

SUPPLEMENTARY MATERIAL

A pair of enantiomeric 5-oxabicyclic[4.3.0]lactam derivatives and one new polyketide from the marine-derived fungus *Penicillium griseofulvum*

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Abstract

A pair of enantiomeric 4-oxabicyclic[4.3.0]lactam derivatives, (+)- and (–)-penicilactam A (**1**), and one new polyketide derivative penicitrinone F (**2**) were isolated from the marine-derived fungus *Penicillium griseofulvum* GT-10. Their structures and absolute configurations were elucidated through extensive spectroscopic analyses combined with the calculated ECD spectra. Penicitrinone F (**2**) had moderate inhibitory activity towards *Bacillus subtilis* with a MIC value of 6.3 μ M.

Keywords: *Penicillium griseofulvum*; bicyclic lactam; polyketide; antibacterial activity

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Jiankun Yang and Bin Zhang contributed equally in this work.

Experimental

General

NMR spectra were recorded on a Bruker Ascend 600 spectrometer (600 MHz for ^1H and 150 MHz for ^{13}C), HRMS analyses were performed with a Bruker Apex-Ultra 7.0T instrument, ECD spectra were measured by Biologic MOS450-SFM300 instrument, OR data measured by British Optical Active Corporation AA-55 automatic rotatory instrument. HPLC separation was performed with Shimadzu Corporation LC 20AT and LC 6AD. A Chiralpak ID semi-preparative HPLC column (10 × 250mm, 5 μm) and a Kromasil C_{18} semi-preparative HPLC column (250 × 10 mm, 5 μm) was used. Sephadex LH-20 (American Pharmacia) and RP- C_{18} (Japan YMC) were used for column chromatography. Precoated silica gel GF254 plates (Yantai Zifu Chemical Group Co.) were used for analytical TLC.

Fungal Material

The fungal strain GT-10 (GenBank access number KT781166) was isolated from marine water, which was collected from the South China Sea, and identified as *Penicillium griseofulvum*. by a molecular biological protocol calling for DNA amplification and ITS region sequence comparison with GenBank database. The strain was deposited in the Key Laboratory of Pharmaceutical Quality Control of Hebei Province, College of Pharmaceutical Sciences, Hebei University, Baoding, People's Republic of China.

Extraction and Isolation

The strain was inoculated in liquid medium (containing 20 g/L glucose, 10 g/L yeast extract, 10 g/L peptone and 33 g/L sea salt) at 28 °C for 3 days. Then medium inoculated with seed culture as scale-up cultivation was carried out under static condition at 28 °C for 43 days in 100 of 500 mL conical flasks containing the liquid medium (100 mL/flask) composed of glucose (10 g/L), maltose (20 g/L), mannitol (20 g/L), monosodium glutamate (10 g/L), KH_2PO_4 (0.5 g/L), $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ (0.3 g/L), corn steep liquor (1 g/L), yeast extract (3 g/L), and seawater. The fermented media were extracted five times with EtOAc. The combined EtOAc layers were evaporated to dryness under reduced pressure to give an EtOAc extract. The EtOAc extract (45.0 g) was subjected to a positive phase silica gel column eluting with petroleum ether-ethyl acetate solvent system at the ration of 100:1, 80:1, 50:1, 20:1, 5:1, 1:1 (v/v), respectively, to yield 6 fractions. Fraction 4 was subjected to Sephadx LH-20 CC

eluting with MeOH to offer three subfractions (Fr4.1-4.3), and then subfraction Fr.4.2 was purified by semi-preparative reversed-phase HPLC eluting with MeOH-H₂O (85:15) to give **1** (12.0 mg). Enantiomers (+)-**1** (6.0 mg) and (–)-**1** (6.0 mg) were prepared by semi-preparative positive-phase HPLC (DACEL, Chiralpak ID, 1.5 mL/min) eluting with PE-IPA (85:15). Fraction 2 was separated by Sephadex LH-20 CC to afford five subfractions. Subfraction Fr.2.2 was purified by a silica gel CC eluting with CHCl₃-MeOH at the ratios of 200:1, 120:1, 100:1, 80:1, 60:1 (v/v) to offer **2** (8.0 mg).

(±)-**Penicilactam A (1)**: a light yellow oil; IR (KBr) ν_{\max} cm⁻¹ 2918, 1731, 1650, 1450, 1434, 1288; UV (CH₂Cl₂) λ_{\max} (log ϵ): 237 (2.975), 246 (2.191), 261 (1.836), 297 (0.176); ¹H-NMR (600 MHz, CDCl₃, δ , ppm, *J*/Hz) δ : 5.43 (1H, m, H-15), 5.41 (1H, m, H-16), 5.21 (1H, dd, *J* = 4.8, 6.0 Hz, H-6), 3.72 (1H, m, H-9a), 3.42 (1H, m, H-9b), 2.30 (1H, m, H-7a), 2.27 (1H, m, H-11a), 2.18 (1H, m, H-11b), 2.12 (1H, m, H-7b), 1.99 (1H, m, H-8a), 1.98 (2H, m, H-14), 1.86 (1H, m, H-8b), 1.80 (3H, s, Me-10), 1.65 (3H, d, *J* = 5.4 Hz, Me-17), 1.52 (2H, m, H-12), 1.37 (2H, m, H-13); ¹³C-NMR (150 MHz, CDCl₃, δ , ppm) δ : 163.6 (C-4), 163.4 (C-2), 131.1 (C-15), 125.1 (C-16), 106.5 (C-3), 87.5 (C-6), 44.3 (C-9), 32.3 (C-14), 31.7 (C-7), 29.7 (C-11), 29.1 (C-13), 26.2 (C-12), 21.9 (C-8), 17.9 (Me-17), 10.1 (Me-10); HRESI-MS, *m/z*: 250.1798 [M + H]⁺ (calcd. for C₁₅H₂₄O₂N: 250.1802).

Penicitrinone F (2): a pale yellow solid; $[\alpha]_{\text{D}}^{20} = -16.3$ (*c* 2.0, CHCl₃); UV (MeOH) λ_{\max} (log ϵ): 190 (0.23), 286 (0.06) nm; ¹H-NMR (600 MHz, CDCl₃, δ , ppm, *J*/Hz) δ : 7.87 (1H, s, 2-OCHO), 5.49 (1H, dq, *J* = 10.2, 6.0 Hz, H-2), 3.07 (1H, dq, *J* = 9.0, 7.0 Hz, H-3), 2.11 (3H, s, Me-2'), 1.92 (3H, s, Me-4'), 1.39 (3H, d, *J* = 6.0 Hz, Me-1), 1.28 (3H, d, *J* = 7.2 Hz, Me-4'); ¹³C-NMR (150 MHz, CDCl₃, δ , ppm) δ : 187.4 (C-3'), 182.9 (C-6'), 150.8 (C-5'), 144.9 (C-2'), 140.2 (C-1'), 116.5 (C-4'), 72.9 (C-2), 40.1 (C-3), 19.1 (Me-1), 15.5 (Me-4), 12.6 (Me-2'), 8.39 (Me-4'); HRESIMS, *m/z*: 253.1074 [M + H]⁺ (calcd. for C₁₃H₁₇O₅, 253.1071)

Biological assays

Antibacterial activity was evaluated by the conventional broth dilution assay. *Bacillus subtilis*, *Micrococcus lysodeikticus* and *Vibrio anguillarum* were used, and ciprofloxacin was used as a positive control.

Computational Methods

Conformational searches were performed using MMFF94S force field for (6*R*)-**1** and (6*S*)-**1**, respectively. All geometries (181 low energy conformers for (6*R*)-**1** and (6*S*)-**1**,

respectively) with relative energy from 0-10.0 kcal/mol were used in optimizations at the B3LYP/6-31G(d) level using Gaussian09 package. The B3LYP/6-31G(d)-optimized conformers (120 low energy conformers for (6*R*)-**1** and 123 for (6*S*)-**1**) with relative energy from 0 to 2.5 kcal/mol were then re-optimized at the B3LYP/6-311+G(d) level. ECD calculations were carried out at the B3LYP/6-311++G(2d,p) level in the gas phase. Boltzmann statistics were performed for ECD simulations with a standard deviation of 0.5 eV using Gibbs free energy.

