Supporting Information

Bacterial AmpD at the Crossroads of Peptidoglycan Recycling and Manifestation of Antibiotic Resistance

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Experimental Procedures

Cloning of the *ampD* gene from *Citrobacter freundii*. The *ampD* gene was amplified by PCR from the chromosome of *C. freundii* (ATCC6879) using two custom-synthesized primers AmpDNdeI: 5′-GCGTCATATGTTGTTAGACGAGGGCTGGCTGGCA-3′ and AmpDHind: 5′- CGATAAGCTTTCA TGTCATCTCCTTGTGTGACGAGGGGGT-3′ (the Nde I and Hind III sites are underlined). The resulting PCR products were digested by the Nde I and Hind III, and ligated into the polylinker of pET24a(+) vector under the T7 promoter. The vectors were transformed into *Escherichia coli* JM83. The selection of transformants was performed on LB agar supplemented with ampicillin (100 μg/mL). The nucleotide sequences of the *ampD* gene from several transformants were verified by sequencing of both DNA strands according to standard procedures. The correct construct was transformed into *E. coli* BL21(DE3) cells for protein expression. In this construct, the *ampD* gene is inducible by isopropyl β-D-thiogalactoside (IPTG).

Protein expression in *E. coli* and purification of AmpD. *E. coli* BL21(DE3) cells harboring the above vector pET-24a$^+$ with the cloned *ampD* gene were grown at 37 °C in 500 mL of LB medium supplemented with kanamycin (50 μg/mL) as the selection agent. The expression of the wild-type AmpD was induced by the addition of IPTG (400 μM, final concentration), when the cell density reached an A_{600} value of 0.7. After 4 h of induction at 30 °C, the cells were harvested by centrifugation (6,000 *g*, 15 min, 4 °C), washed with 150 mM NaCl (in water) and then resuspended in 35 mL of 20 mM phosphate buffer containing 1 mM dithiothreitol pH 7.0 (buffer A). Benzonase (5 units/L of culture) and Pefabloc (0.1 mg/mL in suspension) were added to the cell suspension. Cells were lysed at 4 °C by 30 cycles of sonification (30 s of burst and 20 s of rest for each cycle using a Branson sonifier). The debris was removed by centrifugation at 20,000 *g* for 40 min at 4 °C. The pH of the supernatant was adjusted to 7.0. Then, the supernatant was microfiltered through 0.45 μm Millipore filters and loaded onto a Q-Sepharose column (2.5 cm × 30 cm, 150 mL; Sigma) equilibrated in buffer A (supplemented with 0.05 mg/mL Pefabloc). The column was eluted with a linear NaCl gradient (720 mL) from 0 to 0.35 M in buffer A containing 0.075 mg/mL Pefabloc. The peak fractions containing AmpD (assessed by SDS-PAGE) were pooled, concentrated to 10 mL by ultrafiltration on a 5,000 Da-cutoff Amicon membrane and applied to a molecular sieve column of Sephacryl S100 HR (2.5 cm × 100 cm, 500 mL; Sigma) equilibrated in buffer A (supplemented with 0.05 mg/mL Pefabloc). The fractions containing the protein were collected and concentrated to 4 mL. The solution was added to 4 mL of 2.4 M $(NH_4)_2SO_4$ to make a final concentration of 1.2 M for $(NH_4)_2SO_4$. The enzyme solution (8 mL total) was applied to a Source 15 ISO column (2.5 cm × 30 cm, 60 mL; GE Healthcare Life Science; hydrophobic chromatography). The desired protein was eluted with a linear gradient of 1.2 to 0 M $(NH_4)_2SO_4$ in buffer A. The fractions containing AmpD were collected and dialyzed against 50 mM sodium phosphate buffer, pH 7.0, and concentrated by ultrafiltration to 15~25 mg/mL. After addition of sodium azide (1 mM, final concentration), the enzyme was stored at 4 °C. The protein content from the column fractions was monitored by SDS-PAGE (Figure S1). The AmpD concentration was determined by measuring the absorbance of the solution at 280 nm and using a calculated extinction coefficient of 31,960 $M^{-1}cm^{-1}$.[1] Matrix-assisted laser desorption ionization (MALDI) mass spectrometric analysis revealed a molecular mass of 20 853 ± 20 Da for AmpD, in agreement with that value deduced from the gene sequence (20 847 Da) (Figure S2).

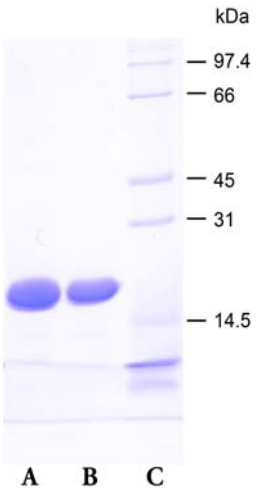
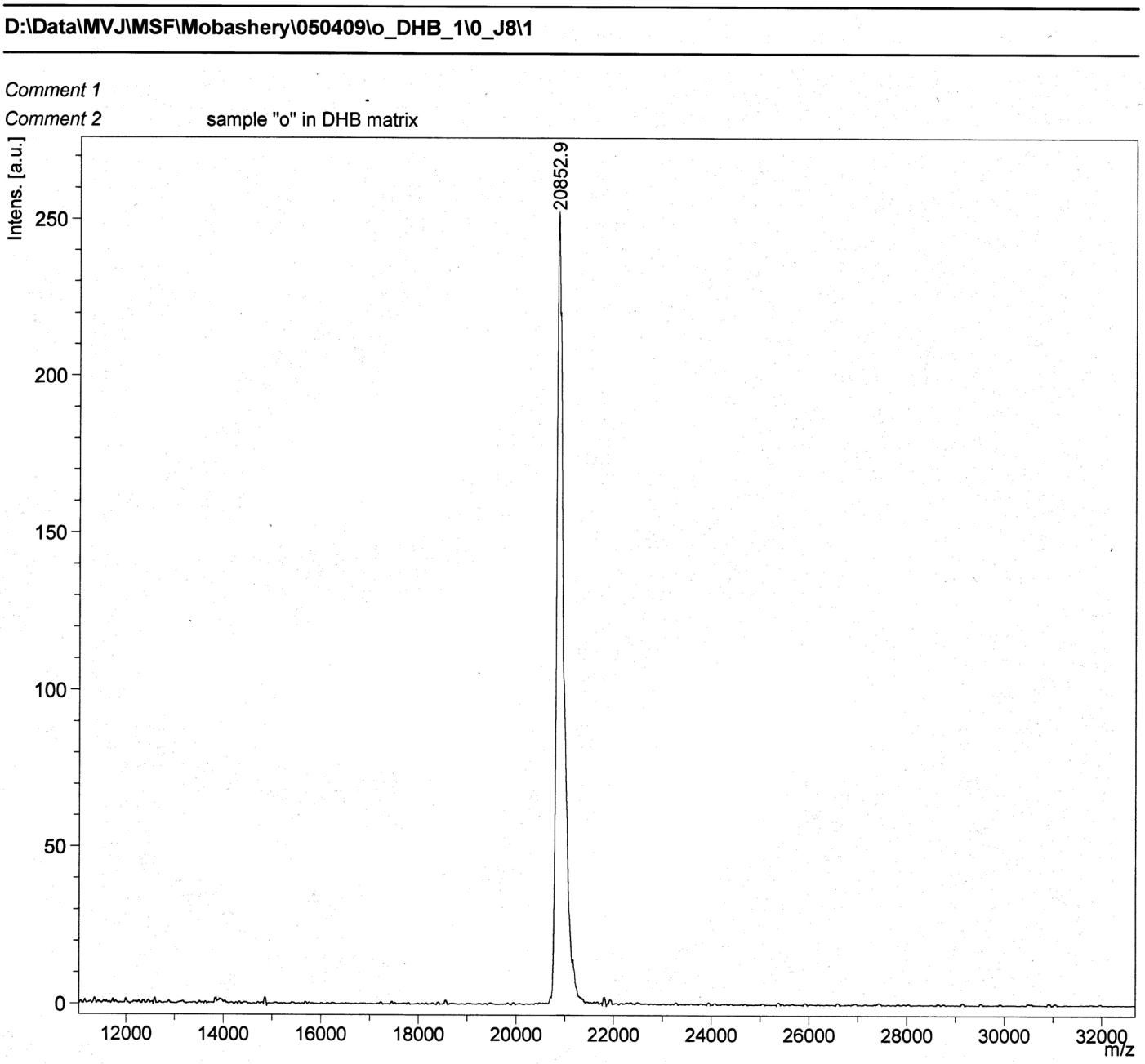


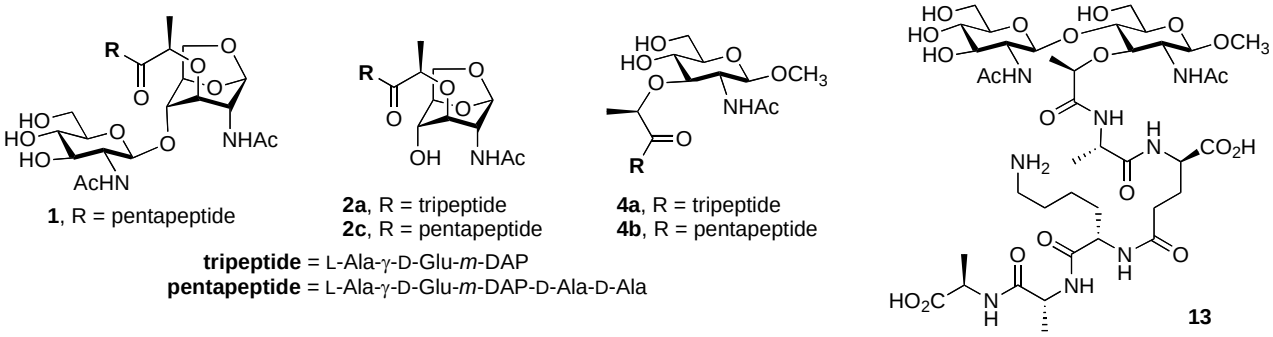
Figure S1. SDS-PAGE analysis of the purified AmpD, (A) 15 μg and (B) 10 μg, (C) molecular mass standards.

[1] Généreux, C.; Dehareng, D.; Devreese, B.; Van Beeumen, J.; Frère, J. M.; Joris, B., *Biochem. J.* **2004,** *377*, 111-120.

Figure S2. MALDI mass spectrum of the purified AmpD.



Kinetic studies. The assays were carried out in 20 mM sodium phosphate buffer, pH 7.0, at 25 °C with substrate concentrations ranging from 50 μM to 3.0 mM and different enzyme concentrations — 50 nM for AnhMurNAc-tripeptide (**2a**), 100 nM for AnhMurNAc-pentapeptide (**2c**), 1.5 μM for GluNAc-AnhMurNAc-pentapeptide (**1**), 500 μM for **4a** and **4b**. The reaction mixtures were incubated at 25 °C for 30 min. The reactions were stopped by the addition of 2 volume of 0.075% TFA in water. The internal standard was GluNAc-MurNAc-pentapeptide (**13**). Reaction products were separated and quantified on a C18 reversed-phase HPLC column (Symmetry Shield RP18, 5 μm, 3.9 mm × 150 mm; Waters) on a PerkinElmer series 200 System. The column was equilibrated with 0.05% trifluoroacetic acid in water and eluted with a linear acetonitrile gradient from 0 to 15% over 40 min with a flow rate of 1 mL/min. The column effluent was monitored at 205 nm. The catalytic activity of the AmpD was quantified from the rate of substrate disappearance and of tripeptide or pentapeptide appearance. The t_R for **2a** was 30.2 min, for **2c** was 34.5 min, for **1** was 36.2 min, for **4a** was 24.6 min and for **4b** was 29.5 min. HPLC chromatograms of AmpD reaction of **2a** is shown in Figure S3 as a representative example.



ESI-MS. Characterization of the reaction products was performed using a Waters Alliance 2695 Separations Module coupled with a Waters 2996 Photodiode Array detector, and Micromass Quattro-LC Triple Quadrupole electrospray ionization (ESI) mass spectrometer (Figure S4). The peaks were initially analyzed using positive ionization mode throughout the *m*/*z* of 100 – 1200. The charge states of the major ions were determined as the reciprocal of the spacing between two adjacent isotopic peaks differing in mass by 1 Da.[2] Analysis of the MS data and fragmentation pattern of the reaction products and of compounds **2a**, **3**, and **12a** allowed us to confirm chemical structure of the reaction products.

Syntheses of Compounds

General Procedures. All organic reagents were purchased from either Sigma-Aldrich Chemical Company or Acros Organics, unless otherwise stated. All reactions were performed under an atmosphere of nitrogen unless noted otherwise. Reactions were monitored by thin-layer chromatography (TLC) carried out on Whatman reagents 0.25 mm silica gel 60-F plates that were visualized using UV light and/or aqueous cerium sulfate staining, followed by heating. Flash chromatography was carried out with silica gel 60, 230-400 mesh (0.040-0.063 mm particle size) purchased from EM Science. NMR spectra, including ^{1}H, ^{13}C, DEPT, H-H COSY, and H-C HETCOR experiments, were recorded on a Varian UnityPlus 300, or a Varian INOVA-500, or Varian DirectDrive 600 spectrometer. Proton and Carbon chemical shifts were referenced to residual solvent peaks. NMR signal assignments for synthesized compounds were performed on the basis of H-H COSY, H-C HETCOR, and DEPT experiments. High-resolution mass spectra were obtained at the Department of Chemistry and Biochemistry, University of Notre Dame via FAB ionization, using a JEOL AX505HA mass spectrometer.

Analytical high performance liquid chromatography (HPLC) was performed on Waters 2414 instrument with SunFire C18 reversed-phased column (Waters) or delta-pak C18 reversed-phased column (Waters) using a linear

[2] Henry, K. D.; McLafferty, F. W. *Org. Mass Spectrom.* **1990,** *25*, 490-492.

gradient of 2-15% acetonitrile in water supplemented with 0.1% TFA over 40 min at 1 mL/min. Detection of the samples was by UV at 205 nm. Preparative HPLC purifications were performed using delta-pak C18 reversed-phased column, 100 Å pore size, 19 × 300 mm.

Crystals were examined under Infineum V8512 oil and placed on a MiTeGen mount, then transferred to the 100 K N_2 stream of a Bruker SMART Apex CCD diffractometer. Unit cell parameters were determined from reflections with $I > 10\sigma(I)$ harvested from three orthogonal sets of 30 0.5° ω scans. Data collection strategy was calculated using COSMO, included in the Apex2 suite of programs[3] to maximize coverage of reciprocal space in a minimum amount of time. Average 4-fold redundancy of measurements was sought. Data were corrected for Lorentz and polarization effects, as well as for absorption. Structure solution and refinement utilized the programs of the SHELXTL software package.[4] Full details of the X-ray structure determinations are in the CIF files included as Supporting Information.

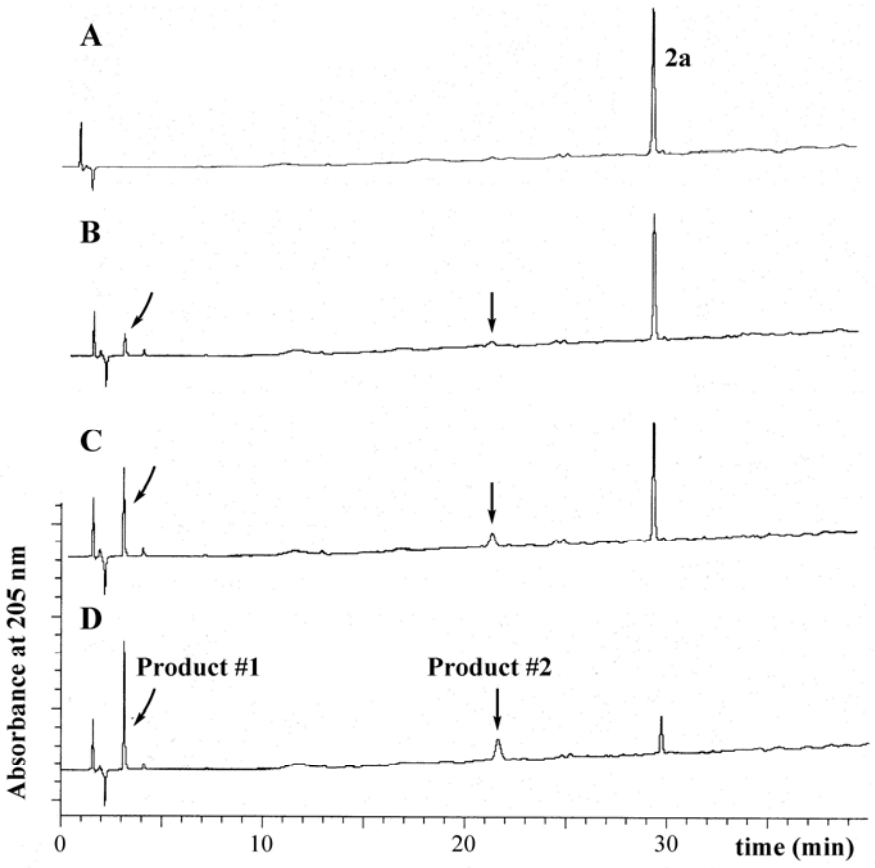


Figure S3. The AmpD reaction with compound **2a**. The single peak is compound **2a** at time zero (A). The time course for conversion of compound **2a** by AmpD to the two products (retention times at 3.1 and 21.5 min) were monitored at 10 min (B), 60 min (C), and 90 min (D) of incubation. The two new peaks correspond to product **1** (the tripeptide) and product **2** (anhMurNAc), whose identities were conformed by LC/MS analysis (Figure S4).

[3] Apex2. Bruker-AXS: Madison, WI, 2008; Vol. 58.
[4] Sheldrick, G. M., *Acta Crystallogr. A.* **2008,** *64*, 112-122.

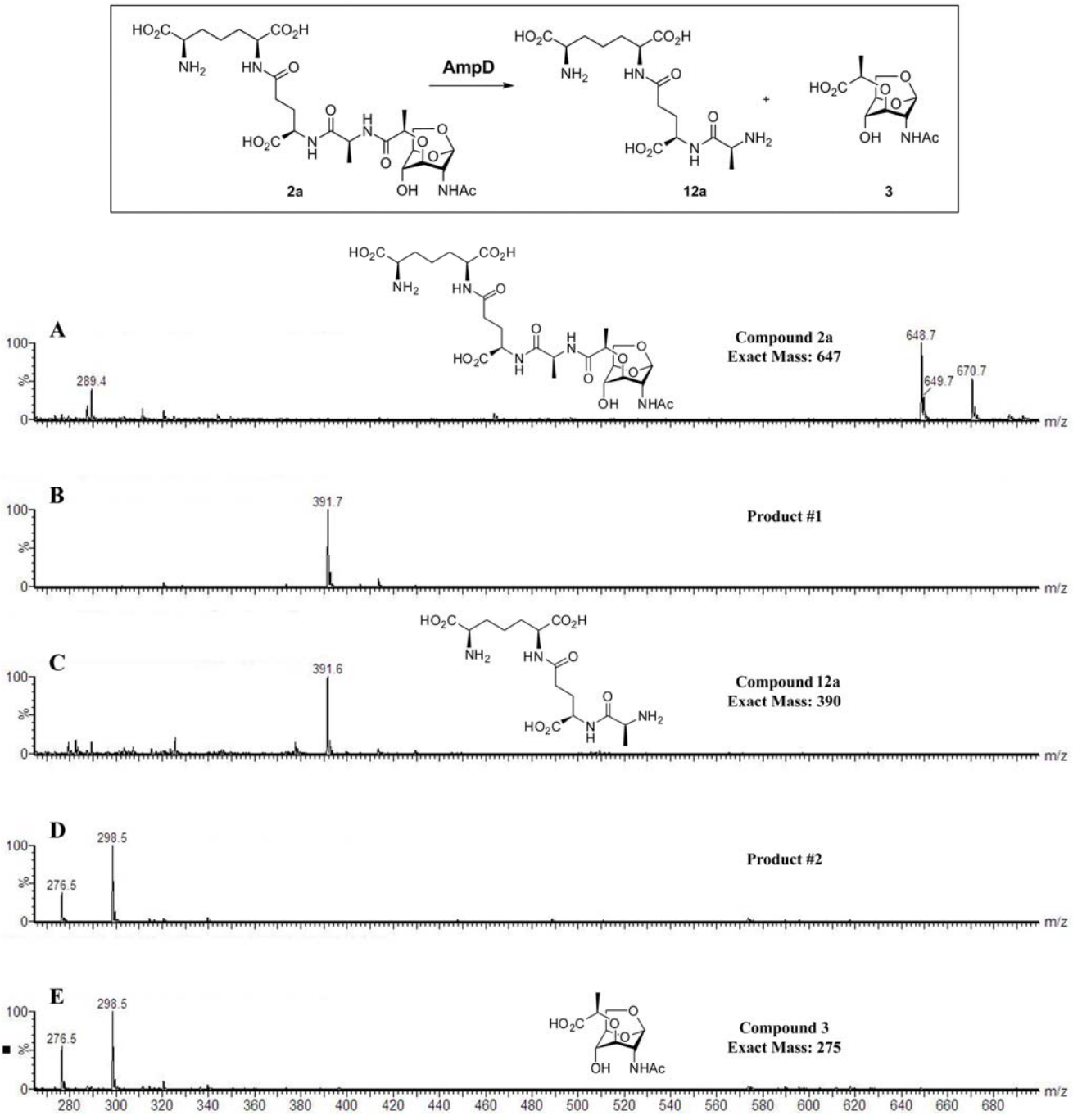


Figure S4. ESI-LC-MS analysis of the AmpD reaction products and comparison to authentic synthetic samples. The spectrum of the synthetic compound **2a** (A), of reaction product #1 (B), authentic compound **12a** (C), of reaction product #2 (D), and of authentic compound **3** (E).

