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Supplementary Information

Discovery of a new PCC-mediated stereoselective oxidative spiroketalization process. An access to a new type of poly-THF spiroketal compounds displaying anticancer activity

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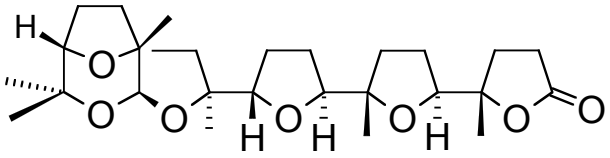
General Methods. All reagents and anhydrous solvents were purchased (Aldrich and Fluka) at the highest commercial quality and used without further purification. Reactions were monitored by thin-layer chromatography carried out on precoated silica gel plates (Merck 60, F_{254}, 0.25 mm thick). Merck silica gel (Kieselgel 40, particle size 0.063-0.200 mm) was used for column chromatography. HPLC separations were carried out on a Varian 2510 apparatus equipped with a Waters R403 dual cell differential refractometer using Phenomenex 250 x 10 mm and 250 x 4.6 mm (both 5μ) columns. NMR experiments were performed on Varian Unity-Inova 500 and Gemini 200 spectrometers in $CDCl_3$. Proton chemical shifts were referenced to the residual $CHCl_3$ signal (7.26 ppm); ^{13}C-NMR chemical shifts were referenced to the solvent (77.0 ppm). J values are in Hz. Abbreviations for signal coupling are as follows: s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet. IR spectra were collected on a Jasco FT-IR-430 spectrometer. ESI mass spectrometric analyses were recorded on an Applied Biosystems API 2000 mass spectrometer equipped with an Electrospray source used in the positive mode. For all the reported products the numeration previously given[1] for the penta-THF **1** is used.

[1] T. Caserta, V. Piccialli, L. Gomez-Paloma, G. Bifulco, *Tetrahedron*, 2005, **61**, 927-939.

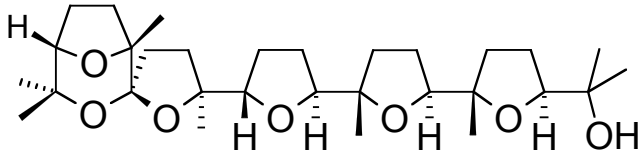
Spirolactone 2



To a solution of **1** (1.19 g, 2.27 mmol) in CH_2Cl_2 (50 mL) was added celite (11.5 g), PCC (2.45 g, 11.34 mmol) and AcOH (9.5 mL, 159 mmol) and the resulting heterogeneous mixture was stirred at room temperature for 4 days. A saturated aqueous $NaHCO_3$ solution (20mL) was added and the mixture extracted with CH_2Cl_2 (3 x 20 mL). The combined extracts were dried (Na_2SO_4) and evaporated in vacuo to give a yellow oil. Filtration on a silica gel pad (eluent $CHCl_3$-MeOH, 95:5) afforded a colourless oil (850 mg) that was separated by HPLC (250x10 mm column; flow: 2.5 mL/min; eluent: hexane-EtOAc, 65:35) to give *spirolactone* **2** (86 mg, 8%, t_R = 11.0 min), bis-lactone **3** (366 mg, 37%, *tr* = 24.5 min) and monolactone **4** (120 mg, 11%, *tr* = 18.5 min).

2: Oil. IR (neat) ν_{max} 1770 cm^{-1}. ^{1}H-NMR (200 MHz, $CDCl_3$) δ_H 3.91-3.71 (3H, m), 3.64 (1H, bt, J = 7.8), 2.78 (1H, dt, J = 17.5, 10.6), 2.54-2.28 (2H, m), 1.46 (3H, s), 1.32 (3H, s), 1.31 (3H, s), 1.27 (3H, s), 1.03 (3H, s), 1.02 (3H, s); ^{13}C-NMR (50 MHz, $CDCl_3$) δ_C 178.1, 109.6, 86.2, 85.9, 85.6, 85.1, 85.0 (two carbons), 82.5, 82.3, 74.4, 36.0, 34.6, 33.6 (two carbons), 32.4, 27.3, 27.2, 26.6, 26.5 (two carbons), 26.1, 25.2, 23.9, 23.4, 21.2. ESIMS: *m/z* 501.3 $[M+Na]^+$.

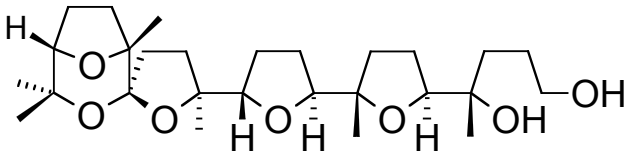
Spiroketal 5



The oxidation of **1** carried out as above described, in the absence of acetic acid, gave by HPLC separation (250×10 mm column; flow: 2.5 mL/min; eluent: hexane-EtOAc, 65:35), *spiroketal* **5** (4%, t_R = 9.5 min), **2** (4%), **3** (9%) and **4** (37%) along with unreacted **1** (ca 40%).

