

Supplementary Information

20-nor-Isopimarane and isopimarane diterpenoids produced by *Aspergillus* sp. WT03

Qiaoqiao Tao,^a Yining Sang,^a Tao Han,^a Hongling Zhou,^a Peng Zhang,^{*a} and Xiaofeng Cai^{*a,b}

^aSchool of Pharmacy, Tongji Medical College of Huazhong University of Science and Technology, Hubei Key Laboratory of Natural Medicinal Chemistry and Resource Evaluation, Wuhan 430030, People's Republic of China.

^bState Key Laboratory of Dao-di Herbs, Beijing 100700, P. R. China.

*Corresponding authors:

E-mail: zhangpeng@hust.edu.cn, caixiaofeng@hust.edu.cn

Contents

Table S1 Energy analysis of 2a/2b at B3LYP/6-311G* level in methanol.....	4
Table S2 Cartesian coordinates of low-energy conformers of 2a/2b optimized at B3LYP/6-311G* level in methanol.....	4
Table S3 Energy analysis of 4a/4b at B3LYP/6-311G* level in methanol.....	5
Table S4 Cartesian coordinates of low-energy conformers of 4a/4b optimized at B3LYP/6-311G* level in methanol.....	6
Fig. S1 Cell viability of cancer cells.....	7
Fig. S2 The morphology and quantity changes of compounds-incubated MCF-7 cells.....	8
Fig. S3 The morphology and quantity changes of compounds-incubated A549 cells.....	8
Fig. S4 The morphology and quantity changes of compounds-incubated HT-29 cells.....	9
Fig. S5 ¹ H-NMR (600 MHz, CD ₃ OD) spectrum of compound 1	10
Fig. S6 ¹³ C-NMR (150 MHz, CD ₃ OD) spectrum of compound 1	11
Fig. S7 DEPT-135 (150 MHz, CD ₃ OD) spectrum of compound 1	12
Fig. S8 HSQC spectrum (CD ₃ OD) of compound 1	13
Fig. S9 ¹ H- ¹ H COSY spectrum (CD ₃ OD) of compound 1	14
Fig. S10 HMBC spectrum (CD ₃ OD) of compound 1	15
Fig. S11 NOESY spectrum (CD ₃ OD) of compound 1	16
Fig. S12 HRTOFMS spectrum of compound 1	17
Fig. S13 IR spectrum of compound 1	18
Fig. S14 UV spectrum of compound 1	19
Fig. S15 CD spectrum of compound 1	20
Fig. S16 ¹ H-NMR (600 MHz, CDCl ₃) spectrum of compound 2	21
Fig. S17 ¹³ C-NMR (150 MHz, CDCl ₃) spectrum of compound 2	22
Fig. S18 DEPT-135 (150 MHz, CDCl ₃) spectrum of compound 2	23
Fig. S19 HSQC spectrum (CDCl ₃) of compound 2	24
Fig. S20 ¹ H- ¹ H COSY spectrum (CDCl ₃) of compound 2	25
Fig. S21 HMBC spectrum (CDCl ₃) of compound 2	26
Fig. S22 NOESY spectrum (CDCl ₃) of compound 2	27
Fig. S23 HRTOFMS spectrum of compound 2	28
Fig. S24 IR spectrum of compound 2	29
Fig. S25 UV spectrum of compound 2	30
Fig. S26 CD spectrum of compound 2	31
Fig. S27 ¹ H-NMR (600 MHz, CD ₃ OD) spectrum of compound 3	32
Fig. S28 ¹³ C-NMR (150 MHz, CD ₃ OD) spectrum of compound 3	33
Fig. S29 DEPT-135 (150 MHz, CD ₃ OD) spectrum of compound 3	34
Fig. S30 HSQC spectrum (CD ₃ OD) of compound 3	35
Fig. S31 ¹ H- ¹ H COSY spectrum (CD ₃ OD) of compound 3	36
Fig. S32 HMBC spectrum (CD ₃ OD) of compound 3	37
Fig. S33 NOESY spectrum (CD ₃ OD) of compound 3	38
Fig. S34 HRTOFMS spectrum of compound 3	39
Fig. S35 IR spectrum of compound 3	40

Fig. S36 UV spectrum of compound 3	41
Fig. S37 CD spectrum of compound 3	42
Fig. S38 ^1H -NMR (600 MHz, CD_3OD) spectrum of compound 4	43
Fig. S39 ^{13}C -NMR (150 MHz, CD_3OD) spectrum of compound 4	44
Fig. S40 DEPT-135 (150 MHz, CD_3OD) spectrum of compound 4	45
Fig. S41 HSQC spectrum (CD_3OD) of compound 4	46
Fig. S42 ^1H - ^1H COSY spectrum (CD_3OD) of compound 4	47
Fig. S43 HMBC spectrum (CD_3OD) of compound 4	48
Fig. S44 NOESY spectrum (CD_3OD) of compound 4	49
Fig. S45 HRTOFMS spectrum of compound 4	50
Fig. S46 IR spectrum of compound 4	51
Fig. S47 UV spectrum of compound 4	52
Fig. S48 CD spectrum of compound 4	53
Fig. S49 ^1H -NMR (600 MHz, CD_3OD) spectrum of compound 5	54
Fig. S50 ^{13}C -NMR (150 MHz, CD_3OD) spectrum of compound 5	55
Fig. S51 DEPT-135 (150 MHz, CD_3OD) spectrum of compound 5	56
Fig. S52 HSQC spectrum (CD_3OD) of compound 5	57
Fig. S53 ^1H - ^1H COSY spectrum (CD_3OD) of compound 5	58
Fig. S54 HMBC spectrum (CD_3OD) of compound 5	59
Fig. S55 NOESY spectrum (CD_3OD) of compound 5	60
Fig. S56 HRTOFMS spectrum of compound 5	61
Fig. S57 IR spectrum of compound 5	62
Fig. S58 UV spectrum of compound 5	63
Fig. S59 CD spectrum of compound 5	64

Table S1 Energy analysis of **2a/2b** at B3LYP/6-311G* level in methanol

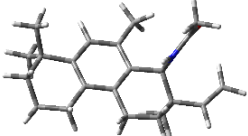
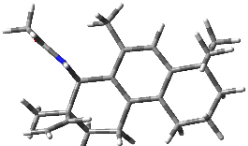
Configuration	Conformer	E (Hartree)	Boltzmann distribution
13R,14S- 2a		-987.046414	100%
13S,14R- 2b		-987.045641	100%

Table S2 Cartesian coordinates of low-energy conformers of **2a/2b** optimized at B3LYP/6-311G* level in methanol

Atom	Conformer 13R,14S- 2a			Conformer 13S,14R- 2b		
	X	Y	Z	X	Y	Z
C	4.150623	-1.752824	-0.601047	4.143683	-1.75831	0.54898
C	4.624287	-0.329831	-0.870221	4.7773	-0.530193	-0.093273
C	3.93163	0.710893	0.037565	3.9594	0.759361	0.143417
C	2.41996	0.4311	0.142453	2.454457	0.500726	-0.058431
C	1.824363	-0.7539	-0.312089	1.898477	-0.782761	-0.148194
C	2.658401	-1.876159	-0.906417	2.771606	-2.023685	-0.069345
C	1.600252	1.393486	0.745221	1.588001	1.592065	-0.163494
C	0.224999	1.252668	0.870009	0.211255	1.472415	-0.313641
C	-0.380119	0.08885	0.350943	-0.357475	0.177795	-0.354263
C	0.418885	-0.912182	-0.219026	0.503959	-0.934246	-0.339954
C	-1.906198	-0.029003	0.350208	-1.884122	0.016758	-0.476739
C	-2.385131	-1.505736	0.440809	-2.409613	-1.454565	-0.337603
C	-1.724189	-2.270953	-0.723126	-1.451018	-2.352121	-1.144298
C	-0.193061	-2.224402	-0.685412	-0.029897	-2.333389	-0.592871
C	-0.563479	2.329838	1.580996	-0.583202	2.750896	-0.477617
C	4.189146	2.104845	-0.574979	4.480711	1.824137	-0.845609
C	4.560438	0.672582	1.450014	4.194411	1.271435	1.583694
N	-2.412588	0.654671	-0.851272	-2.603366	0.880486	0.459769
C	-3.251603	1.728016	-0.842116	-3.591936	1.742794	0.083225
C	-3.591443	2.29929	-2.209656	-4.293429	2.465067	1.222513
O	-3.728384	2.20374	0.179143	-3.884584	1.957708	-1.083018
C	-3.89117	-1.685267	0.356527	-2.38311	-1.8693	1.123439
C	-4.85398	-0.814496	0.644862	-3.400507	-2.315809	1.856824
C	-1.946869	-2.097672	1.802914	-3.816735	-1.552375	-0.940642
H	4.714052	-2.467441	-1.209822	4.786027	-2.635002	0.416308
H	4.338603	-2.02541	0.443279	4.042394	-1.613311	1.630178
H	4.417391	-0.084078	-1.919947	4.863373	-0.704979	-1.173545
H	5.709285	-0.245738	-0.744402	5.797987	-0.376911	0.274361

H	2.51524	-1.898914	-1.996758	2.91006	-2.437518	-1.078993
H	2.294512	-2.843663	-0.548406	2.261703	-2.808378	0.497154
H	2.052406	2.297885	1.139129	1.999889	2.59559	-0.137782
H	-2.320992	0.526868	1.189999	-2.190637	0.372364	-1.464499
H	-2.052009	-3.315925	-0.715499	-1.83282	-3.377905	-1.160337
H	-2.095075	-1.856337	-1.667638	-1.446585	-2.002149	-2.18391
H	0.194334	-2.489364	-1.674625	0.628535	-2.84287	-1.30327
H	0.169122	-3.020509	-0.022102	0.022186	-2.93711	0.32181
H	-1.428353	2.680189	1.016713	-1.398561	2.658322	-1.196037
H	-0.949051	1.970001	2.541706	-1.02698	3.083103	0.465308
H	0.072216	3.191812	1.793191	0.07418	3.551154	-0.82538
H	3.695297	2.207271	-1.545021	4.248539	1.55217	-1.878702
H	3.837551	2.916359	0.0655	4.06135	2.814759	-0.658312
H	5.262987	2.254122	-0.726895	5.568204	1.913303	-0.757755
H	4.097726	1.410824	2.109452	3.634379	2.191064	1.771311
H	4.440955	-0.304064	1.924829	3.877902	0.542038	2.333041
H	5.631228	0.895623	1.399609	5.255561	1.486634	1.74751
H	-1.981276	0.406877	-1.727001	-2.471766	0.700301	1.442573
H	-4.651043	2.119841	-2.406659	-4.003562	2.120259	2.217605
H	-3.44376	3.380102	-2.186187	-4.079773	3.533682	1.144268
H	-3.008838	1.875867	-3.030954	-5.371419	2.342829	1.104035
H	-4.184658	-2.699838	0.081734	-1.414619	-1.779964	1.613143
H	-5.896104	-1.115635	0.591831	-3.258703	-2.593717	2.8967
H	-4.665229	0.215842	0.92414	-4.404259	-2.433703	1.463358
H	-2.419706	-1.550634	2.62202	-4.530707	-0.902747	-0.431878
H	-2.260206	-3.142623	1.884409	-3.802142	-1.258865	-1.992541
H	-0.865872	-2.05679	1.949549	-4.19279	-2.577675	-0.886661

Table S3 Energy analysis of **4a/4b** at B3LYP/6-311G* level in methanol

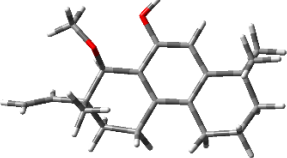
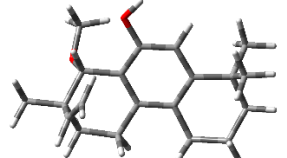
Configuration	Conformer	E (Hartree)	Boltzmann distribution
13 <i>R</i> ,14 <i>R</i> - 4a		-929.444494	100%
13 <i>S</i> ,14 <i>S</i> - 4b		-929.449138	100%

Table S4 Cartesian coordinates of low-energy conformers of **4a/4b** optimized at B3LYP/6-311G* level in methanol

Atom	Conformer 13R,14R-4a			Conformer 13S,14S-4b		
	X	Y	Z	X	Y	Z
C	1.573441	-3.923518	0.361713	0.815847	-3.383789	0.192528
C	2.483319	-3.696937	-0.842572	1.803251	-3.289422	-0.966844
C	3.070081	-2.266507	-0.888115	2.45918	-1.893343	-1.076542
C	1.990741	-1.217692	-0.555703	1.409445	-0.778299	-0.899541
C	0.7391	-1.548272	-0.008923	0.101906	-1.017717	-0.436783
C	0.361905	-2.992296	0.282872	-0.345732	-2.412149	-0.027233
C	2.270119	0.133348	-0.801347	1.785616	0.540839	-1.187615
C	1.357882	1.136943	-0.50274	0.885849	1.591858	-1.070751
C	0.113465	0.832435	0.082832	-0.445648	1.364538	-0.686687
C	-0.199721	-0.522448	0.268753	-0.819525	0.055845	-0.347301
C	-0.824871	1.985425	0.44461	-1.441687	2.516822	-0.672334
C	-2.110029	1.548103	1.219674	-2.551202	2.325629	0.385431
C	-2.606552	0.227406	0.585278	-3.2006	0.951993	0.099928
C	-1.592395	-0.907804	0.733672	-2.206348	-0.210165	0.216294
C	3.643424	-2.045921	-2.306503	3.125381	-1.801455	-2.468505
O	1.631401	2.445859	-0.808331	1.270275	2.892046	-1.314576
C	-3.194286	2.588347	1.003687	-2.026766	2.373491	1.809251
C	-3.892852	3.24914	1.928655	-0.808004	2.700257	2.241843
O	-0.095227	2.935718	1.223413	-2.12452	2.622458	-1.933863
C	-0.221905	4.28305	0.806889	-1.353359	3.102073	-3.016554
C	-1.795527	1.347077	2.712095	-3.614742	3.439454	0.253022
C	4.226551	-2.14215	0.134119	3.556194	-1.743395	0.00582
H	1.238898	-4.967546	0.39972	0.431484	-4.406625	0.288934
H	2.123996	-3.738197	1.293256	1.320776	-3.15101	1.139117
H	1.902362	-3.876224	-1.759198	1.268464	-3.509541	-1.90231
H	3.310525	-4.418584	-0.853192	2.593473	-4.046012	-0.875153
H	-0.321448	-3.363937	-0.498373	-1.017145	-2.824789	-0.797672
H	-0.204745	-3.051236	1.219712	-0.949462	-2.357526	0.886192
H	3.223274	0.418447	-1.242811	2.802041	0.757913	-1.51065
H	-1.138974	2.465735	-0.496074	-0.909978	3.459275	-0.486736
H	-3.56138	-0.060306	1.04204	-4.038817	0.78141	0.788298
H	-2.808318	0.394951	-0.482746	-3.617298	0.98718	-0.911842
H	-1.942557	-1.770812	0.154377	-2.640811	-1.101239	-0.253571
H	-1.566687	-1.250485	1.777984	-2.08387	-0.469395	1.279095
H	2.846032	-2.034669	-3.058044	2.375022	-1.824773	-3.266658
H	4.202533	-1.108307	-2.392906	3.718417	-0.889014	-2.590523
H	4.335374	-2.859249	-2.558134	3.802782	-2.652038	-2.614527
H	2.499672	2.480571	-1.238884	2.213206	2.894551	-1.540355
H	-3.433455	2.769187	-0.047508	-2.790822	2.138886	2.554459
H	-4.672467	3.951931	1.646267	-0.583152	2.728729	3.305122

H	-3.721126	3.124027	2.993643	0.011549	2.94503	1.572576
H	0.387209	4.876933	1.495124	-2.046826	3.233612	-3.852721
H	0.160195	4.421556	-0.213422	-0.879526	4.066891	-2.78424
H	-1.260688	4.636028	0.860935	-0.567549	2.395071	-3.31613
H	-1.536302	2.296421	3.184136	-4.392292	3.317566	1.016928
H	-2.659354	0.917574	3.233092	-3.161022	4.427478	0.392754
H	-0.94246	0.677652	2.853343	-4.085694	3.412014	-0.732154
H	4.658035	-1.135448	0.119533	4.040718	-0.762754	-0.055571
H	3.886654	-2.336406	1.156716	3.146382	-1.841633	1.016404
H	5.025837	-2.85643	-0.10172	4.330501	-2.510095	-0.124554

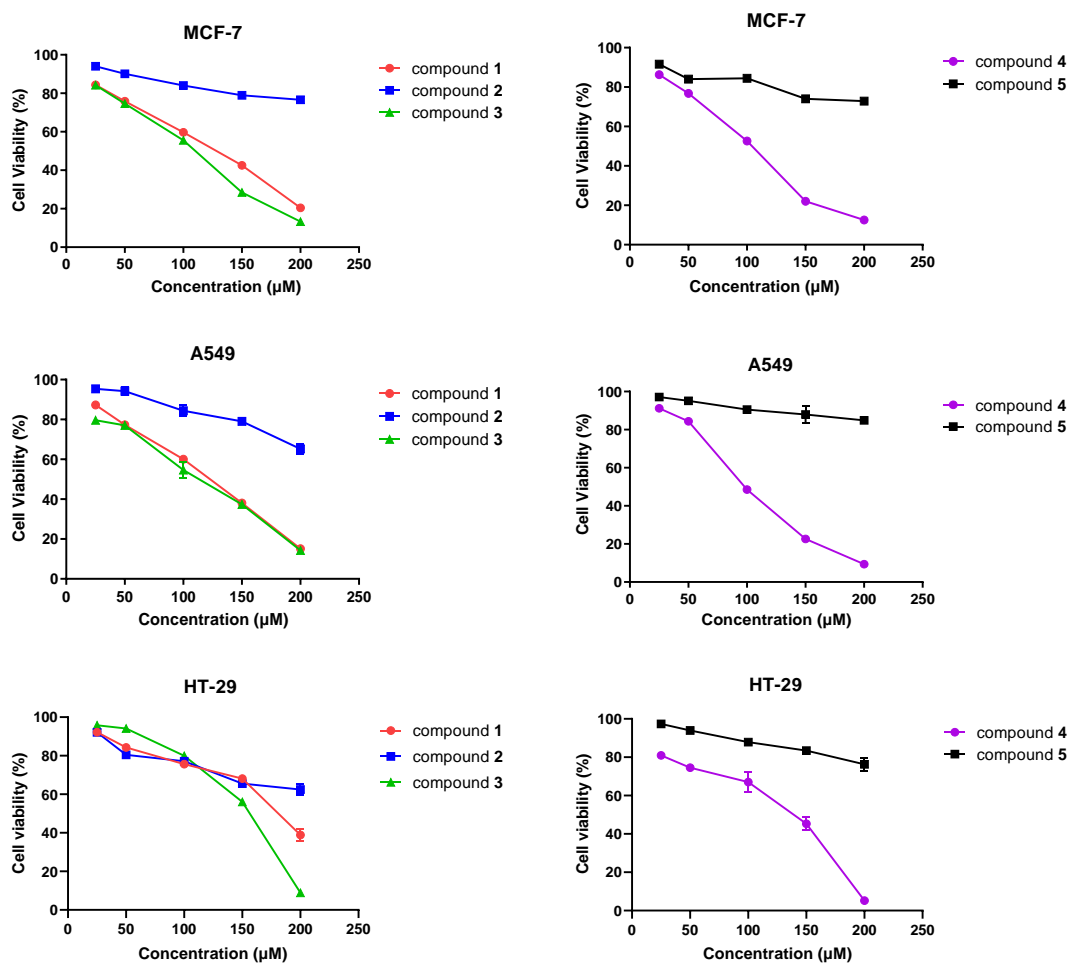
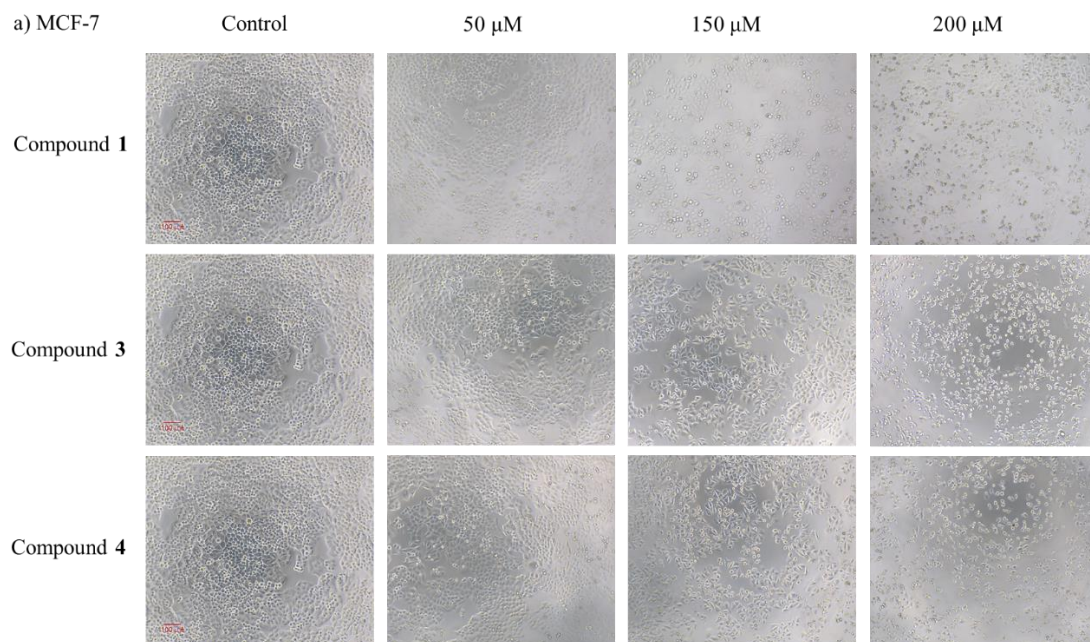
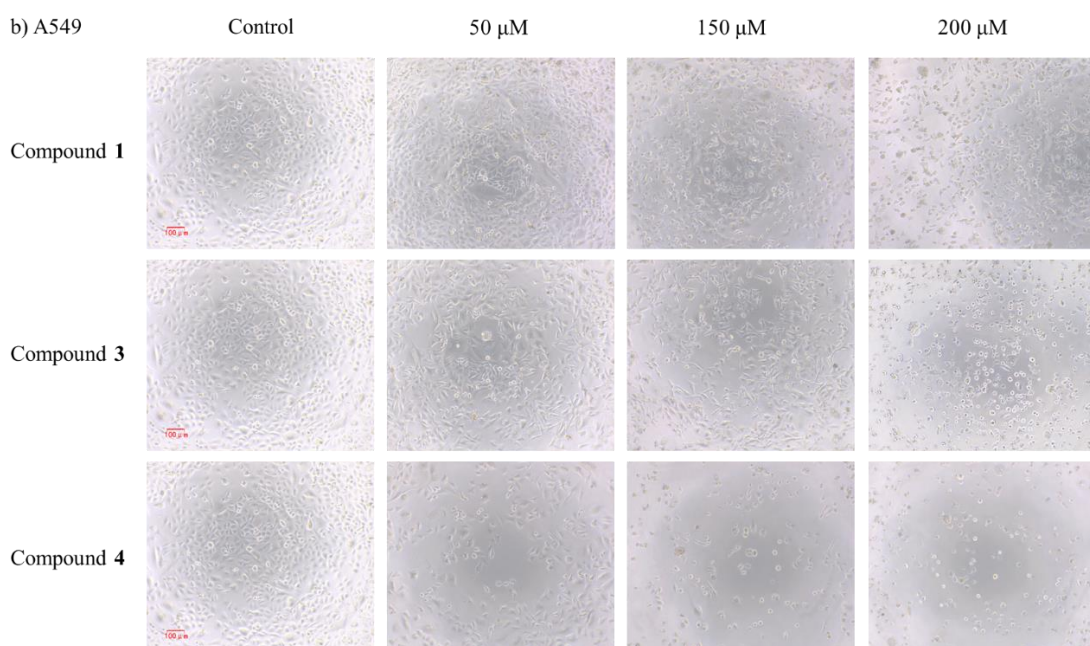


Fig. S1 Cell viability of cancer cells. Each dot represents a mean value and SEM of three dependent experiments (n = 3).



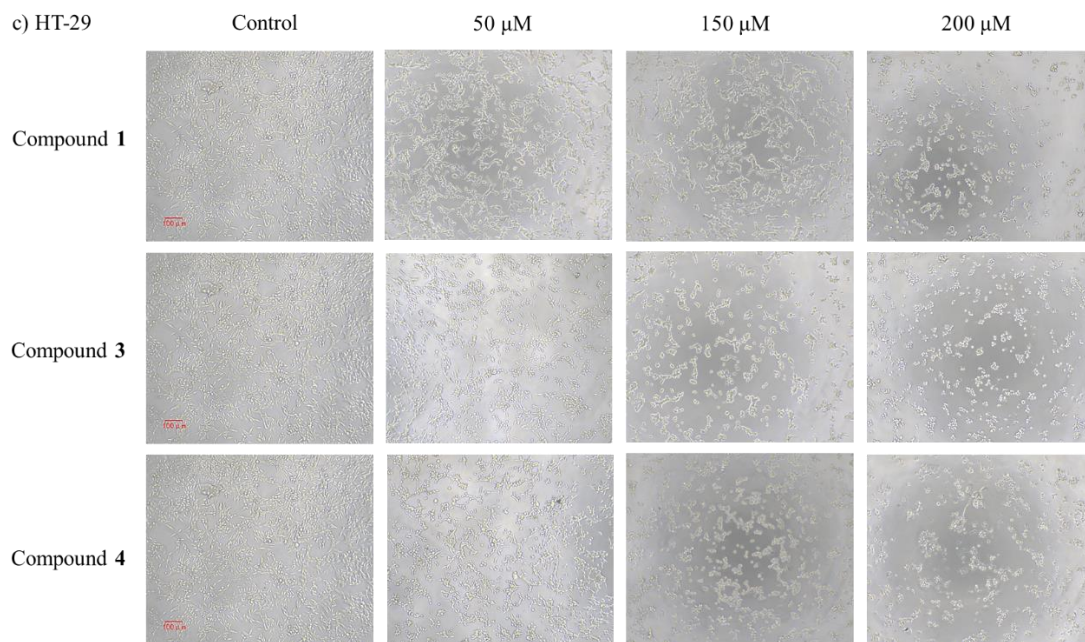
Scale bar: 100 μm

Fig. S2 The morphology and quantity changes of compounds-incubated MCF-7 cells.



