Supporting Information (SI)

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Croton Insularis Introduces the *seco*-Casbane class with EBC-329 and the First Casbane *endo*-Peroxide EBC-324

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General Experimental Procedures. Optical rotations were measured using a Jasco P-2000 spectrophotometer. The CD spectrum of EBC-324 (**8**) was measured at 25 ºC on a Jasco J-715 spectrometer in 0.5 mg/mL CH_3CN solution. IR spectra were obtained using an ATR FT-IR spectrometer Perkin Elmer SPECTRUM 2000. 1D and 2D NMR spectra were recorded on a Bruker DRX500 (^{1}H NMR - 500.13 MHz; ^{13}C NMR 125.77 MHz) in $CDCl_3$: δ in ppm, J in Hz. Low resolution electrospray ionisation mass spectrometry measurements (LRESIMS) were recorded in positive or negative ionization mode on a Bruker Esquire HCT (High Capacity 3D ion trap) instrument with a Bruker ESI source. High resolution electrospray ionisation (HRESIMS) accurate mass measurements were recorded in positive mode on a Bruker MicrOTOF-Q (quadrupole – Time of Flight) instrument with a Bruker ESI source. Accurate mass measurements were carried out with external calibration using sodium formate as reference calibrant.
HPLC instruments include HPLC Agilent 1190, Gilson Fraction collector FC204, semi-preparative reverse phase (RP) C18 column [Phenomenex Luna 5 μ C18(2), 250×10.00 mm] column and analytical RP C18 column [Phenomenex Luna C18(2) 100A, 250×4.6 mm, 5 μ]. Column chromatography (CC): Silica gel 60 (200-300 mesch, 15-40 μ, Merck, Germany).

Plant material. Stems (less than 2cm in diameter) of *Croton insularis* Baill. (family Euphorbiaceae) were sampled from trees growing in an arboretum in the grounds of the Ecobiotics Limited research laboratories at Yungaburra in north Queensland. The arboretum specimens had been established from vegetatively propagated *Croton insularis* that were originally collected (EcoBiotics herbarium voucher specimen Reddell 1714) from a natural population of the species growing in a semi-deciduous vine forest at Iron Range (12°38.33'S, 143°22.76'E), Queensland.

Extraction and isolation. The stem of *Croton insularis* (1.6 kg) collected from Yungabura in October 2012, was mulched and extracted three times (3×24 h) with ethanol (3×5 L) at ambient temperature. The ethanol extracts were evaporated under reduced pressure to yield a dark brown residue (100 ml). The residue was suspended with water and consequently partitioned between water and pet. spirit, chloroform and ethyl acetate layers. The pet. spirit layer was concentrated to give a green viscous residue (6.5 g), which was applied to silica gel column chromatography (70g, 5×8.2 cm) and eluted with pet. spirit-EtOAc gradient system (9:1, 8:1, 4:1, 2:1, 1:1, 1:2, 1:4, EtOAc) and then with EtOAc-MeOH gradient system (99:1, 19:1, 9:1, MeOH) to give 24 fractions each 80 ml. Fraction 8 (0.203 g), containing compound EBC-324 (**8**), was eluted with 2:1 (pet. Spirit-EtOAc), and fraction 13 (0.187 g) containing compound EBC-329 (**9**) with 1:4 solvent mixtures (pet. Spirit-EtOAc). Fraction 8 was subjected to semi-preparative RP HPLC (MeCN (**B**)-H_2O, 80%: (80% **B** - 7 min; from 80% **B** to 90% **B** for 20 min and kept at 90% **B** for 3 min; flow rate 2 ml/min, collection 0.5 ml/tube) to afford compound EBC-324 (**8**) (0.46 mg, R_t 15.6 min).

Fraction 13 (0.187 g) was separated on semi-preparative HPLC using the same method, but starting from MeCN (**B**)-H_2O 75 % to afford compound EBC-329 (**9**) (1.51 mg, R_t 14.0 min).

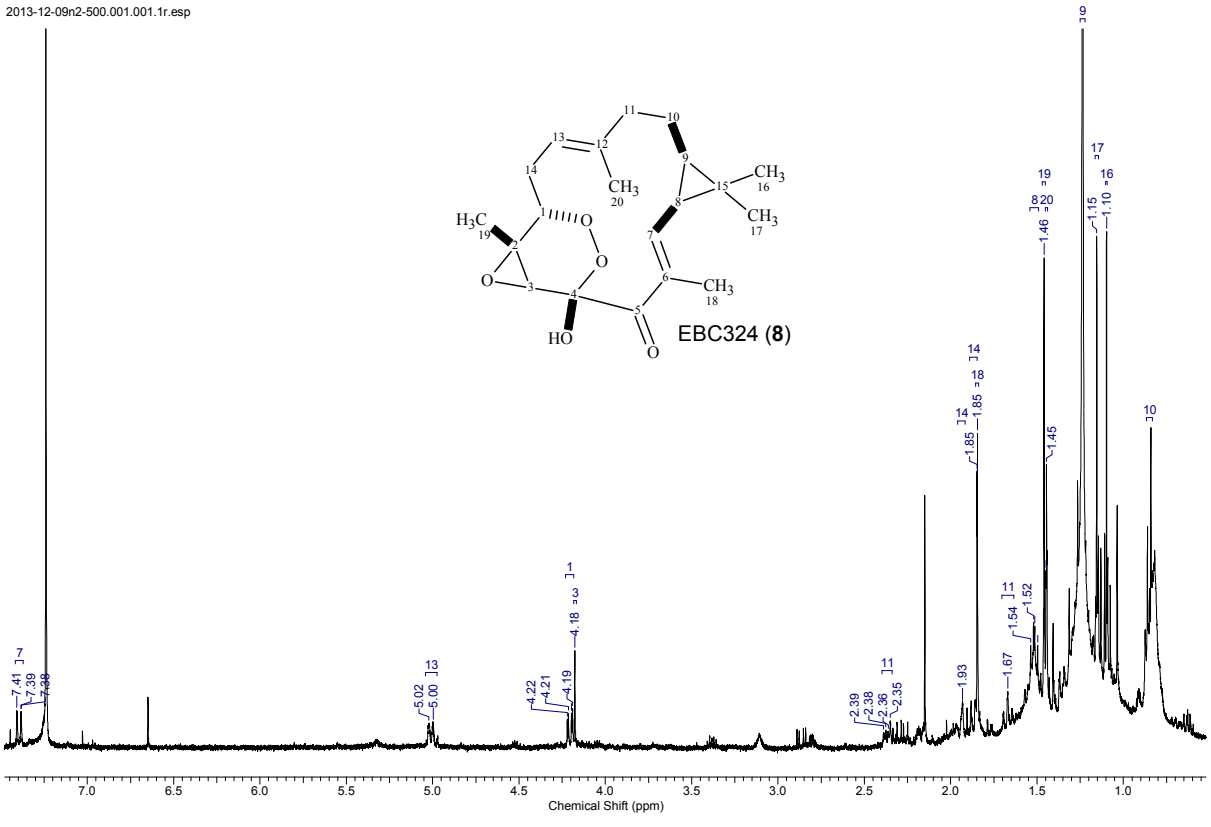
EBC-324 (**8**): Isolated as a colourless oil; $[\alpha]^{26.8}_{D}$ –130.7° (c 0.044, $CDCl_3$); ^{1}H NMR and ^{13}C NMR see Table 1 below on page 4 and copies of the spectra on pages 4-10; IR cm^{-1}: 3396, 2953, 2922, 2856, 1734, 1693, 1654, 1617, 1457, 1379, 1260, 1081, 970, 820; positive ion HRESIMS $[M+Na]^{+}$*m/z* 371.1813 (calcd for $C_{20}H_{28}O_5Na$ – 371.1829).

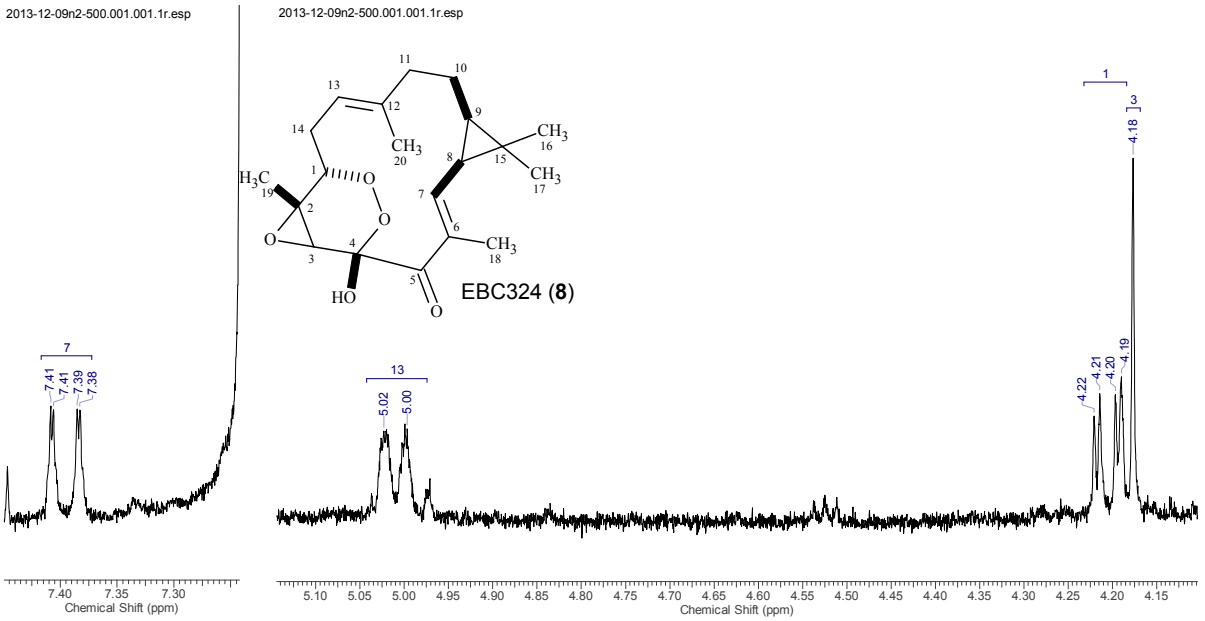
EBC-329 (**9**): Isolated as a colourless oil; $[\alpha]^{25.3}_{D}$ –18.2° (c 0.144, $CDCl_3$); ^{1}H NMR and ^{13}C NMR see Table 2 below on page 11 and copies of the spectra on pages 11-19; IR cm^{-1}: 3410, 3106, 3062, 2925, 2857, 1743, 1675, 1635, 1594, 1378, 1346, 1274, 1120, 929, 853; positive ion HRESIMS $[M+Na]^{+}$*m/z* 353.1732 (calcd for $C_{20}H_{26}O_4Na$ – 353.1723).

Materials and methods for growing cells and cell screening: Cells (3000-5000/well) were seeded in triplicate in 96-well plates in Roswell Park Memorial Institute 1640 medium containing 10% fetal calf serum and treated with the compounds. After 6 days in culture at 37 degrees, the growth of treated K562 cells was compared with untreated controls by addition of MTS, and the colour change read at 490 nm in an ELISA reader. The other cell lines were fixed with ethanol *in situ*, stained with sulforhodamine B and absorbance at 564 nm determined in the ELISA reader. IC50 were determined by interpolation of a plot of absorbance expresses as % of untreated controls. Cells were seeded at the following densities: HeLa: 3000/well, HT29: 3000/well, MCF7: 4000/well, MM96L: 3000/well, NFF: 5000/well, K562: 3500/well.

Table 1. ^{1}H and ^{13}C NMR data for compound EBC-324 (**8**) recorded in $CDCl_3$.

Position	^{1}H, δ (ppm)	multiplicity	J, Hz	^{13}C, δ (ppm)
1	4.21	1 (dd)	12.1, 3.3	81.82
2				65.44
3	4.18	1 (s)		64.4
4				102.74
5				195.37
6				130.87
7	7.40	1 (dq)	11.7, 1.3	151.24
8	1.52	1 (dd)	11.7, 8.4	30.49
9	1.24	1 (m)		37.36
10a	0.85	1 (m)		22.70
10b	1.26	1 (m)		
11a	1.67	1 (m)		37.59
11b	2.36	1 (m)		
12				139.69
13	5.01	1 (m)		122.50
14a	1.87	1 (m)		31.28
14b	1.94	1 (m)		
15				26.31
16	1.10	3 (s)		15.83
17	1.15	3 (s)		29.33
18	1.85	3 (d)	1.3	11.61
19	1.46	3 (s)		13.18
20	1.45	3 (t)	1.5	19.57

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5.00
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4.21
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4.18
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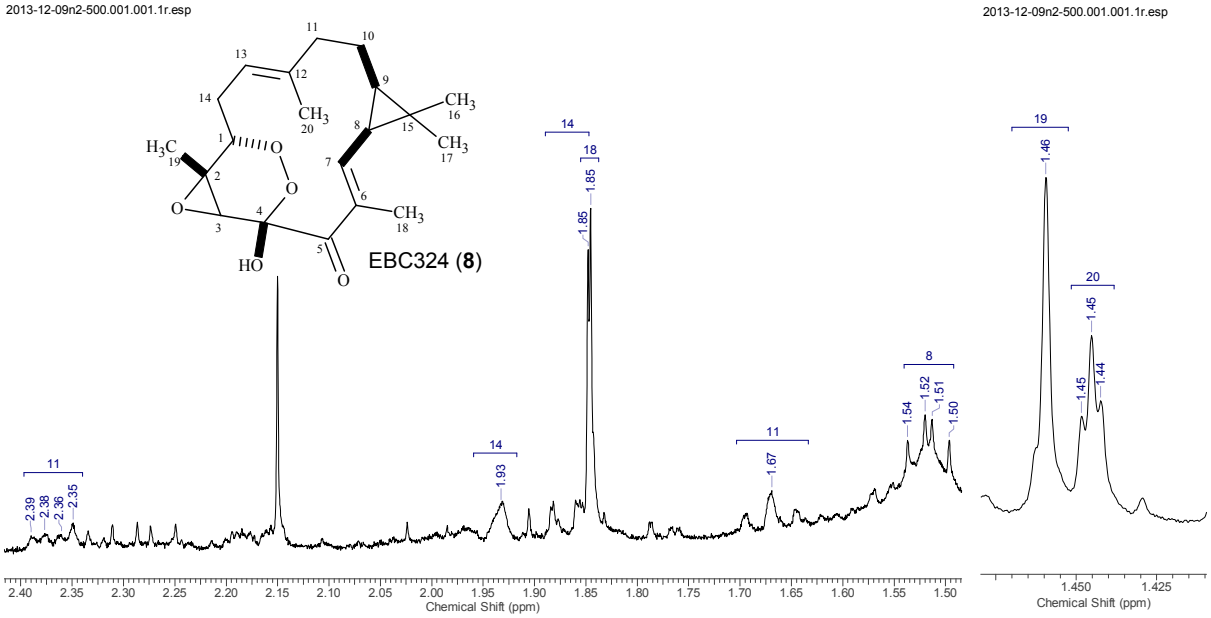
Chemical Shift (ppm)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1.52
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1.45
1.44
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Chemical Shift (ppm)