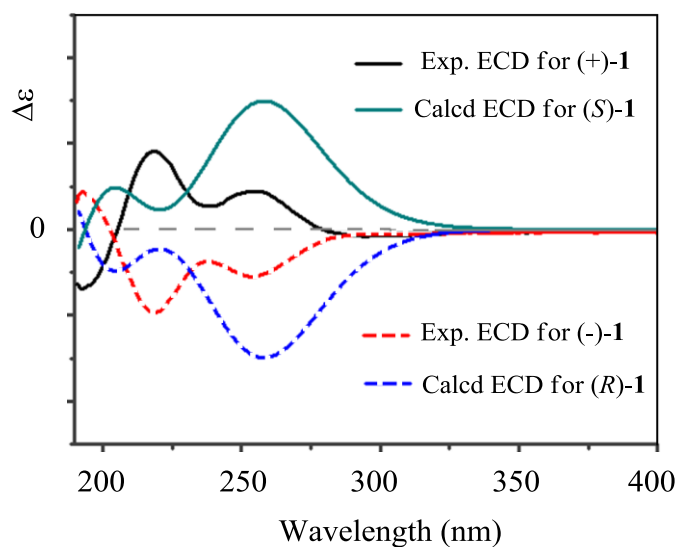


Figure S1. Comparison of experiment ECD for (\pm)-**1** and calculated ECD for 6*R*/6*S*-**1**.

