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AND REACTIVITY OF ORGANIC MOLECULES

NEW OXIDATIVE PROCESSES IN ORGANIC CHEMISTRY AND THEIR SYNTHETIC APPLICATIONS

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List of Publications

Portions of this thesis have been adapted from the following articles that were co-written by the author :

1. **Investigation of the minor reaction products of the pyridinium chlorochromate-mediated oxidation of a polycyclic polyether compound. A deeper comprehension of the PCC chemistry**
   Vincenzo Piccialli, Sabrina Zaccaria, Roberto Centore, Giorgia Oliviero, Stefano D'Errico and Nicola Borbone,
   Manuscript in preparation.

2. **A general synthesis of bis-α-acyloxy 1,4- and 1,5-diketones through catalytic oxidative opening of acylated THF and THP diols**
   Vincenzo Piccialli, Stefano D'Errico, Nicola Borbone, Giorgia Oliviero, Roberto Centore and Sabrina Zaccaria
   Eur. J. Org. Chem., **2013,** 1781-1789.

3. **Synthesis and biological evaluation of unprecedented ring-expanded nucleosides (RENs) containing the imidazo[4,5-d][1,2,6]oxadiazepine ring system**
   Stefano D'Errico, Giorgia Oliviero, Jussara Amato, Nicola Borbone, Vincenzo Cerullo, Akseli Hemminki, Vincenzo Piccialli, Sabrina Zaccaria, Luciano Mayol and Gennaro Piccialli
   Chem. Commun. **2012**, *48*, 9310–9312.

4. **Insight into pyridinium chlorochromate chemistry: catalytic oxidation of tetrahydrofuran compounds and synthesis of umbelactone**
   Vincenzo Piccialli, Sabrina Zaccaria, Giorgia Oliviero, Stefano D'Errico, Valentina D'Atri, Nicola Borbone
   Eur. J. Org. Chem. **2012**, 4293-4305.

5. **Isolation of a bis-iodurated tetra-THF as a trace product from the oxidation of squalene with RuO_4 and its double ring expansion to a novel bis-THF-bis-THP compound**
   Vincenzo Piccialli, Sabrina Zaccaria, Roberto Centore, Angela Tuzi, Nicola Borbone and

Giorgia Oliviero
Molecules **2011**, *16,* 5362-5373.

6. **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 antitumor activity**
   V. Piccialli, S. Zaccaria, N. Borbone, G. Oliviero, S. D'Errico, A. Hemminki, V. Cerullo, V. Romano, A. Tuzi, R. Centore
   Tetrahedron **2010**, *66*, 9370-9378.

Other papers co-written by the author not included in this thesis:

1. **Tautomerism in the fused N-rich triazolo-triazole heterocyclic system**
   Roberto Centore, Sandra Fusco, Amedeo Capobianco, Vincenzo Piccialli, Sabrina Zaccaria and Andrea Peluso,
   Eur. J. Org. Chem, **2013**, in press.

2. **Insight into the conformational arrangement of a bis-THF diol compound through 2D-NMR studies and X-ray structural analysis**
   Vincenzo Piccialli, Sabrina Zaccaria, Roberto Centore, Angela Tuzi, Nicola Borbone, Giorgia Oliviero, Stefano D'Errico, Valentina D'Atri
   J. Chem. Cristallogr. **2012**, *42*(4), 360-365.

3. **Synthesis, stereostructure and H-bonding patterns of a tris-THF compound**
   Roberto Centore, Angela Tuzi, Sabrina Zaccaria, Vincenzo Piccialli
   J. Chem. Cristallogr. **2011**, *41*(9), 1370-1375.

Abstract

Saturated ether rings of various sizes are part of the structure of a variety of natural substances, as well as of non-natural substances characterized by important biological properties. In particular, 2,5-disubstituted tetrahydrofurans are structural fragments commonly found in natural substances such as polyether antibiotics, e.g. monensin A,[1] squalene-derived metabolites such as glabrescol,[2] and annonaceous acetogenins such as goniocin and mucocin, [3] just to mention some representative categories. Interest toward isolation, synthesis and structural modification of the basic backbone of these substances is still very keen as witnessed by the numerous researches devoted to these targets. Indeed, a variety of methods have been developed to produce or modify the oxacycle portions of these molecules, in order to obtain substances belonging to the above mentioned classes, but also to synthesize new analogues of these molecules to be used in biological assay or in structure activity relationship (SAR) studies. In the past years the laboratory where this PhD thesis has been carried out has focused on the synthesis of new THF-containing compounds using transition metal oxo-species such as RuO_4, PCC, $MeReO_3$ and OsO_4. In this regard this PhD thesis focuses on the development of new oxidative processes mediated by transition metal oxo-species. In particular, new types of useful oxidative transformations involving the THF ring have been disclosed.

In the first year of my PhD programme four novel C_{30} polyether bis-spiroketals, displaying selective cell killing effect on 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.[4] 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 also been disclosed.[5] Moreover a novel bis-iodurated polyether compound, based on an unprecedented tetra-THF backbone, has been isolated as a trace by-product of the oxidation of squalene.[6] The double *erythro* configuration of the central portion of the molecule furnishes the first indirect support of the previously postulated pathway operating in the oxidative pentacyclization of the isoprenoid substrate.[7] A bidirectional double oxidative bis-cyclization is invoked to explain the formation of this compound. The isolated substance was also successfully subjected to a double rearrangement-ring expansion to give a novel bis-THF-bis-THP compound.

As part of our efforts toward the understanding of the oxidizing behavior of transition metal oxo-species, in the second year of my PhD programme PCC and the catalytic system PCC(cat.)/H_5IO_6

have been employed to oxidize mono- and poly-tetrahydrofuran compounds.[8] Novel oxidative pathways have been disclosed. 2,2,5-trisubstituted THF rings lead to dicarbonyl compounds via oxidative cleavage of the C2-C3 bond. Cyclic enolethers appear to be intermediate species in this process. Oxidation of 2,2,5-trisubstituted α-keto-THF compounds proceeds with the oxidative cleavage of the C2(THF)-C=O bond to give 1,4-diketones possessing a degraded carbon backbone. Attack of the oxidant to 2,5-disubstituted THF rings leads to 1,4-diketones possessing the intact THF carbon framework. Oxidation of complex poly-THF substrates, embodying up to five THF rings, allows the access to novel poly-THF compounds via diastereoselective THF oxidation along the poly-THF backbone. The collected results provide a deeper comprehension of the reactivity of the PCC and also suggest a new mechanistic hypothesis of the PCC-mediated oxidative cleavage of α-hydroxy-THF compounds to γ-lactones. The proposed mechanism well agrees with that reported for the oxidative cleavage of 8-hydroxy-neoisocedranol oxide by RuO_4 [9] and is in line with the similar oxidizing behaviour shown by these two oxo-species. The synthesis of racemic umbelactone,[10] an antiviral natural butenolide metabolite, has also been carried out by using the developed chemistry. [8b]

On the basis of this results, in the third year of my PhD programme, the first general synthesis of bis-α-acyloxy 1,4- and 1,5-diketones has been accomplished by CCP catalytic oxidative opening of bis acylated THF and THP diols, in turn synthesized by osmium- or ruthenium-catalyzed oxidative cyclization of 1,5- and 1,6-dienes.[11] The overall sequence corresponds to the regioselective double ketoacyloxylation of the starting diene. To test the breadth of our methodology acylated *cis*-reticulatacin,[12] a representative mono-THF *Annonaceous* acetogenin,[13] has also been successfully oxidized with our procedure, thus opening the way to the preparation of new non-THF analogues of these active substances, to be used in biological assays. Moreover, among the conceivable synthetic uses the bis-α-acyloxy-1,5-dicarbonyl compounds obtained have been transformed into pyridine-based oxido pincer ligands or pyrazine dimethanol substances, leading to the discovery of unprecedented aromatization routes.

In the frame of a project joined in collaboration with the group of prof. L. Mayol from the "Dipartimento di Chimica delle Sostanze Naturali", oxidative processes have been applied to the synthesis of new nucleoside analogues possessing different base modifications. The first general approach that allows either the synthesis of ring-expanded nucleosides (RENs), containing the unprecedented bis-alkylated imidazo[4,5-d][1,2, 6]oxadiazepine heterocyclic ring system,[14] or the 2,6-dialkyl(aril)purine moiety has been developed. This method entails first of all the formation of a purine N1-oxide by treatment of the purine nucleoside with $MeReO_3$ followed by addition of a

Grignard reagent to the electrophilic C-6 carbon of the substrate.[15] The reactivity of the C-6-substituted purine nucleosides towards a second Grignard reagent afforded new 4,5-disubstituted imidazo-nucleosides from which we obtained imidazo[4,5-d][1,2,6]oxadiazepine nucleosides, if treated with *t*-BuOOH, or 2,6-disubstituted purine nucleosides by treatment with Ac_2O in pyridine. Using this procedure a small collection of ring-expanded nucleosides (RENs) has been synthesized and subjected to preliminary cytotoxicity tests on breast (MCF-7) and lung (A549) cancer cell lines. We are also synthesizing a collection of 2,6-disubstituted purine nucleosides to be subjected to biological assay.

Eventually, some preliminary experiments have been done to develop RuO_4 conditions for the synthesis of bis-α-acyloxy 1,4-diketones, in order to extend the procedure to acid- or PCC-sensitive molecules. As part of our efforts working toward the synthesis of poly-THF compounds some experiments have been carried out to assess the osmium ability to mediate the bis-cyclization of polyenes with a repetitive 1,5-diene structural motif. These finding deserve further studies.

Riassunto

Eteri ciclici di varie dimensioni fanno parte della struttura di numerose sostanze naturali sia di origine terrestre che marina, come anche di sostanze non naturali. Uno o più anelli tetraidrofuranici (THF) 2,5-disostituiti ed anelli tetraidropiranici sono comunemente presenti in molecole naturali dotate di importanti attività biologiche. Fra queste sono da ricordare gli antibiotici polieterei come la monensina A[1], alcuni metaboliti derivanti dallo squalene, come il glabrescolo[2], e le acetogenine da *Annonaceae* come la goniocina e la mucocina[3] (Figura 1).

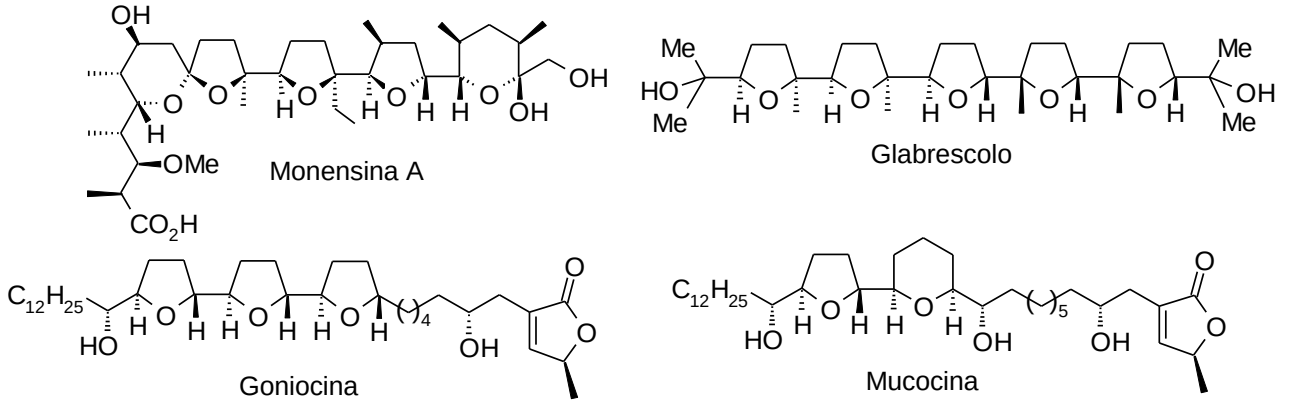
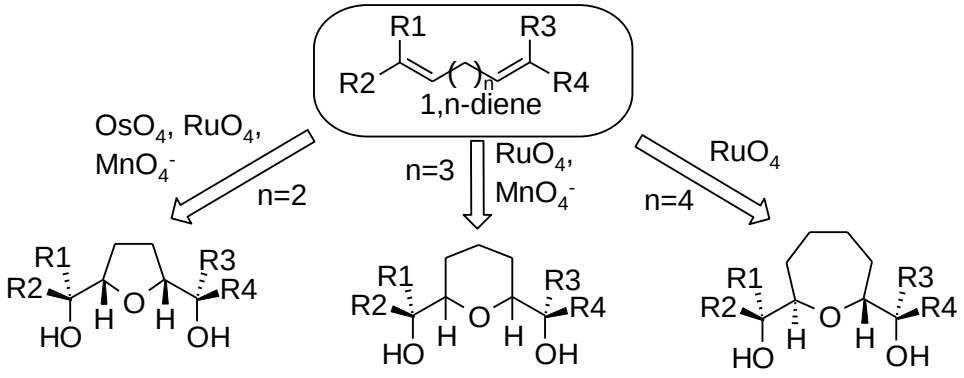


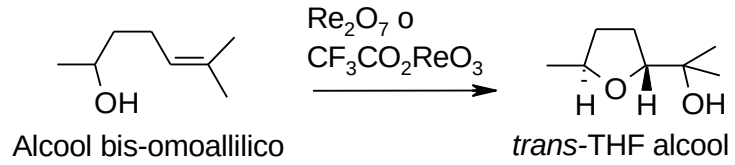
Figura 1. Alcuni esempi di molecole naturali contenenti anelli THF e THP.

Negli ultimi anni numerosi gruppi di ricerca, impegnati nella sintesi totale di molecole naturali, hanno indirizzato i loro sforzi verso la messa a punto di metodi stereoselettivi per la costruzione della porzione ossaciclica di tali tipi di sostanze e di loro analoghi non naturali.

Accanto a metodi classici è stato sviluppato, soprattutto nell'ultimo decennio, un approccio totalmente diverso che coinvolge la reazione di osso-specie di metalli di transizione come RuO_4, OsO_4, MnO_4^- e RuO_4^- con 1,5- 1,6- e 1,7-dieni o di alcoli bis-omoallilici con ossidi del renio (VII) (Schema 1 e Schema 2).[16]

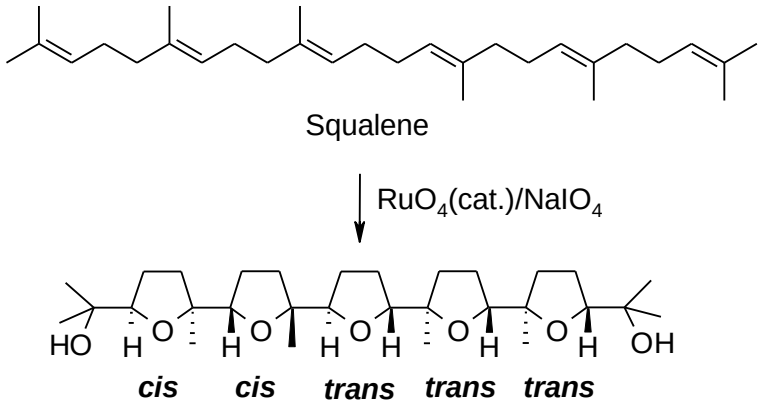


Schema 1. Monociclizzazione ossidativa di dieni mediata da osso-specie di metalli di transizione.



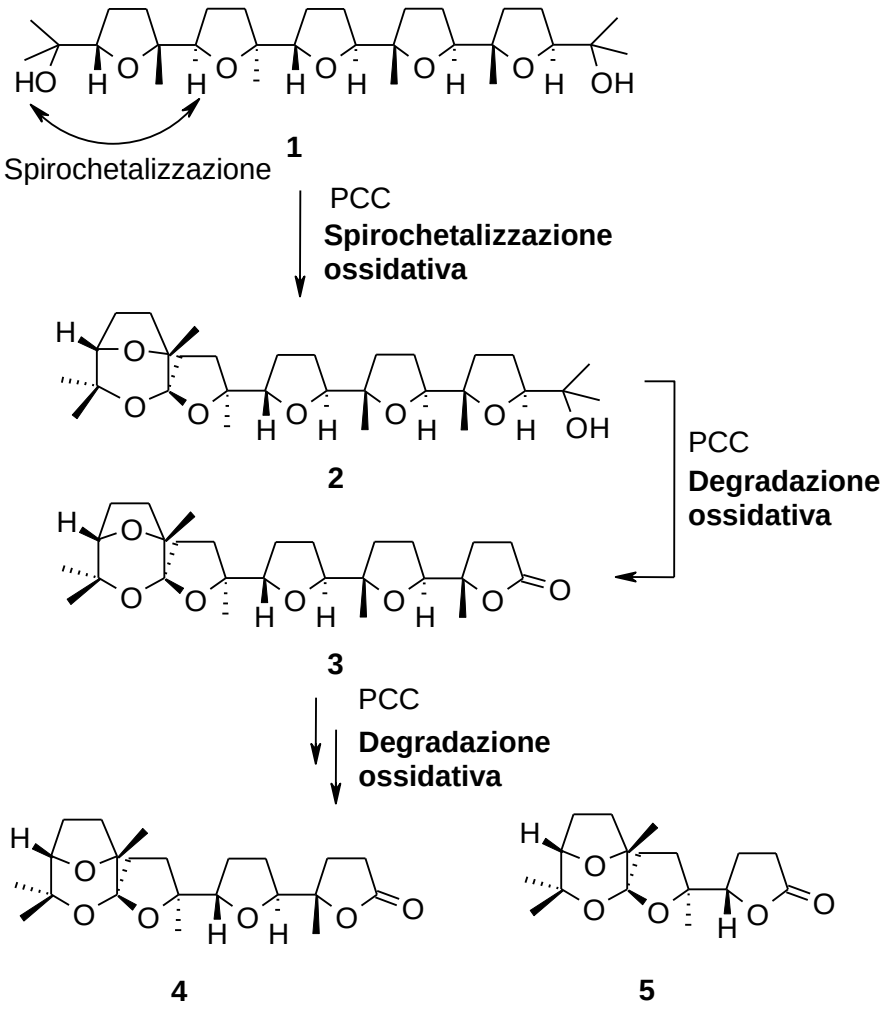
Schema 2. Ciclizzazione ossidativa di alcoli bis-omoallilici mediata da renio (VII).

Il laboratorio di ricerca presso il quale è stato condotto questo lavoro di tesi, nell'ambito di studi su polieni isoprenoidici e lineari, caratterizzati da un'unità 1,5-dienica ripetuta, come il farnesil acetato, il geranilgeranil acetato e lo squalene, ha messo a punto un processo di policiclizzazione ossidativa a cascata, catalizzato dal tetrossido di rutenio,[17] che consente la sintesi in un unico step di prodotti poli-tetraidrofuranici complessi, del tipo mostrato nello Schema 3. La potenzialità del metodo è stata inoltre saggiata nella sintesi del "core" bis-THF diolico di due acetogenine da *Annonaceae* molto attive: la rollimembrina e la rollinastatina-1.[17]



Schema 3. Policiclizzazione ossidativa dello squalene mediata da RuO_4.

Studi recenti condotti dallo stesso gruppo di ricerca, rivolti alla degradazione ossidativa del prodotto penta-THF **1** in presenza di piridinio clorocromato (PCC) (schema 4), hanno portato alla scoperta di un nuovo processo di spirochetalizzazione ossidativa mediato da PCC[5] che conduce alla formazione di un nuovo tipo di sostanze poli-THF (**2** e **3**) caratterizzate da una porzione terminale spirochetalica mai sintetizzata in precedenza. Studi preliminari hanno mostrato che tali sostanze, ed alcuni loro derivati (**3** e **4**), sintetizzati per degradazione mediata da PCC, posseggono attività antitumorale su linee cellulari del cancro delle ovaie (HEY) e del seno (BT474) a concentrazioni μM.

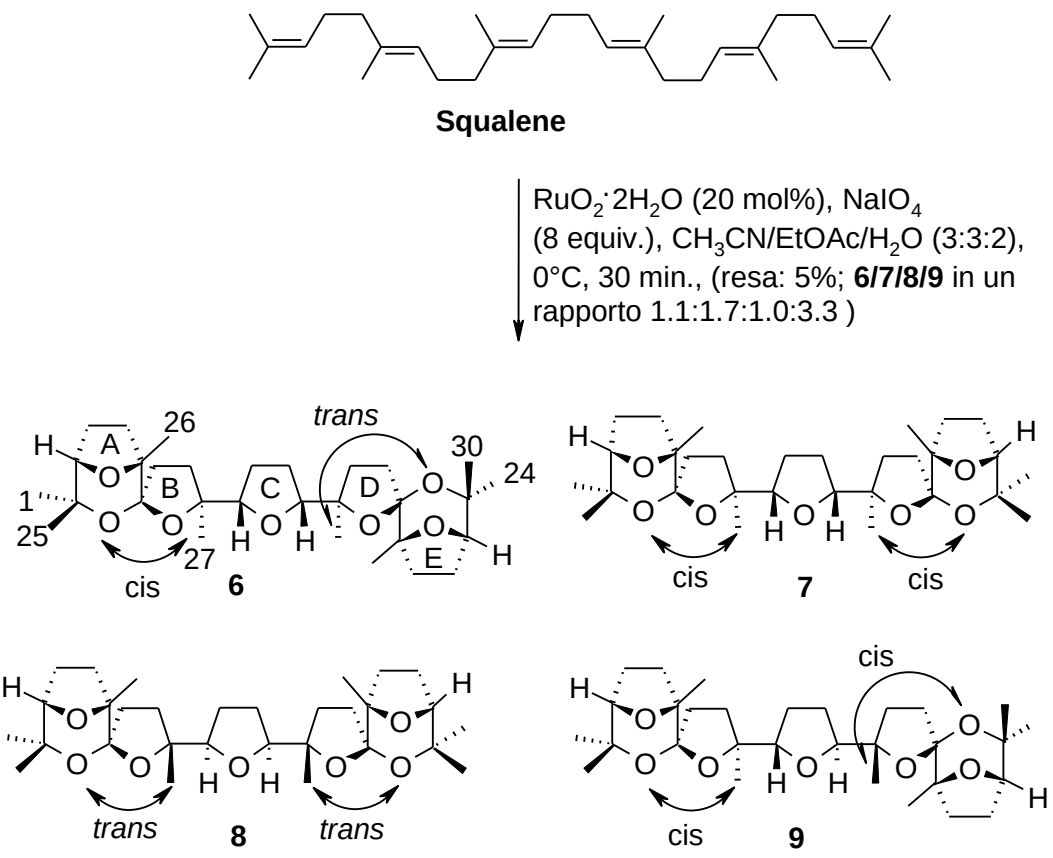


Schema 4. Scissione ossidativa del penta-THF **1** con PCC e degradazione dei prodotti spirochetalici ottenuti.

Nel corso del I anno di dottorato è stato affrontato lo studio della policiclizzazione ossidativa dello squalene catalizzata da RuO_4 in nuove condizioni sperimentali. L'esigenza di ottenere tale sostanza in maggiori quantità per studi sintetici correlati, ci ha spinto ad effettuare il processo su quantità più elevate del substrato (50 g). Tali quantità di squalene avrebbero però richiesto un volume troppo elevato di solvente per la completa dissoluzione del co-ossidante necessario al processo, che è stato pertanto effettuato in condizioni più concentrate. Il nuovo processo ha condotto alla formazione, in un unico step, di quattro nuovi prodotti polieterei isomerici (Schema 5), caratterizzati da porzioni spirochetaliche terminali strutturalmente simili, attraverso una complessa sequenza di reazioni di ciclizzazione.[4] In particolare, si ritiene che inizialmente si formino prodotti penta-THF isomerici del tipo **1,** i quali sottostanno ad una doppia spirochetalizzazione ossidativa, che coinvolge i terminali bis-THF, generando le porzioni spirochetaliche terminali.

La determinazione della struttura dei nuovi prodotti è stata effettuata mediante studi 2D-NMR su

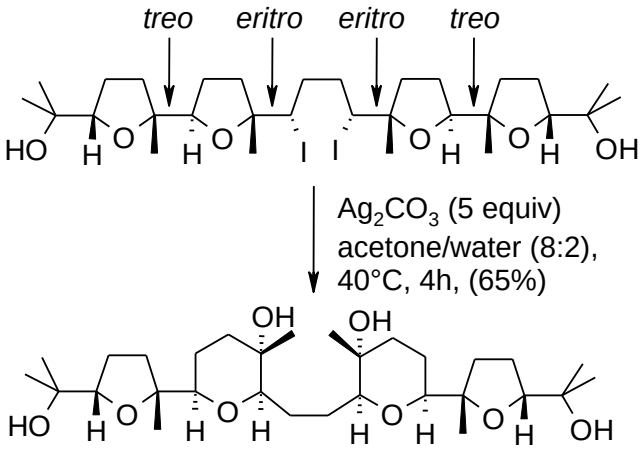
spettrometri ad alto campo ed infine confermata mediante esperimenti di diffrazione dei raggi X. E' stato, inoltre, dimostrato che gli step di spirochetalizzazione ossidativa avvengono con ritenzione di configurazione. Alcuni dei prodotti hanno mostrato attività di inibizione selettiva della crescita di cellule derivanti da tumore al seno (BT474) e alle ovaie (HEY).



Schema 5. Formazione di quattro nuovi prodotti spirochetalici per ossidazione dello squalene con RuO_4 nelle nuove condizioni sperimentali.

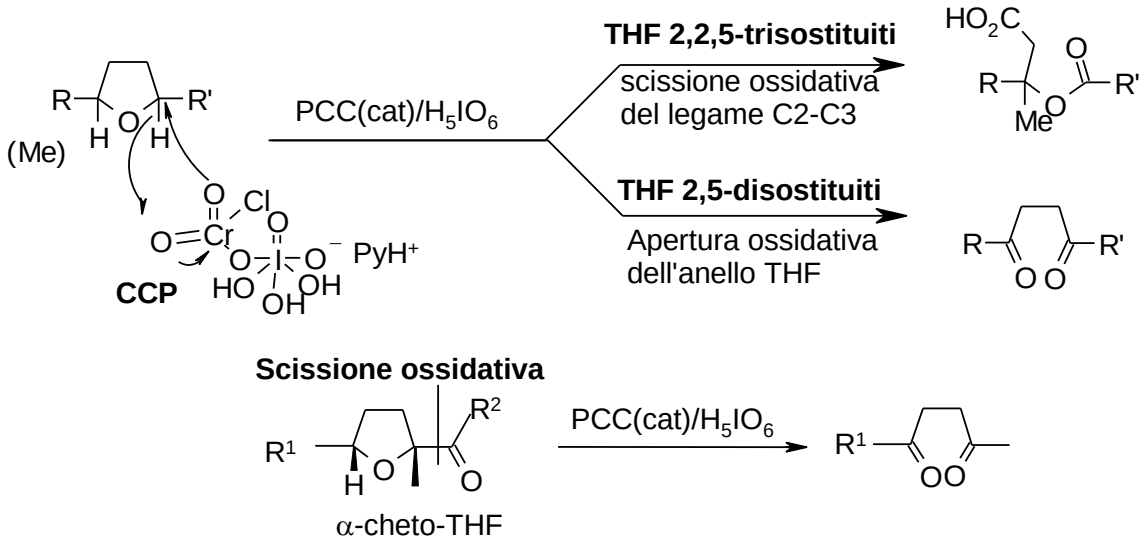
Un'accurata analisi HPLC della miscela di reazione dell'ossidazione dello squalene nelle nuove condizioni sperimentali ha consentito l'isolamento di un nuovo prodotto tetra-THF bis-iodurato la cui stereostruttura è stata determinata mediante diffrazione dei raggi X su cristallo singolo. ottenuto per lenta evaporazione da una soluzione di cloroformio.[6] La doppia configurazione *eritro* della porzione centrale della molecola fornisce supporto indiretto all'ipotesi meccanicistica precedentemente postulata per la pentaciclizzazione ossidativa dello squalene.[7] Tale prodotto è stato, inoltre, sottoposto con successo ad un doppio riarrangiamento con espansione d'anello, ottenendo un nuovo composto bis-THF-bis-THP (Schema 6). Sebbene tale tipo di reazione fosse stata utilizzata nella sintesi di composti a struttura polieterea complessa come la salinomicina, la

reazione effettuata rappresenta il primo esempio di una doppia espansione di anello di tale tipo.



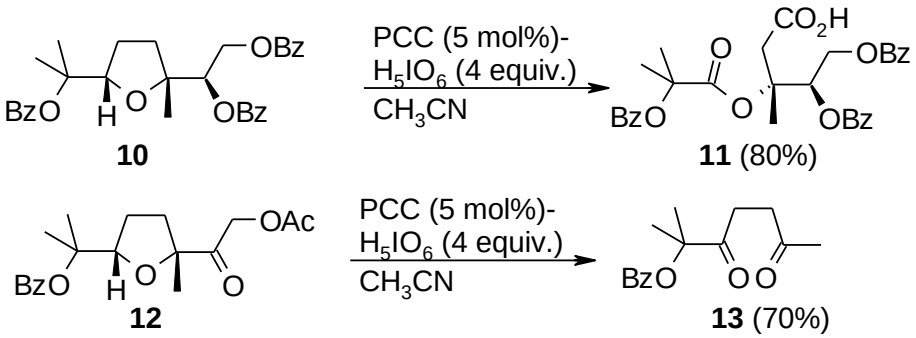
Schema 6: Prodotto minore poli-THF bis-iodurato dalla policiclizzazione ossidativa dello squalene catalizzata da RuO_4 e suo doppio riarrangiamento ad un nuovo composto bis-THF-bis-THP.

Nel corso del II anno di dottorato di ricerca è stato affrontato lo studio dell'ossidazione di alcuni substrati mono- e poli-THF ad opera del sistema catalitico PCC(cat.)/H_5IO_6 (clorocromatoperiodato, CCP)[18] scoprendo nuove utili trasformazioni ossidative.[8] In particolare, è stato dimostrato, per la prima volta, che il CCP è in grado indurre la scissione ossidativa del legame C2-C3 di anelli THF 2,2,5-trisostituiti, portando alla formazione di composti dicarbonilici (Schema 7). Si ritiene che prodotti enoleterei ciclici siano intermedi di tale processo. La presenza di una funzione chetonica adiacente all'anello THF 2,2,5-trisostituito conduce alla formazione di 1,4-dichetoni con scheletro carbonioso degradato, attraverso la scissione del legame C(THF)2-C=O. E' stato inoltre osservato che quando l'anello THF è 2,5-disostituito si ottengono 1,4-dichetoni mediante l'apertura ossidativa del ciclo con ossidazione di entrambi i carboni angolari. Si ritiene che il primo evento in ciascun processo sia costituito dall'attacco del CCP al legame C-H angolare del THF coinvolto. Vi è da sottolineare che, fino ad ora, il PCC in forma catalitica è stato utilizzato solo in rari casi.



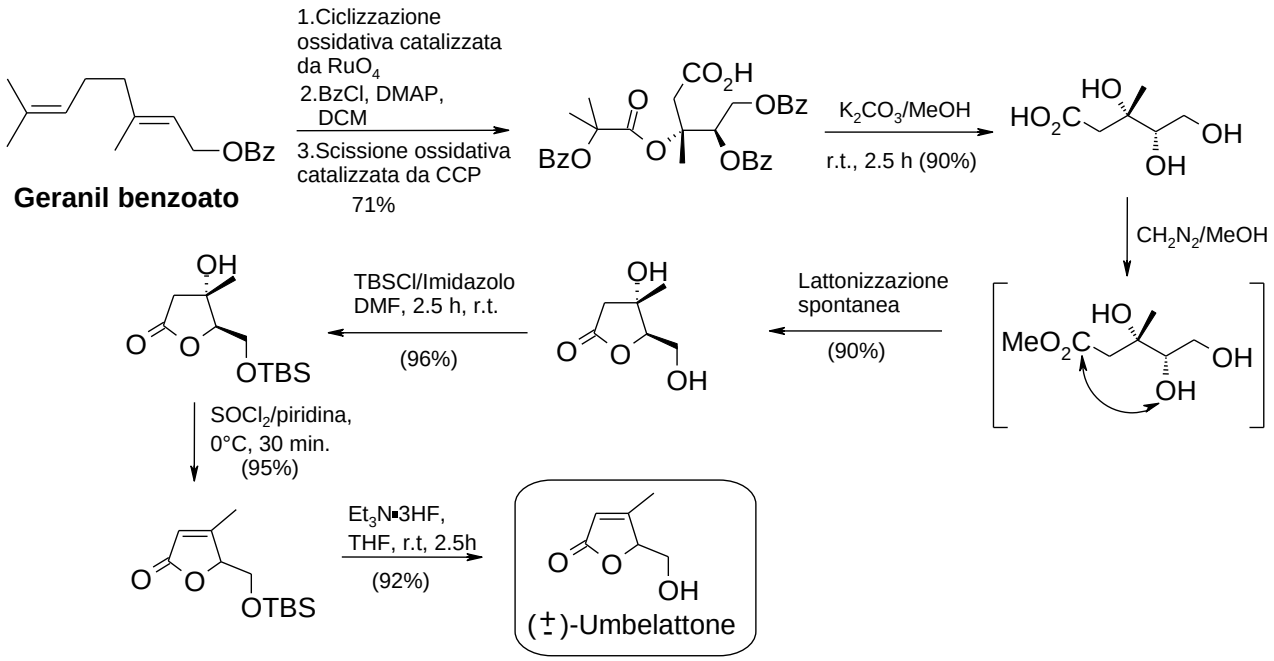
Schema 7: Reattività di anelli THF 2,2,5-trisostituiti e 2,5-disostituiti in presenza di CCP.

E' stata quindi ottimizzata la scissione dei mono-THF trisostituiti, scegliendo il composto **10** come modello. Le migliori rese (80%) sono state ottenute conducendo il processo a t.a. con il 5 % mol di PCC e 4 equiv. di H_5IO_6. Tali condizioni sono state poi applicate all'ossidazione di altri composti mono-THF trisostituiti, opportunamente sintetizzati, ottenendo i corrispondenti prodotti dicarbonilici con rese elevate (65-80%, Schema 7).



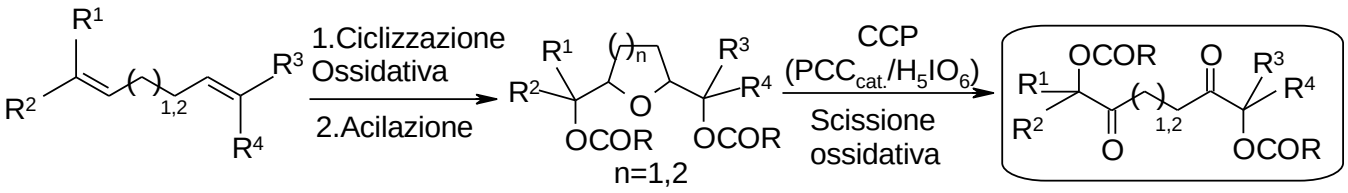
Schema 8: Esempi di ossidazione di substrati tetraidrofuranici 2,2,5-trisostituiti con CCP.

Il nuovo processo è stato poi utilizzato nella sintesi dell'umbelattone (Schema 9), un prodotto naturale a struttura γ-butenolidica isolato dagli estratti etanolici del *Memeycelon umbellatum* Brum,[10] che mostra diverse attività biologiche tra cui quella antivirale contro il virus responsabile del morbo di Ranikhet.



Schema 9 Sintesi dell'umbelattone.

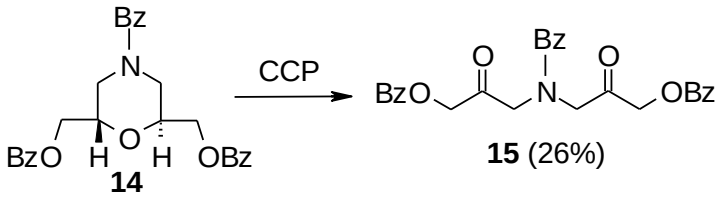
Nel corso del III anno di dottorato di ricerca è stata studiata la scissione ossidativa di mono-THF 2,5-disostituiti e mono-THP 2,6-disostituiti ad opera del sistema catalitico PCC(cat.)/H_5IO_6 (clorocromatoperiodato, CCP).[11] Tale processo consente di ottenere bis α-acilossi-1,4- ed 1,5-dichetoni. E' noto che α-acilossi chetoni sono utili "buildings blocks" in sintesi organica. La presenza di due funzioni α-acilossi-chetoniche nella stessa molecola amplia ulteriormente le potenzialità sintetiche di tali sostanze. In particolare, sono stati sintetizzati diversi substrati tetraidrofuranici e tetraidropiranici, mediante ciclizzazione ossidativa catalizzata da RuO_4 o OsO_4 di opportuni 1,5- ed 1,6-dieni,[16] i quali sono stati sottoposti a scissione ossidativa con CCP (Schema 10).



Schema 10. Sintesi di bis α-acilossi-1,4- ed 1,5-dichetoni a partire da 1,5- ed 1,6-dieni.

E' stato, inoltre, sintetizzato il substrato morfolinico **14**, che per reazione con il sistema catalitico PCC/H_5IO_6 ha dato il dichetone atteso **15**, sebbene in basse rese (Schema 11). Ciò è probabilmente dovuto al carattere di doppio legame della funzione ammidica, che induce la formazione di un

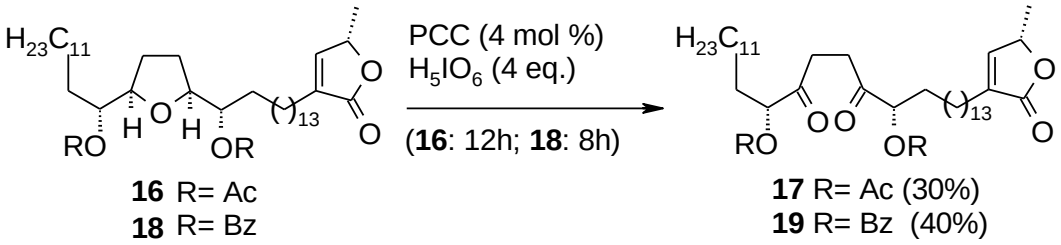
intermedio morfolinico insaturo, il quale viene poi scisso dal CCP dando prodotti collaterali.



Schema 11. Scissione ossidativa di un derivato morfolinico con CCP.

E' stato dimostrato che il gruppo benzoilico, quello acetilico, il tosilato e la funzione ammidica resistono alle condizioni di scissione ossidativa dell'anello etereo. Dagli esperimenti effettuati è, inoltre, risultato che centri chirali adiacenti alle funzioni carboniliche generate non subiscono epimerizzazione. Ciò suggerisce che utilizzando il metodo sviluppato è possibile sintetizzare bis-α-acilossi-1,4-dichetoni aciclici chirali.

Per saggiare la nuova procedura su substrati strutturalmente più complessi è stata utilizzata la *cis*-reticulatacina,[12] una molecola appartenente alla classe delle acetogenine da *Annonaceae*,[13] un gruppo di metaboliti aventi un ampio spettro di proprietà biologiche. Tale molecola è stata protetta sia come acetato che benzoato (**16** e **18**, rispettivamente) e sottoposta a scissione ossidativa con CCP fornendo i dichetoni attesi (**17** e **19**, Schema 12).

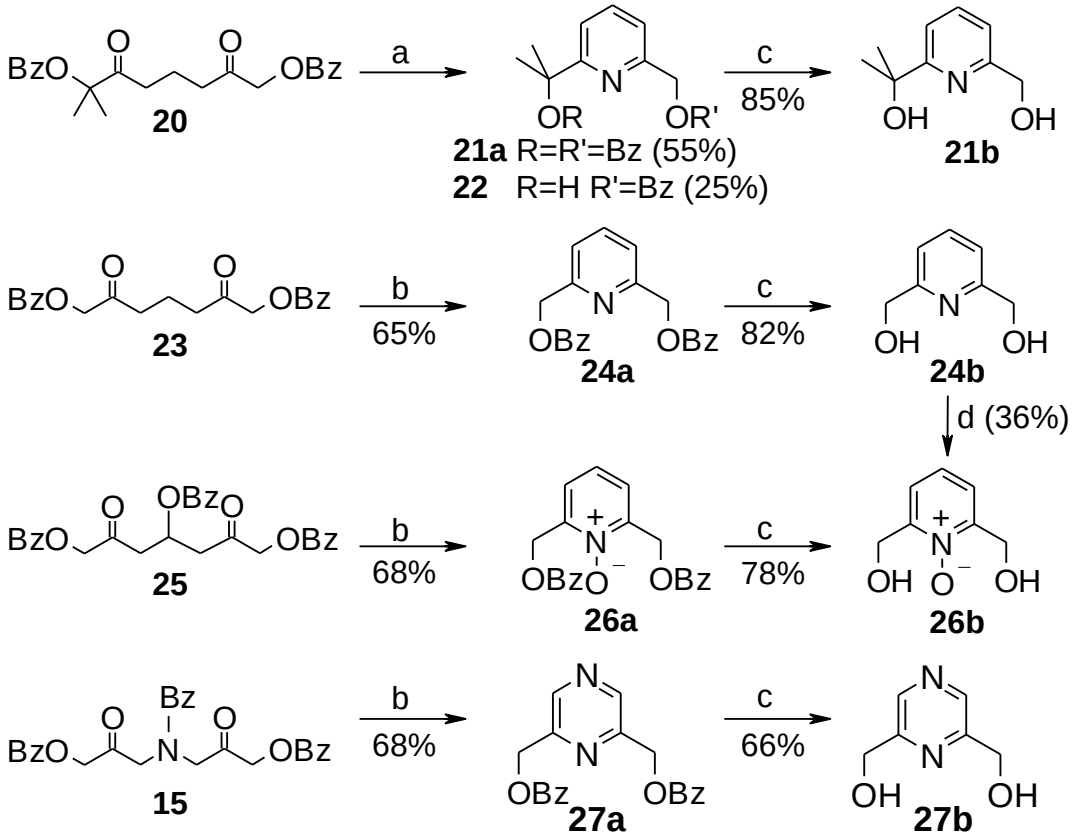


Schema 12. Ossidazione della *cis*-reticulatacina bis-acilata.

L'analisi ^{1}H NMR dei prodotti minori di tali reazioni ha mostrato che la funzione lattonica insatura terminale è preservata (presenza di segnali caratteristici a circa 5 e 7 ppm) e che pertanto anche tale funzione tollera le condizione di reazione. I risultati ottenuti suggeriscono che la procedura messa a punto possa essere utilizzata per la scissione di anelli THF in molecole complesse per ottenere nuovi analoghi "non-tetraidrofuranici" di molecole biologicamente attive, da usare in saggi biologici e studi SAR.

Dato che gruppi carbonilici α-ossigenati sono suscettibili di varie trasformazioni, è stata esplorata la possibilità di convertire i composti 1,5-dicarbonilici ottenuti in eterocicli aromatici azotati (Schema 13). In particolare, utilizzando NH_4OAc/AcOH per il dichetone **20** e le classiche

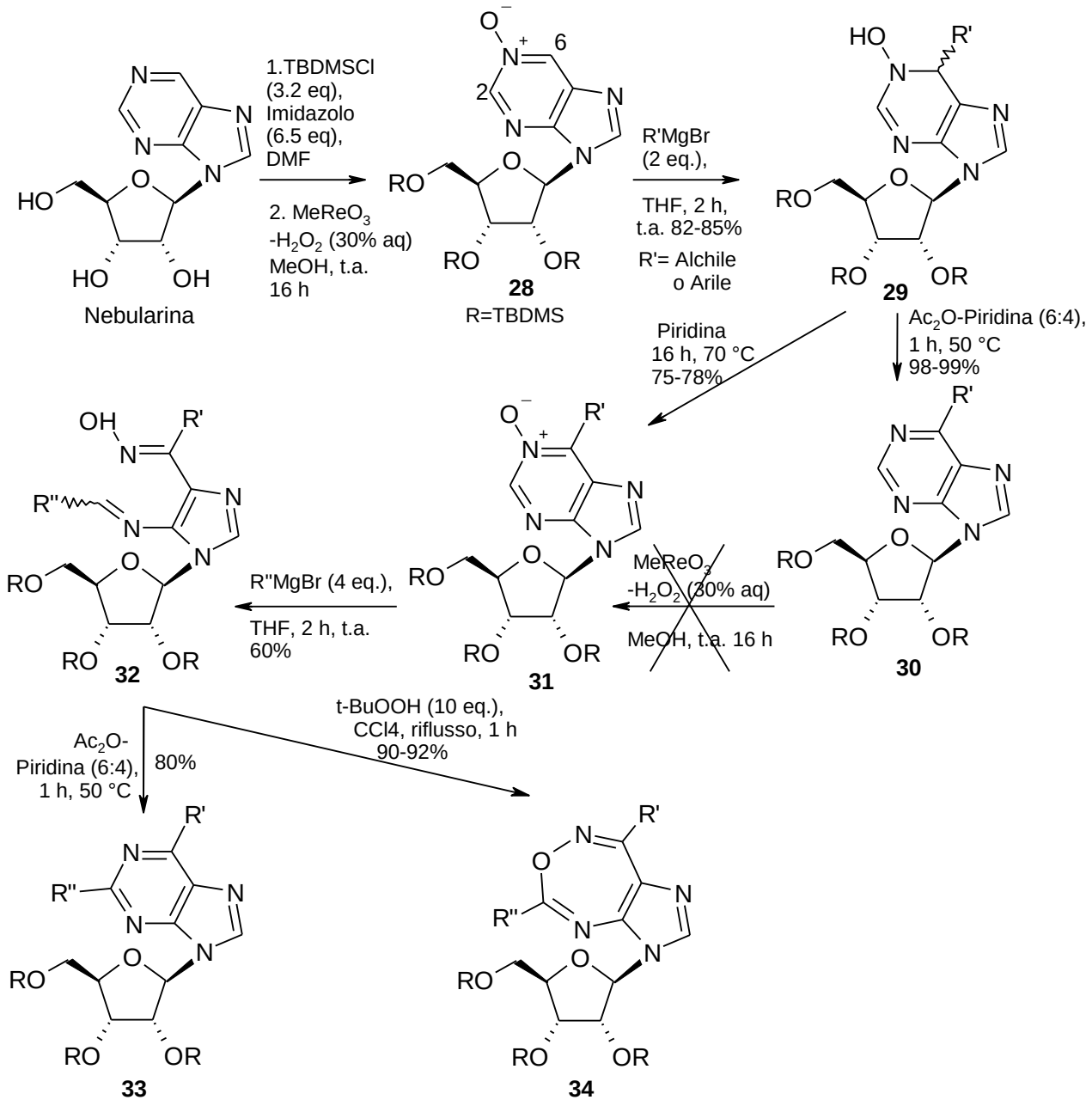
condizioni di Knoevenagel per i composti **23**, **25** e **15**, seguite da metanolisi dei gruppi protettori, è stato possibile ciclizzare gli 1,5-dichetoni sintetizzati ottenendo, rispettivamente, dimetanol-piridine (**21b** e **24b**) appartenenti alla famiglia degli "oxido-pincer ligands", sostanze caratterizzate dalle spiccate proprietà chelanti, una piridina N-ossido (**26b**) ed una dimetanol-pirazina (**27**). In particolare, la conversione di **25** in **26b** rappresenta il primo caso di formazione diretta di una piridina N-ossido da un 1,5-dichetone, mentre la trasformazione di **15** in **27b** potrebbe avere interesse non solo da un punto di vista sintetico, ma anche farmacologico, in quanto rappresenta nel complesso la conversione di un composto morfolinico in uno pirazinico (conversione di **14** in **27b**).



Schema 13. Sintesi di piridine e pirazine dimetanoliche da bis α–benzoilossi–1,5-dichetoni. a) NH_4OAc (7 eq.), AcOH (6.5 eq.), dry MeOH, 24 h t.a. 48 h 55°C; b) $NH_2OH{\cdot}HCl$ (3.5 eq.), EtOH anidro, 6 h-15 h, riflusso; c) 10 mol % K_2CO_3, MeOH, t.a., 30 min; d) MCPBA (1.2 eq.), CHCl3, t.a., 90 min.

Un'ulteriore linea di ricerca ha riguardato lo sviluppo di processi ossidativi per la sintesi di nuovi analoghi nucleosidici modificati. Negli ultimi anni, infatti, molti gruppi di ricerca hanno indirizzato la loro attenzione alla preparazione di nuovi nucleosidi e nucleotidi modificati, sulla base e/o sullo zucchero, con lo scopo di ottenere nuove molecole con potenziali attività antineoplastiche, antiipertensive e antivirali. Basi puriniche e nucleosidi recanti un C- o N-sostituente al C-6 rappresentano un'importante classe di composti che possiedono un ampio spettro di attività

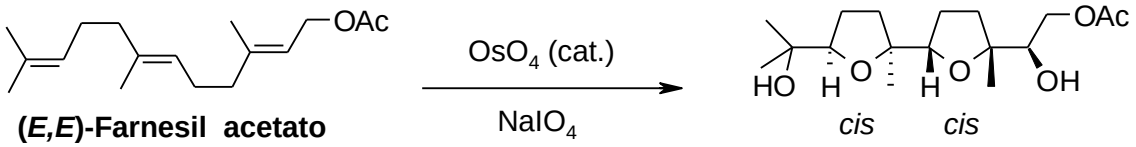
biologiche.[14,15] Di recente, nell'ambito di uno studio in collaborazione con il gruppo di ricerca del professor L. Mayol del Dipartimento di Chimica delle Sostanze Naturali della Federico II, è stata messa a punto una nuova via sintetica del tutto generale per la preparazione di nucleosidi C-6 C-sostituiti. In particolare è stata studiata la reattività della porzione nitronica C6-*N*1-O^- della 9-ribosil-purina (nebularina) N1-ossido nei confronti dei reattivi di Grignard (Schema 14).[15] L'iniziale attacco di questi ultimi sulla posizione più reattiva C-6, fornisce l'addotto N1-idrossi C6-sostituito, che poi aromatizza per trattamento con anidride acetica in piridina. Contrariamente a quanto atteso, il trattamento dei nucleosidi C-6-sostituiti con $MeReO_3$ non permette di ottenere nucleosidi purinici N1-ossido C-6-alchil (aril)-sostituiti **31** con rese soddisfacenti.



Schema 14. Sintesi di nuovi nucleosidi modificati.

L'osservazione che il trattamento dell'addotto **29** con la sola piridina ad elevata temperatura porta alla formazione di nucleosidi purinici N1-ossido C-6-alchil (aril)-sostituiti voluti (**31**), ha aperto la strada allo studio di un'ulteriore funzionalizzazione della base purinica, in posizione C-2. Su tali basi, in collaborazione con il suddetto gruppo di ricerca, è stata realizzata una collezione di nucleosidi purinici C-6-alchil (aril)-sostituiti N1-ossido (**31**) da funzionalizzare al C-2. In particolare, gli studi effettuati hanno mostrato che il secondo attacco del reattivo di Grignard in posizione C-2 fornisce l'intermedio aperto **32** che può ciclizzare e aromatizzare fornendo nucleosidi 2,6-disostituiti se trattato con anidride acetica in piridina, oppure può fornire "ring-expanded nucleosides" (RENs), contenenti il nuovo sistema biciclico imidazo[4,5-d][1,2,6]-ossadiazepinico, per trattamento con *t*-BuOOH (10 eq.) in CCl_4 a riflusso.[6b] Con tale procedura sono stati ottenuti diversi nucleosidi purinici 2,6-alchil (aril) sostituiti. Inoltre, i RENs sintetizzati hanno mostrato interessanti attività antitumorali nei confronti di cellule tumorali del seno (MCF-7) e del polmone (A549).[14]

Infine, nel corso dell'ultimo anno di ricerca sono stati condotti studi preliminari riguardo la scissione ossidativa di anelli tetraidrofuranici con il sistema catalitico $RuO_4/NaIO_4$ ed è stata saggiata la bis-ciclizzazione ossidativa mediata dall'OsO_4 di substrati trienici tipo farnesil acetato (Schema 15).



(*E,E*)-Farnesil acetato

Schema 15. Bis-ciclizzazione ossidativa mediata da OsO_4.

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Chapter 1

The use of transition metal oxo-species in organic chemistry

1.1. Introduction

Oxidation is one of the most fundamental reactions in synthetic organic chemistry and there is always demand for selective and mild oxidation methods. Indeed, over the past few years, numerous research groups have directed their efforts toward the development of novel and useful oxidation processes. In particular significant progress has been achieved within the area of catalytic oxidations, which has led to a variety of selective and mild methods. These reactions may be based on organocatalysis, metal catalysis or biocatalysis. In this regard transition metal oxo-species are of particular interest since they can catalyze a wide spectra of oxidative reactions. This thesis is focused on the use of some of these species namely RuO_4, PCC, $MeReO_3$ and OsO_4, with the aim of establishing new general and synthetically useful oxidation methods.

1.2. RuO_4-catalyzed oxidation of organic compounds

Ruthenium (VIII) tetroxide, RuO_4, is a tetrahedral 16-electron d^0 species, which is isoelectronic to the well-known OsO_4. It was introduced as an organic oxidant in 1953 by Djerassi,[1] who oxidized several sulfides to sulfoxides and sulfones. Since then it has been used for a long time almost for the cleavage of C=C bonds and aromatic compounds leading to its reputation of being a reagent too reactive to be selective. RuO_4 can be generated on treatment of catalytic amount of $RuCl_3$ or RuO_2 with a stoichiometric oxidant such as $NaIO_4$, HIO_4, NaOCl, or $NaBrO_3$, or under electrochemical conditions (Figure 1).[2]

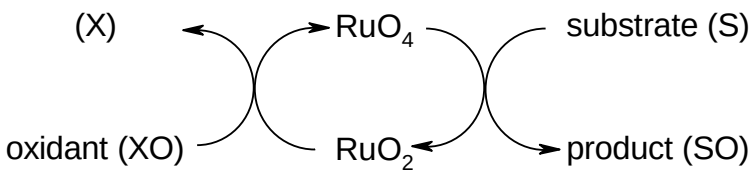


Figure 1. RuO_4 catalytic cycle.

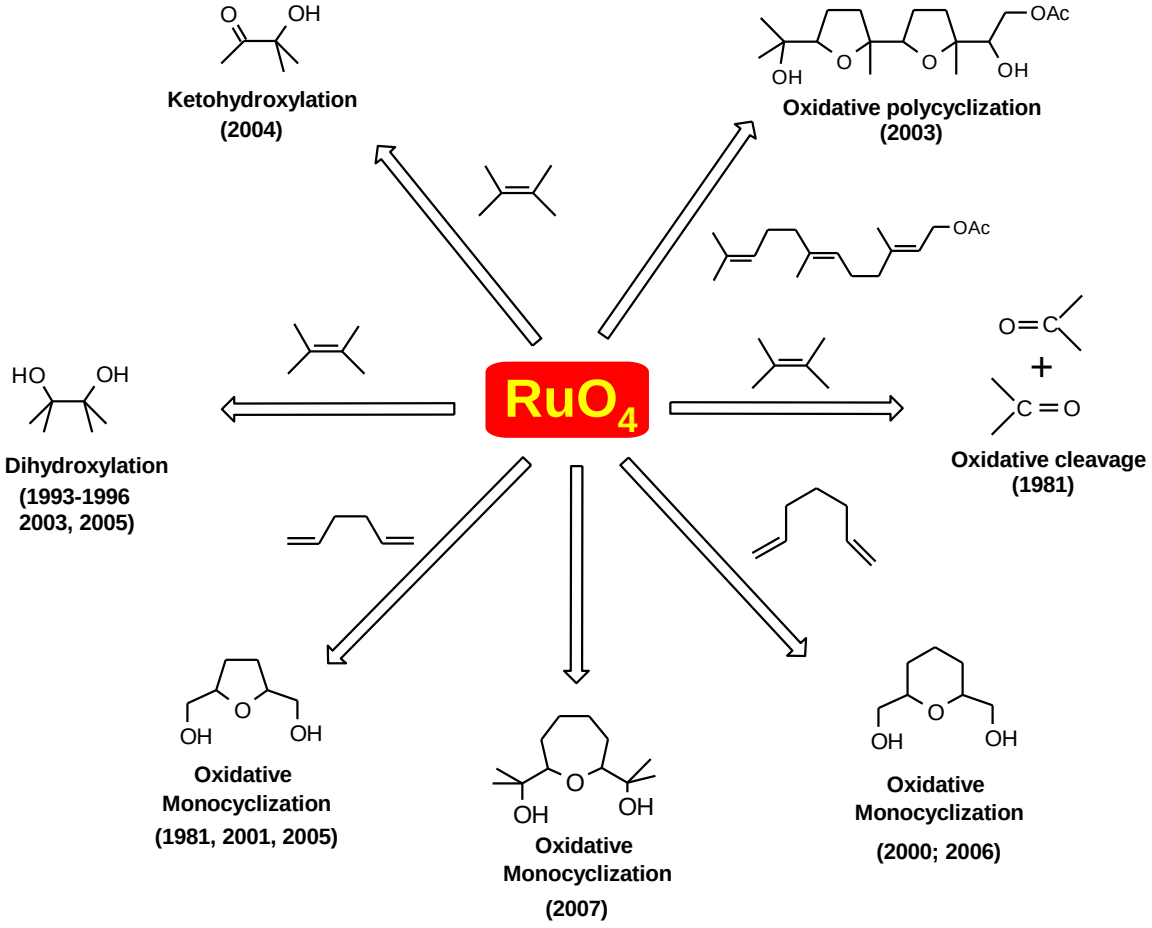
At the beginning the reactions were performed in a biphasic system made up of organic solvent and water, leading to sluggish suspensions that made reactions very slow and incomplete. These problems were due to inactivation of ruthenium catalysts because of the formation of low-valent ruthenium carboxylate complexes, which inhibit the catalyst activity by acting as strongly coordinating ligands.[3] This trouble was overcome by Sharpless,[4] who noticed that the addition of CH_3CN as a co-solvent led to a more selective and faster oxidation even reducing the amount of catalyst. Thus, various oxidations with RuO_4 are improved considerably by employing a solvent system consisting of CCl_4-H_2O-CH_3CN. However, it was only in 1993, with the study made by the research group where this thesis have been carried out, that RuO_4 was found to be a powerful and selective catalyst for the non-destructive oxidation of olefins.[5] Indeed, they managed to get *syn*-diols through a process similar to the reaction of the isoelectronic and less reactive OsO_4 with alkenes. Next Shing *at al.*[6] reported the dihydroxylation of a wide range of olefines with RuO_4 calling that process "flash dihydroxylation" because of the reduced reaction time. With subsequent studies, it was clear that RuO_4 was able to give dihydroxylation even on substrates unreactive with OsO_4 o MnO_4^-.

