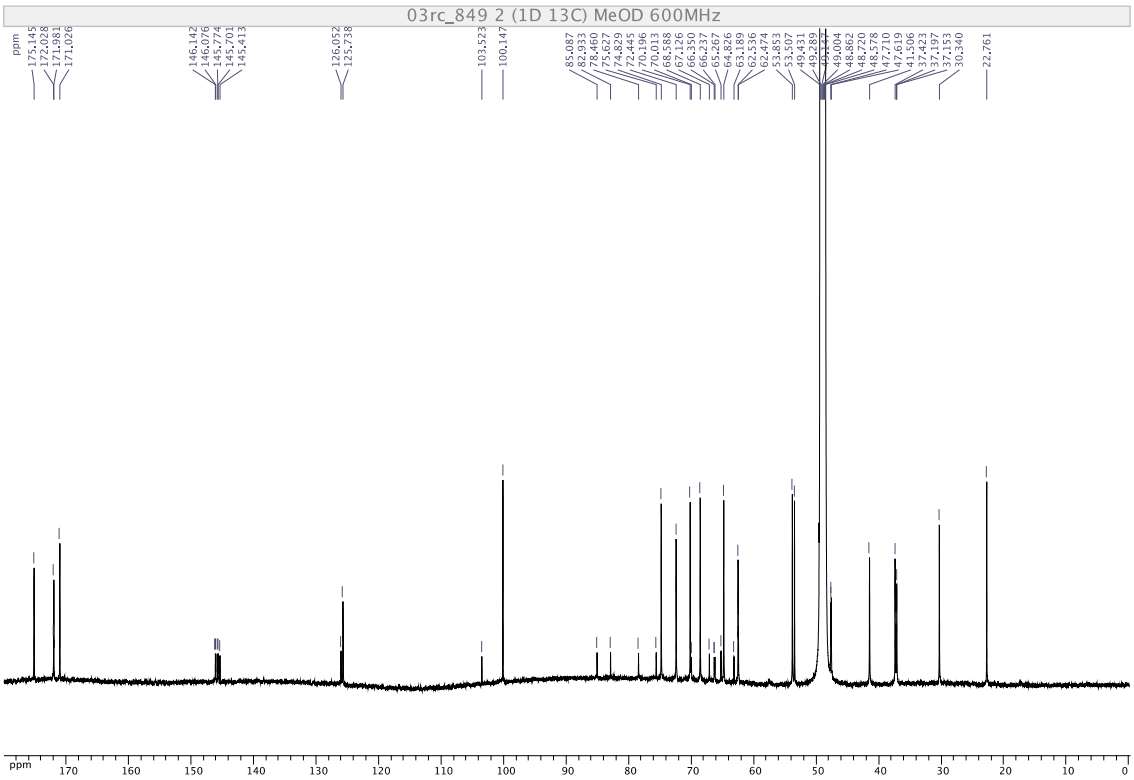
Compound 29



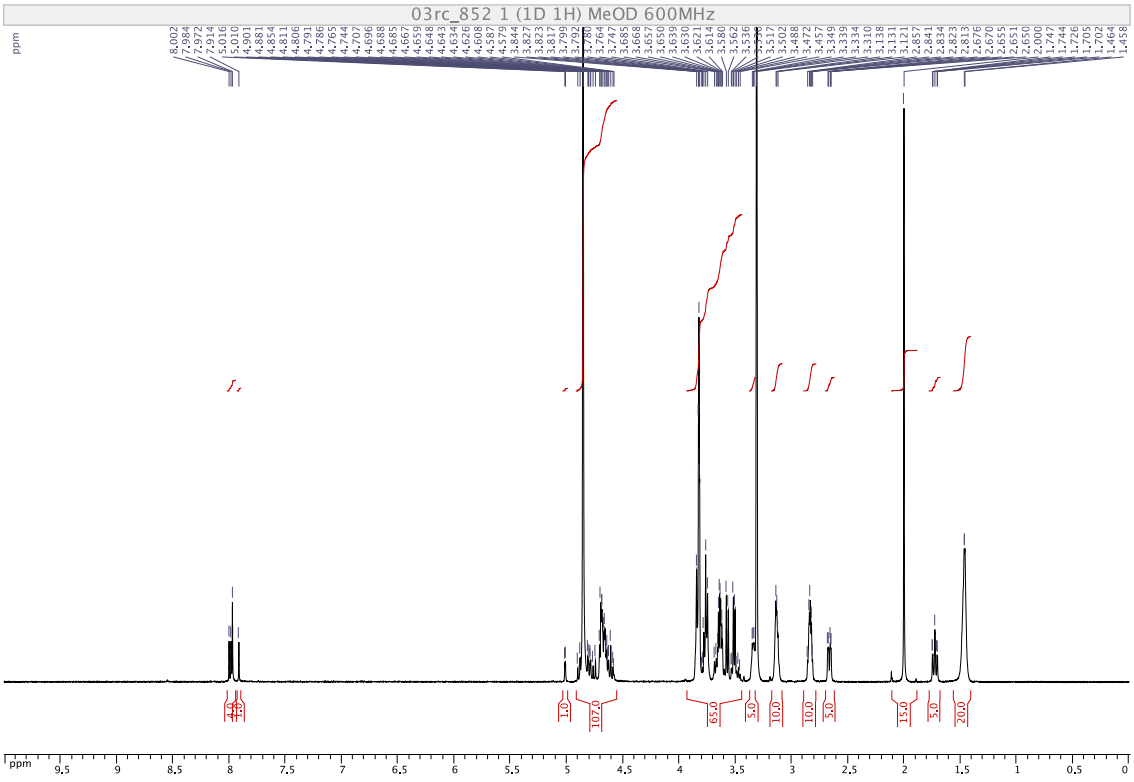


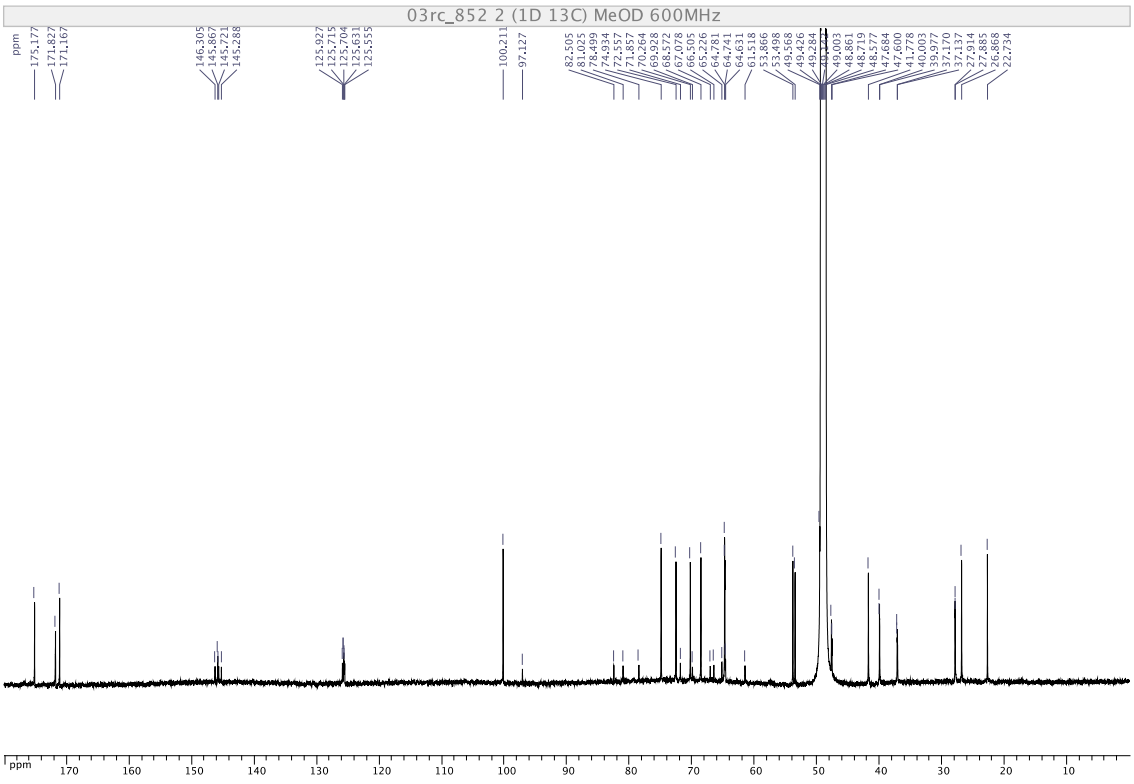
Compound 30



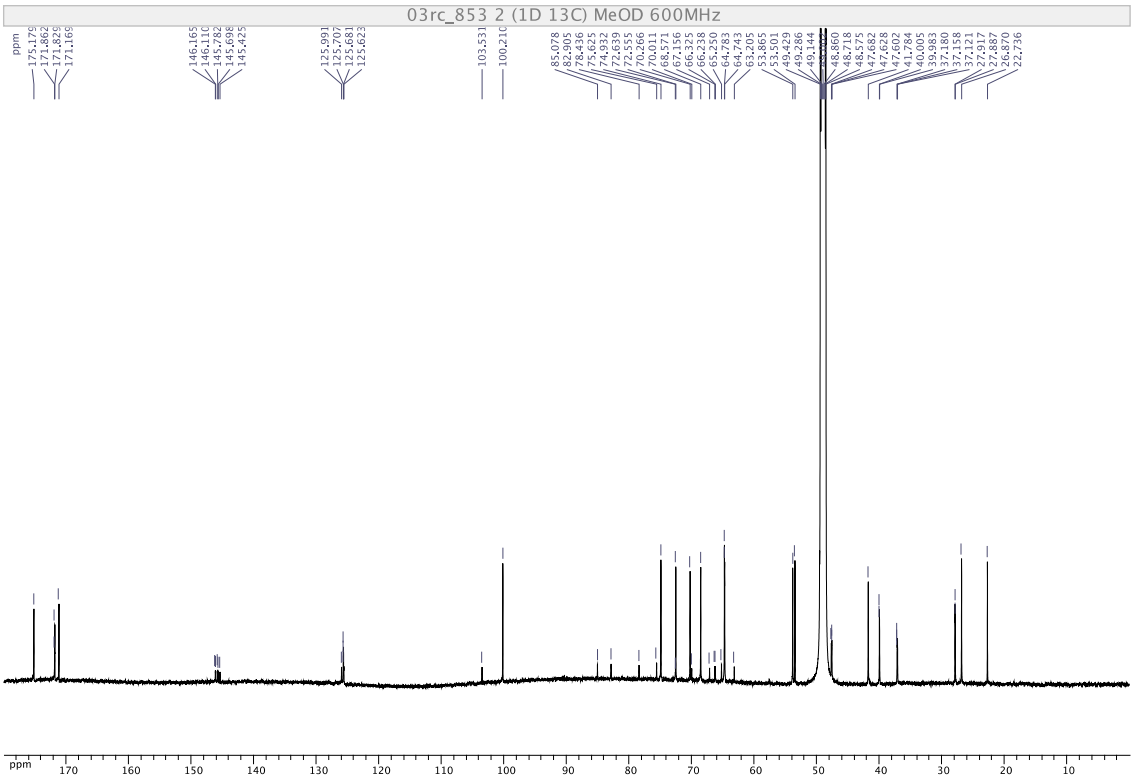


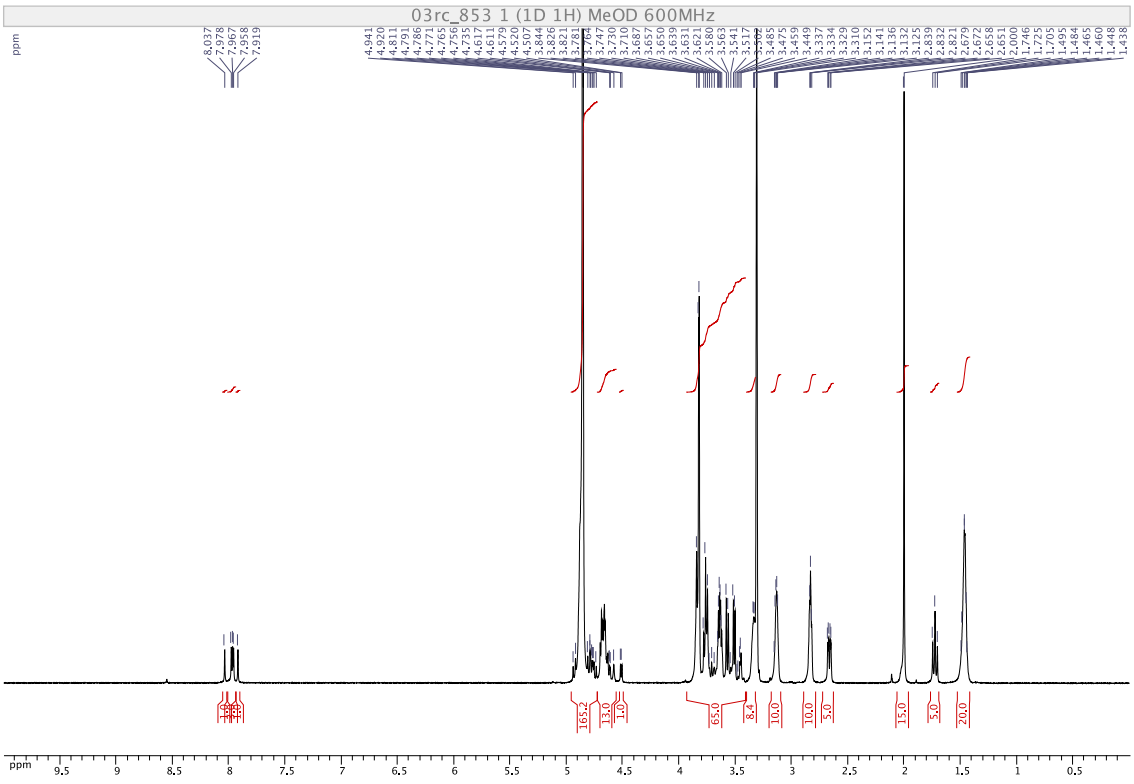
Compound 31


03rc_852 1 (1D 1H) MeOD 600MHz
ppm

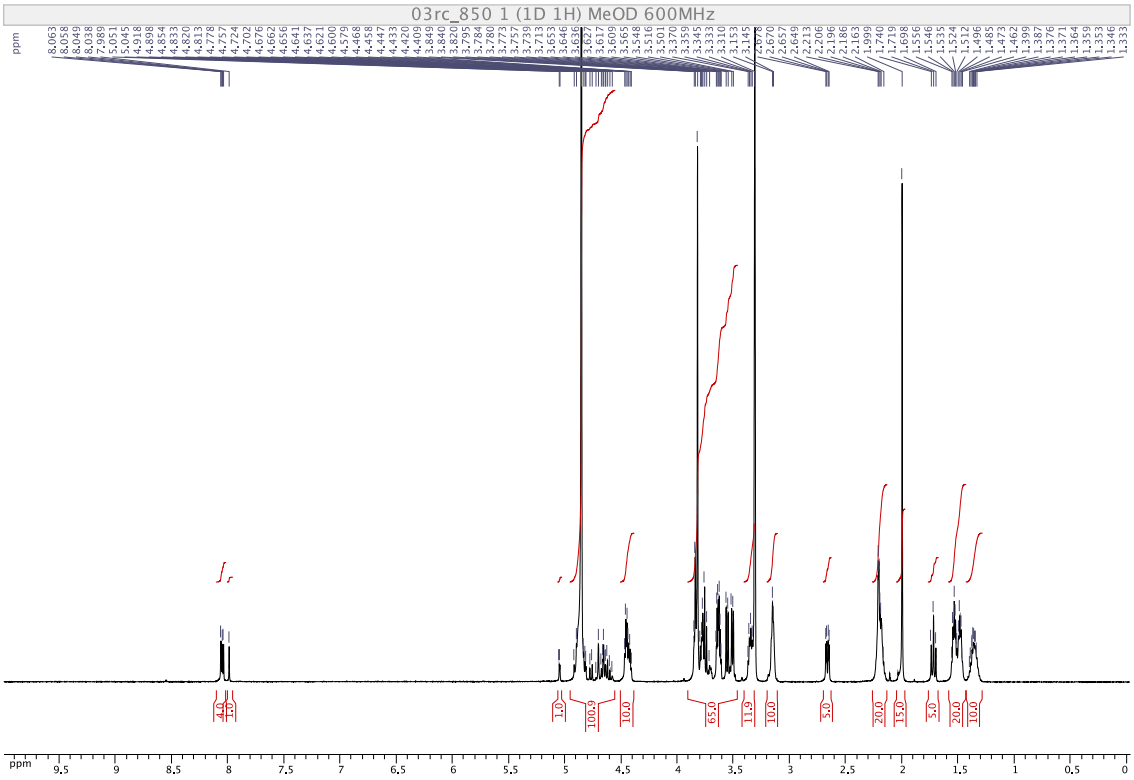

