Electronic Supplementary Material (ESI) for RSC Advances.

Daldinone Derivatives from the Mangrove-Derived Endophytic Fungus *Annulohypoxylon* sp.

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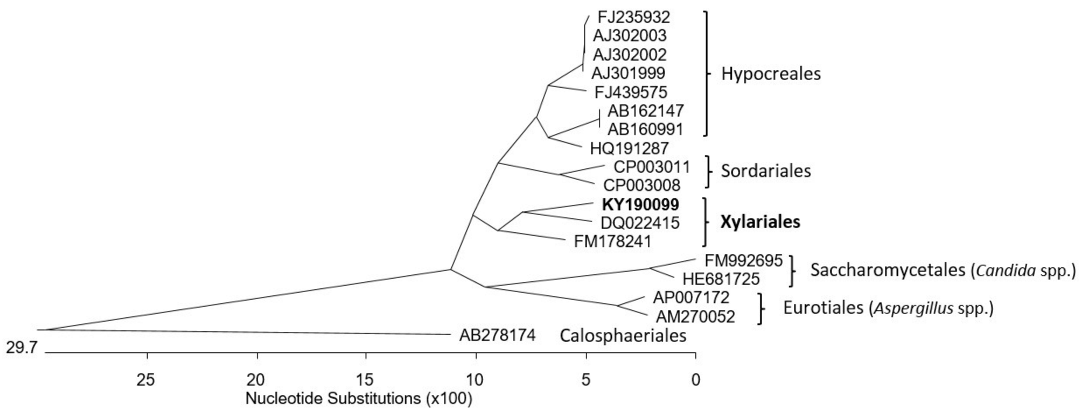
Contents

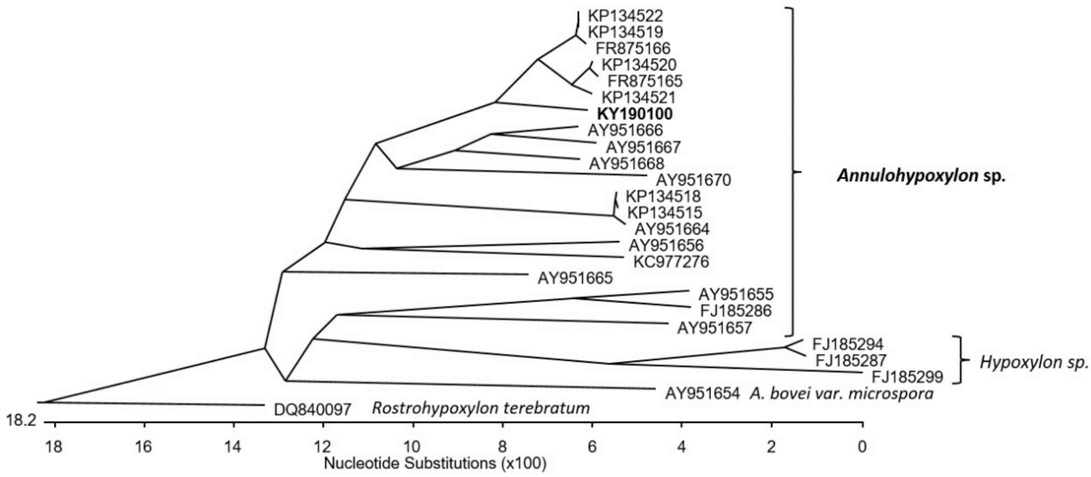
Fig. S1. Phylogenetic tree of *Annulohypoxylon* sp.

A. rDNA-derived phylogenetic tree



rDNA-derived phylogenetic tree, based on alignment of rDNA sequences, which were restricted to bp 28-3357 of the rDNA sequence of the new fungus (acc.-no. KY190099)

B. beta-tubulin-derived phylogenetic tree



beta-tubulin-based phylogenetic tree, based on alignment of beta-tubulin sequences, which were restricted to bp 1-1415 of the beta-tubulin gene sequence of the new fungus (KY190100).

Fig. S2 ^{1}H NMR (600 MHz, CH_3OH-d_4,) spectrum of the new compound **1**

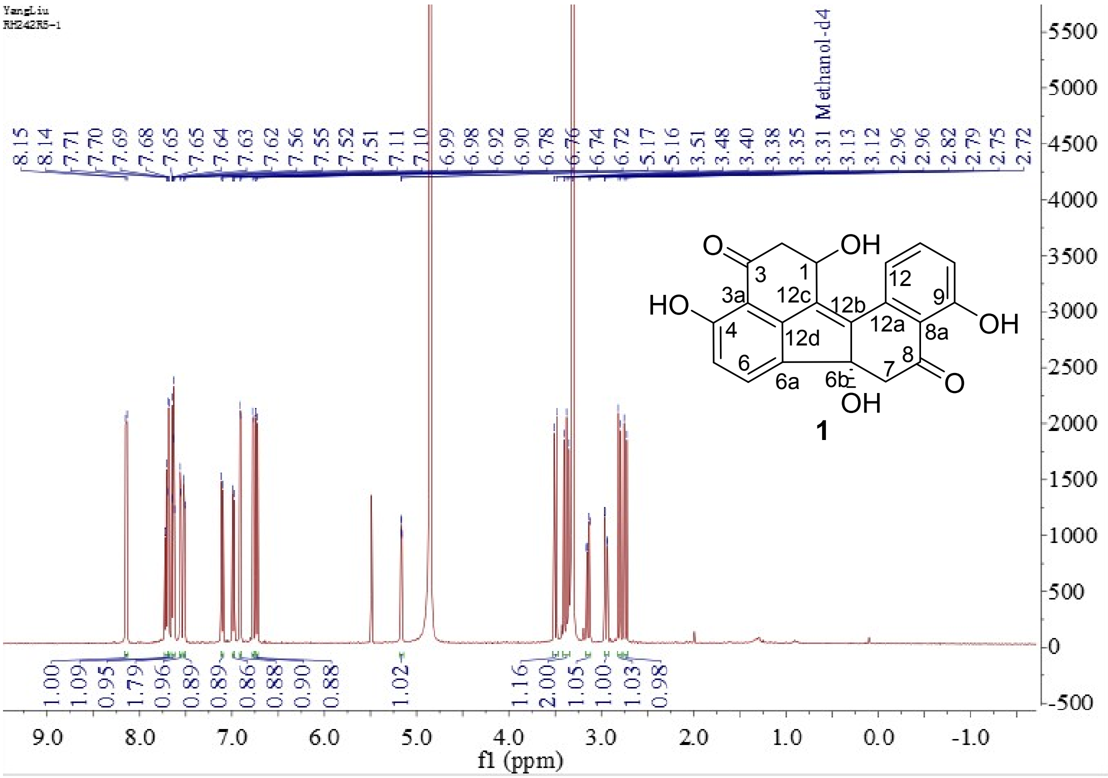
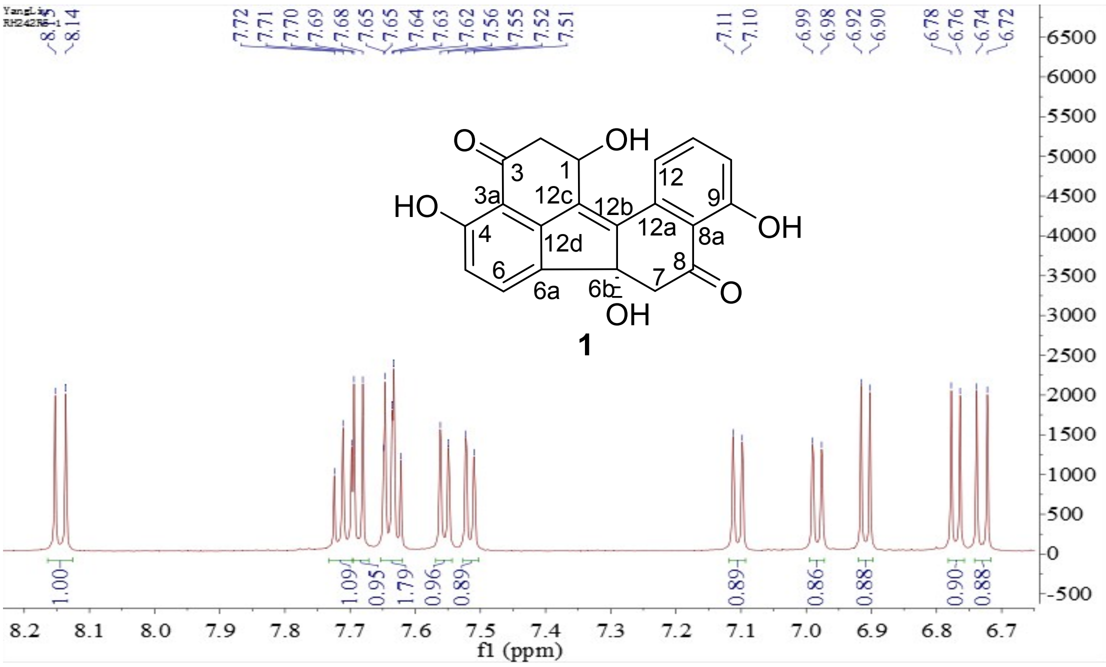


Fig. S3 Expanded ^{1}H NMR (600 MHz, CH_3OH-d_4,) spectrum of the new compound **1**



