Supporting information

D-glucose based bisacrylamide crosslinker: Synthesis and study of homogeneous biocompatible glycopolymeric hydrogels

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Contents

Materials

Methanol, dichloromethane, pyridine, triethylamine, were purified and dried before use. The n-hexane used was the fraction distilling between 40–60 ºC. All the chemicals including acryloyl chloride were procured from either Aldrich or Fluka. DMEM cell cuture medium, Penicillin and Streptomycin were purchased from HiMedia, Mumbai, India. Fetal Bovine Serum (FBS) was procured from Invitrogen BioServices India Pvt. Ltd. MTT was from Sigma Aldrich. Water, with conductivity 0.6 $\mu S\ cm^{-1}$, from Millipore Milli-Q system, was used for the preparation of aqueous solutions.

Methods

The melting points were recorded with a Thomas Hoover Capillary melting point apparatus and are uncorrected. The IR spectra were recorded using diamond single reflectance ATR in IR Affinity-1 spectrometer. The samples were analyzed over the range of 400-4000 cm^{-1}, operating at 4 cm^{-1} resolution. The ^{1}H (200 MHz, 600 MHz, 700 MHz) and ^{13}C (50 MHz, 126 MHz, 176 MHz) NMR spectra were recorded with a Brüker Oxford instrument in $CDCl_3$, CD_3OD or D_2O as solvents. The elemental analysis was carried out with a Thermo-Electron Corporation CHNS analyzer model Flash-EA 1112. The optical rotations were measured using a Jasco P1020 polarimeter. Mass of the materials was carried out with a MS (70 eV). The thin layer chromatography was performed on pre-coated plates (0.25 mm, silica gel 60 F254), and the spots were visualized by UV light or by spraying with 3.5% solution of 2,4-dinitrophenylhydrazine in ethanol/H_2SO_4 or with basic aqueous $KMnO_4$ solution followed by heating. A Co-60 gamma radiation source was used with a dose rate of 1.23 KGy/h. Irradiation was carried out in 1 cm x 1 cm closed glass vials in nitrogen atmosphere.

Experimental

3-Azido-3-deoxy-5-hydroxy-1,2-*O*-isopropylidene-6-*O*-tosyl-α-D-*gluco*-furanose (4).

To a stirred solution of the azido diol **3** (3.50 g, 14.27 mmol) and pyridine (1.38 mL, 17.12 mmol) in CH_2Cl_2 (50 mL), at 0 °C was added tosyl chloride (2.91 g, 15.27 mmol) dissolved in CH_2Cl_2 (15 mL) dropwise and DMAP (0.08 g, 0.71 mmol). The reaction mixture was stirred at same temperature for 1 h, slowly brought to 25 ºC and stirred for additional 2 h. After completion of reaction (*cf.* TLC), water (50 mL) was added and extracted with EtOAc (100 mL). The organic layer was washed, sequentially, with cold 1N HCl (2 x 20 mL), saturated $NaHCO_3$ (1 x 20 mL), brine (1 x 20 mL), water (1 x 50 mL), and dried over Na_2SO_4. Filtration and evaporation in vacuo gave a residue, which on column chromatography afforded **4** (4.96 g, 87%) as a thick liquid: R_f = 0.48 (30% EtOAc/hexane); $[\alpha]_D^{25}$ −7.27 (c 1.1, $CHCl_3$); ν_{max}/cm^{-1} 1176, 1367; δ_H(600 MHz; $CDCl_3$) 7.81 (d, *J* = 8.2 Hz, 2H), 7.36 (d, *J* = 8.2 Hz, 2H), 5.81 (d, *J* = 3.4 Hz, 1H), 4.61 (d, *J* = 3.4 Hz, 1H), 4.31 (d, *J* = 8.8 Hz, 1H), 4.17 (s, 1H), 4.11 – 4.07 (m, 3H), 2.74 (d, *J* = 3.7 Hz, 1H, exchangeable with D_2O), 2.46 (s, 3H), 1.47 (s, 3H), 1.31 (s, 3H); δ_C(50 MHz; $CDCl_3$) 145.8, 132.9, 130.7, 128.7, 113.1, 105.6, 83.8, 78.1, 73.1, 68.2, 66.8, 27.2, 26.9, 22.3. Elem. Anal.Calcd. for $C_{16}H_{21}N_3O_7S$: C, 48.11; H, 5.30. Found: C, 48.15; H, 5.37; ESI-MS: Calcd. for $[C_{16}H_{21}N_3O_7S+ Na]^+$: 422.01 Da, Obsd: 421.85 Da.

3,6-Diazido-3,6,-dideoxy-5-hydroxy-1,2-*O*-isopropylidene-α-D-*gluco*-furanose (5).

To a solution of tosylate (**4**) (4.51 g, 11.29 mmol) in DMF (20 mL) was added sodium azide (1.83 g, 28.25 mmol) and heated at 80 °C for 3 h. After completion of reaction (*cf.* TLC), DMF was removed under vacuo, and the residue was extracted with EtOAc (3 x 50 mL). The combined organic layer was dried over Na_2SO_4, concentrated and purified using column

chromatography to give diazide (**5**) as thick liquid (2.63 g, 86%): R_f = 0.49 (25% EtOAc/hexane); $[\alpha]_D^{25}$ –40.05 (c 1.2, $CHCl_3$); ν_{max}/cm^{-1} 2100; δ_H(700 MHz; $CDCl_3$) 5.87 (d, *J* = 3.5 Hz, 1H), 4.65 (d, *J* = 3.5 Hz, 1H), 4.12 – 4.17 (m, 2H), 4.03 – 3.96 (m, 1H), 3.67 (dd, *J* = 12.6, 2.8 Hz, 1H), 3.51 (dd, *J* = 12.6, 6.3 Hz, 1H), 2.38 – 2.32 (m, 1H, exchangeable with D_2O), 1.51 (s, 3H), 1.33 (s, 3H); δ_C(176 MHz, $CDCl_3$) 113.1, 105.6, 83.9, 79.8, 69.3, 66.8, 55.5, 27.2, 26.8. Elem. Anal. Calcd. for $C_9H_{14}N_6O_4$: C, 40.00; H, 5.22. Found: C, 40.07; H, 5.18.

3,6-Bisacrylamido-3,6,-dideoxy-5-hydroxy-1,2-*O*-isopropylidene-α-D-*gluco*-furanose (6).

To a solution of diazido alcohol (**5**) (2.45 g, 9.06 mmol) in MeOH (30 mL), was added 10% Pd/C (0.17 g) and hydrogenated (80 psi) for 12 h at 25 ºC. The catalyst was filtered through a pad of Celite 545 using MeOH (4 x 10 mL). The filterate was concentrated and dried under vaccum. The vaccum dried diamine was dissolved in CH_2Cl_2 (35 mL) and DIEA (7.89 mL, 45.30 mmol), cooled to –40 ºC, acryloyl chloride (1.64 mL, 20.11 mmol) was added and stirred at same temperature for 20 min. After completion of reaction (*cf.* TLC), reaction mixture was diluted with cold water (5 mL), and extracted using CH_2Cl_2 (3 x 30 mL). The combined organic layer was kept over anhydrous Na_2SO_4, concentrated under vacuo, and purified using column chromatography to afford (**6**) as a thick liquid (2.36 g, 79% (over two steps)): R_f = 0.15 (80% EtOAc/hexane); $[\alpha]_D^{25}$ +90.35 (c 1.50, $CHCl_3$); ν_{max}/cm^{-1}1685, 1665, 1551; δ_H(600 MHz; CD_3OD) 7.85 (s, 1H, exchangeable with D_2O), 6.30 – 6.12 (m, 4H), 5.84 (d, *J* = 3.6 Hz, 1H), 5.66 (dd, *J* = 8.4, 3.5 Hz, 1H), 5.59 (dd, *J* = 10.1, 1.8 Hz, 1H), 4.48 (d, *J* = 3.6 Hz, 1H), 4.43 (d, *J* = 3.3 Hz, 1H), 3.98 (dd, *J* = 8.6, 3.3 Hz, 1H), 3.70 – 3.62 (m, 2H), 3.27 (s, 1H, exchangeable with D_2O), 3.15 (dd, *J* = 14.4, 8.6 Hz, 1H), 1.44 (s, 3H), 1.26 (s, 3H); δ_C(126 MHz; CD_3OD)

167.1, 167.0, 130.6, 129.9, 126.7, 125.5, 111.7, 104.7, 84.0, 79.8, 67.2, 55.8, 42.9, 25.5, 25.1. Elem. Anal. Calcd. for $C_{15}H_{22}N_2O_6$: C, 55.21; H, 6.79. Found: C, 55.19; H, 6.85; ESI-MS: Calcd. for $[C_{15}H_{22}N_2O_6 + Na]^+$: 349.12 Da, Obsd: 348.98 Da.

(2R,3S,4S,5S)-4-acrylamido-6-(acrylamidomethyl)-tetrahydro-2H-Pyran2,3,5-triol (2a).

A pre-cooled solution of TFA-H_2O (3:2, 10 mL) was added dropwise to a RB charged with bisacrylamide (**6**) (1.30 g, 3.98 mmol) at 0 ^oC. The reaction mixture was stirred at same temperature for 30 min, slowly brought to 25 ^oC and stirred for additional 10 h. After completion of reaction (*cf.* TLC) TFA was coevaporated with toluene and dried under vaccum. The residue was precipitated using dry EtOAc (20 mL) and washed well with EtOAc (5 x 10 mL). The residue was vaccum dried, redissolved in double distilled water, filtered through Millex (25 mm, 5 μm) and lyophilized to afford bisacrylamide (**2a**) as a white amorphous powder (0.78 g, 68%): ν_{max}/cm^{-1} 1651, 1640, 1552; *^{1}H NMR revealed the formation of anomeric mixture in the ratio of 55:45 in favor of α-isomer*. δ_H(500 MHz; D_2O) 6.41 – 6.31 (m, 2H), 6.29 – 6.21 (m, 2H), 5.88 – 5.78 (m, 2H), 5.27 (d, *J* = 3.6 Hz, H1*e*), 4.76 (d, *J* = 7.8 Hz, H1*a*), 4.22 (t, *J* = 10.3 Hz, 1H), 4.09 – 3.97 (m, 1H), 3.73 – 3.65 (m, 2H), 3.61 – 3.50 (m, 1H), 3.45 – 3.33 (m, 1H); δ_C(126 MHz; D_2O) (only major peaks are mentioned) 169.5, 168.8, 129.9, 129.7, 127.7, 127.5, 91.5, 69.9, 69.7, 69.5, 53.8, 40.0. Elem. Anal. Calcd. for $C_{12}H_{18}N_2O_6$: C, 50.35; H, 6.34. Found: C, 50.41; H, 6.38; ESI-MS: Calcd. for. $[C_{12}H_{18}N_2O_6 + Na]^+$: 309.09 Da, Obsd: 308.90 Da.

{[1,2,],[5,6]}-Di-*O*-isopropylidene-3-*O*-*tert*-butyldiphenylsilyl-α-D-*gluco*-furanose (8).

To a cooled (0 ^oC) solution of diacetone D-glucose (**7**) (5.00 g, 19.21 mmol) and imidazole (2.61 g, 38.42 mmol) in DMF (25 mL) was added TBDPSCl (6.16 mL, 24.01 mmol) dropwise,

followed it with DMAP (0.12 g, 0.96 mmol). The reaction mixture was slowly brought to 30 °C, and stirred for additional 24 h. After the completion of reaction (*cf.* TLC), usuall work up using EtOAc (200 mL) afforded a thick residue which on coloumn purification afforded (**8**) as a thick liquid (8.30 g, 86%): R_f = 0.55 (10% EtOAc/hexane); $[\alpha]_D^{25}$ –10.08 (c 1.0, $CHCl_3$); ν_{max}/cm^{-1} 1211, 1093; δ_H(600 MHz; $CDCl_3$) 7.98 – 7.64 (m, 4H), 7.52 – 7.32 (m, 6H), 5.81 (d, *J* = 3.2 Hz, 1H), 4.48 – 4.42 (m, 2H), 4.19 – 4.15 (m, 1H), 4.06 (d, *J* = 3.1 Hz, 1H), 4.05 – 3.97 (m, 2H), 1.42 (s, 3H), 1.39 (s, 3H), 1.33 (s, 3H), 1.09 (s, 9H), 1.08 (s, 3H); δ_C(176 MHz; $CDCl_3$) 136.7, 136.4, 134.7, 133.2, 130.6, 130.2, 128.5, 128.3, 112.3, 109.8, 105.7, 85.2, 83.2, 77.3, 72.9, 68.6, 27.6, 27.5, 27.3, 26.7, 25.9, 20.1. Elem. Anal. Calcd. for $C_{28}H_{38}O_6Si$: C, 67.44; H, 7.68. Found: C, 67.47; H, 7.62.

5,6-Dihydroxy-1,2-*O*-isopropylidene-3-*O*-*tert*-butyldiphenylsilyl-α-D-*gluco*-furanose (9).

To a solution of (**8**) (8.00 g, 16.04 mmol) in THF (20 mL) at 0 °C was slowly added 30% perchloric acid (8 mL). The reaction mixture was stirred at same temperature until it showed complete conversion (*cf.* TLC), neutralized using K_2CO_3 (saturated) solution, concentrated, and extracted using EtOAc (3 x 50 mL). The combined organic layer was dried over Na_2SO_4, concentrated, to afford a thick liquid which was purified using coloum chromatography to yield diol (**9**) as a white solid (5.72 g, 77%): Mp 120 °C; R_f = 0.30 (30% EtOAc/hexane); $[\alpha]_D^{25}$ –17.50 (c 1.1, $CHCl_3$); ν_{max}/cm^{-1} 3355br, 1227, 1064; δ_H(700 MHz; $CDCl_3$) 7.73 (d, *J* = 7.7 Hz, 2H), 7.68 (d, *J* = 7.7 Hz, 2H), 7.47 (t, *J* = 7.3 Hz, 2H), 7.42 (t, *J* = 7.3 Hz, 4H), 5.84 (d, *J* = 3.5 Hz, 1H), 4.48 (s, 1H), 4.28 (d, *J* = 3.5 Hz, 1H), 4.06 – 3.97 (m, 2H), 3.88 – 3.78 (m, 1H), 3.73 (dd, *J* = 11.2, 5.3 Hz, 1H), 1.67 – 1.61 (m, 2H, exchangeable with D_2O), 1.40 (s, 3H), 1.14 (s, 3H),

1.10 (s, 9H); δ_C(176 MHz; $CDCl_3$) 136.5, 136.4, 134.5, 133.2, 130.9, 130.8, 128.7, 128.6, 112.4, 105.5, 85.1, 81.9, 77.4, 69.2, 65.2, 27.7, 27.3, 26.7, 20.2. Elem. Anal. Calcd for $C_{25}H_{34}O_6Si$: C, 65.47; H, 7.47. Found: C, 65.44; H, 7.45; ESI-MS: Calcd. for $[C_{25}H_{34}O_6Si + Na]^+$: 481.19 Da, Obsd: 481.02 Da.

6-Azido-6-deoxy-5-hydroxy-1,2-*O*-isopropylidene-3-*O*-*tert*-butyldiphenylsilyl-α-D-*gluco*-furanose (10).

