Electronic Supplementary Material (ESI) for Organic & Biomolecular Chemistry.

Supporting Information

Improvement on binding of chondroitin sulfate derivatives to midkine by increasing hydrophobicity

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Fluorescence polarization assays

Fluorescence polarization measurements were performed in 384-well microplates (black polystyrene, non-treated, Corning). The fluorescence polarization was recorded using a TRIAD multimode microplate reader (from Dynex), with excitation and emission wavelengths of 485 and 535 nm, respectively. The fluorescent probe (a fluorescein labelled heparin-like hexasaccharide previously prepared in our lab[1]) was dissolved in PBS buffer (10 mM, pH 7.4). The protein (recombinant human midkine, FGF-2 or pleiotrophin, from Peprotech) was dissolved in PBS buffer (10 mM, pH 7.4) containing 1% BSA. Synthetic oligosaccharides **2, 3** and **7** were dissolved in PBS buffer (10 mM, pH 7.4). Compounds **1**, **4-6** were dissolved in PBS buffer at 1 mM concentration in the presence of 10% (v/v) DMSO.

For the determination of the IC_{50} values, we recorded the fluorescence polarization from wells containing 20 µL of protein solution (125 nM midkine solution/325 nM pleiotrophin solution/194nM FGF-2 solution) and 10 µL of a 40 nM probe solution in the presence of 10 µL of inhibitor solution. The total sample volume in each well was 40 µL and the final buffer composition was PBS + 0.5% BSA. The final concentration of fluorescent probe in each well was 10 nM while the final protein concentration was 63, 163 and 97 nM for midkine, pleiotrophin and FGF-2, respectively. The average polarization values of three replicates were plotted against the logarithm of inhibitor concentration. The curve was fitted to the equation for a one-site competition: $y = A_2 + (A_1-A_2)/[1+10\text{^}(x-\log IC_{50})]$ where A_1 and A_2 are, respectively, the maximal and minimal values of polarization, and IC_{50} is the concentration that results in 50% inhibition.

For direct binding assay between the fluorescent probe and pleiotrophin, 15 µL of a 20 nM probe solution and 15 µL of a series of pleiotrophin solutions, ranging from 1.3 µM to 20 nM, were transferred to the microplate wells. The final sample volume in each well was 30 µL. All samples were performed in replicates of three. The microplate was shaked in the dark for 5 min, before reading. Blank wells contained 15 µL of pleiotrophin solution (650 nM) and 15 µL of PBS buffer and their measurements were substracted from all values. Wells containing 15 µL of the fluorescent probe solution (20 nM) and 15 µL of PBS buffer plus 1% BSA gave the background polarization of the probe solution, in the absence of protein. The change in the fluorescence polarization (ΔP) was calculated by subtracting the background polarization of probe from the polarization value of the sample solutions. The ΔP values were plotted against pleiotrophin concentrations and the obtained binding curve was fitted to the equation for a one-site binding model $y = \Delta P_{max}x/K_D+x$ where ΔP_{max} is the maximum value approached with increasing concentrations of pleiotrophin and K_D is the dissociation constant of the probe-pleiotrophin interaction.

For the screening assay at a fixed concentration (25 µM) of inhibitor, 10 µL of a 40 nM probe solution and 20 µL of a 325 nM pleiotrophin solution were mixed with 10 µL of a 100 µM

inhibitor solution. The total sample volume in each well was 40 μL and the final buffer composition was PBS + 0.5% BSA. The final concentrations of inhibitor, fluorescent probe and protein in each well were 25 μM, 10 nM and 163 nM, respectively. After stirring for 5 min in the dark, fluorescence polarization was recorded. Two control samples were included in the experiment. The first one only contained fluorescent probe and afforded the expected value for 100% inhibition; the second one contained pleiotrophin and probe, in the absence of inhibitor, and gave the polarization value corresponding to 0% inhibition. Blank wells contained 20 μL of pleiotrophin solution (325 nM) and 20 μL of PBS buffer and their measurements were subtracted from all values. All samples were performed in replicates of three.

Competition assay using heparin-functionalized microtiter plates

1) Preparation of heparin-coated microplate wells: A heparin hexasaccharide containing an amine functionalized linker, previously synthesized in our group,[2] was dissolved in sodium bicarbonate buffer (50 mM, pH 9.6) containing hexadecyltrimethylammonium bromide (1 mM) to afford 10 μM sugar solutions. These solutions were transferred to the wells of Nunc Immobilizer Amino™ 384 microtiter plates (from Thermo Scientific) (20 μL/well). Blank wells were incubated with 100 mM ethanolamine in sodium bicarbonate buffer (50 mM, pH 9.6) (80 μL/well). The plate was shaken overnight at room temperature and the wells were emptied and washed with water. All wells were then blocked by 1 h incubation with 100 mM ethanolamine in sodium bicarbonate buffer, washed with water and stored at 4ºC until use.

2) Competition experiment: Heparin-coated wells were filled with 20 μL of a solution containing midkine (23 nM) and potential inhibitor (**dp18** at 10 μM; **1** and **3** at 100 μM) in PBS buffer (10 mM, pH 7.6) containing 1% BSA. A solution of midkine (23 nM) without competitor was used as positive control and also transferred to heparin-funcionalized wells. Ethanolamine-coated blank wells were also filled with midkine solution. The microplate was incubated with gentle agitation at room temperature for 1 h and then washed with PBS containing 1% Tween 20 and 0.1 % BSA, and water. Rabbit anti-human midkine antibody (from Peprotech, 5 μg/mL in PBS containing 1% BSA, 20 μL/well) was added to the wells. The plate was shaken for 1 h and washed with PBS containing 1% Tween 20 and 0.1 % BSA, and water. The primary anti-midkine antibody was detected by using Alexa Fluor 488 labelled anti-rabbit IgG (from Invitrogen, 20 μg/mL in PBS containing 1% BSA, 20 μL/well). The plate was incubated with shaking in the dark for 1 h and then washed with PBS containing 1% Tween 20 and 0.1 % BSA, and water. The fluorescence was read at 535 nm using a TRIAD multimode microplate reader (from Dynex). The blank well measurements were subtracted from all values. All samples were performed in six replicates.

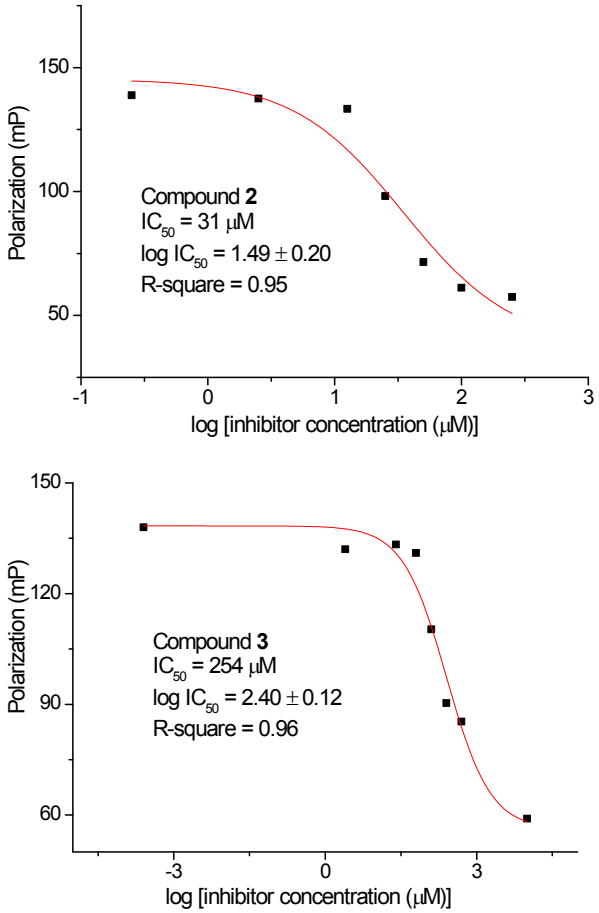
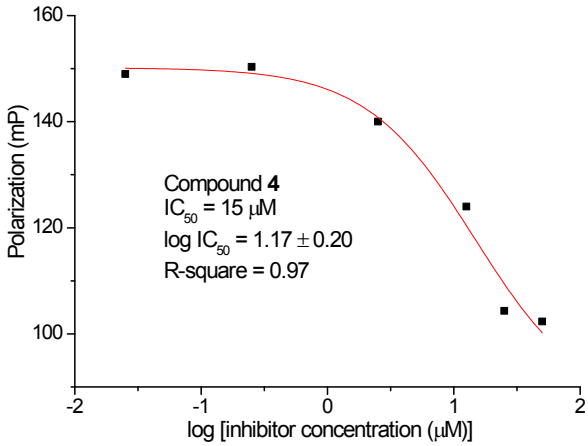
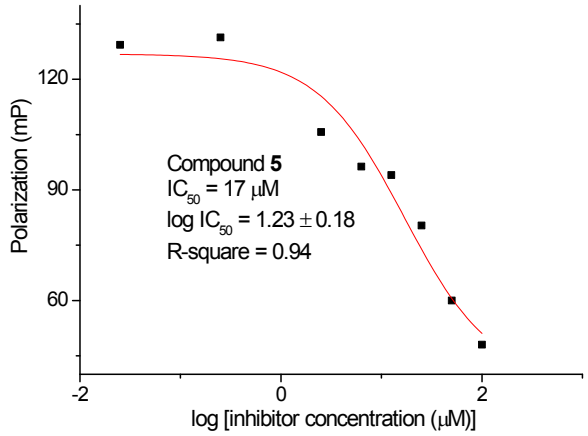


Figure S1. Inhibition curves showing the ability of compounds **2** and **3** to inhibit the interaction between midkine (63 nM) and fluorescent probe (10 nM). All the P values are the average of three replicate wells.





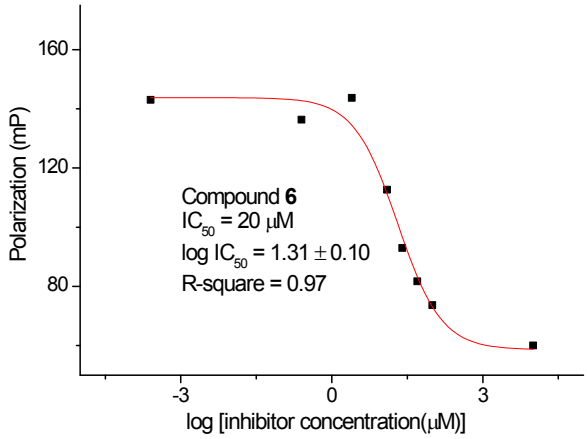


Figure S2. Inhibition curves showing the ability of compounds **4**, **5** and **6** to inhibit the interaction between midkine (63 nM) and fluorescent probe (10 nM). All the P values are the average of three replicate wells.

