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Supporting Information for

Caesalpinimin A, a novel rearranged furanoditerpene with an unprecedented carbon skeleton from the seeds of *Caesalpinia minax* Hance

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Contents of Supporting Information

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Detailed experimental procedures

General Experimental Procedures. Ultraviolet (UV) spectra were determined by V-550 UV/vis spectrophotometer. Infrared (IR) spectra was measured on a Jasco FT/IR-480 plus Fourier Transform infrared spectrometer using KBr pellet. HR-ESI-MS data were obtained on an Agilent 6210 ESI/TOF mass spectrometer. Optical rotation was recorded in CH_3Cl_3 on Jasco P-1020 polarimeter at room temperature. Nuclear magnetic resonance (NMR) spectra were measured on Bruker AV-300 spectrometers. Thin-layer chromatography (TLC) analyses were carried out using pre-coated silica gel GF_{254} plates (Qingdao Marine Chemical Plant, Qingdao, People's Republic of China). Column chromatographies were performed on silica gel (200–400 mesh, Qingdao Marine Chemical Plant, Qingdao, *P. R.* China), reverse-phase C_{18} silica gel (Merck, Darmstadt, Germany) and Sephadex LH-20 (Pharmacia Biotec AB, Uppsala, Sweden). All solvents used in column chromatography and high-performance liquid chromatography (HPLC) were of analytical (Shanghai Chemical Plant, Shanghai, People's Republic of China) grade and chromatographic grade (Fisher Scientific, NJ, USA), respectively.

Extraction and Isolation. The seeds of *C. minax* were collected in in Guangdong Province, People's Republic of China, in September 2008. The seeds were authenticated by Prof. Guang-Xiong Zhou (Jinan University). A voucher specimen (No. 20090824) was deposited in the Institute of Traditional Chinese Medicine and Natural Products, Jinan University, *P. R.* China.

The dried ground seeds (5 kg) were extracted with Methanol three times. The

extract was combined, filtered, and then concentrated under reduced pressure to afford a crude residue (664 g). which was suspended in water and partitioned with cyclohexane, EtOAc and *n*-BuOH to afford the cyclohexane fraction, EtOAc fraction (65g), *n*-BuOH fraction and water soluble, respectively. The EtOAc fraction was subjected to silica gel (200-300 mesh), eluted with cyclohexane-acetic ether (50:1, 40:1, 30:1, 20:1, 15:1, 10:1, 5:1, 3:1 and 1:1) to give 10 fractions (Fr.1 to Fr.10). Fr. 6 was chromatographed on reverse-phase C_{18} silica gel eluted with MeOH–H_2O (20:80 to 90:10) to give Fr. 6a-6e. Fr. 6b was subjected to column chromatography over silica gel followed by preparative HPLC using CH_3CN–H_2O (50:50) as the eluent to yield **1** (3.4 mg) and compound **2**[1] (4.1mg).

Caesalpinimin A (**1**): an amorphous solid; $[\alpha]_D^{30}$ +5.3° (c 0.1, $CHCl_3$); λ_{max} (log ε): 213 (0.64), 258 (0.20) nm;IR (KBr) ν_{max}: 3573, 1737 cm^{-1}; ^1H and ^{13}C NMR data are shown in Table 1. positive ESIMS m/z 487.5 $[M + Na]^+$, 951.1$[2M + Na]^+$; HR-ESI-MS: *m/z* 487.1920 $[M+Na]^+$ (calcd for $C_{24}H_{32}NaO_9$: 487.1936).

COX-2 Inhibitory Activity Assay. Compound **1** was evaluated for COX-2 inhibitory activity using an enzyme immunoassay (EIA) kit (catalog no. 560131, Cayman Chemical, Ann Arbor, MI) according to the manufacturer's instructions. Briefly, reactions mixtures were prepared Tris–HCl buffer and COX-2, and the reaction was initiated by the addition of arachidonic acid. After 2 min the reaction was terminated by adding HCl and PGE2 was quantitated by an ELISA method. The test compound was dissolved in DMSO and a series of concentrations were prepared. Following transfer to a 96-well plate coated with a mouse anti-rabbit IgG, the tracer

prostaglandin acetylcholine esterase and primary antibody (mouse anti PGE2) were added. Plates were then incubated at room temperature overnight, reaction mixtures were removed, and wells were washed. Ellman's reagent was added to each well and the plate was incubated for about 60 min, until the control wells yielded an OD = 0.3–0.8 at 412 nm. Results were expressed as a percentage relative to a control (solvent-treated samples). All determinations were performed in duplicate, and values generally agreed within 10%.

Cytotoxicity assay. The MTT assay was performed as described previously[2] with taxol served as the positive control. Briefly, cells were plated onto 96-well plates at 3×10^3 cells per well for DU145 and PC3 cell lines. Cells were maintained in RPMI-1640 medium supplemented with 10% fetal bovine serum (Gibco, Rockville, MD, USA), 100 units/ml penicillin and streptomycin at 37°C in a 5% CO2 humidified atmosphere. Following incubation for 48 h, 20 μL of the MTT solution [5 mg/mL in phosphate buffered saline (PBS)] was added to each well, and the cells were further incubated for 4 h. Then the medium was removed and replaced by 150 μL of DMSO in each well to dissolve the formazan crystals. The relative cell viability was determined by measuring the optical densities at 570 nm on microplate reader (SPECTRAmax 250, Molecular Devices, Minnesota, USA), and was expressed as a percentage relative to the control. The experiments were performed three times, each in triplicate.

Notes and references

1 S. K. Kalauni, S. Awale, Y. Tezuka, A. H. Banskota, T. Z. Linn, S. Kadota, *Chem. Pharm. Bull.* 2005, **53**, 214–218.

2 Reference 17 in the manuscript.

3 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., Jr. Montgomery, J. E. Peralta, F. Ogliaro, M. Bearpark, J. J. Heyd, E. Brothers, K. N. Kudin, V. N. Staroverov, 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, Ö. Farkas, J. B. Foresman, J. V. Ortiz, J. Cioslowski, D. J. Fox, *Gaussian 09, Revision A.1; Gaussian Inc.: Wallingford CT* **2009** (Reference 10 in the manuscript).

Figure S1. ^{1}H NMR spectrum (full spectrum) of Caesalpinimin A (**1**) in $CDCl_3$ (300 MHz)

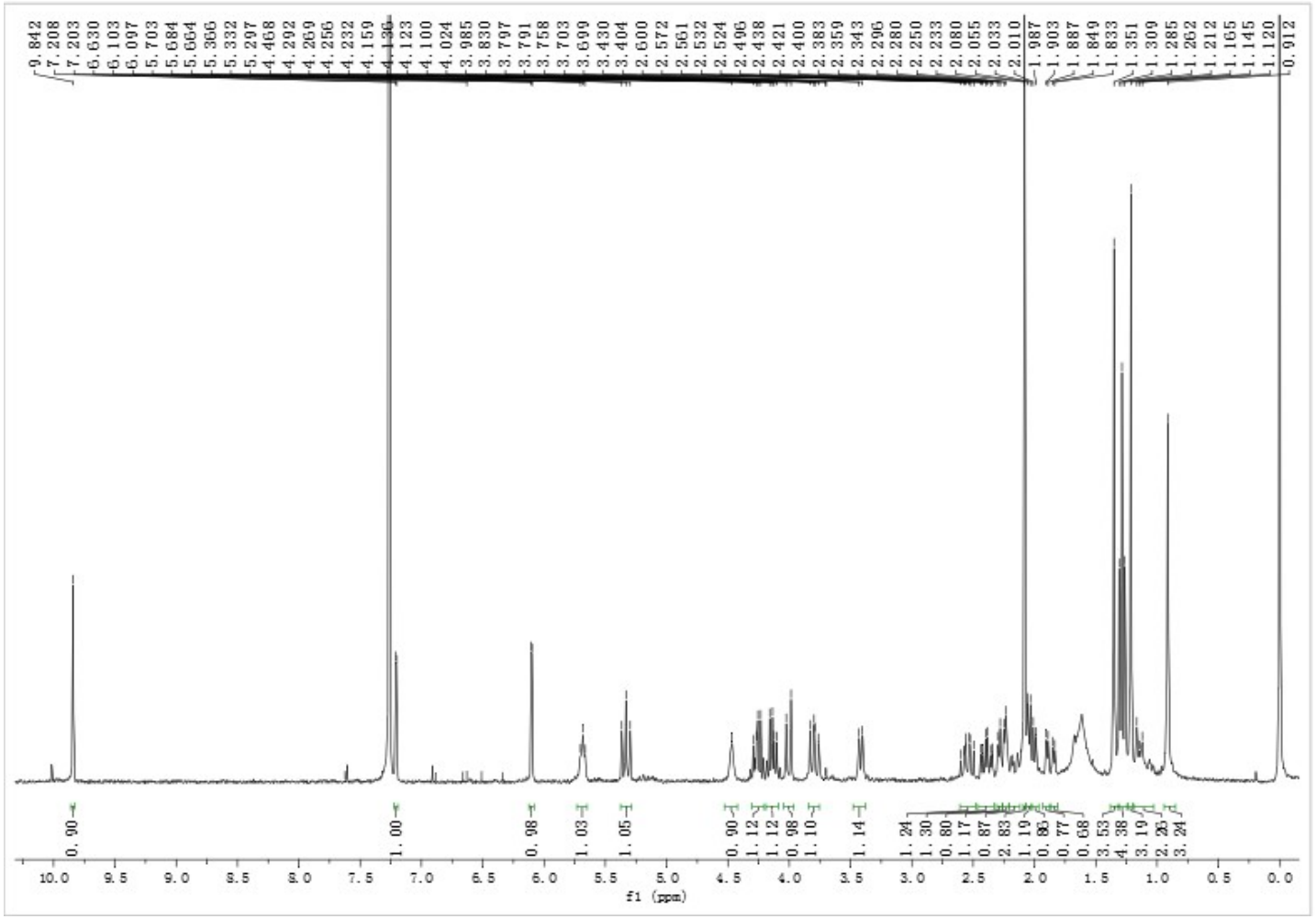

Figure S2. ^{1}H NMR spectrum (0-4.5 ppm) of Caesalpinimin A (**1**) in $CDCl_3$ (300 MHz)