To a solution of diol (**9**) (5.52 g, 12.03 mmol) in CH_2Cl_2 (60 mL) at 0 °C was added triethyl amine (2.01 mL, 14.42 mmol) followed it with dropwise addition of methane sulfonyl chloride (0.98 mL, 12.63 mmol) in CH_2Cl_2 (15 mL) over 30 min. The reaction was stirred at same temperature for 1 h, then brought to 25 °C and stirred for additional 1 h. The reaction was quenched using cold water (20 mL) and extracted using CH_2Cl_2 (3 x 25 mL). The combined organic layers were dried over Na_2SO_4, concentrated and dried under vaccum to afford mesylate (crude) as a thick liquid. To the solution of mesylate (crude) in DMF (25 mL), was added sodium azide (5.47 g, 84.21 mmol) and heated at 70-80 °C for 3 h. The usual workup and coloum purification afforded azide (**10**) (3.10 g, 53%): R_f = 0.70 (20% EtOAc/hexane); $[\alpha]_D^{25}$ –22.25 (c 1.1, $CHCl_3$); ν_{max}/cm^{-1} 2098, 1215, 1093; δ_H(700 MHz; $CDCl_3$) 7.74 (d, *J* = 7.7 Hz, 2H), 7.70 (d, *J* = 7.7 Hz, 2H), 7.48 – 7.45 (m, 2H), 7.44 – 7.39 (m, 4H), 5.90 (d, *J* = 3.5 Hz, 1H), 4.48 (d, *J* = 3.5 Hz, 1H), 4.32 (t, *J* = 2.7 Hz, 1H), 4.22 (q, *J* = 4.7 Hz, 1H), 4.12 – 4.08 (m, 1H), 3.40 (dd, *J* = 12.8, 4.8 Hz, 1H), 3.33 (dd, *J* = 12.8, 4.0 Hz, 1H), 3.19 (d, *J* = 3.5 Hz, 1H, exchangeable with D_2O), 1.49 (s, 3H), 1.31 (s, 3H), 1.09 (s, 9H); δ_C(176 MHz; $CDCl_3$) 136.4, 136.3, 134.2, 133.1, 131.0, 130.9, 128.8, 128.7, 112.5, 105.6, 85.2, 81.7, 77.1, 68.5, 55.5, 27.7, 27.4, 26.8, 20.1. Elem. Anal. Calcd. for $C_{25}H_{33}N_3O_5Si$: C, 62.09; H, 6.88. Found: C, 62.15; H, 6.93.

6-Acrylamido-6-deoxy-5-hydroxy-1,2-*O*-isopropylidene-3-*O*-*tert*-butyldiphenylsilyl-α-D-*gluco*-furanose (11).

The hydroxyl azide (**10**) (3.30 g, 6.82 mmol) was subjected to hydrogenation (10% Pd/C (0.20 g), H_2 (20 psi), 5 h) and acrylation (acryloyl chloride (0.58 mL, 7.17 mmol), DIEA (1.42 mL, 8.18 mmol)) sequentially, as mentioned earlier for the synthesis of bisacrylamide (**6**), to afford acrylamide (**11**) as a thick liquid (2.90 g, 83% (over two steps)): R_f = 0.20 (30% EtOAc/hexane); $[\alpha]_D^{25}$ +24.21 (c 1.2, $CHCl_3$); ν_{max}/cm^{-1} 3490br, 1680; δ_H(700 MHz; $CDCl_3$) 7.76 – 7.74 (m, 2H), 7.70 – 7.67 (m, 2H), 7.48 – 7.42 (m, 2H), 7.40 (q, *J* = 7.2 Hz, 4H), 6.34 – 6.29 (m, 1H), 6.19 (s, 1H, exchangeable with D_2O), 6.13 (dd, *J* = 17.0, 10.3 Hz, 1H), 5.80 (d, *J* = 3.4 Hz, 1H), 5.69 (dd, *J* = 10.3, 1.1 Hz, 1H), 4.50 (d, *J* = 2.2 Hz, 1H), 4.14 – 4.11 (m, 2H), 3.84 (ddd, *J* = 14.4, 6.1, 2.7 Hz, 1H), 3.79 (bs, 1H), 3.46 (dt, *J* = 14.4, 6.1 Hz, 1H), 1.61 (s, 1H, exchangeable with D_2O), 1.36 (s, 3H), 1.09 (s, 12H); δ_C(176 MHz; $CDCl_3$) 168.6, 136.7, 136.4, 134.8, 133.1, 130.9, 130.8, 130.7, 128.7, 128.6, 127.9, 112.3, 105.5, 85.1, 82.6, 77.1, 69.1, 45.5, 27.6, 27.5, 26.8, 20.2. Elem. Anal. Calcd. for $C_{28}H_{37}NO_6Si$: C, 65.72; H, 7.29; Found: C, 65.75; H, 7.36; ESI-MS: Calcd. for $[C_{28}H_{37}NO_6Si + Na]^+$: 534.22 Da, Obsd: 534.09 Da

6-Acrylamido-6-deoxy-3,5-dihydroxy-1,2-*O*-isopropylidene-α-D-*gluco*-furanose (12).

To a solution of acrylamide (**11)** (1.00 g, 1.93 mmol) in THF at 0 ^oC was added TBAF (1M in THF) (2.51 mL, 2.51 mmol). The reaction mixture was stirred to 30 ^oC for 1.5 h. After completion of reaction (*cf*. TLC) the reaction mixture was concentrated under vaccum, and extracted using EtOAc (6 x 20 mL). The resultant thick liquid, was purified using coloumn chromatography to afford the diol (**12**) as a thick liquid (0.44 g, 83 %): R_f = 0.25 (EtOAc);

$[\alpha]_D^{25}$ +4.00 (c 1.1, MeOH); ν_{max}/cm^{-1} 3500br, 1687, 1671, 1545; δ_H(600 MHz; CD_3OD) 6.34 – 6.19 (m, 2H), 5.87 (d, *J* = 2.1 Hz, 1H), 5.65 (d, *J* = 10.0 Hz, 1H), 4.47 (s, 1H), 4.20 (s, 1H), 3.98 – 3.90 (m, 2H), 3.68 (d, *J* = 13.9 Hz, 1H), 3.32 – 3.27 (m, 2H), 1.44 (s, 3H), 1.29 (s, 3H); δ_C(176 MHz; CD_3OD) 167.4, 130.7, 125.4, 111.3, 105.1, 85.6, 81.4, 73.9, 67.3, 43.4, 25.7, 25.0. Elem. Anal. Calcd. for $C_{12}H_{19}NO_6$: C, 52.74; H, 7.01. Found: C, 52.81; H, 6.97; ESI-MS: Calcd. for $[C_{12}H_{19}NO_6 + Na]^+$: 296.10 Da, Obsd: 295.92 Da.

***N*-(((3S,4S,5S,6R)-tetrahydro-3,4,5,6-tetrahydroxy-2H-pyran-2-yl)methyl)acrylamide (2b)**. A pre-cooled solution of TFA-H_2O (3:2, 10 mL) was added dropwise to RB charged with acrylamide (**12**) (1.30 g, 4.75 mmol) at 0 ^oC. The reaction mixture was stirred at same temperature for 30 min, slowly brought to 25 ^oC and was stirred for additional 10 h. After completion of reaction (*cf.* TLC) the reaction was worked up as mentioned for the synthesis of bisacrylamide (**2a**) to get (**2b**) as a white amorphous powder (0.73 g, 66%): ν_{max}/cm^{-1} 1658, 1610, 1539; *^{1}H NMR revealed the formation of anomeric mixture in the ratio of 55:45 in favor of α-isomer*. δ_H(200 MHz; D_2O) 6.61 – 6.42 (m, 2H), 5.85 – 5.73 (m, 1H), 5.20 (d, *J* = 3.6 Hz, H1*e*), 4.62 (d, *J* = 7.0 Hz, H1*a*), 3.79 – 3.40 (m, 4H), 3.38 – 3.17 (m, 2H); δ_C(50 MHz; D_2O) (*peaks due to both α-and β-isomer are listed*) δ 168.9, 168.8, 129.6 (strong), 127.6,127.5, 95.9, 92.0, 75.4, 74.1, 73.9, 72.5, 71.4, 71.1, 70.9, 69.7, 40.1, 39.9. Elem. Anal. Calcd. for $C_9H_{15}NO_6$: C, 46.35; H, 6.48. Found: C, 46.32; H, 6.44; ESI-MS: Calcd. for $[C_9H_{15}NO_6+ Na]^+$: 256.07 Da, Obsd: 255.91 Da.

***In vitro* cell cytotoxicity test**

The cell lines INT407 (human intestinal epithelial cell line) and L929 (mouse fibroblast cell line) were obtained from National Centre for Cell Sciences (NCCS), Pune, India. The cells were grown in DMEM medium with 10% fetal bovine serum, penicillin (100 U/mL) and streptomycin (100 µg/mL). Both the qualitative and quantitative *in vitro* cytotoxicity studies towards the test samples, were performed, respectively by microscopically observing the growth of the cells and by MTT, (3-[4,5-dimethylthiazol-2-yl]-2,5 diphenyl tetrazolium bromide) assay. In the former 15,000 cells were plated per well in a 24 well plate and allowed to adhere overnight. The test samples Glc-acryl, Glc-bis and Glc-gel (obtained directly after gamma irradiation) were added to the appropriate wells next day. Photograph of the cells were taken at regular intervals employing an inverted microscope with an attached camera (Leica EC3 type, Switzerland) at 40 × magnification. In the MTT assay the procedure mentioned above was followed and at the end of 48 h of incubation with the test samples, the number of viable cells in each well was quantified by incubation with MTT (0.5 mg/mL) for 4 h, followed by solubilisation buffer (10% SDS in 0.01N HCl) overnight. The plate was read in a plate reader at 550 nm. In both the experiments the samples were in triplicates and each experiment was conducted twice.

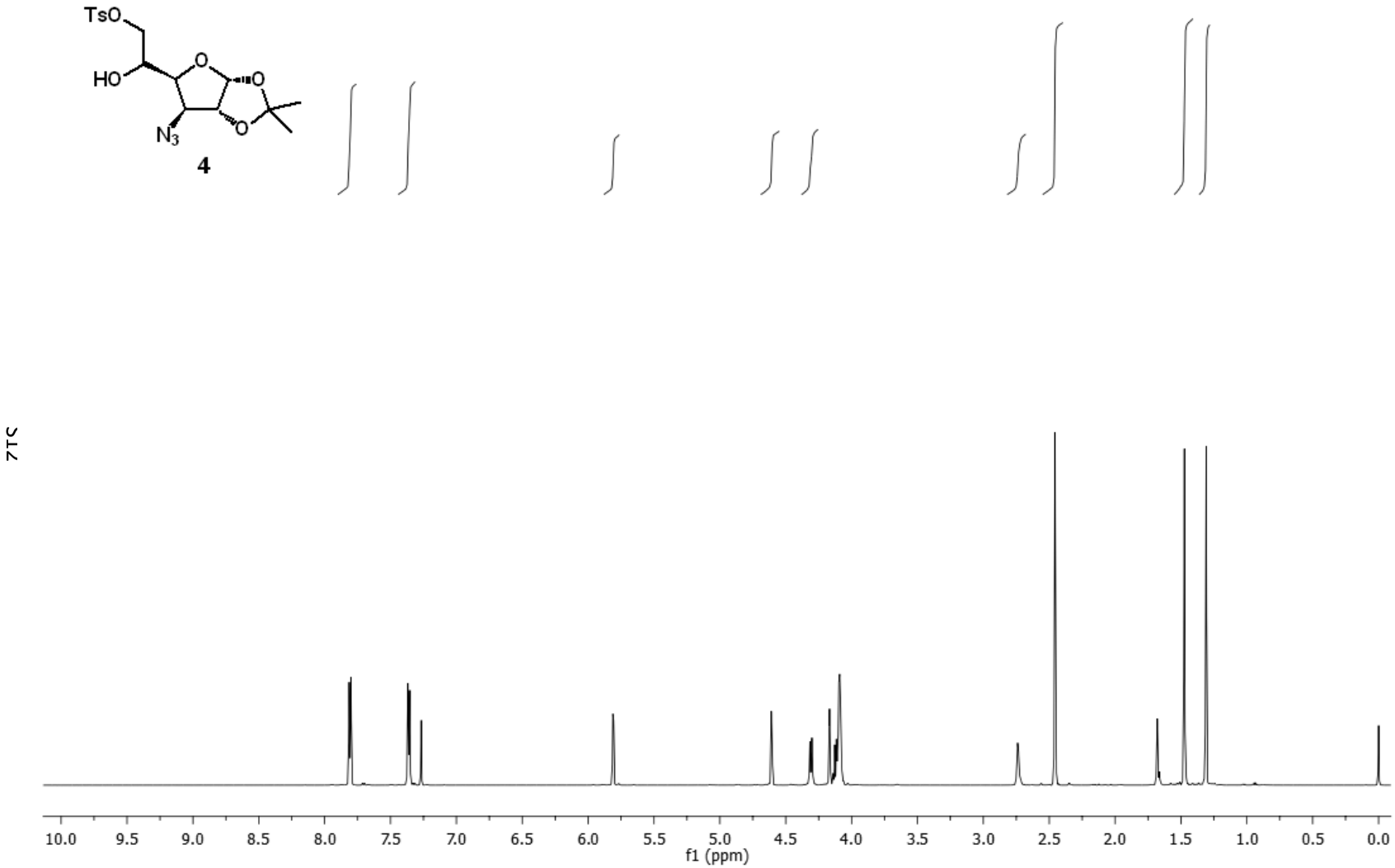
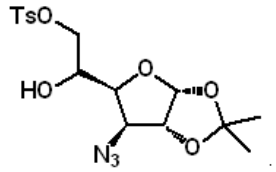


Figure 1: ^{1}H NMR (600 MHz, $CDCl_3$) spectrum of compound **4**



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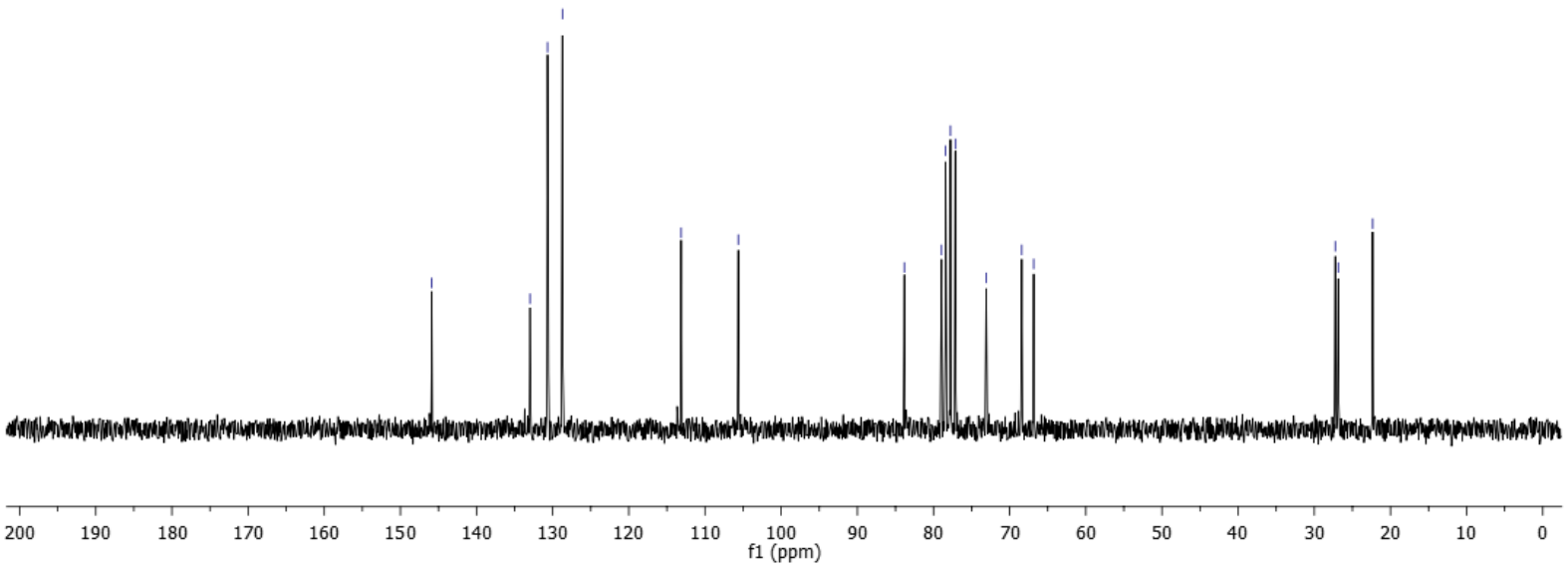

