Supporting Information

Expansinine, a novel indole alkaloid-ergosteroid conjugate from the endophytic fungus *Penicillium expansum* of *Aconitum carmichaelii*

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Figure S1a ^{1}H-NMR spectrum ($CDCl_3$, 400 MHz) of **1**.

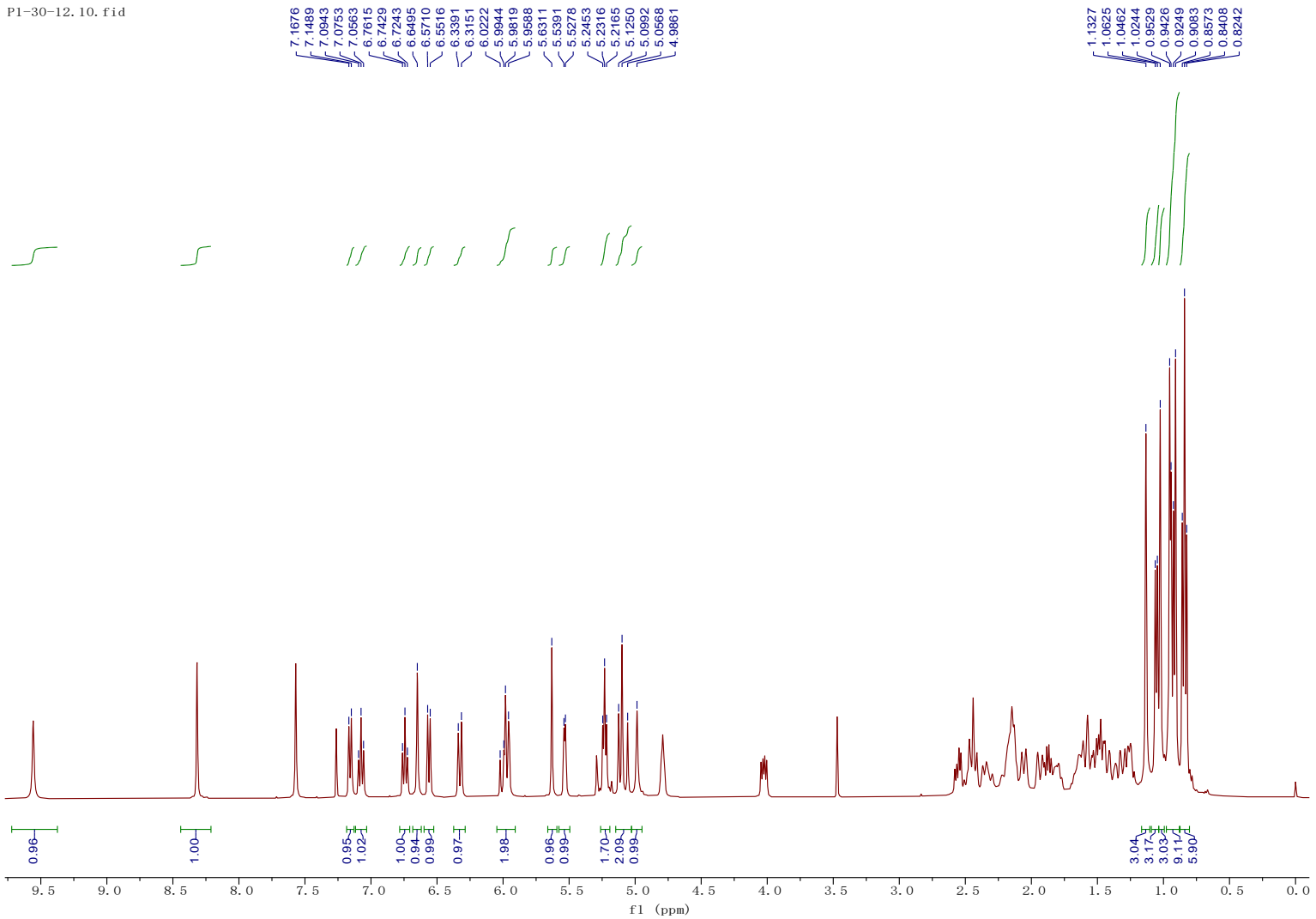


Figure S1b ^{1}H-NMR spectrum ($CDCl_3$, 600 MHz) of **1**.

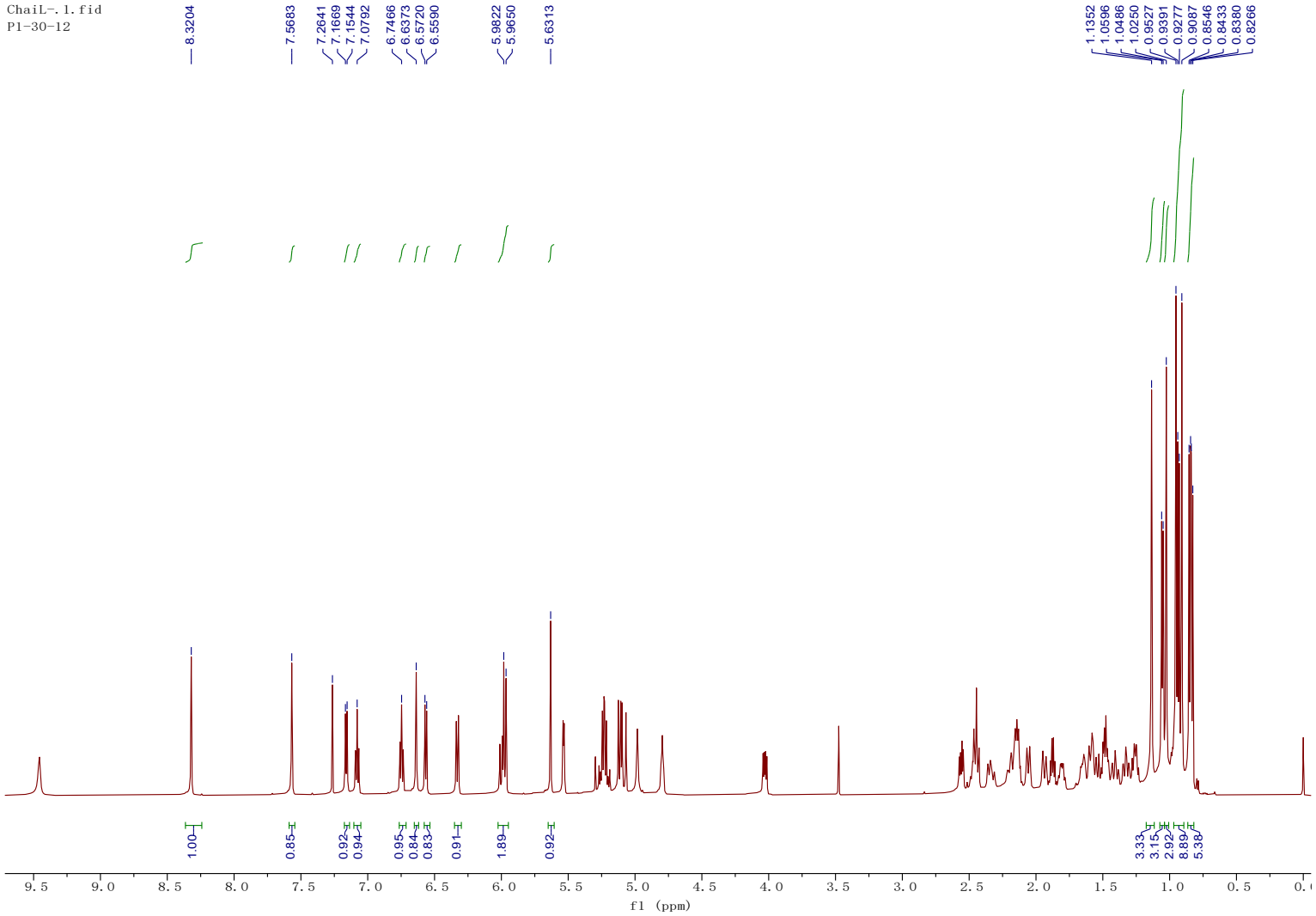


Figure S2 ^{13}C-NMR spectrum ($CDCl_3$, 400 MHz) of **1**.