5: Oil. IR (neat) ν_{max} 3467 m^{-1}. ^{1}H-NMR (200 MHz, $CDCl_3$) δ_H 3.92-3.72 (4H, m), 1.48 (3H, s), 1.32 (3H, s), 1.27 (3H, s), 1.20 (3H, s), 1.10 (6H, s), 1.06 (3H, s), 1.02 (3H, s). ESIMS: *m/z* 545.2. $[M+Na]^+$.

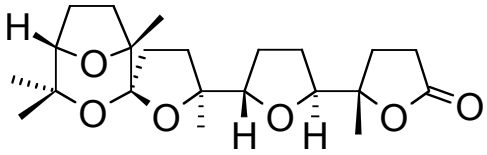
Spirodiol 9



To a solution of *spirolactone* **2** (12.7 mg, 0.026 mmol) in anhydrous ethyl ether (2 mL) was added $LiAlH_4$ (a tip of spatula) at room temperature under stirring. After 10 min the mixture was diluted with wet ether (3 mL) and water was dropwise added. The mixture was filtered and the solid was thoroughly washed with ether. The organic phase was dried (Na_2SO_4) and taken to dryness under reduced pressure to give *spirodiol* **9** (9.0 mg, 72%) as a colourless oil.

9: ^{1}H-NMR (200 MHz, $CDCl_3$) δ_H 3.89-3.59 (6H, overlapped m's), 1.47, 1.31, 1.26, 1.08, 1.07, 1.02 (3H each, s's, methyls). ESIMS: *m/z* 505.3 $[M+Na]^+$.

Degraded spirolactone 10



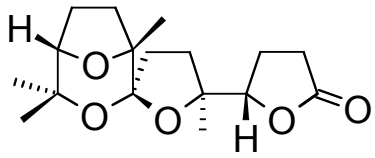
To spirodiol **9** (9.0 mg, 0.019 mmol) Ac_2O and pyridine (ten drops each) were added and the solution kept at 75°C for 3h, after which time the mixture was taken to dryness to give an essentially pure monoacetate product (9.0 mg, 92%). ^{1}H-NMR (200 MHz, $CDCl_3$) δ 4.10-4.00 (2H, bdt, *J*= 7.5, 1.9), 3.88-3.62 (4H, overlapped m's), 2.04 (3H, s, acetate), 1.47, 1.31, 1.25, 1.07, 1.04, 1.02 (3H each, s's, methyls).

To the crude acetate (9.0 mg, 0.017 mmol) in CH_2Cl_2 (1 mL) PCC (2.5 equiv., 9.3 mg, 0.043 mmol) and AcOH (35 equiv., 0.6 mmol, 36 μL) were added and the mixture was refluxed for 3h. Then a saturated aqueous $NaHCO_3$ solution (1 mL) was added and the mixture extracted with CH_2Cl_2 (3x 5 mL). The organic phase was filtered, dried (Na_2SO_4) and taken to dryness. The crude was filtered through a short pad of silica gel eluting with $CHCl_3$-CH_3OH (95:5) and then purified by HPLC (250x 10 mm column, flow=2.5 mL/min, eluent: hexane-EtOAc, 65:35, *tr* = 15.5 min) to give pure *spirolactone* **10** (6.0 mg, 95%) as an oil.

10: Oil. IR (neat) ν_{max} 1772 cm^{-1}. ^{1}H-NMR (200 MHz, $CDCl_3$) δ_H 3.94-3.81 (2H, m), 3.77-3.65

(1H, m), 3.74 (1H, ddd, J = 17.2, 10.0, 10.1, 1.46 (3H, s, methyl), 1.31 (6H, s, two methyls), 1.23, 1.02 (3H each, s's, methyls). ESIMS: *m/z* 417.2 $[M+Na]^+$.

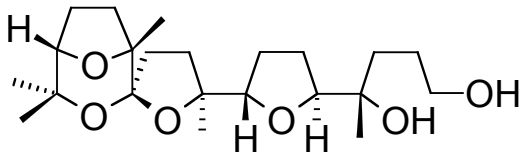
Double degraded spirolactone 8



Spirolactone **8** was obtained from **10** according to the three-step sequence used to convert **2** in **10**. In particular, starting from 4.1 mg (0.010 mmol) of **10**, 4.4 mg of crude intermediate spirodiol were obtained. Acetylation of 3.7 mg of this material under standard conditions proceeded in a quantitative yield giving the monoacetate derivative (3.5 mg, 97% from **10**) that was oxidized with PCC to eventually deliver crude *spirolactone* **8** (3.7 mg). Purification by HPLC (250x4.6 mm column, flow=1.0 mL/min, eluent: hexane-EtOAc, 80:20, *tr* = 30.5 min) gave pure *spirolactone* **8** (2.4 mg, 75% from **10**) as an oil. A 92% yield of the PCC-mediated step was estimated by ^{1}H-NMR.

