SUPPLEMENTARY MATERIAL

Chemical constituents from the deep sea-derived *Streptomyces xiamenensis* MCCC 1A01570 and their effects on RXRα transcriptional regulation

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1. Experimental

1.1. General experimental procedures

NMR spectra were recorded on a Bruker DRX 500 MHz spectrometer using tetramethylsilane as the internal reference. HRESIMS data were measured on a Xevo G2 Q-TOF mass spectrometer (Waters). Column chromatography (CC) was performed on ODS (50 μm, Daiso), silica gel (200–300 mesh, Qingdao Marine Chemistry Co. Ltd.), and Sephadex LH-20 (Amersham Pharmacia Biotech AB). High-performance liquid chromatography (HPLC)-grade solvents were purchased from Merck KGaA (Darmstadt, Germany). Plasmids (pBind RXRα LBD and pG5 luc) were provided by Dr. Xiao-kun Zhang from the Burnham Institute for Medical Research, Cancer Center, La Jolla, CA, USA. Dual Luciferase Reporter Assay System Kit was purchased from

Promega Corporation. Lipofectamine 2000 reagent was bought from Invitrogen Co., Ltd.

1.2. Fungal material

The strain was isolated from a deep sea-sediment sample of the west Pacific Ocean (W147.98°, N 12.99°) at the depth of 2721 m in 2005. It was identified as *Streptomyces xiamenensis* according to 99% similarity of the 16s rRNA gene sequence to *Streptomyces xiamenensis* MCCC 1A01550(T) (EF012099). A voucher strain of this strain was preserved at the Marine Culture Collection of China (MCCC) with the accession number of 1A01570.

1.3. Cultivation, extraction, and isolation

S. xiamenensis was inoculated into Erlenmeyer flasks (5 L) containing 1.8 L of A3 broth (1.5% starch, 1.5% glycerin, 0.5% potato peptone, 1.5% bacteriological peptone, 3.0% sea salt, and 0.2% CaCO3, pH 7.4) to fermentation on a rotary shaker at 180 rpm for 2 days. Then 20 mL fermented broth was transferred to 1 L Erlenmeyer flasks containing 380 mL A3 broth to fermentation for 10 days at 28 °C, 180 rpm. The fermentation broth was extracted with EtOAc three times to afford a dark crude extract (10.8 g). The extract was eluted with petroleum ether (PE)-acetone (500:1→3:1) to provide the five fractions (Fr.1−Fr.5) by column chromatography (CC) on silica gel. Fr.2 (1.1 g) was firstly subjected to CC over silica gel (PE-EtOAc, 50:1→5:1) to give four fractions, and then Fr.2.4 was purified by CC over semi-preparative high-performance liquid chromatography (pre-HPLC) with MeCN-H_2O (25:75) to provide **11** (3.0 mg) and **10** (26.0 mg). Fr.3 (360.3 mg) was separated by CC over silica gel (PE-EtOAc, 50:1→5:1) and Pre-HPLC with MeCN-H_2O (20:80) to **12** (1.1 mg). Fr.4 (1.3 g) was subjected to Sephadex LH-20 (acetone) to provide four subfractions (Fr.4.1–Fr.4.4). Compounds **3** (13.2 mg) and **5** (1.7 mg) was isolated from Fr.4.1 (63.1 mg) by Pre-HPLC with MeCN-H_2O (20:80). Similarly, compound **1** (4.0 mg), **2** (12.1 mg), **4** (19.4 mg), **8** (3.1 mg) were obtained from Fr.4.2 (247.0 mg) by semi-preparative HPLC with MeOH-H_2O (28:72). Compounds **7** (4.2 mg) and **13** (1.7 mg) were obtained from

Fr.4.3 (221.2 mg) by pre-HPLC with MeOH-H_2O (35:65), and **5** (52.3 mg) was obtained from Fr.4.4 (294.5 mg) by Pre-HPLC with MeOH-H_2O (50:50). Fr.5 (1.8 g) was purified by subjected to Sephadex LH-20 (acetone) to provide **9** (52.0 mg) and subfraction5.2. Then **6** (28.5 mg) was isolated by pre-HPLC with MeOH-H_2O (55:45).

2. Biological activity

2.1. Cell culture and dual-luciferase reporter gene assay

As reported previously (Duan et al. 2010), human embryonic kidney 293T cells were cultured in Dulbecco's Modified Eagle's Medium (DMEM) medium supplemented with 10% fetal bovine serum (FBS) for 24 h before transfection. Cells were seeded at approximately 5×10^4 cells/well in 96-well plates and transfected with two plasmids, 30 ng pBind RXRα LBD and 60 ng pG5 luc using Lipofectamine 2000 (Invitrogen). After the cell adheres to the wall in 12 h, cells were exposed to tested compounds for 16 h. Then, the cells were washed with PBS and lysed with passive lysis buffer (1 × PLB) 40 μL/well on rocking platform for 15 min. The activities of firefly luciferase and rellina luciferase were examined according to the introduction of Dual-Luciferase Reporter Assay System Kit. Relative luciferase activities were obtained as the ratio between activities of firefly luciferase and rellina luciferase. The fold Activities were calculated as the relative luciferase activities ratio between sample and blank control. DMEM culture containing 0.5% DMSO was regarded as blank control and 9-*cis*-retinoid acid (0.1 μM) was used as a positive control.

2.2. MTT assay

The *in vitro* cytotoxic bioassay was conducted according to the previously reported protocol (Luo et al. 2008). Briefly, three human cancer cell lines ECA-109, Hela-S3 and PANC-1 were seeded to 96-well plates to incubate for 24 h. Then the tested compounds and *cis*-DDP were added to each well, and the incubation was continued for 48 h. Subsequently, MTT was added and incubated for another 4 h. The liquid supernatant was removed softly and DMSO was added. The absorbencies were measured at 490 nm.

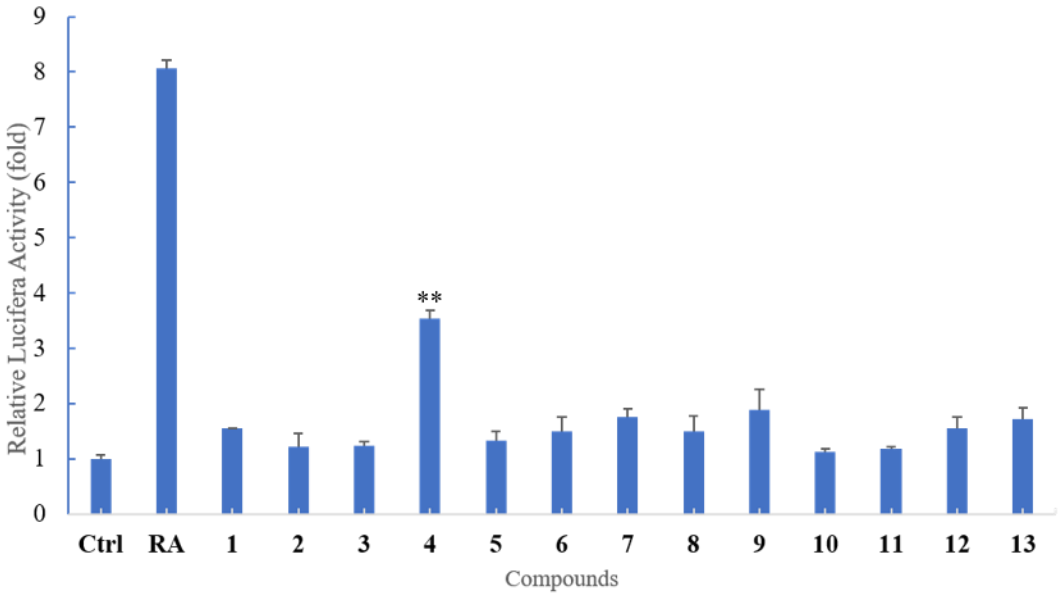

Figure S1. Promoting effects of isolated compounds on reporter transcription activities of RXRα

Transfected 293T cells were treated with compounds (20 μM) or 9-cis-retinoid acid for 16 h. The activities of firefly luciferase and rellina luciferase were measured. Relative luciferase activities were calculated as the ratio between activities of firefly luciferase and rellina luciferase. The fold activities were calculated as the relative luciferase activities ratio between sample and blank control. DMEM culture containing 0.5% DMSO was regarded as blank control and RA (0.1 μM) was used as a positive control. Data were presented as mean ± SD (n = 3). $^{**}P < 0.01$ compared to RA group.

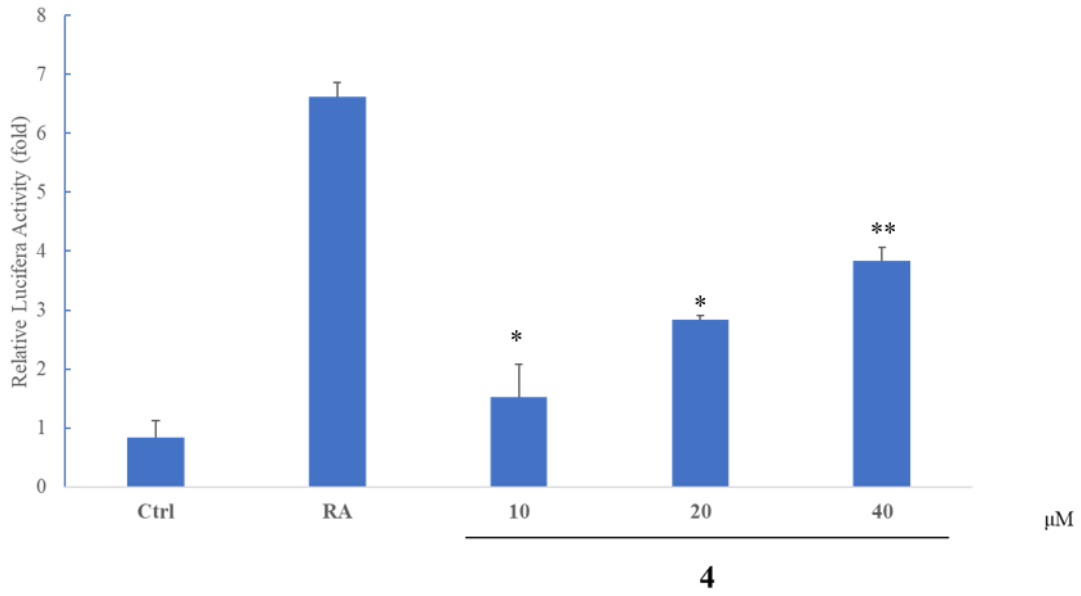

Figure S2. Promoting effect of **4** on reporter transcription activities of RXRα. Data were presented as mean ± SD (n = 3). *P < 0.05, **P < 0.01 compared to RA group.

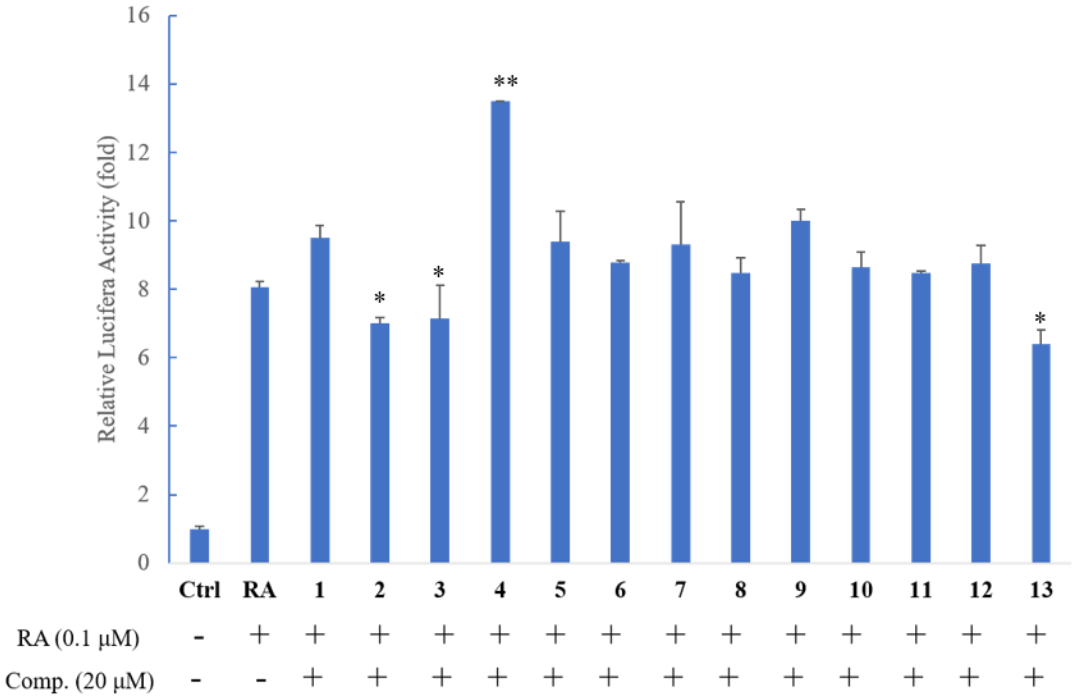

Figure S3. Inhibitory effects of isolated compounds on reporter transcription activities of RXRα.Transfected 293T cells were treated with RA (0.1 μM) with or without compounds (20 μM) for 16 h. The activities of firefly luciferase and rellina luciferase were measured and relative luciferase activities were calculated. The fold activities were calculated as the relative luciferase activities ratio between sample and blank control. DMEM culture containing 0.5% DMSO was regarded as blank control and RA (0.1 μM) was used as a positive control. Data were presented as mean ± SD (n = 3). *P < 0.05, **P < 0.01 compared to RA group.