Figure 2: ^{13}C NMR (50 MHz, $CDCl_3$) spectrum of compound **4**

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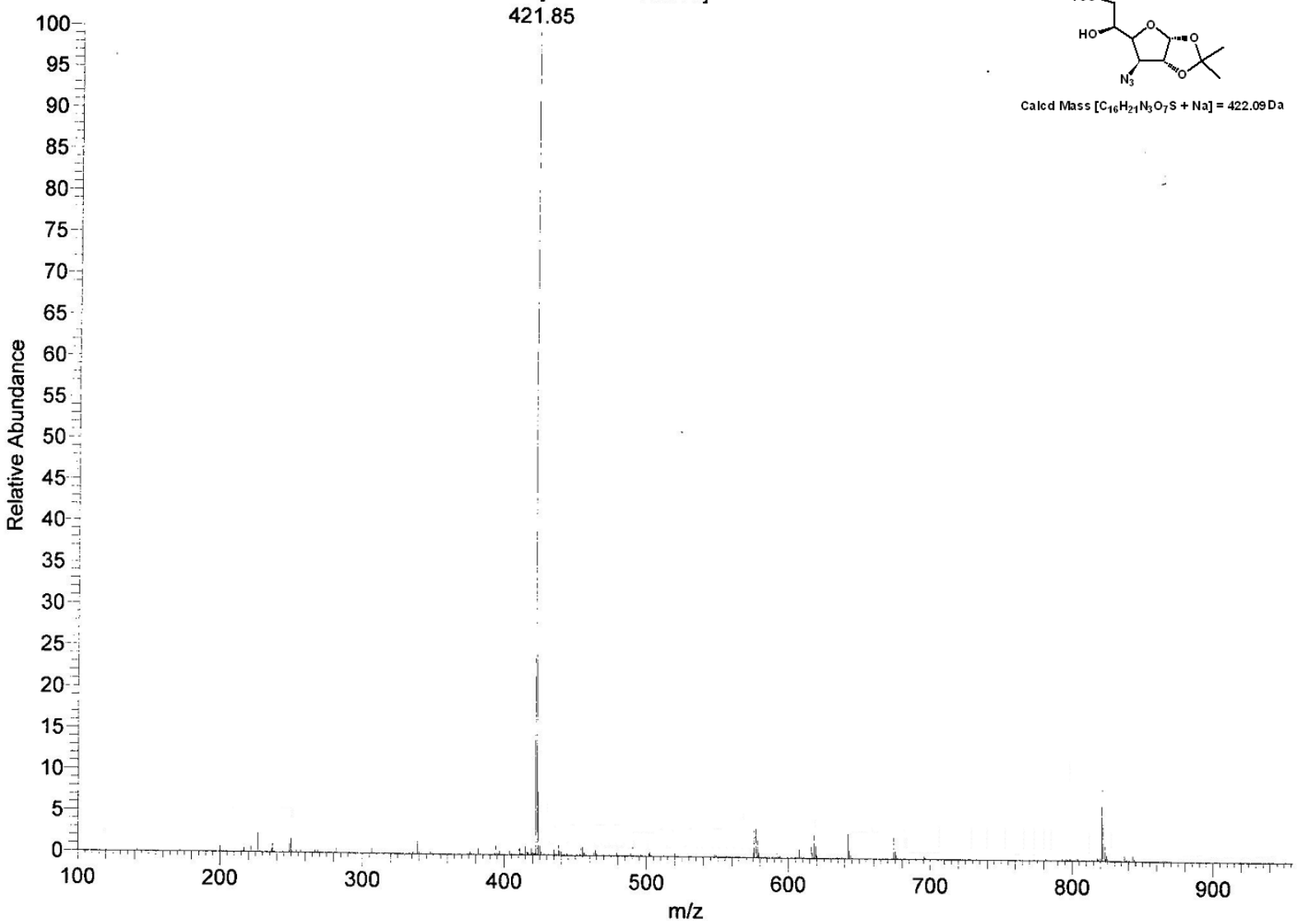


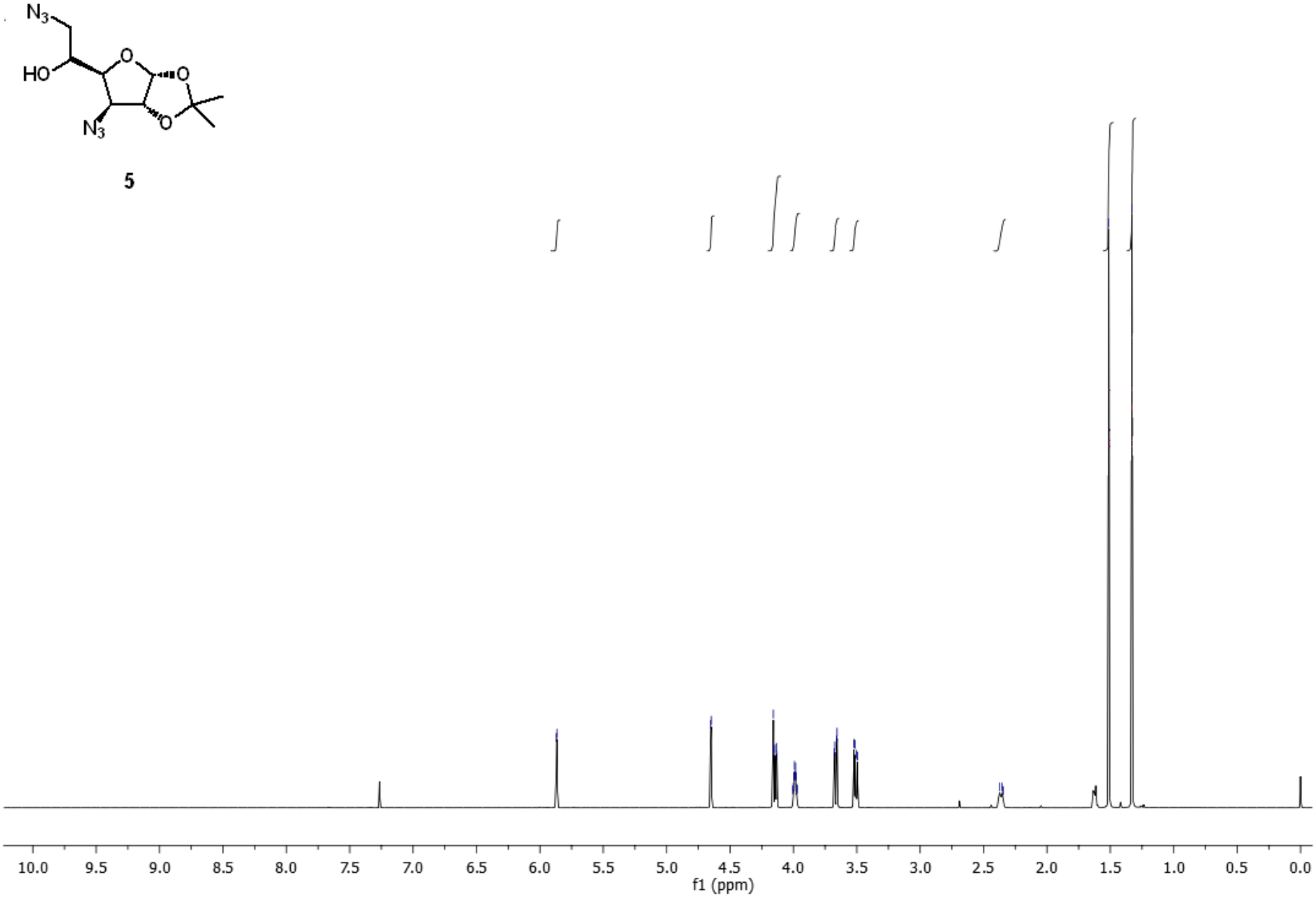
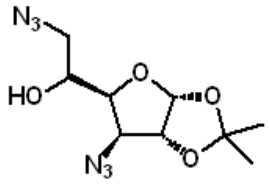


Figure 3: ^{1}H NMR (700 MHz, $CDCl_3$) spectrum of compound **5**



5

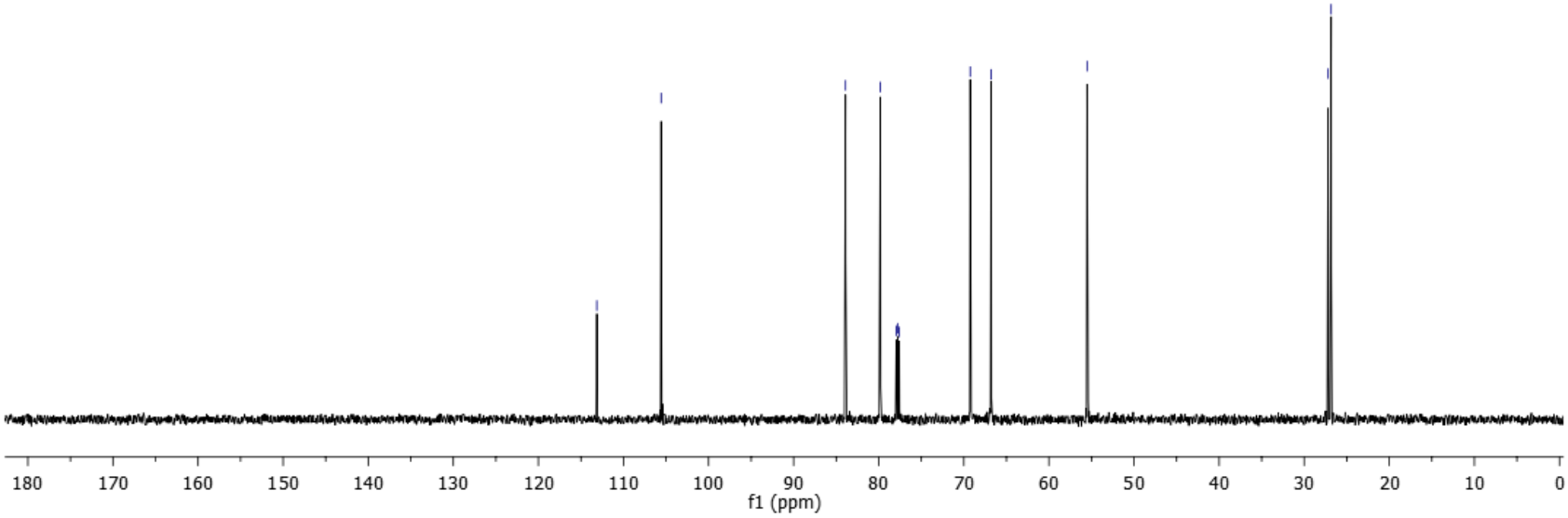


Figure 4: ^{13}C NMR (176 MHz, $CDCl_3$) spectrum of compound **5**

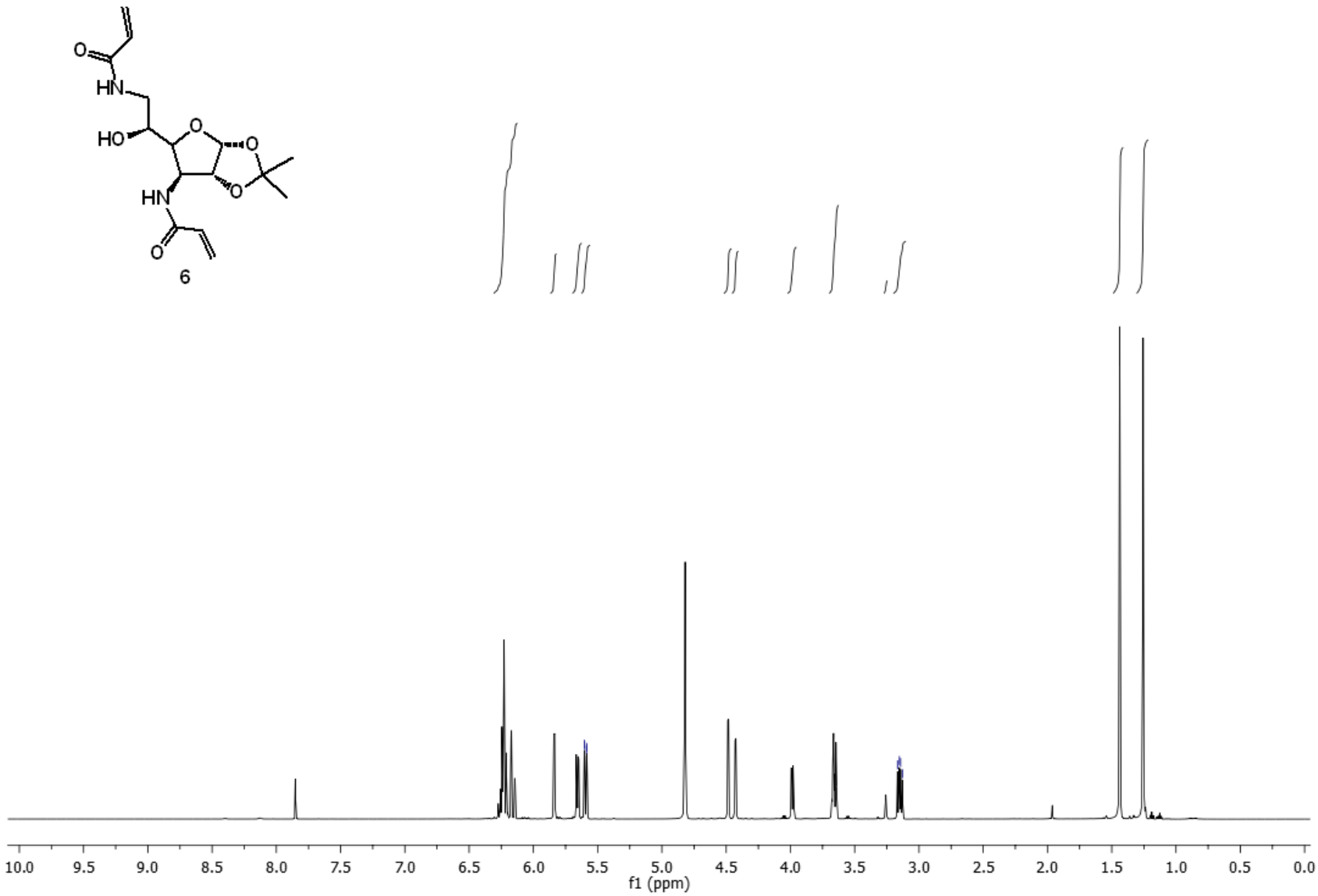
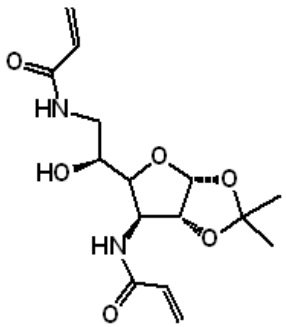