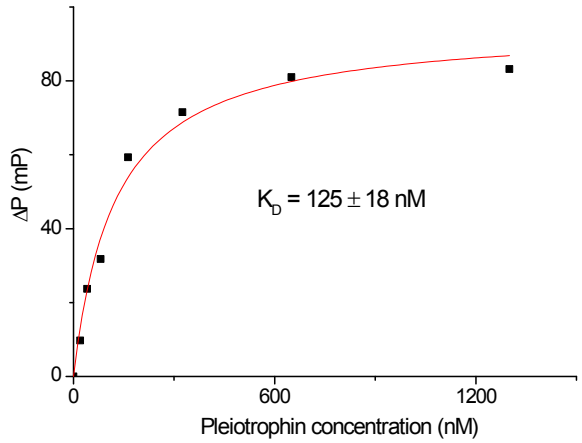


Figure S3. Direct binding assay for the interaction of pleiotrophin with the fluorescent probe. ΔP was plotted against protein concentration and the binding curve was fitted to the equation for a one-site binding model. The plotted data are the averages of three replicates.

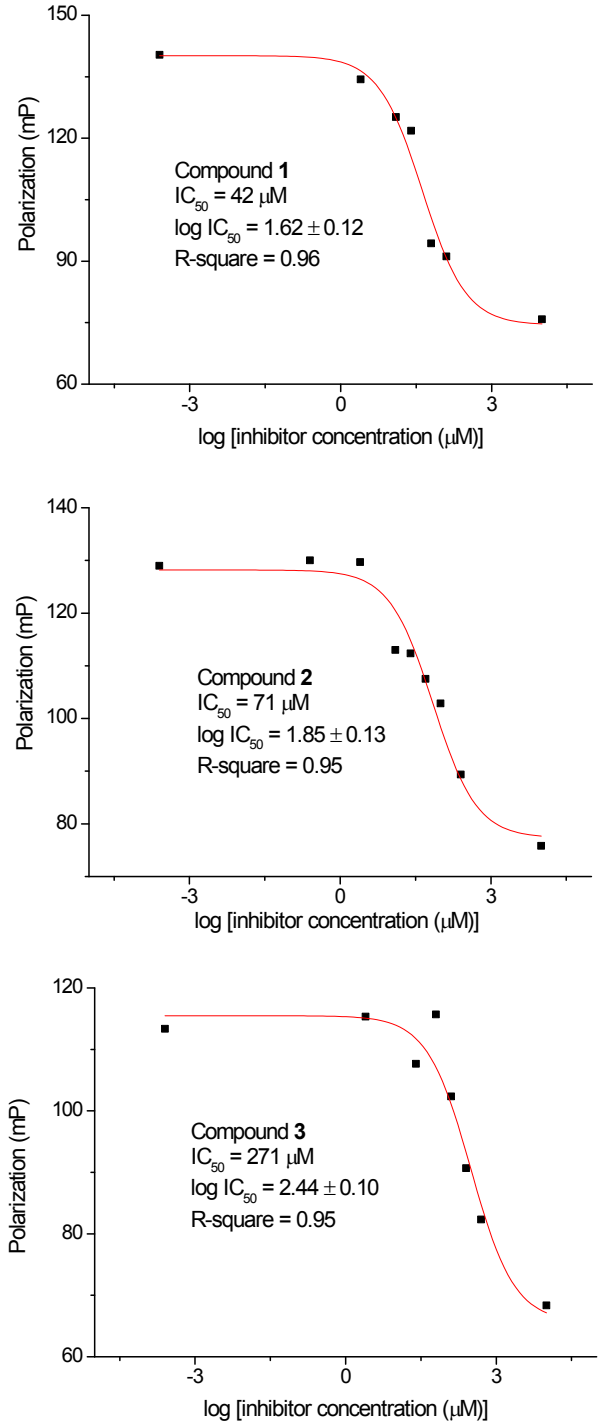
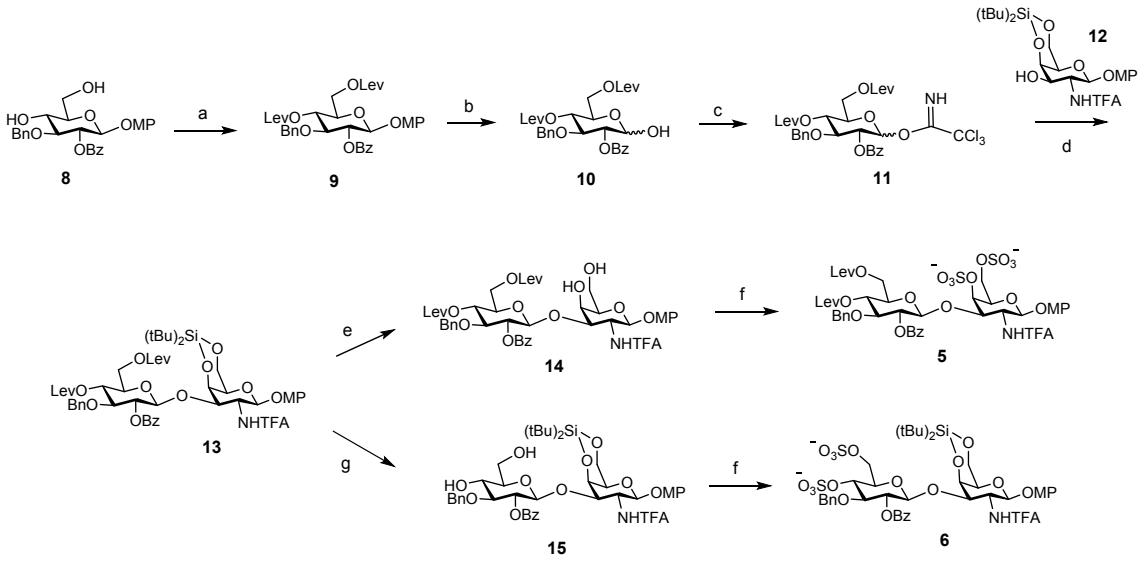


Figure S4. Inhibition curves showing the ability of compounds **1**, **2** and **3** to inhibit the interaction between FGF-2 (97 nM) and fluorescent probe (10 nM). All the P values are the average of three replicate wells.



Scheme S1. Reagents and conditions: a) Lev_2O, CH_2Cl_2, DMAP, 78%; b) CAN, $CH_2Cl_2/CH_3CN/H_2O$, 79%; c) Cl_3CCN, K_2CO_3, CH_2Cl_2, 99%; d) TMSOTf, CH_2Cl_2, 0ºC, 80%; e) $(HF)_n \cdot Py$, THF, 0ºC, 93%; f) $SO_3 \cdot Me_3N$, DMF, 100ºC, MW, 30 min, 89% for **5**, 96% for **6**; g) $NH_2NH_2 \cdot H_2O$, Py/AcOH, CH_2Cl_2, 88%.

General synthetic procedures: Thin layer chromatography (TLC) analyses were performed on silica gel 60 F_{254} precoated on aluminium plates (Merck) and the compounds were detected by staining with sulfuric acid/ethanol (1:9), with cerium (IV) sulfate (10 g)/phosphomolybdic acid (13 g)/sulfuric acid (60 mL) solution in water (1 L), or with anisaldehyde solution [anisaldehyde (25 mL) with sulfuric acid (25 mL), ethanol (450 mL) and acetic acid (1 mL)], followed by heating at over 200ºC. Column chromatography was carried out on silica gel 60 (0.2-0.5 mm, 0.2-0.063 mm or 0.040-0.015 mm; Merck). Optical rotations were determined with a Perkin-Elmer 341 polarimeter. ^{1}H- and ^{13}C-NMR spectra were acquired on Bruker DPX-300 and Avance III-400 spectrometers. Electrospray mass spectra (ESI MS) were carried out with an Esquire 6000 ESI-Ion Trap from Bruker Daltonics. High resolution mass spectra (HR MS) were carried out by CITIUS (Universidad de Sevilla). Microwave-based sulfation reactions were performed using a Biotage Initiator Eight synthesizer in sealed reaction vessels.

4-Methoxyphenyl 2-*O*-benzoyl-3-*O*-benzyl-4,6-di-*O*-levulinoyl-β-D-glucopyranoside (9):

Lev_2O preparation: LevOH (1.8 mL, 18.2 mmol) was added at 0ºC to a solution of 1,3-dicyclohexylcarbodiimide (1.88 g, 9.1 mmol) in CH_2Cl_2 (25 mL). After stirring 5 min at room temperature, the mixture was cooled and filtered, and the urea precipitate was washed with additional CH_2Cl_2 (7 mL), to give 32 mL of a 0.29 M Lev_2O solution.

Lev_2O (32 mL of a 0.29 M solution in CH_2Cl_2) was added at room temperature to a mixture of **8**[3] (730 mg, 1.52 mmol) and DMAP (56 mg, 0.46 mmol). The mixture was stirred for 2 h, diluted with CH_2Cl_2, and washed with saturated aqueous $NaHCO_3$, and H_2O. The organic phase was

dried ($MgSO_4$), filtered and concentrated to dryness. The residue was suspended in hexane/EtOAc (1:1, 20 mL) and the mixture was filtered. The precipitate was washed with additional hexane/EtOAc (1:1, 10 mL) to give **9** as a white amorphous solid (800 mg, 78%). TLC (toluene-EtOAc 2:1) R_f 0.26; $[\alpha]^{20}_D$ +14° (*c* 1.0, $CHCl_3$); ^{1}H-NMR (400 MHz, $CDCl_3$): δ 8.04 (m, 2H, Ar), 7.61 (m, 1H, Ar), 7.47 (m, 2H, Ar), 7.18 (m, 5H, Ar), 6.92 (m, 2H, Ar), 6.77 (m, 2H, Ar), 5.57 (dd, 1H, $J_{1,2}$ = 7.9 Hz, $J_{2,3}$ = 9.2 Hz, H-2), 5.25 (t, 1H, $J_{3,4} = J_{4,5}$ = 9.5 Hz, H-4), 5.05 (d, 1H, H-1), 4.66 (2d, 2H, CH_2(Bn)), 4.28 (m, 2H, H-6), 3.97 (t, 1H, H-3), 3.83 (m, 1H, H-5), 3.74 (s, 3H, Me (OMP)), 2.81-2.45 (m, 8H, CH_2(Lev)), 2.20-2.18 (2s, 6H, CH_3(Lev)); ^{13}C-NMR (100 MHz, $CDCl_3$): δ 206.6, 206.2, 172.3, 171.5, 165.0 (5 x CO), 155.6-114.5 (Ar), 100.7 (C-1), 79.6 (C-3), 74.0 (CH_2(Bn)), 73.1 (C-2), 72.4 (C-5), 70.2 (C-4), 62.7 (C-6), 55.6 (Me (OMP)), 37.9, 37.8 (CH_2(Lev)), 29.8, 29.7 (CH_3(Lev)), 27.94, 27.87 (CH_2(Lev)); HR MS: *m/z*: calcd for $C_{37}H_{40}O_{12}Na$: 699.2412; found: 699.2392 [*M*+Na]$^+$.

