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# Supporting Information

# Noduliprevenone, a Novel Heterodimeric Chromanone with Cancer Chemopreventive Potential

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**Table S1:** NMR spectral data for compound **1.**

| no.[a] | $d^{13}$C (mult.)[b] | $d^{1}$H (*J* in Hz)[b] | COSY[b,d] | HMBC[b,e] | NOESY[b,d] | sel. NOE[c,d] |
|---|---|---|---|---|---|---|
| 1´ | 161.7 (C) | | | | | |
| 2´ | 111.0 (CH) | 6.49 (s) | 11´ | 1´, 4´, 4a´, 9´, 9a´, 11´ | OH-1´, 11´ | OH-1´, 11´ |
| 3´ | 151.6 (C) | | | | | |
| 4´ | 115.0 (C) | | | | | |
| 4a´ | 157.8 (C) | | | | | |
| 5´ | 81.4 (CH) | 4.89 (br t, 5.5) | 6´ | 4a´, 6´, 7´, 8´, 8a´, 10a´ | 6´, 8a´α | 6´, 8a´α |
| 6´ | 22.4 ($CH_2$) | 2.37 (m) | 5´, 7´α, 7´β | 5´, 7´, 8´, 10a´ | 5´ | 5´, 7´α, 7´β, 8a´β |
| 7´ | 26.9 ($CH_2$) | 2.13 (Hβ m) | 6´, 7´α | 5´, 6´, 8´ | | 6´, 7´α |
| | | 1.85 (Hα t, 9.9) | 6´, 7´β | 5´, 6´, 8´ | OH-1 | OH-1, 7´β |
| 8´ | 176.0 (C) | | | | | |
| 8a´ | 40.0 ($CH_2$) | 3.37 (Hβ d, 17.2) | 8a´α | 5´, 9´, 9a´, 10a´, 12´ | | 8a´α |
| | | 2.97 (Hα d, 17.2) | 8a´β | 5´, 9´, 9a´, 10a´, 12´ | 5´ | 5´, 8a´β |
| 9´ | 195.4 (C) | | | | | |
| 9a´ | 106.7 (C) | | | | | |
| 10a´ | 86.2 (C) | | | | | |
| 11´ | 20.8 ($CH_3$) | 1.96 (s) | 2´ | 2´, 3´, 4´, 4a´, 9a´ | 2´, OH-1 | 2´, 11 |
| 12´ | 169.8 (C) | | | | | |
| 13´ | 53.5 ($CH_3$) | 3.70 (s) | | 12´ | 11 | 11, 8a´α |

| | | | | | | |
|---|---|---|---|---|---|---|
| OH-1´ | | 11.47 (s) | | 1´, 2´, 3´, 9´, 9a´ | 2´ | 2´ |
| 1 | 159.5 (C) | | | | | |
| 2 | 116.6 (C) | | | | | |
| 3 | 151.6 (C) | | | | | |
| 4 | 109.8 (CH) | 6.43 (s) | 11 | 1, 2, 9, 9a, 11 | 11 | 11 |
| 4a | 159.8 (C) | | | | | |
| 5 | 73.7 (CH) | 4.14 (br d, 10.6) | OH-5, 6 | 6, 7, 8a, 10a | 6, 7, 8aα, 8aβ | 6, 7, 8aα, 13 |
| 6 | 26.7 ($CH_2$) | 1.73 (m) | 5, 7 | 5, 7, 8 | 5 | 5 |
| 7 | 30.9 ($CH_2$) | 2.54 (m) | 6 | 5, 6, 8 | 5 | 5 |
| 8 | 173.8 (C) | | | | | |
| 8a | 37.9 ($CH_2$) | 3.33 (Hα d, 17.2) | 8aβ | 5, 9, 9a, 10a, 12 | 5 | 8aβ |
| | | 3.04 (Hβ d, 17.2) | 8aα | 5, 9, 9a, 10a, 12 | 5, 6 | 6, 8aα |
| 9 | 197.7 (C) | | | | | |
| 9a | 105.9 (C) | | | | | |
| 10a | 88.1 (C) | | | | | |
| 11 | 21.0 ($CH_3$) | 2.12 (s) | 4 | 2, 3, 4, 4a, 9a | 4, 13´ | 4, 11´, 13´ |
| 12 | 170.9 (C) | | | | | |
| 13 | 53.5 ($CH_3$) | 3.77 (s) | | 12 | | |
| 14 | 51.6 ($CH_3$) | 3.63 (s) | | 8 | | |
| OH-1 | | 11.94 (s) | | 1, 2, 3, 9, 9a | 7´a, 11´ | |
| OH-5 | | 5.05 (br d, 4.8) | 5 | | 5 | |

[a]Position of carbon atom. [b] [$D_6$]Acetone, 300/75.5 MHz. [c] [$D_6$]Acetone, 500/75.5 MHz. [d] Numbers refer to proton resonances. [e] Numbers refer to carbon resonances.

Supporting information – General Experimental

All NMR spectra were recorded on Bruker Avance 500 DRX or 300 DPX spectrometers in [$D_6$]acetone or $CDCl_3$. Spectra were referenced to residual solvent signals. UV and IR spectra were obtained employing Perkin-Elmer Lambda 40 and Perkin-Elmer Spectrum BX instruments, respectively. HRESIMS were recorded on Bruker Daltonics' micrOTOF-Q instrument. Optical rotation was measured on a Jasco DIP 140 polarimeter.

**Circular Dichroism Spectroscopic Measurements.** CD spectra of compound **1** and **2** were recorded at room temperature on a Jasco J-810 spectrophotometer. Samples were measured in 1 cm-cuvettes at concentration of 0.3 mmol $L^{-1}$.

**Modified Mosher Derivatization**

**Preparation of the acid chlorides of (*R*)- and (*S*)-MPA.** Oxalyl chloride (103.7 µL, 1.2 mmol) was added to a solution of the corresponding MPA (20 mg, 0.12 mmol) and DMF (0.94 µL, 0.012 mmol) in hexane at room temperature. After one day, the solvent was vacuum-baked to give 100% of the product MPA-Cl (22.2 mg, 0.12 mmol).

