Electronic Supplementary Material (ESI) for Organic & Biomolecular Chemistry

Supplementary Information

2,6,9-Triazabicyclo[3.3.1]nonanes as overlooked amino-modification products by acrolein

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Materials and methods. All commercially available reagents were used without further purification. ^{1}H and ^{13}C NMR spectra were recorded on JEOL AL400 NMR spectrometer and chemical shifts were represented as δ-values relative to the internal standard TMS. Structural analysis of **8a**, including the 2D NMR analysis, was especially performed using JEOL JNM-ECA600 spectrometer. ESI-mass spectra including the high resolution mass spectra (HRMS) were recorded on JEOL JMS-T100LC mass spectrometer. HeLa cells, RCB0007, was provided by the RIKEN BRC through the National Bio-Resource Project of the MEXT, Japan.

Representative procedure of 2,6,9-triazabicyclo[3.3.1]nonane: preparation of 8a. To a solution of *N*-methyl-1,3-diaminopropane (**8**) (12 μL, 113 μmol) in either $CHCl_3$, H_2O, or PBS buffer solution (400 μL) was slowly added acrolein (5.1 μL, 76 μmol) at room temperature. After stirring for 5 min at this temperature, the mixture was concentrated *in vacuo* to give **8a** as a white solid (13 mg). Direct NMR analysis without any purification process (see Figs SI-2 and 3 below) confirmed the quantitative production of **8a** by this procedure: ^{1}H NMR (600 MHz, $CDCl_3$) δ 3.08 (broad d, 2H, J = 13.2 Hz: 2-H_1 and 2'-H_1), 2.96 (broad d, 2H, J = 14.0 Hz: 4-H_1 and 4'-H_1), 2.93-2.92 (m, 2H: 5-H and 5'-H), 2.74 (td, 2H, J = 6.8, 2.0 Hz: 10-H), 2.62 (ddd, 2H, J = 13.2, 13.2, 2.8 Hz: 2-H_2 and 2'-H_2), 2.51-2.44 (m, 4H: 6-H and 6'-H), 2.40 (td, 2H, J = 6.8, 2.0 Hz: 8-H), 2.33 (ddd, 2H, J = 12.4, 12.4, 2.0 Hz: 4-H_2 and 4'-H_2), 2.23 (s, 3H: 11-H), 2.22 (s, 6H: 1-H and 1'-H), 1.90-1.84 (m, 2H: 7-H_1 and 7'-H_1), 1.74-1.66 (m, 2H: 3-H_1 and 3'-H_1), 1.63 (tt, 2H, J = 6.8, 6.8 Hz: 9-H), 1.59-1.53 (m, 2H: 7-H_2 and 7'-H_2), 1.49 (broad d, 2H, J = 13.2 Hz: 3-H_2, 3'-H_2); ^{13}C NMR (150 MHz, $CDCl_3$), δ 77.6 (2C, C-5, C-5'), 56.1 (2C, C-4, C-4'), 55.6 (C-8), 53.8 (2C, C-6, C-6'), 45.2 (2C, C-2, C-2'), 42.2 (2C, C-1, C-1'), 40.9 (C-11), 40.5 (C-10), 31.0 (C-9), 30.8 (2C, C-7, C-7'), 26.6 (2C, C-3, C-3'); HRESI-MS *m/z* calcd for $C_{18}H_{42}N_6$ $[M+H]^+$ 341.3393, found 341.3397.

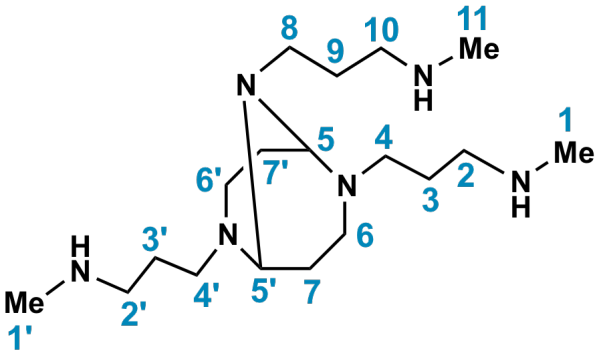

Fig. SI-1 Structure of **8a**. Carbon atoms were arbitrary numbered to assign the ^{1}H and ^{13}C signals described above.

Data for **3a**: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.29-7.16 (m, 15H), 3.50-3.35 (m, 2H), 3.05-2.83 (m, 2H), 2.73 (t, 3H, *J* = 6.8 Hz), 2.74-2.34 (m, 13H), 2.02-1.73 (m, 7H), 1.28 (broad s, 3H); Characteristic ^{13}C NMR signal at bridged acetal carbons (150 MHz, $CDCl_3$), δ 69.0; HRESI-MS *m/z* calcd for $C_{33}H_{44}N_3$ $[M+H]^+$ 482.3535, found 482.3491.