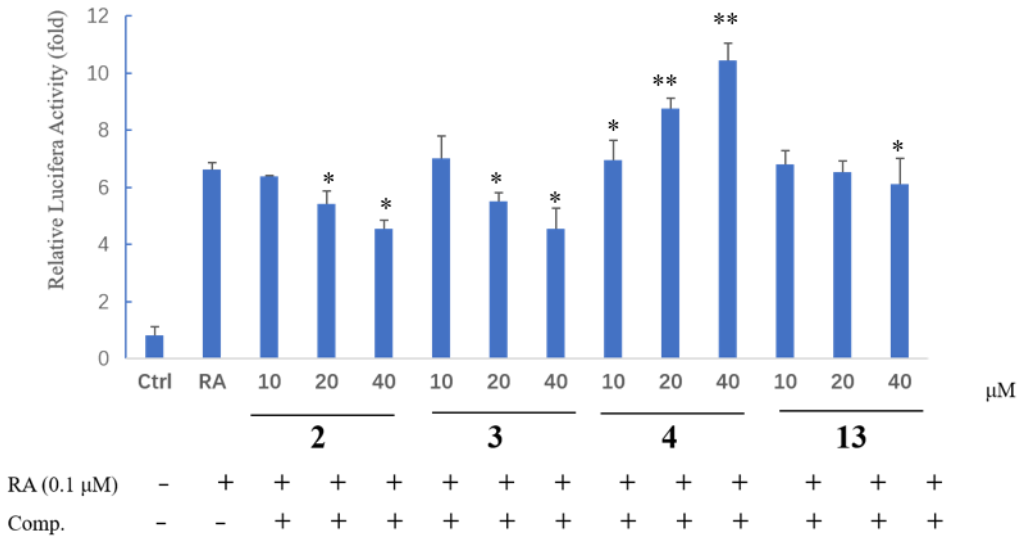

Figure S4. Inhibitory effects of compounds **2**, **3**, **4**, **13**, and **14** on reporter transcription activities of RXRα in concentration-dependent manner. Data were presented as mean ± SD (n = 3). * $P < 0.05$, ** $P < 0.01$ compared to RA group.

Table S1. The inhibitory effects of compounds **1**–**13** (20 μM) from *Streptomyces xiamenensis* MCCC 1A01570 on ECA-109, Hela-S3 and PANC-1 tumor cell lines

	Inhibition rate (%)		
Compounds	ECA-109	Hela-S3	PANC-1
1	38	51	31
2	60	60	37
3	50	45	56
4	44	52	55
5	55	41	14
6	54	47	42
7	30	40	34
8	42	36	50
9	32	11	20
10	58	65	60
11	49	34	37
12	54	52	63
13	46	34	42
cis-DDP (2 μM) [a]	58	65	60

[a] *cis*-DDP: *cis*-Dichlorodiamine platinum, the positive control.

