Supplementary Material

Bioassay-Guided Isolation of Hepatoprotective Lignans from *Vernonia cinerea*: DeepSAT-Driven Structural Elucidation and Predictive Mechanistic Insights Against Drug-Induced Liver Injury

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List of Supporting Information

Fig. S1. The therapeutic efficacy of crude fractions (A-J) in alleviating DILI induced by APAP in AML-12 cells. Data are expressed as mean ± SD. *P < 0.05, **P < 0.01, ***P < 0.001.

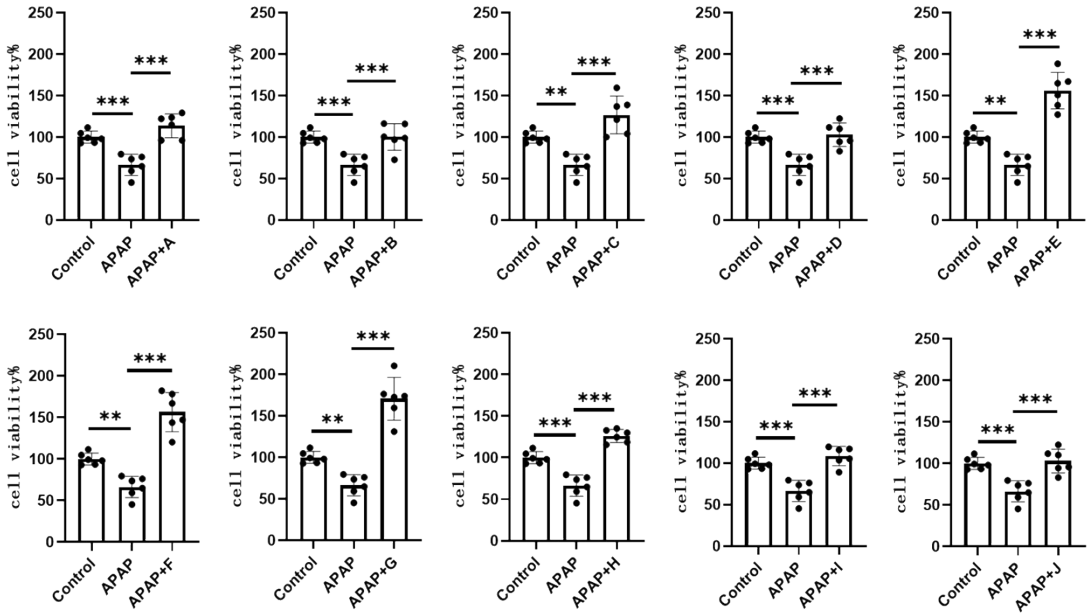


Fig. S2. The therapeutic efficacy of crude fractions (F2-F7) in alleviating DILI induced by APAP in AML-12 cells. Data are expressed as mean ± SD. *P < 0.05, **P < 0.01, ***P < 0.001.

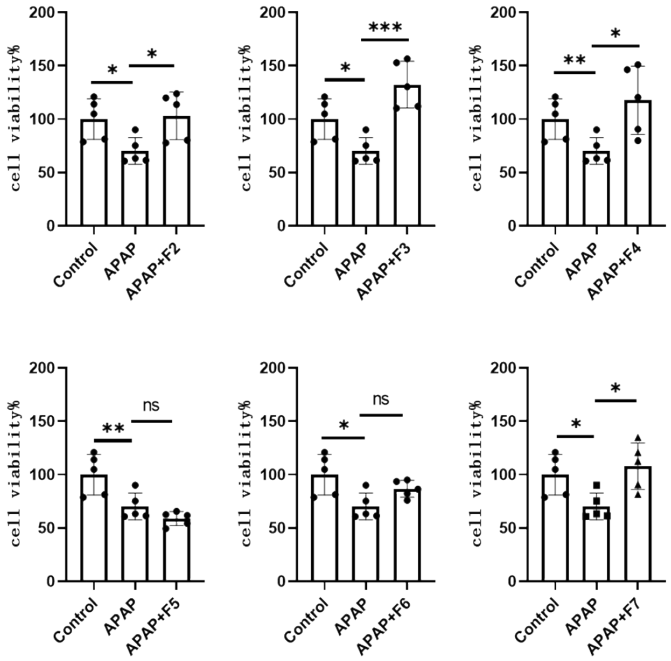
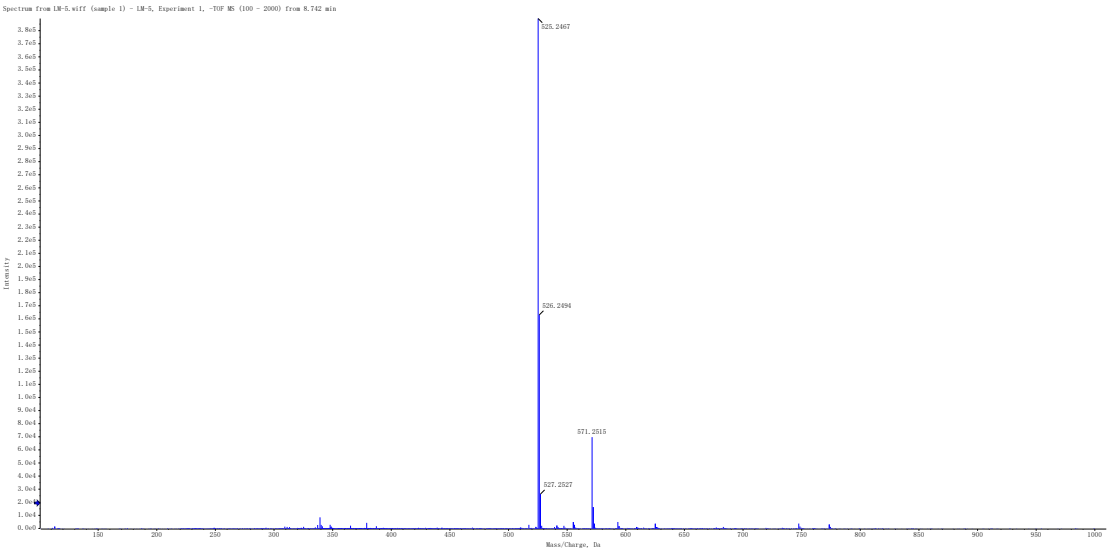


Fig. S3. HR-ESI-MS spectrum of **1**.



m/z 525.2467 $[M - H]^-$ (calcd for $C_{30}H_{37}O_8^-$ 525.2493).

Fig. S4. ^1H NMR spectrum of **1** (400 MHz, CD_3OD).

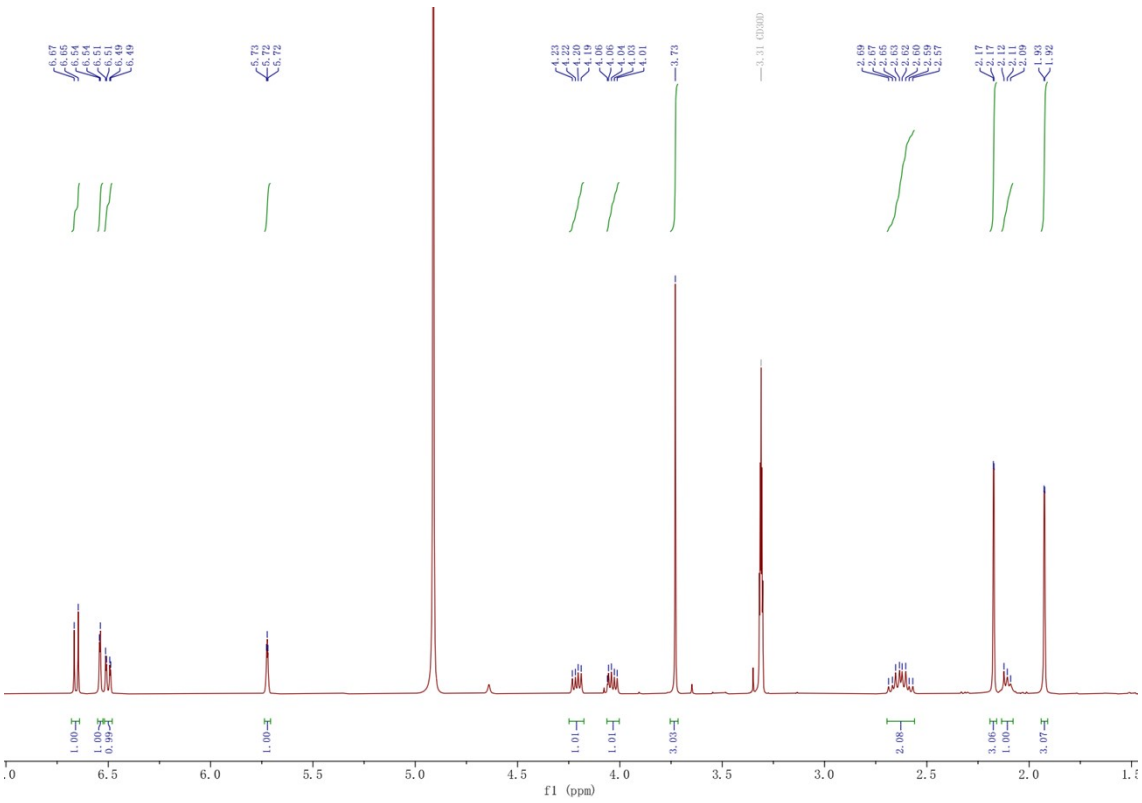


Fig. S5. ^{13}C NMR spectrum of **1** (150 MHz, CD_3OD).

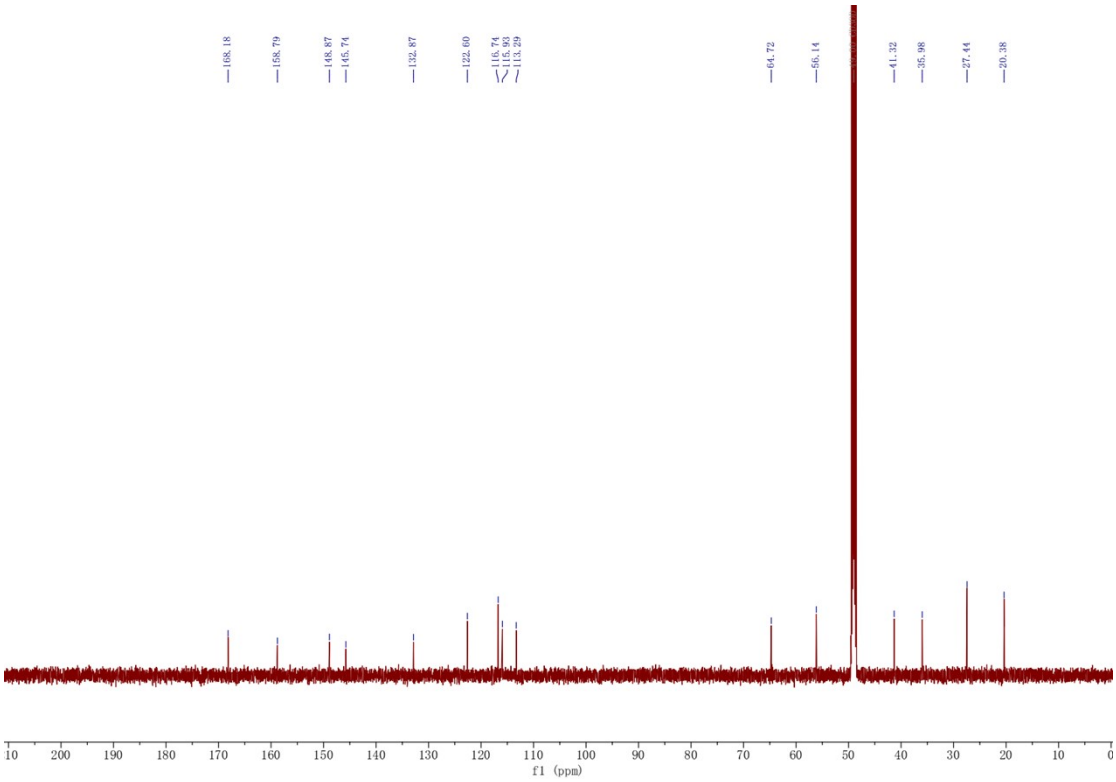


Fig. S6. ^{1}H-^{1}H COSY spectrum of **1** (600 MHz, CD_3OD).

