

Supporting Information

Caspase-1 Inhibitors from an Extremophilic Fungus that Target Specific Leukemia Cell Lines

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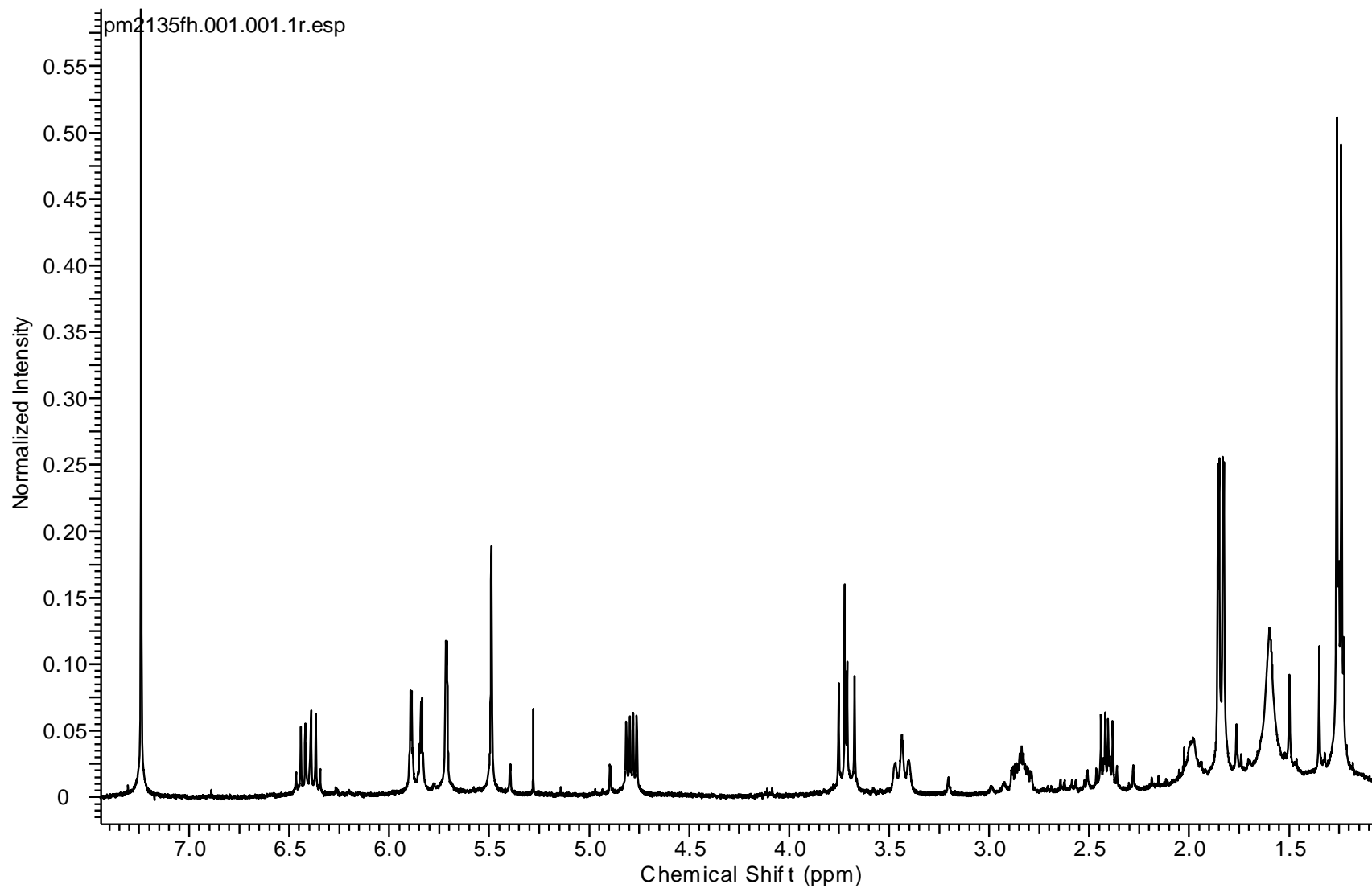
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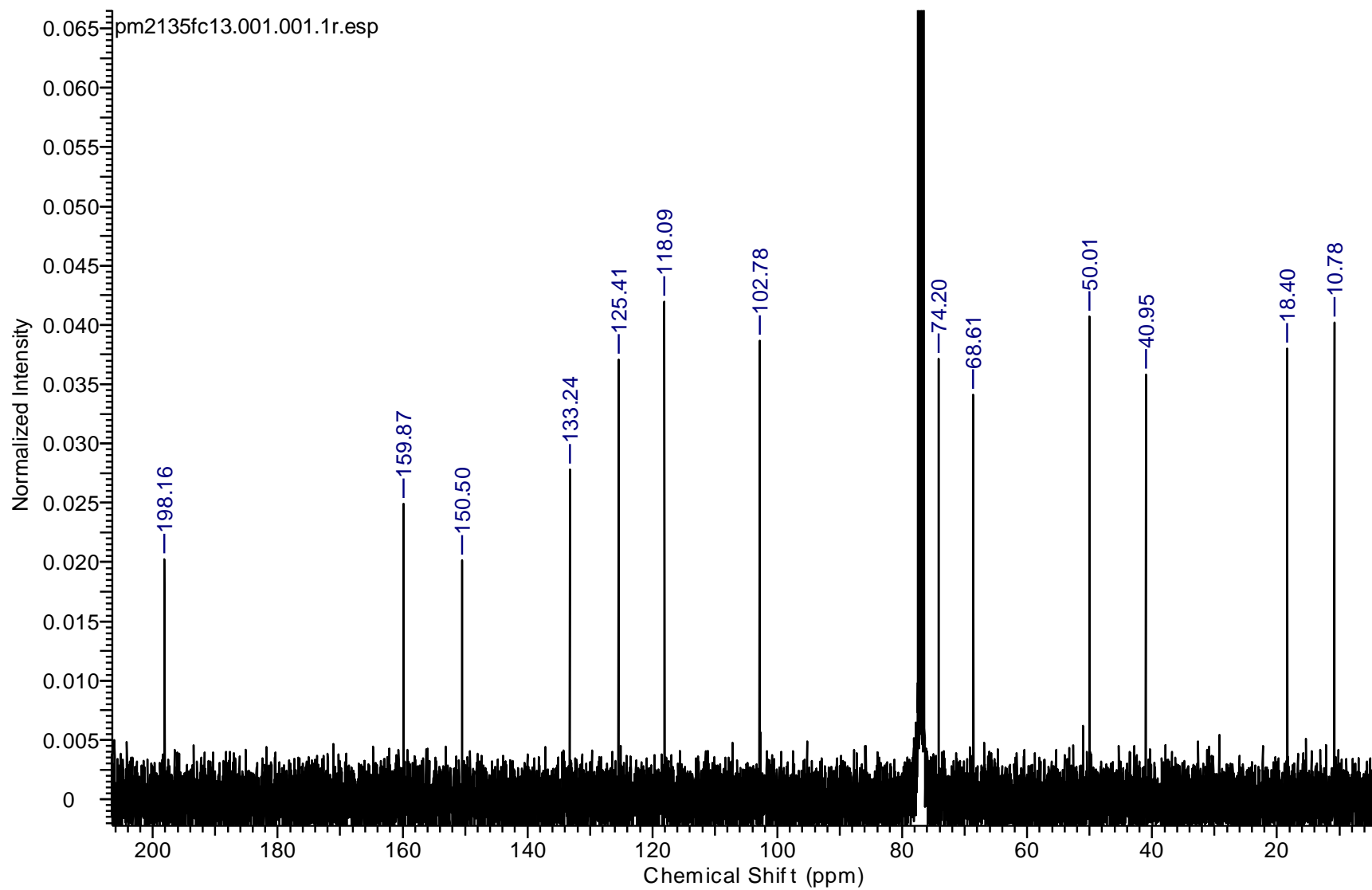
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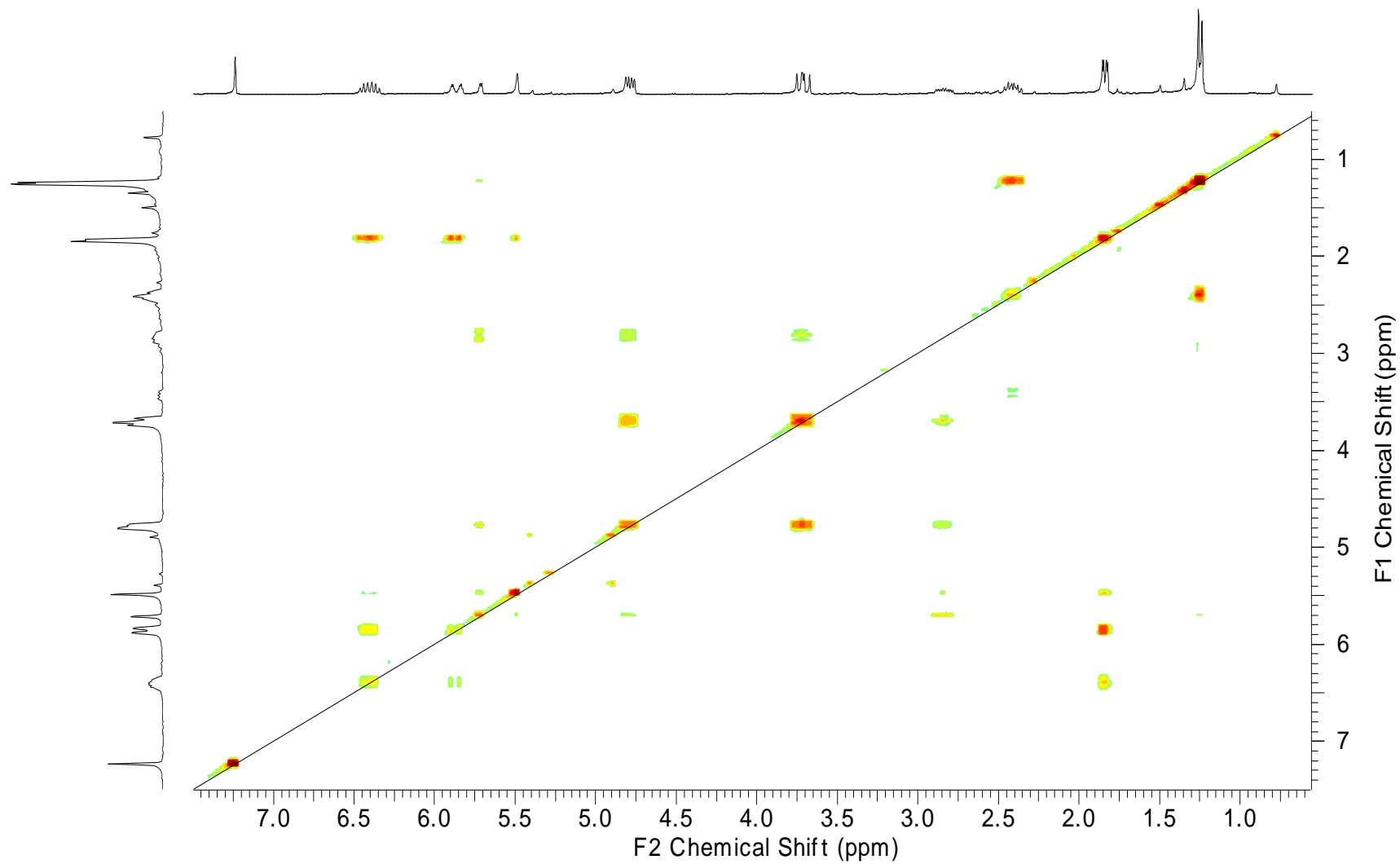
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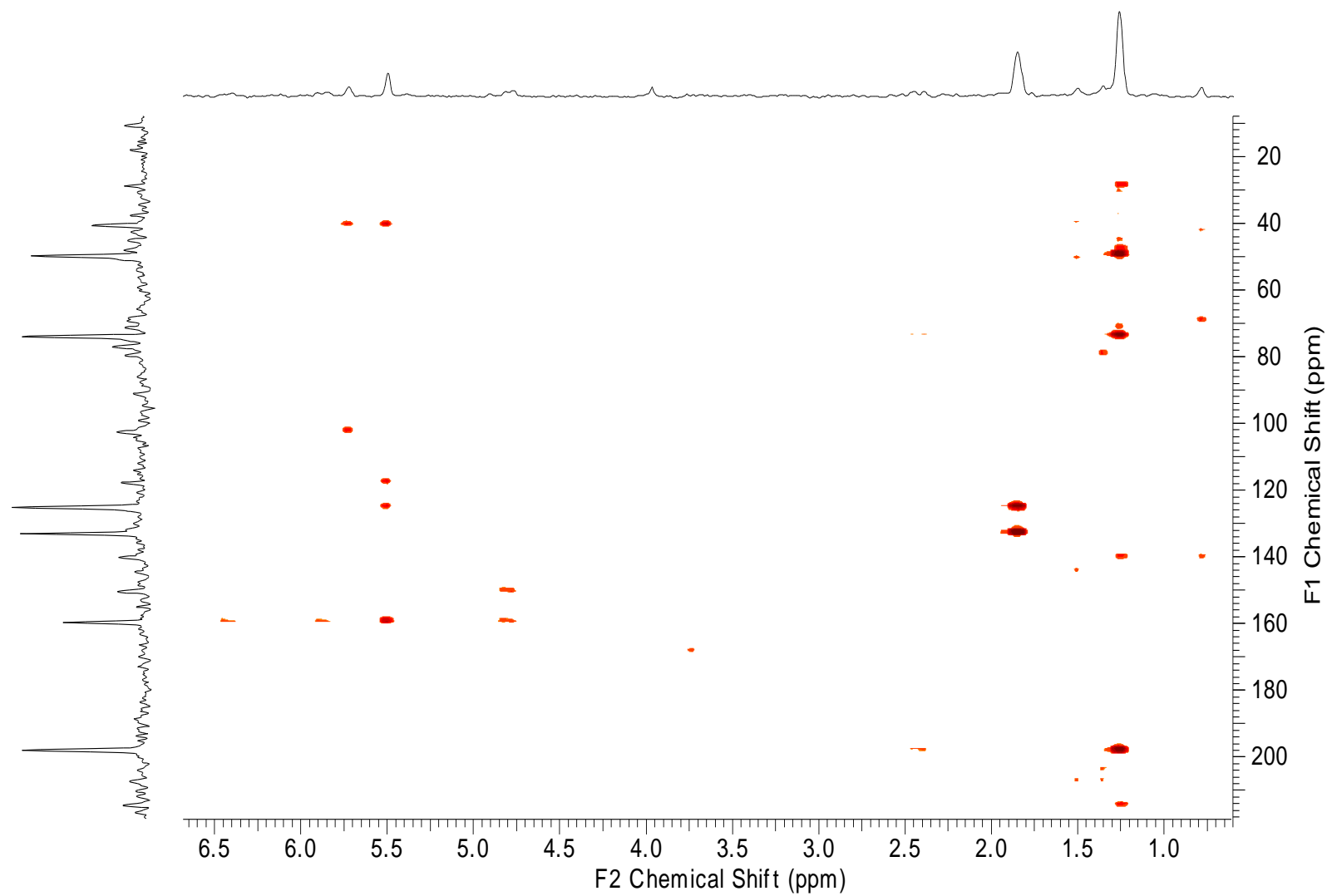
S3 ^1H NMR of Berkazaphilone A (**1**), 300 MHz, CDCl_3