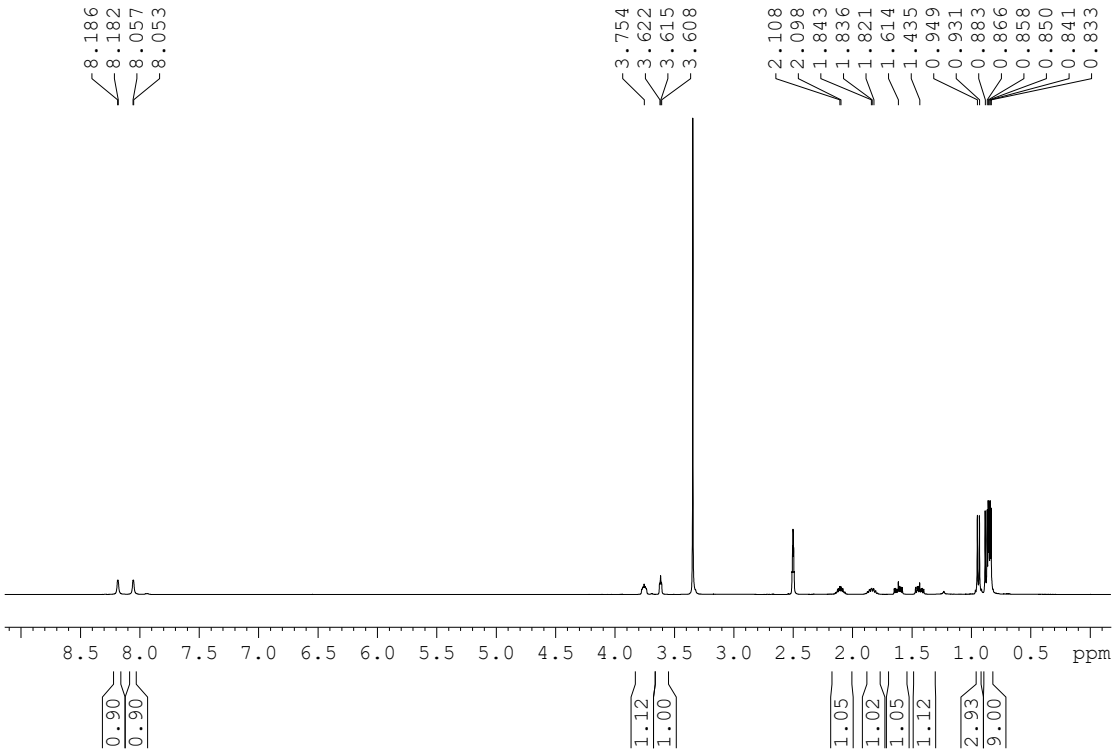

Figure S5 ^{1}H-NMR (500 MHz, DMSO-d_6) spectrum of compound **1**

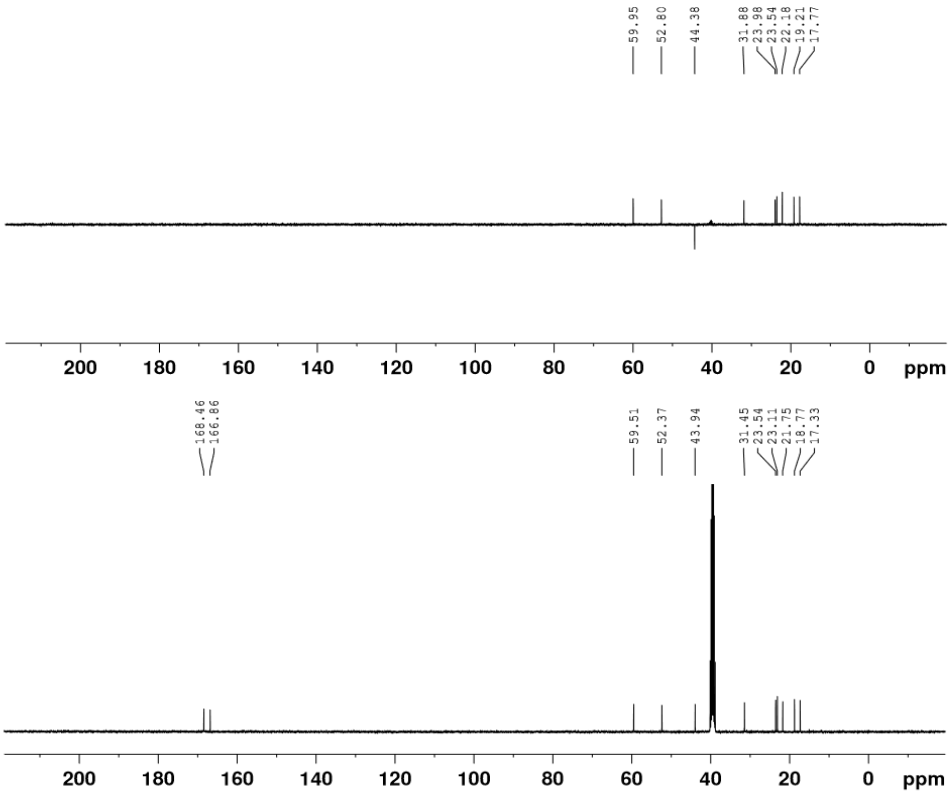

Figure S6 ^{13}C-NMR (125 MHz, DMSO-d_6) spectrum of compound **1**

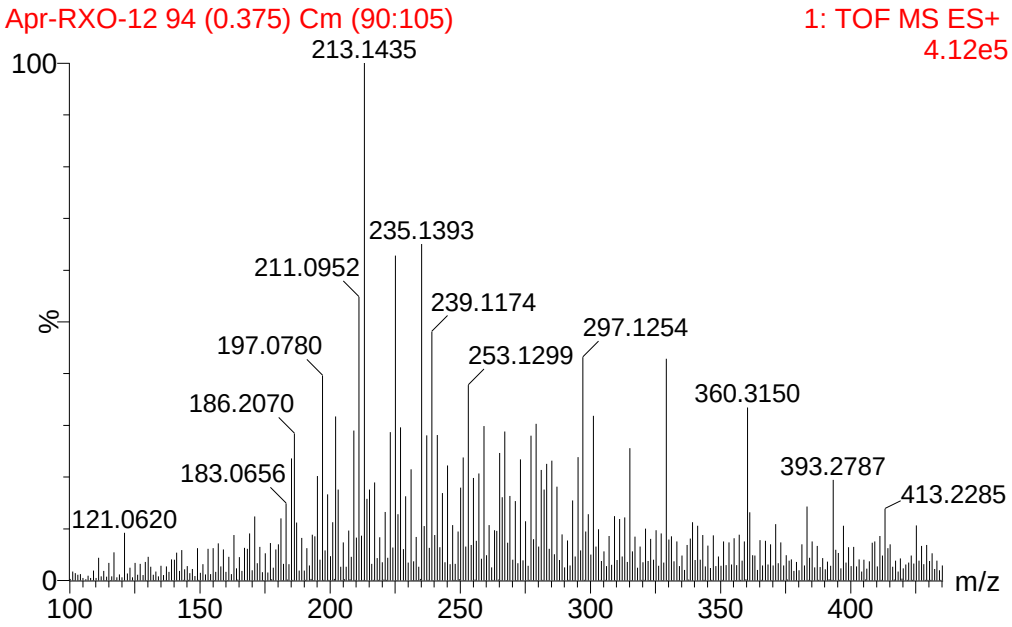

Figure S7 HRESIMS spectrum of compound **1**

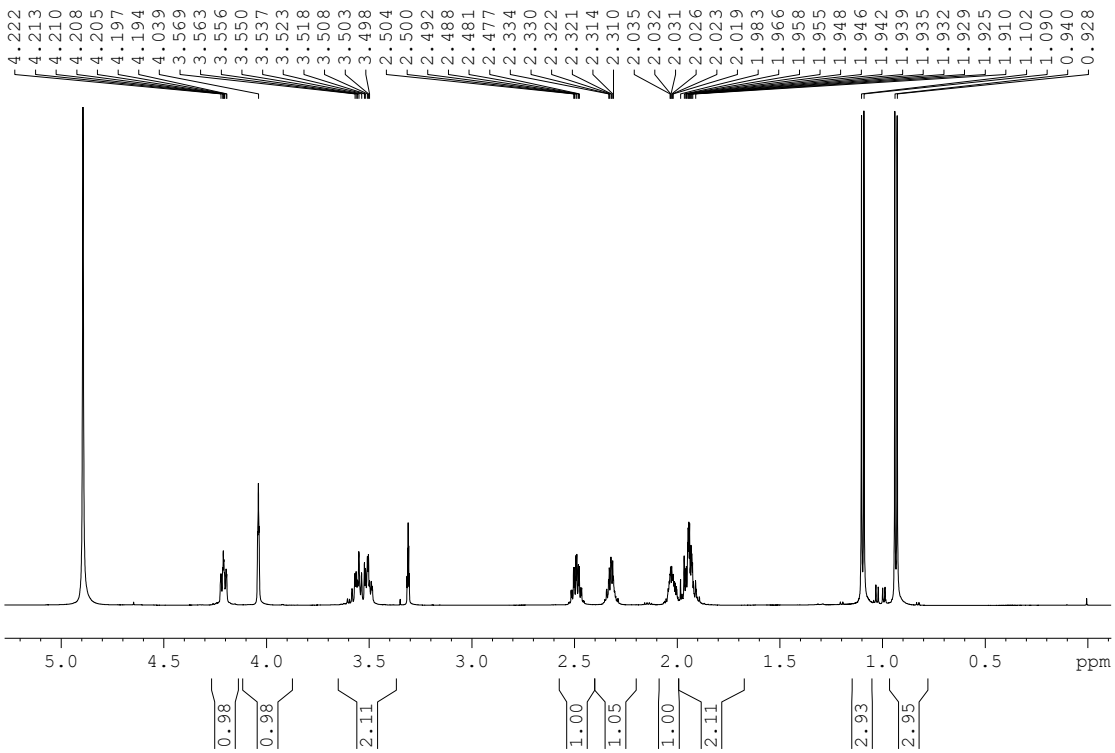

Figure S8 ^{1}H-NMR (500 MHz, CD_3OD) spectrum of compound **2**

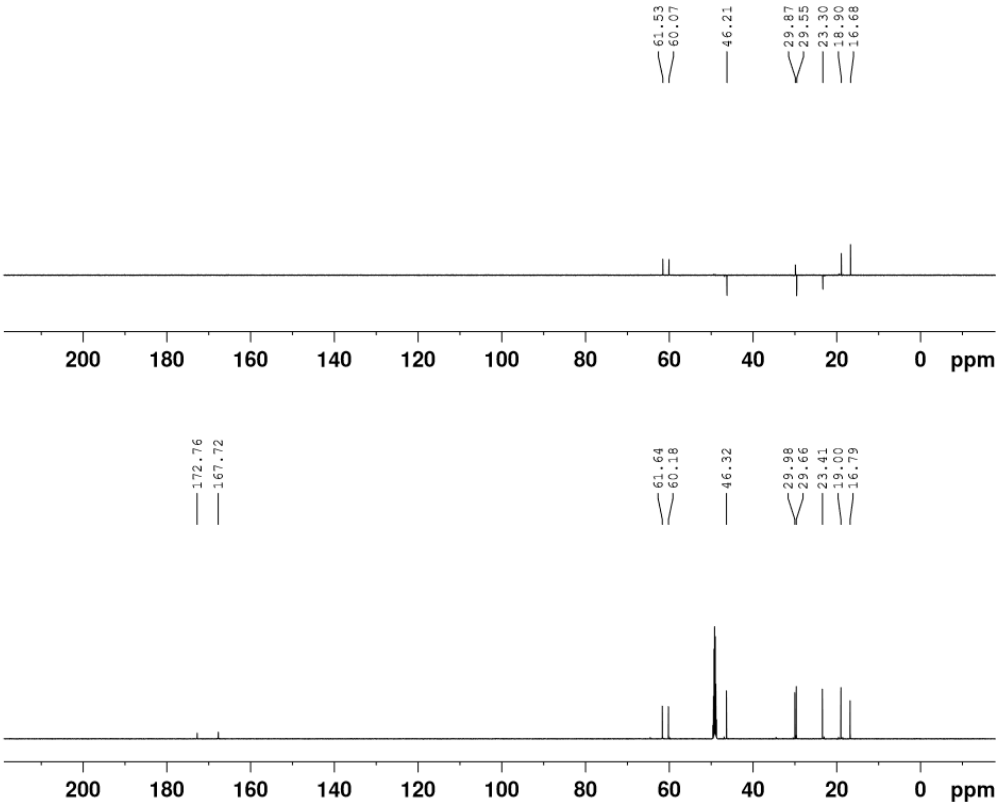

Figure S9 ^{13}C-NMR (125 MHz, CD_3OD) spectrum of compound **2**

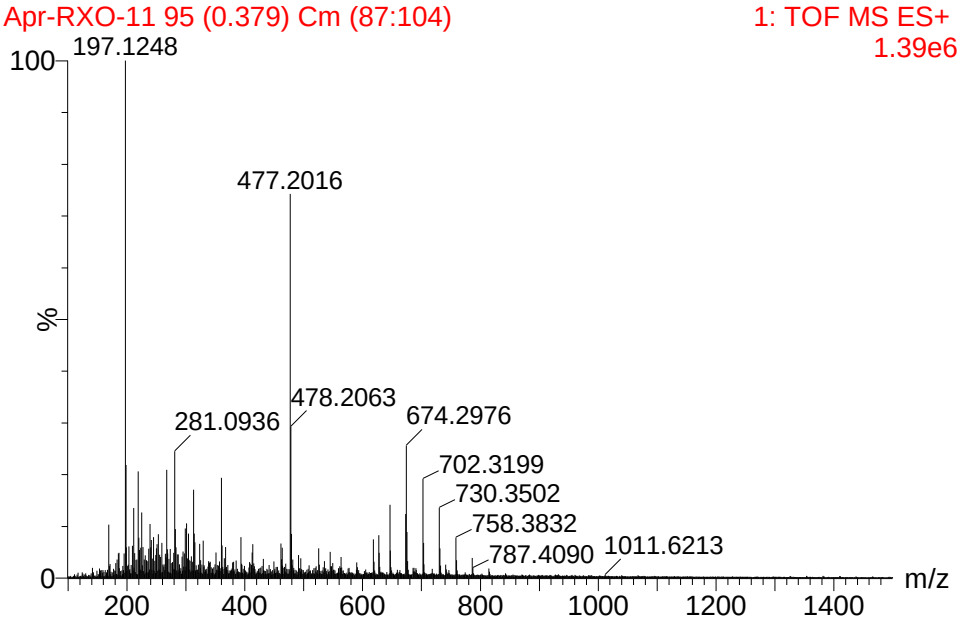

Figure S10 HRESIMS spectrum of compound **2**

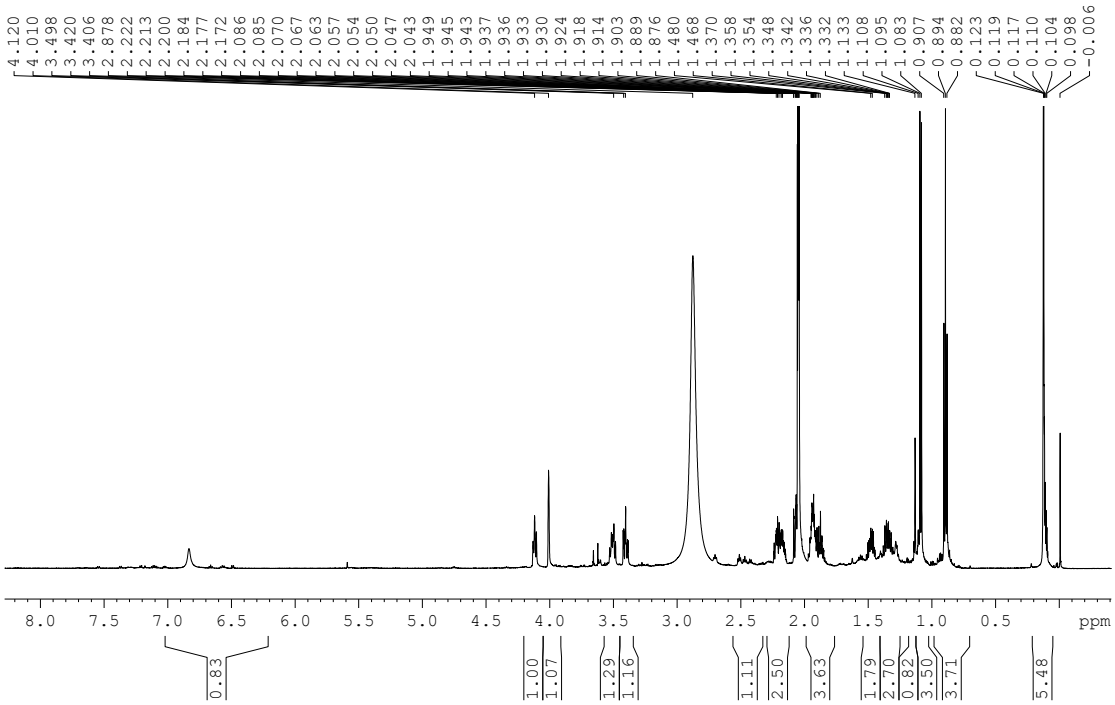

