Supplementary Information

Synthesis of amino core compounds of galactosyl phytosyl ceramide analogs for developing iNKT-cell inducers

Yin-Cheng Huang,[a] Li-Wu Chiang,[b] Kai-Shiang Chang,[b] Wen-Chin Su,[b] Yi-Hsian Lin,[b] Kee-Ching Jeng,[c] Kun-I Lin,[b,d] Kuo-Yen Liao,[b] Ho-Lein Huang,[b] Chung-Shan Yu[b,e*]

[a] Department of Neurosurgery, Chang Gung Memorial Hospital and department of medicine, Chang Gung University, Taoyuan, Taiwan

[b] Department of Biomedical Engineering and Environmental Sciences, National Tsing-Hua University, No. 101 sec.2, Guang-Fu Rd., Hsinchu 30043, Taiwan

[c] Department of Medical Research, Taichung Veterans General Hospital, Taichung 40705, Taiwan

[d] Department of Obstetrics & Gynecology, Chang Bing Show Chwan Memorial Hospital, Lukang Zhen, Changhua County, Taiwan

[e] Institute of Nuclear Engineering and Science, National Tsing-Hua University, Hsinchu 30043, Taiwan.

*Corresponding author; E-mail: csyu@mx.nthu.edu.tw

List of contents:

1. General methods……p.2

2. Synthesis of the compounds……p.2 - p.16

3. Carboxylic acids used for amide preparation……p.16 - p.17

4. Preparation of cell lines and the MTT assay……p.18 - p.19

5. Invariant nature killer cell quantification……p.19

6. ^{1}H- and ^{13}C-NMR, ESI-MS spectra and HPLC chromatogram……p.20 - p.63

1. General Methods

All reagents and solvents were purchased from Sigma-Aldrich, Malingkrodt, Acros, Alfa, Tedia, or Fluka. All preparations for nonradioactive compounds were routinely conducted in dried glassware under a positive pressure of nitrogen at room temperature unless otherwise noted. CH_2Cl_2, toluene, CH_3CN, and pyridine were dried over CaH_2 and MeOH was dried over Mg and distilled prior to reaction. DMF and NEt_3 were distilled under reduced pressure. Reagents and solvents were of reagent grade. Dimethyl amino pyridine (DMAP) was purified through recrystallization from the combination of EtOAc and *n*-hexane before use. The eluents for chromatography: EtOAc, acetone, and *n*-hexane were reagent grade and distilled prior to use; MeOH and $CHCl_3$ were reagent grade and used without further purification. NMR spectroscopy including ^{1}H-NMR (500 MHz) and ^{13}C-NMR (125MHz, DEPT-135) was meadured on Varian UnityInova 500 MHz. D-solvents employed for NMR including CD_3OD, $CDCl_3$, C_6D_6, and DMSO-d^6 were purchased from Cambridge Isotope Laboratories, Inc. Low-resolution mass spectrometry (LRMS) was performed on a ESI-MS spectrometry employing VARIAN 901-MS Liquid Chromatography Tandem Mass Q-Tof Spectrometer was performed at the department of chemistry of National Tsing-Hua University (NTHU). High-resolution mass spectrometry (HRMS) was performed using a varian HPLC (prostar series ESI/APCI) coupled mass dector of varian 901-MS (FT-ICR Mass) and triple quadrpole. Elemental analysis was performed using Foss Heraeus elemental analysis; CHN-O-RAPID. Thin layer chromatography (TLC) was performed with MERCK TLC Silica gel 60 F_{254} precoated plates. The starting materials and products were visualized with UV light (254 nm). Further confirmation was carried out by using staining with 5% *p*-anisaldehyde, ninhydrin or ceric ammonium molybdate under heating. Flash chromatography was performed using Geduran Si 60 silica gel (230-400 mesh). Melting points were measured with MEL-TEMP and were uncorrected. Flowcytometry was carried out by using BD calibur.

2. Synthesis of the compounds

(2S,3S,4R)-2-azido-3,4-bis(benzyloxy)heptadecan-1-ol (4): A solution of NaN_3 (6 g, 90 mmol, 15 eq) in water (15 mL) and CH_2Cl_2 (15 mL) was stirred vigorously at 0 ^oC. An ice-cold solution of Tf_2O (5 mL, 30 mmol, 5 eq) in CH_2Cl_2 (5 mL) was added to NaN_3 (aq) within 1 min. The solution was vigorously stirred for 2 hr and the water phase turned pale yellow. The organic layer was collected and the aqueous phase was further washed with CH_2Cl_2 (7 mL×2). The organic layer combined was washed with saturated Na_2CO_3 (15 mL). To a solution of compound **3** (2.8 g, 6.2 mmol) in MeOH (40 mL) was added a solution of K_2CO_3 (2 eq, 0.012 mol, 1.7 g) and $CuSO_4 \cdot 5H_2O$ (15 mg, 0.06 mmol, 0.01eq) in H_2O (40 mL), sequentially. The solution of TfN_3 described above was added and the color turned to blue-green. The stirring at rt was lasted for 16 h. TLC (MeOH/$CHCl_3$ = 1/19) indicated the consumption of starting material **3** (R_f = 0.29) and the formation of the product **4** (R_f = 0.79). The mixture was extracted with EtOAc (40 mL×3). The organic layer collected was dried with Na_2SO_4 and concentrated under reduced pressure. The residue was purified by flash chromatography using silica gel (140 g) with eluents of EtOAc/*n*-hexane = 1:19 to provide pleasant-odor colorless oil in 83% yield (2.43 g). ^{1}H-NMR (500 MHz, C_6D_6): δ 0.91 (t, J = 7.0 Hz, 3H, $H_{aliphatic}$), 1.20-1.40 (m, 21H, $H_{aliphatic}$), 1.44-1.56 (m, 2H, $H_{aliphatic}$), 1.64 (bs, 1H, H_{OH}), 1.70-1.80 (m, 1H, $H_{aliphatic}$), 3.48 (q, J = 5.0 Hz, 1H, H_4), 3.60-3.76 (m, 4H, H_1, H_2, H_3), 4.37 (d, J_{gem} = 12.0 Hz, 1H, H_{Bn}), 4.46 (t, J_{ge}

$_m$ =12.0 Hz, 1H, H_{Bn}), 4.48 (t, J_{gem} =12.0 Hz, 1H, H_{Bn}), 4.56 (d, J_{gem} = 11.5Hz, 1H, H_{Bn}), 6.98-7.32 (m, 10H, H_{Bn}) ; ^{13}C-NMR (125 MHz, C_6D_6): δ 14.33 (CH_3); CH_2: 23.08, 25.89, 29.37, 29.52, 29.80, 30.16, 30.37, 32.31; 62.67 (CH_2, C_1), 63.80 (CH, C_2), 72.43 (CH_2, $\underline{C}H_2Ph$), 73.76 ($\underline{C}H_2$, CH_2Ph), 79.58 (CH, C_4), 80.03 (CH, C_3), 127.90 (CH, Ph), 128.03 (CH, Ph), 128.12 (CH, Ph), 128.25 (CH, Ph), 128.32 (CH, Ph), 128.63 (CH, Ph), 128.65 (CH, Ph), 138.52 (C, Ph), 138.79 (C, Ph); LRMS (***m/z***) for $C_{31}H_{47}N_3O_3$: M (calcd.) = 509.4 (m/z), ESI+Q-TOF: M = 509.3 (m/z), $[M\text{-}N_2\text{-}Ph^-]^+$ = 404.3 (100%), 405.4 (28%), 406.4 (4%); $[M+H]^+$ = 510.3 (41%), 511.4 (10%); $[M+Na]^+$ = 532.3 (56%), 533.3 (18%), equivalent to the calculated isotopic ratio; analysis (calcd., found for $C_{31}H_{47}N_3O_3$): C (73.05, 72.74), H (9.29, 9.09), N (8.24, 8.21).

(2R,3S,4S)-4-azido-3-(benzyloxy)-2-tridecyltetrahydrofuran (6): Compound **4** (7 mg, 0.013 mmol) was coevaporated with toluene for three times, followed by dissolving in CH_2Cl_2 (1 mL). Upon the cooling down to -50 ^{o}C, pyridine (5μL, 0.06 mmol, 5eq) and Tf_2O (4μL, 0.03 mmol, 2eq) were added sequentially. The reaction was lasted for 30 min. TLC (EtOAc : *n*-hexane = 1 : 9) indicated the consumption of the starting material 4 (R_f = 0.21) and the formation of the product **6** (R_f = 0.55). CH_2Cl_2 (10 mL) was added and the mixture was extracted by saturated aqueous NH_4Cl (5 mL) and H_2O (5 mL×2). The organic layer collected was dried with Na_2SO_4 and concentrated under reduced pressure. The residue was purified with flash chromatography using eluents of EtOAc/*n*-hexane 1:19 and silica gel (4 g) to provide product **6** in 60% yield (3 mg). For analytical purpose, a small amount sample (20 mg) was obtained via another route as described for the preparation of compound **8**. In rare cases, we were able to isolate the triflate **5**. The fragment peaks appeared in ESI-MS spectrum such as 479.3 amu (27%), 493.4 amu (2.4%) and 595.6 amu (2.4%) indicated that the instability of triflate could lead to a number of intermediates. Satisfactory ^{1}H-NMR spectra were, however, not available due to the complex patterns. ^{1}H-NMR (500 MHz, C_6D_6): δ 0.91 (t, J = 7.0 Hz, 3H, $H_{aliphatic}$), 1.23-1.36 (m, 21H, $H_{aliphatic}$), 1.38-1.49 (m, 3H, $H_{aliphatic}$), 3.17 (ddd, $J_{4,3}$ = 6.0, $J_{4,5a}$ = 5.5, $J_{4,5b}$ = 5.5 Hz, 1H, H_4), 3.31 (dd, 1H, $J_{3,2}$ = 6.5, $J_{3,4}$ = 6.0 Hz, 1H, H_3), 3.60 (dd, $J_{1a,1b}$ = 10.0, $J_{1a,2}$ = 5.5 Hz, 1H, H_{1a}), 3.68 (dd, $J_{1b,1a}$ = 10.0, $J_{1b,2}$ = 3.5 Hz, 1H, H_{1a}), 3.96 (ddd, $J_{2,3}$ = 6.5, $J_{2,1a}$ = 5.5, $J_{2,1b}$ = 3.5 Hz, 1H, H_2), 4.21 (d, 1H, J_{gem} = 11.5 Hz, OC$\underline{H}$HPh),

4.48 (d, 1H, J_{gem} = 11.5 Hz, OCH*H*Ph), 7.08-7.11 (m, 1H, Ph), 7.17-7.19 (m, 2H, Ph), 7.29-7.31 (m, 2H, Ph); ^{13}C-NMR (125 MHz, C_6D_6): δ 14.33 (CH_3); CH_2: 23.08, 26.17, 29.79, 30.01, 30.10, 30.13, 32.30, 34.05; 60.69 (CH, C_2), 69.79 (CH_2, C_1), 72.81 ($\underline{C}H_2$, CH_2Ph), 81.13 (CH, C_4), 84.03 (CH, C_3), 128.04 (CH, Ph), 128.11 (CH, Ph), 128.29 (CH, Ph), 128.65 (CH, Ph), 138.08 (C, Ph); LRMS (***m/z***) for $C_{24}H_{39}N_3O_2$: M (calcd.) = 401.3 (m/z); ESI+Q-TOF: M = 401.3 (m/z), M^+-N_2-Ph+H^- = M', $[2M'+H]^+$ = 595.59; $[M\text{-}OTf\cdot+H]^+$ = 493.4; $[M\text{-}OTf\cdot\text{-}N\cdot+H]^+$ = 479.3.

(2S,3S,4R)-2-azido-3,4-bis(benzyloxy)heptadecyl-4-methyl benzenesulfonate (7): Before carrying out the tosylation, TsCl was purified by partition between toluene and 10% NaOH (aq). Compound **4** (2.42 g, 4.75 mmol) was azeotropically distilled with toluene for three times, followed by dissolving in CH_2Cl_2 (75 mL) under N_2 at 0 ^{o}C. Pyridine (75mL) and *p*-TsCl (1.81 g, 9.5 mmol) were added, sequentially, and the mixture was stirred for 10 min, followed by stirring at rt for 16 h. TLC (EtOAc/*n*-hexane = 1:9) indicated the consumption of the starting material **4** (R_f = 0.19) and the formation of the product 7 (R_f = 0.40). Following the addition of H_2O (100 mL), the aqueous phase was extracted with CH_2Cl_2 (40 mL×3). The organic layer collected was dried with Na_2SO_4 and concentrated under reduced pressure. The residue was purified using flash chromatography with eluents of EtOAc : *n*-hexane = 1 : 19 and silica gel (100 g) to provide colorless oil **7** in 93% yield (2.94 g). ^{1}H-NMR (500 MHz, C_6D_6): δ 0.91 (t, *J* = 7.0 Hz, 3H, $H_{aliphatic}$), 1.19-1.38 (m, 22H), 1.38-1.46 (m, 1H), 1.61-1.68 (m, 1H), 1.79 (s, 3H), 3.48 (dd, $J_{3,4}$ = 5.0, $J_{3,2}$ = 5.0 Hz, 1H, H_3), 3.53 (ddd, 1H, $J_{4,5a}$ = 7.0, $J_{4,5b}$ = 6.5, $J_{4,3}$ = 5.0 Hz, 1H, H_4), 3.80 (ddd, $J_{2,1a}$ = 7.5, $J_{2,3}$ = 5.0, $J_{2,1b}$ = 2.5 Hz, 1H, H_2), 4.28 (dd, $J_{1a,1b}$ = 10.5, $J_{1a,2}$ = 7.5 Hz, 1H, H_{1a}), 4.31 (d, 1H, J_{gem} = 12.0 Hz, OC*H*HPh), 4.35 (d, 1H, J_{gem} = 12.0 Hz, OCH*H*Ph), 4.39 (s, 2H, 2×OC*H*HPh), 4.46 (dd, $J_{1b,1a}$ = 10.5, $J_{1b,2}$ = 2.5 Hz, 1H, H_{1b}), 6.63-6.64 (m, 2H, Ph), 7.06-7.13 (m, 4H, Ph), 7.16-7.21 (m, 4H, Ph), 7.26-7.27 (m, 2H, Ph), 7.74-7.57 (m, 2h, Ph); ^{13}C-NMR (125 MHz, C_6D_6): δ 14.33 (CH_3); 21.11 (CH_3); CH2: 23.07, 25.39, 29.79, 30.01, 30.09, 30.14, 30.27, 32.29; 61.60 (CH, C_2), 69.92 (CH_2, C_1), 72.26 (CH_2, $\underline{C}H_2$Ph), 73.52 ($\underline{C}H_2$, CH_2Ph), 78.75 (CH, C_4), 79.67 (CH, C_3), 127.87 (CH, Ph), 128.04 (CH, Ph), 128.23 (CH, Ph), 128.29 (CH, Ph), 128.62 (CH, Ph), 129.86 (CH, Ph), 133.80 (C, tosyl),

138.08 (C, Ph), 138.64 (C, Ph), 144.44 (C, tosyl); LRMS for $C_{38}H_{53}N_3O_5S$: M (calcd.) = 663.4 (m/z), MW = 663.9, ESI+Q-TOF: M = 663.3 (m/z), $[M+Na]^+$ = 686.3.

((2*S*,3*S*,4*R*)-1,2-diazidoheptadecane-3,4-diyl)bis(oxy)bis(methylene) dibenzene (8): An aqueous solution of LiN_3 (10.55 g, 43.1 mmol, 20%wt in water) was azeotropically distilled with DMF (2 mL) under reduced pressure for two times. The residue was dissolved in DMF (75 mL) and transferred to a two-necked bottom flask containing a solution of starting material **7** (2.86 g, 4.31 mmol) in DMF (75 mL) under N_2 at rt. The mixture was then stirred at 80 ^oC for 2h. TLC (EtOAc : *n*-hexane = 1 : 9) indicated the consumption of the starting material **7** (R_f = 0.42) and the formation of the product 8 (R_f = 0.55). The mixture was transferred to a funnel for partition between H_2O (75 mL) and EtOAc (75 mL). The organic layer separated was dried with Na_2SO_4 and filtered through celite pad. The filtrate was concentrated under reduced pressure. The residue obtained was purified with flash chromatography using eluents of EtOAc/*n*-hexane 1:39 and silica gel (110 g) to provide colorless oil **8** in 80% yield (1.81 g) and compound **6** in 9% yield (155 mg). ^{1}H-NMR (500 MHz, C_6D_6): δ 0.91 (t, *J* = 6.5 Hz, 3H, $H_{aliphatic}$), 1.22-1.38 (m, 21H, $H_{aliphatic}$), 1.38-1.52 (m, 2H, $H_{aliphatic}$), 1.66-1.78 (m, 1H, $H_{aliphatic}$), 3.17 (d, *J* = 5.0 Hz, 2H, H_3, H_4), 3.46-3.56 (m, 3H, H_1, H_2), 4.35 (d, J_{gem} = 11.5 Hz, 1H, OC*H*HPh), 4.38 (d, J_{gem} = 11.5 Hz, 1H, OC*H*HPh), 4.41 (d, J_{gem} = 11.5 Hz, 1H, OC*H*HPh), 4.51 (d, J_{gem} = 11.5 Hz, 1H, OC*H*HPh), 7.07-7.12 (m, 2H, Ph), 7.16-7.20 (m, 4H, Ph), 7.21-7.25 (m, 2H, Ph), 7.26-7.27 (m, 2H, Ph); ^{13}C-NMR (125 MHz, C_6D_6): δ 14.33 (CH_3), CH_2: 23.08, 25.66, 29.79, 30.03, 30.09, 30.15, 30.28, 32.30; 52.22 (CH_2, C_1), 62.69 (CH, C_2), 72.20 (CH_2, O<u>C</u>H_2Ph), 73.75 (CH_2, O<u>C</u>H_2Ph), 79.05 (CH, C_4), 79.82 (CH, C_3), 127.97 (CH, Ph), 128.07 (CH, Ph), 128.13 (CH, Ph), 128.22 (CH, Ph), 128.29 (CH, Ph), 128.63 (CH, Ph), 128.65 (CH, Ph), 138.30 (C, Ph), 138.66 (C, Ph); LRMS for $C_{31}H_{46}N_6O_2$: M (calcd.) = 534.3 (m/z), MW = 534.7, ESI+Q-TOF: M = 534.3 (m/z), $[M+Na]^+$ = 557.3 (100%), 558.3 (42%), 559.3 (4%), equivalent to the calculated isotopic ratio; analysis (calcd., found for $C_{31}H_{46}N_6O_2$): C (69.63, 69.40), H (8.67, 8.53), N (15.72, 15.83).

(2*S*,3*S*,4*R*)-1-amino-2-azidoheptadecane-3,4-diol (9):

Starting material **8** (38 mg, 0.071 mmol) after coevaporation with toluene for three times was dissolved in CH_2Cl_2 (1 mL) under N_2. The mixture was cooled down to -78 ^oC. BCl_3/CH_2Cl_2 (1M, 35µL, 0.35 mmol, 5 eq) was added within 2 min. The mixture was stirred at -78 ^oC for 2 h followed by slow warming to rt within 20 min and the stirring was lasted for further 10 hr. TLC (EtOAc : *n*-hexane = 1 : 9) indicated the consumption of the starting material 8 (R_f = 0.75) and the formation of the product **9** (R_f = 0.07). Upon the addition of MeOH (0.1 mL), the mixture became an opaque light brown solution. It was then concentrated under reduced pressure to provide a yellow oily residue. The purification of the residue using flash chromatography with eluents of MeOH/$CHCl_3$ 1:9 and silica gel (1 g) afforded product **9** in 60% yield (13 mg). ^{1}H-NMR (500 MHz, CD_3OD): δ 0.89 (t, J = 7.0 Hz, 3H, $H_{aliphatic}$), 1.24-1.43 (m, 22H, $H_{aliphatic}$), 1.50-1.62 (m, 1H, $H_{aliphatic}$), 1.68-1.78 (m, 1H, $H_{aliphatic}$), 3.11 (dd, $J_{1a,1b}$ = 13.0 Hz, $J_{1a,2}$ = 8.0 Hz, 1H, H_{1a}), 3.17 (dd, $J_{1b,1a}$ = 13.0, $J_{1b,2}$ = 3.5 Hz, 1H, H_{1b}), 3.49 (td, $J_{2,1a}$ = 8.0, $J_{2,3}$ = 8.0, $J_{2,1b}$ = 3.5 Hz, 1H, H_2), 3.67 (dd, $J_{3,2}$ = 8.0, $J_{3,4}$ = 3.0 Hz, 1H, H_3), 3.92 (ddd, J = 8.5, J = 4.0, $J_{4,3}$ = 3.0 Hz, 1H, H_4); ^{13}C-NMR (125 MHz, CD_3OD): δ 14.43 (CH_3), CH_2: 23.73, 26.61, 30.47, 30.79, 33.07, 34.89; 40.18 (CH_2, C_1), 61.88 (CH, C_2), 72.92 (CH, C_4), 76.17 (CH, C_3); LRMS for $C_{17}H_{38}N_4O_2$, M (calcd.) = 328.3 (m/z), ESI+Q-TOF: M = 328.4 (m/z), $[M+H]^+$ = 329.4 (100%), 330.4 (22%), equivalent to the calculated isotopic ratio (100% : 18.9%).

(2S,3S,4R)-1,2-diaminoheptadecane-3,4-diol (1):

Starting material **8** (812 mg, 1.5 mmol) was distilled azeotropically with toluene for three times followed by dissolving in CH_2Cl_2 (20 mL) under N_2. The mixture was cooled down to -78 ^oC. BCl_3 (15 mL, 15 mmol, 1M in CH_2Cl_2, 10 eq) was added within 3 min. The mixture was stirred at -78 ^oC for 2 h, followed by warming to rt within 20 min and the stirring was lasted for further 10 hr. TLC (MeOH : $CHCl_3$ = 2 : 8) indicated the consumption of the starting material **8** (R_f = 0.88) and the formation of the product **1** (R_f = 0.05). Upon the addition of MeOH (5 mL), the pale yellow solution became a milky white mixture. It was then concentrated under reduced pressure to provide a pale yellow solid. After recrystallization from hot $CHCl_3$, the amorphous precipitate was washed with cold *n*-hexane and dried under reduced pressure to provide the yellow solid **1** in quantitative yield (445 mg). The chemical shifts of protons from C_1 to C_4

in ^{1}H-NMR were slightly upfield. Interestingly, the two ammonium protons were no longer observable between 8 and 9 ppm, indicating the presence of neutral amine rather than the ammonium ion. The protons of the ammonium complex with HCl could be observed in ^{1}H-NMR. By contrast, no peaks could be found in ESI-MS. HCl is easier evaporated during electrospraying step and thereby only the neutral amino form emerged as the base peak, 303.4 (m/z). In contrast, a substantial amount of the ammonium hydroxide form would be preserved during ESI thereby appearing as the base peak. The patterns of peak clustering around 389.3 (m/z) implied the presence of chloro-containing molecular ion. mp: 96-100 ^{o}C, ^{1}H-NMR (500 MHz, CD_3OD): δ 0.88 (t, J = 7.0 Hz, 3H, $H_{aliphatic}$), 1.20-1.47 (m, 22H, $H_{aliphatic}$), 1.49-1.62 (m, 1H, $H_{aliphatic}$), 1.64-1.77 (m, 1H, $H_{aliphatic}$), 3.30 (dd, $J_{1a,1b}$ = 14.5, $J_{1a,2}$ = 4.0 Hz,1H, H_{1a}), 3.49 (dd, $J_{1b,1a}$ = 14.5, $J_{1b,2}$ = 5.0 Hz, 1H, H_{1b}), 3.66 (ddd, $J_{4,5a}$ = 8.0, $J_{4,5b}$ = 8.0, $J_{4,3}$ = 3.0 Hz, 1H, H_4), 3.77 (dd, $J_{3,2}$ = 7.0, $J_{3,4}$ = 3.0 Hz, 1H, H_3), 3.83 (ddd, $J_{2,3}$ = 7.0, $J_{2,1b}$ = 5.0, $J_{2,1a}$ = 4.0 Hz,1H, H_2), 8.26 (bs, 1H, NH), 8.47 (bs, 1H, NH); ^{13}C-NMR (125 MHz, CD_3OD):δ 14.44 (CH_3), CH_2: 23.69, 26.57, 30.44, 30.73, 30.76, 33.03, 34.76; 39.15 (CH_2, C_1), 51.87 (CH, C_2), 73.76 (CH, C_4), 74.01 (CH, C_3); LRMS for $C_{17}H_{38}N_2O_2$: M = 302.3 (calcd.); ESI+Q-TOF: M = 302.4 (m/z), $[M+H]^+$ = 303.4 (100%), 304.4 (20%), equivalent to the calculated isotopic ratio; $[M+Na]^+$ = 325.3, $[2M+H]^+$ = 605.6. A sample was further purified with anionic ion exchange resin (OH^-). Following the gentle stirring of the mixture in MeOH for 2 min, it was filtered by paper. The filtrate collected was concentrated to provide white solid for subsequent analysis with ^{1}H-NMR and ESI-MS. ^{1}H-NMR (500 MHz, CD_3OD): δ 0.89 (t, J = 7.0 Hz, 3H, $H_{aliphatic}$), 1.20-1.41 (m, 24H, $H_{aliphatic}$), 1.45-1.65 (m, 1H, $H_{aliphatic}$), 1.70-1.76 (m, 1H, $H_{aliphatic}$), 2.69 (bs, 1H), 2.89 (bs, 2H), 3.30 (bs, 1H), 3.45-3.52 m, 1H), 3.77 (dd, $J_{3,2}$ = 7.0, $J_{3,4}$ = 3.0 Hz, 1H, H_3); LRMS for $C_{17}H_{38}N_2O_2$: ESI+Q-TOF: M = 302.4 (m/z), $[M+H]^+$ = 303.4 (25%), 304.4 (5%), $[M+H_2O+Na]^+$ = 343.4 (100%), 344.4 (29%), 345.4 (4), roughly equivalent to the calculated isotopic ratio (100:18.4:1.6); $[M+2H_2O+K]^+$ = 377.4.

2-Azido-3,4-di-*O*-benzyl-1-*O*-(6-azido-2,3,4-tri-*O*-benzyl-*α*-D-galactopyranosyl)-D-ribo-heptadecan-1-ol (14α): To a solution of donor 10 (954 mg, 1.64 mmol) and acceptor **4** (601 mg, 1.12 mmol) in CH_2Cl_2 (10 mL) under N_2 was added 4Å Molecular sieve (1.8 g). The stirring at rt was lasted

for 30 min, followed by cooling down to 0 ^{o}C. To the mixture was added *N*-iodosuccinimide (1.56 g, 7.0 mmol) and TfOH (13 mg, 0.09 mmol), prepared by dissolving TfOH (0.5 mL) in CH_2Cl_2 (10 mL). The stirring was lasted for 30 min. TLC (EtOAc : *n*-hexane = 1 : 9) indicated the consumption of the acceptor **4** (R_f = 0.22) and the formation of the product **14α** (R_f = 0.50) and product **14β** (R_f = 0.34). When adding CH_2Cl_2 (20 mL) and saturated aqueous $Na_2S_2O_3$ (20 mL) for partition, the solution turned from dark violet to white. The organic layer was further extracted with saturated aqueous $NaHCO_3$ (20 mL). After drying the organic layer with Na_2SO_4, the solution was filtered through celite pad and concentrated under reduced pressure. The residue obtained was purified by flash chromatography with eluents of EtOAc/*n*-hexane = 1:19 to provide compound **14α** in 51% yield (583 mg) and compound **14β** in 44% yield (501 mg), both in their oily appearance. ^{1}H-NMR (500 MHz, C_6D_6): δ 0.91 (t, 3H, CH_3), 1.22-1.36 (m, 20H, CH_2), 1.32-1.48 (m, 1H, C<u>H</u>H), 1.50-1.58 (m, 1H, C<u>H</u>H), 1.58-166 (m, 1H, C<u>H</u>H), 1.84-1.91 (m, 1H, C<u>H</u>H), 2.74 (dd, $J_{6'a,6'b}$ = 12.5, $J_{6'a,5}$ = 4.0 Hz, 1H, H-6'a), 3.45 (s, 1H, H-4'), 3.47 (dd, $J_{6'b,6'a}$ = 12.5, $J_{6'b,5'}$ = 8.0 Hz, 1H, H-6'b), 3.72-3.78 (m, 3H, H_{1a}, H_2, H_4), 3.82 (dd, $J_{5',6b}$ = 8.0, $J_{5',6a}$ = 4.0 Hz, 1H, $H_{5'}$), 3.87 (t, *J* = 4.3 Hz, 1H, H_3), 4.04 (dd, $J_{2',3'}$ = 10.5, $J_{2',1'}$ = 3.5 Hz, 1H, $H_{2'}$), 4.16 (dd, $J_{3',2'}$ = 10.5, $J_{3',4'}$ = 4.0 Hz, 1H, $H_{3'}$), 4.20 (dd, $J_{1b,1a}$ = 13.0, $J_{1b,2}$ = 6.0 Hz,1H, H_{1b}), 4.42-4.48 (m, 3H, C<u>H</u>$_2$Ph), 4.57-4.64 (m, 4H, C<u>H</u>$_2$Ph), 4.71 (d, *J* = 11.5 Hz, 1H, C<u>H</u>$_2$Ph), 4.78 (d, *J* = 11.5 Hz, 1H, C<u>H</u>$_2$Ph), 4.88 (d, $J_{1',2'}$ = 3.5 Hz, 1H, $H_{1'}$), 4.98 (d, *J* = 11.5, 1H, C<u>H</u>2Ph), 7.00-7.05 (m, 1H, H_{Bn}), 7.08-7.12 (m, 6H, H_{Bn}), 7.16-7.21 (m, 8H, H_{Bn}), 7.28-7.30 (m, 4H, H_{Bn}), 7.31-7.33 (m, 2H, H_{Bn}), 7.34-7.37 (m, 4H, H_{Bn}); ^{13}C-NMR (125 MHz, C_6D_6): δ 14.33 (CH_3), CH_2: 23.08, 26.01, 29.79, 30.15, 30.22, 30.35, 32.30, 51.84; 62.43 (CH), 68.69 (CH_2), 70.91 (CH), 72.26 (<u>C</u>H$_2$Ph), 73.36 (<u>C</u>H$_2$Ph), 73.62 (<u>C</u>H$_2$Ph), 74.01 (<u>C</u>H$_2$Ph), 75.04 (<u>C</u>H$_2$Ph), 76.22 (CH), 77.08 (CH), 78.88 (CH), 79.09 (CH), 79.98 (CH), 98.75 (CH),127.66 (CH, Ph), 127.80 (CH, Ph), 128.00 (CH, Ph), 128.19 (CH, Ph), 128.29 (CH, Ph), 128.44 (CH, Ph), 128.49 (CH, Ph), 128.57 (CH, Ph), 128.62 (CH, Ph), 138.85 (C, Ph), 138.99 (C, Ph), 139.15 (C, Ph), 139.19 (C, Ph), 139.30 (C, Ph) ; LRMS for $C_{58}H_{74}N_6O_7$: M (calcd.) = 966.6 (m/z), ESI+Q-TOF: M = 966.6 (m/z), $[M\text{-}H \cdot +H]^+$ = 966.6, M' = M-H^++NH_4^+, $[2M' + H]^+$ = 1967.0; analysis (calcd., found for $C_{58}H_{74}N_6O_7$): C (72.02, 72.11), H (7.71, 7.42), N (8.69, 8.66).