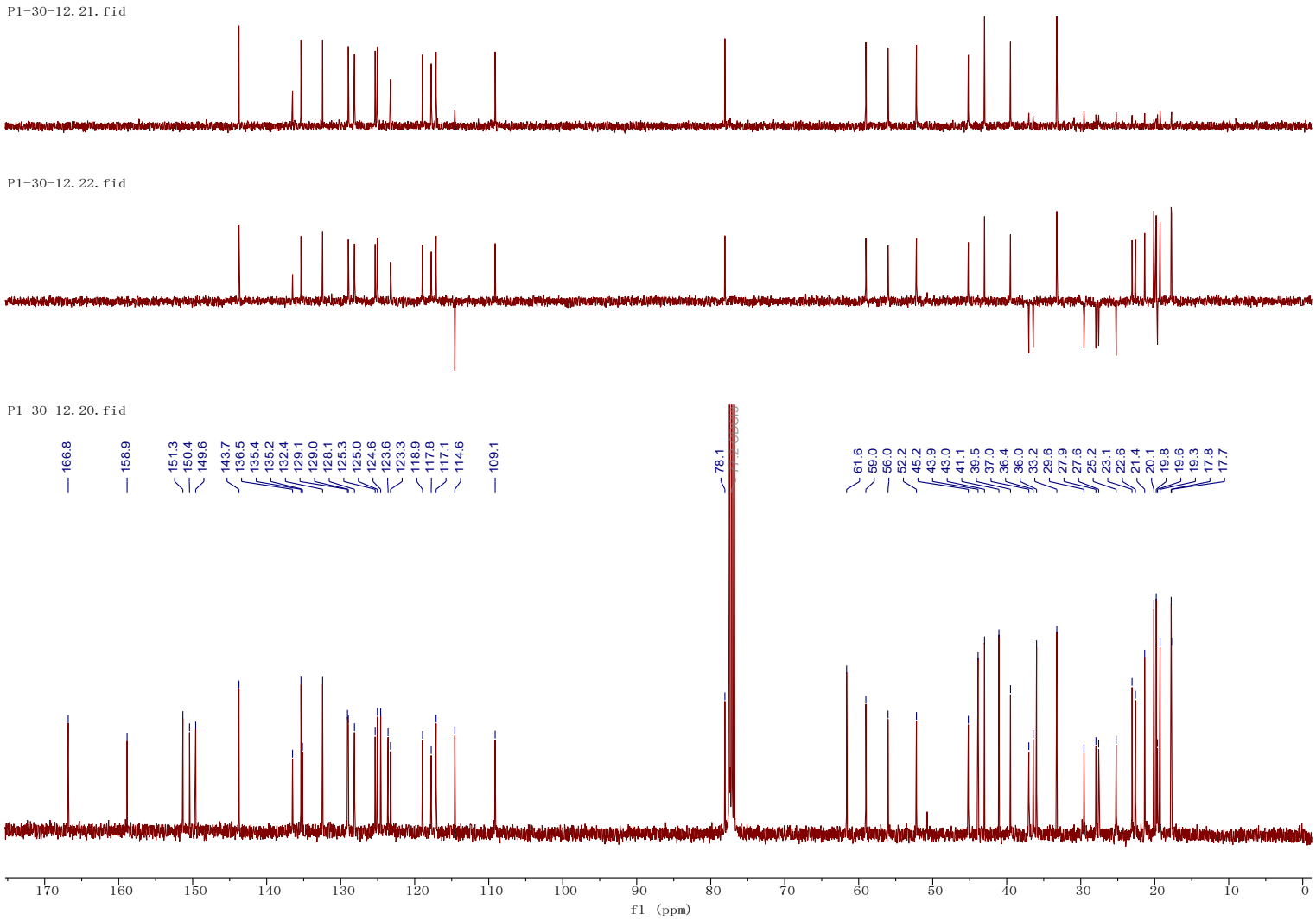


Figure S3 ^{1}H-^{1}H COSY spectrum ($CDCl_3$, 400 MHz) of **1**.

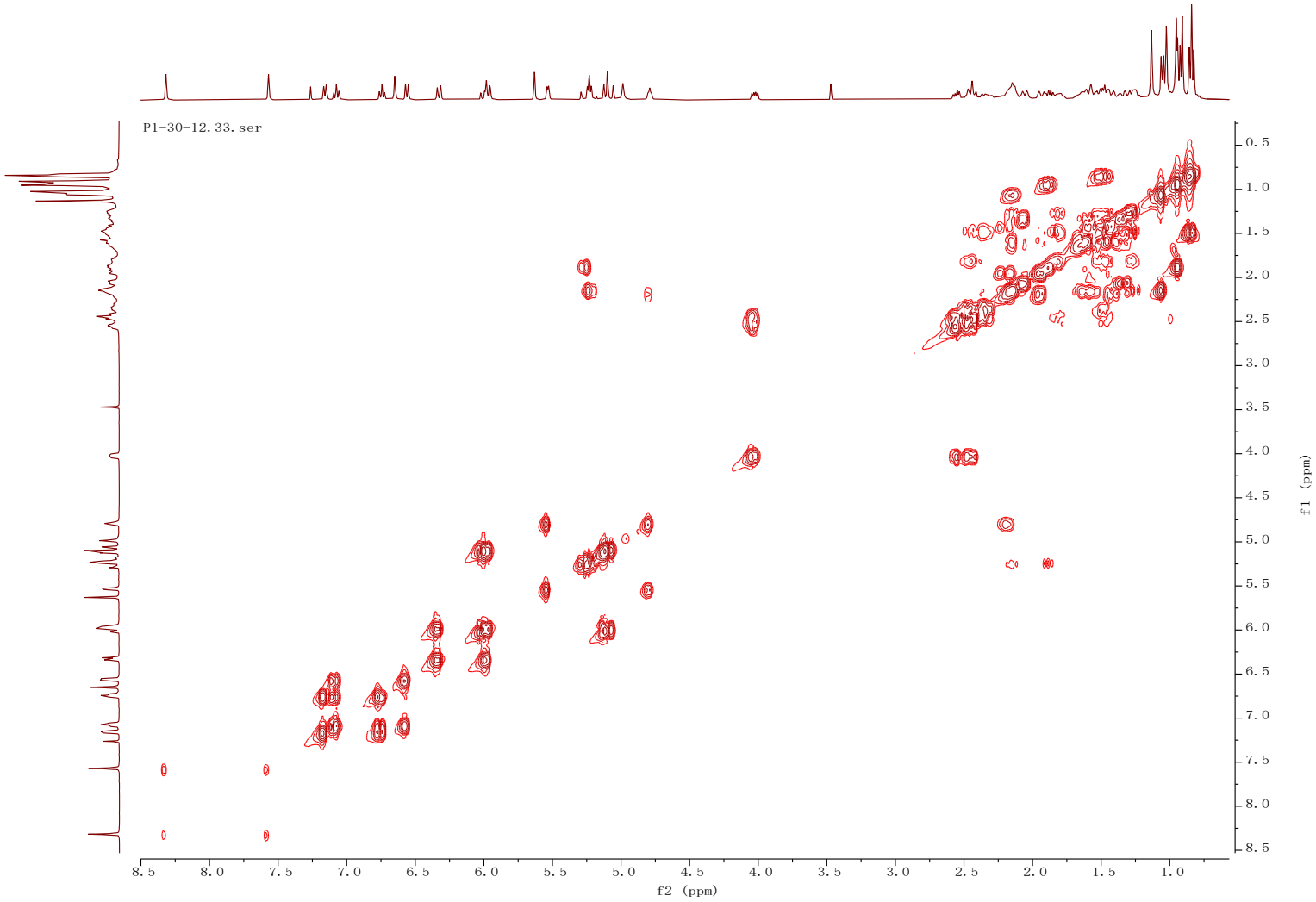


Figure S4a HMBC spectrum ($CDCl_3$, 600 MHz) of **1**.

