Electronic Supplementary Material (ESI) for ChemComm.

Supporting information

Encoding Matter with Regiospecific $^{12}C/^{13}C$ Isotopic Labels

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A. General synthetic methods. Chemical reagents were purchased from Cambridge Isotope Laboratories, Sigma-Aldrich, VWR or Fischer Scientific. Anhydrous *N*,*N*-dimethylformamide was obtained by passage over activated molecular sieves and a subsequent NaOCN column to remove traces of dimethylamine. All reactions were performed under positive pressure of Ar in oven-dried glassware sealed with septa, with stirring from a Teflon coated stir bars using an IKAMAG RCT-basic mechanical stirrer (IKA GmbH). Analytical Thin Layer Chromatography (TLC) was performed on Silica Gel 60 F254 precoated glass plates (EM Sciences). Visualization was achieved with UV light and/or ceric ammonium molybdate (CAM) staining. Flash chromatography was carried out Geduran Silica Gel 60 (40-63 mesh) from EM Biosciences. Yields and characterization data correspond to isolated, chromatographically and spectroscopically homogeneous materials. NMR spectra were recorded on a Varian VX500 spectrometer equipped with an Xsens Cold probe Varian VS500. Chemical shifts for NMR analyses were referenced using signals from the residual solvent (acetone-d6) with ^{1}H and ^{13}C spectra at 2.050 and 29.084 ppm, respectively. Chemical shift δ values for ^{1}H and ^{13}C spectra are reported in parts per million (ppm) relative to these referenced values, and multiplicities are abbreviated as s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, br = broad. All ^{13}C NMR spectra were recorded with complete proton decoupling. FID files were processed using MestraNova 11.0 (MestreLab Research). Electrospray (ESI) mass spectrometric analyses were performed using a ThermoFinnigan LCQ Deca spectrometer, and high-resolution analyses were conducted using a ThermoFinnigan MAT900XL mass spectrometer with electron impact (EI) ionization. A Thermo Scientific LTQ Orbitrap XL mass spectrometer was used for high-resolution electrospray ionization mass spectrometry analysis (HR-ESI-MS).

B. Synthesis of ^{12}C-4'-methylapigenenin (0). Apigenin (24.2 mg, 0.090 mmol) was dissolved in dry DMF (0.5 mL). Solid K_2CO_3 (27.2 mg, 0.197 mmol) followed by ^{12}C-methyliodide (6.7 µL, 0.107 mmol). After 18h at rt, EtOH (1 mL) was added and the mixture was dried by airflow. The residue was purified by flash chromatography (hexanes to 1:1 hexanes/EtOAc) to afford 20.1 mg of **0** (79%), as a colorless solid. Spectroscopic properties matched that of natural samples of acacetin.

^{12}C-4'-methylapigenenin (**0**): ^{1}H-NMR (500 MHz, acetone-d_6) δ 13.00 (s, 1H), 8.03 (d, *J* = 9.0 Hz, 1H), 7.13 (d, *J* = 8.9 Hz, 1H), 6.69 (d, *J* = 1.1 Hz, 1H), 6.56 (t, *J* = 1.7 Hz, 1H), 6.26 (dd, *J* = 0.9, 2.1 Hz, 1H), 3.92 (s, 3H); HR-ESI-MS *m/z* calcd. $C_{16}H_{13}O_5$ $[M+H]^+$: 285.0763, found 285.0761.

C. Synthesis of ^{13}C-4'-methylapigenenin (1). Apigenin (25.8 mg, 0.095 mmol) was dissolved in dry DMF (0.5 mL). Solid K_2CO_3 (29.0 mg, 0.210 mmol) followed by ^{13}C-methyliodide (7.2 µL, 0.115 mmol). After 18h at rt, EtOH (1 mL) was added and the mixture was dried by airflow. The residue was purified by flash chromatography (hexanes to 1:1 hexanes/EtOAc) to afford 21.4 mg of **1** (79%), as a colorless solid. Spectroscopic properties matched that of natural samples of acacetin with the exception of ^{13}C labeling at the methoxy-group.

^{13}C-4'-methylapigenenin (**1**): ^{1}H-NMR (500 MHz, acetone-d_6) δ 13.00 (s, 1H), 8.03 (d, *J* = 9.0 Hz, 1H), 7.13 (d, *J* = 8.9 Hz, 1H), 6.69 (d, *J* = 1.1 Hz, 1H), 6.56 (t, *J* = 1.7 Hz, 1H), 6.26 (dd, *J* = 0.9, 2.1 Hz, 1H), 3.93 (d, *J* = 145.4 Hz, 3H); HR-ESI-MS *m/z* calcd. C_{15} $^{13}C_1H_{13}O_5$ $[M+H]^+$: 286.0796, found 286.0794.

D. Synthesis of ^{12}C-4',^{12}C-7-methylapigenenin (00). ^{12}C-4'-methylapigenenin (25.2 mg, 0.089 mmol) dissolved in dry DMF (0.5 mL). Solid K_2CO_3 (39.2 mg, 0.284 mmol) followed by ^{12}C-methyliodide (12.1 µL, 0.195 mmol). After 18h at rt, EtOH (1 mL) was added and the mixture

was dried by airflow. The residue was purified by flash chromatography (hexanes to 1:1 hexanes/EtOAc) to afford 19.2 mg of **00** (73%), as a colorless solid.

^{12}C-4',^{12}C-7-methylapigenenin (**00**): ^{1}H-NMR (500 MHz, acetone-d_6) δ 12.96 (s, 1H), 8.05 (d, *J* = 9.0 Hz, 1H), 7.14 (d, *J* = 9.0 Hz, 1H), 6.73 (s, 1H), 6.71 (d, *J* = 2.3 Hz, 1H), 6.34 (d, *J* = 2.2 Hz, 1H), 3.93 (s, 3H), 3.92 (s, 3H); δ: 183.1,166.6, 165.0, 163.8, 163.0, 158.7, 129.0, 124.2, 115.4, 106.0, 104.6, 98.7, 93.2, 56.4, 56.0; HR-ESI-MS *m/z* calcd. $C_{17}H_{15}O_5$ $[M+H]^+$: 299.0914, found 299.0912.

E. Synthesis of ^{12}C-4',^{13}C-7-methylapigenenin (01). ^{12}C-4'-methylapigenenin (10.4 mg, 0.037 mmol) dissolved in dry DMF (0.5 mL). Solid K_2CO_3 (16.2 mg, 0.117 mmol) followed by ^{13}C-methyliodide (5.0 µL, 0.080 mmol). After 18h at rt, EtOH (1 mL) was added and the mixture was dried by airflow. The residue was purified by flash chromatography (hexanes to 1:1 hexanes/EtOAc) to afford 8.1 mg of **01** (74%), as a colorless solid.

^{12}C-4',^{13}C-7-methylapigenenin (**01**): ^{1}H-NMR (500 MHz, acetone-d_6) δ 12.96 (s, 1H), 8.05 (d, *J* = 9.1 Hz, 1H), 7.14 (d, *J* = 9.1 Hz, 1H), 6.73 (s, 1H), 6.71 (d, *J* = 2.2 Hz, 1H), 6.33 (d, *J* = 2.3 Hz, 1H), 3.93 (d, *J* = 145.4 Hz, 3H), 3.92 (s, 3H); ^{13}C-NMR (125 MHz, acetone-d_6) δ: 183.2,166.6, 165.0, 163.8, 163.0, 158.7, 129.1, 124.2, 115.4, 106.0, 104.6, 98.7, 93.2, 56.4, 56.0*; HR-ESI-MS *m/z* calcd. $C_{16}{}^{13}C_1H_{15}O_5$ $[M+H]^+$: 300.0947, found 300.0944.

F. Synthesis of ^{13}C-4',^{12}C-7-methylapigenenin (10). ^{13}C-4'-methylapigenenin (11.1 mg, 0.039 mmol) dissolved in dry DMF (0.5 mL). Solid K_2CO_3 (17.3 mg, 0.125 mmol) followed by ^{12}C-methyliodide (5.3 µL, 0.086 mmol). After 18h at rt, EtOH (1 mL) was added and the mixture was dried by airflow. The residue was purified by flash chromatography (hexanes to 1:1 hexanes/EtOAc) to afford 8.3 mg of **01** (71%), as a colorless solid.

^{13}C-4',^{12}C-7-methylapigenenin (**10**): ^{1}H-NMR (500 MHz, acetone-d_6) δ 12.96 (s, 1H), 8.05 (d, *J* = 9.1 Hz, 1H), 7.14 (d, *J* = 9.0 Hz, 1H), 6.73 (s, 1H), 6.71 (d, *J* = 2.3 Hz, 1H), 6.34 (d, *J* = 2.2 Hz, 1H), 3.93 (s, 3H), 3.92 (d, *J* = 144.8 Hz, 3H); ^{13}C-NMR (125 MHz, acetone-d_6) δ: 183.2,166.6, 165.0, 163.8, 163.1, 158.7, 129.1, 124.2, 115.4, 106.0, 104.6, 98.8, 93.3, 56.5*, 56.0; HR-ESI-MS *m/z* calcd. $C_{16}{}^{13}C_1H_{15}O_5$ $[M+H]^+$: 300.0948, found 300.0944.

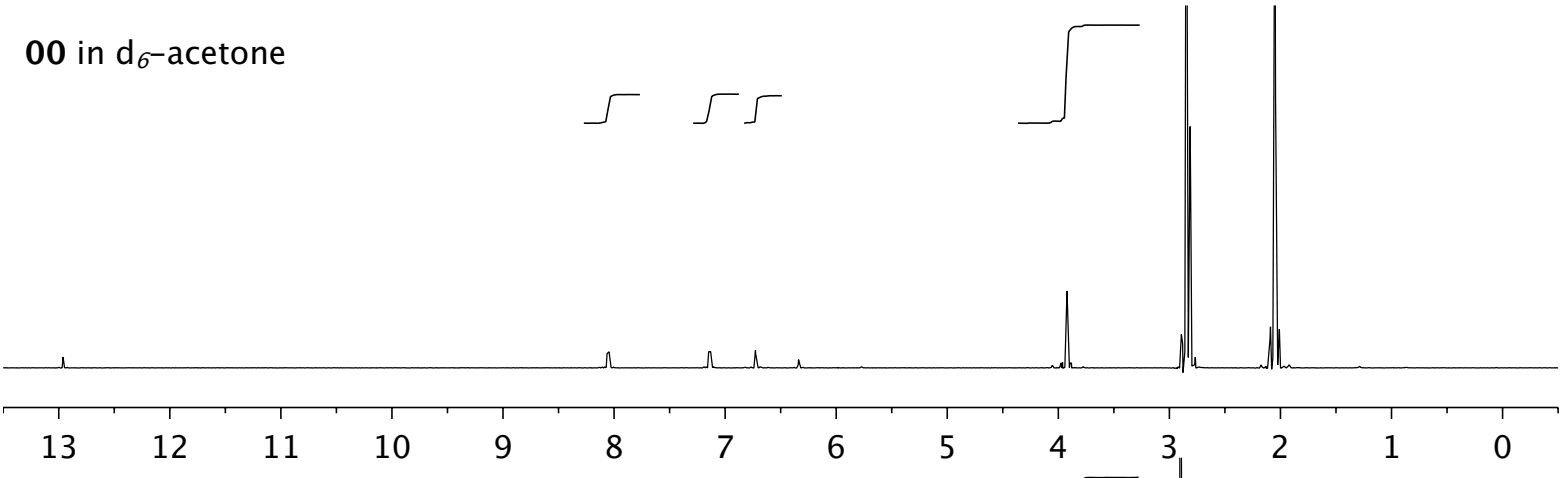
G. Synthesis of ^{13}C-4',^{13}C-7-methylapigenenin (11). ^{13}C-4'-methylapigenenin (10.2 mg, 0.039 mmol) dissolved in dry DMF (0.5 mL). Solid K_2CO_3 (15.9 mg, 0.115 mmol) followed by ^{12}C-methyliodide (4.9 µL, 0.079 mmol). After 18h at rt, EtOH (1 mL) was added and the mixture was dried by airflow. The residue was purified by flash chromatography (hexanes to 1:1 hexanes/EtOAc) to afford 7.8 mg of **01** (73%), as a colorless solid.

