Bioorganic & Medicinal Chemistry 21 (2013) 242–256

Contents lists available at SciVerse ScienceDirect

Bioorganic & Medicinal Chemistry

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

Synthesis of cembranoid analogues and evaluation of their potential as quorum sensing inhibitors

Edisson Tello [a], Leonardo Castellanos [a], Carmenza Duque [a,*]

[a] Departamento de Química, Universidad Nacional de Colombia, AA 14490, Bogotá, Colombia

ARTICLE INFO

Article history:
Received 29 August 2012
Revised 10 October 2012
Accepted 18 October 2012
Available online 31 October 2012

Keywords:
Eunicea knighti
Pseudoplexaura flagellosa
Cembranoids
Antifouling
Quorum sensing inhibition
Antipathogenic drugs

ABSTRACT

Natural cembranoids have shown Quorum Sensing Inhibitory (QSI) activity, but their structure–function interactions are not well understood. Thirty-four cembranoid analogues were synthesized using six natural cembranoids (**1**–**6**) previously isolated from the Colombian Caribbean octocorals *Eunicea knighti* and *Pseudoplexaura flagellosa* as lead compounds. The analogues (**7**–**40**) obtained through the selected chemical transformations were tested in vitro against the QS systems of a *Chromobacterium violaceum* biosensor. Half of the cembranoid analogues assayed showed superior QSI activity to the lead compounds; three (**8**, **13**, and **18**) displayed remarkable potency up to three times higher than the natural compounds. Thereby, we have synthesized a pool of cembranoid QS inhibitors that can be used in concert with natural compounds to develop antipathogenic drugs and antifouling agents.

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1. Introduction

Quorum Sensing (QS) involves the cell control of bacterial populations through communication using chemical signaling molecules and a complex network of genetic circuits with feedback regulation. Sensing these chemical signals, bacteria can respond as groups and detect the 'quorum' of a population in order to regulate different phenotypes.[1,2] In many bacteria, QS regulates phenotypes such as bioluminescence, the transfer of tumor-inducing plasmids (Ti plasmids), antibiotic production, swarming motility, biofilm maturation and the production of virulence factors. Many bacteria do not express virulence factors until the population density is high enough to overwhelm host defense and establish infection.[3] This topic has been a focus of high pharmaceutical or chemical research because of its implications for human health. Quorum sensing inhibitors could prevent communication within bacterial communities and suppress some virulence factors. Compounds capable of this type of interference have been termed anti-pathogenic drugs. Some QSI compounds make biofilms more susceptible to antimicrobial treatments and are capable of reducing mortality and virulence in experimental animal models of infection.[2] QS phenomena have also been discovered in fungi and cancer cells, opening a wider range of applications for QS inhibitors.[4,5]

QSI may act in four different ways on bacterial systems: first, by inhibiting signaling molecule biosynthesis (i.e., AHLs); second, by inducing degradation of these signaling molecules; third, by blocking specific AHL binding sites for to LuxR type proteins; and finally, by inhibiting DNA transcription.[6] Most known QS inhibitors block binding sites, were obtained from natural products, and a few are analogues of AHLs.[6,7] As general revision, we suggested Chan et al., 2004;[8] Dobretsov et al., 2009;[6] Konaklieva & Plotkin, 2006;[9] McDougald et al., 2007;[10] Ni et al., 2009,[11] and Rasmussen et al., 2006.[2] Some SAR studies are well known for natural and derivative bromo-furanones, but structural relationship for other natural occurring compounds have not been well studied.[12]

Some QSI compounds has been recognized as antifoulants,[13] as biofilm development is the first biotic step in the sequential and complex process of marine fouling and is based on the adhesion and irreversible fixation of bacterial. QS inhibitors prevent the formation of multi-species biofilms and indirectly affect larval attachment to modified biofilms.[13,14] Thus, QSI compounds can be considered not only leads to antipathogenic drugs but also fouling controllers;[1,15] furthermore, the QSI assays have been included in the searching for antifoulants.[14]

In our recent research on marine metabolites, we have focused on finding compounds, mainly from octocorals, that exhibit antifouling properties, that is, that keep their surfaces free of fouling organisms[1,6,13] using their own metabolites as chemical defenses. Among these compounds, the naturally occurring *Eunicea* and *Pseudoplexaura* cembranoids that we recently isolated have shown in vitro QSI activity.[16–18] In this work, we selected six lead compounds (**1**–**6**) based on the large quantities that these could be recovered from the octoral and by their reactive functional groups.

* Corresponding author. Tel.: +57 1 3165000x14477; fax: +57 1 3165220.
E-mail addresses: cduqueb@unal.edu.co, cduqueb@etb.net.co (C. Duque).

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http://dx.doi.org/10.1016/j.bmc.2012.10.022

Further, the compound **5** has activity as Quorum Sensing inhibitor and all the lead compounds have biofilm inhibition properties, which are related to QS phenomenon.[16–18] The latter property made them suitable templates for the synthesis of analogues with uncommon structural features that could induce or enhance their QSI activity and have applications in the development of antipathogenic drugs. In this paper, we report the synthesis of a large series of cembranoid analogues (**7**–**40**) using simple, concise and selective functional group interconversions, such as epoxide ring opening, oxidation, cyclization, intramolecular rearrangement, to yield thirty-four cembranoid analogues that were evaluated in a QSI assay.

2. Results and discussion

The natural lead compounds (**1**–**6**) (Fig. 1) selected for this work were recently isolated from the Colombian octocorals *Eunicea knighti* and *Pseudoplexaura flagellosa* using chromatographic methods, and their stereostructures were elucidated by means of spectroscopic features, as reported in Tello et al., 2009,[16] 2011,[17] and 2012.[18] The lead compounds were chosen by considering their high concentration in the gorgonians; and the large variety of reactive functional groups present in their structures, that is, epoxide groups in the C-7 and C-8, hydroxy groups in the C-2 and C-18, reactive double bonds between the C-3/C-4 and C-11/C-12, and keto or hydroxy reactive groups in C-3, C-6 and C-11. All these compounds showed high values of QSI and/or biofilm inhibition in our laboratory assays.[16–18]

To obtain a wide range of cembranoid analogue structures and to significantly induce or improve their QSI activity, we envisaged a group of regioselective, straightforward, fast, reproducible and high-yield reactions to afford synthetic analogues of cembranoids (**7**–**40**). The reactions used in this work are grouped for the discussion as follows: epoxide ring opening, oxidation reactions, treatment with iodine, photochemical reactions, methylation and acetylation, and synthesis of cyclic hemiketals. Since the lead compounds were optically pure, without ambiguity in the absolute configuration, we assumed that the absolute configuration of the analogues obtained was as depicted.

2.1. Epoxide ring opening of compounds 1–3 and 16

According to our previous studies, when the epoxide ring is opened, QSI activity increases, particularly when new double bonds are formed.[16] Hence, our first attempt at structural modification was to treat the epoxide functionality of compounds **1** and **3** under acidic conditions to obtain a hydroxy group at the C-7 position and a mixture of alkenes formed by nucleophilic attack on the tertiary carbocation C-8, using the procedure described in Tello et al., 2009.[16]

In this way, we took advantage of the regioselectivity of the C-7,8 epoxide function in **3**, that react with acids to predominantly give the C-7(*R*) diastereomer.[16,18] Compound **3** was treated with acidified $CDCl_3$ and stirred for 36 h to yield a 2.2:1.0:2.2:1.5:1.0 mixture of C-7 hydroxylated compounds **7**–**11** (scheme 1) in 47.5% overall yield with leftover starting material **3**. The HRESIMS and NMR spectral data of **7**–**9** indicated that these compounds possess the same molecular formula and functionality, consistent with the disappearance of the epoxide ring, the introduction of a hydroxy group at C-7 and the formation of a new double bond at C-8. The ^{13}C NMR spectrum of **7** revealed the lack of the epoxide moiety, displaying signals for only three methyl groups. The olefinic methylene carbon resonating at δ_C 110.8 ppm, correlating with two broad singlets at δ_H 5.02 and 5.00 in the HMBC experiment, allowed assignment as an *exo*-methylene at C-19. The ^{13}C NMR spectrum of **8** revealed signals for four methyl groups and a methyl resonance ascribed to Me-19 at a high field of 11.4 ppm. This observation implied an (*E*)-configuration for the C-8,9 trisubstituted double bond. ^{13}C NMR spectrum signals of compound **9** showed four methyl groups also, with the methyl resonance of Me-19 appearing downfield at 17.2 ppm, indicating the (*Z*)-configuration of the C-8,9 trisubstituted double bond. The stereochemistry of C-7 in alcohols **7**–**9** was determined to be (*R*) using the method reported in Tello et al., 2009,[16] with the aid of NOESY spectra and the Mosher's ester method using MPA as a derivatization reagent. These data allowed us to establish the structure of **7** as 7*R*-hydroxy-C8(19)-en-cembrane, **8** as 7*R*-hydroxy-*8E*-8-en-cembrane and **9** as 7*R*-hydroxy-*8Z*-8-en-cembrane, as expected for the epoxide ring opening.[16]

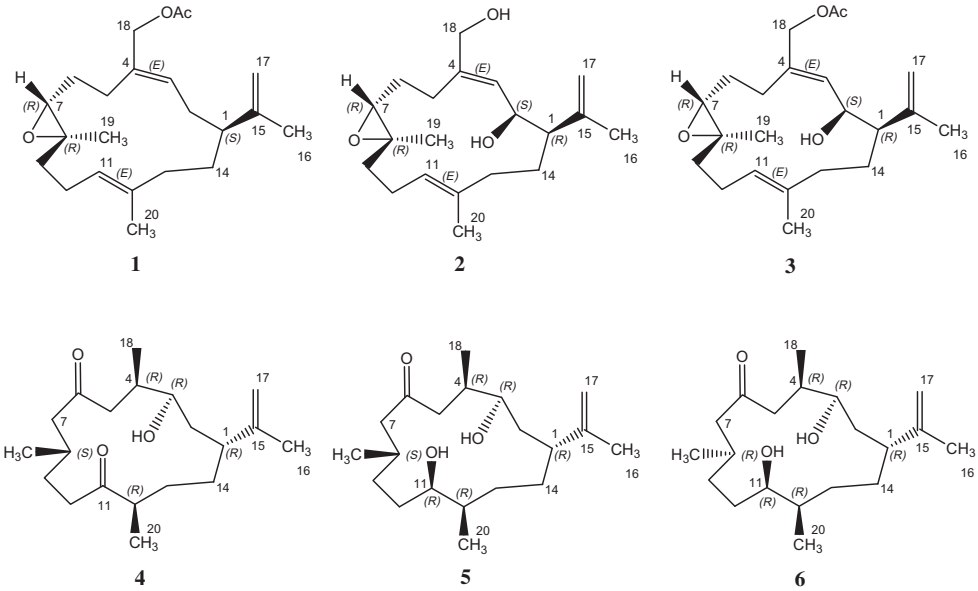
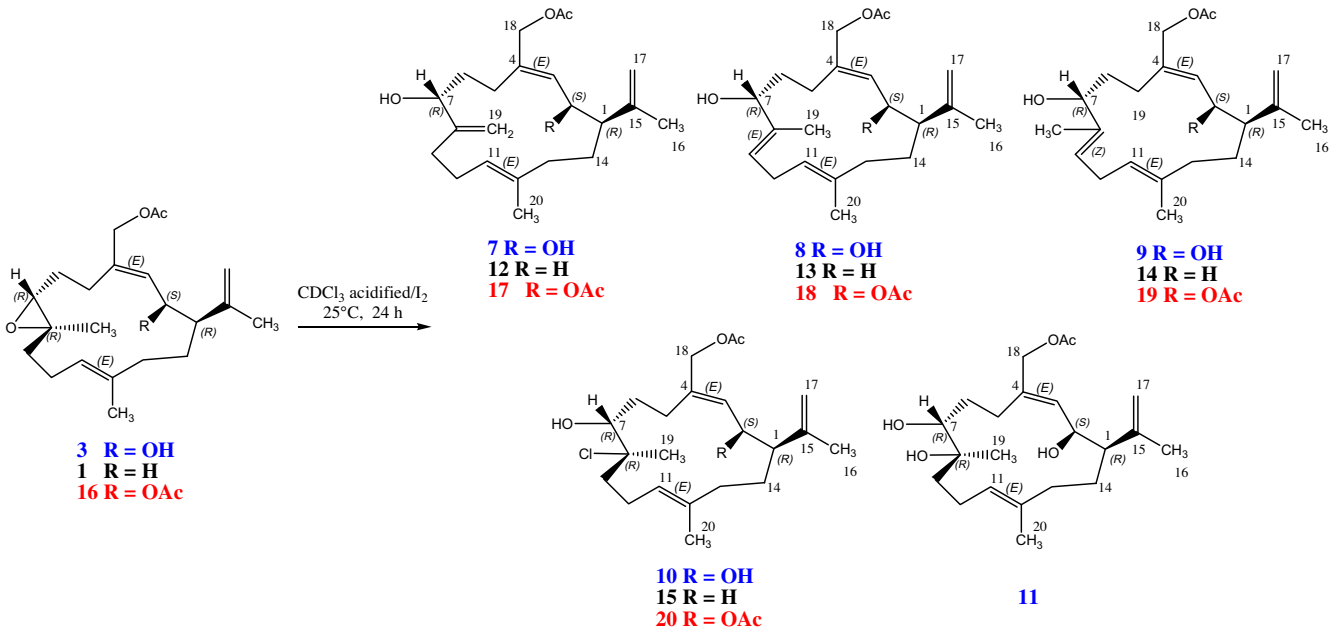


Figure 1. Lead compounds used in this work, isolated from *E. knighti* (**1**, **2**, and **3**) and *P. flagellosa* (**4**, **5**, and **6**).



Scheme 1. Epoxide ring opening of compounds **1**–**3** and **16**.

The presence of chlorine in compound **10** was evident. The HRESIMS showed an adduct specific chloride isotope mass pattern with (3:1) intensities at *m*/*z* 421.2128 $[M+Na]^+$ and *m*/*z* 423.2125 $[M+2+Na]^+$, and the ^{13}C NMR exhibited a signal for C-8 (δ_C 83.1) at lower field than that observed for **3** (δ_C 60.3). This evidence clearly supports epoxide ring opening and the introduction of chlorine at C-8. The configuration of C-8 in **10** was determined on the basis of strong NOE correlations observed between methyl signal H_3-19 with H_2-6 but not with H-7 in a phase-sensitive NOESY experiment, which indicates a *syn* orientation for H_3-19 and H_2-6. H-7 correlates with H_2-9, indicating the same orientation. These data clearly demonstrate the existence of a *trans* relationship for CH_3-19 and H-7, allowing us to assign the *R* configuration at C-8 and establish the structure of **10** as the *trans* chlorohydrin derivative of cembrane **3**.