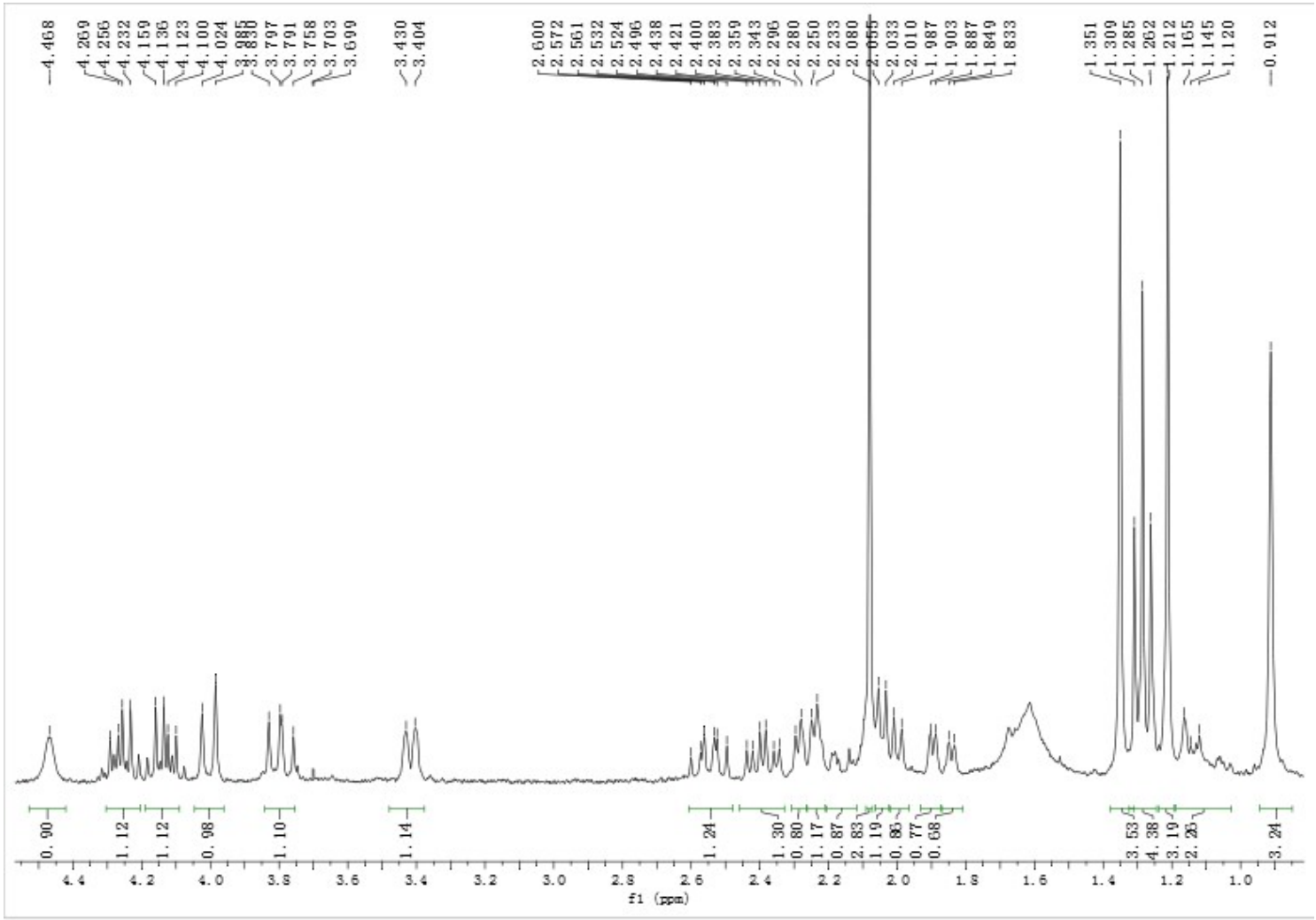

Figure S3. ^{1}H NMR spectrum (4.5-10.5 ppm) of Caesalpinimin A (**1**) in $CDCl_3$ (300 MHz)

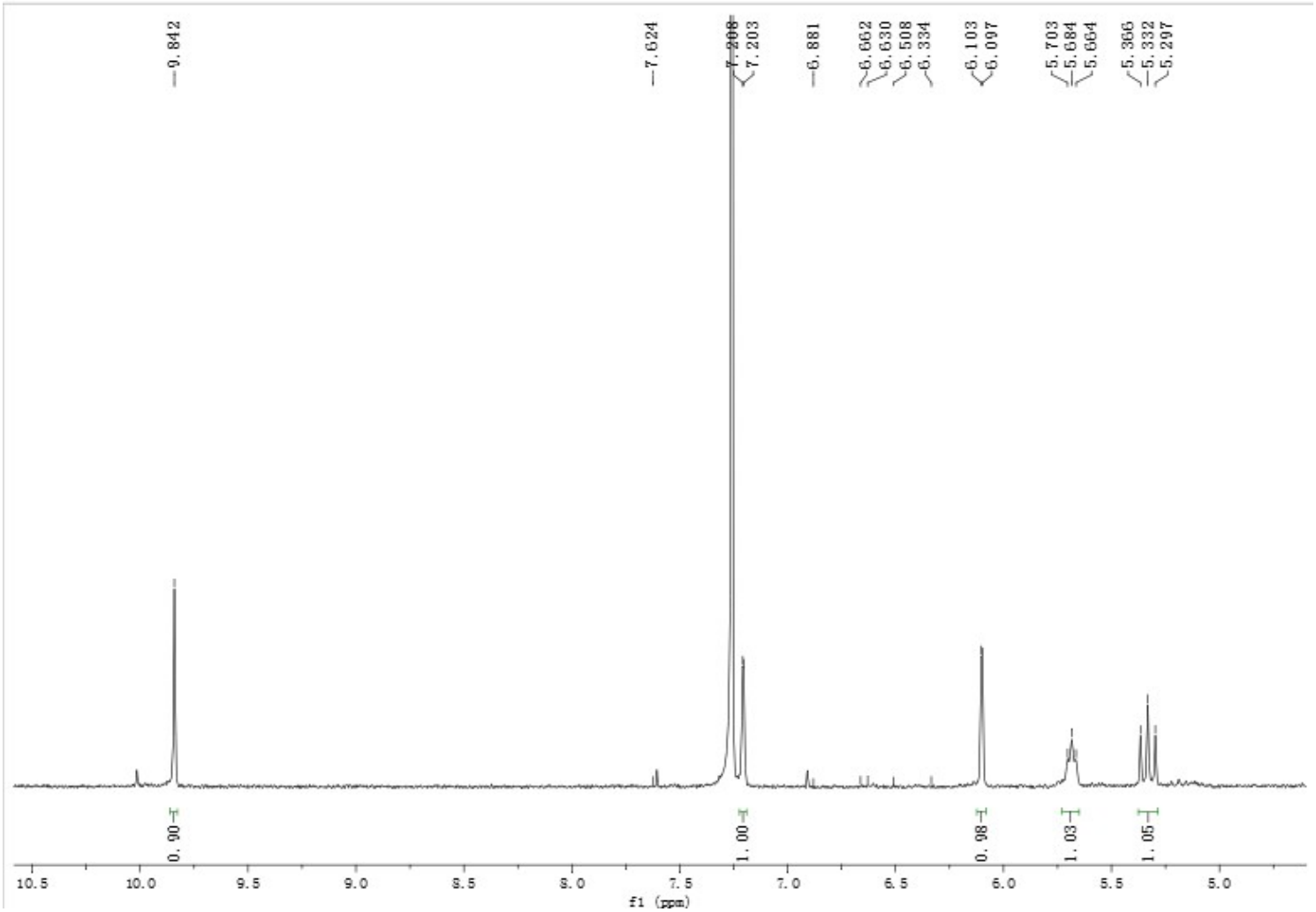

Figure S4. ^{13}C spectra (full spectra) of Caesalpinimin A (**1**) in $CDCl_3$ (75 MHz)

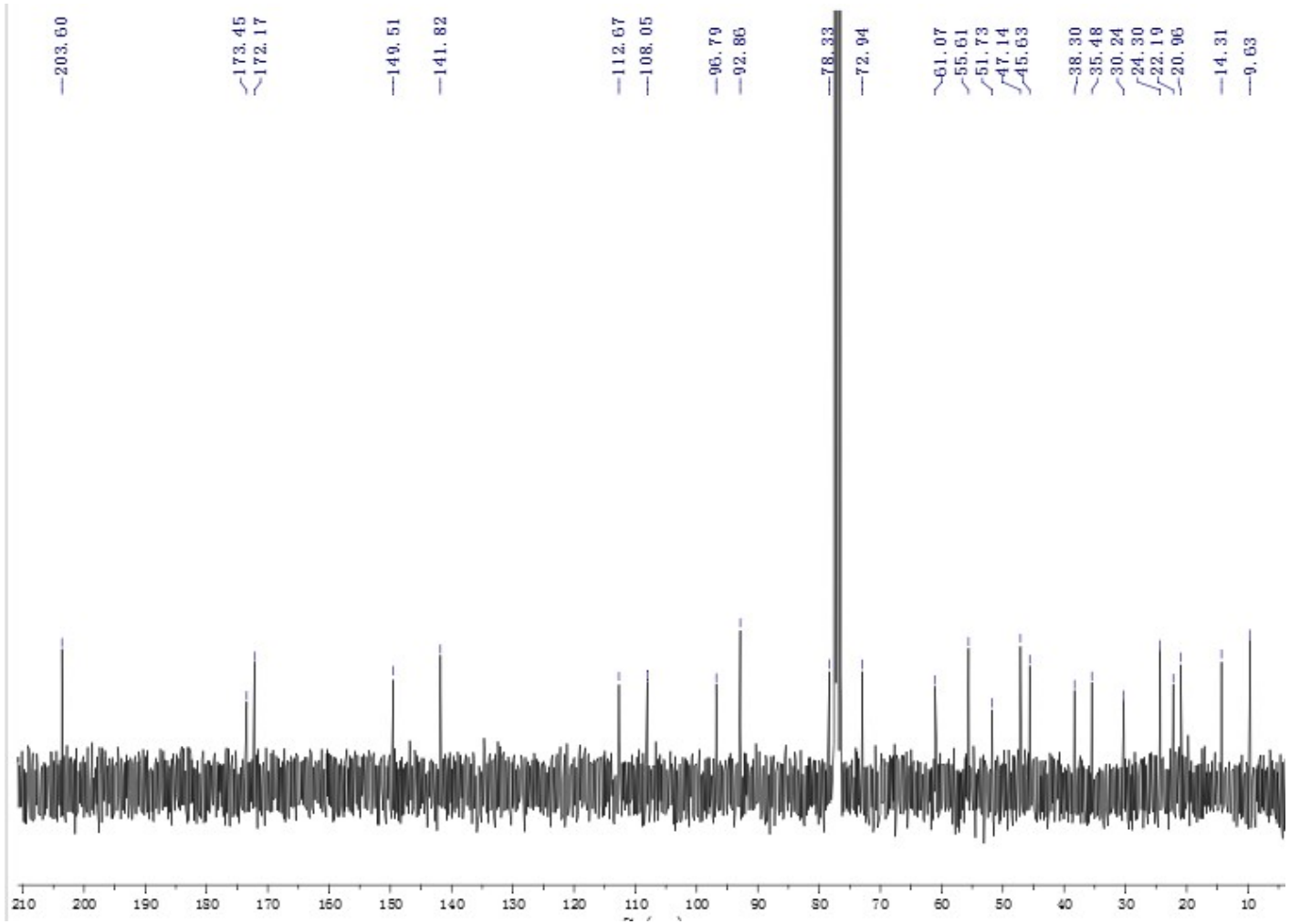

Figure S5. ^{13}C and DEPT NMR spectra (full spectra) of Caesalpinimin A (**1**) in $CDCl_3$ (75 MHz)