The interest towards the oxidation not only of unsaturated substrates but also of a great variety of functional groups has grown over the time and now RuO_4-mediated oxidations are an indispensable and irreplaceable tool for organic chemists (Scheme 1), as reported in a recent review.[2]

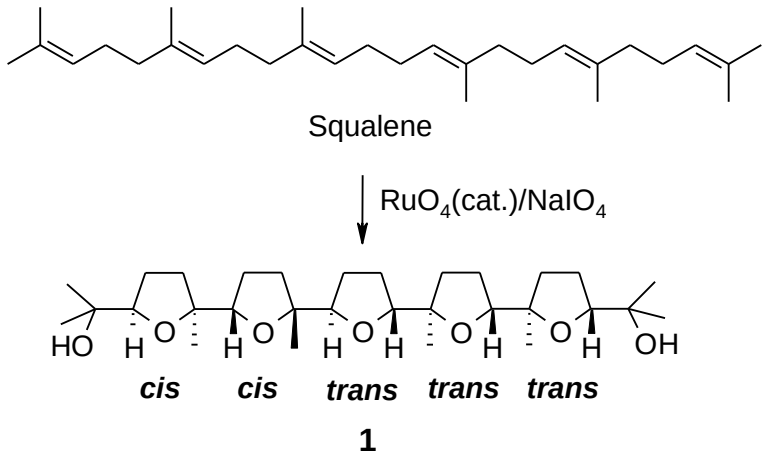
Recently the dihydroxylation reaction has been further improved and conditions for α-chetols have been developed.[7] Lately our research group was interested in the oxidative cyclization of 1,n-dienes showing that stereoselective synthesis of 2,5-THF-diols, 2,6-THP-diols, 2,7-oxepan-diols from 1,5-dienes, 1,6-dienes e 1,7-dienes, respectively, is possible.[8]

Moreover the same group has also discovered the stereoselective oxidative polycyclization of polyenes characterized by a repetitive 1,5-diene structural motif.[8a] This process allows access to adjacently linked polytetrahydrofuran products in a single step using catalytic amounts of RuO_4 in the presence of $NaIO_4$ as co-oxidant. Among the reported experiments, the pentacyclization of squalene is noteworthy, not only because it generates five THF rings in a single step with a remarkable yield of 40-50%, but also because ten new chiral centers are formed and the product is obtained as a single diastereoisomer (Scheme 2).[8a, b] This process is useful in organic synthesis, since it can be used for the preparation of the THF portions of numerous natural and non-natural biological active substances, such as polyether antibiotics,[9] squalene-derived metabolites[10] and annonaceous acetogenins,[11] containing one or more 2,5-disubstituted THF rings adjacently linked.

The ruthenium-mediated oxidative polycyclization process seems to be closely related to the oxidative polycyclization of polyenic bis-homoallylic alchohols carried out with rhenium(VII) oxo-species that allows the formation of either bis- or tris-tetrahydrofuran compounds.[12]



Scheme 1. RuO_4 mediated transformations'overview.



Scheme 2. RuO_4 mediated pentacyclization of squalene.

In this thesis we have further explored the potential applications of RuO_4 in the organic synthesis. In particular we have reinvestigated the pentacyclization reaction of squalene in new conditions, discovering new and unexpected results (see Chapter 4). Some preliminary experiments have been also carried out to find alternative conditions for the oxidation of 2,5-substitued THF ring, a PCC oxidative process developed during my PhD (see Chapters 3 and 6).

1.3. From Pyridinium Chlorochromate (PCC) to Chlorochromatoperiodate (CCP)

Pyridinium chlorochromate (PCC, also known as Corey's reagent) is a well known reagent employed in an array of oxidising processes.[13] It is a red-orange solid salt with the formula $C_5H_5NH[CrO_3Cl]$.

PCC was synthesized for the first time in 1899 by Meyer,[14] but only after the studies of Corey[15] and co-workers in 1975 organic chemists began to use it for the oxidation of primary and secondary alchools to aldehyde and chetones.

For many years, researchers have worked to find a mild, versatile and selective reagent for the operationally simple oxidation of alcohols to carbonyl compounds. Many reagents containing the chromium (VI) ion have been studied, but a large portion of them may not be used in modern organic synthesis due to difficulties in their use, toxicity or because too poorly selective.[13]

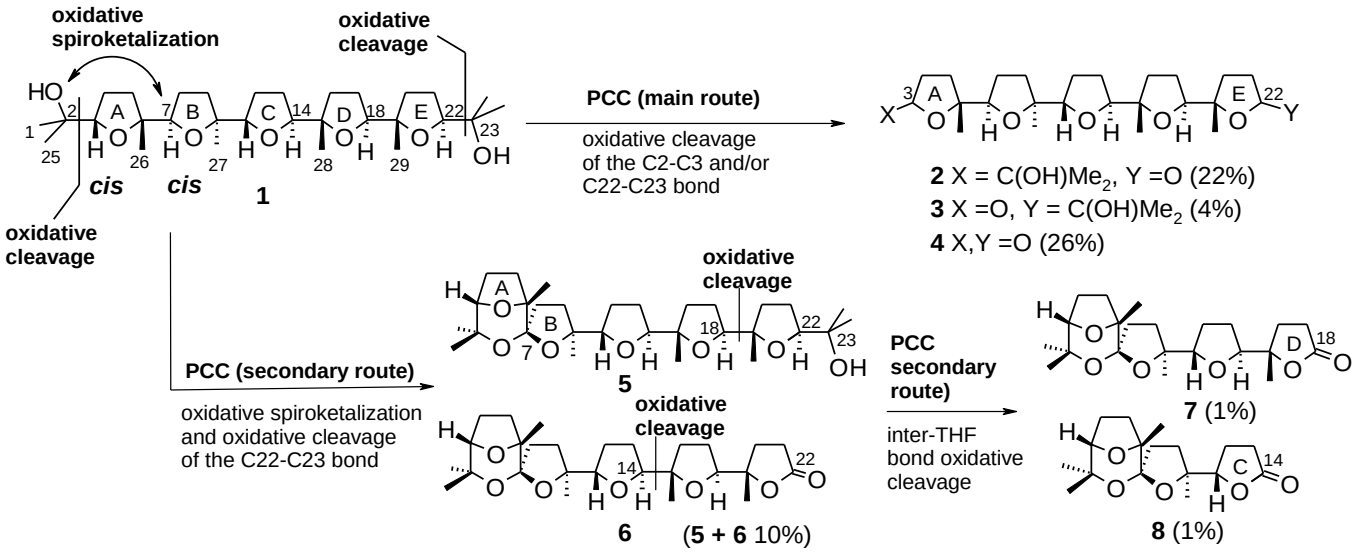
PCC have almost replaced the Jones and Collins reagents in the oxidation of alcohols. Indeed, when compared with the Jones reagent, which is a solution of chromium trioxide in diluted sulfuric acid, PCC is milder, less acidic and does not promote the over-oxidation of obtained aldehydes to carboxylic acids. Both the Collins reagent, which is a chromium trioxide/pyridine complex, and PCC are able to stop primary alcohols oxidation at aldehyde level, but PCC is easier and safer to prepare (Collins reagent can inflame during preparation), self-stable, and more efficient, since up to a sixfold excess of Collins reagent may be required to effect a complete reaction.[16]

PCC can be prepared easly and safely by addition of chromium trioxide to 6 N hydrochloric acid, then pyridine is added at 0 °C to the unstable chlorochromic acid formed and immediately PCC precipitates as a red-orange solid which is not appreciable hygroscopic. [13]

An improved procedure for PCC preparation has been described by Agarwal in 1990.[17] In the same paper is reported that synthetic utility of the reagent increases in the presence of anhydrous acetic acid, used for the first time as catalyst, for the oxidation of alcohols.

Though the most popular process mediated by PCC is the oxidation of primary and secondary alcohols, many other functional groups undergo PCC oxidation. As a continuation of the interest in oxidative processes mediated by transition metal oxo-species as well as in the synthesis and

derivatization of new THF-containing compounds,[8] the research group where this thesis have been carried out recently focused on oxidation processes mediated by PCC.[18] These studies led to the discovery of a new interesting oxidative spiroketalization process. In particular, it has been reported[18] that penta-THF **1,** obtained by the Ru(VIII)-catalysed oxidative pentacyclization of squalene (Scheme 2),[8b-d] when treated with PCC furnishes four new compounds (**2**-**5**) representative of a novel class of cytotoxic antitumor poly-THF spiroketals, besides poly-THF γ-lactones **6**-**8**, the main products of the process (Scheme 3).

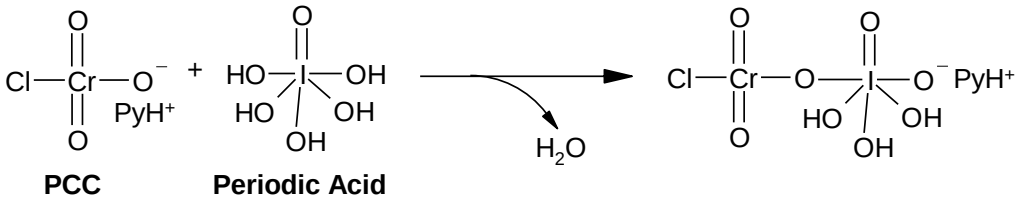


Scheme 3. Spiroketal and degradation products obtained by PCC-mediated oxidation of a penta-THF compound

Inspection of the structure of these substances reveals that compound **1** undergoes three types of oxidative processes all involving the interaction of the oxidant with different THF ether methine groups. In particular, lactones **2** and **4**, the main oxidation products, are produced by oxidative removal of one or both the terminal three-carbon chains of **1**. The spiroketal-containing compound **5**, possessing the intact carbon skeleton of **1**, originates from an oxidative spiroketalization process formally involving the C(2)OH group and the C-7 carbon at the *cis*-*cis* bis-THF terminus of the poly-THF chain. The terminal lactone in the related spiro-compound **6** in turn arises from the further oxidative cleavage of the C22-C23 bond in **5** as it happens when compounds **2-4** are generated from **1**. Finally, an inter-THF C-C bond cleavage in **5** and/or **6** is responsible of the formation of the degraded, minor, spiro-compounds **7** and **8**.

During my PhD programme this process has been further explored leading to the discovery of related novel oxidative pathways.

PCC is usually used in stoichiometric or in excess amounts to oxidise a variety of functional groups. In contrast, the use of PCC in catalytic amounts has received little attention to date,[19,20] though chromium(VI) species are known to be carcinogenic and environmentally hazardous and the use of substoichiometric amounts of such reagent would therefore be highly desirable. In this respect, the catalytic system PCC (cat.)/H_5IO_6 is particularly appealing. From previous studies[21] it is thought that the combination of PCC and H_5IO_6 generates chlorochromatoperiodate (CCP, Scheme 4), an oxidising agent more powerful than PCC itself.



Scheme 4. Chlorochromate periodate (CCP) formation.

CCP has been employed in only a few cases so far, in particular in 2005 Hunsen described a pyridinium chlorochromate catalyzed (2 mol%) oxidation of alcohols to ketones and aldehydes using 1.05 equiv of H_5IO_6 in acetonitrile.[19a] In 2008, Stark and Roth reported the catalytic cleavage of cyclic β-hydroxy ethers, which were converted to the corresponding γ-butyrolactones and δ-valerolactones within minutes and isolated in excellent yield.[20]

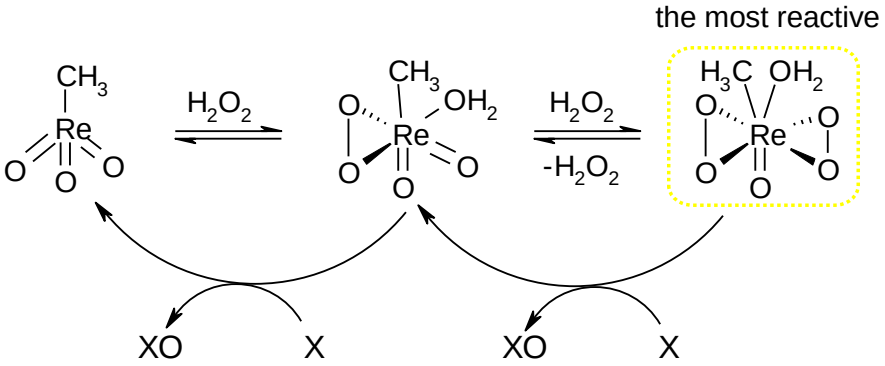
As a part of our ongoing interest in oxidative processes mediated by PCC a substantial part of my thesis work was devoted to the study of new oxidative processes mediated by catalytic amounts of PCC, testing the CCP's ability to interact with THF methane bonds and finding new useful oxidative transformation involving various THF ring systems (see Chapters 2 and 3).

1.4. Methyltrioxorhenium, a versatile oxidant in organic synthesis

Methyltrioxorhenium (MTO for short) is one of the most versatile oxidation catalyst known to date and its efficient synthesis and catalytic utility have made it one of the most studied organometallic compound. It has been widely studied expecially during the last 30 years as an oxygen transfer reagent.[22] Actually it has been used for the oxidation of a number of functionalized compounds, including alkenes, alkynes, arenes, phenols, benzaldehydes, sulfides, anilines, amines, phosphines, as well as for insertion into C-H bonds.

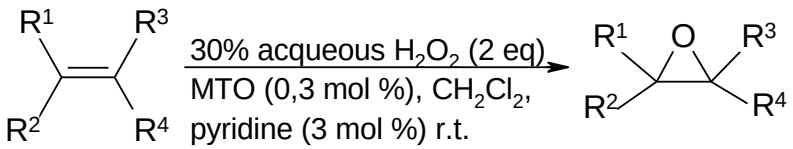
MTO was synthesized for the first time in 1979 by Beattie and Jones.[23] Later, in 1991, Herrmann developed a more simple synthesis featuring the reaction of dirhenium heptoxide with methyltributyltin.[24] Herrmann was the first to demonstrate the ability of MTO to catalyze the

epoxidation of alkenes. In 1993 Espenson *et al.* began to study MTO from a mechanistic point of view so they have fully explained the mechanism of oxygen transfer in the MTO/H_2O_2 system.[25] H_2O_2 is usually used as the stoichiometric oxidant in reaction with MTO. MTO reacts with H_2O_2 to give equilibria with formation of monoperoxo- and diperoxo-rhenium(VII) species (Scheme 5). Both peroxorhenium complexes are able to react with oxygen-accepting substrates. However the latter confers a characteristic yellow color to the solution and it is the most reactive.



Scheme 5. General mechanism of oxygen transfer from hydrogen peroxide catalyzed by MTO.

Until the discovery of the MTO/H_2O_2 system, epoxidation were performed almost exclusively using *meta*-chloroperoxybenzoic acid (*m*-CPBA). In contrast with the *m*-CPBA, MTO has many advantages: it is safer; it has greater scope and selectivity, as even acid sensitive substrates can be oxidized; it is more reactive, requires less solvent and involves easier product work up and isolation; water is the only byproduct. Moreover Sharpless and coworkers discovered that the addition of at least 3 mol % catalytic amounts of pyridine to the MTO/H_2O_2 system improves olefins epoxidation by increasing the rate, preventing epoxide hydrolysis and extending the catalyst lifetime (Scheme 6).[26]



Scheme 6. Alkenes epoxidation catalyzed by the MTO/H_2O_2 system.

In 1998 as a continuation of the interest in oxidative processes mediated by transition metal oxo-species, the research group where this thesis has been carried out tested the reactivity of some conjugated diene steroids with MTO, using H_2O_2 as a co-oxidant.[27]

Another fundamental reaction catalyzed by MTO is the oxidation of secondary amines. This reaction is an important means to obtain nitrones, which are precious substrates in organic synthesis for the assembly of structurally complex nitrogen-containing molecules. The oxidation of amines can be performed either with MTO/H_2O_2 system or with urea-hydrogen peroxide complex (UHP) as the stoichiometric oxidant in reactions carried out in non-aqueous media.[28]

More recently a convenient method for the selective N-oxidation of purine catalyzed by the MTO/H_2O_2 system.[29] Inspired by these findings, part of the current PhD project has also been devoted to the achievement of a novel approach to the synthesis of nucleoside analogues possessing new base modifications. The developed method is based on the formation of a C6-N1-O^- aldonitrone moiety of 9-ribosyl-purine (nebularine) *N*1-oxide in the presence of MTO which is then prone to be attacked at the electrophilic C-6 carbon by a Grignard (see Chapter 5).

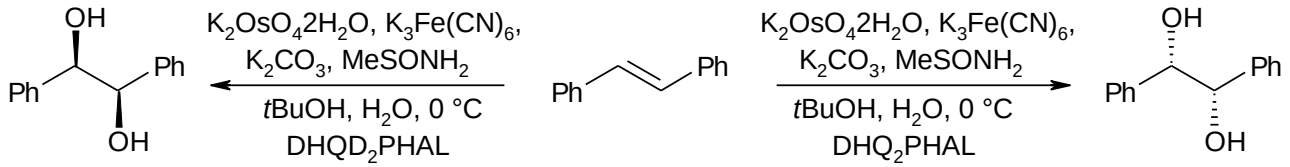
1.5. OsO_4, a Nobel prize oxidant

Among osmium compounds, OsO_4 is the most important and easy to prepare. As well as ruthenium, osmium is octavalent (of all the elements, they are the only two that reach such a high oxidation state), but in contrast with the first, it is a more controllable and milder oxidizing agent and most of its applications derive from this property.[30]

One of the osmium tetroxide distinguishing feature is that the solid is volatile at room temperature. Its vapors are poisonous, but OsO_4 carries its own warning system, a characteristic rather ozone-like smell, that makes accidents with it rare. However, despite its toxicity, an aqueous solutions of osmium tetroxide is used to treat refractory rheumatoid arthritis in humans by local administration.[31] Overall OsO_4 should be regarded as no more dangerous than many reagents daily used in laboratories. Osmium tetroxide is also expensive and this, together with the safety concerns, led to the development of catalytic methods.

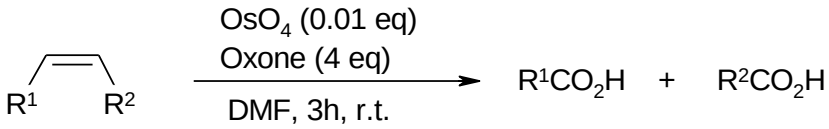
OsO_4 was discovered in 1803 by Smithson Tennant, who also isolated metallic osmium from it.[32] Since then osmium tetroxide applications have increased over the time, both in the organic chemistry and in biochemistry. Osmium tetroxide capability of oxidizing olefins is known and applied since 1913,[30] but the process has been widely studied from the 70s by Sharpless and many others chemists.[33] However it was only during the 90s that the Sharpless'group developed the optimized conditions for the catalytic asymmetric dihydroxylation (AD) process (Scheme 7). They found that best *ee* were obtained using K_2OsO_4•$2H_2O$, as source of OsO_4, $K_3Fe(CN)_6$, as the stoichiometric oxidant, K_2CO_3 and methanesulfonamide ($MeSO_2NH_2$), to increase the rate of the reaction, and cinchona alkaloid derivatives as chiral ligands. Together, these reagents form a premix

which is now commercially available under the name of "AD-mix". For his work on AD, Sharpless was awarded the Nobel Prize in 2001.



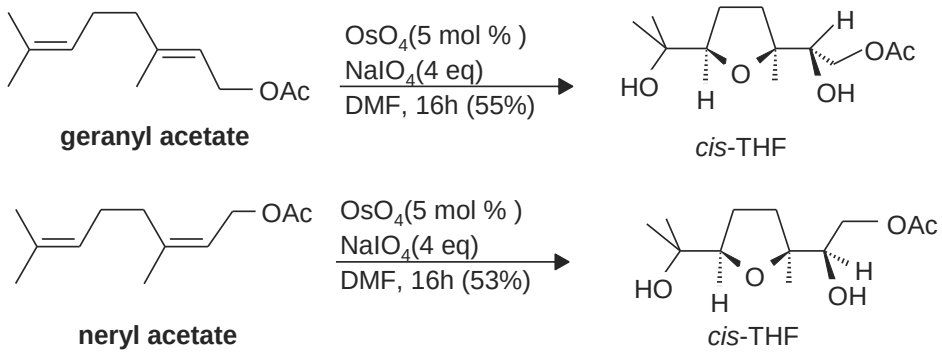
Scheme 7. Sharpless catalytic asymmetric dihydroxylation. DHQD and DHQ are the alkaloid ligands dihydroquinidine and dihydroquinine, respectively. PHAL is the phthalazine based group attached to the ligands.

Another important OsO_4-catalyzed reaction is the oxidative cleavage of olefins to aldeydes and/or ketones. The standard method for the direct oxidative cleavage of olefins is ozonolysis, but due to safety concerns alternative procedures have been developed. There are different methods employing OsO_4, the most common are Johnson-Lemieux oxidation ($NaIO_4$, OsO_4 (cat.))[34] and the more recent and popular Upjohn dihydroxylation/diol cleavage (NMO, OsO_4 (cat).; $NaIO_4$).[35] Both methods are based on the olefins dihydroxylation by catalytic OsO_4, followed by $NaIO_4$ cleavage of the resulting 1,2-diol, but Johnson-Lemieux oxidation employed $NaIO_4$ also as co-oxidant for osmium tetroxide, whereas in the Upjohn oxidation NMO is used. Borhan and co-workers have recently developed new procedures (an example is reported in scheme 8) involving the use of oxone (a triple salt containing two parts of $KHSO_5$, one part of $KHSO_4$, and one part of K_2SO_4)[36] or of H_2O_2[37] as the co-oxidant for oxidative cleavage of olefins with OsO_4, proceeding without the intermediacy of 1,2-diols.



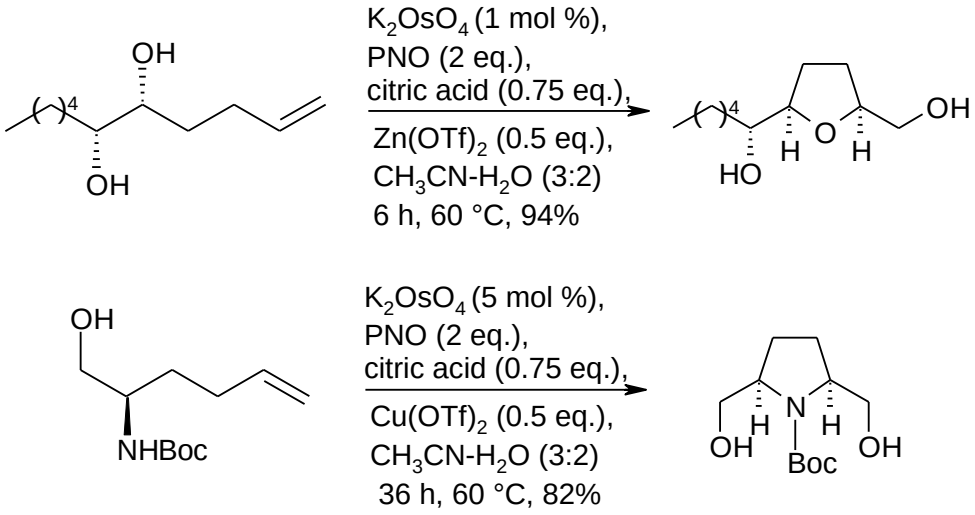
Scheme 8. Osmium tetroxide-promoted cleavage of olefins (Borhan's procedure).

At this point, we should mention another fundamental osmium tetroxide application that is the cyclization of dienes to THF compounds, that was first reported in 1998 by the research group where this thesis have been carried out.[38] Indeed professor Piccialli found that using catalytic osmium tetroxide with sodium periodate as a reoxidant it was possible to cyclize geranyl acetate and neryl acetate into their corresponding THFs in moderate yields (see Scheme 9).[38]



Scheme 9. The first example of OsO_4–mediated cyclization of 1,5-dienes by V. Piccialli.[38]

Then Donohoe and co-workers have widely studied the osmium–mediated cyclization of 1,5-dienes.[39] They continuously developed the method until it has been transformed into an extremely efficient and powerful new catalytic reactions for the stereoselective formation of THFs and pyrrolidines (Scheme 10). In particular they found that by performing a regioselective asymmetric dihydroxylation of certain polyenes and treating the resulting enantioenriched diols (bearing a pendant alkene) with Os^{VI} rather than the more familiar Os^{VIII}, more satisfactory results in terms of yields and stereoselectivity were obtained. All their efforts in this area are recorded in a recent perspective. [39]



Scheme 10. Donohoe's conditions for THFs and pyrrolidines synthesis.

As a continuation of our efforts toward the synthesis and modification of THFs containing compounds, during the third year of my PhD programme some preliminary experiments have been carried out to evaluate the possibility of inducing the OsO_4-catalized oxidative bis-cyclization of farnesol-like substrates (see Chapter 7).

1.6. References

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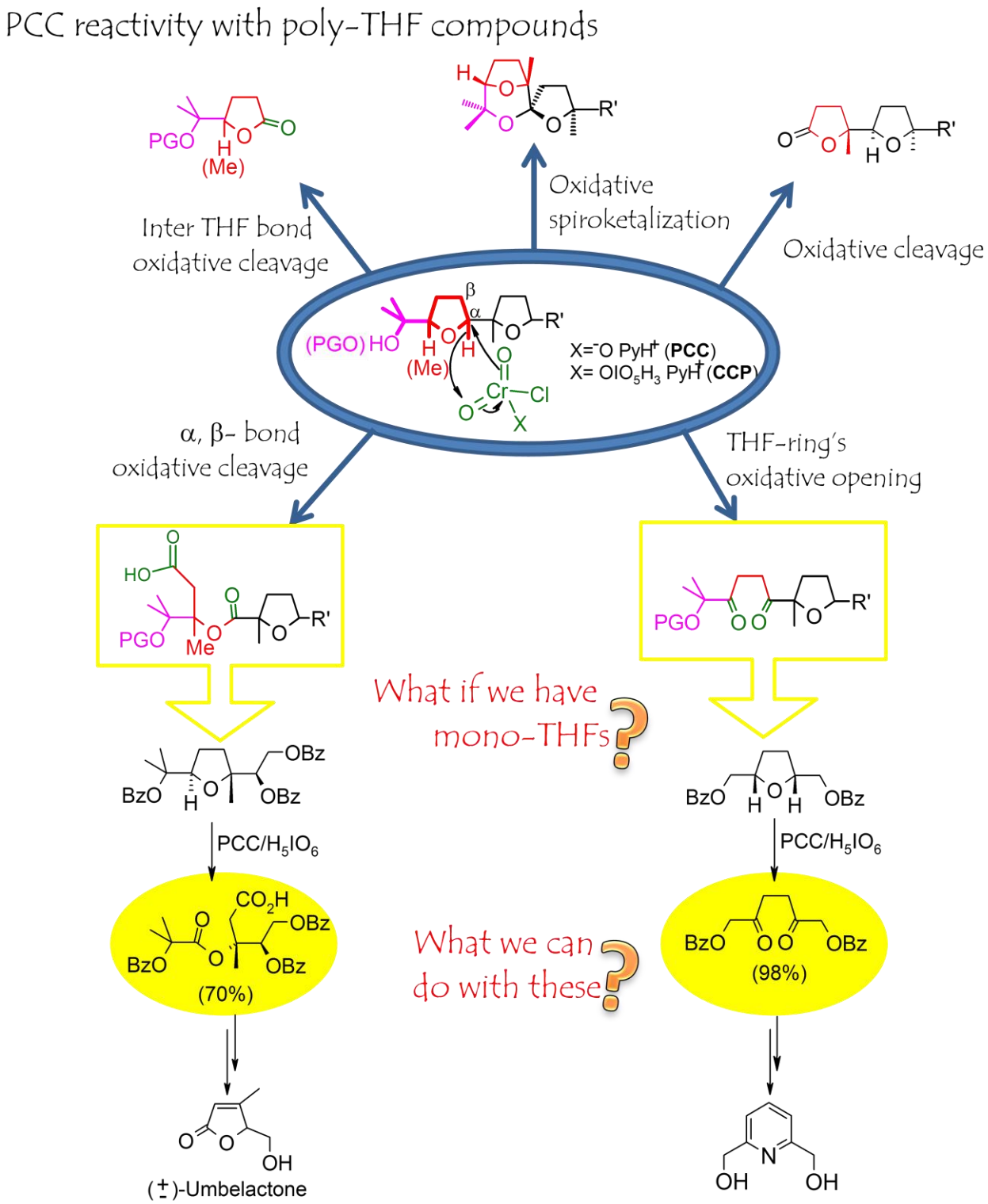
Part 1. *Piridinium Chlorocromate Chemistry*



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Chapter 2

Pyridinium chlorochromate-mediated oxidation of mono- and poly-tetrahydrofurans. Disclosure of novel oxidative pathways

2.1. Introduction

Tetrahydrofuran-containing compounds are widely distributed in nature. In particular 2,5-disubstituted THF fragments are commonly found in a great variety of biologically active substances such as polyether antibiotics, e.g. monensin A,[1] squalene-derived polycyclic polyether metabolites (oxasqualenoids) such as glabrescol,[2] and annonaceous acetogenins, such as goniocin and mucocin (Figure 1).[3] Interest toward isolation, synthesis and structural modification of the basic backbone of these substances is still very keen as witnessed by the numerous researches devoted to these targets. Synthesis of non-natural poly-THF or THF-containing substances represents a related area of research aimed at obtaining materials displaying new important biological properties.

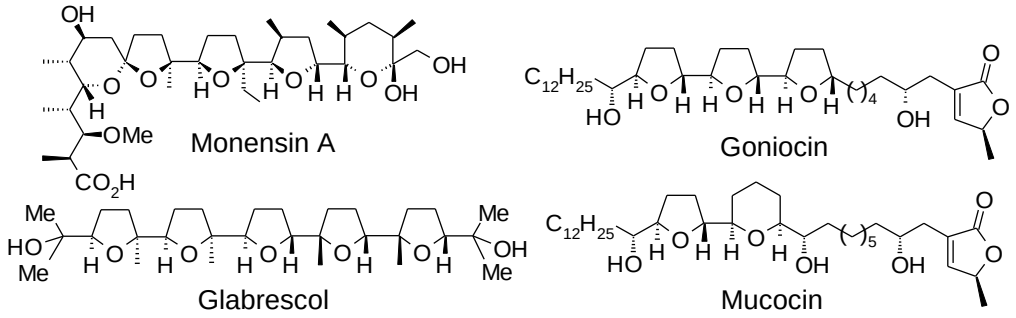
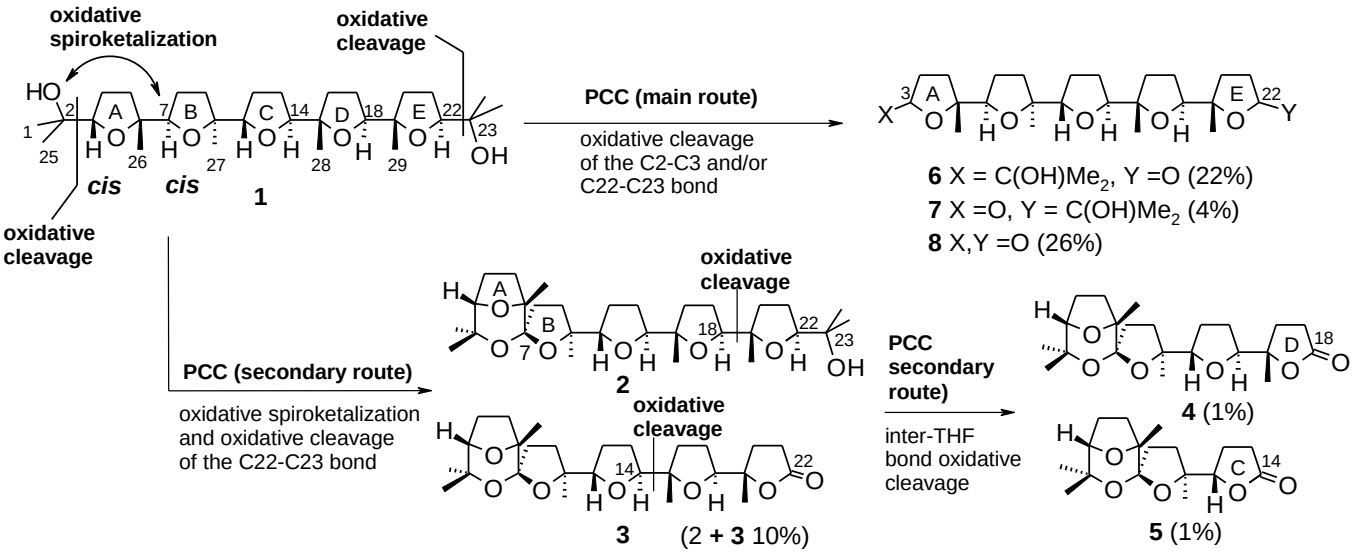


Figure 1. Some bio-active THF-containing natural compounds.

As a part of our ongoing interest in oxidative processes mediated by transition metal oxo-species[4] and in particular in the synthesis and derivatization of mono and poly-THF compounds,[5] we report here the results of a study concerning the oxidation of some mono- and poly-THF compounds with pyridinium chlorochromate (PCC)[6] as well as with the catalytic system PCC (cat.)/H_5IO_6 (Chlorocromatoperiodate, CCP)[7, 8, 9] (for PCC and CCP see Chapter 1).The present investigation started from some observations made during the oxidation of a poly-THF compound with PCC. In particular, we have recently reported[10] that compound **1**, the penta-THF product obtained by the Ru(VIII)-catalysed oxidative pentacyclization of squalene,[5g, 5j] when treated with PCC (Scheme 1, see also Chapter 1, paragraph 1.3) furnishes compounds **2-5**, four representative of a novel class of cytotoxic poly-THF spiroketals, besides poly-THF γ-lactones **6-8**, the main products of the process.



Scheme 1. Summary of the products obtained by PCC-mediated oxidative spiroketalization/oxidative cleavage of penta-THF compound **1**.

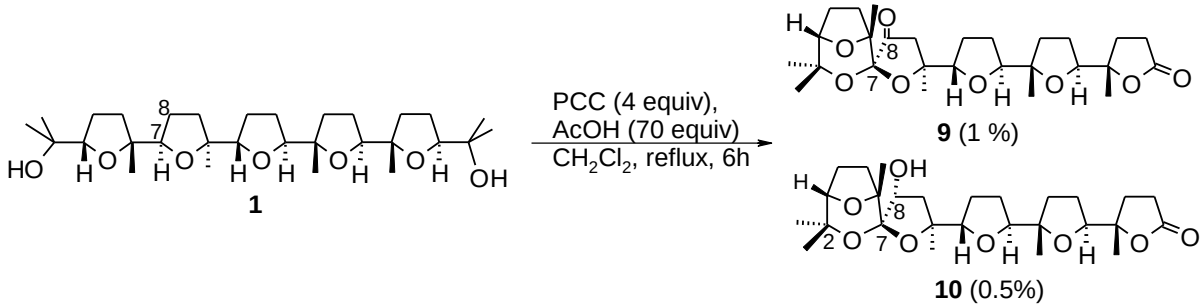
Whereas the PCC-mediated conversion of α-hydroxy mono- and poly-THF compounds to γ-lactones (see for example the conversion of **2** to **3** or **1** to **6-8**, scheme 1) is well-documented,[11] PCC-mediated cleavage of inter-THF bonds is unprecedented. In order to collect further evidences on the oxidative routes leading to compounds **2-8**, we preliminarily undertook a careful analysis of some persistent minor side-products formed during the oxidation of **1** with PCC. This survey allowed to confirm that PCC is indeed able to cleave the inter-THF bonds in **1** providing a deeper insights into the mode of action of PCC with THF-containing compounds. Prompted by these results we next examined the oxidation of some less structurally complex poly- and mono-THF substances with PCC, extending our investigation to the related PCC(cat.)-H_5IO_6 system as well. Overall, the acquired data leaded to disclose novel oxidative pathways on the oxidation of differently substituted and configurated tetrahydrofurans with PCC or allowed to confirm previously formulated mechanistic hypotheses on some PCC-mediated processes.[10] A new plausible hypothesis on the PCC-mediated oxidative cleavage of THF rings bearing an α tertiary alcohol function,[5d, 5g, 11] such as **1**, **6**, **7** or **2**, to the corresponding γ-lactones, that well agrees with the mechanism proposed for the analogous process mediated by RuO_4, has also been formulated.

2.2. Results and Discussion

2.2.1 Identification of minor products arisen from the oxidation of penta-THF 1 with PCC

C-8 oxygenated spiroketals

Penta-THF **1** was synthesized from squalene according to a previously described procedure[5g] and thoroughly purified by preparative direct and reversed-phase HPLC to exclude the presence of minor impurities. Its purity was further checked by 700 MHz ^{1}H NMR analysis. The oxidation of **1** in the presence of PCC was carried out in CH_2Cl_2 at reflux as previously described.[10] The crude reaction mixture was subjected to a careful preparative and then, when required, analytical HPLC to give the new C-8 oxygenated spiroketals **9** and **10** (Scheme 2) as well as some dilactone compounds derived from the degradation of the carbon backbone of **1** (see later, Scheme 4), besides previously isolated compounds **2**-**8**.

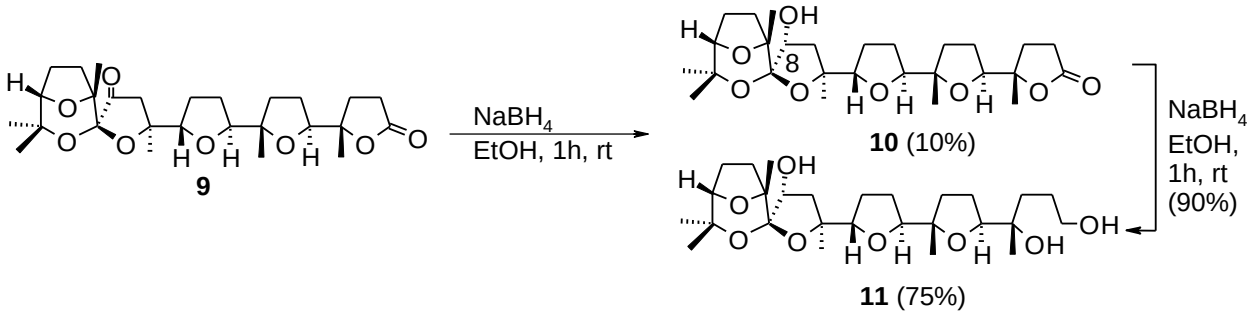


Scheme 2. Minor C-8 oxygenated spiroketals obtained by PCC-mediated oxidation of **1**

Determination of the stereostructure of the C-8 keto-compound **9** was accomplished by registration of a full set of high-field 2D-NMR spectra while the structure of the corresponding C-8 alcohol **10** was inferred by chemical correlation with **9** and NMR evidence. In particular, the configuration of the C-7 spiro-centre belonging to the tricyclic spiroketal subunit in **9** was established to be the same found in related spiro-compounds **2**-**5** (Scheme 1) through unambiguous ROESY evidence.

The structural relationship between **9** and the corresponding alcohol **10** was proven by sodium borohydride reduction of **9** that gave alcohol **10** (10%, Scheme 3) along with the corresponding triol **11** (75%) derived from the further reduction of the γ-lactone function in **9** through attack of the reducing agent to the carbonyl plane from its upper face. Configuration at C-8 in **10** was established on the basis of 2D-NMR studies carried out on the corresponding triol **11**, available in higher

amounts, having ascertained the streostructural relationship between the two compounds by borohydride reduction of the former to the latter. In particular, a significant ROESY correlation peak between Me-26 and H-8 (Figure 1) established the α-orientation of the OH group in this compound and thus its *cis* relationship with the oxygen bridging C-2 and C-7 in the 1,4-dioxane ring, a fact having important mechanistic implications, as discussed later.



Scheme 3. Borohydride reduction of the C-8 keto-spirocompound **9**.

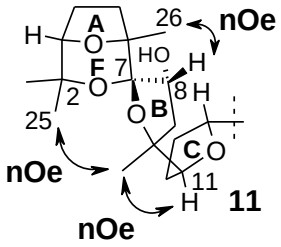
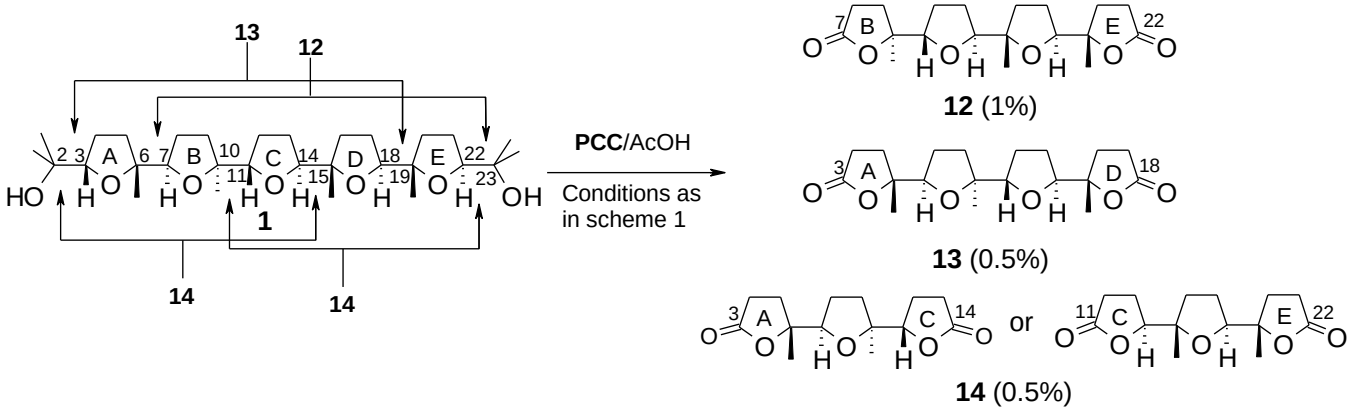


Figure 1. A stereo-view of the spiroketal-containing portion of **11** showing significant ROESY correlations.

Dilactones

Dilactones **12-14** (Scheme 4), derived from the cleavage of the poly-THF backbone of **1** at different hydrogen-carrying angular positions, were also isolated as minor products (overall 2% yield) of the oxidation of **1** with PCC. The stereostructures of **12** and **13** was determined by 2D-NMR studies. The *trans* configuration of both the THF rings in the most abundant among these substances (**12**, 1%) indicated that it came from the oxidative cleavage of **1** at the C6-C7 bond interconnecting A and B THF rings accompanied by the oxidative removal of the terminal 2-hydroxypropyl moiety at the other end of the molecule (C22-C23 bond cleavage). Dilactone **13** (0.5 %) is the *threo-cis-threo-trans-threo* isomer of **12** originating from the analogous oxidative cleavage of the C18-C19 bond connecting D and E THF rings and the oxidative cleavage of the C2-C3 bond. Conflicting NMR evidences prevented assignment of the configuration for the remaining

tricyclic dilactone **14** (0.5%). Consequently, both a *threo-trans-threo* or *threo-cis-threo* configuration is possible for this substances according to the two cleavage patterns conducting to it (cleavage of C10-C11/C22-C23 or C2-C3/C14-C15 bond pairs).



Scheme 4. Minor polycyclic dilactone products from the PCC-mediated degradation of **1** and relevant cleavage patterns.

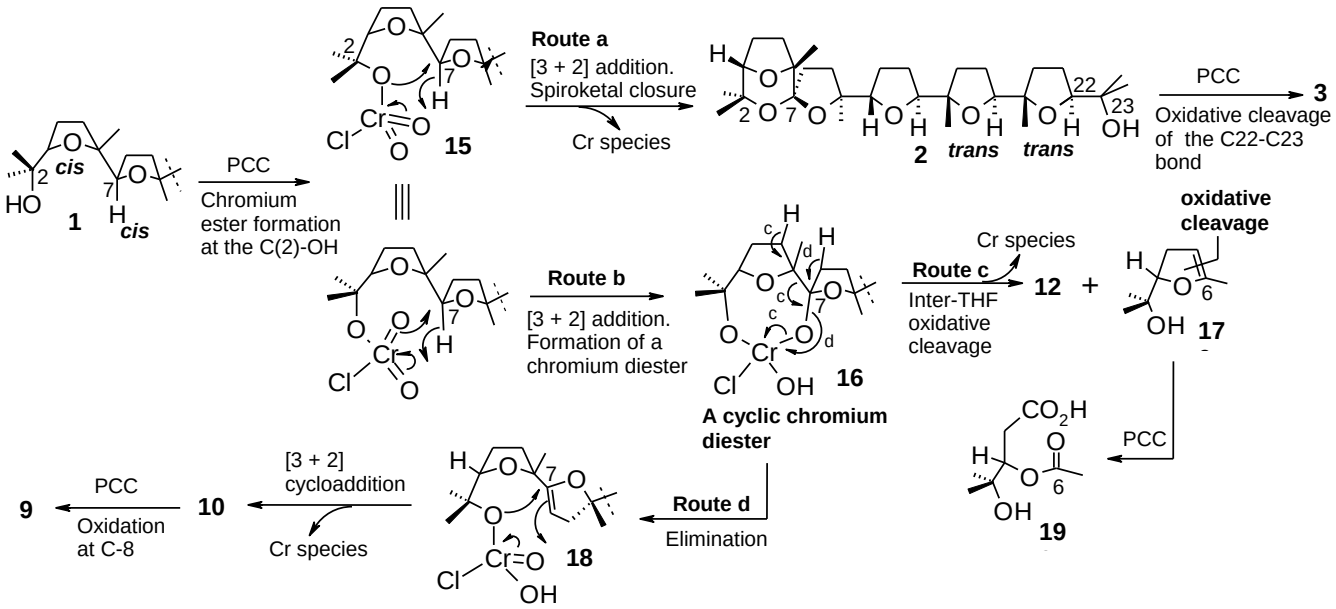
2.2.2 A mechanistic rationalisation for the formation of spiroketals 9, 10 and dilactones 12-14

Isolation of the above products suggested intriguing new oxidative pathways. A plausible mechanistic rationalisation for the formation of above spiroketal and dilactone substances, that agrees with reported reactivity for PCC[6] and previously developed chemistry by our own group[10] is given in schemes 5 and 6.

Thus, after the initial formation of a chromium ester **15** (Scheme 5) by interaction of PCC with the C(2)-OH, the oxo-chromium appendage tethered to C-2 may either attack the close-in-space C7-H bond, causing the closure of the spiroketal function in **2** (route a), or generate a cyclic chromium diester intermediate **16** (route b) through a path involving the a [3+2] addition of a O=Cr=O portion to the same C7-H bond.

Formation of chromate ester **15** is a reasonable assumption since there is evidence for the very rapid formation of such esters by reaction of PCC with primary, secondary and tertiary alcohols.[12] In addition, attack of transition metal oxo-species such as OsO_4[13] and RuO_4[14] to C-H bonds of alkanes has been suggested to proceed through a [3+2] addition of a C-H bond across an O=M=O unit through a mechanism analogous to the one now widely accepted for alkene bishydroxylation. Moreover, evidence is reported in the literature that RuO_4 oxidation of neoisocedrane oxide,[15] a sesquiterpene containing a THF ring, proceeds through the insertion of an oxoruthenium bond into

the angular C-H bond of the THF (see later Scheme 9), much in the same way postulated in the transformation of **15** to **16**. On this ground the proposed [3+2] attack of the C-2 oxo-chromium portion to the CH-7 bond in **15** to give **16** seems a very logical event.



Scheme 5. A mechanistic explanation for the formation of compounds **2**, **3**, **9**, **10** and **12**

As previously pointed out,[10] the closure of the spiroketal (conversion of **15** to **2**) is made possible by the *cis-threo-cis* configuration of the bis-THF terminus in **1**, that resulted in the vicinity in the space of the C7-H bond and the oxochromium appendage tethered to C-2. In fact, closure of a similar spiroketal at the other end of **1** is not possible due to the *trans-threo-trans* configuration of the terminal bis-THF portion (Figure 2) and an alternative path, leading to the cleavage of the C22-C23 bond, is observed (see conversion of **2** to **3**). Formation of diester **16** appears essential to explain the generation of all the other isolated substances. Indeed, **16** can evolve through two paths (route c and d) both conducting to cyclic enolether products (**17** and **18**). In particular, route c would be responsible for the direct formation of dilactone **12** and enolether **17** *via* oxidative cleavage of the C6-C7 bond connecting A and B rings. It was anticipated that the C-C double bond in the latter would likely undergo oxidative cleavage to the dicarbonyl compound **19** based on previous evidence on the reactivity of enolethers with PCC.[16] Evidence for the existence of such an intermediate was subsequently gained when the dibenzoate of **1** was oxidised in the same conditions (see later Schemes 10 and Figure 3) and we succeeded in the isolation of the benzoate of **19**. A chromium diester intermediate strictly similar to **16** has been postulated in the oxidative ring fission of 2,5-dialkylfuranes with PCC to give α,β-unsaturated 1,4-dicarbonyl compounds.[17] Alternatively, diester **16** can generate the ring-B enolether intermediate **18** (route d), where the chromium-

containing appendage is still linked to C-2 and the carbon backbone of **1** is preserved, *via* an elimination step. Formation of spiroketal **10** would then follow *via* a cycloaddition step, possibly a [3+2] process involving the attack of the oxochromium appendage still tethered to C-2 on the Δ^7 double bond in **18**. In this way the spiroketal system of **10** is formed with the simultaneous delivery of an hydroxyl group at C-8.

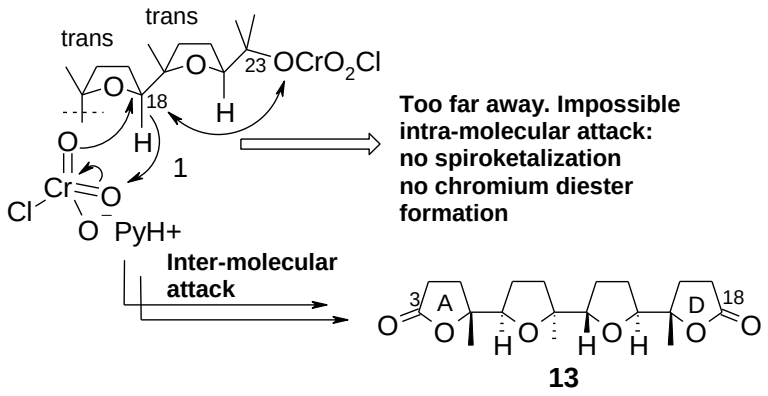


Figure 2. Intra-molecular *versus* inter-molecular attack of PCC to the terminal *trans-trans* bis-THF portion of **1.**

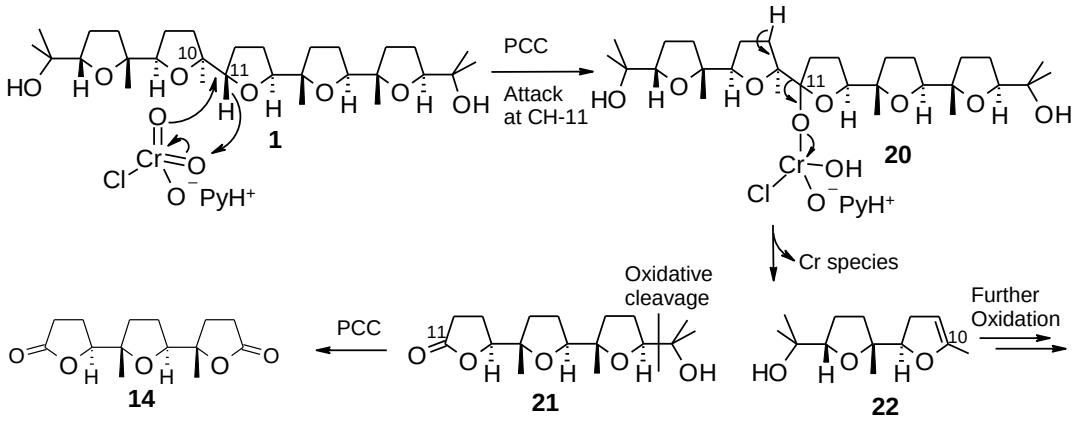
Conversion of **18** to **10** is a likely transformation because the PCC-mediated oxidative cyclization of bishomoallylic tertiary alcohols to THF-alcohols has previously been reported.[18] It is worth noting that such a cycloaddition step also explains the observed *cis* relationship between the ring-F spiroketal oxygen and the hydroxyl on C-8 (see Figure 1 for derivative **11**). Finally, keto-spiroketal **9** would derive from the corresponding alcohol **10** by further PPC oxidation at C-8 in the reaction medium. It is to be noted that **9**, **10** and **12** (Schemes 2 and 4) all possess ring-D terminal lactone moieties originating by a further oxidative cleavage step of the C22-C23 bond of the type involved in the conversion of **2** to **3** (Scheme 7 and 8)

Our results on the conversion of **18** to **10** well agree with the Schlecht *et al*[18c] and McDonald *et al*[18b] mechanistic proposals. This transformation is strongly reminiscent of the oxidative spirocyclization of cyclic enolethers mediated by rhenium (VII) oxides[19] reported by Boyce and Kennedy and represents the first example of the PCC-induced closure of a cyclic spiroketal involving an enolether double bond. It is to be noted that this transformation involves the chromium ester of a tertiary alcohol (C2-OH) while the rhenium-mediated process is reported to induce the sole spiroketalization of primary alcohols. This transformation is certainly worth of further experimentation using *ad hoc* devised substrates.

Formation of dilactone **13**, the *threo-cis-threo-trans-threo* isomer of **12**, (not shown in Scheme 5) would proceed in a way strictly analogous to that conducting to **12**, *via* cleavage of C2-C3 and

C18-C19 bonds, the former one once again generating fragment **17**. However, in this case, an intermolecular attack of PCC to C18-H bond should be invoked because a C23-tethered oxochromium appendage and the C18-H bond cannot be brought near in the space due to the *threo-trans-threo* configuration of the bis-THF terminus (Figure 2), as above pointed out for the spiroketalization at this terminus.

Formation of dilactone **14** from **1** is depicted in Scheme 6. It requires the cleavage of one of the bonds adjacent to the central THF ring (C10-C11 or C14-C15) to take place (scheme 6 shows the attack at C11-H). An the inter-molecular attack of PCC is required in this case as well, to give the chromium ester intermediate **20**. Lactone **21** would then originate from **20** through a route strictly similar to the one by which **16** is transformed into bis-lactone **12** (see route c, Scheme 5). A bis-THF enolether-containing species (**22**) would also be produced in this step, the fate of which we were unable to follow further though it is presumable that its C-C double bond could underwent oxidative cleavage as hypothesised for **17** in scheme 5. Finally, PCC-mediated oxidative cleavage of the terminal side chain in **21** would give rise to the second lactone function of **14**.

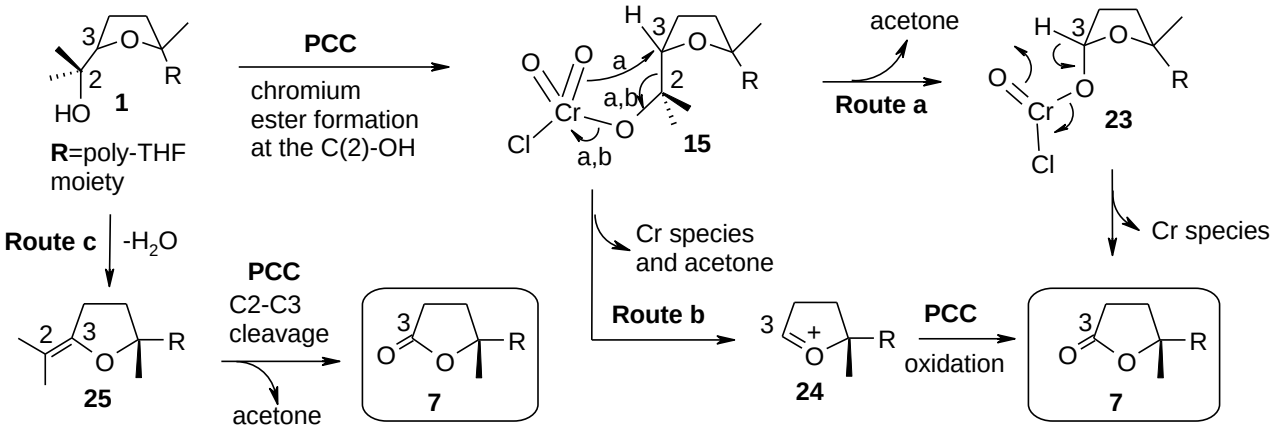


Scheme 6. Proposed mechanism for the formation of dilactone **14**

Summarising, the above results clearly established the ability of PCC to cleave inter-THF bonds in **1**. The main oxidative routes, leading to γ-lactones **6**-**8**,[5g, 10] spiro-compounds **2** and **3** as well as minor C-8 oxygenated spiro-compounds **9** and **10** and bis-lactone **12**, proceed through the preliminary formation of chromium esters by interaction of PCC with the two hydroxyl groups of **1**. Secondary routes leading to truncated spirolactones **4** and **5** (Scheme 1) and dilactones **13** and **14**, likely proceed through inter-molecular attack of PCC to the suitable THF CH bonds followed by inter-THF C-C cleavage. There is evidence indicating formation of cyclic enolethers.

2.2.3 On the oxidative cleavage of α-hydroxy-THF compounds. Conversion of 1 to 6-8

The PCC-mediated oxidative cleavage of α-hydroxy mono- and poly-THF compounds to γ-lactones (see for example conversion of **1** to **6-8** or **2** to **3** in Scheme 1) is a well-documented process[11] interesting both from a mechanistic and applicative point of view. No definitive evidence on the real path of this transformation has been provided to date although plausible speculative reasoning have been put forward.[5g,11] In the light of the above collected evidences a comment to this transformation, with reference to THF bearing α-tertiary alcoholic moieties, seems appropriate. Reasoning for example for the transformation of **1** to **7** (Scheme 1), a summary of the reported routes, explaining the oxidative cleavage of the C2-C3 bond, is shown in Scheme 7.

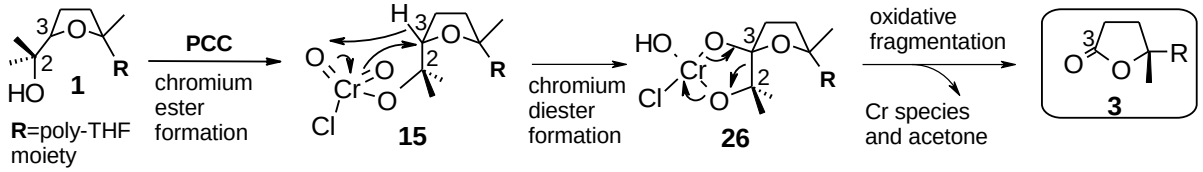


Scheme 7. Oxidative cleavage of the α-hydroxy-THF portion of **1** according to previously formulated mechanistic hypotheses.[11]

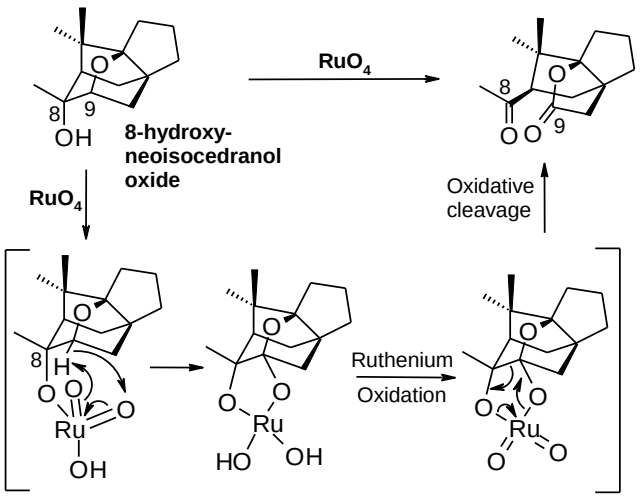
In particular, the great part of the reported hypotheses, including our own, supposes a preliminary coordination of PCC to the alcohol group α to the THF ring. Thus, the C-2 tethered oxochromium appendage in the first-formed species **15** may attack the C-3 carbon though route a, with formation of a C-3 chromium ester intermediate **23**, that in a successive oxidation step would generate the lactone function in **7**. Alternatively, a cation species **24** may be formed (route b) that is then further oxidised by PCC to give **7**. Another reported route (route c)[11d] supposes the involvement of an enolether species such as **25**, formed by dehydration, that is then cleaved by PCC. However, such a type of substance could not be detected among the reaction products, as the authors pointed out.[11d] In line with our reasoning on the conversion of **15** to **16** shown in Scheme 5.