^{13}C-4',^{13}C-7-methylapigenenin (**11**): ^{1}H-NMR (500 MHz, DMSO-d_6) δ ^{1}H-NMR (500 MHz, acetone-d_6) δ 12.96 (s, 1H), 8.05 (d, *J* = 9.0 Hz, 1H), 7.14 (d, *J* = 9.0 Hz, 1H), 6.72 (s, 1H), 6.71 (d, *J* = 2.2 Hz, 1H), 6.34 (d, *J* = 2.3 Hz, 1H), 3.93 (d, *J* = 145.4 Hz, 3H), 3.92 (d, *J* = 144.8 Hz, 3H); δ: 183.2,166.6, 165.0, 163.8, 163.1, 158.7, 129.1, 124.2, 115.4, 106.0, 104.5, 98.7, 93.2, 56.6*, 56.0*; HR-ESI-MS *m/z* calcd. $C_{15}{}^{13}C_2H_{15}O_5$ $[M+H]^+$: 301.0981, found 301.0979.

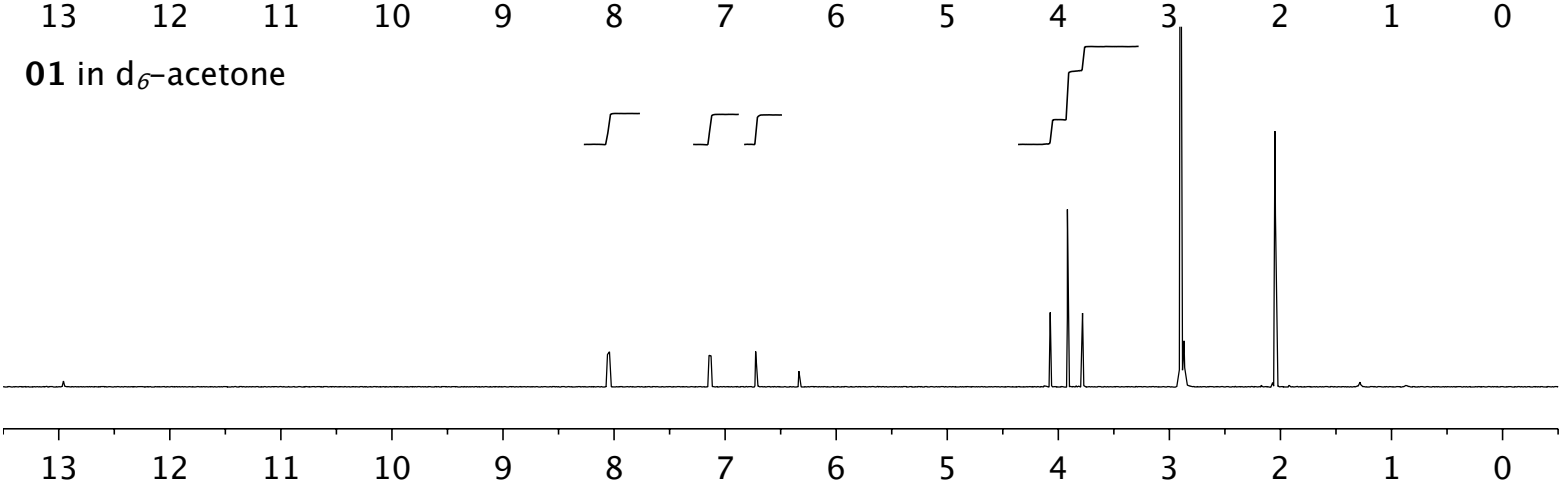
H. Mixture preparation and analysis. Solutions of compounds were prepared in volumetric flasks at 100.0±0.1 µM in CH_2Cl_2. A volume of the minor components was added to a second 100 mL volumetric flask using a syringe with accuracy to ±0.1 mL. The volumetric flask was then filled with the major component and mixed. The resulting solution was then dried on a rotary evaporator. The resulting mixtures were conducted by NMR analyses in acetone-d_6.

^{1}H–NMR (500 MHz) spectra of **00**, **01**, **10**, and **11**

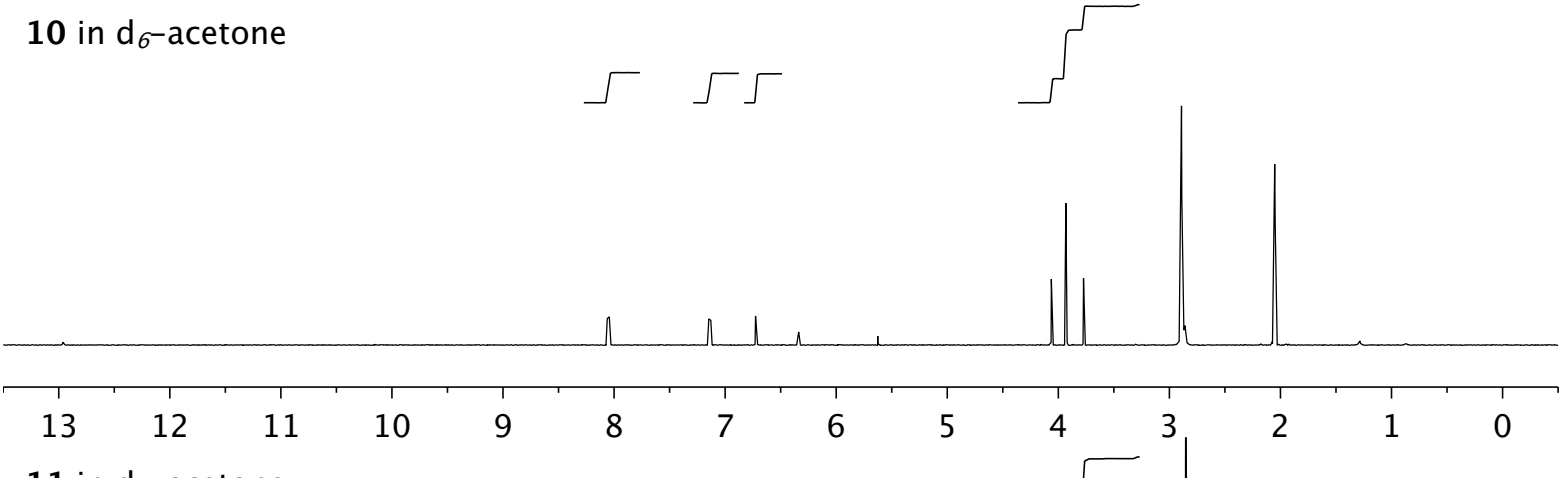
00 in d_6–acetone



01 in d_6–acetone



10 in d_6–acetone



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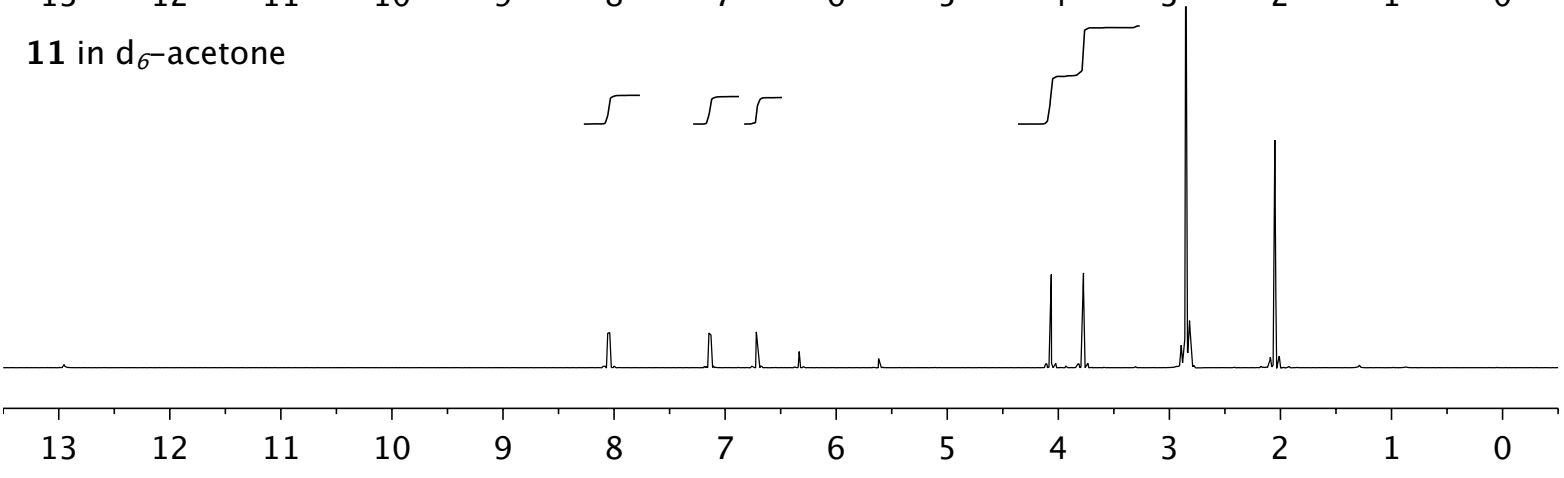
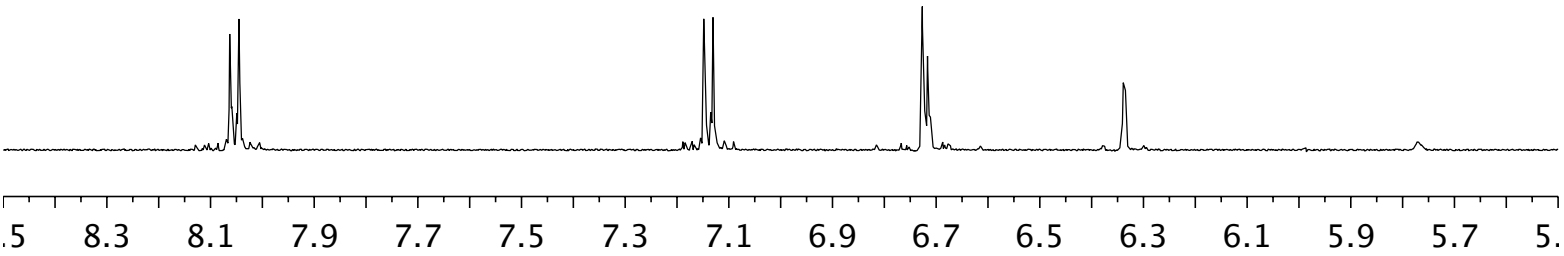
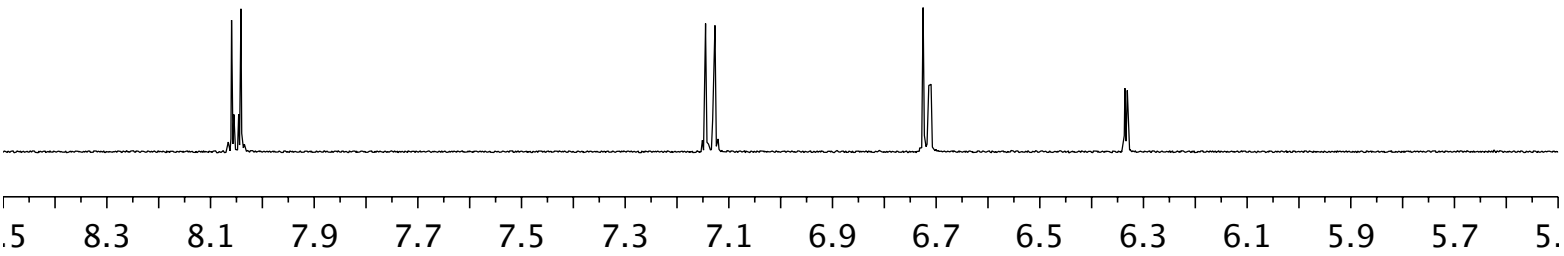