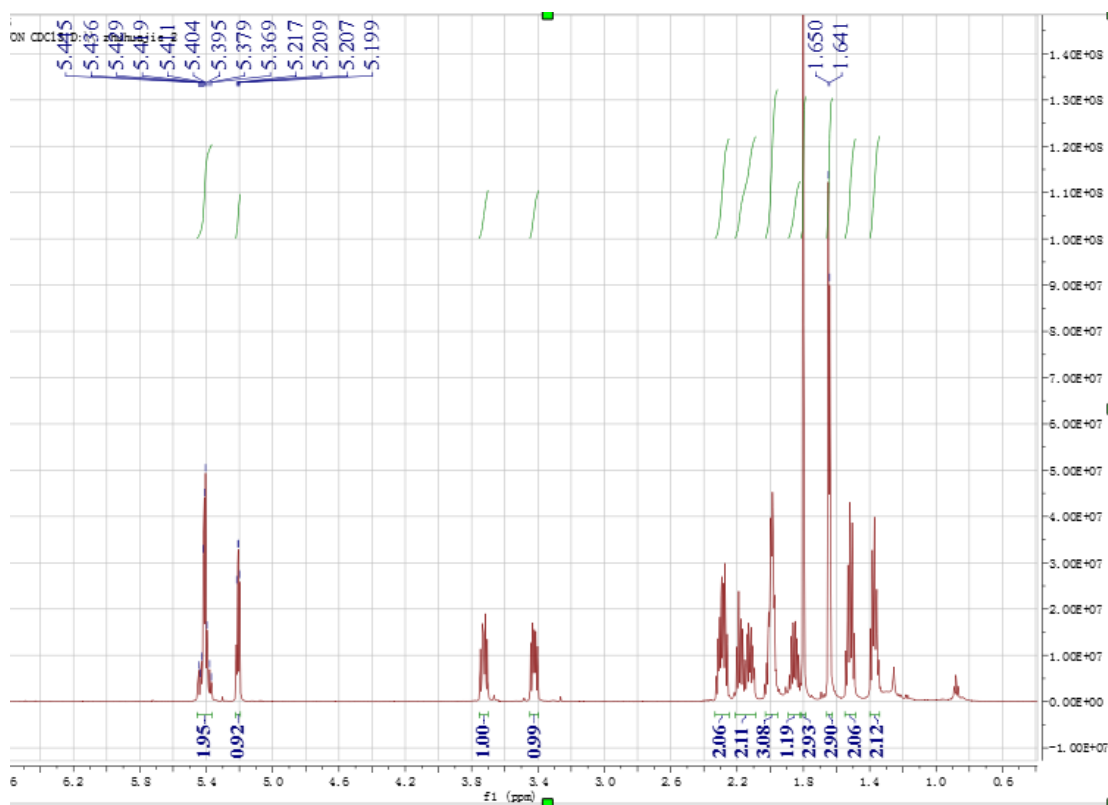


Figure S2. ^1H NMR in CDCl_3 for compound **1**

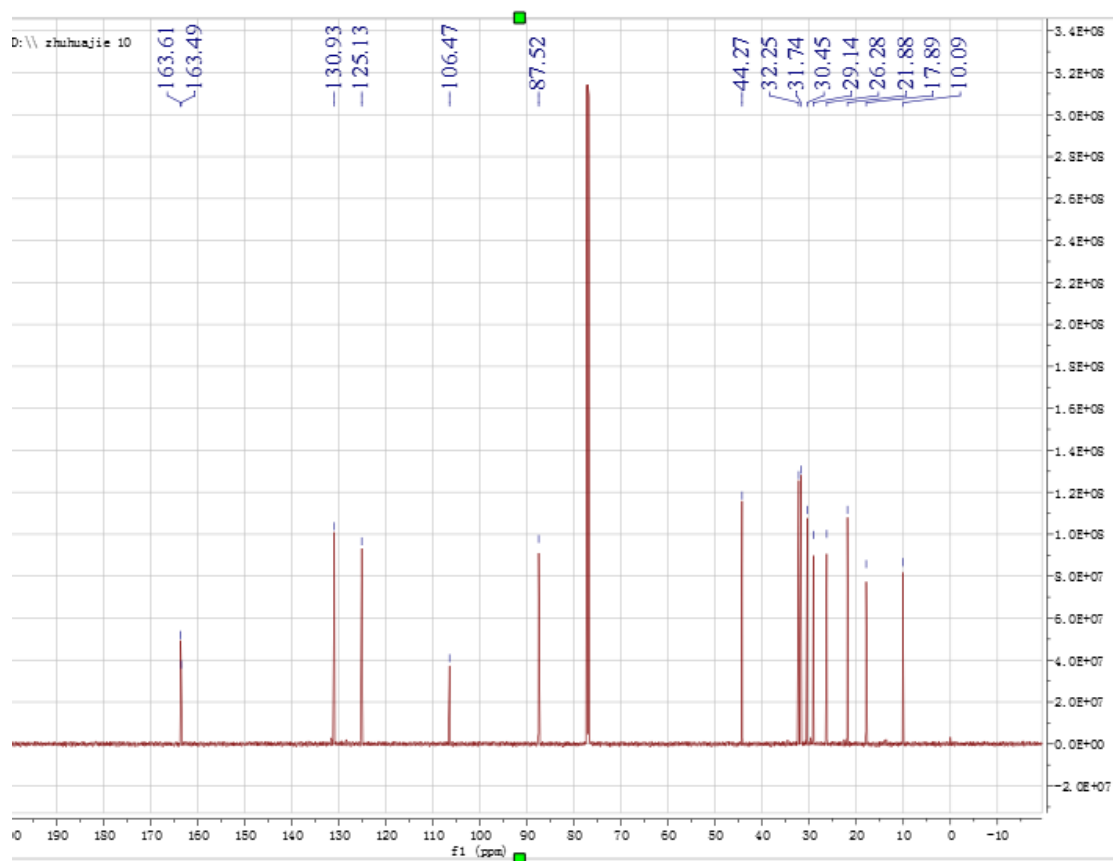


Figure S3. ^{13}C NMR in CDCl_3 for compound **1**

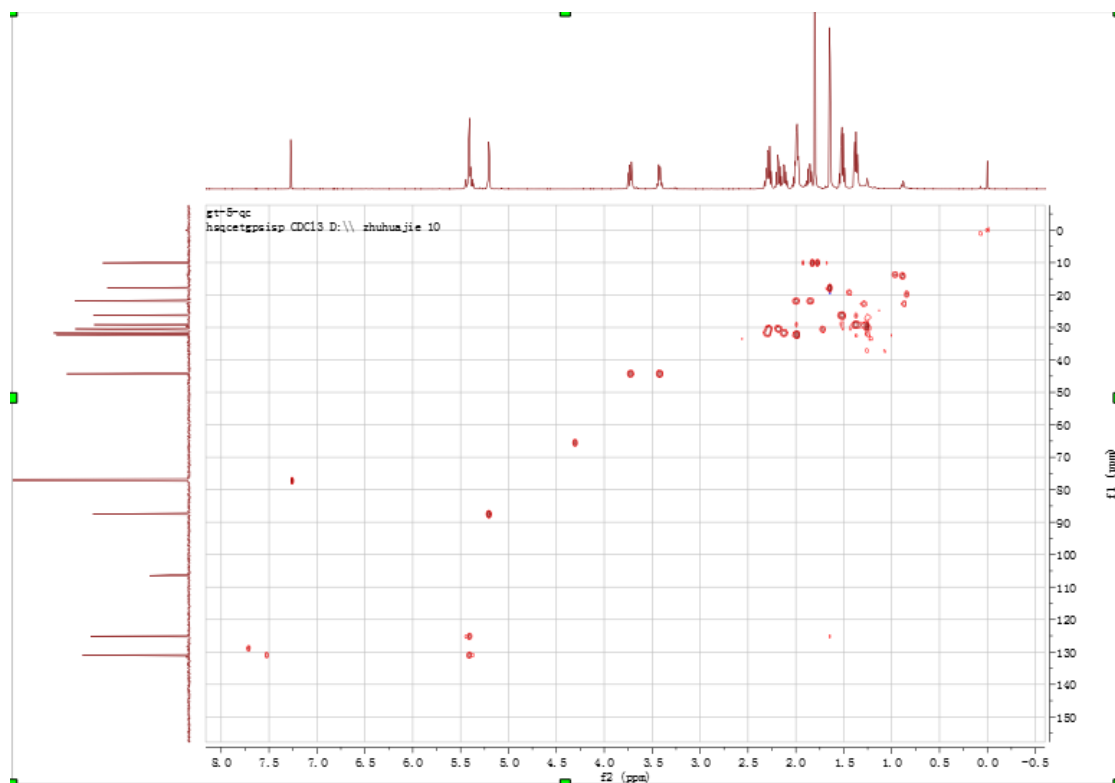


Figure S4. HMBC in CDCl_3 for compound **1**

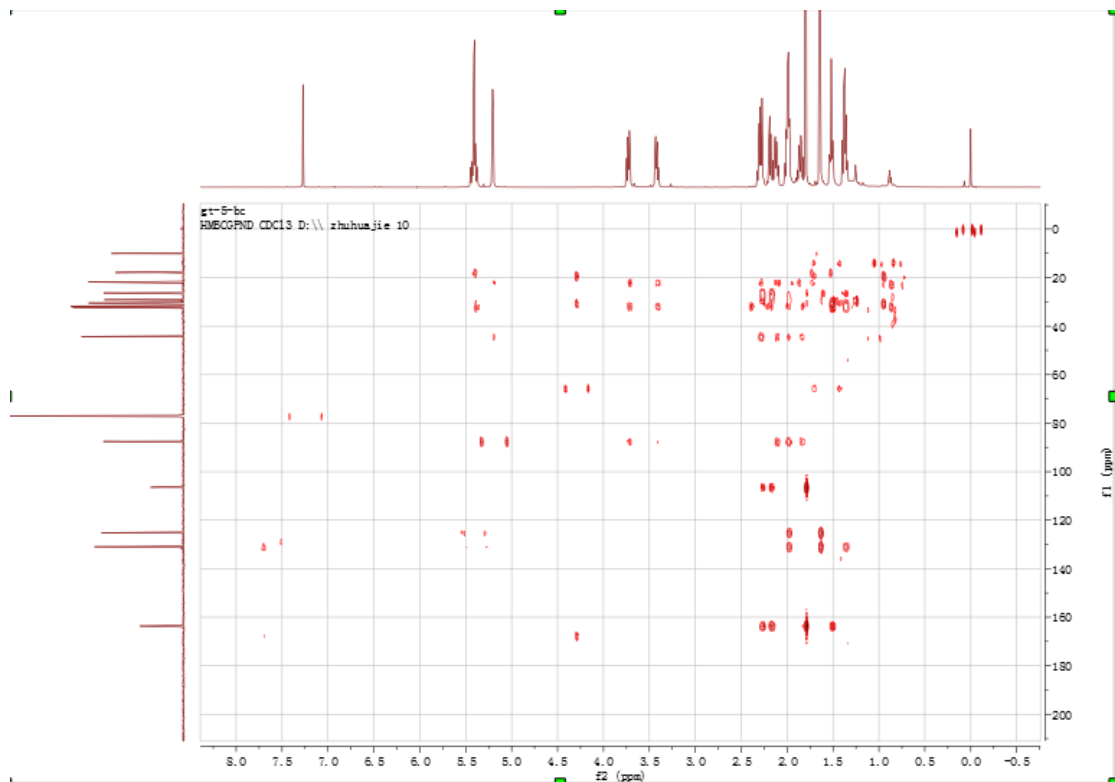


Figure S5. HMBC in CDCl_3 for compound **1**

2061230-GT-5_161230153859 #11 RT: 0.16 AV: 1 NL: 1.24E8
T: FTMS + p ESI Full ms [150.00-1000.00]

