Tetrahedron 66 (2010) 9370–9378

Contents lists available at ScienceDirect

Tetrahedron

journal homepage: www.elsevier.com/locate/tet

Discovery of a novel one-step RuO_4-catalysed tandem oxidative polycyclization/double spiroketalization process. Access to a new type of polyether bis-spiroketal compound displaying antitumour activity

Vincenzo Piccialli [a,*], Sabrina Zaccaria [a], Nicola Borbone [b], Giorgia Oliviero [b], Stefano D'Errico [b], Akseli Hemminki [c], Vincenzo Cerullo [c], Valentina Romano [c], Angela Tuzi [d], Roberto Centore [d]

[a] Dipartimento di Chimica Organica e Biochimica, Università degli Studi di Napoli 'Federico II', Via Cynthia 4, 80126 Napoli, Italy
[b] Dipartimento di Chimica delle Sostanze Naturali, Università degli Studi di Napoli 'Federico II', Via D. Montesano, 49, 80131 Napoli, Italy
[c] Department of Molecular Cancer Biology, University of Helsinki, Biomedicum, Haartmaninkatu 8, Helsinki, Finland
[d] Dipartimento di Chimica, Università degli Studi di Napoli 'Federico II', Via Cynthia 4, 80126 Napoli, Italy

ARTICLE INFO

Article history:
Received 19 May 2010
Received in revised form 13 September 2010
Accepted 4 October 2010
Available online 25 October 2010

Keywords:
RuO_4
Squalene
Oxidative pentacyclization
Double oxidative spyroketalization
C_{30} polyether bis-spiroketals
Antitumor activity

ABSTRACT

Four novel C_{30} polyether bis-spiroketals, displaying selective inhibition of the BT474 breast-derived cancer cell line, have been obtained from squalene through an unprecedented one-step, RuO_4-catalysed, cascade process characterised by a tandem oxidative pentacyclization/double oxidative spiroketalization sequence. Preliminary studies indicate that the Ru-mediated spiroketalization steps proceed with retention of configuration at the forming spirocentres. A similarity with the oxidative behaviour of PCC has been disclosed.

1. Introduction

The ruthenium tetroxide mediated oxidative polycyclization of polyenes characterised by a repetitive 1,5-diene structural motif is a unique stereoselective process discovered a few years ago in our laboratories.[1] It allows adjacently linked poly-tetrahydrofuran products to be obtained in a single step employing catalytic amounts of ruthenium tetroxide in the presence of $NaIO_4$ as co-oxidant. In particular, the pentacyclization of squalene (Scheme 1)[1a,c] is a remarkable transformation in terms of stereoselectivity, overall yield and stereochemical complexity of the final penta-THF product (**1**) that includes ten newly-generated chiral centres. The cheapness and the availability of the starting material[2] allowed the straightforward preparation of multi-gram amounts of this substance.

More recently, we have discovered that penta-THF **1** when treated with PCC undergoes a stereoselective oxidative spiroketalization process to give compounds **2** and **3** (Scheme 1) characterised by an unprecedented terminal tricyclic (A/B/F rings) spiroketal moiety.[3] In addition, we have devised a high-yielding route leading to small-sized spiroketals of the same type (e.g., **4** and **5**, Scheme 1) starting from **2** and/or **3**. Preliminary cytotoxicity tests carried out on **2** and **3** showed that both displayed a significant inhibition on HEY ovarian-derived cancer cell line and BT474 breast-derived cancer cell line.[3]

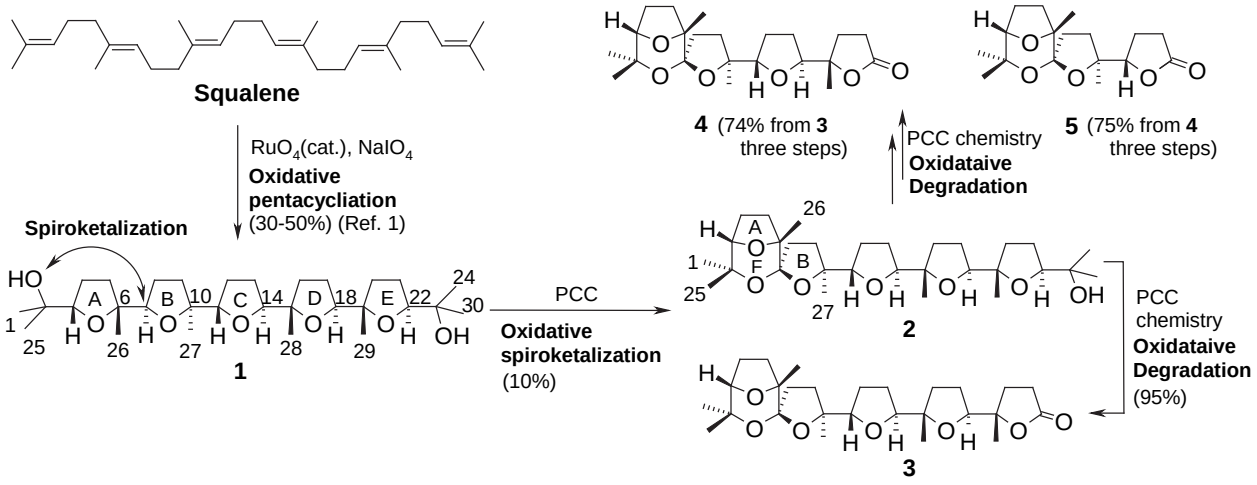
In continuing our studies in this field we report here the discovery of a novel RuO_4-catalysed tandem oxidative polycyclization/double oxidative spiroketalization process, a transformation partly related to the above PCC-mediated process, that allows the one-step assembly of the new structurally complex polyether bis-spiroketals **6**–**9** (Scheme 2) starting from squalene.

2. Results and discussion

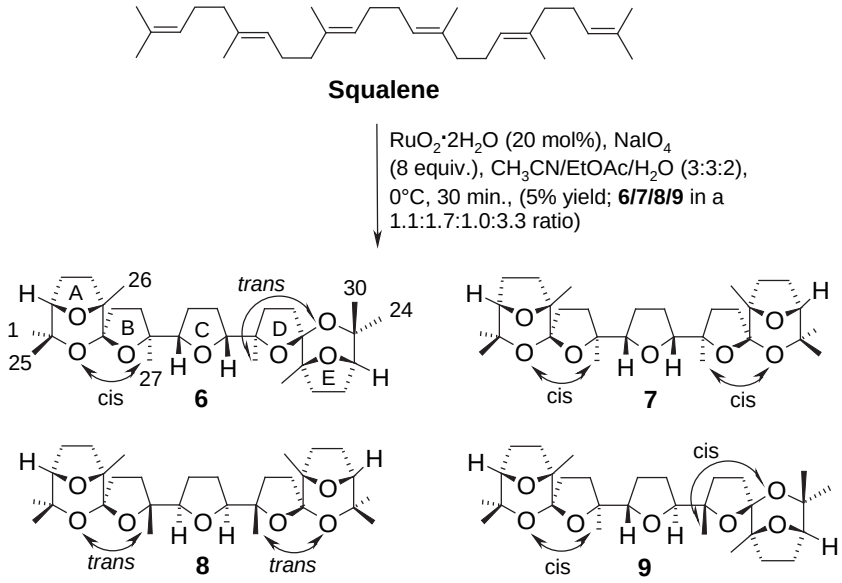
Aimed at preparing greater amounts of spiro-compounds **2**–**5** (Scheme 1) to gain further insight into the chemistry and anticancer activity of this new type of substance, we planned to prepare a large amount of penta-THF **1** (Scheme 1) by scaling-up the RuO_4-mediated oxidative cyclization of squalene. A problem encountered on this road was the too large volume of solvent (14 L of a 3:3:1, $CH_3CN/EtOAc/H_2O$, mixture) required to completely dissolve the

* Corresponding author. Tel.: +39 081 674111; fax: +39 081 674393; e-mail address: vinpicci@unina.it (V. Piccialli).

