# SUPPORTING INFORMATION

## Juniperolide A: A New Polyketide Isolated from a Terrestrial Actinomycete, *Streptomyces* sp.

Ritesh Raju, Oleksandr Gromyko,Viktor Fedorenko, Andriy Luzhetskyy, Alberto Plaza and Rolf Müller*

*Department of Microbial Natural Products, Helmholtz-Institute for Pharmaceutical Research Saarland (HIPS), Helmholtz Centre for Infection Research (HZI) and Pharmaceutical Biotechnology, Saarland University, Campus C2 3, 66123 Saarbrücken, Germany and Department of Genetics and Biotechnology of Ivan Franko National University of L'viv, Grushevskogo st. 4, L'viv 79005, Ukraine*

## Experimental Section

### General

Chiroptical measurements ($[\alpha]_D$) were obtained on a Perkin Elmer (Model 341) polarimeter in a 100 × 2mm cell at 20°C. UV spectrum was recorded on an Agilent 8453 spectrophotometer. NMR spectra were obtained on a Bruker Ascend 500 and 700 MHz spectrometer equipped with a cryoprobe system (Bruker Biospin GmbH, Germany), in the solvents indicated and referenced to residual $^1$H signals in deuterated solvents. (ESI-MS) were acquired using an Agilent 1100 Series separations module equipped with an Agilent 1100 Series LC/MSD mass detector in both positive and negative ion modes under the following conditions (Zorbax $C_8$ column, 150 × 4.6 mm, eluting with 0.4 mL/min 95% $H_2O$/MeCN to 5% $H_2O$/MeCN (with isocratic 0.01% TFA) over 22 mins, then held for 5 min. HRMS was carried out using an UltiMate 3000$^{TM}$ rapid separation liquid chromatography system (Dionex RSLC) coupled to an UHR-TOF mass spectrometer (Bruker Daltonik maxis) operating in the positive ESI mode.

### Isolation and Identification of strain 1-48

Sampling was performed in Crimean Mountains (Ukraine) at the base of Mount Kishka. The soil was collected from the rhizosphere of *Juniperus excelsa* and resuspended in sterile water (20 ml). An oatmeal agar was used as the isolation medium. To sequence the 16S rDNA the chromosomal DNA of Lv 1-48 was isolated according to the protocol described in Kieser et al (2000). Based on the 16S rDNA sequence analysis strain Lv 1-48 was classified to belong to the genus *Streptomyces.*

## 16S rRNA gene sequence

CACGTAGTTAGCCGGCGCTTCTTCTGCAGGTACCGTCCTTTCGCTTCTTCCCTGCTGAAAGAGGTTTACAACCCGAAGGCCGTCATCCCTCACGCGGCGTCGCTGC
ATCAGGCTTTCGCCCATTGTGCAATATTCCCCACTGCTGCCTCCCGTAGGAGTCTGGGCCGTGTCTCAGTCCCAGTGTGGCCGGTCGCCCTCTCAGGCCGGCTACC
CGTCGTCGCCTTGGTAGGCCATTACCCCACCAACAAGCTGATAGGCCGCGGGCTCATCCTTCACCGCCGGAGCTTTTAACCTCTTCAGATGCCTGAAGAAGTGTTA
TCCGGTATTAGACCCCGTTTCCAGGGCTTGTCCCAGAGTGAAGGGCAGATTGCCCACGTGTTACTCACCCGTTCGCCACTAATCCACCCCGAAGGGCTTCATCGTT
CGACTTGCATGTGTTAAGCACGCCGCCAGCGTTCGTCCTGAGCCAGGATCAAAA

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LOCUS       NR_043353               1494 bp    rRNA    linear   BCT 10-AUG-2011
DEFINITION  Streptomyces chryseus strain NRRL B-12347 16S ribosomal RNA,
            partial sequence.
ACCESSION   NR_043353
VERSION     NR_043353.1  GI:343202860
DBLINK      Project: 33175
            BioProject: PRJNA33175
KEYWORDS    .
SOURCE      Streptomyces chryseus
  ORGANISM  Streptomyces chryseus
            Bacteria; Actinobacteria; Actinobacteridae; Actinomycetales;
            Streptomycineae; Streptomycetaceae; Streptomyces.
REFERENCE   1  (bases 1 to 1494)
  AUTHORS   Goodfellow,M., Labeda,D.P., Liu,Z. and Swings,J.
  TITLE     Finally a phylogeny for Streptomyces
  JOURNAL   Unpublished
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**Analytical Cultivation and Chemical Profiling**

Strain Lv 1-48 was cultivated in (250 mL) Schott flask containing M1 (1% starch, 0.4% yeast extract, and 0.2% peptone) prepared in distilled water (80 mL). The strains were shaken at 160 rpm for 8 d at 30 °C, extracted with EtOAc (50 mL), and the organic phase concentrated *in vacuo* to yield a crude extract of 5.6 mg. The crude extracts were redissolved in MeOH generating a concentration of 1 $mgmL^{-1}$ and analysed by HPLC-DAD-ESI(±)MS.

**Preparative Cultivation and Isolation**

Six 5 L Erlenymeyer flasks containing M1 broth (1.2 L) were inoculated with starter culture (20 mL) of *Streptomyces* sp. The flasks were incubated at 30 °C on a rotary shaker at 160 rpm for 8 d, extracted with EtOAc (2 × 500 mL per flask), and the organic phases concentrated *in vacuo* to yield a combined EtOAc extract (197.8 mg). The EtOAc extract was sequentially triturated with hexane, $CH_2Cl_2$ and MeOH (50 mL aliquots), which were concentrated *in vacuo*, to yield 29.5 mg, 138.9 mg and 9.8 mg partitions respectively. The $CH_2Cl_2$ soluble material was further fractionated by HPLC (Zorbax, $C_8$ column, 250 × 9.4 mm, 5 μm, 3 mL $min^{-1}$, gradient from 10 – 100% ACN – $H_2O$ over 45 min) to afford Juniperolide A (**1**) ($t_R$ = 28.2 min, 2.6 mg).

**Juniperolide A (1)**: clear oil; $[\alpha]_D$ +7.0 (*c* 0.2, MeOH); UV (MeOH) $\lambda_{max}$ (log ε) 240 (4.69), ; NMR (500 MHz, MeOH-$d_4$) see Table 1; HRESI(+)MS *m/z* 732.4648 (calcd for $C_{39}H_{67}NO_{10}Na$, 732.4663)

## Juniperolide A Mosher analysis

A solution of **1** (2.0 mg, 2.8 μmol), (*S*)-MTPA (4.6 mg, 19.6 μmol), DCC (3.5 mg, 17.0 μmol) and DMAP (1.4 mg, 11.4 μmol) in dry $CH_2Cl_2$ (1 mL) was stirred at room temperature for 18 h. A second reaction was performed in an analogous manner using (*R*)-MTPA in place of (*S*)-MTPA. The products were semi-purified by HPLC (Phenomenex, Synergi 4μm, Fusion-RP, 250 × 10 mm, gradient from 10 – 100% ACN – $H_2O$ over 30 mins with a hold at 100% ACN for 20 mins) to afford juniperolide A (*S*)-Mosher ester (**1a**) ($t_R$ = 37.6 , 0.7 mg) and juniperolide A (*R*)-Mosher ester (**1b**) ($t_R$ = 38.0 , 0.9 mg). (Note: A repeated batch fermentation (6 L) and isolation of juniperolide A was performed to generate juniperolide A (*R*)-Mosher ester (1b)) under similar conditions.)

## Biological assays

**Cytotoxic activity:** Human HCT-116 colon carcinoma cells (DSMZ, ACC 581) were seeded at 1.2 x $10^4$ cells per well of 96-well plates (Corning, CellBind) in 180 μl complete medium and directly treated with compound **1** at 1 and 10 μg/ml to assess acute cytotoxicity. After 2 d incubation, 20 μl of 5 mg/ml MTT (thiazolyl blue tetrazolium bromide) in PBS was added per well and it was further incubated for 2 h at 37°C. The medium was then discarded and cells were washed with 100 μl PBS before adding 100 μl 2-propanol/10 N HCl (250:1) in order to dissolve formazan granules. The absorbance at 570 nm was measured using a microplate reader (EL808, Bio-Tek Instruments Inc.), and cell viability was expressed as percentage relative to the respective control. Juniperolide A showed no cytotoxic activity up to a concentration of 10 μg/ml.

**Antimicrobial activity.** All microorganisms were handled under standard conditions recommended by the depositor. Overnight cultures of bacteria were prepared in EBS medium (0.5% peptone casein, 0.5% proteose peptone, 0.1% peptone meat, 0.1% yeast extract; pH 7.0) and of yeast and fungi in Myc medium (1% phytone peptone, 1% glucose, 50 mM HEPES, pH 7.0) by inoculation either from cryocultures or of single colonies on agar plates. The next day, $OD_{600}$ was measured on a photometer. Overnight cultures of microorganisms were diluted to $OD_{600}$ 0.01 (bacteria) or 0.05 (yeast/fungi) in the respective medium. Juniperolide A was tested in a serial dilution starting from 111.1 μg/ml. Growth inhibition was assessed after overnight incubation by visual inspection and $OD_{600}$ measurement.