Compounds **1**, **4a**, **4b**, and **13** were prepared according to the literature methods developed by our laboratory.[5]
Compound 9. EDCI (0.18 g, 0.94 mmol) was added to a mixture of *N*-hydroxysuccinimde (0.11 g, 0.96 mmol) and **6** (0.28 g, 0.76 mmol) in CH_2Cl_2 (5 mL) in an ice-water bath. The mixture was stirred at room temperature for 20 h. Meanwhile, the Boc-protected tripeptide (0.72 g, 0.91 mmol) in CH_2Cl_2 (5 mL) was treated with trifluoroacetic acid (2 mL) in an ice-water bath. The temperature was gradually increased to room temperature over 1 h. The reaction mixture was evaporated to dryness under reduced pressure and the residue (**7**) was dissolved in toluene. This was followed by evaporation to dryness. The residue was dissolved in *i*Pr_2NEt (0.4 mL, 2.3 mmol) and DMF (5 mL), and the solution was then added to the NHS-ester of the anhydrosugar, prepared above. The resulting mixture was stirred at room temperature for 20 h. The mixture was diluted with CH_2Cl_2 and water was added. Layers were separated. The organic layer was dried over $MgSO_4$, filtered, concentrated and the sample was subjected to column chromatography on silica gel (CH_2Cl_2/MeCN/MeOH, 10:3:0.5) to give the title compound (0.57 g, 72%). ^{1}H NMR (500 MHz, CD_3OD) δ 1.29 (d, *J* = 6.8 Hz, 3H), 1.34 (d, *J* = 7.0 Hz, 3H), 1.36 - 1.45 (m, 2H), 1.60 - 1.71 (m, 2H), 1.75 - 1.84 (m, 2H), 1.95 - 2.05 (m, 1H), 2.12 - 2.21 (m, 1H), 2.29 (t, *J* = 6.9 Hz, 2H), 3.49 (d, *J* = 1.2 Hz, 1H, H-4), 3.52 (m, 1H, H-3), 3.68 (dd, *J* = 7.5, 5.7 Hz, 1H, H-6a), 3.85 (s, 1H, H-2), 4.02 (dd, *J* = 8.2, 5.2 Hz, 1H, DAP-H-6), 4.17 (q, *J* = 6.8 Hz, 1H, Lac-α-H), 4.23 (d, *J* = 7.6 Hz, 1H, H-6b), 4.30 - 4.46 (m, 3H, Ala-α-H, Glu-α-H, DAP-H-2), 4.61, 4.71 (AB, 2H, O*CH*$_2$Ph), 4.67 (m, H-5), 5.08 - 5.22 (m, 6H, O*CH*$_2$Ph), 5.38 (s, 1H, H-1), 7.28 - 7.40 (m, 20H); ^{13}C NMR (126 MHz, CD_3OD) δ 18.5, 18.6, 22.7 (3 × q), 23.1, 28.2, 31.8, 32.7 (5 × t), 49.9 (d, C-2), 50.2 (d, Ala-α-C), 53.4 (d, DAP-C-2), 53.7 (d, Glu-α-C), 62.9 (d, DAP-C-6), 66.3 (t, C-6), 68.0, 68.4, 72.6 (4 × t, O*CH*$_2$Ph), 75.4 (d, C-5), 76.9 (d, Lac-α-C), 77.6 (2d, C-3 and C-4), 101.6 (d, C-1), 129.1, 129.2, 129.3, 129.5, 129.6, 129.7, 129.7, 136.8, 137.1, 137.1, 139.2, 164.8, 171.7, 172.6, 172.7, 173.3, 174.7, 174.8, 174.9; HRMS (FAB), calcd for $C_{54}H_{64}N_7O_{14}$ ($M+H^+$), 1034.4511, found 1034.4525.

1,6-Anhydro-β-D-*N*-acetylmuramyl-L-Ala-γ-D-Glu-*meso*-DAP (2a). Compound **9** (0.50 g, 0.43 mmol) was dissolved in MeOH (5 mL) and stirred in the presence of 10% Pd/C (0.1 g) under an atmosphere of hydrogen at 50 ºC for 3 h. The reaction mixture was filtered through a layer of Celite and the residue was washed with MeOH. The combined filtrate was concentrated to dryness under reduced pressure. The crude product was subjected to HPLC purification to afford compound **2a** (0.18 g, 66%). Preparative HPLC purifications were performed on delta-pak C18 reversed-phased column, 100 Å pore size, 19 × 300 mm using a linear gradient of 5-15% acetonitrile in water supplemented with 0.1% TFA over 0.5 h. ^{1}H NMR (600 MHz, D_2O) δ 1.30 (d, *J* = 6.1 Hz, 3H), 1.35 (d, *J* = 5.6 Hz, 3H), 1.56 - 2.12 (m, 7H), 1.91 - 1.93 (m, 3H), 1.94 - 1.98 (m, 3H), 2.15 - 2.36 (m, 3H), 3.35 (br.s., 1H, H-3), 3.66 (m, 1H), 3.73 (t, *J* = 6.2 Hz, 1H, H-6a), 3.84 (br.s., 1H, H-2), 3.80 (br.s., 1H, H-4), 4.04 - 4.16 (m, 3H), 4.19 (d, *J* = 7.3 Hz, 1H, H-6b), 4.31 (br.s, 1H), 4.60 (d, *J* = 4.2 Hz, 1H, H-5), 5.40 (s, 1H, H-1); ^{13}C NMR (151 MHz, D_2O) δ 17.2, 18.1, 21.9 (3 × q), 21.2, 27.7, 30.2, 31.1, 32.0 (5 × t), 49.5 (d, C-2), 49.5 (d), 65.3 (t, C-6), 68.2 (d, C-4), 76.0 (d, C-5, Lac-α-C), 78.5 (d, C-3), 100.0 (d, C-1); HRMS (FAB), calcd for $C_{26}H_{42}N_5O_{14}$ ($M+H^+$), 648.2728, found 648.2719.

1,6-Anhydro-β-D-*N*-acetylmuramyl-L-Ala-γ-D-Glu-*meso*-DAP-D-Ala-D-Ala (2c). This material was prepared in the same manner as described for **2a**, with the exception that pentapeptide **8** was used in place of **7**. **Compound 10**. ^{1}H NMR (600 MHz, CD_3CN) δ 1.29 (d, *J* = 7.0 Hz, 3H), 1.32 (d, *J* = 6.7 Hz, 3H), 1.34 (d, *J* = 7.3 Hz, 3H), 1.34 - 2.00 (m, 7H), 1.37 (d, *J* = 7.3 Hz, 3H), 1.89 (s, 3H), 2.15 - 2.27 (m, 3H), 3.48 (s, 1H, H-4), 3.55 (s, 1H, H-3), 3.71 (dd, *J* = 7.6, 6.2 Hz, 1H, H-6a), 3.91 (d, *J* = 8.8 Hz, 1H, H-2), 4.06 (dd, *J* = 8.7, 4.8 Hz, 1H, DAP-H-6), 4.07 - 4.11 (m, 1H), 4.13 (q, *J* = 6.9 Hz, 1H, Lac-α-H), 4.16 (d, *J* = 7.6 Hz, 1H, H-6b), 4.27 - 4.43 (m, 4H), 4.64, 4.68 (AB, *J* = 12.0 Hz, 2H, O*CH*$_2$Ph), 4.69 (br. s., 1H, H-5), 5.07 - 5.15 (m, 4H, O*CH*$_2$Ph), 5.21 (s, 2H, O*CH*$_2$Ph), 5.39 (s, 1H, H-1), 6.55 (d, *J* = 8.8 Hz, 1H, NH), 7.31 - 7.44 (m, 20H), 7.52 (t, *J* = 7.6 Hz, 1H, NH), 7.90 (d, *J* = 6.5 Hz, 1H, NH); ^{13}C NMR (151 MHz, CD_3CN) δ 17.6, 17.9, 18.0, 18.6, 23.1 (5 × q), 22.9, 27.7, 31.7, 32.1 (5 × t), 49.3 (d, C-2), 49.3, 49.8, 50.6 (3 × d, Ala-α-C), 52.1 (d, Glu-α-C)), 55.4 (d, DAP-C-2),

[5] Hesek, D.; Lee, M.; Zhang, W.; Noll, B. C.; Mobashery, S., *J. Am. Chem. Soc.* **2009,** *131*, 5187-5193; Hesek, D.; Suvorov, M.; Morio, K.; Lee, M.; Brown, S.; Vakulenko, S. B.; Mobashery, S., *J. Org. Chem.* **2004,** *69*, 778-784.

62.7 (d, DAP-C-6), 66.0 (t, C-6), 67.3, 67.6, 68.1, 72.1 (4 × t, O*C*H$_2$Ph), 75.1 (d, C-5), 76.6 (d, Lac-α-C), 77.0 (d, C-4), 77.4 (d, C-3), 101.4 (d, C-1), 128.8, 128.9, 129.0, 129.1, 129.2, 129.4, 129.5, 129.6, 136.7, 137.0, 137.2, 139.2, 170.7, 171.4, 172.3, 173.2, 173.2, 173.5, 173.8, 174.0, 174.4; HRMS (FAB), calcd for $C_{60}H_{73}N_9O_{16}$ (M^+), 1176.5254, found 1176.5288.

Compound 2c. ^{1}H NMR (600 MHz, CD_3OD) δ 1.39 (d, *J* = 6.5 Hz, 3H), 1.39 (d, *J* = 7.0 Hz, 3H), 1.42 (d, *J* = 7.3 Hz, 3H), 1.44 (d, *J* = 7.3 Hz, 3H), 1.51 - 2.03 (m, 7H), 1.99 (s, 3H), 2.22 - 2.39 (m, 3H), 3.42 (s, 1H, H-3), 3.72 (s, 1H, H-4), 3.76 (dd, *J* = 7.3, 5.6 Hz, 1H, H-6a), 3.88 (s, 1H, H-2), 4.01 (t, *J* = 6.0 Hz, 1H), 4.21 (q, *J* = 6.7 Hz, 1H, Lac-α-H), 4.24 - 4.27 (m, 1H), 4.28 (d, *J* = 7.3 Hz, 1H, H-6b), 4.33 - 4.45 (m, 4H), 4.59 (d, *J* = 5.0 Hz, 1H, H-5), 5.40 (s, 1H, H-1); ^{13}C NMR (126 MHz, CD_3OD) δ 17.7, 18.0, 18.3, 18.7, 22.8 (5 × q), 22.6, 28.5, 31.2, 32.2, 32.7 (5 × t), 50.0 (d, C-2), 49.5, 50.6, 50.9 (3 × d, Ala-α-C), 52.7 (d, Glu-α-C), 53.9, 55.4 (2 × d, DAP-C-2, DAP-C-6), 66.4 (t, C-6), 70.8 (d, C-4), 77.1 (d, Lac-α-C), 77.7 (d, C-5), 81.0 (d, C-3), 101.9 (d, C-1), 171.9, 173.1, 174.4, 174.6, 174.8, 175.1, 175.3, 175.7, 175.9; HRMS (FAB), calcd for $C_{32}H_{52}N_7O_{16}$ ($M+H^+$), 790.3471, found 790.3455.

2-Acetamido-1,6-anhydro-4-*O*-benzyl-2-deoxy-3-*O*-[(1*R*)-1-(methoxycarbonyl)ethyl]-β-D-glucopyranose (11). Compound **3** (0.28 g, 1.0 mmol) in anhydrous MeOH (2 mL) was stirred in the presence of AcOH (2 mL) at 60 ºC for 10 h. The resulting mixture was concentrated to dryness. Crystals were grown from mixed solvents of hexanes and Et_2O and used for determination of X-ray crystal structure. ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.42 (d, *J* = 7.0 Hz, 3H), 2.06 (s, 3H), 3.43 (s, 1H), 3.48 (s, 3H), 3.73 (d, *J* = 3.2 Hz, 2H), 3.75 (s, 3H), 4.09 (d, *J* = 8.4 Hz, 1H), 4.19 (d, *J* = 7.2 Hz, 1H), 4.31 (q, *J* = 6.9 Hz, 1H), 4.54 (d, *J* = 5.2 Hz, 1H), 5.36 (s, 1H), 6.91 (d, *J* = 8.4 Hz, 1H); ^{13}C NMR (126 MHz, $CDCl_3$) δ 18.3 (q), 22.9 (q), 50.5 (d), 52.2 (q, OCH_3), 65.4 (t, C-6), 70.1 (d), 74.2 (d), 76.4 (d), 78.1 (d), 95.9 (d), 100.8 (d), 170.6, 173.5.

L-Ala-γ-D-Glu-*meso*-DAP (12a). This material was prepared in the same manner as described for **2a**, with the exception that the tripeptide **7** was used in place of **9**. ^{1}H NMR (300 MHz, D_2O) δ 1.46 - 2.30 (m, 8H), 1.56 (d, *J* = 7.2 Hz, 3H), 2.38 - 2.49 (m, 2H), 3.90 (t, *J* = 5.9 Hz, 1H), 4.13 (q, *J* = 7.0 Hz, 1H), 4.37 (m, 2H); ^{13}C NMR (126 MHz, D_2O) δ 17.1, 20.9, 21.4, 27.1, 30.1, 30.6, 31.9, 49.6, 53.2, 53.2, 54.2, 171.3, 173.9, 175.5, 175.8, 176.4, 177.2; HRMS (FAB), calcd for $C_{15}H_{27}N_4O_8$ ($M+H^+$), 391.1829, found 391.1839.

X-ray structures of compounds 11. Crystals of compound **11** was obtained and their X-ray crystal structure were determined to confirm the structure of 1,6-anhydromuramic acid. Compound **11** keeps a typical anhydropyranose structure, where all substitutens are in axial positions. Two-H bonds were present in compound **11**. The O4 atom engages two H-bonds. One is the intramolecular H-bond between N1–H1N–O4 and the other is the intermolecular H-bond between O4–H4O–O8. Detailed information on H-bonds is given in Table S2. Structural details are given in Figures S5 and S6.

Table S1. Crystallographic Details of Compound **11**.

	11
chemical formula	$C_{12}H_{19}NO_7$
formula weight	289.28
space group	$P2_1$
a (Å)	6.3338(1)
b (Å)	15.4559(3)
c (Å)	7.2741(2)
α (°)	90.00
β (°)	108.775(1)
γ (°)	90.00
V Å^3	674.20(3)
Z	2
T (°C)	100(2)
λ (Å)	1.54178
D_{obsd} (g cm^{-3})	1.425
μ (cm^{-1})	1.006
R1(F^2, $I > 2\sigma(I)$)	0.0264
wR2(F^2)	0.0684
S	1.060

$$wR2 = \sqrt{\frac{\sum[w(F_o^2 - F_c^2)^2]}{\sum[w(F_o^2)^2]}} \;;\; R1 = \frac{\sum||F_o| - |F_c||}{\sum|F_o|} \;;\; GooF = S = \sqrt{\frac{\sum[w(F_o^2 - F_c^2)^2]}{(n-p)}}$$

n= number of reflections, *p*= number of parameters refined

Table S2. Hydrogen-bond geometry of compound **11**.

D–H···*A*	*D*–H (Å)	H···*A* (Å)	*D*···*A* (Å)	*D*–H ···*A* (º)
N1–H1N···O4^{i}	0.83(2)	2.54(2)	3.0638(17)	122.7(10)
O4–H4O···O8^{ii}	0.840	1.914	2.7035(16)	156

*symmetry codes: (i) x, y, z; (ii) x-1, y, z-1.

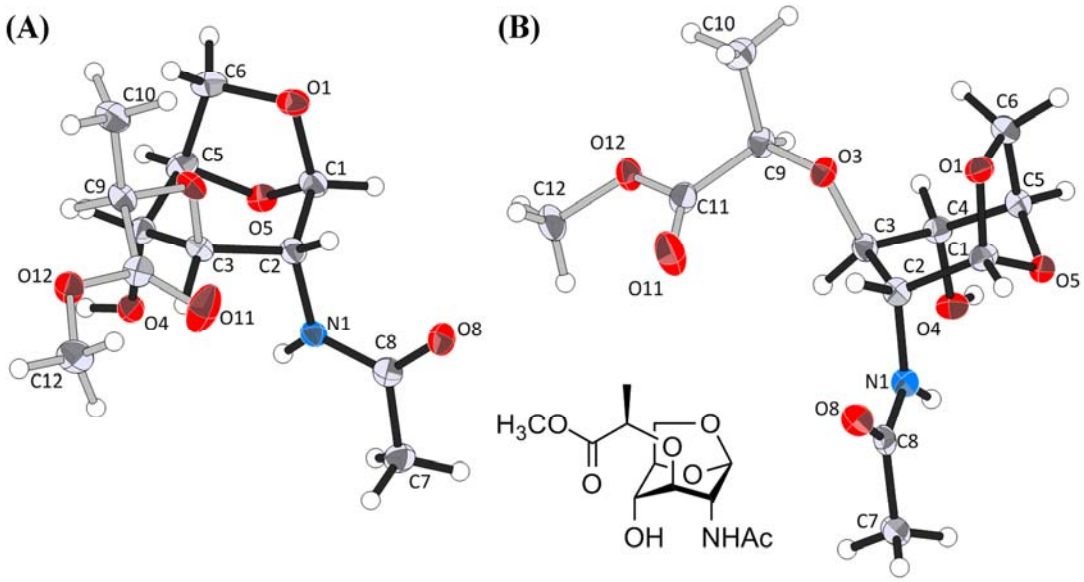


Figure S5. (A) The molecular structure of compound **11**, showing the atom-numbering scheme. The ORTEP diagram is shown at 50% probability level. Bonds of lactate side chain at C-3 are shown in gray, while other bonds in anhydrosugar backbone are shown in black. Hydrogen atoms are shown as small spheres of arbitrary radii. (B) An alternative view.