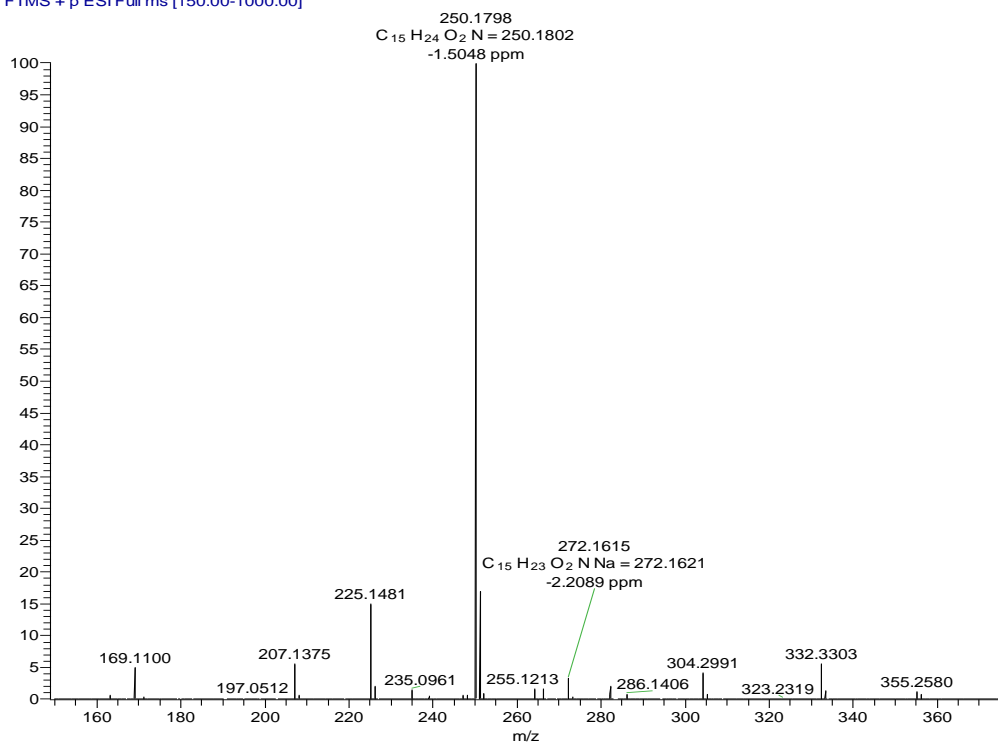


Figure S6. HRESIMS for compound 1

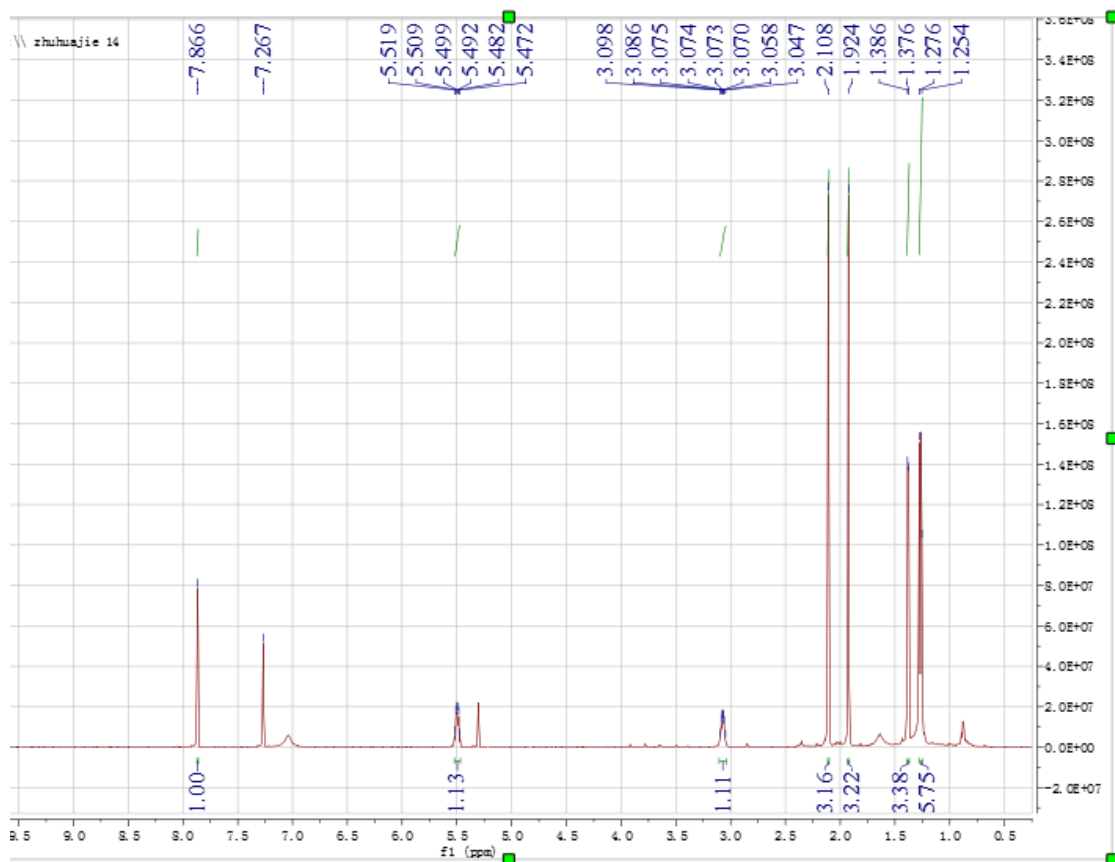


Figure S7. 1H NMR in $CDCl_3$ for compound 2

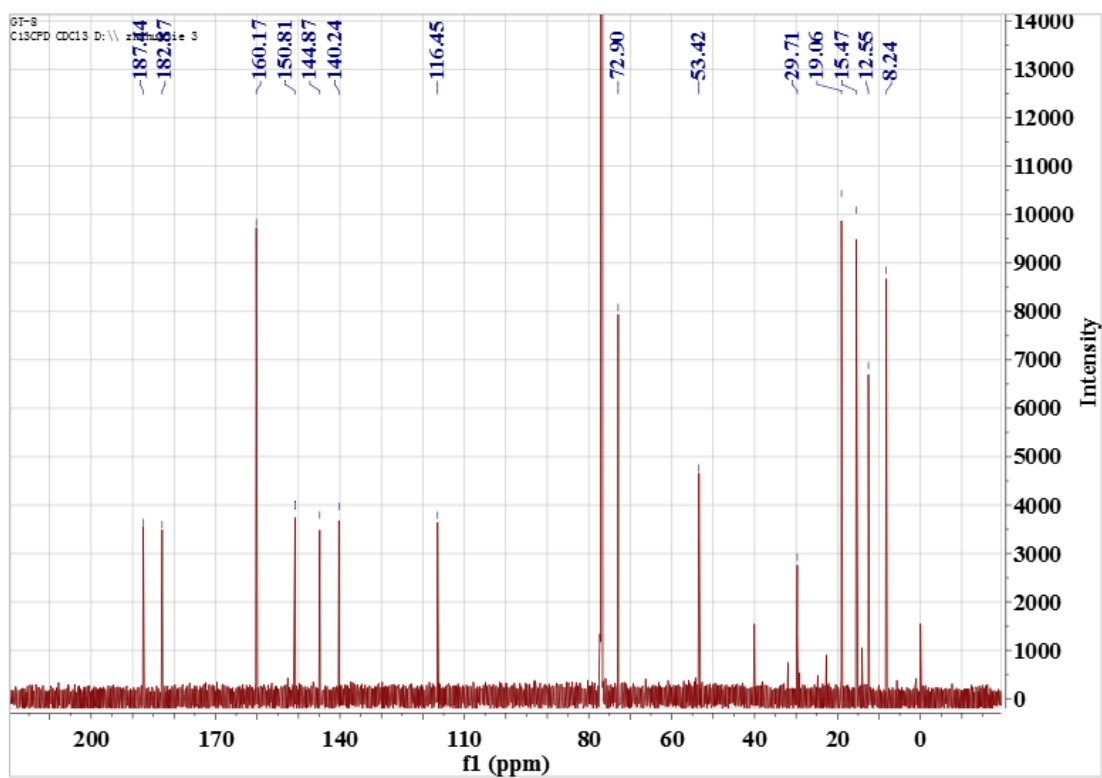


Figure S8. ^{13}C NMR in CDCl_3 for compound 2

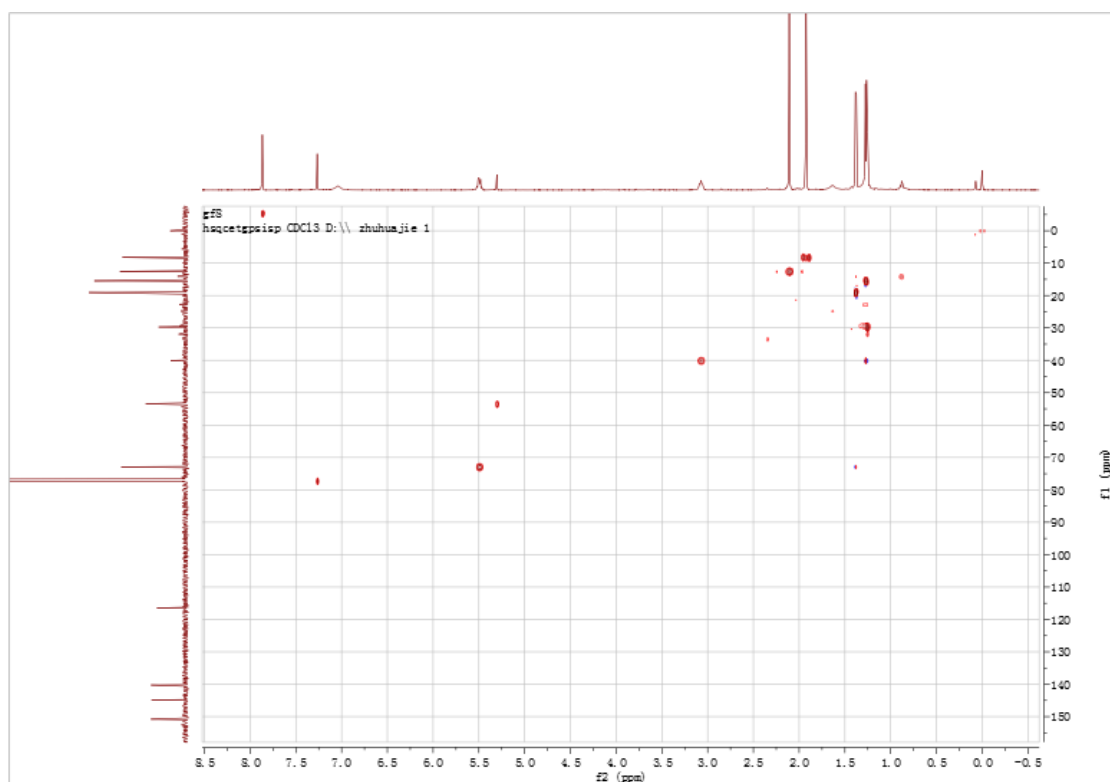


