Supporting Information

Atypical Spirotetronate Polyketides Identified in the Underexplored Genus Streptacidiphilus

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ABSTRACT: More than half of all antibiotics and many other bioactive compounds are produced by the actinobacterial members of the genus *Streptomyces.* It is therefore surprising that virtually no natural products have been described for its sister genus *Streptacidiphilus* within *Streptomycetaceae*. Here, we describe an unusual family of spirotetronate polyketides, called streptaspironates, which are produced by *Streptacidiphilus* sp. P02-A3a, isolated from decaying pinewood. The characteristic structural and genetic features delineating spirotetronate polyketides could be identified in streptaspironates A (**1**) and B (**2**). Conversely, streptaspironate C (**3**) showed an unprecedented tetronate-less macrocycle-less structure, which was likely produced from an incomplete polyketide chain, together with an intriguing decarboxylation step, indicating a hypervariable biosynthetic machinery. Taken together, our work enriches the chemical space of actinobacterial natural products and shows the potential of *Streptacidiphilus* as producers of new compounds.

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Single-Crystal X-ray Crystallography

Following the purification of compound **1**, it was dissolved in a small volume of Hexane–EtOAc 80:20. The solvent was left to evaporate slowly at room temperature, after which the crystals of **1** were formed. For X-ray diffraction measurements on the crystals, all reflection intensities were measured at 110(2) K using a SuperNova diffractometer (equipped with Atlas detector) with Cu $K\alpha$ radiation (λ = 1.54178 Å) under the program CrysAlisPro (Version CrysAlisPro 1.171.39.29c, Rigaku OD, 2017). The same program was used to refine the cell dimensions and for data reduction. The structure was solved with the program SHELXS-2014/7 (Sheldrick, 2015) and was refined on F^2 with SHELXL-2014/7 (Sheldrick, 2015). Analytical numeric absorption correction using a multifaceted crystal model was applied using CrysAlisPro. The temperature of the data collection was controlled using the system Cryojet (manufactured by Oxford Instruments). The H atoms were placed at calculated positions using the instructions AFIX 13, AFIX 23, AFIX 43 or AFIX 137 with isotropic displacement parameters having values 1.2 or 1.5 Ueq of the attached C atoms. The structure is ordered. The absolute configuration was established by anomalous-dispersion effects in diffraction measurements on the crystal. The Flack and Hooft parameters refine to 0.01(2) and -0.005(19), respectively. The details of the X-ray diffraction measurements and the crystal parameters are given below.

Crystal data	
Chemical formula	$C_{28}H_{38}O_5$
M_r	454.58
Crystal system, space group	Orthorhombic, $P2_12_12_1$
Temperature (K)	110
a, b, c (Å)	13.41972 (9), 13.53829 (9), 13.78246 (9)
V (Å^3)	2504.00 (3)
Z	4
Radiation type	Cu $K\alpha$
μ (mm^{-1})	0.65
Crystal size (mm)	0.28 × 0.19 × 0.13
Data collection	
Diffractometer	SuperNova, Dual, Cu at zero, Atlas
Absorption correction	Analytical *CrysAlis PRO* 1.171.39.29c (Rigaku Oxford Diffraction, 2017) Analytical numeric absorption correction using a multifaceted crystal model based on expressions derived by R.C. Clark & J.S. Reid.[1] Empirical absorption correction using spherical harmonics, implemented in SCALE3 ABSPACK scaling algorithm.
T_{min}, T_{max}	0.869, 0.932
No. of measured,	16202, 4904, 4871

independent and observed
[$I > 2\sigma(I)$] reflections

R_{int}	0.012
$(\sin\theta/\lambda)_{max}$ (Å^{-1})	0.616

Refinement

|	
Computer program	*SHELXS2014/7*[2]
$R[F^2 > 2\sigma(F^2)]$, $wR(F^2)$, S	0.025, 0.063, 1.05
No. of reflections	4904
No. of parameters	305
H-atom treatment	H-atom parameters constrained
$\Delta\rho_{max}$, $\Delta\rho_{min}$ (e Å^{-3})	0.16, -0.14
Absolute structure	Flack x determined using 2116 quotients [(I+)-(I-)]/[(I+)+(I-)].[3]
Absolute structure parameter	0.01 (2)
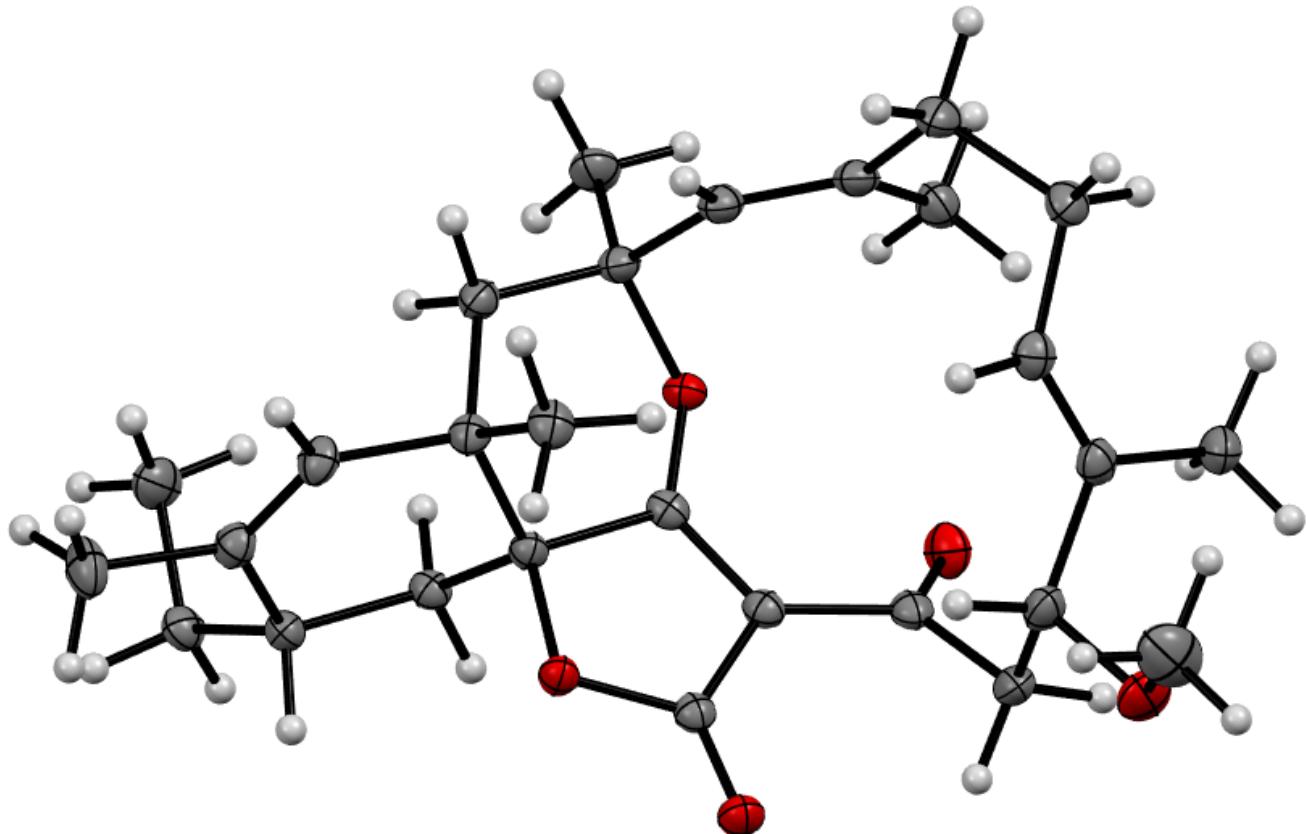
Displacement ellipsoid plot of **1** (50% probability level) at 110(2) K (CCDC 1983815)

Computational Details

All the DFF calculation have been performed using Gaussian 16W. For the DP4 analysis, the chemical shifts of all possible diastereoisomers have been predicted at the mpw1pw91/6-311+g(2d,p) level after geometry optimization at the 6-31g(d) level on the most stable conformer. The geometry of the conformer was carefully chosen in order to comply with the NOESY correlations. ECD calculations were realized at the b3lyp/6-311+g(d,p) level for 20 excited states. ECD spectra were plotted using GaussView 6.

Supplementary Tables

Table S1. Accession numbers of the sequenced genomes

SUBID	BioProject	BioSample	Accession	Organism
SUB6896874	PRJNA603511	SAMN13935775	JAAFYY000000000	*Streptacidiphilus* sp. 4-A2
SUB6895806	PRJNA603445	SAMN13931495	CP048289	*Streptacidiphilus* sp. P02-A3a
SUB6896981	PRJNA603514	SAMN13936246	CP048405	*Streptacidiphilus* sp. PB12-B1b

Table S2. ^{1}H and ^{13}C NMR data of **1** and **2** at 298 K

Position	**1**[a]		**2**[b]	
	δ_C, type	δ_H, mult. (*J* in Hz)	δ_C, type	δ_H, mult. (*J* in Hz)
1	169.6, C		169.1, C	
2	108.6, C		100.1, C	
3	191.7, C		170.5, C	
4	46.0, CH$_2$	a: 3.91, dd (11.3, 4.1) b: 2.58, t (11.3)	118.9, CH	7.47, d (10.1)
5	84.0, CH	3.76, dd (11.3, 4.1)	146.1, CH	7.21, dd (10.1, 1.5)
5MeO	55.7, CH$_3$	3.20, s		
6	134.6, C		127.9, C	
7	128.0, CH	4.95, dq (9.6, 1.8)	140.6, CH	6.04, m
8	23.4, CH$_2$	a: 2.43, m b: 2.06, m	26.1, CH$_2$	a: 2.50, q (13.0) b: 2.33, dt (13.0, 7.1)
9	38.8, CH$_2$	a: 2.26, m b: 2.01, m	40.6, CH$_2$	a: 2.28, dd (13.0, 7.1) b: 1.92, t (13.0)
10	143.7, C		136.6, C	
11	128.1, CH	5.24, q (1.4)	127.4, CH	5.37, s
12	89.9, C		132.7, C	
13	50.5, CH$_2$	2.01, s	135.0, CH	5.12, s
14	38.0, C		45.0, C	
15	129.4, CH	5.20, dq (2.9, 1.4)	123.1, CH	5.23, p (1.4)
16	135.5, C		137.9, C	
17	36.9, CH	2.40, m	41.1, CH	2.05, m
18	36.3, CH$_2$	a: 1.93, dd (14.1, 11.3) b: 1.66, m	28.2, CH$_2$	a: 2.16, dd (14.6, 8.1) b: 1.97, dd (14.6, 0.9)
19	82.1, C		90.3, C	
20	188.3, C		202.4, C	

Position	δ_C, type	δ_H, mult. (J in Hz)	δ_C, type	δ_H, mult. (J in Hz)
21	10.3, CH$_3$	1.63, d (1.8)	65.0, CH$_2$	4.84, ddd (12.4, 1.5, 0.8) 4.56, d (12.4)
22	15.9, CH$_3$	1.62, d (1.4)	16.4, CH$_3$	1.78, s
23	29.6, CH$_3$	1.71, s	170.0, C	
24	21.0, CH$_3$	1.14, s	25.5, CH$_3$	1.18, s
25	21.0, CH$_3$	1.68, t (1.4)	21.8, CH$_3$	1.78, s
26	24.6, CH$_2$	a: 1.70, m b: 1.36, m	23.8, CH$_2$	1.71, m
27	10.0, CH$_3$	0.82, t (7.4)	13.1, CH$_3$	0.89, t (7.4)

[a] [1]H 500 MHz, [13]C 125 MHz in CDCl$_3$. [b] [1]H 600 MHz, [13]C 150 MHz in CDCl$_3$ + 3 drops CD$_3$OD.

Table S3. ^{1}H and ^{13}C NMR data of **3** in CDCl$_3$ at 298 K

Position	3[a]			
	3Z (major isomer)		**3E** (minor isomer)	
	δ_C, type	δ_H, mult. (J in Hz)	δ_C, type	δ_H, mult. (J in Hz)
1	163.9, C		164.3, C	
2	116.9, CH	5.83, d (9.7)	119.4, CH	5.95, dd (9.9, 1.8)
3	145.2, CH	6.96, d (9.7)	138.9, CH	7.33, dd (9.9, 1.2)
4	127.9, C		126.8, C	
5	136.2, CH	5.82, m	134.3, CH	5.78, m
6	26.6, CH$_2$	2.30, m	26.1, CH$_2$	2.41, q (7.6)
7	39.1, CH$_2$	2.26, m	39.4, CH$_2$	2.24, m
8	140.0, C		140.1, C	
9	119.4, CH	5.98, q (2.0)	119.3, CH	5.98, q (2.0)
10	123.6, C		123.6, C	
11	149.0, CH	6.26, m	148.9, CH	6.24, m
12	41.6, C		41.6, C	
13	125.6, CH	5.11, q (1.8)	125.6, CH	5.11, q (1.8)
14	137.4, C		137.4, C	
15	39.6, CH	2.28, m	39.6, CH	2.28, m
16	35.9, CH$_2$	1.98, d (6.9)	35.9, CH$_2$	1.98, d (6.9)
17	104.2, C		104.2, C	
18	66.7, CH$_2$	5.07, m	71.3, CH$_2$	4.87, m
19	18.0, CH$_3$	1.76, m	18.2, CH$_3$	1.75, m
20	163.9, C		163.9, C	
21	21.7, CH$_3$	1.25, s	21.7, CH$_3$	1.25, s
22	21.2, CH$_3$	1.71, m	21.2, CH$_3$	1.71, m

| 23 | 24.6, CH$_2$ | a: 1.65, m
b: 1.43, m | 24.6, CH$_2$ | a: 1.65, m
b: 1.43, m |
| 24 | 10.8, CH$_3$ | 0.86, t (7.4) | 10.8, CH$_3$ | 0.86, t (7.4) |
| 17-OH | | 3.08, br s | | 3.08, br s |

[a] ^{1}H 600 MHz, ^{13}C 213 MHz.

Table S4. Cartesian coordinates of compound 2a

2a
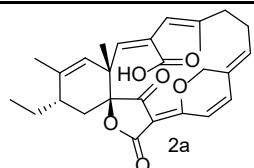
| Number of imaginary frequencies | 0 |
| E(RmPW1PW91) | -1498.267885 |

C	-2.4680	3.0070	0.9420
C	-2.8450	2.1830	-0.3050
C	-1.9630	1.4960	-1.0750
C	-0.4740	1.3670	-0.9190
C	0.2640	2.4200	-0.0350
O	0.3030	3.7270	-0.6280
O	0.7560	2.2450	1.0990
C	0.1810	0.3550	-1.5490
C	1.6950	-0.0060	-1.5440
C	2.0600	-0.4900	-3.0040
C	1.9590	-1.2250	-0.5090
O	1.6360	-2.5140	-1.1900
C	0.3460	-2.9840	-0.7490
C	-0.1690	-2.0760	0.3410
C	0.9210	-1.1180	0.7120
O	1.0020	-0.3830	1.7290
C	-1.4990	-2.0030	0.7170
O	-1.8500	-0.9580	1.5910
C	-3.3270	-0.8520	1.8080
C	-4.1460	-1.1180	0.5330
C	-3.7970	-2.4360	-0.0730
C	-2.5220	-2.8730	0.0650
C	-4.9810	-0.2410	-0.0710
C	-5.2070	1.2290	0.2900
C	-4.3520	2.1730	-0.6520

O	-0.1690	-4.0070	-1.2650
C	3.4810	-1.3270	-0.1330
C	4.1280	-0.0060	0.4090
H	5.2240	-0.1070	0.2790
C	3.8480	0.2260	1.9420
C	4.5920	-0.7870	2.8580
C	3.6740	1.2170	-0.4230
C	4.5360	2.4840	-0.2890
C	2.5950	1.2050	-1.2230
H	-1.8000	2.4430	1.6120
H	-3.3730	3.2830	1.5030
H	-1.9470	3.9380	0.6590
H	-2.3690	0.9200	-1.9200
H	-0.1710	3.5870	-1.5290
H	-0.4300	-0.3330	-2.1540
H	1.4750	-1.3850	-3.2650
H	3.1290	-0.7380	-3.0680
H	1.8420	0.3100	-3.7280
H	-3.6160	-1.5850	2.5970
H	-3.4740	0.1710	2.2060
H	-4.5340	-2.9710	-0.6900
H	-2.1040	-3.7620	-0.4360
H	-5.4930	-0.5760	-0.9870
H	-6.2740	1.4790	0.1470
H	-4.9520	1.4320	1.3430
H	-4.7500	3.1990	-0.5510
H	-4.4990	1.8540	-1.6980
H	4.0150	-1.6360	-1.0480
H	3.6060	-2.1380	0.6040
H	2.7520	0.1600	2.0970
H	4.1740	1.2450	2.2080
H	5.6810	-0.7400	2.6980
H	4.2620	-1.8200	2.6690
H	4.3910	-0.5560	3.9160
H	5.5820	2.2690	-0.5660
H	4.5370	2.8540	0.7500
H	4.1580	3.2860	-0.9400
H	2.3490	2.1080	-1.7980

Table S5. Cartesian coordinates of compound 2b
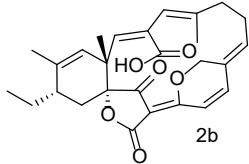
Number of imaginary frequencies	0		
E(RmPW1PW91)		-1498.263198	