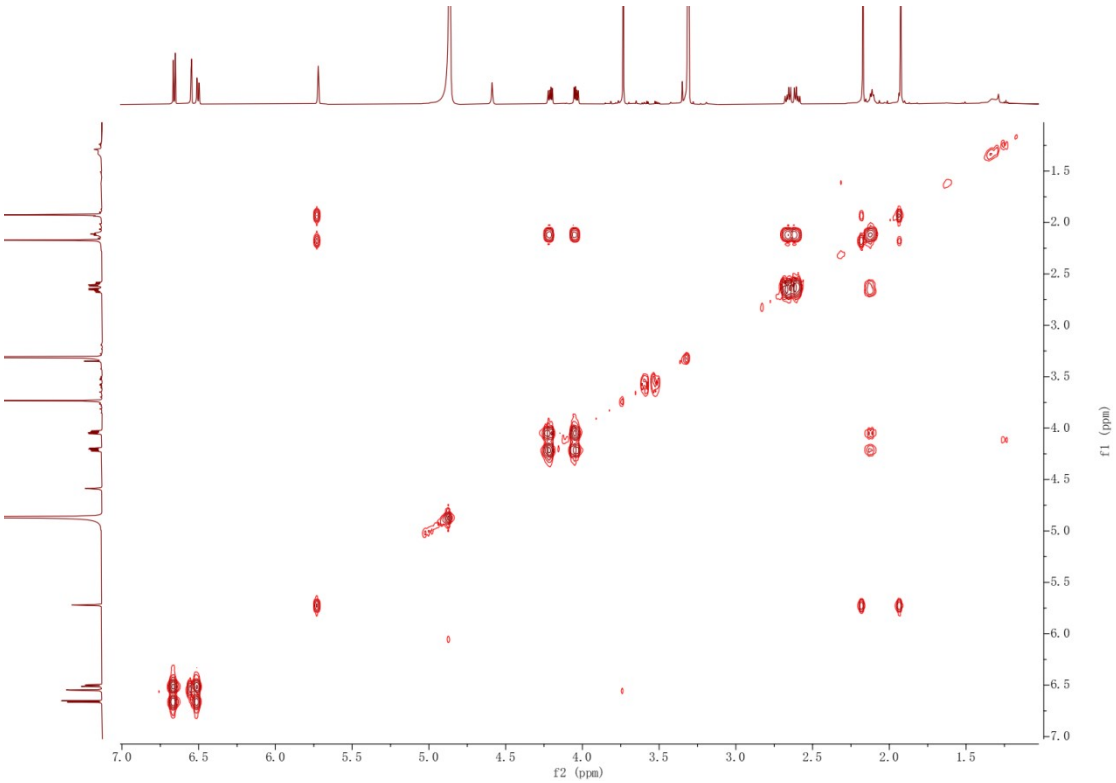


Fig. S7. HSQC spectrum of **1** (600 MHz, CD_3OD).

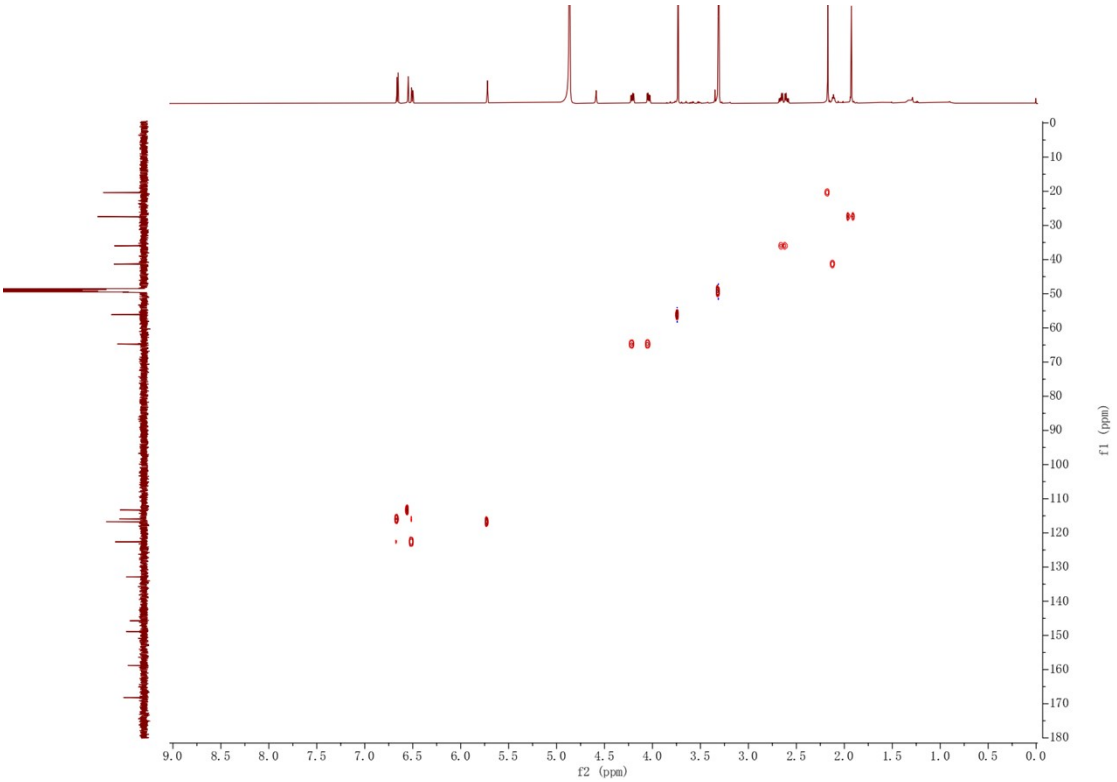


Fig. S8. HMBC spectrum of **1** (600 MHz, CD_3OD).

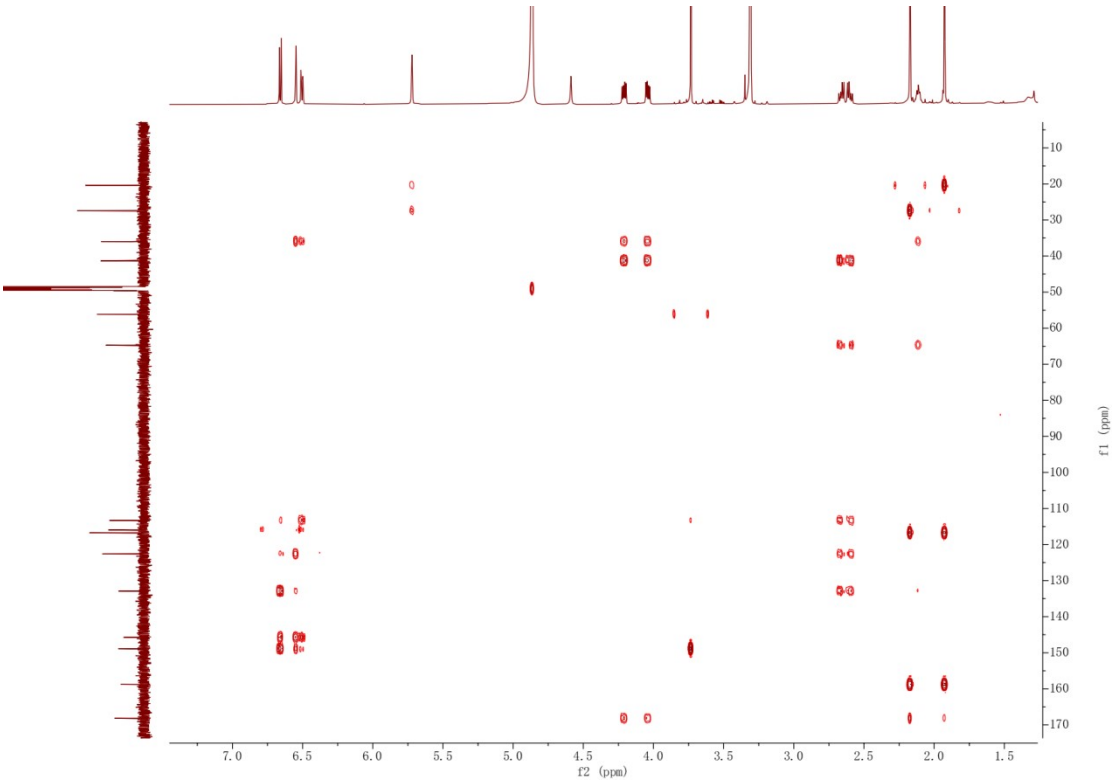


Fig. S9. Experimented ECD Spectrum of **1**.

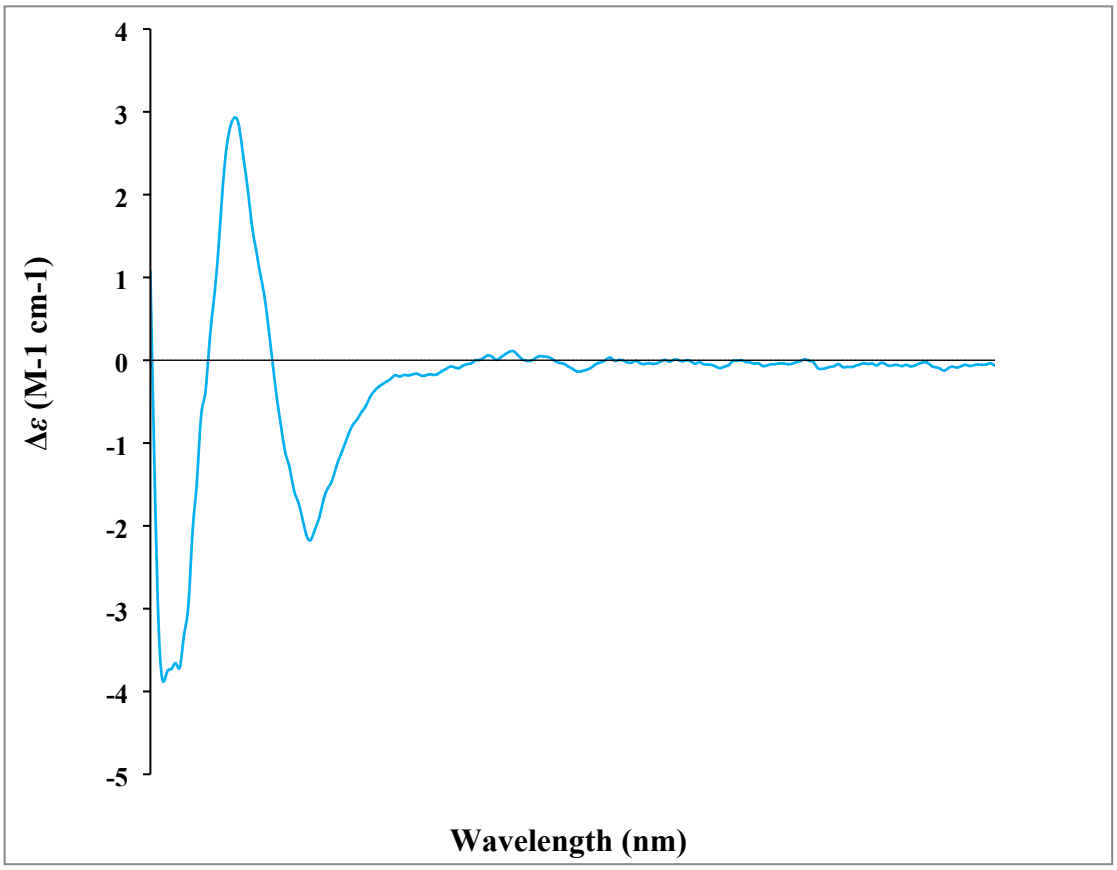
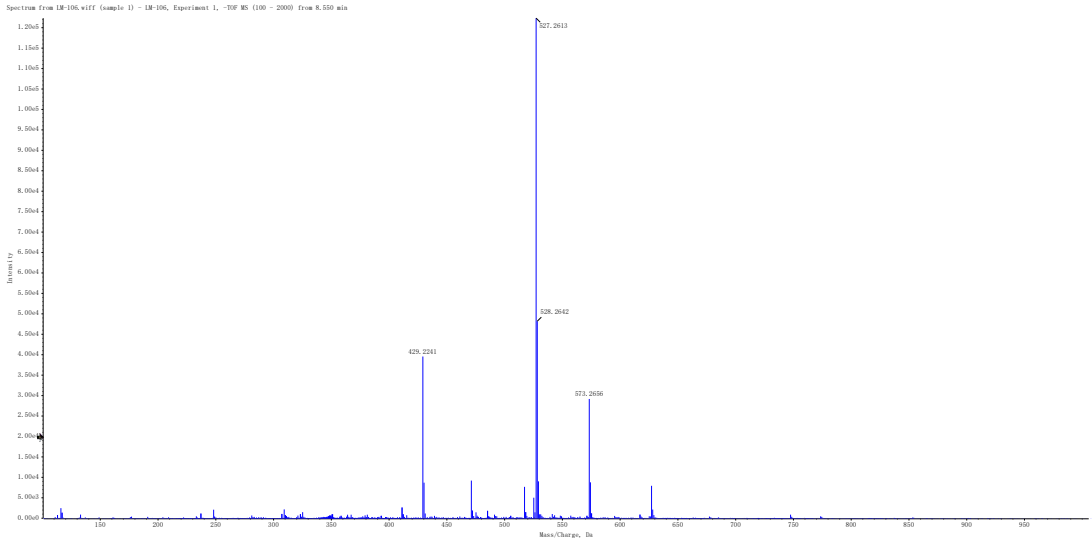


Fig. S10. HR-ESI-MS spectrum of **2**.



m/z 527.2613 $[M - H]^-$ (calcd for $C_{30}H_{39}O_8^-$ 527.2650).

Fig. S11. ^{1}H NMR spectrum of **2** (600 MHz, CD_3OD).