Scale bar: 100 μm

Fig. S3 The morphology and quantity changes of compounds-incubated A549 cells.



Scale bar: 100 μm

Fig. S4 The morphology and quantity changes of compounds-incubated HT-29 cells.

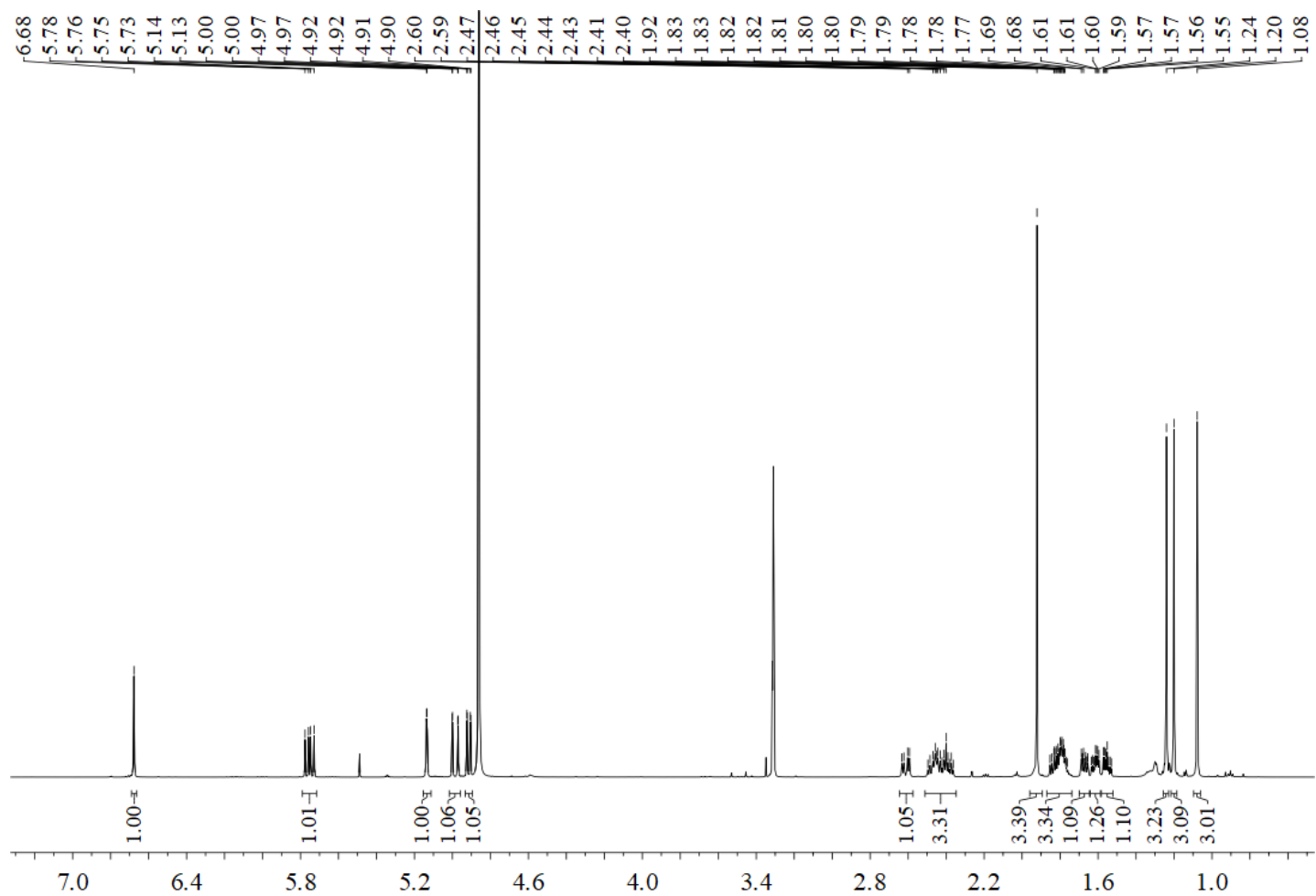


Fig. S5 ¹H-NMR (600 MHz, CD₃OD) spectrum of compound 1.

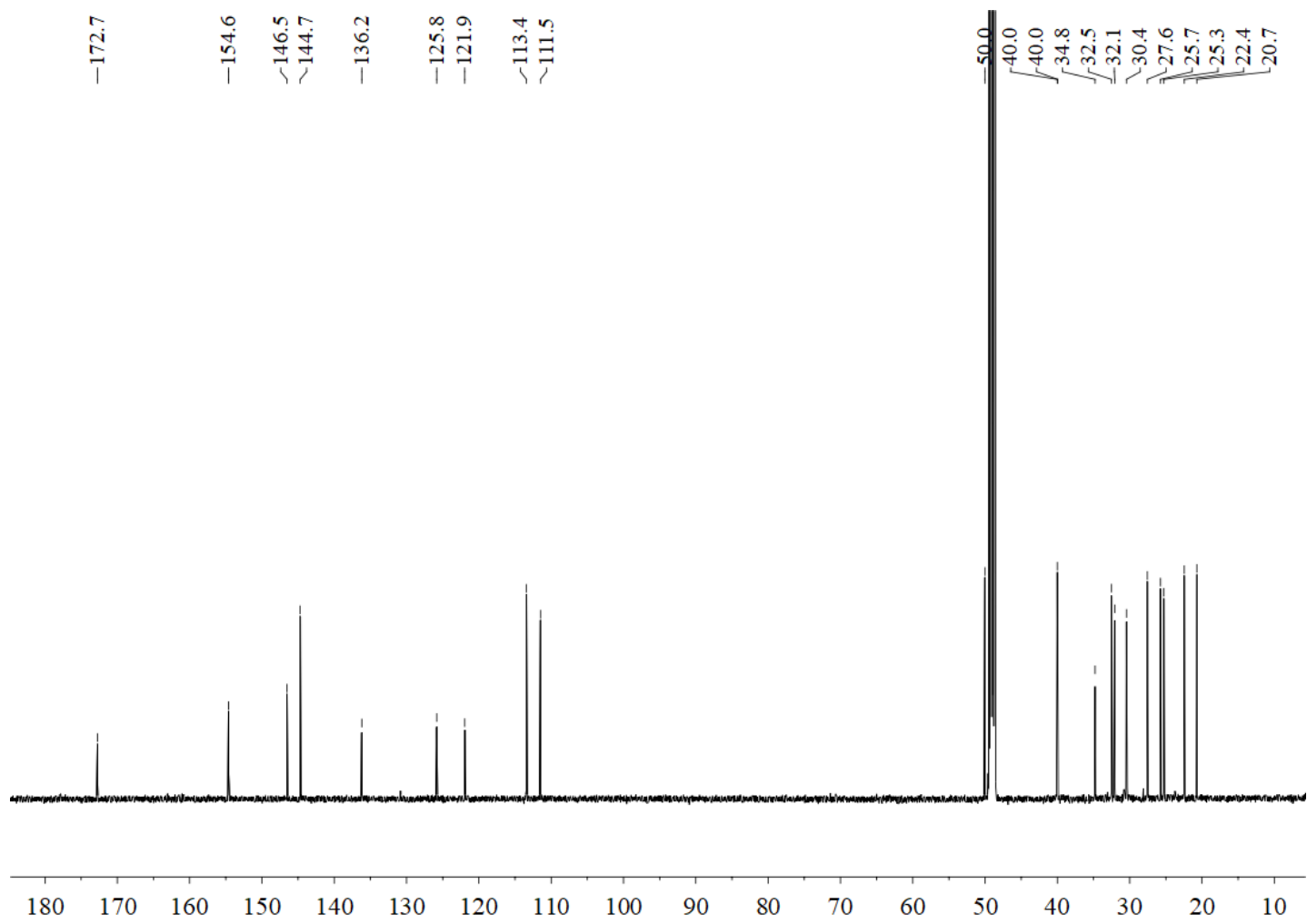


Fig. S6 ^{13}C -NMR (150 MHz, CD_3OD) spectrum of compound 1.

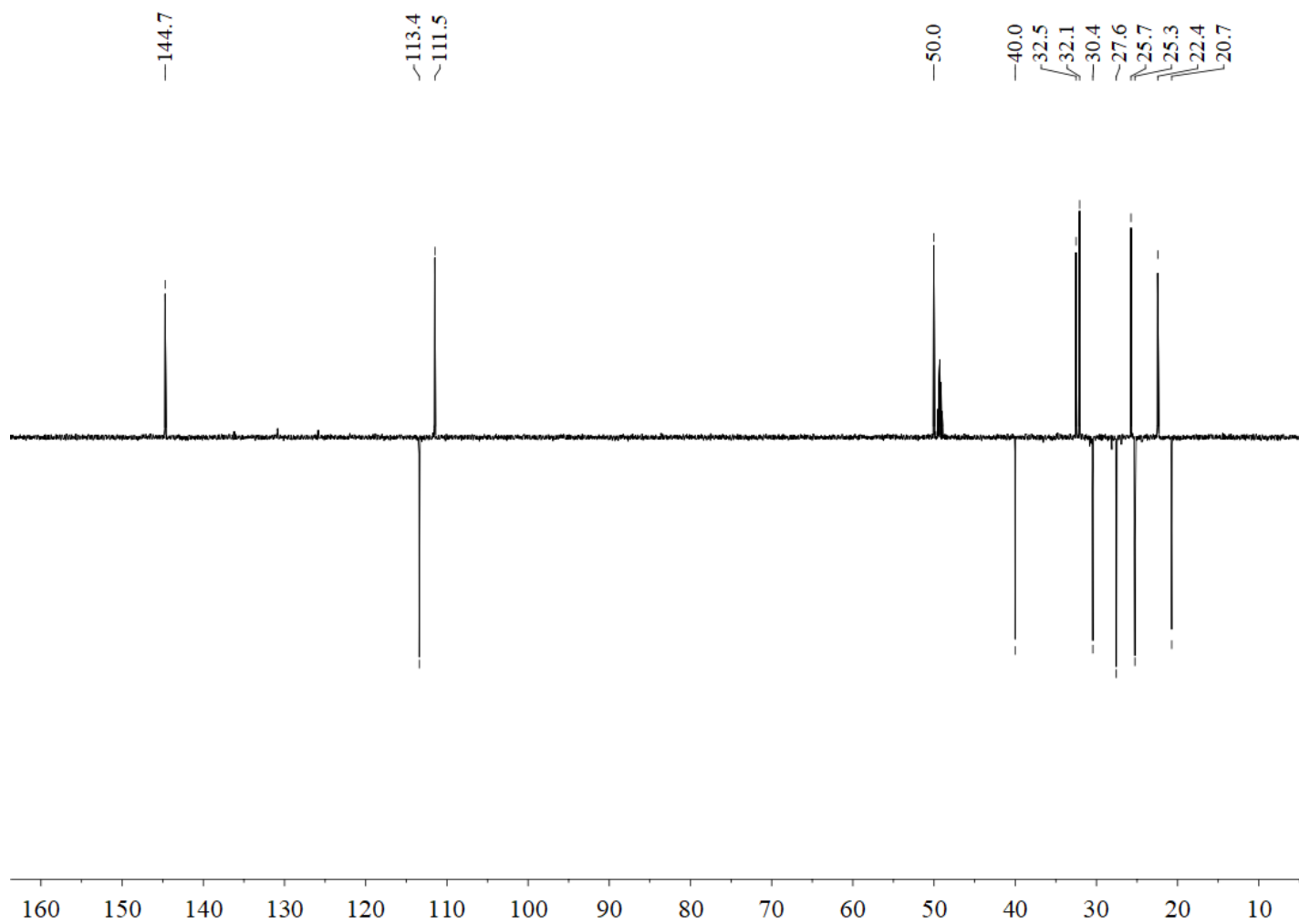


Fig. S7 DEPT-135 (150 MHz, CD₃OD) spectrum of compound **1**.

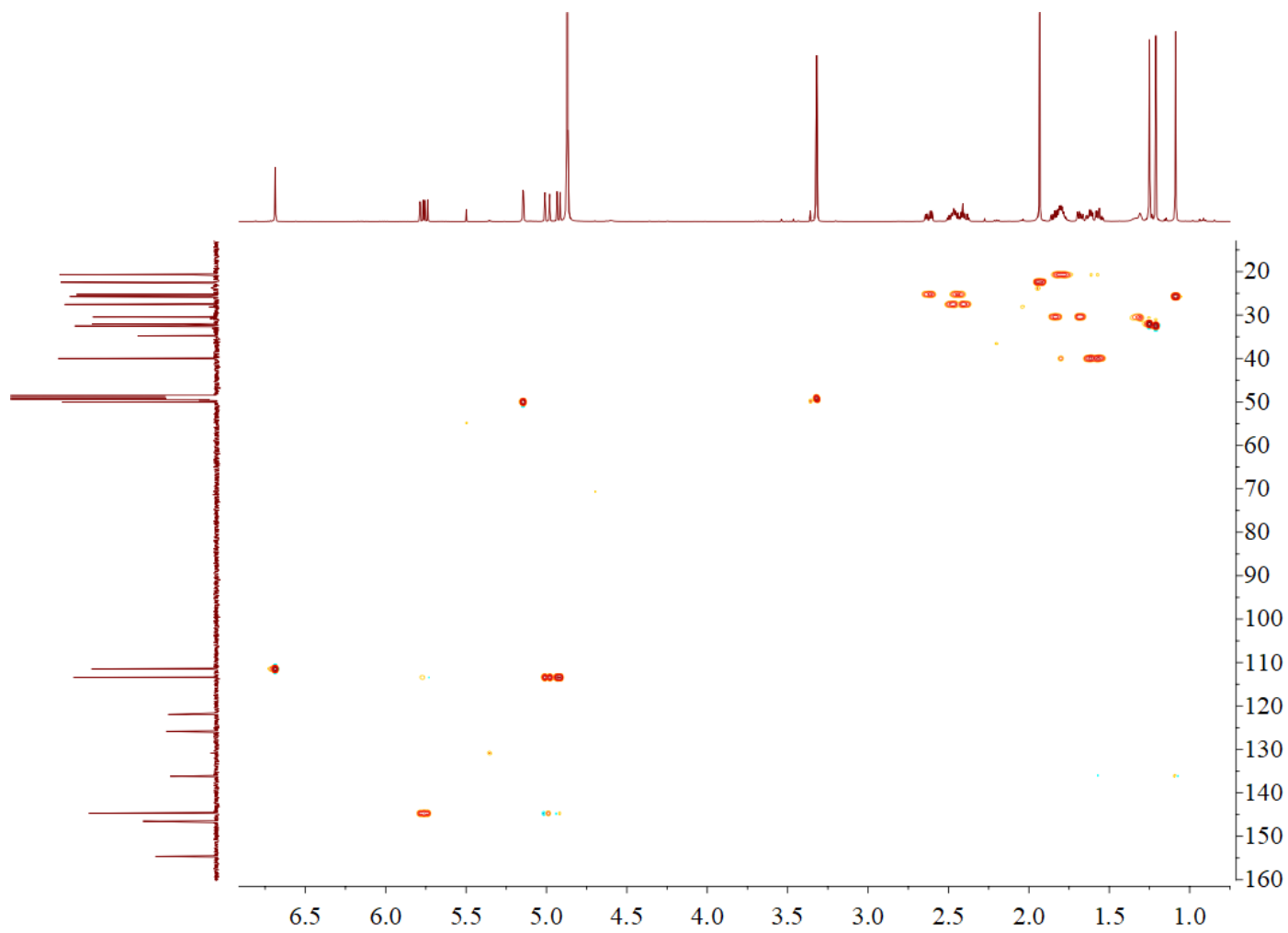


Fig. S8 HSQC spectrum (CD₃OD) of compound **1**.

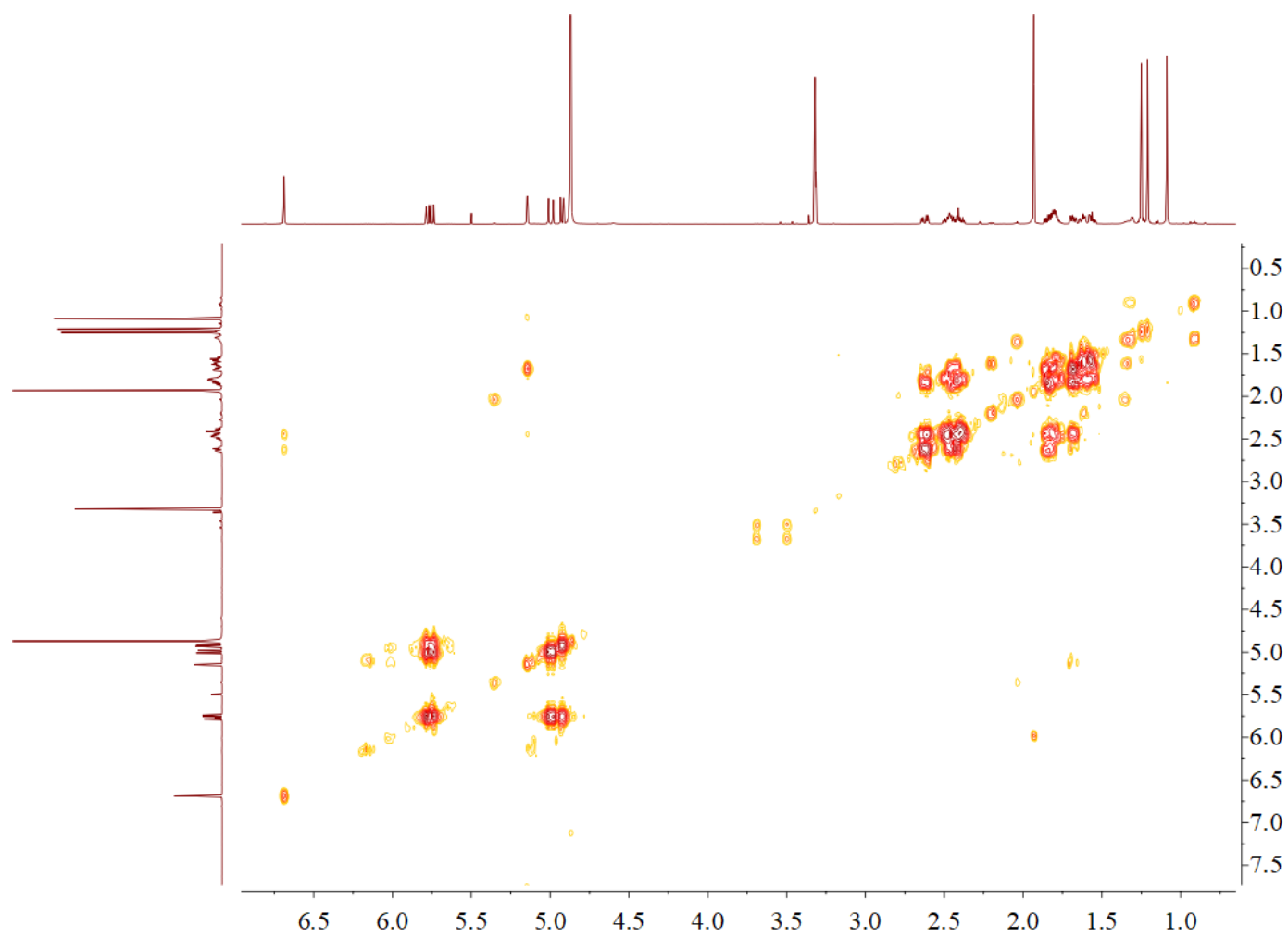


Fig. S9 ^1H - ^1H COSY spectrum (CD_3OD) of compound **1**.

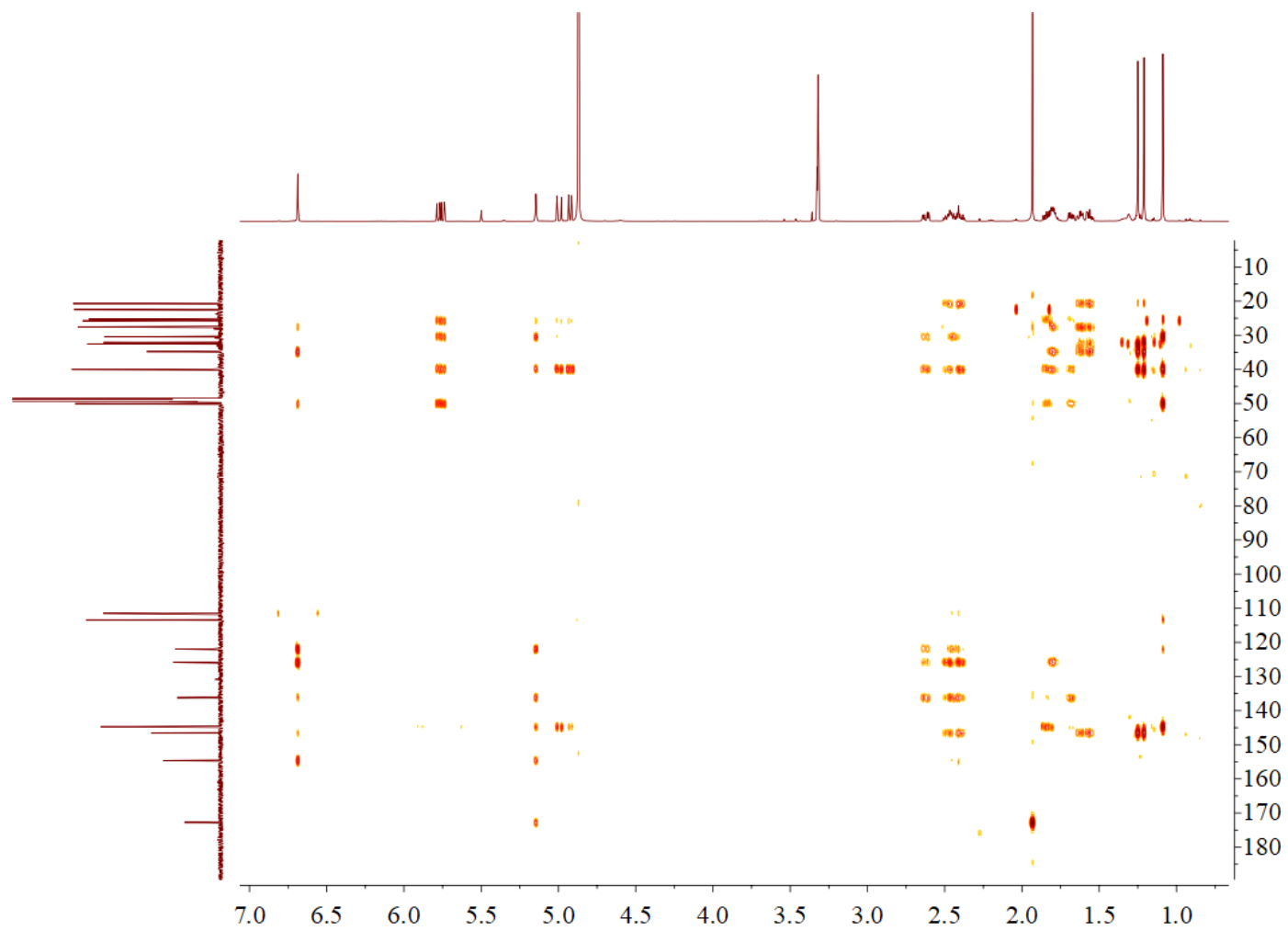


Fig. S10 HMBC spectrum (CD₃OD) of compound **1**.

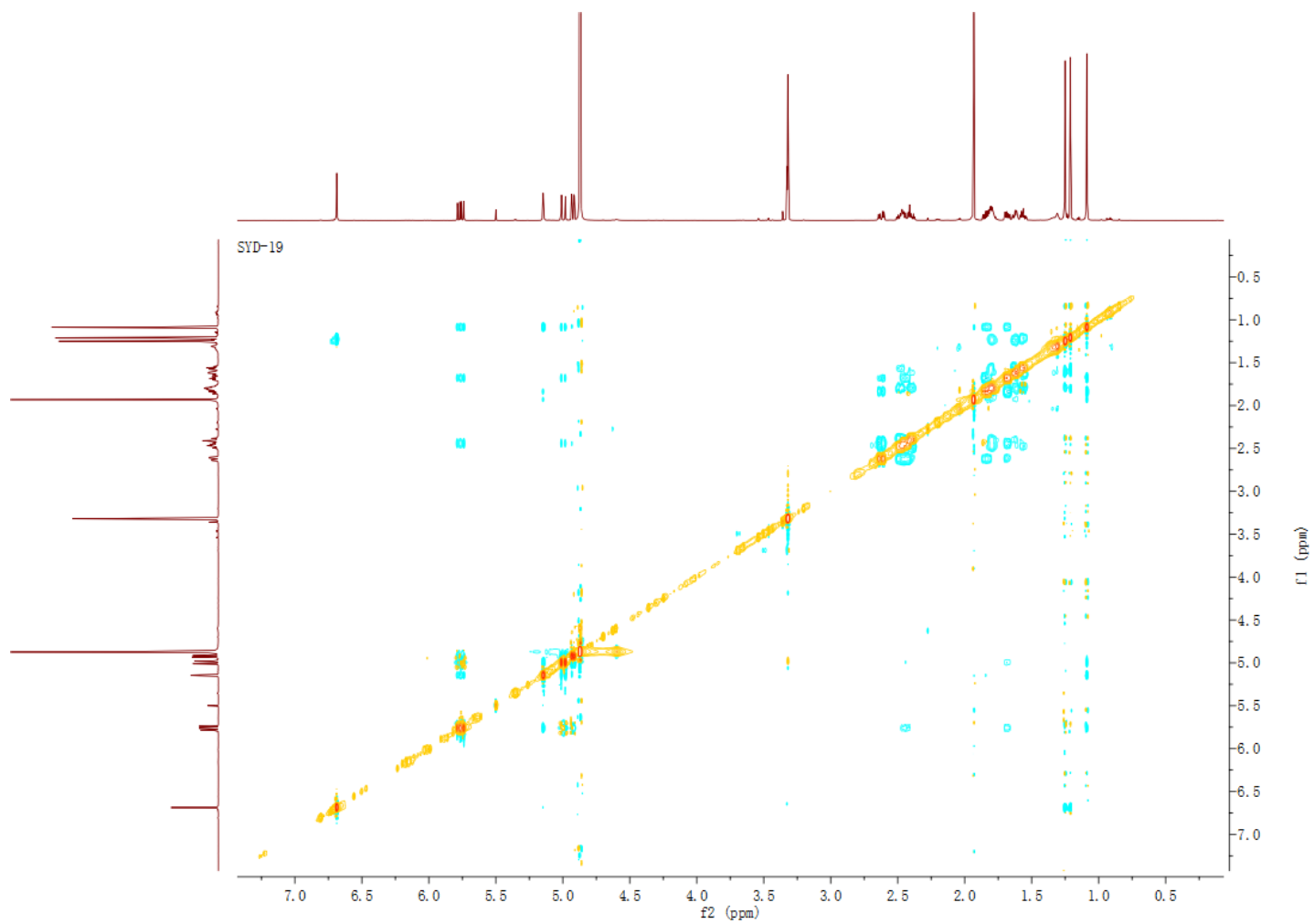


Fig. S11 NOESY spectrum (CD_3OD) of compound **1**.