0040-4020/$ – see front matter © 2010 Elsevier Ltd. All rights reserved.
doi:10.1016/j.tet.2010.10.004



Scheme 1. Structurally complex poly-THF and spiroketal compounds through RuO_4-catalysed and PCC-mediated oxidative chemistry starting from squalene.

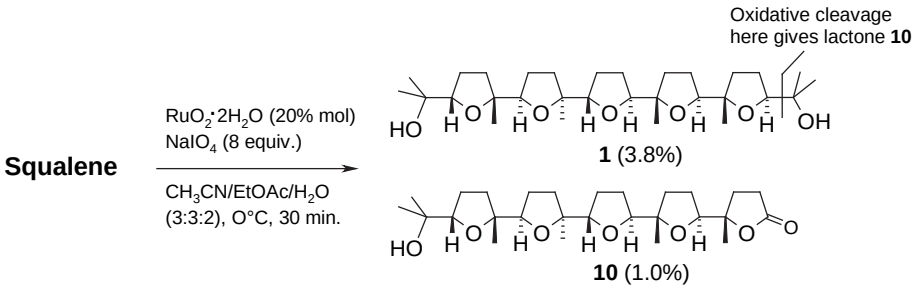


Scheme 2. Novel C_{30} bis-spiroketals by RuO_4-catalysed tandem oxidative polycyclization/double oxidative spiroketalization of squalene. Letters marking THF rings in **6** highlight the structural relationship with compounds **1** and **2** of Scheme 1.

co-oxidant ($NaIO_4$) under standard polycyclization conditions.[1a,c] Therefore, in one of the experiments devised to this end, squalene was oxidized with the same (RuO_2 (cat.)/$NaIO_4$) oxidising system using about one tenth of the above volume in the new 3:3:2, $CH_3CN/EtOAc/H_2O$, solvent mixture, at 0 °C, where the co-oxidant was in part undissolved. We reasoned that the complete dissolution of periodate could nonetheless take place during the process due to the precipitation of sodium iodate concomitant to the oxidation of the substrate.[4] The new conditions allowed to oxidise a 50 g amount (122 mmol) of squalene using a volume of solvent (1.6 L) acceptable on a laboratory scale. Interestingly, squalene oxidation under the new conditions followed in part a new path. In particular, though under the new conditions squalene was completely consumed, penta-THF **1**, usually produced in good yields under standard conditions, was only obtained in a less than 4% yield besides to a ca. 1% amount of the terminal monolactone **10**[1c] (Scheme 3), likely derived from **1** by oxidative cleavage of the hydroxypropyl terminus bonded to the terminal *trans*-THF ring, as previously observed for PCC (Scheme 1, see conversion of **2** to **3**). Formation of such a terminal lactone had also been observed in the oxidative polycyclization of digeranyl with the same oxide.[1f] However, contrary to what observed under the previously employed conditions,[1] an abundant less polar fraction was now produced along with a very abundant more polar fraction comprising most of the recovered material.

HPLC separation of the less polar fraction, revealed it to be composed of a mixture of spiroketal compounds, structurally related to compounds **2**–**5** (Scheme 1) on the basis of the characteristic chemical shifts of the methyl proton signals pertinent to the spiroketal moieties (1.46–1.47 and 1.01 ppm: Me-1/Me-25 and/or Me-24/Me-30, Scheme 2; numbering in these compounds follows the one of penta-THF **1** shown in Scheme 1). In particular, the four isomeric polyether bis-spiroketals **6**–**9** were isolated as the main products belonging to this fraction, in an overall 5% yield (their approximate ratio is **6**/**7**/**8**/**9**, 1.1:1.7:1.0:3.3).

The stereostructure of the non-symmetric compound **6** was determined by X-ray diffraction analysis (Fig. 1) carried out on a single crystal of the substance obtained from hexane/EtOAc (9:1)



Scheme 3. Further poly-THF products derived from the oxidation of squalene.

by slow evaporation of the solvent. It is made up of a central *cis*-THF ring flanked by two structurally similar terminal tricyclic spiroketal moieties only differing by the configuration of the THF rings (B and D) engaged into the spiroketal junctions. In particular, O2/Me-27 are cis and O7/Me-28 are trans (Fig. 1 and Scheme 2). The two bridging THF rings (A and E) involved into the spiroketal portions both possess cis configuration and, in addition, an all-*threo* relationship subsists between the central THF and the flanking THFs, a fact that has significant mechanistic implications (see later Scheme 4 and Scheme 5).[1a,c]

and O2/Me-27 and O7/Me-28 are both cis as well. Compound **8** still possesses the same three A, C and E *cis*-THF rings but O2/Me-27 and O7/Me-28 are now trans. Compound **9** possesses a C_2 symmetry differing from **7** only for the trans nature of the central THF ring. In addition, a *threo* relationship exists between B,C and C,D pair of rings in compounds **7**–**9** as well.

Interestingly, contrary to what expected on the basis of the similarity of the RuO_4- and PCC-mediated spiroketalization processes, formation of mono-spiroketals **2** and/or **3**, derived from the sole spiroketalization of the penta-THF **1** at its cis–cis-terminus

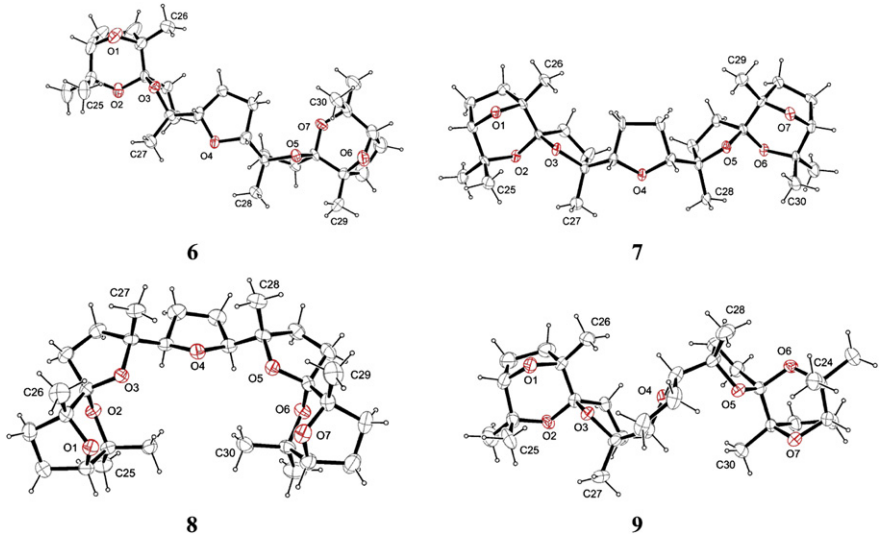
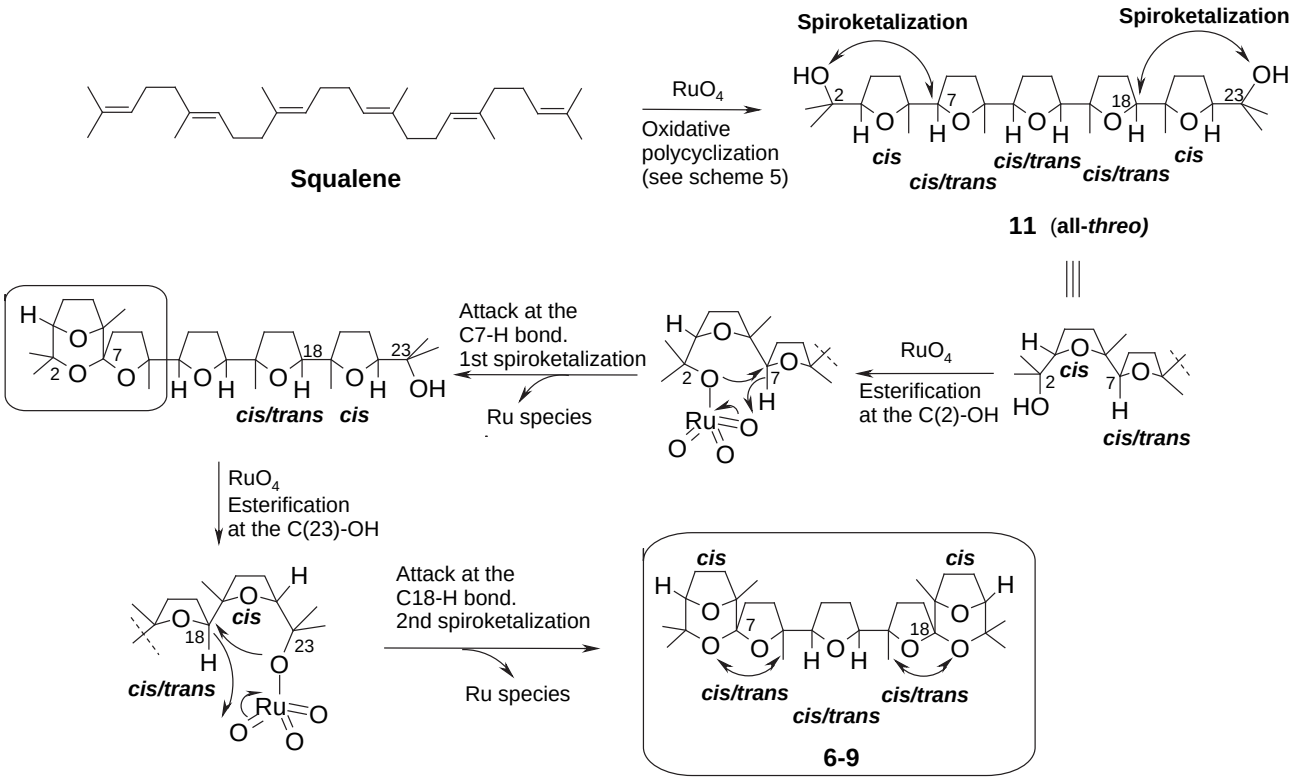


