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Synthesis of Bioactive Sesterterpenolides from *ent*-Halimic Acid. 15-Epi-*ent*-cladocoran A and B

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The bioactive sesterterpenoid γ-hydroxybutenolides 15,18-bisepi-*ent*-Cladocoran A and B, **1** and **2**, and 15-epi-*ent*-Cladocoran A and B, **57** and **55**, were synthesized from *ent*-halimic acid. The synthesized sesterterpenolids **2**, **55**, **57**, and **59** inhibited cellular proliferation ($IC_{50} \cong 2\ \mu M$) of a number of human leukaemic and solid tumor cell lines.

1. Introduction

The study of the marine metabolites has been a subject of great interest in recent years, due to the large number of such compounds that present very interesting biological properties. Among these, a considerable number of sesterterpenoids[1] have been isolated, many of which possess a γ-hydroxybutenolide[2−5] as a significant structural feature, and in many cases this group is involved in the biological activities of these compounds.

Cladocoran A and B are sesterterpenolides isolated from the mediterranean coral[6] *Cladocora cespitosa* (L) by Fontana et al. These authors proposed structures **1** and **2** on the basis of spectroscopic data. Since the new carbon skeleton proposed for **1** and **2** was an isoprenyl *ent*-halimane, we decided to synthesize it along with some analogues and to test the biological activity of these compounds.

Sesterterpenolides **1** and **2** are structural analogues of the natural sesterterpenolide dysidiolide, isolated in 1996,[7] an inhibitor of protein phosphatase cdc25A ($IC_{50} = 9.4\mu M$) and cdc25B ($IC_{50} = 87\mu M$), which are essential for cell proliferation. Dysidiolide[8] inhibits the growth of A-549 human lung carcinoma and P388 murine leukaemia cell lines at low micromolar concentrations.[9,18] Because of its unusual structure and its potentially important physiological activity, dysidiolide has attracted considerable attention from chemists, biologists, and pharmacologists.[8−19]

We have previously communicated the synthesis of **1** and **2** and the epimers of these compounds at C_{18}, showing that the structures proposed by Fontana et al. for cladocoran A and B (**1** and **2**) should be revised.[20] Recently, during the preparation of this paper, Yamada et al. reported the synthesis of the enantiomers of **1** and **2**, and their epimers at C_{18} (optical rotation for enantiomers of **1** and **2**:[21] *ent*-**1**, $[\alpha]_D$ − 7.2 (*c* 0.25, $CHCl_3$); *ent*-**2**, $[\alpha]_D$ − 64.3 (*c* 0.28, $CHCl_3$). They went on to describe the synthesis of the correct structures for cladocoran A and B (see Figure 1), establishing cladocoran B as an olefinic regioisomer of dysidiolide and cladocoran A as its acetate.[21]

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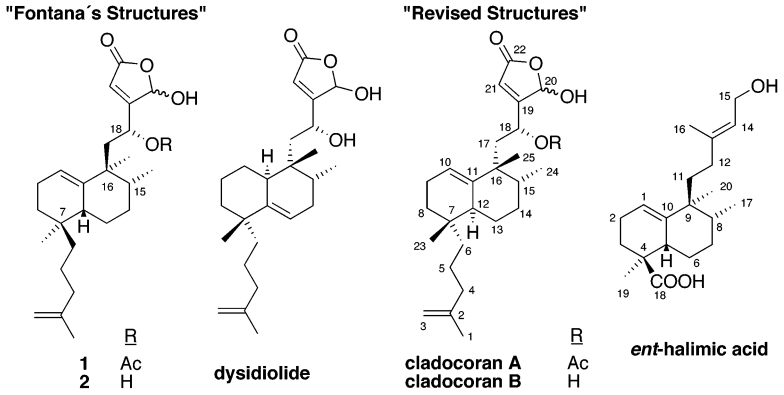

FIGURE 1. Chemical structures for sesterterpenolides and *ent*-halimic acid

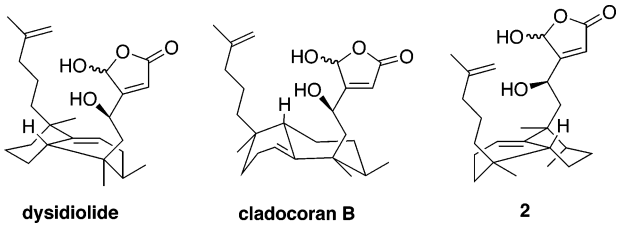

FIGURE 2. Structures for dysidiolide, cladocoran B, and **2**.

The X-ray study shows that dysidiolide, in the crystalline state, adopts a conformation with both chains on the same side of the Decalin ring system.[7] Molecular modeling of dysidiolide, **2**, and cladocoran B shows that these compounds are able to adopt analogous conformations to that of dysidiolide,[7] with both chains parallel on the same side of the Decalin, as is shown in Figure 2.

The molecular modeling studies[22] performed for dysidiolide, compound **2**, and cladocoran B show a correspondence between the main functional groups and, additionally, the occupied volumes for these molecules are similar (Figure 3). Since dysidiolide, compound **2**, and cladocoran B have the same key functional groups for an interaction with a potential biological substrate, it was thought probable that **2** and cladocoran B would show similar biological properties to dysidiolide.