**Preparation of the (*R*)- and (*S*)- MPA esters.** The corresponding MPA-Cl (4.27 mg, 23.15 µmol) was dissolved in 5 mL of $CH_2Cl_2$ and added to a solution of compound **1** (3.1 mg/ 4.63 µmol for *R*-derivatization; 2.4 mg/ 3.6 µmol for *S*-derivatization), $Et_3N$ (7.70 µL, 55.56 µmol) and DMAP (0.57 mg, 4.63 µmol) as catalyst. After 20 min reaction time the obtained products were evaporated under vacuum and further purified by HPLC using a Merck-Hitachi system consisting of a L-6200 A pump, a L-4500 A photodiode array detector and a D-6000 A interface. The separation was performed with a RP18 column (Macherey-Nagel Nucleodur Sphinx RP, 5 µm, 250 x 4.6 mm) and a mobile phase (1.2 mL/min) consisting of MeCN/$H_2O$ 64/36. The main peaks in the

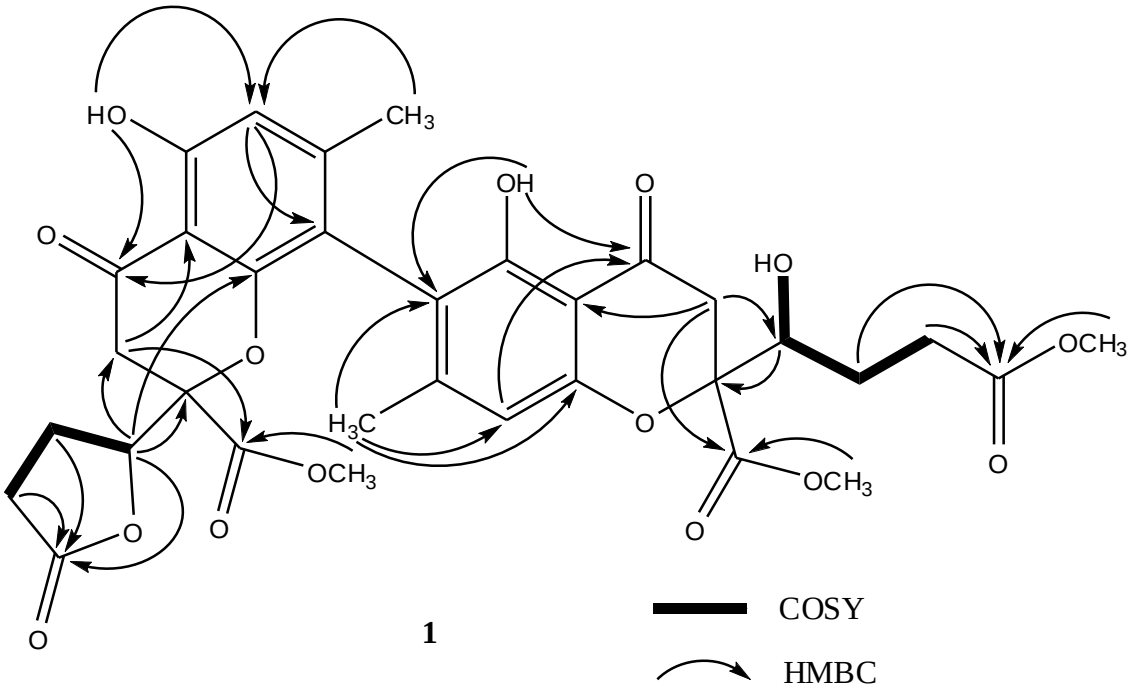
chromatograms revealed the pure MPA esters (2 mg = 52.8 % of *R*-MPA ester and 1.6 mg = 54.6 % of *S*-MPA ester, respectively).

**Molecular Modeling.** All models were calculated employing conformation search (Boltzman jump) and a standard force field as implemented in the Cerius2 4.0 (MSI) molecular modeling software package. Models were further refined with 1500 iterations of minimization. Calculations were performed using a Silicon Graphics O2 workstation (Irix 6.5.6).

**Determination of Potential Cancer Chemopreventive Activities.** Homogenates of H4IIE rat hepatoma cells induced for 39 h with the CYP1A inducer β-naphthoflavone (β-NF) at a concentration of 10 μM were used as an enzyme source to measure CYP1A activity. The rate of time-dependent dealkylation of 3-cyano-7-ethoxycoumarin (CEC) to 3-cyano-7-hydroxycoumarin (CHC) was determined fluorimetrically in 96-well plates for 45 min at 37°C using a SpectraMax Gemini XS fluorescence reader (Molecular Devices, excitation 409 nm, emission 451 nm, cutoff 435 nm).[8] Activity of solvent control C: 36 ± 7 pmol $min^{-1}$ $mg^{-1}$ of protein (*n*=3-4). Means significantly different from control (**P* < 0.01, **P < 0.001) using ANOVA with Holm-Sidak test for multiple comparison with *n*=3-4. Inhibition constants were generated from Lineweaver-Burk-, Dixon- and Cornish-Bowden plots of the results of kinetic experiments with 2.5 μM, 5 μM and 10 μM CEC, respectively, as a substrate. The $IC_{50}$ value of α-naphthoflavone (a-NF), a known CYP1A inhibitor employed as a positive control, was 0.005 ± 0.001 μM (*n*=4).
For the detection of phase 2 enzyme inducers, QR activity was measured in cultured Hepa 1c1c7 murine hepatoma cells (2 x $10^4$ cells $mL^{-1}$) after a 48 h induction period by the NADPH-dependent menadiol-mediated reduction of MTT [3-(4,5-dimethylthiazo-2-yl)-2,5-diphenyltetrazolium bromide] to a blue formazan as described previously.[8] Induction of QR activity was calculated from the ratio of specific enzyme activities of compound-treated cells in comparison with a solvent control, and CD values (concentration required to double the specific QR activity in μM) were generated. Sulforaphane, an isothiocyanate from broccoli, was used as a positive control with a CD value of 0.19 ± 0.05 μM (*n*=3). Specific activities of untreated controls were 26 ± 3 nmol $min^{-1}$ $mg^{-1}$ protein (n=4).