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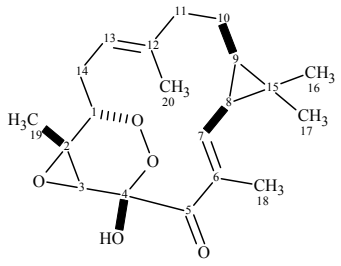

EBC324 (**8**)

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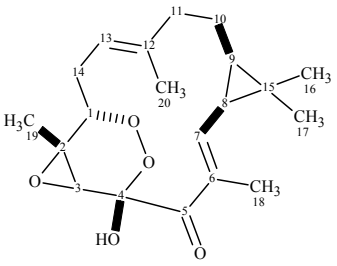

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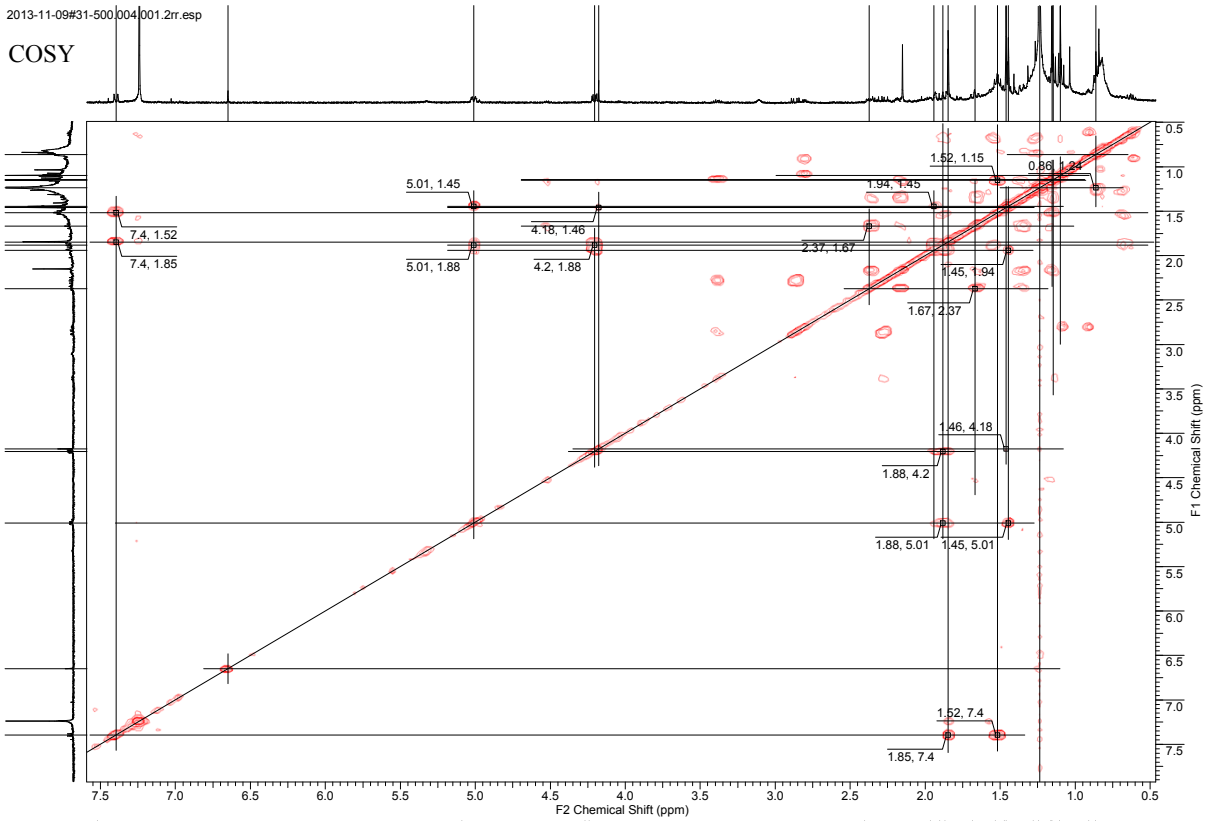

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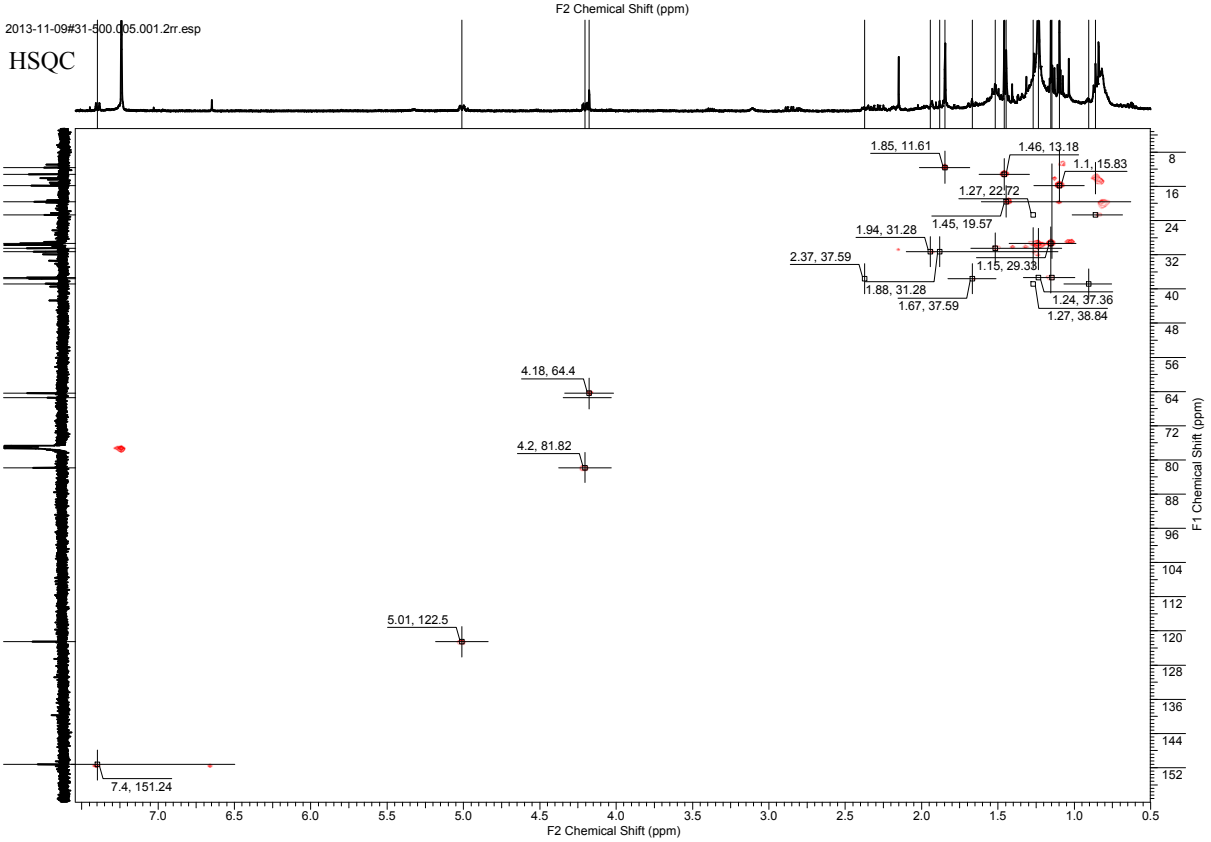

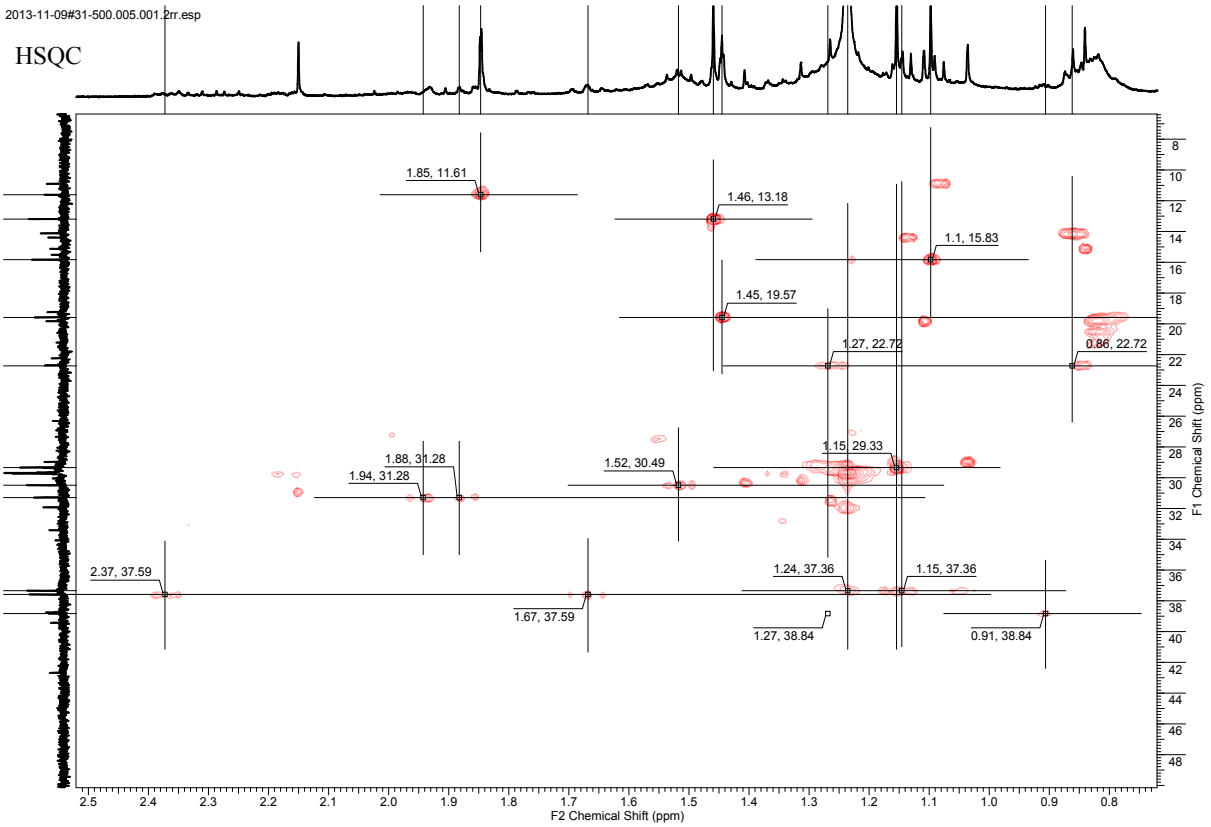
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1.94, 31.28
1.52, 30.49
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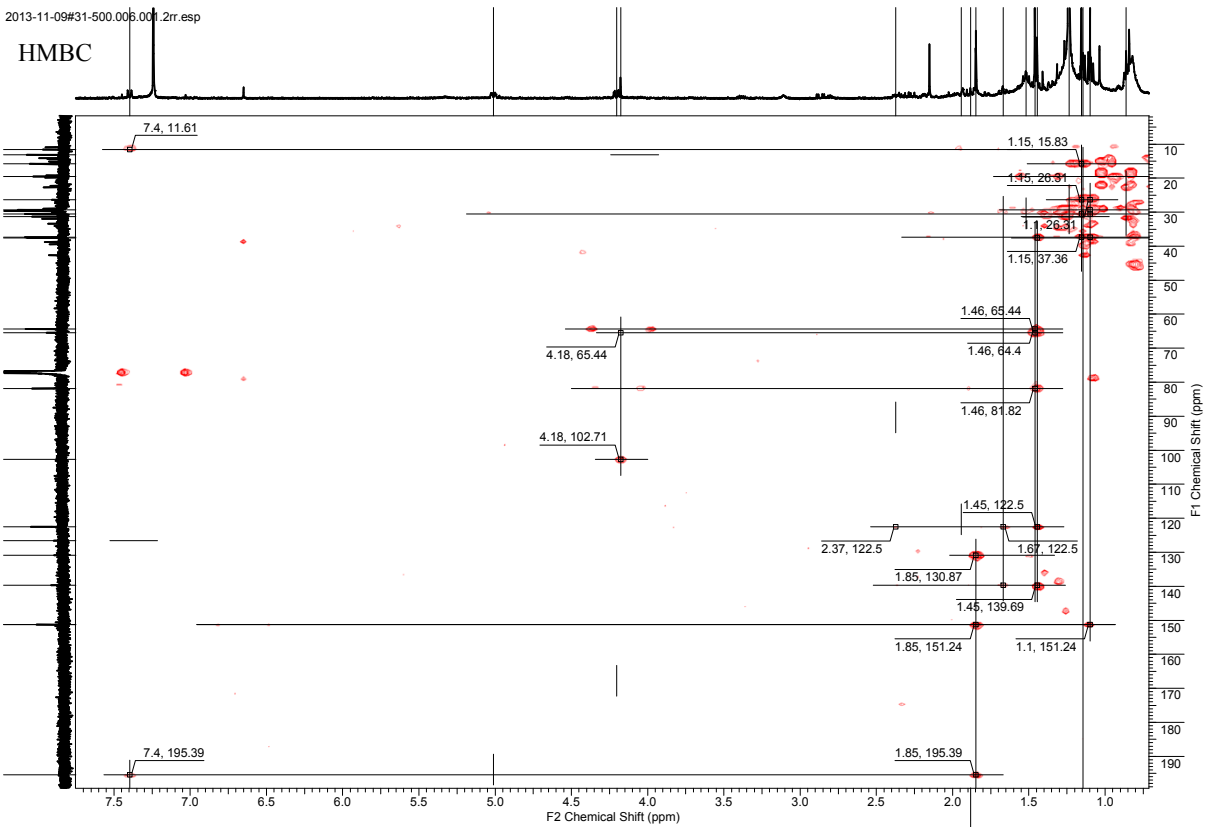
F1 Chemical Shift (ppm)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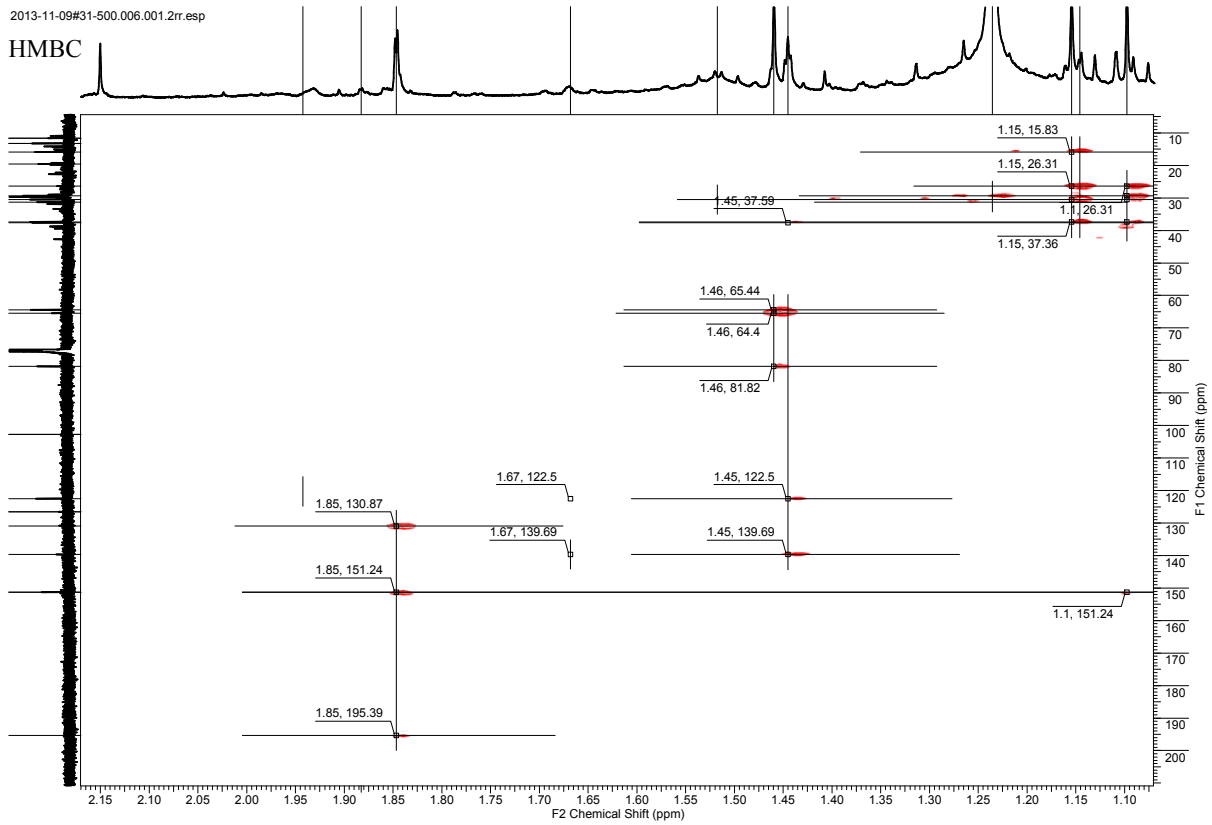
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1.46, 81.82
1.67, 122.5
1.45, 122.5
1.85, 130.87
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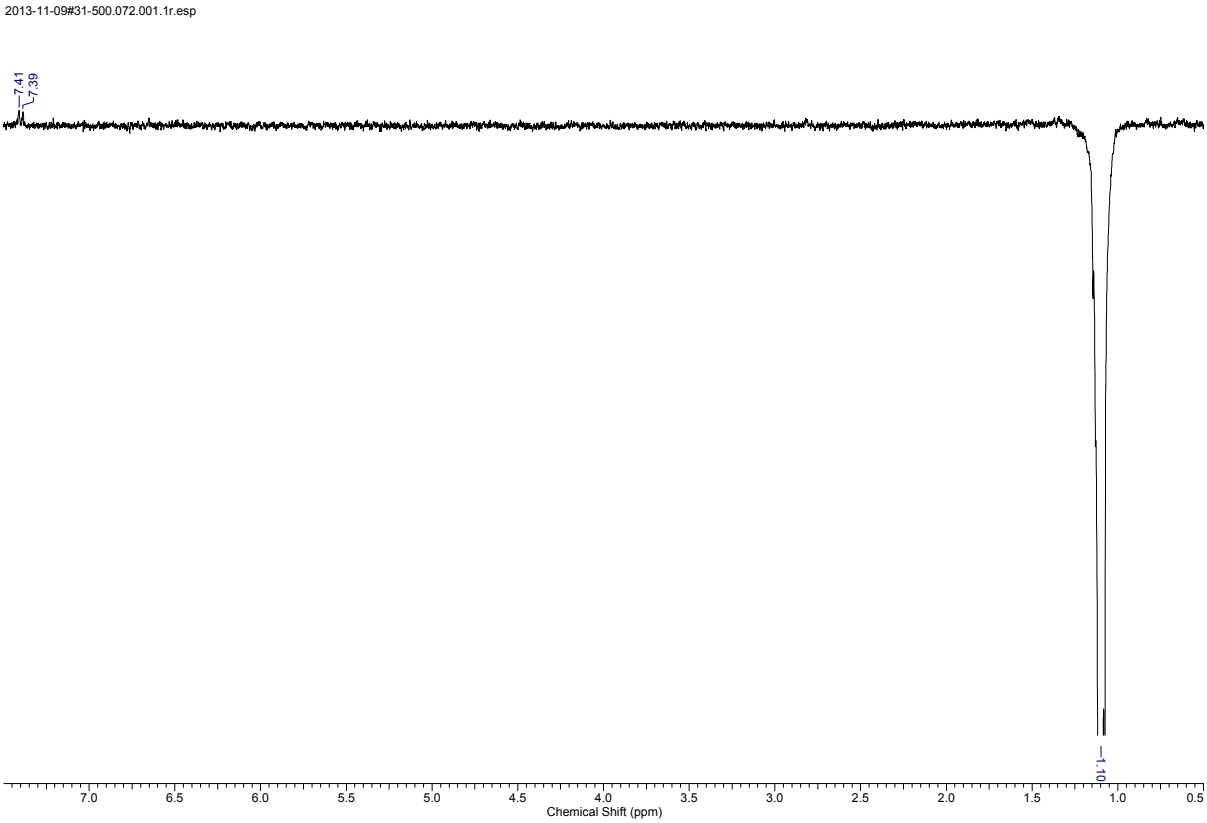
F2 Chemical Shift (ppm)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7.39
1.10
Chemical Shift (ppm)