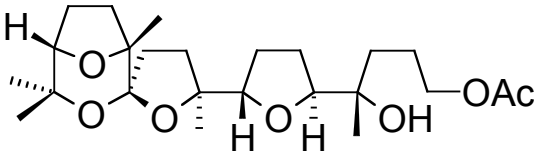
8: Oil. IR (neat) ν_{max} 1770 cm^{-1}. ^{1}H-NMR (200 MHz, $CDCl_3$) δ_H 4.32 (1H, t, J = 6.9), 3.86 (1H, d, J = 6.9), 1.47, 1.33, 1.26, 1.03 (3H each, s's, methyls). ESIMS: *m/z* 333.1 $[M+Na]^+$.

Intermediate spirodiol



^{1}H-NMR (200 MHz, $CDCl_3$) δ_H 3.91-3.74 (2H, m), 3.65 (1H, bt, J = 6.0), 1.47, 1.31, 1.26, 1.07, 1.02 (3H each, s's, methyls). ESIMS: *m/z* 421.3 $[M+Na]^+$.

Intermediate spirodiol acetate



^{1}H-NMR (200 MHz, $CDCl_3$) δ_H 4.05 (2H, bt, J = 7.6), 3.90-3.73 (3H, overlapped m's), 2.03 (3H, s, acetate), 1.47, 1.30, 1.25, 1.03, 1.02 (3H each, s's, methyls). ESIMS: *m/z* 463.2 $[M+Na]^+$.

Antitumor activity of poly-THF spiroketal compounds. Ovarian cancer cell line (HEY) and breast cancer cell line (BT474) were treated with different concentrations (0.1μM, 1μM and 10μM) of spiro-compounds **2**, **5** and **9**. At different time points the viability of the cells was assessed measuring the mitochondrial activity.[2] We found that all the compounds showed to increase mitochondrial activity at day 3. This phenomenon is often observed and can be explained as "starving state" of the cells before cell death. According to this hypothesis at later time points cell viability decreased in all treated conditions compared with control group (Fig. S1). Already at week 1 compound **2** showed a 19% cell death at the concentration of 0.1μM, 20% at 1μM and 13% at 10μM in BT474 cell line (Fig. S1 panels A, B, C. White circle), and 33% cell death at the concentration of 0.1μM, 30% at 1μM, 11% at 10μM in HEY cell line (Fig. S1 panels D, E, F. White circle). At week 2, the same compound showed 20% of cell death at 0.1μM, 42% at 1μM, 67% at 10μM in BT474 cell line, and 39%, 60%, 66% at 0.1μM, 1μM, 10μM in HEY cell line, respectively. The same trend was also observed with the two other analyzed compounds. At week 1 compound **9** showed 23%, 29%, 27% cell death at the concentration of 0.1 μM, 1 μM, 10μM, respectively, in BT474 cell line (Fig. S1 panels A, B, C. Black circle), and 57%, 52%, 20% at the concentration of 0.1 μM, 1 μM, 10μM, respectively, in HEY cell line (Fig. S1 panels D, E, F. Black circle). At week 2 the same compound showed 16%, 30%, 55% cell death at 0.1 μM, 1 μM, 10μM, respectively, in BT474 cell line (Fig. S1 panels A, B, C. Black circle), and 41%, 59%, 67% at the concentration of 0.1μM, 1μM, 10μM, respectively, in HEY cell line (Fig. S1 panels D, E, and F. Black circle). The compound that resulted to be the most effective in our model resulted compound **5**. At week 1 this compound showed 45%, 18%, 30% cell death at the concentration of 0.1μM, 1μM, 10μM, respectively, in BT474 cell line (Fig. S1 panels A, B, C. Grey circle), and 61%, 55%, 38% at the concentration of 0.1μM, 1μM, 10μM, respectively, in HEY cell line (Fig. S1 panels D, E, and F. Grey circle). At week 2 the same compound showed 22%, 35%, 76% cell death at the concentration of 0.1μM, 1μM, 10μM, respectively, in BT474 cell line (Fig. S1 panels A, B, C. Grey circle), and 51%, 61%, 70% at the concentration of 0.1μM, 1μM, 10μM, respectively, in HEY cell line (Fig. S1 panels D, E, and F. Grey circle). Interestingly, at latest time-point, both HEY and BT474 cell lines seemed to have acquired resistance to low concentrations of drugs while the same effect was not observed at higher concentration.