Figure 5: ^{1}H NMR (600 MHz, CD_3OD) spectrum of compound **6**



6

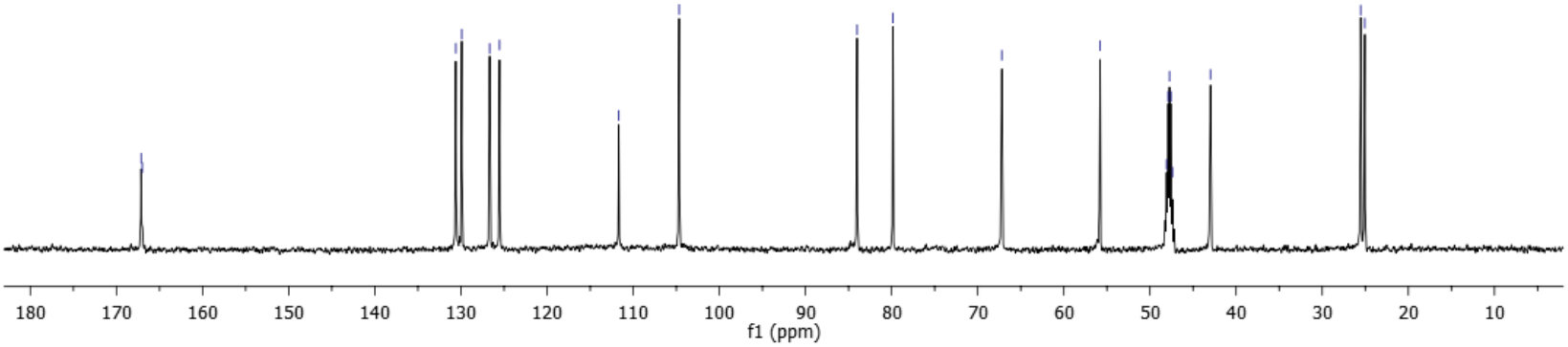
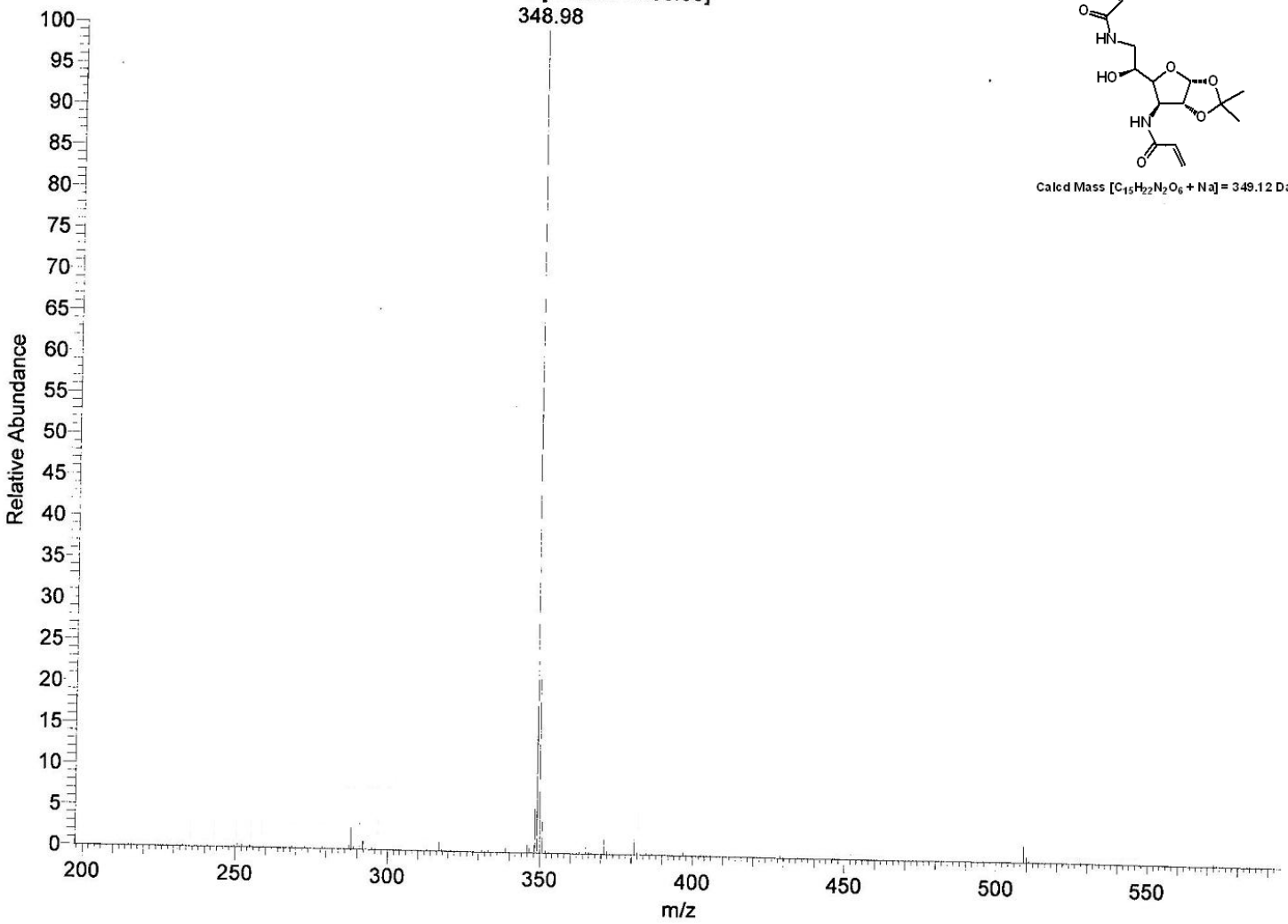


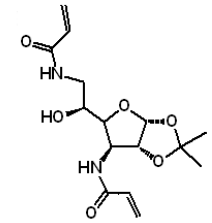
Figure 6: ^{13}C NMR (126 MHz, CD_3OD) spectrum of compound **6**

F:\DATA\June-2013\21\AKS-III

6/21/2013 12:50:53 PM

AKS-III #9-20 RT: 0.14-0.33 AV: 12 NL: 7.94E7
T: {0,0} + c ESI corona sid=80.00 det=1600.00 Full ms [100.00-2000.00]





Calcd Mass [$C_{15}H_{22}N_2O_6$ + Na] = 349.12 Da

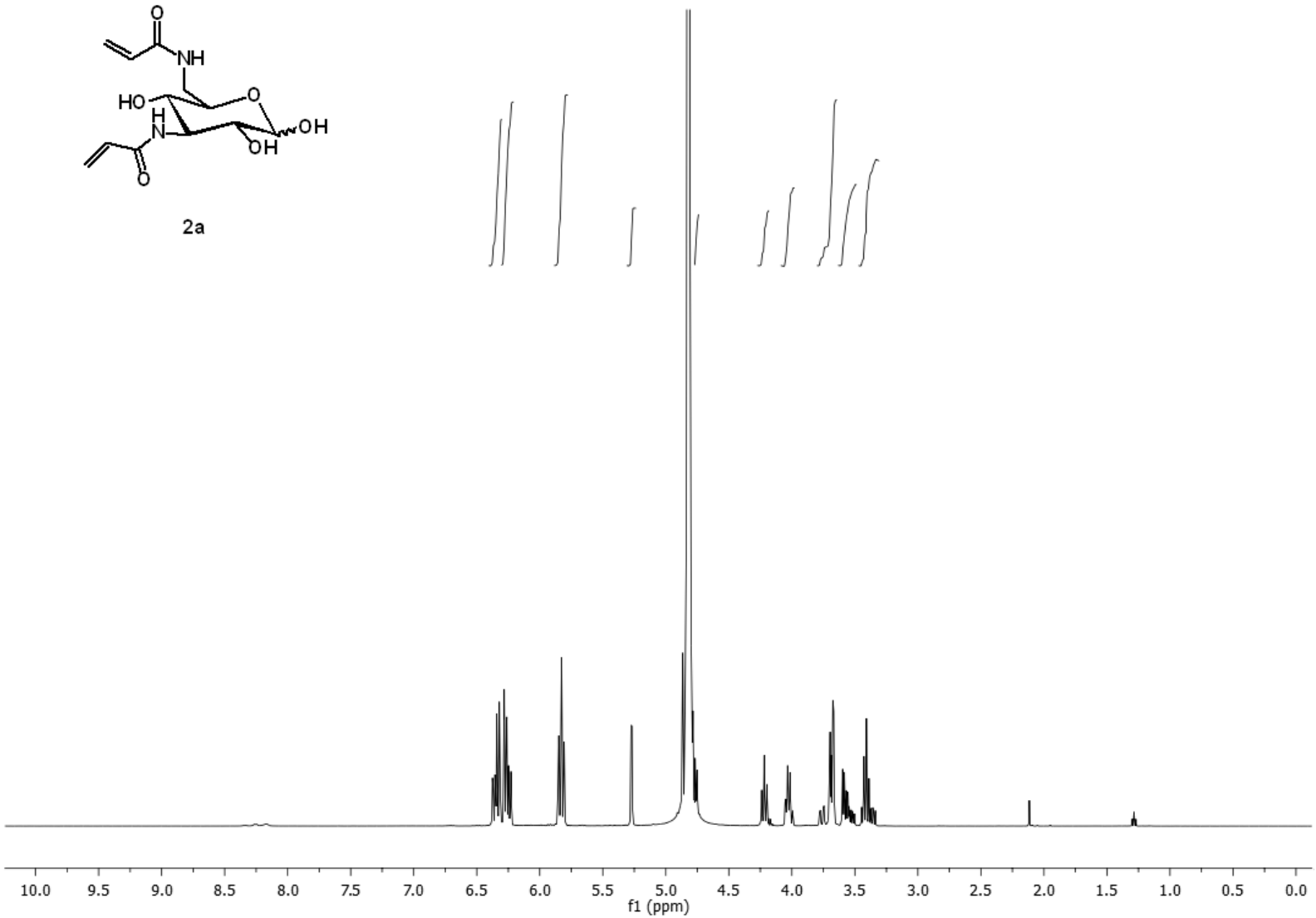
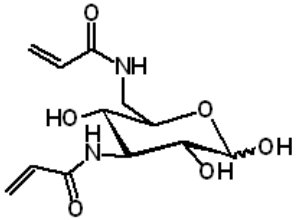


Figure 7: ^{1}H NMR (500 MHz, D_2O) spectrum of compound **2a**



2a

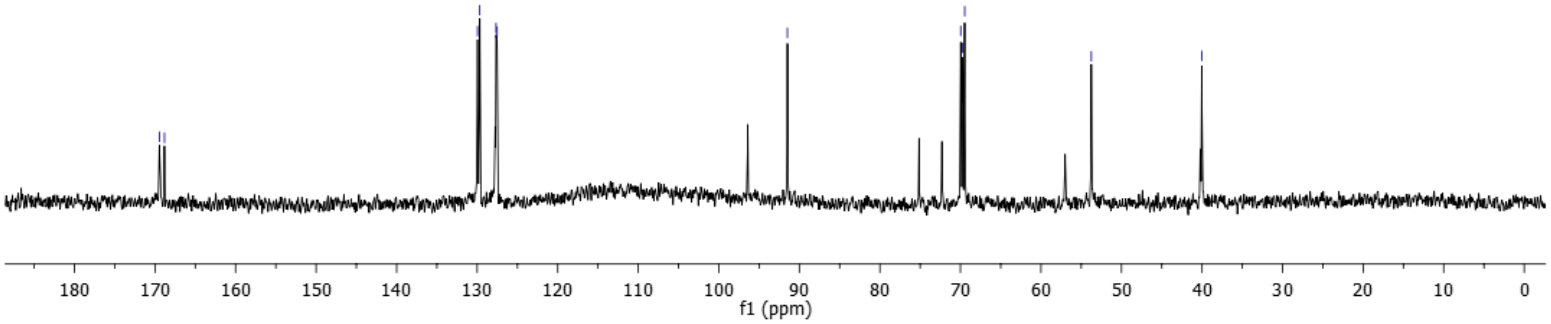
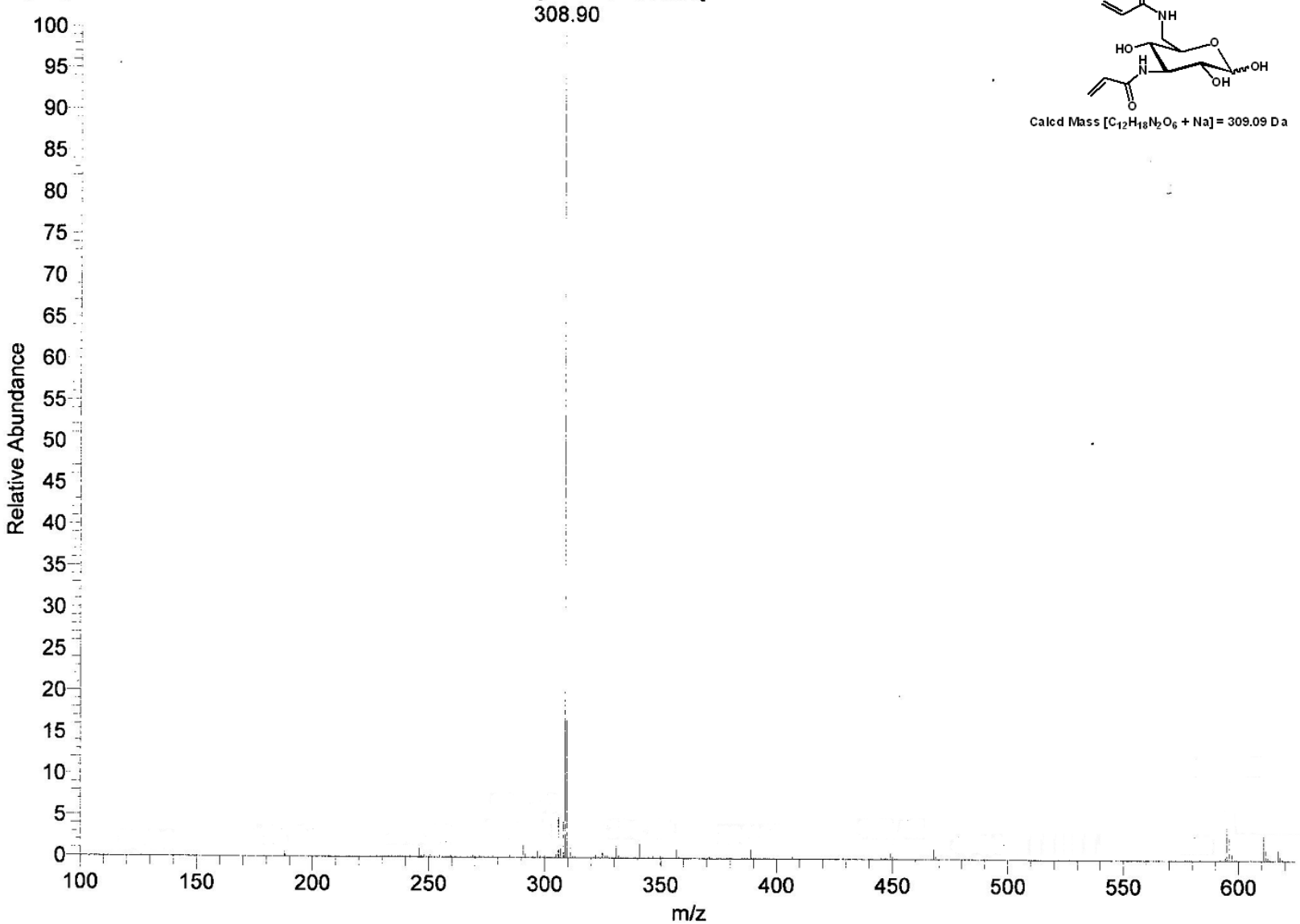
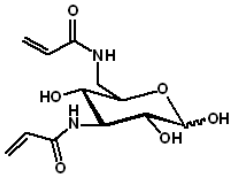


Figure 8: ^{13}C NMR (126 MHz, D_2O) spectrum of compound **2a**

F:\DATA\June-2013\24\AKS-4 6/24/2013 12:48:12 PM

AKS-4 #9-21 RT: 0.14-0.35 AV: 13 NL: 3.28E7
T: {0,0} + c ESI corona sid=80.00 det=1600.00 Full ms [100.00-2000.00]