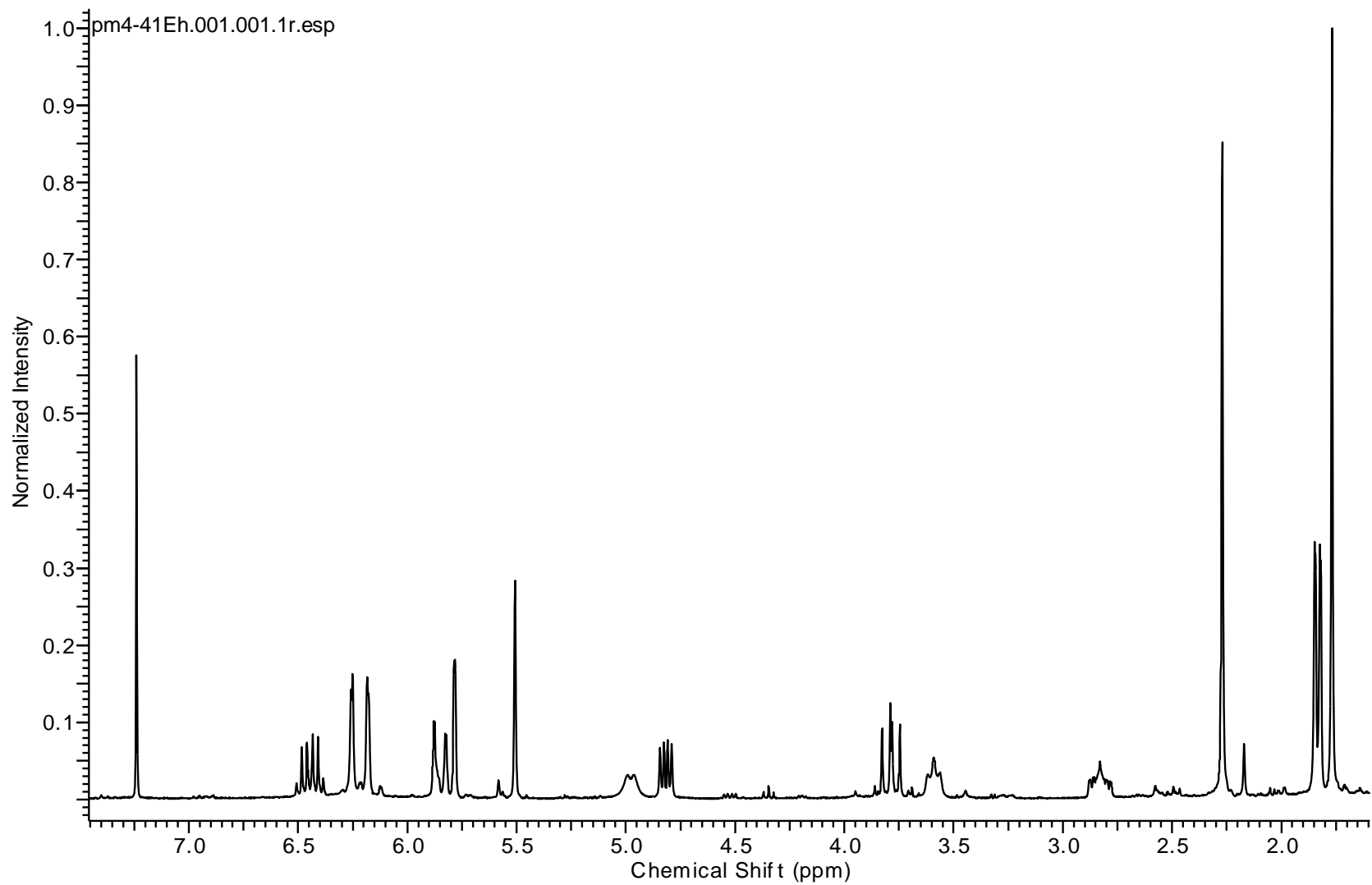
S4 ^{13}C NMR of Berkazaphilone A (1), 75 MHz, CDCl_3



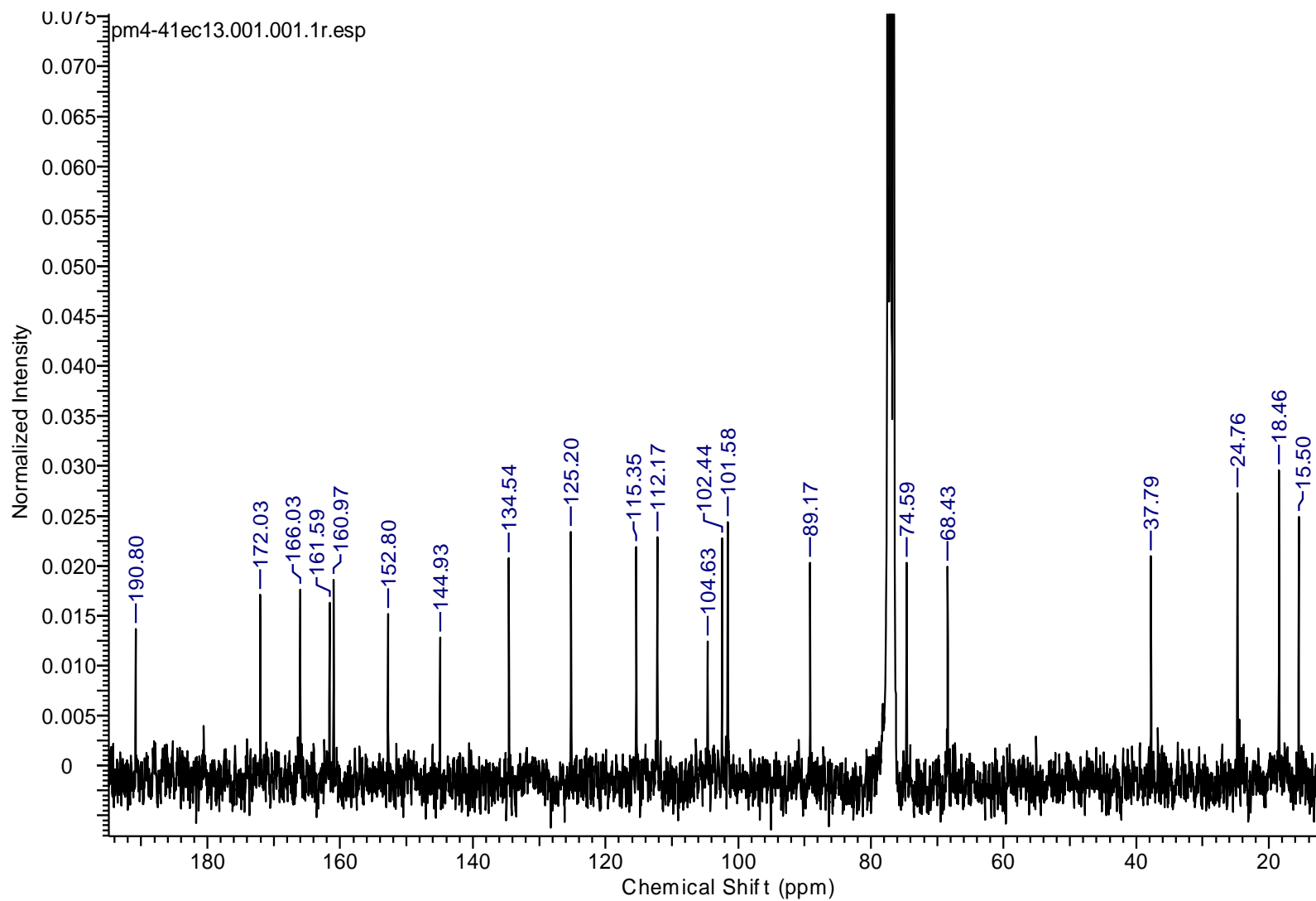
S5 COSY of Berkazaphilone A (1)