[2] A. H. Cory, T. C. Owen, J. A. Barltrop, J. G. Cory, *Cancer Commun.*, 1991, **3**, 207-212.

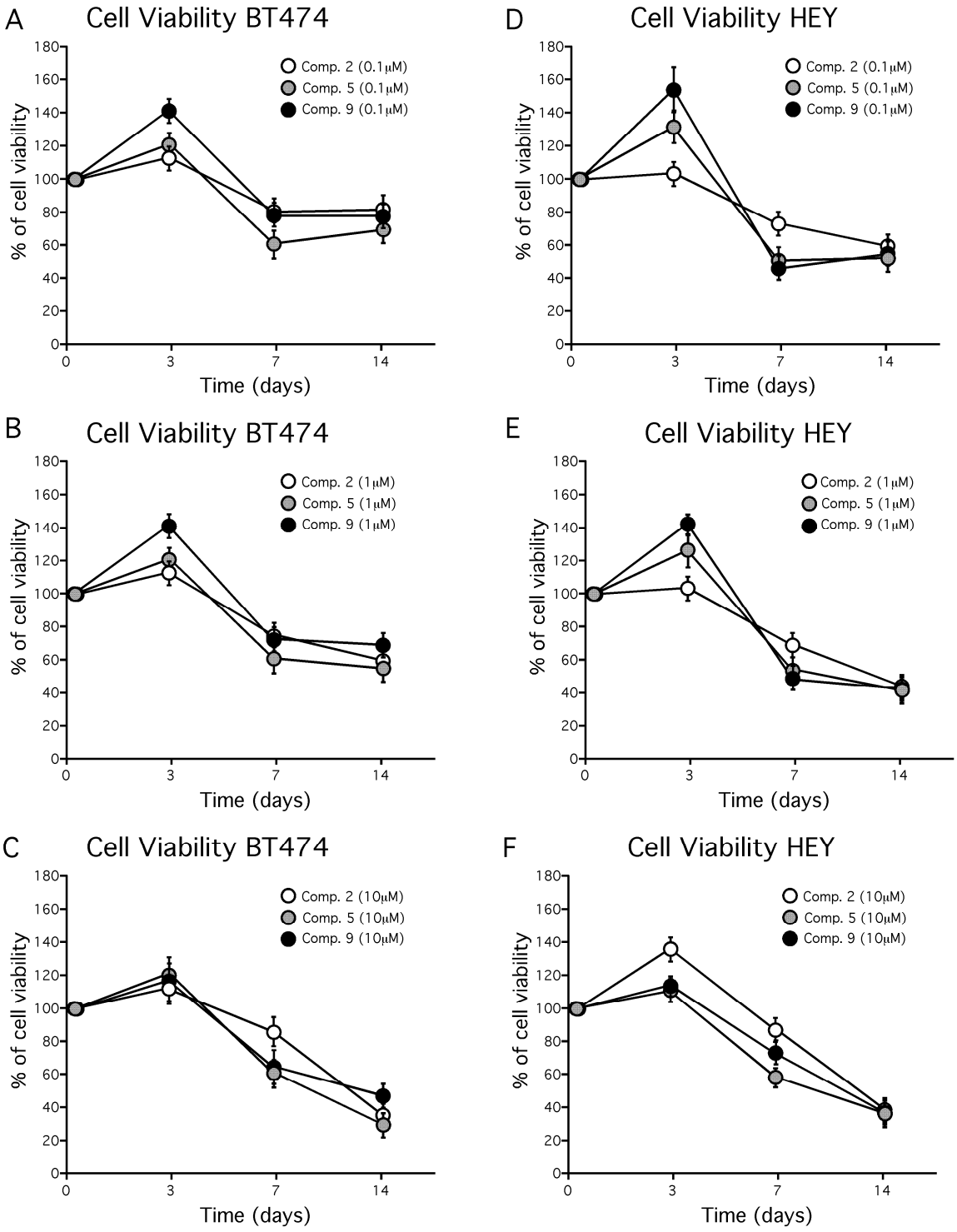


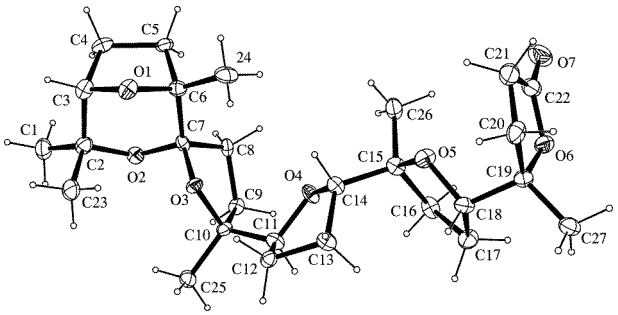
Fig. S1 Cytotoxic effect of spiro-compounds 2, 5 and 9 in two different tumor-derived cell lines. Breast cancer-derived cell line (BT474) was treated with three different concentrations (0.1μM, Panel A; 1μM Panel B; 10μM Panel C) of compound **2** (white circle, ○) compound **9** (black circle, ●) and compound **5** (grey circle, ◍) and cell viability was assessed over time. Ovarian cancer-derived cell line (HEY) was treated with three different concentrations (0.1μM, Panel D;

1μM Panel E; 10μM Panel F) of compound **2** (white circle) compound **9** (black circle) and compound **5** (grey circle) and cell viability was assessed over time.

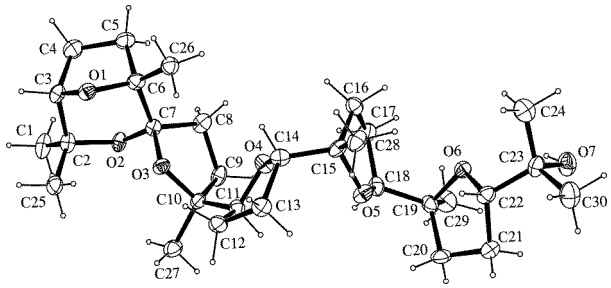
Description of the Assay

Cell viability was assessed by MTS assay as described elsewhere.[2] Briefly, ovarian (HEY cell line) and breast (BT474 cell line) cancer cells were seeded at the concentration of 0.5×10^5 cells per well on 96-well plate and maintained under appropriate condition (RPMI 1640 or DMEM, completed with 10% FCS, 2 mmol L-glutamine and 100 units/mL of penicillin (all from Sigma, St. Luis MO). Every second day cells were washed with PBS and media containing different concentrations of tested compounds (0.1μM, 1μM and 10μM) were replaced in all wells except the controls. Control wells only received media and solvent used for the dissolution of the compounds. At the indicated time point cell viability was assessed using CellTiter 96 Aqueous One Solution Cell Proliferation Assay (Promega), i.e., the 3-(4,5,dimethylthiazol-2-yl)-5-(3-carboxymethoxy-phenyl)-2-(4-sulfophenyl)-2H-tetrazolium, inner salt (MTS) assay and compared with their control.