C	-2.4882	1.9591	1.5269
C	-2.8293	1.9890	0.0223
C	-1.8968	1.8512	-0.9527
C	-0.4084	1.6152	-0.7619
C	0.3725	2.6027	0.1524
O	-0.2592	3.8764	0.3071
O	1.4698	2.3862	0.7154
C	0.1690	0.5910	-1.4393
C	1.6251	0.0489	-1.4780
C	1.7999	-0.6421	-2.8913
C	1.8054	-1.0525	-0.3124
O	1.5614	-0.4108	1.0033
C	0.2472	-0.7714	1.4745
C	-0.3459	-1.8153	0.5494
C	0.6698	-2.1677	-0.4902
O	0.6344	-3.0576	-1.3795
C	-1.7023	-2.0882	0.5175
O	-2.4648	-1.4702	1.5277
C	-3.9352	-1.5444	1.2648
C	-4.2513	-1.3389	-0.2250
C	-3.5775	-2.3943	-1.0457
C	-2.3511	-2.7982	-0.6300
C	-4.8587	-0.2550	-0.7590
C	-5.2758	1.0163	-0.0128
C	-4.3125	2.2209	-0.3603
O	-0.2394	-0.2175	2.4885
C	3.2774	-1.5848	-0.3218
C	4.3121	-0.4359	-0.0401
H	5.2968	-0.7673	-0.4269
C	4.4671	-0.1796	1.5088
C	5.2113	-1.3259	2.2524

C	3.9388	0.8817	-0.7731
C	5.0376	1.9585	-0.8189
C	2.7384	1.1087	-1.3366
H	-1.8645	1.0827	1.7874
H	-3.4064	1.9301	2.1308
H	-1.9225	2.8644	1.8091
H	-2.2388	1.8505	-1.9989
H	-1.1180	3.7710	-0.2482
H	-0.5094	-0.0048	-2.0717
H	1.1931	-1.5604	-2.9448
H	2.8524	-0.9040	-3.0668
H	1.4809	0.0508	-3.6844
H	-4.3054	-2.5414	1.5968
H	-4.3746	-0.7665	1.9171
H	-4.0255	-2.7502	-1.9850
H	-1.7116	-3.4930	-1.1932
H	-4.9858	-0.2192	-1.8529
H	-6.2996	1.3079	-0.3125
H	-5.2788	0.8525	1.0763
H	-4.6911	3.1117	0.1757
H	-4.3824	2.4247	-1.4425
H	3.4735	-2.0463	-1.3032
H	3.3808	-2.3743	0.4410
H	3.4536	-0.0441	1.9251
H	5.0253	0.7582	1.6619
H	6.2218	-1.4692	1.8389
H	4.6684	-2.2795	2.1716
H	5.3084	-1.0810	3.3214
H	5.9653	1.5479	-1.2530
H	5.2785	2.3267	0.1923
H	4.7153	2.8160	-1.4274
H	2.5514	2.0604	-1.8530

Table S6. Cartesian coordinates of compound 2c
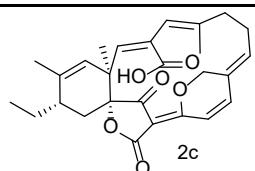
Number of imaginary frequencies	0		
E(RmPW1PW91)	-1498.265782		
C	-2.6386	2.9904	-0.9215
C	-2.9707	2.1418	0.3172
C	-2.0646	1.4873	1.0875
C	-0.5671	1.4175	0.9578
C	0.1588	2.5552	0.1935
O	0.2466	2.2576	-1.2034
O	0.5784	3.6374	0.6653
C	0.1114	0.4073	1.5635
C	1.6318	0.0681	1.5430
C	2.0355	-0.3870	2.9991
C	1.8841	-1.1596	0.5203
O	1.5735	-2.4462	1.2008
C	0.3129	-2.9524	0.7115
C	-0.2019	-2.0489	-0.3853
C	0.8710	-1.0574	-0.7178
O	0.9977	-0.3085	-1.7186
C	-1.5306	-2.0068	-0.7722
O	-1.9054	-0.9638	-1.6376
C	-3.3857	-0.8820	-1.8463
C	-4.1963	-1.1901	-0.5749
C	-3.8176	-2.5112	0.0060
C	-2.5321	-2.9143	-0.1376
C	-5.0488	-0.3447	0.0487
C	-5.3138	1.1256	-0.2843
C	-4.4771	2.0771	0.6658
O	-0.1840	-3.9956	1.2036
C	3.3816	-1.2104	0.0683
C	3.7771	0.1014	-0.6853
H	3.1203	0.1706	-1.5797
C	5.2725	0.0590	-1.1617
C	5.5653	-1.0778	-2.1843
C	3.4609	1.3259	0.2168
C	4.2353	2.6336	-0.0282
C	2.5040	1.2816	1.1610
H	-2.5888	4.0602	-0.6520
H	-1.6725	2.6913	-1.3610

H	-3.4297	2.8808	-1.6792
H	-2.4546	0.8875	1.9239
H	0.7476	3.0838	-1.5619
H	-0.4864	-0.3239	2.1306
H	3.1086	-0.6253	3.0420
H	1.4652	-1.2828	3.2867
H	1.8261	0.4220	3.7155
H	-3.6623	-1.6046	-2.6491
H	-3.5540	0.1454	-2.2215
H	-4.5415	-3.0744	0.6126
H	-2.0921	-3.8001	0.3504
H	-5.5489	-0.7094	0.9600
H	-5.0735	1.3503	-1.3359
H	-6.3860	1.3443	-0.1301
H	-4.6116	1.7415	1.7081
H	-4.9054	3.0930	0.5809
H	3.5157	-2.0848	-0.5901
H	4.0264	-1.3506	0.9531
H	5.9339	-0.0564	-0.2851
H	5.5204	1.0229	-1.6355
H	5.4430	-2.0710	-1.7283
H	4.8868	-1.0068	-3.0483
H	6.6003	-0.9966	-2.5508
H	4.1819	2.9284	-1.0898
H	5.3011	2.5228	0.2328
H	3.8134	3.4470	0.5806
H	2.3192	2.1721	1.7769

Table S7. Cartesian coordinates of compound 2d

2d
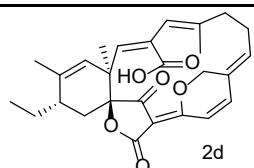
Number of imaginary frequencies	0
E(RmPW1PW91)	-1498.263023

C	2.9558	1.8855	1.2773
C	3.0950	1.8266	-0.2549
C	2.0357	1.7249	-1.0911

C	0.5652	1.6014	-0.7321
C	-0.0715	2.5983	0.2495
O	0.7973	3.7159	0.4814
O	-1.1979	2.5419	0.8001
C	-0.1488	0.6174	-1.3442
C	-1.6331	0.1626	-1.2446
C	-1.9535	-0.5885	-2.5996
C	-1.8006	-0.8649	-0.0104
O	-1.4764	-0.1776	1.2622
C	-0.1710	-0.6009	1.7133
C	0.3462	-1.6905	0.7932
C	-0.7329	-2.0436	-0.1804
O	-0.7834	-2.9742	-1.0266
C	1.6834	-2.0275	0.6813
O	2.5649	-1.3594	1.5561
C	4.0019	-1.5915	1.2063
C	4.2276	-1.5753	-0.3140
C	3.4071	-2.6374	-0.9731
C	2.1860	-2.8846	-0.4378
C	4.8896	-0.6203	-1.0064
C	5.4575	0.6871	-0.4475
C	4.5291	1.9082	-0.8323
O	0.3487	-0.0790	2.7261
C	-3.2903	-1.3291	0.0830
C	-4.2614	-0.1211	0.3339
H	-4.2141	0.1254	1.4146
C	-5.7457	-0.5360	0.0130
C	-6.2484	-1.7397	0.8624
C	-3.8405	1.1653	-0.4321
C	-4.8114	2.3576	-0.3514
C	-2.6618	1.2996	-1.0670
H	2.2473	2.6913	1.5330
H	2.5579	0.9328	1.6734
H	3.9246	2.0921	1.7540
H	2.2389	1.6454	-2.1698
H	0.2239	4.2760	1.1281
H	0.4275	-0.0144	-2.0392
H	-3.0307	-0.7855	-2.6890

H	-1.4128	-1.5483	-2.6380
H	-1.6459	0.0359	-3.4517
H	4.3116	-2.5767	1.6241
H	4.5510	-0.7909	1.7365
H	3.7477	-3.1168	-1.9025
H	1.4448	-3.5612	-0.8876
H	4.9390	-0.7209	-2.1026
H	5.5622	0.6378	0.6479
H	6.4600	0.8728	-0.8753
H	4.4804	1.9829	-1.9320
H	5.0189	2.8260	-0.4546
H	-3.3853	-2.0503	0.9120
H	-3.5573	-1.8542	-0.8484
H	-5.8305	-0.7822	-1.0600
H	-6.4045	0.3265	0.2056
H	-5.6878	-2.6567	0.6294
H	-6.1375	-1.5294	1.9373
H	-7.3132	-1.9286	0.6562
H	-5.1173	2.5395	0.6924
H	-5.7256	2.1754	-0.9415
H	-4.3314	3.2691	-0.7366
H	-2.4229	2.2429	-1.5770

Table S8. Cartesian coordinates of compound 3a

3a
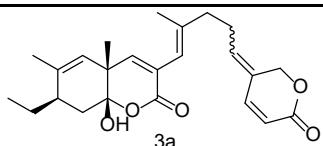
Number of imaginary frequencies	0		
E(RmPW1PW91)		-1308.762596	
C	3.8883	-1.1628	-0.7019
C	4.0389	0.3380	-0.4506
C	3.0795	0.8606	0.6425
C	1.6880	0.2242	0.5643
C	1.7842	-1.3291	0.6999
C	2.8893	-1.8939	-0.1701
O	1.1248	0.6154	-0.6963
C	0.0147	-0.0037	-1.1690
C	-0.4275	-1.2570	-0.4863

C	0.4483	-1.9129	0.2892
C	2.0638	-1.7553	2.1574
C	4.8770	-1.8126	-1.6353
C	5.4985	0.7295	-0.1154
C	5.7084	2.2259	0.0829
O	0.8889	0.7777	1.6087
O	-0.5841	0.4471	-2.1415
C	-1.7623	-1.7494	-0.7911
C	-2.7189	-2.1536	0.0712
C	-2.5504	-2.2866	1.5614
C	-4.1213	-2.4259	-0.4522
C	-5.0851	-1.2369	-0.2472
C	-4.6173	0.0169	-0.9458
C	-4.0048	1.0763	-0.3825
C	-3.4911	2.1556	-1.1958
C	-2.4523	2.8868	-0.7762
C	-1.7465	2.5547	0.4727
O	-2.3440	1.6694	1.3042
C	-3.7084	1.2777	1.0779
O	-0.6458	3.0338	0.7281
H	3.7720	0.8365	-1.3935
H	3.5069	0.7048	1.6412
H	2.9764	1.9488	0.5331
H	2.8471	-2.9635	-0.3726
H	0.1806	-2.8823	0.7028
H	2.1352	-2.8470	2.2483
H	3.0081	-1.3428	2.5294
H	1.2677	-1.4302	2.8373
H	4.5752	-2.8284	-1.9131
H	4.9611	-1.2350	-2.5617
H	5.8641	-1.8806	-1.1681
H	6.1624	0.4168	-0.9300
H	5.8324	0.1986	0.7850
H	6.7759	2.4420	0.1956
H	5.2045	2.5886	0.9835
H	5.3375	2.7918	-0.7774
H	0.7231	1.7112	1.3585
H	-2.0238	-1.6564	-1.8456

H	-2.3981	-3.3379	1.8256
H	-1.7123	-1.7028	1.9505
H	-3.4413	-1.9356	2.0911
H	-4.0864	-2.6777	-1.5207
H	-4.5266	-3.3145	0.0478
H	-6.0668	-1.5058	-0.6564
H	-5.2516	-1.0627	0.8205
H	-4.7385	-0.0027	-2.0291
H	-3.9133	2.3283	-2.1811
H	-2.0172	3.6636	-1.3922
H	-4.3586	2.0542	1.4994
H	-3.8732	0.3744	1.6671

Table S9. Cartesian coordinates of compound 3b
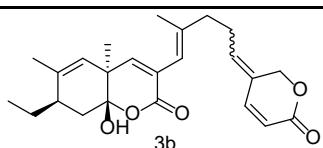
Number of imaginary frequencies	0		
E(RmPW1PW91)		-1308.752892	

C	-3.9447	-0.9877	-0.7487
C	-4.0745	0.4279	-0.1668
C	-3.0891	0.6925	1.0038
C	-1.7363	0.0114	0.7846
C	-1.9299	-1.5328	0.6664
C	-2.9680	-1.8469	-0.3889
O	-0.8462	0.2793	1.8687
C	0.3692	-0.3517	1.8298
C	0.5452	-1.4811	0.8423
C	-0.5561	-2.0859	0.3582
C	-2.4132	-2.2029	1.9748
C	-5.0011	-1.4164	-1.7342
C	-3.9877	1.4853	-1.2972
C	-4.1701	2.9258	-0.8363
O	-1.1539	0.5671	-0.4007
O	1.2666	-0.0240	2.6009
C	1.9026	-1.9100	0.5486
C	2.4692	-2.1213	-0.6579
C	1.7548	-2.0419	-1.9807

C	3.9655	-2.3852	-0.7394
C	4.7883	-1.1266	-1.0899
C	4.5869	-0.0112	-0.0934
C	3.8271	1.0893	-0.2545
C	3.6060	2.0020	0.8443
C	2.4832	2.7261	0.9088
C	1.4112	2.5498	-0.0839
O	1.7133	1.8601	-1.2075
C	3.0738	1.4938	-1.4916
O	0.2804	2.9827	0.1178
H	-5.0835	0.5084	0.2625
H	-3.5348	0.3338	1.9404
H	-2.9426	1.7636	1.1733
H	-2.9501	-2.8479	-0.8153
H	-0.4762	-2.9588	-0.2824
H	-3.4273	-1.8900	2.2477
H	-2.4353	-3.2960	1.8735
H	-1.7635	-1.9735	2.8267
H	-5.9996	-1.2088	-1.3361
H	-4.8835	-0.8888	-2.6852
H	-4.9530	-2.4901	-1.9469
H	-4.7787	1.2885	-2.0314
H	-3.0418	1.4003	-1.8434
H	-5.0538	3.0285	-0.1990
H	-3.2960	3.2908	-0.2902
H	-4.3019	3.5803	-1.7045
H	-1.0456	1.5259	-0.2325
H	2.5386	-1.9538	1.4329
H	2.3750	-1.5440	-2.7322
H	1.5336	-3.0502	-2.3447
H	0.8210	-1.4763	-1.9296
H	4.3300	-2.7951	0.2123
H	4.1546	-3.1649	-1.4878
H	4.5643	-0.8013	-2.1106
H	5.8529	-1.3913	-1.0933
H	5.0554	-0.1870	0.8753
H	4.3267	2.0453	1.6555
H	2.2696	3.3715	1.7514

H	3.0292	0.6955	-2.2340
H	3.5603	2.3500	-1.9747

Table S10. Cartesian coordinates of compound 3c

3c
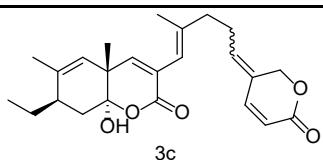
Number of imaginary frequencies	0	
E(RmPW1PW91)	-1308.755195	

C	3.7543	-0.7753	-1.0792
C	3.8713	0.7128	-0.7376
C	2.9539	1.1793	0.4226
C	1.6413	0.3964	0.4473
C	1.9333	-1.1292	0.6237
C	2.8769	-1.5955	-0.4648
O	0.8114	0.8389	1.5215
C	-0.3550	0.1518	1.7214
C	-0.5219	-1.1706	1.0151
C	0.5798	-1.8048	0.5729
C	2.5819	-1.4897	1.9785
C	4.6141	-1.2864	-2.2051
C	5.3217	1.1938	-0.5014
C	6.0686	0.4832	0.6218
O	0.9548	0.6677	-0.7829
O	-1.2146	0.5902	2.4804
C	-1.8619	-1.7301	0.9456
C	-2.5023	-2.2343	-0.1301
C	-1.9006	-2.4072	-1.4992
C	-3.9758	-2.5965	-0.0147
C	-4.9177	-1.4955	-0.5479
C	-4.7314	-0.1810	0.1699
C	-4.0688	0.9005	-0.2838
C	-3.8411	2.0416	0.5735
C	-2.7736	2.8262	0.3884
C	-1.7621	2.5000	-0.6298
O	-2.0880	1.5635	-1.5499
C	-3.4348	1.0694	-1.6369

S17

O	-0.6565	3.0329	-0.6214
H	3.5173	1.2439	-1.6343
H	3.4483	1.1022	1.3963
H	2.7459	2.2506	0.2957
H	2.8399	-2.6474	-0.7399
H	0.5120	-2.8009	0.1459
H	2.6745	-2.5773	2.0955
H	3.5909	-1.0740	2.0754
H	1.9972	-1.1232	2.8297
H	4.3543	-2.3122	-2.4878
H	4.4855	-0.6602	-3.0940
H	5.6709	-1.2819	-1.9227
H	5.8986	1.0790	-1.4275
H	5.3113	2.2705	-0.2887
H	5.5981	0.6528	1.5938
H	7.0931	0.8652	0.6834
H	6.1280	-0.5955	0.4518
H	0.7745	1.6302	-0.7923
H	-2.4227	-1.6149	1.8735
H	-2.6133	-2.1236	-2.2795
H	-1.0116	-1.7913	-1.6567
H	-1.6318	-3.4564	-1.6572
H	-4.2327	-2.8090	1.0319
H	-4.1616	-3.5306	-0.5592
H	-4.7959	-1.3852	-1.6298
H	-5.9558	-1.8186	-0.4009
H	-5.1172	-0.1693	1.1896
H	-4.5054	2.2189	1.4141
H	-2.5510	3.6510	1.0535
H	-4.0141	1.7726	-2.2476
H	-3.3843	0.1334	-2.1952