2-Azido-3,4-di-*O*-benzyl-1-*O*-(6-azido-2,3,4-tri-*O*-benzyl-*β*-D-galactopyranosyl)-D-ribo-heptadecan-1-ol (14β): ^{1}H-NMR (500 MHz, C_6D_6): δ 0.91 (t, 3H, CH_3), 1.19-1.35 (m, 20H, CH_2), 1.36-1.46 (m, 1H, C<u>H</u>H), 1.48-1.56 (m, 1H, C<u>H</u>H), 1.58-164 (m, 1H, C<u>H</u>H), 1.83-1.90 (m, 1H, C<u>H</u>H), 2.70 (dd, $J_{6'a,6'b}$ = 12.5, $J_{6'a,5}$ = 4.0 Hz, 1H, $H_{6'a}$), 2.94 (dd, $J_{3,2}$ = 7.5, $J_{3,4}$ = 4.0 Hz, 1H, H_3), 3.22 (dd, $J_{3',2'}$ = 9.5 Hz, $J_{3',4'}$ = 3.0 Hz, 1H, $H_{3'}$), 3.28 (dd, $J_{4',3'}$ = 3.0, $J_{4',5'}$ = 2.5 Hz, 1H, $H_{4'}$), 3.38 (dd, $J_{6'b,6'a}$ = 12.5, $J_{6'b,5'}$ = 7.5 Hz, 1H, $H_{6'b}$), 3.73 (ddd, $J_{2,3}$ = 7.5, $J_{2,1a}$ = 3.0, $J_{2,1b}$ = 2.5 Hz, 1H, H_2), 3.80 (ddd, $J_{5',6b'}$ = 7.5, $J_{5',6a'}$ = 4.0, $J_{5',4'}$ = 2.5 Hz, 1H, $H_{5'}$), 3.82-3.86 (m, 1H, H_4), 3.94 (dd, $J_{1a,1b}$ = 10.5, $J_{1a,2}$ = 2.5 Hz, 1H, H_{1a}), 4.06 (dd, $J_{2',3'}$ = 9.5, $J_{2',1'}$ = 7.5 Hz, 1H, $H_{2'}$), 4.26 (d, $J_{1',2'}$ = 7.5 Hz, 1H, $H_{1'}$), 4.39 (dd, $J_{1b,1a}$ = 10.5, $J_{1b,2}$ = 6.5 Hz, 1H, H_{1b}), 4.42 (dd, *J* = 12.0 Hz, 1H, C<u>H</u>$_2$Ph), 4.44 (dd, *J* = 12.0 Hz, 1H, C<u>H</u>$_2$Ph), 4.53 (dd, *J* = 12.0 Hz, 1H, C<u>H</u>$_2$Ph), 4.55 (dd, *J* = 11.5 Hz, 1H, C<u>H</u>$_2$Ph), 4.64-4.70 (m, 3H), 4.76 (d, *J* = 11.0 Hz, 1H, C<u>H</u>$_2$Ph), 4.94 (d, *J* = 11.5 Hz, 1H, C<u>H</u>$_2$Ph), 5.09 (d, *J* = 11.5 Hz, 1H, C<u>H</u>$_2$Ph), 7.07-7.13 (m, 7H, H_{Bn}), 7.16-7.21 (m, 8H, H_{Bn}), 7.25-7.26 (m, 2H, H_{Bn}), 7.32-7.34 (m, 4H, H_{Bn}), 7.36-7.37 (m, 2H, H_{Bn}), 7.45-7.46 (m, 2H, H_{Bn}); ^{13}C-NMR (125 MHz, C_6D_6):δ 14.33 (CH_3), CH_2: 23.08, 25.95, 29.79, 30.11, 30.15, 30.22, 30.30, 32.30, 51.40; 62.64 (CH), 69.15 (CH_2), 72.12 (<u>C</u>H$_2$Ph), 73.42 (<u>C</u>H$_2$Ph), 73.86 (<u>C</u>H$_2$Ph),74.20 (CH), 74.75 (CH), 74.89 (<u>C</u>H$_2$Ph), 75.29 (<u>C</u>H$_2$Ph), 78.93 (CH), 79.82 (CH), 82.01 (CH), 104.08 (CH), 127.61 (CH, Ph), 127.70 (CH, Ph), 127.81 (CH, Ph), 127.92 (CH, Ph), 128.00 (CH, Ph), 128.19 (CH, Ph), 128.29 (CH, Ph), 128.38 (CH, Ph), 128.45 (CH, Ph), 128.57 (CH, Ph), 128.65 (CH, Ph), 138.82 (C, Ph), 139.07 (C, Ph), 139.13 (C, Ph), 139.59 (C, Ph).

2-azido-3,4-di-*O*-benzoyl-1-*O*-(6-azido-2,3,4-tri-O-benzyl-α-D-galactopyranosyl)-D-ribo-octadecan-1-ol (15α,β): A mixture of donor **10** (50 mg, 0.86 mmol) and acceptor **12** (79 mg, 0.14 mmol, 1.5 equiv) was azeotropically distilled with toluene (10 mL) for three times. CH_2Cl_2 (1.5 mL) and powdered 4 Å MS (150 mg) were added, sequentially, under N_2. After stirring for 30 min, the mixture was moved to ice bath. Following the addition of NIS (126 mg, 0.56 mmol, 6.2 equiv), the flask was stirred at -78 ℃for 5 min. TfOH (0.56 μL, 0.006 mmol, 0.1 equiv) was added, while the mixture turned dark red. The stirring was warmed to - 20℃ during 10 min. After 1 h, TLC (EtOAc: *n*-hexane = 1:9) indicated the formation

of the products **15α,β** (R_f = 0.66) and the consumption of the acceptor **12** (R_f = 0.26) and the donor **10** (R_f = 0.66). The mixture were filtered through celite pad and the filtrate obtained was concentrated under reduced pressure. The residue was dissolved in EtOAc and treated with $Na_2S_2O_{3(aq)}$ (3 mL), followed by extraction with $NaHCO_{3(aq)}$ (5 mL). The organic phase was collected and dried with Na_2SO_4, followed by filtration with celite pad. The filtrate was concentrated under reduced pressure and the resultant residue was purified by flash chromatography using eluents of EtOAc: *n*-hexane =1:39 to provide the colorless product mixtures **15** in 65% yield (60 mg) and α/β ratio of 2:1. Each of the two anomers could be collected in its pure form from the fractions. **Data of 15α:** ^{1}H-NMR (500 MHz, $CDCl_3$): δ 0.86 (t, J =7.0 Hz, 3H, $H_{aliphatic}$), 1.19-1.40 (m, 24H, $H_{aliphatic}$), 1.83-1.85 (m, 2H, $H_{aliphatic}$), 2.91(dd, $J_{6a',6b'}$= 12.5 Hz, $J_{6a',5'}$ = 5.0 Hz, 1H, H-6a'), 3.43 (dd, $J_{6b',6a'}$ = 12.5, $J_{6b',5'}$ = 8.5 Hz, 1H, H-6b'), 3.68 (dd, $J_{1a,1b}$ = 10.5, $J_{1a,2}$ = 7.5 Hz, 1H, H-1a), 3.73 (bs, 1H, H-4'), 3.82 (dd, $J_{5',6b'}$ = 8.5, $J_{5',6a'}$ = 5.0 Hz, 1H, H-5'), 3.90 (dd, $J_{3',2'}$ = 10.0, $J_{3',4'}$ = 3.0 Hz, 1H, H-3'), 3.98 (dd, $J_{2',3'}$ = 10.0, $J_{2',1'}$ = 4.0 Hz, 1H, H-2'), 4.00 (dd, $J_{2,1a}$ = 7.5, $J_{2,1b}$ = 3.0 Hz, 1H, H-2), 4.03 (dd, $J_{1b,1a}$ = 10.5, $J_{1b,2}$ = 3.0 Hz, 1H, H-1b), 4.55 (d, 1H, J = 11.5Hz, C$\underline{H}_2$Ph), 4.62 (d, 1H, J = 12.5Hz, C$\underline{H}_2$Ph), 4.67-4.70 (d, J = 11.5Hz, 2H, C$\underline{H}_2$Ph), 4.80 (d, 1H, $J_{1',2'}$ = 4.0Hz, $H_{1'}$), 4.83 (d, 1H, J = 11.5Hz, C$\underline{H}_2$Ph), 4.95 (d, 1H, J = 11.5 Hz, C$\underline{H}_2$Ph), 5.50-5.53 (m, 2H, H-3+H-4), 7.14-7.44 (m, 19H, ArH), 7.52-7.86 (m, 2H, ArH), 7.96-8.01 (m, 4H, ArH) ; ^{13}C-NMR (125MHz, $CDCl_3$): δ 14.11 (CH_3), CH_2: 22.68, 25.31, 29.35, 29.38, 29.41, 29.50, 29.58, 29.64, 29.67, 29.69, 29.87, 31.92, 51.40; 61.36 (CH), 68.54 (CH_2) , 70.32 (CH), 72.86 (CH), 72.90 (CH), 73.12 (CH_2, $\underline{C}H_2$Ph), 73.65 (CH_2, $\underline{C}H_2$Ph), 74.58 (CH_2, $\underline{C}H_2$Ph), 75.20 (CH), 76.21 (CH), 78.42 (CH), 98.80 (CH); arom. CH: 127.55, 127.59, 127.83, 127.89, 128.27, 128.38, 128.43, 128.47, 128.56, 129.73, 129.87, 133.16, 133.48; arom. quaternary C: 129.32, 129.79, 138.10, 138.51, 138.70; 165.15 (CO), 165.73 (CO). Data of **15β**: ^{1}H-NMR (500 MHz, $CDCl_3$): δ 0.86 (t, 3H, J = 7.0Hz, $H_{aliphatic}$), 1.16-1.43 (m, 22H, $H_{aliphatic}$), 1.57 (bs, 2H, $H_{aliphatic}$), 1.78-1.86 (m, 2H, $H_{aliphatic}$), 2.82 (dd, $J_{6a',6b'}$ = 12.0 Hz, $J_{6a',5'}$ = 4.5 Hz, 1H, $H_{6a'}$), 3.39 (dd, $J_{6b',6a'}$ = 12.0, $J_{6b',5'}$ = 7.5 Hz, 1H, H-6b'), 3.43 (dd, $J_{5',6b'}$ = 7.5, $J_{5',6a'}$ = 4.5 Hz, 1H, H-5'), 3.47 (dd, $J_{3',2'}$ = 9.5, $J_{3',4'}$ = 2.5 Hz, 1H, H-3'), 3.65 (d, J = 1.0 Hz, 1H, H-4'), 3.83 (dd, $J_{2',3'}$ = 9.5, $J_{2',1'}$ = 8.0 Hz, 1H, H-2'), 3.90 (dd,

$J_{1a,1b}$ = 10.5, $J_{1a,2}$ = 1.5 Hz, 1H, H-1a), 3.96 (bs, 1H, H-2), 4.14 (dd, $J_{1b,1a}$ = 10.5, $J_{1b,2}$ = 8.5 Hz, 1H, H-1b), 4.36 (d, $J_{1',2'}$ = 8.0 Hz, 1H, H_1'), 4.58 (d, *J* = 11.5 Hz, 1H, C$\underline{H}_2$Ph), 4.70 (d, *J* = 11.5 Hz, 1H, C$\underline{H}_2$Ph), 4.73 (d, *J* = 10.5 Hz, 1H, C$\underline{H}_2$Ph), 4.77 (d, *J* = 10.5 Hz, 1H, C$\underline{H}_2$Ph), 4.89 (d, 1H, *J* = 11.0 Hz, H_1), 4.96 (d, 1H, *J* = 11.5 Hz, C$\underline{H}_2$Ph), 5.48-5.54(m, 2H, H-3+H-4), 7.20-7.36 (m, 14H, ArH), 7.36-7.43 (m, 5H, ArH), 7.51-7.58 (m, 2H, ArH), 7.96-8.00 (m, 4H, ArH); ^{13}C-NMR (125MHz, $CDCl_3$): δ 14.07 (CH_3), CH_2: 22.67, 25.28, 29.34, 29.39, 29.49, 29.57, 29.62, 30.10, 31.90, 51.0; 61.42 (CH), 68.50 (CH_2), 72.81 (CH), 72.97 (CH), 73.42 (CH_2, $\underline{C}H_2$Ph), 73.53 (CH), 74.20 (CH), 74.34 (CH_2, $\underline{C}H_2$Ph), 75.30 (CH_2, $\underline{C}H_2$Ph), 79.23 (CH), 81.89 (CH), 103.43(CH), arom. CH: 127.47, 127.58, 127.71, 127.84, 127.98, 128.22, 128.33, 128.43, 128.46, 129.74, 129.92, 133.12, 133.34; arom. quaternary C:129.39, 129.78, 138.05, 138.22,138.63; 165.05 (CO), 165.71 (CO); LRMS for $C_{59}H_{72}N_6O_9$: M (calcd.) = 1008.5 (m/z), ESI+Q-TOF: M = 1008.6 (m/z) , $[M+Na]^+$=1031.6 (3.67%), 1032.6 (2.54%), approximately equivalent to the calculated isotopic ratio (100% : 65%). Coupling of **11** and **12** afforded only the undesired silylated product. ^{1}H-NMR (300 MHz, $CDCl_3$): δ 0.10 (s, 9H, CH_3), 0.90 (t, 3H, $CH_{3(aliphatic)}$), 1.15-1.50 (m, 24H, $H_{aliphatic}$), 1.80-2.00 (m, 2H, $H_{aliphatic}$), 3.75-4.00 (m, 3H), 5.46-5.56 (m, 2H, H_3, H_4), 7.40-7.50 (m, 4H, ArH), 7.53-7.63 (m, 2H, ArH), 7.98-8.00 (m, 4H, ArH).

Attempt to synthesize compound (16) by coupling (10) and (13)

Anal. $C_{83}H_{124}N_4O_8$, M (calcd.) = 1304.9 (m/z); ESI+Q-TOF: M = 1304.8 (m/z), $[M+Na]^+$ = 1327.8 (8.7%), 1328.6 (7.8%), 1329.5 (3.6%), approximately equivalent to the calculated isotopic ratio (100% : 91.5%: 43.0%).

2-Amino-1-*O*-(6-amino-*β*-D-galactopyranosyl)-D-ribo-heptadecan-1,3,4-ol (17β): To a solution of β-anomer **14β** (40 mg, 0.41 mmol) in $CHCl_3$ (0.5 mL) was added MeOH (2 mL). AcOH (20 μL) and $Pd(OH)_2$ (81 mg) were added to the stirred mixture, sequentially. It was then sealed with septa and parafilm. The glassware was evacuated with syringe, followed by charging with hydrogen gas provided by a balloon. Repeating the procedure twice, a mixed solution of MeOH/$CHCl_3$ (1 mL, 4/1) was added to compensate for the solvent reduced by evaporation. The mixture was then stirred under an atmosphere of a balloon filling with hydrogen for 23 h. TLC (NH_3/MeOH/$CHCl_3$ = 1/5/5) indicated the consumption of

the starting material **14β** (R_f = 0.95) and the formation of the product **17β** (R_f = 0.14). The mixture was then filtered through celite pad, followed by washing with $CHCl_3$ and MeOH, sequentially. The filtrates were combined and concentrated under reduced pressure to provide an off-white solid which was followed by washing with $CHCl_3$ to remove some colored impurities. The wet solid was filtered and collected. The residue was dried under reduced pressure to afford a white solid in 86% yield (16 mg). Recrystallization of a sample from water was unsuccessful. Instead, after concentration under reduced pressure, the solid become pale yellow. ^{1}H NMR (500MHz, D_2O): δ 0.82 (bs, 3H, CH_3), 1.23 (bs, 22H, CH_2), 1.47 (bs, 1H), 1.72 (bs, 1H), 3.28 (bs, 2H), 3.54 (bs, 2H), 3.69 (bs, 2H), 3.81 (bs, 1H), 3.93 (bs, 2H), 4.05 (bs, 1H), 4.13 (bs, 1H), 4.50 (bs, 1H); ^{13}C NMR (125 MHz, D_2O): δ 14.10 (CH_3), CH_2: 22.86, 25.51, 29.74, 30.21, 32.21, 34.02, 40.46; 53.45 (CH), 65.56 (CH_2), 69.29 (CH), 70.65 (CH), 71.04 (CH), 71.97 (CH), 72.20 (CH), 72.39 (CH), 102.48 (CH); LRMS for $C_{23}H_{48}N_2O_7$: MW = 464.6, M (calcd.) = 464.4 (m/z), ESI+Q-TOF: M = 464.4 (m/z), $[M+H]^+$ = 465.4 (13.4%), 466.4 (4.2%), approximately equivalent to the calculated isotopic ratio (100% : 25.1%).

2-amino-1-*O*-(6-amino-α-D-galactopyranosyl)-D-ribo-octadecan-3,4-diol (2): To a mixture of starting material **15α** and MeOH (8 mL) was added NaOMe (3 mg, 0.05 mmol, 0.5 eq). The stirring was allowed for 1 h. TLC (EtOAc/*n*-hexane = 1:4) indicated the consumption of the starting material **15α** (R_f = 0.90) and the formation of the product (R_f = 0.58). After adding the cationic exchange resin (H+), the pH was adjusted to neutral range. The mixture was filtered through celite pad. The filtrate was concentrated and the residue obtained was further purified using column chromatography (MeOH/$CHCl_3$ 1:9) to afford white solid in 90% yield (57 mg). **2-azido-1-*O*-(6-azido-2,3,4-tri-O-benzyl-α-D-galactopyranosyl)-D-ribo-octadecan-3,4-diol** ^{1}H-NMR (500 MHz, CD_3OD): δ 0.88 (t, 3H, *J* = 7.0 Hz, $H_{aliphatic}$), 1.26-1.36 (m, 24H, $H_{aliphatic}$), 1.60-1.80 (m, 2H, $H_{aliphatic}$), 3.14 (dd, 1H, $J_{6a',6b'}$= 12.5 Hz, $J_{6a',5'}$ = 4.5 Hz, $H_{6a'}$), 3.48 (dd, $J_{6b',6a'}$= 12.5 Hz, $J_{6b',5'}$= 8.5 Hz, 1H, $H_{6b'}$), 3.52-3.57 (m, 1H), 3.59 (dd, *J* = 7.0, 4.5 Hz, 1H), 3.70-3.73 (m, 1H), 3.75 (dd, *J* = 10.5, 6.5 Hz, 1H), 3.94 (bs, 1H), 3.96 (dd, *J* = 10.0, 2.5 Hz, 1H), 4.00 (dd, *J* = 10.0, 3.5 Hz, 1H), 4.10 (dd, *J* = 10.0, 2.5 Hz, 1H), 4.55 (d, *J* = 11.5 Hz,1H, C$\underline{H_2}$Ph),

4.73-4.77 (m, 4H, C$\underline{H}_2$Ph), 4.90 (d, J =11.0 Hz, 1H, C$\underline{H}_2$Ph), 4.94 (d, 1H, $J_{1,2}$ = 3.5 Hz, H_1), 7.26-7.38 (m, 15H, ArH); ^{13}C-NMR (125MHz, CD_3OD): δ 14.46 (CH_3), CH_2: 23.74, 26.74, 30.48, 30.76, 30.79, 33.07, 34.14, 52.61; 63.68 (CH), 68.98 (CH_2), 71.66 (CH), 73.01 (CH), 74.18 (CH_2, $\underline{C}H_2$Ph), 74.28 (CH_2, $\underline{C}H_2$Ph), 75.99 (CH_2, $\underline{C}H_2$Ph), 76.46 (CH), 76.96 (CH), 77.30 (CH), 79.85(CH), 99.68(CH), arom. CH: 128.68, 128.79, 128.89, 129.17, 129.33, 129.36, 129.39, 129.42; arom. quaternary C: 139.70, 139.80, 139.96; LRMS for $C_{45}H_{64}N_6O_7$: M (calcd.) = 800.5 (m/z), ESI+Q-TOF: M = 800.3 (m/z), $[M+Na]^+$ = 823.3. The similar procedure as described for compound **17β** was employed. The benzoyl-group-removed compound (57 mg, 0.07 mmol), a cosolvent of MeOH (8 mL) and $CHCl_3$ (2 mL), glacial AcOH (30 μL) and $Pd(OH)_2$ (114 mg) were employed. TLC (NH_3/MeOH/$CHCl_3$ = 0.2/1/1) indicated the formation of the product (R_f = 0.13) and the consumption of the starting material (R_f = 0.95). After 30 h, the mixture was filtered through celite pad and washed with MeOH to obtain the filtrate. After concentration under reduced pressure, a white solid of product **2** was obtained in 90% yield (30 mg). LRMS for $C_{24}H_{50}N_2O_7$: M (calcd.) = 478.4 (m/z), ESI+Q-TOF: M = 478.36 (m/z), $[M+H]^+$= 479.3 (94.0%), 480.3 (28.9%), 481.3 (5.8%), approximately equivalent to the calculated isotopic ratio (100% : 26.8% : 4.9%).

***N*-((2S,3S,4R)-2-amino-3,4-dihydroxyheptadecyl)-4-butylbenzamide (18):** Compound **1** (15 mg, 0.05 mmol), 4-butylbenzoic acid (1 eq) and HBTU (1.2 eq) were used, respectively. Purification used column chromatography with eluents of MeOH/$CHCl_3$ 1:19→1:12 to afford the product mixtures, which were observed to be pure in TLC. Further purification using HPLC as described above but with eluents of MeOH/$CHCl_3$ 1:13 to collect the fraction under the area between 8.5 and 10.5 min. Product **18** was obtained in 35 % yield (8 mg). Reinjection of the concentrated fraction into HPLC showed two peaks in chromatogram. It was suspected to be two conformers due to rotation. Miscellenuous small unidentified peaks in ^{1}H-NMR were impurities, which were also observable in HPLC chromatogram. The impurities were suspected to be the unremoved diisopropylethyl amine, which was confirmed from the spectrum of ESI-MS: $[M+H]^+$ = 130.2 (28%), 131.2 (3%); ^{1}H-NMR (500 MHz, C_6D_6): δ 0.90-0.96 (m, 6H, $H_{aliphatic}$), 1.25-1.50 (m, 22H, $H_{aliphatic}$), 1.62-1.80 (m, 4H, $H_{aliphatic}$), 2.02-2.12 (m, 2H), 2.44-2.47 (m, 2H), 3.99-

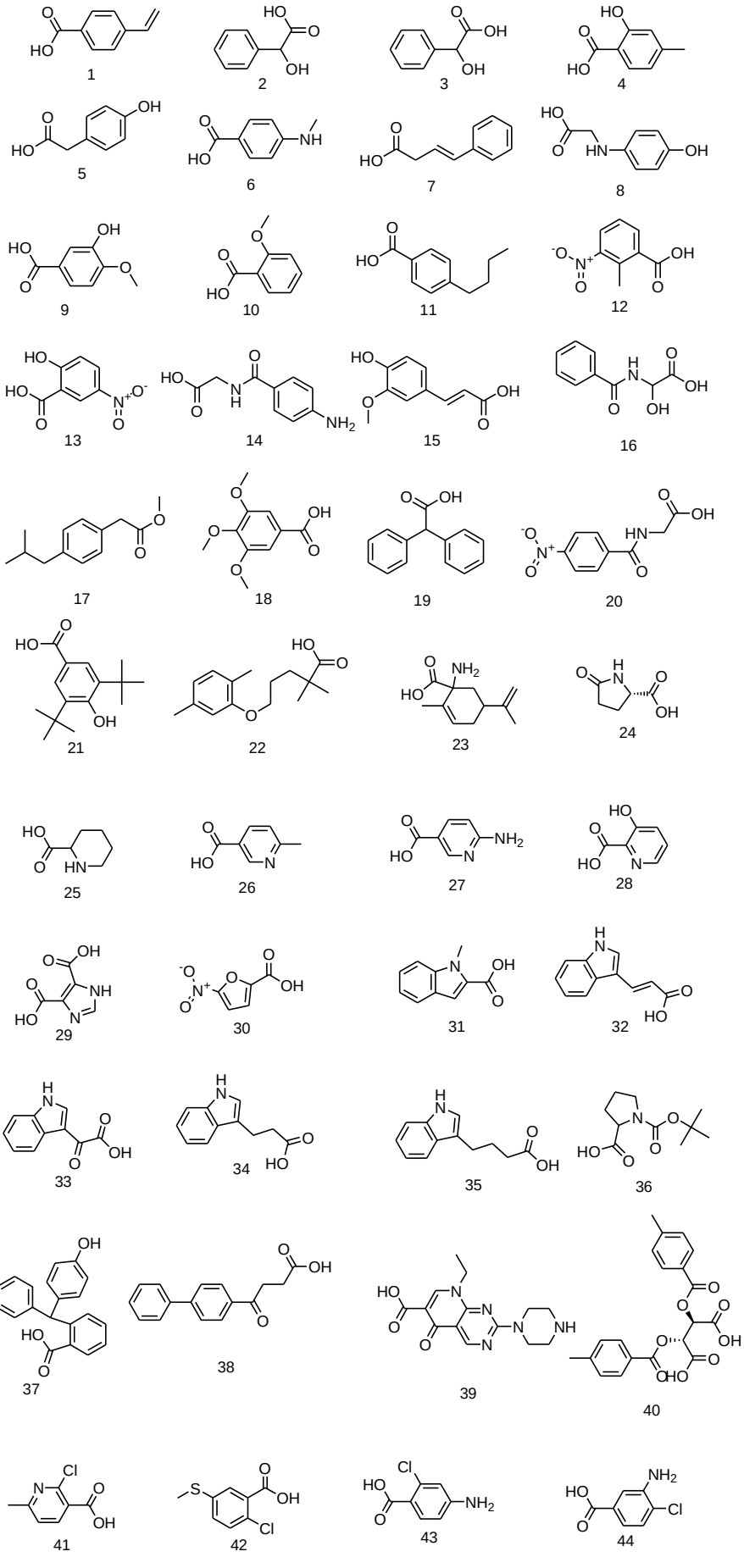
4.24 (m, 5H), 7.13-7.15 (m, 2H, ArH), 8.04-8.06 (m, 2H, ArH), 8.29 (bs, 1H, amide); ^{13}C-NMR(125MHz, C_6D_6): δ 8.13, 14.10, 14.37, 22.78, 23.14, 26.36, 29.92, 30.27, 30.38, 30.45, 30.49, 30.53, 32.39, 33.45, 35.83, 46.62, 54.92, 66.20, 72.60, 74.10, 127.47, 129.01, 130.67, 147.83, 170.38; LRMS for $C_{28}H_{50}N_2O_3$: M (calcd.) = 462.4 (m/z), ESI+Q-TOF: M = 462.4 (m/z), $[M+H]^+$ = 463.4 (100%), 464.4 (29%), 465.4 (5%), $[M+Na]^+$ = 485.3 (8%), 486.4 (2%), approximately equivalent to the calculated isotopic ratio (100% : 31% : 5.3%).

4-butyl-*N*-(((2R,3R,4S,5R,6S)-6-(((2S,3S,4R)-2-(4-butylbenzamido)-3,4-dihydroxyoctadecyl)oxy)-3,4,5-trihydroxytetrahydro-2H-pyran-2-yl)methyl)benzamide (**19):** To a mixture of 4-butylbenzoic acid (23 mg, 0.13 mmol, 2.1 equiv), HBTU (57 mg, 0.15 mmol, 2.4 equiv) and DMF (6 mL) was added diisopropyl ethyl amine (14 μL, 0.08 mmol, 1.3 equiv) under N_2. After stirring for 10 min, TLC (EtOAc: *n*-hexane =1:3) indicated the formation of the ester intermediate (R_f = 0.73) and consumption of the starting 4-butyl benzoic acid (R_f = 0.12). To this mixture was added the solution of compound **2** (30 mg, 0.06 mmol) in DMF (4 mL). After stirring for 30 h, TLC (NH_3/MeOH/$CHCl_3$ =0.2/1/1) indicated the formation of the product **19** (R_f = 0.89) and consumption of the starting compound **2** (R_f = 0.14). The mixture was concentrated under reduced pressure. The residue obtained was purified using column chromatography (EtOAc : *n*-hexane=1:4) to afford the white solid **19** in 60% yield (31 mg). The sample was further purified using HPLC (0.9 cm×20 cm, Si-100) with eluents MeOH/$CHCl_3$ =1/29 at a flow rate of 3 mL/min to afford white solid (5 mg). t_R = 19.2 min; t_R = 11.9 min (aromatic impurities). Anal. $C_{46}H_{74}N_2O_9$, M (calcd.) = 798.5 (m/z), ESI+Q-TOF: M = 798.6 (m/z), $[M+H]^+$ = 799.6 (19.04%), 800.6 (10.99%), $[M+Na]^+$= 821.6 (100%), 822.6 (50.09%), 823.6 (11.33%), equivalent to the calculated isotopic ratio 100:50.8:12.7; HRMS (ESI) M (calcd.) = 798.53943 (m/z), M (found) = 798.53975 (m/z),^{1}H-NMR (500 MHz, CD_3OD): δ 0.87-0.94 (m, 9H, $H_{aliphatic}$), 1.21-1.40 (m, 28H, $H_{aliphatic}$), 1.60-1.70 (m, 5H, $H_{aliphatic}$), 2.61-2.67 (m, 4H), 3.44-3.48 (m, 1 H), 3.56-3.60 (m, 1 H), 3.67-3.82 (m, 6H), 3.93-4.00 (m, 2H), 4.40-4.44 (dd, 1H, *J* = 10.5, *J* = 5.0 Hz), 4.93-4.94 (d, 1H, *J* = 3.5 Hz, H_1), 7.20-7.25 (m, 4 H, ArH), 7.67-7.71 (m, 4 H, ArH); ^{13}C-NMR (125 MHz, CD_3OD): δ 14.18 (CH_3), 14.36 (CH_3), 23.29 (CH_2), 23.32 (CH_2), 23. 67 (CH_2), 26.77 (CH_2), 30.41(CH_2), 30.71 (CH_2), 30.75 (CH_2), 33.03

(CH_2), 33.47 (CH_2), 34.54 (CH_2), 36.46 (CH_2), 41. 69 (CH_2), 52.60 (CH_2), 67.96 (CH_2), 70.18 (CH), 70.52 (CH), 71.29 (CH), 71.46 (CH), 73.04 (CH), 75.85 (CH_3), 101.13 (CH), arom: 128.38, 128.46, 129.57, 132.93, 133.19, 148.34, 148.27; 169.93 (amide), 170.76 (amide).

3. Carboxylic acids used for amide preparation

Five amide products described in the maintext were generated from acid components: a) 4-Butyl-benzoic acid, b) 2-Hydroxy-5-nitro-benzoic acid, c)3-Bis-(4-methyl-benzoyloxy)-succinic acid, d) (3-Chloro-4-hydroxy-phenyl)-acetic acid , e) (3-Fluoro-4-hydroxy-phenyl)-acetic acid.


1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44

4. Preparation of cell lines and the MTT assay

Cell Culture

Adherent normal human fibroblast and U87 cells were maintained at 37℃ in a humidified CO_2-controlled atmosphere in Minimum Essential Medium (MEM) (Sigma-Aldrich) supplemented with 10% heat-inactivated fetal bovine serum (FBS) (Biological Industries). In addition, adherent A549 and C26 cells were maintained at 37 ℃ in a humidified CO_2-controlled atmosphere in RPMI 1640 (Sigma-Aldrich) supplemented with 10% heat-inactivated fetal bovine serum (FBS) (Biological Industries).

MTT Assay of amide-bond formation products

1. Plating cell

Briefly, 3000 cells per well were plated in 96-well microtiter plates with 100 μL MEM/10%FBS and incubated at 37 ℃ in a humidified CO_2-controlled atmosphere for 1 day.

2. Construction of amide bonding libraries and their cytotoxicity screening

We used 44 carboxylic acids to construct amide bonding libraries. Every carboxylic acid (1 eq, dissolved in 25 μL DMSO) was activated by HBTU (1.1 eq, 4.1 mg, dissolved in 23 μL DMSO) and DIEA (1.2 eq, 0.012 mmol, 2 μL). The amide bonding reaction was carried out by coupling a portion of crude active ester (10 μL, 0.2 M, dissolved in DMSO) with amine (10 μL, 0.2 M, dissolved in DMSO). After completion of amide bond formation, a portion of the crude product (0.1 M, dissolved in 4 μL DMSO) was diluted by de-ionized sterilized water (396 μL) and filtrated with 0.2 μm filter. The filtrate (1 mM crude product in 10 μL water containing 1 % (v/v) DMSO) was diluted with 100 μL culture medium in the previous cell-plated microtiter plates so that the concentration of DMSO was less than 0.1% (v/v), and the crude product was less than 100 μM. These microtiter plates was further incubated at 37 ℃ in a humidified CO_2-controlled atmosphere for 2 days. 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (0.5 mg dissolved in 1 mL PBS buffer) was added to previous microtiter plates and incubated for 4 h. After removing the culture medium from microtiter plates and dissolving insoluble formazan

with 100 μL DMSO, cytotoxicity screening data was obtained by dectecting the absorbance of 570 nm with microtiter plate reader (Plate CHAMELEON^{TM}). The MTT assay results were shown below.