Data for **4a**: ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.29-7.16 (m, 15H), 3.45 (d, 1H, *J* = 3.9 Hz), 3.38 (m, 1H), 2.84-2.80 (m, 2H), 2.71 (t, 3H, *J* = 6.8 Hz), 2.65-2.57 (m, 7H), 2.45-2.38 (m, 2H), 1.96 (m, 1H), 1.76-1.62 (m, 8H), 1.54-1.45 (m, 6H), 1.17 (broad s, 3H); Characteristic ^{13}C NMR signal at bridged acetal carbons (150 MHz, $CDCl_3$), δ 69.1; HRESI-MS *m/z* calcd for $C_{36}H_{50}N_3$ $[M+H]^+$ 524.4005, found 524.4055.

Data for **5a**: ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.50 (d, 1H, *J* = 4.4 Hz), 3.36 (m, 1H), 2.93 (m, 1H), 2.85-2.82 (m, 2H), 2.68 (t, 1H, *J* = 7.3 Hz), 2.56 (m, 1H), 1.96 (m, 1H), 1.77 (m, 1H), 1.47-1.40 (m, 5H), 1.29 (broad s, 19H), 0.88 (t, 9H, *J* = 6.8 Hz); Characteristic ^{13}C NMR signal at bridged acetal carbons (150 MHz, $CDCl_3$), δ 68.8; HRESI-MS *m/z* calcd for $C_{24}H_{50}N_3$ $[M+H]^+$ 380.4005, found 380.3981.

Data for **6a**: ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.49 (d, 1H, *J* = 3.9 Hz), 1.14 (m, 1H), 2.83 (m, 1H), 2.67 (t, 2H, *J* = 6.8 Hz), 2.42-2.39 (m, 2H), 1.57-1.26 (m, 75H), 0.88 (t, 9H, *J* = 6.8 Hz); Characteristic ^{13}C NMR signal at bridged acetal carbons (150 MHz, $CDCl_3$), δ 68.8; HRESI-MS *m/z* calcd for $C_{45}H_{92}N_3$ $[M+H]^+$ 674.7291, found 674.7245.

Data for **7a**: ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.17-4.07 (m, 2H), 3.70-2.82 (m, 6H), 3.14 (m, 1H), 2.96 (ddd, 4H, *J* = 9.7, 6.3, 3.9 Hz), 2.92 (m, 1H), 2.84 (ddd, 2H, *J* = 8.2, 5.8, 2.9 Hz), 2.71 (ddd, 2H, *J* = 8.7, 6.8, 1.9 Hz), 2.57-2.52 (m, 3H), 1.73-1.64 (m, 9H), 1.36 (m, 1H); Characteristic ^{13}C NMR signal at bridged acetal carbons (150 MHz, $CDCl_3$), δ 67.5; HRESI-MS *m/z* calcd for $C_{15}H_{32}N_3O_3$ 302.2444, found 302.2454.

Data for **9a**: ^{1}H NMR (400 MHz, $CDCl_3$) δ 4.39-4.35 (m, 3H), 3.39-3.35 (m, 2H), 3.32 (s x 2, 3H x 2), 3.31 (s x 4, 3H x 4), 2.86-2.82 (m, 2H), 2.71 (ddd, 5H, *J* = 12.1, 7.3, 1.9 Hz), 2.47-2.35 (m, 3H), 1.66-1.46 (m, 14H), 1.25 (broad s, 2H); Characteristic ^{13}C NMR signal at bridged acetal carbons (150 MHz, $CDCl_3$), δ 69.2; HRESI-MS *m/z* calcd for $C_{24}H_{50}N_3O_6$ $[M+H]^+$ 476.3700, found 476.3698.

Data for **10a**: ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.74-3.50 (m, 41H), 3.40-38 (m, 7H), 3.16-2.44 (m, 7H), 2.25-1.77 (m, 3H); Characteristic ^{13}C NMR signal at bridged acetal carbons (150 MHz, $CDCl_3$), δ 70.0; HRESI-MS *m/z* calcd for $C_{30}H_{59}N_{12}O_9$ $[M+H]^+$ 731.4528, found 731.4551.

Cell culture and MTS assay. A549 and HeLa cells were cultured in DMEM medium (Sigma) supplemented with 10% (v/v) FBS at 37 °C in a 5% CO_2-incubator. A549 cells were seeded at 500 cells/well in 50 μL of DMEM, and placed for 6 h. Solution of the caged compounds **7a** and **8a** with various concentrations was prepared in advance by diluting their stock solution in DMSO with the culture medium. Following the addition of the fresh culture medium (40 μL), the compound diluents (30 μL) were also added to the cell cultures. The final volume of DMSO in the medium was equal to 0.1% (v/v). The cells under compound treatment were incubated for 72 h at 37 °C under 5% CO_2 atmosphere. The wells in the plates were washed twice with the cultured medium, which did not contain phenol-red. After 1 h incubation with 100 μL of this medium, the cell culture in each well was supplemented with the MTS solution (20 μL),* and then incubated for 40 min according to the established procedure.[17] Absorbance at 490 nm at each well was measured using a Wallac 1420 ARVO SX multilabel counter (Perkin Elmer).