03rc_852 2 (1D 13C) MeOD 600MHz
ppm

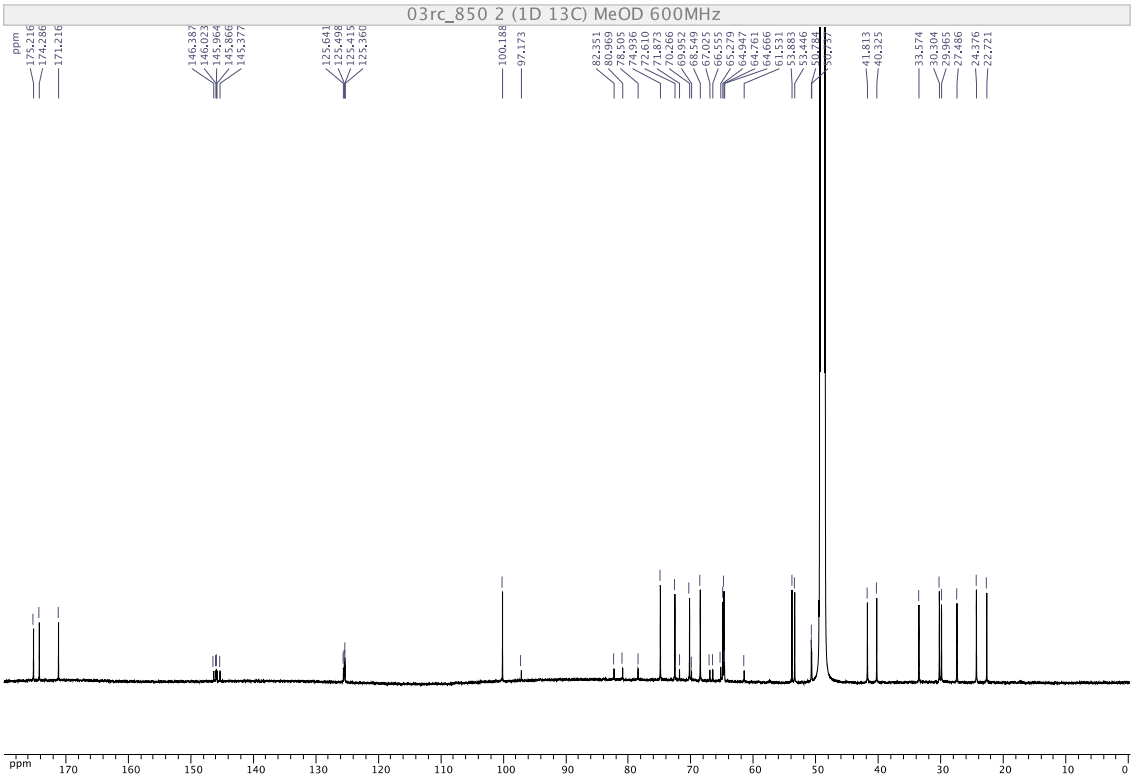
Compound 32


03rc_853 2 (1D 13C) MeOD 600MHz
ppm

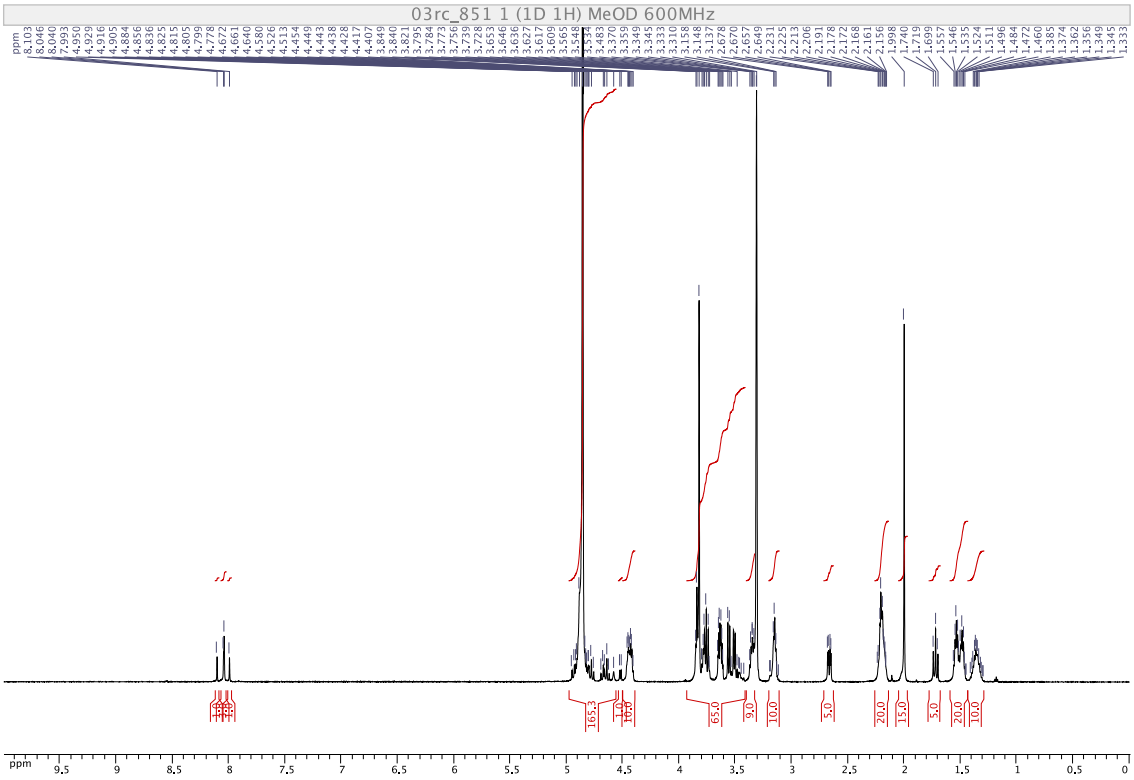

03rc_853 1 (1D 1H) MeOD 600MHz
ppm

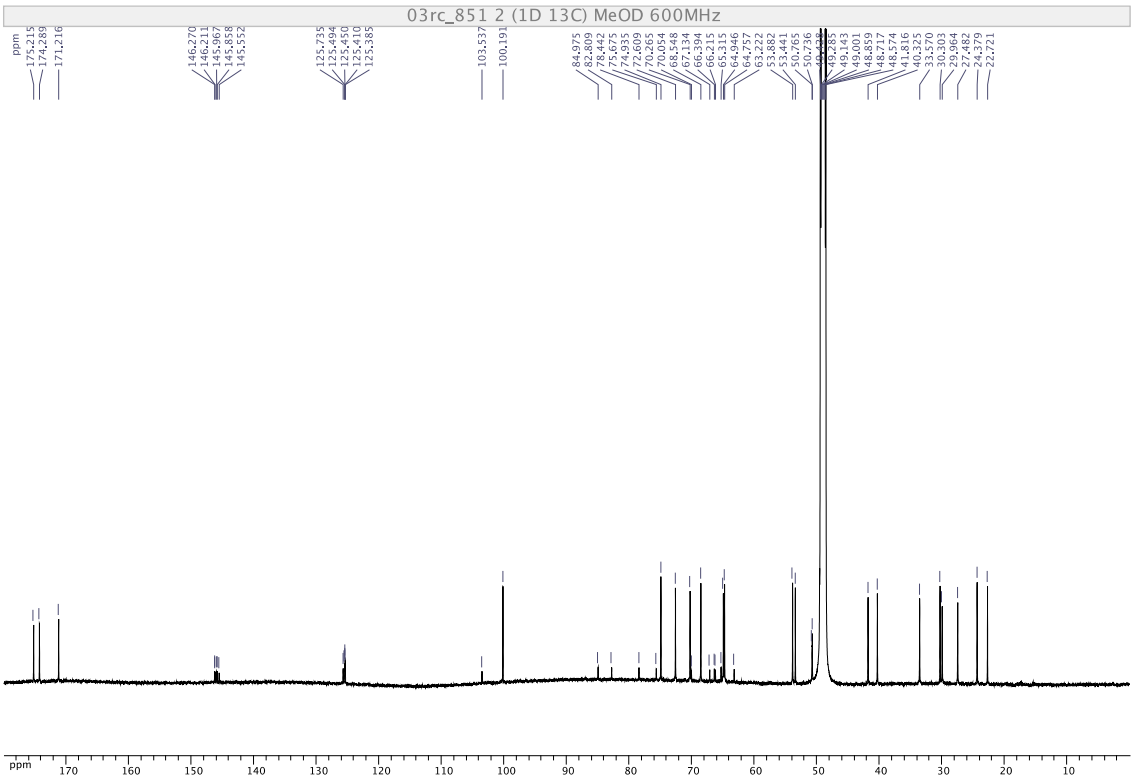
Compound 33



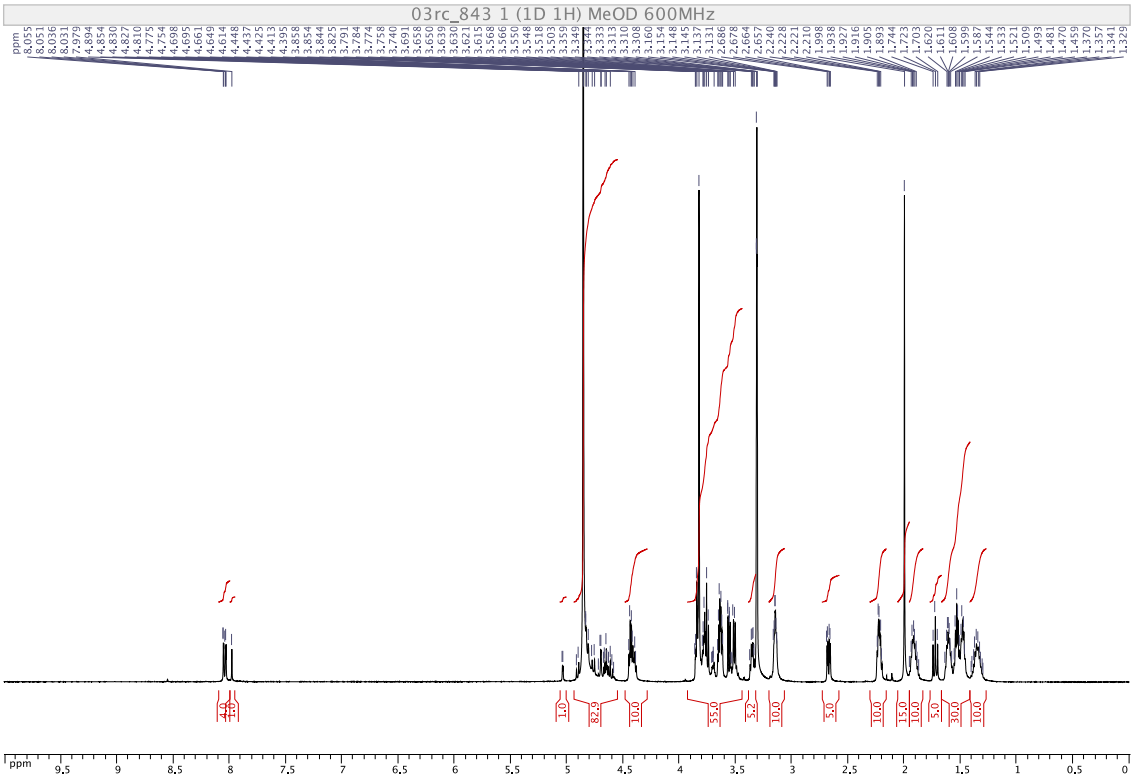


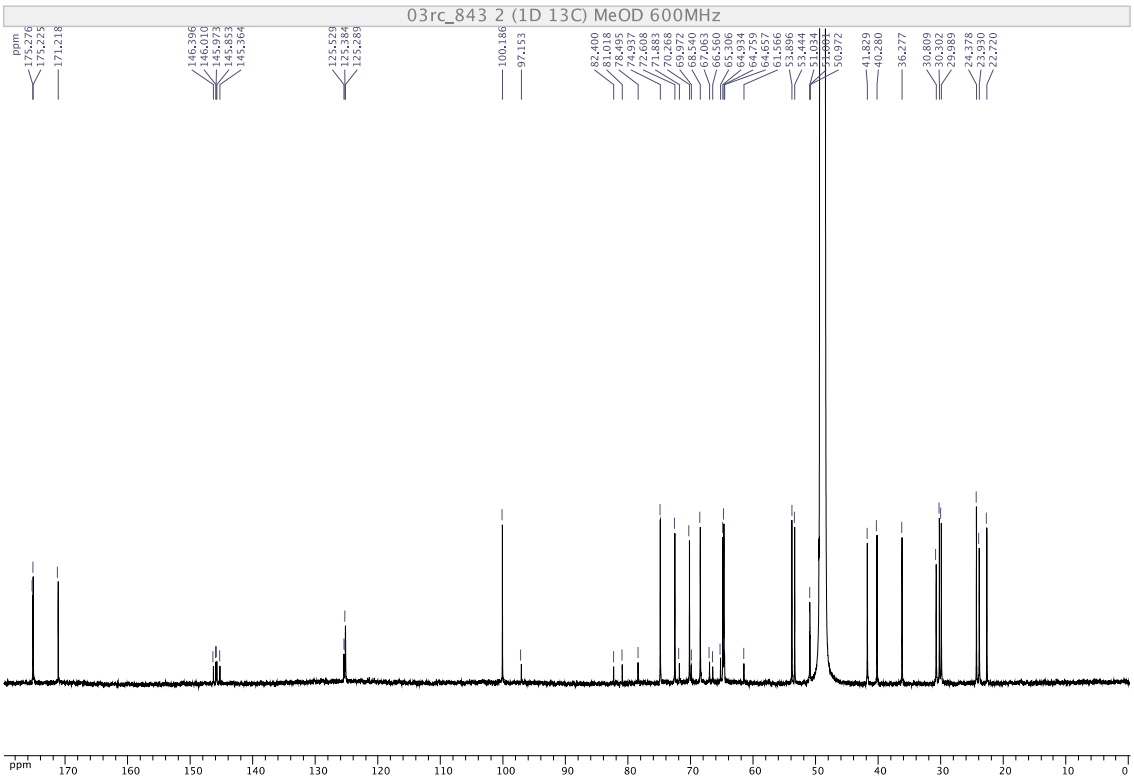