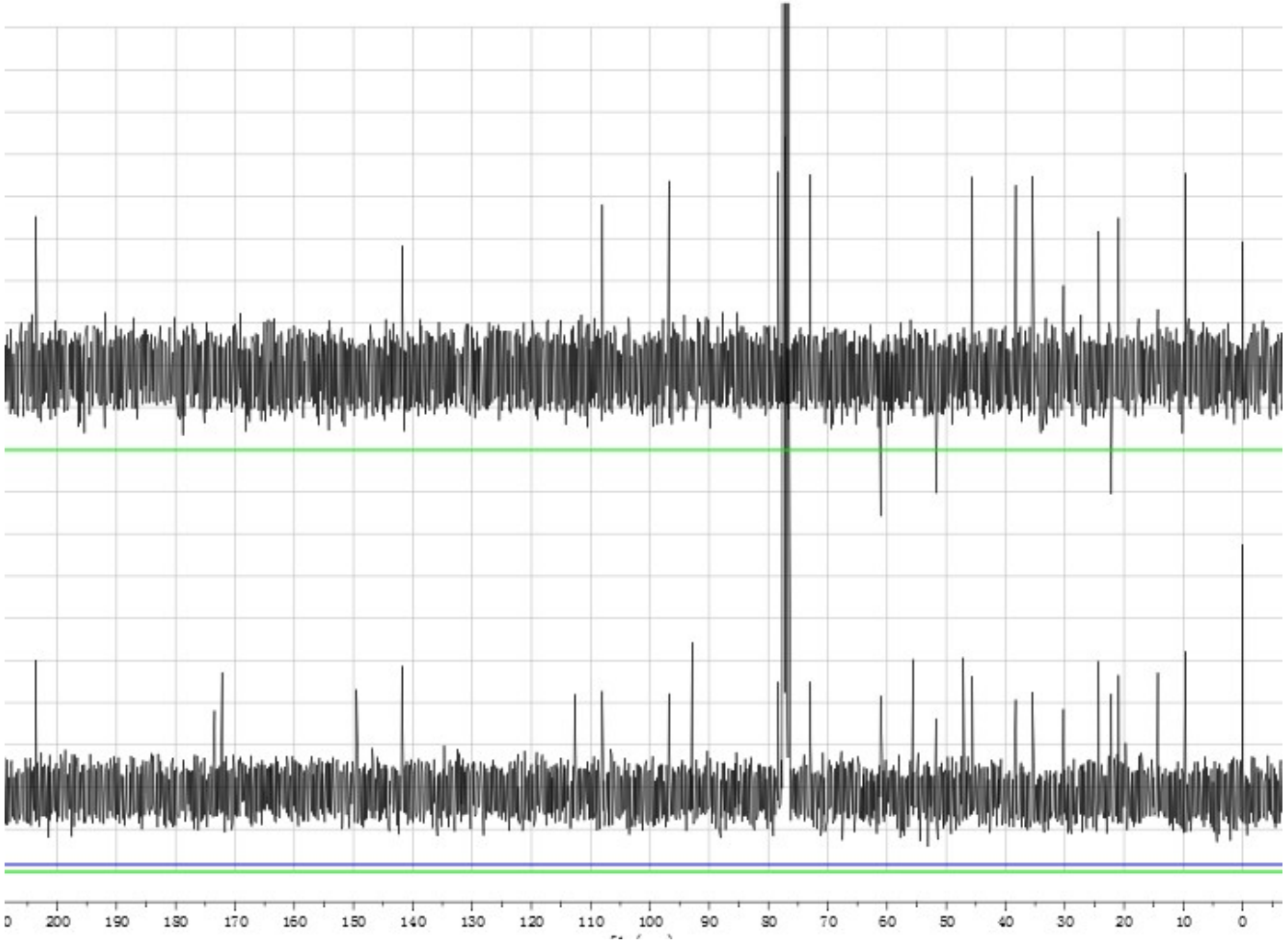

Figure S6. ^{13}C and DEPT NMR spectra (0-80 ppm) of Caesalpinimin A (**1**) in $CDCl_3$ (75 MHz)

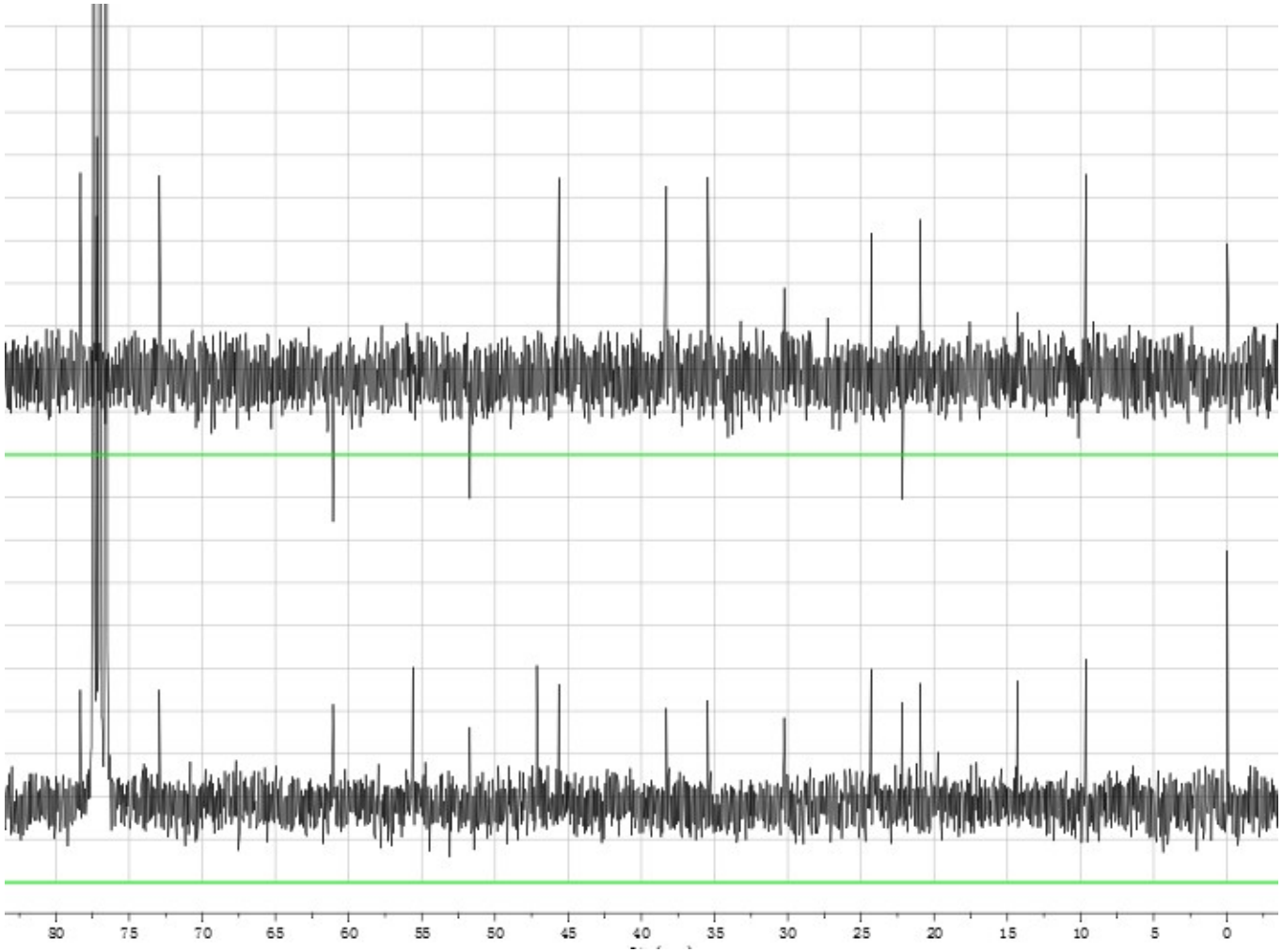

Figure S7. ^{1}H-^{1}H COSY spectrum (full spectra) of Caesalpinimin A (**1**) in $CDCl_3$