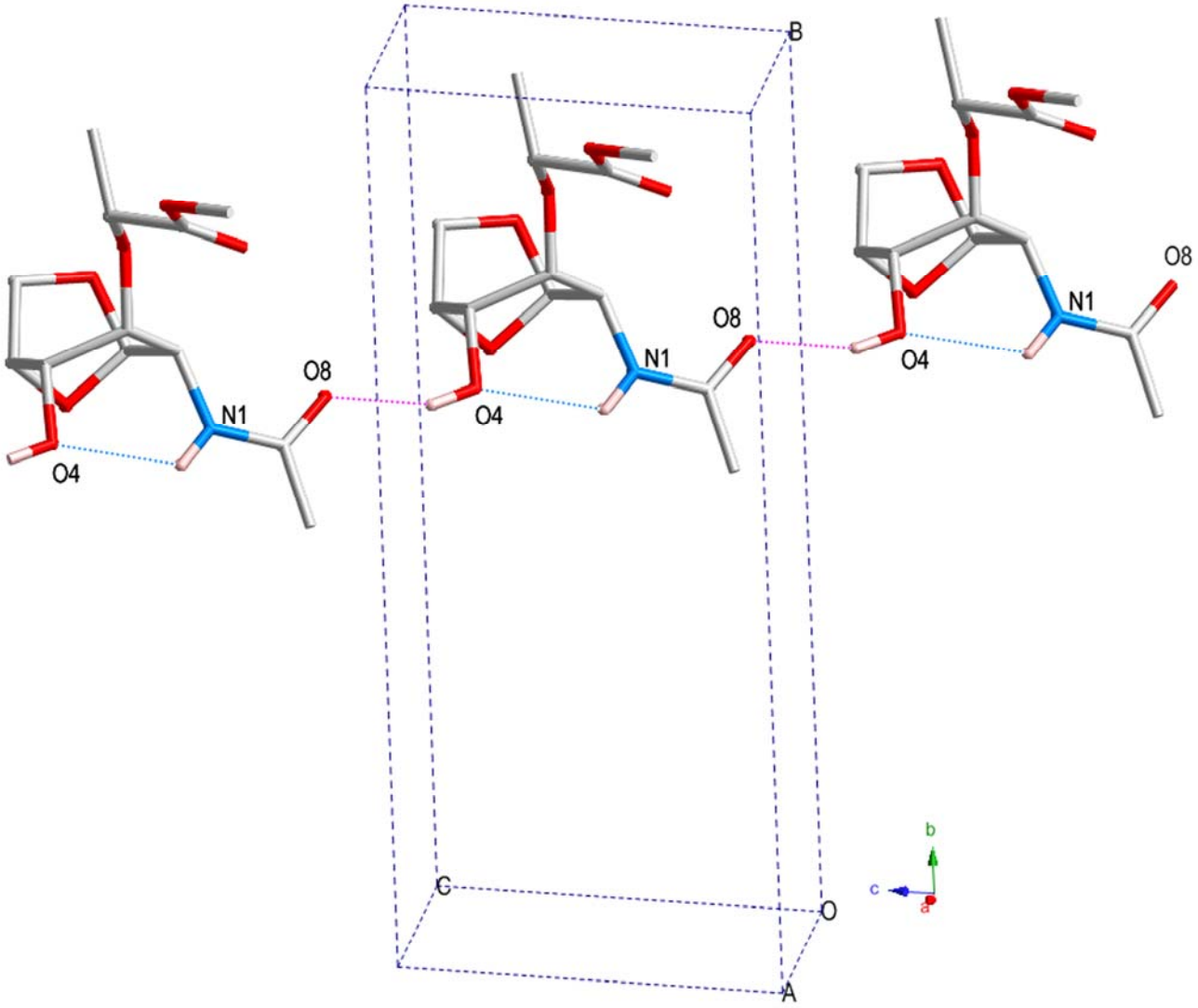
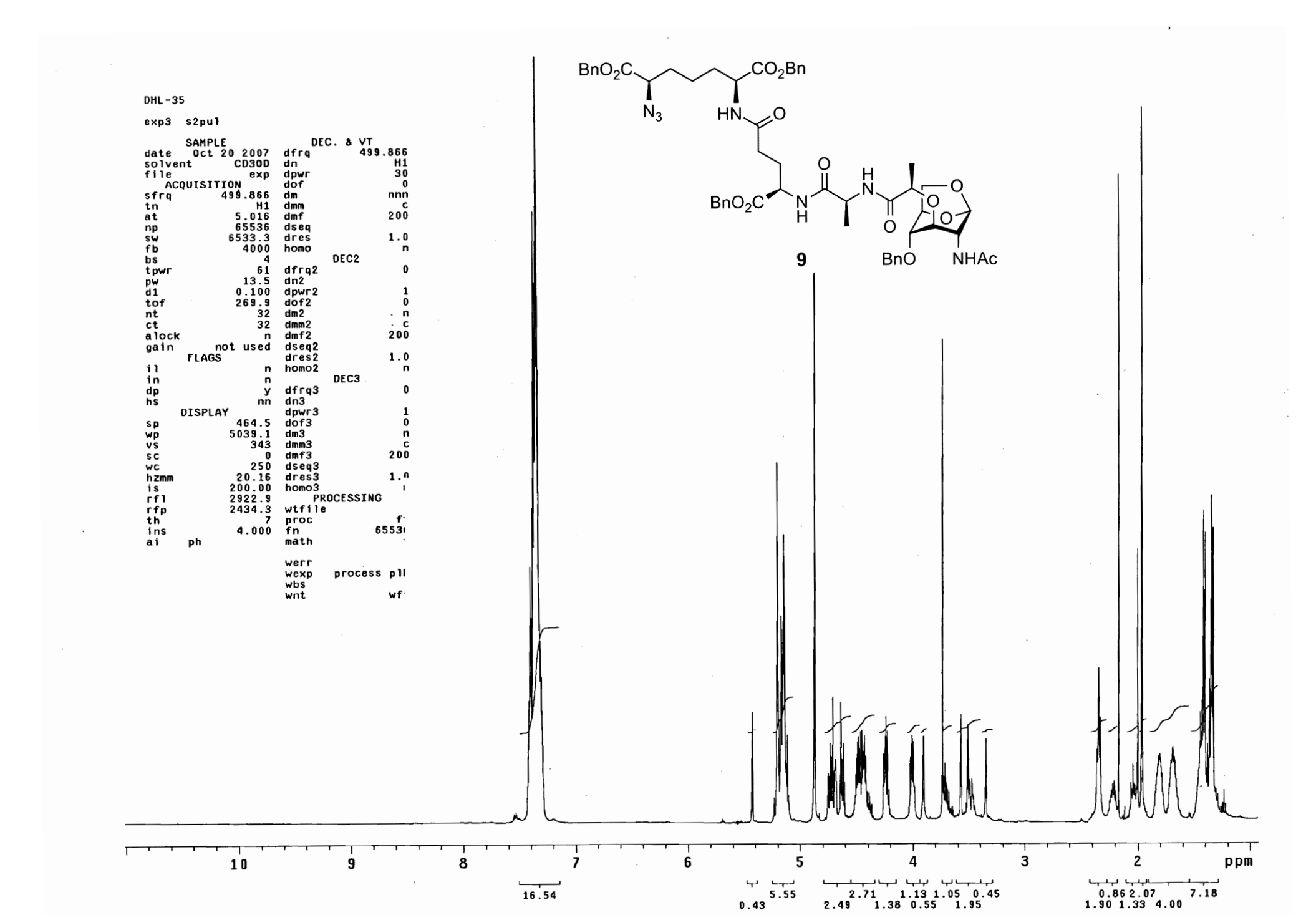
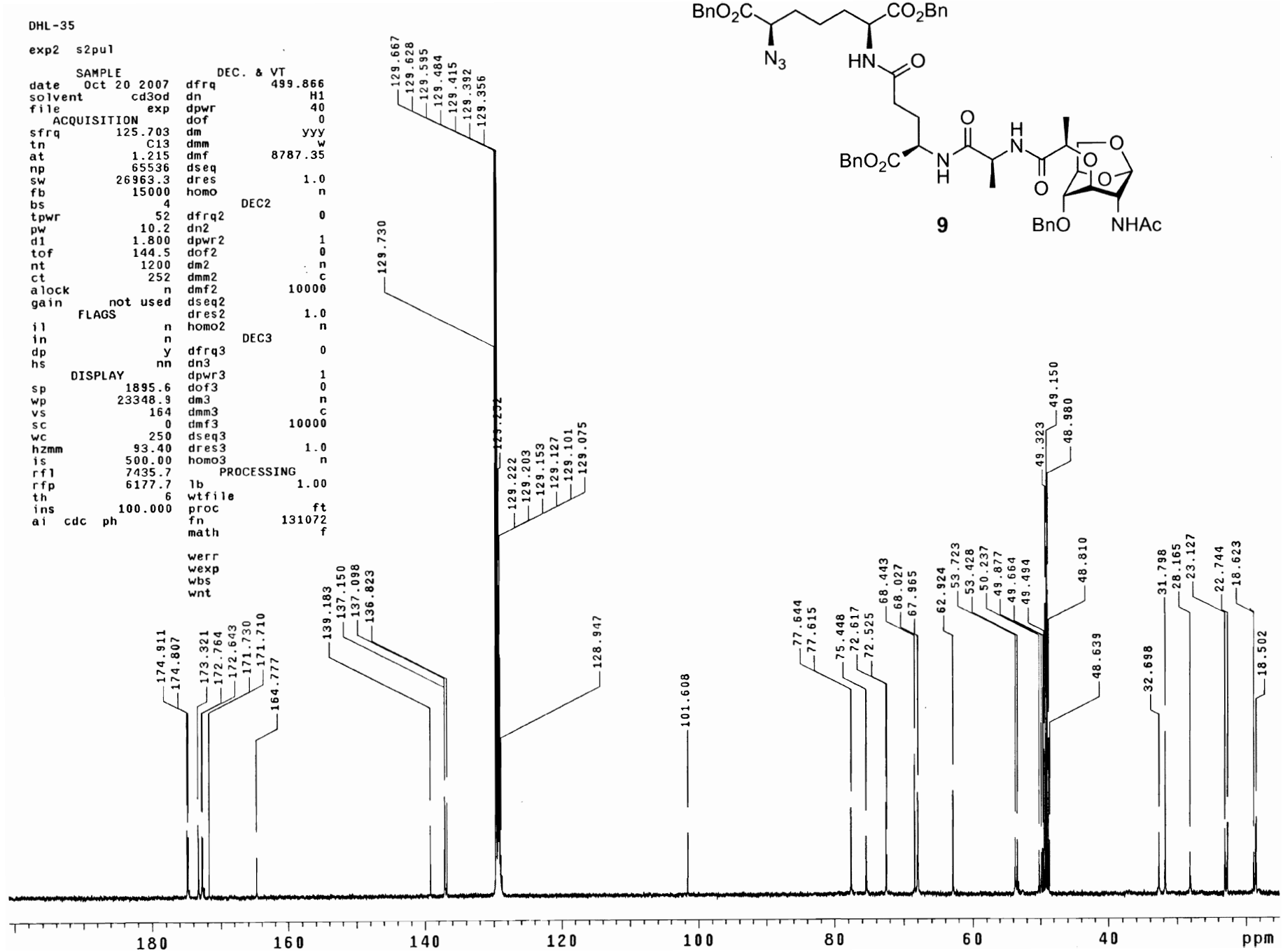


Figure S6. Two-H bonds in compound **11** are shown in dotted lines. One is the intramolecular H-bond between N1–H1N–O4 (blue) and the other is the intermolecular H-bond between O4–H4O–O8 (pink). A unit cell is shown in dotted lines (dark blue).

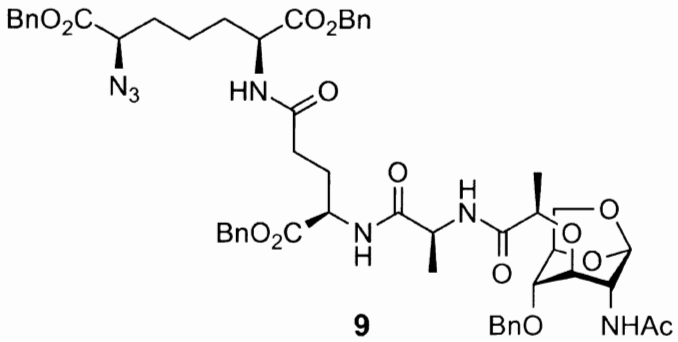

DHL-35
exp3 s2pul
SAMPLE
date Oct 20 2007
solvent CD3OD
file exp
ACQUISITION
sfrq 499.866
tn H1
at 5.016
np 65536
sw 6533.3
fb 4000
bs 4
tpwr 61
pw 13.5
d1 0.100
tof 269.9
nt 32
ct 32
alock n
gain not used
FLAGS
il n
in n
dp y
hs nn
DISPLAY
sp 464.5
wp 5039.1
vs 343
sc 0
wc 250
hzmm 20.16
is 200.00
rfl 2922.9
rfp 2434.3
th 7
ins 4.000
ai ph
DEC. & VT
dfrq 499.866
dn H1
dpwr 30
dof 0
dm nnn
dmm c
dmf 200
dseq
dres 1.0
homo n
DEC2
dfrq2 0
dn2
dpwr2 1
dof2 0
dm2 n
dmm2 c
dmf2 200
dseq2
dres2 1.0
homo2 n
DEC3
dfrq3 0
dn3
dpwr3 1
dof3 0
dm3 n
dmm3 c
dmf3 200
dseq3
dres3 1.0
homo3
PROCESSING
wtfile
proc f
fn 65536
math
werr
wexp process pll
wbs
wnt wf
BnO2C
CO2Bn
N3
HN
O
BnO2C
N
H
H
N
O
O
O
O
O
BnO
NHAc
9
10
9
8
7
6
5
4
3
2
ppm
16.54
0.43
5.55
2.49
2.71
1.38
1.13
0.55
1.05
1.95
0.45
1.90
0.86
1.33
2.07
4.00
7.18


DHL-35
exp2 s2pul
SAMPLE
date Oct 20 2007
solvent cd3od
file exp
ACQUISITION
sfrq 125.703
tn C13
at 1.215
np 65536
sw 26963.3
fb 15000
bs 4
tpwr 52
pw 10.2
d1 1.800
tof 144.5
nt 1200
ct 252
alock n
gain not used
FLAGS
il n
in n
dp y
hs nn
DISPLAY
sp 1895.6
wp 23348.9
vs 164
sc 0
wc 250
hzmm 93.40
is 500.00
rfl 7435.7
rfp 6177.7
th 6
ins 100.000
ai cdc ph
DEC. & VT
dfrq 499.866
dn H1
dpwr 40
dof 0
dm yyy
dmm w
dmf 8787.35
dseq
dres 1.0
homo n
DEC2
dfrq2 0
dn2
dpwr2 1
dof2 0
dm2 n
dmm2 c
dmf2 10000
dseq2
dres2 1.0
homo2 n
DEC3
dfrq3 0
dn3
dpwr3 1
dof3 0
dm3 n
dmm3 c
dmf3 10000
dseq3
dres3 1.0
homo3 n
PROCESSING
lb 1.00
wtfile
proc ft
fn 131072
math f
werr
wexp
wbs
wnt
9
BnO
NHAc
ppm

DHL-35

Pulse Sequence: relayh
Solvent: cd3od
Ambient temperature
INOVA-500 "nmr2a.chem.nd.edu"

Relax. delay 1.300 sec
COSY 90-90
Acq. time 0.149 sec
Width 3435.5 Hz
2D Width 3435.5 Hz
8 repetitions
516 increments
OBSERVE H1, 499.8631218 MHz
DATA PROCESSING
Sine bell 0.075 sec
F1 DATA PROCESSING
Sine bell 0.037 sec
FT size 1024 x 1024
Total time 1 hr, 45 min, 45 sec



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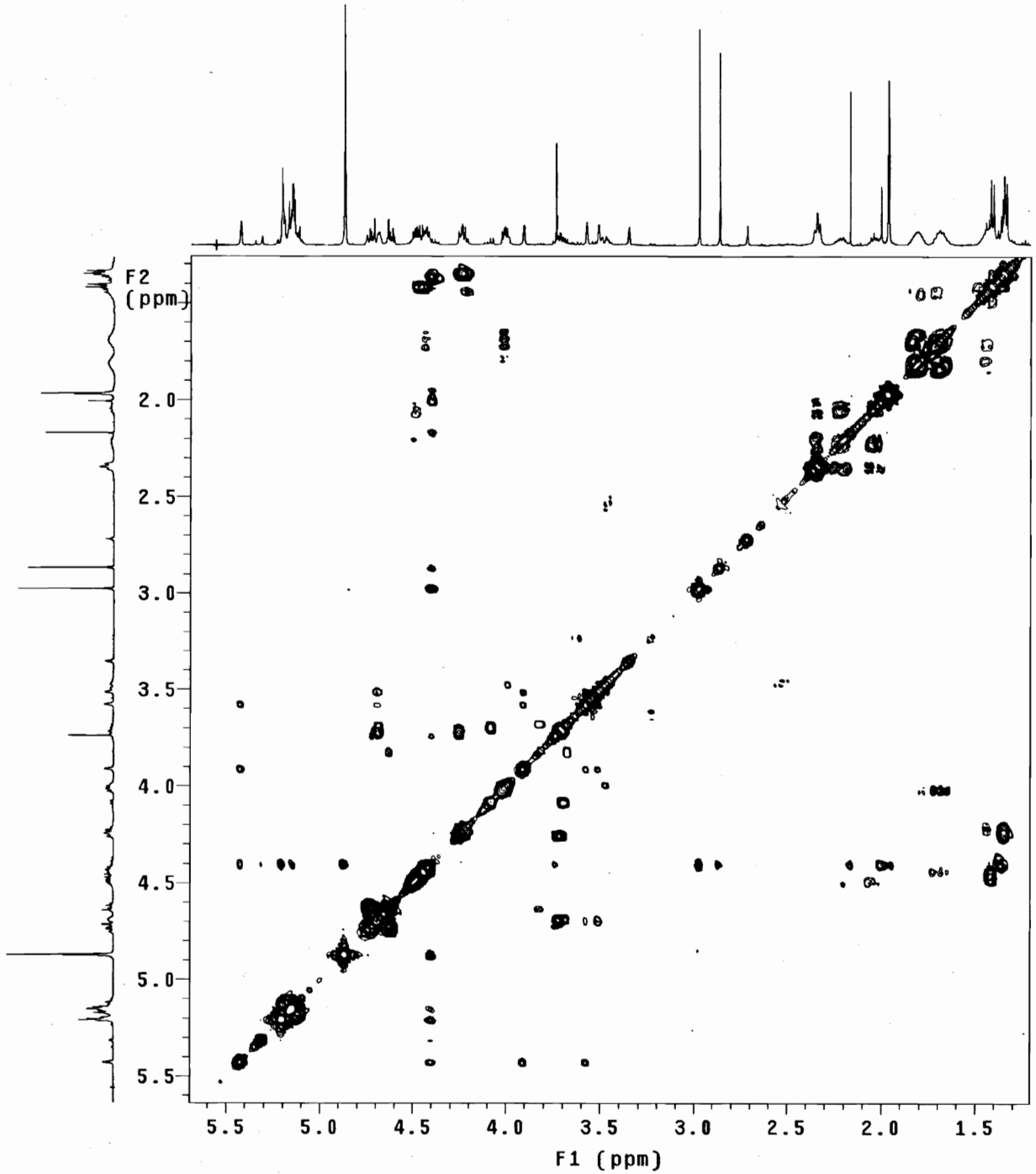
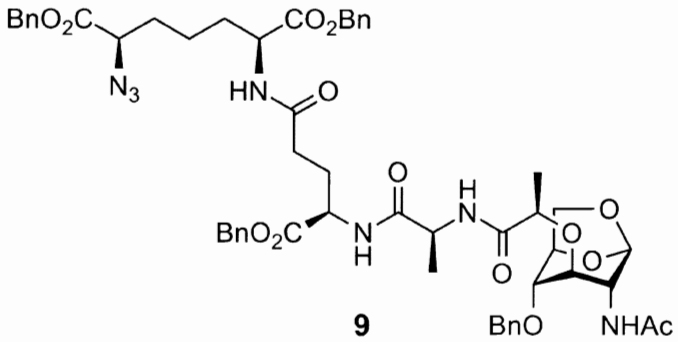


DHL-35

Pulse Sequence: relayh
Solvent: cd3od
Ambient temperature
INOVA-500 "nmr2a.chem.nd.edu"

Relax. delay 1.300 sec
COSY 90-90
Acq. time 0.149 sec
Width 3435.5 Hz
2D Width 3435.5 Hz
8 repetitions
516 increments
OBSERVE H1, 499.8631218 MHz
DATA PROCESSING
Sine bell 0.075 sec
F1 DATA PROCESSING
Sine bell 0.037 sec
FT size 1024 x 1024
Total time 1 hr, 45 min, 45 sec



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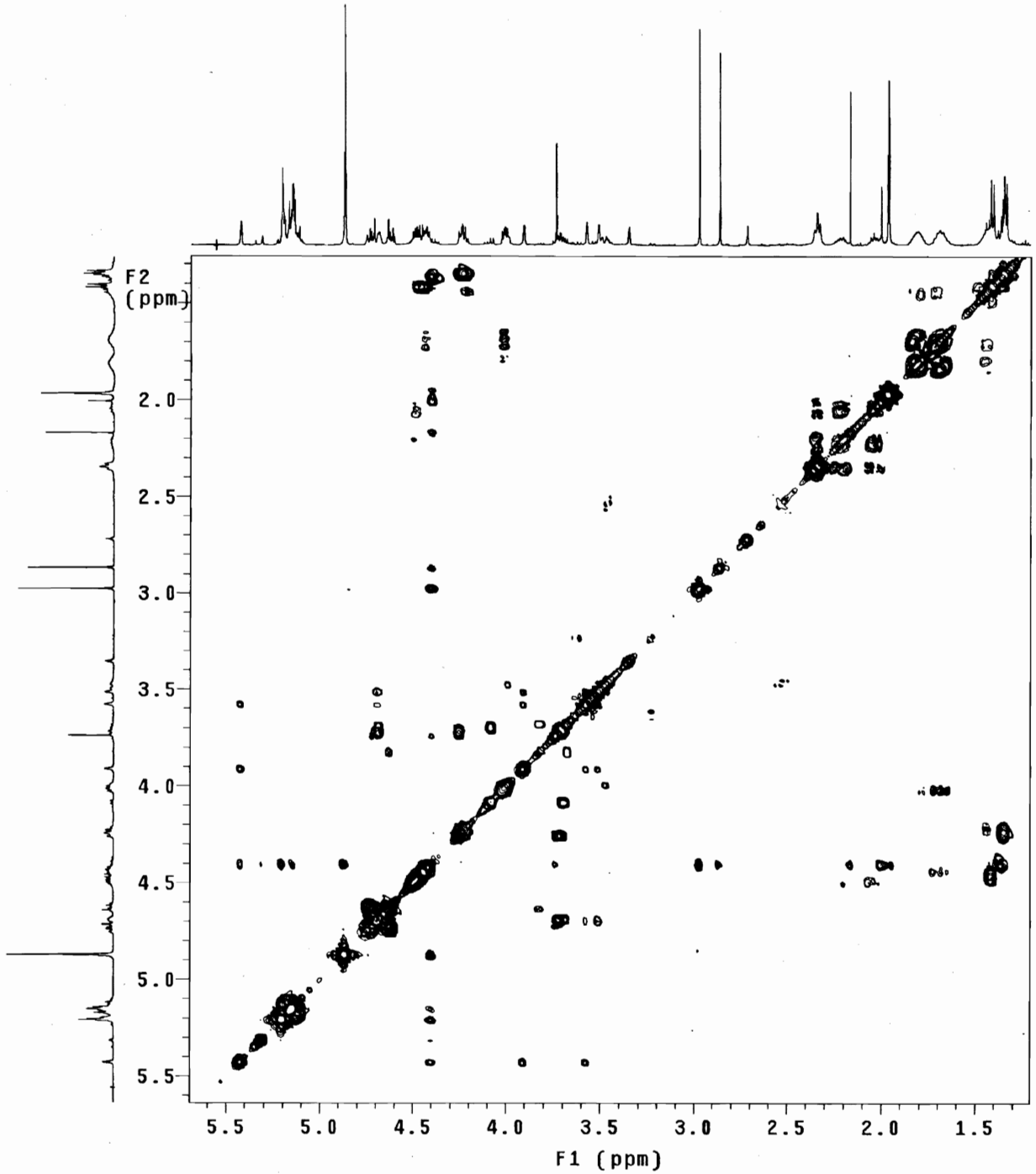
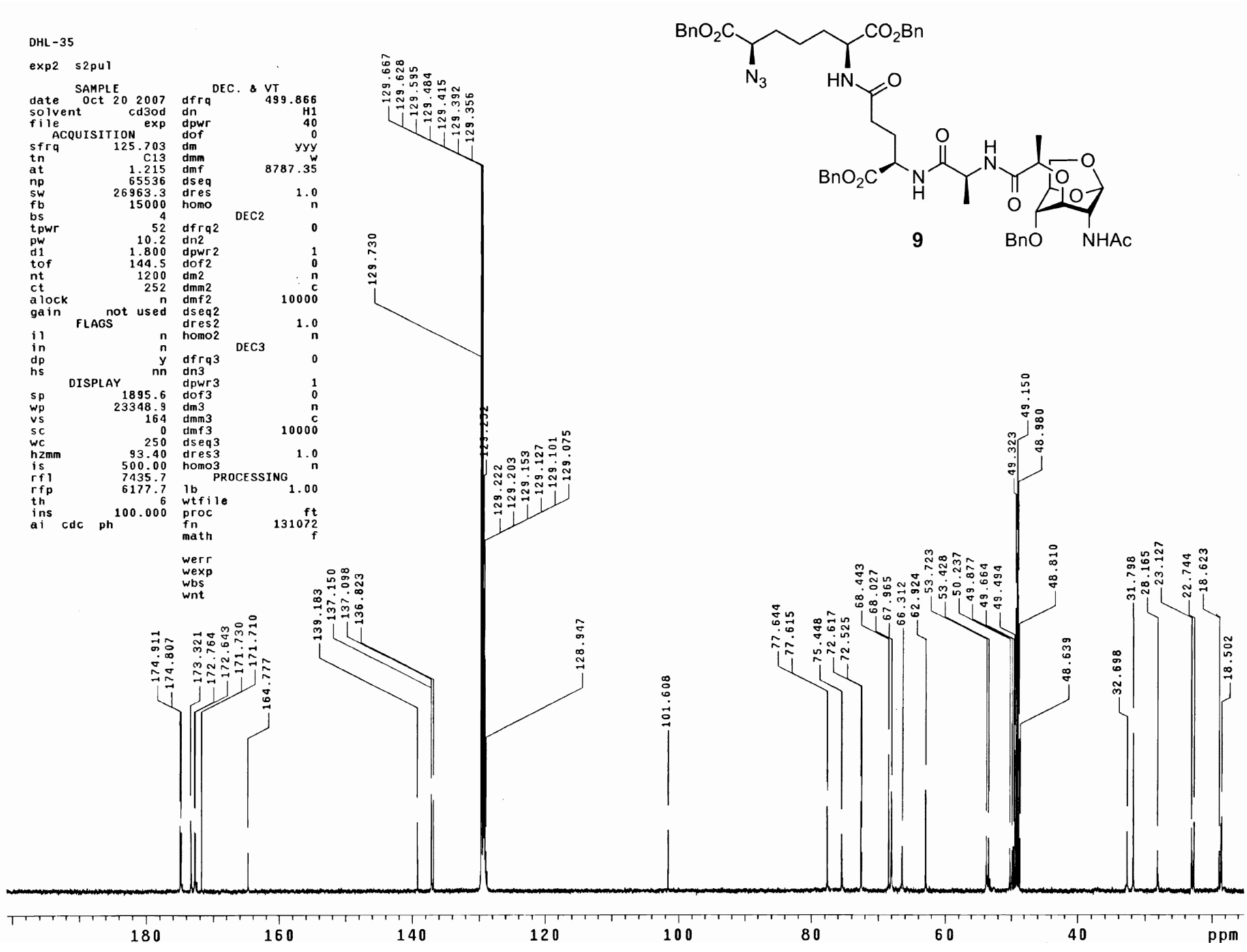
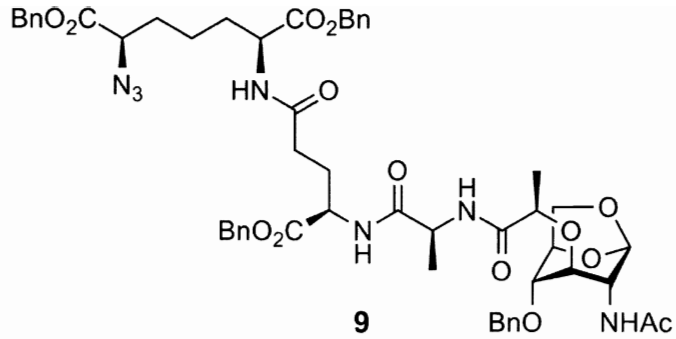