FIGURE 3. 3 Overlay of **2**, cladocoran B, and dysidiolide. The carbon atoms of **2** are magenta, those of cladocoran B are black, and those of dysidiolide are brown; oxygens and hydrogens are red and blue, respectively, in both structures.[22]

In this paper we describe the synthesis of **1** and **2** and their epimers at C_{18}, **55** and **57**. Physical properties of the compounds synthesized by us do not correspond with those reported for cladocoran A and B.[6,20]

2. Results and Discussion

The synthesis of compounds **1** and **2** was planned starting from *ent*-halimic acid methyl ester **3**,[23] of known absolute configuration, due to its structural similarity to the targets. At present, *ent*-halimic acid is being employed in the synthesis of *ent*-halimanolides,[24] and furan diterpenoids such as chettaphanin I and II.[25]

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(22) Analysis of molecular models of both the structures (Yamada and Fontana) described for Cladocorans A and B shows that these compounds are able to adopt conformations analogous to that observed crystallographically for dysidiolide, with both "floppy" side chains lying parallel in the same hemisphere relative to the Decalin ring. A conformation of each molecule was selected based on low energy and geometric similarity both to each other and to the crystal structure of dysidiolide, and these conformers were then overlaid on dysidiolide by using the oxygen atoms and sp^2 hybridized carbon atoms as matchpoints for the Sybyl "MATCH" command. The resulting overlay displayed an RMS error for these atoms of 0.81 and 0.72 Å for Cladocoran B and **2**, respectively, the maximal distance between matching atoms being just under 1.2 Å for both structures. The structures are shown in the graphic with heavy atoms and hydrogens attached to heteroatoms displayed. Clearly the major functional groups correspond closely and indeed the volumes occupied by the molecules are similar. Since the key functional groups likely to form interactions with any biological target are in virtually identical relative orientations in these molecules as overlaid in the figure, it is highly likely that cladocoran B will show biological properties similar to those already observed for dysidiolide and reported here for **2**. This is particularly probable as the more orientation-dependent features (hydrogen bonding sites) are able to align very closely. Even the relatively polarizable double-bond regions correspond closely between the two structures, and the hydrophobic zones are sufficiently similar to lead to a high expectation that similar interactions could be formed with a biological target. This is to a limited extent offset by the less clear conformational preference of **2**, but although only the diaxial form might be expected to contribute to dysidiolide-like activity for this compound, this is expected to be a major, if not necessarily the major, conformation in aqueous medium.

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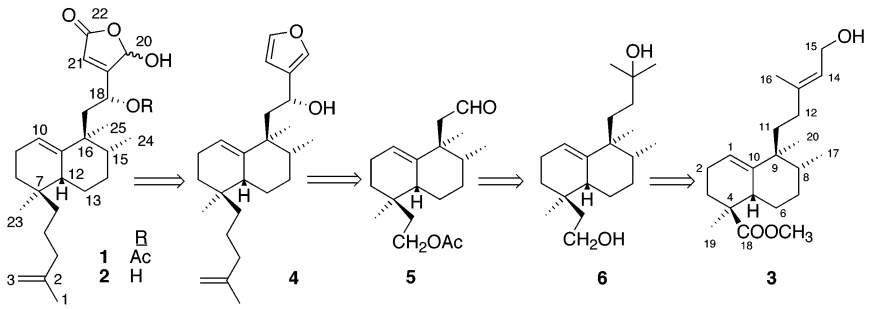
SCHEME 1



The synthesis of **1** and **2** from *ent*-halimic acid methyl ester **3** presented two main problems: manipulation of side chains on C_{18} (south chain: *ent*-halimic acid numbering), to introduce a new isoprenic unit, and on C_9 (north chain), to achieve the introduction of the γ-hydroxybutenolide group and control of stereochemistry for the hydroxyl group to be placed at C_{12} of *ent*-halimic acid methyl ester **3**.

The retrosynthetic route for **1** and **2** from *ent*-halimic acid methyl ester **3** is presented in Scheme 1.

The γ-hydroxybutenolide moiety of **1** and **2** was obtained from **4** following Faulkner[26] methodology. Compound **4** could be obtained by addition of furyllithium to an aldehyde such as **5** and elongation of the south chain of **5**. The elongation of the south chain by an isoprene unit was done in two steps: adding one carbon by Wittig condensation to give an intermediate such as **6**, and then adding the four remaining carbons by S_N2 substitution, due to the difficulty of achieving substitution at a neopentyl carbon.

Degradation of the north chain had to take account of the annular double bond of **3**, so the oxidation of the Δ^{13} olefin would have to be chemoselective. Initially, attention was focused on degradation of **3** and elongation by one carbon unit on C_{18} to obtain a compound such as **6**.

Hence, the synthesis of **1** and **2** could be described in four main chapters: synthesis of diol **6**, aldehyde **5**, furan **4**, and the final compounds **1** and **2**.

2.1. Synthesis of Diol 6. The transformation of *ent*-halimic acid methyl ester **3** into diol **6** could be addressed in two different ways: Route 1, extension by one carbon atom at C_{18} and modification of the north side chain, or Route 2, modification of the north side chain and then elongation of south side chain.

Route 1: First of all *ent*-halimic methyl ester **3** was protected as its methoxy derivative **7**, which was transformed into tosylate **10** by reduction of the ester group of **7** with LAH and tosylation of the resulting alcohol **8** under the usual conditions (Scheme 2). Substitution at C_{18} was tested with various simple nucleophiles (BuBr/Li, MeLi, INa, ...), with no success. Due to the failure of these model experiments, extension by one isoprenic unit at C_{18} of the *ent*-halimane skeleton requires two steps: first the introduction of a new carbon, C_{18a}, and second a subsequent addition of the remaining four carbon atoms. Hence, diol **6** was obtained from the methyl ester of *ent*-halimic acid **3** (Scheme 2) in two ways, route 1a and route 1b, with extension by one carbon atom at C_{18} in both cases leading to **16** and degradation of the side chain to give **6**. The main difference between the routes is the sequence in which the homologation at C_{18} took place.

The synthesis of **16**, precursor of **6** (Scheme 2), was accomplished by two different procedures: in route 1a, Arndt−Eistert[27] synthesis using the acid chloride **12,** and in route 1b, TPAP oxidation of **8** followed by Wittig[28] reaction and hydrolysis to obtain the aldehyde **19**. In both cases we used **7** as the starting material.

Route 1a: Alkaline hydrolysis of **7** led to acid **11,** which, on reaction with $SOCl_2$, gave **12**, which in turn was transformed into **13** by reaction with CH_2N_2. Heating of **13** with MeOH or EtOH in the presence of Ag_2O gave esters **14** or **15** in low yield, which, on reduction with LAH, both produce **16**.

Route 1b: As the yield in the Wolff rearrangement of **13** was not satisfactory, the extension by Wittig condensation was tested[28] starting from aldehyde **9** (Scheme 2), which was obtained in an excellent yield by reduction of **7** with LAH and subsequent TPAP oxidation of **8**.