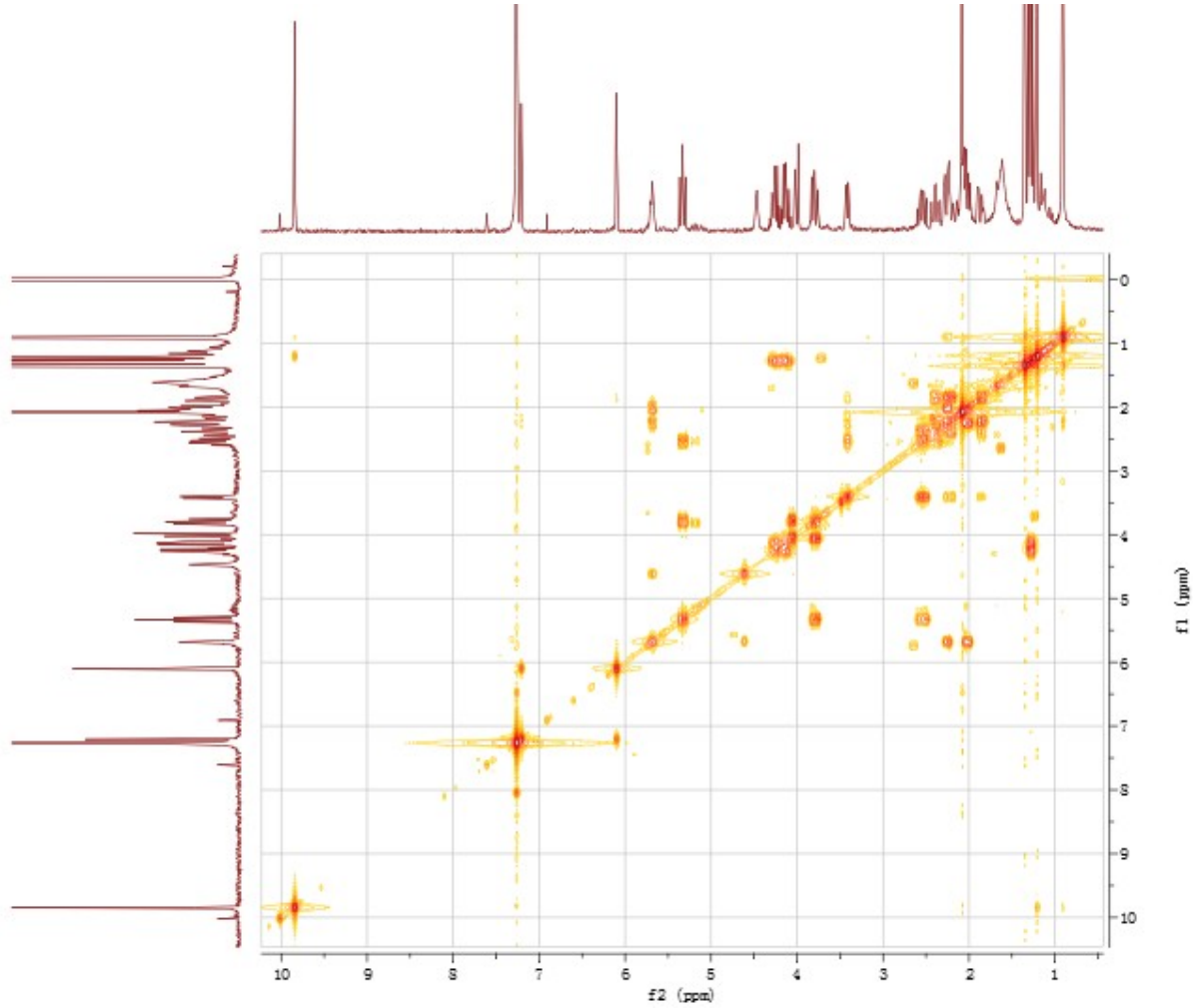

Figure S8. ^{1}H-^{1}H COSY spectrum (0-6.0 ppm) of Caesalpinimin A (**1**) in $CDCl_3$