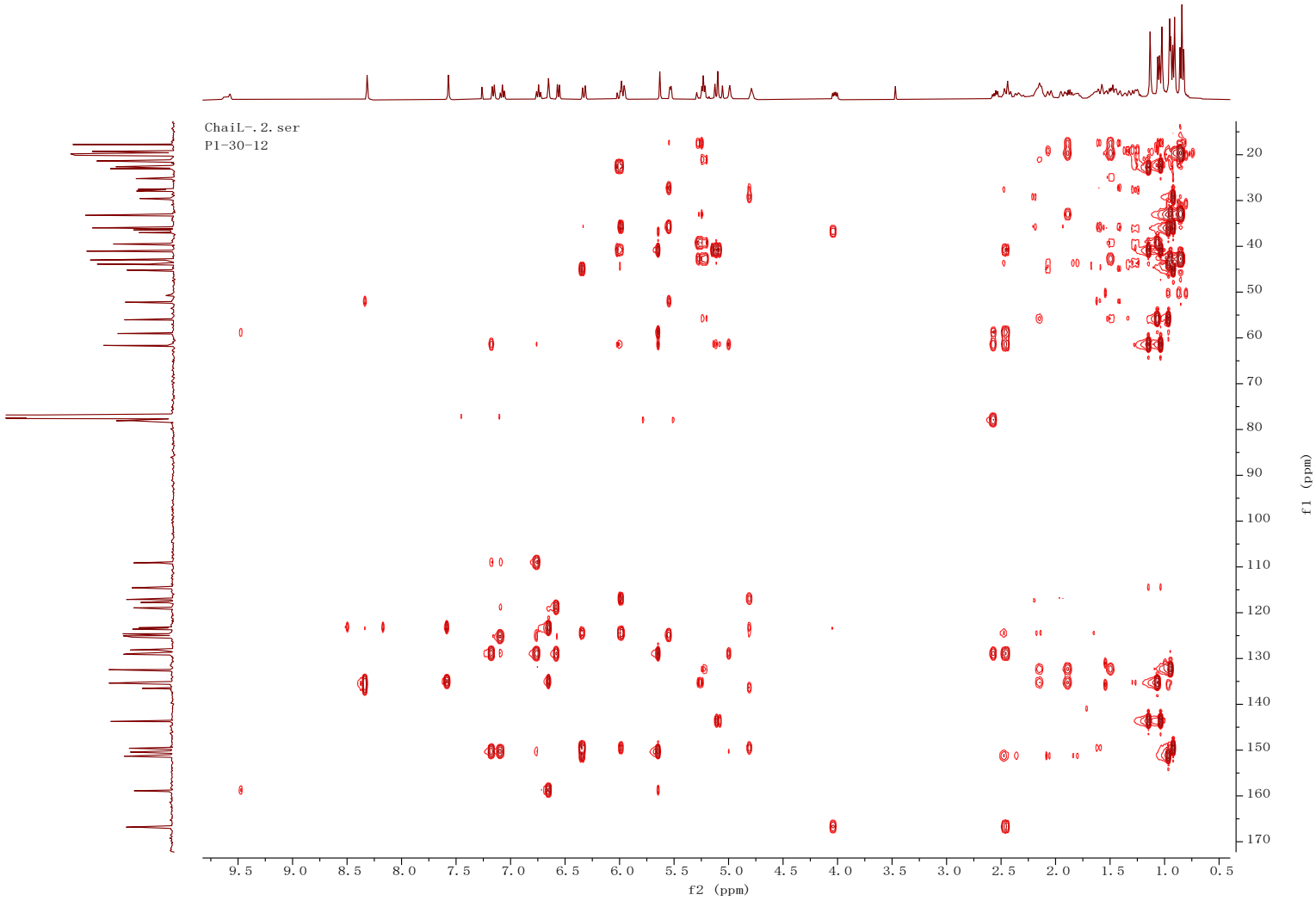


Figure S4b Partial HMBC spectrum ($CDCl_3$, 600 MHz) of **1**.

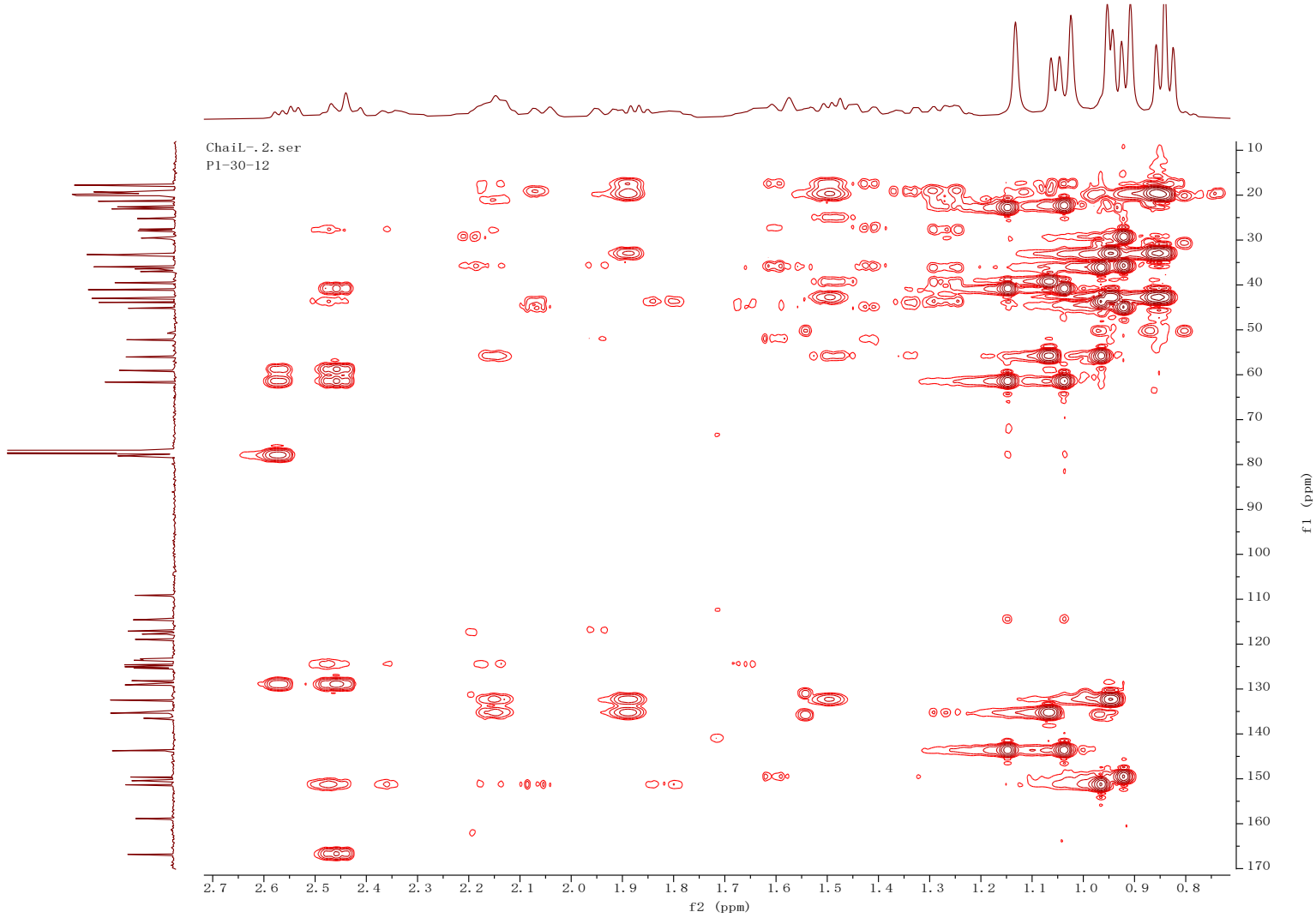


Figure S4c Partial HMBC spectrum ($CDCl_3$, 600 MHz) of **1**.