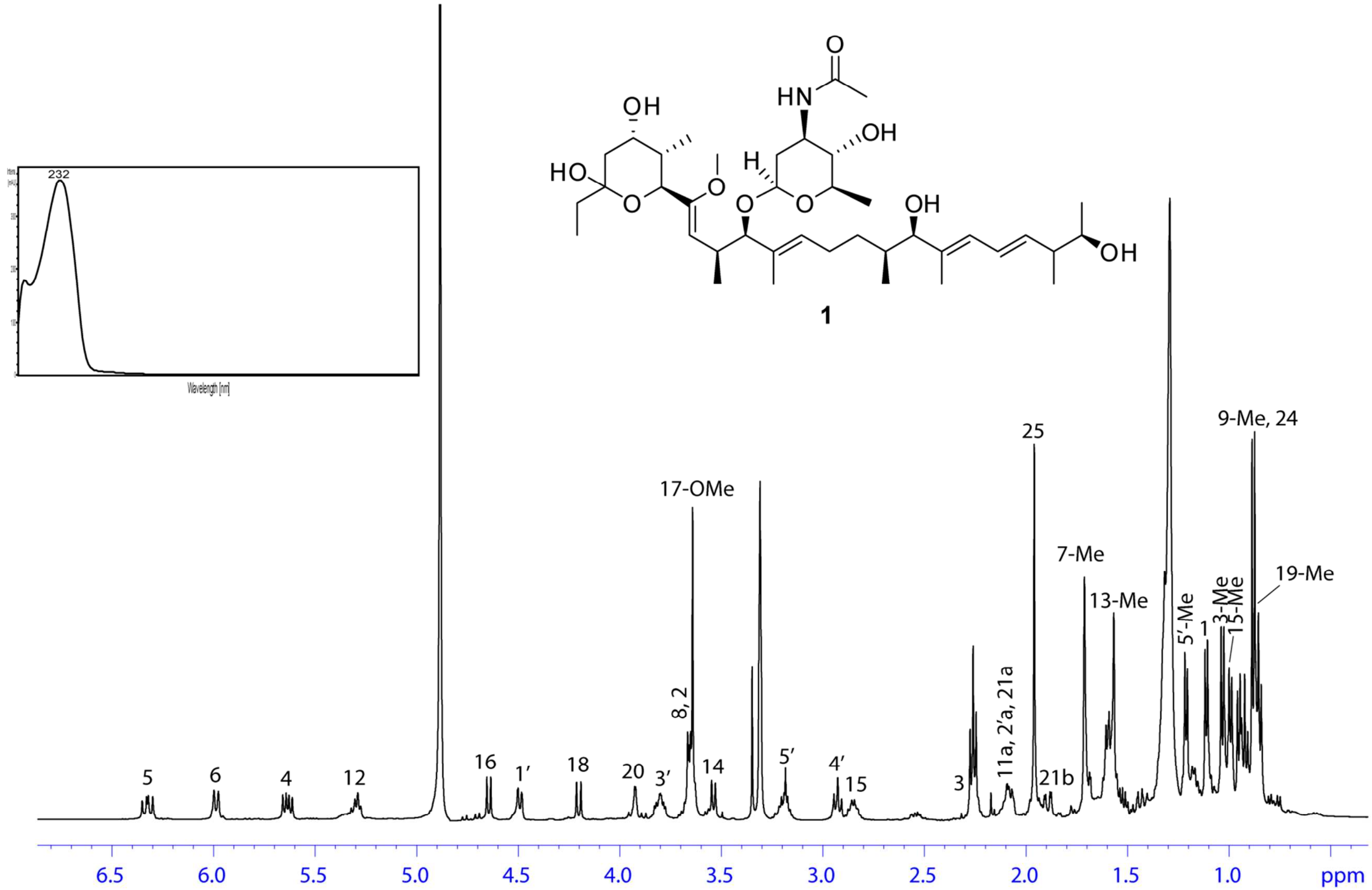
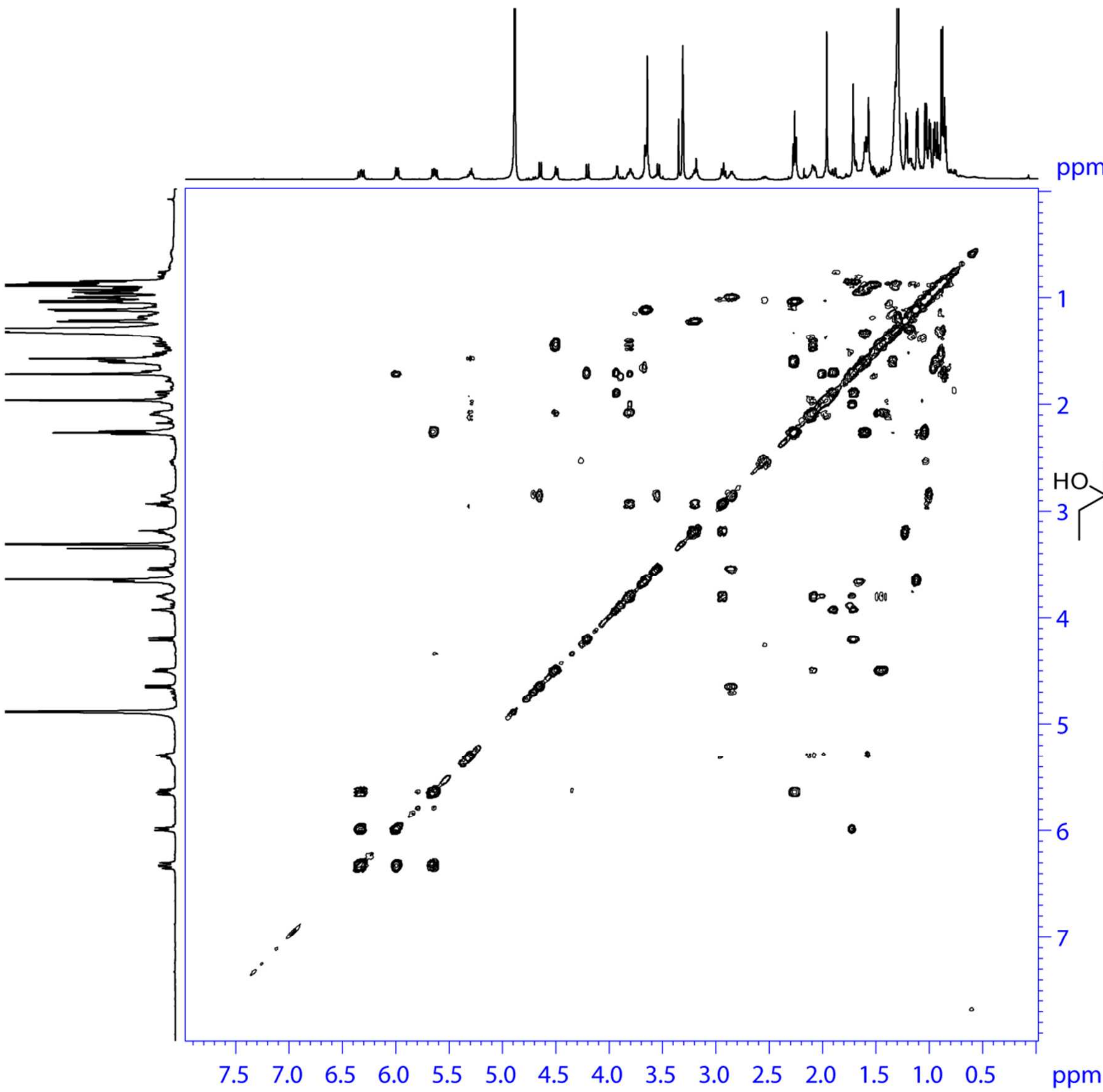
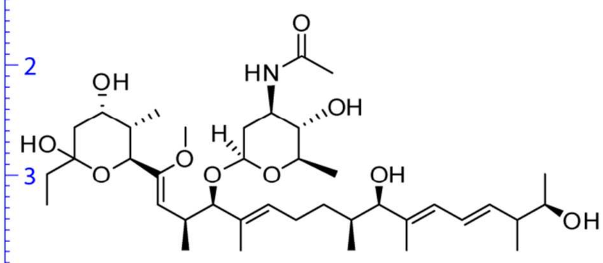