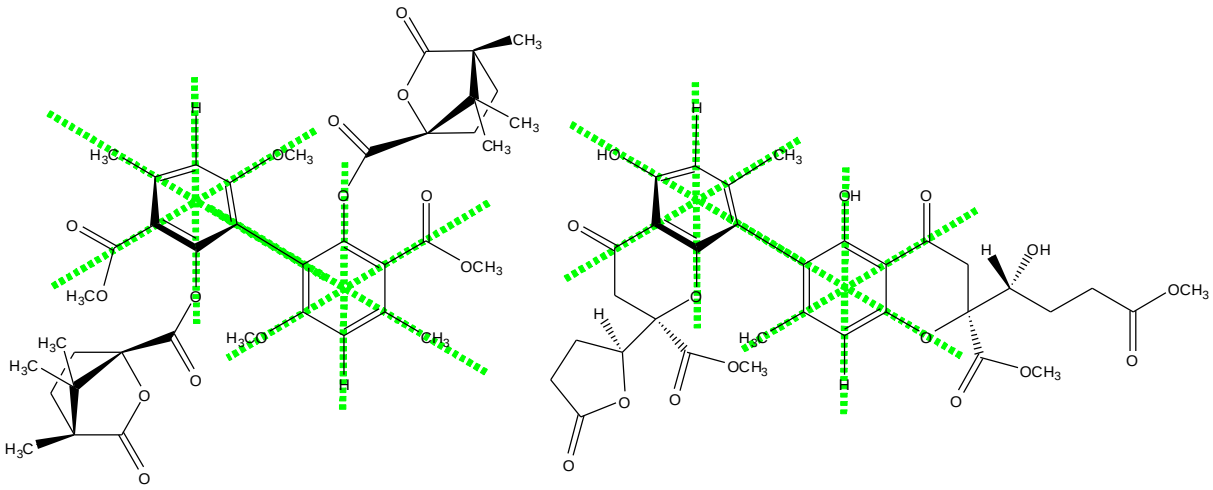
Supporting information

**Figure S1.** Important $^{1}H,^{1}H$-COSY and $^{1}H,^{13}C$ long range (HMBC) correlations of compound **1**.




**1**

**Figure S2.** CD spectra of compound **1** and the reference (*M*)-orsellinic acid camphanate **(2)** in MeCN with sector rule for the $^{1}L_{b}$-Cotton effect (drawn through the ring carbons)[10,11] for the benzene chromophores.



**2** **1**

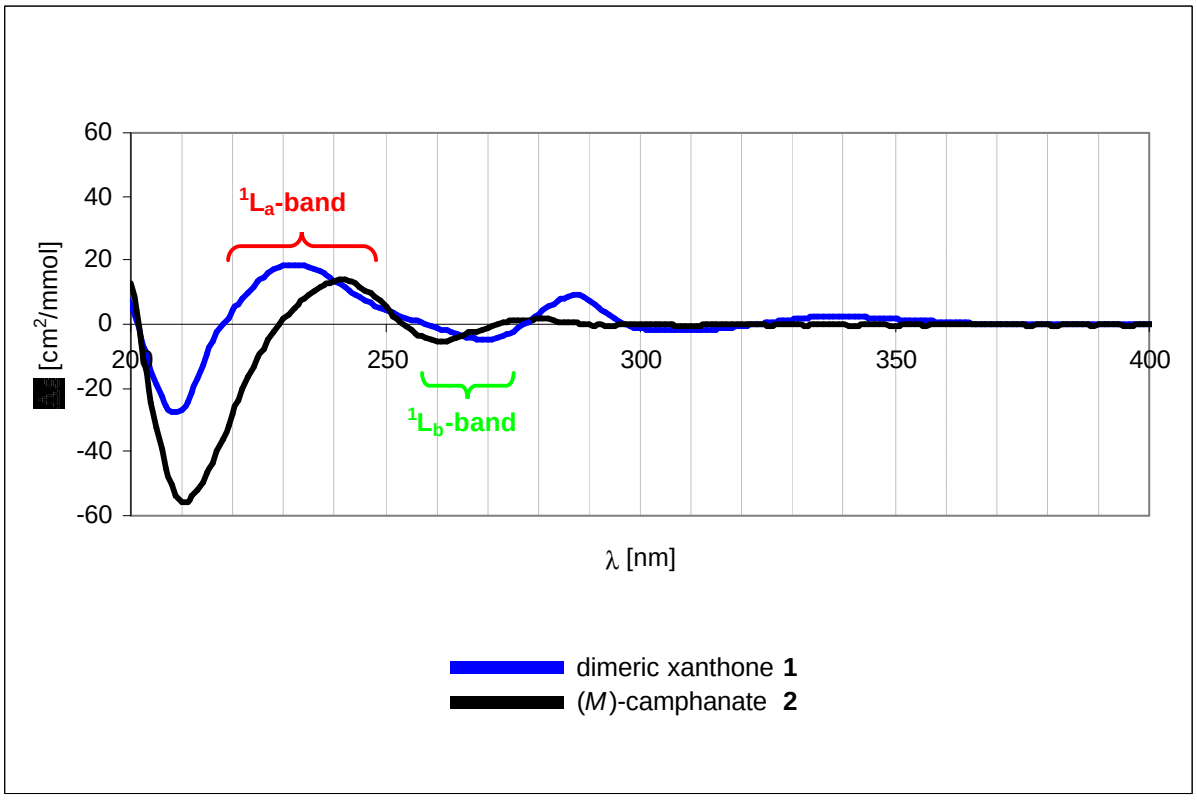


**Figure S3.** Selective gradient NOEs (purple arrows) depicted in autonomous 3D models (Cerius2) of both monomeric sub-units disconnected at the biphenyl axis (brownish bond).