DHL-35
exp2 s2pul
SAMPLE
date Oct 20 2007
solvent cd3od
file exp
ACQUISITION
sfrq 125.703
tn C13
at 1.215
np 65536
sw 26963.3
fb 15000
bs 4
tpwr 52
pw 10.2
d1 1.800
tof 144.5
nt 1200
ct 252
alock n
gain not used
FLAGS
il n
in n
dp y
hs nn
DISPLAY
sp 1895.6
wp 23348.9
vs 164
sc 0
wc 250
hzmm 93.40
is 500.00
rfl 7435.7
rfp 6177.7
th 6
ins 100.000
ai cdc ph
DEC. & VT
dfrq 499.866
dn H1
dpwr 40
dof 0
dm yyy
dmm w
dmf 8787.35
dseq
dres 1.0
homo n
DEC2
dfrq2 0
dn2
dpwr2 1
dof2 0
dm2 n
dmm2 c
dmf2 10000
dseq2
dres2 1.0
homo2 n
DEC3
dfrq3 0
dn3
dpwr3 1
dof3 0
dm3 n
dmm3 c
dmf3 10000
dseq3
dres3 1.0
homo3 n
PROCESSING
lb 1.00
wtfile
proc ft
fn 131072
math f
werr
wexp
wbs
wnt
9
ppm

DHL-35

Pulse Sequence: dept



9

CH3 carbons

CH2 carbons

CH carbons

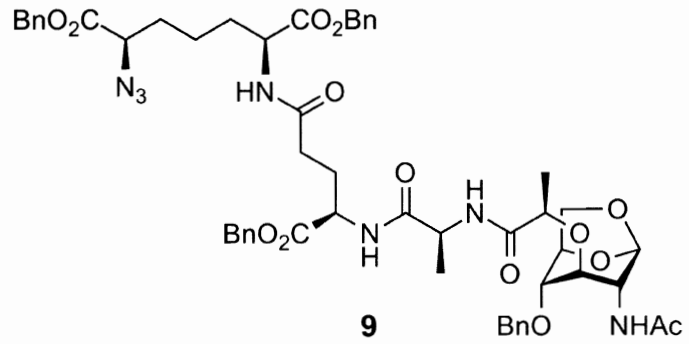
all protonated carbons

100 90 80 70 60 50 40 30 ppm

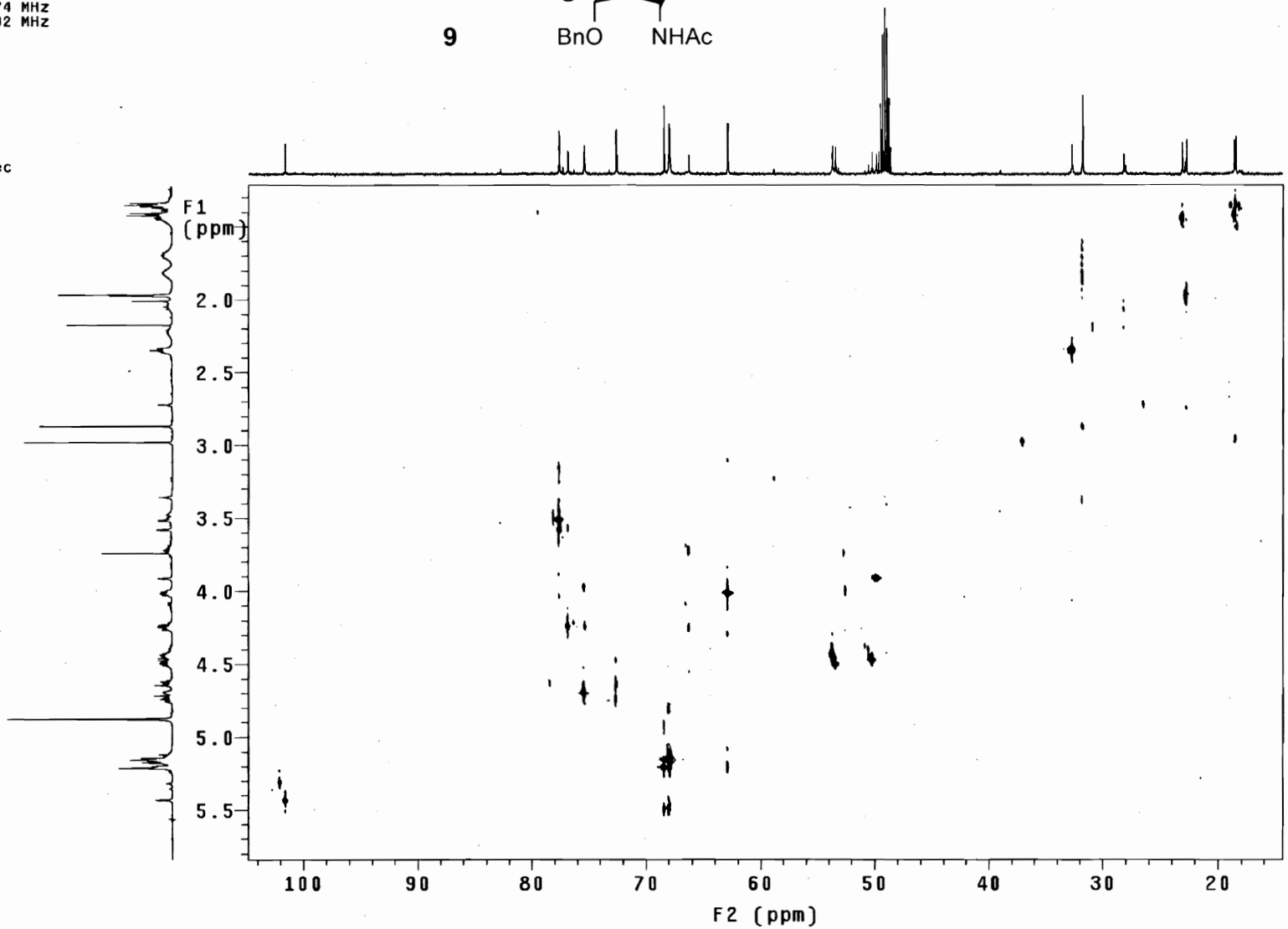
DHL-35

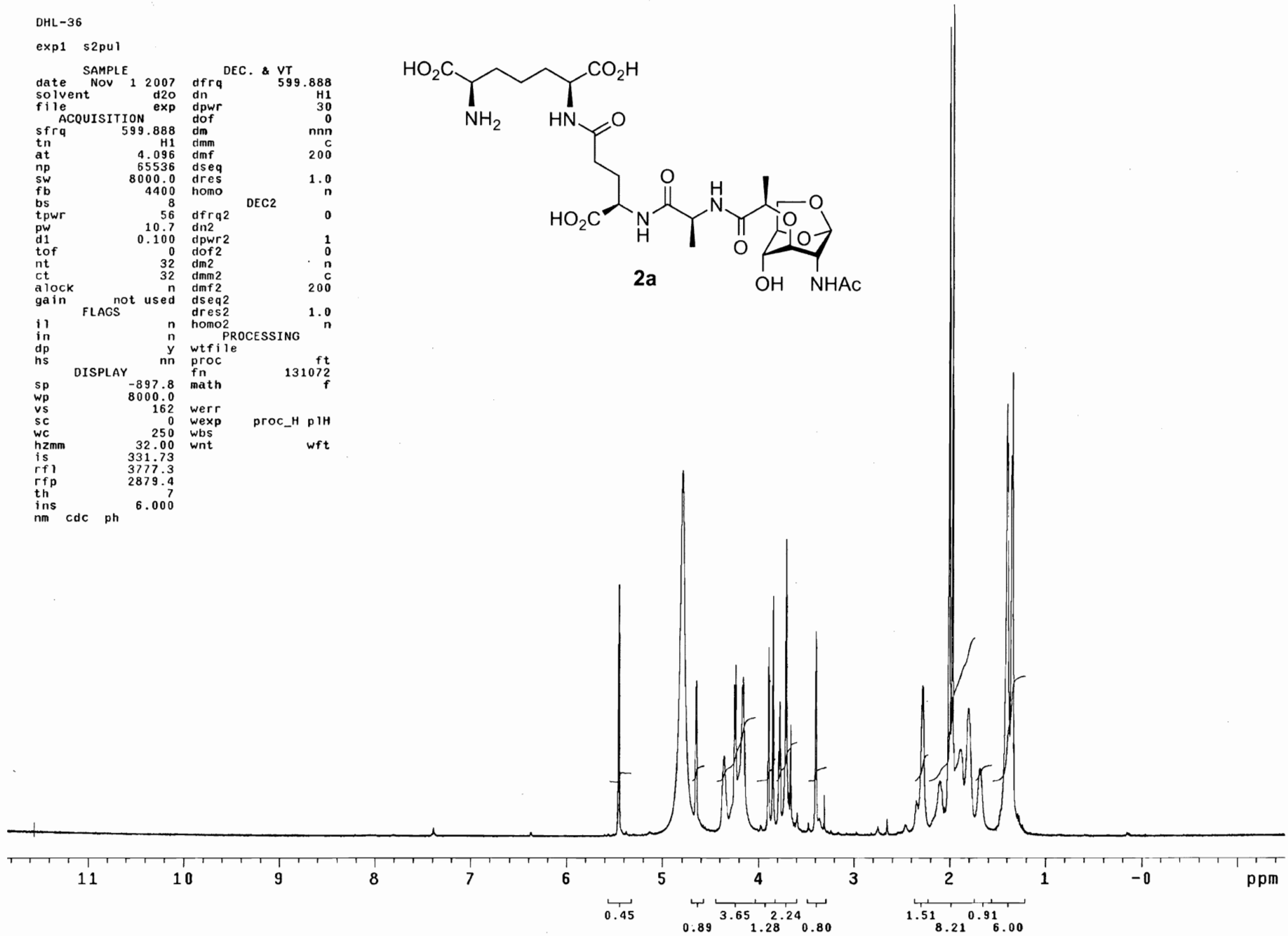
Pulse Sequence: hetcor
Solvent: cd3od
Ambient temperature
User: 1-14-87
INOVA-500 "nmr2a.chem.nd.edu"

Relax. delay 1.500 sec
Acq. time 0.111 sec
Width 18403.5 Hz
2D Width 3247.3 Hz
8 repetitions
256 increments
OBSERVE C13, 125.6905174 MHz
DECOUPLE H1, 499.8653292 MHz
Power 40 dB
on during acquisition
off during delay
WALTZ-16 modulated
DATA PROCESSING
Line broadening 1.0 Hz
F1 DATA PROCESSING
Line broadening 0.3 Hz
FT size 4096 x 512
Total time 57 min, 16 sec



9

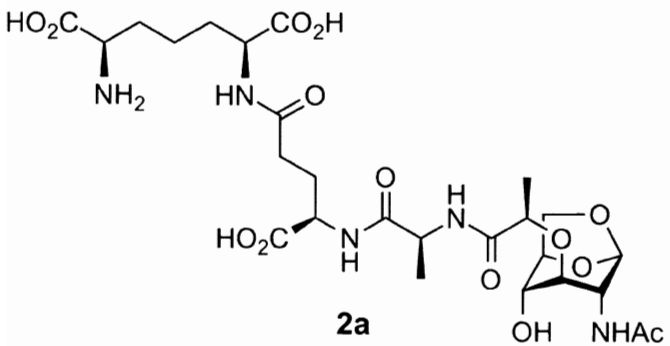



DHL-36
exp1 s2pul
SAMPLE
date Nov 1 2007
solvent d2o
file exp
ACQUISITION
sfrq 599.888
tn H1
at 4.096
np 65536
sw 8000.0
fb 4400
bs 8
tpwr 56
pw 10.7
d1 0.100
tof 0
nt 32
ct 32
alock n
gain not used
FLAGS
il n
in n
dp y
hs nn
DISPLAY
sp -897.8
wp 8000.0
vs 162
sc 0
wc 250
hzmm 32.00
is 331.73
rfl 3777.3
rfp 2879.4
th 7
ins 6.000
nm cdc ph
DEC. & VT
dfrq 599.888
dn H1
dpwr 30
dof 0
dm nnn
dmm c
dmf 200
dseq
dres 1.0
homo n
DEC2
dfrq2 0
dn2
dpwr2 1
dof2 0
dm2 n
dmm2 c
dmf2 200
dseq2
dres2 1.0
homo2 n
PROCESSING
wtfile
proc ft
fn 131072
math f
werr
wexp proc_H plH
wbs
wnt wft
HO2C
CO2H
NH2
HN
O
HO2C
N
H
H
N
O
O
O
O
O
OH
NHAc
2a
11
10
9
8
7
6
5
4
3
2
1
-0
ppm
0.45
0.89
3.65
1.28
2.24
0.80
1.51
8.21
0.91
6.00

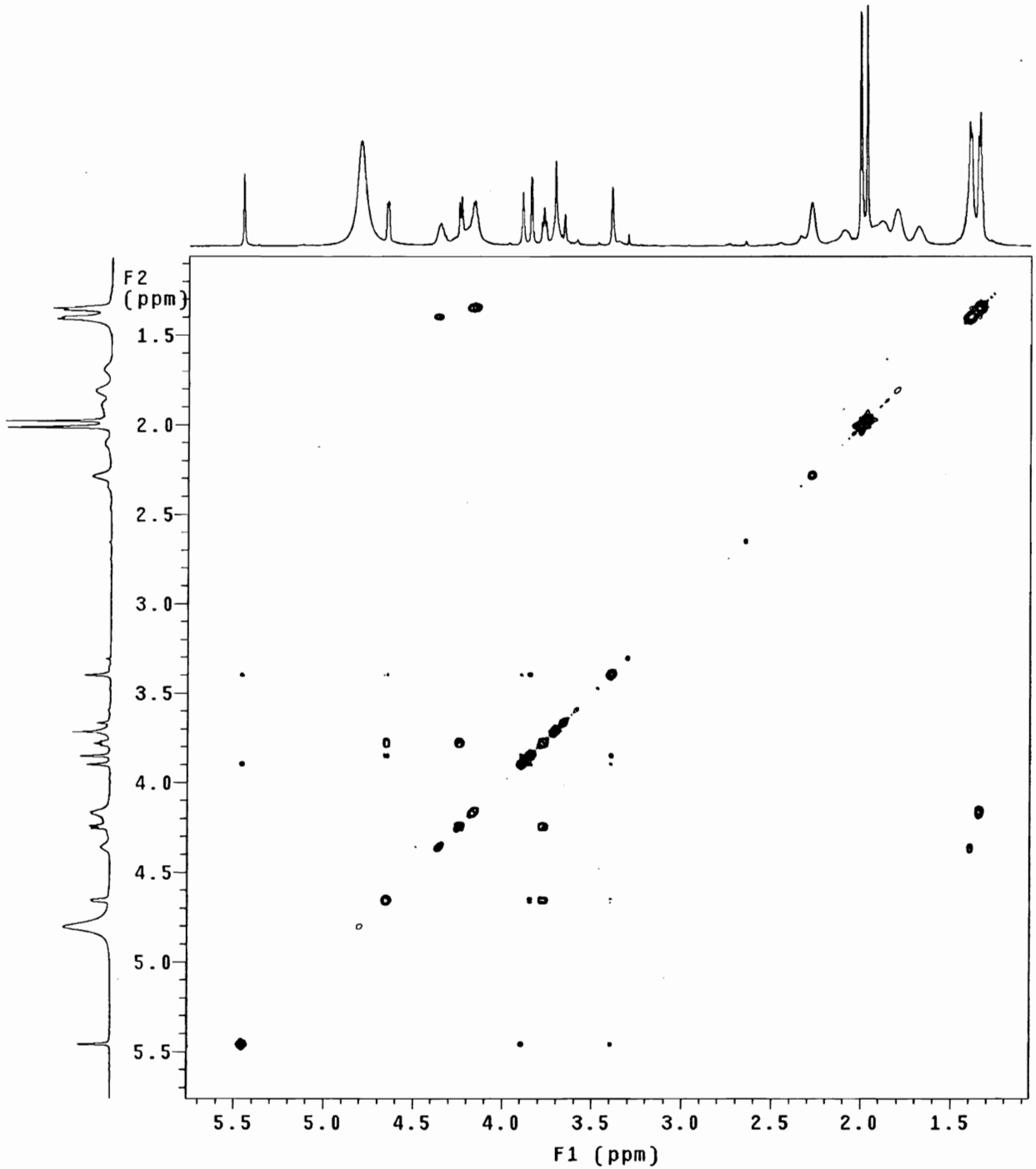
DHL-36

Pulse Sequence: relayh
Solvent: d2o
Ambient temperature
UNITYplus-600 "tesla"

Relax. delay 1.000 sec
COSY 90-90
Acq. time 0.182 sec
Width 2817.3 Hz
2D Width 2817.3 Hz
16 repetitions
512 increments
OBSERVE H1, 599.8853710 MHz
DATA PROCESSING
Sine bell 0.091 sec
F1 DATA PROCESSING
Sine bell 0.045 sec
FT size 1024 x 1024
Total time 2 hr, 55 min, 27 sec