**Figure S1.** $^{1}$H NMR (500 MHz, methanol-$d_4$) and UV-vis (inset) spectra of juniperolide A (**1**)




**1**

**Figure S2.** COSY spectrum (500 MHz, methanol-$d_4$) of juniperolide A (**1**)

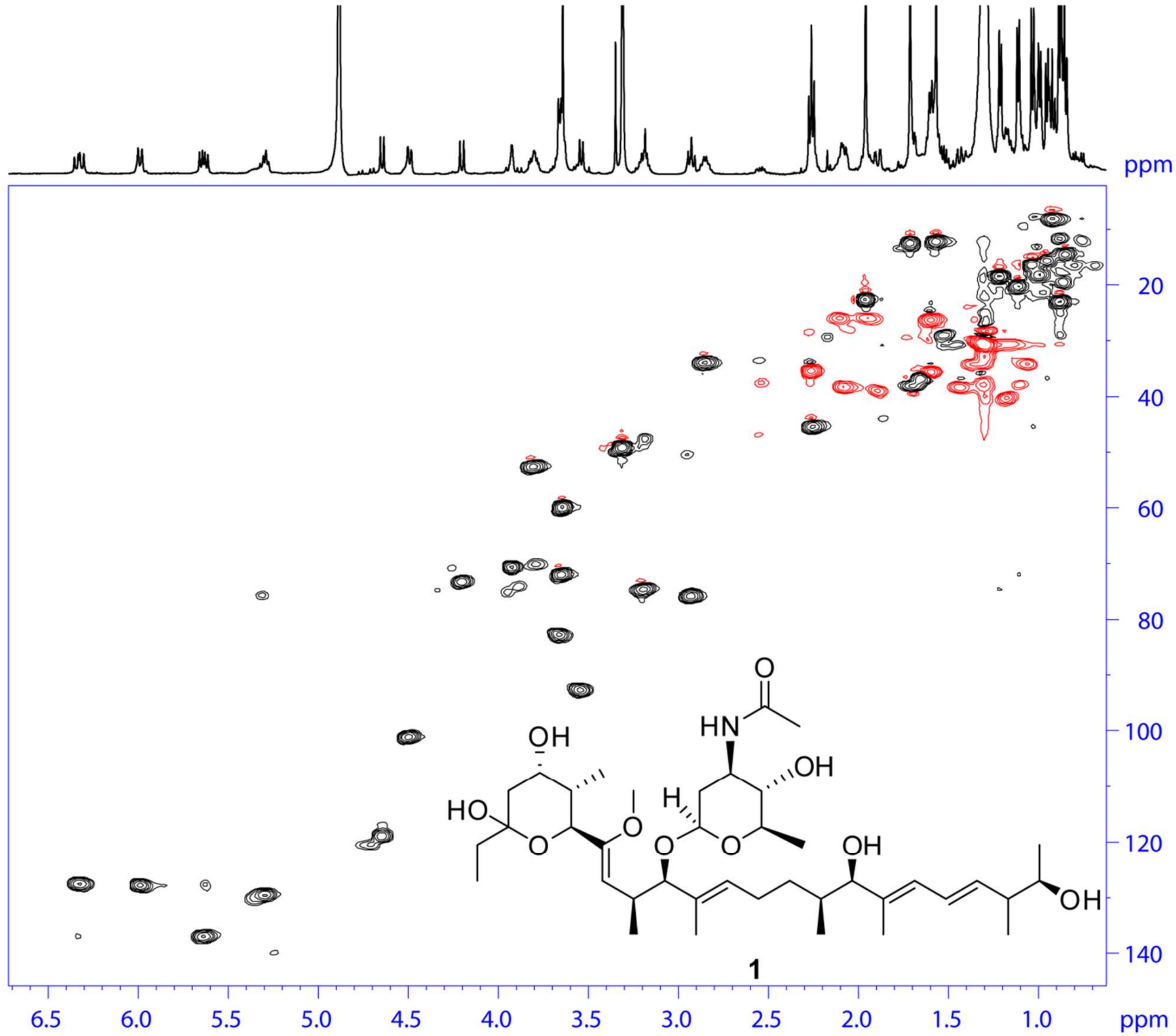
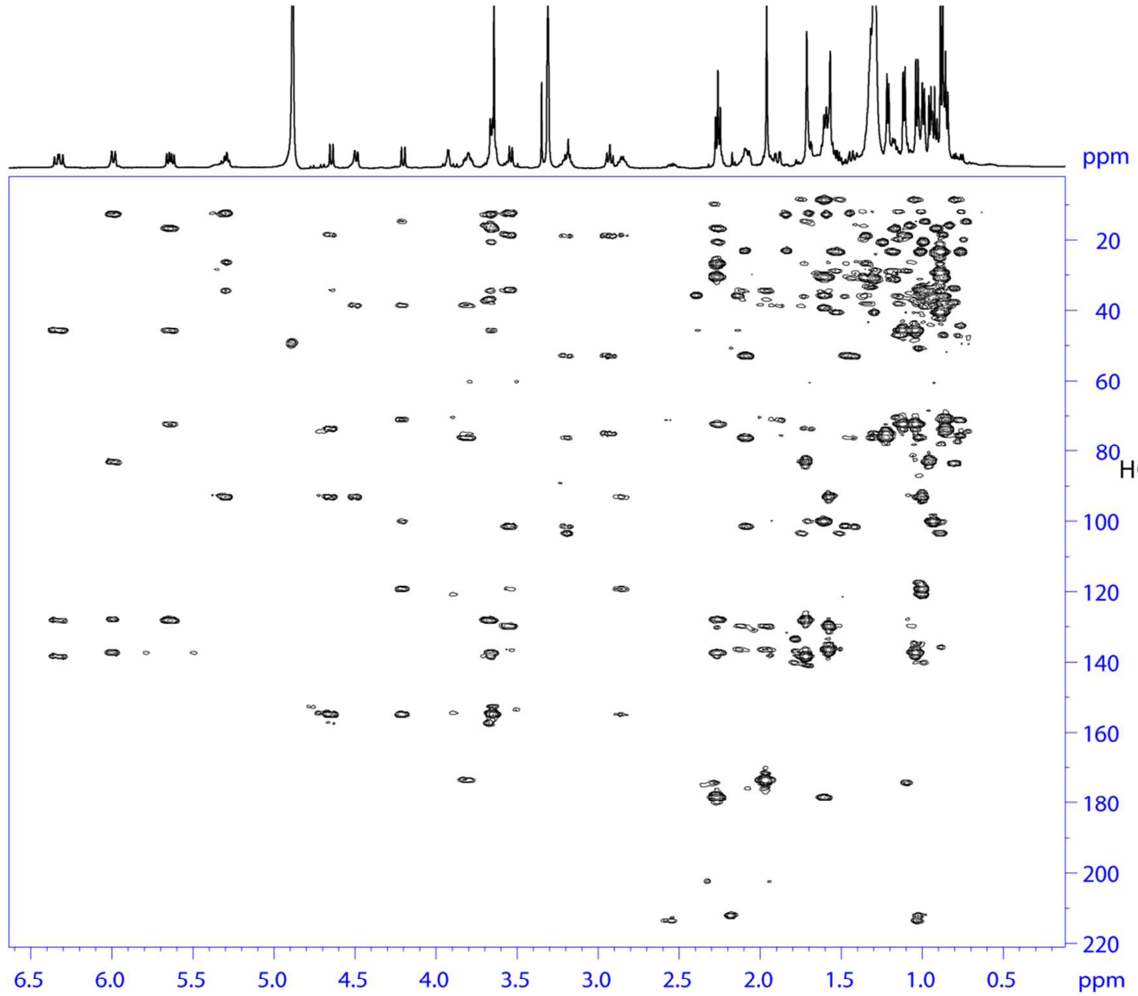
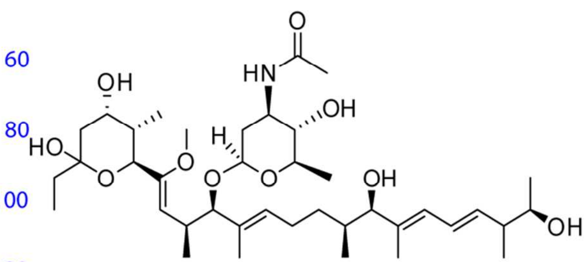


