Supporting Information:

Fidelity and Promiscuity of a Mycobacterial Glycosyltransferase

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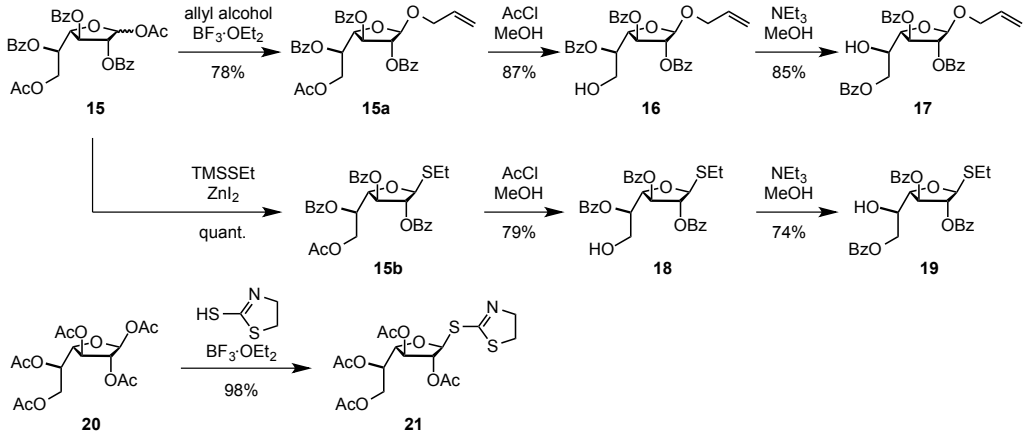
Supporting Information

1. Experimental procedures

1.1. General

All compounds were purchased from Sigma Aldrich (Milwaukee, WI). Tetrahydrofuran (THF) and toluene were distilled from sodium/benzophenone ketyl. All reactions were run under nitrogen atmosphere unless otherwise stated. Analytical thin layer chromatography (TLC) was carried out on E. Merck (Darmstadt) TLC plates pre-coated with silica gel 60 F254 (250 μm layer thickness). Flash chromatography was performed on Scientific Adsorbents Incorporated silica gel (32-63 μm, 60 Å pore size). ^{1}H and ^{13}C nuclear magnetic resonance (NMR) spectra were recorded on Bruker AC-300 or Varian Mercury Plus 300 spectrometers, and chemical shifts are reported relative to residual solvent peaks in parts per million ($CHCl_3$: ^{1}H: 7.24, ^{13}C: 77.16; MeOH: ^{1}H: 3.31, ^{13}C: 49.15). Peak multiplicity is reported as singlet (s), doublet (d), doublet of doublets (dd), triplet (t), doublet of triplets (dt), etc. High resolution electrospray ionization mass spectra (HRESI-MS) were obtained on a Micromass LCT.

1.2. Synthesis of oligosaccharide acceptors



Scheme S1 Synthesis of monosaccharide building blocks.

Allyl 2,3,6-tri-*O*-benzoyl-β-D-galactofuranoside (17)

Compound **16**[1] (152 mg, 0.29 mmol), under argon, was dissolved in MeOH (4.9 mL) and CH_2Cl_2 (4.9 mL). Triethylamine (142 μL, 1.03 mmol) was added, and the solution was stirred for 6 h. The solvent was removed under reduced pressure, and the product was purified by silica gel column chromatography (SiO_2 = 20 mL, 6/1 to 2/1 gradient hexanes/EtOAc) to provide 129 mg (85%) of **17** as a white solid.

^{1}H NMR ($CDCl_3$): δ 8.09–8.03 (m, 6H), 7.59–7.39 (m, 9H), 5.99–5.86 (m, 1H), 5.66 (dd, J = 4.8, 0.8 Hz, 1H), 5.55 (d, J = 1.3 Hz, 1H), 5.32 (s, 1H), 5.36–5.28 (m, 1H), 5.22–5.17 (m, 1H), 4.65–4.58 (dd, J = 12.8, 8.0 Hz, 1H), 4.52–4.46 (m, 2H), 4.41 (dd, J = 4.8, 2.1 Hz, 1H), 4.29–4.22 (m, 1H), 4.12–4.04 (m, 1H), 2.71 (d, J = 8.2 Hz, 1H).

^{13}C NMR ($CDCl_3$): δ 166.7, 166.4, 165.6, 133.8, 133.3, 130.3, 130.1, 129.9, 129.3, 129.2, 128.8, 128.7, 128.6, 117.8, 105.1, 100.7, 83.4, 81.8, 78.4, 69.2, 68.2, 66.4. HRESI MS m/z calculated for $[M+Na]^+$ $C_{30}H_{28}O_9Na$: 555.1626. Found: 555.1623.

Ethyl 6-*O*-acetyl-2,3,5-tri-*O*-benzoyl-1-thio-β-D-galactofuranoside (15b)

(Ethylthio)trimethylsilane (150 μL, 0.93 mmol) was added to a stirring solution of **15**[1] (267 mg, 0.46 mmol) in CH_2Cl_2 (1.2 mL) under argon. Zinc iodide (74 mg, 0.23 mmol) was added to the solution as a solid. After 1 h, the reaction mixture was quenched by the addition of triethylamine (0.2 mL). The solvent was removed under reduced pressure and the residue purified by silica gel column chromatography (1/0 to 9/1 gradient hexanes/EtOAc) to give 268 mg of **15b** in quantitative yield.

^{1}H NMR ($CDCl_3$): δ 8.09–8.06 (m, 4H), 7.93–7.89 (m, 2H), 7.62 (m, 5H), 7.37–7.28 (m, 4H), 5.93 (m, 1H), 5.65 (br s, 1H), 5.59 (dd, *J* = 5.2, 1.1 Hz, 1H), 5.48 (t, *J* = 1.3, 1H), 4.74 (dd, *J* = 4.6, 3.7 Hz, 1H), 4.54 (dd, *J* = 11.8, 4.8 Hz, 1H), 4.46 (dd, *J* = 11.8, 7.2 Hz, 1H), 2.75 (m, 2H), 2.01 (s, 3H), 1.36 (t, *J* = 7.3 Hz, 3H). ^{13}C NMR ($CDCl_3$): δ 170.6, 165.8, 165.6, 165.5, 133.7, 133.6, 133.4, 130.1, 130.0, 129.6, 129.1, 129.0, 128.6, 88.4, 83.0, 81.1, 77.9, 70.3, 62.9, 25.4, 20.8, 15.1. HRESI-MS *m*/*z* calculated for $[M+Na]^+$ $C_{31}H_{30}O_9SNa$: 601.1503. Found: 601.1514.

Ethyl 2,3,5-tri-*O*-benzoyl-1-thio-β-D-galactofuranoside (18)

Protected sugar **15b** (375 mg, 0.65 mmol) was dissolved in MeOH (23 mL) and CH_2Cl_2 (0.6 mL). The reaction nuxtyre, under argon, was placed in an ice bath. Acetyl chloride (56 μL, 0.78 mmol) was added drop-wise. The reaction was allowed to warm to room temperature overnight. After 15 h, the reaction was quenched by the addition of pyridine and concentrated. The crude residue was purified by silica gel column chromatography (1/0 to 4/1 gradient hexanes/EtOAc) to give 276 mg of thioglycoside **18** in 79% yield.

^{1}H NMR ($CDCl_3$): δ 8.10–8.07 (m, 4H), 8.00–7.96 (m, 2H), 7.62–7.29 (m, 9H), 5.70–5.62 (m, 3H), 5.52 (t, *J* = 1.3 Hz, 1H), 4.81 (t, *J* = 4.5 Hz, 1H), 4.07 (t, *J* = 5.2 Hz, 2H), 2.85–2.66 (m, 2H), 2.43 (t, *J* = 6.2 Hz, 1H), 1.36 (t, *J* = 7.6 Hz, 3H). ^{13}C NMR ($CDCl_3$): δ 166.6, 166.0, 165.7, 133.9, 1.7, 133.5, 130.2, 130.1, 130.0, 129.8, 129.2, 129.1, 128.7, 128.6, 88.5, 83.1, 82.2, 78.3, 73.7, 62.9, 25.6, 15.2. HRESI-MS *m*/*z* calculated for $[M+Na]^+$ $C_{29}H_{28}O_8SNa$: 559.1398. Found: 559.1390.

Ethyl 2,3,6-tri-*O*-benzoyl-1-thio-β-D-galactofuranoside (19)

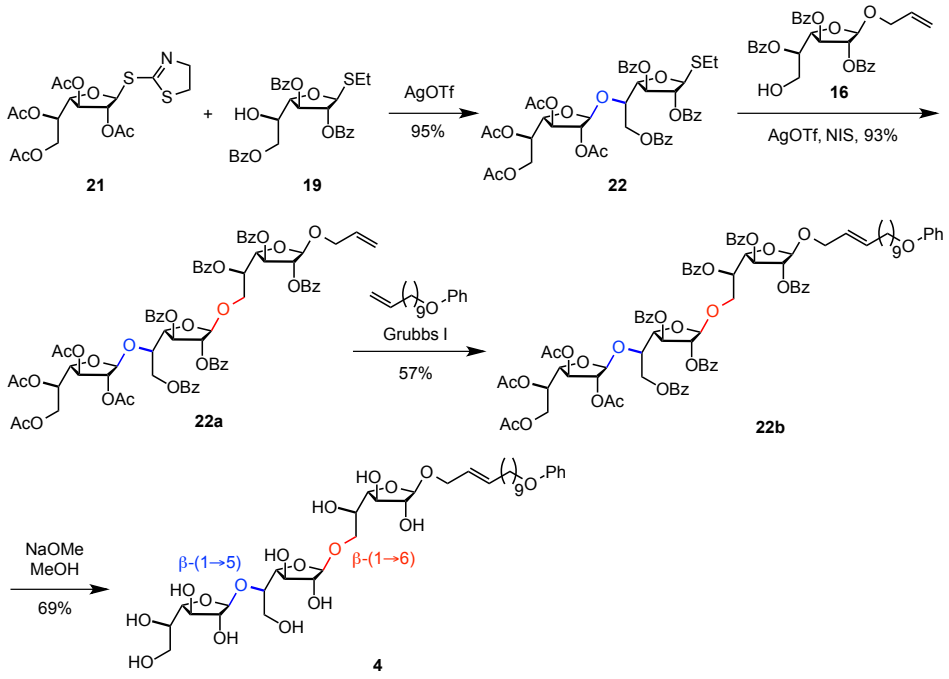
Triethylamine (481 μL, 3.5 mmol) was added to a stirring solution of monosaccharide **18** (454 mg, 0.85 mmol) in MeOH (14.1 mL) and CH_2Cl_2 (14.1 mL). The reaction mixture was stirred for 3 h. The solvent was removed under reduced pressure, and the residue was purified by silica gel chromatography (SiO_2 = 80 mL, 6/1 to 2/1 gradient hexanes/EtOAc] to provide 334 mg (74%) of **19** as a white solid.

^{1}H NMR ($CDCl_3$): δ 8.10–8.03 (m, 6H), 7.61–7.53 (m, 3H), 7.48–7.39 (m, 6H), 5.71 (d, *J* = 5.2 Hz, 1H), 5.63 (s, 1H), 5.56 (t, *J* = 0.3 Hz, 1H), 4.62–4.47 (m, 4H), 2.81 (d, *J* = 1.6 Hz, 1H), 2.70 (m, 2H), 1.30 (t, *J* = 7.4 Hz, 3H). ^{13}C NMR ($CDCl_3$): δ 166.7, 166.2, 165.6, 133.9, 133.8, 133.3, 130.2, 130.1, 130.0, 129.9, 129.2, 128.7, 128.6, 88.6, 82.9, 82.5, 78.6, 69.1, 66.3, 25.5, 15.0. HRESI-MS *m*/*z* calculated for $[M+Na]^+$ $C_{29}H_{28}O_8SNa$: 559.1398. Found: 559.1395.

Thiazolinyl 2,3,5,6-tetra-*O*-acetyl-1-thio-β-D-galactofuranoside (26)

Gal*f* pentaacetate **25**[2] (500 mg, 1.3 mmol) was dissolved in CH_2Cl_2 (6.4 mL), and 4 Å molecule sieve (192 mg) and HSTaz (305 mg, 1.6 mmol) were added to the solution. The mixture was cooled in an ice bath. $BF_3{\cdot}OEt_2$ (474 µL, 3.8 mmol) was added, and the ice bath was removed. After 2 h of stirring, the reaction mixture was filtered through celite and diluted with EtOAc. The organic phase was washed with saturated $NaHCO_3$ solution, followed by brine, dried over Na_2SO_4, filtered, and concentrated. The crude residue was purified by silica gel column chromatography (SiO_2 = 100 mL, 1/0 to 50/1 gradient CH_2Cl_2/MeOH) to yield 563 mg (98%) of **26** as a colorless oil.

^{1}H NMR ($CDCl_3$): δ 6.21 (s, 1H), 5.39 (dt, *J* = 7.0, 4.2 Hz, 1H), 5.29 (t, *J* = 1.7 Hz, 1H), 5.12–5.09 (m, 1H), 4.39–4.15 (m, 5H), 3.41 (t, *J* = 7.9 Hz, 2H), 2.12 (three singlet, 9H), 2.05 (s, 3H). ^{13}C NMR ($CDCl_3$): δ 170.6, 170.1, 169.7, 169.4, 162.9, 88.5, 81.8, 81.0, 76.5, 69.3, 64.3, 62.7, 35.5, 20.9, 20.8, 20.8. HRESI-MS *m*/*z* calculated for $[M+Na]^+$ $C_{17}H_{23}NO_9S_2Na$: 472.0707. Found: 472.0718.



Scheme S2 Synthesis of trisaccharide **4**

Ethyl 2,3,6-tri-*O*-benzoyl-1-thio-β-D-galactofuranosyl-(1,5)-2,3,5,6-tetra-*O*-acetyl-β-D-galactofuranoside (22)

Glycosyl acceptor **19** (105 mg, 0.20 mmol) and donor **21** (114 mg, 0.25 mmol) were transferred to a flask and dried by three successive coevaporations with toluene, followed by rotary evaporation under pump vacuum at 40 °C for 30 min. The flask, under argon, was charged with CH_2Cl_2 (6.5 mL) and 4 Å molecule sieve beads (200 mg), and stirred for 20 min. The flask was transferred to an ice bath for 10 min before silver triflate (101 mg, 0.39 mmol) was added to the solution. The reaction mixture was stirred at room temperature for 1.5 h. The reaction was

filtered through a plug of Celite, and the organic phase was washed sequentially with saturated $NaHCO_3$ solution, water, and brine. The organic phase was dried over $MgSO_4$, filtered, and concentrated. The pure product was obtained by silica gel column chromatography (1/0 to 7/3 gradient hexanes/EtOAc) to provide 161 mg (95%) of **22** as a white solid.

^{1}H NMR ($CDCl_3$): δ 8.13–8.02 (m, 6H), 7.63–7.41 (m, 9H), 5.78 (dd, *J* = 4.9, 0.7 Hz, 1H), 5.60 (br s, 1H), 5.55 (t, *J* = 1.8 Hz, 1H), 5.51 (s, 1H), 5.33 (dt, *J* = 7.0, 4.2 Hz, 1H), 5.24 (dd, *J* = 2.4, 0.9 Hz, 1H), 5.01 (dd, *J* = 5.8, 2.3 Hz, 1H), 4.66–4.57 (m, 4H), 4.45 (dd, *J* = 5.6, 3.8 Hz, 1H), 4.30 (dd, *J* = 11.8, 4.5 Hz, 1H), 4.14 (dd, *J* = 11.8, 7.2 Hz, 1H), 2.83–2.63 (m, 2H), 2.04 (s, 3H), 2.01 (s, 3H), 1.96 (s, 3H), 1.81 (s, 3H), 1.33 (t, *J* = 7.3 Hz, 3H). ^{13}C NMR ($CDCl_3$): δ 170.6, 170.2, 169.6, 166.2, 165.7, 165.6, 133.8, 133.8, 133.4, 130.2, 130.1, 129.9, 129.3, 128.9, 128.8, 128.6, 105.1, 88.2, 82.6, 82.1, 81.4, 81.0, 77.7, 77.4, 76.6, 72.9, 69.6, 64.6, 62.8, 25.4, 20.9, 20.8, 20.6, 15.1. HRESI-MS *m*/*z* calculated for $[M+Na]^+$ $C_{43}H_{46}O_{17}SNa$: 889.2547. Found: 889.2556.

Allyl 2,3,5-tri-*O*-benzoyl-β-D-galactofuranosyl-(1,6)-2,3,6-tri-*O*-benzoyl-β-D-galactofuranosyl-(1,5)-2,3,5,6-tetra-*O*-acetyl-β-D-galactofuranoside (22a)

Glycosyl acceptor **16** (81 mg, 0.15 mmol) and disaccharide donor **22** (88 mg, 0.10 mmol) were dissolved in CH_2Cl_2 (2.0 mL) and 4 Å molecule sieve (324 mg) was added. The mixture was cooled to 4 ^{o}C and *N*-iodosuccinimide (34 mg, 0.15 mmol) and silver triflate (6.5 mg, 0.025 mmol) were added to the solution. The reaction mixture was stirred at the same temperature for 45 min. The reaction was filtered through a plug of Celite, and the organic phase was washed sequentially with 1.5 M $Na_2S_2O_3$ solution, saturated $NaHCO_3$ solution, and brine. The organic phase was dried over Na_2SO_4, filtered, and concentrated. The product was purified by silica gel column chromatography (SiO_2 = 30 mL, 2/1 to 1/1 gradient hexanes/EtOAc), providing 127 mg (93%) of **22a** as a colorless oil.

^{1}H NMR ($CDCl_3$): δ 8.06–8.00 (m, 10H), 7.91–7.88 (m 2H), 7.61–7.18 (m, 18H), 5.96–5.83 (m, 2H), 5.68 (d, *J* = 4.2 Hz, 1H), 5.59 (d, *J* = 5.3 Hz, 1H), 5.50 (s, 1H), 5.47 (s, 1H), 5.45 (s, 1H), 5.35–5.27 (m, 4H), 5.21–5.15 (m, 2H), 4.98 (dd, *J* = 5.8, 2.5 Hz, 1H), 4.70 (dd, *J* = 4.9, 3.9 Hz, 1H), 4.65–4.58 (m, 4H), 4.38 (dd, *J* = 6.0, 3.8 Hz, 1H), 4.31–3.99 (m, 6H), 2.02 (s, 3H), 1.97 (s, 3H), 1.94 (s, 3H), 1.75 (s, 3H). ^{13}C NMR ($CDCl_3$): δ 170.6, 170.2, 169.6, 166.2, 166.0, 165.9, 165.7, 165.4, 133.8, 133.7, 133.6, 133.6, 133.5, 133.3, 130.1, 130.0, 129.9, 129.9, 129.3, 129.2, 128.8, 128.7, 128.6, 117.7, 106.3, 105.1, 104.9, 83.1, 82.4, 81.9, 81.7, 81.4, 80.7, 77.4, 76.5, 72.7, 71.4, 69.6, 68.2, 66.2, 65.0, 62.8, 20.9, 20.8, 20.5. HRESI-MS *m*/*z* calculated for $[M+Na]^+$ $C_{71}H_{68}O_{26}Na$: 1359.3892. Found: 1359.3866.

12-Phenoxy-dodec-2-enyl 2,3,5-tri-*O*-benzoyl-β-D-galactofuranosyl-(1,6)-2,3,6-tri-*O*-benzoyl-β-D-galactofuranosyl-(1,5)-2,3,5,6-tetra-*O*-acetyl-β-D-galactofuranoside (22b)

Grubbs first generation catalyst (11.2 mg, 0.014 mmol) was added to a solution of trisaccharide **22a** (65.1 mg, 0.049 mmol) and 11-phenoxy-1-undecene (34 mg, 0.14 mmol) in CH_2Cl_2 (0.81 mL), and the mixture was heated at reflux for 3 h. The mixture was concentrated under reduced pressure, and the residue was

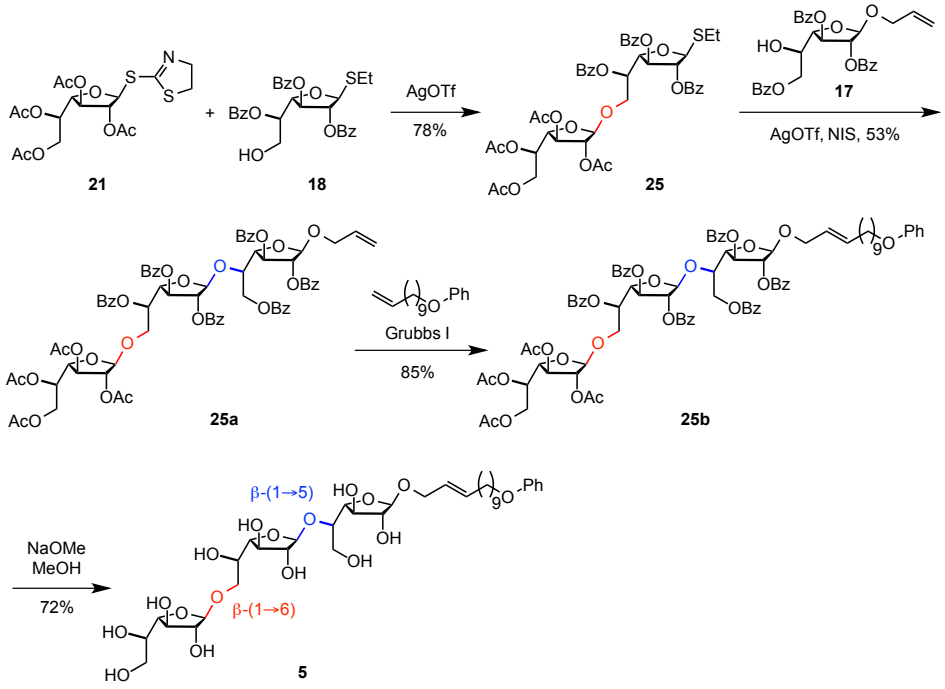
purified by silica gel column chromatography (SiO_2 = 15 mL, 2/1 to 1/1 gradient hexanes/EtOAc) to yield 43 mg (57%) of **22b** as a brown oil.

^{1}H NMR ($CDCl_3$): δ 8.10-7.95 (m, 10H), 7.90-7.85 (m, 2H), 7.60-7.20 (m, 20H), 6.93-6.86 (m, 3H), 5.89-5.86 (m, 1H), 5.73-5.65 (m, 2H), 5.56-5.44 (m, 5H), 5.31-5.24 (m, 3H), 5.19 (br s, 1H), 4.96 (dd, *J* = 6.0, 2.7 Hz, 1H), 4.68-4.57 (m, 5H), 4.36 (dd, *J* = 5.7, 3.6 Hz, 1H), 4.29-4.07 (m, 4H), 4.03-3.97 (m, 2H), 3.94-3.90 (m, 2H), 2.04-1.90 (m, 11H), 1.80-1.65 (m, 5H), 1.40-1.19 (m, 12H). ^{13}C NMR ($CDCl_3$): δ 170.4, 170.0, 169.4, 166.1, 165.8, 165.7, 165.5, 165.2, 159.2, 135.7, 133.6, 133.5, 133.4, 133.3, 133.2, 133.1, 130.0, 130.0, 130.0, 129.9, 129.8, 129.7, 129.5, 129.2, 129.2, 129.1, 129.1, 128.7, 128.6, 128.5, 128.5, 128.4, 128.4, 125.0, 120.5, 114.6, 106.1, 104.8, 104.5, 83.0, 82.3, 81.8, 81.5, 81.2, 80.6, 77.7, 77.2, 76.3, 72.6, 71.4, 69.4, 67.9, 67.9, 66.2, 64.9, 62.7, 32.4, 29.5, 29.5, 29.4, 29.3, 29.2, 26.1, 20.8, 20.6, 20.4. HRESI-MS *m*/*z* calculated for $[M+Na]^+$ $C_{86}H_{90}O_{27}Na$: 1577.5562. Found: 1577.5582.

12-Phenoxy-dodec-2-enyl β-D-galactofuranosyl-(1,6)-β-D-galactofuranosyl-(1,5)-β-D-galactofuranoside (4)

Sodium methoxide solution (0.28 mL, 0.5 M in MeOH) was added to a stirring solution of **22b** (43 mg, 0.028 mmol) in MeOH (0.41 mL) and CH_2Cl_2 (0.14 mL). The reaction was stirred for 2 h at room temperature and neutralized with Amberlite (IR-120 H^+) ion exchange resin, filtered, and concentrated under reduced pressure. Purification by silica gel column chromatography (SiO_2 = 3 mL, 20/1 to 1/1 gradient EtOAc/MeOH) provided 15 mg (69%) of compound **4** as a colorless film.

^{1}H NMR (CD_3OD): δ 7.23 (dd, *J* = 9.0, 8.1 Hz, 2H), 6.90-6.86 (m, 3H), 5.70-5.50 (m, 2H), 5.16 (s, 1H), 4.88 (s, 1H), 4.87 (s, 1H), 4.20-3.80 (m, 19H), 3.64-3.60 (m, 2H), 3.60-3.50 (m, 1H), 2.06-2.03 (m, 2H), 1.77-1.71 (m, 2H), 1.52-1.29 (m, 12H). ^{13}C NMR (CD_3OD): δ 160.7, 136.0, 130.5, 127.3, 121.6, 115.7, 109.9, 109.3, 108.3, 85.0, 84.8, 84.3, 83.6, 83.4, 82.8, 79.1, 78.9, 78.8, 77.4, 72.4, 71.2, 69.2, 69.0, 64.4, 62.9, 59.1, 35.5, 30.8, 30.7, 30.6, 30.6, 30.4, 27.3. HRESI-MS *m*/*z* calculated for $[M+NH_4]^+$ $C_{36}H_{62}NO_{17}$: 780.4013. Found: 780.3979.



