Discovery of a novel one-step RuO₄-catalysed tandem oxidative polycyclization/double spiroketalization process. Access to a new type of polyether bis-spiroketal compound displaying antitumour activity

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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.⁴ It allows adjacentely linked poly-tetrahydrofuran products to be obtained in a single step employing catalytic amounts of ruthenium tetroxide in the presence of NaIO₄ as co-oxidant. In particular, the pentacyclization of squalene (Scheme 1)¹ ¹ ³, ⁴ 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 material⁵ 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.² 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 RuO₄-catalysed tandem oxidative pentacyclization/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 RuO₄-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, CH₂CN/EtOAc/H₂O mixture) required to completely dissolve the...
Therefore, in one of the experiments devised to this end, squalene was oxidized with the same (RuO₂ (cat.)/NaIO₄) oxidising system using about one tenth of the above volume in the new 3:3:2, CH₃CN/EtOAc/H₂O, 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. 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, 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 (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. However, contrary to what observed under the previously employed conditions, 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 1 and 2 of Scheme 1. 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)
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-threeo 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

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 13C 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 C3-symmetric. The former possesses the three THF rings of the molecule (A, C and E) in a cis configuration 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 C2 symmetry differing from 7 only for the trans nature of the central THF ring. In addition, a threeo 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 RuO4- 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 (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 threeo 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,c 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-spiroketalts 6-9 from squalene.

Scheme 5. Ru-catalysed cascade sequence leading to the intermediate penta-THF 11 shown in Scheme 4.
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 spirocenres 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 RuO₄ to attack the γ-hydrogens of tetrahydrofuran (oxidation of tetrahydrofuran to γ-butyrolactone) is known, 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 RuO₄ 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 RuO₄, OsO₄ and MnO₄ as well as for the RuO₄-catalysed polycyclizations of both linear and isoprenoid polyenes. Thus, a cis-three 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 (Δ⁶) 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 NaIO₄, 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 (Δ¹⁸) double bond in the carbon chain, to give the bis-three, bis cyclised, intermediate 16. Usually, this second cyclization step is cis-selective as well but in one case, the oxidation of digeranyl, we observed a trans selectivity. Reiteration of the cyclization/ruthenium oxidation sequence then occurs with a Ru-containing portion (likely RuO₂) 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 stereo-selectivity with all the other THF being formed in a non stereo-selective 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 spiroketalization 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.

As for this reaction, the same RuO₄(cat)/NaIO₄ 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). Therefore, very interestingly, the chemical behaviour of RuO₄ 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).

Pleasingly, the yield of 9 raised to 51% when the reaction was conducted using the catalytic system RuCl₃(10%)/NaIO₄ (6 equiv) in the biphasic mixture CH₃CN/EtOAc/H₂O (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-spiroketalts 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 to occur as the first, rate
determining, step in the oxidation of tetrahydrofuran by ruthenium tetroxide in aqueous perchloric acid solutions, leading to an oxo-nium ion intermediate. Further studies are currently ongoing to collect evidence on the mechanism of the above Ru-mediated spiroketalization process.

![Diagram](image)

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-spiroketal 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 \(\mu\)M, 1.0 \(\mu\)M and 10 \(\mu\)M) of spiroketalts 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 \(\mu\)M, while it doubled its activity (40% of cell death) at the concentration of 10 \(\mu\)M. 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 \(\mu\)M and 10 \(\mu\)M. However, it is worth comparing the activities of 17 and the diastereomeric mono-spiroketal 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 \(\mu\)M 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-spiroketal 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 \(\mu\)M that increased to 70% at 10 \(\mu\)M). Compound 8 was scarcely active at 0.1 \(\mu\)M and 1.0 \(\mu\)M but a 65% of cell death was observed at 10 \(\mu\)M. Isomer 9 caused a 60% cell death at 10 \(\mu\)M 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 \(\mu\)M and 1.0 \(\mu\)M while a 30% cell death was observed at 10 \(\mu\)M. 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 \(\mu\)M.

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-spiroketalts 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.