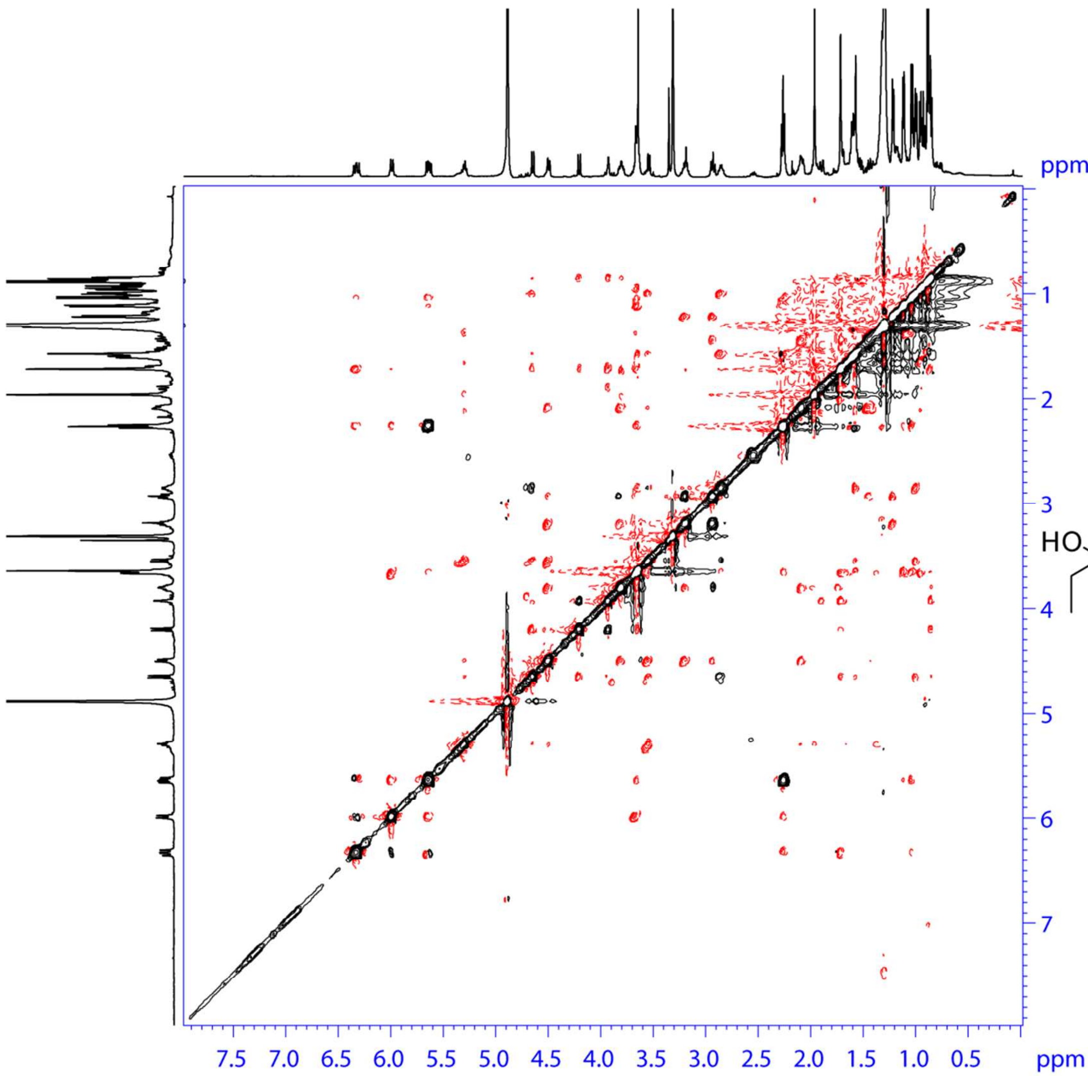

**Figure S3.** $^{1}H - ^{13}C$ HSQC spectrum (500 MHz, methanol-$d_4$) of juniperolide A (**1**)

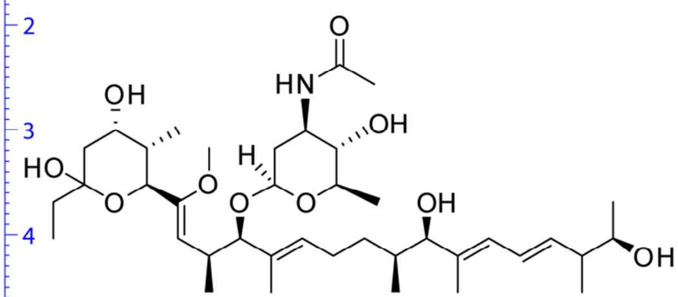



**1**

**Figure S4.** $^{1}H$ – $^{13}C$ HMBC spectrum (500 MHz, methanol-$d_4$) of juniperolide A (**1**)




**1**

**Figure S5.** ROESY spectrum (500 MHz, methanol-$d_4$) of juniperolide A (**1**)

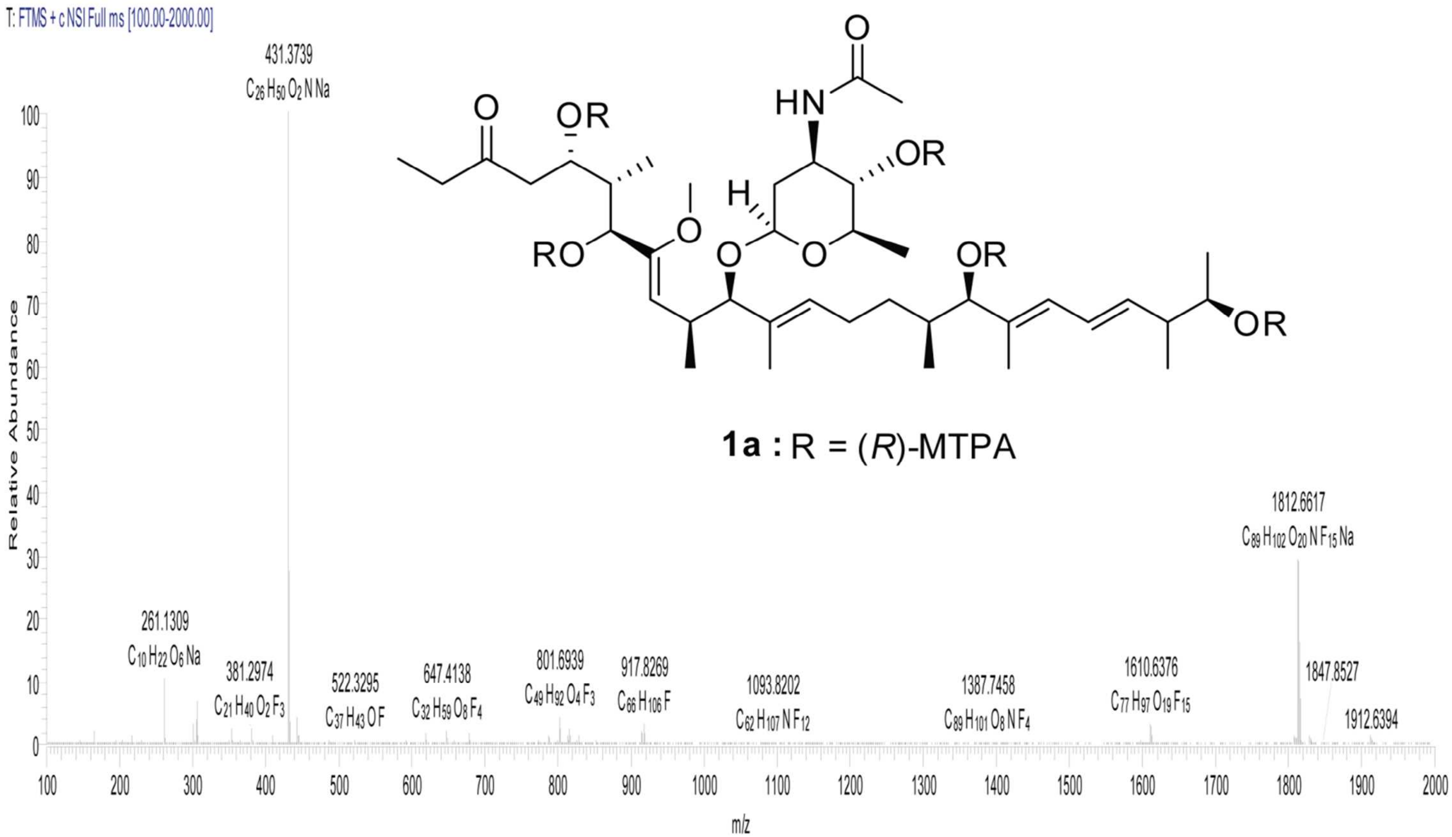



**Figure S6.** HRMS of juniperolide A (*R*)-penta-MTPA ester (**1a**)

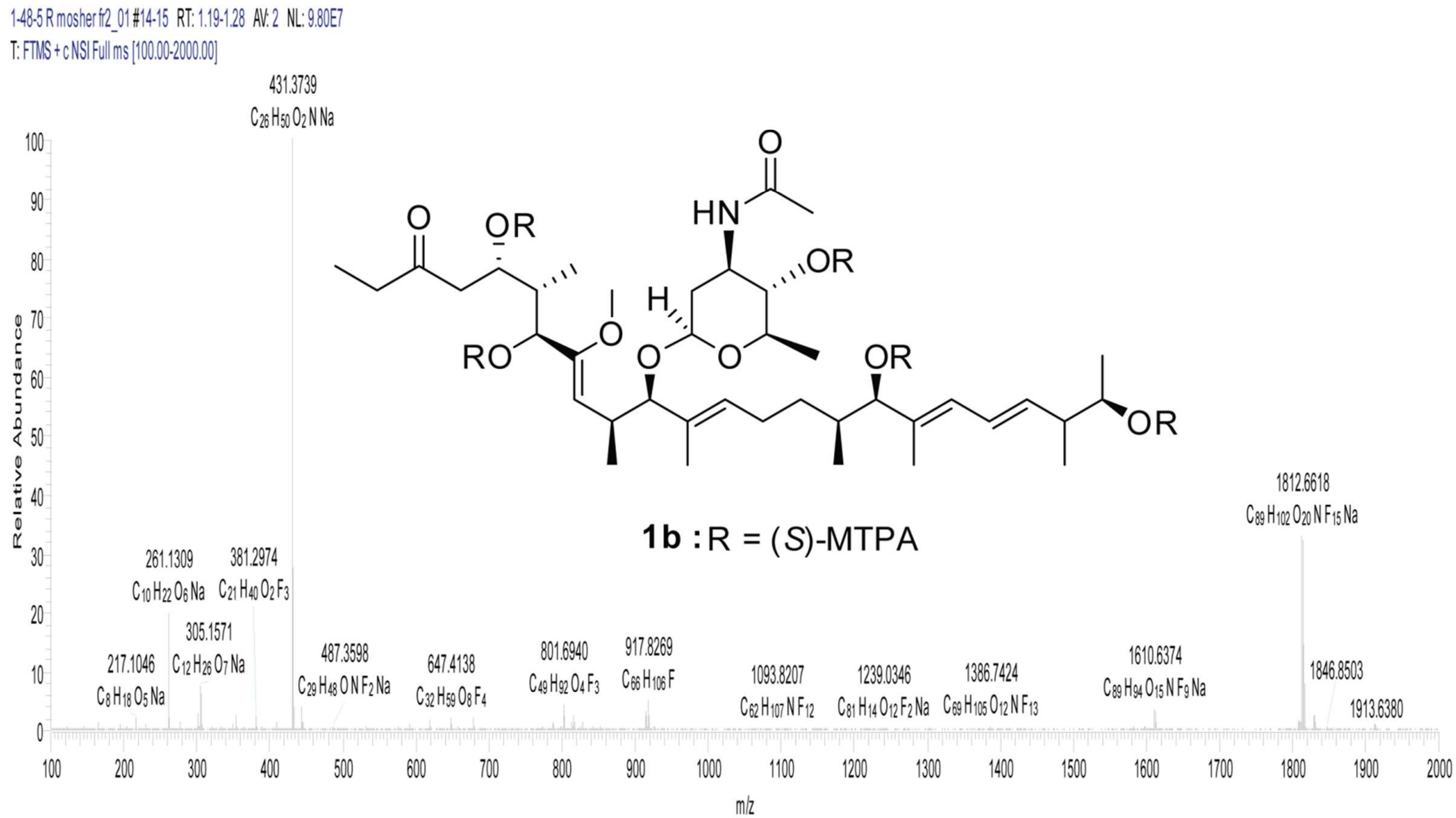



**Figure S7.** HRMS of juniperolide A (*S*)-penta-MTPA ester (**1b**)

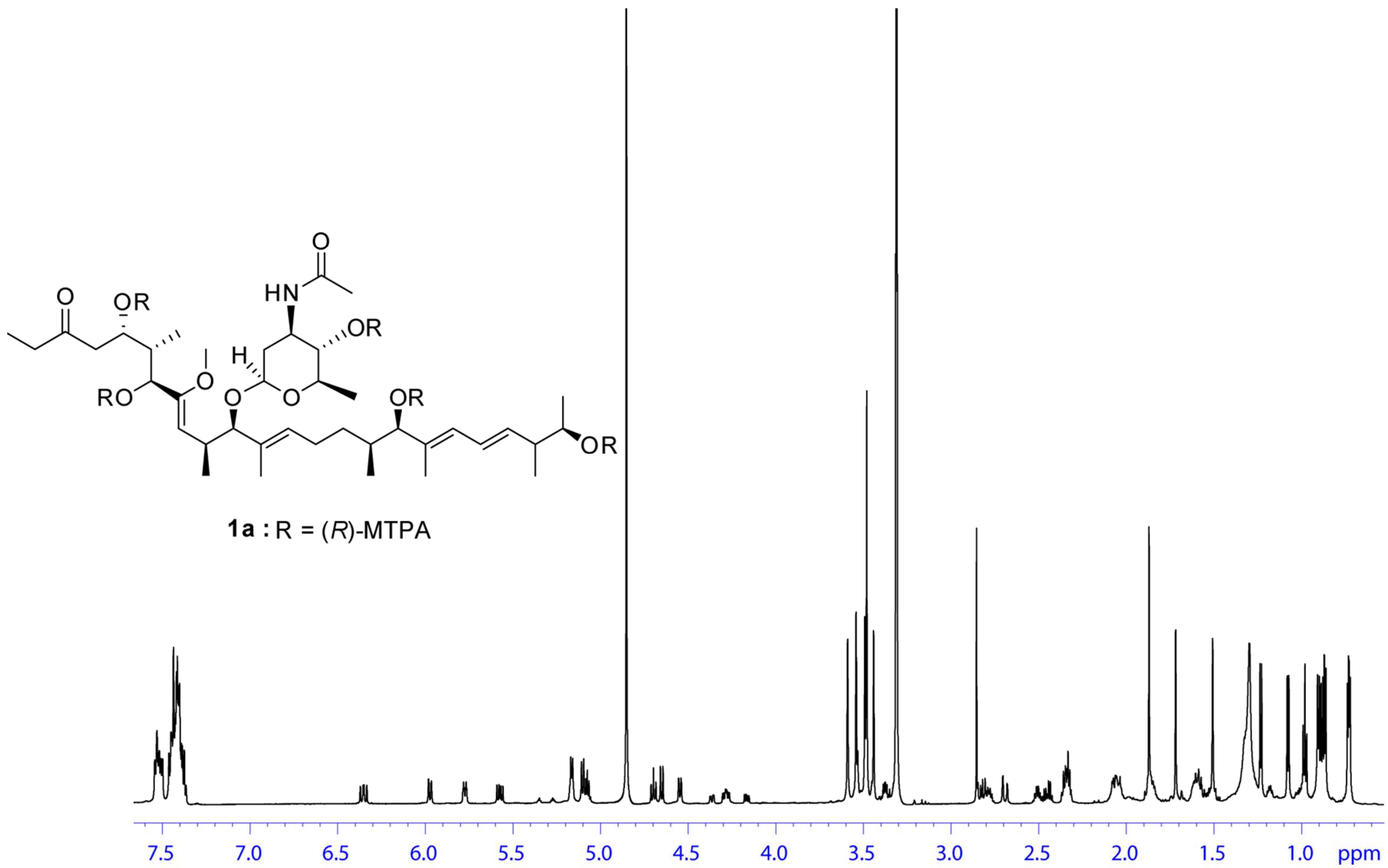