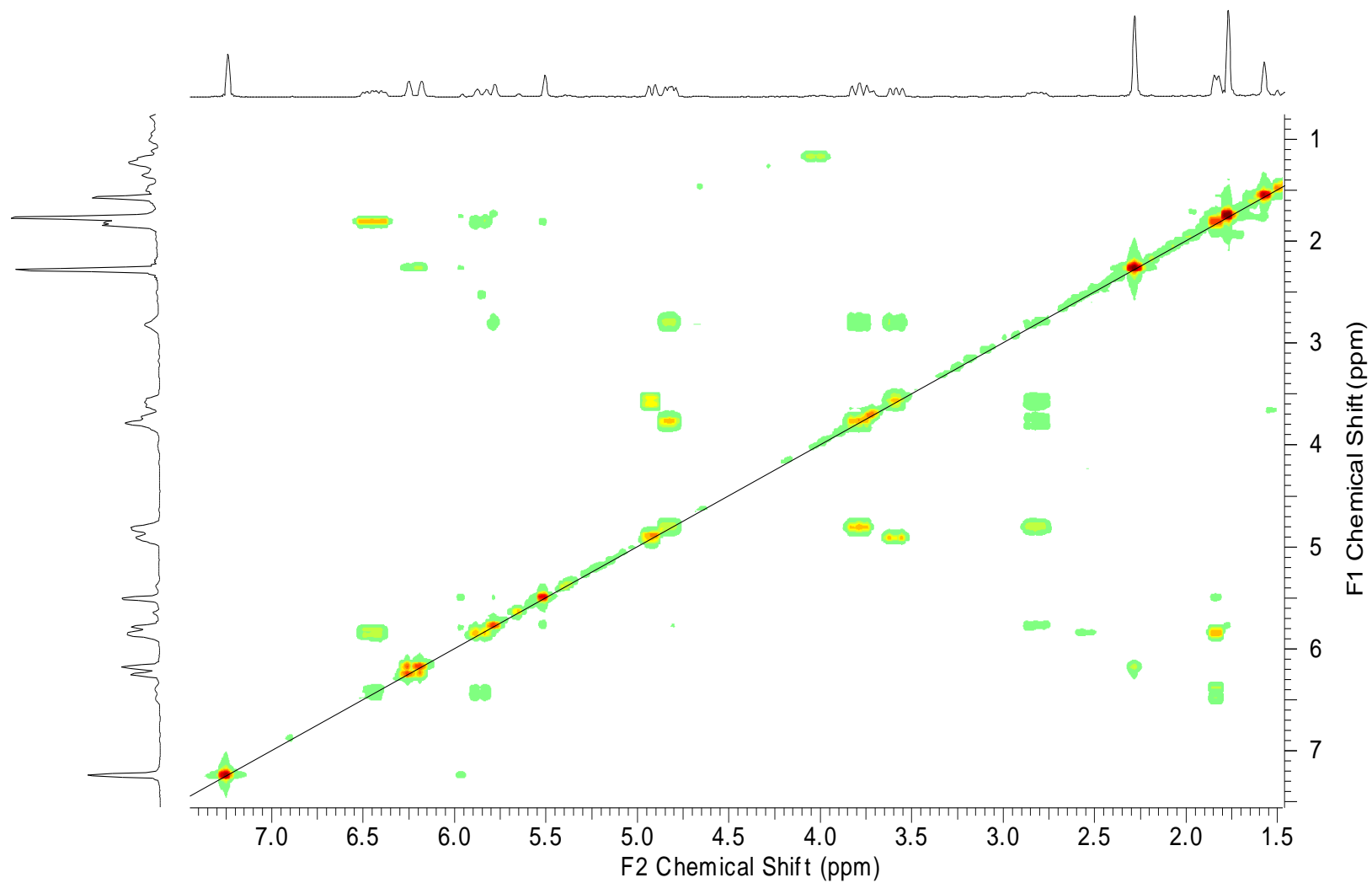
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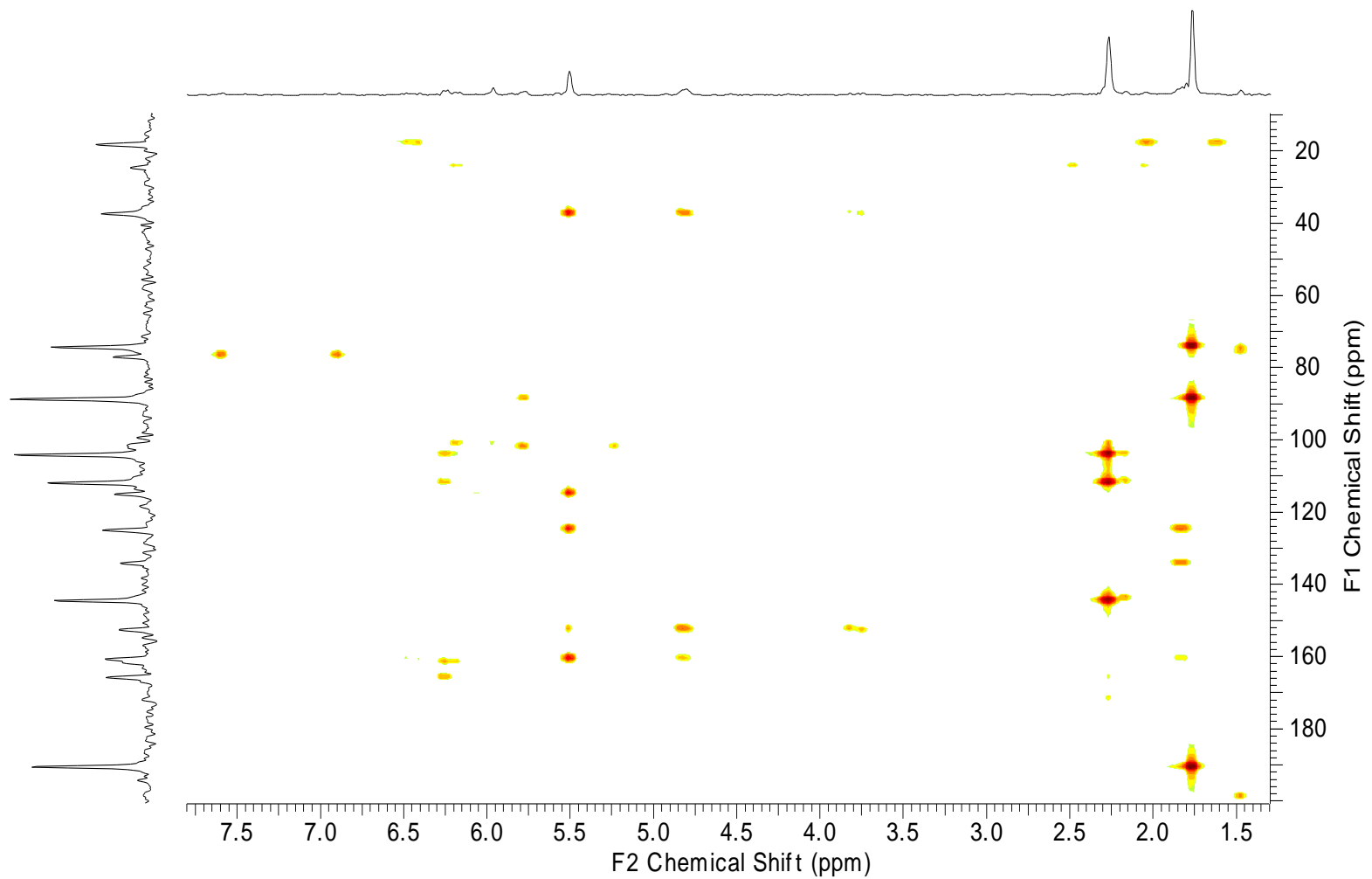
S7 ^1H NMR of Berkazaphilone B (**2**), 300 MHz, CDCl_3