Scheme S3 Synthesis of trisaccharide **5**.

Ethyl 2,3,5-tri-*O*-benzoyl-1-thio-β-D-galactofuranosyl-(1,6)-2,3,5,6-tetra-*O*-acetyl-β-D-galactofuranoside (25)

Glycosyl acceptor **18** (22 mg, 0.041 mmol) and donor **21** (24 mg, 0.054 mmol) were dissolved in CH_2Cl_2 (1.4 mL) and 4 Å molecule sieve (38 mg) was added. The mixture was cooled to 4 ^oC and silver triflate (21 mg, 0.083 mmol) was added to the solution. The reaction mixture was stirred at room temperature for 1 h. The reaction was filtered through a plug of Celite, and the organic phase was washed sequentially with saturated $NaHCO_3$ solution and brine. The organic phase was dried over Na_2SO_4, filtered, and concentrated. The pure product was obtained by silica gel column chromatography (SiO_2 = 6.5 mL, 2/1 to 1/1 gradient hexanes/EtOAc) to provide 28 mg (78%) of **25** as a white solid.

^1H NMR ($CDCl_3$): δ 8.10–8.06 (m, 4H), 7.90–7.87 (m, 2H), 7.62–7.43 (m, 5H), 7.37–7.28 (m, 4H), 5.85 (dt, *J* = 6.2, 3.8 Hz, 1H), 5.63–5.59 (m, 2H), 5.50 (t, *J* = 1.6 Hz, 1H), 5.38 (dt, *J* = 7.3, 4.0 Hz, 1H), 5.09 (s, 1H), 4.99 (t, *J* =1.5 Hz, 1H), 4.95 (dd, *J* = 5.5, 1.6 Hz, 1H), 4.76 (dd, *J* = 4.5, 3.9 Hz, 1H), 4.31 (dd, *J* = 11.8, 4.2 Hz, 1H), 4.31 (dd, *J* = 5.3, 3.6 Hz, 1H), 4.18 (dd, *J* = 11.9, 7.4 Hz, 1H), 4.05 (dd, *J* = 10.3, 5.9 Hz, 1H), 3.90 (dd, *J* = 10.3, 6.6 Hz, 1H), 2.86–2.66 (m, 2H), 2.10 (s, 3H), 2.02 (s, 3H), 2.01 (s, 3H), 1.96 (s, 3H), 1.36 (t, *J* = 7.3 Hz, 3H). ^{13}C NMR ($CDCl_3$): δ 170.7, 170.2, 169.6, 166.0, 165.7, 133.8, 133.6, 133.4, 130.2, 130.0, 129.8, 129.3, 129.1, 128.7, 128.6, 106.0, 88.4, 83.2, 81.4, 81.1, 80.9, 78.1, 77.4, 76.6, 71.3, 69.6, 66.0, 62.9, 25.6, 21.0, 20.9, 20.8, 20.7, 15.2. HRESI-MS *m/z* calculated for $[M+Na]^+$ $C_{43}H_{46}O_{17}SNa$: 889.2547. Found: 889.2549.

Allyl 2,3,6-tri-*O*-benzoyl-β-D-galactofuranosyl-(1,5)-2,3,5-tri-*O*-benzoyl-β-D-galactofuranosyl-(1,6)-2,3,5,6-tetra-*O*-acetyl-β-D-galactofuranoside (25a)

Glycosyl acceptor **17** (107 mg, 0.20 mmol) and disaccharide donor **25** (116 mg, 0.13 mmol) were dissolved in CH_2Cl_2 (2.7 mL) and 4 Å molecule sieve (429 mg) was added. The mixture was cooled to 4 ^oC and *N*-iodosuccinimide (45 mg, 0.20 mmol) and silver triflate (8.6 mg, 0.034 mmol) were added to the solution. The reaction mixture was stirred at the same temperature for 45 min. The reaction was filtered through a plug of celite, and the organic phase was washed sequentially with 1.5 M $Na_2S_2O_3$ solution, saturated $NaHCO_3$ solution, and brine. The organic phase was dried over Na_2SO_4, filtered, and concentrated. The product was purified by silica gel column chromatography (SiO_2 = 30 mL, 2/1 to 1/1 gradient hexanes/EtOAc), providing 98 mg (53%) of **25a** as a white solid.

^1H NMR ($CDCl_3$): δ 8.02–7.98 (m, 8H), 7.87–7.77 (m, 4H), 7.58–7.19 (m, 18H), 5.97–5.81 (m, 3H), 5.75 (s, 1H), 5.64 (s, 1H), 5.58–5.53 (m, 2H), 5.36–5.28 (m, 3H), 5.21–5.17 (m, 1H), 4.98 (s, 1H), 4.91–4.84 (m, 3H), 4.80–4.67 (m, 3H), 4.54 (dd, *J* = 5.9, 4.3 Hz, 1H), 4.29–4.21 (m, 3H), 4.17–3.97 (m, 3H), 3.87 (dd, *J* = 11.3, 7.5 Hz, 1H), 2.06 (s, 3H), 1.98 (s, 3H), 1.94 (s, 3H), 1.87 (s, 3H). ^{13}C NMR ($CDCl_3$): δ 170.6, 170.2, 169.4, 166.3, 166.0, 165.9, 165.7, 165.6, 165.4, 133.9, 133.7, 133.4, 133.2, 130.1, 130.0, 129.9, 129.3, 129.2, 129.1, 128.8, 128.7, 128.5, 128.4, 117.6, 106.5, 105.7, 105.0, 82.7, 82.4, 82.2, 82.1, 81.3, 80.6, 78.0, 77.4, 76.5, 73.4, 71.9, 69.6, 68.1, 67.5, 64.9, 62.9, 21.0, 20.9, 20.8, 20.6, 20.0, 19.5. HRESI-MS *m*/*z* calculated for $[M+Na]^+$ $C_{71}H_{68}O_{26}Na$: 1359.3892. Found: 1359.3962.

12-Phenoxy-dodec-2-enyl 2,3,6-tri-*O*-benzoyl-β-D-galactofuranosyl-(1,5)-2,3,5-tri-*O*-benzoyl-β-D-galactofuranosyl-(1,6)-2,3,5,6-tetra-*O*-acetyl-β-D-galactofuranoside (25b)

Grubbs first-generation catalyst (10.5 mg, 0.013 mmol) was added to a solution of trisaccharide **25a** (61 mg, 0.046 mmol) and 11-phenoxy-1-undecene (31 mg, 0.13 mmol) in CH_2Cl_2 (0.76 mL), and the mixture was heated at reflux for 3 h. The mixture was concentrated under reduced pressure, and the residue was purified by silica gel column chromatography (SiO_2 = 10 mL, 2/1 to 1/1 gradient hexanes/EtOAc) to yield 60 mg (85%) of **25b** as a brown oil.

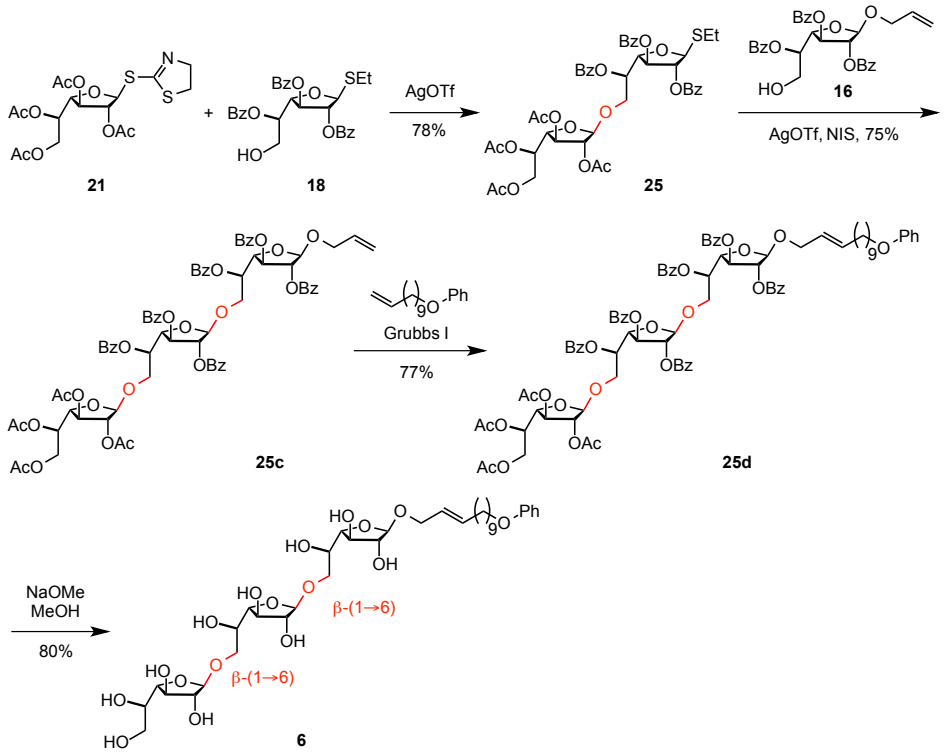
^1H NMR ($CDCl_3$): δ 8.00-7.94 (m, 10H), 7.85-7.74 (m, 4H), 7.55-7.16 (m, 18H), 6.93-6.86 (m, 3H), 5.83-5.60 (m, 4H), 5.59-5.48 (m, 3H), 5.31-5.25 (m, 2H), 4.95 (m, 1H), 4.92-4.81 (m, 3H), 4.78-4.64 (m, 3H), 4.54-4.48 (m, 1H), 4.28-4.06 (m, 5H), 4.02-3.80 (m, 5H), 2.14-1.91 (m, 11H), 1.85 (s, 3H), 1.78-1.70 (m, 2H), 1.45-1.32 (m, 12H). ^{13}C NMR ($CDCl_3$): δ 170.4, 170.0, 169.9, 169.2, 166.1, 165.7, 165.6, 165.5, 165.4, 165.1, 159.1, 135.5, 133.4, 133.2, 133.0, 129.9, 129.9, 129.7, 129.7, 129.6, 129.3, 129.1, 129.0, 128.9, 128.8, 128.5, 128.4, 128.3, 128.2, 128.2, 125.0, 120.4, 114.4, 106.2, 105.5, 104.4, 82.3, 82.2, 81.9, 81.0, 80.3, 77.8, 77.2, 76.3, 73.1, 71.7, 69.4, 67.8, 67.7, 67.2, 64.8, 62.6, 32.3, 29.5, 29.4, 29.2, 29.2, 29.0, 26.0, 20.7, 20.6, 20.5, 20.3. HRESI-MS *m*/*z* calculated for $[M+Na]^+$ $C_{86}H_{90}O_{27}Na$: 1577.5562. Found: 1577.5605.

12-Phenoxy-dodec-2-enyl β-D-galactofuranosyl-(1,5)-β-D-galactofuranosyl-(1,6)-β-D-galactofuranoside (5)

Sodium methoxide solution (0.39 mL, 0.5 M in MeOH) was added to a stirring solution of **25b** (60 mg, 0.039 mmol) in MeOH (0.58 mL) and CH_2Cl_2 (0.19 mL). The reaction was stirred overnight at room temperature and

neutralized with Amberlite (IR-120 H^+) ion exchange resin, filtered, and concentrated under reduced pressure. Purification by silica gel column chromatography (SiO_2 = 3 mL, 20/1 to 1/1 gradient EtOAc/MeOH) provided 21 mg (72%) of compound **5** as a colorless film.

^{1}H NMR (CD_3OD): δ 7.23 (dd, *J* = 8.1, 8.1 Hz, 2H), 6.91-6.85 (m, 3H), 5.77-5.49 (m, 2H), 5.20 (s, 1H), 4.93 (s, 1H), 4.88 (s, 1H), 4.16-3.81 (m, 16H), 3.76-3.71 (m, 3H), 3.66-3.52 (m, 3H), 2.10-2.01 (m, 2H), 1.78-1.71 (m, 2H), 1.48-1.26 (m, 12H). ^{13}C NMR (CD_3OD): δ 160.7, 136.1, 130.5, 127.3, 121.6, 115.7, 110.1, 109.0, 108.4, 86.1, 85.1, 84.1, 83.8, 83.0, 82.5, 79.1, 79.1, 77.3, 77.3, 72.7, 71.3, 70.5, 69.2, 69.0, 64.6, 62.8, 33.5, 30.8, 30.7, 30.6, 30.6, 30.4, 27.3. HRESI-MS *m*/*z* calculated for $[M+Na]^+$ $C_{36}H_{58}O_{17}Na$: 785.3567. Found: 785.3534.



Scheme S4 Synthesis of trisaccharide **6**.

Allyl 2,3,5-tri-*O*-benzoyl-β-D-galactofuranosyl-(1,6)-2,3,5-tri-*O*-benzoyl-β-D-galactofuranosyl-(1,6)-2,3,5,6-tetra-*O*-acetyl-β-D-galactofuranoside (25c)

Glycosyl acceptor **16** (25 mg, 0.047 mmol) and disaccharide donor **25** (53 mg, 0.061 mmol) were transferred to a flask and dried by coevaporation with toluene (3 times), followed by rotary evaporation under pump vacuum at 40 °C for 30 min. The flask, under argon, was charged with CH_2Cl_2 (1.6 mL) and 4 Å molecule sieve beads (150 mg), and stirred for 15 min. The flask was transferred to an ice bath, and *N*-iodosuccinimide (15 mg, 0.066 mmol) and silver triflate (3 mg, 0.011 mmol) were added to the solution. The reaction mixture was stirred at room temperature for 30 min. The reaction was filtered through a plug of celite, and the organic phase was washed sequentially with 10% $Na_2S_2O_3$, saturated $NaHCO_3$ solution, and brine. The organic phase was dried over $MgSO_4$,

filtered, and concentrated. The product was purified by silica gel column chromatography (1/0 to 7/3 gradient touene/EtOAc), providing 47 mg (75%) of **25c** as a white solid.

^{1}H NMR ($CDCl_3$): δ 8.07–7.99 (m, 8H), 7.91–7.88 (m, 2H), 7.75–7.72 (m, 2H), 7.55–7.45 (m, 6H), 7.40–7.16 (m, 12H), 5.98–5.82 (m, 3H), 5.61 (d, *J* = 5.0 Hz, 1H), 5.53 (d, *J* = 4.9 Hz, 1H), 5.49 (d, *J* = 0.9 Hz, 1H), 5.37–5.30 (m, 5H), 5.29 (s, 1H), 5.20–5.15 (m, 1H), 5.06 (s, 1H), 4.95–4.91 (m, 2H), 4.76 (dd, *J* = 5.3, 3.5 Hz, 1H), 4.67 (dd, *J* = 4.6, 3.3 Hz, 1H), 4.33–3.91 (m, 8H), 2.07 (s, 3H), 1.98 (s, 3H), 1.96 (s, 3H), 1.90 (s, 3H). ^{13}C NMR ($CDCl_3$): δ 170.6, 170.2, 170.2, 169.5, 166.0, 166.0, 165.9, 165.8, 165.7, 165.4, 133.8, 133.6, 133.4, 133.3, 130.1, 130.1, 130.0, 130.0, 129.9, 129.8, 129.3, 129.2, 129.2, 129.0, 128.6, 128.5, 117.7, 106.4, 106.2, 105.0, 82.5, 82.2, 82.1, 81.5, 81.2, 80.6, 77.8, 77.4, 76.5, 71.6, 71.3, 69.6, 68.2, 66.7, 66.2, 62.8, 21.0, 20.8, 20.8, 20.6. HRESI-MS m/z calculated for $[M+Na]^+$ $C_{71}H_{68}O_{26}Na$: 1359.3892. Found: 1359.3888.

12-Phenoxy-dodec-2-enyl 2,3,5-tri-*O*-benzoyl-β-D-galactofuranosyl-(1,6)-2,3,5-tri-*O*-benzoyl-β-D-galactofuranosyl-(1,6)-2,3,5,6-tetra-*O*-acetyl-β-D-galactofuranoside (25d)

Grubbs first generation catalyst (11.4 mg, 0.014 mmol) was added to a solution of trisaccharide **25c** (66 mg, 0.049 mmol) and 11-phenoxy-1-undecene (34 mg, 0.14 mmol) in CH_2Cl_2 (0.82 mL), and the mixture was heated at reflux for 2.5 h. The mixture was concentrated under reduced pressure, and the residue was purified by silica gel column chromatography (SiO_2 = 15 mL, 2/1 to 1/1 gradient hexanes/EtOAc) to yield 59 mg (77%) of **25d** as a brown oil.

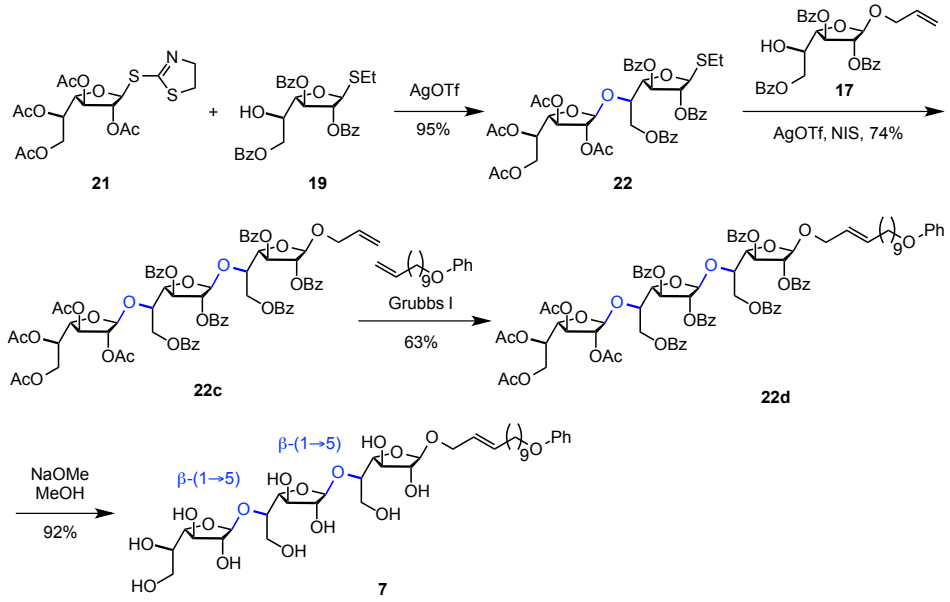
^{1}H NMR ($CDCl_3$): δ 8.05-7.98 (m, 10H), 7.89-7.86 (m, 2H), 7.72-7.69 (m, 2H), 7.52-7.16 (m, 18H), 6.93-6.86 (m, 3H), 5.90-5.80 (m, 2H), 5.78-5.66 (m, 1H), 5.60-5.44 (m, 5H), 5.38-5.30 (m, 4H), 5.04 (br s, 1H), 4.92-4.89 (m, 2H), 4.74-4.72 (m, 1H), 4.65-4.63 (m, 1H), 4.32-3.99 (m, 7H), 3.96-3.88 (m, 3H), 2.12-1.91 (m, 11H), 1.88 (s, 3H), 1.78-1.62 (m, 2H), 1.45-1.18 (m, 12H). ^{13}C NMR ($CDCl_3$): δ 170.4, 170.0, 169.9, 169.2, 165.8, 165.7, 165.7, 165.5, 165.4, 165.1, 159.1, 135.6, 133.3, 133.2, 133.2, 133.0, 129.9, 129.9, 129.9, 129.8, 129.8, 129.7, 129.7, 129.6, 129.4, 129.1, 129.0, 128.8, 128.4, 128.4, 128.3, 128.2, 124.9, 120.4, 114.5, 106.0, 104.5, 100.3, 82.3, 82.0, 81.9, 81.2, 81.0, 80.4, 77.6, 77.2, 76.3, 71.4, 71.2, 69.4, 67.8, 66.5, 66.1, 62.6, 32.3, 29.5, 29.4, 29.3, 29.2, 28.9, 26.0, 20.7, 20.6, 20.5, 20.4. HRESI-MS *m*/*z* calculated for $[M+Na]^+$ $C_{86}H_{90}O_{27}Na$: 1577.5562. Found: 1577.5616.

12-Phenoxy-dodec-2-enyl β-D-galactofuranosyl-(1,6)-β-D-galactofuranosyl-(1,6)-β-D-galactofuranoside (6)

Sodium methoxide solution (0.38 mL, 0.5 M in MeOH) was added to a stirring solution of **S4** (59 mg, 0.038 mmol) in MeOH (0.57 mL) and CH_2Cl_2 (0.19 mL). The reaction was stirred overnight at room temperature and neutralized with Amberlite (IR-120 H^+) ion exchange resin, filtered, and concentrated under reduced pressure. Purification by silica gel column chromatography (SiO_2 = 3 mL, 20/1 to 1/1 gradient EtOAc/MeOH) provided 23 mg (80%) of compound **6** as a colorless film.

^{1}H NMR (CD_3OD): δ 7.23 (dd, *J* = 9.0, 8.1 Hz, 2H), 6.90-6.86 (m, 3H), 5.78-5.54 (m, 2H), 4.92 (s, 1H), 4.91 (s, 1H), 4.90 (s, 1H), 4.18-4.12 (m, 1H), 4.04-3.78 (m, 15H), 3.76-3.70 (m, 2H), 3.65-3.61 (m, 2H), 3.57-3.51 (m, 2H), 2.09-2.02 (m, 2H), 1.80-1.71 (m, 2H), 1.50-1.28 (m, 12H). ^{13}C NMR (CD_3OD): δ 160.7, 136.0, 130.5, 127.3,

121.6, 115.7, 111.3, 110.1, 108.3, 85.6, 85.1, 84.9, 83.6, 83.1, 83.1, 79.1, 79.1, 79.0, 72.7, 71.3, 70.7, 70.7, 69.2, 69.0, 64.6, 33.5, 30.8, 30.7, 30.6, 30.6, 30.4, 27.3. HRESI-MS *m/z* calculated for $[M+Na]^+$ $C_{36}H_{58}O_{17}Na$: 785.3567. Found: 785.3539.



Scheme S5 Synthesis of trisaccharide **7**.

Allyl 2,3,6-tri-*O*-benzoyl-β-D-galactofuranosyl-(1,5)-2,3,6-tri-*O*-benzoyl-β-D-galactofuranosyl-(1,5)-2,3,5,6-tetra-*O*-acetyl-β-D-galactofuranoside (22c)

Glycosyl acceptor **17** (81 mg, 0.15 mmol) and disaccharide donor **22** (88 mg, 0.10 mmol) were dissolved in CH_2Cl_2 (2.0 mL) and 4 Å molecule sieve beads (324 mg) was added. The mixture was cooled to 4 ^oC and *N*-iodosuccinimide (34 mg, 0.15 mmol) and silver triflate (6.5 mg, 0.025 mmol) were added to the solution. The reaction mixture was stirred at the same temperature for 30 min. The reaction was filtered through a plug of Celite, and the organic phase was washed sequentially with 1.5 M $Na_2S_2O_3$ solution, saturated $NaHCO_3$ solution, and brine. The organic phase was dried over Na_2SO_4, filtered, and concentrated. The product was purified by silica gel column chromatography (SiO_2 = 30 mL, 2/1 to 1/1 gradient hexanes/EtOAc), providing 101 mg (74%) of **22c** as a white solid.

^1H NMR ($CDCl_3$): δ 8.08-7.94 (m, 12H), 7.85-7.82 (m, 2H), 7.60-7.21 (m, 16H), 5.93-5.79 (m, 2H), 5.75 (s, 1H), 5.71-5.69 (m, 2H), 5.48 (d, *J* = 6.0 Hz, 2H), 5.32-5.24 (m, 3H), 5.20-5.13 (m, 2H), 4.94 (dd, *J* = 6.6, 2.7 Hz, 1H), 4.79 (dd, *J* = 4.2, 3.0 Hz, 1H), 4.74-4.59 (m, 5H), 4.56-4.51 (m, 2H), 4.36 (dd, *J* = 6.0, 3.9 Hz, 1H), 4.27-4.17 (m, 2H), 4.13-4.00 (m, 2H), 1.97 (s, 3H), 1.96 (s, 3H), 1.88 (s, 3H), 1.70 (s, 3H). ^{13}C NMR ($CDCl_3$): δ 170.3, 170.3, 170.0, 169.4, 166.1, 165.9, 165.7, 165.6, 165.4, 165.2, 133.7, 133.6, 133.5, 133.4, 133.3, 133.1, 133.1, 130.0, 129.8, 129.8, 129.8, 129.7, 129.2, 129.0, 128.9, 128.7, 128.6, 128.4, 128.4, 128.3, 117.4, 105.3, 104.8, 104.7, 83.1,

82.6, 81.9, 81.9, 81.2, 80.5, 77.3, 77.3, 77.2, 76.3, 73.3, 72.6, 69.4, 67.9, 64.8, 64.5, 62.7, 20.7, 20.6, 20.6, 20.3. HRESI-MS *m/z* calculated for $[M+Na]^+$ $C_{71}H_{68}O_{26}Na$: 1359.3892. Found: 1359.3895.