* *MTS solution used here was prepared in advance by mixing 500 mL MTS solution (1 g dissolved in PBS(-), G111A, Promega)[17] with 25 mL PES solution (25 mg, dissolved in PBS(-), 26711-61, Nacalai).*

Cell lysate and Western Blotting. After cells (6.0×10^5) were seeded and placed on the plate for 18 h, they were treated with the compound **8a** (60 μM) for 48 h at 37 °C under 5% CO_2 atmosphere. Cells were pelleted and suspended in ice-cold lysis buffer (1% Triton X-100/50 mM β-glycerophosphate/1.5 mM EGTA/0.5 mM EDTA/5% glycerol/25 mM Tris HCl, pH 7.4) in the presence of protease inhibitors cocktail (Complete; Roche, Diagnostic, Germany), and the resulting lysates were cleared by centrifugation. Resulting proteins were separated by 4-20% gradient SDS-PAGE and then transferred to PVDF membranes. After incubation with 5% non-fat dried milk in TBS containing 0.1% Tween 20, the membranes were incubated with anti-HO-1 antibody (ab13248, Abcam), followed by HRP-conjugated anti-mouse IgG (GE Healthcare). Protein bands were detected by treatment with Supersignal West Femto Maximum Sensitivity Substrate (Thermo Scientific) using LAS-1000P lus·MAC analyzer (Fujifilm). After treating the PVDF membranes with the Pestore TM western Blot Stripping Buffer (Thermo Scientific), anti-GAPDH antibody (clone 6C5/MAB374, Millipore), and then HRP-conjugated anti-mouse IgG were continuously applied to detect the GAPDH.