Reaction of **9** with methoxymethylenetriphenylphosphonium chloride in the presence of LDA gave **17/18** (*Z*:*E*, 1:1) in low yield, but if NaHMDS was used as base the yield was 92% (*Z*:*E*, 8:1). The increase in the ratio of the *Z* isomer is in concordance with salt-free reaction conditions, and additionally the latter base used favors kinetic control and hence an increase in the proportion of the *Z* isomer, **17**.[29]

The synthesis of **19** from **17**/**18** did not take place under Levine conditions,[30] but could be achieved with *p*-TsOH in acetone/water.[30] The best conditions, to avoid the formation of **20** and **21**, were to run the reaction with **17**/**18** in a 0.03 M solution of acetone/H_2O (45:1) in the presence of a small quantity of *p*-TsOH (0.3 mol/mol). Optimization of the reaction conditions for the preparation of **19** was necessary to avoid the formation of byproducts **20** and **21**, tricyclic compounds epimeric at

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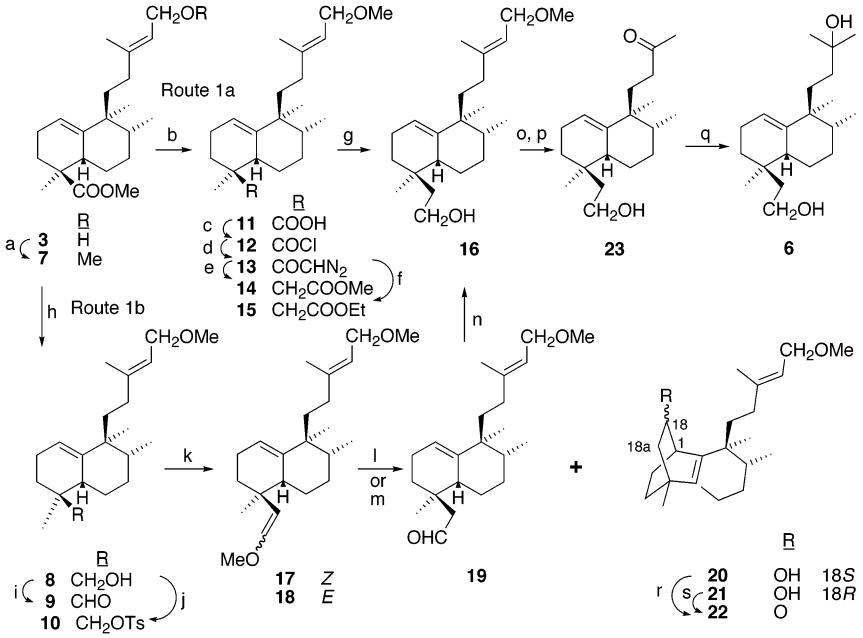
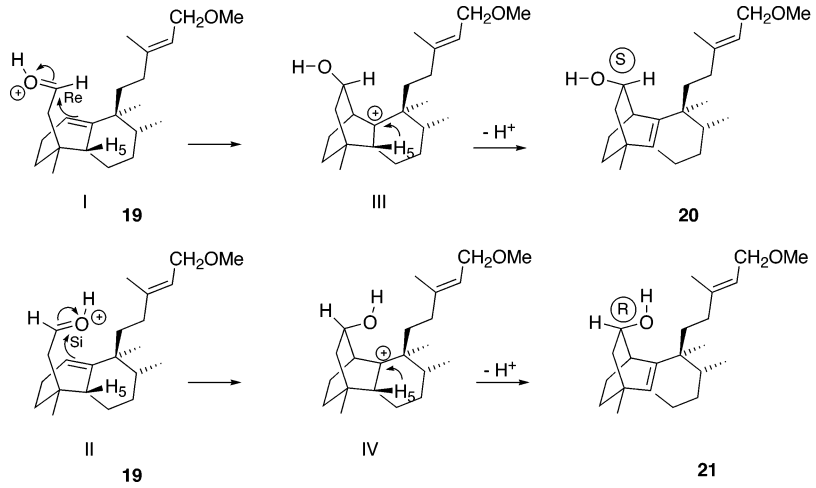
(30) (a) Levine, S. J. *J. Am. Chem. Soc.* **1958**, *80*, 6150. (b) To avoid compounds **20** and **21** in this reaction, the best conditions founded were to conduct the reaction with a solution of **17**/**18** in 0.03 M acetone/H_2O (45:1) and *p*-TsOH (0.3 mol/mol).

SCHEME 2 [a]



[a] Reagents and conditions: (a) NaH, MeI, THF, 2 h (92%); (b) NaOH, MeOH, 80 °C, 3 h (99%); (c) $SOCl_2$, C_6H_6, 1 h (99%); (d) CH_2N_2, Et_2O (94%); (e) Ag_2O, MeOH, 60 °C, 8 h (20%); (f) Ag_2O, EtOH, 60 °C, 8 h (20%); (g) LAH, Et_2O, 45 min (92%); (h) LAH, Et_2O, 1 h (96%); (i) TPAP, NMO, CH_2Cl_2, 15 min (90%); (j) TsCl, pyridine, 16 h (96%); (k) $(MeOCH_2PPh_3)^+ Cl^-$, NaHMDS, THF, −78 °C, 20 min (92%); (l) **17**/**18** 0.03 M acetone/H_2O, *p*-TsOH (0.3 mol/mol), 4 h (**19**: 98%); (m) **17**/**18** 0.14 M acetone/H_2O, *p*-TsOH (1.0 mol/mol), 20 min (**19**: 19%; **20**: 30%; **21**: 18%); (n) LAH, Et_2O, 30 min (96%); (o) OsO_4, NMO, *t*-BuOH/THF/H_2O (7:2:1), 24 h (99%); (p) $Pb(OAc)_4$, C_6H_6, 20 min (95%); (q) MeMgBr, Et_2O, −78 °C, 1 h 30 min (91%); (r) TPAP, NMO, CH_2Cl_2, 3 h (82%); (s) TPAP, NMO, CH_2Cl_2, 3 h (83%).