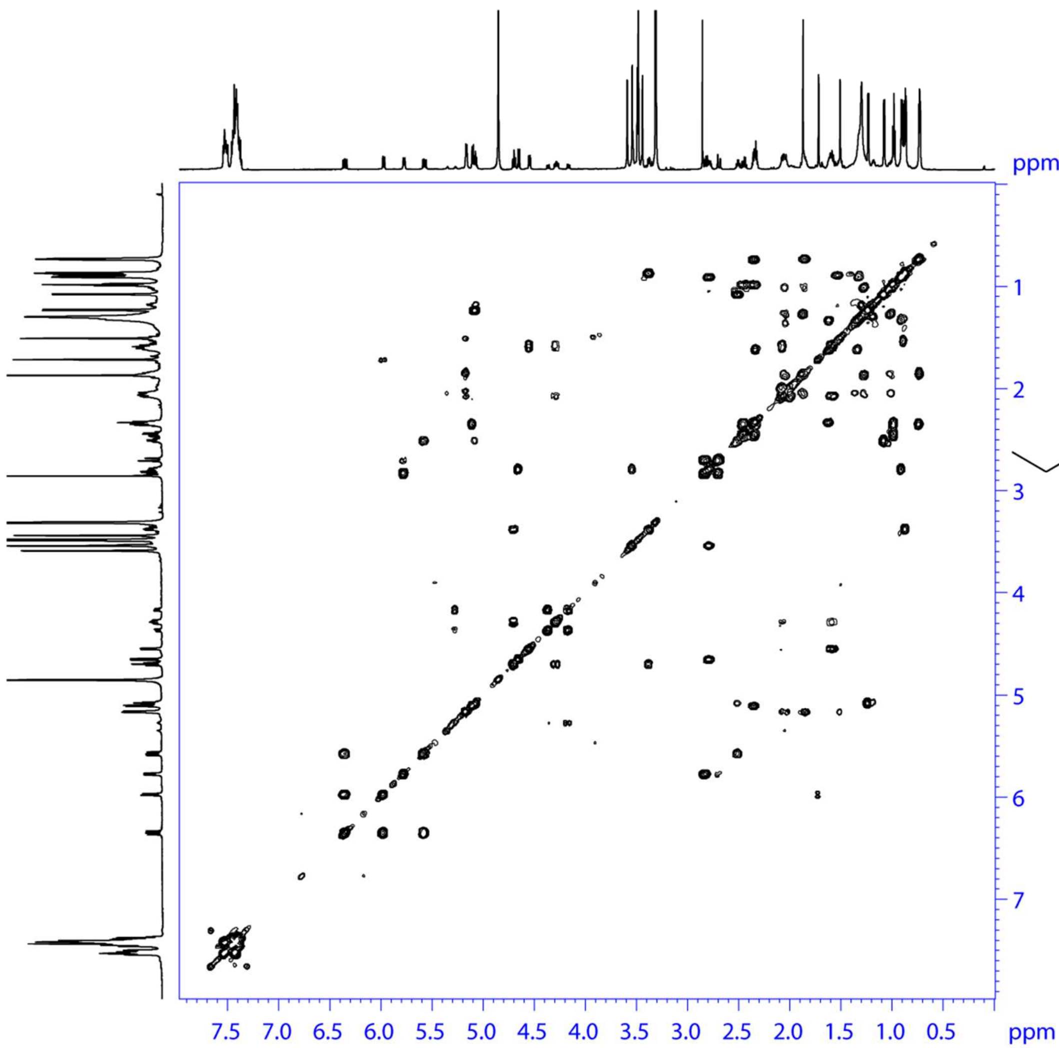
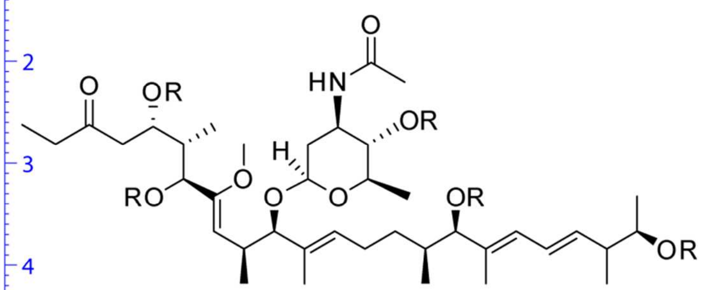


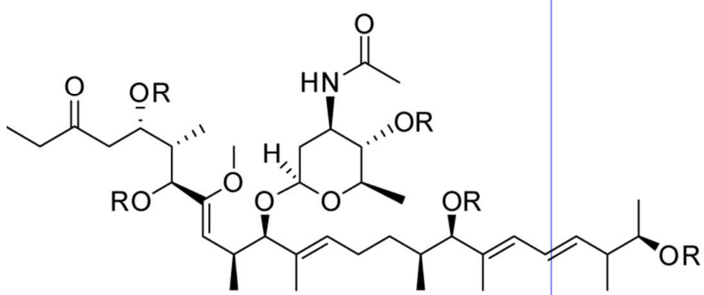

**Figure S8.** [1]H NMR (700 MHz, methanol-$d_4$) spectrum of juniperolide A (*R*)-penta-MTPA ester (**1a**)




**1a** : R = (*R*)-MTPA

**Figure S9.** COSY spectrum (700 MHz, methanol-$d_4$) of juniperolide A (*R*)-penta-MTPA ester (**1a**)



**1a :** R = (*R*)-MTPA

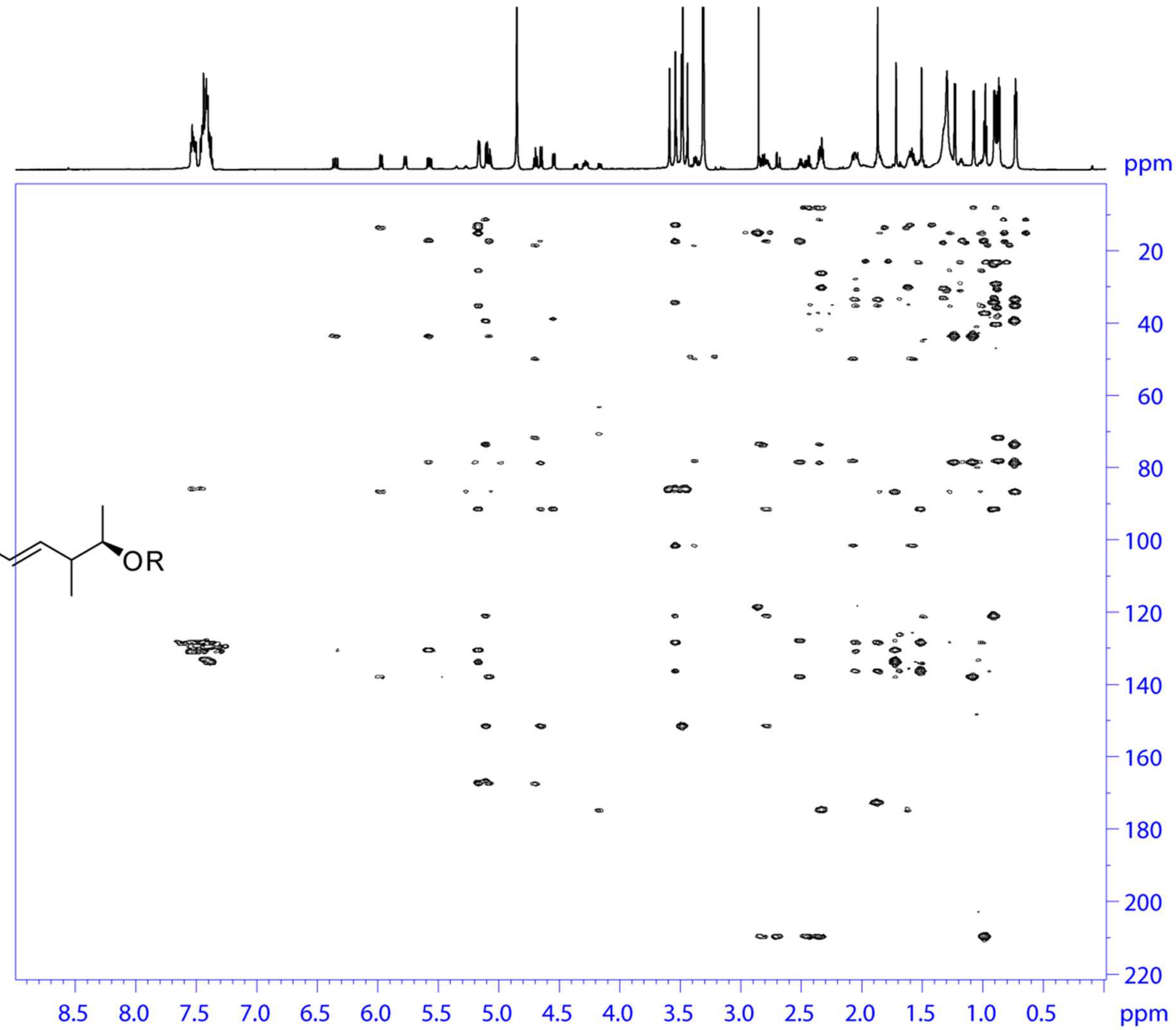



**Figure S10.** $^{1}H$ – $^{13}C$ HMBC spectrum (700 MHz, methanol-$d_4$) of juniperolide A (*R*)-penta-MTPA ester (**1a**)