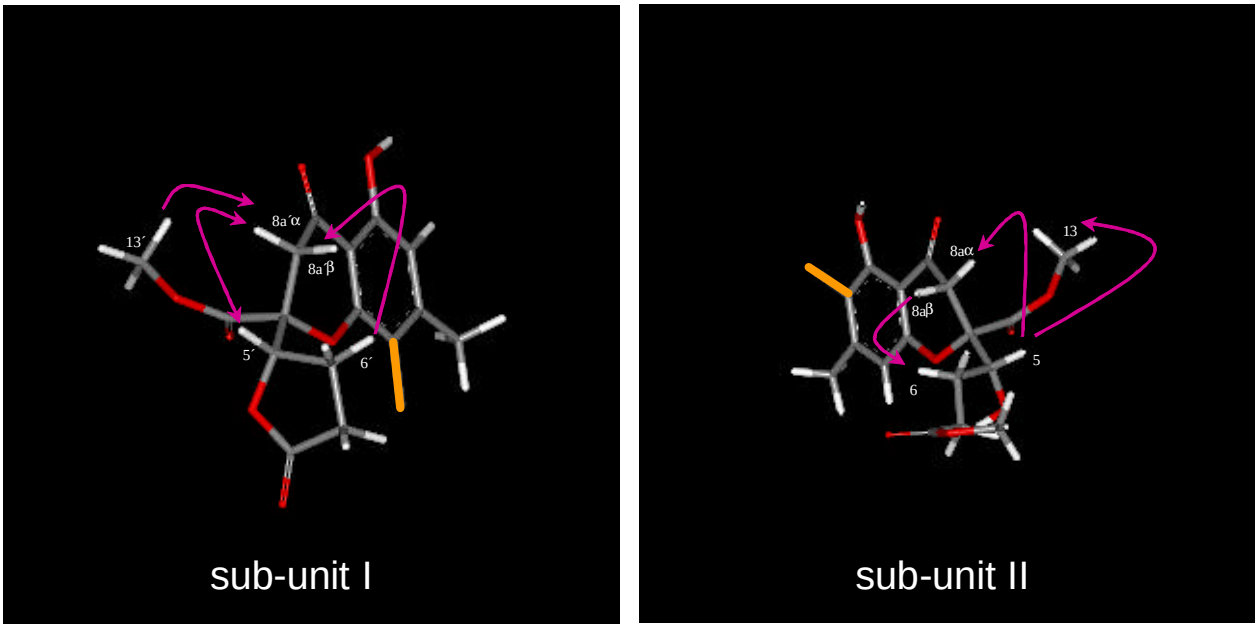
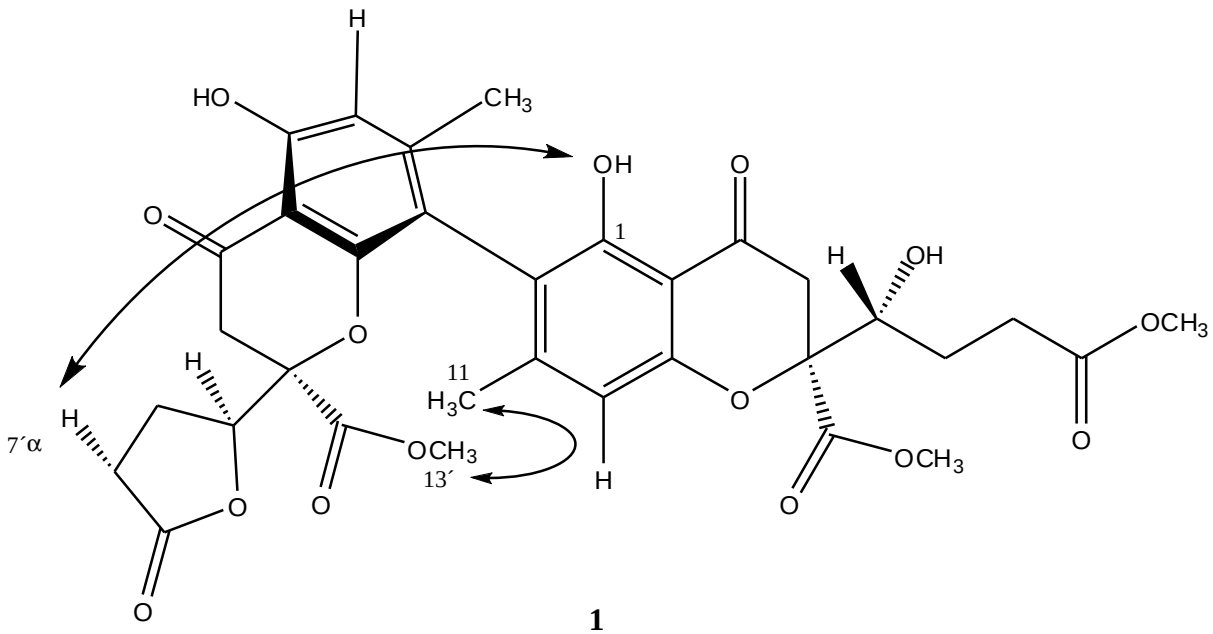





**Figure S4.** Selective gradient NOEs (arrows) between the two sub-units of **1**.




**1**

**Figure S 5.** Deduction of the absolute configuration at C-5 using modified Mosher's method with ? $d^{RS}$-values of MPA esters of compound **1**.