Figure S11 ^{1}H-NMR (500 MHz, acetone-d_6) spectrum of compound **3**

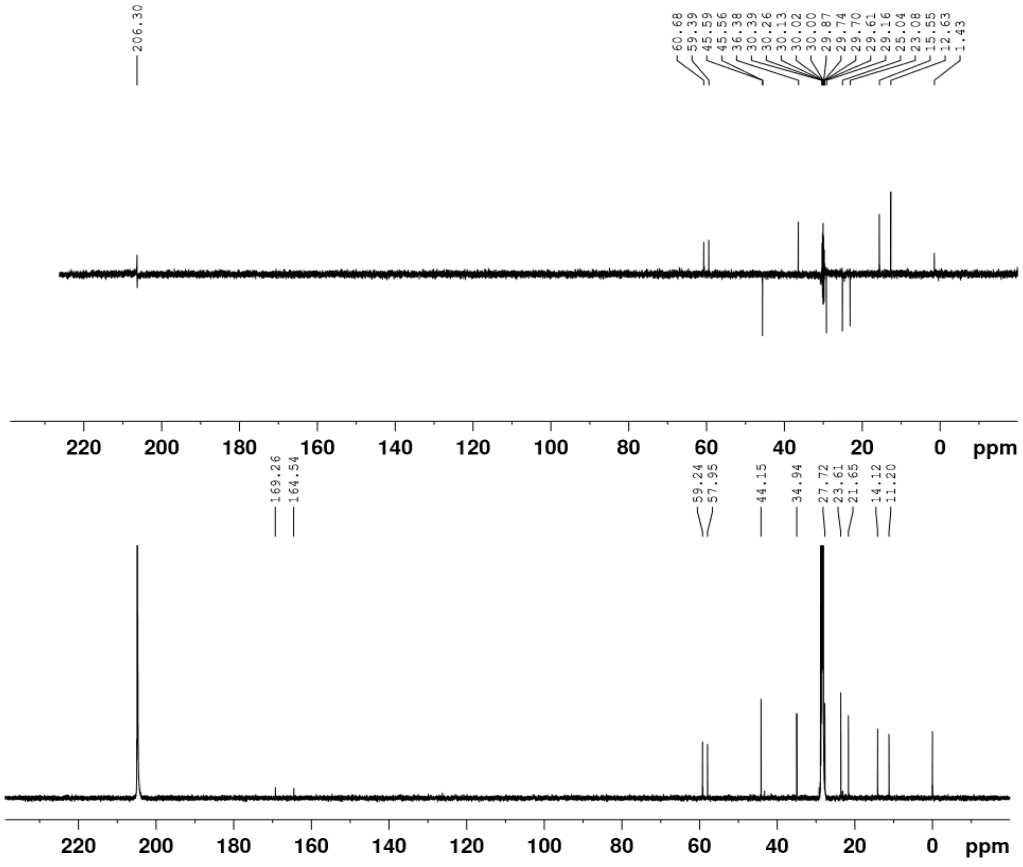

Figure S12 ^{13}C-NMR (125 MHz, acetone-d_6) spectrum of compound **3**

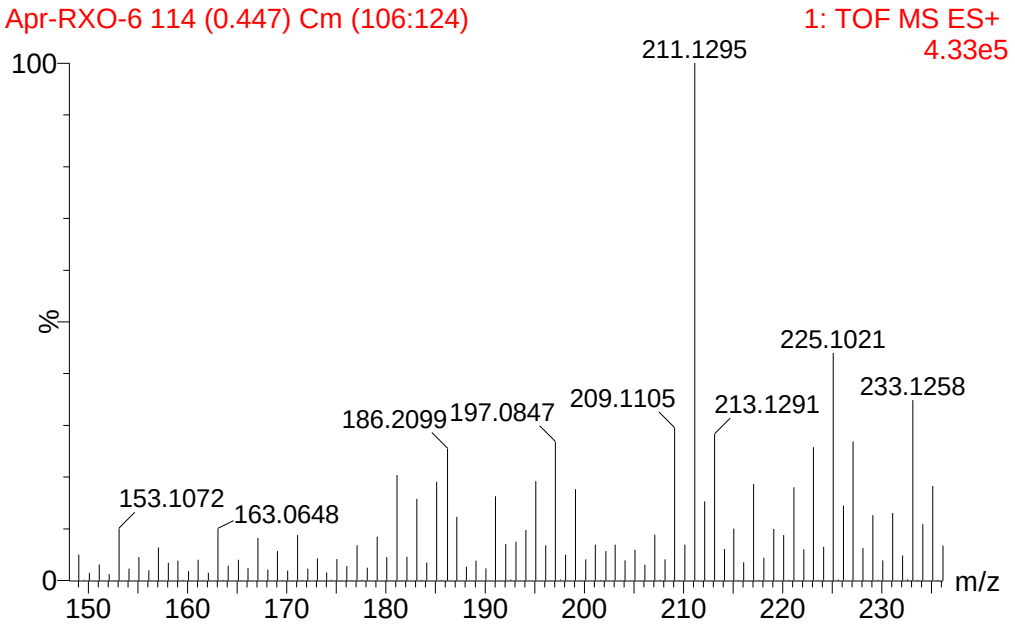

Figure S13 HRESIMS spectrum of compound **3**

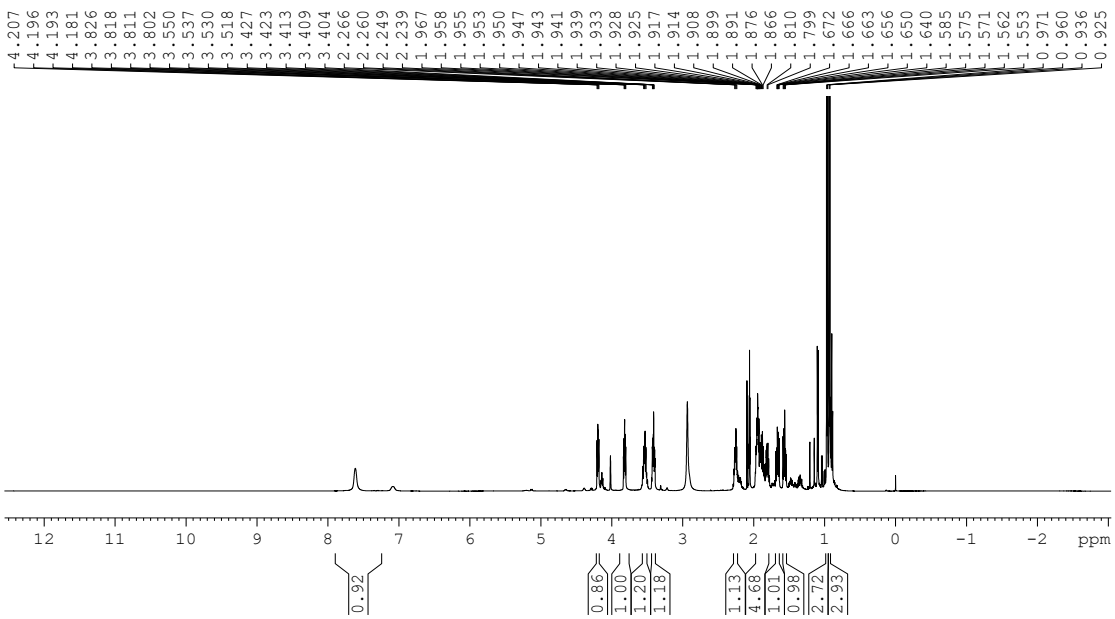

Figure S14 ^{1}H-NMR (500 MHz, acetone-d_6) spectrum of compound **4**

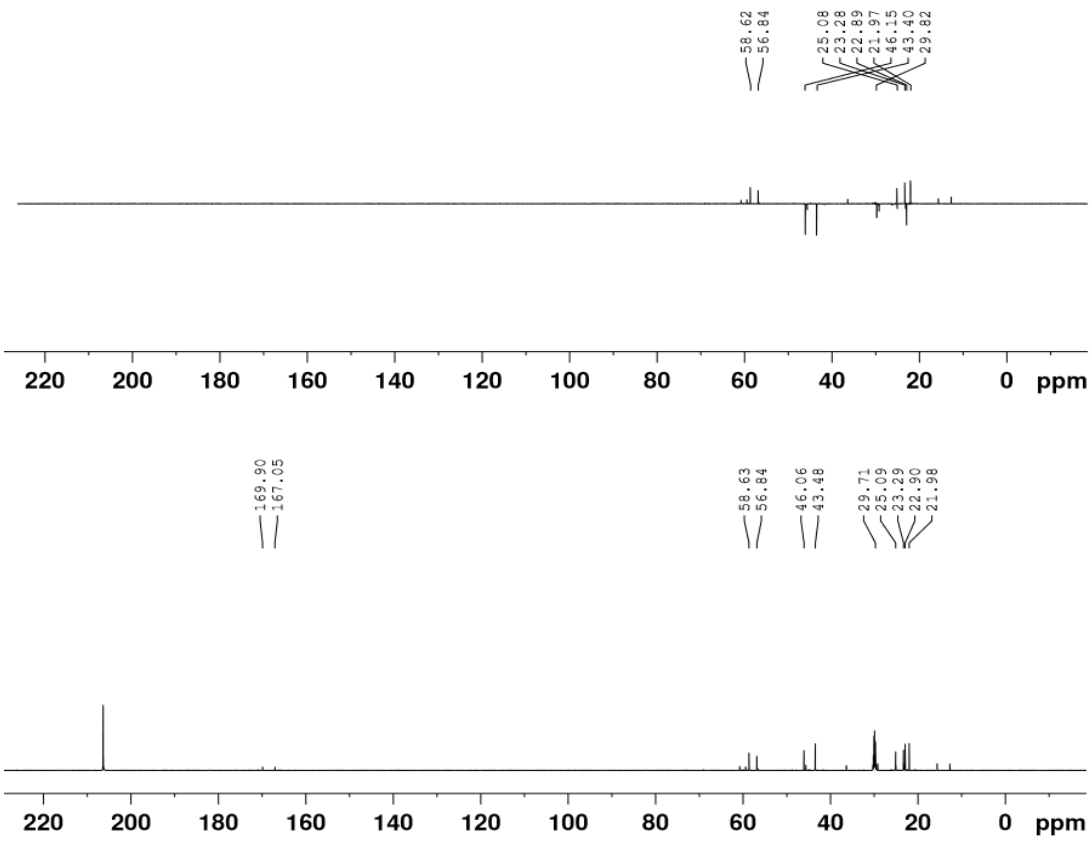

Figure S15 ^{13}C-NMR (125 MHz, acetone-d_6) spectrum of compound **4**

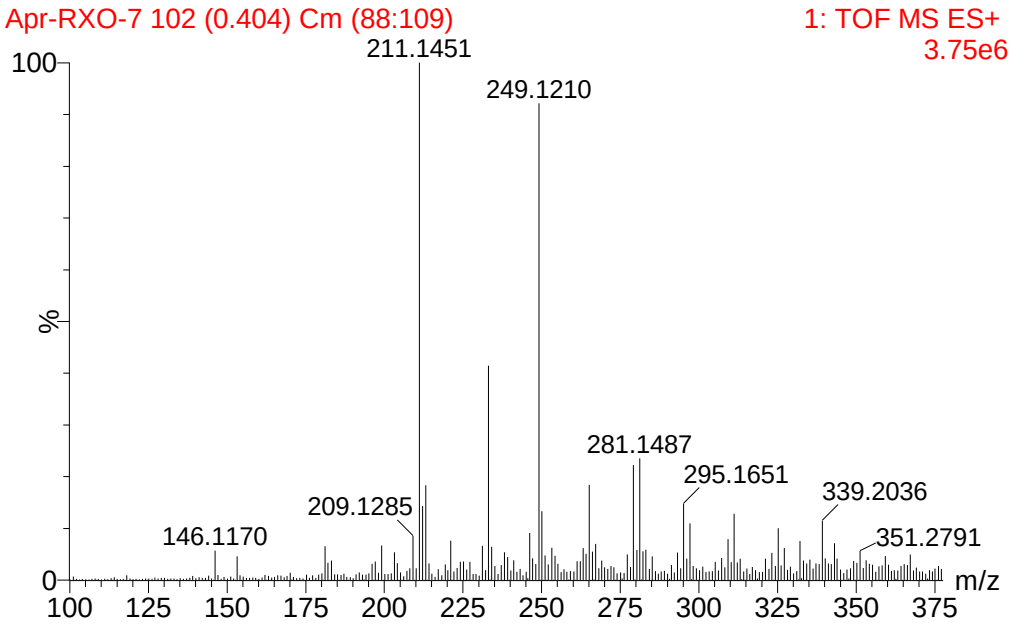

Figure S16 HRESIMS spectrum of compound **4**

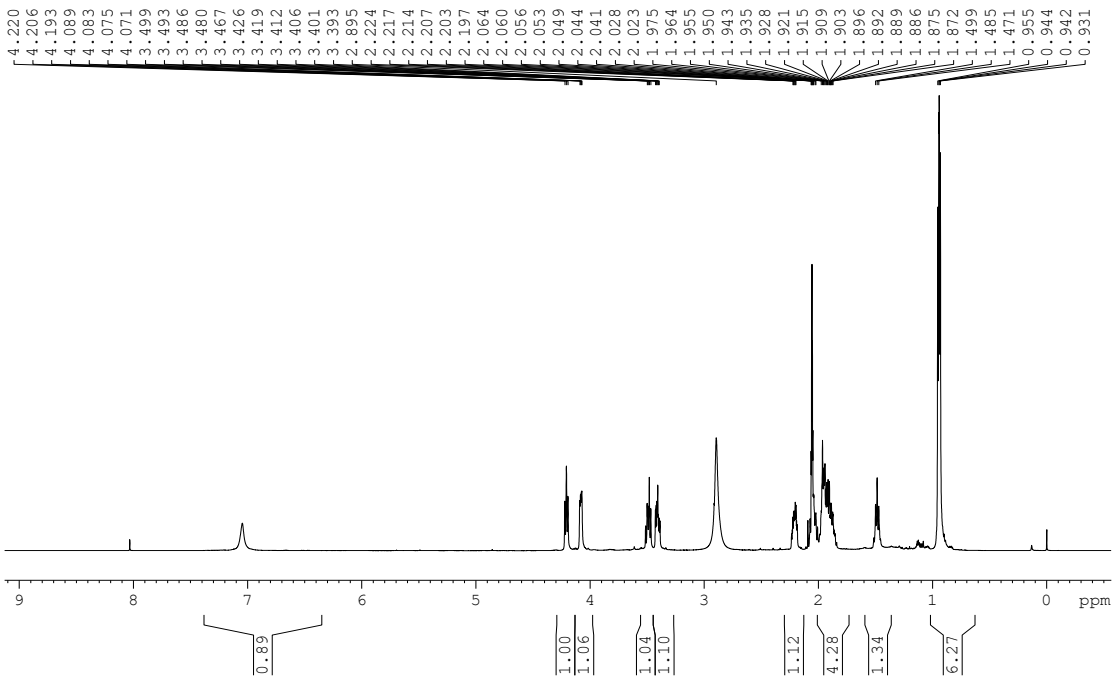

Figure S17 ^{1}H-NMR (500 MHz, CD_3OD) spectrum of compound **5**