Figure S9. HMQC in CDCl_3 for compound 2

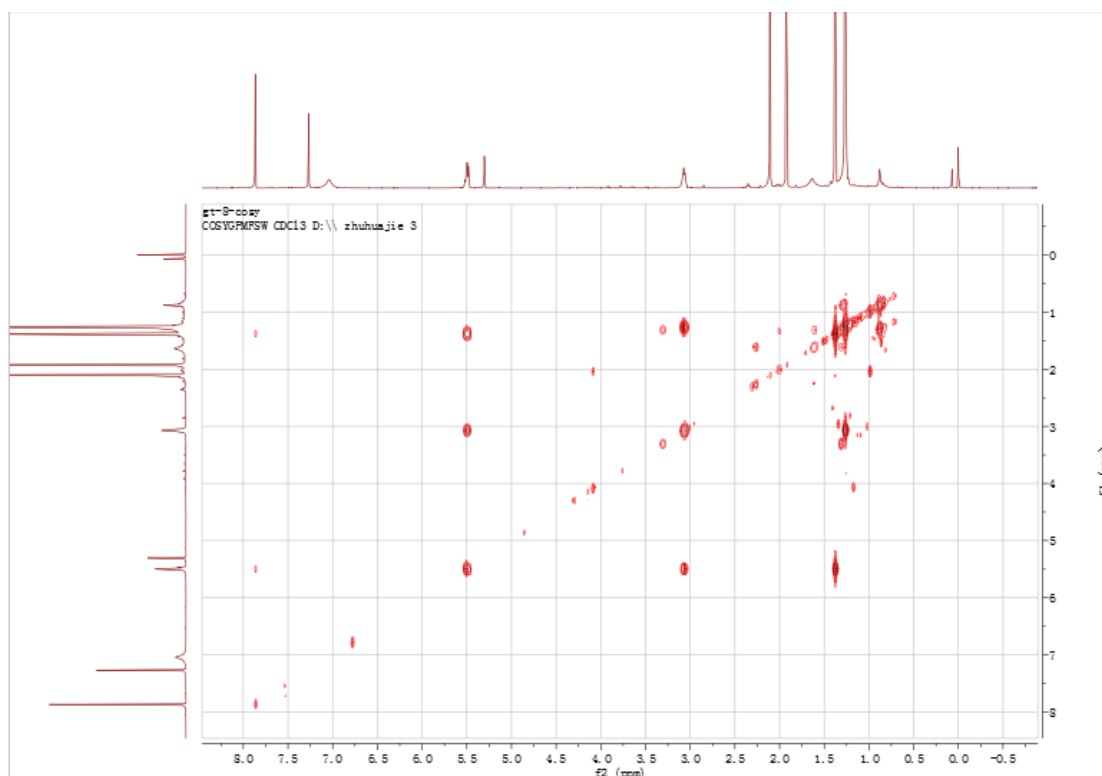


Figure S10. ^1H - ^1H COSY in CDCl_3 for compound 2

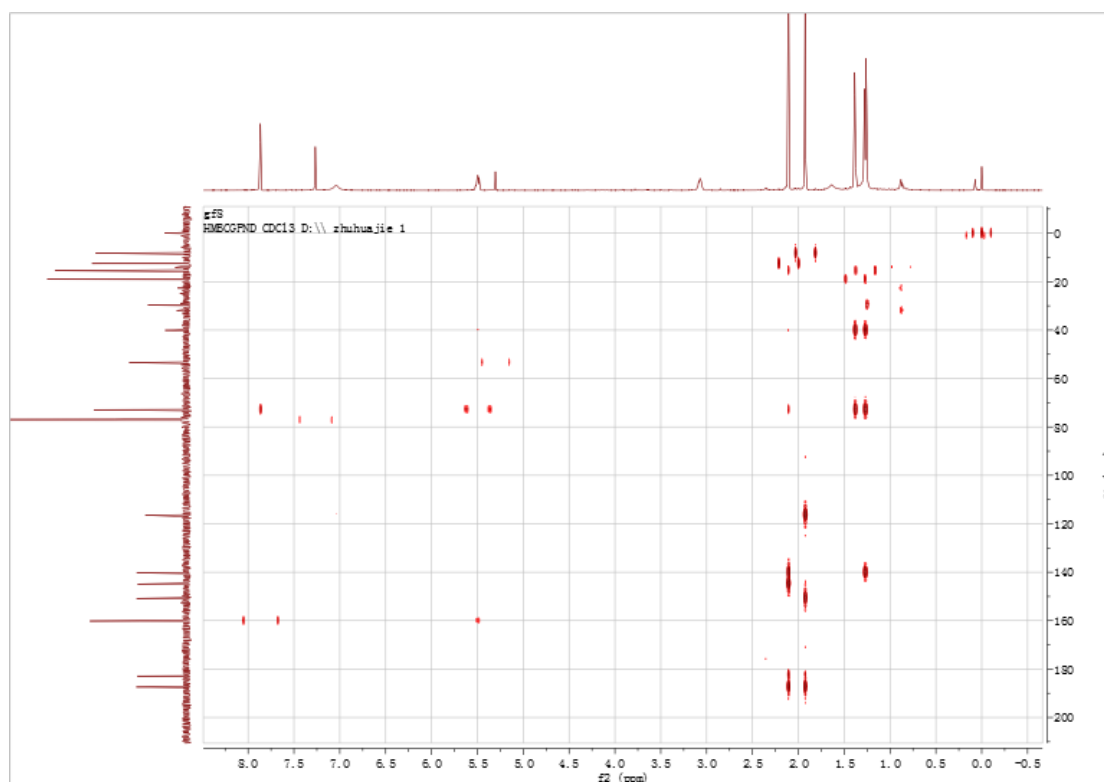


Figure S11. HMBC in CDCl_3 for compound 2

20160921-GF6_160921155120 #59.60 RT: 0.49-0.50 AV: 2 NL: 2.50E5
T: FTMS + p ESI Full ms [100.00-2000.00]