Compound 34



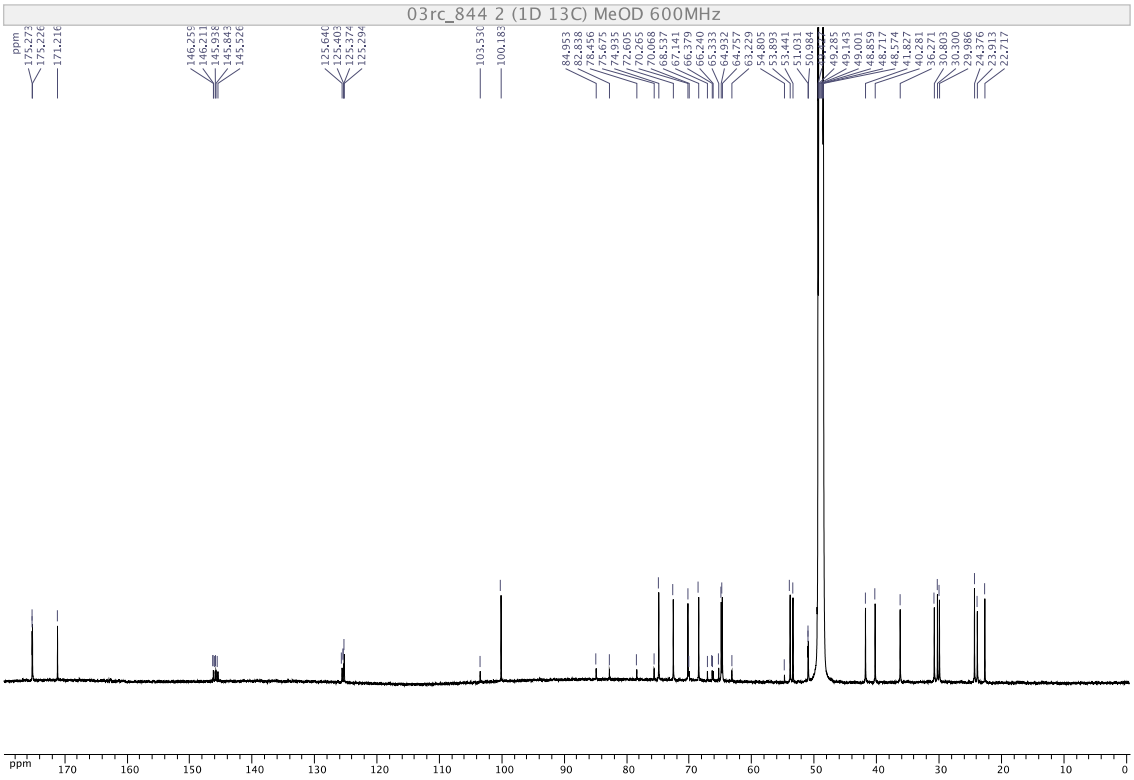


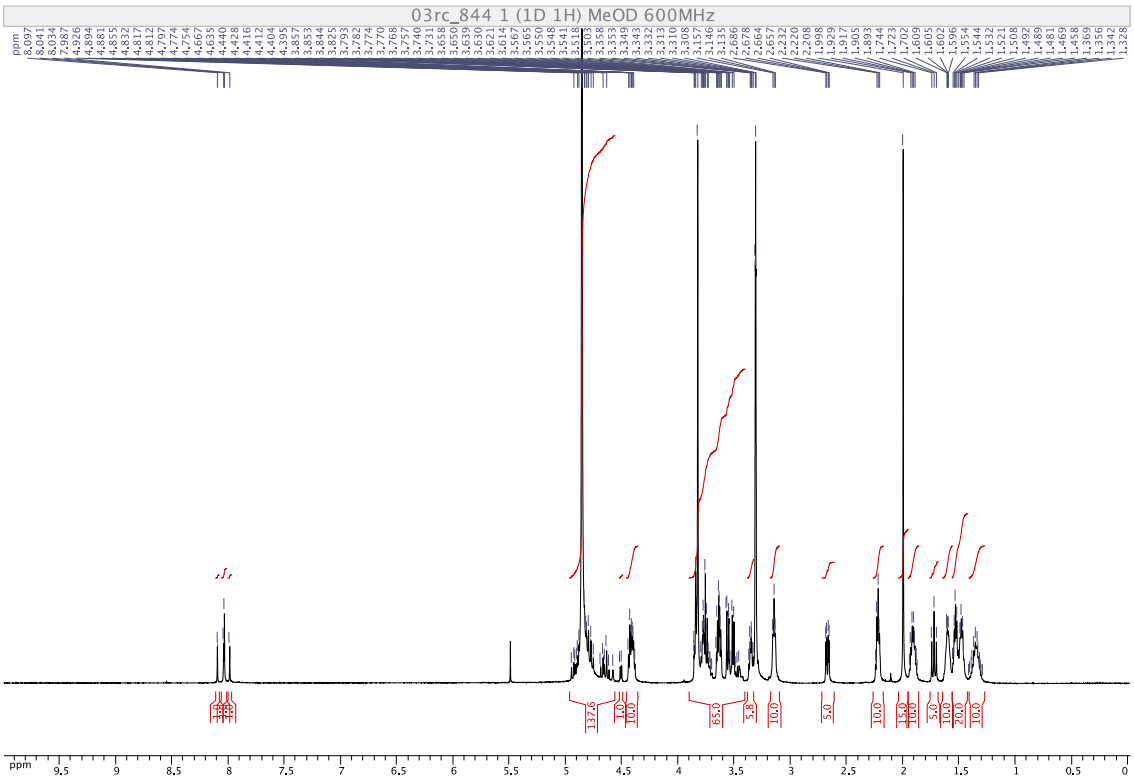
Compound 35



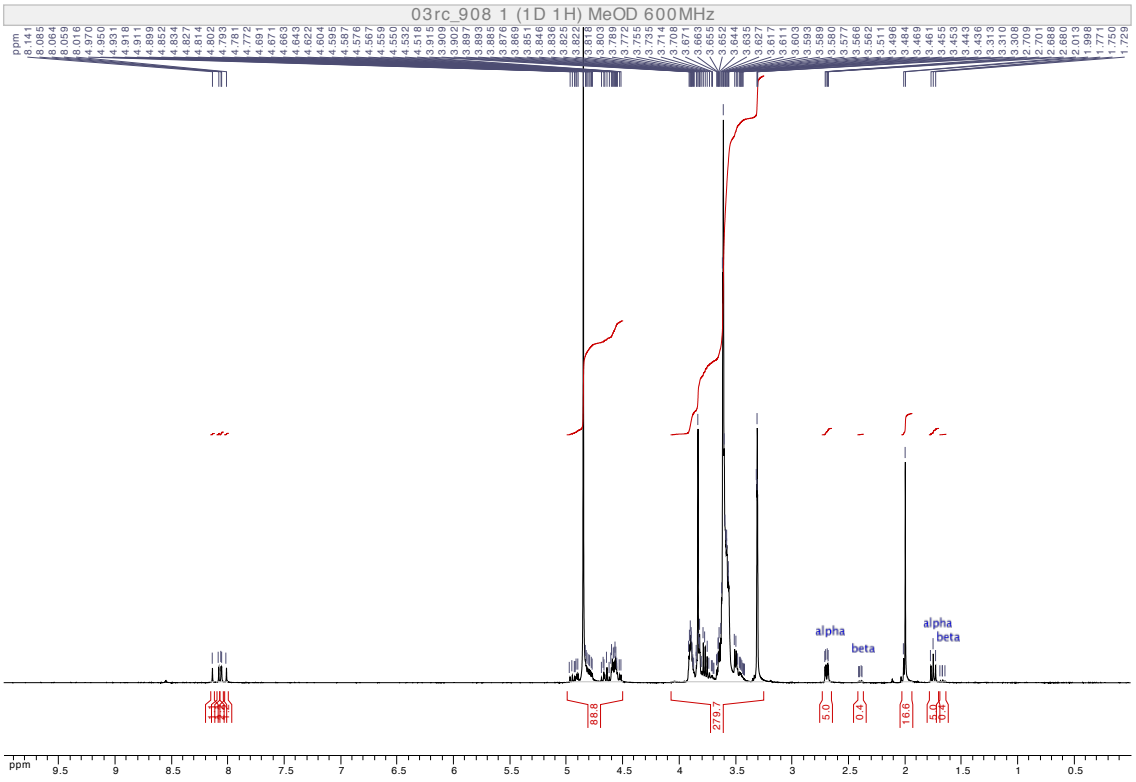


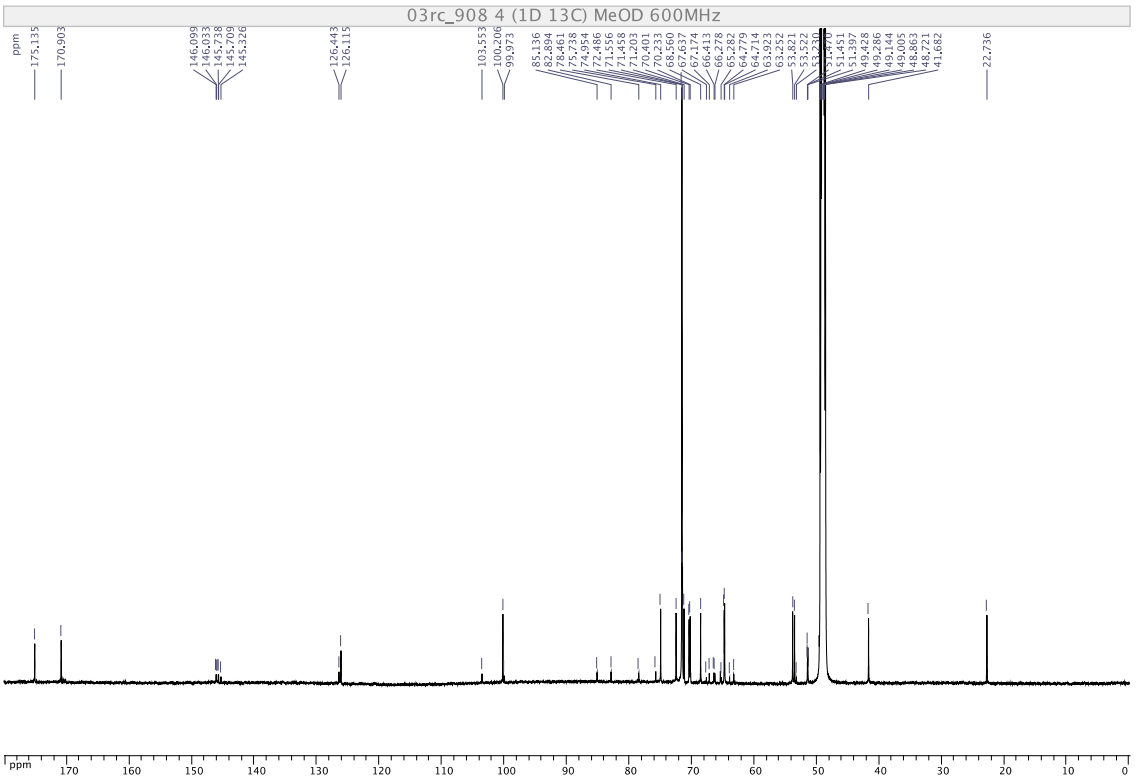
Compound 36



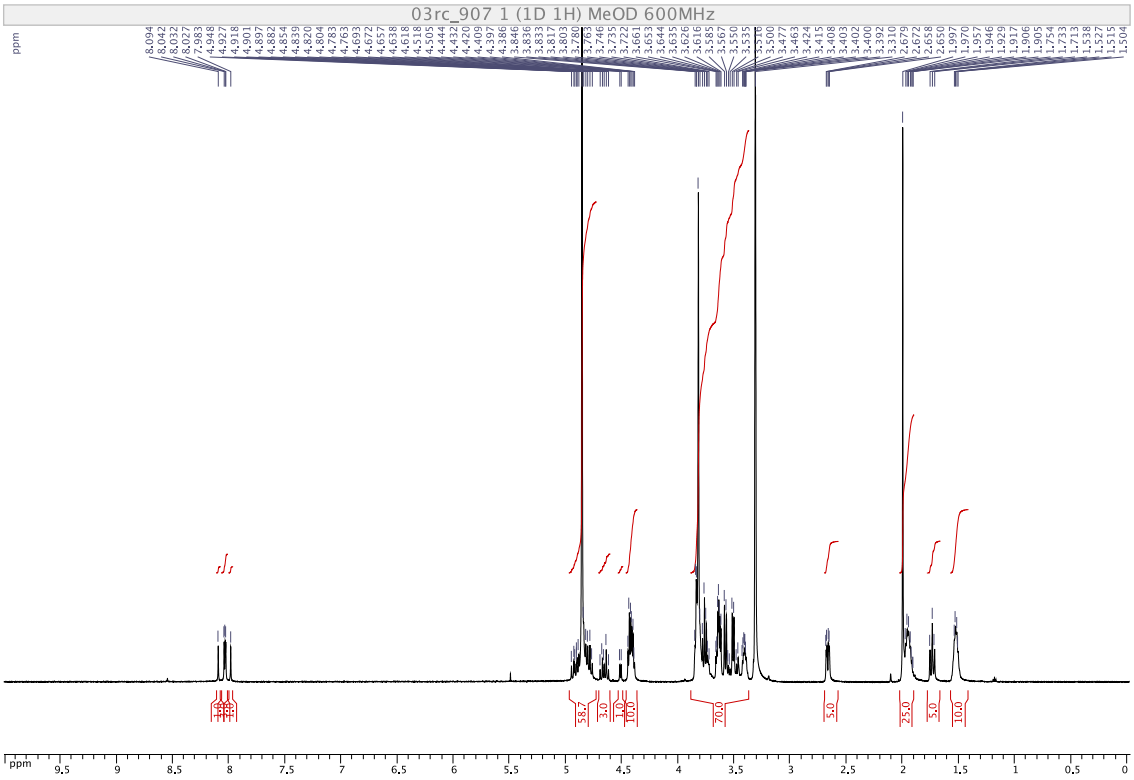


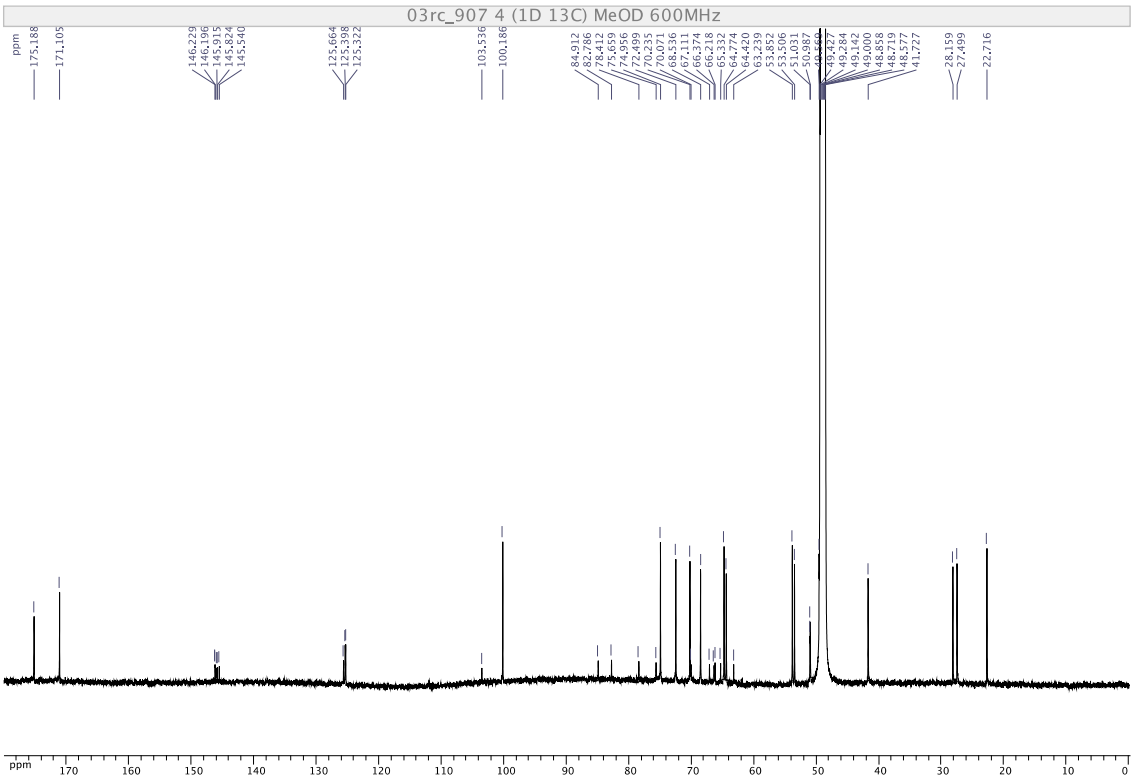
Compound 37


03rc_908 1 (1D 1H) MeOD 600MHz
