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Supporting Information

Innate Immune Responses of Synthetic Lipid A Derivatives of *Neisseria meningitidis*

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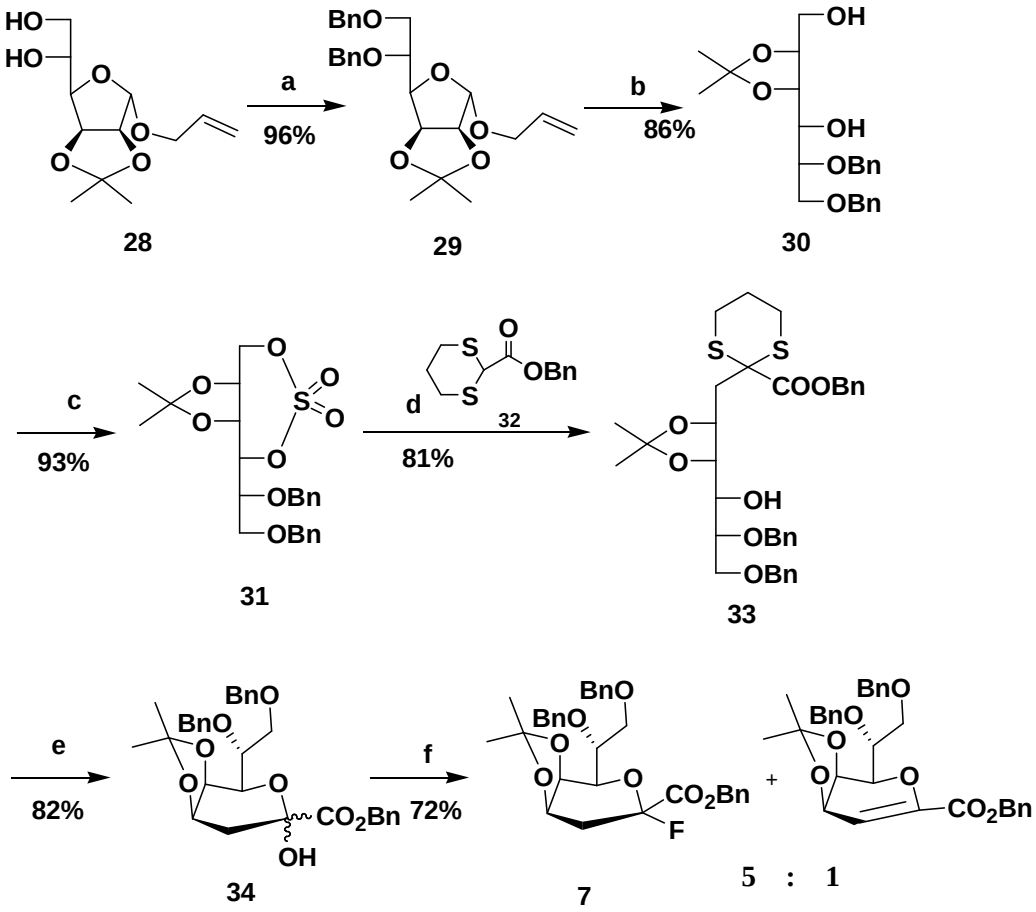
General procedures

Column chromatography was performed on silica gel 60 (EM Science, 70-230 mesh). Reactions were monitored by thin-layer chromatography (TLC) on Kieselgel 60 F254 (EM Science), and compounds were detected by examination under UV light and by charring with 10% sulfuric acid in MeOH. Solvents were removed under reduced pressure at <40ºC. CH_2Cl_2 was distilled from NaH and stored over molecular sieves (3 Å). Tetrahydrofuran (THF) was distilled from sodium directly prior to application. MeOH was dried by refluxing with magnesium methoxide and then was distilled and stored under argon. Pyridine was dried by heating under refluxing over CaH_2 and then distilled and stored over molecular sieves (3 Å). Molecular sieves (3 and 4 Å) used for reactions, were crushed and activated in vacuo at 390ºC during 8 h and then for 2-3 h at 390ºC directly prior to application.

^{1}H NMR and ^{13}C NMR spectra were recorded with Varian spectrometers (models Inova300, Inova500 and Inova600) equipped with Sun workstations. ^{1}H NMR spectra were recorded in $CDCl_3$ and referenced to residual $CHCl_3$ at 7.24 ppm, and ^{13}C NMR spectra were referenced to the central peak of $CDCl_3$ at 77.0 ppm. Assignments were made by standard gCOSY and gHSQC. High resolution mass spectra were obtained on a Bruker model Ultraflex MALDI-TOF mass spectrometer.

Synthesis of the KDO donor



Scheme SI1. Reagents and conditions: (a) BnBr, NaH, DMF; (b) 1: Pd/C, CH_3OH, reflux; 2: I_2, pyridine, H_2O, THF; 3: $NaBH_4$, EtOH; (c) 1: $SOCl_2$, Et_3N, DCM, –15ºC; 2: $NaIO_4$, $RuCl_3$, H_2O, CH_3CN, DCM; (d) BuLi, HMPA, THF, –40ºC; then H_2SO_4, H_2O, THF, 50ºC; (e) NBS, $NaHCO_3$, H_2O, acetone; (f) DAST, MS 4 Å, DCM, –60ºC → rt.

Allyl 5,6-di-*O*-benzyl-2,3-di-*O*-isopropylidene-⟨-D-mannofuranoside (**29**): NaH (1.27 g, 53.0 mmol) was added portionwise to a stirred solution of **28** (2.50 g, 10.6 mmol) in dry DMF (20 mL). After stirring the reaction mixture for 30 min, it was cooled (0ºC) and then BnBr (5.0 mL, 42.4 mmol) was added. The reaction mixture was stirred at room temperature for 10 h, after which it was quenched by addition of methanol (5 mL), diluted with ethyl acetate (50 mL), and washed with brine (2 x 30 mL). The organic phase was dried ($MgSO_4$), filtered, and the filtrate was concentrated in vacuo. The residue was purified by silica gel column chromatography

(eluent: hexane/ethyl acetate, 10/1, v/v) to afford **29** as a colorless oil (4.29 g, 92%). R_f = 0.65 (hexane/ethyl acetate, 6/1, v/v); ^{1}H NMR (300 MHz, $CDCl_3$): ™ 7.39-7.20 (m, 10H, aromatic), 5.86 (m, 1H, OCH_2CH=CH_2), 5.23 (dd, 1H, J = 17.4 Hz, J = 1.5 Hz, OCH_2CH=CH*H*), 5.15 (dd, 1H, J = 17.4 Hz, 10.2 Hz, OCH_2CH=CH*H*), 5.00 (s, 1H, H-1), 4.84 (dd, 1H, J = 3.3 Hz, J = 5.7 Hz, H-3), 4.80 (d, 1H, J = 11.1 Hz, CH*H*Ph), 4.69 (d, 1H, J = 11.1 Hz, CH*H*Ph), 4.65-4.54 (m, 3H, H-2, 2 x CH*H*Ph), 4.11-4.05 (m, 2H, H-4, OCH*H*CH=CH_2), 4.00-3.81 (m, 3H, H-4, H-6a, OCH*H*CH=CH_2), 3.65 (dd, 1H, $J_{5,6b}$ = 5.4 Hz, $J_{6a,6b}$ = 16.5 Hz, H-6b), 1.44 (s, 3H, CH_3), 1.36 (s, 3H, CH_3); HR MS (m/z) calcd for $C_{26}H_{32}O_6$[M+Na]$^+$, 463.2091; found, 463.2118.

5,6-di-*O*-Benzyl-2,3-di-*O*-isopropylidene-D-mannitol (**30**): A suspension of **29** (3.20 g, 7.27 mmol) and Pd/C (50 mg) in methanol (70 mL) was refluxed for 16 h, after which the catalyst was removed by filtration, and the filtrate was concentrated in vacuo to afford the isomerization product as a pale yellow. The obtained intermediate was dissolved in a mixture of THF (50 mL), pyridine (2 mL) and H_2O (10 mL) at 0ºC, and then I_2 (2.77 g, 10.9 mmol) was added portion wise. After stirring the reaction mixture for 30 min, it was diluted with ethyl acetate (100 mL), washed with aqueous NaS_2O_3 (2 x 50 mL, 15%), saturated aqueous $NaHCO_3$ (2 x 50 mL) and brine (2 x 50 mL), successively. The organic phase was dried ($MgSO_4$) and filtered. Next, the filtrate was concentrated in vacuo. The residue was purified by silica gel column chromatography (eluent: hexane/ethyl acetate, 4/1-3/1, v/v) to afford a lactol as a colorless oil (2.18 g, 75%). R_f = 0.65 (hexane/ethyl acetate, 2/1, v/v); HR MS (m/z) calcd for $C_{23}H_{28}O_6$[M+Na]$^+$, 423.1778; found, 423.2083. The above obtained lactol (2.00 g, 5.00 mmol) was dissolved in ethanol (30 mL), and then $NaBH_4$ (285 mg, 7.50 mmol) was added portionwise. After stirring the reaction mixture for 10 h, it was cooled (0ºC), quenched with acetic acid (15 mL), and diluted with ethyl acetate (80 mL). The solution was washed with saturated aqueous $NaHCO_3$ (2 x 50 mL) and brine (2 x 40 mL). The organic phase was dried ($MgSO_4$), filtered, and the filtrate was concentrated in vacuo. The residue was purified by silica gel column chromatography (eluent: hexane/ethyl acetate, 5/2-3/2, v/v) to afford **30** as an amorphous solid (1.89 g, 94%). R_f = 0.45 (hexane/ethyl acetate, 3/2, v/v); ^{1}H NMR (300 MHz, $CDCl_3$): ™ 7.39-7.22 (m, 10H, aromatic), 4.73 (d, 1H, J = 11.7 Hz, CH*H*Ph), 4.58-4.54 (m, 3H, CH*H*Ph), 4.45 (dd, 1H, J = 1.5 Hz, J = 6.9 Hz, H-3), 4.22 (m, 1H, H-2), 3.86-3.71 (m, 5H, 2 x H-1, H-4, H-5,

H-6b); 3.63 (dd, 1H, *J* = 3.9 Hz, *J* = 8.1 Hz, H-6a), 1.56 (s, 3H, C*H*$_3$), 1.38 (s, 3H, C*H*$_3$). HR MS (m/z) calcd for $C_{23}H_{30}O_6$[M+Na]$^+$, 425.1935; found, 425.1886.

5,6-di-*O*-Benzyl-2,3-di-*O*-isopropylidene-1,4-di-*O*-sulfate-D-mannitol (**31**): To a cooled (–15ºC) solution of **30** (1.44 g, 3.53 mmol) and Et_3N (2.0 mL, 14.2 mmol) in DCM (20 mL) was added dropwise thionyl chloride (387 ⌈l, 5.30 mmol). After stirring the reaction mixture for 30 min, it was diluted with DCM (30 mL), and then washed with saturated aqueous $NaHCO_3$ (2 x 40 mL) and brine (2 x 40 mL). The organic phase was allowed to pass through a pad of silica gel, which was then eluted with ethyl acetate (50 mL). The combined eluents were concentrated in vacuo to afford the crude cyclic sulfite as a slightly colored oil. The above obtained crude product was dissolved in a mixture of DCM (10 mL) and acetonitrile (10 mL), and then $RuCl_3 \cdot H_2O$ (14.7 mg, 71 ⌈mol), $NaIO_4$ (1.13 g, 5.30 mmol) and H_2O (15 mL) were added, successively. After stirring the reaction mixture for 20 min, it was diluted with ethyl acetate (40 mL), and then washed with saturated aqueous $NaHCO_3$ (2 x 40 mL) and brine (2 x 40 mL). The organic phase was dried ($MgSO_4$), filtered, and the filtrate was concentrated in vacuo. The residue was purified by silica gel column chromatography (eluent: hexane/ethyl acetate, 10/1, v/v) to afford **31** as an amorphous solid (1.89 g, 94%). R_f = 0.45 (hexane/ethyl acetate, 4/1, v/v); ^{1}H NMR (300 MHz, $CDCl_3$): ™ 7.40-7.22 (m, 10H, aromatic), 4.99 (d, $J_{4,5}$ = 9.0 Hz, H-4), 4.77-4.72 (m, 2H, H-3, CH*H*Ph), 4.66 (d, 1H, *J* = 12.0 Hz, CH*H*Ph), 4.59 (d, 1H, *J* = 11.4 Hz, CH*H*Ph), 4.53 (d, 1H, *J* = 12.0 Hz, CH*H*Ph), 4.43-4.26 (m, 3H, 2 x H-1, H-2); 3.95 (ddd, 1H, *J* = 1.8 Hz, *J* = 3.9 Hz, *J* = 9.0 Hz, H-5), 3.82 (dd, 1H, $J_{5,6a}$ = 1.8 Hz, $J_{6a,6b}$ = 10.5 Hz, H-6a), 3.82 (dd, 1H, $J_{5,6b}$ = 3.9 Hz, $J_{6a,6b}$ = 10.5 Hz, H-6b),1.54 (s, 3H, CH_3), 1.48 (s, 3H, CH_3). HR MS (m/z) calcd for $C_{23}H_{28}O_8S$[M+Na]$^+$, 487.1397; found, 487.1464.

