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

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A new type of cytotoxic poly-tetrahydrofuran compound, embodying a novel tricyclic spiroketal moiety, has been obtained through an unprecedented PCC-mediated stereoselective oxidative spiroketalization process.

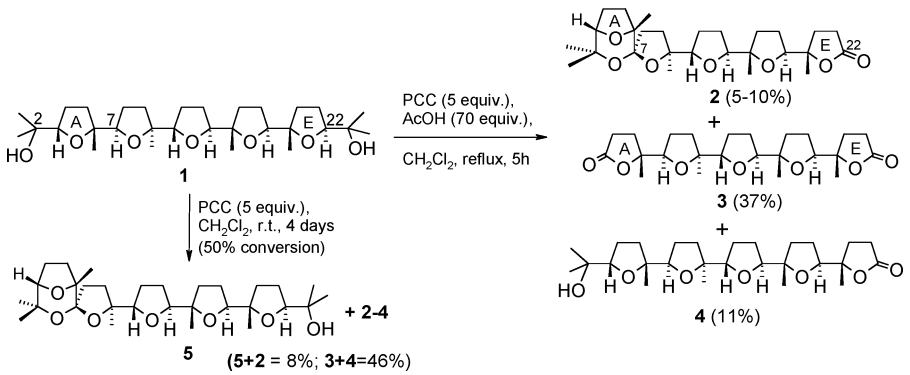
The oxidation of squalene with catalytic amounts of ruthenium tetroxide and sodium periodate as co-oxidant, discovered some years ago in our laboratories, furnishes a penta-THF diol product (**1**, Scheme 1) through a one-step *quintuple* oxidative polycyclization process.[1] This is a remarkable transformation in terms of stereoselectivity, overall yield (45–50% for five consecutive cyclization steps) and stereochemical complexity of the final poly-THF product. The cheapness and the availability of the starting material have allowed us to straightforwardly prepare multi-gram amounts of this substance.

In the course of the PCC[2]-mediated oxidative degradation of **1**[1c,3] to obtain small-sized poly-THF, we isolated a minor side product of the process displaying the novel tricyclic spiroketal structure (**2**, Scheme 1). In particular, prolonged reaction (4 days) of **1** with excess PPC/AcOH in DCM, in the presence of celite, constantly gave, besides bislactone **3** and monolactone **4** (**3** + **4** 48%, Scheme 1),[1c] the new compound **2** (5–10%) embodying an unprecedented dihydro-3′*H*-3,8-dioxaspiro[bicyclo[3.2.1]octane-2,2′-furan] moiety. Pure **2** was obtained by HPLC separation (hexane/EtOAc, 65:35) of the reaction mixture and good crystals suitable for X-ray analysis (Fig. 1) were obtained from ethanol by slow evaporation of the solvent.

Compound **2** was the sole crystalline derivative of penta-THF **1** ever obtained. Its structure deduced from the X-ray analysis was in full agreement with the stereostructure of the parent product previously determined by our group through a combination of 2D-NMR data and chemical degradation work on the parent molecule.[1c]

We observed that the oxidation of **1** with PCC could be carried out in DCM at reflux (5 h) in the absence of celite as well, providing products **2–4** with no substantial change of yields. Similar results were obtained by refluxing **1** in acetic acid (4 h) in the absence of celite. In a further experiment, the previously synthesized bis-*p*-bromobenzoate of **1**[1c] was subjected to the same conditions employed for the oxidation of **1**. After two days the product was shown to be unaltered (^{1}H-NMR and HPLC) indicating that the presence of the free hydroxyl at C-2 is crucial to both processes leading to spiroketal **2** and degraded γ-lactones **3** and **4**.

To gain further insight into the formation of **2**, the oxidation of **1** was carried out under slightly modified reaction conditions. In particular, oxidation of **1** in the absence of acetic acid was shown to also be effective but, as generally observed for other PCC-mediated oxidations, the process was slower[4] and after four days at r.t. an approximately 50% conversion was observed.