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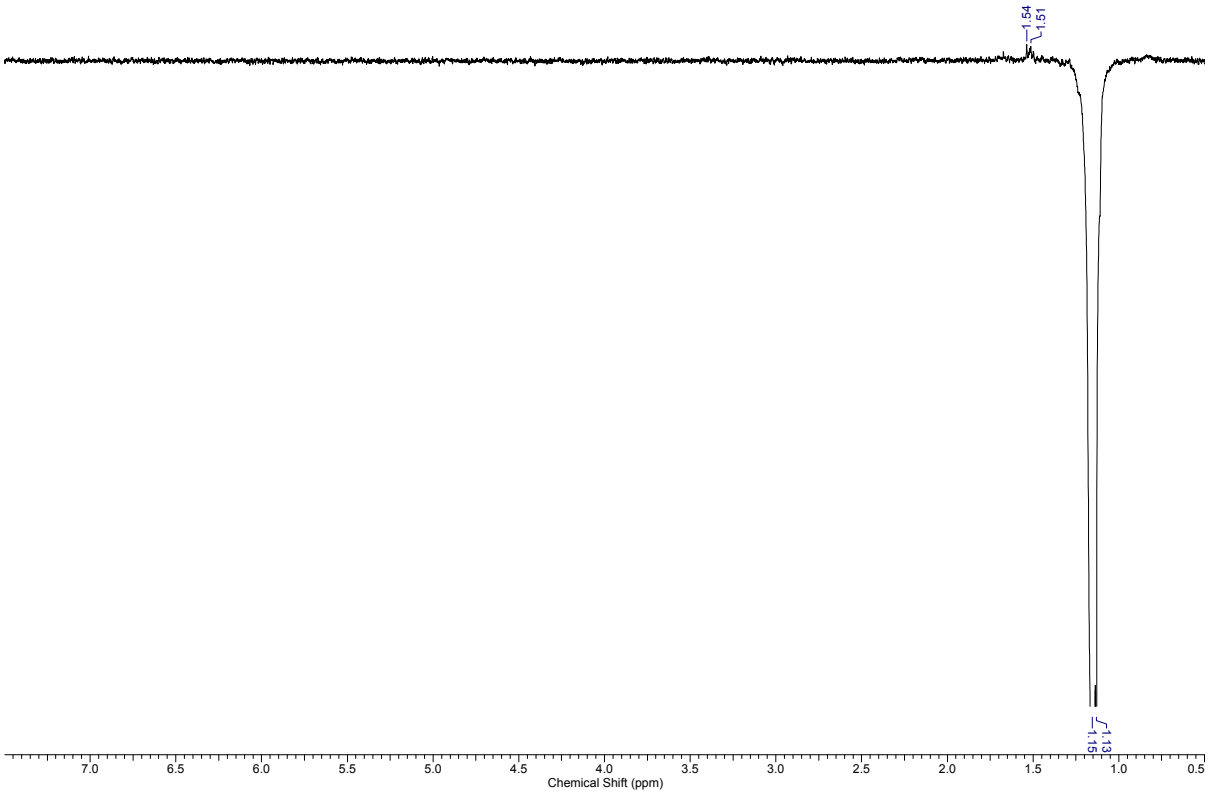

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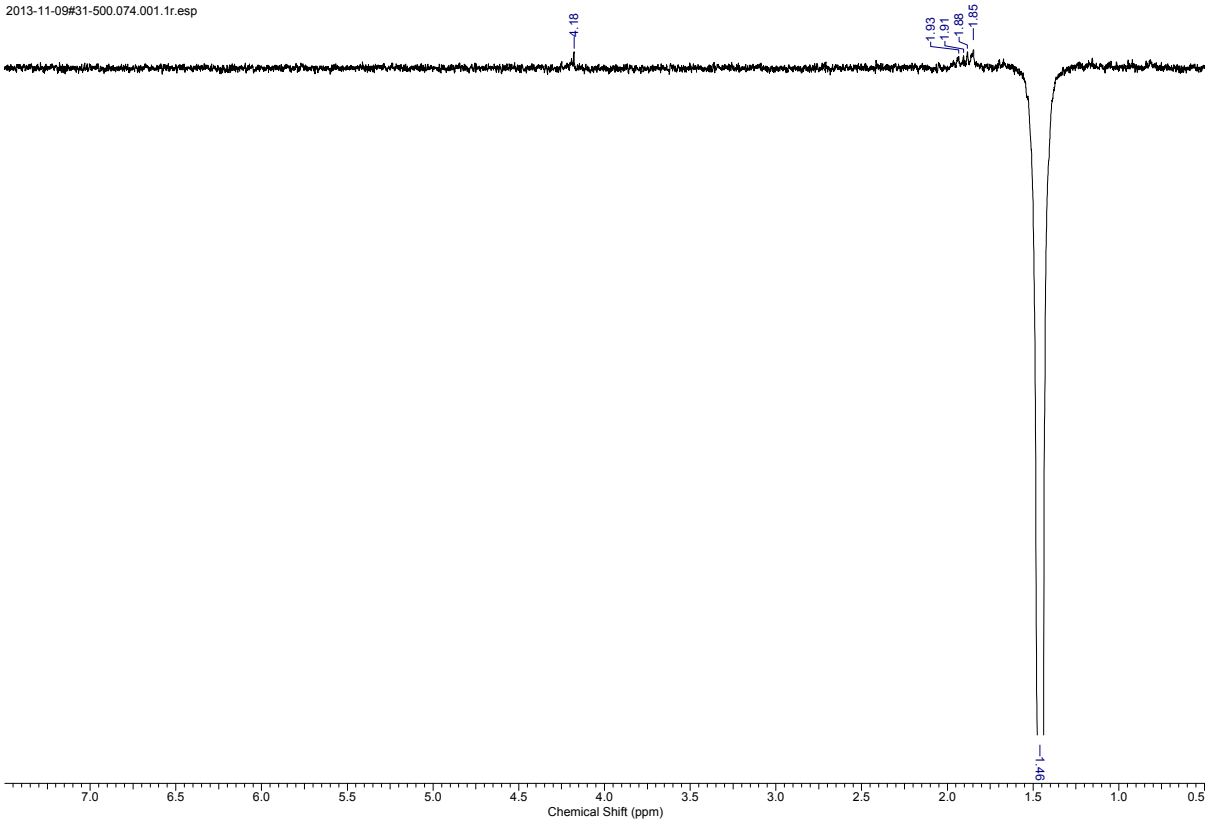