We believe that a fourth plausible path (Scheme 8) can be proposed where the C3-H bond is attacked by the close-in-space C-2 tethered oxidant, to give the cyclic chromium diester **26**. Successive oxidative fragmentation of this intermediate, would proceed in the usual manner with

expulsion of acetone, generating the lactone function of **7**. This route would compete with the one where the C7-H is attacked by the same C-2 tethered O-Cr=O portion to give **16** (Scheme 5). Cyclic esters such as **26** are thought to be involved into the oxidative cleavage of alkenes or vicinal diols with related oxo-species RuO_4, OsO_4, RuO_4^- and MnO_4^-. Importantly, our path is strictly similar to the proposed route for the oxidative cleavage observed for 8-hydroxy-neoisocedranol oxide with RuO_4 shown in Scheme 9[15] and further supports our hypothesis on the similarity between the RuO_4 and PCC in such processes. On this ground it seems also conceivable that this route could work with related α-hydroxy-THF substances.[11]



Scheme 8. Our proposed route for the oxidative cleavage of the α-hydroxy-THF portion in **1**.

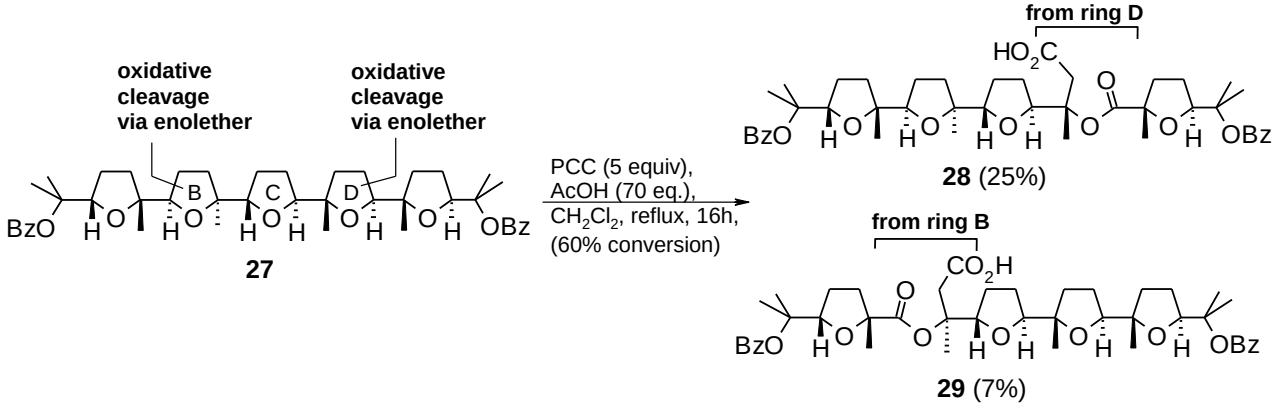


Scheme 9. Reported oxidative cleavage of 8-hydroxy-neoisocedranol oxide with RuO_4 and postulated mechanism.[15]

2.2.4 Minor oxidation products from the PCC-mediated oxidation of penta-THF dibenzoate 27

Intrigued by these results, we decided to collect further evidences on the oxidative pathways depicted in Schemes 5 and 6 by investigating the reactivity of the bis-benzoate of **1** (**27**, Scheme 10) in the same oxidative conditions. Based on the above mechanistic hypothesis, the absence of the C-

2 and C-23 hydroxyl groups in **27** would depress the main oxidative route thus enhancing, in principle, the intermolecular attack of PCC to the differently substituted THF rings embodied in **1**.



Scheme 10. Major products from the oxidation of penta-THF dibenzoate **27** with PCC/AcOH.

Dibenzoate **27** was prepared from **1** under standard benzoylation conditions (BzCl, DMAP, CH_2Cl_2) and thoroughly purified by preparative direct and reversed-phase HPLC and then subjected to oxidation with PCC in CH_2Cl_2. After 16 h at reflux, a 60% conversion was reached and the process was stopped. Careful HPLC separation of the reaction mixture, both in a direct and reverse-phase mode, gave as main products the two isomeric acids **28** and **29** in 25% and 7% yields, respectively (Scheme 10), characterised by strictly similar dicarbonyl moieties, clearly originating from the degradation of D or B rings in **27**. Minor products (Figure 3) included ketol **30** (2%) embodying a ring-B oxygenated moiety, lactone **31** (2%) lacking one of the terminal rings of **27**, two isomeric small-sized lactones **32** and **33** (major isomer 4%; minor isomer 2%), conceivably originating by cleavage of the central THF ring in **27**, as well as the two isomeric unsaturated aldehydes **34** (1% each) and fragment **35** (2%)**,** the benzoate derivative of previously hypothesised **19** (Scheme 5). The latter embodies a dicarbonyl moiety analogous to that present in compounds **28** and **29**. The rest of material for mass balance was mostly made up of very polar substances likely originating by over-oxidation of some of the isolated substances or by other oxidation side processes.

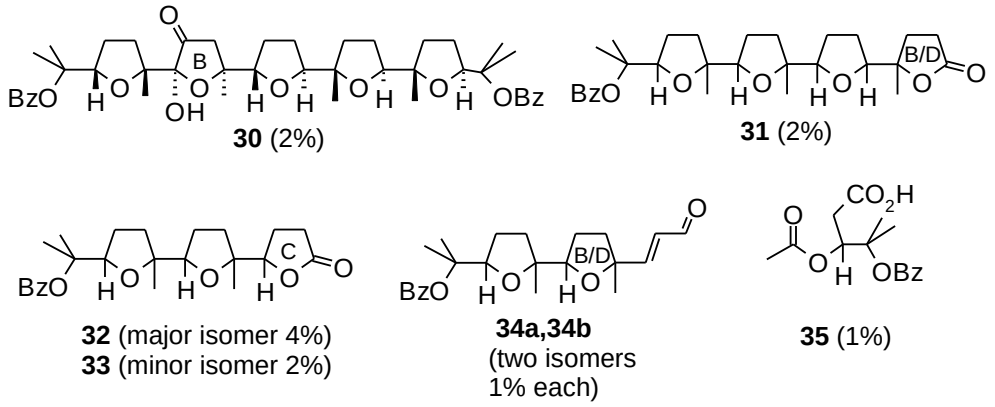
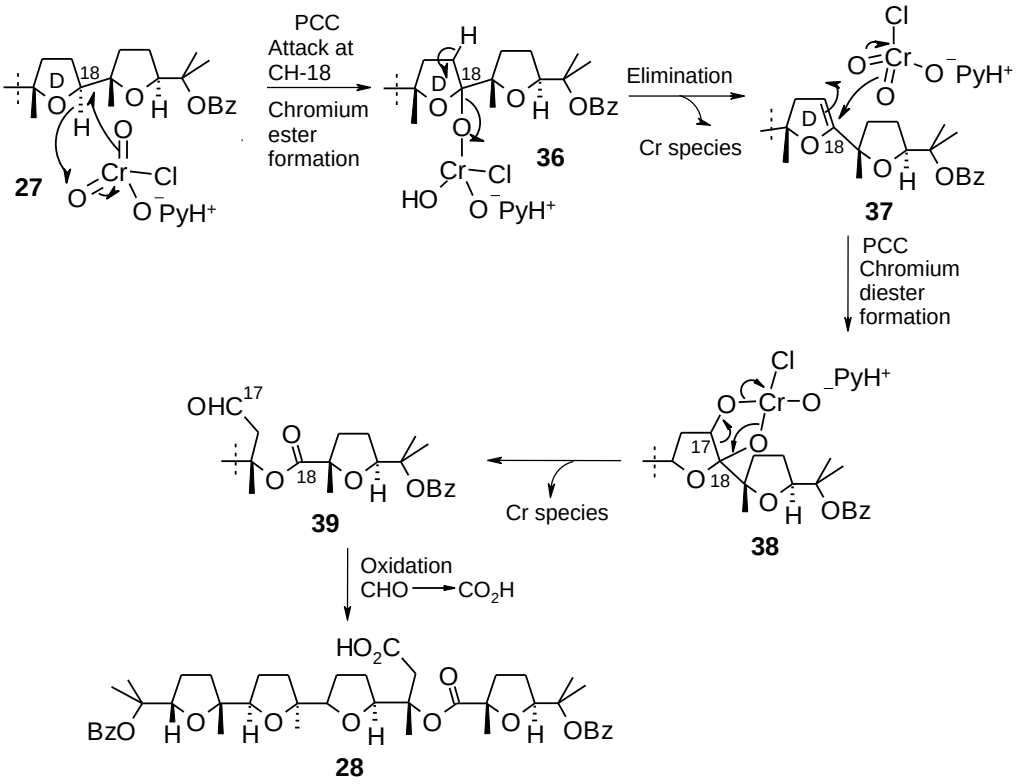


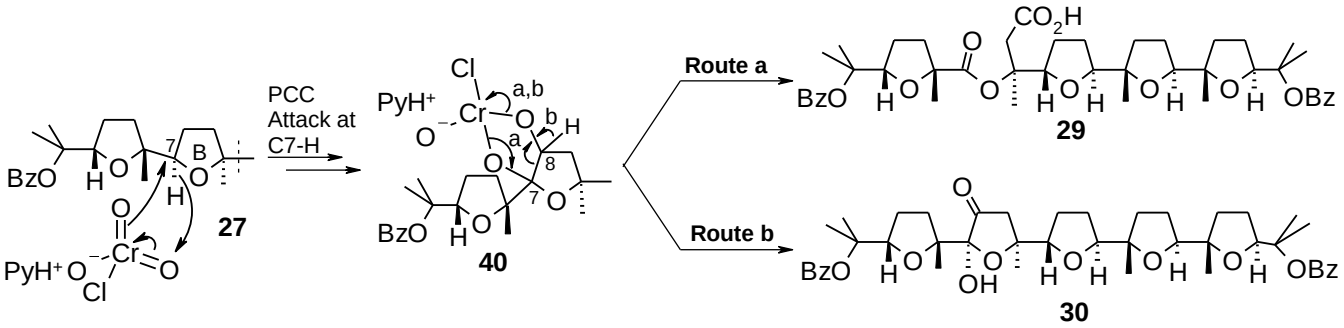
Figure 3. Minor products from the oxidation of penta-THF dibenzoate **27** with PCC.

The structure of **28-35** indicated that the D-ring was preferably attacked by PCC, furnishing acid **28** as the main substance whereas attack at B and C rings took place to a less extent. B ring oxidation gives **29** and **30** whereas degradation products **32**-**34** originate from the oxidation of the C ring. Lactone **31**, the configuration of which could not be determined, can result from oxidation of either B or D ring.

The reactivity of **27** can be rationalised as shown in Schemes 11 and 12 following the lines traced in schemes 5 and 6 for the corresponding debenzoylated analogous. In particular, following a backward reasoning for the formation of **28**, it seems clear that the dicarbonyl portion in this substance can be traced back to the oxidative cleavage of an enolether function embodied in the D ring of **27** (see intermediate **37**, scheme 11) *via* formation of chromium diester **38** and its successive oxidative cleavage to aldehyde **39** that is eventually oxidised to acid **28**. Enolether **37** is in turn formed through an elimination step involving the first-formed chromium ester **36** that originates from the attack of PCC to the C18-H bond, in a manner similar to that priviously shown in Scheme 6 (see conversion of **1** to **22**). A similar path would give rise to isomer **29** by attack of PCC to the C7-H bond in ring B *via* chromium diester **40** (Scheme 12, route a), analogous to **38**. Corroboration for this path came from the isolation of ring-B ketol **30**, likely formed by the competing oxidative opening (oxidation at C-8) of the chromium diester intermediate **40**. Isolation of **30** furnishes the first indirect support to the previously hypothesised[16] formation of a chromium diester intermediate in the oxidative cleavage of enolethers.



Scheme 11. An hypothesis for the formation of compounds **28** from **27**.

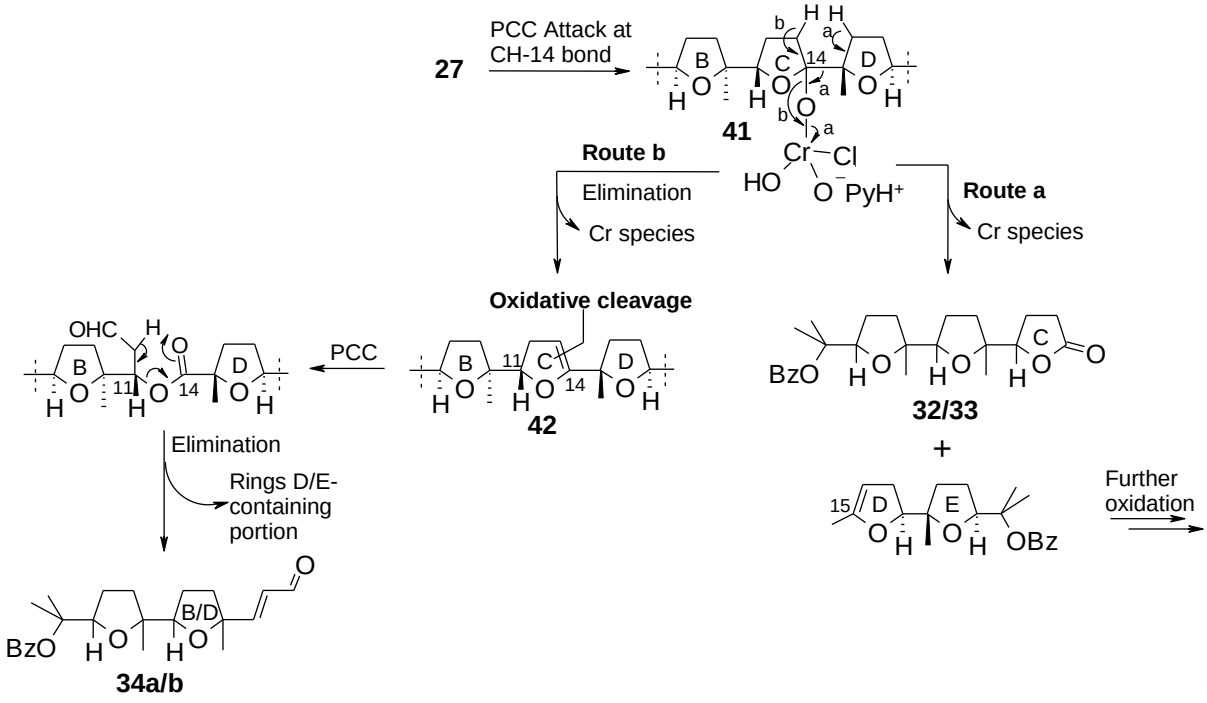


Scheme 12. Formation of compounds **29** and **30** from **27**.

Whereas PCC is unable to cleave isolated (unactivated) carbon-carbon double bonds, the oxidative cleavage of both cyclic and acyclic enolethers to esters with PCC has been reported.[16] In particular, the above reactivity (conversion of **37** to **39**, scheme 11) well agrees with the previously observed oxidative cleavage of cyclic enolethers lacking α hydrogens.[16a,16c]

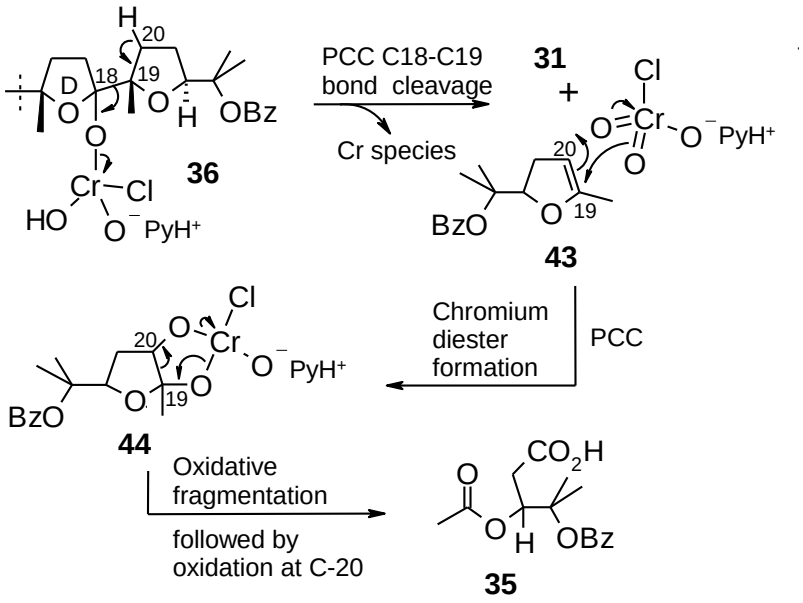
Formation of degraded compounds **32**-**34** is rationalised through attack of PCC to both C11-H and C14-H in the central THF (Scheme 13). Reasoning for the attack at C14-H, in line with the previously developed hypothesis, lactones **32** and **33** would derive from the monoester intermediate

41 by oxidative cleavage of C14-C15 bond (route a) whereas an alternative path (route b) would conduct to the ring-C enolether **42**, by elimination of a chromium species (see also conversion of **36** to **37**, Scheme 11). Oxidative cleavage of the enolether double bond in the latter, followed by the further elimination of the rings D/E-containing portion of the molecule, would give rise to the conjugated aldehydes **34a** and **34b**.



Scheme 13. Formation of **32-34** from **27**.

Finally, structurally complementary compounds **31** and **35** are formed from **27** (Scheme 14) through cleavage of the C18-C19 bond in the chromium ester **36** (shown in Scheme 11). Meanwhile, a cyclic enolether species (**43**) is once again delivered and it gives rise to the dicarbonyl compound **35** when its olefin function is oxidatively cleaved through formation and successive oxidative fragmentation of a cyclic chromium diester (**44**). This route is analogous to that conducting to structurally similar **12** and **17** when **1** was oxidised (Scheme 5). Since configuration of **27** remained undetermined its formation can either follow the depicted route, through attack at C18-H, or the analogous path where the symmetric B ring is oxidised by attack of C7-H.



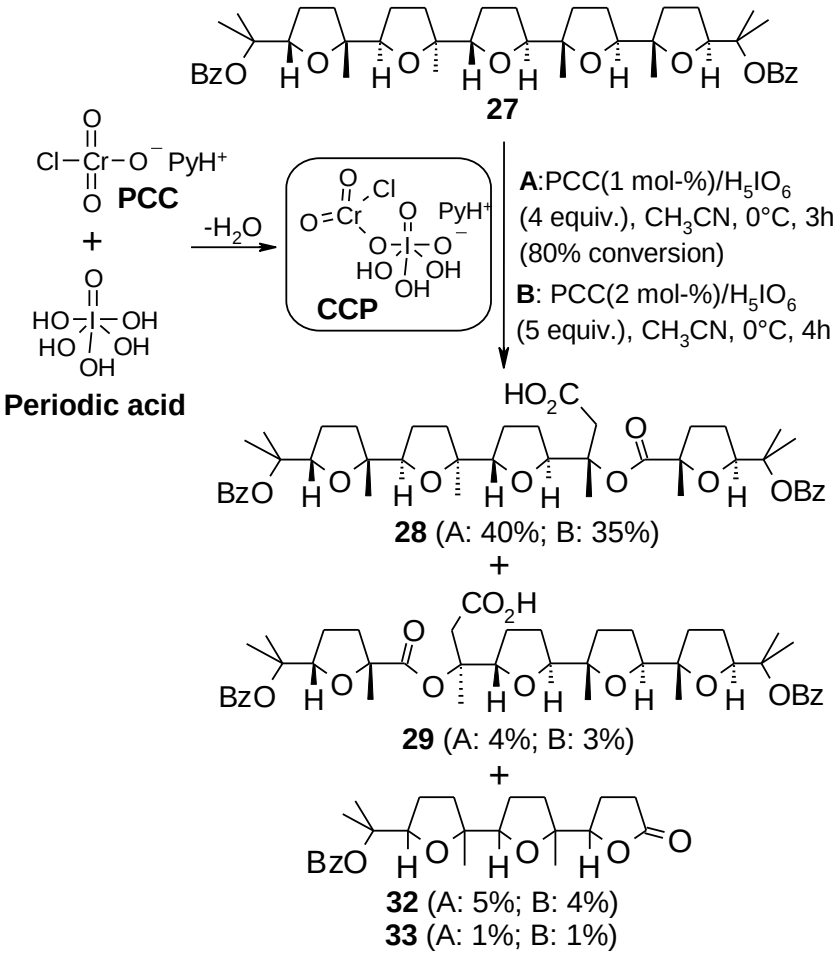
Scheme 14. A mechanistic explanation for the formation of compounds **31** and **35** from **27**.

Thus, the oxidation of dibenzoate **27** provided confirmatory evidences on the reactivity of its debenzoylated counterpart **1** with PCC. The absence of free hydroxyl groups on terminal side chains eliminated both the spiroketalization and oxidative cleavage of terminal moieties, caused by the preliminary coordination of PCC to them. The ability of PCC to cleave inter-THF bonds was still observed as indicated by the isolation of terminal lactones **31**-**33**. In the meantime, a new oxidative route, leading to the oxidative cleavage of the α,β-bond of B and D THF rings, not observed during the oxidation of **1**, was disclosed. This path, as well as isolation of ketol **30** and aldehydes **34**, strongly support the involvement of cyclic enolether intermediates. Worth mentioning is also the sole formation of the B-ring ketol **30**. The analogous, possible, ring-D ketol could not be detected even after careful HPLC analysis. As expected, due to the presence of five THF rings this process is scarcely selective though a propensity for the attack at B and D rings is observed.

2.2.5 Oxidations of poly-THF compounds with PCC/H_5IO_6

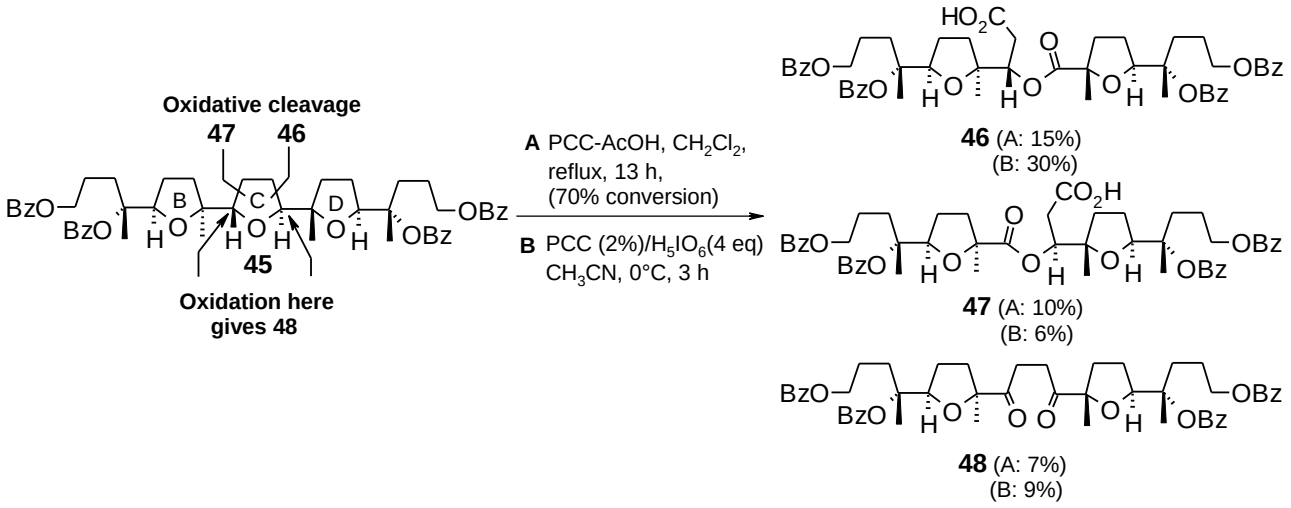
We next decided to test the reactivity of **27** in the presence of the catalytic system PCC (cat.)-H_5IO_6 (Scheme 15; see also Chapter 1, pargraph 1.3).This oxidising combination has received to date little attention.[7-9] Based on previous studies[9] it is thought that the combination of the two reagents generates chlorochromatoperiodate (CCP), an oxidising agent more powerful than PCC. It

was hoped that the use of a more reactive and sterically demanding reagent such as this, might improve the regioselectivity of the attack of the oxidant to the poly-THF system of **27** and could also reduce reaction times and force the process to completion. The oxidation was initially carried out in CH_3CN at 0°C using 1% of PCC and four equiv. of periodic acid (Scheme 12, conditions A). The process was indeed much cleaner under this conditions and overall improved yields of acids **28** and **29** (**28**: 40%; **29** 4%) were obtained in a shorter time (3h at 0°C). Importantly, the two isomers were now obtained in a ca. 10:1 ratio in favour of **28**, indicating a preferred attack of the oxidant at the D ring. However, under these conditions the process was still incomplete and stopped at 80% conversion. Attack of the oxidant at the central THF ring was the second most important route giving isomeric lactones **32** and **33** in 6% overall yield (major isomer: 5%; minor isomer: 1%). Addition of a further 1% PCC and 1 equiv of H_5IO_6 (conditions B) forced the process to completion at the expense of a slight reduction in the yield of **28** whereas yields of acid **29** and lactones **32**/**33** were scarcely affected.



Scheme 15. Oxidation of penta-THF dibenzoate **27** with PCC(cat.)-H_5IO_6.

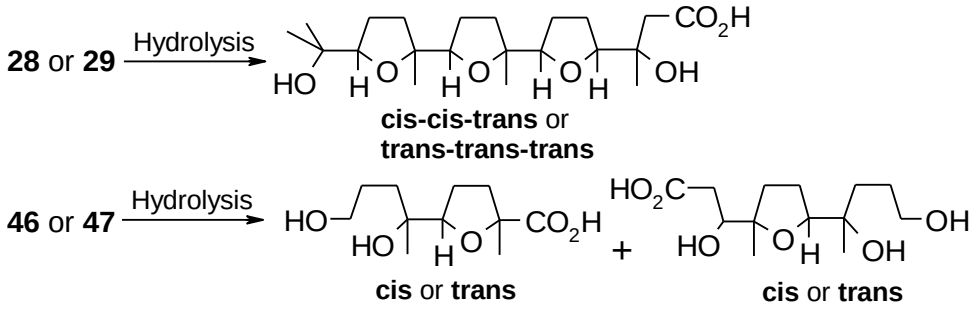
Oxidation of tris-THF tetrabenzoate **45** (Scheme 16), a structurally "simplified" substrate, was then carried out. Compound **45** was obtained by benzoylation of the corresponding tetrol in turn prepared from penta-THF **1** through a short degradative sequence set up in a previous study conducted by our group.[5g] For a comparative purpose, the process was conducted both with PCC-AcOH and PCC(cat.)-H_5IO_6. This compound was obtained by benzoylation of the corresponding tetrol in turn prepared from penta-THF **1** through a short sequence set up in a previous study.[6e] Interestingly, in this case the three major products **46**-**47**, two of them, acids **46** and **47**, structurally related to compounds **28** and **29**, and all originating from the attack of the oxidant at the central THF ring, were obtained. Once again better results were obtained with the PCC(cat.)-H_5IO_6 system though a 2% amount of PCC was required to force the process to completion (conditions B). A new interesting feature of the process was formation of the 1,4-diketone **48** through a third oxidative route featuring the oxidation of both the angular carbons of the central THF ring in **45**. This route did not operate in previous instances. In addition, the oxidative route leading to the inter-THF bond cleavage seems not to operate with this substrate. Since 1,4-diketones are easily transformed into five-membered heterocycles, substances such as **48** may be synthetically useful to access new types of mixed poly-THF/heterocycle compounds.



Scheme 16. Oxidation of tris-THF tetrabenzoate **45**.

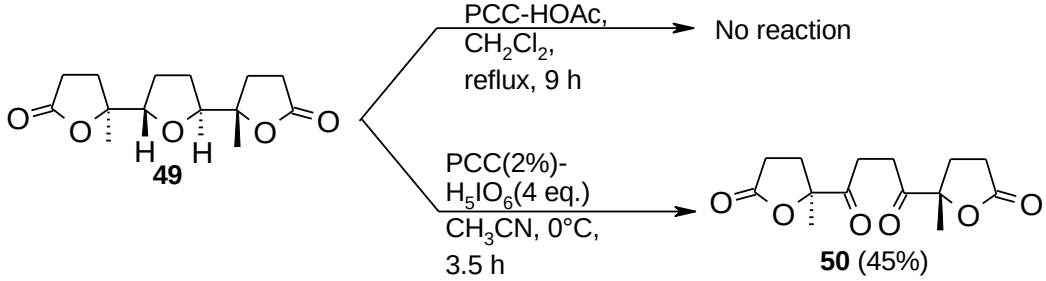
The parent penta-THF **1** can be obtained in a single step in multi-gram amounts from cheap and commercially available squalene, so the oxidative degradation of its penta-THF backbone can allow the facile access to stereochemically definite and functionalised *cis-cis-trans* and *trans-trans-cis* tris-THF compounds by hydrolysis of the inter-THF ester function in either **28** or **29** (Scheme 17). Likewise, new functionalised *cis* or *trans* mono-THF can originate from **46** and **47** by hydrolysis

(Scheme 5). Given literature precedents, it can be presumed that at least some of these substances may be useful for further citotoxic activity studies and also for synthetic purposes.[20]



Scheme 17. Functionalised new all-*threo* mono- and tris-THF systems accessible trough hydrolysis of poly-THF compounds **28**, **29**, **46** and **47**.

A further simplification of the poly-THF substrate was achieved by conversion of the tetrol corresponding to **45** to bis-lactone **49** (Scheme 18) through a previously set up sequence.[5g] This compound proved unreactive with PCC under standard conditions while gave 1,4-diketone **50** in an interesting 45% yield with PCC(cat.)-H_5IO_6. This behaviour can be tentatively explained assuming that an electron density deprivation of the angular CH bonds in the THF would result due to the withdrawing effect of the two lactone moieties. As a result the weakly electrophilic PCC was no longer able to attack these bonds. In accord with this reasoning, the strongly Lewis-acidic chromium species CCP was capable of attacking the THF ring of this compound, even though a longer reaction time is required compared to the oxidation of tris-THF **45** with the same reactive.



Scheme 18. Oxidation of dilactone **49**.

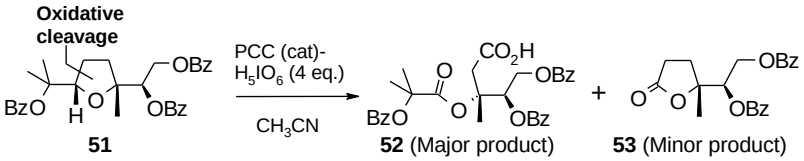
2.2.6 Oxidation of mono-THF compounds with PCC/H_5IO_6

The above collected results indicated that the oxidation of complex poly-THF compounds containing both di- and trisubstituted THF rings, proceeds through two main routes. Oxidative cleavage of the C2-C3 bond is preferred when a 2,2,5-trisubstituted THF ring is involved as in the

oxidation of **27**. A second route leads to 1,4-diketones, such as **48** and **50,** through the attack of the oxidant at a 2,5-disubstituted THF ring and oxidation of both its angular carbons, as in the oxidation of **45** and **49**.

With the aim of rendering the above process synthetically useful, the oxidation of some mono-THF compounds, based on the di- and trisubstituted THF motifs present in previously studied poly-THF compounds, was tested. c*is*-THF **51** (Table 1), obtained by benzoylation of the corresponding diol, in turn synthesized by RuO_4-catalyzed oxidative cyclization of geranyl benzoate,[21] was initially chosen as a model compound to find the best conditions for the oxidative process. Oxidation of **51** under previously employed conditions (PCC 2 mol %, H_5IO_6 4 eq., 0 °C) gave acid **52,** analogous to compounds **28**/**29** and **46**/**47**, obtained from more complex substrates, in a 54% yield (Table 1, entry 1) although longer reaction times were required. The process was then carried out at room temp. with the same amounts of catalysts and co-oxidant, leading to **51** with a similar 55% yield in only 3 h (entry 2). Improved yields (80%) and further reduced reaction times (45 min.) were achieved by increasing the catalyst amount to 5% (entry 3). Increasing of the catalyst amount to 10% resulted in a similar yield and further reduced times (78%, 30 min., entry 4). Further increasing of the catalyst up to stoichiometric amounts (50-100%) only resulted in diminished yields (entries 5 and 6). Finally, the effect of addition of water to the process was tested. Although the related system CrO_3(cat.)/H_5IO_6 has been successfully used to oxidise alcohols cleanly in wet CH_3CN (0.75 vol-% water)[22] in our case we have observed that addition of water (2-5 vol-%) is detrimental, causing the process to stop at 10-20% conversion.

Table 1. Optimization of the process on a model 2,2,5-trisubstituted THF compound.

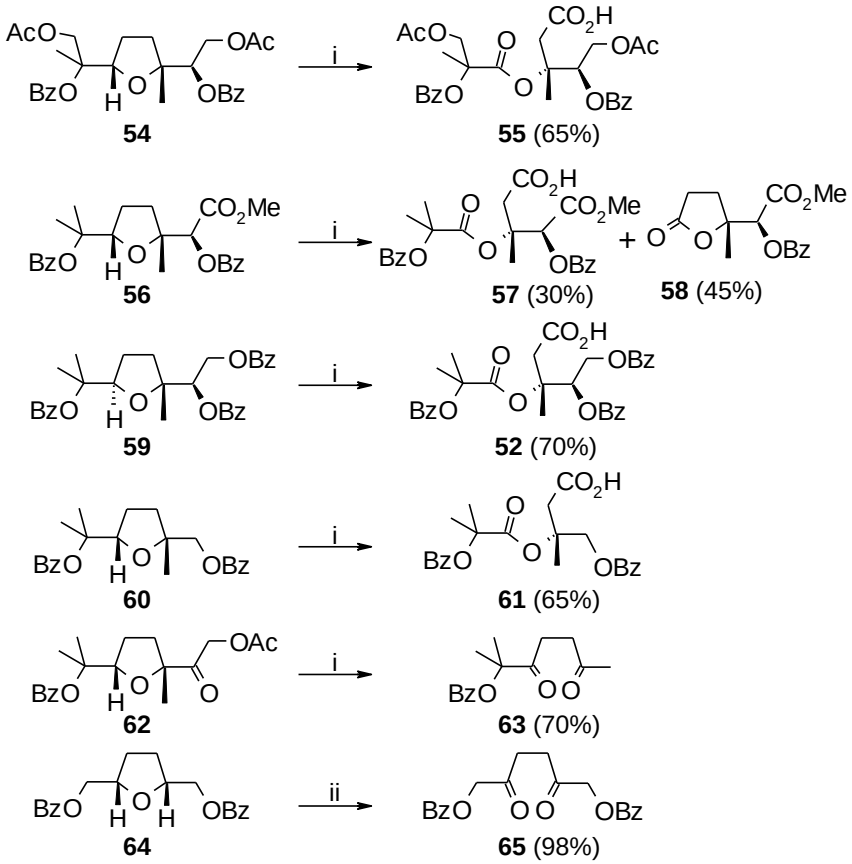


51 **52** (Major product) **53** (Minor product)

Entry	PCC (mol-%)	H_5IO_6 (equiv.)	Time	Yield of **12**[a] (%)
1[b]	2	4	19 h	54
2	2	4	3 h	55
3	**5**	**4**	**45 min.**	**80**[c]
4	10	4	30 min.	78
5	50	4	30 min.	62
6	100	4	30 min.	37

[a] Estimated by ^{1}H-NMR of the crude mixture. [b] Carried out at 0° C. [c] Isolated yield.

Oxidation of mono-THF compounds structurally related to **51** under optimized conditions was then carried out (Scheme 19). c*is*-THF **54**, **56** and **64** were synthesized by benzoylation of the corresponding THF diols, in turn obtained by RuO_4-catalysed oxidative cyclization of *trans trans*-2,6-dimethyl-2,6-octadiene-1,8-diol diacetate,[5k] methyl geranate[5k] and hexa-1,5-diene,[21] respectively, as reported. t*rans*-THF **59** was obtained by benzoylation of the corresponding THF diol, a minor product from the oxidative cyclization of geranyl benzoate.[23] THF **60** was synthesized from **51** through a short sequence. In particular, selective removal of both the primary and secondary benzoates in **51** with K_2CO_3 in MeOH, proceeded cleanly to give the corresponding diol. Oxidative cleavage of the diol system by treatment with silica-supported $NaIO_4$[24] in CH_2Cl_2, followed by borohydride reduction and benzoylation, afforded dibenzoate **60** in 43% yield (over four steps). Ketone **62** was obtained by TPAP-catalysed oxidative cyclization of geranyl acetate[5h] followed by benzoylation.



Scheme 19. Oxidation of functionalised 2,2,5-trisubstituted and 2,5-disubstituted THF compounds. i) PCC (5 mol-%), H_5IO_6 (4 equiv.), CH_3CN, r.t., 1h; ii) PCC (2 mol-%), H_5IO_6 (4 equiv.), CH_3CN, r.t., 40 min.

CCP oxidation of *cis*-THF diacetate **54** and *trans*-THF **59** gave acids **55** and **52**, respectively, in 65-70% yields. The methoxycarbonyl derivative **56** unexpectedly afforded the corresponding acid in a diminished 30% yield, the major product of the process being lactone **58**, analogous to **53**, derived from the oxidative removal of the dimethylated left-hand side chain. This side process is unusual and is reminiscent of the oxidative cleavage of a similar substrate.[8] This type of lactone is also the main side-product of the process carried out on all the other substrates. Similarly, the THF derivative **60** gave acid **61** in 65 % yield.

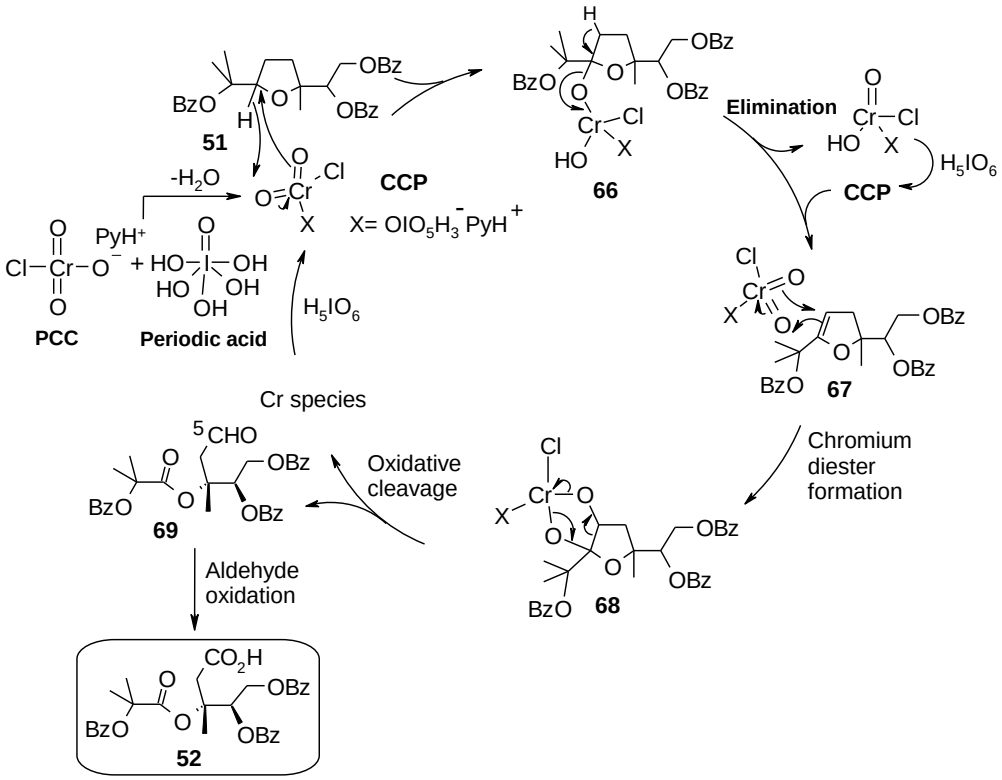
In another experiment, α-keto THF **62** was oxidised. In this case the presence of a keto group adjacent to the THF ring induced removal of the side chain through the oxidative cleavage of the C(THF)2-C=O bond to give 1,4-diketones **63** in good yields.

Finally, the reactivity of the 2,5-disubstituted *cis* mono-THF **64**, was tested. Pleasingly, 1,4-diketone **65** was obtained in a nearly quantitative yield (98%) even when the amount of PCC was reduced to 2 mol-% amount, This process is particularly appealing because it allows, when coupled with the oxidative cyclization of 1,5-dienes, the transformation of the latter into bis-ketols in a regioselective manner. This experiment and the oxidation of the more complex disubstituted mono-THF **49** demonstrated that for substrates containing only a 2,5-disubstituted THF ring the preferred route is the one leading to 1,4-diketones.

2.2.7 Mechanistic considerations on the oxidation of mono-THFs compounds

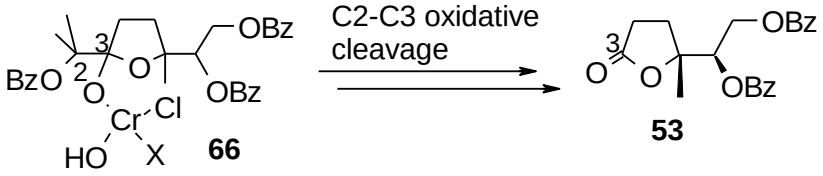
Based on the known reactivity of PCC, a rational mechanistic route explaining the formation of the above compounds was hypothesised. In particular, a path leading to compound **52**, but that also applies to the formation of the analogous compounds **55** and **57**, is shown in scheme 20. It is similar to that postulated for the formation of the compounds **28**/**29** and **46**/**47**. It is likely that the process begins with the attack of CCP, formed by elimination of water from PCC and periodic acid, to the angular C-H bond of the THF ring to give the mixed chromium ester intermediate **66**. This step is conceivably a [3+2] addition of the O=Cr=O portion of the oxidant to the angular C-H bond of the THF ring as seen before in Scheme 5.

In the next step chromium ester **66** would give rise to cyclic enolether **67** through an elimination step delivering a low-valent chromium species that was then reoxidised to CCP by H_5IO_6.



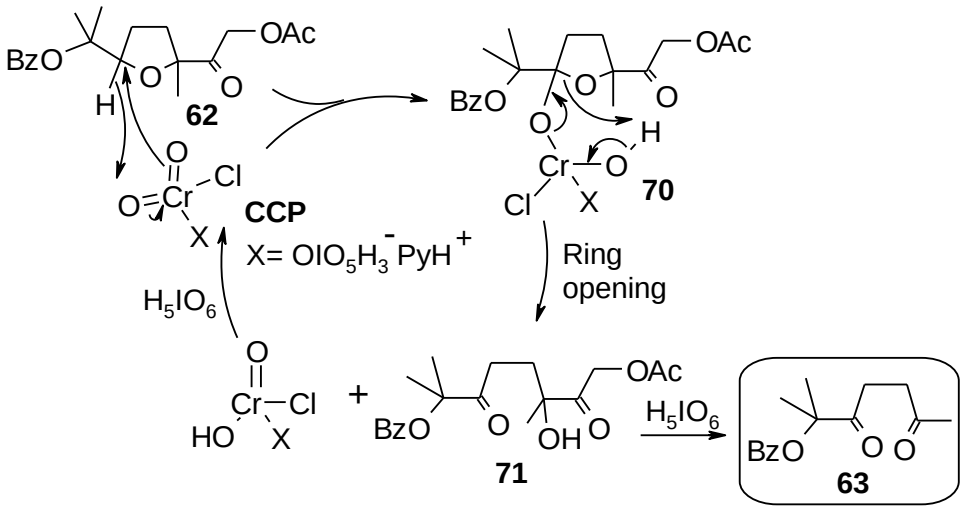
Scheme 20. A plausible catalytic cycle explaining formation of acid **52**.

The conversion of **67** to the final dicarbonyl compound **52** is thought to proceed through the formation of the chromium diester intermediate **68**, *via* a second [3+2] addition step in a manner similar to that seen before for conversion of **37** to **39** in Scheme 11. Oxidative cleavage to aldehyde **69**, followed by oxidation of the latter eventually generates acid **12**. Finally, formation of lactone **53**, the main side-product of the oxidation of **51**, can be seen to originate from the oxidative removal of the dimethyl benzoate side-chain. It has previously been observed that THF rings flanked by a tertiary (free) alcohol function undergo a similar oxidative cleavage with CCP to give γ-lactones[8] such as **13**, *via* a C-2 chromium ester intermediate. It is conceivable that a similar route could be responsible of the C2-C3 oxidative cleavage in a chromium ester intermediate possibly formed by further evolution of **66** (Scheme 21). This interaction is supported by the complete absence of hydrolysis of primary and secondary benzoate functions co-existing in the same molecule.



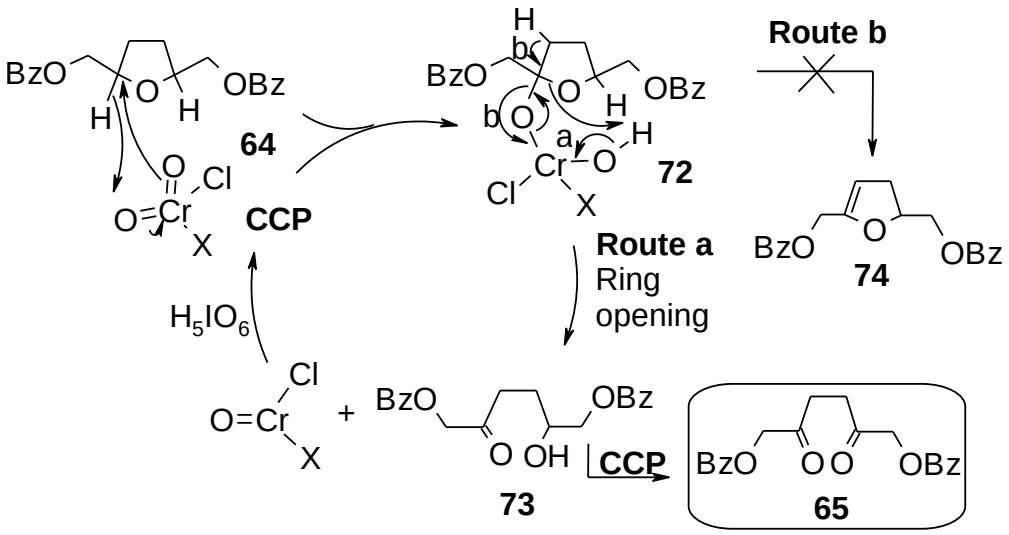
Scheme 21. Formation of lactone **53** by side-chain removal in intermediate **66**.

Along the same line of reasoning, formation of diketone **63** from **62** (Scheme 22) can be explained assuming that the first-formed chromium ester **70** collapses to the dicarbonyl species **71** the α-ketol portion of which undergoes oxidative cleavage to give **63** through a known process.



Scheme 22. A catalytic cycle for the formation of diketone **63**.

Formation of 1,4-diketone **65** from **64** is shown in Scheme 23. In particular, CCP would attack one of the angular CH bonds in **64** in the usual way to give chromium ester **72**. Opening of the latter would then follow to give ketol **73** (route a) with simultaneous delivery of a chromium species the oxidation of which would regenerate CCP closing the catalytic cycle. CCP oxidation of the alcohol function in **73**[7a] would eventually give 1,4-diketone **63**. Formation of related 1,4-diketones **48** (Scheme 16) and **50** (Scheme 18) can be explained in a similar manner. It is to be noted that the possible competing path giving rise to an enolether species (**74**, Scheme 23, route b) from **72** is completely suppressed in this case. It is worth noting that the chemical behaviour of PCC in this transformation parallels the one displayed by RuO_4. In fact, in a similar transformation, 2,5-dimethyl-tetrahydrofuran is reported to give the oxidative opening to 2,5-hexanedione by the RuO_2(cat.)/$NaIO_4$ system.[25]

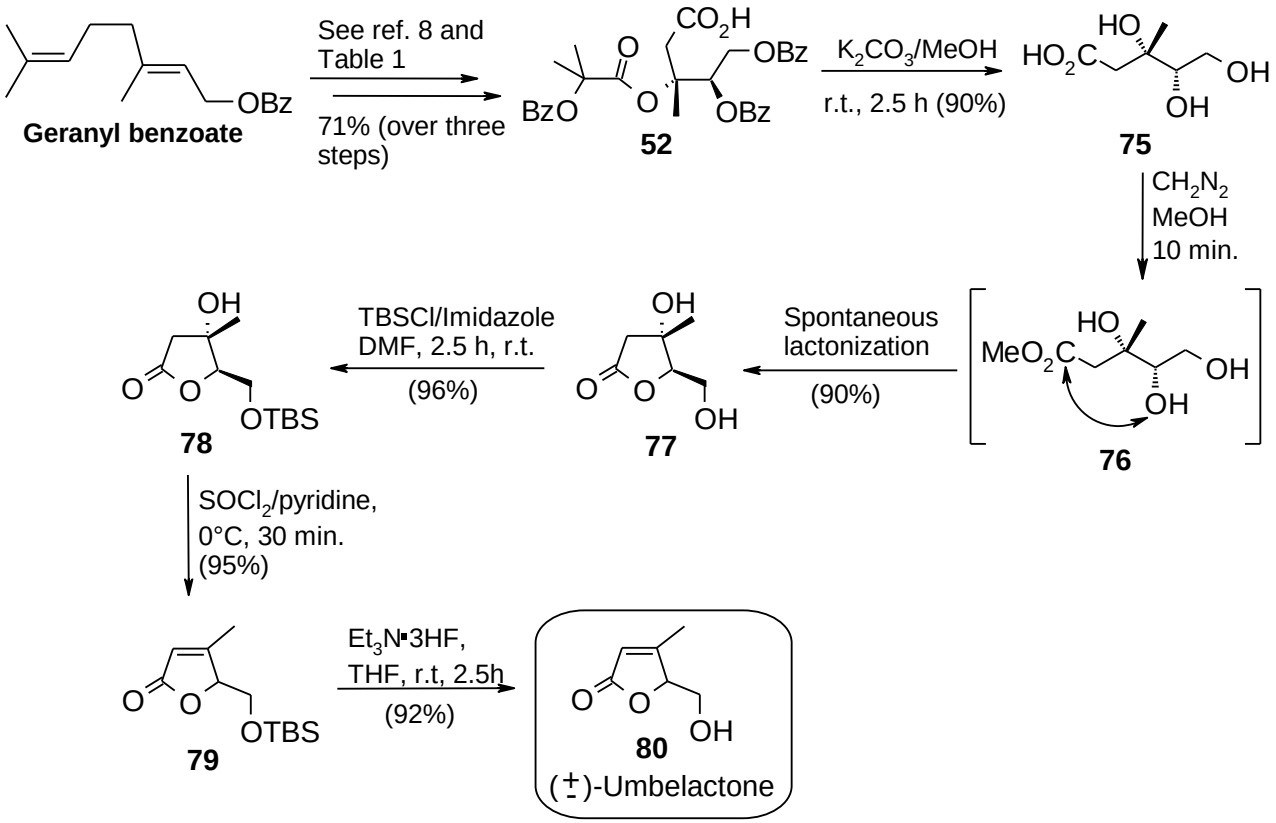


Scheme 23. A catalytic cycle for the formation of 1,4-diketone **65**.

2.2.8 Synthesis of umbelactone

As an application of the developed oxidative procedure, a short synthesis of racemic umbelactone was carried out. Umbelactone is a γ-butenolide natural product isolated from the ethanolic extracts of *Memecylon umbellatum* Brum,[26] that exhibited antiviral activity against the Ranikhet disease virus as well as spasmolytic and antiamphetamine activity. This substance has been the subject of various synthesis both in racemic and enantiopure form.[27]

Our above described oxidation of trisubstituted THFs such as **51** allows the access to elaborated 3,4,5-tryoxygenated acids. In particular, compound **52** derived from **51** was envisaged to be a good starting product to synthesize umbelatone (Scheme 24). Thus, tribenzoate **52** was converted into trihydroxy acid **75** in high yield by treatment with K_2CO_3 in MeOH. Successive treatment of crude **75** with CH_2N_2 gave transient methyl ester **76** that spontaneously cyclised to the γ-lactone **77** in 90% yield (81% from **51**). The primary hydroxyl group in the latter was then protected as the TBS ether **78** by treatment with TBSCl/Imidazole in DMF (96% yield).[28] Dehydration of **78** was accomplished with $SOCl_2$-pyridine to give silylated umbelactone **79** in 95% yield. Eventually, desilylation of **79** with Et_3N•3HF in THF cleanly gave umbelactone **80** (92% yield).



Scheme 24. Synthesis of umbelactone.

Our synthetic route to umbelactone is short and highly efficient and does not require carbon-carbon bond forming steps. In addition, it can be rendered enantioselective to give both the umbelactone enantiomers because mono-THF tribenzoate **51** (Table 1), the immediate precursor of **52**, can be obtained in both enantiomeric forms from commercially available geraniol in high yields and enantiomeric purity.[29] Studies towards this goal as well exploitation of the above chemistry for the preparation of further functionalised butenolides are ongoing.

2.3. Conclusions

In conclusion, in the present study we have examined the oxidative behaviour of some poly- and mono-THF compounds with PCC under classical conditions or with PCC(cat.)-H_5IO_6. Penta-THF **1** and penta-THF dibenzoate **27** revealed as good model compounds to study the action of PCC on adjacently linked poly-THF compounds. Novel oxidative pathways leading to the modification-degradation of the THF ring, as well as of the poly-THF backbone, have been disclosed. Plausible hypotheses, consistent with the known reactivity of PCC, have been put forward to explain the formation of all isolated substances. In particular, attack of the oxidant at the angular C-H bond of the target THF ring and formation of a mixed chromium ester, is thought to be the first event. Two main routes then follow. Oxidative carbon-carbon bond cleavage, to give dicarbonyl-containing products, occurs when 2,2,5-trisubstituted THFs are oxidised. Isolation of some minor side products of the process strongly supports the formation of cyclic enolether intermediates in this transformation. THF ring opening, with no C-C bond cleavage, is observed with 2,5-disubstituted mono-THFs. 1,4-Diketones, useful building blocks in organic synthesis[30] are obtained in this case. The latter transformation proceeds with a remarkable, nearly quantitative yield. At the moment the factors governing the switch of the process toward one of the above routes are unclear and further work is needed to reach this goal. A third route, leading to the oxidative cleavage of a C-2 side-chain, works when the THF ring is flanked by a ketone function. Isolation of minor C-8 oxygenated spiroketal compounds from the oxidation of **1** suggests that cyclic enolethers with α-tethered tertiary alcohol portions can be used in a chromium-mediated spiroketalization process. Further clarification of this issue could come from theoretical studies.

2.4. Experimental Section

2.4.1 General experimental methods

All reagents were purchased 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. Na_2SO_4 was used as a drying agent for aqueous work-up. 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) and LiChrosorb RP-18 250 x 4.0 mm columns. NMR experiments were performed on Varian Unity Inova 700, Varian Unity-Inova 500, Varian Mercury Plus 400, 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 Hz. Abbreviations for signal coupling are as follows: s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet. IR spectra were recorded on a Jasco FT-IR 430 spectrophotometer. 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[6e] for the penta-THF **1** is used.

2.4.2 Oxidation of 1 with PCC/AcOH. Isolation of minor products 9, 10 and 12-14

Penta-THF **1** was synthetized as previously described.[5g] To a solution of **1** (332 mg, 0.63 mmol) in CH_2Cl_2 (5 mL) was added PCC (5 equiv. 3.16 mmol, 682 mg) and AcOH (70 equiv., 2.5 mL) and the resulting heterogeneous mixture was stirred at room temperature for 6h. A saturated aqueous $NaHCO_3$ solution was added and the mixture extracted with CH_2Cl_2. The combined extracts were dried and evaporated *in vacuo* to give a yellow oil. Filtration on a silica gel pad (eluent $CHCl_3$-MeOH, 9:1) afforded a colourless oil (310 mg) that was separated by HPLC (250x10 mm column; flow: 2.5 mL/min; eluent: hexane-EtOAc, 65:35) to give spirolactone **4** (2.5 mg, 1%, t_R = 13.5 min), spirolactone **5** (1.9 mg, 1%, t_R = 18.5 min), spiroketone **9** (3.1 mg, 1%, t_R = 15.0 min), spiroalcohol **10** (1.6 mg, 0.5%, *tr* = 17.5 min), bislactone **12** (2.1 mg, 1 %, t_R = 41.0 min). The fraction eluted in the range 26-38 min contained a mixture of bislactones **13** and **14**. A further HPLC run of this fraction (eluent: hexane-EtOAc, 3:7) gave *ca.* 80% pure **13** (t_R = 17.0 min) and **14** (t_R = 15.0 min). Final purification of these substances was carried out by reversed-phase HPLC (250

x 4.6 mm column; flow: 1.0 mL/min; **13**: eluent CH_3CN/H_2O, 6:4, t_R = 4.5 min; **14**: eluent CH_3CN/H_2O, 7:3, t_R = 3.0 min) to give **13** (1.0 mg, 0.5%) and **14** (0.8 mg, 0.5%).

9: Amorphous solid; IR (neat) ν_{max} 1763, 1699, 1045 cm^{-1}. ^{1}H-NMR (500 MHz, $CDCl_3$) δ 3.91 (1H, d, J= 7.0), 3.87-3.79 (3H, m), 2.88 (1H, ddd, *J*=13.0, 9.7, 5.4), 2.86 (1H, d, *J*= 17.6, H_a-9), 2.76 (1H, *J*= 17.6, 10.6, 9.4), 2.45 (*J*= 17.6, 10.3, 3.3), 2.36 (1H, d, *J*=7.6, H_b-9) 2.35 (1H, m, partially overlapped to the H_b-9 signal), 2.14-2.00 (3H, overlapped multiplets), 2.00-1.82 (7H, overlapped multiplets), 1.60 (1H, ddd, *J*= 12.1, 7.8, 4.8), 1.40 (1H, ddd, *J*= 12.8, 12.8, 4.2), 1.52, 1.35, 1.32, 1.21, 1.06, 1.04 (3H each, s's, 6xMe); ^{13}C-NMR (125 MHz, $CDCl_3$) δ 208.9, 178.0, 100.9, 86.3, 85.8, 85.3, 84.9, 83.7, 82.6, 81.3, 78.7, 76.0, 44.9, 34.4, 32.4, 30.3, 30.0, 27.6, 26.7, 26.5, 26.2, 26.1, 25.6, 25.0, 23.9, 23.4, 21.2; HRMS (ESI) *m/z* calcd for $C_{27}H_{40}NaO_8$ $[M+Na]^+$ 515.2621, found 515.2630.

10. Amorphous solid;. IR (neat) ν_{max} 3398 (br), 1763 cm^{-1}; ^{1}H-NMR (300 MHz, $CDCl_3$) δ 4.09 (1H, dt, *J*= 11.1, 8.7, 8.7), 3.90 (1H, d, *J*= 6.9), 3.83 (1H, dd, *J*=7.3, 7.3), 3.75 (1H, m) 3.57 (1H, m), 2.77 (1H, dt, *J*=16.9, 9.3, 9.3), 1.54, 1.33, 1.30, 1.26, 1.09, 1.02 (3H each, s's, 6xMe); HRMS (ESI) *m/z* calcd for $C_{27}H_{42}NaO_8$ $[M+Na]^+$ 517.2777, found 517.2789.

