Supporting Information

# The 3-Deoxy Analogue of α-GalCer: Disclosing the Role of the 4-Hydroxyl Group for CD1d-Mediated NKT Cell Activation

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## *Table of Contents*



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## A. Figures

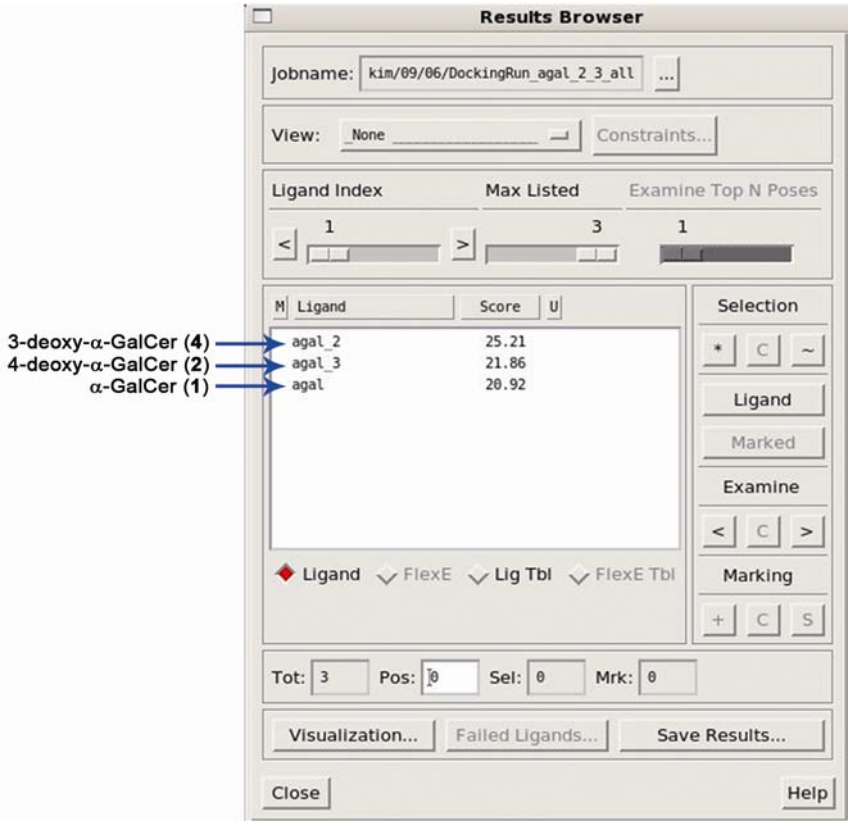



**Figure S1**. Surflex-Dock docking scores of compounds **1**, **2**, and **4**.

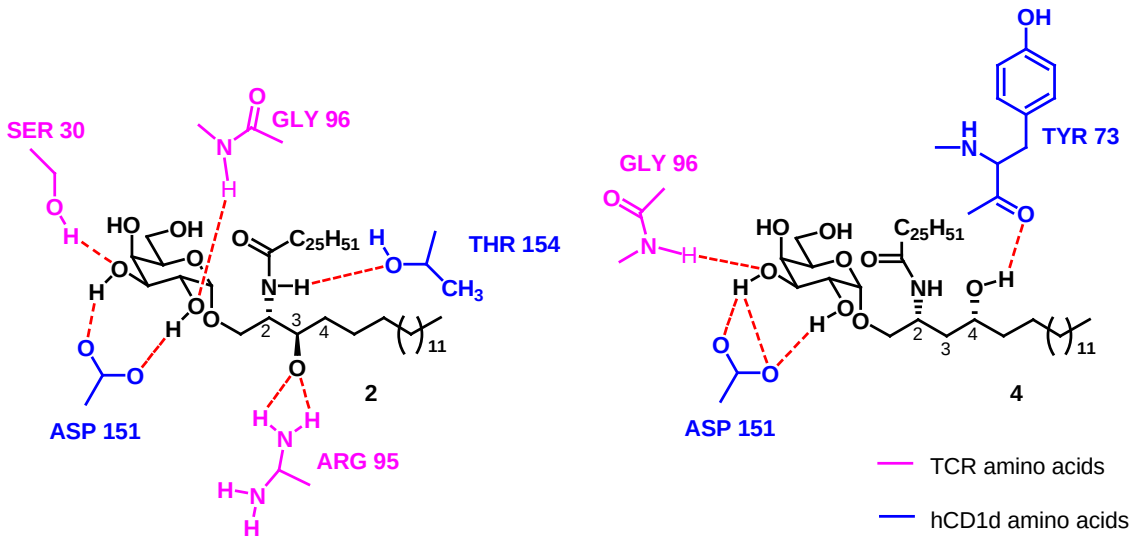



**Figure S2**. The hydrogen bonding modes of the docked compounds **2** and **4**.

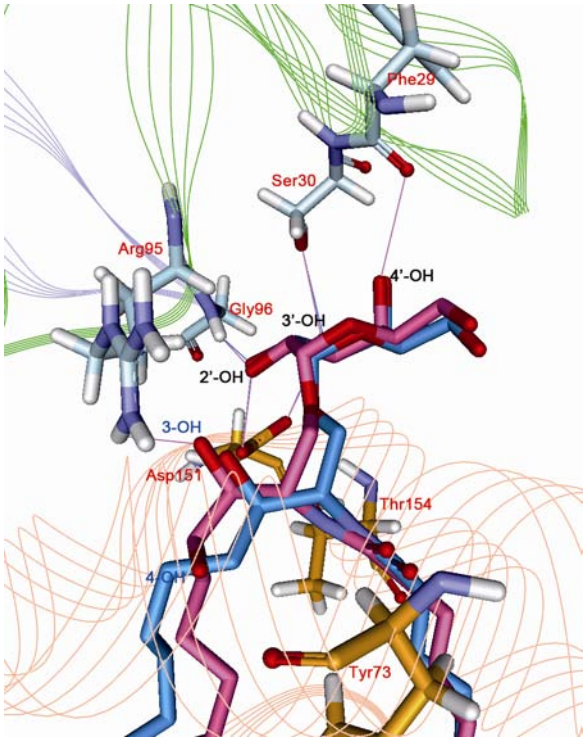



**Figure S3**. Comparison of the docking model of 4-deoxy-α-GalCer **2** (blue backbone) with the crystal structure of α-GalCer **1** (pink backbone, Protein Data Bank code 2PO6). The key amino acid residues are shown in light blue (TCR residues) and orange (CD1d residues).

## B. Experimental Methods

### (a) Biological methods

Glycolipids were dissolved in a 0.5% solution of Tween 20 in saline as a vehicle at a concentration of 200 μg/mL, and diluted with sterile PBS immediately prior to use.

**Determination of the stimulating activity for NKT hybridoma cells**[1,2]

Mouse CD1d-transfected rat basophilic leukemia (RBL) cells were loaded with 8 ng/mL α-GalCer (**1**), 4-deoxy-α-GalCer (**2**) or 3-deoxy-α-GalCer (**4**). After 4 h, free glycolipids were removed by washing three times with PBS 3, and the RBL cells were incubated with DN32.D3 NKT hybridoma cells for 16 h. The level of IL-2 secreted into the supernatant was determined by an Enzyme-Linked Immunosorbent Assay (ELISA).

**Evaluation of the cytokine levels produced by primary splenocytes**[3]

Splenocytes from naïve C57BL/6 mice were cultured in the presence of 0.5 ng/mL α-GalCer (**1**), 4-deoxy-α-GalCer (**2**)[4] or 3-deoxy-α-GalCer (**4**) for 72 h. The levels of IFN-γ and IL-4 secreted into the supernatant were determined by ELISA.

### (b) Molecular modeling methods

In order to perform docking studies of α-GalCer analogues with hCD1d of NKT, the Surflex-Dock program in Sybyl version 8.1.1 (Tripos Associates) was operated on an IBM computer (Intel Pentium 4, 2.8GHz CPU, 1GB memory) running the Red Hat Linux 4.0 operating system. The X-ray crystallographic structure of hCD1d complex (PDB code 2PO6) was used for docking analysis. The structures of the α-GalCer

analogues were drawn into the Sybyl package with standard bond lengths and angles and minimized using the conjugate gradient method. The Gasteiger-Hückel charge, with a distance-dependent dielectric function, was applied for the minimization process.

Surflex-Dock docks the molecules into a receptor-binding site that is defined to a protomol. The protomol is a unique and important factor of the docking algorithm and is a computational representation of assumed ligands that interact with the binding site. Surflex-Dock's scoring function contains hydrophobic, polar, repulsive, entropic, and solvation terms. After running Surflex-Dock on the α-GalCer derivatives, the scores of 20 docked conformers were ranked in a molecular spreadsheet, and the best total score conformer was chosen as the binding mode of the ligand to the active site.

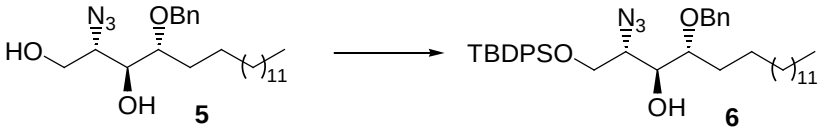
**(c) Synthetic procedures and compound characterization**

**General methods**

All chemicals were reagent grade and used as purchased. All reactions were performed under an inert atmosphere of dry argon or nitrogen and using dry distilled solvents. Reactions were monitored by TLC analysis using silica gel 60 F-254 thin layer plates. Flash column chromatography was carried out on silica gel (230–400 mesh). Optical rotations were measured using a polarimeter set for sodium D light (589.3 nm). $^{1}H$ NMR (500, 400 or 300 MHz) and $^{13}C$ NMR (100 or 75 MHz) spectra were recorded in δ units relative to the non-deuterated solvent as an internal reference. High-resolution mass spectra (HRMS) were recorded using fast atom bombardment (FAB). The purities of the products were >95% based on proton NMR spectra.

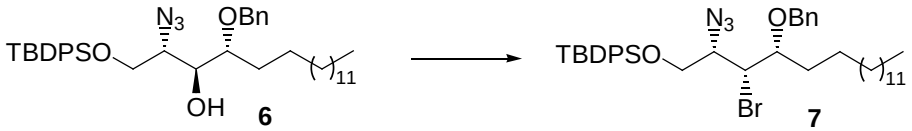
**Compound synthesis**

**(2*S*,3*S*,4*R*)-2-Azido-4-(benzyloxy)-1-(*tert*-butyldiphenylsilyloxy)octadecan-3-ol (6)**




Triethylamine (0.2 mL, 1.40 mmol) and TBDPS-Cl (0.29 mL, 1.13 mmol) were added to a cooled (0 °C) solution of **5** (405 mg, 0.93 mmol) in $CH_2Cl_2$ (10 mL). The reaction mixture was stirred at room temperature for 12 h and quenched with saturated aqueous $NaHCO_3$ solution. The mixture was then extracted with EtOAc. The organic extracts were washed with brine, dried over $Na_2SO_4$, and then concentrated under reduced pressure. The residue was purified using silica gel column chromatography (hexane/EtOAc, 20:1) to give **6** (622 mg, 99%) as a yellow liquid: $^1$H NMR (400 MHz, $CDCl_3$) δ 0.87 (t, *J* = 6.4 Hz, 3H), 1.07 (s, 9H), 1.13–1.32 (m, 24H), 1.39–1.64 (m, 2H), 2.36 (d, *J* = 3.7 Hz, 1-OH), 3.42–3.53 (m, 2H), 3.82–3.90 (m, 2H), 4.02 (dd, *J* = 2.7, 10.8 Hz, 1H), 4.45 (dd, *J* = 11.3, 18.0 Hz, 2H), 7.22–7.30 (m, 4H), 7.37–7.45 (m, 6H), 7.66–7.70 (m, 5H); $^{13}$C NMR (100 MHz, $CDCl_3$) δ 14.1, 19.1, 22.7, 25.2, 26.7, 28.3, 29.3, 29.57, 29.61, 29.65, 29.7, 31.9, 63.4, 64.9, 70.3, 71.8, 79.5, 127.77, 127.83, 128.4, 129.8, 132.80, 132.84, 135.6, 138.1; HRMS (FAB) calcd for $C_{41}H_{62}O_3N_3Si$ 672.4560 ($[M+H]^+$), found 672.4537.