Single-crystal X-ray diffraction report for compounds 2 and 5. Ellipsoids are drawn at 30% probability level.



2



5

Prismatic colourless crystal of **2** (0.3 x 0.3 x 0.1 mm) and a plate-like colourless crystal of **5** (0.3 x 0.2 x 0.02 mm) were selected for X-ray analysis. Single crystal X-ray diffraction data were collected using graphite monochromated MoKα radiation (λ = 0.71073 Å) on a Bruker-Nonius kappaCCD diffractometer, equipped with an Oxford Cryostream N_2 open-flow cooling device. Data were collected at 173(2) K for both compounds. The data were collected to a maximum resolution of 0.76 Å for **2** and 0.84 Å for **5**. Cell parameters and intensity data were processed using Dirax/lsq[3] and Collect programs.[4]

Both structures were solved by direct methods[5] and refined by the full-matrix least squares method on *F* using SHELXL program.[6] Intensities were corrected for absorption effects by the multi-scan method using SADABS program.[7] All non-H atoms were refined with anisotropic displacement parameters. Although all H atoms were clearly located in difference Fourier maps, their positions were determined on stereochemical grounds and refinement was performed with the riding model with U_{iso} in the range 1.0-1.5 times U_{eq} of the carrier atom. The only exception was the hydroxyl H atom of **5** whose position was determined by difference Fourier map. Also in this case refinement was by the riding model.

Crystals of **2** are twinned according to the twinning law [-1, 0, 0 / 0, -1, 0 / 0, 0 1] so HKLF5 was used in SHELXL refinement and no average of equivalent reflections was performed. Crystals of **5** were poorly diffracting.