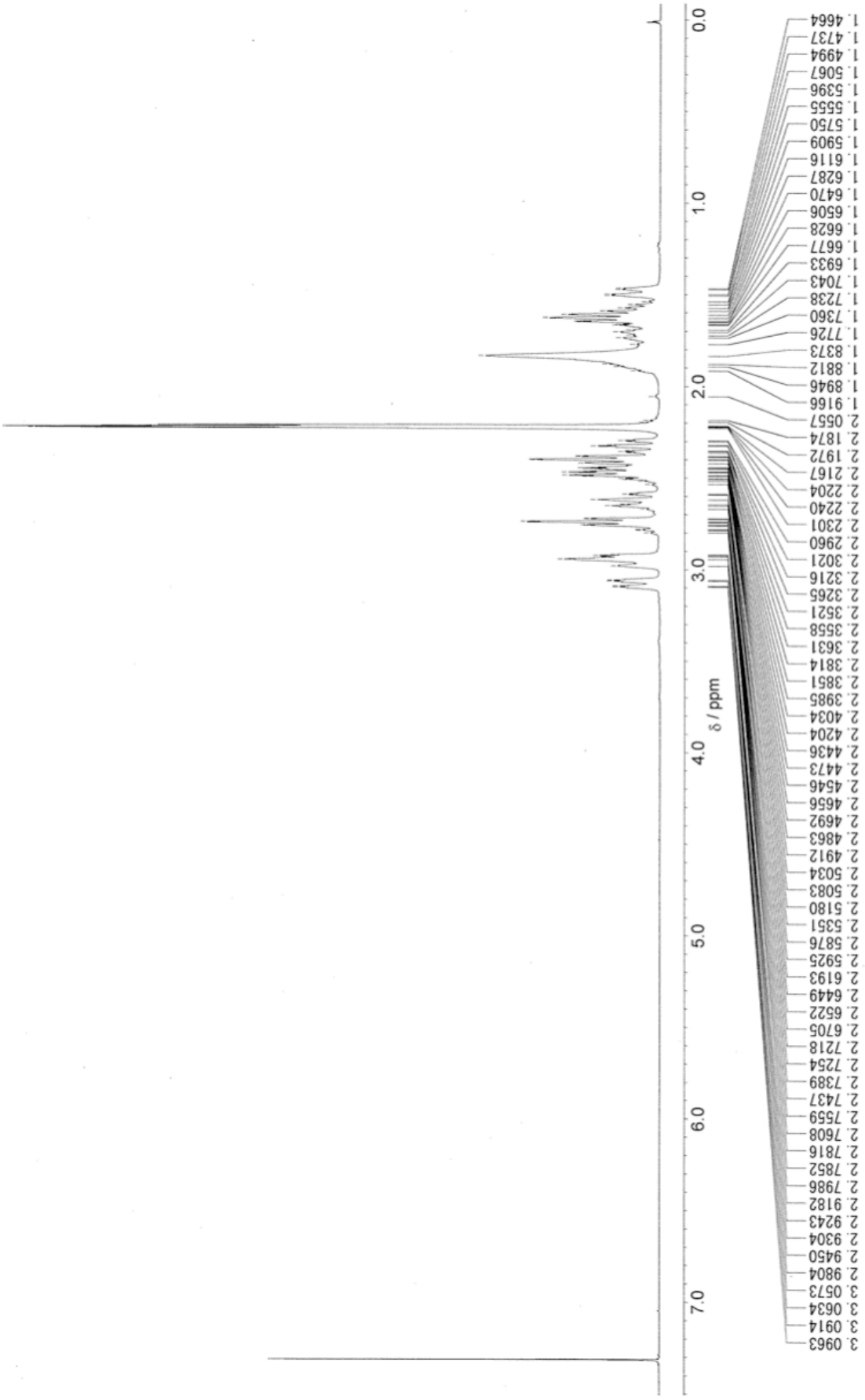

Fig. SI-2 ^{1}H NMR spectrum of **8a**.

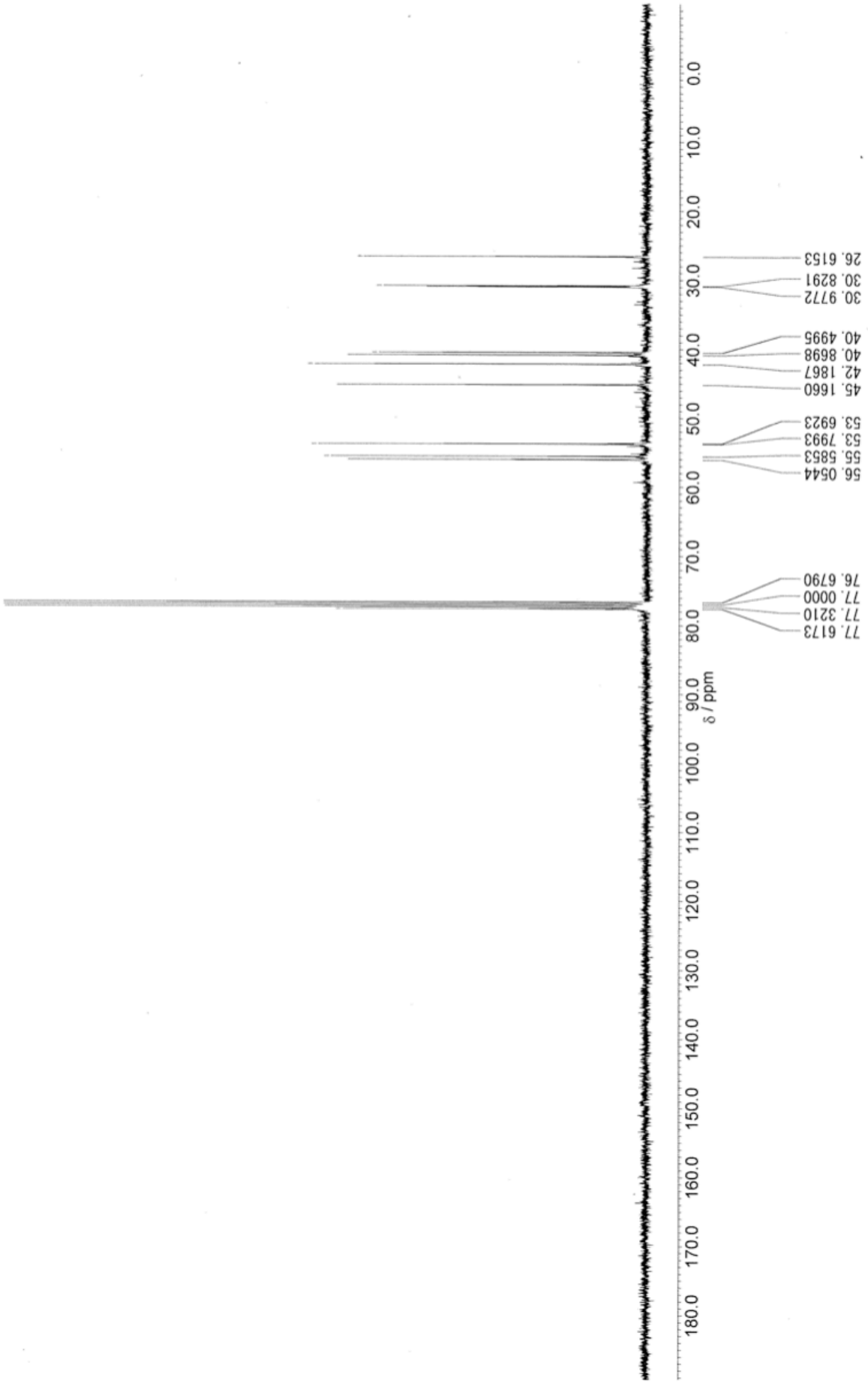

Fig. SI-3 ^{13}C NMR spectrum of **8a**.