2-*O*-benzoyl-3-*O*-benzyl-4,6-di-*O*-levulinoyl-α,β-D-glucopyranose (10): CAN (4.7 mL of a 1 M solution in H_2O) was added to a solution of **9** (780 mg, 1.15 mmol) in CH_2Cl_2/MeCN (1:2; 42 mL). After stirring for 2.5 h at room temperature, the reaction mixture was diluted with EtOAc, washed with H_2O, saturated aqueous $NaHCO_3$, and H_2O. The organic phase was dried ($MgSO_4$), filtered and concentrated to dryness. The residue was purified by column chromatography (hexane-EtOAc 2:3) to afford **10** as a yellow oil (515 mg, 79%, mixture of α/β anomers). TLC (hexane-EtOAc 2:3) Rf 0.25, 0.17; ^{1}H-NMR (300 MHz, $CDCl_3$) (data for α anomer): δ 8.04 (m, 2H, Ar), 7.57 (m, 1H, Ar), 7.43 (m, 2H, Ar), 7.19 (m, 5H, Ar), 5.56 (d, 1H, $J_{1,2}$ = 3.3 Hz, H-1), 5.12-5.04 (m, 2H, H-2, H-4), 4.69 (2d, 2H, CH_2(Bn)), 4.26-4.09 (m, 4H, H-3, H-5, H-6), 2.78-2.35 (m, 8H, CH_2(Lev)), 2.17-2.14 (2s, 6H, CH_3(Lev)); ^{13}C-NMR (75 MHz, $CDCl_3$) (data for α anomer): δ 208.2, 206.8, 172.7, 172.0, 166.1 (5 x CO), 138.4-128.0 (Ar), 90.6 (C-1), 77.3 (C-3), 75.3 (CH_2(Bn)), 74.4, 70.9 (C-2, C-4), 67.7 (C-5), 63.0 (C-6), 38.5, 38.2 (CH_2(Lev)), 30.2, 30.1 (CH_3(Lev)), 28.4, 28.2 (CH_2(Lev)); HR MS: *m/z*: calcd for $C_{30}H_{34}O_{11}Na$: 593.1993; found: 593.1975 [*M*+Na]$^+$.

***O*-(2-*O*-benzoyl-3-*O*-benzyl-4,6-di-*O*-levulinoyl-α,β-D-glucopyranosyl) trichloroacetimidate (11):** Trichloroacetonitrile (0.2 mL, 2.0 mmol) and K_2CO_3 (33 mg, 0.24 mmol) were added to **10** (113 mg, 0.20 mmol) in dry CH_2Cl_2 (1.5 mL) under an argon atmosphere. After stirring at room temperature for 4 h, the mixture was filtered and concentrated in vacuo to give **11** as a colorless oil (140 mg, 99%, mixture of α/β anomers). TLC (toluene-EtOAc 4:1) Rf 0.55 and 0.42; ^{1}H-NMR (400 MHz, $CDCl_3$) (data for a 0.4:0.6 α/β mixture): δ 8.66, 8.60 (2s, 1H, N*H*α and N*Hβ*), 8.00-7.19 (m, 10H, Ar), 6.65 (d, 0.4H, $J_{1,2}$ = 3.6 Hz, H-1α), 5.98 (d, 0.6H, $J_{1,2}$ = 7.7 Hz, H-1β), 5.61 (dd, 0.6H, $J_{2,3}$ = 8.7 Hz, H-2β), 5.40 (dd, 0.4H, $J_{2,3}$ = 9.9 Hz, H-2α), 5.33-5.25 (m, 1H, H-4α/β), 4.73-4.66 (2s, 2H, CH_2(Bn)), 4.30-4.21 (m, 2.8H, H-3α, H-5α, H-6α/β), 3.98 (t, 0.6H, H-3β), 3.92 (m, 0.6H, H-5β), 2.80-2.48 (m, 8H, CH_2(Lev)), 2.22-2.17 (4s, 6H, CH_3(Lev)); ^{13}C-NMR (100 MHz, $CDCl_3$) (data for a 0.4:0.6 α/β mixture): δ 206.6, 206.5, 206.2, 172.3,

171.4, 165.3, 164.7 (CO), 161.2, 160.3 (C=NH), 137.6-127.6 (Ar), 96.0 (C-1β), 93.4 (C-1α), 90.8, 90.3 (CCl_3), 79.0 (C-3β), 76.5 (C-3α), 74.9 73.9 (CH_2(Bn)), 73.1 (C-5β), 72.2 (C-2α), 71.7 (C-2β), 70.7 (C-5α), 69.5, 69.4 (C-4α/β), 62.2, 61.9 (C-6α/β), 37.9-27.8 (CH_2, CH_3(Lev)); ESI MS: *m/z*: calcd for $C_{32}H_{34}Cl_3NO_{11}Na$: 736.1; found: 736.4 $[M+Na]^+$.

4-Methoxyphenyl 3-*O*-(2-*O*-benzoyl-3-*O*-benzyl-4,6-di-*O*-levulinoyl-β-D-glucopyranosyl)-4,6-*O*-di-*tert*-butylsilylene-2-deoxy-2-trifluoroacetamido-β-D-galactopyranoside (13): Donor **11** (138 mg, 0.19 mmol) and aceptor **12**[1] (72 mg, 0.14 mmol) were coevaporated with toluene, concentrated in vacuo and dissolved in dry CH_2Cl_2 (2 mL) in the presence of freshly activated 4Å molecular sieves (150 mg). After stirring for 15 min at 0 ºC, TMSOTf (100 µL of a 0.19 M solution in dry CH_2Cl_2) was added under an argon atmosphere. After stirring for 30 min at 0ºC, the reaction mixture was neutralized with Et_3N, filtered, and concentrated to dryness. The residue was purified by column chromatography (hexane-EtOAc 1:1) to afford **13** as a white foam (118 mg, 80%). TLC (hexane-EtOAc 1:1) Rf 0.26; $[\alpha]^{20}_D$ +17º (*c* 1.0, $CHCl_3$); ^{1}H-NMR (400 MHz, $CDCl_3$): δ 8.02 (m, 2H, Ar), 7.60 (m, 1H, Ar), 7.53 (d, 1H, $J_{2,NH}$ = 8.2 Hz, NH), 7.46 (m, 2H, Ar), 7.18-7.08 (m, 5H, Ar), 6.98 (m, 2H, Ar), 6.80 (m, 2H, Ar), 5.36 (dd, 1H, $J_{1,2}$ = 8.2 Hz, $J_{2,3}$ = 9.3 Hz, H-2'), 5.20 (m, 2H, H-1, H-1'), 5.07 (t, 1H, $J_{3,4}$ = $J_{4,5}$ = 9.7 Hz, H-4'), 4.66 (d, 1H, $J_{3,4}$ = 2.3 Hz, H-4), 4.59-4.51 (m, 4H, H-3, H-6'a, CH_2(Bn)), 4.36 (m, 1H, H-2), 4.28-4.17 (m, 2H, H-6), 4.06 (dd, 1H, $J_{5,6b}$ = 5.6 Hz, $J_{6a,6b}$ = 12.4 Hz, H-6'b), 3.83-3.77 (m, 4H, H-3', Me (OMP)), 3.71 (m, 1H, H-5'), 3.56 (br s, 1H, H-5), 2.93-2.36 (m, 8H, CH_2(Lev)), 2.18-2.16 (2s, 6H, CH_3(Lev)), 1.09-0.94 (2s, 18H, $C(CH_3)_3$); ^{13}C-NMR (100 MHz, $CDCl_3$): δ 207.8, 206.3, 172.5, 171.5, 165.1 (5 x CO), 157.5 (q, $J_{C,F}$ = 36.6 Hz, *C*OCF_3), 155.7-114.4 (Ar), 100.2, 99.2 (C-1, C-1'), 80.3 (C-3'), 74.3 (CH_2(Bn)), 73.5, 73.1, 72.9, 72.6 (C-3, C-4, C-2', C-5'), 71.4 (C-5), 69.6 (C-4'), 67.0 (C-6), 62.2 (C-6'), 55.6 (Me (OMP)), 52.5 (C-2), 38.0, 37.8 (CH_2(Lev)), 29.70, 29.68 (CH_3(Lev)), 28.3, 27.8 (CH_2(Lev)), 27.6, 27.4 (C(*C*H_3)$_3$), 23.2, 20.8 (*C*$(CH_3)_3$); HR MS: *m/z*: calcd for $C_{53}H_{66}F_3NO_{17}NaSi$: 1096.3944; found: 1096.3962 $[M+Na]^+$.

4-Methoxyphenyl 3-*O*-(2-*O*-benzoyl-3-*O*-benzyl-4,6-di-*O*-levulinoyl-β-D-glucopyranosyl)-2-deoxy-2-trifluoroacetamido-β-D-galactopyranoside (14): An excess of $(HF)_n$·Py (170 µL, 6.5 mmol) was added at 0°C under an argon atmosphere to a solution of **13** (35 mg, 0.033 mmol) in dry THF (1.0 mL). After 20 h at 0ºC the mixture was diluted with CH_2Cl_2 and washed with H_2O and saturated $NaHCO_3$ solution until neutral pH. The organic layers were dried ($MgSO_4$), filtered and concentrated in vacuo to give **14** (28 mg, 93%) as a white amorphous solid. TLC (CH_2Cl_2-MeOH 20:1) Rf 0.34; ^{1}H-NMR (400 MHz, $CDCl_3$/CD_3OD 5:1): δ 8.73 (d, 1H, $J_{2,NH}$ = 8.0 Hz, NH), 7.96 (m, 2H, Ar), 7.59 (m, 1H, Ar), 7.44 (m, 2H, Ar), 7.13-7.04 (m, 5H, Ar), 6.87 (m, 2H, Ar), 6.73 (m, 2H, Ar), 5.24 (t, 1H, H-2'), 5.05 (m, 2H, H-1, H-4'), 4.78 (d, 1H, $J_{1,2}$ = 8.0 Hz, H-1'), 4.56 (2d, 2H, CH_2(Bn)), 4.36 (dd, 1H, $J_{5,6a}$ = 1.8 Hz, $J_{6a,6b}$ = 12.1 Hz, H-6'a), 4.27 (dd, 1H, $J_{2,3}$ = 10.9 Hz, $J_{3,4}$ = 2.8 Hz, H-3), 4.20 (d, 1H, H-4), 4.08-4.00 (m, 2H, H-2, H-6'b), 3.91-3.80 (m, 3H, H-3', 2xH-6), 3.76-3.71 (m, 4H, H-5', Me (OMP)), 3.63 (t, 1H, H-5), 2.84-

2.35 (m, 8H, CH_2(Lev)), 2.20-2.15 (2s, 6H, CH_3(Lev)); ^{13}C-NMR (100 MHz, $CDCl_3$/CD_3OD 5:1): δ 208.9, 207.2, 172.7, 171.8, 165.7 (5 x CO), 157.9 (q, $J_{C,F}$ = 36.7 Hz, *C*OCF_3), 155.4-114.1 (Ar), 101.1 (C-1'), 99.8 (C-1), 79.9 (C-3'), 77.6 (C-3), 74.8, 74.6 (CH_2(Bn), C-5), 73.3 (C-2'), 72.0 (C-5'), 70.2 (C-4'), 67.8 (C-4), 62.2 (C-6'), 61.3 (C-6), 55.5 (Me (OMP)), 52.9 (C-2), 37.9, 37.7 (CH_2(Lev)), 29.6, 29.4 (CH_3(Lev)), 27.8, 27.7 (CH_2(Lev)); HR MS: *m/z*: calcd for $C_{45}H_{50}F_3NO_{17}Na$: 956.2923; found: 956.2905 [*M*+Na]$^+$.