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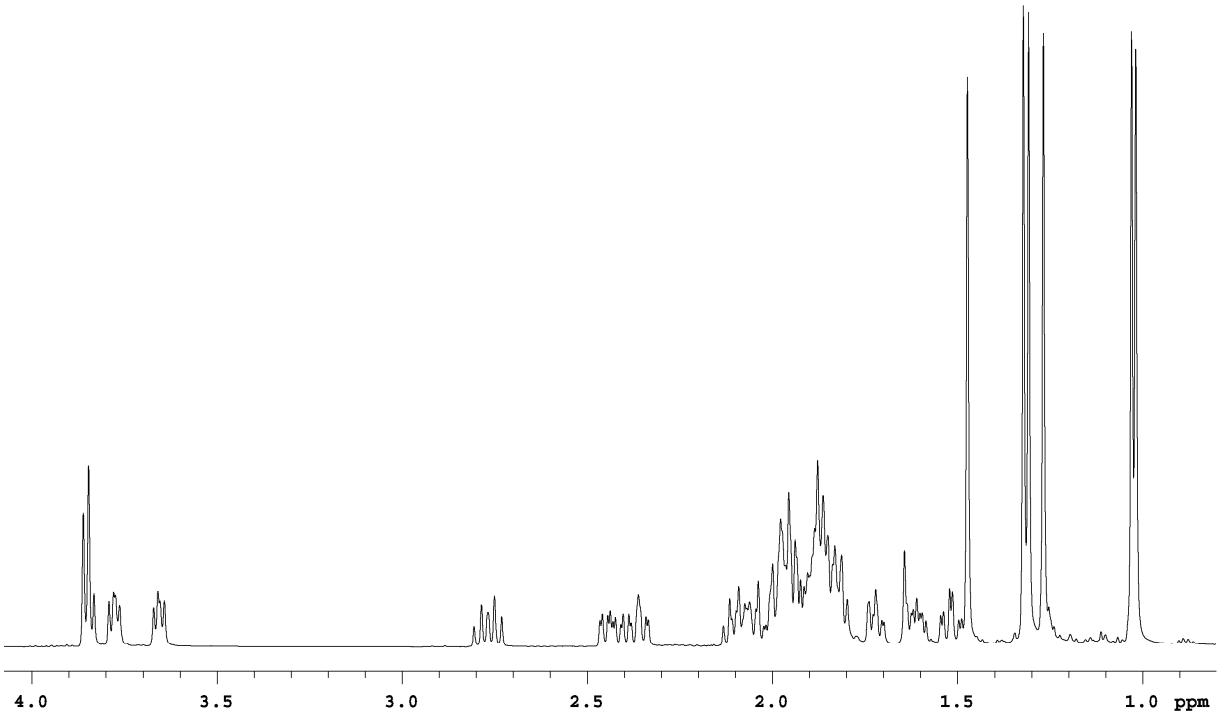
[3] A. J. M. Duisenberg, R. W. W. Hooft, A. M. M. Schreurs, J. Kroon, *J. Appl. Cryst.*, 2000, **33**, 893-898.
[4] Nonius, COLLECT. Nonius BV, Delft, The Netherlands, 1999.
[5] A. Altomare, M. C. Burla, M. Camalli, G. L. Cascarano, C. Giacovazzo, A. Guagliardi, A. G. G. Moliterni, G. Polidori, R. Spagna, *J. Appl. Cryst.*, 1999, **32**, 115-119.
[6] G. M. Sheldrick, *Acta Cryst.*, 2008, **A64**, 112-122.
[7] Bruker-Nonius, SADABS. Bruker-Nonius, Delft, The Netherlands, 2002.