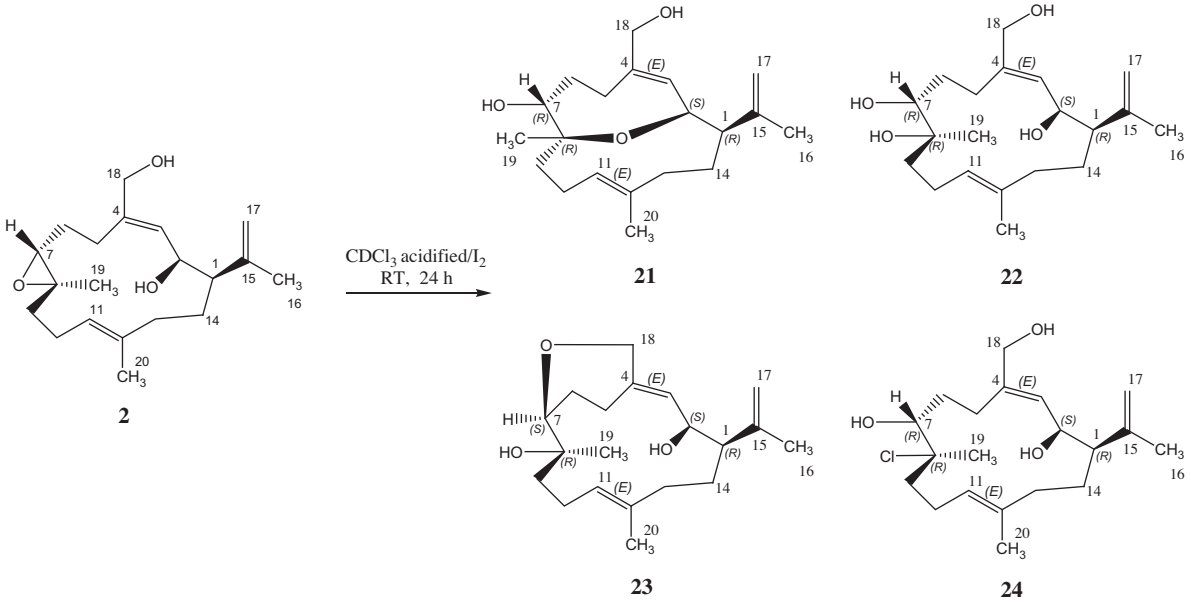
Compound **11**, obtained from **3**, shows different structural features. The HRESIMS spectrum presents an ion in *m*/*z* 403.2460 $[M+Na]^+$ (Calcd for $C_{22}H_{36}O_5Na$, 403.2460) that indicates the presence of a new oxygen atom. The ^{13}C NMR revealed as well an extra signal for oxygenated carbon at δ_C 80.2 that can be assigned to a hydroxy quaternary carbon (C-8); the signal of Me-19 was shifted downfield at δ_C 27.3 and linked to singlet δ_H 1.59 in the HMQC experiment, supporting the introduction of a hydroxy group at C-8. The *trans* relationship between CH_3-19 and H-7 was deduced from the NOE correlations.

The epoxide ring opening, in the natural compound knightol acetate (**1**), was performed under the same conditions as described for **3**.[16,18] A mixture of compounds **12**–**15** (1.2:1.0:1.1:1.0) in 69.2% overall yield from **1** were obtained (scheme 1). The structural elucidation for all compounds (**12**–**15**) was conducted using 1D/2D NMR and HRESMS analysis. All compounds presented C-7*R* hydroxy functionalities, and the geometry of the new double bond was deduced by the chemical shift of C-19 together with a NOE correlation between H-9 and Me-19, and the presence of a chlorine atom was deduced by HRESIMS. The structure of compounds **12**–**15** showed the same reaction pattern as the epoxide ring opening of compound **3**. Notably, compound **12** present an exocyclic double bond, compound **13** present an *E*-endocyclic double bond, and compound **14** present a *Z*-endocyclic double bond. Finally, compound **15** was *trans* chlorohydrin derivative.

Finally, the lead compound **2** was converted into **16**, and the previous procedure was applied over this compound to get the compounds **17**–**20**, compounds previously described in Tello et al., 2009,[16] but synthetized again to evaluate its QSI activy against *Chromobacterium violaceum*.

When the asperdiol (**2**) was treated in the same acidic conditions as **1** and **3**, a mixture of compounds **21**-**24** (2.6:3.2:2.1:1.0) were obtained in 91% yield (scheme 2). The total absence of *endo*- and *exo*-cyclic double bonds in the products is notable.

Thus, the HRESIMS of compound **21** *m*/*z* 359.2238 $[M+Na]^+$ established the molecular formula of $C_{20}H_{32}O_3$ with five degrees of unsaturation, and the ^{13}C NMR to establish a C-7*R* hydroxy functionality (δ_C 76.8; δ_H 3.36) as described for compounds **1** and **3**. The quaternary carbon C-8 was shifted downfield at δ_C 79.8, implying an addition of an oxygen to this carbon. Additionally, the long-range coupling observed in the HMBC experiment between H-2 at δ_H 4.51 ppm with the carbon signal ascribed to C-8 indicated a nucleophilic attack from the hydroxy group located at C-2 (δ_C 79.8; δ_H 4.51) on C-8 to form an eight-membered oxacyclic feature. The remaining part of the molecule showed the same structure as the starting material. The HRESIMS of **22** *m*/*z* 361.2351 $[M+Na]^+$ corroborated the extra 18 Da, which corresponded to the introduction of an H_2O molecule. The NMR data for compound **22** was reminiscent of compound **11**, but with an epoxy ring opening to producing the known C-7*R* hydroxy function and the addition of –OH to C-8 to form the C-8*R* hydroxy group assigned by the NOESY experiment and confirming the structure of diol **22** (scheme 2). Compound **23** exhibited a similar HRESIMS *m*/*z* 359.2362 $[M+Na]^+$ to **21**, suggesting that they are structural isomers and possess the same five degrees of unsaturation. In this case, the oxa ring was formed between C-7, resonating at δ_C 77.0; δ_H 3.44 and C-18, shifted to downfield at δ_C 74.3; δ_H 4.46, H-18a; 4.13, H-18b. The long-range cross-peak observed in the HMBC experiment between H-18a and C-7 confirmed the location of the oxa ring. The stereochemistry was established as C-7*S* and C-8*R* by the strong NOE correlation between H-7 and Me-19 that implies a *syn* relationship. The HRESIMS *m*/*z* 361.1889 $[M-H_2O+Na]^+$ for compound **24** is consistent with a molecular formula $C_{20}H_{31}ClO_2$, corroborating the presence of chlorine in this compound, and the NMR data including the NOESY correlation were in agreement with the



Scheme 2. Epoxide ring opening of compound **2**.

stereostructures of compounds **10**, **15**, and **20**. The formation of compounds **21**, **22**, and **24** could be explained by the nucleophilic attack on the carbocation formed at C-8, obtained from the epoxide ring aperture under acid conditions; in the case of compound **21**, the nucleophilic reaction occurred in an intramolecular way. The formed compound **23** was produced by an intramolecular nucleophilic attack of the hydroxy group at C-18 to the less hindered epoxide carbon located at C-7. These mechanistic considerations are in agreement with the pattern reaction proposed for the derivatives of the natural compounds **1** and **3**, as described before.

2.2. Oxidation reactions

In a parallel study we could establish that oxygenated cembranoids have a better QSI activity than those non-oxygenated (unpublished results), in this way we attempted to oxidize the natural cembranoid **2** and **3** in order to obtain analogues with QSI activity. Thus, we selected PCC and P_2O_5 to effect carbonyl derivatives, SeO_2 to oxidize allylic positions, and m-CPBA to obtain the epoxide derivatives.

2.3. Reaction of asperdiol acetate (3) with P_2O_5

The treatment of compound **3** with P_2O_5 at room temperature in CH_2Cl_2 for 3.5 h led to a complex mixture from which we only could separate compounds **25** and **26** using a HPLC RP-18 column (scheme 3). Comparison of the ^{13}C NMR data of compound **25** to **3** showed major differences in the C-6/C-9 fragment. The ^{13}C NMR of **25** has a signal at δ_C 206.0, indicating the presence of an aldehyde functionality with HMQC correlations to the proton signal at δ_H 9.37. On the other hand, HMBC correlations were observed from H-9 protons (δ_H 1.25), and the Me-19 (δ_H 1.05) to the aldehyde signal at C-7. Another HMBC cross-peak was observed from the H_2-5 protons (δ_H 1.81/1.46), H_2-6 protons (δ_H 1.83/1.63), and H-9 protons (δ_H 1.25) to the quaternary carbon at C-8 (δ_C 49.4). All of these correlations were in agreement with the presence of aldehyde groups linked to the quaternary carbon at 49.4 ppm. The HRESIMS data for **25** showed a $[M+Na]^+$ pseudomolecular ion at 385.2295 corresponding to the molecular formula of $C_{22}H_{34}O_4$, with six degrees of unsaturation in the molecule. The lack of NOE correlations for the Me-19 precluded the determination of the relative stereochemistry of the chiral center at C-8. We established a rearrangement in the cembranoid core of analogue **25**, forming a new C-6 and C-8 single bond and an aldehyde function at C-7.

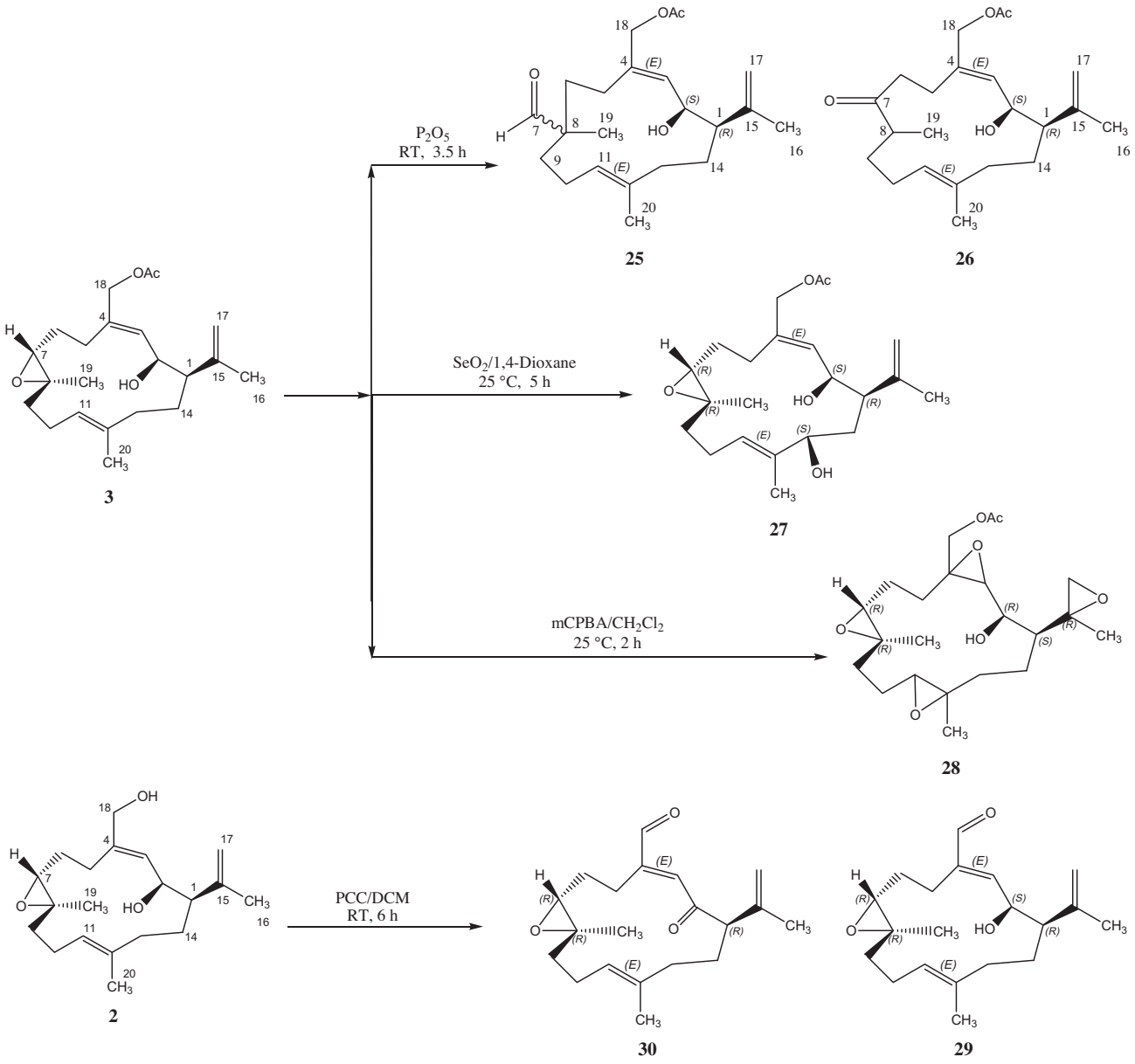
The structure of **26** was established by comparing the NMR data with that of **3**, whose main differences were located at C-7 with a signal at δ_C 213.9 ppm, corresponding to a keto function, and at C-8 with a signal at δ_C 45.5, corresponding to a methine functionality, which correlated in the HMQC with the signal at δ_H 2.53 m. The H-8 proton correlated with the doublet signal in the COSY ascribed to Me-19 at δ_C 1.10. In the HMBC experiment, we observed cross-peaks between the proton of Me-19 with C-7 and C-8 that lead us to fix its position at C-8. As mentioned for **25**, the lack of a key NOE correlation of Me-19 prevented us from establishing the relative configuration of this chiral center. In this way, compound **26** is a keto derivative at C-7 of the natural cembranoid **3**.

2.4. Reaction of asperdiol acetate (3) with SeO_2

When compound **3** was treated with SeO_2 and *t*-BuOOH, the reaction afforded compound **27** in 17.6% together with a large amount of an intractable mixture of highly polar compounds (scheme 3). The HRESIMS of compound **27** *m*/*z* 401.2298 $[M+Na]^+$ led us to establish the same five degrees of unsaturation and extra sixteen Da of mass than those found in the lead compound **3**, which was ascribed to an oxygen atom in the molecule. The NMR data for compound **27** were similar to the starting material, except for the chemical shift of C-13 (the signals of the methylene group in **3** were shifted downfield at δ_C 76.5 in ^{13}C NMR and at δ_H 4.03 in the ^1H NMR), implying the addition of a hydroxy group to this carbon, data which were corroborated in the DEPT experiment. All remaining data were consistent with the counterpart in the starting material. The stereochemistry of the new chiral center C-13*S* was deduced by the *syn* relationship between H-2 and H-13, as demonstrated by the NOE correlation observed in the NOESY experiment.

2.5. Reaction of asperdiol acetate (3) with m-chloroperbenzoic acid to produce 28

The conversion of **3** to the tetraepoxide **28** was performed with m-CPBA in dry dichloromethane at room temperature in 56.9%



Scheme 3. Oxidation reactions in compounds **2** and **3**.