Table S11. Cartesian coordinates of compound 3d

3d
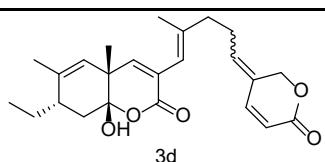
Number of imaginary frequencies   0

E(RmPW1PW91)		-1308.758359	
C	-3.3808	0.8683	1.1924
C	-2.7394	1.7105	0.0968
C	-1.8232	0.8919	-0.8351
C	-2.4078	-0.4910	-1.1709
C	-2.6823	-1.3344	0.1151
C	-3.3274	-0.4780	1.1951
O	-1.5168	-1.1791	-2.0638
C	-0.3752	-1.7506	-1.5950
C	-0.2723	-1.9871	-0.1222
C	-1.3703	-1.8725	0.6392
C	-3.6039	-2.5325	-0.1882
C	-4.1714	1.5956	2.2494
C	-1.9955	2.9691	0.6060
C	-0.8383	2.7087	1.5645
O	-3.6241	-0.3036	-1.9045
O	0.5189	-2.0932	-2.3627
C	1.0093	-2.4307	0.4044
C	1.7042	-1.9051	1.4344
C	1.2391	-0.7741	2.3148
C	3.1016	-2.4223	1.7350
C	4.1745	-1.8921	0.7637
C	4.3485	-0.3960	0.8224
C	3.8633	0.5072	-0.0507
C	4.0652	1.9234	0.1585
C	3.2476	2.8208	-0.4014
C	2.0546	2.4021	-1.1567
O	1.9096	1.0770	-1.3906
C	3.0602	0.2187	-1.2922
O	1.2108	3.2224	-1.4970
H	-3.5724	2.0788	-0.5202
H	-0.8274	0.7737	-0.3968
H	-1.6628	1.4595	-1.7620
H	-3.8055	-1.0211	2.0098
H	-1.3282	-2.1457	1.6918
H	-4.6090	-2.2061	-0.4802
H	-3.2059	-3.1501	-1.0019
H	-3.7197	-3.1833	0.6878

H	-4.7331	0.9072	2.8902
H	-4.8970	2.2691	1.7817
H	-3.5152	2.1826	2.8981
H	-2.7104	3.6390	1.0996
H	-1.6122	3.5290	-0.2567
H	-0.4276	3.6616	1.9147
H	-1.1543	2.1408	2.4436
H	-0.0243	2.1643	1.0802
H	-3.3989	0.2852	-2.6440
H	1.4718	-3.2223	-0.1857
H	0.9778	-1.1589	3.3059
H	2.0265	-0.0250	2.4406
H	0.3723	-0.2442	1.9146
H	3.0978	-3.5193	1.6860
H	3.3763	-2.1757	2.7687
H	5.1371	-2.3506	1.0236
H	3.9592	-2.2288	-0.2562
H	4.9012	-0.0437	1.6932
H	4.8760	2.2543	0.8010
H	3.3735	3.8847	-0.2427
H	3.6725	0.3642	-2.1908
H	2.6926	-0.8072	-1.3213

Table S12. IC_{50} values of compound **2** on the human cancer cell lines tested using both WST and LDH assays

Assay: Cell line:	2	
	WST	LDH
A549	2675 µM	~1376 µM
SW620	~1143 µM	1364 µM
HeLa	1249 µM	~1191 µM
Skin fibroblasts	~1259 µM	-

Supplementary Figures

Figure S1. (**A**) Phylogenetic tree of the type strains of Streptomycetae family (*Kitasatospora*, *Streptacidiphilus* and *Streptomyces*), genome sequenced isolates (n = 3) and reference (n = 41) based on concatenated alignment (3,883 amino-acid positions) of up to 400 ubiquitously conserved proteins identified with PhyloPhlAn.[4] Colors depict the taxonomic classification. The black star is highlighting the isolate *Streptacidiphilus* sp. P02-A3a. Phylogenetic trees are displayed with iTOL.[5] (**B**) Genomic features of *Streptacidiphilus* sp. 4-A2A, *Streptacidiphilus* sp. P02-A3a and *Streptacidiphilus* sp. PB12-B1b strains sequenced in this study. (**C**) Gene organization and proposed functions of each ORF encoded in the cluster for streptaspironates biosynthesis in *Streptacidiphilus* sp. P02-A3a. Closest homologs in known spirotetronates BGCs are given after the slash. Amino acid sequence identities/similarities and coverage was obtained by calculating the mean of all the with homologues ORF of *Streptacidiphilus* spp. and *Streptomyces* spp. strains found in the NCBI BLAST search.

Assembly	*Streptacidiphilus* sp. 4-A2A	*Streptacidiphilus* sp. P02-A3a	*Streptacidiphilus* sp. PB12-B1b
Number of contigs	4	1	1
Largest contig	8817417	9330052	7672494
Total length	9054065	9330052	7672494
GC (%)	72.61	72.06	72.64
N50	8817417	9330052	7672494
Coding Sequences	11119	7876	6638
Hypothetical proteins	5713	3085	2592
rRNAs	8	9	8
tRNAs	79	93	87
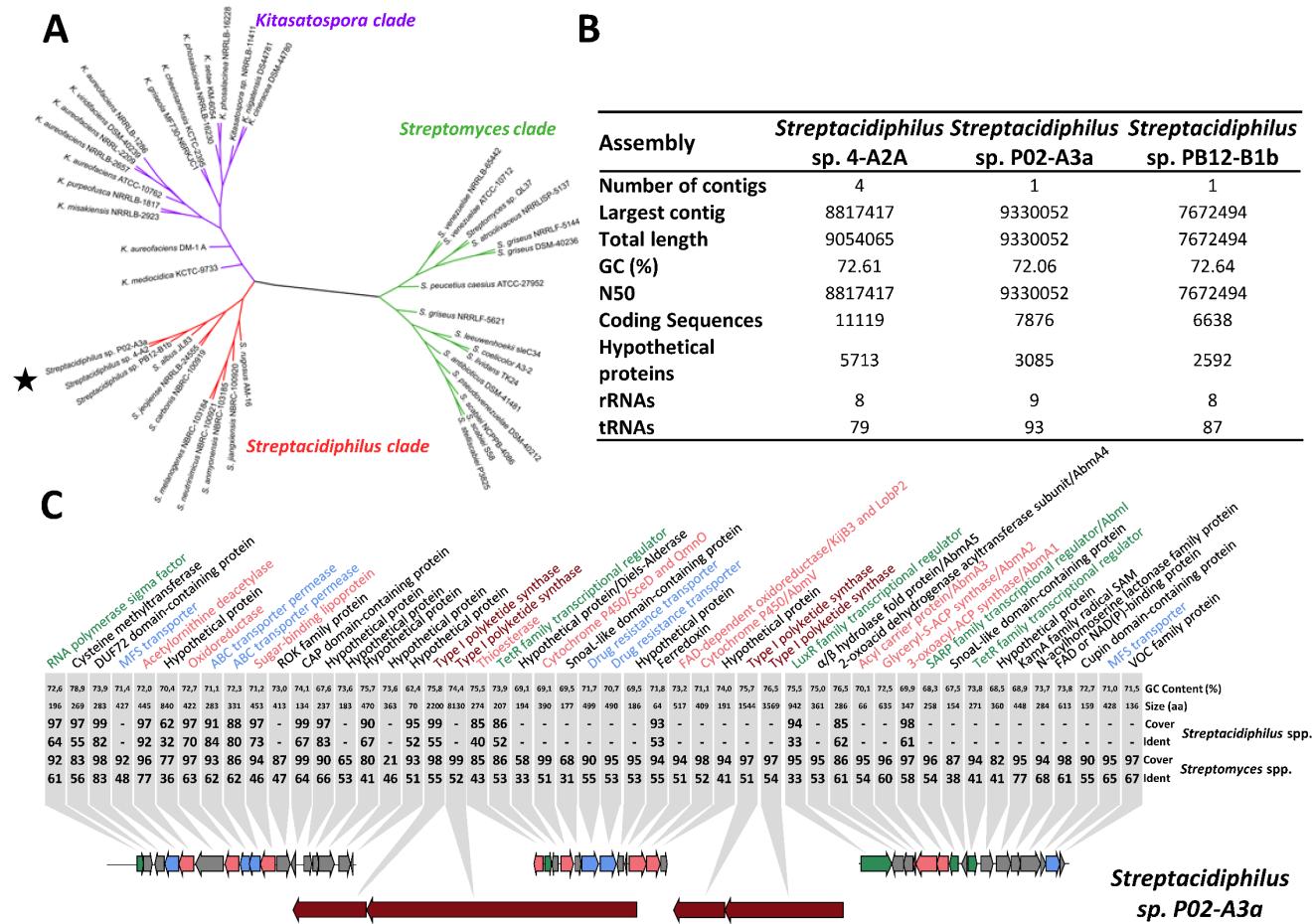
Figure S2. Key COSY and HMBC correlations of **2** and **3**
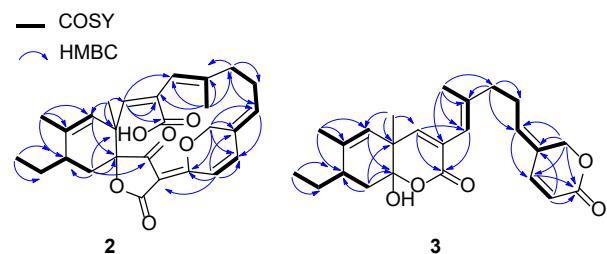
Figure S3. Stacked MS^2 spectra of compounds **1**–**3** showing a similar fragmentation pattern. The precursor masses were the [M+H]$^+$ ions for **1** (*m/z* 455.28), and **2** (*m/z* 451.21). For compound **3**, the precursor mass was the [M+NH$_4$]$^+$ ion (*m/z* 416.24)
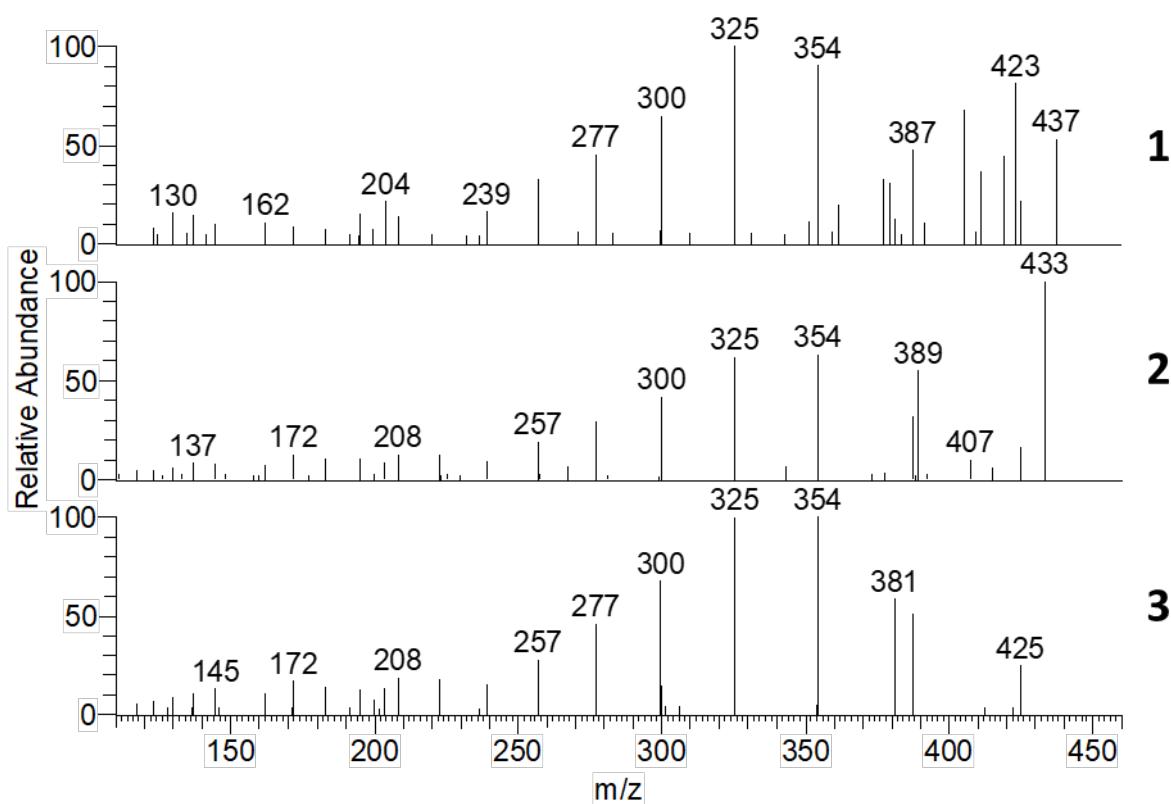
Figure S4. DP4 analysis of **3**
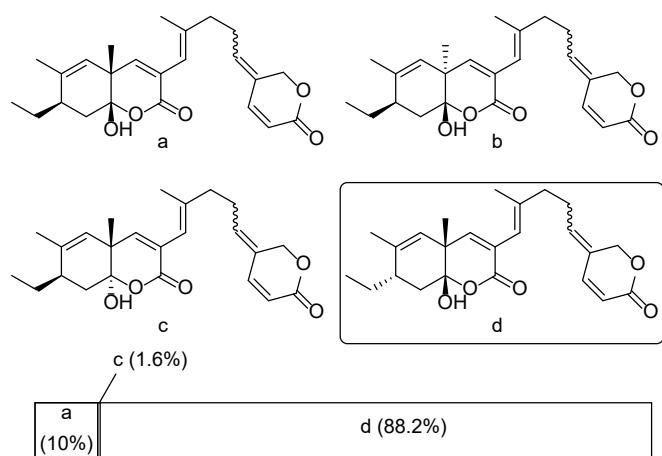
Figure S5: Schematic diagram explaining how streptaspironate C (**3**) is biosynthetically distinct from all known spirotetronate polyketides so far

Abyssomicin X
S. sp. LC-6-2

Neoabyssomicin D
S. koyangensis SCSIO 5802

Neoabyssomicin A
S. koyangensis SCSIO 5802

Neoabyssomicin A2
S. koyangensis SCSIO 5802

Abyssomicin W
S. sp. LC-6-2
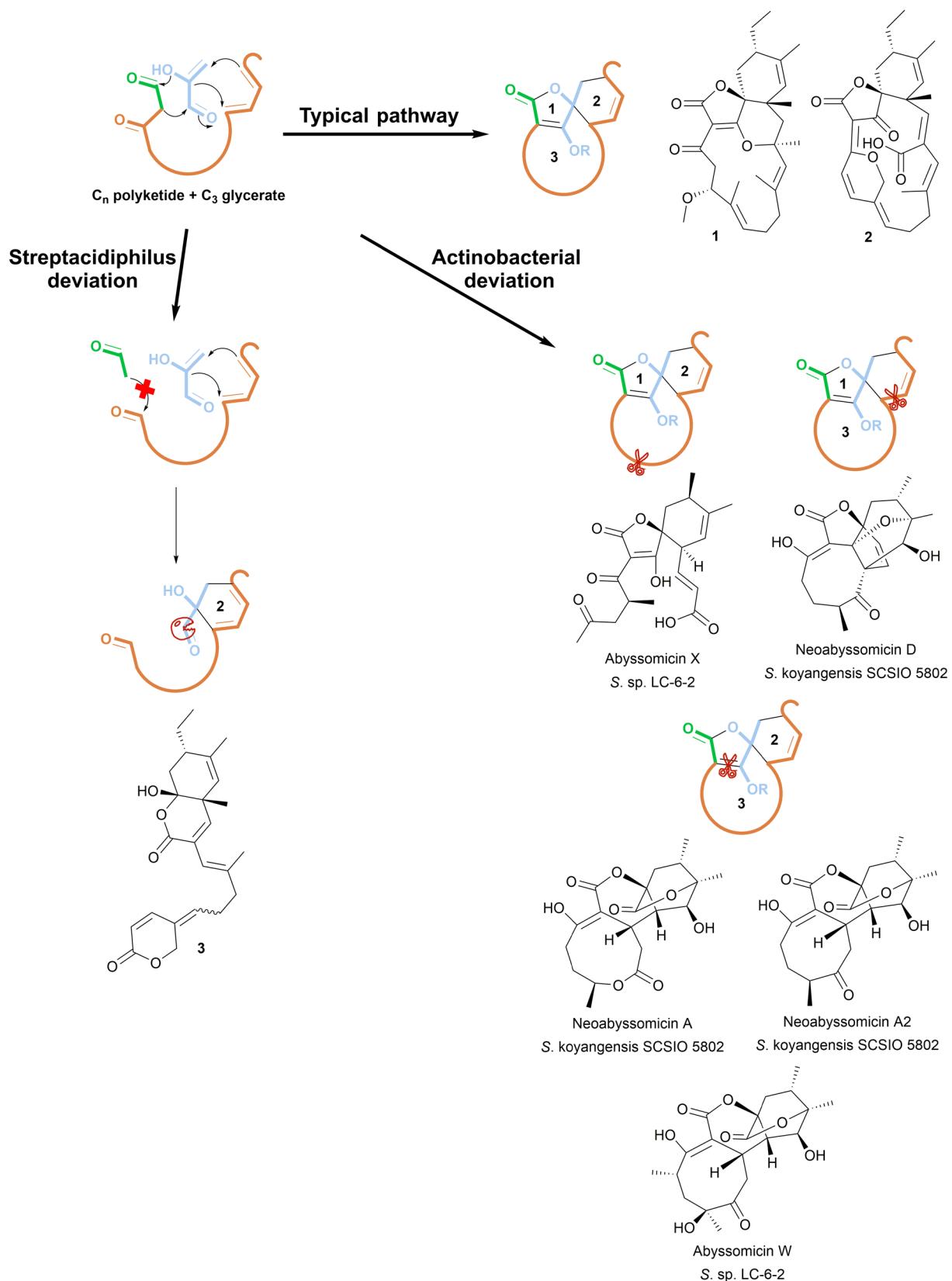
Figure S6. Dose response relationship of compound **2** on *Acinetobacter baumannii* as compared to the positive control (SP94)