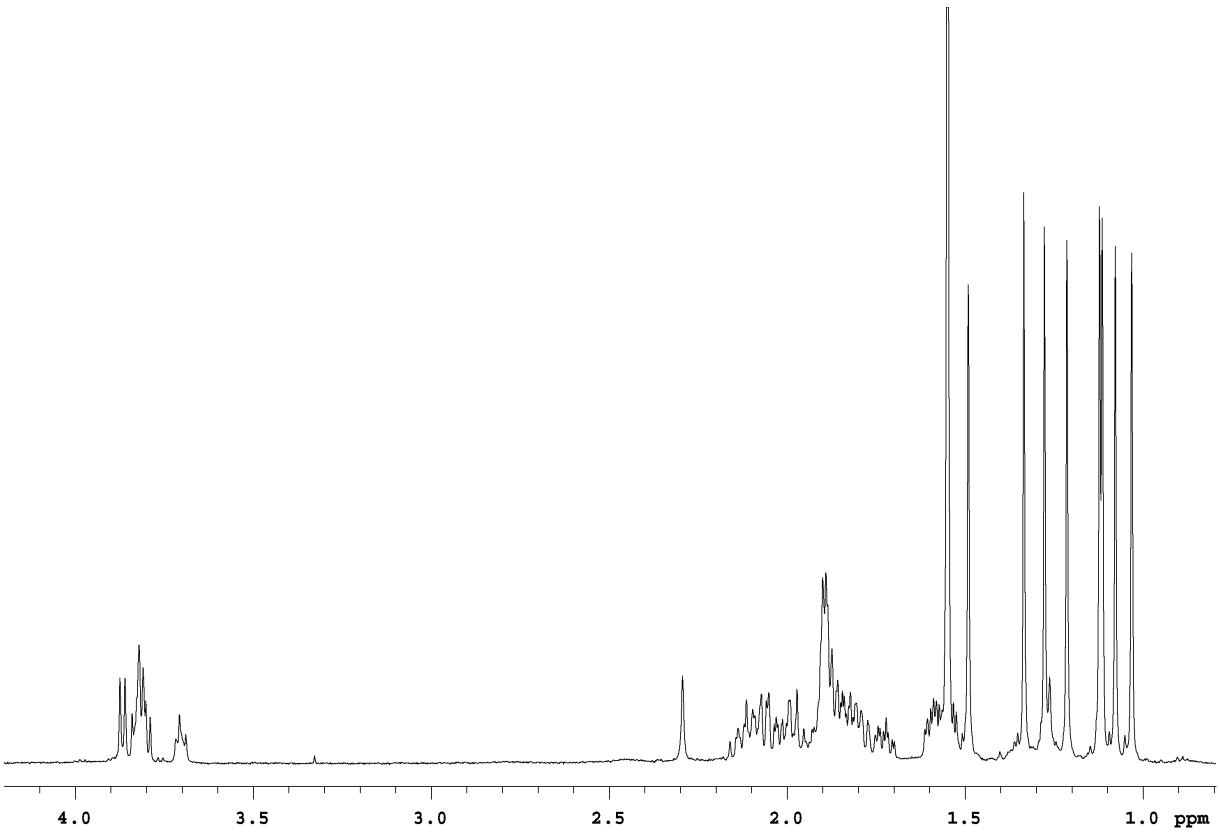
Single crystal X-ray diffraction data for **2**. $C_{27}H_{42}O_7$; Mr = 478.61; monoclinic (P 2_1/c); a = 13.209(1), b = 7.494(1), c = 25.505(5) Å; β = 91.45(1)°; V = 2523.9(6) $Å^3$; T = 173(2) K; Z = 4; μ = 0.089 mm^{-1}; Dcalc = 1.260 g/cm^3; reflections collected 18190; R values [I > 3σ(I), 5205 reflections]: R_1 = 0.0575, wR_2 = 0.2048. Crystallographic data (excluding structure factors) for compound **2** have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication CCDC 717142. Copies of the data can be obtained, free of charge, on application to CCDC, 12 Union Road, Cambridge CB2 1EZ, UK, (fax: +44-(0)1223-336033 or e-mail: deposit@ccdc.cam.ac.uk).

Single crystal X-ray diffraction data for **5**. $C_{30}H_{50}O_7$; Mr = 522.70; monoclinic (P 2_1/c); a = 15.162(4), b = 8.489(9), c = 24.593(4) Å; β = 111.634(9)°; V = 2942(3) $Å^3$; T = 173(2) K; Z = 4; μ = 0.082 mm^{-1}; Dcalc = 1.180 g/cm^3; reflections collected 22393; independent reflections 5101; R values [I > 3σ(I), 1593 reflections]: R_1 = 0.0687, wR_2 = 0.2574. Crystallographic data (excluding structure factors) for compound **5** have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication CCDC 717143. Copies of the data can be obtained, free of charge, on application to CCDC, 12 Union Road, Cambridge CB2 1EZ, UK, (fax: +44-(0)1223-336033 or e-mail: deposit@ccdc.cam.ac.uk).