Benzyl 2-deoxy-4,5-di-*O*-isopropylidene-7,8-di-*O*-benzyl-D-glycero-D-galacto-octulosonate 1,3-propylene dithioacetal (**33**): To a cooled solution (–45ºC) of **32** (330 mg, 1.3 mmol) in a mixture of THF (2 mL) and HMPA (0.8 mL) was added BuLi (2.5 M in hexane, 0.56 mL, 1.4 mmol). The reaction mixture was stirred for 2 h, after which a solution of **31** (470 mg, 1.0 mmol) in THF (1 mL) was added dropwise. The stirring continued at room temperature for another 2 h till TLC analysis showed compound **31** nearly completely disappeared. Then, the reaction mixture was first neutralized with sulfuric acid (1 M in THF, 1 mL) followed by the addition of

H_2O (15 ⌈L), after which another portion of sulfuric acid (1 M in THF, 1 mL) was added till pH 3. After heating the mixture (50ºC) for 1 h, it was cooled (25ºC), diluted with ethyl acetate (30 mL), and then washed with saturated aqueous $NaHCO_3$ (2 x 40 mL) and brine (2 x 30 mL). The organic phase was dried ($MgSO_4$), filtered, and the filtrate was concentrated in vacuo. The residue was purified by silica gel column chromatography (eluent: toluene/ethyl acetate, 30/1, v/v) to afford **33** as a colorless oil (510 mg, 78%). R_f = 0.55 (hexane/ethyl acetate, 3/1, v/v); ^{1}H NMR (300 MHz, $CDCl_3$): ™ 7.40-7.21 (m, 15H, aromatic), 5.24 (d, 1H, *J* = 12.6 Hz, CO_2CH*H*Ph), 5.14 (d, 1H, *J* = 12.6 Hz, CO_2CH*H*Ph), 4.74 (d, 1H, *J* = 11.4 Hz, CH*H*Ph), 4.63-4.53 (m, 4H, H-4, 2 x CH*H*Ph), 4.39 (dd, 1H, *J* = 1.2 Hz, *J* = 6.9 Hz, H-5), 3.85 (dd, 1H, *J* = 3.0 Hz, *J* = 10.5 Hz, H-8a), 3.75-3.67 (m, 2H, H-6, H-8b), 3.60-3.54 (m, 1H, H-7), 3.25 (ddd, 1H, *J* = 2.7 Hz, *J* = 14.6 Hz, CH_{2axi} of SCH_2), 3.06 (ddd, 1H, *J* = 2.4 Hz, *J* = 14.6 Hz, CH'_{2axi} of SCH'_2), 2.75-2.60 (m, 3H, H-3a, CH_{2equo} of SCH_2, CH'_{2equo} of SCH'_2), 2.43 (dd, 1H, $J_{3a,3b}$ = 15.0 Hz, $J_{3b,4}$ = 3.0 Hz, H-3b), 2.09-2.03 (m, 1H,) 1.92-1.78 (m, 1H), 1.39 (s, 3H, CH_3 of isopropylidene), 1.29 (s, 3H, CH_3 of isopropylidene). HR MS (m/z) calcd for $C_{32}H_{42}O_7S_2[M+Na]^+$, 661.2264; found, 661.2397.

Benzyl 3-deoxy-4,5-di-*O*-isopropylidene-7,8-di-*O*-benzyl-⟨,®-D-manno-2-octulopyranosonate (**34**): To a stirred suspension of **33** (1.06 g, 1.66 mmol) and $NaHCO_3$ (1 g, 11.9 mmol) in a mixture of CH_3COCH_3 (20 mL) and H_2O (1 mL) was added NBS (1.77 g, 9.96 mmol) at 0ºC. After stirring the reaction mixture for 10 min, it was quenched with aqueous $Na_2S_2O_3$ (15%, 100 mL), diluted with ethyl acetate (50 mL), and then washed with saturated aqueous $NaHCO_3$ (2 x 40 mL) and brine (2 x 40 mL). The organic phase was dried ($MgSO_4$), filtered, and the filtrate was concentrated in vacuo. The residue was purified by silica gel column chromatography (eluent: hexane/ethyl acetate, 6/1-4/1, v/v) to afford **34** as a colorless oil (1.89 g, 94%). R_f = 0.35 (hexane/ethyl acetate, 4/1, v/v). HR MS (m/z) calcd for $C_{32}H_{36}O_8$ $[M+Na]^+$, 571.2302; found, 571.3219.

Benzyl 3-deoxy-4,5-di-*O*-isopropylidene-7,8-di-*O*-benzyl-⟨,®-D-manno-2-octulopyranosyl fluoride (**7**): A suspension of **34** (700 mg, 1.28 mmol) and molecular sieves (4 Å, 100 mg) in DCM (6 mL) was stirred at room temperature for 1 h. The mixture was cooled (–60ºC) and then DAST (220 ⌈L, 1.66 mmol) was added dropwise. After stirring the reaction mixture at room

temperature for 30 min, it was cooled (–30ºC) and then quenched by stirring with acetic acid (150 ⌈L) for 2 min. Then, the solids were removed by filtration, and the filtrate was washed with saturated aqueous $NaHCO_3$ (2 x 40 mL) and brine (2 x 40 mL). The organic phase was dried ($MgSO_4$), filtered, and the filtrate was concentrated in vacuo. The residue was purified by silica gel column chromatography (eluent: hexane/ethyl acetate, 6/1, v/v) to afford a mixture (631 mg) of **7** (75%) and its elimination product (15%). R_f = 0.60 (hexane/ethyl acetate, 5/1, v/v). HR MS (m/z) calcd for $C_{32}H_{35}FO_7$ $[M+Na]^+$, 573.2259; found, 573.2516.

Synthesis of compound 1

***t*-Butyldimethylsilyl 3-*O*-allyloxycarbonyl-6-*O*-benzyl-2-deoxy-4-*O*-(1,5-dihydro-3-oxo-3⌊^{5}-3H-2,4,3-benzodioxaphosphepin-3yl)-2-[(*R*)-3-dodecanoyloxy-tetradecanoylamino]-®-D-glucopyranosyl-(1 6)-4-*O*-benzyl-2-[(*R*)-3-benzyloxy-dodecanoylamino]-3-*O*-[(*R*)-3-dodecanoyloxy-dodecanoyl]-2-deoxy-®-D-glucopyranoside** (**24**): A suspension of **23** (180 mg, 0.111 mmol), zinc (< 10 micron, 72 mg, 1.11 mmol), and acetic acid (25 ⌈L, 0.444 mmol) in DCM (5 mL) was stirred at room temperature for 12 h, after which it was diluted with ethyl acetate (20 mL), the solids removed by filtration and the residue washed with ethyl acetate (2 x 2 mL). The combined filtrates were washed with saturated aqueous $NaHCO_3$ (2 x 15 mL) and brine (2 x 15 mL). The organic phase was dried ($MgSO_4$), filtered, and the filtrate was concentrated in vacuo. The residue was purified by silica gel column chromatography (eluent: hexane/ethyl acetate, 5/2, v/v) to afford an amine as a pale yellow syrup (160 mg, 90%). R_f = 0.35 (hexane/ethyl acetate, 2/1, v/v); HR MS (m/z) calcd for $C_{89}H_{137}N_2O_{19}PSi[M+Na]^+$, 1619.9220; found, 1620.1069. A reaction mixture of (*R*)-3-dodecanoyl-tetradecanoic acid **9** (31 mg, 73 ⌈mol) and DCC (20 mg, 98 ⌈mol) in DCM (2 mL) was stirred at room temperature for 10 min, and then the above obtained amine (78 mg, 49 ⌈mol) was added. The reaction mixture was stirred at room temperature for 10 h, after which the insoluble materials were removed by filtration, and the residue was washed with DCM (2 x 1 mL). The combined filtrates were concentrated in vacuo and the residue was purified by preparative silica gel TLC (eluent: hexane/ethyl acetate, 5/1, v/v) to give **24** as an amorphous solid (82 mg, 84%). R_f = 0.51 (hexane/ethyl acetate, 2/1, v/v). $[\langle]^{26}_D$ = -3.0º (c = 1.0, $CHCl_3$). ^{1}H NMR (500 MHz, $CDCl_3$): ™ 7.37-7.18 (m, 19H, aromatic), 5.85 (d, 1H, $J_{NH',2'}$ = 7.5 Hz, N*H*'), 5.86-5.79 (m, 1H, OCH_2CH=CH_2), 5.65 (d, 1H, $J_{NH,2}$ = 9.0 Hz, N*H*), 5.45 (t, 1H, $J_{2',3'} = J_{3',4'}$ = 10.0 Hz, H-3'), 5.28 (d, 1H, *J* = 16.0Hz, OCH_2CH=CH*H*), 5.16 (d, 1H, *J* = 11.0 Hz, OCH_2CH=CH*H*), 5.04-4.92 (m, 8H, H-1', H-3, 2 x H-3_L, *o*-$C_6H_4(CH_2O)_2P$), 4.63 (d, 1H, $J_{1,2}$ = 7.5 Hz, H-1), 4.55-4.35 (m, 9H, H-4', 3 x C*H*$_2$Ph, OC*H*$_2$CH=CH_2), 3.92 (d, 1H, $J_{6a,6b}$ = 10.5 Hz, H-6a), 3.78-3.71 (m, 3H, H-2, H-6'a, H-3_S), 3.67-3.62 (m, 3H, H-5, H-6b, H-6'b), 3.50-3.48 (m, 2H, H-4, H-5'), 3.39-3.32 (m, 1H, H-2'), 2.48-2.13 (m, 10H, 2 x H-2_L, H-2_S, 2 x H-$2_{L'}$), 1.63-1.42 (m, 10H, 2 x H-4_L, H-4_S, 2 x H-$3_{L'}$), 1.26 [bs, 82H, H-(5_S-11_S), 2 x H-(5_L-13_L), 2 x H-($4_{L'}$-$11_{L'}$)], 0.90-0.86 [m, 24H, 2 x H-12_S, 2 x H-14_L, 2 x H-$12_{L'}$, SiC(C*H*$_3$)$_3$], 0.11 (s, 3H, SiC*H*$_3$), 0.09 (s, 3H, SiC*H*$_3$). ^{13}C NMR (75

MHz, $CDCl_3$): ™ 173.66 (C=O), 173.51 (C=O), 170.10 (C=O), 169.19 (C=O), 154.47 (C=O), 138.51-127.48 (aromatic, $OCH_2CH=CH_2$), 118.78 ($OCH_2CH=CH_2$), 99.13 (C-1'), 96.11 (C-1), 76.02-74.85 (m, C-3, C-3', C-4, C-4', C-3_S), 74.31, 74.17, 73.47 (C-5, C-5', CH_2Ph), 71.26 (CH_2Ph), 70.99 (C-3_L), 70.72 (C-3_L), 68.93-68.01 (m, C-6, C-6', $OCH_2CH=CH_2$, 2 x CH_2Ph), 56.37 (C-2), 56.11 (C-2'), 41.69 (C-2_L), 39.52 (C-2_S), 34.51-14.10 [m, $SiC(CH_3)_3$, C-(4_S-12_S), 2 x C-(4_L-14_L), 2 x C-($2_{L'}$-$12_{L'}$)], -3.86 ($SiCH_3$), -5.15 ($SiCH_3$). HR MS(m/z) for calcd for $C_{115}H_{185}N_2O_{22}PSi[M+Na]^+$, 2028.2824; found, 2028.2843.