12-Phenoxy-dodec-2-enyl 2,3,6-tri-*O*-benzoyl-β-D-galactofuranosyl-(1,5)-2,3,6-tri-*O*-benzoyl-β-D-galactofuranosyl-(1,5)-2,3,5,6-tetra-*O*-acetyl-β-D-galactofuranoside (22d)

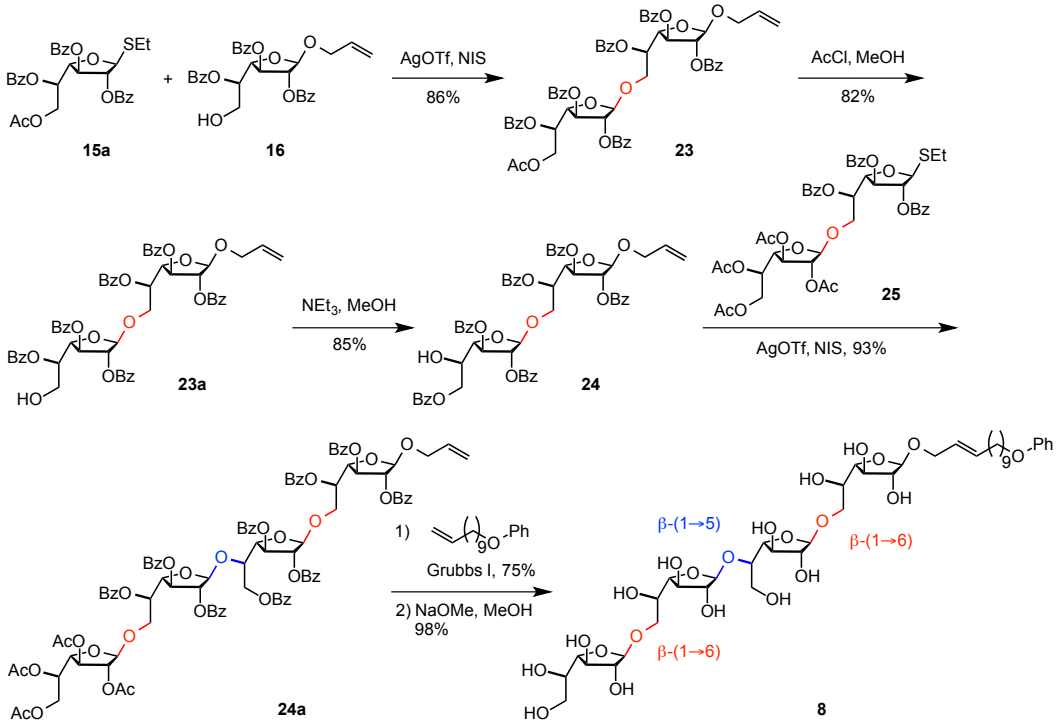
Grubbs first generation catalyst (11.6 mg, 0.014 mmol) was added to a solution of trisaccharide **22c** (68 mg, 0.051 mmol) and 11-phenoxy-1-undecene (35 mg, 0.14 mmol) in CH_2Cl_2 (0.84 mL), and the mixture was heated at reflux for 3 h. The mixture was concentrated under reduced pressure, and the residue was purified by silica gel column chromatography (SiO_2 = 15 mL, 2/1 to 1/1 gradient hexanes/EtOAc) to yield 49 mg (63%) of **22d** as a brown oil.

^{1}H NMR ($CDCl_3$): δ 8.05-7.94 (m, 12H), 7.85-7.81 (m, 2H), 7.60-7.20 (m, 18H), 6.93-6.86 (m, 3H), 5.79-5.64 (m, ,5H), 5.56-5.47 (m, 3H), 5.32-5.19 (m, 3H), 4.94 (dd, *J* = 6.3, 2.4 Hz, 1H), 4.88-4.80 (m, 1H), 4.78-4.56 (m, 5H), 4.52-4.49 (m, 2H), 4.36 (dd, *J* = 5.7, 3.3 Hz, 1H), 4.27-4.05 (m, 3H), 4.01-3.90 (m, 3H), 2.15-1.95 (m, 8H), 1.88 (s, 3H), 1.80-1.65 (m, 5H), 1.45-1.20 (m, 12H). ^{13}C NMR ($CDCl_3$): δ 170.4, 170.1, 170.0, 169.4, 166.2, 165.9, 165.8, 165.6, 165.4, 165.2, 159.2, 135.6, 133.6, 133.5, 133.4, 133.3, 133.1, 133.1, 130.0, 130.0, 129.9, 129.8, 129.8, 129.7, 129.4, 129.2, 129.1, 129.0, 128.7, 128.6, 128.4, 128.3, 125.0, 120.5, 114.5, 105.4, 104.8, 104.4, 83.1, 82.5, 82.0, 81.9, 81.2, 80.5, 77.4, 77.2, 76.3, 73.3, 72.6, 69.4, 67.9, 67.7, 64.8, 64.6, 62.7, 32.3, 29.5, 29.4, 29.3, 29.2, 29.1, 26.1, 20.7, 20.6, 20.6, 20.3. HRESI-MS *m/z* calculated for $[M+Na]^+$ $C_{86}H_{90}O_{27}Na$: 1577.5562. Found: 1577.5594.

12-Phenoxy-dodec-2-enyl β-D-galactofuranosyl-(1,5)-β-Dgalactofuranosyl-(1,5)-β-D-galactofuranoside (7)

Sodium methoxide solution (0.32 mL, 0.5 M in MeOH) was added to a stirring solution of **S6** (59 mg, 0.038 mmol) in MeOH (0.47 mL) and CH_2Cl_2 (0.16 mL). The reaction was stirred overnight at room temperature and neutralized with Amberlite (IR-120 H^+) ion exchange resin, filtered, and concentrated under reduced pressure. Purification by silica gel column chromatography (SiO_2 = 3 mL, 20/1 to 1/1 gradient EtOAc/MeOH) provided 22 mg (92%) of compound **7** as a colorless film.

^{1}H NMR (CD_3OD): δ 7.23 (dd, *J* = 8.1, 7.5 Hz, 2H), 6.90-6.86 (m, 3H), 5.74-5.48 (m, 2H), 5.17 (s, 2H), 4.81 (s, 1H), 4.19-3.85 (m, 15H), 3.76-3.70 (m, 5H), 3.68-3.60 (m, 2H), 2.11-2.01 (m, 2H), 1.80-1.71 (m, 2H), 1.52-1.30 (m, 12H). ^{13}C NMR (CD_3OD): δ 160.7, 136.1, 130.5, 127.3, 121.6, 115.7, 109.1, 109.0, 108.3, 84.9, 84.1, 83.8, 83.6, 83.4, 83.0, 78.9, 78.8, 78.6, 77.3, 77.1, 72.4, 69.3, 69.0, 64.4, 62.9, 62.5, 33.5, 30.8, 30.7, 30.6, 30.6, 30.4, 27.3. HRESI-MS *m/z* calculated for $[M+NH_4]^+$ $C_{36}H_{62}NO_{17}$: 780.4013. Found: 780.3976.



Scheme S6 Synthesis of tetrasaccharide **8**.

Allyl 2,3,5-tri-*O*-benzoyl-β-D-galactofuranosyl-(1,6)-6-*O*-acetyl-2,3,5-tri-*O*-benzoyl-β-D-galactofuranoside (23)

Glycosyl acceptor **16** (130 mg, 0.24 mmol) and donor **15a** (113 mg, 0.20 mmol) were dissolved in CH_2Cl_2 (3.9 mL) and 4 Å molecule sieve (520 mg) were added to the solution. The solution was cooled to 4 ^oC and *N*-iodosuccinimide (55 mg, 0.24 mmol) and silver triflate (13 mg, 0.049 mmol) were added as solids. Stirring was continued at 4 °C for 1 h. The crude reaction mixture was filtered through a pad of celite, and the organic phase was washed sequentially with 1.5 M $Na_2S_2O_3$ solution, saturated $NaHCO_3$ solution, and brine. The organic phase was dried over Na_2SO_4, filtered, and concentrated. The pure product was obtained by silica gel column chromatography (SiO_2 = 22 mL, 20/1 to 12/1 gradient toluene/EtOAc) to provide 178 mg (86%) of **23** as a white solid.

^1H NMR ($CDCl_3$): δ 8.07–8.00 (m, 8H), 7.92–7.89 (m, 2H), 7.81–7.78 (m, 2H), 7.53–7.23 (m, 18H), 5.97–5.84 (m, 3H), 5.61 (d, *J* = 5.4 Hz, 1H), 5.52 (d, *J* = 4.9, 1H), 5.49 (s, 1H), 5.38 (s, 1H), 5.36 (s, 1H), 5.35 (s, 1H), 5.31–5.30 (m, 1H), 5.17 (dd, *J* = 10.5, 0.9, 1H), 4.74 (dd, *J* = 5.1, 3.9 Hz, 1H), 4.67 (dd, *J* = 4.8, 3.3 Hz, 1H), 4.52 (dd, *J* = 11.7, 4.4 Hz, 1H), 4.45 (dd, *J* = 11.7, 7.2 Hz, 1H), 4.30–4.01 (m, 4H), 1.96 (s, 3H). ^{13}C NMR ($CDCl_3$): δ 170.6, 165.9, 165.8, 165.7, 165.5, 165.3, 133.6, 133.5, 133.4, 133.4, 133.3, 133.2, 130.0, 130.0, 129.9, 129.9, 129.7, 129.5, 129.2, 129.1, 129.1, 129.0, 128.9, 128.5, 128.5, 128.5, 128.5, 128.4, 128.3, 125.4, 117.6, 106.1, 105.0, 82.4, 82.0, 81.8, 81.4, 77.5, 77.4, 71.3, 70.3, 68.2, 65.9, 62.9, 20.7. HRESI-MS *m/z* calculated for $[M+Na]^+$ $C_{59}H_{52}O_{18}Na$: 1071.3046. Found: 1071.3008.

Allyl 2,3,5-tri-*O*-benzoyl-β-D-galactofuranosyl-(1,6)-2,3,5-tri-*O*-benzoyl-β-D-galactofuranoside (23a)

Disaccharide **23** (143 mg, 0.14 mmol) was dissolved in MeOH (3.9 mL) and CH_2Cl_2 (0.65 mL). Acetyl chloride (15 μL, 0.20 mmol) was added drop-wise at 4 ^oC. The reaction was allowed to warm to room temperature overnight. At 16 h, the reaction was quenched by the addition of pyridine (17 μL) and concentrated. The crude residue was purified by silica gel column chromatography (SiO_2 = 30 mL, 4/1 to 2/1 gradient hexanes/EtOAc) to yield 112 mg (82%) of disaccharide **23a**.

^1H NMR ($CDCl_3$): δ 8.08–7.95 (m, 9H), 7.89–7.83 (m, 4H), 7.57–7.24 (m, 17Ha, 6.03–5.98 (m, 1H), 5.96–5.85 (m, 1H), 5.64–5.56 (m, 2H), 5.54 (d, *J* = 5.1 Hz, 1H), 5.49 (d, *J* = 1.1 Hz, 1H), 5.41 (d, *J* = 1.1 Hz, 1H), 5.40 (s, 1H), 5.38–5.30 (m, 2H), 5.20–5.16 (m, 1H), 4.82 (dd, *J* = 5.1, 3.1 Hz, 1H), 4.69 (dd, *J* = 4.9, 3.6 Hz, 1H), 4.27 (ddt, *J* = 13.1, 4.9, 1.4 Hz, 1H), 4.21–3.99 (m, 5H). ^{13}C NMR ($CDCl_3$): δ 166.4, 166.2, 166.1, 166.0, 165.7, 165.5, 133.8, 133.7, 133.6, 133.4, 130.2, 130.1, 130.0, 130.0, 129.8, 129.8, 129.2, 129.1, 128.7, 128.6, 117.8, 106.9, 105.1, 82.4, 82.0, 78.0, 77.8, 77.4, 73.5, 72.0, 68.3, 67.3, 62.3, 61.4. HRESI-MS *m*/*z* calculated for [M+Na]+ $C_{57}H_{50}O_{17}Na$: 1029.2941. Found: 1029.2914.

Allyl 2,3,5-tri-*O*-benzoyl-β-D-galactofuranosyl-(1,6)-2,3,6-tri-*O*-benzoyl-β-D-galactofuranoside (24)

Disaccharide **23a** (112 mg, 0.11 mmol) was dissolved in MeOH (1.9 mL) and CH_2Cl_2 (1.9 mL). Triethylamine (60 μL, 0.43 mmol) was added, and the solution was stirred at room temperature for 12 h. The solvent was removed under reduced pressure, and the residue was purified by silica gel column chromatography (SiO_2 = 20 mL, 4/1 to 2/1 gradient hexanes/EtOAc) to provide 96 mg (85%) of **24** as a white solid.

^1H NMR ($CDCl_3$): δ 8.08-7.90 (m, 12H), 7.58-7.20 (m, 18H), 5.94-5.81 (m, 2H), 5.59-5.55 (m, 2H), 5.44 (dd, *J* = 5.4, 0.9 Hz, 2H), 5.35-5.26 (m, 3H), 5.16 (ddd, *J* = 11.1, 2.7, 1.2 Hz, 1H), 4.67 (dd, *J* = 5.1, 3.9 Hz, 1H), 4.61-4.38 (m, 4H), 4.26-3.96 (m, 4H), 2.67 (d, *J* = 8.4 Hz, 1H). ^{13}C NMR ($CDCl_3$): δ 166.6, 166.2, 165.9, 165.8, 165.6, 165.2, 133.7, 133.6, 133.6, 133.5, 133.4, 133.2, 133.1, 130.1, 130.0, 129.9, 129.9, 129.9, 129.8, 129.7, 129.2, 129.1, 129.1, 129.0, 128.6, 128.5, 128.5, 128.5, 128.4, 117.6, 106.4, 105.0, 83.7, 82.3, 81.6, 81.5, 78.1, 77.6, 77.4, 71.4, 69.3, 68.1, 66.4. HRESI-MS *m*/*z* calculated for $[M+Na]^+$ $C_{57}H_{50}O_{17}Na$: 1029.2941. Found: 1029.2946.

Allyl 2,3,5-tri-*O*-benzoyl-β-D-galactofuranosyl-(1,6)-2,3,6-tri-*O*-benzoyl-β-D-galactofuranosyl-(1,5)-2,3,5-tri-*O*-benzoyl-β-D-galactofuranosyl-(1,6)-2,3,5,6-tetra-*O*-acetyl-β-D-galactofuranoside (24a)

Glycosyl acceptor **24** (96 mg, 0.095 mmol) and disaccharide donor **25** (84 mg, 0.096 mmol) were dissolved in CH_2Cl_2 (1.9 mL) and 4 Å molecule sieve (382 mg) was added. The mixture was cooled to 4 ^oC and *N*-iodosuccinimide (24 mg, 0.10 mmol) and silver triflate (4.4 mg, 0.017 mmol) were added to the solution. The reaction mixture was stirred at the same temperature for 1 h. The reaction was filtered through a plug of Celite, and the organic phase was washed sequentially with 1.5 M $Na_2S_2O_3$ solution, saturated $NaHCO_3$ solution, and brine. The organic phase was dried over Na_2SO_4, filtered, and concentrated. The product was purified by silica gel column chromatography (SiO_2 = 20 mL, 3/2 to 1/1 gradient hexanes/EtOAc), providing 161 mg (93%) of **24a** as a colorless oil.

^{1}H NMR ($CDCl_3$): δ 8.05-7.95 (m, 10H), 7.89-7.86 (m, 4H), 7.82-7.73 (m, 4H), 7.53-7.16 (m, 27H), 5.92-5.75 (m, 4H), 5.63 (s, 1H), 5.58-5.55 (m, 2H), 5.46 (s, 2H), 5.34-5.28 (m, 4H), 5.15 (m, 1H), 4.96 (s, 1H), 4.89-4.85 (m, 2H), 4.82-4.77 (m, 1H), 4.73-4.63 (m, 4H), 4.60-4.55 (m, 1H), 4.26-3.96 (m, 9H), 3.87-3.83 (m, 1H), 2.03 (s, 3H), 1.95 (s, 3H), 1.91 (s, 3H), 1.83 (s, 3H).^{13}C NMR ($CDCl_3$): δ 170.3, 169.9, 169.9, 169.1, 166.0, 165.7, 165.6, 165.4, 165.1, 165.1, 133.7, 133.4, 133.4, 133.2, 133.1, 133.1, 133.0, 130.0, 130.0, 130.0, 129.9, 129.8, 129.8, 129.7, 129.7, 129.2, 129.1, 129.0, 129.0, 128.9, 128.5, 128.5, 128.5, 128.4, 128.4, 128.4, 128.3, 128.2, 117.5, 106.3, 106.1, 105.6, 104.9, 83.0, 82.3, 82.1, 81.9, 81.7, 81.5, 81.1, 80.4, 77.7, 77.3, 76.3, 73.2, 71.8, 71.2, 69.5, 68.1, 67.3, 66.0, 65.1, 62.7, 20.8, 20.7, 20.6, 20.4. HRESI-MS *m*/*z* calculated for $[M+Na]^+$ $C_{98}H_{90}O_{34}Na$: 1833.5206. Found: 1833.5289.

12-Phenoxy-dodec-2-enyl 2,3,5-tri-*O*-benzoyl-β-D-galactofuranosyl-(1,6)-2,3,6-tri-*O*-benzoyl-β-D-galactofuranosyl-(1,5)-2,3,5-tri-*O*-benzoyl-β-D-galactofuranosyl-(1,6)-2,3,5,6-tetra-*O*-acetyl-β-D-galactofuranoside (24b)

Grubbs first generation catalyst (16 mg, 0.020 mmol) was added to a solution of tetrasaccharide **24a** (129 mg, 0.071 mmol) and 11-phenoxy-1-undecene (49 mg, 0.20 mmol) in CH_2Cl_2 (1.2 mL), and the mixture was heated at reflux for 3 h. The mixture was concentrated under reduced pressure, and the residue was purified by silica gel column chromatography (SiO_2 = 40 mL, 2/1 to 1/1 gradient hexanes/EtOAc) to yield 109 mg (75%) of **24b**, the compound **8** precursor, as a brown oil.

^{1}H NMR ($CDCl_3$): δ 8.04-7.95 (m, 10H), 7.90-7.85 (m, 4H), 7.82-7.73 (m, 4H), 7.48-7.18 (m, 29H), 6.89-6.86 (m, 3H), 5.89-5.83 (m, 1H), 5.80-5.74 (m, 3H), 5.62 (s, 1H), 5.56-5.53 (m, 3H), 5.45 (m, 2H), 5.30-5.26 (m, 3H), 4.96 (s, 1H), 4.88-4.84 (m, 2H), 4.82-4.80 (m, 1H), 4.69-4.63 (m, 4H), 4.61-4.60 (m, 1H), 4.26-3.80 (m, 12H), 2.04-1.90 (m, 11H), 1.83 (s, 3H), 1.78-1.69 (m, 2H), 1.45-1.15 (m, 12H). ^{13}C NMR ($CDCl_3$): δ 170.3, 170.0, 169.9, 169.1, 166.0, 165.7, 165.7, 165.7, 165.4, 165.4, 165.1, 165.1, 165.1, 159.1, 135.6, 133.5, 133.4, 133.4, 133.3, 133.1, 133.1, 133.0, 130.1, 130.0, 130.0, 129.9, 129.9, 129.9, 129.8, 129.7, 129.5, 129.3, 129.2, 129.1, 129.1, 129.0, 129.0, 128.6, 128.5, 128.5, 128.4, 128.4, 128.4, 128.3, 125.0, 120.5, 114.6, 106.3, 106.2, 105.7, 104.5, 83.0, 82.4, 82.2, 82.0, 81.8, 81.4, 81.2, 80.5, 77.8, 77.7, 77.3, 76.4, 73.3, 71.8, 71.3, 69.5, 67.9, 67.9, 67.3, 66.1, 65.2, 62.7, 32.4, 29.6, 29.5, 29.4, 29.3, 29.1, 26.1, 20.9, 20.8, 20.7, 20.6. HRESI-MS *m*/*z* calculated for $[M+2Na]^{2+}$ $C_{113}H_{112}O_{35}Na_2$: 1037.3385. Found: 1037.3375.

12-Phenoxy-dodec-2-enyl β-D-galactofuranosyl-(1,6)-β-D-galactofuranosyl-(1,5)-β-D-galactofuranosyl-(1,6)-β-D-galactofuranoside (8)

Sodium methoxide solution (30 μL, 0.5 M in MeOH) was added to a stirring solution of the protected trisaccharide precursor (6.1 mg, 0.0030 mmol) in MeOH (0.09 mL) and CH_2Cl_2 (0.03 mL). The reaction was stirred for 4 h at room temperature and neutralized with Amberlite (IR-120 H^+) ion exchange resin, filtered, and concentrated under reduced pressure. Purification by silica gel column chromatography (SiO_2 = 0.3 mL, 20/1 to 1/1 gradient EtOAc/MeOH) provided 2.7 mg (98%) of compound **8** as a colorless film.

^{1}H NMR (CD_3OD): δ 7.23 (dd, *J* = 8.4, 7.5 Hz, 2H), 6.90-6.86 (m, 3H), 5.78-5.50 (m, 2H), 5.19 (s, 1H), 4.93 (s, 1H), 4.90 (s, 1H), 4.90 (s, 1H), 4.20-3.70 (m, 24H), 3.64-3.60 (m, 2H), 3.57-3.49 (m, 2H), 2.08-2.01 (m, 2H), 1.77-1.70 (m, 2H), 1.49-1.28 (m, 12H). ^{13}C NMR (CD_3OD): δ 160.7, 136.0, 130.5, 127.3, 121.6, 115.7, 110.0, 110.0, 109.1, 108.3, 86.1, 85.1, 84.9, 84.7, 83.6, 83.4, 83.1, 82.5, 79.1, 79.1, 79.1, 79.0, 77.5, 72.7, 71.3, 71.2, 70.7, 70.5, 69.2, 69.0, 64.6, 62.8, 33.5, 30.8, 30.7, 30.6, 30.6, 30.4, 27.3. HRESI-MS *m*/*z* calculated for $[M+Na]^+$ $C_{42}H_{68}O_{22}Na$: 947.4095. Found: 947.4101.

1.3. Production and purification of His_6-GlfT2

Production and purification of His_6-GlfT2 were performed as reported in the literature.[1] Purity of the isolated GlfT2 was analyzed by SDS-PAGE followed by Coomassie Brilliant Blue staining. A representative gel image is shown in Figure S1.

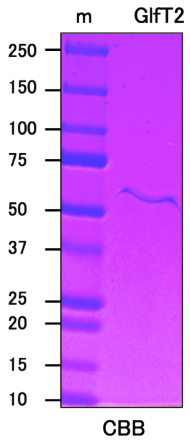


Figure S1 CBB stain gel image of the isolated GlfT2.

1.4. GlfT2-catalyzed polymerization of the oligosaccharide acceptors

Polymerization reactions consisted of 30 µL total volume containing final concentrations of 0.2 µM His_6-GlfT2, 40 µM acceptor, 2.0 mM UDP-Gal*f* in 50 mM Hepes, pH 7.0, 25 mM $MgCl_2$, and 100 mM NaCl. Reactions were incubated at room temperature for 20 h, then quenched with 30 µL of a 1:1 mixture of $CHCl_3$/MeOH. Quenched reaction mixtures were evaporated to dryness under vacuum in a SpeedVac SC100 (Varian) then resuspended in 30 µL of 50% MeCN in MQ water for MALDI MS analysis. Samples for MALDI MS analysis were spotted as a 1:3 mixture with α-cyano-4-hydroxycinnamic acid matrix and spectra were recorded in positive linear mode using a Bruker Ultraflex III mass spectrometer.

1.5. Continuous assay to measure UDP production by His_6-GlfT2

In a quartz cuvette, the following were mixed to give a total volume of 120µL: buffer (50 mM Hepes, 25 mM $MgCl_2$, and 100 mM NaCl (pH 7.0); diluted from a 10X stock), 300 units of pyruvate kinase (Sigma), 20 units of lactate dehydrogenase (Sigma), 250 µM NADH, 500 µM phosphoenolpyruvate, and 0.2 µM His_6-GlfT2.

Absorbance at 340 nm was monitored over time in a Cary 50 Bio UV-Visible Spectrophotometer (Varian) until a steady baseline was reached (usually 2 min), then UDP-Gal*f* was added to 1.25 mM. Absorbance at 340 nm was again monitored, then acceptor substrate was added to the desired concentration (Table S1). Absorbance at 340 nm was monitored over time. The steady-state rate was calculated from the slope of the linear portion of the decrease in absorbance over time by using $\varepsilon = 6{,}300\ M^{-1}\cdot cm^{-1}$ for NADH.[1]

For determination of the kinetic parameters, data were fit to the Michaelis–Menten equation,

$$v = (V_{max} \cdot S)/(K_m + S),$$

where the value of v is the observed rate and S is the corresponding acceptor substrate concentration. Because products of elongation of the acceptor substrate serve as substrates for additional elongation during the polymerization reaction, we used the parameter K_m to refer to the initial concentration of acceptor substrate that results in half-maximal steady-state velocity.

For the time course experiment shown in Figure 6, 200 μM acceptor substrates were used.