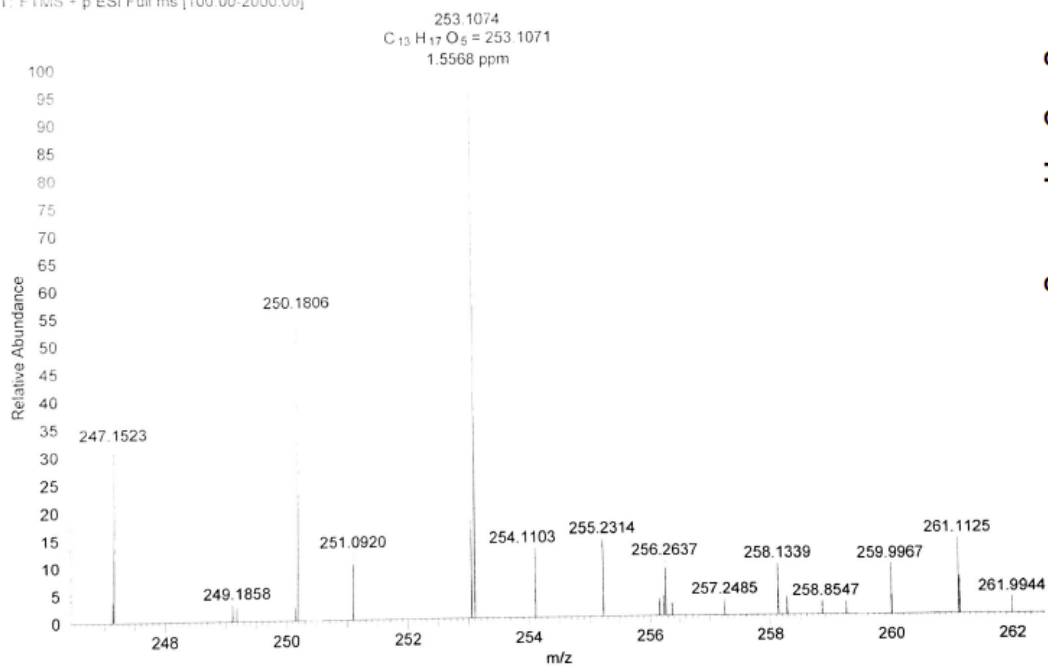


Figure S12. HRESIMS for compound **2**

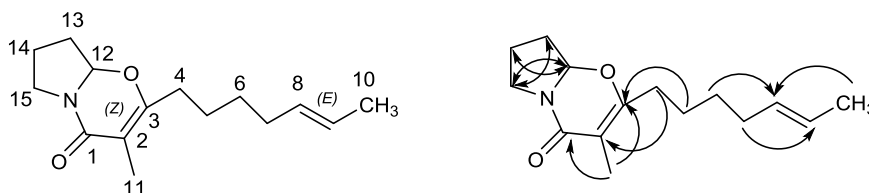


Figure S13 Key HMBC (\rightarrow) correlation of **1**

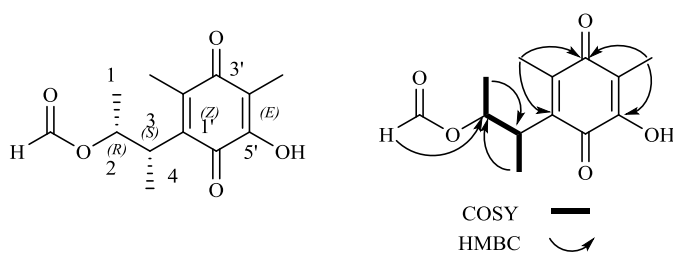


Figure S14 COSY (—) and key HMBC (\rightarrow) correlation of **2**

Table S1. Calculated ECD for 1a

Wavenumber	ECD	Wavelength	ECD	Wavelength	ECD
190.74483	-2.19308	202.19201	3.70146	215.10087	2.30725
190.97988	-2.01682	202.45614	3.73869	215.39983	2.26033
191.21552	-1.84048	202.72096	3.77131	215.69962	2.21524
191.45173	-1.66422	202.98648	3.79938	216.00024	2.17211
191.68853	-1.48822	203.25269	3.82294	216.30171	2.13108
191.92591	-1.31262	203.51960	3.84205	216.60402	2.09226
192.16389	-1.13759	203.78721	3.85679	216.90717	2.05579
192.40245	-0.96330	204.05553	3.86722	217.21118	2.02177
192.64161	-0.78992	204.32456	3.87342	217.51604	1.99032
192.88136	-0.61760	204.59429	3.87549	217.82175	1.96155
193.12171	-0.44652	204.86474	3.87352	218.12833	1.93555
193.36266	-0.27684	205.13590	3.86761	218.43577	1.91243
193.60422	-0.10872	205.40779	3.85788	218.74407	1.89228
193.84637	0.05767	205.68039	3.84443	219.05325	1.87517
194.08914	0.22217	205.95372	3.82739	219.36331	1.86121
194.33251	0.38462	206.22778	3.80689	219.67424	1.85046
194.57649	0.54486	206.50257	3.78306	219.98605	1.84300
194.82109	0.70274	206.77809	3.75603	220.29876	1.83889
195.06630	0.85809	207.05434	3.72595	220.61235	1.83820
195.31213	1.01078	207.33134	3.69296	220.92684	1.84098
195.55858	1.16066	207.60908	3.65722	221.24222	1.84730
195.80565	1.30757	207.88756	3.61888	221.55851	1.85719
196.05335	1.45138	208.16679	3.57810	221.87570	1.87070
196.30168	1.59195	208.44677	3.53504	222.19380	1.88788
196.55063	1.72915	208.72751	3.48986	222.51281	1.90875
196.80022	1.86286	209.00900	3.44274	222.83275	1.93335
197.05045	1.99295	209.29126	3.39383	223.15360	1.96169
197.30131	2.11930	209.57427	3.34332	223.47538	1.99381
197.55280	2.24181	209.85806	3.29137	223.79809	2.02971
197.80495	2.36037	210.14261	3.23816	224.12173	2.06942
198.05773	2.47487	210.42794	3.18386	224.44631	2.11293
198.31116	2.58523	210.71404	3.12865	224.77183	2.16025
198.56525	2.69136	211.00092	3.07269	225.09829	2.21138
198.81998	2.79318	211.28858	3.01618	225.42571	2.26631
199.07537	2.89061	211.57703	2.95927	225.75408	2.32504
199.33142	2.98359	211.86627	2.90215	226.08341	2.38755
199.58812	3.07207	212.15630	2.84498	226.41370	2.45382
199.84549	3.15598	212.44712	2.78794	226.74495	2.52384
200.10352	3.23529	212.73874	2.73119	227.07718	2.59757
200.36222	3.30997	213.03117	2.67490	227.41038	2.67499
200.62159	3.37998	213.32440	2.61924	227.74456	2.75607
200.88163	3.44530	213.61844	2.56436	228.07973	2.84076
201.14234	3.50592	213.91329	2.51043	228.41588	2.92903
201.40374	3.56184	214.20895	2.45761	228.75303	3.02083
201.66581	3.61306	214.50543	2.40604	229.09117	3.11611
201.92857	3.65959	214.80274	2.35587	229.43031	3.21482
292.13982	3.66817	321.20244	0.40758	356.68625	0.01434

292.69155	3.54498	321.86952	0.38463	357.50905	0.01321
293.24536	3.42424	322.53939	0.36280	358.33567	0.01216
293.80128	3.30599	323.21204	0.34203	359.16611	0.01119
294.35931	3.19025	323.88751	0.32229	360.00041	0.01030
294.91946	3.07705	324.56581	0.30354	360.83859	0.00947
295.48175	2.96640	325.24696	0.28573	361.68069	0.00870
296.04618	2.85833	325.93097	0.26884	362.52673	0.00799
296.61278	2.75283	326.61786	0.25282	363.37673	0.00733
297.18154	2.64993	327.30766	0.23763	364.23073	0.00673
297.75250	2.54961	328.00037	0.22324	365.08875	0.00617
298.32565	2.45188	328.69602	0.20962	365.95083	0.00566
298.90101	2.35674	329.39463	0.19674	366.81698	0.00518
299.47860	2.26417	330.09622	0.18455	367.68725	0.00474
300.05842	2.17416	330.80080	0.17303	368.56165	0.00434
300.64050	2.08670	331.50840	0.16215	369.44023	0.00397
301.22483	2.00177	332.21903	0.15187	370.32300	0.00363
301.81144	1.91934	332.93271	0.14218	371.21000	0.00332
302.40034	1.83941	333.64946	0.13304	372.10126	0.00303
302.99154	1.76192	334.36931	0.12442	372.99681	0.00277
303.58506	1.68687	335.09227	0.11630	373.89668	0.00252
304.18091	1.61422	335.81836	0.10866	374.80091	0.00230
304.77911	1.54393	336.54761	0.10147	375.70952	0.00210
305.37966	1.47597	337.28003	0.09471	376.62254	0.00191
305.98258	1.41030	338.01565	0.08835	377.54001	0.00174
306.58788	1.34689	338.75448	0.08238	378.46197	0.00158
307.19559	1.28569	339.49655	0.07678	379.38843	0.00144
307.80571	1.22666	340.24188	0.07152	380.31945	0.00131
308.41826	1.16976	340.99048	0.06658	381.25504	0.00119
309.03325	1.11495	341.74239	0.06196	382.19525	0.00108
309.65070	1.06218	342.49762	0.05763	383.14011	0.00098
310.27062	1.01140	343.25620	0.05357	384.08965	0.00089
310.89303	0.96258	344.01815	0.04977	385.04391	0.00081
311.51794	0.91565	344.78348	0.04622	386.00293	0.00073
312.14537	0.87058	345.55223	0.04290	386.96673	0.00066
312.77533	0.82732	346.32441	0.03980	387.93536	0.00060
313.40784	0.78581	347.10006	0.03691	388.90885	0.00054
314.04291	0.74602	347.87918	0.03421	389.88723	0.00049
314.68056	0.70789	348.66181	0.03169	390.87056	0.00044
315.32080	0.67138	349.44797	0.02934	391.85885	0.00040
315.96366	0.63643	350.23768	0.02715	392.85216	0.00036
316.60914	0.60300	351.03097	0.02511	393.85051	0.00033
317.25727	0.57104	351.82787	0.02322	394.85395	0.00030
317.90805	0.54050	352.62839	0.02145	395.86252	0.00027
318.56151	0.51135	353.43255	0.01981	396.87625	0.00024
319.21766	0.48352	354.24040	0.01829	397.89519	0.00022
319.87652	0.45698	355.05195	0.01687	398.91937	0.00020
320.53811	0.43168	355.86722	0.01556	399.94884	0.00018
400.98364	0.00016	457.84394	0.00000	533.49458	0.00000
402.02380	0.00014	459.20052	0.00000	535.33739	0.00000

403.06938	0.00013	460.56516	0.00000	537.19298	0.00000
404.12040	0.00011	461.93793	0.00000	539.06148	0.00000
405.17693	0.00010	463.31891	0.00000	540.94302	0.00000
406.23899	0.00009	464.70817	0.00000	542.83774	0.00000
407.30664	0.00008	466.10579	0.00000	544.74578	0.00000
408.37991	0.00007	467.51184	0.00000	546.66728	0.00000
409.45885	0.00007	468.92640	0.00000	548.60239	0.00000
410.54351	0.00006	470.34955	0.00000	550.55124	0.00000
411.63393	0.00005	471.78136	0.00000	552.51399	0.00000
412.73016	0.00005	473.22191	0.00000	554.49079	0.00000
413.83224	0.00004	474.67129	0.00000	556.48178	0.00000
414.94023	0.00004	476.12957	0.00000	558.48712	0.00000
416.05416	0.00003	477.59684	0.00000	560.50696	0.00000
417.17409	0.00003	479.07318	0.00000	562.54147	0.00000
418.30007	0.00003	480.55868	0.00000	564.59080	0.00000
419.43214	0.00002	482.05342	0.00000	566.65512	0.00000
420.57035	0.00002	483.55749	0.00000	568.73459	0.00000
421.71476	0.00002	485.07097	0.00000	570.82937	0.00000
422.86542	0.00002	486.59396	0.00000	572.93965	0.00000
424.02237	0.00002	488.12654	0.00000	575.06558	0.00000
425.18567	0.00001	489.66880	0.00000	577.20736	0.00000
426.35537	0.00001	491.22084	0.00000	579.36514	0.00000
427.53152	0.00001	492.78275	0.00000	581.53912	0.00000
428.71418	0.00001	494.35463	0.00000	583.72947	0.00000
429.90340	0.00001	495.93656	0.00000	585.93639	0.00000
431.09924	0.00001	497.52865	0.00000	588.16006	0.00000
432.30174	0.00001	499.13100	0.00000	590.40067	0.00000
433.51098	0.00001	500.74370	0.00000	592.65841	0.00000
434.72700	0.00001	502.36686	0.00000	594.93349	0.00000
435.94986	0.00001	504.00057	0.00000	597.22611	0.00000
437.17962	0.00000	505.64494	0.00000	599.53646	0.00000
438.41634	0.00000	507.30008	0.00000	601.86476	0.00000
439.66007	0.00000	508.96609	0.00000	604.21121	0.00000
440.91088	0.00000	510.64308	0.00000	606.57603	0.00000
442.16883	0.00000	512.33116	0.00000	608.95943	0.00000
443.43398	0.00000	514.03043	0.00000	611.36164	0.00000
444.70639	0.00000	515.74102	0.00000	613.78287	0.00000
445.98612	0.00000	517.46302	0.00000	616.22336	0.00000
447.27323	0.00000	519.19657	0.00000	618.68333	0.00000
448.56780	0.00000	520.94177	0.00000	621.16303	0.00000
449.86988	0.00000	522.69874	0.00000	623.66268	0.00000
451.17955	0.00000	524.46760	0.00000	626.18253	0.00000
452.49686	0.00000	526.24847	0.00000	628.72282	0.00000
453.82189	0.00000	528.04148	0.00000	631.28381	0.00000
455.15470	0.00000	529.84675	0.00000	633.86575	0.00000
456.49536	0.00000	531.66441	0.00000	636.46889	0.00000
639.09351	0.00000	796.81324	0.00000		
641.73986	0.00000	800.93114	0.00000		
644.40821	0.00000	805.09182	0.00000		