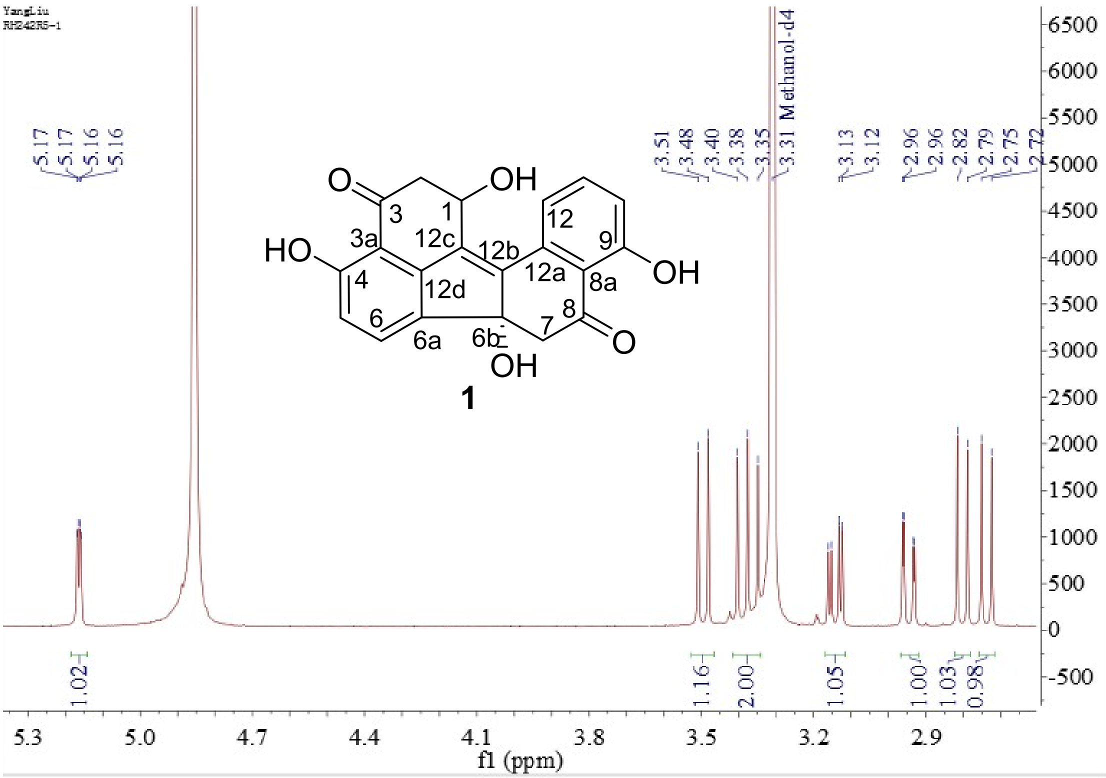


Fig. S4 COSY spectrum of the new compound **1**

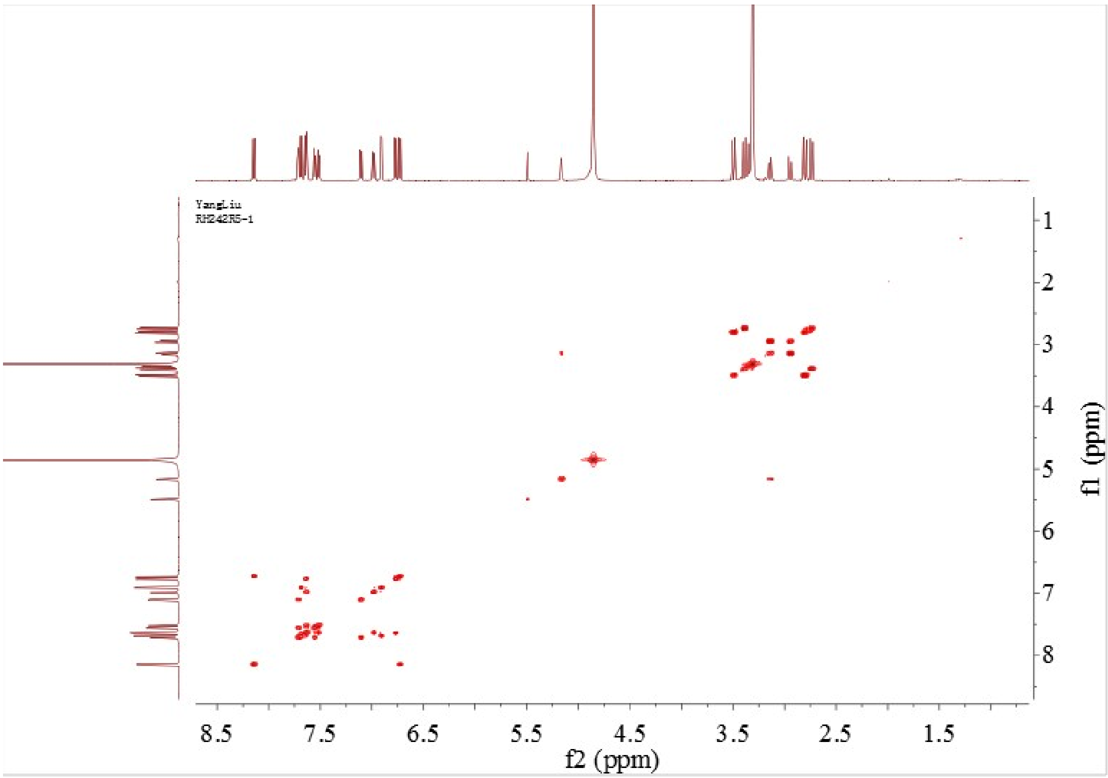


Fig. S5 HSQC spectrum of the new compound **1**

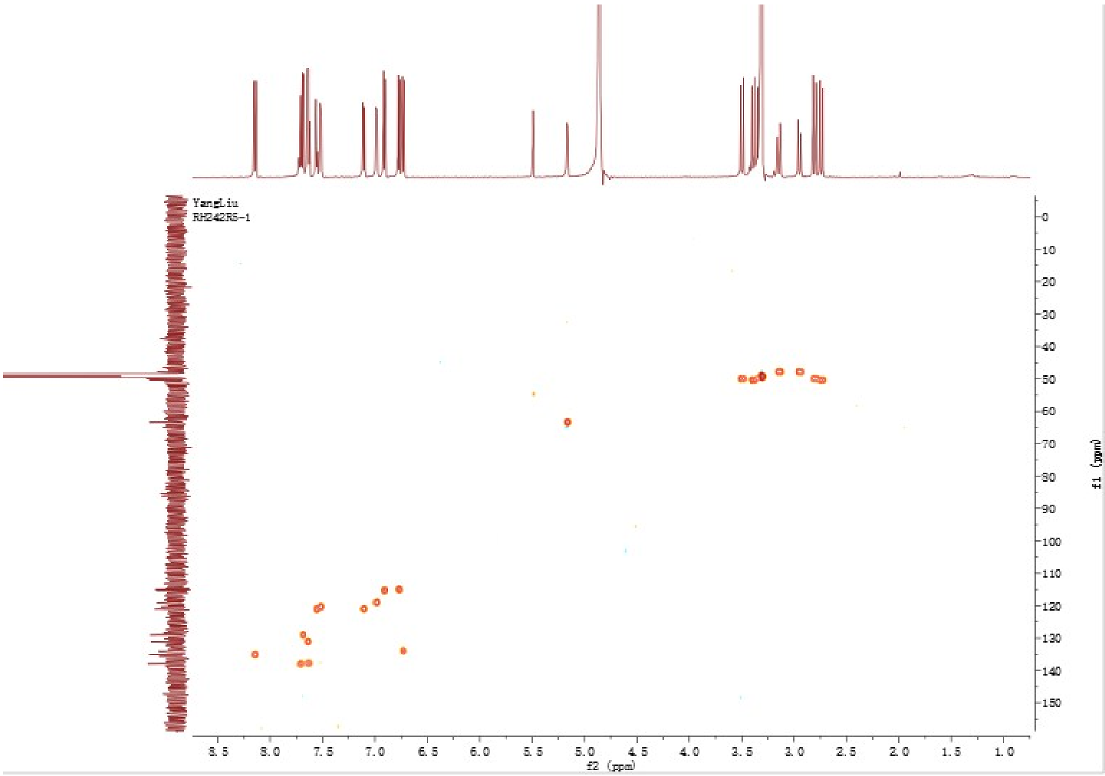


Fig. S6 HMBC spectrum of the new compound **1**

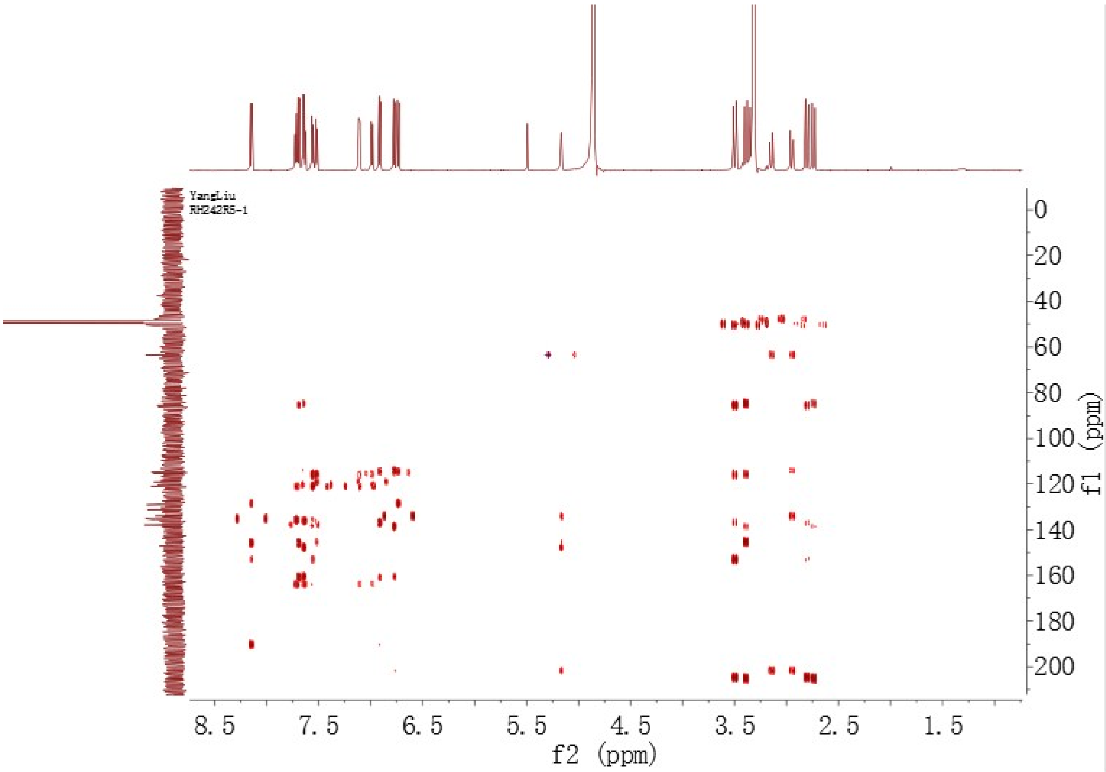
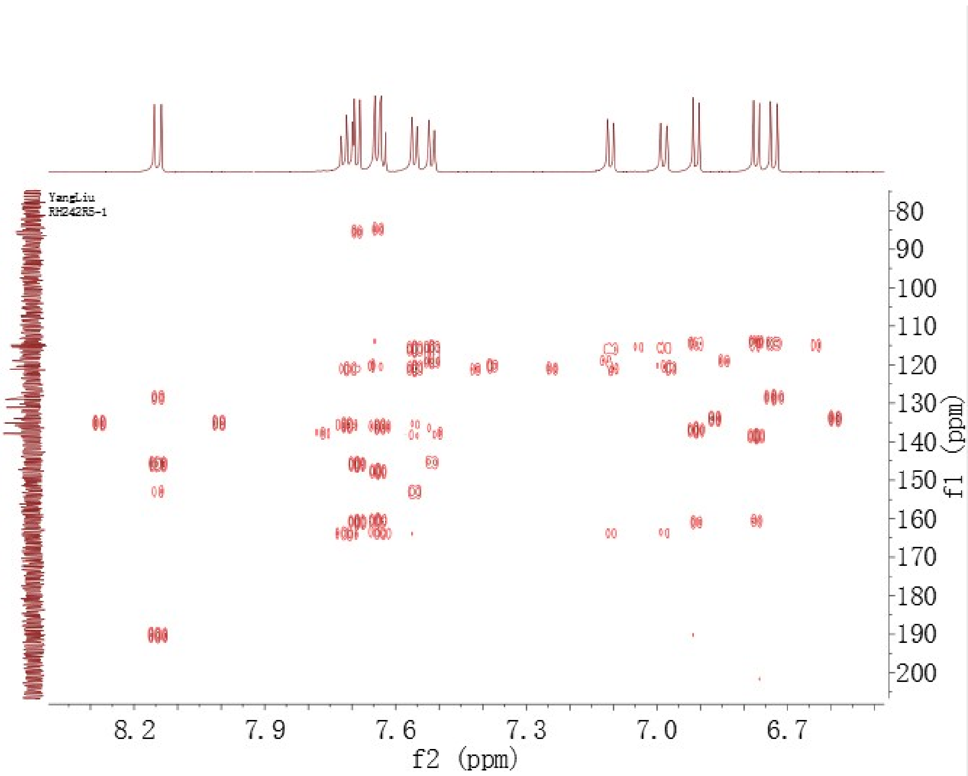


Fig. S7 Expanded HMBC spectrum of the new compound **1**



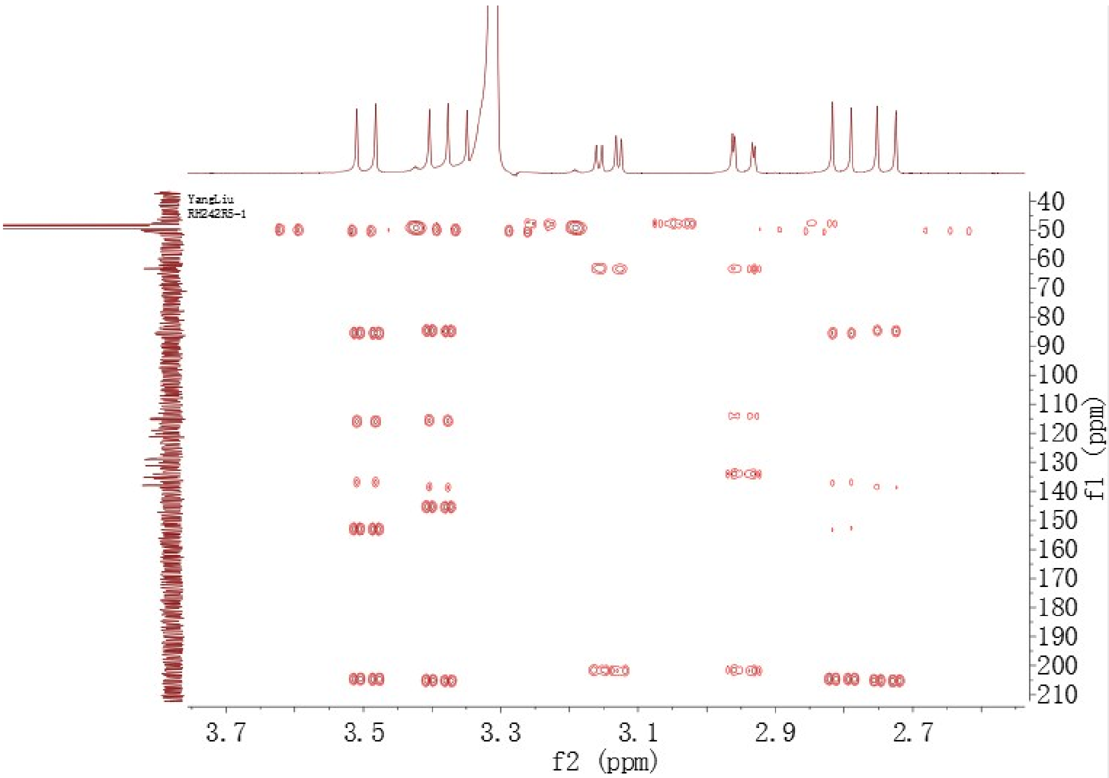


Fig. S8 HRESIMS spectrum of the new compound **1**

Mass Spectrum SmartFormula Report

Analysis Info

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Method	tune_low.m	Operator	Peter Tommes
Sample Name	Yang Liu RH242R5-1 (CH3OH)	Instrument	maXis 288882.20213
Comment	5 ul in 1000 ul		

Acquisition Parameter

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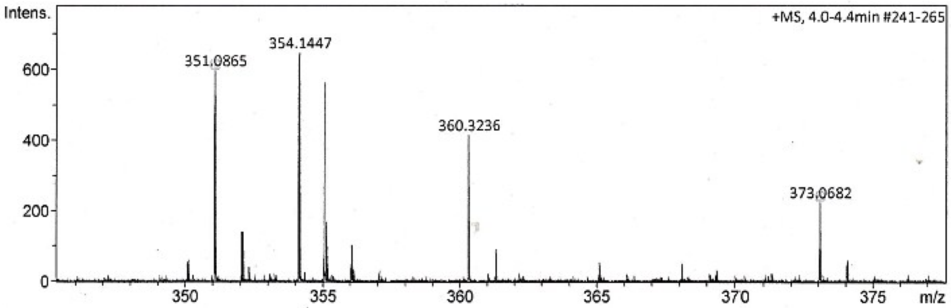

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373.0682	1	C20H14NaO6	373.0683	0.1	n.a.	1	100.00	13.5	even	ok
	2	C5H10N12NaO7	373.0688	1.4	n.a.	2	79.93	6.5	even	ok

Fig. S9 Structure and population of the low-energy B3LYP/6-31G(d) *in vacuo* conformers (>2%) of (1*R*,6b*R*)-**1** diastereomer