alpha
beta
alpha
beta
88.8
279.7
5.0
0.4
16.6
5.0
0.4

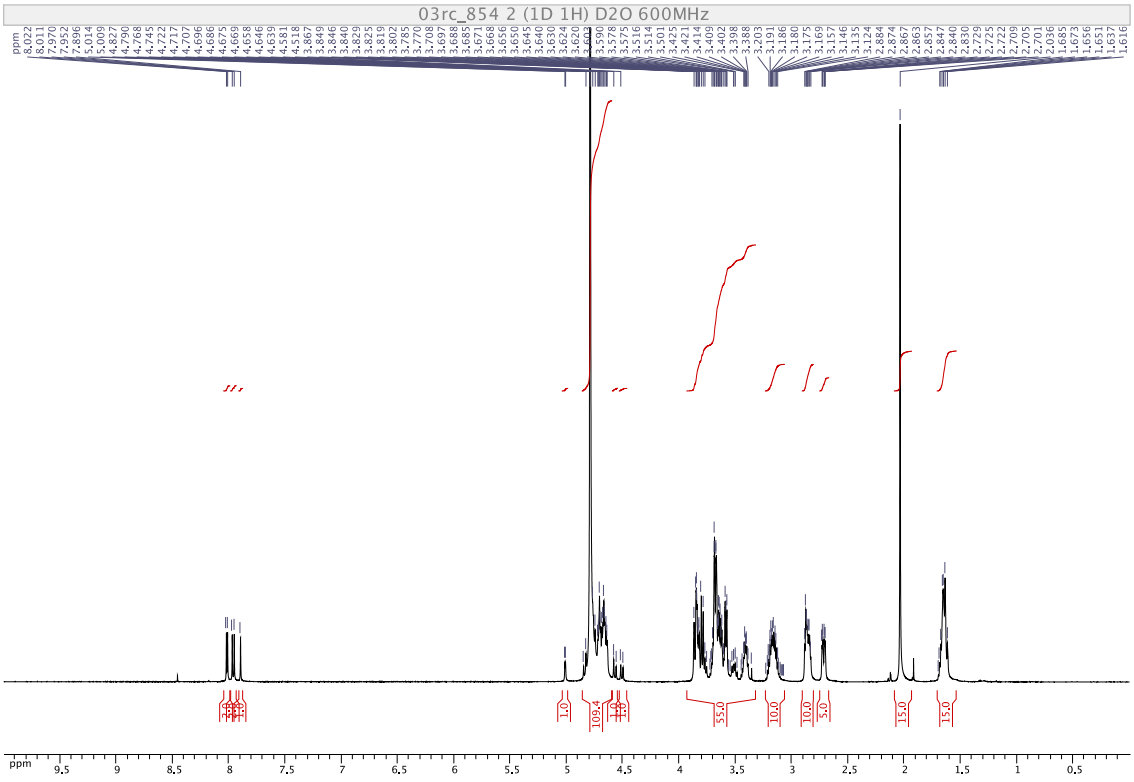

03rc_908 4 (1D 13C) MeOD 600MHz

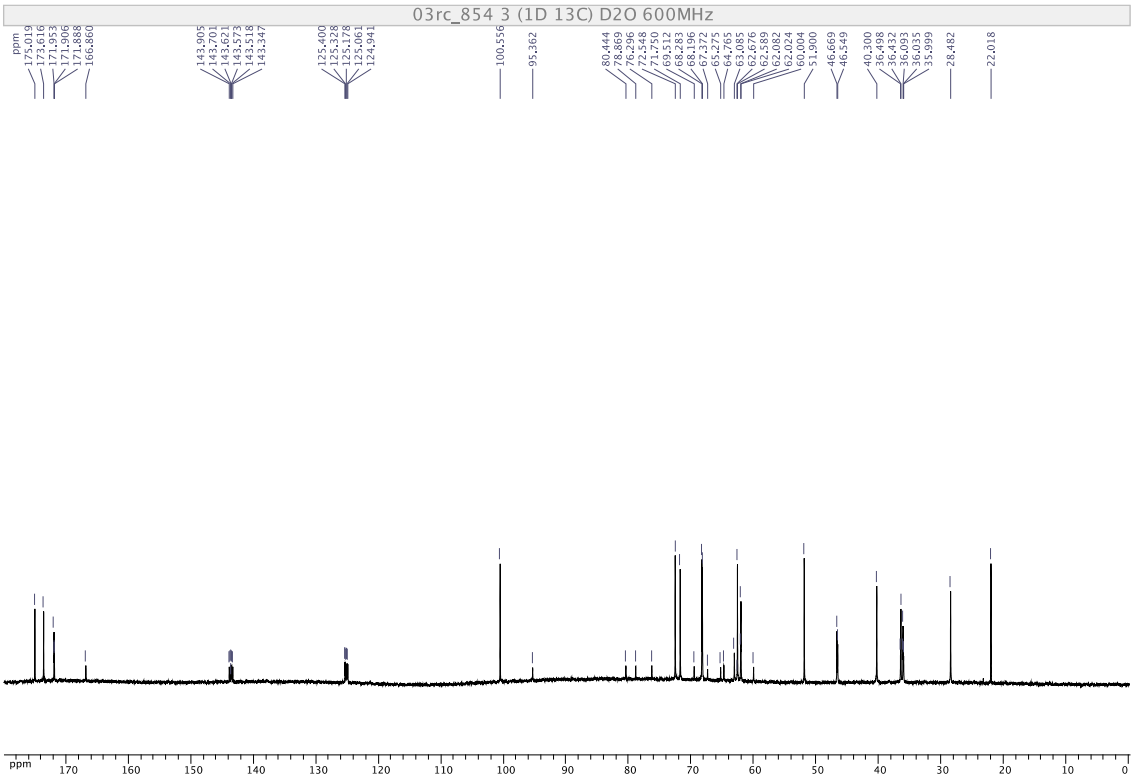
Compound 38


03rc_907 1 (1D 1H) MeOD 600MHz
ppm
9.5
9
8.5
8
7.5
7
6.5
6
5.5
5
4.5
4
3.5
3
2.5
2
1.5
1
0.5
0
58.7
3.0
1.0
10.0
70.0
5.0
25.0
5.0
10.0