^{1}H–NMR (500 MHz) spectra of **00**, **01**, **10**, and **11**

00 in d_6–acetone



01 in d_6–acetone



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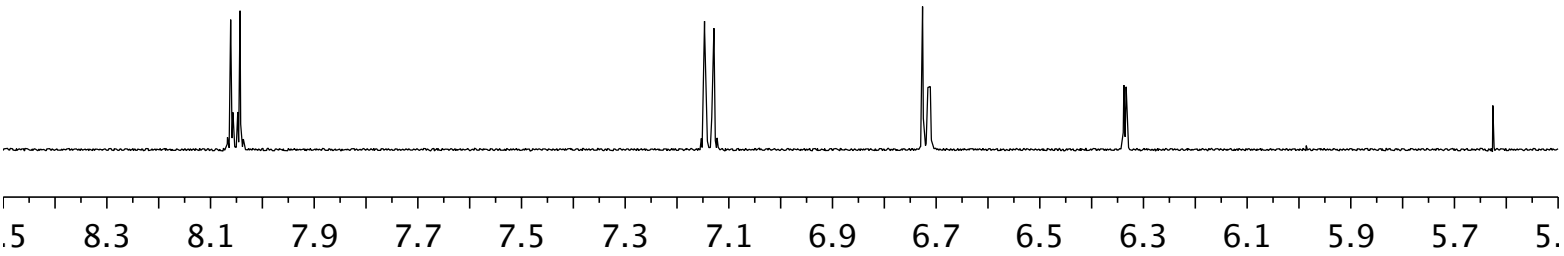
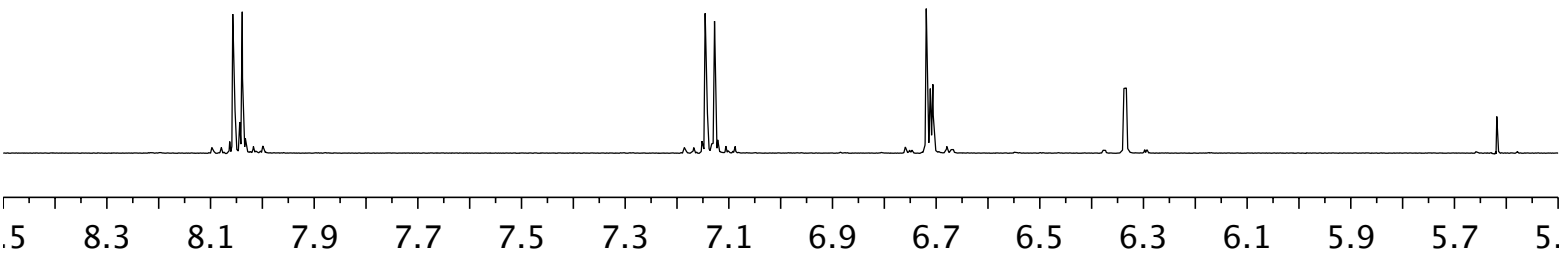
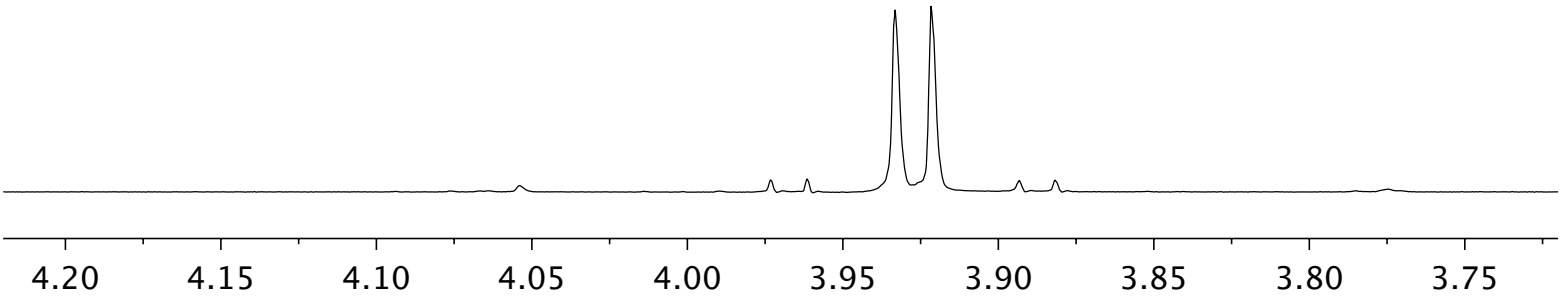