Scheme 1 PCC-mediated degradation–spiroketalization of penta-THF **1**.

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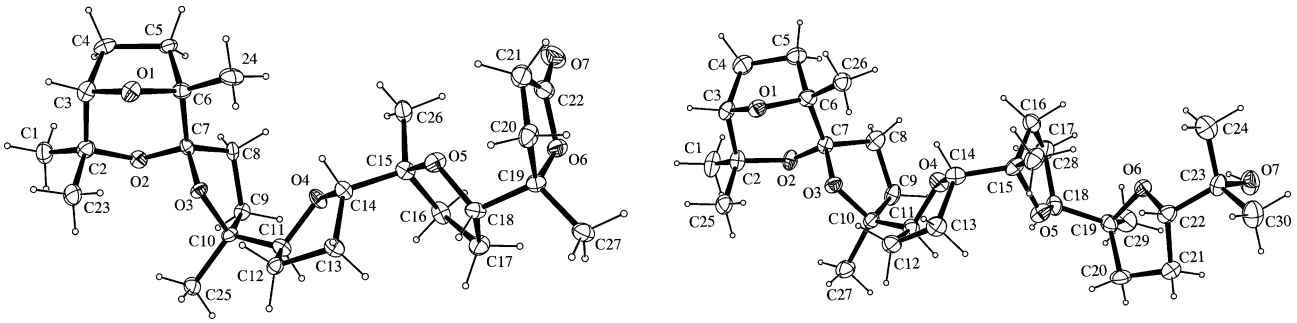


Fig. 1 ORTEP drawings of spiro-compounds **2** (left) and **5** (right). Ellipsoids are drawn at 30% probability level.

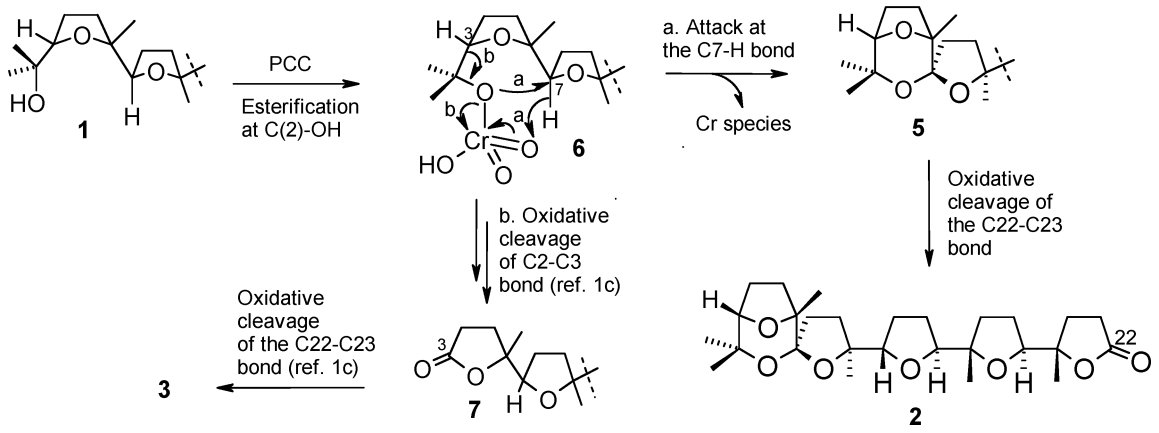
Under these conditions compound **5**, derived from **1** by spiroketalization without further degradation of the terminal E THF ring, could be isolated in addition to **2** (**2**/**5** *ca.* 1:1; **2**+**5** 8%). Correspondingly, degradation of **1** was incomplete as well and a higher amount of monolactone **4** (25%) was isolated besides bislactone **3** (**3**+**4** 50%). Pleasingly, crystals of **5** suitable for X-ray analysis (Fig. 1) could be obtained from MeOH by slow evaporation of the solvent. Bond lengths and angles from X-ray data showed that both in **2** and **5** the bicyclo-spiroketal moiety suffered no steric strain. The THF and the dioxane rings composing the bicyclo unit displayed envelope (with oxygen out of the plane) and chair conformations, respectively, while the other THF rings were all in distorted envelope conformations in both compounds. It is worth noting that the X-ray analysis of **2** and **5** allowed the definitive confirmation of the stereostructure of the parent penta-THF compound **1** thus putting on a firmer basis the previously formulated mechanistic hypothesis for the RuO_4-mediated formation of this substance from squalene.[1c]