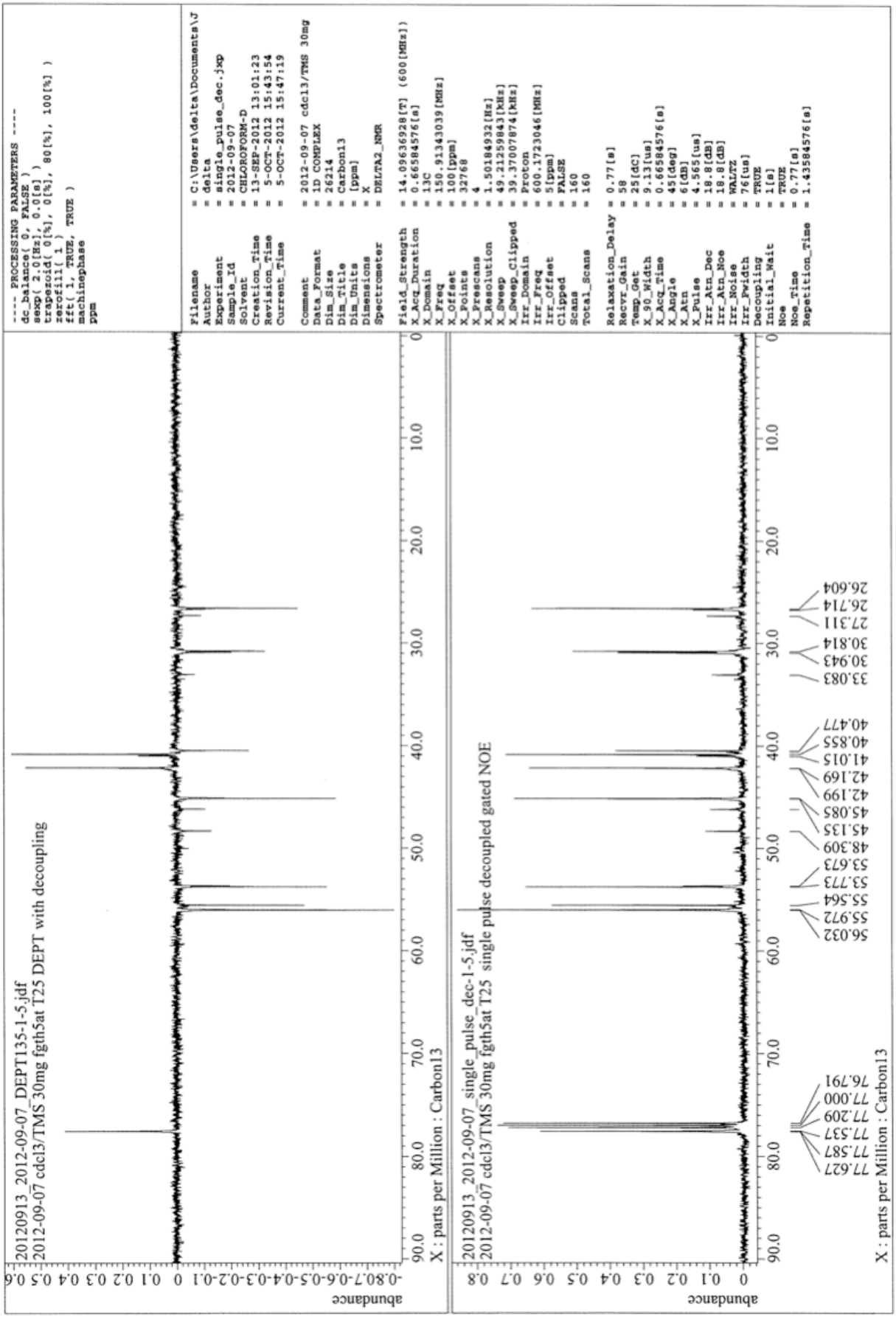

Fig. SI-4 DEPT of **8a**.