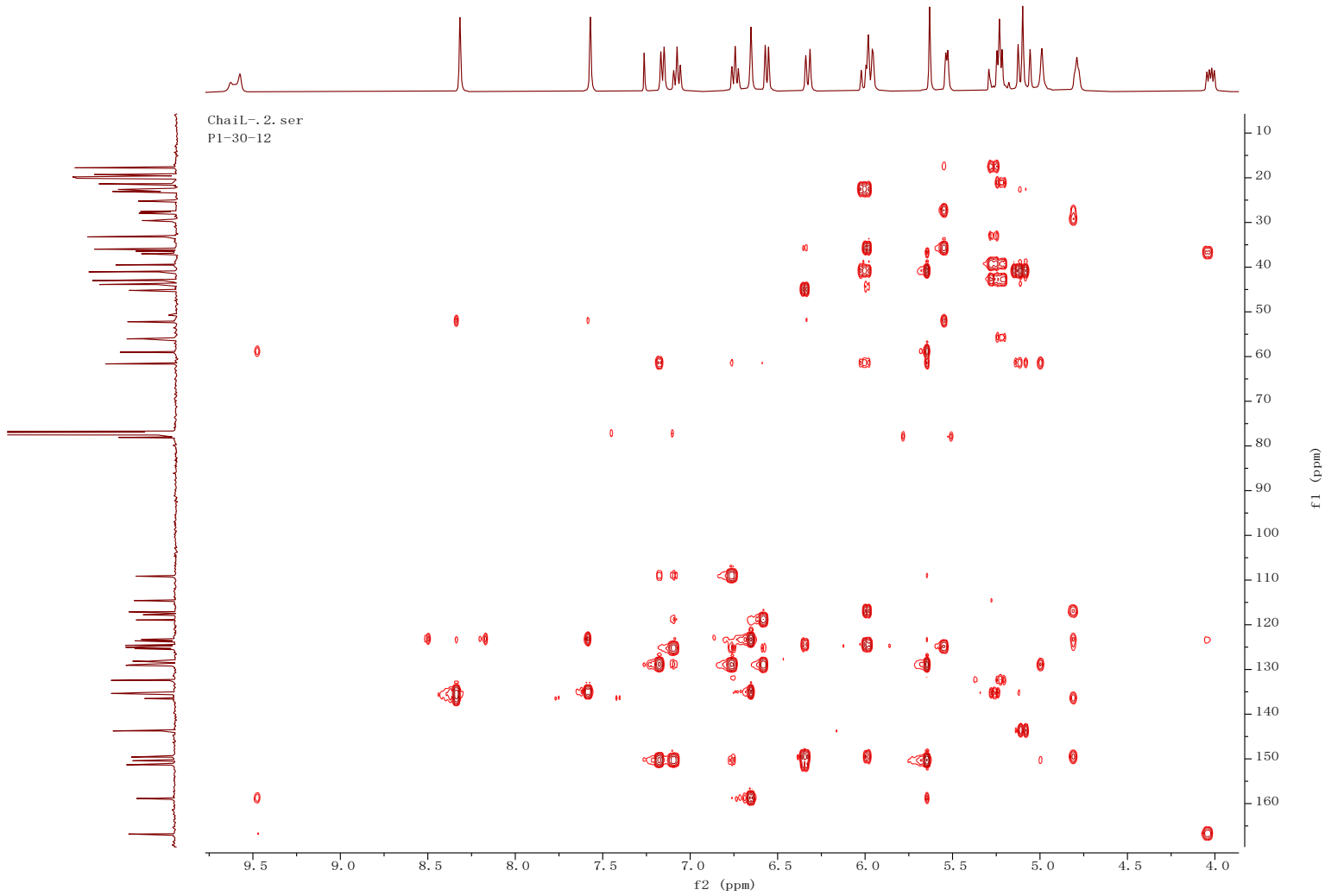


Figure S5 HSQC spectrum ($CDCl_3$, 400 MHz) of **1**.

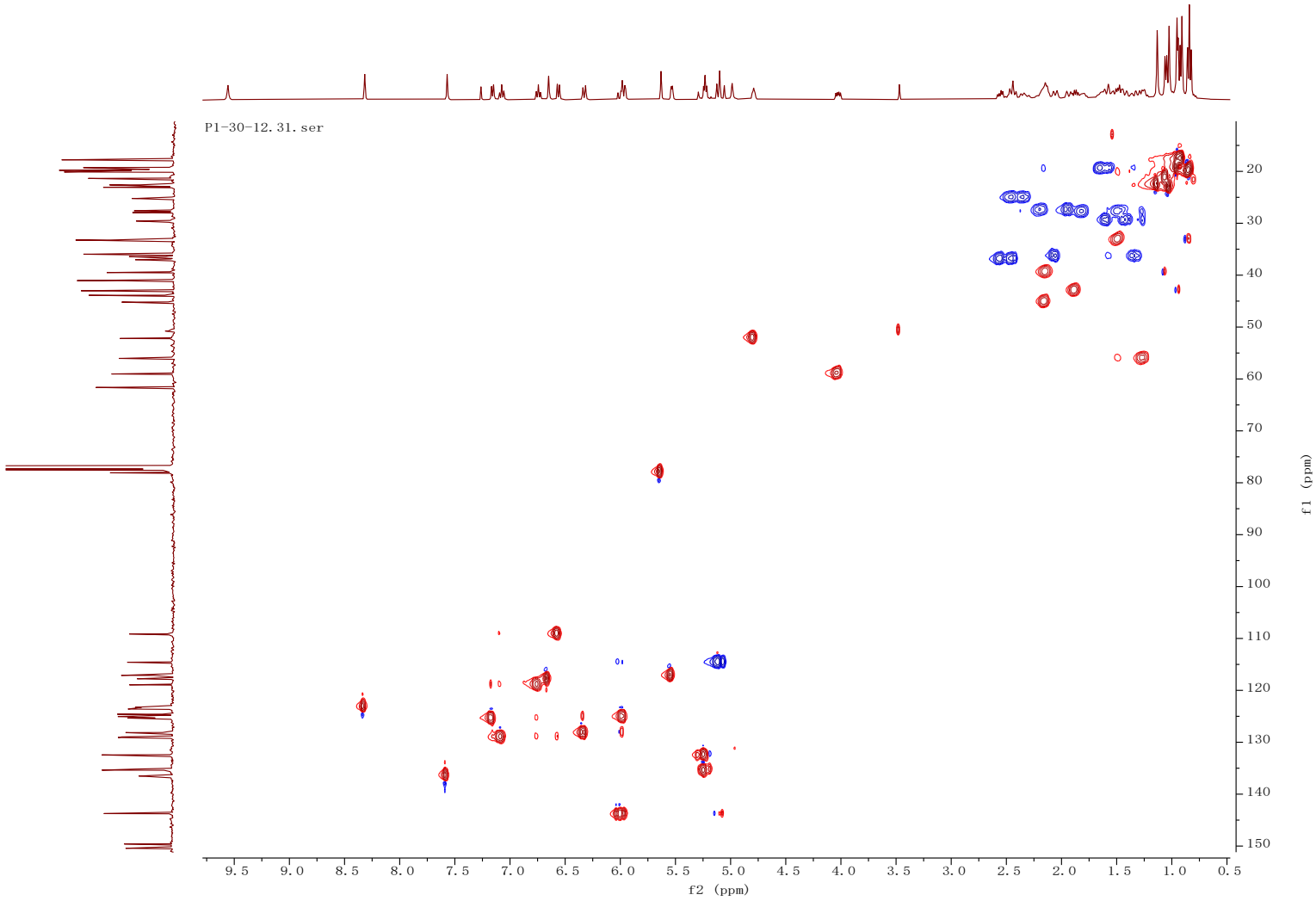


Figure S6 ROSEY spectrum ($CDCl_3$, 600 MHz) of **1**.