It is to be noted that formation of the spiroketal centre in **2** and **5** proceeded with retention of configuration at the C-7 centre of **1** giving the most stable spiroketal (C7-O(THF) bond axial) benefiting by the anomeric effect. Attempted acid-catalyzed equilibration of spiroketal **2** was carried out with PTSA (10 mol%) both in DCM and $CDCl_3$ (^{1}H-monitoring). This experiment, aimed at obtaining a specimen of the C-7 epimer of **2** whose presence in the reaction mixture could suggest the involvement of an oxonium ion species in the process, only returned unaltered **2** even after several days.

Formation of the spiroketals **2** and **5** poses an intriguing mechanistic problem. It seems likely that formation of both bis-γ-lactone **3** and spiroketal **5** can initially proceed through a common C-2 chromium ester intermediate[5] (**6**, Scheme 2). Two routes would be possible at this stage. Attack of the C(7)-H bond by a close-in-space oxochromium appendage tethered to C-2 (route a), in a manner similar to the one hypothesized for the analogous oxidation of THF's to γ-lactones with RuO_4,[6] would well explain the selective formation of the spiroketal at the *cis*-*cis* bis-THF terminus of the poly-THF chain. The H-abstraction step could occur with formation of an intermediate oxonium ion involving C-7, that would then be attacked by the C-2 oxygen with concomitant loss of a chromium species. At the moment, no evidence supporting the involvement of such an intermediate species in the process is available and in principle other hypotheses are equally possible.

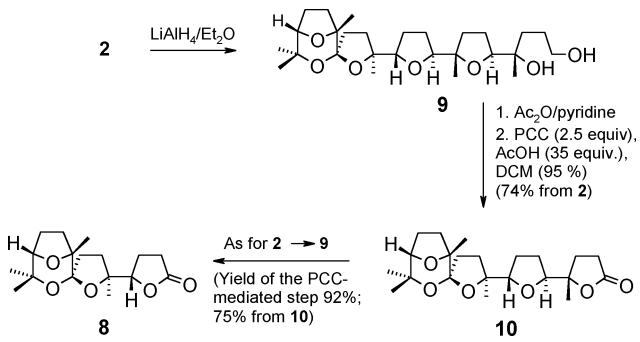
Formation of the C-3 lactone function in **3** could in turn proceed from the same intermediate **6** through the oxidative cleavage of the C2-C3 bond (route b), possibly *via* an oxonium ion involving C-3, followed by a further oxidation at C-3 to give a ring-A monolactone species **7**. Compound **3** is eventually obtained through a second oxidative fission of the C22-C23 bond at the other terminus (E ring) of the molecule.[1c] In the same manner, spiroketal **2** and monolactone **4** would be formed from the first-formed spiroketal **5** and from penta-THF **1**, respectively, by the oxidative degradation of ring E.

Searching for minor side-products of the process that could throw light on the formation of **2** and **5**, small amounts of two



Scheme 2 A mechanistic route rationalising the formation of spiroketals **2** and **5** and lactones **3** and **4**.

further spiro-compounds (*ca.* 1% each), tentatively identified as **8** and **10** (Scheme 3) by ^{1}H-NMR and MS data, were isolated by HPLC from the oxidation of **1** conducted both in the presence and in the absence of AcOH.



Scheme 3 Chemical correlation of spiroketals **2**, **8** and **10**.