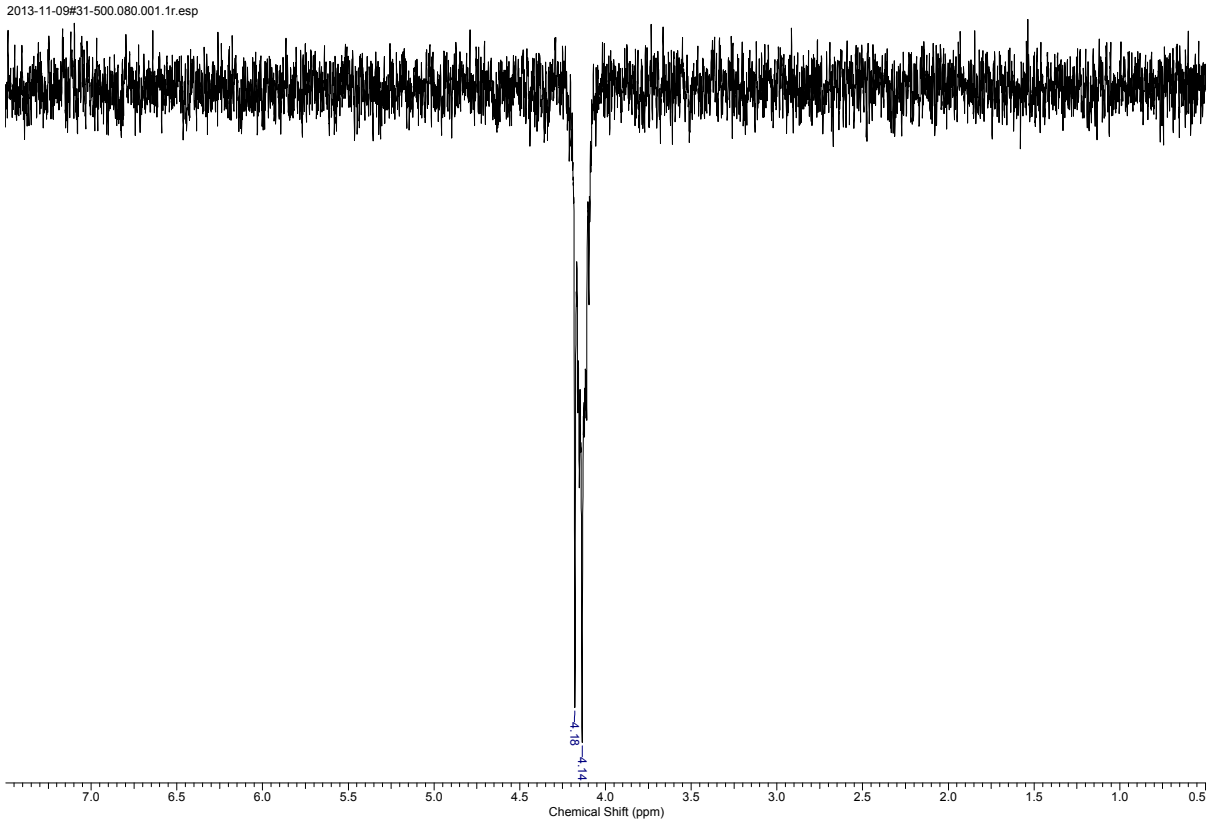
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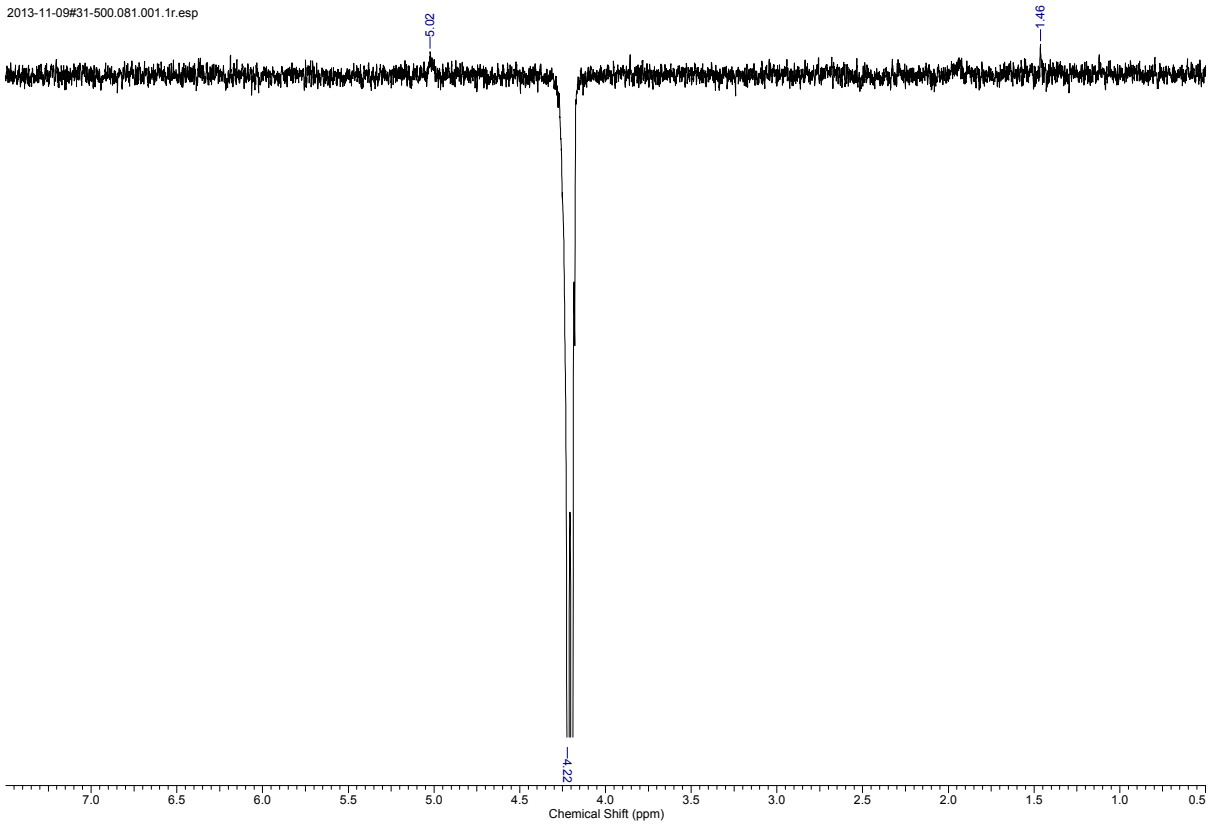
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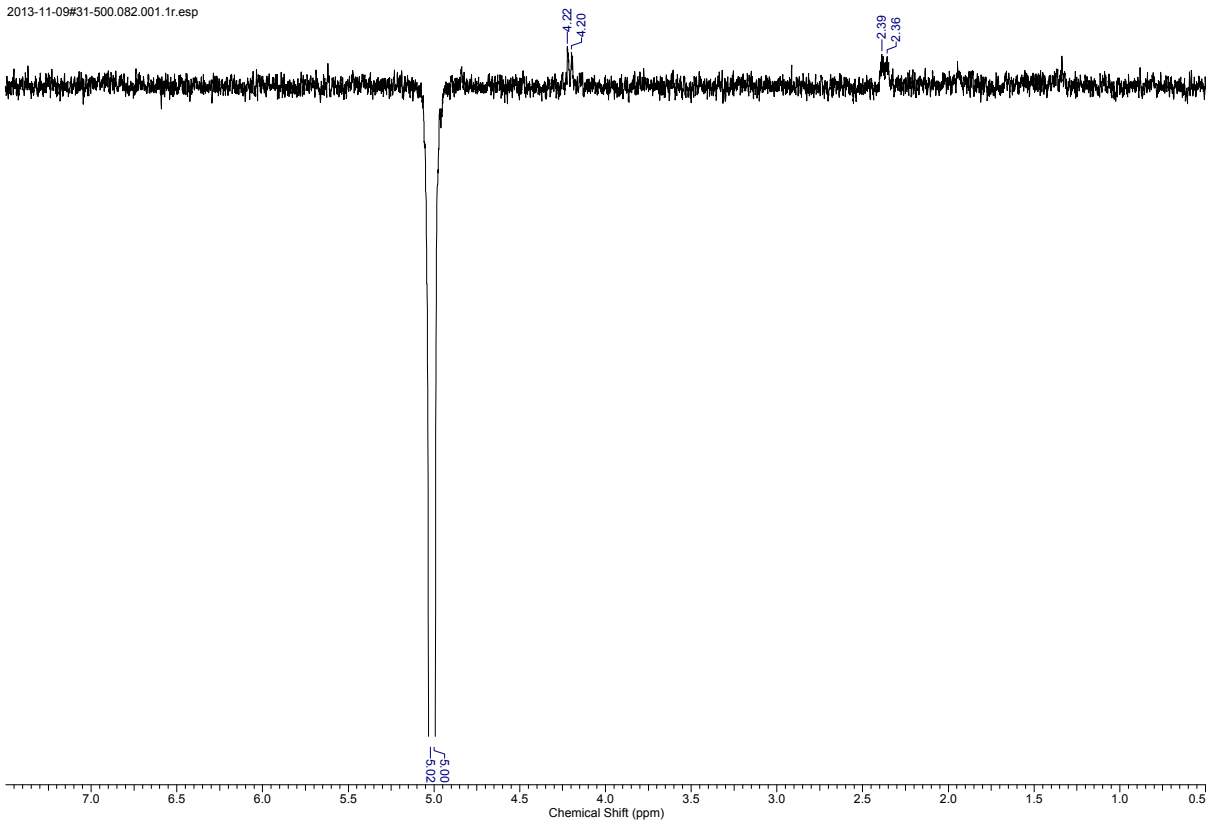
Chemical Shift (ppm)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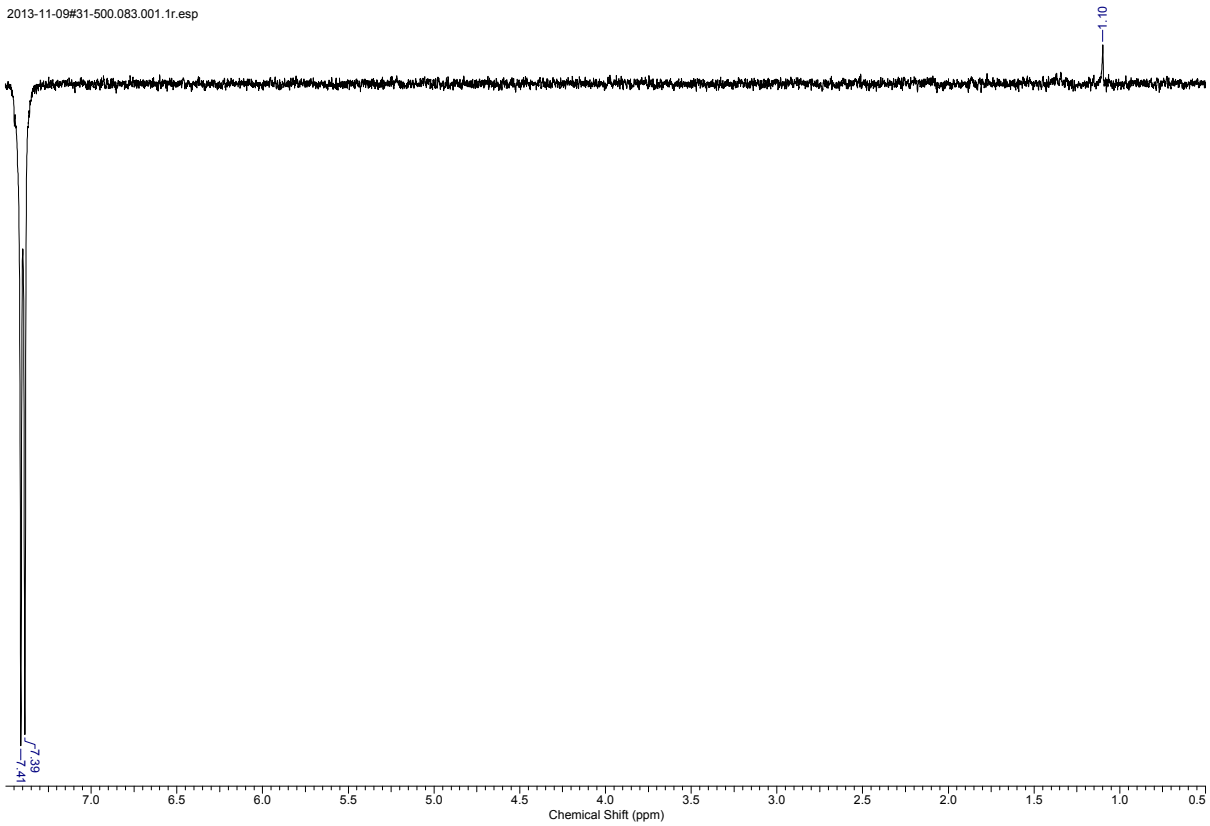
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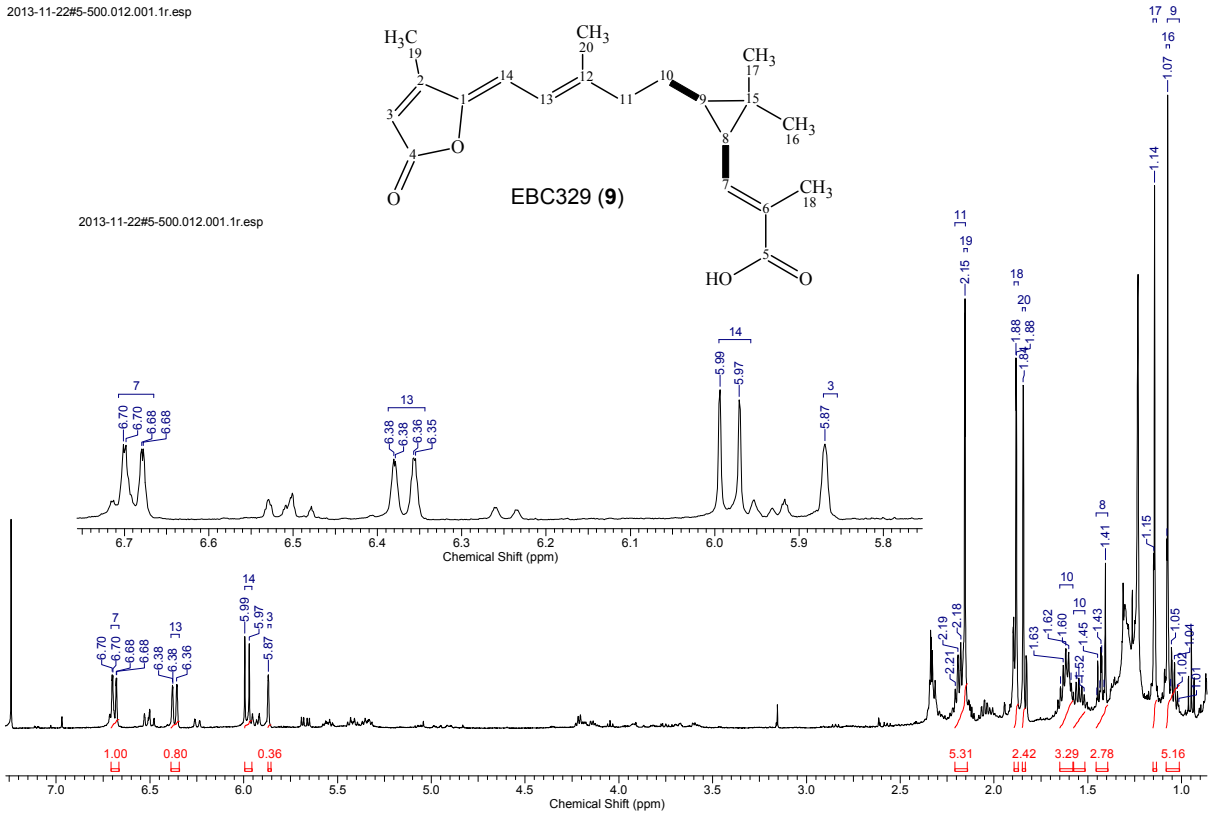
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
Chemical Shift (ppm)

Table 2. ^{1}H and ^{13}C NMR data for compound EBC-329 (**9**) recorded in $CDCl_3$.