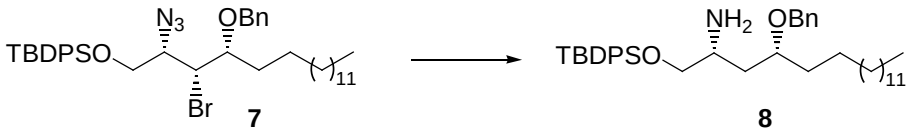
**((2*S*,3*R*,4*R*)-2-Azido-4-(benzyloxy)-3-bromooctadecyloxy)(*tert*-butyl)diphenylsilane (7)**




Triphenylphosphine (498 mg, 1.92 mmol) and carbon tetrabromide (630 mg, 1.92 mmol) were added to a solution of **6** (318 mg, 0.48 mmol) in $CH_2Cl_2$ (10 mL). The

reaction mixture was stirred at room temperature for 1 day. After the addition of EtOAc, the solid was filtered. The filtrate was evaporated and the crude product was purified using silica gel column chromatography (hexane/EtOAc, 20:1) to give **7** (250 mg, 72%) as a yellow liquid; $^{1}$H NMR (400 MHz, $CDCl_3$) δ 0.81 (t, *J* = 6.7 Hz, 3H), 1.02 (s, 9H), 1.12–1.25 (m, 24H), 1.32–1.76 (m, 2H), 3.47–3.51 (m, 1H), 3.68–3.82 (m, 3H), 4.20–4.23 (m, 1H), 4.47 (dd, *J* = 11.2, 19.6 Hz, 2H), 7.16–7.20 (m, 4H), 7.26–7.40 (m, 6H), 7.57–7.62 (m, 5H); $^{13}$C NMR (100 MHz, $CDCl_3$) δ 14.1, 19.1, 22.7, 25.5, 26.8, 26.82, 26.84, 29.36, 29.43, 29.51, 29.55, 29.6, 29.65, 29.69, 31.9, 55.6, 63.9, 65.0, 72.1, 79.9, 127.7, 127.76, 127.81, 127.84, 127.9, 128.4, 130.0, 135.57, 135.61, 135.63, 135.7, 137.9; HRMS (FAB) calcd for $C_{41}H_{61}BrO_2N_3Si$ 734.3716 ($[M+H]^+$), found 734.3742.

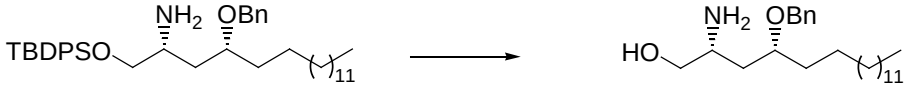
**(2*R*,4*R*)-4-(Benzyloxy)-1-(*tert*-butyldiphenylsilyloxy)octadecan-2-amine (8)**




Nikel(II) chloride (79 mg, 0.61 mmol) and sodium borohydride (46 mg, 1.22 mmol) were added to a solution of **7** (225 mg, 0.31 mmol) in EtOH (6 mL) at room temperature. The reaction mixture was stirred for 12 h and the solid was removed through a pad of Celite. The filtrate was extracted with EtOAc and water. The organic layer was washed with brine, dried over $Na_2SO_4$, and then concentrated under reduced pressure. The residue was purified using silica gel column chromatography ($CH_2Cl_2$/MeOH, 10:1) to give **8** (164 mg, 85%) as a white solid: $^{1}$H NMR (300 MHz, $CDCl_3$) δ 0.83 (t, *J* = 6.7 Hz, 3H), 1.01 (s, 9H), 1.12-1.27 (m, 24H), 1.32–1.60 (m, 2H), 2.00–2.18 (m, 2H), 2.97–3.05 (m, 1H), 3.38–3.44 (m, 2H), 3.56–3.64 (dd, *J* = 4.1, 9.9 Hz, 1H), 4.45 (d, *J* = 11.6

Hz, 1H), 4.31 (d, *J* = 11.3 Hz, 1H), 7.16–7.26 (m, 4H), 7.28–7.40 (m, 6H), 7.58–7.63 (m, 5H); $^{13}$C NMR (100 MHz, $CDCl_3$) δ 14.1, 19.3, 22.6, 25.0, 25.1, 26.9, 29.3, 29.5, 29.7, 29.8, 31.9, 33.8, 38.0, 51.0, 68.6, 70.5, 77.5, 127.4, 127.56, 127.59, 127.67, 127.70, 127.73, 128.3, 129.7, 133.5, 135.55, 135.58, 135.7, 138.7; HRMS (FAB) calcd for $C_{41}H_{64}O_2NSi$ 630.4706 ($[M+H]^+$), found 630.4706.

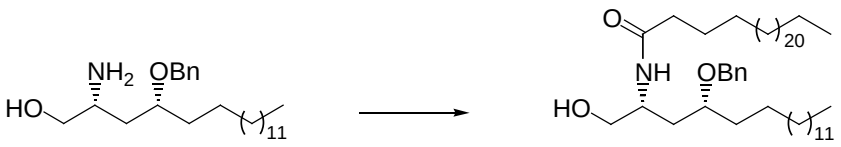
**(2*R*,4*R*)-2-Amino-4-(benzyloxy)octadecan-1-ol (9)**




**8** **9**

TBAF (0.36 mL, 1.0 M solution in THF, 0.36 mmol) was added to a solution of **8** (112 mg, 0.178 mmol) in THF (3 mL) at room temperature. The reaction mixture was stirred for 12 h and then diluted with water and extracted with $CH_2Cl_2$. The organic layer was washed with brine, dried over $Na_2SO_4$, and then concentrated under reduced pressure. The crude product was purified using silica gel column chromatography ($CH_2Cl_2$/MeOH, 10:1 to 5:1) to give **9** (56 mg, 80%) as a white solid: $^1$H NMR (400 MHz, $CDCl_3$) δ 0.85 (t, *J* = 6.7 Hz, 3H), 1.15–1.31 (m, 24H), 1.33–1.49 (m, 3H), 1.57–1.76 (m, 2H), 1.82–1.90 (m, 1H), 3.25–3.30 (m, 1H), 3.40–3.43 (m, 1H), 3.57–3.64 (m, 2H), 3.73 (dd, *J* = 2.8, 12.0 Hz, 1H), 4.43 (d, *J* = 11.5 Hz, 1H), 4.55 (d, *J* = 11.4 Hz, 1H), 7.20–7.31 (m, 5H); $^{13}$C NMR (100 MHz, $CDCl_3$) δ 14.1, 19.7, 22.7, 24.0, 24.6, 29.3, 29.56, 29.65, 29.7, 29.8, 31.9, 33.1, 33.6, 52.9, 58.9, 62.0, 70.3, 127.9, 128.2, 128.7, 137.7; HRMS (FAB) calcd for $C_{25}H_{46}O_2N$ 392.3529 ($[M+H]^+$), found 392.3530.

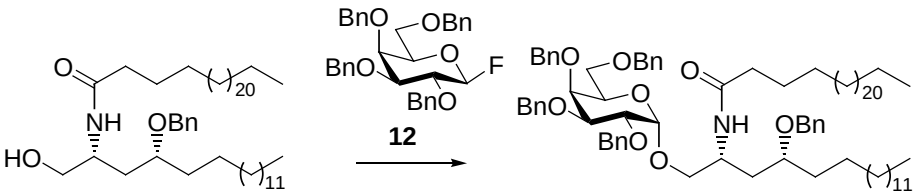
***N*-((2*R*,4*R*)-4-(Benzyloxy)-1-hydroxyoctadecan-2-yl)hexacosanamide (10)**




**9** **10**

EDCI (197 mg, 0.26 mmol), DMAP (2 mg, 0.013 mmol), and hexacosanoic acid (49 mg, 0.26 mmol) were added to a stirred solution of **9** (50 mg, 0.13 mmol) in $CH_2Cl_2$ (2 mL). The reaction mixture was stirred under a nitrogen atmosphere for 6 h. Solvent was removed *in vacuo* and the resulting residue was purified using silica gel column chromatography (hexane/EtOAc, 1:1) to afford the desired products **10** (77 mg, 78%) as a white solid; $^1$H NMR (300 MHz, $CDCl_3$) δ 0.86 (t, *J* = 6.7 Hz, 6H), 1.11–1.32 (m, 68H), 1.41–1.52 (m, 2H), 1.58–1.81 (m, 4H), 1.88–1.93 (m, 2H), 3.50–3.58 (m, 3H), 3.89–3.98 (m, 1H), 4.32 (d, *J* = 11.4 Hz, 1H), 4.63 (d, *J* = 11.2 Hz, 1H), 6.33 (d, *J* = 5.3 Hz, 1-NH), 7.27–7.35 (m, 5H); $^{13}$C NMR (100 MHz, $CDCl_3$) δ 14.1, 22.7, 24.6, 25.7, 29.2, 29.29, 29.35, 29.5, 29.60, 29.64, 29.7, 29.8, 31.9, 33.2, 35.2, 36.6, 52.3, 66.9, 70.5, 77.9, 127.7, 127.8, 128.5, 138.0, 174.5; HRMS (FAB) calcd for $C_{51}H_{96}O_3N$ 770.7390 ($[M+H]^+$), found 770.7375.