SCHEME 3



the secondary alcohol center, which were both transformed into ketone **22** by TPAP[31] oxidation. The correlation present between C_1 of **20** or **21** (38.3 or 38.1 ppm) and H_{18} (3.87 or 3.83 ppm) observed in the HMBC experiment can only be explained by the existence of a bond between the relevant carbon atoms with the formation of a new ring.

Formation of **20** and **21** proceeded by a Prins[32] reaction of the annular olefin with the aldehyde that results from the acidic hydrolysis of **17**/**18,** as indicated in Scheme 3. Conformers I and II of **19** would thus be transformed into III and IV, respectively, followed by loss of H_5 to give **20** and **21**, respectively, depending on whether the cyclization takes place initially on the Re (I) or Si (II) side of the protonated aldehyde. Furthermore, the more stable conformer I would give the major isomer **20**, so it may be deduced that the stereochemistry at C_{18} is *S* for **20** and so *R* for **21**; this stereochemistry will be confirmed later on.

Reduction of **19** with LAH gives **16** (Scheme 2), the side chain of which was shortened by two carbons by chemoselective epoxidation with *m*-CPBA[33] and oxidation[34] with H_5IO_6, giving **23** in a 64% yield for the two steps. If the sequence $OsO_4/Pb(OAc)_4$[35] is used, the yield increases to

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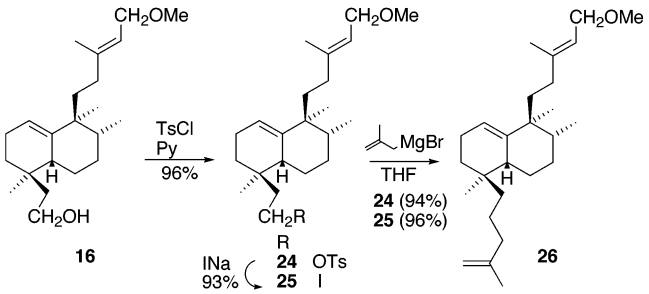
SCHEME 4



95%. The Baeyer−Villiger reaction cannot be used without affecting the annular double bond,[33] and for this reason diol **6** was synthesized by treatment of **23** with MeMgBr.[36]

To test the efficiency of the sequence for full extension of the south chain, compound **16** was transformed as indicated in Scheme 4 into **24**[37] and this compound further transformed under the usual conditions into iodide **25**. Either of these compounds, on reaction with 2-methylallylmagnesium bromide, gave **26** in good yield.

Route 2: The second route for the synthesis of **6** (Scheme 5) consisted first of all of shortening the side chain by two carbons followed by elongation by one carbon at C_{18}. It is not necessary to protect the hydroxyl on C_{15} for the degradation, and for this reason compound **27**, obtained directly by reduction of **3**, was chosen as the starting material.

Treatment of **27** with *m*-CPBA (mol/mol), followed by oxidation with H_5IO_6 gives **28** in a 61% yield, which can be increased (to 90%) by hydroxylation with OsO_4 followed by treatment with $Pb(OAc)_4$.

It is convenient to transform the ketone of **28** into the tertiary alcohol **29** by addition of MeMgBr to avoid any problems with the elongation at C_{18}.

Extension by one carbon atom at C_{18} of **29** was performed following the synthetic route given in Scheme 5. Aldehyde **30**, obtained by TPAP oxidation of **29**, reacted with the phosphonium salt $(MeOCH_2PPh_3)Cl$ in the presence of NaHMDS to give the mixture of vinyl ethers **31** and **32** in a 6:1 ratio.

The coupling constants between H_{18} and H_{18a} in the ^1H NMR spectra of **31** and **32** establish *Z* stereochemistry ($J = 7.0$ Hz) for **31** and *E* stereochemistry ($J = 12.9$ Hz) for **32**. Hydrolysis of the mixture **31/32** under the conditions described earlier gave **33** (80%), **34** (10%), and a small amount (2%) of recovered **32**. The absolute configuration of **34** (Figure 4) has been established by X-ray crystallography.[38] The conformation of the aldehyde in the Prins reaction is fixed by a hydrogen bond to the hydroxyl group of the side chain, so the addition of the alkene can only be on the Si side of the aldehyde (Scheme 6), which corresponds with the product observed.

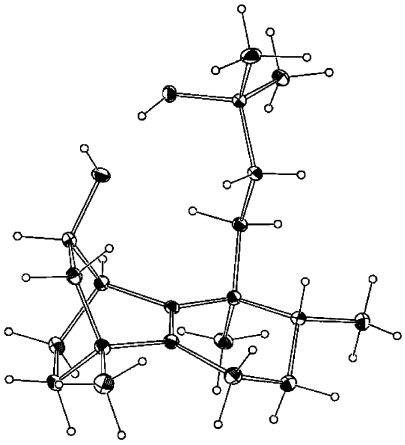


FIGURE 4. ORTEP view of compound **34**.

Taking into account the fact that the structural differences of **34**, **20**, and **21** are only in the side chains, and that the influence of these differences on the rotation value should be relatively small, we propose the absolute configuration 18*R* for **21** and 18*S* for **20**, due to the similarity of the $[\alpha]_D$ of **21** with that of **34**, of known absolute configuration.

LAH reduction of aldehyde **33** gave diol **6** (Scheme 5), the compound at the intersection of routes 1 and 2, and the key objective of the first part of the proposed synthesis.

Route 1b gives diol **6** in 9 steps with a 60% overall yield and Route 2 gives the same diol in 8 steps but with a 47% overall yield.

2.2. Synthesis of Aldehyde 5. The synthesis of **5**, as planned in the retrosynthetic scheme (Scheme 1), requires shortening of the side chain of the *ent*-halimane by removal of two carbon atoms, C_{13} and C_{16}, from **6**. Since it was impossible to acheive this directly it was necessary to study the dehydration of the hydroxy group on C_{13}, to give a Δ^{12} derivative.