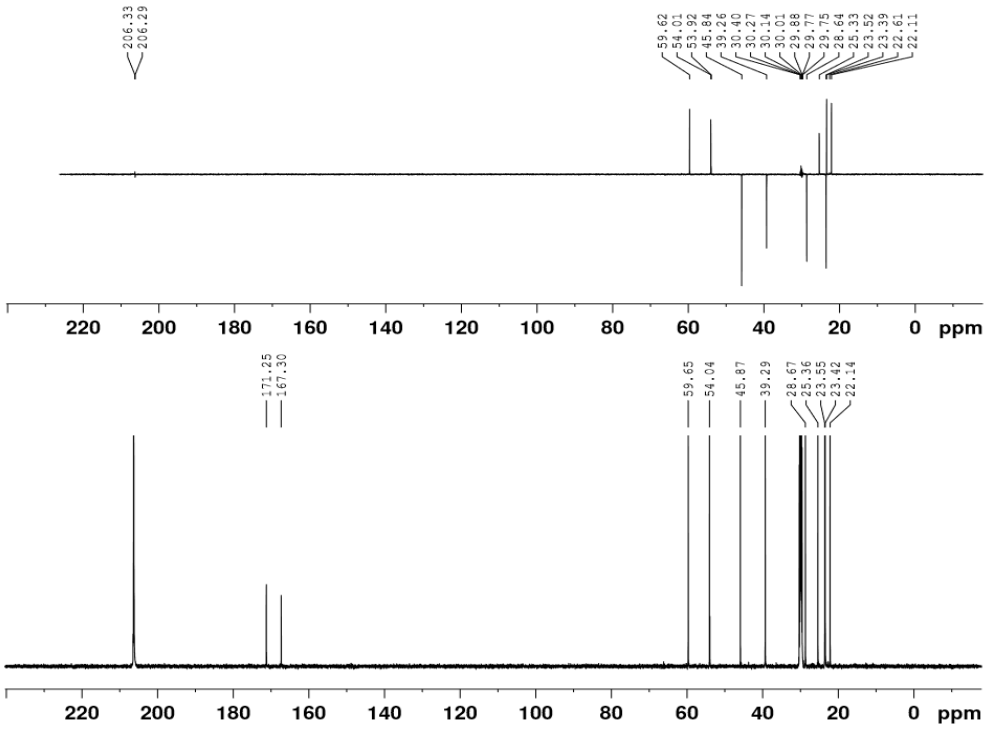

Figure S18 ^{13}C-NMR (125 MHz, CD_3OD) spectrum of compound **5**

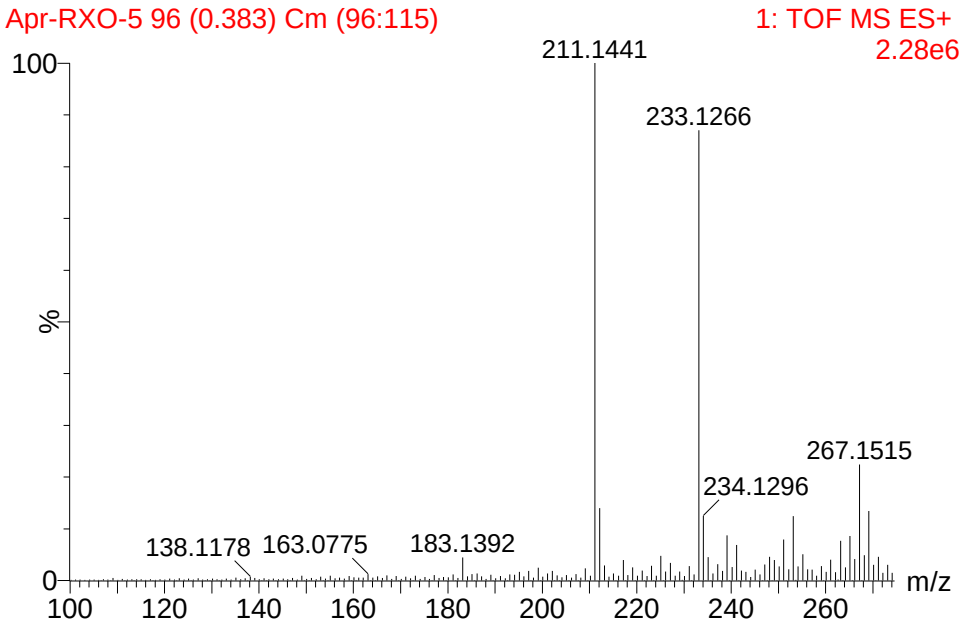

Figure S19 HRESIMS spectrum of compound **5**

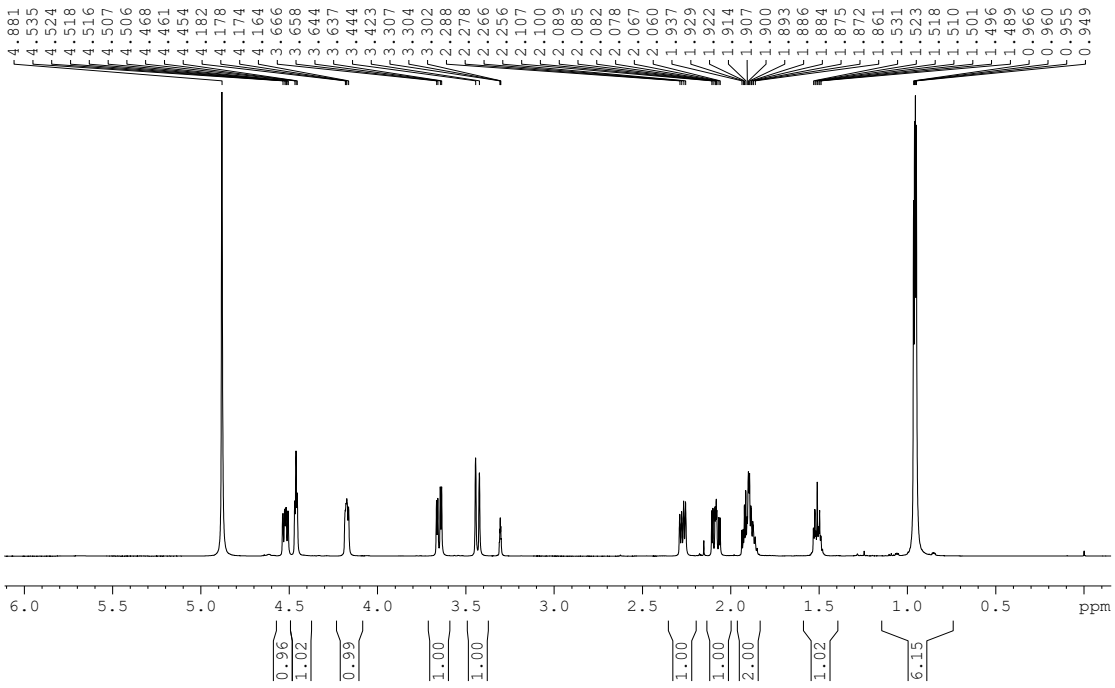

Figure S20 ^{1}H-NMR (500 MHz, CD_3OD) spectrum of compound **6**

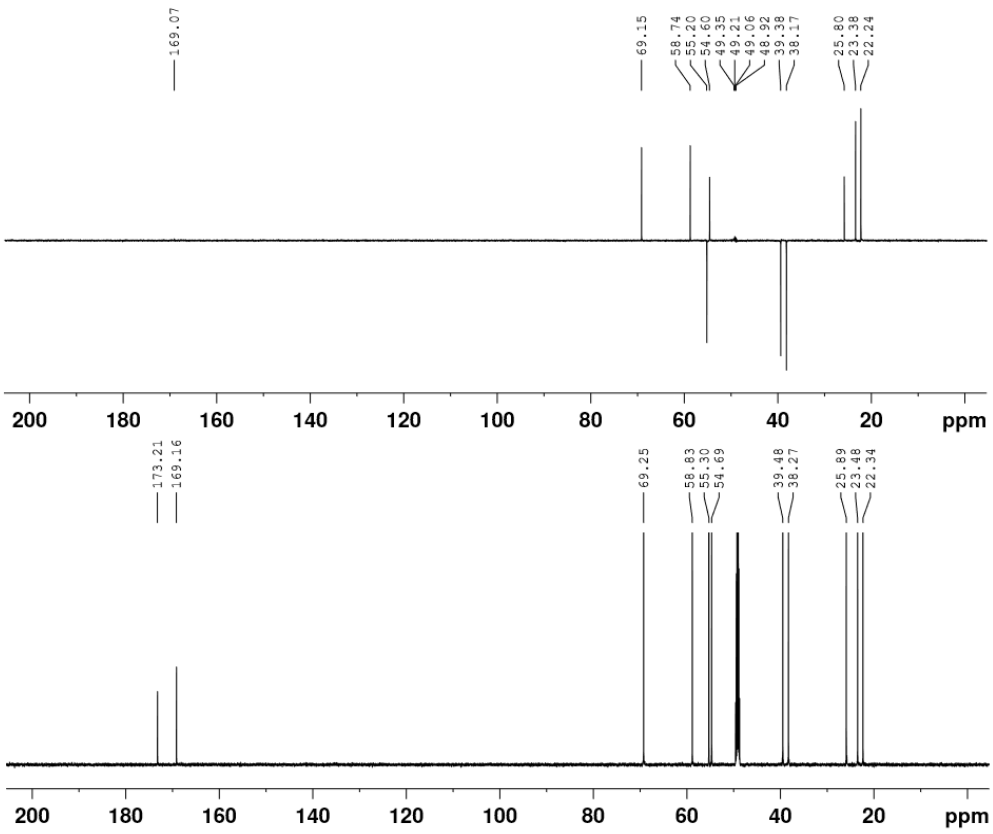

Figure S21 ^{13}C-NMR (125 MHz, CD_3OD) spectrum of compound **6**

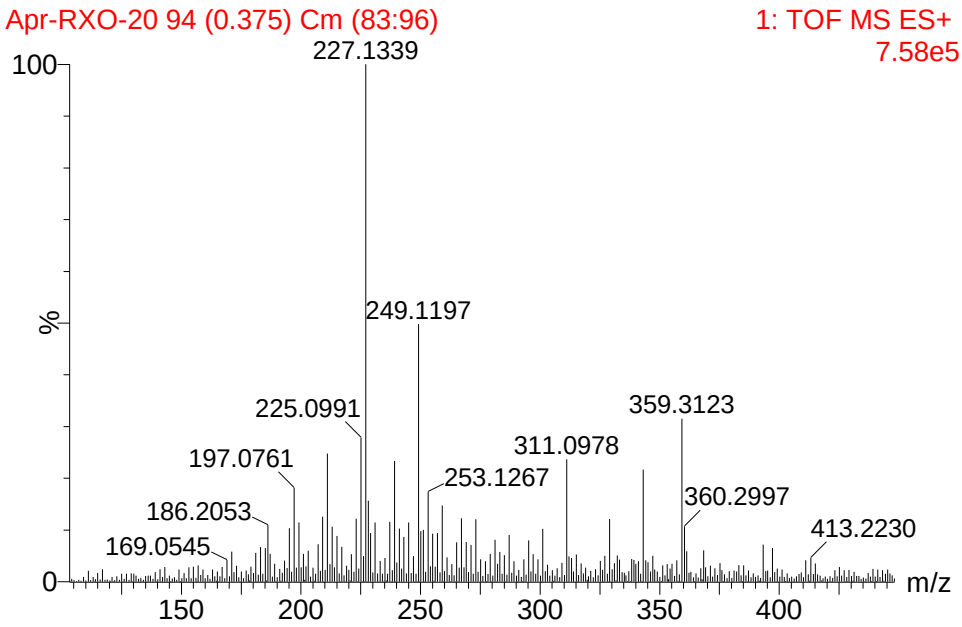

Figure S22 HRESIMS spectrum of compound **6**

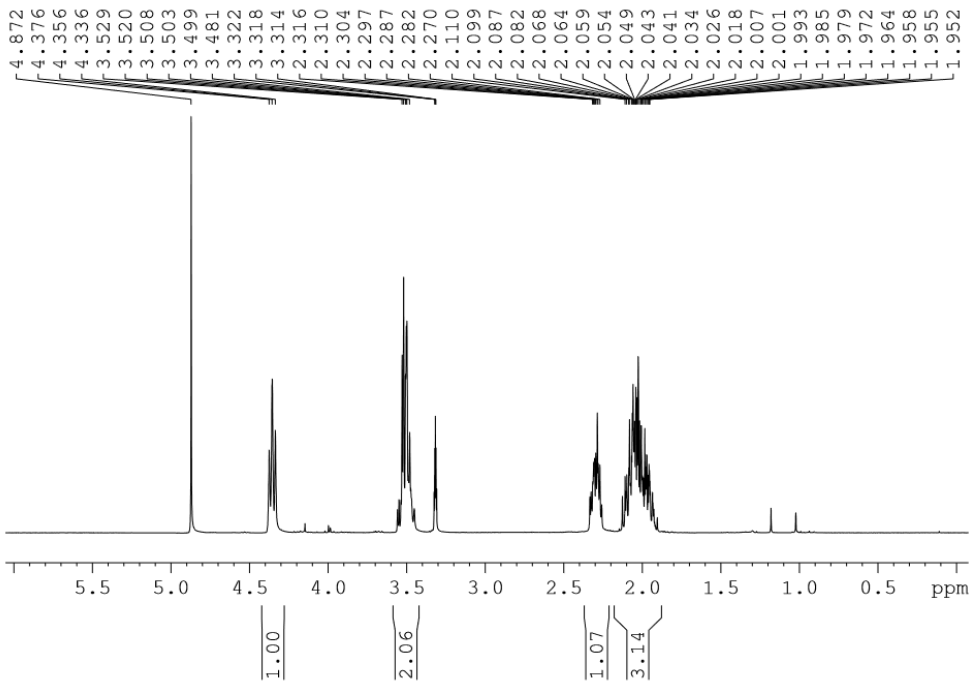

Figure S23 ^{1}H-NMR (500 MHz, CD_3OD) spectrum of compound **7**

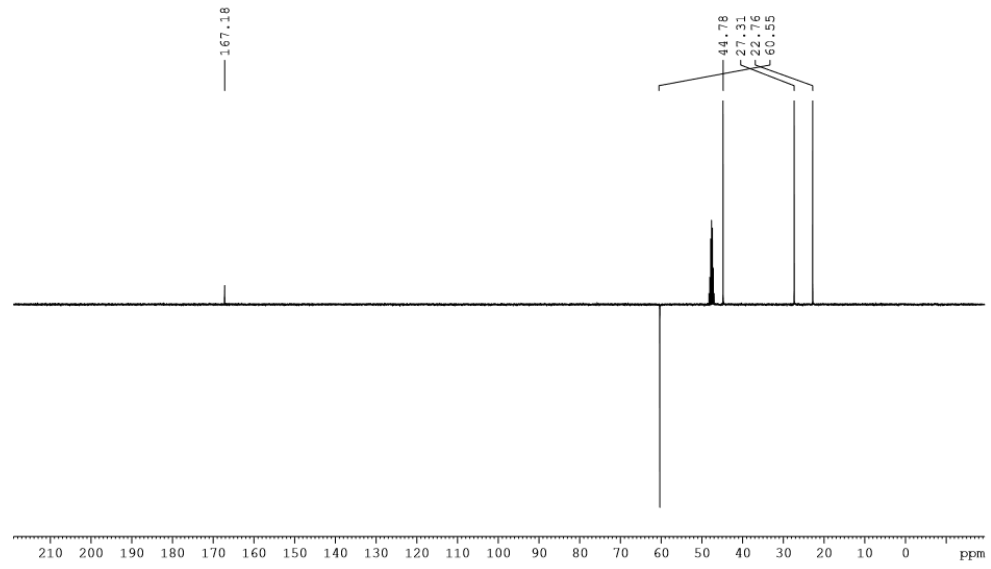