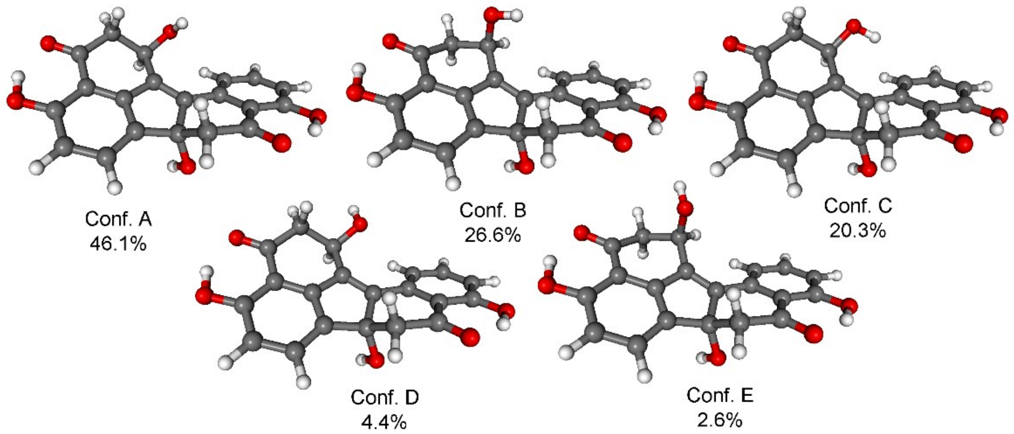


Fig. S10 Experimental ECD spectrum of **1** compared with the Boltzmann-weighted ECD spectra computed for the B3LYP/6-31G(d) *in vacuo* low-energy conformers of (1*R*,6b*R*)-**1** at various levels

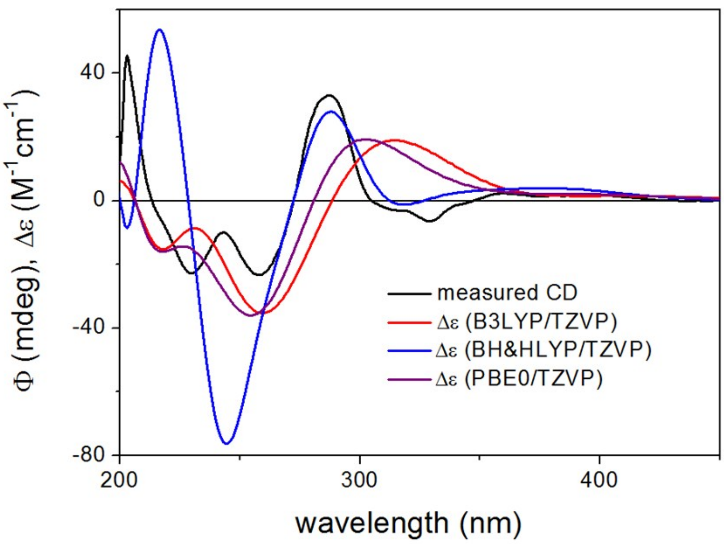


Fig. S11 Structure and population of the low-energy B3LYP/6-31G(d) *in vacuo* conformers (>2%) of (1*S*,6b*R*)-**1** diastereomer

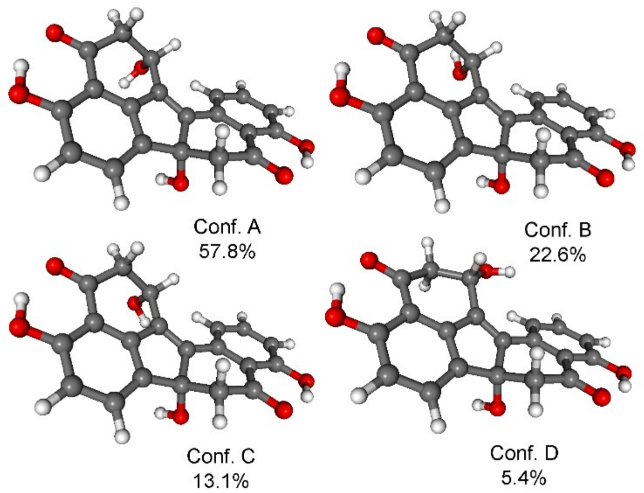


Fig. S12 Experimental ECD spectrum of **1** compared with the Boltzmann-weighted ECD spectra computed for the B3LYP/6-31G(d) *in vacuo* low-energy conformers of (1*S*,6b*R*)-**1** at various levels

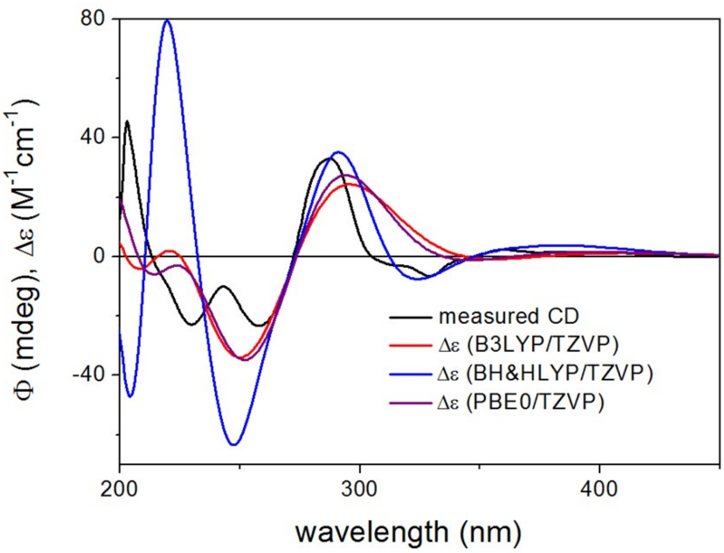


Fig. S13 Experimental ECD spectrum of **1** compared with the Boltzmann-weighted ECD spectra computed for the B3LYP/TZVP PCM/MeCN low-energy conformers of (1*R*,6b*R*)-**1** at various levels

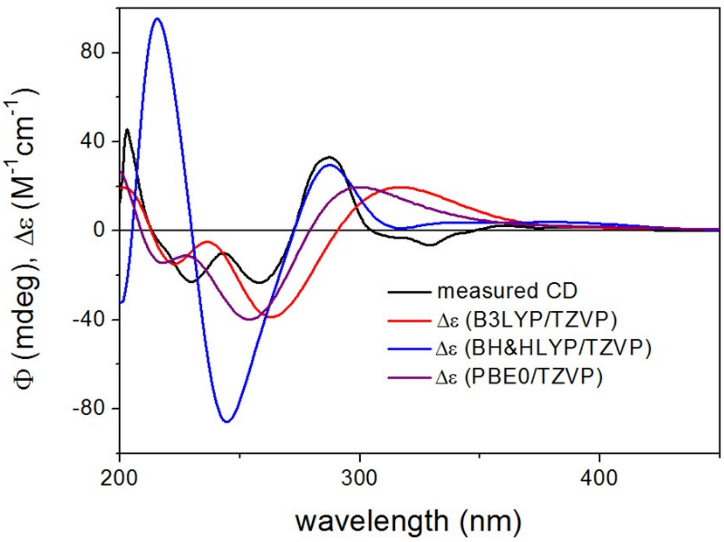


Fig. S14 Experimental ECD spectrum of **1** compared with the Boltzmann-weighted ECD spectra computed for the B3LYP/TZVP PCM/MeCN low-energy conformers of (1*S*,6b*R*)-**1** at various levels

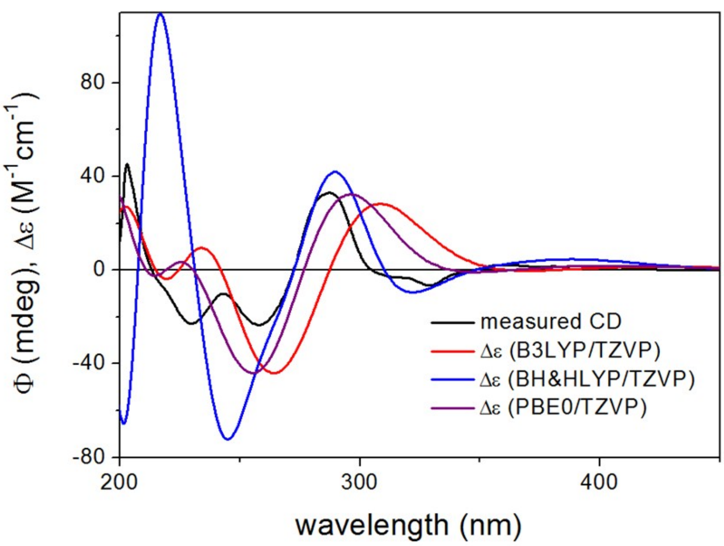


Fig. S15 Experimental ECD spectrum of **1** compared with the Boltzmann-weighted ECD spectra computed for the CAM-B3LYP/TZVP PCM/MeCN low-energy conformers of (1*R*,6b*R*)-**1** at various levels

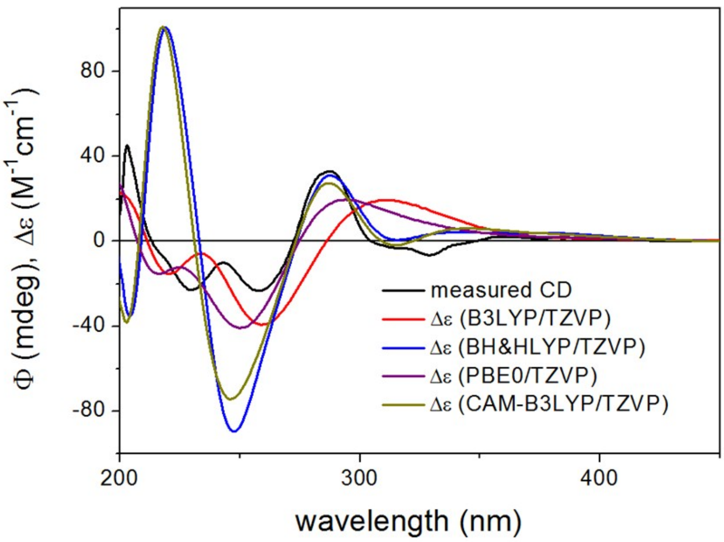


Fig. S16 Experimental ECD spectrum of **1** compared with the Boltzmann-weighted ECD spectra computed for the CAM-B3LYP/TZVP PCM/MeCN low-energy conformers of (1*S*,6b*R*)-**1** at various levels

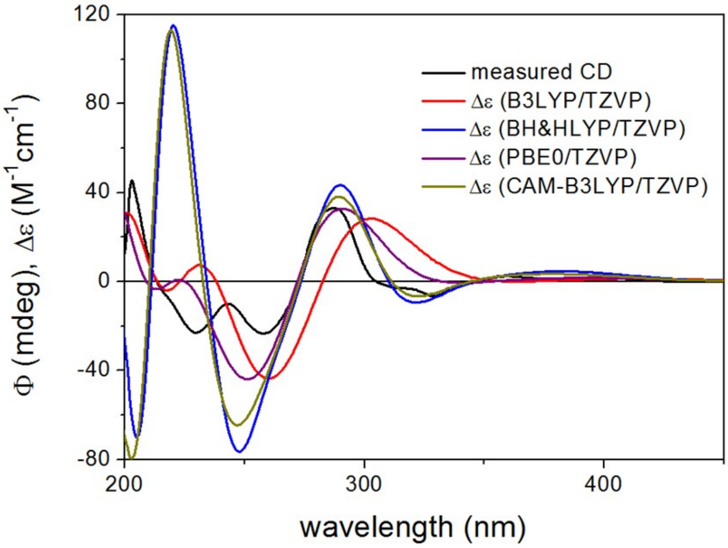


Table S1 Populations of conformers with equatorial and axial 1-H for (1*R*,6b*R*)-**1** at various levels of theory

	B3LYP/6-31G(d) Boltzmann population / ax. or eq.	B3LYP/TZVP PCM/MeCN Boltzmann population / ax. or eq.	B97D/TZVP PCM/MeCN Boltzmann population / ax. or eq.	CAM-B3LYP/TZVP PCM/MeCN Boltzmann population / ax. or eq.
Conf. A	46.1% / ax.	65.5% / eq.	68.9% / eq.	63.4% / eq.
Conf. B	26.6% / eq.	20.5% / eq.	15.1% / eq.	21.0% / eq.
Conf. C	20.3% / ax.	7.0% / ax.	8.0% / ax.	7.3% / ax.
Conf. D	4.4% / ax.	3.8% / ax.	5.8% / ax.	4.3% / ax.
Conf. E	2.6% / eq.	3.2% / ax.	2.2% / ax.	4.0% / ax.
ax./eq.	71/29	14 / 86	16/84	16 / 84

Table S2 Populations of conformers with equatorial and axial 1-H for (1*S*,6b*R*)-**1** at various levels of theory

	B3LYP/6-31G(d) Boltzmann population / ax. or eq.	B3LYP/TZVP PCM/MeCN Boltzmann population / ax. or eq.	B97D/TZVP PCM/MeCN Boltzmann population / ax. or eq.	CAM-B3LYP/TZVP PCM/MeCN Boltzmann population / ax. or eq.
Conf. A	57.8% / eq.	47.2% / eq.	47.4% / eq.	53.2% / eq.
Conf. B	22.6% / eq.	33.0% / eq.	30.6% / eq.	25.9% / eq.
Conf. C	13.1% / eq.	18.4% / eq.	21.2% / eq.	19.0% / eq.
Conf. D	5.4% / ax.	0.8% / ax.	0.6% / eq.	1.1% / eq.
Conf. E	1.1% / ax.	0.7% / ax.	0.1% / eq.	0.8% / eq.
ax./eq.	6/94	1 / 99	1/99	2 / 98