![Graph](image)

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 \(\mu\)M (black bars), 1.0 \(\mu\)M (white bars), 10 \(\mu\)M (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 Ru(II)-catalysed oxidation of squalene, characterised by a tandem pentacyclisation/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-spiroketalts 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 and the process can be carried out on a large scale allowing the access, in a single step, to hundred milligrams of the final mixture EtOAc/CH3CN/H2O (3:3:2, 1.6 L). The solution was cooled to 0 °C and NaOJ (80 mmol, 576 mmol, 209 g) and RuO2-H2O (20 mol %, 24.4 mmol, 3.25 g) were subsequently added under vigorous stirring. A voluminous amount of a grey solid formed within a few minutes. After 30 min excess Na2S2O3·5H2O 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 (Na2SO4) 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)/EtOAc mixtures (from 7:3 to 100% ether) and then with CHCl3/MeOH mixtures (up to CHCl3/MeOH 8:2) to give three fractions: fraction A (7.40 g) eluted before penta-THF; fraction B (4.75 g) containing penta-THF and lactone; fraction C (35.18 g) eluted after penta-THF.

A sample (500 mg) of the less polar fraction A was separated by HPLC (250 × 10 mm column, eluent: hexane/MeOH, 65:35, flow 2.5 mL/min, 30 mg/injection) to give bis-spiroketal 9 (133 mg, 2.3%, tR = 7.0 min), a mixture of bis-spiroketalts 6 and 7 in an approximate ratio of 2:3 (113 mg, 1.98%, tR = 8.5 min); 6: 0.8%; 7: 1.2%), bis-spiroketal 8 (38 mg, 0.7%, tR = 9.6 min) and mono-spiroketal 17 (40 mg) impure of lactone. Their separation was eventually achieved by reverse-phase HPLC (eluent: MeOH/H2O, 8:2, flow 2.5 mL/min, 17 mg/injection, lR = 11.6 min). A sample of 6 was obtained from the above mixture of 6 and 7 by reverse-phase HPLC (eluent: MeOH/H2O, 8:2, flow 2.5 mL/min, 17 mg/injection, tR = 11.6 min). A sample of 6 still contaminated by 6 was obtained from the same separation (tR = 12.0 min). A further HPLC run was necessary to obtain pure 7 (250 × 4 mm column, hexane/MeOH, 85:15, tR = 13.8 min).

Fraction B was chromatographed on silica gel (50 × 5 cm column) eluting with petroleum ether (40–70)/EtOAc (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: 10%). Further elution with CHCl3/MeOH (9:1) gave 2.18 g of a more polar material that was no further investigated.

5. General methods

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 chromatography carried out on precoated silica gel plates (Merck 60, F254, 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 CDCl3. Proton chemical shifts were referenced to the residual CHCl3 signal (7.26 ppm). 13C 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 conducted on a Bruker APEX II FT-ICR mass spectrometer using electron spray ionization (ESI) technique. For all the reported products the numbering previously given11 for the penta-THF 1 is used.

5.2. Bis-spiroketalts 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 of EtOAc/CH3CN/H2O (3:2.1:1.6 L). The solution was cooled to 0 °C and NaOJ (80 mmol, 576 mmol, 209 g) and RuO2-H2O (20 mol %, 24.4 mmol, 3.25 g) were subsequently added under vigorous stirring. A voluminous amount of a grey solid formed within a few minutes. After 30 min excess Na2S2O3·5H2O 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 (Na2SO4) 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)/EtOAc mixtures (from 7:3 to 100% ether) and then with CHCl3/MeOH mixtures (up to CHCl3/MeOH 8:2) to give three fractions: fraction A (7.40 g) eluted before penta-THF; fraction B (4.75 g) containing penta-THF and lactone; fraction C (35.18 g) eluted after penta-THF.

A sample (500 mg) of the less polar fraction A was separated by HPLC (250 × 10 mm column, eluent: hexane/MeOH, 65:35, flow 2.5 mL/min, 30 mg/injection) to give bis-spiroketal 9 (133 mg, 2.3%, tR = 7.0 min), a mixture of bis-spiroketalts 6 and 7 in an approximate ratio of 2:3 (113 mg, 1.98%, tR = 8.5 min); 6: 0.8%; 7: 1.2%), bis-spiroketal 8 (38 mg, 0.7%, tR = 9.6 min) and mono-spiroketal 17 (40 mg) impure of lactone. Their separation was eventually achieved by reverse-phase HPLC (eluent: MeOH/H2O, 8:2, flow 2.5 mL/min, 17 mg/injection, tR = 11.6 min). A sample of 6 was obtained from the above mixture of 6 and 7 by reverse-phase HPLC (eluent: MeOH/H2O, 8:2, flow 2.5 mL/min, 17 mg/injection, tR = 11.6 min). A sample of 6 still contaminated by 6 was obtained from the same separation (tR = 12.0 min). A further HPLC run was necessary to obtain pure 7 (250 × 4 mm column, hexane/MeOH, 85:15, tR = 13.8 min).