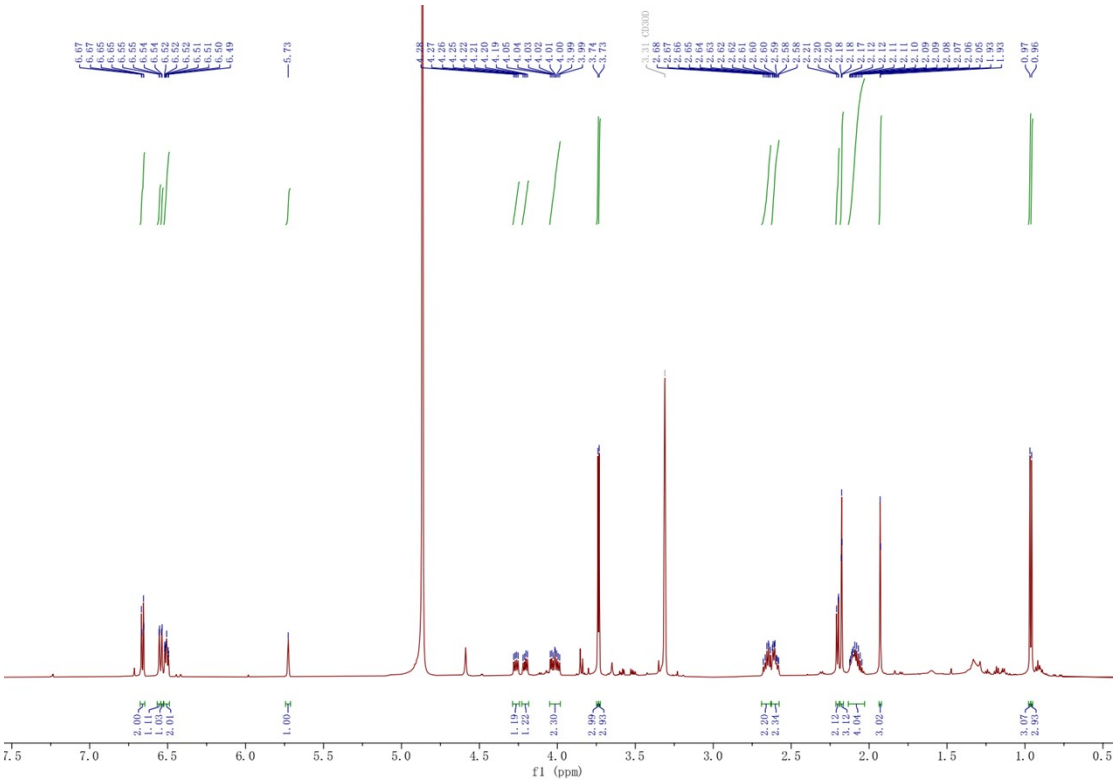


Fig. S12. ^{13}C NMR spectrum of **2** (150 MHz, CD_3OD).

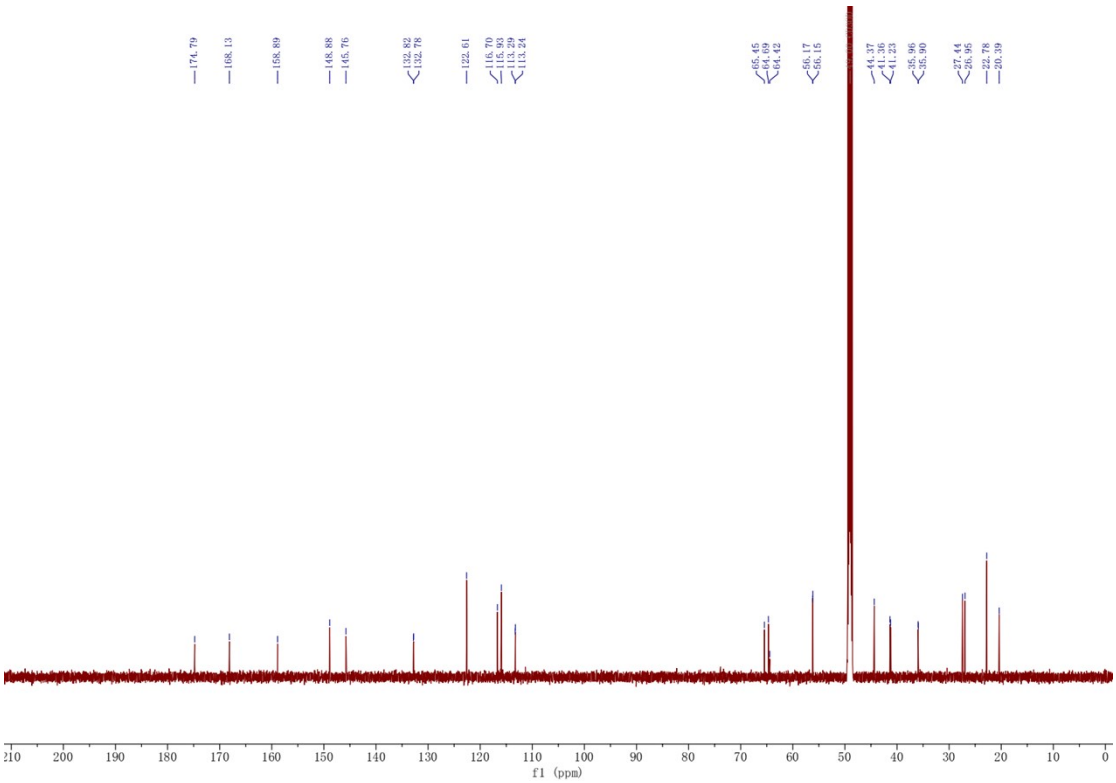


Fig. S13. ^{1}H-^{1}H COSY spectrum of **2** (600 MHz, CD_3OD).

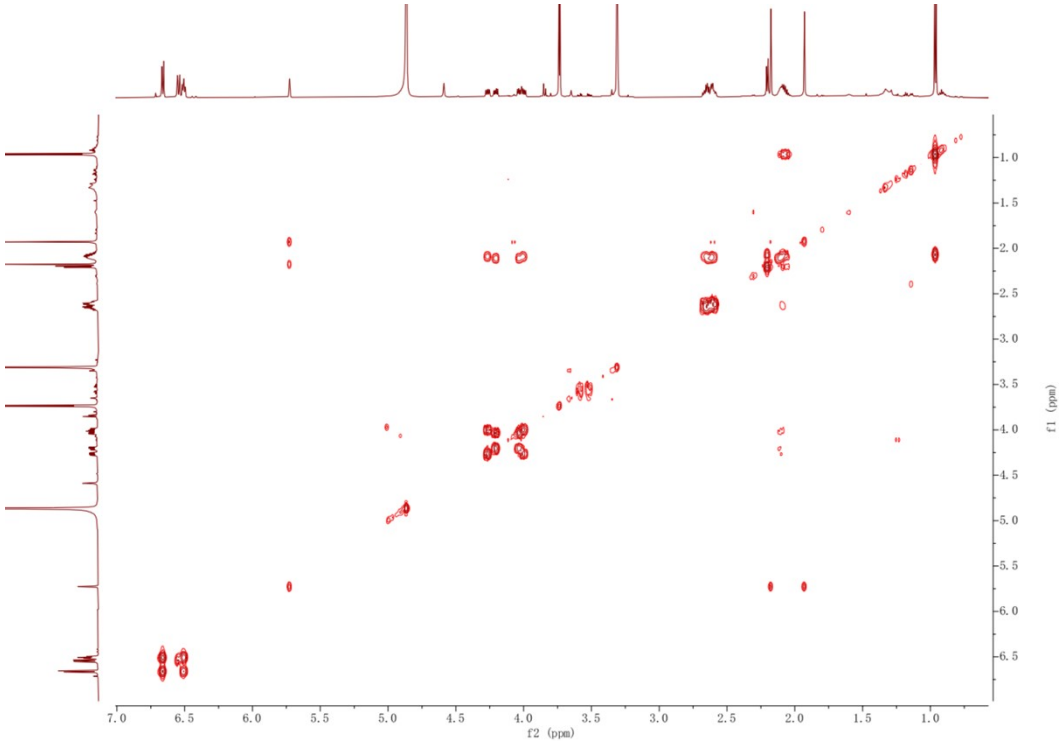


Fig. S14. HSQC spectrum of **2** (600 MHz, CD_3OD).

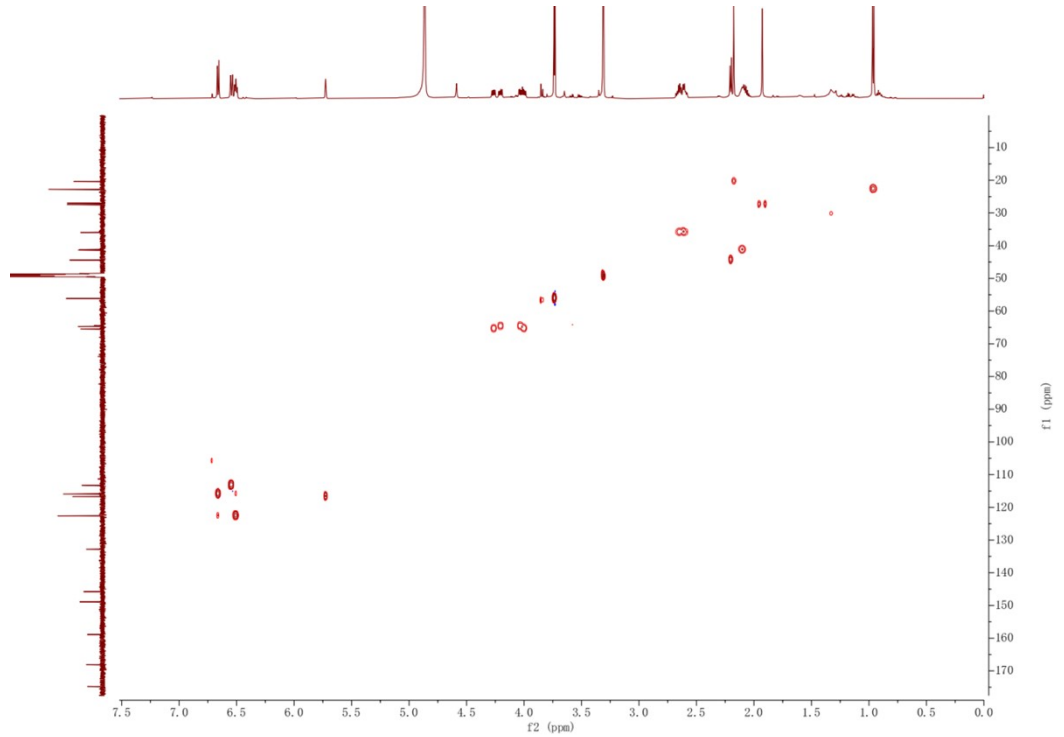


Fig. S15. HMBC spectrum of **2** (600 MHz, CD_3OD).

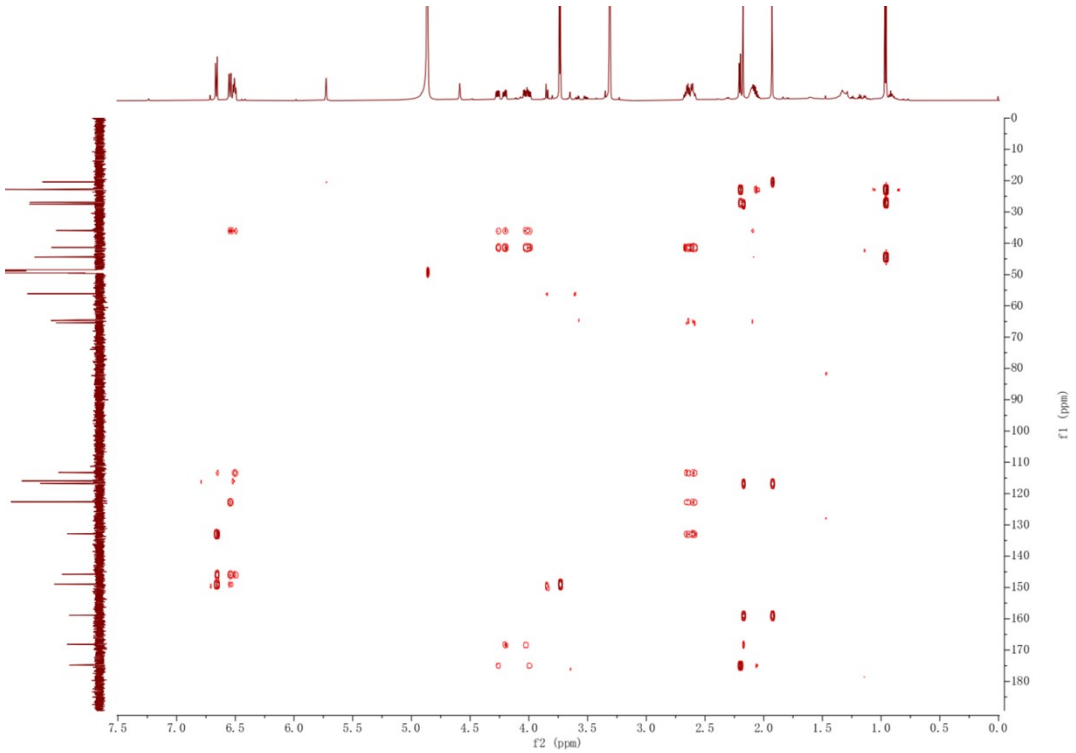


Fig. S16. Experimented ECD Spectrum of **2**.