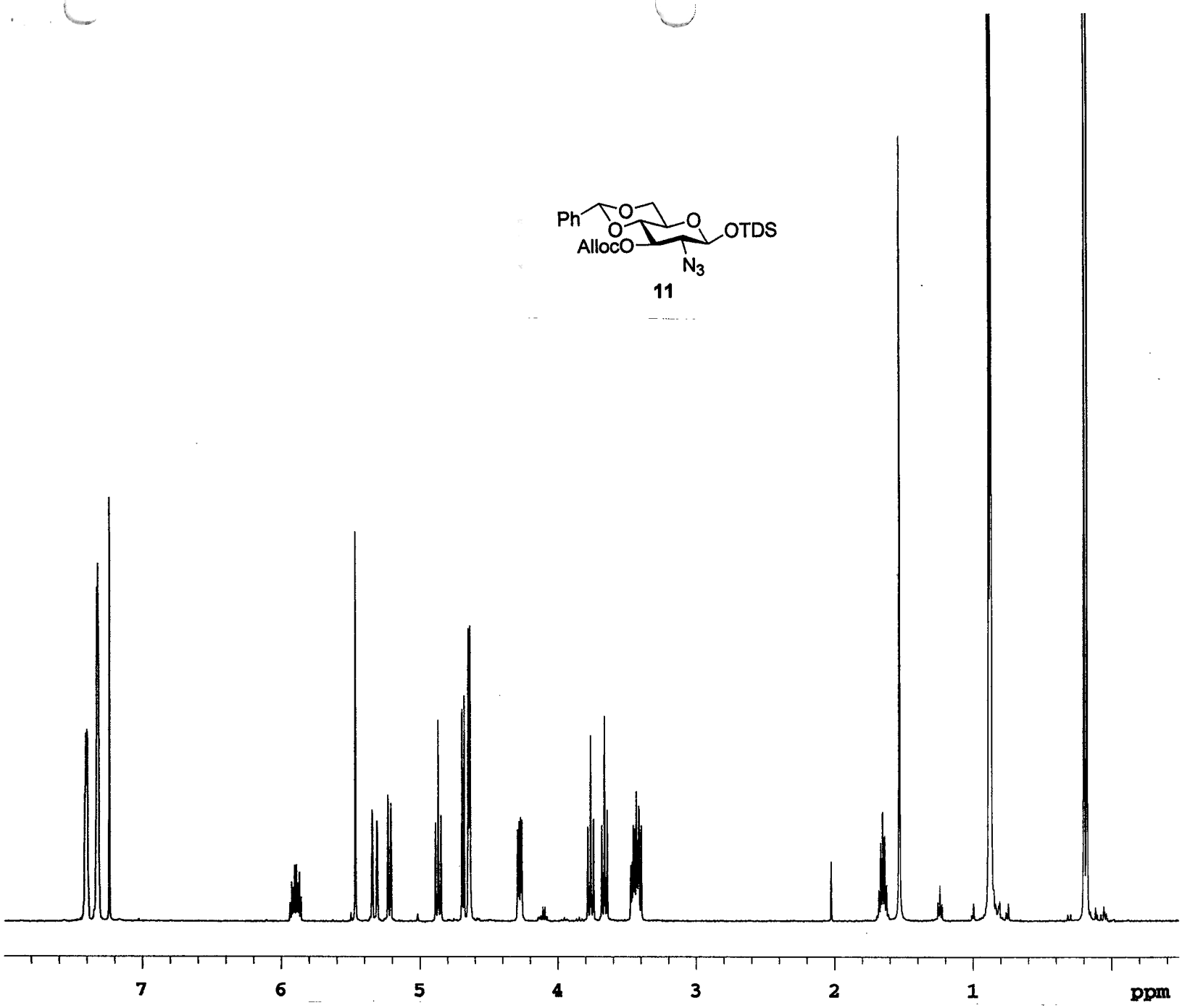
***t*-Butyldimethylsilyl 6-*O*-benzyl-3-*O*-[(*R*)-3-benzyloxy-dodecanoyl]-2-deoxy-4-*O*-(1,5-dihydro-3-oxo-3⌊^{5}-3H-2,4,3-benzodioxaphosphepin-3yl)-2-[(*R*)-3-dodecanoyloxy-tetradecanoylamino]-®-D-glucopyranosyl-(1 6)-4-*O*-benzyl-2-[(*R*)-3-benzyloxy-dodecanoylamino]-3-*O*-[(*R*)-3-dodecanoyloxy-dodecanoyl]-2-deoxy-®-D-glucopyranoside** (**26**): Tetrakis(triphenylphosphine)palladium (6.9 mg, 6 ⌈mol) was added to a solution of **24** (62 mg, 31 ⌈mol), n-$BuNH_2$ (6.1 ⌈L, 62 ⌈mol), and HCOOH (2.3 ⌈L, 62 ⌈mol) in THF (5 mL). After stirring the reaction mixture at room temperature for 20 min, it was diluted with DCM (10 mL), and washed with water (20 mL), saturated aqueous $NaHCO_3$ (2 x 20 mL) and brine (2 x 20 mL), successively. The organic phase was dried ($MgSO_4$), filtered, and the filtrate was concentrated in vacuo. The residue was purified by silica gel column chromatography (eluent: hexane/ethyl acetate, 4/3, v/v) to give **25** as a colorless syrup. A solution of (*R*)-3-benzyloxy-dodecanoic acid **8** (14 mg, 47 ⌈mol) and DCC (13 mg, 62 ⌈mol) in DCM (2 mL) was stirred at room temperature for 10 min, and then the above obtained intermediate **25** and DMAP (1.8 mg, 15 ⌈mol) were added. The reaction mixture was stirred for another 10 h, after which the solids were removed by filtration and washed with DCM (2 x 1 mL). The combined filtrates were concentrated in vacuo. The residue was purified by silica gel column chromatography (eluent: hexane/ethyl acetate, 4/1, v/v) to afford **26** as an amorphous white solid (49 mg, 72%, 2 steps). R_f = 0.45 (hexane/ethyl acetate, 2/1, v/v). ^{1}H NMR (600 MHz, $CDCl_3$): ™ 7.37-7.10 (m, 24H, aromatic), 5.69 (d, 1H, $J_{NH,2}$ = 8.4 Hz, N*H*), 5.63 (d, 1H, $J_{NH',2'}$ = 7.8Hz, N*H'*), 5.59 (t, 1H, $J_{2',3'} = J_{3',4'}$ = 9.6 Hz, H-3'), 5.10 (t, 1H, $J_{2,3} = J_{3,4}$ = 9.6 Hz, H-3), 5.07 (1H, $J_{1',2'}$ = 8.4 Hz, H-1'), 5.04-4.85 (m, 6H, 2 x H-3_L, *o*-$C_6H_4(CH_2O)_2P$), 4.69 (t, 1H, $J_{1,2}$ = 7.8Hz, H-1), 4.63-4.41 (m, 9H, H-4', 4 x CH_2Ph,), 3.97 (d, 1H, $J_{6a,6b}$ = 10.8 Hz, H-6a), 3.88-3.78 (m, 4H, H-2, H-5, 2 x H-3_S), 3.72-3.67 (m, 3H, H-5', H-6b, H-6'a), 3.58-3.54 (m, 2H, H-4, H-6'b), 3.29-3.25 (m, 1H, H-2'), 2.66-2.01 (m, 12H, 2 x H-2_L, 2

x H-2_S, 2 x H-$2_{L'}$), 1.58-1.54 (m, 12H, 2 x H-4_L, 2 x H-4_S, 2 x H-$3_{L'}$), 1.24 [bs, 96H, 2 x H-(5_S-11_S), 2 x H-(5_L-13_L), 2 x H-($4_{L'}$-$11_{L'}$)], 0.87-0.84 [m, 27H, 2 x H-12_S, 2 x H-14_L, 2 x H-$12_{L'}$, SiC(CH_3)$_3$], 0.09 (s, 3H, SiCH_3), 0.07 (s, 3H, SiCH_3). ^{13}C NMR (75 MHz, $CDCl_3$): ™ 173.64 (C=O), 171.63 (C=O), 171.40 (C=O), 169.89 (C=O), 168.15 (C=O), 138.63-127.48 (aromatic), 99.45 (C-1'), 96.16 (C-1), 75.89 (C-4), 75.57 (C-3_S), 75.38 (C-3_S), 74.92 (C-4'), 74.38 (C-3), 74.19 (CH_2Ph), 73.79 (C-6'), 73.50 (CH_2Ph), 71.99 (C-3'), 71.33 (CH_2Ph), 71.28 (CH_2Ph), 70.80 (C-3_S), 70.54 (C-3_S), 68.93-68.18 (m, C-5, C-5', C-6, 2 x CH_2Ph), 56.26 (C-2'), 56.31 (C-2), 41.68 (C-2_L), 41.42 (C-2_L), 39.51 (C-2_S), 38.92 (C-2_S), 34.50-14.10 [m, SiC(CH_3)$_3$, 2 x C-(4_S-12_S), 2 x C-(4_L-14_L), 2 x C-($2_{L'}$-$12_{L'}$)], -3.81 (SiCH_3), -5.10 (SiCH_3). HR MS (m/z) calcd for $C_{130}H_{209}N_2O_{22}PSi[M+Na]^+$, 2232.4702; found, 2232.5168.

Bis(benzyloxy)phosphoryl 6-*O*-benzyl-3-*O*-[(*R*)-3-benzyloxy-dodecanoyl]-2-deoxy-4-*O*-(1,5-dihydro-3-oxo-3⌊^{5}-3H-2,4,3-benzodioxaphosphepin-3yl)-2-[(*R*)-3-dodecanoyloxy-tetradecanoylamino]-®-D-glucopyranosyl-(1 6)-4-*O*-benzyl-2-[(*R*)-3-benzyloxy-dodecanoylamino]-3-*O*-[(*R*)-3-dodecanoyloxy-dodecanoyl]-2-deoxy-⟨-D-glucopyranose (**27**): HF/pyridine (40 ⌈L) was added dropwise to a stirred solution of **26** (31 mg, 14 ⌈mol) in THF (2 mL). The reaction mixture was stirred at room temperature for 5 h, after which it was diluted with ethyl acetate (15 mL), and washed with saturated aqueous (2 x 20 mL) and brine (2 x 20 mL). The organic phase was dried ($MgSO_4$), filtered, and the filtrate was concentrated in vacuo. The residue was purified by silica gel column chromatography (eluent: hexane/ethyl acetate, 3/1-4/3, v/v) to give a lactol intermediate as an amorphous solid (25.8 mg, 88%). R_f = 0.39 (hexane/ethyl acetate, 1/1, v/v); ^{1}H NMR (600 MHz, $CDCl_3$): ™ 7.38-6.81 (m, 24H, aromatic), 5.90 (d, 1H, $J_{NH,2}$ = 9.0 Hz, NH), 5.83 (d, 1H, $J_{NH',2'}$ = 7.2 Hz, NH'), 5.53 (t, 1H, $J_{2',3'}$ = $J_{3',4'}$ = 9.6 Hz, H-3'), 5.48 (d, 1H, $J_{1',2'}$ = 8.4 Hz, H-1'), 5.34 (t, 1H, $J_{2,3}$ = $J_{3,4}$ = 9.6 Hz, H-3), 5.12-5.10 (m, 2H, H-1, H-3_L), 5.03-4.84 (m, 5H, H-3_L, *o*-C_6H_4(CH_2O)$_2$P), 4.63-4.37 (m, 9H, H-4', 4 x CH_2Ph), 4.14-4.11 (m, 1H, H-2), 4.05-4.02 (m, 1H, H-5), 3.88-3.80 (m, 4H, H-6a, H-6'a, 2 x H-3_S), 3.80-3.68 (m, 3H, H-5', H-6b, H-6'b), 3.29 (t, 1H, $J_{3,4}$ = $J_{4,5}$ = 9.6 Hz, H-4), 3.17-3.13 (m, 1H, H-2'), 2.71-2.12 (m, 12H, 2 x H-2_L, 2 x H-2_S, 2 x H-$2_{L'}$), 1.62-1.51(broad, 12H, 2 x H-4_L, 2 x H-4_S, 2 x H-$3_{L'}$), 1.23 [bs, 96H, 2 x H-(5_S-11_S), 2 x H-(5_L-13_L), 2 x H-($4_{L'}$-$11_{L'}$)], 0.87-0.85 (m, 18H, 2 x H-12_S, 2 x H-14_L, 2 x H-$12_{L'}$). HR MS (m/z) calcd for $C_{124}H_{195}N_2O_{22}PSi[M+Na]^+$, 2118.3837; found, 2118.5320. To a cooled (–78ºC) solution of the above obtained lactol (14 mg,

6.7 ⌈mol) and tetrabenzyl diphosphate (18 mg, 34 ⌈mol) in anhydrous THF (2 mL) was added dropwise lithium bis(trimethylsilyl)amide in THF (1.0 M, 20 ⌈L, 20 ⌈mol). The reaction mixture was stirred for 1 h, and then allowed to warm up to –20ºC. After stirring the reaction mixture for 1 h, it was quenched with saturated aqueous $NaHCO_3$ (10 mL), and extracted with ethyl acetate (15 mL). The organic phase was washed with brine (2 x 15 mL), dried ($MgSO_4$), filtered, and the filtrate was concentrated in vacuo. The residue was purified by Iatrobeads column chromatography (hexane/ethyl acetate, 5/1 3/1 1/1, v/v) to give **27** as a colorlesssyrup (13 mg, 81%).

3-*O*-[(*R*)-3-Hydroxy-dodecanoyl]-2-deoxy-2-[(*R*)-3-dodecanoyloxy-tetradecanoylamino]-®-D-glucopyranosyl-(1 6)-2-[(*R*)-3-hydroxy-dodecanoylamino]-3-*O*-[(*R*)-3-dodecanoyloxy-dodecanoyl]-2-deoxy-⟨-D-glucopyranoside 1, 4'-bisphosphate (**1**): A reaction mixture of **27** (10 mg, 4.2 ⌈mol) and Pd black (15 mg) in anhydrous THF (5 mL) was shaken under an atmosphere of H_2 (60 psi) at room temperature for 30 h, after which it was neutralized with triethylamine (10 ⌈L), and the catalyst was removed by filtration and the residue was washed with THF (2 x 1 mL). The combined filtrates were concentrated in vacuo to afford **1** as a colorless film (5.4 mg, 74%). ^{1}H NMR (500 MHz, $CDCl_3$): ™ 5.13 (bs, 1H, H-1), 4.84 (bs, 4H, H-3, H-3', 2 x H-3_L), 3.93 (m, 1H, H-2), 3.68 (m, 1H, H-3_S), 3.66 (m, 1H, H-3_S), 3.51 (m, H-2'), 3.17 (m, H, H-4), 2.33-1.95 (m, 12H, 2 x H-2_L, 2 x H-2_S, 2 x H-$2_{L'}$), 1.24 (bs, 12H, 2 x H-4_L, 2 x H-4_S, 2 x H-3_L), 0.91 [bs, 96H, 2 x H-(5_S-11_S), 2 x H-(5_L-13_L), 2 x H-($4_{L'}$-$11_{L'}$)], 0.54-0.52 (m, 18H, 2 x H-12_S, 2 x H-14_L, 2 x H-$12_{L'}$). HR MS (m/z) (negative) for $C_{88}H_{166}N_2O_{25}P_2$, 1713.1255; found, 1712.2797[M-H], 1713.2834[M].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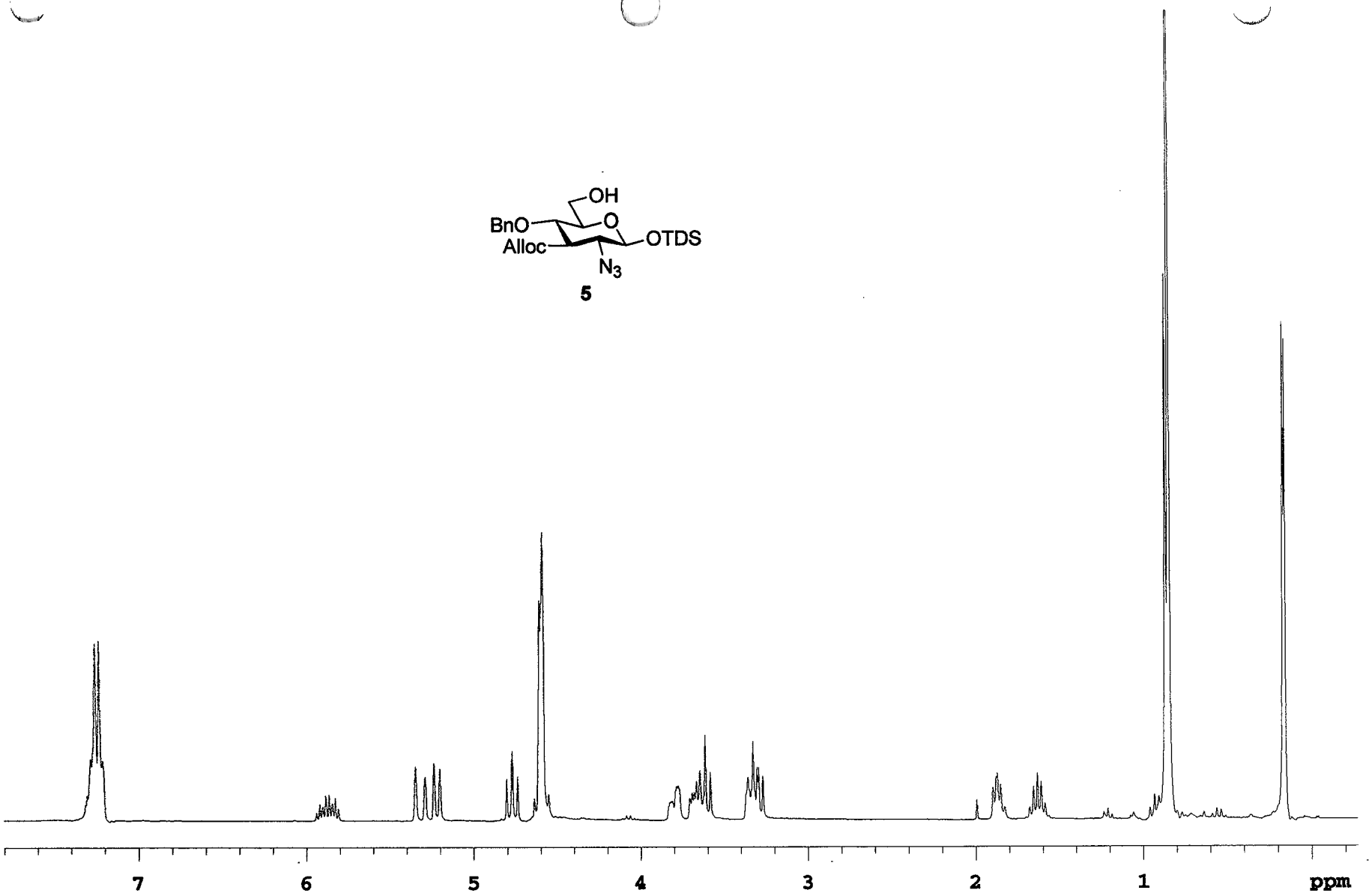
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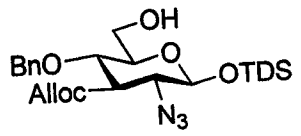