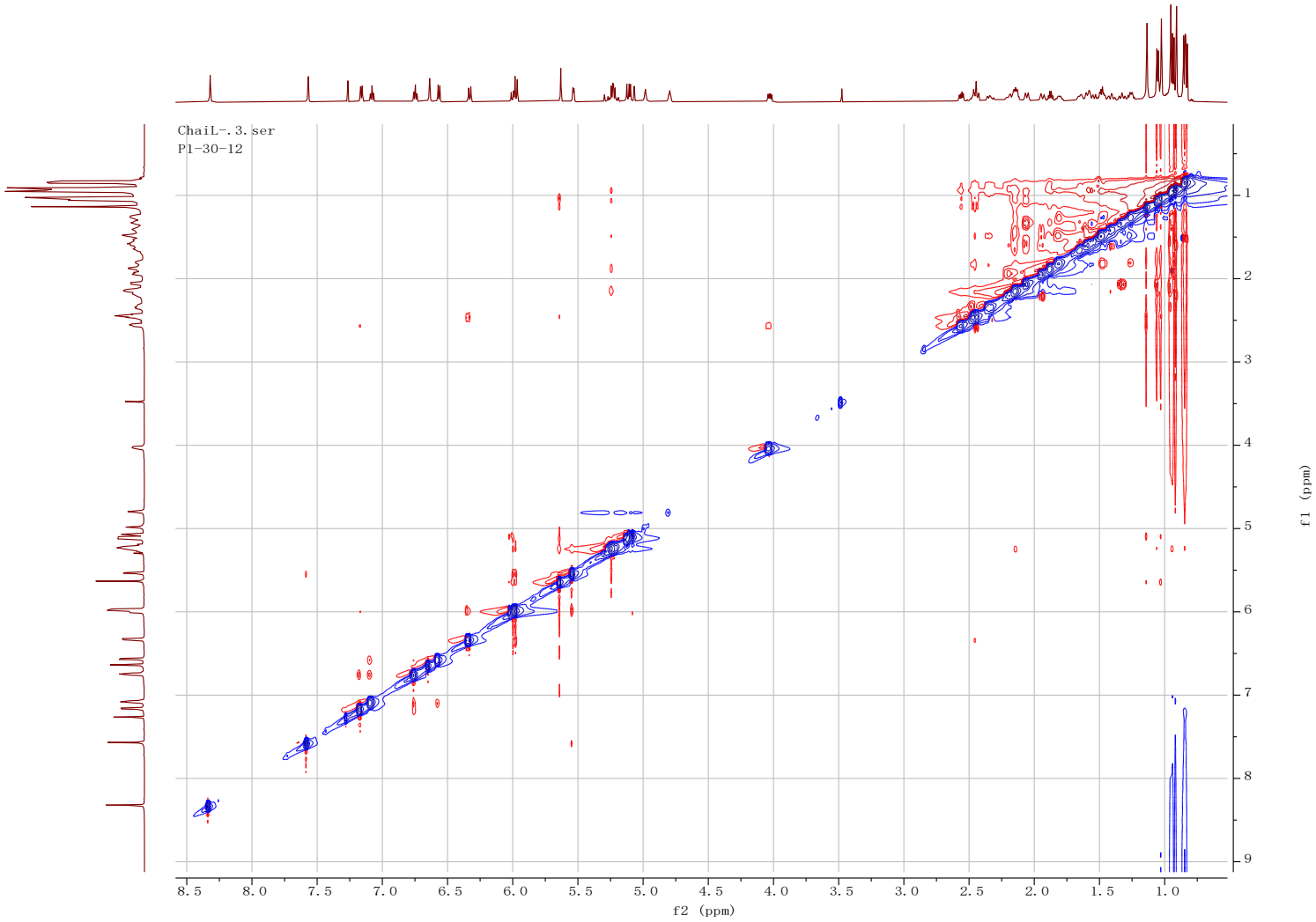


Figure S7 (+)-HR-ESI-MS of **1**.

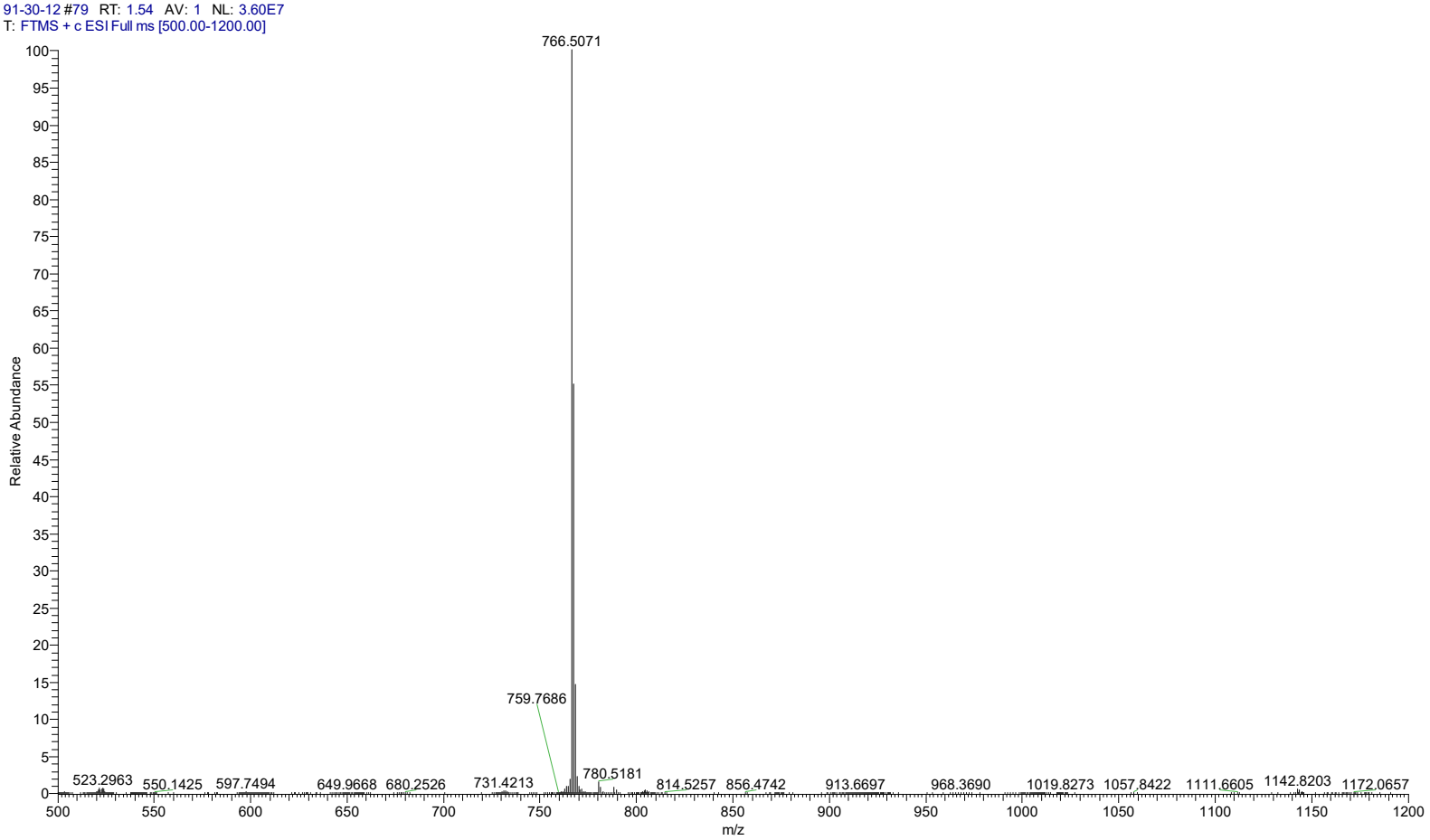


Figure S8 IR spectrum of **1**.

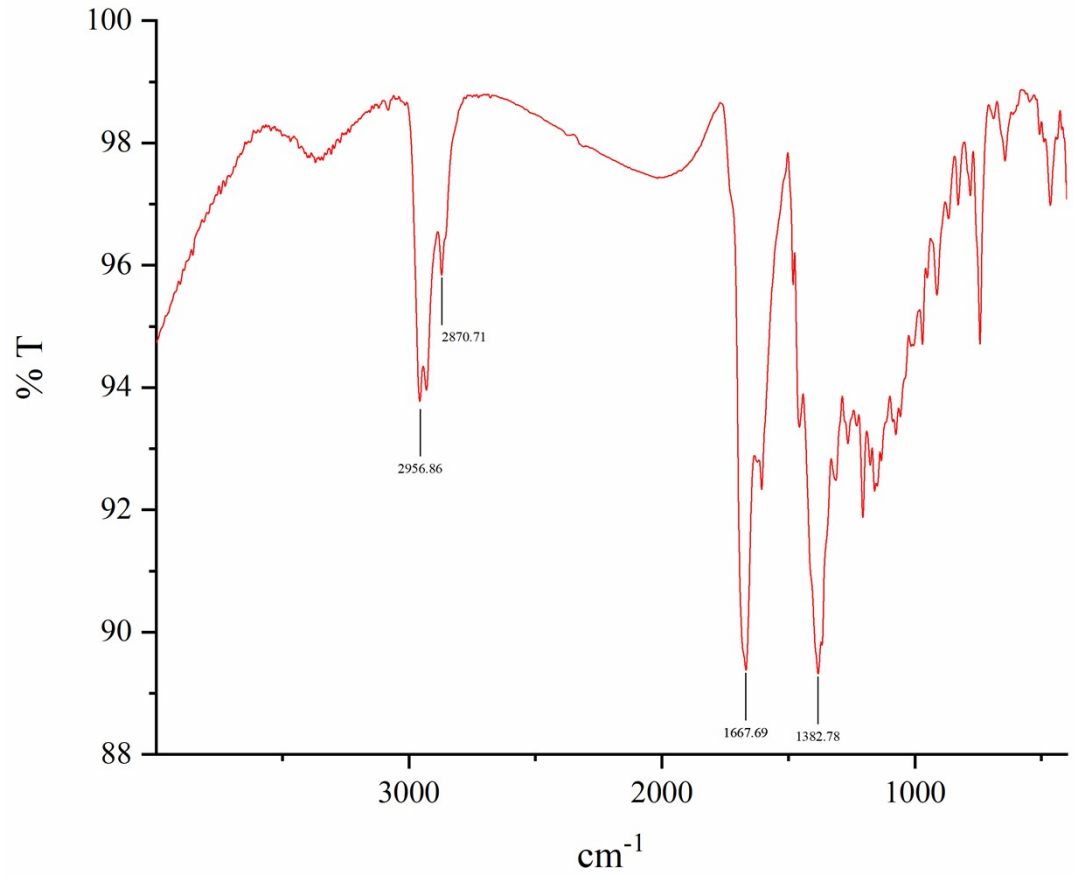


Figure S9 UV spectrum of **1**.