Fig. 1. ORTEP drawings of bis-spiro-compounds **6**–**9**. Ellipsoids are drawn at 30% probability level.

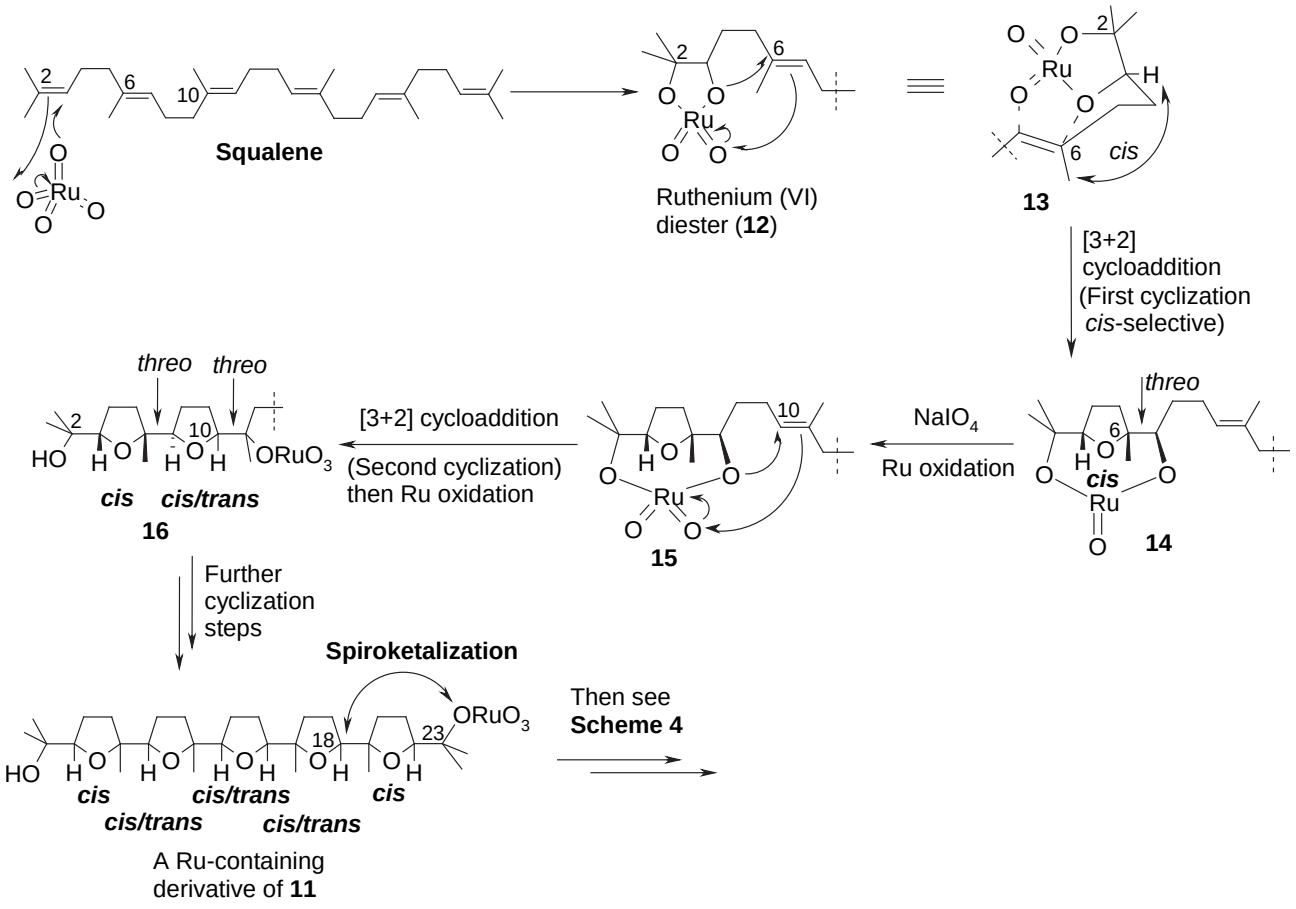
Bis-spiroketals **7**–**9** (Fig. 1 and Scheme 2) proved to be isomeric with **6** and possessed symmetric structures as indicated by the halving of the ^1H and ^{13}C NMR signals. 2D NMR data could give unambiguous information on the spiroketal moiety in all these compounds. In particular, as previously observed for related mono-spiroketals **2** and **3** (Scheme 1), the rigidity of the spiroketal portions invariably produced strong NOE correlations between Me-25 and Me-27 when B ring is cis, as in **6**, **7** and **9** or, alternatively, between Me-26 and Me-27 when B ring is trans as in **8** (Fig. 2). However, NMR data could not settle the cis/trans identity of the central THF ring in these compounds due to their symmetry. The complete stereostructure of **7**–**9** was eventually determined by X-ray diffraction analysis for these compounds as well (Fig. 1). Crystals suitable for these experiments were obtained for all three substances from hexane by slow evaporation of the solvent. Compounds **7** and **8** are both C_S-symmetric. The former possesses the three THF rings of the molecule (A, C and E) in a cis configuration

(see Scheme 1) was not observed. However, it cannot be excluded that these compounds once formed could undergo further oxidative transformations.

From a mechanistic point of view, seven consecutive cyclization steps should take place to built up the structure of spiroketals **6**–**9** from squalene (Scheme 4), simply taking into account the closure of the seven rings composing these compounds. Actually, given the catalytic nature of the overall process, the reoxidation of ruthenium bonded to the various intermediates (see Scheme 5 later) takes place more or less doubling the involved steps. The *threo* nature of all inter-THF relationships in all four bis-spiroketals suggests that a penta-THF backbone (see **11**) was preliminarily assembled through a cascade process (five consecutive THF-forming steps) where the *syn*-addition of two oxygens across each reacting trans double bond takes place according to the mechanism previously hypothesised for the formation of penta-THF **1**.[1a,e] The configuration of each THF shown in **11** reflects the ones found in bis-spiroketals **6**–**9** to which it



Scheme 4. A plausible mechanistic hypothesis explaining the formation of bis-spiroketals **6**–**9** from squalene.



Scheme 5. Ru-catalysed cascade sequence leading to the intermediate penta-THF **11** shown in Scheme 4.