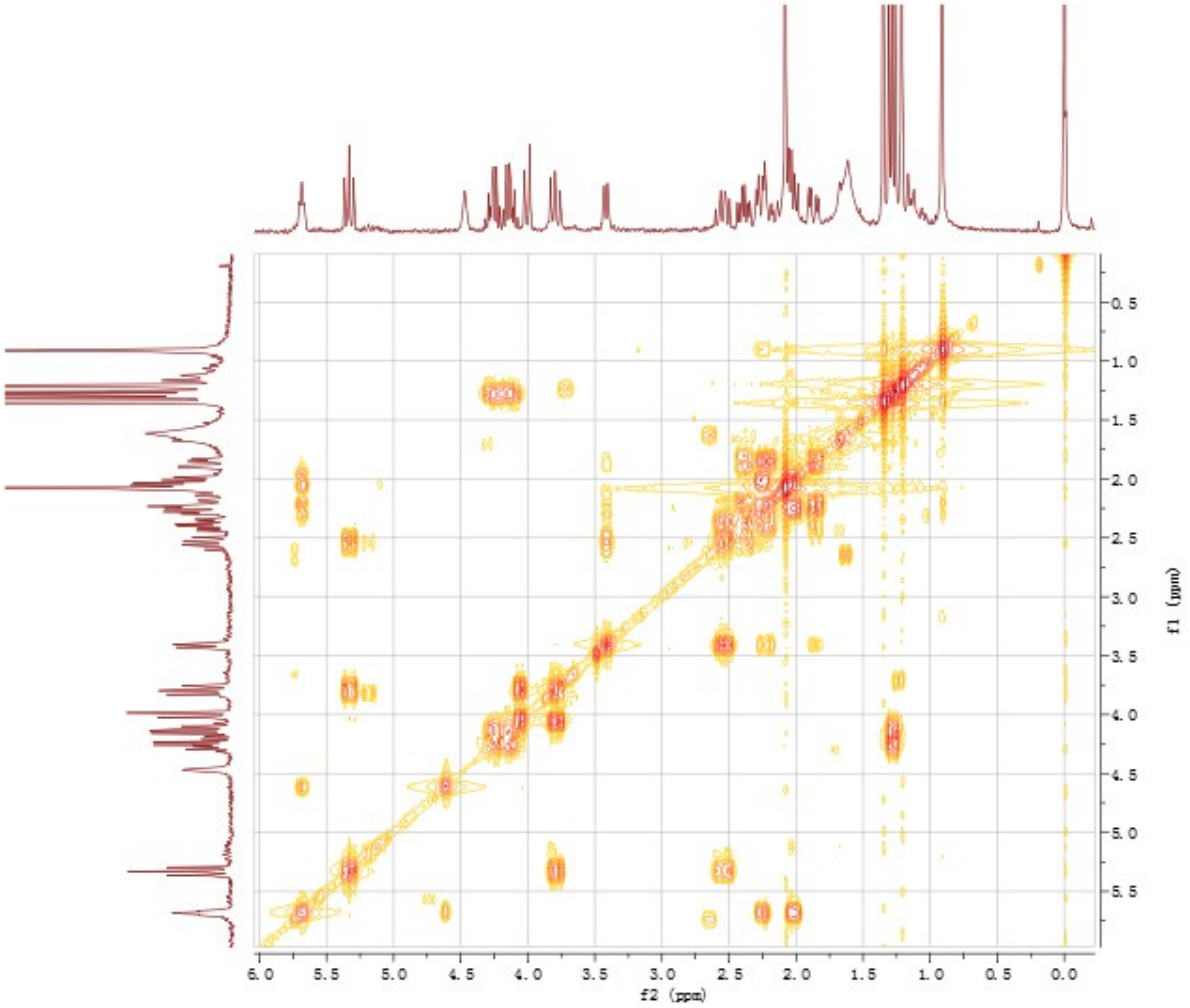

Figure S9. HSQC spectrum (full spectra) of Caesalpinimin A (**1**) in $CDCl_3$

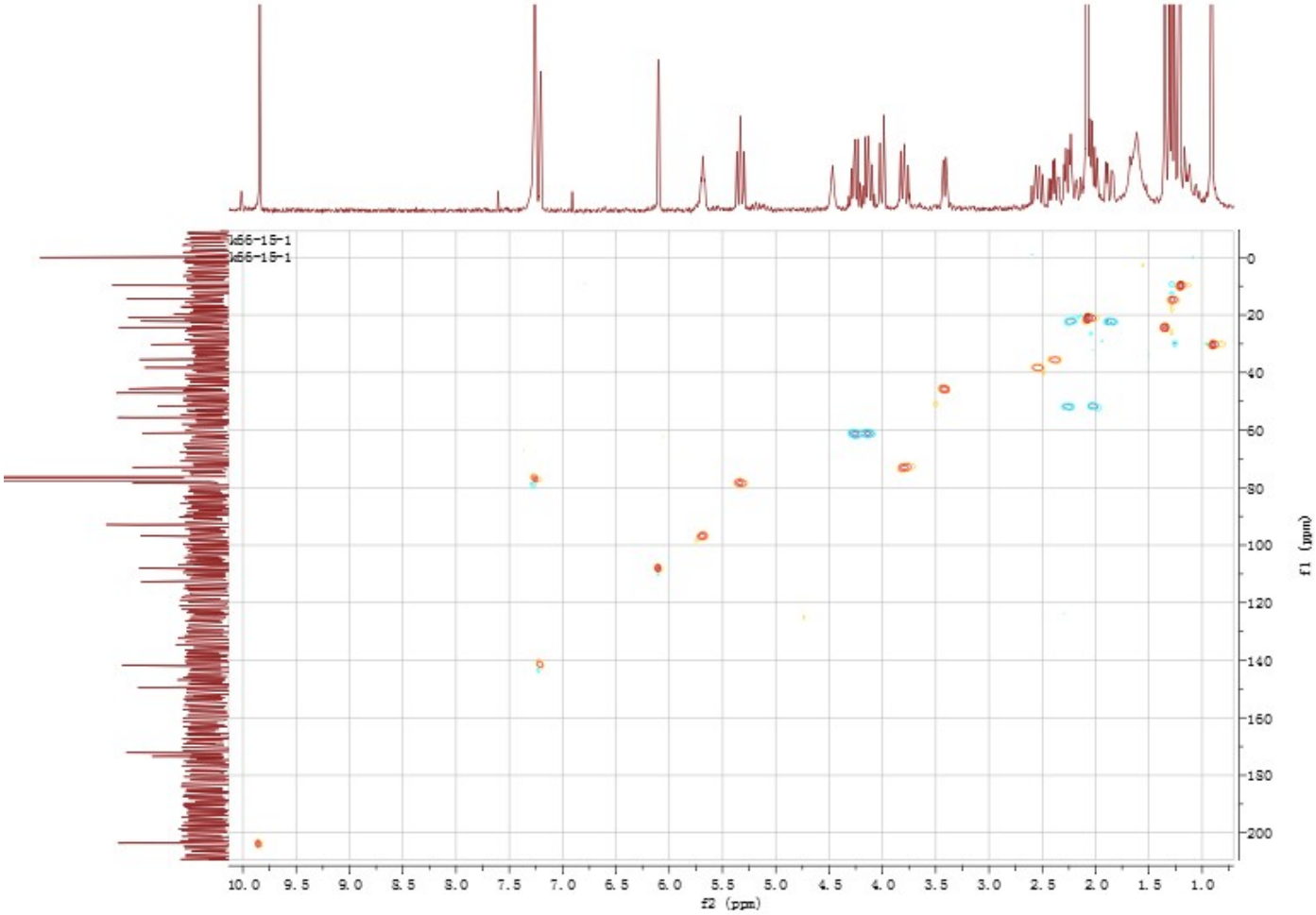

Figure S10. HMBC spectrum (full spectra) of Caesalpinimin A (**1**) in $CDCl_3$

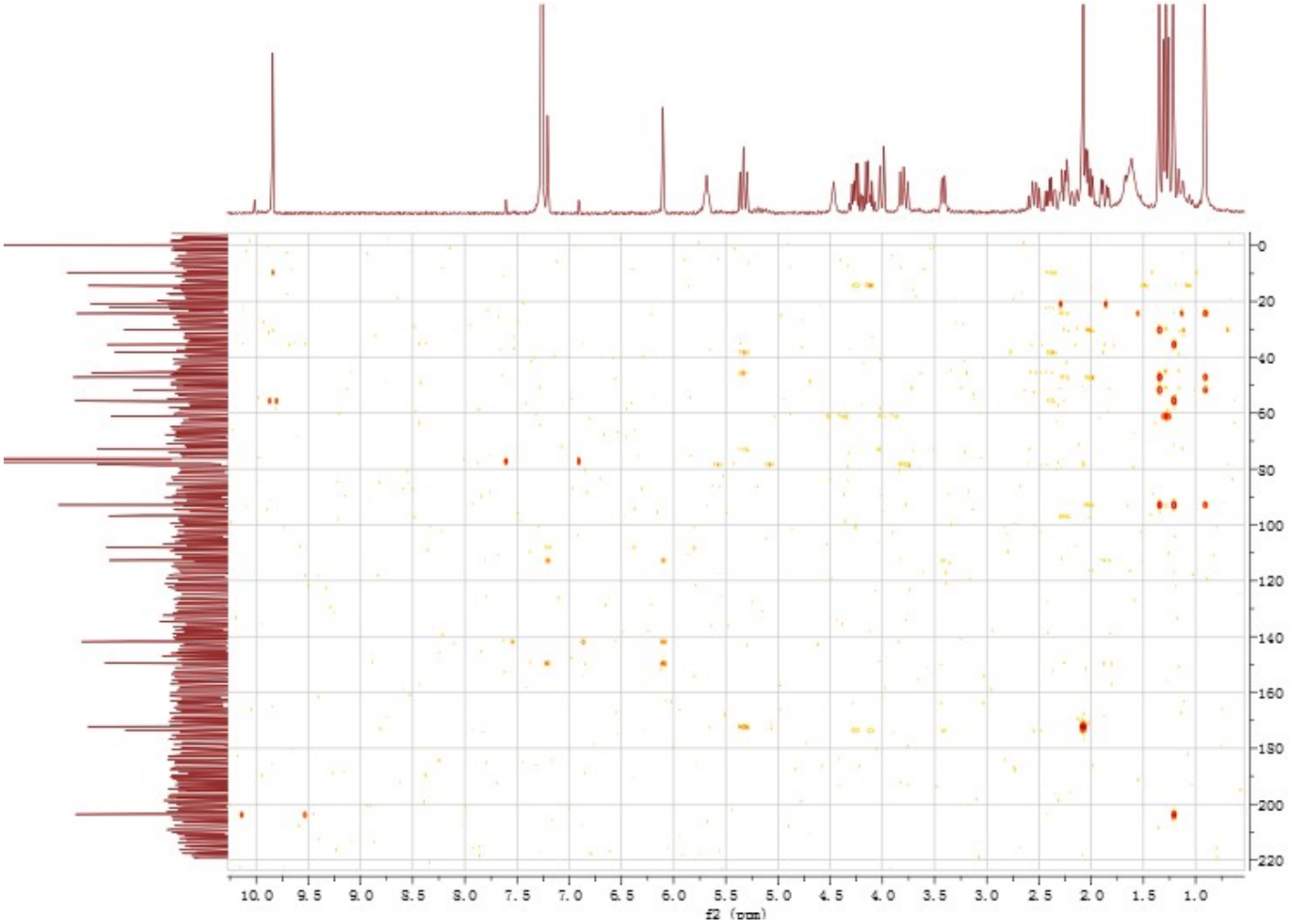

Figure S11. HMBC spectrum (δ_H 0.5-2.8 ppm and δ_C 0-210 ppm) of Caesalpinimin A (**1**) in $CDCl_3$

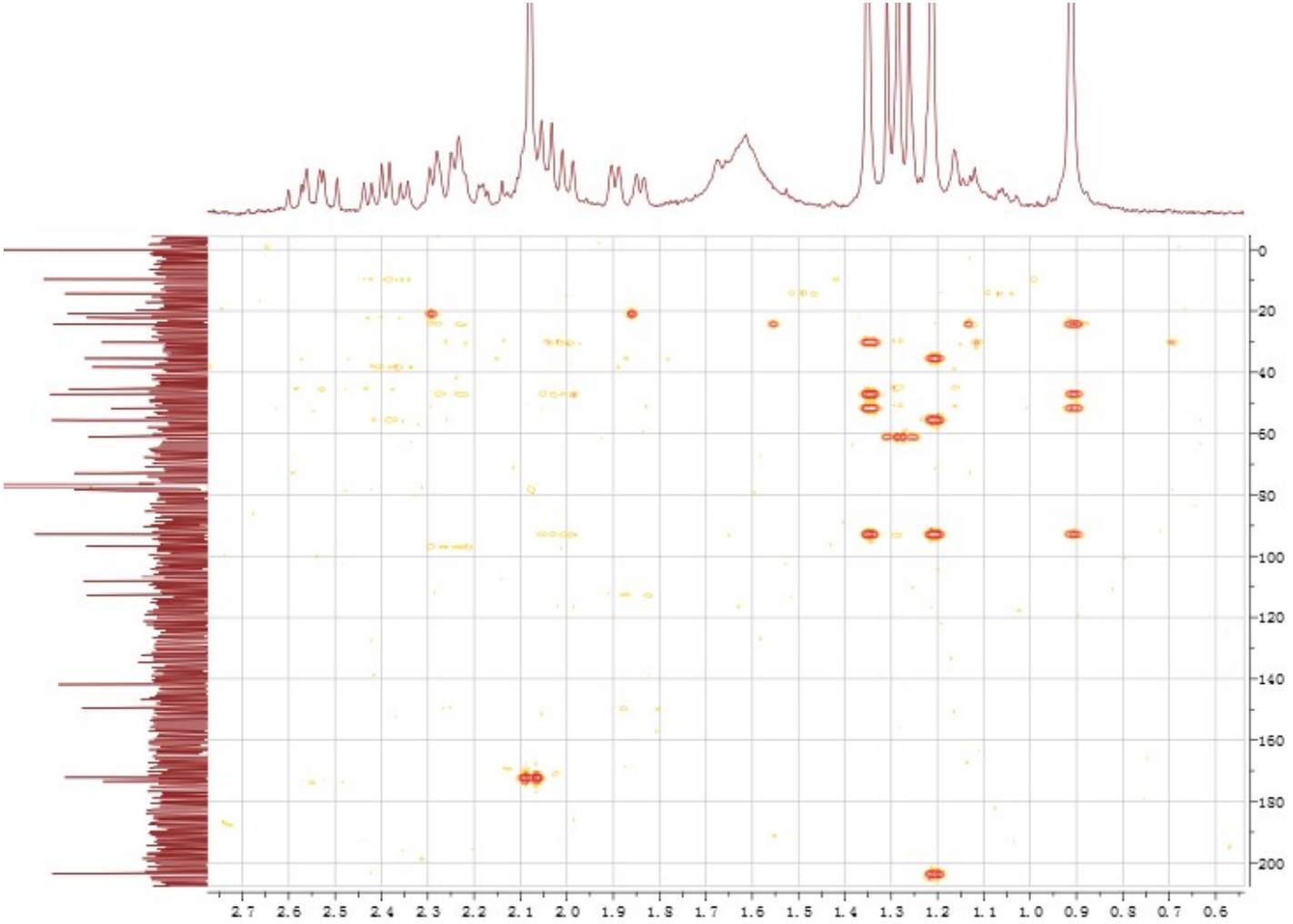

Figure S12. HMBC spectrum (δ_H 2.0-7.5 ppm and δ_C 0-180 ppm) of Caesalpinimin A (**1**) in $CDCl_3$