11 in d_6–acetone

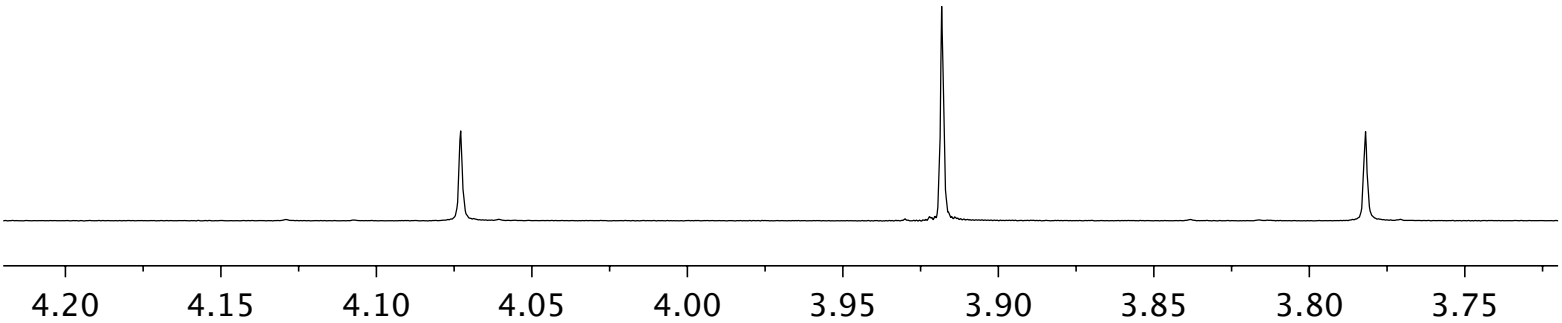


^{1}H–NMR (500 MHz) spectra of **00**, **01**, **10**, and **11**

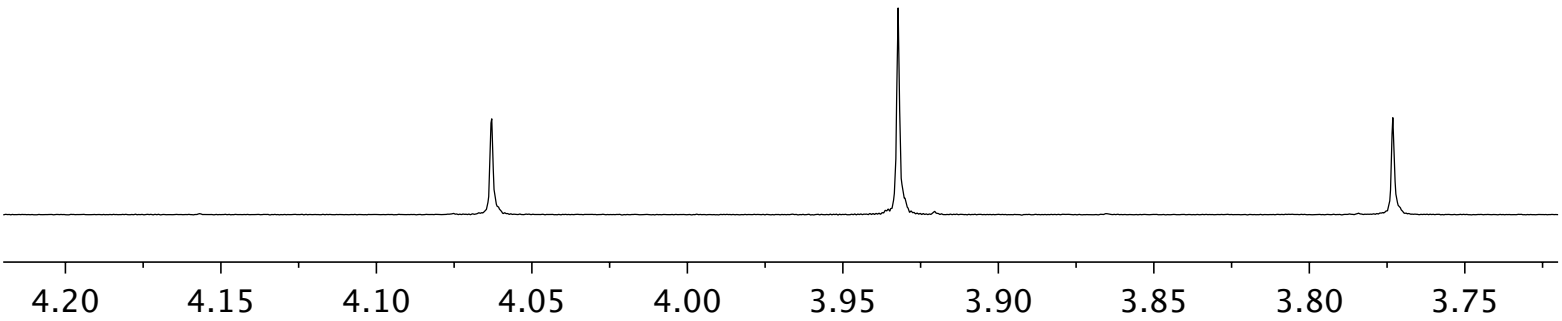
00 in d_6–acetone



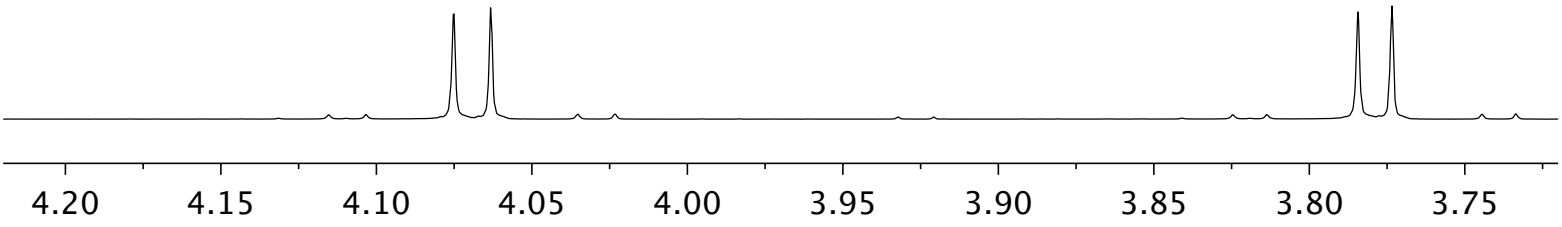
01 in d_6–acetone



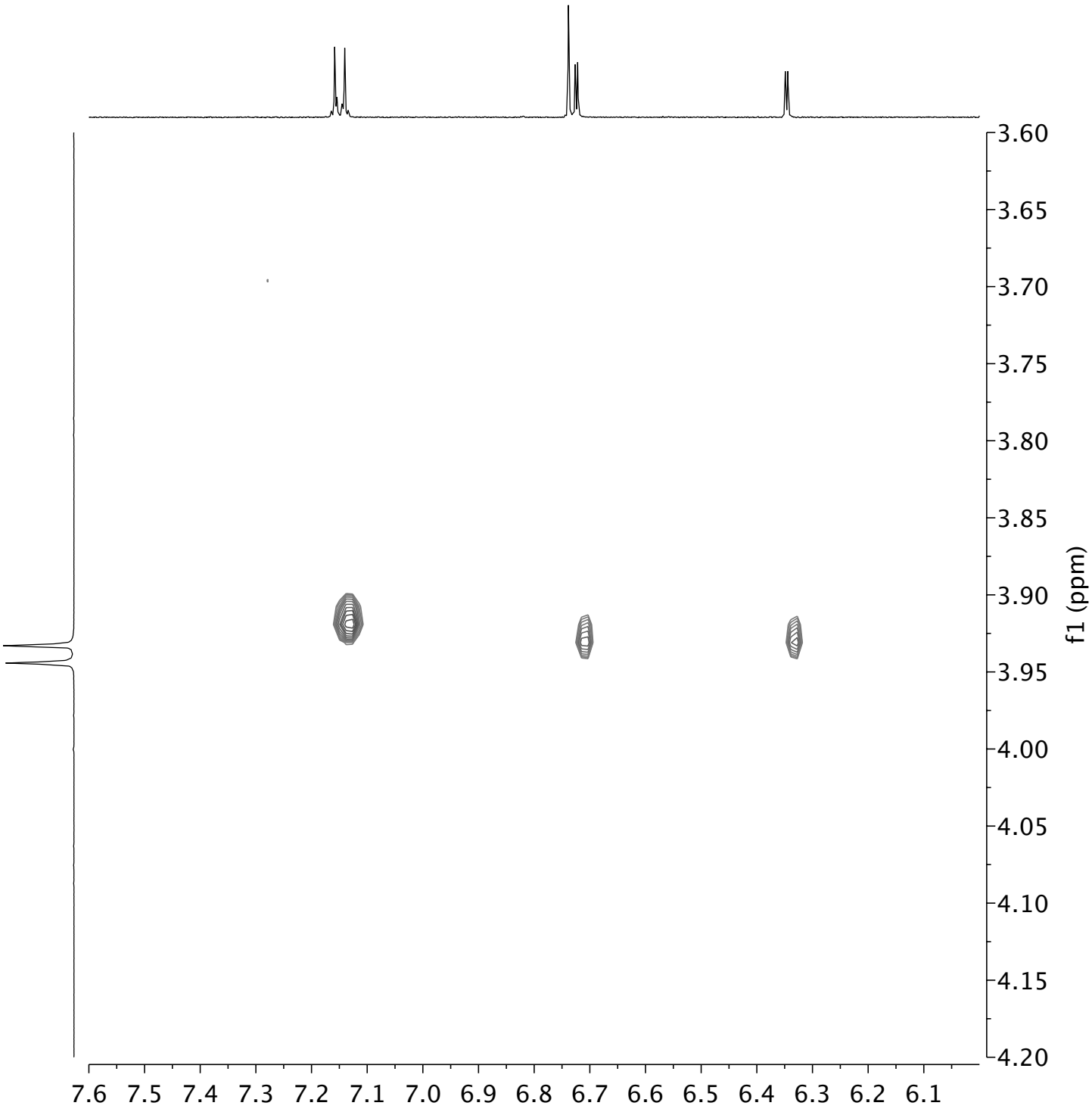
10 in d_6–acetone



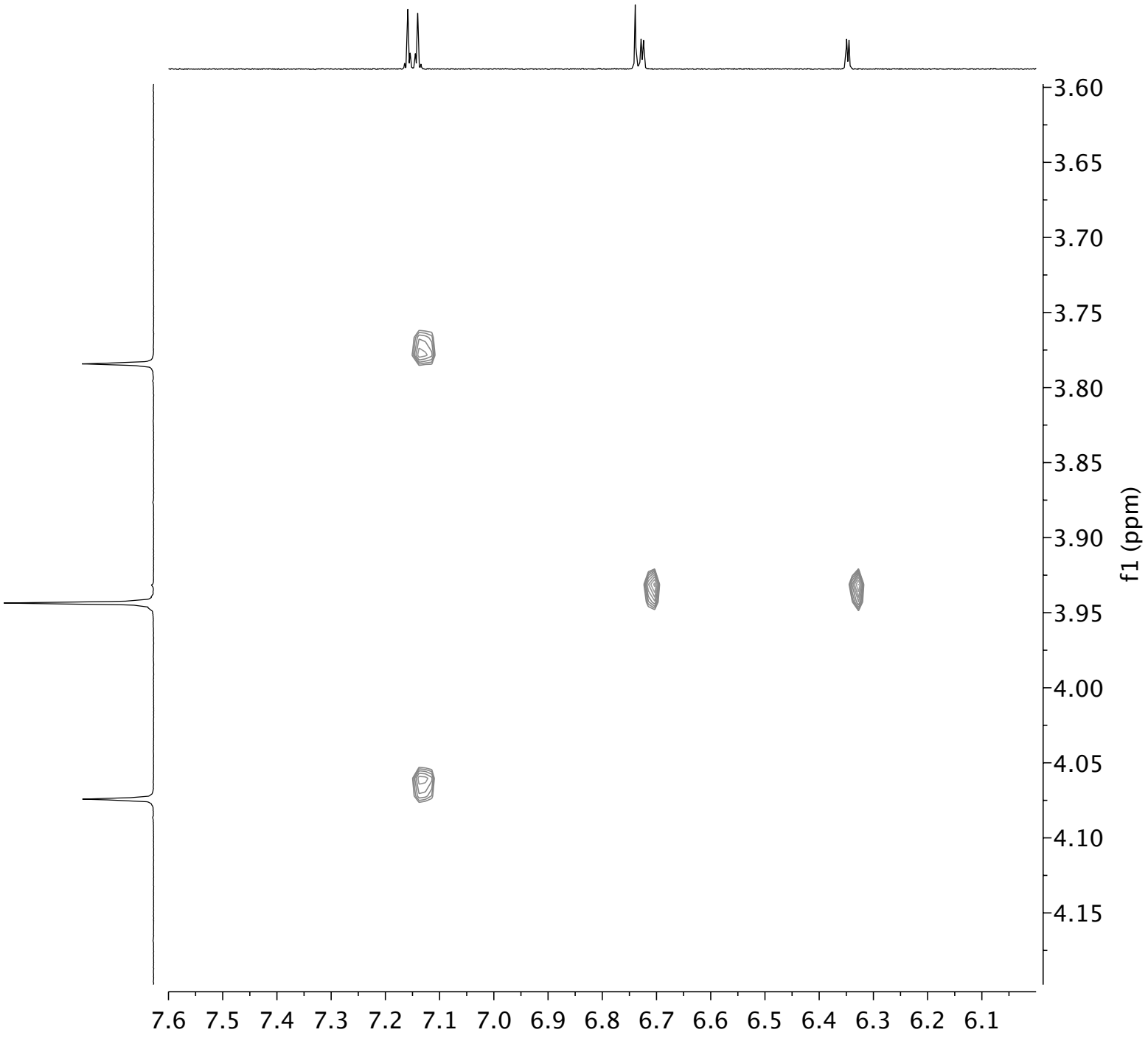
11 in d_6–acetone



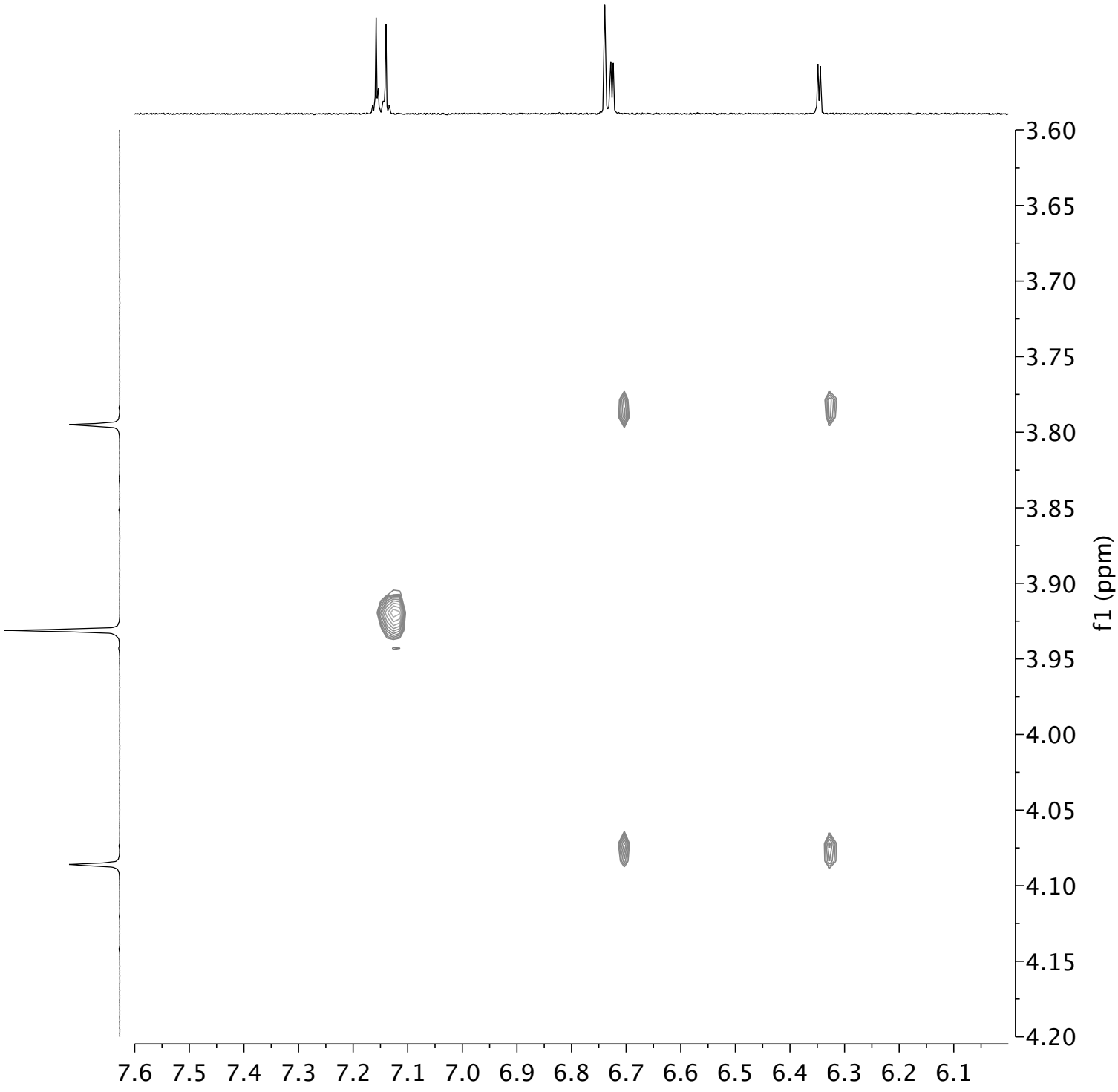
An expansion of the $^{1}H,^{1}H$-NOESY (500 MHz) spectrum of **00** in d_6-acetone