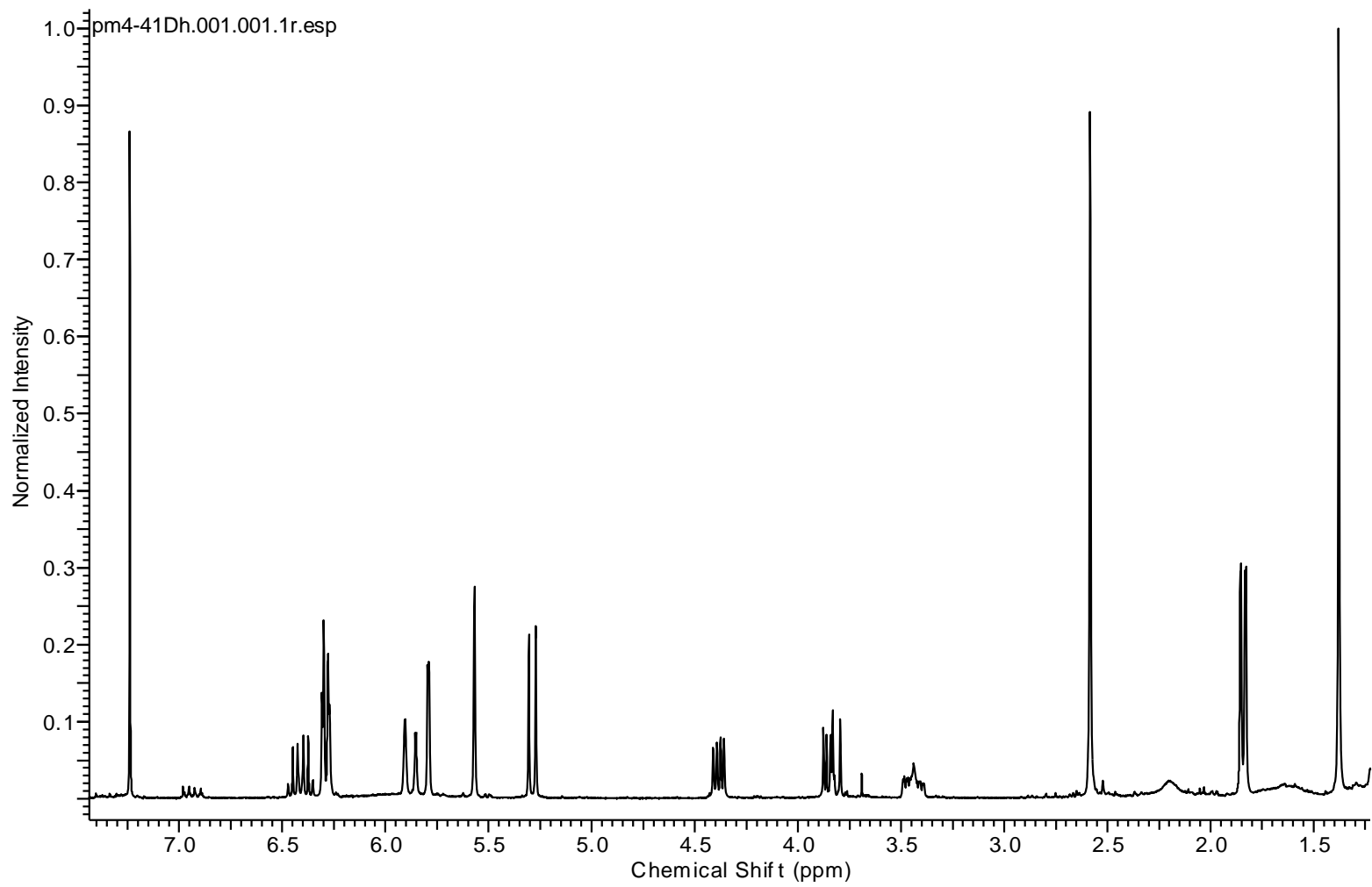
S8 ^{13}C NMR of Berkazaphilone B (**2**), 75 MHz, CDCl_3



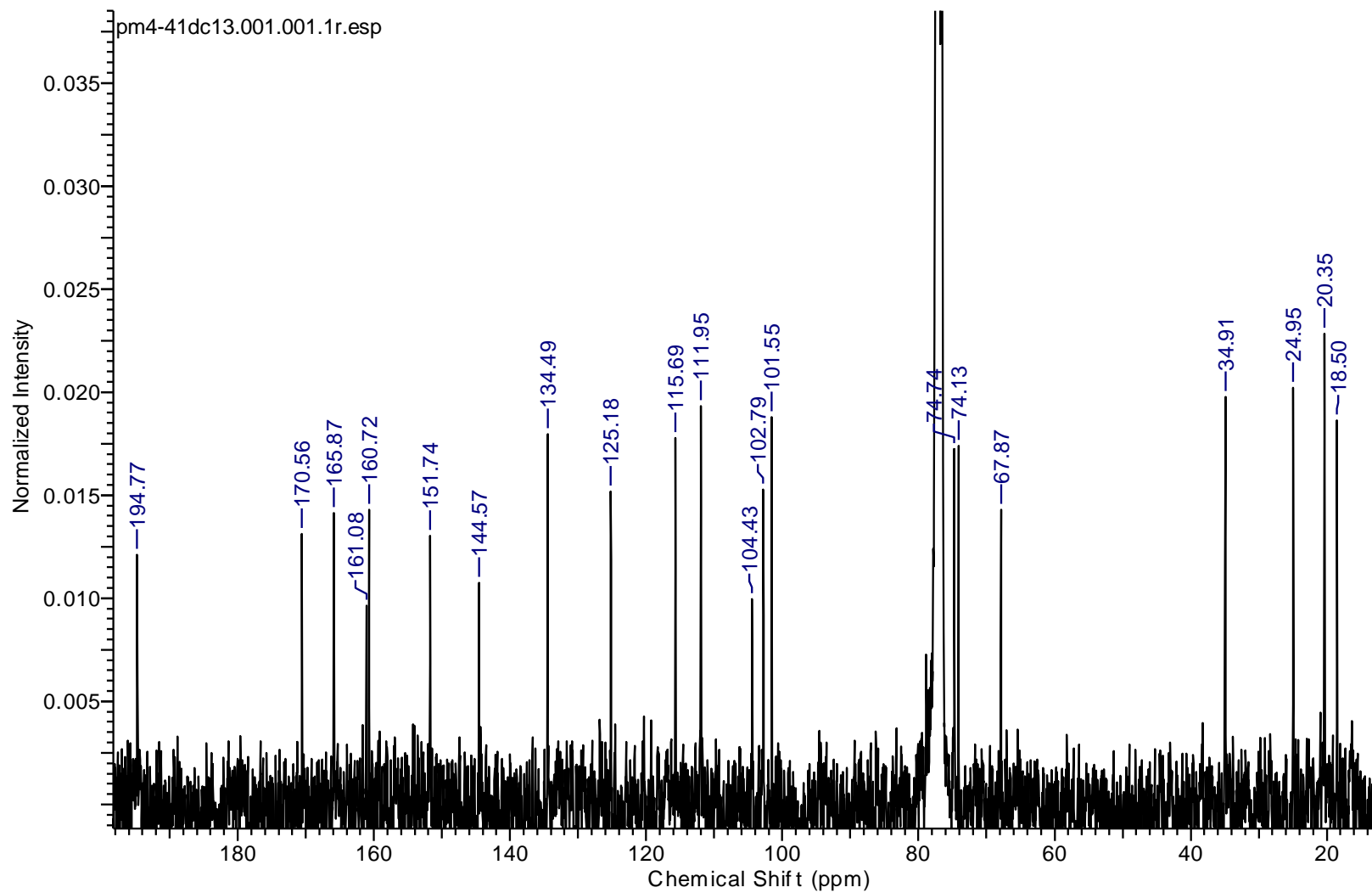
S9 COSY of Berkazaphilone B (2)



S10 HMBC of Berkazaphilone B (2)



S11 ^1H NMR of Berkazaphilone C (**4**), 300 MHz, CDCl_3



S12 ^{13}C NMR of Berkazaphilone C(4), 75 MHz, CDCl_3

Spectral Data for Compounds 6, 7 and 8

Vermistatin (6): $[\alpha]_D^{25}$ -10.5° (c 0.0154, MeOH/CHCl₃, 1:1).

Dihydrovermistatin (7): yellow solid, $[\alpha]_D^{25}$ -28.6° (c 0.0091 CHCl₃); UV (MeOH) λ_{\max} (log ϵ) 305 (3.35), 249 (3.57); IR (CHCl₃) ν_{\max} 3013, 2966, 1770, 1668, 1506, 1238, 1120, 911 cm⁻¹; ¹H NMR (CDCl₃): δ 7.41 (s, 1H, H-2'), 6.96 (d, J = 2.0 Hz, H-7), 6.66 (d, 1H, J = 2.0 Hz, H-5), 6.44 (s, 1H, H-3), 6.17 (s, 1H, H-5'), 3.85 (s, 3H, C-6 OCH₃), 3.77 (s, 3H, C-4 OCH₃), 2.45 (t, J = 7.4 Hz, H-7'), 1.62 (m, 1H, J = 7.4 Hz, H-8'), 0.95 (t, 1H, J = 7.4 Hz, H-9'); ¹³C NMR (CDCl₃): δ 177.0 (C-4'), 170.0 (C-1), 169.4 (C-6'), 163.0 (C-6), 154.8 (C-4), 154.4 (C-2'), 129.3 (C-8), 127.7 (C-9), 123.3 (C-3'), 114.4 (C-5'), 105.1 (C-5), 98.9 (C-7), 73.5 (C-3), 56.0 (C-6 OCH₃), 55.8 (C-4 OCH₃), 35.2 (C-7'), 19.9 (C-8'), 13.4 (C-9'); EIMS m/z [M]⁺ 330(100), 271(40), 217 (70); HREIMS m/z 330.1108 [M]⁺ (calcd for C₁₈H₁₈O₆, 330.1103).

Penisimplicissin (8): yellow solid, $[\alpha]_D^{25}$ -78.2° (c 0.0118, CHCl₃); UV (MeOH) λ_{\max} (log ϵ) 448 (3.97), 310 (3.45); IR (CHCl₃) ν_{\max} 3011, 1768, 1668, 1509, 1362, 1156, 910 cm⁻¹; ¹H NMR (CDCl₃) δ 7.41 (s, 1H, H-2'), 6.95 (d, J = 2.0 Hz, H-7), 6.65 (d, J = 2.0 Hz, H-5), 6.43 (s, 1H, H-3), 6.17 (s, 1H, H-5'), 3.85 (s, 3H, C-6 OCH₃), 3.76 (s, 3H, C-4 OCH₃), 2.44 (s, 3H, H-7'); ¹³C NMR (CDCl₃) δ 176.9 (C-4'), 170.4 (C-1), 166.0 (C-6'), 163.0 (C-6), 154.9 (C-4), 154.4 (C-2'), 129.3 (C-8), 127.7 (C-9), 123.3 (C-3'), 115.0 (C-5'), 105.1 (C-5), 98.9 (C-7), 73.5 (C-3), 56.0 (C-6 OCH₃), 55.8 (C-4 OCH₃), 19.6 (C-7'); EIMS m/z [M]⁺ 302(100), 259 (30), 217 (30), 165 (32); HREIMS m/z 302.0787 [M]⁺ (calcd for C₁₆H₁₄O₆, 302.0790).