Position	^{1}H, δ (ppm)	multiplicity	J, Hz	^{13}C, δ (ppm)
1				148.68
2				154.37
3	5.87	1 (br. s)		115.38
4				169.36
5				171.83
6				126.68
7	6.69	1 (dd)	10.6, 1.2	143.37
8	1.43	1 (m)		28.05
9	1.05	1 (m)		33.56
10a	1.54	1 (dd)	14.6, 6.5	23.90
10b	1.62	1 (dt)	14.6, 7.1	
11	2.15-2.21	2 (m)		40.50
12				146.91
13	6.37	1 (dd)	11.7, 1.0	118.78
14	5.98	1 (d)	11.7	107.28
15				25.15
16	1.08	3 (s)		15.78
17	1.14	3 (s)		29.13
18	1.88	3 (d)	1.2	12.24
19	2.16	3 (d)	1.0	11.65
20	1.84	3 (s)		17.30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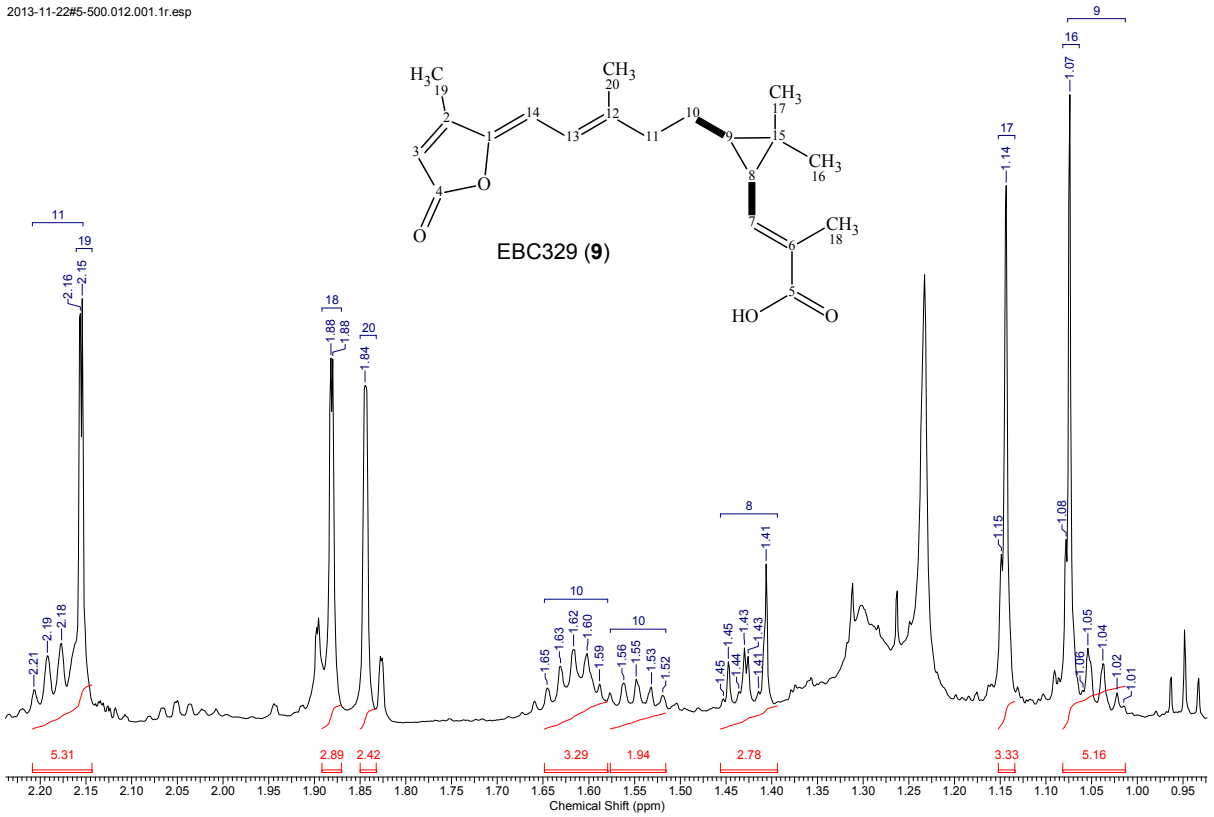
EBC329 (9)
Chemical Shift (ppm)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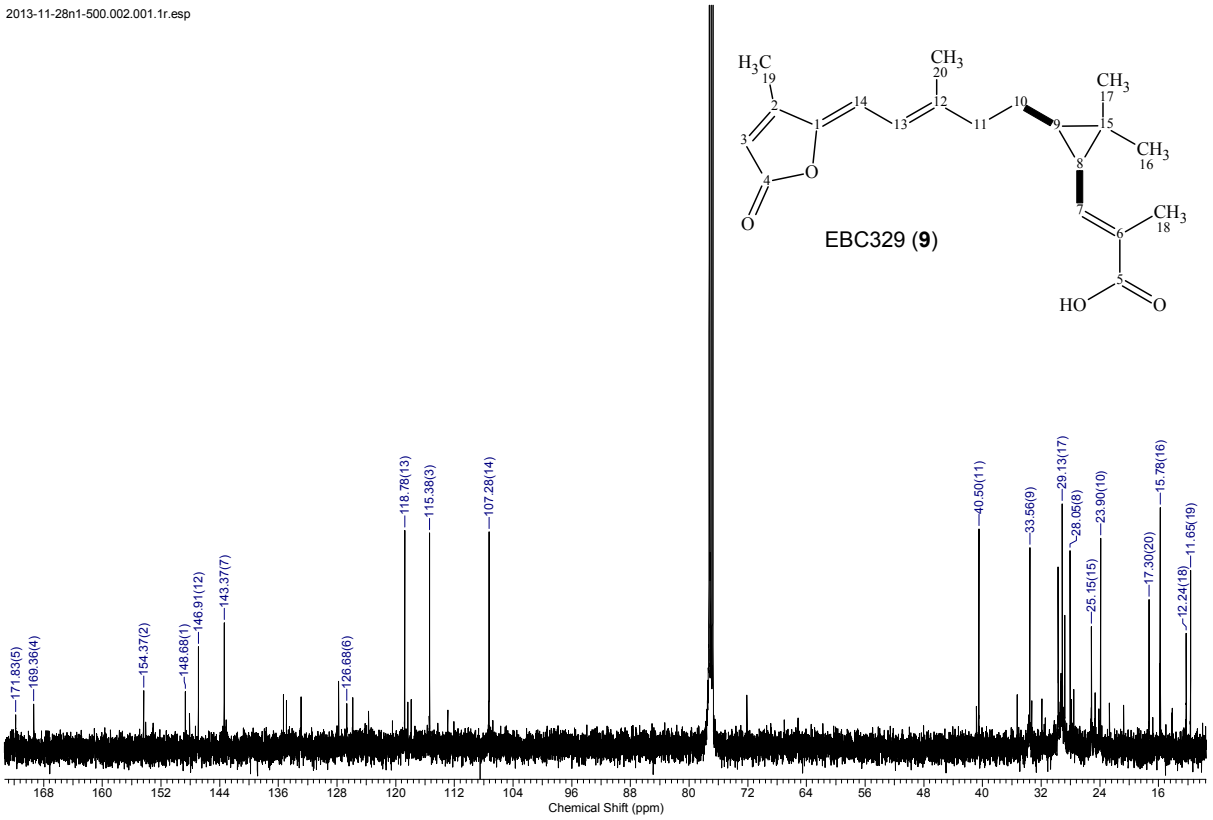
Chemical Shift (ppm)



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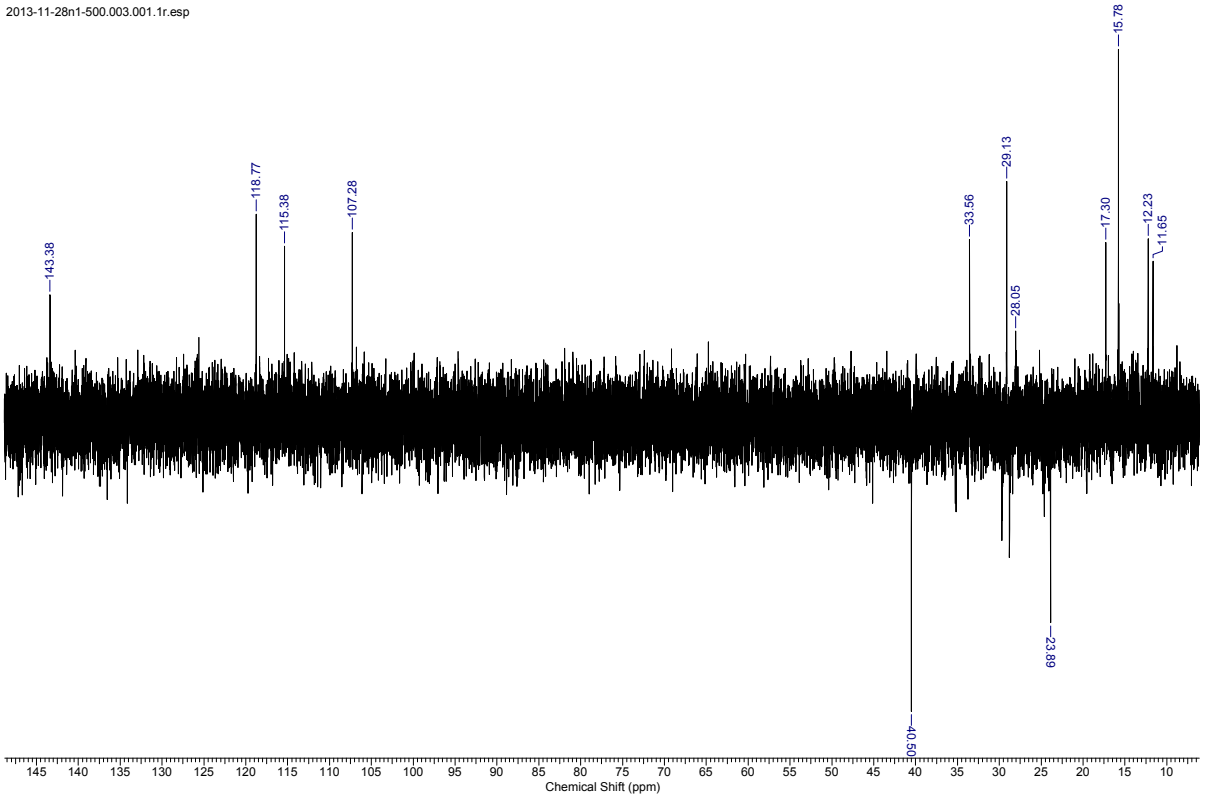