Bearing in mind not only the dehydration of the tertiary hydroxy group but also the need to have a good leaving group on C_{18}, compound **6** was treated with Tf_2O (Scheme 7) to give **35** in 24% yield (the tertiary hydroxy

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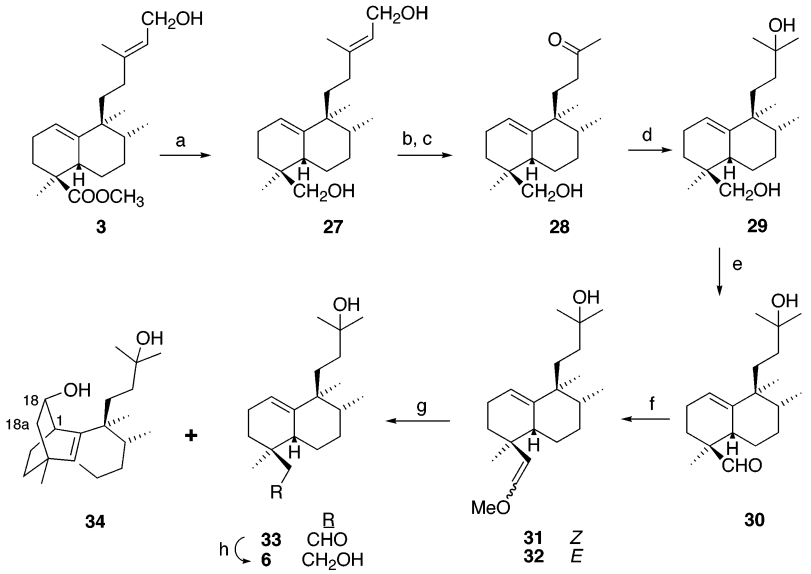
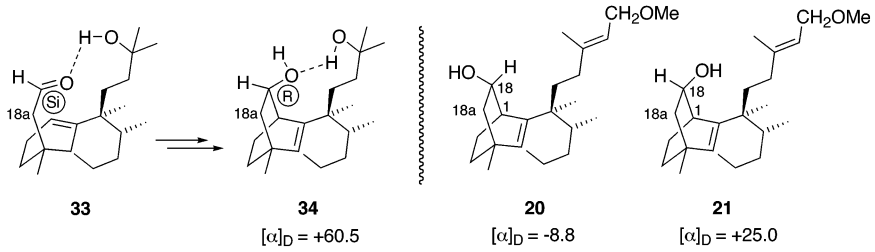
(38) Crystal data for **34**: $C_{20}H_{34}O_2$, MW 306.5, orthorhombic, space group *P*212121, $a = 6.9511(3)$ Å, $b = 11.9573(7)$ Å, $c = 22.330(2)$ Å, $\alpha = 90°$, $\beta = 103.38(1)°$, $\gamma = 90°$, $V = 1856.0(2)$ Å^3, $Z = 2$, $D_c = 1.097$ Mg/m^3, μ(Cu Kα) = 0.523 mm^{-1}, $F(000) = 680$. Data (1841 independent reflections, θ range 3.15 to 65.00°) were measured on a rotating anode diffractometer with Cu Kα radiation (graphite monochromator), using $2\theta-\omega$ scans at 268 K. The sturture was solved by direct methods and the non-hydrogen atoms were refined anisotropically by full-matrix least squares based on F_2 to give $R_1 = 0.0325$ and $wR_2 = 0.0479$ [$I > 2\sigma(I)$]. The positions of the hydrogen atoms were located from the difference Fourier method and refined isotropically. The absolute chirality was determined by internal reference. Computations were carried on a Digital 300-MHz workstation with the XRAY80 and SHELX93 programs. Crystallographic data for the structure reported in this paper have been deposited at the Cambridge Crystallographic Data Center as Supporting Information, N*o*. CCDC 145502.

SCHEME 5 [a]

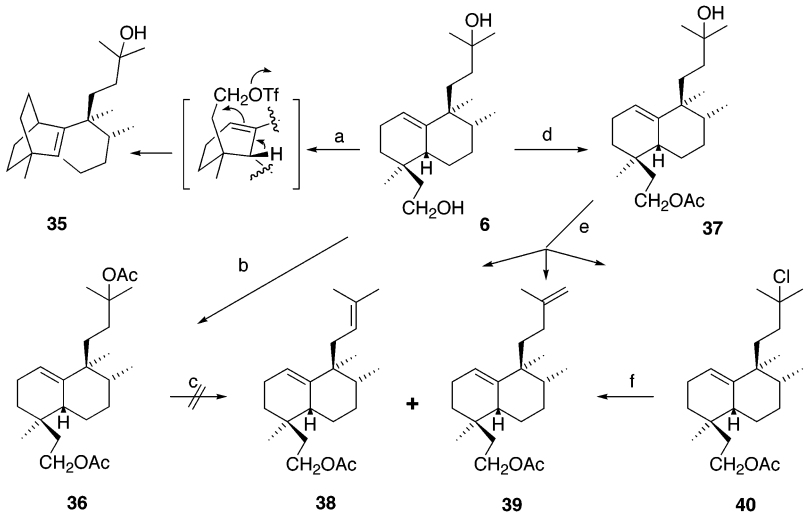


[a] Reagents and conditioins: (a) LAH, Et_2O, 1 h (97%); (b) OsO_4, NMO, *t*-BuOH/THF/H_2O (7:2:1), 24 h (97%); (c) $Pb(OAc)_4$, C_6H_6, 20 min (93%); (d) MeMgBr, Et_2O, −78°C, 2 h (97%); (e) TPAP, NMO, CH_2Cl_2, 2 h (92%); (f) $(MeOCH_2PPh_3)^+ Cl^-$, HMDSNa, THF, −78 °C, 20 min (81%; *Z*:*E*, 6:1); (g) *p*-TsOH, acetone, H_2O (**33**: 80%; **34**: 10%); (h) LAH, Et_2O, 30 min (90%).