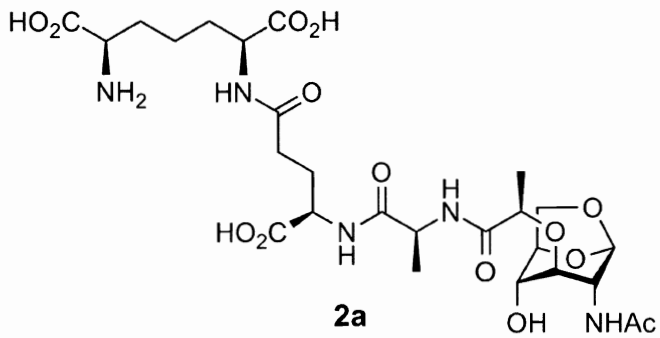
2a



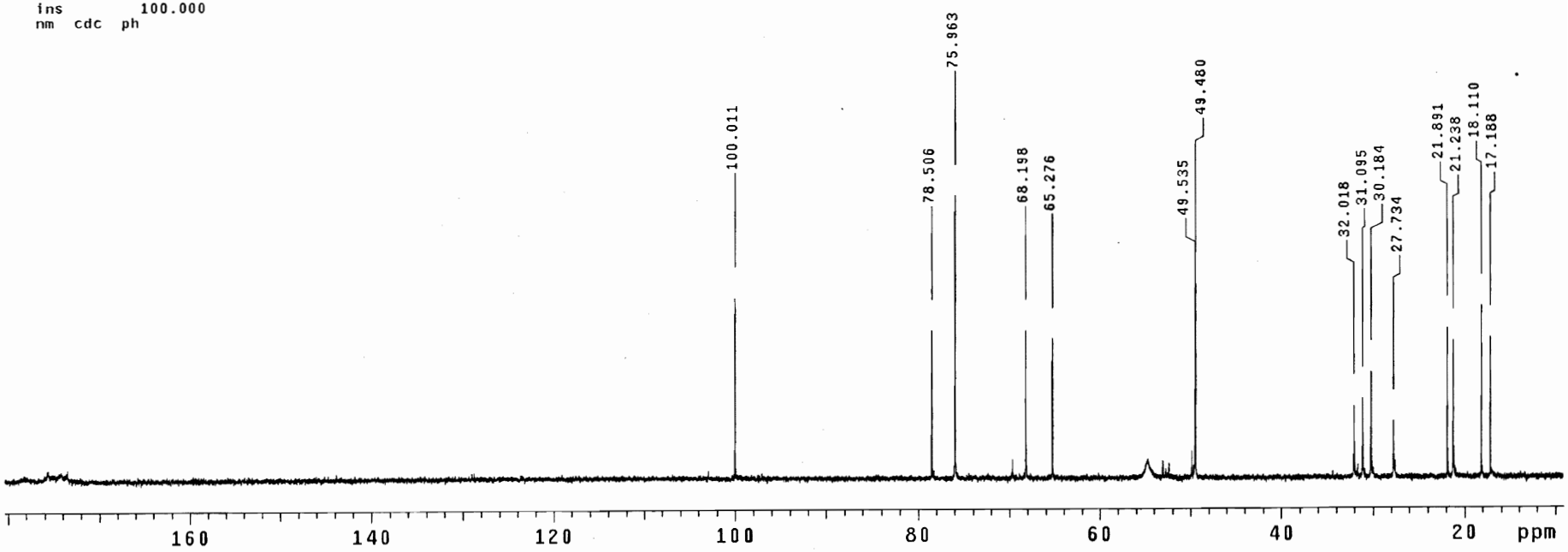
DHL-36

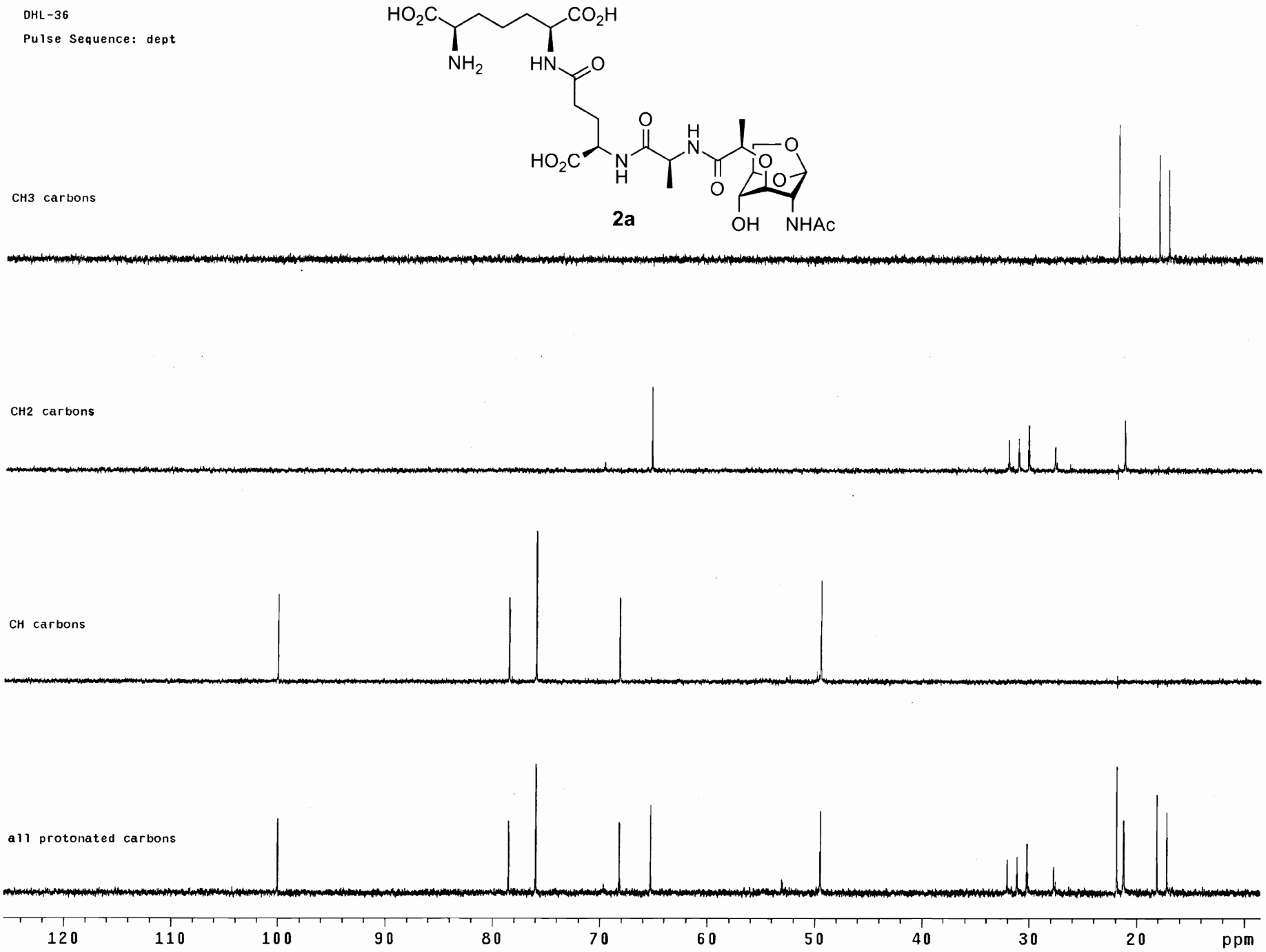
exp5 s2pul

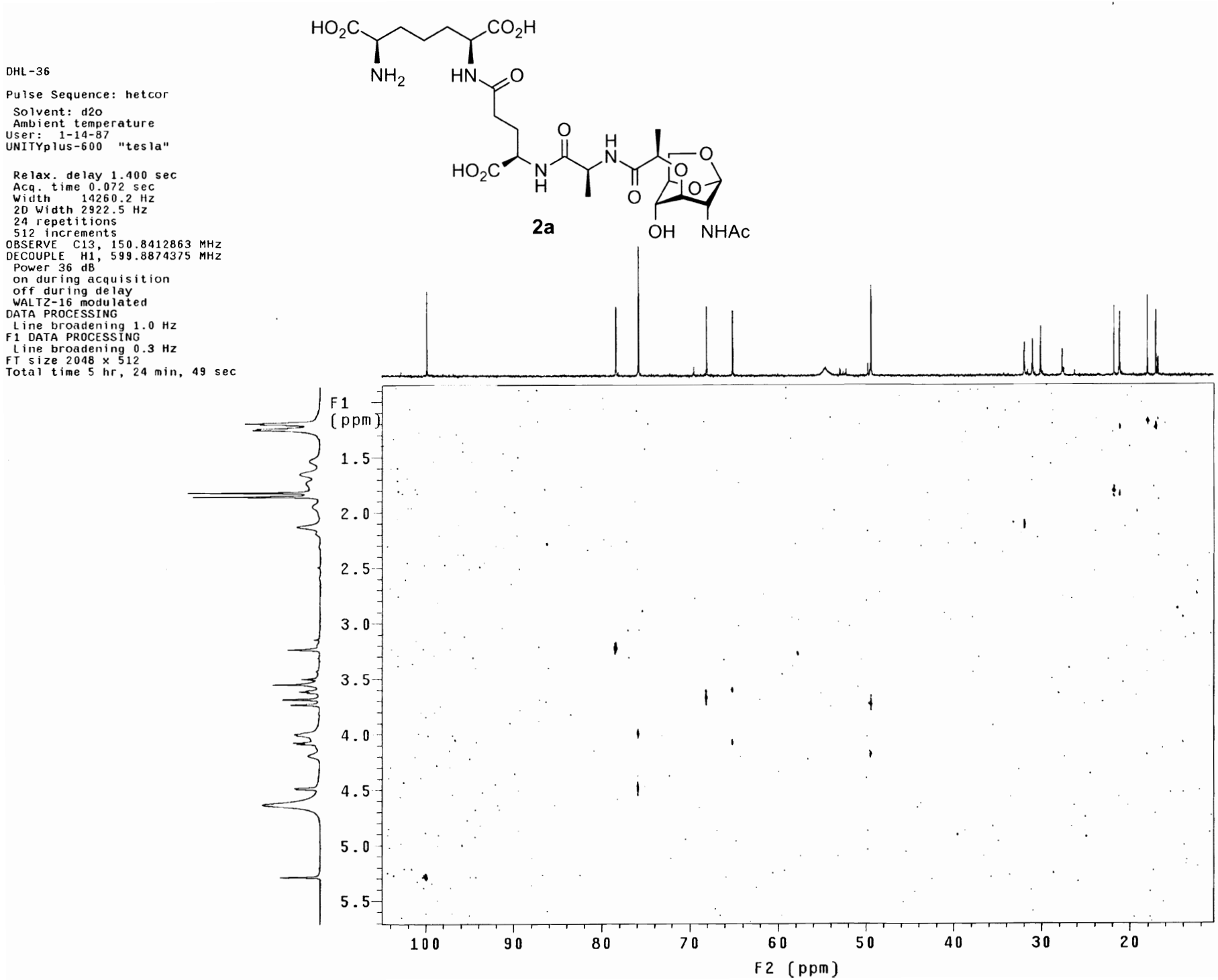
SAMPLE		DEC. & VT	
date	Nov 1 2007	dfrq	599.888
solvent	d2o	dn	H1
file	exp	dpwr	36
ACQUISITION		dof	0
sfrq	150.856	dm	yyy
tn	C13	dmm	w
at	0.963	dmf	9708
np	65536	dseq	
sw	34013.6	dres	1.0
fb	18800	homo	n
bs	4	DEC2	
tpwr	51	dfrq2	0
pw	8.0	dn2	
d1	2.000	dpwr2	1
tof	1576.9	dof2	0
nt	12000	dm2	n
ct	9342	dmm2	c
alock	n	dmf2	15202
gain	not used	dseq2	
FLAGS		dres2	1.0
il	n	homo2	n
in	n	PROCESSING	
dp	y	lb	1.00
hs	nn	wtfile	
DISPLAY		proc	ft
sp	1376.1	fn	131072
wp	25833.9	math	f
vs	45		
sc	0	werr	
wc	250	wexp	process p1C
hzmm	103.34	wbs	wft
is	500.00	wnt	
rfl	1931.1		
rfp	0		
th	6		
ins	100.000		
nm cdc ph			



2a



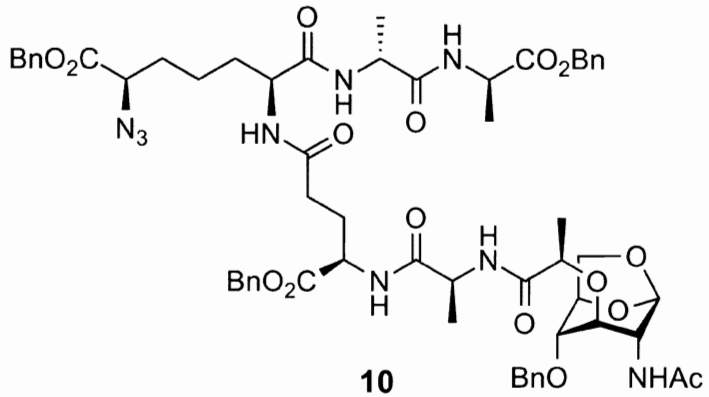

DHL-36
Pulse Sequence: dept
CH3 carbons
CH2 carbons
CH carbons
all protonated carbons
2a
120
110
100
90
80
70
60
50
40
30
20
ppm


DHL-36
Pulse Sequence: hetcor
Solvent: d2o
Ambient temperature
User: 1-14-87
UNITYplus-600 "tesla"
Relax. delay 1.400 sec
Acq. time 0.072 sec
Width 14260.2 Hz
2D Width 2922.5 Hz
24 repetitions
512 increments
OBSERVE C13, 150.8412863 MHz
DECOUPLE H1, 599.8874375 MHz
Power 36 dB
on during acquisition
off during delay
WALTZ-16 modulated
DATA PROCESSING
Line broadening 1.0 Hz
F1 DATA PROCESSING
Line broadening 0.3 Hz
FT size 2048 x 512
Total time 5 hr, 24 min, 49 sec
HO2C
CO2H
NH2
HN
O
HO2C
N
H
H
N
O
O
O
O
O
OH
NHAc
2a
F1 (ppm)
1.5
2.0
2.5
3.0
3.5
4.0
4.5
5.0
5.5
100
90
80
70
60
50
40
30
20
F2 (ppm)

DHL-42

exp7 Proton

SAMPLE		SPECIAL	
date	Jan 15 2008	temp	22.0
solvent	cd3cn	gain	not used
file	exp	spin	not used
ACQUISITION		hst	0.008
sw	9615.4	pw90	11.100
at	2.049	alfa	10.000
np	39396	**FLAGS**	
fb	4000	il	n
bs	32	in	n
ss	2	dp	y
d1	1.000	hs	nn
nt	16	**PROCESSING**	
ct	16	fn	65536
TRANSMITTER		**DISPLAY**	
tn	H1	sp	652.0
sfrq	599.880	wp	5281.3
tof	599.9	rfl	1208.4
tpwr	61	rfp	0
pw	5.550	rp	-192.9
DECOUPLER		lp	2.9
dn	C13	**PLOT**	
dof	0	wc	250
dm	nnn	sc	0
dmm	c	vs	328
dpwr	38	th	12
dmf	35088	ai cdc ph	



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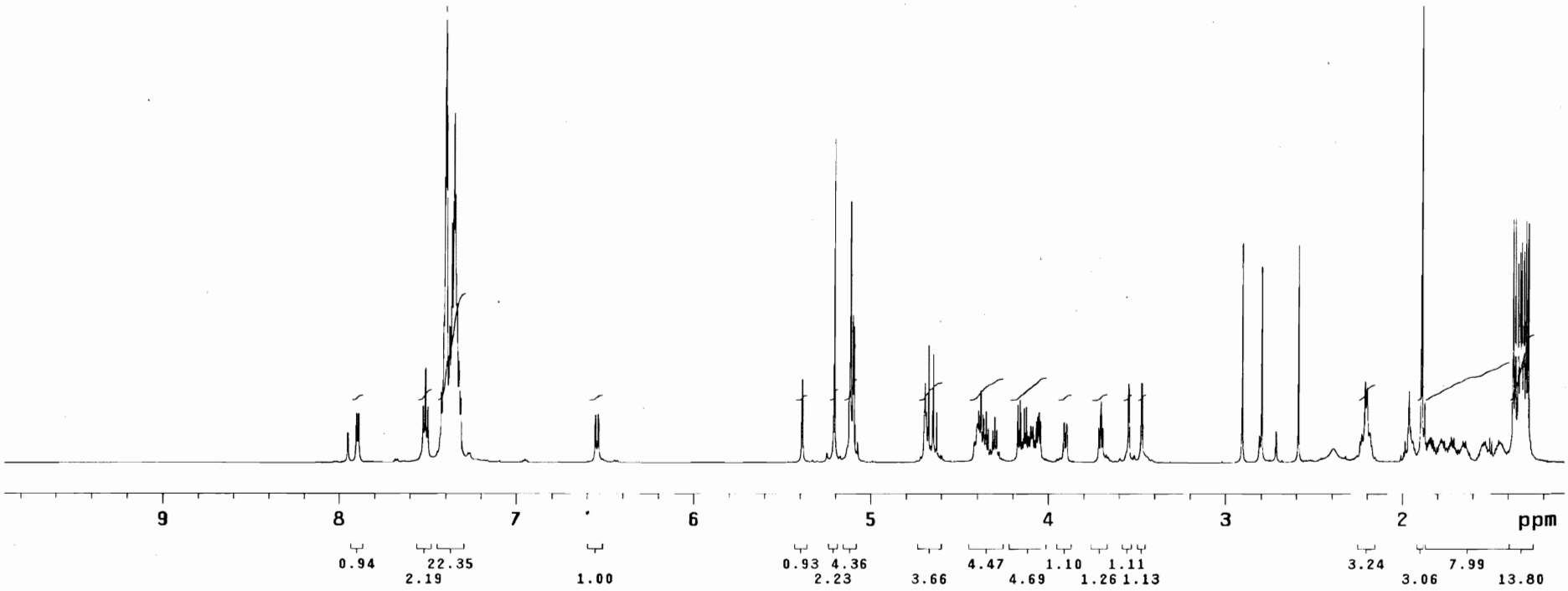


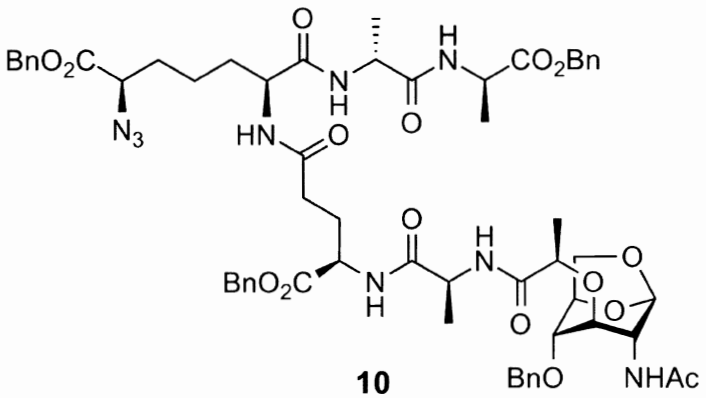
DHL-42

File: xp

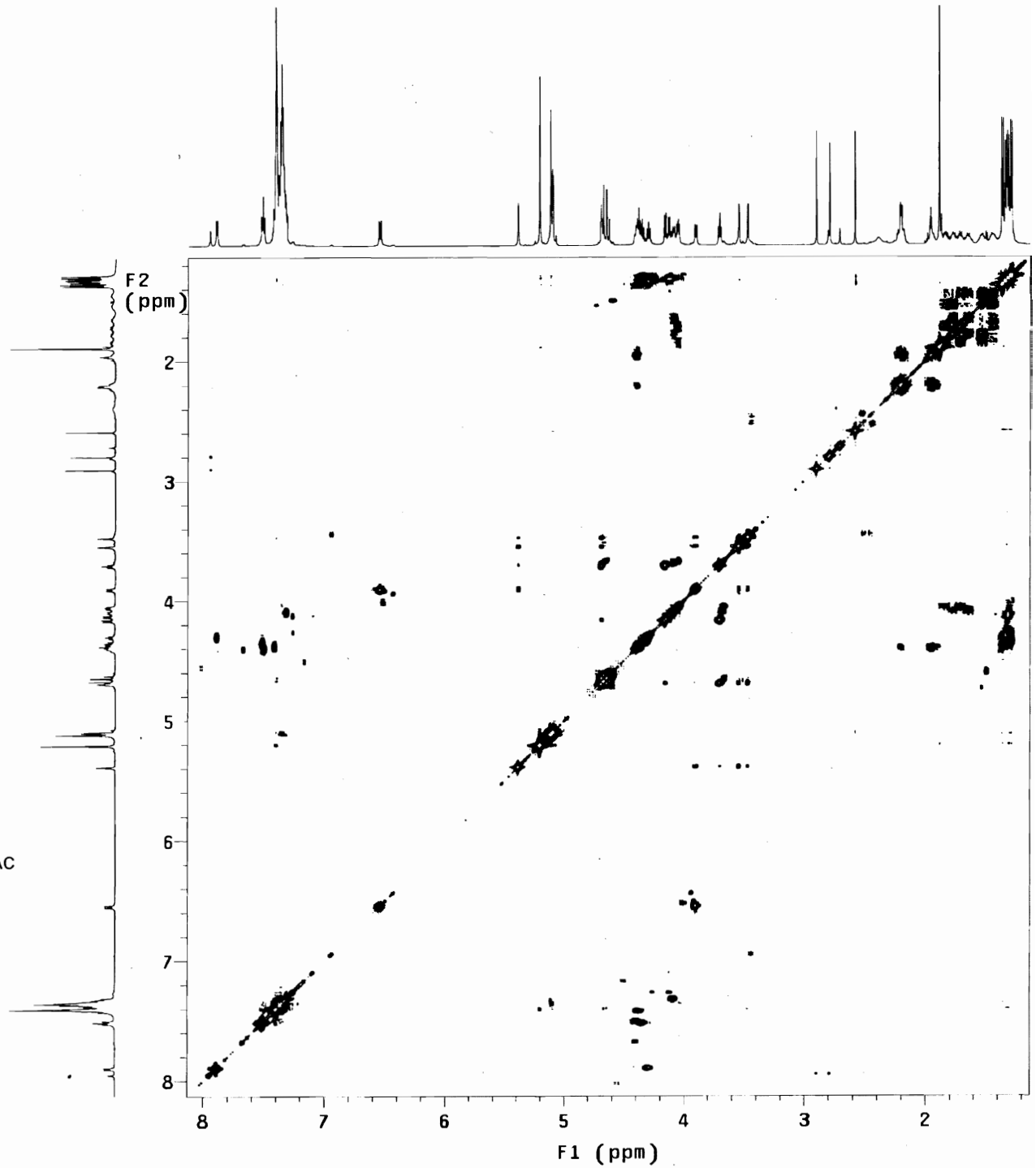
Pulse Sequence: gCOSY

Solvent: cd3cn
Temp. 22.0 C / 295.1 K
Operator: dhesek
VNMRS-600 "nmr600"