12: Oil;. IR (neat) ν_{max} 1769 cm^{-1}; ^{1}H-NMR (400 MHz, $CDCl_3$) δ 3.88-3.81 (2H, m), 3.79 (1H, dd, *J*= 9.6, 5.5), 2.83-2.70 (2H, m), 2.52-2.32 (4H, overlapped multiplets), 2.09-1.76 (9H, overlapped multiplets), 1.68-1.60 (1H, m), 1.33 (6H, s, 2 x Me), 1.05 (3H, s, Me); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 177.9, 177.6, 86.3, 86.1, 85.74, 85.68, 85.3, 84.7, 34.5, 32.6, 32.3, 29.9, 29.8, 27.2, 26.9, 26.5, 23.9, 23.5, 23.0; HRMS (ESI) *m/z* calcd for $C_{19}H_{29}O_6$ $[M+H]^+$ 353.1964, found 353.1948.

13: Oil;. IR (neat) ν_{max} 1769 cm^{-1}; ^{1}H-NMR 400 MHz, $CDCl_3$) δ 3.94 (1H, dd, *J* = 7.4, 7.4), 3.84 (1H, dd, *J* = 7.5, 7.5), 3.72 (1H, dd, *J* = 9.7, 4.4), 2.82-2.67 (2H, m), 2.54 (1H, bt, *J* = 12.7), 2.48-2.37 (3H, overlapped multiplets), 2.10 (1H, m), 1.35, 1.31, 1.09 (3H each, s's, 3 x Me); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 178.1, 177.7, 86.7, 85.7, 84.89, 84.81, 84.0, 83.7, 34.8, 32.6, 32.2, 30.04, 30.00, 27.1, 26.7, 26.6, 24.6, 24.0, 23.3; HRMS (ESI) *m/z* calcd for $C_{19}H_{29}O_6$ $[M+H]^+$ 353.1964, found 353.1951.

14: Oil;. IR (neat) ν_{max} 1762 cm^{-1}; ^{1}H-NMR (200 MHz, $CDCl_3$) δ 4.36 (1H, dd, *J* = 6.8, 6.8), 3.90 (1H, dd, *J* = 7.8, 7.8), 2.74-1.87 (11H, overlapped multiplets), 1.80-1.72 (1H, m), 1.32 (3H, s, Me), 1.15 (3H, s, Me); ^{13}C-NMR (100 MHz, $CDCl_3$) δ 177.7, 177.3, 86.9, 85.8, 85.3, 84.5, 34.0, 32.4, 29.9, 28.6, 26.1, 23.8, 23.0, 22.8; HRMS (ESI) *m/z* calcd for $C_{14}H_{21}O_5$ $[M+H]^+$ 269,1389, found 269,1380.

2.4.3 Borohydride reduction of 9

To a solution of **9** (3.0 mg, 0.0061 mmol) in anhydrous ethanol (1 mL) was added $NaBH_4$ (a tip of spatula) at room temperature under stirring. After 1h the mixture was diluted with ethanol (1 mL) and AcOH (two drops) was added. The mixture was filtered and the solid was thoroughly washed with ethanol. The organic phase was dried (Na_2SO_4) and taken to dryness under reduced pressure to give a colourless oil (3 mg). HPLC separation (250 x 4.6 mm column; flow: 1.0 mL/min; hexane/EtOAc, 1:1) gave pure samples of **10** (0.3 mg, 10%, t_R = 6.8 min) and **11** (2.5 mg, 75%, t_R = 35.5 min).

11: Oil;. IR (neat) ν_{max} -3400 (br) cm^{-1}; ^{1}H-NMR (500 MHz, $CDCl_3$) δ 4.11 (1H, q, *J*= 9.1), 3.90 (1H, bd *J*= 7.5), 3.78 (1H), 3.72-3.80 (2H, m), 3.65 (2H, t *J*=5.4), 3.60 (1H, dd *J*= 9.1, 4.8), 2.57 (1H, ddd, *J*= 12.9, 9.9, 4.7), 2.48 (1H, m), 2.14 (1H, bddd, *J*= 10.0, 10.0, 10.0), 2.00 (1H, ddd, *J*= 13.0, 9.5, 5.4), 1.42 (1H, ddd, *J*= 12.6, 12.6, 4.8), 1.54, 1.52, 1.31, 1.25, 1.085, 1.080, 1.077 (3H each, s's, 6xMe); ^{13}C NMR ($CDCl_3$, data from 500 MHz HMBC) δ 104.1, 86.1, 85.7, 84.7, 84.3, 81.9, 81.7, 80.0, 75.4, 72.7, 72.1, 63.6, 44.2, 37.2 (two carbons), 34.5, 31.2, 27.0 (two carbons), 26.7, 26.6, 26.3, 26.0, 25.0, 23.9, 21.4, 21.2; HRMS (ESI) *m/z* calcd for $C_{27}H_{46}NaO_8$ $[M+Na]^+$ 521.3090, found 521.3075.

2.4.4 Synthesis of poly-THF substrates 27, 45 and 49

Penta-THF **1**, the tetrol precursor of **45** and dilactone **49** were synthesized as previously described.[5g] Compound **1** was benzoylated as follows. To **1** (236 mg, 0.45 mmol) dissolved in CH_2Cl_2 (10 mL) was added benzoyl chloride (15 equiv., 6.75 mmol, 785 μL) and DMAP (30 equiv., 3.5 mmol, 11.6 g) and the mixture was stirred at r.t for 30 h. Water (2 mL) was added and the mixture stirred for 15 min. in a water bath and then taken to dryness. The residue was taken up in CH_2Cl_2 and washed with a sat. $NaHCO_3$ solution and water. The organic phase was dried, filtered and evaporated *in vacuo* to give an oily product that was chromatographed on silica gel eluting with (40-70) petroleum ether/Et_2O (8:2) to give 264 mg (80 %) of dibenzoyl penta-THF **27**. Further purification of **27** was carried out by HPLC (250x10 mm column; flow: 2.5 mL/min, hexane/EtOAc, 75:25).

Penta-THF dibenzoate 27: Oil. IR (neat): ν_{max} = 1712, 1285, 710 cm^{-1}. ^{1}H NMR (200 MHz, $CDCl_3$): δ = 8.05-7.92 (4H, m), 7.58-7.35 (6H, m), 4.25-4.15 (2H, m), 4.02-3.75 (4H, overlapped multiplets,), 1.64 (3H, s), 1.60 (6H, s), 1.58, 1.23, 1.16, 1.15, 1.11 (3H each, s's, 5 x Me) ppm. ^{13}C

NMR (50 MHz, $CDCl_3$): δ = 165.54, 165.45, 132.3, 132.2, 131.9, 131.7, 129.2, 128.0, 86.1, 85.7, 85.1, 84.9, 84.4, 84.3, 83.83, 83.78, 83.5, 83.3, 82.5, 34.4, 34.3, 34.1, 32.5, 27.6, 26.9, 26.8, 26.7, 26.5, 24.7, 24.0, 23.4, 23.2, 23.0, 22.6, 21.3, 21.1 ppm. HRMS (ESI) *m/z* calcd for $C_{44}H_{60}NaO_9$ $[M+Na]^+$ 755.4135, found 755.4144.

The tris-THF tetrol corresponding to **45** was benzoylated as described for **1**. To 77 mg of the tetrol (0.173 mmol) dissolved in CH_2Cl_2 (2.0 mL) was added benzoyl chloride (15 equiv., 2.6 mmol, 305 μL) and DMAP (60 equiv., 1.3 g, 10.4 mmol) and the mixture was refluxed for 10 h. The mixture was worked-up as for **1** to give an oily product. Column chromatography on silica gel eluting with petroleum ether (40-70)/Et_2O (8:2) gave 108 mg (73 %) of tetrabenzoyl tris-THF **45**.

Tris-THF tetraBz, 45: Oil. IR (neat): ν_{max} = 1714, 1275, 710 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$): δ = 8.16-7.95 (8H, m), 7.64-7.45 (4H, m), 7.45-7.32 (8H, m), 4.54 (1H, dd, J = 6.6, 6.6 Hz), 4.32 (5H, m), 3.89-3.74 (2H, m), 1.63, 1.57, 1.12, 1.08 (3H each, s's, 4xMe) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 166.50, 166.46, 165.6, 132.80, 132.78, 132.50, 132.46, 131.7, 131.6, 130.26, 130.24, 129.5, 129.4, 128.26, 128.24, 128.16, 128.15, 85.9, 85.3, 85.2, 84.7, 84.4, 83.9, 83.7, 81.4, 65.2, 65.1, 34.6, 34.4, 31.9, 31.5, 27.5, 27.2, 26.92, 26.86, 24.3, 23.7, 23.5, 23.1, 20.4, 20.0ppm. HRMS (ESI) *m/z* calcd for $C_{52}H_{60}NaO_{11}$ $[M+Na]^+$ 883.4033, found 883.4028.

2.4.5 Oxidation of 27 with PCC/AcOH

To a solution of **27** (365 mg, 0.50 mmol) in CH_2Cl_2 (16 mL) was added PCC (5 equiv., 537 mg, 2.5 mmol) and AcOH (70 equiv., 35 mmol, 2 mL) and the resulting mixture was refluxed for 16h. A saturated aqueous $NaHCO_3$ solution was added and the organic phase was washed with water, dried and evaporated *in vacuo* to give a yellow oil. Filtration on a silica gel pad (eluent $CHCl_3$-MeOH, 9:1) afforded an oily product (350 mg). Further elution with $CHCl_3$-MeOH (8:2) gave a mixture of polar products (57 mg) that was no further studied. The first-eluted fraction was separated by HPLC (250x10 mm column; flow: 2.5 mL/min; eluent: hexane-EtOAc, 75:25) to give still impure compounds **28-35**. Analytical HPLC (250 x 4.6 mm column; flow: 1.0 mL/min; 3 mg/injection, hexane/EtOAc, 75:25) gave pure major acid **28** (58.4 mg, 25%, t_R = 11.5 min) and minor acid **29** (16.3 mg, 7%, t_R = 10.0 min) along with slightly impure ketol **30** (t_R = 5.0 min), lactone **37** (t_R = 18.0 min), lactone **33** (t_R = 13.0 min), lactone **31** (t_R = 13.5 min) and **35** (t_R = 16.5 min). Pure aldehydes **34a** (1.2 mg, 1%, t_R = 13.8 min) and **34b** (1.2 mg, 1%, t_R = 10.0 min) were obtained by HPLC on the same column by using hexane/EtOAc, 85:15. A further reversed-phase HPLC run

(250 x 4.0 mm column; flow: 1.0 mL/min; 2 mg/injection) was required to obtain pure **30** (MeCN/H_2O, 9:1, 4.6 mg, 2%, t_R = 14.0 min), **31** (MeCN/H_2O, 85:15, 3.0 mg, 2%, t_R = 5.2 min), **32** (MeCN/H_2O, 8:2, 5.0 mg, 4%, t_R = 5.0 min), **33** (MeCN/H_2O, 85:15, 2.5 mg, 2%, t_R = 4.5 min) and **35** (MeCN/H_2O, 8:2, 1.8 mg, 2%, t_R = 2.0 min).

Acid 28 (major isomer): Oil; ν_{max} 1714, 1288, 712 cm^{-1}**;** ^{1}H-NMR: (700 MHz, $CDCl_3$) δ 7.95 (4H, bd, *J*= 7.6, phenyl *orto* protons), 7.52 (2H, bt, *J*= 7.4, phenyl *para* protons), 7.41 (4H, bt, *J*= 7.7, phenyl *meta* protons), 4.42 (1H, dd, *J*= 6.8, 6.8), 4.27 (1H, dd, *J*= 7.1, 7.1), 4.10 (1H, dd, *J*= 8.4, 6.5), 4.06 (1H, dd, *J*= 10.0, 5.3), 3.95 (1H, dd, *J*= 8.4, 6.0), 3.58 (1H, bs), 3.30 (1H, d, *J*=14.9), 2.99 (1H, d, *J*= 14.9), 2.36 (1H, ddd, *J*= 13.8, 8.4, 5.5), 1.62, 1.60, 1.59, 1.56, 1.55, 1.50, 1.20, 1.14 (3H each, s's, 8xMe); ^{13}C-NMR (175 MHz, $CDCl_3$): 174.4, 171.9, 165.7, 165.6, 132.6, 132.5, 131.85, 131. 78, 129.42, 129.37, 128.25, 128.20, 86.4, 85.5, 85.3, 84.6, 84.5, 83.9, 83.6 (two carbons), 83.2, 82.8, 81.9, 42.,2, 36.5, 34.5, 32.9, 27.9, 27.2, 26.7, 26.6, 26.0, 24.3, 23.14, 23.09, 22.8, 22.3, 21.5, 21.41, 21.38; HRMS (ESI) *m/z* calcd for $C_{44}H_{58}NaO_{12}$ $[M+Na]^+$ 801.3826, found 801.3819.

Acid 29 (minor isomer): Oil; ν_{max} 1712, 1287, 712 cm^{-1}**;** ^{1}H-NMR: (400 MHz, $CDCl_3$) δ 7.9 (4H, bd, *J*= 7.4, phenyl *orto* protons), 7.51 (2H, bt, *J*= 7.3, phenyl *para* protons), 7.40 (4H, bt, *J*= 7.4, phenyl *meta* protons), 4.35 (1H, dd, *J*= 7.2, 7.2), 4.24 (1H, dd, *J*= 9.3, 5.9), 4.07-3.99 (2H, m), 3.55 (1H, dd, *J*= 7.2, 7.2), 3.31 (1H, d, *J*=15.0), 2.98 (1H, d, *J*= 15.0), 2.61-2.53 (1H, m), 2.19-2.10 (1H, m), 1.64, 1.63, 1.60, 1.59, 1.48, 1.47, 1,15, 1.12 (3H each, s's, 8xMe); ^{13}C-NMR (100 MHz, $CDCl_3$): δ 174.8, 172.9, 165.8, 165.7, 132.6, 132.4, 132.0, 131.7, 129.5, 129.4, 128.19, 128.16, 86.3, 86.0, 85.8, 85.5, 85.1, 84.3, 83.9, 83.8, 83.7, 83.4, 81.5, 43.0, 36.4, 34.7, 34.4, 26.95, 26.88 (two carbons), 26.82 (two carbons), 25.2, 24.1, 23.0, 22.7 (two carbons), 21.7 (three carbons); HRMS (ESI) *m/z* calcd for $C_{44}H_{58}NaO_{12}$ $[M+Na]^+$ 801.3826, found 801.3835.

Ketol 30: Oil; ν_{max} 3301, 1712, 1287, 712 cm^{-1}**;** ^{1}H-NMR: (700 MHz, $CDCl_3$) δ 7.99 (2H, d, *J*= 6.9, phenyl *orto* protons), 7.98 (2H, d, *J*= 6.5, phenyl *orto* protons), 7.52 (2H, t, *J*=7.3, phenyl *para* protons), 7.43 (2H, t, *J*=7.7, phenyl *meta* protons), 7.40 (2H, t, *J*=7.6, phenyl *meta* protons), 4.16 (1H, dd, *J*= 9.2, 6.0), 4.10 (1H, dd, *J*= 8.2, 7.0), 3.89 (1H, m), 3.72 (2H, m), 2.66 (1H, ddd, *J*= 12.7, 10.5, 5.4), 2.55 (1H, d, *J*= 16.9), 2.44 (1H, d, *J*= 16.9), 2.18 (1H, ddd, *J*= 12.3, 9.3, 8.2), 1.60 (6H, s, 2 x Me),1.58, 1.55, 1.36, 1.29, 1.14, 1.07 (3H each, s's, 6xMe); ^{13}C-NMR (175 MHz, $CDCl_3$): δ 212.7, 165.8, 165.7, 132.6, 132.4, 132.0, 131.6, 129.6 (two carbons), 129.4 (two carbons), 128.2 (two carbons), 128.1 (two carbons), 101.7, 86.4, 85.9, 85.70, 85.68, 85.65, 84.3, 83.51, 83.46, 83.2, 82.9, 77.8, 48.4, 35.1, 34.4, 31.8, 27.0, 26.86, 26.83, 26.81, 26.0, 24.6, 24.4, 22.82, 22.79, 22.74, 22.2, 21.6, 20.1; HRMS (ESI) *m/z* calcd for $C_{44}H_{58}NaO_{11}$ $[M+Na]^+$ 785.3877, found 785.3884.

Lactone 31: Oil; IR (neat): ν_{max} 1773, 1711, 1288, 1071, 713 cm^{-1}**;** ^{1}H-NMR: (500 MHz, $CDCl_3$) δ 7.96 (2H, bd, *J*= 7.0, phenyl *orto* protons), 7.53 (H, bt, *J*= 7.4, phenyl *para* proton), 7.41 (2H, bt, *J*= 7.5, phenyl *meta* protons), 4.25 (1H, dd, *J*=7.0, 7.0), 3.94 (1H, dd, *J*=9.0, 6.2), 3.88 (1H, dd, *J*=7.2, 7.2), 3.78 (1H, dd, *J*= 9.4, 5.1), 2.80 (1H, ddd, *J*= 17.4, 10.1, 10.2), 2.47 (1H, ddd, *J*= 17.4, 10.6, 3.5), 2.39 (1H, ddd, *J*= 13.9, 10.7, 3.5), 1.63, 1.58, 1.31, 1.22, 1.11 (3H each, s's, 5XMe); ^{13}C-NMR (100 MHz, $CDCl_3$): δ 177.6, 165.7, 132.5, 131.9, 129.4 (two carbons), 128.2 (two carbons) 86.5, 85.7, 85.2, 84.6, 84.0, 83.6, 83.4, 82.7, 35.0, 32.7, 29.8, 27.8, 27.3, 26.8, 26.6, 23.5, 23.3, 23.2, 23.0, ,22.9, 21.3; HRMS (ESI) *m/z* calcd for $C_{29}H_{40}NaO_7$ $[M+Na]^+$ 523.2672, found 523.2668.

Lactone 32 (major isomer): Oil; IR (neat): ν_{max} 1775, 1712, 1288, 713 cm^{-1}**;** ^{1}H-NMR: (500 MHz, $CDCl_3$) δ 7.96 (2H, dd, *J*= 8.0, 1.0, phenyl *orto* protons), 7.52 (H, dddd, *J*= 7.4, 7.4, 1.2, 1.2, phenyl *para* proton), 7.42 (2H, bt, *J*= 7.8, phenyl *meta* protons), 4.36 (1H, dd, *J*=7.9, 6.1), 4.19 (1H, dd, *J*=7.0, 7.0), 3.95 (1H, dd, *J*=9.2, 6.1), 2.56 (1H, ddd, *J*= 17.7, 10.1, 6.3), 2.40 (1H, ddd, *J*= 17.5, 10.3, 7.1), 2.32-2.24 (1H, m), 2.20-2.09 (2H, overlapped multiplets), 2.08-2.0 (1H, m), 1.95-1.96 (2H, overlapped multiplets), 1.84-1.75 (1H, m), 1.61, 1.59, 1.20, 1.19 (3H each, s's, 4xMe); ^{13}C-NMR (100 MHz, $CDCl_3$): δ 177.7, 165.7, 132.5, 131.8, 129.4 (two carbons), 128.2 (two carbons), 84.4, 84.3, 83.8 (two carbons), 83.1, 83.0, 34.3, 32.3, 28.7, 27.6, 26.6, 23.1 (two carbons), 22.9, 22.6, 21.5; HRMS (ESI) *m/z* calcd for $C_{24}H_{32}NaO_6$ $[M+Na]^+$ 439.2097, found 439.2106.

Lactone 32 (minor isomer): Oil; IR (neat): ν_{max} 1776, 1712, 1288, 714 cm^{-1}**;** ^{1}H-NMR: (400 MHz, $CDCl_3$) δ 7.98 (2H, bd, *J*= 8.2, phenyl *orto* protons), 7.52 (H, bt, *J*= 7.0, phenyl *para* proton), 7.41 (2H, bt, *J*= 7.5, phenyl *meta* protons), 4.38 (1H, dd, *J*=7.4, 4.8), 4.19 (1H, dd, *J*=9.3, 5.6), 3.85 (1H, dd, *J*=9.5, 5.8), 2.65 (1H, ddd, *J*= 17.2, 9.8, 6.8), 2.45 (1H, ddd, *J*= 17.1, 10.6, 6.3), 1.60 (6H s, 2xMe), 1.19, 1.13 (3H each, s's, 2xMe); ^{13}C-NMR (100 MHz, $CDCl_3$): δ 177.7 165.7, 132.4, 129.4 (two carbons), 128.1 (two carbons), 87.0, 85.9, 84.8, 83.6, 83.5, 83.3, 34.6, 34.1, 28.6, 26.9, 26.5, 24.5, 23.5, 22.9, 22.8, 21.6; HRMS (ESI) *m/z* calcd for $C_{24}H_{32}NaO_6$ $[M+Na]^+$ 439.2097, found 439.2100.

α,β-unsaturated aldehydes

34a: Oil; IR (neat): ν_{max} 1712, 1288, 712 cm^{-1}**;** ^{1}H-NMR: (500 MHz, $CDCl_3$) δ 9.41 (1H, d, *J*= 7.8), 7.98 (2H, d, *J*= 8.0, phenyl *orto* protons), 7.52 (H, bt, *J*= 7.8, phenyl *para* proton), 7.40 (2H, bt, *J*= 8.0, phenyl *meta* protons), 6.88 (1H, d, *J*=15.6), 6.17 (1H, dd, *J*= 15.6, 7.8), 4.21 (1H, dd, *J*=6.8, 6.8), 4.06 (1H, dd, *J*=6.8, 6.8), 1.62, 1.61, 1.37, 1.22 (3H each, s's, 4XMe); HRMS (ESI) *m/z* calcd for $C_{23}H_{30}NaO_5$ $[M+Na]^+$ 409.1991, found 409.1996.

34b: Oil; IR (neat): ν_{max} 1712, 1288, 712 cm^{-1}; ^{1}H-NMR: (500 MHz, $CDCl_3$) δ 9.58 (1H, d, *J*= 7.9), 7.98 (2H, d, *J*= 8.2, phenyl *orto* protons), 7.52 (H, bt, *J*= 7.4, phenyl *para* proton), 7.41 (2H, bt, *J*= 8.0, phenyl *meta* protons), 6.84 (1H, d, *J*=15.6), 6.27 (1H, dd, *J*= 15.6, 7.9), 4.23 (1H, dd, *J*=9.6, 5.6), 3.94 (1H, dd, *J*=6.0, 6.0), 1.61 (6H, s, 2xMe), 1.42, 1.18 (3H each, s's, 2XMe); HRMS (ESI) *m/z* calcd for $C_{23}H_{30}NaO_5$ $[M+Na]^+$ 409.1991, found 409.1990.

Acid 35: Oil; ν_{max} 1744, 1715, 1283, 712 cm^{-1}**;** ^{1}H-NMR: (500 MHz, $CDCl_3$) δ 7.97 (2H, bd, *J*= 7.3, phenyl *orto* protons), 7.55 (H, bt, *J*= 7.3, phenyl *para* proton), 7.44 (2H, bt, *J*= 7.7, phenyl *meta* protons), 5.62 (1H, dd, *J*= 9.3, 3.1), 2.86 (1H, dd, *J*= 16.1, 3.2), 2.74 (1H, dd, *J*= 16.1, 9.4), 2.11 (3H, s, acetate), 1.66, 1.65 (3H each, s's, 2x Me); ^{3}C-NMR (100 MHz, $CDCl_3$): δ 175.2, 170.1, 165.1, 133.0, 131.1, 129.5 (two carbons), 128.4 (two carbons), 82.4, 73.9, 34.9, 22.4, 21.7, 20.9; HRMS (ESI) *m/z* calcd for $C_{15}H_{18}NaO_6$ $[M+Na]^+$ 317.1001, found 317.1009.

2.4.6 Oxidation of 27 with PCC(cat.)/H_5IO_6

To a suspension of H_5IO_6 (4 equiv., 78.8 mg, 0.35 mmol) in acetonitrile (1.5 mL) at 0°C was added 1.0 mol% PCC (87 μL of a 0.01 M stock solution in acetonitrile) under vigorous stirring. After 5 min compound **27** (61 mg, 0.083 mmol) dissolved in acetonitrile (650 μL) was added. After 3h CH_2Cl_2 (1.5 mL) was added followed by ethanol (40 μL) and taken to dryness. Filtration through a short pad of sodium thiosulphate adsorbed on silica[8] ($CHCl_3$-MeOH, 9:1) gave an oily product (50 mg). Further elution with $CHCl_3$-MeOH (8:2) gave a mixture of polar products (14 mg) that was no further studied. The first-eluted fraction was separated as above described for the analogous process with PCC/AcOH to give compounds **28** (20.6 mg, 40%), **29** (2.2 mg, 4%), **32** (1.4 mg, 5%), **33** (0.3 mg, 1%).

2.4.7 Oxidation of 45 with PCC/AcOH

To a solution of **45** (21.8 mg, 0.025 mmol) in CH_2Cl_2 (2 mL) was added PCC (4 eq., 21.8 mg, 0.10 mmol) and AcOH (60 eq., 1.52 mmol, 87 μL) and the resulting mixture was refluxed for 13h. A saturated aqueous $NaHCO_3$ solution was added and the organic phase was washed with water, dried and evaporated *in vacuo* to give a yellow oil. Filtration on a silica gel pad (eluent $CHCl_3$-MeOH, 9:1) afforded an oily product (14 mg). Further elution with $CHCl_3$-MeOH (8:2) gave a mixture of polar products (9.0 mg) that was no further studied. The first-eluted fraction was separated by HPLC (250 x 4.6 mm column; flow: 1.0 mL/min; 1 mg/injection, hexane/EtOAc,

75:25) to give **46** (2.4 mg, 15%, *tr* = 30.5 min), **47** (1.6 mg, 10%, *tr* = 33.5 min) and **48** (1.1 mg, 7%, *tr* = 10.0 min).

Acid 46 (**major isomer**)**:** Oil; IR (neat): ν_{max} 1717, 1279, 1119, 710 cm^{-1}**;** ^{1}H-NMR: (400 MHz, $CDCl_3$) δ 8.02 (4H, d, *J* =8.2, phenyl *orto* protons), 7.96 (4H, d, *J* =8.0, phenyl *orto* protons), 7.55-7.45 (4H, m, phenyl *para* protons), 7.44-7.38 (8H, m, phenyl *meta* protons), 5.40 (1H, dd, *J* =9.7, 3.1), 4.49 (1H, dd, *J* =8.5, 6.2), 4.44 (1H, dd, *J* =7.4, 7.4), 4.40-4.28 (4H, overlapped multplets), 2.73 (1H, dd, *J* = 15.8, 3.0), 2.64 (1H, dd, *J* = 15.8, 9.8), 2.37 (1H, ddd, *J* = 12.6, 7.9, 6.0), 2.29-2.19 (2H, m), 2.17-2.00 (2H, m), 1.57, 1.55, 1.45, 1.19 (3H each, s's, 4x Me); ^{13}C-NMR (100 MHz, $CDCl_3$): δ 174.2, 173.4, 166.8, 166.6, 165.8, 165.6, 132.93, 132. 87, 132.7 (two carbons), 131.5, 131.4, 130.26, 130.21, 129.6, 129.55, 129.50 (two carbons), 128.32 (three carbons), 128.27, 85.2, 85.0, 83.9, 83.5, 83.3, 81.3, 75.0, 65.3, 65.0, 36.1, 35.7, 33.7, 31.6, 31.5, 26.9, 25.9, 24.1, 23.5, 23.2, 22.7, 20.4, 20.2; HRMS (ESI) *m/z* calcd for $C_{52}H_{58}NaO_{14}$ $[M+Na]^+$ 929.3724, found 929.3736.

Acid 47 (**minor isomer**)**:** Oil; IR (neat): ν_{max} 1714, 1277, 1113, 711 cm^{-1}**;** ^{1}H-NMR: (400 MHz, $CDCl_3$) δ 8.04 (2H, bd, *J* =7.0, phenyl *orto* protons), 8.02 (2H, bd, *J* =5.5, phenyl *orto* protons), 7.98 (4H, bd, *J* =8.0, phenyl *orto* protons), 7.53 (4H, m, phenyl *para* protons), 7.41 (8H, m, phenyl *meta* protons), 5.41 (1H, dd, *J* =6.1, 6.1), 4.64 (1H, dd, *J* =7.4, 7.4), 4.49-4.44 (5H, m), 2.63 (1H, dd, *J* = 15.7, 7.3), 2.56 (1H, dd, *J* = 15.8, 5.9), 2.44 (1H, bddd, *J* = 12.5, 5.5, 5.5), 1.64, 1.53, 1.44, 1.28 (3H each, s's, 4x Me); ^{13}C-NMR (100 MHz, $CDCl_3$): δ 172.9, 171.3, 167.2, 167.0, 165.8, 165.7, 133.03, 132.98, 132.7, 132.6, 131.6, 131.4, 130.1 (two carbons) 129.64 (two carbons), 129.57, 129.4, 128.34 (three carbons), 128.29, 85.3, 84.6, 84.3, 84.1, 83.9, 83.5, 74.9, 65.5, 65.3, 36.5, 35.0, 33.4, 32.0, 31.1, 26.9, 26.3, 24.8, 24.4, 23.6, 23.2, 20.8, 20.6; HRMS (ESI) *m/z* calcd for $C_{52}H_{58}NaO_{14}$ $[M+Na]^+$ 929.3724, found 929.3742.

Dketone 48: Oil; IR (neat): ν_{max} 1710, 1274, 1111, 710 cm^{-1}**;** ^{1}H-NMR: (200 MHz, $CDCl_3$) δ 8.10-7.91 (8H, m, phenyl *orto* protons), 7.62-7.32 (12H, m, phenyl *meta* and *para* protons), 7.54-7.25 (6H, overlapped multiplets, 2 x THF and 2 x CH_2O- protons), 1.63 (3H, s, 2 x Me), 1.37, 1.34 (3H each, s's, 2 x Me); ^{13}C-NMR (100 MHz, $CDCl_3$): δ 214.0, 212.7, 166.5 (two carbons), 165.7 (two carbons), 132.8, 132.7, 131.6, 131.4, 130.35, 130.31, 129.55, 129.53, 129.48, 129,43, 128.35, 128.31, 88.9, 88.7, 84.93, 84.92, 84.2, 83.4, 65.0 (two carbons), 35.2, 34.8, 32.3, 31.8, 30.8, 29.9, 26.3 (two carbons), 24.2, 23.7, 23.55, 23.51, 20.6, 20.4; HRMS (ESI) *m/z* calcd for $C_{52}H_{58}NaO_{12}$ $[M+Na]^+$ 897.3826, found 897.3815.

2.4.8 Oxidation of 45 with PCC(cat.)/H_5IO_6

To a suspension of H_5IO_6 (4 eq., 34.2 mg, 0.15 mmol) in acetonitrile (600 μL) at 0°C was added 1.0 mol% PCC (38 μL of a 0.01 M stock solution in acetonitrile) under vigorous stirring. After 5 min compound **45** (32.3 mg, 0.037 mmol) dissolved in acetonitrile (150 μL) was added. After 2h a further 1% PCC was added and the mixture kept at 0°C for an additional 1h. Then CH_2Cl_2 (700 μL) was added followed by ethanol (40 μL) and taken to dryness. Filtration through a short pad of sodium thiosulphate adsorbed on silica gave an oily product (19 mg). Further elution with $CHCl_3$-MeOH (8:2) gave a mixture of polar products (3.2 mg) that was no further studied. The first-eluted fraction was separated by HPLC as above described for the analogous process with PCC/AcOH to give compounds **46** (10.0 mg, 30%), **47** (2.0 mg, 6%) and **48** (2.9 mg, 9%).

2.4.9 Oxidation of 49 with PCC(cat.)/H_5IO_6

To a suspension of H_5IO_6 (4 equiv., 35.4 mg, 0.15 mmol) in acetonitrile (600 μL) at 0°C was added 2.0 mol% PCC (80 μL of a 0.01 M stock solution in acetonitrile) under vigorous stirring. After 5 min compound **49** (10.4 mg, 0.039 mmol) dissolved in acetonitrile (150 μL) was added. After 3.5h CH_2Cl_2 (750 μL) was added followed by ethanol (40 μL) and taken to dryness. Filtration through a short pad of sodium thiosulphate adsorbed on silica gave almost pure **50** as an oil. HPLC (250 x 4.6 mm column; flow: 1.0 mL/min; $CHCL_3$/MeOH 98:2, *tr* = 4.0 min) gave pure **50** (4.3 mg, 45%).

50: Oil; IR (neat): ν_{max} 1774, 1717 cm^{-1}; ^{1}H-NMR: (500 mhz, $CDCl_3$) δ 3.01-2.92 (2H, m), 2.92-2.82 (2H, m), 2.65-2.53 (6H, m), 2.113-2.04 (2H, m), 1.55 (6H, s, 2x Me); ^{13}C-NMR (125 MHz, $CDCl_3$): δ 209.2, 175.9, 89.2, 31.3, 31.0, 28.2, 23.6; HRMS (ESI) *m/z* calcd for $C_{14}H_{18}NaO_6$ $[M+Na]^+$ 305.1001, found 305.1008.

2.4.10 Synthesis of mono-THF 51, 54, 56, 59, 62, 64

The title compounds were obtained by benzoylation of the corresponding THF diols in turn synthesized as previously described.[5h,5k, 21,23] Purification of these compounds was achieved by column chromatography with the following eluent: **51** (40/70 petroleum ether-ethyl ether, 9:1), **54** (40/70) petroleum ether-ethyl ether, 1:1), **56** (40/70 petroleum ether-ethyl ether, 6:4), **59** (40/70 petroleum ether-ethyl ether, 1:1), **62** (40/70 petroleum ether-ethyl ether, 8:2), **64** (hexane-EtOAc,

8:2).

51: Oil. IR (neat): ν_{max} = 1719, 1281, 1111, 709 cm^{-1}. ^{1}H NMR: (200 MHz, $CDCl_3$): δ = 8.13 – 7.88 (6H, m), 7.68 -. 7.28 (9H, m), 5.65 (1H, dd, *J* = 2.9, 8.8 Hz), 4.80 (1H, dd, *J* = 2.9, 12.2 Hz), 4.59 (1H, dd, *J* = 12.2, 8.8 Hz), 4.33 (1H, dd, *J* = 6.3 Hz), 2.24 – 1.88 (4H, m), 1.67 (3H, s), 1.64 (3H, s), 1.43 (3H, s) ppm. ^{13}C NMR (50 MHz, $CDCl_3$): δ 166.4, 166.0, 165.6, 133.0, 132.9, 132.5, 131.6, 129.8, 129.6, 129.5, 129.3, 128.4, 128.3, 128.2, 83.5, 83.0, 82.8, 76.2, 63.9, 34.6, 26.9, 23.6, 23.2, 21.3 ppm. HRMS (ESI) *m/z* calcd for $C_{31}H_{32}NaO_7$ $[M+Na]^+$ 539.2046, found 539.2038.

54: Oil. IR (neat): ν_{max} = 1740, 1721, 1275, 712 cm^{-1}. ^{1}H NMR (200 MHz, $CDCl_3$): δ = 8.00 (4H, m), 7.66 – 7.30 (6H, m), 5.46 (1H, dd, *J* = 2.6, 8.8 Hz), 4.78 – 4.20 (5H, overlapped multiplets), 2.02 (3H, s), 1.96 (3H, s), 1.67 (3H, s), 1.36 (3H, s) ppm. ^{13}C NMR (50 MHz, $CDCl_3$): δ = 170.9, 170.5, 166.1, 165.6, 133.1, 132.8, 131.0, 129.7, 129.6, 128.5, 128.3, 83.2, 83.0, 80.4, 76.1, 65.2, 63.3, 34.5, 26.8, 23.5, 20.8, 18.9 ppm. HRMS (ESI) *m/z* calcd for $C_{28}H_{32}NaO_9$ $[M+Na]^+$ 535.1944, found 535.1963.

56: Oil. IR (neat): ν_{max} = 1713, 1285, 1113, 710 cm^{-1}. ^{1}H NMR (200 MHz, $CDCl_3$): δ = 8.07 (2H, d, *J* = 8.5 Hz), 7.96 (2H, d, *J* = 7.1 Hz), 7.64-7.29 (6H, m), 5.20 (1H, s), 4.33 (1H, dd, *J* = 6.9, 7.7 Hz), 3.72 (3H, s), 2.56-2.83 (1H, m), 2.19-1.75 (3H, m), 1.61 (3H, s), 1.59 (3H, s), 1.46 (3H, s) ppm. ^{13}C NMR (50 MHz, $CDCl_3$): δ = 168.6, 166.0, 165.7, 133.3, 132.5, 131.6, 129.8, 129.4, 128.4, 128.2, 83.5, 83.3, 82.8, 78.0, 52.2, 34.3, 26.5, 23.4, 23.0, 21.3 ppm. HRMS (ESI) *m/z* calcd for $C_{25}H_{28}NaO_7$ $[M+Na]^+$ 463.1733, found 463.1717.

59: Oil. IR (neat): ν_{max} = 1712, 1278, 1109, 708 cm^{-1}. ^{1}H NMR (200 MHz, $CDCl_3$): δ = 8.07 (2H, d, *J* = 7.7 Hz), 7.96 (4H, d, *J* = 8.2 Hz), 7.64–7.30 (9H, m), 5.65 (1H, dd, *J* = 2.7, 8.6 Hz), 4.75 (1H, dd, *J* = 2.8, 11.9 Hz), 4.55 (1H, dd, *J* = 8.6, 11.9 Hz), 4.32 (1H, dd, *J* = 6.6, 8.1 Hz), 2.27–1.68 (4H, m), 1.60 (6H, s), 1.45 (3H, s) ppm. ^{13}C NMR (50 MHz, $CDCl_3$): δ 166.5, 166.0, 165.7, 133.0, 132.4, 131.9, 130.1, 129.7, 129.6, 129.4, 128.4, 128.3, 128.2, 85.5, 83.0, 76.1, 64.0, 34.8, 26.7, 24.1, 22.6, 21.5 ppm. HRMS (ESI) *m/z* calcd for $C_{31}H_{32}NaO_7$ $[M+Na]^+$ 539.2046, found 539.2055.

62: Oil. IR (neat): ν_{max} = 1735, 1716, 1289, 712 cm^{-1}. ^{1}H NMR (200 MHz, $CDCl_3$): δ = 7.96–7.87 (2H, d, *J* = 6.9 Hz), 7.59–7.35 (3H, m), 5.04 (2H, AB system, *J* = 17.8 Hz), 4.20 (1H, dd, *J* = 7.18 Hz), 2.54–2.39 (1H, m), 2.08 (3H, s), 1.71 (3H, s), 1.61 (3H, s), 1.41 (3H, s) ppm. ^{13}C NMR (50 MHz, $CDCl_3$): δ = 206.5, 170.2, 165.7, 132.7, 131.4, 129.3, 128.4, 88.5, 85.9, 82.7, 65.7, 35.4, 26.0, 23.6, 22.8, 22.6, 20.4 ppm. HRMS (ESI) *m/z* calcd for $C_{19}H_{24}NaO_6$ $[M+Na]^+$ 371.1471, found 371.1489.

64: Oil. IR (neat): ν_{max} = 1715, 1268, 709 cm^{-1}. ^{1}H NMR (500 MHz, $CDCl_3$): δ = 8.05 (4H, bd, *J* = 8.4 Hz), 7.55 (2H, bt, *J* = 7.4 Hz), 7.42 (4H, bt, *J* = 7.8 Hz), 4.42 (2H, overlapped multiplets), 4.39-4.32 (4H, overlapped multiplets), 2.12 (2H, m), 1.89 (2H, m) ppm. ^{13}C NMR (50 MHz, $CDCl_3$): δ = 166.4, 132.9, 130.0, 129.6, 128.3, 77.5, 66.6, 27.8 ppm. HRMS (ESI) *m/z* calcd for $C_{20}H_{20}NaO_5$ $[M+Na]^+$ 363.1208, found 363.1220.

2.4.11 Synthesis of 60

To compound **51** (106 mg, 0.20 mmol) dissolved in MeOH (5 mL) was added K_2CO_3 (excess) and the mixture stirred at r. t. for 1.5 h. Water (1 mL) was added followed by acetic acid up to neutrality. The mixture was taken to dryness in vacuo and the solid partitioned between a sat. $NaHCO_3$ solution and EtOAc. The organic phase was washed with water, dried and evaporated to give 65.9 mg of an oily product.

To the crude obtained as above, dissolved in CH_2Cl_2 (5 mL), was added $NaIO_4$ supported on wet silica (2.4 equiv., 0.47 mmol, 746 mg; 0.64 mmol/g) and the mixture was stirred fro 30 min. at r.t. The reaction mixture was filtered, the solid was thoroughly washed with CH_2Cl_2 and the filtrate was taken to dryness to give an oily product.

The above oil (55.1 mg) dissolved in EtOH (4 mL) sodium borohydride (two spatula tips) was added. After 30 min. acetic acid was dropwise added until no gas evolution was observed. The mixture was filtered and the filtrate taken to dryness to give an oily product.

The above oil (33.0 mg, 0.12 mmol) was dissolved in pyridine (500 μL) and benzoyl chloride (1.1 equiv., 0.13 mmol, 15 μL) was added. After 1 h water (100 μL) was added and the mixture was taken to dryness. Purification by preparative TLC (hexane/EtOAc, 1:1) yielded pure **20** (33.0 mg, 43% over four steps).

60: Oil. IR (neat): ν_{max} = 1712, 1277, 1112, 709 cm^{-1}. ^{1}H NMR (200 MHz, $CDCl_3$): δ = 8.06 (2H, d, *J* = 7.0 Hz), 7.98 (2H, d, *J* = 6.9 Hz), 7.61-7.33 (6H, overlapped multiplets), 4.38 (1H, d, *J* = 11.1 Hz), 4.31 (1H, m), 4.20 (1H, d, *J* = 11.1), 2.20-1.73 (4H, m), 1.64 (3H, s), 1.61 (3H, s), 1.38 (3H, s) ppm. ^{13}CNMR (50 MHz, $CDCl_3$): δ = 166.4, 165.7, 133.0, 132.5, 131.7, 130.1, 129.5, 129.4, 128.3, 128.2, 83.8, 83.4, 81.8, 69.7, 34.5, 26.8, 24.1, 23.0, 21.4 ppm. HRMS (ESI) *m/z* calcd for $C_{23}H_{26}NaO_5$ $[M+Na]^+$ 405.1678, found 405.1670.

2.4.12 General reaction procedure for the oxidation of mono-THF compounds 51, 54, 56, 59, 60, 62, 64

To a suspension of H_5IO_6 (4 eq.) in acetonitrile at r.t. was added 5.0 mol % PCC (from a 0.01 M stock solution in acetonitrile) under vigorous stirring. After 5 min. the mono-THF compound (1 eq.) dissolved in acetonitrile was added. The overall volume of acetonitrile was such that the final concentration of the solution was 0.05 M. After complete consumption of the starting material (TLC control, usually 45-60 min), ethanol (excess) was added and stirring continued until the colour of the solution turned from yellow to green. Then silica (excess) was added and the solvent evaporated in vacuo to give a fine powder that was loaded on the top of a silica gel column. Elution with $CHCl_3$-MeOH (9:1) allowed recovery of the acid that resulted pure enough for successive spectral studies and/or synthetic steps.

Compound **51** (112.0 mg, 0.22 mmol) was subjected to the standard oxidative procedure to give the crude acid as a yellow oil which was purified by column chromatography (CH_2Cl_2-MeOH, 95: 5) to yield **52** (99 mg, 80%) and lactone **53** (8.0 mg, 10%) as oils.

52: Oil. IR (neat): ν_{max} = 1717, 1282, 1107, 709 cm^{-1}. ^1H NMR: (200 MHz, $CDCl_3$): δ = 8.00 (2H, d, *J* = 7.7 Hz), 7.87 (4H, m), 7.57-7.28 (9H, m), 5.89 (1H, dd, *J* = 7.8, 3.1 Hz), 4.74 (1H, dd, *J* = 3.1, 12.2 Hz), 4.45 (1H, dd, *J* = 7.8, 12.2 Hz), 3.24 (2H, AB system, *J* = 15.6 Hz), 1.86 (3H, s), 1.71 (6H, s) ppm. ^{13}C NMR (50 MHz, $CDCl_3$): δ = 174.1, 171.1, 166.0, 165.5, 165.2, 133.15, 133.12, 133.0, 129.8, 129.5, 129.3, 128.3, 82.0, 79.0, 74.6, 62.8, 39.2, 24.55, 24.46, 21.3 ppm. HRMS (ESI) *m/z* calcd for $C_{31}H_{30}NaO_{10}$ $[M+Na]^+$ 585.1737, found 585.1723.

53: Oil. IR (neat): ν_{max} = 1778, 1721, 1259, 709 cm^{-1}. ^1H NMR (200 MHz, $CDCl_3$): δ = 8.04 (2H, d, *J* = 7.9 Hz), 7.92 (2H, d, *J* = 8.0 Hz), 7.67-7.31 (6H, m), 5.66 (1H, dd, *J* = 8.3, 3.2 Hz), 4.85 (1H, dd, *J* = 12.1, 3.2 Hz), 4.52 (1H, dd, *J* = 12.1, 8.3 Hz), 2.61 (2H, t, *J*=7.6 Hz), 2.37 (1H, ddd, *J* = 13.1, 7.6, 7.6 Hz), 2.08 (1H, ddd, *J*=13.1, 9.5, 9.5 Hz), 1.66 (3H, s) ppm. ^{13}C NMR (50 MHz, $CDCl_3$): δ = 176.2, 166.2, 165.5, 133.7, 133.2, 129.9, 129.6, 129.4, 128.9, 128.7, 128.4, 85.2, 75.6, 62.8, 31.5, 28.9, 24.2 ppm. HRMS (ESI) *m/z* calcd for $C_{21}H_{20}NaO_6$ $[M+Na]^+$ 391.1158, found 391.1167.

Compound **54** (13.5 mg, 0.026 mmol) was subjected to the standard oxidative procedure to give the crude acid as an oil which was purified by column chromatography (CH_2Cl_2-MeOH, 95: 5) to yield **15** (9.4 mg, 65%) as an oil.

55: Oil. IR (neat): ν_{max} = 1721, 1273, 1226, 711 cm^{-1}. ^1H NMR (200 MHz, $CDCl_3$): δ = 8.0 (4H,

m), 7.65-7.29 (6H, m), 5.75 (1H, dd, *J* = 7.8, 2.9 Hz), 4.67-4.49 (3H, m), 4.21 (1H, dd, *J* = 12.1, 7.8 Hz), 3.2 (2H, AB system, *J* = 15.7 Hz), 2.09 (3H, s), 1.94 (3H, s), 1.85 (3H, s), 1.73 (3H, s) ppm. ^{13}C NMR (50 MHz, $CDCl_3$): δ = 172.4, 172.3, 170.5, 170.3, 165.24, 165.17, 82.8, 79.4, 74.3, 66.0, 62.2, 38.9, 21.1, 20.71, 20.68, 19.7 ppm. HRMS (ESI) *m/z* calcd for $C_{28}H_{30}NaO_{12}$ $[M+Na]^+$ 581.1635, found 581.1651.

Compound **56** (23.8 mg, 0.050 mmol) was subjected to the standard oxidative procedure to give the crude acid as an oil which was purified by column chromatography (CH_2Cl_2 to CH_2Cl_2-MeOH, 95: 5) to yield **57** (7.3 mg, 30%) and lactone **58** (6.4 mg, 45%) as oils.

57: Oil. IR (neat): ν_{max} = 1719, 1287, 1110, 712 cm^{-1}. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 8.02-7.89 (4H, m), 7.61-7.44 (2H, m), 7.44-7.29 (4H, m), 5.69 (1H, s), 3.64 (3H, s), 3.42 (1H, d, *J* = 16.2 Hz), 3.27 (1H, d, *J* = 16.2 Hz), 1.91 (3H, s), 1.65 (3H, s), 1.63 (3H, s) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 171.4, 171.0, 167.4, 165.4, 165.2, 133.4, 133.1, 129.8, 129.6, 128.4 128.2, 81.1, 78.8, 75.8, 52.4, 39.0, 24.6, 24.1, 20.7 ppm. HRMS (ESI) *m/z* calcd for $C_{25}H_{26}NaO_{10}$ $[M+Na]^+$ 509.1424, found 509.1418.

58: Oil. IR (neat): ν_{max} = 1781, 1726, 1271, 1112, 714 cm^{-1}. ^{1}H NMR (200 MHz, $CDCl_3$): δ = 8.06, (2H, d, *J* = 7.0 Hz), 7.63 (1H, t, *J* = 6.5 Hz), 7.48 (2H, t, *J* = 7.7 Hz), 5.26 (1H, s), 3.80 (3H, s), 2.76 – 2.52 (3H, m), 2.20-2.01 (1H, m), 1.65 (3H, s) ppm. ^{13}C NMR (50 MHz, $CDCl_3$): δ = 175.6, 167.3, 165.5, 133,8, 129.9, 128.6, 84.3, 77.2, 52.9, 30.9, 28.7, 23.8 ppm. HRMS (ESI) *m/z* calcd for $C_{15}H_{16}NaO_6$ $[M+Na]^+$ 315.0845, found 315.0853.

Compound **59** (9.7 mg, 0.019 mmol) was subjected to the standard oxidative procedure to give the crude acid as an oil which was purified by column chromatography (CH_2Cl_2-MeOH, 95: 5) to yield **52** (7.3 mg, 70%).

Compound **60** (31.0 mg, 0.081 mmol) was subjected to the standard oxidative procedure to give the crude acid as an oil which was purified by column chromatography (CH_2Cl_2-MeOH, 95: 5) to yield **61** (21.5 mg, 65%) as an oil.

61: Oil. IR (neat): ν_{max} = 1716, 1284, 1109, 710 cm^{-1}. ^{1}H NMR (200 MHz, $CDCl_3$) δ = 7.94 (4H, m), 7.60-7.44 (2H, m), 7.42-7.28 (4H, m), 4.60 (2H, AB system, *J* = 11.7 Hz), 3.12 (2H, bs), 1.71 (3H, s), 1.63 (3H,s) ppm. ^{13}C NMR (50 MHz, $CDCl_3$): δ = 174.9, 171.3, 165.8, 165.4, 133.05, 133.02, 129.8, 129.65, 129.60, 129.5, 128.35, 128.3, 80.4, 78.9, 67.5, 39.9, 24.5, 21.0 ppm. HRMS (ESI) *m/z* calcd for $C_{23}H_{24}NaO_8$ $[M+Na]^+$ 451.1369, found 451.1360.

Compound **62** (10.1 mg, 0.029 mmol) was subjected to the standard oxidative procedure to give the crude acid as an oil which was purified by HPLC (hexane-EtOAc, 7:3) to yield 1,4-diketone **63** (5.3 mg, 70%), as an oil.

63: Oil. IR (neat): ν_{max} = 1715, 1287, 714 cm^{-1}. ^{1}H NMR (200 MHz, $CDCl_3$) δ = 8.04 (2H, d, *J* = 8.3 Hz), 7.59 (1H, t, *J* = 7.2 Hz), 7.45 (2H, t, *J* = 7.7 Hz), 2.78 (4H, m), 2.19 (3H, s), 1.65 (6H, s) ppm. ^{13}C NMR (50 MHz, $CDCl_3$): δ = 207.8, 207.3, 165.9, 133.4, 129.8, 128.4, 84.2, 37.0, 30.1, 30.0, 23,8 ppm. HRMS (ESI) *m/z* calcd for $C_{15}H_{18}NaO_4$ $[M+Na]^+$ 285.1103, found 285.1109.

Compound **64** (107.0 mg, 0.313 mmol) was subjected to the standard oxidative procedure at 0°C, using a 2 mol % of PCC, to give 1,4-diketone **65** (109 mg, 98%).

65: Amorphous solid. IR (neat): ν_{max} = 1713, 1275, 715 cm^{-1}. ^{1}H NMR (200 MHz, $CDCl_3$): δ = 8.09 (4H, d, *J* = 7.2 Hz), 7.58 (2H, t, *J* = 7.5 Hz), 7.45 (4H, t, *J* = 7.7 Hz), 4.95 (4H, s), 2.87 (4H, s) ppm. ^{13}C NMR (50 MHz, $CDCl_3$): δ = 202.7, 165.8, 133.5, 129.9, 129.0, 128.4, 68.3, 32.0 ppm. HRMS (ESI) *m/z* calcd for $C_{20}H_{18}NaO_6$ $[M+Na]^+$ 377.1001, found 377.1015.

2.4.13 Synthesis of umbelactone

Trihydroxiacid 75: To acid **52** (118.1 mg, 0.21 mmol) dissolved in MeOH (5 mL) was added K_2CO_3 (excess) and the mixture stirred at r. t. for 2.5 h. Water (1 mL) was added followed by acetic acid up to neutrality. The mixture was taken to dryness in vacuo and the solid partitioned between a sat. $NaHCO_3$ solution and EtOAc. The organic phase was washed with water, dried and evaporated to give 31.2 mg (90%) of **75** that was used in the next step without further purification.

75: ^{1}H-NMR (500 MHz, CD_3OD): δ = 3.78 (1H, dd, *J* =10.9, 2.5), 3.58 (1H, dd, *J* =10.9, 7.9 Hz), 3.53 (1H, dd, *J* =7.9, 2.5 Hz), 2.49 (1H, d, *J* =14.9 Hz), 2.53 (1H, d, *J* =14.9 Hz), 1.36 (3H, s) ppm. ^{13}C NMR (125 MHz, CD_3OD): δ = 180.6, 79.0, 73.5, 63.9, 45.6, 24.7 ppm. HRMS (ESI) *m/z* calcd for $C_6H_{12}NaO_5$ $[M+Na]^+$ 187.0582, found 187.0590.

Dihydroxylactone 77: To Trihydroxiacid **75** (30.0 mg, 0.18 mmol) dissolved in MeOH (4 mL) was added axcess CH_2N_2 in Et_2O until a yellow colour persisted. The mixture was stirred for further 10 min. and then the excess of CH_2N_2 was destroyed by dropwise addition of acetic acid until the solution became colourless. The mixture was taken to dryness to give an oil which was purified by column chromatography (CH_2Cl_2-MeOH, 9:1) to yield lactone **77** (23.5 mg, 90%) as a clear oil.

77: Oil. IR (neat): ν_{max} = 3408, 1771 cm^{-1}. ^{1}H NMR (200 MHz, $CDCl_3$): δ = 4.30 (1H, dd, *J* = 6.0,

4.4 Hz), 3.86 (2H, m), 2.77 (1H, d, *J* = 17.2 Hz), 2.50 (1H, d, *J* = 17.2 Hz), 1.44 (3H, s) ppm.^{13}C NMR (50 MHz, $CDCl_3$): δ = 177.9, 90.1, 75.7, 61.0, 45.6, 24.5 ppm. HRMS (ESI) *m/z* calcd for $C_6H_{10}NaO_4$ $[M+Na]^+$ 169.0477, found 169.0472.

Silylated lactone 78: To lactone **77** (21.3 mg, 0.14 mmol), dissolved in DMF (300 μL), were added imidazole (3.0 equiv., 28.6 mg, 0.42 mmol) and *tetr*-butyldimethylsilyl chloride (1.5 equiv., 31.5 mg, 0.21 mmol) and the mixture was stirred for 2.5 h at r.t.. Then MeOH (500 μL) was added and stirring continued for 30 min. The reaction mixture was concentrated onto silica and purified by column chromatography (hexane-EtOAc, 1:1) to yield compound **78** (34.9 mg, 96%) as a clear oil.

78: Oil. IR (neat): ν_{max} = 3447, 1782 cm^{-1}. ^{1}H NMR (200 MHz, $CDCl_3$): δ = 4.19 (1H, dd, *J* = 3.8, 2.8 Hz), 4.12 (1H, dd, *J* = 11.8, 2.8 Hz), 4.02 (1H, dd, *J* = 11.8, 3.8 Hz), 3.66 (1H, bs), 2.73 (1H, d, *J* = 17.6 Hz), 2.57 (1H, d, *J* = 17.6 Hz), 1.49 (3H, s), 0.90 (9H, s), 0.12 (6H, s) ppm. ^{13}C NMR (50 MHz, $CDCl_3$): δ = 174.7, 85.5, 75.4, 61.8, 44.9, 26.7, 25.7, 18.1, -5.6, -5.7 ppm. HRMS (ESI) *m/z* calcd for $C_{12}H_{24}NaO_4Si$ $[M+Na]^+$ 283.1342, found 283.1339.

Unsaturated lactone 79: To lactone **78** (30.0 mg, 0.11 mmol), dissolved in pyridine (500 μL), was added thionyl chloride (5 equiv., 0.55 mmol, 40 μL,), and the mixture was stirred at 0°C for 30 min. Water (500 μL) was added and the reaction mixture was evaporated in vacuo to give an oily product. Column chromatography (hexane-EtOAc, 8:2) yielded compound **79** (25.3 mg, 95%) as a clear oil.

79: Oil. IR (neat): ν_{max} = 1759, 1132, 837, 778 cm^{-1}. ^{1}H NMR (500 MHz, $CDCl_3$): δ = 5.83 (1H, bs), 4.82 (1H, bs), 3.94 (1H, dd, *J* =11.3, 3.7 Hz), 3.90 (1H, dd, *J* =11.3, 3.5 Hz), 2.10 (3H, s), 0,85 (9H, s), 0.06 (3H, s), 0.05 (3H, s) ppm. ^{13}C NMR (125 MHz, $CDCl_3$): δ = 173.2, 166.7, 118.1, 84.7, 61.7, 25.7, 18.1, 14.1, -5.5, -5.6 ppm. HRMS (ESI) *m/z* calcd for $C_{12}H_{22}NaO_3Si$ $[M+Na]^+$ 265.1236, found 265.1244.

Umbelactone 80: To lactone **79** (20.2 mg, 0.083 mmol), dissolved in dry THF (1 mL), $Et_3N{\cdot}3HF$ (20 eq., 270 μL) was added and the mixture stirred for 2.5 h at r.t. Et_3N (500 μL) was added and the mixture taken to dryness. The residue was co-evaporated with Et_3N (2x500 μL) and MeOH (3x 500 μL) and evaporated to give an oil which was purified by column chromatography (EtOAc) to yield umbelactone **80** (9.8 mg, 92%) as a clear oil.

80: Oil. IR (neat): ν_{max} = 3404, 1727, 1050 cm^{-1}. ^{1}H NMR (200 MHz, $CDCl_3$): δ = 5.89 (1H, bs), 4.91 (1H, bs), 4.07 (1H, dd, *J* = 2.9, 12.7 Hz), 3.77 (1H, dd, *J* = 12.7, 3.9 Hz), 2.11 (3H, s) ppm. ^{13}C

NMR (50 MHz, $CDCl_3$): δ = 172.8, 165.8, 118.3, 85.0, 61.5, 14.0 ppm. HRMS (ESI) *m/z* calcd for $C_6H_8NaO_3$ $[M+Na]^+$ 151.0371, found 151.0375.