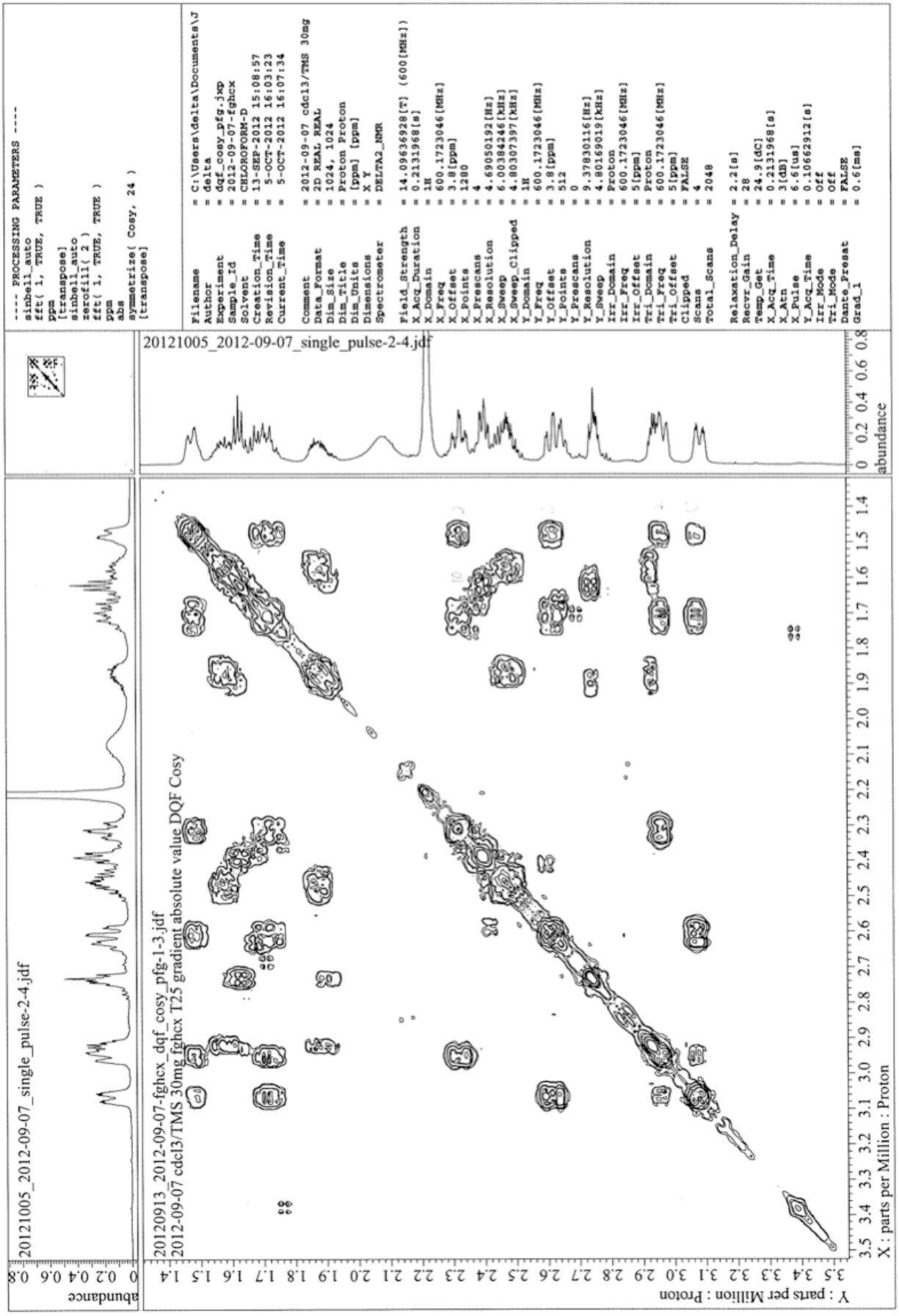

Fig. SI-5 COSY of **8a**.