***N*-((2*R*,4*R*)-4-(Benzyloxy)-1-((2*S*,3*R*,4*S*,5*S*,6*R*)-3,4,5-tris(benzyloxy)-6-(benzyloxymethyl)tetrahydro-2*H*-pyran-2-yloxy)octadecan-2-yl)hexacosanamide (11)**




**10** **11**

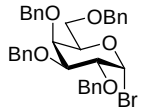
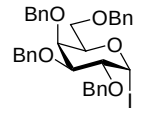
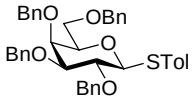
Freshly ground molecular sieves (4 Å, 50 mg), silver perchlorate (42 mg, 0.198 mmol), and tin(II) chloride (51 mg, 0.198 mmol) were added to a solution of compound **10** (51

mg, 0.066 mmol) in dry THF (2 mL) and stirred for 30 min. The mixture was cooled to –10 °C, and a solution of *β*-tetrabenzylgalactosyl fluoride **12** (54 mg, 0.099 mmol) in dry THF (2 mL) was added dropwise. After 10 min, the mixture was warmed to room temperature and stirred for 2 h. The mixture was filtered through a pad of Celite and the filter cake was washed with EtOAc. The filtrate was washed with brine, dried over $Na_2SO_4$, and concentrated under reduced pressure. The residue was purified using silica gel column chromatography (hexane/EtOAc, 6:1) to give **11** as a white solid (8 mg, 10%): $^1$H NMR (500 MHz, $CDCl_3$) δ 0.86 (t, *J* = 6.9 Hz, 6H), 1.15–1.37 (m, 68H), 1.46–1.55 (m, 4H), 1.72–1.81 (m, 2H), 1.88–1.98 (m, 2H), 3.37 (dd, *J* = 5.7, 9.4 Hz, 1H), 3.40–3.46 (m, 1H), 3.51 (dd, *J* = 6.8, 9.3 Hz, 1H), 3.56 (dd, *J* = 3.6, 11.0 Hz, 1H), 3.73 (dd, *J* = 3.6, 11.1 Hz, 1H), 3.84–3.89 (m, 2H), 3.92 (t, *J* = 6.3 Hz, 1H), 4.01 (dd, *J* = 3.7, 9.9 Hz, 1H), 4.10–4.13 (m, 1H), 4.36 (dd, *J* = 7.5, 11.6 Hz, 2H), 4.46 (dd, *J* = 8.8, 11.6 Hz, 2H), 4.55 (d, *J* = 11.6 Hz, 1H), 4.62 (d, *J* = 11.8 Hz, 1H), 4.70 (d, *J* = 11.7 Hz, 1H), 4.75-4.77 (m, 2H), 4.80 (d, *J* = 11.7 Hz, 1H), 4.90 (d, *J* = 11.6 Hz, 1H), 6.17 (d, *J* = 8.1 Hz, 1-NH), 7.21-7.36 (m, 25H); $^{13}$C NMR (100 MHz, $CDCl_3$) δ 14.2, 19.7, 22.7, 24.9, 25.7, 27.1, 29.4, 29.6, 29.7, 29.9, 30.0 (2C), 30.1, 31.9, 32.8, 33.5, 36.7, 37.1, 53.5, 59.0, 60.1, 70.4, 73.2 (2C), 73.5, 74.5, 74.9, 75.1, 79.0, 95.8, 98.1, 120.0, 127.5, 127.6, 127.7 (2C), 127.9 (2C), 128.3 (2C), 128.4 (2C), 138.2, 138.4, 138.6, 138.7, 138.8, 138.9, 173.9; HRMS (FAB) calcd for $C_{85}H_{130}O_8N$ 1292.9796 ($[M+H]^+$), found 1292.9763.

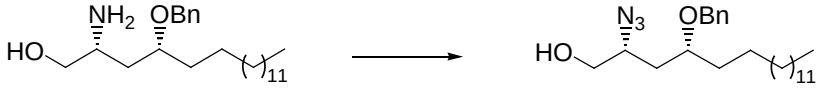
Attempts to increase the yield of **11** by varying the glycosyl donor and reaction conditions were not successful (Table S1, not optimized).

**Table S1.**

| entry | donor | conditions | yield (%) | ref. |
|---|---|---|---|---|

| | | | | |
|---|---|---|---|---|
| 1 |  | $Bu_4NBr$, TMU, $CH_2Cl_2$, rt | < 10 | 5 |
| 2 | | *n*-$Hex_4NBr$, 4Å MS, $CH_2Cl_2$, rt | < 10 | |
| 3 |  | $Bu_4NI$, DIPEA, $CH_2Cl_2$, rt | 0 | 6 |
| 4 |  | 1-Benzenesulfinylpiperidine, $Tf_2O$, $CH_2Cl_2$, –78 °C to 0 °C | < 5 | 7 |

**(2*R*,4*R*)-2-Azido-4-(benzyloxy)octadecan-1-ol (13)**

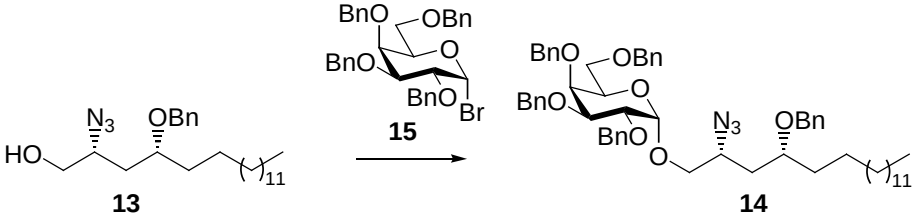



Synthesis of $TfN_3$ solution: A solution of $NaN_3$ (167 mg, 2.58 mmol) in $H_2O$ (2 mL) was cooled in an ice bath and treated with 4 mL of $CH_2Cl_2$. The resulting biphasic mixture was stirred vigorously and treated with $Tf_2O$ (0.09 mL, 0.52 mmol). After stirring for 2 h at 0 °C, the organic phase was separated and the aqueous phase was extracted twice with $CH_2Cl_2$. The combined organic layers were washed with saturated $Na_2CO_3$ solution and used without further purification.

Compound **9** (84 mg, 0.21 mmol) was dissolved in $H_2O$ (2 mL) and treated with $K_2CO_3$ (44 mg, 0.32 mmol) and $CuSO_4$ hydrate (4 mg, 0.02 mmol). MeOH (4 mL) and the $TfN_3$ solution were added to the reaction mixture, followed by addition of more MeOH until homogeneity was observed. The reaction mixture was stirred for 12 h and the solvent was removed under reduced pressure. The crude product was purified using silica gel column chromatography (hexane/EtOAc, 6:1) to give **13** (71 mg, 79%) as a white solid: $^1$H NMR (300 MHz, $CDCl_3$) δ 0.86 (t, *J* = 6.7 Hz, 3H), 1.59–1.30 (m, 24H), 1.48–1.65 (m, 2H), 1.70–1.89 (m, 2H), 2.15–2.19 (m, 1H), 3.50–3.68 (m, 4H), 4.43 (d,

*J* = 11.5 Hz, 1H), 4.55 (d, *J* = 11.4 Hz, 1H), 7.25–7.36 (m, 5H); $^{13}$C NMR (100 MHz, $CDCl_3$) δ 14.1, 22.7, 25.0, 29.3, 29.55, 29.63, 29.66, 29.70, 31.9, 33.3, 34.6, 60.8, 64.6, 70.8, 75.7, 127.8, 127.9, 128.4, 138.1; HRMS (FAB) calcd for $C_{25}H_{44}O_2N_3$ 418.3434 ($[M+H]^+$), found 418.3427.

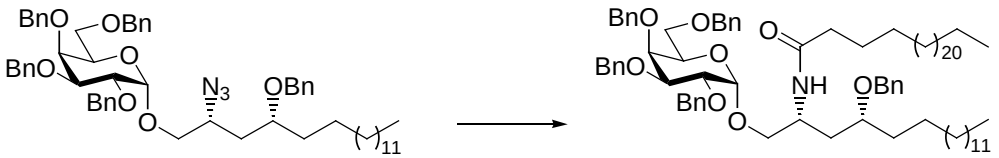
**(2*S*,3*R*,4*S*,5*S*,6*R*)-2-((2*R*,4*R*)-2-Azido-4-(benzyloxy)octadecyloxy)-3,4,5-tris(benzyloxy)-6-(benzyloxymethyl)tetrahydro-2*H*-pyran (14)**[8]




Triphenylphosphine (226 mg, 0.86 mmol) and carbon tetrabromide (285 mg, 0.86 mmol) were added to a solution of tetrabenzyl galactose (155 mg, 0.29 mmol) in dry $CH_2Cl_2$ (4 mL) and the resulting mixture was stirred at room temperature for 3 h. (*in situ* generation of compound **15**). *N*,*N*-tetramethylurea (0.06 mL, 0.48 mmol), $Bu_4NBr$ (93 mg, 0.29 mmol) and acceptor **13** (40 mg, 0.10 mmol) dissolved in dry $CH_2Cl_2$ (3 ml) were then added by syringe. The reaction was stirred at room temperature for 8 days until complete consumption of the starting material observed by TLC. The reaction mixture was filtered through a pad of Celite and the filter cake was washed with $CH_2Cl_2$. The organic mixture was washed with saturated aqueous $NaHCO_3$ solution and brine, dried over $Na_2SO_4$, and concentrated under reduced pressure. The residue was purified using silica gel column chromatography (hexane/EtOAc, 8:1) to give **14** (75 mg, 83%) as a yellow liquid: $^1$H NMR (300 MHz, $CDCl_3$) δ 0.85 (t, *J* = 6.5 Hz, 3H), 1.17–1.32 (m, 24H), 1.46–1.54 (m, 2H), 1.64–1.85 (m, 2H), 3.44–3.47 (m, 4H), 3.55–3.63 (m, 1H), 3.74 (dd, *J* = 3.1, 10.4 Hz, 1H), 3.90–3.93 (m, 3H), 4.01 (dd, *J* = 3.3, 9.5 Hz, 1H), 4.33–

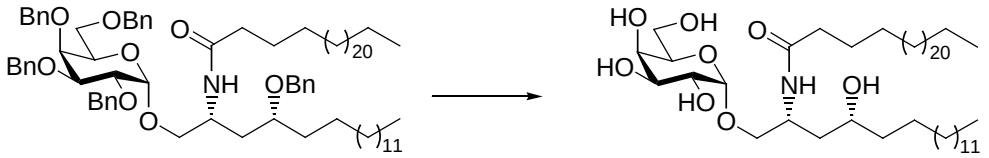
4.47 (m, 4H), 4.53 (d, *J* = 15.4 Hz, 1H), 4.61–4.66 (m, 1H), 4.72 (d, *J* = 12.5 Hz, 1H), 4.78–4.81 (m, 3H), 4.90 (d, *J* = 11.4 Hz, 1H), 7.22–7.32 (m, 25H) ); $^{13}$C NMR (100 MHz, $CDCl_3$) δ 14.1, 21.1, 22.7, 24.9, 29.4, 29.7, 29.8, 31.9, 35.6, 34.7, 58.6, 60.4, 69.0 (4C), 69.4, 70.7, 73.0, 73.2 (2C), 73.4, 74.7, 75.0, 75.1, 79.2, 96.0, 98.4, 127.4, 127.5, 127.6, 127.7 (2C), 127.8 (2C), 128.2 (2C), 128.3, 128.4, 137.4, 138.0, 138.5, 138.6, 138.7, 138.8, 138.9; HRMS (FAB) calcd for $C_{59}H_{78}O_7N_3$ 940.5840 ($[M+H]^+$), found 940.5822.