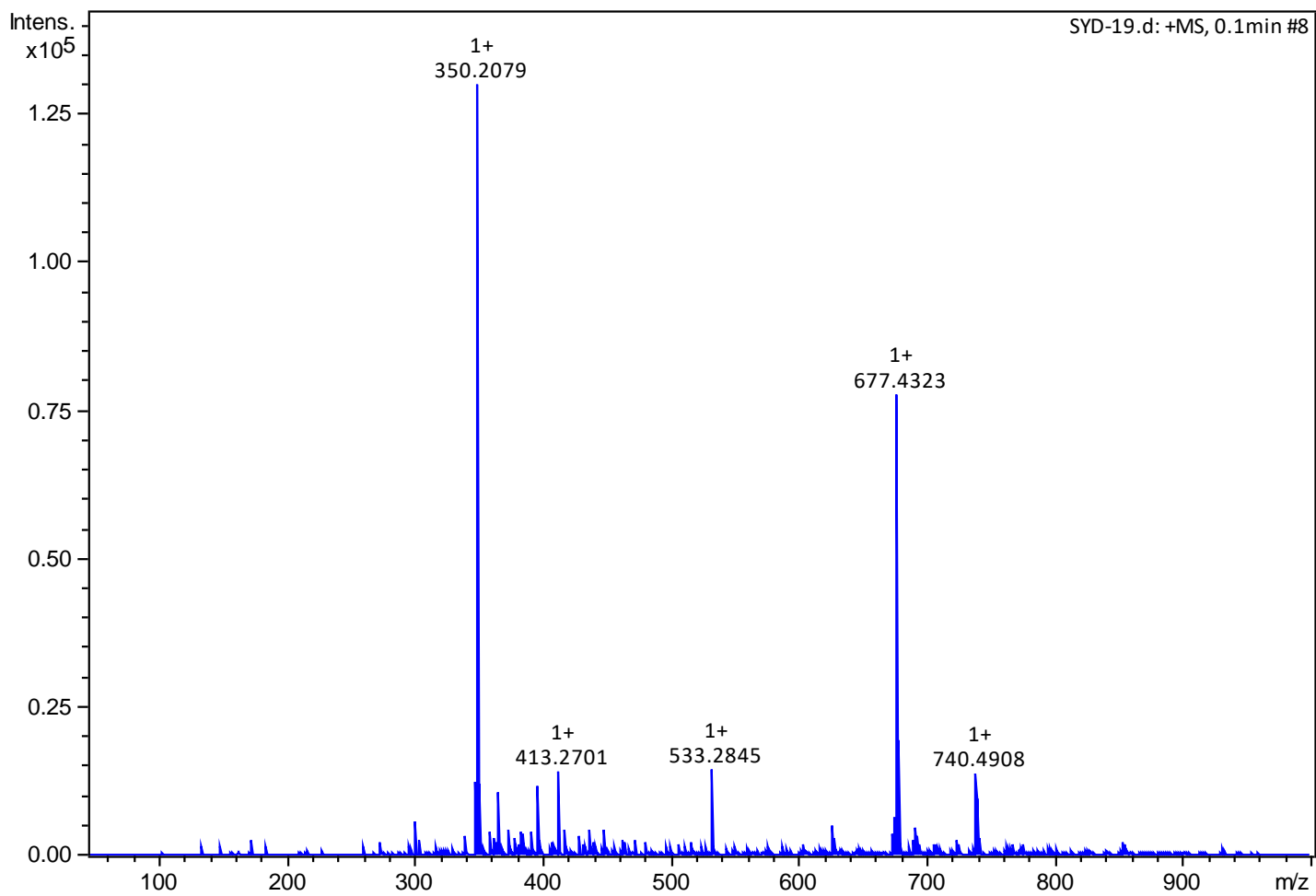


Fig. S12 HRTOFMS spectrum of compound **1**.

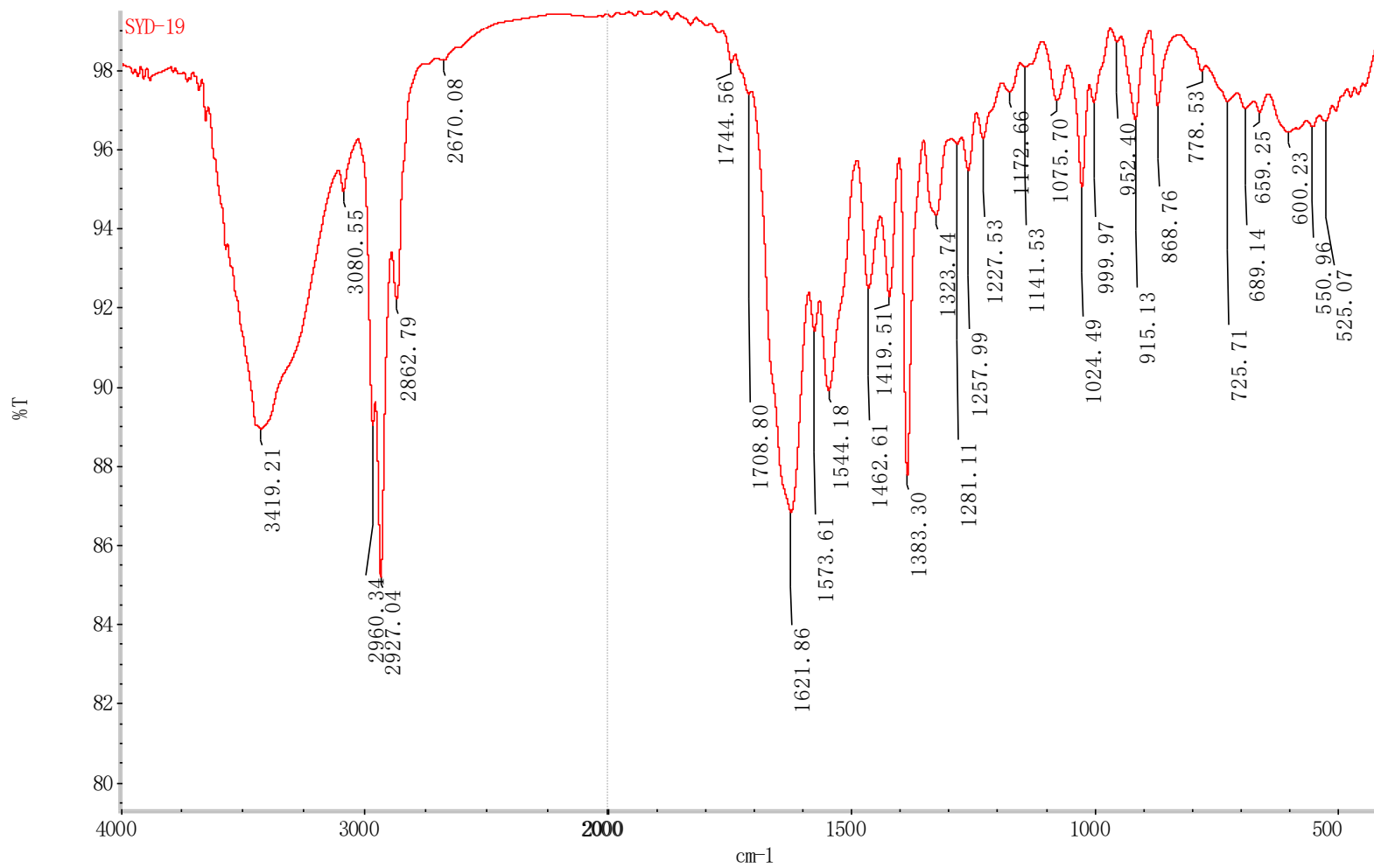


Fig. S13 IR spectrum of compound 1.

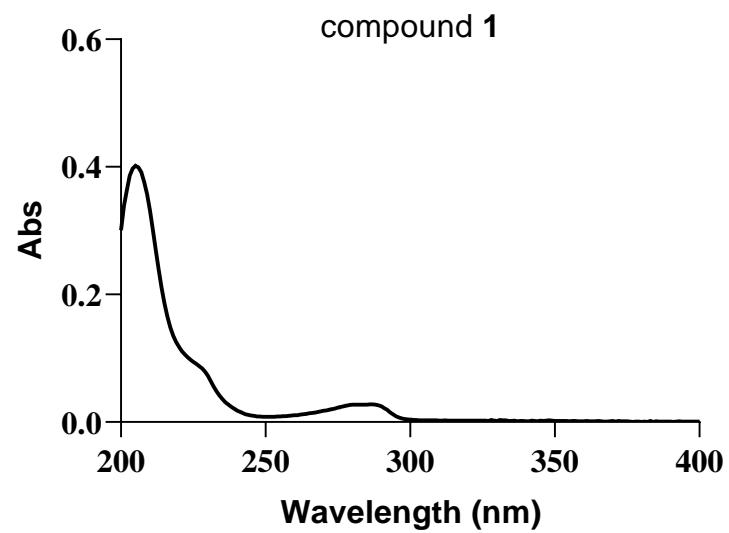


Fig. S14 UV spectrum of compound 1.

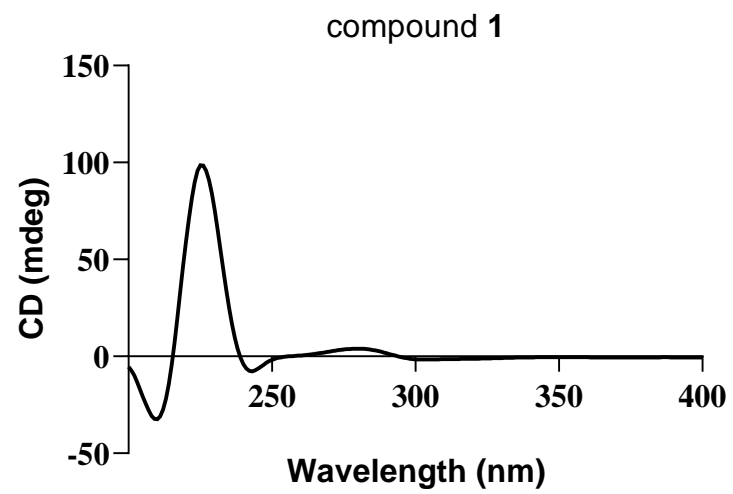


Fig. S15 CD spectrum of compound 1.

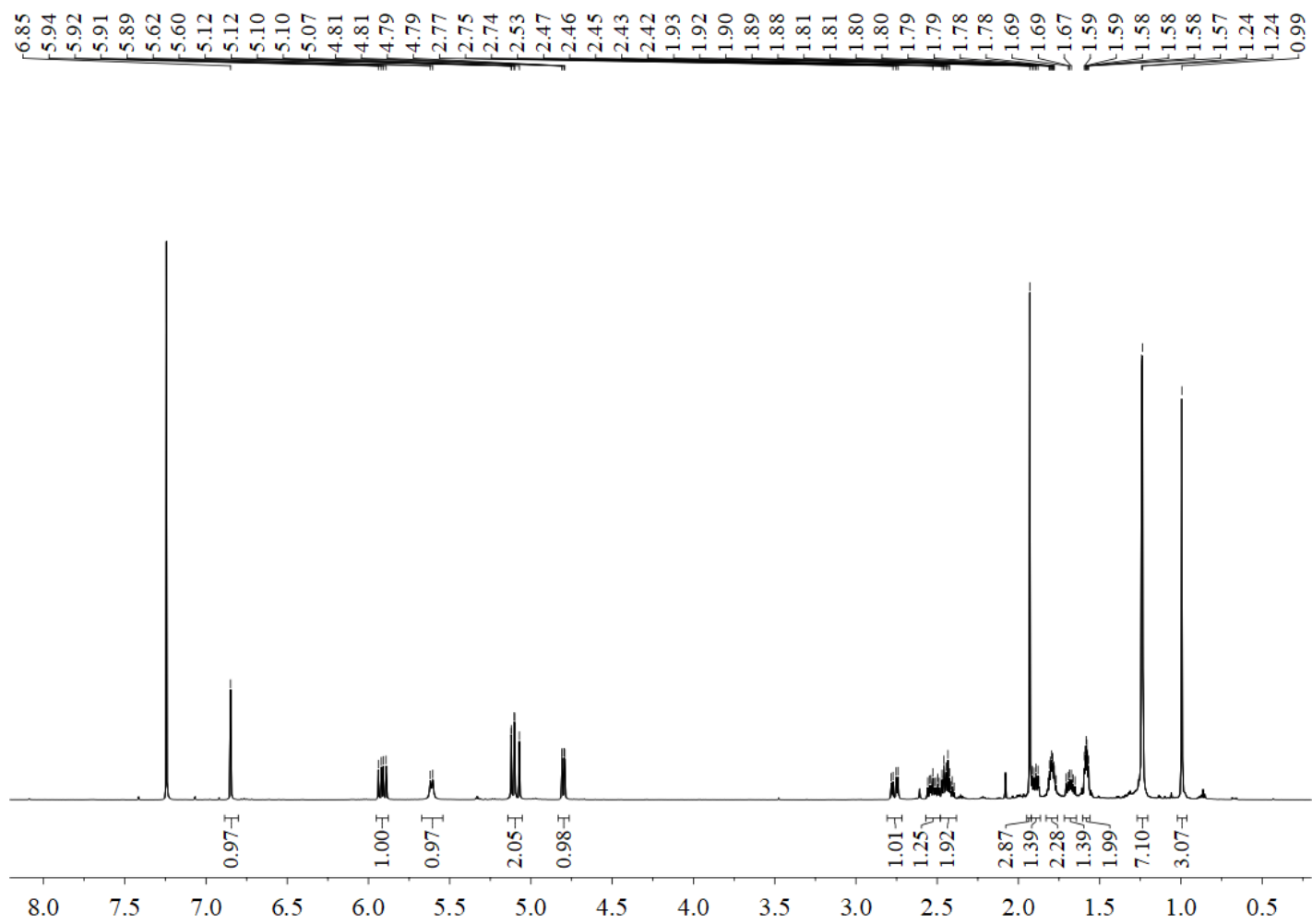


Fig. S16 ¹H-NMR (600 MHz, CDCl₃) spectrum of compound 2.

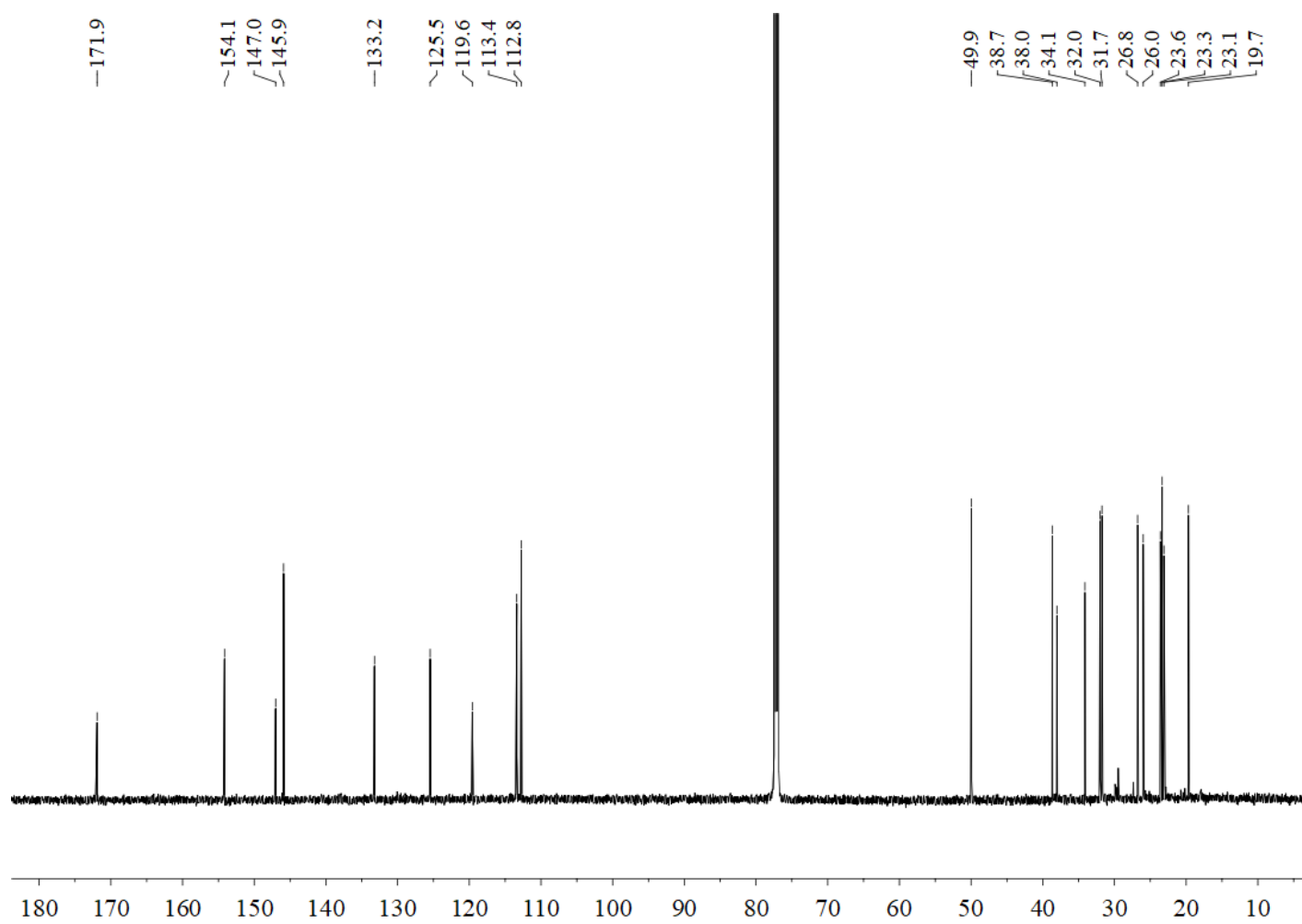


Fig. S17 ^{13}C -NMR (150 MHz, CDCl_3) spectrum of compound 2.

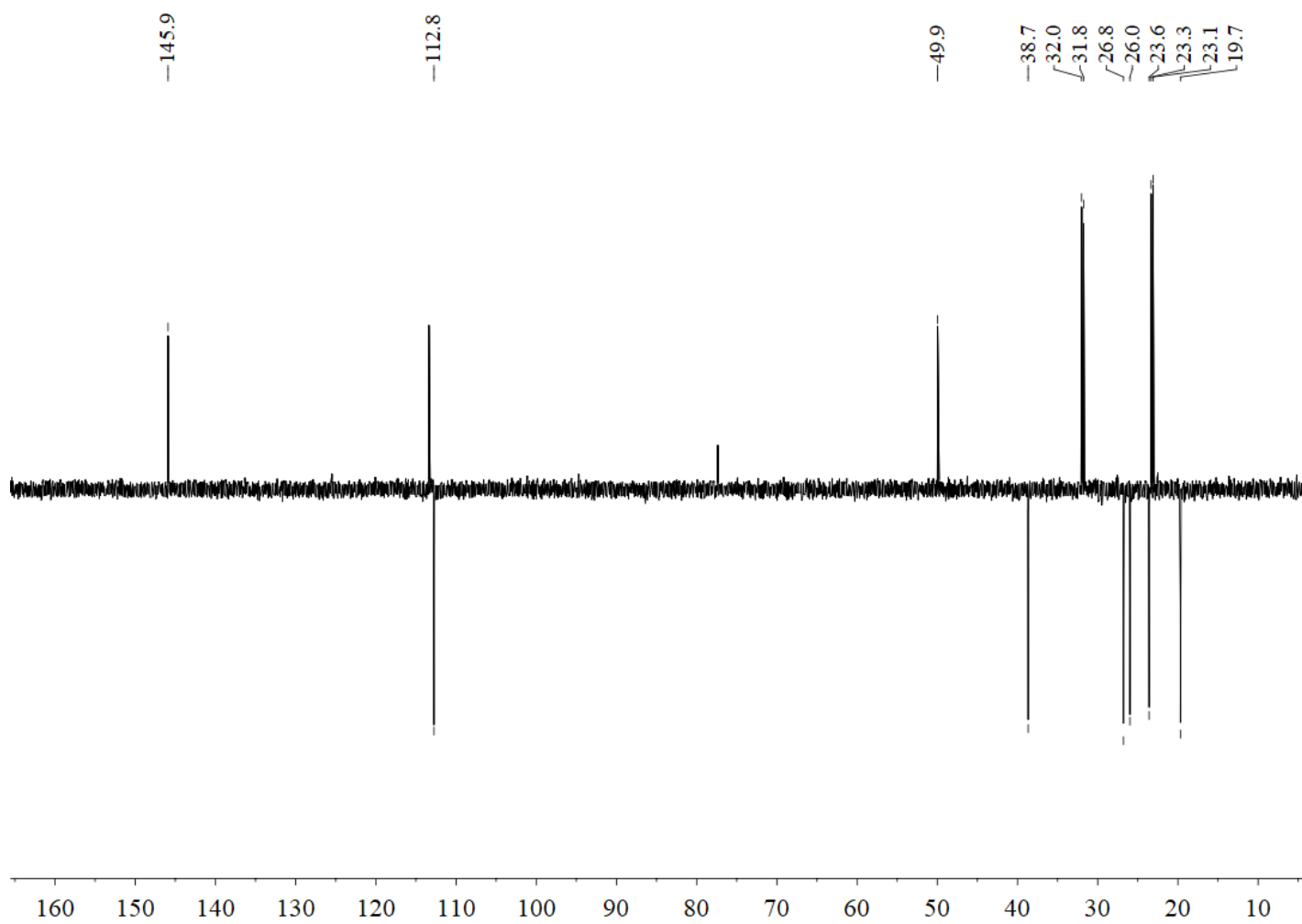


Fig. S18 DEPT-135 (150 MHz, CDCl₃) spectrum of compound **2**.

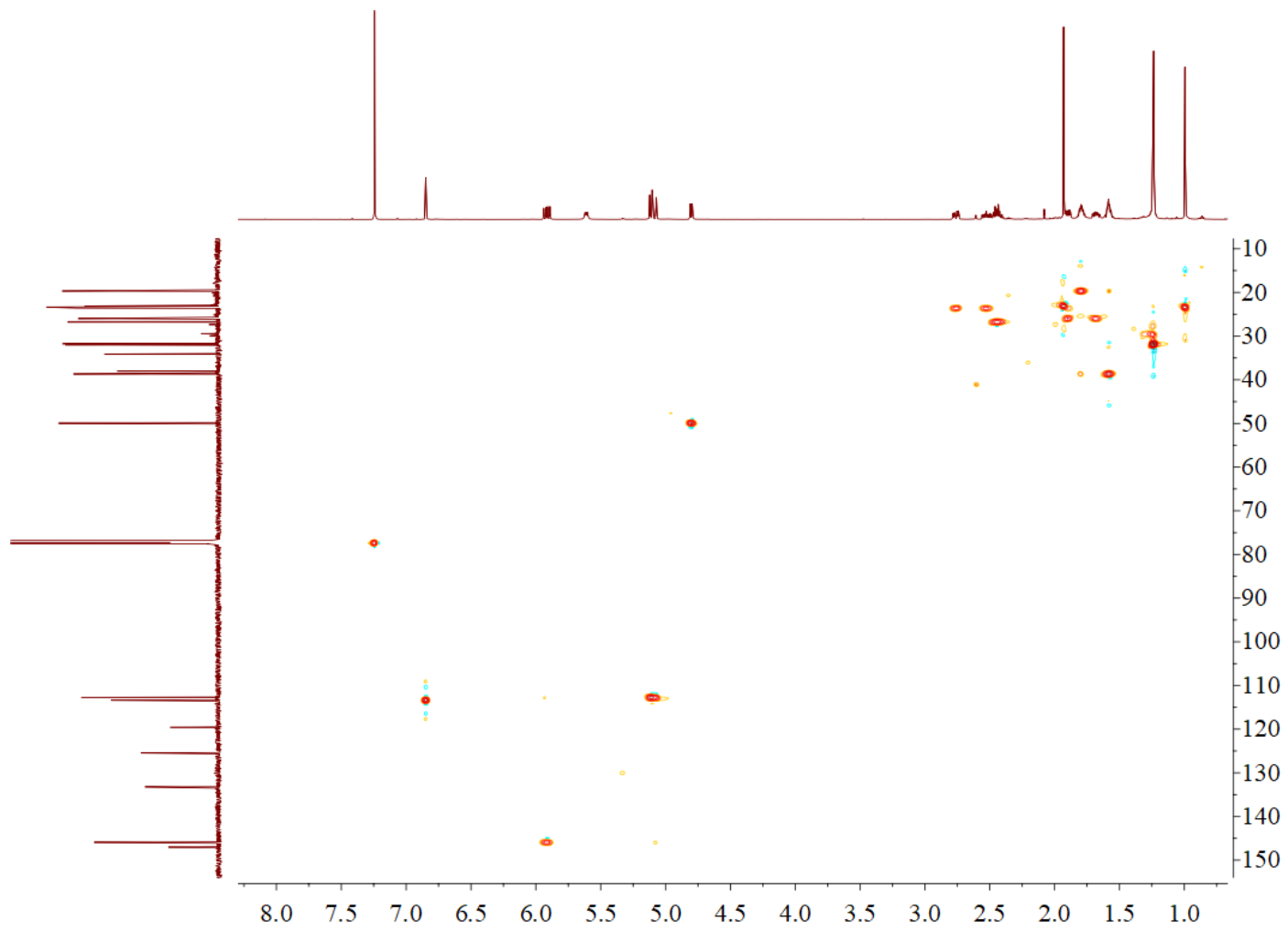


Fig. S19 HSQC spectrum (CDCl₃) of compound **2**.