The stereostructure of **8** and **10** was proven by degradation of **2** (Scheme 3). In particular, LAH reduction of **2** gave spirodiol **9** whose acetylation, followed by treatment with PCC (2.5 equiv.)/AcOH (35 equiv.), gave the degraded spirolactone **10** in an overall 74% yield (for three steps). It is worth noting that the oxidative cleavage step in this sequence proceeded with 95% yield. Compound **10** was then subjected to another degradation cycle as for the conversion of **2** to **10** to give a compound indistinguishable from **8** in an overall 75% yield from **10**; the PCC-mediated step proceeded in high yields (92%) in this case as well. This sequential double degradation of **2** proved that substrates possessing more complex tertiary alcoholic portions α to a THF ring can also be subjected to the PCC-mediated degradation to give γ-lactones in very high yields and opened up the way to the easy preparation of small-sized spiro-compounds of the above type.

We believe that the presence of compounds **8** and **10** among the oxidation products of **1** cannot be explained through the PCC-mediated oxidative fission of C14-C15 and C18-C19 bonds in **2** and/or **5**, successive to their formation. Chromium (VI) reagents are known to oxidize ether to esters. For example chromic acid oxidizes THF to γ-lactones;[7a] anhydrous chromium trioxide in acetic acid converts methyl ethers into the corresponding formats;[7b] *t*-butyl chromate oxidizes spiroethers to spirolactones.[7c] However, though PCC is able to oxidize cyclic benzylic and allylic ethers[2b] to the corresponding lactones, as far as we know, its ability to oxidize α-carbons in THF rings has never been reported. In fact, when compound **2** was treated with PCC (1.2 equiv, DCM, reflux, 4 h) no trace of spirocompounds **8** and **10** was detected (^{1}H-NMR and analytical HPLC) and **2** was recovered unaltered. Moreover, as seen above, the bis-*p*-bromobenzoate of **1** was unaffected by reaction with PCC as well. These evidences seem to exclude the direct attack of PCC to angular THF methines in **2**/**5** though it cannot be excluded that a further chromium species, delivered in the medium along the path leading to γ-lactones or spiro-compounds, could operate the oxidative cleavage of the C14-C15 and C18-C19 bonds thus producing the degraded spiroketals **8** and **10**.

It has been reported that poly-THF[8] and spiroketal[9] compounds display cytotoxic activity. Based on these precedents cytotoxicity tests were carried out treating the ovarian cancer cell line (HEY) and breast cancer cell line (BT474) with different concentrations (0.1 μM, 1 μM and 10 μM) of spiroketals **2**, **5**, and **9**. These compounds share the same spiroketal moiety and differ only in the right-hand terminal portions. At different time points the viability of the cells was assessed measuring the mitochondrial activity.[10] Interestingly, we found that all the tested compounds displayed cytotoxicity at the lowest concentration already after the first week of treatment (Fig. S1†) though the cell killing effect was more prominent at higher concentrations. After two weeks of treatment the experiment was ended and all the compounds showed a significant cell killing effect at all tested concentrations on both cell lines (Fig. S1: panels A, B and C, BT474 cell line; panels D, E and F, HEY cell line†).

To conclude, this paper reports the discovery of an unprecedented PCC-mediated oxidative spiroketalization process that allowed us to obtain a new type of structurally complex poly-THF spiroketal compound that could hardly be accessed through a different synthetic route. A good yielding route, allowing us to obtain further degraded spiroketal compounds (**8** and **10**), has been set-up that also highlights the potential of the PPC-mediated degradation of THF rings bearing α tertiary alcoholic portions. Moreover, we have reported that all the tested compounds (**2**, **5** and **9**) possess antitumor activity on breast and ovarian cancer cell lines and that a minimum amount of these compounds is required for a significant reduction of cell viability. Further studies on this novel transformation, as well as further experiments to evaluate the biological activity of the above spiroketals, including degraded spirolactones **8** and **10**, and derivatives thereof, are currently underway. Further experiments to elucidate the minimum structural moiety required for the cytotoxic activity will be carried out as well.

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