4-Methoxyphenyl 3-*O*-(2-*O*-benzoyl-3-*O*-benzyl-4,6-di-*O*-levulinoyl-β-D-glucopyranosyl)-2-deoxy-4,6-di-*O*-sulfo-2-trifluoroacetamido-β-D-galactopyranoside (5): Compound **14** (26 mg, 28 μmol) and sulfur trioxide–trimethylamine complex (77 mg, 0.56 mmol) were dissolved in dry DMF (1.5 mL) and heated at 100ºC for 30 min using microwave radiation (20 W average power). The reaction vessel was cooled and Et_3N (300 μL), MeOH (1 mL) and CH_2Cl_2 (1 mL) were added. The solution was layered on the top of a Sephadex LH 20 chromatography column which was eluted with CH_2Cl_2-MeOH (1:1) to obtain **5** as triethylammonium salt (32 mg, 89%). TLC (EtOAc/pyridine/H_2O/AcOH 12:5:3:1) Rf 0.30; ^{1}H-NMR (400 MHz, CD_3OD): δ 8.00 (m, 2H, Ar), 7.63 (m, 1H, Ar), 7.48 (m, 2H, Ar), 7.16-7.06 (m, 5H, Ar), 7.01 (m, 2H, Ar), 6.83 (m, 2H, Ar), 5.37 (t, 1H, $J_{1,2}$ = 7.8 Hz, $J_{2,3}$ = 9.6 Hz, H-2'), 5.16 (t, 1H, $J_{3,4}$ = $J_{4,5}$ = 9.6 Hz, H-4'), 5.04 (d, 1H, $J_{3,4}$ = 2.9 Hz, H-4), 4.94 (d, 1H, H-1'), 4.89 (d, 1H, H-1), 4.64 (d, 1H, *J* = 11.2 Hz, CH_2(Bn)), 4.50 (m, 2H, H-6a, CH_2(Bn)), 4.38-4.12 (m, 6H, H-2, H-3, H-5, H-6b, H-6'x2), 3.99 (t, 1H, H-3'), 3.84 (m, 1H, H-5'), 3.74 (s, 3H, Me (OMP)), 3.23 (q, 12H, Et_3NH^+), 2.94-2.45 (m, 8H, CH_2(Lev)), 2.24-2.17 (2s, 6H, CH_3(Lev)), 1.32 (t, 18H, Et_3NH^+); ^{13}C-NMR (100 MHz, CD_3OD): δ 209.4, 207.7, 173.1, 172.0, 165.1 (5 x CO), 155.5-114.2 (Ar), 101.5 (C-1'), 100.8 (C-1), 80.5 (C-3'), 76.1 (C-5 or C-3), 75.5 (C-4), 73.9 (CH_2(Bn)), 73.4, 73.3 (C-2', C-5 or C-3), 72.0 (C-5'), 70.4 (C-4'), 68.1 (C-6), 62.3 (C-6'), 54.6 (Me (OMP)), 52.0 (C-2), 46.6 (Et_3NH^+), 37.5, 37.1 (CH_2(Lev)), 28.5, 28.2 (CH_3(Lev)), 27.7, 27.6 (CH_2(Lev)), 7.9 (Et_3NH^+); ESI MS: *m/z*: calcd for $C_{45}H_{49}F_3NO_{23}S_2$: 1092.2; found: 1092.2 [*M*+H]$^-$.

4-Methoxyphenyl 3-*O*-(2-*O*-benzoyl-3-*O*-benzyl-β-D-glucopyranosyl)-4,6-*O*-di-*tert*-butylsilylene-2-deoxy-2-trifluoroacetamido-β-D-galactopyranoside (15): Compound **13** (83 mg, 0.077 mmol) was dissolved in CH_2Cl_2 (1 mL) and hydrazine monohydrate (0.62 mL of a 0.5 M solution in Py/AcOH 3:2) was added. After stirring at room temperature for 2 h, the reaction mixture was quenched with acetone (0.2 mL). The mixture was diluted with CH_2Cl_2 and washed with 1 M HCl aqueous solution, saturated $NaHCO_3$ aqueous solution and H_2O. The organic layer was dried ($MgSO_4$), filtered and concentrated in vacuo. The residue was purified by column chromatography (toluene-EtOAc 1:1) to afford **15** (60 mg, 88%) as a white amorphous solid. TLC (toluene-EtOAc 1:1) Rf 0.30; $[\alpha]^{20}_D$ +17º (*c* 1.0, $CHCl_3$); ^{1}H-NMR (400 MHz, $CDCl_3$): δ 8.05 (m, 2H, Ar), 7.63 (m, 1H, Ar), 7.48 (m, 2H, Ar), 7.21-7.16 (m, 6H, Ar, NH), 6.92 (m, 2H, Ar), 6.79 (m, 2H, Ar), 5.32 (d, 1H, $J_{1,2}$ = 7.6 Hz, H-1'), 5.25 (t, 1H, H-2'), 5.08 (d, 1H, $J_{1,2}$ = 7.9 Hz, H-1), 4.69 (2d, 2H, CH_2(Bn)), 4.62 (d, 1H, $J_{3,4}$ = 2.1 Hz, H-4), 4.32 (m, 1H, H-2), 4.23-4.18

(m, 3H, H-6, H-3), 3.95 (dd, 1H, $J_{5,6a}$ = 2.7 Hz, $J_{6a,6b}$ = 12.0 Hz, H-6'a), 3.80-3.76 (m, 5H, H-4', H-6'b, Me (OMP)), 3.66 (t, 1H, $J_{2,3} = J_{3,4}$ = 9.0 Hz, H-3'), 3.44 (m, 1H, H-5'), 3.38 (br s, 1H, H-5), 1.11-1.08 (2s, 18H, $C(CH_3)_3$); ^{13}C-NMR (100 MHz, $CDCl_3$): δ 165.5 (CO), 158.0 (q, $J_{C,F}$ = 36.9 Hz, $COCF_3$), 155.8-114.2 (Ar), 115.6 (q, $J_{C,F}$ = 288.4 Hz, $COCF_3$), 100.3 (C-1), 99.9 (C-1'), 82.5 (C-3'), 76.3 (C-5'), 75.8 (C-3), 74.5 (CH_2(Bn)), 73.9 (C-2'), 73.1 (C-4), 71.3 (C-5), 70.2 (C-4'), 67.0 (C-6), 62.0 (C-6'), 55.6 (Me (OMP)), 53.1 (C-2), 27.6 ($C(CH_3)_3$), 23.3, 20.8 ($C(CH_3)_3$); HR MS: *m/z*: calcd for $C_{43}H_{54}F_3NO_{13}NaSi$: 900.3209; found: 900.3207 $[M+Na]^+$.

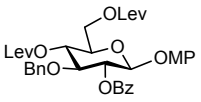
4-Methoxyphenyl 3-*O*-(2-*O*-benzoyl-3-*O*-benzyl-4,6-di-*O*-sulfo-β-D-glucopyranosyl)-4,6-*O*-di-*tert*-butylsilylene-2-deoxy-2-trifluoroacetamido-β-D-galactopyranoside (6): Compound **15** (18 mg, 21 μmol) and sulfur trioxide–trimethylamine complex (57 mg, 0.41 mmol) were dissolved in dry DMF (1.5 mL) and heated at 100ºC for 30 min using microwave radiation (20 W average power). The reaction vessel was cooled and Et_3N (300 μL), MeOH (1 mL) and CH_2Cl_2 (1 mL) were added. The solution was layered on the top of a Sephadex LH 20 chromatography column which was eluted with CH_2Cl_2-MeOH (1:1) to obtain **6** as triethylammonium salt (24 mg, 96%). TLC (EtOAc/pyridine/H_2O/AcOH 15:5:3:1) Rf 0.30; ^{1}H-NMR (400 MHz, CD_3OD): δ 7.96 (m, 2H, Ar), 7.64 (m, 1H, Ar), 7.48 (m, 2H, Ar), 7.11-6.98 (m, 5H, Ar), 6.91 (m, 2H, Ar), 6.82 (m, 2H, Ar), 5.21 (t, 1H, $J_{1,2} = J_{2,3}$ = 8.6 Hz, H-2'), 5.08 (d, 1H, $J_{3,4}$ = 2.6 Hz, H-4), 5.08 (d, 1H, J = 10.9 Hz, CH_2(Bn)), 4.91-4.80 (m, 3H, H-1, H-1', H-6'a), 4.55 (d, 1H, CH_2(Bn)), 4.51-4.43 (m, 2H, H-2, H-6a), 4.32 (t, 1H, $J_{3,4} = J_{4,5}$ = 9.2 Hz, H-4'), 4.14 (br d, 1H, $J_{6a,6b}$ = 11.2 Hz, H-6b), 4.06 (dd, 1H, H-6'b), 3.97 (m, 2H, H-5', H-3), 3.89 (t, 1H, H-3'), 3.75 (s, 3H, Me (OMP)), 3.69 (br s, 1H, H-5), 3.21 (q, 12H, Et_3NH^+), 1.32 (t, 18H, Et_3NH^+), 1.12-1.10 (2s, 18H, $C(CH_3)_3$); ^{13}C-NMR (100 MHz, CD_3OD): δ 165.3 (CO), 155.5-114.1 (Ar), 102.3 (C-1'), 101.1 (C-1), 81.3 (C-3'), 79.0 (C-3 or C-5'), 77.0 (C-4'), 74.8 (CH_2(Bn)), 73.6, 73.4 (C-2', C-3 or C-5'), 72.2 (C-4), 71.5 (C-5), 67.7 (C-6'), 66.8 (C-6), 54.6 (Me (OMP)), 50.9 (C-2), 46.5 (Et_3NH^+), 27.0, 26.9 ($C(CH_3)_3$), 22.6, 20.3 ($C(CH_3)_3$), 7.9 (Et_3NH^+); ESI MS: *m/z*: calcd for $C_{43}H_{52}F_3NO_{19}S_2Si$: 517.6; found: 517.7 $[M]^{2-}$.

References

(1) Maza, S.; Mar Kayser, M.; Macchione, G.; Lopez-Prados, J.; Angulo, J.; de Paz, J. L.; Nieto, P. M. *Org. Biomol. Chem.* **2013**, *11*, 3510.