***N*-((2*R*,4*R*)-4-(Benzyloxy)-1-((2*S*,3*R*,4*S*,5*S*,6*R*)-3,4,5-tris(benzyloxy)-6-(benzyloxymethyl)tetrahydro-2*H*-pyran-2-yloxy)octadecan-2-yl)hexacosanamide (11)**




Triphenylphosphine (24 mg, 0.12 mmol) was added to a solution of **14** (56 mg, 0.06 mmol) in a mixture of benzene (3 mL) and water (30 μL). The mixture was stirred overnight at 60 °C and concentrated under reduced pressure. The residue was dissolved in $CH_2Cl_2$ (3 mL) followed by addition of EDCI (27 mg, 0.12 mmol), DMAP (1 mg, 10 mol%) and hexacosanoic acid (44 mg, 0.12 mmol). The mixture was stirred overnight at room temperature and then concentrated under reduced pressure. The residue was purified using silica gel column chromatography (hexane/EtOAc, 3:1) to give **11** (42 mg, 54%) as a white solid: All of the spectral data for compound **11** were in agreement for the products of the two synthetic routes (synthesized via intermediates **10** and **12**, respectively).

***N*-((2*R*,4*R*)-4-Hydroxy-1-((2*S*,3*R*,4*S*,5*R*,6*R*)-3,4,5-trihydroxy-6-(hydroxymethyl)tetrahydro-2*H*-pyran-2-yloxy)octadecan-2-yl)hexacosanamide (4)**




**11** **4**

The compound **11** (15 mg, 0.01 mmol) and $Pd(OH)_2$ (7 mg, 50 wt%) were suspended in a solution of $EtOH/CH_2Cl_2$ (3:1, 2 mL). The reaction mixture was hydrogenated (1 atm) at room temperature. After 2 days, the palladium catalyst was removed by filtration through a syringe filter (PTFE, 0.50 μm, hydrophobic) and rinsed with $EtOH/CH_2Cl_2$ (9:1). The filtrate was concentrated *in vacuo*. The residual solid was triturated with hexane/EtOAc (1:1) to give **4** (7 mg, 72%) as a white solid: $[\alpha]^{25}_D$ +43.6 (*c* 1.0, pyridine); $^1$H NMR (300 MHz, $C_5D_5N$) δ 0.85 (t, *J* = 6.0 Hz, 6H), 1.16–1.48 (m, 68H), 1.57–1.88 (m, 4H), 2.12–2.28 (m, 2H), 2.45 (t, *J* = 7.3 Hz, 2H), 4.34–4.42 (ddd, *J* = 5.0, 10.4, 44.7 Hz, 2H), 4.32–4.62 (m, 6H), 4.62–4.73 (m, 1H), 4.78–4.86 (m, 1H), 5.35–5.45 (m, 1H), 5.42 (d, *J* = 3.8 Hz, 1H), 8.65 (d, *J* = 8.8 Hz, 1-NH) ); $^{13}$C NMR (100 MHz, $C_5D_5N$) δ 14.8, 21.7, 23.4, 26.2, 26.9, 30.1, 30.2, 30.3, 30.5, 32.6, 34.7, 37.2, 37.3, 47.4, 63.3, 71.0, 71.5, 72.1, 72.3, 72.8, 73.6, 102.4, 171.2, 173.6; HRMS (FAB) calcd for $C_{50}H_{100}O_8N$ 842.7449 ($[M+H]^+$), found 842.7462.

## C. References of Supporting Information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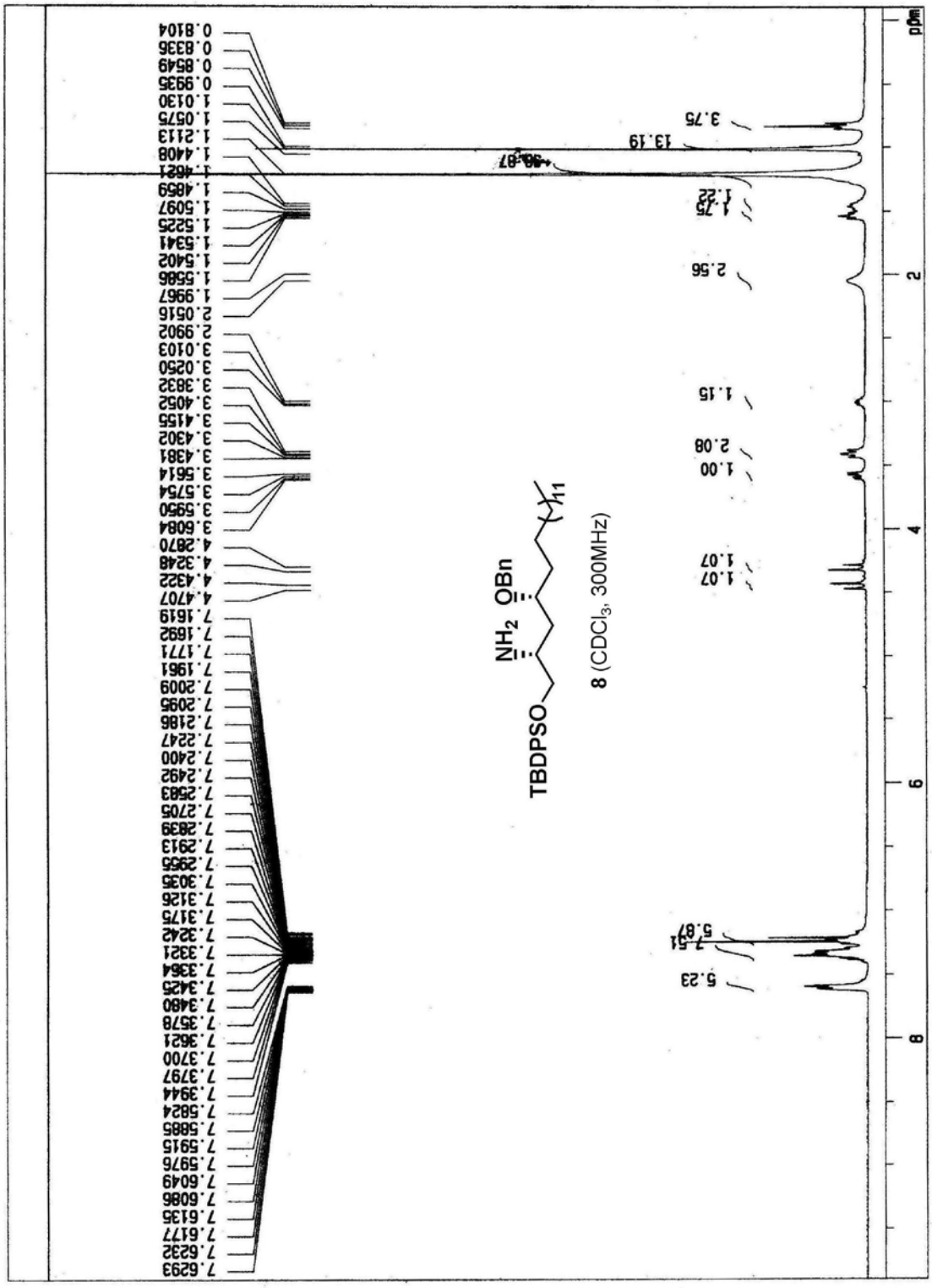
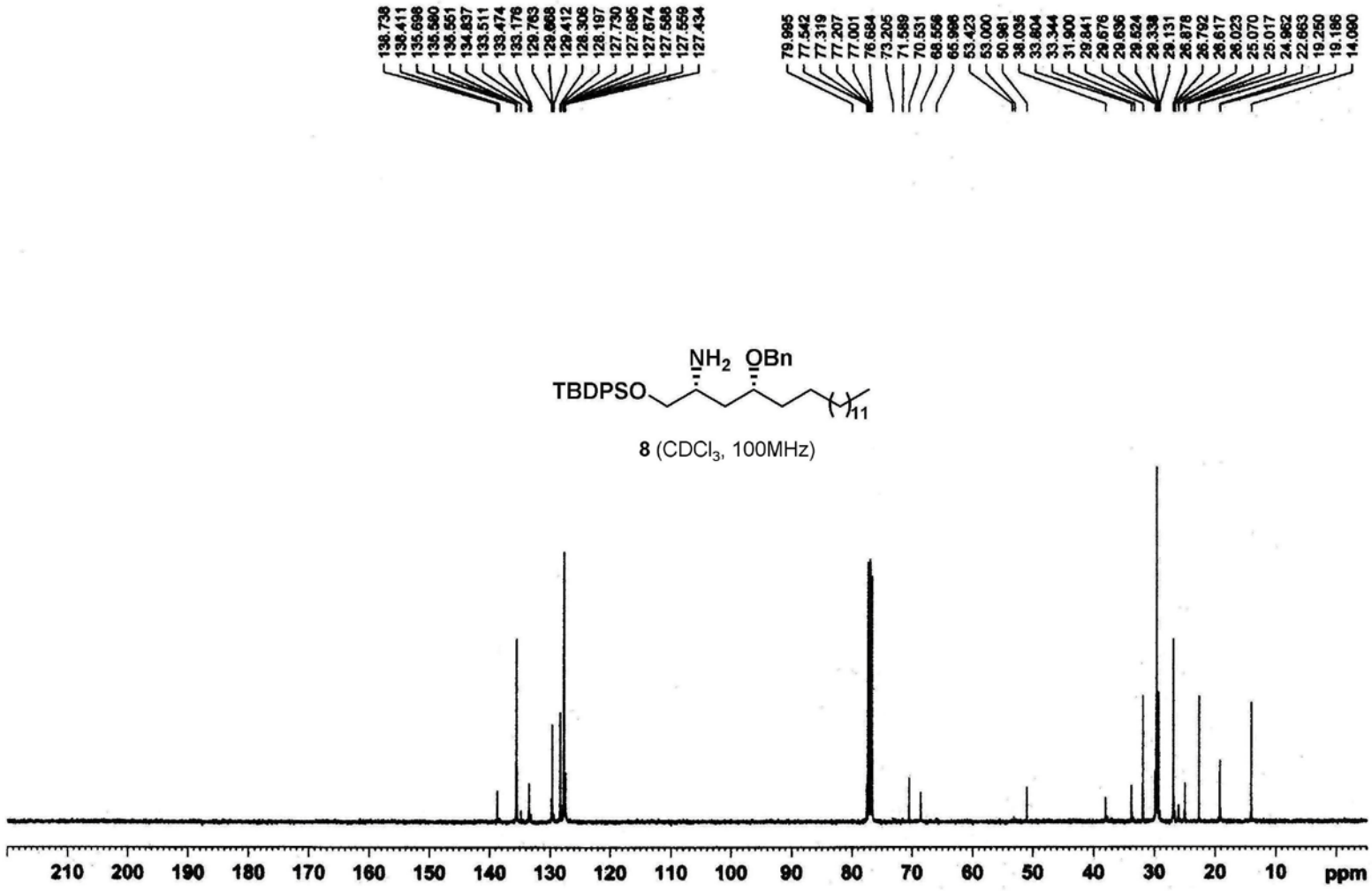
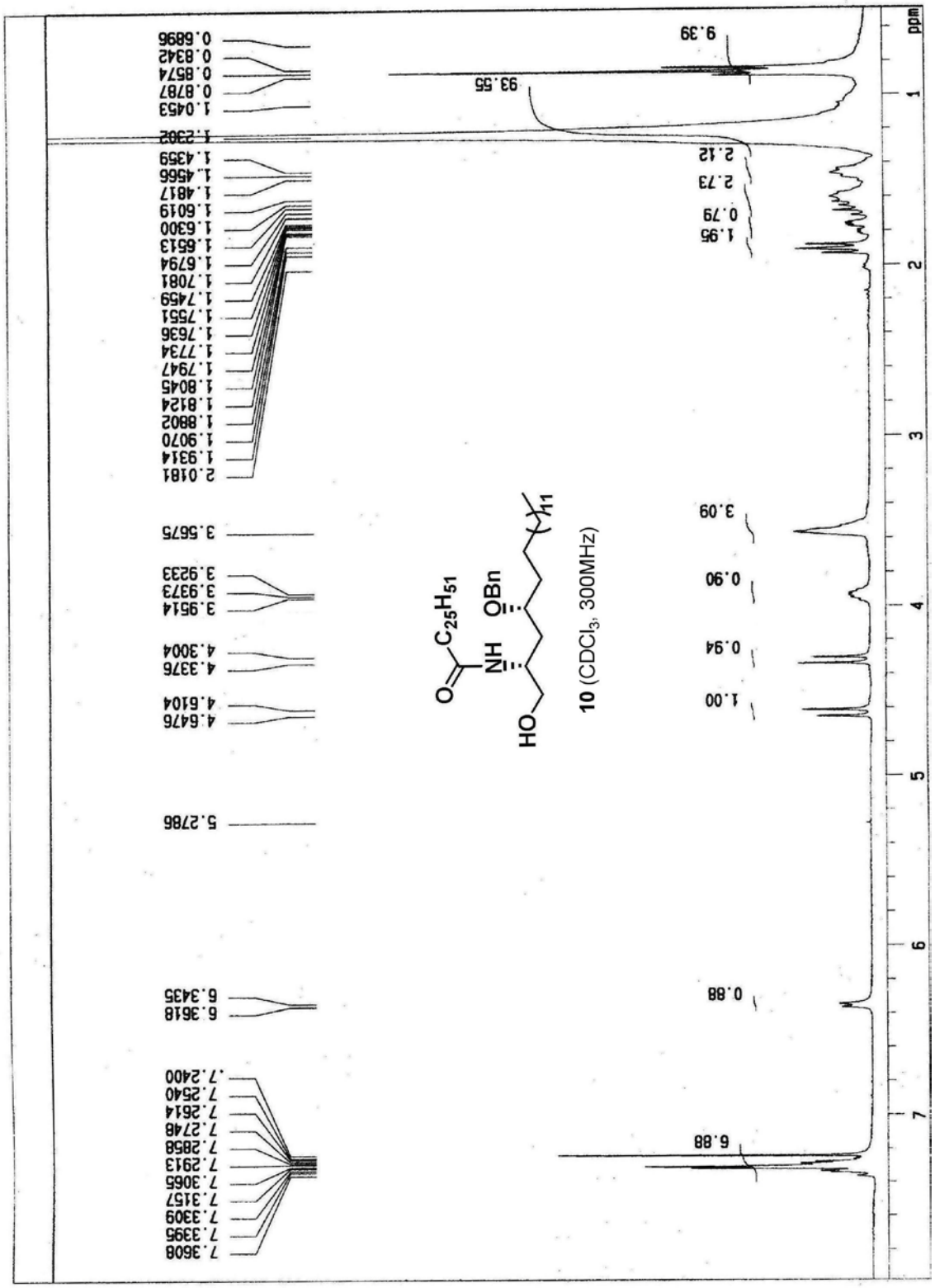
## D. $^{1}$H and $^{13}$C NMR Spectra for Selected Compounds