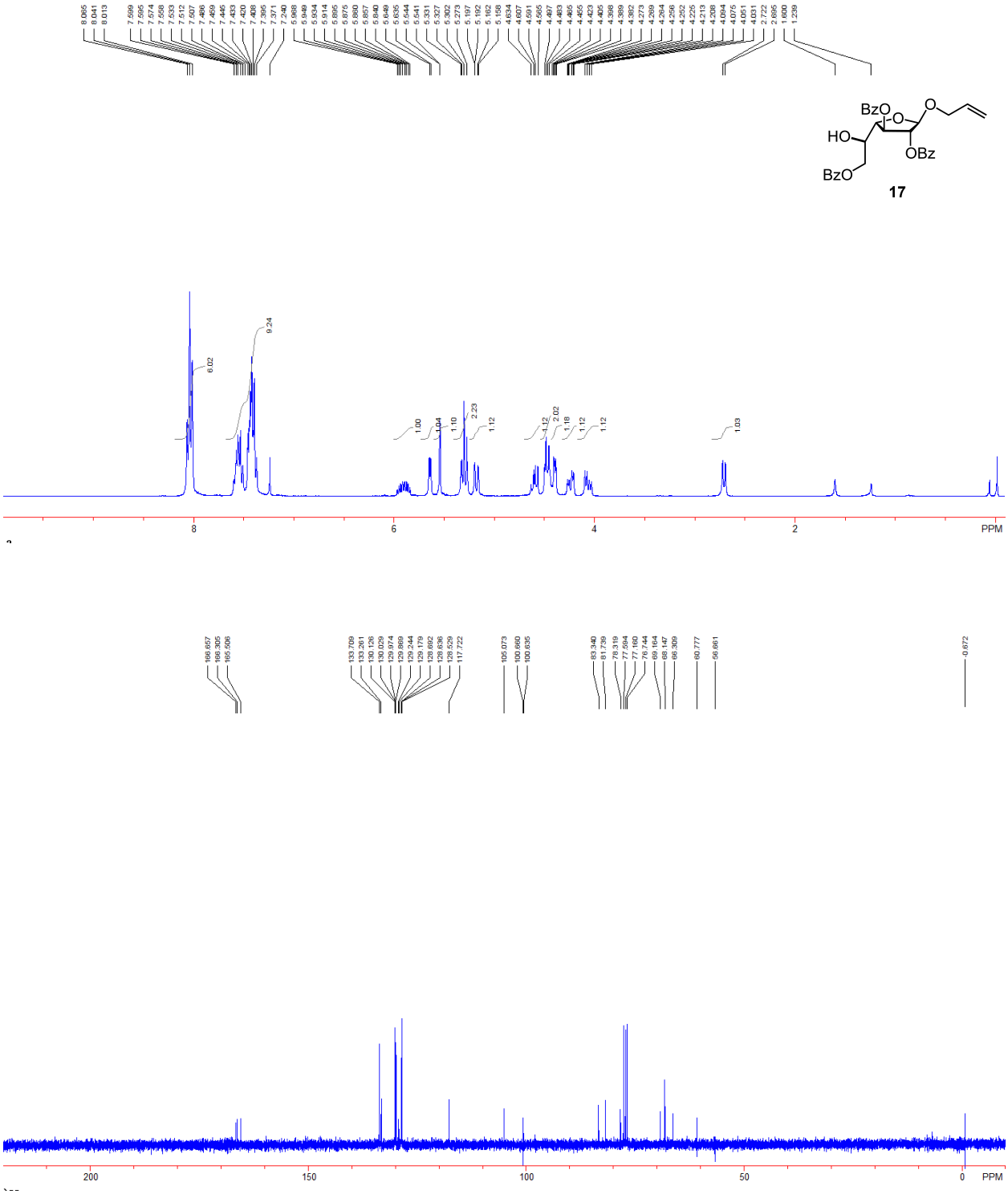
Table S1. A list of acceptor substrate concentration used to determine the kinetic parameters.

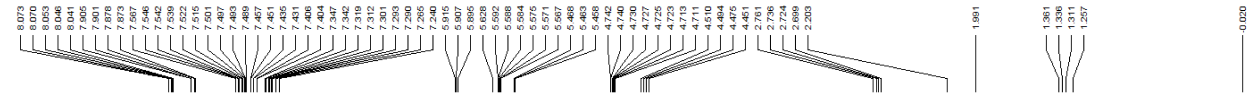
Acceptor	Concentration (μM)
4 Gal*f*β(1-5)Gal*f*(1-6)Gal*f*	2.5 / 5 / 10 / 20 / 40 / 80
5 Gal*f*β(1-6)Gal*f*(1-5)Gal*f*	100 / 200 / 400 / 800 / 1600
6 Gal*f*β(1-6)Gal*f*(1-6)Gal*f*	5/ 10 / 20 / 35 / 50 / 100
7 Gal*f*β(1-5)Gal*f*(1-5)Gal*f*	12.5 / 25 / 50 / 100 / 200
8 Tetrasaccharide	5 / 10 / 20 / 40 / 80 / 200

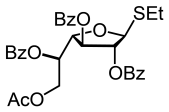
1.6. References

(1) May, J. F.; Splain, R. A.; Brotschi, C.; Kiessling, L. L. *Proc. Natl. Acad. Sci. U.S.A.* **2009**, *106*, 11851.
(2) Chittenden, G. J. F. *Carbohydr. Res.* **1972**, *25*, 35.

2. Spectral data



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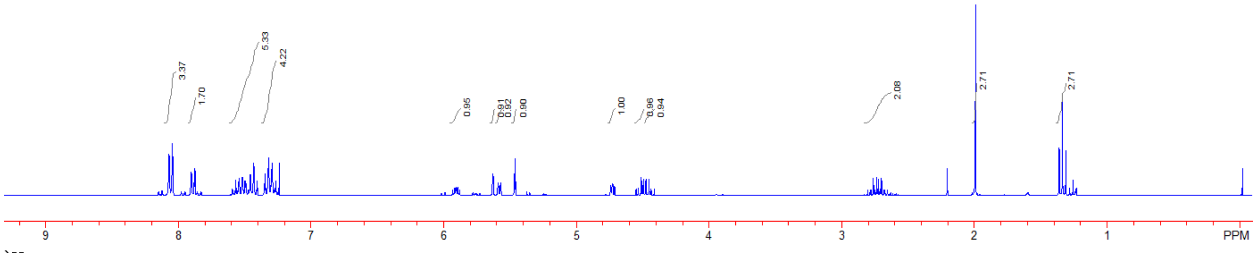
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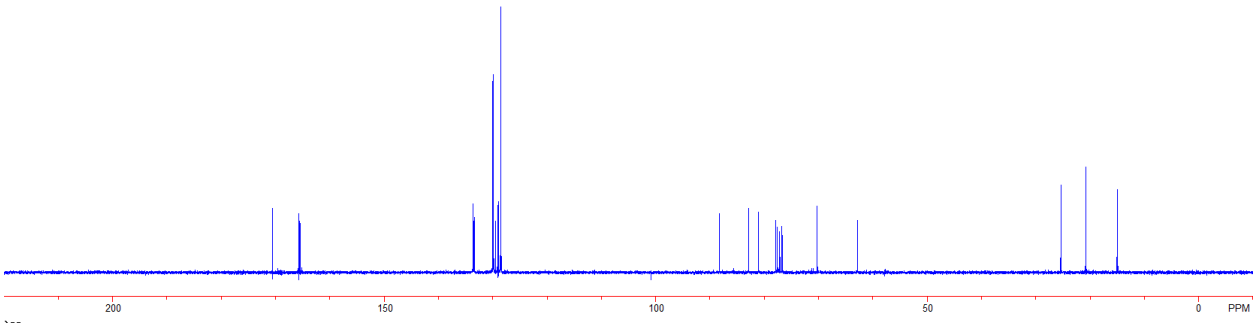
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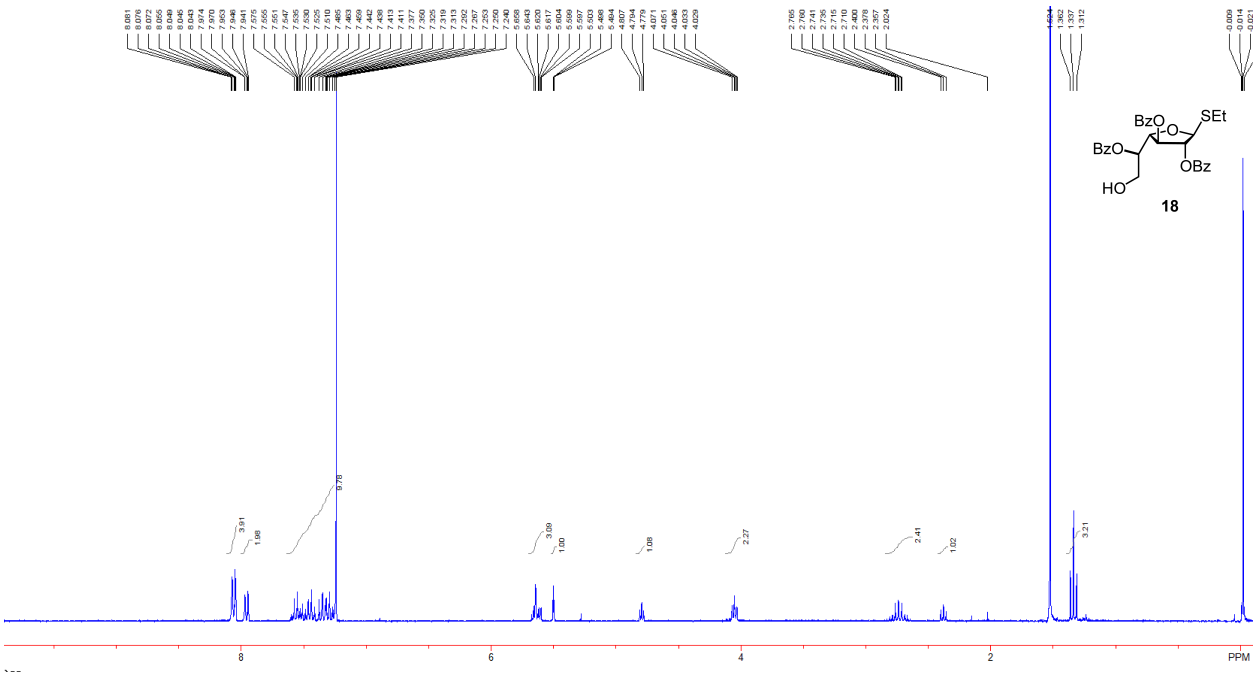
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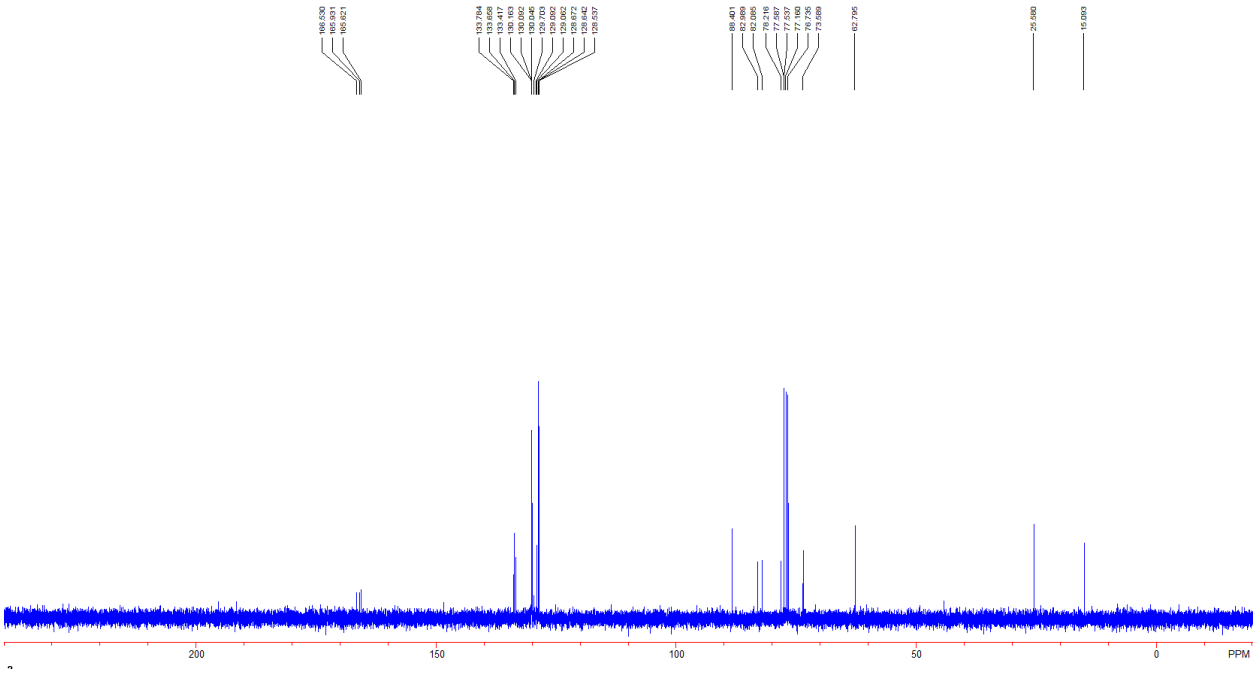
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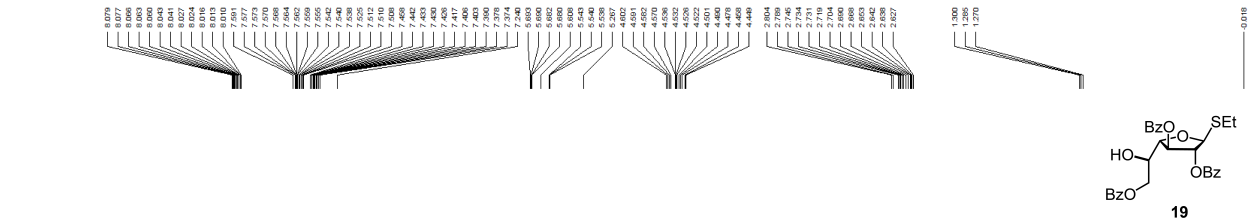

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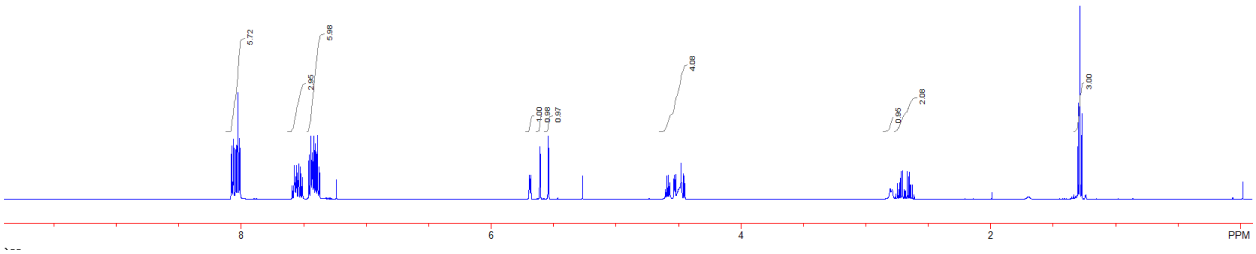
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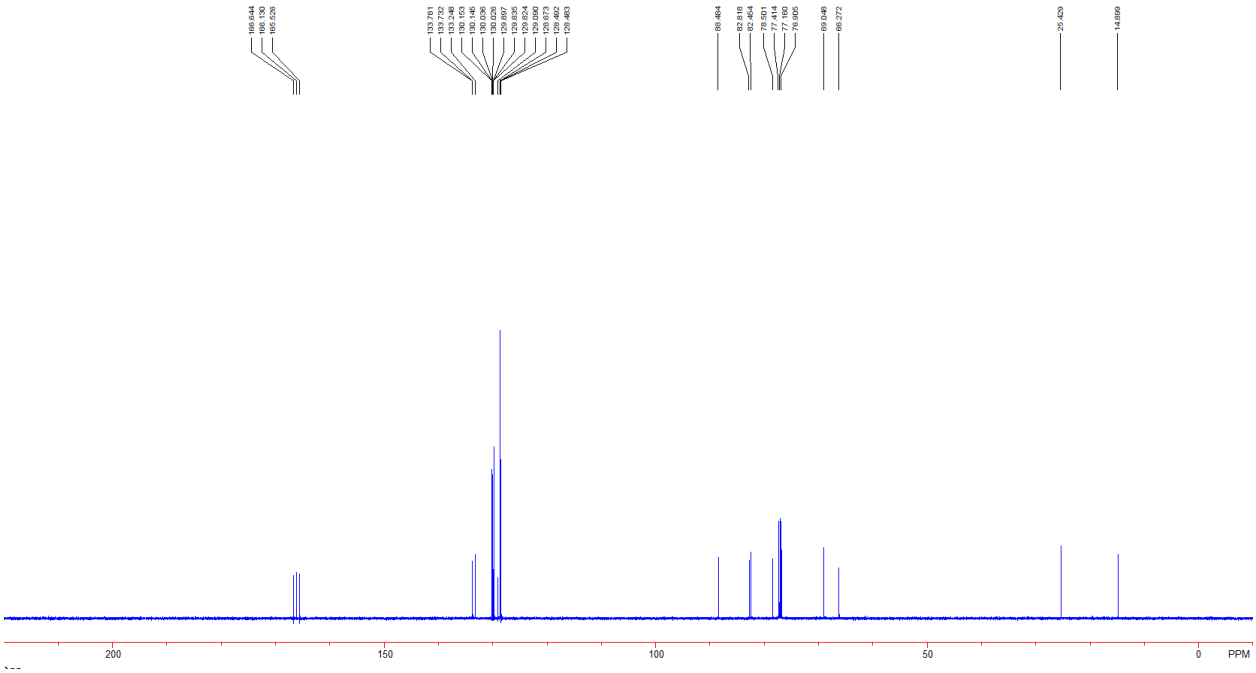
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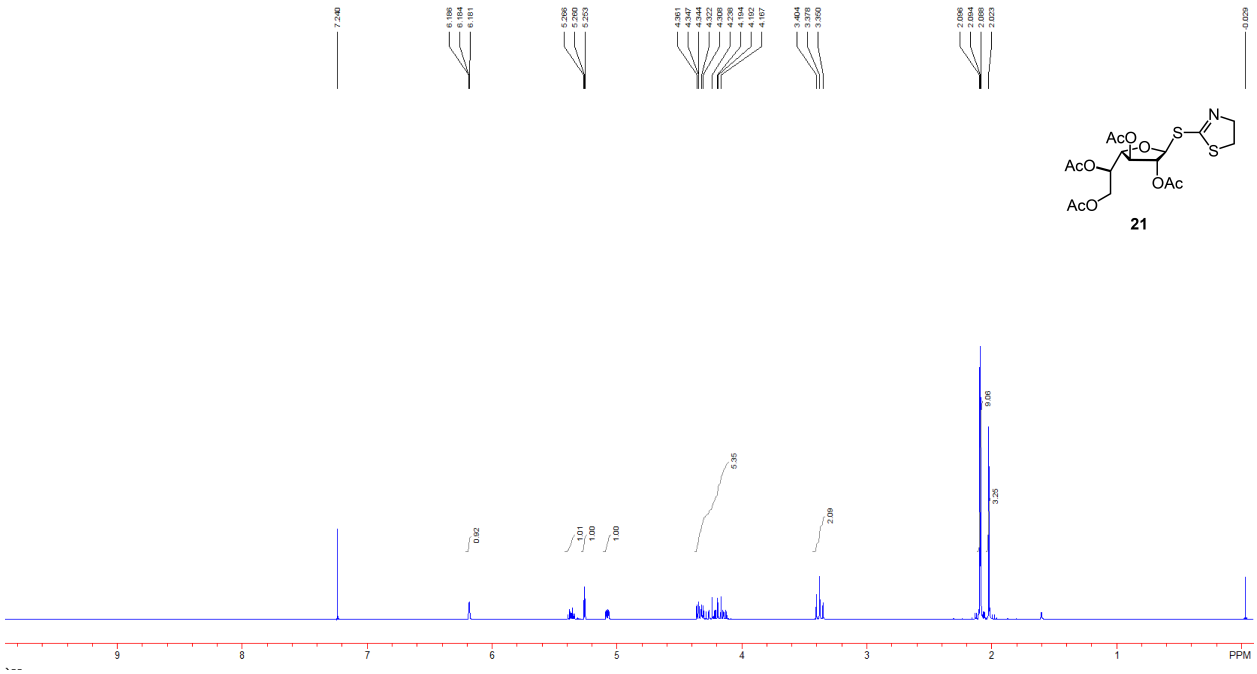
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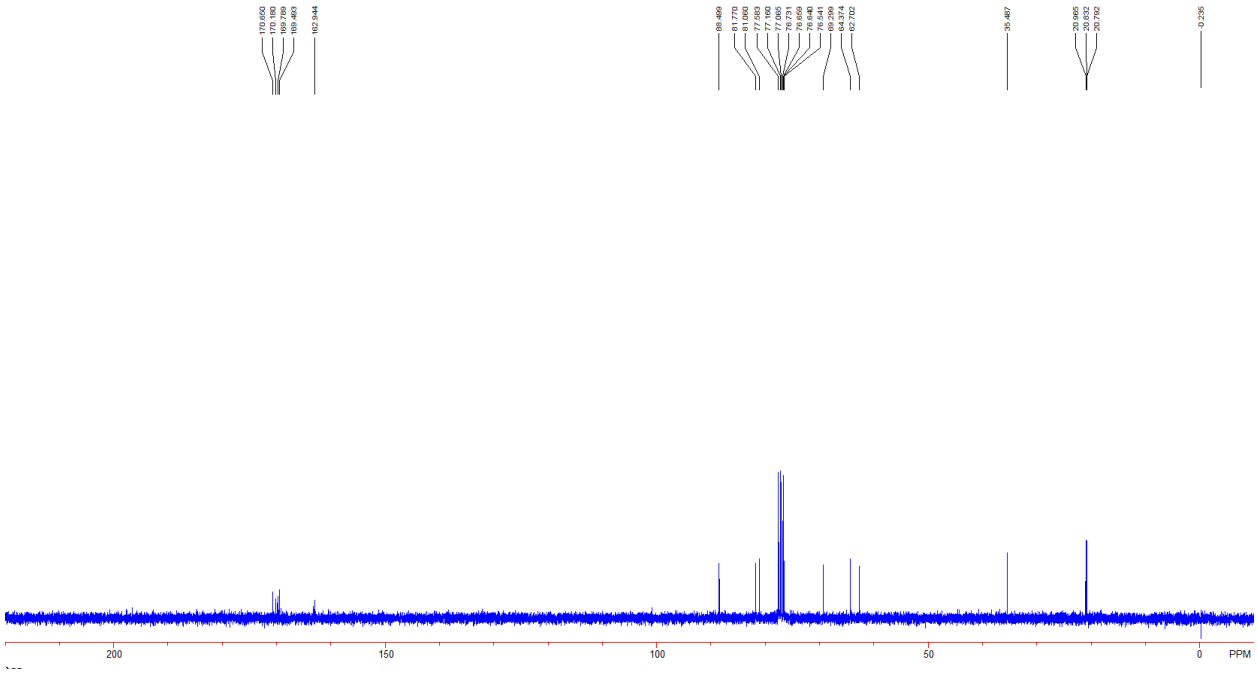
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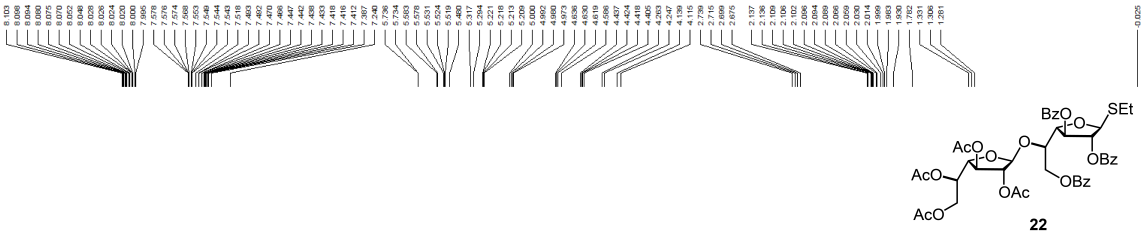
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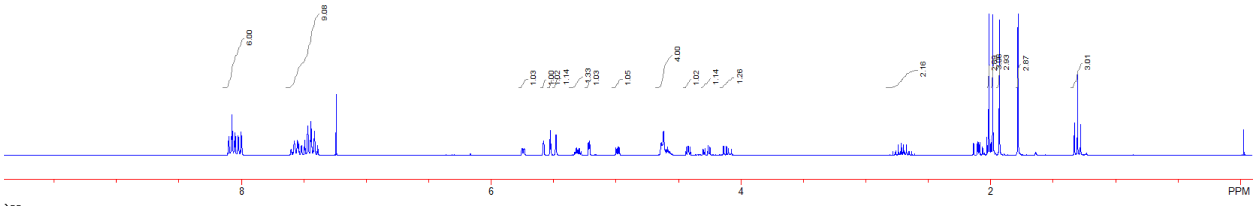
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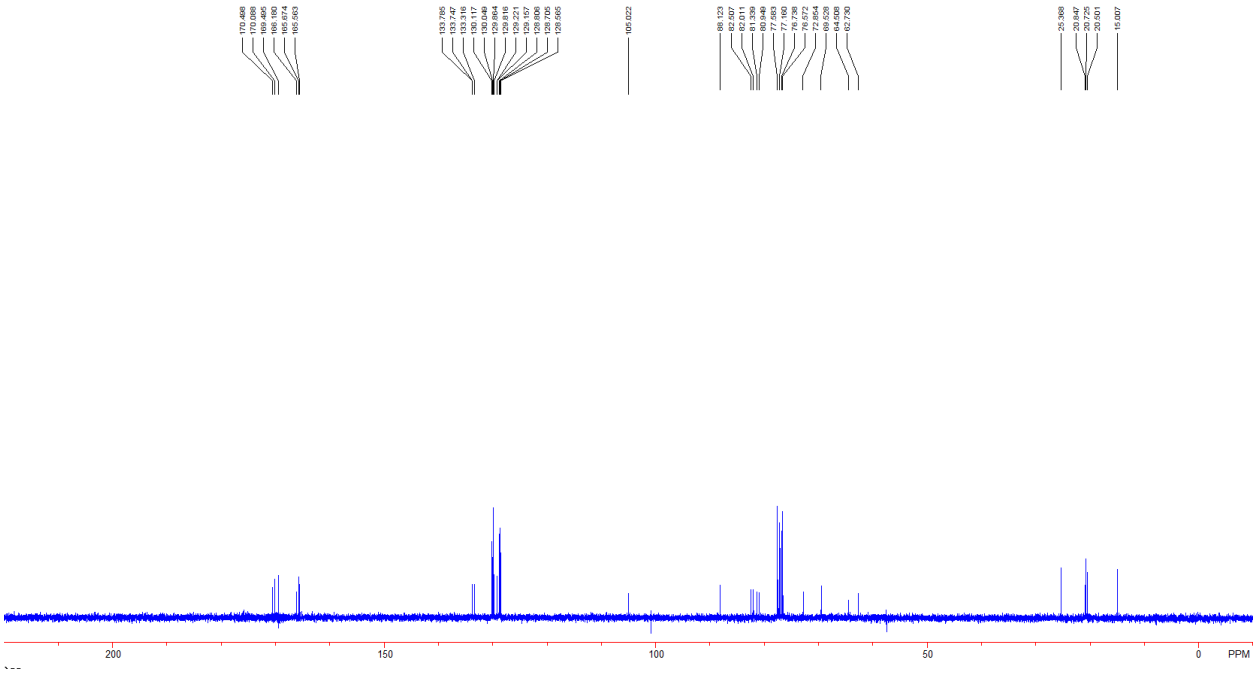
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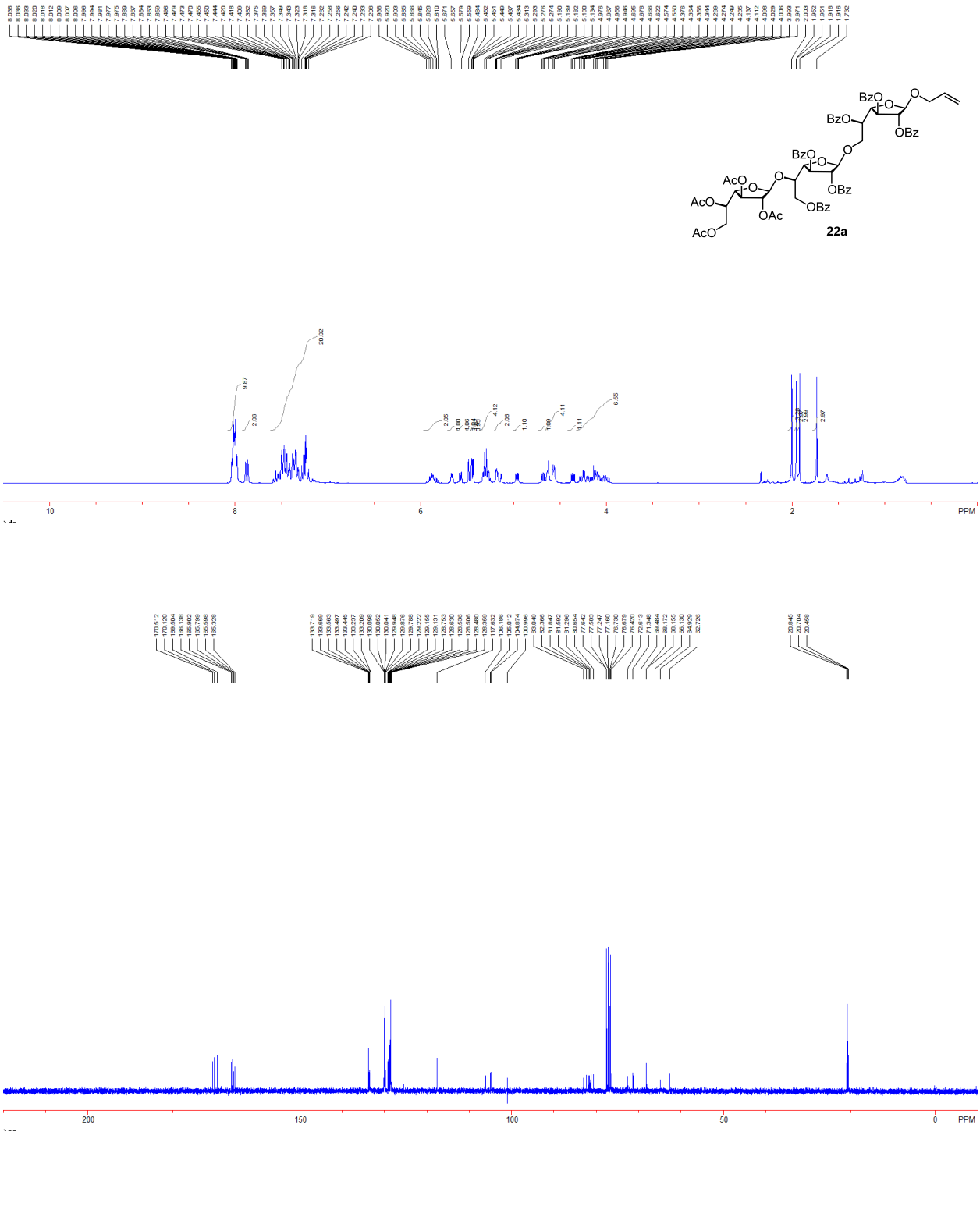
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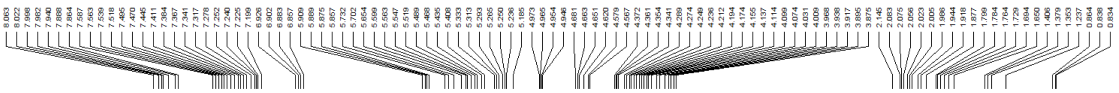