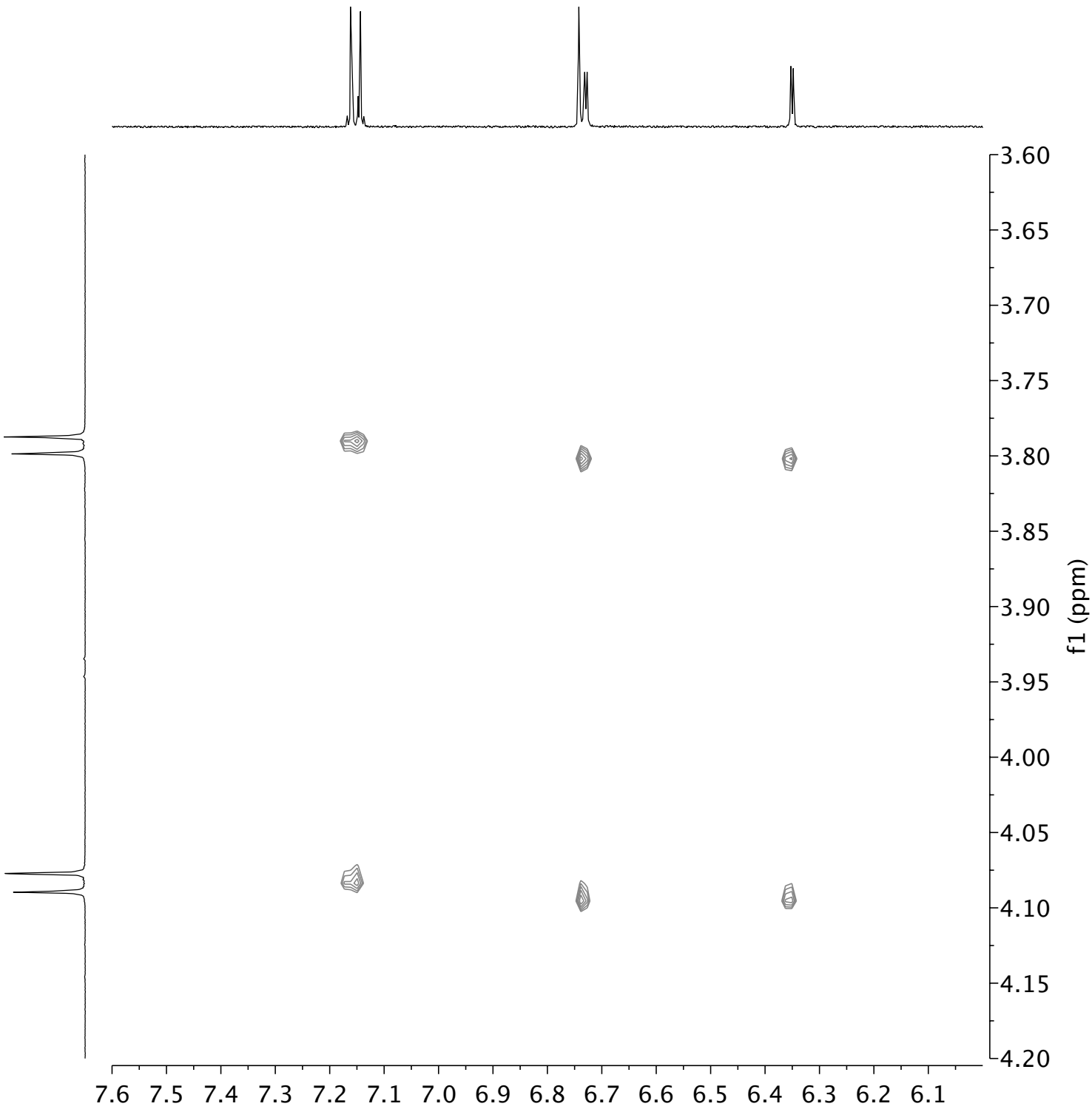
An expansion of the $^{1}H,^{1}H$-NOESY (500 MHz) spectrum of **01** in d_6-acetone



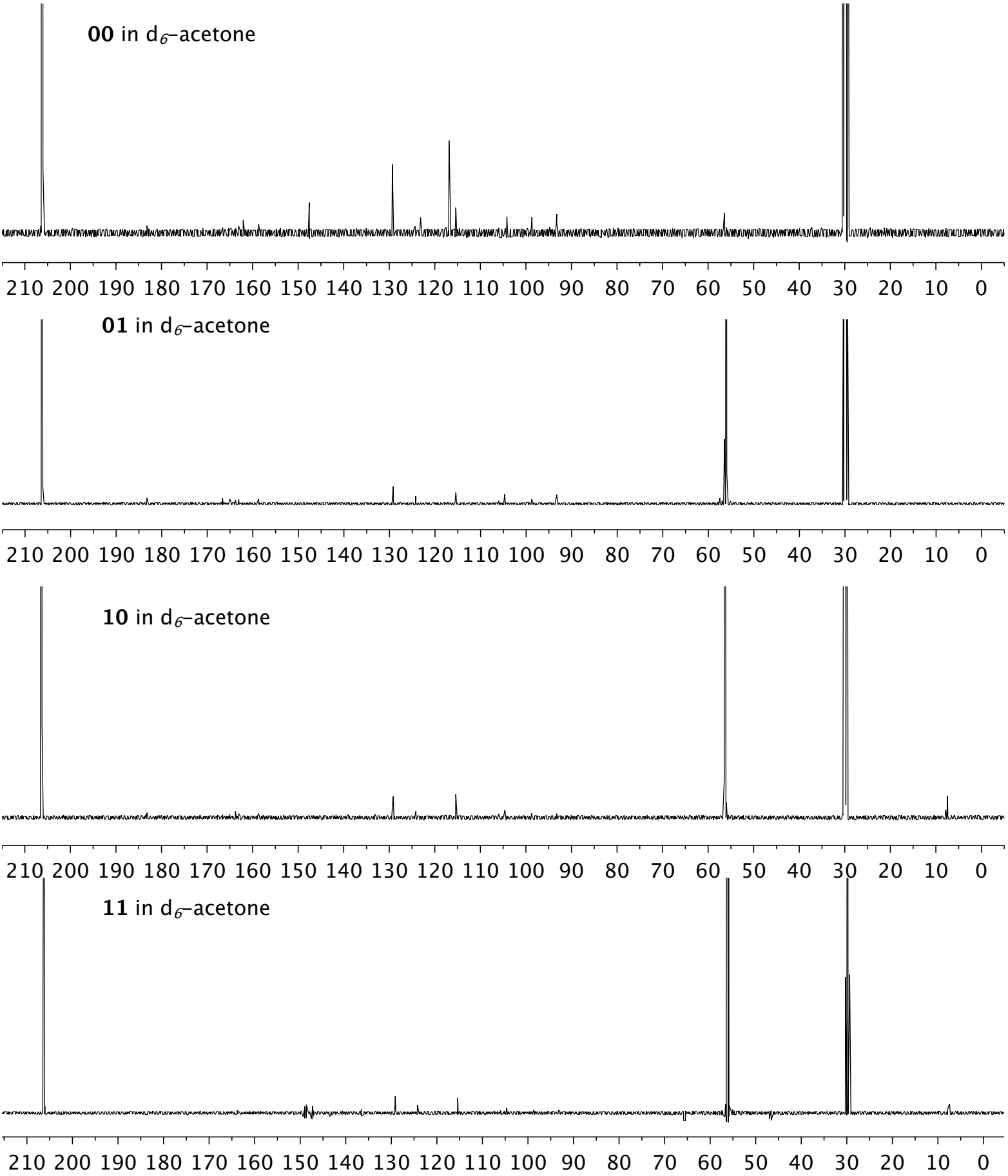
An expansion of the $^{1}H,^{1}H$-NOESY (500 MHz) spectrum of **00** in d_6-acetone



An expansion of the $^{1}H,^{1}H$-NOESY (500 MHz) spectrum of **11** in d_6-acetone