647.09885	0.00000	809.29595	0.00000		
649.81206	0.00000	813.54423	0.00000		
652.54811	0.00000	817.83734	0.00000		
655.30729	0.00000	822.17600	0.00000		
658.08992	0.00000	826.56093	0.00000		
660.89627	0.00000				
663.72666	0.00000				
666.58140	0.00000				
669.46080	0.00000				
672.36518	0.00000				
675.29488	0.00000				
678.25022	0.00000				
681.23154	0.00000				
684.23918	0.00000				
687.27350	0.00000				
690.33486	0.00000				
693.42360	0.00000				
696.54011	0.00000				
699.68476	0.00000				
702.85794	0.00000				
706.06002	0.00000				
709.29142	0.00000				
712.55253	0.00000				
715.84376	0.00000				
719.16555	0.00000				
722.51830	0.00000				
725.90246	0.00000				
729.31847	0.00000				
732.76679	0.00000				
736.24786	0.00000				
739.76217	0.00000				
743.31019	0.00000				
746.89241	0.00000				
750.50932	0.00000				
754.16144	0.00000				
757.84927	0.00000				
761.57334	0.00000				
765.33420	0.00000				
769.13238	0.00000				
772.96845	0.00000				
776.84298	0.00000				
780.75655	0.00000				
784.70975	0.00000				
788.70318	0.00000				
792.73747	0.00000				

Table S2. Calculated ECD for **1b**

Wavenumber	ECD	Wavenumber	ECD	Wavenumber	ECD
190.74483	2.19308	202.19201	-3.70146	215.10087	-2.30725
190.97988	2.01682	202.45614	-3.73869	215.39983	-2.26033
191.21552	1.84048	202.72096	-3.77131	215.69962	-2.21524
191.45173	1.66422	202.98648	-3.79938	216.00024	-2.17211
191.68853	1.48822	203.25269	-3.82294	216.30171	-2.13108
191.92591	1.31262	203.51960	-3.84205	216.60402	-2.09226
192.16389	1.13759	203.78721	-3.85679	216.90717	-2.05579
192.40245	0.96330	204.05553	-3.86722	217.21118	-2.02177
192.64161	0.78992	204.32456	-3.87342	217.51604	-1.99032
192.88136	0.61760	204.59429	-3.87549	217.82175	-1.96155
193.12171	0.44652	204.86474	-3.87352	218.12833	-1.93555
193.36266	0.27684	205.13590	-3.86761	218.43577	-1.91243
193.60422	0.10872	205.40779	-3.85788	218.74407	-1.89228
193.84637	-0.05767	205.68039	-3.84443	219.05325	-1.87517
194.08914	-0.22217	205.95372	-3.82739	219.36331	-1.86121
194.33251	-0.38462	206.22778	-3.80689	219.67424	-1.85046
194.57649	-0.54486	206.50257	-3.78306	219.98605	-1.84300
194.82109	-0.70274	206.77809	-3.75603	220.29876	-1.83889
195.06630	-0.85809	207.05434	-3.72595	220.61235	-1.83820
195.31213	-1.01078	207.33134	-3.69296	220.92684	-1.84098
195.55858	-1.16066	207.60908	-3.65722	221.24222	-1.84730
195.80565	-1.30757	207.88756	-3.61888	221.55851	-1.85719
196.05335	-1.45138	208.16679	-3.57810	221.87570	-1.87070
196.30168	-1.59195	208.44677	-3.53504	222.19380	-1.88788
196.55063	-1.72915	208.72751	-3.48986	222.51281	-1.90875
196.80022	-1.86286	209.00900	-3.44274	222.83275	-1.93335
197.05045	-1.99295	209.29126	-3.39383	223.15360	-1.96169
197.30131	-2.11930	209.57427	-3.34332	223.47538	-1.99381
197.55280	-2.24181	209.85806	-3.29137	223.79809	-2.02971
197.80495	-2.36037	210.14261	-3.23816	224.12173	-2.06942
198.05773	-2.47487	210.42794	-3.18386	224.44631	-2.11293
198.31116	-2.58523	210.71404	-3.12865	224.77183	-2.16025
198.56525	-2.69136	211.00092	-3.07269	225.09829	-2.21138
198.81998	-2.79318	211.28858	-3.01618	225.42571	-2.26631
199.07537	-2.89061	211.57703	-2.95927	225.75408	-2.32504
199.33142	-2.98359	211.86627	-2.90215	226.08341	-2.38755
199.58812	-3.07207	212.15630	-2.84498	226.41370	-2.45382
199.84549	-3.15598	212.44712	-2.78794	226.74495	-2.52384
200.10352	-3.23529	212.73874	-2.73119	227.07718	-2.59757
200.36222	-3.30997	213.03117	-2.67490	227.41038	-2.67499
200.62159	-3.37998	213.32440	-2.61924	227.74456	-2.75607
200.88163	-3.44530	213.61844	-2.56436	228.07973	-2.84076
201.14234	-3.50592	213.91329	-2.51043	228.41588	-2.92903
201.40374	-3.56184	214.20895	-2.45761	228.75303	-3.02083
201.66581	-3.61306	214.50543	-2.40604	229.09117	-3.11611
201.92857	-3.65959	214.80274	-2.35587	229.43031	-3.21482