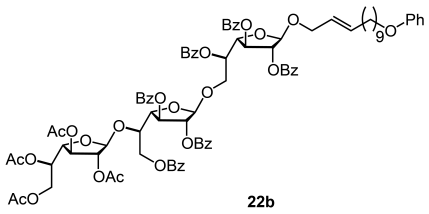
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OBz
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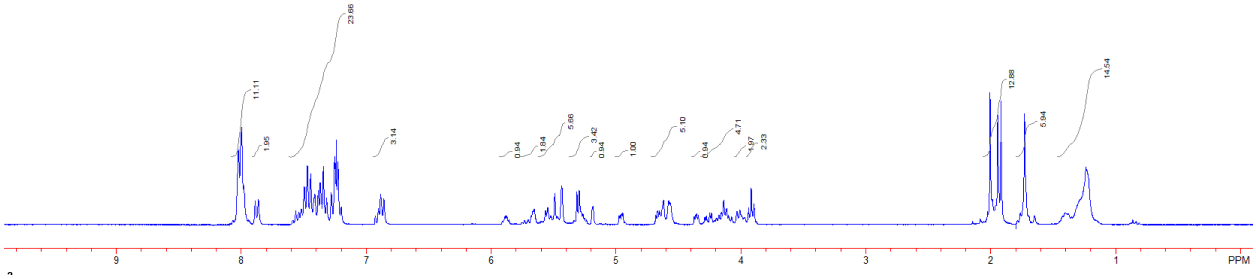
OBz
AcO
AcO
OBz
OAc
AcO
Ph
g

22b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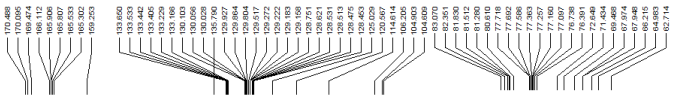
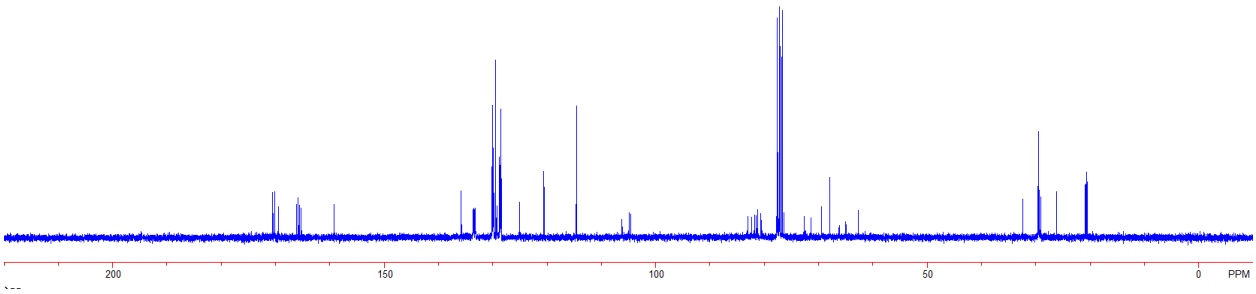
14.54
PPM

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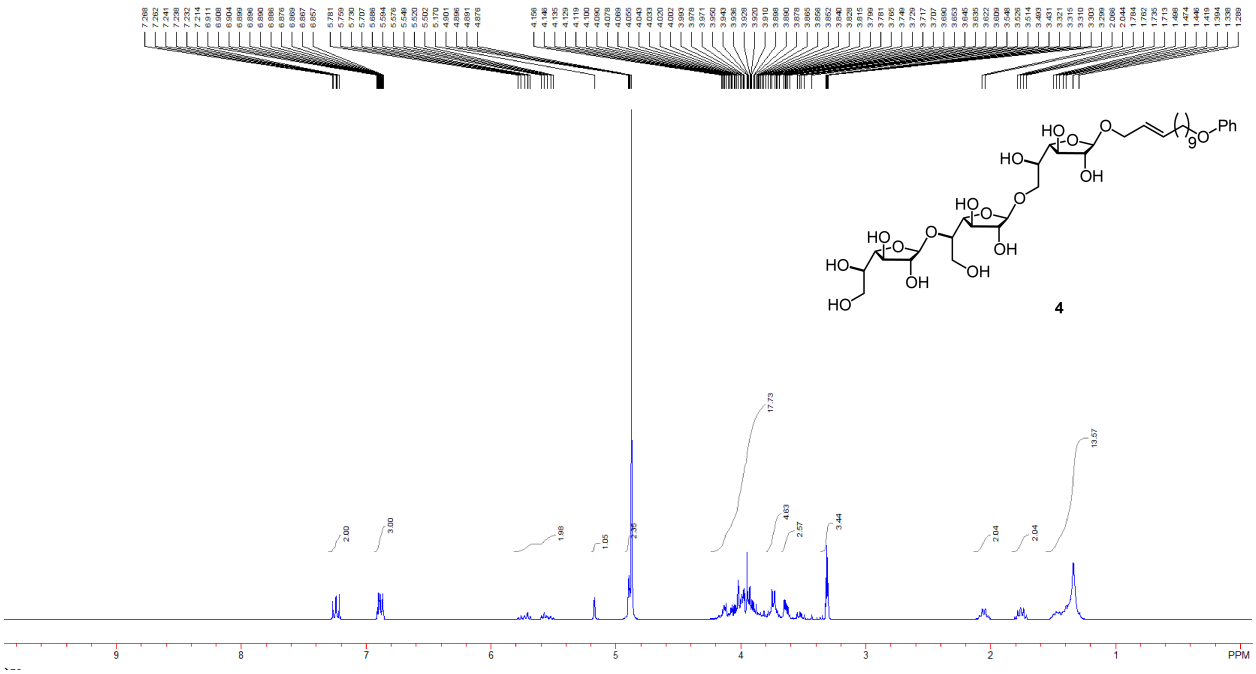
HO
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g
O
Ph
OH
HO
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OH
HO
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HO
OH
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3.00
1.98
1.05
2.35
17.73
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13.57
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7
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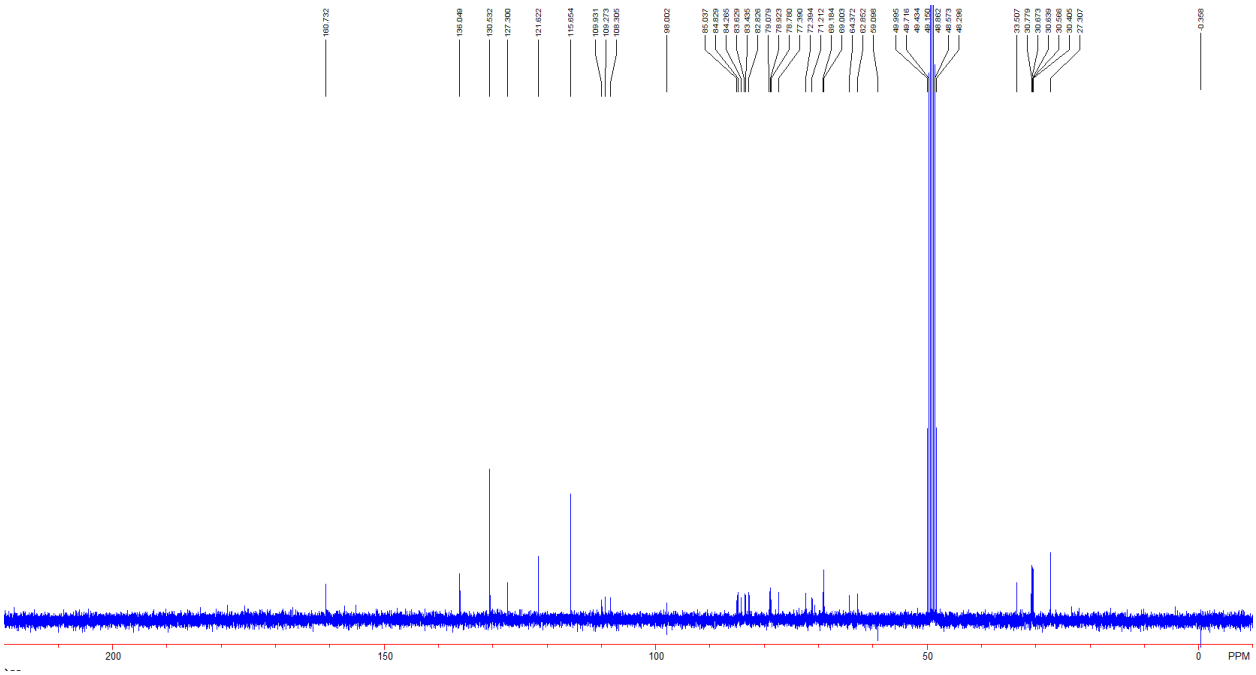
1
PPM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82.829
82.709
78.760
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77.264
71.212
69.184
69.003
64.472
62.952
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49.966
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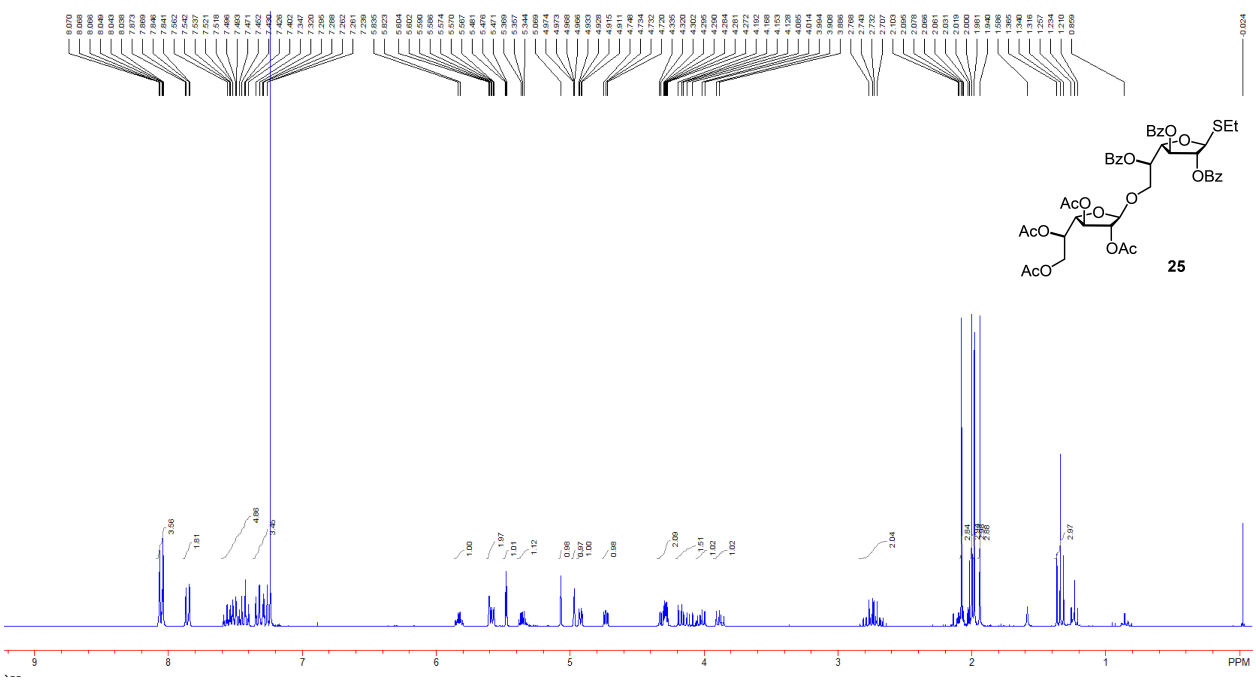
BzO
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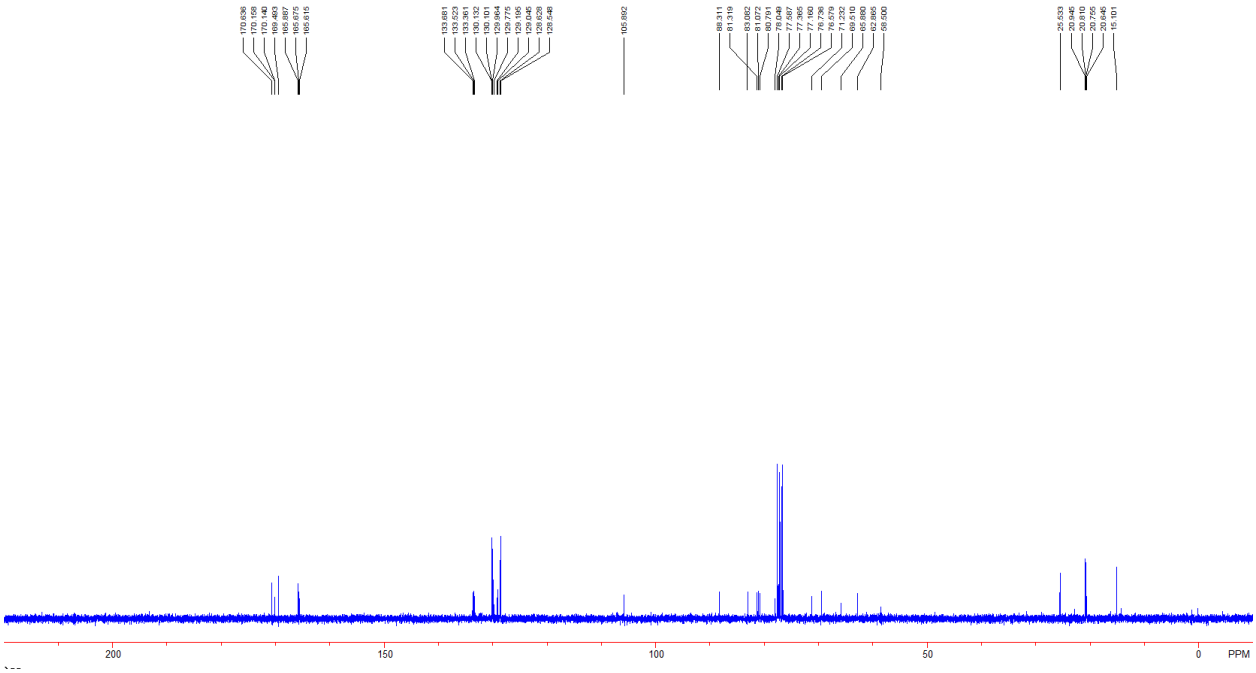
OAc
AcO
25
PPM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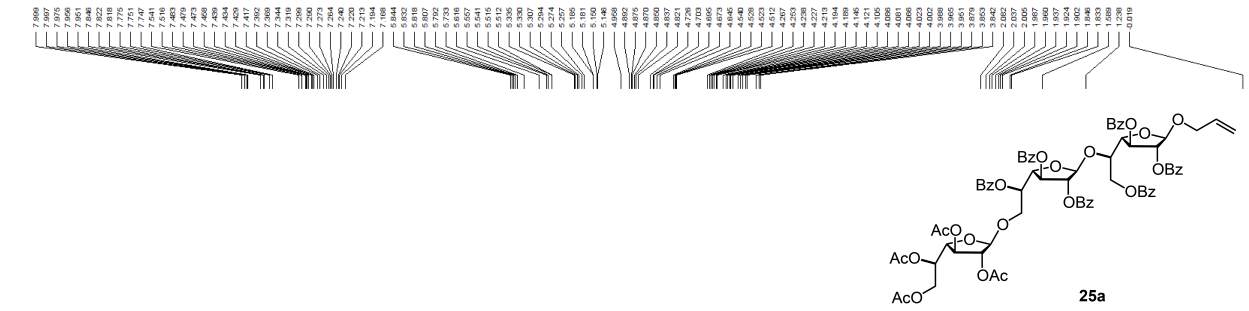
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BzO
OBz
OBz
OBz
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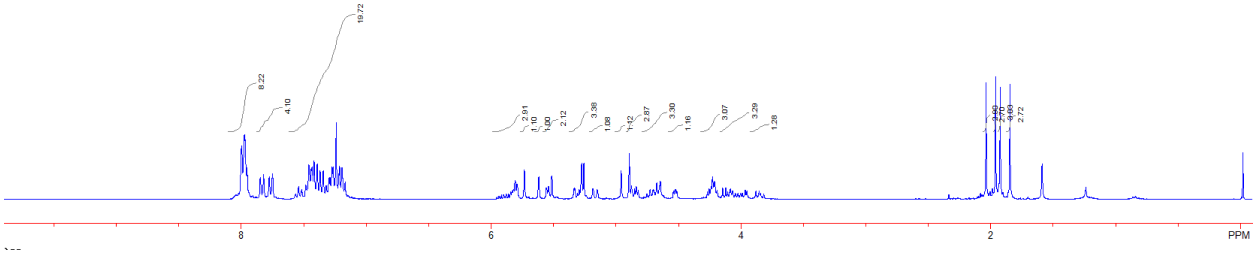
AcO
AcO
OAc
25a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1.00
2.12
3.38
1.08
1.12
2.87
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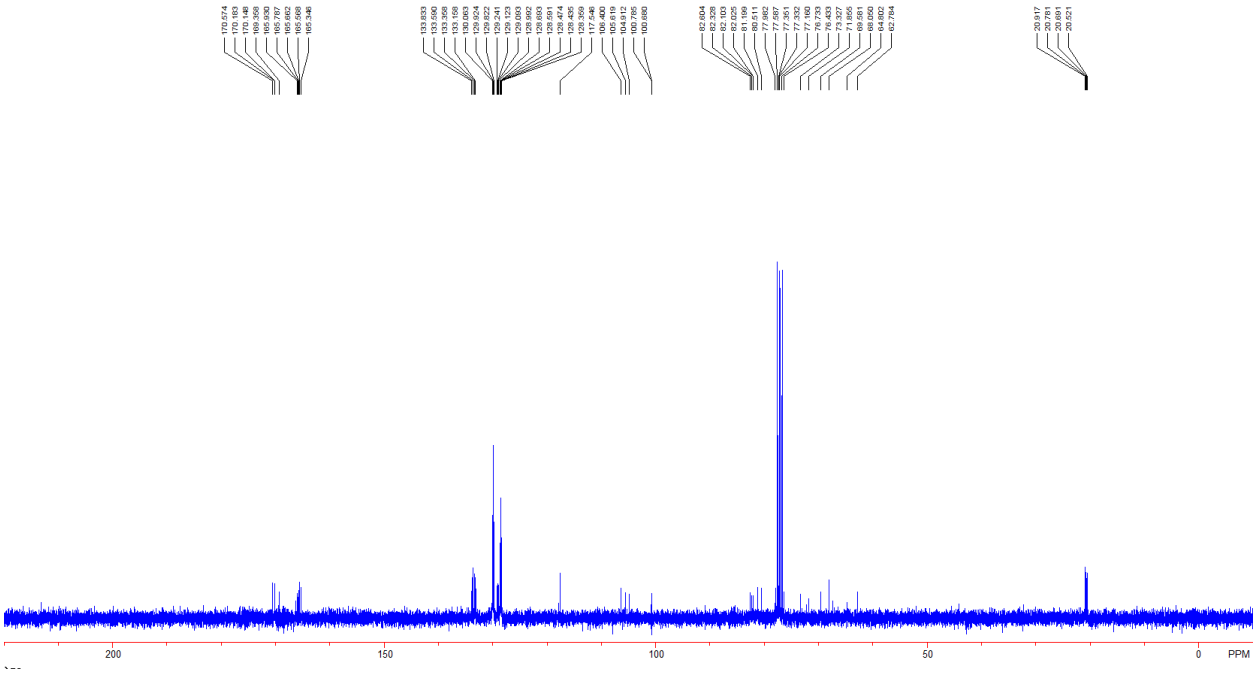
1.16
3.07
3.29
1.26
2.70
2.70
4.93
2.72
8
6
4
2
PPM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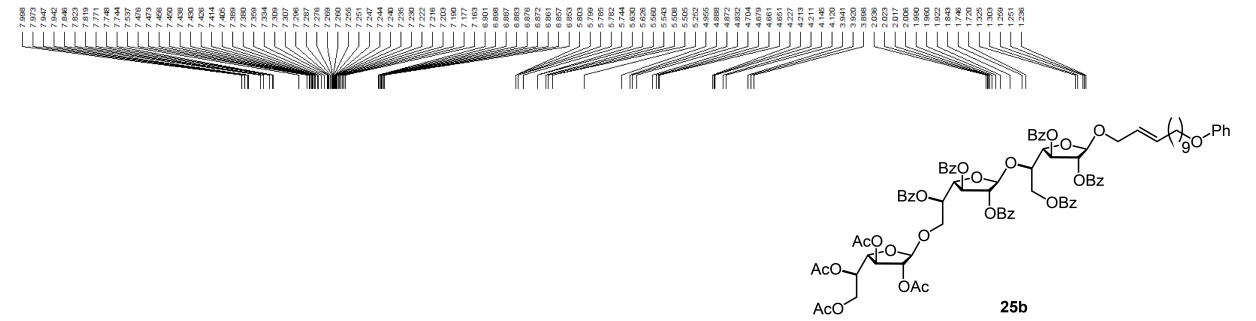
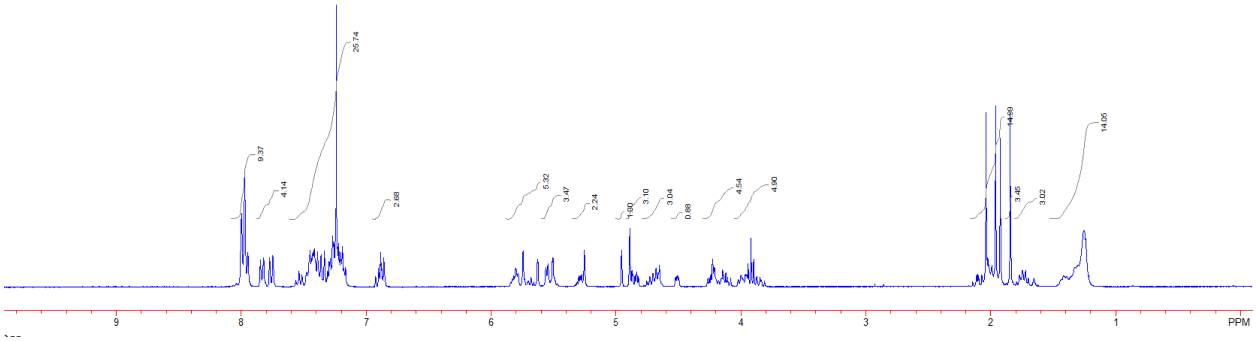
PPM