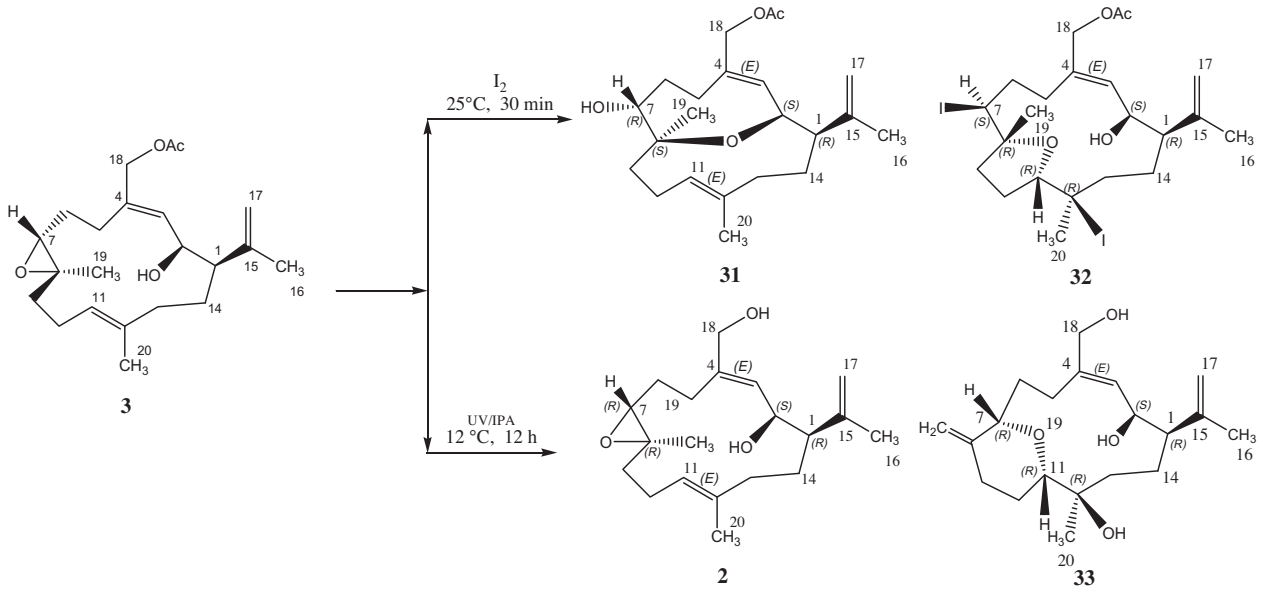
yield after purification using column chromatography. The molecular formula $C_{20}H_{30}O_5$ and six degrees of unsaturation for **28** were deduced from the HRESIMS data of the pseudomolecular ion $[M+Na]^+$ 433.2197. The differences between **28** and **3** were three extra oxygens appearing in compound **28** and three additional pairs of signals in the ^{13}C NMR spectra at δ_C 64.0 (CH-3)/57.2 (C-4), δ_C 64.4 (CH-11)/62.6 (C-12), and δ_C 61.0 (C-15)/53.6 (CH_2-17), attributed to three additional epoxide groups in compound **28** instead of the Δ^3, Δ^{11}, and Δ^{15} double bonds presents in **3**. Furthermore, the occurrence of proton signals at δ_H 3.08 (H-3), δ_H 2.66 (H-11), and δ_H 2.60 (H-17a)/2.47 (H-17b) in the ^1H NMR spectrum confirmed two trisubstituted and one disubstituted epoxide functions, respectively.

2.6. Reaction of asperdiol (2) with PCC to produce 29 and 30

The last oxidation reaction was the oxidation of the two available hydroxy groups at C-2 and C-18 of compound **2** using PCC in dry dichloromethane to produce, after considerable purification, mono- (**30**) and diketo (**29**) compounds in a 22.5% and 38.7% yield, respectively. The ^{13}C NMR spectrum of compound **30** showed two signals at δ_C 199.9 and δ_C 195.0 ascribed to carbonyl groups located at C-2 and C-18, respectively. These carbonyl groups together with the lack of signals from the hydroxy groups at C-2 (δ_C 68.4, d) and C-18 (δ_C 65.6, t) in its counterpart **2**, established an aldehyde function in C-18 and a keto function in C-2. The stereostructure of **30** was determined, as shown in scheme 3. Similar to compound **30**, the ^{13}C and ^1H NMR signals located at δ_C 194.7 and at δ_H 9.42 (correlating in the HMQC experiment) were assigned to the aldehyde group ascribed to C-18. As before, the lack of signals for the hydroxymethylene group (δ_C 65.6; δ_H 4.02/4.10) ascribed to C-18 for the lead compound **2** established the structure of **29**, as depicted in scheme 3.

2.7. Treatment with iodine

The next attempt was the treatment of asperdiol acetate (**3**) with iodine to obtain an unusual cembranoid compounds, and in



Scheme 4. Treatment with iodine and photochemical reactions in compound **3**.

this way incorporates chemical diversity to the derivates. Exposure of **3** to iodine in $CDCl_3$ yielded a complex mixture of products from which compounds **31** and **32** were isolated in 13.6% and 11.1%, respectively (scheme 4), together with an intractable polar mixture (60.1%). The formation of an oxa bridge function at the C-2 and C-8 of **31** was suggested by the chemical shifts of the carbon NMR at C-2 (δ_C 67.1) and C-8 (δ_C 75.8). The formation of the bridged ether core of **31** must proceed via epoxide ring opening in **3**, as described for compound **21**. Additionally, a *trans* relationship between Me-19 and H-7 was established for compound **31** by the NOESY experiment (as established for compound **11**), implying C-7*R* and C-8*S* configurations. The NMR data of compound **32** were in agreement with a hydrofuranether functionality between C-8 (δ_C 78.0) and C-11 (δ_C 74.8) and were supported by the cross-peak observed in the HMBC experiment between the signal occurring at δ_H 3.37 (H-11) and C-8. The HRESIMS established the presence of two iodine atoms and the molecular formula $C_{22}H_{34}I_2O_4$. Additionally, the downfield-shifted chemical shifts of carbon C-7 (δ_C 39.0) and C-12 (δ_C 49.6) in the ^{13}C NMR spectrum suggested the location of these iodine atoms. The stereostructure of **32** was established based on a mechanistic proposal and the key NOE correlations observed in a NOESY experiment. For compound **32**, a *trans* relationship between H-7 and β-Me-19 was established via the key NOE correlations as for the compound **11**; in turn, the β-Me-19 showed a strong NOE correlation with H-11, indicating a *syn* orientation, and finally, H-11 and Me-20 were located *trans* to each other, establishing the configuration of C-7*S*, C-8*R*, C-11*R* and C-12*R*. The transformation of compound **3** to diiodide **32** can be explained by nucleophilic attack at C-7, with inversion of the configuration, of an iodide anion to give iodohydrin at C-7 and C-8 and the attack from the β-face of the C-8 alkoxy group to the C-11 of the cyclic iodonium previously formed from the attack of iodine to Δ^{11} olefinic group.

2.8. Photochemical reactions

The photochemical reaction of asperdiol acetate (**3**) in isopropanol over 12 h produced a 4:1:2 mixture of compound **2**, compound **33** and the starting material **3** (scheme 4), which were separated using CC-SiO_2. The ^1H and ^{13}C NMR data of compound **33** were quite different from starting material **3**, including the absence of the epoxide ring, which opened during the reaction to form a C-7*R* hydroxy function and an *exo*-methylene function at C-8 reminiscent of the structures of **7**, **12**, and **17**, as deduced by the signals observed in the ^1H and ^{13}C NMR spectra: δ_C 84.5/δ_H 3.50 ascribed to C-7; δ_C 145.2 ascribed to C-8 and δ_C 115.4/δ_H 5.05, 4.90 ascribed to C-19. Its position was fixed by the cross-peak observed in a HMBC experiment between H_2-19 and the carbon signals of C-7 at δ_C 84.5, and C-9 at δ_C 29.7. The formation of an oxa bridge function in the C-7 and C-11 position was unexpected and was deduced by the lack of signals corresponding to a double bond between C-11 and C-12; by the signals shifted to high field at δ_C 80.1/δ_H 4.42, ascribed to C-11 in compound **33**; by the signal observed in ^{13}C NMR at δ_C 84.7 ascribed to C-12, which by its shift was assigned to a quaternary hydroxy carbon; and by the C-7 hydroxy signal (scheme 4). The stereochemistry of **33** was deduced by the mechanistic approach and NOE correlations observed between H-7 and H-11 in the NOESY experiment, establishing a *syn* relationship over the α-face of the molecule. In addition, the orientation of the oxa bridge in the β-face was suggested by mechanistic considerations establishing the configuration of C-7*R* and C-11*R*. Finally, the chiral center at C-12*R* was determined by the NOE relationship between H-2 and Me-20 over the α-face of the molecule.

2.9. Methylation and acetylation

With some methylations and acetylations we are trying just to incorporate chemical diversity to the different leads compounds.

2.10. Reaction of asperdiol (2) with MeOTf to produce 34

The methylated compound **34** was obtained by the reaction of compound **2** with methyltriflate and triethylamine in dichloromethane overnight. After purification of the residue by HPLC-RP-18, the pure compound **34** was afforded in a 52.6% overall yield. The HRESIMS of compound **34** *m*/*z* 339.2285 $[M-H_2O+Na]^+$ was consistent with the molecular formula $C_{21}H_{34}O_3$ and indicated the introduction of a methyl group whose signal was shifted downfield in the ^1H NMR δ_H 3.16 (H-21). In addition, this methyl group showed cross-peaks with δ_C 56.7 in the HMQC experiment, confirming the methylation of the hydroxy group. The signals corresponding to diasterotopic protons H_2-18 shifted at δ_H 4.23 (H-18a) and 3.53 (H-18b) correlating with the signal at δ_C 75.0 in the HMQC experiment that indicated methylation at C-18. The

NMR of **34** showed a signal at δ_C 216.0, consistent with a carbonyl group and established at C-7 by the relationship with H_3-19 (δ_H 1.01) as observed in the HMBC experiment. All of the above established the structure of **34** as shown in scheme 5.

Reaction of 8*S*-plexaurolone (**4**), 8*S*-dihydroplexaurolone (**5**) and 8*R*-dihydroplexaurolone (**6**) with acetic anhydride to produce compounds **35**–**37**.

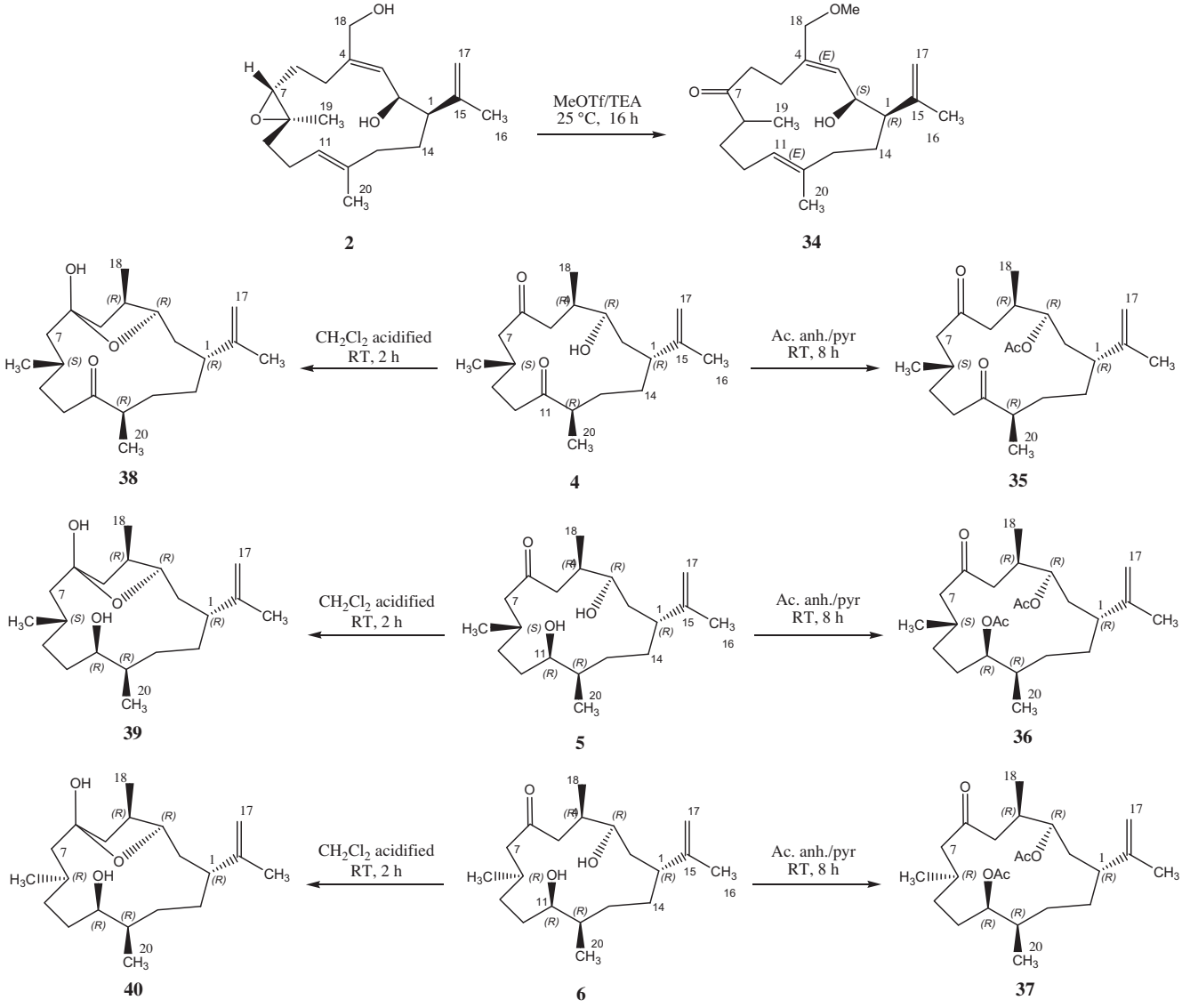
Compounds **4**, **5**, and **6** were treated with acetic anhydride in dry pyridine to afford compounds **35**, **36**,[19] and **37** in 96.8%, 97.6% and 98.0%, respectively. The HRESIMS of compounds **35** *m*/*z* 387.2508 $[M+Na]^+$, **36** *m*/*z* 431.2773 $[M+Na]^+$, and **37** *m*/*z* 431.2768 $[M+Na]^+$ were consistent with the molecular formulas of $C_{22}H_{36}O_4$, $C_{24}H_{40}O_5$, and $C_{24}H_{40}O_5$, respectively. The above-indicated monoacetylation of compound **35** and diacetylation of compounds **36** and **37** were performed. The NMR data for compounds **35**–**37** clearly indicated acetylation and established the structures of these compounds as shown in scheme 5.

2.11. Synthesis of cyclic hemiketals

In a previous work[17] we observed some compounds that could form hemiketals in solution, showing different QSI properties than those that cannot form them. In this way, we want to obtain some hemiketals to establish their QSI activity.

Reaction of 8*S*-plexaurolone (**4**), 8*S*-dihydroplexaurolone (**5**) and 8*R*-dihydroplexaurolone (**6**) with CH_2Cl_2 acidified to produce **38**, **39** and **40**.