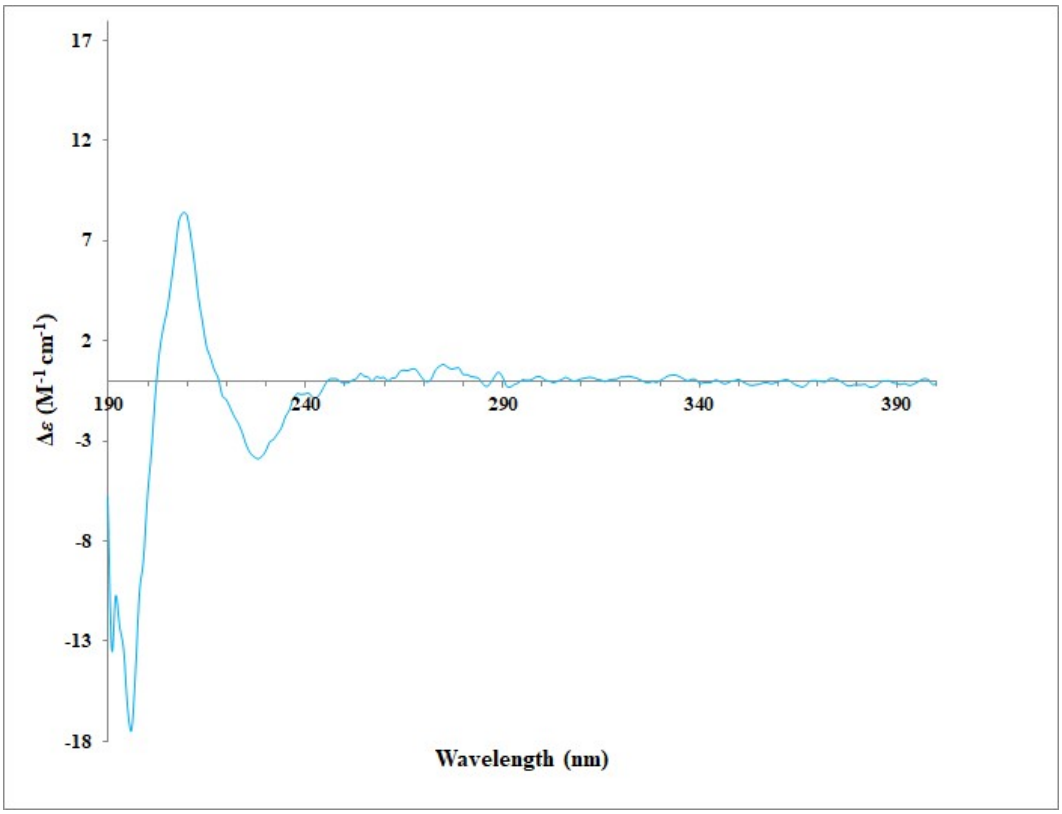
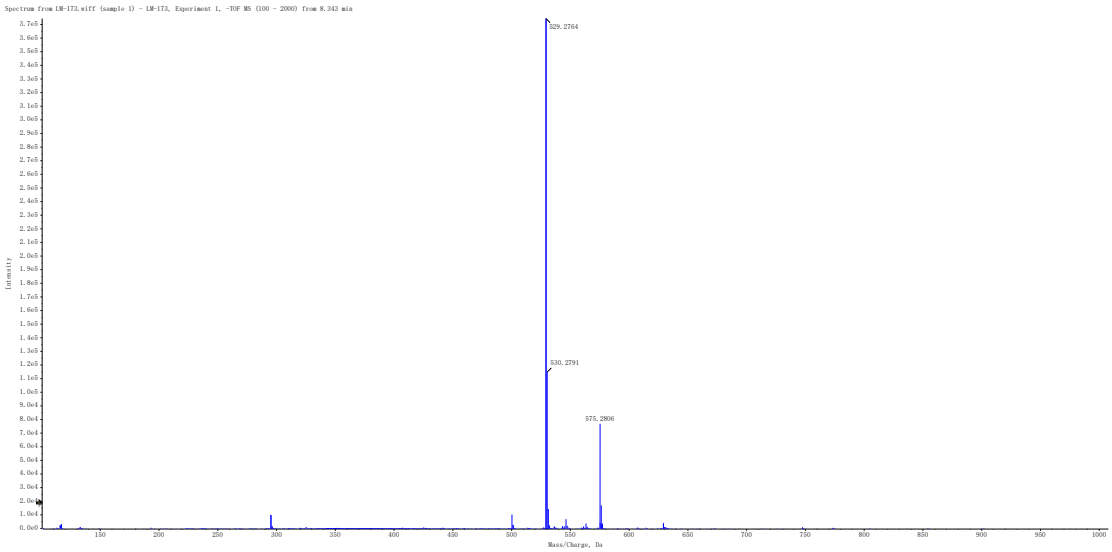


Fig. S17. HR-ESI-MS spectrum of **3**.



m/z 529.2764 $[M - H]^-$ (calcd for $C_{30}H_{41}O_8^-$ 529.2807).

Fig. S18. ^1H NMR spectrum of **3** (600 MHz, CD_3OD).

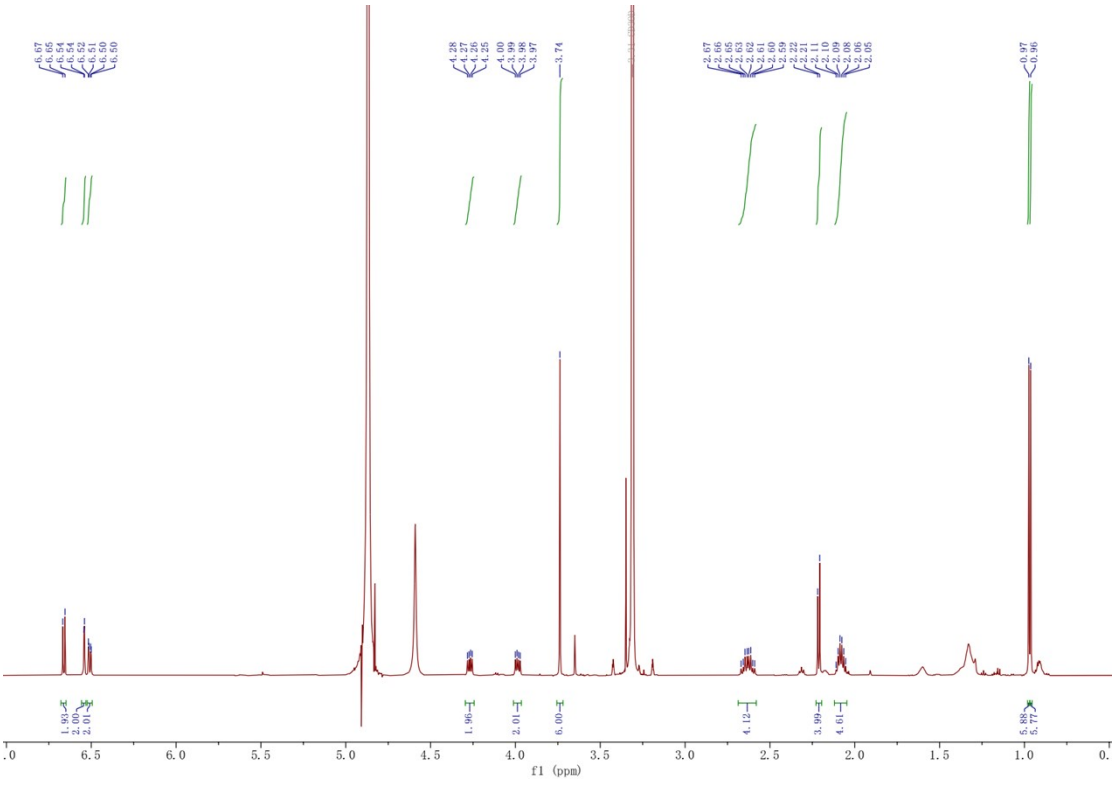


Fig. S19. ^{13}C NMR spectrum of **3** (150 MHz, CD_3OD).

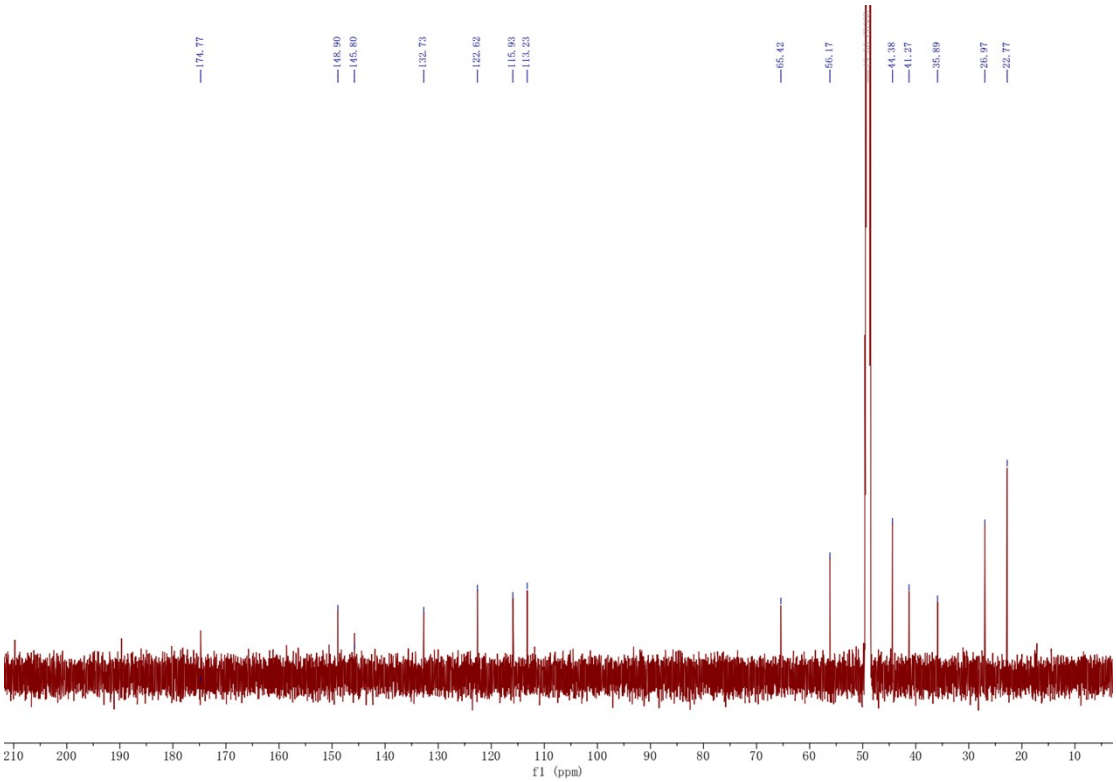


Fig. S20. ^{1}H-^{1}H COSY spectrum of **3** (600 MHz, CD_3OD).

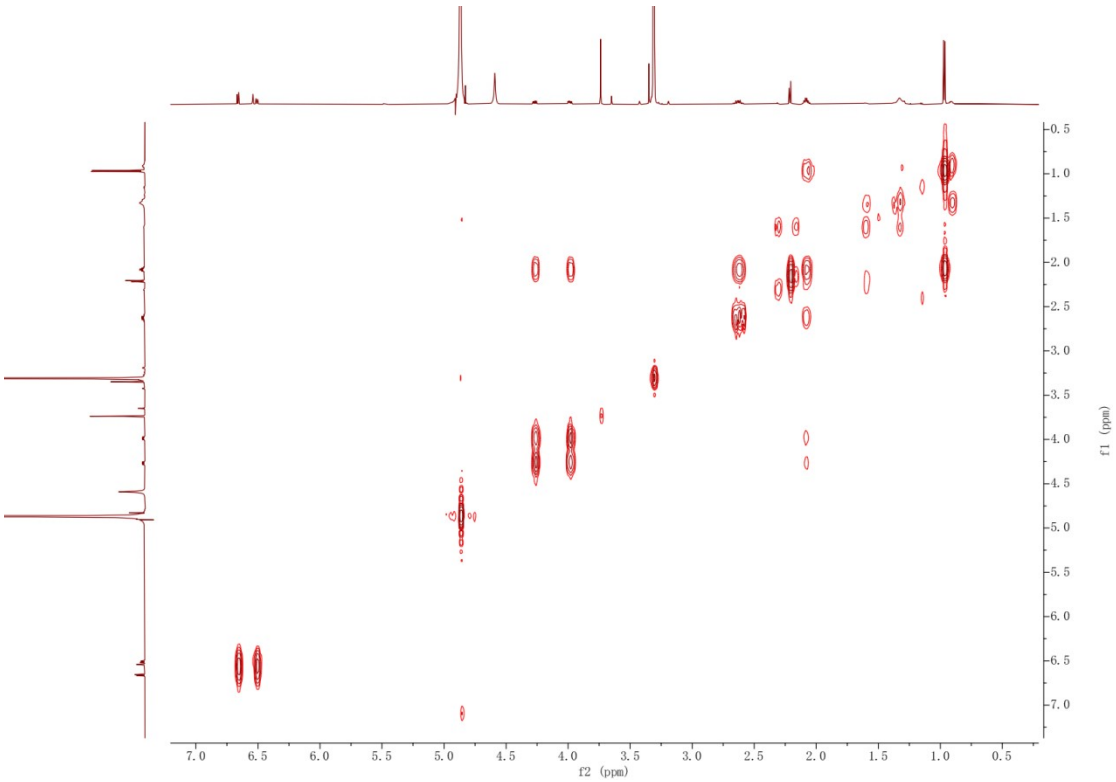


Fig. S21. HSQC spectrum of **3** (600 MHz, CD_3OD).