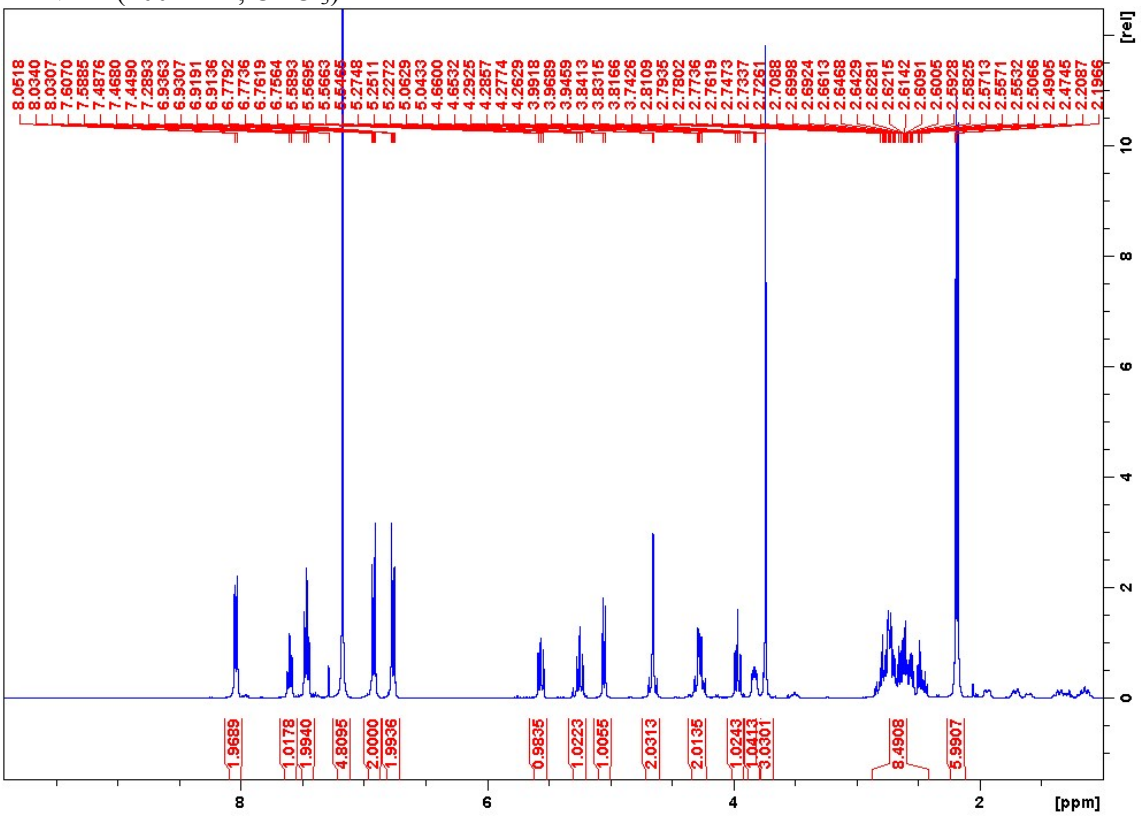
(2) Maza, S.; Macchione, G.; Ojeda, R.; Lopez-Prados, J.; Angulo, J.; de Paz, J. L.; Nieto, P. M. *Org. Biomol. Chem.* **2012**, *10*, 2146.

(3) Karst, N.; Jacquinet, J. C. *Eur. J. Org. Chem.* **2002**, 815.

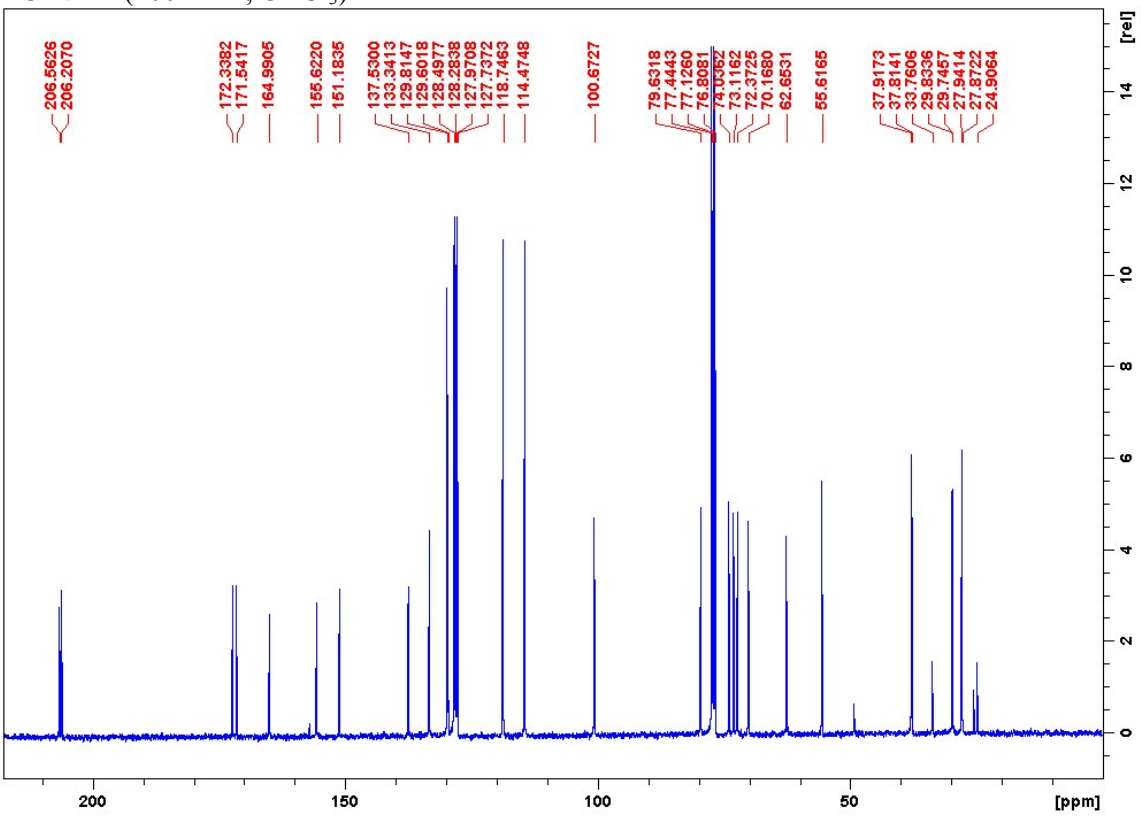


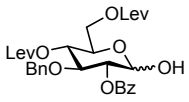
9

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



^{13}C-NMR (100 MHz, $CDCl_3$)





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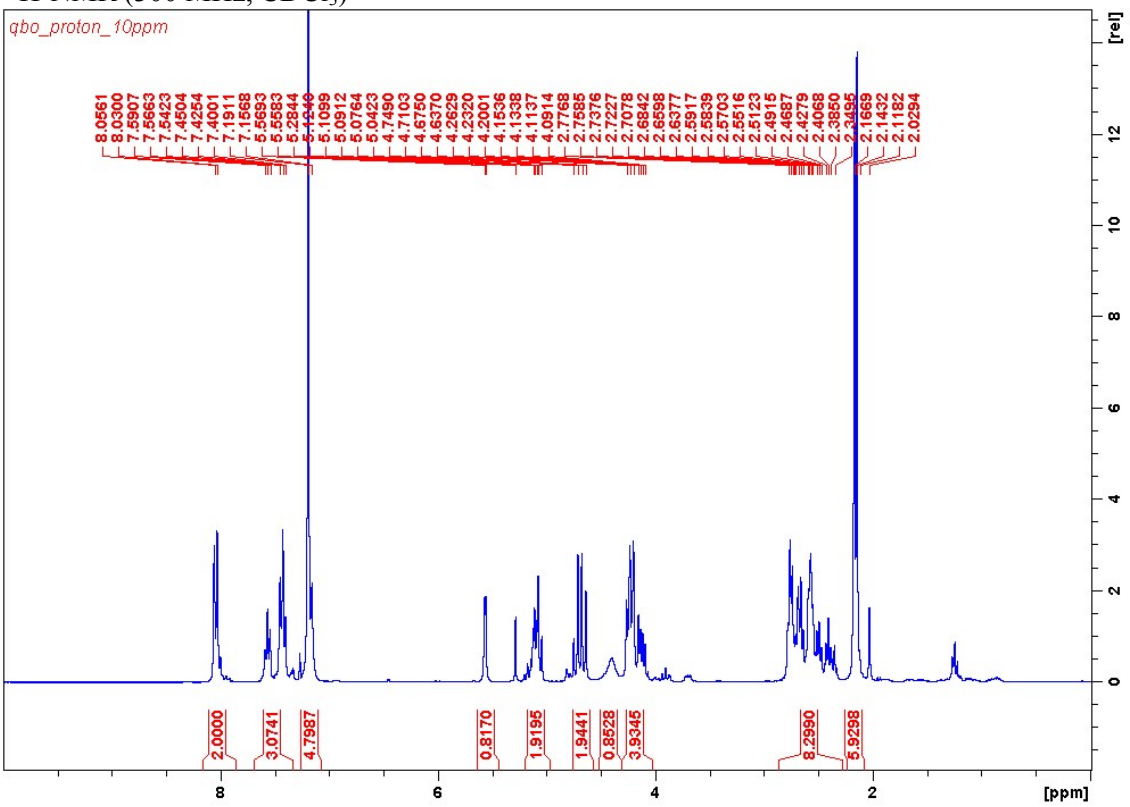
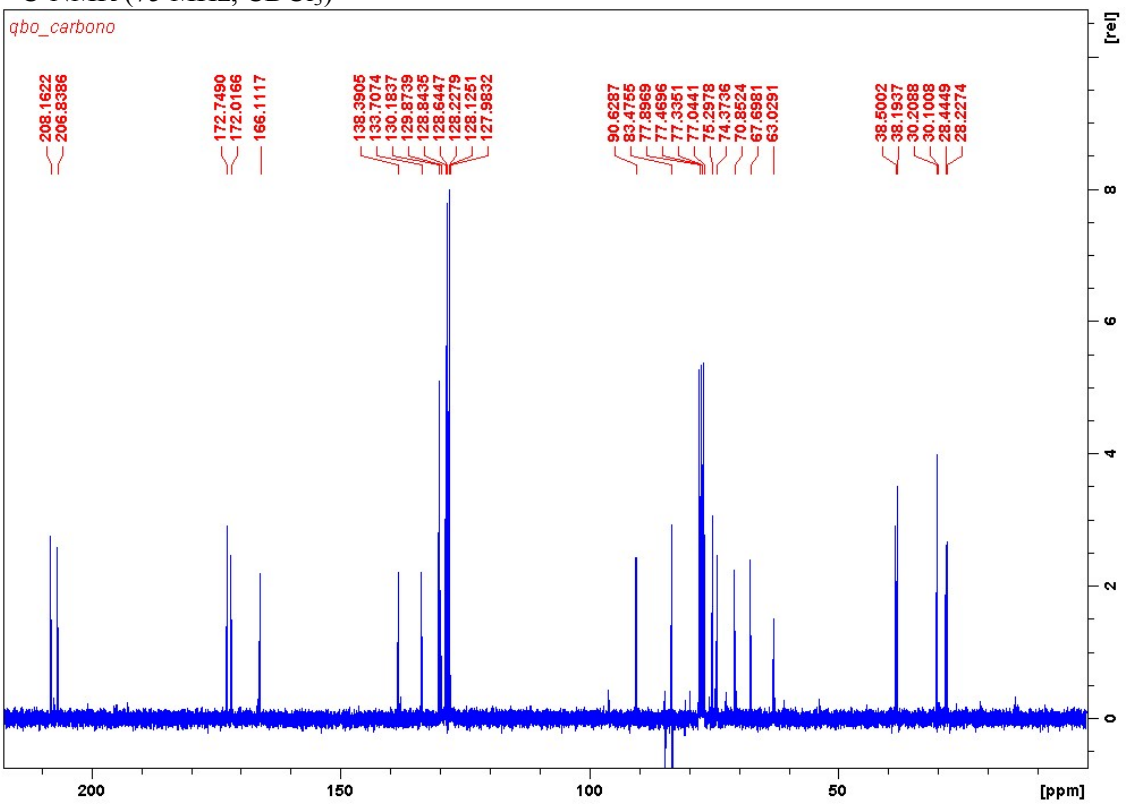
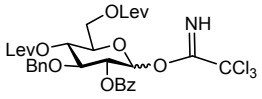
^{1}H-NMR (300 MHz, $CDCl_3$)