Spectral Data for Compounds 9, 10, 11, and 12

Compound 9: solid, $[\alpha]_D^{25} +20.8^\circ$ (c 0.014, MeOH); $^1\text{H NMR}$ (CD_3OD) δ 9.77 (1H, s, H-12), 6.22 (1H, s, H-5), 4.22 (1H, m, H-10), 4.04 (2H, s, H-7), 2.72 (1H, m, H-9), 2.61 (1H, m, H-9), 2.01 (3H, s, H-13), 1.19 (3H, d, $J = 6.3$ Hz, H-11); $^{13}\text{C NMR}$ (CD_3OD) δ 208.8 (C, C-8), 195.1 (CH, C-12), 165.2 (C, C-2), 164.6 (C, C-4), 139.1 (C, C-6), 113.8 (C, C-1), 111.9 (C, C-3), 111.5 (CH, C-5), 65.3 (CH, C-10), 52.1 (CH_2 , C-9), 47.7 (CH_2 , C-7), 23.8 (CH_3 , C-11), 7.4 (CH_3 , C-13); EIMS m/z 252 (4), 234 (25), 166 (100); HREIMS m/z 252.1003 $[\text{M}]^+$ (calcd for $\text{C}_{13}\text{H}_{16}\text{O}_5$, 252.0998).

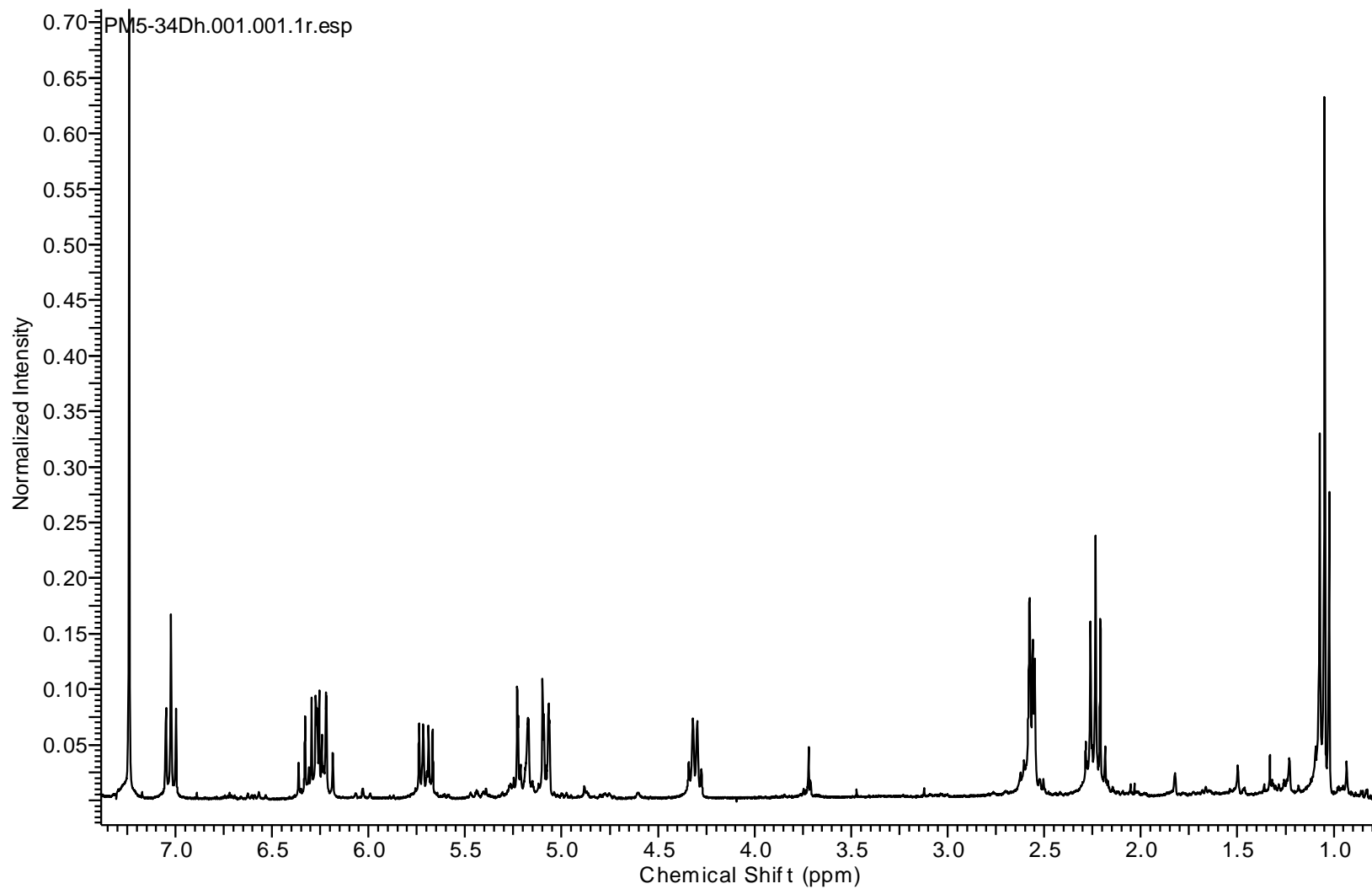
Chiral derivatization of compound 9. Compound 9 (1.0 mg) was dissolved in dry pyridine (100 μL) and either the R or S stereoisomer of α -methoxy- α -trifluoromethylphenylacetyl chloride (6 μL) added. The mixtures were stirred for 24 hours under nitrogen. After that time, MeOH (400 μL), was added and the solvents removed. The reaction mixtures were then each passed through a small Si gel column and eluted with CH_2Cl_2 to give the R- and S- MTPA esters, 10. **(R)-MTPA ester-** $^1\text{H NMR}$ (CDCl_3) δ 9.60 (1 H, s), 7.45 (5 H, m, aromatics), 6.85 (1 H, s), 5.55 (1 H, m), 3.60 (2H, s), 3.51 (3 H, s, OCH_3), 3.03 (1 H, dd, $J = 17.2, 7.9$ Hz), 2.85 (1 H, dd, $J = 17.2, 5.0$ Hz), 1.81 (3 H, s), 1.32 (3 H, d, $J = 6.4$ Hz).

(S)-MTPA ester- $^1\text{H NMR}$ (CDCl_3) δ 9.57 (1 H, s), 7.41 (5 H, m, aromatics), 6.80 (1 H, s), 5.59 (1 H, m), 3.590 (2 H, s), 3.50 (3H, s, OCH_3), 2.96 (1 H, dd, $J = 17.2, 8.2$ Hz), 2.80 (1 H, dd, $J = 17.2, 4.7$ Hz), 1.80 (3 H, s), 1.39 (1 H, d, $J = 6.4$ Hz).

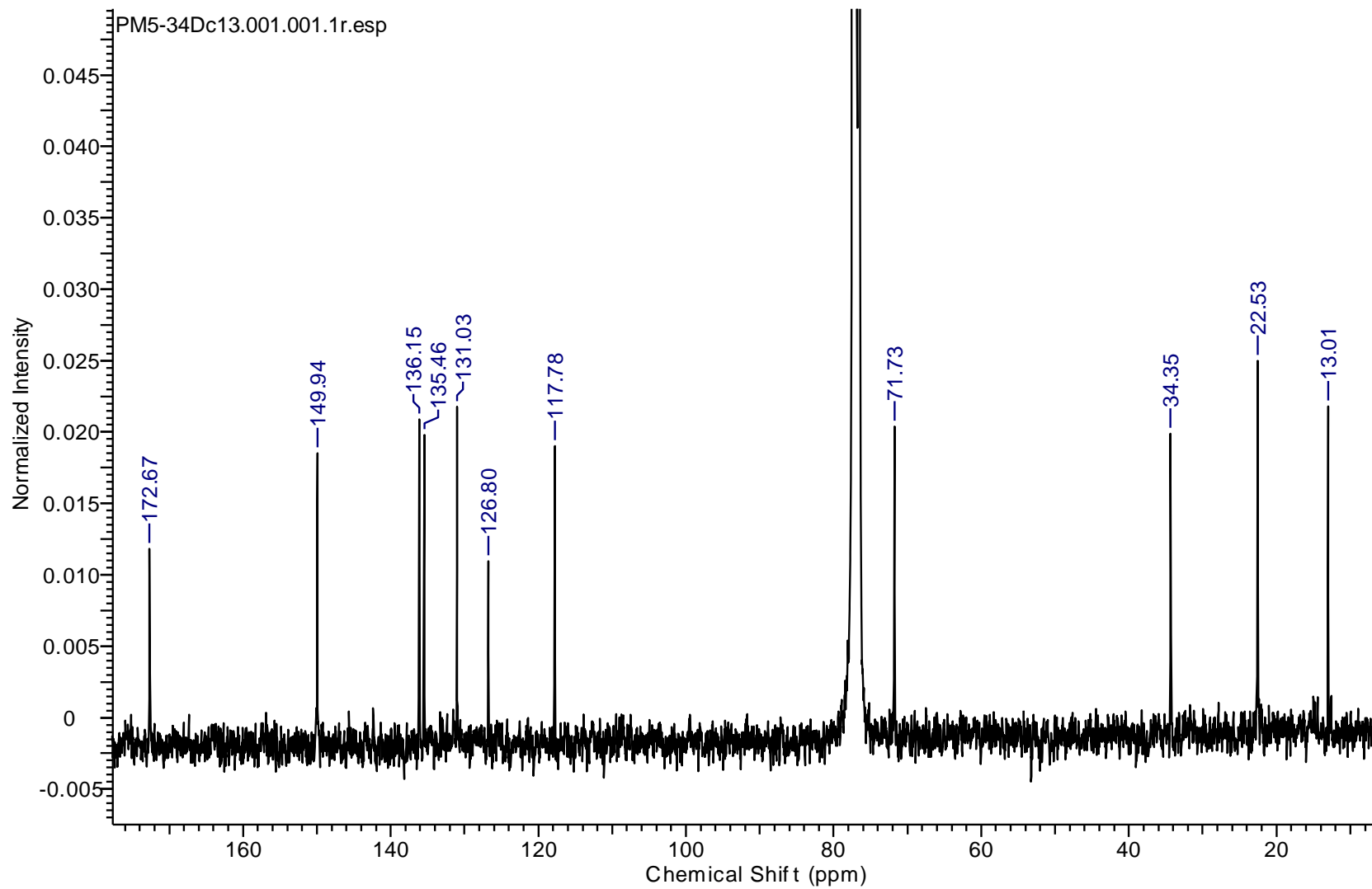
Methyl paraconic acid (11): solid, $[\alpha]_D^{25} +29.1^\circ$ (c 0.0088, CHCl_3); IR (CHCl_3) ν_{max} 3027 (broad), 2981, 2939, 1774, 1716, 1178, 1025 cm^{-1} ; ^1H NMR (CDCl_3) δ 4.50 (1H, t, $J = 9.0$ Hz, H-5), 4.29 (1H, t, $J = 9.0$ Hz, H-5), 3.12 (1H, q, $J = 9.0$ Hz, H-3), 2.88 (1H, m, H-2), 1.37 (3H, d, $J = 6.9$ Hz, H-6); ^{13}C NMR (CDCl_3) δ 177.5 (C, C-1), 175.6 (C, C-4), 66.4 (CH_2 , C-5), 47.7 (CH, C-3), 37.8 (CH, C-2), 14.6 (CH_3 , C-6) ; EIMS m/z 144 $[\text{M}]^+$.