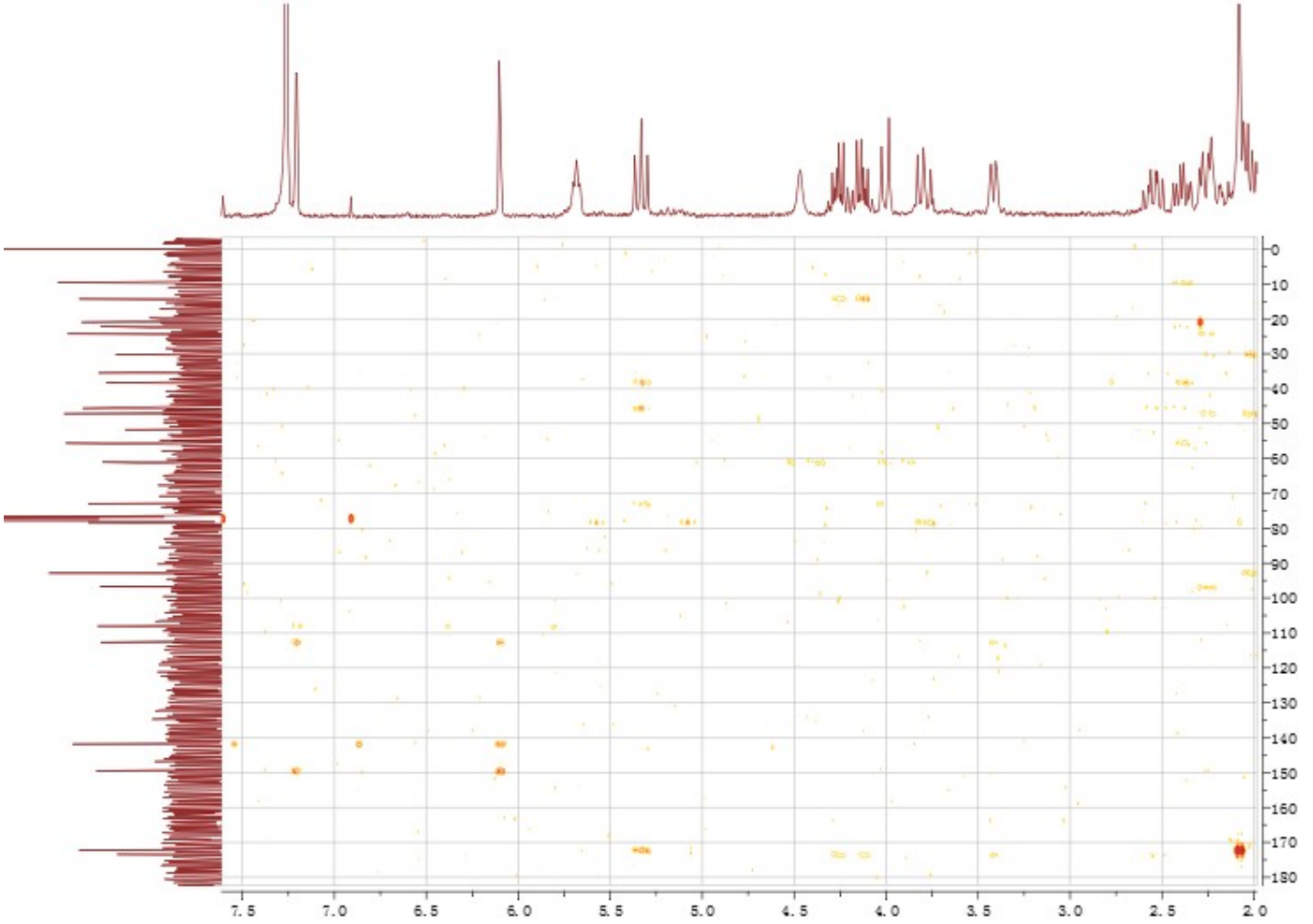

Figure S13. ROESY spectrum (full spectra) of Caesalpinimin A (**1**) in $CDCl_3$

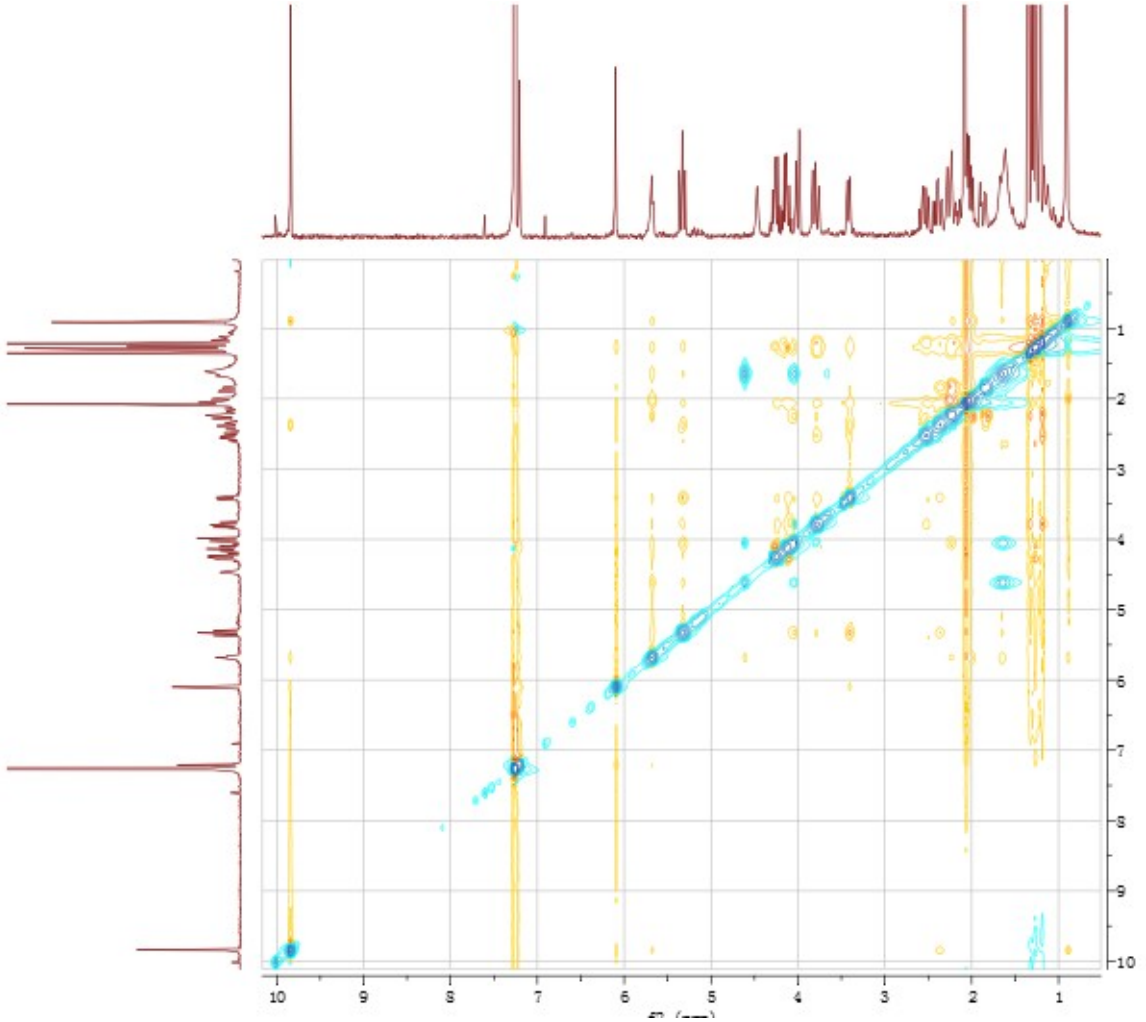

Figure S14. Positive ESIMS spectrum of Caesalpinimin A (**1**)

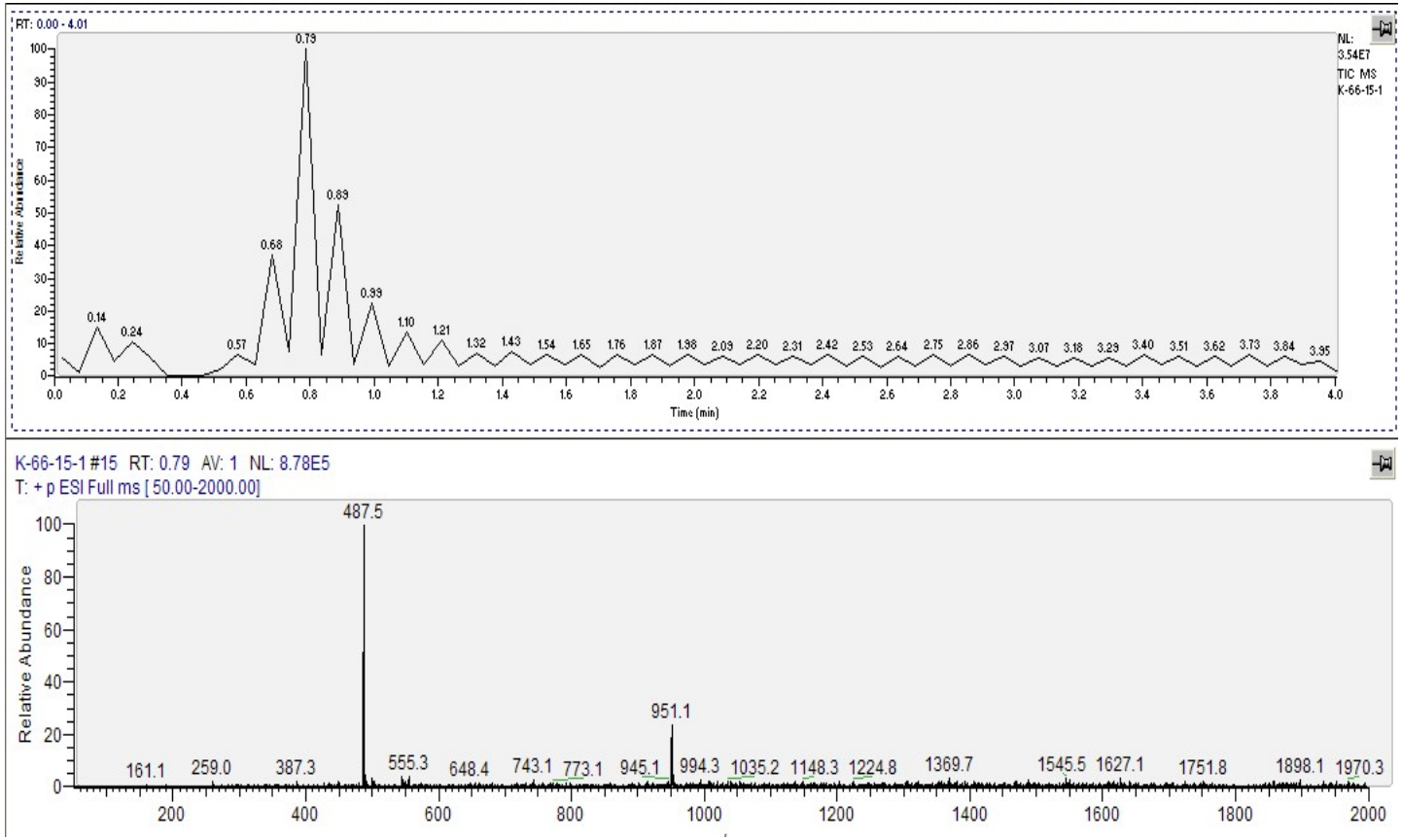

Figure S15. Positive HRESIMS spectrum of Caesalpinimin A (**1**)

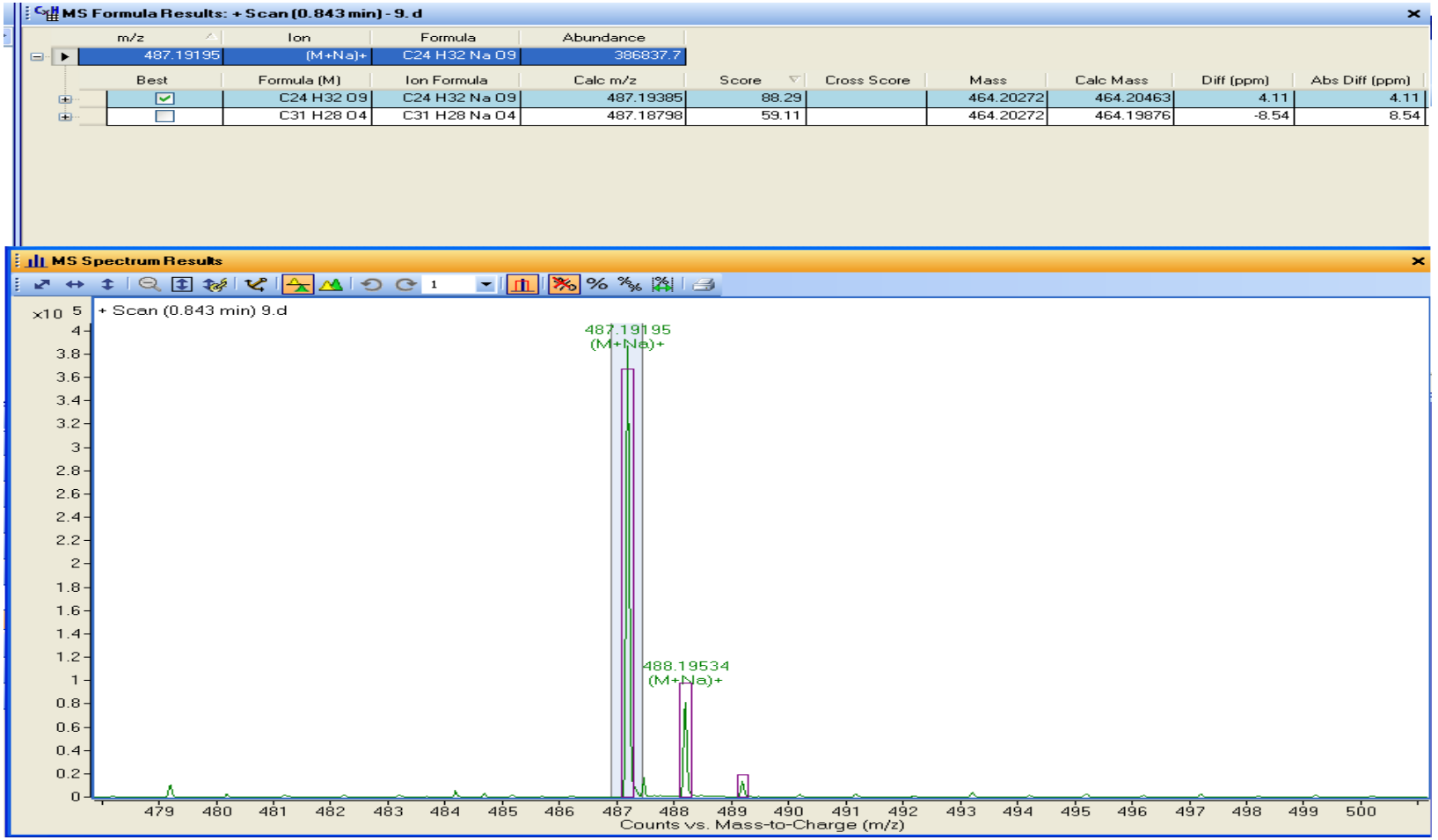

Figure S16. IR spectrum of Caesalpinimin A (**1**)