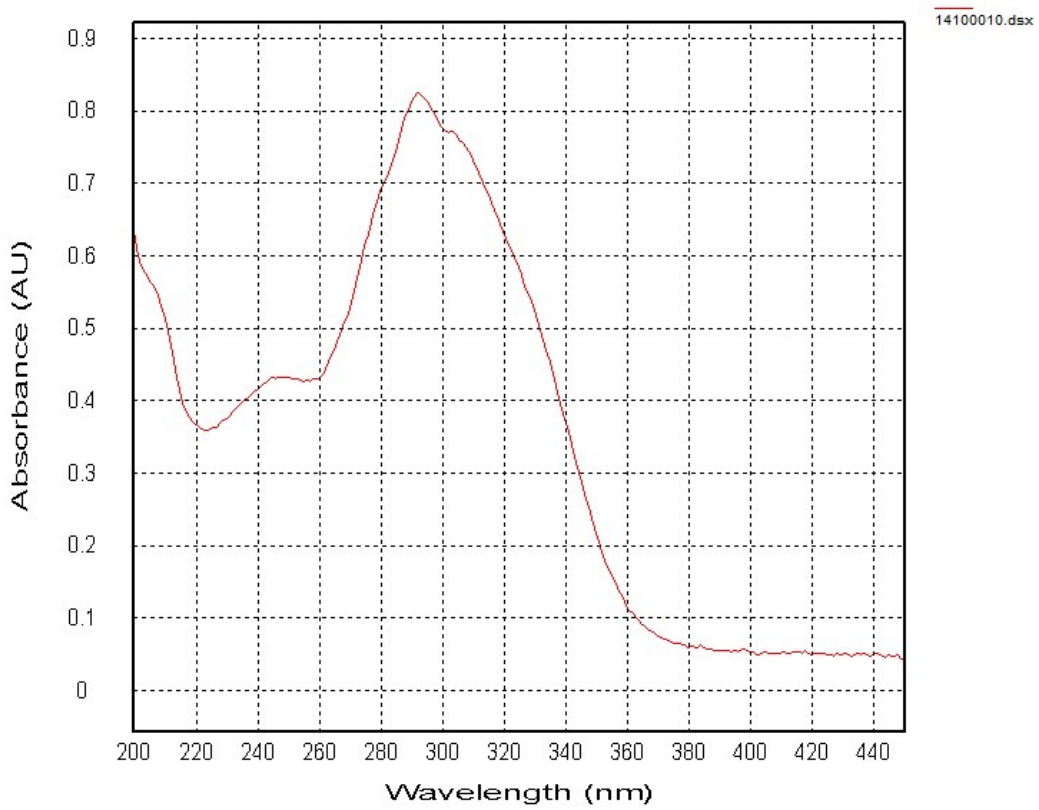


Figure. S10. Optical rotation value (MeOH) of **1**

Rudolph Research Analytical

This sample was measured by Autopol IV, Serial Number: 83650

Manufactured by Rudolph Research Analytical, Hackettstown, NJ, USA.

Measurement Date : 2024/6/26

Method Name : Specific Rotation @25C

Set Temperature : 25.0°C

Time Delay : 10

Delay between measurement : 1 Sec

N	Avg.	Std.Dev.	%RSD	Min	Max
5	-15.927	0.651	-4.08	-16.909	-15.364

S.No	Sample ID	Time	Result	Scale	OR °Arc	WLG.nm	Lg.mm	Conc.g/100mL	Temp
1	20240602-P1-30-12 2	16:21:52	-15.545	SR	-0.0171	589	100	0.110	25.0°C
2	20240602-P1-30-12 2	16:21:59	-15.545	SR	-0.0171	589	100	0.110	25.0°C
3	20240602-P1-30-12 2	16:22:06	-16.273	SR	-0.0179	589	100	0.110	25.0°C
4	20240602-P1-30-12 2	16:22:12	-16.909	SR	-0.0186	589	100	0.110	25.0°C
5	20240602-P1-30-12 2	16:22:19	-15.364	SR	-0.0169	589	100	0.110	25.0°C

TDDFT-ECD calculations

Conformational searches for **1a** and **1b** were performed using CREST and xtb software at the GFN2-xTB level with a search of the top ten advantageous conformations in search results. The optimized conformers of **1a** and **1b** were subjected to ECD calculations using the time-dependent density functional theory (TDDFT) methodology at the CAM-B3LYP/6-311+G(d,p) level in methanol with the polarizable continuum model (PCM) of the solvent (methanol).

NMR computational methods

Stable conformers of isomer of **1** (**1a** and **1b**) with the search of the top ten advantageous conformations in search results were obtained. The calculations in solution were performed using the polarizable continuum model (PCM) of the solvent (chloroform) used in the experiment. Then, **1a** and **1b** were first optimized at the PCM/mPW1PW91/6-311+G(d,p) level of theory and subjected to ^{1}H and ^{13}C NMR chemical shift calculations using the GIAO method at the PCM/mPW1PW91/6-311+G(d,p) level of theory for DP4+ calculations. The calculated chemical shifts of the aforementioned diastereomers of **1a** and **1b** were compared with the experimental values using total absolute deviation (TAD), mean absolute error (MAE), and DP4+ probability analyses.

Figure S11. Total absolute deviation (TAD), mean absolute error (MAE), and DP4+ probability analyses for expansinine (**1**).

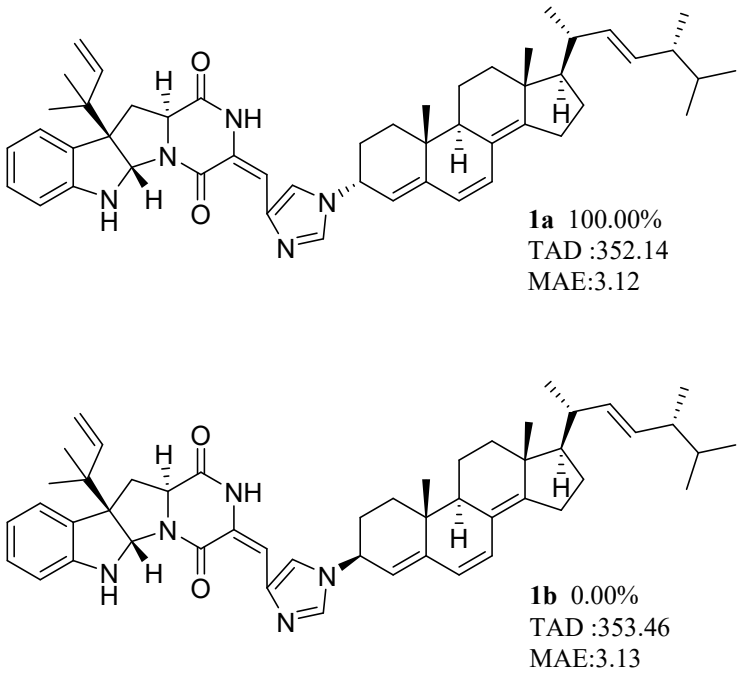
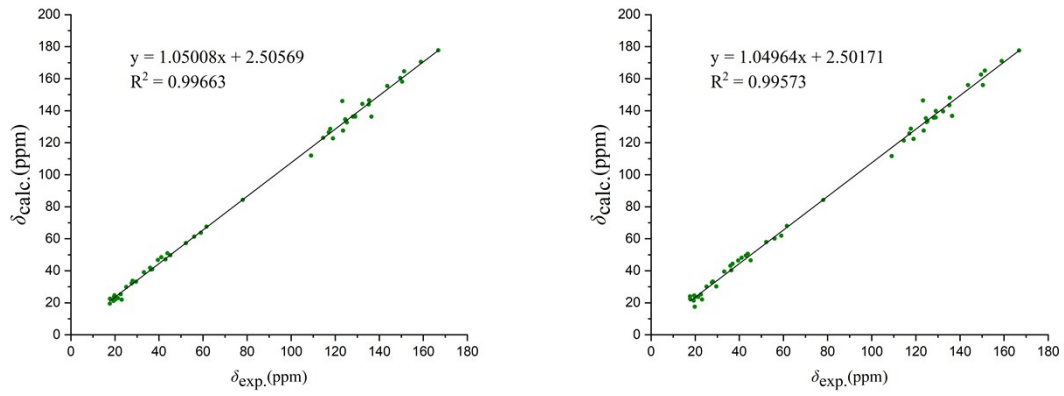


Figure S12. Linear correlations between the experimental and calculated ^{13}CNMR chemical shifts for **1** at the PCM/mPW1PW91/6-311 G(d,p) level.