Figure S24 ATP (125 MHz, CD_3OD) spectrum of compound **7**

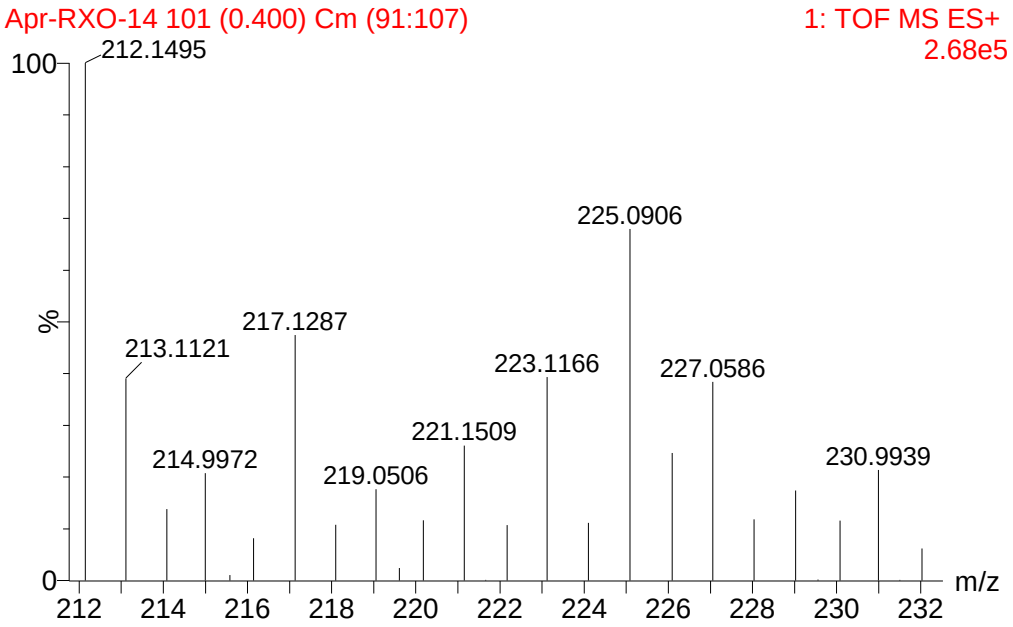

Figure S25 HR-ESIMS spectrum of compound **7**

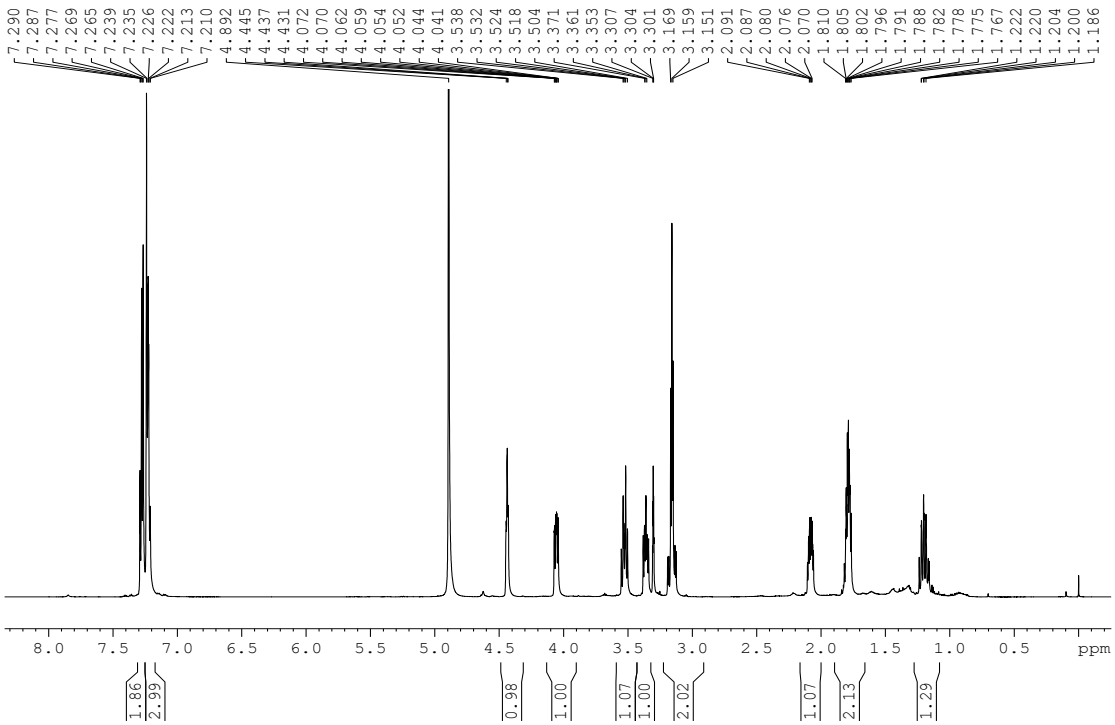

Figure S26 ^{1}H-NMR (500 MHz, CD_3OD) spectrum of compound **8**

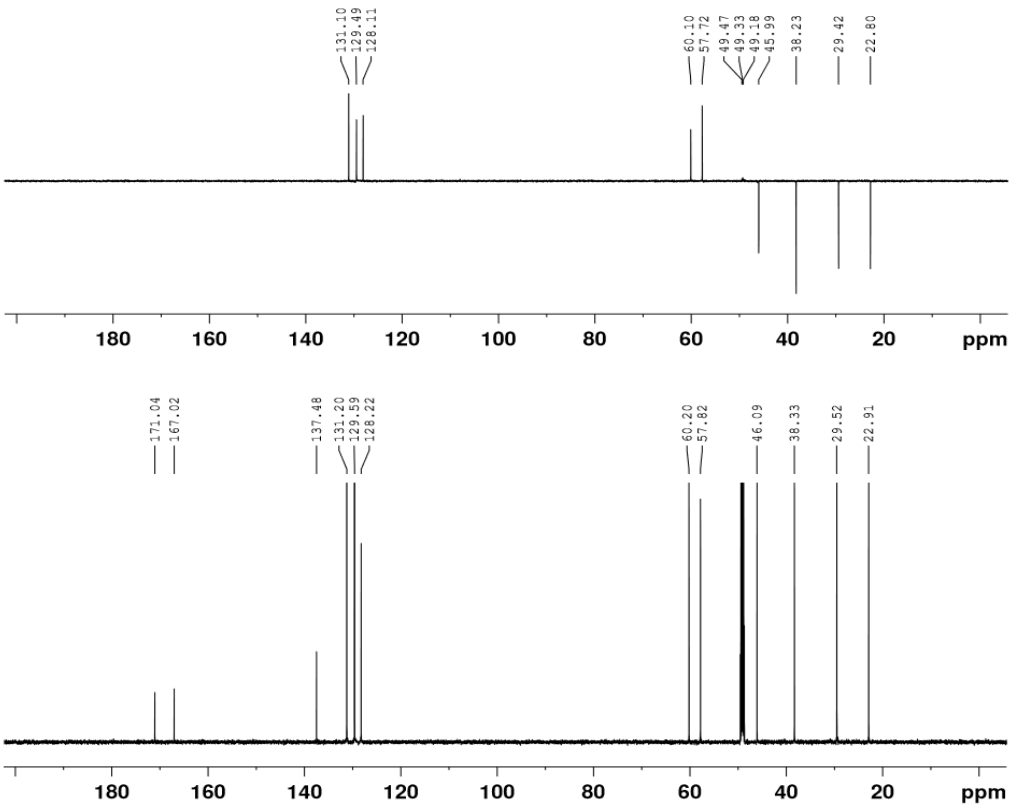

Figure S27 ^{13}C-NMR (125 MHz, CD_3OD) spectrum of compound **8**

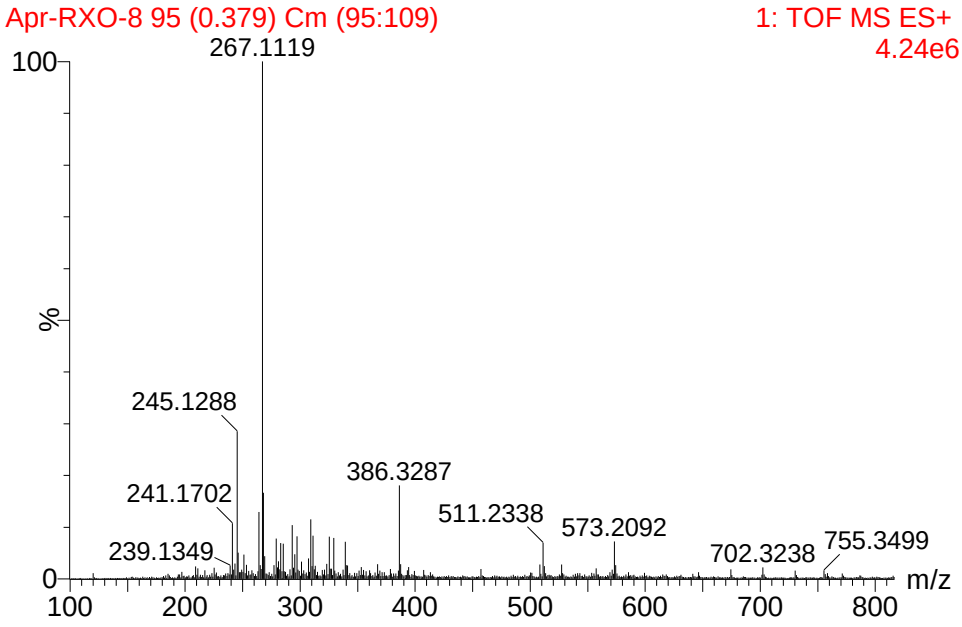

Figure S28 HRESIMS spectrum of compound **8**

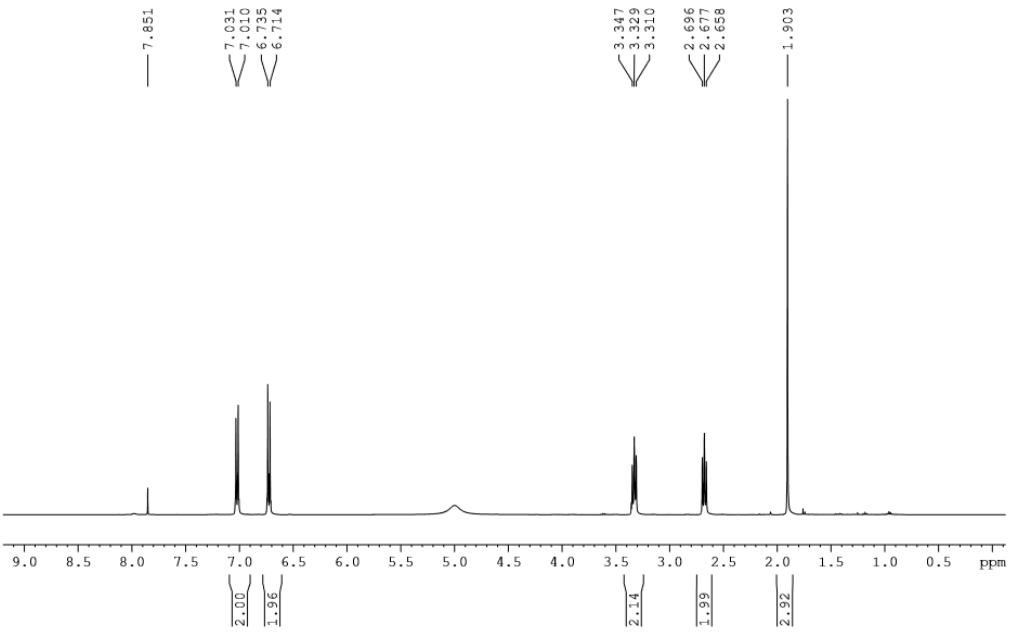

Figure S29 [1]H-NMR (500 MHz, CD_3OD) spectrum of compound **9**

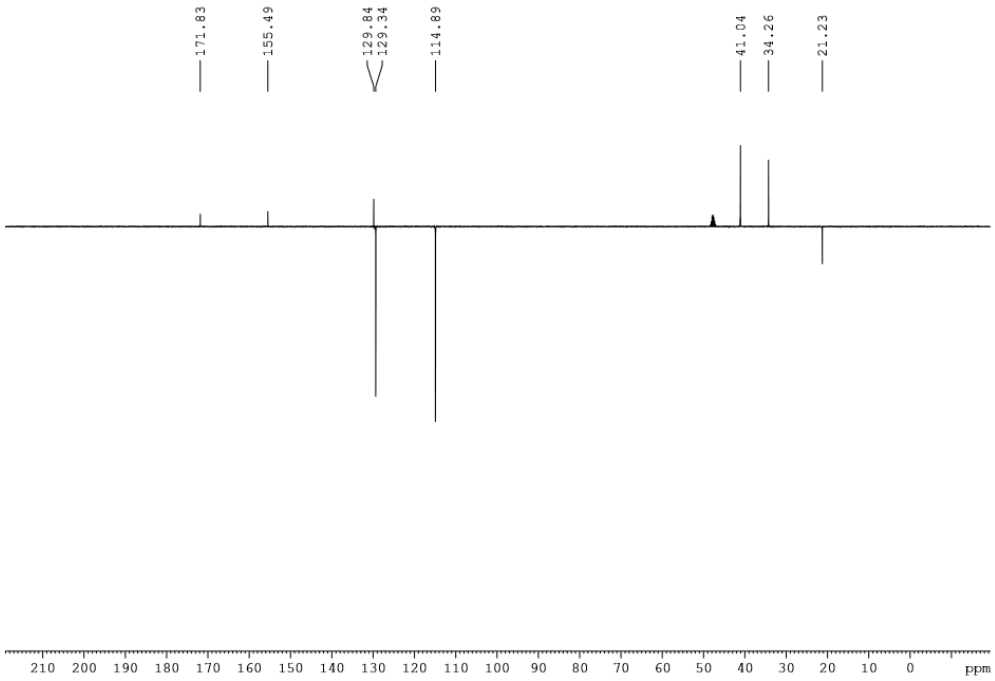

Figure S30 ATP (125 MHz, CD_3OD) spectrum of compound **9**

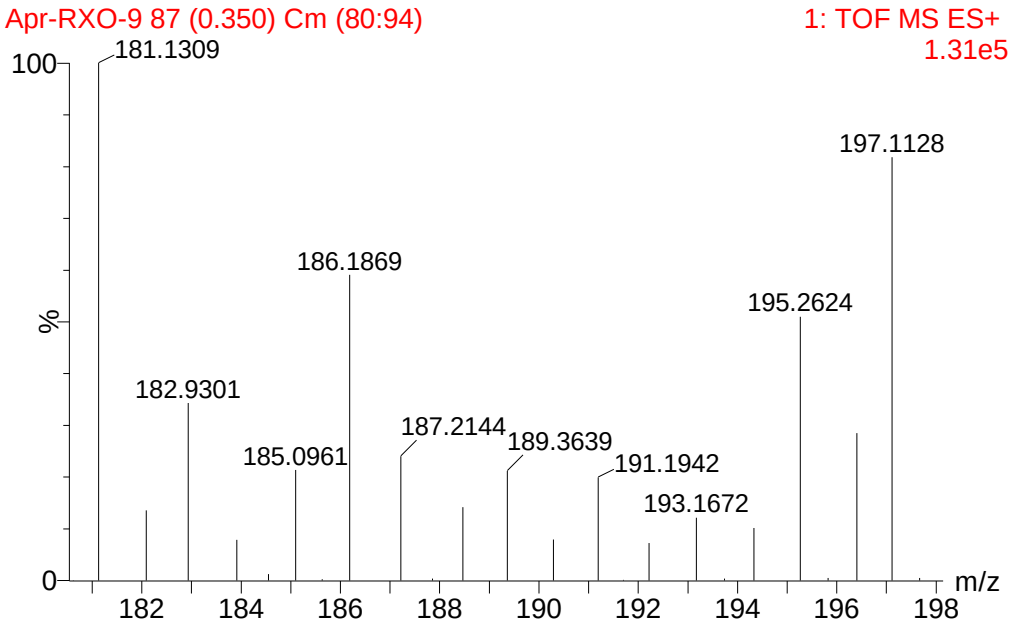