Fig. S17 ^{1}H NMR (600 MHz, DMSO-d_6) spectrum of the new compound **2**

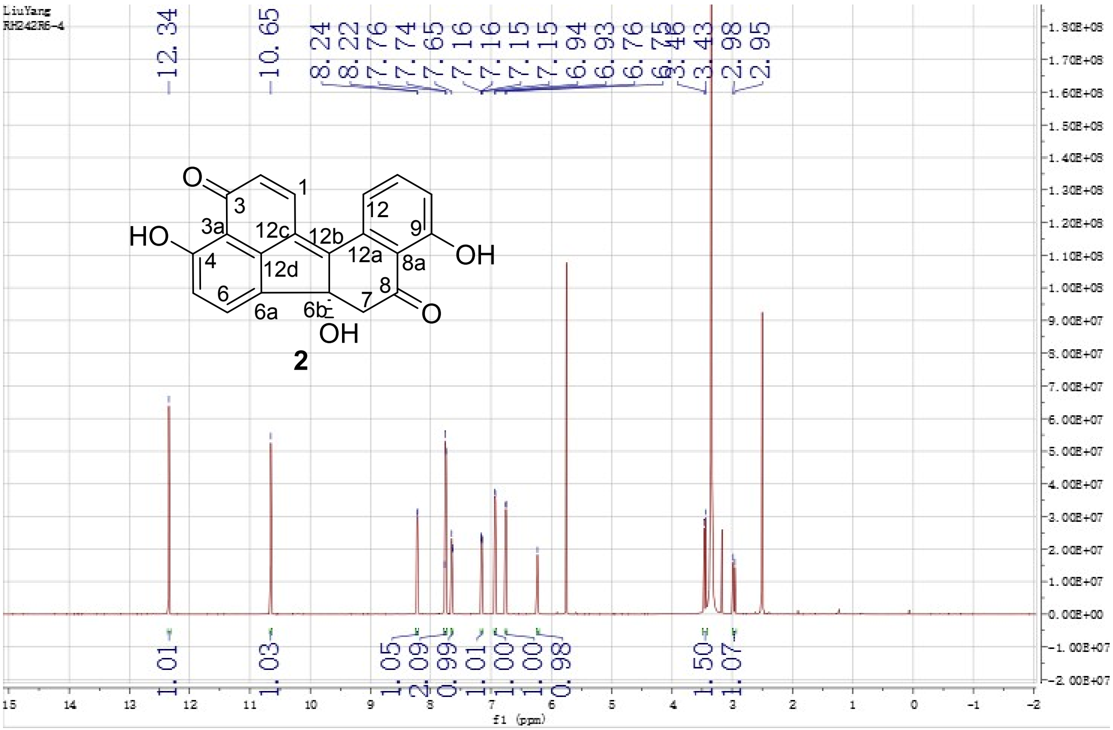
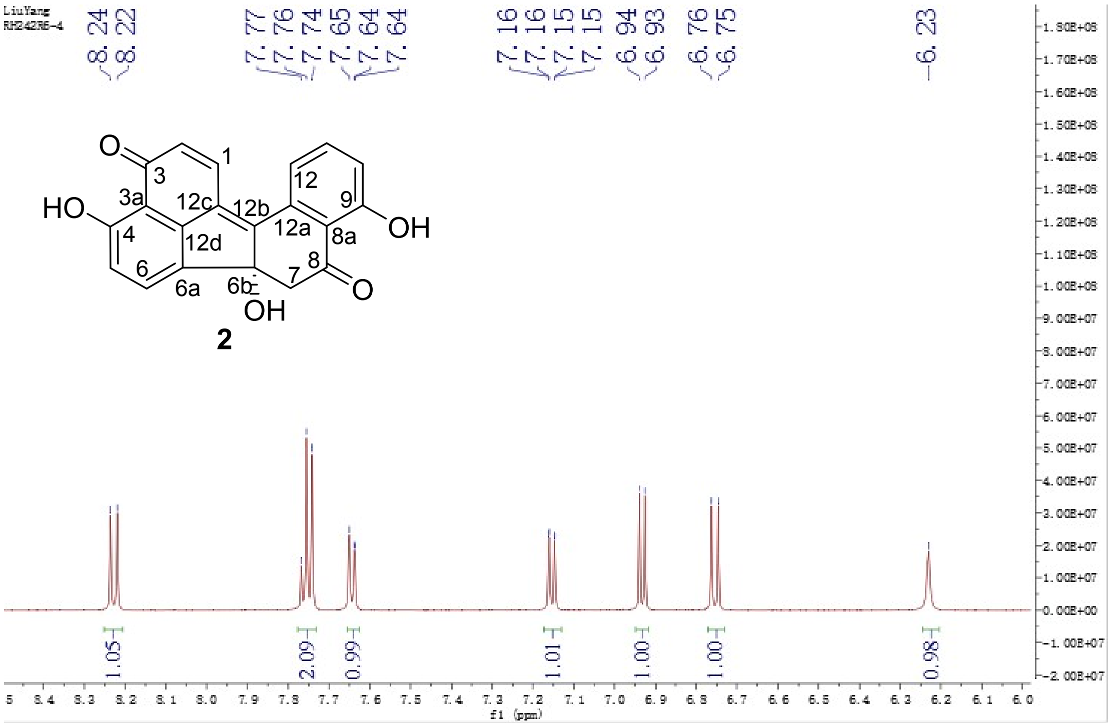


Fig. S18 Expanded ^{1}H NMR (DMSO-d_6, 600 MHz) spectrum of the new compound **2**



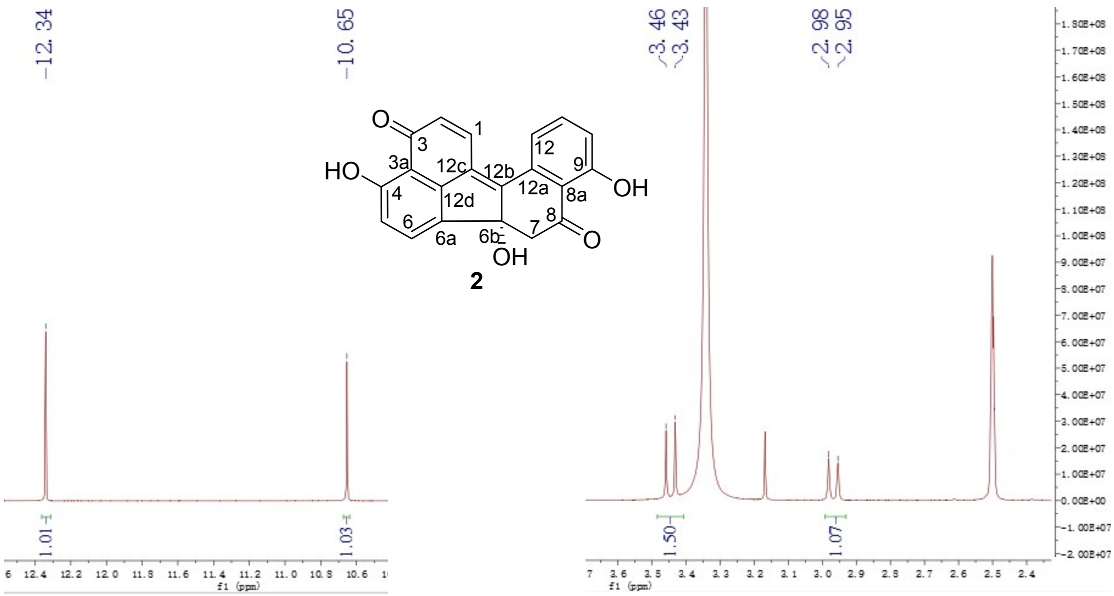


Fig. S19 COSY spectrum of the new compound **2**

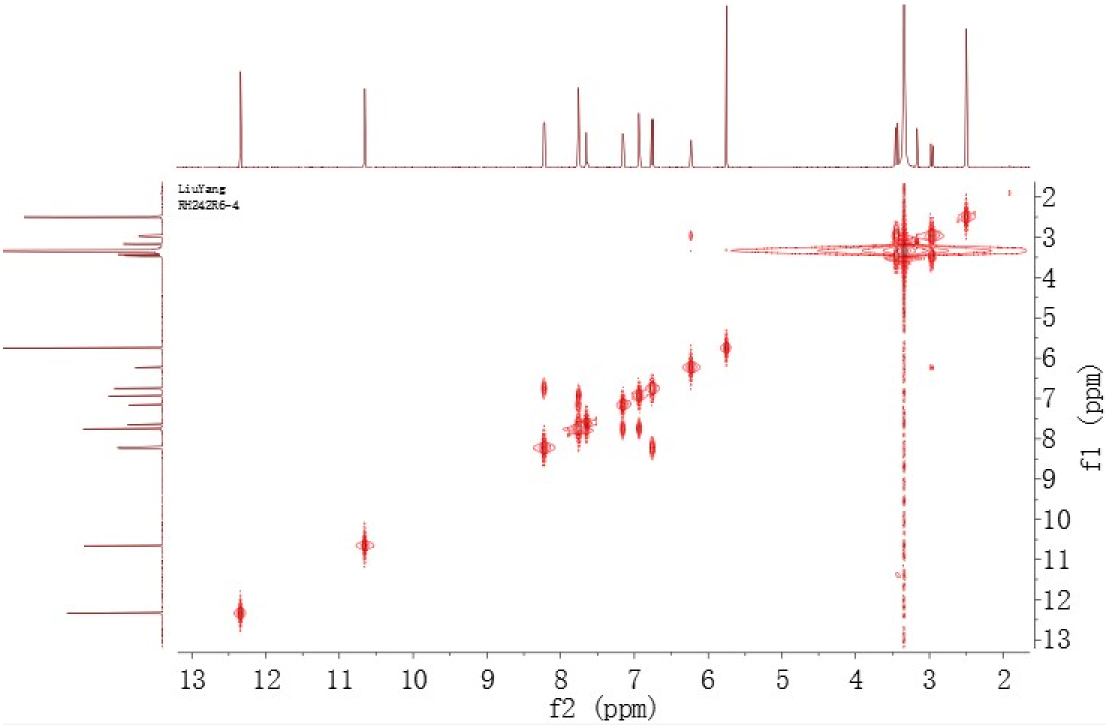


Fig. S20 ^{13}C NMR (DMSO-d_6, 150 MHz) spectrum of the new compound **2**

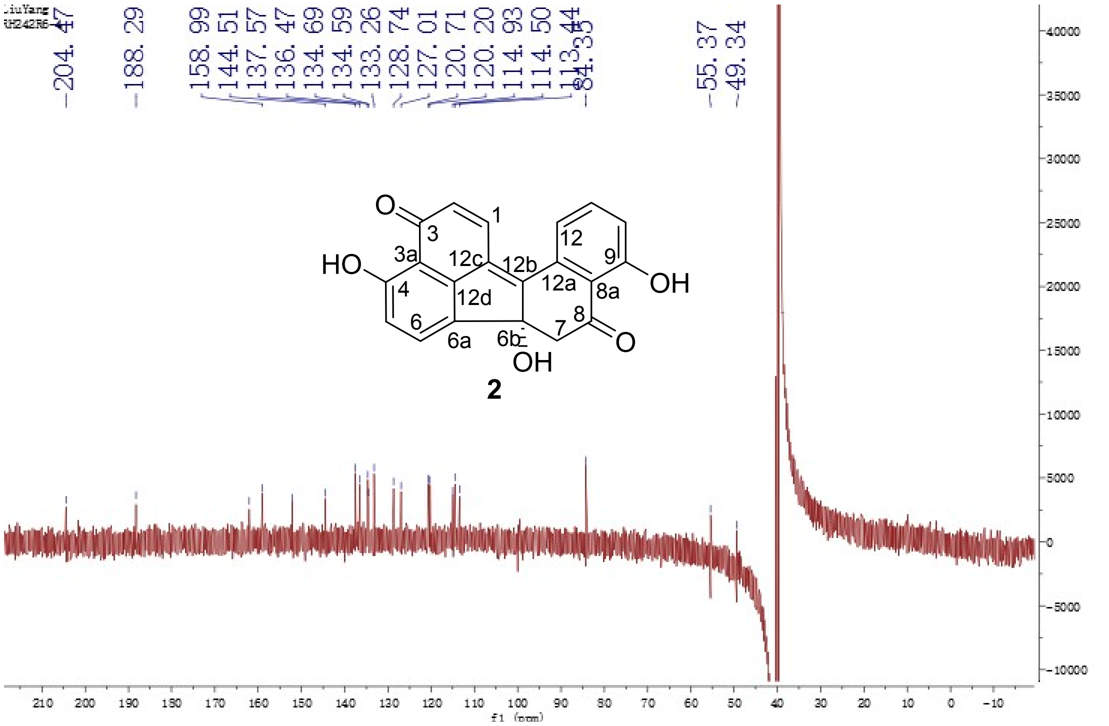


Fig. S21 Expanded ^{13}C NMR (150 MHz, DMSO-d_6) spectrum of the new compound **2**