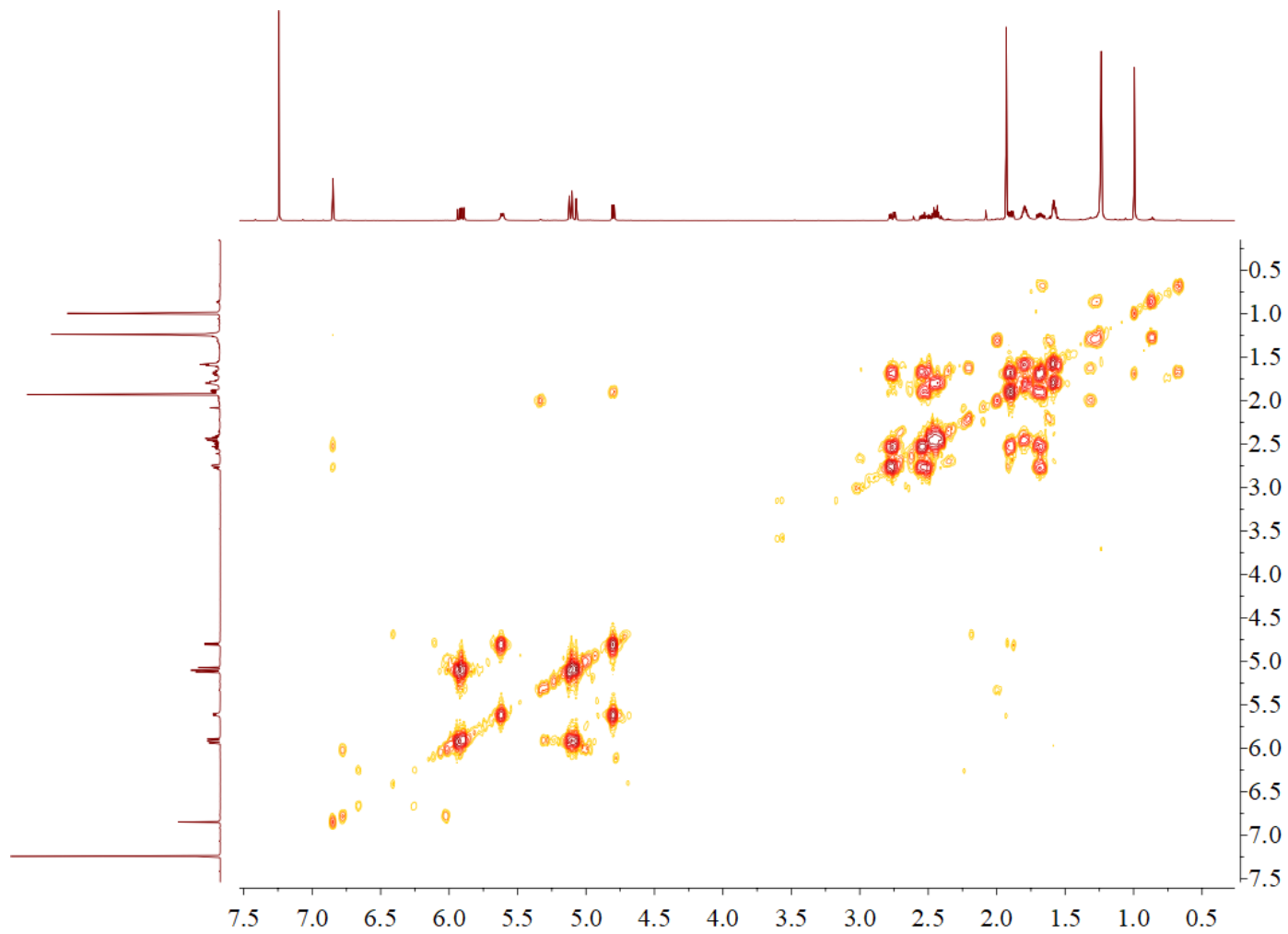


Fig. S20 ^1H - ^1H COSY spectrum (CDCl_3) of compound **2**.

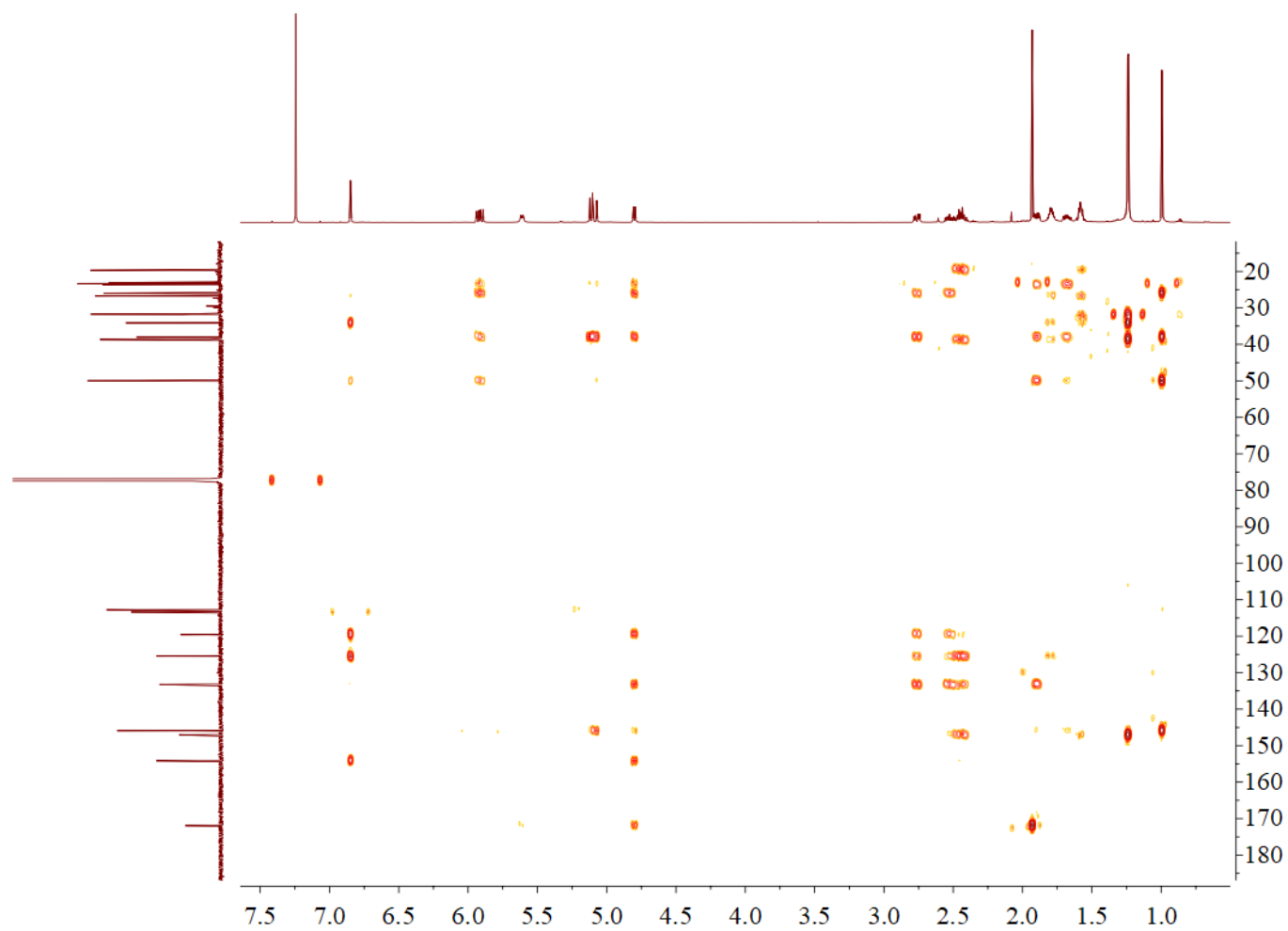


Fig. S21 HMBC spectrum (CDCl_3) of compound **2**.

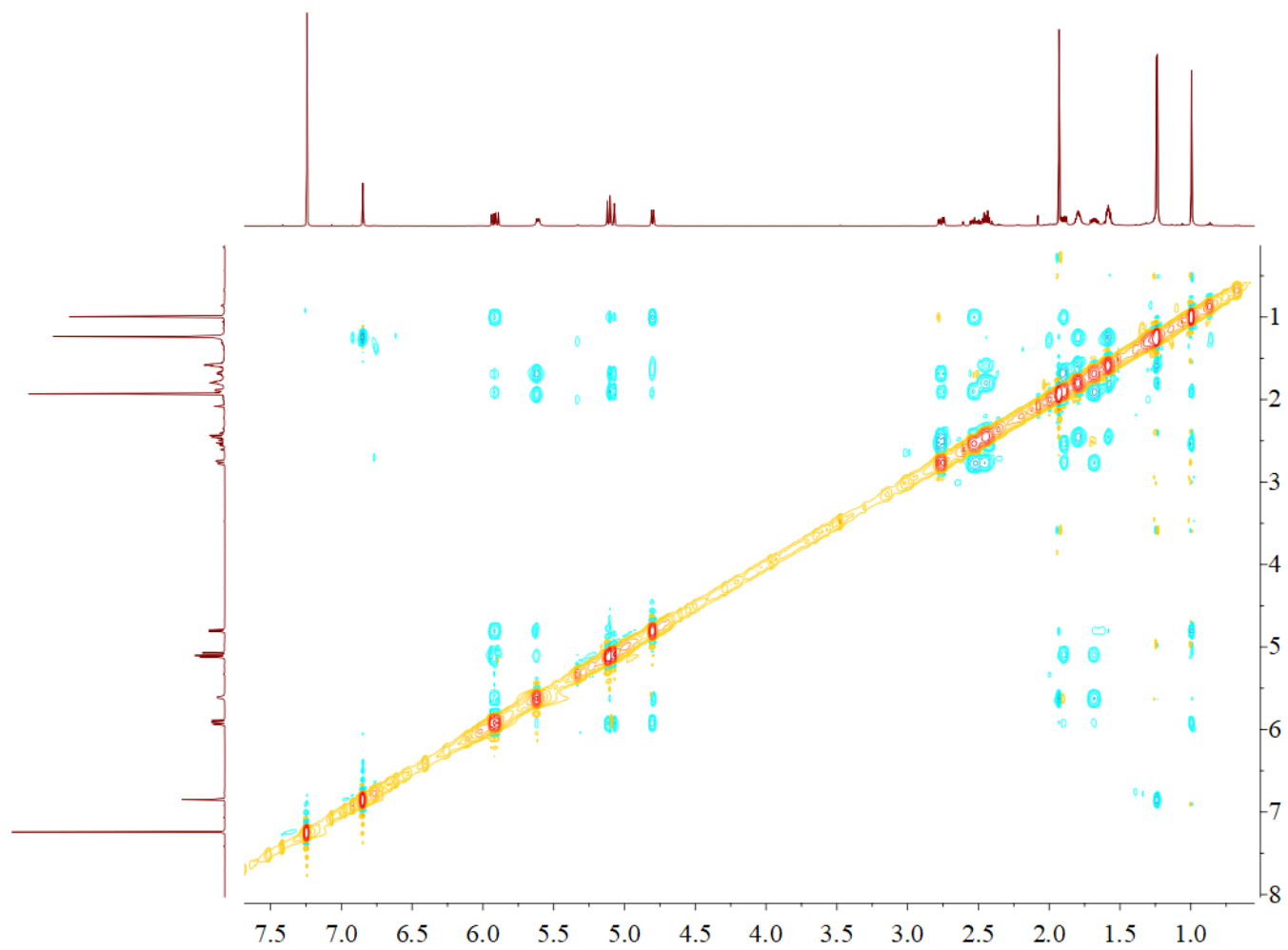


Fig. S22 NOESY spectrum (CDCl_3) of compound **2**.

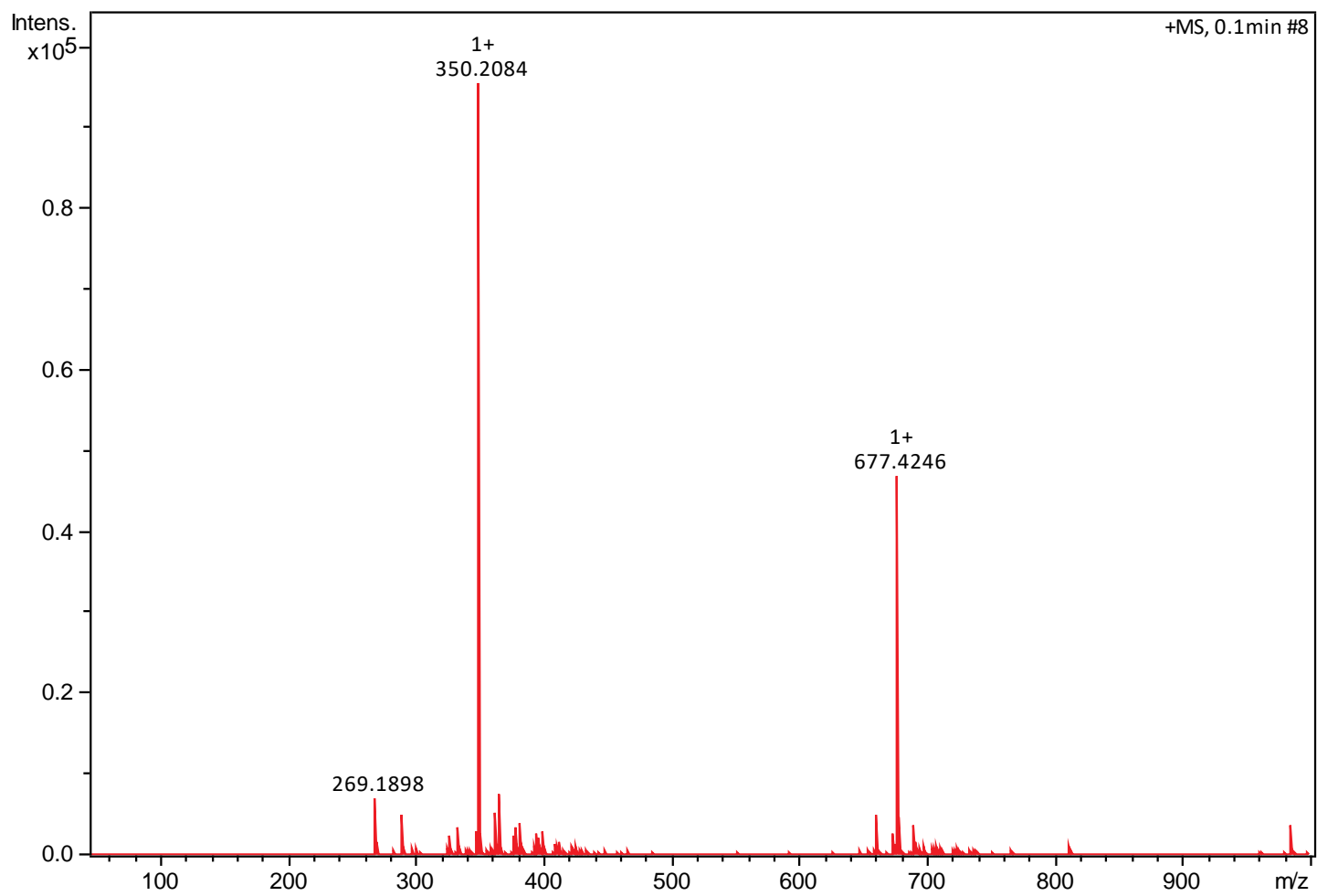


Fig. S23 HRTOFMS spectrum of compound **2**.

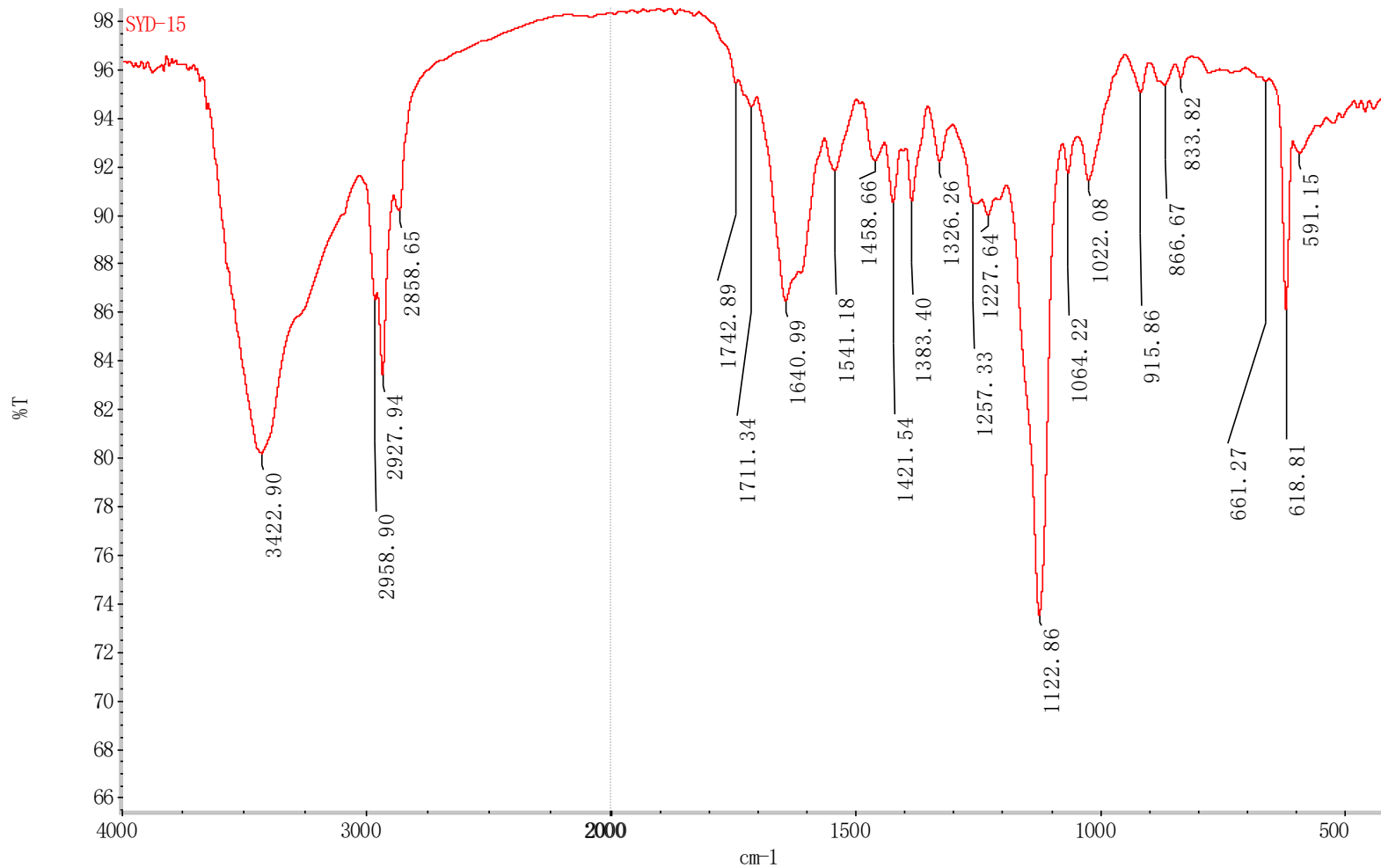


Fig. S24 IR spectrum of compound 2.

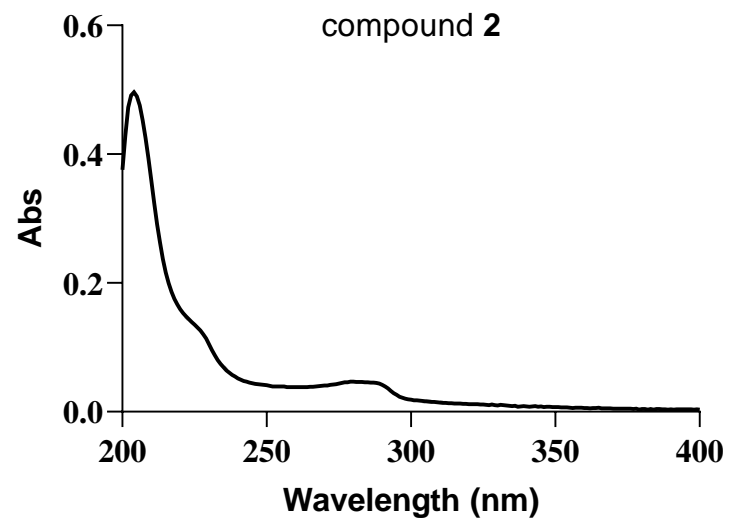


Fig. S25 UV spectrum of compound 2.

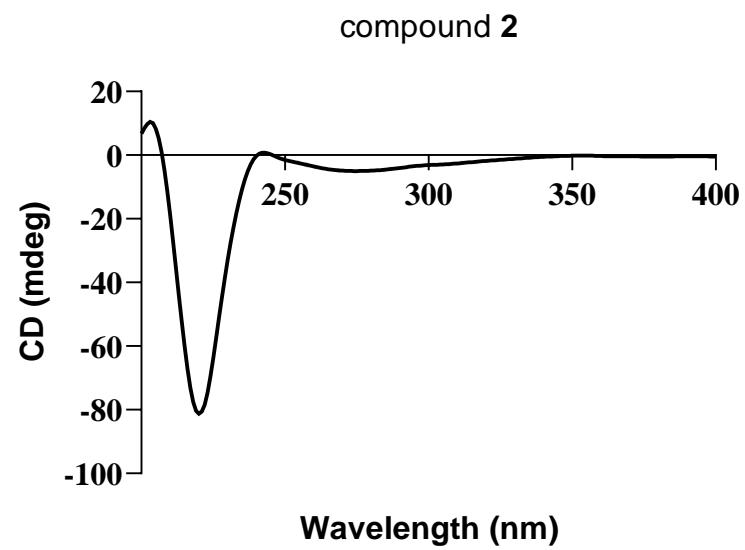


Fig. S26 CD spectrum of compound 2.

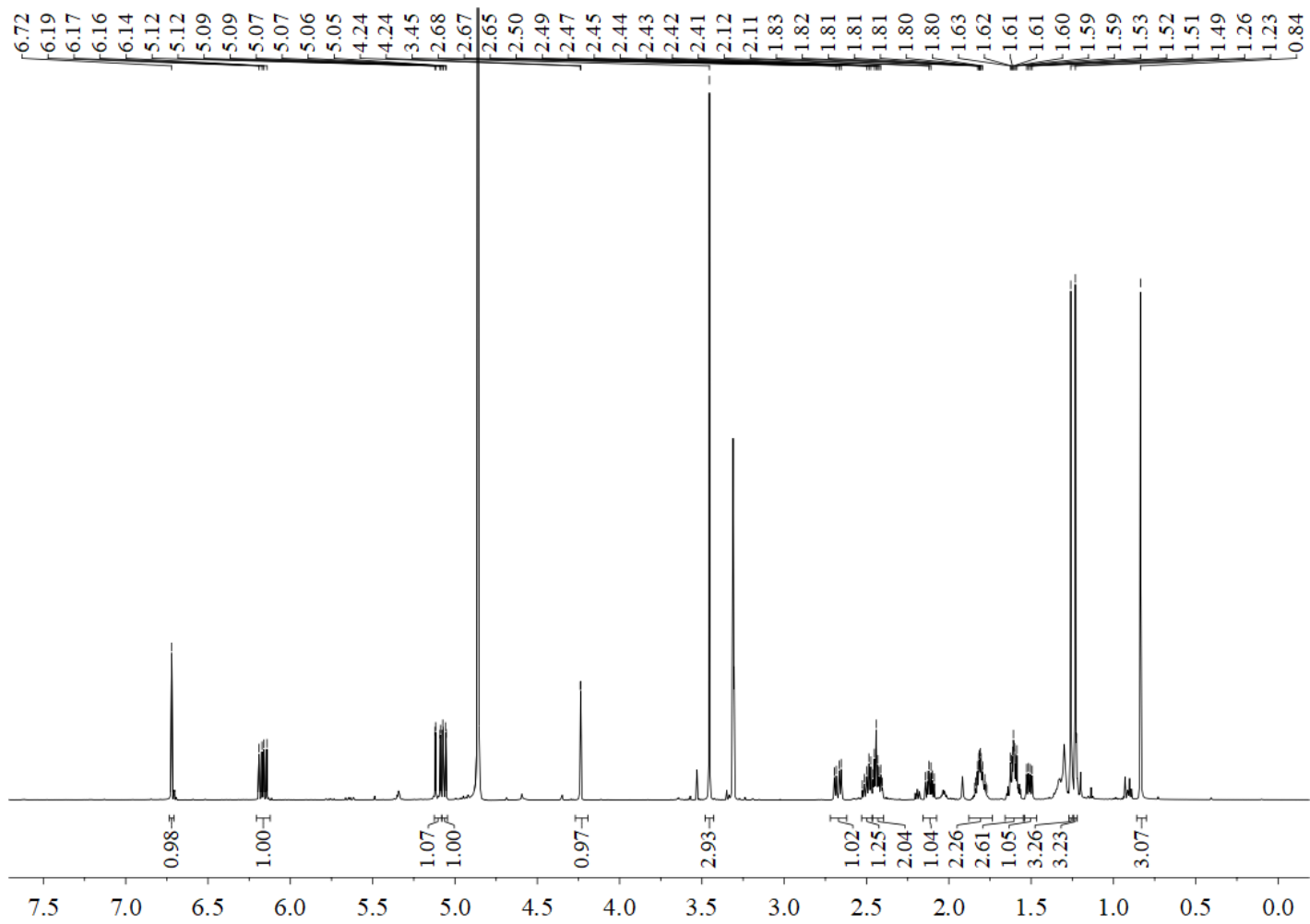


Fig. S27 $^1\text{H-NMR}$ (600 MHz, CD_3OD) spectrum of compound **3**.