Our last synthetic work attempted to induce an intramolecular cyclization between the hydroxy group and the available keto groups for compounds **4**, **5**, and **6**, using the methodology described in Tello et al. 2011.[17] Each was treated with acidified CH_2Cl_2 to afford, after purification, compounds **38** (93.3%), **39** (86.6%),[19] and **40** (74%). The HRESIMS of compounds **38** *m*/*z* 345.2396 $[M+Na]^+$, **39** *m*/*z* 347.2544 $[M+Na]^+$, and **40** *m*/*z* 347.2546 $[M+Na]^+$ were consistent with the molecular formulas $C_{20}H_{34}O_3$ (IHD = 4), $C_{20}H_{36}O_3$ (IHD = 3), and $C_{20}H_{36}O_3$ (IHD = 3), respectively. For compound **38**, the lack of a keto group together with NMR signals at δ_C 110.8 ascribed to C-6 and δ_H 3.68/δ_C 75.7 for C-3 suggested the formation of the cyclic hemiketal **38** (Scheme 5), probably due to the intramolecular nucleophilic addition of hydroxy at C-3 to the carbonyl ketone group at C-6. The remaining compounds **39** and **40** showed the same reaction pattern. Thus, for **39**, the signal for C-6 at δ_C 109.7 and the signals for C-3 at δ_H 3.95/δ_C 79.0 and, for **40**, the signal for C-6 at δ_C 109.5 and the signals for C-3 at δ_H 3.79/δ_C 79.0, together



Scheme 5. Methylations, acetilations and obtention of cyclic hemiketals.

with further detailed analyses of COSY and gHMBC, suggested the formation of cyclic hemiketals as shown in scheme 5. The position of the cyclic hemiketals produced was established by differences in NMR data compared with their counterparts, particularly by the signals ascribed to carbonyl and hydroxy groups as is depicted in scheme 5.

3. QSI studies

Table 1 presents all compounds tested in the Quorum sensing inhibition assay using *C. violaceum* as biosensor; each compound was evaluated at 2.5, 5.0, 7.5, 15.0, and 30.0 μg/disk. We used kojic acid, a known inhibitor of Quorum sensing systems, as a positive control.[1] The natural compounds **1**–**6** were used as lead compounds for the synthesis of 34 cembranoid analogues (**7**–**40**). These precursors did not show activity even at the highest tested quantity (30 μg/disk), except for the active compound **5** (7.5 μg/disk), but they display a biofilm inhibition properties (data not shown), activity related to QS; also, some similar cembranoids have shown good QSI activity (e.g., knightol, knightal, 11(*R*)-hydroxy-12(20)-en-knightal, and 11(*R*)-hydroxy-12(20)-en-knightol acetate).[16,18] We were pleased when half of the cembranoid analogues showed QSI activity without toxicity against the biosensor bacteria, mainly, because sixteen active analogues were obtained from five non active natural compounds (in QSI assay), and two analogues (**36**, **39**) were afforded from the active natural compound **5**. Additionally, as *C. violaceum* growth was not inhibited by these compounds, it is possible to establish that the inhibition on violacein pigment production is caused by disruption of the QS systems. Thus, if there not bactericidal effect, there is no selective pressure for the development of resistance in bacteria. The synthetic compounds with the best QSI activity were **8** (2.5 μg/disk), **13** (5.0 μg/disk), and **18** (2.5 μg/disk), which present similar structure features: a C-7*R* hydroxy methine group, a double bond with *E* configuration between C-8 and C-9, and an acetyl group at C-18, the above allows us to infer that the presence of a *E*-hydroxy allylic moiety is highly relevant for the activity. Other active compounds (**7**, **10**, **17**, **19**, **25**, **26**, **35**, and **39**; all showed QSI activity at 7.5 μg/disk) also had an oxygenated core, particularly at C-2, C-7, and C-18 as in the above compounds (except **35** and **39**), but differed in the position and geometry of the C-8 double bond. The remaining active compounds act as QSI at 15.0 and 30.0 μg/disk, a higher dose than needed for the most active compounds but less than needed for the positive control kojic acid (90.0 μg/disk). Overall, the synthesis reactions yielded improved QSI activity of compounds, and the most active cembranoid analogues (**8**, **13**, and **18**) were more potent than the most active natural products yet isolated, for example, knightal (7.5 μg/disk), 11(*R*)-hydroxy-12(20)-en-knightal (7.5 μg/disk), and 11(*R*)-hydroxy-12(20)-en-knightol acetate (15.0 μg/disk),[18] achieving our main goal. Extensive studies of the QSI structure–activity relationship for these compounds are being conducted by our group.

Table 1
Quorum Sensing Inhibition of compounds **1**–**40**

Assay	Quorum sensing inhibition[a] (quantity in μg/disk)[b]		
Compound	*Chromobacterium violaceum*	Compound no.	*Chromobacterium violaceum*
1	—	**21**	30.0
2	—	**22**	—
3	—	**23**	—
4	—	**24**	30.0
5	7.5	**25**	7.5
6	—	**26**	30.0
7	7.5	**27**	30.0
8	2.5	**28**	—
9	—	**29**	—
10	7.5	**30**	—
11	7.5	**31**	—
12	—	**32**	—
13	5.0	**33**	—
14	—	**34**	—
15	—	**35**	7.5
16	—	**36**	30.0
17	7.5	**37**	15.0
18	2.5	**38**	—
19	7.5	**39**	7.5
20	—	**40**	—
Kojic acid	90.0	Kojic acid	90.0
Solvent	—	Solvent	—

[a] Activity was established by the inhibition of violacein pigment.
[b] Minimum quantity in μg per disk of compound required to inhibit violacein pigment. —No zone of inhibition observed, even at 30 μg/disk.

In summary, we selected six natural compounds as lead compounds (**1**–**6**) to attempt to induce or improve QSI activity by means of chemical transformations applied selectively to various positions on the cembranoid core. Thirty-four cembranoid analogues (**7**–**40**) were afforded; half showed remarkable QSI activity against *C. violaceum* without interfering with bacterial growth, thereby achieving a pool of cembranoids that are structurally related as inhibitors of QS and that might be used as anti-pathogenic drugs or antifouling agents. This pool of cembranoid analogues, together with the natural compounds, could be useful for further studies of biological activities, including anti-cancer, anti-inflammatory, and acetylcholinesterase inhibition, because others cembranoid compounds have previously exhibited these activities[20] and the database here reported may be also useful in future SAR studies.

4. Experimental section

4.1. General experimental procedures

Optical rotation was measured on a Polartronic ADP440+, Bellinghan + Stanley polarimeter. IR spectra were recorded on a PERKIN–ELMER FT-IR Paragon 500 Series 1000 spectrophotometer. ^{1}H NMR spectra were recorded on a Bruker AVANCE 400 (400 MHz), Bruker AV 400 (400 MHz) or Bruker DPX 400 (400 MHz) spectrometer as dilute solutions in deuterochloroform at room temperature, unless stated otherwise. The chemical shifts are quoted in parts per million (ppm) relative to residual solvent peaks, and the multiplicity of each signal is designated by the following abbreviations: s (singlet), d (doublet), t (triplet), q (quartet), br (broad), m (multiplet). All coupling constants are in hertz. ^{13}C NMR spectra were recorded using a Bruker AVANCE 400 (100 MHz), Bruker AV 400 (100 MHz) or Bruker DPX 400 (100 MHz) instrument as dilute solutions in deuteriochloroform, unless stated otherwise. Chemical shifts of ^{13}C were quoted in parts per million (ppm) downfield of tetramethylsilane; spectra use a residual protonated solvent (δ = 77.0 ppm for $CDCl_3$) as a reference. Assignments were made on the basis of chemical shift using the DEPT sequence. Mass spectra were recorded on a Bruker MicroTOF system using electrospray (ESI) techniques and a LCMS-2010EV- Shimadzu with a MS-2010 mass spectrometer in the positive mode. Preparative HPLC was conducted using a Merck-Hitachi instrument with a UV/Vis L-4250 detector (detected at 210 nm) and a LichroCART RP-18 (250 × 10 mm i.d., 10 μm) column, with MeOH/H_2O (50:50 v/v and 80:20 v/v) as the eluent at a flow rate of 2.5 ml/min.

All reactions were monitored by thin layer chromatography (TLC) using aluminum plates precoated with Merck silica gel $60F_{254}$, which were visualized with ultraviolet light (λ_{max} = 254 nm) and either basic potassium permanganate solution

or acid cerium sulfate ammonium. Unless stated otherwise, reactions requiring anhydrous conditions were conducted in an inert atmosphere of nitrogen or argon in a flame- or oven-dried apparatus. Dry organic solvents were routinely stored under a nitrogen atmosphere and/or dried over sodium wire. Dichloromethane, triethylamine and pyridine were distilled from calcium hydride. Dry tetrahydrofuran was distilled from sodium and benzophenone. Solvents were removed in vacuo at approx. 20 mmHg using a Buchi rotary evaporator.

4.2. Animal material

The octocorals *E. knighti* and *P. flagellosa* were collected in Santa Marta Bay (Colombian Caribbean Sea) by scuba diving. The fresh colonies were frozen immediately after collection and remained frozen until extraction. The animals were identified by Professor Dr. S. Zea and Professor Dr. Mónica Puyana, and voucher specimens were deposited at the invertebrate collection of ICN (Instituto de Ciencias Naturales de la Universidad Nacional de Colombia), coded as ICN-MHN-CO No 0106 (*E. knighti*) and ICN-MHN-PO 0257 (*P. flagellosa*). The octocorals *E. knighti* (650 g) and *P. flagellosa* (360 g) were each extracted, concentrated, partitioned, and subjected to silica gel vacuum column chromatography and reverse-phase HPLC to afford pure lead compounds **1**–**6** (Fig. 1), as reported by Tello et al., 2009[16], 2011[17], and 2012.[18]

4.3. Synthesis of cembranoid analogues

4.3.1. Reaction of asperdiol acetate (3) with acidified $CDCl_3$

Compound **3** (130 mg, 0.36 mmol) was dissolved in $CDCl_3$ (6 mL), and an iodine crystal was added. Then, 250 μL of acidified $CDCl_3$ ($HCl_{(g)}$ bubbled into 5 mL of $CDCl_3$ for 30 s) was added to the mixture. After 24 h, the reaction mixture was quenched and purified on a silica gel column, eluting with hexane/EtOAc (90:10 v/v) to yield a mixture of **7** and **8** (27 mg, 20.7%) and pure compounds **9** (*Z* isomer) (12 mg, 0.033 mmol, 9.2%), **10** (18 mg, 0.045 mmol, 12.6%) and **11** (8 mg, 0.021 mmol, 5.8%). The mixture of **7** and **8** was subjected to HPLC RP-18, MeOH/H_2O (50:50 v/v), to obtain pure **7** (*exo*-methylene) (8 mg, 0.022 mmol, 6.1%) and pure **8** (*E* isomer) (18 mg, 0.050 mmol, 13.8%).

4.3.1.1. (−)-(1*R*,2*S*,7*R*,3*E*,11*E*)-18-Acetoxycembra-3,8(19),11,15(17)-tetraen-2,7-diol 7. Colorless oil; $[\alpha]_D^{25}$ −61.5 (*c* 0.13, $CHCl_3$); IR (CH_2Cl_2) ν_{max} 3436, 1732, 1024, 736 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz), δ 2.07 (m, 1H, H-1), 4.52 (dd, *J* = 8.6, 3.6 Hz, 1H, H-2), 5.40 (d, *J* = 8.5 Hz, 1H, H-3), 2.30 (m, 1H, H-5a), 1.85 (m, 1H, H-5b), 1.76 (m, 1H, H-6a), 1.48 (m, 1H, H-6b), 4.14 (dd, *J* = 8.7, 4.9 Hz, 1H, H-7), 1.95 (m, 1H, H-9a), 1.89 (m, 1H, H-9b), 2.43 (dt, *J* = 15.3, 7.7 Hz, 1H, H-10a), 2.22 (m, 1H, H-10b), 5.36 (t, *J* = 6.9 Hz, 1H, H-11), 2.11 (m, 2H, H-13), 2.20 (m, 1H, H-14a), 1.95 (m, 1H, H-14b), 1.73 (s, 3H, H-16), 4.86 (br s, 1H, H-17a), 4.69 (br s, 1H, H-17b), 4.56 (d, *J* = 12.7 Hz, 1H, H-18a), 4.44 (d, *J* = 12.7 Hz, 1H, H-18b), 5.02 (br s, 1H, H-19a), 5.00 (br s, 1H, H-19b), 1.61 (s, 3H, H-20), 2.07 (s, 3H, H-22); ^{13}C NMR ($CDCl_3$, 100 MHz), δ 48.8 (d, C-1), 67.2 (d, C-2), 132.6 (d, C-3), 134.6 (s, C-4), 25.7 (t, C-5), 33.6 (t, C-6), 77.0 (d, C-7), 146.1 (s, C-8), 26.9 (t, C-9), 24.1 (t, C-10), 125.0 (d, C-11), 133.8 (s, C-12), 36.0 (t, C-13), 30.4 (t, C-14), 149.0 (s, C-15), 23.9 (q, C-16), 112.9 (t, C-17), 67.4 (t, C-18), 110.8 (t, C-19), 15.1 (q, C-20), 170.8 (s, C-21), 21.0 (q, C-22); HRESIMS *m*/*z* 385.2350 $[M+Na]^+$ (Calcd for $C_{22}H_{34}O_4Na$, 385.2354).

4.3.1.2. (−)-(1*R*,2*S*,7*R*,3*E*,8*E*,11*E*)-18-Acetoxycembra-3,8,11,15(17)-tetraen-2,7-diol 8. Colorless oil; $[\alpha]_D^{25}$ −42.0 (*c* 0.29, $CHCl_3$); IR (CH_2Cl_2) ν_{max} 3455, 1739, 1234, 736 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz), δ 1.97 (m, 1H, H-1), 4.49 (m, 1H, H-2), 5.56 (t, *J* = 7.9 Hz, 1H, H-3), 1.93 (m, 1H, H-5a), 1.90 (m, 1H, H-5b), 1.81 (m, 1H, H-6a), 1.74 (m, 1H, H-6b), 4.02 (dd, *J* = 10.1, 2.9 Hz, 1H, H-7), 5.15 (dd, *J* = 10.0, 5.2 Hz, 1H, H-9), 2.96 (m, 1H, H-10a), 2.58 (td, *J* = 12.6, 6.2 Hz, 1H, H-10b), 5.32 (d, *J* = 7.4 Hz, 1H, H-11), 2.20 (m, 1H, H-13a), 2.11 (m, 1H, H-13b), 1.95 (m, 2H, H-14), 1.71 (s, 3H, H-16), 4.79 (br s, 1H, H-17a), 4.63 (br s, 1H, H-17b), 4.53 (d, *J* = 12.8 Hz, 1H, H-18a), 4.43 (d, *J* = 12.8 Hz, 1H, H-18b), 1.61 (s, 3H, H-19), 1.69 (s, 3H, H-20), 2.06 (s, 3H, H-22); ^{13}C NMR ($CDCl_3$, 100 MHz), δ 47.9 (d, C-1), 68.1 (d, C-2), 132.7 (d, C-3), 136.4 (s, C-4), 24.3 (t, C-5), 32.3 (t, C-6), 77.1 (d, C-7), 132.2 (s, C-8), 123.6 (d, C-9), 26.4 (t, C-10), 125.8 (d, C-11), 133.3 (s, C-12), 36.3 (t, C-13), 26.9 (t, C-14), 146.2 (s, C-15), 23.8 (q, C-16), 112.6 (t, C-17), 67.2 (t, C-18), 11.4 (q, C-19), 15.2 (q, C-20), 170.7 (s, C-21), 21.0 (q, C-22); HRESIMS *m*/*z* 385.2345 $[M+Na]^+$ (Calcd for $C_{22}H_{34}O_4Na$, 385.2349).