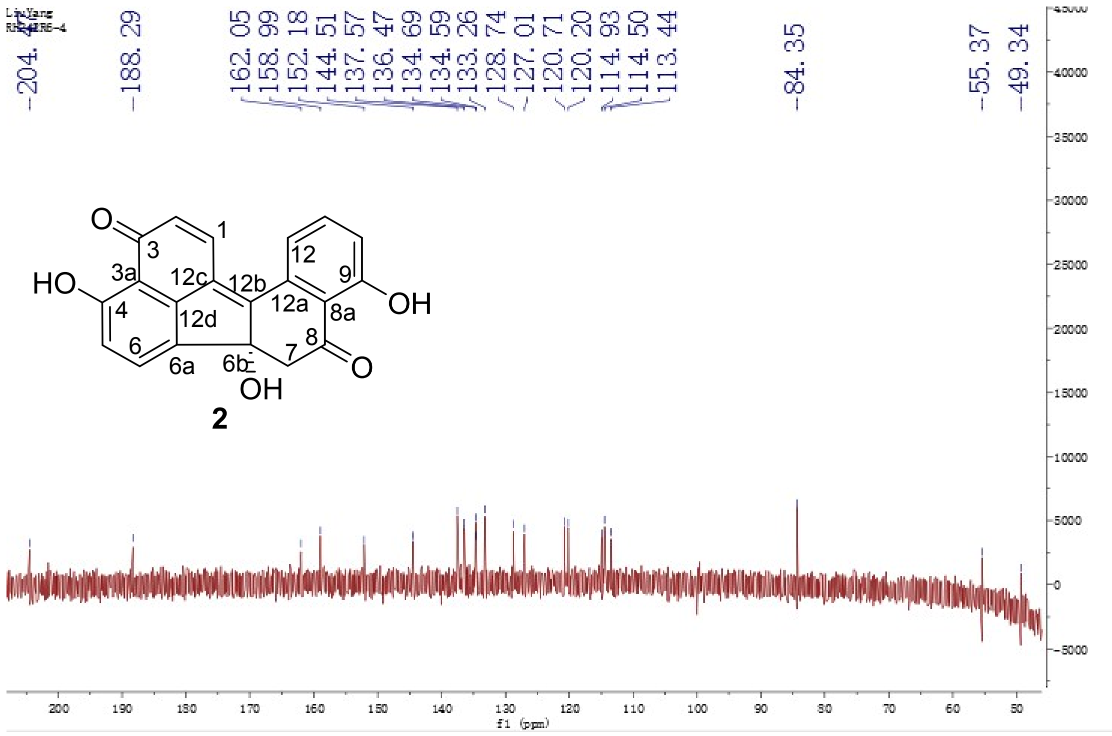


Fig. S22 HSQC spectrum of the new compound **2**

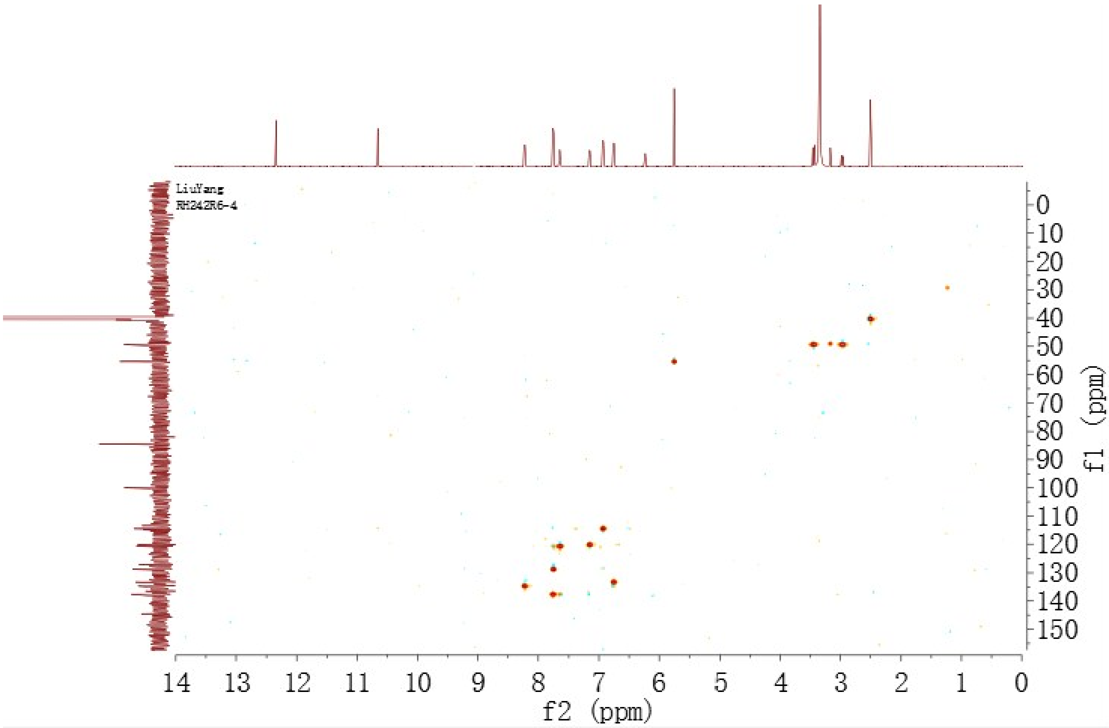


Fig. S23 HMBC spectrum of the new compound **2**

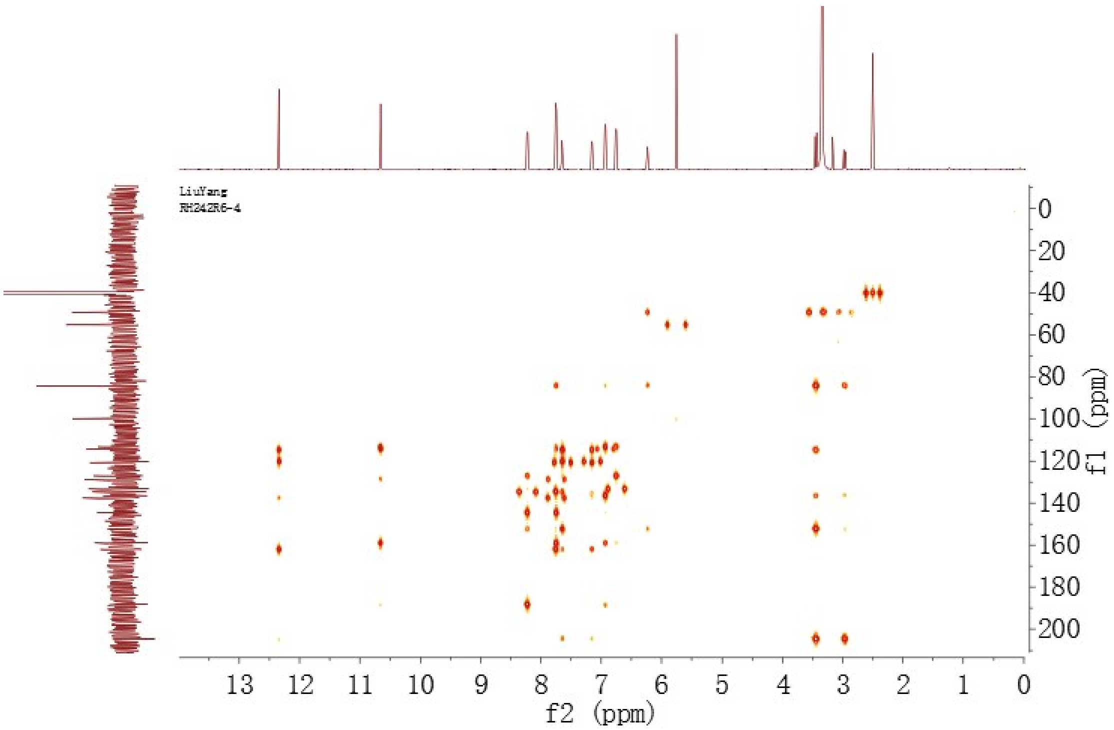


Fig. S24 Expanded HMBC spectrum of the new compound **2**

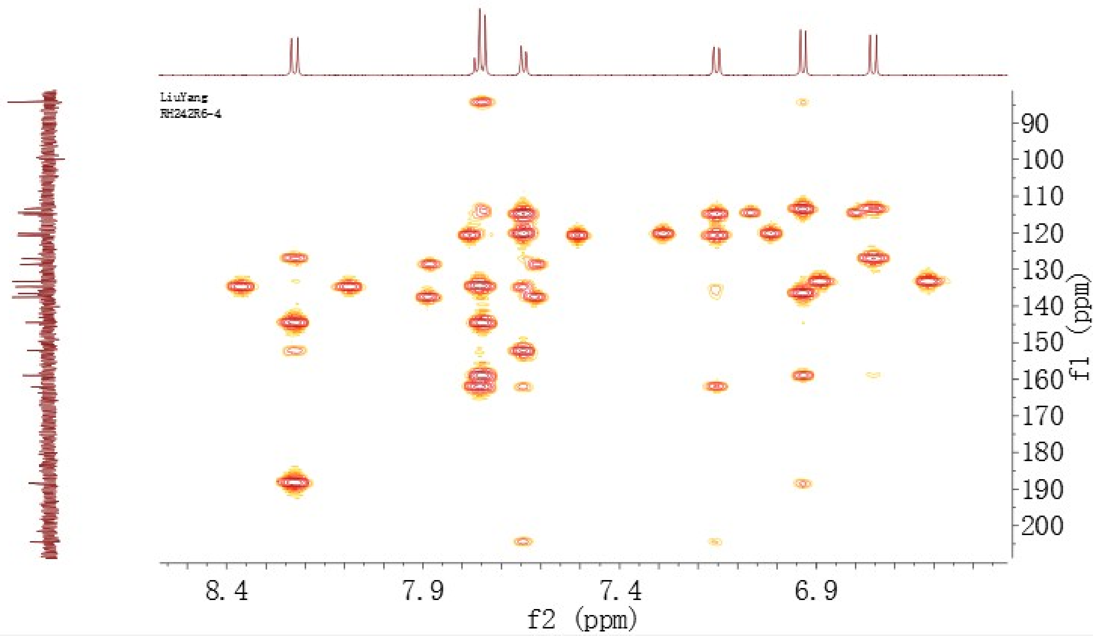


Fig. S25 HRESIMS spectrum of the new compound **2**

Mass Spectrum SmartFormula Report

Analysis Info

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Sample Name	Yang Liu RH242R6-4 (CH3OH)	Instrument	maXis 288882.20213
Comment			

Acquisition Parameter

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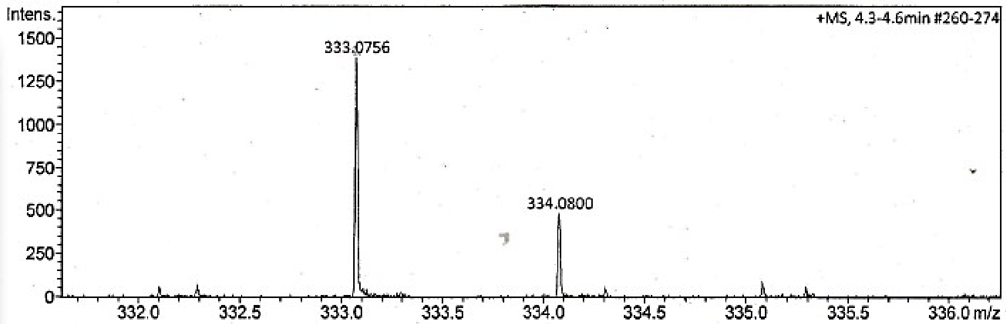
Scan End	1500 m/z	Set Collision Cell RF	600.0 Vpp	Set Divert Valve	Source



Meas. m/z	#	Ion Formula	m/z	err [ppm]	mSigma	# mSigma	Score	rdb	e⁻ Conf	N-Rule
333.0756	1	C20H13O5	333.0757	0.4	75.0	1	100.00	14.5	even	ok
	2	C5H9N12O6	333.0763	1.9	141.2	2	2.81	7.5	even	ok