2.5. References and notes

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Chapter 3

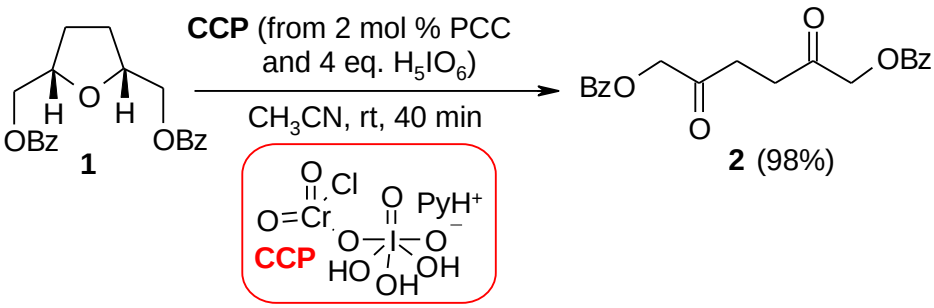
A general synthesis of bis-α-acyloxy 1,4- and 1,5-diketones through catalytic oxidative opening of acylated THF and THP

3.1. Introduction

α-Acyloxy ketones are important intermediates in organic synthesis, and their structural motif is found in a variety of natural products (e.g. taxol) and pharmaceutical agents. They can be seen as protected α-hydroxy ketones, themselves structural subunits of several active natural substances and useful synthetic building blocks.[1]

The keen interest of synthetic organic chemists in α-acyloxy ketones is demonstrated by the number of procedures that have been developed to obtain these substances.[2] Generally, their synthesis features the introduction of the acyloxy group α to the ketone functional group. Recently, various asymmetric α-oxybenzoylation methods have been reported.[3] A thallium promoted preparation of α-formyloxy ketones is also known.[2f] However, despite the potential uses of the products in organic synthesis, to the best of our knowledge, no general method for the synthesis of bis-α-acyloxy diketones has been developed to date, and the chemistry of these compounds and that of the corresponding free bis-ketols is still largely unexplored.[4]

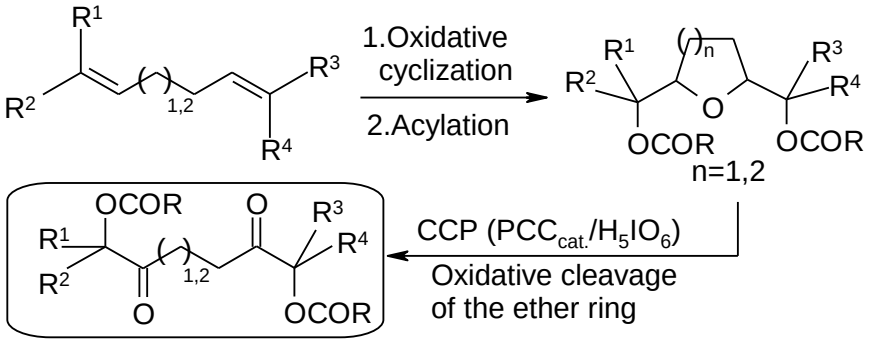
Recent work in our laboratory has focused on the catalytic use of chlorochromatoperiodate (CCP for short, Scheme 1), generated by the condensation of PCC and periodic acid,[5] as a powerful reagent capable of oxidising THF-containing compounds of various structural complexity[6] (for a deeper insight see sections 1.3, 2.2.6 and 2.2.7). In a single case, we observed that the oxidative opening of a 2,5-bis-benzoyloxymethyl THF compound (**1**, Scheme 1) led to a bis-α-benzoyloxy 1,4-diketone **2** in an excellent yield.



Scheme 1. Oxidative THF opening under previous conditions.[6]

This result was particularly interesting, since it suggested that the THF ring and possibly other ether rings, flanked by acylated hydroxymethyl groups, could be seen as masked bis-α-acyloxy diketones. With the above result in mind, we envisaged that 1,5- and 1,6-dienes could be transformed into bis-α-acyloxy 1,4- and 1,5-diketones through a short sequence consisting of transition metal-mediated oxidative cyclization of the diene[7] to give THF or THP diols, followed by hydroxyl acylation and CCP-catalysed opening of the ether ring (Scheme 2).

Since formation of the two ketone functionalities occurs at the ether carbons, the projected sequence should allow the bis-ketoacyloxylation of the starting diene to occur regioselectively, which is hardly achievable in a direct way. In a previous investigation by Smith III and Scarborough,[8] the system RuO_2 (cat.)/$NaIO_4$ was used to accomplish the transformation of unfunctionalised THF or THP ring, mostly to give lactones. In a single case, a 2,5-disubstituted THF was converted into a 1,4-diketone, but the process required long reaction times (typically 24) and there were no chiral centres adjacent to the reaction centre. In this chapter we report the accomplishment of the above idea and the use of some of the synthesized 1,5-diketones to obtain six-membered nitrogen heterocycles.

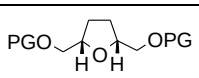
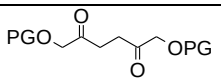
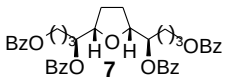
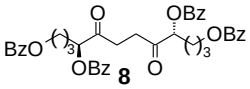
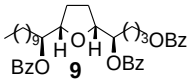
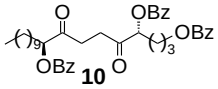
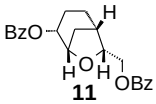
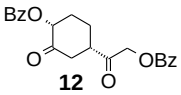
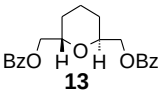
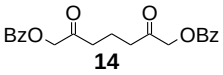
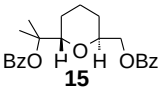
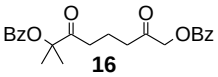
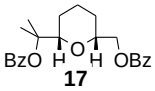
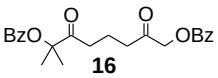
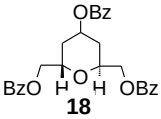
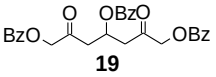
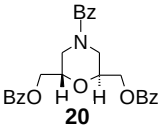
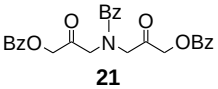


Scheme 2. Projected general synthesis of bis-α-acyloxy diketones from dienes.

3.2. Results and Discussion

To test the substrate scope of the projected transformation, a range of tetrahydrofuran and tetrahydropyran diols were synthesised mostly through well-established ruthenium[9] or osmium[10]-catalysed oxidative cyclization of suitable 1,5- and 1,6-dienes. The diols were then acylated (in one case tosylated) and subjected to our oxidative procedure (Table 1).

Table 1. CCP-catalyzed oxidative opening of protected 2,5-disubstituted THF and 2,6-disubstituted THP diols.[a]

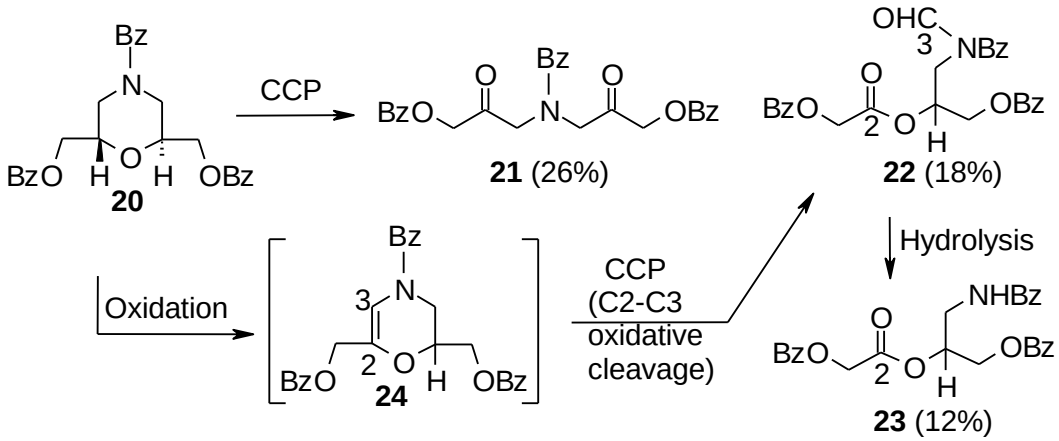
Entry	Substrate	Product	Yield (%)[c]
1[a]			
	1 (PG=Bz)	**2**	97
2	**3** (PG=Ac)	**4**	80
3[b]	**5** (PG=Ts)	**6**	85
4	 **7**	 **8**	74
5	 **9**	 **10**	61
6	 **11**	 **12**	75
7	 **13**	 **14**	85
8	 **15**	 **16**	95
9	 **17**	 **16**	83
10	 **18**	 **19**	78
11	 **20**	 **21**	26[d]

[a] Reagents and conditions. THF oxidations: 0.05 M substrate in CH_3CN, 2 mol % PCC, 3.2 eq. H_5IO_6, 30-40 min, rt. THP oxidations: 0.05 M substrate in CH_3CN, 4 mol % PCC, 4 eq. H_5IO_6, 6-8 h, rt. [b] This process required 24 h. [c] Isolated yields. [d] Isolated HPLC yield.

Optimization of the oxidative protocol was carried out with THF **1** and THP **13** as model compounds. In particular, we found that the oxidation of **1** (entry 1, Table 1) proceeded in excellent

yields and short reaction times with 2 mol % of PCC, even reducing the amount of the co-oxidant to 3 equiv. However, the opening of the THP ring of **13** proceeded very slowly or the reaction was incomplete under these conditions. A screening of the reaction conditions for this substrate showed that the best results were obtained with 4 mol % of catalyst and 4 equiv. of periodic acid.

Oxidation of all the other substrates was then carried out under optimized conditions. As summarised in Table 1 both THF and THP-derivatives reacted well to give the expected diketones in generally good to excellent yields.[11] The process was shown to be tolerant of various substitution patterns and of structural complexity in the substrate. Even bicyclic THF **11** (entry 6) gave a good yield of the corresponding diketone **12**. Isomeric *trans* and *cis*[9d] THPs **15** and **17**, respectively, gave the same product **16** in comparable yields. In an attempt to further expand the utility of this reaction, morpholine derivative **20** (entry 11, Scheme 3) was oxidised. However, a more complex reactivity pattern resulted in this case. The expected diketone **21** was obtained, albeit in a diminished 26% yield (HPLC)[12] along with a non-negligible amount of by-products **22** (18%) and **23** (12%). We believe that the Achilles' heel of this substrate is the double bond character of the benzamide group, which favours the formation of the unsaturated morpholine intermediate **24**, which is then oxidatively cleaved by CCP itself (Scheme 3).[13] Presumably that higher yields of **21** could be obtained by tuning the nitrogen-protecting group in **20**. As can be seen, benzoate (entry 1), acetate, tosylate (entries 2 and 3) and amide functions (entry 11) were compatible with the ether-cleavage reaction conditions.



Scheme 3. Oxidation of a morpholine-protected substrate. Reaction conditions as in Table 1

An important aspect of the above process that must be addressed concernes the fate of the potentially epimerizable chiral centers adjacent to the newly-formed ketone functionalities in compounds such as **8**, **10** and **12**. Considering the oxidation of **11**, NMR spectroscopic analysis of the by-products of the process ruled out the presence of stereoisomers of diketone **12**. In addition,

an X-ray diffraction analysis of this compound (Figure 1)[14] was carried out, and the results showed that the relative stereochemistry of the THF precursor **11** had been preserved in **12**. This suggests that the synthesis of chiral, acyclic bis-α-acyloxy 1,4-diketones such as **8** and **10** is feasible, given the easy access to chiral THF-dimethanol substances such as **7** or **9** according to the OsO_4-catalyzed protocol developed by Donohoe and Butterworth.[15]

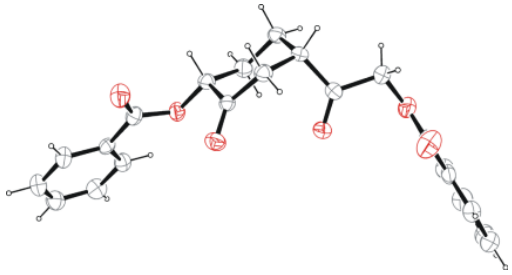
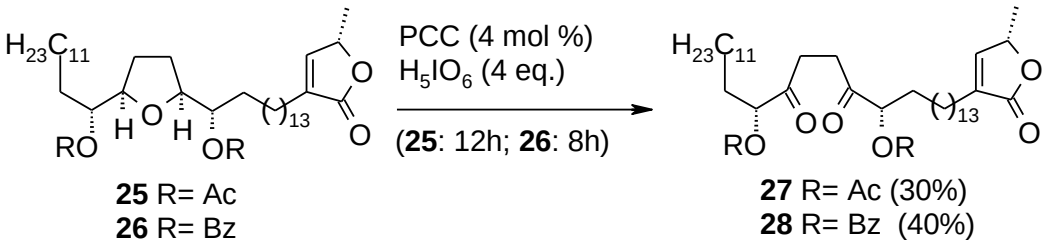


Figure 1. ORTEP drawing of diketone 12. Ellipsoids are drawn at 30% probability level.

To test our procedure on a more elaborated substrate, acetylated and benzoylated *cis*-reticulatacins **25** and **26**, respectively were oxidised (Scheme 4).[16,17] *cis*-Reticulatacin belongs to the *Annonaceous* acetogenins class, a group of plant-derived metabolites having a wide array of biological properties such as antitumor, immunosuppressive, antimicrobial and insecticidal activities.[18] Several syntheses of some members of this group as well as preparation of analogues have been reported.[19] We envisaged that our process could be used to cleave the THF ring in this compound to obtain non-THF analogues such as recently synthesized dihydro-coibin[20] or other derivatives thereof.

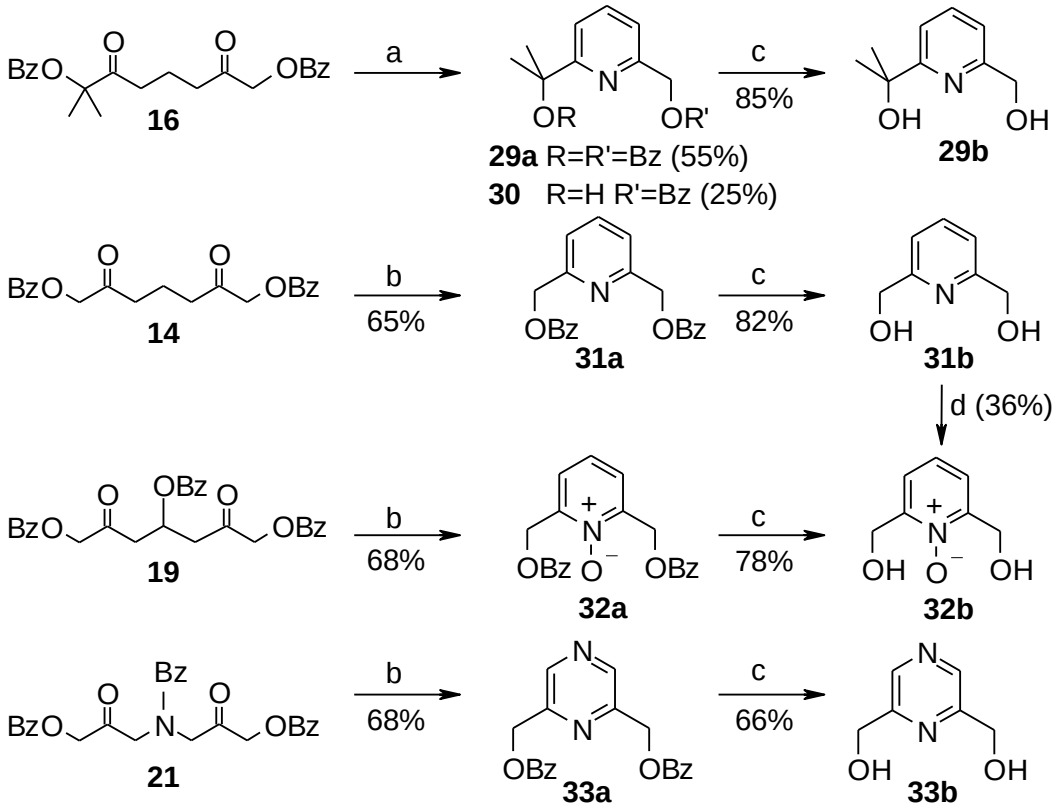


Scheme 4. Oxidation of bis-acylated *cis*-reticulatacin.

Indeed, reaction of **25** and **26** under slightly modified conditions gave the expected reticulatacin-derived diketones **27** and **28**, respectively, (Scheme 4) though in lower yields (30-40%). Although the structures of the by-products (HPLC separation) of this process were not thoroughly investigated, ^{1}H NMR spectroscopic data showed that they all incorporated the terminal unsaturated lactone functionality (characteristic proton signals at ca. 5 and 7 ppm) which indicates that even this

moiety is tolerant to the oxidising conditions. This transformation also highlights that the method may be useful to prepare analogues of a variety of biologically active natural substances, where saturated ether rings of various sizes are often flanked by hydroxyl groups.

It is well known[1b,2] that the α-oxygenated carbonyl groups are susceptible of several transformations. The presence in the same molecule of two of these structural subunits further increases the synthetic utility of these compounds. Among the various conceivable synthetic uses of the synthesized substances, we planned to probe the transformation of the 1,5-dicarbonyl compounds into the corresponding pyridinedimethanol derivatives[21,22] (Scheme 5). These substances have notable coordination properties, and belong to the oxido pincer ligands class.[23]



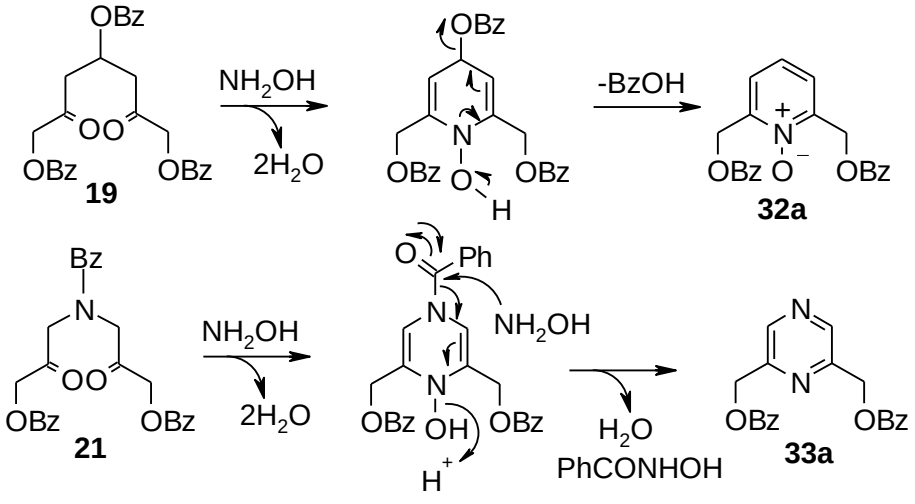
Scheme 5. Pyridine and pyrazine diol synthesis from bis α-benzoyloxy-1,5-diketones. a) NH_4OAc (7 eq.), AcOH (6.5 eq.), dry MeOH, 24 h rt and 48 h 55 °C; b) $NH_2OH{\cdot}HCl$ (3.5 eq.), dry EtOH, 6 h-15 h, reflux; c) 10 mol % K_2CO_3, MeOH, rt, 30 min; d) MCPBA (1.2 eq.), CHCl3, rt, 90 min.

Reaction of **16** with ammonium acetate and AcOH gave, in a reproducible manner, the expected pyridine **29a** together with the corresponding debenzoylated compound **30** in a ca 2:1 ratio. Usually, it is believed that under these conditions, 1,4-dihydropyridine intermediates disproportionate to give the pyridine compounds and products with higher hydrogen content such as piperidines or tetra-hydropyridines. However, our pr ocess was very clean, and no by-products of this type could be

detected. Thus, in agreement with Rodriguez et al.,[24] we presume that atmospheric oxygen could be responsible for the oxidation step leading to aromatisation.

Compound **14** failed to cyclize under these conditions. In an alternative approach, good yields of pyridine **31a** were obtained by reaction of **14** with hydroxylamine hydrochloride under classical Knoevenagel conditions. Analogously, reaction of diketone **19** under the conditions employed to cyclize **14** led to pyridine oxide **32a**. Even diketo-benzamide **21** could be cyclized to give 2,6-bis(hydroxymethyl)pyrazine **33a** in good yields and in a short time when compared with pyridine cyclizations. Formation of the pyrazine ring through cyclization of a diketoamide such as **21** has never been accomplished before and it is likely that variants of this process will be feasible. Debenzoylation of the mixture of **29a**/**30**,[25] and of compounds **31a**-**33a** was then carried out with K_2CO_3 (cat.) in MeOH to give the corresponding diols. Compounds **29b** and **31b** are known. The structural relationship between **31b** and **32b** was proven by MCPBA (*m*-chloroperbenoic acid) oxidation of the former to the latter.

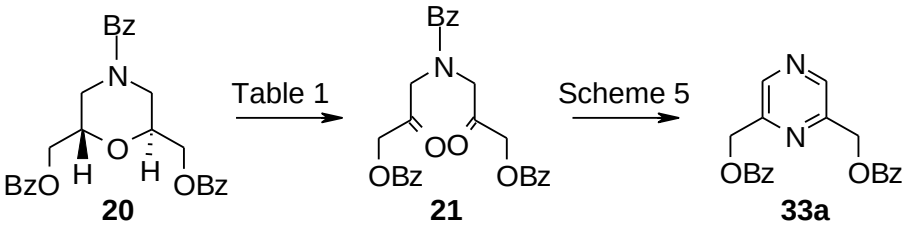
The cyclizations of **19** and **21** are particularly interesting and deserve a comment. As for transformation of **19** to **32a** (Scheme 6) the benzoate group on C-4 acts as a leaving group and, after formation of the intermediate N-hydroxydihydropyridine, aromatization occurs by elimination of benzoic acid. This is the first example of the synthesis of the pyridine nucleus using a 3-benzoyloxy-1,5-diketone. An interesting new feature of this reaction is the direct formation of a pyridine oxide.



Scheme 6. Plausible pathways leading to pyridine oxide **32a** and pyrazine **33a**.

A pathway leading to pyrazine **33a** is shown in Scheme 6. In this case, the initial closure of the *N*-hydroxy-dihydropyrazine ring is followed by the elimination of the benzoyl portion, likely through a transamidation step driven by aromatization. Overall, the oxidative opening of the

morpholine ring in **20** to give diketone **21** (Scheme 7), followed by cyclization of the latter compound to pyrazine **33a**, namely the transformation of a morpholine derivative into a pyrazine one, may have synthetic and pharmacological value.[26]



Scheme 7. Overall transformation of morpholine **20** into pyrazine **33a**.

3.3. Conclusions

In conclusion, we have developed the first general procedure to synthesize bis-α-acyloxy 1,4- and 1,5-diketones from 1,5- and 1,6-dienes, respectively, in a regioselective manner. An interesting feature of our procedure is the timing of the introduction of the acyloxy and ketone functionalities onto the diene, that allows for a variety of acyloxy moieties to be incorporated. In addition, it is reasonable that also THF or THP substrates that lack one or both of the neighbouring acyloxy groups could be cleaved using our procedure, which would give a wider spectrum of 1,4- and 1,5-diketones. We have demonstrated that the process can be applied to acylated *cis*-reticulatacin, a representative mono-THF *Annonaceous* acetogenin, thus opening the way to the preparation of new non-THF analogues of these active substances to be used in biological assays. The 1,5-diketone products have been successfully cyclized to pyridine dimethanol derivatives, opening a new route to pyridine-based oxido pincer ligands, and, in one case, to a pyrazinedimethanol derivative. The direct conversion of a 3-benzoyloxy-1,5-diketone into a pyridine N-oxide is also unprecedented. The synthetic utility of the bis-α-acyloxy diketone products is currently under investigation.

3.4. Experimental Section

3.4.1 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. Na_2SO_4 was used as a drying agent for aqueous work-up. 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 700, Varian Unity Inova 500, Varian Mercury Plus 400 and Gemini 200 spectrometers in $CDCl_3$ or CD_3OD. Proton chemical shifts were referenced to the residual $CHCl_3$ (7.26 ppm) or CD_2HOD (3.31 ppm) signals. ^{13}C-NMR chemical shifts were referenced to the solvents ($CDCl_3$ 77.0; CD_2HOD 49.0 ppm). Coupling constants, *J*, values are given in Hz. Abbreviations for signal coupling are as follows: s=singlet, d=doublet, dd=double doublet, ddd=double double doublet, t=triplet, dt=double triplet, q=quartet, quin=quintet, m=multiplet, app=apparent, br=broad. Optical rotations were recorded on a Jasco P-1010 polarimeter (using the sodium D line, 589 nm) and $[\alpha]_D$ are given in units of $degcm^3g^{-1}dm^{-1}$. IR spectra were collected on a Jasco FT-IR-430 spectrometer. Melting points were obtained with a Gallenkamp melting point apparatus. High Resolution MS were recorded on a Bruker APEX II FT-ICR mass spectrometer using electron spray ionization (ESI) technique in positive mode.

Crystals of **12** were grown by slow evaporation from a $CHCl_3$-toluene (1:1) solution. A prismatic colourless crystal of **12** was 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 at room temperature. Owing to the poorly diffracting features of the crystal, data were collected up to θ_{max} = 25 °. Unit cell parameters were determined by least squares refinement of the θ angles of 35 strong reflections in the range 3.785° < θ < 11.308°. Data reduction and semiempirical absorption correction were done using SADABS program.[27] The structure was solved by direct methods (SIR97 program[28]) and refined by the full matrix least-squares method on F^2 using SHELXL-97 program. [29] All non-hydrogen atoms were refined anisotropically. H atoms were determined stereochemically and refined by the riding model with $U_{iso} = 1.2 \cdot U_{eq}$ of the carrier atom.

3.4.2 Synthesis of substrates

The substrates **1**,[9c] **3**,[9c] **5**,[9c] **7**,[30] **9**,[31] **11**,[32] **13**,[9d] **15**,[9d] **17**,[9d] and **20**[9e] were obtained by acetylation, benzoylation and tosylation of the corresponding THF, THP or morpholine polyols under standard conditions. The latter were synthesized according to literature procedures. **1**[6] is a known compound. Compound **18** was synthesized from hepta-1,6-dien-4-yl benzoate according to a literature procedure[9e] and belzoylated under standard conditions.

(±)-((2*S*,5*R*)-tetrahydrofuran-2,5-diyl)bis(methylene) diacetate (3): Oil; IR: ν_{max}= 1742s, 1372w, 1235br s, 1041m cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ= 4.30 – 4.06 (m, 4H), 3.97 (dd, *J* = 12.3, 7.2 Hz, 2H), 2.07 (s, 6H), 2.16 – 1.89 (m, 2H), 1.83 – 1.55 (m, 2H); ^{13}C NMR (50 MHz, $CDCl_3$) δ= 170.5, 77.0, 66.1, 27.3, 20.5; HRMS (ESI) *m/z* $C_{10}H_{16}NaO_5$ requires $[M+Na]^+$ 239.0895, found 239.0884.

(±)-((2*S*,5*R*)-tetrahydrofuran-2,5-diyl)bis(methylene) bis(4-methylbenzenesulfonate) (5): Oil; IR: ν_{max}= 2957m, 2922m, 2855w, 1597w, 1356m, 1260w, 1189m, 1174s, 1094m, 969m, 812m, 665m cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$): δ= 7.77 (d, *J* = 8.1 Hz, 4H), 7.34 (d, *J* = 8.1 Hz, 4H), 4.17 – 4.02 (m, 2H), 3.93 (m, 4H), 2.45 (s, 6H), 2.03 – 1.85 (m, 2H), 1.84 – 1.64 (m, 2H); ^{13}C NMR (50 MHz, $CDCl_3$): δ=144.9, 132.8, 129.9, 127.9, 77.2, 71.1, 27.5, 21.6; HRMS (ESI) *m/z* $C_{20}H_{24}NaO_7S_2$ requires $[M+Na]^+$ 463.0861, found 463.0859.

(±)-(4*S*)-4-(benzoyloxy)-4-[(2*S*,5*R*)-5-[(1*R*)-1,4-bis(benzoyloxy)butyl]oxolan-2-yl]butyl benzoate (7): Oil; IR: ν_{max}= 2956br w, 2877br w, 1716 br s, 1601w, 1584w, 1451w, 1314w, 1272br s, 1176w, 1112m, 1069w, 1025w, 710m cm^{-1}; ^1H NMR (200MHz, $CDCl_3$): δ= 8.09 (d, *J* = 7.6, 4H), 8.01 (d, *J* = 7.0, 4H), 7.60 – 7.27 (m, 12H), 5.49 – 5.24 (m, 2H), 4.47 – 4.24 (br s, 4H), 4.24 – 4.04 (m, 2H), 2.11 – 1.67 (m, 12H); ^{13}C NMR (50 MHz, $CDCl_3$): δ= 166.3, 166.1, 132.8, 132.7, 130.1, 130.0, 129.5, 129.4, 128.2, 128.1, 79.8, 75.0, 64.4, 27.7, 27.4, 24.7; HRMS (ESI) *m/z* $C_{40}H_{40}NaO_9$ requires $[M+Na]^+$ 687.2570, found 687.2566.

(±)-(*R*)-1-((2*R*,5*S*)-5-((*S*)-1-(benzoyloxy)undecyl)tetrahydrofuran-2-yl)butane-1,4-diyl dibenzoate (9): Oil; IR: ν_{max}= 2924m, 2852w, 1718s, 1271s, 1112m, 1069w, 1026w, 710m cm^{-1}; ^1H NMR: (200MHz, $CDCl_3$): δ= 8.18 – 7.91 (m, 6H), 7.60 – 7.31 (m, 9H), 5.42 – 5.14 (m, 2H), 4.30 (t, *J* = 5.6 Hz, 2H), 4.21 – 4.06 (m, 2H), 2.07 – 1.51 (m, 12H), 1.46 – 1.05 (m, 10H), 0.84 (t, *J* = 6.8 Hz, 3H), ^{13}C NMR (50 MHz, $CDCl_3$): δ= 166.5, 166.3, 132.9, 132.81, 132.80, 130.4, 130.2,

130.1, 129.8, 129.7, 129.5, 128.35, 128.30, 80.1, 79.8, 75.9, 75.2, 64.6, 31.9, 30.9, 29.55, 29.53, 29.50, 27.8, 27.5, 25.4, 24.9, 22.6, 14.1; HRMS (ESI) *m/z* $C_{40}H_{50}NaO_7$ requires $[M+Na]^+$ 665,3454, found 665.3458.

(±)-[(1*R*,4*R*,5*S*,7*R*)-4-(benzoyloxy)-6-oxabicyclo[3.2.1]octan-7-yl]methyl benzoate (11): Oil; IR: ν_{max}= 2953br w, 2874br w, 1716s, 1450w, 1335w, 1313w, 1271s, 1176w, 1114w, 1098w, 1069w, 1026w, 941w, 711m cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$): δ= 8.13 – 8.02 (d, *J* = 7.3 Hz, 2H), 7.63 – 7.35 (m, 6H), 4.94 (dd, *J* = 9.2, 6.7 Hz, 1H), 4.75 – 4.51 (m, 3H), 4.40 (br dd, *J* = 9.6, 6.1 Hz, 1H), 2.40 (br s, 1H), 2.34 – 1.84 (m, 4H), 1.78 (d, *J* = 11.8 Hz, 1H), 1.73 – 1.53 (m, 1H); ^{13}C NMR (50 MHz, $CDCl_3$): δ= 166.5, 166.2, 133.0, 132.9, 130.3, 130.1, 129.9, 129.8, 128.3, 128.2, 80.3, 78.0, 75.0, 63.8, 37.1, 35.8, 25.3, 24.0; HRMS (ESI) *m/z* $C_{22}H_{22}NaO_5$requires $[M+Na]^+$ 389.1365, found 389.1362.

(±)-((2*S*,6*S*)-tetrahydro-2H-pyran-2,6-diyl)bis(methylene) dibenzoate (13): Oil; IR: ν_{max}= 2940br w, 1717s, 1601w, 1584w, 1451w, 1315w, 1275s, 1118br m, 1070w, 1026w, 710s cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$): δ= 7.98 (d, *J* = 7.1 Hz, 4H), 7.52 (t, *J* = 7.4 Hz, 2H), 7.33 (t, *J* = 7.6 Hz, 4H), 4.58 (dd, *J* = 12.5, 9.0 Hz, 2H), 4.35 – 4.18 (m, 4H), 1.96 – 1.39 (m, 6H); ^{13}C NMR (50 MHz, $CDCl_3$): δ= 166.5, 132.8, 130.0, 129.6, 128.3, 69.6, 65.2, 26.3, 18.5; HRMS (ESI) *m/z* $C_{21}H_{22}NaO_5$ requires $[M+Na]^+$ 377.1365, found 377.1368.

(±)-2-[(2*S*,6*S*)-6-[(benzoyloxy)methyl]oxan-2-yl]propan-2-yl benzoate (15): Oil; IR: ν_{max}= 2867w, 2940br w, 1720s, 1602w, 1583w, 1451m, 1386w, 1366w,1314m, 1275br s, 1113br m, 1070w, 1026w, 710s cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$): δ= 8.00 (d, *J* = 8.1 Hz, 2H), 7.95 (d, *J* = 8.1 Hz, 2H), 7.57 – 7.46 (m, 2H), 7.35 (td, *J* = 7.9, 3.3 Hz, 4H), 4.81 (dd, *J* = 10.4, 7.8 Hz, 1H), 4.45 – 4.30 (m, 2H), 4.45 – 4.24 (m, 2H), 4.07 (d, *J* = 10.8 Hz, 1H), 1.97 – 1.36 (m, partly overlapped to methyl signals, 6H), 1.59 (s, 3H), 1.57 (s, 3H); ^{13}C NMR (50 MHz, $CDCl_3$): δ=166.4, 165.5, 132.9, 132.4, 131.8, 130.1, 129.6, 129.4, 128.3, 128.1, 84.0, 74.5, 71.0, 62.9, 25.5, 25.0, 22.9, 21.5, 19.0; HRMS (ESI) *m/z* $C_{23}H_{26}NaO_5$ requires $[M+Na]^+$ 405.1678, found 405.1676.

(±)-2-[(2*S*,6*R*)-6-[(benzoyloxy)methyl]oxan-2-yl]propan-2-yl benzoate (17): Oil; IR: ν_{max}= 2983w, 2942br w, 2858w, 1716br s, 1602w, 1583w, 1451m, 1384w, 1367w, 1314m, 1290s, 1274s, 1118m, 1069w, 1027w, 711s cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$): δ= 7.98 (m, 4H), 7.58 – 7.45 (m, 2H), 7.37 (t, *J* = 7.6 Hz, 4H), 4.30 (app d, *J* = 5.3 Hz, 2H), 3.91 – 3.71 (m, 2H), 2.08 – 1.91 (m, 1H), 1.62 (s, 3H), 1.57 (s, 3H), 1.80 – 1.21 (m, partly overlapped to methyl signals, 6H); ^{13}C NMR

(50 MHz, $CDCl_3$): δ= 166.4, 165.5, 132.8, 132.4, 131.8, 130.2, 129.5, 129.4, 128.2, 128.1, 83.8, 81.4, 75.9, 67.6, 27.7, 24.9, 23.0, 21.6; HRMS (ESI) *m/z* $C_{23}H_{26}NaO_5$ requires $[M+Na]^+$ 405.1678, found 405.1677.

(±)-((2*S*,6*S*)-4-(benzoyloxy)-tetrahydro-2H-pyran-2,6-diyl)bis(methylene) dibenzoate (19): Oil; IR: ν_{max}=2857br w, 1720s, 1601w, 1583w, 1451m, 1315m, 1272br s, 1176w, 1110br m, 1026m, 710m cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$): δ= 8.13 – 7.94 (m, 6H), 7.64 – 7.41 (m, 3H), 7.34 (t, *J* = 7.6 Hz, 6H), 5.57 – 5.40 (m, 1H), 4.84 (dd, *J* = 11.5, 8.0 Hz, 1H), 4.74 – 4.49 (m, 2H), 4.50 – 4.21 (m, 3H), 2.27 (dt, *J* = 13.7, 4.3 Hz, 1H), 2.05 (t, *J* = 5.5 Hz, 2H), 1.85 (dt, *J* = 13.8, 7.0 Hz, 1H); ^{13}C NMR (50 MHz, $CDCl_3$): δ= 166.3, 165.7, 133.2, 132.9, 129.9, 129.85, 129.80, 129.6, 129.5, 128.5, 128.3, 68.9, 67.6, 67.1, 65.3, 65.1, 31.6, 31.4; HRMS (ESI) *m/z* $C_{28}H_{26}NaO_7$ requires $[M+Na]^+$ 497.1576, found 497.1573.

(±)-((2*S*,6*S*)-4-benzoylmorpholine-2,6-diyl)bis(methylene) dibenzoate (20): Oil; IR: ν_{max}= 2955br w, 1721s, 1639br s, 1602w, 1451m, 1273br s, 1114m, 1070w, 1027w, 711s cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ= 8.14 – 7.73 (br m, 6H), 7.53 (t, *J* = 7.3 Hz, 3H), 7.48 – 7.27 (m, 6H), 4.76 – 4.43 (br m, 2H), 4.43 – 4.17 (br m, 4H), 4.17 – 3.75 (br m, 2H), 3.75 – 3.38 (br m, 2H); ^{13}C NMR (50 MHz, $CDCl_3$) δ= 171.1, 166.0, 134.7, 133.1, 130.0, 129.6, 129.5, 128.6, 128.3, 127.0, 68.8, 62.8, 47.8, (br) 43.4 (br); HRMS (ESI) *m/z* $C_{27}H_{25}NNaO_6$ requires $[M+Na]^+$ 482.1580, found 482.1583.

(1*R*)-1-[(2*R*,5*S*)-5-[(1*S*)-1-(acetyloxy)-15-[(5*S*)-5-methyl-2-oxo-2,5-dihydrofuran-3-yl]pentadecyl]oxolan-2-yl]tridecyl acetate (25): Oil; IR: ν_{max}= 2922br s, 2852m, 1735br s, 1459w, 1370w, 1318w, 1237s, 1073w, 1025m, 949w cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$): δ= 6.98 (br q, *J* = 1.3 Hz, 1H), 4.99 (br q, *J* = 6.7 Hz, 1H), 4.94 – 4.81 (m, 3H), 4.01 – 3.89 (m, 2H), 2.26 (t, *J* = 7.6 Hz, 2H), 2.07 (s, 6H), 2.01 – 1.16 (m, 48H), 1.40 (d, *J* = 6.8 Hz, 3H), 0.87 (t, *J* = 6.1 Hz, 5H); ^{13}C NMR (50 MHz, $CDCl_3$): δ= 173.8, 170.7, 148.8, 134.3, 79.8, 77.4, 75.4, 31.9, 30.8, 29.6, 29.6, 29.3, 29.3, 29.2, 27.8, 27.4, 25.3, 25.2, 22.7, 21.1, 19.2, 14.1; HRMS (ESI): *m/z* $C_{41}H_{72}NaO_7$ requires $[M+Na]^+$ 699.5176, found 699.5180.

(1*R*)-1-[(2*R*,5*S*)-5-[(1*S*)-1-(benzoyloxy)-15-[(5*S*)-5-methyl-2-oxo-2,5-dihydrofuran-3-yl]pentadecyl]oxolan-2-yl]tridecyl benzoate (26): Oil; IR: ν_{max}= 2917m, 2850w, 1716s, 1599w, 1585w, 1451m, 1361w, 1311w, 1272s, 1248m, 1121m, 1023w, 771w, 714m cm^{-1}; ^1H NMR (200

MHz, $CDCl_3$) δ= 8.07 (d, *J* = 7.3 Hz, 4H), 7.53 (t, *J* = 7.4 Hz, 2H), 7.38 (t, *J* = 7.4 Hz, 4H), 6.98 (s, 1H), 5.23 (app q, *J* = 5.5 Hz, 2H), 4.99 (br q, *J* = 7.3 Hz, 1H), 4.17 – 4.03 (m, 2H), 2.26 (t, *J* = 7.9 Hz, 2H), 2.05 – 1.09 (m, 48H), 1.40 (d, *J* = 6.8 Hz, 3H), 0.87 (t, *J* = 6.0 Hz, 3H); ^{13}C NMR (50 MHz, $CDCl_3$) δ= 174.5, 166.3, 166.2, 148.8, 134.3, 132.7, 130.5, 129.7, 128.3, 79.9, 77.4, 76.0, 31.9, 30.8, 29.63, 29.56, 29.50, 29.3, 29.2, 27.9, 27.4, 25.5, 25.2, 22.7, 19.2, 14.1; HRMS (ESI): *m/z* $C_{51}H_{76}NaO_7$ requires $[M+Na]^+$ 823.5489, found 823.5491.

3.4.3 Oxidative cleavage of substrates

Procedure A for the oxidative cleavage of tetrahydrofuran substrates

To a suspension of H_5IO_6 (3.2 eq.) in CH_3CN at rt was added PCC (2 mol % from a 0.01 M stock solution in CH_3CN) under vigorous stirring. After 5 min the THF compound (1 eq.) dissolved in CH_3CN was added. The overall volume of CH_3CN was such that the final concentration of the solution was 0.05 M. After complete consumption of the starting material (TLC control, typically 30-40 min), EtOH (excess) was added and stirring continued until the colour of the solution turned from yellow to green (ca. 5 min). Then silica (excess) was added and the solvent evaporated under reduced pressure. The resulting powder was loaded on the top of a silica gel column. Flash chromatography eluting with $CHCl_3$/MeOH (100:0 to 9:1) gave the desired product.

Procedure B for the oxidative cleavage of tetrahydropyran substrates

To a suspension of H_5IO_6 (4.0 eq.) in CH_3CN at rt was added PCC (4 mol % from a 0.01 M stock solution in CH_3CN) under vigorous stirring. After 5 min the THP compound (1 eq.) dissolved in CH_3CN was added. The overall volume of CH_3CN was such that the final concentration of the solution was 0.05 M. After complete consumption of the starting material (TLC control, typically 6-8 h), EtOH (excess) was added and stirring continued until the colour of the solution turned from yellow to green (ca. 5 min). Then silica (excess) was added and the solvent evaporated under reduced pressure. The resulting powder was loaded on the top of a silica gel column. Flash chromatography eluting with chloroform/methanol (100:0 to 9:1) gave the desired product.

2,5-dioxohexane-1,6-diyl dibenzoate (2): Benzoate-protected tetrahydrofuran **1** (22.0 mg, 0.065 mmol) was subjected to General Procedure A to furnish the title compound **2** (22.3 mg, 0.063 mmol, 97 %) as a colorless solid. m.p. 138-140 °C; IR (thin film): ν_{max} = 2949w, 1724s, 1600w,

1451w, 1408m, 1271s, 1128w, 1101w, 1063w, 1028w, 988w, 702m; ^{1}H NMR and ^{13}C NMR see ref. 6.

2,5-dioxohexane-1,6-diyl diacetate (4): Acetate-protected tetrahydrofuran **3** (24.5 mg, 0.113 mmol) was subjected to General Procedure A to furnish the title compound **4** (20.8 mg, 0.090 mmol, 80%) as a colorless solid. m.p. 86-88 °C; IR (thin film): ν_{max}= 3004w, 2934w, 1763s, 1722s, 1415s, 1367m, 1304w, 1281m, 1232br s, 1102w, 1038br m, 1034w, 960w, 841w, 755br w, 713w, 682w, 619w cm^{-1}; ^{1}H NMR (200 MHz, $CDCl_3$): δ= 4.69 (s, 4H), 2.74 (s, 4H), 2.15 (s, 6H); ^{13}C NMR (50 MHz, CDCl3): δ= 202, 170.2, 67.9, 31.9, 20.4; HRMS (ESI) $C_{10}H_{14}NaO_6$ requires $[M+Na]^+$ 253.0688, found 253.0691.

2,5-dioxohexane-1,6-diyl bis(4-methylbenzenesulfonate) (6): Tosylate-protected tetrahydrofuran **5** (15.7 mg, 0.036 mmol) was subjected to General Procedure A to furnish the title compound **6** (13.8 mg, 0.030 mmol, 85 %) as an oil. IR (thin film): ν_{max}= 2922br w, 2852w, 1732m, 1597w, 1359m, 1189m, 1176s, 1091w, 1003br m, 814m, 667m cm^{-1}; ^{1}H NMR (200 MHz, $CDCl_3$): δ= 7.81 (d, J = 8.3 Hz, 4H), 7.37 (d, J = 8.1 Hz, 4H), 4.52 (s, 4H), 2.80 (s, 4H), 2.46 (s, 6H); ^{13}C NMR (50 MHz, $CDCl_3$): δ= 201.7, 145.6, 132.1, 130.1, 128.1, 71.7, 32.2, 21.7; HRMS (ESI) $C_{20}H_{22}NaO_8S_2$ requires $[M+Na]^+$ 477.0654, found 477.0658.

(±)-(4*R*,9*S*)-5,8-dioxododecane-1,4,9,12-tetrayl tetrabenzoate (8): Benzoate-protected tetrahydrofuran **7** (122.0 mg, 0.184 mmol) was subjected to General Procedure A to furnish the title compound **8** (92.3 mg, 0.136 mmol, 74 %) as a white solid. m.p. 123-125 °C; IR (thin film): ν_{max}= 2960br w, 2917w, 1716br s, 1600w, 1584w, 1451w, 1315w, 1272br s, 1112br m, 1070w, 1026w, 710m cm^{-1}; ^{1}H NMR (200 MHz, $CDCl_3$) δ= 8.06 (t, J = 8.3 Hz, 8H), 7.82 – 7.26 (m, 12H), 5.36 (t, J = 5.9 Hz, 2H), 4.39 (t, J = 5.8 Hz, 4H), 3.15 – 2.62 (m, 4H), 2.33 – 2.07 (m, 2H), 2.07 – 1.87 (m, 2H); ^{13}C NMR (50 MHz, $CDCl_3$): δ= 205.7, 166.5, 166.0, 133.5, 132.8, 130.1, 129.8, 129.5, 129.1, 128.5, 128.3, 78.3, 64.1, 31.8, 27.4, 24.5; HRMS (ESI) $C_{40}H_{38}NaO_{10}$ requires $[M+Na]^+$ 701.2363, found 701.2367.

(±)-(4*R*,9*S*)-5,8-dioxononadecane-1,4,9-triyl tribenzoate (10): Benzoate-protected tetrahydrofuran **9** (11.6 mg, 0.018 mmol) was subjected to General Procedure A to furnish the title compound **10** (7.2 mg, 0.011 mmol, 61 %) as an oil. IR (thin film): ν_{max}= 2923br m, 2853w, 1717br s, 1602w, 1451w, 1315w, 1271br s, 1176w, 1112m, 1070w, 1026w, 711m cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$) δ= 8.08 (d, J = 7.6 Hz, 4H), 8.03 (d, J = 7.7 Hz, 2H), 7.59 (dd, J = 7.9, 5.9 Hz, 2H), 7.54 (t, J = 7.4 Hz, 1H), 7.46 (t, J = 7.7 Hz, 4H), 7.42 (t, J = 7.7 Hz, 2H), 5.35 (dd, J = 8.0, 4.5 Hz,

1H), 5.25 (dd, *J* = 6.9, 5.9 Hz, 1H), 4.43 – 4.34 (m, 2H), 2.95 (dt, *J* = 14.9, 6.0 Hz, 2H), 2.85 – 2.75 (m, 2H), 2.23 – 2.08 (m, 2H), 1.96 (dq, *J* = 13.4, 6.2 Hz, 4H), 1.48 (s, 2H), 1.28 (t, *J* = 23.1 Hz, 14H), 0.87 (t, *J* = 6.9 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) δ= 206.2, 205.8, 166.5, 166.2, 166.1, 133.5, 133.4, 132.9, 130.1, 129.83, 129.81, 129.6, 129.4, 129.1, 128.54, 128.49, 128.34, 79.0, 78.4, 64.2, 31.87, 31.83, 30.8, 29.7, 29.53, 29.51, 29.35, 29.29, 29.21, 27.5, 25.3, 24.6, 22.7, 14.1; HRMS (ESI) $C_{40}H_{48}NaO_8$ requires $[M+Na]^+$ 679.3247, found 679.3245.

(±)-2-[(1*S*,4*R*)-4-(benzoyloxy)-3-oxocyclohexyl]-2-oxoethyl benzoate (12): Benzoate-protected tetrahydrofuran **11** (17.7 mg, 0.048 mmol) was subjected to General Procedure A to furnish the title compound **12** (13.7 mg, 0.036 mmol, 75 %) as white needles. m.p. 156-157 °C; IR (thin film): ν_{max}= 2934br w, 1721br s, 1601w, 1584w, 1451m, 1414w, 1316m, 1274br s, 1177w, 1114m, 1065m, 1026w, 996w, 710m cm^{-1}; ^{1}H NMR (200 MHz, $CDCl_3$) δ= 8.17 – 8.00 (m, 4H), 7.75 – 7.34 (m, 6H), 5.38 (dd, *J* = 8.9, 6.2 Hz, 1H), 5.12 (d, *J* = 16.8 Hz, 1H), 4.85 (d, *J* = 16.8 Hz, 1H), 3.64 – 3.09 (m, 1H), 2.85 (ddd, *J* = 14.5, 4.4, 1.5 Hz, 1H), 2.58 (ddd, *J* = 14.5, 5.7, 0.6 Hz, 1H), 2.49 – 2.09 (m, 4H); ^{13}C NMR (50 MHz, $CDCl_3$): δ= 204.0, 201.6, 165.9, 165.5, 133.6, 133.3, 130.0, 129.9, 129.4, 128.9, 128.5, 128.4, 75.7, 67.0, 45.4, 40.0, 28.9, 24.5; HRMS (ESI): *m/z* $C_{22}H_{20}NaO_6$ requires $[M+Na]^+$ 403.1158, found 403.1161. *Single crystal X-ray diffraction data for* **12** (Fig 1): $C_{22}H_{20}O6$, M = 380.38, monoclinic, *a* = 5.300(5), *b* = 10.946(4), *c* = 32.574(9) Å, β = 97.37(3)°, *V* = 1874(2) Å^3, *T* = 298 K, space group P21/c, Z = 4, μ(Mo-Kα) = 0.098 mm^{-1}, 10511 reflections measured, 3248 unique (R(int) = 0.0690) which were used in all calculations. Final agreement indices were *R* = 0.0573 ($I>2\sigma(I)$). and $wR(F^2)$ = 0.1421 (all data).

2,6-dioxoheptane-1,7-diyl dibenzoate (14): Benzoate-protected tetrahydropyran **13** (24.1 mg, 0.068 mmol) was subjected to General Procedure B to furnish the title compound **14** (21.3 mg, 0.058 mmol, 85 %) as a white solid. m.p. 123-125 °C; IR (thin film): ν_{max}= 2949br w, 2918w, 2850w, 1724br s, 1600w, 1451w, 1408m, 1271br s, 1128w, 1101m, 1063m, 1028m, 988w, 702m cm^{-1}; ^{1}H NMR (200 MHz, $CDCl_3$) δ= 8.09 (d, *J* = 7.0 Hz, 4H), 7.60 (t, *J* = 7.3 Hz, 2H), 7.46 (t, *J* = 7.4 Hz, 4H), 4.87 (s, 4H), 2.61 (t, *J* = 6.8 Hz, 4H), 2.01 (quin, *J* = 6.8 Hz, 2H); ^{13}C NMR (50 MHz, $CDCl_3$): δ= 203.6, 165.9, 133.4, 129.9, 129.6, 128.5, 68.4, 37.2, 16.7; HRMS (ESI): *m/z* $C_{21}H_{20}NaO_6$ requires $[M+Na]^+$ 391.1158, found 391.1159.

7-methyl-2,6-dioxooctane-1,7-diyl dibenzoate (16): Benzoate-protected tetrahydropyrans **15** (26.4 mg, 0.068 mmol) and **17** (21.0 mg, 0.055 mmol) were subjected to General Procedure B to furnish the title compound **16** (25.7 mg, 0.065 mmol, 95 % from **15**; 18.2 mg, 0.046 mmol, 83 % from **17**)

as an oil. IR (thin film): ν_{max}= 2990w, 2937br w, 1721br s, 1601w, 1584w, 1451m, 1416w, 1367w, 1316m, 1286br s, 1113m, 1070m, 1026m, 712s cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ= 8.07 (d, *J* = 7.2 Hz, 2H), 8.01 (d, *J* = 7.2 Hz, 2H), 7.60 – 7.54 (m, 2H), 7.45 (t, *J* = 7.4 Hz, 2H), 7.43 (t, *J* = 7.4 Hz, 2H), 4.88 (s, 2H), 2.66 – 2.50 (q, 4H), 1.97 (quin, *J* = 6.8 Hz, 2H), 1.59 (s, 6H); ^{13}C NMR (50 MHz, $CDCl_3$): δ= 208.4, 203.8, 165.8, 133.3, 129.9, 129.8, 129.2, 128.4, 84.1, 68.4, 37.2, 33.8, 23.7, 16.9; HRMS (ESI): *m/z* $C_{23}H_{24}NaO_6$ requires $[M+Na]^+$ 419.1471, found 419.1473.

2,6-dioxoheptane-1,4,7-triyl tribenzoate (19): Benzoate-protected tetrahydropyran **18** (27.0 mg, 0.057 mmol) was subjected to General Procedure B to furnish the title compound **19** (21.5 mg, 0.044 mmol, 78 %) as a white solid. m.p. 148-150 °C; IR (thin film): ν_{max}= 3068w, 2929w, 1720s, 1601w, 1583w, 1451w, 1406br w, 1314w, 1275s, 1177w, 1112w, 1069w, 1026w, 711m cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ= 8.18 – 7.91 (m, 6H), 7.67 – 7.52 (m, 3H), 7.50 – 7.34 (m, 6H), 5.86 (quin, *J* = 6.0 Hz, 1H), 4.96 (d, *J* = 16.9 Hz, A part of an AB system, 2H), 4.87 (d, *J* = 16.9 Hz, B part of an AB system, 2H), 3.23 – 2.97 (m, 4H); ^{13}C NMR (50 MHz, $CDCl_3$): δ= 201.0, 165.8, 165.6, 133.5, 133.3, 129.9, 129.7, 129.6, 129.0, 128.5, 68.7, 66.5, 41.9; HRMS (ESI): *m/z* $C_{28}H_{24}NaO_8$ requires $[M+Na]^+$ 511.1369, found 511.1366.

3,3'-benzamidobis(2-oxopropane-3,1-diyl) dibenzoate (21): Benzoate-protected morpholine **20** (20.0 mg, 0.044 mmol) was subjected to General Procedure B. The reaction mixture was filtered, taken to dryness and separated by HPLC (hexane/EtOAc, 6:4) to give the title compound **21** (5.2 mg, 0.011 mmol, 26 %), compounds **22** (3.9 mg, 0.008 mmol, 18 %) and **23** (2.3 mg, -0.005 mmol, 12 %). **21**: Oil; IR (thin film): ν_{max}= 2921w, 2848w, 1725br s, 1644m, 1601w, 1451m, 1414w, 1315w, 1275s, 1112m, 1069m, 1027w, 712m cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ= 8.10 (d, *J* = 7.6 Hz, 2H), 7.99 (d, *J* = 7.7 Hz, 2H), 7.60 (t, *J* = 7.3 Hz, 2H), 7.54 – 7.31 (m, 9H), 5.04 (s, 2H), 4.74 (s, 2H), 4.52 (s, 2H), 4.42 (s, 2H); ^{13}C NMR (50 MHz, $CDCl_3$): δ= 200.0, 199.8, 172.3, 166.0, 165.7, 134.2, 133.7, 133.6, 130.5, 129.94, 129.87, 128.80, 128.5, 126.8, 67.5, 67.0, 56.8, 52.6; HRMS (ESI): *m/z* $C_{27}H_{23}NNaO_7$ requires $[M+Na]^+$ 496.1372, found 496.1373.

1-(benzoyloxy)-3-(*N*-formyl-1-phenylformamido)propan-2-yl 2-(benzoyloxy)acetate (22): Oil; IR (thin film): ν_{max}= 2949br w, 2926br w, 2849w, 1762w, 1727s, 1673s, 1602w, 1451w, 1275br s, 1206br m, 1117br m, 1071w cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ= 8.96 (s, 1H), 8.06 (d, *J* = 7.7 Hz, 2H), 8.03 (d, *J* = 8.4 Hz, 2H), 7.62 – 7.53 (m, *J* = 8.1 Hz, 5H), 7.51 – 7.39 (m, 6H), 5.72 (d, *J* = 3.4 Hz, 1H), 4.85 (d, *J* = 16.0 Hz, A part of an AB system, 1H), 4.79 (d, *J* = 16.0 Hz, B part of an AB system, 1H), 4.60 (dd, *J* = 12.2, 3.8 Hz, 1H), 4.54 – 4.43 (m, 3H), 4.15 (dd, *J* = 14.0, 2.9 Hz, 1H);

^{13}C NMR (50 MHz, $CDCl_3$): δ= 172.1, 167.7, 166.0, 165.8, 164.2, 133.4, 133.3, 133.0, 132.3, 129.9, 129.8, 129.4, 129.3, 129.0, 128.9, 128.5, 128.4, 70. 6, 63.6, 61.1, 40.5; HRMS (ESI) *m/z* $C_{27}H_{23}NNaO_8$ requires $[M+Na]^+$ 512.1321, found 512.1320.

1-(benzoyloxy)-3-(phenylformamido)propan-2-yl 2- (benzoyloxy)acetate (23): Oil; IR (thin film): ν_{max}= 3380br w, 3300br w, 3061w, 2934w, 1726s, 1645w, 1537br w, 1451w, 1275s, 1203w, 1117m, 709m cm^{-1}; ^{1}H NMR (200 MHz, $CDCl_3$) δ= 8.06 – 7.95 (m, 4H), 7.83 (d, *J* = 7.9 Hz, 2H), 7.65 – 7.35 (m, 9H), 6.75 (br t, *J* = 5.4 Hz, 1H), 5.59 – 5.42 (m, *J* = 7.3 Hz, 1H), 4.92 (d, *J* = 15.8 Hz, A part of an AB system, 1H), 4.82 (d, *J* = 15.8 Hz, B part of an AB system, 1H), 4.60 (dd, *J* = 12.2, 4.1 Hz, 1H), 4.49 (dd, *J* = 12.2, 5.5 Hz, 1H), 3.99 (ddd, *J* = 14.5, 6.5, 3.9 Hz, 1H), 3.67 (ddd, *J* = 14.5, 7.2, 5.6 Hz, 1H); ^{13}C NMR (101 MHz, $CDCl_3$) δ= 167.7, 167.4, 166.7, 166.2, 133.8, 133.7, 133.3, 131.6, 129. 9, 129.7, 129.6, 129.3, 128.555, 128.548, 128.5, 127.1, 126.6, 72.2, 63.3, 61.7, 40.1; HRMS (ESI): *m/z* $C_{26}H_{23}NNaO_7$ requires $[M+Na]^+$ 484.4531, found 484.4520.

(13*R*,18*S*)-18-(acetyloxy)-32-[(5*S*)-5-methyl-2-oxo-2,5-dihydrofuran-3-yl]-14,17-dioxodotriacontan-13-yl acetate (27): *Cis*-reticulatacin diacetate **25** (5.4 mg, 0.008 mmol) was subjected to General Procedure B. After being stirred for 12h, the reaction mixture was filtered, taken to dryness and separated by HPLC (hexane/EtOAc, 8:2) to give the title compound **27** (1.4 mg, 0.002 mmol, 30 %) as a colorless oil. IR (thin film): ν_{max}= 2924s, 2853m, 1747br s, 1374w, 1235br m, cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ= 6.99 (s, 1H), 5.04 – 4.92 (m, 3H), 2.88 – 2.75 (m, 2H), 2.75 – 2.62 (m, 2H), 2.26 (t, *J* = 7.6 Hz, 2H), 2.14 (s, 6H), 1.90 – 1.65 (m, 6H), 1.66 – 1.17 (m, 38H), 1.40 (d, *J* = 6.8 Hz, 3H), 0.88 (t, *J* = 6.7 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ= 206.1, 173.9, 170.7, 148.8, 134.3, 78.6, 77.4, 31.9, 31.7, 30.5, 29.6, 29.5, 29.4, 29.2, 27.4, 25.21, 25.20, 22.7, 20.7, 19.2, 14.1; HRMS (ESI): *m/z* $C_{41}H_{70}NaO_8$ requires $[M+Na]^+$ 713.4968, found 713.4966.