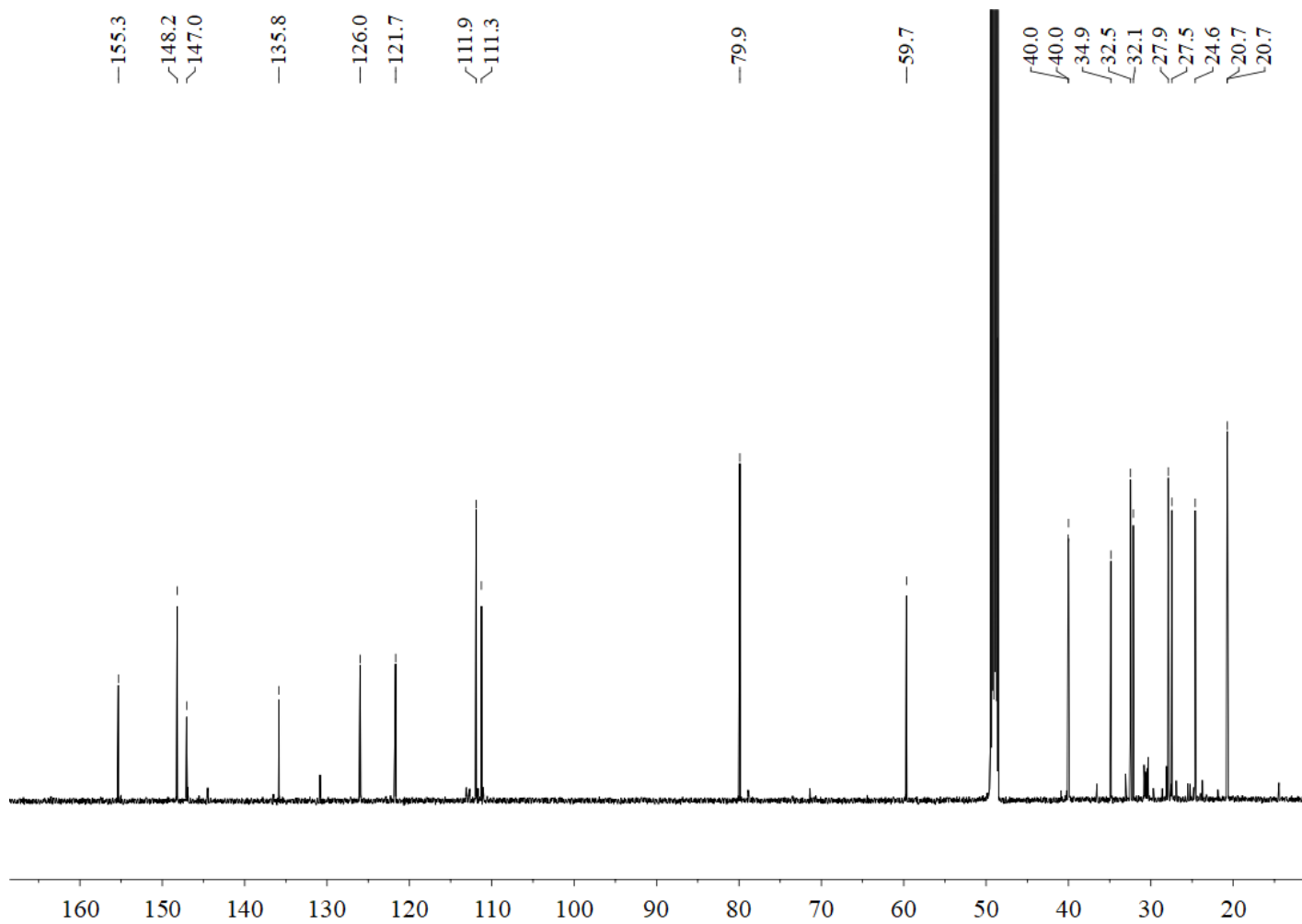


Fig. S28 ^{13}C -NMR (150 MHz, CD_3OD) spectrum of compound 3.

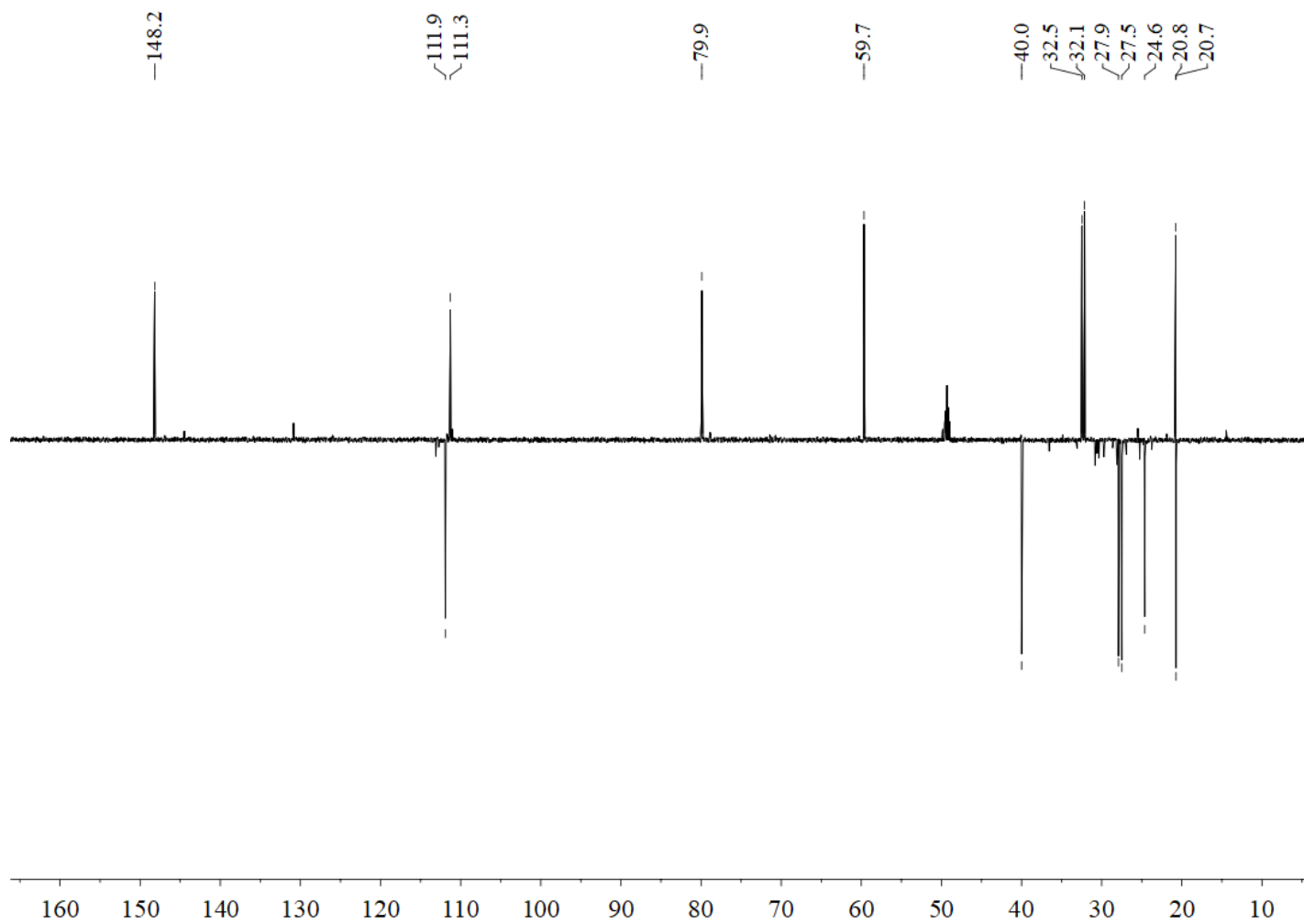


Fig. S29 DEPT-135 (150 MHz, CD₃OD) spectrum of compound 3.

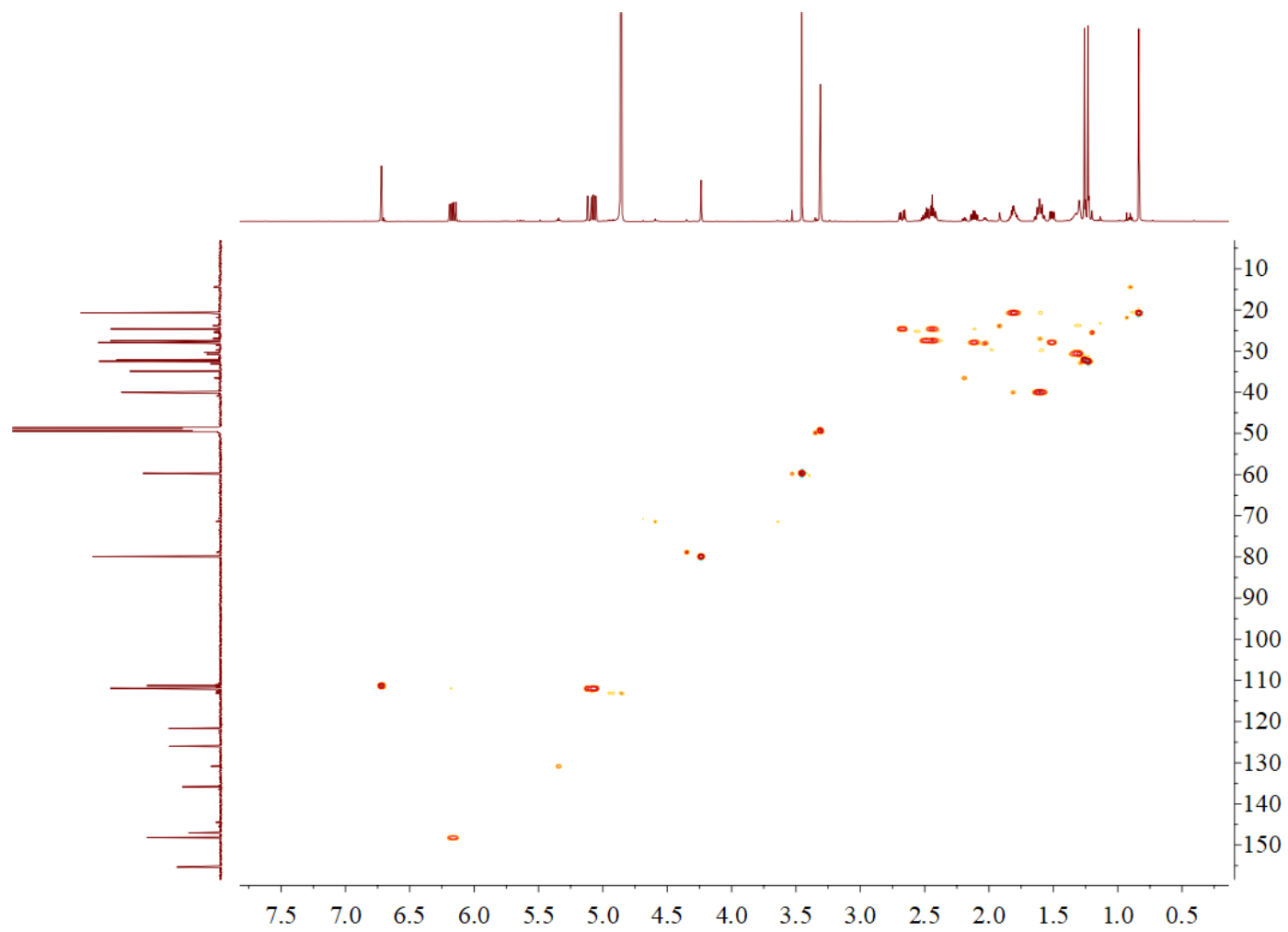


Fig. S30 HSQC spectrum (CD₃OD) of compound **3**.

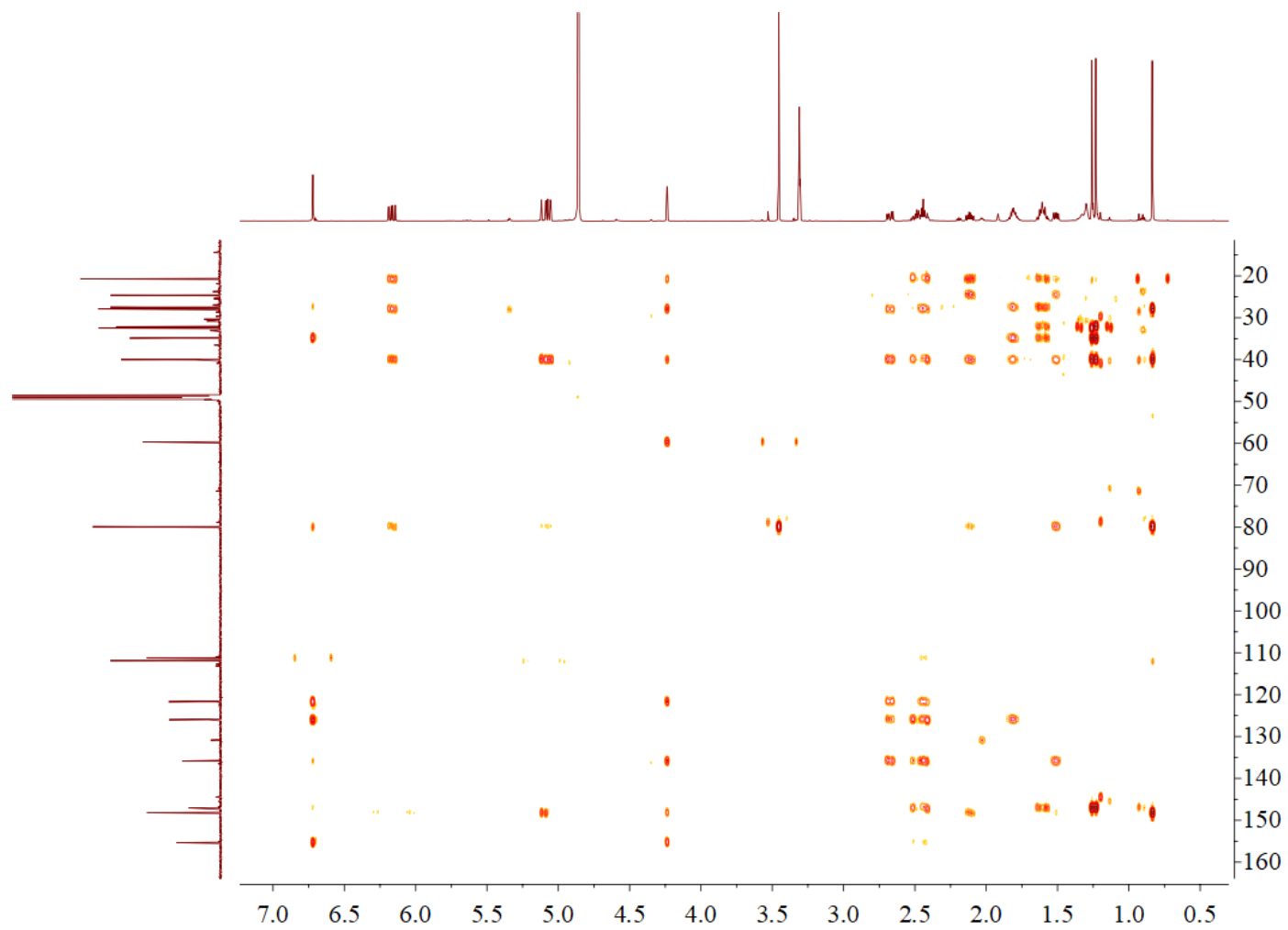


Fig. S32 HMBC spectrum (CD₃OD) of compound **3**.

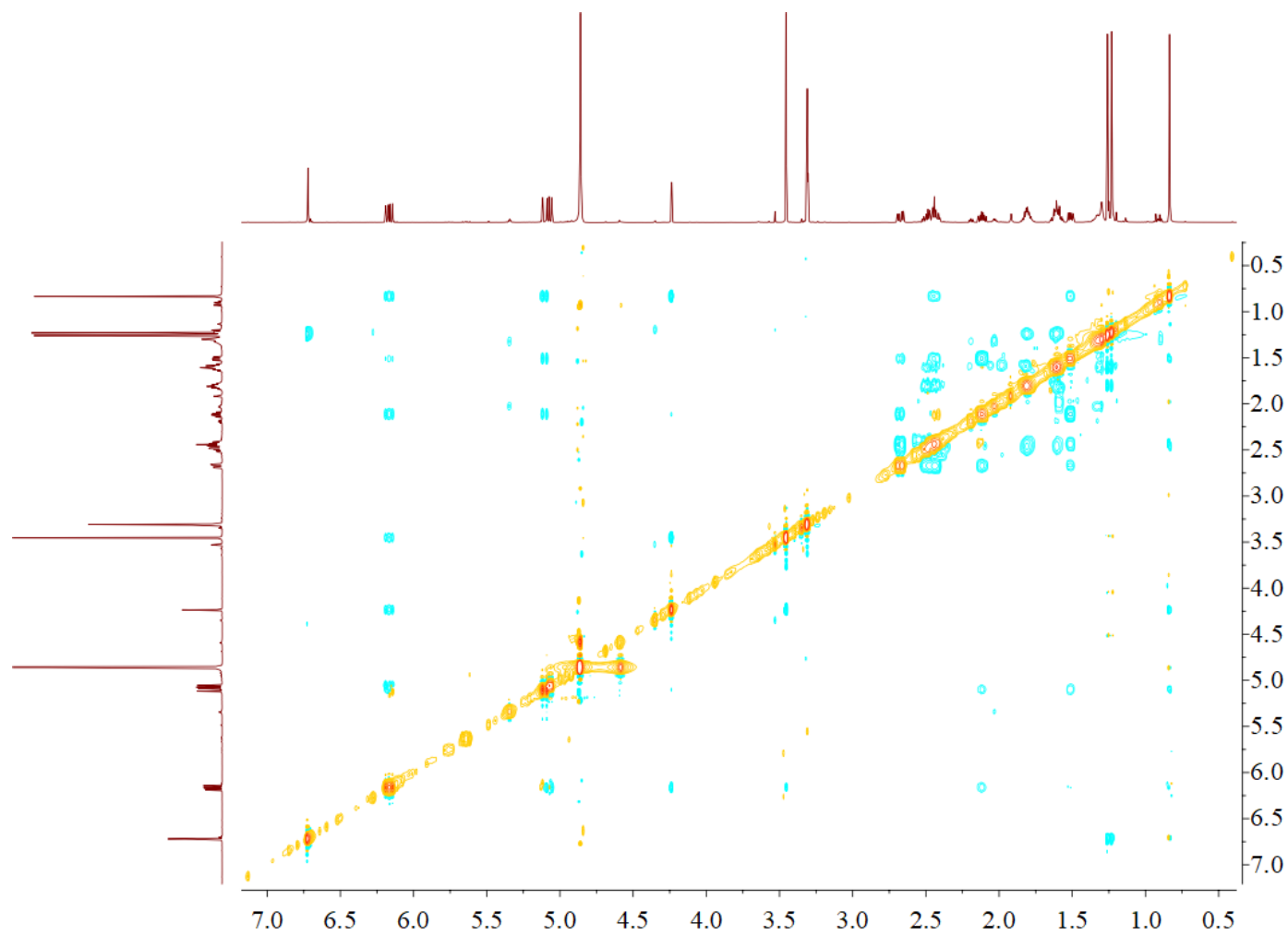


Fig. S33 NOESY spectrum (CD₃OD) of compound **3**.

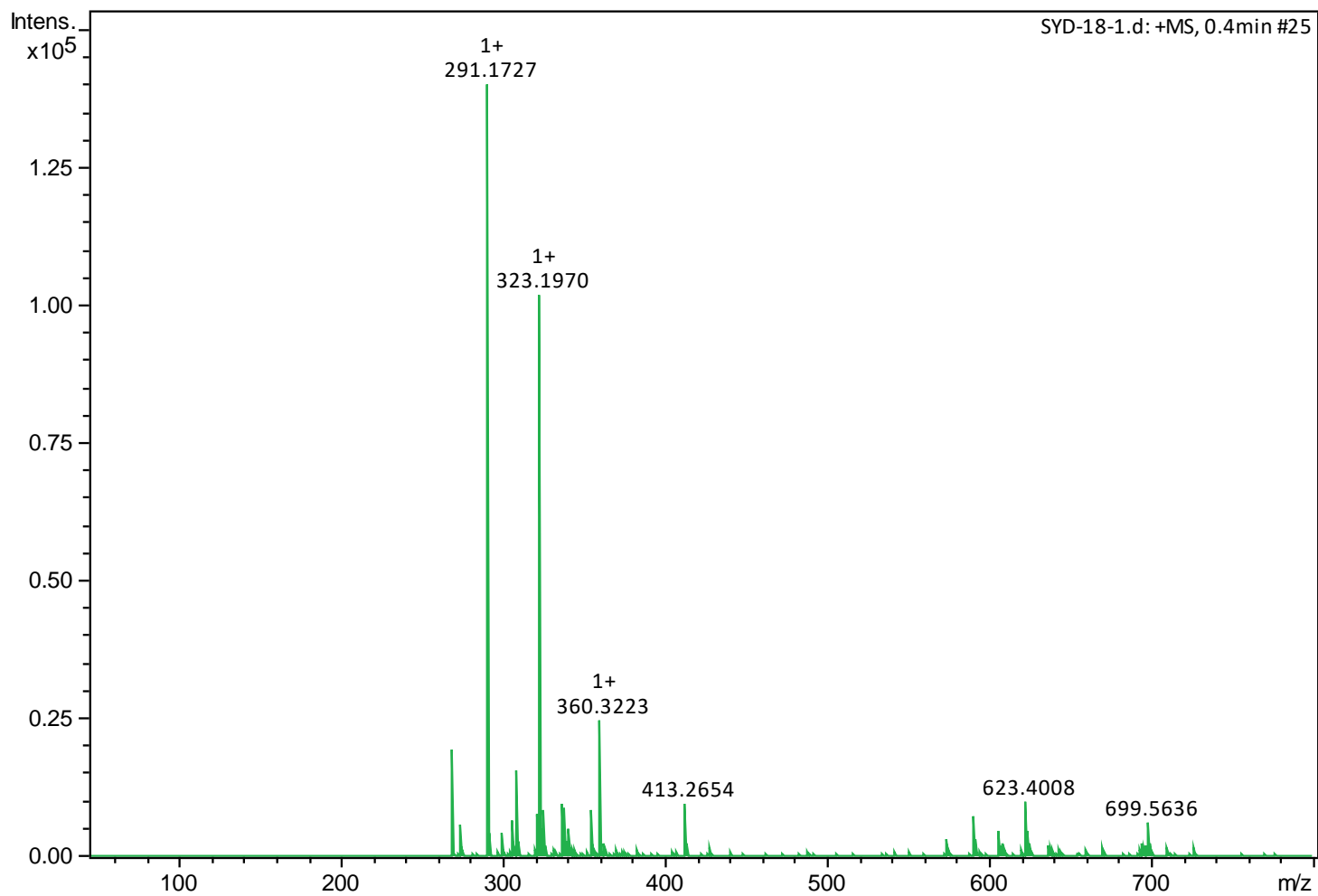


Fig. S34 HRTOFMS spectrum of compound 3.

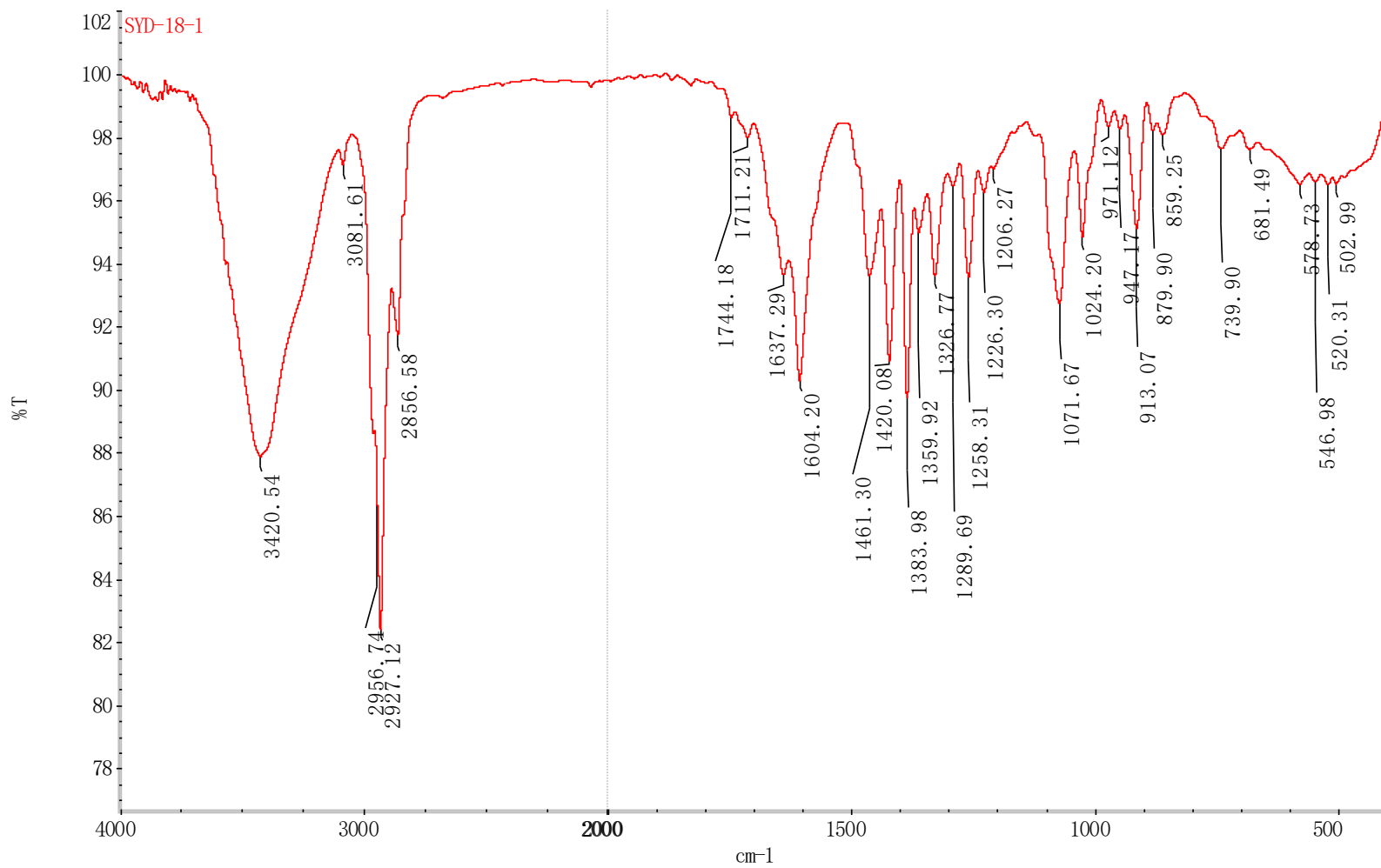


Fig. S35 IR spectrum of compound 3.