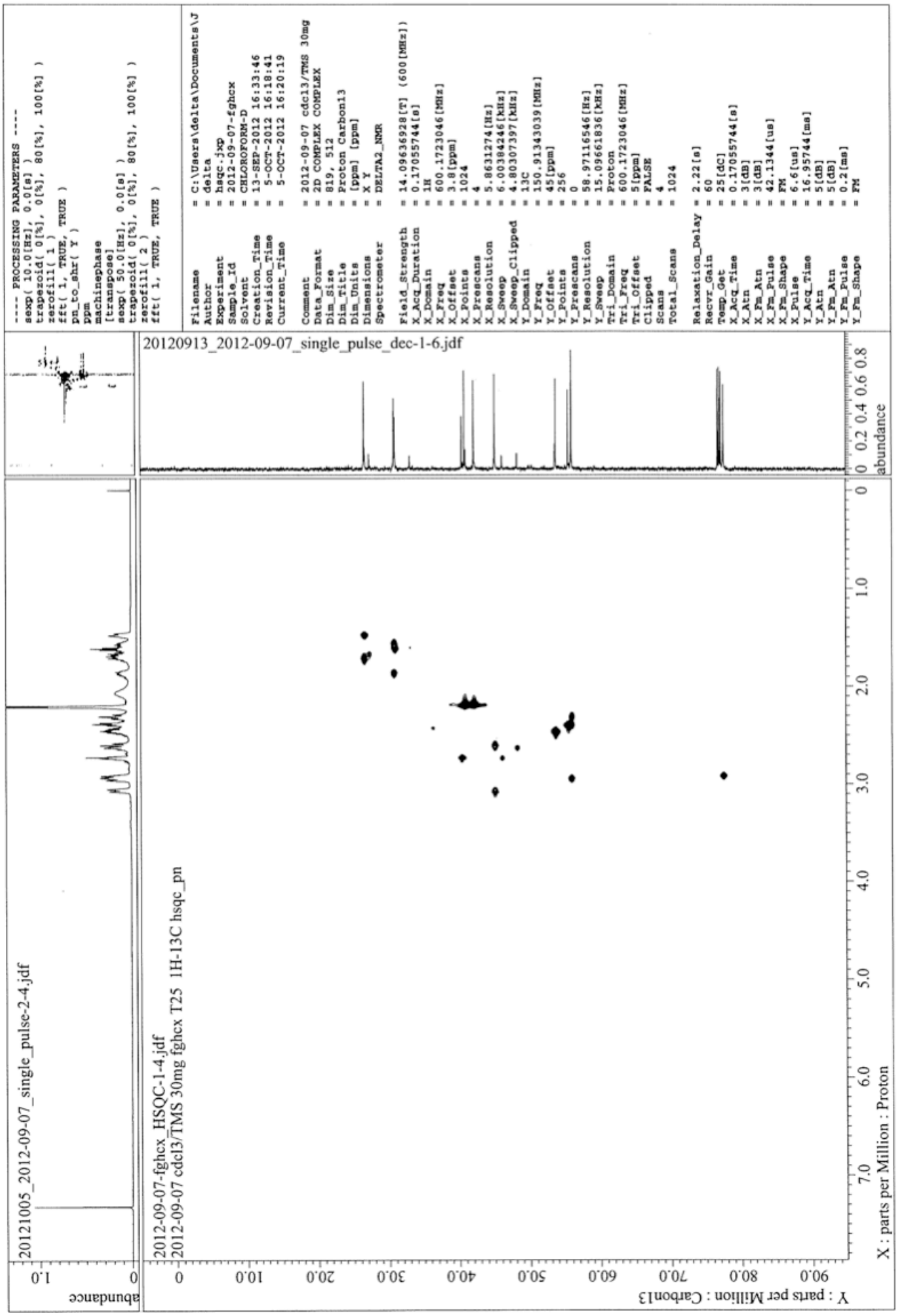

Fig. SI-6 HSQC of **8a**.