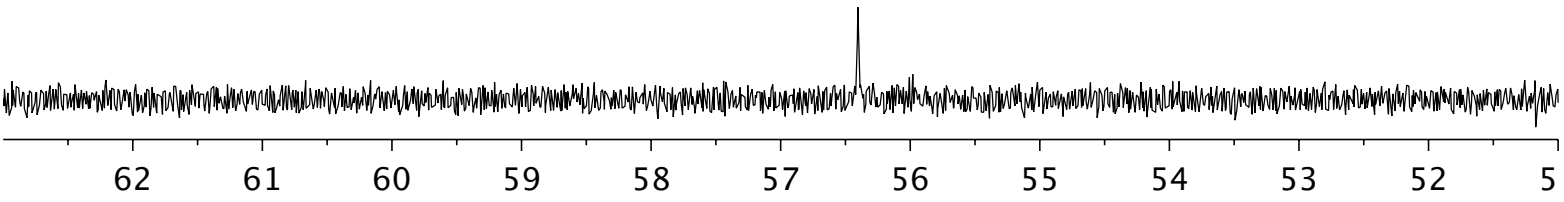


^{13}C–NMR (125 MHz) spectra of **00**, **01**, **10**, and **11**

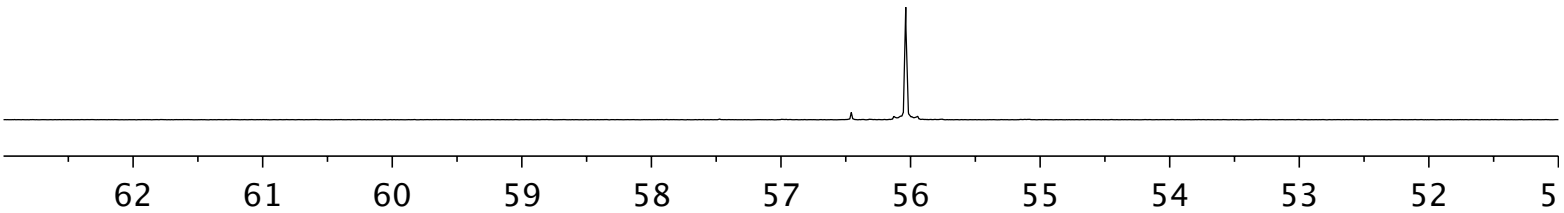


^{13}C–NMR (125 MHz) spectra of **00**, **01**, **10**, and **11**

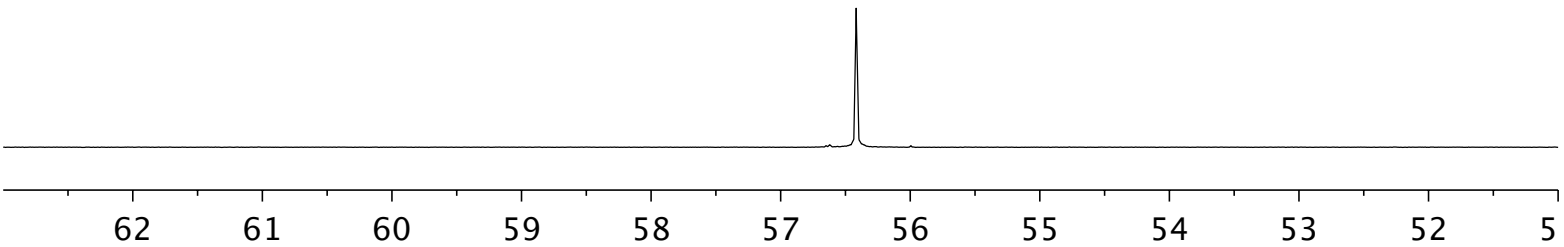
00 in d$_6$–acetone



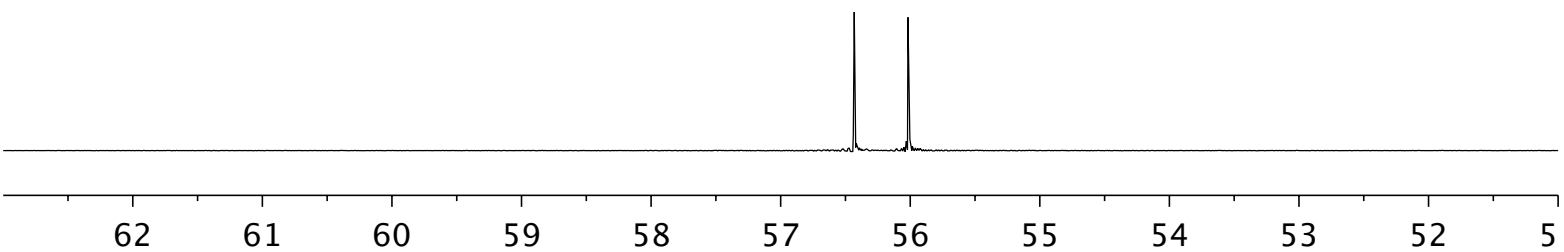
01 in d$_6$–acetone



10 in d$_6$–acetone

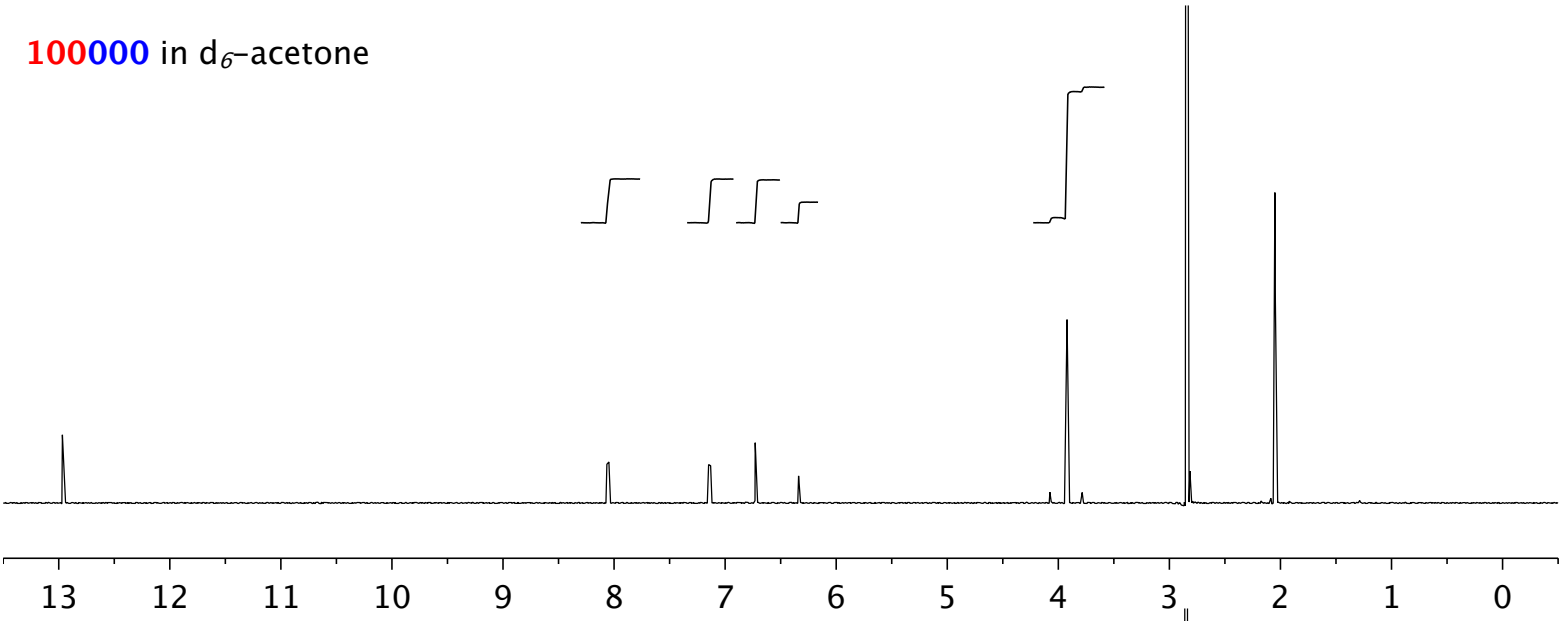


11 in d$_6$–acetone

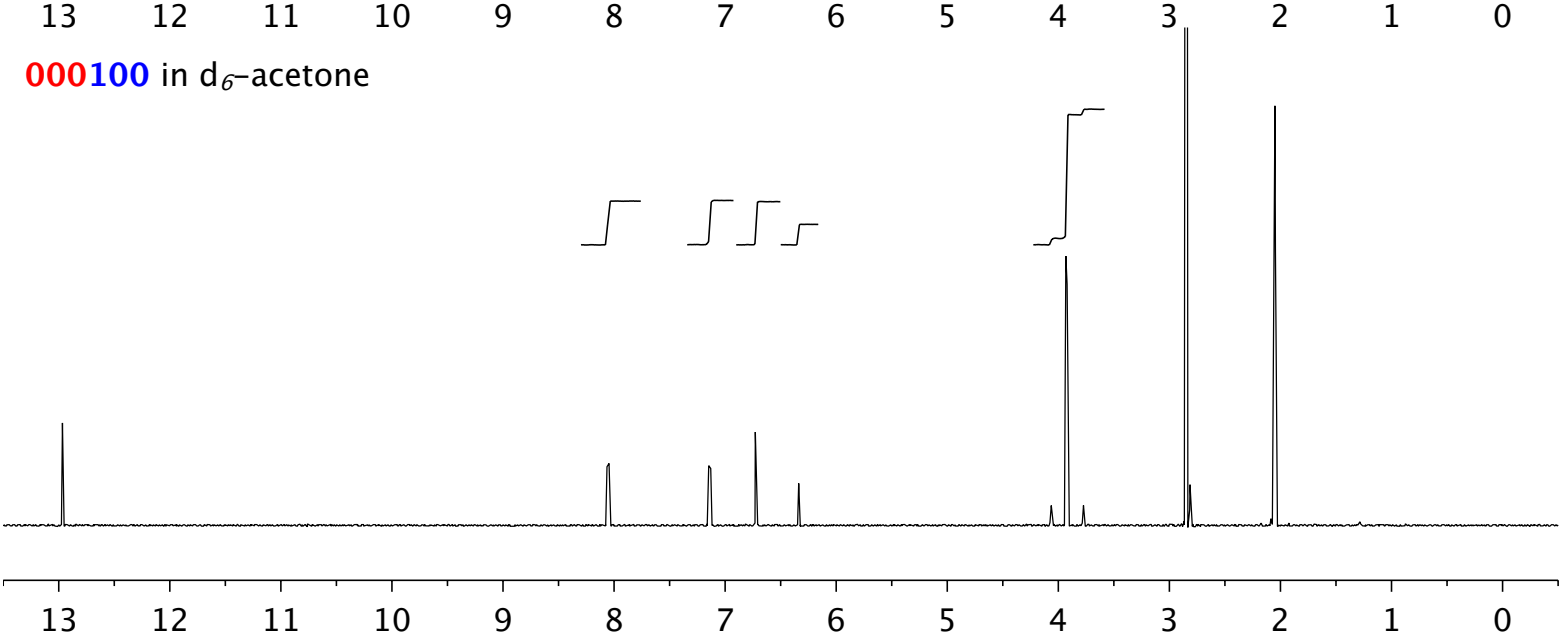


^{1}H-NMR (500 MHz) spectra of **100000**, **000100**, and **100100** as shown in Fig. 4