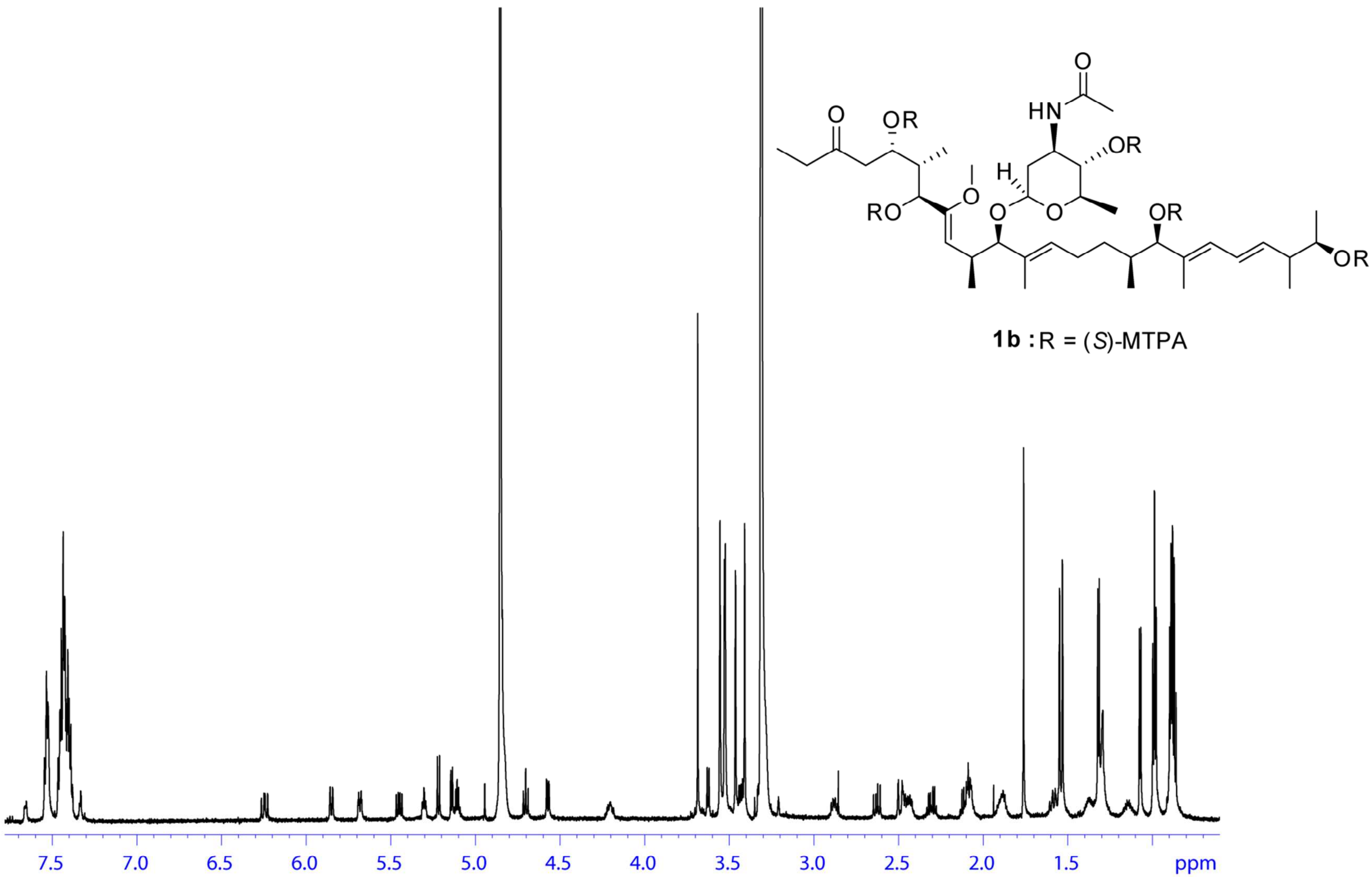



**Figure S11.** $^{1}$H NMR (500 MHz, methanol-$d_4$) spectrum of juniperolide A (*S*)-penta-MTPA ester (**1b**)

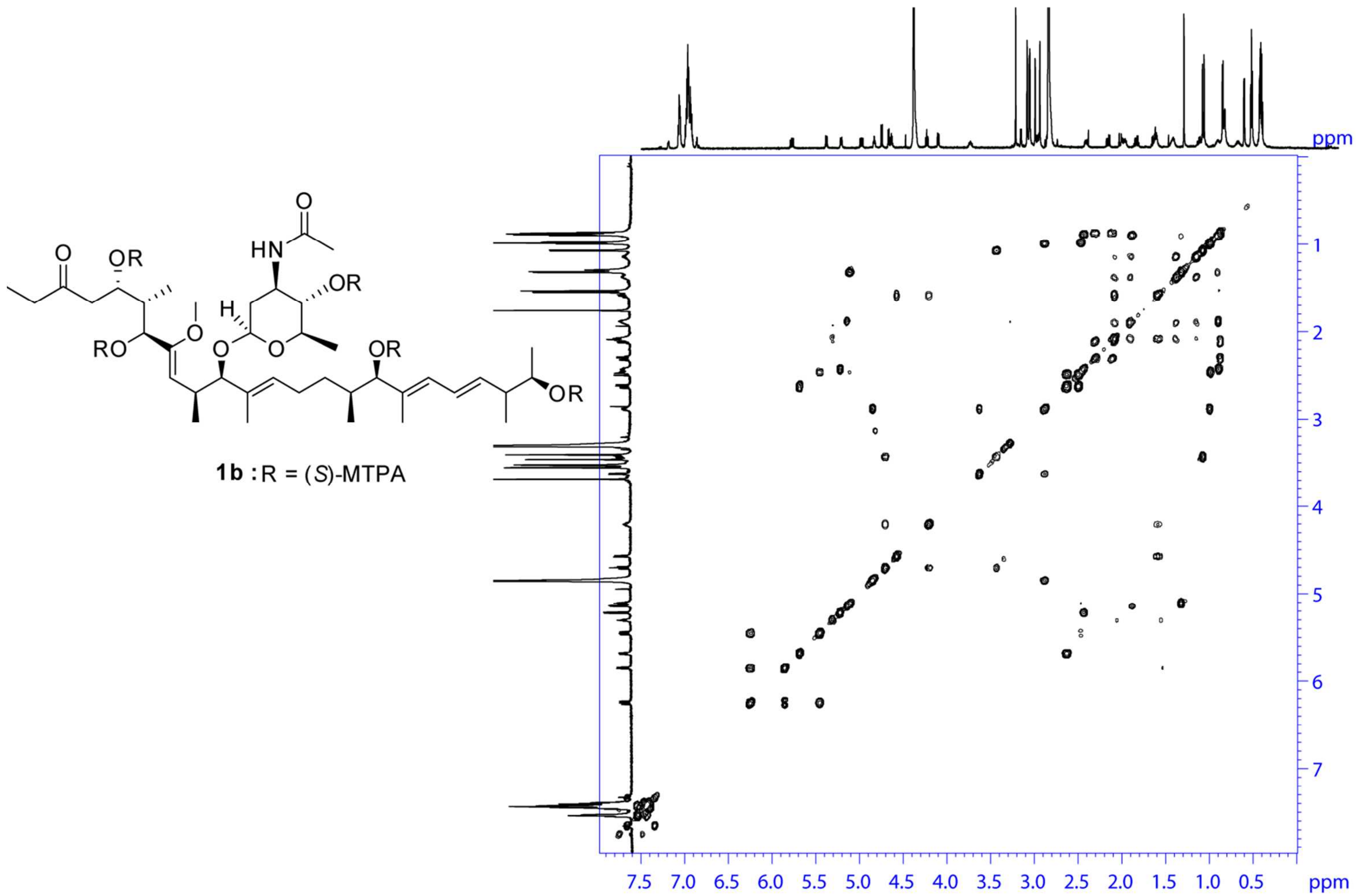
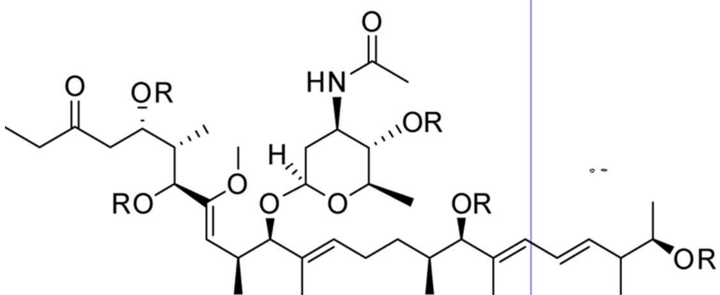