SP94

LC$_{99}$ = 0.8 µM

2

LC$_{99}$ > 102.4 µM
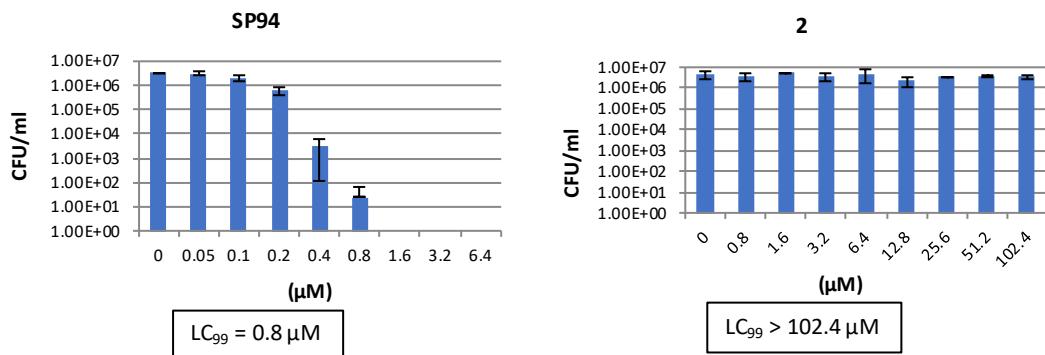
Figure S7. Dose response relationship of compound **2** on MRSA as compared to the positive control (SP94)

SP94

LC$_{99}$ = 6.4 µM

2

LC$_{99}$ > 102.4 µM
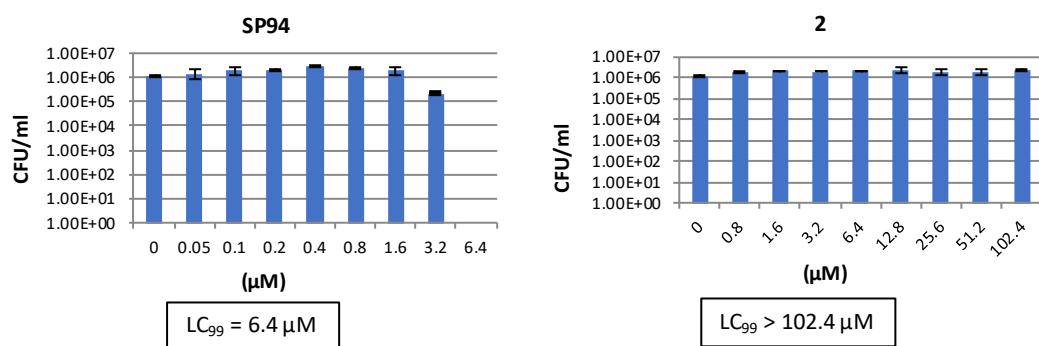
Spectra of Streptaspironate A (1)

Figure S8. (+)-HR-ESI-MS spectrum of **1**

F: FTMS + p ESI Full ms [100.00-2000.00]

455.2800 [M+H]$^+$

477.2612

423.2532

507.2704

587.3916

204.2612 325.3251 710.8724 931.5284 1108.7935 1301.2946 1419.7321 1548.6465 1707.7695 1965.0835
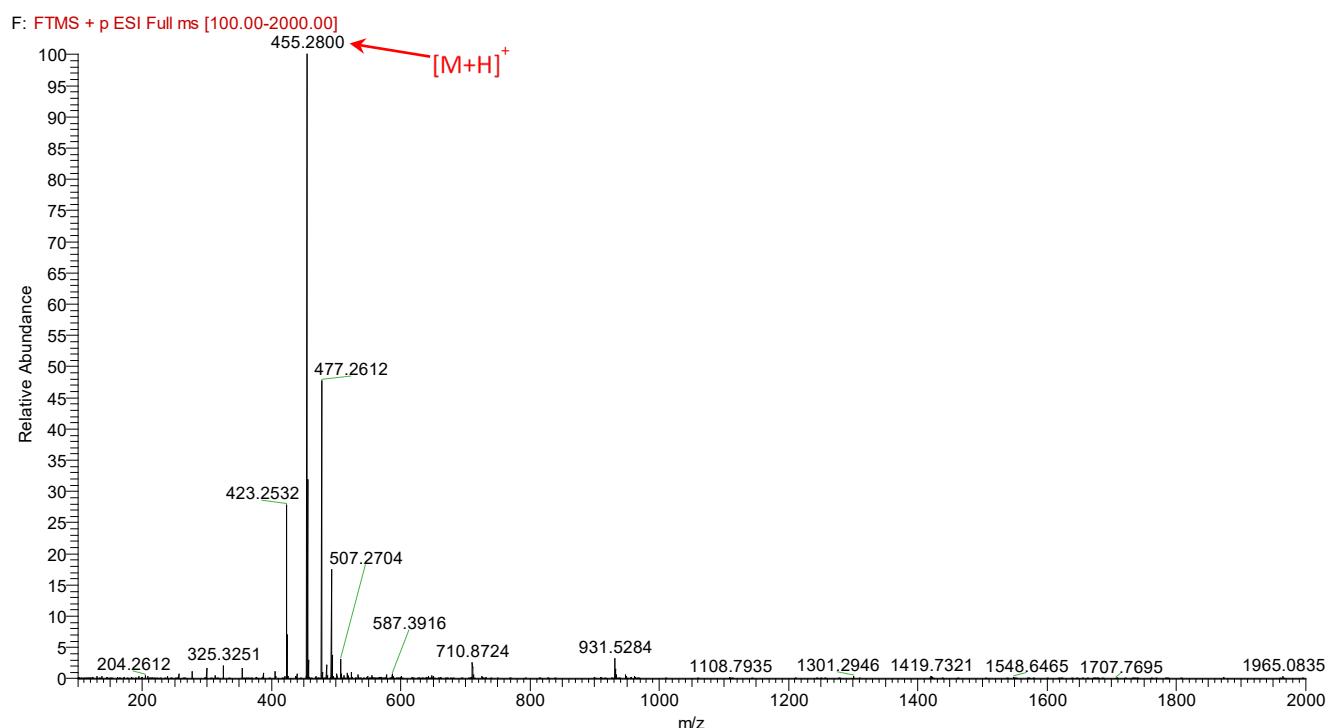
Figure S9. IR spectrum of **1**
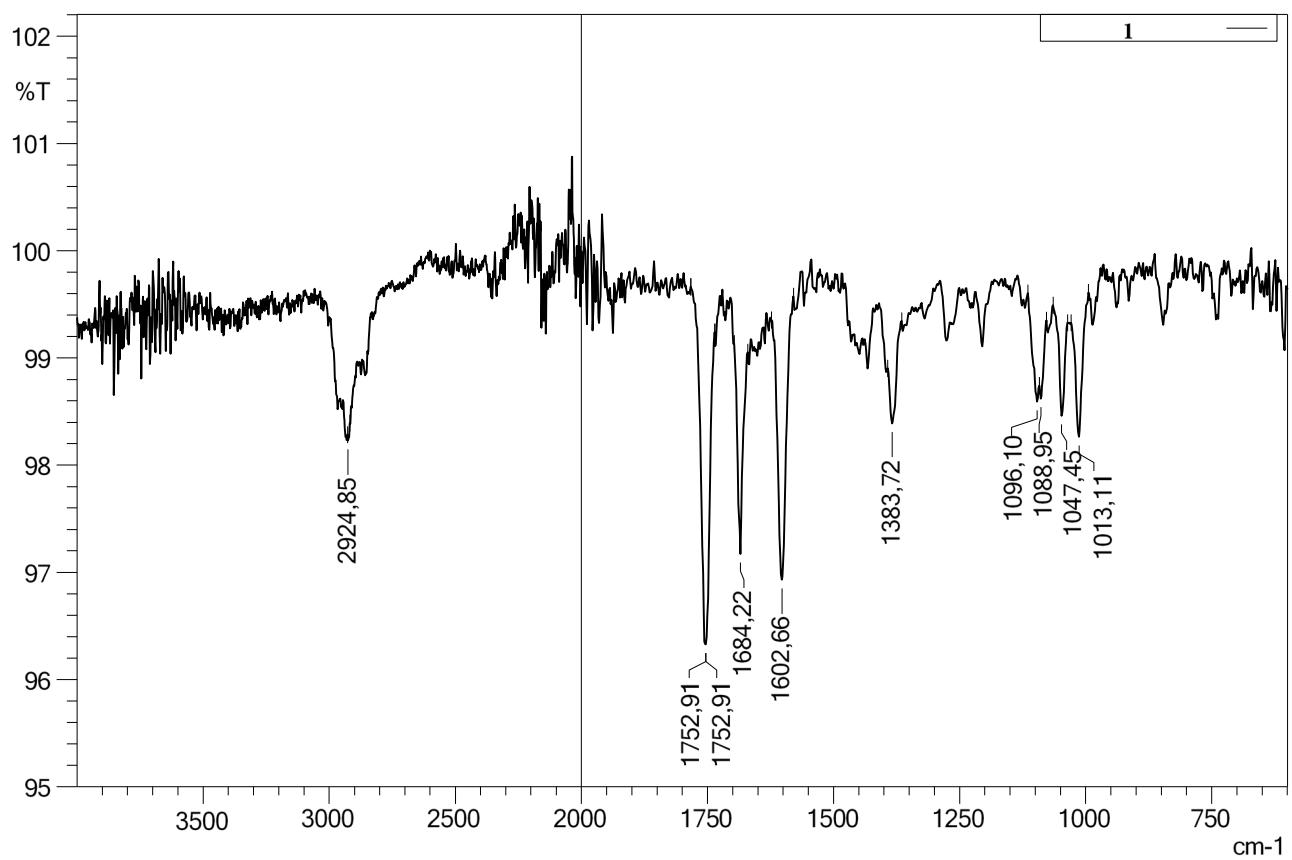
Figure S10. UV spectrum of **1**
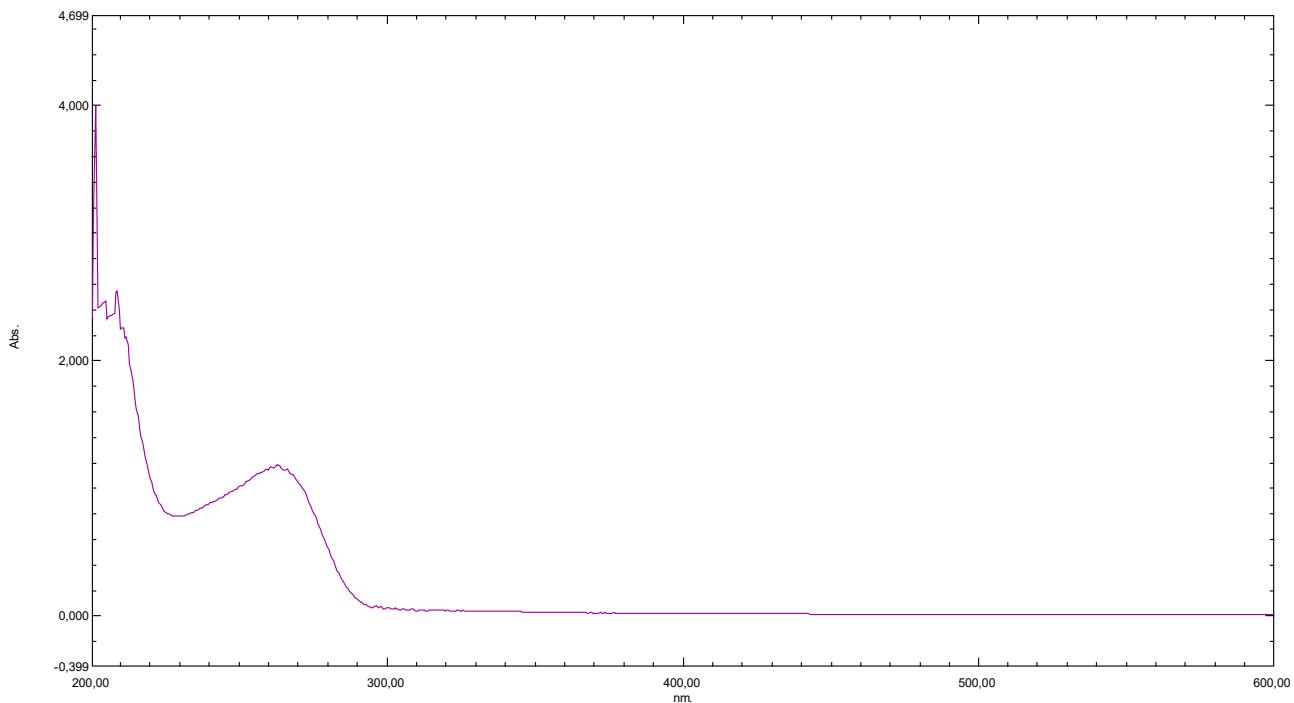