SCHEME 6



SCHEME 7 [a]

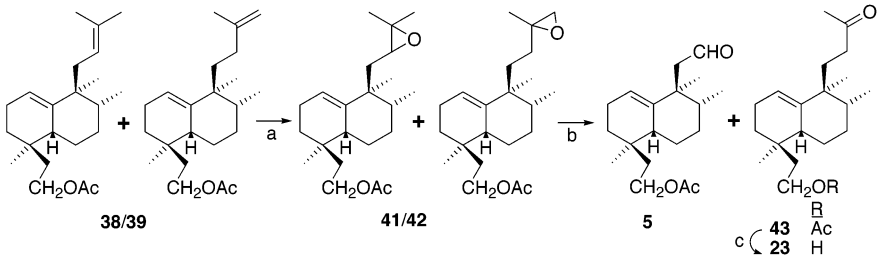


[a] Reagents and conditions: (a) Tf_2O, Et_3N, 20 min (24%); (b) Ac_2O, DMAP, pyridine, 16 h (35%); (c) SiO_2, 100 °C, 4 h; (d) Ac_2O, pyridine, 5 h (95%); (e) $POCl_3$, pyridine, rt, 1 h (**38**/**39**: 1:1, 75%; **40**: (10%); (f) Li_2CO_3, DMF, 100 °C, 24 h (**38**/**39**: 25:75, 99%).

group remaining in place). The formation of **35** can be explained as indicated in Scheme 7. Treatment of **6** with Ac_2O in pyridine gave the monoacetyl derivative **37** in 95% yield; if DMAP was added to an otherwise unaltered mixture of reagents, the diacetyl derivative **36** was obtained in a 35% yield. Pyrolysis of **36** on silica gel gave decomposition. Reaction of **37** with $POCl_3$/Py[39] at room temperature gave the mixture **38**/**39** (1:1) in a 75% yield and 10% of the chloro derivative **40**. Upon treatment of

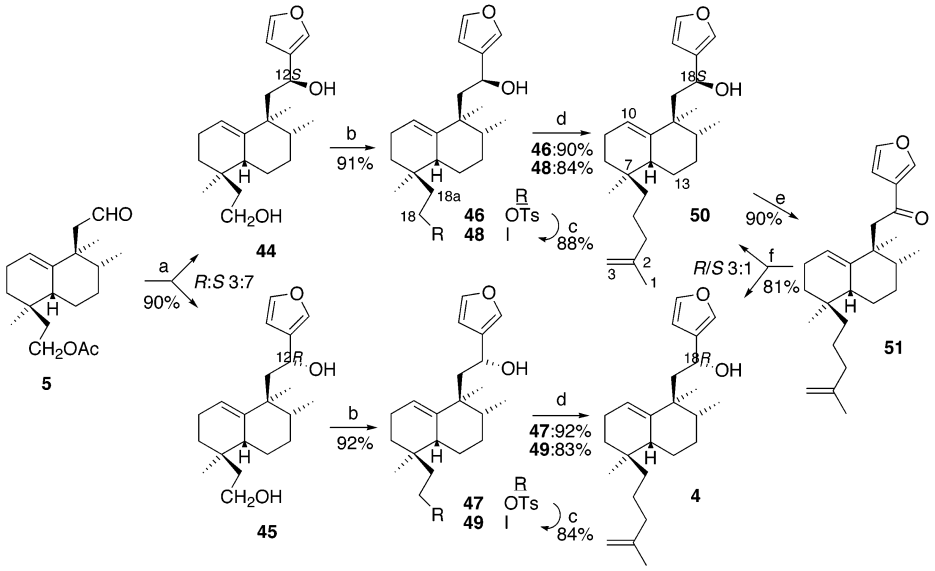
(39) (a) Mehta, G.; Murthy, A. N.; Reddy, A. V. *J. Am. Chem. Soc.* **1986**, *108*, 3443. (b) Giner, J. L.; Margot, C.; Djerassi, C. *J. Org. Chem.* **1989**, *54*, 369.

SCHEME 8 [a]



[a] Reagents and conditions: (a) *m*-CPBA, Cl_2CH_2, 0 °C to rt, 2 h (90%); (b) H_5IO_6, THF, H_2O, 15 min (**5**: 46%; **43**: 45%); (c) K_2CO_3, MeOH, 20 min (98%).

SCHEME 9 [a]



[a] Reagents and conditions: (a) 3-bromofurane, *n*-BuLi, THF, −78 °C, 20 min; (b) TsCl, pyridine, 4 h; (c) NaI, acetone; (d) $CH_2{=}C(CH_3){-}CH_2MgCl$, THF, 12 h; (e) TPAP, NMO, CH_2Cl_2, 45 min; (f) LAH, Et_2O, 30 min.

the latter compound with Li_2CO_3 in DMF,[40] the mixture of olefins **38**/**39** (1:3) was obtained quantitatively.

With this mixture **38**/**39** in hand, the synthesis of the 18a-homo-tetranorderivative **5** was started (Scheme 8). Reaction of **38**/**39** (1:1) with *m*-CPBA (0.5 mol/mol) gave recovered **39** (42%) and a mixture of epoxides **41**/**42** in a 52% yield, which upon cleavage with H_5IO_6 gave **5** (85%) and **43** (6%).

This epoxidation, employing 0.5 equiv of oxidant, provided **39** for the synthesis of dysidiolide analogues with one carbon atom less in one of the side chains. If the epoxidation of **38**/**39** is carried out with 1 equiv of *m*-CPBA (1 mol/mol) it gives rise to the mixture **41**/**42**, which, upon cleavage under the usual conditions, leads to **5** (46%) and **43** (45%).

Dehydration of **6** is not regioselective, but the methyl ketone **43** could be recycled through its hydrolysis product **23** to give **6**, and so increase the overall yield of **5**.

2.3. Synthesis of the Furan Derivative 4: Furyllithium Addition, Absolute Configuration Determination at C_{12}, and Elongation at C_{18}. Treatment of **5** (Scheme 9) with an excess of 3-furyllithium[41] obtained by reaction of 3-bromofuran with BuLi gives a 7:3 mixture of diastereoisomers **44** and **45**.