BzO
BzO
BzO
OBz
OBz
OBz
AcO
AcO
AcO
OAc
O
Ph
9

25b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1.80
3.10
3.04
0.88
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14.05
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6
5
4
3
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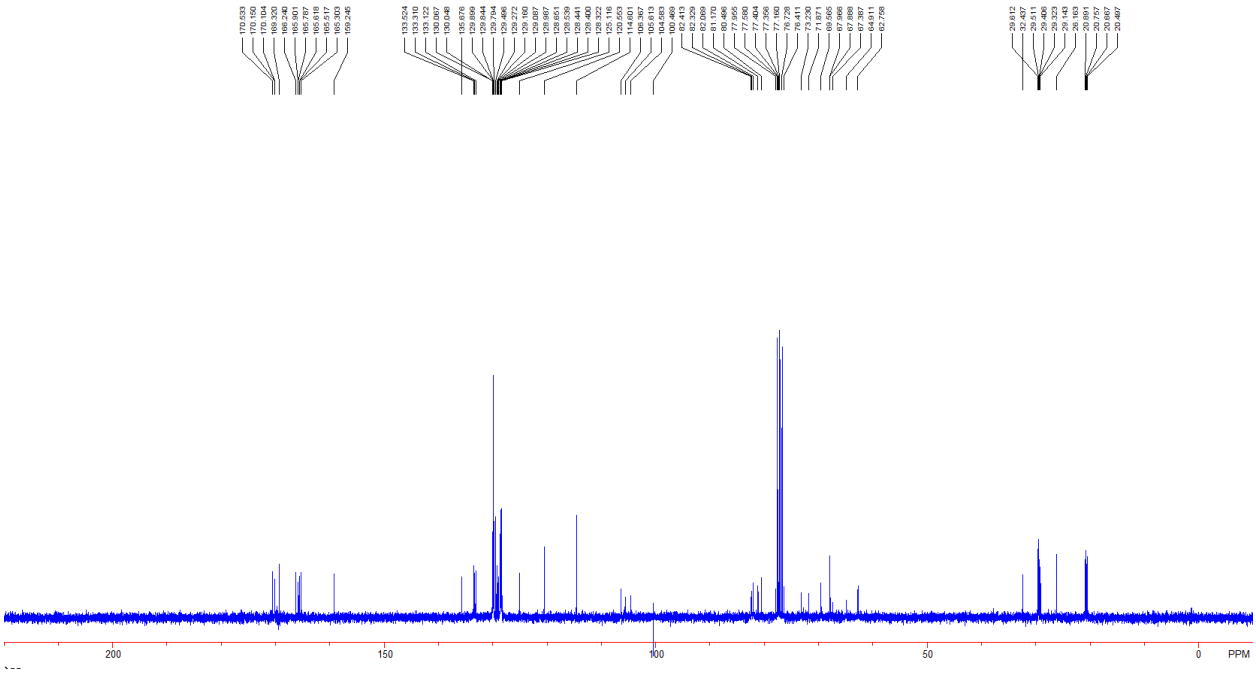
1
PPM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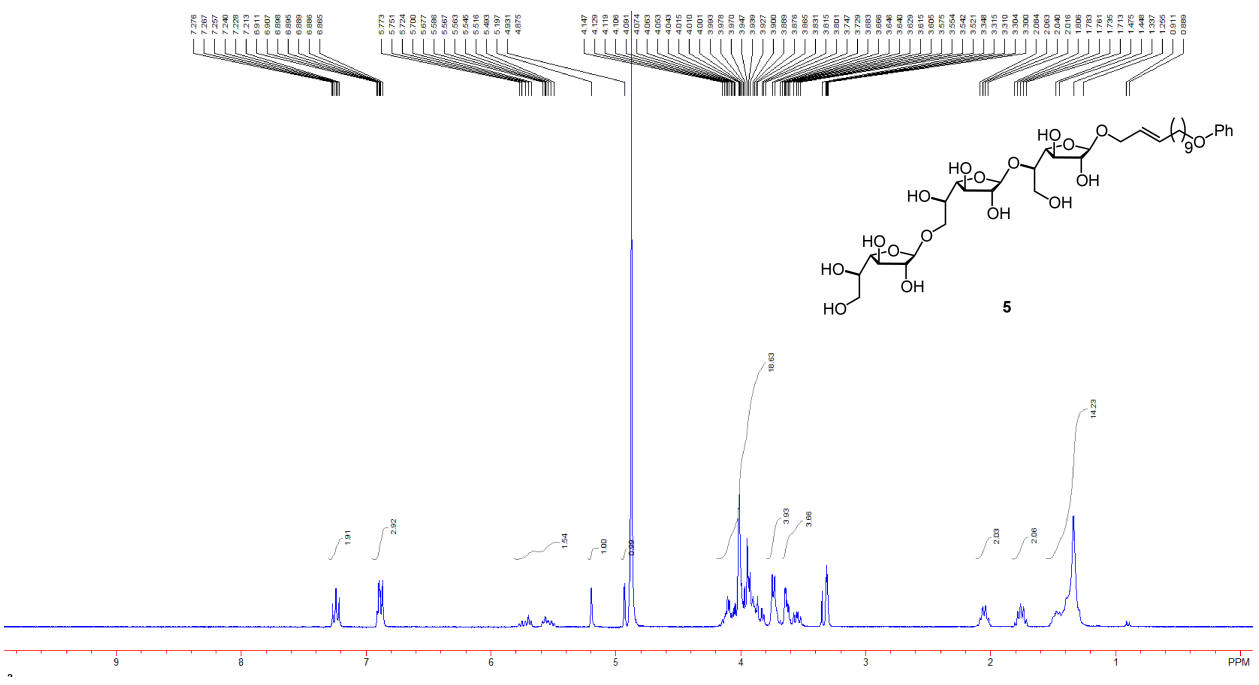
PPM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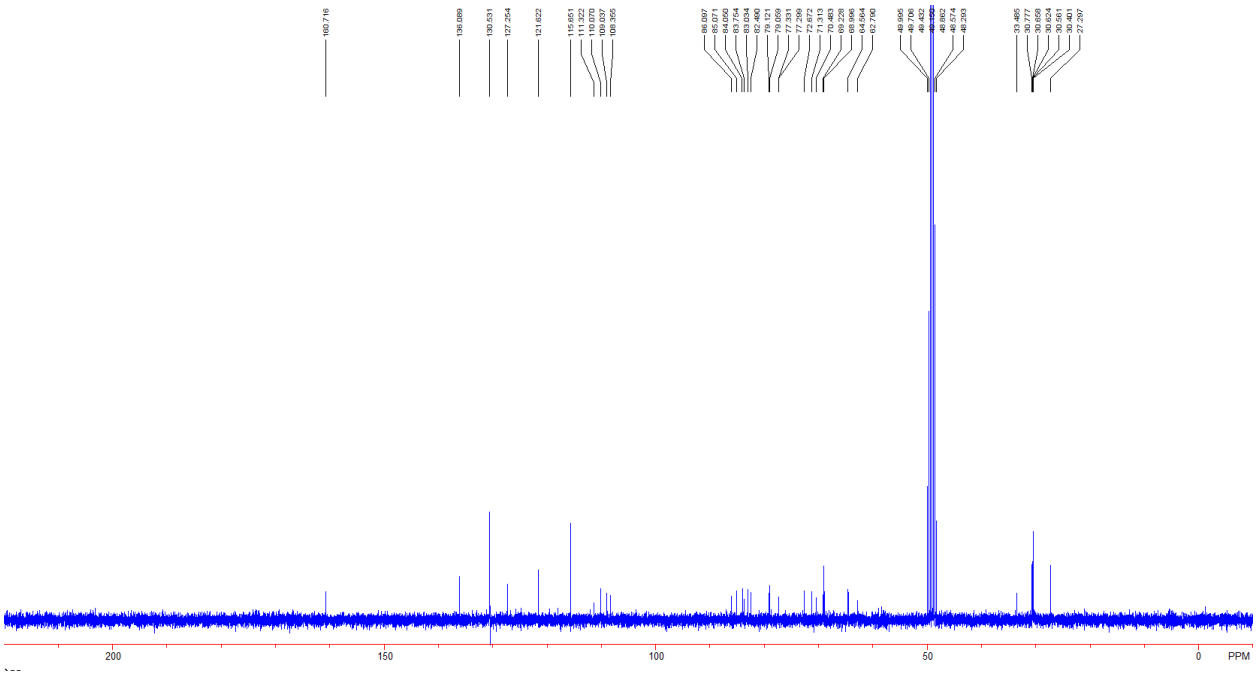
Ph
g
OH
5
PPM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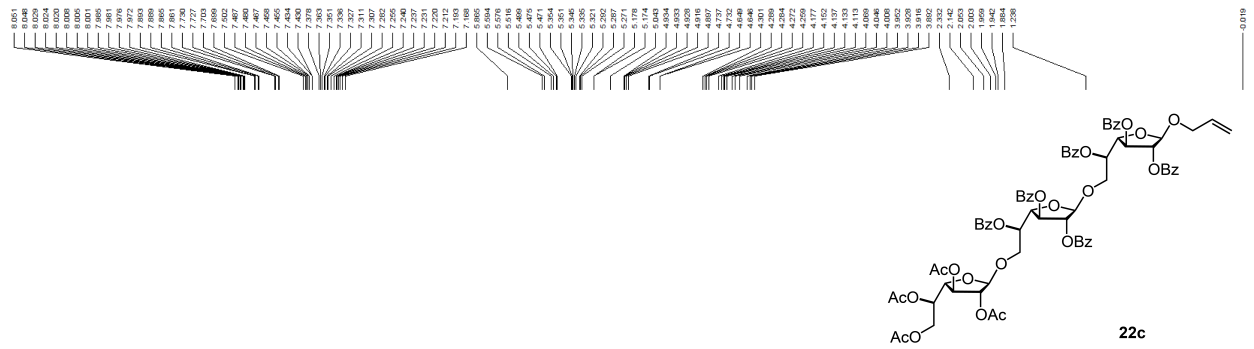
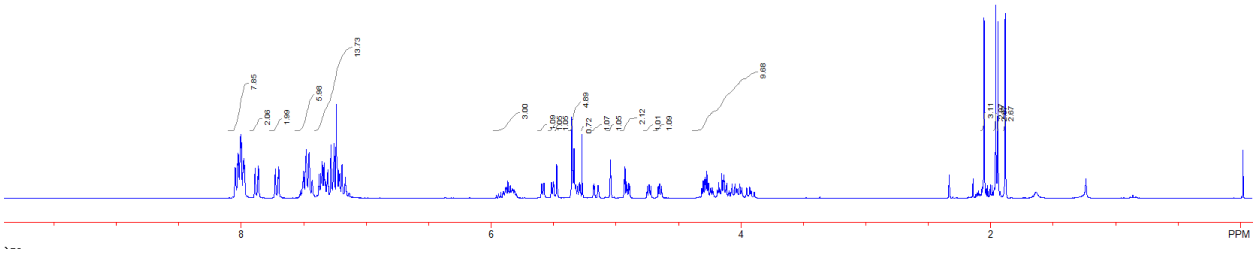

BzO
BzO
OBz
BzO
BzO
OBz
AcO
AcO
OAc
AcO

22c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3.00
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0.72
1.07
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2.12
1.01
1.00
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2.07
2.07
2.07
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6
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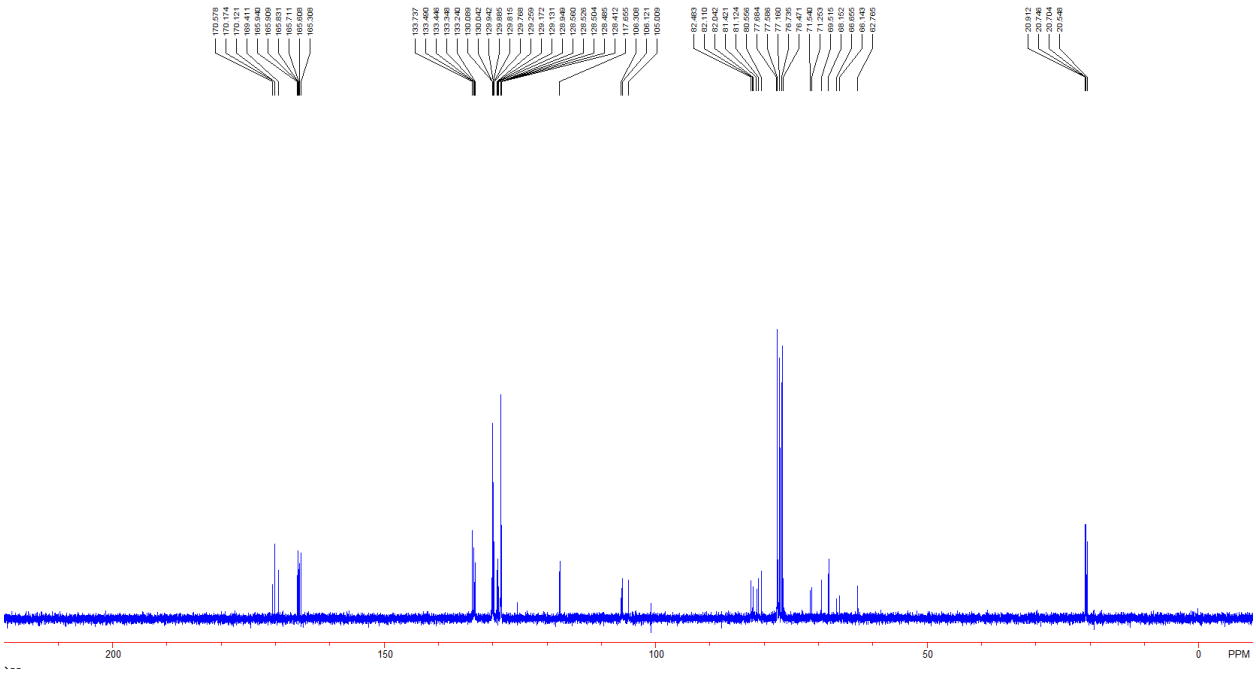
PPM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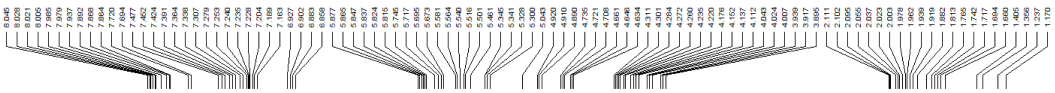
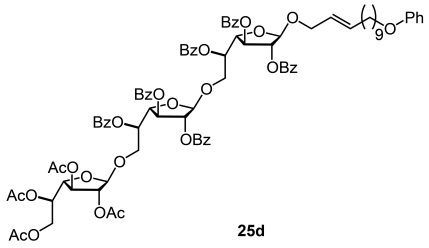
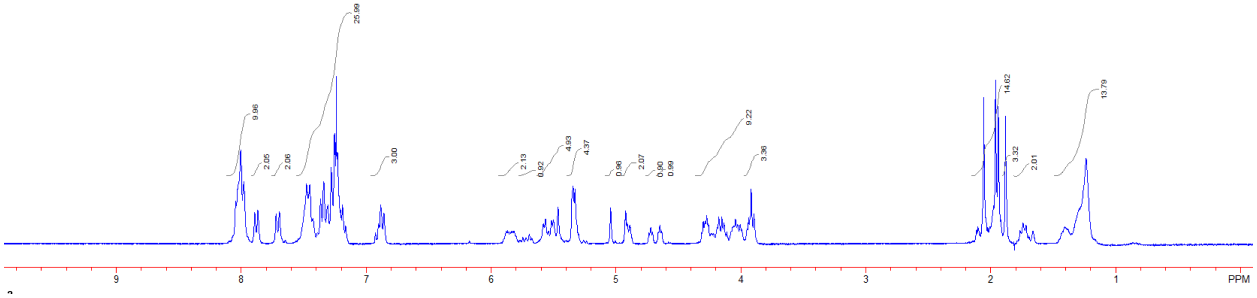



BzO
BzO
BzO
BzO
AcO
AcO
AcO
OBz
OBz
OAc
g
Ph

25d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2.06
3.00
2.13
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4.93
4.87
0.96
2.07
0.90
0.99
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3.36
14.02
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13.79
9
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7
6
5
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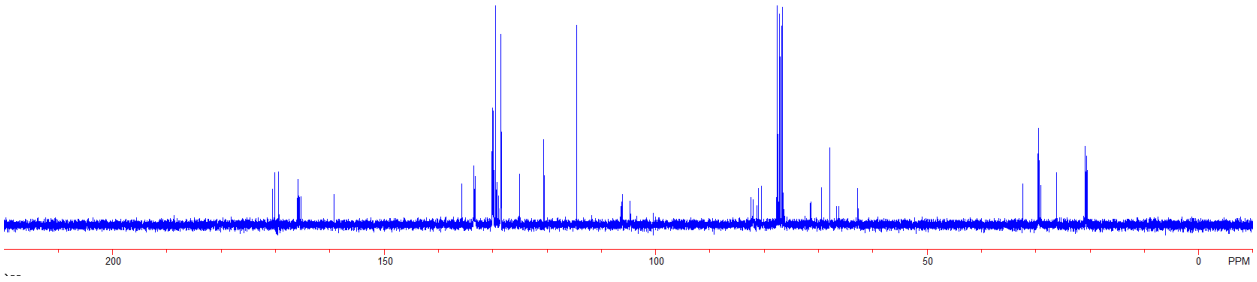
3
2
1
PPM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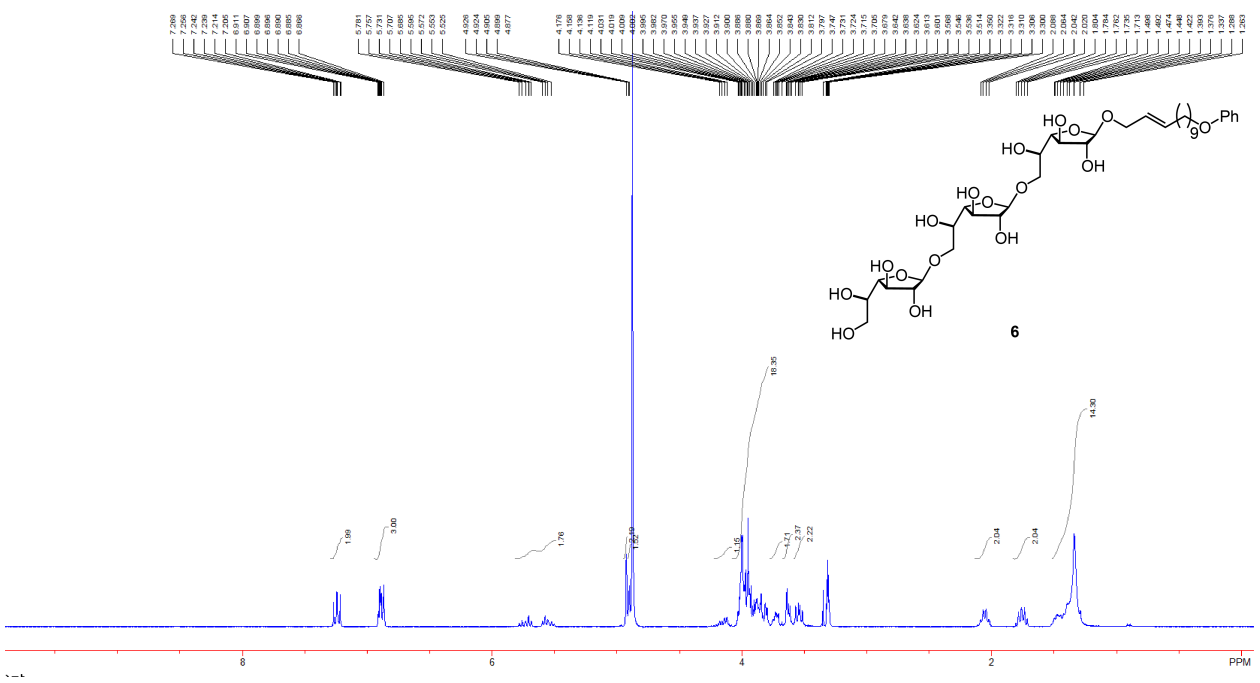
PPM