Methylation of 11: Compound 11, (2.0 mg) was dissolved in Et_2O (200 μL) and a solution of CH_2N_2 in Et_2O was added drop wise until the yellow color persisted. The solution was stirred for 5 minutes and the solvent removed under a stream of N_2 to yield the methyl ester **12** (2.0 mg).

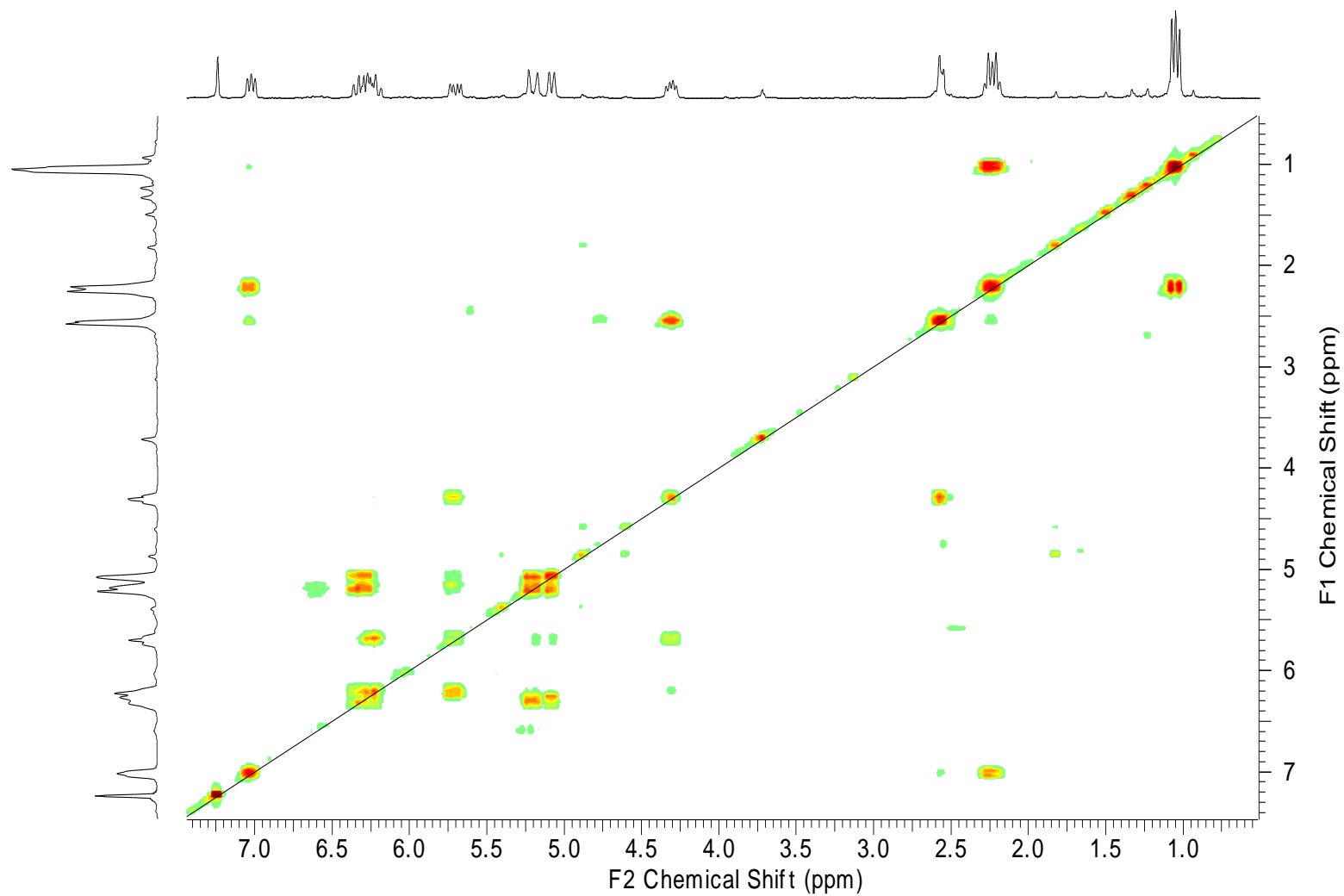
Compound 12: ^1H NMR (CDCl_3) δ 4.46 (1H, t, $J = 9.2$ Hz, H-5), 4.24 (1H, t, $J = 9.2$ Hz, H-5), 3.76 (3H, s, OCH_3), 3.07 (1H, dt, $J = 10.2, 9.2$ Hz, H-3), 2.85 (1H, dq, $J = 10.2, 7.1$ Hz, H-2), 1.34 (3H, d, $J = 7.1$ Hz, H-6) ; HREIMS m/z 159.0657 $[\text{M}+\text{H}]^+$ (calcd for $\text{C}_7\text{H}_{11}\text{O}_4$, 159.0686).



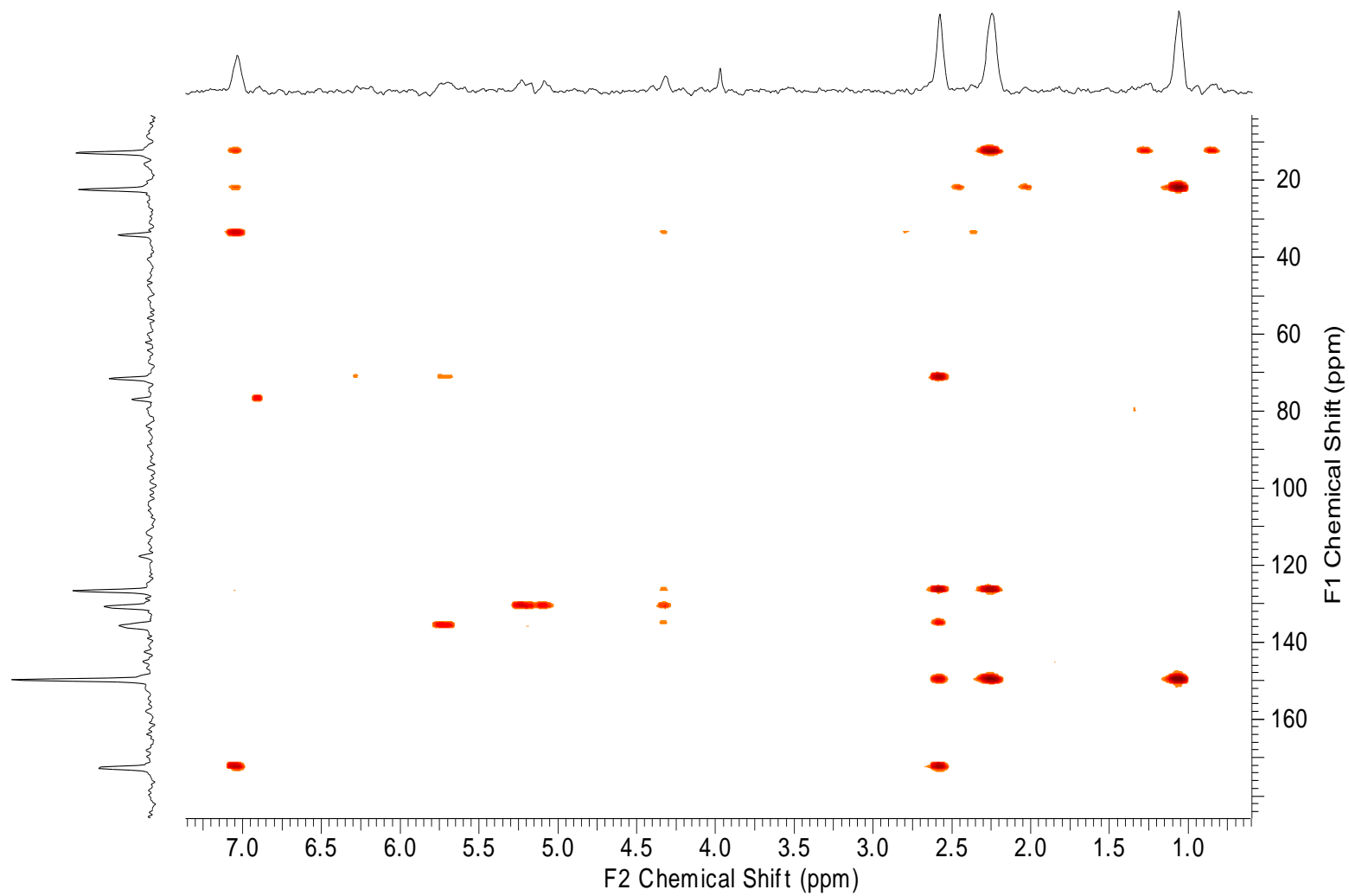
S16 ^1H NMR of Berkedienoic acid(**13**), 300 MHz, CDCl_3



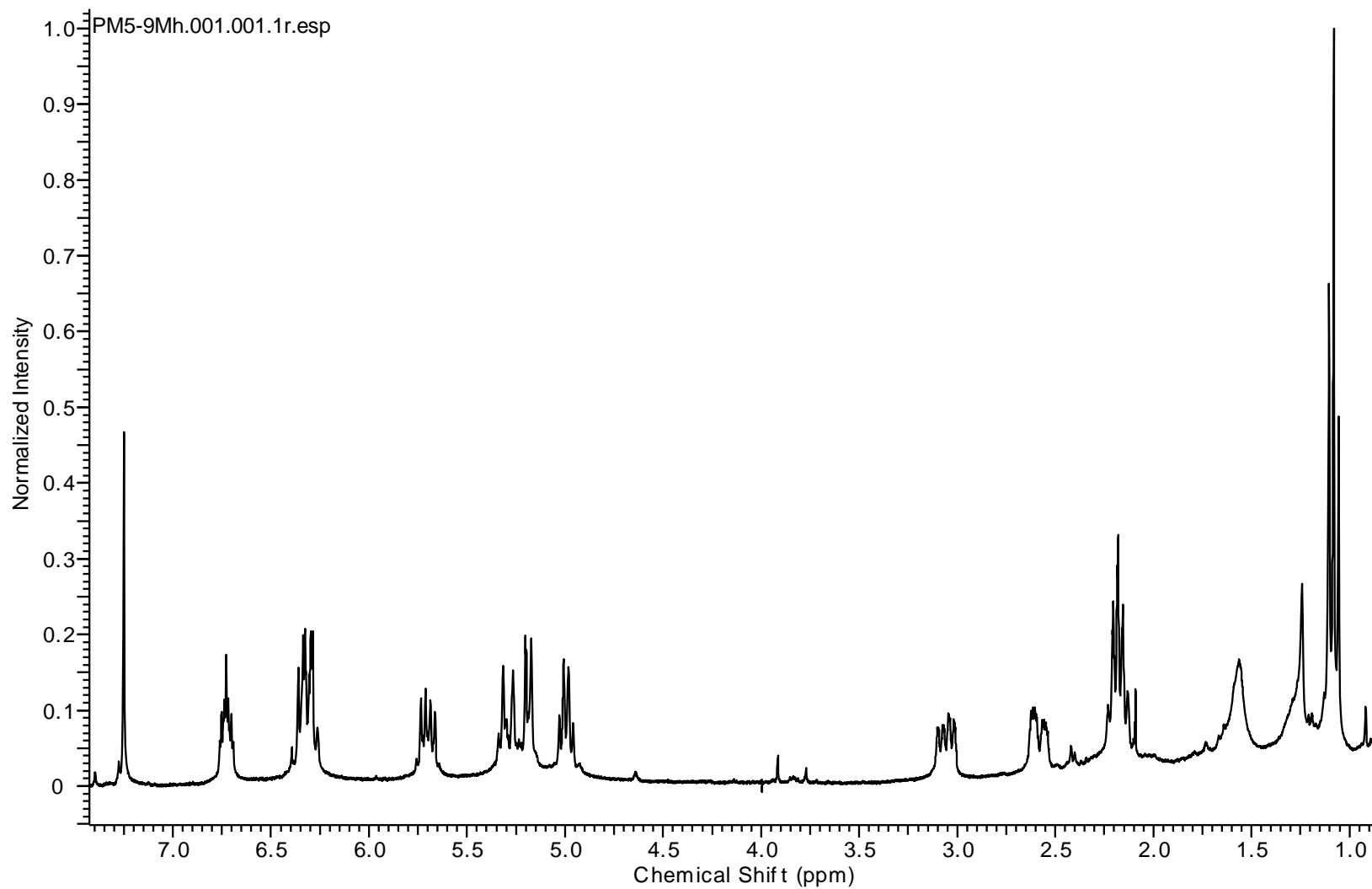
S17 ^{13}C NMR of Berkedienoic acid(**13**), 75 MHz, CDCl_3