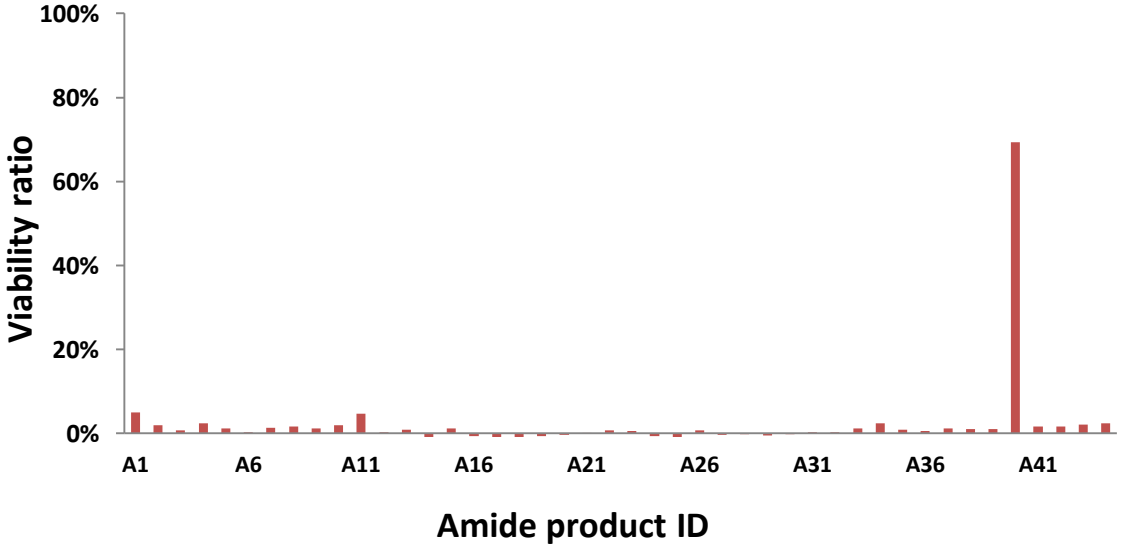
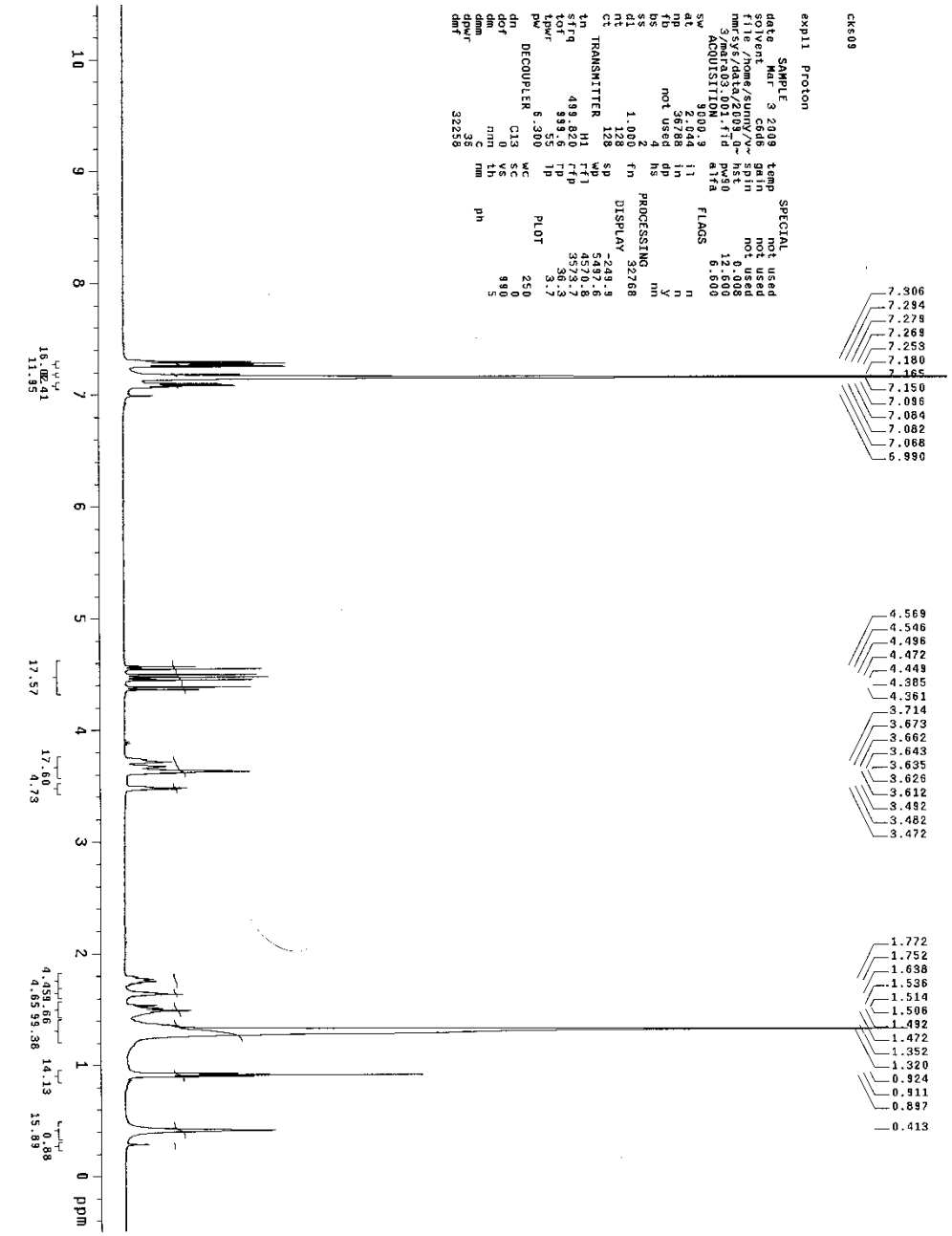


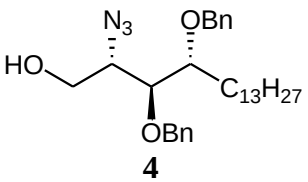
Figure 1. Analog **18** (A11) showed the less cytotoxicites against normal human fibroblasts (50% in U87 cells). A40 was obtained from (2R,3R)-2,3-bis(4-methylbenzoyloxy)succinic acid. Purification of the product mixtures of A40 with HPLC generated a number of unidentified peaks in chromatogram.

5. Invariant nature killer cell quantification

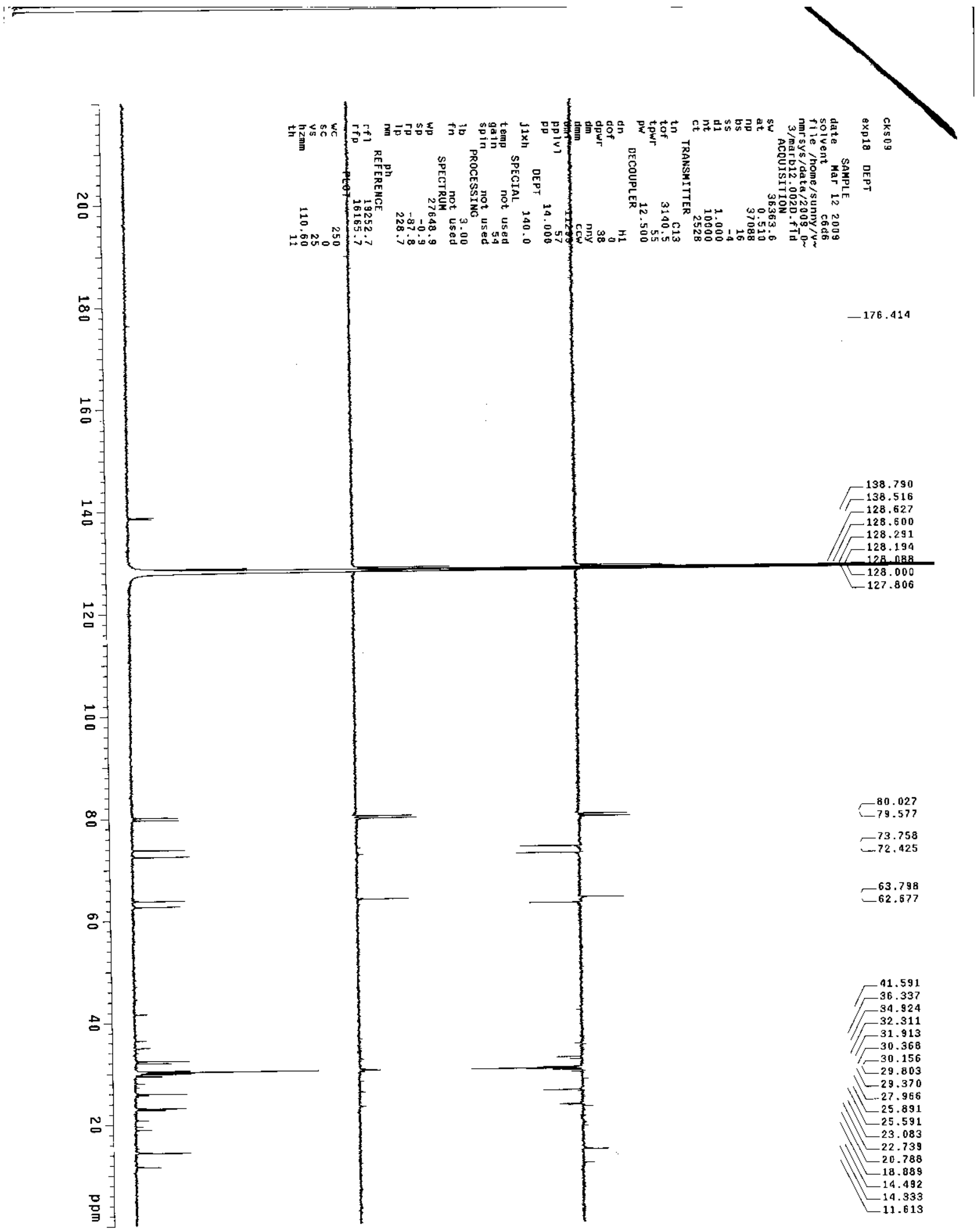
The iNKT was obtained from peripheral blood monocytes (PBMC) of healthy donors after gradient-separated at 400g, 30 minutes with Ficoll-Hypaque^{TM} plus (GE Healthcare, CA, USA). The cells were cultured and enriched in RPMI with L-glutamin (Gibco, NY, USA) with supplement of 10% fetal calf serum and 1% penicillin-streptomycin. α-Galactosylceramide (α-GalCer, Kirin, Gunma, Japan) was added to the medium at defined concentration every 3 days. The iNKT population was either identified or sorted (fluorescence-activated cell sorted (FACS), magnetic cell seperation) with antibodies against $V\alpha24^{+}/V\beta11^{+}$. The antibodies for staining T-cell receptors (TCR, $V\alpha24^{+}/V\beta11^{+}$) were purchased from Beckman Coulter (CA, USA), BD bioscience (NJ, USA) and Miltenyi-Biotec (CA, USA). Briefly, after 7-10 days of incubation, the cultured cells were analyzed with flow cytometry.

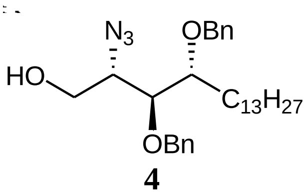
6. ^{1}H- and ^{13}C-NMR, ESI-MS spectra and HPLC chromatogram



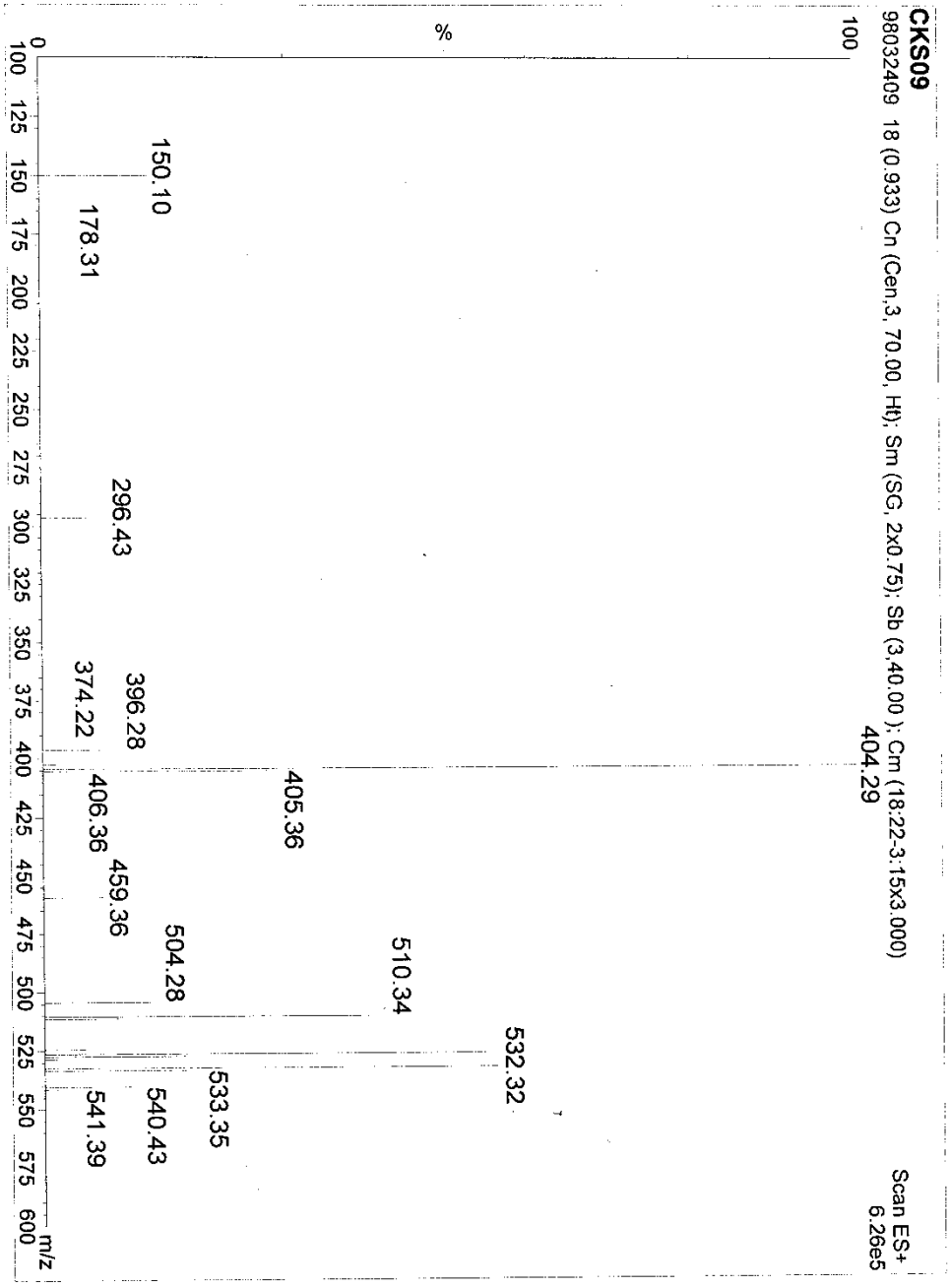


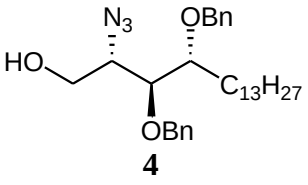
4





4


CKS09
98032409 18 (0.933) Cn (Cen,3, 70.00, Ht); Sm (SG, 2x0.75); Sb (3,40.00); Cm (18:22-3:15x3.000)
Scan ES+
6.26e5
%
100
0
100 125 150 175 200 225 250 275 300 325 350 375 400 425 450 475 500 525 550 575 600
m/z
150.10
178.31
296.43
374.22
396.28
404.29
405.36
406.36
459.36
504.28
510.34
532.32
533.35
540.43
541.39


N3
OBn
HO
C13H27
OBn

4

國立交通大學應用化學系

元素分析儀 Heraeus CHN-O Rapid 服務報告書

使用者姓名：張凱翔　　中心編號：980357

服務單位：清大原科 俞鐘山實驗室　樣品名稱或代號：CKS09

收件日期： 98 年 3 月 24 日 完成日期： 98 年 3 月 26 日

分析結果：

實驗值：	N%	C%	H%
1.	8.10	72.85	8.49
2.	8.21	72.74	9.09
3.			
4.			
推測值：	8.24	73.05	9.29

本日所使用之 Standard：A

(A)Acetanilide (B)Atropin (C)N-Anilin

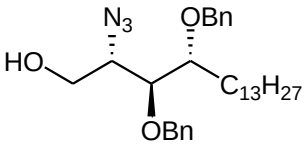
	N%	C%	H%
理論值：	10.36	71.09	6.71
測出值：	10.42	70.86	6.55

備註：

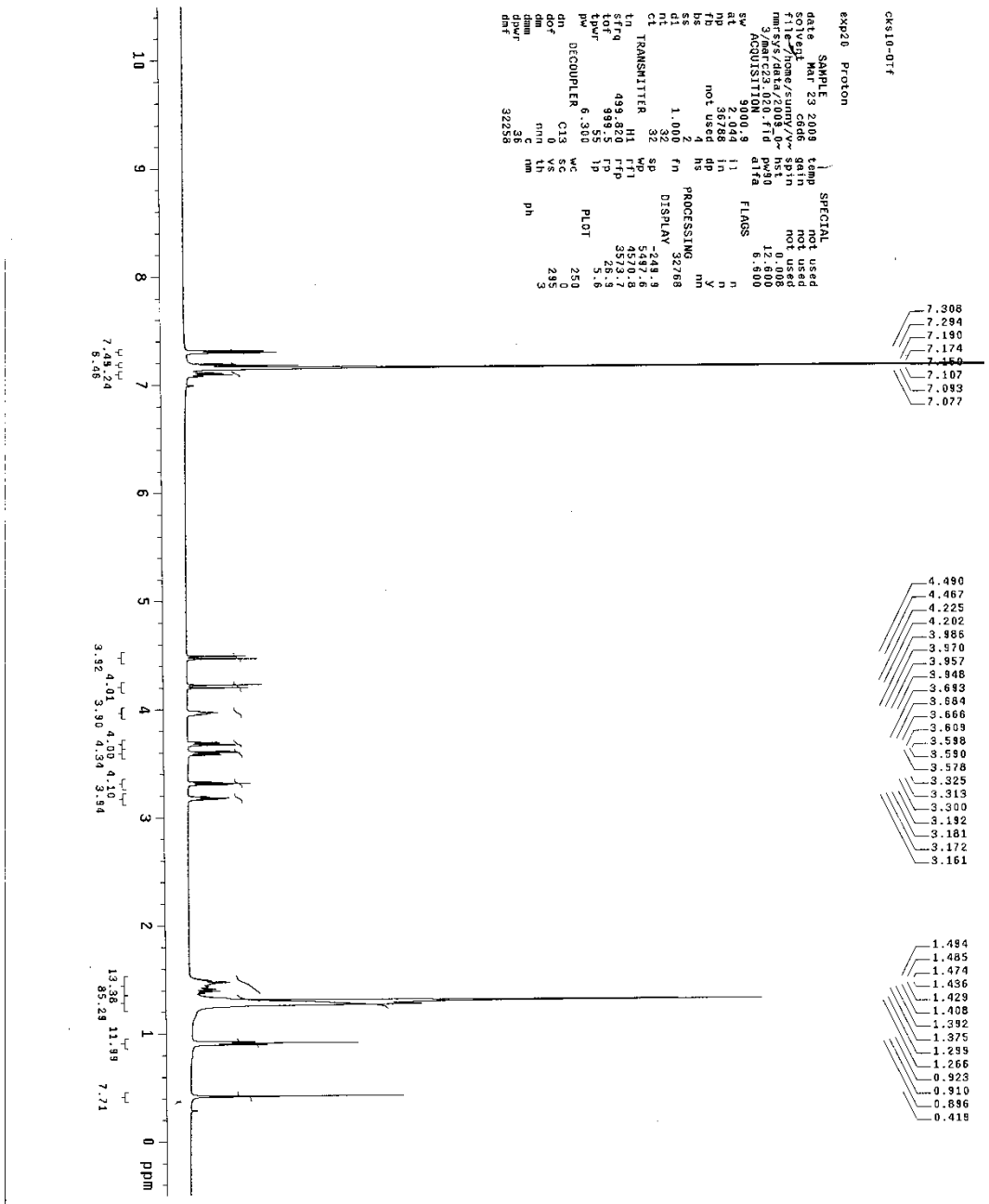
費用核算：NCH：800 元

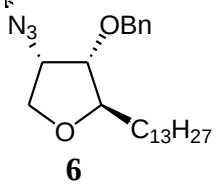
報告日期： 98 年 3 月 27 日

儀器負責人簽章：　　技術員簽章：技士李蘊明

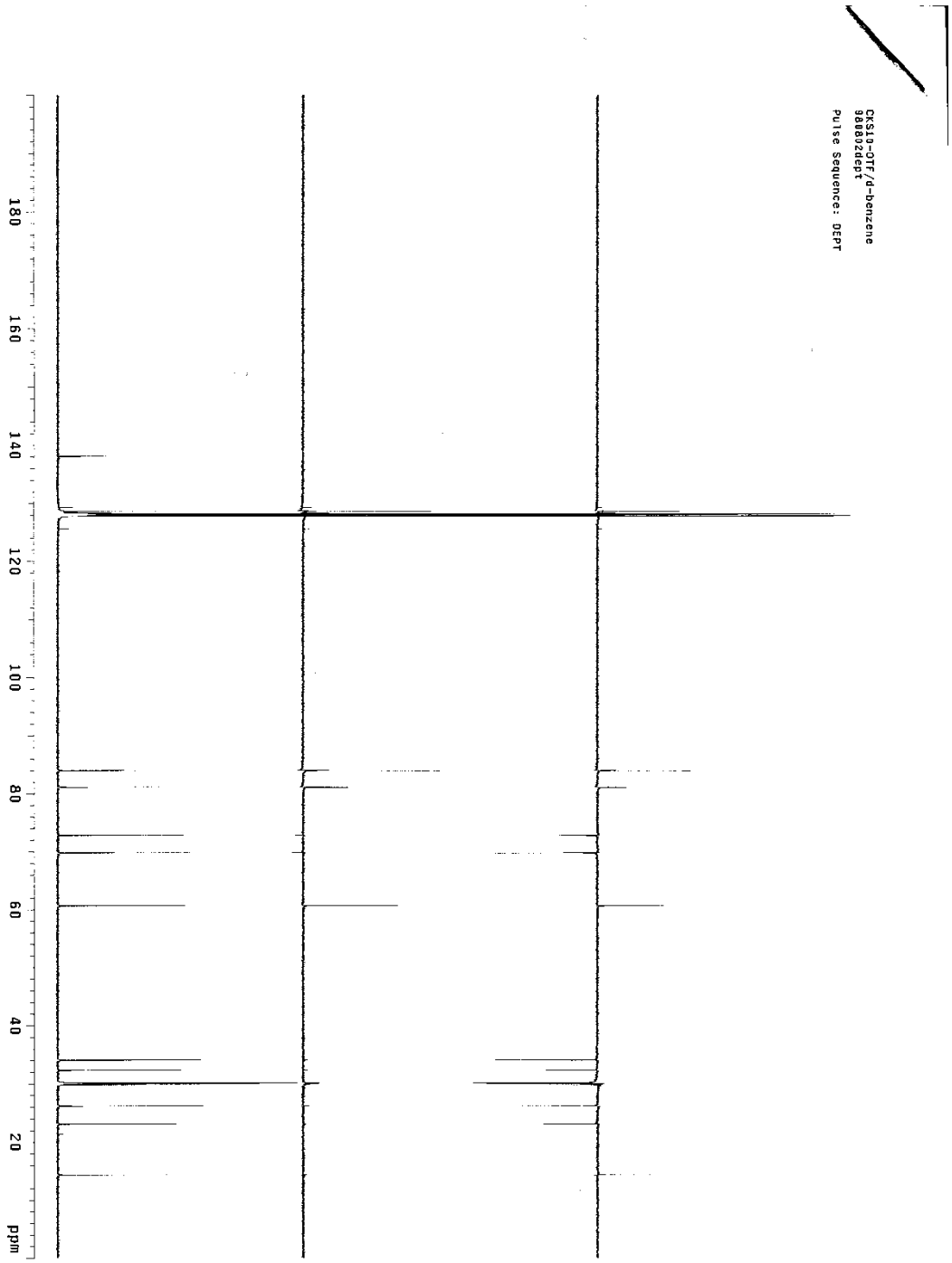


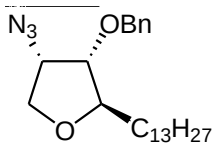
4


gks10-0Tf
exp20 Proton
7.308
7.294
7.190
7.174
7.159
7.107
7.093
7.077
4.490
4.467
4.225
4.202
3.986
3.970
3.957
3.948
3.693
3.684
3.666
3.609
3.598
3.590
3.578
3.325
3.313
3.300
3.192
3.181
3.172
3.161
1.494
1.485
1.474
1.436
1.429
1.408
1.392
1.375
1.299
1.266
0.923
0.910
0.896
0.419
ppm

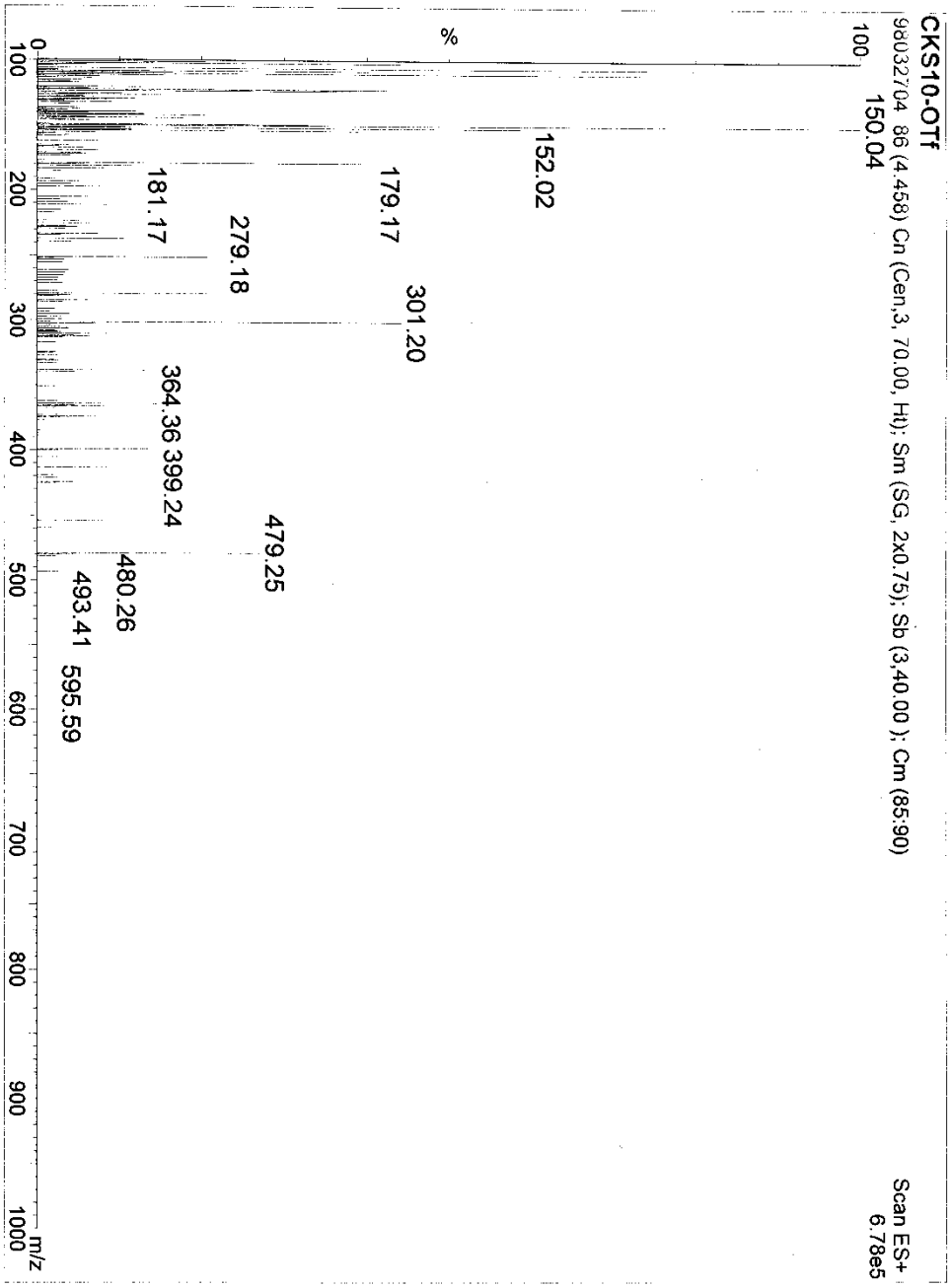

N3
OBn
O
C13H27

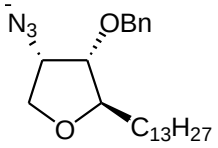
6


CKS1g-DTF/d-benzene
980802dept
Pulse Sequence: DEPT
180
160
140
120
100
80
60
40
20
ppm

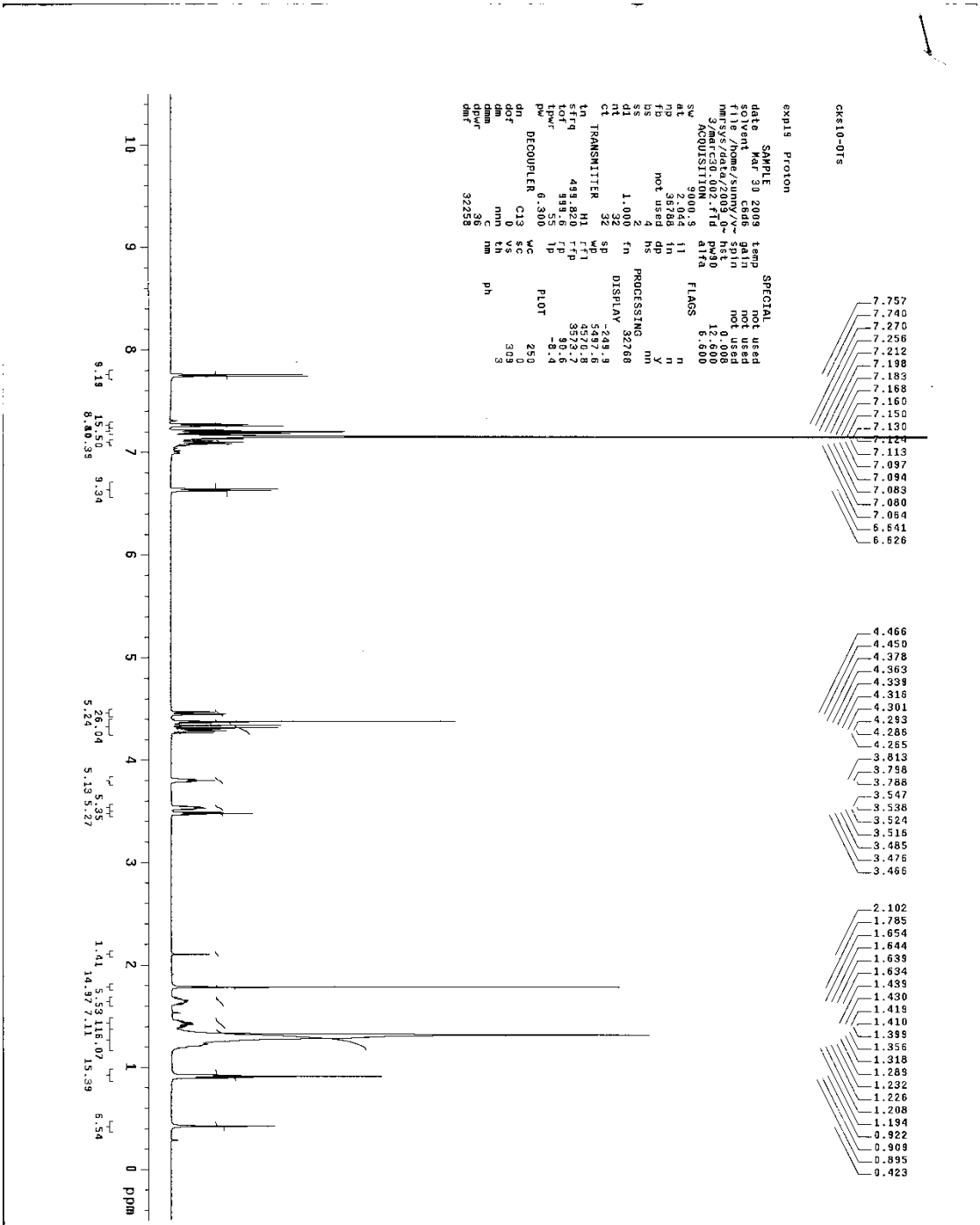

N3
OBn
O
C13H27

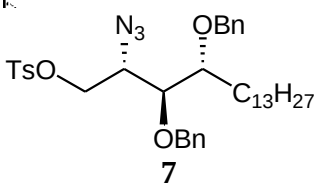
6


CKS10-OTf
98032704 86 (4.458) Cn (Cen,3, 70.00, Hi); Sm (SG, 2x0.75); Sb (3,40.00); Cm (85.90)
Scan ES+
6.78e5
%
100
0
150.04
152.02
179.17
181.17
279.18
301.20
364.36
399.24
479.25
480.26
493.41
595.59
100
200
300
400
500
600
700
800
900
1000
m/z