The NMR data of compound **8** indicated the formation of a new olefinic double bond between C-8 (δ_C 132.2) and C-9 (δ_C 123.6, δ_H 5.15, 1H, dd, *J* = 10.0, 5.2 Hz), with an (*E*) configuration deduced from the highfield signal of Me-19 located at δ_C 11.4.

4.3.1.3. (−)-(1*R*,2*S*,7*R*,3*E*,8*Z*,11*E*)-18-Acetoxycembra-3,8,11,15(17)-tetraen-2,7-diol 9. Colorless oil; $[\alpha]_D^{25}$ −63.8 (*c* 0.04, $CHCl_3$); IR (CH_2Cl_2) ν_{max} 3436, 1730, 1025, 736 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz), δ 2.11 (m, 1H, H-1), 4.45 (m, 1H, H-2), 5.43 (d, *J* = 7.1 Hz, 1H, H-3), 1.74 (m, 2H, H-5), 1.32 (m, 2H, H-6), 4.46 (m, 1H, H-7), 5.04 (d, *J* = 8.9 Hz, 1H, H-9), 2.98 (m, 1H, H-10a), 2.53 (m, 1H, H-10b), 5.52 (t, *J* = 7.6 Hz, 1H, H-11), 2.18 (m, 1H, H-13a), 2.08 (m, 1H, H-13b), 1.72 (m, H, H-14a), 1.36 (m, H, H-14b), 1.74 (s, 3H, H-16), 4.91 (br s, 1H, H-17a), 4.69 (br s, 1H, H-17b), 4.53 (d, *J* = 12.7 Hz, 1H, H-18a), 4.44 (d, *J* = 12.3 Hz, 1H, H-18b), 1.64 (s, 3H, H-19), 1.70 (s, 3H, H-20), 2.07 (s, 3H, H-22); ^{13}C NMR ($CDCl_3$, 100 MHz), δ 49.3 (d, C-1), 70.8 (d, C-2), 133.0 (d, C-3), 139.7 (s, C-4), 27.1 (t, C-5), 35.2 (t, C-6), 71.6 (d, C-7), 133.5 (s, C-8), 124.3 (d, C-9), 26.2 (t, C-10), 125.5 (d, C-11), 134.6 (s, C-12), 36.5 (t, C-13), 27.8 (t, C-14), 145.1 (s, C-15), 23.9 (q, C-16), 113.7 (t, C-17), 67.5 (t, C-18), 17.2 (q, C-19), 15.8 (q, C-20), 170.9 (s, C-21), 21.0 (q, C-22); HRESIMS *m*/*z* $C_{22}H_{34}O_4Na$, 385.2346 $[M+Na]^+$ (Calcd for $C_{22}H_{34}O_4Na$, 385.2349).

The (*Z*) configuration of the double bond located at C-8 (δ_C 133.5) and C-9 (δ_C 124.3; δ_H 5.04, d, *J* = 8.9 Hz, 1H) in **9** was deduced from the chemical shift of Me-19, which was resonant downfield (δ_C 17.2), and by the NOE correlation between H-9 and Me-19.

4.3.1.4. (−)-(1*R*,2*S*,7*R*,8*R*,3*E*,11*E*)-8-Chloro-18-acetoxycembra-3,11,15(17)-trien-2,7-diol 10. Colorless oil; $[\alpha]_D^{25}$ −66,2 (*c* 0.10, $CHCl_3$); IR (CH_2Cl_2) ν_{max} 3457, 1730, 1372, 1235, 737 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz), δ 2.00 (m, 1H, H-1), 4.56 (dd, *J* = 8.7, 3.4 Hz, 1H, H-2), 5.46 (d, *J* = 8.8 Hz, 1H, H-3), 2.36 (m, 2H, H-5), 1.70 (m, 2H, H-6), 3.79 (d, *J* = 10.6 Hz, 1H, H-7), 2.31 (m, 2H, H-9), 2.22 (m, 2H, H-10), 5.34 (t, *J* = 7.9 Hz, 1H, H-11), 2.22 (m, 1H, H-13a), 2.00 (m, 1H, H-13b), 1.98 (m, 2H, H-14), 1.77 (s, 3H, H-16), 4.93 (br s, 1 H, H-17a), 4.74 (br s, 1H, H-17b), 4.51 (d, *J* = 13.0 Hz, 1H, H-18a), 4.48 (d, *J* = 13.0 Hz, 1H, H-18b), 1.52 (br s, 3H, H-19), 1.61 (br s, 3H, H-20), 2.08 (s, 3H, H-22); ^{13}C NMR ($CDCl_3$, 100 MHz), δ 49.1 (d, C-1), 66.9 (d, C-2), 132.0 (d, C-3), 136.2 (s, C-4), 23.1 (t, C-5), 30.5 (t, C-6), 75.5 (d, C-7), 83.1 (s, C-8), 42.2 (t, C-9), 26.7 (t, C-10), 125.9 (d, C-11), 134.8 (s, C-12), 35.8 (t, C-13), 27.2 (t, C-14), 146.0 (s, C-15), 24.0 (q, C-16), 113.1 (t, C-17), 67.3 (t, C-18), 25.2 (q, C-19), 15.3 (q, C-20), 170.8 (s, C-21), 21.0 (q, C-22); HRESIMS *m*/*z* 423.2125/421.2128 $[M+Na]^+$ (Calcd for $C_{22}H_{35}ClO_4Na$, 421.2121).

4.3.1.5. (−)-(1*R*,2*S*,7*R*,8*R*,3*E*,11*E*)-18-Acetoxycembra-3,11,15(17)-trien-2,7,8-triol 11. Colorless oil; $[\alpha]_D^{25}$ −4.4 (*c* 0.59, $CHCl_3$); IR (CH_2Cl_2) ν_{max} 3457, 1730, 1372, 1235, 737 cm^{-1}; ^1H

NMR ($CDCl_3$, 400 MHz), δ 2.05 (m, 1H, H-1), 4.52 (dd, J = 8.8, 4.0 Hz, 1H, H-2), 5.48 (d, J = 8.6 Hz, 1H, H-3), 2.38 (m, 1H, H-5a), 2.35 (m, 1H, H-5b), 1.68 (m, 2H, H-6), 3.45 (m, 1H, H-7), 2.27 (m, 2H, H-9), 2.24 (m, 2H, H-10), 5.23 (t, J = 7.6 Hz, 1H, H-11), 2.17 (m, 1H, H-13a), 1.99 (m, 1H, H-13b), 1.96 (m, 1H, H-14a), 1.69 (m, 1H, H-14b), 1.78 (s, 3H, H-16), 4.95 (br s, 1 H, H-17a), 4.76 (br s, 1H, H-17b), 4.58 (d, J = 12.7 Hz, 1H, H-18a), 4.47 (d, J = 12.7 Hz, 1H, H-18b), 1.59 (s, 3H, H-19), 1.71 (s, 3H, H-20), 2.08 (s, 3H, H-22); ^{13}C NMR ($CDCl_3$, 100 MHz), δ 49.4 (d, C-1), 67.1 (d, C-2), 133.7 (d, C-3), 135.1 (s, C-4), 25.8 (t, C-5), 27.4 (t, C-6), 76.2 (d, C-7), 80.2 (s, C-8), 42.8 (t, C-9), 25.8 (t, C-10), 132.4 (d, C-11), 133.6 (s, C-12), 35.9 (t, C-13), 32.1 (t, C-14), 145.8 (s, C-15), 23.7 (q, C-16), 113.3 (t, C-17), 67.6 (t, C-18), 27.3 (q, C-19), 15.2 (q, C-20), 170.8 (s, C-21), 21.0 (q, C-22); HRESIMS m/z 403.2460 $[M+Na]^+$ (Calcd for $C_{22}H_{36}O_5Na$, 403.2460).

4.3.2. Reaction of knightol acetate (1) with acidified $CDCl_3$

Compound **1** (200 mg, 0.58 mmol) was treated as described for **3**. After 24 h, the reaction mixture was quenched and purified on a silica gel column, eluting with hexane/EtOAc (90:10 v/v) to yield **12** (36 mg, 0.10 mmol, 17.2%), **13** (40 mg, 0.11 mmol, 19.0%), **14** (37 mg, 0.08 mmol, 14.0%) and **15** (42 mg, 0.11 mmol, 19.0%).

4.3.2.1. (−)-(1*S*,7*R*,3*E*,11*E*)-18-Acetoxycembra-3,8(19),11,15(17)-tetraen-7-ol 12. Colorless oil; $[\alpha]_D^{25}$ −26.7 (c 0.16, $CHCl_3$); IR (CH_2Cl_2) ν_{max} 3457, 1730, 1372, 1235, 737 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz), δ 2.00 (m, 1H, H-1), 2.38 (m, 1H, H-2a), 1.82 (m, 1H, H-2a), 5.56 (d, J = 7.9 Hz, 1H, H-3), 2.36 (m, 2H, H-5), 1.70 (m, 2H, H-6), 4.17 (d, J = 10.6 Hz, 1H, H-7), 2.31 (m, 2H, H-9), 2.22 (m, 2H, H-10), 5.34 (t, J = 7.9 Hz, 1H, H-11), 2.22 (m, 1H, H-13a), 2.00 (m, 1H, H-13b), 1.98 (m, 2H, H-14), 1.77 (s, 3H, H-16), 4.93 (br s, 1 H, H-17a), 4.74 (br s, 1H, H-17b), 4.51 (d, J = 13.0 Hz, 1H, H-18a), 4.48 (d, J = 13.0 Hz, 1H, H-18b), 5.06 (br s, 1H, H-19a), 5.03 (br s, 1H, H-19b), 1.61 (br s, 3H, H-20), 2.08 (s, 3H, H-22); ^{13}C NMR ($CDCl_3$, 100 MHz), δ 45.1 (d, C-1), 29.7 (t, C-2), 129.2 (d, C-3), 136.2 (s, C-4), 23.1 (t, C-5), 30.5 (t, C-6), 77.0 (d, C-7), 145.2 (s, C-8), 42.2 (t, C-9), 26.7 (t, C-10), 125.9 (d, C-11), 134.8 (s, C-12), 35.8 (t, C-13), 27.2 (t, C-14), 146.0 (s, C-15), 24.0 (q, C-16), 113.1 (t, C-17), 67.3 (t, C-18), 111.0 (t, C-19), 15.3 (q, C-20), 170.8 (s, C-21), 21.0 (q, C-22); HRESIMS m/z 369.2486 $[M+Na]^+$ (Calcd for $C_{22}H_{34}O_3Na$, 369.2405).

For **12**, the formation of *exo*-methylene function at C-8 and C-19 was evident by the appearance of signals at δ_C 111.0, δ_H 5.06/5.03 and the simultaneous disappearance of the methyl signal located at δ_C 16.9 δ_H 1.20.

4.3.2.2. (−)-(1*S*,7*R*,3*E*,8*E*,11*E*)-18-Acetoxycembra-3,8,11,15(17)-tetraen-7-ol 13. Colorless oil; $[\alpha]_D^{25}$ −7.4 (c 0.34, $CHCl_3$); IR (CH_2Cl_2) ν_{max} 3457, 1730, 1372, 1235, 737 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz), δ 2.00 (m, 1H, H-1), 2.38 (m, 1H, H-2a), 1.82 (m, 1H, H-2a), 5.56 (d, J = 7.9 Hz, 1H, H-3), 2.36 (m, 2H, H-5), 1.70 (m, 2H, H-6), 4.04 (dd, J = 10.0, 2.9 Hz, 1H, H-7), 5.20 (m, 1H, H-9), 2.22 (m, 2H, H-10), 5.34 (t, J = 7.9 Hz, 1H, H-11), 2.22 (m, 1H, H-13a), 2.00 (m, 1H, H-13b), 1.98 (m, 2H, H-14), 1.77 (s, 3H, H-16), 4.93 (br s, 1 H, H-17a), 4.74 (br s, 1H, H-17b), 4.51 (d, J = 13.0 Hz, 1H, H-18a), 4.48 (d, J = 13.0 Hz, 1H, H-18b), 1.64 (s, 3H, H-19), 1.61 (br s, 3H, H-20), 2.08 (s, 3H, H-22); ^{13}C NMR ($CDCl_3$, 100 MHz), δ 45.1 (d, C-1), 29.7 (t, C-2), 129.2 (d, C-3), 136.2 (s, C-4), 23.1 (t, C-5), 30.5 (t, C-6), 77.0 (d, C-7), 131.6 (s, C-8), 124.6 (t, C-9), 26.7 (t, C-10), 125.9 (d, C-11), 134.8 (s, C-12), 35.8 (t, C-13), 27.2 (t, C-14), 146.0 (s, C-15), 24.0 (q, C-16), 113.1 (t, C-17), 67.3 (t, C-18), 11.6 (q, C-19), 15.3 (q, C-20), 170.8 (s, C-21), 21.0 (q, C-22); HRESIMS m/z 369.2477 $[M+Na]^+$ (Calcd for $C_{22}H_{34}O_3Na$, 369.2405).

The geometry of the new olefinic double bond between C-8 and C-9 with (*E*) configuration was deduced from the highfield signal of Me-19 located at δ_C 11.6.

4.3.2.3. (−)-(1*S*,7*R*,3*E*,8*Z*,11*E*)-18-Acetoxycembra-3,8,11,15(17)-tetraen-7-ol 14. Colorless oil; $[\alpha]_D^{25}$ −6.2 (c 0.12, $CHCl_3$); IR (CH_2Cl_2) ν_{max} 3457, 1730, 1372, 1235, 737 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz), δ 2.00 (m, 1H, H-1), 2.38 (m, 1H, H-2a), 1.82 (m, 1H, H-2a), 5.56 (d, J = 7.9 Hz, 1H, H-3), 2.36 (m, 2H, H-5), 1.70 (m, 2H, H-6), 4.50 (m, 1H, H-7), 5.09 (dd, J = 8.3, 2.4 Hz, 1H, H-9), 2.98 (m, 1H, H-10a), 2.55 (m, 1H, H-10b), 5.34 (t, J = 7.9 Hz, 1H, H-11), 2.22 (m, 1H, H-13a), 2.00 (m, 1H, H-13b), 1.98 (m, 2H, H-14), 1.77 (s, 3H, H-16), 4.93 (br s, 1 H, H-17a), 4.74 (br s, 1H, H-17b), 4.51 (d, J = 13.0 Hz, 1H, H-18a), 4.48 (d, J = 13.0 Hz, 1H, H-18b), 1.64 (s, 3H, H-19), 1.61 (br s, 3H, H-20), 2.08 (s, 3H, H-22); ^{13}C NMR ($CDCl_3$, 100 MHz), δ 45.1 (d, C-1), 29.7 (t, C-2), 129.2 (d, C-3), 136.2 (s, C-4), 23.1 (t, C-5), 30.5 (t, C-6), 73.7 (d, C-7), 133.5 (s, C-8), 124.3 (t, C-9), 26.7 (t, C-10), 125.9 (d, C-11), 134.8 (s, C-12), 35.8 (t, C-13), 27.2 (t, C-14), 146.0 (s, C-15), 24.0 (q, C-16), 113.1 (t, C-17), 67.3 (t, C-18), 17.4 (q, C-19), 15.3 (q, C-20), 170.8 (s, C-21), 21.0 (q, C-22); HRESIMS m/z 369.2415 $[M+Na]^+$ (Calcd for $C_{22}H_{34}O_3Na$, 369.2405).