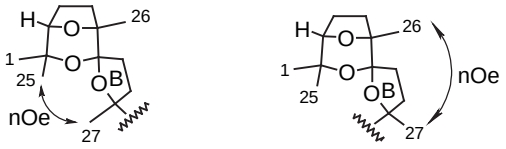
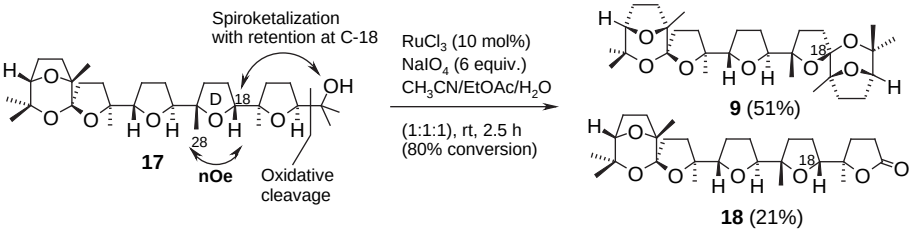


Fig. 2. Characteristic nOe correlations within the spiroketal moiety, including a cis (left) or trans (right) ring-B THF, observed for **6**–**9**.

eventually give rise. Once formed each bis-THF terminus of the proper penta-THF diol **11** is involved into an oxidative spiroketalization step where the C-7 and C-18 spirocentres are formed very likely through the action of a close-in-space oxoruthenium appendage tethered to C-2 or C-23 on the C(7)-H or C(18)-H bonds, respectively. Though the ability of RuO_4 to attack the α-hydrogens of tetrahydrofuran (oxidation of tetrahydrofuran to γ-butyrolactone) is known,[5] this type of reactivity is unprecedented.

As for the formation of the penta-THF diol intermediate **11** (Scheme 4), the first steps of the cascade process leading to this species are shown in Scheme 5. In particular, the sequence begins with the attack of RuO_4 at the terminal double bond of squalene to give a ruthenium(VI) diester (**12**). Closure of the first THF ring proceeds with cis stereocontrol, as usually observed in the oxidative mono-cyclizations of 1,5-dienes mediated by all three related oxo-species RuO_4,[6] OsO_4[7] and MnO_4[8] as well as for the RuO_4-catalysed polycyclizations of both linear and isoprenoid polyenes.[1a–f] Thus, a *cis-threo* mono-THF intermediate species (**14**) is obtained through a [3+2] cycloaddition of an O–Ru=O portion of the ruthenium bis-glycolate **12** across the second (Δ^6) C–C double bond, with the molecule adopting the chair-like conformation **13** in the transition state. Then, the metal, is oxidised at an 'active' oxidation state (see intermediate **15**) by $NaIO_4$, thereby allowing for the second cyclization step to take place via another [3+2] cycloaddition reaction once again involving an O–Ru=O portion and the successive (Δ^{10}) double bond in the carbon chain, to give the bis-*threo*, bis cyclised, intermediate **16**. Usually, this second cyclization step is cis-selective as well but in one case, the oxidation of digeranyl,[1f] we observed a trans selectivity. Reiteration of the cyclization/ruthenium oxidation sequence then occurs with a Ru-containing portion (likely RuO_3) migrating along the carbon chain to eventually deliver penta-THF **11** diol by hydrolysis or more likely this species is not formed and the Ru-containing appendage tethered to its terminus C(23)-OH initiate the first spiroketalization as shown in Scheme 4. Therefore, in the new process leading to bis-spiroketals **6**–**9**, only the first THF-forming step proceeds with the usual cis stereoselectivity with all the other THF being formed in a non stereoselective manner with a cis or trans configuration.

We have evidence indicating that the spiroketal-forming steps involved in the sequence shown in Scheme 4 proceeds with retention of configuration at the involved (C-7 or C-18) carbon centres. In particular, besides bis-spiroketals **6**–**9**, mono-spiroketal **17** (Scheme 6), possessing the same constitution of **2** (see Scheme 1), was isolated from the reaction mixture as well (0.5% yield), likely originating from a single spiroketalisation of the suitable penta-THF precursor according to the mechanism shown in Scheme 4. Its structure, was deduced by 2D NMR. In particular, *inter alia*, a strong NOE correlation (shown in Scheme 6) was observed between the angular Me-28 and the C(18)–H that unambiguously settled the cis configuration of the D THF ring in this compound. Compound **17** is likely the immediate precursor of the bis-spiroketal **9** along the path highlighted in Scheme 4. We decided, therefore, to study the possible spiroketalization of **17** to **9** both to confirm the hypothesis postulated in Scheme 4 and to disclose the stereochemical course of this transformation.



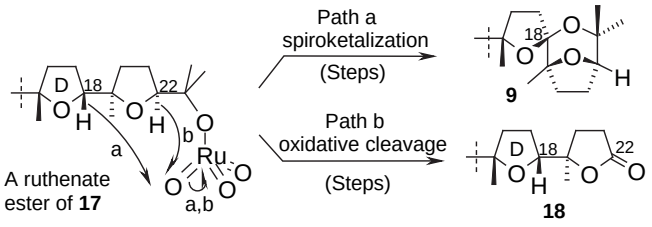
Scheme 6. RuO_4-mediated oxidative mono-spiroketalization and the competing oxidative cleavage.

As for this reaction, the same RuO_2(cat)/$NaIO_4$ system employed to convert squalene into compounds **6**–**9** was initially used, but 4 equiv of the co-oxidant were now employed. In this way compound **9** was obtained in a 25% yield from **17** along with a 25% of the terminal lactone **18** derived from a competing process where the hydroxypropyl terminus in **17** is oxidatively cleaved (Scheme 6). This result indicated that the transformation of **17** to **9** proceeds with retention of the configuration at the C18-forming spirocentre, as previously observed for the analogous PCC-mediated process (Scheme 1).[3] Therefore, very interestingly, the chemical behaviour of RuO_4 in this transformation parallels that displayed by PCC, which is able to both induce spiroketalization and cause the oxidative scission of the hydroxypropyl terminus in **1** (Scheme 1).[1c,f,3,9]

Pleasingly, the yield of **9** raised to 51% when the reaction was conducted using the catalytic system $RuCl_3$(10%)/$NaIO_4$ (6 equiv) in the biphasic mixture CH_3CN/EtOAc/H_2O (1:1:1). Meanwhile, a diminished yield (21%) of lactone **18** was obtained, though the process stopped at 80% conversion. The above transformation is still unoptimised and its synthetic potential towards mono- or bis-spiroketals of the above type is to be further evaluated on a broader range of substrates. However, it is worth noting that the effectiveness of this process is higher than that displayed by the analogous PCC-mediated reaction previously tested on penta-THF **1** (Scheme 1).

With a sample of lactone **18** in hand we were also able to isolate a small amount (ca. 0.1%) of this substance in the crude derived from the initial oxidation of squalene. This is further evidence that indeed in the course of the oxidation of squalene the above spiroketalization of **17** takes place along the path leading to **9**. The concomitant formation of **9** and **18** seems to suggest that the above hypothesised oxoruthenium appendage tethered to the C-23 OH (see Scheme 4) could both attack the H-18 and the H-22 through competing, and possibly strictly related, paths, as shown in Scheme 7. This step is reminiscent of the hydride abstraction postulated by Lee and Van den Engh[5] to occur as the first, rate

determining, step in the oxidation of tetrahydrofuran by ruthenium tetroxide in aqueous perchloric acid solutions, leading to an oxonium ion intermediate. Further studies are currently ongoing to collect evidence on the mechanism of the above Ru-mediated spiroketalization process.



Scheme 7. Possible first step of the competing Ru-mediated oxidative cleavage and oxidative spiroketalization of **17**.