Relax. delay 1.000 sec
Acq. time 0.244 sec
Width 4194.6 Hz
2D Width 4194.6 Hz
8 repetitions
256 increments
OBSERVE H1, 599.8760308 MHz
DATA PROCESSING
Sine bell 0.122 sec
F1 DATA PROCESSING
Sine bell 0.122 sec
FT size 4096 x 4096
Total time 44 min, 26 sec



10



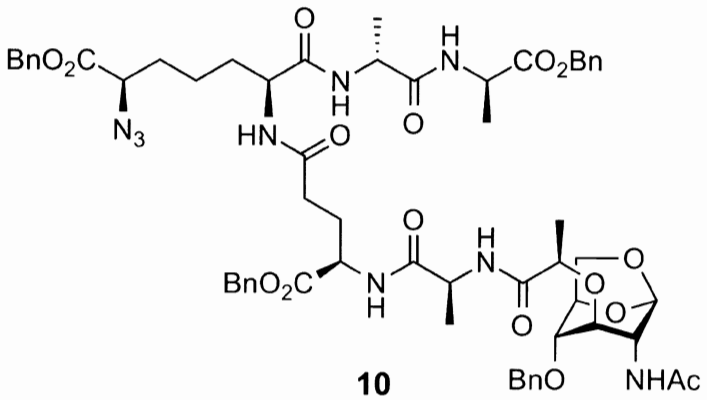
DHL-42

File: xp

Pulse Sequence: gCOSY

Solvent: cd3cn
Temp. 22.0 C / 295.1 K
Operator: dhesek
VNMRS-600 "nmr600"

Relax. delay 1.000 sec
Acq. time 0.244 sec
Width 4194.6 Hz
2D Width 4194.6 Hz
8 repetitions
256 increments
OBSERVE H1, 599.8760308 MHz
DATA PROCESSING
Sine bell 0.122 sec
F1 DATA PROCESSING
Sine bell 0.122 sec
FT size 4096 x 4096
Total time 44 min, 26 sec



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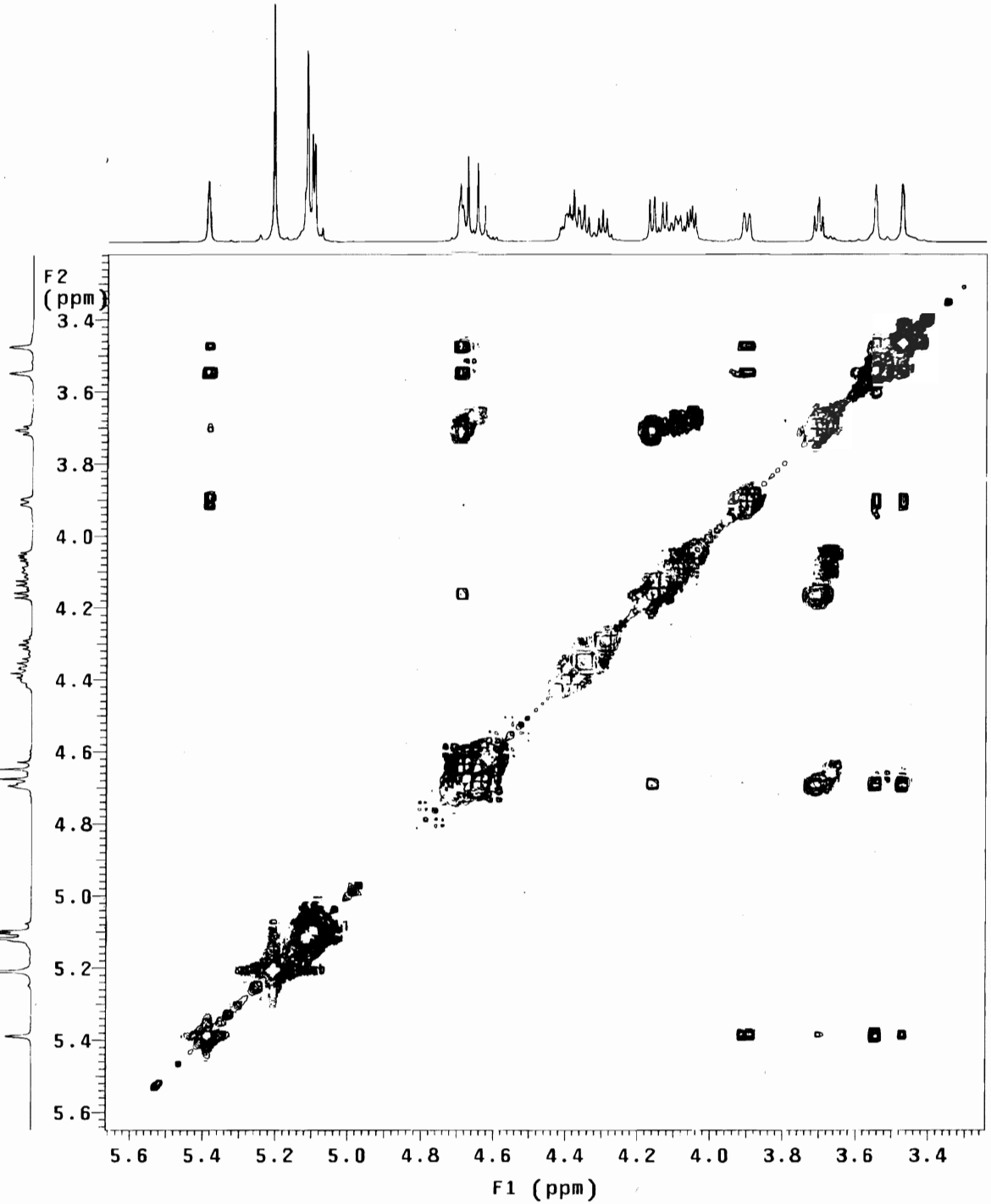
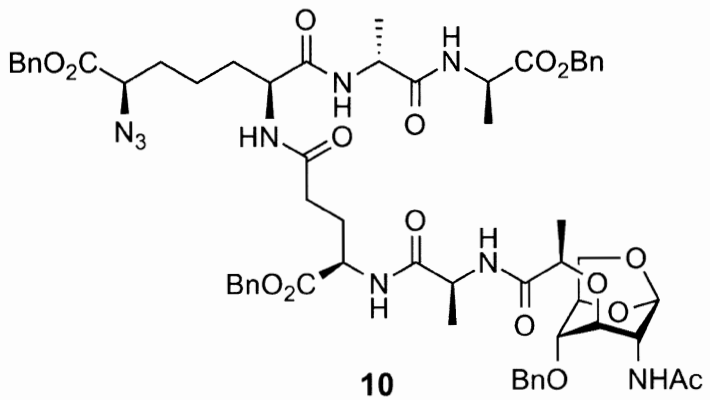


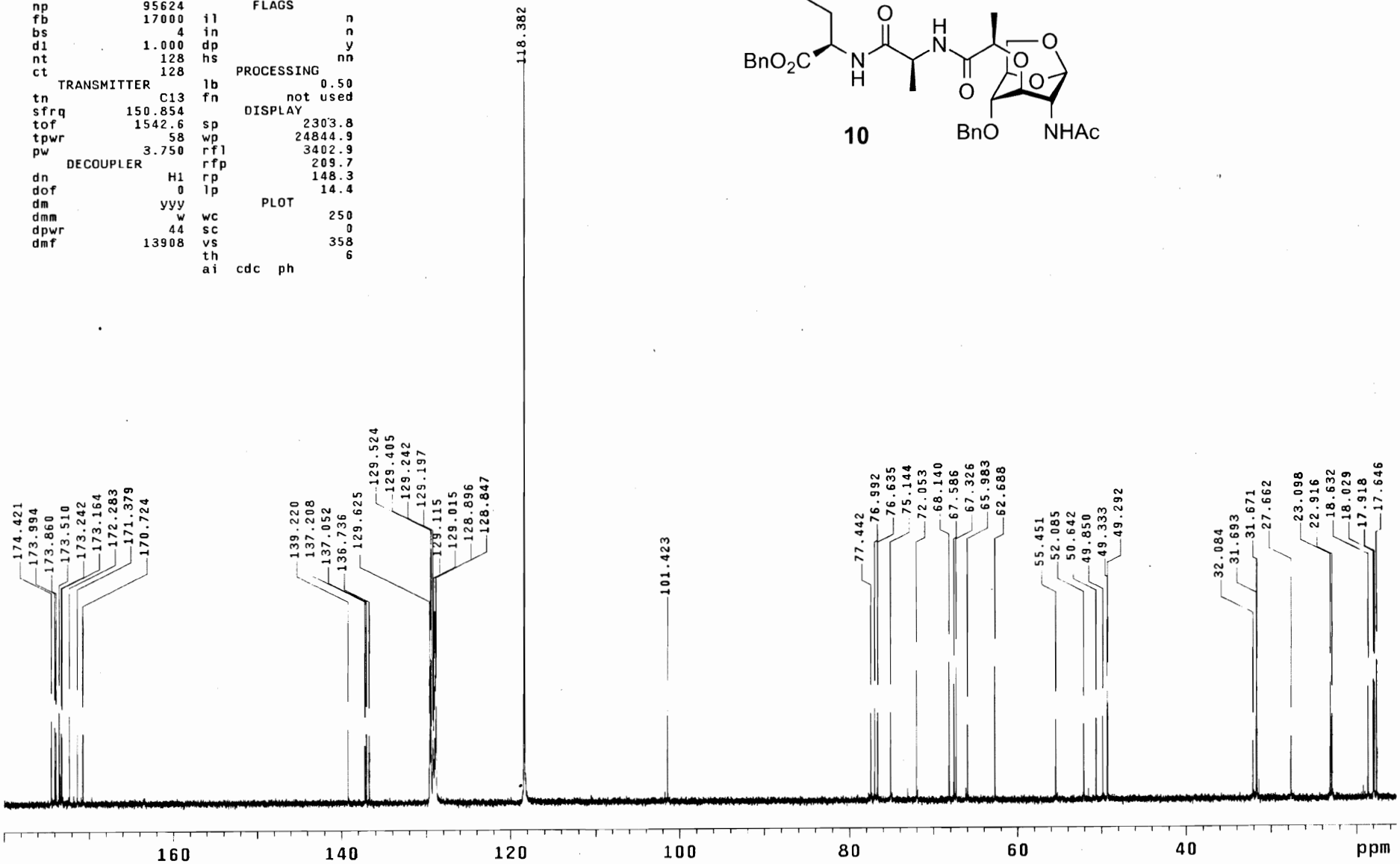
DHL-42

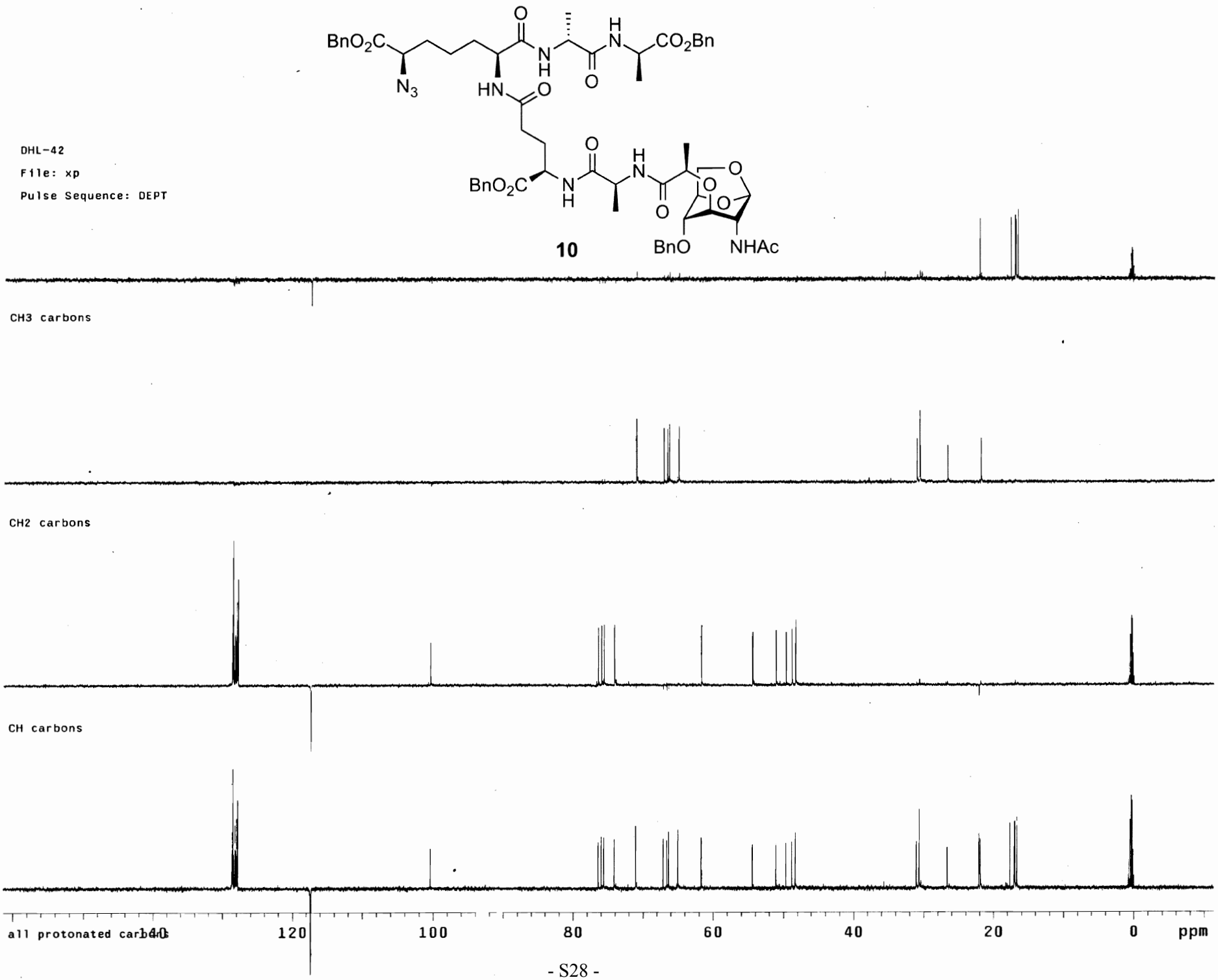
exp2 Carbon

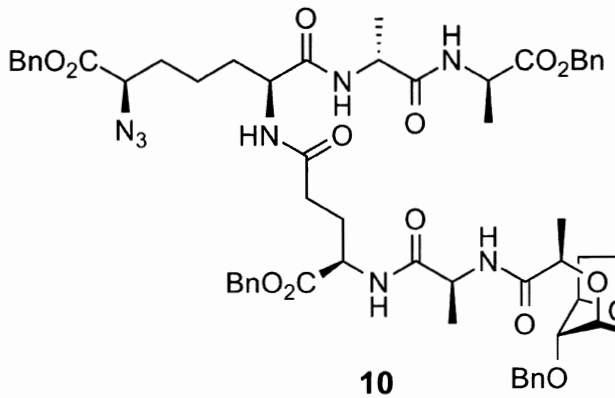
SAMPLE		SPECIAL	
date	Jan 18 2008	temp	22.0
solvent	CDCl3	gain	not used
file	exp	spin	not used
ACQUISITION		hst	0.008
sw	36764.7	pw90	7.500
at	1.300	alfa	10.000
np	95624	FLAGS	
fb	17000	il	n
bs	4	in	n
d1	1.000	dp	y
nt	128	hs	nn
ct	128	PROCESSING	
TRANSMITTER		lb	0.50
tn	C13	fn	not used
sfrq	150.854	DISPLAY	
tof	1542.6	sp	2303.8
tpwr	58	wp	24844.9
pw	3.750	rfl	3402.9
DECOUPLER		rfp	209.7
dn	H1	rp	148.3
dof	0	lp	14.4
dm	yyy	PLOT	
dmm	w	wc	250
dpwr	44	sc	0
dmf	13908	vs	358
		th	6
		ai	cdc ph



10




DHL-42
File: xp
Pulse Sequence: DEPT
10
CH3 carbons
CH2 carbons
CH carbons
all protonated carbons
140
120
100
80
60
40
20
0
ppm



10

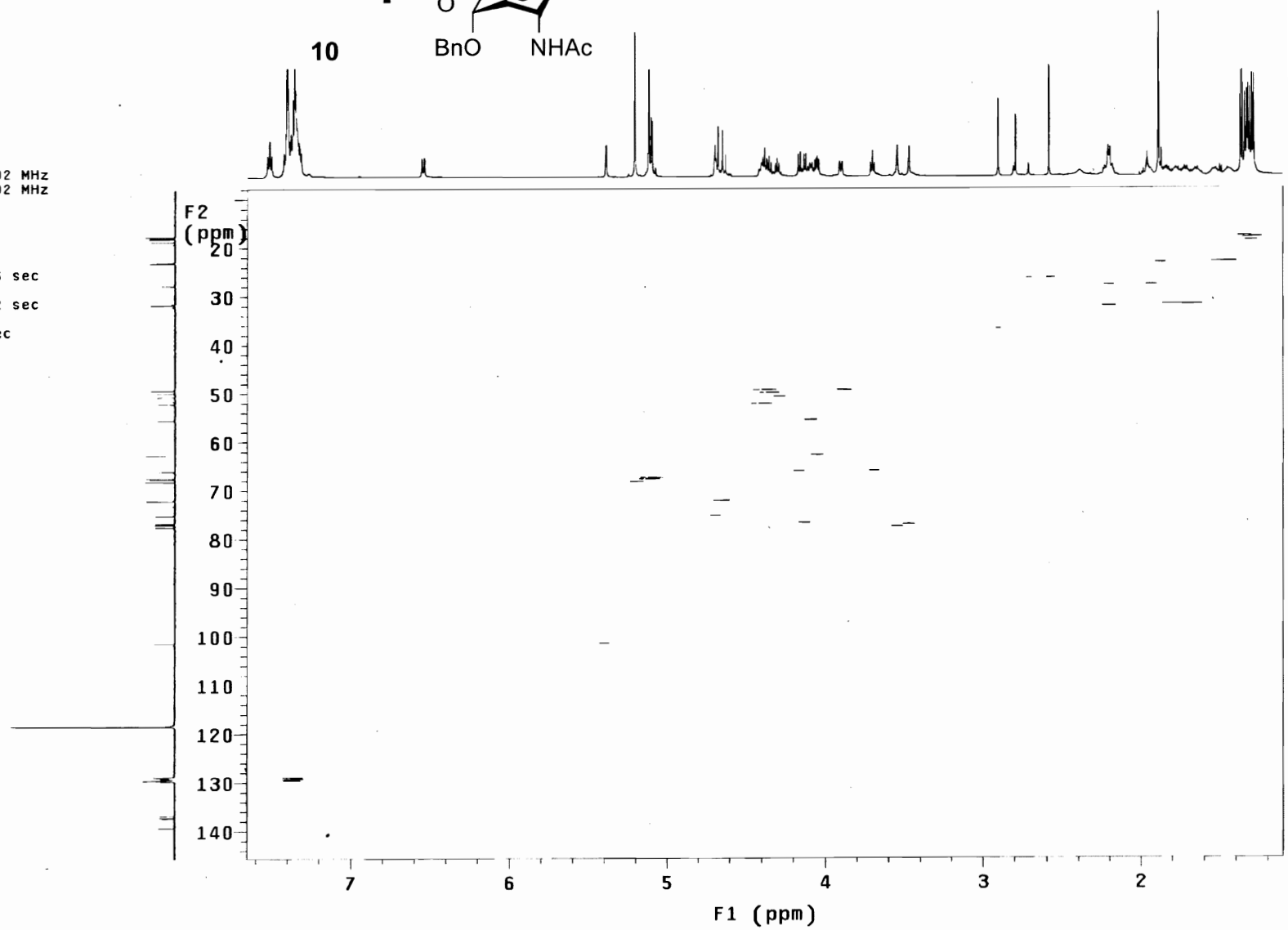
DHL-42

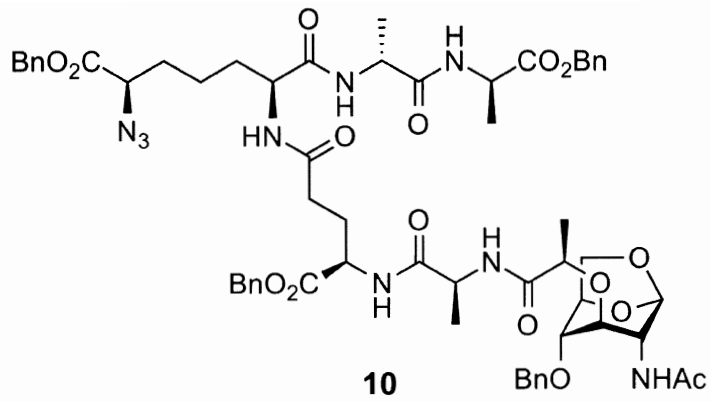
File: xp

Pulse Sequence: HETCOR

Solvent: cd3cn
Temp. 22.0 C / 295.1 K
Operator: dhesek
VNMRS-600 "nmr600"