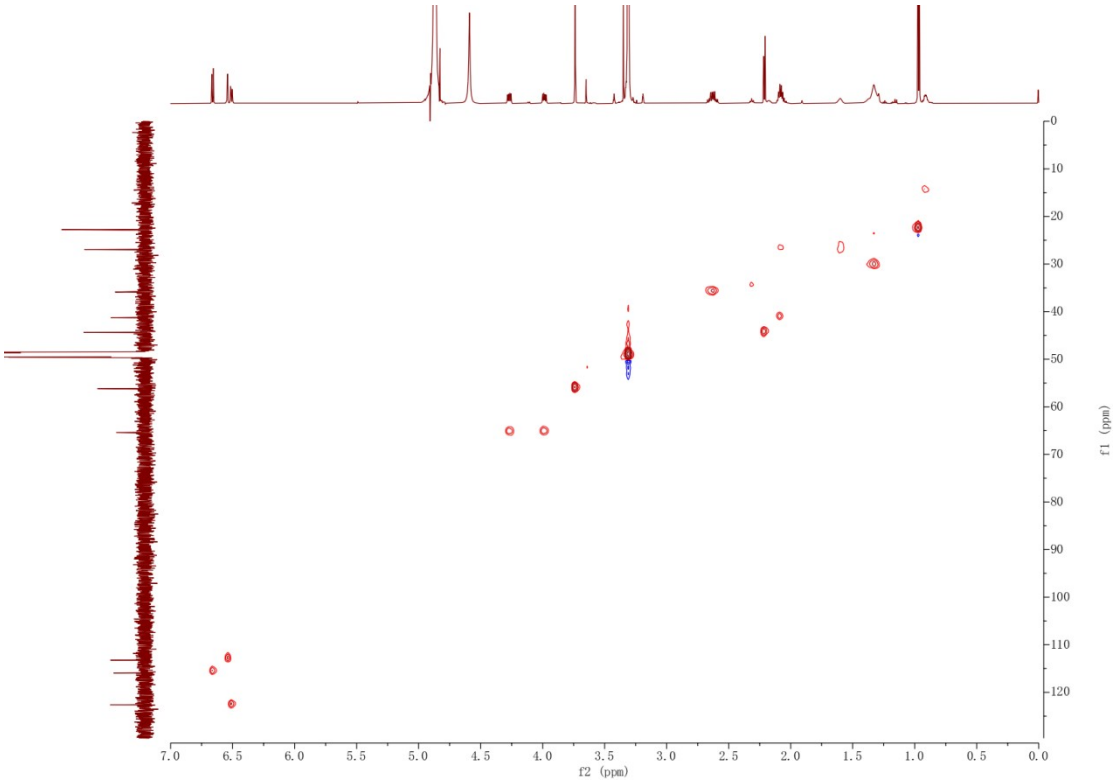


Fig. S22. HMBC spectrum of **3** (600 MHz, CD_3OD).

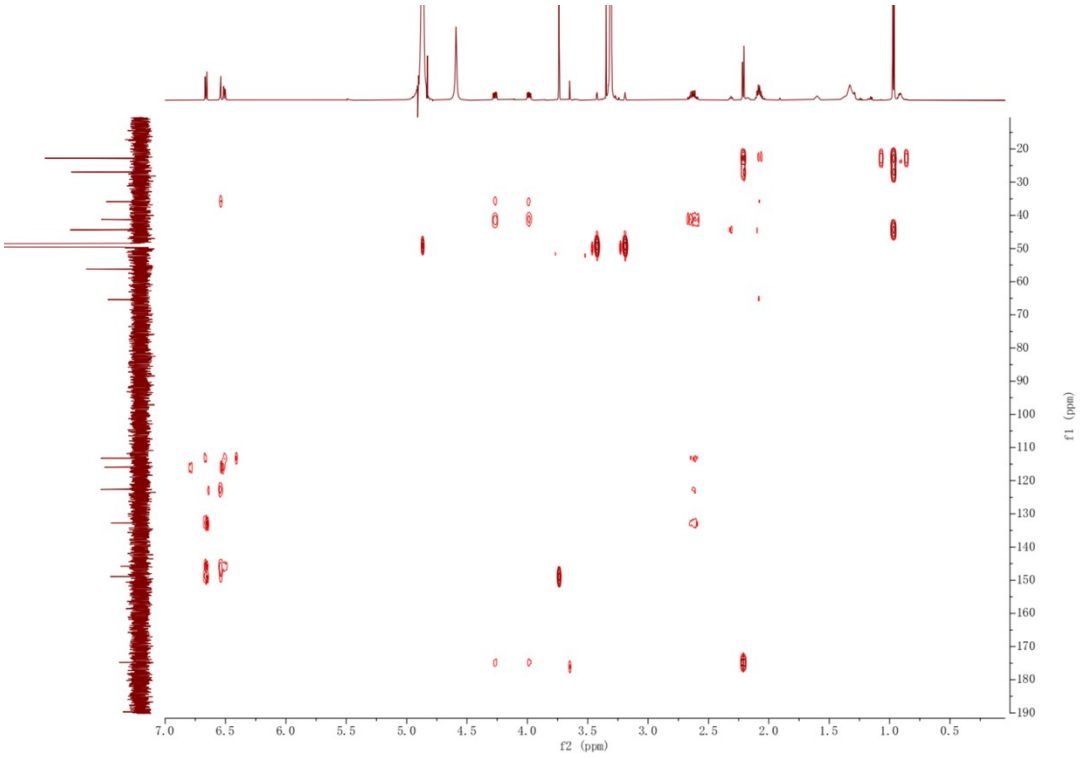


Fig. S23. Experimented ECD spectrum of **3**.

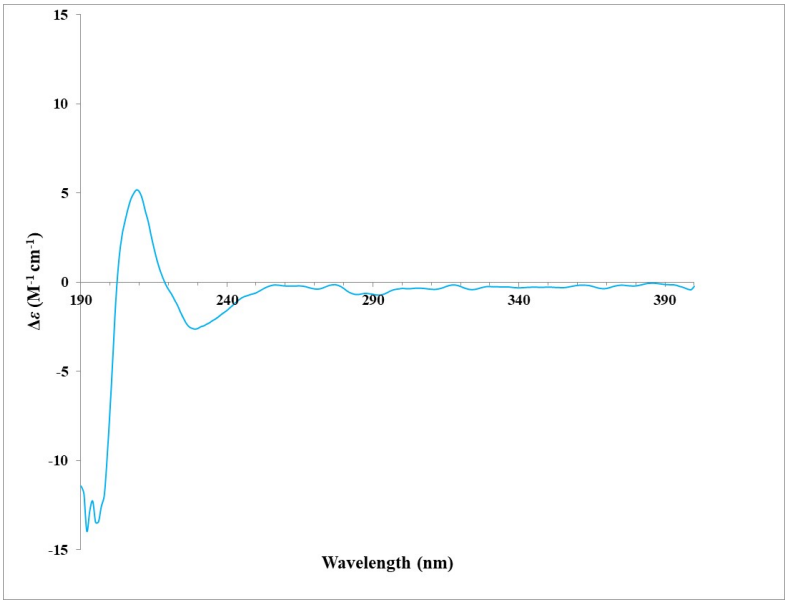


Fig. S24. Vernolignan A-C (**1**-**3**) fragments observed by HR-ESI-MS. Fragments are highlighted and color coded in the MS/MS spectrum, and the corresponding fragments are illustrated.

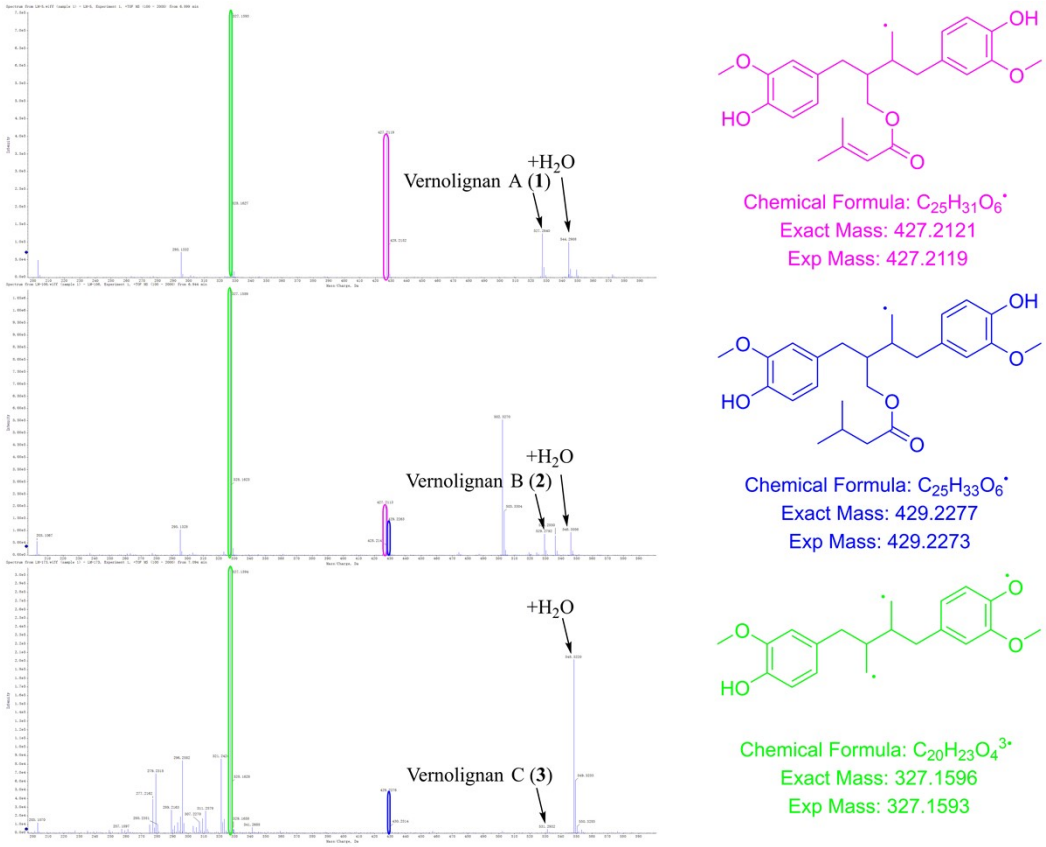


Fig. S25. ^{1}H NMR spectrum of **4** (400 MHz, CD_3OD).

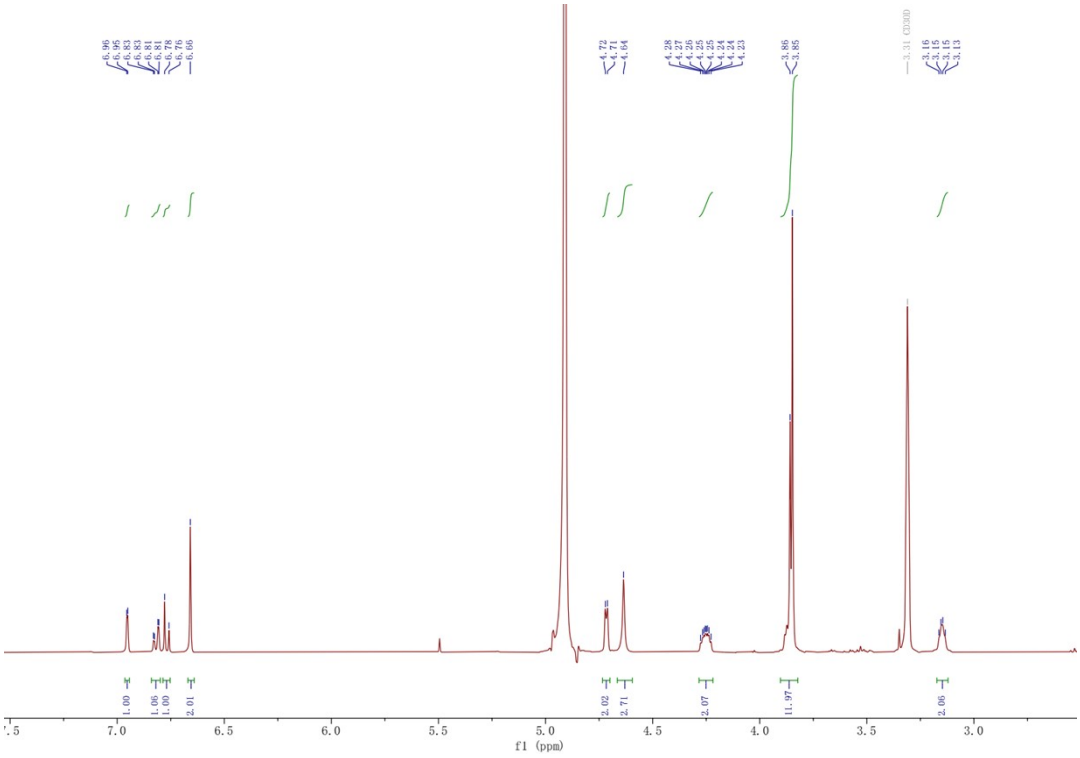


Fig. S26. ^{13}C NMR spectrum of **4** (150 MHz, CD_3OD).

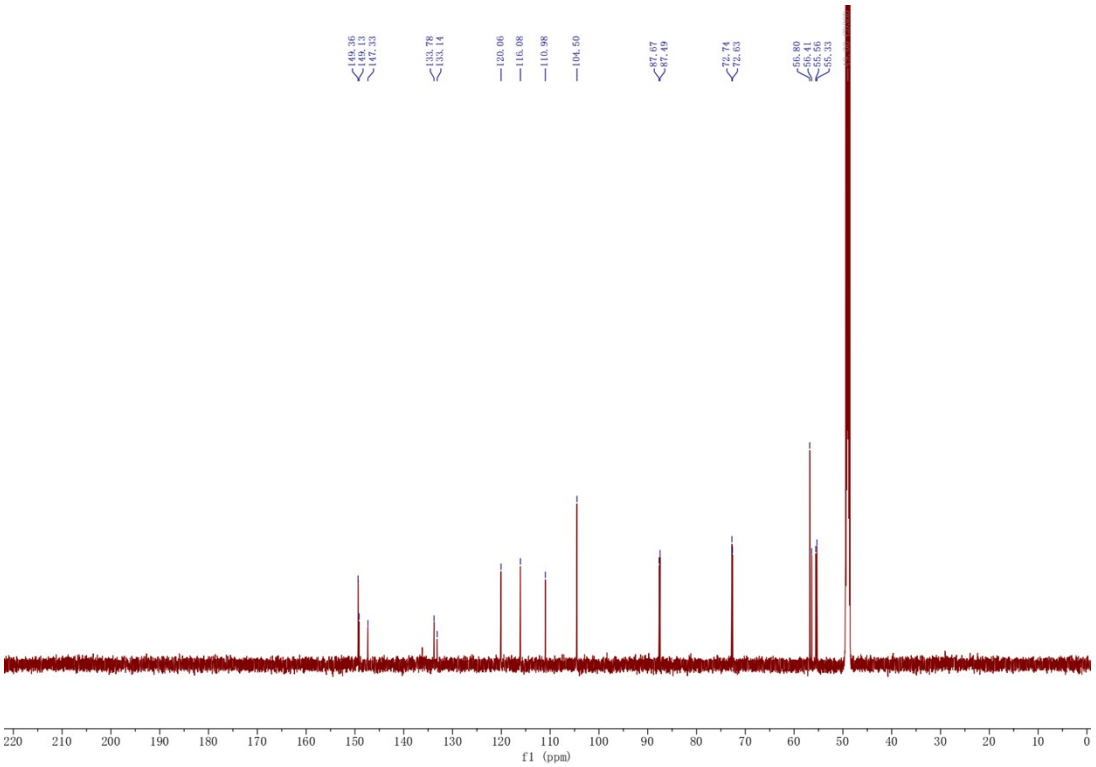


Fig. S27. ^{1}H NMR spectrum of **5** (400 MHz, $CDCl_3$).