3. Antitumor activity of spiroketal compounds 6–9 and 17

It has been reported that poly-THF[10] and spiroketal[11] compounds display cytotoxic activity. Based on these precedents and the significant inhibition exhibited by mono-spiroketals **2** and **3** on HEY ovarian-derived cancer cell line and BT474 breast-derived cancer cell line,[3] cytotoxicity tests on the same cellular lines were carried out by using different concentrations (0.1 μM, 1.0 μM and 10 μM) of spiroketals **6**–**9** and **17**. After 2 weeks of treatment the viability of the cells was assessed measuring the mitochondrial activity[12] using the phosphate buffer saline (PBS) medium as negative control (Fig. 3). In the HEY cell line (Fig. 3, panel A) compound **9** showed the highest activity killing around 20% of cells already at the concentration of 0.1 μM, while it doubled its activity (40% of cell death) at the concentration of 10 μM. A lower activity was observed for its isomers **7** and **8** (20–25% of cell death). Interestingly, non-symmetric compound **6** differing from **7** and **8** only for the configuration of the D or B THF rings, respectively, proved to be inactive thus highlighting the importance of symmetry in maintaining the antitumor activity. Likewise, mono-spiroketal **17**, the precursor of bis-spiroketal **9** (Scheme 6), was scarcely active (5–10% cell death) at both 1.0 μM and 10 μM. However, it is worth comparing the activities of **17** and the diastereomeric mono-spioroketal **2** (Scheme 1 and Fig. 3), previously tested on the same HEY cells,[3] differing from **17** only for the configuration of the terminal bis-THF moiety (trans–trans in **2** and cis–cis in **17**; Fig 3 right). Compound **2** caused a 70% cell death after 14 days at a 10 μM concentration. This remarkable difference between the two diastereomers indicated that the configuration of the terminal bis-THF portion in these compounds plays an important role in determining the significantly higher activity of **2**.

Higher activities were observed when the BT474 cell line (Fig. 3, panel B) was treated with symmetric bis-spiroketals **7**–**9** at the same concentrations, suggesting that some degree of specificity exists for these compounds. In particular, compound **7** possesses the strongest inhibition activity on BT474 cell line (a 60% of cell death was observed already at the lowest concentration of 0.1 μM that increased to 70% at 10 μM). Compound **8** was scarcely active at 0.1 μM and 1.0 μM but a 65% of cell death was observed at 10 μM. Isomer **9** caused a 60% cell death at 10 μM though a 30–40% cell death was already observed at lower concentrations. As observed in the HEY cell line, non-symmetric **6** was inactive at 0.1 μM and 1.0 μM while a 30% cell death was observed at 10 μM. In addition, compound **17** showed no activity on this cell type as well. Once again, its diastereomer **2** showed a good activity causing a 76% cell death at 10 μM.

In summary, in the bis-spiroketal series the activity seems to be related to the configuration of the spiroketal moiety with symmetric compounds **7**–**9** possessing a higher activity. On the other hand, the activity of mono-spiroketals **2** and **17** appears to be due to not only to the spiroketal portion but also to the presence of a terminal poly-THF portion of suitable configuration.

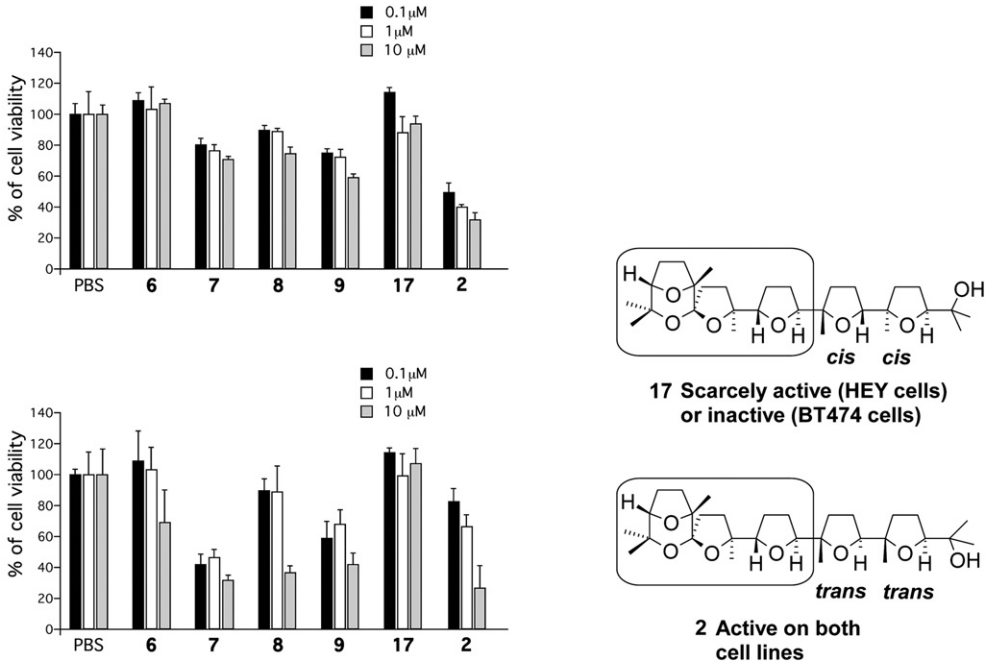


Fig. 3. Cytotoxic effect of spiro-compounds **6**–**9** and **17** in two different tumour-derived cell lines. Ovarian cancer-derived cell line (HEY; Panel A) and breast cancer-derived cell line (BT474; Panel B) were treated with compounds **6**, **7**, **8**, **9** and **17** at the concentration of 0.1 μM (black bars), 1.0 μM (white bars), 10 μM (grey bars) and cell viability was assessed by MTS assay. Phosphate buffer saline (PBS) was used as control. Data for **2** are from Ref. 3. The structure of **2** and **17** are shown on side. The structural portion in the boxes is common to the two substances.

4. Conclusions

In conclusion, a novel RuO_4-catalysed oxidation of squalene, characterised by a tandem pentacyclization/double oxidative spiroketalization, has been discovered. Preliminary experiments conducted on the mono-spiroketalization step of the sequence leading to bis-spiroketal **9** highlighted the potential of this transformation for the synthesis of the spiroketal moiety included in substances of the above type. The symmetric bis-spiroketals **7**–**9** showed the highest antitumor activity and a selectivity for the breast cancer-derived cell line. Though the yield of these bis-spiro compounds is low it should not be forgotten that these products are formed through a complex cascade process made up of at least seven steps each proceeding with an overall average yield of about 65%. In addition, the starting product, squalene, is a commercially available, cheap material[2] and the process can be carried out on a large scale allowing the access, in a single step, to hundred milligrams of new materials characterised by a remarkable sterostructural complexity, hardly accessible through alternative synthetic routes. The process allows to obtain an almost complete set of stereoisomeric substances thus opening up the way to further structure–activity relationship studies concerning, for example, their citotoxicity as well as the possible metal-binding ability or ionophoric aptitude.[13] The present study has also evidenced a close similarity in the oxidative chemical behaviour (oxidative spiroketalization/oxidative cleavage) of PCC and RuO_4 towards poly-THF substances possessing terminal tertiary alcoholic portions of suitable configuration, that is, worth of further investigation. As far as we know this is the most complex process involving RuO_4 ever discovered.[14]

5. Experimental section

5.1. General methods

All reagents 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 250×10 mm and 250×4.6 mm (both 5 μ) and NUCLEOSIL C18 250/10 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 given in hertz. 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. The High Resolution MS were recorded on a Bruker APEX II FT-ICR mass spectrometer using electron spray ionization (ESI) technique. For all the reported products the numbering previously given[1c] for the penta-THF **1** is used.

5.2. Bis-spiroketals 6–9, mono-spiroketal 17 and lactone 18

Squalene (50 g, 122 mmol) was placed into a 5 L round-bottomed flask equipped with a mechanical stirrer and dissolved in the biphasic mixture EtOAc/CH_3CN/H_2O (3:3:2, 1.6 L). The solution was cooled to 0 °C and $NaIO_4$ (8 equiv, 976 mmol, 209 g) and $RuO_2 \cdot 2H_2O$ (20 mol %, 24.4 mmol, 3.25 g) were sequentially added under vigorous stirring. A voluminous amount of a grey solid formed within a few minutes. After 30 min excess $Na_2S_2O_3 \cdot 5H_2O$ was added and the mixture was stirred for further 10 min and then filtered through a Buchner funnel. The solid left on the Buchner was thoroughly washed with EtOAc and the resulting biphasic solution was concentrated in vacuo. The aqueous suspension was extracted with EtOAc (3×300 mL). The combined organic phase was dried (Na_2SO_4) and evaporated in vacuo to give an oily product that was chromatographed on silica gel (50×8 cm column) eluting with petroleum ether (40–70)/Et_2O mixtures (from 7:3 to 100% ether) and then with $CHCl_3$/MeOH mixtures (up to $CHCl_3$/MeOH 8:2) to give three fractions: fraction A (7.40 g) eluted before penta-THF **1**; fraction B (4.75 g) containing penta-THF **1** and lactone **10**; fraction C (35.18 g) eluted after penta-THF **1**.