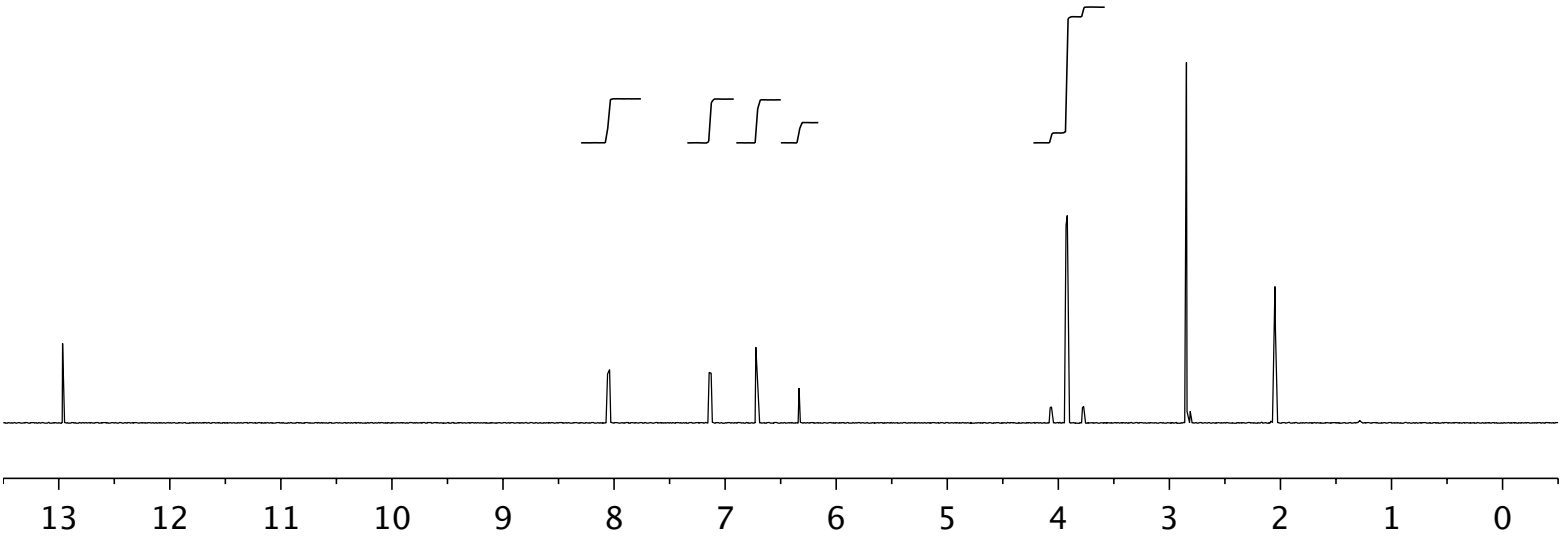
100000 in d_6-acetone



000100 in d_6-acetone

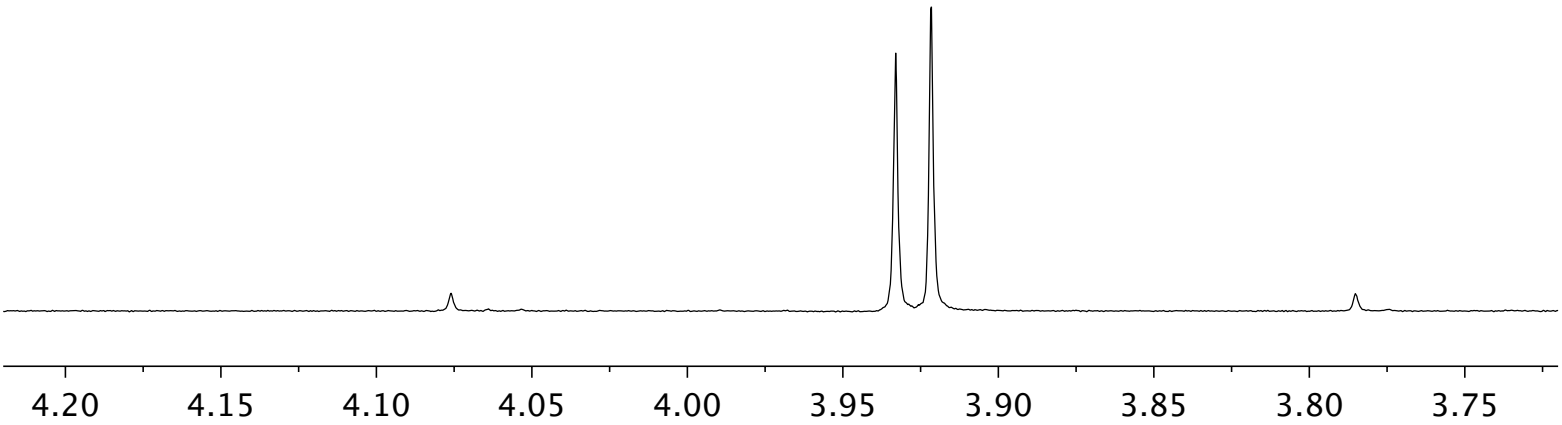


100100 in d_6-acetone

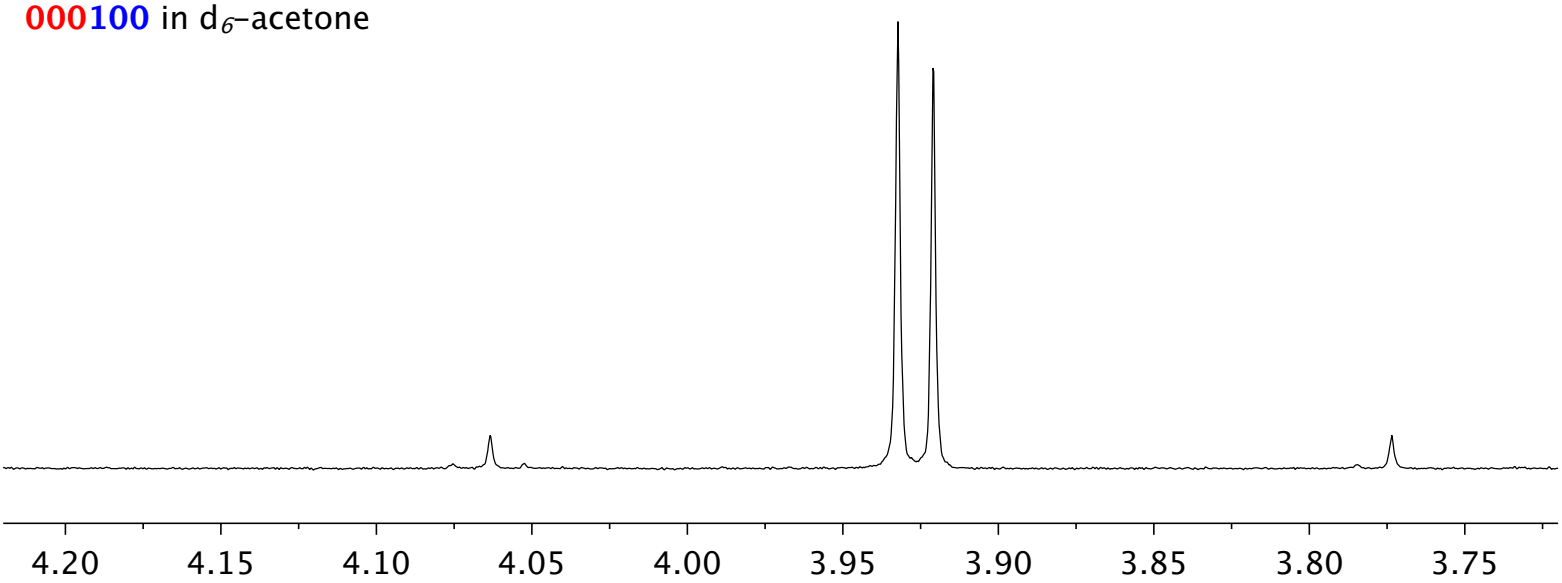


^{1}H–NMR (500 MHz) spectra of **100000**, **000100**, and **100100** as shown in Fig. 4

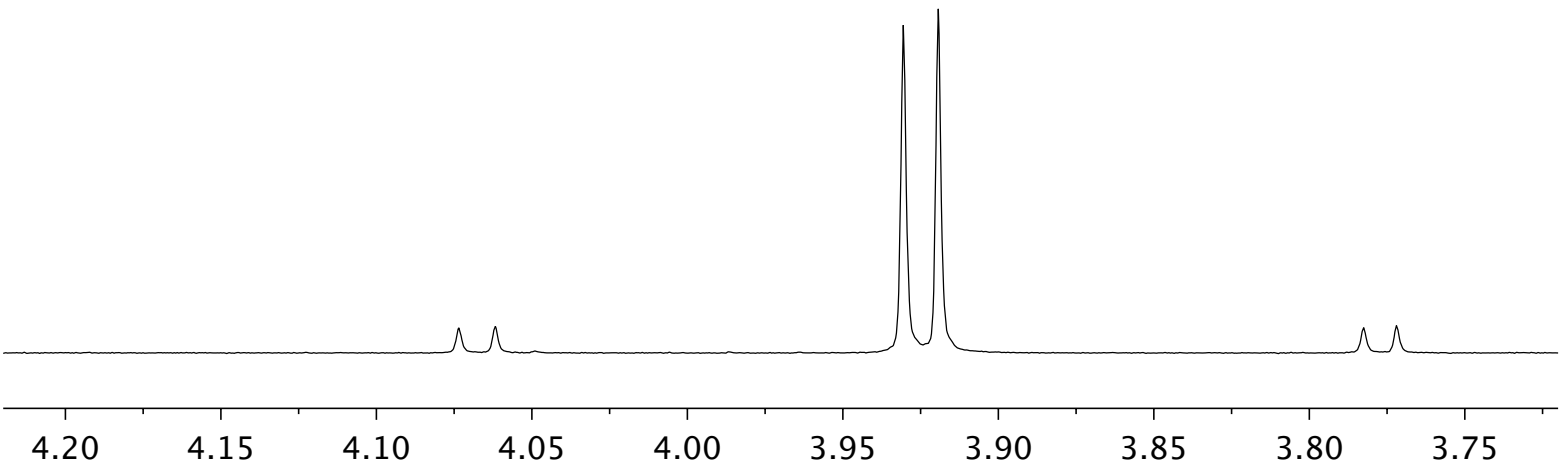
100000 in d_6–acetone



000100 in d_6–acetone

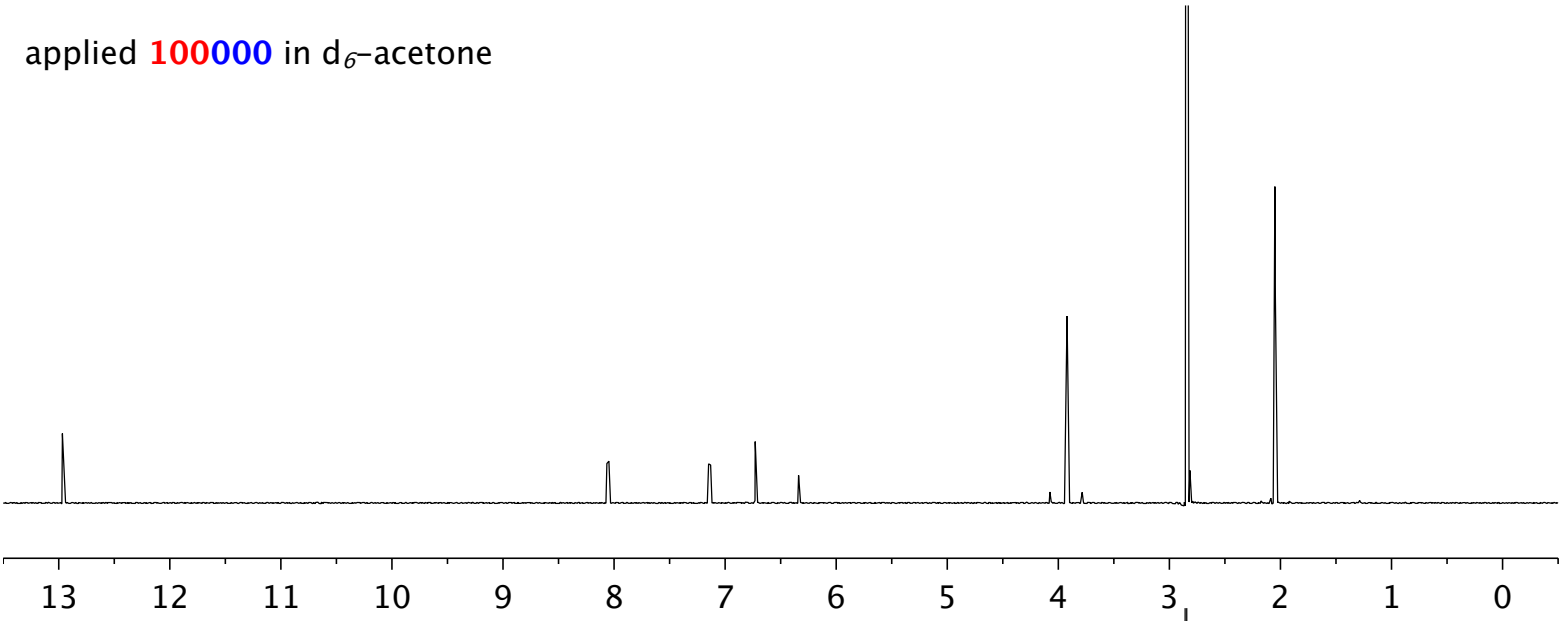


100100 in d_6–acetone

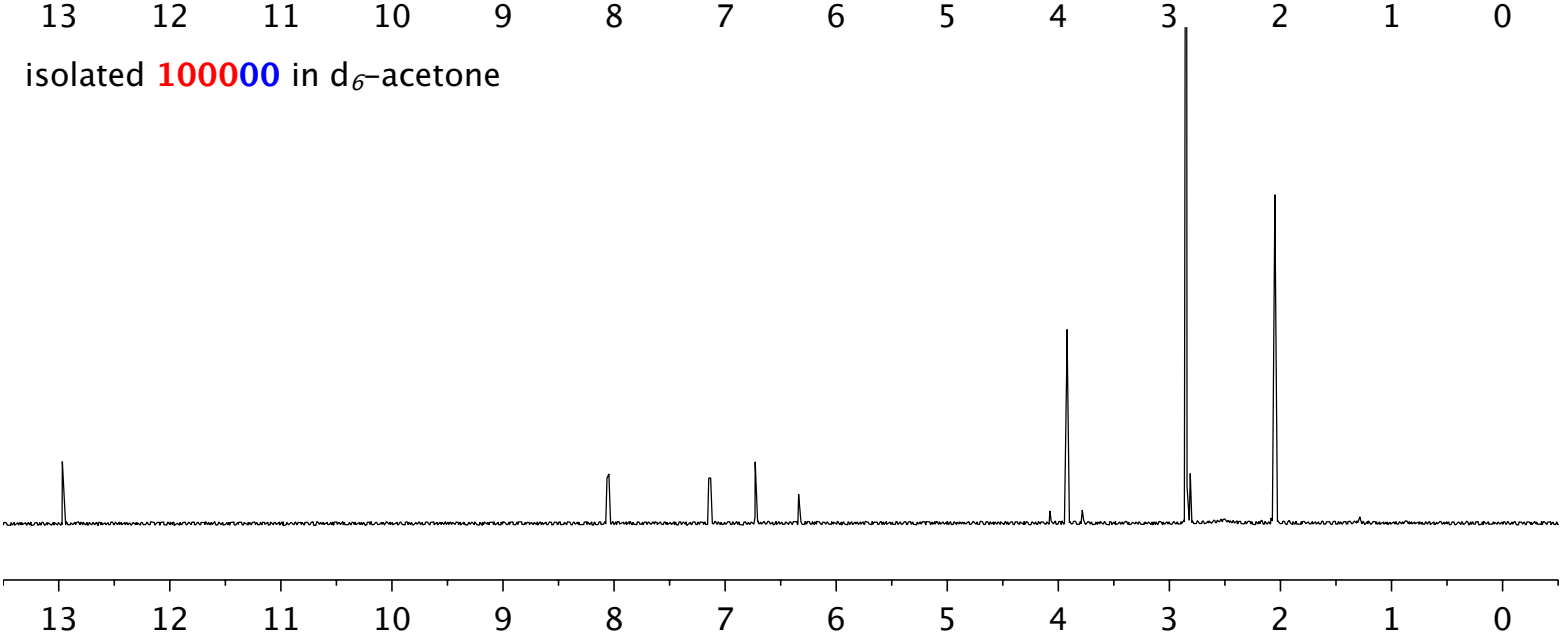


^{1}H-NMR (500 MHz) spectra of applied **100000** and isolated **000100** as shown in Fig. 5