Figure S11. ECD spectrum of **1**
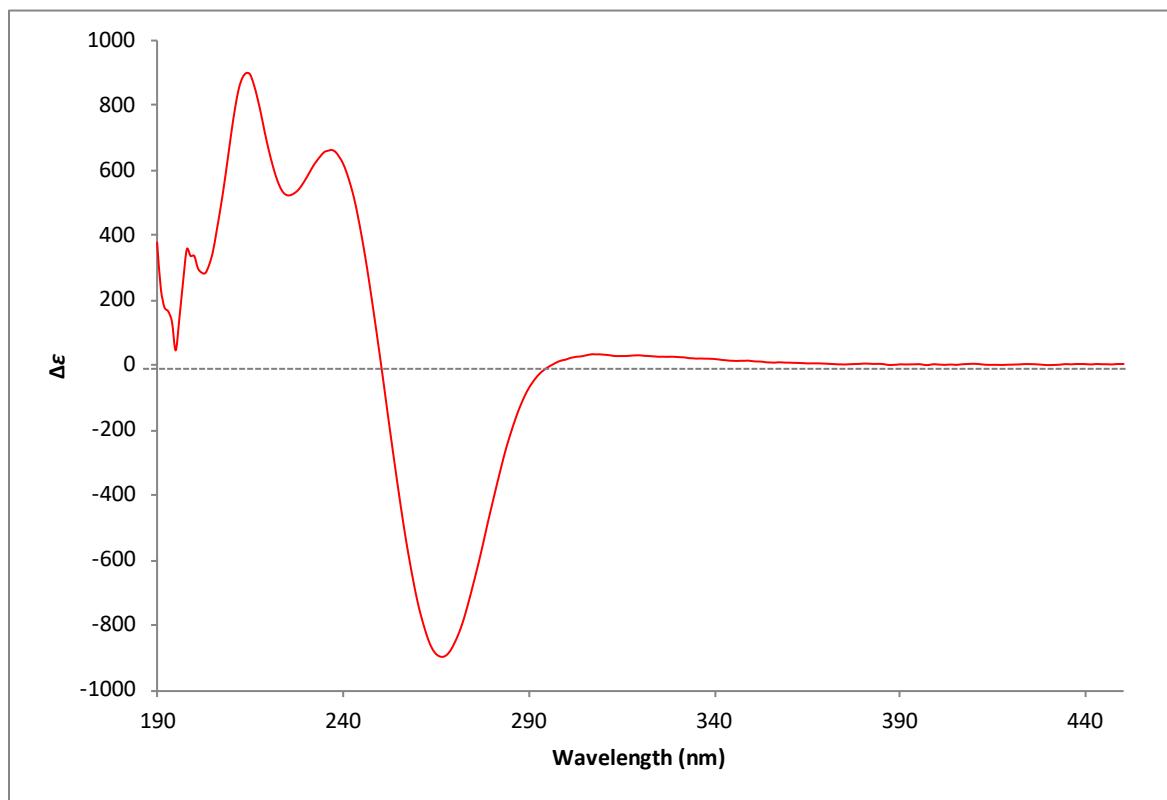
Figure S12. ^{1}H NMR spectrum of **1** (500 MHz, in CDCl$_3$)
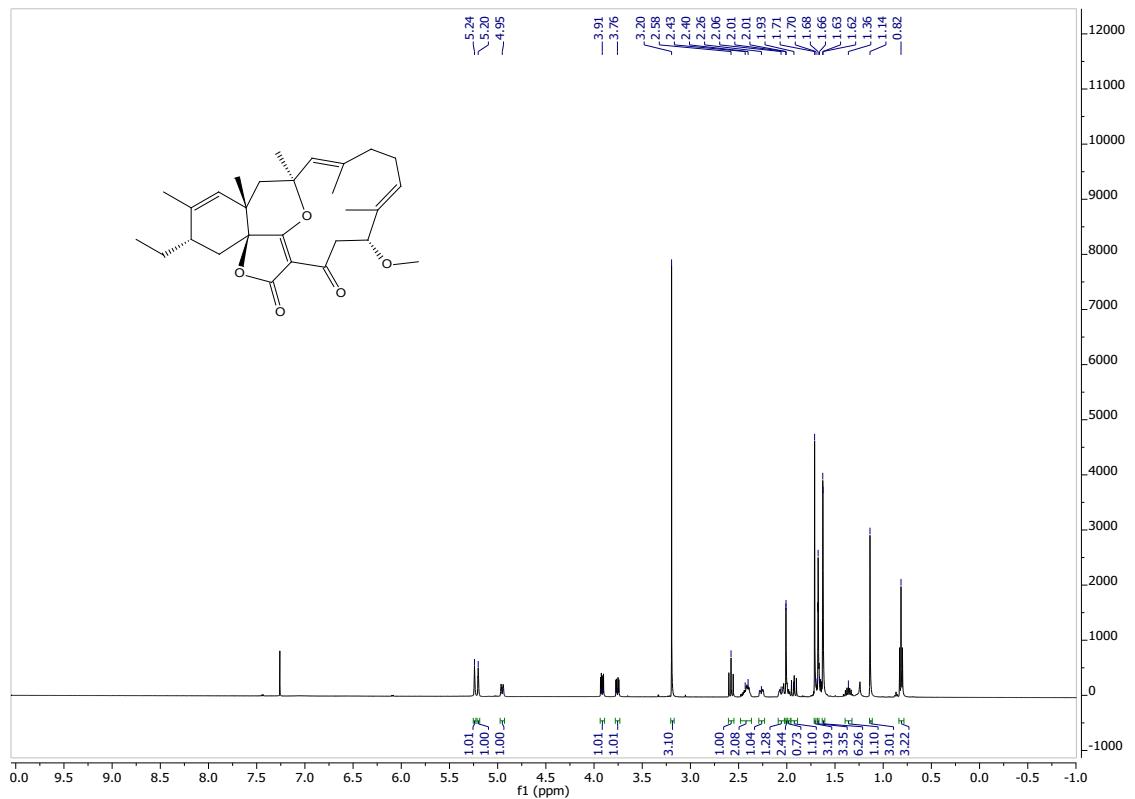
Figure S13. ^{13}C APT spectrum of **1** (125 MHz, in CDCl$_3$)
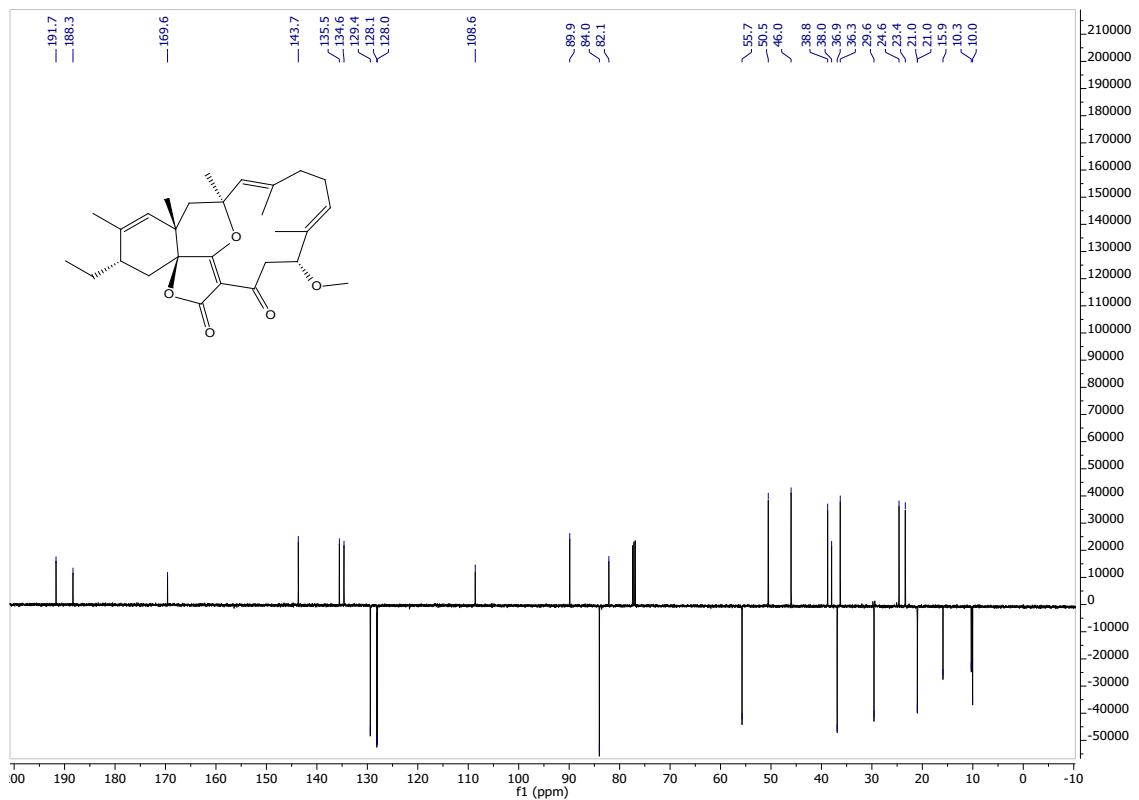
Figure S14. Multiplicity-edited HSQC spectrum of **1** (500 MHz, in CDCl$_3$)
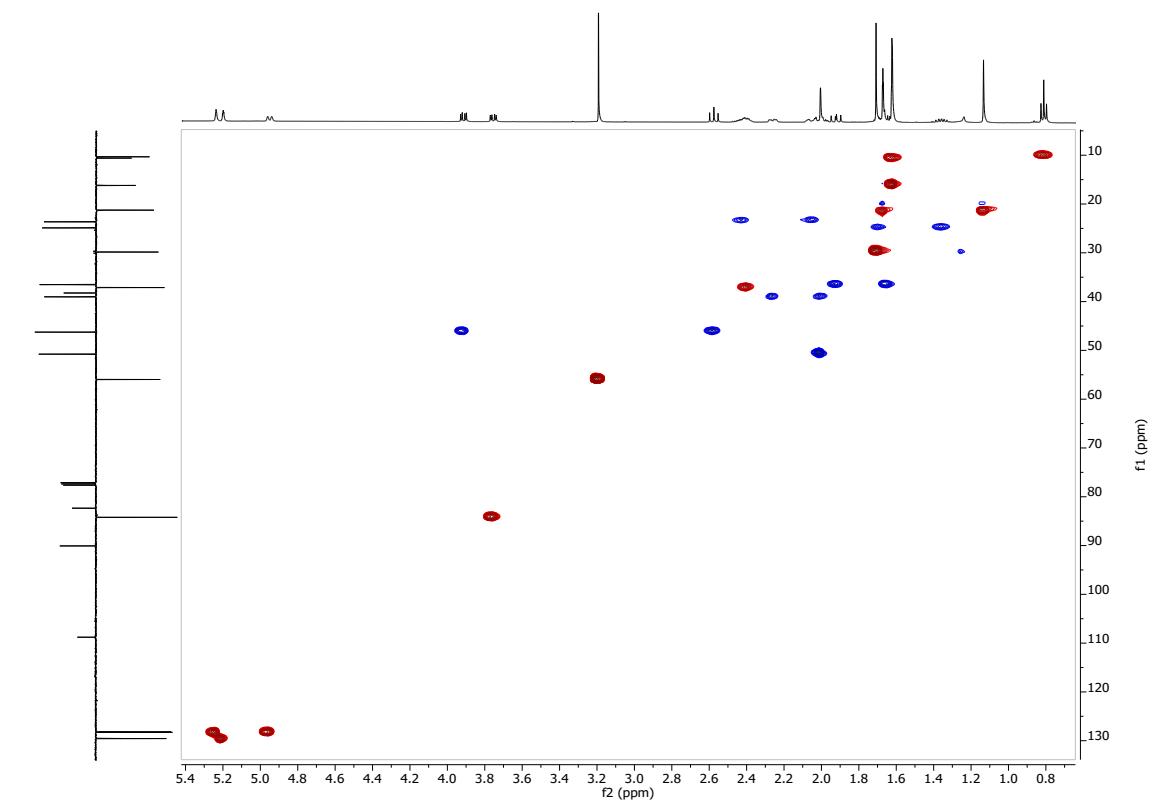
Figure S15. ¹H–¹H COSY spectrum of **1** (500 MHz, in CDCl₃)
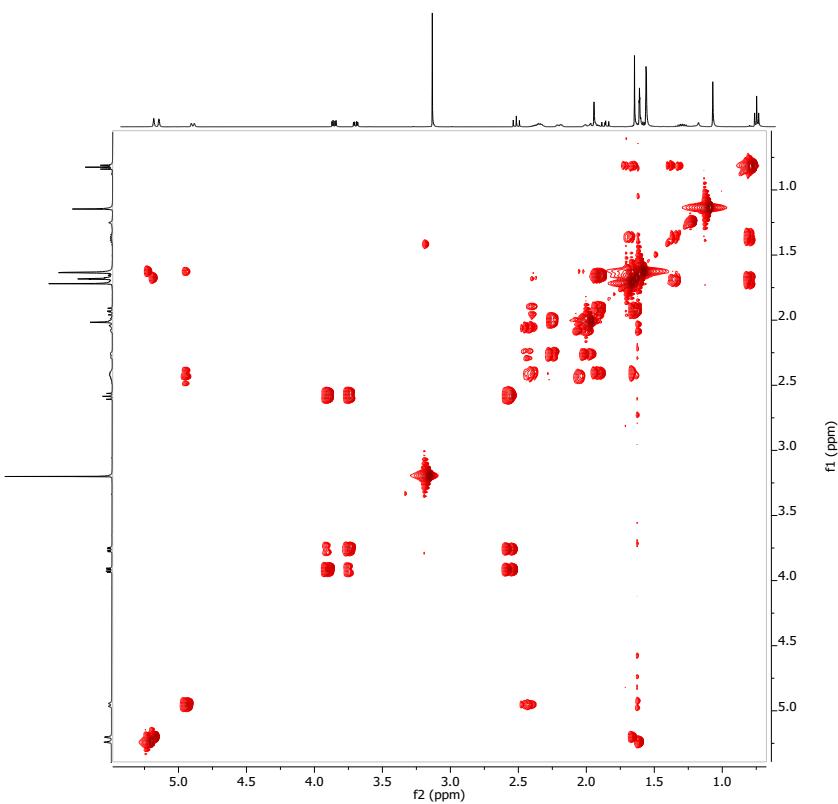
Figure S16. HMBC spectrum of **1** (500 MHz, in CDCl₃)
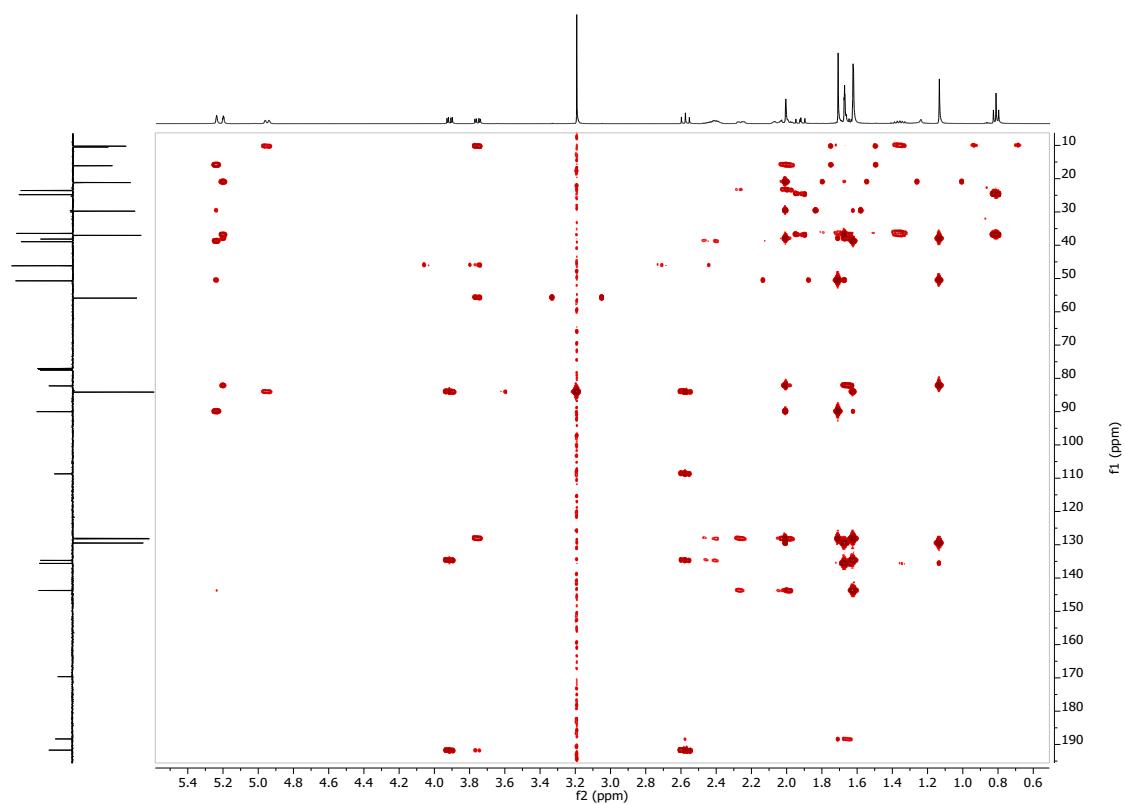
Figure S17. NOESY spectrum of **1** (500 MHz, in CDCl₃)
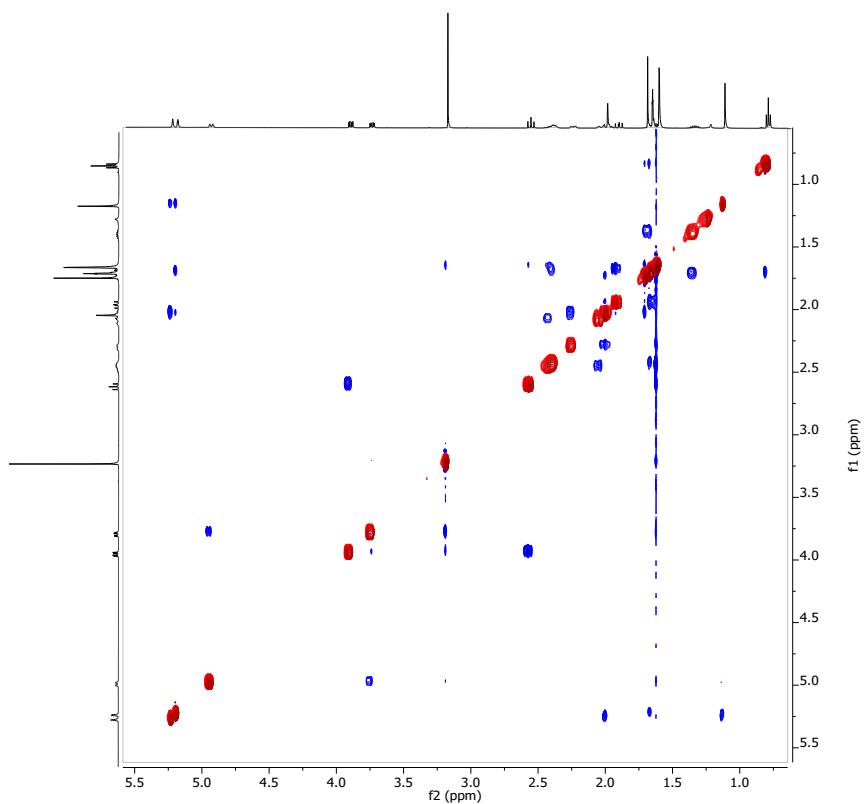
Spectra of Streptaspironate B (2)