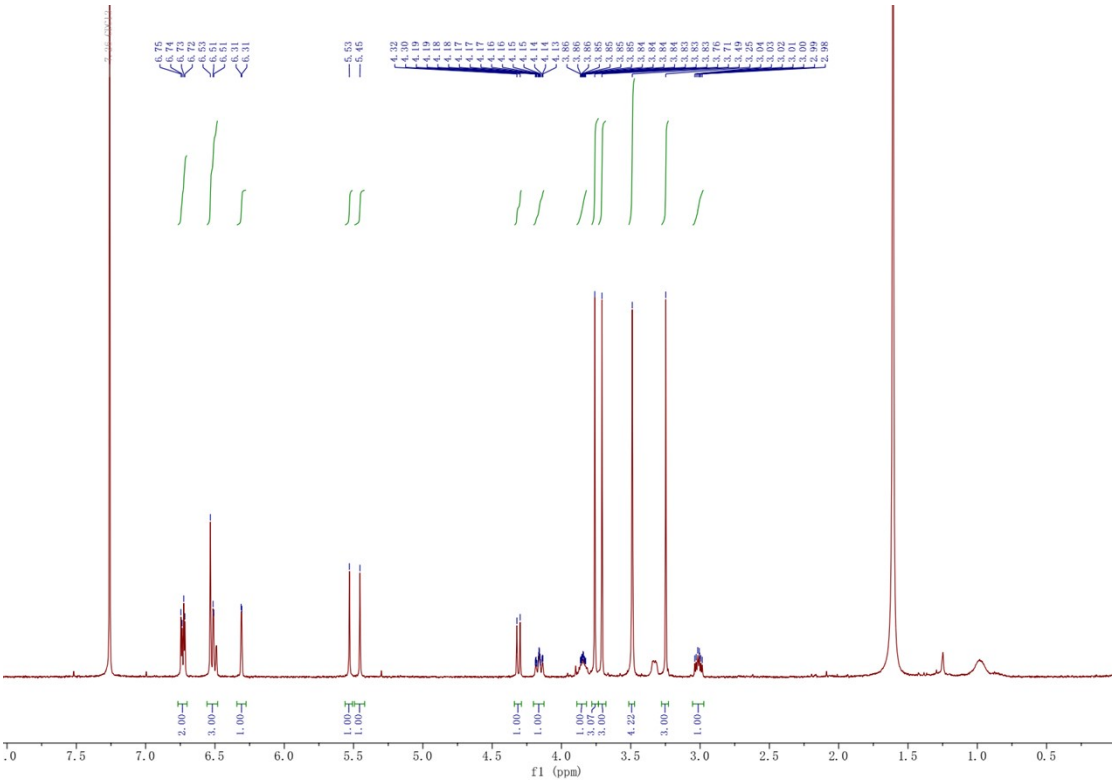


Fig. S28. ^{13}C NMR spectrum of **5** (150 MHz, $CDCl_3$).

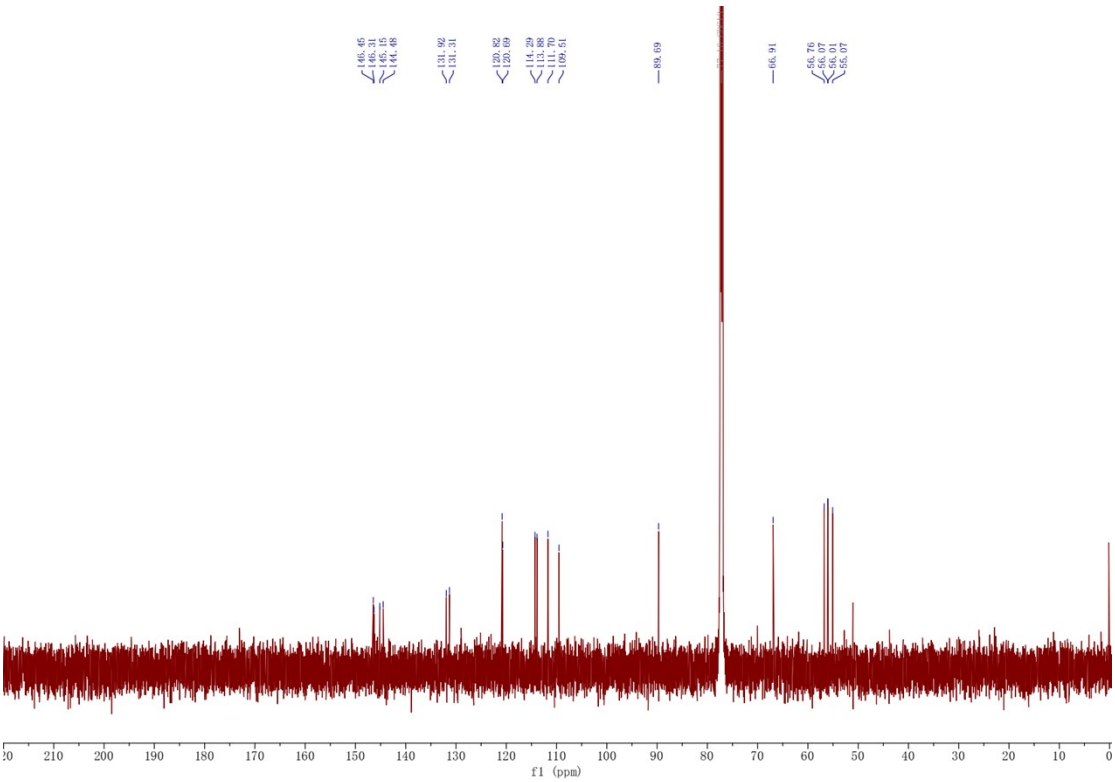


Fig. S29. ^{1}H NMR spectrum of **6** (400 MHz, CD_3OD).

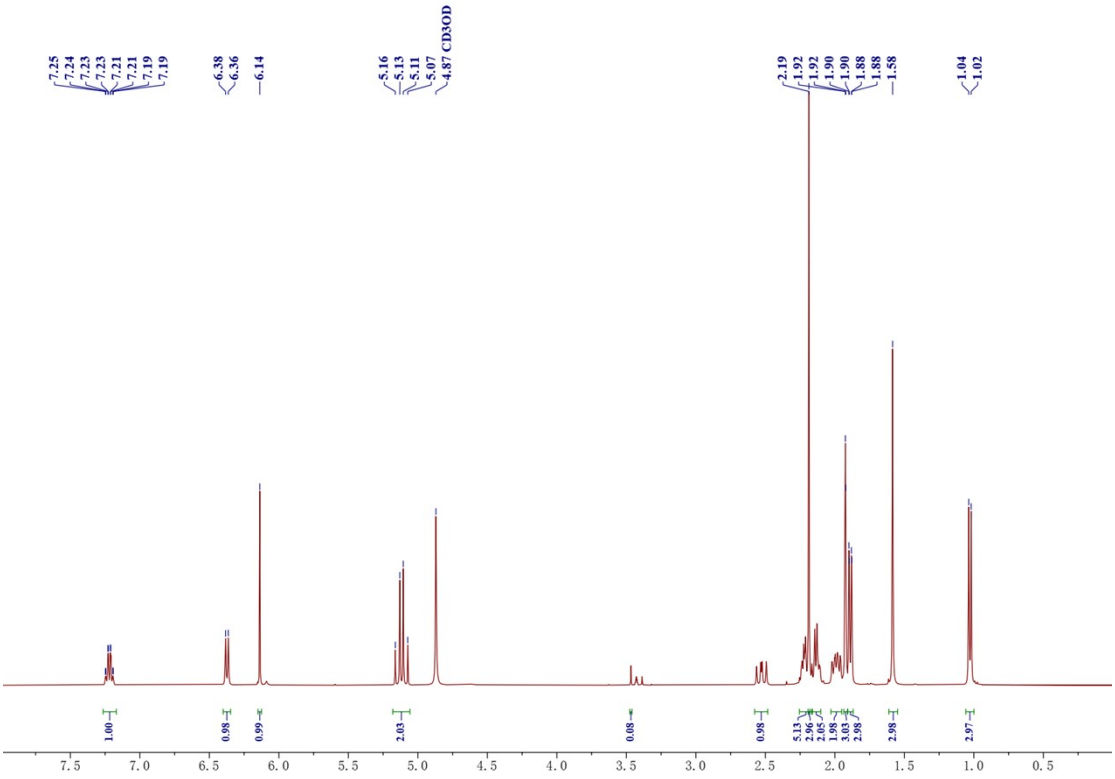


Fig. S30. ^{13}C NMR spectrum of **6** (150 MHz, CD_3OD).

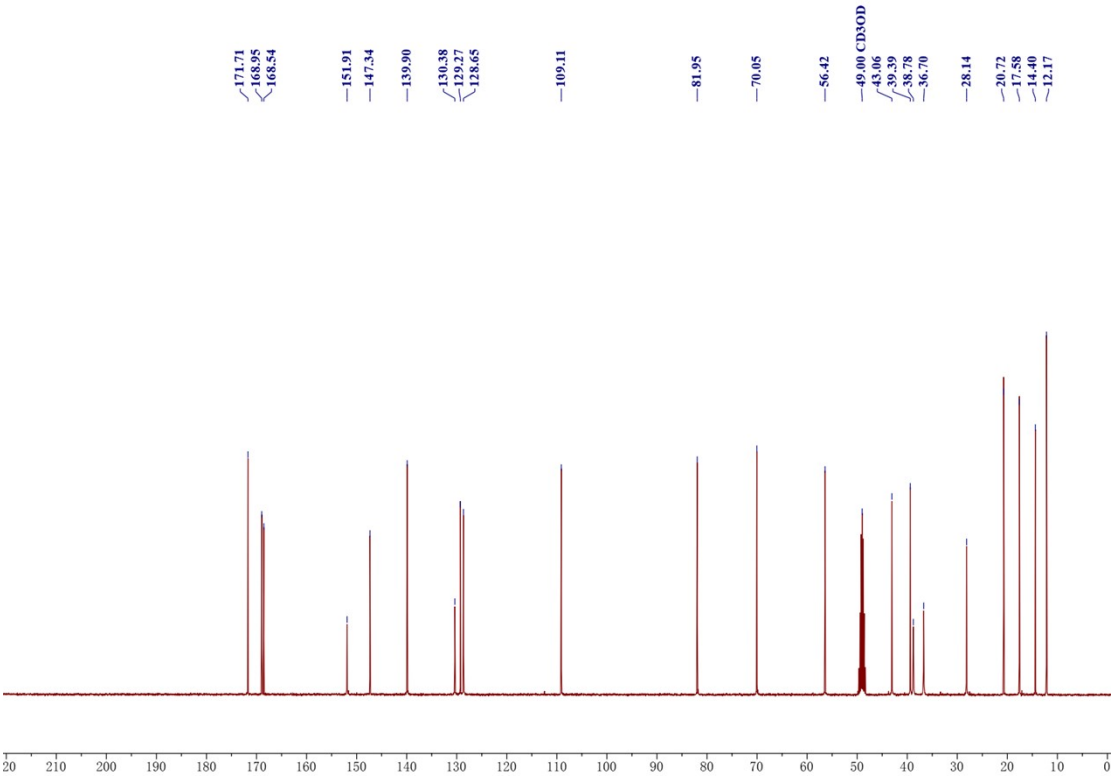


Fig. S31. Gene Ontology (GO) analysis for targets of **1**.

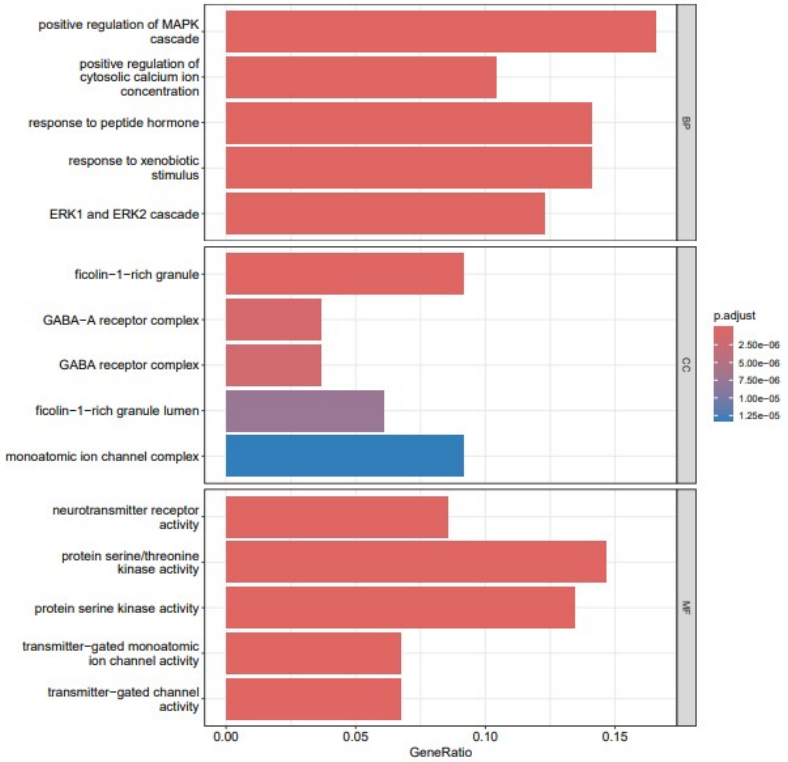


Fig. S32. Kyoto Encyclopedia of Genes and Genomes (KEGG) analysis for targets of **1**.