Calcd Mass [$C_{12}H_{18}N_2O_6$ + Na] = 309.09 Da

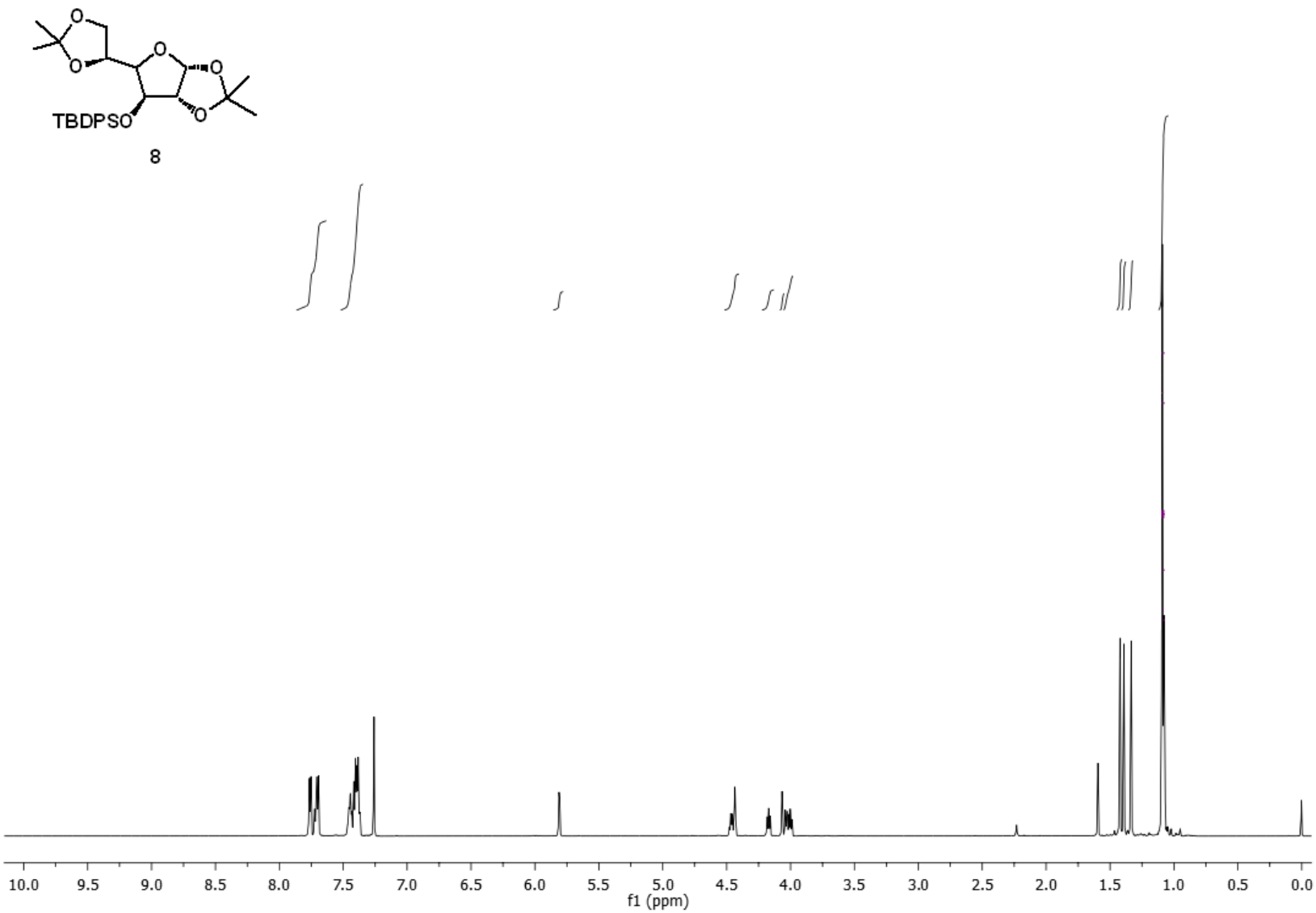
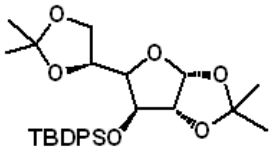


Figure 9: ^{1}H NMR (600 MHz, $CDCl_3$) spectrum of compound **8**



8

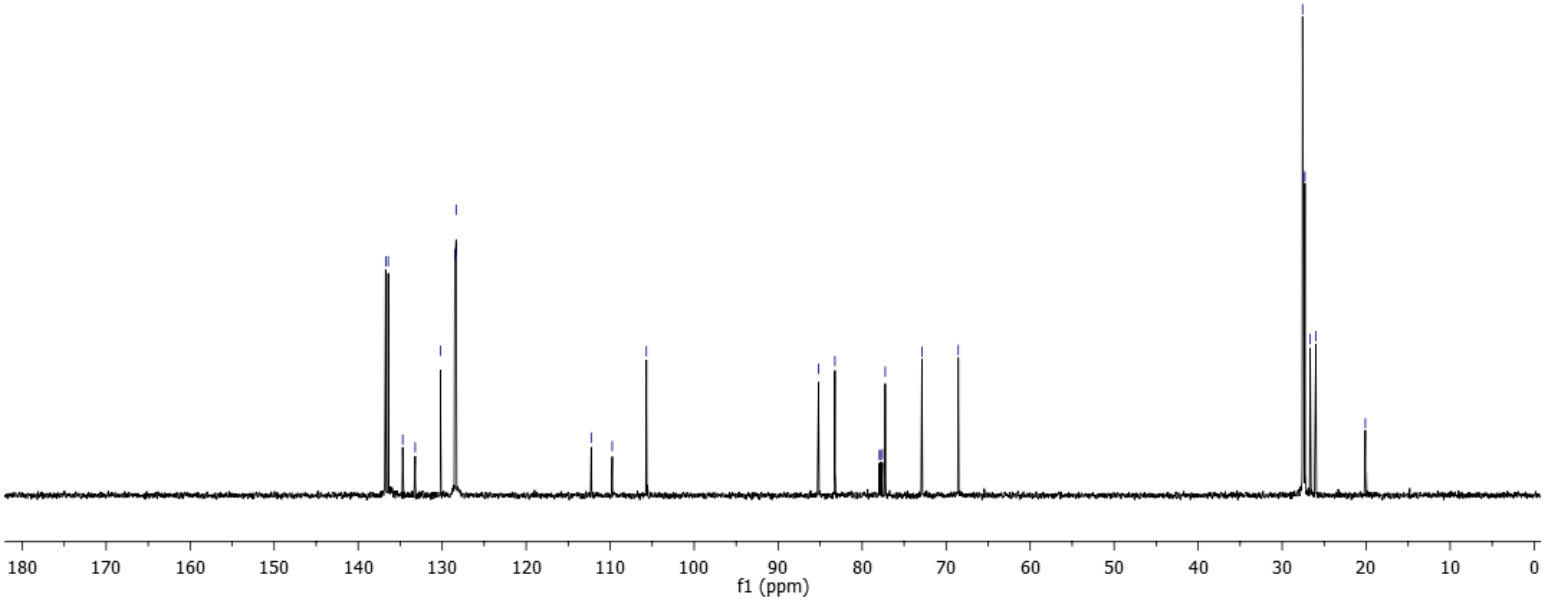


Figure 10: ^{13}C NMR (176 MHz, $CDCl_3$) spectrum of compound **8**

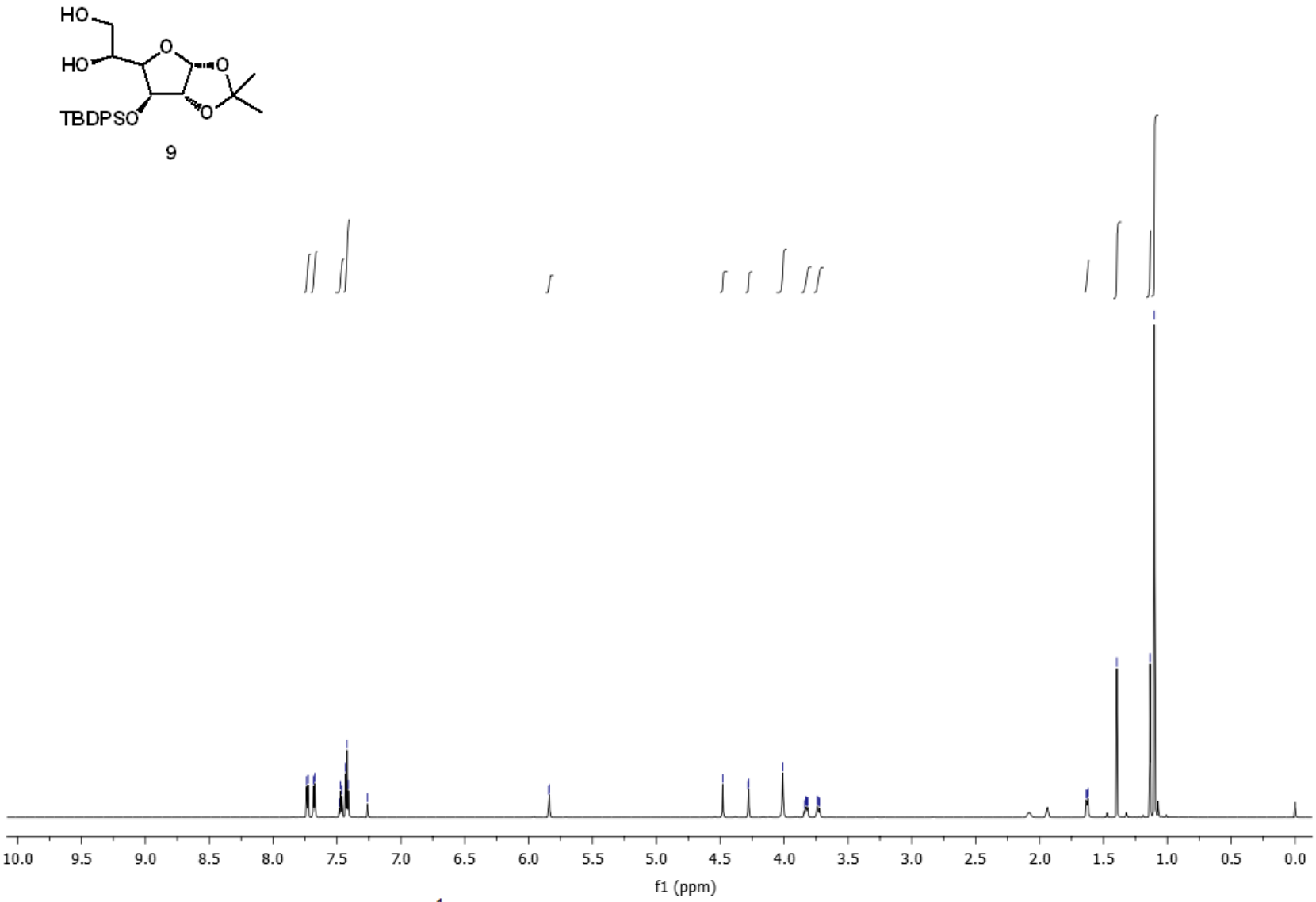
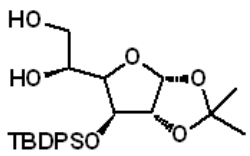


Figure 11: ^{1}H NMR (700 MHz, $CDCl_3$) spectrum of compound **9**



9

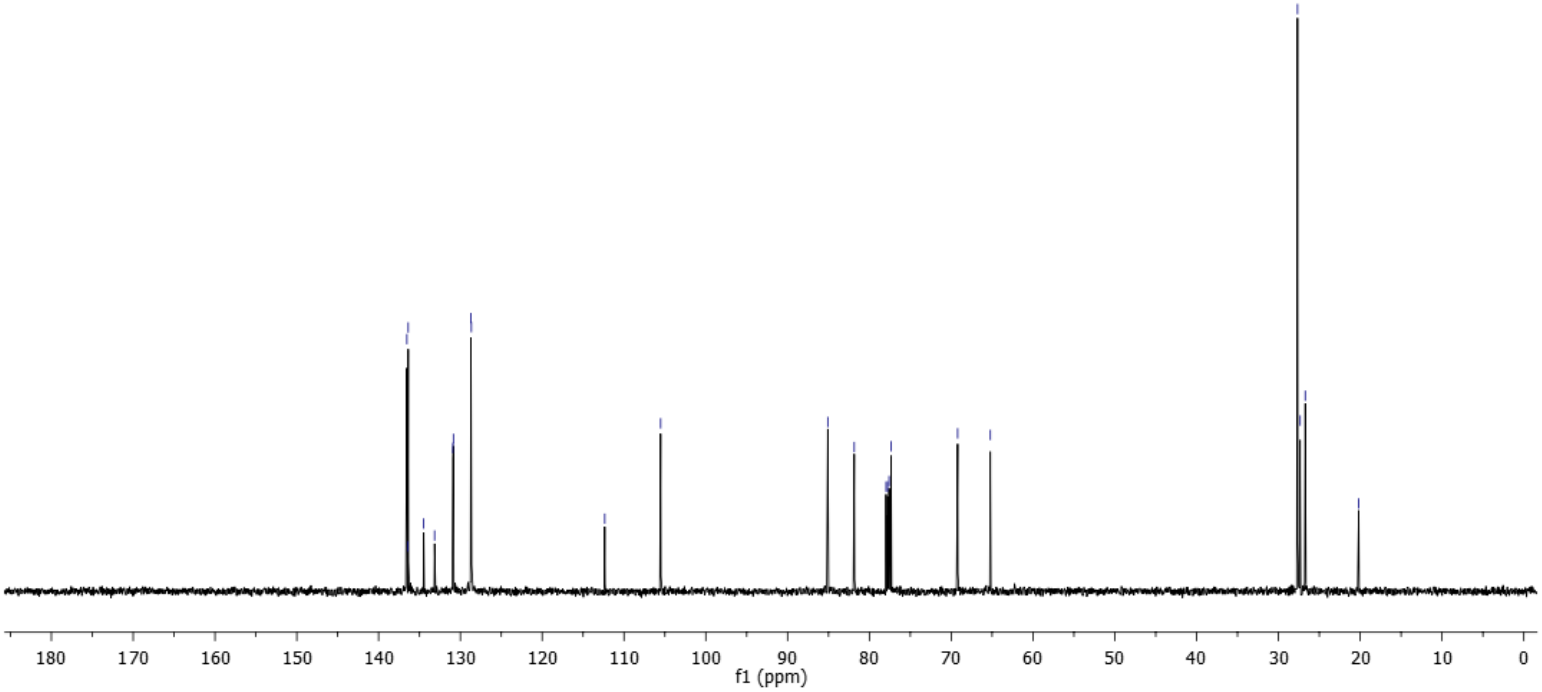
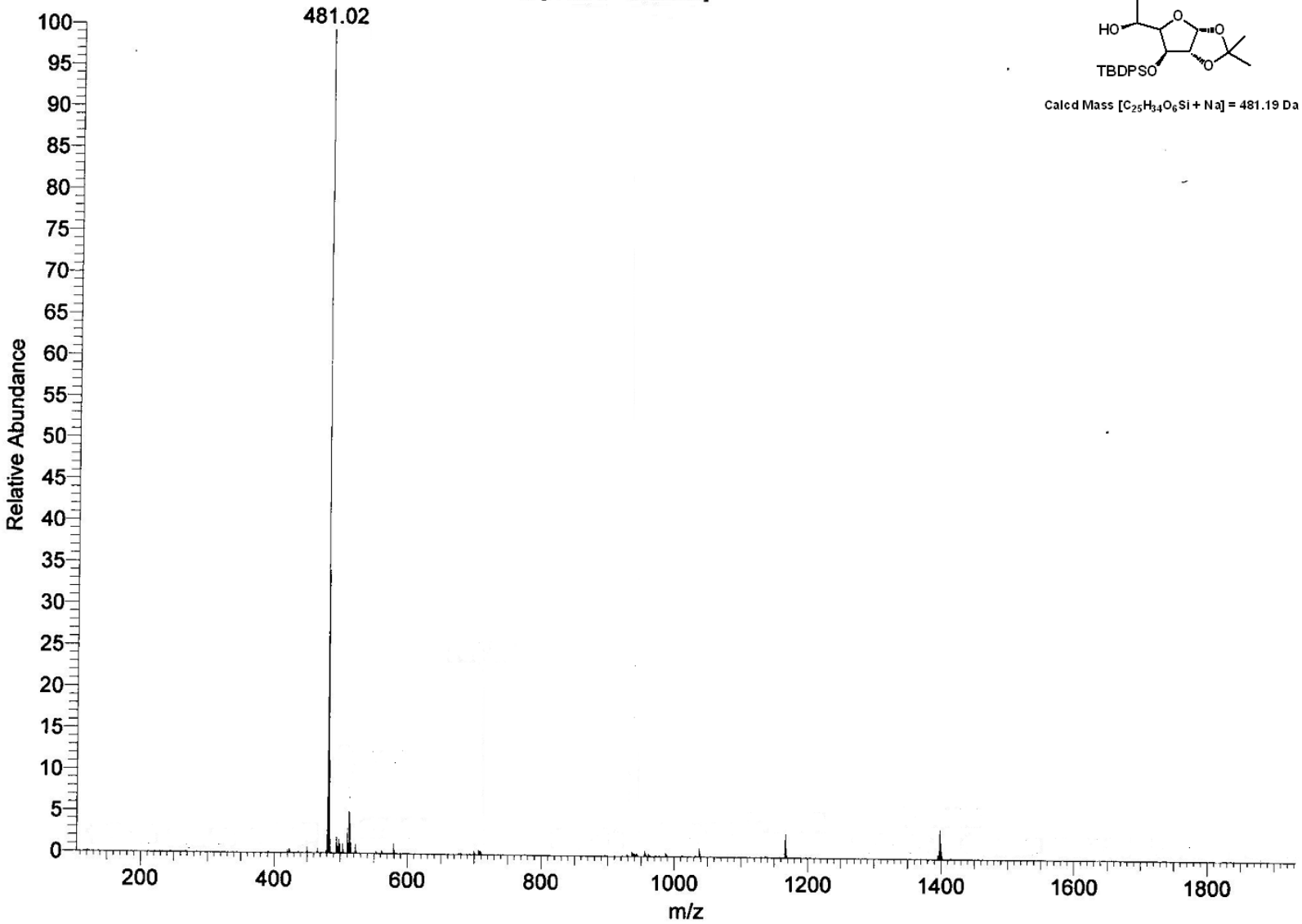