**Figure S12.** COSY spectrum (500 MHz, methanol-$d_4$) of juniperolide A (*S*)-penta-MTPA ester (**1b**)



**1b :** R = (*S*)-MTPA

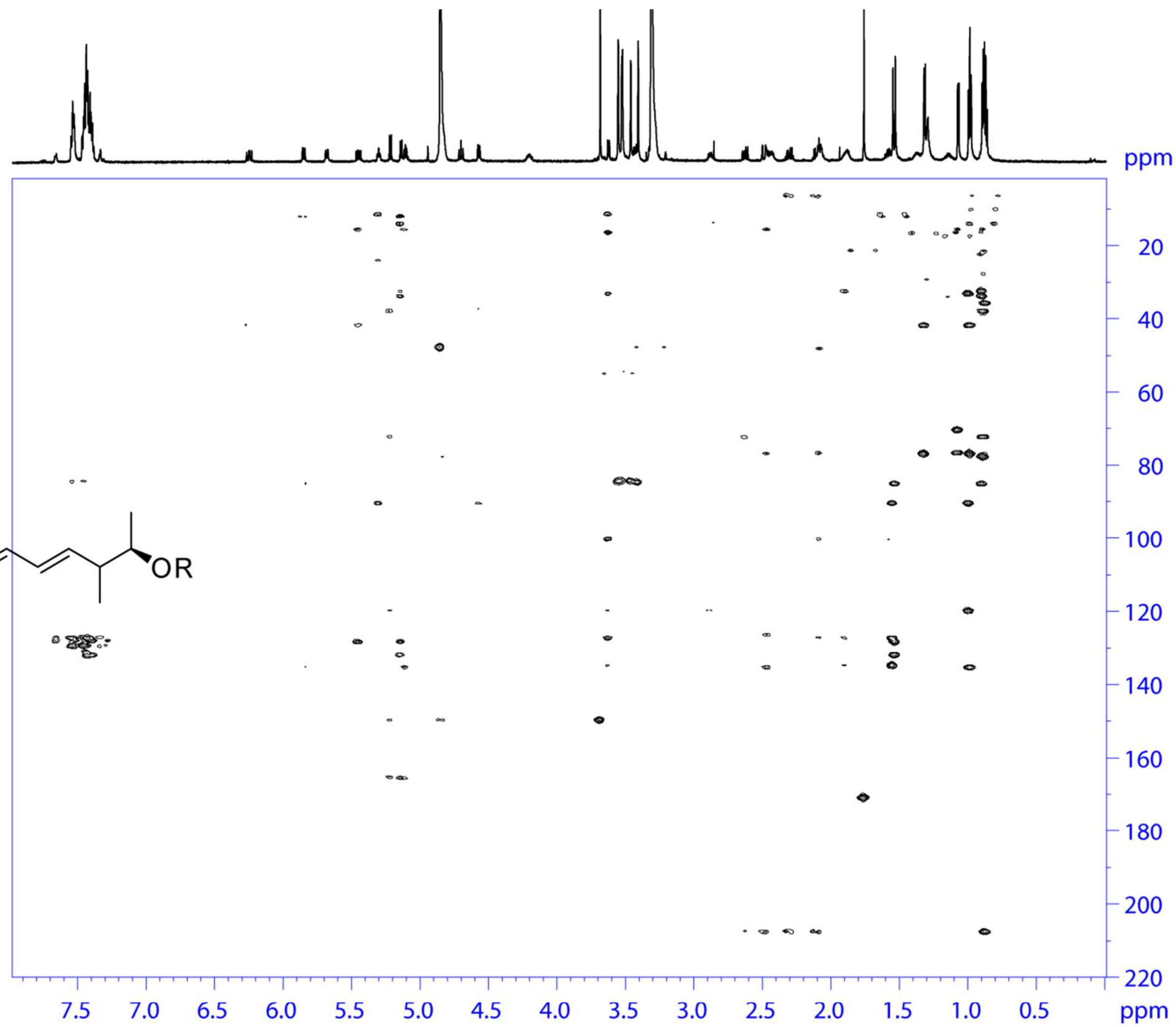


**Figure S13.** $^{1}H - ^{13}C$ HMBC spectrum (500 MHz, methanol-$d_4$) of juniperolide A (*S*)-penta-MTPA ester (**1b**)