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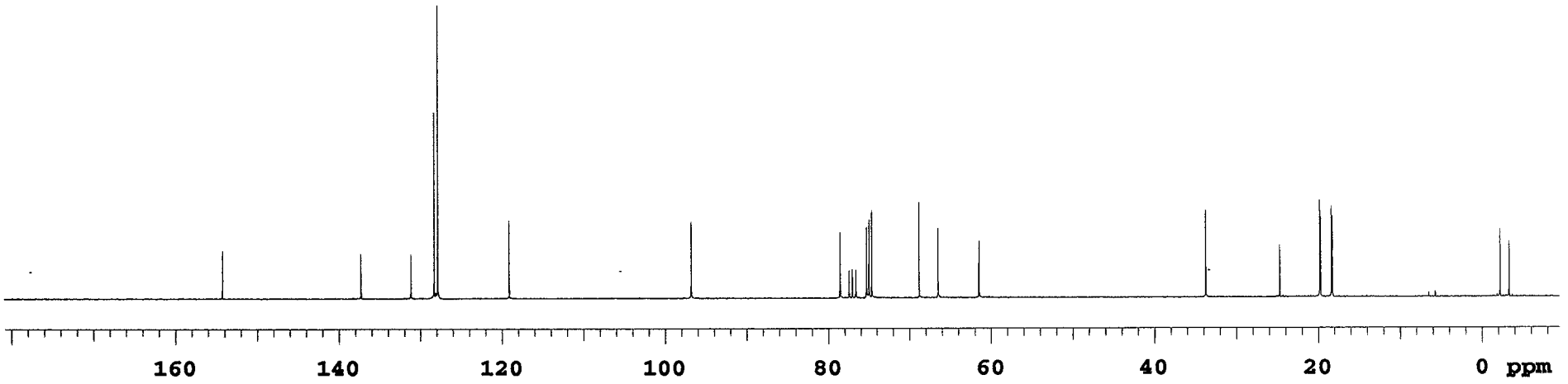