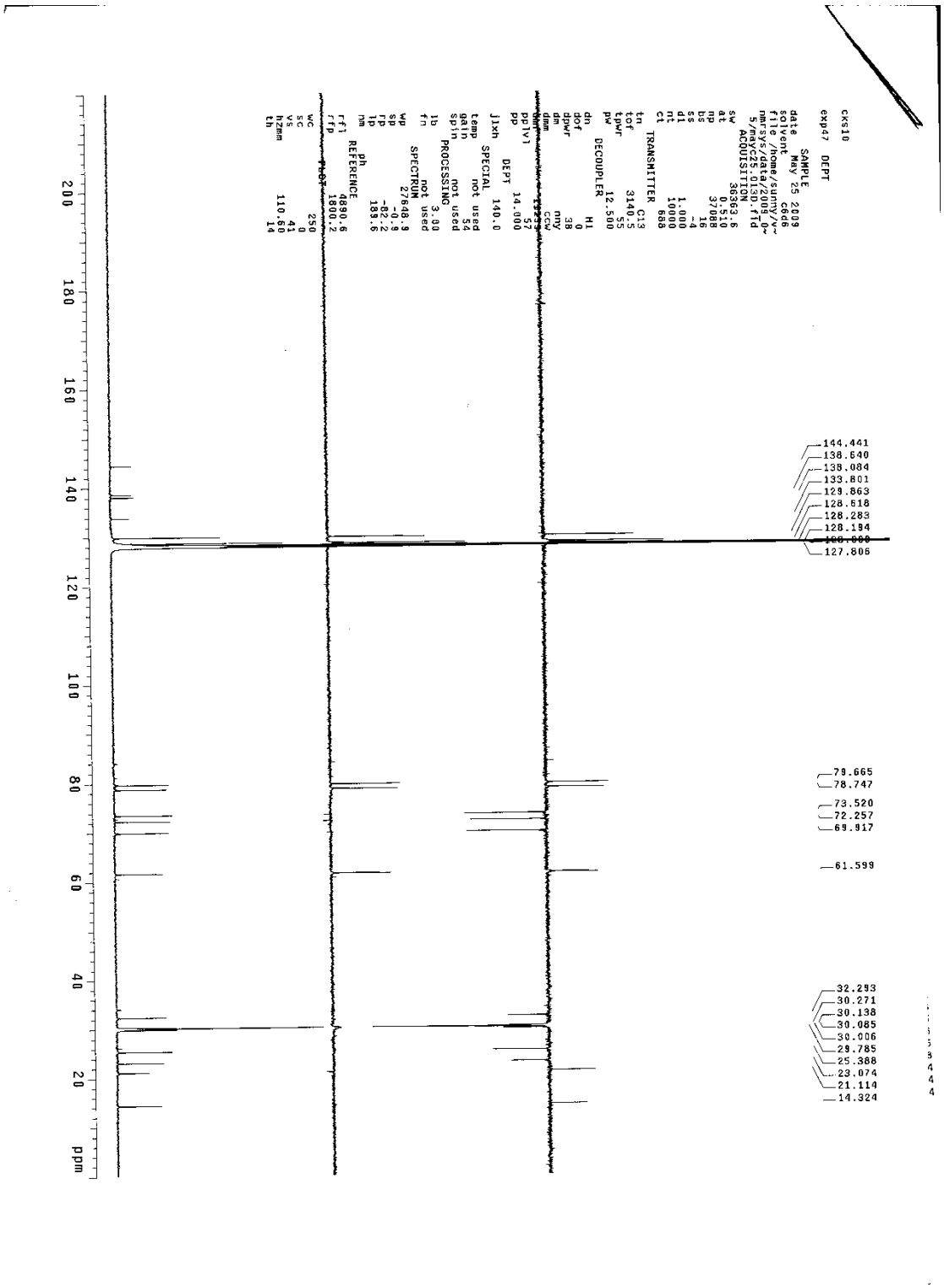

N3
OBn
O
C13H27

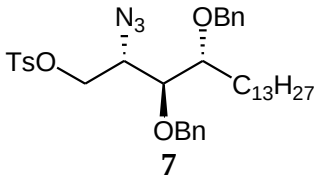
6


gks10-OTs
expts Proton
SAMPLE
date Mar 30 2009
solvent cdcl3
file /home/sunny/v~
nmrsys/data/2009_0~
3/mail30_02.fid
ACQUISITION
sw 9000.9
at 2.044
np 36788
fb not used
bs 4
ss 2
d1 1.000
nt 32
ct 32
TRANSMITTER
tn H1
sfrq 499.820
tof 999.6
tpwr 55
pw 6.300
DECOUPLER
dn C13
dof 0
dm nnn
dmm c
dmf 32258
SPECIAL
temp
gain
spin
hst
pw90
d1fa
FLAGS
not used
not used
not used
12.000
6.600
n
n
y
nn
PROCESSING
DISPLAY 32768
-249.9
5497.6
4570.8
3573.7
90.6
-8.4
PLOT
250
0
309
3
ph
7.757
7.740
7.270
7.256
7.212
7.198
7.183
7.168
7.160
7.150
7.130
7.124
7.113
7.097
7.094
7.083
7.080
7.064
6.641
6.626
4.466
4.450
4.378
4.363
4.339
4.316
4.301
4.293
4.286
4.265
3.813
3.798
3.788
3.547
3.538
3.524
3.516
3.485
3.476
3.466
2.102
1.785
1.654
1.644
1.639
1.634
1.439
1.430
1.419
1.410
1.399
1.356
1.318
1.289
1.232
1.226
1.208
1.194
0.922
0.909
0.895
0.423
ppm

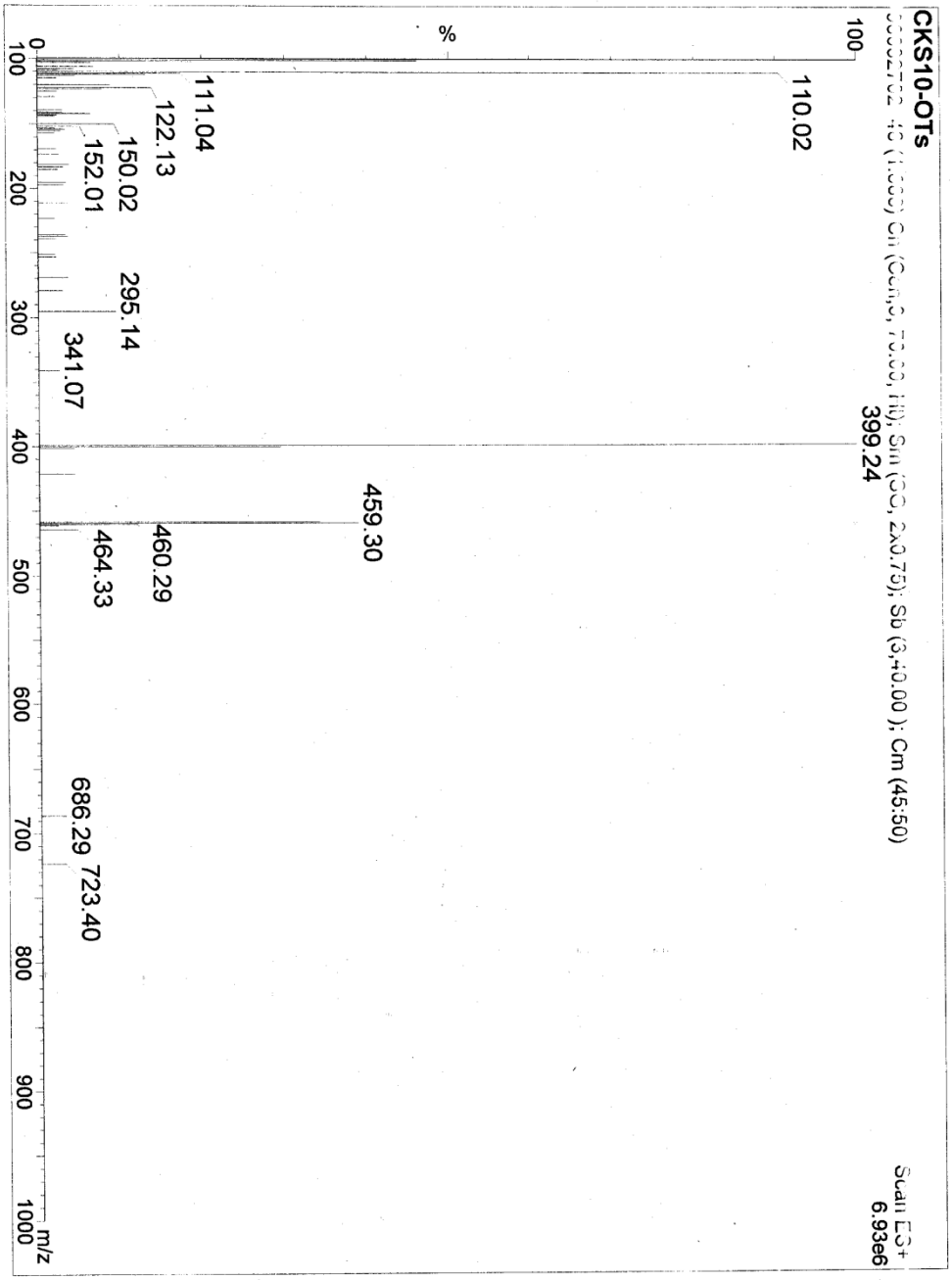

N3
OBn
TsO
C13H27
OBn

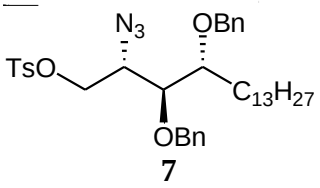
7


cks10
exp47 DEPT
SAMPLE
date May 25 2009
solvent cdcl3
file /home/sunny/v~
nmrsys/data/2009_0~
5/may25.013D.fid
ACQUISITION
sw 6369.6
at 0.510
np 32688
bs 16
ss -4
d1 1.000
nt 10000
ct 668
tn TRANSMITTER C13
tof 3140.5
tpwr 55
pw 12.500
DECOUPLER
dn H1
dof 0
dpwr 39
dm nny
dmm ccw
pp1v1 57
pp 14.000
DEPT
jlxh 140.0
SPECIAL
temp not used
gain 54
spin not used
PROCESSING
lb 3.00
fn not used
SPECTRUM
wp 2768.9
sp -0.9
rp -82.2
lp 189.6
nm
ph
REFERENCE
rf1 1890.6
rfp 1800.2
wc 250
sc 0
vs 41
hzmm 110.60
th 14
144.441
138.640
138.084
133.801
129.863
128.618
128.283
128.194
127.806
79.665
78.747
73.520
72.257
69.917
61.599
32.293
30.271
30.138
30.085
30.006
29.785
25.388
23.074
21.114
14.324
200
180
160
140
120
100
80
60
40
20
ppm

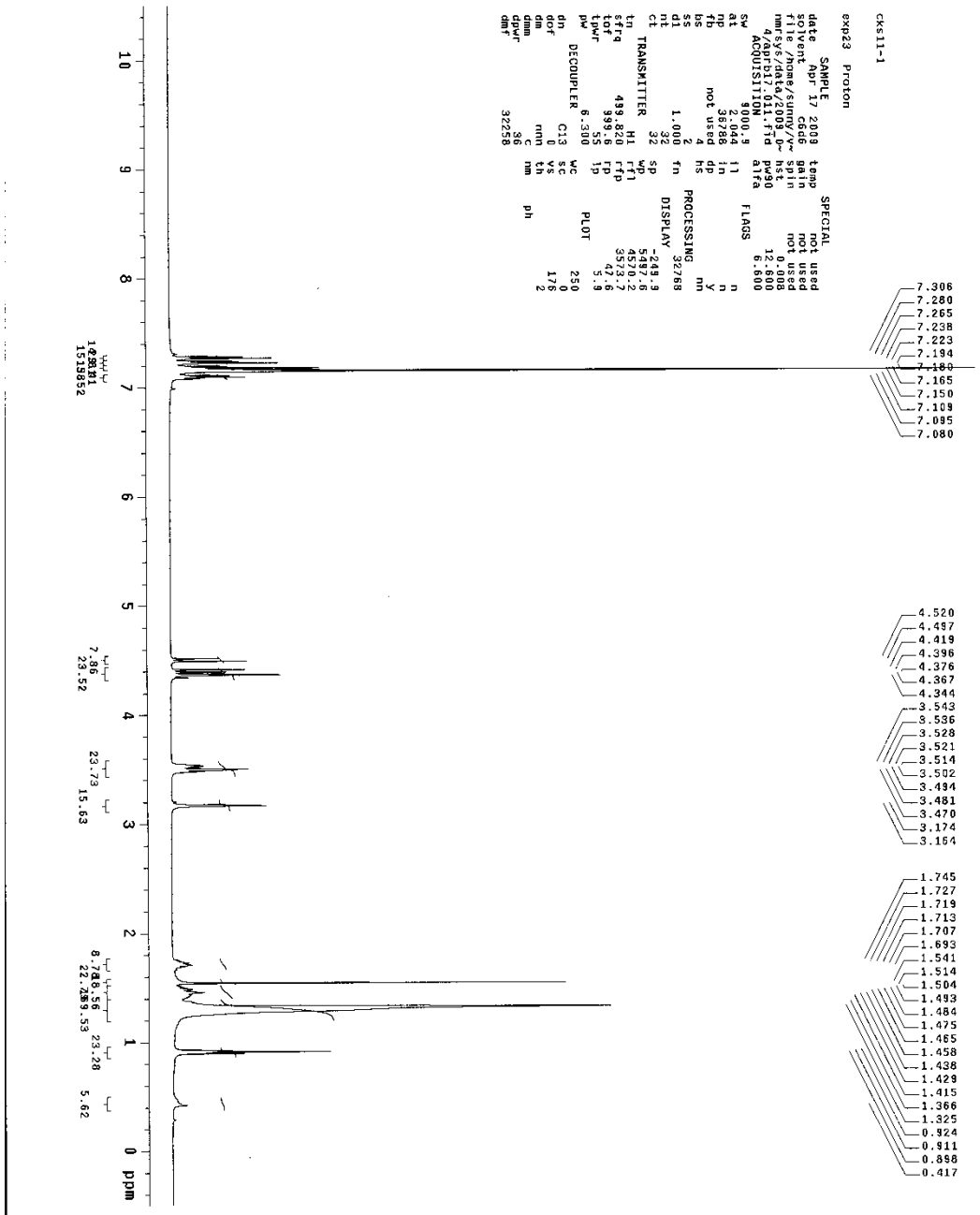

N3
OBn
TsO
C13H27
OBn

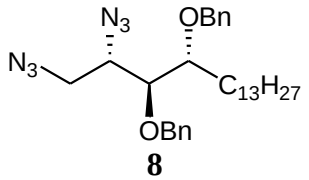
7


CKS10-OTs
Scan ES+
6.93e6
%
m/z
110.02
111.04
122.13
150.02
152.01
295.14
341.07
399.24
459.30
460.29
464.33
686.29
723.40

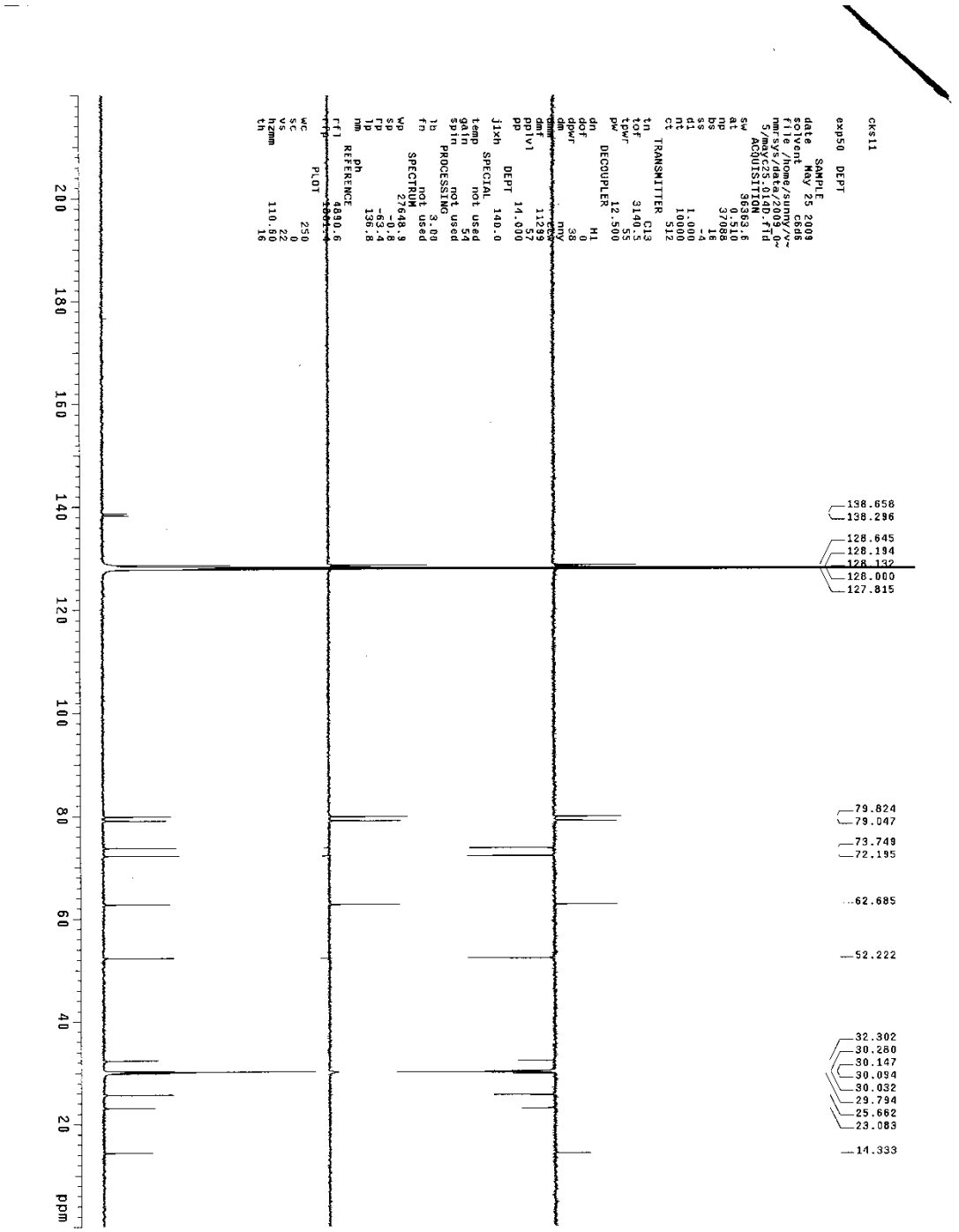

N3
OBn
TsO
C13H27
OBn

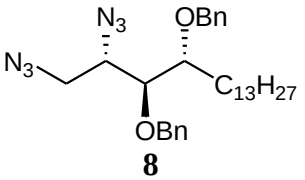
7


cks11-1
exp23 Proton
7.306
7.280
7.265
7.238
7.223
7.194
7.180
7.165
7.150
7.109
7.095
7.080
4.520
4.497
4.419
4.396
4.376
4.367
4.344
3.543
3.536
3.528
3.521
3.514
3.502
3.494
3.481
3.470
3.174
3.164
1.745
1.727
1.719
1.713
1.707
1.693
1.541
1.514
1.504
1.493
1.484
1.475
1.465
1.458
1.438
1.429
1.415
1.366
1.325
0.924
0.911
0.898
0.417
ppm

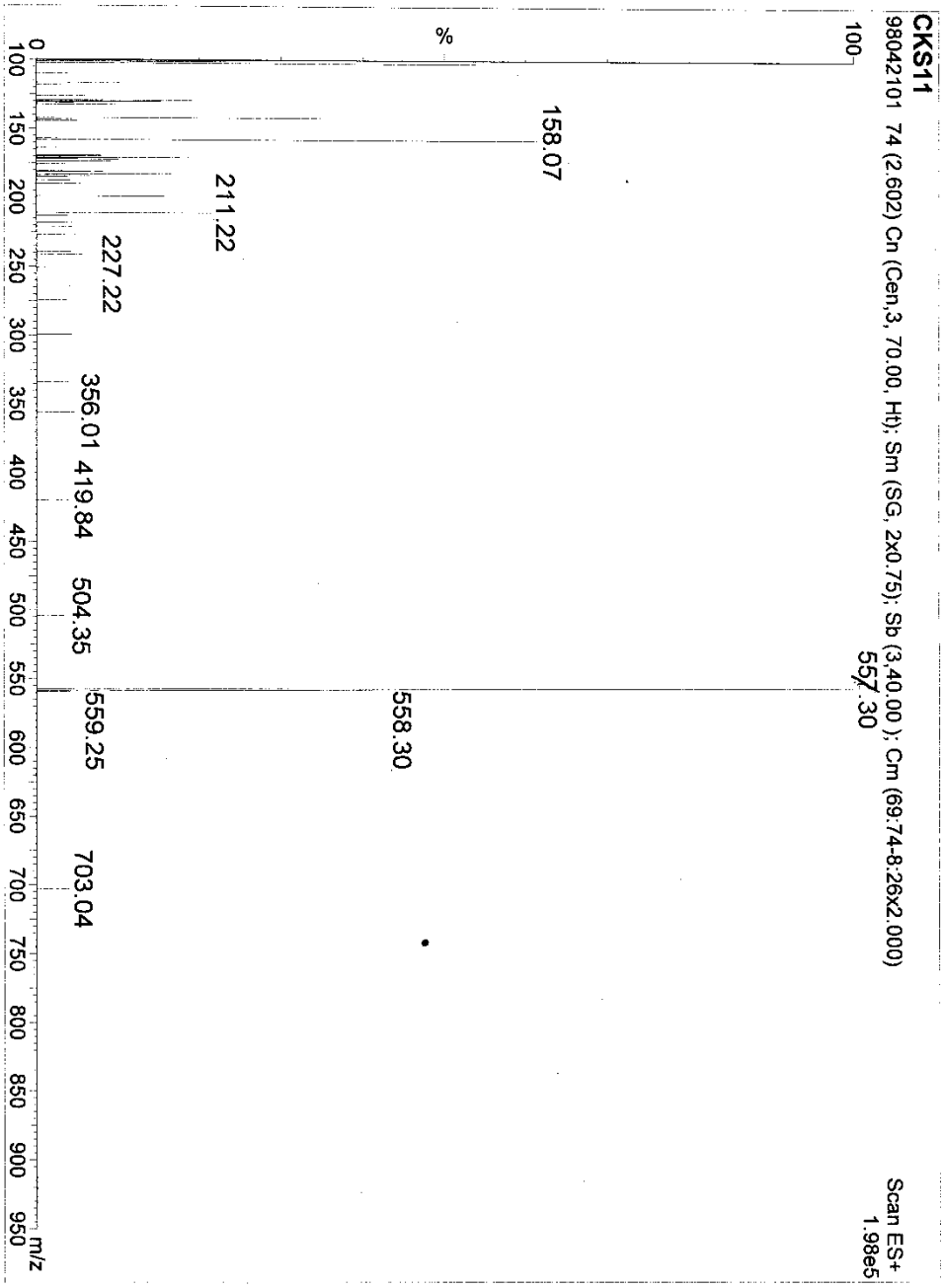

N3
OBn
N3
C13H27
OBn

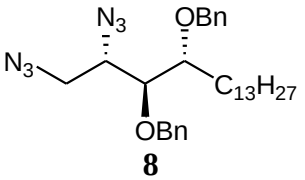
8


cks11
exp50 DEPT
SAMPLE
date May 25 2009
solvent cdcl3
file /home/sunny/v~
nmrsys/data/2009_0~
5/may25.01.fid
ACQUISITION
sw 36963.6
at 0.510
np 37088
bs 16
ss -4
d1 1.000
nt 10000
ct 512
TRANSMITTER
tn C13
tof 3140.5
tpwr 55
pw 12.500
DECOUPLER
dn H1
dof 0.0
dpwr 38
dm nny
dmm ccw
dmf 11299
ppl(v) 57
pp 14.000
DEPT
jlxh 140.0
SPECIAL
temp not used
gain 54
spin not used
PROCESSING
lb not used
fn 8.00
SPECTRUM
wp 27648.9
sp -0.8
rp -63.4
lp 136.8
nm ph
REFERENCE
rfl 4890.6
rfp 1881.4
PLOT
wc 250
sc 0
vs 22
hzmm 110.60
th 16
138.658
138.296
128.645
128.194
128.132
128.000
127.815
79.824
79.047
73.749
72.195
62.685
52.222
32.302
30.260
30.147
30.094
30.032
29.794
25.662
23.083
14.333
ppm


N3
OBn
N3
C13H27
OBn

8


CKS11
98042101 74 (2.602) Cn (Cen,3, 70.00, Ht); Sm (SG, 2x0.75); Sb (3.40.00); Cm (69:74-8:26x2.000)
Scan ES+
1.98e5
%
100
0
158.07
211.22
227.22
356.01
419.84
504.35
557.30
558.30
559.25
703.04
100 150 200 250 300 350 400 450 500 550 600 650 700 750 800 850 900 950 m/z


N3
OBn
N3
C13H27
OBn

8

國立交通大學應用化學系

元素分析儀 Heraeus CHN-O Rapid 服務報告書

使用者姓名：張凱翔　　中心編號：980530

服務單位：清大原科 俞鐘山實驗室　樣品名稱或代號：CKS11

收件日期：98 年 5 月 14 日完成日期：98 年 5 月 18 日

分析結果：

實驗值：	N%	C%	H%
1.	16.03	69.96	8.24
2.	15.83	69.40	8.53
3.			
4.			
推測值：	15.72	69.63	8.67

本日所使用之 Standard：A

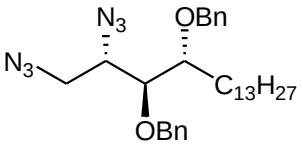
(A)Acetanilide	(B)Atropin	(C)N-Anilin
	N%	C%	H%
理論值：	10.36	71.09	6.71
測出值：	10.34	71.01	7.09