The (*Z*) configuration of the double bond located at C-8 and C-9 was deduced by the downfield resonance of the Me-19 at δ_C 17.4 and the NOE correlation between H-9 and Me-19.

4.3.2.4. (+)-(1*S*,7*R*,8*R*,3*E*,11*E*)-8-Cloro-18-acetoxycembra-3,11,15(17)-trien-7-ol 15. Colorless oil; $[\alpha]_D^{25}$ +5.3 (c 0.17, $CHCl_3$); IR (CH_2Cl_2) ν_{max} 3457, 1730, 1372, 1235, 737 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz), δ 2.00 (m, 1H, H-1), 2.38 (m, 1H, H-2a), 1.82 (m, 1H, H-2a), 5.56 (d, J = 7.9 Hz, 1H, H-3), 2.36 (m, 2H, H-5), 1.70 (m, 2H, H-6), 3.79 (d, J = 10.6 Hz, 1H, H-7), 2.31 (m, 2H, H-9), 2.22 (m, 2H, H-10), 5.34 (t, J = 7.9 Hz, 1H, H-11), 2.22 (m, 1H, H-13a), 2.00 (m, 1H, H-13b), 1.98 (m, 2H, H-14), 1.77 (s, 3H, H-16), 4.93 (br s, 1 H, H-17a), 4.74 (br s, 1H, H-17b), 4.51 (d, J = 13.0 Hz, 1H, H-18a), 4.48 (d, J = 13.0 Hz, 1H, H-18b), 1.52 (br s, 3H, H-19), 1.61 (br s, 3H, H-20), 2.08 (s, 3H, H-22); ^{13}C NMR ($CDCl_3$, 100 MHz), δ 45.1 (d, C-1), 29.7 (t, C-2), 129.2 (d, C-3), 136.2 (s, C-4), 23.1 (t, C-5), 30.5 (t, C-6), 75.5 (d, C-7), 83.1 (s, C-8), 42.2 (t, C-9), 26.7 (t, C-10), 125.9 (d, C-11), 134.8 (s, C-12), 35.8 (t, C-13), 27.2 (t, C-14), 146.0 (s, C-15), 24.0 (q, C-16), 113.1 (t, C-17), 67.3 (t, C-18), 25.2 (q, C-19), 15.3 (q, C-20), 170.8 (s, C-21), 21.0 (q, C-22); HRESIMS m/z 405.2184 $[M+Na]^+$ (Calcd for $C_{22}H_{35}ClO_3Na$, 405.2172).

4.3.3. Reaction of compound (2) with acetic anhydride

Asperdiol (**2**) (150 mg, 0.47 mmol) was treated with acetic anhydride (1 mL) in dry pyridine (2 mL) for 24 h under stirring at room temperature. The product was purified by column chromatography (hexane/EtOAc (90:10 v/v)) to obtain a colorless oil corresponding to pure diacetylated compound **16** (180 mg, 0.44 mmol, 94.8 %) as a colorless oil.

4.3.3.1. (−)-Diacetoxy asperdiol 16. Colorless oil; $[\alpha]_D^{25}$ −80.0 (c 0.40, $CHCl_3$); IR, NMR, and MS data are consistent with literature values.[16]

4.3.4. Reaction of asperdiol diacetate (7) with acidified $CDCl_3$

Compound **16** (174 mg, 0.43 mmol) was treated as described for **3**. After 24 h, the reaction mixture was quenched and purified on a silica gel column, eluting with hexane/EtOAc (9:1 v/v) to yield **17** (11 mg, 0.027 mmol, 6.3%), a mixture of **18** and **19** (29 mg, 16.6%), and **20** (71 mg, 0.16 mmol, 37.5%). The mixture of 1**8** and **19** was subjected to HPLC RP-18, MeOH/H_2O (50:50 v/v), to obtain pure **18** (*E* isomer) (19 mg, 0.047, 10.9%) and pure **19** (*Z* isomer) (7 mg, 0.017, 4.0%).

4.3.4.1. (−)-(1*R*,2*S*,7*R*,3*E*,11*E*)-2,18-Diacetoxycembra-3,8(19), 11, 15(17)-tetraen-7-ol 17. Colorless oil; $[\alpha]_D^{25}$ −61.5 (c 0.13,

$CHCl_3$); IR, NMR, and MS data are consistent with literature values.[16]

4.3.4.2. (−)-(1*R*,2*S*,7*R*,3*E*,8*E*,11*E*)-2,18-Diacetoxycembra-3,8,11, 15(17)-tetraen-7-ol 18. Colorless oil; $[\alpha]_D^{25}$ −160.0 (*c* 0.10, $CHCl_3$); IR, NMR, and MS data are consistent with literature values.[16]

4.3.4.3. (−)-(1*R*,2*S*,7*R*,3*E*,8*Z*,11*E*)-2,18-Diacetoxycembra-3,8,11, 15(17)-tetraen-7-ol 19. Colorless oil; $[\alpha]_D^{25}$ −55.9 (*c* 0.14, $CHCl_3$); IR, NMR, and MS data are consistent with literature values.[16]

4.3.4.4. (−)-(1*R*,2*S*,7*R*,8*R*,3*E*,11*E*)-8-Chloro-2,18-diacetoxycembra-3,11,15(17)-trien-7-ol 20. Colorless oil; $[\alpha]_D^{25}$ −66.2 (*c* 0.27, $CHCl_3$); IR, NMR, and MS data are consistent with literature values.[16]

4.3.5. Reaction of asperdiol (2) with acidified $CDCl_3$

Compound **2** (175 mg, 0.55 mmol) was treated as described for **1**, **3**, and **716**. After 24 h, the reaction mixture was quenched and purified on a silica gel column, eluting with hexane/EtOAc (90:10 v/v) to yield **21** (50 mg, 0.15 mmol, 27.2%), **22** (60 mg, 0.18 mmol, 32.7%), **23** (40 mg, 0.12 mmol, 21.8%), and **24** (19 mg, 0.056 mmol, 10.2%).

4.3.5.1. (−)-(1*R*,2*S*,7*R*,8*R*,3*E*,11*E*)-2,8-Oxa-cembra-7,18-diol 21. Colorless oil; $[\alpha]_D^{25}$ −3.3 (*c* 0.13, $CHCl_3$); IR (CH_2Cl_2) ν_{max} 3069, 1739, 1232, 736 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz), δ 1.99 (m, 1H, H-1), 4.51 (dd, *J* = 8.7, 4.4 Hz, 1H, H-2), 5.40 (d, *J* = 8.7 Hz, 1H, H-3), 1.85 (m, 1H, H-5a), 1.69 (m, 1H, H-5b), 1.48 (m, 2H, H-6), 3.36 (dd, *J* = 10.1, 2.1 Hz, 1H, H-7), 2.13 (m, 1H, H-9a), 1.77 (m, 1H, H-9b), 2.28 (m, 1H, H-10a), 1.52 (m, 1H, H-10b), 5.25 (t, *J* = 7.6 Hz, 1H, H-11), 1.34 (m, 1H, H-13a), 1.29 (m, 1H, H-13b), 1.34 (m, 1H, H-14a), 1.27 (m, 1H, H-14b), 1.77 (s, 3H, H-16), 4.91 (br s, 1H, H-17a), 4.75 (br s, 1H, H-17b), 4.07 (d, *J* = 13.4 Hz, 1H, H-18a), 4.02 (d, *J* = 13.4 Hz, 1H, H-18b), 1.58 (s, 3H, H-19), 1.29 (s, 3H, H-20); ^{13}C NMR ($CDCl_3$, 100 MHz), δ 49.3 (d, C-1), 67.4 (d, C-2), 128.5 (d, C-3), 140.8 (s, C-4), 27.6 (t, C-5), 30.8 (t, C-6), 76.8 (d, C-7), 79.8 (s, C-8), 36.0 (t, C-9), 26.0 (t, C-10), 125.9 (d, C-11), 135.6 (s, C-12), 34.2 (t, C-13), 22.5 (t, C-14), 146.2 (s, C-15), 23.4 (q, C-16), 113.2 (t, C-17), 66.3 (t, C-18), 15.3 (q, C-19), 19.4 (q, C-20); HRESIMS *m*/*z* 359.2238 $[M+K]^+$ (calcd for $C_{20}H_{32}O_4K$, 359.2198).

4.3.5.2. (−)-(1*R*,2*S*,7*R*,8*R*,3*E*,11*E*)-7,8-Dihydroxy-asperdiol 22. Colorless oil; $[\alpha]_D^{25}$ −15.5 (*c* 0.10, $CHCl_3$); IR (CH_2Cl_2) ν_{max} 3069, 1739, 1232, 736 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz), δ 1.99 (m, 1H, H-1), 4.52 (dd, *J* = 8.6, 3.2 Hz, 1H, H-2), 5.38 (d, *J* = 8.6 Hz, 1H, H-3), 2.15 (m, 1H, H-5a), 1.56 (m, 1H, H-5b), 1.64 (m, 1H, H-6a), 1.50 (m, 1H, H-6b), 3.39 (d, *J* = 10.5 Hz, 1H, H-7), 1.76 (m, 1H, H-9a), 1.53 (m, 1H, H-9b), 2.34 (m, 1H, H-10a), 2.29 (m, 1H, H-10b), 5.22 (t, *J* = 7.5 Hz, 1H, H-11), 2.15 (m, 1H, H-13a), 2.04 (m, 1H, H-13b), 2.00 (m, 1H, H-14a), 1.52 (m, 1H, H-14b), 1.76 (s, 3H, H-16), 4.90 (br s, 1H, H-17a), 4.73 (br s, 1H, H-17b), 4.09 (d, *J* = 13.1 Hz, 1H, H-18a), 3.97 (d, *J* = 13.1 Hz, 1H, H-18b), 1.31 (s, 3H, H-19), 1.57 (s, 3H, H-20); ^{13}C NMR ($CDCl_3$, 100 MHz), δ 49.3 (d, C-1), 67.1 (d, C-2), 129.4 (d, C-3), 140.1 (s, C-4), 25.5 (t, C-5), 30.4 (t, C-6), 76.2 (d, C-7), 76.0 (s, C-8), 39.6 (t, C-9), 22.9 (t, C-10), 125.7 (d, C-11), 135.6 (s, C-12), 36.0 (t, C-13), 27.4 (t, C-14), 146.2 (s, C-15), 23.7 (q, C-16), 113.0 (t, C-17), 66.4 (t, C-18), 25.1 (q, C-19), 15.1 (q, C-20); HRESIMS *m*/*z* 361.2351 $[M+Na]^+$ (calcd for $C_{20}H_{34}O_4Na$, 361.2354).

4.3.5.3. (+)-(1*R*,2*S*,7*S*,8*R*,3*E*,11*E*)-7,18-Oxa-cembra-2,8-diol 23. Colorless oil; $[\alpha]_D^{25}$ +6.0 (*c* 0.12, $CHCl_3$); IR (CH_2Cl_2) ν_{max} 3069, 1739, 1232, 736 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz), δ 1.97 (m, 1H, H-1), 4.19 (d, *J* = 9.6 Hz, 1H, H-2), 5.28 (d, *J* = 8.9 Hz, 1H, H-3), 2.54 (dd, *J* = 11.0, 9.4 Hz, 1H, H-5a), 2.20 (m, 1H, H-5b), 1.99 (m, 1H, H-6a), 1.74 (m, 1H, H-6b), 3.44 (dd, *J* = 11.6, 3.5 Hz, 1H, H-7), 1.57 (m, 2H, H-9), 2.10 (m, 1H, H-10a), 1.64 (m, 1H, H-10b), 5.16 (t, *J* = 7.5 Hz, 1H, H-11), 1.99 (m, 1H, H-13a), 1.74 (m, 1H, H-13b), 1.34 (m, 1H, H-14a), 1.24 (m, 1H, H-14b), 1.76 (s, 3H, H-16), 4.97 (br s, 1H, H-17a), 4.90 (br s, 1H, H-17b), 4.46 (d, *J* = 11.4 Hz, 1H, H-18a), 4.13 (d, *J* = 11.8 Hz, 1H, H-18b), 1.24 (s, 3H, H-19), 1.59 (s, 3H, H-20); ^{13}C NMR ($CDCl_3$, 100 MHz), δ 55.2 (d, C-1), 69.0 (d, C-2), 127.5 (d, C-3), 138.7 (s, C-4), 22.9 (t, C-5), 23.9 (t, C-6), 77.0 (d, C-7), 74.0 (s, C-8), 36.7 (t, C-9), 23.3 (t, C-10), 123.6 (d, C-11), 136.6 (s, C-12), 38.2 (t, C-13), 30.0 (t, C-14), 144.9 (s, C-15), 18.9 (q, C-16), 114.6 (t, C-17), 74.3 (t, C-18), 24.4 (q, C-19), 15.1 (q, C-20); HRESIMS *m*/*z* 359.2362 $[M+K]^+$ (calcd for $C_{20}H_{32}O_4K$, 359.1988).

4.3.5.4. (−)-(1*R*,2*S*,7*R*,8*R*,3*E*,11*E*)-8-Chloro-7-hydroxy-asperdiol 24. Colorless oil; $[\alpha]_D^{25}$ −18.3 (*c* 0.05, $CHCl_3$); IR (CH_2Cl_2) ν_{max} 3069, 1739, 1232, 736 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz), δ 1.99 (m, 1H, H-1), 4.53 (dd, *J* = 8.6, 4.1 Hz, 1H, H-2), 5.43 (d, *J* = 8.6 Hz, 1H, H-3), 2.15 (m, 1H, H-5a), 1.56 (m, 1H, H-5b), 1.64 (m, 1H, H-6a), 1.50 (m, 1H, H-6b), 3.39 (d, *J* = 11.3 Hz, 1H, H-7), 1.76 (m, 1H, H-9a), 1.53 (m, 1H, H-9b), 2.34 (m, 1H, H-10a), 2.29 (m, 1H, H-10b), 5.23 (t, *J* = 7.4 Hz, 1H, H-11), 2.15 (m, 1H, H-13a), 2.04 (m, 1H, H-13b), 2.00 (m, 1H, H-14a), 1.52 (m, 1H, H-14b), 1.76 (s, 3H, H-16), 4.91 (br s, 1H, H-17a), 4.74 (br s, 1H, H-17b), 4.09 (d, *J* = 13.0 Hz, 1H, H-18a), 4.00 (d, *J* = 13.0 Hz, 1H, H-18b), 1.69 (s, 3H, H-19), 1.58 (s, 3H, H-20); ^{13}C NMR ($CDCl_3$, 100 MHz), δ 49.6 (d, C-1), 67.1 (d, C-2), 129.3 (d, C-3), 140.1 (s, C-4), 25.3 (t, C-5), 31.9 (t, C-6), 76.2 (d, C-7), 76.0 (s, C-8), 35.9 (t, C-9), 23.6 (t, C-10), 124.9 (d, C-11), 134.9 (s, C-12), 38.2 (t, C-13), 27.4 (t, C-14), 146.0 (s, C-15), 23.6 (q, C-16), 113.1 (t, C-17), 66.2 (t, C-18), 27.4 (q, C-19), 15.3 (q, C-20); HRESIMS *m*/*z* 361.1889 $[M-H_2O+Na]^+$ (calcd for $C_{20}H_{31}O_2ClNa$, 361.1910).