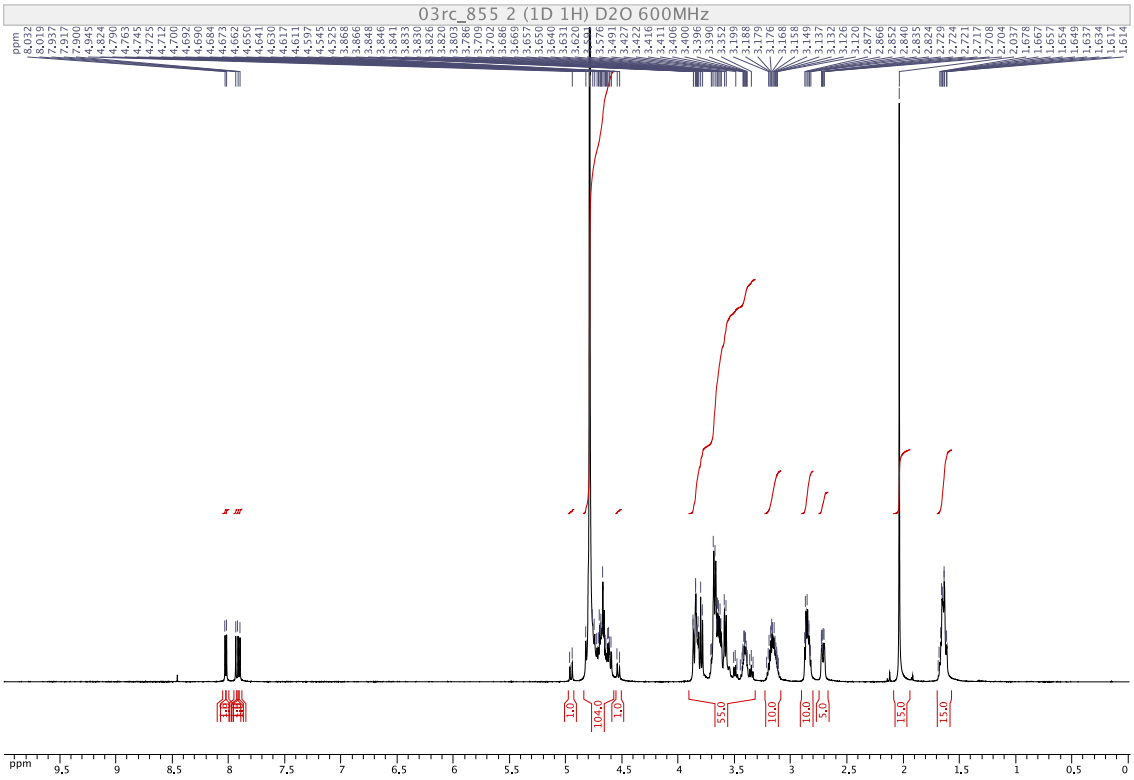

03rc_907 4 (1D 13C) MeOD 600MHz
ppm
175.188
171.105
146.229
146.196
145.915
145.824
145.540
125.664
125.398
125.322
103.536
100.186
84.912
82.786
78.412
75.518
74.956
72.499
70.235
70.071
68.536
67.111
66.274
66.218
65.332
64.774
64.420
63.239
53.852
53.506
53.191
50.987
49.427
49.284
49.142
49.000
48.858
48.719
48.577
41.727
28.159
27.499
22.716
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0