1a **1b**

ECD calculation details for 1

The ECD spectra were simulated by overlapping Gaussian functions for each transition according to:

$$\Delta\varepsilon(E)=\frac{1}{2.296\times10^{-39}\sqrt{\pi}\sigma}\sum_{i}^{A}\Delta E_i R_i e^{[-(E-\Delta Ei)^2/\sigma^2]}$$

The σ represented the width of the band at 1/e height, and ΔE_i and R_i were the excitation energies and rotational strengths for transition *i*, respectively. R_{vel} had been used in this work.

Table S1 Energy (298.15K) analysis for **1a**, **1b**, of **1**

conf.	G (Hartree)	△G (Kcal/mol)	Boltzmann Distribution	conf.	G (Hartree)	△G (Kcal/mol)	Boltzmann Distribution
1a1	-2365.37	0.003223	0.02481635	1b1	-2365.37	0.00776	0.00024495
1a2	-2365.37	0.006542	0.000736792	1b2	-2365.37	0.00627	0.001189134
1a3	-2365.37	0	0.75500894	1b3	-2365.37	0.00545	0.002835255
1a4	-2365.37	0.002958	0.032861702	1b4	-2365.37	0.00643	0.000997328
1a5	-2365.37	0.006729	0.000604349	1b5	-2365.37	0.00643	0.000997328
1a6	-2365.37	0.004958	0.003947267	1b6	-2365.36	0	0.909543975
1a7	-2365.37	0.003367	0.021304413	1b7	-2365.37	0.00652	0.000911426
1a8	-2365.37	0.003906	0.012034374	1b8	-2365.37	0.00553	0.002602053
1a9	-2365.37	0.003907	0.012021629	1b9	-2365.37	0.00775	0.00024573
1a10	-2365.37	0.001613	0.136664183	1b10	-2365.36	0.00229	0.080432821

Table S2. Calculated (calc.) and experimental (exp.) ^{1}H and ^{13}C NMR chemical shift values of **1a**, **1b** of **1** at the mPW1PW91/6-311 G(d,p) level in chloroform and total absolute deviation (TAD) and mean absolute error (MAE).

No.	exp.	cacl.1a	|cacl.1a-exp.|	cacl.1b	|cacl.1b-exp.|
1	166.8	177.7249	10.92491857	177.6338	10.83375849
2	59	63.66614	4.666142444	61.88741	2.887410394
3	158.9	170.4242	11.52419011	170.9889	12.08894744
4	78.1	84.30814	6.208140933	84.2296	6.129601034
5	150.4	158.0914	7.69144451	155.9629	5.562852204
6	109.1	111.9168	2.816834138	111.6075	2.507499894
7	129	136.7078	7.70780844	135.6646	6.664584014
8	119	122.7345	3.734489265	122.3429	3.342892152
9	125.3	132.6696	7.369585788	133.4506	8.150561514
10	129.1	136.2529	7.152901901	139.7498	10.64980082
11	61.6	67.52152	5.921520142	68.04782	6.447816974
12	41.1	48.37859	7.278587152	48.10177	7.001774502
13	22.6	25.33982	2.739824339	25.22456	2.624559566
14	23.1	21.97484	1.125161397	22.0878	1.012201661
15	143.7	155.4463	11.74633211	156.0243	12.32425331
16	114.6	123.0881	8.488132238	121.3131	6.713104138
17	37	40.95148	3.951480194	44.40285	7.402848453
18	123.6	127.5132	3.913233289	127.5238	3.923757647
19	117.8	128.5646	10.76455615	128.6567	10.85668357
20	135.2	143.857	8.656976757	143.3699	8.16989773
21	123.3	145.971	22.67097955	146.3219	23.0218997
22	136.5	136.3053	0.194711801	136.7547	0.25466155
23	52.2	57.30165	5.101645618	57.91806	5.718061056
24	27.6	32.10271	4.502710711	32.70241	5.102412928
25	29.6	33.16796	3.567958181	30.24273	0.642729075
26	36	41.835	5.835003288	43.05712	7.057122569
27	17.7	19.45939	1.759392391	24.01774	6.317736463
28	149.6	160.4227	10.8226983	162.5369	12.93686487
29	125	133.227	8.226951681	132.9096	7.909580334
30	128.1	136.2383	8.138294571	135.5934	7.493352696
31	124.6	134.6421	10.04213029	135.2147	10.61471767
32	151.3	164.5551	13.25508303	165.0479	13.74790456
33	25.2	29.89554	4.695543332	30.03888	4.838881579
34	28	33.6655	5.665501541	33.22518	5.225176425
35	56	61.34387	5.34386652	60.09086	4.090855722
36	39.5	46.77109	7.271091832	46.39685	6.896853957
37	135.4	146.481	11.08103106	148.1018	12.70180773

38	132.4	144.2464	11.84640739	139.6022	7.202227106
39	43	47.02061	4.02061423	49.48285	6.482847525
40	17.8	22.45744	4.657436151	22.37483	4.5748294
41	33.2	39.08553	5.885531356	39.50167	6.301667259
42	19.8	24.62873	4.828728891	17.57625	2.223747523
43	20.1	21.96875	1.868748405	23.65908	3.559079208
44	21.4	22.86948	1.469480252	23.67779	2.277785377
45	43.9	50.90381	7.003810075	50.63532	6.73532423
46	19.3	21.1811	1.88109827	21.38008	2.080083315
47	36.4	40.73843	4.338428651	40.29609	3.896088313
48	19.6	23.58575	3.985751463	24.53275	4.932746797
49	45.2	49.69772	4.497724984	46.55086	1.350861033
50	117.1	126.477	9.376977935	125.729	8.62904298
51	4.02	4.21797	0.197969781	4.382546	0.362546447
52	5.63	5.660449	0.030449201	5.75028	0.120280331
53	4.98	5.11551	0.135509858	5.739944	0.759944364
54	5.57	6.830578	1.260578438	6.78069	1.21069016
55	7.08	7.529902	0.44990199	7.488036	0.408036383
56	6.75	7.080569	0.33056928	7.014484	0.264484319
57	7.16	7.630201	0.470201368	7.536916	0.376915696
58	1.14	0.974331	0.165668715	0.690782	0.449217938
59	1.14	1.562268	0.422267999	1.144873	0.004873403
60	1.14	1.454877	0.314877006	0.887665	0.252335354
61	1.03	0.771158	0.258841688	1.206856	0.176856026
62	1.03	0.703707	0.326292839	1.424373	0.394373217
63	1.03	1.49337	0.463370309	1.077558	0.047557724
64	5.98	6.816246	0.836246153	6.886363	0.906363289
65	5.11	5.698596	0.588596126	5.732677	0.622677302
66	5.08	5.605913	0.525912737	5.627342	0.547341792
67	2.56	2.665807	0.10580749	2.881251	0.321251457
68	2.47	2.682829	0.212829412	2.373175	0.096825082
69	9.46	6.842078	2.617922439	6.785578	2.674422453
70	6.63	6.741033	0.111032546	6.770856	0.140856497
71	8.31	7.82241	0.487589534	7.856621	0.453378918
72	7.56	9.55325	1.993250461	9.630567	2.070567343
73	4.8	4.946628	0.146628364	4.928685	0.128685205
74	1.94	1.769756	0.170244374	1.783176	0.156823525
75	2.19	2.327869	0.137869333	2.00831	0.181689745
76	1.59	1.748776	0.158776319	1.50744	0.082560334
77	1.41	1.613516	0.203516407	2.028388	0.618387574
78	0.9	1.181996	0.281995659	1.382856	0.482855525
79	0.9	1.031933	0.131932778	1.215863	0.315863464
80	0.9	1.030456	0.130456321	1.181991	0.281991011