A sample (500 mg) of the less polar fraction A was separated by HPLC (250×10 mm column, eluent: hexane/EtOAc, 65:35, flow 2.5 mL/min, 30 mg/injection) to give bis-spiroketal **9** (133 mg, 2.3%, t_R=7.0 min), a mixture of bis-spiroketals **6** and **7** in an approximate ratio of 2:3 (113 mg, 1.98%, t_R=8.5 min; **6**: 0.8%; **7**: 1.2%), bis-spiroketal **8** (38 mg, 0.7%, t_R=9.6 min) and mono-spiroketal **17** (40 mg) impure of lactone **18**. Their separation was eventually achieved by reverse-phase HPLC (eluent: MeOH/H_2O, 8:2, flow 2.5 mL/min; **17**: t_R=10 min; **18**: t_R=8.5 min) to give 28 mg (0.5%) of **17** and 5 mg (0.1%) of **18**. A pure samples of **6** was obtained from the above mixture of **6** and **7** by reverse-phase HPLC (eluent: MeOH/H_2O, 8:2, flow 2.5 mL/min, 17 mg/injection. **6**: t_R=11.6 min). A sample of **7** still contaminated by **6** was obtained from the same separation (t_R=12.0 min). A further HPLC run was necessary to obtain pure **7** (250×4 mm column, hexane/EtOAc, 85:15, t_R=13.8 min).

Fraction B was chromatographed on silica gel (50×5 cm column) eluting with petroleum ether (40–70)/Et_2O (1:1) to give 2.93 g of a mixture of **1** and the corresponding lactone **10** in an approximate ratio of 4:1 (**1**: 3.8%; **10**: 1.0%). Further elution with $CHCl_3$/MeOH (9:1) gave 2.18 g of a more polar material that was no further investigated.

*5.2.1. Compound **6**.* Amorphous solid. IR (neat) ν_{max} 2969, 2870, 1457, 1378, 1364, 1216, 1174, 1104, 1042 (str), 993, 962, 918, 889, 870, 846 cm^{-1}. ^1H NMR (500 MHz, $CDCl_3$) δ_H 3.92 (1H, t, *J*=7.1), 3.850 (1H, d, *J*=7.0), 3.846 (1H, d, *J*=7.0), 3.64 (1H, dd, *J*=9.7, 5.5), 1.47 (3H, s), 1.45 (3H, s), 1.36 (3H, s), 1.33 (3H, s), 1.31 (3H, s), 1.18 (3H, s), 1.01 (3H, s), 1.00 (3H, s); ^{13}C NMR (75 MHz, $CDCl_3$) δ_C 109.44, 109.39, 86.9, 85.3, 84.9, 84.6, 82.58, 82.50, 82.4, 82.1, 74.6, 74.5, 36.2, 35.1, 33.73, 33.67, 31.8, 31.6, 27.2, 26.7, 26.5 (3×C), 26.3, 26.2, 25.2, 25.1, 22.5, 21.3, 21.0; ESIMS: *m*/*z* 543 $[M+Na]^+$, 559 $[M+K]^+$; HRESIMS: *m*/*z* 543.3278 ($[M+Na]^+$, $C_{30}H_{48}O_7Na$, requires 543.3298).

*5.2.2. Compound **7**.* Amorphous solid. IR (neat) ν_{max} 2969, 2870, 1457, 1376, 1218, 1174, 1101, 1041 (str), 993, 961, 919, 888, 869, 848, 754 cm^{-1}. ^1H NMR (500 MHz, $CDCl_3$) δ_H 3.85 (1H, d, *J*=7.2), 3.65 (1H, br t, *J*=5.3), 1.47 (3H, s), 1.36 (3H, s), 1.30 (3H, s), 1.01 (3H, s); ^{13}C NMR (125 MHz, $CDCl_3$) δ_C 109.3, 85.5, 84.2, 82.5, 82.4, 74.5, 36.3, 33.6, 31.8, 26.65, 26.56, 26.51, 26.1, 25.2, 21.2; ESIMS: *m*/*z* 543 $[M+Na]^+$, 559 $[M+K]^+$; HRESIMS: *m*/*z* 543.3283 ($[M+Na]^+$, $C_{30}H_{48}O_7Na$, requires 543.3298).

*5.2.3. Compound **8**.* Amorphous solid. IR (neat) 2969, 2870, 1457, 1374, 1213, 1174, 1105, 1042 (str), 989, 963, 906, 889, 869, 845 ν_{max} cm^{-1}. ^1H NMR (500 MHz, $CDCl_3$) δ_H 3.94 (1H, br t, *J*=5.3), 3.84 (1H, d, *J*=7.0), 1.45 (3H, s), 1.34 (3H, s), 1.19 (3H, s), 1.00 (3H, s); ^{13}C NMR (50 MHz, $CDCl_3$) δ_C 109.4, 86.9, 85.1, 82.5, 82.0, 74.5, 34.9, 33.6, 31.6, 27.4, 26.5, 26.3, 25.0, 22.3, 21.0; ESIMS: *m*/*z* 543 $[M+Na]^+$, 559 $[M+K]^+$; HRESIMS: *m*/*z* 543.3307 ($[M+Na]^+$, $C_{30}H_{48}O_7Na$, requires 543.3298).

*5.2.4. Compound **9**.* Amorphous solid. IR (neat) ν_{max} 2968, 2867, 1456, 1375, 1218, 1175, 1105, 1043 (str), 998, 962, 933, 915, 904, 889, 867, 846 cm^{-1}. ^1H NMR (500 MHz, $CDCl_3$) δ_H 3.85 (1H, d, *J*=7.1), 3.74 (1H, br dd, *J*=8.0, 6.1), 1.47 (3H, s), 1.32 (3H, s), 1.23 (3H, s), 1.02 (3H, s); ^{13}C NMR (125 MHz, $CDCl_3$) δ_C -109.6, 85.3, 85.2, 82.5, 82.3, 74.4,

36.0, 33.7, 33.6, 27.2, 26.5 (2×C), 26.0, 25.2, 21.3; ESIMS: *m*/*z* 543 $[M+Na]^+$, 559 $[M+K]^+$; HRESIMS: *m*/*z* 543.3272 ($[M+Na]^+$, $C_{30}H_{48}O_7Na$, requires 543.3298).

5.2.5. Compound ***17***. Amorphous solid. IR (neat) ν_{max} 3470 (OH), 2969, 2871, 1455, 1372, 1217, 1176, 1150, 1104, 1065, 1042 (str), 996, 961 cm^{-1}. ^1H NMR (500 MHz, $CDCl_3$) δ_H 3.85 (4H, overlapped m's), 3.80 (1H, t, *J*=6.8), 3.1 (1H, br s, OH), 1.47 (3H, s), 1.31 (3H, s), 1.24 (3H, s), 1.21 (3H, s), 1.15 (3H, s), 1.11 (3H, s), 1.07 (3H, s), 1.01 (3H, s); ^{13}C NMR (125 MHz, $CDCl_3$) δ_C 109.5, 85.5, 85.0, 84.8, 84.2, 83.9, 83.6, 82.6, 82.29, 82.26, 74.4, 71.6, 36.0, 34.1, 33.8, 33.7, 33.2, 27.8, 27.6, 27.17, 27.12, 26.51, 26.49, 26.0, 25.7, 25.2, 24.6, 23.5, 23.3, 21.2; ESIMS: *m*/*z* 545 $[M+Na]^+$; HRESIMS: *m*/*z* 545.3465 ($[M+Na]^+$, $C_{30}H_{50}O_7Na$, requires 545.3454).