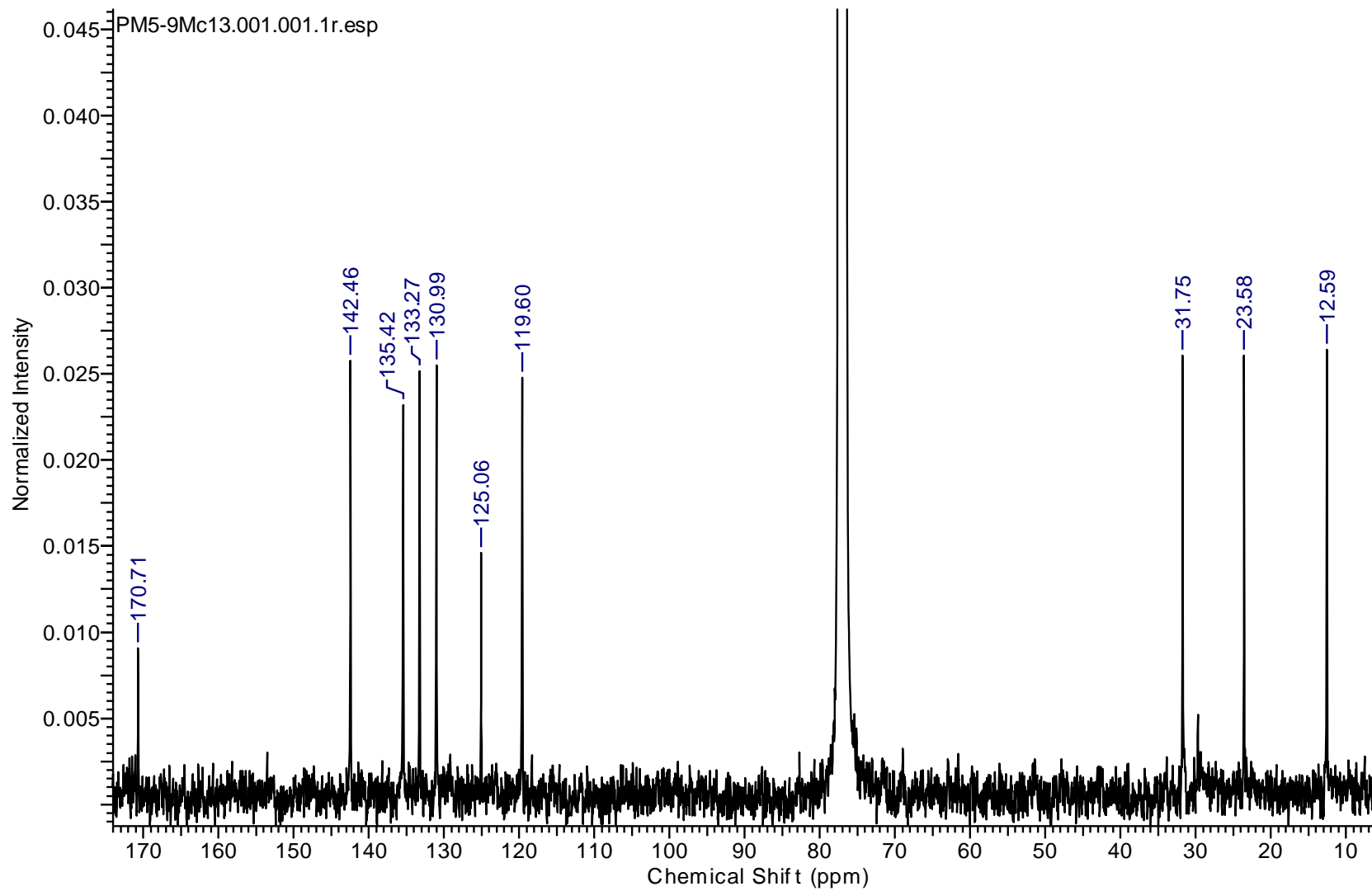
S18 COSY of Berkedienoic acid (**13**), 300 MHz, CDCl₃



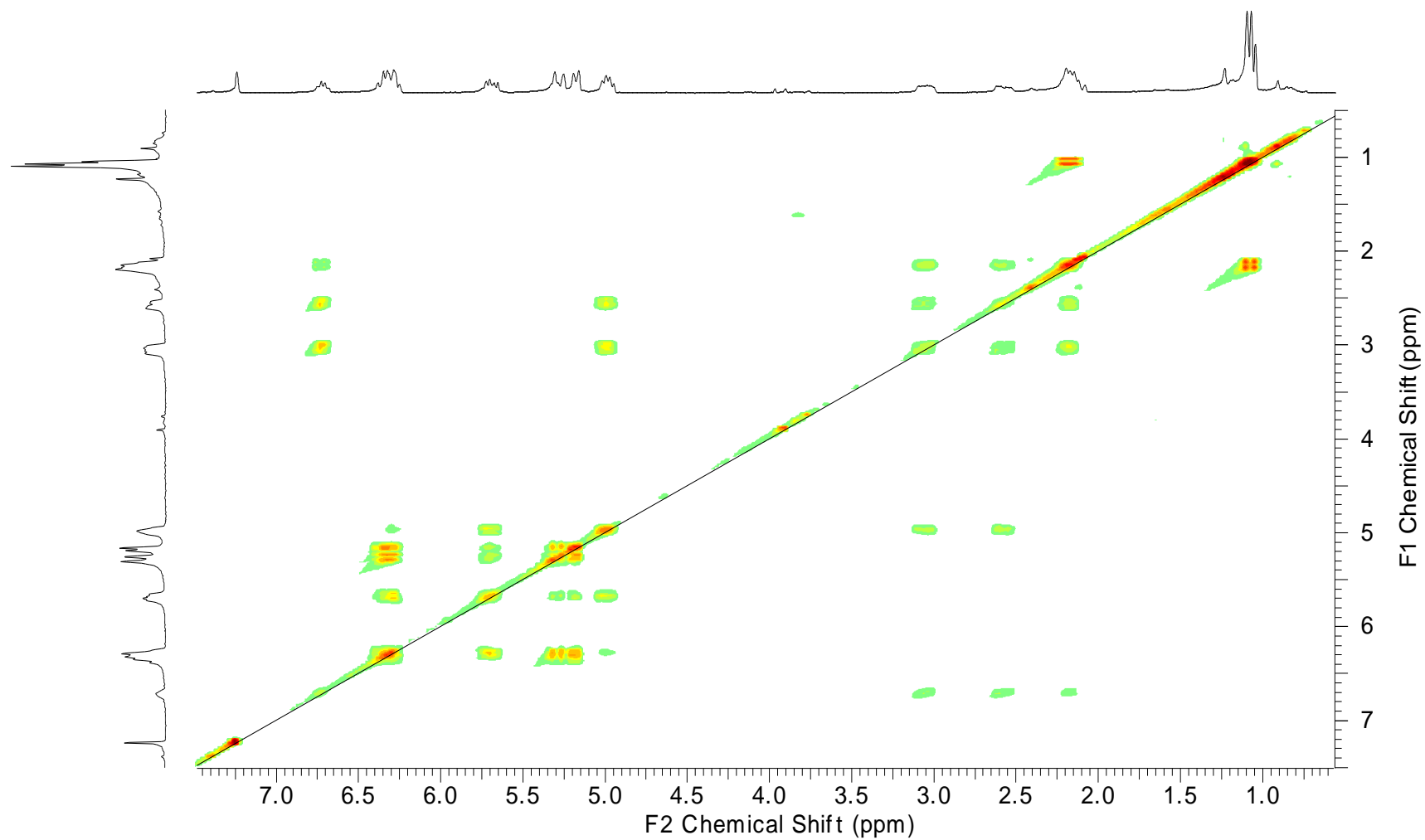
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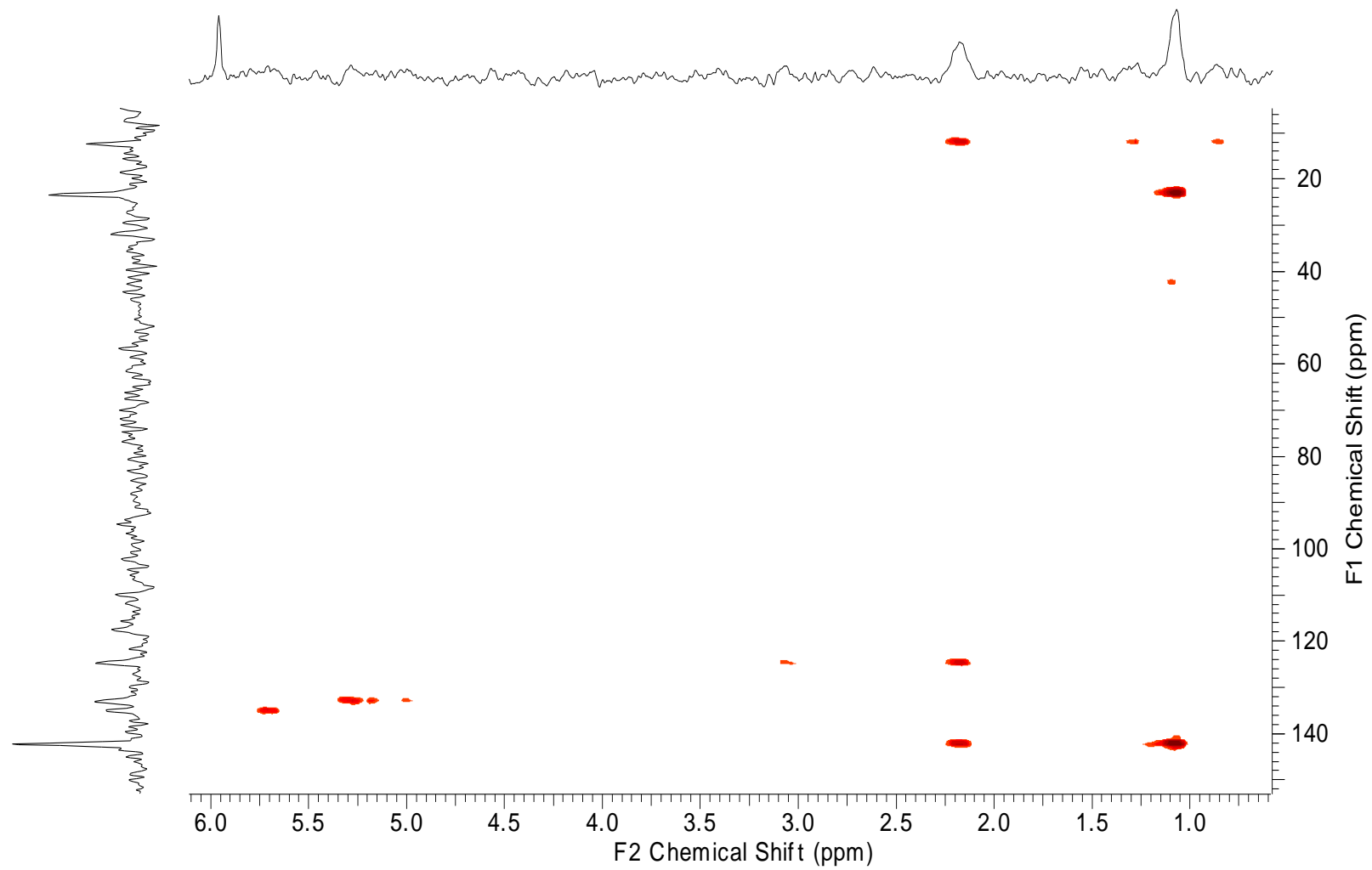
S20 ^1H NMR of Berkedienolactone (**15**), 300 MHz, CDCl_3