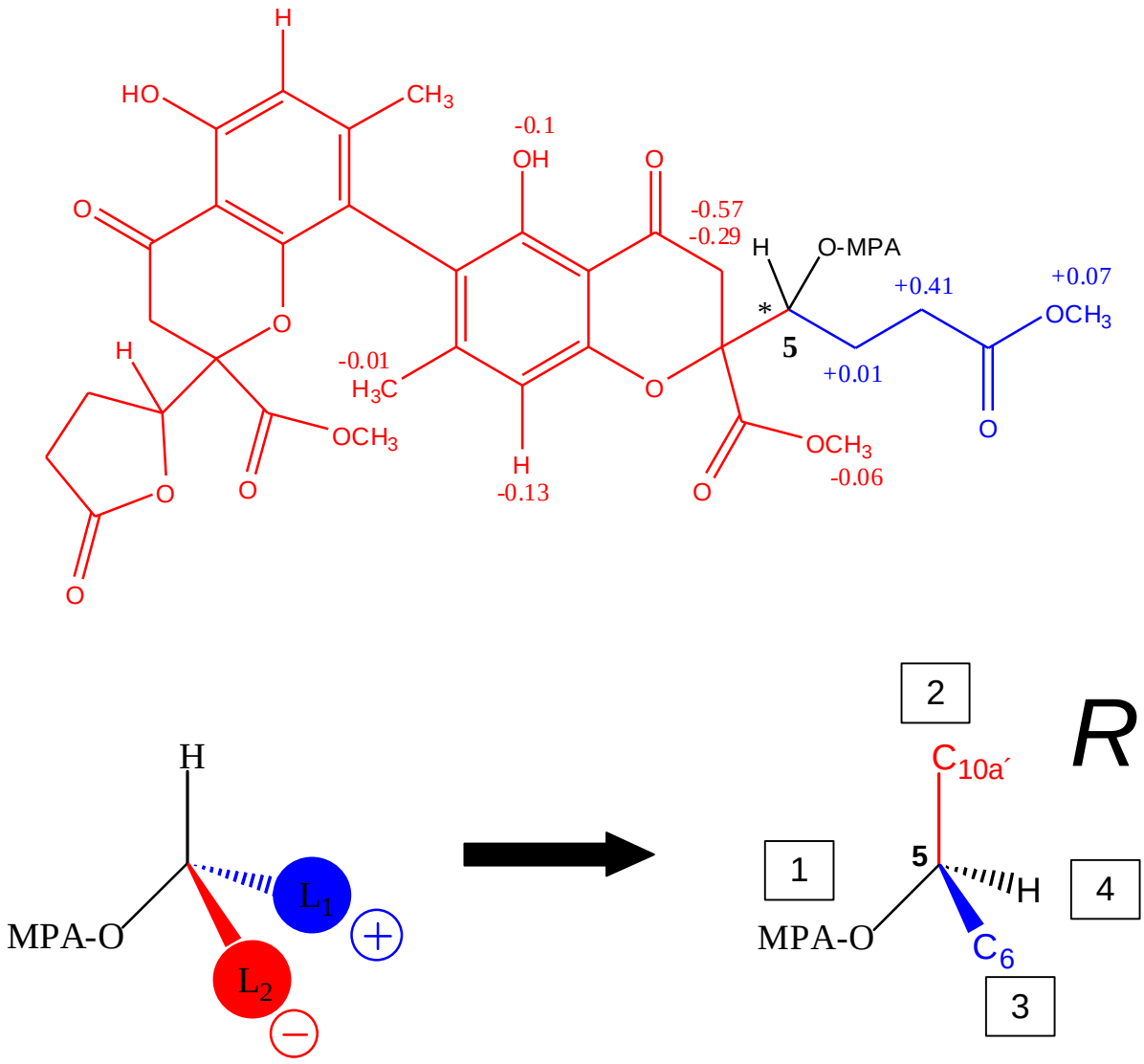


**Figure S6.** Proposed biosynthesis for compound **1**.

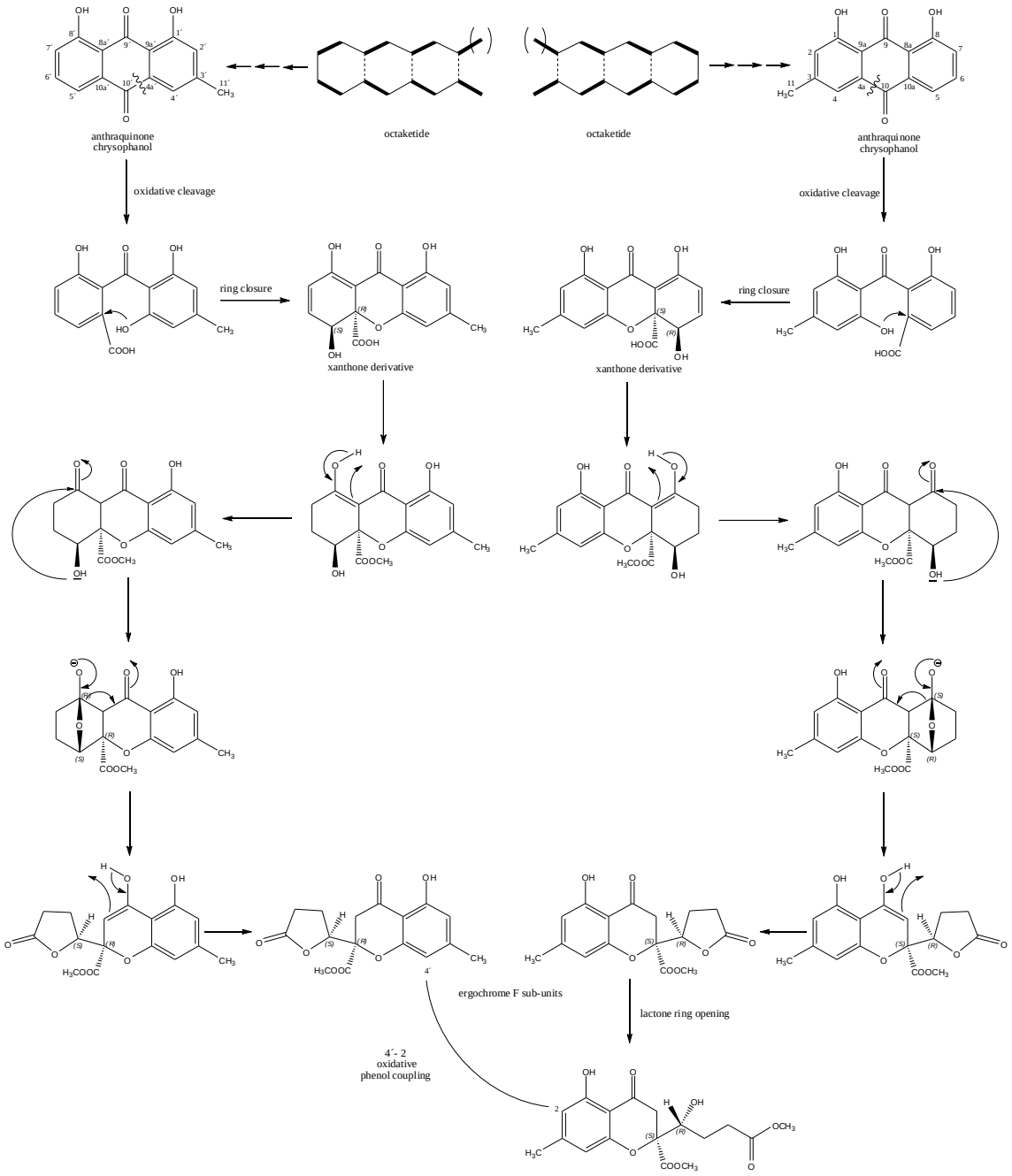
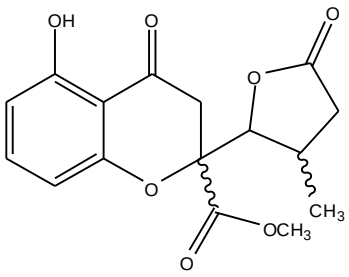
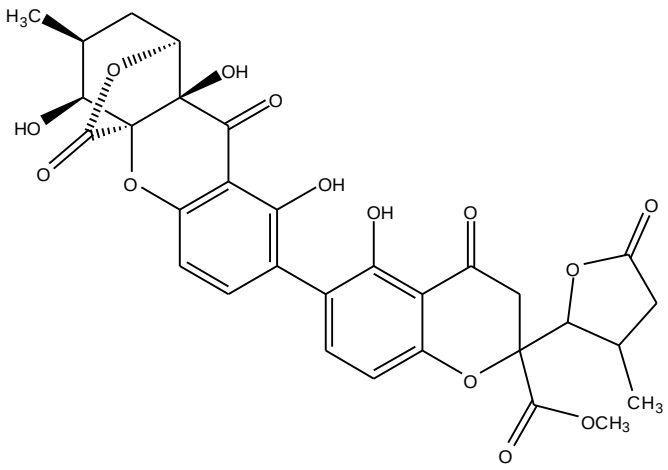