Relax. delay 1.313 sec
Acq. time 0.187 sec
Width 21929.8 Hz
2D Width 5398.9 Hz
8 repetitions
2 x 128 increments
OBSERVE C13, 150.8387992 MHz
DECOUPLE H1, 599.8787302 MHz
Power 44 dB
on during acquisition
off during delay
WALTZ-16 modulated
DATA PROCESSING
Gauss apodization 0.086 sec
F1 DATA PROCESSING
Gauss apodization 0.022 sec
FT size 8192 x 2048
Total time 52 min, 33 sec





10

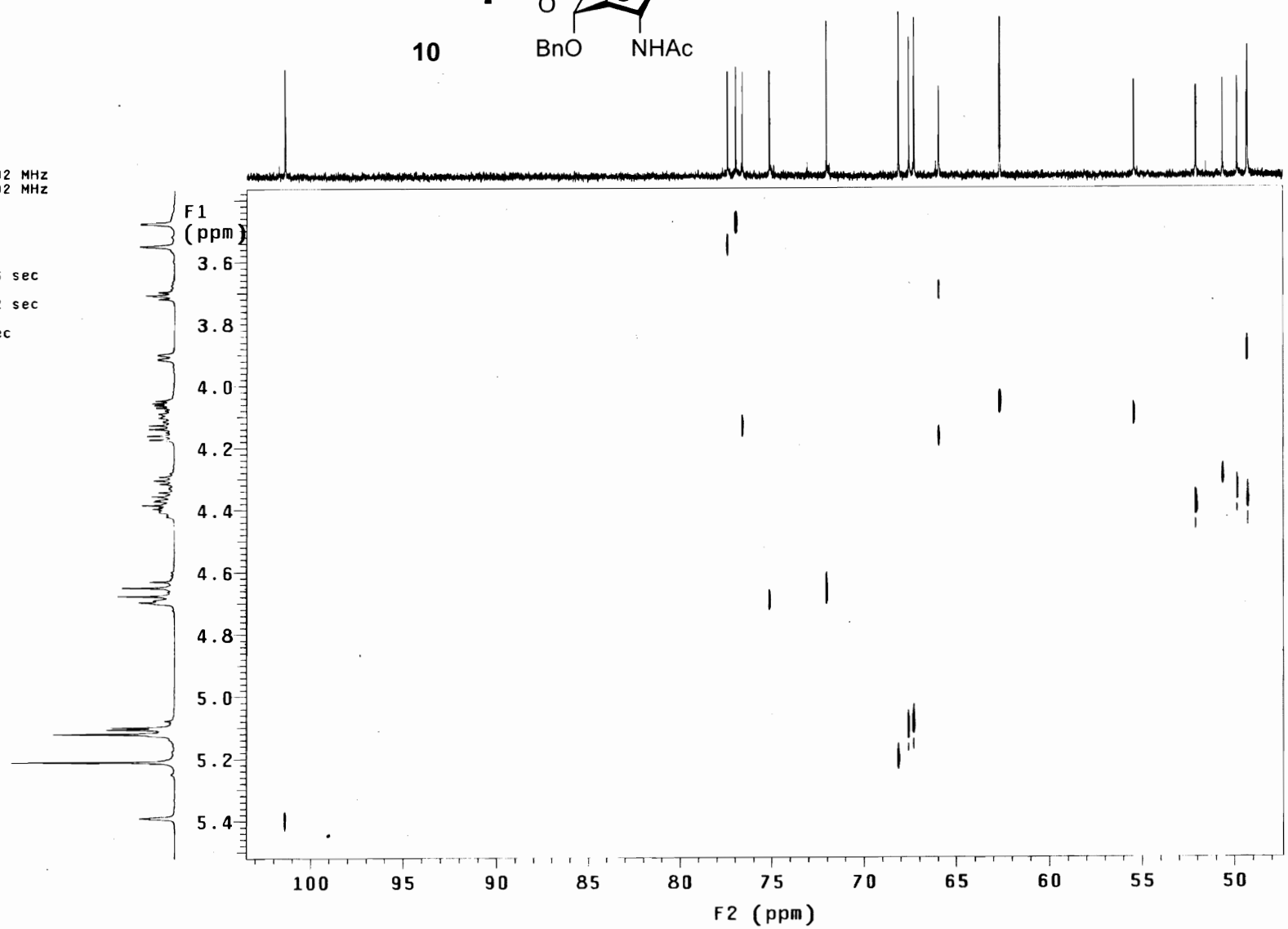
DHL-42

File: xp

Pulse Sequence: HETCOR

Solvent: cd3cn
Temp. 22.0 C / 295.1 K
Operator: dhesek
VNMRS-600 "nmr600"

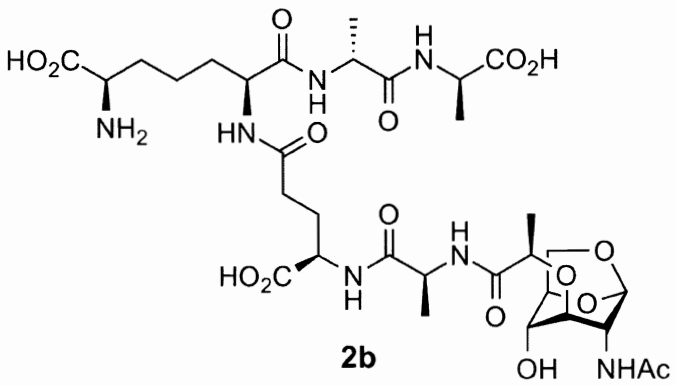
Relax. delay 1.313 sec
Acq. time 0.187 sec
Width 21929.8 Hz
2D Width 5398.9 Hz
8 repetitions
2 x 128 increments
OBSERVE C13, 150.8387992 MHz
DECOUPLE H1, 599.8787302 MHz
Power 44 dB
on during acquisition
off during delay
WALTZ-16 modulated
DATA PROCESSING
Gauss apodization 0.086 sec
F1 DATA PROCESSING
Gauss apodization 0.022 sec
FT size 8192 x 2048
Total time 52 min, 33 sec



DHL-51

exp1 Proton

SAMPLE		SPECIAL	
date	Feb 16 2008	temp	22.0
solvent	cd3od	gain	not used
file	exp	spin	not used
ACQUISITION		hst	0.008
sw	9615.4	pw90	11.100
at	3.408	alfa	10.000
np	65536	**FLAGS**	
fb	4000	il	n
bs	4	in	n
ss	2	dp	y
d1	1.000	hs	nn
nt	16	**PROCESSING**	
ct	16	fn	131072
TRANSMITTER		**DISPLAY**	
tn	H1	sp	257.7
sfrq	599.879	wp	4455.3
tof	599.8	rfl	1208.5
tpwr	61	rfp	0
pw	11.100	rp	-43.9
DECOUPLER		lp	3.3
dn	C13	**PLOT**	
dof	0	wc	250
dm	nnn	sc	0
dmm	c	vs	200
dpwr	38	th	50
dmf	35088	ai cdc ph	



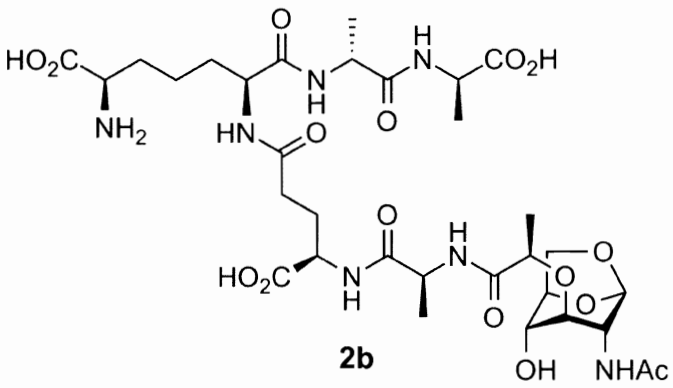
2b

DHL-51

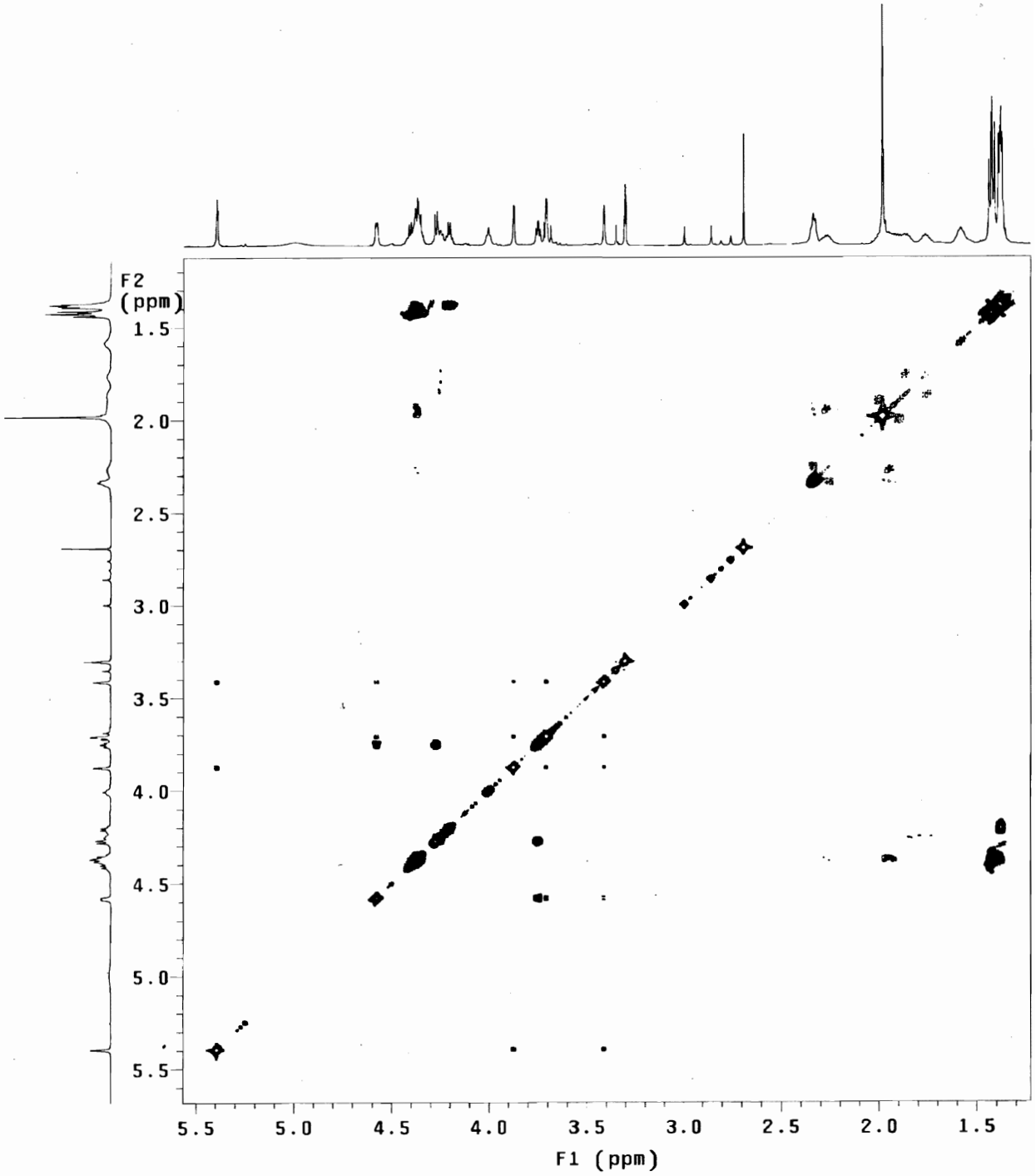
File: xp

Pulse Sequence: gCOSY
Solvent: cd3od
Temp. 22.0 C / 295.1 K
Operator: vnmr1
VNMRS-600 "nmr600"

Relax. delay 1.300 sec
Acq. time 0.213 sec
Width 5411.3 Hz
2D Width 5411.3 Hz
32 repetitions
256 increments
OBSERVE H1, 599.8752210 MHz
DATA PROCESSING
Sine bell 0.107 sec
F1 DATA PROCESSING
Sine bell 0.095 sec
FT size 4096 x 4096
Total time 3 hr, 32 min, 33 sec



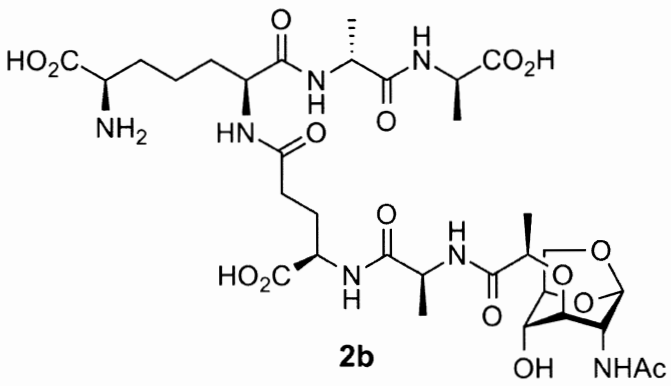
2b



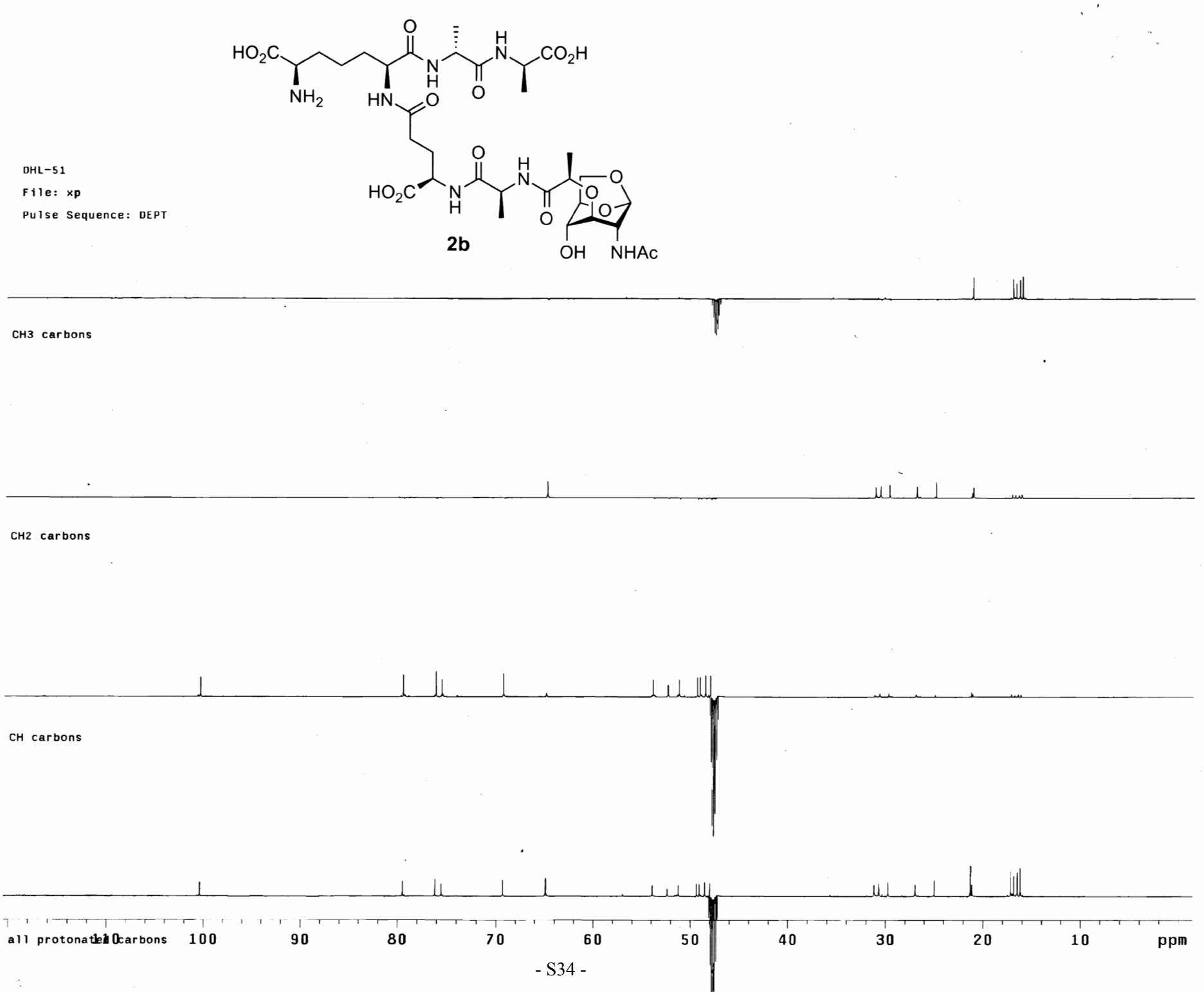
DHL-51

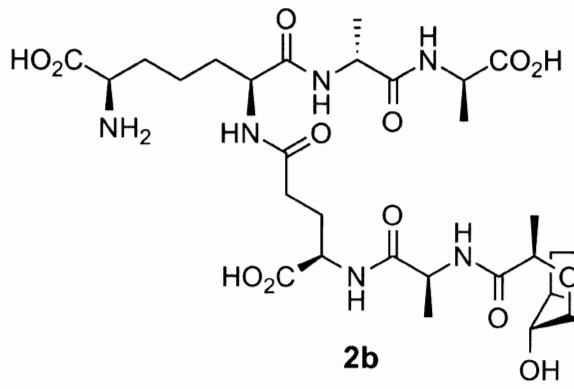
exp2 Carbon

SAMPLE		SPECIAL	
date	Feb 16 2008	temp	22.0
solvent	cd3od	gain	not used
file	exp	spin	not used
ACQUISITION		hst	0.008
sw	36764.7	pw90	7.500
at	1.783	alfa	10.000
np	131072	**FLAGS**	
fb	17000	il	n
bs	8	in	n
d1	1.220	dp	y
nt	2400	hs	nn
ct	184	**PROCESSING**	
TRANSMITTER		lb	0.50
tn	C13	fn	262144
sfrq	150.855	**DISPLAY**	
tof	1542.6	sp	2091.6
tpwr	58	wp	25062.8
pw	7.500	rfl	9730.6
DECOUPLER		rfp	7413.7
dn	H1	rp	56.0
dof	0	lp	2.7
dm	yyy	**PLOT**	
dmm	w	wc	250
dpwr	44	sc	0
dmf	13908	vs	403
		th	3
		ai cdc ph	



2b


DHL-51
File: xp
Pulse Sequence: DEPT
2b
CH3 carbons
CH2 carbons
CH carbons
all protonated carbons
100
90
80
70
60
50
40
30
20
10
ppm



2b

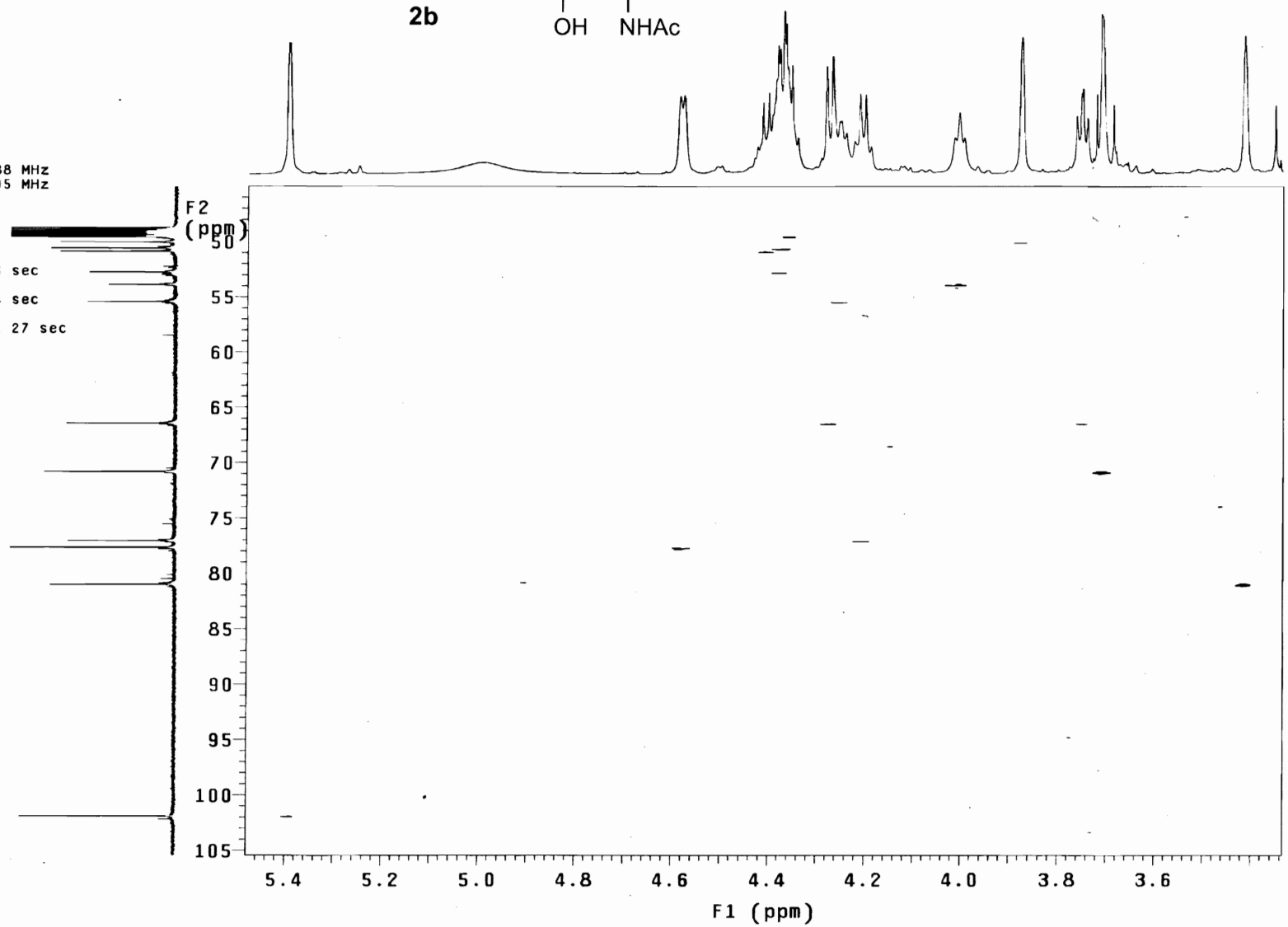
DHL-51

File: xp

Pulse Sequence: HETCOR

Solvent: cd3od
Temp. 22.0 C / 295.1 K
Operator: vnmr1
VNMRS-600 "nmr600"