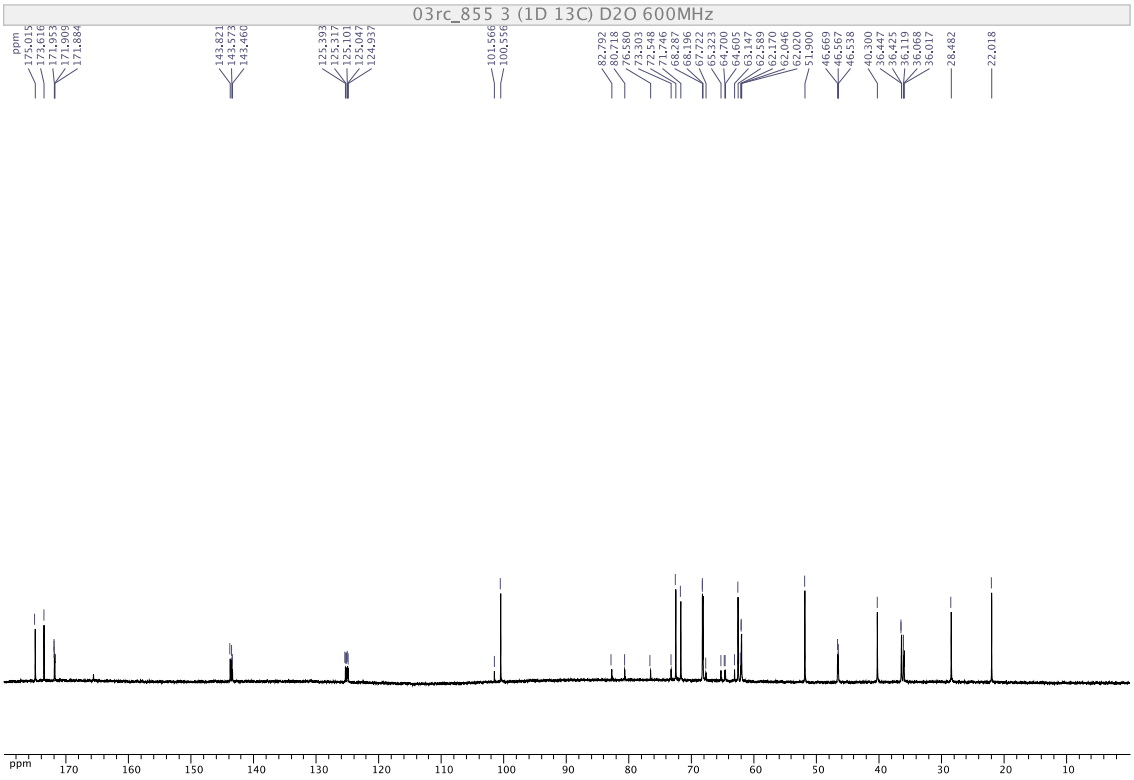
Compound 39



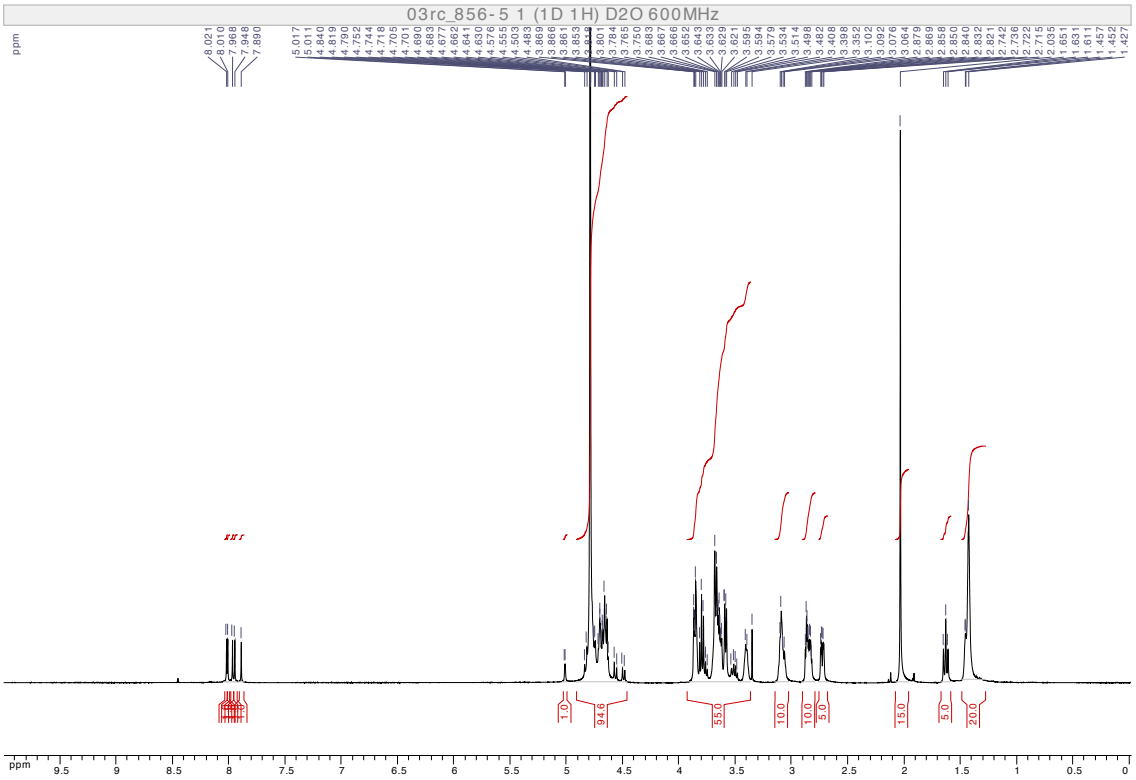


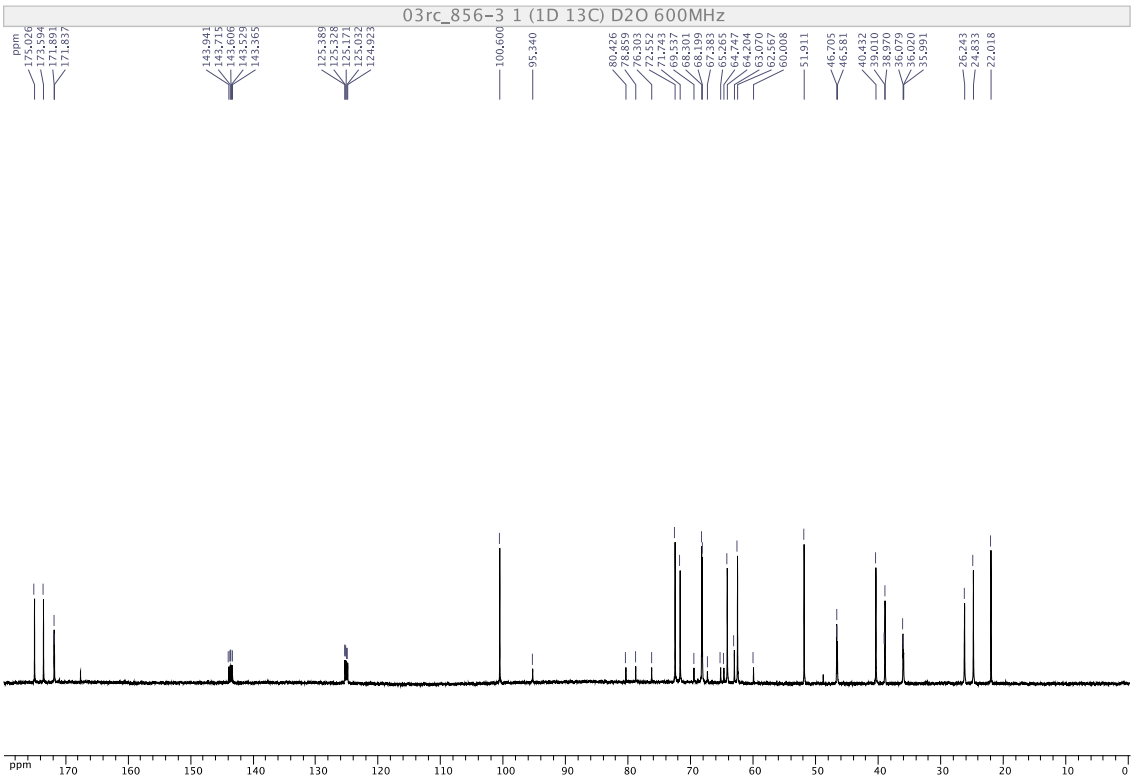
Compound 40



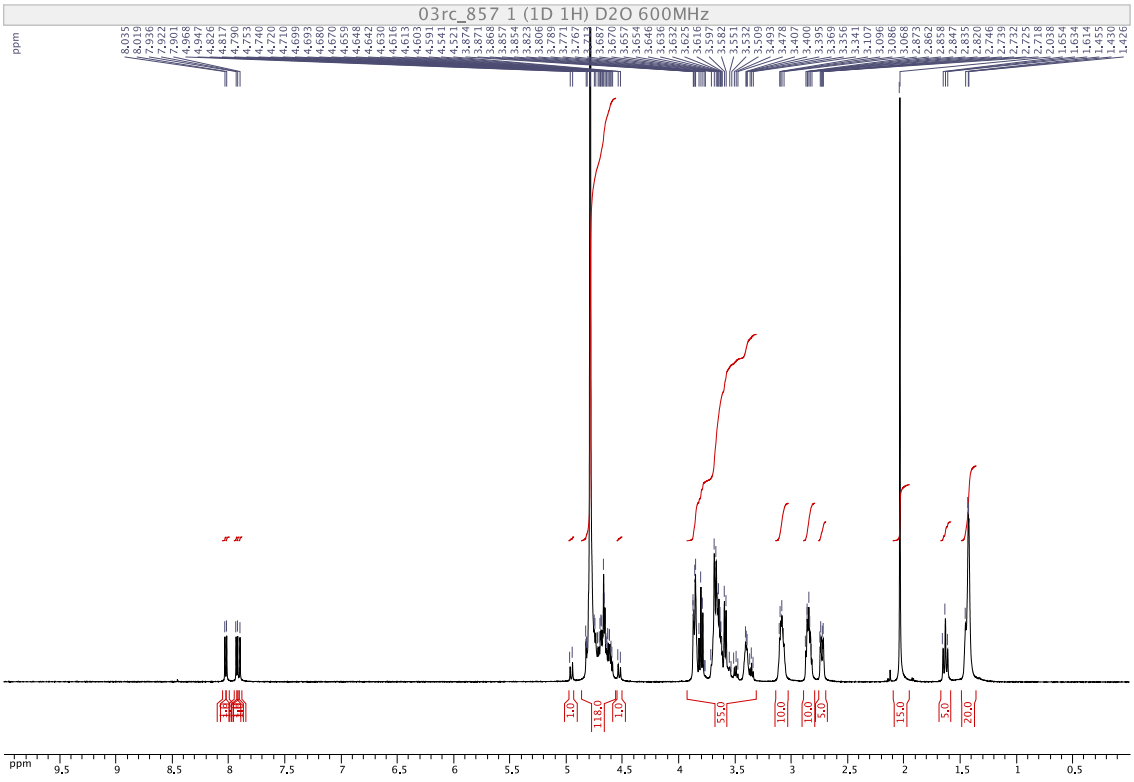


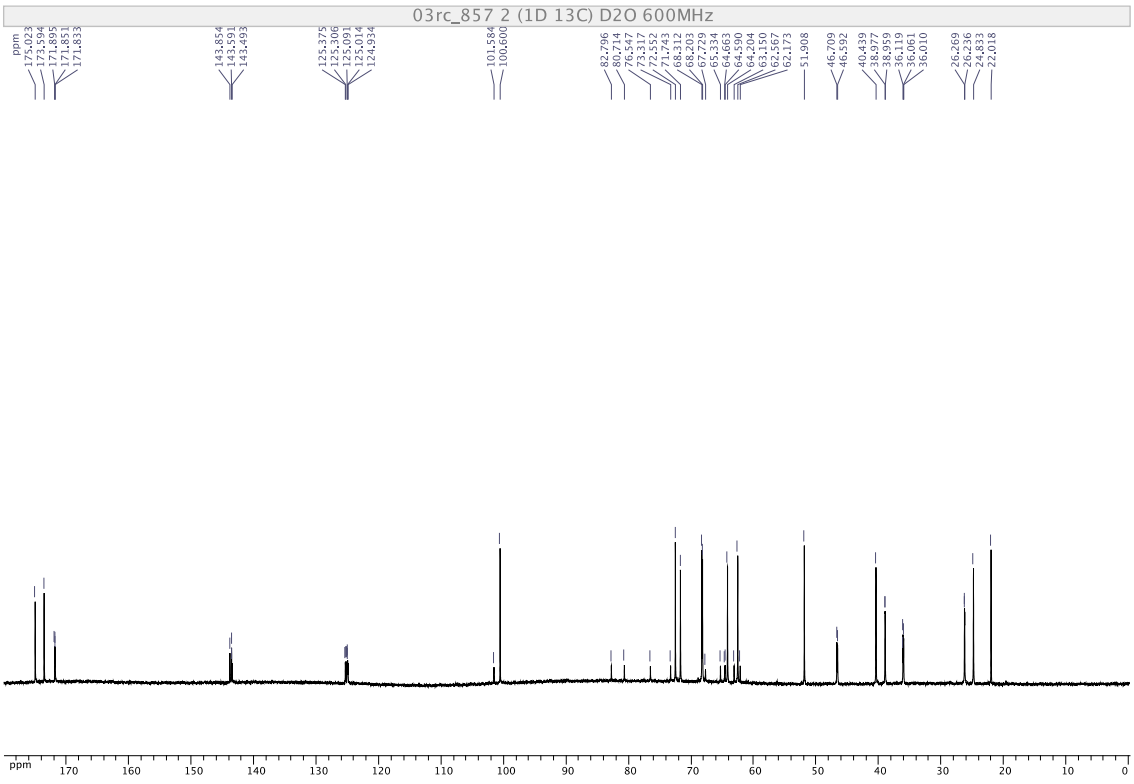
Compound 41



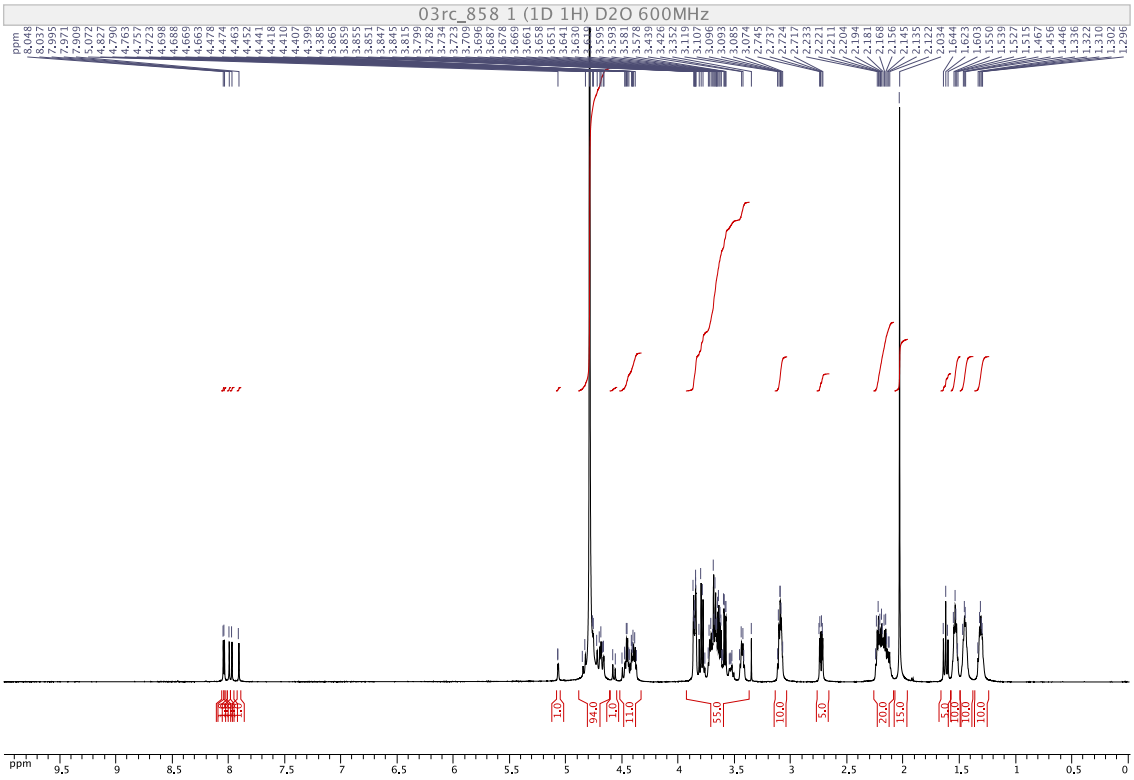


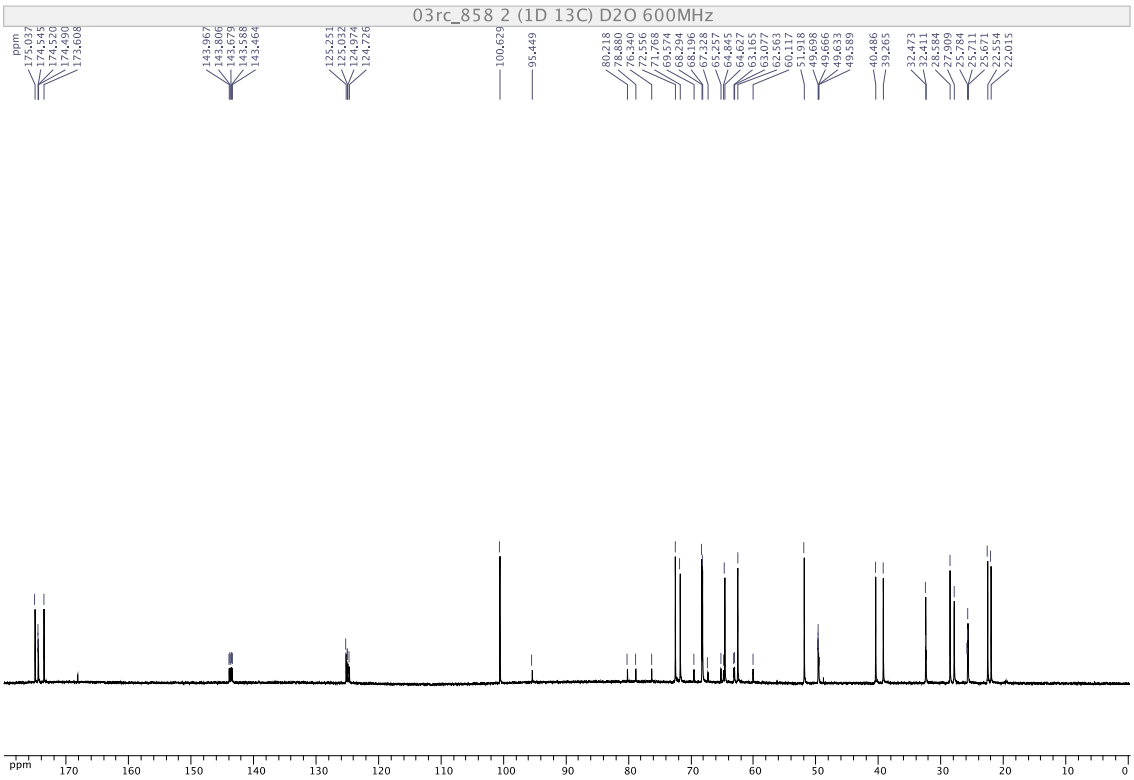
Compound 42


03rc_857 1 (1D 1H) D2O 600MHz
ppm
1.0
118.0
1.0
55.0
10.0
10.0
5.0