### (a) $^{1}$H NMR and $^{13}$C NMR spectra of compound 8



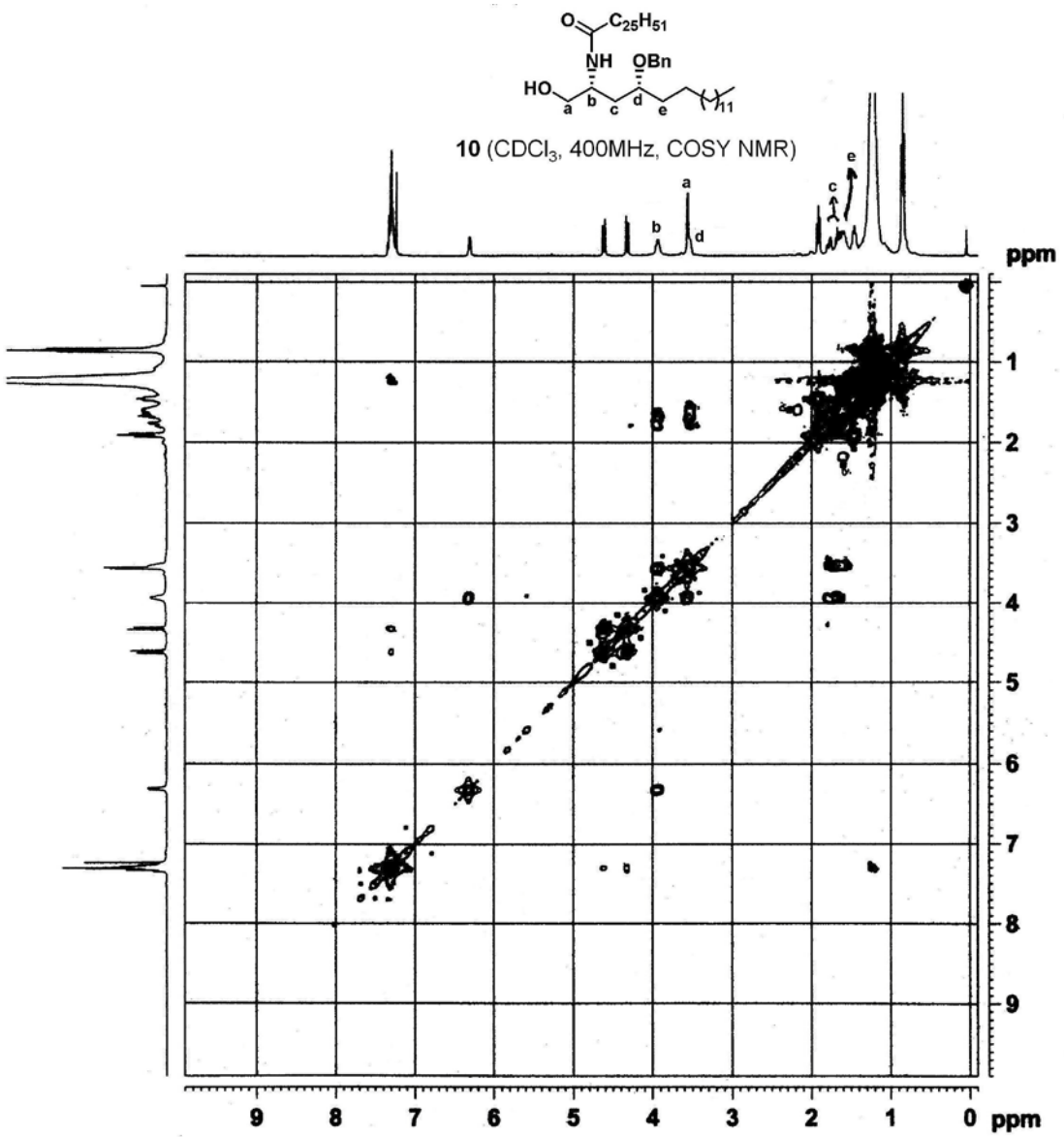

138.738
138.411
135.698
135.560
135.551
134.837
133.511
133.474
133.176
129.763
129.668
128.412
128.308
128.197
127.730
127.695
127.674
127.588
127.559
127.434
79.995
77.542
77.319
77.207
77.001
76.684
73.205
71.589
70.531
68.556
65.986
53.423
53.000
50.981
38.035
33.804
33.344
31.900
29.841
29.676
29.636
29.524
29.338
29.131
26.878
26.792
26.617
26.023
25.070
25.017
24.962
22.663
19.250
19.186
14.060
NH2
OBn
TBDPSO
11
8 (CDCl3, 100MHz)
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
ppm

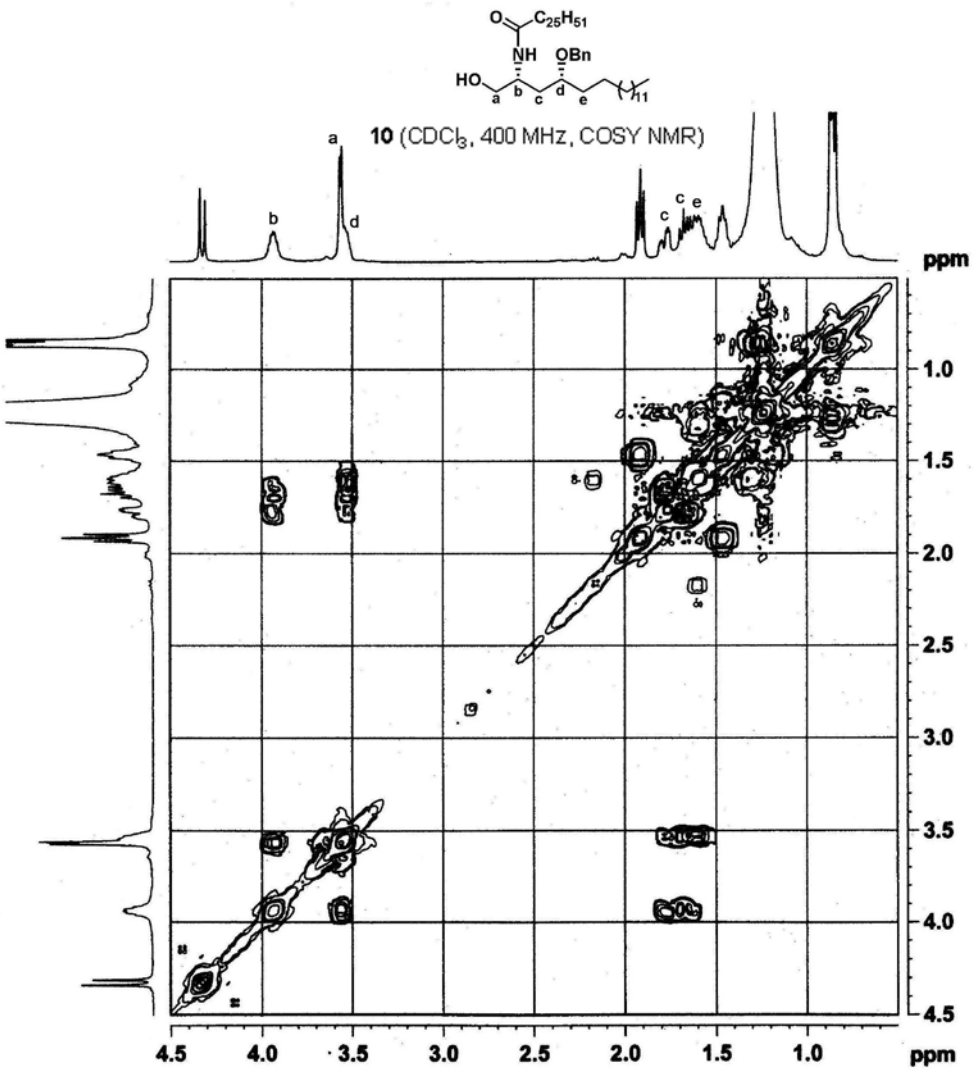
**(b) $^{1}$H NMR and $^{13}$C NMR spectra of compound 10**