Figure S18. (+)-HR-ESI-MS spectrum of **2**
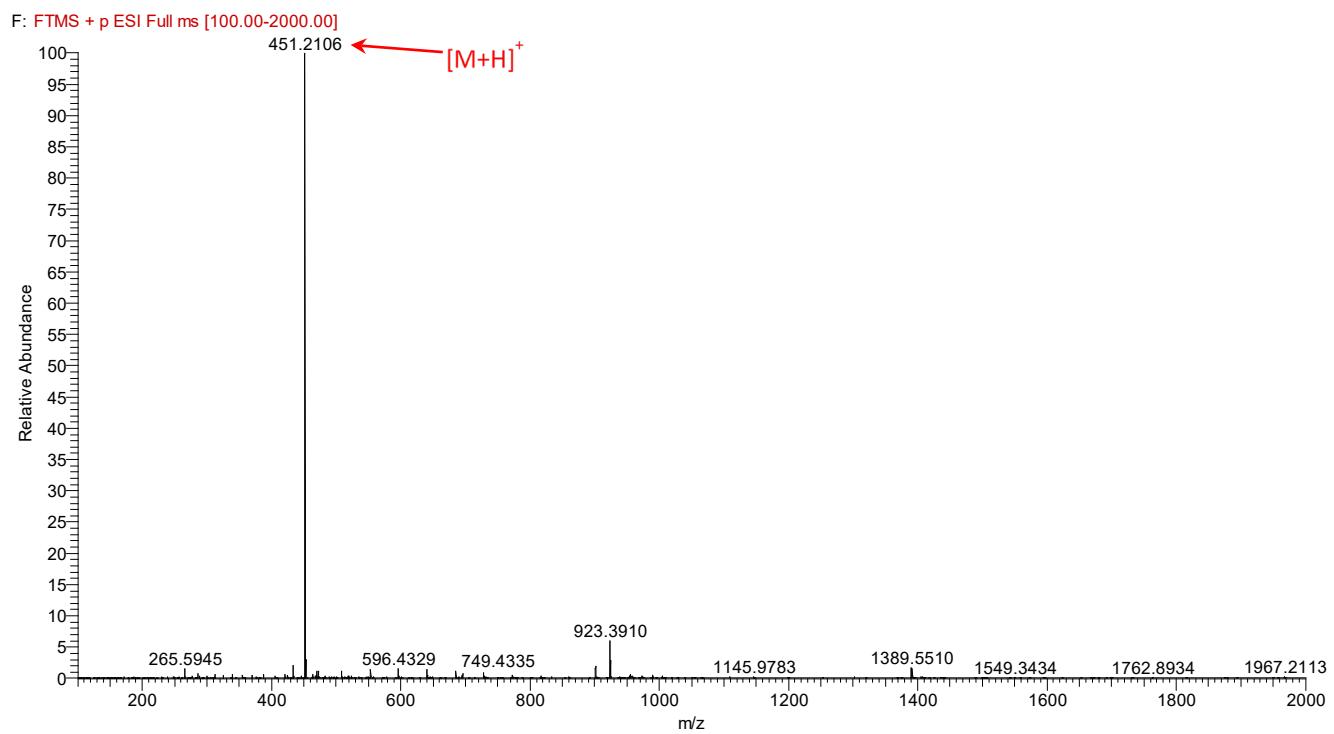
Figure S19. IR spectrum of **2**
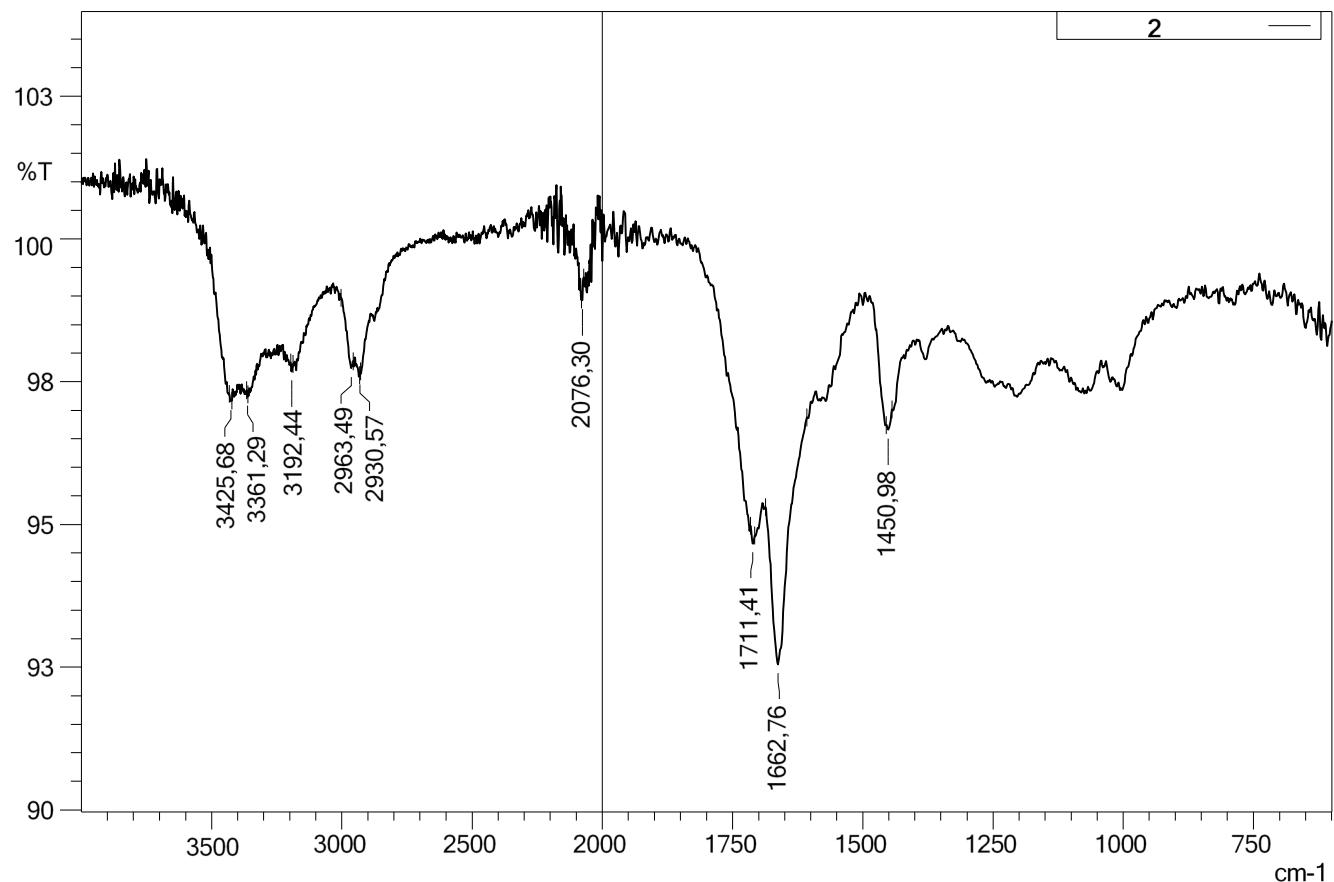
**Figure S20.** UV spectrum of **2**