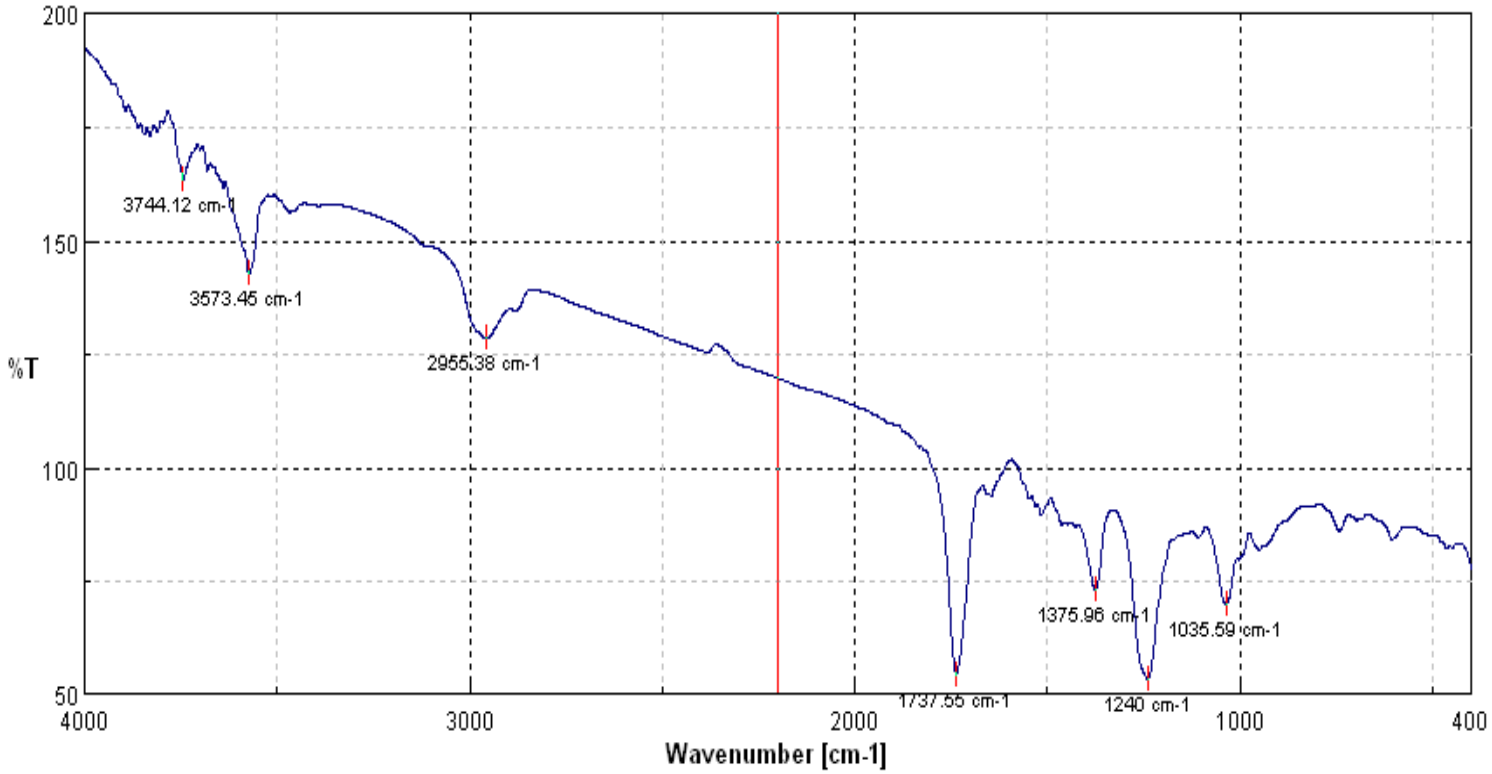

Figure S17. UV spectrum of Caesalpinimin A (**1**)

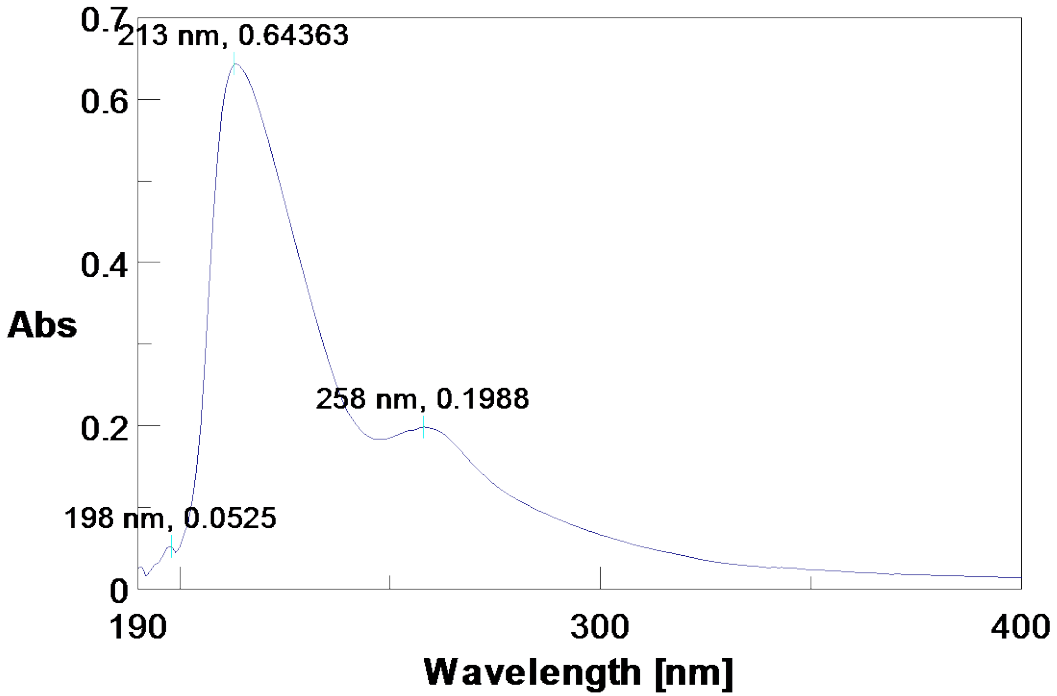

Figure S18. ECD spectrum of Caesalpinimin A (**1**)

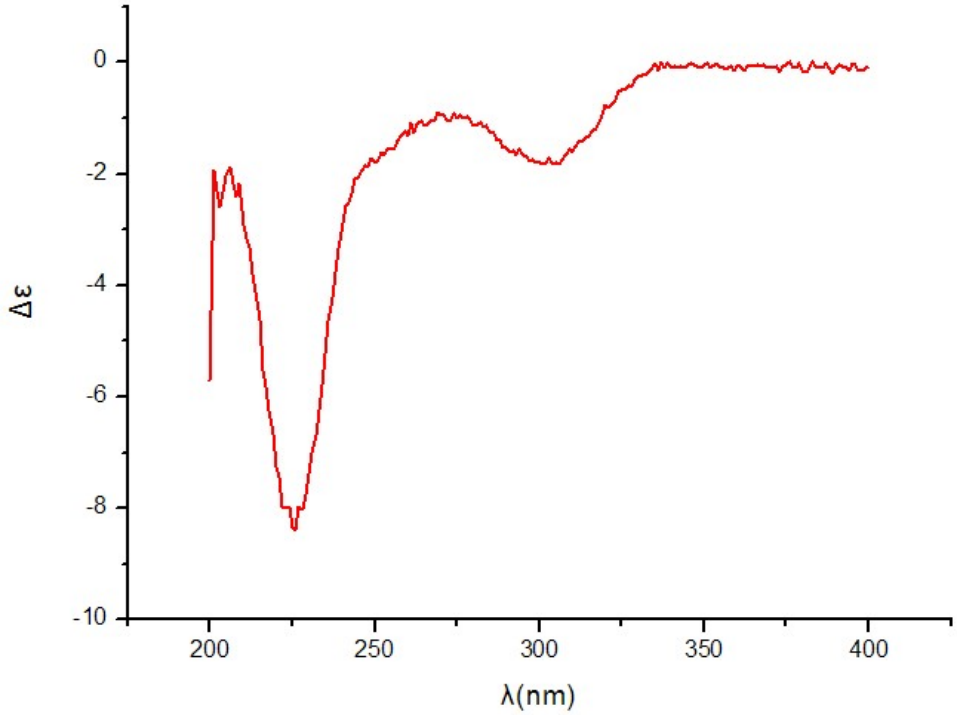

Table S1. Optimized Z-Matrixes of model A and its transition state and product as mentioned in Figure 4, as well as the model B, at the B3LYP/6-31+(d,p) level in the gas phase.