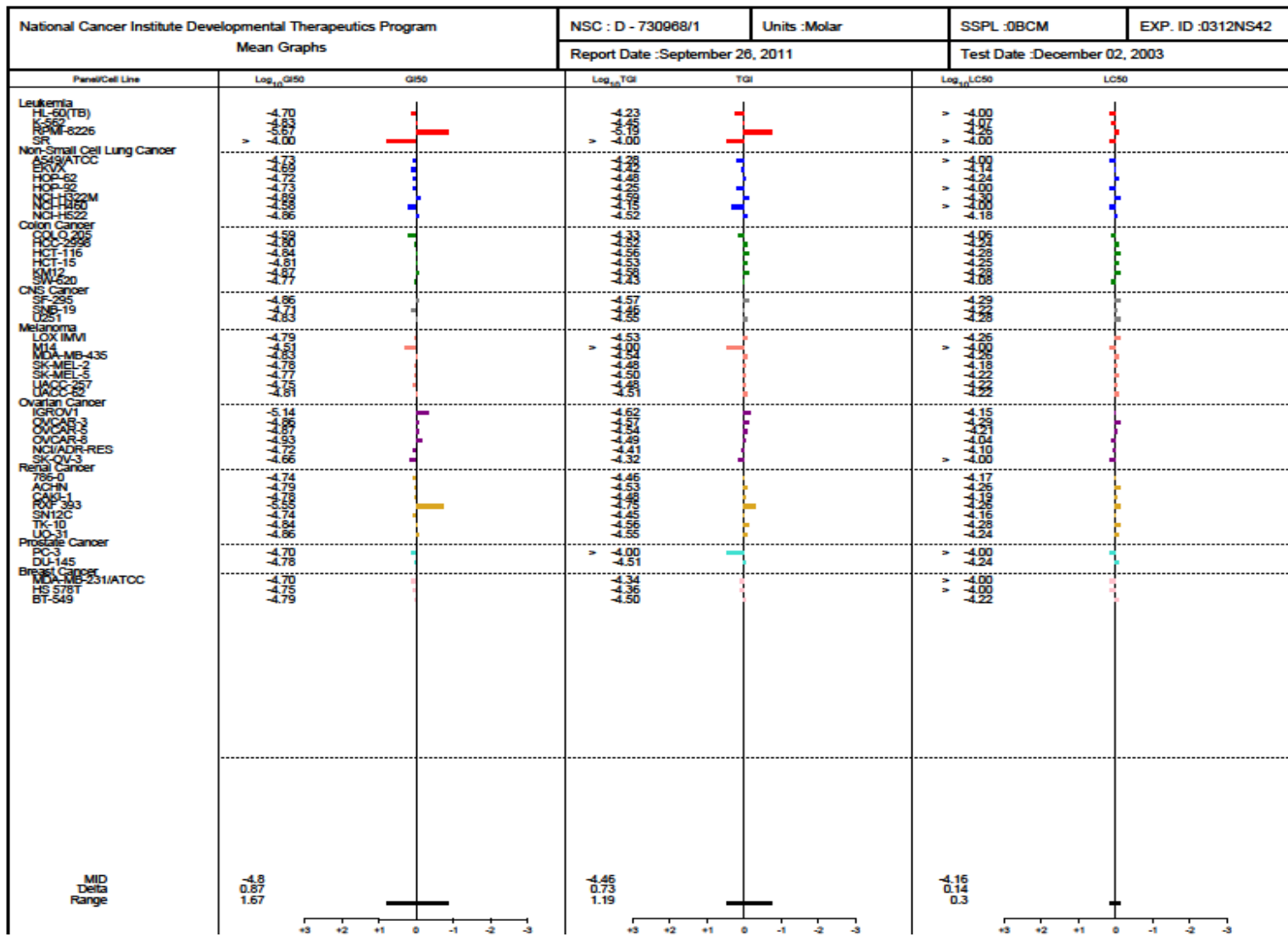
S21 ^{13}C NMR of Berkedienolactone (**15**), 75 MHz, CDCl_3



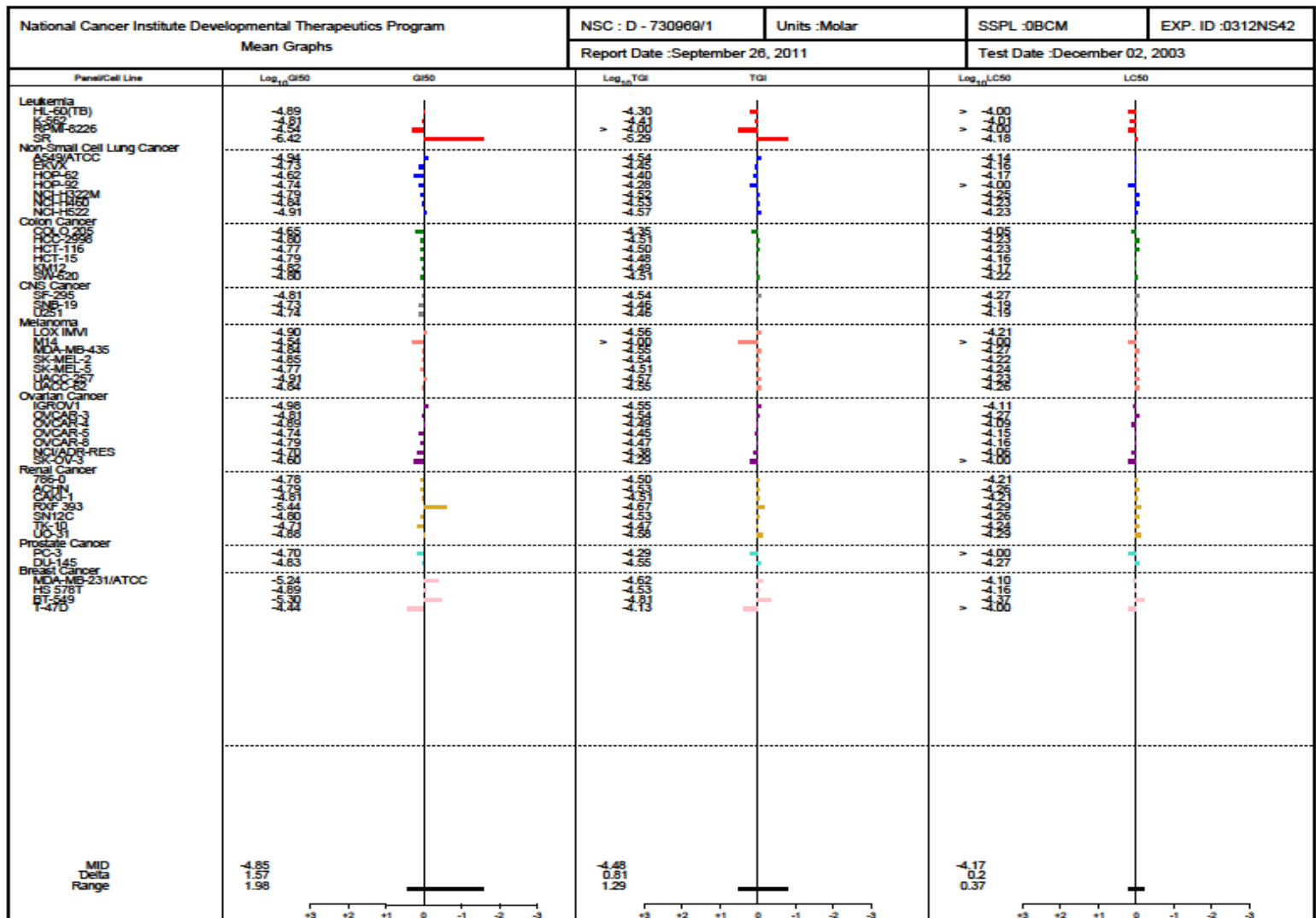
S22 COSY of Berkedienolactone (**15**), 300 MHz, CDCl₃



S23 HMBC of Berkedienolactone (**15**), 300 MHz, CDCl₃



S 24 NCI cell line data for berkazaphilone B (2)



S 25 NCI cell line data for berkazaphilone C (4)