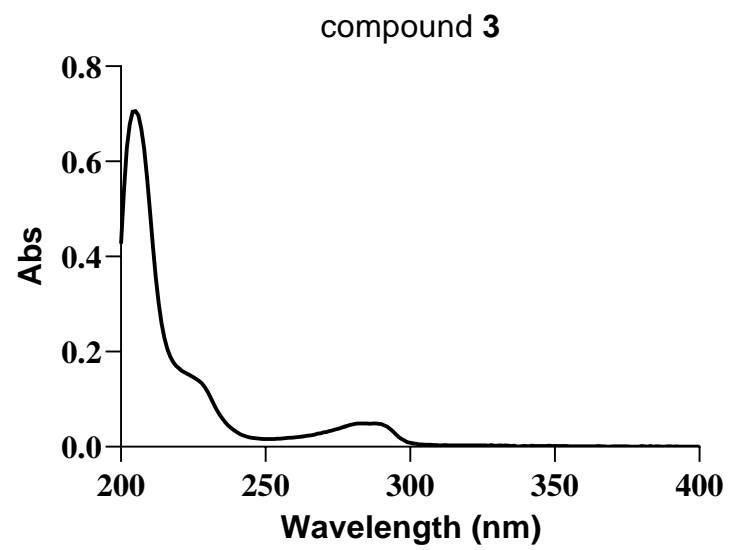


Fig. S36 UV spectrum of compound 3.

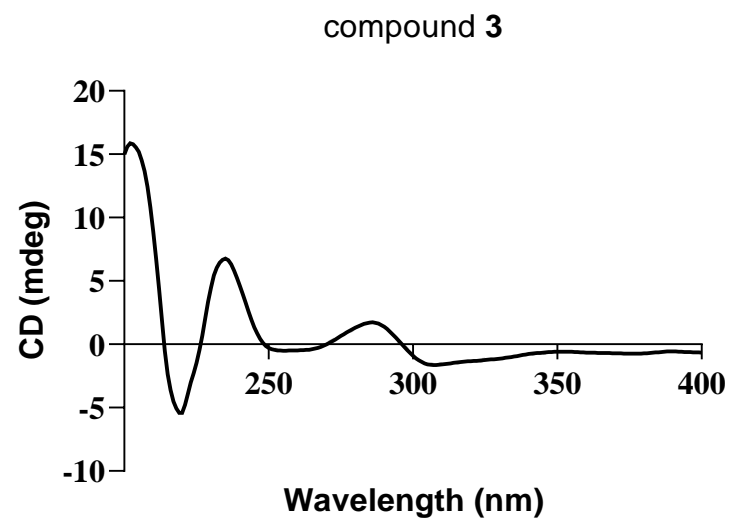


Fig. S37 CD spectrum of compound 3.

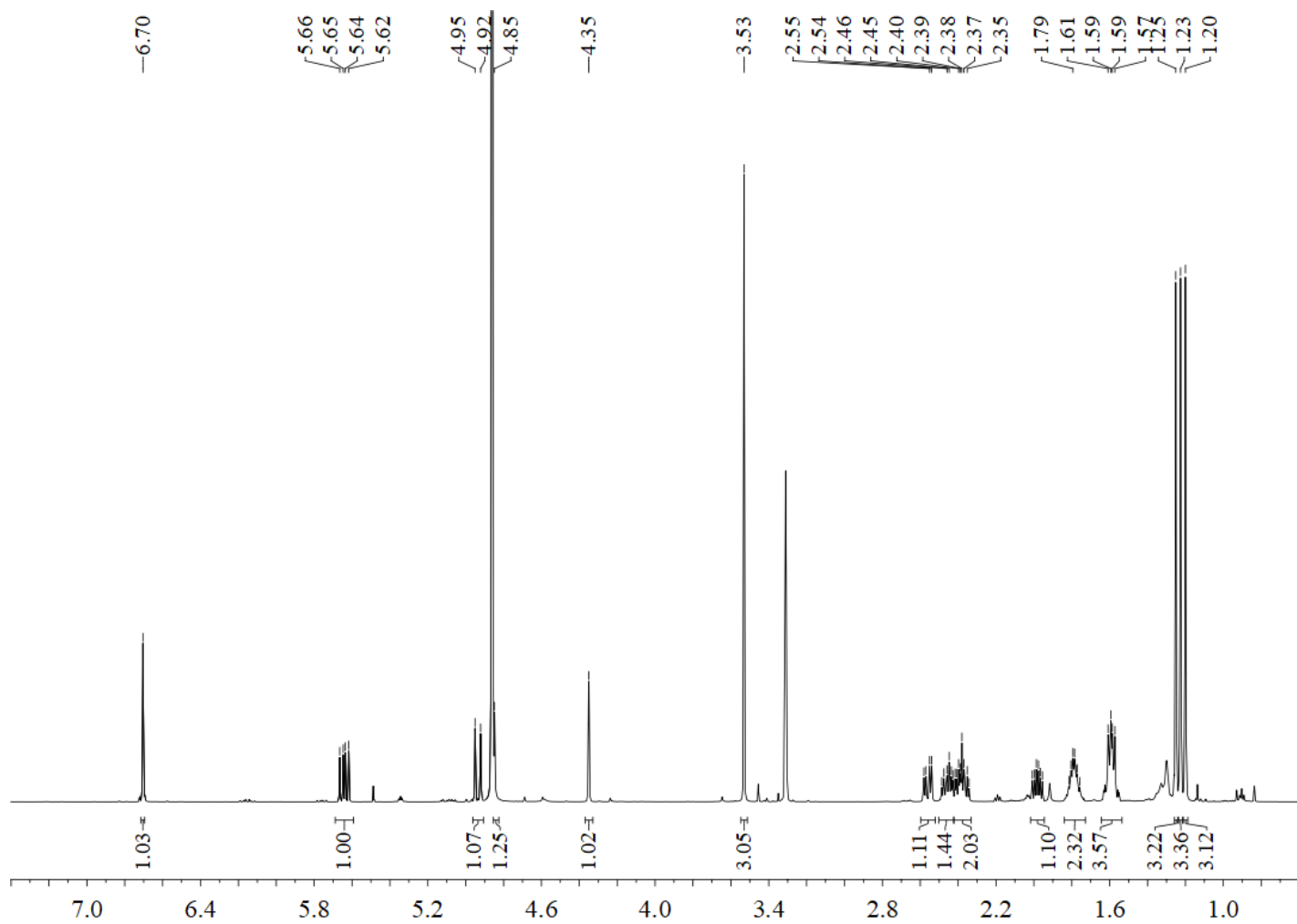


Fig. S38 $^1\text{H-NMR}$ (600 MHz, CD_3OD) spectrum of compound 4.

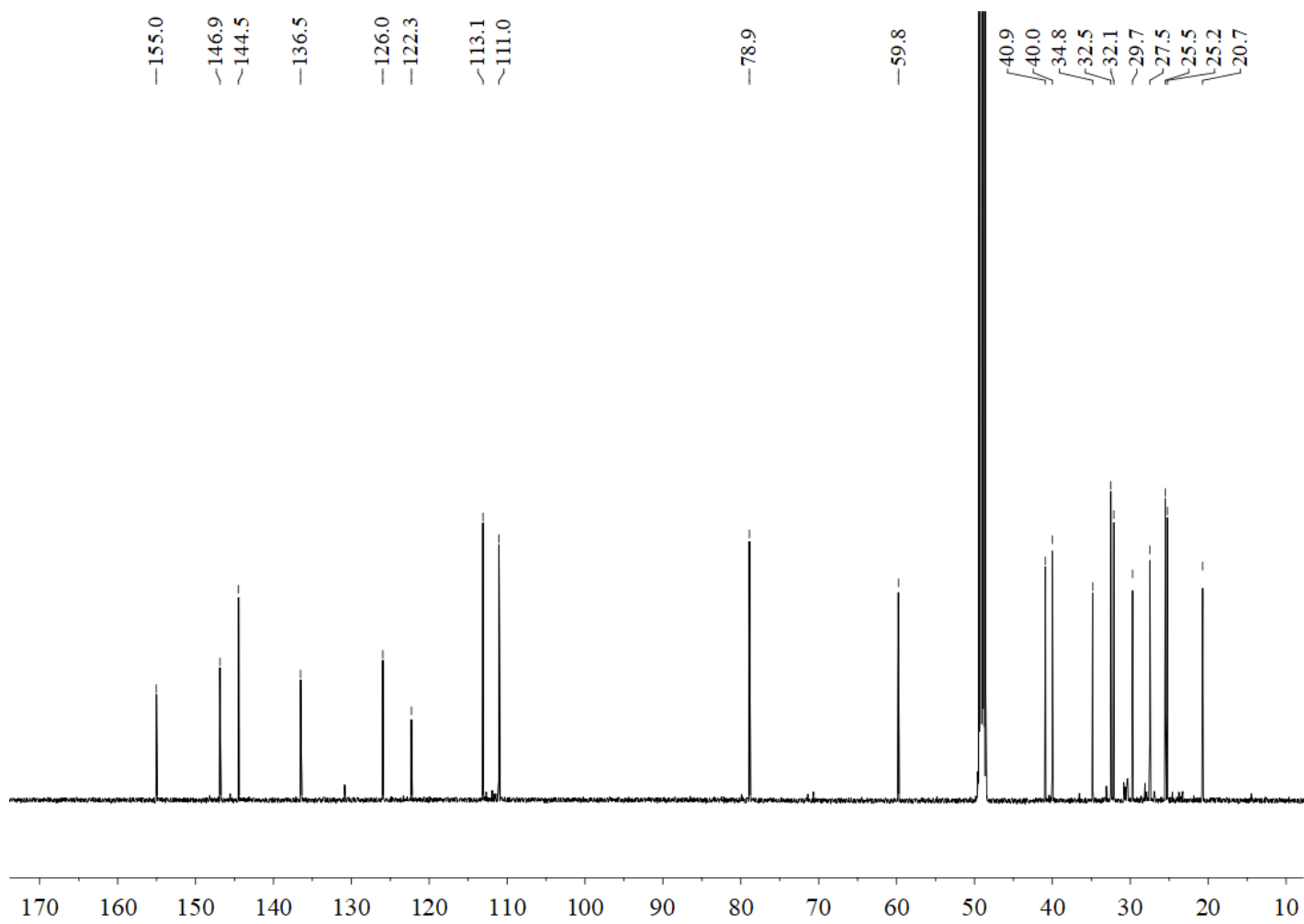


Fig. S39 ^{13}C -NMR (150 MHz, CD_3OD) spectrum of compound 4.

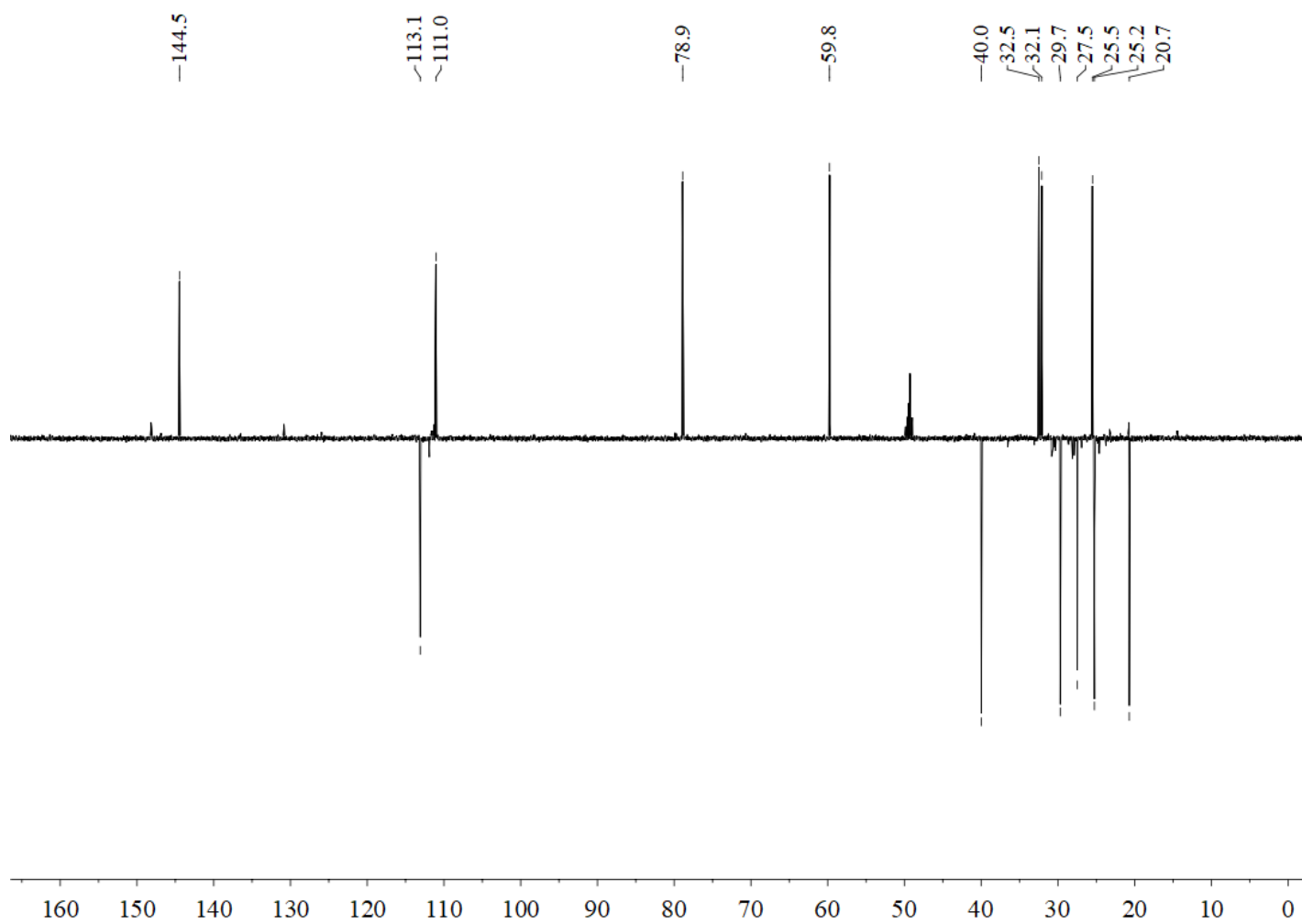


Fig. S40 DEPT-135 (150 MHz, CD₃OD) spectrum of compound **4**.

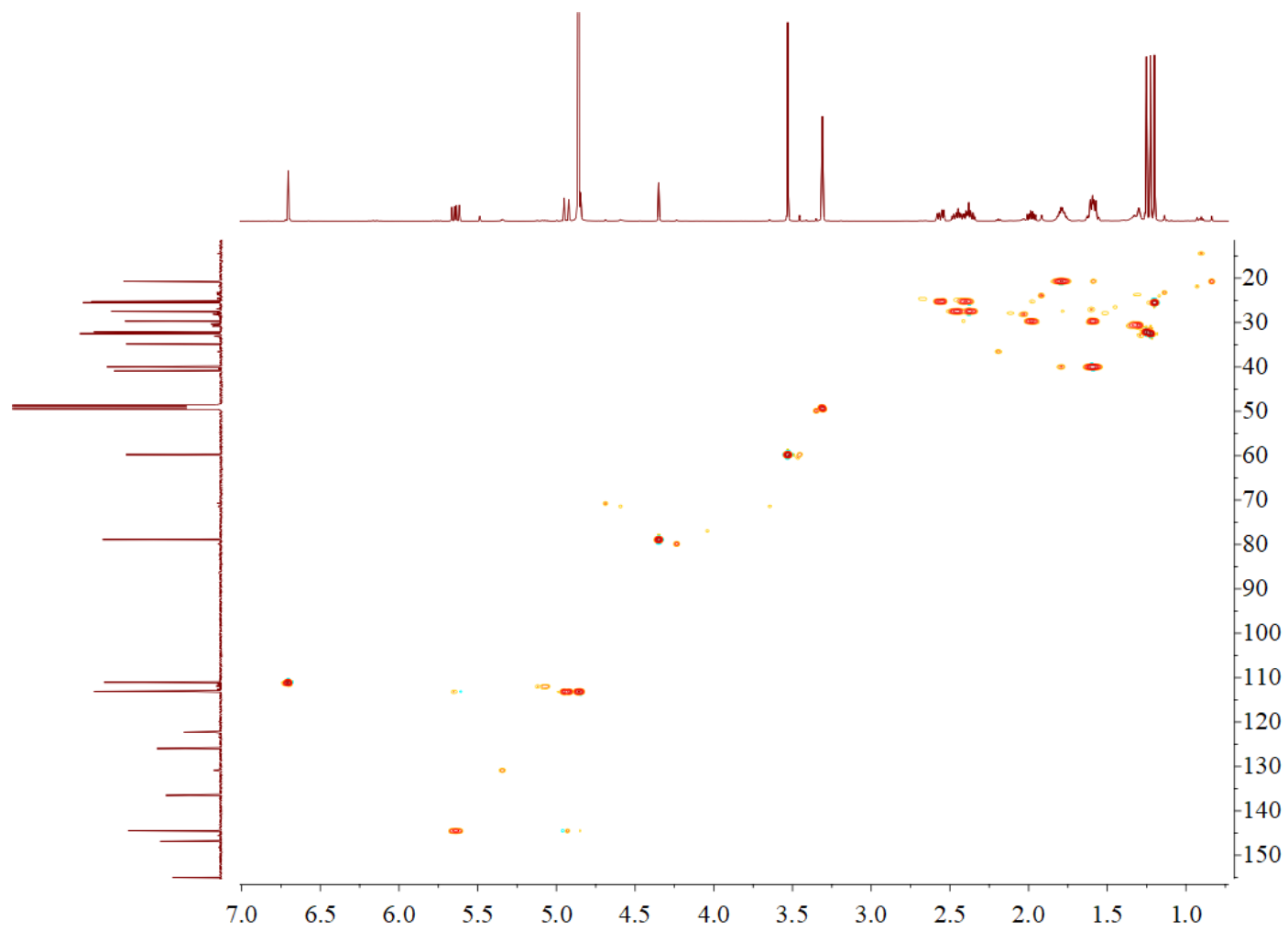


Fig. S41 HSQC spectrum (CD₃OD) of compound **4**.

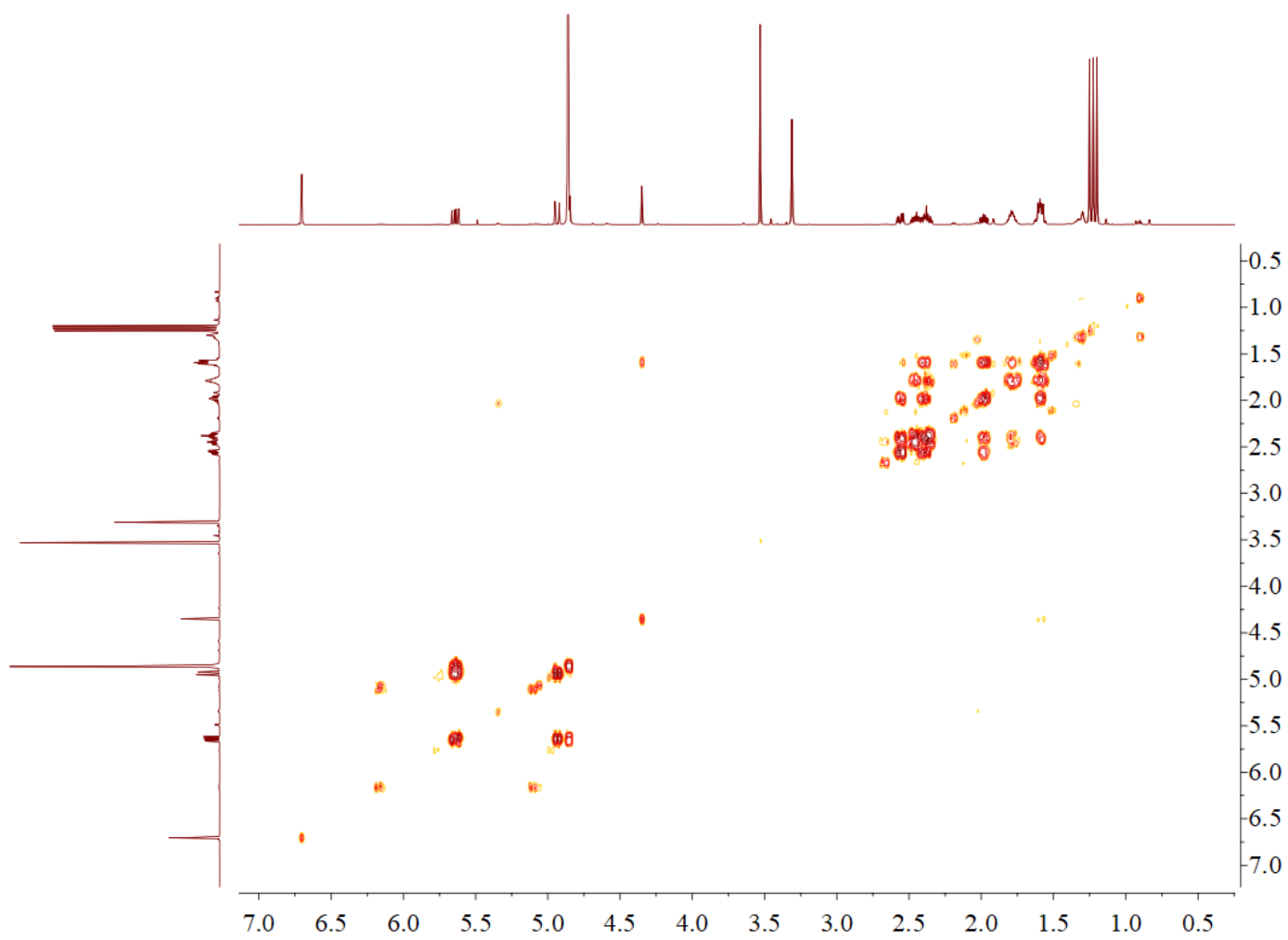


Fig. S42 ^1H - ^1H COSY spectrum (CD_3OD) of compound 4.

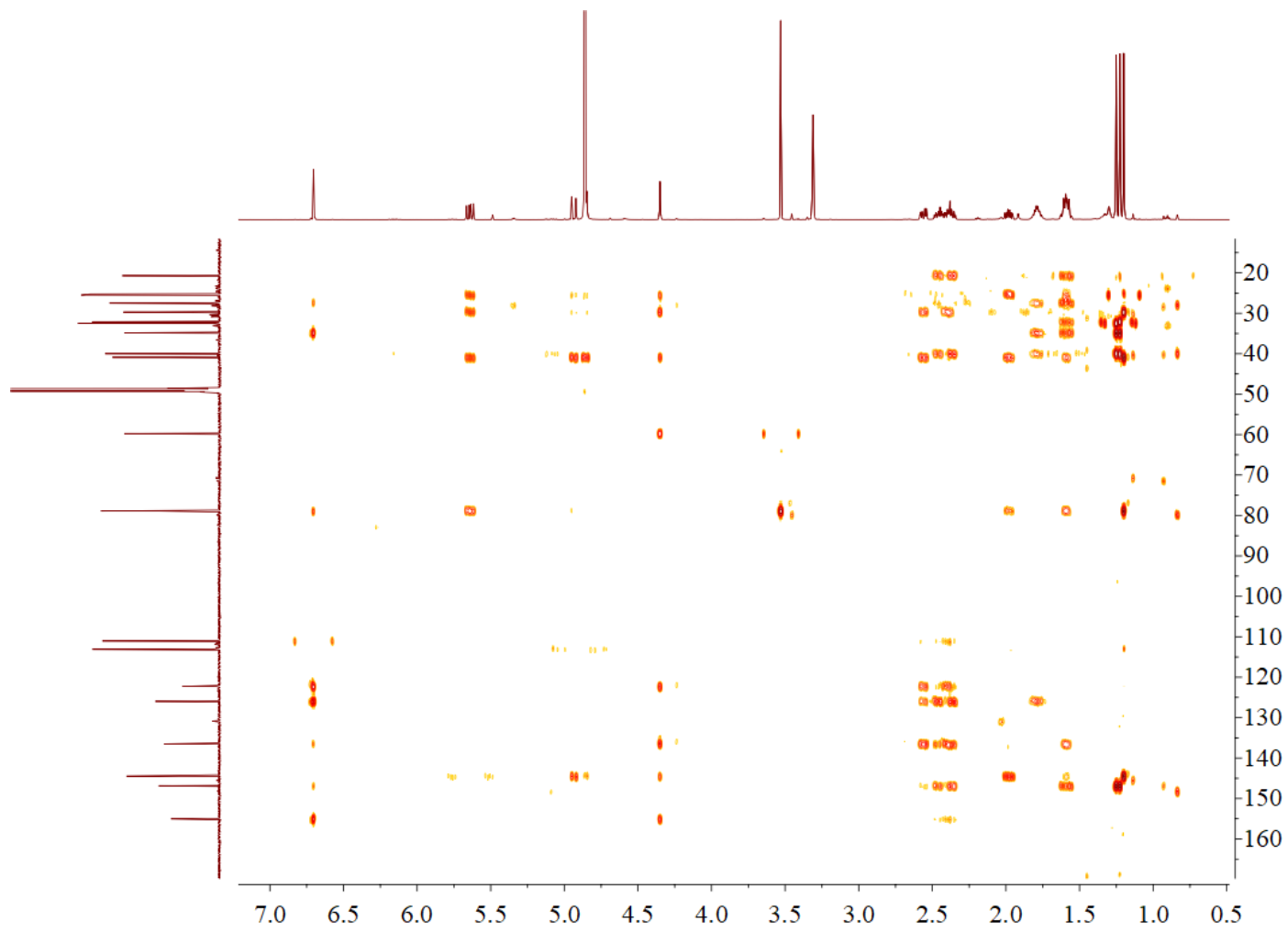


Fig. S43 HMBC spectrum (CD₃OD) of compound **4**.