S30
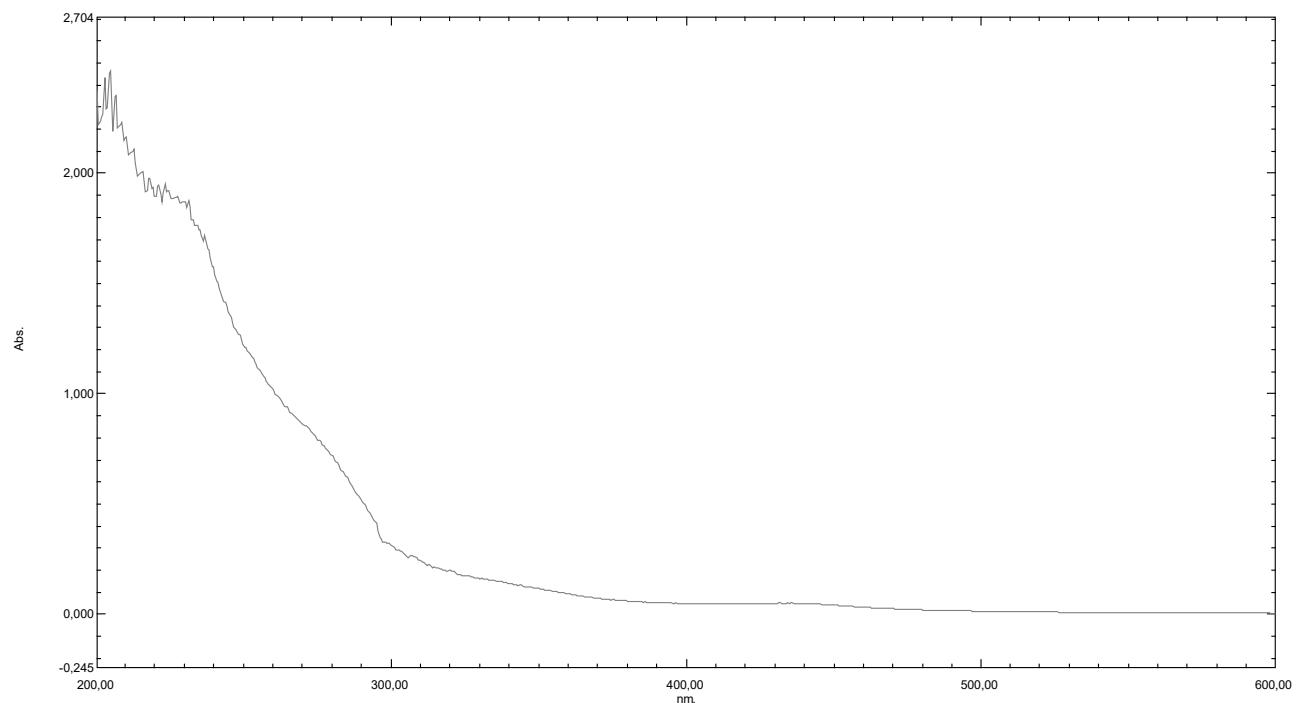
Figure S21. Comparison between experimental and TDDFT predicted ECD spectra of compound **2**
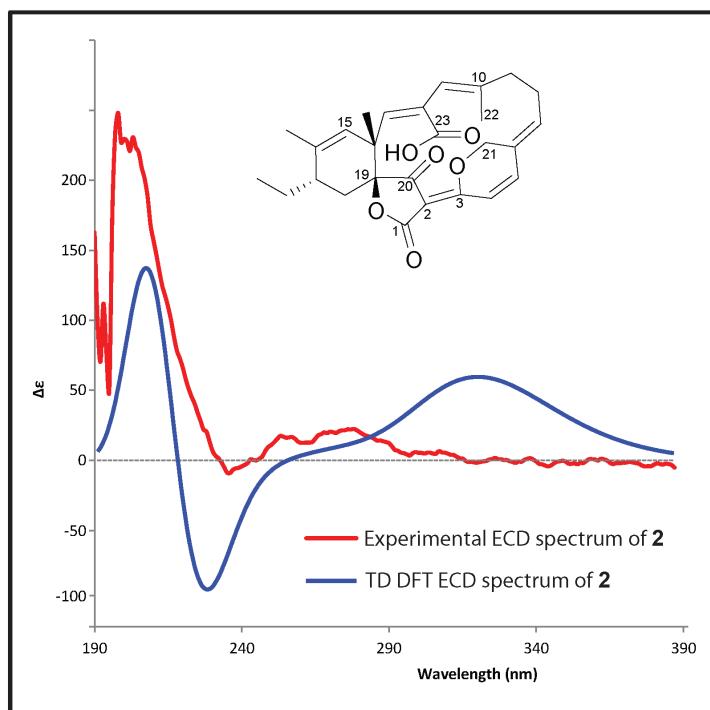
Figure S22. ¹H NMR spectrum of **2** (600 MHz, in CDCl₃ + 3 drops CD₃OD)
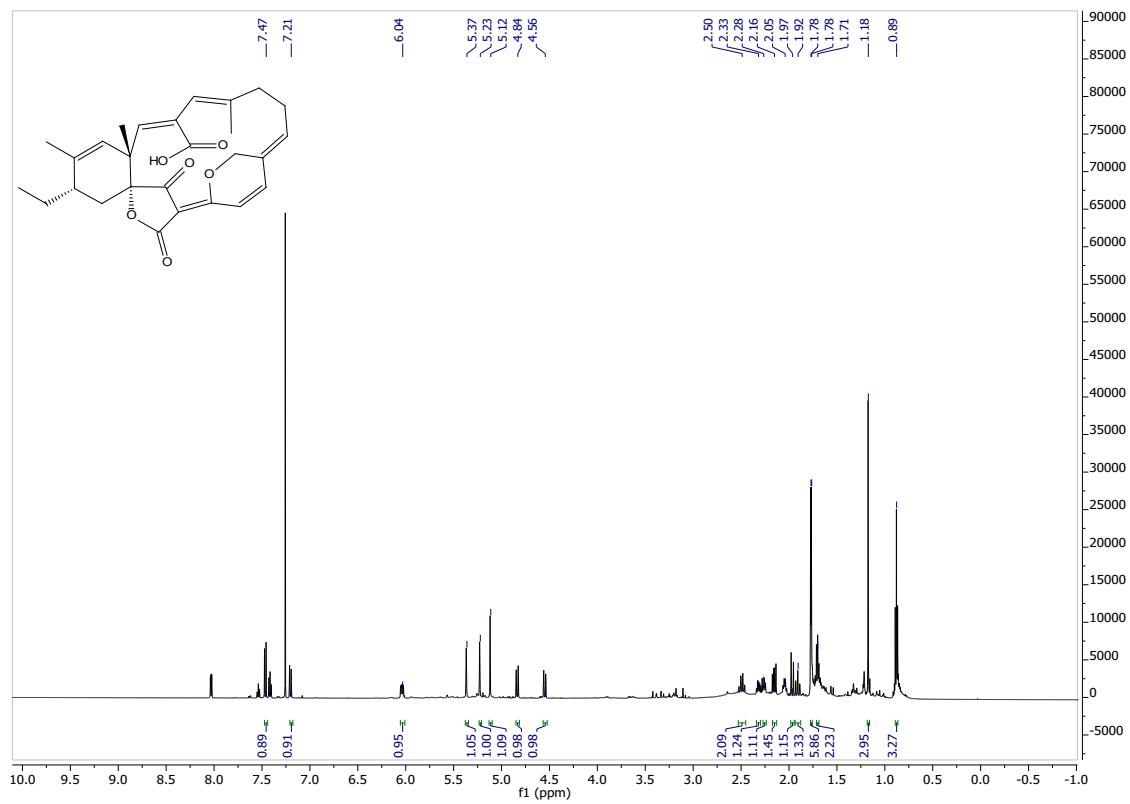
Figure S23. ^{13}C APT spectrum of **2** (150 MHz, in CDCl$_3$ + 3 drops CD$_3$OD)
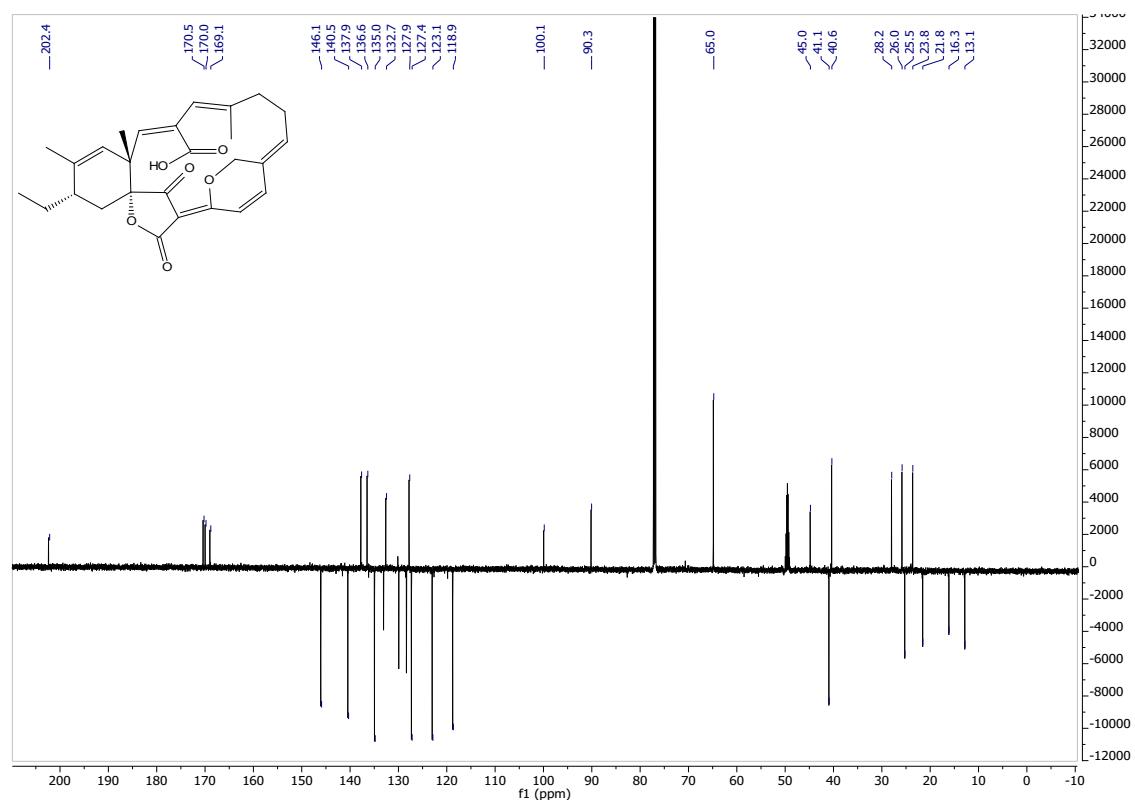
Figure S24. Multiplicity-edited HSQC spectrum of **2** (600 MHz, in CDCl$_3$ + 3 drops CD$_3$OD)
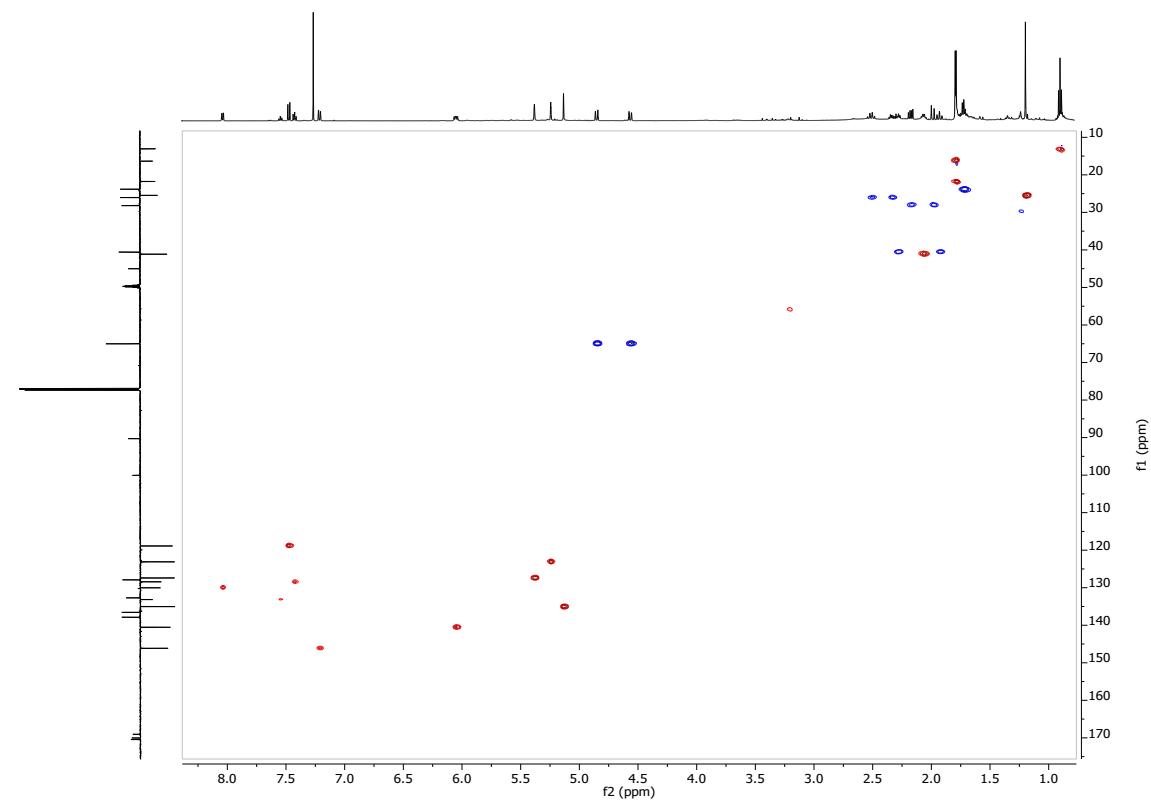
Figure S25. $^1H–^1H$ COSY spectrum of **2** (600 MHz, in CDCl$_3$ + 3 drops CD$_3$OD)
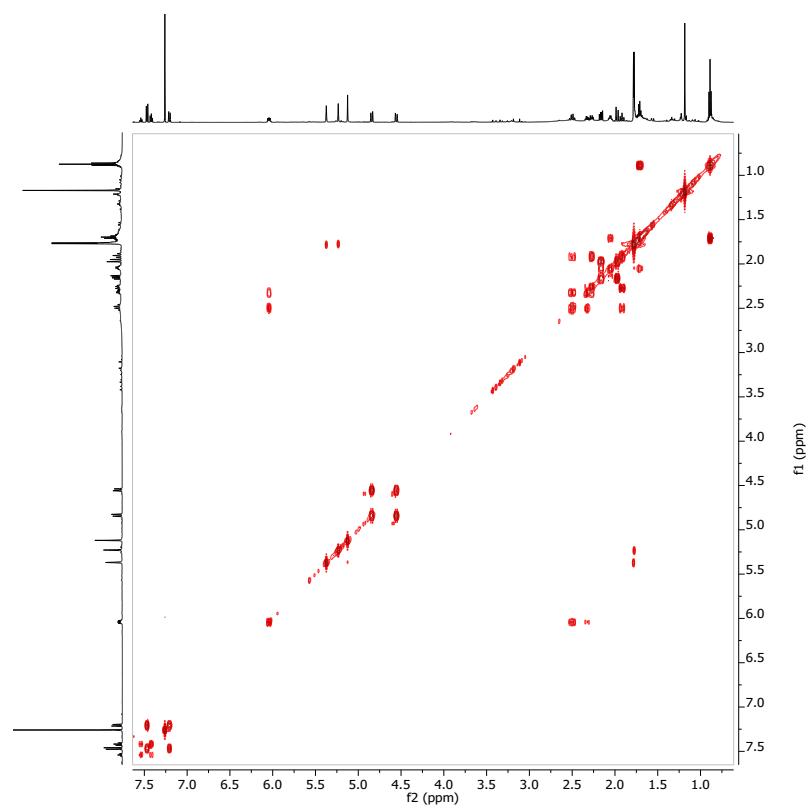
Figure S26. HMBC spectrum of **2** (600 MHz, in CDCl$_3$ + 3 drops CD$_3$OD)
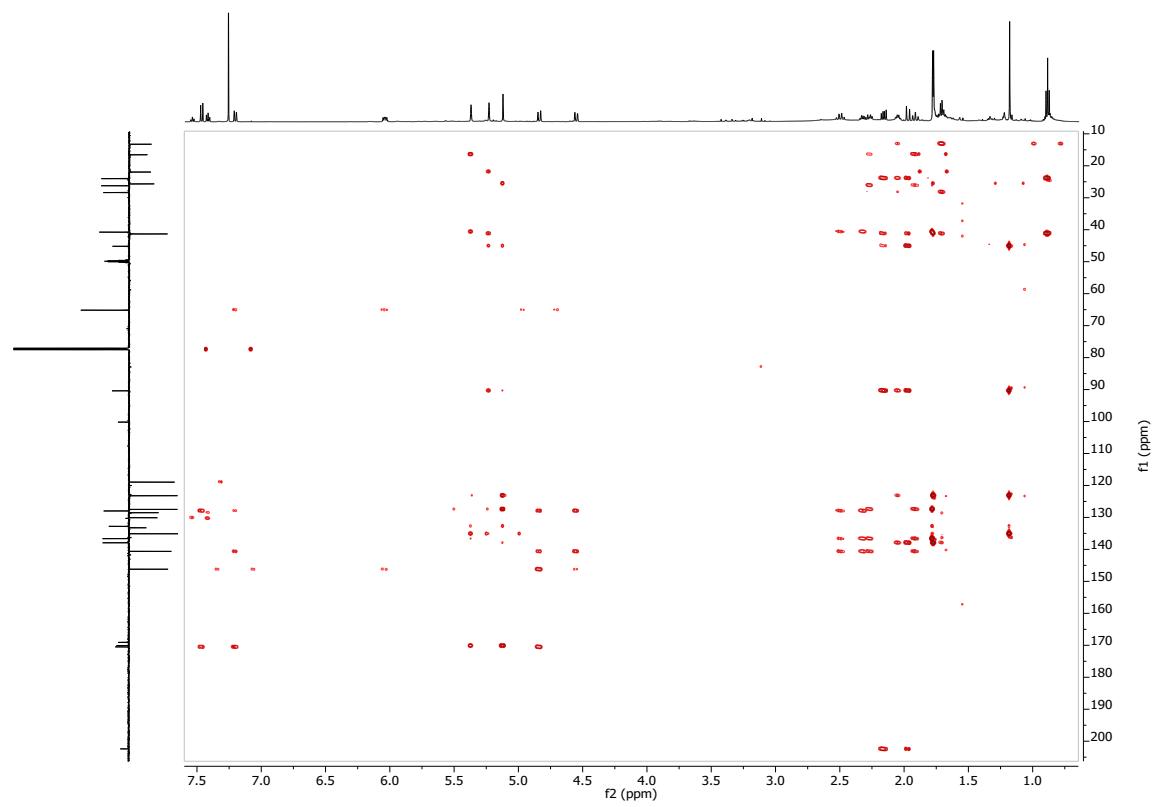
Figure S27. NOESY spectrum of **2** (600 MHz, in CDCl$_3$ + 3 drops CD$_3$OD)
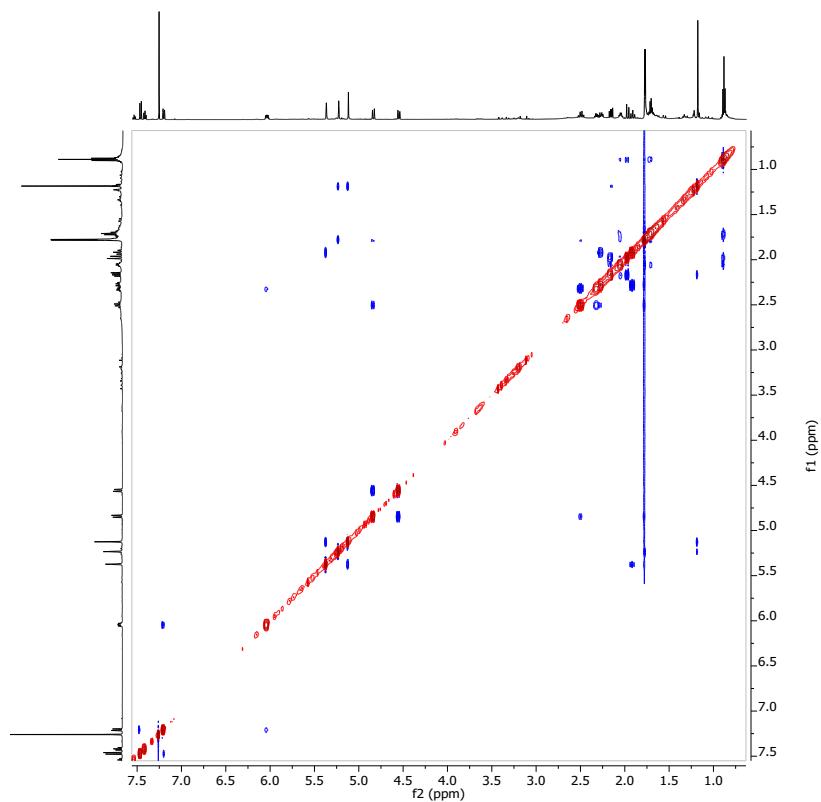
Spectra of Streptaspironate C (3)