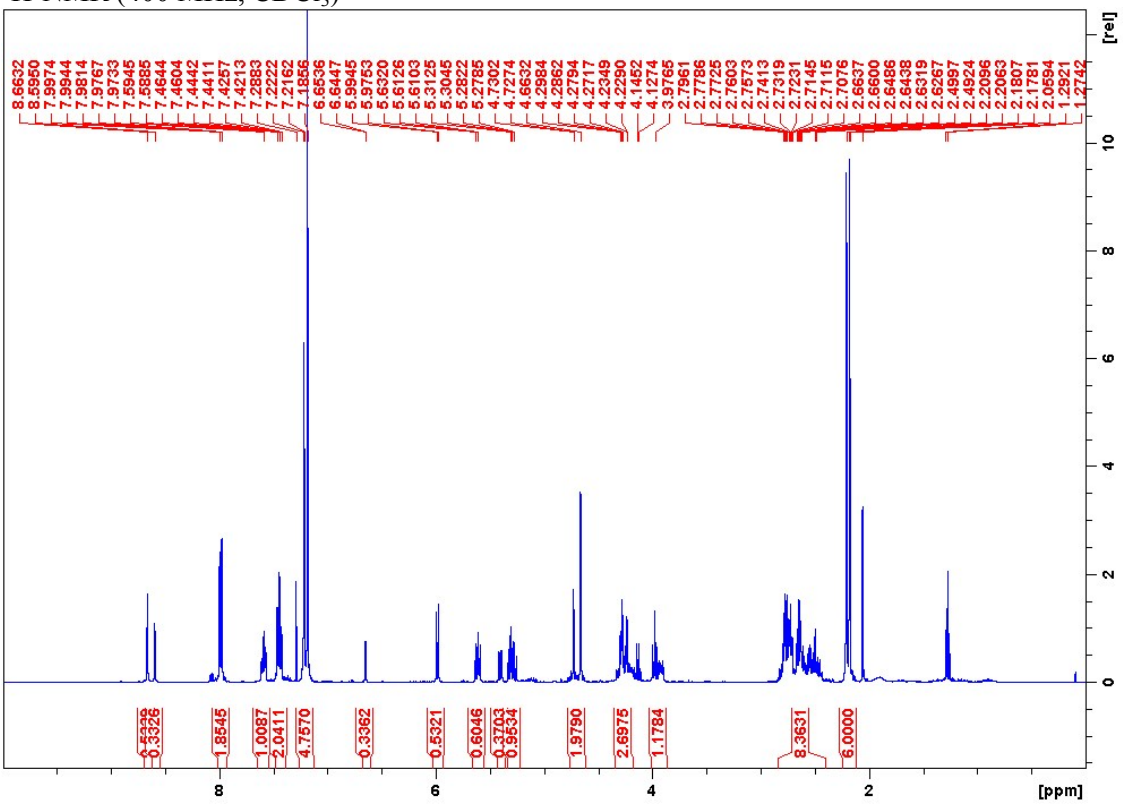
^{13}C-NMR (75 MHz, $CDCl_3$)





11

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



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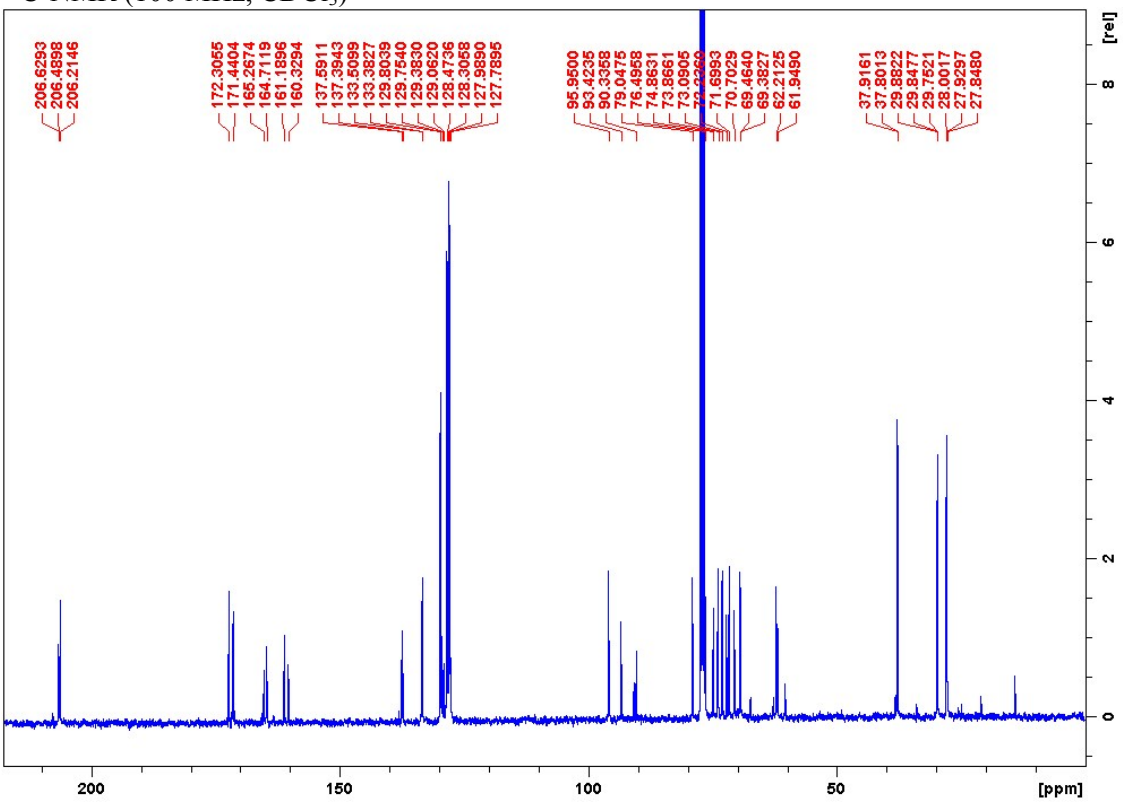

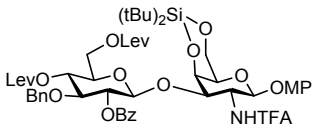

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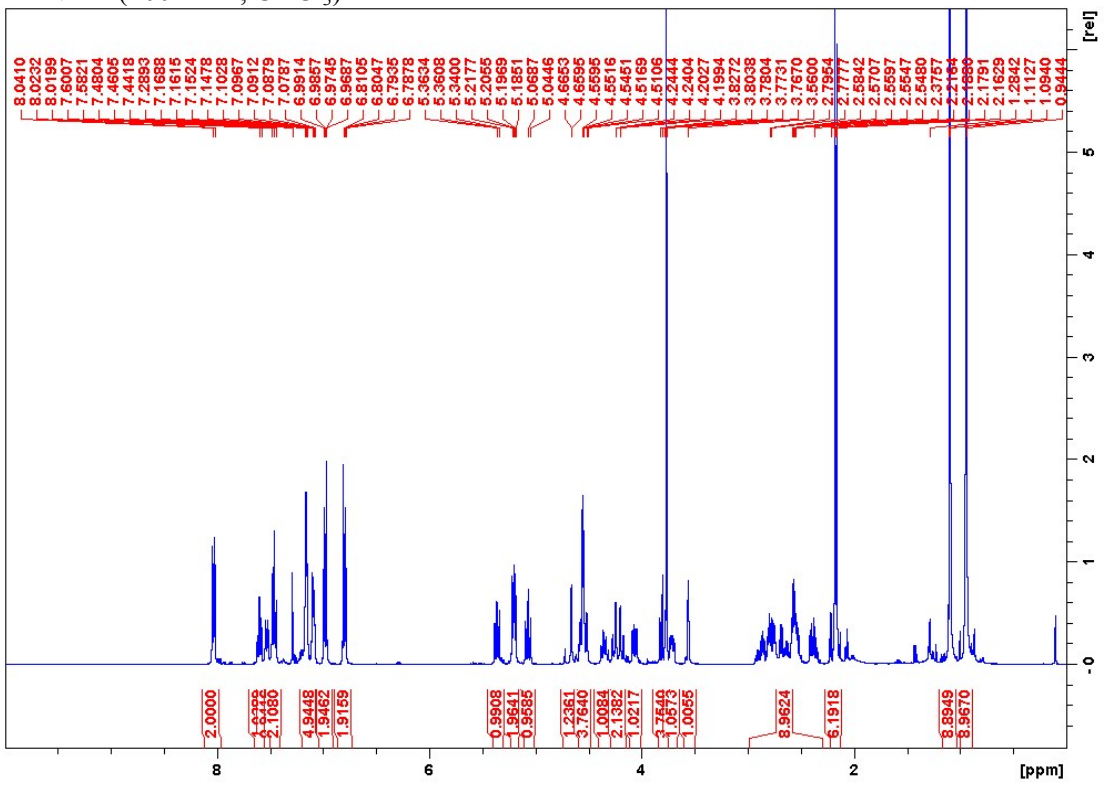