**Figure S7.** Structures of fungal metabolites related to **1**.

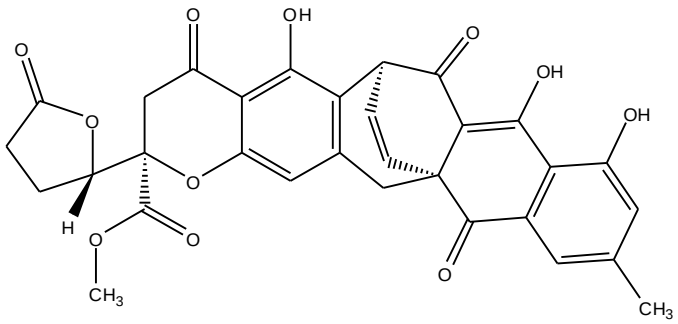
ergochrome F unit[15]:



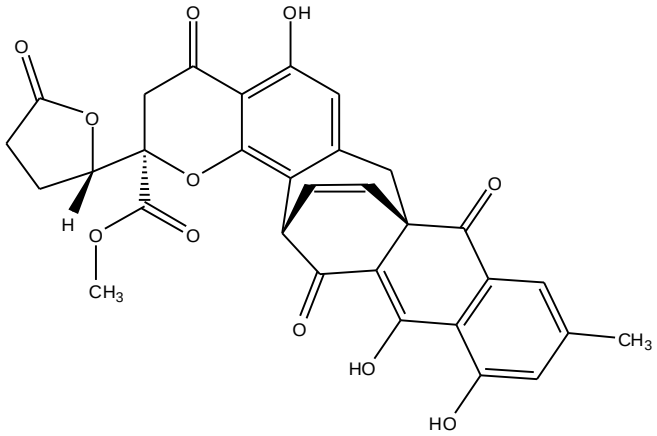

ergoxanthin[15]:



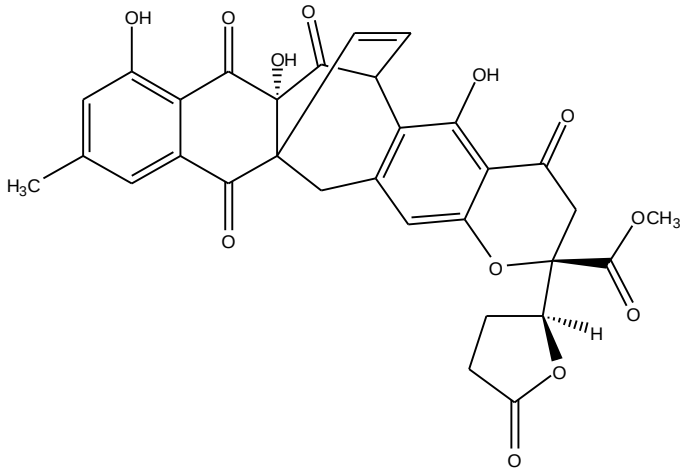

xanthoquinodin A3 [14]:



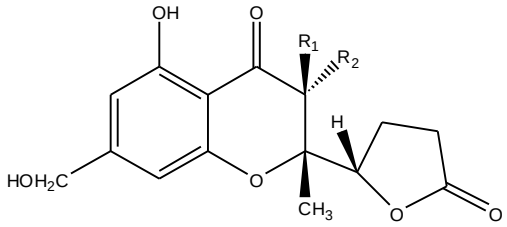
xanthoquinodin B3 [14]:



chaetomanone[16]:



lachnone 3, 4 and 5 [17]:

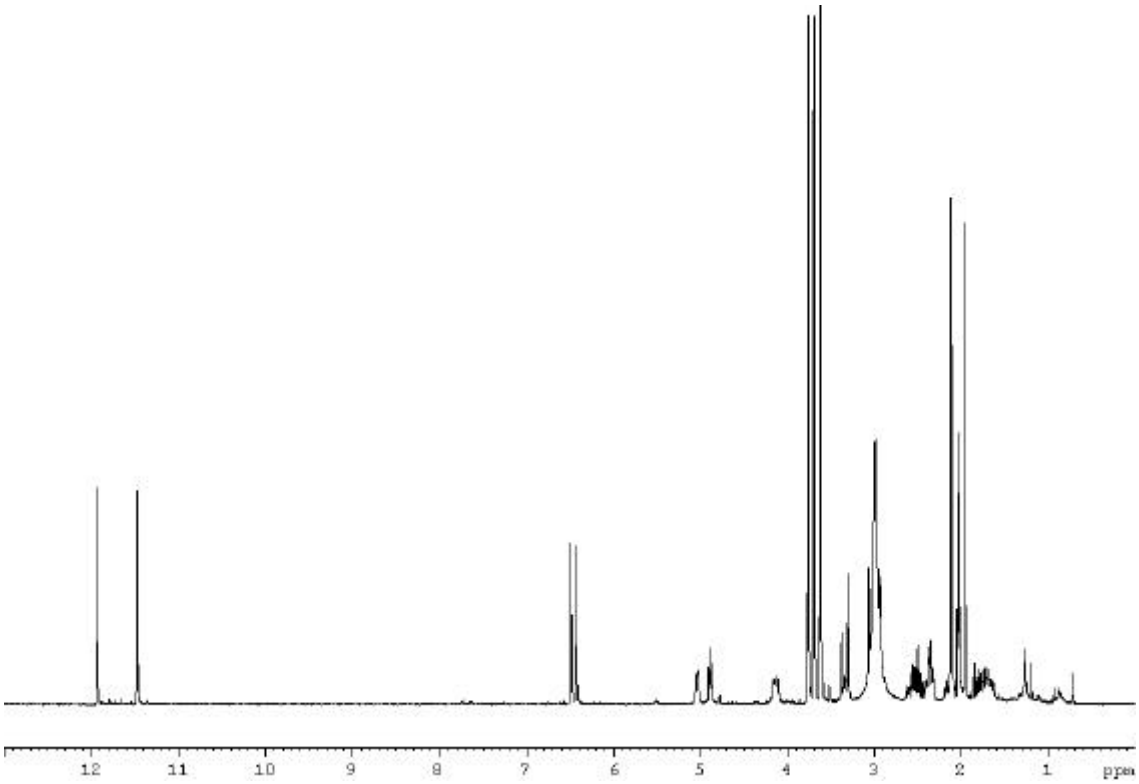


3: $R_1 = R_2 = H$

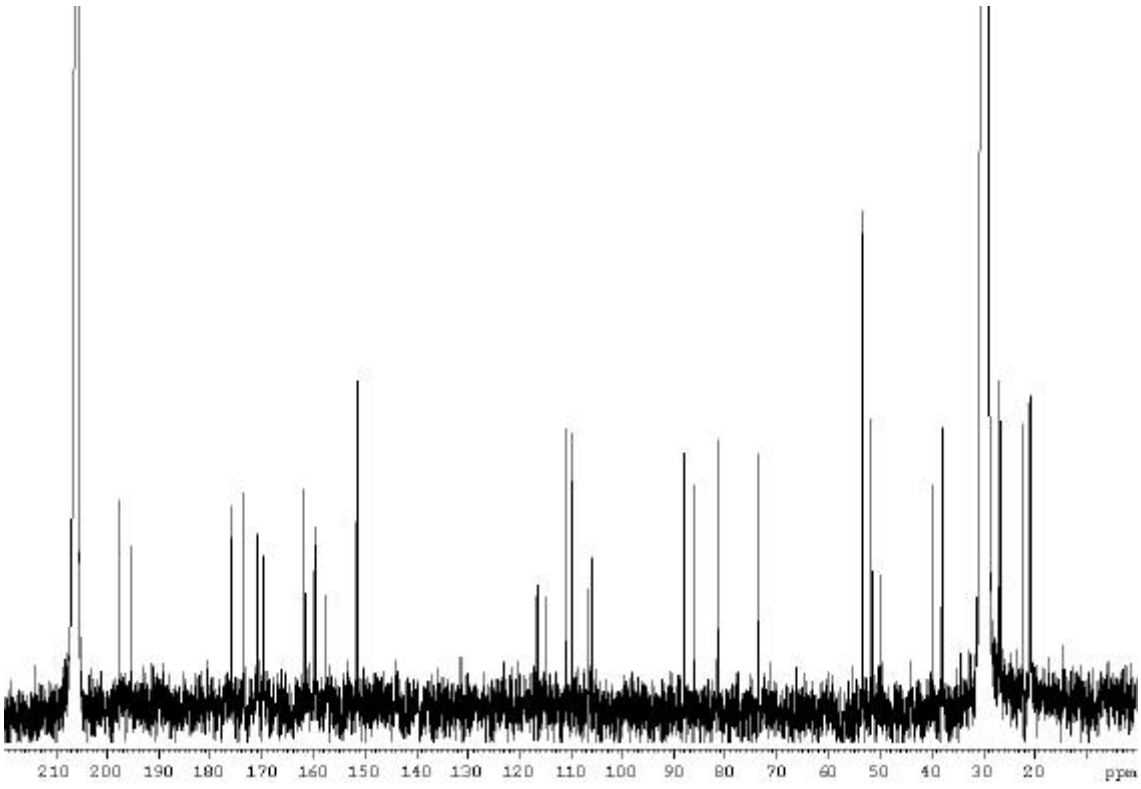
4: $R_1 = OH$; $R_2 = H$

5: $R_1 = -CH_2COCH_3$; $R_2 = OH$

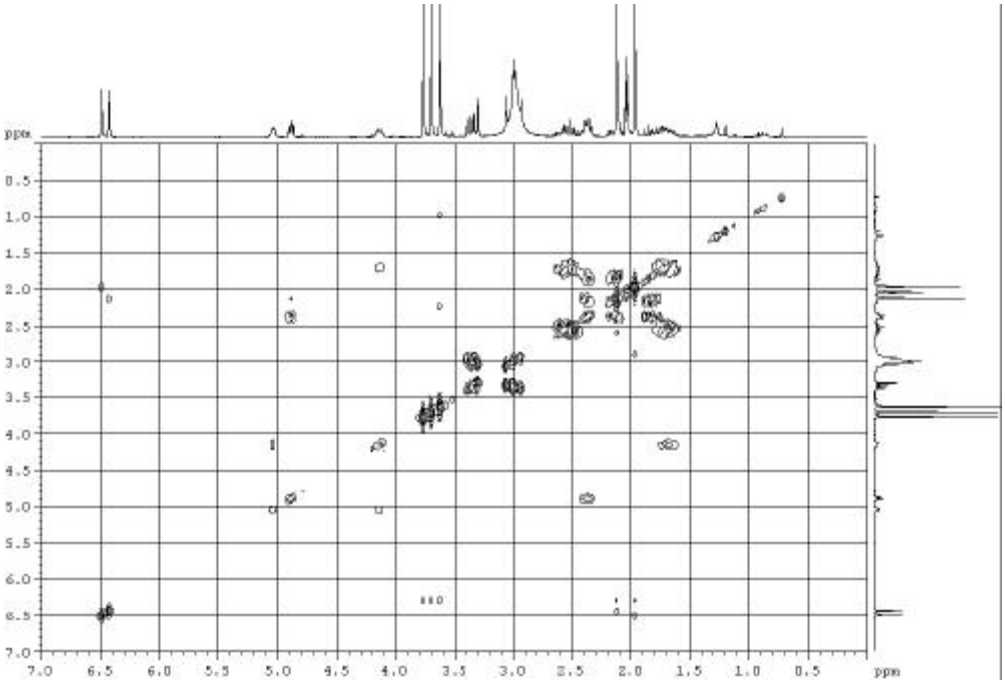
Supporting information – $^{1}H$ NMR spectrum (300 MHz in $[D_6]$acetone) of the new compound **1**.