4.3.6. Reaction of asperdiol acetate (3) with P_2O_5

A mixture of **3** (120 mg, 0.33 mmol) and P_2O_5 (42 mg, 0.35 mmol) in dry CH_2Cl_2 (5 mL) was refluxed gently for 3.5 h before it was quenched with saturated $NaHCO_3$ (10 mL). The reaction mixture was extracted with CH_2Cl_2 (3 x 5 mL), concentrated, and purified with reversed-phase HPLC, eluting with MeOH-H_2O (80:20 v/v) to yield **25** (43.4 mg, 0.12 mmol, 34%) and **26** (27.1 mg, 0.075 mmol, 23%) as colorless oils.

4.3.6.1. (−)-(1*R*,2*S*,3*E*,11*E*)-18-Acetoxycembra-2-hydroxy-3,11, 15(17)-trien-8-al 25. Colorless oil; $[\alpha]_D^{25}$ −6.3 (*c* 0.12, $CHCl_3$); IR (CH_2Cl_2) ν_{max} 3457, 1730, 1372, 1235, 737 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz), δ 1.82 (m, 1H, H-1), 4.61 (d, *J* = 8.0 Hz, 1H, H-2), 5.41 (d, *J* = 8.7 Hz, 1H, H-3), 1.81 (m, 1H, H-5a), 1.46 (m, 1H, H-5b), 1.83 (m, 1H, H-6a), 1.63 (m, 1H, H-6b), 9.37 (s, 1H, H-7), 1.25 (m, 2H, H-9), 2.33 (m, 1H, H-10a), 2.12 (m, 1H, H-10b), 5.36 (d, *J* = 10.3 Hz, 1H, H-11), 2.23 (m, 1H, H-13a), 2.16 (m, 1H, H-13b), 1.60 (m, 1H, H-14a), 1.46 (m, 1H, H-14b), 1.75 (s, 3H, H-16), 4.89 (br s, 1 H, H-17a), 4.72 (br s, 1H, H-17b), 4.54 (d, *J* = 12.8 Hz, 1H, H-18a), 4.42 (d, *J* = 12.9 Hz, 1H, H-18b), 1.05 (s, 3H, H-19), 1.63 (s, 3H, H-20), 2.06 (s, 3H, H-22); ^{13}C NMR ($CDCl_3$, 100 MHz), δ 49.6 (d, C-1), 65.9 (d, C-2), 132.7 (d, C-3), 133.7 (s, C-4), 24.6 (t, C-5), 26.3 (t, C-6), 206.0 (d, C-7), 49.4 (s, C-8), 28.9 (t, C-9), 22.1 (t, C-10), 128.5 (d, C-11), 133.3 (s, C-12), 36.5 (t, C-13), 32.8 (t, C-14), 146.2 (s, C-15), 24.7 (q, C-16), 112.7 (t, C-17), 67.2 (t, C-18), 21.5 (q, C-19), 14.2 (q, C-20), 170.7 (s, C-21), 20.9 (q, C-22); HRESIMS *m*/*z* $[M+Na]^+$ 385.2295 (Calcd for $C_{22}H_{34}O_4Na$, 385.2354).

4.3.6.2. (+)-(1*R*,2*S*,3*E*,11*E*)-18-Acetoxycembra-7-keto-3,11,15 (17)-trien-2-ol 26. Colorless oil; $[\alpha]_D^{25}$ +5.3 (*c* 0.17, $CHCl_3$); IR (CH_2Cl_2) ν_{max} 3457, 1730, 1372, 1235, 737 cm^{-1}; ^1H NMR ($CDCl_3$, 400 MHz), δ 2.09 (m, 1H, H-1), 4.49 (m, 1H, H-2), 5.48 (d,

J = 8.1 Hz, 1H, H-3), 2.19 (m, 1H, H-5a), 2.07 (m, 1H, H-5b), 1.67 (m, 2H, H-6), 2.53 (m, 1H, H-8), 2.48 (m, 2H, H-9), 2.38 (m, 1H, H-10a), 2.20 (m, 1H, H-10b), 5.09 (t, J = 7.1 Hz, 1H, H-11), 2.67 (m, 1H, H-13a), 1.99 (m, 1H, H-13b), 1.60 (m, 2H, H-14), 1.76 (s, 3H, H-16), 4.97 (br s, 1 H, H-17a), 4.77 (br s, 1H, H-17b), 4.51 (d, J = 12.1 Hz, 1H, H-18a), 4.45 (d, J = 12.2 Hz, 1H, H-18b), 1.10 (d, J = 6.8 Hz, 3H, H-19), 1.59 (s, 3H, H-20), 2.06 (s, 3H, H-22); ^{13}C NMR ($CDCl_3$, 100 MHz), δ 50.0 (d, C-1), 69.2 (d, C-2), 133.0 (d, C-3), 136.4 (s, C-4), 25.9 (t, C-5), 27.6 (t, C-6), 213.9 (s, C-7), 45.5 (d, C-8), 39.1 (t, C-9), 23.9 (t, C-10), 125.2 (d, C-11), 134.1 (s, C-12), 35.9 (t, C-13), 34.1 (t, C-14), 145.0 (s, C-15), 23.1 (q, C-16), 114.1 (t, C-17), 67.4 (t, C-18), 16.6 (q, C-19), 15.7 (q, C-20), 170.7 (s, C-21), 21.0 (q, C-22); HRESIMS m/z [M+Na]$^+$ 385.2301 (Calcd for $C_{22}H_{34}O_4Na$, 385.2354).

4.3.7. Reaction of asperdiol acetate (3) with SeO_2

SeO_2 (3.66 mg, 0.033 mmol) was added to dry CH_2Cl_2 (5 mL) and tert-butylhydroperoxide (0.063 mL, 0.66 mmol), and the mixture was stirred for 30 min at room temperature. Compound **3** (120 mg, 0.33 mmol) was added to the solution, and the reaction mixture was stirred at room temperature for 24 h. The mixture was partitioned between water and ethyl acetate, and the organic phase was purified by HPLC-RP (MeOH/H_2O 50:50 v/v) to obtain compound **27** as a colorless oil (39 mg, 0.116 mmol, 17.6%).

4.3.7.1. (−)-(1*R*,2*S*,7*R*,8*R*,13*S*,3*E*,11*E*)-13-Hydroxy-asperdiol acetate 27. Colorless oil; $[\alpha]_D^{25}$ −45.0 (c 0.35, $CHCl_3$); IR (CH_2Cl_2) ν_{max} 3457, 1730, 1372, 1235, 737 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz), δ 2.20 (m, 1H, H-1), 4.54 (m, 1H, H-2), 5.52 (d, J = 8.5 Hz, 1H, H-3), 1.80 (m, 1H, H-5a), 1.56 (m, 1H, H-5b), 2.25 (m, 1H, H-6a), 1.67 (m, 1H, H-6b), 2.61 (t, J = 5.8 Hz, 1H, H-7), 1.83 (m, 1H, H-9a), 1.76 (m, 1H, H-9b), 2.30 (m, 1H, H-10a), 2.10 (m, 1H, H-10b), 5.36 (t, J = 7.2 Hz, 1H, H-11), 4.03 (dd, J = 7.3, 4.4 Hz, 1H, H-13), 1.52 (m, 2H, H-14), 1.78 (s, 3H, H-16), 4.94 (br s, 1 H, H-17a), 4.76 (br s, 1H, H-17b), 4.52 (m, 1H, H-18a), 4.49 (m, 1H, H-18b), 1.26 (s, 3H, H-19), 1.65 (s, 3H, H-20), 2.07 (s, 3H, H-22); ^{13}C NMR ($CDCl_3$, 100 MHz), δ 49.0 (d, C-1), 69.9 (d, C-2), 132.0 (d, C-3), 137.1 (s, C-4), 25.5 (t, C-5), 25.6 (t, C-6), 63.3 (d, C-7), 61.0 (s, C-8), 35.9 (t, C-9), 23.0 (t, C-10), 126.2 (d, C-11), 134.3 (s, C-12), 76.5 (d, C-13), 36.7 (t, C-14), 145.4 (s, C-15), 22.2 (q, C-16), 114.0 (t, C-17), 67.0 (t, C-18), 17.4 (q, C-19), 11.8 (q, C-20), 170.6 (s, C-21), 21.0 (q, C-22); HRESIMS m/z [M+Na]$^+$ 401.2298 (Calcd for $C_{22}H_{34}O_5Na$, 401.2303).

4.3.8. Reaction of asperdiol acetate (3) with *m*-chloroperbenzoic acid

A solution of compound **3** (120 mg, 0.33 mmol) in dry CH_2Cl_2 (5 mL) was stirred vigorously with *m*-CPBA (177 mg, 1.02 mmol) at room temperature for 2 h and concentrated to leave a residue that was chromatographed on silica gel. Eluting with hexane-EtOAc (50:50 v/v) yielded 77 mg (0.19 mmol, 56.9%) of tetraepoxide **28** as a colorless oil.

4.3.8.1. (−)-(1*S*,2*R*,7*R*,8*R*,15*R*)-3,4-11,12-15,17-Triepoxy-asperdiol acetate 28. Colorless oil; $[\alpha]_D^{25}$ −1.5 (c 0.55, $CHCl_3$); IR (CH_2Cl_2) ν_{max} 3457, 1730, 1372, 1235, 737 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz), δ 1.65 (m, 1H, H-1), 3.70 (t, J = 7.8 Hz, 1H, H-2), 3.08 (d, J = 8.4 Hz, 1H, H-3), 2.09 (m, 1H, H-5a), 2.02 (m, 1H, H-5b), 1.50 (m, 1H, H-6a), 1.39 (m, 1H, H-6b), 2.75 (dd, J = 7.9, 3.4 Hz, 1H, H-7), 2.00 (m, 1H, H-9a), 1.37 (m, 1H, H-9b), 1.65 (m, 2H, H-10), 2.66 (m, 1H, H-11), 2.08 (m, 1H, H-13a), 1.42 (m, 1H, H-13b), 1.95 (m, 1H, H-14a), 1.39 (m, 1H, H-14b), 1.34 (s, 3H, H-16), 2.60 (d, J = 4.6 Hz, 1H, H-17a), 2.47 (d, J = 4.7 Hz, 1H, H-17b), 4.40 (d, J = 12.4 Hz, 1H, H-18a), 3.89 (d, J = 12.4 Hz, 1H, H-18b), 1.30 (s, 3H, H-19), 1.25 (s, 3H, H-20), 2.10 (s, 3H, H-22); ^{13}C NMR ($CDCl_3$, 100 MHz), δ 46.6 (d, C-1), 72.1 (d, C-2), 64.0 (d, C-3), 57.2 (s, C-4), 24.9 (t, C-5), 26.6 (t, C-6), 61.9 (d, C-7), 59.6 (s, C-8), 35.3 (t, C-9), 24.3 (t, C-10), 64.4 (d, C-11), 62.6 (s, C-12), 35.0 (t, C-13), 25.4 (t, C-14), 61.0 (s, C-15), 16.8 (q, C-16), 53.6 (t, C-17), 66.1 (t, C-18), 17.3 (q, C-19), 17.7 (q, C-20), 170.6 (s, C-21), 20.8 (q, C-22); HRESIMS m/z [M+Na]$^+$ 433.2197 (Calcd for $C_{22}H_{34}O_7Na$, 433.2202).

4.3.9. Reaction of asperdiol (2) with PCC

Compound **2** (100 mg, 0.31 mmol) was treated with PCC (120 mg) in dry CH_2Cl_2 (4 mL) for about 2 h under stirring at room temperature. The reaction was quenched by addition of water (3 mL) and the organic layer was concentrated to dryness. The crude product was dried and purified by column chromatography and eluting with hexane/EtOAc (90:10 v/v) to obtain pure compounds **30** (35 mg, 0.12 mmol, 38.7%) and **29** (22 mg, 0.07 mmol, 22.5%) as colorless oils.

4.3.9.1. (−)-1*R*,7*R*,8*R*-2,18-Diketo-asperdiol 30. Colorless oil; $[\alpha]_D^{25}$ −65,9 (c 0.20, $CHCl_3$); IR (CH_2Cl_2) ν_{max} 3069, 1739, 1232, 736 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz), δ 2.67 (dd, J = 9.9, 3.0 Hz, 1H, H-1), 6.80 (s, 1H, H-3), 1.75 (m, 1H, H-5a), 1.62 (m, 1H, H-5b), 2.26 (m, 1H, H-6a), 1.98 (m, 1H, H-6b), 3.20 (dd, J = 10.0, 2.8 Hz, 1H, H-7), 2.26 (m, 1H, H-9a), 1.71 (m, 1H, H-9b), 2.00 (m, 1H, H-10a), 1.84 (m, 1H, H-10b), 4.95 (t, J = 6.8 Hz, 1H, H-11), 3.38 (ddd, J = 13.9, 11.5, 5.2 Hz, 1H, H-13a), 2.57 (ddd, J = 13.4, 10.9, 5.1 Hz, 1H, H-13b), 2.17 (m, 1H, H-14a), 1.64 (m, 1H, H-14b), 1.70 (s, 3H, H-16), 4.98 (br s, 1 H, H-17a), 4.89 (br s, 1H, H-17b), 9.45 (s, 1H, H-18), 1.57 (s, 3H, H-19), 1.05 (s, 3H, H-20); ^{13}C NMR ($CDCl_3$, 100 MHz), δ 60.8 (d, C-1), 199.9 (s, C-2), 141.3 (d, C-3), 143.0 (s, C-4), 36.6 (t, C-5), 38.0 (t, C-6), 61.0 (d, C-7), 60.5 (s, C-8), 25.9 (t, C-9), 21.5 (t, C-10), 127.2 (d, C-11), 134.3 (s, C-12), 21.1 (t, C-13), 26.2 (t, C-14), 149.9 (s, C-15), 20.4 (q, C-16), 115.5 (t, C-17), 195.0 (d, C-18), 15.3 (q, C-19), 18.1 (q, C-20); HRESIMS m/z 339.1921 [M+Na]$^+$ (calcd for $C_{20}H_{28}O_3Na$, 339.1931).