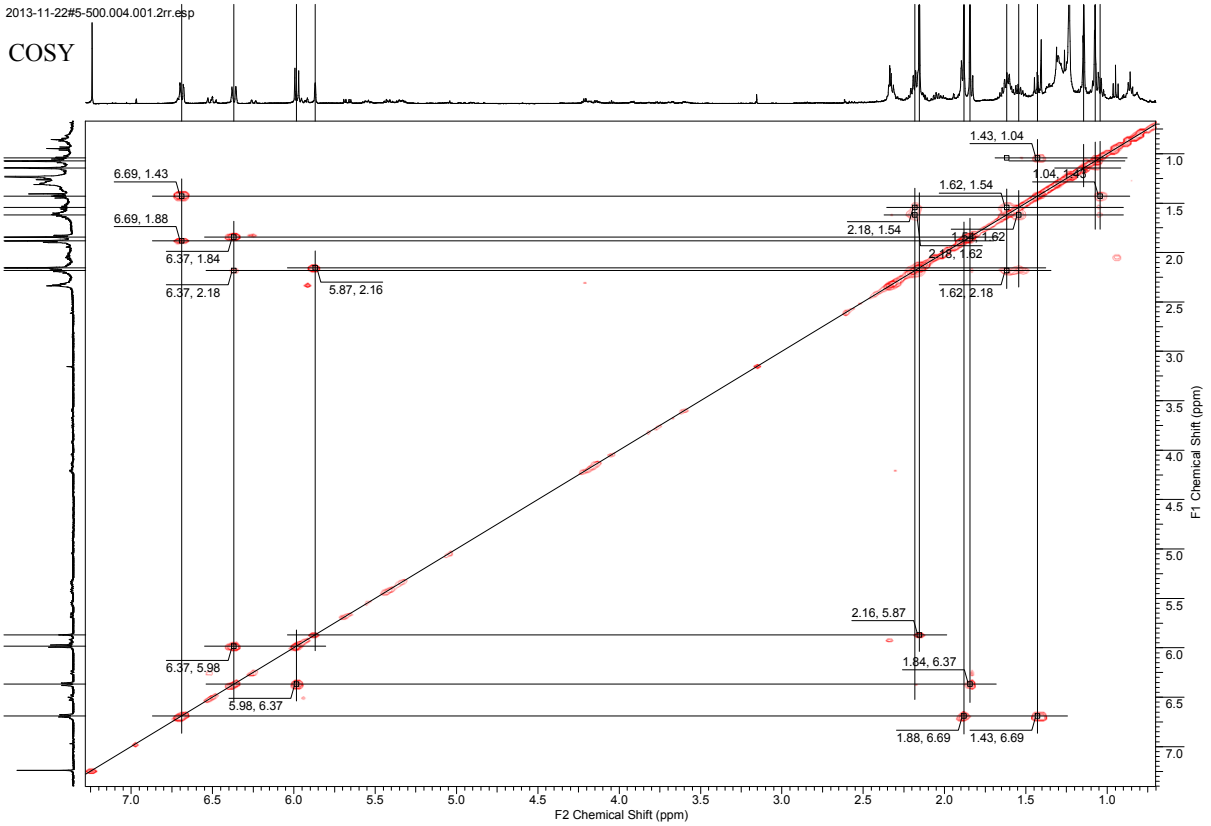
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1.62, 2.18
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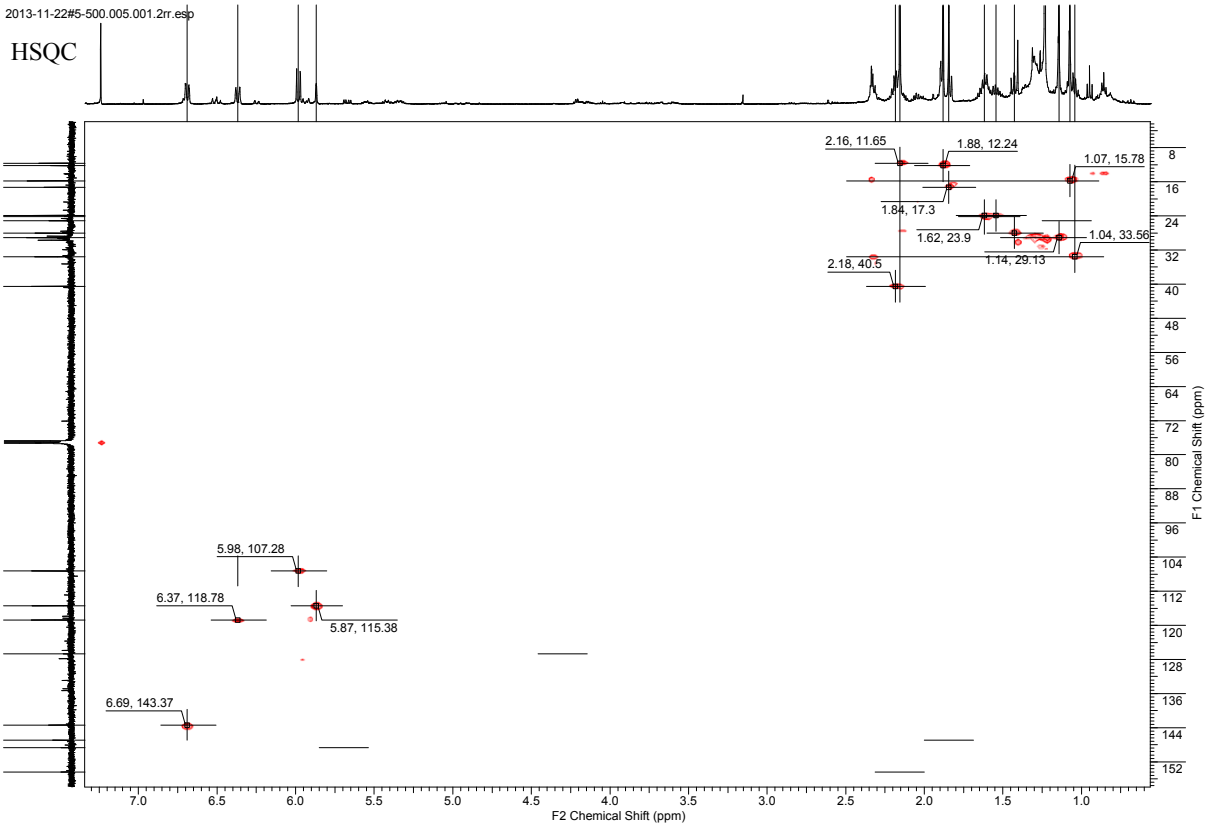
1.43, 6.69
F2 Chemical Shift (ppm)
F1 Chemical Shift (ppm)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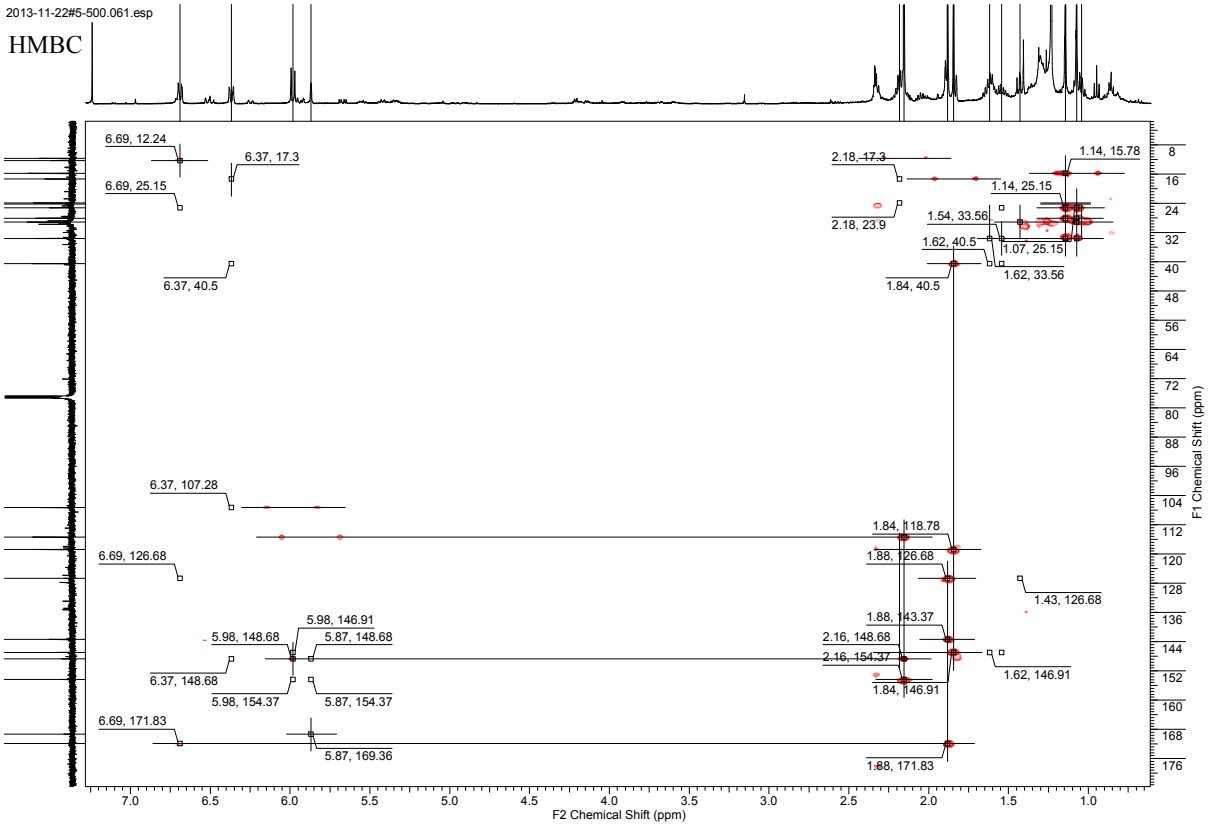
F1 Chemical Shift (ppm)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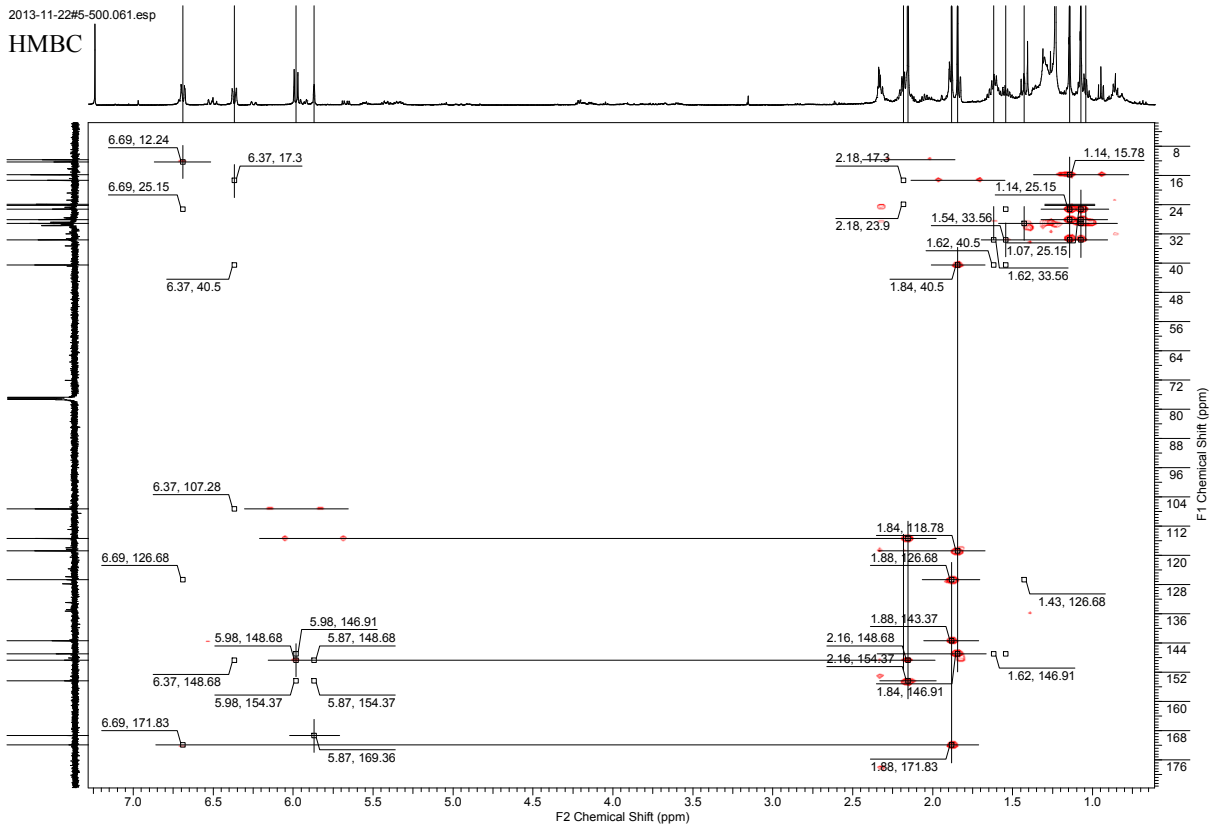
F2 Chemical Shift (ppm)
F1 Chemical Shift (ppm)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2.18, 23.9
1.54, 33.56
1.62, 40.5
1.07, 25.15
1.62, 33.56
1.84, 40.5
6.37, 107.28
1.84, 118.78
6.69, 126.68
1.88, 126.68
1.43, 126.68
1.88, 143.37
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5.98, 148.68
5.87, 148.68
2.16, 148.68
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5.98, 154.37
5.87, 154.37
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6.69, 171.83
5.87, 169.36
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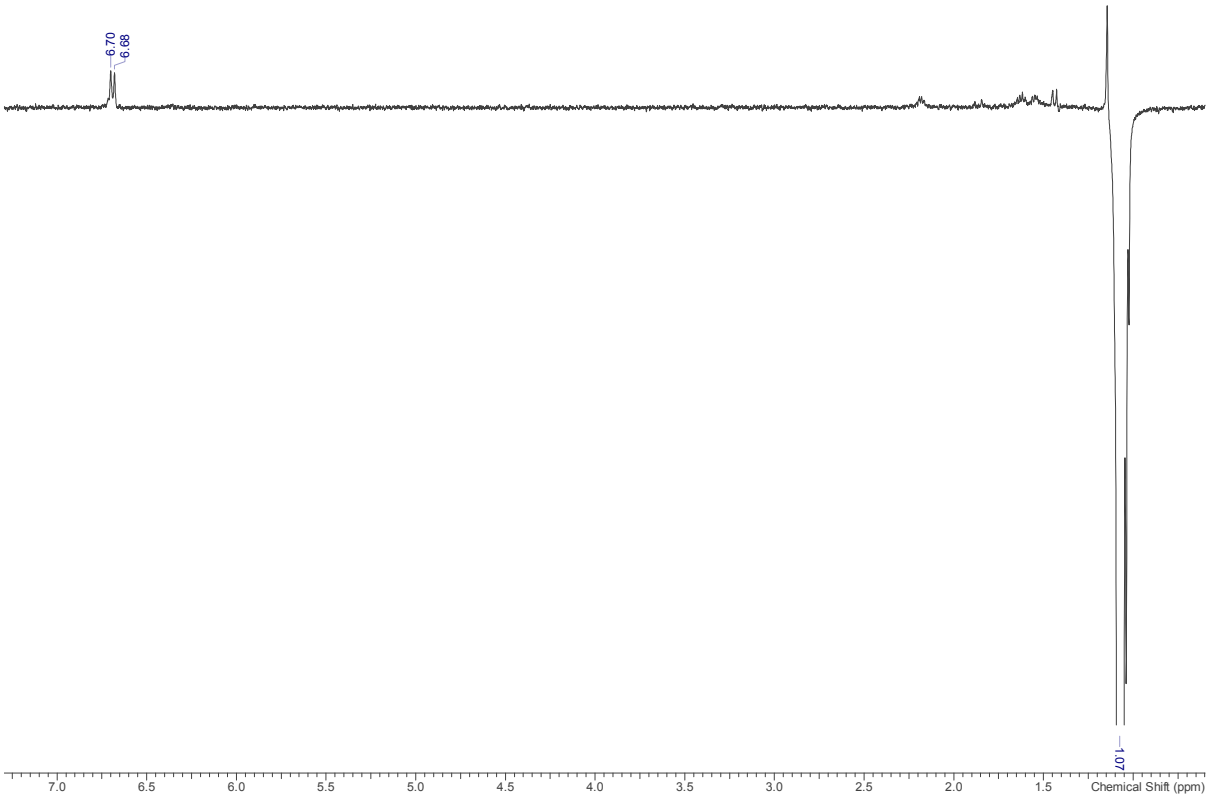
F2 Chemical Shift (ppm)
F1 Chemical Shift (ppm)

2013-11-22#5-500.072.001.1r.esp



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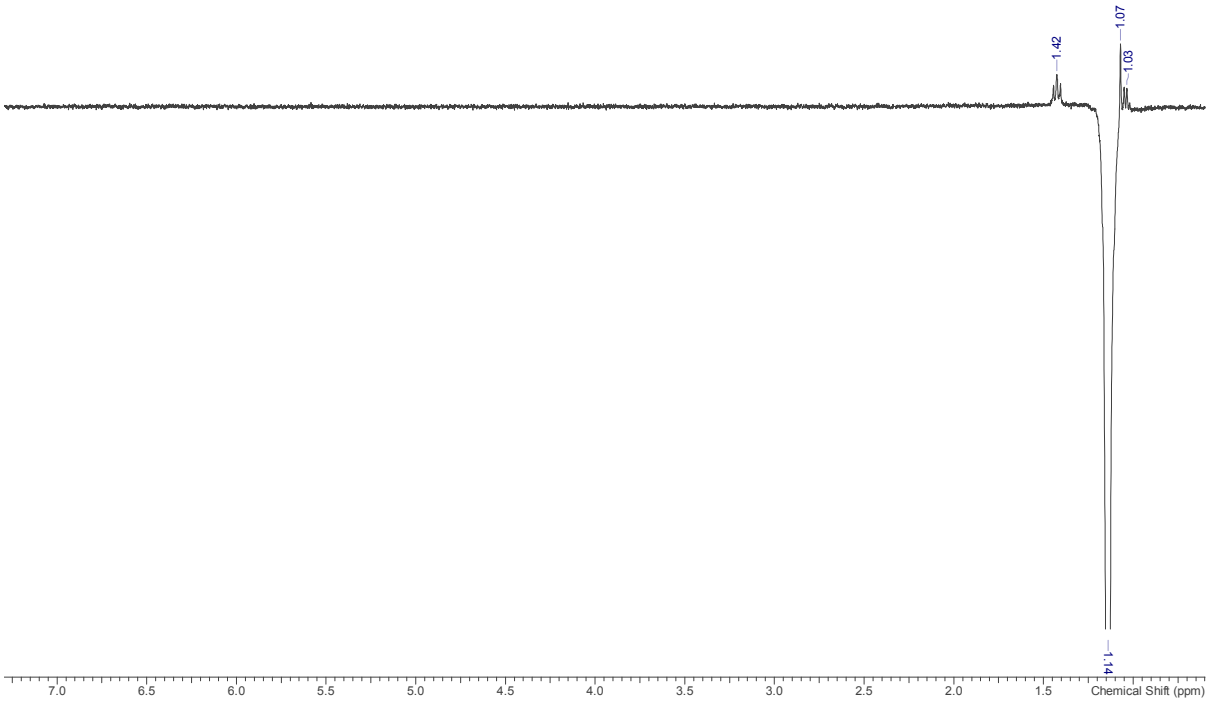