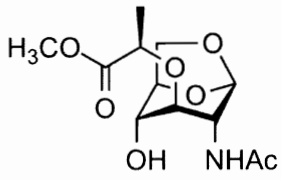
Relax. delay 1.313 sec
Acq. time 0.187 sec
Width 21929.8 Hz
2D Width 5398.9 Hz
16 repetitions
2 x 258 increments
OBSERVE C13, 150.8385088 MHz
DECOUPLE H1, 599.8773205 MHz
Power 44 dB
on during acquisition
off during delay
WALTZ-16 modulated
DATA PROCESSING
Gauss apodization 0.086 sec
F1 DATA PROCESSING
Gauss apodization 0.044 sec
FT size 8192 x 2048
Total time 3 hr, 33 min, 27 sec



DHL-27_2

exp1 s2pul

SAMPLE		DEC. & VT	
date	Nov 30 2007	dfrq	499.864
solvent	CDCl3	dn	H1
file	exp	dpwr	30
ACQUISITION		dof	0
sfrq	499.864	dm	nnn
tn	H1	dmm	c
at	5.016	dmf	200
np	65536	dseq	
sw	6533.3	dres	1.0
fb	4000	homo	n
bs	4	DEC2	
tpwr	61	dfrq2	0
pw	13.5	dn2	
d1	0.100	dpwr2	1
tof	269.9	dof2	0
nt	16	dm2	n
ct	16	dmm2	c
alock	n	dmf2	200
gain	not used	dseq2	
FLAGS		dres2	1.0
il	n	homo2	n
in	n	DEC3	
dp	y	dfrq3	0
hs	nn	dn3	
DISPLAY		dpwr3	1
sp	639.9	dof3	0
wp	4455.5	dm3	n
vs	102	dmm3	c
sc	0	dmf3	200
wc	250	dseq3	
hzmm	17.82	dres3	1.0
is	200.00	homo3	n
rfl	510.6	PROCESSING	
rfp	0	wtfile	
th	7	proc	ft
ins	1.000	fn	65536
ai ph		math	f
		werr	
		wexp	process plH
		wbs	
		wnt	wft

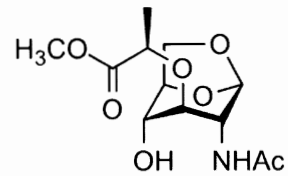


11

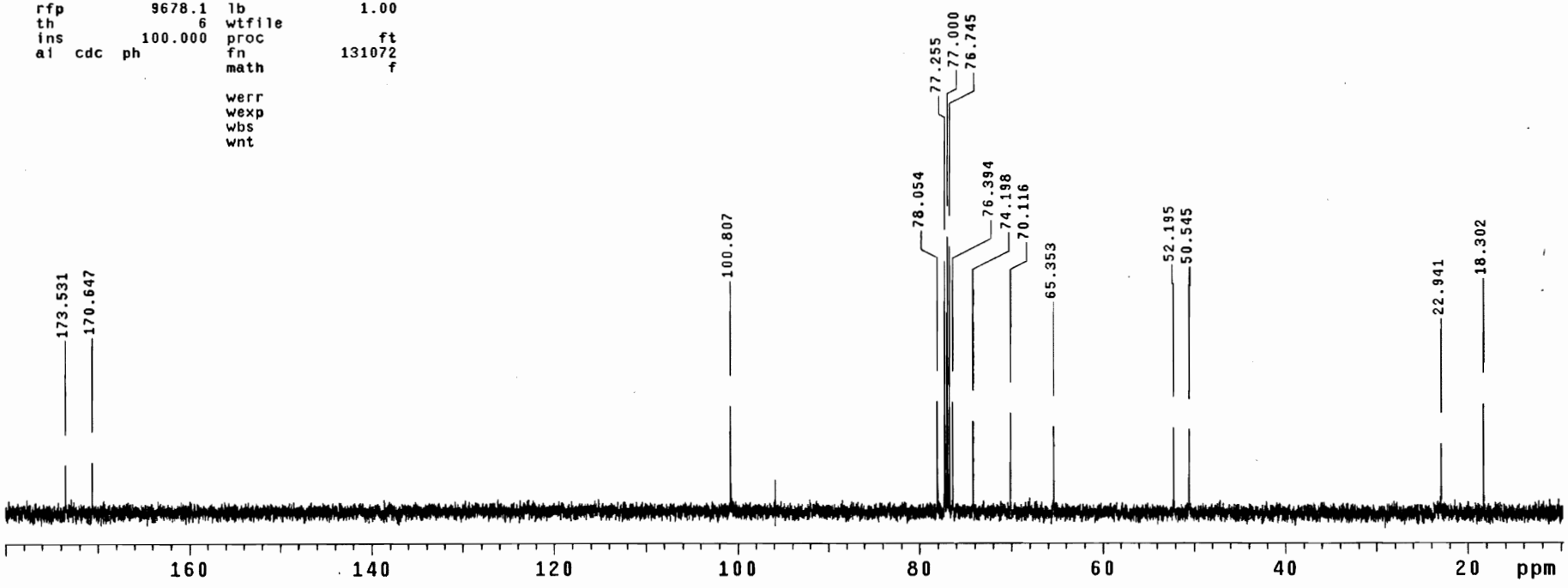
DHL-27_2

exp2 s2pul

SAMPLE		DEC. & VT	
date	Nov 30 2007	dfrq	499.864
solvent	CDCl3	dn	H1
file	exp	dpwr	40
ACQUISITION		dof	0
sfrq	125.702	dm	yyy
tn	C13	dmm	w
at	1.215	dmf	8787.35
np	65536	dseq	
sw	26963.3	dres	1.0
fb	15000	homo	n
bs	4	DEC2	
tpwr	52	dfrq2	0
pw	10.2	dn2	
d1	1.800	dpwr2	1
tof	144.5	dof2	0
nt	1200	dm2	n
ct	80	dmm2	c
alock	n	dmf2	10000
gain	not used	dseq2	
FLAGS		dres2	1.0
il	n	homo2	n
in	n	DEC3	
dp	y	dfrq3	0
hs	nn	dn3	
DISPLAY		dpwr3	1
sp	1225.0	dof3	0
wp	21400.0	dm3	n
vs	304	dmm3	c
sc	0	dmf3	10000
wc	250	dseq3	
hzmm	85.60	dres3	1.0
is	500.00	homo3	n
rfl	11110.6	PROCESSING	
rfp	9678.1	lb	1.00
th	6	wtfile	
ins	100.000	proc	ft
ai cdc ph		fn	131072
		math	f
		werr	
		wexp	
		wbs	
		wnt	

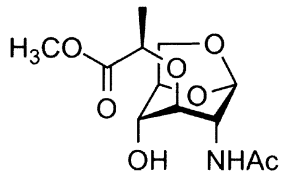


11



DHL-27_2

Pulse Sequence: dept



11

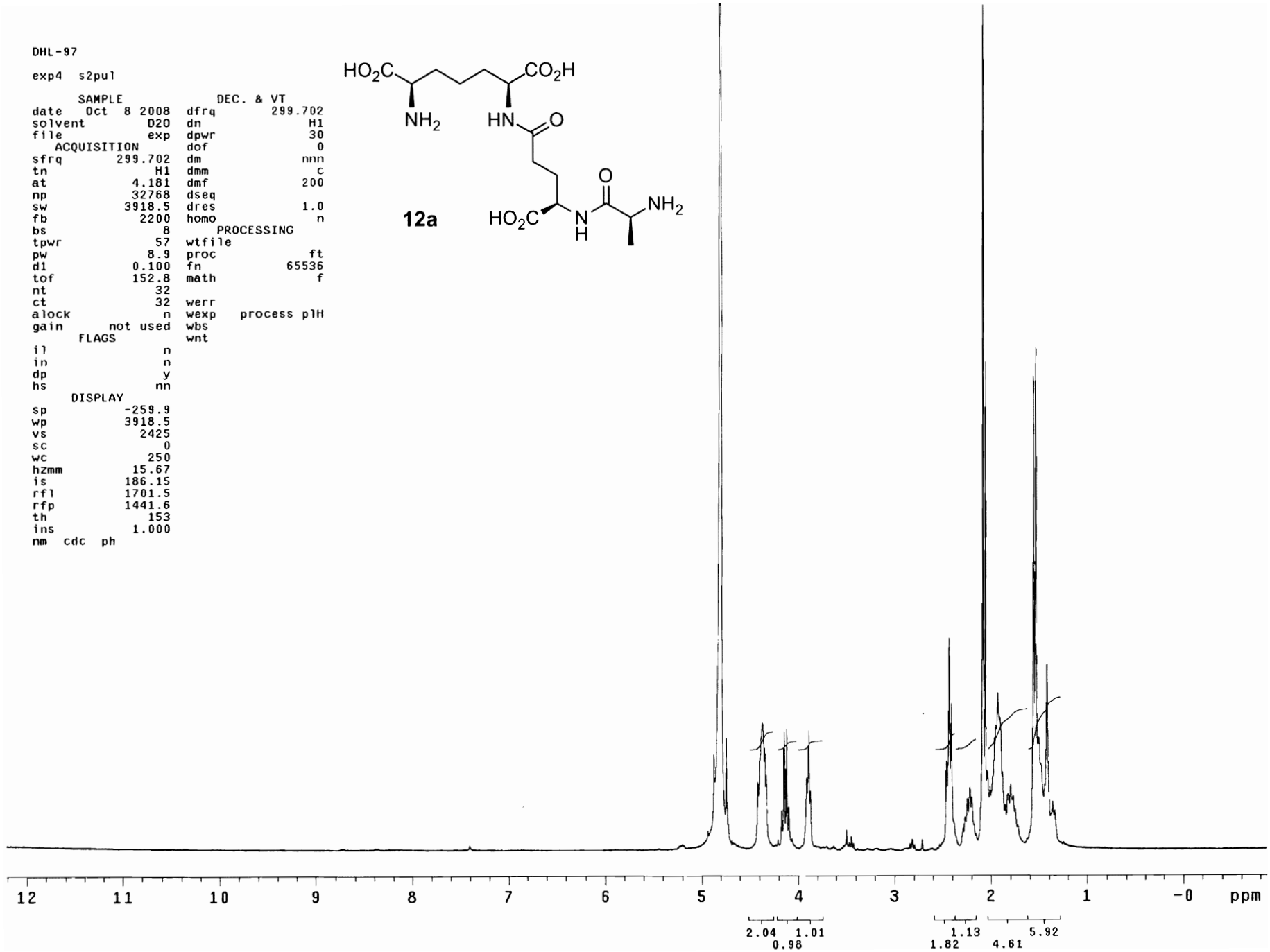
CH3 carbons

CH2 carbons

CH carbons

all protonated carbons

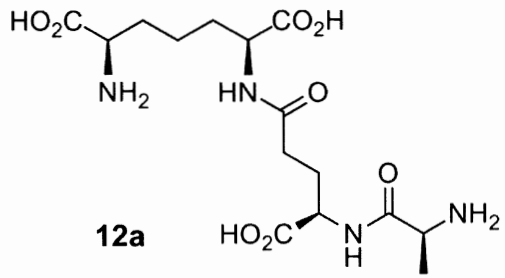
110 100 90 80 70 60 50 40 30 20 ppm


DHL-97
exp4 s2pul
SAMPLE
date Oct 8 2008
solvent D2O
file exp
ACQUISITION
sfrq 299.702
tn H1
at 4.181
np 32768
sw 3918.5
fb 2200
bs 8
tpwr 57
pw 8.9
d1 0.100
tof 152.8
nt 32
ct 32
alock n
gain not used
FLAGS
il n
in n
dp y
hs nn
DISPLAY
sp -259.9
wp 3918.5
vs 2425
sc 0
wc 250
hzmm 15.67
is 186.15
rfl 1701.5
rfp 1441.6
th 153
ins 1.000
nm cdc ph
DEC. & VT
dfrq 299.702
dn H1
dpwr 30
dof 0
dm nnn
dmm c
dmf 200
dseq
dres 1.0
homo n
PROCESSING
wtfile
proc ft
fn 65536
math f
werr
wexp process plH
wbs
wnt
12a
12 11 10 9 8 7 6 5 4 3 2 1 -0 ppm
2.04 1.01 0.98
1.13 1.82
5.92 4.61

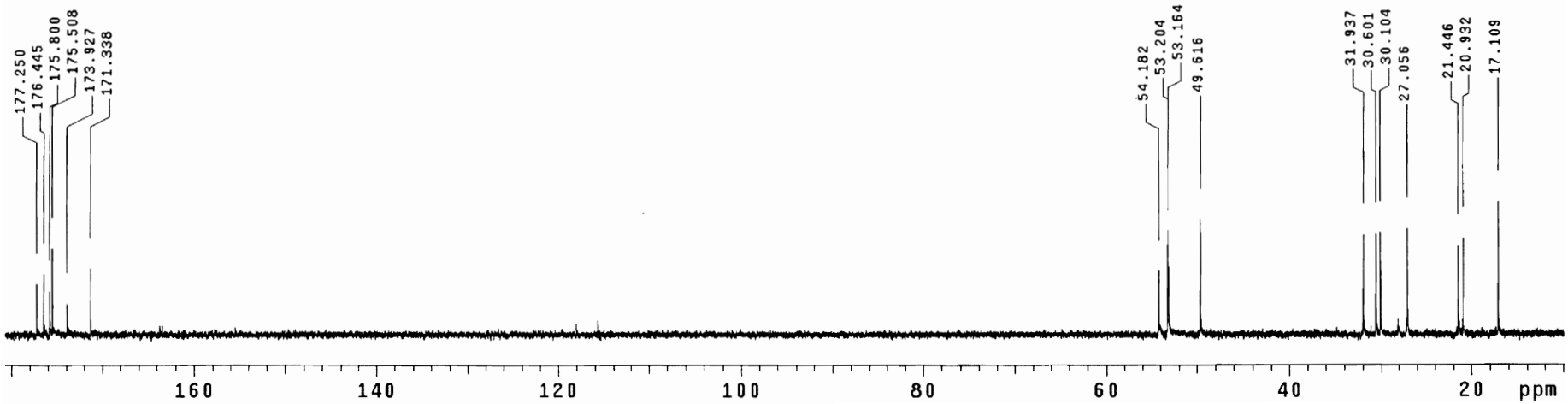
DHL-97

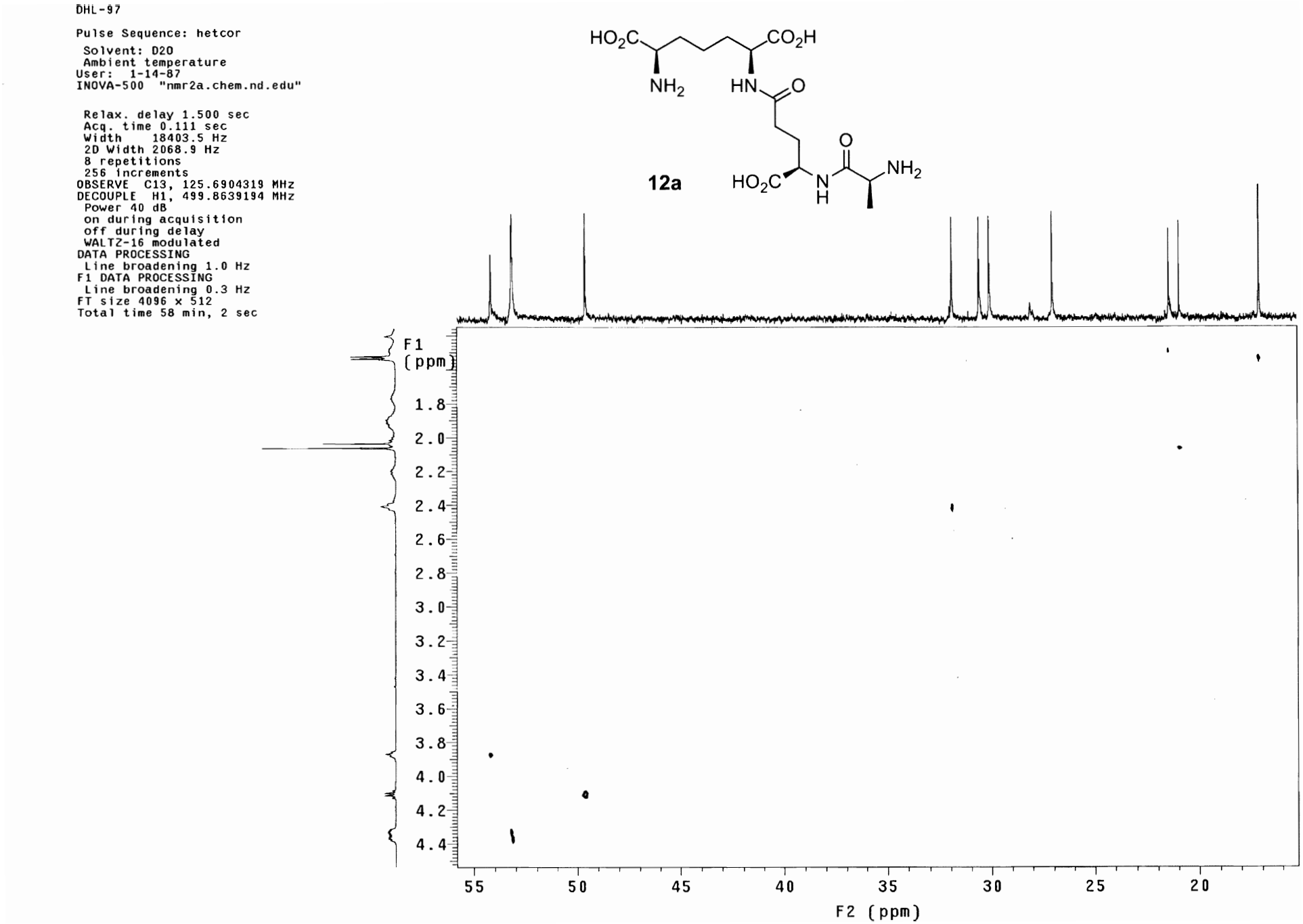
exp2 s2pul

SAMPLE		DEC. & VT	
date	Oct 10 2008	dfrq	499.865
solvent	D2O	dn	H1
file	exp	dpwr	40
ACQUISITION		dof	0
sfrq	125.703	dm	yyy
tn	C13	dmm	w
at	1.215	dmf	8787.35
np	65536	dseq	
sw	26963.3	dres	1.0
fb	15000	homo	n
bs	4	DEC2	
tpwr	52	dfrq2	0
pw	10.2	dn2	
d1	1.800	dpwr2	1
tof	144.5	dof2	0
nt	2400	dm2	n
ct	1608	dmm2	c
alock	n	dmf2	10000
gain	not used	dseq2	
FLAGS		dres2	1.0
il	n	homo2	n
in	n	DEC3	
dp	y	dfrq3	0
hs	nn	dn3	
DISPLAY		dpwr3	1
sp	1242.0	dof3	0
wp	21470.7	dm3	n
vs	636	dmm3	c
sc	0	dmf3	10000
wc	250	dseq3	
hzmm	85.88	dres3	1.0
is	500.00	homo3	n
rfl	1519.4	PROCESSING	
rfp	174.7	lb	1.00
th	4	wtfile	
ins	100.000	proc	ft
ai cdc ph		fn	131072
		math	f
		werr	
		wexp	
		wbs	
		wnt	



12a




DHL-97
Pulse Sequence: hetcor
Solvent: D2O
Ambient temperature
User: 1-14-87
INOVA-500 "nmr2a.chem.nd.edu"
Relax. delay 1.500 sec
Acq. time 0.111 sec
Width 18403.5 Hz
2D Width 2068.9 Hz
8 repetitions
256 increments
OBSERVE C13, 125.6904319 MHz
DECOUPLE H1, 499.8639194 MHz
Power 40 dB
on during acquisition
off during delay
WALTZ-16 modulated
DATA PROCESSING
Line broadening 1.0 Hz
F1 DATA PROCESSING
Line broadening 0.3 Hz
FT size 4096 x 512
Total time 58 min, 2 sec
12a
F1 (ppm)
1.8
2.0
2.2
2.4
2.6
2.8
3.0
3.2
3.4
3.6
3.8
4.0
4.2
4.4
55
50
45
40
35
30
25
20
F2 (ppm)