(13*R*,18*S*)-18-(benzoyloxy)-32-[(5*S*)-5-methyl-2-oxo-2,5-dihydrofuran-3-yl]-14,17-dioxodotriacontan-13-yl benzoate (28): *Cis*-reticulatacin dibenzoate **26** (3.6 mg, 0.004 mmol) was subjected to General Procedure B. After being stirred for 8h, the reaction mixture was filtered, taken to dryness and separated by HPLC (hexane/EtOAc, 85:15) to give the title compound **28** (1.6 mg, 0.002 mmol, 40 %) as a colorless oil. $[\alpha]_D^{30}$ = -23.3 (c=0.03, $CHCl_3$); IR (thin film): ν_{max}= 2924s, 2853m, 1756m, 1720s, 1453w, 1316w, 1271m, 1112br w, 1028w, 712m cm^{-1}; ^{1}H NMR (200 MHz, $CDCl_3$) δ= 8.08 (d, *J* = 7.1 Hz, 4H), 7.60 (t, *J* = 7.3 Hz, 2H), 7.46 (t, *J* = 7.4 Hz, 4H), 6.98 (br q, *J* = 1.7 Hz, 1H), 5.24 (t, *J* = 6.4 Hz, 2H), 4.99 (br q, *J* = 6.8 Hz, 1H), 3.05 – 2.67 (m, 4H), 2.26 (t, *J* = 7.6 Hz, 2H), 1.94 (app q, *J* = 6.5 Hz, 4H), 1.64 – 1.15 (m, 40H), 1.40 (d, *J* = 6.8 Hz, 3H), 0.87 (t, *J*

= 6.4 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$): δ= 206.2, 173.9, 166.9, 166.2, 148.9, 134.3, 133.4, 129.8, 129.3, 128.5, 79.0, 77.4, 31.9, 31.8, 30.7, 29.7, 29.6, 29.5, 29.3, 29.2, 27.4, 25.3, 25.1, 22.7, 19.2, 14.1; HRMS (ESI): *m/z* $C_{51}H_{74}NaO_8$ requires $[M+Na]^+$ 837.5281, found 837.5284.

3.4.4 Synthesis of six-membered nitrogen heterocycles

2-[6-(hydroxymethyl)pyridin-2-yl]propan-2-ol (29b): To a solution of diketone **16** (12.0 mg, 0.030 mmol) in dry MeOH (300 μL) were added NH_4OAc (4.0 mg, 0.052 mmol) and AcOH (3 μL). After being stirred for 24 h at rt and 48 h at 55°C the reaction mixture was diluted with EtOAc (2 mL) and washed with a sat. aqueous $NaHCO_3$ solution and water. The organic phase was dried, filtered and concentrated under reduced pressure. Separation by preparative TLC (hexane/EtOA, 3:7) gave **29a** (6.3 mg, 55%) and **30** (2.1 mg, 25%) as colorless oils. **29a**: oil; IR (thin film): ν_{max}= 2923br w, 2849w, 1716s, 1594w, 1450w, 1314w, 1265br s, 1151w, 1108s, 1070w, 1026w, 709s cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$) δ= 8.11 (d, *J* = 7.2 Hz, 2H), 8.06 (d, *J* = 7.2 Hz, 2H), 7.71 (t, *J* = 7.8 Hz, 1H), 7.60 – 7.54 (m, 2H), 7.48 – 7.42 (m, 4H), 7.39 (d, *J* = 8.0 Hz, 1H), 7.32 (d, *J* = 7.6 Hz, 1H), 5.52 (s, 2H), 1.94 (s, 6H); ^{13}C NMR (50 MHz, $CDCl_3$): δ= 166.3, 165.3, 155.3 154.9, 137.2, 133.1, 132.7, 129.7, 129.6, 128.4, 128.3, 119.3, 117.9, 83.3, 67.2, 27.6; HRMS (ESI): *m/z* $C_{23}H_{21}NNaO_4$ requires $[M+Na]^+$ 398.1368, found 398.1367.

To a solution of **30** (2.1 mg, 0.008 mmol) in MeOH, was added K_2CO_3 (1.0 mg, 10 mol %). After being stirred for 30 min at rt, AcOH was added up to neutrality and the mixture was evaporated under reduced pressure. The residue was redissolved in $CHCl_3$ and filtered to give pyridine diol **29b** (1.2 mg, 0.007 mmol, 85 %) as an oil. **29b**: IR (thin film): ν_{max}= 3420br m, 2920br m, 2853m, 1736br m, 1462br w, 1376br w, 1260br w, 1169br w, 1099br w, 1022br w, 802w cm^{-1}; ^{1}H NMR (200 MHz, $CDCl_3$): δ= 7.72 (t, *J* = 7.7 Hz, 1H), 7.32 (d, *J* = 7.8 Hz, 1H), 7.19 (d, *J* = 7.8 Hz, 1H), 4.79 (s, 2H), 1.57 (s, 6H); HRMS (ESI): *m/z* $C_9H_{13}NNaO_2$ requires $[M+Na]^+$ 190.0844, found 190.0842.

[6-(hydroxymethyl)pyridin-2-yl]methanol (31b): To a solution of diketone **14** (9.4 mg, 0.024 mmol) in dry EtOH (1 mL) was added $NH_2OH{\cdot}HCl$ (5.8 mg, 0.084 mmol). After being stirred for 16 h at rt and 8 h at reflux, sat. aqueous $NaHCO_3$ was added up to neutrality and the mixture was concentrated under reduced pressure and filtered through a short pad of silica gel ($CHCl_3$/MeOH, 8:2). Purification by preparative TLC (hexane/EtOA, 3:7) gave **31a** (5.4 mg, 0.016 mmol, 65%) as a colorless oil. **31a**: IR (thin film): ν_{max}= 2917br m, 2850m, 1716m, 1600w, 1585w, 1451w, 1361w,

1311w, 1272s, 1248m, 1173w, 1121m, 1023w, 771w, 714m cm^{-1}; cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ= 8.16 – 8.08 (m, 4H), 7.76 (t, J = 7.7 Hz, 1H), 7.65 – 7.54 (m, 2H), 7.52 – 7.43 (m 4H), 7.41 (d, J = 7.2 Hz, 2H), 5.51 (s, 4H); ^{13}C NMR (50 MHz, $CDCl_3$): δ= 166.2, 155.9, 137.6, 132.2, 129.8, 128.6, 128.5, 120.7, 67.0; HRMS (ESI): *m/z* $C_{21}H_{17}NNaO_4$ requires $[M+Na]^+$ 370.1055, found 370.1053. Debenzoylation of **31a** (4.5 mg, 0.013 mmol) with K_2CO_3 in MeOH as described for **30** gave pyridine-2,6-diyldimethanol **31b** (1.5 mg, 0.011 mmol, 82%) as an oil. Spectroscopic data for **31b** were consistent with those previously reported for this compound.[33]

2,6-bis(hydroxymethyl)-1λ^4-pyridin-1-olate (32b): To a solution of diketone **19** (10.5 mg, 0.021 mmol) in dry EtOH (500 μL) was added $NH_2OH{\cdot}HCl$ (5.1 mg, 0.0073 mmol). After being stirred for 32 h at rt and 15 h at reflux, sat. aqueous $NaHCO_3$ was added up to neutrality and the mixture was concentrated under reduced pressure and filtered through a short pad of silica gel ($CHCl_3$/MeOH, 8:2). Purification by preparative TLC (hexane/EtOA, 1:1) gave **32a** (5.3 mg, 0.014 mmol, 68 %) as a colorless oil. **32a**: IR (thin film): ν_{max}= 2924br m, 2852w, 1717s, 1601w, 1449w, 1368w, 1264s, 1174m, 1115s, 1071m, 1027w, 845m, 769w, 709s cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ= 8.15 (d, J = 7.5 Hz, 4H), 7.62 (t, J = 7.2 Hz, 2H), 7.50 (t, J = 7.7 Hz, 4H), 7.46 (d, J = 7.6 Hz, 2H), 7.34 (t, J = 8.0 Hz, 1H), 5.71 (s, J = 32.9 Hz, 4H); ^{13}C NMR (125 MHz, $CDCl_3$): δ= 165.7, 147.3, 133.5, 129.8, 129.3, 128.6, 125.5, 122.2, 60.8; HRMS (ESI): *m/z* $C_{21}H_{17}NNaO_5$ requires $[M+Na]^+$ 386.1010, found 370.1012. Debenzoylation of **32a** (3.8 mg, 0.010 mmol) with K_2CO_3 in MeOH as described for **30** gave the corresponding diol **32b** (1.2 mg, 0.008 mmol, 78%) as an oil. **32b**: IR (thin film): ν_{max}= 3418br m, 2918w, 2849w, 1644br m, 1416w, 1358w, 1212w, 1166w, 1085w, 1031w cm^{-1};^1H NMR (200 MHz, $CDCl_3$): δ= 7.36 (br s, 3H), 4.84 (br s, 4H); ^{13}C NMR (175 MHz, CD_3OD): δ= 157.6, 130.1, 122.4, 59.7; HRMS (ESI): *m/z* $C_7H_9NNaO_3$ requires $[M+Na]^+$ 178.0480, found 178.0481.

Converson of 31b to 32b: To a solution of pyridine diol **31b** (3.5 mg, 0.025 mmol) in $CHCl_3$ (500 μL)) was added MCPBA (1.2 eq., 0.030 mmol, 500 μL,) from a stock solution (0.060 mM) in $CHCl_3$. The mixture was stirred for 90 min at rt and concentrated under reduced pressure. Separation by preparative TLC ($CHCl_3$/MeOH, 8:2) gave **32b** (1.4 mg, 0.009 mmol, 36%) as a colorless oil.

[6-(hydroxymethyl)pyrazin-2-yl]methanol (33b): To a solution of diketone **21** (10.8 mg, 0.023 mmol) in dry EtOH (500 μL) was added $NH_2OH{\cdot}HCl$ (4.8 mg, 0.069 mmol). After being stirred for 6 h at rt, sat. aqueous $NaHCO_3$ was added up to neutrality and the mixture was concentrated under

reduced pressure and filtered through a short pad of silica gel ($CHCl_3$/MeOH, 8:2). Purification by HPLC (hexane/EtOA, 6:4) gave **33a** (5.6 mg, 0.016 mmol, 68 %) as a colorless oil. **33a**: ν_{max}= 2917m, 2849w, 1719s, 1601w, 1451w, 1315w, 1266s, 1176w, 1110m, 1071w, 1025w, 709s cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ= 8.74 (s, 2H), 8.11 (d, J = 7.0 Hz, 4H), 7.61 (t, J = 7.3 Hz, 2H), 7.47 (t, J = 7.3 Hz, 4H), 5.54 (s, 4H); ^{13}C NMR (50 MHz, $CDCl_3$): δ= 166.1, 150.9, 142.6, 133.5, 129.8, 129.4, 128.6, 65.1; HRMS (ESI): *m/z* $C_{20}H_{16}N_2NaO_4$ requires $[M+Na]^+$ 371.3418, found 371.3419. Debenzoylation of **33a** (6.5 mg, 0.019 mmol) with K_2CO_3 in MeOH as described for **30** gave the corresponding diol **33b** (1.7 mg, 0.012 mmol, 66%) as an oil. **33b**: IR (thin film); ν_{max}= 3364br m, 2924s, 2854m, 1723br m, 1641br m, 1576w, 1539w, 1490w, 1456w, 1418br w, 1275br m, 1160br w, 1083br m, 1022w, 713m cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$): δ= 8.58 (2H, br s), 4.86 (4H, br s); ^{13}C NMR (50 MHz, $CDCl_3$): δ= 153.6, 141.5, 62.9; HRMS (ESI): *m/z* $C_6H_8N_2NaO_2$ requires $[M+Na]^+$ 163.0483, found 163.0485.

3.5. References and notes

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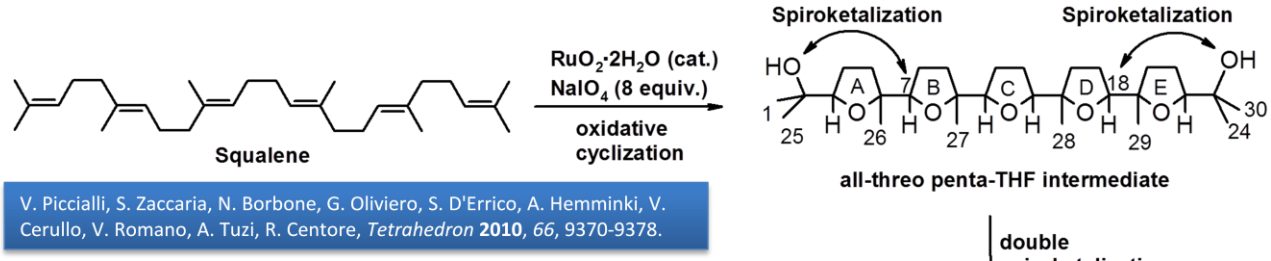
Part 2. *Ruthenium Tetroxide Chemistry*



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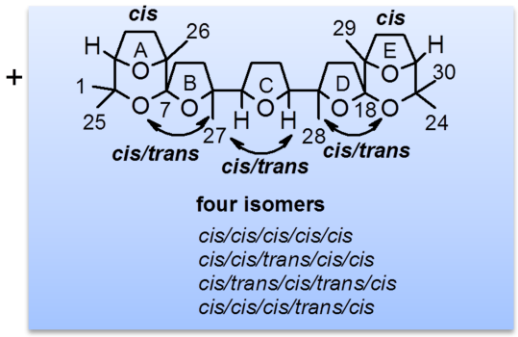

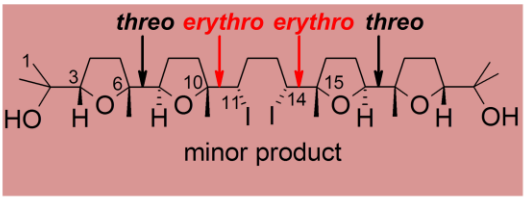

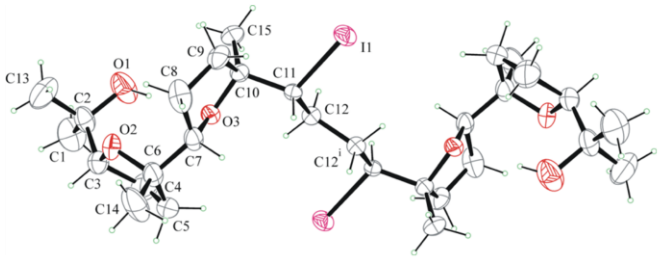

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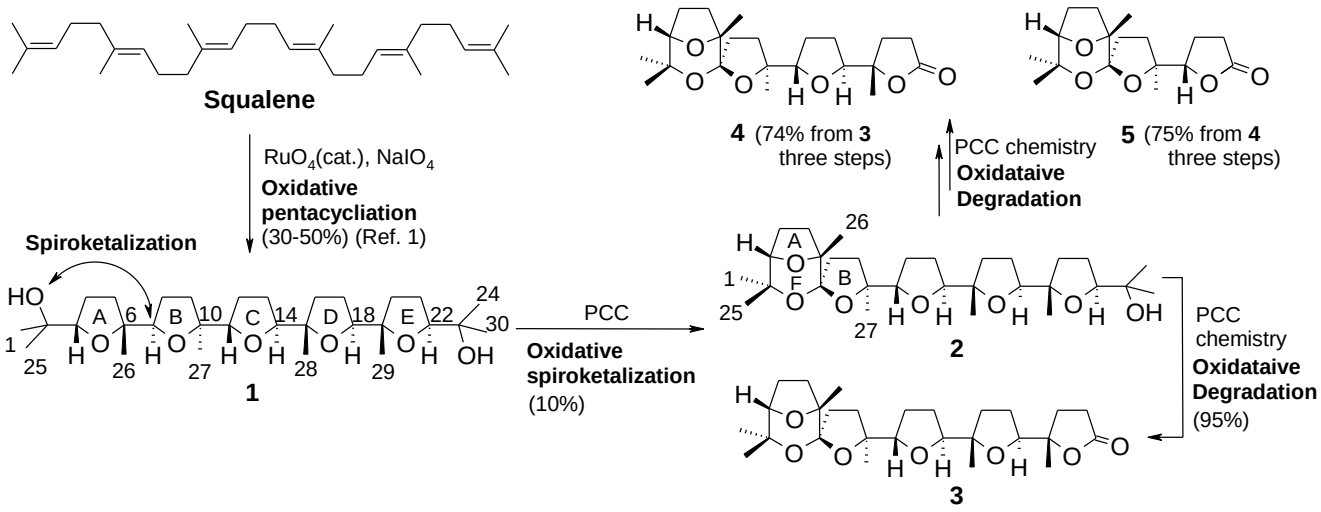
Chapter 4

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

4.1. Introduction

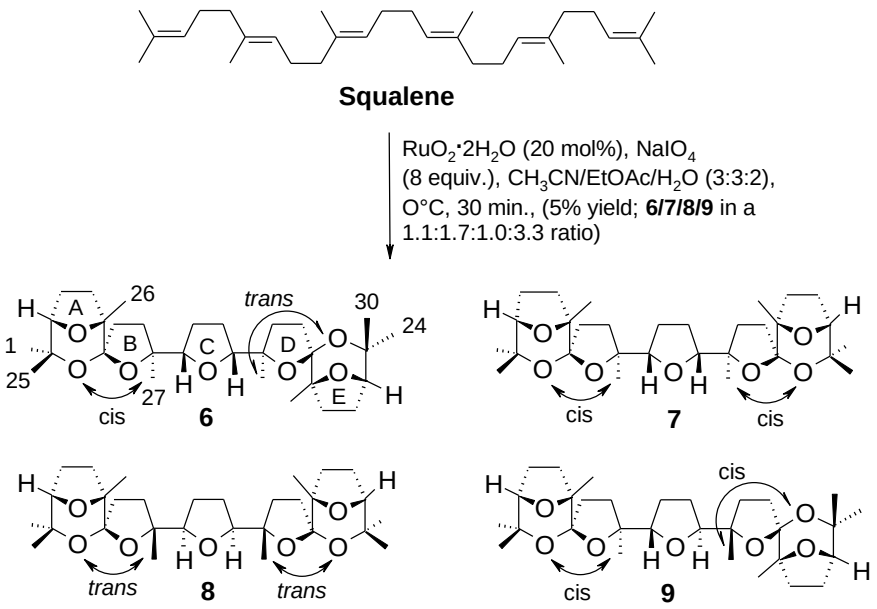
The ruthenium tetroxide mediated oxidative polycyclization of polyenes characterised by a repetitive 1,5-diene structural motif is a unique stereoselective process discovered a few years ago in our laboratories[1] (see § 1.2). It allows to obtain adjacently linked poly-tetrahydrofuran products in a single step employing catalytic amounts of ruthenium tetroxide in the presence of $NaIO_4$ as co-oxidant. In particular, the penta-cyclization of squalene (Scheme 1)[1a,1c] 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.



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

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, see also § 1.3 and 2.1) 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 (**4** and **5**, Scheme 1) starting from **2** and/or **3**. Preliminary citotoxicity 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.

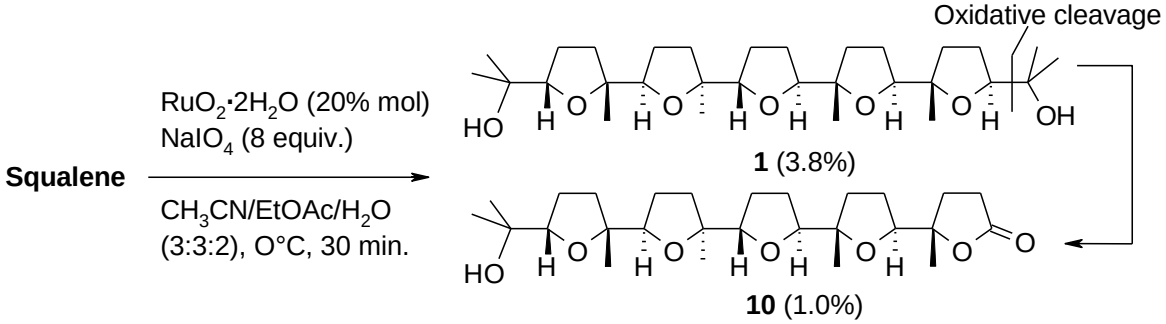


Scheme 2. Novel C_{30} bis-spiroketals by RuO_4-catalysed tandem oxidative polycyclization/double oxidative spiroketalization of squalene. Letters marking THF rings in **6** highlight the structural relationship with compounds **1** and **2** of scheme 1.

4.2. Results and Discussion

4.2.1 Isolation of new spiroketal compounds

Aimed at preparing higher 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 co-oxidant ($NaIO_4$) under standard poly-cyclization conditions.[1a,1c] 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** and § 2.2.2 for a mechanistic rationalisation). 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 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.



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

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 (Figure 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* (Figure 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 Schemes 4 and 5).[1a,1c]

Bis-spiroketals **7**-**9** (Figure 1 and Scheme 2) proved to be isomeric with **6** and possessed symmetric structures as indicated by the halving of the ^{1}H 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** (Figure 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 (Figure 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 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.

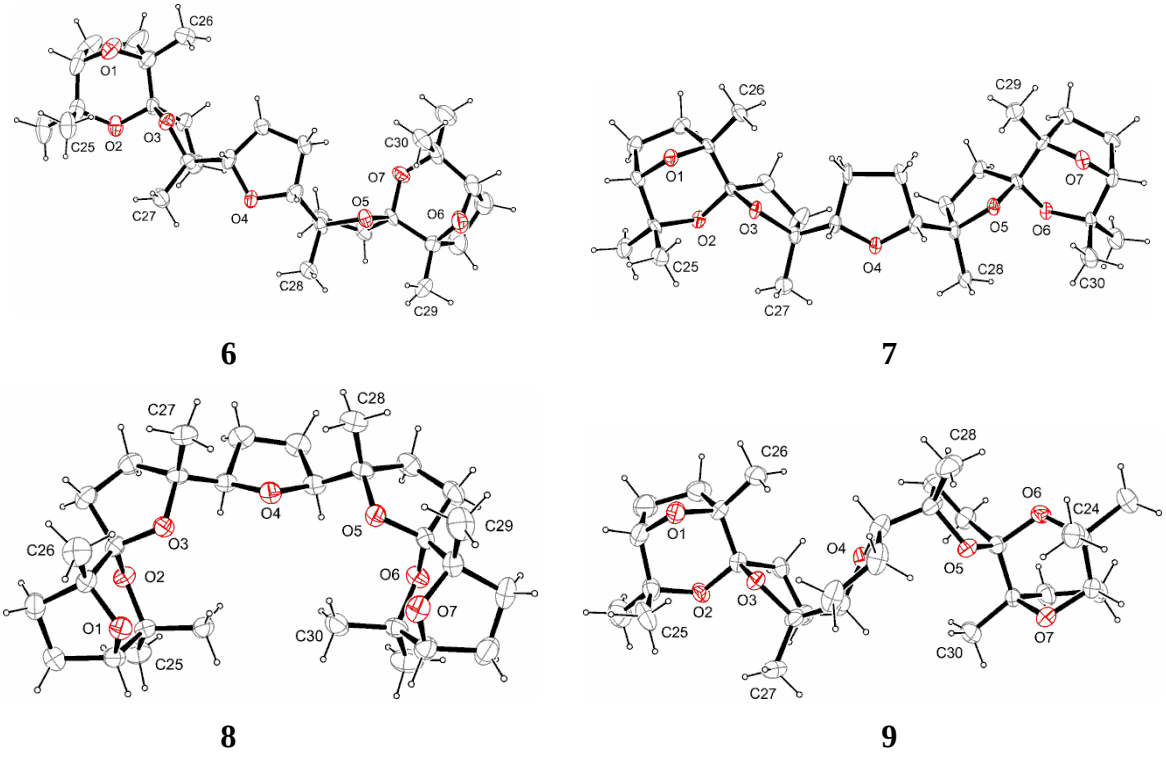
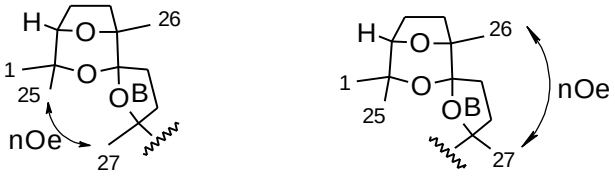


Figure 1. ORTEP drawings of bis-spiro-compounds **6-9**. Ellipsoids are drawn at 30% probability level.



Spiroketal arrangement as in **6**,**7** and **9**

Spiroketal arrangement as in **8**

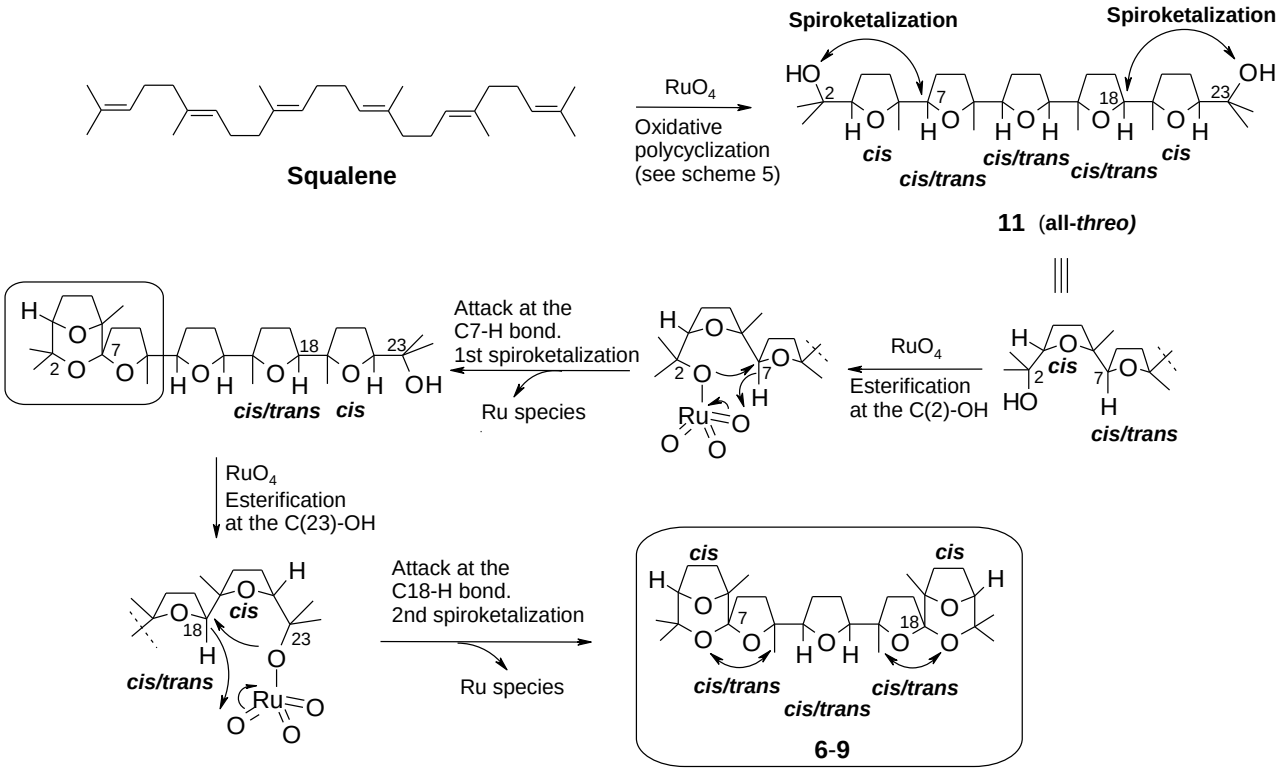
Figure 2 Characteristic nOe correlations within the spiroketal moiety, including a *cis* (left) or *trans* (right) ring-B THF, observed for **6-9**.

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 (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

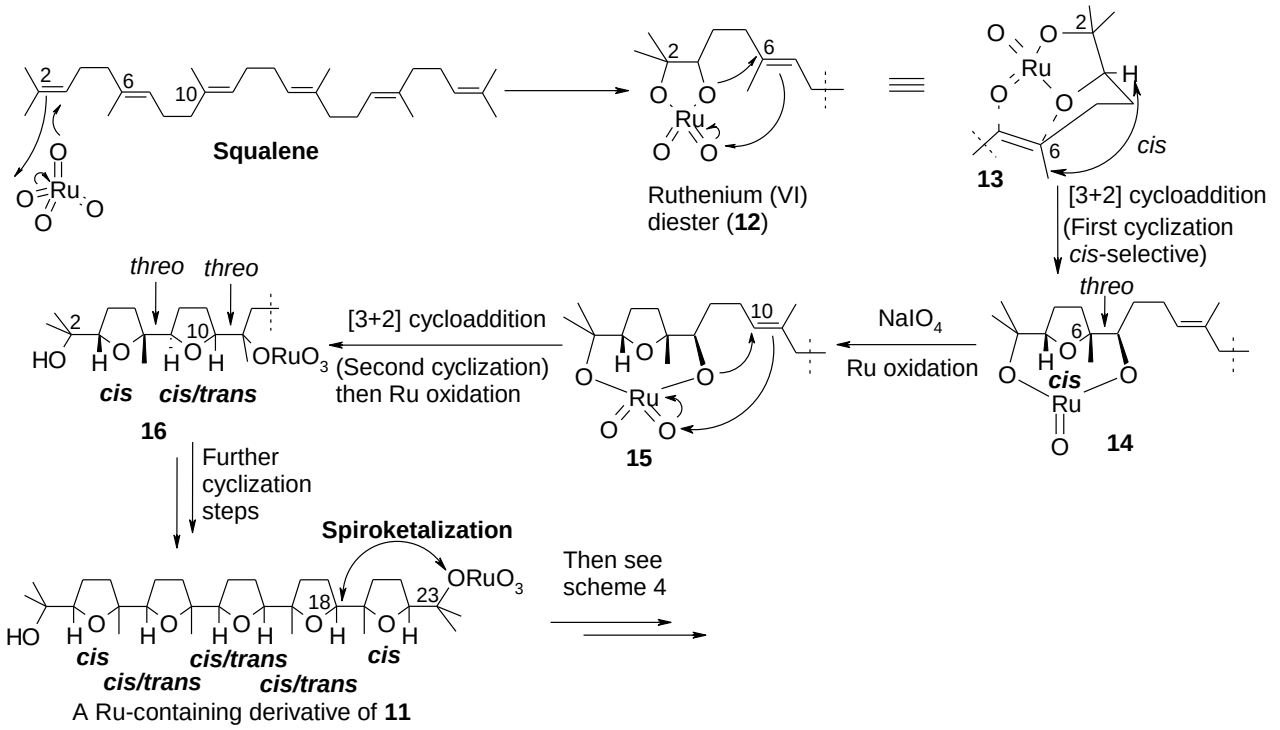
process, the reoxidation of ruthenium bonded to the various intermediates (see scheme 5 later) takes place more or less doubling the involved steps. The *threo* nature of all inter-THF relationships in all four bis-spiroketals suggests that a penta-THF backbone (see **11**) was preliminarily assembled 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,1e] The configuration of each THF shown in **11** reflects the ones found in bis-spiroketals **6**-**9** to which it 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 a 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.

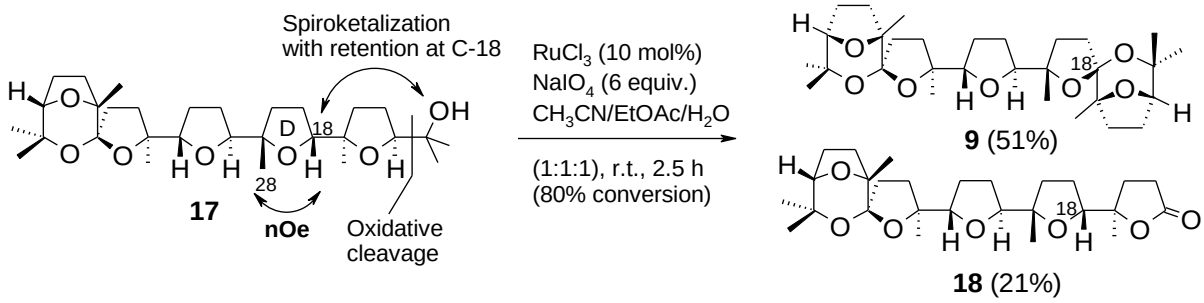


Scheme 4. A plausible mechanistic hypothesis explaining the formation of bis-spiroketals **6**-**9** from squalene

We have evidence indicating that the spiroketal-forming steps involved in the sequence shown in Scheme 4 proceed 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 spectroscopic evidences. 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 5. Ru-catalysed cascade sequence leading to the intermediate penta-THF **11** shown in Scheme 4.

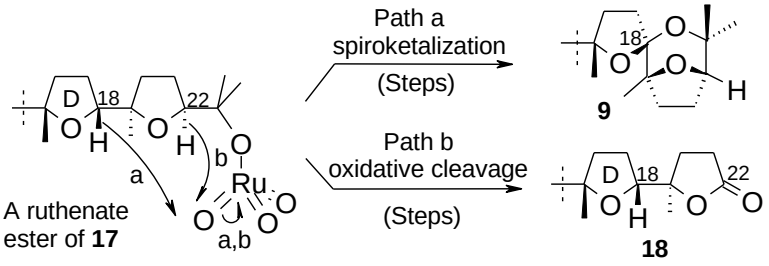


Scheme 6. RuO_4-mediated oxidative mono-spiroketalization and the competing oxidative cleavage.

As for this reaction, the same RuO_2(cat)/$NaIO_4$ system employed to convert squalene into compounds **6**-**9** was initially used, but four equiv. of the co-oxidant were employed now. 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,1f,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 little 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**

4.2.2 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 cell killing effect 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 two 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 (Figure 3). In the HEY cell line (Figure 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 Figure. 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**; Figure 3 right). Compound **2** caused a 70% cell death after 14 days at a 10 μM concentration. This remarkable difference between the two diastreomers 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 (Figure 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 cell killing 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.

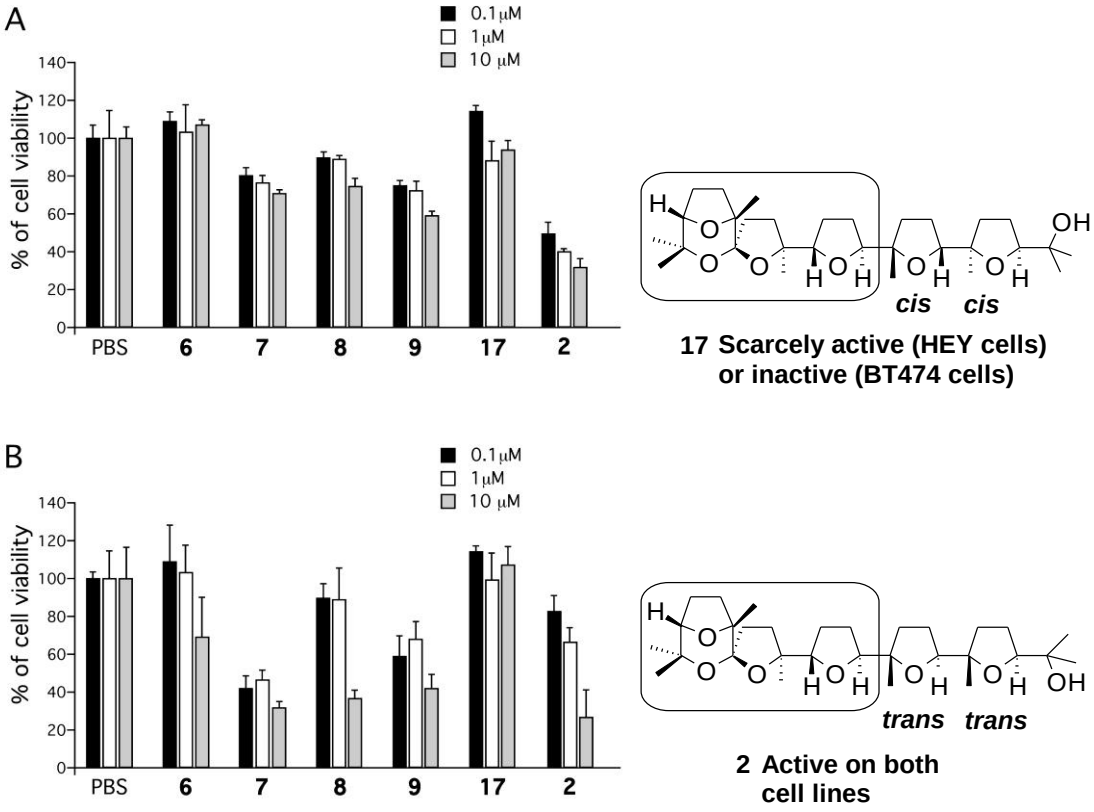


Figure 3. ***Cytotoxic effect of spiro-compounds 6-9 and 17 in two different tumor-derived cell lines.*** Ovarian cancer-derived cell line (HEY; Panel A) and breast cancer-derived cell line (BT474; Panel B) were treated with compounds **6**, **7**, **8**, **9** and **17** at the concentration of 0.1μM (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.

4.2.3 Isolation of a bis-iodurated tetra-THF as a trace product

Careful further HPLC separation of the reaction mixture allowed also the isolation of tetra-THF **19** (Figure 4), a very minor side-product of the process, possessing a C_S-symmetric structure embodying two terminal *cis-threo-trans* bis-THF moieties connected by a central bis-iodurated tetracarbonious segment.

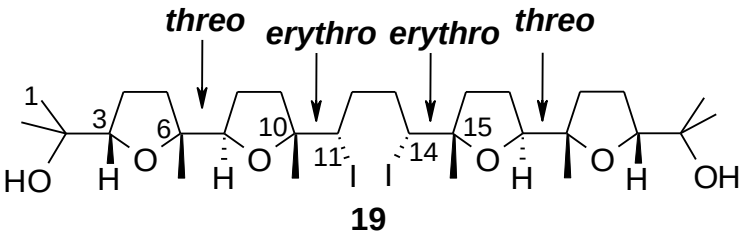


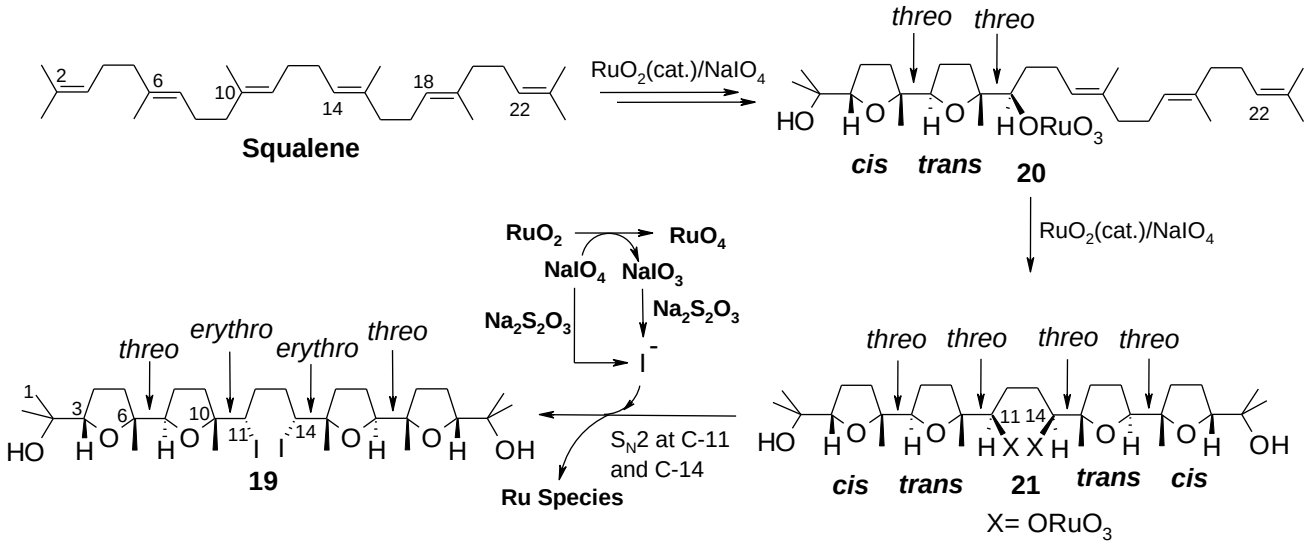
Figure 4. Bis-iodurated tetra-THF isolated as a trace product from the reaction mixture.

The structure of compound **19** was determined by X-ray diffraction analysis carried out on a single crystal of the substance obtained by slow evaporation of a chloroform solution. The most interesting, and unusual feature of this compound is the double *erythro* configuration around the C10/C11 and C14/C15 bonds. In fact, previous studies from our group had demonstrated that the oxidation of both linear and isoprenoid polyenes constantly furnishes poly-THF compounds possessing *threo* inter-THF relationships (see Scheme 5).

Based on these precedents, formation of **19** was intriguing and could be rationalised through a double *cis*/*trans*-selective oxidative bis-cyclization process (Scheme 8). Each bis-cyclization event involves three consecutive double bonds of the polyene chain starting from the terminal ones. In particular, attack of RuO_4 to the Δ^2 double bond induces two successive cyclization steps giving rise to bis-THF intermediate **20**, in the same manner as shown for the synthesis of **1** (see intermediate **16**, Scheme 5). A second bis-cyclization would then occur at the other side of the molecule by attack of RuO_4 at the terminal, Δ^{22}, double bond to give the all-*threo* tetra-THF **21** still possessing two oxoruthenate appendages linked to C-11 and C-14. It can be presumed that a double substitution of the ruthenium-containing portions, with inversion of configuration at involved carbon centres, would then occur during the reductive quenching of the process, by iodide ions probably generated *in situ* by the action of tiosulphate on iodate in turn produced during the oxidation of RuO_2 to RuO_4. It cannot be excluded that iodide could originate from reduction of periodate itself not completely consumed in the reaction medium. It is probable that such a side-process could be due to the higher concentration of the reaction medium in the new experimented conditions.

In order to enlarge the range of polyether substances accessible through the Ru-mediated polycyclization process we began an exploration of the possible post-synthetic modifications of some of the poly-THF backbones obtained through the above process. We have previously demonstrated that progressive structural simplification of compound **1** to small-sized poly-THF compounds can be achieved *via* an iterative PCC-mediated oxidative cleavage/reduction sequence.[1c] The entire process is possible due to the two alcohol functionalities adjacent to the terminal THF rings that are prone to be intercepted by PCC.[20] In addition, a new type of cytotoxic spyroketal poly-THF compound, strictly related to bis-spiroketals **2-5**, could be accessed through a PCC-mediated oxidative spiroketalization process starting from **1** (Scheme1, § 1.3 and 2.1). [3] In a more recent study we have also shown that the same oxidant, or the related system PCC-H_5IO_6, is able to attack the angular CH position of the THF ring in various mono and poly-THF substrates leading to either the oxidative opening of the THF ring or the oxidative cleavage of suitable inter-THF bonds

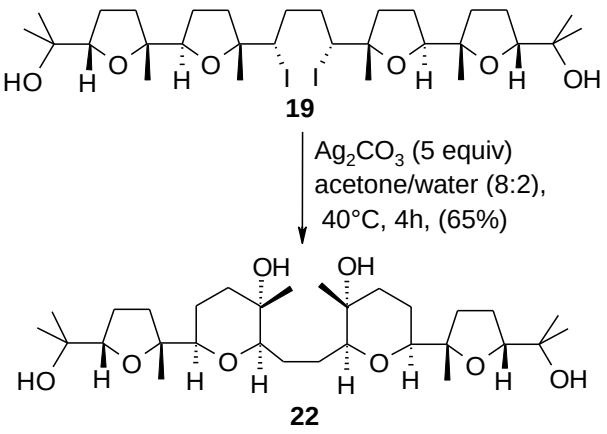
(see § 2.2.4 and 2.2.5).[21]



S**cheme 8.** A plausible path for the formation of tetra-THF **19**.

As a continuation of this project, we envisaged that compound **19**, possessing a central bis-(α-iodo-THF) portion, could be a good model compound to probe a double rearrangement-ring expansion process involving the two internal THF rings as a means to access a new type of mixed THF-THP polyether compounds further functionalised for successive synthetic manipulations providing access to new polyether polycyclic materials. This type of reaction has previously been carried out on substances containing a single α-iodo-THF subunit[22] but has never been attempted on a substrate containing two α-iodo-THF moieties and, in particular, to the best of our knowledge, the double rearrangement-ring expansion of a bis-THF substance has never been accomplished. Related chemistry has been successfully employed for example in the synthesis of salynomicin[23] as well as in the synthesis of a bis-oxepane portion of hemibrevetoxin.[24] Pleasingly, when compound **19** was reacted with excess Ag_2CO_3 (5 equiv.) in acetone/water (8:2, 40 °C, 4 h), compound **22** was obtained in a 65% yield demonstrating the feasibility of the projected transformation (Scheme 9).

Proof for the structure **22** was gained by chemical and high-field 2D NMR evidence. Attempted acetylation and benzoylation under standard conditions only delivered unreacted **21** indicating, as expected, the presence of tertiary hydroxyl groups in this compound.



Scheme 9. Double rearrangement-ring expansion of compound **19**.

A $^{1}H^{1}H$ COSY experiment at 700 MHz indicated the presence in **22** of the two five-proton spin systems H-3/H_2-4/H_2-5 and H-7/H_2-8-H_2-9 belonging to the two adjacent rings as well as the H-11/H_2-12 spin system. Assignment of each of these spin systems to the proper ring came from considerations of spectral data and comparison with strictly related THF- and THP-containing substances. In particular, the signals resonating at δ 3.86 and 2.36 were assigned, respectively, to the angular THF proton (H-3) and to the H_α-5 proton based on the good agreement of their chemical shift values with those typically exhibited by these protons in strictly analogous poly-THF substances including the same *cis*-THF-containing substructure, previously synthesised in our laboratories.[1] This deduction suggested that the two higher field one-proton resonances at δ 3.26 (H-7) and 3.16 (H-11) could be ascribable to the angular hydrogens in the THP ring.

The good proton dispersion of the signals in the ^{1}H NMR spectrum of **22** allowed us to fully analyse some crucial signals. In particular, the presence of a THP ring in **22** was corroborated by *J* values (*J* = 12.5, 3.6 Hz) of the H-9 equatorial proton resonating as a clean double triplet at δ 1.89, as expected for an equatorial proton next to a quaternary centre (C-10) in a six-membered ring possessing a chair conformation. In addition, a W coupling observed between the signal at δ 1.54 for H_{ax}-9 and the singlet methyl resonance at δ 1.19, ascribable to the C-10 methyl group, also pointed to the presence of a THP ring and the axial nature of that methyl. W-type long-range couplings were also observed between the singlet resonances at δ 1.23 and 1.05 allowing assignment of these signals to the two methyls belonging to the terminal 2-hydroxyisopropyl group. Similarly, a long-range coupling between the methyl signal at δ 1.12 and the H_a-5 resonance at δ 2.36 allowed to assign the former resonance to the angular methyl of the THF ring (C6-Me).

These conclusions were reinforced by data from a very informative 700 MHz NOESY experiment (Figure 5) that also provided conclusive information on the relative configuration of the

C-7, C-10 and C-11 centres belonging to the THP ring in **22**. In particular, the *cis* nature of the THP ring was inferred by the presence of a strong correlation peak between signals for the H-7 and H-11 angular protons. Similarly, the axial nature of the C-10 methyl, was further corroborated by a nOe correlation between its resonance at δ 1.19 and that of the H_{ax}-8 proton at δ 1.74. The rest of nOe cross peaks shown in Figure 5 were in full agreement with the given stereostructure.

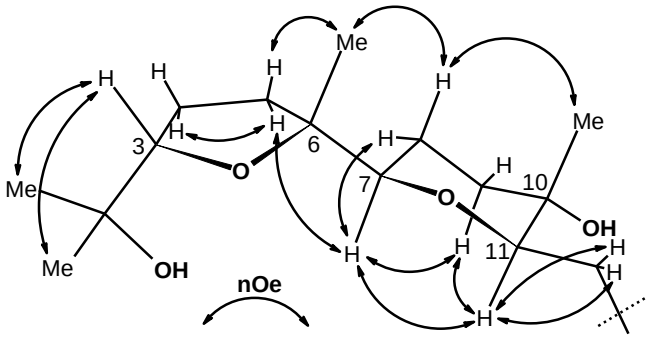


Figure 5. Summary of some significant 700 MHz NOESY correlations for compound **22** (due to the symmetry, half molecule is shown).

X-ray crystallography of compound 19

Molecules of **19** in the crystals are centrosymmetric (C_i point group) as they lie on crystallographic inversion centres (Figure 6). The molecules have a stretched winding shape, which is due to the double *cis-trans* sequence of the 2,5-disubstituted THF rings and to the *trans*-planar conformation of the carbon chain.

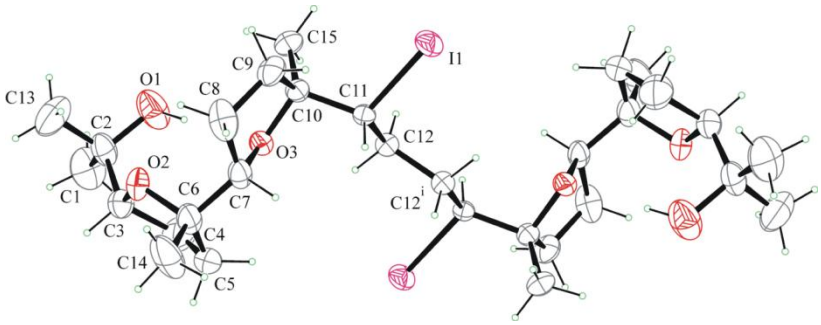


Figure 6. ORTEP view of **19**.

The molecular conformation is stabilized by an intramolecular H bonding between O–H donor and the oxygen acceptor of the inner THF ring (O1−H···O3 0.983, 2.224, 3.175(9) Å, 163°). Ring puckering coordinates of the inner THF ring are q_2 = 0.356(6) Å φ_2 = 211(1)°, and of the outer are q_2 = 0.316(7) Å φ_2 = 324(1)°. On the basis of the calculated phase angles, it can be argued that both

are basically in envelope conformation, with C7 and C4 atoms out of the envelope plane.

The packing of molecules is accomplished through weak H bonding interactions between iodine atoms as bifurcated acceptors and methyne C–H donors.[25] This is clearly shown in Figure 7. Chains of H-bonded molecules are formed which run along **b** + **c** and **b** – **c** lattice directions. The weak H-bonding leads to the formation of ring patterns having graph set descriptor $R_2^1(7)$. Along **a** molecules are stacked in layers through van der Waals contacts.

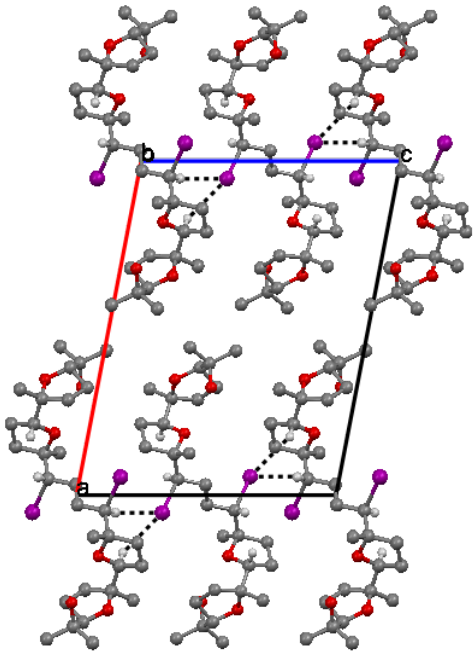


Figure 7. Crystal packing of **19** viewed down **b**.

4.3. Conclusions

In conclusion, a novel RuO_4-catalysed oxidation of squalene, characterised by a tandem penta-cyclization/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] Eventually, the isolation of bis-iodocompound **19** was interesting from both a mechanistic and a synthetic point of view. Its existence among the oxidation products of squalene with RuO_4 was indicative of the existence of an intermediate species (see **21**, Scheme 8), carrying oxoruthenium substituents, likely $ORuO_3$ groups, adjacent to the two internal THF rings, able to undergo a facile nucleophilic displacement, that enforces the mechanistic hypothesis previously put forward to explain the formation of penta-THF **1** from the same substrate (Scheme 5). In addition, the postulated mechanism for the formation of **19** also suggests a new possible use of the RuO_4-catalysed polycyclization process where suitable polyenes can be induced to undergo bidirectional poly-THF-forming oxidative sequences. The facile access to a novel type of bis-THF-bis-THP compound (**22**) has been demonstrated *via* a double ring-enlargement process. Studies are in progress to further develop the chemistry presented here toward the synthesis of new THP-containing polyether compounds.

4.4. Experimental Section

4.4.1 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 x 10 mm and 250 x 4.6 mm (both 5μ) 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 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. 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.

4.4.2 Bis-spiroketals 6-9, monospiroketal 17 and lactone 18

Squalene (50 g, 122 mmol), placed into a 5L round-bottomed flask equipped with a mechanical stirrer, was 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•$2H_2O$ (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 $Na_2S_2O_3$•$5H_2O$ 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 x 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 x 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 x 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 x 4 mm column, hexane-EtOAc, 85:15, t_R = 13.8 min).

Fraction B was chromatographed on silica gel (50 x 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.

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}. ^{1}H-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 (3xC), 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).

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}. ^{1}H-NMR (500 MHz, $CDCl_3$) δ_H 3.85 (1H, d, *J*= 7.2), 3.65 (1H, bt, *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).

8: Amorphous solid. IR (neat) 2969, 2870, 1457, 1374, 1213, 1174, 1105, 1042 (str), 989, 963, 906,

889, 869, 845 ν_{max} cm^{-1}. ^{1}H-NMR (500 MHz, $CDCl_3$) δ_H 3.94 (1H, bt, *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).

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}. ^{1}H-NMR (500 MHz, $CDCl_3$) δ_H 3.85 (1H, d, *J*= 7.1), 3.74 (1H, bdd, *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 (2xC), 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).

17: Amorphous solid. IR (neat) ν_{max} 3470 (OH), 2969, 2871, 1455, 1372, 1217, 1176, 1150, 1104, 1065, 1042(str), 996, 961cm^{-1}. ^{1}H-NMR (500 MHz, $CDCl_3$) δ_H 3.85 (4H, overlapped m's), 3.80 (1H, t, *J*= 6.8), 3.1 (1H, bs, OH) 1.47, 1.31, 1.24, 1.21, 1.15, 1.11, 1.07, 1.01 (3H each, s's, 8x Me); ^{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).

18: Oil; IR (neat) ν_{max} 2968, 2870, 1774, 1460, 1375, 1237, 1158, 1016, 962 cm^{-1}. ^{1}H-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, 1.35, 1.31, 1.25, 1.11, 1.01 (3H each, s's, 6x Me); ^{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).

4.4.3 Mono-spiroketalization of 17 to 9

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, $Na_2S_2O_3$ $5H_2O$ (excess) was added and the mixture was extracted with EtOAc (3x 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 ^{1}H-NMR. Yields based on reacted **17** at 70% conversion are as follows: bis-spiroketal **9** (25%), lactone **18** (25%).

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 $2H_2O$ (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.

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 x 1mL) 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%).

4.4.4 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 $0.5X10^5$ 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)-2H-tetrazolium, inner salt (MTS) assay and compared with their control.

4.4.5 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 MoKα 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.

***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}, 11495 reflections measured, 4948 unique which were used in all calculations. Final agreement indices were $wR(F^2)$ = 0.2810 (all data) and *R* = 0.0633 ($I>3\sigma(I)$). 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).

***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 *Aba*2 (no. 41), Z = 8, 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 ($I>3\sigma(I)$). 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).

***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}, 15654 reflections measured, 5021 unique which were used in all calculations. Final agreement indices were $wR(F^2)$ = 0.1304 (all data) and R = 0.0424 ($I>3\sigma(I)$). 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).

***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}, 13483 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).

4.4.6 Isolation of the bis-iodurated tetra-THF 19

Compound **19** was isolated from the less polar fraction A by HPLC separation (250×10 mm column, eluent: hexane-EtOAc, 8:2, flow 2.5 mL/min). The fraction eluted in the range 20-30 min

was subjected to a further reversed-phase HPLC separation (250×10 mm column; flow: 1.0 mL/min, eluent: MeOH/H_2O, 8:2, t_R = 14.5 min) to give pure **19** (2.5 mg, 0.03%).

19: IR (neat): ν_{max} 3706, 3780, 1054, 1013 cm^{-1}; ^{1}H-NMR: (400 MHz, $CDCl_3$) δ 4.00 (1H, bd, *J* = 9.7), 3.91 (1H, m), 3.85 (1H, dd, *J* = 7.7, 5.2), 2.32 (1H, m), 2.23 (1H, ddd, *J* = 12.1, 8.7, 8.7), 1.45, 1.25, 1.13, 1.09 (3H each, s's, 4xMe); ^{13}C-NMR (50 MHz, $CDCl_3$): δ 85.6, 85.0, 84.4, 83.0, 71.9, 47.9, 39.9, 36.4, 34.7, 27.9, 27.2, 25.8, 24.9, 24.3, 22.9; HRMS (ESI) *m/z* calcd for $C_{30}H_{52}I_2NaO_6$ $[M+Na]^+$ 785.1751, found 785.1748.

***Ring expansion of* 19 *to* 22**

To compound **19** (1.5 mg, 0.02 mmol) dissolved in acetone-water (4:1, 500 μL) was added silver carbonate (16.8 mg, 0.1 mmol) and the mixture stirred at 40 °C. After 4 h, the mixture was filtered and the solid thoroughly washed with acetone. The organic phase was taken to dryness to give an oily product. HPLC purification (250 × 4.6 mm column; flow: 1.0 mL/min; $CHCl_3$MeOH, 98:2) gave pure **22** (0.7 mg, 65%, t_R =16.5 min).

22: Oil; IR (neat): ν_{max} 3440 cm^{-1}; ^{1}H-NMR: (700 MHz, $CDCl_3$) δ 3.86 (1H, dd, *J* = 8.4, 3.7), 3.26 (1H, bdd, *J* = 7.0, 7.0), 3.16 (1H, bd, *J* = 6.3), 2.36 (1H, ddd, *J* = 10.0, 10.0, 10.0), 2.05 (1H, dddd, *J* = 12.7, 9.6, 3.7, 3.7), 2.03-1.94 (2H, m), 1.89 (1H, ddd, *J* = 12.5, 3.6, 3.6), 1.75 (2H, m), 1.54 (2H, m), 1.31 (1H, m), 1.23, 1.19, 1.12, 1.05 (3H each, s's, 4 × Me); HRMS (ESI) *m/z* calcd for $C_{30}H_{54}NaO_8$ $[M+Na]^+$ 565.3716, found 565.3710.