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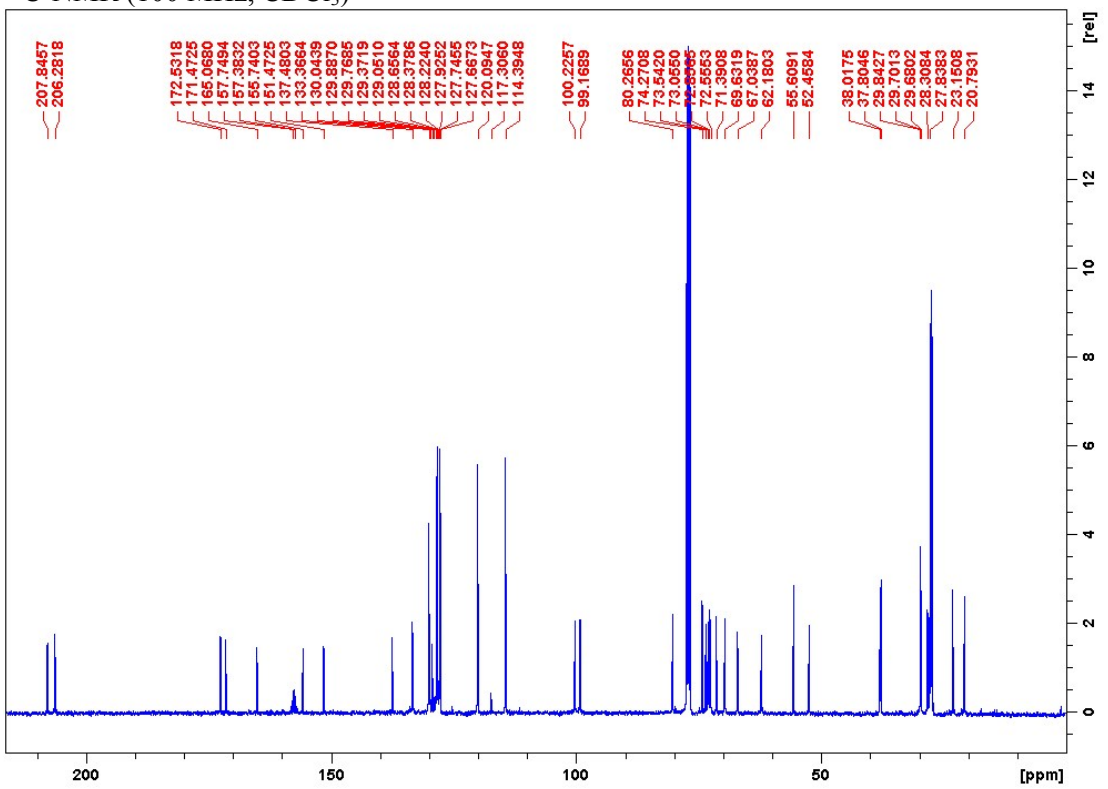

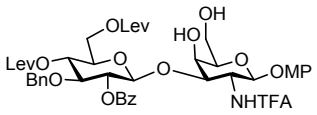

14

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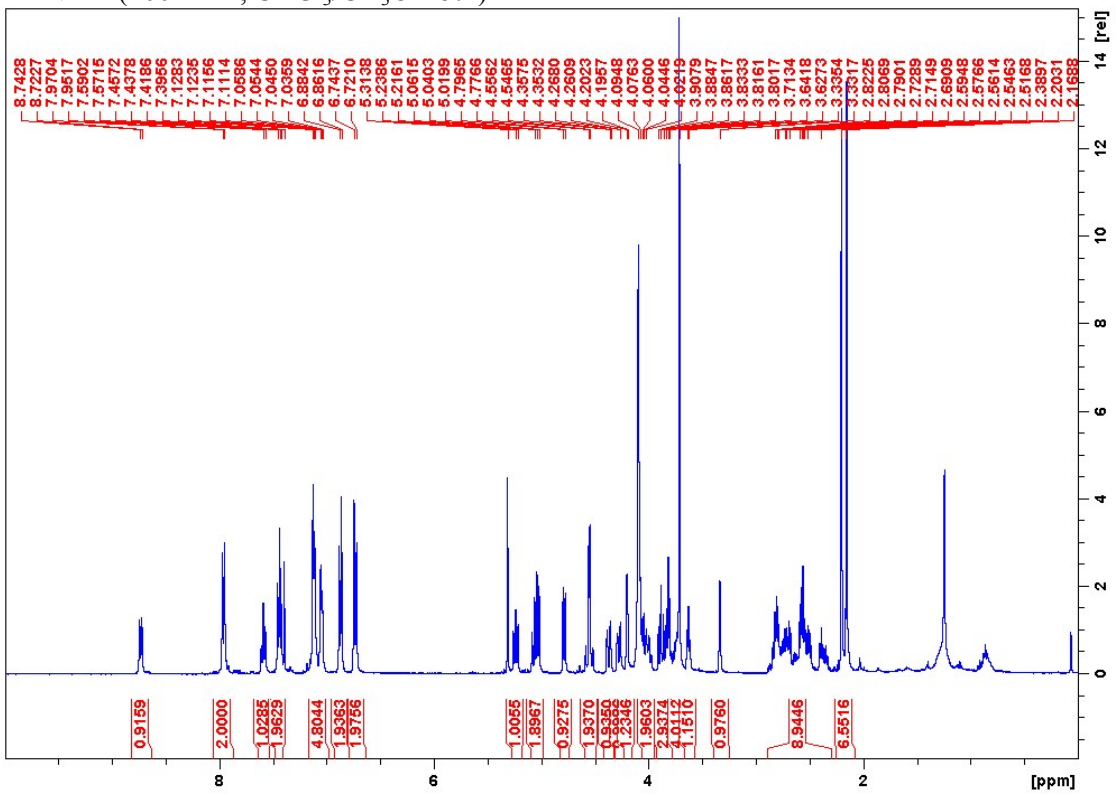

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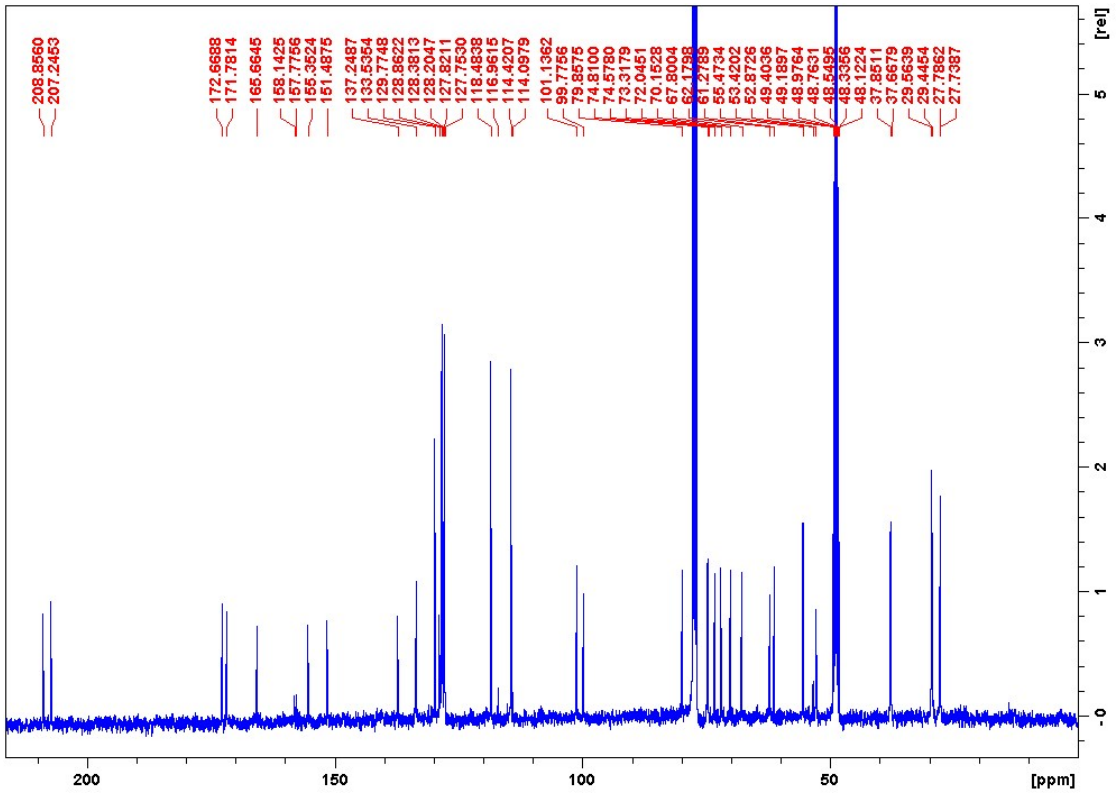
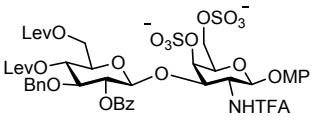
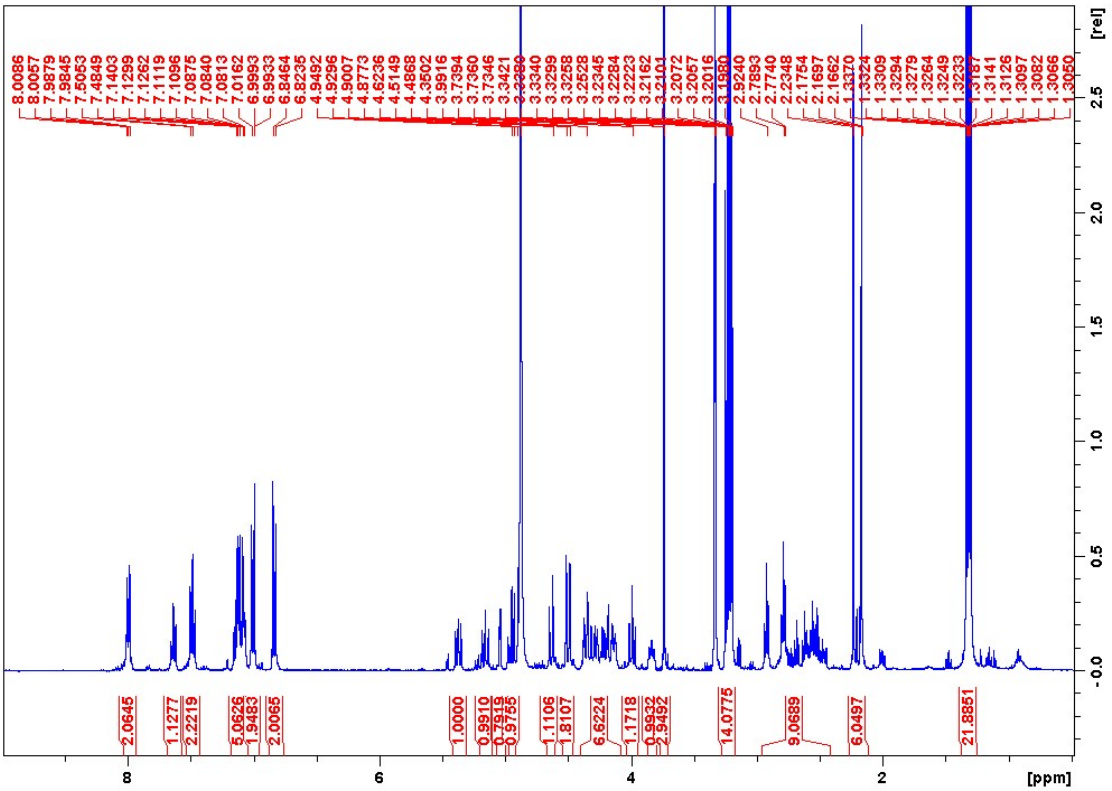