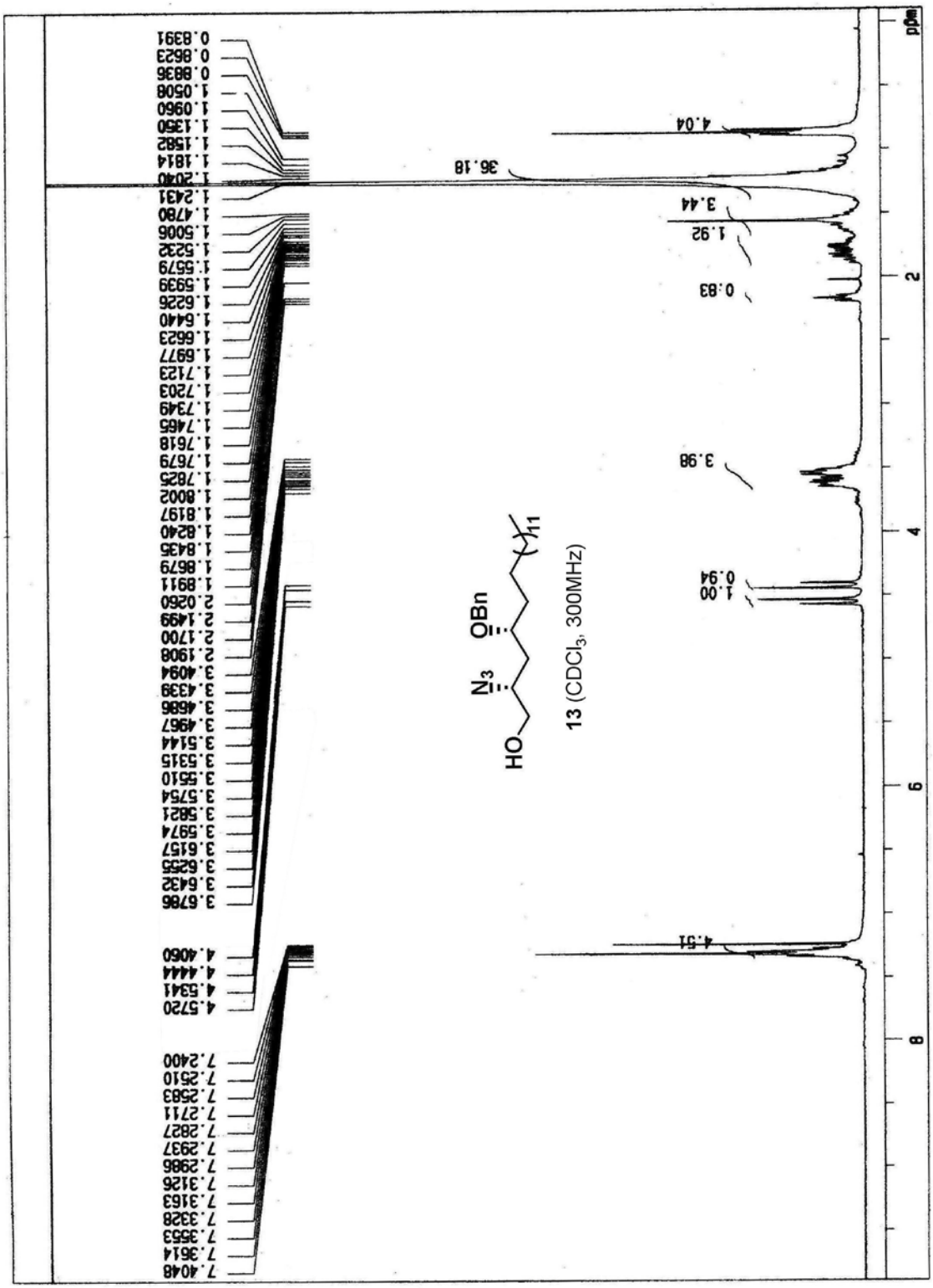

14.093
22.670
24.812
25.884
29.215
29.291
29.345
29.499
29.596
29.644
29.687
29.802
31.906
33.192
35.150
36.630
52.263
66.944
70.490
76.681
76.999
77.204
77.316
77.940
127.733
127.864
128.541
137.994
174.467
O
C25H51
NH
OBn
HO
11
10 (CDCl3, 100MHz)
ppm
10
20
30
40
50
60
70
80
90
100
110
120
130
140
150
160
170
180
190
200
210

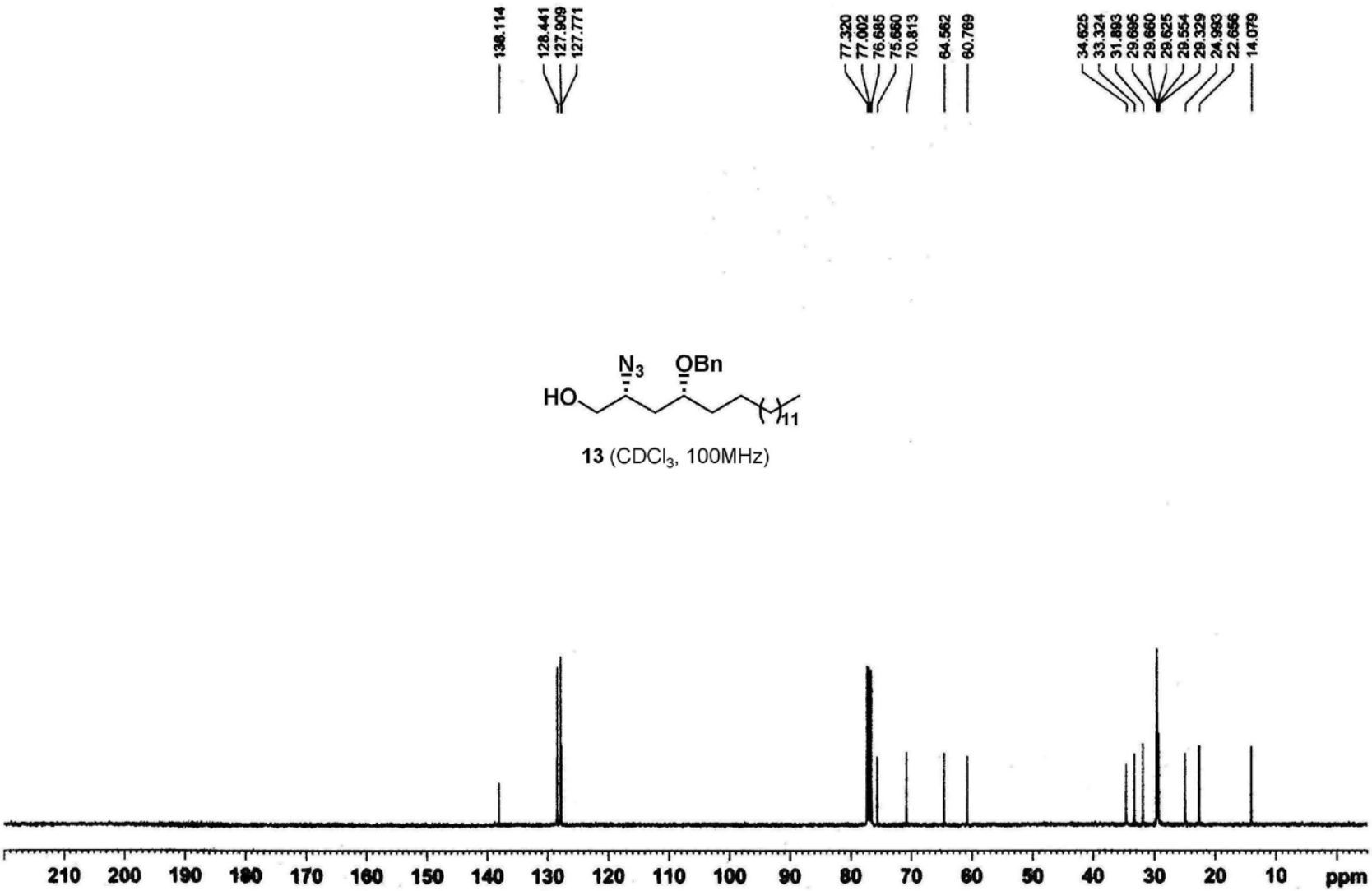
**(c) COSY NMR spectra of compound 10**




O
C25H51
NH
OBn
HO
a
b
c
d
e
11
10 (CDCl3, 400 MHz, COSY NMR)
a
b
d
c
c
e
ppm
1.0
1.5
2.0
2.5
3.0
3.5
4.0
4.5
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
ppm

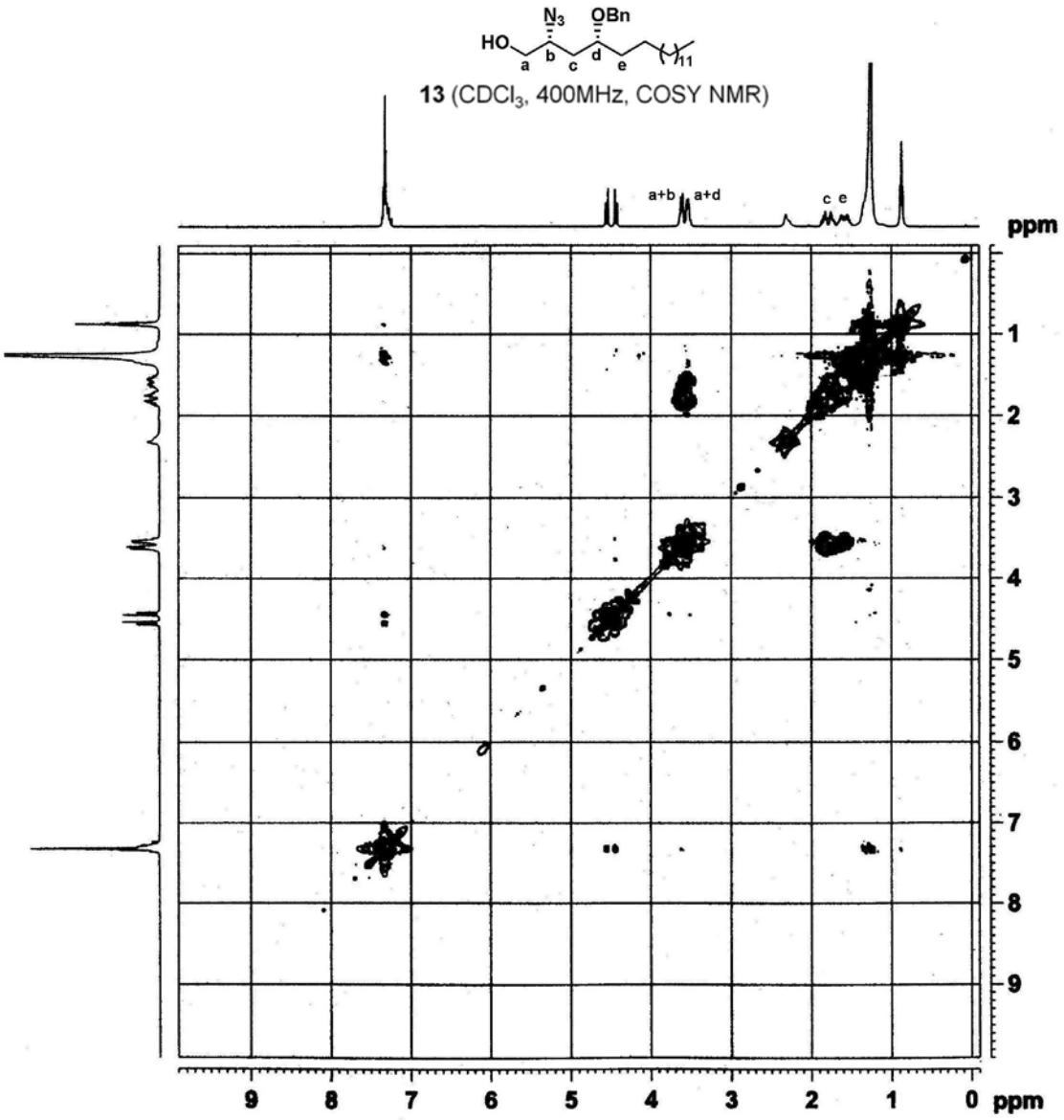
**(d) $^{1}$H NMR and $^{13}$C NMR spectra of compound 13**