5.2.6. Compound ***18***. Oil. IR (neat) ν_{max} 2968, 2870, 1774, 1460, 1375, 1237, 1158, 1016, 962 cm^{-1}. ^1H NMR (500 MHz, $CDCl_3$) δ_H 3.85 (2H, m), 3.77 (2H, m), 2.75 (1H, m), 2.48–2.38 (2H, m), 1.47 (3H, s), 1.35 (3H, s), 1.31 (3H, s), 1.25 (3H, s), 1.11 (3H, s), 1.01 (3H, s); ^{13}C NMR (125 MHz, $CDCl_3$) δ_C 177.5, 109.6, 85.9, 85.39, 85.38, 84.8, 84.3, 83.6, 82.6, 82.3, 74.3, 36.0, 34.0, 33.6, 33.4, 31.8, 29.8, 27.5, 27.1, 26.6, 26.52, 26.50, 25.7, 25.2, 24.5, 23.2, 21.2; ESIMS: *m*/*z* 501 $[M+Na]^+$; HRESIMS: *m*/*z* 501.2840 ($[M+Na]^+$, $C_{27}H_{42}O_7Na$, requires 501.2828).

5.3. Mono-spiroketalization of 17 to 9

5.3.1. Catalytic procedure I. To mono-spiroketal **17** (6.6 mg, 0.0126 mmol) dissolved in a mixture of CH_3CN/H_2O (3:2, 60 μL/40 μL) was added $NaIO_4$ (4 equiv, 18 mg, 0.083 mmol) followed by 60 μL (20 mol %) of a RuO_4 stock solution (0.032 mM) in EtOAc at 0 °C. After 30 min stirring, $Na_2S_2O_3 \cdot 5H_2O$ (excess) was added and the mixture was extracted with EtOAc (3×1 mL), dried (Na_2SO_4) and taken to dryness to give 5.3 mg of an oily product. The crude was chromatographed on silica gel using hexane/EtOAc mixtures as eluent to give 1.2 mg of pure **9** and 2.5 mg of a mixture of unreacted **17** and lactone **18** in a ratio of 1.3/1 as estimated by 500 MHz ^1H NMR. Yields based on reacted **17** at 70% conversion are as follows: bis-spiroketal **9** (25%), lactone **18** (25%).

5.4. Preparation of the RuO_4 stock solution

The RuO_4 stock solution employed in the above experiment was prepared as follows. To a solution of $NaIO_4$ (4 equiv, 17.7 mg) in H_2O (1 mL) was added $RuO_2 \cdot 2H_2O$ (2.8 mg) and the mixture was vigorously stirred until all the black dioxide was converted in RuO_4. The latter was then extracted in EtOAc (0.5 mL) and used in the above process.

5.4.1. Catalytic procedure II. Mono-spiroketal **17** (6.0 mg, 0.011 mmol) was dissolved in a mixture of $CH_3CN/EtOAc/H_2O$ (1:1:1; 0.5 mL each) and $NaIO_4$ (6 equiv, 15 mg, 0.068 mmol) were added followed by $RuCl_3$ (10 mol %, 12 μL) from a stock solution in EtOAc, at room temperature. After 2.5 h stirring, *iso*-propanol (excess) was added and the mixture stirred for further 10 min. The mixture was taken to dryness and partitioned between EtOAc and water (1 mL each). The aqueous layer was further extracted with EtOAc (2×1 mL) and the combined organic phase was dried (Na_2SO_4) and evaporated in vacuo. The crude (6.4 mg) was analysed at 700 MHz and integration of the well separated methyl resonances allowed to determine the following yields (based on reacted **17** at 80% conversion): bis-spiroketal **9** (51%) and lactone **18** (21%).

5.4.2. Description of the assay. Cell viability was assessed by MTS assay as described elsewhere.[12] 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 concentration of compounds were replaced in every well, except for the controls where only the solvent used to dissolve the compounds was present. 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)-2*H*-tetrazolium, inner salt (MTS) assay and compared with their control.

5.4.3. Single-crystal X-ray diffraction report for compounds ***6***–***9***. Prismatic colourless crystals of **6**, **7**, **8** and **9** were selected for X-ray analysis. Single crystal X-ray diffraction data were collected using graphite monochromated Mo Kα radiation (λ=0.71073 Å) on a Bruker-Nonius kappaCCD diffractometer at room temperature. Owing to the poorly diffracting features of the crystals, data were collected up to θ_{max}=25° for all the structures. Cell parameters and intensity data were processed using Dirax/lsq[15] and Collect programs.[16]

The structures were solved by direct methods[17] and refined by the full-matrix least squares method on F^2 using SHELXL program.[18] Intensities were corrected for absorption effects by the multi-scan method using SADABS program.[19] All non-H atoms were refined with anisotropic displacement parameters; H atoms were determined stereochemically and are fined by the riding model with U_{iso} in the range 1.2–1.5 times U_{eq} of the carrier atom.

5.4.4. Single crystal X-ray diffraction data for ***6***. $C_{30}H_{48}O_7$, *M*=520.68, triclinic, *a*=5.954(4), *b*=14.236(8), *c*=17.250(8) Å, α=99.08(5), β=99.10(7), γ=92.48(6)°, *V*=1422(1) $Å^3$, *T*=293 K, space group $P\bar{1}$ (no. 2), *Z*=2, μ(Mo Kα)=0.085 mm^{-1}, 11,495 reflections measured, 4948 unique, which were used in all calculations. Final agreement indices were $wR(F^2)$=0.2810 (all data) and *R*=0.0633 ($I>3\sigma(I)$). Crystallographic data (excluding structure factors) for compound **6** have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication CCDC 767599. 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).

5.4.5. Single crystal X-ray diffraction data for **7**. $C_{30}H_{48}O_7$, *M*=520.68, orthorhombic, *a*=26.501(8), *b*=21.742(7), *c*=9.795(4) Å, *V*=5644(3) $Å^3$, *T*=293 K, space group Aba2 (no. 41), *Z*=8, μ(Mo Kα)=0.085 mm^{-1}, 10,335 reflections measured, 4508 unique, which were used in all calculations. Final agreement indices were $wR(F^2)$=0.1745 (all data) and *R*=0.0576 ($I>3\sigma(I)$). Crystallographic data (excluding structure factors) for compound **7** have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication CCDC 767600. 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).

5.4.6. Single crystal X-ray diffraction data for ***8***. $C_{30}H_{48}O_7$, *M*=520.68, monoclinic, *a*=19.089(8), *b*=13.070(6), *c*=11.780(6) Å, β=101.32(4), *V*=2882(2) $Å^3$, *T*=293 K, space group $P2_1/c$ (no. 14), *Z*=4, μ(Mo Kα)=0.084 mm^{-1}, 15,654 reflections measured, 5021 unique, which were used in all calculations. Final agreement indices were $wR(F^2)$=0.1304 (all data) and *R*=0.0424 ($I>3\sigma(I)$). Crystallographic data (excluding structure factors) for compound **8** have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication CCDC 767601. 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).

5.4.7. Single crystal X-ray diffraction data for **9**. $C_{30}H_{48}O_7$, M=520.68, triclinic, a=8.295(2), b=12.299(4), c=14.972(5) Å, α=107.96(3), β=90.65(2), γ=97.83(3)°, V=1437.2(8) Å^3, T=293 K, space group $P\bar{1}$ (no. 2), Z=2, μ(Mo Kα)=0.084 mm^{-1}, 13,483 reflections measured, 5039 unique, which were used in all calculations. Final agreement indices were $wR(F^2)$=0.1565 (all data) and R=0.0503 ($I>3\sigma(I)$). Crystallographic data (excluding structure factors) for compound **9** have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication CCDC 767602. 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).

Acknowledgements

We are grateful to MURST, Italy (PRIN 2007), for financial support in favour of this investigation. We are also grateful to the 'Centro di Metodologie Chimico-Fisiche' and the 'Centro di Servizio Interdipartimentale di Analisi Strumentale' of the University of Napoli 'Federico II' for X-ray and NMR facilities. The authors are thankful to Dr. Luisa Cuorvo for technical assistance.