Figure 12: ^{13}C NMR (176 MHz, $CDCl_3$) spectrum of compound **9**

F:\DATA\Aug-2013\12\AKS-6 8/12/2013 12:10:41 PM

AKS-6 #8-39 RT: 0.12-0.66 AV: 32 SB: 47 0.00-0.10 , 0.50-1.18 NL: 2.69E7
T: {0,0} + c ESI corona sid=75.00 det=1600.00 Full ms [100.00-2000.00]



Calcd Mass [$C_{25}H_{34}O_6Si + Na$] = 481.19 Da

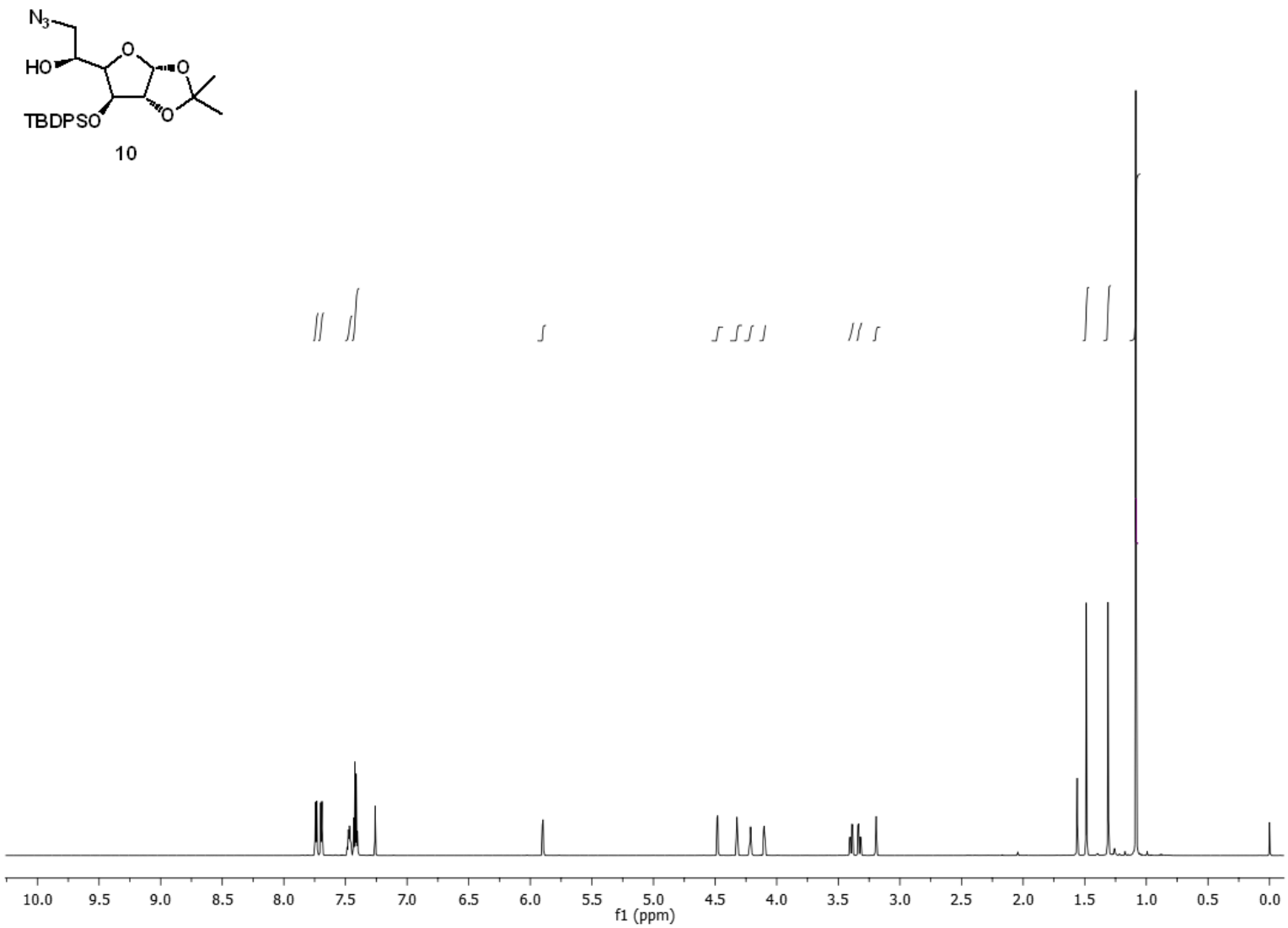
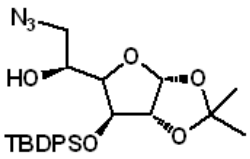


Figure 13: ^{1}H NMR (700 MHz, $CDCl_3$) spectrum of compound **10**



10

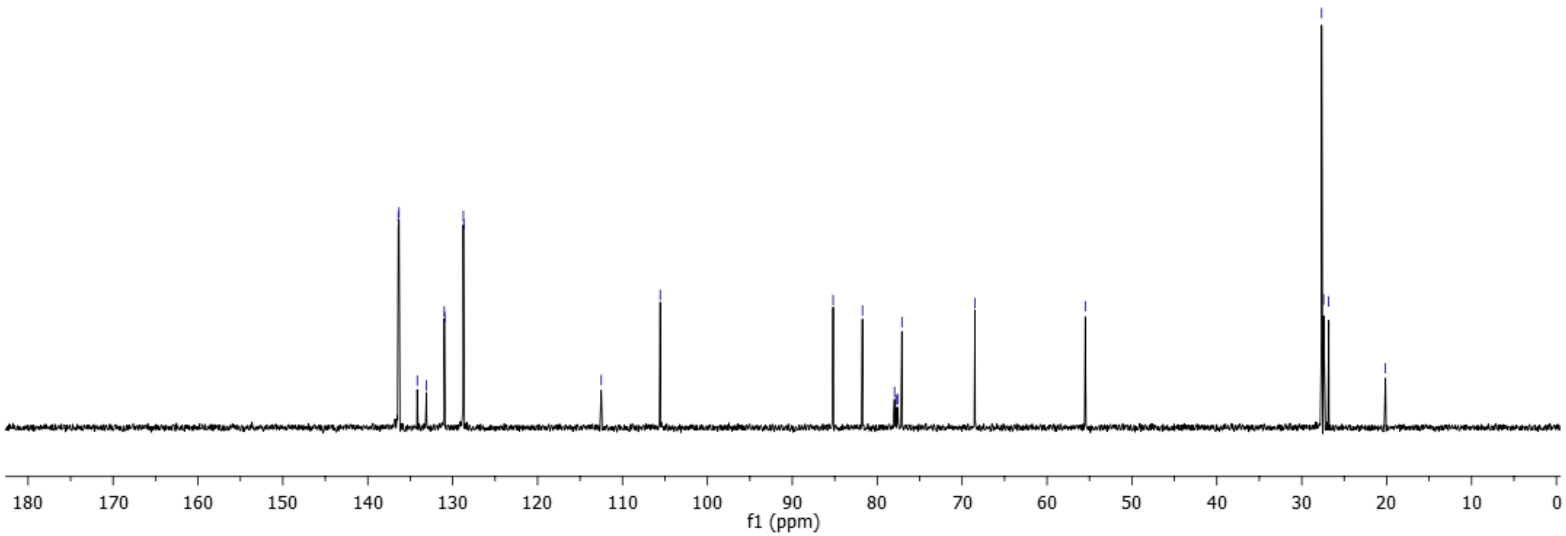


Figure 14: ^{13}C NMR (176 MHz, $CDCl_3$) spectrum of compound **10**

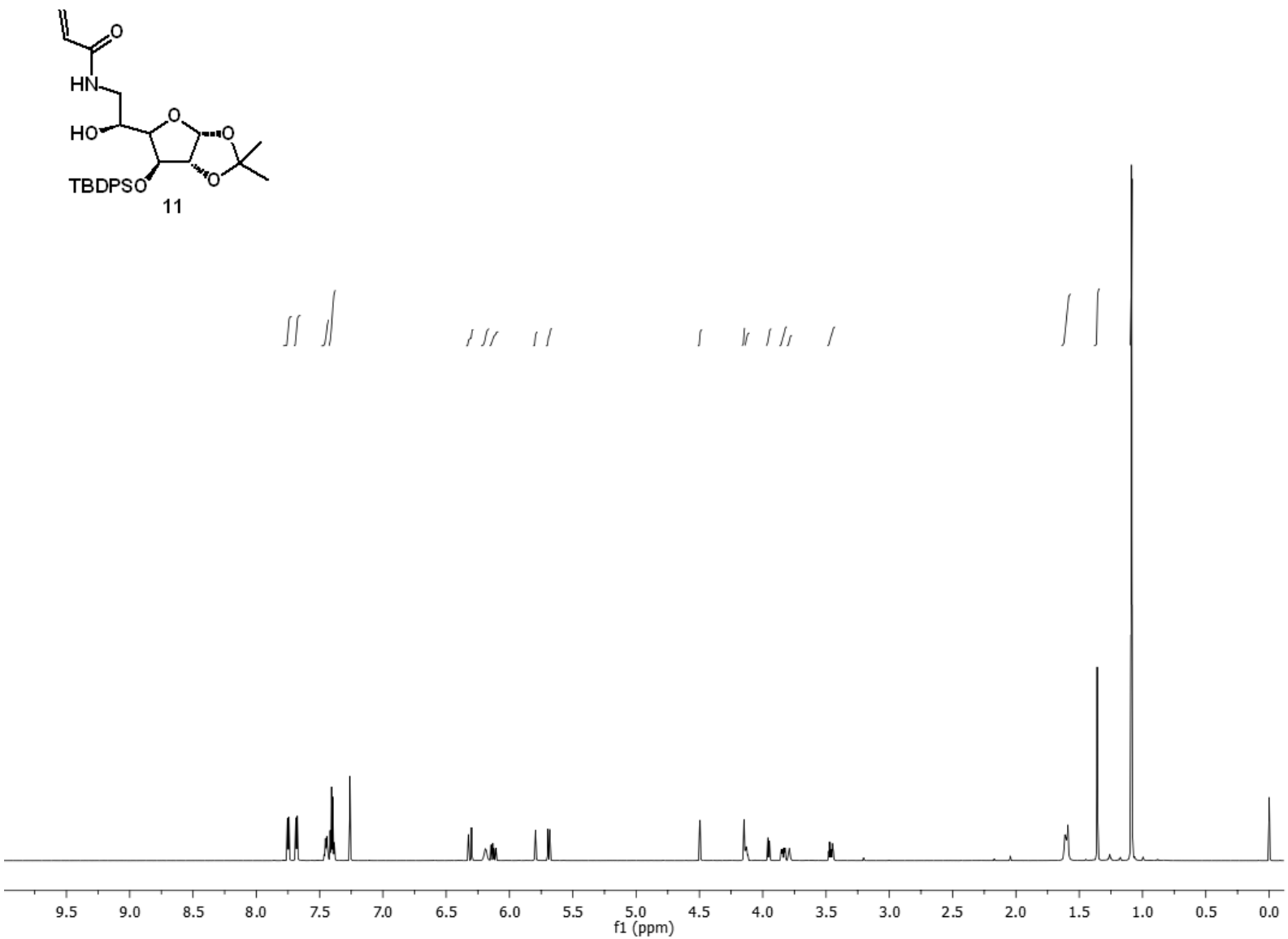
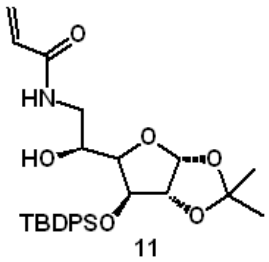


Figure 15: ^{1}H NMR (700 MHz, $CDCl_3$) spectrum of compound **11**



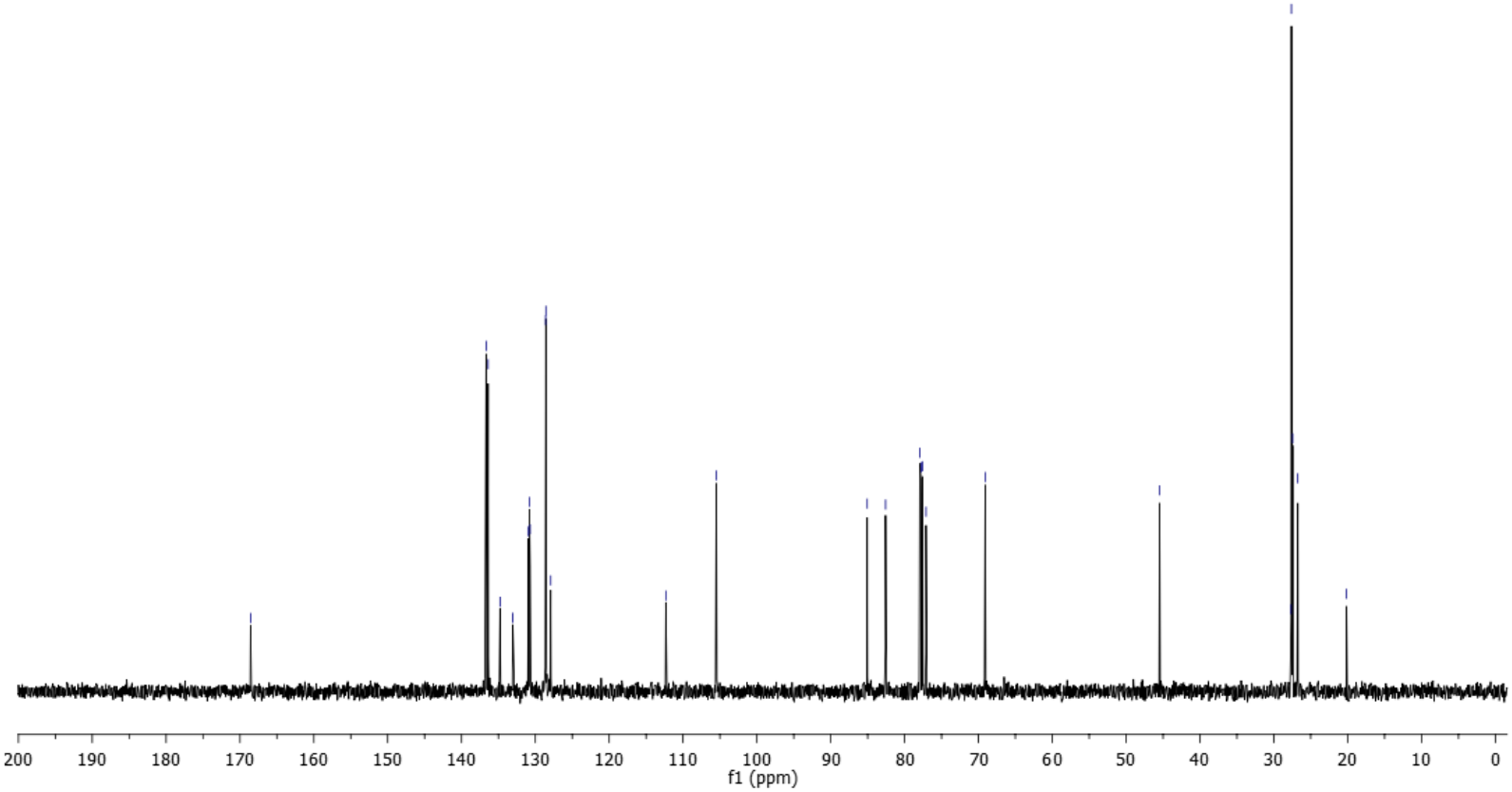
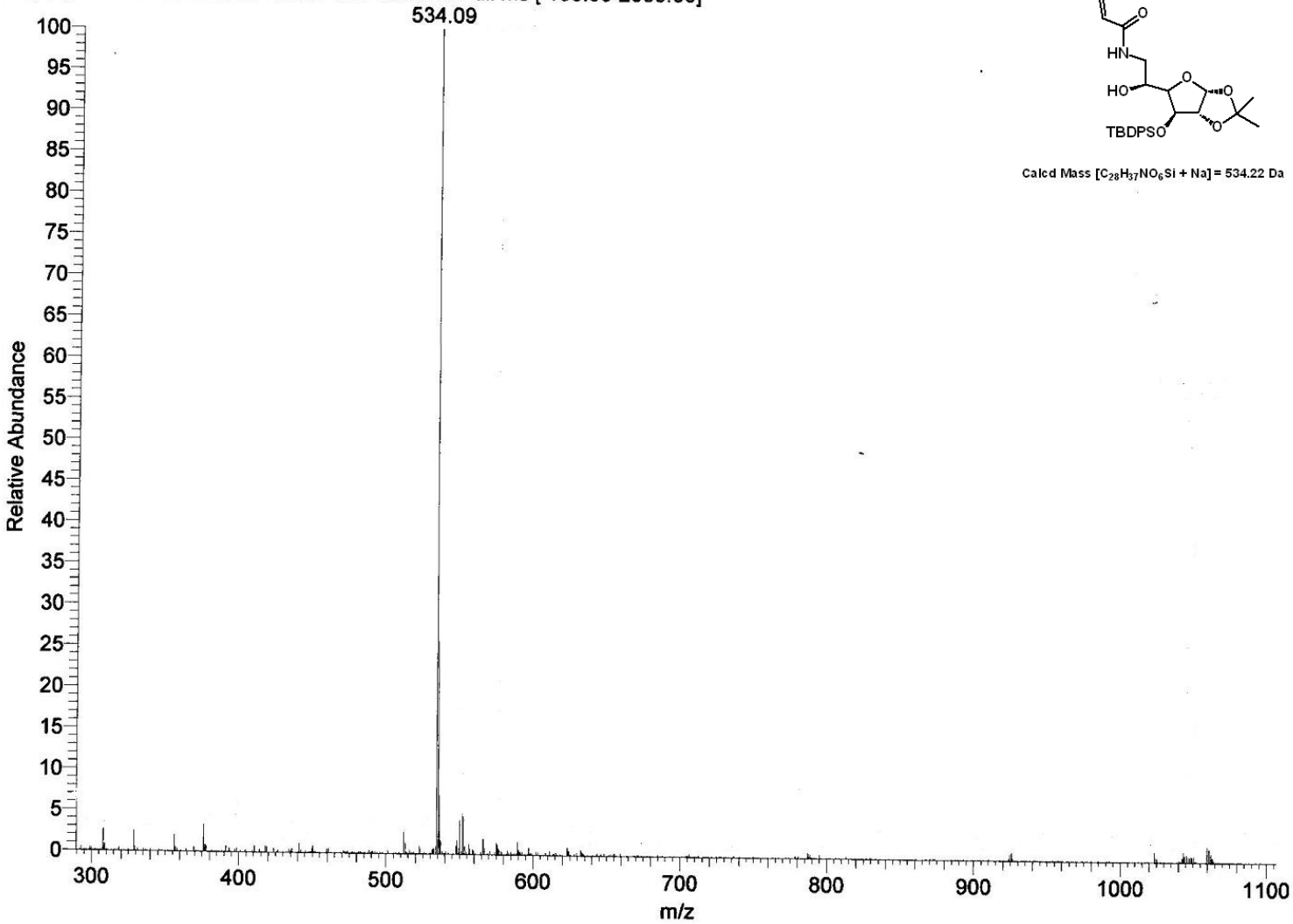