15.0
5.0
20.0

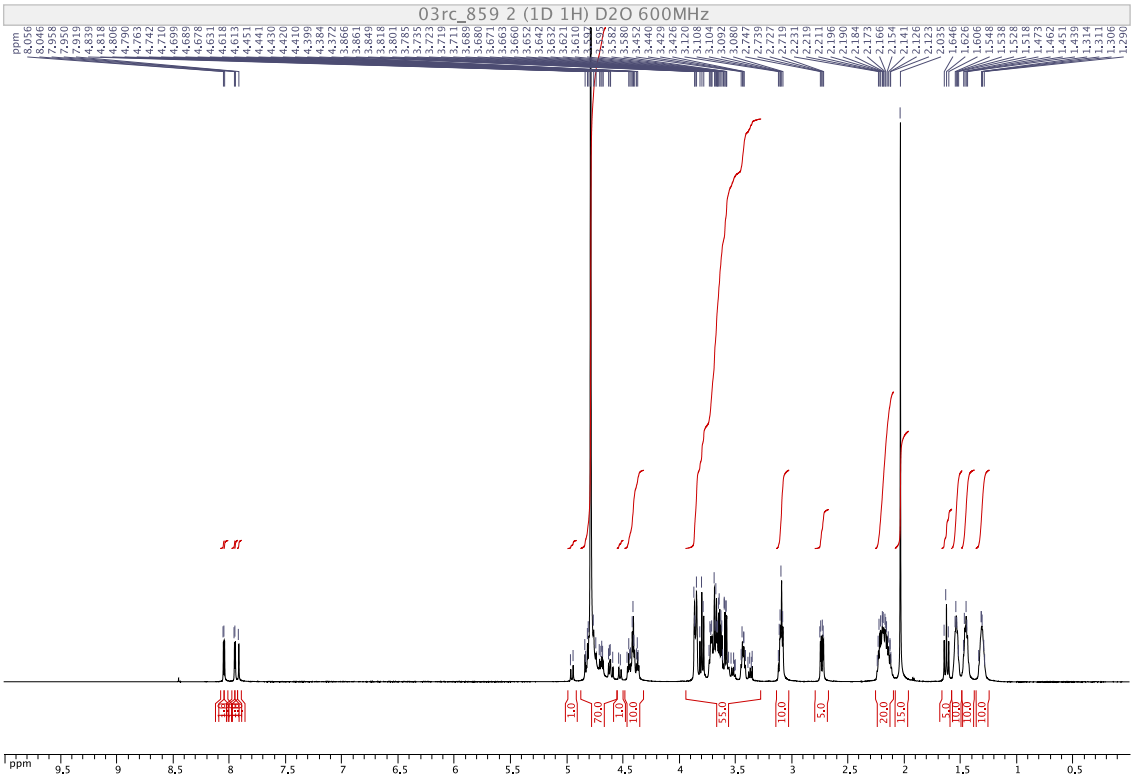

03rc_857 2 (1D 13C) D2O 600MHz
ppm

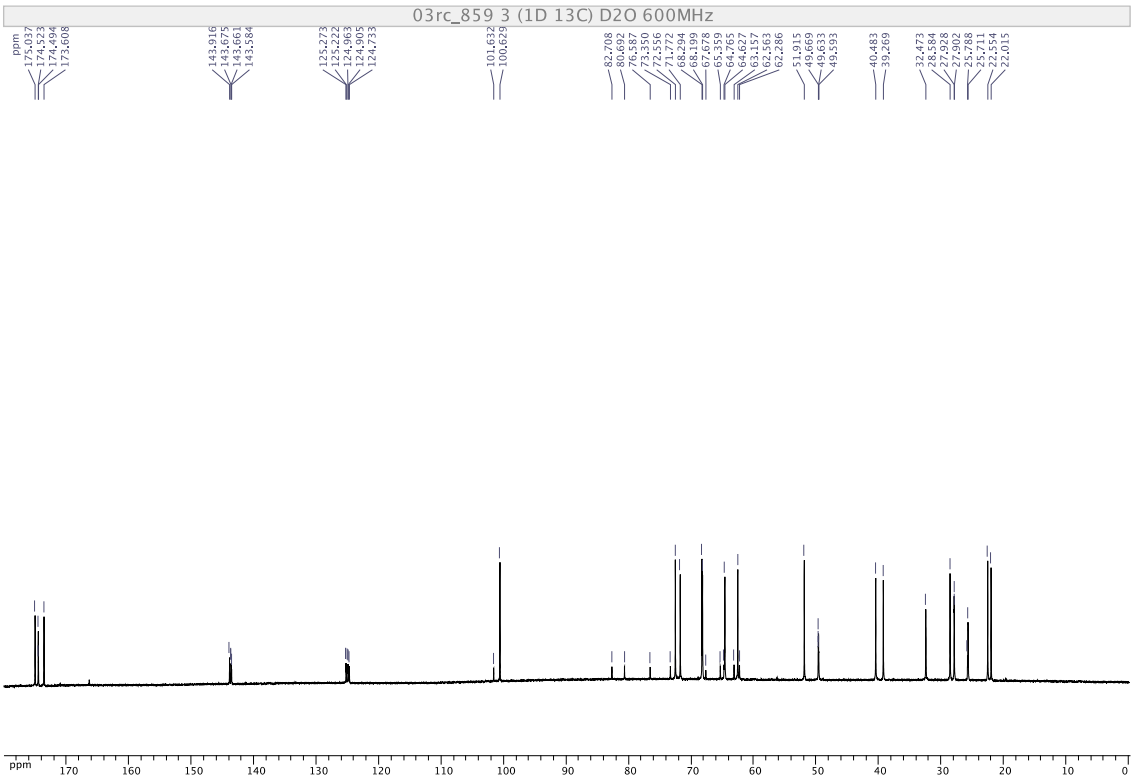
Compound 43



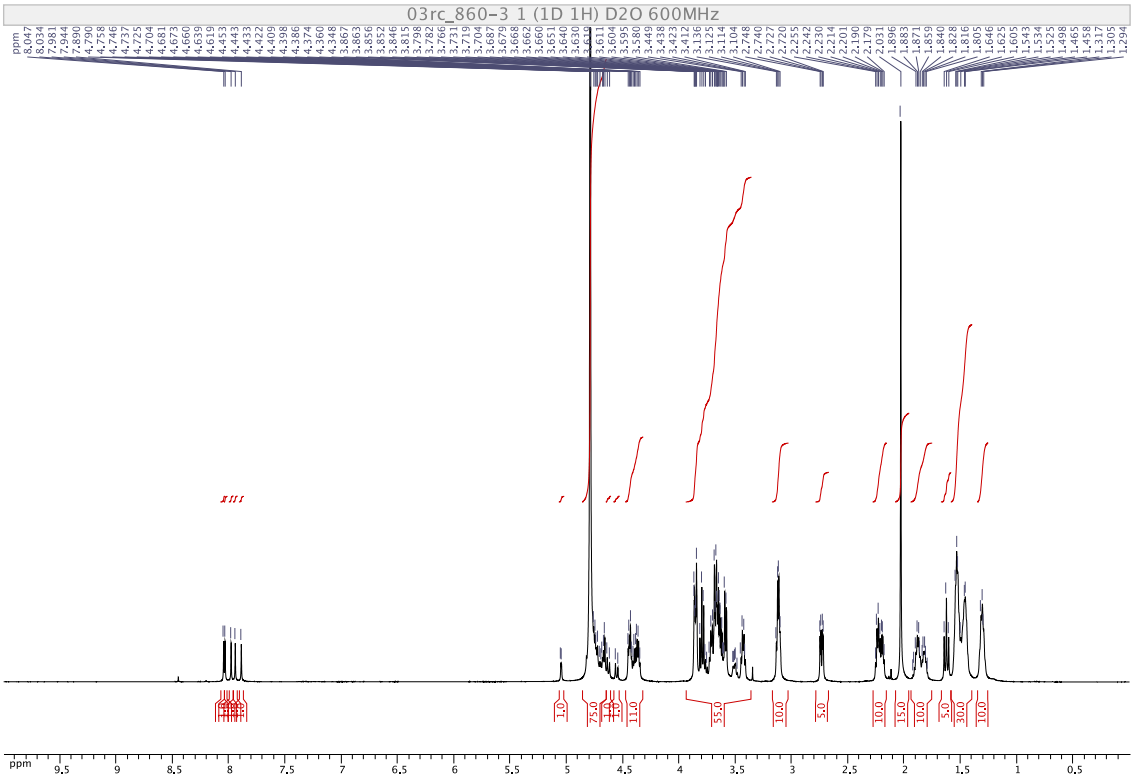


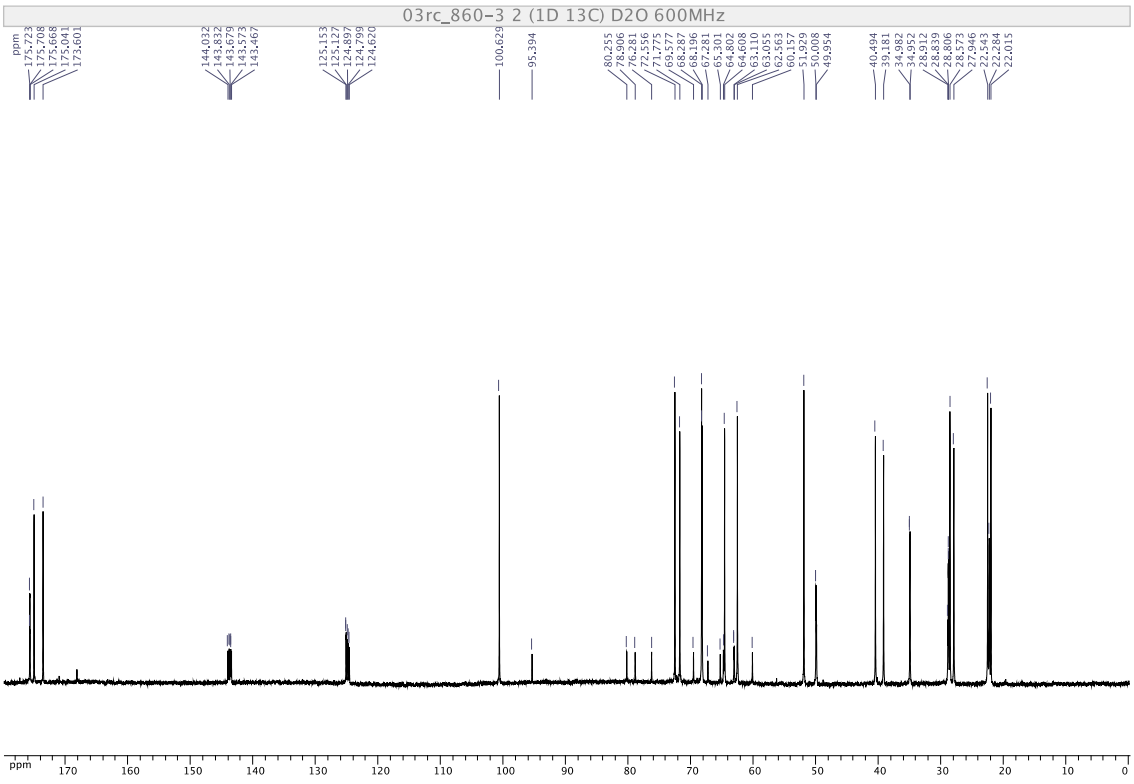
Compound 44



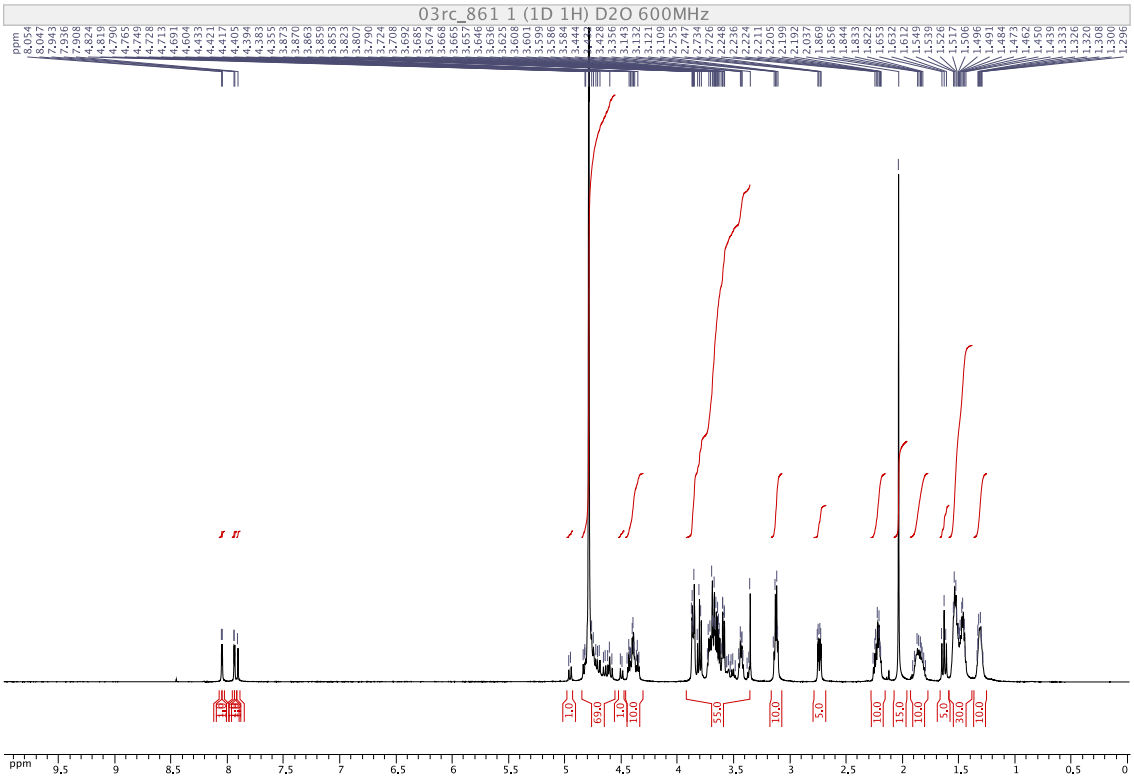


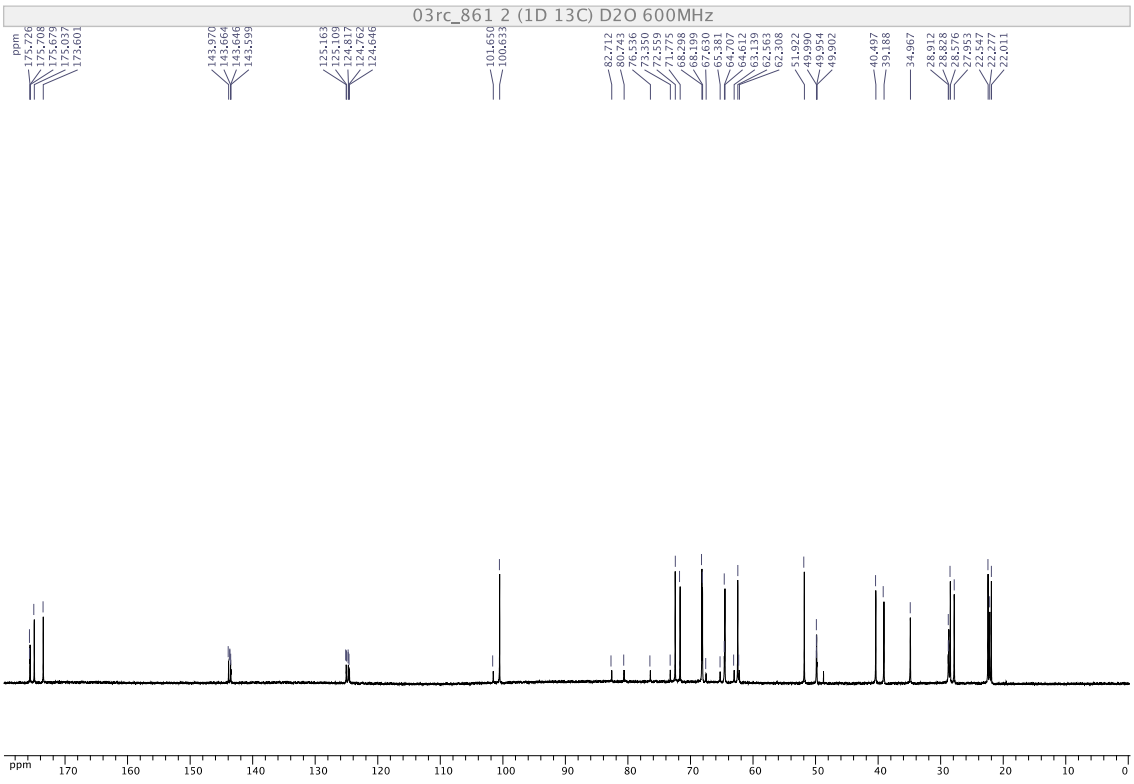
Compound 45



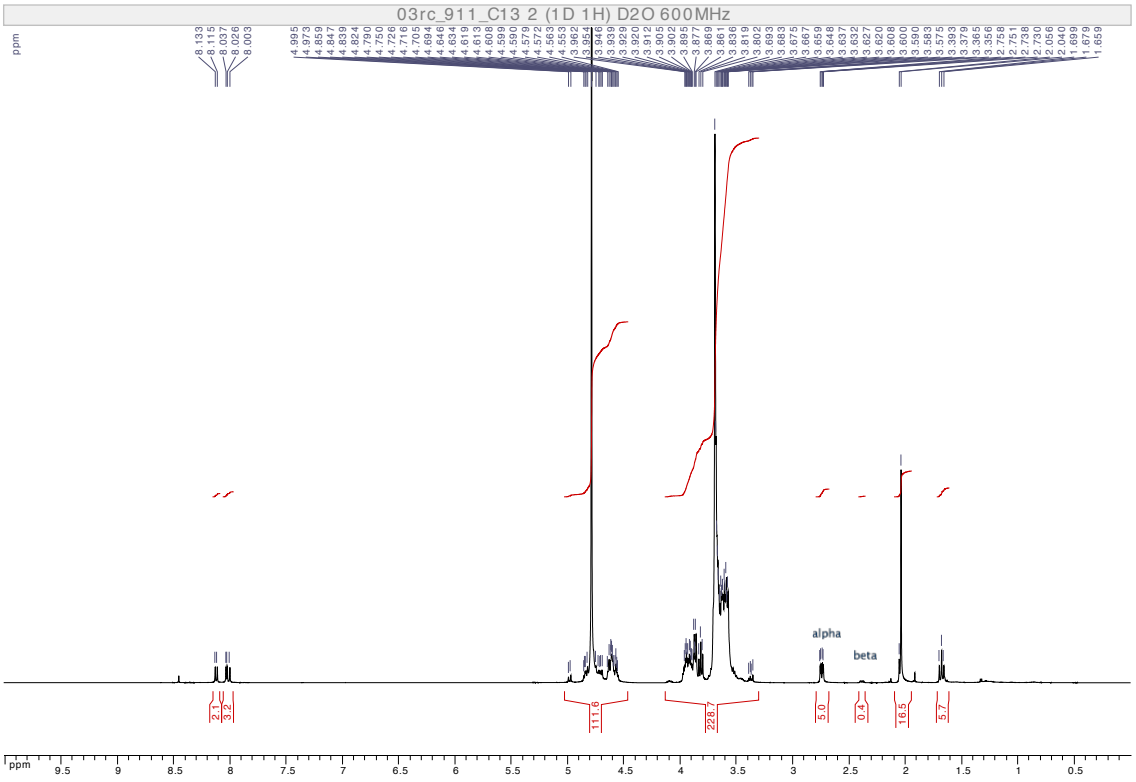


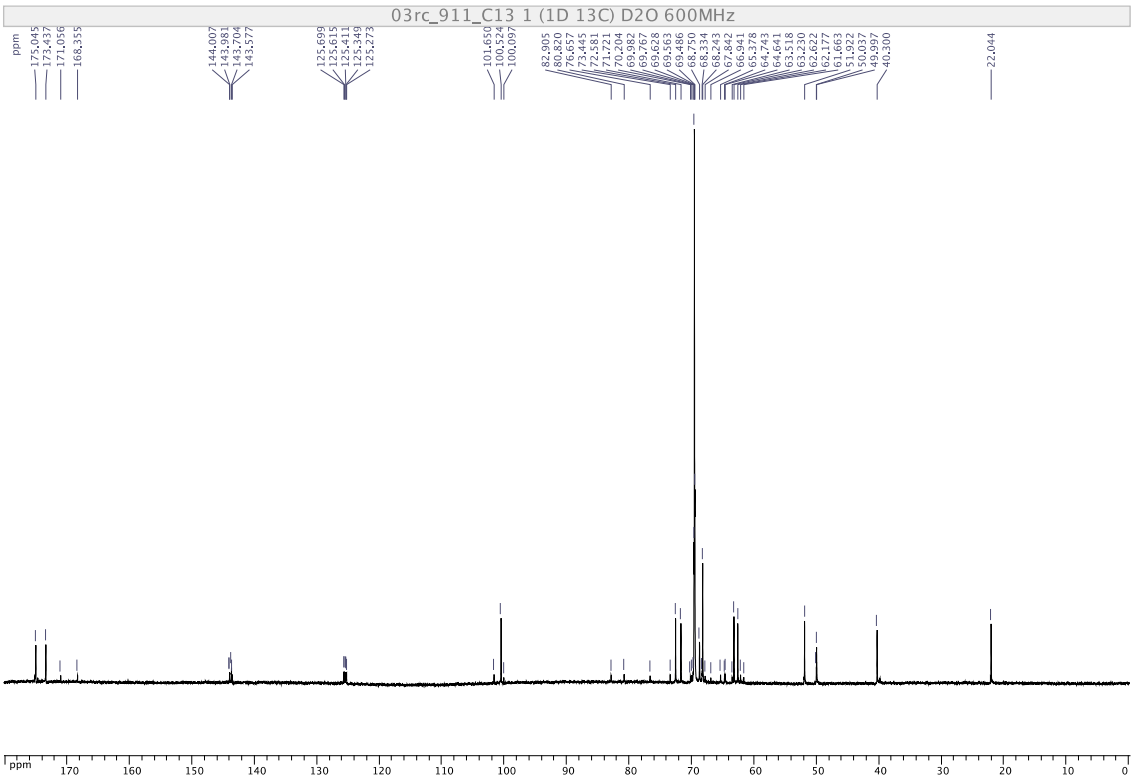