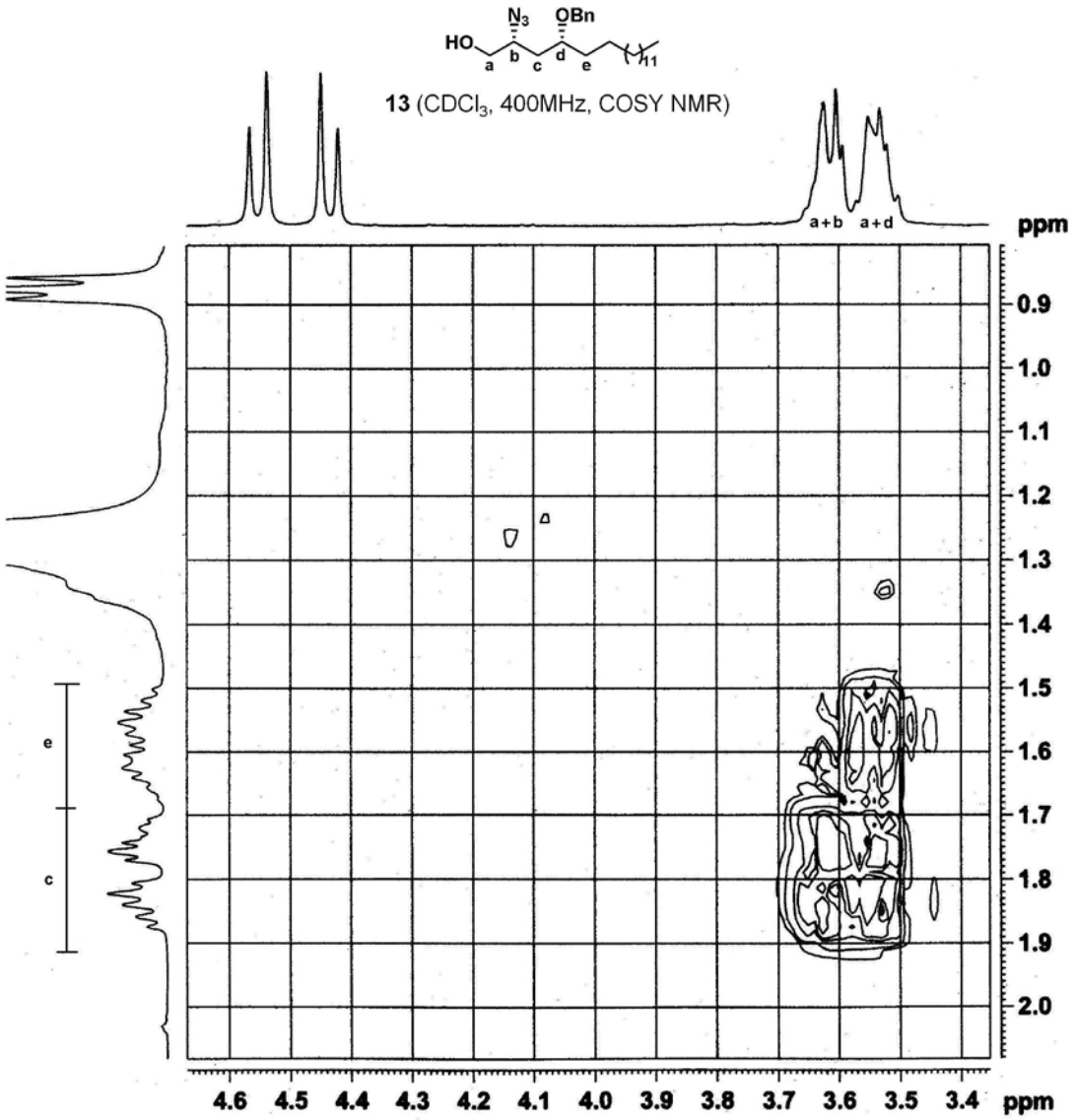




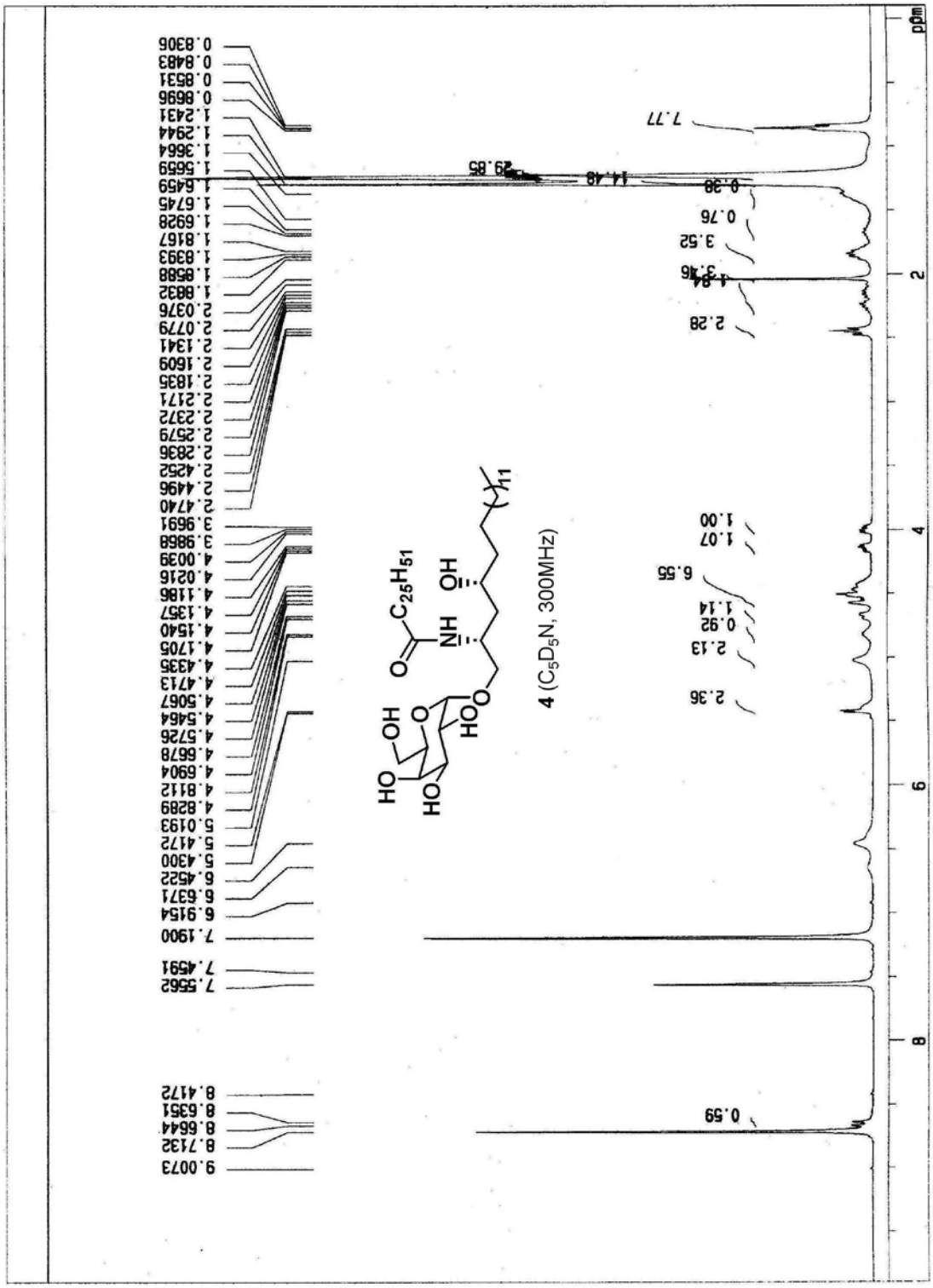

**13** ($CDCl_3$, 100MHz)

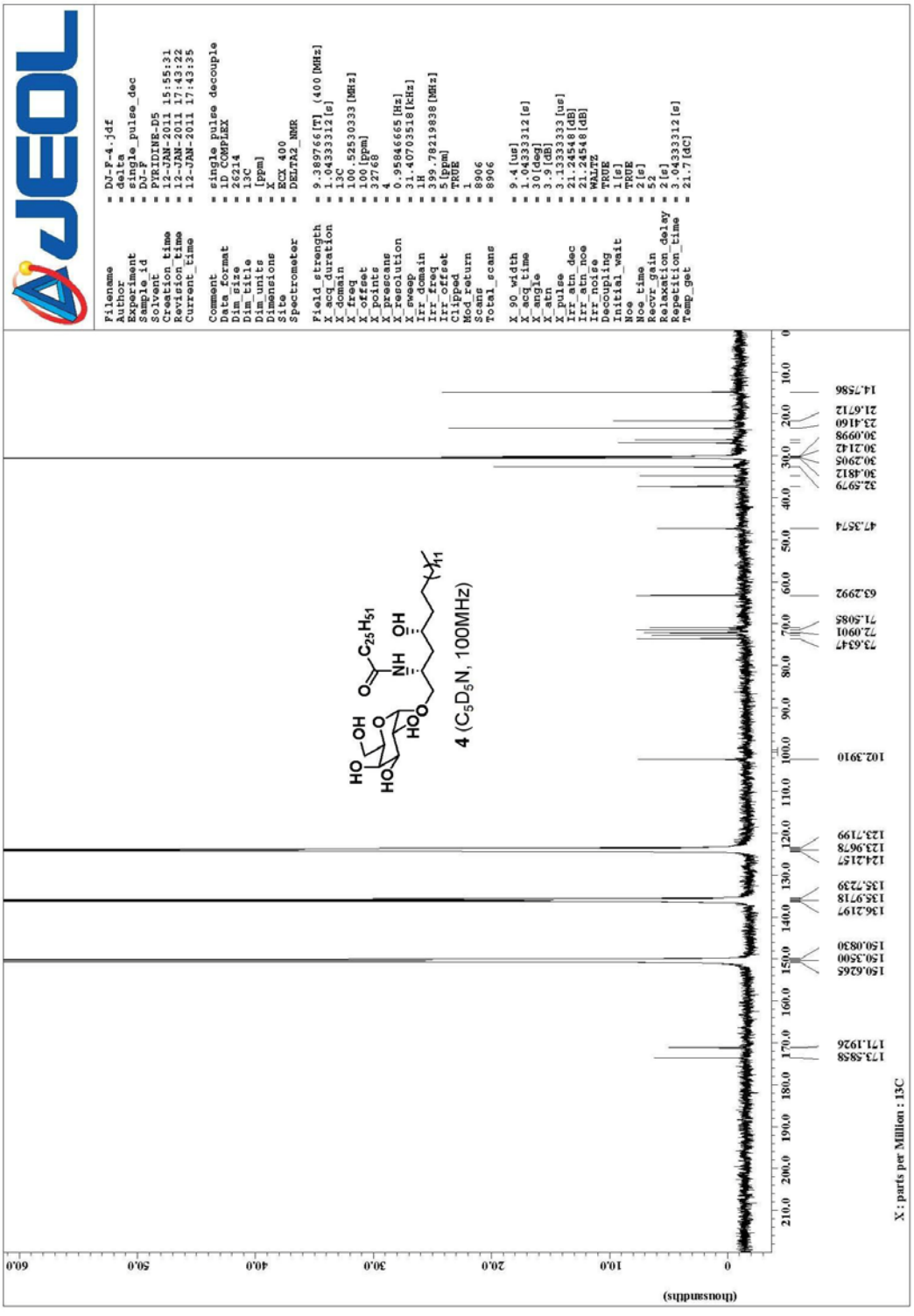
**(e) COSY NMR spectra of compound 13**




N3
OBn
HO
a
b
c
d
e
11
13 (CDCl3, 400MHz, COSY NMR)
a+b
a+d
ppm
0.9
1.0
1.1
1.2
1.3
1.4
1.5
1.6
1.7
1.8
1.9
2.0
e
c
4.6
4.5
4.4
4.3
4.2
4.1
4.0
3.9
3.8
3.7
3.6
3.5
3.4
ppm

**(f) $^{1}$H NMR and $^{13}$C NMR spectra of compound 4**

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4 (C5D5N, 100MHz)
X : parts per Million : 13C
(thousandths)
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23.4160
30.0998
30.2142
30.2905
30.4812
32.5979
47.3574
63.2992
71.5085
72.0901
73.6347
102.3910
123.7199
123.9678
124.2157