Figure 16: ^{13}C NMR (176 MHz, $CDCl_3$) spectrum of compound **11**

F:\DATA\Aug-2013\16\AKS-7 8/16/2013 12:57:50 PM

AKS-7 #9-26 RT: 0.14-0.43 AV: 18 SB: 19 0.00-0.12 , 0.40-0.57 NL: 4.59E6
T: {0,0} + c ESI corona sid=75.00 det=1600.00 Full ms [100.00-2000.00]



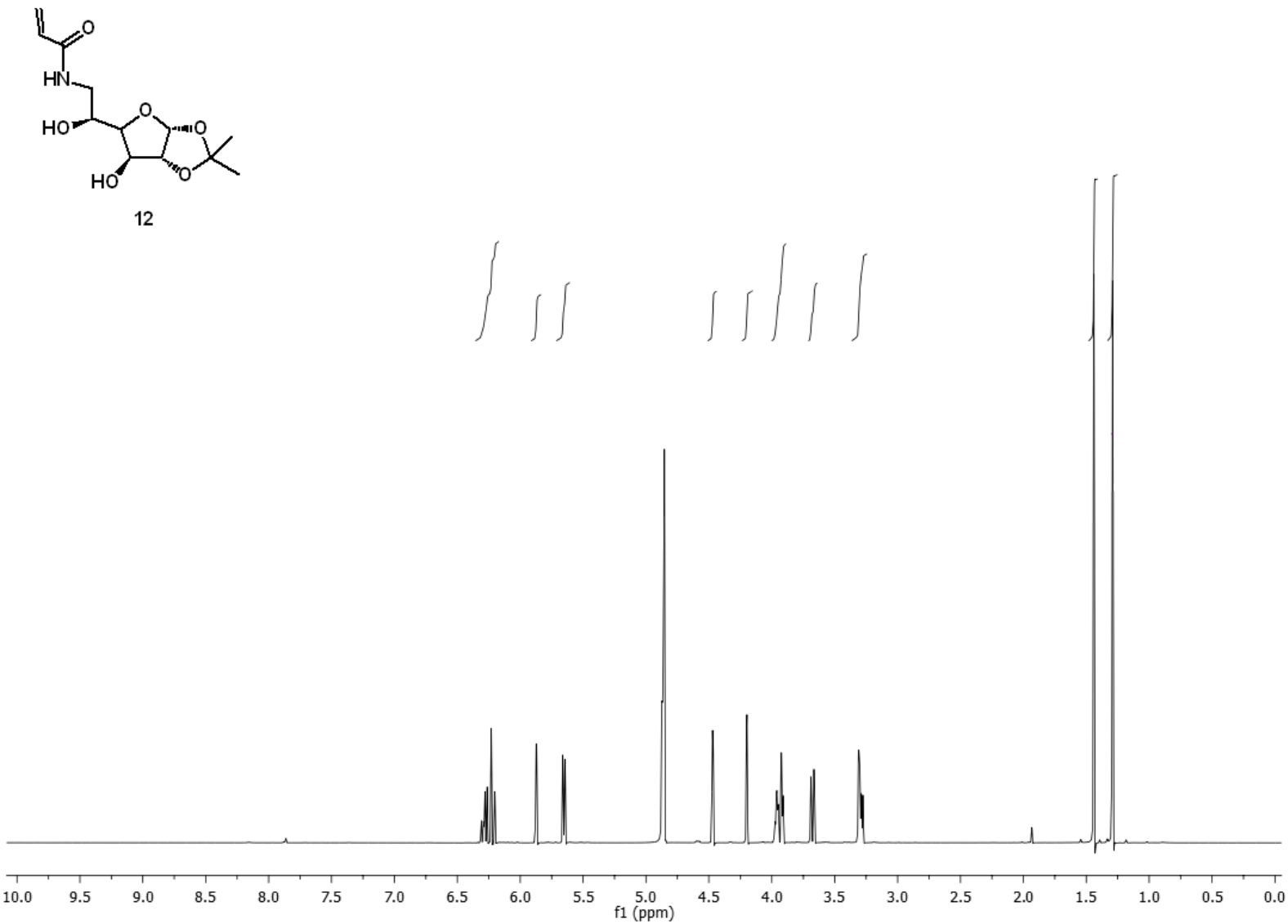
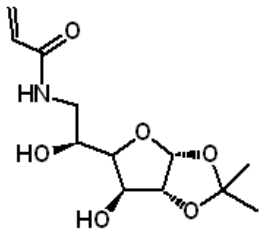


Figure 17: ^{1}H NMR (600 MHz, CD_3OD) spectrum of compound **12**



12

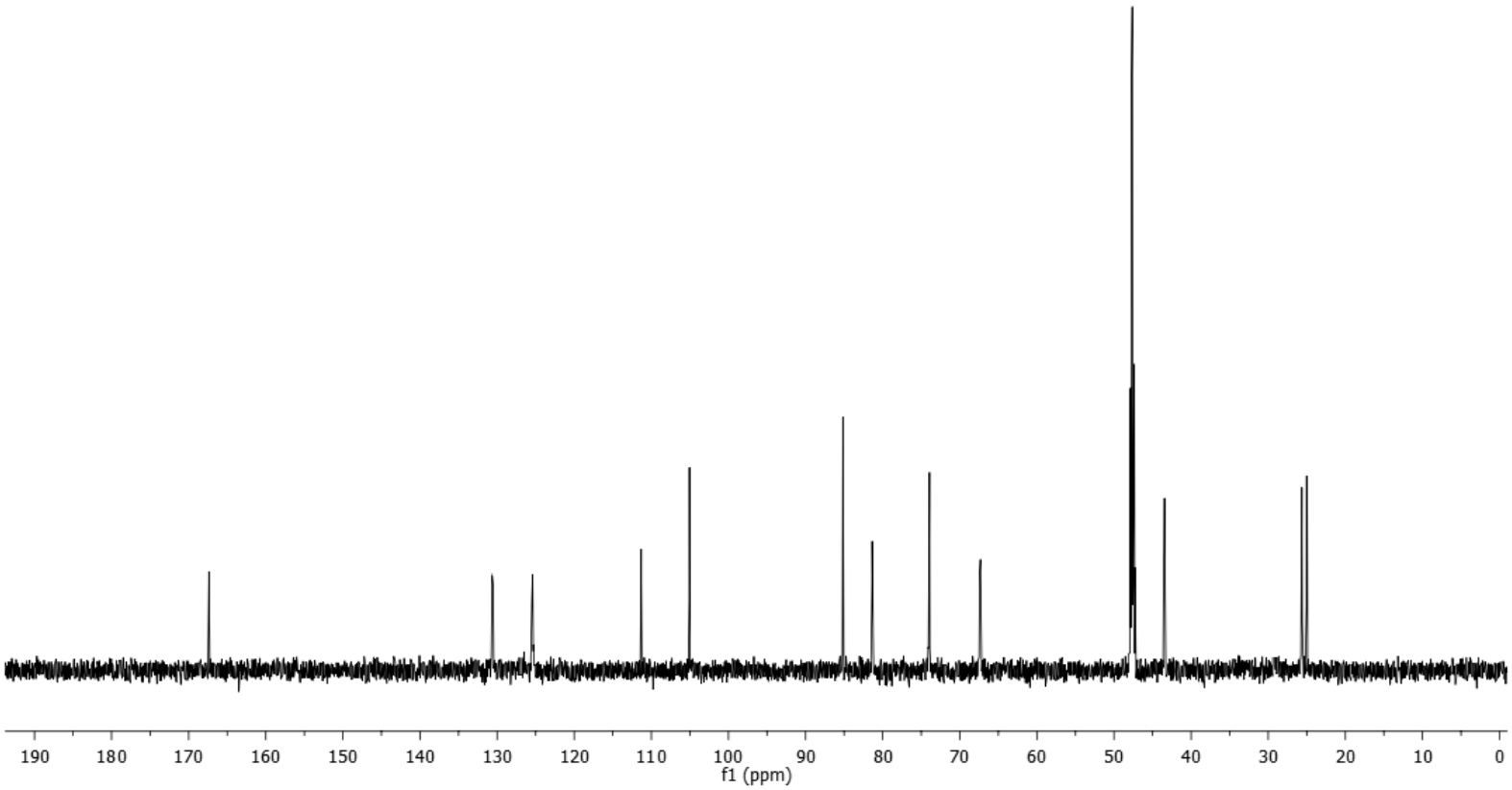
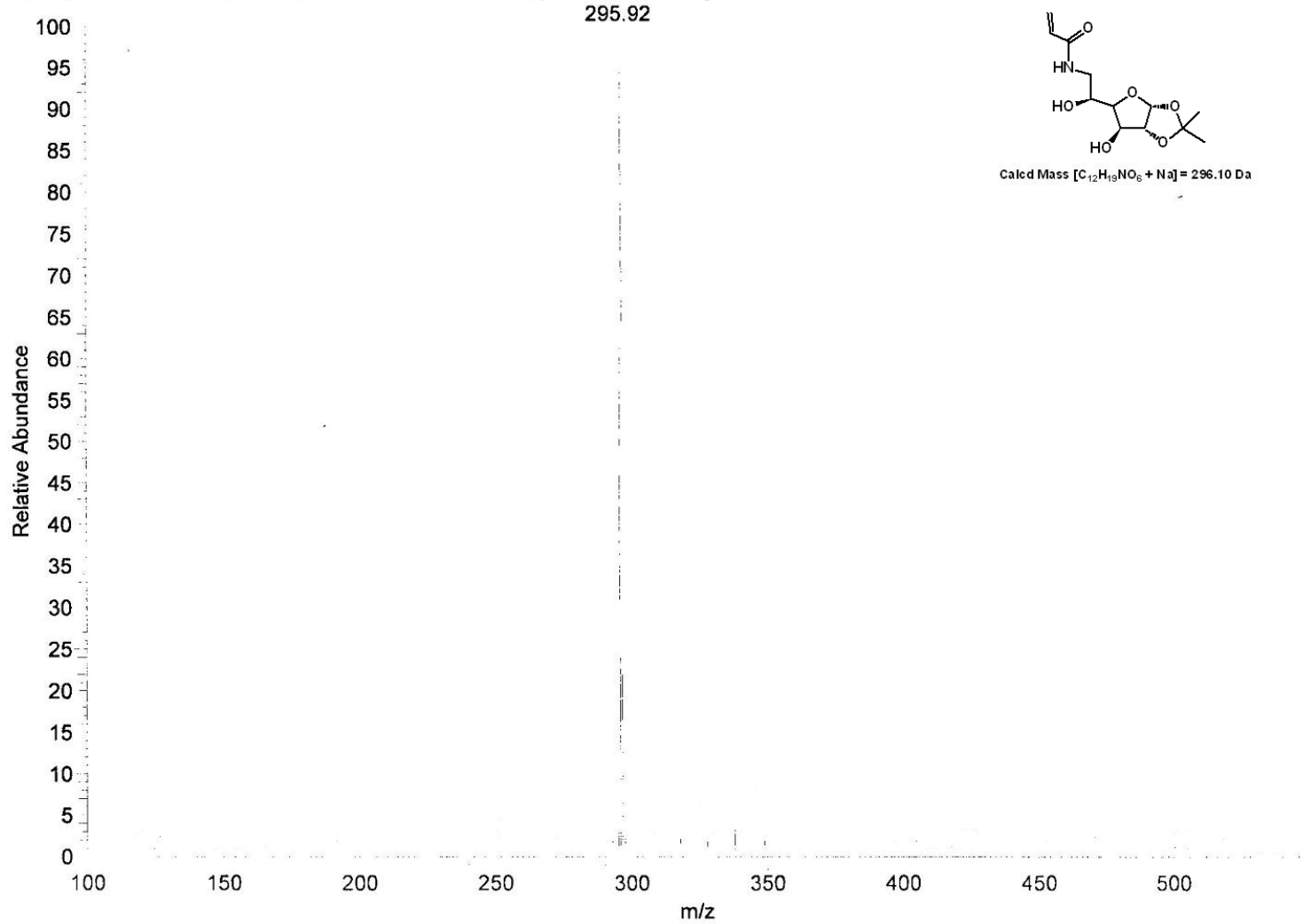


Figure 18: ^{13}C NMR (176 MHz, CD_3OD) spectrum of compound **12**

AKS-19

T: {0,0} + c ESI corona sid=75.00 det=1600.00 Full ms [100.00-2000.00]