Structures of **44** and **45** were determined by NMR studies and the configuration of C_{12} was established by X-ray crystallography as *R* for **45**.[20]

The minor isomer **45** (12*R*) is the one required for the synthesis of the proposed structure of the natural compounds, although the synthesis will also be performed with the mixture of epimers at C_{12} with the aim of providing analogues for SAR studies.

To introduce the remaining four carbon atoms, the hydroxyl group at C_{18} was transformed into a good leaving group, first to a tosylate and then to an iodide (Scheme 9). Selective tosylation[42] of **44** and **45** gives the monotosyl derivatives **46** and **47**, respectively, which were easier to separate by column chromatography than the corresponding alcohols. Reaction of **46** and **47** with NaI in acetone at room temperature gave the corresponding iodides **48** and **49**.

(40) Chae, M.-Y.; Cherian, X. M.; Czarnik, A. W. *J. Org. Chem.* **1993**, *58*, 5797.

(41) (a) Ihara, M.; Suzuki, S.; Taniguchi, N.; Fukumoto, K. *J. Chem. Soc., Perkin Trans. 1* **1993**, 2251. (b) Tokoroyama, T.; Fujimori, K.; Shimizu, T.; Yamagiwa, Y.; Monden, M.; Iio, H. *Tetrahedron* **1988**, *44*, 6607. (c) Bock, I.; Bornowski, H.; Ranft, A.; Theis, H. *Tetrahedron* **1990**, *46*, 1199.

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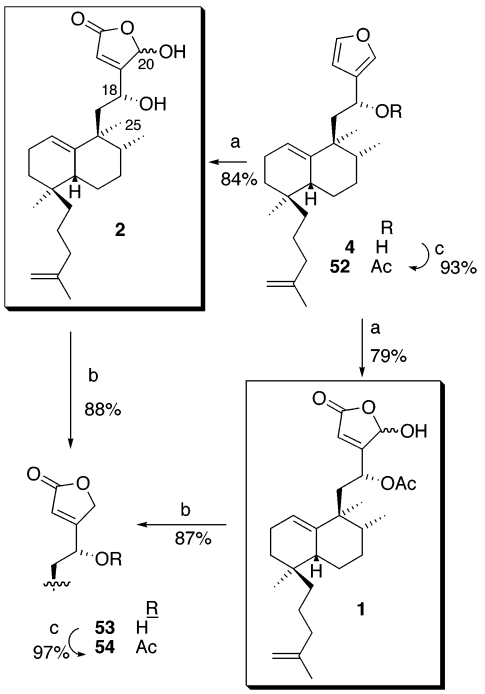
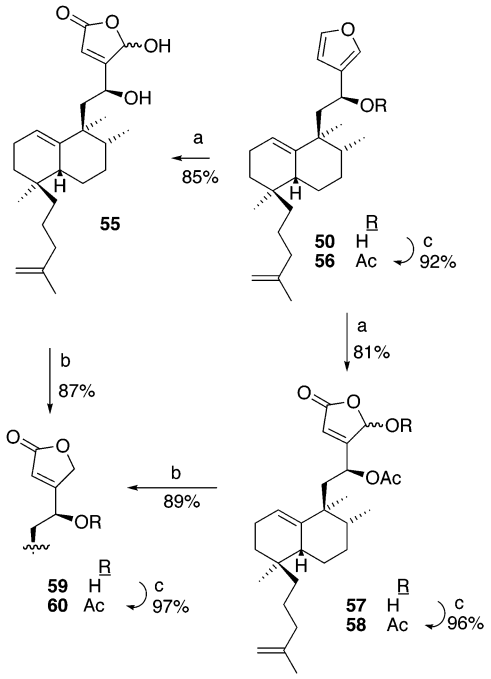
SCHEME 10 [a]



[a] Reagents and conditions: (a) $^1O_2$, *hν*, Rose Bengal, DIPEA, CH_2Cl_2, −78 °C, 2 h 30 min; (b) $NaBH_4$, EtOH, 10 min; (c) Ac_2O, pyridine, 8 h.

SCHEME 11 [a]



[a] Reagents and conditions: (a) $^1O_2$, *hν*, Rose Bengal, DIPEA, CH_2Cl_2, −78°C, 2 h 30 min; (b) $NaBH_4$, EtOH, 10 min; (c) Ac_2O, pyridine, 8 h.

Reaction of **46** or **48** with 2-methylallylmagnesium chloride gave the sesterterpene **50** and reaction of **47** and **49** with the same reagent led to **4** (Scheme 9).

The major isomer **50** can be transformed in good yield to its epimer **4**, which displays the required configuration for the synthesis of the natural product. TPAP oxidation of **50** gives ketone **51**, which, upon reduction with LAH, gives compounds **4** and **50** in a 3:1 ratio. The reduction of the ketone with LAH proceeds with inverse stereoselectivity with respect to that observed for the addition of furyllithium to aldehyde **5**, due to the fact that the Si side of ketone **51** is more accessible to the hydride, giving rise to the hydroxy derivative **4**. The presence of the furyl group in the north side chain of **51** and the pseudoaxial disposition of the south side chain determine that the most stable conformation of the molecule will be the one that directs the Si side of the ketone as the more accessible face.

2.4. Synthesis of 1 and 2. The final transformation for the synthesis of **1** and **2** is the furan oxidation to a *γ*-hydroxybutenolide, which can be done following Faulkner methodology.[26] Photochemical oxidation of **4** gave **2** in 84% yield. Acetylation of **4** under the usual conditions gave the acetyl derivative **52**, which upon $^1O_2$ oxidation under the same conditions gives **1** (Scheme 10).

The physical and spectroscopic properties of the synthesized compounds, **1** and **2** ($[\alpha]_D$ +20.0 and +98.9, respectively), do not coincide with those described for Fontana et al. for the natural compounds[6] cladocoran A and B ($[\alpha]_D$ −25.8 and −59.9, respectively). These natural products[6] were isolated as a 1:1 mixture of epimers at C_{20} and show simple spectra, while from the ^1H NMR spectra of **1** and **2** it can be deduced that the ratio of epimers is 7:3. The signals corresponding to the major and minor epimer can be deduced from the spectrum of **1** and similarly for **2**, and the same can be done for the ^1H NMR spectrum of **2** in C_6D_6. Bidimensional experiments ($^1H/^{13}C$) on **1** and **2** confirmed the structures of these compounds and the signals for ^1H and ^{13}C spectra can be assigned unequivocally.