X-Ray Crystallography of compound 19

Crystals of **19** suitable for X-ray analysis were obtained from $CHCl_3$ by slow evaporation of the solvent. Data were collected at 298 K on a Bruker-Nonius Kappa-CCD diffractometer using graphite monochromated MoK*α* radiation (*λ* = 0.71073 Å). Data reduction and multi-scan absorption correction were done using SADABS program.[26] The structure was solved by direct methods (SIR97 program)[27] and refined by the full matrix least-squares method on F^2 using SHELXL-97 program [28] with the aid of the program WinGX [40]. Non-hydrogen atoms were refined anisotropically. H atoms of the hydroxy group was located in difference Fourier maps and refined with $U_{iso} = 1.2 \cdot U_{eq}$ of the carrier atom. The positions of the other H atoms were determined stereochemically and refined by the riding model with $U_{iso} = 1.2 \cdot U_{eq}$ of the carrier atom ($1.5 \cdot U_{eq}$ for

H atoms of methyl groups). Ring puckering coordinates [29] were determined using the program PARST.[30] The analysis of the crystal packing and the drawing of the molecule were performed using the programs Mercury [31] and ORTEP[32]. Crystal and refinement data are summarized in Table 1. CCDC reference number 821334 contains the supplementary crystallographic data for **19**.

4.5. References and notes

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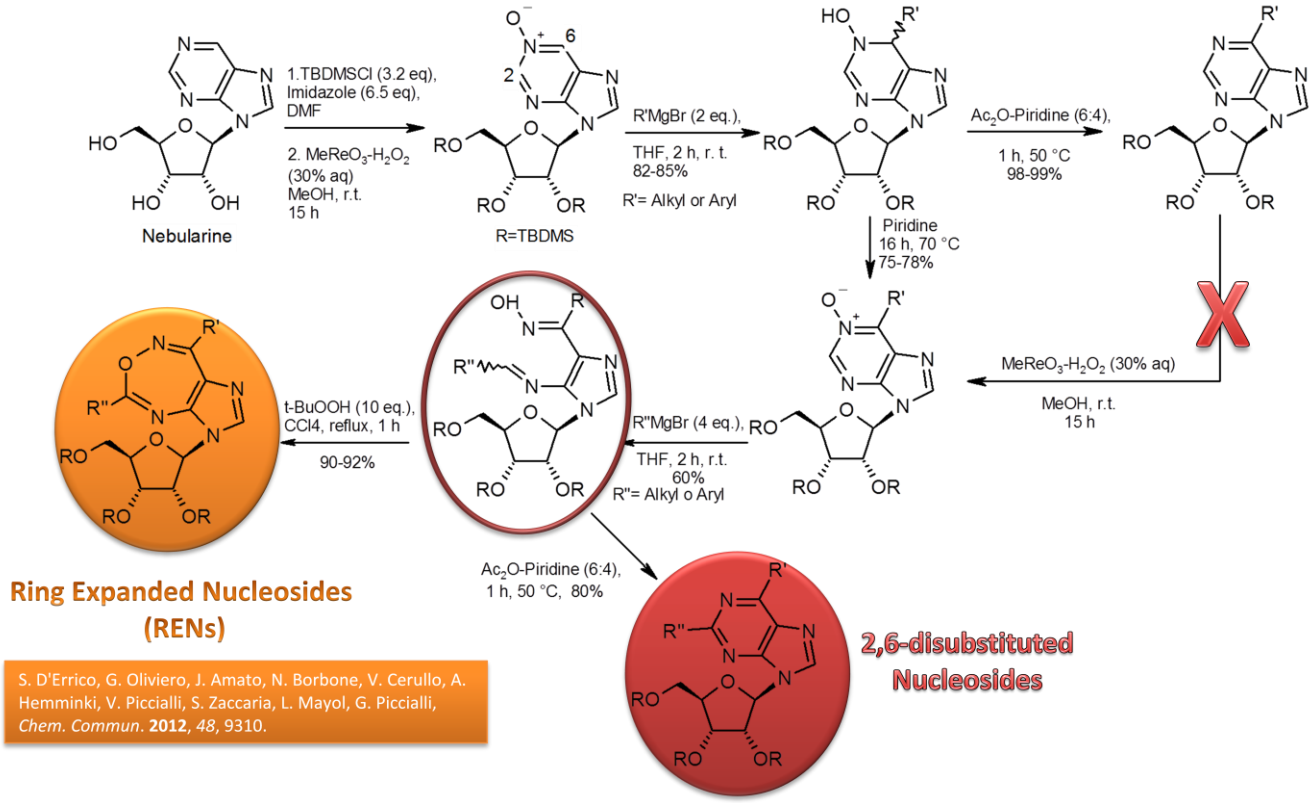
Part 3. *New synthetic routes for the preparation of nucleoside analogues*



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Chapter 5

Synthesis of new nucleoside analogues

5.1. Introduction

Besides being involved in nucleic acid replication and therefore in the transfer of genetic heritage from generation to generation, nucleosides take part in many metabolic processes by interacting with specific enzymes such as polymerases, kinases, reductases, motor proteins, membrane receptors and structural proteins, all targets of biological importance. No wonder then that nucleosides are among the most studied biological molecules and that they are currently used as antiviral,[1,2] antineoplastic[3-6] as well as antifungal[7-9] agents.

Recently, an increasing number of research groups have focused their attention on the preparation of new nucleoside and nucleotide analogues in order to widen the pool of these molecules with potential biological activity. To that end, structural modifications were made on the heterocyclic base and/or on the sugar. Most active derivatives contain modification on sugar portion so a large number of nucleobase analogues have been synthesized and employed against cancer and viral diseases. An important class of nucleoside analogues is that of purine bases and nucleosides bearing a C- or N-substituent at C-6. These molecules posses a wide range of biological effects including cytostatic, antiviral, antibacterial as well as receptor-modulation activity.[10]

The C-6 halogenation of the nucleo-base has opened the way to the construction of new libraries of C-6 modified nucleosides, generally through direct aromatic nucleophilic substitution (S_NAr),[11] or metal-mediated cross-coupling processes[12, 13] (Figure 1). The most reliable methods to access C-6 C-substituted nucleosides use metal or organometal-mediated reactions.[12]

The need for new methods for the C-6 derivatization led us to consider the possibility to exploit the reactivity of nitrones to reach this goal. Indeed, nitrones are precious substrates used in organic synthesis for the assembly of structurally complex nitrogen-containing compounds. Their most well-studied reactions are the 1,3-dipolar cycloadditions (1,3-DC) and the nucleophilic addition of organometallic reagents.[14]

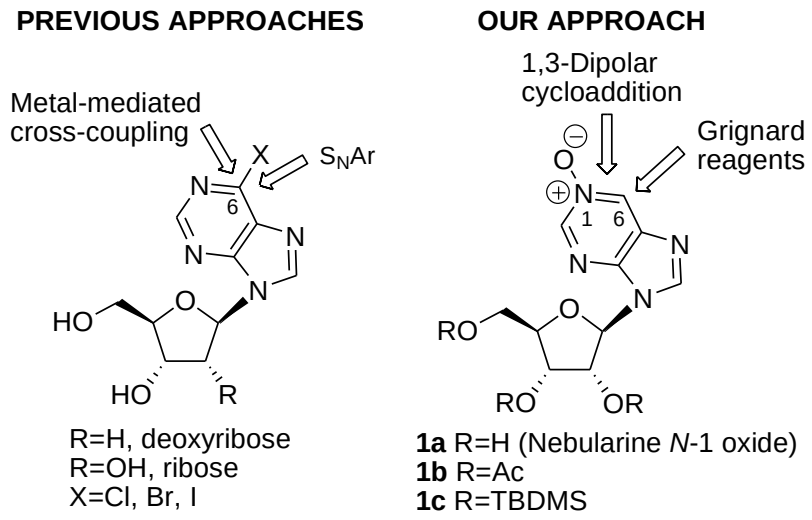


Figure 1. Approaches to C-6 functionalization of purine nucleosides

In the frame of a project joined in collaboration with the group of prof. L. Mayol from the "Dipartimento di Chimica delle Sostanze Naturali", oxidative processes have been applied to the synthesis of new nucleoside analogues possessing different base modifications. We reasoned that the formation of a purine N1-oxide by treatment of the purine with $MeReO_3$ followed by addition of a Grignard reagent to the electrophilic C-6 carbon of the substrate could allow the C-6 functionalization of the purine base (Figure 1).[15] Using this unprecedented approach new C-6 substituted purine nucleosides have been synthesized, without using metal catalysts. Inspired by these findings we envisaged that C-6 substituted nucleosides could be further derivatized at C-2 through formation of a new reactive C6-*N*1-O^- nitrone moiety and successive reaction of Grignard reagent to the electrophilic C-2 carbon. During our early experiments, serendipitously, unprecedented bis-alkylated imidazo[4,5-d][1,2,6]oxadiazepine nucleosides were obtained[16] instead of expected 2,6-disubstituted purine nucleosides. A small collection of these compounds have been synthesized and subjected to preliminary cytotoxicity tests on breast (MCF-7) and lung (A549) cancer cell lines. However, in subsequent experiments, our initial goal was successfully reached and a colletion of 2,6-dialkyl(aryl) purine nucleosides has been synthesized. In this chapter is therefore described the first general approach that allows either the synthesis of ring-expanded nucleosides (RENs), containing the unprecedented bis-alkylated imidazo[4,5-d][1,2, 6]oxadiazepine heterocyclic ring system, or the 2,6-dialkyl(aril)purine moiety.

5.1.1 Ring-expanded nucleosides (RENs) and 2,6-disubstituted purine nucleosides

Nucleosides containing a 5:7-fused ring system as nucleobase, also known as ring-expanded nucleosides (RENs), are very interesting molecules from several points of view. They are mimics of purine nucleosides and most of them possess significant biological activities due to their interference with the enzymes involved in the purine metabolism.[17] Coformycin and pentostatin (2'-deoxycoformycin) are two naturally occurring RENs containing an imidazo[4,5-*d*][1,3]diazepine ring system. They show a potent antitumor activity due to the strong inhibition of adenosine deaminase (ADA), acting as transition-state analogues.[18,19] Pentostatin is a drug approved in 1991 by FDA for the treatment of hairy cell leukemia. However, it is characterized by a severe toxicity that limits its use in therapy.[17] A number of coformycin analogues have been synthesized, both to investigate the structure-activity relationship and to produce less toxic derivatives.[20-25] Furthermore, imidazo[4,5-*c*]azepine nucleosides, ring-expanded derivatives of xanthosine, guanosine and inosine, have been investigated and their cytotoxic activity has been reported.[26] RENs have shown antiviral properties as well. Coformycin and some of its derivatives are able to enhance the activity of therapeutic antiviral drugs such as 2′,3′-dideoxyadenosine (ddA) and 2'-fluoro-2',3'-dideoxyadenosine (FddA). Other RENs, for example those containing the 6-alkylamino-substituted imidazo[4,5-*e*][1,3]diazepine or the imidazo[4,5-*e*][1,2,4]triazepine moiety, show direct antiviral activity against human hepatitis B virus and West Nile Virus.[24]

Due to their noncanonical steric hindrance, RENs have been inserted in oligonucleotides to study conformational and stability changes in duplex, triplex and other DNA secondary structures, as well as H-bonding profile, base stacking, *endo/exo* sugar puckering and α/β anomeric ribose configuration.[27] From a synthetic point of view, the construction of a nucleoside containing a 5:7-fused ring base system can be performed using a sugar unit bearing a functionalized imidazole (or other 5-terms cycles), on which the seven-membered ring closure can be accomplished by inserting an appropriate molecular moiety.[25, 26, 28]

Alternatively, a pre-synthesized 5:7-terms heterocyclic system can be joined to a sugar moiety by reaction with an activated glycosyl donor.[23, 29, 30] The latter approach is longer but it does not suffer from problems due to the weakness of N-glycosidic bond and it also offers the chance to synthesize both α/β nucleoside isomers.

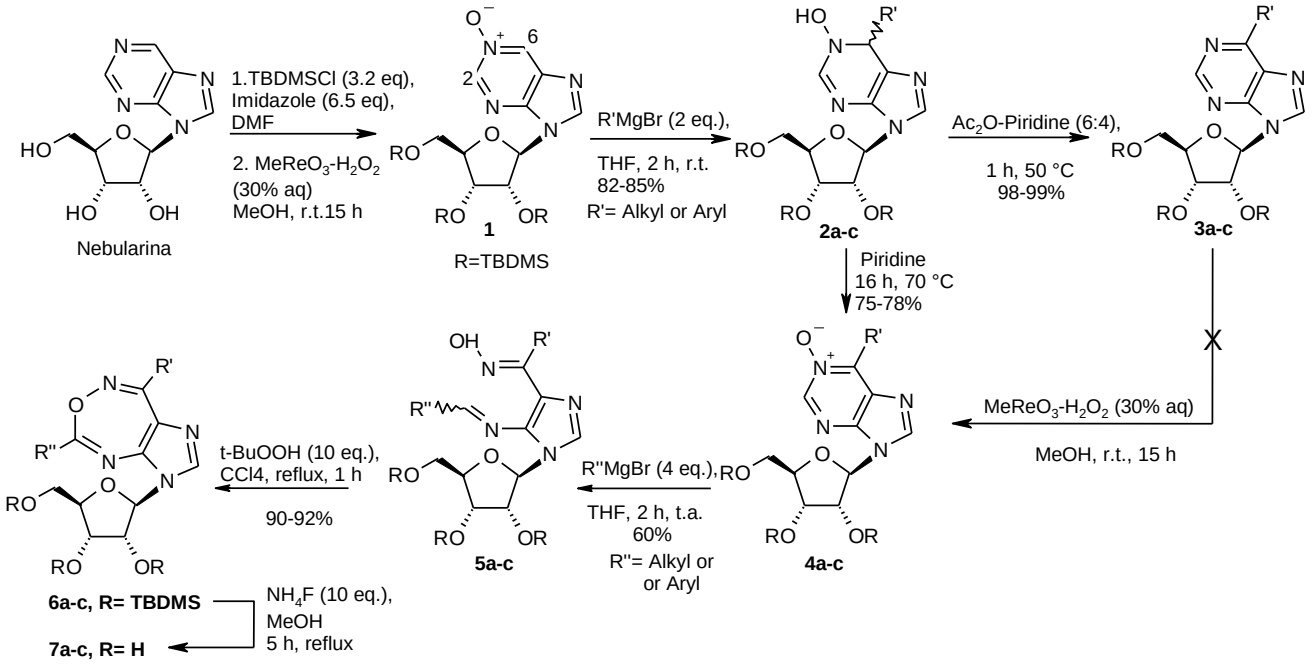
A considerable number of 2,6-disubstituted purines, their nucleosides, and nucleotides exert inhibitory activity in various biological systems. [31] In a recent paper by Hocek, the synthesis of

2-substituted 6-phenylpurine has been reported. Two alternative approaches for the synthesis of these compounds have been used. The first is based on the suzuki-Miayaura cross-coupling reaction of 2,6-dihalopurines with phenylboronic acid, the second features the deaminative transformation of 2-amino-6-phenylpurine nucleosides.

5.2. Results and Discussion

5.2.1 Synthesis and biological evaluation of unprecedented ring-expanded nucleosides (RENs) containing the imidazo[4,5-d][1,2,6]oxadiazepine ring system

We have developed the synthesis of new ring-expanded nucleosides (Scheme 1) containing the unprecedented imidazo[4,5-*d*][1,2,6]oxadiazepine heterocyclic system as the base moiety through a new approach featuring the opening/reclosure of the pyrimidine ring system of 2',3',5'-tri-*O*-(*tert*-butyldimethylsilyl) nebularine N1-oxide **1**. Our synthetic strategy allowed us to prepare a small set of 5,8-dialkyl(aryl)-substituted imidazo[4,5-*d*][1,2,6]oxadiazepine nucleosides (**7a-c**, Table 1), in high yields, exploiting the previously reported reactivity of **1** towards Grignard reagents.[16]



Scheme 1. Synthesis of new ring-expanded nucleosides from Nebularine.

Table 1. 5,8-dialkyl(aryl)-substituted imidazo[4,5-*d*][1,2,6]oxadiazepine nucleosides synthesized.

Entry	R'	R''	7a-c*
a	-Et	-Me	34
b	-Me	-Et	34
c	-Ph	-Et	32

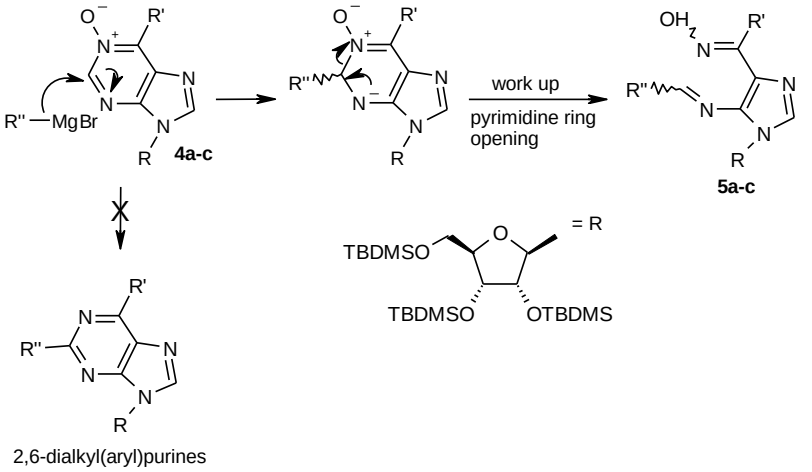
*Reaction yields (%) from **1**.

The reactivity of α-carbons towards Grignard reagents in aromatic nitrones has previously

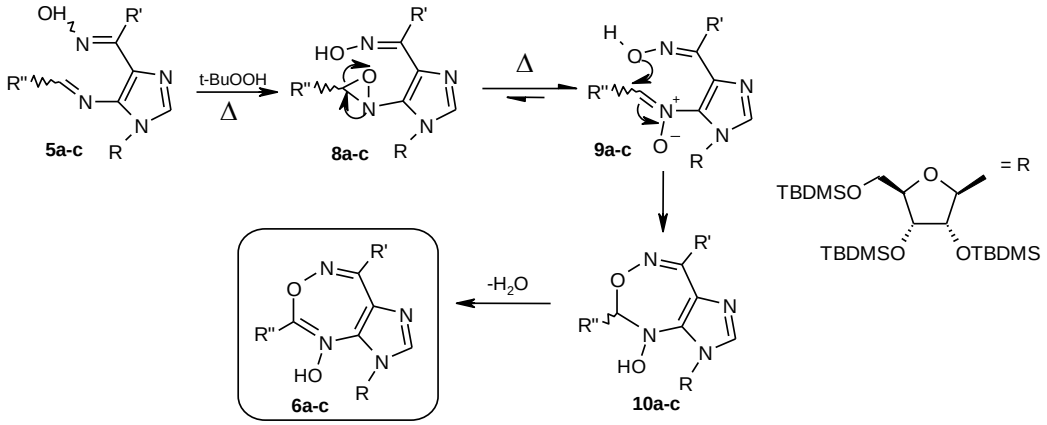
been observed for pyridine N-oxide[32-34] which results in cleavage of the N-Cα bond with the concomitant formation of oxime and *trans* alkene functions. In our previous studies[16] we observed that reaction of nitrone **1** with a Grignard reagent leads to addition at C6 furnishing the adducts **2**, as a mixture of C6 diastereomers, which slowly decomposed on standing, partly giving dehydration to C6-alkyl(aryl)nebularines **3**. This process was pushed towards aromatization by treatment of the crude adduct **2** with Ac_2O in pyridine at 50°C, to give **3** in nearly quantitative yields.[35]

We reasoned that the introduction of a N1-nitrone function on **3** could induce an electrophilic behaviour on C2 as well, thus allowing to obtain, at least in priciple, 2,6-dialkylpurine. To probe this hypothesis the representative compound **3a** was treated with catalytic amounts of methyltrioxorhenium ($MeReO_3$) in the presence of H_2O_2, the same oxidizing mixture used to obtain nitrone **1** from silylated nebularine. Under these conditions, **3a** produced only traces of N1-oxide **4a**. Alternatively, the oxidation of the N1-hydroxy derivative **2a** with air in pyridine at 70 °C afforded the C6-ethylnebularine N1-oxide **4a** in 78% yield. The similar procedure using DMF as a solvent was reported for 2,6-alkyl(aryl)-substituted N-hydroxypyridine derivatives.[34]

The successive reaction of **4a** with Grignard reagents confirmed the electrophilic reactivity of C2 but, in this case, the addition was followed by the breaking of the N1/C2 bond leading to compound **5a** (60% yield) as a 1:1 mixture of (*E/Z*) isomers at the imine double bond (Scheme 2). In particular, 2D NMR experiments and HRMS data confirmed the structure of **5a**. The HMBC experiment supported the presence of the aldimine moiety showing clear correlations between the aldimine proton and both the aldimine carbon and the Grignard residue R". Furthermore, the absence of correlation between aldimine proton and C6 of the purine ring strongly suggested the opening of the cycle at the N1/C2 bond. Next, we tested the reactivity of the open system of **5a** as for the possible ring reclosure. In particular, treatment of **5a** with ^tBuOOH (10 equiv.) in refluxing CCl_4 gave nucleoside **6a**, containing the imidazo[4,5-*d*][1,2,6]oxadiazepine framework (Scheme 3) in a 92% yield.



Scheme 2. Proposed mechanism for the formation of compounds **5a-c**.



Scheme 3. Proposed mechanism for the formation of compounds **6a-c**.

The obtainment of the seven-membered ring could be explained invoking the formation of the oxaziridine **8a** (Scheme 3) through oxidation of the aldimine functionality.[36]

It is reported that nucleophiles usually open the oxaziridine ring by attack at the nitrogen or oxygen[37] atom rather than on the carbon atom. However, it is known that oxaziridines containing aromatic substituents could easily rearrange to nitrones on heating,[38] making the carbon the preferred attacked site. Accordingly, nitrone **9a**, derived from **8a**, would undergo a fast ring reclosure by nucleophilic attack of the oxime hydroxyl function[‡,39] on the nitrone carbon,[40] to give intermediate **10a** (not isolable) which would lead to the oxadiazepine cycle of **6a** by water loss.

Previous mechanistic studies on the reactivity of 2-haloquinazoline 3-oxide system with alkali[41] showed that the opening of the pyrimidine ring by nucleophilic attack of the hydroxyl

ion at C2 afforded an *anti*-oxime. In our case, 2D-NMR studies on compound **5a** performed in pyridine-d_5 did not give conclusive information on the oxime configuration, although a (*Z*) configuration is needed for the ring closure (**9a** → **10a**).

The same reaction sequence leading to **6a** furnished compounds **6b-c** in comparable yields to **6a** (Scheme 1). Compounds **5a-c** are rather unstable and decompose on standing within few hours. Therefore, reactions on **5b** and **5c** were performed on the crude reaction mixture without observing lowering of the yields.

Finally, nucleosides **6a-c** were deprotected at ribose using NH_4F in MeOH[42] to give, after HPLC purification, compounds **7a-c** (94-97% yield, Scheme 1).

The structure of nucleosides **6a-c** and **7a-c** was confirmed by 2D-NMR experiments and HRMS data. Moreover, the disappearance of the characteristic purine band around 260 nm in the UV spectra of **6a-b**[43] provided further evidence of the 7-membered ring formation, excluding the presence of the isomeric 2,6-disubstituted purine N1-oxide system.

Considering that RENs significantly inhibit the growth of several cancer cell lines,[17] we tested the cytotoxicity of the novel RENs by treating lung A549 and breast MCF-7 cancer cell lines with different concentrations (0.1, 1.0, 10 and 100 μM) of **7a-c**. At different time points for each concentration the cell viability was determined by measuring the mitochondrial activity.[44] Cisplatin was used as the control since its activity in these cell lines has been extensively studied.[45, 46] We found that at day 7 all tested compounds significatively reduced the cell viability of breast cancer derived MCF-7 cells at the highest tested concentration (also at 10 μM in the case of **7a**). An explanation for such a delayed activity could be the slow formation of an active metabolite during the assay. At the same time point the inhibition of A549 cells growth, albeit observed, was only marginal (Figure 2 MCF-7 cell line; Figure 3 A549 cell line, see also § 5.4.3 and 5.4.4).

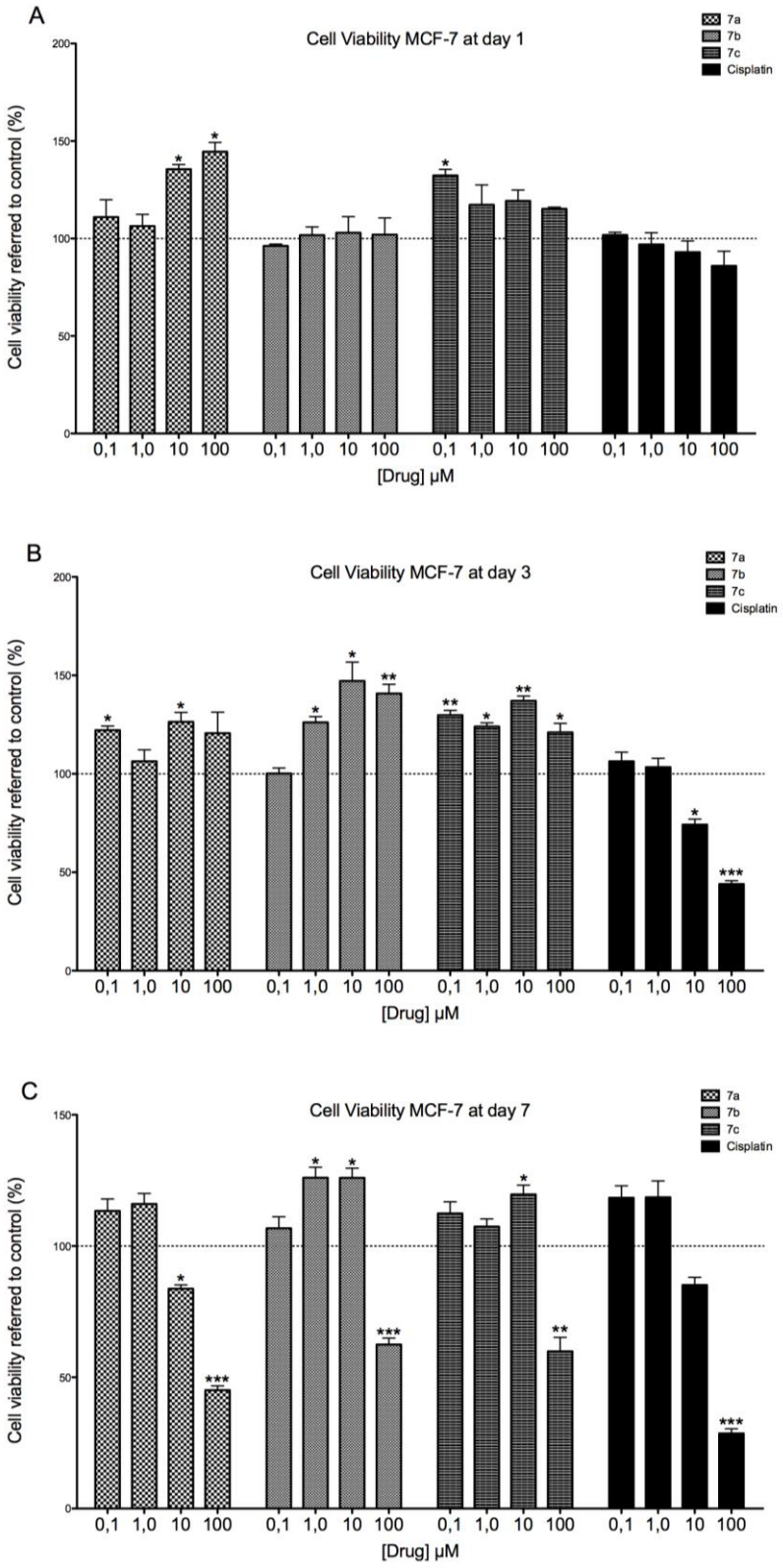


Figure 2. Cytotoxic effect of RENs 7a-c and cisplatin in MCF-7 breast cancer-derived cell line. MCF-7 cell line was treated with four different concentrations (0.1 µM, 1 µM, 10 µM and 100 µM) of compound **7a-c** and cisplatin. Cell viability (% referred to untreated cells) was assessed at day 1 (Panel A), day 3 (Panel B) and day 7 (Panel C). Columns, mean of quadruplicates; bars, SE (*$p < 0.05$; **$p < 0.01$;***$p < 0.001$).

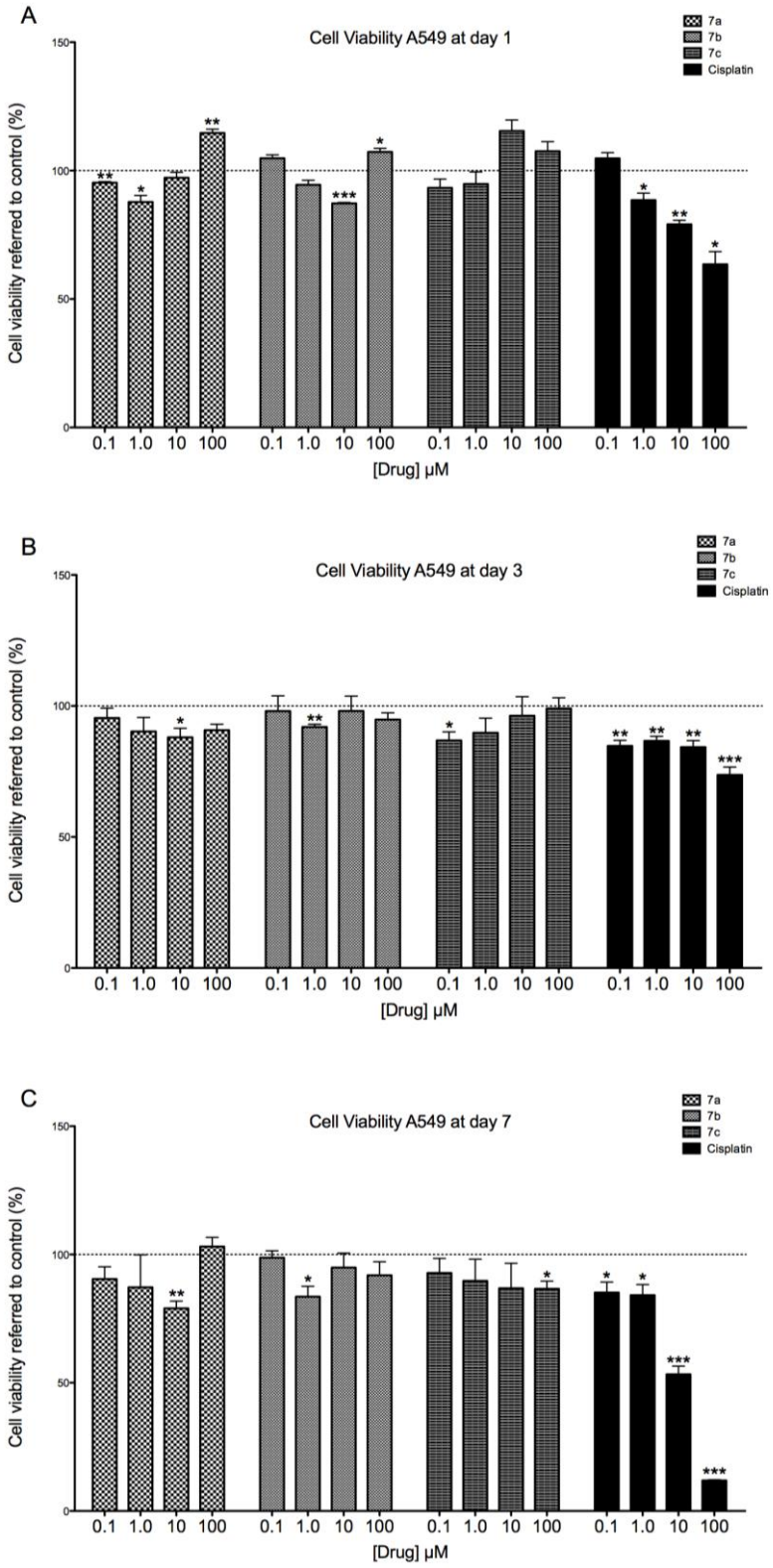
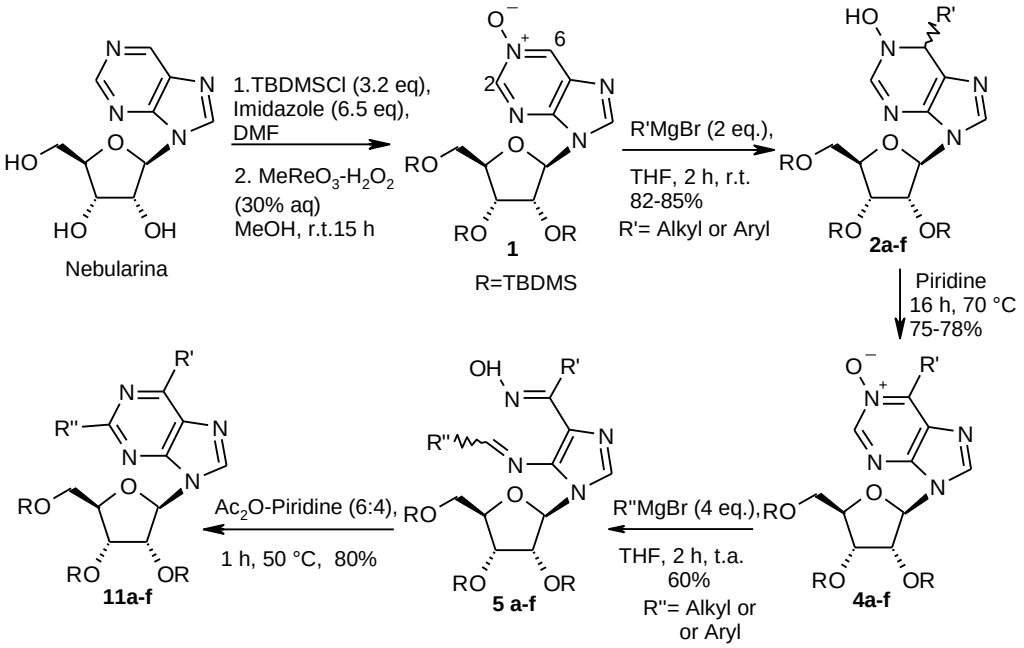


Figure 3. Cytotoxic effect of RENs 7a-c and cisplatin in A549 lung cancer-derived cell line. A549 cell line was treated with four different concentrations (0.1 µM, 1 µM, 10 µM and 100 µM) of compound **7a-c** and cisplatin. Cell viability (% referred to untreated cells) was assessed at day 1 (Panel A), day 3 (Panel B) and day 7 (Panel C). Columns, mean of quadruplicates; bars, SE (*$p < 0.05$; **$p < 0.01$;***$p < 0.001$).

5.2.2 Synthesis of 2,6-disubstituted purine nucleosides

The synthesis of 2,6-disubstituted purine nucleosides, our initial goal, has been achieved in subsequent experiments. Indeed, it was found that by treating intermediate **5** (Scheme 4) with Ac_2O in pyridine the expected compounds were obtained in good yields (60-83%). The scope of the process has been successfully proven using various Grignard reagents pairs carrying both alkyl and aryl substituents (Table 2). In this way, a collection of six 2,6-disubstituted nucleoside analogues has been synthesized. We are currently planning to synthesize other compounds of this type, which will be then deprotected and used in biological assays and SAR studies.



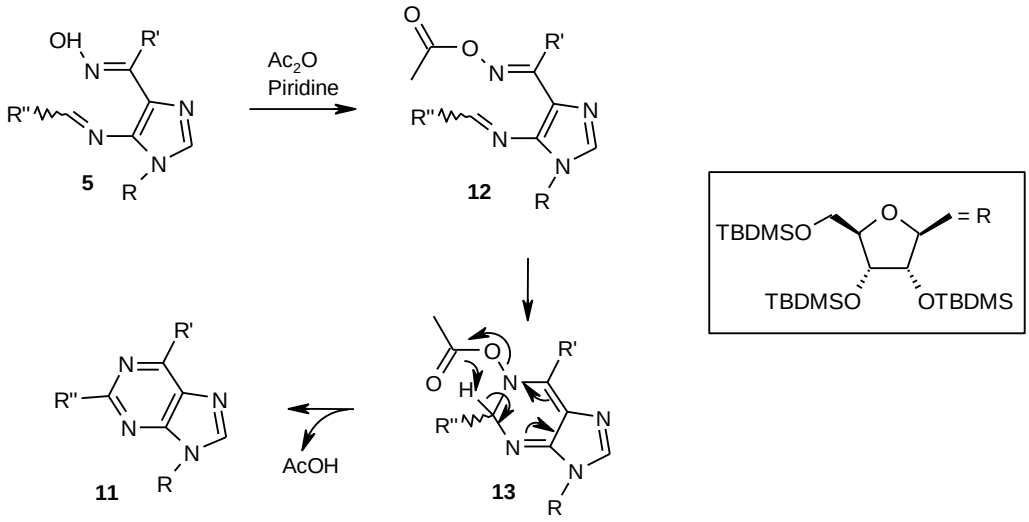
Scheme 4. Synthesis of 2,6-disubstitued purine.

Table 2. 2,6-dialkyl(aryl)-substituted nucleosides synthesized.

Entry	R'	R"	Yield* (%)
a	CH_2CH_3	$-CH_3$	80
b	$-CH_2CH_3$	- Ph	75
c	$-CH_3$	$-CH_2CH_3$	80
d	-Ph	- Ph	83
e	$-(CH_2)_3OTBDPS$	-Ph	60
f	$-(CH_2)_3OTBDPS$	-Et	61

*Reaction yields (%) from **1**.

From a mechanistic point of view we assume that the open system of **5**, once acylated at the ketoxime functionality (**12**), undergoes an electrocyclic process with ring reclosure (**13**) and successive rearomatization by elimination of acetic acid (Scheme 5).



Scheme 5. Mechanistic hypotesis for the formation of 2,6-disubstituted purine nucleosides.

5.3. Conclusions

In conclusion, we have developed new reactions on the purine nucleobase enlarging the synthetic toolbox to obtain novel nucleoside analogues. The reactivity of the herein proposed C6-alkyl(aryl)purine-N1-oxides towards Grignard reagents afforded the new 4,5-disubstituted imidazo-nucleosides **5** from which the unprecedented imidazo[4,5-*d*][1,2,6]oxadiazepine nucleosides were built in good yields by treatment with ^{t}BuOOH. It is to be noted that this heterocyclic system has not been so far reported and the proposed synthetic strategy allows the introduction of C-substituents on C5 and C8 of the oxadiazepine cycle. We have also reported that new RENs are able to inhibit the cell growth of breast cancer derived MCF-7 cells. The observed cytotoxic effects of **7a-c** were dose-dependent and comparable to those of cisplatin.

In addition, we have also reported that, treating the same intermediate **5** with Ac_2O-pyridine it is possble to synthetize 2,6-dialkyl(aryl)-substituted purine nucleosides.

Further studies to enlarge the collection of the above nucleoside analogues, to investigate the reactivity of nebularine N1-oxide toward other C-nucleophiles, including organo-lithium reagents, as well as the reactivity of the open intermediates **5a-c**, will be carried out.

5.4. Experimental section

5.4.1 General methods

All the reagents were obtained from commercial sources (Sigma-Aldrich) and were used without further purification. ^{1}H and ^{13}C-NMR spectra were acquired on a Varian Mercury Plus 400 MHz and on a Varian Unit Inova 700 MHz in CD_3OD or $CDCl_3$. Chemical shifts are reported in parts per million (δ) relative to the residual solvent signals: CD_2HOD 3.31 and $CHCl_3$ 7.27 for ^{1}H-NMR; CD_2HOD 49.0 and $CHCl_3$ 77.0 for ^{13}C-NMR. ^{1}H-NMR chemical shifts were assigned by 2D NMR experiments. The abbreviations s, bs, d, dd and m stand for singlet, broad singlet, doublet, doublet of doublets and multiplet, respectively. HPLC analyses and purifications were carried out on a Jasco UP-2075 Plus pump equipped with a Jasco UV-2075 Plus UV detector using a 4.60 x 150 mm LUNA (Phenomenex) silica column (particle size 5 µm) eluted with a linear gradient of MeOH in AcOEt (from 0 to 5% in 15 min, flow 1.0 mL min^{-1}, system A), with a linear gradient of AcOEt in *n*-hexane (from 0 to 100% in 30 min, flow 1.0 mL min^{-1}, system B) or using a 4.8 x 150 mm C-18 reverse-phase column (particle size 5 µm) eluted with a linear gradient of MeOH in H_2O (from 0 to 100% in 30 min, flow 1.3 mL min^{-1}, system C). UV spectra were recorded on a Jasco V-530 UV spectrophotometer. High Resolution MS spectra were recorded on a Bruker APEX II FT-ICR mass spectrometer using electrospray ionization (ESI) technique in positive mode. Elemental analyses were performed on a Thermo Finnigan Flash EA 1112 CHN analyser. IR spectra were recorded on a Jasco FT-IR 430 spectrophotometer. Optical rotations were determined on a Jasco polarimeter using a 1 dm cell at 25 °C; concentrations are in g/100 mL. Preparative PLC chromatography was performed using F254 silica gel plates (0.5 mm thick, Merck). Analytical TLC analyses were performed using F254 silica gel plates (0.2 mm thick, Merck). TLC spots were detected under UV light (254 nm). For MTS assays the UV absorbance at 490 nm was read using a B**e**ckman Anthos 96 well Microplate Reader.

5.4.2 Experimental procedures for preparation of RENs 7a-c

For the general procedure for the preparation of compounds **3a-c** see ref. 15.

General procedure for the preparation of compounds 4a-c

The mixtures of diastereomers **2a-c** (0.030 mmol), prepared as previously described[15] were dissolved in pyridine (0.5 mL) and stirred at 70 °C for 18 h. Pyridine was evaporated under reduced pressure and the crudes were purified on silica gel plates (developing system: AcOEt/MeOH, 95:5). The bands were scratched from the plates and the products were eluted with AcOEt/MeOH, 7:3 (50 mL), affording compounds **4a-c**, the purity of which was checked by HPLC (system A, see General Methods).

2',3',5'-Tri-*O*-(*tert*-butyldimethylsilyl)-6-ethyl nebularine N-1 oxide 4a

Oil (15.0 mg, 0.023 mmol, 78%). $[\alpha]_D$ -14.0 (*c* =0.9 ,CH_3OH). ^{1}H-NMR (400 MHz, CD_3OD) ppm 8.98 (s, 1H, H-2), 8.80 (s, 1H, H-8), 6.11 (d, *J* = 5.0 Hz, 1H, H-1'), 4.80-4.75 (m, 1H, H-2'), 4.46-4.42 (m, H-3'), 4.20-4.15 (m, 1H, H-4'), 4.05 (dd, *J* = 11.5, 4.3 Hz, H-5'$_a$), 3.87 (dd, *J* = 11.5, 2.9 Hz, H-5'$_b$), 3.37 (q, *J* = 7.5 Hz, 2H, C*H*$_2$CH$_3$), 1.14 (t, *J* = 7.5 Hz, 3H, CH$_2$C*H*$_3$), 0.97 (s, 9H, C(CH$_3$)$_3$), 0.96 (s, 9H, C(CH$_3$)$_3$), 0.81 (s, 9H, C(CH$_3$)$_3$), 0.17 (s, 3H, CH$_3$), 0.16 (s, 3H, CH$_3$), 0.15 (s, 6H, 2 x CH$_3$), 0.020 (s, 3H, CH$_3$), -0.21 (s, 3H, CH$_3$). ^{13}C-NMR (100 MHz, $CDCl_3$) ppm 154.5, 145.6 (two C), 141.6, 132.9, 88.3, 86.0, 76.1, 72.0, 62.5, 26.1, 25.8, 25.6, 19.8, 18.5, 18.0, 17.8, 10.3, -4.4, -4.6, -4.7, -5.2, -5.4. *m/z* 661.3620 (HRESIMS) ($[M+Na]^+$, $C_{30}H_{58}N_4NaO_5Si_3$, requires 661.3613). IR (neat) ν_{max} 2923, 1470, 1251, 1122, 834, 781 cm^{-1}; UV (MeOH) λ_{max} 226, 325 nm, shoulders 240, 267 nm.

2',3',5'-Tri-*O*-(*tert*-butyldimethylsilyl)-6-methyl nebularine N-1 oxide 4b

Amorphous white solid (14.4 mg, 0.023 mmol, 78%). $[\alpha]_D$ -19.0 (*c* =0.2 ,$CHCl_3$). ^{1}H-NMR (400 MHz, CD_3OD) ppm 8.99 (s, 1H, H-2), 8.80 (s, 1H, H-8), 6.11 (d, *J* = 5.0 Hz, 1H, H-1'), 4.78-4.74 (m, 1H, H-2'), 4.45-4.40 (m, H-3'), 4.19-4.15 (m, 1H, H-4'), 4.05 (dd, *J* = 11.5, 4.3 Hz, H-5'$_a$), 3.86 (dd, *J* = 11.5, 2.8 Hz, H-5'$_b$), 2.85 (s, 3H, CH$_3$), 0.96 (s, 9H, C(CH$_3$)$_3$), 0.95 (s, 9H, C(CH$_3$)$_3$), 0.81 (s, 9H, C(CH$_3$)$_3$), 0.16 (s, 3H, CH$_3$), 0.15 (s, 6H, 2 x CH$_3$), 0.14 (s, 3H, CH$_3$), 0.020 (s, 3H, CH$_3$), -0.21 (s, 3H, CH$_3$). ^{13}C-NMR (100 MHz, CD_3OD) ppm 152.0, 148.5, 146.2, 144.0, 134.5, 90.0, 87.2, 77.2, 73.2, 63.5, 26.5, 26.4, 26.2, 19.3, 18.9, 18.7, 12.3, -4.1, -4.3, -4.8, -5.2, -5.3 *m/z* (HRESIMS) 647.3463 ($[M+Na]^+$, $C_{29}H_{56}N_4NaO_5Si_3$, requires 647.3456). IR (neat) ν_{max} 2927, 1475, 1248, 1126, 831, 787 cm^{-1}; UV (MeOH) λ_{max} 240, 328 nm, shoulders 226, 266 nm.

2',3',5'-Tri-*O*-(*tert*-butyldimethylsilyl)-6-phenyl nebularine N-1 oxide 4c

Pale yellow oil (15.0 mg, 0.022 mmol, 75%). $[\alpha]_D$ -38.2 (*c* =1.9, CH_3OH). ^{1}H-NMR (400 MHz, CD_3OD) ppm 9.01 (s, 1H, H-2), 8.81 (s, 1H, H-8), 8.20-8.16 (m, 2H, HPh), 7.65-7.60 (m, 3H, HPh), 6.15 (d, *J* = 4.9 Hz,1H, H-1'), 4.81-4.76 (m, 1H, H-2'), 4.48-4.43 (m, 1H, H-3'), 4.21-4.16 (m, 1H, H-4'), 4.07 (dd, *J* = 11.5, 4.2 Hz, 1H, H-5'$_a$), 3.87 (dd, *J* = 11.5, 2.7 Hz, 1H, H-5'$_b$), 0.98 (s, 9H, $C(CH_3)_3$), 0.95 (s, 9H, $C(CH_3)_3$), 0.84 (s, 9H, $C(CH_3)_3$), 0.17 (s, 3H, CH_3), 0.15 (s, 9H, 3 x CH_3), 0.050 (s, 3H, CH_3), -0.15 (s, 3H, CH_3). ^{13}C-NMR (100 MHz, CD_3OD) ppm 152.4, 149.3, 148.9, 147.5, 145.3, 133.6, 132.6, 132.2 (two C), 129.2 (two C), 127.8, 90.0, 87.1, 77.3, 73.1, 63.4, 26.5, 26.3, 26.2, 19.3, 18.9, 18.7, -4.1, -4.3, -4.8, -5.2, -5.3. *m/z* (HRESIMS) 709.3624 ($[M+Na]^+$, $C_{34}H_{58}N_4NaO_5Si_3$, requires 709.3613). IR (neat) ν_{max} 2950, 2928, 2851, 1253, 1157, 836, 776 cm^{-1}; UV (MeOH) λ_{max} 258, 346 nm, shoulder 300 nm.

General procedure for the preparation of compounds 6a-c

In a flamed round bottom flask charged with dry nitrogen, **4a-c** (0.020 mmol), dissolved in dry THF (0.5 mL), were added via cannula. To the flasks, fresh Grignard reagents (0.080 mmol) were quickly added in one portion and the mixtures were stirred for 2 h (TLC monitoring: AcOEt/MeOH, 95:5) at room temperature. The reactions were quenched by addition of a 1 M solution of NH_4Cl (1 mL), diluted with AcOEt (10 mL) and extracted with brine (2 x 10 mL). The organic layers were separated, dried (Na_2SO_4), filtered and concentrated under evaporation. Compound **5a** was purified as indicated for **4a-c**, while compounds **5b-c** were used for the next reaction step without further purification. **5a-c** were dissolved CCl_4 (0.5 mL) and then ^{t}BuOOH (10 equiv.) was added in one portion. The systems were gently refluxed for 1 h (TLC monitoring: AcOEt/MeOH, 95:5) and then evaporated under reduced pressure. The crudes were purified by HPLC (system A for **6a-b**, system B for **6c**, see General Methods) affording compounds **6a-c**.

5-(*E,Z*)-Ethylideneamino-1-[2,3,5-tri-O-(tert-butyldimethylsilyl)-β-D-ribofuranosyl]-3H-imidazo-4-(*E,Z*)-propanoxime 5a (major isomer)

Oil (7.8 mg, 0.012 mmol, 60%). Element. Anal. Calcd. for $C_{31}H_{62}N_4O_5Si_3$ C, 56.84; H, 9.54; N, 8.55; found C, 56.81; H, 9.57; N, 8.59; ^{1}H-NMR (500 MHz; pyridine-d_5): δ 12.80 (s, 1H, NOH), 8.91 (q, *J* = 4.8 Hz, 1H, N=C*H*CH_3), 8.31 (s, 1H, H-2), 6.25 (d, *J* = 5.0 Hz, 1H, H-1'), 4.81-4.77 (m, 1H, H-2'), 4.56-4.52 (m, 1H, H-3'), 4.33-4.29 (m, 1H, H-4'), 4.07 (dd, *J* = 11.3, 4.1 Hz, 1H, H-5'$_a$), 3.91 (dd, *J* = 11.3, 2.7 Hz, 1H, H-5'$_b$), 3.35 (q, *J* = 7.4 Hz, 2H, C$H_2$$CH_3$), 1.93 (d, *J* = 4.8 Hz, 3H,

N=CHC*H*$_3$), 1.38 (t, *J* = 7.4 Hz, 3H, CH_2CH_3), 0.98 (s, 18H, 2 x $C(CH_3)_3$), 0.95 (s, 9H, $C(CH_3)_3$), 0.19 (s, 3H, CH_3), 0.18 (s, 3H, CH_3), 0.17 (s, 3H, CH_3), 0.16 (s, 3H, CH_3), 0.13 (s, 3H, CH_3), 0.06 (s, 3H, CH_3). ^{13}C-NMR (175 MHz; pyridine-d_5) ppm 168.0, 157.4, 139.5, 133.5, 125.9, 88.8, 85.6, 77.2, 73.0, 63.6, 26.60 (two C), 26.58, 26.43 (four C), 26.38 (two C), 23.6, 21.3, 19.0, 18.8, 18.6, 12.0, -3.8, -4.1, -4.2, -4.3, -4.89, -4.93. *m*/*z* 654.4032 (HRESIMS) ($[M+Na]^+$, $C_{31}H_{62}N_4NaO_5Si_3$, requires 654.4028). UV (MeOH) λ_{max} 232 nm, shoulder 264 nm.

8-Ethyl-5-methyl-3-[2,3,5-tri-*O*-(*tert*-butyldimethylsilyl)-β-D-ribofuranosyl]-3*H*-imidazo[4,5-*d*][1,2,6]oxadiazepine 6a

Amorphous white solid (7.1 mg, 0.011 mmol, 92%). Element. Anal. Calcd. for $C_{31}H_{60}N_4O_5Si_3$ C, 57.01; H, 9.26; N, 8.58; found C, 57.02; H, 9.28; N, 8.60. $[\alpha]_D$ -37.7 (*c* = 0.3 ,CH_3OH). ^{1}H-NMR (400 MHz, CD_3OD) ppm 8.72 (s, 1H, H-2), 6.10 (d, *J* = 4.9 Hz, H-1'), 4.80-4.76 (m, 1H, H-2'), 4.45-4.42 (m, 1H, H-3'), 4.19-4-14 (m, 1H, H-4'), 4.07 (dd, *J* = 11.9, 5.4 Hz, 1H, H-5'$_a$), 3.86 (dd, *J* = 11.9, 2.6 Hz, 1H, H-5'$_b$), 3.37 (q, *J* = 7.6 Hz, 2H, C*H*$_2$CH$_3$), 2.82 (s, 3H, CH_3), 1.41 (t, *J* = 7.6 Hz, 3H, CH_2CH_3), 0.97 (s, 9H, $C(CH_3)_3$), 0.95 (s, 9H, $C(CH_3)_3$), 0.82 (s, 9H, $C(CH_3)_3$), 0.17 (s, 3H, CH_3), 0.15 (s, 9H, 3 x CH_3), 0.019 (s, 3H, CH_3), -0.20 (s, 3H, CH_3). ^{13}C-NMR (100 MHz, CD_3OD) ppm 157.3, 156.2, 147.8, 143.7, 133.0, 90.3, 87.2, 77.4, 73.3, 63.7, 26.8, 26.6, 26.4, 21.3 (two C), 19.6, 19.1, 18.9, 11.1, -3.8, -4.2, -4.6, -4.9, -5.1 . *m*/*z* 675.3764 (HRESIMS) ($[M+Na]^+$, $C_{31}H_{60}N_4NaO_5Si_3$, requires 675.3769). IR (neat) ν_{max} 2923, 1470, 1251, 1119, 834, 773 cm^{-1}. UV (MeOH) λ_{max} 226, 324 nm.

5-Ethyl-8-methyl-3-[2,3,5-tri-*O*-(*tert*-butyldimethylsilyl)-β-D-ribofuranosyl]-3*H*-imidazo[4,5-*d*][1,2,6]oxadiazepine 6b

Amorphous white solid (7.2 mg, 0.011 mmol, 55% over two steps). Element. Anal. Calcd. for $C_{31}H_{60}N_4O_5Si_3$ C, 57.01; H, 9.26; N, 8.58; found C, 57.04; H, 9.29; N, 8.61. $[\alpha]_D$ -40.2 (*c* = 0.1 ,CH_3OH). ^{1}H-NMR (400 MHz, CD_3OD) ppm 8.74 (s, 1H, H-2), 6.12 (d, *J* = 5.4 Hz, H-1'), 4.86-4.81 (m, 1H, H-2'), 4.44-4.39 (m, 1H, H-3'), 4.19-4-14 (m, 1H, H-4'), 4.06 (dd, *J* = 11.5, 5.3 Hz, 1H, H-5'$_a$), 3.87 (dd, *J* = 11.5, 3.0 Hz, 1H, H-5'$_b$), 3.23 (q, *J* = 7.4 Hz, 2H, C*H*$_2$CH$_3$), 2.85 (s, 3H, CH_3), 1.43 (t, *J* = 7.4 Hz, 3H, CH_2CH_3), 0.97 (s, 9H, $C(CH_3)_3$), 0.96 (s, 9H, $C(CH_3)_3$), 0.80 (s, 9H, $C(CH_3)_3$), 0.17 (s, 3H, CH_3), 0.16 (s, 3H, CH_3), 0.15 (s, 3H, CH_3), 0.14 (s, 3H, CH_3), 0.0040 (s, 3H, CH_3), -0.23 (s, 3H, CH_3). ^{13}C-NMR (100 MHz, CD_3OD) ppm 160.2, 151.6, 147.6, 143.1, 132.9, 89.6, 86.9, 76.6, 73.2, 63.6, 27.1, 26.3, 26.2, 25.9, 19.1, 18.7, 18.5, 12.7, 10.4, -4.3, -4.5, -4.9, -5.3, -5.5. *m*/*z* (HRESIMS) 675.3760 ($[M+Na]^+$, $C_{31}H_{60}N_4NaO_5Si_3$, requires 675.3769). IR (neat) 2925,

1472, 1254, 1118, 835, 773 ν_{max} cm^{-1}. UV (MeOH) λ_{max} 226, 320 nm

5-Ethyl-8-phenyl-3-[2,3,5-tri-*O*-(*tert*-butyldimethylsilyl)-*β*-D-ribofuranosyl]-3*H*-imidazo[4,5-*d*][1,2,6]oxadiazepine 6c

Oil (7.7 mg, 0.011 mmol, 54% over two steps). Element. Anal. Calcd. for $C_{36}H_{62}N_4O_5Si_3$ C, 60.46; H, 8.74; N, 7.83; found C, 60.48; H, 8.72; N, 7.81. $[\alpha]_D$ -26.3 (*c* = 0.2 ,CH_3OH). ^{1}H-NMR (400 MHz, CD_3OD) ppm 8.73 (s, 1H, H-2), 8.08-8.04 (m, 2H, HPh), 7.63-7.59 (m, 3H, HPh), 6.17 (d, *J* = 5.3 Hz, H-1'), 4.87-4.84 (m, 1H, H-2'), 4.45-4.41 (m, 1H, H-3'), 4.20-4.16 (m, 1H, H-4'), 4.07 (dd, *J* = 11.3, 4.9 Hz, 1H, H-5'$_a$), 3.88 (dd, *J* = 11.3, 3.0 Hz, 1H, H-5'$_b$), 3.27 (q, *J* = 7.4 Hz, 2H, C*H*$_2$CH$_3$), 1.47 (t, *J* = 7.4 Hz, 3H, CH$_2$C*H*$_3$), 0.98 (s, 9H, C(CH$_3$)$_3$), 0.95 (s, 9H, C(CH$_3$)$_3$), 0.82 (s, 9H, C(CH$_3$)$_3$), 0.18 (s, 3H, CH$_3$), 0.16 (s, 3H, CH$_3$), 0.15 (s, 6H, 2 x CH$_3$), 0.030 (s, 3H, CH$_3$), -0.18 (s, 3H, CH$_3$). ^{13}C-NMR (100 MHz, CD_3OD) ppm 161.4, 149.5, 148.3, 144.4, 132.3 (two C), 132.1 (two C), 129.2 (two C), 128.6, 89.8, 87.1, 76.9, 73.4, 63.7, 27.6, 26.5, 26.4, 26.2, 19.4, 18.9, 18.8, 10.7, -4.1, -4.3, -4.7, -5.1, -5.2. *m*/*z* 737.3942 (HRESIMS) ($[M+Na]^+$, $C_{36}H_{62}N_4NaO_5Si_3$, requires 737.3926). IR (neat) ν_{max} 2955, 2931, 2858, 1255, 1160, 1124, 1075, 836, 777 cm^{-1}.UV (MeOH) λ_{max} 252, 296, shoulder 346 nm.

General procedure for the desilylation of compounds 6a-c. Synthesis of 7a-c

Compounds **6a-c** (0.010 mmol) were dissolved in 1.0 mL of MeOH, and then NH_4F (0.10 mmol) was added in one portion. The systems were refluxed for 5 h (TLC monitoring: AcOEt/MeOH, 7:3). The solvent was removed under reduced pressure and the crudes were dissolved in water, filtered and then purified by HPLC (system C, see General Methods) affording compounds **7a-c**.