5

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



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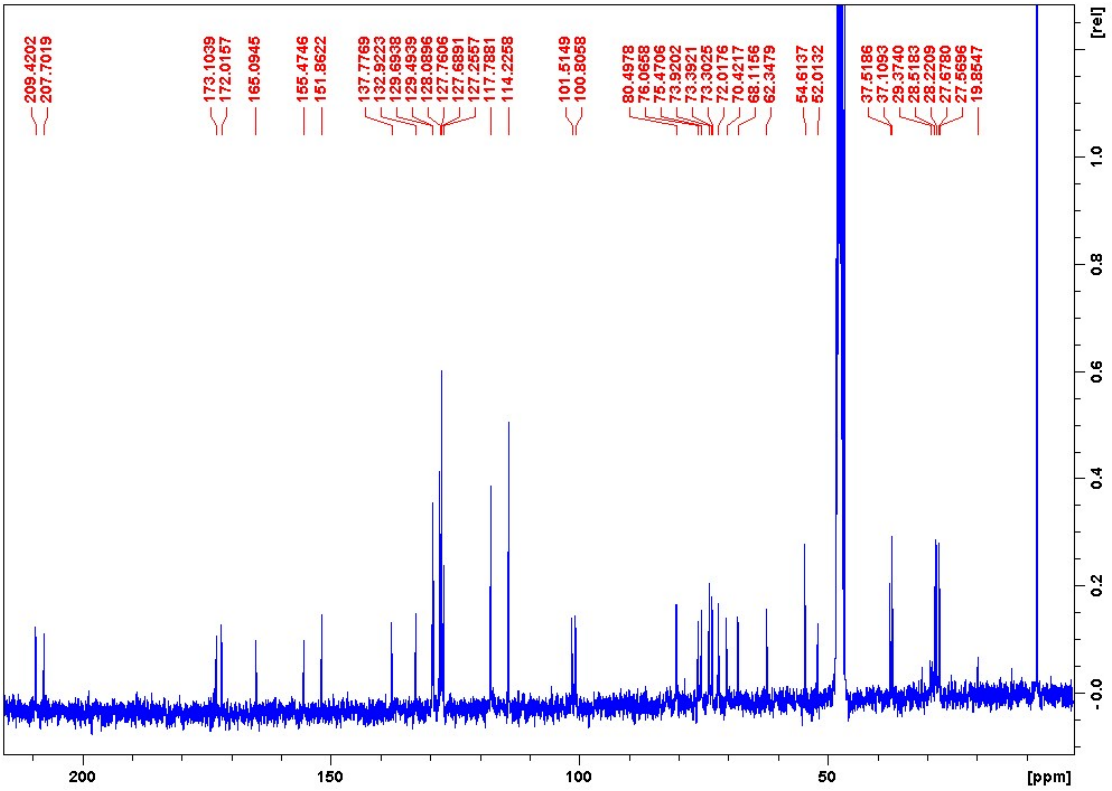

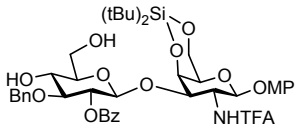

15

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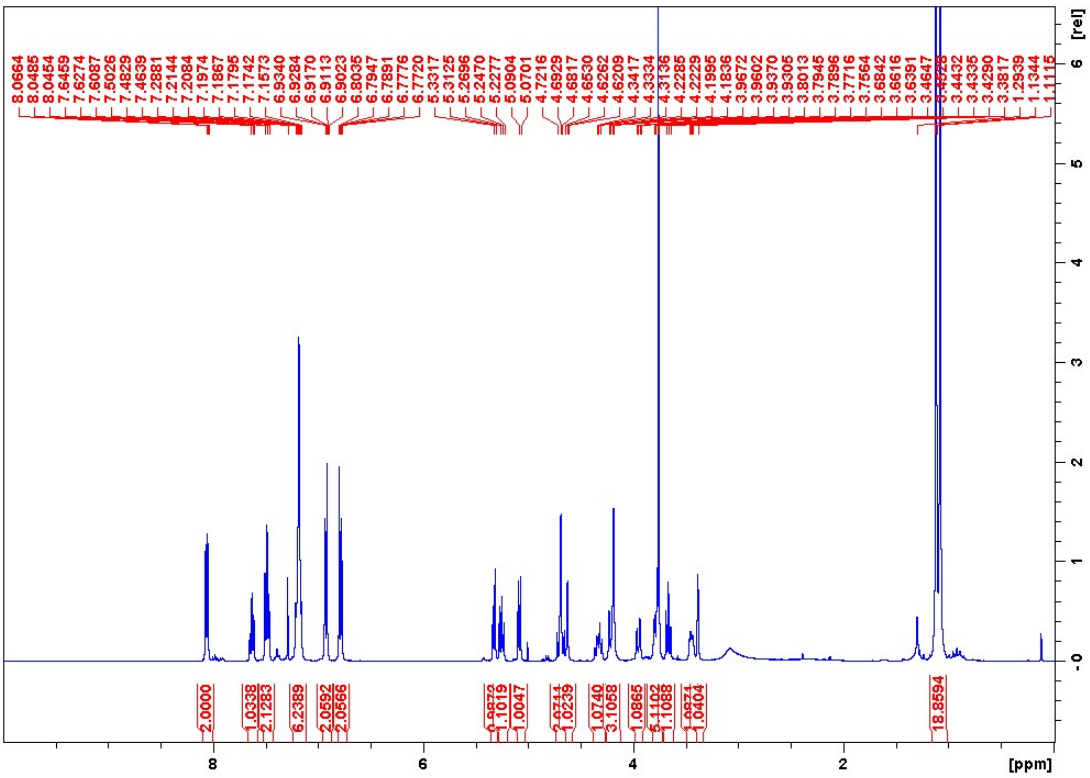

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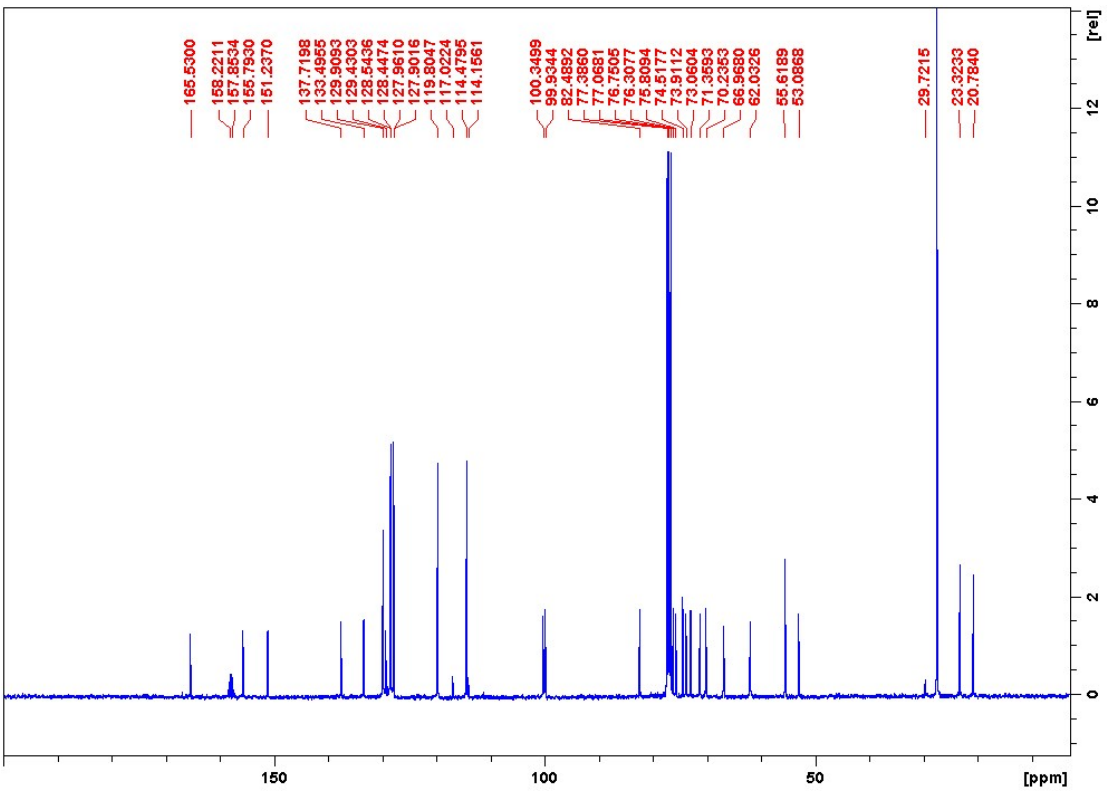

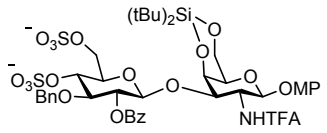

6

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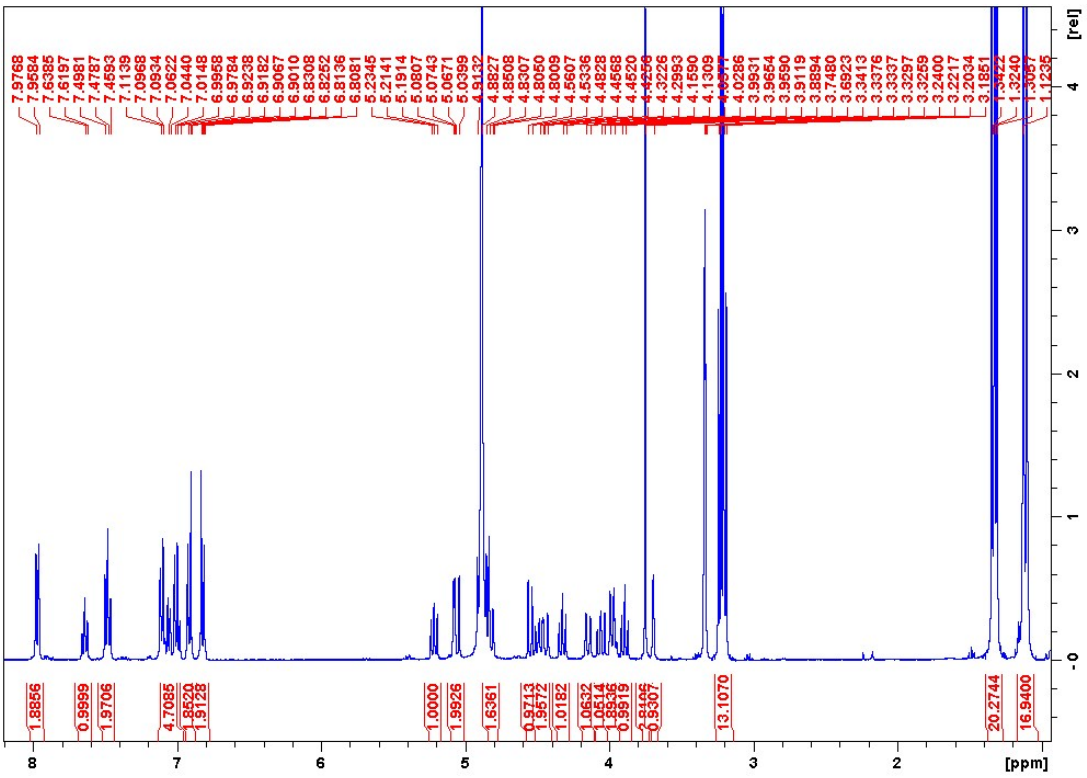

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