To corroborate that ring B of cladocoran A was not that of an *ent*-halimane, and to synthesize compounds for biological testing, some analogues were obtained of the supposed natural products **1** and **2**.

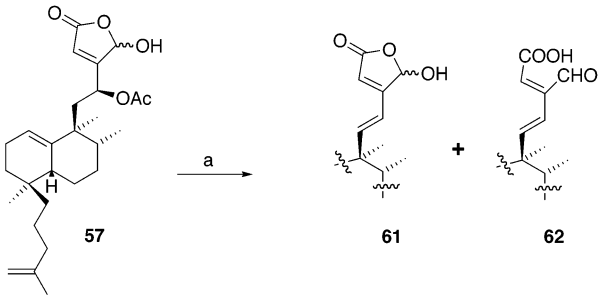
To this end, **55** and **57** (epimers at C_{18} of **1** and **2**) were synthesized, along with the *γ*-hydroxybutenolide reduction products **53**, **54**, **59**, and **60**. (Schemes 10 and 11).

The synthesis of **55** and **57** (Scheme 11) was started from **50**, the epimer of **4** at C_{18}. Photochemical oxidation of **50** gives **55** ($[\alpha]_D$ +26.6) and, in the same way, the oxidation of **56** (obtained by acetylation of **50**) gives **57** ($[\alpha]_D$ +2.0). Acetylation of **57** gives the diacetyl derivative **58**, and it is easy to deduce from the ^1H NMR spectrum of this compound that the ratio of epimers at C_{20} is 7:3.

Reduction of **2** and **1** with $NaBH_4$ gives butenolides **53** and **54**, respectively (Scheme 10), which were correlated by acetylation. In the same way, reduction of **55** and **57** with $NaBH_4$ gives butenolides **59** and **60**, respectively, which were correlated by acetylation of **59** (Scheme 11). However, the physical properties of **54** ($[\alpha]_D$ +25.9) and **60** ($[\alpha]_D$ +20.7) do not coincide with those reported for the reduction product of cladocoran A[6] ($[\alpha]_D$ −38.3).

As indicated in our previous communication, the spectroscopic studies for cladocoran A, cladocoran B, **1**, **2**, **55**, and **57** showed that the two side chains are present in the natural compounds, so the difference must be in the Decalin fragment. This fragment cannot correspond with the *ent*-halimic acid Decalin, but must be an epimer, as resolved by Yamada et al.[20] They conclude that

SCHEME 12 [a]



[a] Reagents and conditions: (a) K_2CO_3, MeOH, 1%, 10 days (**61**: 7%; **62**: 7%).

cladocoran B is an olefinic regioisomer of dysidiolide and that cladocoran A is its acetate, and so **1** and **2** are 15,-18-bisepi-enantio-cladocoran A and B, and its epimers at C_{18} **57** and **55** are the corresponding 15-epi-enantio-cladocoran A and B, respectively.

The well-differentiated presence of the α and β epimers at C_{20} in the ^1H and ^{13}C NMR spectra of **1** and **2** and its epimers **57** and **55** could be attributed to a hydrogen bond in the side chain that will stabilize both epimers. To study the equilibrium of α/β epimers of the γ-hydroxybutenolides, the behavior of the major compound **57** was studied in acidic and basic media.

No variation in the α/β epimer ratio was observed upon treatment of **57** with a saturated solution of oxalic acid. Reaction of **57** and **58** with K_2CO_3/MeOH 3% only gave decomposition products, and when **57** was treated with K_2CO_3/MeOH 1% (Scheme 12) for several days the hydroxylactone **61** and the aldehyde **62** were isolated.

3. Activities

The in vitro antitumor activity for compounds **2**, **55**, **57**, and **59** was determined by measurement of their cytostatic and cytotoxic properties in human tumor cell lines by XTT assays, in which the metabolic activity of viable cells was measured (Table 1). The cell lines used were HL-60 (human acute myeloid leukaemia), HeLa (human cervix cancer), HT-29 (human colon carcinoma), and A549 (human lung carcinoma).

Cells were incubated in RPMI (HL-60) or DMEM (HeLa, HT-29, A549) culture medium containing 10% fetal calf serum in the absence and presence of the indicated compounds at a concentration range of 10^{-4} to 10^{-8} M in a 96-well plate, and following 72 h of incubation at 37°C in a humidified atmosphere of air/CO_2 (19/1) the XTT assay was performed. Measurements were done in triplicate, and the IC_{50} value, defined as the drug concentration required to cause 50% inhibition in the cellular proliferation with respect to the untreated controls, was determined for each compound. Values shown are means ± SE of three independent determinations.

TABLE 1. Inhibition of Proliferation of Human Tumor Cell Lines by Dysidiolide Analogues

	IC_{50} (μM)			
compd	HL-60	HeLa	HT-29	A549
2	0.9 ± 0.1	2.6 ± 0.3	2.3 ± 0.3	1.3 ± 0.2
55	1.9 ± 0.6	2.3 ± 0.5	2.4 ± 0.9	1.7 ± 0.8
57	2.2 ± 0.4	2.9 ± 0.2	2.2 ± 0.4	2.0 ± 0.3
59	7.9 ± 3.4	2.5 ± 0.1	4.7 ± 1.9	2.5 ± 0.8

The proliferation inhibition data show that compounds **2**, **55**, **57**, and **59** have an IC_{50} in the range of 2 μM against various human tumor cells, including leukaemic and solid tumor cells. These data indicate that these compounds are slightly more potent, and act in the same range of concentrations as dysidiolide (IC_{50}, 7.1 μM in HL-60 cells and 5.4 μM on the human lung cancer cell line SBC-5).[43]

These assays reinforce the molecular modeling studies and it seems reasonable to think that cladocorans A and B should also be active compounds.

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Supporting Information Available: ^1H and ^{13}C NMR spectra for all compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

JO034663L

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