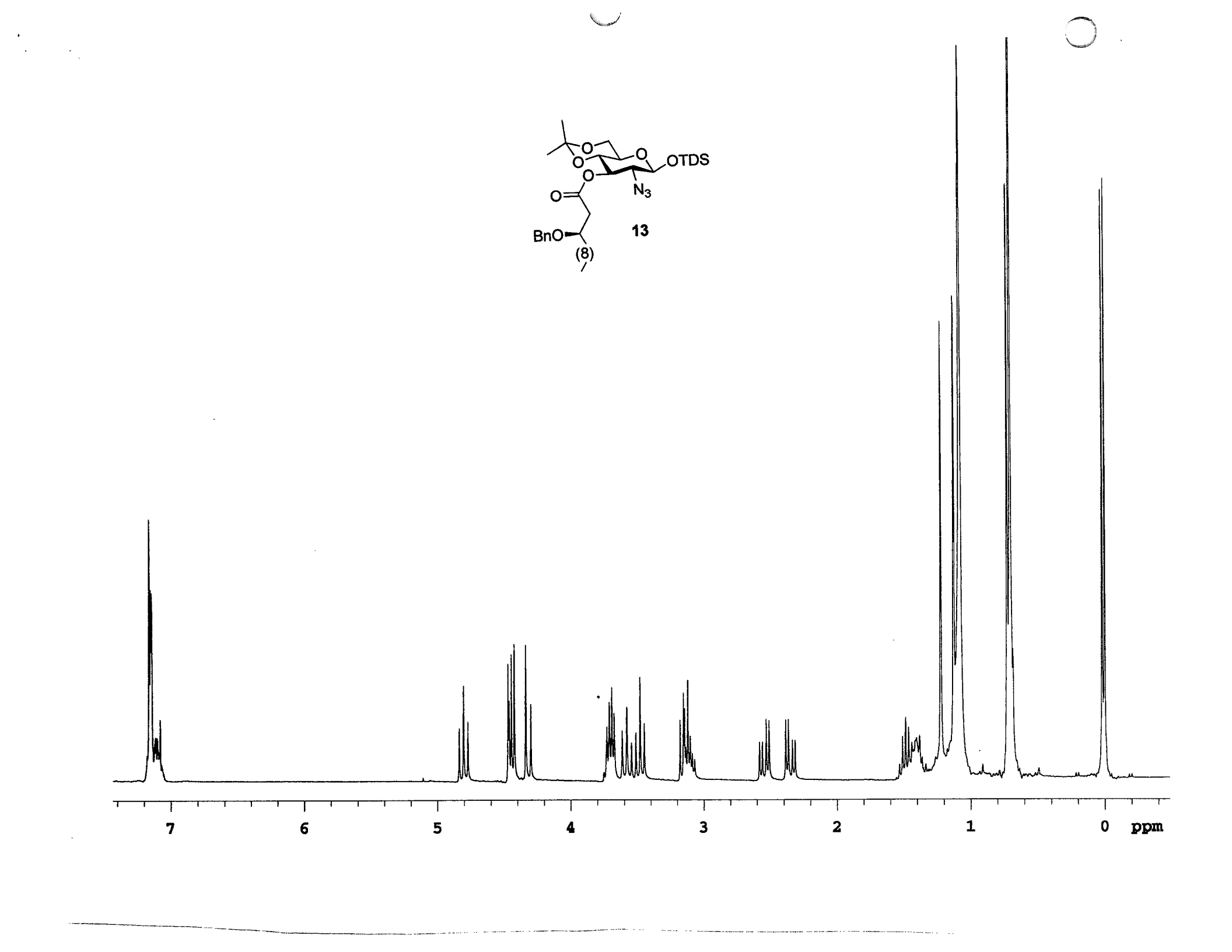
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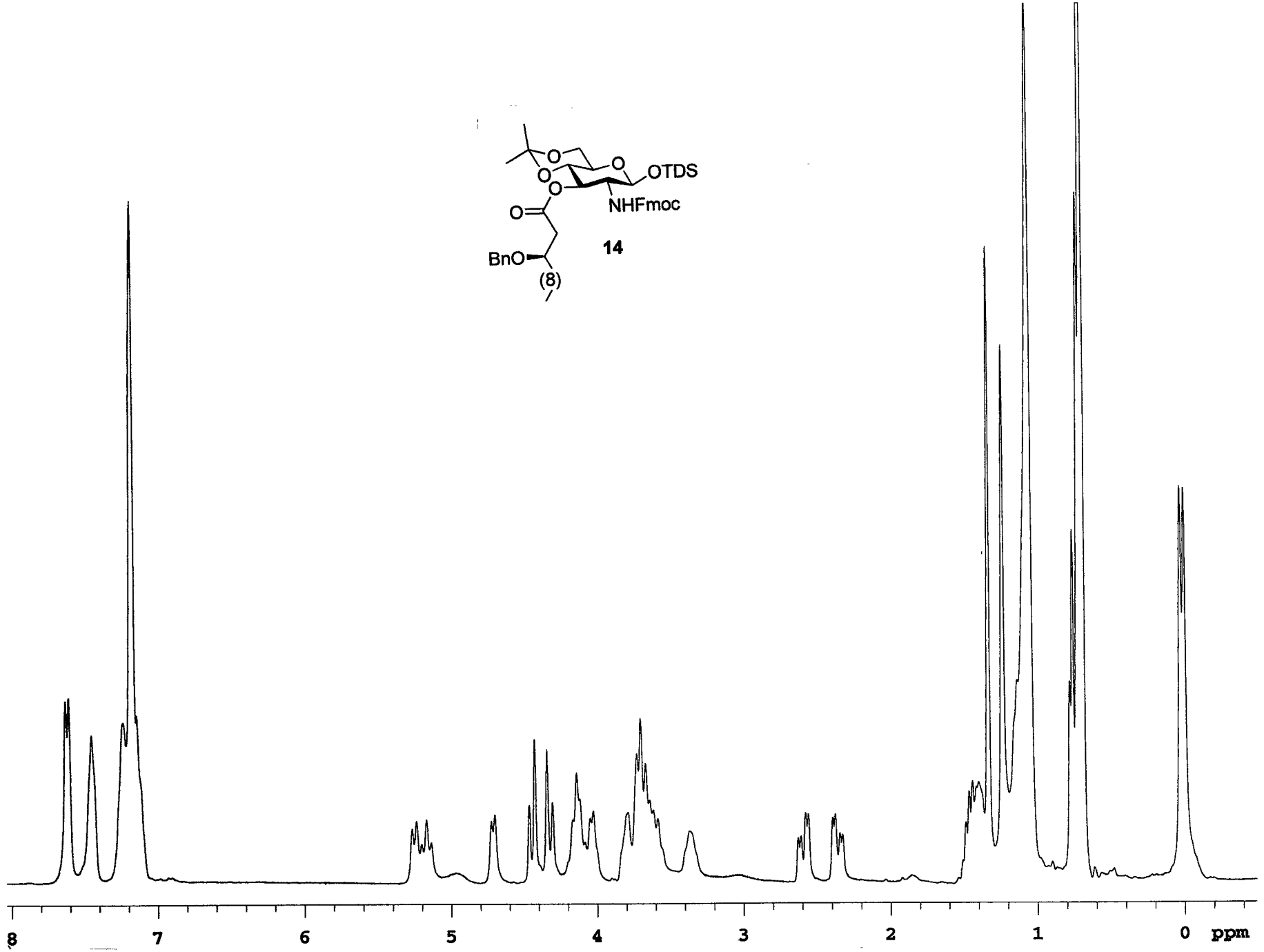
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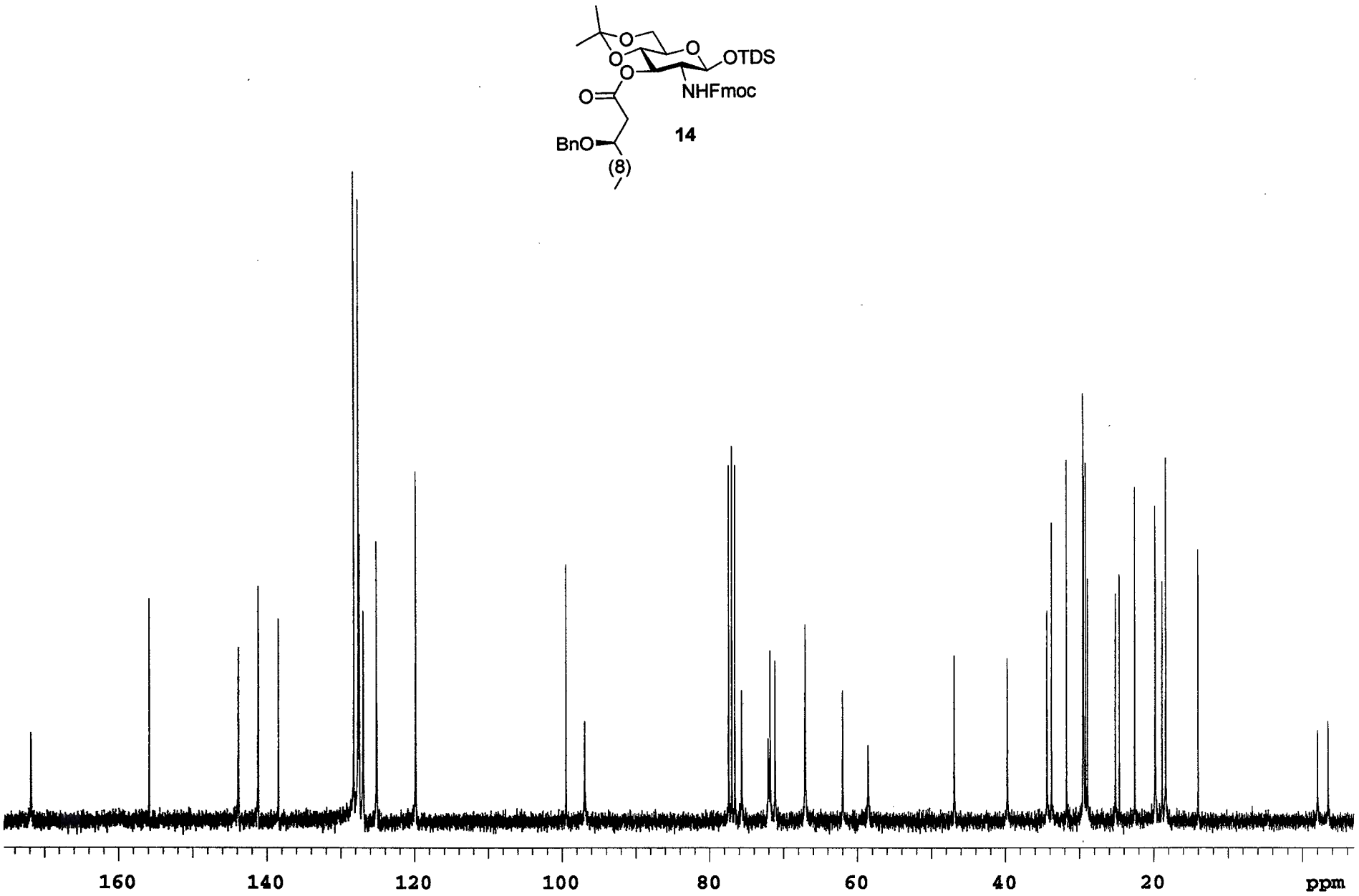
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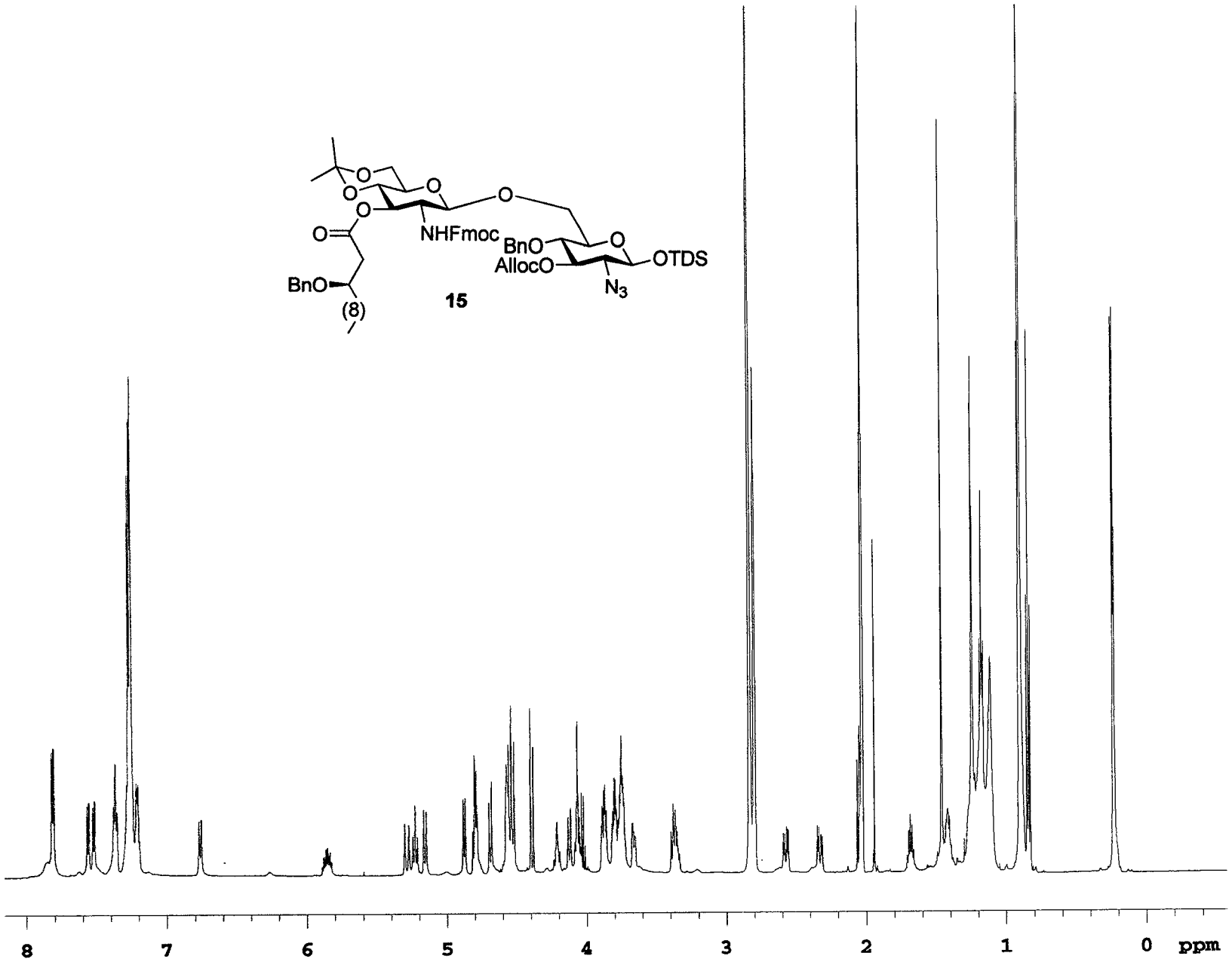
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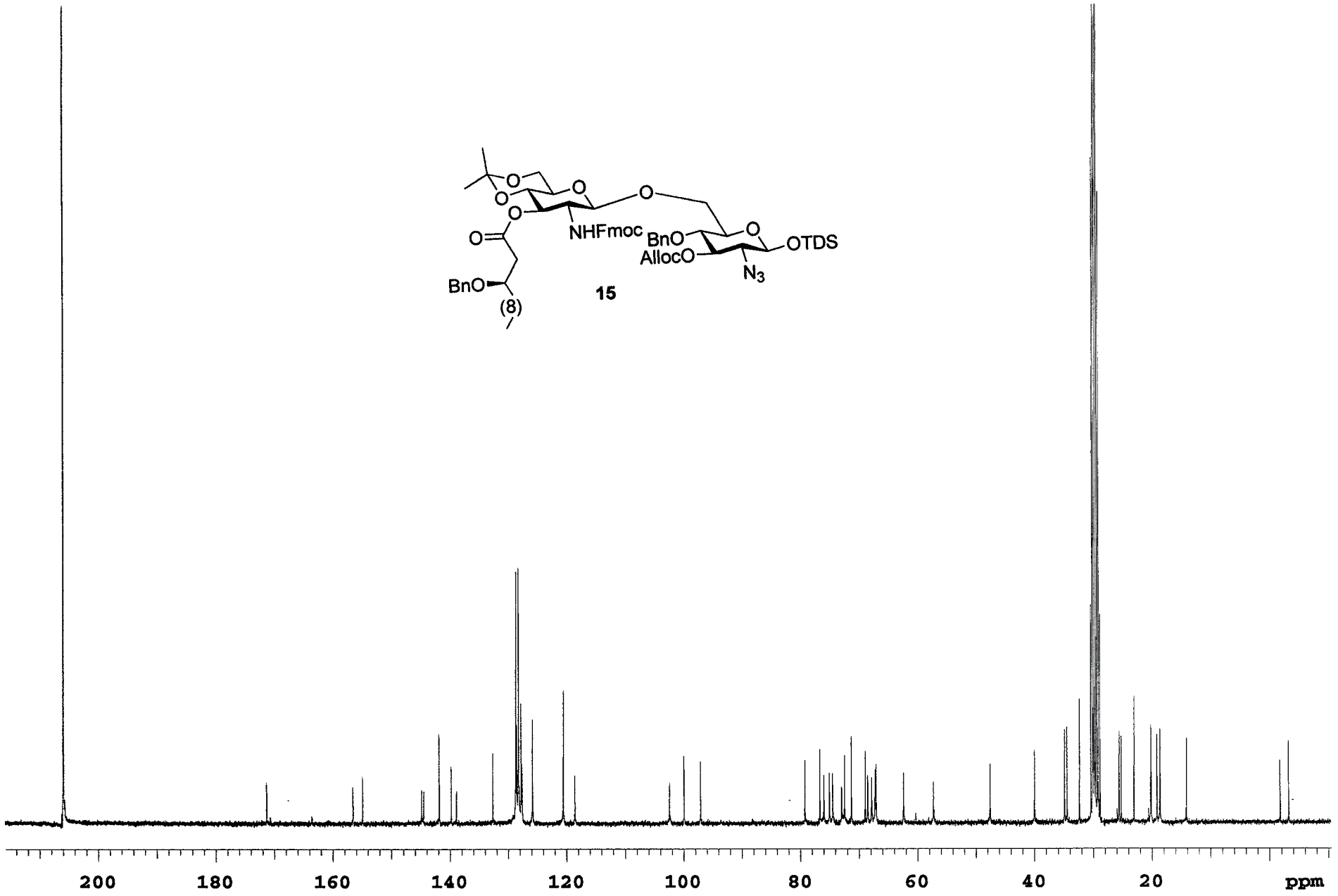