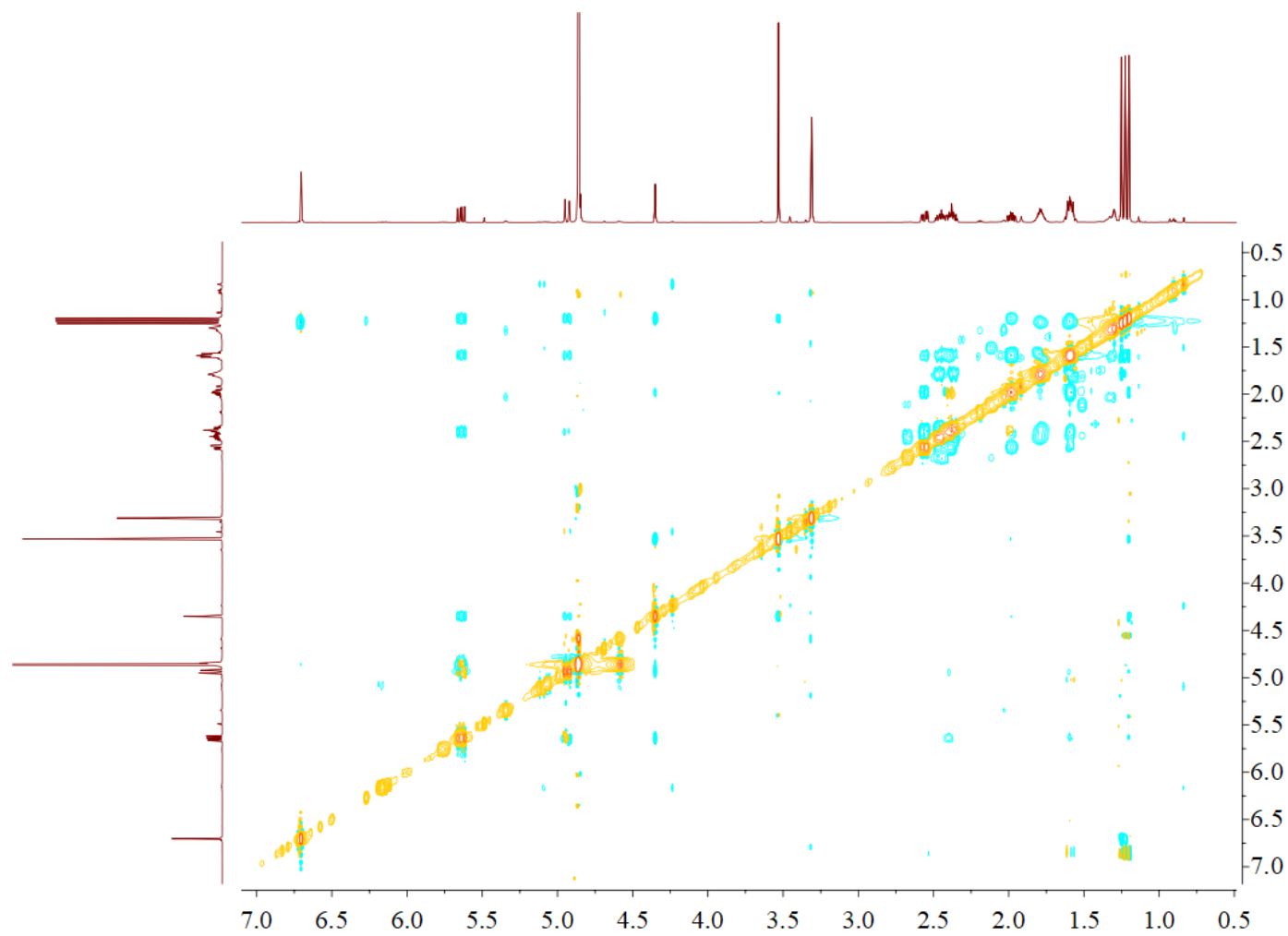


Fig. S44 NOESY spectrum (CD₃OD) of compound **4**.

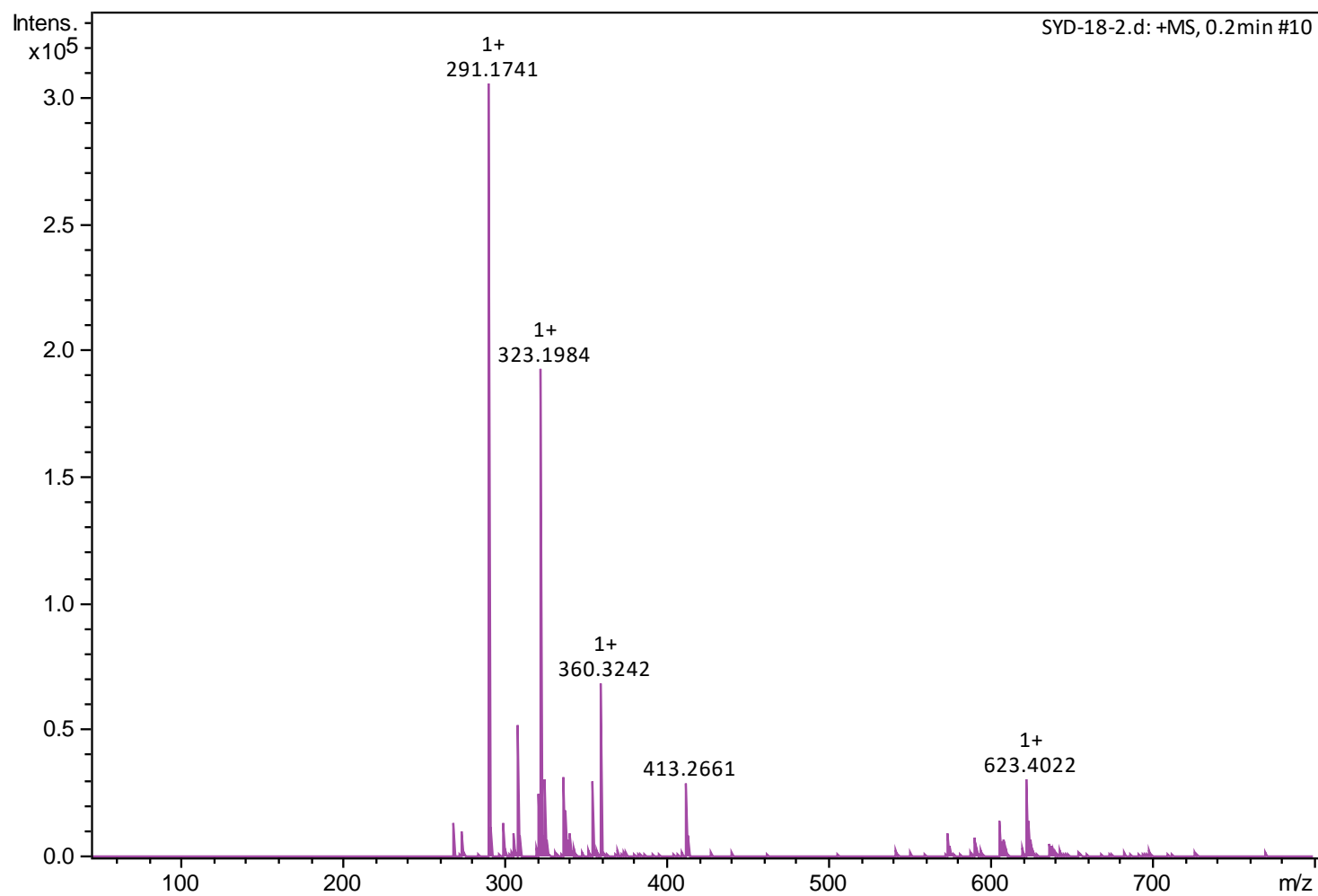


Fig. S45 HRTOFMS spectrum of compound 4.

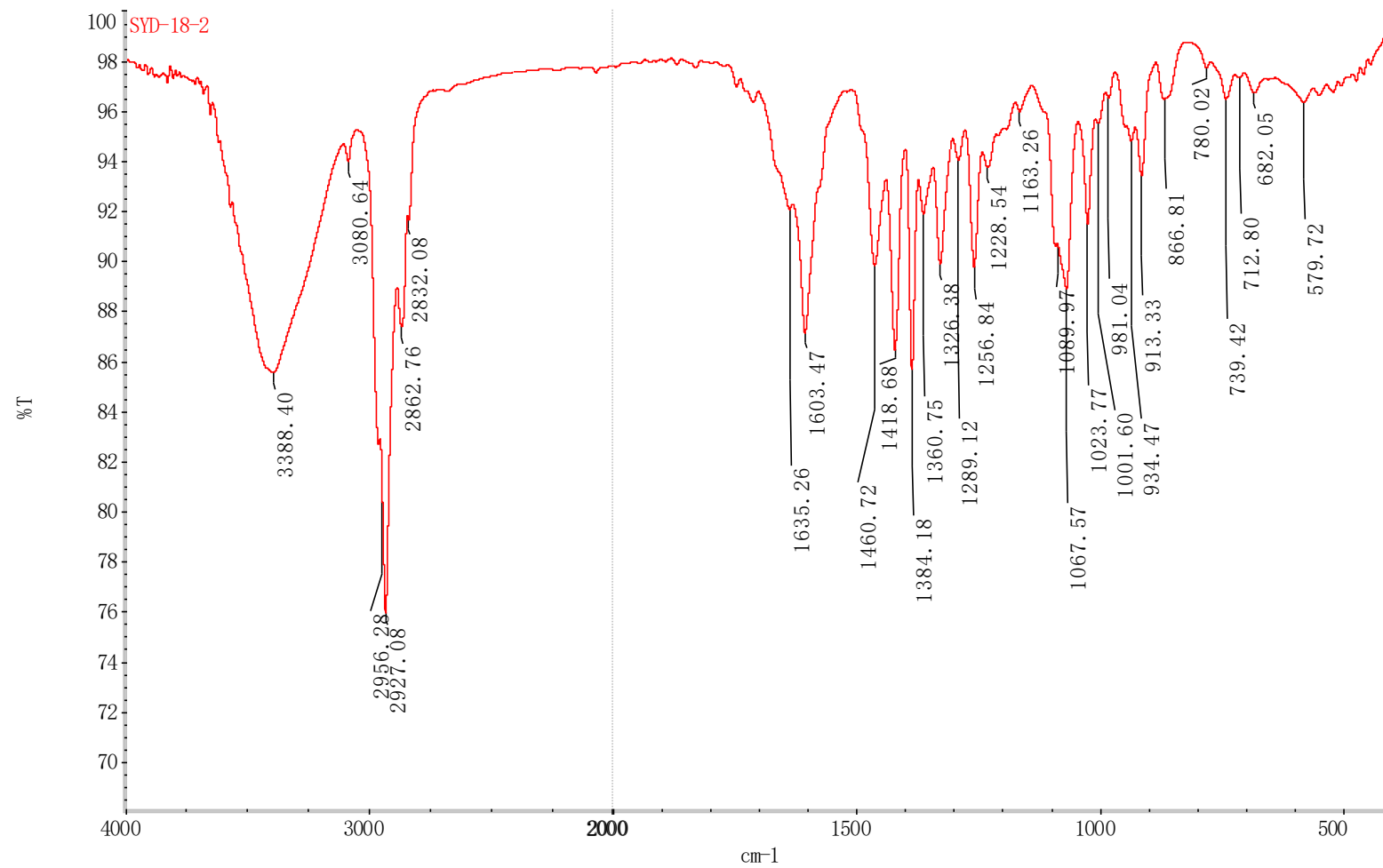


Fig. S46 IR spectrum of compound 4.

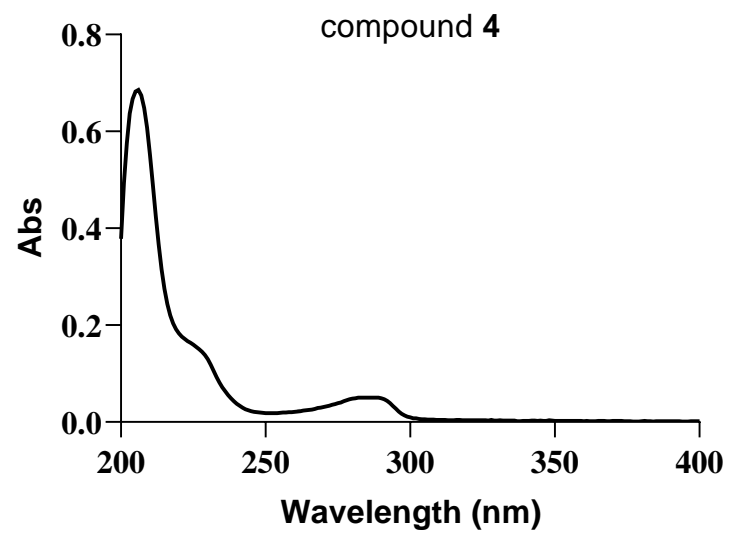


Fig. S47 UV spectrum of compound 4.

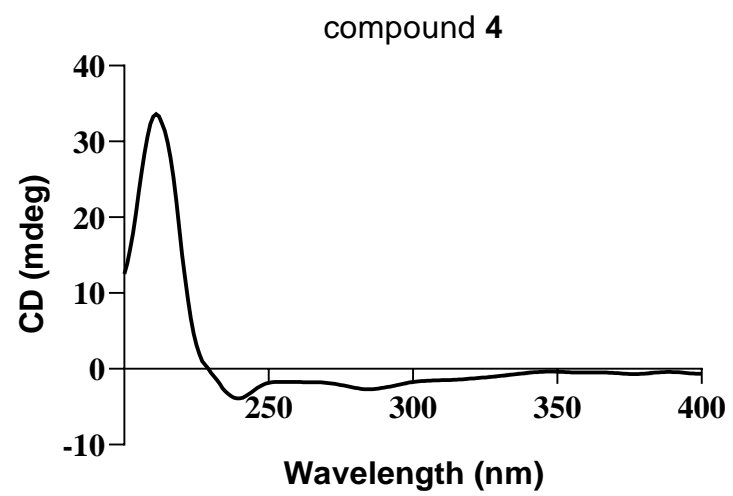


Fig. S48 CD spectrum of compound 4.

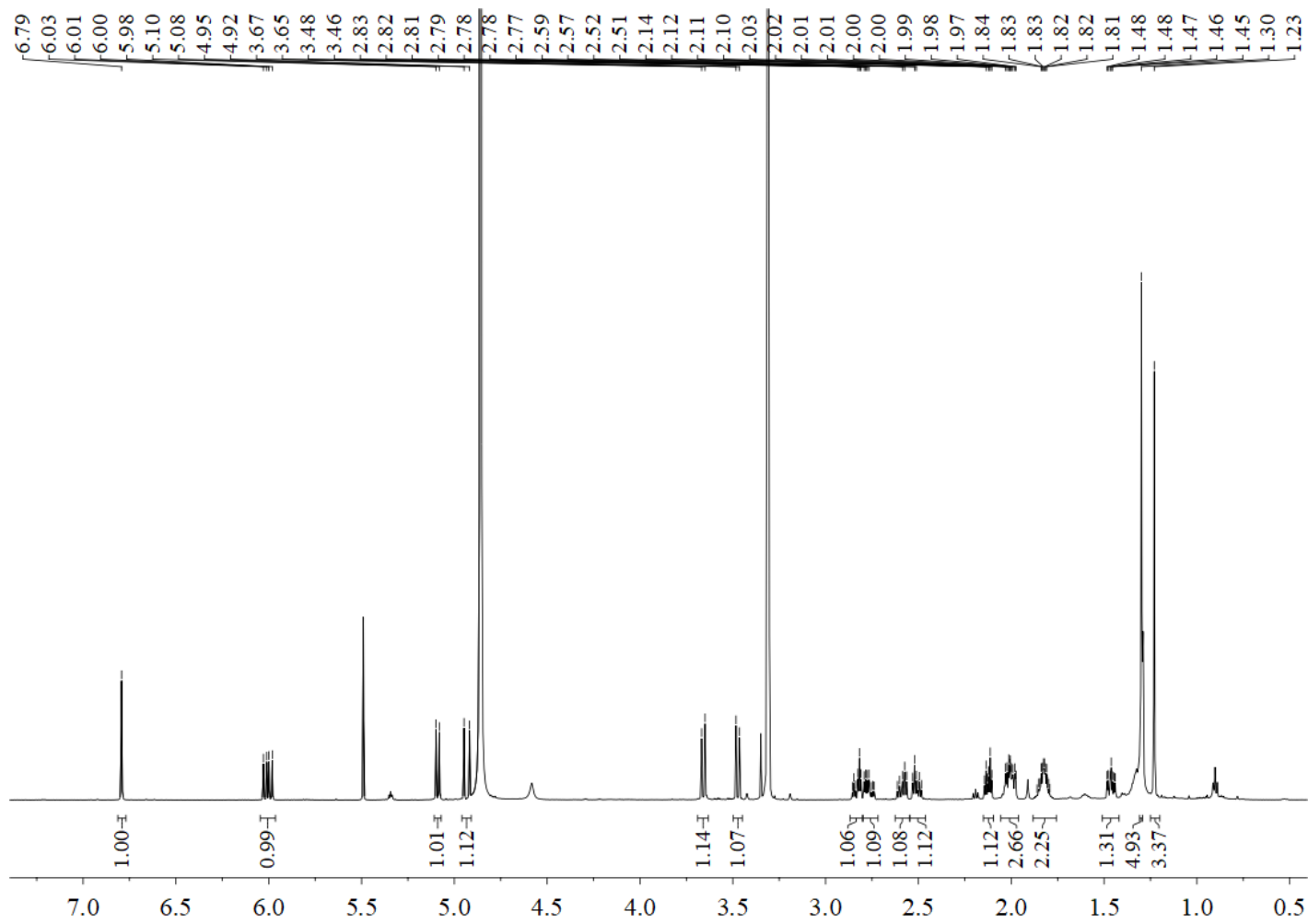


Fig. S49 $^1\text{H-NMR}$ (600 MHz, CD_3OD) spectrum of compound 5.

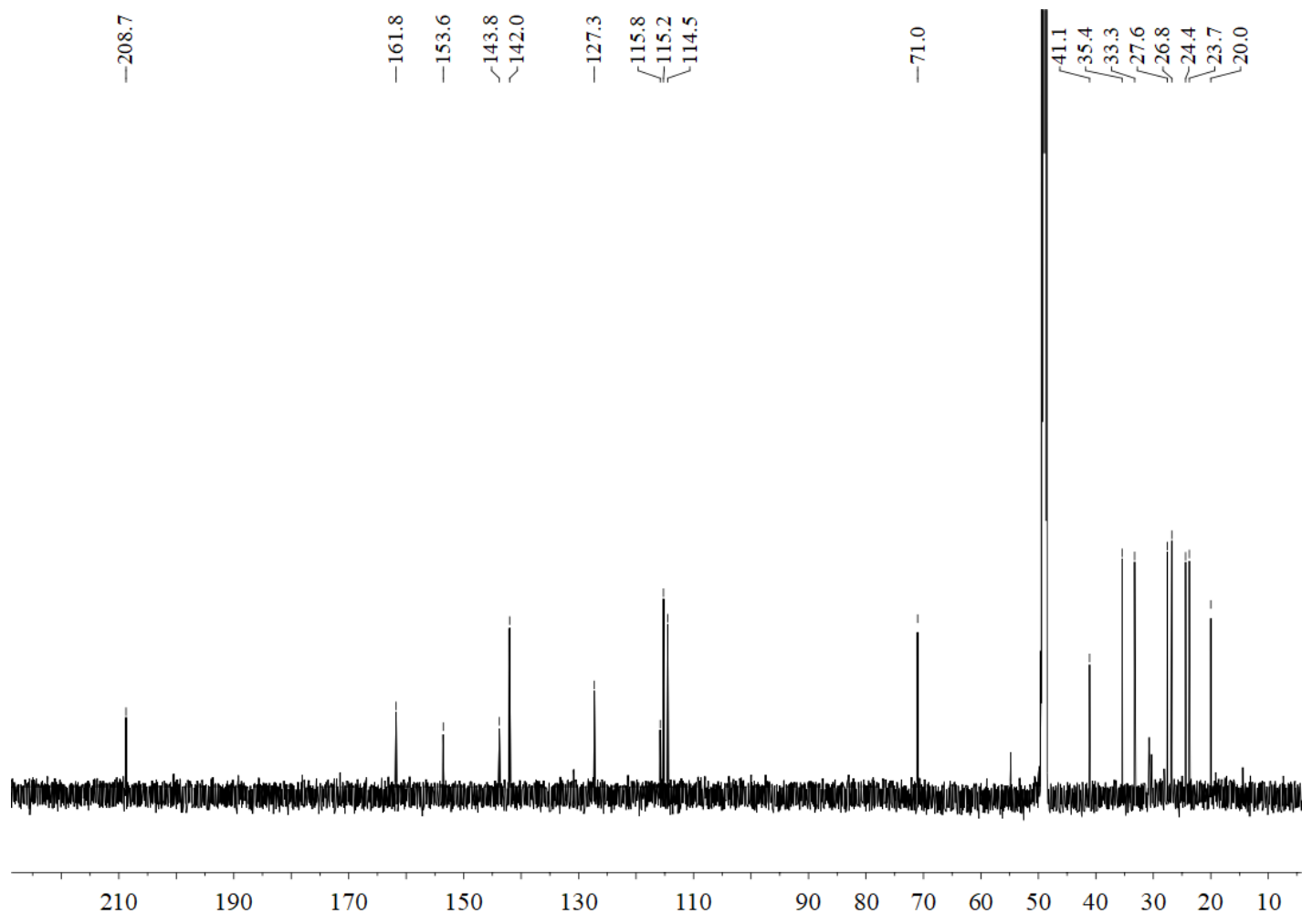


Fig. S50 ^{13}C -NMR (150 MHz, CD_3OD) spectrum of compound 5.

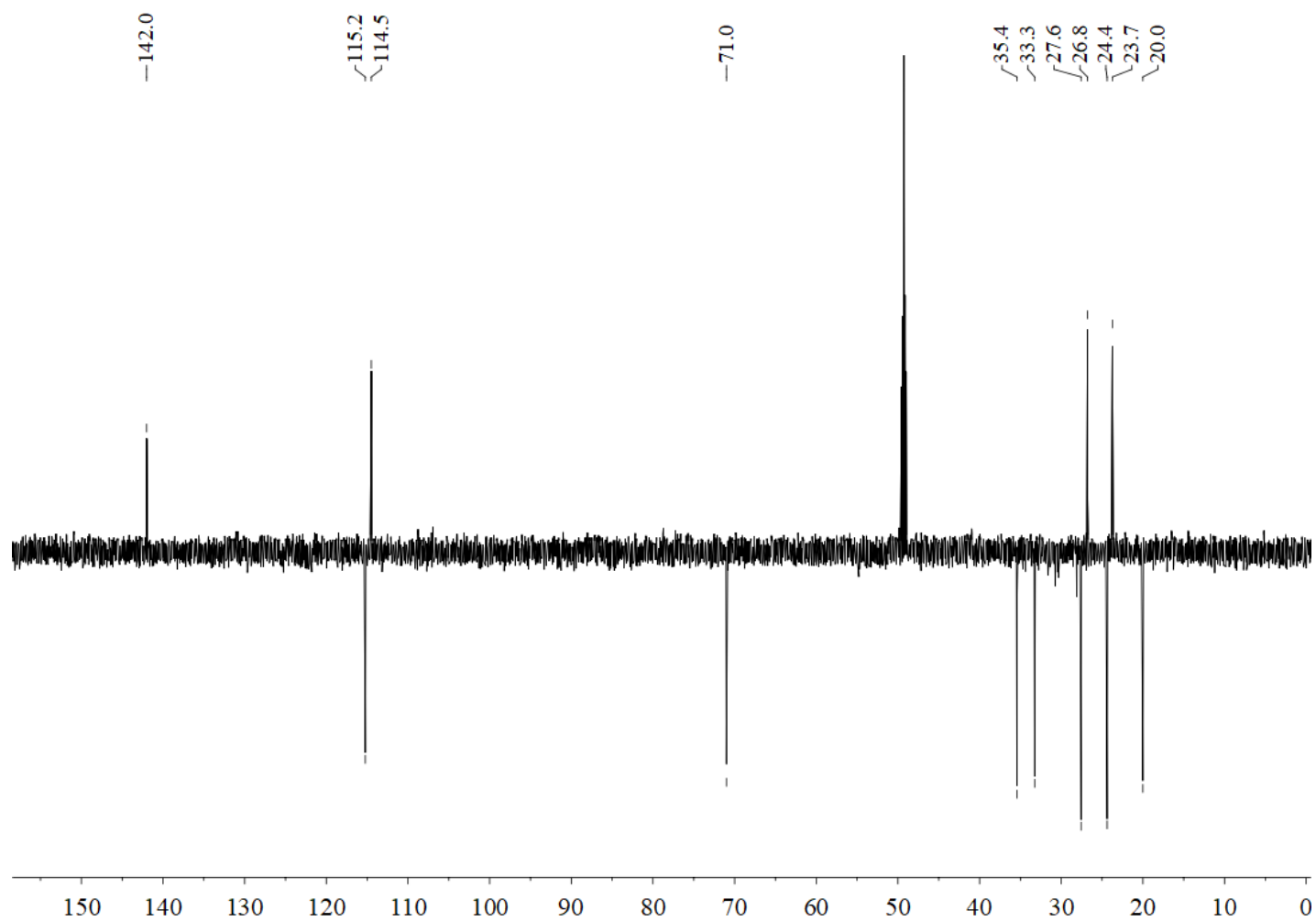


Fig. S51 DEPT-135 (150 MHz, CD₃OD) spectrum of compound 5.