8-Ethyl-5-methyl-3-(*β*-D-ribofuranosyl)-3*H*-imidazo[4,5-*d*][1,2,6]oxadiazepine 7a

Amorphous white solid (3.0 mg, 0.0097 mmol, 97%). Element. Anal. Calcd. for $C_{13}H_{18}N_4O_5$ C, 50.32; H, 5.85; N, 18.06; found C, 50.30; H, 5.83; N, 18.05. $[\alpha]_D$ -3.2 (*c* = 0.1 ,CH_3OH). ^{1}H-NMR (400 MHz, CD_3OD) ppm 8.78 (s, 1H, H-2), 6.09 (d, *J* = 5.1 Hz, 1H, H-1'), 4.70-4.65 (m, 1H, H-2'), 4.38-4.34 (m, 1H, H-3'), 4.17-4.11 (m, 1H, H-4'), 3.88 (dd, *J* = 12.2, 3.0 Hz, 1H, H-5'$_a$), 3.77 (dd, *J* = 12.2, 3.4 Hz, 1H, H-5'$_b$), 3.36 (q, *J* = 7.5 Hz, 2H, C*H*$_2$CH$_3$), 2.81 (s, 3H, CH$_3$), 1.41 (t, *J* = 7.5 Hz, 3H, CH$_2$C*H*$_3$). ^{13}C-NMR (100 MHz, CD_3OD) ppm 157.1, 155.8, 148.0, 143.7, 132.8, 90.4, 87.3, 75.9, 71.8, 62.6, 21.0 (two C), 10.9. *m*/*z* 333.1170 (HRESIMS) ($[M+Na]^+$, $C_{13}H_{18}N_4NaO_5$, requires 333.1175). IR (neat) ν_{max} 3329, 2917, 2848, 1245, 1117 cm^{-1}. UV (MeOH) λ_{max} 226, 325 nm.

5-Ethyl-8-methyl-3-(*β*-D-ribofuranosyl)-3*H*-imidazo[4,5-*d*][1,2,6]oxadiazepine 7b

Amorphous white solid (3.0 mg, 0.0096 mmol, 96%). Element. Anal. Calcd. for $C_{13}H_{18}N_4O_5$ C, 50.32; H, 5.85; N, 18.06; found C, 50.33; H, 5.82; N, 18.04. $[\alpha]_D$ -7.9 (*c* = 0.1, CH_3OH). ^{1}H-NMR (400 MHz, CD_3OD) ppm 8.76 (s, 1H, H-2), 6.11 (d, *J* = 5.1 Hz, 1H, H-1'), 4.77-4.73 (m, 1H, H-2'), 4.42-4.38 (m, 1H, H-3'), 4.15-4.11 (m, 1H, H-4'), 3.87 (dd, *J* = 12.2, 3.3 Hz, 1H, H-5'$_a$), 3.77 (dd, *J* = 12.2, 3.9 Hz, 1H, H-5'$_b$), 3.21 (q, *J* = 7.4 Hz, 2H, C*H*$_2$CH$_3$), 2.84 (s, 3H, CH_3), 1.43 (t, *J* = 7.4 Hz, 3H, CH_2CH_3). ^{13}C-NMR (100 MHz, CD_3OD) ppm 160.3, 151.7, 148.1, 143.5, 133.0, 90.3, 87.1, 75.7, 71.8, 62.7, 27.2, 12.9, 10.3. *m*/*z* 333.1187 (HRESIMS) ($[M+Na]^+$, $C_{13}H_{18}N_4NaO_5$, requires 333.1175). IR (neat) ν_{max} 3331, 2920, 2852, 1246, 1118 cm^{-1}. UV (MeOH) λ_{max} 225, 320 nm

5-Ethyl-8-phenyl-3-(*β*-D-ribofuranosyl)-3*H*-imidazo[4,5-*d*][1,2,6]oxadiazepine 7c

Amorphous white solid (3.5 mg, 0.0094 mmol, 94%). Element. Anal. Calcd. for $C_{18}H_{20}N_4O_5$ C, 58.06; H, 5.41; N, 15.05; found C, 58.03; H, 5.40; N, 15.07. $[\alpha]_D$ = -10.4 (*c* = 0.1 ,CH_3OH). ^{1}H-NMR (700 MHz, CD_3OD) ppm 8.78 (s, 1H, H-2), 8.08-8.05 (m, 2H, HPh), 7.61-7.59 (m, 3H, HPh), 6.16 (d, *J* = 5.0 Hz, 1H, H-1'), 4.80-4.77 (m, 1H, H-2'), 4.43-4.41 (m, 1H, H-3'), 4.16-4.13 (m, 1H, H-4'), 3.88 (dd, *J* = 12.1, 3.3 Hz, 1H, H-5'$_a$), 3.79 (dd, *J* = 12.1, 3.8 Hz, 1H, H-5'$_b$), 3.26 (q, *J* = 7.4 Hz, 2H, C*H*$_2$CH$_3$), 1.47 (t, *J* = 7.4 Hz, 3H, CH_2CH_3). ^{13}C-NMR (175 MHz, CD_3OD) ppm 161.3, 149.4, 148.7, 144.7, 132.3, 133.2, 132.1 (two C), 129.2 (two C), 128.8, 94.4, 87.1, 75.8, 71.9, 62.7, 27.5, 10.4. *m*/*z* 395.1323 (HRESIMS) ($[M+Na]^+$, $C_{18}H_{20}N_4NaO_5$, requires 395.1331). IR (neat) ν_{max} 3335, 2914, 2846, 1245, 1124 cm^{-1}. UV (MeOH) λ_{max} 252, 296, 342 nm.

5.4.3 Experimental procedures for preparation of 2,6-disubstituted purine nucleosides 10a-f

Compounds **2a-f – 4a-f** have been prepared as described in § 5.4.2 for analogous compounds. 3-bromopropanol was silylated according to a literature procedure[47] to obtain 3-bromopropoxy-*tert*-butyl-diphenylsilane.

Preparation of 3-(tert-butyl-diphenyl-silanoxy)-propylmagnesium bromide

To a mixture of Mg turnings (59.2 mg, 2.57 mmol, 1.3 eq.), iodine (catalytic amount) in anhydrous THF (5 mL) was slowly added (3-bromopropoxy)(*tert*-butyl)diphenylsilane (500 mg, 1.98 mmol) in THF (5 mL) at room temperature under argon. After the reaction initiated, the speed of addition of (3-bromopropoxy)(*tert*-butyl)diphenylsilane solution was controlled to mantain the

temperature of the reaction mixture at 30-35 °C. After the addition was completed the resulting mixture was stirred at 40 °C for 1 h to afford a 0.9 M solution of 3-(tert-butyl-diphenyl-silanoxy)-propylmagnesium bromide. The Grignard reagent was titrated according to a literature procedure[48]

2',3',5'-Tri-*O*-(*tert*-butyldimethylsilyl)-6-(3-((*tert*-butyldiphenylsilyl)oxy)propyl) nebularine ossido 4e,f

Oil (27.6 mg, 0.036 mmol, 60%). ^{1}H NMR (400 MHz, CD_3OD) δ = 8.97 (s, 1H, H-2), 8.82 (s, 1H, H-8), 7.62-7.59 (m, 4H, HPh), 7.43-7.34 (m, 6H, HPh), 6.09 (d, *J* = 4.6 Hz, 1H, H-1'), 4.70 (t, *J* = 4.2 Hz, 1H, H-2'), 4.43 (t, *J* = 4.2 Hz, 1H, H-3'), 4.18 (dd, *J* = 6.7, 3.9 Hz, 1H, H-4'), 4.08 (dd, *J* = 11.5, 4.1 Hz, 1H, H-5'$_a$), 3.87 (dd, *J* = 11.6, 2.7 Hz, 1H, H-5'$_b$), 3.80 (t, *J* = 6.6 Hz, 2H, $CH_2CH_2\mathit{CH_2}$OTBDPS), 3.47 (t, *J* = 6.6 Hz, 2H, $\mathit{CH_2}CH_2CH_2$OTBDPS), 2.19 (quint, *J* = 6.6 Hz, 2H, $CH_2\mathit{CH_2}CH_2$OTBDPS), 0.97 (s, 9H, $C(CH_3)_3$), 0.96 (s, 9H, $C(CH_3)_3$), 0.94 (s, 9H, $C(CH_3)_3$), 0.82 (s, 9H, $C(CH_3)_3$), 0.17 (s, 3H, CH_3), 0.165 (s, 3H, CH_3), 0.160 (s, 3H, CH_3), 0.13 (s, 3H, CH_3), 0.02 (s, 3H, CH_3), -0.19 (s, 3H, CH_3). ^{13}C NMR (100 MHz, CD_3OD) δ = 155.1, 148.3, 146.5, 144.2, 136.6, 134.65, 134.60, 130.9, 128.8, 90.1, 87.0, 77.3, 73.0, 64.8, 63.4, 29.8, 27.3, 26.6, 26.4, 26.2, 24.6, 19.9, 19.4, 19.0, 18.8, -4.0, -4.3, -4.7, -5.1, -5.2. ESI+/MS: 767 $[M+H]^+$, 789 $[M+Na]^+$, 805 $[M+K]^+$.

General procedure for the preparation of compounds 10a-f

In a flamed round bottom flask charged with dry nitrogen, compound **4a-f** (0.020 mmol), dissolved in dry THF (0.5 mL), was added via cannula. To the flask, fresh Grignard reagent (0.080 mmol) was quickly added in one portion and the mixtures was stirred for 2 h (TLC monitoring: AcOEt/MeOH, 95:5) at room temperature. The reactions was quenched by addition of a 1 M solution of NH_4Cl (1 mL), diluted with AcOEt (10 mL) and extracted with brine (2 x 10 mL). The organic layers were separated, dried (Na_2SO_4), filtered and concentrated under reduced pressure. Compound **5a-f** was dissolved in a mixture of Ac_2O-pyridine (6:4, v/v; 1 mL) and stirred at 50 °C for 16 and then evaporated under reduced pressure. The crude was purified over PLC plates (*n*-hexane/AcOEt, 4:6).

2',3',5'-Tri-*O*-(*tert*-butyldimethylsilyl)-6-ethyl-2-methyl- nebularine 10a

Amorphous white solid (6.1 mg, 0.0096 mmol, 80% over four steps). ^{1}H NMR (400 MHz, CD_3OD) δ = 8.57 (s, 1H, H-8), 6.11 (d, *J* = 5.0 Hz, 1H, H-1'), 4.92 (m, 1H, H-2'), 4.44 (dd, *J* = 4.1, 3.0 Hz, 1H, H-3'), 4.17-4.14 (m, 1H, H-4'), 4.08 (dd, *J* = 11.3, 4.9 Hz, 1H, H-5'$_a$), 3.85 (dd, *J* = 11.3, 2.9

Hz, H-5'$_b$), 3.14 (q, *J* = 7.3 Hz, 2H, C*H*$_2$CH$_3$), 2.74 (s, 3H, CH$_3$), 1.38 (t, *J* = 7.3 Hz, 3H, CH$_2$C*H*$_3$), 0.98 (s, 9H, C(CH$_3$)$_3$), 0.95 (s, 9H, C(CH$_3$)$_3$), 0.78 (s, 9H, C(CH$_3$)$_3$), 0.18 (s, 3H, CH$_3$), 0.16 (s, 3H, CH$_3$), 0.151 (s, 3H, CH$_3$), 0.150 (s, 3H, CH$_3$), -0.03 (s, 3H, CH$_3$), -0.30 (s, 3H, CH$_3$). ^{13}C NMR (100 MHz, CD$_3$OD) δ = 164.9, 163.4, 152.5, 145.0, 131.3, 89.7, 87.4, 76.8, 73.8, 63.9, 27.25, 26.6, 26.4, 26.2, 25.6, 19.4, 19.0, 18.8, 13.5, -4.1, -4.3, -5.0, -5.20, -5.25. ESI+/MS: 637 [M+H]$^+$, 659 [M+Na]$^+$, 675 [M+K]$^+$.

2',3',5'-Tri-*O*-(*tert*-butyldimethylsilyl)-6-ethyl-2-phenyl nebularine 10b

Amorphous white solid (6.3 mg, 0.009 mmol, 75% over four steps). ^{1}H NMR (400 MHz, CD$_3$OD) δ = 8.61 (s, 1H, H-8), 8.49 (m, 2H, HPh), 7.49 (m, 3H, HPh), 6.20 (d, *J* = 6.0 Hz, 1H, H-1'), 5.11 (dd, *J* = 5.9, 4.4 Hz, 1H, H-2'), 4.46 (dd, *J* = 4.1, 3.0 Hz, 1H, H-3'), 4.17 (m, 1H, H-4'), 4.09 (dd, *J* = 11.0, 5.5 Hz, 1H, H-5'$_a$), 3.88 (dd, *J* = 11.1, 3.3 Hz, H-5'$_b$), 3.24 (q, *J* = 7.6 Hz, 2H, C*H*$_2$CH$_3$), 1.48 (t, *J* = 7.6 Hz, 3H, CH$_2$C*H*$_3$), 0.99 (s, 9H, C(CH$_3$)$_3$), 0.95 (s, 9H, C(CH$_3$)$_3$), 0.75 (s, 9H, C(CH$_3$)$_3$), 0.19 (s, 3H, CH$_3$), 0.17 (s, 3H, CH$_3$), 0.14 (s, 3H, CH$_3$), 0.13 (s, 3H, CH$_3$), -0.05 (s, 3H, CH$_3$), -0.31 (s, 3H, CH$_3$). ^{13}C NMR (100 MHz, CD$_3$OD) δ = 164.8, 160.4, 152.6, 145.7, 139.3, 132.2, 131.3, 129.5, 129.4, 89.8, 87.46, 76.3, 73.9, 64.1, 27.4, 26.5, 26.4, 26.2, 19.3, 19.0, 18.8, 12.9, -4.1, -4.2, -4.3, -4.9, -5.1, -5.2. ESI+/MS: 699 [M+H]$^+$, 721 [M+Na]$^+$, 737 [M+K]$^+$.

2',3',5'-Tri-*O*-(*tert*-butyldimethylsilyl)-2-ethyl-6-methyl nebularine 10c

Amorphous white solid (6.1 mg, 0.0096 mmol, 80% over four steps). ^{1}H NMR (400 MHz, CD$_3$OD) δ = 8.58 (s, 1H, H-8), 6.11 (d, *J* = 6.0 Hz, 1H, H-1'), 4.99 (dd, *J* = 4.4, 5.8 Hz, 1H, H-2'), 4.42 (dd, *J* = 4.4, 3.0 Hz, 1H, H-3'), 4.17-4.14 (m, 1H, H-4'), 4.10 (dd, *J* = 11.3, 5.4 Hz, 1H, H-5'$_a$), 3.85 (dd, *J* = 11.1, 3.1 Hz, H-5'$_b$), 3.01 (q, *J* = 7.6 Hz, 2H, C*H*$_2$CH$_3$), 2.79 (s, 3H, CH$_3$), 1.39 (t, *J* = 7.6 Hz, 3H, CH$_2$C*H*$_3$), 0.98 (s, 9H, C(CH$_3$)$_3$), 0.96 (s, 9H, C(CH$_3$)$_3$), 0.78 (s, 9H, C(CH$_3$)$_3$), 0.18 (s, 3H, CH$_3$), 0.157 (s, 3H, CH$_3$), 0.155 (s, 3H, CH$_3$), 0.15 (s, 3H, CH$_3$), -0.04 (s, 3H, CH$_3$), -0.30 (s, 3H, CH$_3$). ^{13}C NMR (100 MHz, CD$_3$OD) δ = 167.6, 160.1, 152.4, 145.3, 132.3, 89.8, 87.4, 76.4, 73.9, 64.0, 33.2, 26.5, 26.4, 26.2, 19.3, 19.05, 19.0, 18.8, 13.9, -4.1, -4.3, -4.9, -5.2, -5.3. ESI+/MS: 637 [M+H]$^+$, 659 [M+Na]$^+$, 675 [M+K]$^+$.

2',3',5'-Tri-*O*-(*tert*-butyldimethylsilyl)-2,6-diphenyl nebularine 10d

Amorphous white solid (7.5 mg, 0.010 mmol, 83% over four steps). ^{1}H NMR (400 MHz, CD$_3$OD) δ = 8.86 (dd, *J* = 8.0, 1.7 Hz, 2H, HPh), 8.65 (s, 1H, H-8), 8.61 (dd, *J* = 8.0, 1.8 Hz, 2H, HPh), 7.60 (m, 3H, HPh), 7.52 (m, 3H, HPh), 6.26 (d, *J* = 6.0 Hz, 1H, H-1'), 5.13 (dd, *J* = 5.9, 4.4 Hz, 1H, H-

2'), 4.48 (dd, *J* = 3.9, 2.8 Hz, 1H, H-3'), 4.18 (m, 1H, H-4'), 4.10 (dd, *J* = 11.0, 5.4 Hz, 1H, H-5'$_a$), 3.90 (dd, *J* = 11.0, 3.2 Hz, H-5'$_b$), 1.00 (s, 9H, C(CH_3)$_3$), 0.96 (s, 9H, C(CH_3)$_3$), 0.76 (s, 9H, C(CH_3)$_3$), 0.20 (s, 3H, CH_3), 0.18 (s, 3H, CH_3), 0.16 (s, 3H, CH_3), 0.14 (s, 3H, CH_3), -0.04 (s, 3H, CH_3), -0.29 (s, 3H, CH_3). ^{13}C NMR (100 MHz, CD_3OD) δ = 160.0, 155.8, 154.6, 146.2, 139.4, 137.2, 132.2, 131.4, 130.9, 129.6, 129.5, 129.4, 89.7, 87.4, 76.4, 74.0, 64.2, 26.6, 26.5, 26.2, 19.3, 19.0, 18.8, -4.0, -4.2, -4.3, -4.8, -5.1, -5.2. ESI+/MS: 747 $[M+H]^+$, 769 $[M+Na]^+$, 785 $[M+K]^+$.

2',3',5'-Tri-*O*-(*tert*-butyldimethylsilyl)-6-(3-((*tert*-butyldiphenylsilyl)oxy)propyl)-2-phenyl nebularine 10e

Amorphous white solid (6.0 mg, 0.0072 mmol, 60%). ^{1}H NMR (400 MHz Acetone-d6) δ = 8.60 (s, 1H, H-8), 8.59-8.56 (m, 2H, HPh), 7.69 (d, *J* = 4.6 Hz, 4H, HPh), 7.51-7.50 (m, 3H, HPh), 7.41-7.34 (m, 6H, HPh), 6.24 (d, *J* = 5.2 Hz, 1H, H-1'), 5.14 (t, *J* = 3.7 Hz, 1H, H-2'), 4.58 (t, *J* = 3.6 Hz, 1H, H-3'), 4.23-4.10(m, 2H, H-4' e H-5'$_a$), 3.95 (dd, *J* = 10.4, 2.6 Hz, 1H, H-5'$_b$), 3.87 (t, *J* = 6.6 Hz, 2H, $CH_2CH_2\mathit{CH_2}$OTBDPS), 3.39 (t, *J* = 6.6 Hz, 2H, $\mathit{CH_2}CH_2CH_2$OTBDPS), 2.32 (quint, *J* = 6.8 Hz, 2H, $CH_2\mathit{CH_2}CH_2$OTBDPS), 1.03 (s, 9H, C(CH_3)$_3$), 1.00 (s, 9H, C(CH_3)$_3$), 0.96 (s, 9H, C(CH_3)$_3$), 0.80 (s, 9H, C(CH_3)$_3$), 0.22 (s, 3H, CH_3), 0.19 (s, 3H, CH_3), 0.16 (s, 3H, CH_3), 0.15 (s, 3H, CH_3), 0.00 (s, 3H, CH_3), -0.20 (s, 3H, CH_3). ^{13}C NMR (100 MHz, Acetone-d6) δ = 162.9, 159.0, 152.5, 144.9, 139.3, 136.3, 136.2, 134.6, 130.9, 130.5, 129.2, 129.1, 128.6, 89.3, 86.2, 75.8, 73.1, 64.2, 63.6, 31.4, 27.2, 26.5, 26.3, 26.1, 19.8, 19.1, 18.7, 18.5, -4.0, -4.3, -4.7, -5.10, -5.15. ESI+/MS: 843 $[M+H]^+$, 865 $[M+Na]^+$, 881 $[M+K]^+$.

2',3',5'-Tri-*O*-(*tert*-butyldimethylsilyl)-2-ethil-6-(3-((*tert*-butyldiphenylsilyl)oxy)propyl) nebularine 10f

Amorphous white solid (5.8 mg, 0.0073 mmol, 61%). ^{1}H NMR (400 MHz Acetone-d6) δ = 8.48 (s, 1H, H-8), 7.68 (d, *J* = 7.0 Hz, 4H, HPh), 7.47 – 7.35 (m, 6H, HPh), 6.11 (d, *J* = 5.4 Hz, 1H, H-1'), 5.09 (t, *J* = 3.9 Hz, 1H, H-2'), 4.54 (t, *J* = 3.9 Hz, 1H, H-3'), 4.17-4.13 (m, 2H, H-4' e H-5'$_a$), 3.89 (dd, *J* = 13.2, 5.7 Hz, 1H, H-5'$_b$), 3.82 (t, *J* = 6.6 Hz, 2H, $CH_2CH_2\mathit{CH_2}$OTBDPS), 3.27 (t, *J* = 6.6 Hz, 2H, $\mathit{CH_2}CH_2CH_2$OTBDPS), 2.97 (q, *J* = 7.6 Hz, 2H, $\mathit{CH_2}CH_3$), 2.24 (quint, *J* = 6.6 Hz, 2H, $CH_2\mathit{CH_2}CH_2$OTBDPS), 1.36 (t, *J* = 7.6 Hz, 3H, $CH_2\mathit{CH_3}$), 1.02 (s, 9H, C(CH_3)$_3$), 0.98 (s, 9H, C(CH_3)$_3$), 0.96 (s, 9H, C(CH_3)$_3$), 0.80 (s, 9H, C(CH_3)$_3$), 0.20 (s, 3H, CH_3H), 0.17 (s, 3H, CH_3), 0.15 (s, 6H, 2CH_3), -0.02 (s, 3H, CH_3), -0.24 (s, 3H, CH_3). ^{13}C NMR (100 MHz, Acetone-d6) δ = 166.4, 162.7, 152.1, 144.0, 136.3, 134.7, 132.4, 130.5, 128.6, 89.3, 86.2, 75.6, 73.2, 64.2, 63.5, 33.3, 31.5, 27.2, 26.4, 26.3, 26.1, 19.8, 19.1, 18.7, 18.5, 13.7, -4.1, -4.4, -4.8, -5.1, -5.2. ESI+/MS: 795

$[M+H]^+$, 817 $[M+Na]^+$, 833 $[M+K]^+$.

5.4.4 Procedures used for the MTS assays

Cell viability was assessed by MTS assay as described elsewhere. Breast (MCF-7 cell line) and lung (A549 cell line) cancer cells were seeded at the concentration of 0.5×10^4 cells per well on 96-well plate and maintained overnight under appropriate condition (1% sodium pyruvate MEM or DMEM, respectively, completed with 10% FCS, 2 mmol L-glutamine and 100 units/mL of penicillin. Cells in quadruplicated were than cultured in 5% FCS media containing different concentrations of tested compounds (0.1 μM, 1 μM, 10 μM and 100 μM) except for control wells that only received 5% FCS media. Every second day cells were washed with PBS and media were replaced. At the indicated time point cell viability was assessed reading the absorbance of treated and control cells at 490 nm using the 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.

5.4.5 Antitumor activity of compounds 7a-c

MCF-7 breast cancer cell line and A549 lung cancer cell line were treated with different concentrations (0.1 μM, 1 μM, 10 μM and 100 μM) of novel RENs **7a-c**. Cisplatin was used as control since its activity in these cell lines has been extensively studied.[27,28] Cell viability was assessed by measuring the mitochondrial activity at day 1, 3 or 7 (MTS assay) *in vitro*.[26] We found that all tested RENs increased significantly the mitochondrial activity of treated MCF-7 cells at day 1 and 3, with the exception of **7b** that resulted inactive at day 1 (Fig. S1, panels A and B). This phenomenon is often reported as "starving state" leading up to cell death at later time points and/or at higher concentrations. Accordingly, at day 7 RENs **7b** and **7c** increased the mitochondrial activity at concentrations up to 10 μM, while at 100 μM they showed 38% and 40% cell death, respectively (Figure 2, panel C in § 5.2.1). At the same time point **7a** increased the mitochondrial activity at 0.1 μM and 1 μM, but showed 16% cell killing effect already at 10 μM and 55% cell death at 100 μM. It is noteworthy that at day 7 at 10 μM compound **7a** proved to be as cytotoxic as cisplatin (16% and 15% cell death, respectively). **7a-c** resulted less active in A549 cell line at almost all tested conditions (Figure 3, panels A-C in § 5.2.1) and, in agreement with the "starving state" hypothesis, we did not observe any increased mitochondrial activity at day 3 and 7. The most active compound in A549 cell line was **7a** that showed 21% cell death at day 7 at 10 μM. All

together these data showed that the new synthesized compounds possess some degree of cytotoxic activity, whose manifestation in some cases was slower than the control. Further and more specific experiments would have to be performed in order to fully clarify the reason for such phenomenon. However, at this point we can speculate that only active metabolites formed around day 7 were capable to kill cells.

5.5 References and notes

‡The nitrogen of the oxime should require harsh condition to react, as outlined in reference 22.

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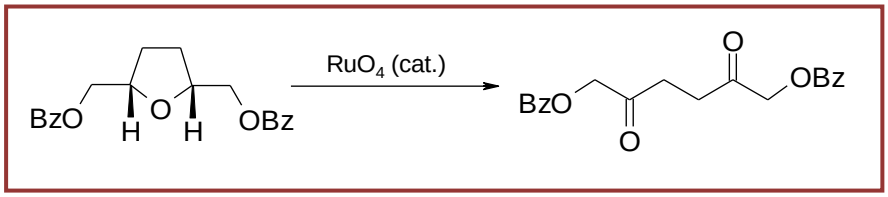
Part 4. *Preliminary experiments…*

…with RuO_4



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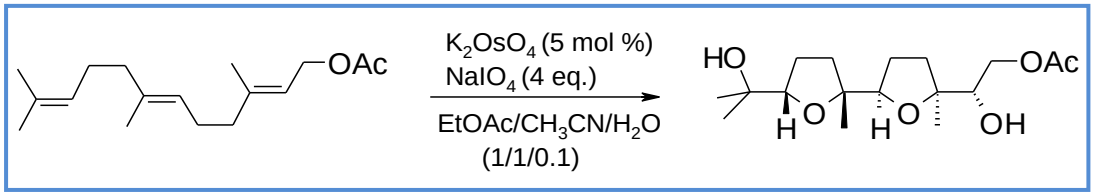
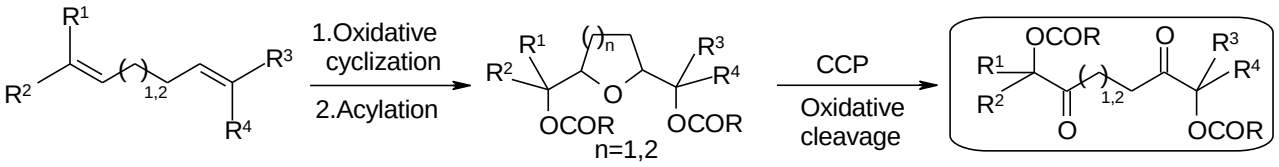


Chapter 6

Preliminary experiments on the RuO_4-catalyzed oxidative opening of acylated THF. An alternative method for the synthesis of bis-α-acyloxy 1,4-diketones

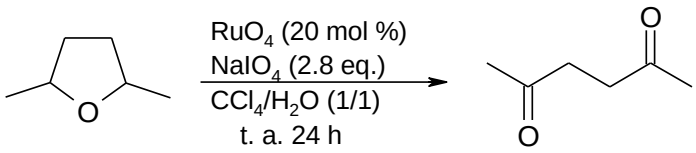
6.1. Introduction

Recently in our laboratory we have focused on the catalytic use of chlorochromatoperiodate (CCP for short), generated by the condensation of PCC and periodic acid,[1] as a powerful reagent capable of oxidising THF-containing compounds of various structural complexity (for a deeper insight on PCC and CCP see § 1.3, and Chapter 2 and 3).[2-4] In particular we have developed the first general synthesis of bis-α-acyloxy 1,4- and 1,5-diketones by a CCP-catalyzed oxidative opening of bis acylated THF and THP diols, in turn synthesized by osmium- or ruthenium-catalyzed oxidative cyclization of 1,5- and 1,6-dienes (Scheme 1, see also Chapter 3).[4] Since α-acyloxy ketones are important building blocks in organic synthesis,[5] the presence in the same molecule of two of these structural subunits further increases the synthetic utility of bis-α-acyloxy diketones.



Scheme 1. Our general synthesis of bis-α-acyloxy diketones from dienes through CCP-catalyzed oxidative opening of bis acylated THF and THP diols.[4a]

In order to extend this synthetic procedure to acid- or PCC- sensitive substrates, during the third year of my PhD programme some preliminary experiments have been carried out on mono-THF compounds using the systems RuO_4 (cat.)/$NaIO_4$ [6] and RuO_4 (cat.)/H_5IO_6[7]. Indeed, in 1980 Smith III and Scarborough used RuO_4 to accomplish the transformation of unfunctionalised THF or THP ring mostly into lactones.[8] In a single case, a 2,5-disubstituted THF was converted into a 1,4-diketone, but the process required long reaction times (typically 24) and there were no chiral centres adjacent to the reaction centres (Scheme 2). In this chapter we report our early experiments aimed at the development of a RuO_4-catalyzed method for the synthesis of bis-α-acyloxy 1,4-diketones.



Scheme 2. Oxidative THF opening under Smith III and Scarborough conditions.[8]

6.2. Results and discussion

Optimization of the oxidative protocol was carried out with THF **1** as model compound (Table 1). In particular, two sets of conditions have been developed, one, employing $NaIO_4$ as the stoichiometric oxidant, designed for acid-sensitive substrates, the other one, with H_5IO_6 as co-oxidant. This would also allow to evaluate the effect of H_5IO_6 compared to $NaIO_4$. Indeed, Tse[7a] and then Sharpless[7b] reported the superiority of periodic acid over sodium periodate in the RuO_4 oxidations of organic compounds.

As regards the conditions for acid-sensitive substrates, we initially tested the oxidation of **1** (entry 1, Table 1) under Smith III and Scarborough conditions (20 mol% RuO_2, 2.8 $NaIO_4$, CCl_4/H_2O, 1:1). However, the process failed to go to completion under these conditions and after 24 h the reaction stopped at a 60% conversion, indicating that new conditions had to be devised for more complex substrates.

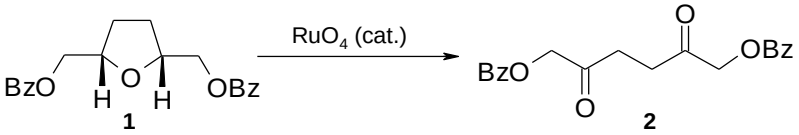
Then the process was accomplished varying the amounts of both oxidant and co-oxidant. In particular, two different solvent mixture, $CCl_4/CH_3CN/H_2O$ (2:2:3) (entries 2-4) and, $^tBuOH/\ H_2O$ (7:3) (entries 5-7) were tested. Best results (complete conversion and 80% yield) were obtained using 10 mol % of $RuCl_3$ and 10 equiv. of $NaIO_4$ in $CCl_4/CH_3CN/H_2O$, 2:2:3 at 40 °C (entry 4). In absence of water, using 5 mol % of $RuCl_3$ as pre-catalist, 3 equiv. of SiO_2-supported $NaIO_4$ [9] and CH_3CN as solvent, a < 50% conversion was reached even after a week (entry 8).

Regarding the oxidation with the system RuO_4 (cat.)/H_5IO_6 (Entry 9-15), screening of different amounts of oxidant and co-oxidant and of various solvent systems was performed. In particular, using 15 mol % of $RuCl_3$ and 13 equiv. of H_5IO_6 in CH_3CN/H_2O (9:1) the reaction went to completion in 48 h with a 70% yield (entry 9). Improved yields (90%) and reduced reaction times (16 h) were achieved using a mixture of $^tBuOH/H_2O$ (7:3) and 10 mol % of $RuCl_3$ with 10 equiv. of H_5IO_6 (entry 12). When the process was carried out a larger scale (0.15 mmol compared to 0.03-0.04 mmol used in all the others reactions) under the same conditions as in entry 12, the expected diketone in an improved 86% yield (entry 13), though in 26h.

Eventually, using the biphasic mixture $EtOAc/CH_3CN/H_2O$ (3:3:2) as the solvent, as reported for

others RuO_4 (cat.)/H_5IO_6 oxidative processes,[7a,b] the reaction did not proceed at all (<10% yield 6 days, entry 15).

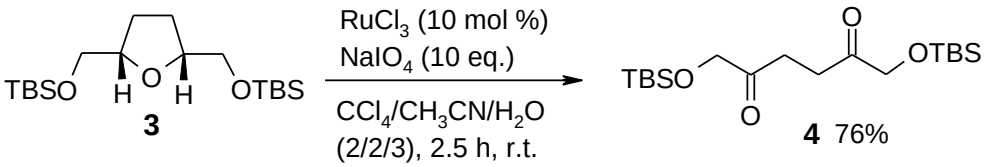
Table 1. Optimization of the process on a model 2,5-disubstituted THF compound.



Exp.	Ox [a] (mol%)	Co-Ox	eq	Solvent	Time	Yield[b] (%)	Conv.[b] (%)
1	20		2.8	CCl_4/H_2O, 1:1	24 h		60
2	5		4.2	$CCl_4/CH_3CN/H_2O$, 2:2:3	6 d	80	90
3	20		8	$CCl_4/CH_3CN/H_2O$, 2:2:3	43 h		80
4	**10**	**$NaIO_4$**	**10**	**$CCl_4/CH_3CN/H_2O$, 2:2:3**	**7 h[c]**	**80**	**100**
5	10		8	$^tBuOH/ H_2O$, 7:3	13 h	66	90
6	10		8	$^tBuOH/ H_2O$, 7:3	24 h	74	100
7	5		8	$^tBuOH/ H_2O$, 7:3	48 h	75	100
8	5	$NaIO_4/SiO_2$	3	CH_3CN	7 d		<50
9	15		13	CH_3CN /H_2O, 9:1	48 h	70	100
10	10		15	CH_3CN/H_2O, 8:2	21 h		75
11	5		10	CH_3CN/H_2O, 7:3	48 h		80
12	**10**	**H_5IO_6**	**10**	**$^tBuOH/ H_2O$, 7:3**	**16 h**	**90**	**100**
13[d]	10		10	$^tBuOH/ H_2O$, 7:3	26	86	90
14	10		10	$^tBuOH/ H_2O$, 1:1	6 h[c]		72
15	5		10	$EtOAc/CH_3CN/H_2O$, 3:3:2	6 d		<10

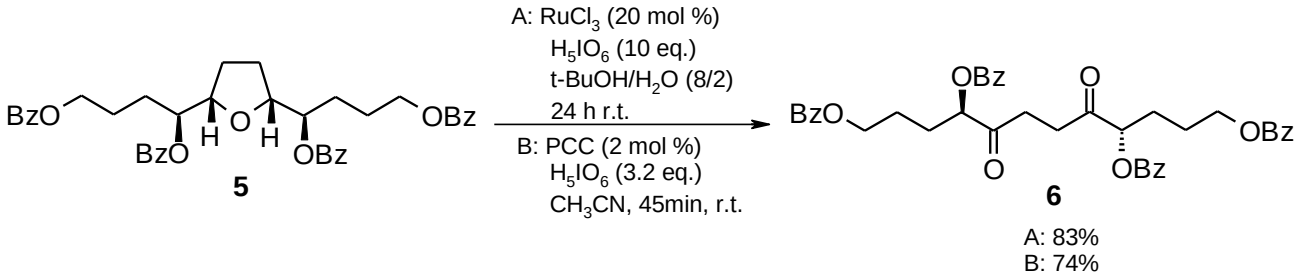
[a] All the experiments, except entry 1, were carried out using $RuCl_3$ as precursor of RuO_4. Experiment 1 was performed using RuO_2 as pre-catalyst, according to the Smith III and Scarborough procedure.[8] [b] Estimated by ^{1}H-NMR of the crude mixture. Yields have been calculated only for reaction with good conversion (≥90%). [c] Carried out at 40 °C. [d] reaction carried out on a 0.15 mmol scale (0.03-0.04 mmol were used in all the others reactions).

Using non-acidic optimized condition a silylated mono-THF (**3**) has been oxidized (Scheme 3) to give the expected bis-α-acyloxy 1,4-diketone (**4**) with a 76% yield. It is to be noted that in this case the reaction went to completion within 2.5 hours, without needing to warm the system. It is likely that this is due to the electron withdrawing character of the benzoyl group, that unlike silyl, makes the substrate less susceptible to the attack of the oxidant.



Scheme 3. Oxidation of a mono-THF substrate with RuO_4 (cat.)/ $NaIO_4$ conditions.

Oxidation of mono-THF **5**, one of the substrate previously oxidized with CCP (see § 3.2)[4a], was then carried out using the optimized H_5IO_6 –conditions (Schema 4). In this case we obtained the expected bis-α-acyloxy 1,4-diketone with an improved 83% yield, using a 20 mol% of co-oxidant, whereas with CCP the yield was 74%.



Scheme 4. Oxidation of a mono-THF substrate with RuO_4 (cat.)/H_5IO_6 and with previously developed CCP conditions (§ 3.2).[4a]

6.3. Conclusions

In conclusion, some preliminary experiments have been carried out to develop RuO_4 conditions for the synthesis of bis-α-acyloxy 1,4-diketones, in order to extend the process to acid- or PCC-sensitive molecules. The procedure has been optimized using both $NaIO_4$ and H_5IO_6 as co-oxidant and it has been used to oxidize two further mono-THF compounds. The first one, a silylated dimethanol THF that could not be cleaved using CCP, has been converted into the corresponding 1,4-diketone with a good yield using the system RuO_2/$NaIO_4$. The second compound, a mono-THF previously oxidized with CCP (74% yield), has been transformed into the expected 1,4-diketone with an increased yield of 83%, using RuO_4 (cat.)/H_5IO_6. These experiments show that the use of RuO_4 might be a viable alternative to the previously developed CCP-mediated ether cleavage. Further experiments are currently ongoing.

6.4. Experimental section

6.4.1 General methods

All reagents and 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. NMR experiments were performed on Varian Unity Inova 700, Varian Unity Inova 500, Varian Mercury Plus 400 and Gemini 200 spectrometers in $CDCl_3$ or CD_3OD. Proton chemical shifts were referenced to the residual $CHCl_3$ (7.26 ppm) signal. ^{13}C-NMR chemical shifts were referenced to the solvents ($CDCl_3$ 77.0 ppm). Coupling constants, *J*, are given in Hz. Abbreviations for signal coupling are as follows: s=singlet, d=doublet, dd=double doublet, ddd=double double doublet, t=triplet, dt=double triplet, q=quartet, quin=quintet, m=multiplet, app=apparent, br=broad.

6.4.2 Synthesis of Substrates

The substrates **1**,[10] **3**, **5**,[11] were obtained by benzoylation and silylation of the corresponding THF polyols under standard conditions. **1**[4b] is a known compound. NMR spectroscopic data of compounds **2**, **5** and **6** have been previously reported (§ 3.4.2).[4a]

3: Oil; ^{1}H NMR (200 MHz, $CDCl_3$) δ= 4.03-3.93 (m, 2H), 3.66-3.59 (dd, *J* = 10.3, 4.7 Hz, 2H), 3.56-3.48 (dd, *J* = 10.3, 5.8 Hz, 2H), 1.97-1.82 (m, 2H), 1.81-1.67 (m, 2H), 0.89 (s, 18H), -0.51 (s, 12H); ^{13}C NMR (50 MHz, $CDCl_3$) δ= 80.1 (2C), 65.8 (2C), 27.6 (2C), 25.9 (6C), 18.4 (2C), -5.3 (4C); ESI+/MS: *m/z* 361 $[M+H]^+$, 383 $[M+Na]^+$ 399 $[M+K]^+$.

6.4.3 Oxidative cleavage of substrates

Procedure A. Oxidative cleavage with the system RuO_4 (cat.)/ $NaIO_4$

$NaIO_4$ (10 eq.) was dissolved in the biphasic mixture $CCl_4/CH_3CN/H_2O$ (2:2:3, 700 µL) at r.t., then $RuCl_3$ (10 mol % from a 0.02 M stock solution in CH_3CN) was added under vigorous stirring. After 5 min the THF compound (1 eq.) was added and the reaction mixture was warmed to 40 °C. After complete consumption of the starting material (TLC control), ^{i}PrOH (excess) was added, and stirring continued until the colour of the solution turned from yellow to black (ca. 5 min). Then the

mixture was diluted with water (2 mL) and extracted with CH_2Cl_2 (3 x 3 mL). The combined organic phase was dried (Na_2SO_4) and evaporated in vacuo to give an oily product. Flash chromatography eluting with $CHCl_3$/MeOH (100:0 to 9:1) gave the desired product.

Benzoate-protected tetrahydrofuran **1** (10.5 mg, 0.031 mmol) was subjected to Procedure A to furnish compound **2** (8.8 mg, 0.025 mmol, 80 %) as a colorless solid. For experimental data see § 3.4.3.[4a]

TBDMS-protected tetrahydrofuran **3** (10.0 mg, 0.028 mmol) was subjected to Procedure A to furnish compound **4** (7.9 mg, 0.021 mmol, 96 %) as an oil.

4: Oil; ^{1}H NMR (200 MHz, $CDCl_3$) δ = 4.22 (s, 4H), 2.79 (s, 4H), 0.91 (s, 18H), 0.08 (s, 12H); ^{13}C NMR (50 MHz, $CDCl_3$) δ= 209.6 (2C), 69.2 (2C), 31.7 (2C), 25.8 (6C), 18.3 (2C), -5.5 (4C); ESI+/MS: *m/z* 375 $[M+H]^+$, 397 $[M+Na]^+$ 413 $[M+K]^+$.

Procedure B. Oxidative cleavage with the system RuO_4 (cat.)/ $NaIO_4$

To a solution of H_5IO_6 (10 eq.) in ^{t}BuOH/ H_2O (1mL, 7:3) at r.t. was added $RuCl_3$ (10 mol % from a 0.02 M stock solution in ^{t}BuOH) under vigorous stirring. After 5 min the THF compound (1 eq.) was added. After complete consumption of the starting material (TLC control), ^{i}PrOH (excess) was added and stirring continued until the colour of the solution turned from yellow to black (ca. 5 min). Then the mixture was diluted with water (2 mL) and extracted with CH_2Cl_2 (3 x 3 mL). The combined organic phase was dried (Na_2SO_4) and evaporated in vacuo to give an oily product. Flash chromatography eluting with $CHCl_3$/MeOH (100:0 to 9:1) gave the desired product.

Benzoate-protected tetrahydrofuran **1** (15.0 mg, 0.044mmol) was subjected to General Procedure B to furnish compound **2** (14.0 mg, 0.04 mmol, 90 %) as a colorless solid. For experimental data see § 3.4.3.[4a] The reaction has also been carried out on 50 mg (0.15 momol) of **1** with the same procedure to give compound **2** with a slightly diminished yiel (45.6 mg, 0.13 mmol, 86%).

Benzoate-protected tetrahydrofuran **5** (20.9 mg, 0.031 mmol) was subjected to General Procedure B (20 mol % RuO_4 was used) to furnish compound **6** (17.4 mg, 0.026 mmol, 83 %) as a white solid. For experimental data see § 3.4.3.[4a]

6.5. References and notes

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Chapter 7

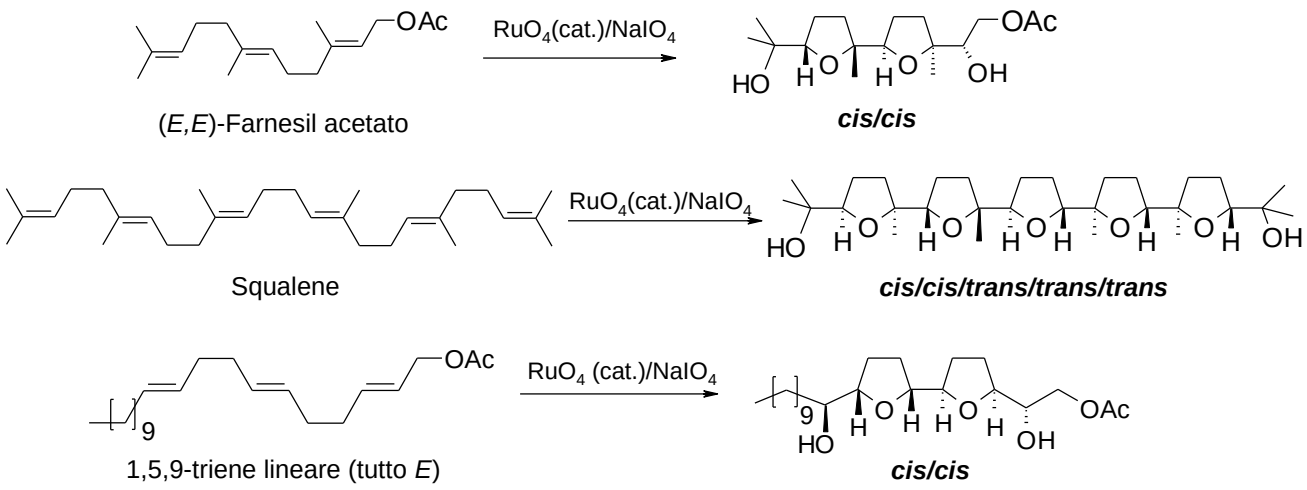
Preliminary experiments on the OsO_4-mediated oxidative bis-cyclization of farnesyl acetate

7.1. Introduction

Saturated ether rings of various sizes are part of the structure of a variety of natural and non-natural substances displaying important biological properties. In particular, 2,5-disubstituted tetrahydrofurans are structural fragments commonly found in natural compounds such as polyether antibiotics, e.g. monensin A,[1] squalene-derived metabolites such as glabrescol,[2] and annonaceous acetogenins such as goniocin and mucocin, [3] just to mention some representative categories. Many research group have focused their efforts toward isolation, synthesis as well as structural modification of the basic backbone of these substances. Indeed, a variety of methods have been developed to produce or modify the oxacycle portions of these molecules, in order to obtain substances belonging to the above mentioned classes, but also to synthesize new analogues of these molecules to be used in biological assay or in structure activity relationship (SAR) studies.

One of the method currently employed to synthesized THF rings features the oxidative cyclization of 1,5-dienes mediated by transition metal oxo-species. Initially, oxidative cyclization of polyenes was restricted to the reactivity of permanganate with 1,5-dienes,[4] next it has been discovered that OsO_4, RuO_4, and MnO_4^- share a common reactivity toward 1,5-dienes and all of them can be used to obtain mono-THF diol compounds.[5]

Some of the findings in this field have been achieved in the laboratory where this PhD thesis has been carried out.[6,7] In particular, besides studies focused on the oxidative mono-cyclization of 1,n-dienes, some years ago, the same group has discovered a new RuO_4-mediated stereoselective cascade process allowing the polycyclization of polyenes characterized by a repetitive 1,5-diene structural motif (Scheme 1).[7a] Employing this procedure it is possible to prepare all-*threo* adjacently linked poly-THF compounds in a single step and a stereoselective manner. This process is useful in organic synthesis, since it can be used for the preparation of the poly-THF portion of natural and non-natural biological active substances containing one or more 2,5-disubstituted THF rings adjacently linked.[8]

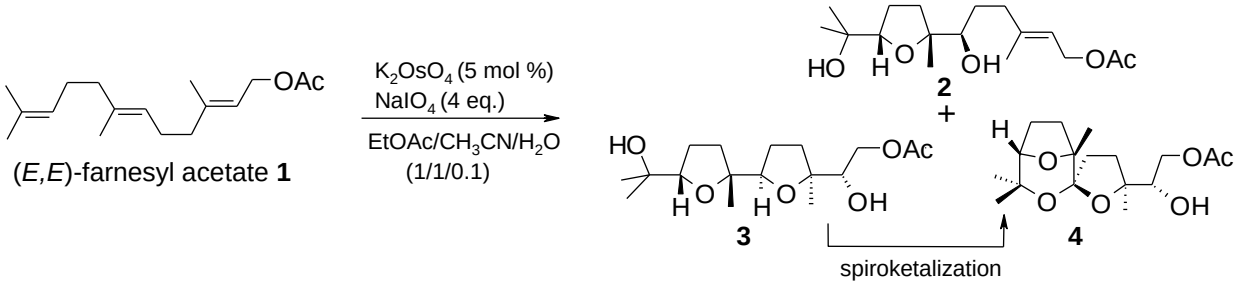


Scheme 1. Representative examples of RuO_4-catalyzed polycyclization of isoprenoid and linear polyenes developed by our own group.

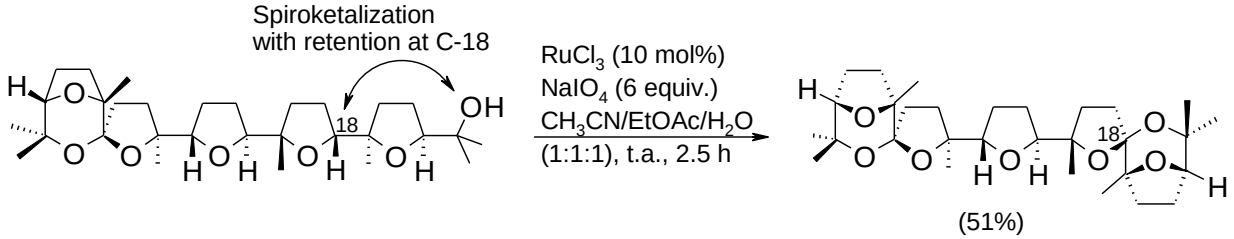
In continuing our studies in this field, during the last year of my PhD programme, some experiments have been carried out to assess the osmium ability to mediate the bis-cyclization of polyenes with a repetitive 1,5-diene structural motif.

7.2. Results and discussion

We envisaged that OsO_4, like ruthenium tetroxide, could be successfully employed to induce the bis-cyclization of proper polyenes, to develop a new alternative and maybe more efficient method to that end. Therefore, farnesyl acetate (**1**) was used to test different reaction conditions. We found that the system $K_2OsO_4/NaIO_4$ is able to induce the bis-cyclization process providing a 30% yield of bis-cyclised compounds. Best results were obtained using 5 mol % of K_2OsO_4, 4 equiv. of $NaIO_4$ and a mixture of EtOAc/MeCN/H_2O (1/1/0.1) as the solvent (Scheme 2). In particular, the main product of the process is the mono-cyclized compound **2** (30%), whereas the product of bis-cyclization **3** is obtained in a 25% yield along with a 5% amount of **4**, a compound arising from the spiroketalizatin of **3**. This process is similar to the RuO_4-catalysed oxidative spiroketalization (see Chapter 4) that we have recently developed (Scheme 3).



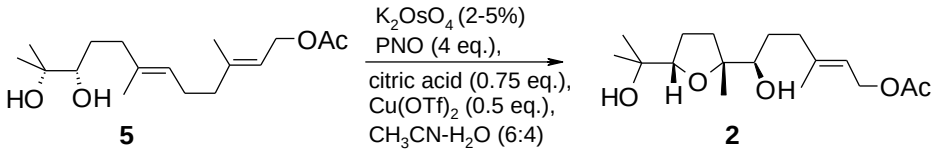
Scheme 2. Reaction of farnesyl acetate with $OsO_{4\,(cat.)}/NaIO_4$.



Scheme 3. RuO_4-mediated spiroketalization.

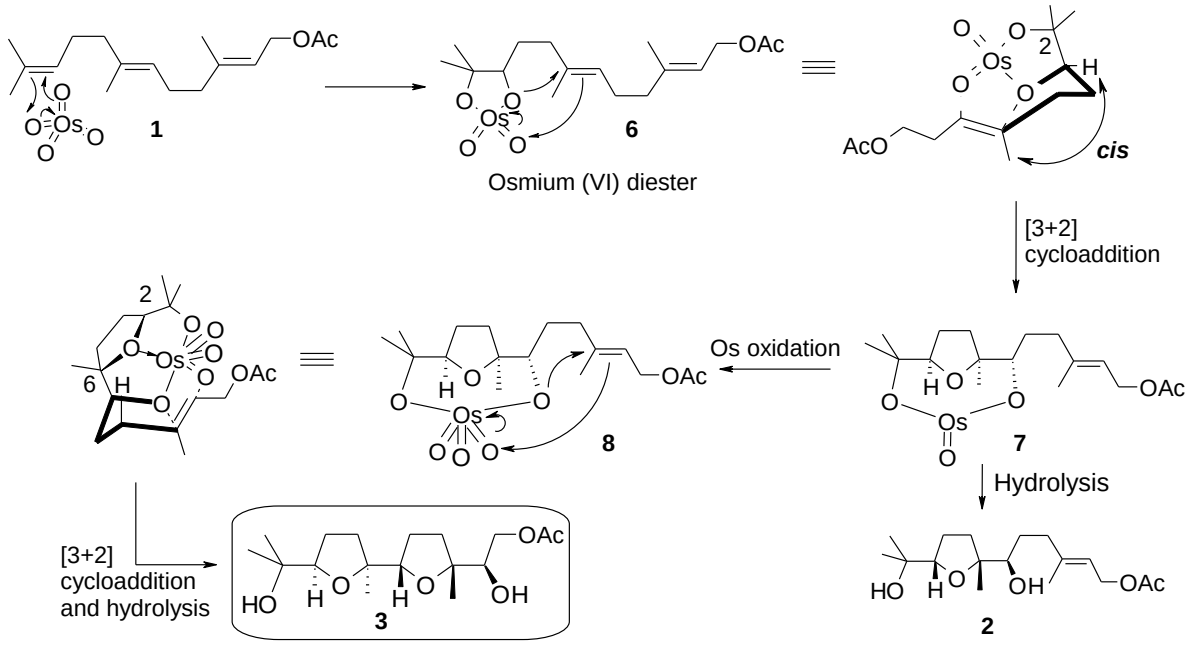
For what concerns the amount of water in the solvent mixture we found that larger quantities turned out to be detrimental for the process of bis-cyclization since water induces the hydrolysis of the osmate diester intermediate **7** (see later Scheme 5) interrupting the process after the mono-cyclization. Once osmium has dissociated from the substrate it is no longer able to re-attack the mono-THF compound. Importantly, a certain amount of water is still required since it is needed to set osmium free at the end of the process in order to close the catalytic cycle.

Therefore, we have demonstrated that the system $K_2OsO_4/NaIO_4$ succeeded in the bis-cyclization process providing a 30% yield of bis-cyclised compounds. On the contrary, using K_2OsO_4 with pyridine *N*-oxide (PNO for short) as co-oxidant (PNO is a milder oxidant compared to $NaIO_4$ and it is employed to avoid oxidation of Os(VI) to Os(VIII)), to cyclise the diol of farnesyl acetate (**5**, Scheme 3) in the conditions developed by Donohoe *et al.* for the mono-cyclization of 1,5-dienes, we only get the mono-cyclized product. This result suggests that possibly an Os^{VIII} species is involved in the second cyclization.



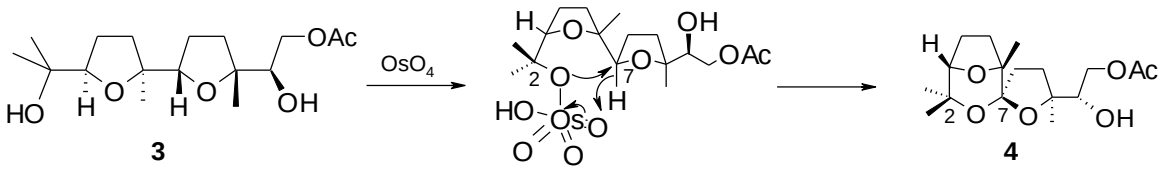
Scheme 4. Oxidative mono-cyclization using Donohoe's conditions.

A plausible mechanistic rationalisation for the formation the bis-cyclized product (Schema 5) is given in Scheme 5 in agreement with the known reactivity of OsO_4 and related transition metal oxo-species as well as the model developed for RuO_4. It is likely that, after the formation of the first THF ring, an osmium species at a low-valent state is bonded to the newly formed diol system (**7**). At this point, if osmium is re-oxidized to Os^{VIII} forming intermediate **8**, a second cycloaddition can occur and a new THF ring is formed, otherwise osmium ester **7** is hydrolyzed and the process stops leaving a mono-THF compound. It is also to be noted that both THF rings are *cis* because of the locked conformation adopted by the osmate ester in the intermediate **6** and **8**.



Scheme 5. A plausible mechanistic explanation for the formation of compound **3**.

For what concerns the formation of the spiroketal moiety in **4**, a mechanistic hypothesis is reported in Scheme 6. As we have seen for the analogue process mediated by RuO_4 (see Chapter 6 and Scheme 3), an oxo-osmium appendage tethered to the C2-OH could attack the C7-H bond leading to formation of the spiroketal.



Scheme 6. A plausible mechanistic hypothesis for the osmium-mediated spiroketalization.

Overall, these results have a certain relevance since osmium ability to mediate the bis-cyclization of a 1,5,9-triene as well as the spiroketalization process have never been reported before and therefore these processes deserve further studies.

7.4. Experimental section

Farnesyl acetate was obtained by acetylation of farnesol under classical condition.

Farnesyl acetate (25.4 mg, 0.096 mmol) was was dissolved in the biphasic mixture EtOAc/CH_3CN/H_2O (1:1:0.1, 2.1 mL). $NaIO_4$ (4 equiv., 0.385 mmol, 82.3 mg) and K_2OsO_4(5 mol %, 0.0043 mmol, 1.77 mg) were sequentially added under vigorous stirring. After 19 h excess $Na_2S_2O_5$ was added and the mixture was stirred for further 10 min and then it was extracted with EtOAc (3 x 5mL). The combined organic phases were dried (Na_2SO_4) and evaporated in vacuo to give an oily product that was separated by HPLC (250 x 10 mm column, eluent: EtOAc-cyclohexane, 6:4, flow 2.5 mL/min, 10 mg/injection) to give spiroketal **4** (1.6 mg, 0.0048 mmol, 5 %, t_R = 14.0 min), mono-THF **2** (9.0 mg, 0.029 mmol, 30 %, t_R = 17.5 min) and bis-THF **3** (7.9 mg, 0.024 mmol, 25 %, t_R = 21.5 min). Spectral data of **3** are in agreement with those reported.[9]

2: Oil; ^{1}H NMR (500 MHz, $CDCl_3$) δ = 5.38 (t, *J* = 7.0 Hz, 1H), 5.38 (t, *J* = 7.0 Hz, 1H), 4.59 (d, *J* = 7.0 Hz, 2H), 3.86 (t, *J* = 6.3 Hz, 1H), 3.41 – 3.35 (m, 1H), 2.34 (dd, *J* = 14.3, 7.1 Hz, 1H), 2.05, 1.71, 1.25, 1.17, 1.13; ESI+/MS: *m/z* 315 $[M+H]^+$, 337 $[M+Na]^+$ 353 $[M+K]^+$.

4: Oil; ^{1}H NMR (500 MHz, $CDCl_3$) δ = 4.59 (d, *J* = 6.9 Hz, 1H), 4.22 (dd, *J* = 11.8, 2.6 Hz, 1H), 4.05 – 4.00 (m, 1H), 3.87 (d, *J* = 7.3 Hz, 1H), 3.65 (dd, *J* = 8.6, 2.7 Hz, 1H), 2.11 (s, 3H), 1.46 (s, 3H), 1.33 (s, 3H), 1.32 (s, 3H), 1.03 (s, 3H); ESI+/MS: *m/z* 331 $[M+H]^+$, 353 $[M+Na]^+$ 369 $[M+K]^+$.

7.5. References

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