Substrate				**Transition state**				**Product**				**Model B**			
C	-4.9468	-1.4348	-1.6448	C	3.8707	-1.1296	1.8544	C	-3.3372	-1.2495	-1.7632	C	2.4058	2.0343	0.5725
C	-4.3674	-1.7205	-0.2877	C	4.3432	-1.5466	0.4762	C	-4.2844	-1.4935	-0.5733	C	2.0884	0.5884	0.3037
C	-3.7559	-0.5707	0.5892	C	3.8678	-0.5180	-0.5933	C	-3.9044	-0.4629	0.5269	C	0.6702	0.4112	-0.3272
C	-2.2705	-0.1874	0.1064	C	2.3588	-0.1552	-0.1402	C	-2.4012	-0.1147	0.1146	C	-0.3798	1.4547	0.1209
C	-1.2262	-1.2158	0.6278	C	1.3192	-1.1714	-0.6819	C	-1.3664	-1.1198	0.6993	C	0.0990	2.9172	-0.0877
C	0.1409	-0.9728	-0.0155	C	-0.0587	-0.9495	-0.0458	C	0.0057	-0.9240	0.0466	C	1.5017	3.0297	0.3808
C	0.7249	0.3926	0.3514	C	-0.6390	0.4292	-0.3623	C	0.5973	0.4518	0.3568	C	0.1431	-0.9845	0.0096
C	-0.2864	1.4938	-0.0747	C	0.3691	1.5105	0.1173	C	-0.3934	1.5354	-0.1542	C	-1.2591	-1.3176	-0.5306
C	-1.7385	1.2712	0.5447	C	1.8248	1.3057	-0.4931	C	-1.8635	1.3469	0.4271	C	-2.3243	-0.2400	-0.1396
C	0.2692	2.9141	0.2193	C	-0.1763	2.9401	-0.1472	C	0.1550	2.9633	0.1131	C	-1.7636	1.1890	-0.5978
C	1.6563	2.9915	-0.3005	C	-1.5742	3.0068	0.3451	C	1.5642	3.0181	-0.3473	O	2.0445	4.2415	0.7009
C	2.5161	1.9685	-0.5392	C	-2.4391	1.9795	0.5443	C	2.4261	1.9836	-0.5215	C	3.3329	3.9990	1.1037
C	2.1450	0.5326	-0.2873	C	-2.0660	0.5497	0.2618	C	2.0351	0.5574	-0.2435	C	3.6091	2.6673	1.0480
C	3.7273	2.5650	-1.0407	C	-3.6586	2.5656	1.0378	C	3.6611	2.5585	-0.9886	C	3.1448	0.0018	-0.6422
C	3.4995	3.9072	-1.0612	C	-3.4296	3.9065	1.0940	C	3.4436	3.9008	-1.0559	O	4.0031	-0.8008	0.0155

C	3.1878	-0.1081	0.6375	C	-3.0963	-0.0641	-0.6960	C	3.0442	-0.0627	0.7320	O	3.2198	0.2558	-1.8259
C	-3.7707	-1.1321	2.0370	C	3.9870	-1.1508	-1.9954	C	-4.0877	-1.0718	1.9324	C	5.0286	-1.4463	-0.7927
C	-4.7484	0.6165	0.5442	C	4.8297	0.6913	-0.5323	C	-4.8678	0.7372	0.3936	C	5.8585	-2.3160	0.1307
C	-1.7560	1.5433	2.0599	C	1.8521	1.6146	-2.0017	C	-1.9255	1.6909	1.9271	H	0.8048	0.4833	-1.4115
C	1.5101	-3.0326	-0.2987	C	-1.4499	-3.0089	0.1596	C	1.4091	-2.9739	-0.1737	H	-0.5454	1.3248	1.1968
C	1.1991	-3.0548	-1.7802	C	-1.1510	-3.0902	1.6411	C	1.2300	-2.9680	-1.6768	C	-3.8470	-0.5469	-0.5676
C	-2.5583	2.3532	-0.1686	C	2.6422	2.3604	0.2697	C	-2.6595	2.3860	-0.3810	C	-4.6093	-0.0979	0.7093
C	5.0618	-1.5760	0.7252	C	-4.9808	-1.5138	-0.8472	C	4.9241	-1.5152	0.9157	C	-3.6557	-0.3926	1.8530
C	5.8639	-2.4370	-0.2305	C	-5.8442	-2.3397	0.0855	C	5.8157	-2.3269	-0.0030	O	1.0593	-1.9398	-0.5642
O	2.1720	-3.8847	0.2511	O	-2.1227	-3.8243	-0.4281	O	2.0190	-3.8361	0.4170	C	1.5267	-3.0495	0.0772
O	2.2340	4.1884	-0.6129	O	-2.1559	4.1964	0.6754	O	2.1626	4.2019	-0.6689	C	1.2356	-3.2327	1.5510
O	-2.1761	-0.2462	-1.3175	O	2.4123	-0.2442	1.2987	O	-2.4147	-0.2382	-1.3206	O	2.1928	-3.8297	-0.5666
O	-4.6367	-0.5197	-2.3931	O	3.1205	-1.9152	2.5782	O	-2.6737	-2.4036	-2.2055	O	-1.5327	-2.5982	0.0348
O	-1.6450	-2.5535	0.3389	O	1.7449	-2.5042	-0.4116	O	-1.7899	-2.4763	0.4801	C	-1.6284	1.3312	-2.1358
O	1.0256	-2.0133	0.4730	O	-0.9332	-1.9731	-0.5761	O	0.8662	-1.9630	0.5760	O	-2.3516	-0.2248	1.3134
O	3.2634	0.0914	1.8315	O	-3.1467	0.1622	-1.8865	O	3.0746	0.1605	1.9237	C	-4.4060	0.2039	-1.7958
O	4.0366	-0.8945	-0.0527	O	-3.9627	-0.8570	-0.0377	O	3.9203	-0.8586	0.0885	C	-4.1556	-2.0444	-0.8219

H	-0.4051	1.4182	-1.1620	H	0.4700	1.4026	1.2055	H	-0.4698	1.4177	-1.2430	O	-3.8710	-1.7115	2.3129
H	0.8642	0.4407	1.4378	H	-0.7659	0.5198	-1.4471	H	0.7072	0.5556	1.4425	C	-2.6646	2.2858	-0.0160
O	-2.9475	3.3784	0.3535	O	3.0030	3.4202	-0.1998	O	-3.0166	3.4623	0.0528	O	-3.2147	3.1643	-0.6478
H	-1.1240	-1.1038	1.7125	H	1.2303	-1.0271	-1.7654	H	-1.2792	-0.9559	1.7794	H	2.1466	0.0291	1.2454
H	0.0298	-1.0526	-1.0965	H	0.0299	-1.0684	1.0355	H	-0.1078	-1.0452	-1.0314	H	0.0202	3.2166	-1.1405
H	2.1758	-0.0108	-1.2393	H	-2.1112	-0.0193	1.1985	H	2.0902	-0.0115	-1.1790	H	-0.5359	3.6113	0.4745
H	0.2648	3.1272	1.2948	H	-0.1430	3.1780	-1.2172	H	0.0994	3.2079	1.1806	H	0.1020	-1.1006	1.0956
H	-0.3523	3.6823	-0.2507	H	0.4363	3.6933	0.3567	H	-0.4419	3.7170	-0.4087	H	-1.1967	-1.3914	-1.6219
H	4.6372	2.0643	-1.3406	H	-4.5743	2.0594	1.3093	H	4.5792	2.0444	-1.2365	H	3.9053	4.8668	1.3911
H	4.0940	4.7591	-1.3511	H	-4.0284	4.7522	1.3931	H	4.0556	4.7408	-1.3444	H	4.5464	2.1951	1.3072
H	-5.7128	-2.1621	-1.9823	H	4.4638	-0.3648	2.3765	H	-3.8271	-0.8429	-2.6507	H	4.5271	-2.0331	-1.5665
H	-5.1796	-2.1844	0.2884	H	5.4381	-1.6023	0.4691	H	-5.3291	-1.3780	-0.8763	H	5.6240	-0.6677	-1.2785
H	-3.6302	-2.5213	-0.4356	H	3.9371	-2.5374	0.2748	H	-4.1597	-2.5173	-0.2167	H	6.6474	-2.8098	-0.4463
H	-3.0439	-0.1145	-1.7477	H	2.2337	-1.2740	1.9083	H	-2.3081	-2.8308	-1.4110	H	5.2410	-3.0904	0.5946
H	-4.5272	1.3752	1.2961	H	4.5942	1.4672	-1.2625	H	-4.6699	1.5371	1.1098	H	6.3312	-1.7208	0.9185
H	-5.7549	0.2390	0.7590	H	5.8419	0.3382	-0.7550	H	-5.8869	0.3817	0.5799	H	-4.8322	0.9731	0.6775
H	-4.7956	1.1046	-0.4312	H	4.8673	1.1565	0.4566	H	-4.8615	1.1658	-0.6129	H	-5.5558	-0.6300	0.8310

H	-3.3003	-0.4595	2.7542	H	3.6892	-0.4629	-2.7910	H	-3.8512	-0.3614	2.7295	H	-3.7292	0.3080	2.6953
H	-3.2743	-2.1027	2.0954	H	3.3965	-2.0640	-2.0806	H	-3.4829	-1.9688	2.0726	H	1.7173	-4.1567	1.8694
H	-4.8090	-1.2679	2.3579	H	5.0341	-1.4156	-2.1783	H	-5.1373	-1.3584	2.0613	H	0.1598	-3.2938	1.7295
H	-1.4225	2.5604	2.2651	H	1.7052	2.6824	-2.1663	H	-1.8061	2.7655	2.0685	H	1.6390	-2.4012	2.1384
H	-1.1139	0.8579	2.6175	H	1.0806	1.0726	-2.5531	H	-1.1522	1.1796	2.5049	H	-1.9678	-3.1605	-0.6139
H	-2.7642	1.4770	2.4671	H	2.8161	1.3665	-2.4442	H	-2.8918	1.4289	2.3567	H	-2.3384	2.0676	-2.5155
H	-1.0487	-3.1495	0.8129	H	1.1375	-3.1030	-0.8680	H	-1.1613	-3.0482	0.9439	H	-1.8035	0.3934	-2.6632
H	1.6620	-3.9476	-2.1989	H	-1.6519	-3.9774	2.0269	H	1.7175	-3.8609	-2.0665	H	-0.6314	1.6682	-2.4248
H	1.6042	-2.1729	-2.2858	H	-1.5245	-2.2115	2.1757	H	1.6952	-2.0852	-2.1255	H	-5.4807	-0.0003	-1.8533
H	0.1206	-3.0807	-1.9606	H	-0.0760	-3.1713	1.8290	H	0.1764	-2.9730	-1.9707	H	-3.9647	-0.1545	-2.7301
H	5.6767	-0.8187	1.2204	H	-5.5525	-0.7425	-1.3714	H	5.4778	-0.7448	1.4599	H	-4.2871	1.2849	-1.7490
H	4.5628	-2.1722	1.4933	H	-4.4715	-2.1327	-1.5904	H	4.4024	-2.1439	1.6421	H	-5.2202	-2.1358	-1.0619
H	6.6529	-2.9570	0.3227	H	-6.6271	-2.8413	-0.4928	H	6.5888	-2.8268	0.5896	H	-3.9573	-2.6654	0.0496
H	6.3347	-1.8308	-1.0109	H	-6.3258	-1.7110	0.8410	H	6.3099	-1.6880	-0.7417	H	-3.6113	-2.4252	-1.6956
H	5.2263	-3.1895	-0.7033	H	-5.2499	-3.1065	0.5905	H	5.2406	-3.0944	-0.5289	H	-3.1111	-1.9713	2.8522
H	-2.7187	2.1825	-1.2509	H	2.8189	2.1265	1.3363	H	-2.8195	2.1218	-1.4423	H	-2.7482	2.2624	1.0885