Fig. S26 ^1H NMR (600 MHz CH_3OH-d_4) spectrum of the new compound **3**

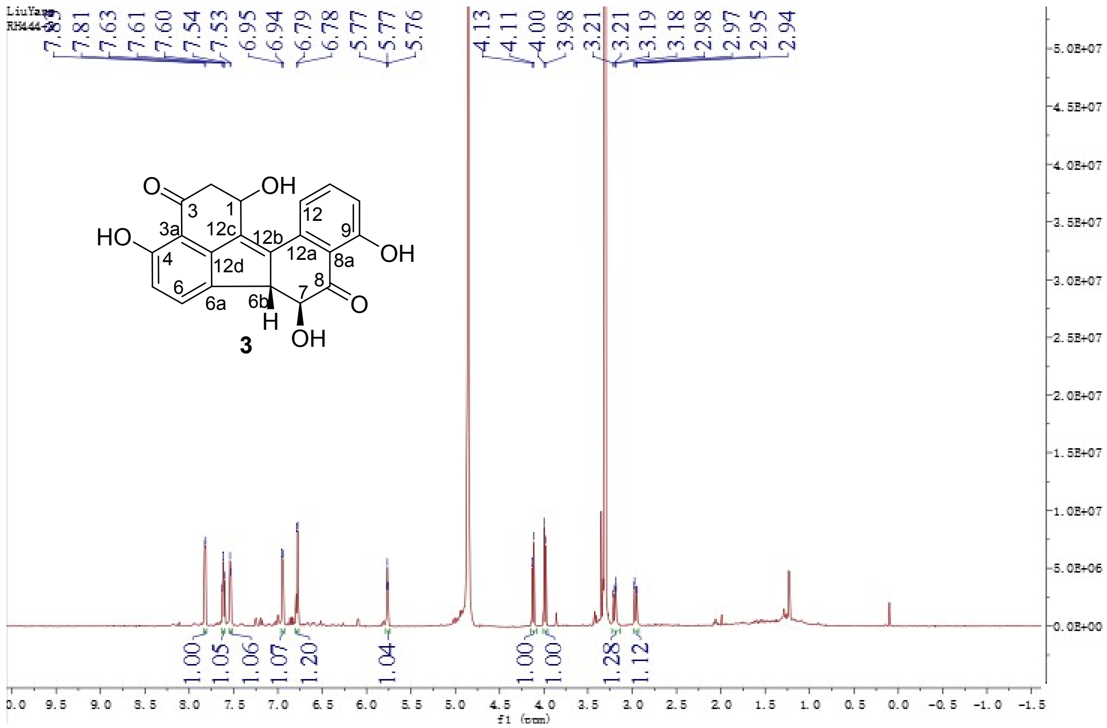
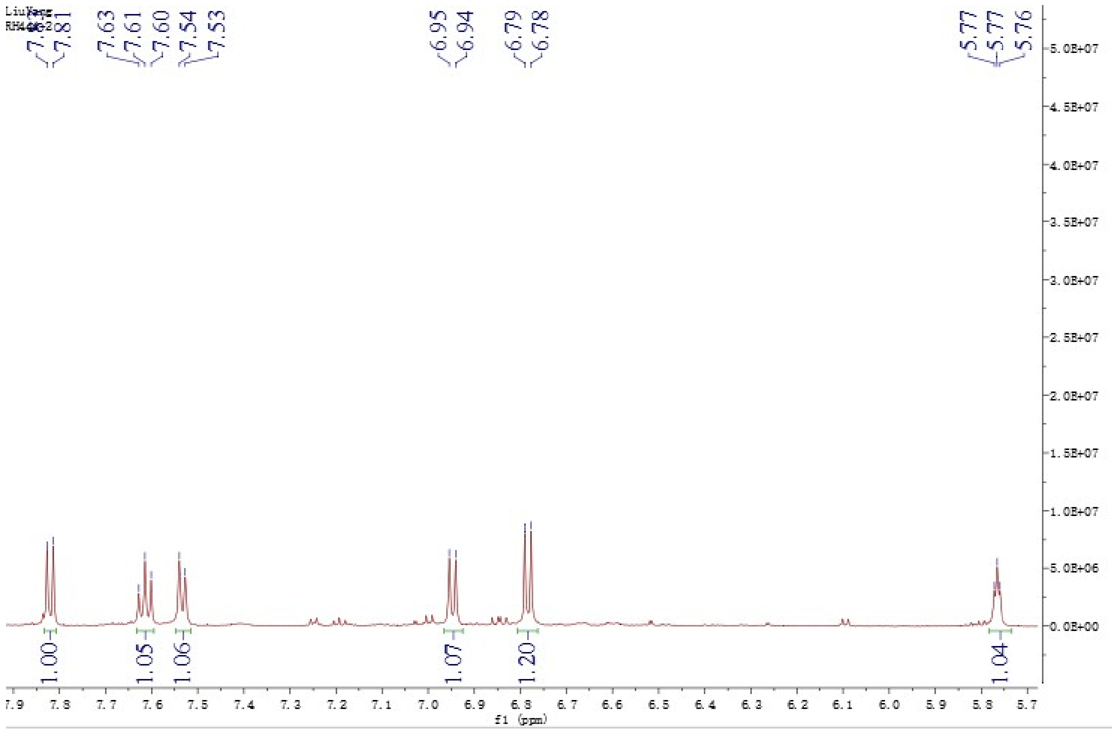


Fig. S27 Expanded ^{1}H NMR (600 MHz, CH_3OH-d_4,) spectrum of the new compound **3**



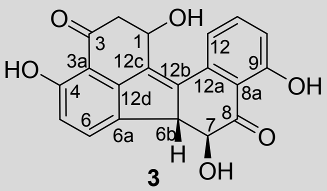


Fig. S28 COSY spectrum of the new compound **3**

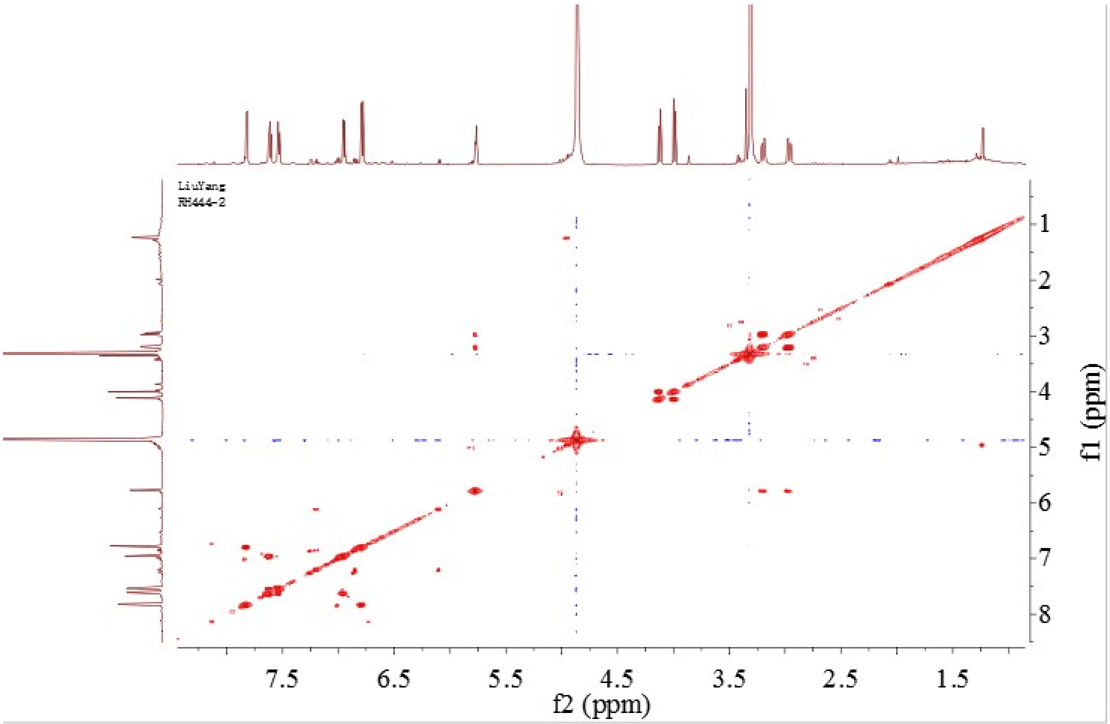


Fig. S29 HSQC spectrum of the new compound **3**

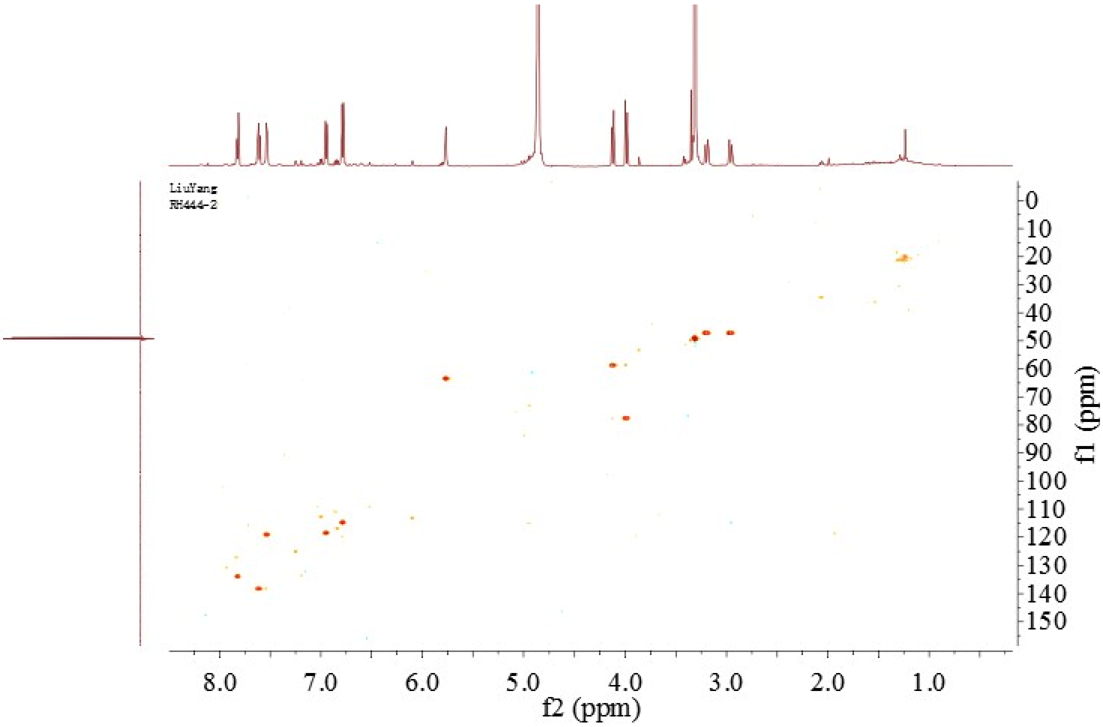


Fig. S30 HMBC spectrum of the new compound **3**

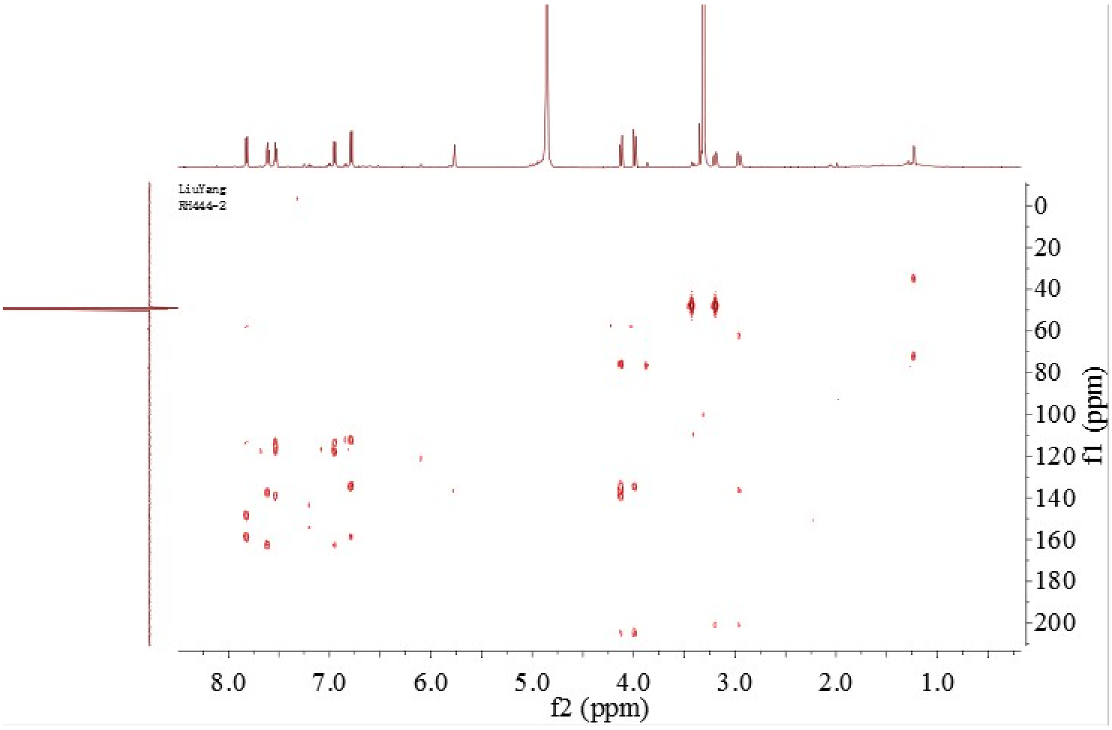


Fig. S31 HRESIMS spectrum of the new compound **3**

Mass Spectrum SmartFormula Report

Analysis Info

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Method tune_low.m

Operator Peter Tommes

Sample Name Yang RH444-2 (CH3OH)

Instrument maXis 288882.20213

Comment

Acquisition Parameter

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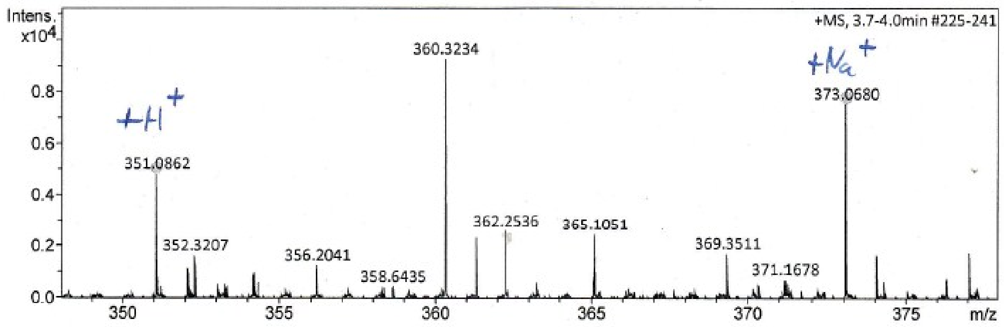
Scan End	1500 m/z	Set Collision Cell RF	600.0 Vpp	Set Divert Valve	Source



Meas. m/z	#	Ion Formula	m/z	err [ppm]	mSigma	# mSigma	Score	rdb	e⁻ Conf	N-Rule
351.0862	1	C20H15O6	351.0863	0.4	10.0	1	100.00	13.5	even	ok
373.0680	1	C20H14NaO6	373.0683	0.6	2.4	1	100.00	13.5	even	ok

Fig. S32 Structure and population of the low-energy B3LYP/6-31G(d) *in vacuo* conformers (>2%) of (1*R*,6b*S*,7*R*)-**3** diastereomer