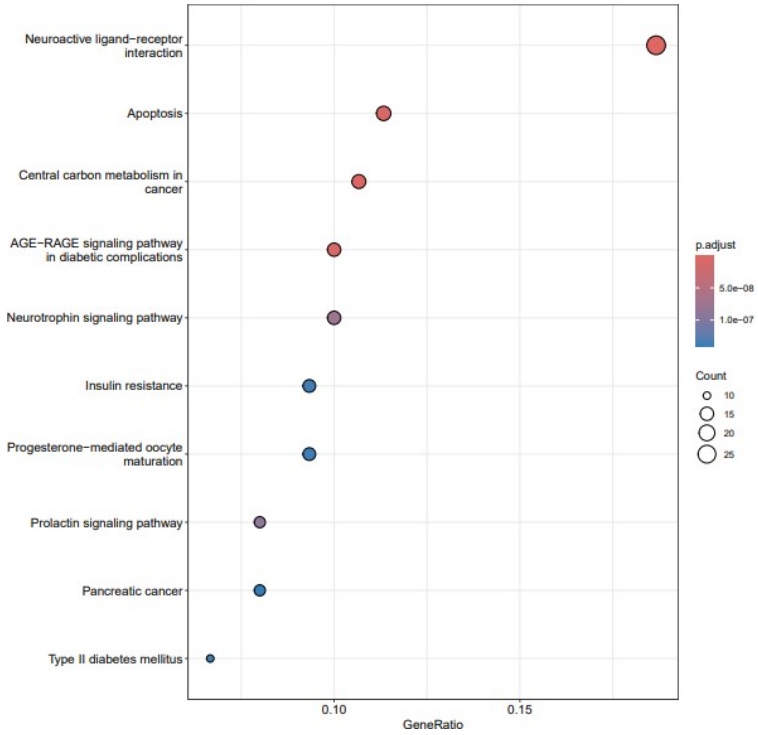


Fig. S33. Protein-Protein Interaction (PPI) analysis for targets of **1**.

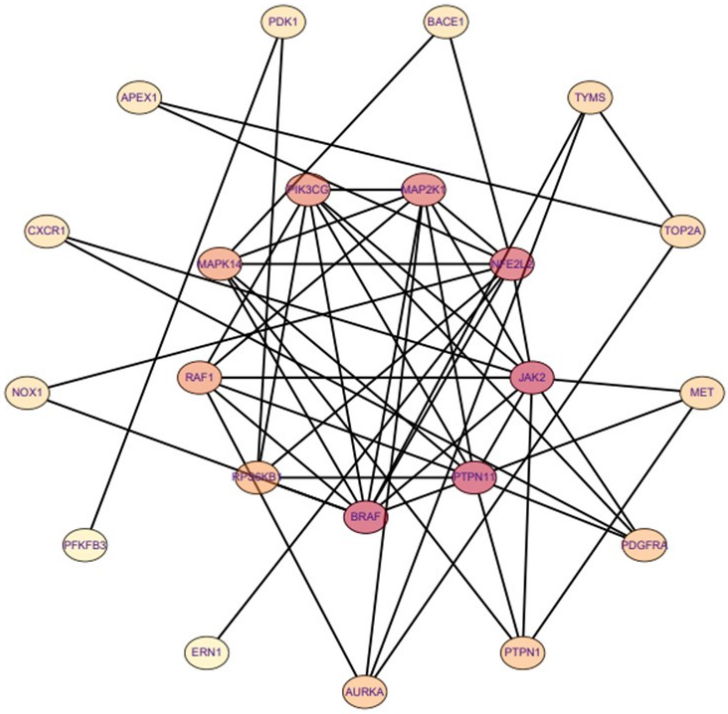
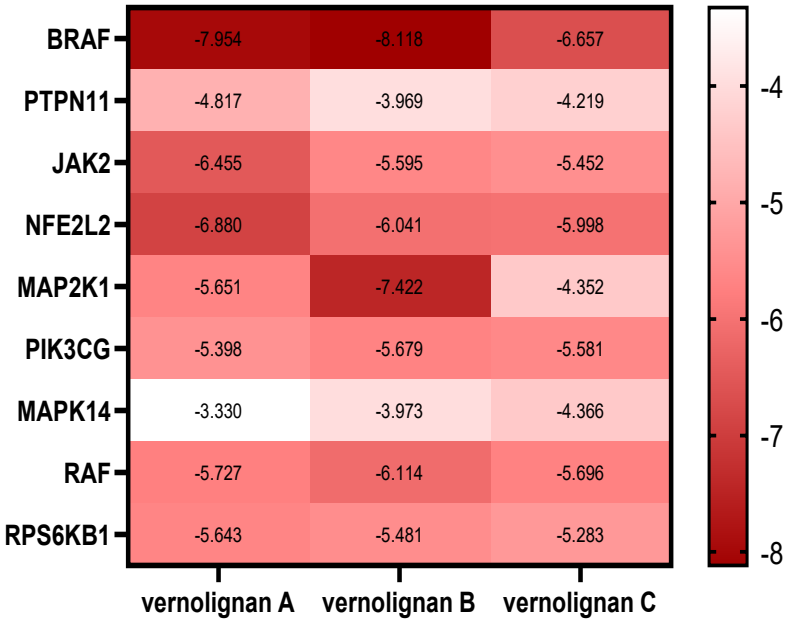


Fig. S34. The docking energy with potential drug targets of **1-3**.



ECD Computational Methods

Conformation search was performed by using Merk Molecular Force Field (MMFF) in Spartan'20. The low energy conformations were optimized by density functional theory (DFT) at B3LYP/6-31G (d,p) level in Gaussian 09. The ECD calculations were calculated at B3LYP/6-311G (2d,p) level for (8*R*, 8'*R*)-**1** based on Time-dependent density functional theory (TDDFT). The calculated ECD curves were averaged according to the Boltzmann distribution theory and their relative Gibbs free energy, which was generated using the SpecDis 1.62 and Origin Pro 9.0 software.

Fig. S35. B3LYP/6-31g (d,p) optimized lowest energy conformers for (8*R*, 8'*R*)-**1**.

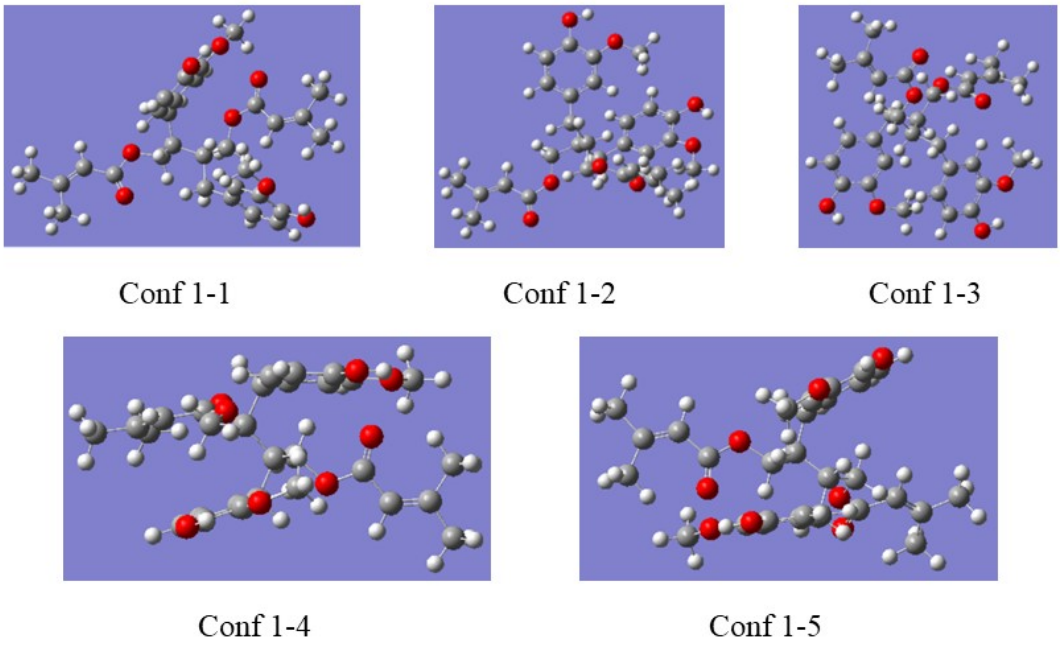


Table S1. Extracted heated and weighting factors of the optimized conformers of (8*R*, 8'*R*)-**1** at B3LYP/6-31g (d,p) level.

Compound	Conformer	Extracted heats(A.U.)	ΔG(kcal/mol)
(8*R*, 8'*R*)-**1**	1-1	-1767.842274	0.44524
	1-2	-1767.842951	0.02065
	1-2	-1767.842984	0
	1-4	-1767.842941	0.02064
	1-5	-1767.842949	0.02226

Table S2. The Cartesian coordinates of the lowest energy conformers for (8*R*, 8'*R*)-**1**.

Conf 1	**X axis(Å)**	**Y axis(Å)**	**Z axis(Å)**	**Conf 2**	**X axis(Å)**	**Y axis(Å)**	**Z axis(Å)**
C	2.63266	-2.71852	1.68401	C	3.24152	-0.06986	-2.36801
C	3.23191	-3.76895	0.96045	C	3.53621	1.27081	-2.68618
C	2.57072	-4.30228	-0.13346	C	2.49619	2.18214	-2.83459
C	1.32876	-3.79003	-0.52045	C	1.17265	1.76279	-2.66781
C	0.73016	-2.74365	0.17736	C	0.86666	0.43595	-2.34995
C	1.39782	-2.21762	1.29817	C	1.92175	-0.48258	-2.20358
C	-0.59440	-2.14728	-0.25085	C	-0.57310	0.00077	-2.15879
C	-0.47597	-0.68479	-0.76603	C	-1.18678	0.42388	-0.79541
C	-1.84046	0.05636	-0.71503	C	-0.50402	-0.24469	0.43058
C	0.22128	-0.68255	-2.13439	C	-2.71336	0.31797	-0.82827
O	0.71142	0.62450	-2.49820	O	-3.08746	-1.04533	-1.09356
C	2.00786	1.01730	-2.36050	C	-4.38010	-1.49292	-1.03192
O	2.28644	2.12967	-2.75515	O	-4.55118	-2.69343	-1.10034
C	2.96362	0.08775	-1.71741	C	-5.44627	-0.47277	-0.91449
C	4.29586	0.09241	-1.91460	C	-6.73532	-0.71286	-0.58953
C	5.16847	-0.88098	-1.16701	C	-7.71238	0.43400	-0.52857
C	5.03156	1.00044	-2.86041	C	-7.31898	-2.06119	-0.26567

C	-1.73221	1.59943	-0.78324	C	-0.84058	0.45676	1.77225
C	-0.92523	2.22887	0.33556	C	-0.53390	1.94076	1.83006
C	-2.82958	-0.45778	-1.77028	C	-0.76911	-1.75488	0.54534
O	-4.17473	-0.00430	-1.49999	O	0.07394	-2.33076	1.57531
C	0.19919	3.01427	0.03594	C	0.76718	2.41616	1.57387
C	0.94259	3.60409	1.05214	C	1.04778	3.77598	1.63961
C	0.58068	3.41531	2.39826	C	0.03473	4.70008	1.97325
C	-0.53386	2.64623	2.69955	C	-1.24836	4.23794	2.23072
C	-1.28103	2.06144	1.67513	C	-1.52611	2.86810	2.15463
C	-4.92415	-0.77441	-0.66487	C	1.17508	-3.02214	1.19578
C	-6.26178	-0.17004	-0.51745	C	1.85620	-3.54480	2.39799
O	-4.49971	-1.79106	-0.15718	O	1.51158	-3.16493	0.02880
C	-7.28333	-0.64483	0.21772	C	2.87365	-4.42910	2.43278
C	-8.58861	0.10554	0.25121	C	3.44123	-4.86010	3.76144
C	-7.25133	-1.90152	1.04187	C	3.51786	-5.06341	1.22910
O	1.31759	3.98930	3.39239	O	0.31946	6.03241	2.03620
O	2.05569	4.38875	0.88528	O	2.26809	4.36263	1.40726
C	2.43719	4.75321	-0.44258	C	3.33705	3.53865	0.95693
O	3.39002	-2.27757	2.73975	O	4.35756	-0.86317	-2.24994
O	4.45573	-4.24003	1.32865	O	4.83315	1.66522	-2.84995
C	2.87103	-1.23672	3.56308	C	4.16701	-2.26333	-2.06650
H	3.03856	-5.11495	-0.67605	H	2.73526	3.20831	-3.09740
H	0.82531	-4.21955	-1.37994	H	0.36945	2.48456	-2.79698
H	0.94139	-1.41070	1.85822	H	1.70034	-1.51113	-1.93897
H	-1.04323	-2.78595	-1.01816	H	-1.18747	0.45073	-2.95074
H	-1.29350	-2.15778	0.59063	H	-0.66175	-1.08207	-2.29096
H	0.18301	-0.15214	-0.07289	H	-0.99276	1.49971	-0.68611
H	-2.27440	-0.20191	0.25624	H	0.57527	-0.13645	0.26213