備註：

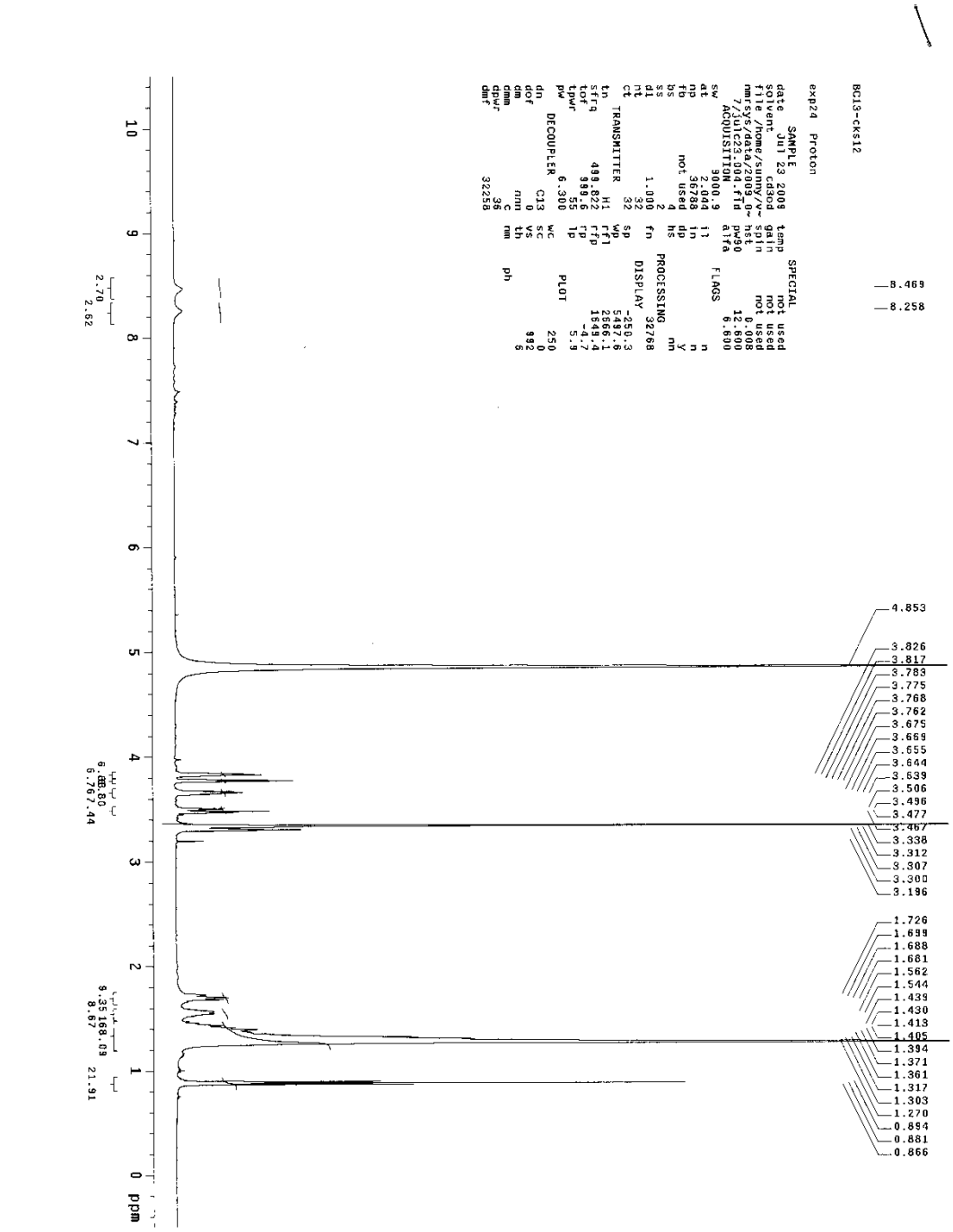
費用核算：NCH：800 元

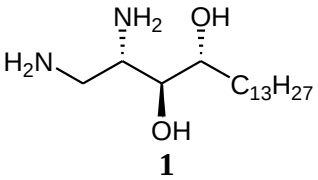
報告日期：98 年 5 月 19 日

儀器負責人簽章：　　技術員簽章：技士李蘊明

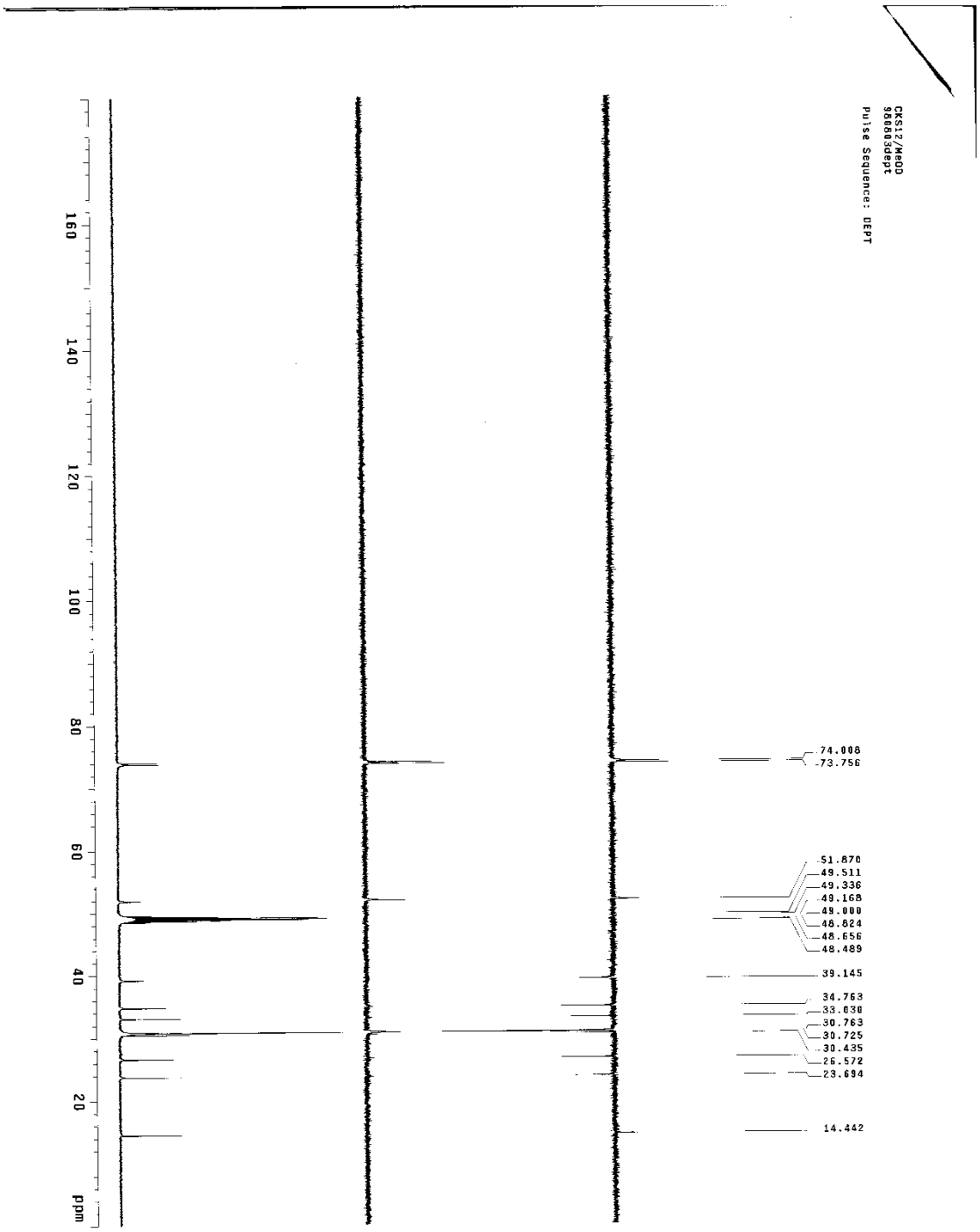


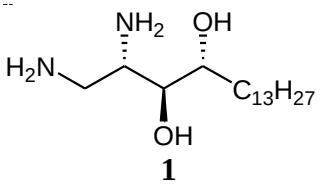
8


BCl3-cks12
exp24 Proton
8.469
8.258
4.853
3.826
3.817
3.783
3.775
3.768
3.762
3.675
3.669
3.655
3.644
3.639
3.506
3.496
3.477
3.467
3.338
3.312
3.307
3.300
3.196
1.726
1.699
1.688
1.681
1.562
1.544
1.439
1.430
1.413
1.405
1.394
1.371
1.361
1.317
1.303
1.270
0.894
0.881
0.866
ppm

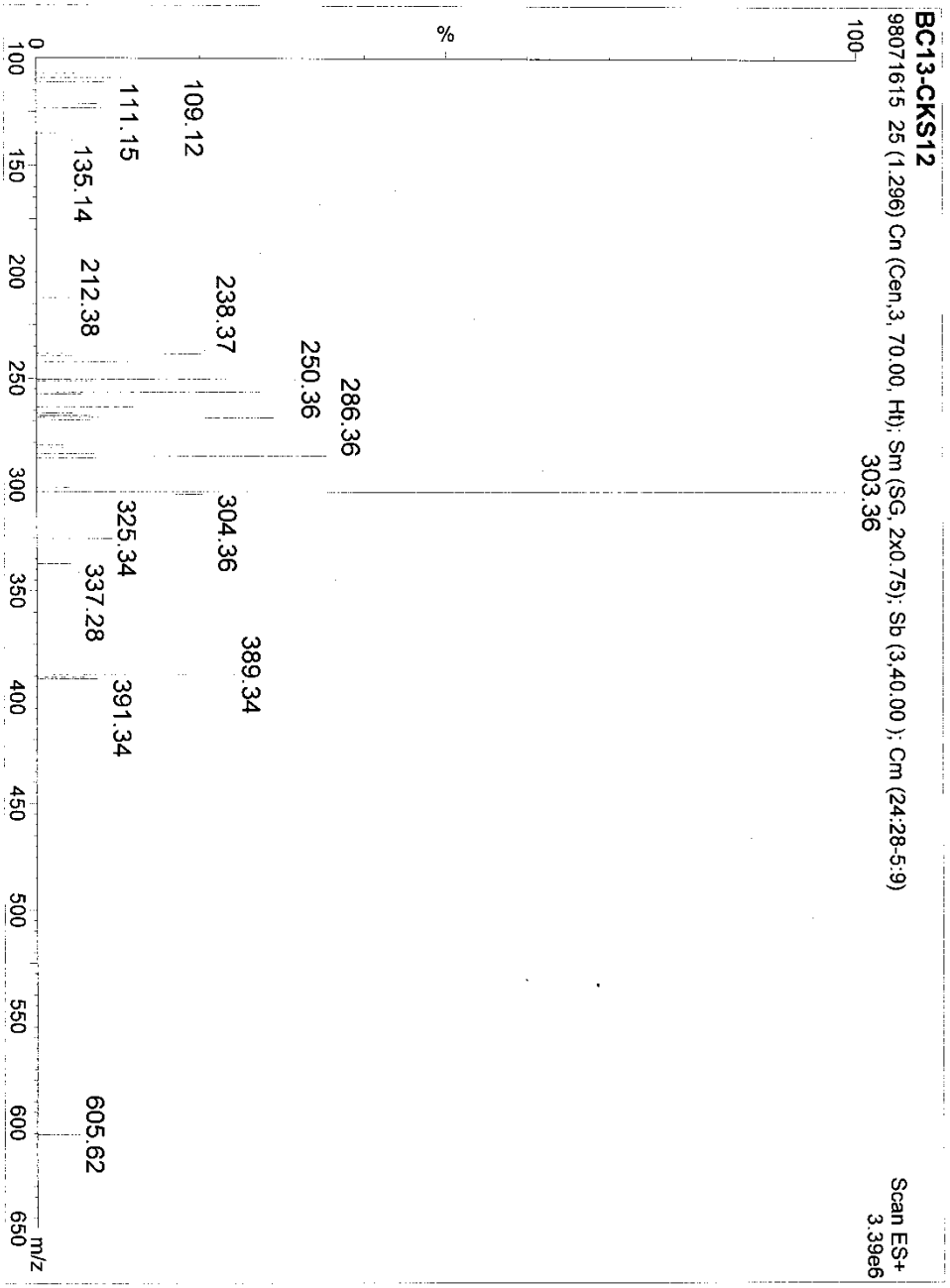

NH2
OH
H2N
C13H27
OH

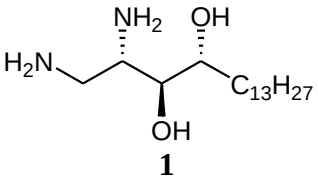
1


CKS12/MeOD
Pulse Sequence: DEPT
74.008
73.756
51.870
49.511
49.336
49.168
49.000
48.824
48.656
48.489
39.145
34.763
33.830
30.763
30.725
30.435
26.572
23.694
14.442
ppm

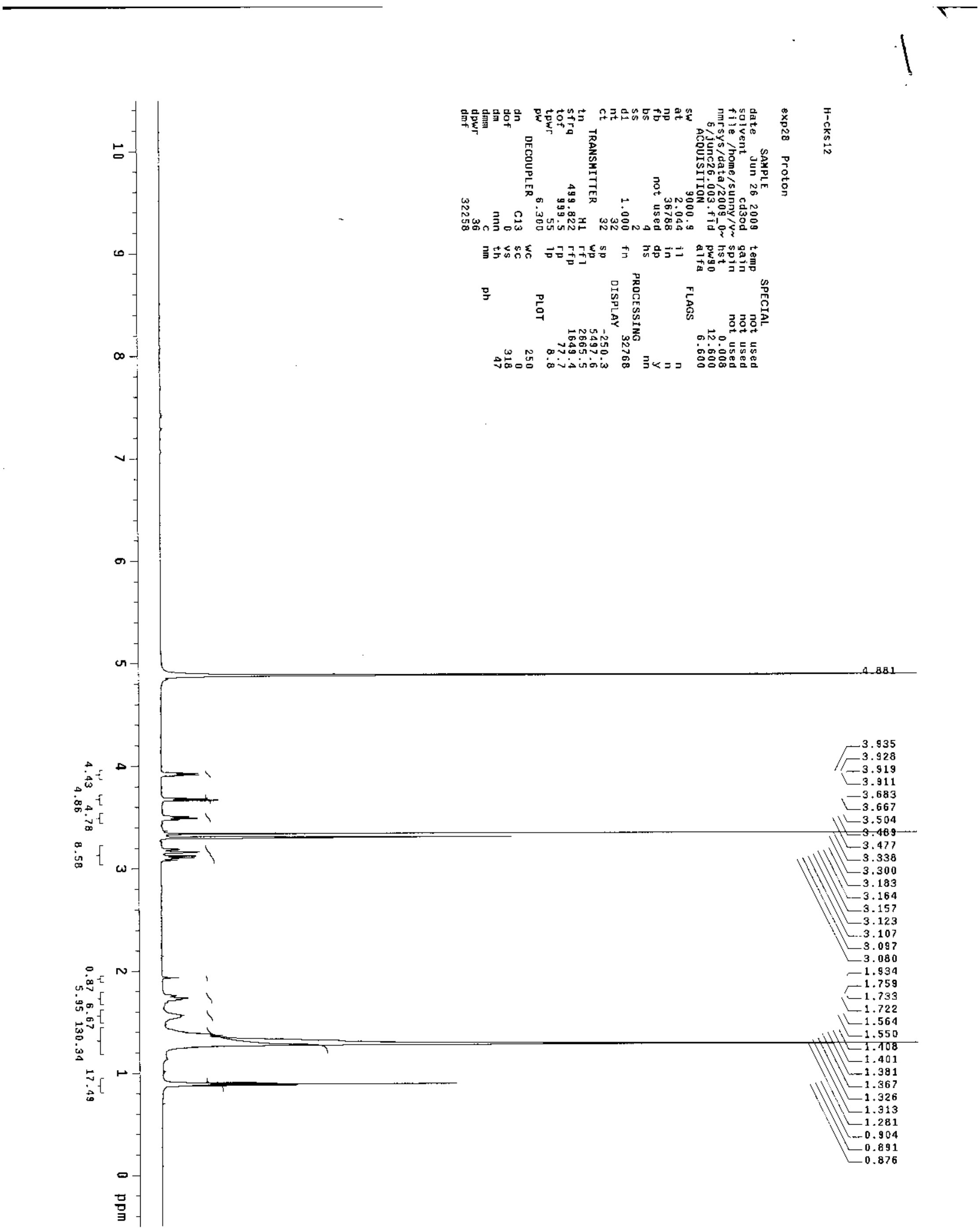

NH2
OH
H2N
C13H27
OH

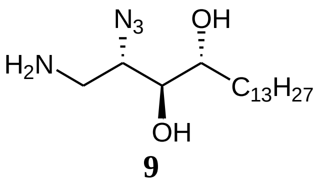
1


BC13-CKS12
98071615 25 (1.296) Cn (Cen,3, 70.00, Ht); Sm (SG, 2x0.75); Sb (3,40.00); Cm (24:28-5:9)
Scan ES+
3.39e6
%
100
0
100
150
200
250
300
350
400
450
500
550
600
650
m/z
109.12
111.15
135.14
212.38
238.37
250.36
286.36
303.36
304.36
325.34
337.28
389.34
391.34
605.62

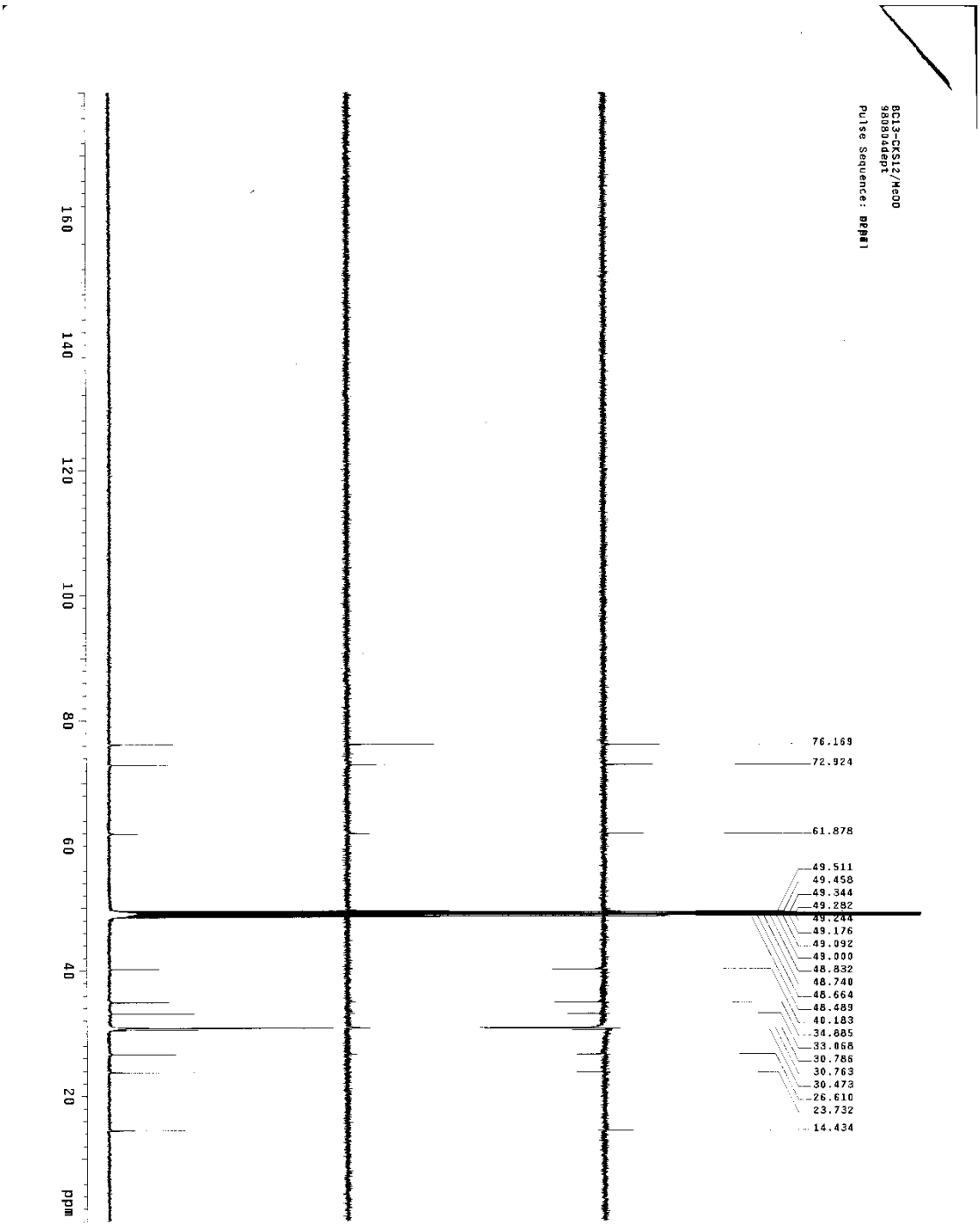

NH2
OH
H2N
C13H27
OH

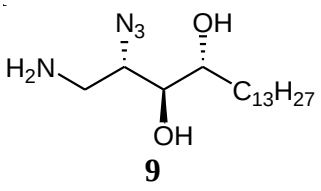
1



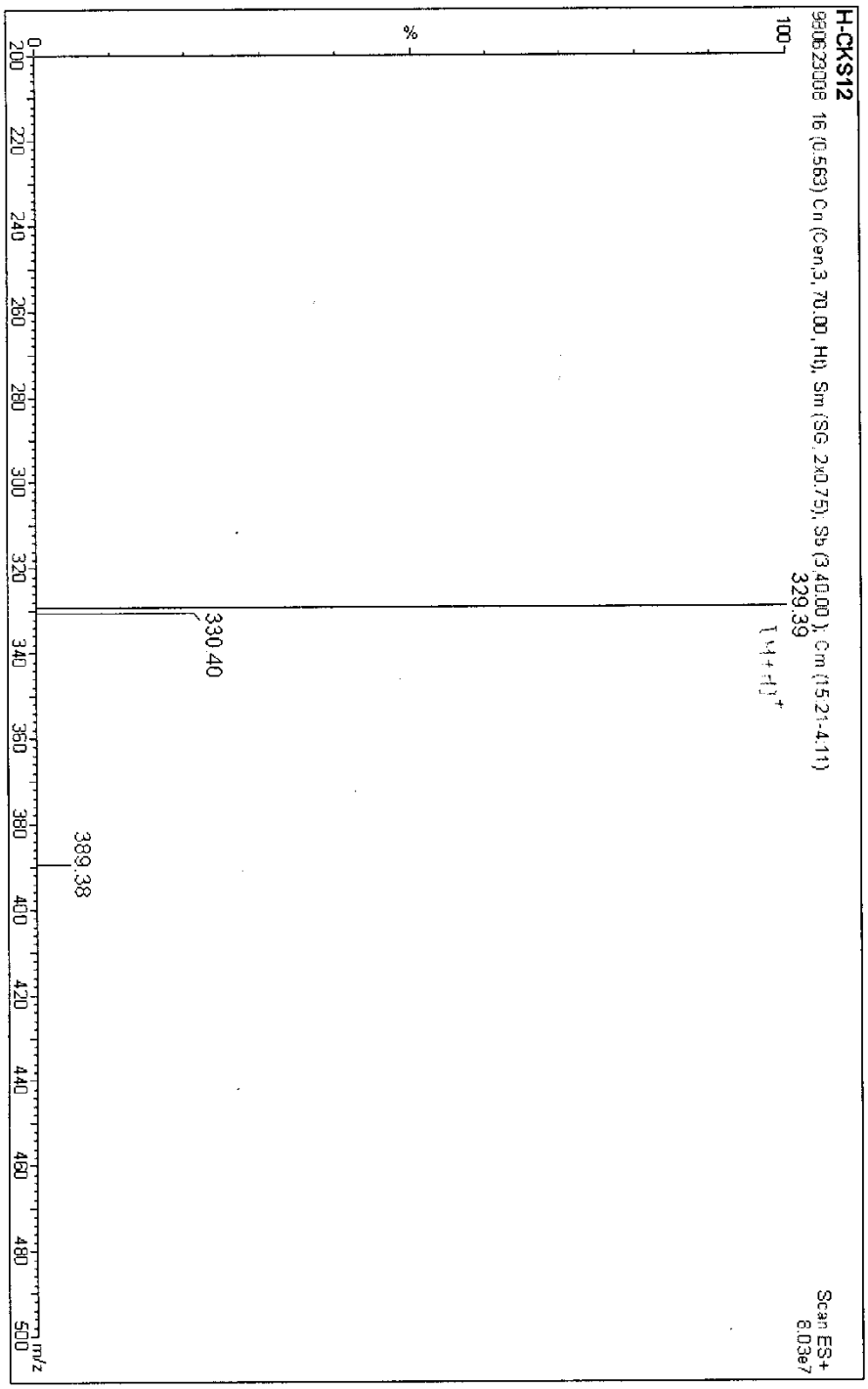


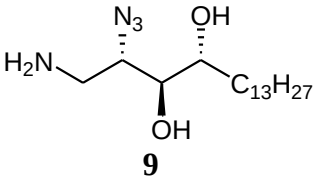
9


BC13-CKS12/MeOD
Pulse Sequence: DEPT
160
140
120
100
80
60
40
20
ppm
76.169
72.924
61.878
49.511
49.458
49.344
49.282
49.244
49.176
49.092
49.000
48.832
48.740
48.664
48.489
40.183
34.885
33.068
30.786
30.763
30.473
26.610
23.732
14.434

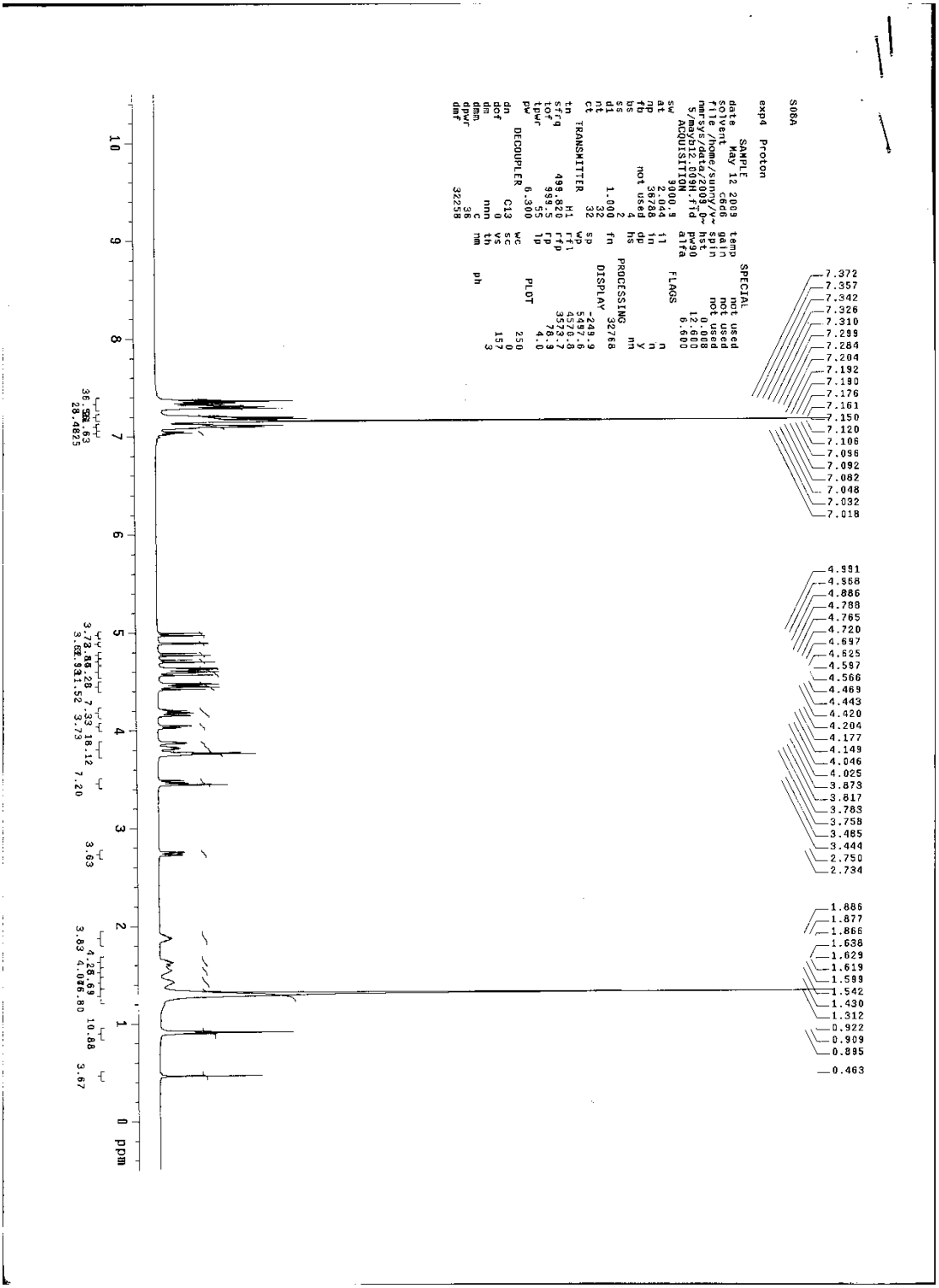

N3
OH
H2N
C13H27
OH

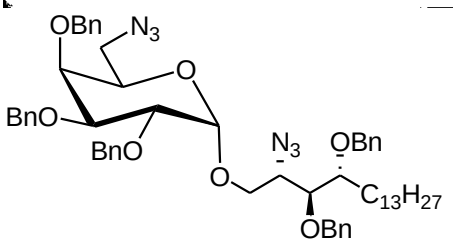
9


H-CKS12
%
100
0
200
220
240
260
280
300
320
329.39
330.40
389.38
340
360
380
400
420
440
460
480
500
m/z
Scan ES+
8.03e7

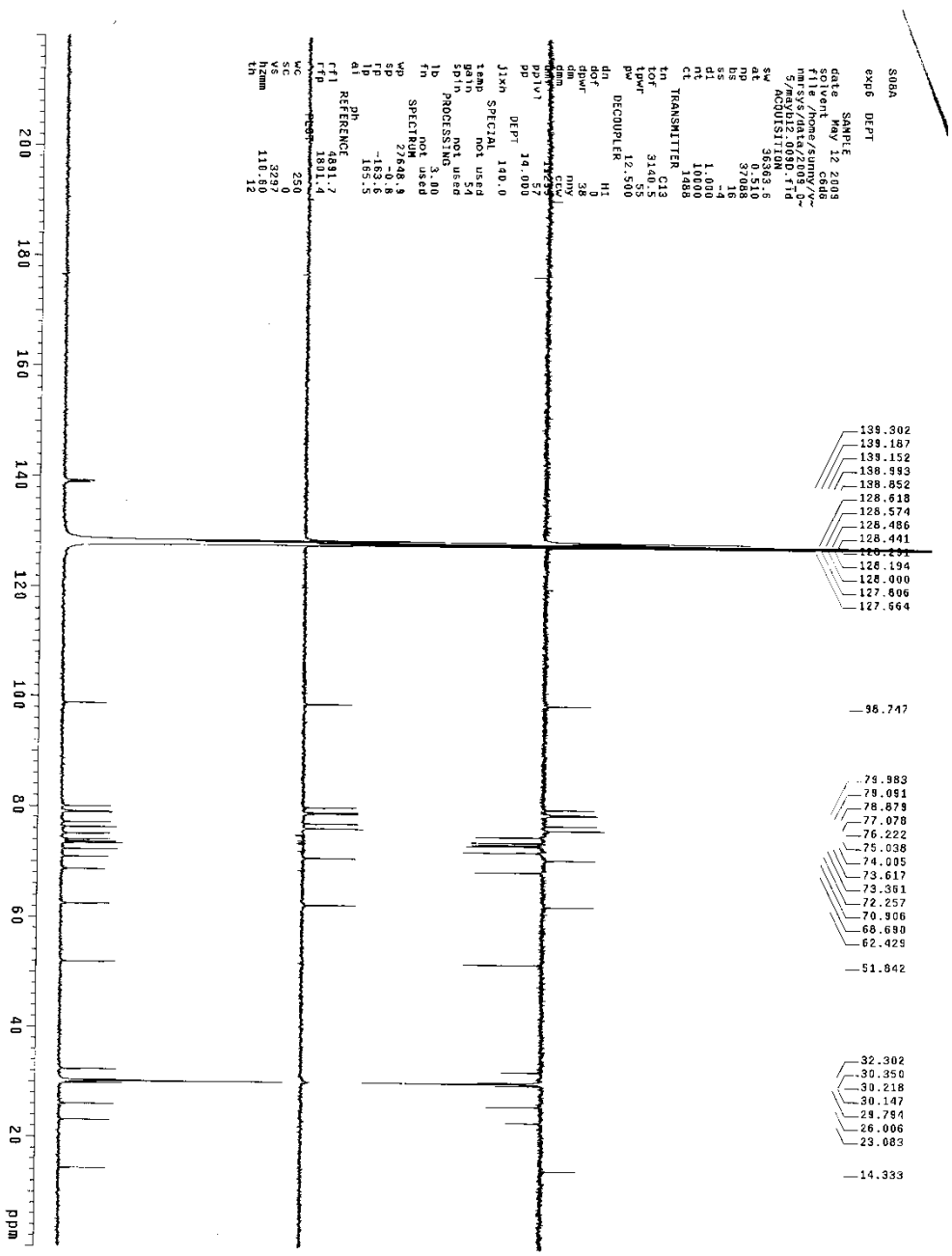

N3
OH
H2N
C13H27
OH

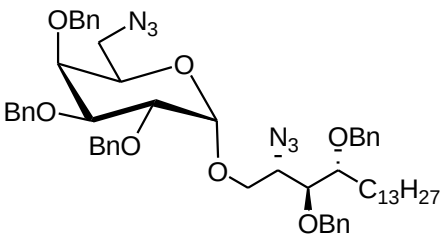
9



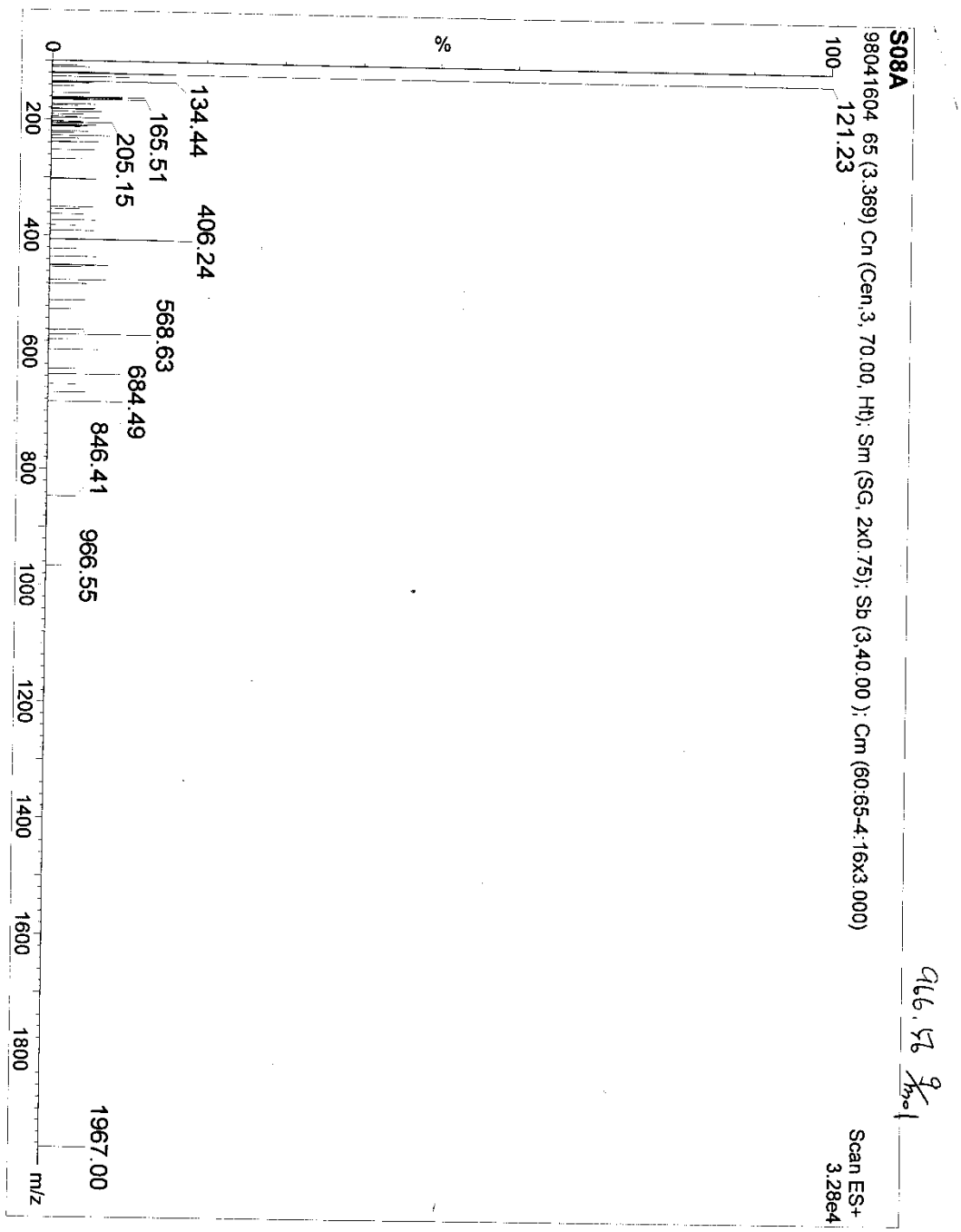


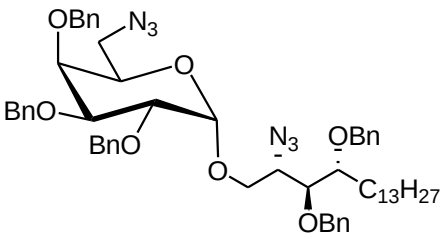
14α


S08A
exp6 DEPT
SAMPLE
date May 12 2009
solvent cdcl3
file /home/sumpy/nmrsys/data/2009/5/may12.009D.fid
ACQUISITION
sw 36363.6
at 0.510
np 37088
bs 16
ss -4
d1 1.000
nt 10000
ct 1488
TRANSMITTER
tn C13
tof 3140.5
tpwr 55
pw 12.500
DECOUPLER
dn H1
dof 0
dpwr 28
dm nny
dmm ccw
PROCESSING
lb not used
fn SPECTRUM
wp 27648.9
sp -0.8
lp -185.6
el 185.5
ph REFERENCE
rfl 4891.7
rfp 1801.4
PLOT
wc 250
sc 250
vs 3297
hzmm 110.60
th 12
DEPT
pplvl 57
pp 14.000
jixn SPECIAL
temp not used
gain 54
spin not used
139.302
139.187
139.152
138.993
138.852
128.618
128.574
128.486
128.441
128.291
128.194
128.000
127.806
127.664
98.747
79.983
79.091
78.879
77.078
76.222
75.038
74.005
73.617
73.361
72.257
70.906
68.690
62.429
51.842
32.302
30.350
30.218
30.147
29.794
26.006
23.083
14.333
ppm