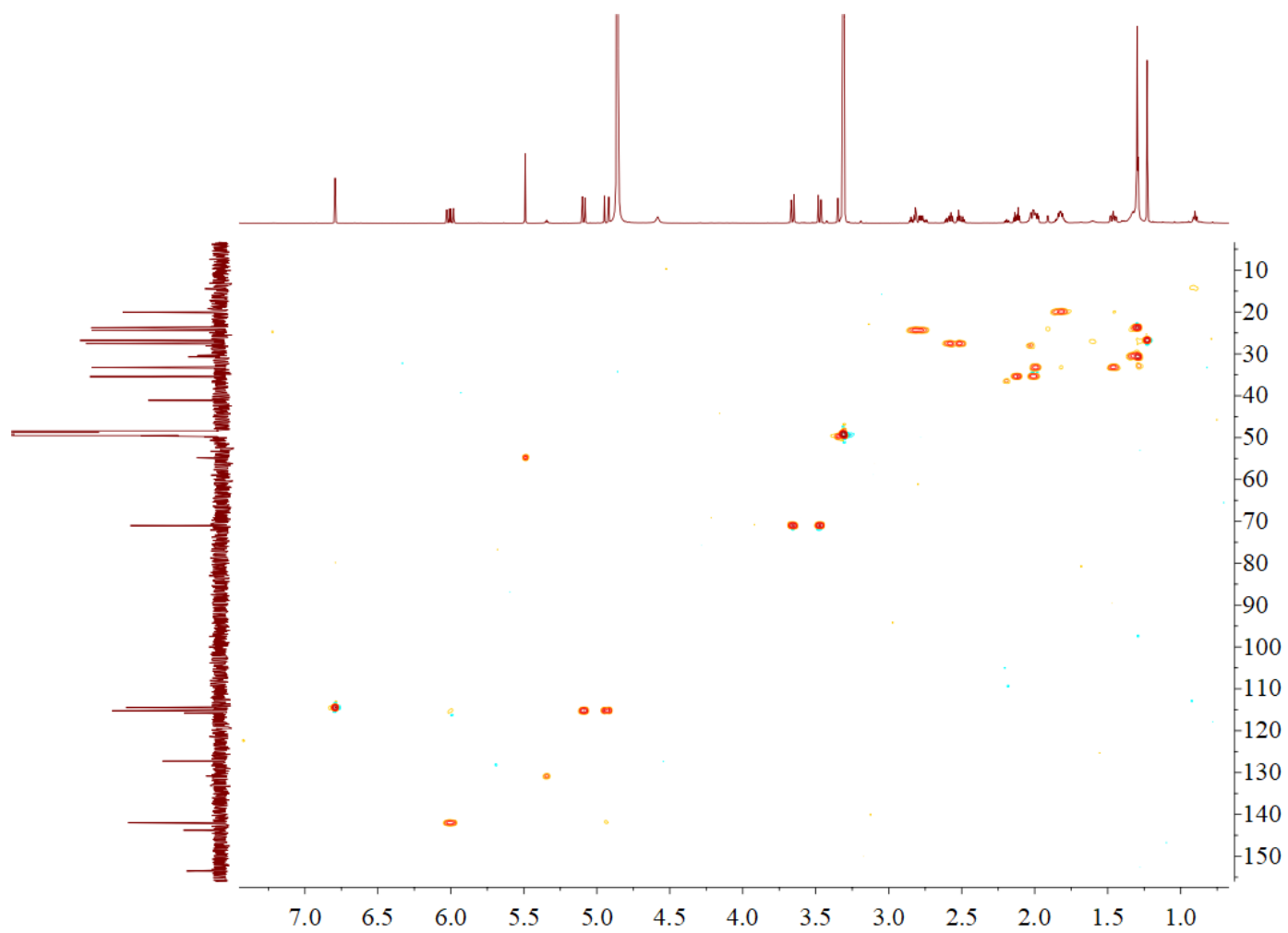


Fig. S52 HSQC spectrum (CD₃OD) of compound 5.

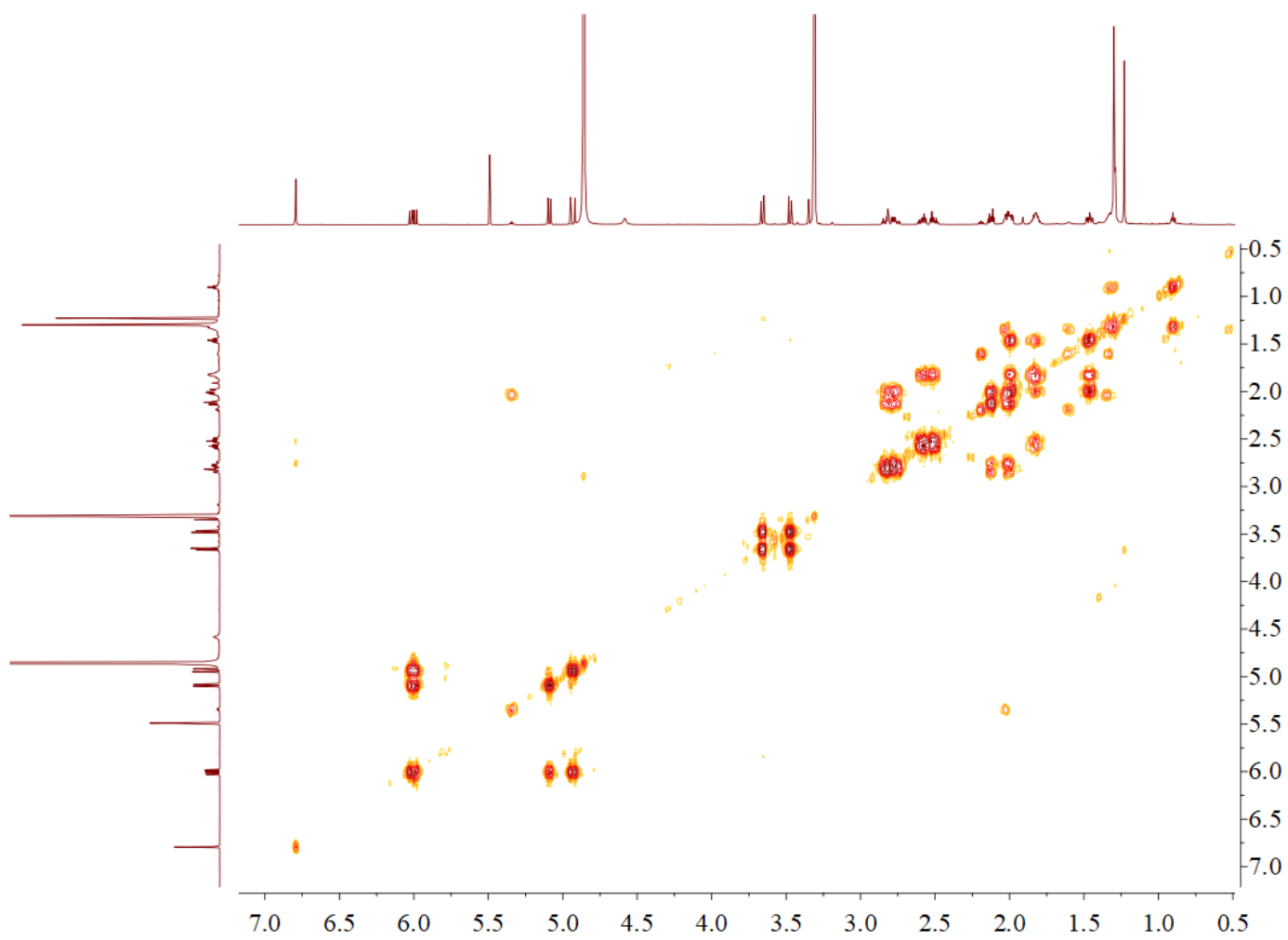


Fig. S53 ^1H - ^1H COSY spectrum (CD_3OD) of compound 5.

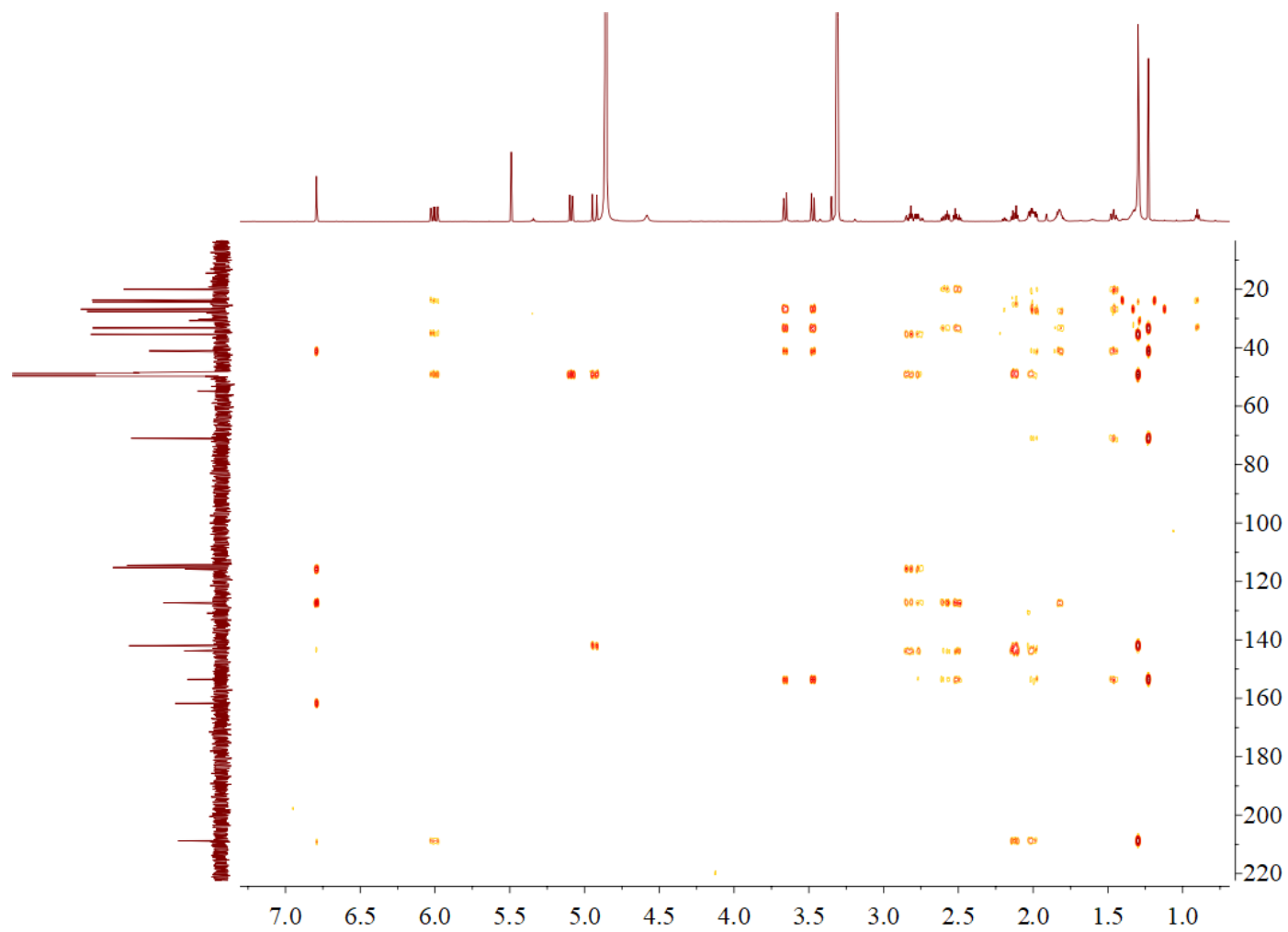


Fig. S54 HMBC spectrum (CD₃OD) of compound 5.

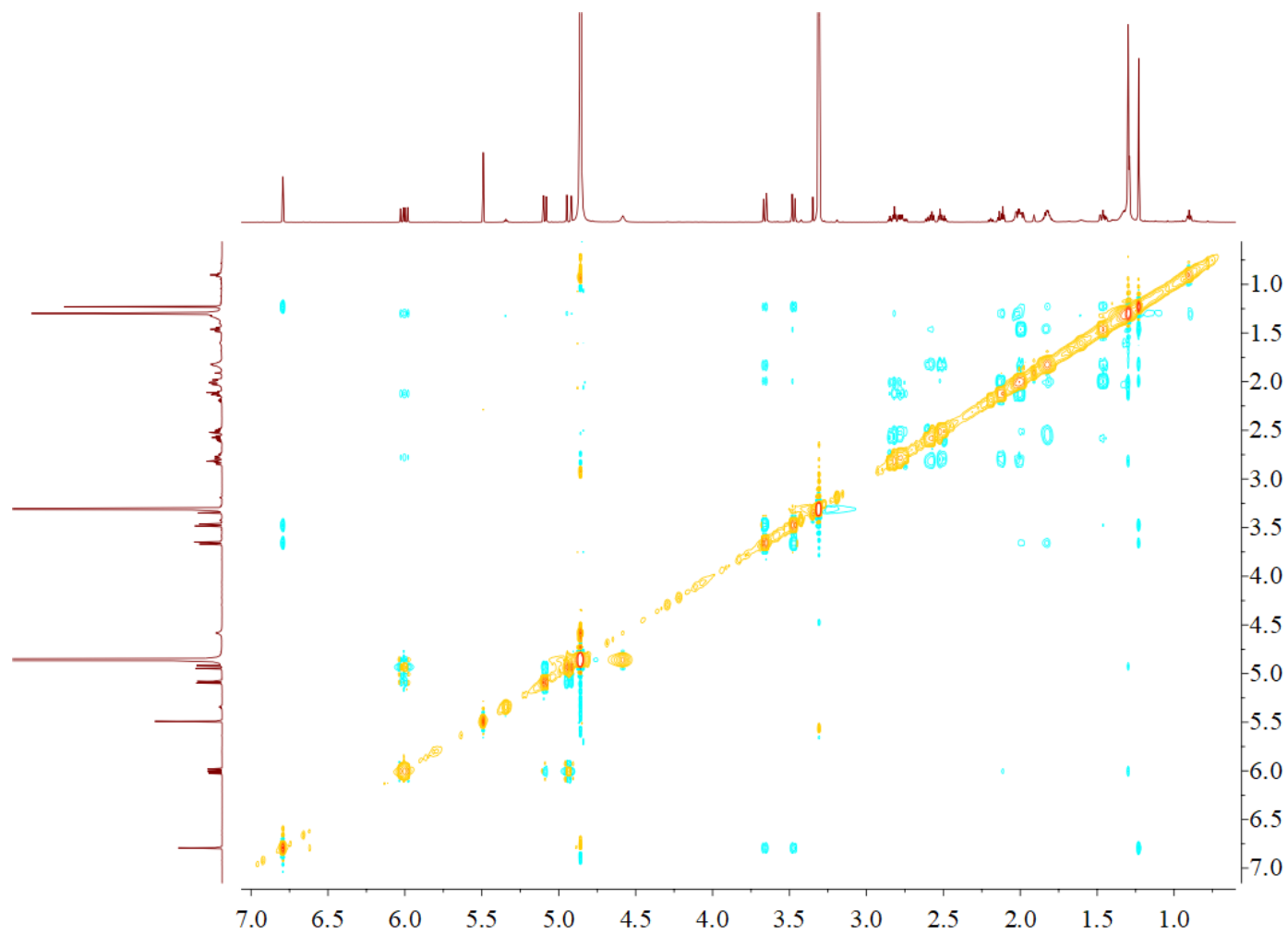


Fig. S55 NOESY spectrum (CD_3OD) of compound **5**.

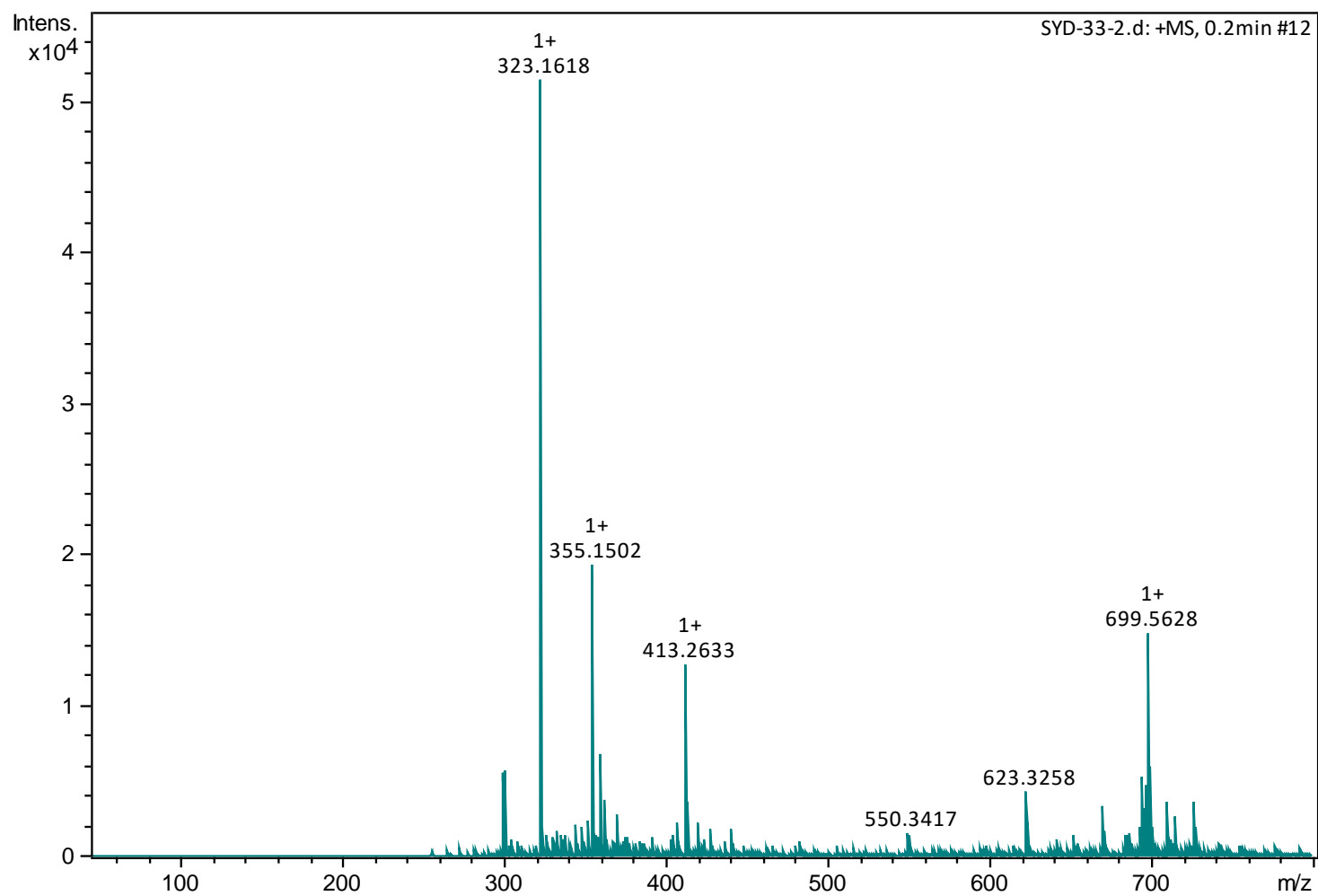


Fig. S56 HRTOFMS spectrum of compound 5.

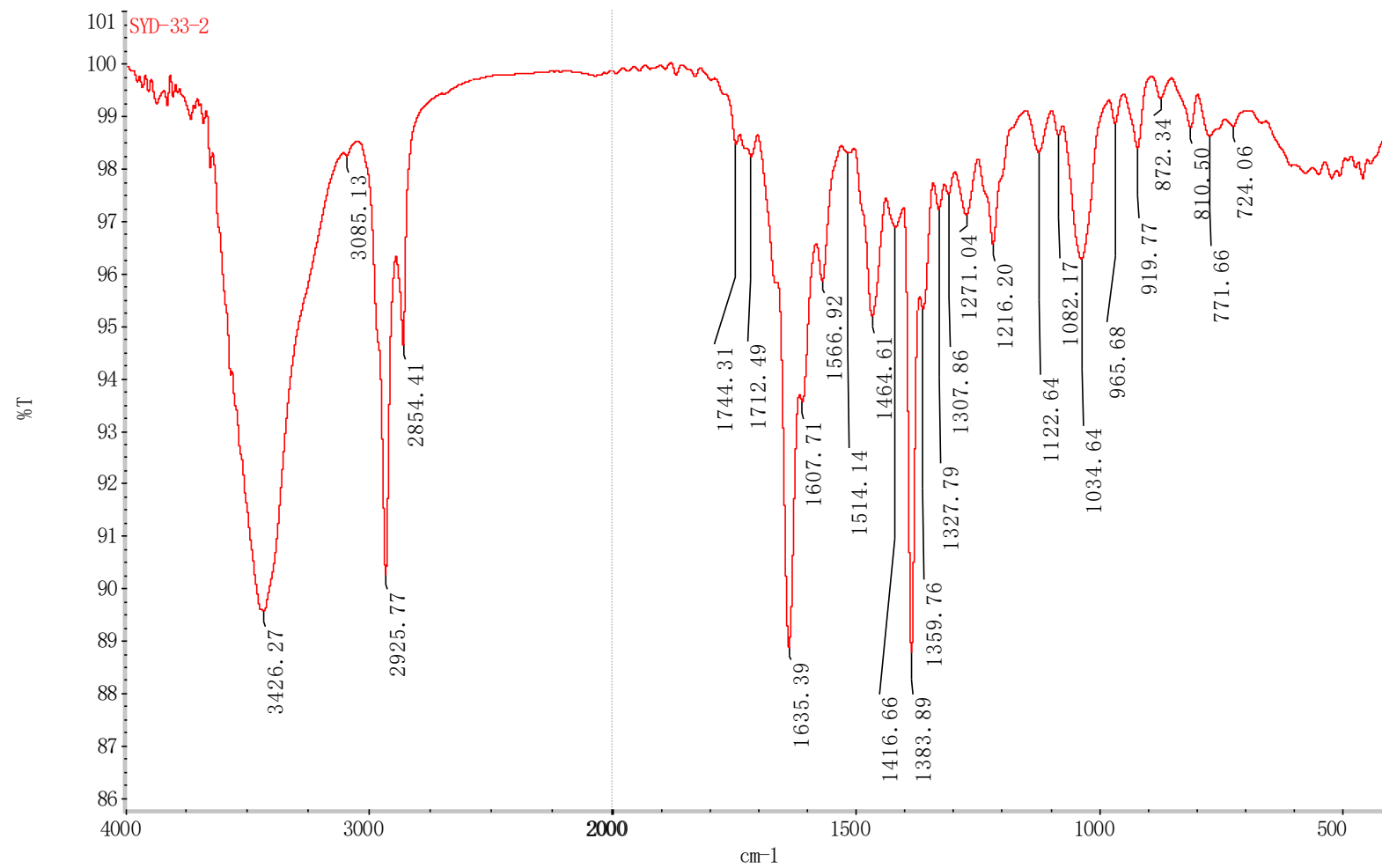


Fig. S57 IR spectrum of compound 5.

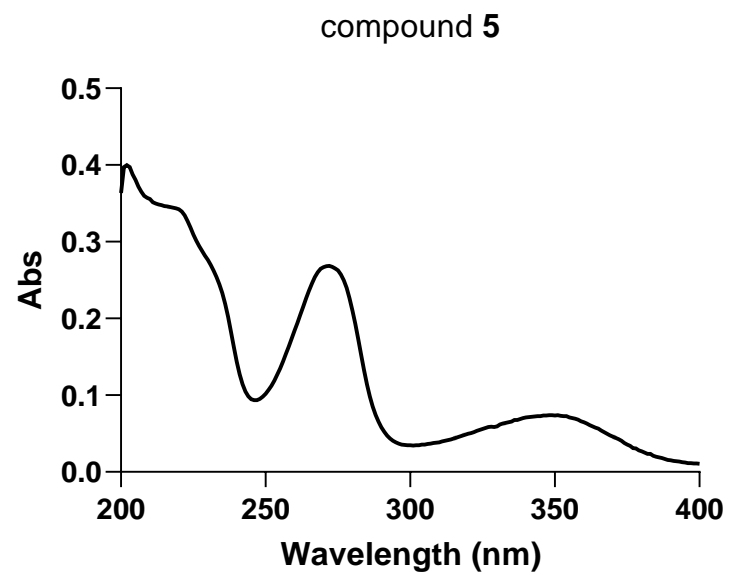


Fig. S58 UV spectrum of compound 5.

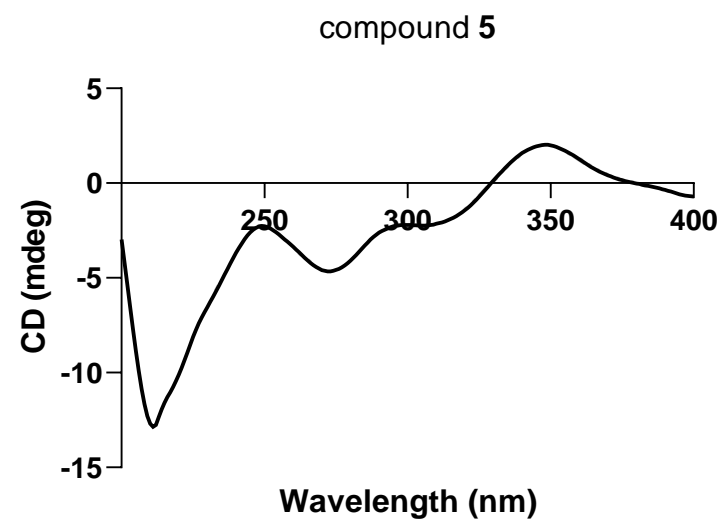


Fig. S59 CD spectrum of compound 5.