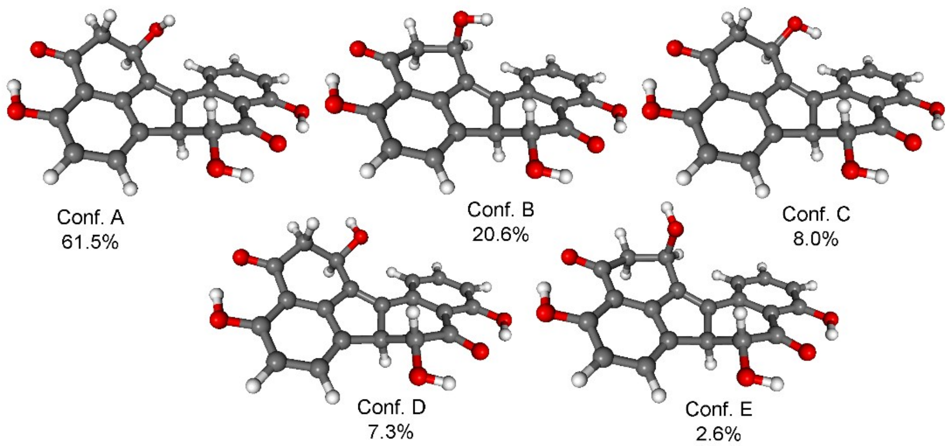


Fig. S33 Experimental ECD spectrum of **3** compared with the Boltzmann-weighted ECD spectra computed for the B3LYP/6-31G(d) *in vacuo* low-energy conformers of (1*R*,6b*S*,7*R*)-**3** at various levels

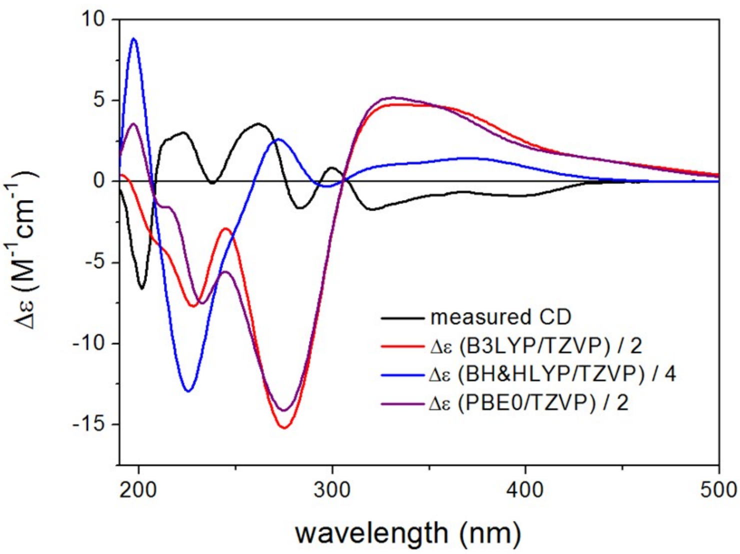


Fig. S34 Structure and population of the low-energy B3LYP/6-31G(d) conformers (>2%) of (1*S*,6b*S*,7*R*)-**3** diastereomer

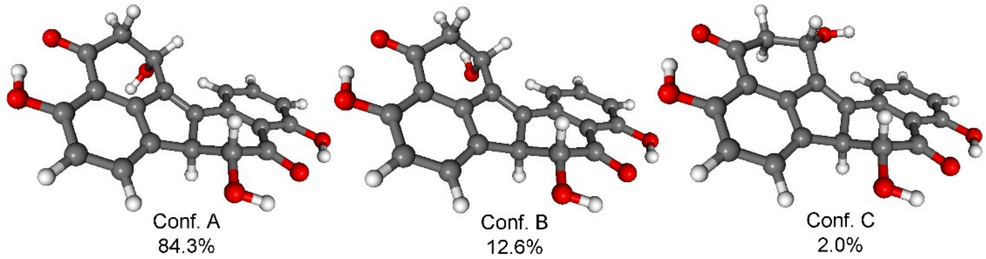


Fig. S35 Experimental ECD spectrum of **3** compared with the Boltzmann-weighted ECD spectra computed for the B3LYP/6-31G(d) *in vacuo* low-energy conformers of (1*S*,6b*S*,7*R*)-**3** at various levels

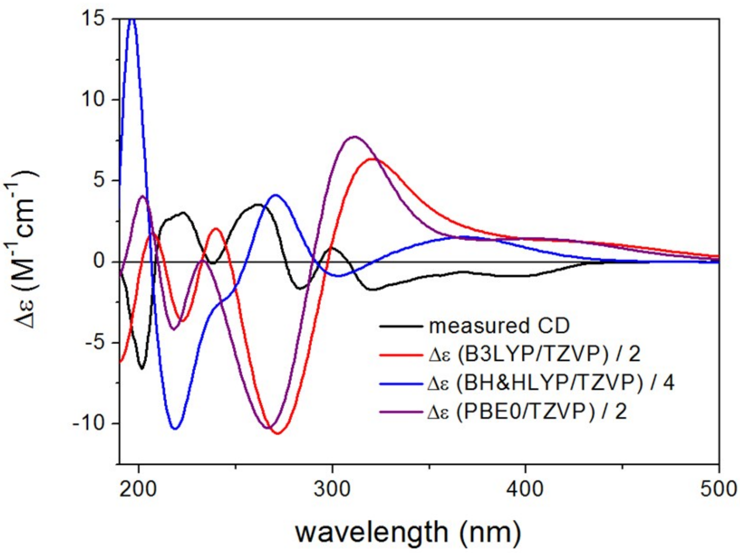


Fig. S36 Experimental ECD spectrum of **3** compared with the Boltzmann-weighted ECD spectra computed for the B3LYP/TZVP PCM/MeCN low-energy conformers of (1*R*,6b*S*,7*R*)-**3** at various levels.

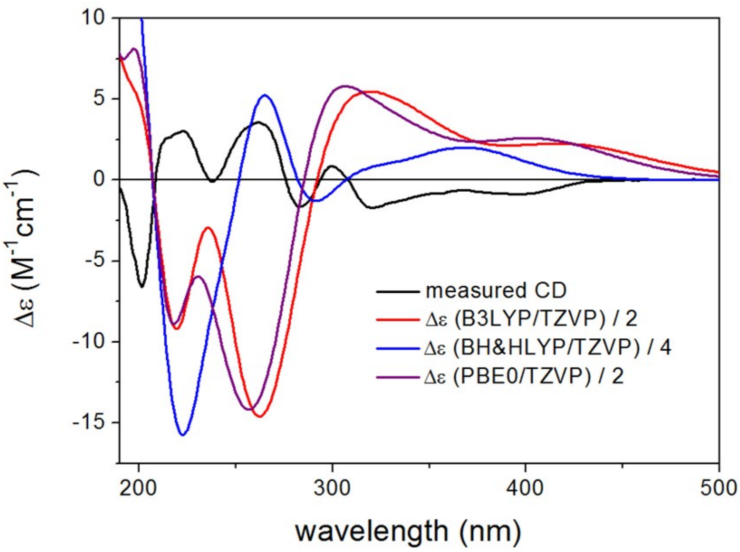


Fig. S37 Experimental ECD spectrum of **3** compared with the Boltzmann-weighted ECD spectra computed for the B3LYP/TZVP PCM/MeCN low-energy conformers of (1*S*,6b*S*,7*R*)-**3** at various levels

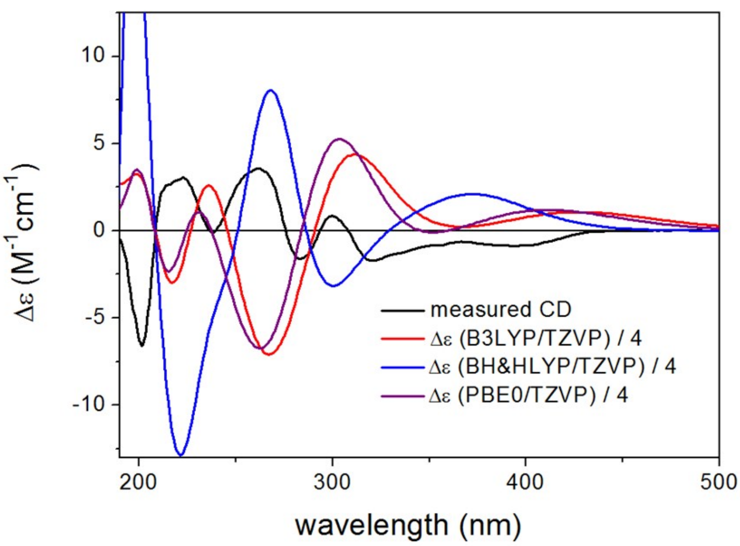


Fig. S38 Experimental ECD spectrum of **3** compared with the Boltzmann-weighted ECD spectra computed for the CAM-B3LYP/TZVP PCM/MeCN low-energy conformers of (1*R*,6b*S*,7*R*)-**3** at various levels

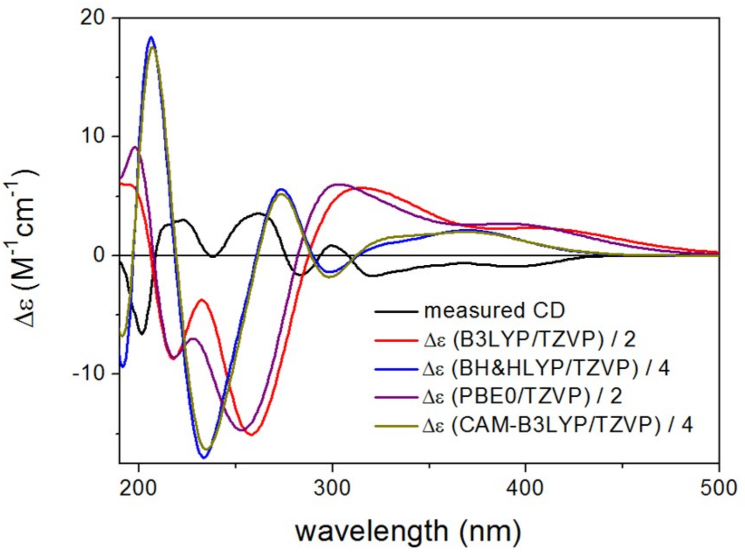


Fig. S39 Experimental ECD spectrum of **3** compared with the Boltzmann-weighted ECD spectra computed for the CAM-B3LYP/TZVP PCM/MeCN low-energy conformers of (1*S*,6b*S*,7*R*)-**3** at various levels.

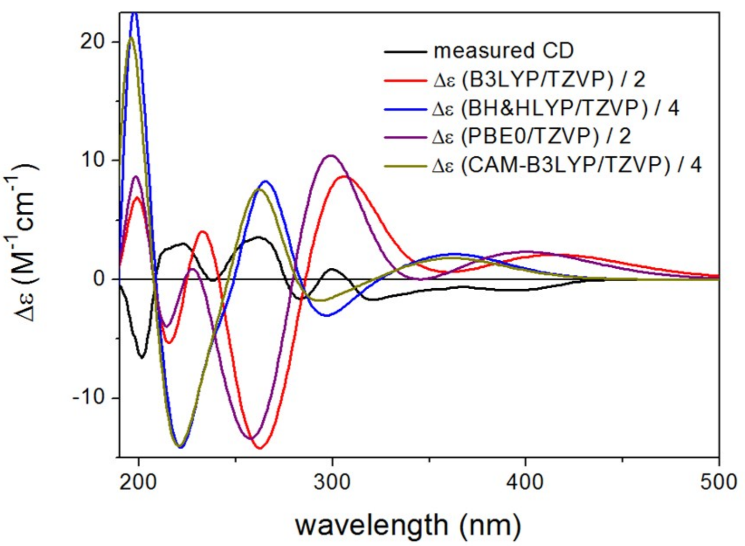
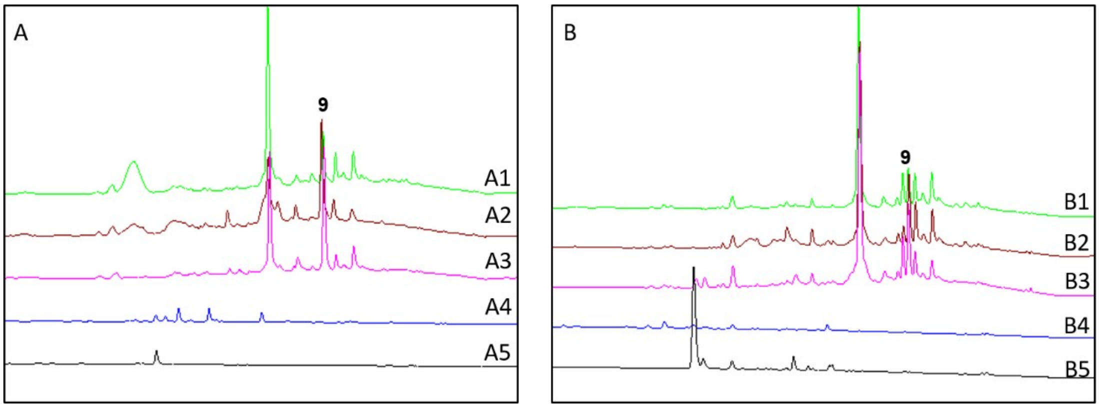


Fig. S40 HPLC chromatograms of EtOAc extracts from co-culture experiments (detection at UV 235 nm)



A: (A1) *Annulohypoxylon* sp. control, (A2) co-cultivation of *Annulohypoxylon* sp. with viable *S. coelicolor*, (A3) co-cultivation of *Annulohypoxylon* sp. with viable *S. lividans*, (A4) *S. coelicolor* control, (A5) *S. lividans* control;