OBn
N3
O
BnO
BnO
O
N3
OBn
C13H27
OBn

14α


S08A
98041604 65 (3.369) Cn (Cen,3, 70.00, Ht); Sm (SG, 2x0.75); Sb (3.40.00); Cm (60:65-4:16x3.000)
Scan ES+
3.28e4
%
m/z
121.23
134.44
165.51
205.15
406.24
568.63
684.49
846.41
966.55
1967.00


OBn
N3
O
BnO
BnO
O
N3
OBn
C13H27
OBn

14α

國立交通大學應用化學系

元素分析儀 Heraeus CHN-O Rapid 服務報告書

使用者姓名：廖國延　　中心編號：980529

服務單位：清大原科 俞鐘山實驗室　樣品名稱或代號：SO8A

收件日期： 98 年 5 月 14 日完成日期： 98 年 5 月 18 日

分析結果：

實驗值：	N%	C%	H%
1.	8.66	72.11	7.42
2.	8.46	71.79	7.27
3.			
4.			
推測值：	8.69	72.02	7.71

本日所使用之 Standard：A

(A)Acetanilide (B)Atropin (C)N-Anilin

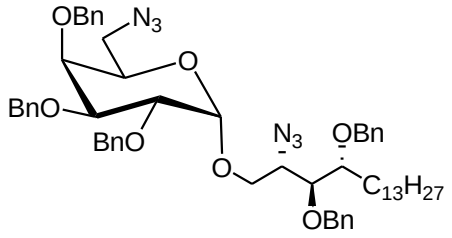
	N%	C%	H%
理論值：	10.36	71.09	6.71
測出值：	10.34	71.01	7.09

備註：

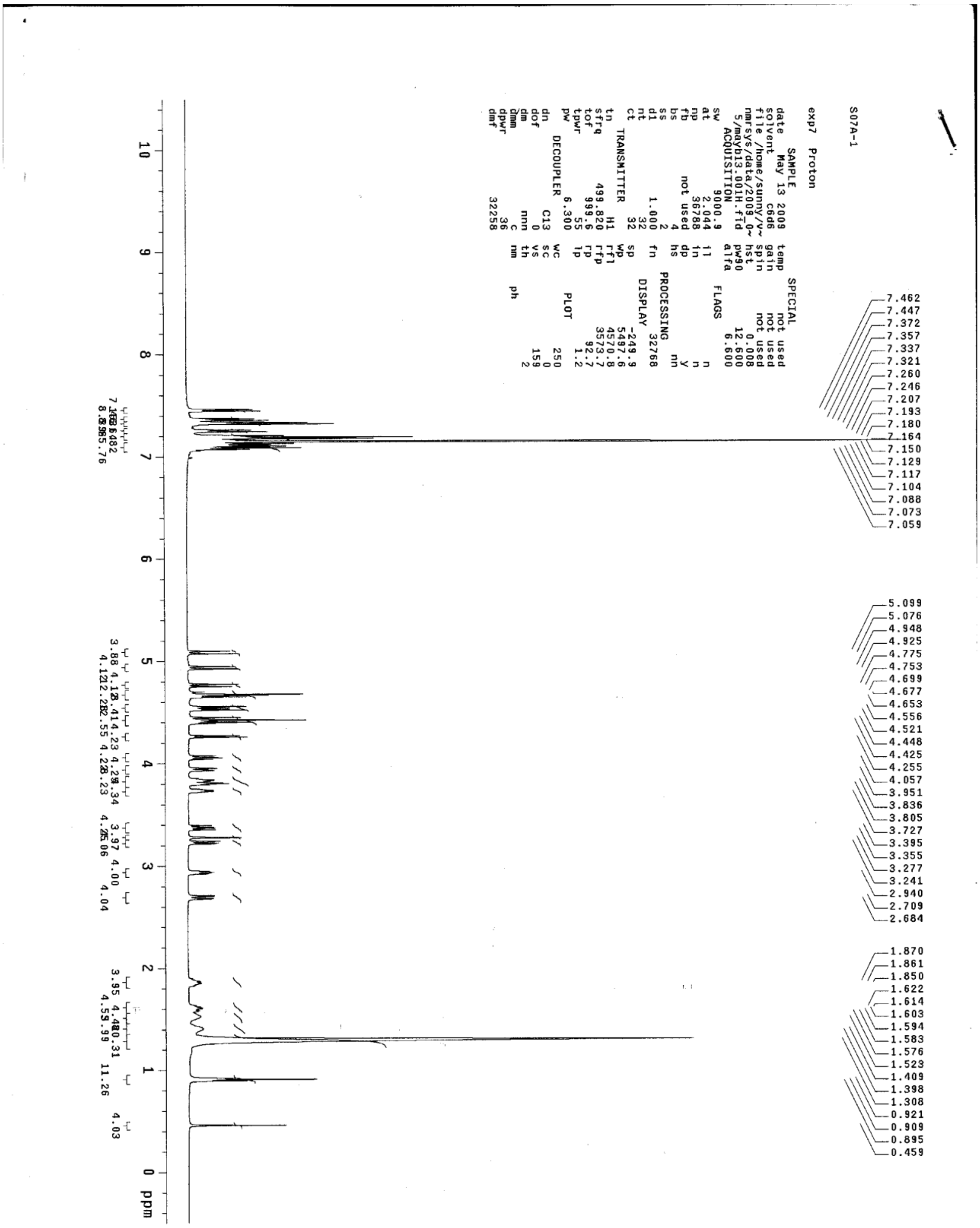
費用核算：NCH：800 元

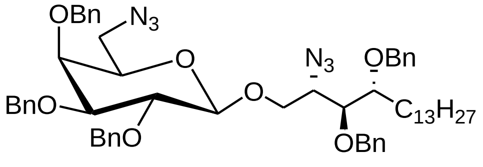
報告日期： 98 年 5 月 19 日

儀器負責人簽章：　　技術員簽章： 技士李蘊明

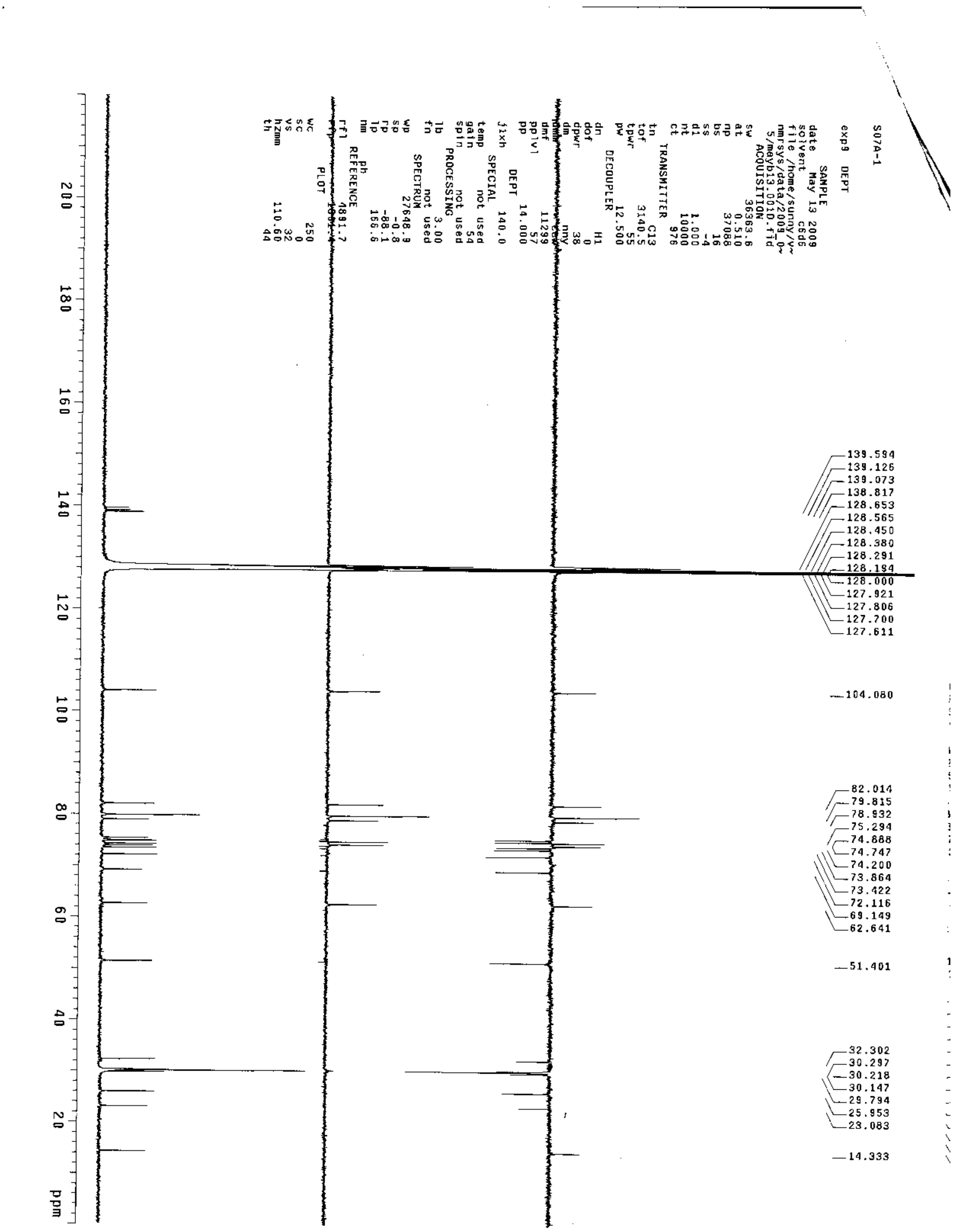


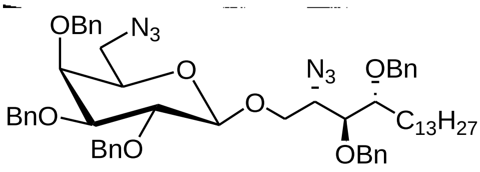
14α



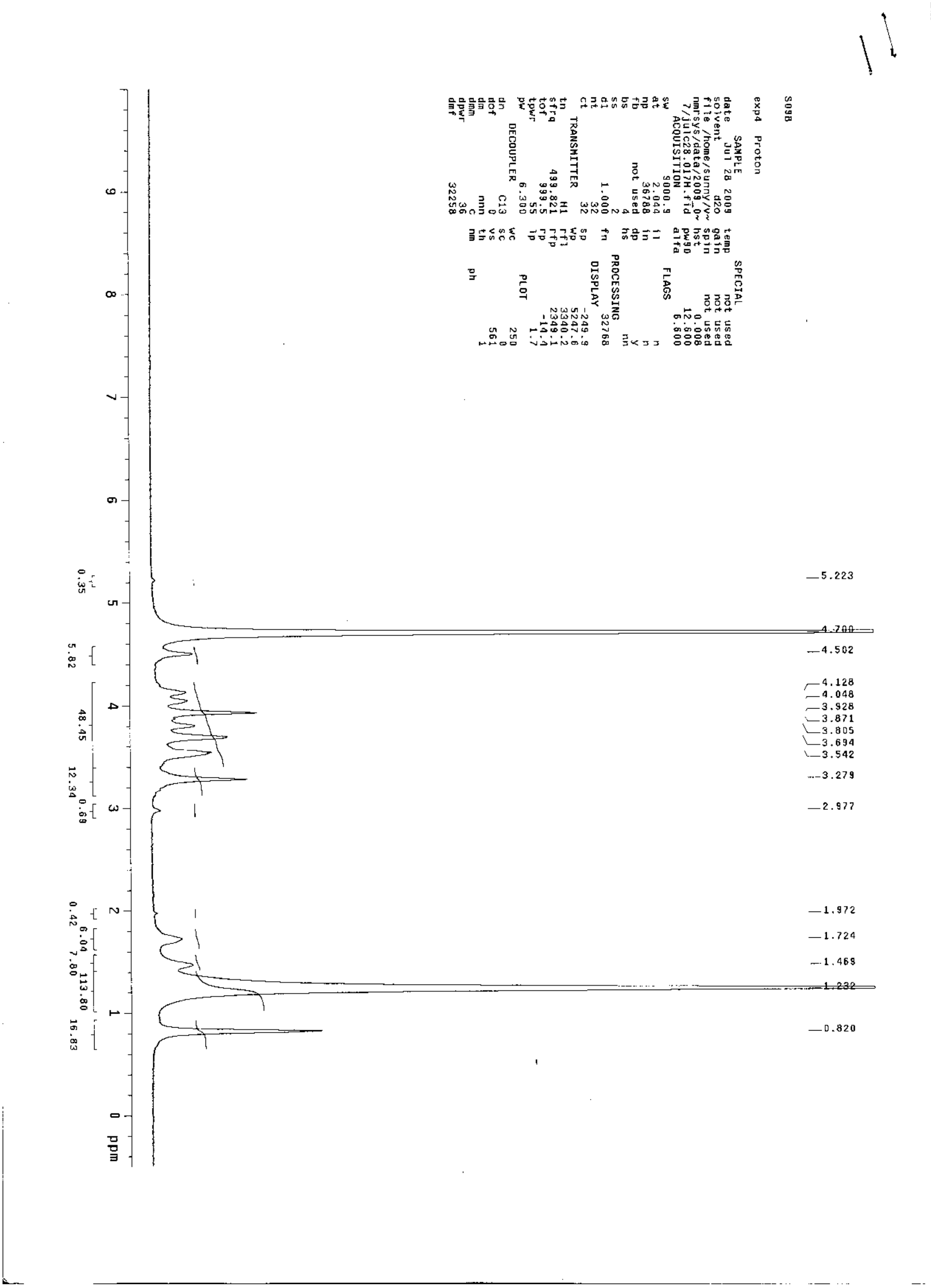


14β

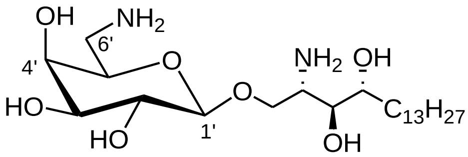

139.594
139.126
139.073
138.817
128.653
128.565
128.450
128.380
128.291
128.194
128.000
127.921
127.806
127.700
127.611
104.080
82.014
79.815
78.932
75.294
74.888
74.747
74.200
73.864
73.422
72.116
69.149
62.641
51.401
32.302
30.297
30.218
30.147
29.794
25.953
23.083
14.333


OBn
N_3
O
BnO
BnO
O
N_3
OBn
$C_{13}H_{27}$
OBn

14β

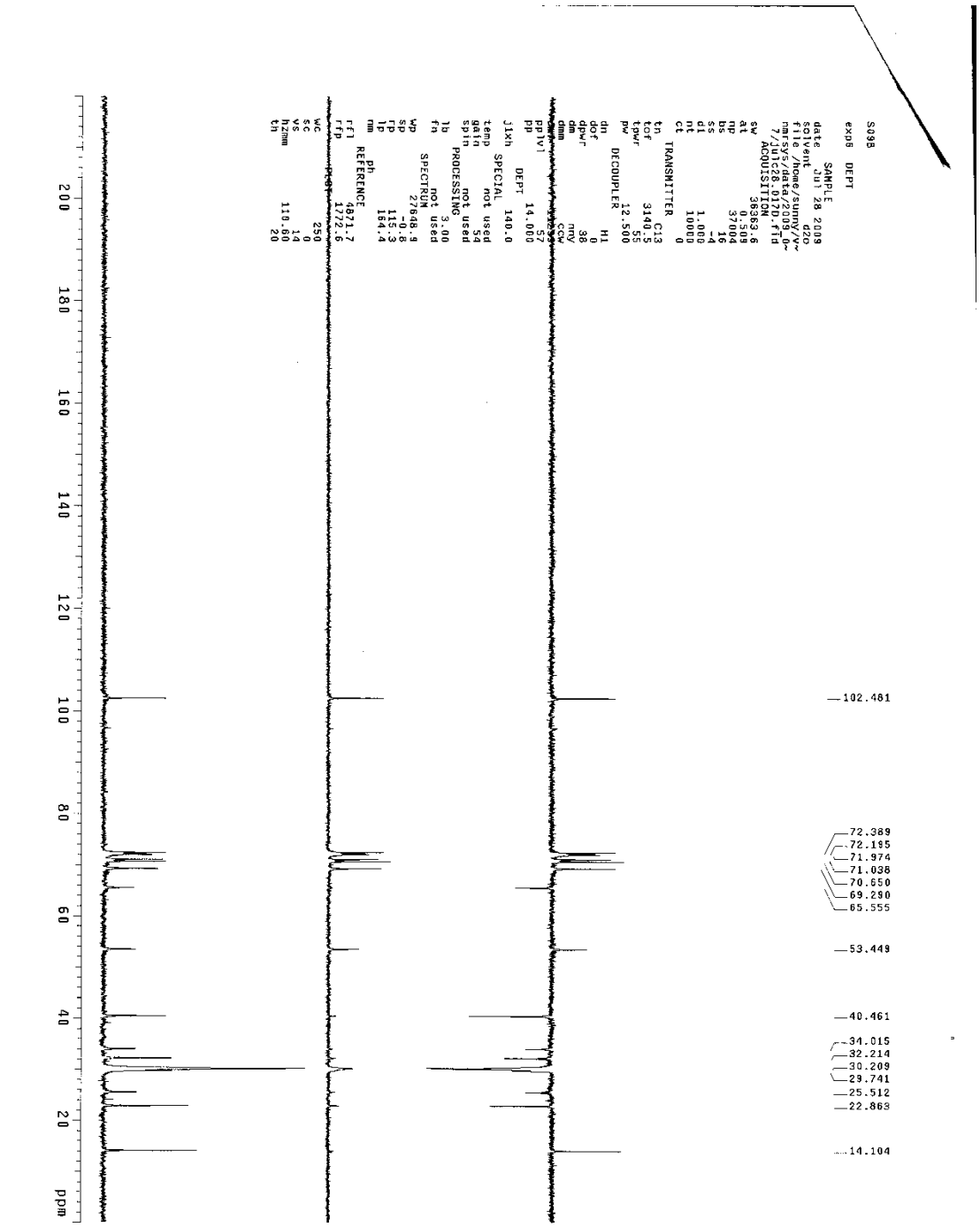


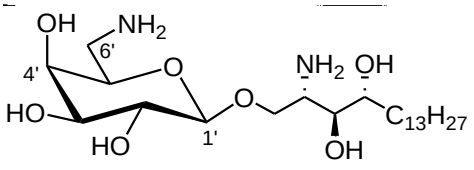
S09B
exp4 Proton
5.223
4.700
4.502
4.128
4.048
3.928
3.871
3.805
3.694
3.542
3.279
2.977
1.972
1.724
1.468
1.232
0.820
ppm



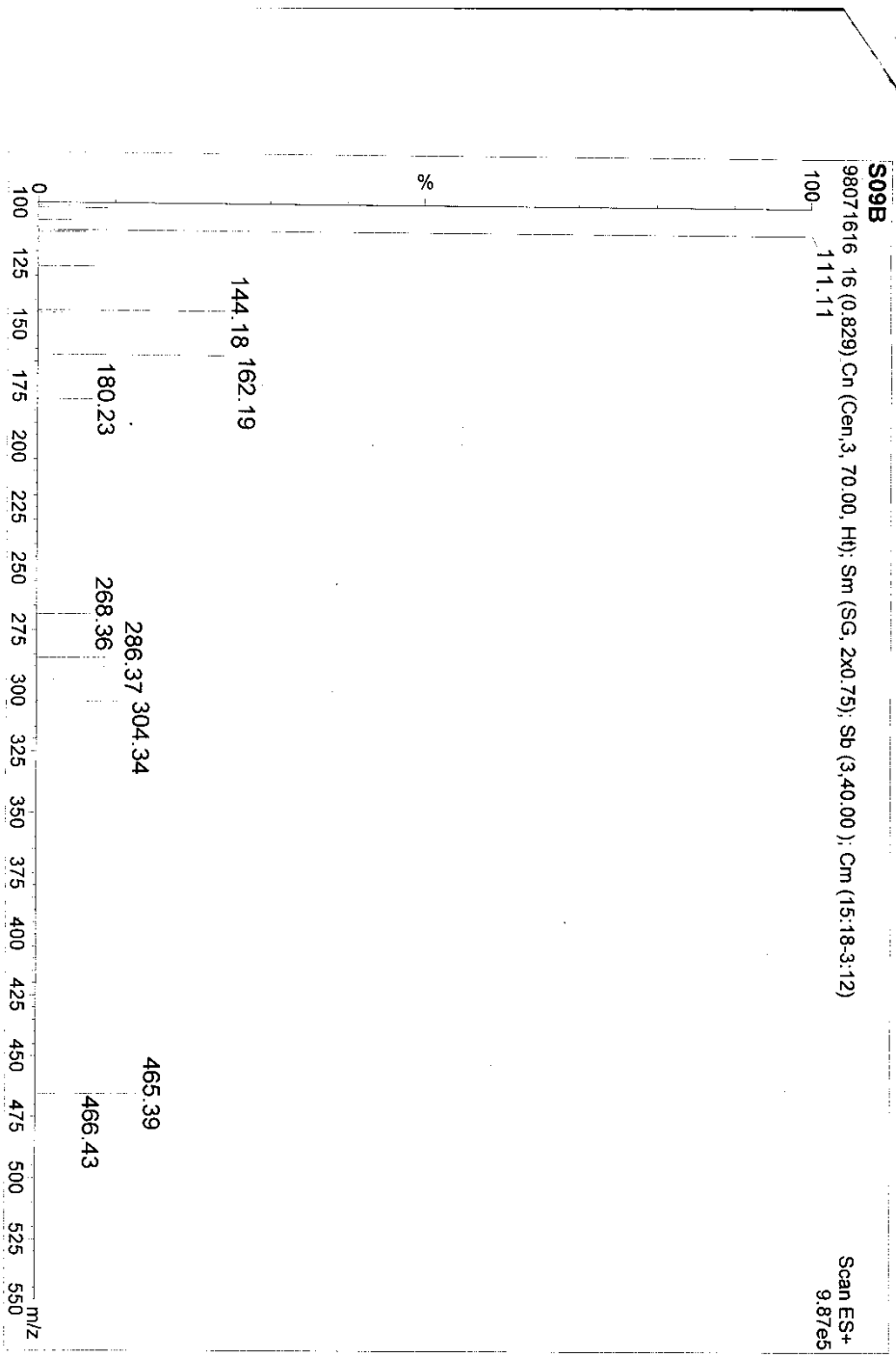
OH
NH2
6'
4'
O
HO
HO
1'
O
NH2
OH
C13H27
OH

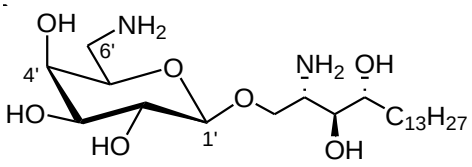
17β


S09S
exp6 DEPT
SAMPLE
date Jul 28 2009
solvent CDCl3
file /home/sunny/d~
nmrsys/data/2009_0~
7/jul28_01?D.fid
ACQUISITION
sw 36363.6
at 0.509
np 37104
bs 16
ss -4
d1 1.000
nt 10000
ct 0
TRANSMITTER
tn C13
to 3140.15
tpwr 55
pw 12.500
DECOUPLER
dn H1
dof 0
dpwr 38
dm nny
dmm ccw
pplvl 57
pp 14.000
DEPT 140.0
jlxh SPECIAL
not used
temp
gain 54
spin not used
PROCESSING 3.00
lb
fn not used
SPECTRUM
wp 2764.9
sp -0.8
rp 115.3
lp 164.4
rm
ph
REFERENCE 4871.7
rfl 1272.6
rfp
wc 250
sc 0
vs 14
hzmm 110.60
th 20
102.481
72.389
72.185
71.974
71.038
70.650
69.290
65.555
53.449
40.461
34.015
32.214
30.209
29.741
25.512
22.863
14.104
200
180
160
140
120
100
80
60
40
20
ppm

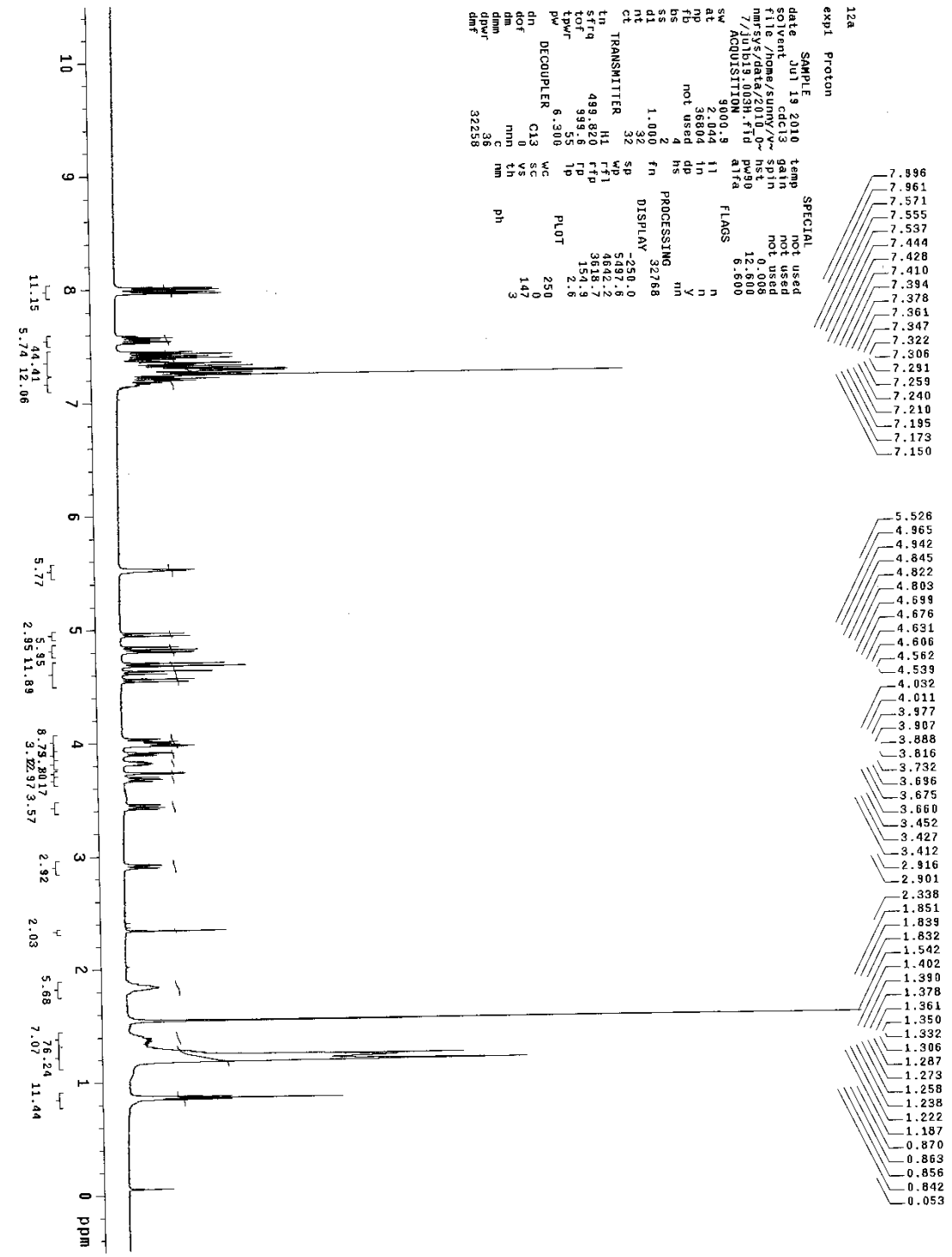

OH
NH2
6'
4'
O
NH2
OH
HO
O
C13H27
HO
1'
OH

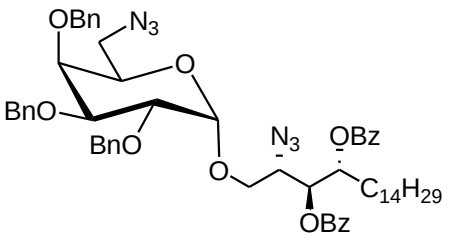
17β


S09B
98071616 16 (0.829) Cn (Cen,3, 70.00, Ht); Sm (SG, 2x0.75); Sb (3,40.00); Cm (15:18-3:12)
111.11
144.18
162.19
180.23
268.36
286.37
304.34
465.39
466.43
Scan ES+
9.87e5
%
m/z