Fraction B was chromatographed on silica gel (50 × 5 cm column) eluting with petroleum ether (40–70)/EtOAc (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: 10%). Further elution with CHCl3/MeOH (9:1) gave 2.18 g of a more polar material that was no further investigated.
aqueous layer was further extracted with EtOAc (2 mL each) and taken to dryness and partitioned between EtOAc and water (1 mL each). The mixture was stirred for further 10 min. The mixture was taken to dryness to give 5.3 mg of an oily product. The crude eluent to give 1.2 mg of pure 961 cm

**5.3.5.** Compound 17. Amorphous solid. IR (neat) $\nu_{\text{max}}$ 3470 (OH), 2969, 2871, 1455, 1372, 1217, 1176, 1150, 1104, 1065, 1042 (str), 996, 944, 868, 829, 724, 74, 15, 74, 76, 78, 27, 27, 27, 27.1, 26.5, 26.2, 26.1, 26.0, 25.7, 24.6, 23.5, 23.3, 21.2; ESIMS: m/z 545 [M+Na]$^+$; HRESIMS: m/z 545.3465 ([M+Na]$^+$, C$_{30}$H$_{48}$O$_7$Na, requires 545.3454). 

**5.3.6.** Compound 18. Oil. IR (neat) $\nu_{\text{max}}$ 2968, 2877, 1774, 1460, 1375, 1237, 1158, 1016, 962 cm

**5.4.1.** Catalytic procedure II. Mono-spiroketalization of 17 to 9

**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 $\theta_{\text{max}} = 25^\circ$ for all the structures. Cell parameters and intensity data were processed using Dirax/Aeq$^2$ and Collect programs. The structures were solved by direct methods and refined by the full-matrix least squares method on $F^2$ using SHELXL program. Intensities were corrected for absorption effects by the multi-scan method using SAADAS program. All non-H atoms were refined with anisotropic displacement parameters; H atoms were determined stereochemically and are fined by the riding model with $U_{	ext{iso}}$ in the range 1.2–1.5 times $U_{	ext{eq}}$ of the carrier atom.

**5.4.4.** Single crystal X-ray diffraction data for 6. C$_{30}$H$_{48}$O$_7$M$^+$, $M$ = 520.68, triclinic, $a$ = 5.954(4), $b$ = 14.236(8), $c$ = 17.250(8) Å, $\alpha$ = 99.08(5), $\beta$ = 99.10(7), $\gamma$ = 92.48(6), $V$ = 1422(1) $A^3$, $Z$ = 293 K, space group $P$T (no. 2), $Z$ = 2, μ(Mo Kα) = 0.085 mm$^{-1}$, 11495 reflections measured, 4948 unique, which were used in all calculations. Final agreement indices were $wR(F^2) = 0.2810$ (all data) and $R = 0.063$ ($I > 2\sigma(I)$). 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), $b$ = 217.427(7), $c$ = 9.795(4) Å, $V$ = 5644(3) $A^3$, $T$ = 293 K, space group $P$2$_1$2$_1$2$_1$ (no. 41), Z = 2, μ(Mo Kα) = 0.085 mm$^{-1}$, 10335 reflections measured, 4508 unique, which were used in all calculations. Final agreement indices were $wR(F^2) = 0.1745$ (all data) and $R = 0.0576$ ($I > 2\sigma(I)$). 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(4) Å, $\beta$ = 101.32(4), $V$ = 2882(2) $A^3$, $T$ = 293 K, space group $P$2$_1$/$c$ (no. 14), Z = 4, μ(Mo Kα) = 0.084 mm$^{-1}$, 15654 reflections measured, 5021 unique, which were used in all calculations. Final agreement indices were $wR(F^2) = 0.1304$ (all data) and $R = 0.0625$ ($I > 2\sigma(I)$). 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
Crystallographic Data Centre as supplementary publication CCDC factors) for compound R9378 deposit@ccdc.cam.ac.uk).

0 1223 336033 or e-mail: deposit@ccdc.cam.ac.uk).

2. Squalene can be purchased from Aldrich at a cost of 271 Eur/L.

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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


2. Squalene can be purchased from Aldrich at a cost of 271 Eur/L.


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 longlione peroxide and teurulene and therefore may possess ionophoric properties. See for example Ref. 10d and references therein.