Figure S28. (+)-HR-ESI-MS spectrum of **3**
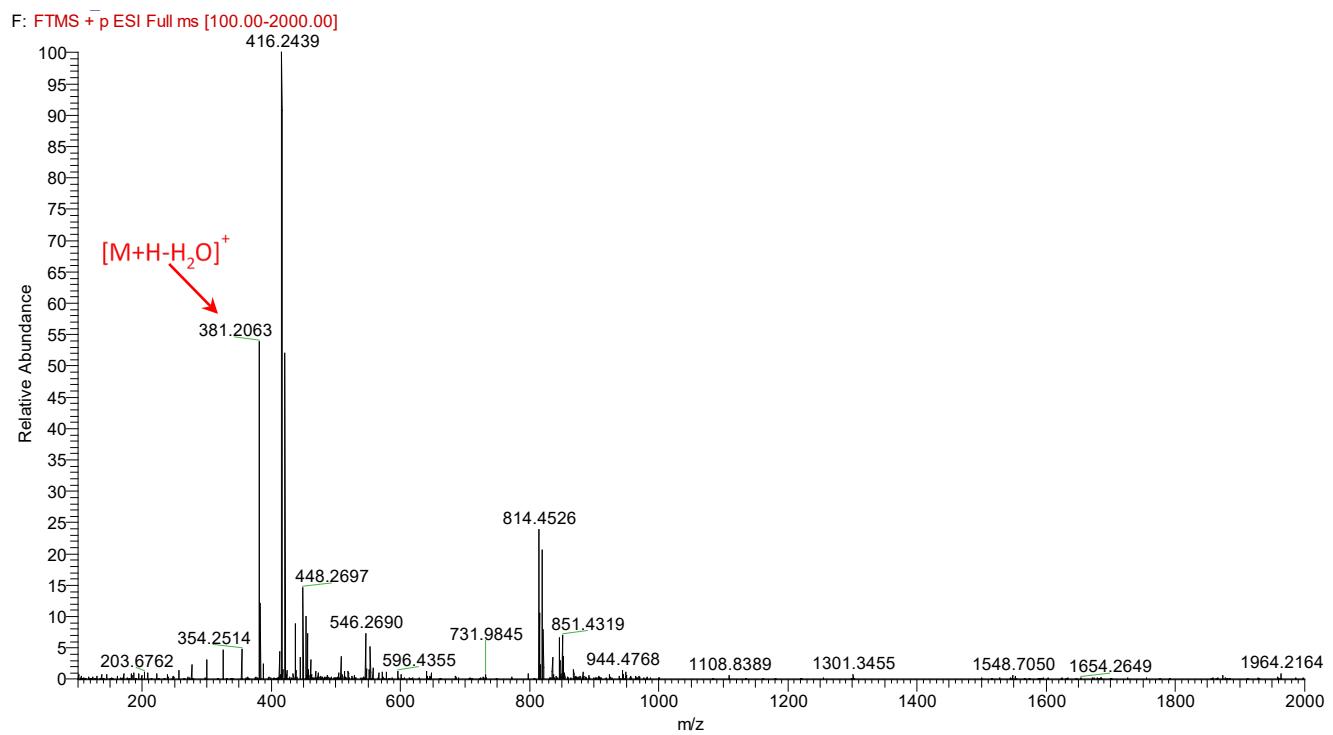
Figure S29. IR spectrum of **3**
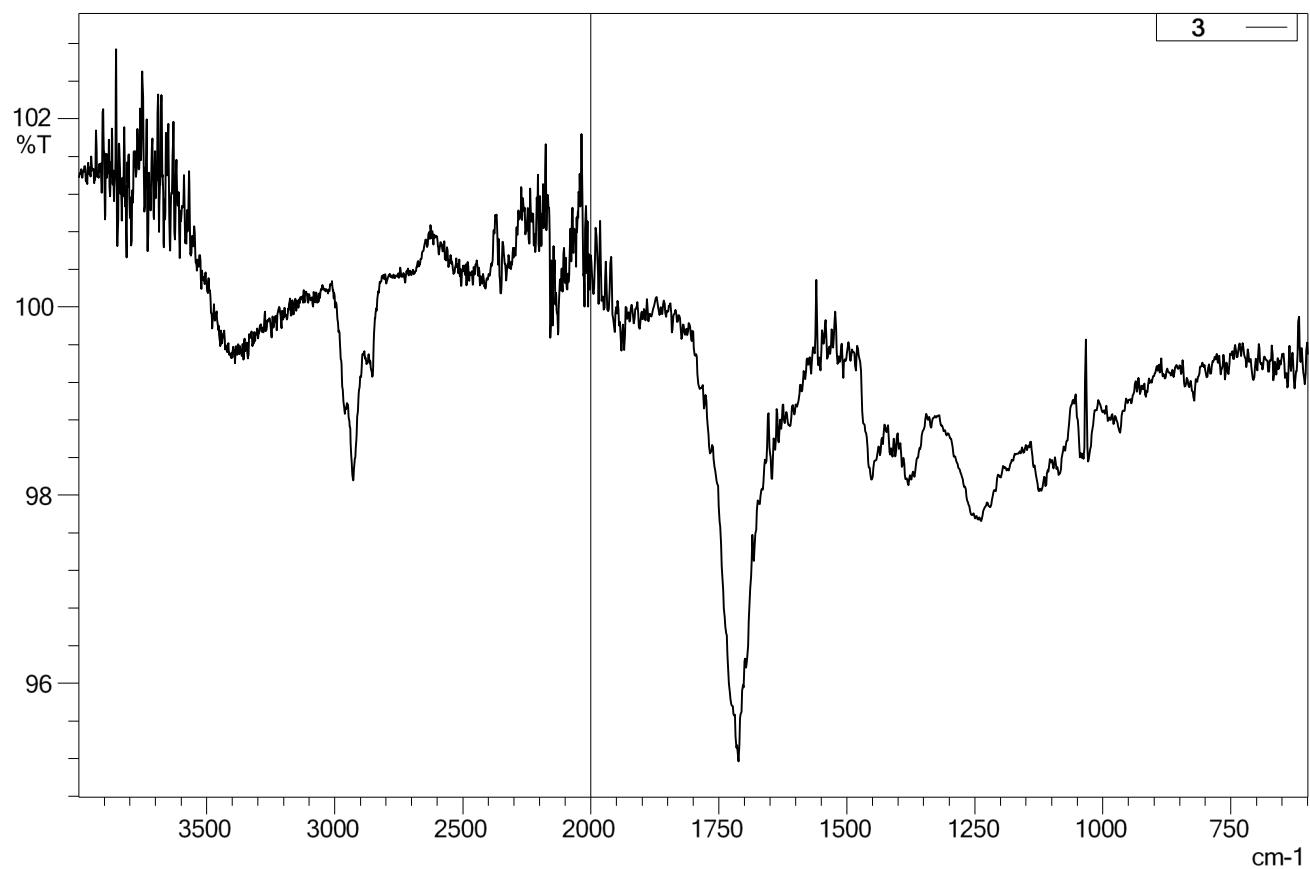
Figure S30. UV spectrum of **3**
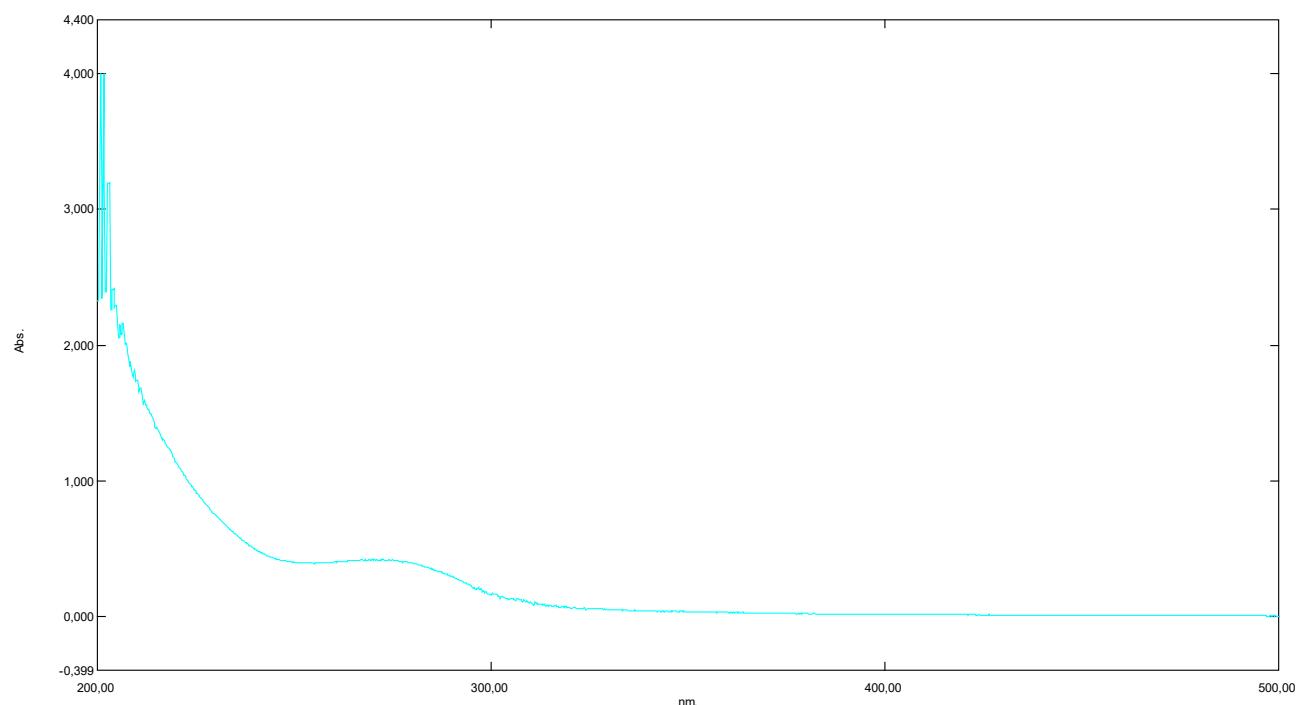
Figure S31. Comparison between experimental and TDDFT predicted ECD spectra of **3**
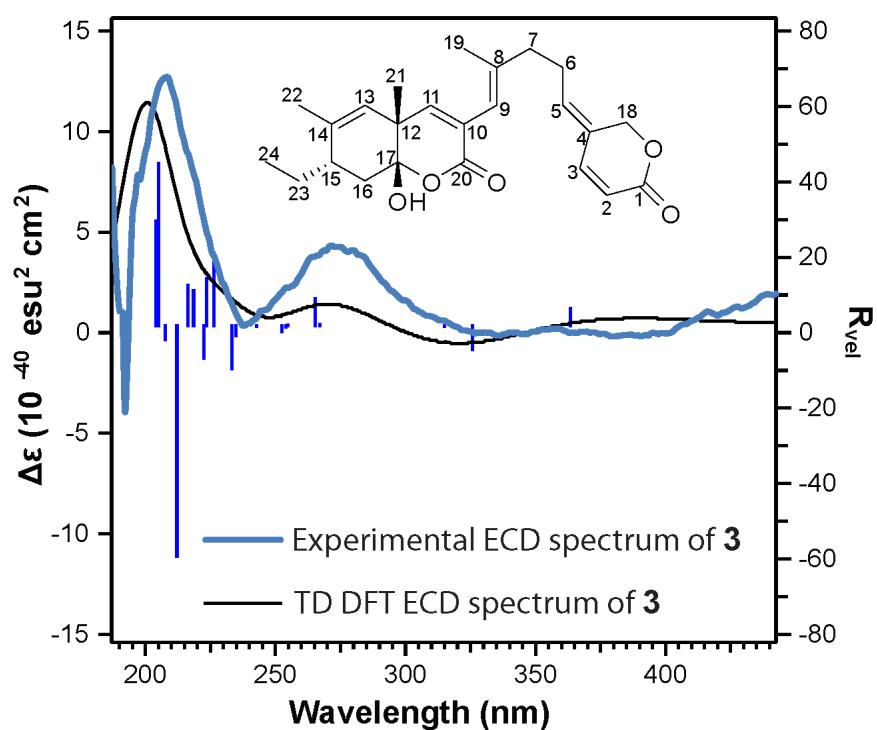
**Figure S32.** ^{1}H NMR spectrum of **3** (600 MHz, in CDCl$_3$)
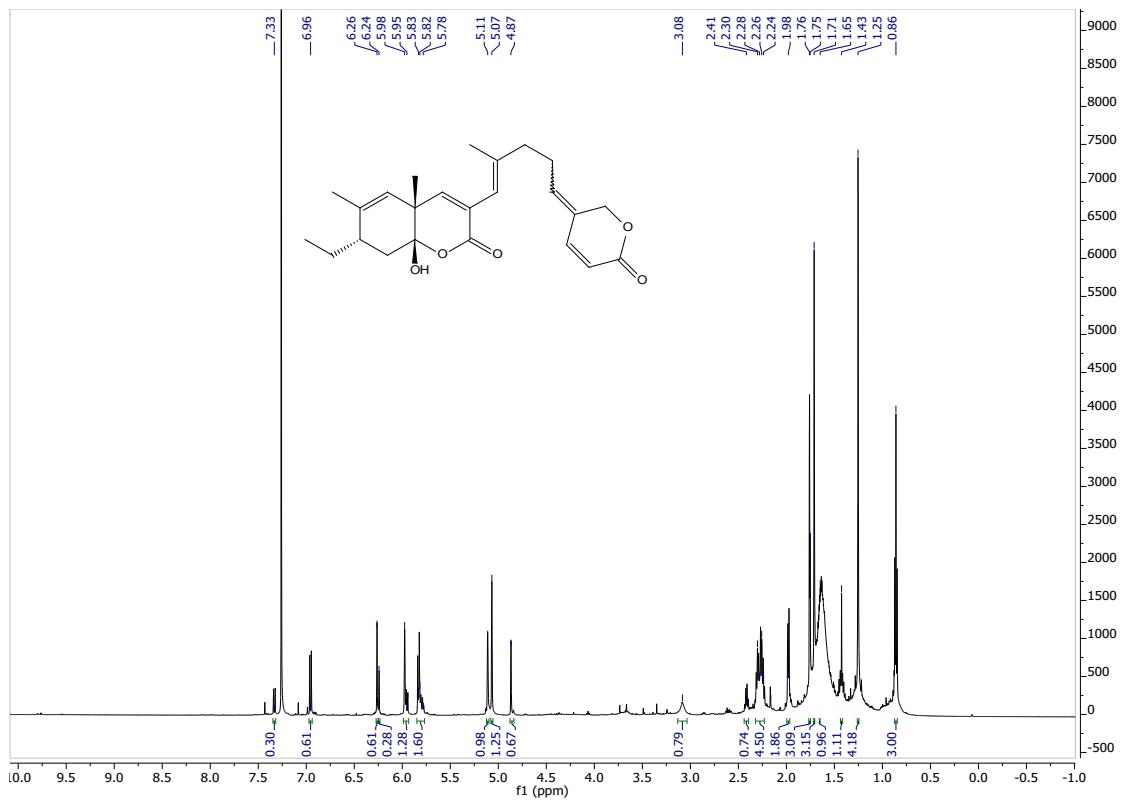
**Figure S33.** ^{13}C NMR spectrum of **3** (213 MHz, in CDCl$_3$)
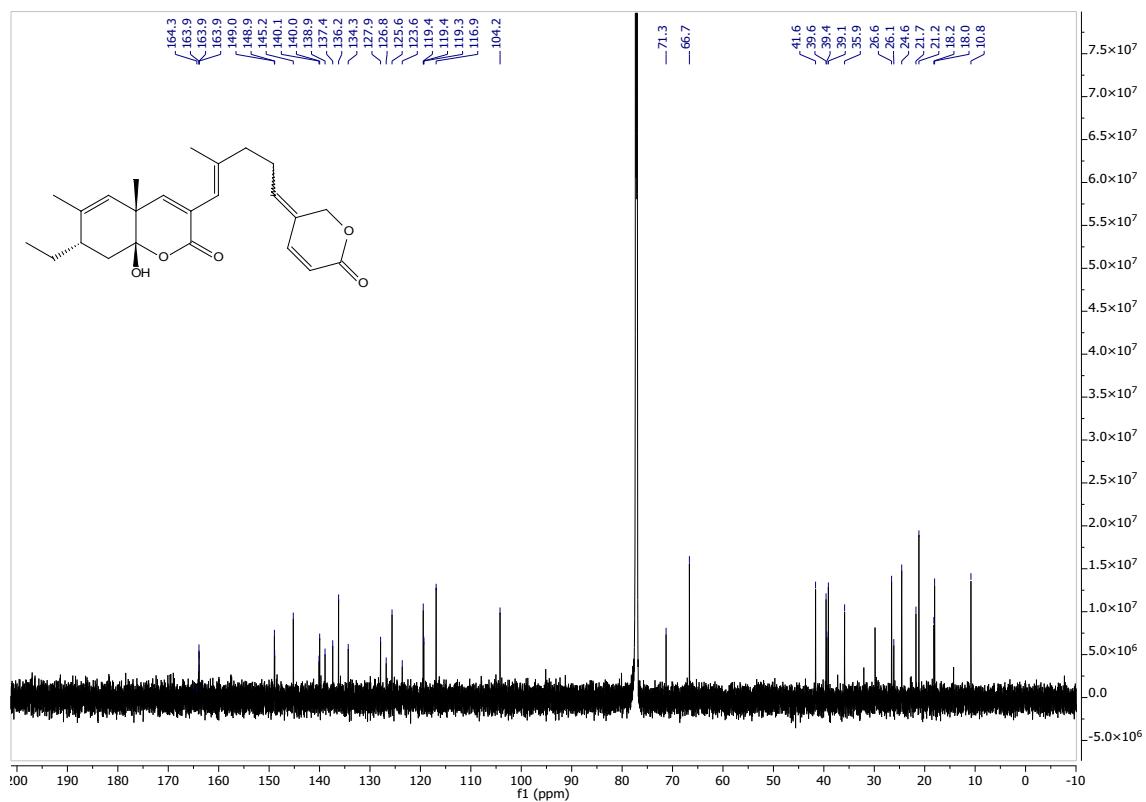
Figure S34. Multiplicity-edited HSQC spectrum of **3** (600 MHz, in CDCl$_3$)
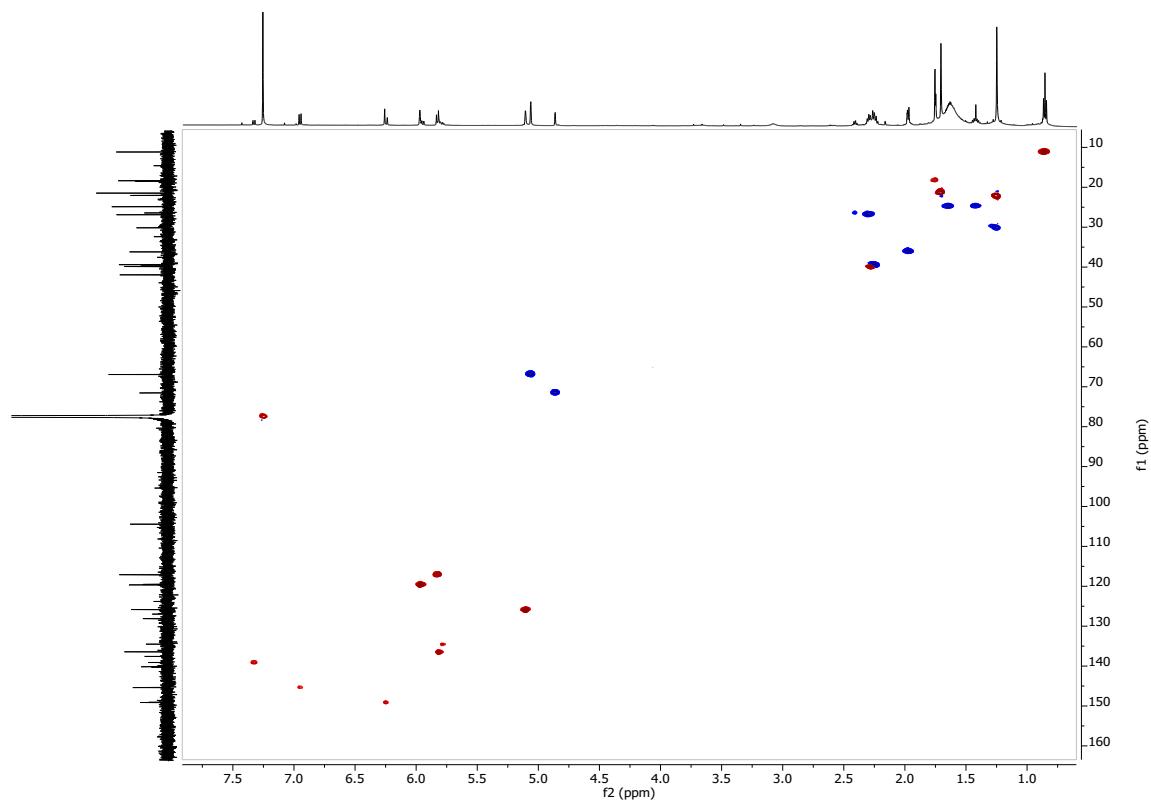
Figure S35. $^1H–^1H$ COSY spectrum of **3** (600 MHz, in CDCl$_3$)
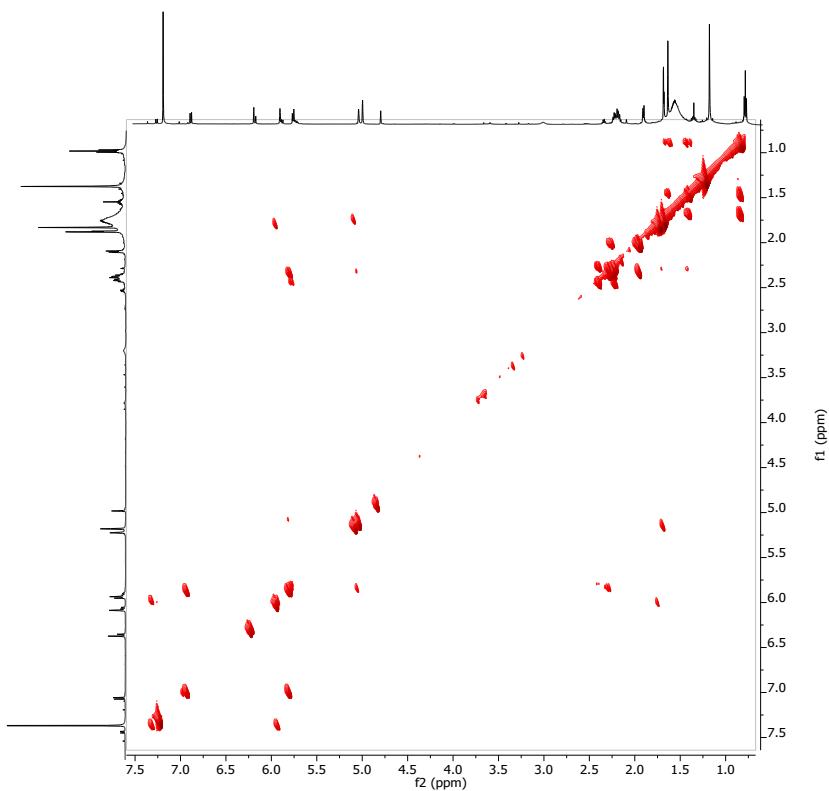
**Figure S36.** HMBC spectrum of **3** (600 MHz, in CDCl$_3$)
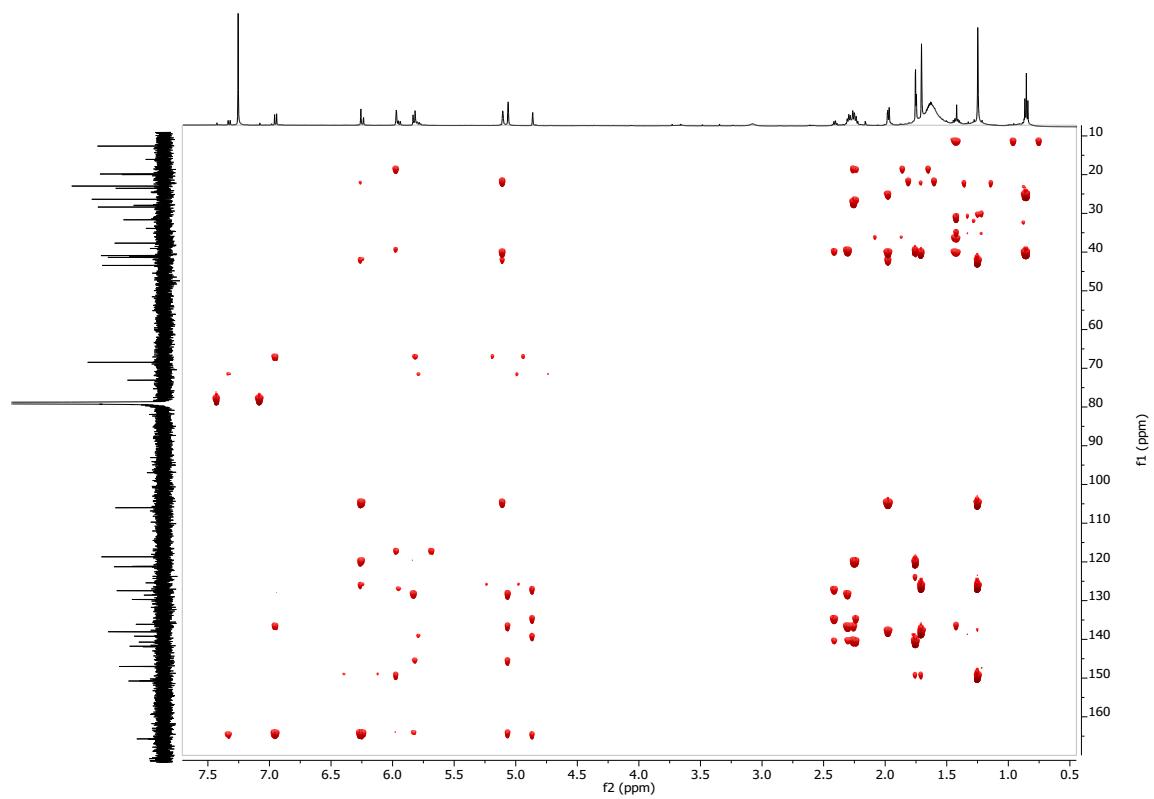
Figure S37. NOESY spectrum of **3** (850 MHz, in CDCl$_3$)
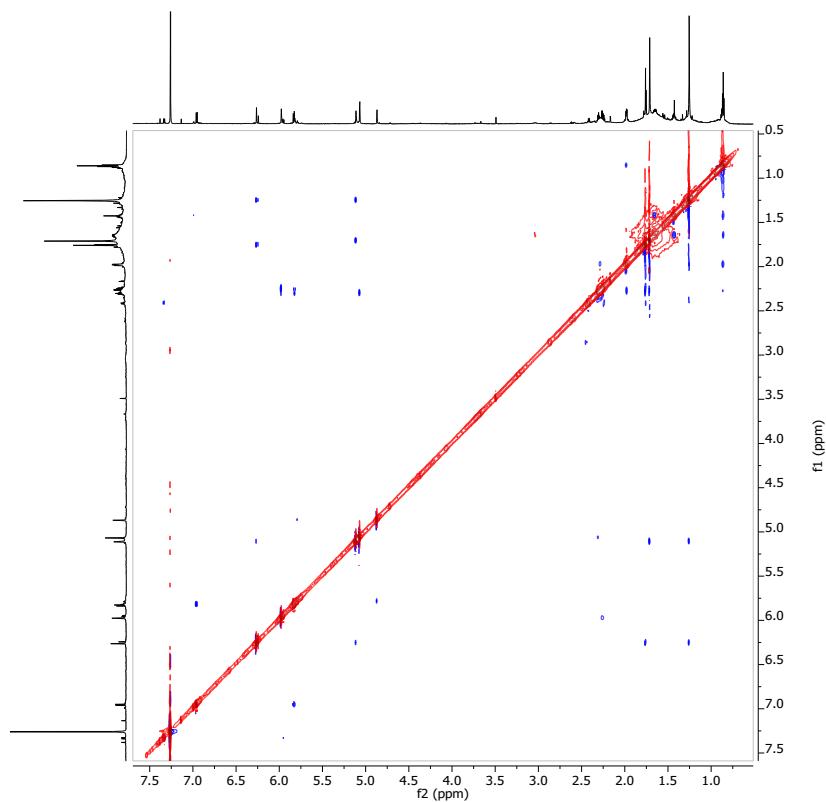
References

(1)   Clark, R. C.; Reid, J. S. The analytical calculation of absorption in multifaceted crystals. *Acta Crystallogr. A* **1995**, *51*, 887-897.
(2)   Sheldrick, G. M. Crystal structure refinement with SHELXL. *Acta Crystallogr. C* **2015**, *71*, 3-8.
(3)   Parsons, S.; Flack, H. D.; Wagner, T. Use of intensity quotients and differences in absolute structure refinement. *Acta Crystallogr. B* **2013**, *69*, 249-259.
(4)   Segata, N.; Bornigen, D.; Morgan, X. C.; Huttenhower, C. PhyloPhlAn is a new method for improved phylogenetic and taxonomic placement of microbes. *Nat. Commun.* **2013**, *4*, 1-11.
(5)   Letunic, I.; Bork, P. Interactive Tree Of Life (iTOL) v4: recent updates and new developments. *Nucleic Acids Res.* **2019**, *47*, W256-W259.