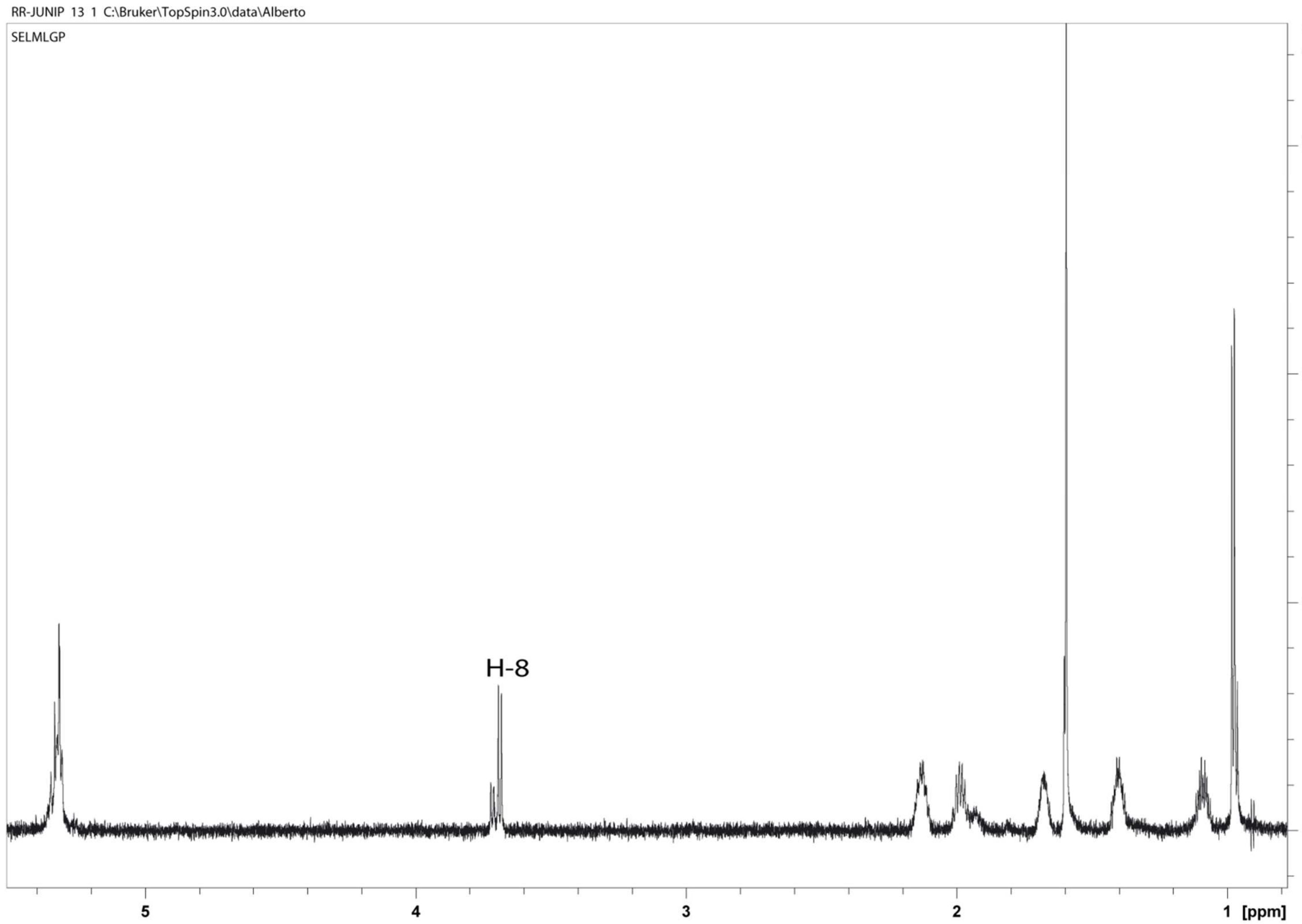



**Figure S14.** 1D TOCSY spectrum (700 MHz, methanol-$d_4$) of juniperolide A (**1**)

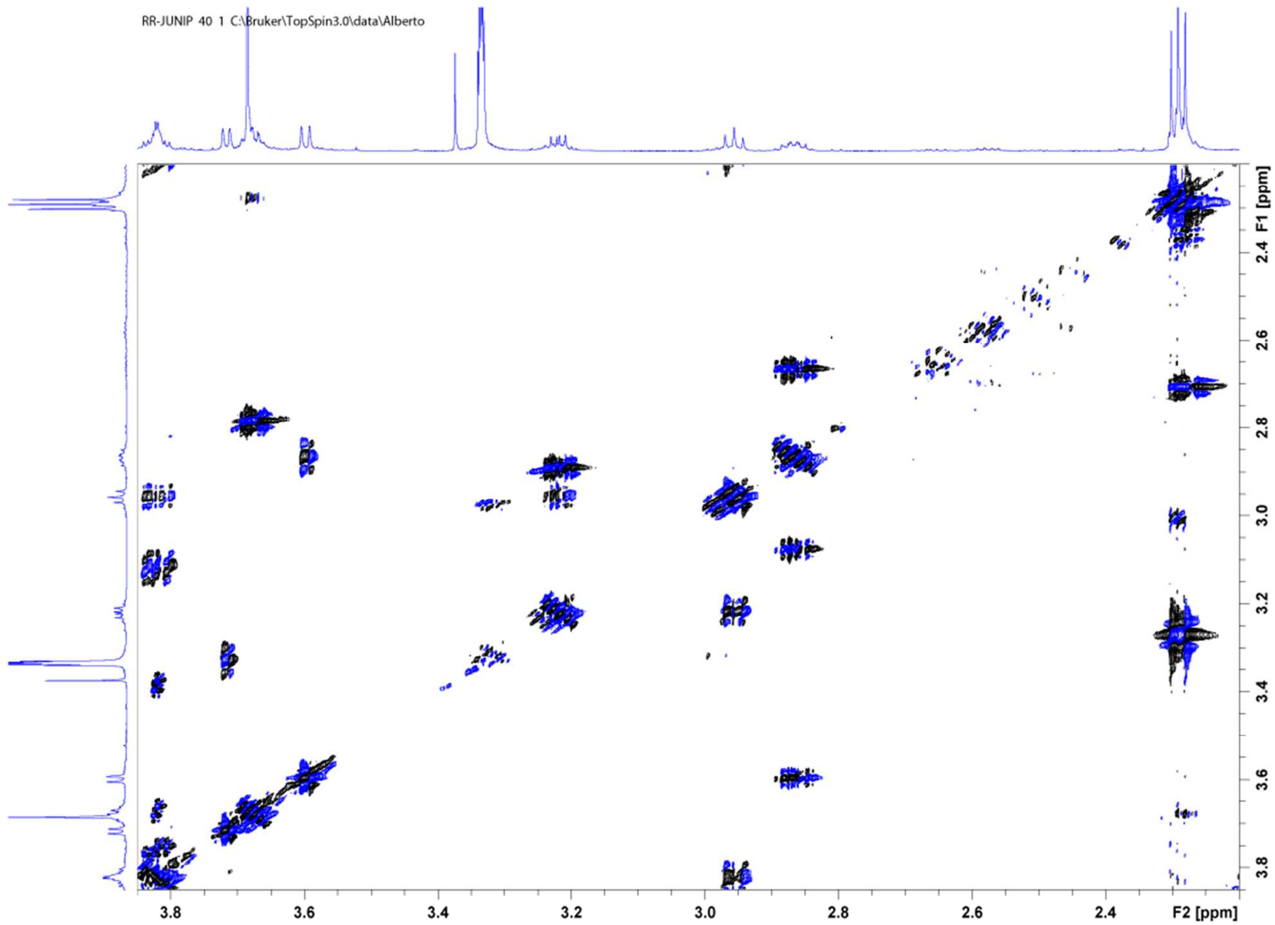



**Figure S15.** E.COSY spectrum (700 MHz, methanol-$d_4$) of juniperolide A (**1**)

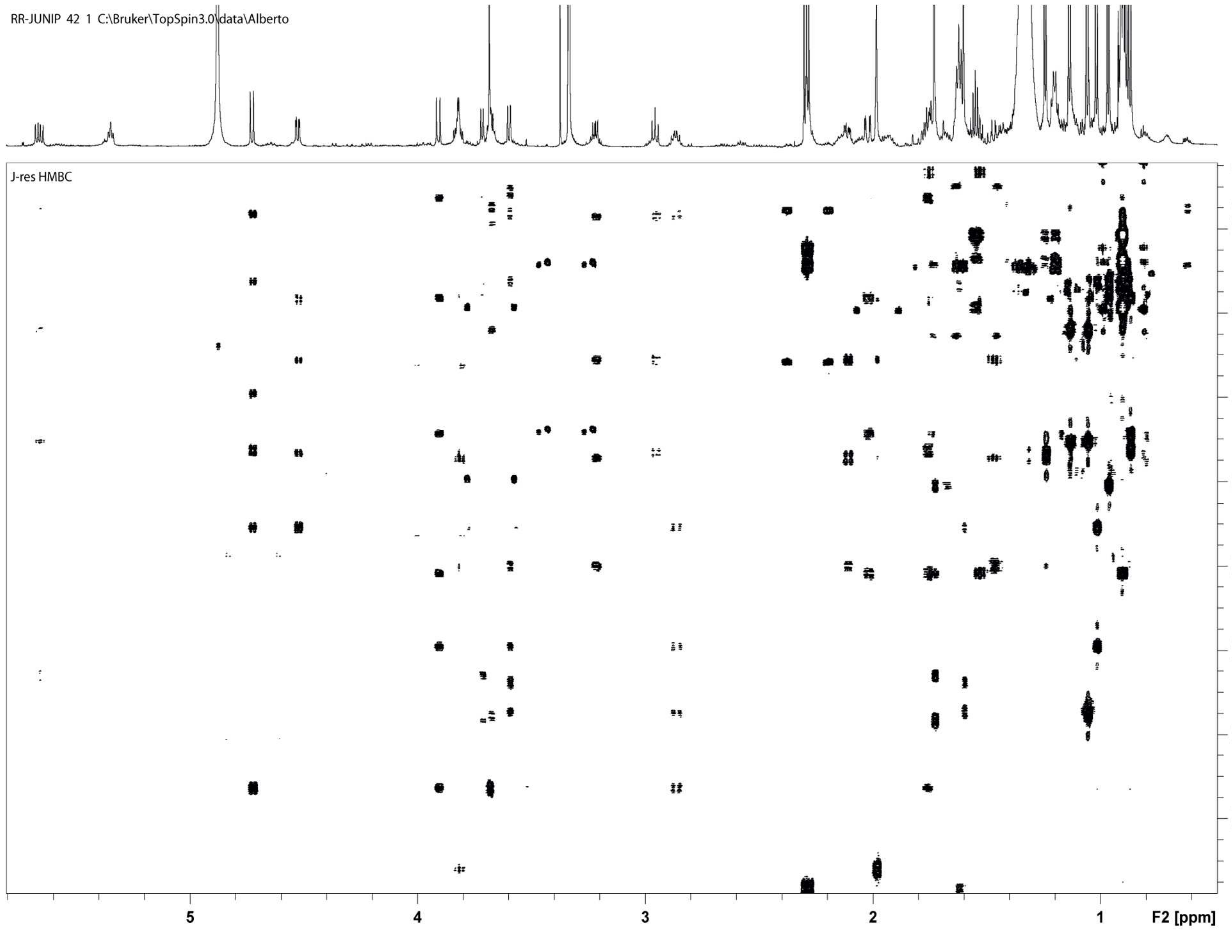



**Figure S16**. J-HMBC spectrum (700 MHz, methanol-$d_4$) of juniperolide A (**1**)

**Table S1.** NMR (700 MHz, methanol-$d_4$) data for **1a**

| pos | $\delta_H$, m *J* in (Hz) | COSY |
|---|---|---|
| 1 | 1.23, d (*6.2*) | 2 |
| 2 | 5.07, m | 1, 3 |
| 3 | 2.51, m | 2, 4, 25 |
| 4 | 5.57, dd (*15.1*, *8.6*) | 3, 5 |
| 5 | 6.35, dd (*15.1*, *10.8*) | 4, 6 |
| 6 | 5.97, d (*10.8*) | 5 |
| 8 | 5.16, m[a] | 9 |
| 9 | 1.84, m | 8, 10b, 27 |
| 10a | 1.27, m | 10b, 11a/b |
| 10b | 1.00, m | 9, 10a, 11a/b |
| 11a | 2.04, m | 10a/b, 11b, 12 |
| 11b | 1.86, m | 10a/b, 11a, 12 |
| 12 | 5.16, m[a] | 11a/b |
| 14 | 3.54, m* | 15 |
| 15 | 2.78, m | 14, 16, 29 |
| 16 | 4.65, d (*10.1*) | 15 |
| 18 | 5.10, d (*8.8*) | 19 |
| 19 | 2.34, m[b] | 18, 20, 30 |
| 20 | 5.77, dt (*10.2*, *2.6*) | 19, 21a/b |
| 21a | 2.83, dd (*17.6*, *10.2*) | 20, 21b |
| 21b | 2.69, dd ((*17.6*, *2.2*) | 20, 21a |
| 23a | 2.44, q (*7.3*) | 23b, 24 |
| 23b | 2.34, m[b] | 23a, 24 |
| 24 | 0.98, t (*7.3*) | 23a/b |
| 25 | 1.08, d (*6.8*) | 3 |
| 26 | 1.72, s | |
| 27 | 0.72, d (*7.0*) | 9 |
| 28 | 1.51, s | |
| 29 | 0.90, d (*6.7*) | 15 |
| 30 | 0.73, d (*7.0*) | 19 |
| 31 | 0.86, d (*6.2*) | 5′ |
| 32 | 1.87, s | |
| 1′ | 4.55, dd (*9.8*, *1.8*) | 2′a/b |
| 2′a | 2.07, m | 1′, 2′b, 3′ |
| 2′b | 1.59, m | 1′, 2′a, 3′ |
| 3′ | 4.28, ddd (*12.5*, *10.4*, *4.9*) | 2′a/b, 4′ |
| 4′ | 4.69, dd (*10.6*, *10.4*) | 3′, 5′ |
| 5′ | 3.37, dq (*10.6*, *6.2*) | 4′, 31 |
| 17-OMe | 3.48, s | |

[a,b] overlapping signals, *obscured by methoxy signal

**Table S2.** NMR (700 MHz, methanol-$d_4$) data for **1b**

| pos | $\delta_H$, m *J* in (Hz) | COSY |
|---|---|---|
| 1 | 1.32, d (*6.2*) | 2 |
| 2 | 5.11, m | 1, 3 |
| 3 | 2.47, m | 2, 4, 25 |
| 4 | 5.45, dd (*15.1*, *8.4*) | 3, 5 |
| 5 | 6.24, dd (*15.1*, *10.8*) | 4, 6 |
| 6 | 5.85, d (*10.8*) | 5 |
| 8 | 5.14, d (*7.3*) | 9 |
| 9 | 1.88, m | 8, 10a/b, 27 |
| 10a | 1.38, m | 9, 10b, 11a/b |
| 10b | 1.15, m | 9, 10a, 11a/b |
| 11a | 2.08, m | 10a/b, 11b, 12 |
| 11b | 1.90, m | 10a/b, 11a, 12 |
| 12 | 5.30, dd (*7.1*, *6.9*) | 11a/b |
| 14 | 3.62, d (*7.8*) | 15 |
| 15 | 2.88, m | 14, 16, 29 |
| 16 | 4.85* | 15 |
| 18 | 5.22, d (*9.2*) | 19 |
| 19 | 2.43, m[a] | 18, 20, 30 |
| 20 | 5.68, dt (*10.1*, *2.7*) | 19, 21a/b |
| 21a | 2.63, dd (*17.5*, *10.3*) | 20, 21b |
| 21b | 2.45, m[a] | 20, 21a |
| 23a | 2.30, m | 23b, 24 |
| 23b | 2.11, m | 23a, 24 |
| 24 | 0.87, t (*7.3*) | 23a/b |
| 25 | 0.98, d (*7.1*) | 3 |
| 26 | 1.53, s | |
| 27 | 0.89, d (*6.9*) | 9 |
| 28 | 1.55, s | |
| 29 | 0.99, d (*6.8*) | 15 |
| 30 | 0.88, d (*6.7*) | 19 |
| 31 | 1.07, d (*6.1*) | 5′ |
| 32 | 1.76, s | |
| 1′ | 4.57, dd (*9.6*, *2.0*) | 2′a/b |
| 2′a | 2.07, m | 1′, 2′b, 3′ |
| 2′b | 1.59, m | 1′, 2′a, 3′ |
| 3′ | 4.20, m | 2′a/b, 4′ |
| 4′ | 4.70, dd (*10.3*, *9.8*) | 3′, 5′ |
| 5′ | 3.43, m | 4′, 31 |
| 17-OMe | 3.68, s | |

[a] overlapping signals, *obscured by $H_2O$ signal