Calcd Mass [$C_{12}H_{19}NO_6$ + Na] = 296.10 Da

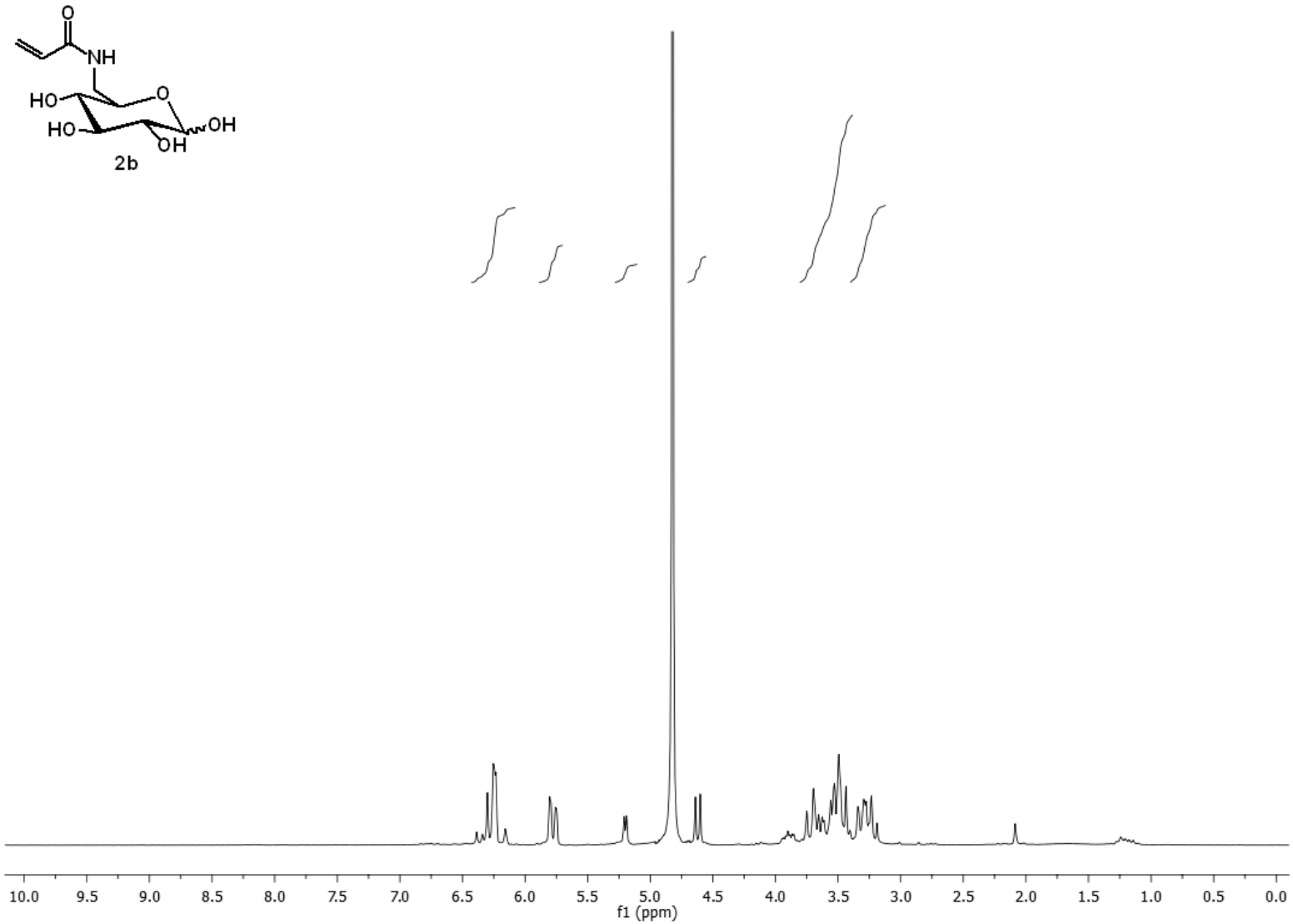
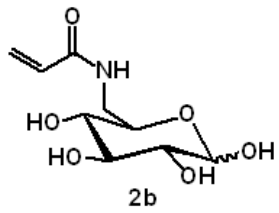


Figure 19: ^{1}H NMR (200 MHz, D_2O) spectrum of compound **2b**



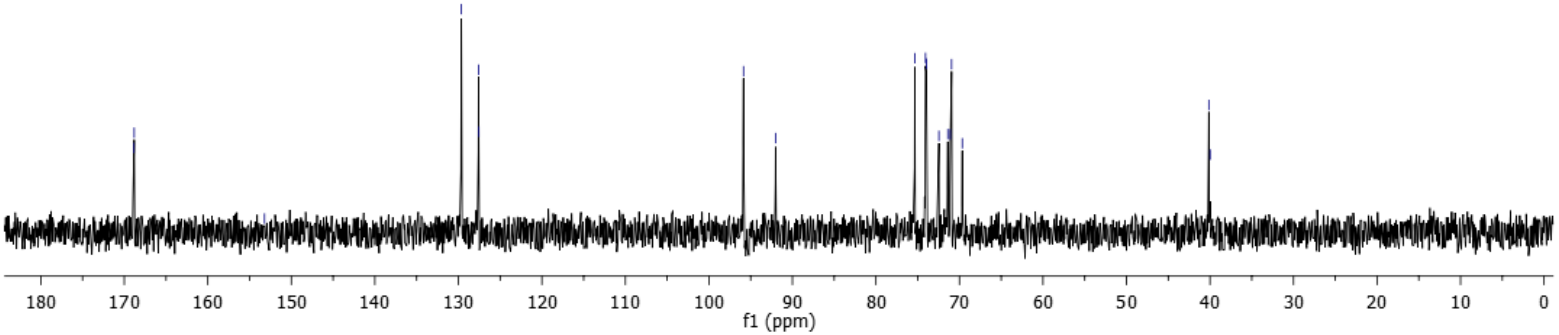
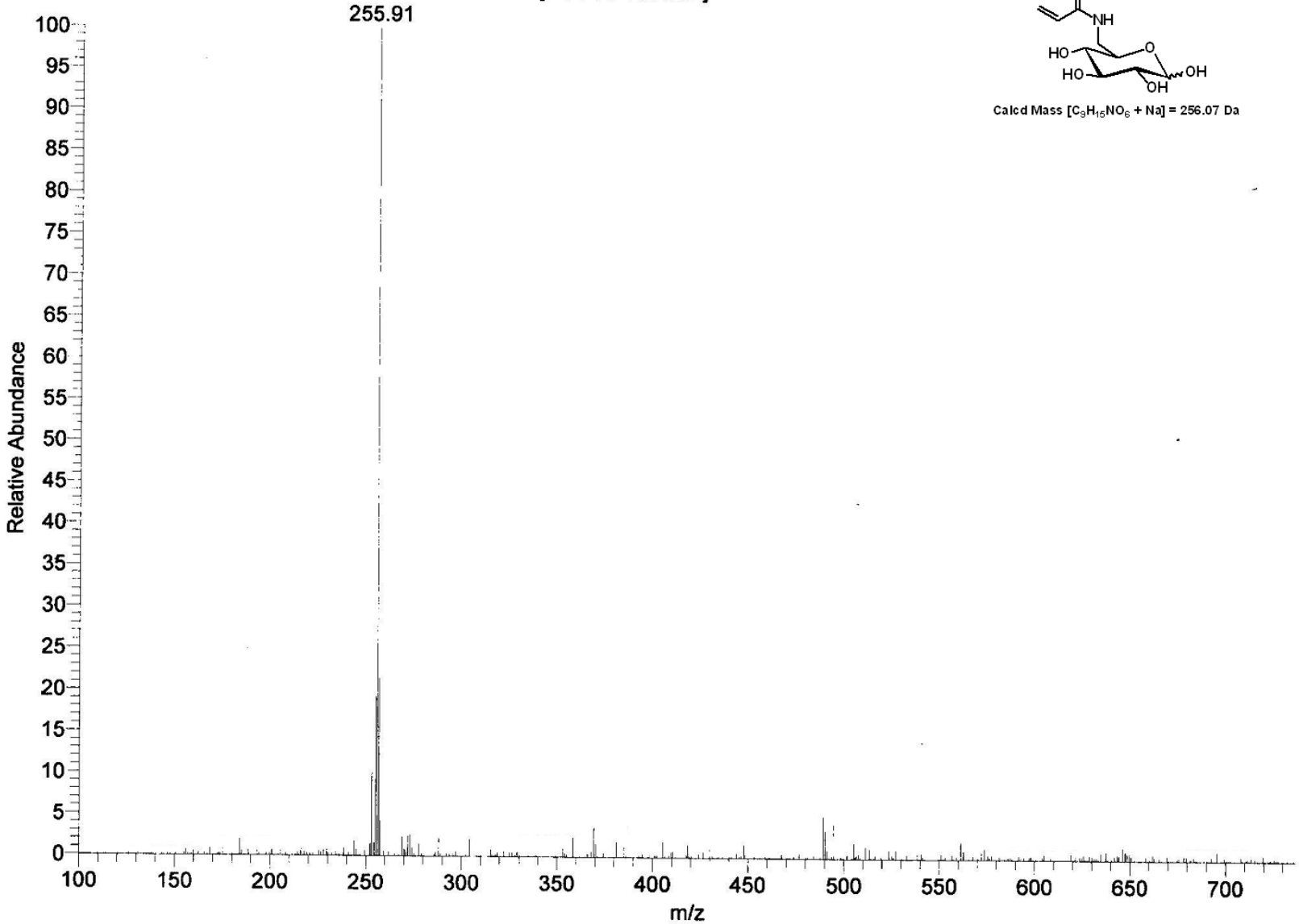
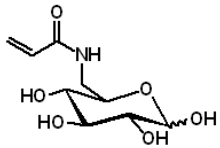


Figure 20: ^{13}C NMR (50 MHz, D_2O) spectrum of compound **2b**

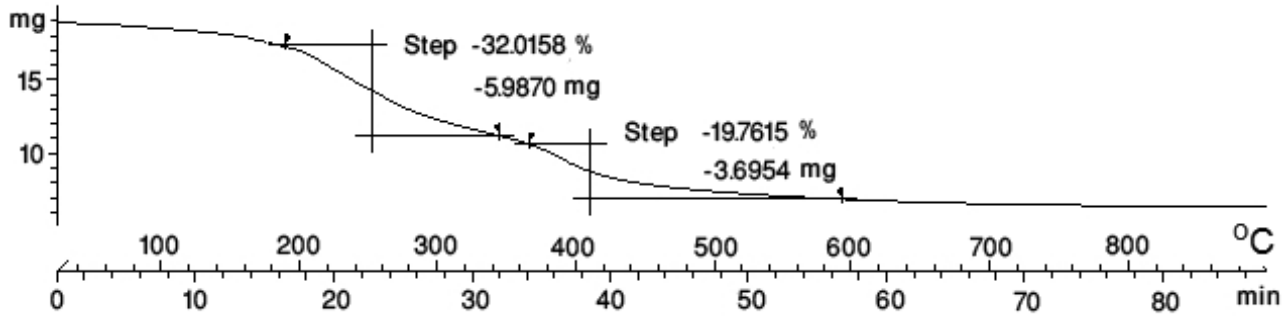
F:\DATA\Oct-2013\17\AKS-1 10/17/2013 11:43:34 AM

AKS-1 #6-22 RT: 0.09-0.37 AV: 17 SB: 45 0.00-0.11 , 0.49-1.14 NL: 3.07E6
T: {0,0} + c ESI corona sid=40.00 det=1600.00 Full ms [100.00-1500.00]



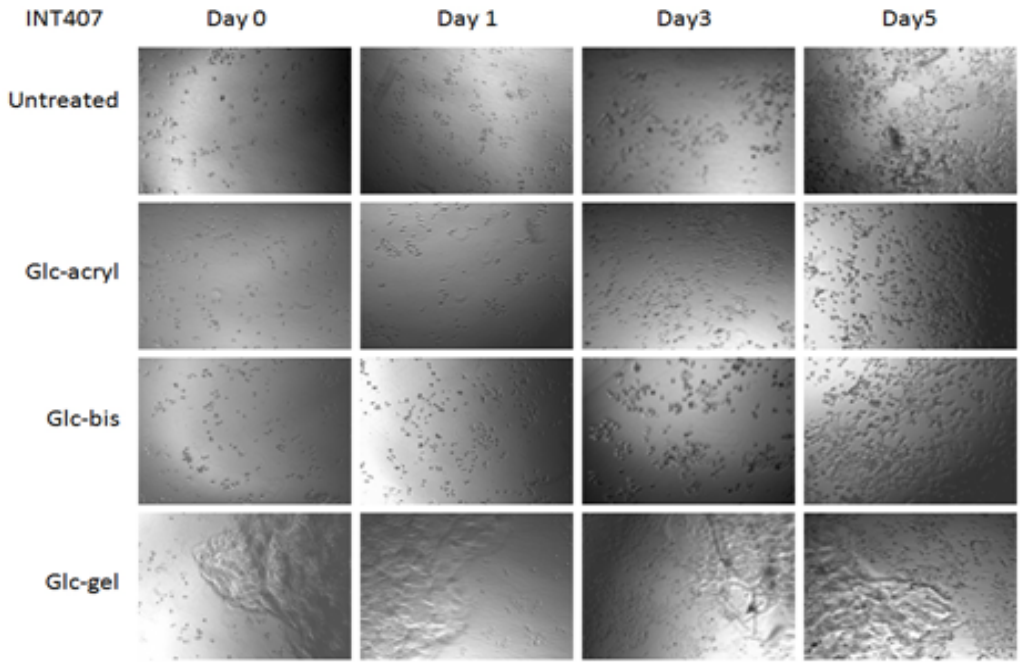


Calcd Mass [$C_9H_{15}NO_6$ + Na] = 256.07 Da

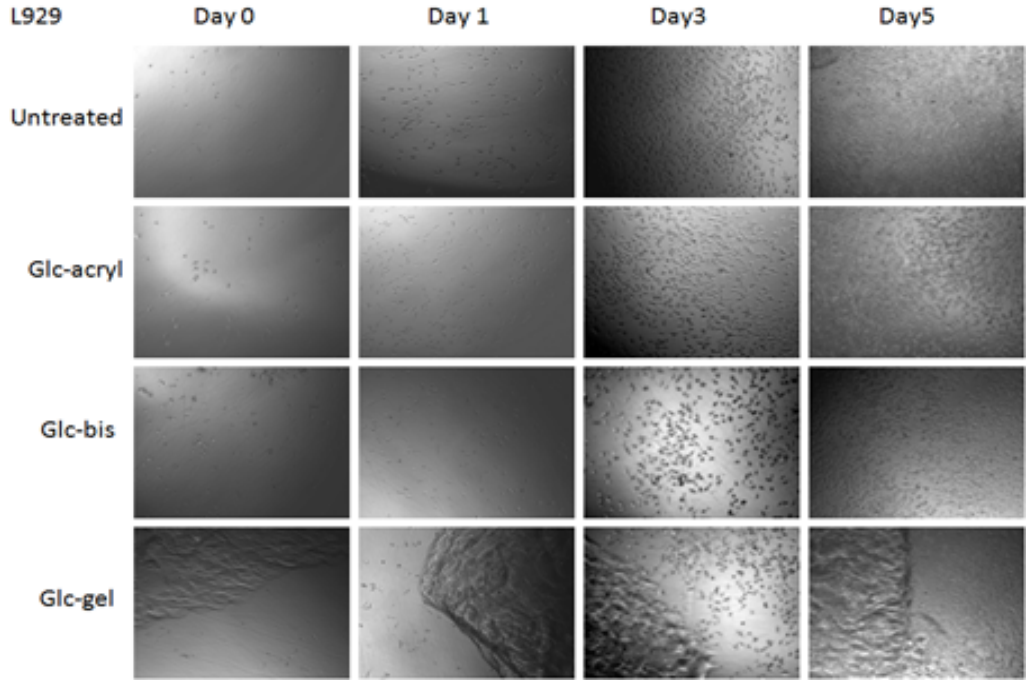


Thermal degradation profile of a typical vaccum dried Glc-gel

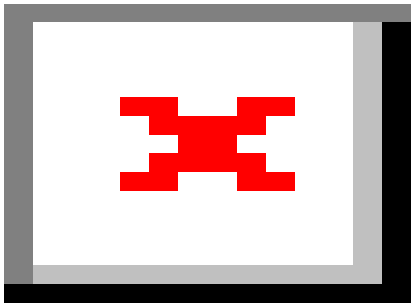
(A)



(B)



Growth of cells monitored in the absence and presence of test samples (1 mg/mL each of Glc-acryl and Glc-bis and 20 mg piece of Glc-gel) under microscope (40 × magnification). (A) INT407 cells and (B) L929 cells.



SEM image showing the porous structure of a typical freeze dried Glc-gel