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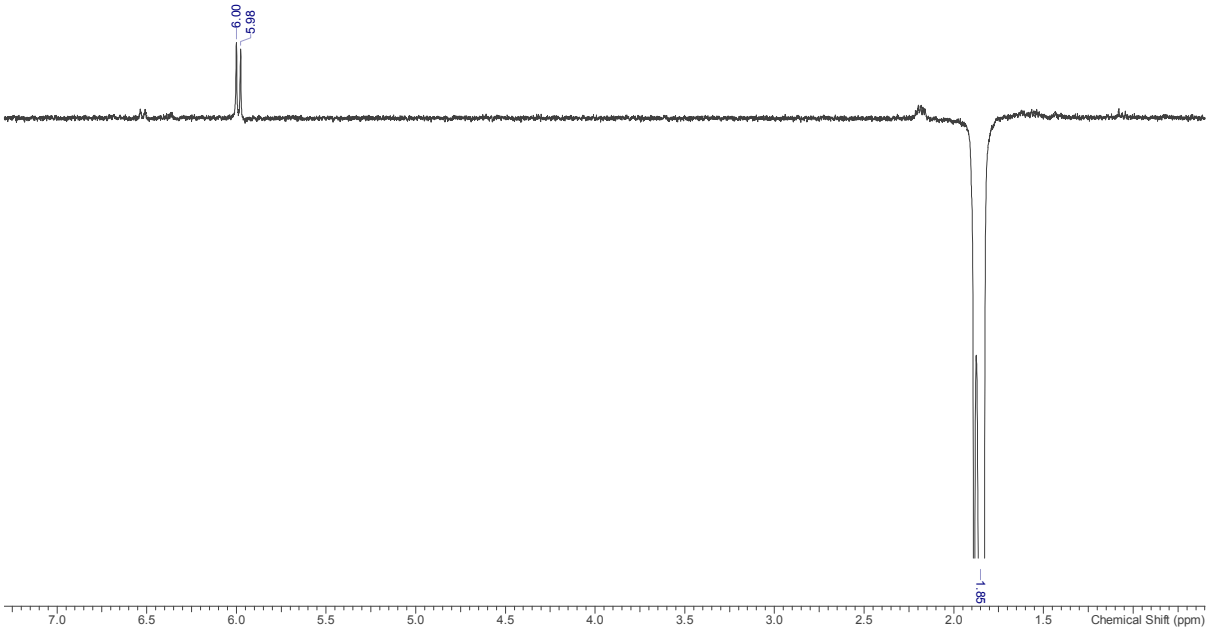

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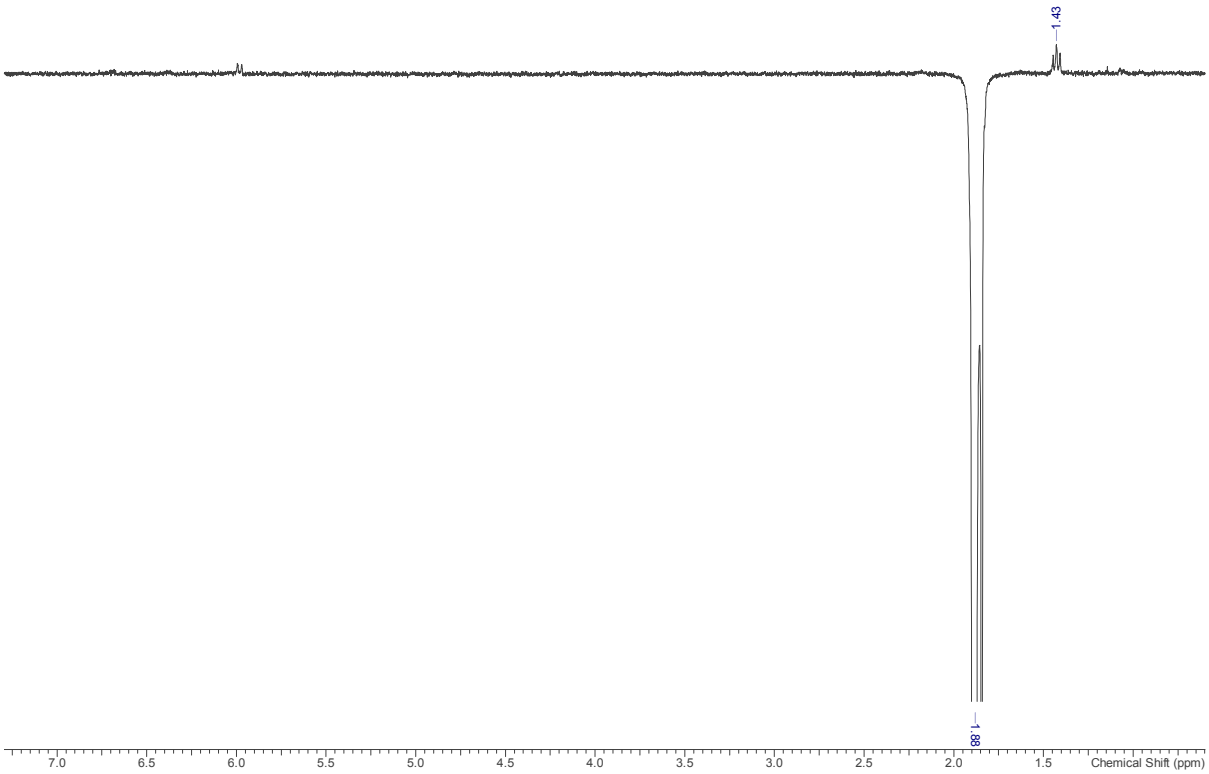

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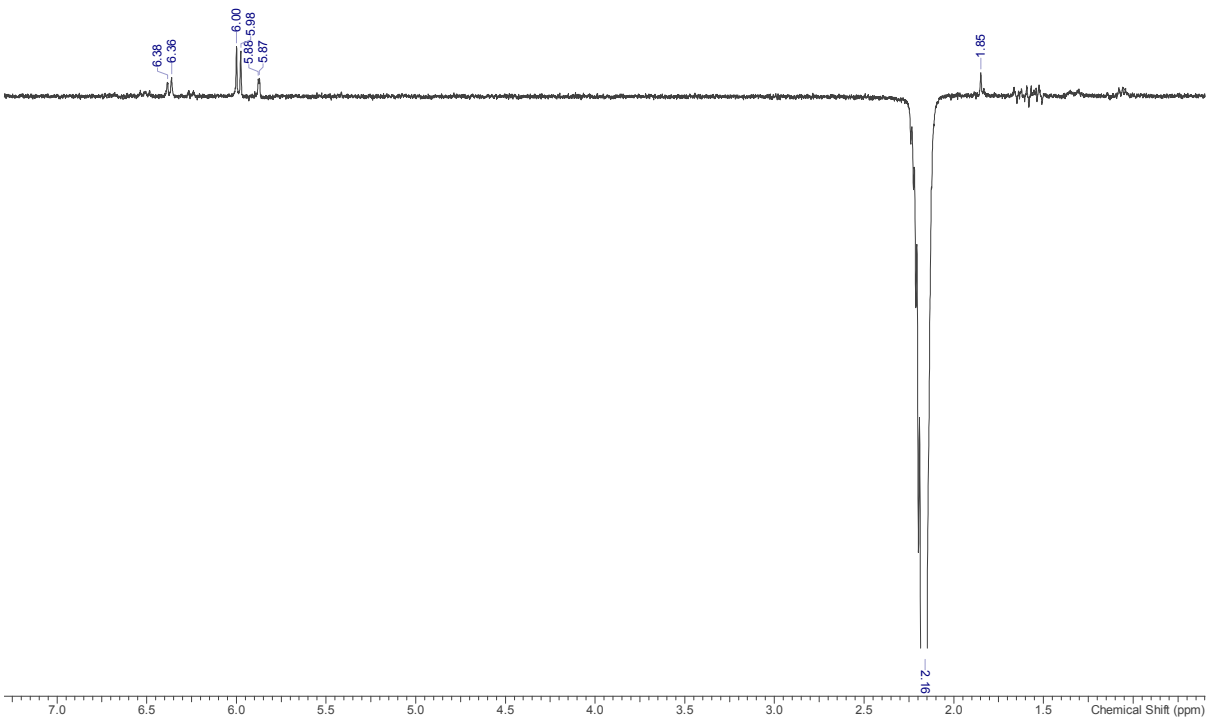

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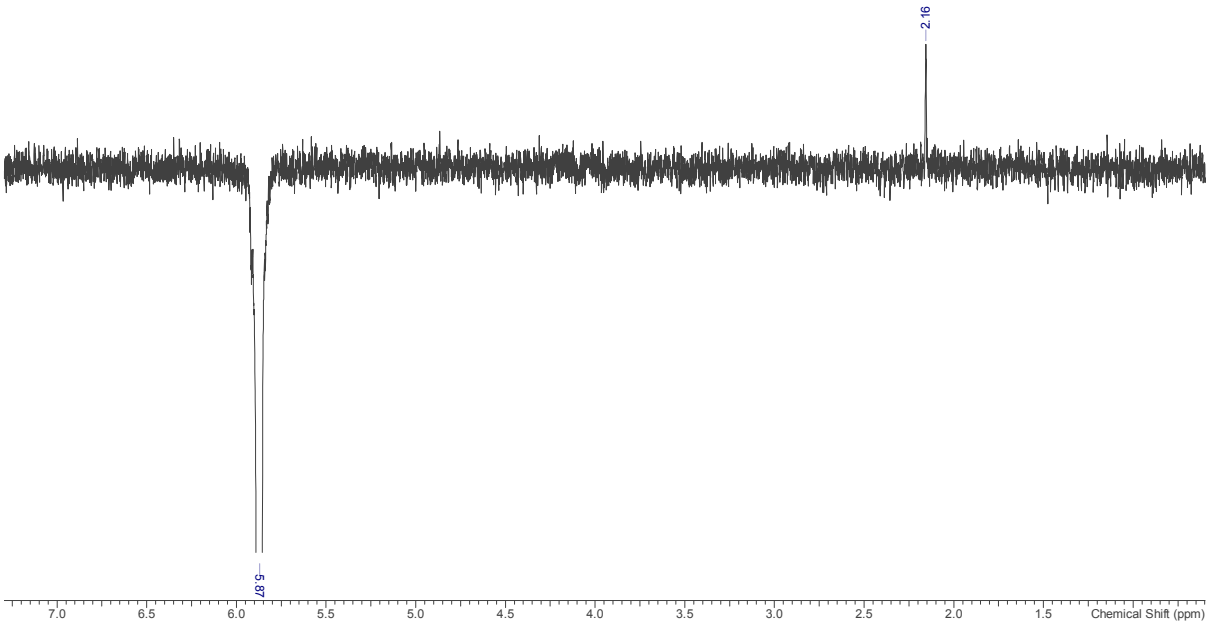

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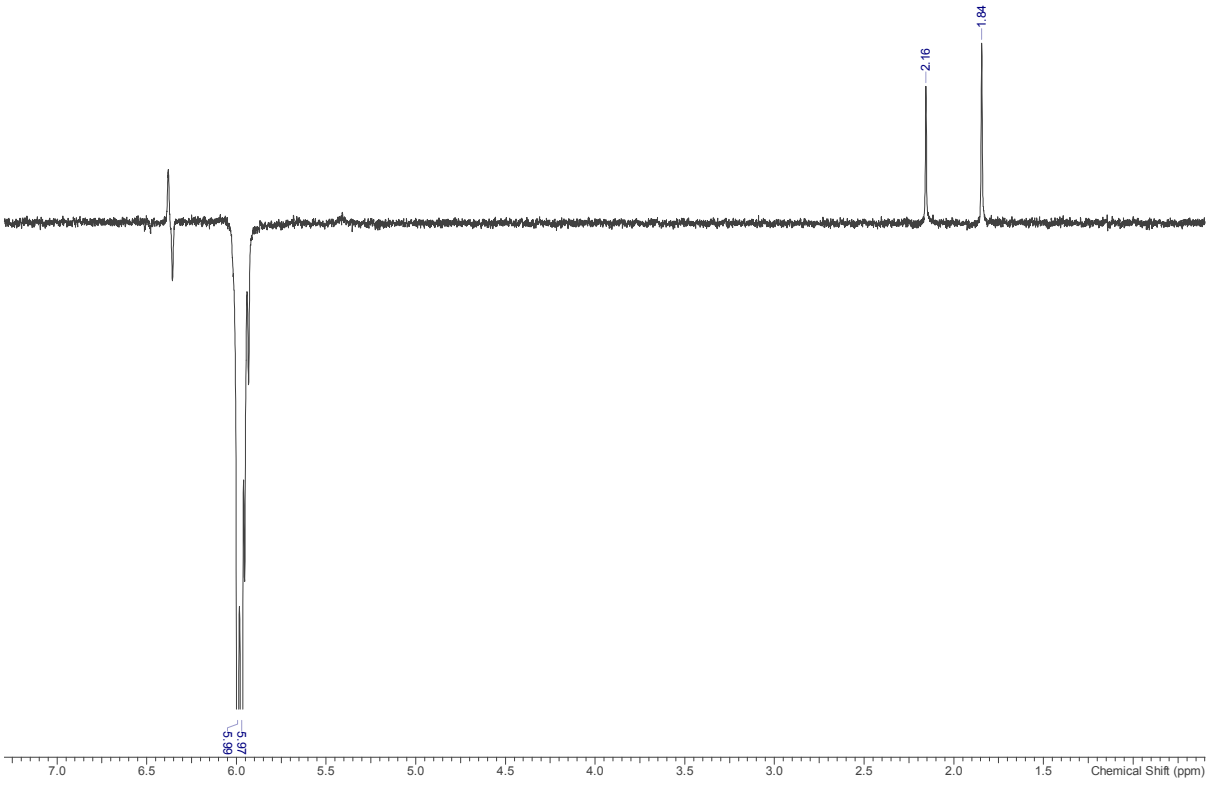