HO
HO
O
OH
HO
HO
O
OH
HO
HO
O
OH
HO
HO
O
g
O
Ph
6
16.35
14.30
1.99
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2.22
2.04
2.04
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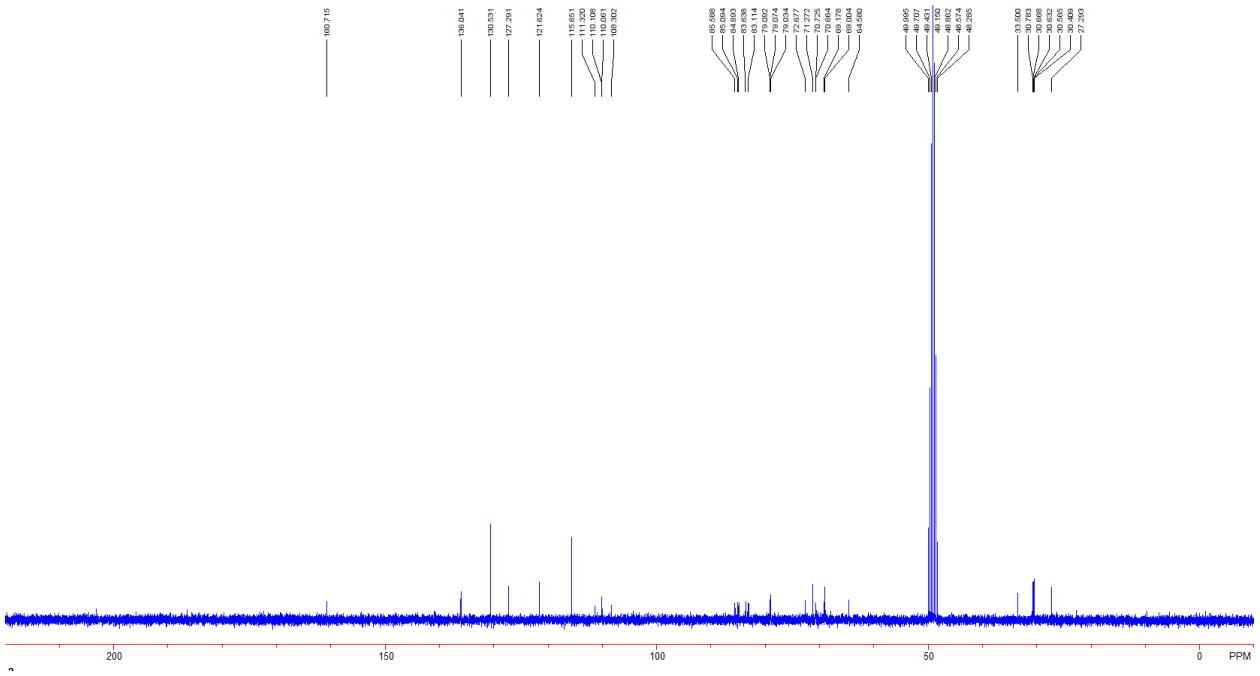
6
4
2
PPM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79.034
77.377
71.272
70.725
70.664
69.076
69.004
64.980
49.995
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49.431
49.155
48.882
48.574
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33.000
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27.393
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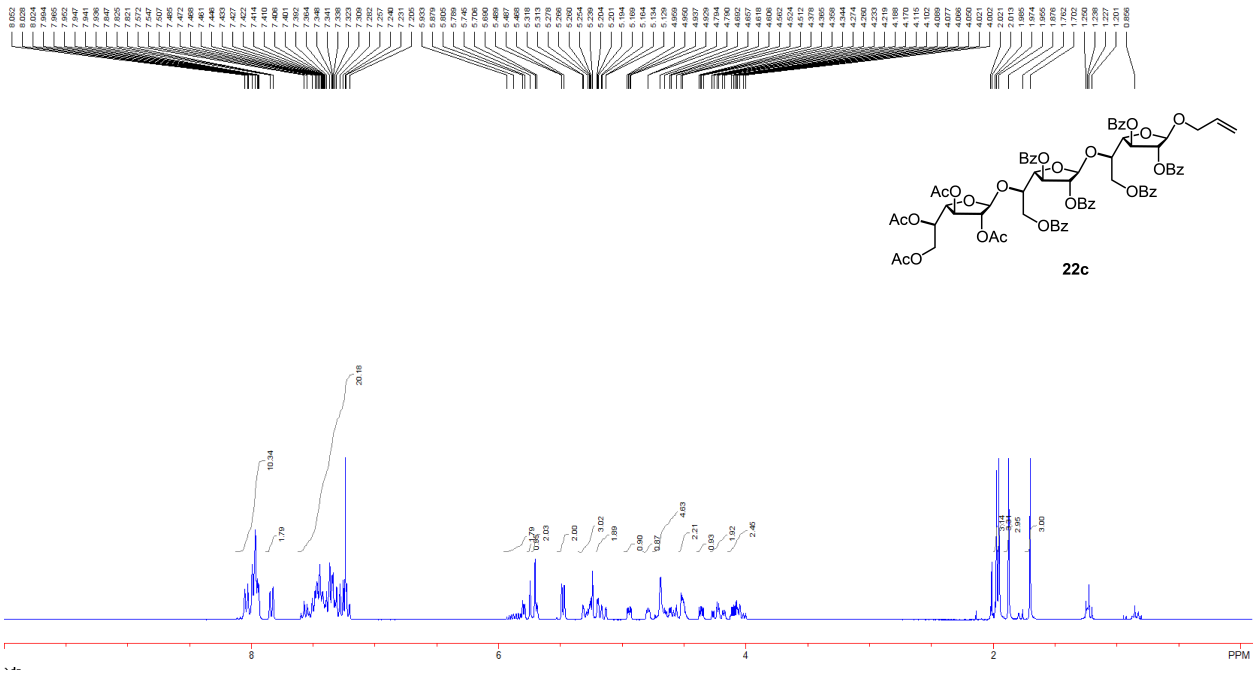
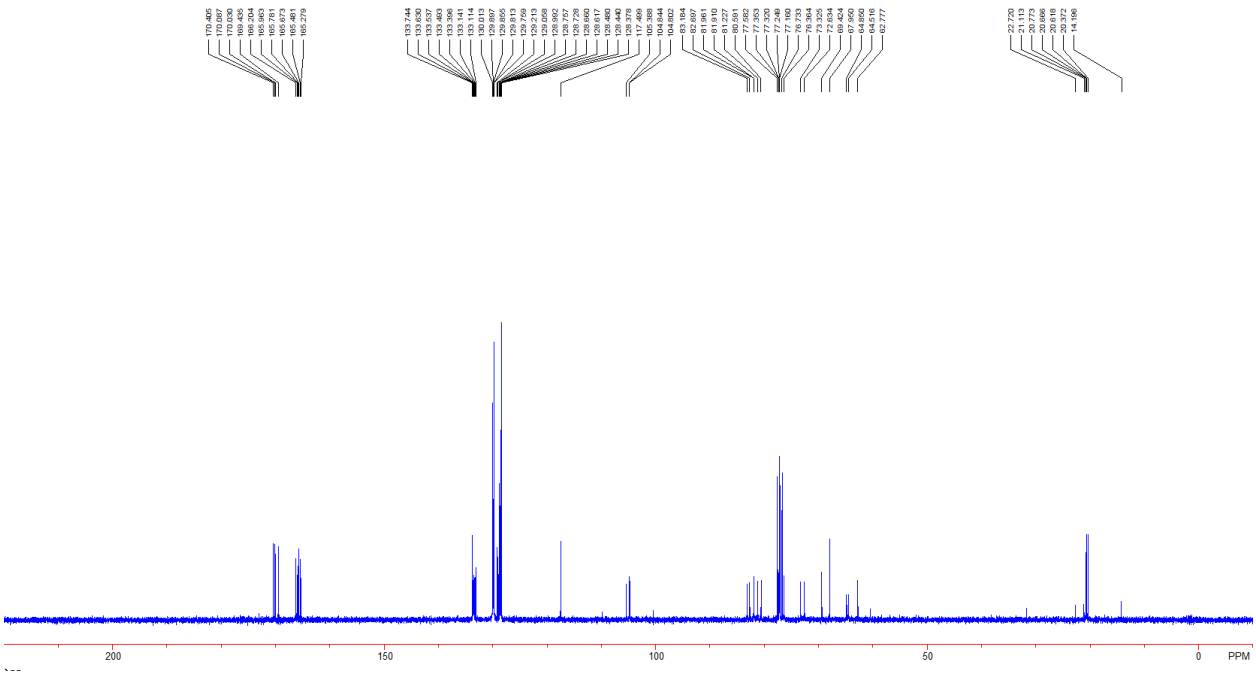
100
50
0
PPM


BzO
BzO
AcO
AcO
AcO
OAc
OBz
OBz
OBz
OBz

22c

20.18
10.34
1.79
3.02
1.89
4.63
2.21
3.00
PPM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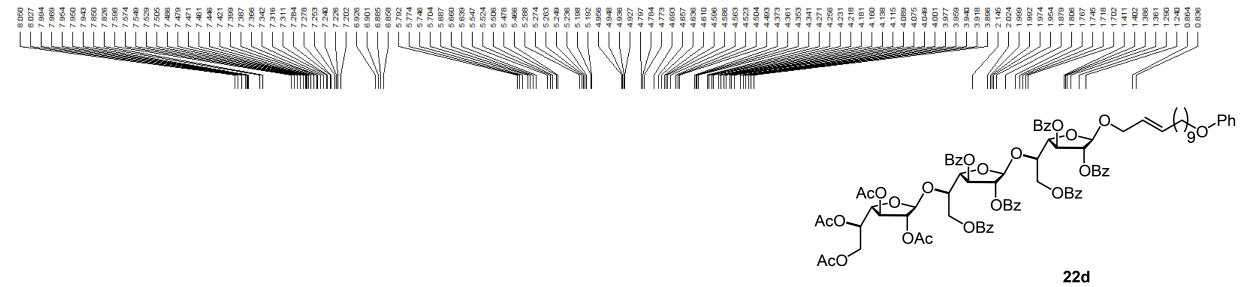
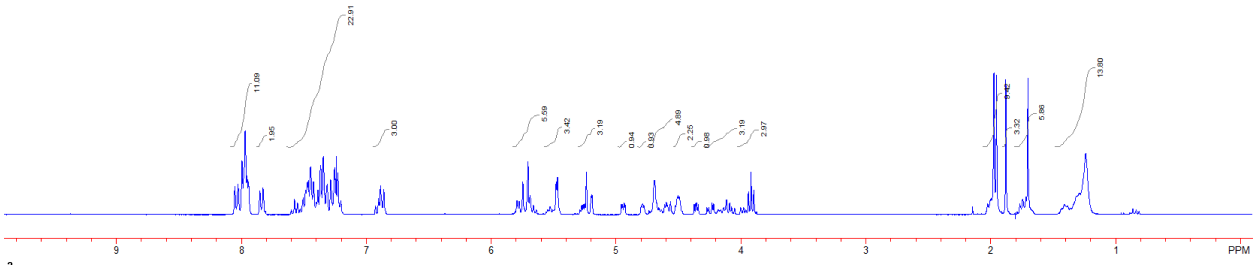

BzO
AcO
OAc
OBz
Ph
22d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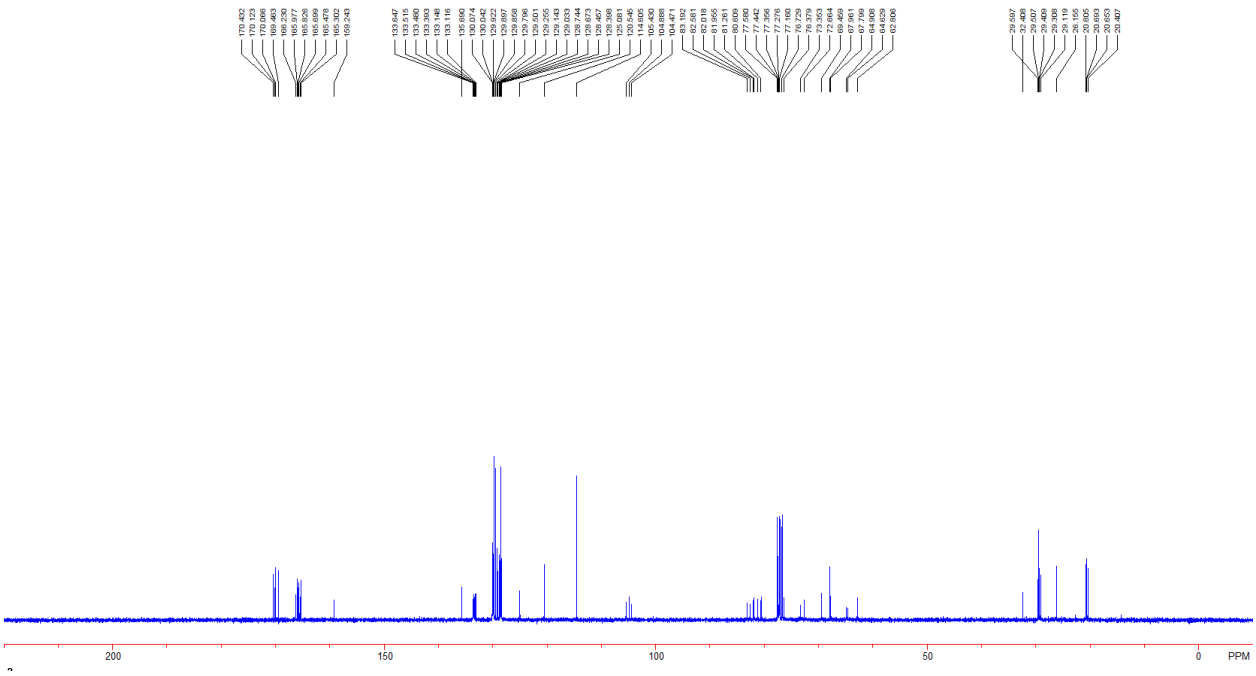
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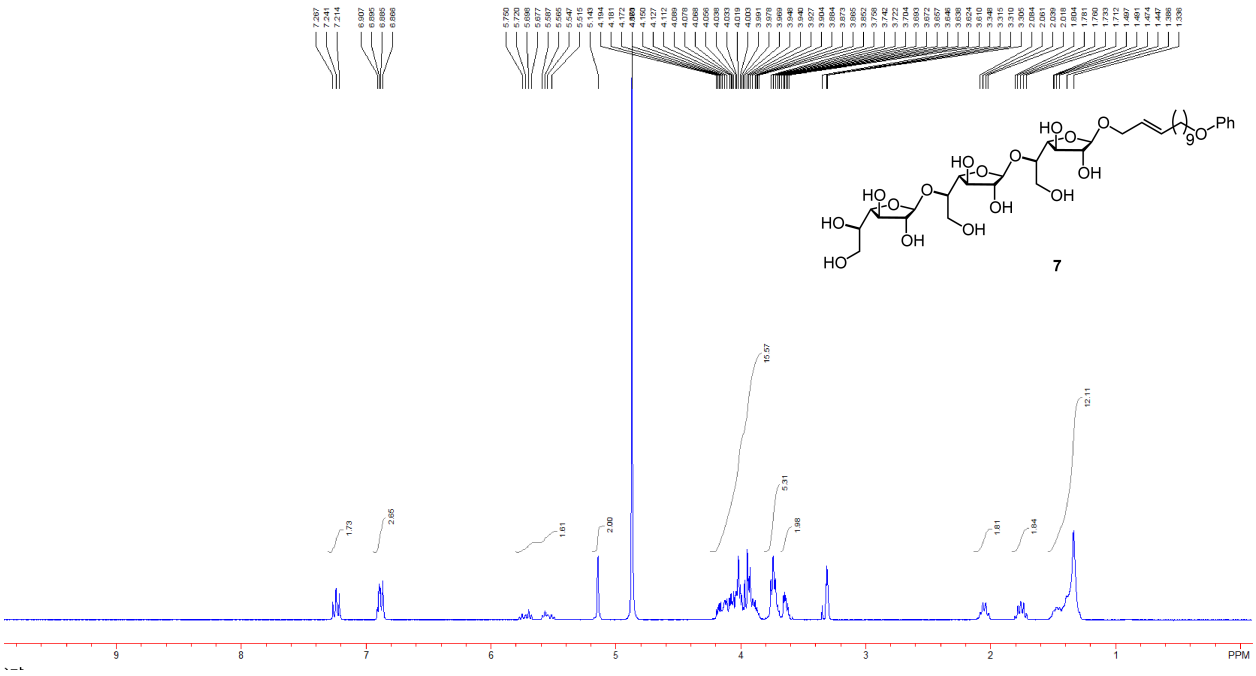
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Ph
g
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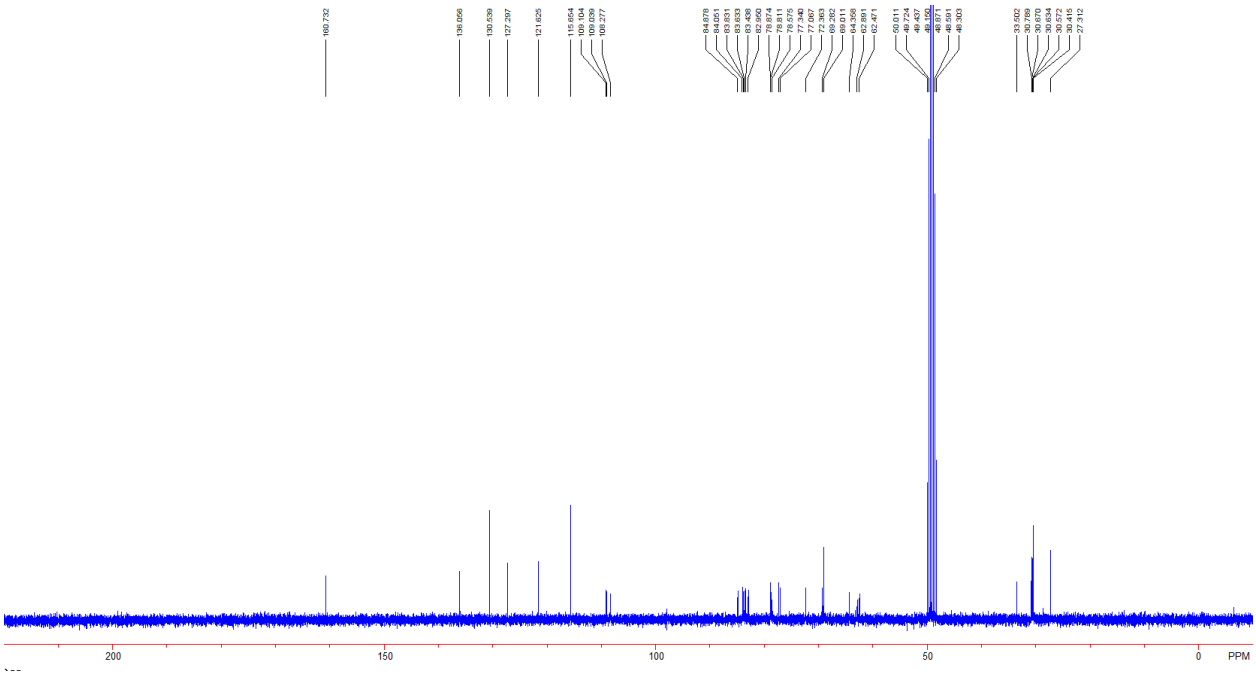
7
PPM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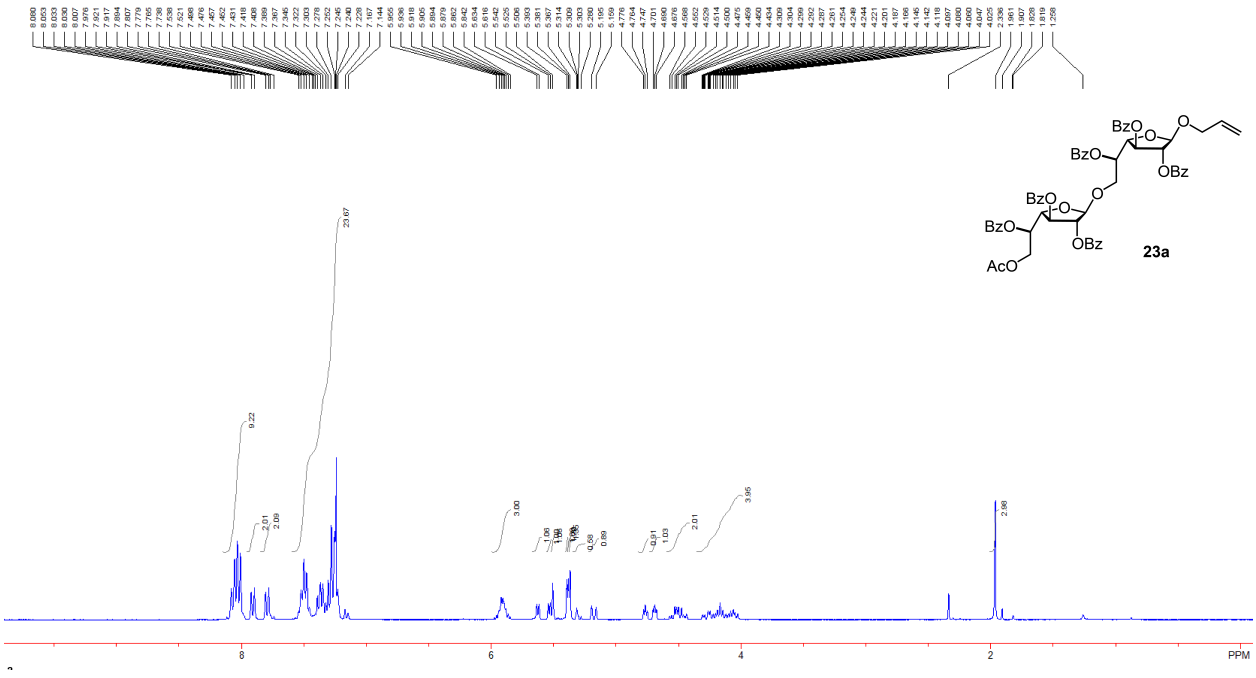
BzO
BzO
OBz
BzO
BzO
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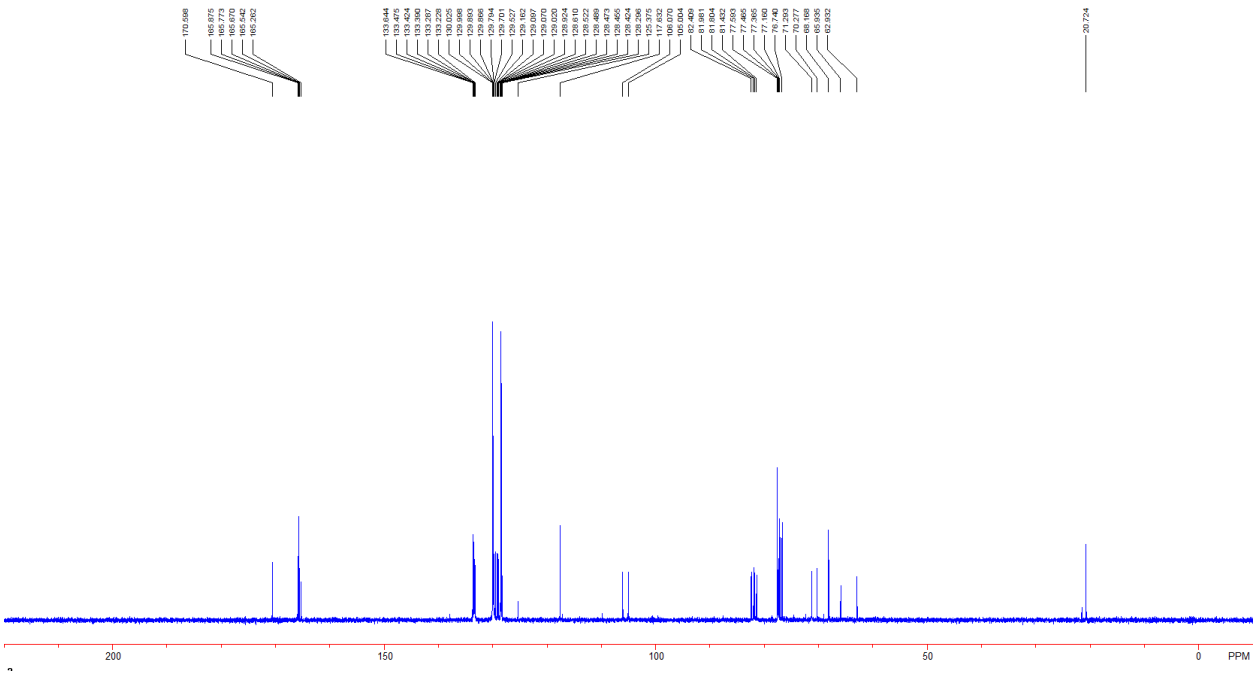
AcO
23a
PPM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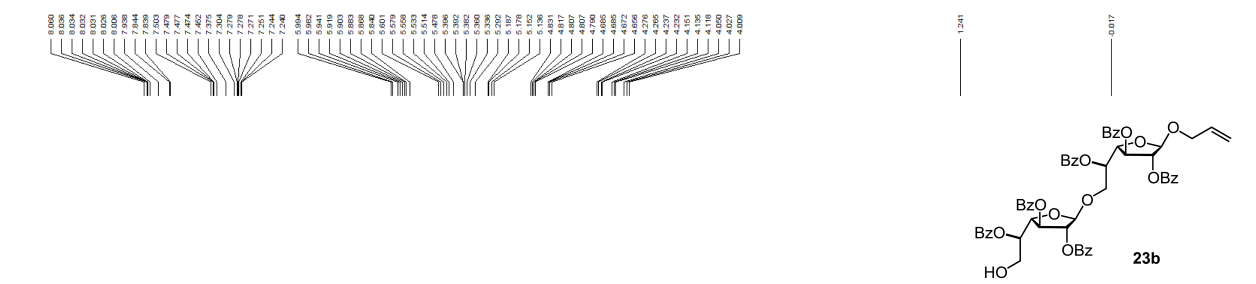
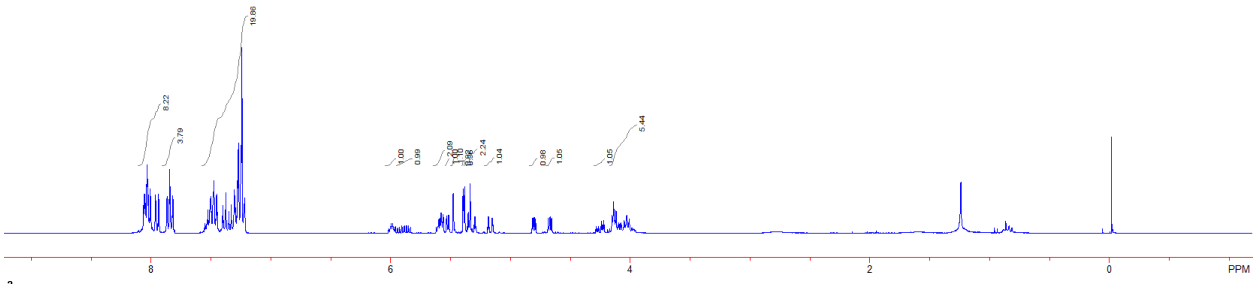