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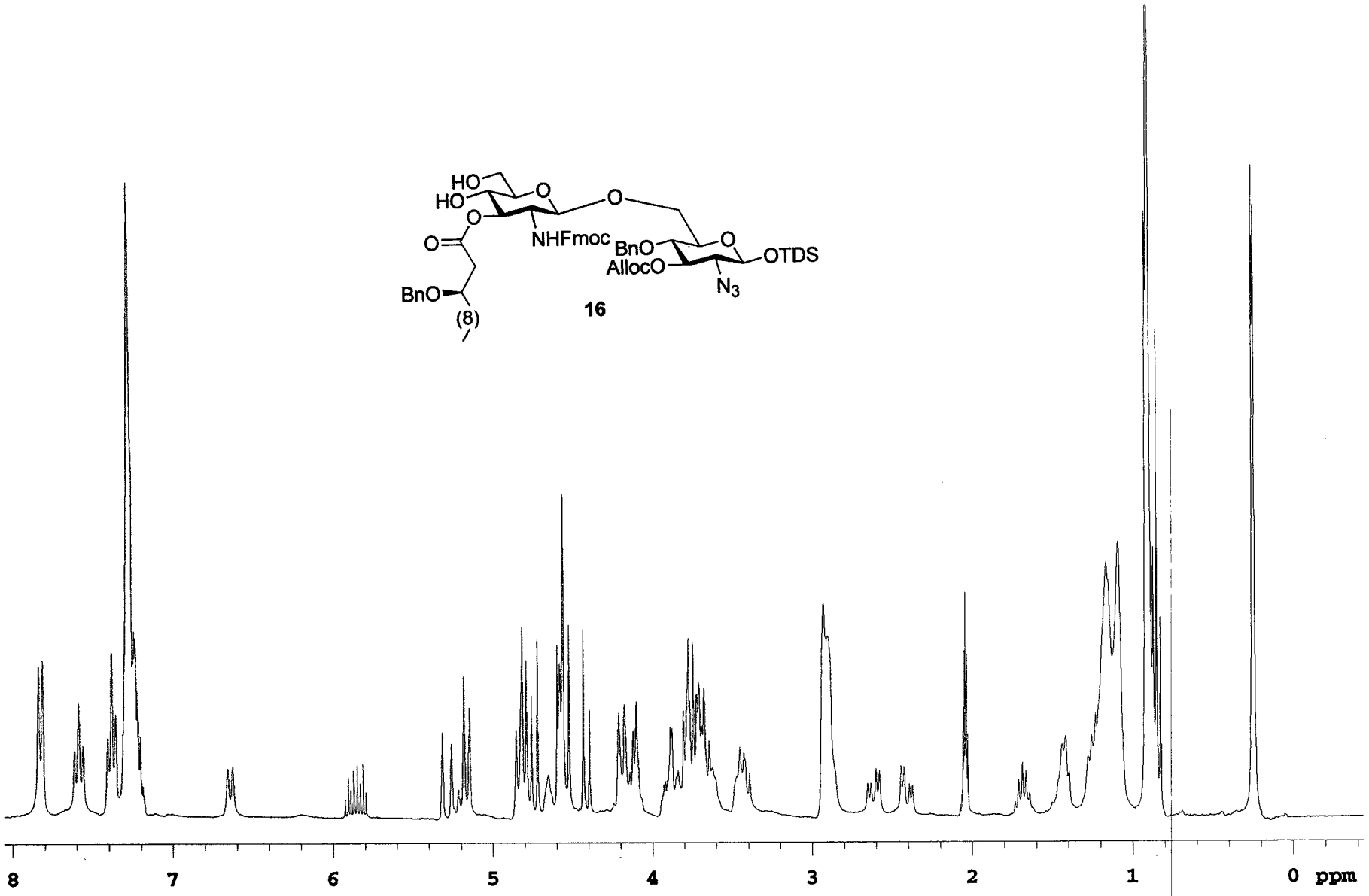
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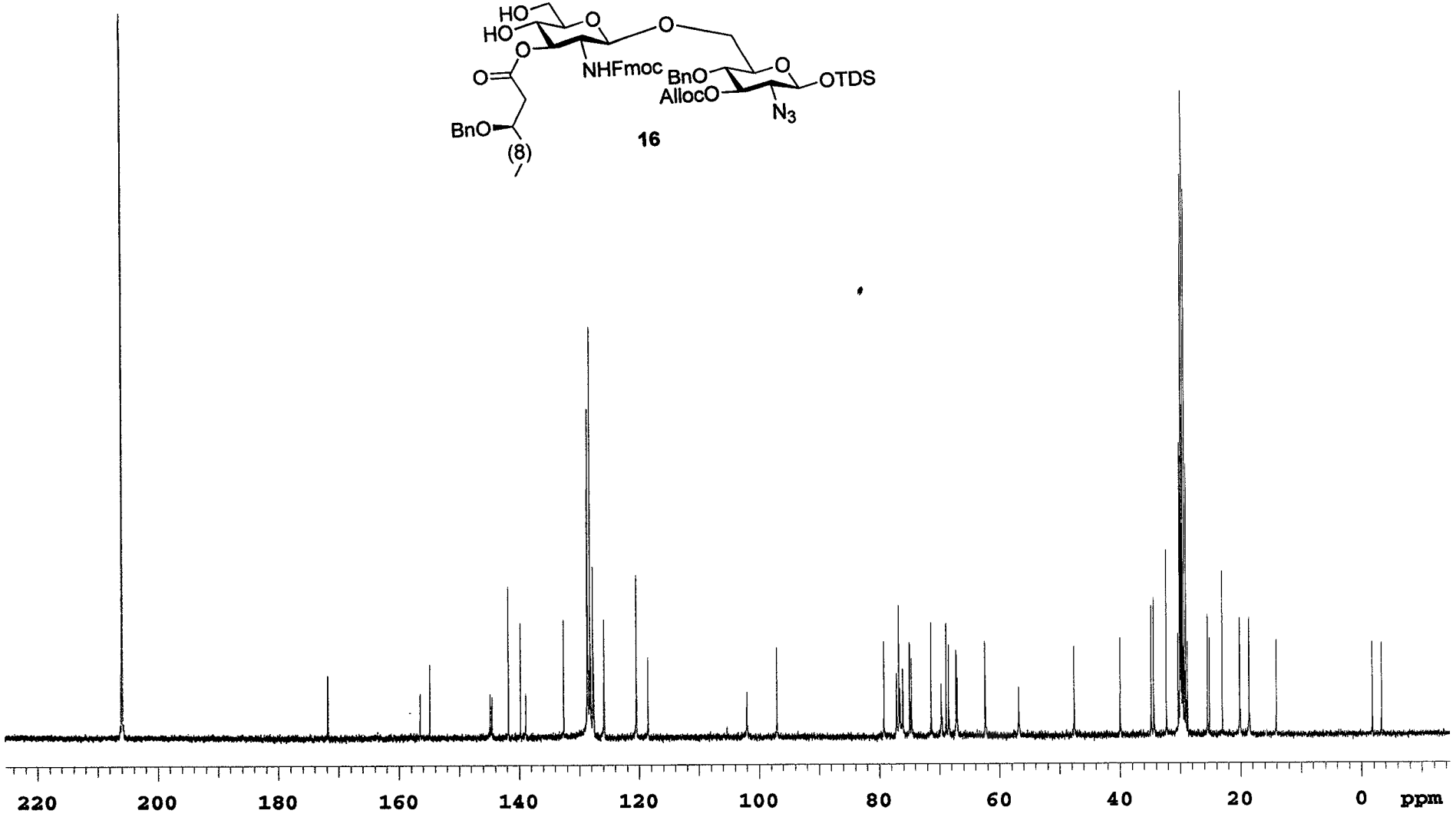
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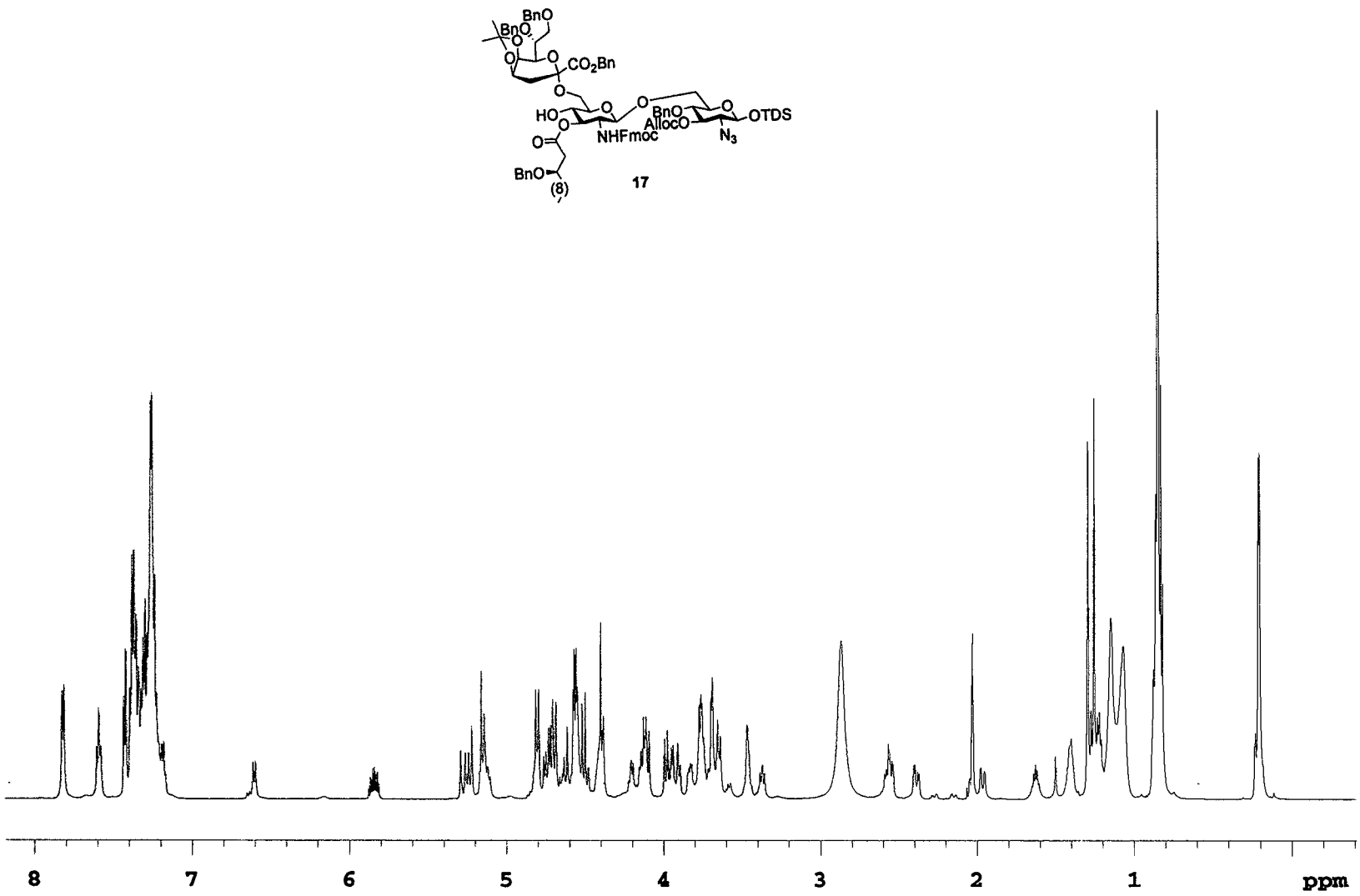
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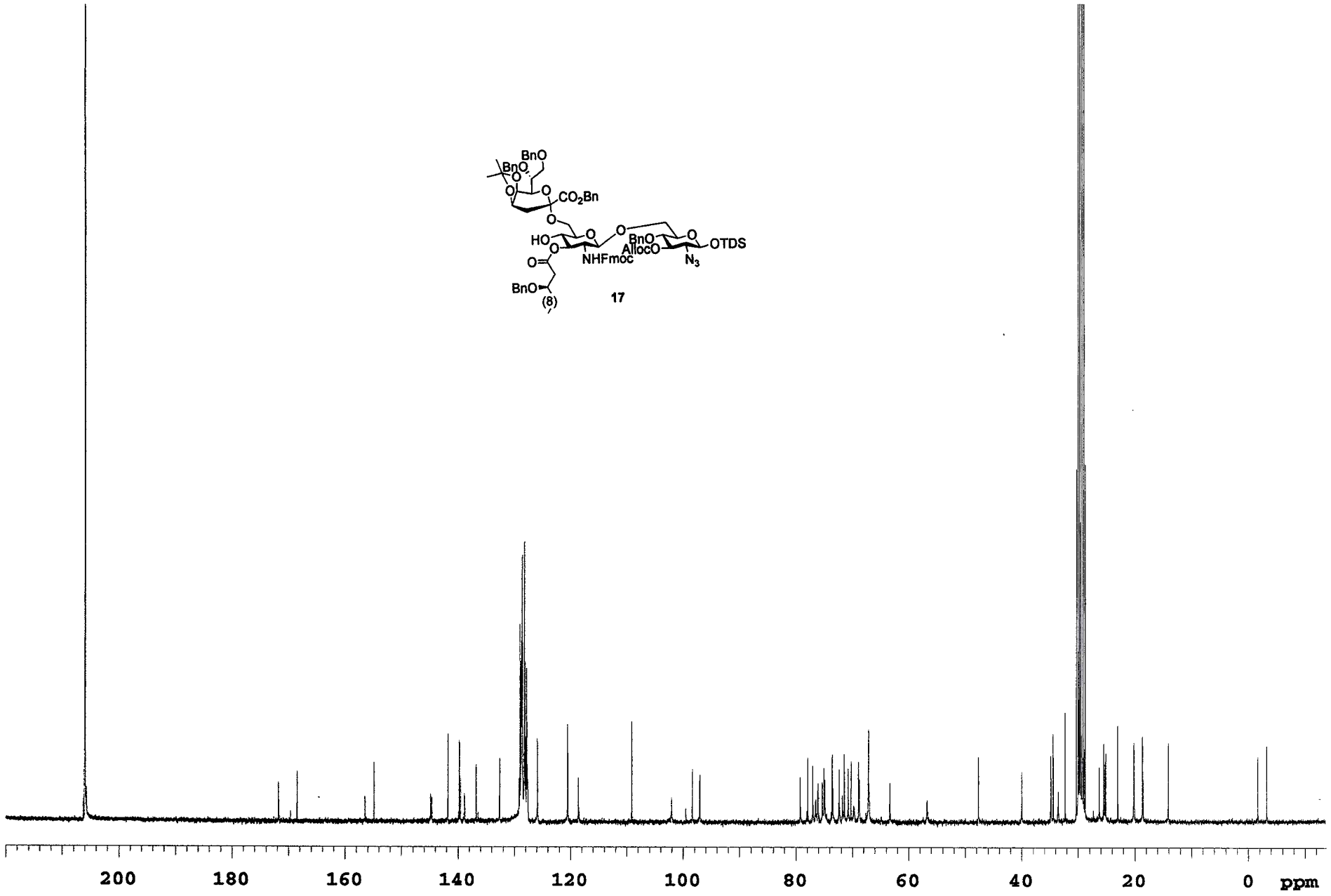
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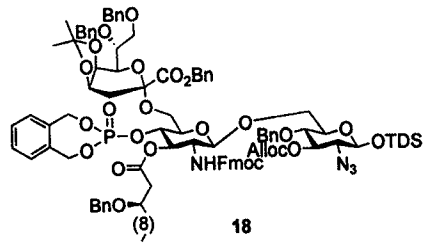