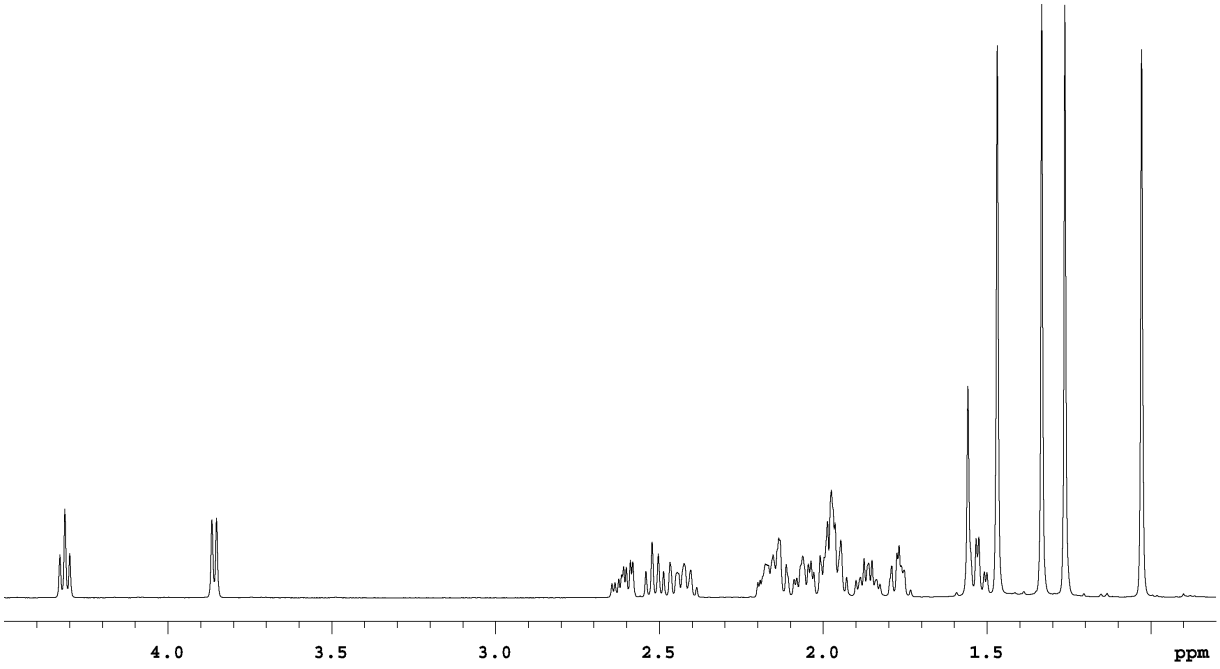
^{1}H-NMR spectrum of 2 (500 MHz, $CDCl_3$).



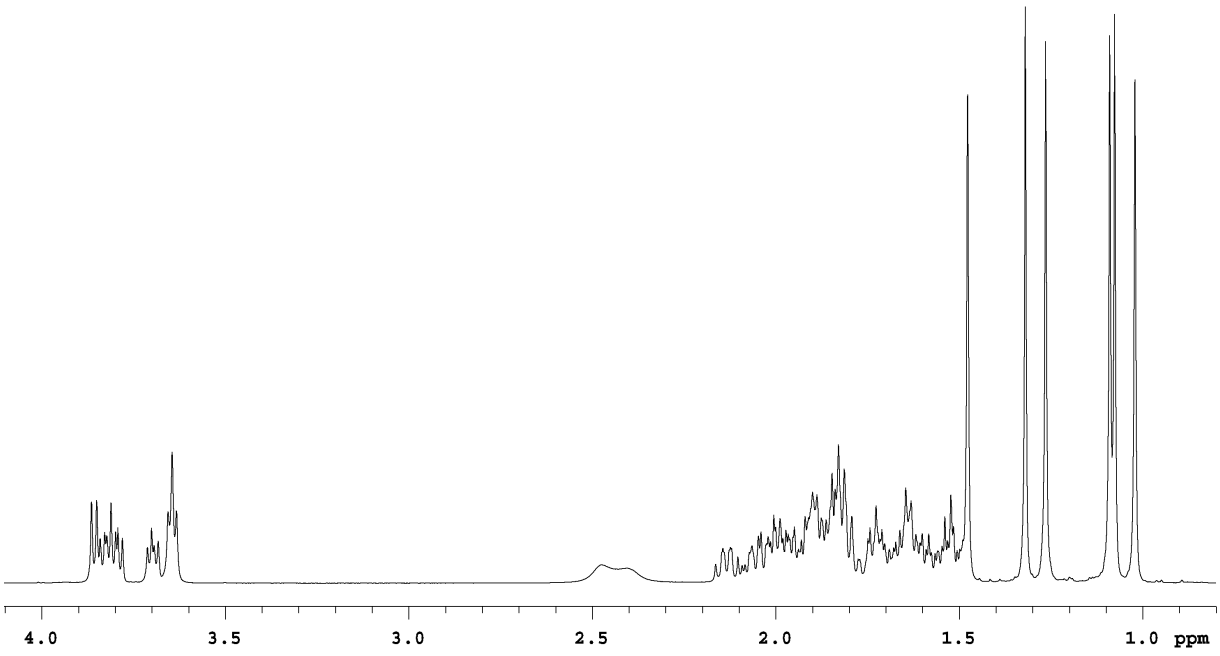
^{1}H-NMR spectrum of 5 (500 MHz, $CDCl_3$).



^{1}H-NMR spectrum of 8 (500 MHz, $CDCl_3$).



^{1}H-NMR spectrum of 9 (500 MHz, $CDCl_3$).



^{1}H-NMR spectrum of 10 (500 MHz, $CDCl_3$).