81	5.98	6.498094	0.518093804	6.634046	0.654046102
82	6.33	6.874499	0.544499381	6.823739	0.493738923
83	2.38	2.613721	0.233720553	2.612012	0.232012408
84	2.45	2.761526	0.311526266	2.728382	0.278381855
85	1.49	1.948874	0.458873679	1.904143	0.414142806
86	1.81	1.697267	0.112732752	1.705515	0.104485026
87	1.26	1.5538	0.293800237	1.449121	0.189121258
88	2.13	2.27687	0.146869546	2.27508	0.145080475
89	5.21	5.842186	0.632185843	5.737792	0.527791663
90	5.26	6.327819	1.067819119	5.746362	0.486361944
91	1.88	1.694446	0.185554068	2.161342	0.281342185
92	0.95	1.21908	0.26908018	1.075083	0.125082506
93	0.95	1.034023	0.084023467	1.29307	0.343069613
94	0.95	0.656895	0.293105129	1.020006	0.07000625
95	1.48	1.642155	0.162154782	1.654492	0.174491563
96	0.83	1.413184	0.583184367	1.288787	0.458787459
97	0.83	0.672462	0.157537642	0.673252	0.156748244
98	0.83	0.911357	0.08135687	0.822184	0.007816342
99	0.85	0.641407	0.208593339	0.966377	0.116377479
100	0.85	0.923387	0.073387082	0.998224	0.148223896
101	0.85	1.181202	0.33120181	0.956438	0.106438395
102	1.05	1.564474	0.514474412	1.549877	0.499877421
103	1.05	1.041002	0.00899778	0.971418	0.078581941
104	1.05	0.940554	0.109446111	0.989112	0.060887724
105	0.95	1.19693	0.246929903	1.144272	0.194272249
106	0.95	1.279331	0.329331178	1.216904	0.266904406
107	0.95	1.097843	0.147842571	1.0154	0.065399878
108	2.06	2.35103	0.291029953	2.218221	0.1582209
109	1.33	1.739345	0.409344879	1.478948	0.148948203
110	1.64	1.853807	0.213807132	1.772309	0.132308853
111	1.57	1.787771	0.217771112	1.784386	0.214386425
112	2.16	2.723245	0.563245377	2.646138	0.486137776
113	5.53	5.985254	0.455253787	6.146213	0.61621281
TAD			352.1414707		353.4580913
MAE			3.116296201		3.127947711

Figure S13. DP4+ evaluation of theoretical and experimental data of **1**

Functional	Solvent?	Basis Set	Type of Data
mPW1PW91	PCM	6-311+G(d,p)	Unscaled Shifts

	Isomer 1	Isomer 2	Isomer 3	Isomer 4	Isomer 5	Isomer 6
sDP4+ (H data)	98.86%	1.14%	–	–	–	–
sDP4+ (C data)	100.00%	0.00%	–	–	–	–
sDP4+ (all data)	100.00%	0.00%	–	–	–	–
uDP4+ (H data)	18.73%	81.27%	–	–	–	–
uDP4+ (C data)	100.00%	0.00%	–	–	–	–
uDP4+ (all data)	100.00%	0.00%	–	–	–	–
DP4+ (H data)	95.22%	4.78%	–	–	–	–
DP4+ (C data)	100.00%	0.00%	–	–	–	–
DP4+ (all data)	100.00%	0.00%	–	–	–	–

Functional	Solvent?	Basis Set	Type of Data
mPW1PW91	PCM	6-311+G(d,p)	Unscaled Shifts

		DP4+	100.00%	0.00%	–	–	–
Nuclei	sp2?	Experimental	Isomer 1	Isomer 2	Isomer 3	Isomer 4	Isomer 5
C	x	166.80	177.724919	177.633759			
C		59.00	63.6661424	61.8874104			
C	x	158.90	170.42419	170.988947			
C		78.10	84.3081409	84.229601			
C	x	150.40	158.091445	155.962852			
C	x	109.10	111.916834	111.6075			
C	x	129.00	136.707808	135.664584			
C	x	119.00	122.734489	122.342892			
C	x	125.30	132.669586	133.450562			
C	x	129.10	136.252902	139.749801			
C		61.60	67.5215201	68.047817			
C		41.10	48.3785872	48.1017745			
C		22.60	25.3398243	25.2245596			
C		23.10	21.9748386	22.0877983			
C	x	143.70	155.446332	156.024253			
C	x	114.60	123.088132	121.313104			
C		37.00	40.9514802	44.4028485			
C	x	123.60	127.513233	127.523758			
C	x	117.80	128.564556	128.656684			
C	x	135.20	143.856977	143.369898			
C	x	123.30	145.97098	146.3219			
C	x	136.50	136.305288	136.754662			
C		52.20	57.3016456	57.9180611			
C		27.60	32.1027107	32.7024129			
C		29.60	33.1679582	30.2427291			
C		36.00	41.8350033	43.0571226			
C		17.70	19.4593924	24.0177365			
C	x	149.60	160.422698	162.536865			
C	x	125.00	133.226952	132.90958			
C	x	128.10	136.238295	135.593353			
C	x	124.60	134.64213	135.214718			
C	x	151.30	164.555083	165.047905			
C		25.20	29.8955433	30.0388816			
C		28.00	33.6655015	33.2251764			
C		56.00	61.3438665	60.0908557			
C		39.50	46.7710918	46.396854			
C	x	135.40	146.481031	148.101808			
C	x	132.40	144.246407	139.602227			
C		43.00	47.0206142	49.4828475			
C		17.80	22.4574362	22.3748294			
C		33.20	39.0855314	39.5016673			
C		19.80	24.6287289	17.5762525			
C		20.10	21.9687484	23.6590792			
C		21.40	22.8694803	23.6777854			
C		43.90	50.9038101	50.6353242			
C		19.30	21.1810983	21.3800833			
C		36.40	40.7384287	40.2960883			
C		19.60	23.5857515	24.5327468			
C		45.20	49.697725	46.550861			
C	x	117.10	126.476978	125.729043			

Functional	Solvent?	Basis Set	Type of Data
mPW1PW91	PCM	6-311+G(d,p)	Unscaled Shifts

		DP4+	100.00%	0.00%	–	–	–
Nuclei	sp2?	Experimental	Isomer 1	Isomer 2	Isomer 3	Isomer 4	Isomer 5
H		4.02	4.217969781	4.382546447			
H		5.63	5.660449201	5.750280331			
H		4.98	5.115509858	5.739944364			
H	x	5.57	6.830578438	6.78069016			
H	x	7.08	7.52990199	7.488036383			
H	x	6.75	7.08056928	7.014484319			
H	x	7.16	7.630201368	7.536915696			
H		1.14	0.974331285	0.690782062			
H		1.14	1.562267999	1.144873403			
H		1.14	1.454877006	0.887664646			
H		1.03	0.771158312	1.206856026			
H		1.03	0.703707161	1.424373217			
H		1.03	1.493370309	1.077557724			
H	x	5.98	6.816246153	6.886363289			
H	x	5.11	5.698596126	5.732677302			
H	x	5.08	5.605912737	5.627341792			
H		2.56	2.66580749	2.881251457			
H		2.47	2.682829412	2.373174918			
H		9.46	6.842077561	6.785577547			
H	x	6.63	6.741032546	6.770856497			
H	x	8.31	7.822410466	7.856621082			
H	x	7.56	9.553250461	9.630567343			
H		4.80	4.946628364	4.928685205			
H		1.94	1.769755626	1.783176475			
H		2.19	2.327869333	2.008310255			
H		1.59	1.748776319	1.507439666			
H		1.41	1.613516407	2.028387574			
H		0.90	1.181995659	1.382855525			
H		0.90	1.031932778	1.215863464			
H		0.90	1.030456321	1.181991011			
H	x	5.98	6.498093804	6.634046102			
H	x	6.33	6.874499381	6.823738923			
H		2.38	2.613720553	2.612012408			
H		2.45	2.761526266	2.728381855			
H		1.49	1.948873679	1.904142806			
H		1.81	1.697267248	1.705514974			
H		1.26	1.553800237	1.449121258			
H		2.13	2.276869546	2.275080475			
H	x	5.21	5.842185843	5.737791663			
H	x	5.26	6.327819119	5.746361944			
H		1.88	1.694445932	2.161342185			
H		0.95	1.21908018	1.075082506			
H		0.95	1.034023467	1.293069613			
H		0.95	0.656894871	1.02000625			
H		1.48	1.642154782	1.654491563			
H		0.83	1.413184367	1.288787459			
H		0.83	0.672462358	0.673251756			
H		0.83	0.91135687	0.822183658			
H		0.85	0.641406661	0.966377479			
H		0.85	0.923387082	0.998223896			
H		0.85	1.18120181	0.956438395			
H		1.05	1.564474412	1.549877421			
H		1.05	1.04100222	0.971418059			
H		1.05	0.940553889	0.989112276			
H		0.95	1.196929903	1.144272249			
H		0.95	1.279331178	1.216904406			
H		0.95	1.097842571	1.015399878			
H		2.06	2.351029953	2.2182209			
H		1.33	1.739344879	1.478948203			
H		1.64	1.853807132	1.772308853			
H		1.57	1.787771112	1.784386425			
H		2.16	2.723245377	2.646137776			
H	x	5.53	5.985253787	6.14621281			