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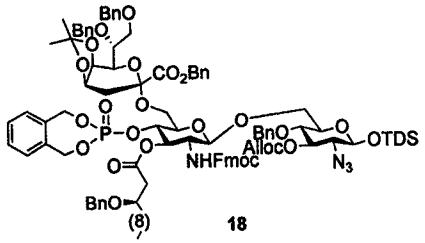

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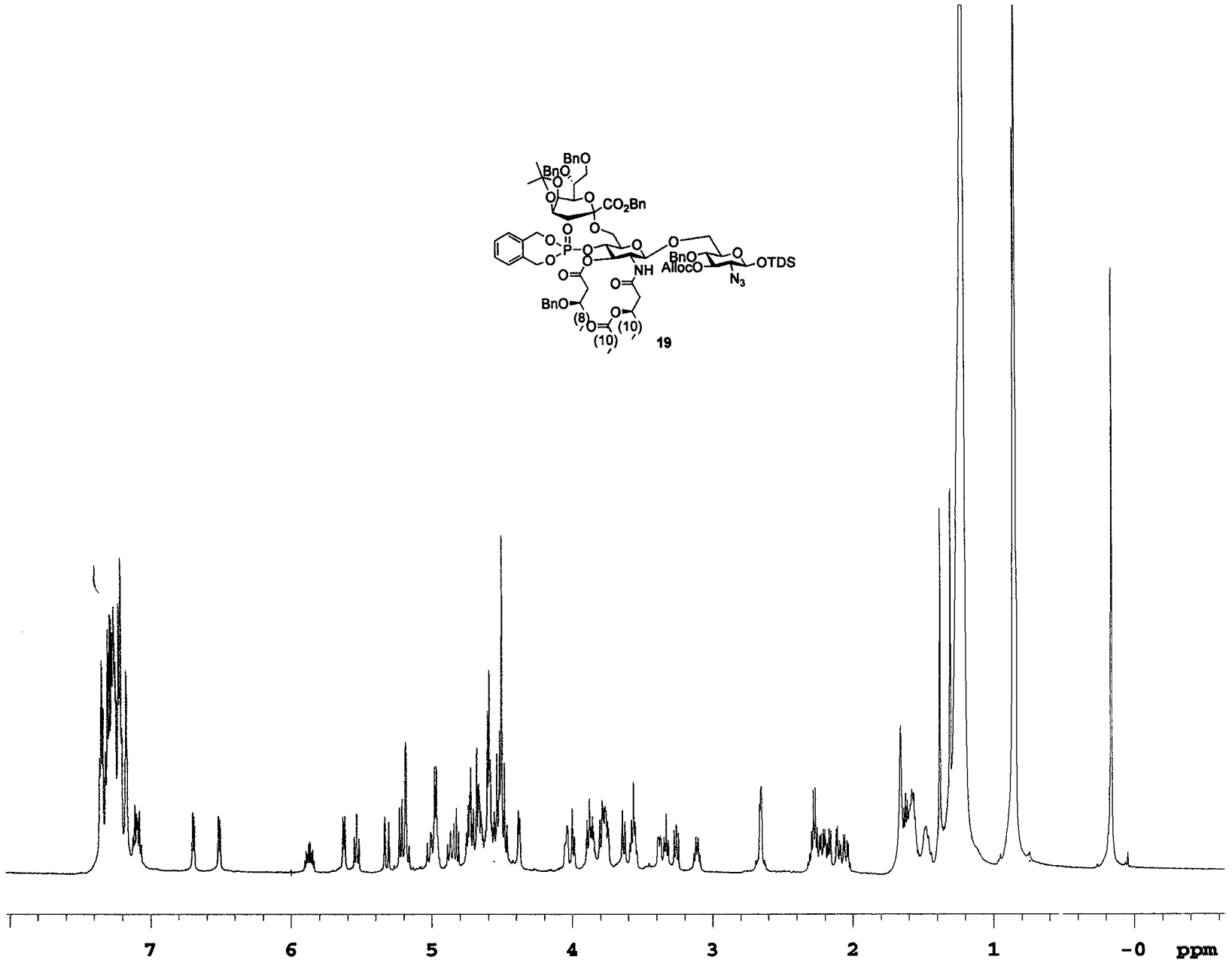
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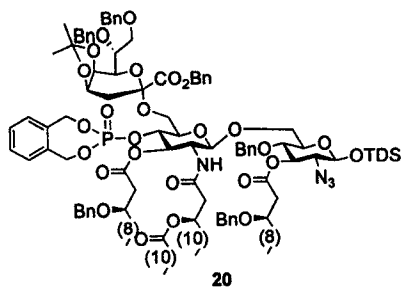
(8)
(10)
(10)
19



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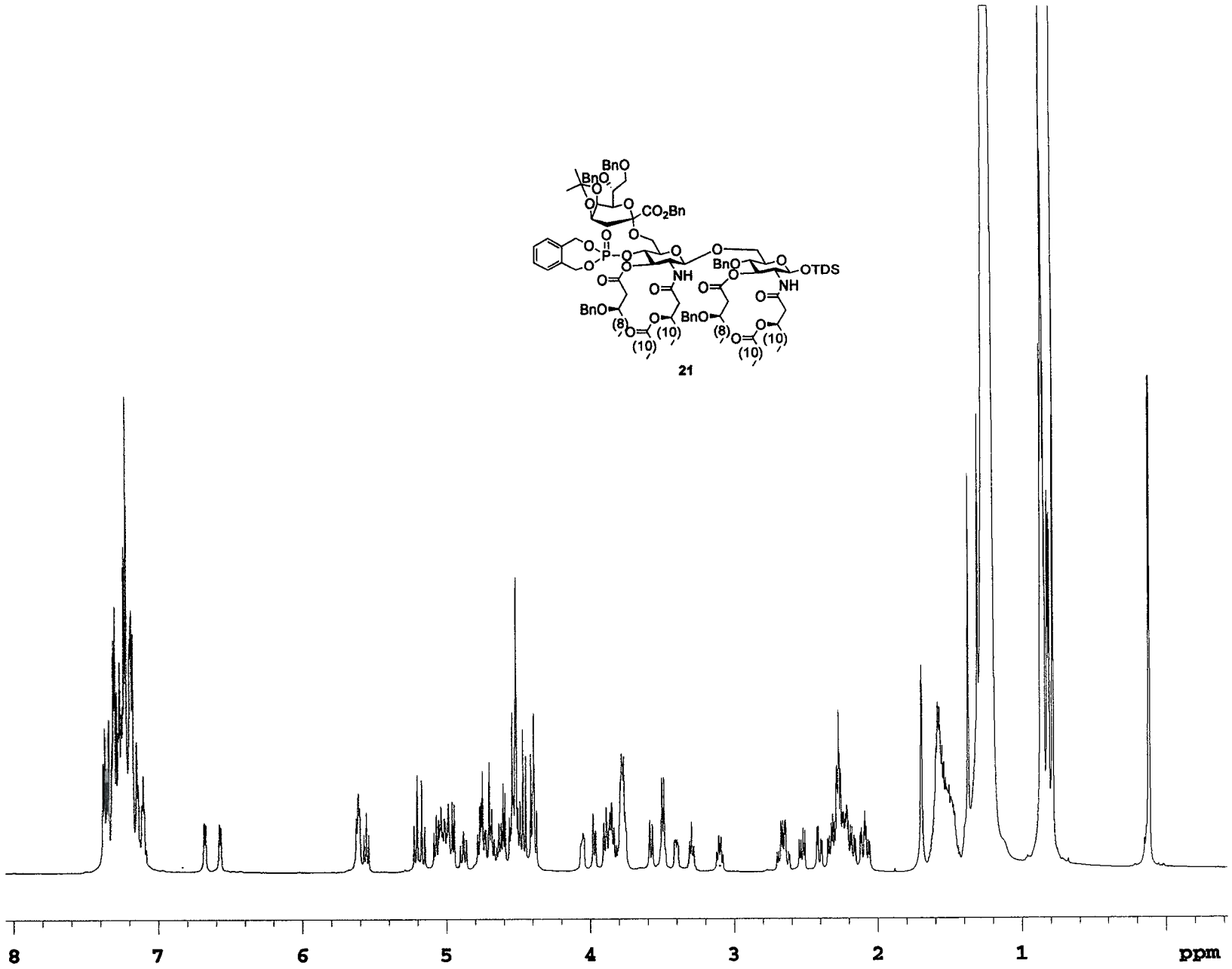

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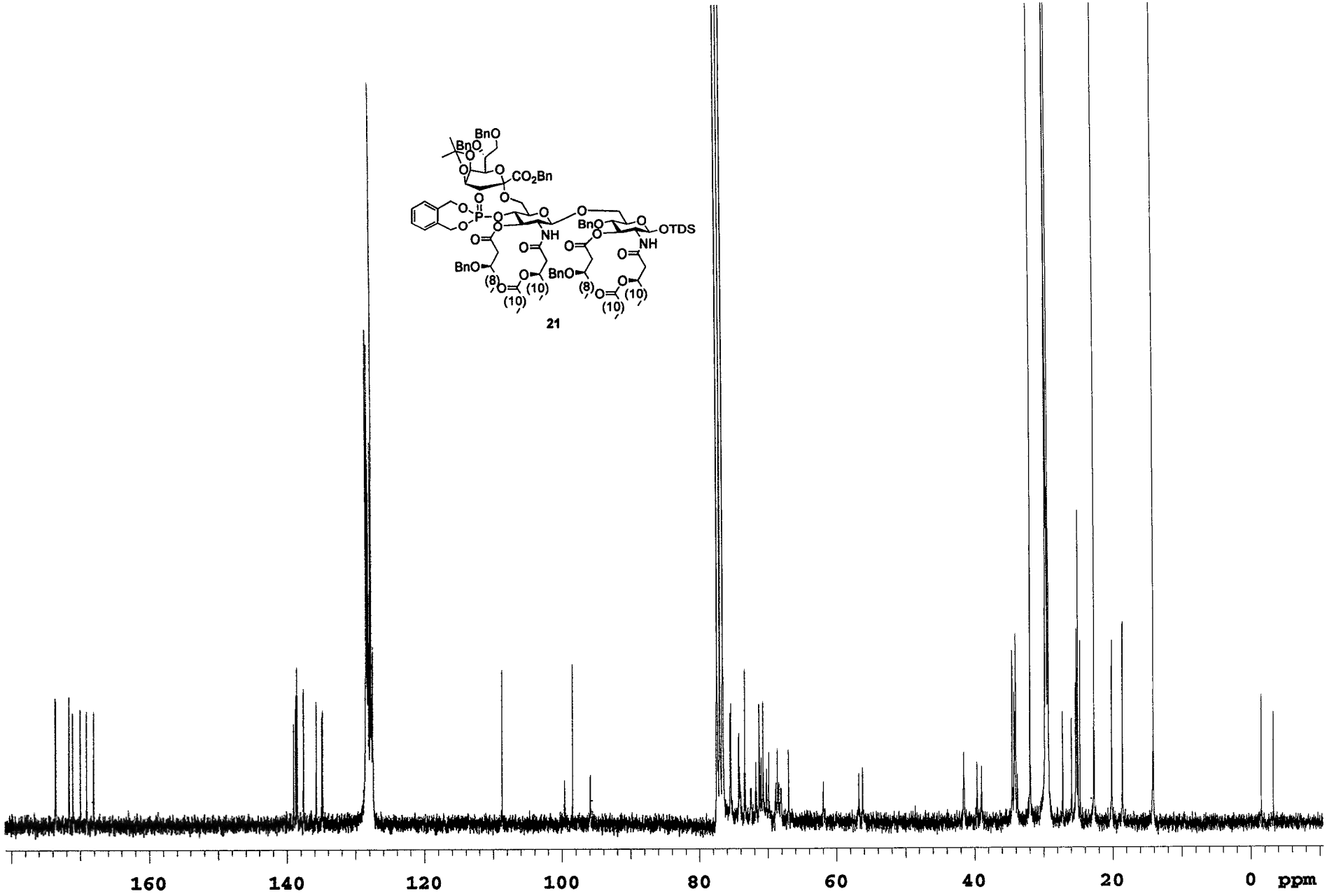
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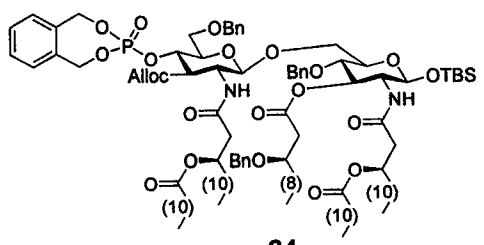
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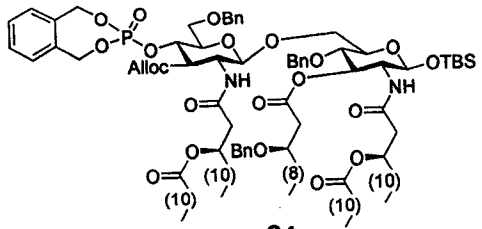
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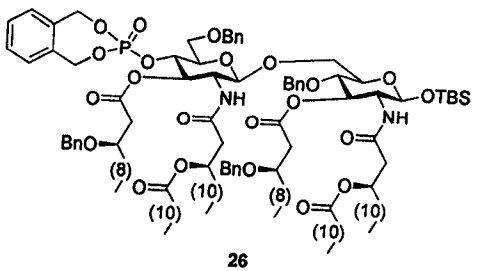

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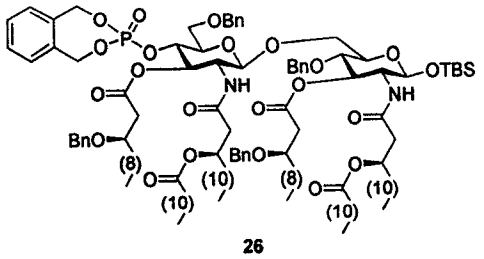
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OTBS
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BnO
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(10)
(10)