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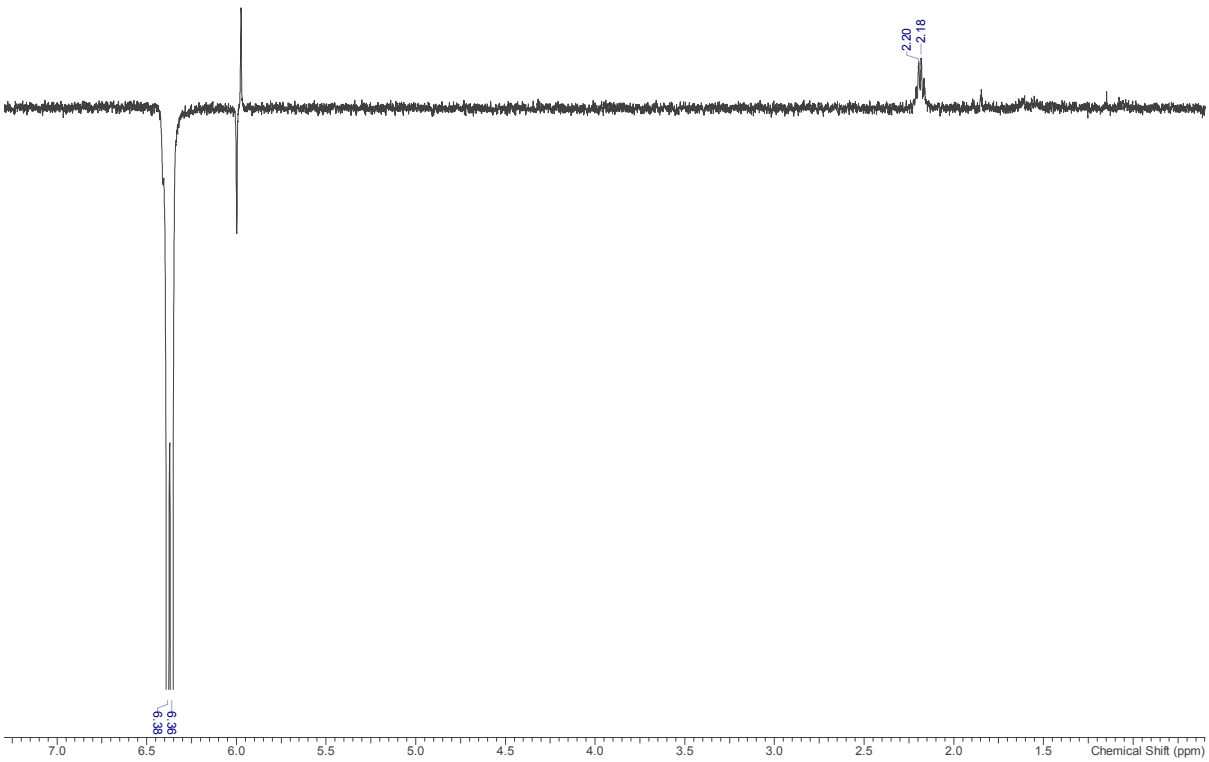

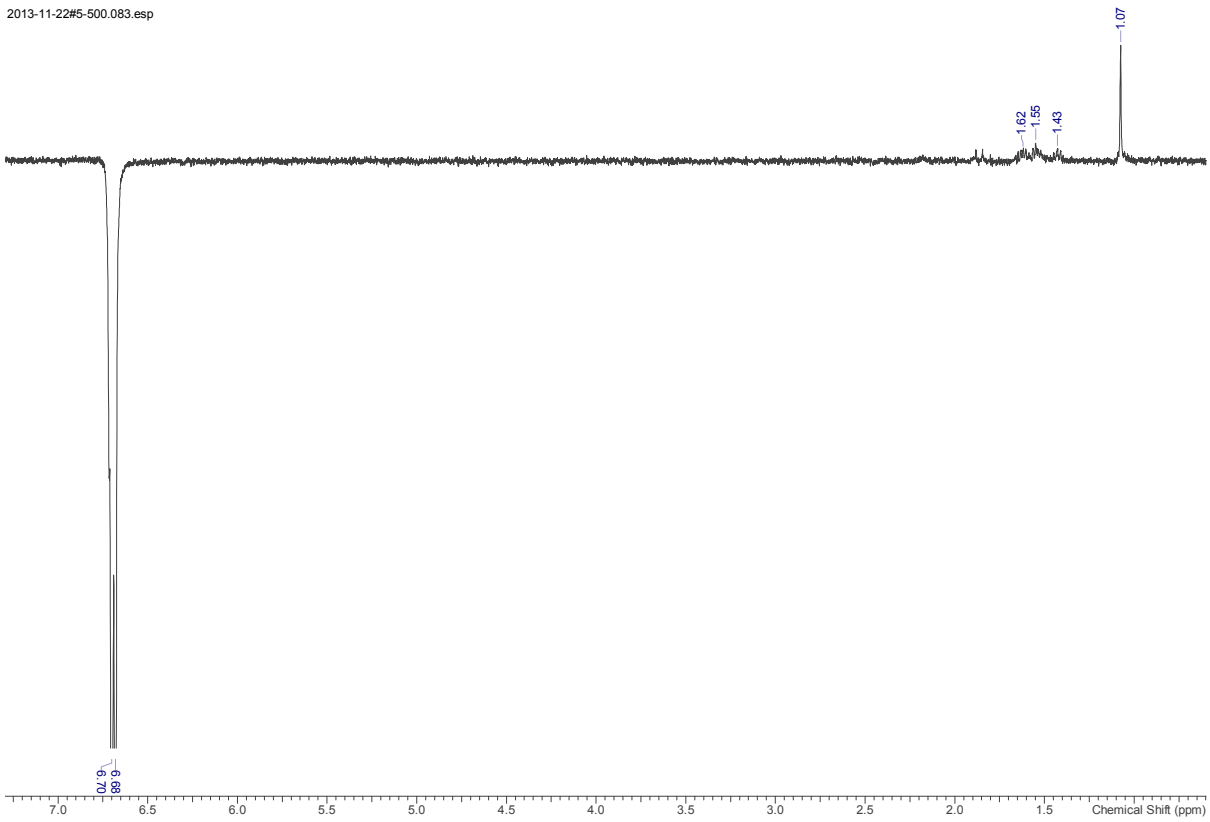
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1.55
1.43
6.70
6.68
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
Chemical Shift (ppm)

Quantum Chemical Calculation of the CD Spectrum of EBC-324 (8)

Quantum chemical calculations were performed in Gaussian 09[1] and ORCA 3.0.1.[2] Geometry optimizations were performed at the B3LYP/6-31G(d) level of theory[3] in the gas phase. The computed harmonic vibrational frequencies gave zero-point energies and thermochemical corrections, which were scaled according to the method of Moran and Radom[4] in order to provide Gibbs free energies in the gas phase. A conformational search of EBC324 (**8**) identified five low-energy conformers ($\Delta G \leq 3.1$ kcal/mol at 25 °C). For each of these five conformers, a time-dependent density functional theory calculation was performed at the TD-RI-B2PLYP/TZVP level of theory[5] using the auxiliary TZV/C correlation fitting basis set. Solvation in acetonitrile was simulated using the COSMO model.[6] Simulation of 50 excitations was found to allow coverage of the wavelength range of the experimental measurements. Boltzmann weighting of the electronic circular dichroism spectra was performed in the program SpecDis,[7] employing Gaussian broadening with $\sigma = 0.32$. The calculated CD spectra were normalized and blue-shifted by 12.4 nm for comparison to experiment.

1. M. J. Frisch, G. W. Trucks, H. B. Schlegel, G. E. Scuseria, M. A. Robb, J. R. Cheeseman, G. Scalmani, V. Barone, B. Mennucci, G. A. Petersson, H. Nakatsuji, M. Caricato, X. Li, H. P. Hratchian, A. F. Izmaylov, J. Bloino, G. Zheng, J. L. Sonnenberg, M. Hada, M. Ehara, K. Toyota, R. Fukuda, J. Hasegawa, M. Ishida, T. Nakajima, Y. Honda, O. Kitao, H. Nakai, T. Vreven, J. A. Montgomery, Jr., J. E. Peralta, F. Ogliaro, M. Bearpark, J. J. Heyd, E. Brothers, K. N. Kudin, V. N. Staroverov, T. Keith, R. Kobayashi, J. Normand, K. Raghavachari, A. Rendell, J. C. Burant, S. S. Iyengar, J. Tomasi, M. Cossi, N. Rega, J. M. Millam, M. Klene, J. E. Knox, J. B. Cross, V. Bakken, C. Adamo, J. Jaramillo, R. Gomperts, R. E. Stratmann, O. Yazyev, A. J. Austin, R. Cammi, C. Pomelli, J. W. Ochterski, R. L. Martin, K. Morokuma, V. G. Zakrzewski, G. A. Voth, P. Salvador, J. J. Dannenberg, S. Dapprich, A. D. Daniels, O. Farkas, J. B. Foresman, J. V. Ortiz, J. Cioslowski, and D. J. Fox, Gaussian 09, Revision D.01, Gaussian, Inc., Wallingford CT, 2013.
2. F. Neese, *WIREs Comput. Mol. Sci.*, 2012, **2**, 73–78.
3. a) C. Lee, W. Yang, R. G. Parr, *Phys. Rev. B*, 1988, **37**, 785–789. b) A. D. Becke, *J. Chem. Phys.*, 1993, **98**, 1372–1377. c) A. D. Becke, *J. Chem. Phys.*, 1993, **98**, 5648–5652. (d) P. J. Stephens, F. J. Devlin, C. F. Chabalowski, M. J. Frisch, *J. Phys. Chem.*, 1994, **98**, 11623–11627.
4. J. P. Merrick, D. Moran, L. Radom, *J. Phys. Chem. A* 2007, **111**, 11683–11700.
5. S. Grimme, *J. Chem. Phys.* 2006, **124**, 034108.
6. a) A. Klamt, G. Schüürmann, *J. Chem. Soc., Perkin Trans 2* 1993, 799–805. b) S. Sinnecker, A. Rajendran, A. Klamt, M. Diedenhofen, F. Neese, *J. Phys. Chem. A* 2006, **110**, 2235–2245.
7. T. Bruhn, A. Schaumlöffel, Y. Hemberger, G. Bringmann, SpecDis, Version 1.60, University of Wuerzburg, Germany, 2012.

Calculated Geometries and Energies of the Lowest-Energy Conformers of EBC-324 (8)

The Cartesian coordinates of the five low-energy conformers of EBC-324 below are listed below, together with the following energies:

B3LYP/6-31G(d) electronic energy (E)
B3LYP/6-31G(d) scaled Gibbs free energy in the gas phase at 298.15 K and 1 atm (G)

Energies are given in Hartree.

8a

C	-2.082869	1.169260	-0.500787
C	-2.574576	0.478913	0.765844
C	-2.700772	-0.987817	0.756073
C	-2.306898	-1.741957	-0.518281
O	-2.034109	-0.867280	-1.623005
O	-1.224639	0.279109	-1.214325
O	-1.634592	-0.321989	1.481410
C	-3.717964	-1.676121	1.640832
C	-1.260837	-2.870656	-0.349667
C	0.044911	-2.498043	0.296479
C	1.293019	-2.764801	-0.117023
C	2.510340	-2.360281	0.714918
C	2.380792	-1.098245	1.608426
C	3.083345	0.183833	1.135169
C	2.333002	1.458495	0.711744
C	0.898123	1.460284	0.422491
C	0.106454	2.562216	0.322738
C	0.568519	3.976936	0.594854
C	-1.253383	2.485764	-0.222082
O	-1.860115	3.517728	-0.517767
C	1.636676	-3.551140	-1.358853
C	3.274318	0.705841	-0.265502
C	4.595450	1.403774	-0.556312
C	2.684560	0.045656	-1.501164
H	0.738534	4.524397	-0.340256
H	-0.200487	4.531779	1.140710
H	1.491188	4.002282	1.177848
H	0.446271	0.501868	0.197551
H	2.741614	2.388800	1.094610
H	4.477955	2.150832	-1.351355
H	4.992311	1.913297	0.328722
H	5.349323	0.678680	-0.890651
H	2.474535	0.803745	-2.265780
H	1.756133	-0.494043	-1.309403
H	3.405928	-0.660198	-1.931582
H	3.933339	0.430386	1.770434
H	2.811537	-1.328930	2.589185
H	1.329490	-0.883224	1.809606
H	3.370513	-2.239565	0.044816
H	2.771459	-3.219529	1.351393
H	2.251614	-2.953419	-2.044353
H	2.233531	-4.436623	-1.097181
H	0.759306	-3.896181	-1.910605
H	-0.077562	-1.977000	1.238654
H	-1.120193	-3.311421	-1.341314
H	-1.749738	-3.647605	0.261807
H	-3.225916	-2.225698	-0.878342
O	-3.212642	1.522680	-1.219429
H	-3.252625	1.075831	1.375887

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H   -3.892369   -1.083925    2.543533
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0 imaginary frequencies
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G = -1154.515385
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8b

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H    2.067255   -4.229323   -0.129304
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H    5.423924   -0.107324   -0.396934
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C    2.757593    0.417494    1.781820
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H    3.826929    1.114773   -1.514161
C    1.809846    1.872253   -1.105055
H    0.902157    1.289705   -1.277073
H    2.012395    2.375087   -2.058125
C    1.479752    2.947695   -0.022780
H    1.889886    2.650435    0.943390
H    1.957281    3.901807   -0.281853
C   -0.027051    3.106505    0.094491
C   -0.665557    3.991884   -0.946295
H   -0.477874    3.603591   -1.957136
H   -1.747557    4.092108   -0.827254
H   -0.231522    5.000494   -0.912714
C   -0.693266    2.368377    0.995462
H   -0.108523    1.762343    1.685869
C   -2.176073    2.201436    1.158902
H   -2.457735    2.406054    2.201280
H   -2.736766    2.915137    0.543927
C   -2.724878    0.785101    0.850894
H   -3.795404    0.802227    1.100311
C   -2.649282    0.340006   -0.611000
C   -2.047601   -0.967927   -0.921630
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O   -2.630903   -2.595929    0.632017
H   -2.266334   -3.506315    0.711099
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C    2.315077   -2.249232   -0.015898
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C    2.458006   -0.248291    1.656137
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C    1.592546    2.105156    0.626049
C    0.224181    1.646624    0.361637
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C    3.760508    2.730333   -0.646365
C    2.406526    0.787725   -1.522563
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H   -1.735433    4.196706    1.233150
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H    3.414382    3.364782   -1.472061
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0 imaginary frequencies
E = -1154.903618
G = -1154.512182

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O	-1.087861	0.343169	1.501558
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C	2.649194	-0.143624	1.756888
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H	5.088380	-1.565358	-0.285485
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H	1.635064	0.239262	1.647717
H	3.262869	0.635250	2.226865
H	3.891980	-0.043474	-1.544882
H	1.113824	0.968947	-1.269716
H	2.410052	1.538823	-2.275719
H	2.752740	2.288843	0.610255
H	2.898473	3.298735	-0.821324
H	0.898109	4.681178	-1.497672
H	0.108627	3.223438	-2.098417
H	-0.730530	4.270391	-0.943144
H	0.808792	2.117970	1.765844
H	-1.765634	3.519383	0.814574
H	-1.375126	2.931255	2.425164
H	-2.894538	1.333067	1.651138
C	-3.247154	2.102445	-1.100396
H	-2.939093	-0.611508	-1.645249
H	-3.494058	1.742668	-2.103173
H	-4.181035	2.222061	-0.538623
H	-2.780423	3.087429	-1.200134

0 imaginary frequencies
E = -1154.903628
G = -1154.510411