applied **100000** in d_6-acetone

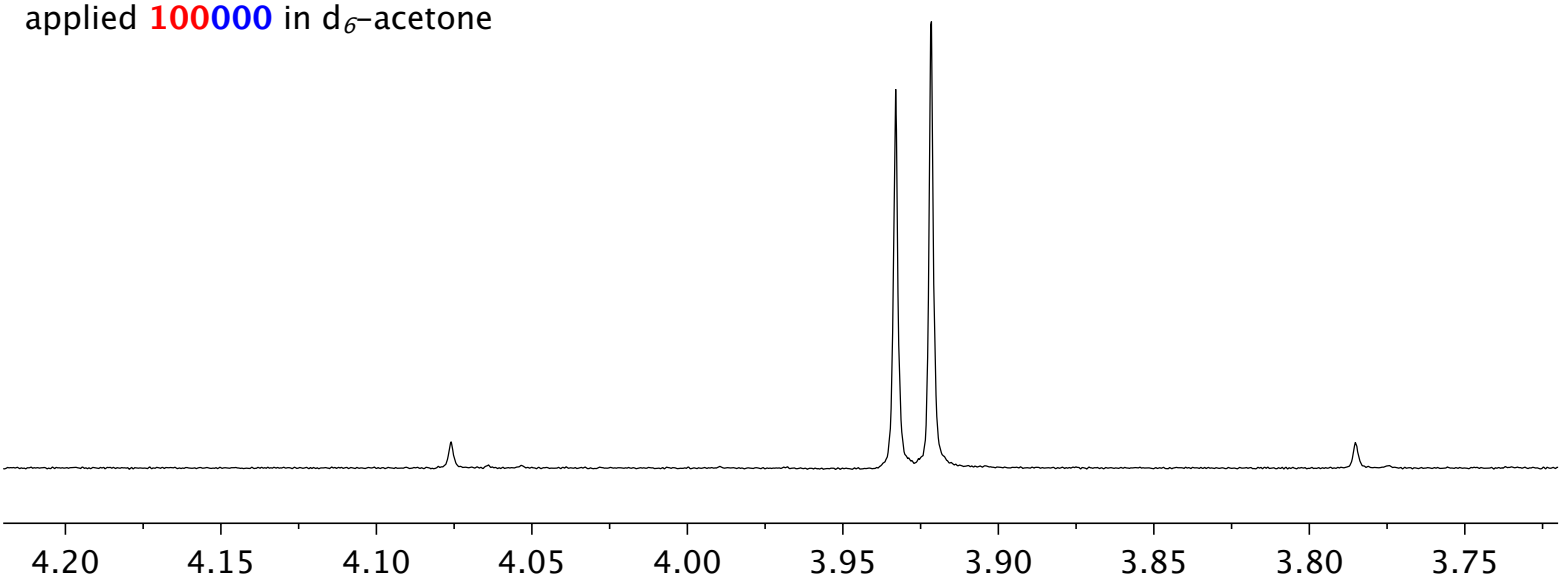


isolated **100000** in d_6-acetone

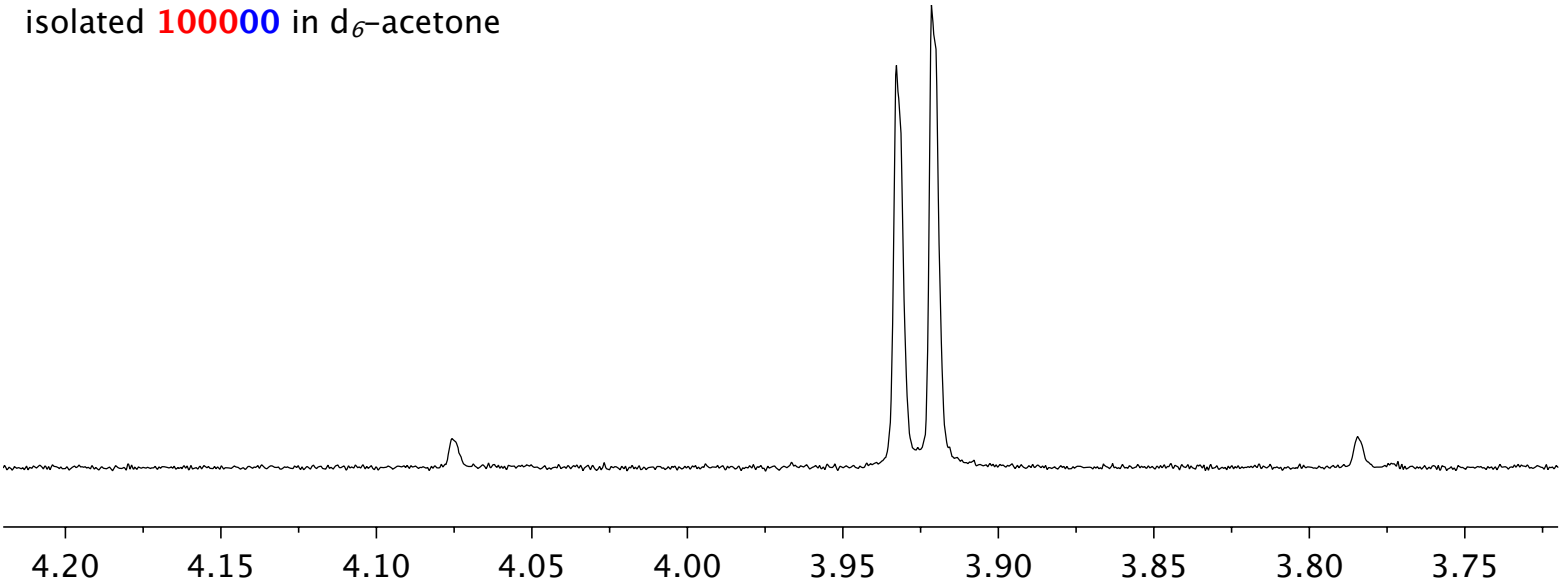


^{1}H-NMR (500 MHz) spectra of applied **100000** and isolated **000100** as shown in Fig. 5

applied **100000** in d_6-acetone

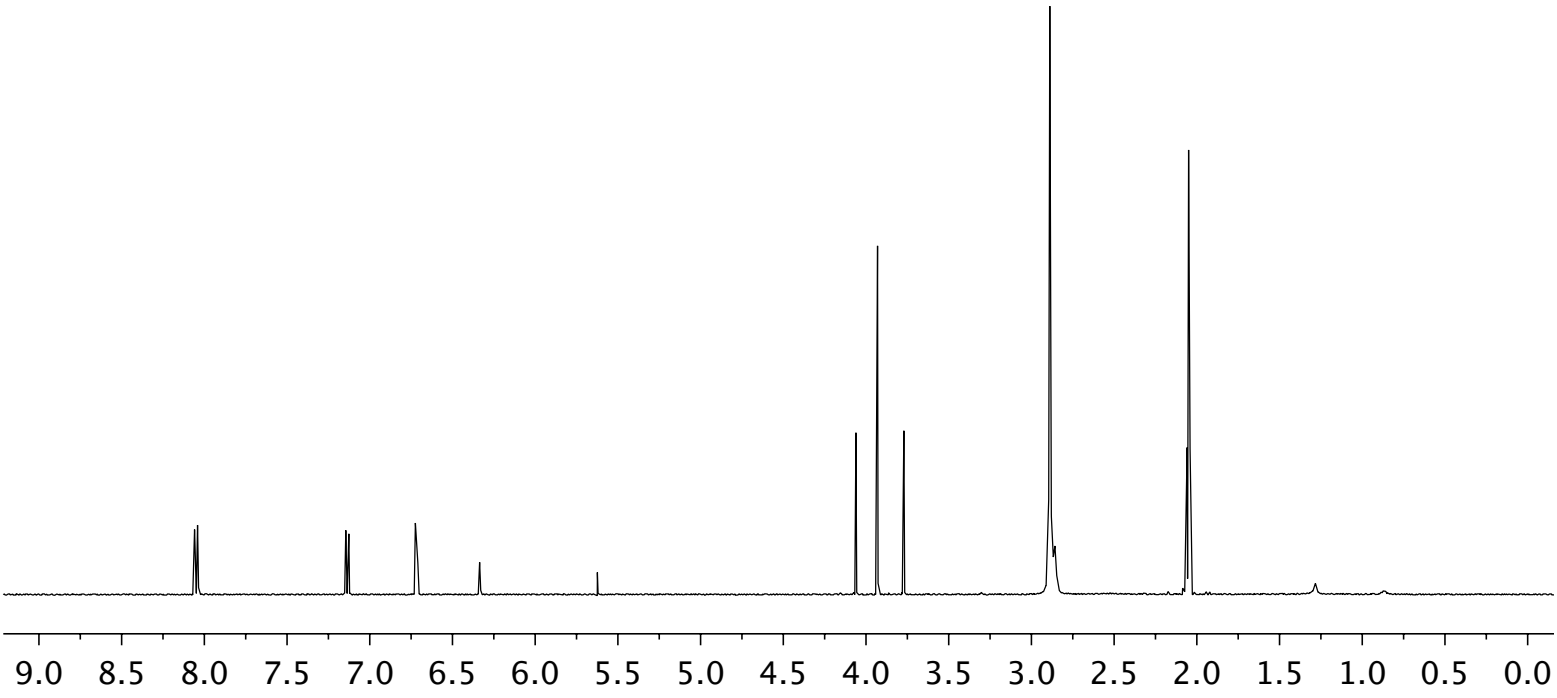


isolated **100000** in d_6-acetone



Stability analyses on materials stored at rt. These spectra were collected on materials prepared in 06/2009 and stored in vials. NMR spectra were collected in 01/2018. These spectra show no decompostion when compared with that presented on page S5.

10 in d_6–acetone



01 in d_6–acetone