135.2739
135.9718
136.2197
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150.3500
150.6265
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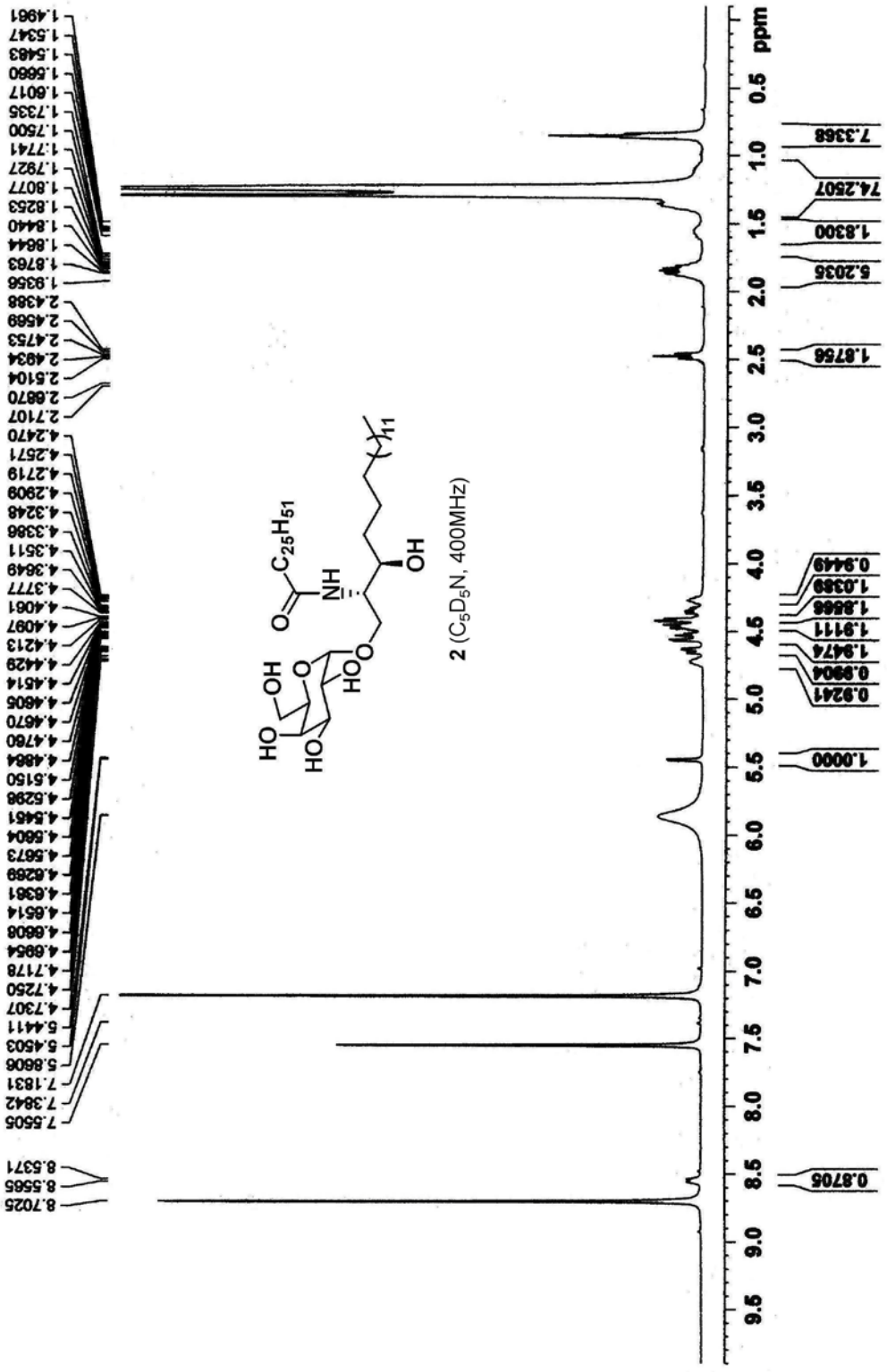
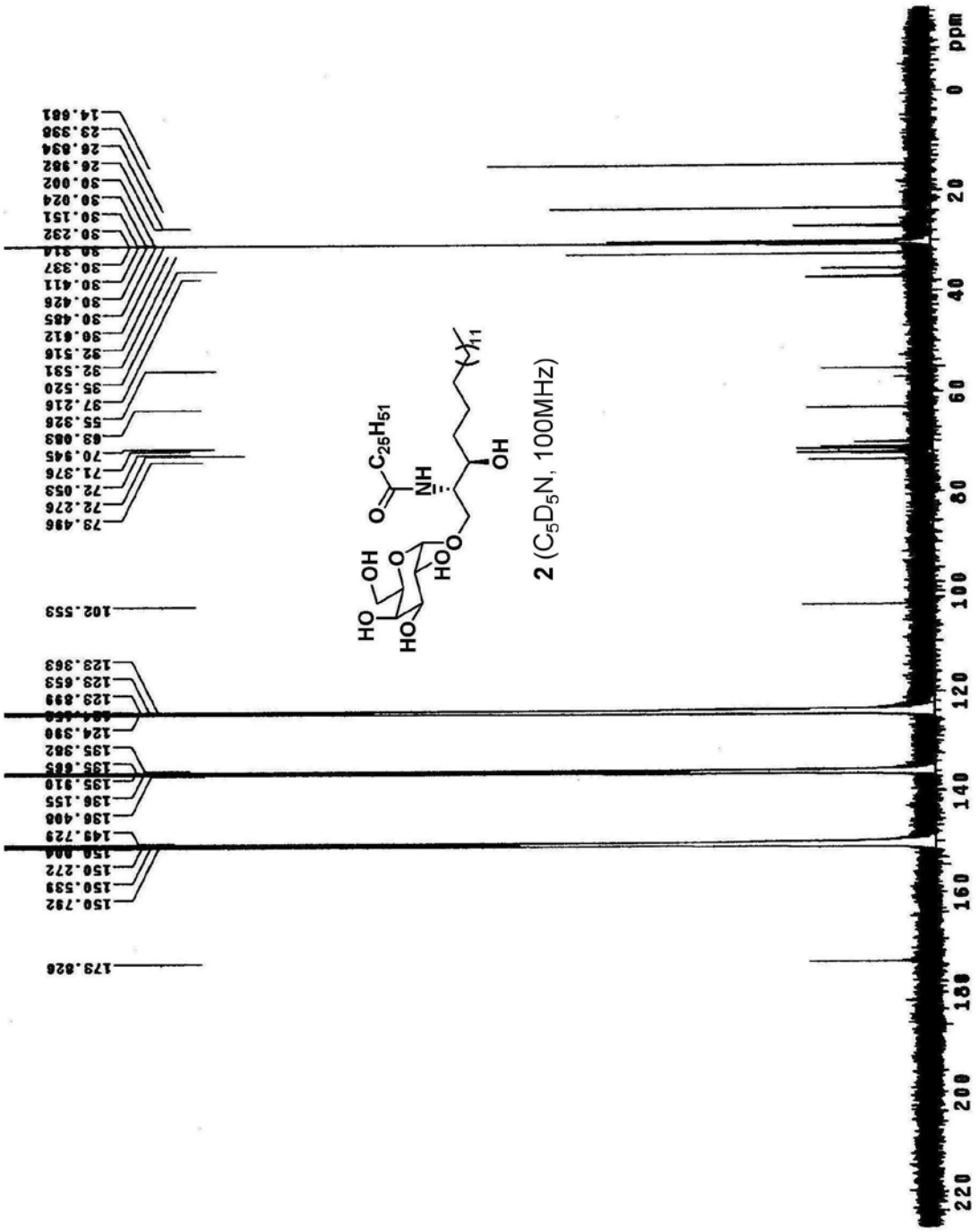
173.5858

**(g) $^{1}$H NMR and $^{13}$C NMR spectra of compound 2**

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30.002
30.024
30.151
30.232
30.314
30.337
30.411
30.426
30.485
30.612
32.516
32.531
35.520
37.216
55.326
63.083
70.945
71.376
72.053
72.276
73.496
102.553
123.363
123.653
123.899
124.390
135.362
135.685
135.910
136.155
136.408
149.729
150.004
150.272
150.539
150.792
179.826
HO
OH
O
HO
HO
O
HO
NH
O
C25H51
OH
11
2 (C5D5N, 100MHz)
ppm
0
20
40
60
80
100
120
140
160
180
200
220