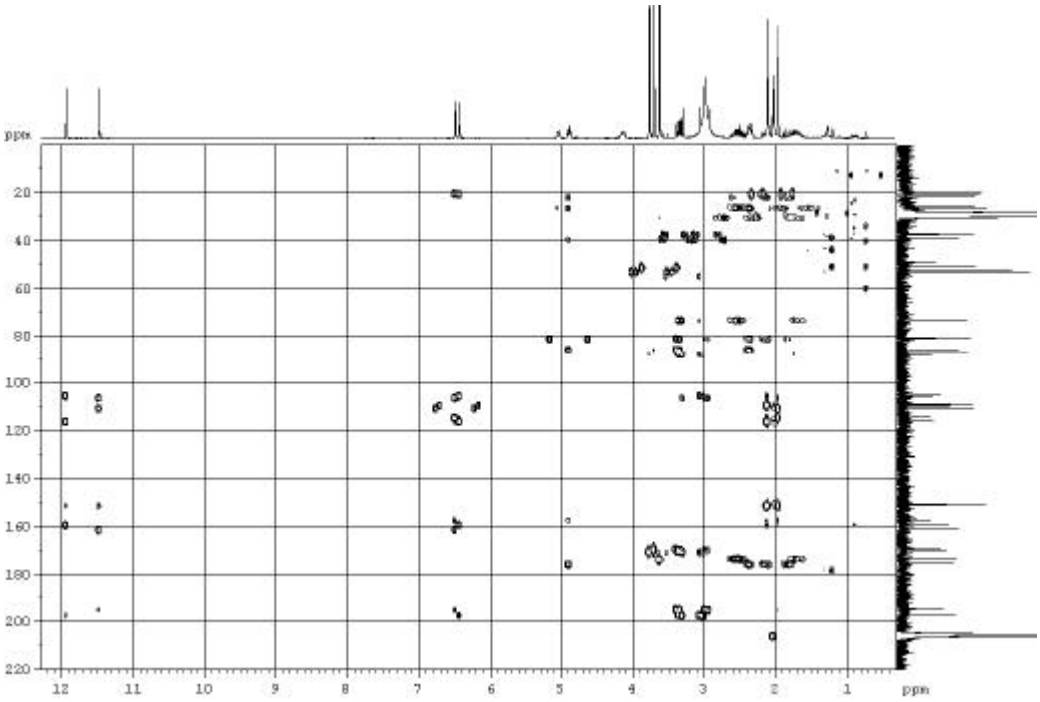

Supporting information – $^{13}C$ NMR spectrum (75 MHz in $[D_6]$acetone) of the new compound **1**.



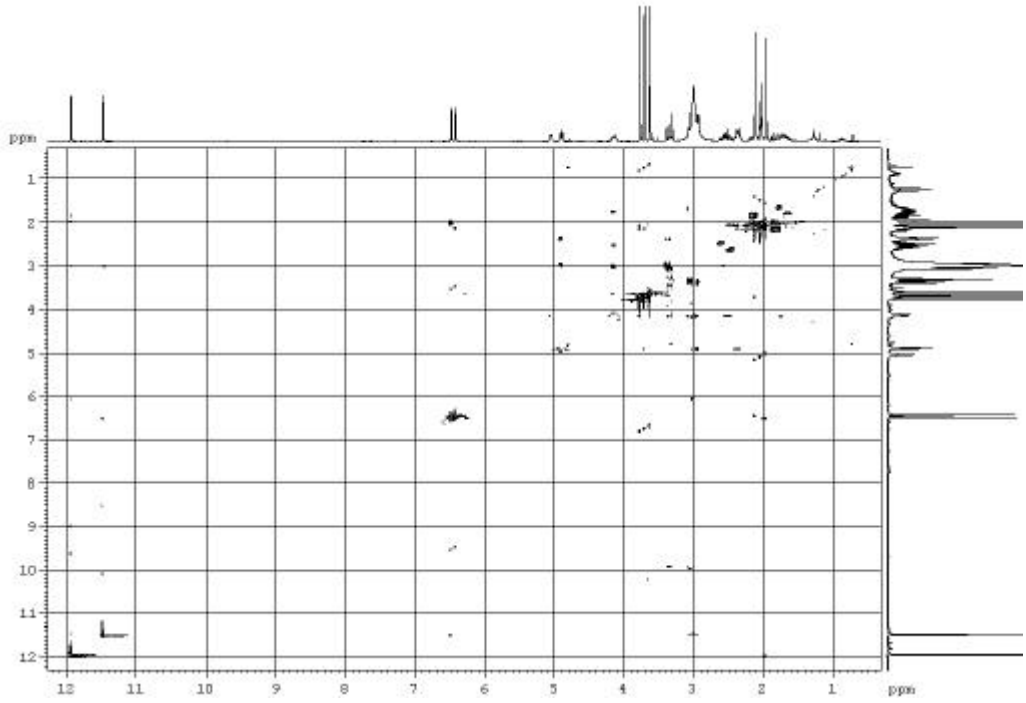
Supporting information –$^{1}$H,$^{1}$H-COSY spectrum (300 MHz in [$D_6$]acetone) of the new compound **1**.




Supporting information –$^{1}$H,$^{13}$C-HMBC spectrum (300 MHz in [$D_6$]acetone) of the new compound **1**.



Supporting information –$^{1}$H,$^{1}$H-NOESY spectrum (300 MHz in [$D_6$]acetone) of the new compound **1**.




Supporting information

**Table S2.** $^{1}$H NMR spectral data for sub-unit II of (*R*) and (*S*)-MPA products of **1** with calculated ? $d^{RS}$ values.

| no.[a] | (*R*)-MPA product<br>$d$ $^{1}$H ppm (mult.)[b] | (*S*)-MPA product<br>$d$ $^{1}$H ppm (mult.)[b] | ? $d^{RS}$ |
|---|---|---|---|
| 4 | 6.27 (s) | 6.40 (s) | -0.13 |
| 5 | 5.61 (d) | 5.59 (d) | +0.02 |
| 6 | 1.77 (m) | 1.76 (m) | +0.01 |
| 7 | 2.40 (m) | 1.99 (m) | +0.41 |
| 8aa | 2.75 (d) | 3.04 (s) | -0.29 |
| 8aß | 2.47 (d) | 3.04 (s) | -0.57 |
| 11 | 2.11 (s) | 2.12 (s) | -0.01 |
| 13 | 3.74 (s) | 3.80 (s) | -0.06 |
| 14 | 3.70 (s) | 3.63 (s) | +0.07 |
| OH-1 | 11.66 (s) | 11.76 (s) | -0.10 |

[a] Position of proton atom. [b] $CDCl_3$, 300/75.5 MHz.