Figure S31 HRESIMS spectrum of compound **9**

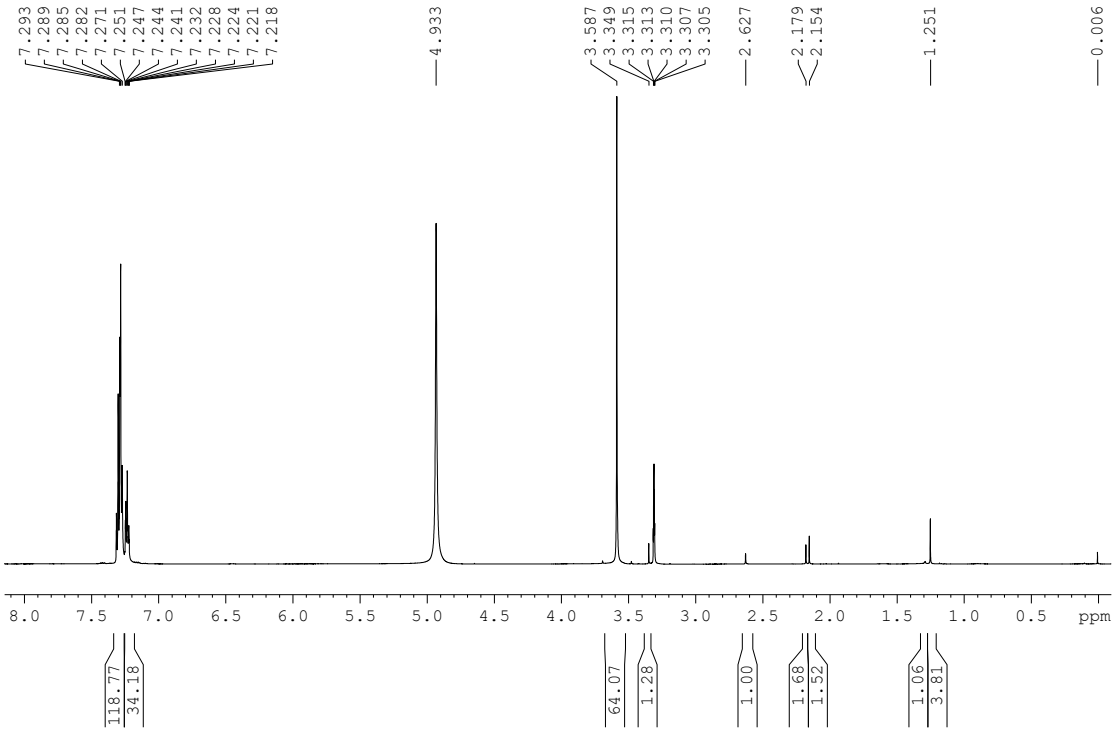

Figure S32 ^{1}H-NMR (500 MHz, CD_3OD) spectrum of compound **10**

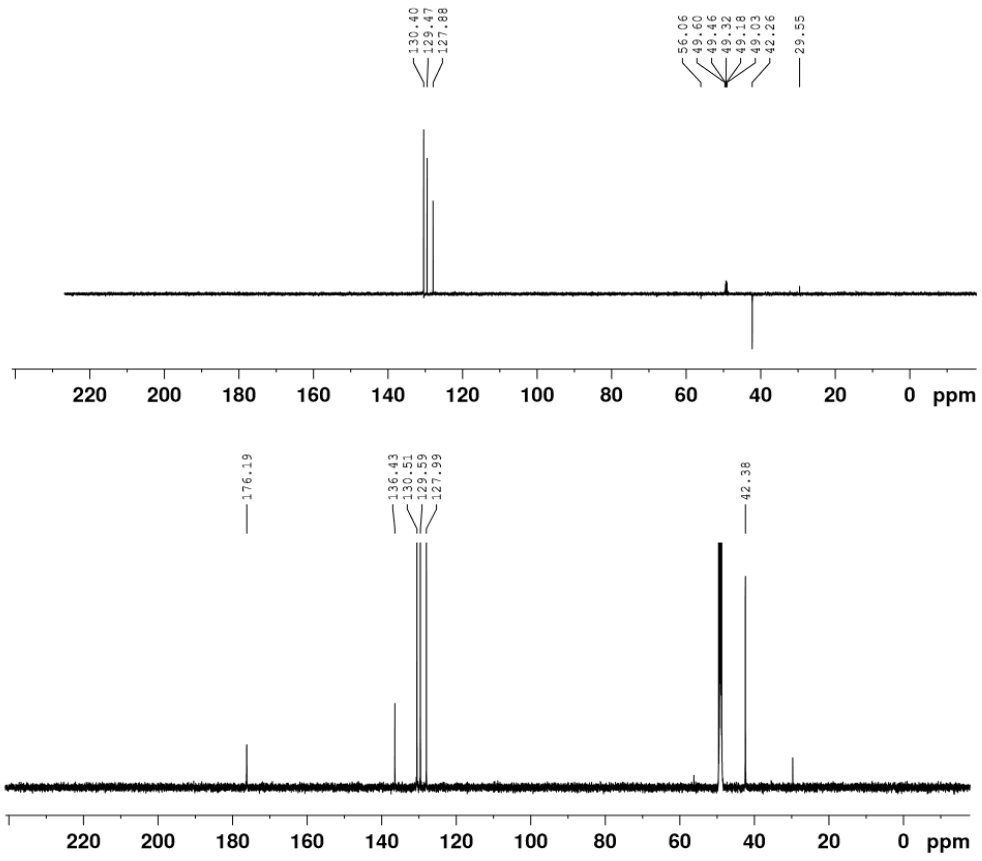

Figure S33 ^{13}C-NMR (125 MHz, CD_3OD) spectrum of compound **10**

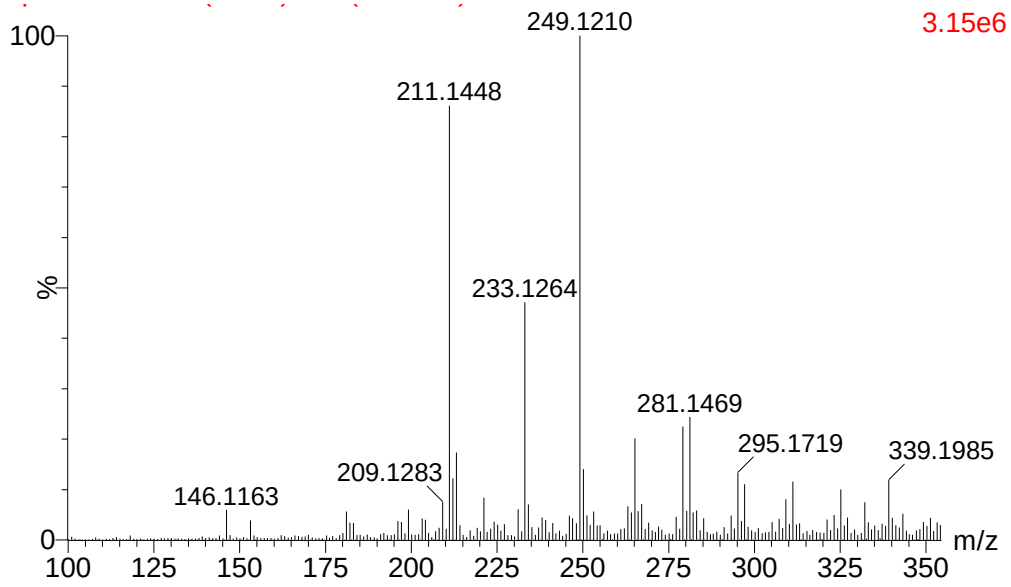

Figure S34 HRESIMS spectrum of compound **10**

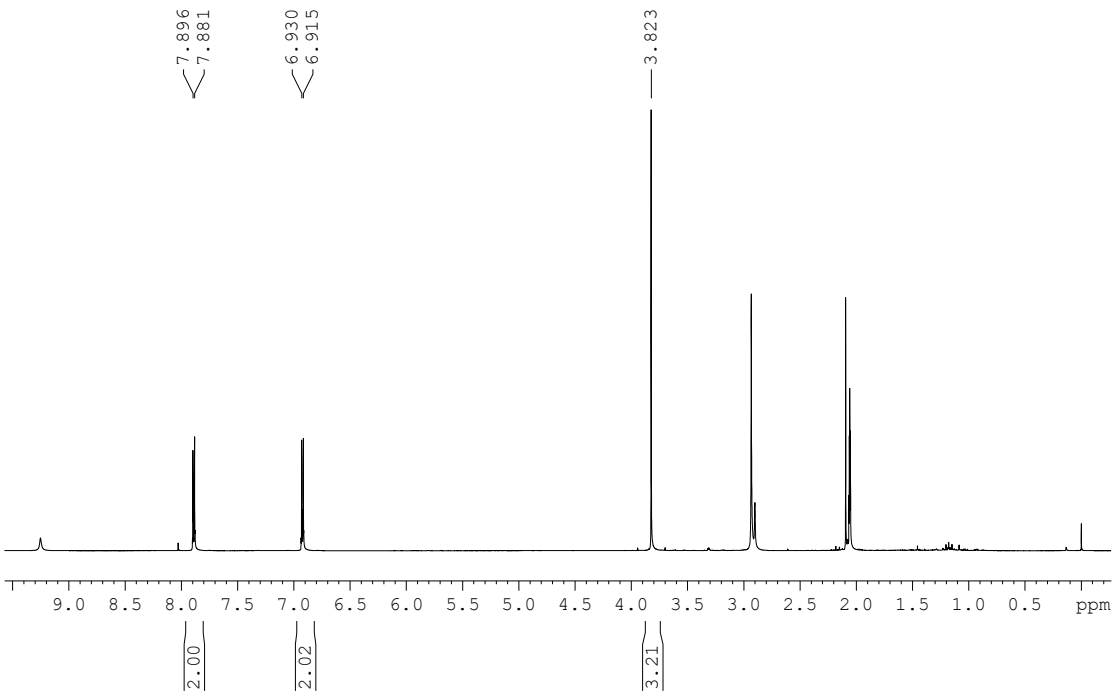

Figure S35 ^{1}H-NMR (500 MHz, CD_3OD) spectrum of compound **11**

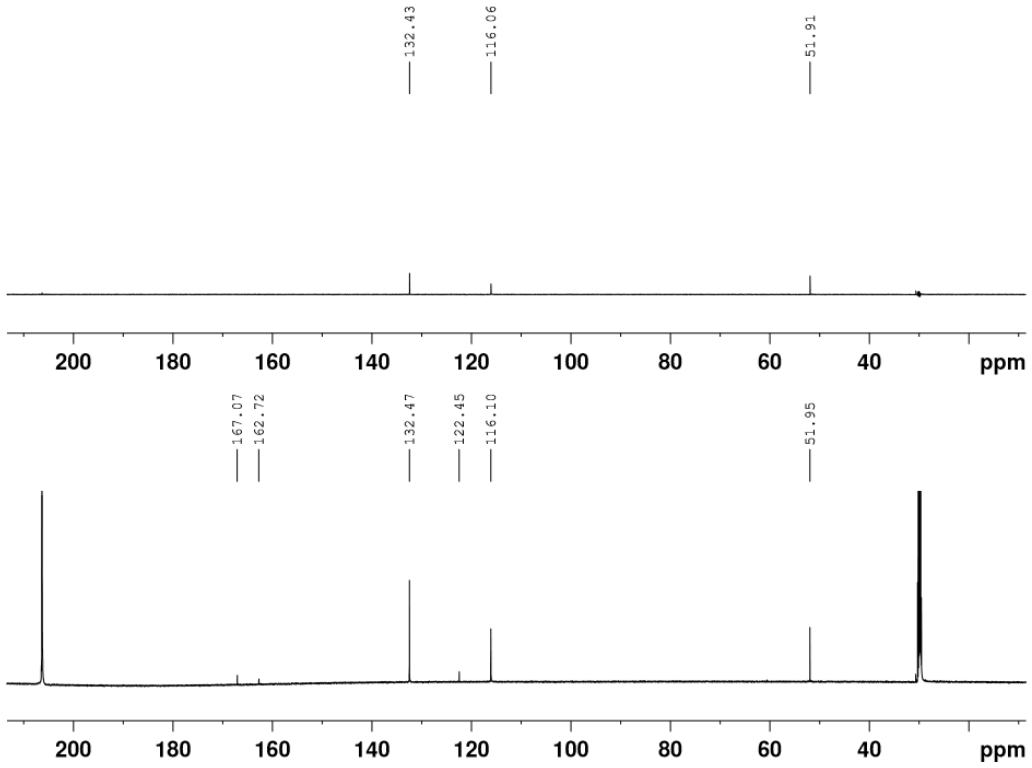

Figure S36 ^{13}C-NMR (125 MHz, CD_3OD) spectrum of compound **11**

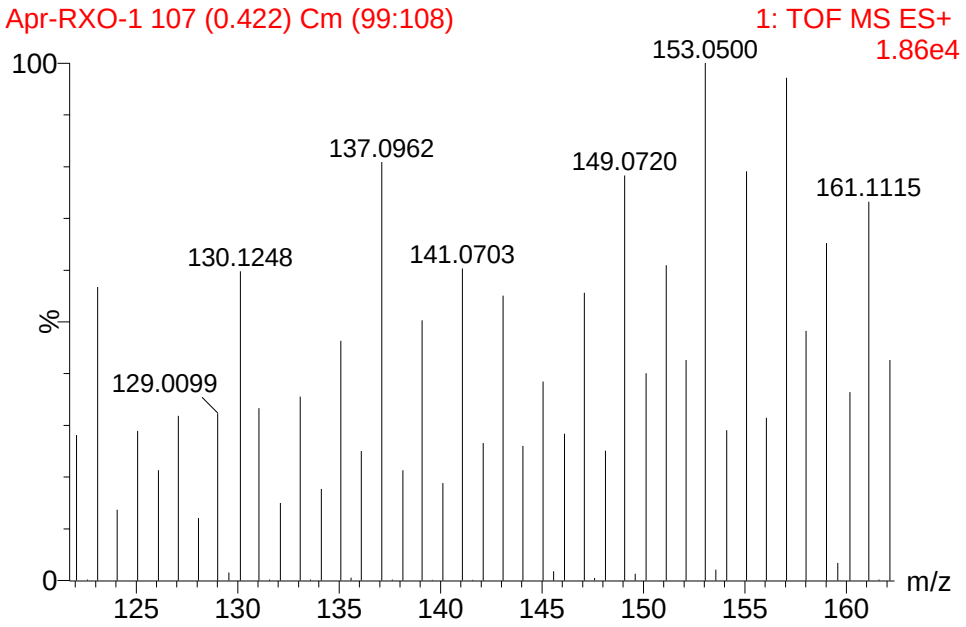

Figure S37 HR-ESIMS spectrum of compound **11**