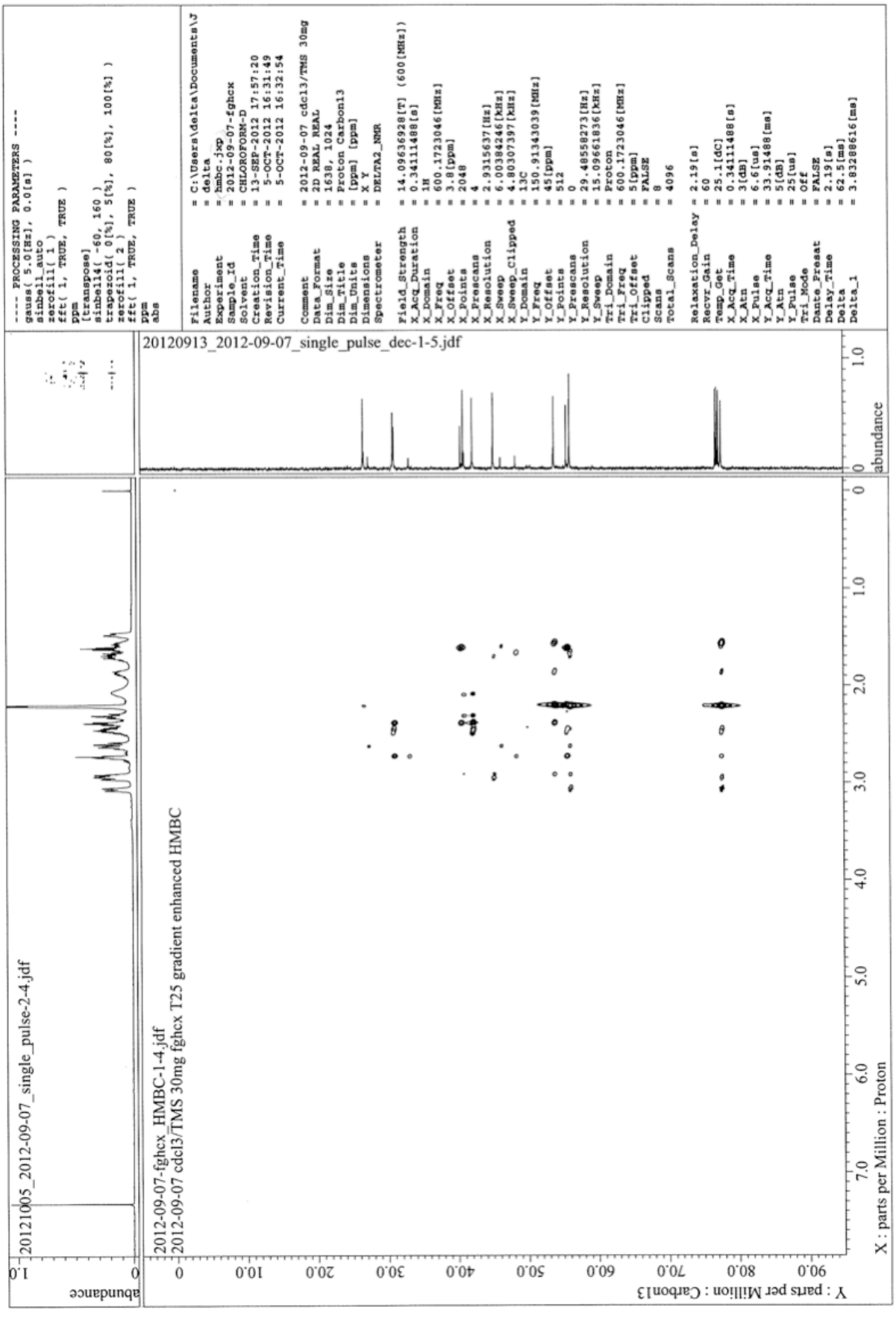

Fig. SI-7 HMBC of **8a**.

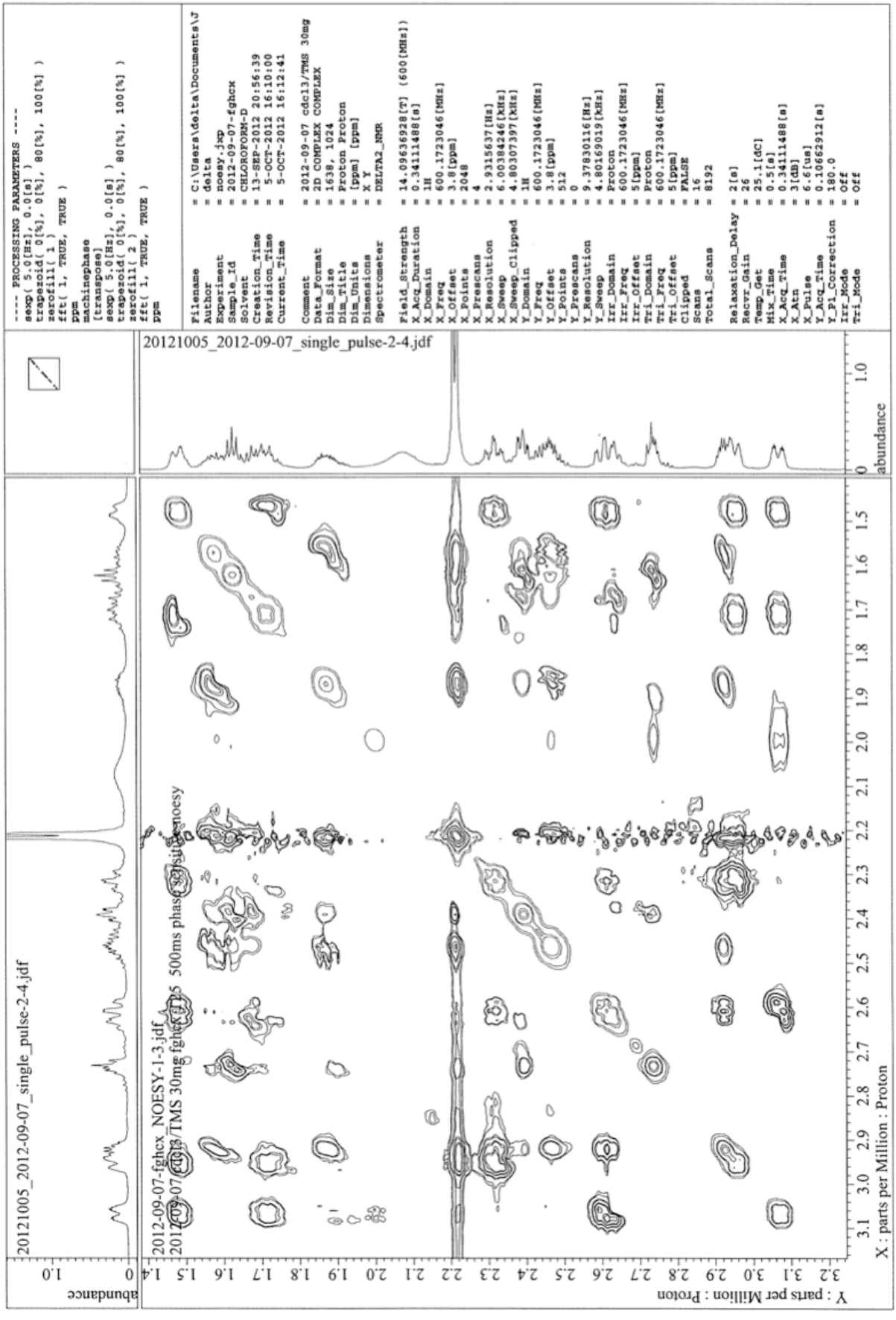

Fig. SI-8 NOESY of **8a**.