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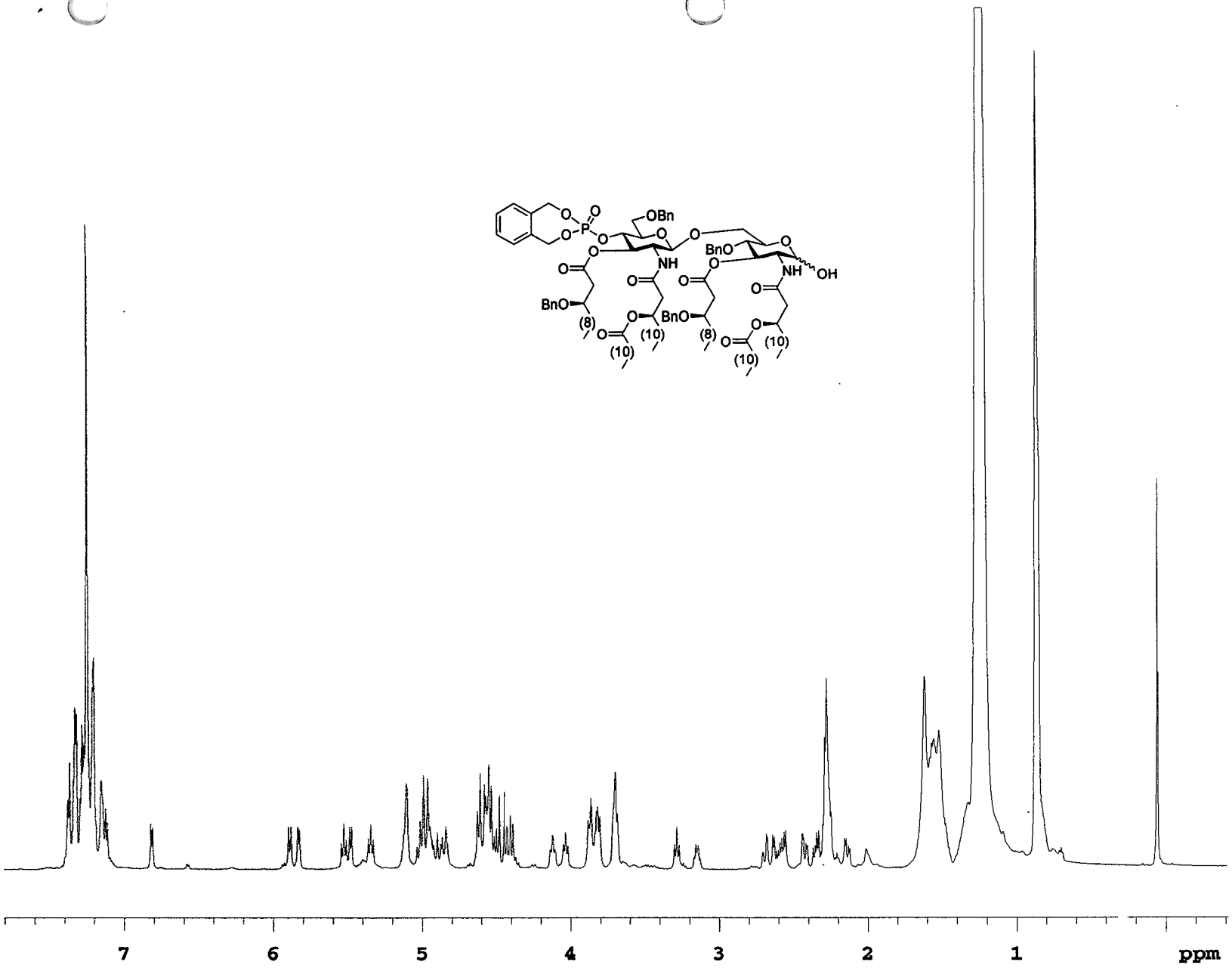
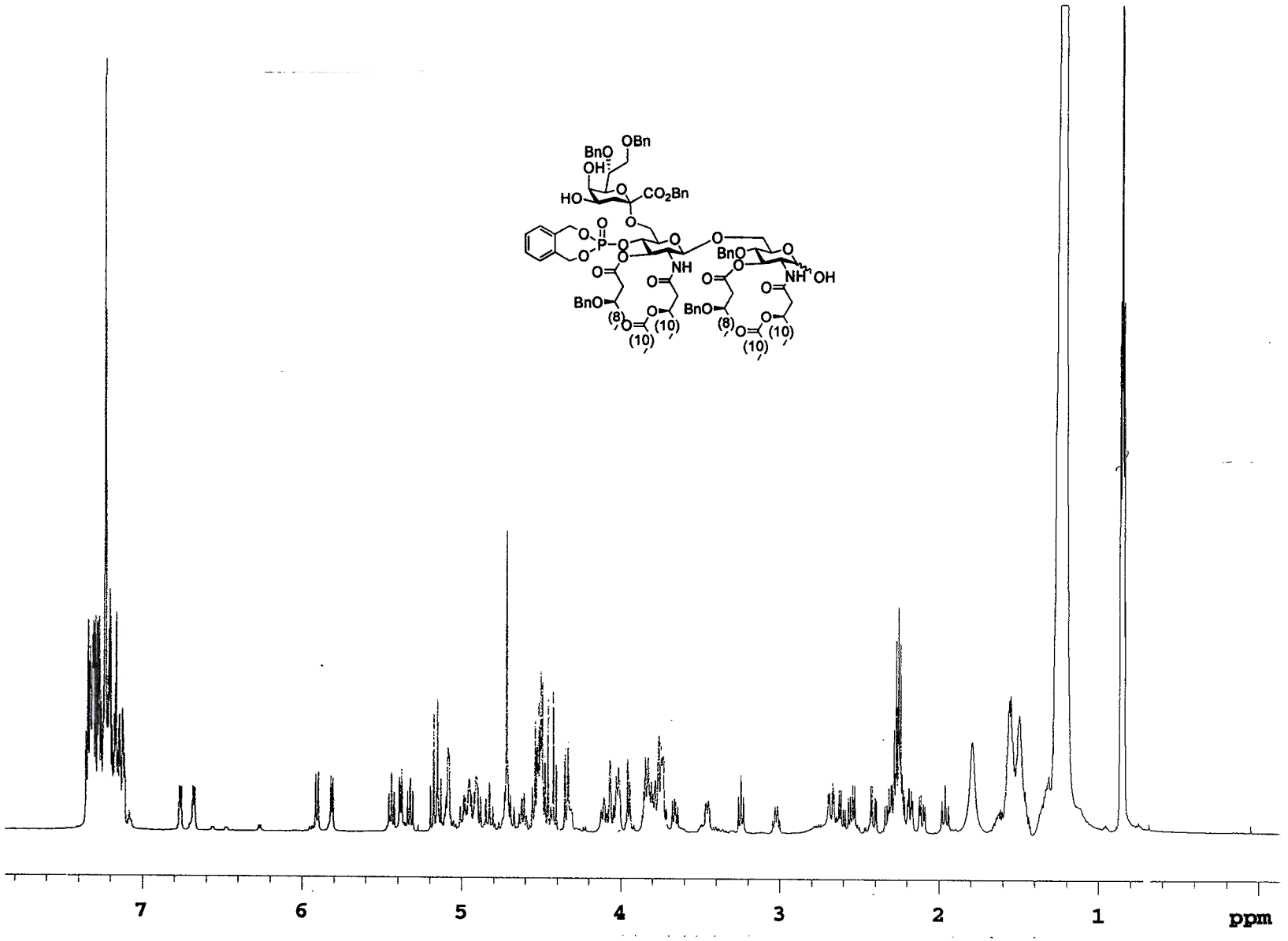
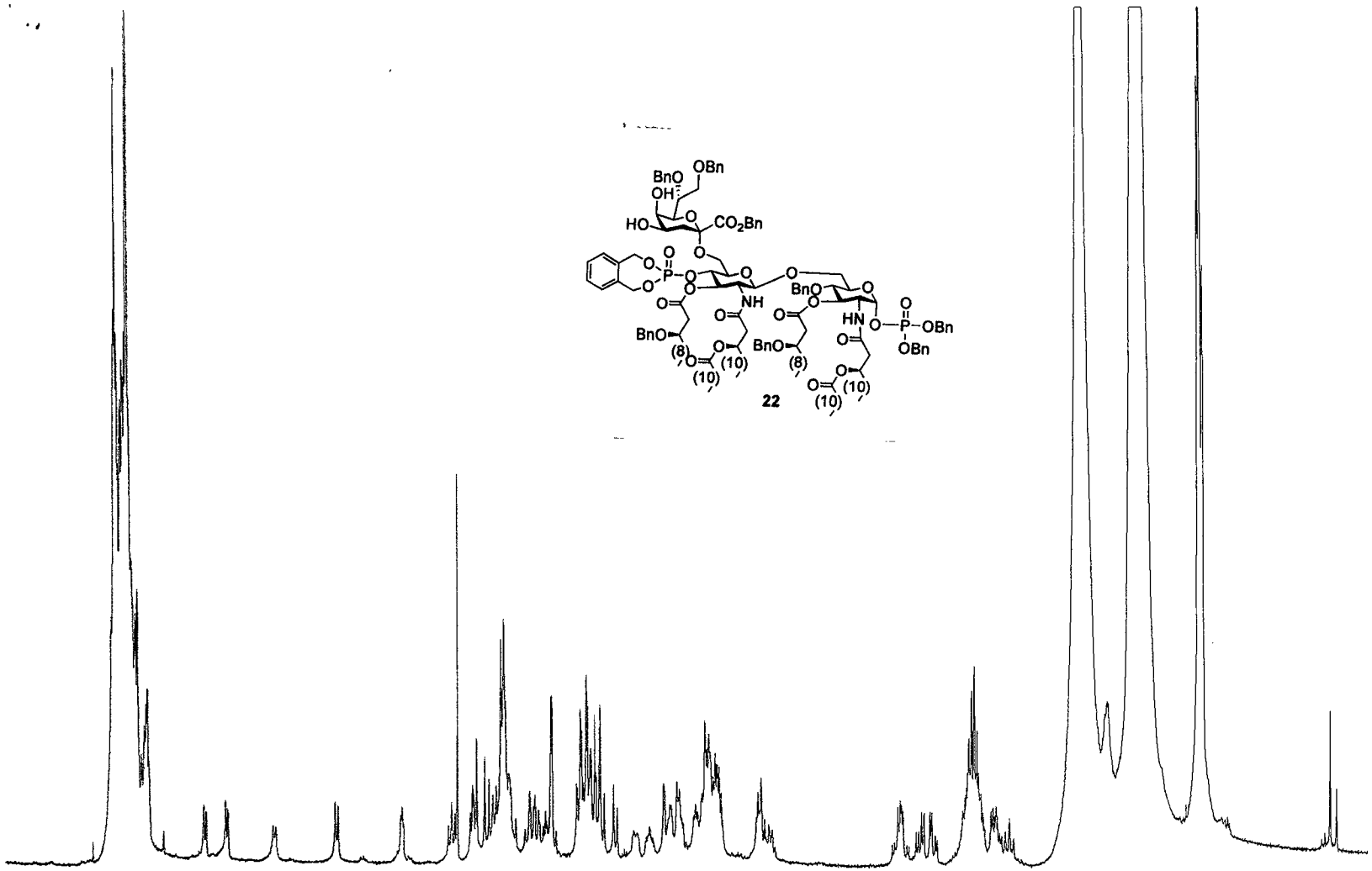
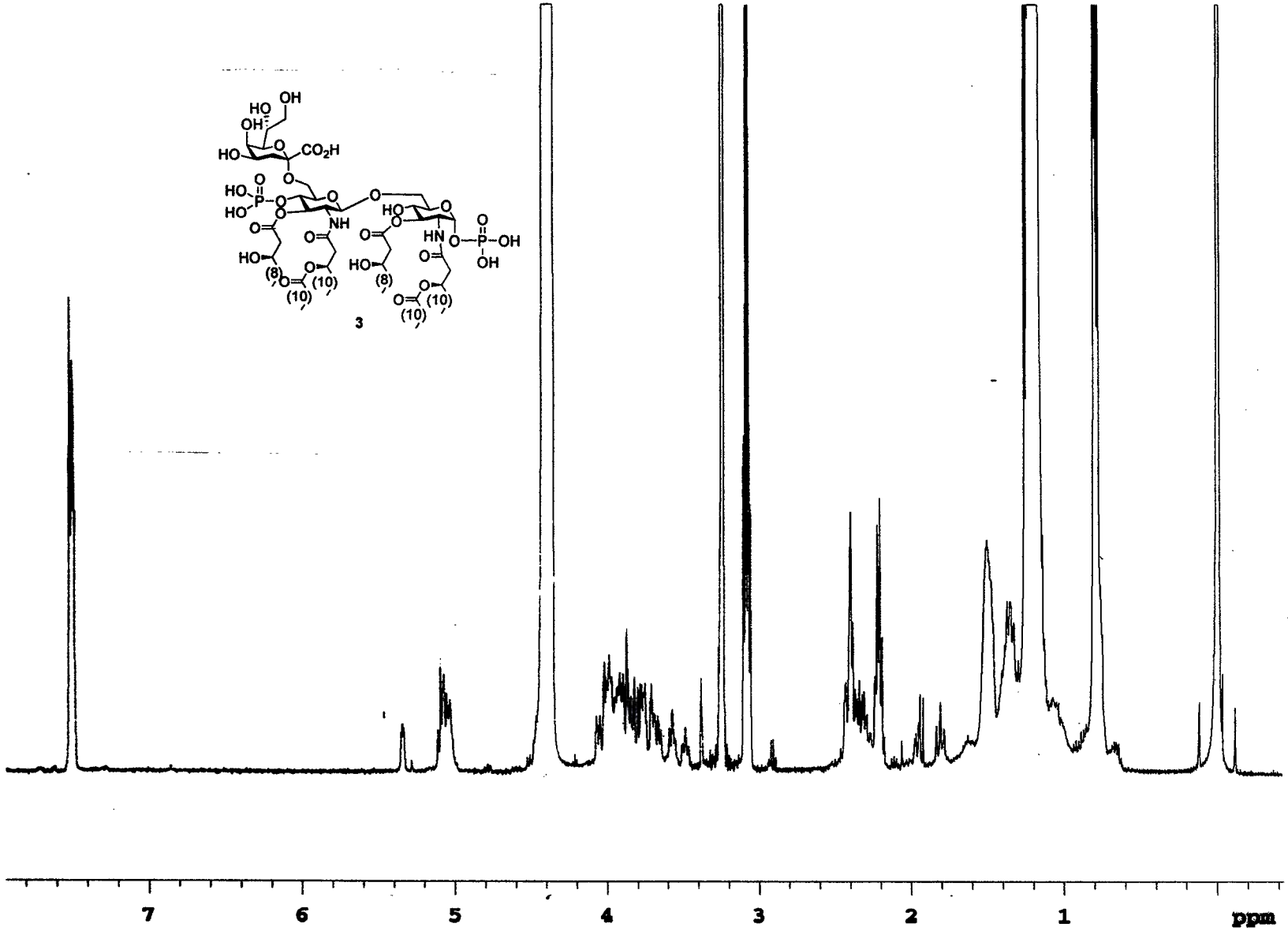

OBn
BnO
NH
OH
BnO
BnO
(8)
(10)
(10)
(8)
(10)
(10)
7
6
5
4
3
2
1
ppm

ppm
0
1
2
3
4
5
6
7
1


BnO
OBn
OH
HO
CO2Bn
O
P
NH
NH
OH
BnO
BnO
(8)
(10)
(10)
(8)
(10)
(10)
7
6
5
4
3
2
1
ppm


22
7
6
5
4
3
2
1
ppm


3
7
6
5
4
3
2
1
ppm