Compound 46



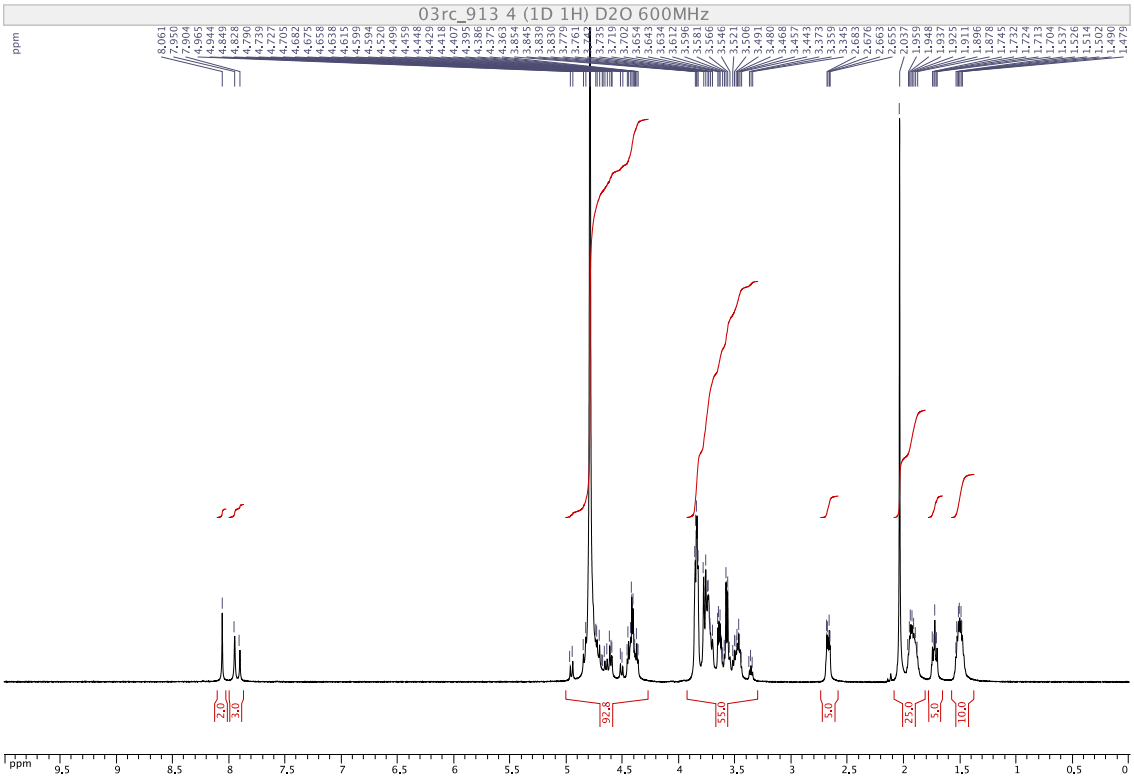


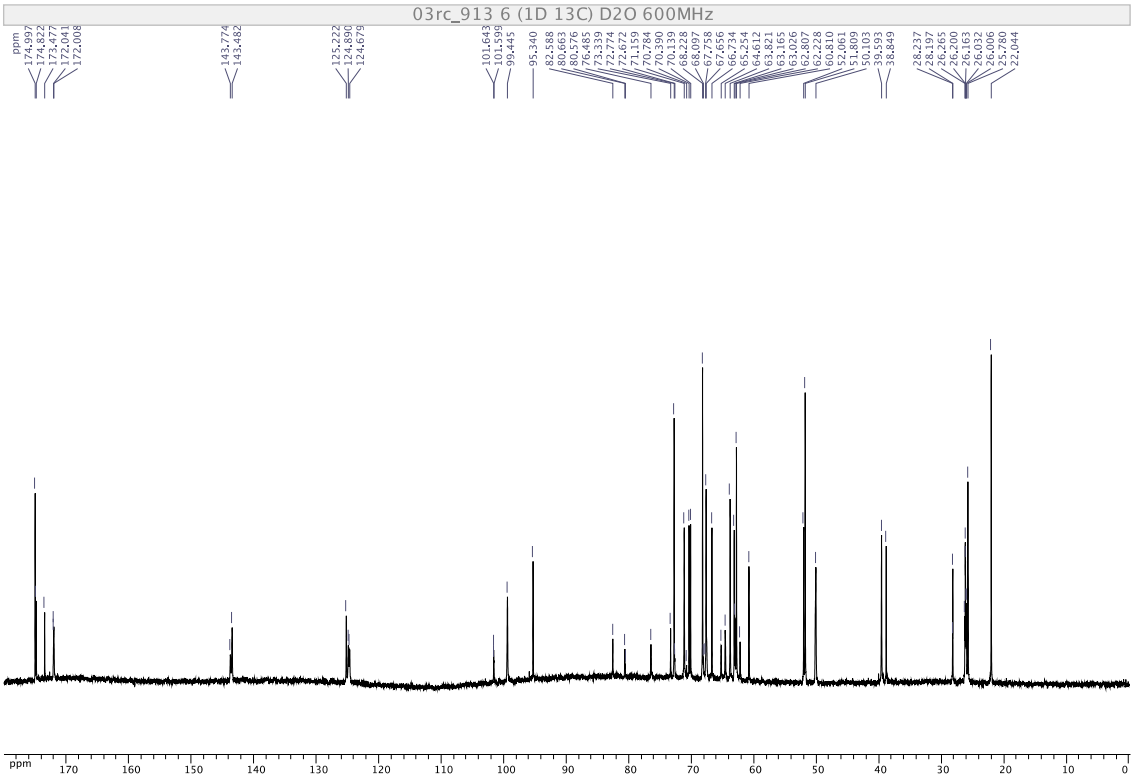
Compound 47


03rc_911_C13 2 (1D 1H) D2O 600MHz
alpha
beta


03rc_911_C13 1 (1D 13C) D2O 600MHz

Compound 48


03rc_913 4 (1D 1H) D2O 600MHz
ppm
2.0
3.0
92.8
35.0
5.0
25.0
5.0
10.0


03rc_913 6 (1D 13C) D2O 600MHz
ppm

Supporting Information References

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