B: (B1) *Annulohypoxylon* sp. control, (B2) co-cultivation of *Annulohypoxylon* sp. with viable *B. cereus*, (B3) co-cultivation of *Annulohypoxylon* sp. with viable *B. subtilis*, (B4) *B. cereus* control, (B5) *B. subtilis* control

Fig. S41 Proposed biogenetic pathway of benzo[*j*]fluoranthene derivatives

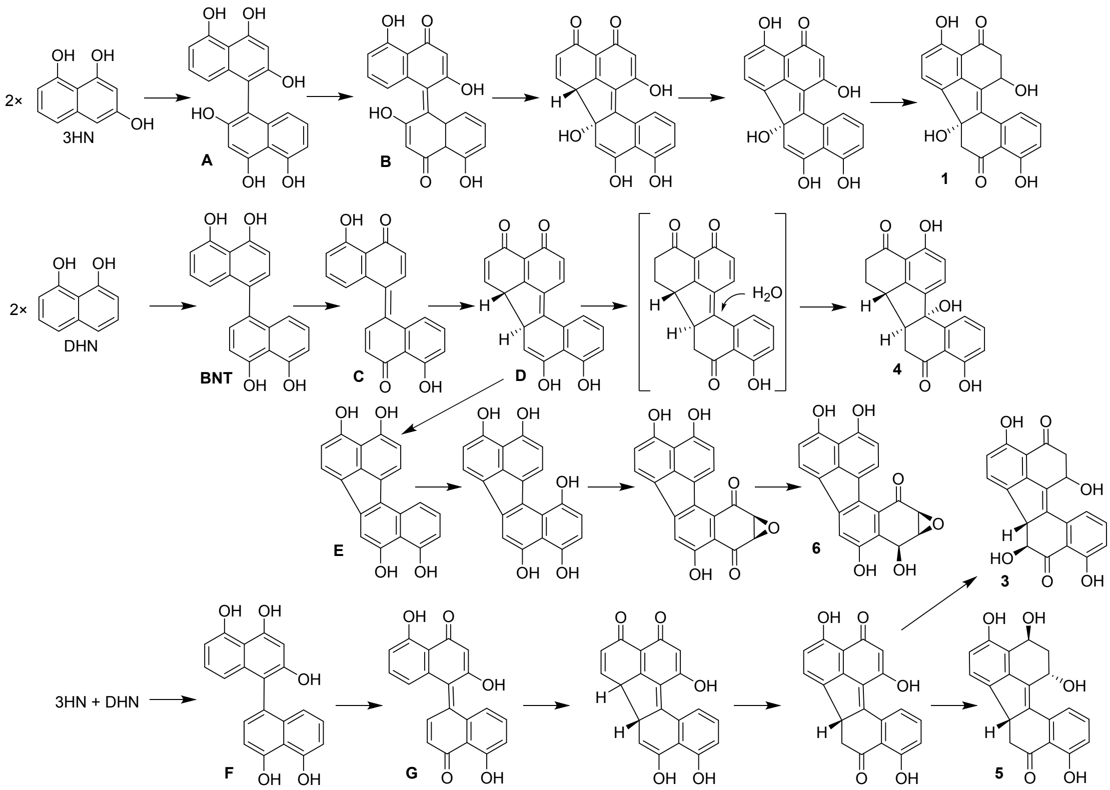
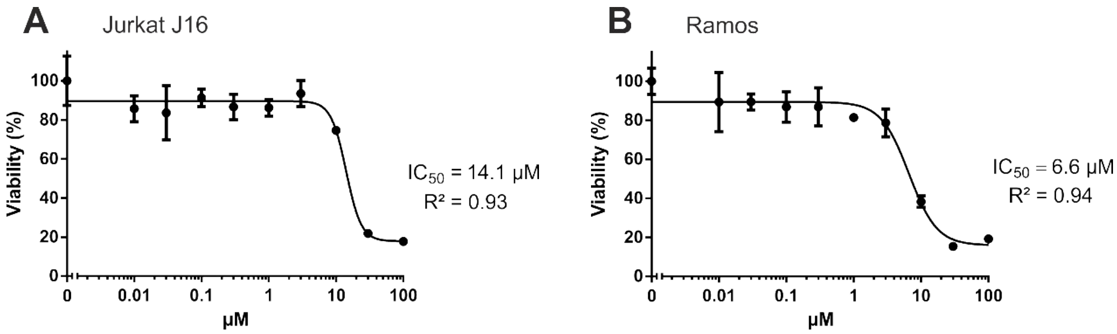
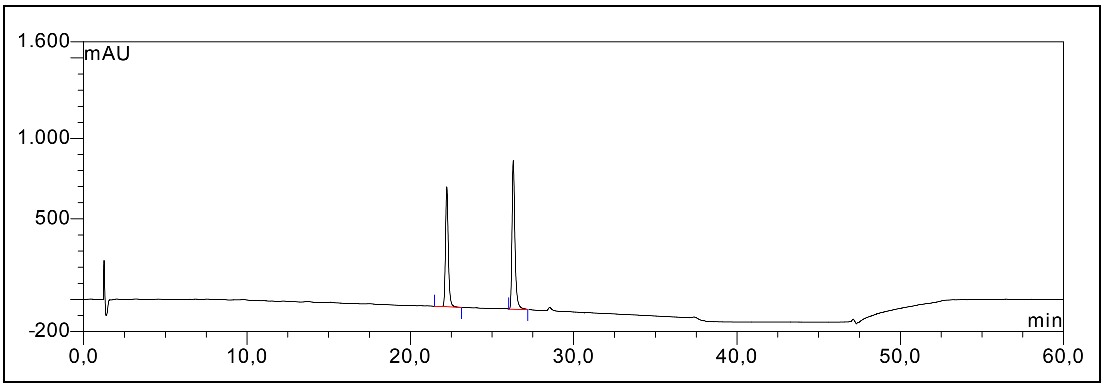


Fig S42. Cytotoxic effect of compound **2** on human leukemia and lymphoma cell lines measured by MTT assay

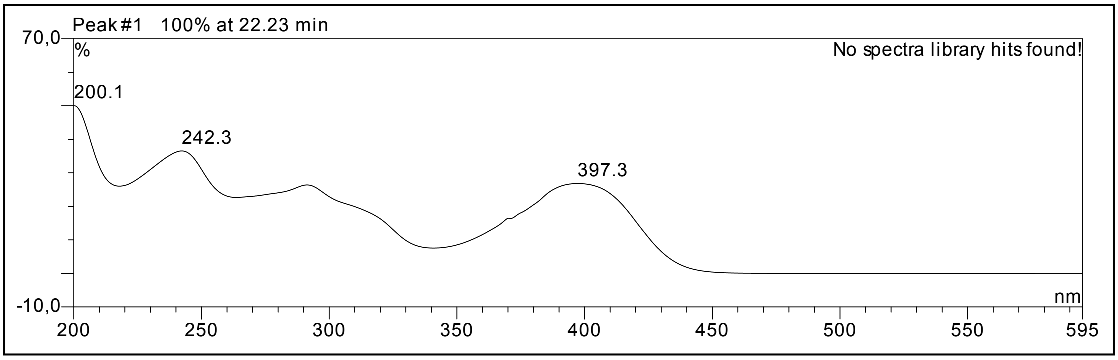


(A) Jurkat J16 cells (acute T cell leukemia cells) and (B) Ramos cells (Burkitt's lymphoma B lymphocytes) were seeded at a density of 5 × 10^5 cells/mL and incubated with increasing concentrations of compound **2**. Cells treated with DMSO (0.1% v/v) for 24 h were used as negative control. After incubation period of 24 h cell viability was monitored using the MTT Assay as described in methods. Relative viability in DMSO treated control cells was set to 100%. Data points shown are the mean of triplicates, error bars = SD. Viability and IC_{50} values (IC_{50} = half maximal inhibitory concentration) were calculated using Prism 6 (GraphPad Software). R^2 = coefficient of determination.

Fig. S43 HPLC Chromatogram of compound **1** (compound **1** was isolated as a 1:1 mixture with compound **2**)



UV absorption of compound **1** (peak 1, retention time: 22.230 min)



UV absorption of compound **2** (peak 2, retention time: 26.303 min)

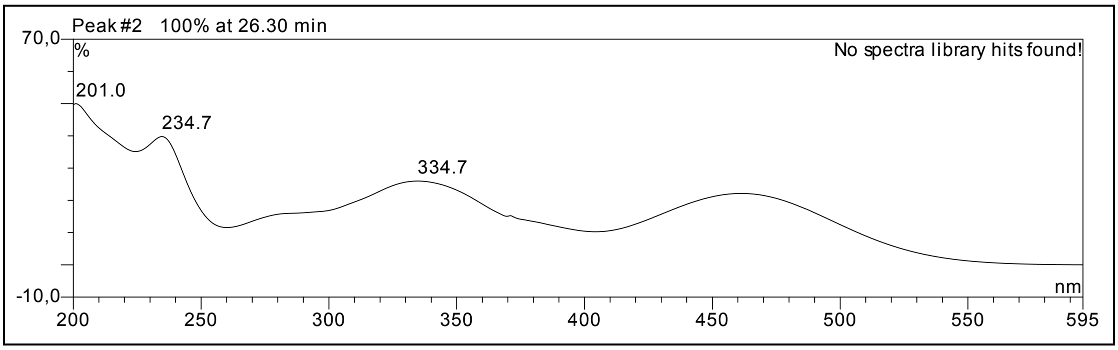
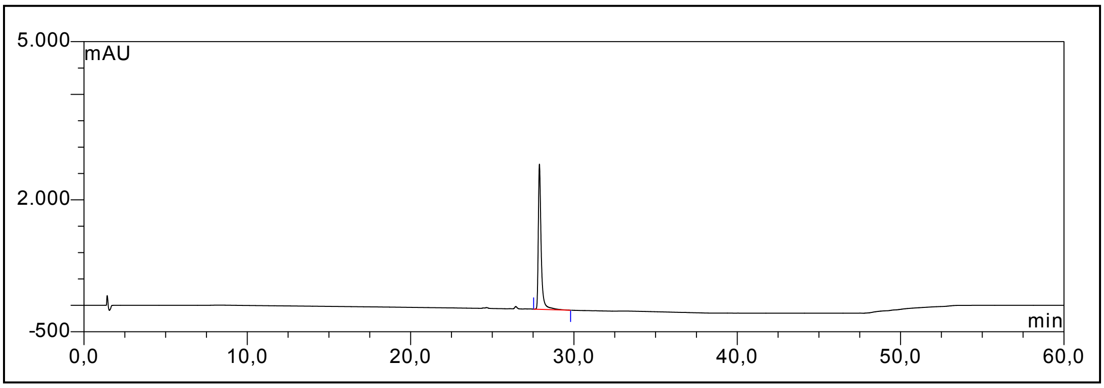


Fig. S44 HPLC Chromatogram of compound **2**



UV absorption of compound **2** (peak 1, retention time: 27.887 min)

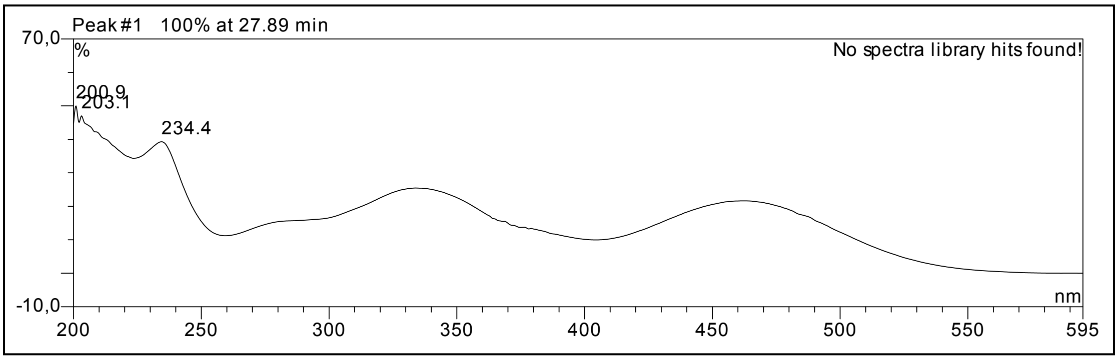
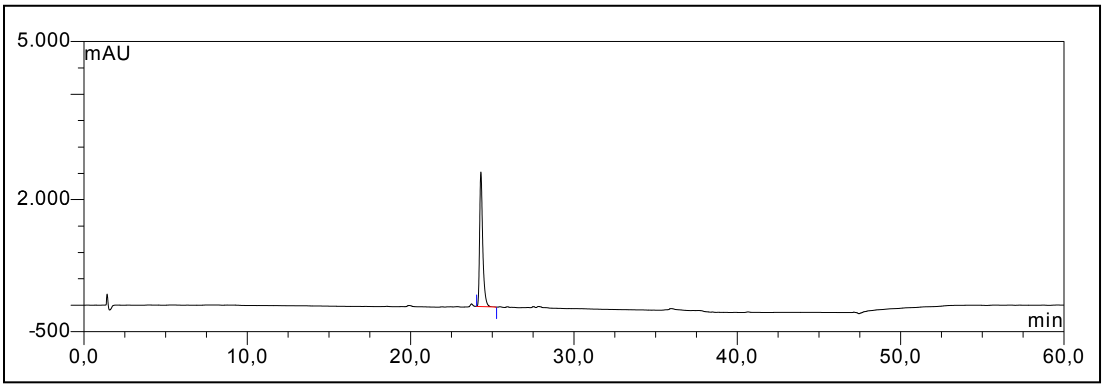


Fig. S45 HPLC Chromatogram of compound **3**



UV absorption of compound **3** (peak 1, retention time: 24.303)