229.77046	-3.31690	247.37458	-10.15830	267.90004	-10.64473
230.11162	-3.42230	247.77006	-10.28324	268.36394	-10.53051
230.45379	-3.53094	248.16681	-10.40474	268.82945	-10.41249
230.79698	-3.64277	248.56484	-10.52264	269.29657	-10.29083
231.14120	-3.75770	248.96414	-10.63678	269.76532	-10.16567
231.48645	-3.87566	249.36472	-10.74702	270.23570	-10.03719
231.83272	-3.99658	249.76660	-10.85321	270.70773	-9.90554
232.18004	-4.12037	250.16977	-10.95520	271.18141	-9.77090
232.52840	-4.24694	250.57425	-11.05287	271.65675	-9.63342
232.87780	-4.37620	250.98004	-11.14607	272.13376	-9.49328
233.22825	-4.50806	251.38715	-11.23469	272.61245	-9.35066
233.57977	-4.64241	251.79557	-11.31859	273.09282	-9.20572
233.93234	-4.77917	252.20533	-11.39767	273.57489	-9.05863
234.28598	-4.91821	252.61642	-11.47182	274.05867	-8.90958
234.64069	-5.05943	253.02886	-11.54093	274.54415	-8.75874
234.99648	-5.20271	253.44264	-11.60491	275.03137	-8.60627
235.35334	-5.34795	253.85778	-11.66366	275.52031	-8.45236
235.71129	-5.49501	254.27428	-11.71711	276.01100	-8.29717
236.07034	-5.64378	254.69215	-11.76517	276.50343	-8.14087
236.43047	-5.79412	255.11140	-11.80778	276.99763	-7.98364
236.79171	-5.94590	255.53203	-11.84487	277.49360	-7.82565
237.15406	-6.09899	255.95405	-11.87640	277.99135	-7.66705
237.51751	-6.25324	256.37746	-11.90231	278.49088	-7.50802
237.88208	-6.40853	256.80228	-11.92258	278.99221	-7.34871
238.24777	-6.56469	257.22851	-11.93716	279.49536	-7.18928
238.61459	-6.72159	257.65615	-11.94604	280.00032	-7.02988
238.98254	-6.87907	258.08522	-11.94921	280.50710	-6.87068
239.35162	-7.03698	258.51572	-11.94665	281.01573	-6.71180
239.72185	-7.19516	258.94766	-11.93837	281.52620	-6.55341
240.09322	-7.35345	259.38105	-11.92438	282.03854	-6.39563
240.46575	-7.51169	259.81588	-11.90470	282.55274	-6.23861
240.83943	-7.66972	260.25218	-11.87935	283.06881	-6.08248
241.21428	-7.82737	260.68995	-11.84838	283.58678	-5.92736
241.59030	-7.98447	261.12919	-11.81181	284.10665	-5.77337
241.96749	-8.14086	261.56992	-11.76971	284.62842	-5.62064
242.34586	-8.29636	262.01213	-11.72214	285.15212	-5.46927
242.72541	-8.45079	262.45584	-11.66914	285.67774	-5.31938
243.10616	-8.60400	262.90106	-11.61082	286.20531	-5.17107
243.48810	-8.75580	263.34779	-11.54723	286.73483	-5.02444
243.87124	-8.90602	263.79604	-11.47847	287.26631	-4.87957
244.25560	-9.05449	264.24582	-11.40464	287.79977	-4.73656
244.64116	-9.20102	264.69714	-11.32583	288.33521	-4.59549
245.02795	-9.34546	265.15000	-11.24216	288.87265	-4.45644
245.41595	-9.48762	265.60441	-11.15373	289.41209	-4.31947
245.80519	-9.62733	266.06039	-11.06067	289.95356	-4.18467
246.19567	-9.76442	266.51793	-10.96311	290.49705	-4.05208
246.58739	-9.89873	266.97705	-10.86118	291.04258	-3.92177
246.98036	-10.03007	267.43775	-10.75500	291.59017	-3.79378
292.13982	-3.66817	321.20244	-0.40758	356.68625	-0.01434

292.69155	-3.54498	321.86952	-0.38463	357.50905	-0.01321
293.24536	-3.42424	322.53939	-0.36280	358.33567	-0.01216
293.80128	-3.30599	323.21204	-0.34203	359.16611	-0.01119
294.35931	-3.19025	323.88751	-0.32229	360.00041	-0.01030
294.91946	-3.07705	324.56581	-0.30354	360.83859	-0.00947
295.48175	-2.96640	325.24696	-0.28573	361.68069	-0.00870
296.04618	-2.85833	325.93097	-0.26884	362.52673	-0.00799
296.61278	-2.75283	326.61786	-0.25282	363.37673	-0.00733
297.18154	-2.64993	327.30766	-0.23763	364.23073	-0.00673
297.75250	-2.54961	328.00037	-0.22324	365.08875	-0.00617
298.32565	-2.45188	328.69602	-0.20962	365.95083	-0.00566
298.90101	-2.35674	329.39463	-0.19674	366.81698	-0.00518
299.47860	-2.26417	330.09622	-0.18455	367.68725	-0.00474
300.05842	-2.17416	330.80080	-0.17303	368.56165	-0.00434
300.64050	-2.08670	331.50840	-0.16215	369.44023	-0.00397
301.22483	-2.00177	332.21903	-0.15187	370.32300	-0.00363
301.81144	-1.91934	332.93271	-0.14218	371.21000	-0.00332
302.40034	-1.83941	333.64946	-0.13304	372.10126	-0.00303
302.99154	-1.76192	334.36931	-0.12442	372.99681	-0.00277
303.58506	-1.68687	335.09227	-0.11630	373.89668	-0.00252
304.18091	-1.61422	335.81836	-0.10866	374.80091	-0.00230
304.77911	-1.54393	336.54761	-0.10147	375.70952	-0.00210
305.37966	-1.47597	337.28003	-0.09471	376.62254	-0.00191
305.98258	-1.41030	338.01565	-0.08835	377.54001	-0.00174
306.58788	-1.34689	338.75448	-0.08238	378.46197	-0.00158
307.19559	-1.28569	339.49655	-0.07678	379.38843	-0.00144
307.80571	-1.22666	340.24188	-0.07152	380.31945	-0.00131
308.41826	-1.16976	340.99048	-0.06658	381.25504	-0.00119
309.03325	-1.11495	341.74239	-0.06196	382.19525	-0.00108
309.65070	-1.06218	342.49762	-0.05763	383.14011	-0.00098
310.27062	-1.01140	343.25620	-0.05357	384.08965	-0.00089
310.89303	-0.96258	344.01815	-0.04977	385.04391	-0.00081
311.51794	-0.91565	344.78348	-0.04622	386.00293	-0.00073
312.14537	-0.87058	345.55223	-0.04290	386.96673	-0.00066
312.77533	-0.82732	346.32441	-0.03980	387.93536	-0.00060
313.40784	-0.78581	347.10006	-0.03691	388.90885	-0.00054
314.04291	-0.74602	347.87918	-0.03421	389.88723	-0.00049
314.68056	-0.70789	348.66181	-0.03169	390.87056	-0.00044
315.32080	-0.67138	349.44797	-0.02934	391.85885	-0.00040
315.96366	-0.63643	350.23768	-0.02715	392.85216	-0.00036
316.60914	-0.60300	351.03097	-0.02511	393.85051	-0.00033
317.25727	-0.57104	351.82787	-0.02322	394.85395	-0.00030
317.90805	-0.54050	352.62839	-0.02145	395.86252	-0.00027
318.56151	-0.51135	353.43255	-0.01981	396.87625	-0.00024
319.21766	-0.48352	354.24040	-0.01829	397.89519	-0.00022
319.87652	-0.45698	355.05195	-0.01687	398.91937	-0.00020
320.53811	-0.43168	355.86722	-0.01556	399.94884	-0.00018

400.98364	-0.00016	457.84394	0.00000	533.49458	0.00000
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402.02380	-0.00014	459.20052	0.00000	535.33739	0.00000
403.06938	-0.00013	460.56516	0.00000	537.19298	0.00000
404.12040	-0.00011	461.93793	0.00000	539.06148	0.00000
405.17693	-0.00010	463.31891	0.00000	540.94302	0.00000
406.23899	-0.00009	464.70817	0.00000	542.83774	0.00000
407.30664	-0.00008	466.10579	0.00000	544.74578	0.00000
408.37991	-0.00007	467.51184	0.00000	546.66728	0.00000
409.45885	-0.00007	468.92640	0.00000	548.60239	0.00000
410.54351	-0.00006	470.34955	0.00000	550.55124	0.00000
411.63393	-0.00005	471.78136	0.00000	552.51399	0.00000
412.73016	-0.00005	473.22191	0.00000	554.49079	0.00000
413.83224	-0.00004	474.67129	0.00000	556.48178	0.00000
414.94023	-0.00004	476.12957	0.00000	558.48712	0.00000
416.05416	-0.00003	477.59684	0.00000	560.50696	0.00000
417.17409	-0.00003	479.07318	0.00000	562.54147	0.00000
418.30007	-0.00003	480.55868	0.00000	564.59080	0.00000
419.43214	-0.00002	482.05342	0.00000	566.65512	0.00000
420.57035	-0.00002	483.55749	0.00000	568.73459	0.00000
421.71476	-0.00002	485.07097	0.00000	570.82937	0.00000
422.86542	-0.00002	486.59396	0.00000	572.93965	0.00000
424.02237	-0.00002	488.12654	0.00000	575.06558	0.00000
425.18567	-0.00001	489.66880	0.00000	577.20736	0.00000
426.35537	-0.00001	491.22084	0.00000	579.36514	0.00000
427.53152	-0.00001	492.78275	0.00000	581.53912	0.00000
428.71418	-0.00001	494.35463	0.00000	583.72947	0.00000
429.90340	-0.00001	495.93656	0.00000	585.93639	0.00000
431.09924	-0.00001	497.52865	0.00000	588.16006	0.00000
432.30174	-0.00001	499.13100	0.00000	590.40067	0.00000
433.51098	-0.00001	500.74370	0.00000	592.65841	0.00000
434.72700	-0.00001	502.36686	0.00000	594.93349	0.00000
435.94986	-0.00001	504.00057	0.00000	597.22611	0.00000
437.17962	0.00000	505.64494	0.00000	599.53646	0.00000
438.41634	0.00000	507.30008	0.00000	601.86476	0.00000
439.66007	0.00000	508.96609	0.00000	604.21121	0.00000
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