BzO
BzO
OBz
BzO
BzO
OBz
HO
23b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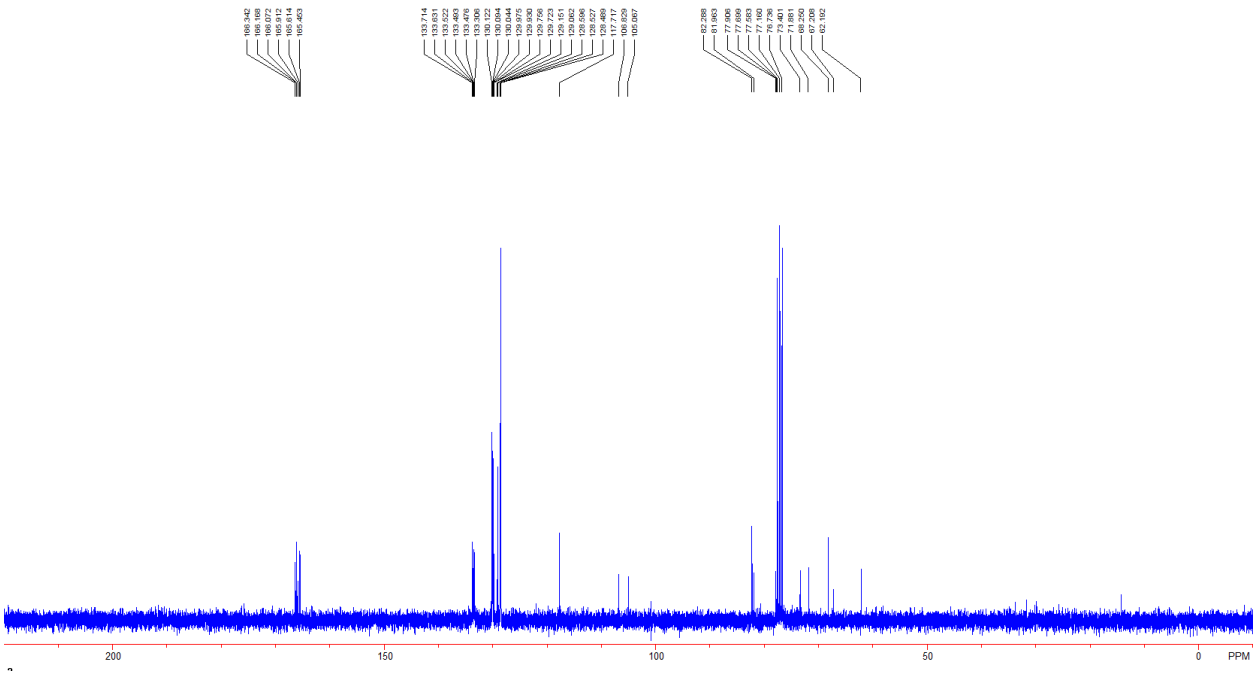
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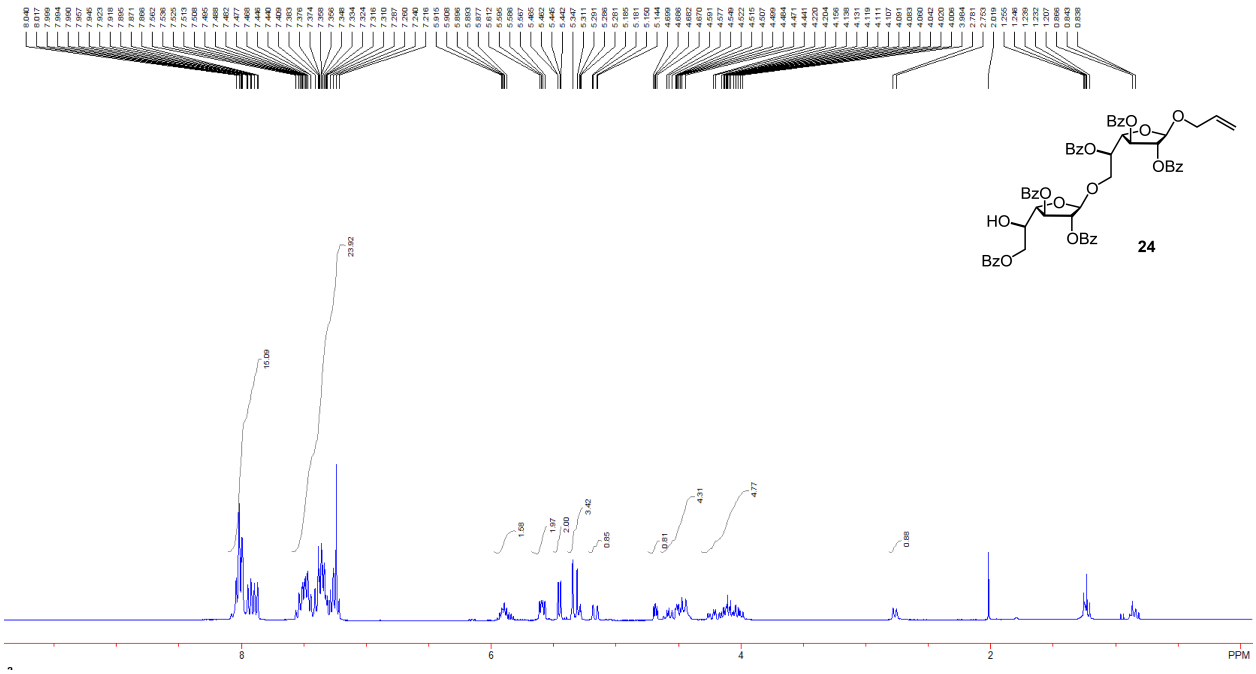
BzO
BzO
OBz
BzO
HO
OBz
BzO
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15.09
23.92
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3.42
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0.81
4.31
4.77
0.88
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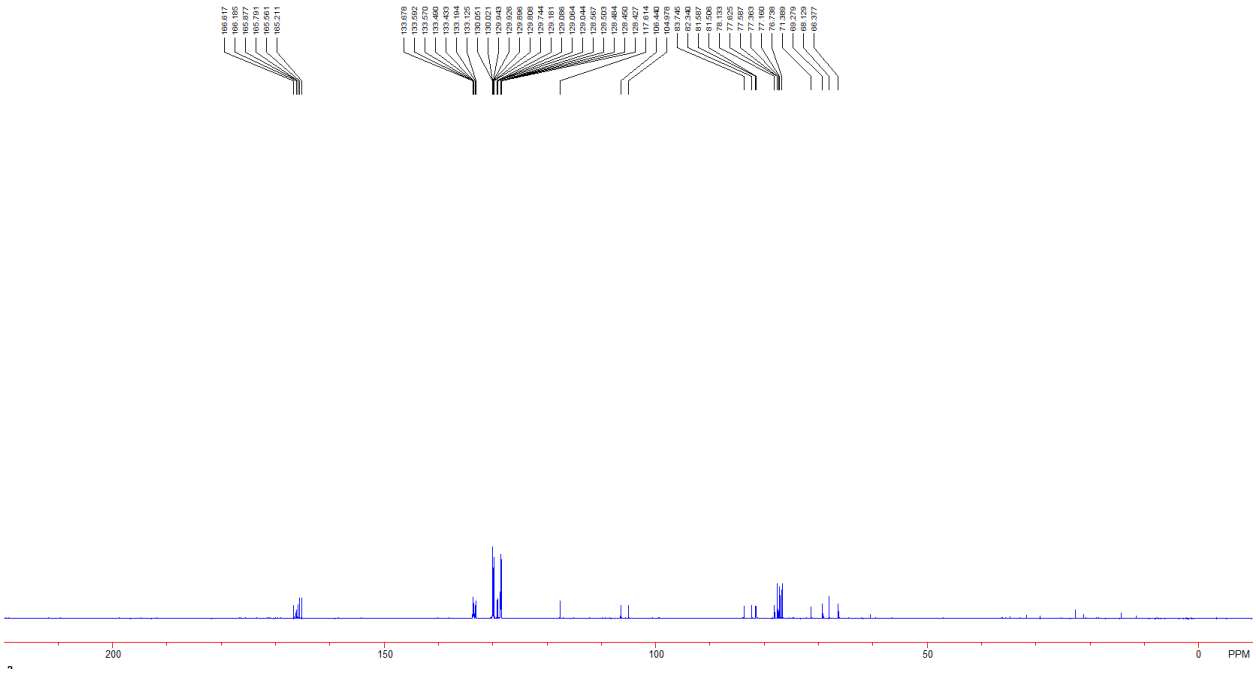
6
4
2
PPM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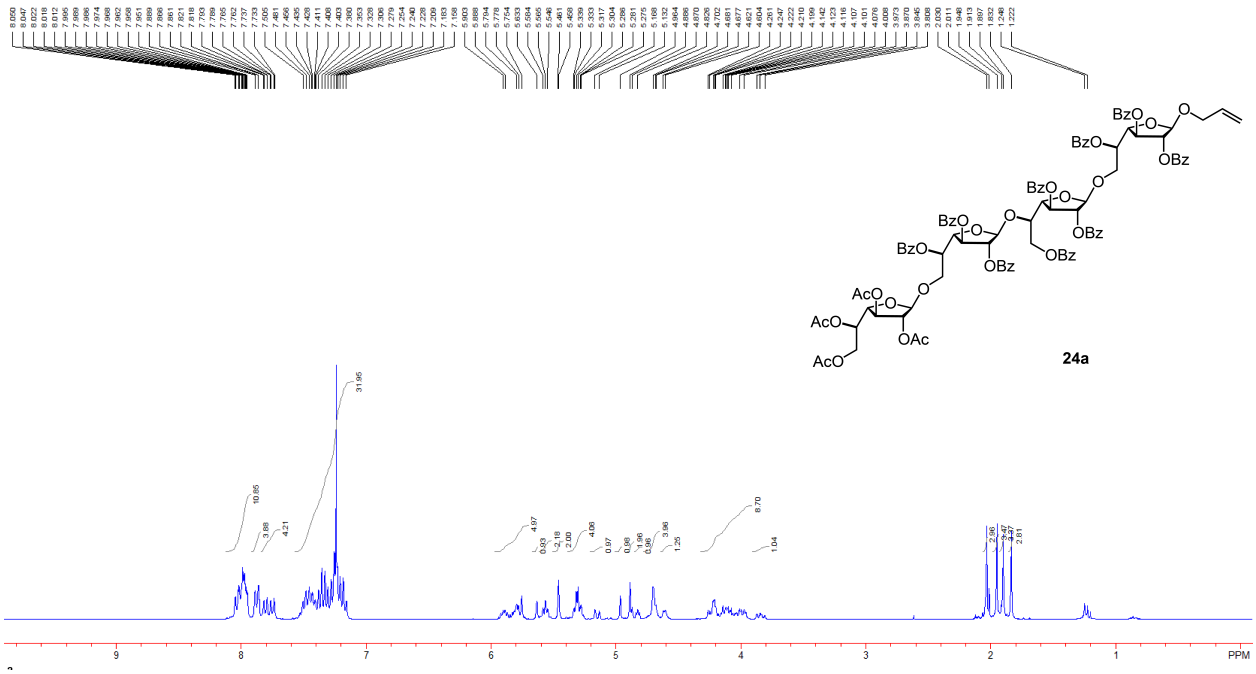
PPM


BzO
OBz
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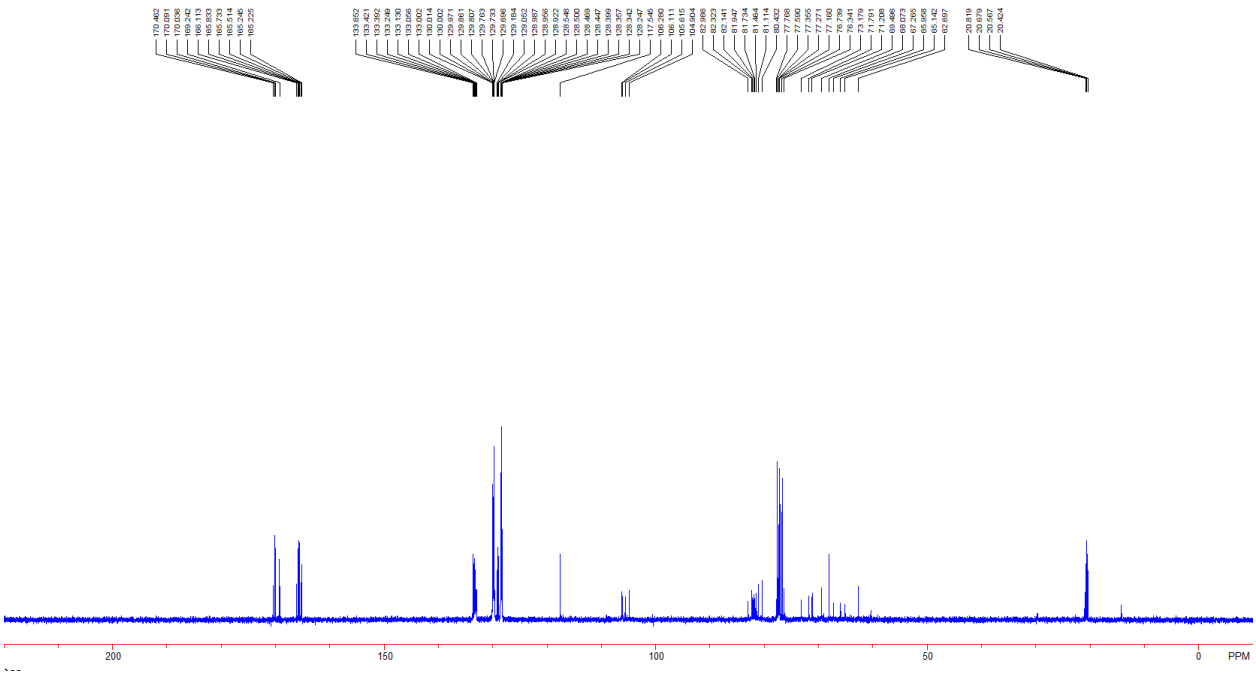
OAc
24a
PPM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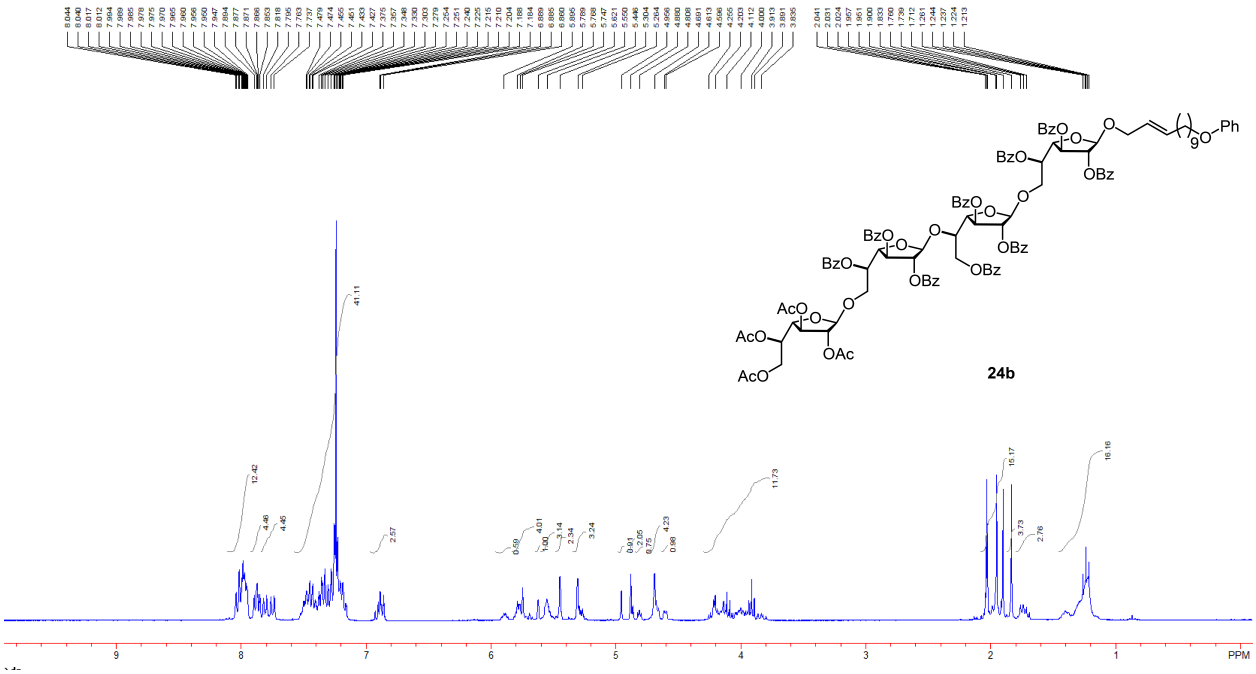
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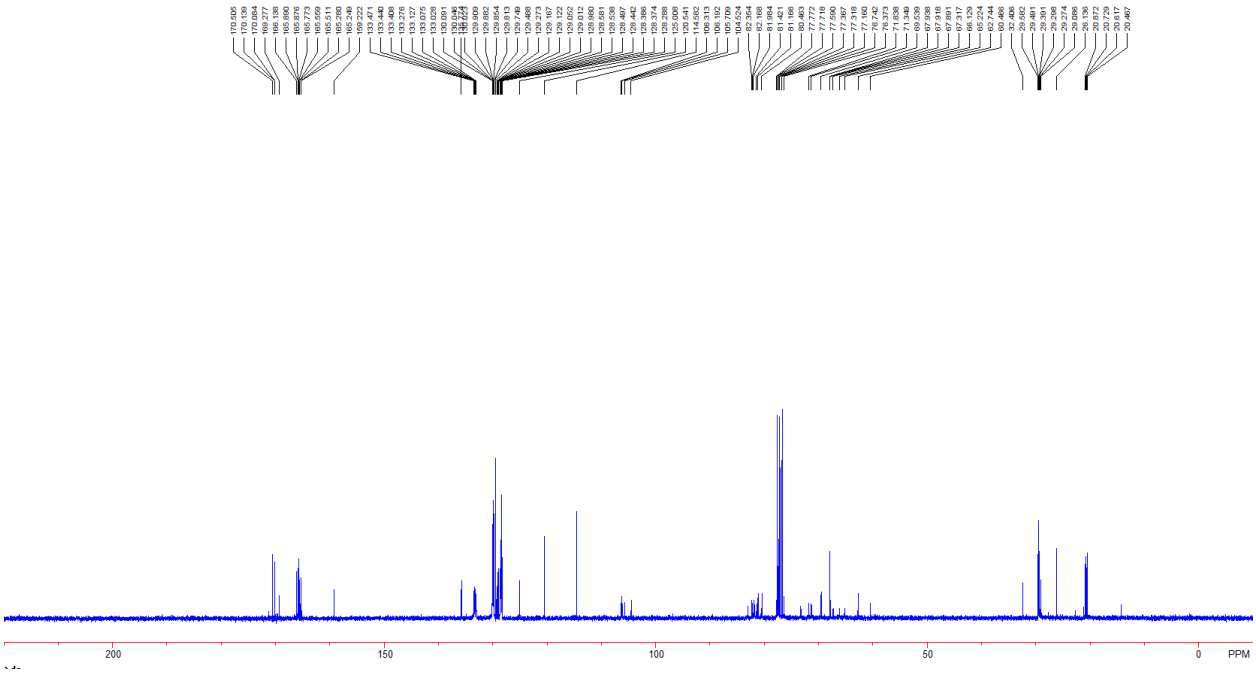
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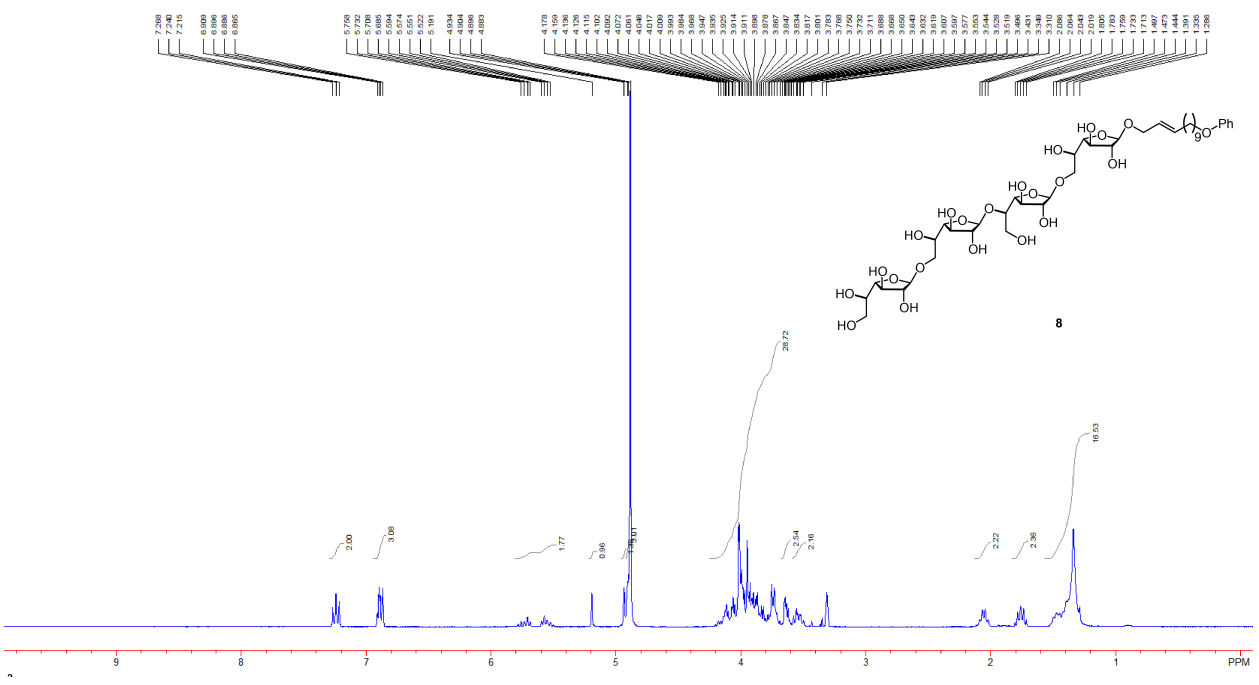
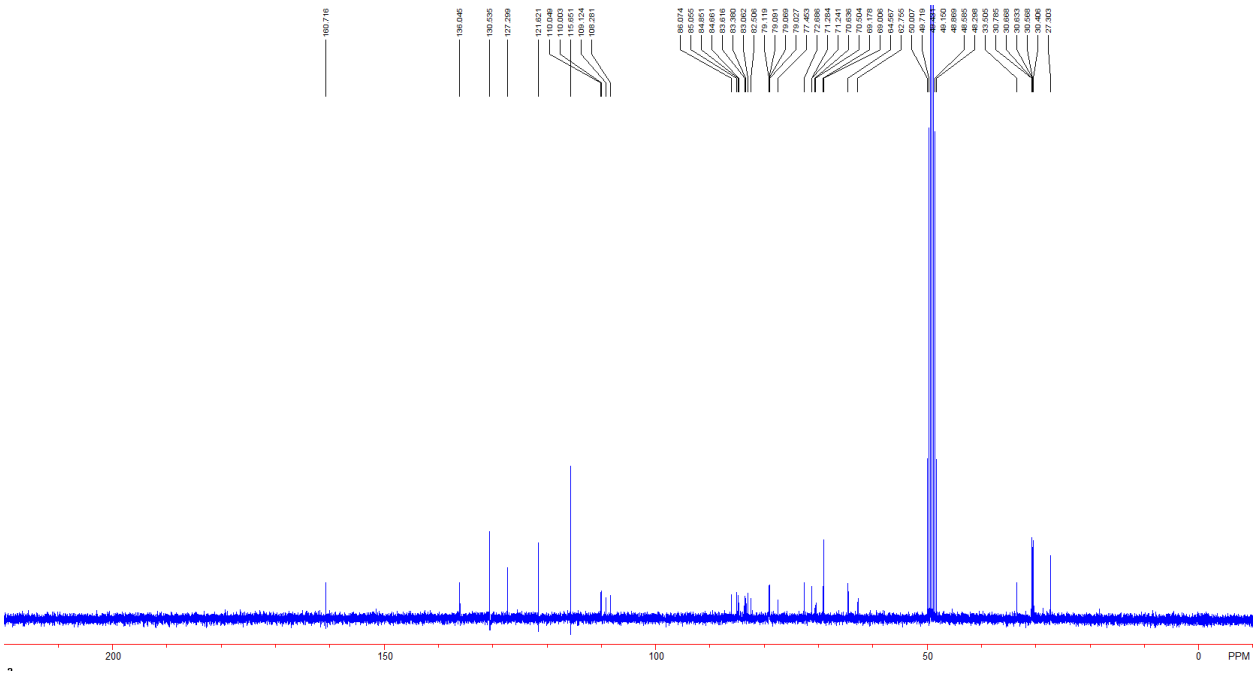

8
PPM


PPM