H	1.03389	-1.40897	-2.15117	H	-3.15281	0.64810	0.12002
H	-0.47048	-0.95372	-2.93476	H	-3.09604	0.96726	-1.62665
H	2.56089	-0.63998	-1.02387	H	-5.16499	0.55647	-1.11183
H	4.61302	-1.47100	-0.43769	H	-7.25596	1.38853	-0.80629
H	5.65691	-1.56984	-1.86536	H	-8.56313	0.24919	-1.19817
H	5.97160	-0.34642	-0.64719	H	-8.13053	0.53102	0.48246
H	5.62911	1.71834	-2.28587	H	-7.91371	-2.00129	0.65524
H	5.73781	0.41783	-3.46138	H	-8.01247	-2.36140	-1.06395
H	4.36749	1.56560	-3.50753	H	-6.55723	-2.83257	-0.17362
H	-1.30151	1.89044	-1.74358	H	-1.90211	0.30487	2.00938
H	-2.75259	1.99648	-0.76084	H	-0.28498	-0.06789	2.55966
H	-2.60573	-0.05402	-2.75880	H	-1.78903	-1.96503	0.87057
H	-2.83554	-1.54733	-1.81063	H	-0.58575	-2.27841	-0.39193
H	0.49067	3.14948	-0.99773	H	1.55441	1.71488	1.31811
H	-0.81262	2.52504	3.73985	H	-2.02055	4.95734	2.48546
H	-2.15885	1.47921	1.93439	H	-2.53565	2.52152	2.36295
H	-6.39504	0.74797	-1.07807	H	1.44802	-3.17383	3.33381
H	-8.56129	1.01112	-0.35625	H	2.96048	-4.35363	4.60310
H	-9.40453	-0.53094	-0.10937	H	3.32468	-5.94390	3.89700
H	-8.84340	0.38419	1.27989	H	4.51990	-4.65680	3.80457
H	-7.47019	-1.66084	2.08875	H	4.56006	-4.72486	1.14118
H	-8.04555	-2.58002	0.70950	H	3.55845	-6.15312	1.35809
H	-6.29507	-2.41179	0.98272	H	2.99341	-4.82449	0.30576
H	2.01360	4.50237	2.95963	H	1.26193	6.12633	1.81107
H	1.64166	5.32605	-0.93050	H	3.08045	3.03418	0.01783
H	3.32121	5.37983	-0.33405	H	4.18403	4.20726	0.79359
H	2.67439	3.87573	-1.04879	H	3.60598	2.79093	1.71399
H	4.72144	-3.75196	2.11971	H	5.37883	0.86626	-2.73998

H	3.62457	-1.05559	4.32732	H	5.16810	-2.69590	-2.01374
H	2.70700	-0.32004	2.98827	H	3.61673	-2.47635	-1.14419
H	1.93413	-1.54246	4.03952	H	3.62378	-2.69827	-2.91512
Conf 3	**X axis(Å)**	**Y axis(Å)**	**Z axis(Å)**	**Conf 4**	**X axis(Å)**	**Y axis(Å)**	**Z axis(Å)**
C	3.47226	1.64938	-2.16114	C	3.24150	-0.07000	-2.36783
C	3.01269	2.95596	-2.41780	C	3.53627	1.27067	-2.68589
C	1.65442	3.17459	-2.61775	C	2.49630	2.18207	-2.83429
C	0.75983	2.10281	-2.56579	C	1.17274	1.76278	-2.66761
C	1.20338	0.80193	-2.31100	C	0.86666	0.43593	-2.34986
C	2.57789	0.58372	-2.11028	C	1.92169	-0.48266	-2.20350
C	0.22299	-0.35263	-2.25380	C	-0.57313	0.00079	-2.15878
C	-0.61843	-0.43227	-0.94582	C	-1.18686	0.42393	-0.79544
C	0.25905	-0.67878	0.31495	C	-0.50406	-0.24454	0.43058
C	-1.74947	-1.44359	-1.17587	C	-2.71343	0.31790	-0.82832
O	-2.66639	-1.39408	-0.06712	O	-3.08744	-1.04544	-1.09351
C	-3.96756	-1.78933	-0.16462	C	-4.38002	-1.49314	-1.03166
O	-4.72694	-1.42371	0.71500	O	-4.55103	-2.69367	-1.09976
C	-4.32361	-2.66514	-1.30202	C	-5.44627	-0.47302	-0.91448
C	-5.57522	-2.93103	-1.73486	C	-6.73528	-0.71312	-0.58943
C	-5.77800	-3.87189	-2.89511	C	-7.71244	0.43369	-0.52876
C	-6.83814	-2.35480	-1.15511	C	-7.31884	-2.06141	-0.26527
C	-0.40331	-0.28208	1.66344	C	-0.84064	0.45695	1.77224
C	-0.94557	1.13184	1.73389	C	-0.53402	1.94096	1.82995
C	0.76347	-2.12753	0.38799	C	-0.76903	-1.75474	0.54541
O	1.74986	-2.28667	1.43199	O	0.07413	-2.33051	1.57533
C	-2.33190	1.34407	1.83875	C	0.76711	2.41631	1.57393
C	-2.84318	2.63549	1.91754	C	1.04770	3.77615	1.63947
C	-1.97988	3.74771	1.89232	C	0.03458	4.70029	1.97277

C	-0.60865	3.54346	1.78717	C	-1.24855	4.23820	2.23008
C	-0.09822	2.24413	1.71138	C	-1.52630	2.86834	2.15418
C	3.05982	-2.29547	1.07926	C	1.17515	-3.02210	1.19573
C	3.88323	-2.47615	2.29122	C	1.85633	-3.54470	2.39794
O	3.44354	-2.17325	-0.07529	O	1.51143	-3.16516	0.02874
C	5.21470	-2.68505	2.34343	C	2.87381	-4.42895	2.43271
C	5.89030	-2.85094	3.68087	C	3.44149	-4.85988	3.76135
C	6.12908	-2.77992	1.15152	C	3.51799	-5.06326	1.22900
O	-2.48980	5.01246	1.97253	O	0.31928	6.03264	2.03555
O	-4.17526	2.96861	2.01790	O	2.26805	4.36276	1.40724
C	-5.11254	1.91341	2.20113	C	3.33703	3.53873	0.95705
O	4.83326	1.56947	-1.97938	O	4.35748	-0.86339	-2.24973
O	3.89651	3.99391	-2.47268	O	4.83324	1.66504	-2.84957
C	5.40803	0.28024	-1.80343	C	4.16684	-2.26357	-2.06663
H	1.31331	4.18555	-2.81783	H	2.73546	3.20824	-3.09700
H	-0.29882	2.28988	-2.72926	H	0.36958	2.48460	-2.79677
H	2.93333	-0.41858	-1.89536	H	1.70023	-1.51121	-1.93893
H	-0.47589	-0.26171	-3.09698	H	-1.18744	0.45075	-2.95076
H	0.76222	-1.29563	-2.41016	H	-0.66178	-1.08205	-2.29094
H	-1.09596	0.54511	-0.80887	H	-0.99294	1.49977	-0.68619
H	1.14236	-0.04081	0.18638	H	0.57523	-0.13623	0.26213
H	-2.28130	-1.18497	-2.09848	H	-3.15292	0.64807	0.11994
H	-1.36129	-2.46531	-1.28686	H	-3.09614	0.96711	-1.62674
H	-3.49802	-3.14161	-1.82222	H	-5.16503	0.55619	-1.11209
H	-4.83767	-4.29424	-3.26092	H	-7.25605	1.38821	-0.80651
H	-6.44041	-4.69914	-2.60696	H	-8.56306	0.24873	-1.19850
H	-6.27317	-3.35511	-3.72818	H	-8.13079	0.53078	0.48218
H	-7.48168	-1.97517	-1.95933	H	-7.91390	-2.00132	0.65541

H	-7.40601	-3.14948	-0.65114	H	-8.01199	-2.36202	-1.06370
H	-6.64220	-1.56999	-0.42745	H	-6.55701	-2.83265	-0.17271
H	-1.21019	-0.98546	1.88781	H	-1.90216	0.30501	2.00938
H	0.35757	-0.42013	2.44169	H	-0.28498	-0.06765	2.55964
H	-0.04335	-2.80973	0.67227	H	-1.78893	-1.96495	0.87069
H	1.20381	-2.45684	-0.55531	H	-0.58572	-2.27828	-0.39186
H	-2.99955	0.48970	1.83916	H	1.55438	1.71497	1.31847
H	0.04707	4.40871	1.77195	H	-2.02077	4.95766	2.48455
H	0.97762	2.10439	1.63762	H	-2.53588	2.52180	2.36237
H	3.31694	-2.44874	3.21763	H	1.44818	-3.17367	3.33375
H	5.19115	-2.74871	4.51545	H	2.96084	-4.35332	4.60301
H	6.37201	-3.83565	3.75051	H	3.32491	-5.94366	3.89699
H	6.68837	-2.10633	3.80407	H	4.52018	-4.65662	3.80437
H	6.84570	-1.94653	1.16489	H	4.56001	-4.72426	1.14074
H	6.72658	-3.69944	1.21134	H	3.55909	-6.15292	1.35820
H	5.58403	-2.76007	0.20962	H	2.99322	-4.82473	0.30573
H	-3.45469	4.91392	2.05541	H	1.26178	6.12654	1.81054
H	-4.90451	1.35882	3.12497	H	3.08047	3.03413	0.01801
H	-6.09158	2.39146	2.27346	H	4.18401	4.20733	0.79366
H	-5.10346	1.21057	1.36059	H	3.60594	2.79109	1.71422
H	4.77882	3.61513	-2.31274	H	5.37888	0.86605	-2.73961
H	6.47936	0.44505	-1.67203	H	5.16790	-2.69623	-2.01407
H	4.99674	-0.22681	-0.92387	H	3.61661	-2.47679	-1.14433
H	5.23970	-0.34894	-2.68706	H	3.62349	-2.69826	-2.91532

Conf 5	**X axis(Å)**	**Y axis(Å)**	**Z axis(Å)**
C	-2.80944	-0.12967	2.68488
C	-3.00489	1.20081	3.10535
C	-1.92634	2.07881	3.11660

C	-0.66303	1.63684	2.71153
C	-0.45594	0.31995	2.28963
C	-1.54925	-0.56480	2.28314
C	0.91692	-0.14160	1.84142
C	1.31018	0.33011	0.41436
C	0.40692	-0.25388	-0.70775
C	2.81554	0.17025	0.18502
O	3.17356	-1.21526	0.33289
C	4.46783	-1.64525	0.45495
O	4.63775	-2.81837	0.71803
C	5.53902	-0.64991	0.22370
C	6.83717	-0.79927	0.56540
C	7.82315	0.29411	0.23981
C	7.42266	-1.99249	1.27068
C	0.52992	0.51520	-2.04936
C	0.25502	2.00460	-1.97617
C	0.60408	-1.76044	-0.94243
O	-0.40913	-2.26244	-1.85017
C	-0.97827	2.48275	-1.49143
C	-1.23226	3.84793	-1.43099
C	-0.26145	4.77567	-1.86489
C	0.95386	4.31122	-2.34822
C	1.20663	2.93530	-2.39797
C	-1.46467	-2.93312	-1.32960
C	-2.34704	-3.37502	-2.42892
O	-1.61947	-3.11884	-0.13086
C	-3.39511	-4.21848	-2.33710
C	-4.18414	-4.56428	-3.57447

C	-3.86958	-4.88336	-1.07258
O	-0.51976	6.11347	-1.80350
O	-2.38660	4.43668	-0.97464
C	-3.38737	3.60307	-0.40219
O	-3.95344	-0.89053	2.72169
O	-4.24352	1.61811	3.50078
C	-3.83932	-2.28380	2.44614
H	-2.08581	3.09662	3.45984
H	0.17268	2.33236	2.73586
H	-1.40828	-1.58440	1.94060
H	1.66929	0.25349	2.53788
H	0.99023	-1.23144	1.90962
H	1.14414	1.41570	0.38539
H	-0.62343	-0.13155	-0.34990
H	3.09631	0.52183	-0.81567
H	3.35922	0.77160	0.92348
H	5.25453	0.27379	-0.26997
H	7.36479	1.12213	-0.30871
H	8.27301	0.69404	1.15859
H	8.65150	-0.10361	-0.36185
H	8.06133	-2.55358	0.57385
H	8.07276	-1.66197	2.09108
H	6.65893	-2.67176	1.64393
H	1.53194	0.35970	-2.47133
H	-0.16398	0.04469	-2.75716
H	1.55241	-1.97253	-1.43865
H	0.55791	-2.33037	-0.01541
H	-1.73320	1.77820	-1.15894

H	1.69403	5.03351	-2.67864
H	2.16237	2.58765	-2.78322
H	-2.07555	-2.97558	-3.40190
H	-3.82077	-4.03654	-4.46074
H	-4.13917	-5.64392	-3.77151
H	-5.24589	-4.31877	-3.43582
H	-3.18979	-4.71786	-0.23879
H	-4.86273	-4.49945	-0.79900
H	-3.99181	-5.96155	-1.24029
H	-1.41312	6.20728	-1.42817
H	-2.99242	3.03892	0.45095
H	-4.17922	4.27309	-0.06287
H	-3.79546	2.90688	-1.14616
H	-4.82526	0.83898	3.44877
H	-4.84771	-2.69065	2.54476
H	-3.45931	-2.46300	1.43508
H	-3.17452	-2.77226	3.16999