Supplementary data

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.tet.2010.10.004.

References and notes

1. (a) Bifulco, G.; Caserta, T.; Gomez-Paloma, L.; Piccialli, V. *Tetrahedron Lett.* **2002**, *43*, 9265–9269; Corrigendum *Tetrahedron Lett.* **2003**, *44*, 3429; (b) Bifulco, G.; Caserta, T.; Gomez-Paloma, L.; Piccialli, V. *Tetrahedron Lett.* **2003**, *44*, 5499–5503; (c) Caserta, T.; Piccialli, V.; Gomez-Paloma, L.; Bifulco, G. *Tetrahedron* **2005**, *61*, 927–939; (d) Piccialli, V.; Caserta, T.; Caruso, L.; Gomez-Paloma, L.; Bifulco, G. *Tetrahedron* **2006**, *62*, 10989–11007; (e) Piccialli, V. *Synthesis* **2007**, *17*, 2585–2607; (f) Piccialli, V.; Borbone, N.; Oliviero, G. *Tetrahedron* **2008**, *64*, 11185–11192.
2. Squalene can be purchased from Aldrich at a cost of 271 Eur/L.
3. Piccialli, V.; Oliviero, G.; Borbone, N.; Tuzi, A.; Centore, R.; Hemminki, A.; Ugolini, M.; Cerullo, V. *Org. Biomol. Chem.* **2009**, *7*, 3036–3039.
4. Wolfe, S.; Hasan, S. K.; Campbell, J. R. *J. Chem. Soc., Chem. Commun.* **1970**, *21*, 1420–1421.
5. Lee, D. G.; Van den Engh, M. *Can. J. Chem.* **1972**, *50*, 3129–3134.
6. (a) Carlsen, P. H. J.; Katsuki, T.; Martin, V. S.; Sharpless, K. B. *J. Org. Chem.* **1981**, *46*, 3936–3938; (b) Piccialli, V.; Cavallo, N. *Tetrahedron Lett.* **2001**, *42*, 4695–4699; (c) Albarella, L.; Musumeci, D.; Sica, D. *Eur. J. Org. Chem.* **2001**, *5*, 997–1003; (d) Roth, S.; Göhler, S.; Cheng, H.; Stark, C. B. W. *Eur. J. Org. Chem.* **2005**, *19*, 4109–4118; (e) Göhler, S.; Cheng, H.; Stark, C. B. W. *Org. Biomol. Chem.* **2007**, *5*, 1605–1614; (f) Göhler, S.; Roth, S.; Cheng, H.; Göksel, H.; Rupp, A.; Haustedt, L. O.; Stark, C. B. W. *Synthesis* **2007**, *17*, 2751–2754.
7. (a) de Champdoré, M.; Lasalvia, M.; Piccialli, V. *Tetrahedron Lett.* **1998**, *39*, 9781–9784; (b) Donohoe, T. J.; Winter, J. J. G.; Helliwell, M.; Stemp, G. *Tetrahedron Lett.* **2001**, *42*, 971–974; (c) Donohoe, T. J.; Butterworth, S. *Angew. Chem., Int. Ed.* **2003**, *42*, 948–951.
8. (a) Klein, E.; Rojahn, W. *Tetrahedron* **1965**, *21*, 2353–2358; (b) Baldwin, J. E.; Crossley, M. J.; Lehtonen, E.-M. M. *J. Chem. Soc., Chem. Commun.* **1979**, 918–919; (c) Walba, D. M.; Wand, M. D.; Wilkes, M. C. *J. Am. Chem. Soc.* **1979**, *101*, 4396–4397; (d) Walba, D. M.; Edwards, P. D. *Tetrahedron Lett.* **1980**, *21*, 3531–3534; (e) Spino, C.; Weiler, L. *Tetrahedron Lett.* **1987**, *28*, 731–734; (f) Walba, D. M.; Przybyla, C. A.; Walker, C. B. J. *J. Am. Chem. Soc.* **1990**, *112*, 5624–5625; (g) Kocienskyi, P. J.; Brown, R. C. D.; Pommier, A.; Procter, M.; Schmidt, B. *J. Chem. Soc., Perkin Trans. 1* **1998**, 9–39; (h) Brown, R. C. D.; Hughes, R. M.; Keily, J.; Kenney, A. *Chem. Commun.* **2000**, 1735–1736; (i) Brown, R. C. D.; Keily, J. F. *Angew. Chem., Int. Ed.* **2001**, *40*, 4496–4498.
9. (a) Ali, S. M.; Ramesh, K.; Borchardt, R. T. *Tetrahedron Lett.* **1990**, *31*, 1509–1512; (b) Baskaran, S.; Chandrasekaran, S. *Tetrahedron Lett.* **1990**, *31*, 2775–2778; (c) Towne, T. B.; McDonald, F. E. *J. Am. Chem. Soc.* **1997**, *119*, 6022–6028; (d) Roth, S.; Stark, C. B. W. *Chem. Commun.* **2008**, 6411–6413.
10. (a) Alali, F. Q.; Liu, X.-X.; McLaughlin, J. L. *J. Nat. Prod.* **1999**, *62*, 504–540; (b) Zeng, L.; Oberlies, N. H.; Shi, G.; Gu, Z.-M.; He, K.; McLaughlin, J. L. *Nat. Prod. Rep.* **1996**, *13*, 275–306; (c) Rupprecht, J. K.; Hui, Y. H.; McLaughlin, J. L. *J. Nat. Prod.* **1990**, *53*, 237–278; (d) Hioki, H.; Yoshio, S.; Motosue, M.; Oshita, Y.; Nakamura, Y.; Mishima, D.; Fukuyama, Y.; Kodama, M.; Ueda, K.; Katsu, T. *Org. Lett.* **2004**, *6*, 961–964; (e) Fernandez, J. J.; Souto, M. L.; Norte, M. *Nat. Prod. Rep.* **2000**, *17*, 235–246; (f) Otake, N.; Sasaki, T. *Agric. Biol. Chem.* **1977**, *41*, 1039–1047; (g) Morimoto, Y.; Iwai, T.; Yoshimura, T.; Kinoshita, T. *Bioorg. Med. Chem. Lett.* **1998**, *8*, 2005–2010.
11. (a) Zinzalla, G.; Milroy, L.-G.; Ley, S. V. *Org. Biomol. Chem.* **2006**, *4*, 1977–2002; (b) Milroy, L. G.; Zinzalla, G.; Loiseau, F.; Qian, Z.; Prencipe, G.; Pepper, C.; Fegan, C.; Ley, S. V. *ChemMedChem* **2008**, *3*, 1922–1935; (c) Perron, F.; Albizati, K. F. *Chem. Rev.* **1989**, *89*, 1617–1661 and references cited therein.
12. Cory, A. H.; Owen, T. C.; Barltrop, J. A.; Cory, J. G. *Cancer Commun.* **1991**, *3*, 207–212.
13. Due to the presence of a central *cis*-THF ring in their structures, compounds 6–8 could adopt a 'horse shoe' conformation in principle able to bind cations as observed for similar squalene-derived substances such as longilene peroxide and teurilene and therefore may possess ionophoric properties. See for example Ref. 10d and references therein.
14. Plietker, B. *Synthesis* **2005**, *15*, 2453–2472.
15. Duisenberg, A. J. M.; Hooft, R. W. W.; Schreurs, A. M. M.; Kroon, J. *J. Appl. Crystallogr.* **2000**, *33*, 893–898.
16. *Nonius, COLLECT. Nonius BV*; Delft: The Netherlands, 1999.
17. Altomare, A.; Burla, M. C.; Camalli, M.; Cascarano, G. L.; Giacovazzo, C.; Guagliardi, A.; Moliterni, A. G. G.; Polidori, G.; Spagna, R. *J. Appl. Crystallogr.* **1999**, *32*, 115–119.
18. Sheldrick, G. M. *Acta Crystallogr.* **2008**, *A64*, 112–122.
19. *Bruker-Nonius, SADABS. Bruker-Nonius*; Delft: The Netherlands, 2002.