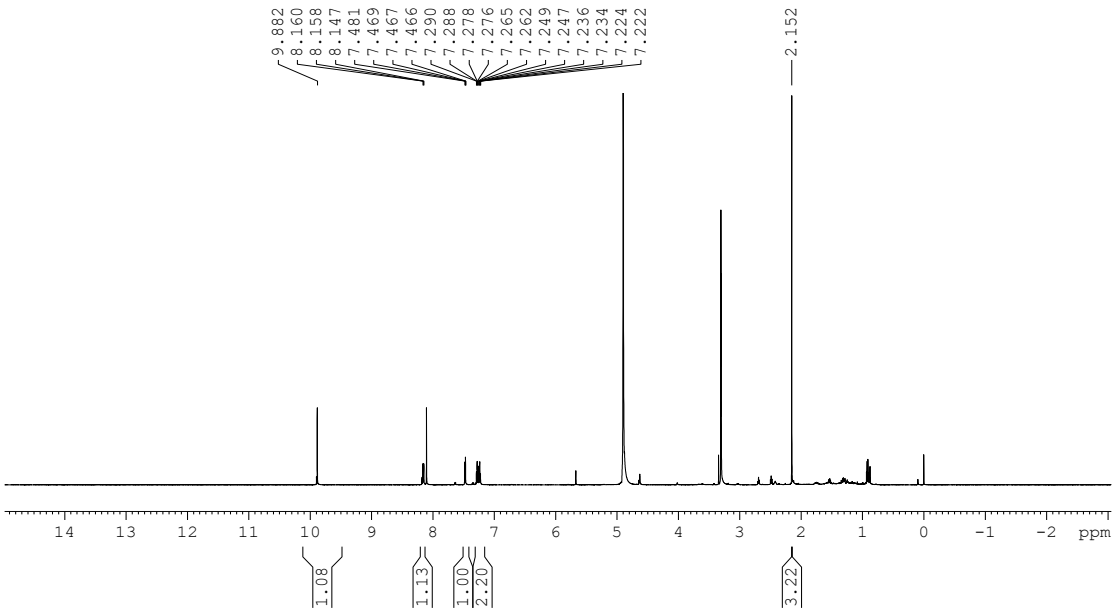

Figure S38 ^{1}H-NMR (500 MHz, CD_3OD) spectrum of compound **12**

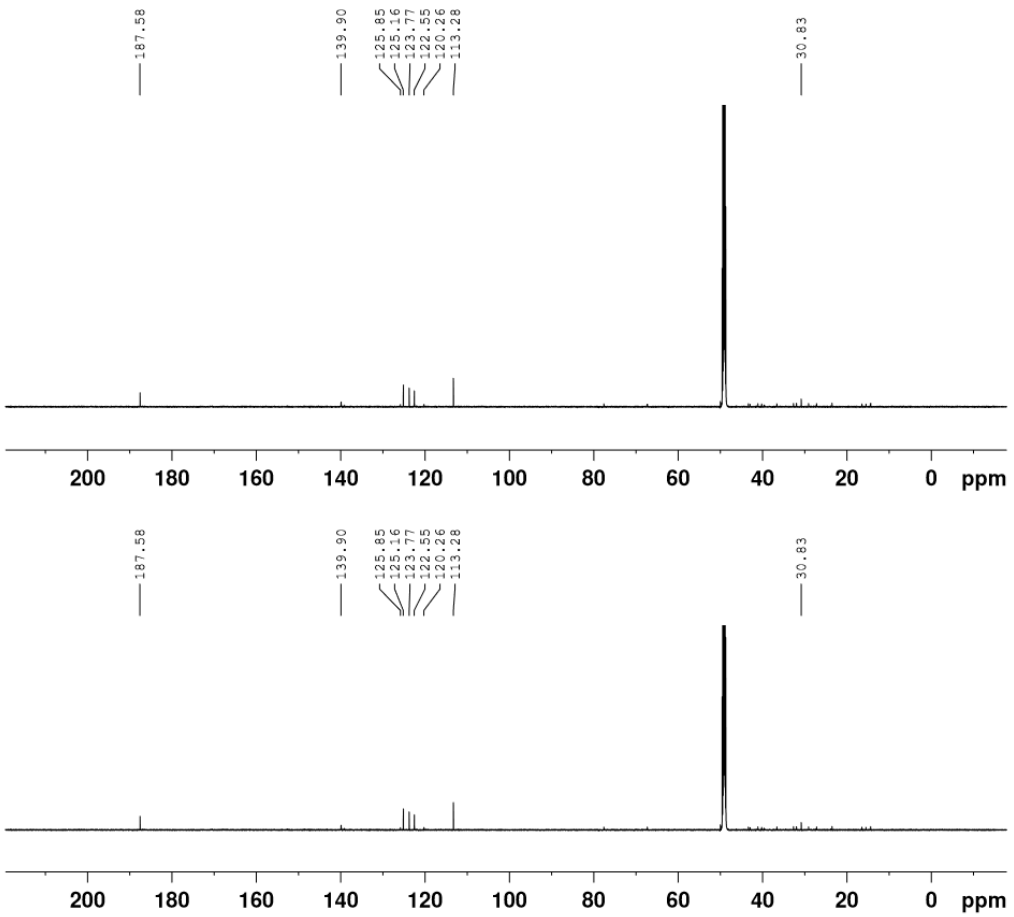

Figure S39 ^{13}C-NMR (125 MHz, CD_3OD) spectrum of compound **12**

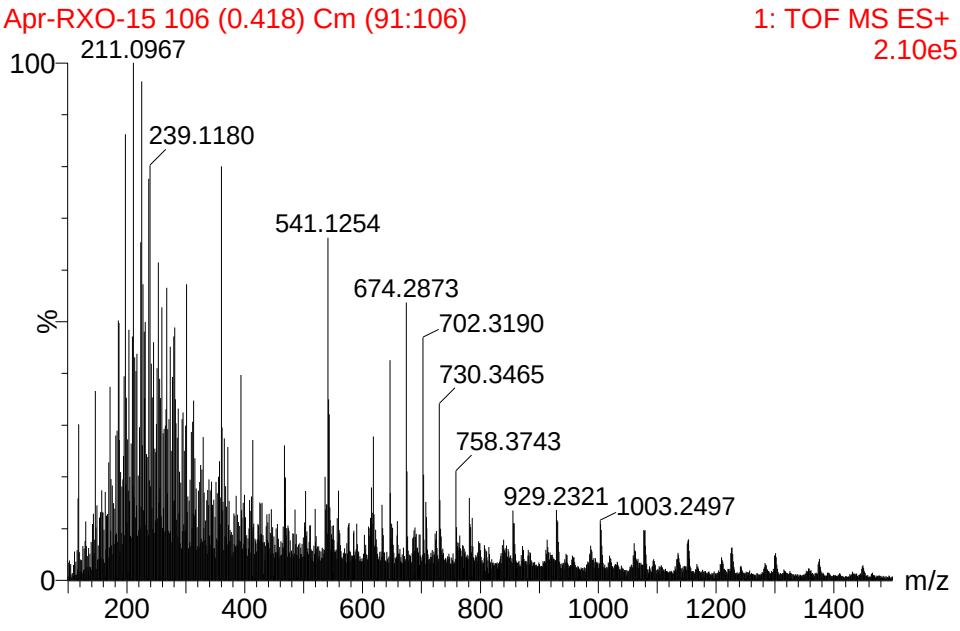

Figure S40 HRESIMS spectrum of compound **12**

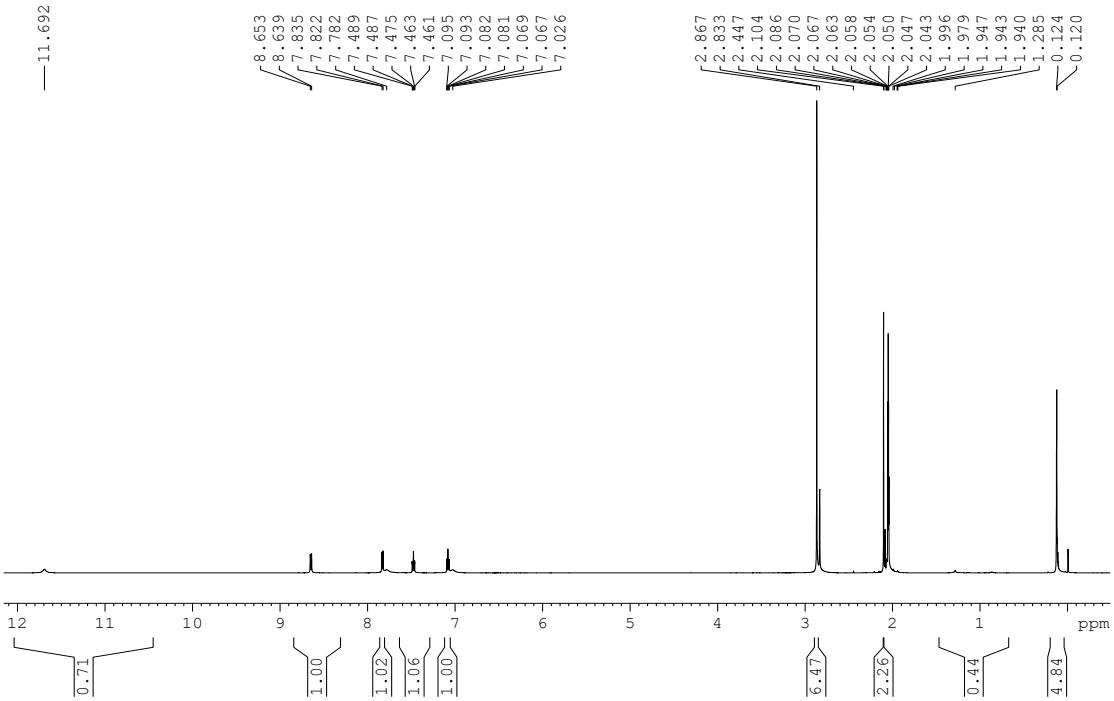

Figure S41 ^{1}H-NMR (500 MHz, CD_3OD) spectrum of compound **13**

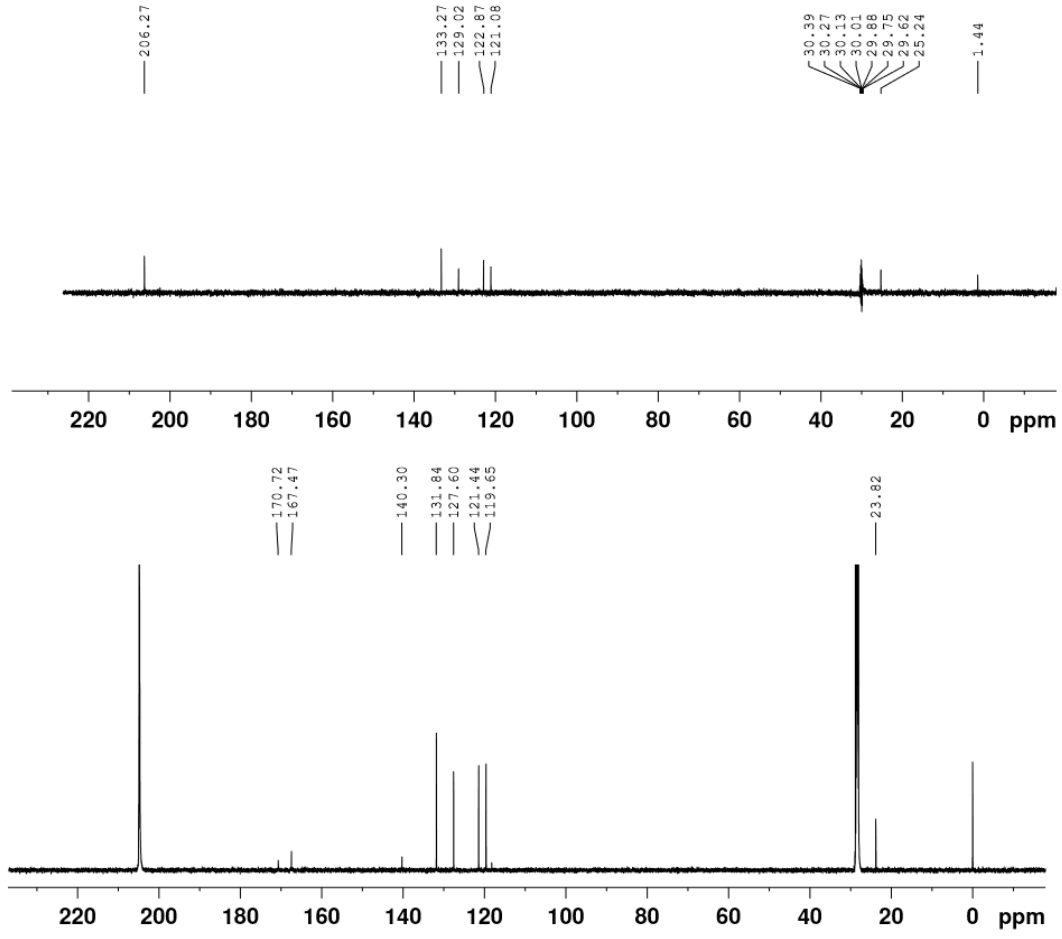

Figure S42 ^{13}C-NMR (125 MHz, CD_3OD) spectrum of compound **13**

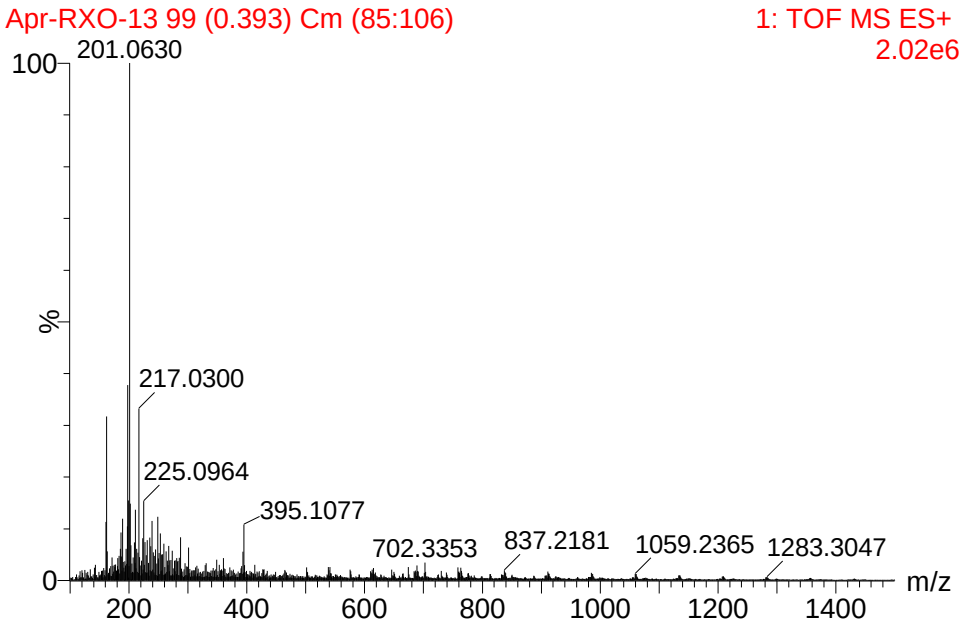

Figure S43 HRESIMS spectrum of compound **13**

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