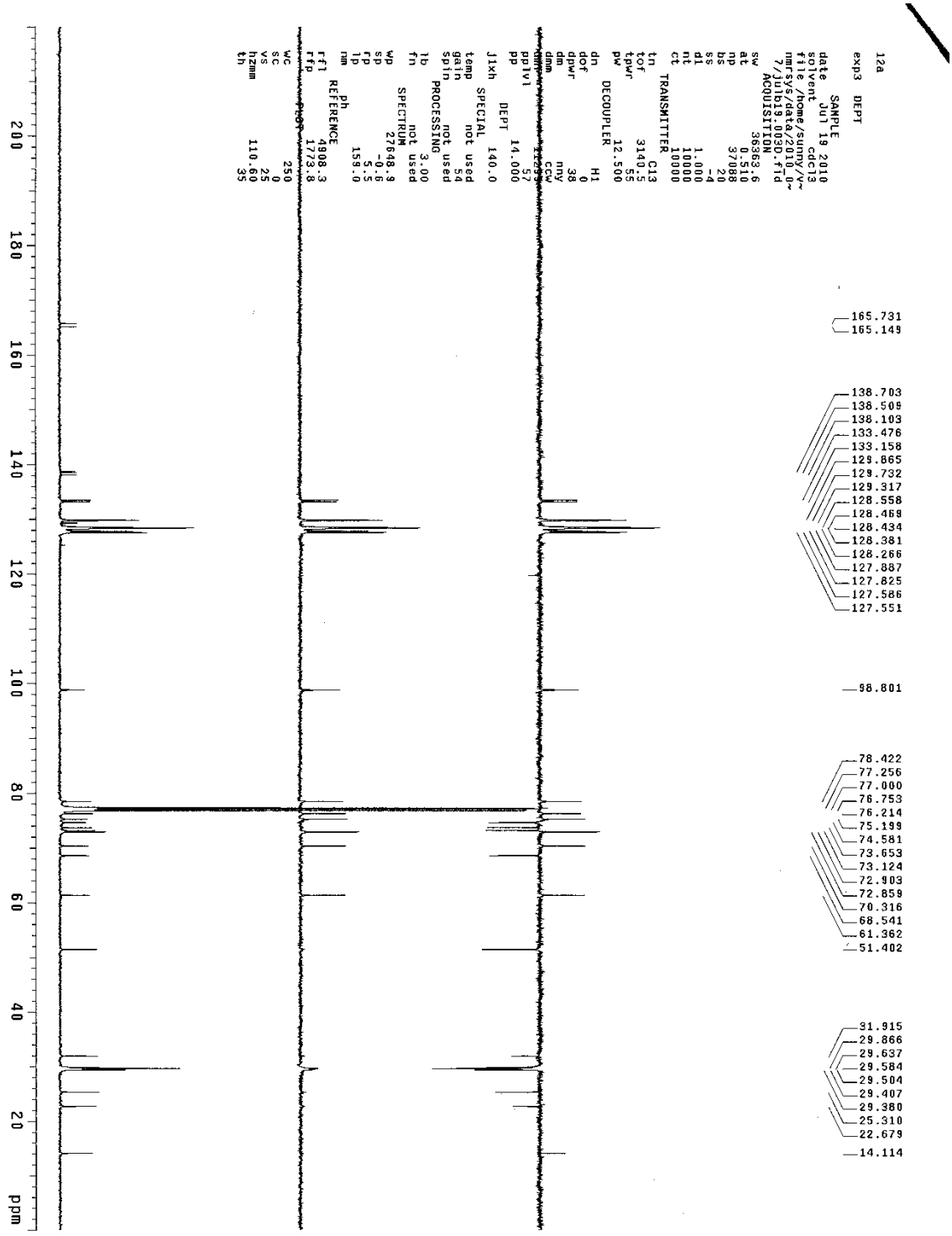

OH
NH2
6'
4'
O
NH2
OH
HO
O
C13H27
HO
1'
OH

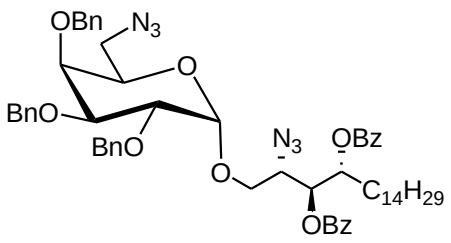
17β



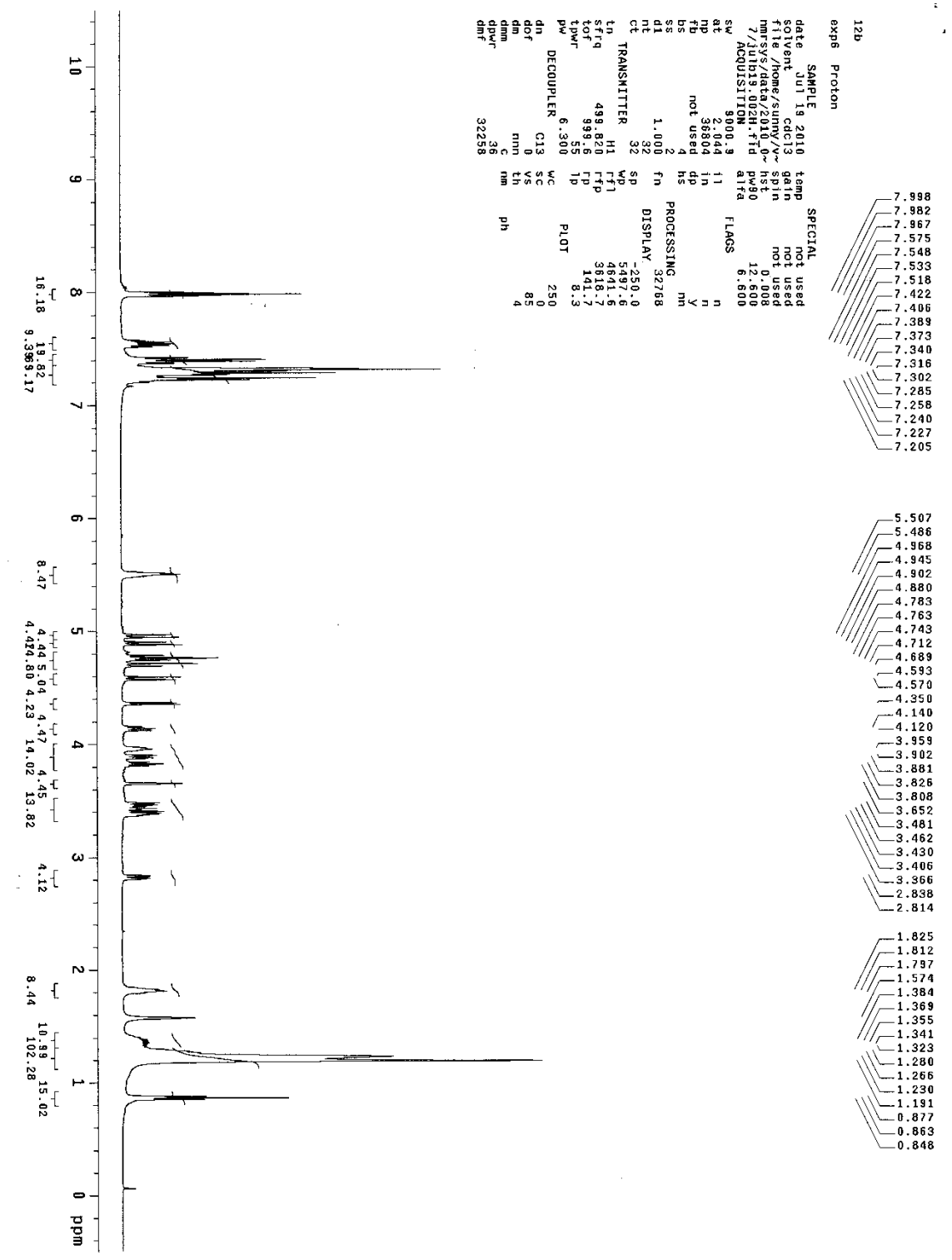


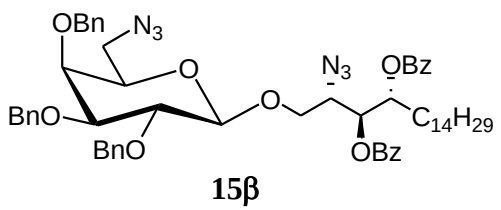
15α


12a
exp3 DEPT
date SAMPLE
Jul 19 2010
solvent cdcl3
file /home/sunny/v~
nmrsys/data/2010_0~
7/jul19.003D.fid
ACQUISITION
sw 36363.6
at 0.510
np 37088
bs 20
ss 4
d1 1.000
nt 10000
ct 10000
TRANSMITTER
tn C13
tof 3140.5
tpwr 55
pw 12.500
DECOUPLER
dn H1
dof 0
dpwr 38
dm nny
dmm ccw
pplvl 57
pp 14.000
DEPT
J1xh 140.0
SPECIAL
temp not used
gain 54
spin not used
PROCESSING
lb 3.00
fn not used
SPECTRUM
wp 27648.9
sp -0.6
rp 5.5
lp 159.0
nm
ph
REFERENCE
rf1 4908.3
rfp 1773.8
PLOT
wc 250
sc 0
vs 25
vsm 110.60
th 35
165.731
165.149
138.703
138.509
138.103
133.476
133.158
129.865
129.732
129.317
128.558
128.469
128.434
128.381
128.266
127.887
127.825
127.586
127.551
98.801
78.422
77.256
77.000
76.753
76.214
75.199
74.581
73.653
73.124
72.903
72.859
70.316
68.541
61.362
51.402
31.915
29.866
29.637
29.584
29.504
29.407
29.380
25.310
22.679
14.114
ppm

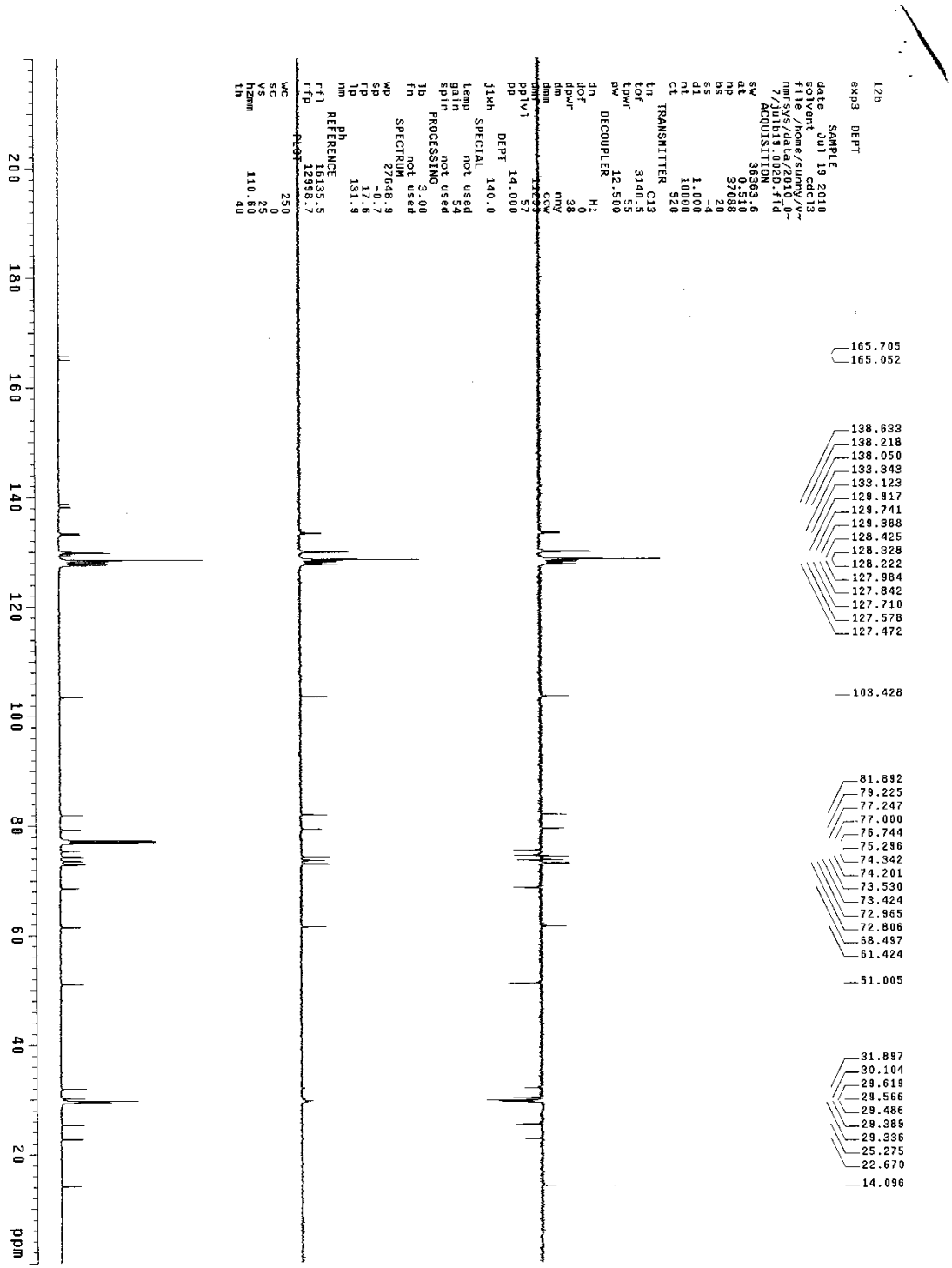

OBn
N3
O
BnO
BnO
O
N3
OBz
C14H29
OBz

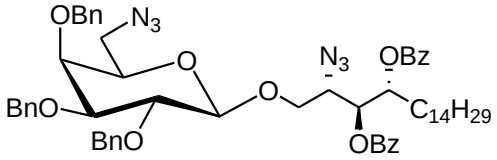
15α


12b
exp6 Proton
SAMPLE
date Jul 19 2010
solvent cdcl3
file /home/sunny/v~
nmrsys/data/2010_0~
7/jul19.002H.fid
ACQUISITION
sw 9000.9
at 2.044
np 36804
fb not used
bs 4
ss 2
d1 1.000
nt 32
ct 32
TRANSMITTER
tn H1
sfrq 499.820
tof 999.6
tpwr 55
pw 6.300
DECOUPLER
dn C13
dof 0
dm nnn
dmm c
dmf 32258
temp
gain
spin
hst
pw90
alfa
FLAGS
SPECIAL
not used
not used
not used
0.008
12.600
6.600
PROCESSING
DISPLAY 32768
sp -250.0
wp 5497.6
rfl 4641.6
rfp 3618.7
rp 141.7
lp 8.3
PLOT
wc 250
sc 0
vs 85
th 4
ph
nm
n
n
y
nn
7.998
7.982
7.967
7.575
7.548
7.533
7.518
7.422
7.406
7.389
7.373
7.340
7.316
7.302
7.285
7.258
7.240
7.227
7.205
5.507
5.486
4.958
4.945
4.902
4.880
4.783
4.763
4.743
4.712
4.689
4.593
4.570
4.350
4.140
4.120
3.959
3.902
3.881
3.826
3.808
3.652
3.481
3.462
3.430
3.406
3.366
2.838
2.814
1.825
1.812
1.797
1.574
1.384
1.369
1.355
1.341
1.323
1.280
1.266
1.230
1.191
0.877
0.863
0.848
ppm

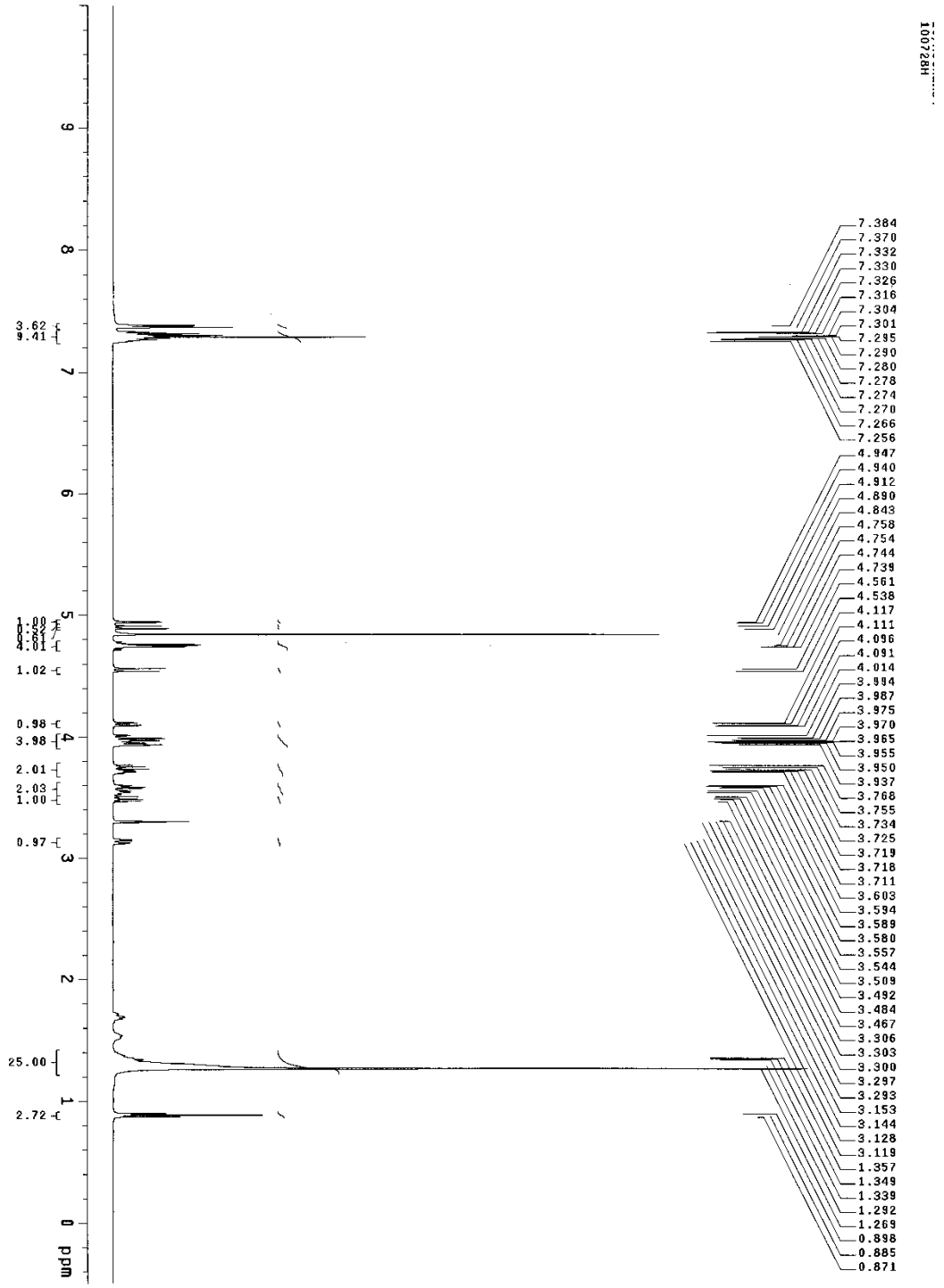

OBn
N3
O
N3
OBz
O
BnO
C14H29
BnO
OBz

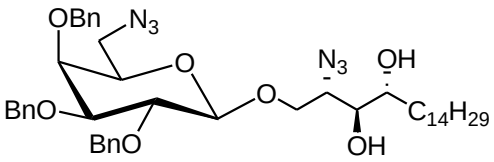
15β

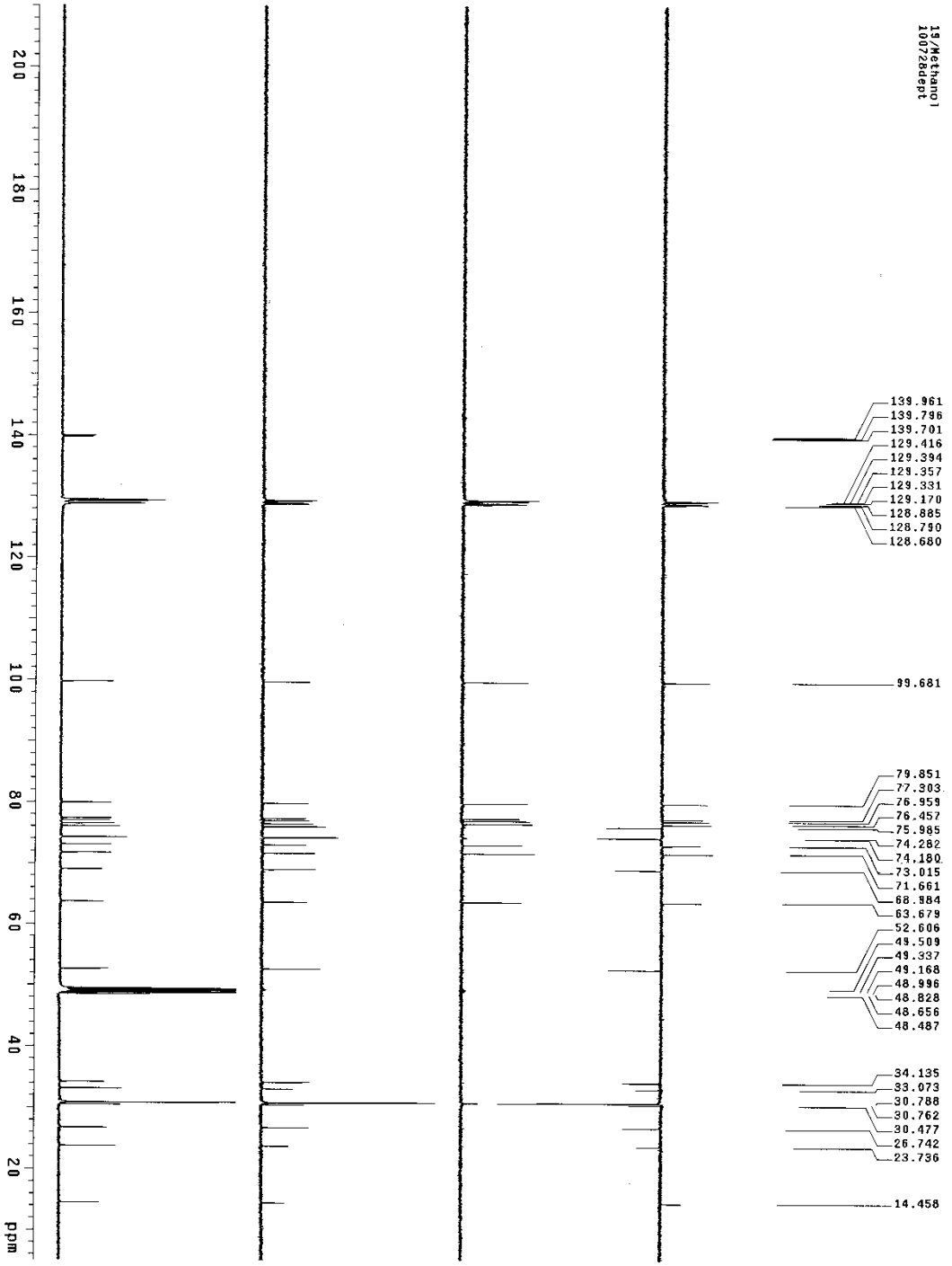

12b
exp3 DEPT
SAMPLE
date Jul 19 2010
solvent c_dcm13
file /home/sunny/v~
nmrsys/data/2010/0~
7/jul19_002D.fid
ACQUISITION
sw 36363.6
at 0.510
np 37088
bs 20
ss -4
d1 1.000
nt 10000
ct 520
TRANSMITTER C13
tn C13
tof 31405.5
tpwr 55
pw 12.500
DECOUPLER
dn
dof 0
dpwr 38
dm nny
dmm ccw
dmf 11693
pplvl 57
pp 14.000
DEPT
J1xh 140.0
SPECIAL
temp not used
gain 54
spin not used
PROCESSING 3.00
lb not used
fn SPECTRUM
wp 27648.9
sp -0.7
rp 17.6
lp 131.9
ph
REFERENCE
rfl 16135.5
rfp 12998.7
PLOT
wc 250
sc 0
vs 25
hzmm 110.60
th 40
165.705
165.052
138.633
138.218
138.050
133.343
133.123
129.917
129.741
129.388
128.425
128.328
128.222
127.984
127.842
127.710
127.578
127.472
103.428
81.892
79.225
77.247
77.000
76.744
75.296
74.342
74.201
73.530
73.424
72.965
72.806
68.497
61.424
51.005
31.897
30.104
29.619
29.566
29.486
29.389
29.336
25.275
22.670
14.096
ppm

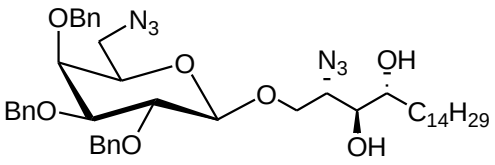

OBn
N3
O
N3
OBz
BnO
O
C14H29
BnO
OBz

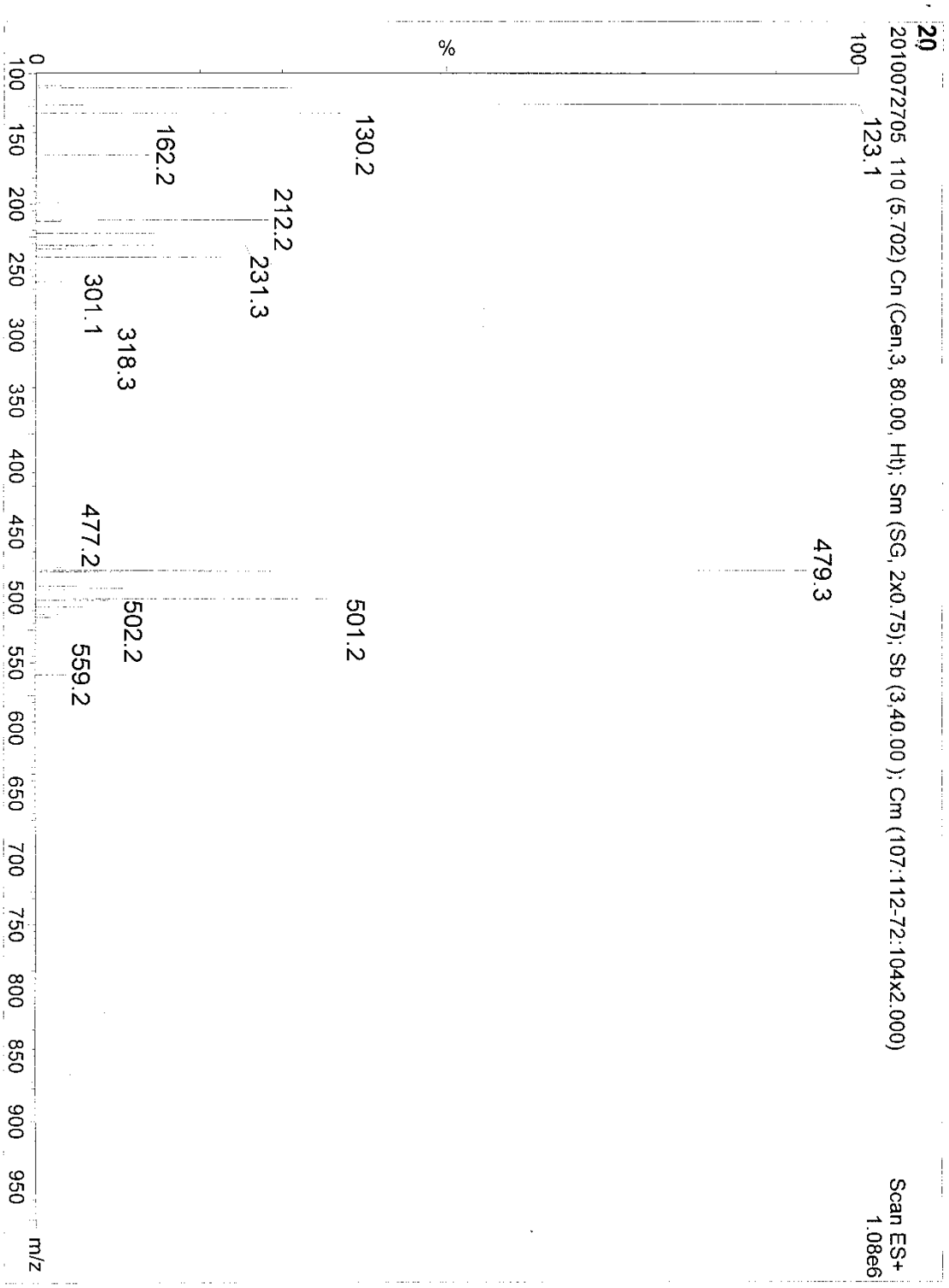
15β

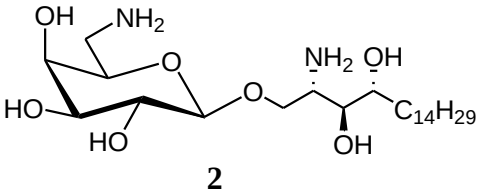

7.384
7.370
7.332
7.330
7.326
7.316
7.304
7.301
7.295
7.290
7.280
7.278
7.274
7.270
7.266
7.256
4.947
4.940
4.912
4.890
4.843
4.758
4.754
4.744
4.739
4.561
4.538
4.117
4.111
4.096
4.091
4.014
3.994
3.987
3.975
3.970
3.965
3.955
3.950
3.937
3.768
3.755
3.734
3.725
3.719
3.718
3.711
3.603
3.594
3.589
3.580
3.557
3.544
3.509
3.492
3.484
3.467
3.306
3.303
3.300
3.297
3.293
3.153
3.144
3.128
3.119
1.357
1.349
1.339
1.292
1.269
0.898
0.885
0.871
3.62
9.41
1.00
4.01
1.02
0.98
3.98
2.01
2.03
1.00
0.97
25.00
2.72
ppm


OBn
N3
O
N3
OH
BnO
O
C14H29
BnO
OH

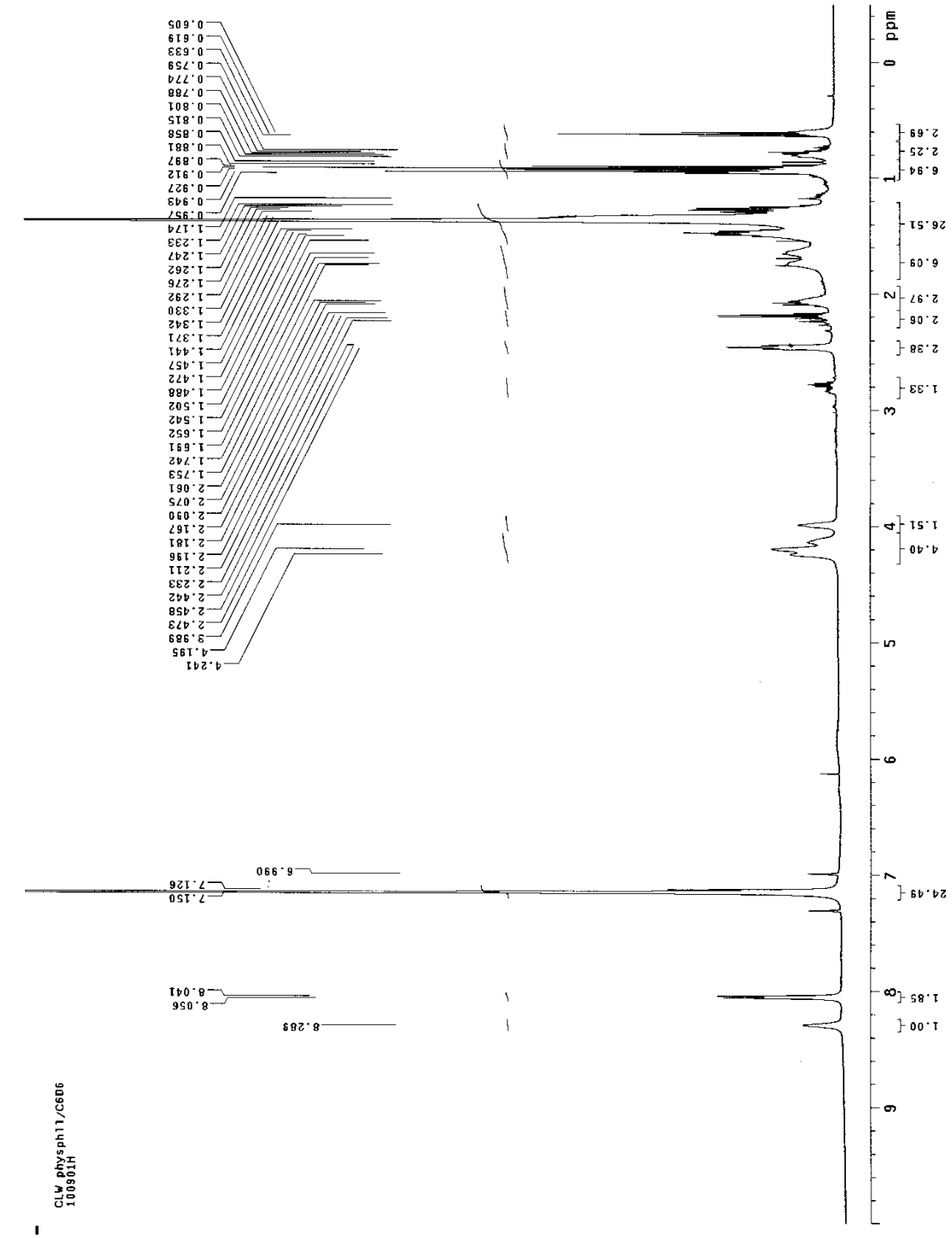

15/Methanol
100728dept
200
180
160
140
120
100
80
60
40
20
ppm
139.961
139.796
139.701
129.416
129.394
129.357
129.331
129.170
128.885
128.790
128.680
99.681
79.851
77.303
76.959
76.457
75.985
74.282
74.180
73.015
71.661
68.984
63.679
52.606
49.509
49.337
49.168
48.996
48.828
48.656
48.487
34.135
33.073
30.788
30.762
30.477
26.742
23.736
14.458

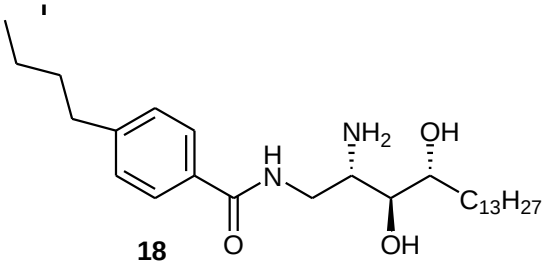

OBn
N3
O
N3
OH
BnO
O
C14H29
BnO
OH


20
20100727O5 110 (5.702) Cn (Cen,3, 80.00, Ht); Sm (SG, 2x0.75); Sb (3,40.00); Cm (107:112-72:104x2.000)
Scan ES+
1.08e6
%
m/z
123.1
130.2
162.2
212.2
231.3
301.1
318.3
477.2
479.3
501.2
502.2
559.2

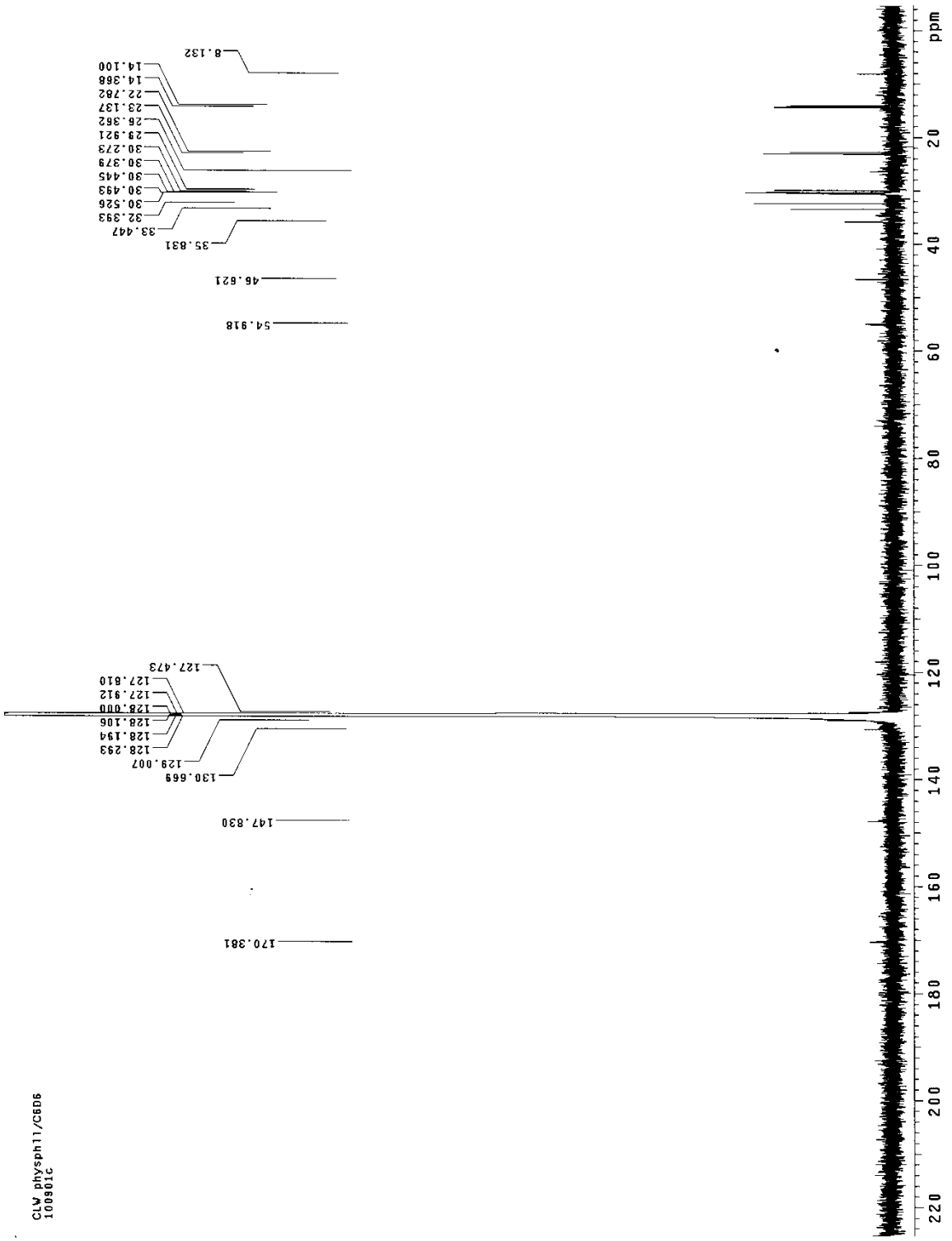

OH
NH2
O
HO
HO
O
NH2
OH
C14H29
OH

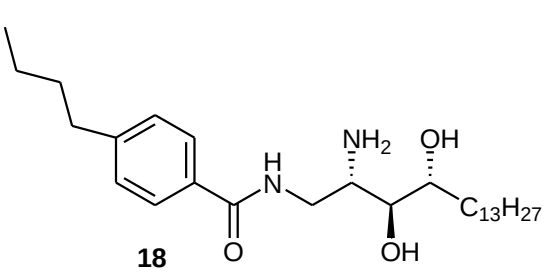
2


CLW physph11/C6D6
1000901H
ppm
0 1 2 3 4 5 6 7 8 9
2.69
2.25
6.94
26.51
6.09
2.97
2.06
2.38
1.33
1.51
4.40
24.49
1.85
1.00
6.990
7.150
7.126
8.056
8.041
8.268
4.241
4.195
3.989
2.473
2.458
2.442
2.233
2.211
2.196
2.181
2.167
2.090
2.075
2.061
1.753
1.742
1.691
1.652
1.542
1.502
1.488
1.472
1.457
1.441
1.371
1.342
1.330
1.292
1.276
1.262
1.247
1.233
1.174
0.957
0.943
0.927
0.912
0.897
0.881
0.858
0.815
0.801
0.788
0.774
0.759
0.633
0.619
0.605

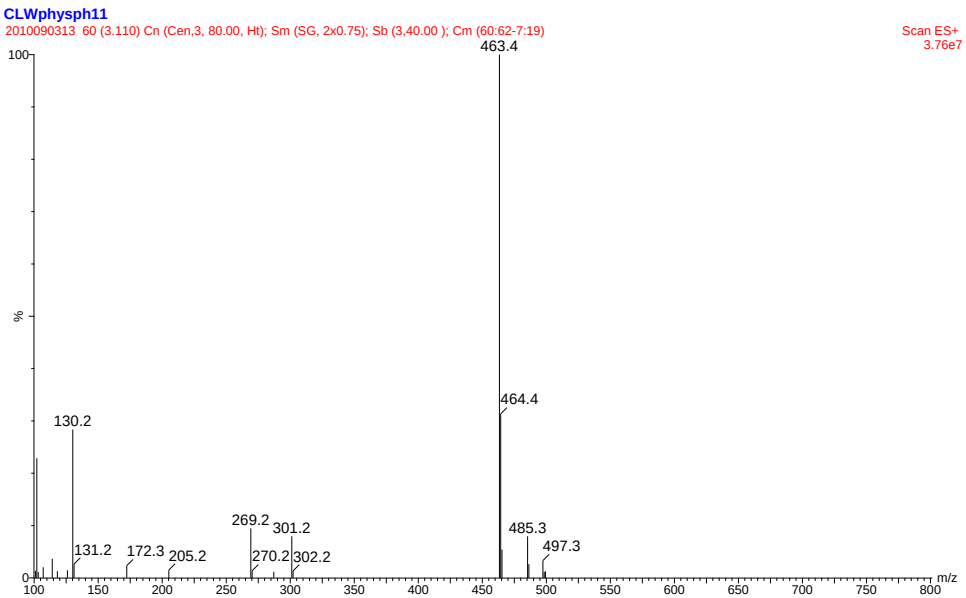

NH2
OH
H
N
O
OH
C13H27

18

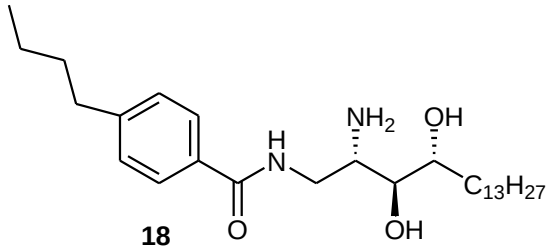

CLW physph11/C6D6
100901c
8.132
14.100
14.366
22.782
23.137
26.362
29.921
30.273
30.379
30.445
30.493
30.526
32.393
33.447
35.831
46.621
54.918
127.473
127.810
127.912
128.000
128.106
128.194
128.293
129.007
130.669
147.830
170.381
ppm
20
40
60
80
100
120
140
160
180
200
220


NH2
OH
H
N
O
OH
C13H27

18



101.0 4.995e5
102.1 8.586e6
103.2 4.086e5
107.0 7.572e5
114.1 1.361e6
118.1 4.668e5
126.0 5.419e5
130.2 1.065e7
131.2 9.776e5
172.3 8.613e5
205.2 5.447e5
269.2 3.542e6
270.2 4.965e5
287.2 4.321e5
301.2 2.977e6
302.2 4.835e5
463.4 3.758e7
464.4 1.177e7
465.4 2.025e6
485.3 2.978e6
486.4 9.812e5
497.3 1.221e6
498.3 4.184e5
499.3 4.683e5



18

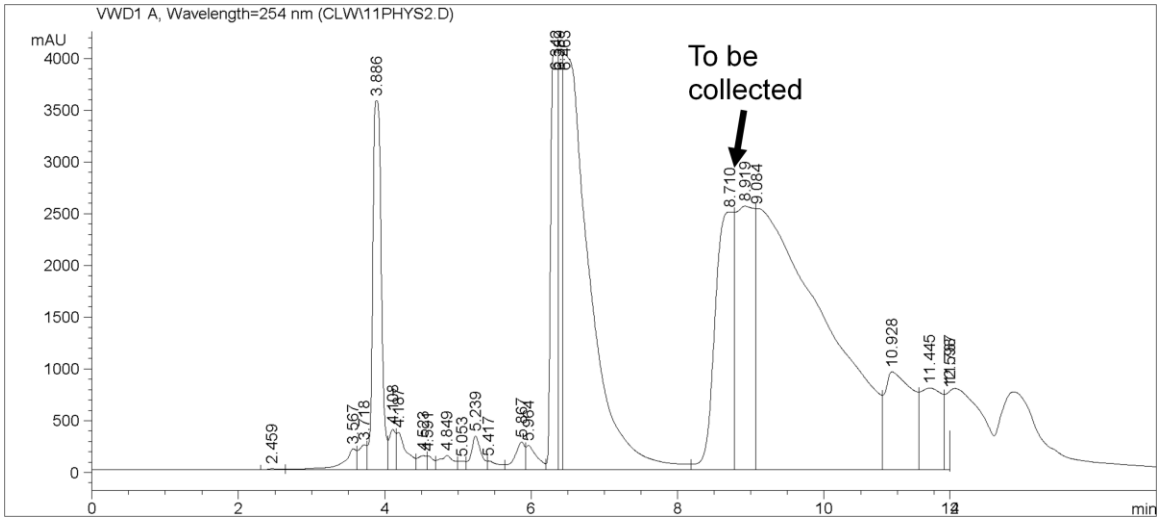


Figure 2. Chromatogram for the mixtures of amide compound **18** after purification with column chromatography.

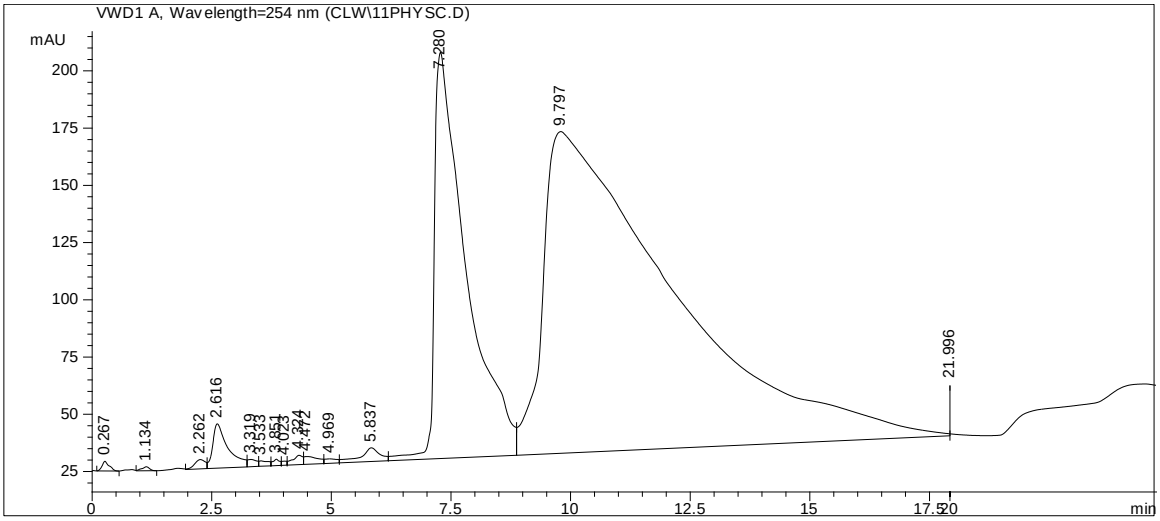
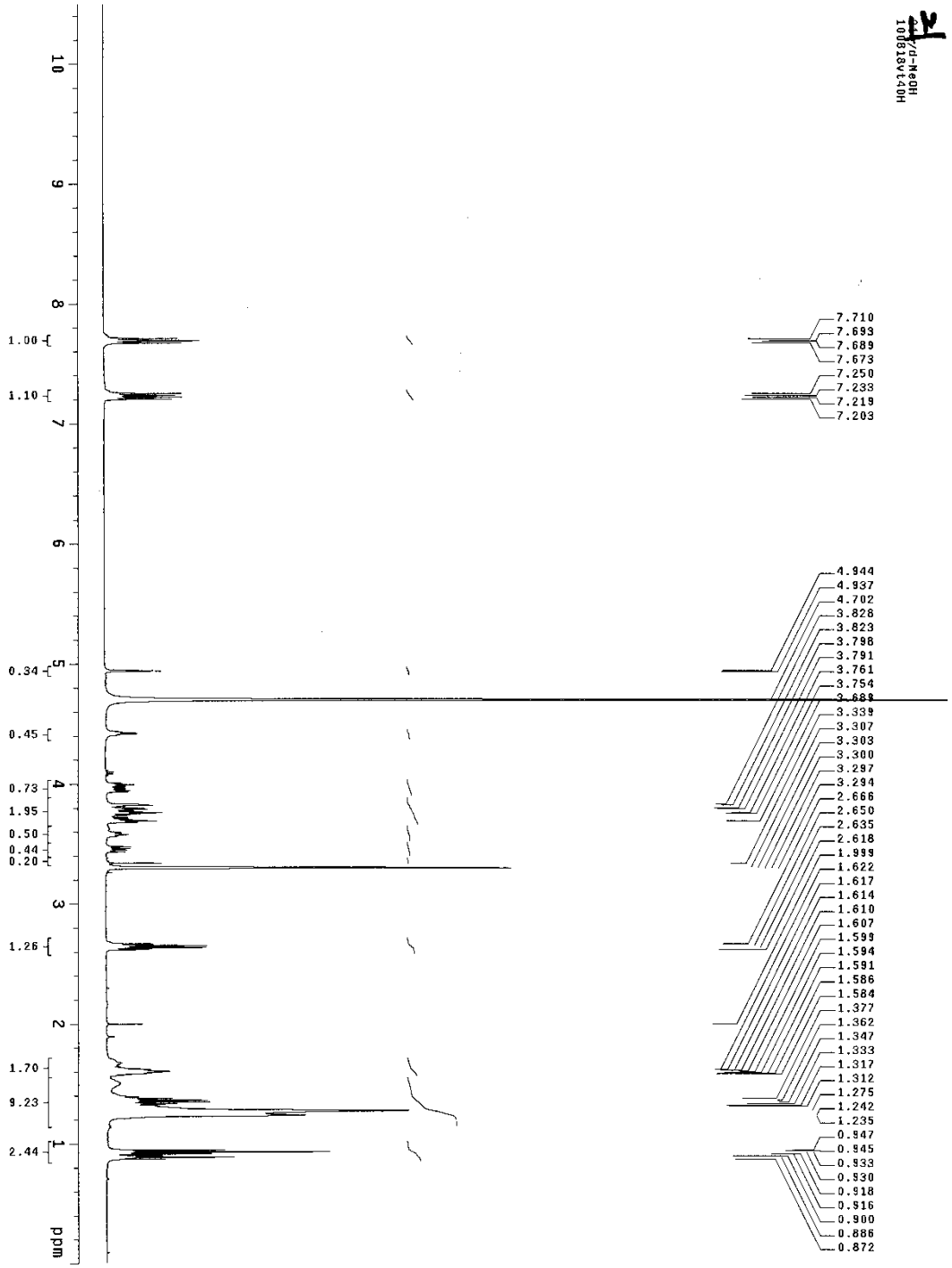
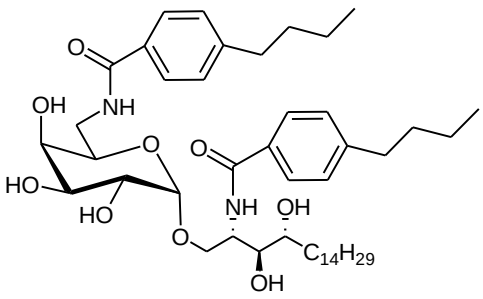
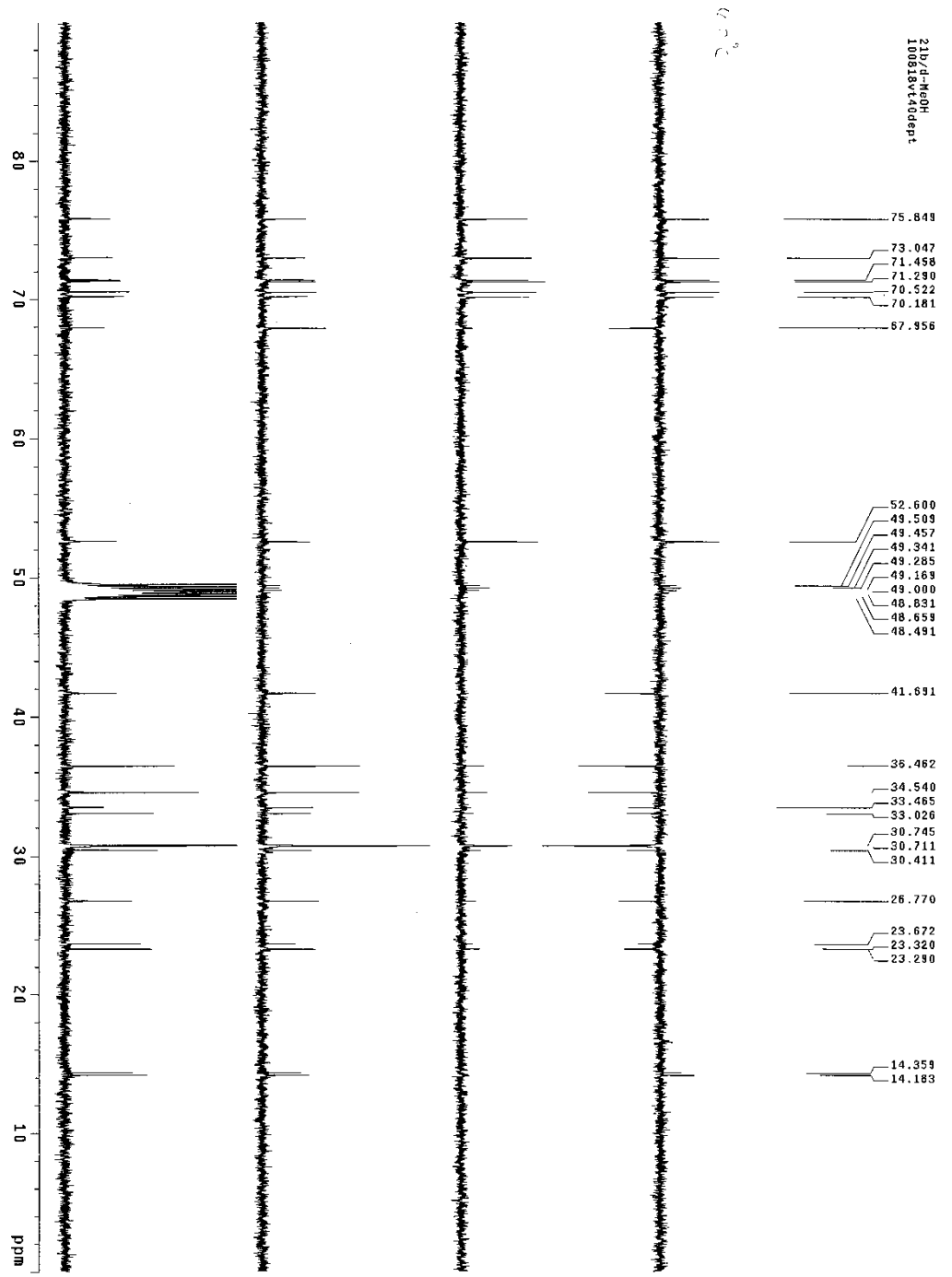


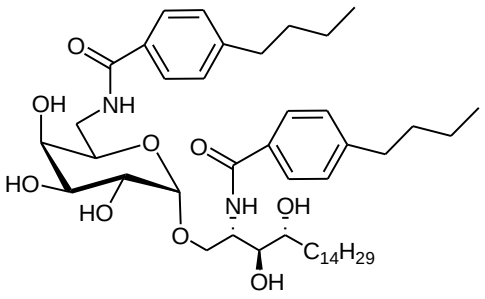
Figure 3. HPLC chromatogram derived from the reinjection of the collected fraction shown in Fig. 1.


10
9
8
7
6
5
4
3
2
1
ppm
1.00
1.10
0.34
0.45
0.73
1.95
0.50
0.44
0.20
1.26
1.70
9.23
2.44
7.710
7.693
7.689
7.673
7.250
7.233
7.219
7.203
4.944
4.937
4.702
3.828
3.823
3.798
3.791
3.761
3.754
3.689
3.339
3.307
3.303
3.300
3.297
3.294
2.666
2.650
2.635
2.618
1.999
1.622
1.617
1.614
1.610
1.607
1.599
1.594
1.591
1.586
1.584
1.377
1.362
1.347
1.333
1.317
1.312
1.275
1.242
1.235
0.947
0.945
0.933
0.930
0.918
0.916
0.900
0.886
0.872

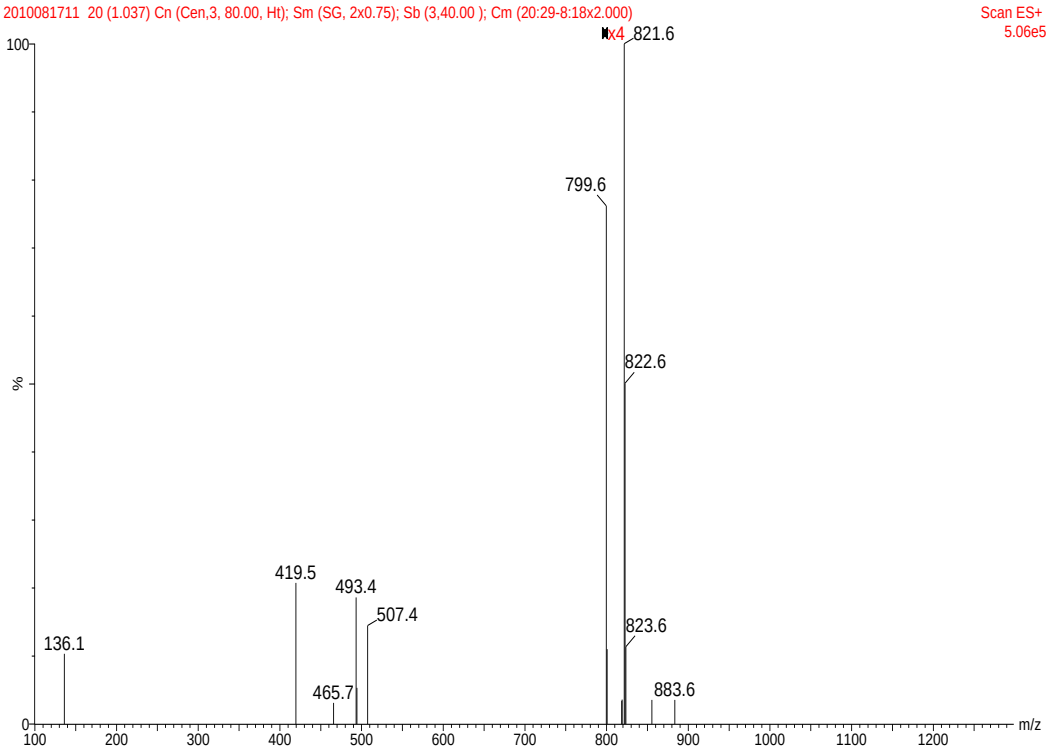

O
NH
OH
O
HO
HO
O
O
NH
OH
O
C14H29
OH

19


21b/d-MeOH
100818vt40dept
75.849
73.047
71.458
71.290
70.522
70.181
67.956
52.600
49.509
49.457
49.341
49.285
49.169
49.000
48.831
48.659
48.491
41.691
36.462
34.540
33.465
33.026
30.745
30.711
30.411
26.770
23.672
23.320
23.290
14.359
14.183
80
70
60
50
40
30
20
10
ppm

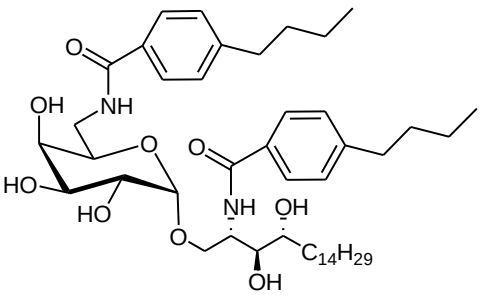

O
NH
OH
O
HO
HO
O
O
NH
OH
C14H29
OH

19



2010081711 20 (1.037) Cn (C

No	Mass	Inten	%BPI	%TIC
1:	136.1	5.21e4	10.30	2.04
2:	419.5	1.05e5	20.73	4.10
3:	465.7	1.56e4	3.09	0.61
4:	493.4	9.40e4	18.59	3.68
5:	494.3	2.68e4	5.30	1.05
6:	507.4	7.31e4	14.44	2.86
7:	508.4	1.22e4	2.41	0.48
8:	799.6	9.63e4	19.04	3.77
9:	800.6	5.56e4	10.99	2.18
10:	818.6	1.72e4	3.40	0.67
11:	819.0	1.80e4	3.56	0.71
12:	819.7	1.31e4	2.60	0.51
13:	821.6	5.06e5	100.00	19.79
14:	822.6	2.53e5	50.09	9.91
15:	823.6	5.73e4	11.33	2.24
16:	835.5	1.02e4	2.03	0.40
17:	855.5	1.78e4	3.51	0.70
18:	883.6	1.78e4	3.51	0.70
19:	889.6	1.18e4	2.34	0.46



19

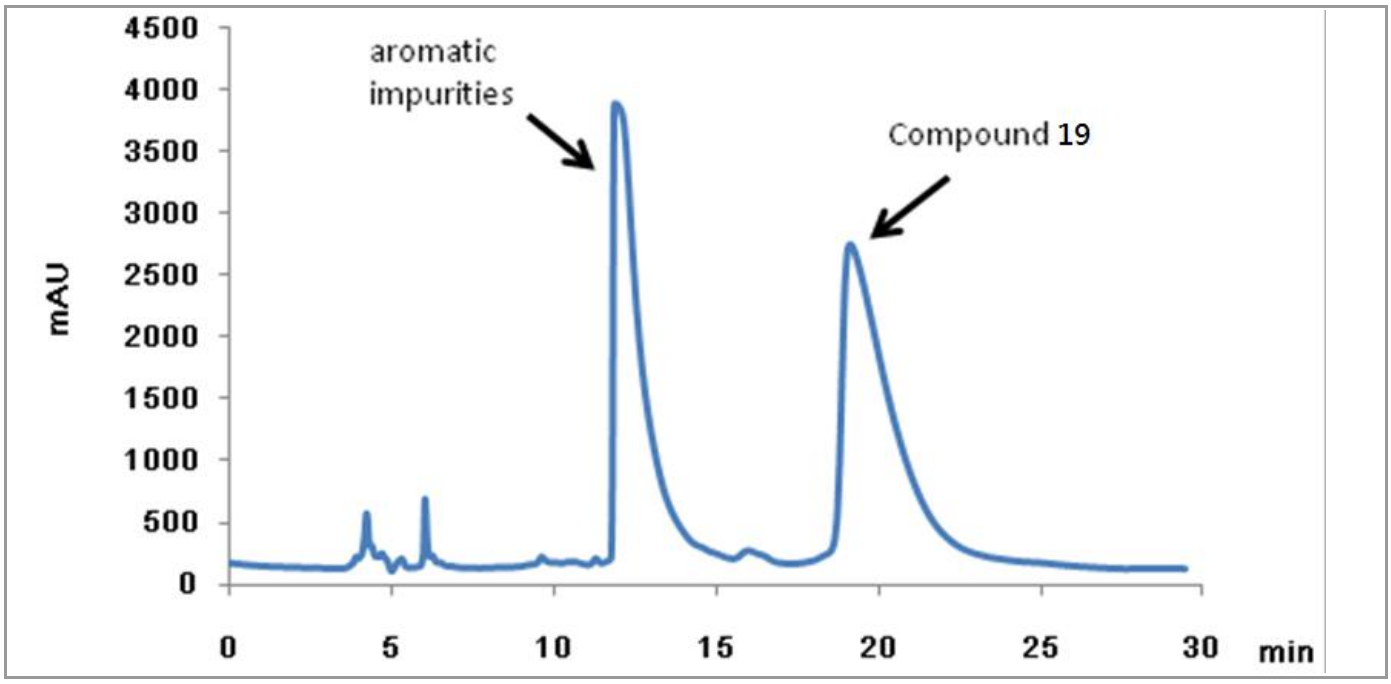


Figure 4. HPLC Chromatogram for the mixtures of amide compound **19** obtained from purification through column chromatography.