4.3.9.2. (−)-1*R*,2*S*,7*R*,8*R*-18-Keto-asperdiol 29. Colorless oil; $[\alpha]_D^{25}$ −4.0 (c 0.73, $CHCl_3$); IR (CH_2Cl_2) ν_{max} 3069, 1739, 1232, 736 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz), δ 2.28 (m, 1H, H-1), 4.72 (t, J = 6.9 Hz, 1H, H-2), 6.43 (d, J = 7.9 Hz, 1H, H-3), 1.98 (m, 1H, H-5a), 1.35 (m, 1H, H-5b), 2.24 (m, 2H, H-6), 2.61 (m, 1H, H-7), 1.86 (m, 1H, H-9a), 1.56 (m, 1H, H-9b), 2.19 (m, 1H, H-10a), 2.10 (m, 1H, H-10b), 5.16 (t, J = 6.9 Hz, 1H, H-11), 1.86 (m, 1H, H-13a), 1.58 (m, 1H, H-13b), 2.61 (m, 1H, H-14a), 2.26 (m, 1H, H-14b), 1.80 (s, 3H, H-16), 5.02 (br s, 1 H, H-17a), 4.81 (br s, 1H, H-17b), 9.42 (s, 1H, H-18), 1.20 (s, 3H, H-19), 1.65 (s, 3H, H-20); ^{13}C NMR ($CDCl_3$, 100 MHz), δ 50.2 (d, C-1), 69.3 (d, C-2), 155.2 (d, C-3), 141.9 (s, C-4), 37.3 (t, C-5), 24.0 (t, C-6), 64.5 (d, C-7), 60.3 (s, C-8), 26.6 (t, C-9), 36.2 (t, C-10), 125.3 (d, C-11), 135.1 (s, C-12), 27.8 (t, C-13), 22.1 (t, C-14), 144.7 (s, C-15), 22.2 (q, C-16), 115.0 (t, C-17), 194.7 (d, C-18), 16.8 (q, C-19), 16.0 (q, C-20); HRESIMS m/z 341.2073 [M+Na]$^+$ (calcd for $C_{20}H_{30}O_3Na$, 341.2087).

4.3.10. Reaction of asperdiol acetate (3) with iodine

A solution of iodine (25 mg, 0.20 mmol) in $CDCl_3$ (2.0 mL) was added dropwise over 45 min to a magnetically stirred solution of **3** (120 mg, 0.33 mmol) in $CDCl_3$ (4.0 mL). The mixture was stirred at 25 °C for 30 min and concentrated, and the resulting oil was purified on silica gel, eluting with hexane/EtOAc (80:20 v/v) to yield **31** (22 mg, 0.045 mmol, 13.6%) and **32** (18 mg, 0.037 mmol, 11.1%) as colorless oils, an intractable mixture of polar compounds and the starting material **3**.

4.3.10.1. (−)-(1*R*,2*S*,7*R*,8*S*,3*E*,11*E*)-18-Acetoxycembra-2,8-oxa-3,11,15(17)-trien-7-ol 31. Colorless oil; $[\alpha]_D^{25}$ −26.7 (c 0.16, $CHCl_3$); IR (CH_2Cl_2) ν_{max} 3457, 1730, 1372, 1235, 737 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz), δ 2.03 (m, 1H, H-1), 4.53 (dd, J = 8.8, 3.9 Hz, 1H, H-2), 5.46 (d, J = 8.8 Hz, 1H, H-3), 2.22 (m, 1H, H-5a), 1.80 (m, 1H, H-5b), 1.60 (m, 2H, H-6), 3.39 (t, J = 8.0 Hz, 1H, H-7),

1.79 (m, 1H, H-9a), 1.62 (m, 1H, H-9b), 2.37 (m, 1H, H-10a), 1.87 (m, 1H, H-10b), 5.23 (t, J = 7.8 Hz, 1H, H-11), 1.84 (m, 1H, H-13a), 1.77 (m, 1H, H-13b), 2.06 (m, 1H, H-14a), 1.62 (m, 1H, H-14b), 1.77 (s, 3H, H-16), 4.94 (br s, 1 H, H-17a), 4.75 (br s, 1H, H-17b), 4.57 (d, J = 12.9 Hz, 1H, H-18a), 4.45 (d, J = 12.9 Hz, 1H, H-18b), 1.59 (s, 3H, H-19), 1.34 (s, 3H, H-20), 2.08 (s, 3H, H-22); ^{13}C NMR ($CDCl_3$, 100 MHz), δ 49.3 (d, C-1), 67.1 (d, C-2), 133.7 (d, C-3), 135.7 (s, C-4), 26.1 (t, C-5), 27.5 (t, C-6), 76.0 (d, C-7), 75.8 (s, C-8), 39.9 (t, C-9), 23.0 (t, C-10), 125.9 (d, C-11), 135.4 (s, C-12), 35.9 (t, C-13), 30.6 (t, C-14), 146.0 (s, C-15), 23.7 (q, C-16), 113.3 (t, C-17), 67.4 (t, C-18), 24.9 (q, C-19), 15.1 (q, C-20), 171.0 (s, C-21), 21.0 (q, C-22); HRESIMS m/z [M+Na]$^+$ 385.2333 (Calcd for $C_{22}H_{34}O_4Na$, 385.2354).

4.3.10.2. (−)-(1*R*,2*S*,7*S*,8*R*,11*R*,12*R*,3*E*)-18-Acetoxycembra-7,12-diiodine-8,11-oxa-3,15(17)-dien-2-ol 32. Colorless oil; $[\alpha]_D^{25}$ −7.4 (c 0.34, $CHCl_3$); IR (CH_2Cl_2) ν_{max} 3457, 1730, 1372, 1235, 737 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz), δ 2.02 (m, 1H, H-1), 4.50 (dd, J = 7.5, 3.6 Hz, 1H, H-2), 5.43 (d, J = 7.5 Hz, 1H, H-3), 1.57 (m, 2H, H-5), 2.74 (dt, J = 15.0, 4.6 Hz, 1H, H-6a), 1.76 (m, 1H, H-6b), 4.12 (dd, J = 9.4, 2.5 Hz, 1H, H-7), 1.69 (m, 1H, H-9a), 1.64 (m, 1H, H-9b), 2.23 (m, 1H, H-10a), 1.54 (m, 1H, H-10b), 3.37 (d, J = 10.0 Hz, 1H, H-11), 2.25 (m, 2H, H-13), 1.78 (m, 2H, H-14), 1.76 (s, 3H, H-16), 4.96 (br s, 1 H, H-17a), 4.87 (br s, 1H, H-17b), 4.68 (d, J = 13.5 Hz, 1H, H-18a), 4.45 (d, J = 13.5 Hz, 1H, H-18b), 1.12 (s, 3H, H-19), 1.40 (s, 3H, H-20), 2.12 (s, 3H, H-22); ^{13}C NMR ($CDCl_3$, 100 MHz), δ 51.8 (d, C-1), 68.7 (d, C-2), 131.6 (d, C-3), 133.8 (s, C-4), 25.4 (t, C-5), 24.4 (t, C-6), 39.0 (d, C-7), 78.9 (s, C-8), 47.2 (t, C-9), 33.3 (t, C-10), 74.8 (d, C-11), 49.6 (s, C-12), 33.7 (t, C-13), 34.8 (t, C-14), 144.5 (s, C-15), 20.0 (q, C-16), 113.1 (t, C-17), 65.7 (t, C-18), 20.6 (q, C-19), 26.3 (q, C-20), 170.0 (s, C-21), 21.1 (q, C-22); HRESIMS m/z [M−I+K]$^+$ 529.1411 (Calcd for $C_{22}H_{35}IO_4K$, 529.1217).

4.3.11. Photolysis of asperdiol acetate (3)

A magnetically stirred solution of **3** (70 mg, 0.19 mmol) in isopropanol (3 mL) placed in a small Pyrex tube was irradiated (254 nm; 50 W) for 12 h at 25 °C. After concentration, the resulting oil was purified on silica gel (hexane/EtOAc 80:20 v/v) to provide the pure compound **33** (40 mg, 0.12 mmol, 62.6%) and the pure compound **2** (10 mg, 0.033 mmol, 17.4%).

4.3.11.1. (+)-(1*R*,2*S*,7*R*,11*R*,12*R*,3*E*)-7,11-Oxacembra-3,8(19),15(17)-trien-2,12,18-triol 33. Colorless oil; $[\alpha]_D^{25}$ +42.2 (c 0.69, $CHCl_3$); IR (CH_2Cl_2) ν_{max} 3457, 1730, 1372, 1235, 737 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz), δ 2.22 (m, 1H, H-1), 4.48 (t, J = 8.7 Hz, 1H, H-2), 5.34 (d, J = 9.2 Hz, 1H, H-3), 2.43 (m, 1H, H-5a), 2.25 (m, 1H, H-5b), 1.83 (m, 1H, H-6a), 1.70 (m, 1H, H-6b), 3.50 (d, J = 9.6 Hz, 1H, H-7), 2.31 (m, 1H, H-9a), 2.21 (m, 1H, H-9b), 1.86 (m, 1H, H-10a), 1.79 (m, 1H, H-10b), 4.42 (t, J = 7.3 Hz, 1H, H-11), 2.31 (m, 2H, H-13), 1.87 (m, 1H, H-14a), 1.70 (m, 1H, H-14b), 1.80 (s, 3H, H-16), 5.09 (br s, 1 H, H-17a), 4.87 (br s, 1H, H-17b), 4.10 (d, J = 14.6 Hz, 1H, H-18a), 4.06 (d, J = 14.0 Hz, 1H, H-18b), 5.05 (br s, 1H, H-19a), 4.90 (br s, 1H, H-19b), 1.19 (s, 3H, H-20); ^{13}C NMR ($CDCl_3$, 100 MHz), δ 52.3 (d, C-1), 67.8 (d, C-2), 125.7 (d, C-3), 144.2 (s, C-4), 26.7 (t, C-5), 26.8 (t, C-6), 84.5 (d, C-7), 145.2 (s, C-8), 29.7 (t, C-9), 31.3 (t, C-10), 80.1 (d, C-11), 84.7 (s, C-12), 36.4 (t, C-13), 29.8 (t, C-14), 145.2 (s, C-15), 22.1 (q, C-16), 114.0 (t, C-17), 66.8 (t, C-18), 115.4 (t, C-19), 20.4 (q, C-20); HRESIMS m/z [M+Na]$^+$ 359.2189 (Calcd for $C_{20}H_{32}O_4Na$, 359.2193).

4.3.12. Reaction of asperdiol (2) with MeOTf

Compound **2** (60 mg, 0.19 mmol) was dissolved in dry CH_2Cl_2 (1 mL), and triethylamine (320 μL) and methyl triflate (150 μL) were added. The reaction mixture was stirred at room temperature overnight and was partitioned against saturated $NaHCO_3$ solution. The organic phase was dried over Na_2SO_4 and purified by HPLC-RP (MeOH-H_2O 70:30 v/v) to afford pure O-methyl asperdiol **34** (35.0 mg, 0.10 mmol, 52.6%).

4.3.12.1. (+)-(1*R*,2*S*,3*E*,11*E*)-7-Ketocembra-18-metoxy-2-ol 34. Colorless oil; $[\alpha]_D^{25}$ +8.3 (c 0.15, $CHCl_3$); IR (CH_2Cl_2) ν_{max} 3069, 1739, 1232, 736 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz), δ 2.06 (m, 1H, H-1), 2.94 (dt, J = 13.2, 4.9 Hz, 1H, H-2), 4.97 (d, J = 11.3 Hz, 1H, H-3), 2.22 (m, 1H, H-5a), 1.99 (m, 1H, H-5b), 2.13 (m, 1H, H-6a), 1.94 (m, 1H, H-6b), 2.11 (m, 1H, H-8), 2.43 (dt, J = 13.2, 5.4 Hz, 1H, H-9a), 1.69 (m, 1H, H-9b), 1.98 (m, 2H, H-10), 3.26 (m, 1H, H-11), 2.18 (m, 1H, H-13a), 1.98 (m, 1H, H-13b), 2.68 (ddd, J = 13.1, 5.1, 3.3 Hz, 1H, H-14a), 1.70 (m, 1H, H-14b), 1.62 (s, 3H, H-16), 4.63 (m, 2H, H-17), 4.23 (d, J = 12.3 Hz, 1H, H-18a), 3.53 (d, J = 12.4 Hz, 1H, H-18b), 1.01 (d, J = 7.6 Hz, 3H, H-19), 1.62 (s, 3H, H-20), 3.16 (s, 3H, H-21); ^{13}C NMR ($CDCl_3$, 100 MHz), δ 44.1 (d, C-1), 49.4 (d, C-2), 134.0 (d, C-3), 131.8 (s, C-4), 33.3 (t, C-5), 38.9 (t, C-6), 216.0 (s, C-7), 40.8 (d, C-8), 32.6 (t, C-9), 28.6 (t, C-10), 134.0 (d, C-11), 128.6 (s, C-12), 26.7 (t, C-13), 22.8 (t, C-14), 149.0 (s, C-15), 19.5 (q, C-16), 110.5 (t, C-17), 75.0 (t, C-18), 19.5 (q, C-19), 19.5 (q, C-20), 56.7 (q, C-21); HRESIMS m/z 339.2285 [M-H_2O+Na]$^+$ (calcd for $C_{21}H_{32}O_2Na$, 339.2300).

4.3.13. Reaction of 8*S*-plexaurolone (4), 8*S*-dihydroplexaurolone (5), and 8*R*-dihydroplexaurolone (6) with acetic anhydride

8*S*-plexaurolone (**4**) (100 mg, 0.31 mmol), 8*S*-dihydroplexaurolone (**5**) (100 mg, 0.31 mmol), and 8*R*-dihydroplexaurolone (**6**) (20 mg, 0.06 mmol) were each treated with acetic anhydride (1.0 mL) in dry pyridine (2.0 mL for **4** and **5** and 0.5 mL for **6**) for 8 h under stirring at room temperature. The products were purified by column chromatography (hexane/EtOAc 90:10 v/v) to obtain the acetylated compound **35** (110 mg, 0.30 mmol, 96.8%) and the diacetylated compounds **36** (126 mg, 0.31 mmol, 97.6%) and **37** (24 mg, 0.06 mmol, 98%).

4.3.13.1. (−)-1*R*,3*R*,4*R*,8*S*,12*R*-3-Acetoxy-plexaurolone 35. White crystals; $[\alpha]_D^{25}$ −7.7 (c 0.37, $CHCl_3$); IR (KBr), ν_{max} 3425, 1710, 1636, 891 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz), δ 1.99 (m, 1H, H-1), 1.55 (m, 1H, H-2a), 1.39 (m, 1H, H-2a), 4.62 (ddd, J = 8.6, 6.3, 2.3 Hz, 1H, H-3), 2.12 (m, 1H, H-4), 2.50 (m, 1H, H-5a), 2.38 (m, 1H, H-5b), 2.50 (m, 1H, H-7a), 2.04 (m, 1H, H-7b), 2.15 (m, 1H, H-8), 1.58 (m, 1H, H-9a), 1.24 (m, 1H, H-9b), 2.42 (m, 1H, H-10a), 2.09 (m, 1H, H-10b), 2.45 (m, 1H, H-12), 1.48 (m, 1H, H-13a), 1.41 (m, 1H, H-13b), 1.65 (m, 1H, H-14a), 1.13 (m, 1H, H-14b), 1.59 (s, 3H, H-16), 4.67 (br s, 1H, H-17a), 4.62 (br s, 1H, H-17b), 0.91 (d, J = 6.8 Hz, 3H, H-18), 0.96 (d, J = 6.7 Hz, 3H, H-19), 1.00 (d, J = 6.9 Hz, 3H, H-20), 2.00 (s, 3H, H-22); ^{13}C NMR ($CDCl_3$, 100 MHz), δ 44.3 (d, C-1), 37.8 (t, C-2), 75.7 (d, C-3), 37.5 (d, C-4), 47.6 (t, C-5), 210.4 (s, C-6), 48.4 (t, C-7), 29.0 (d, C-8), 29.5 (t, C-9), 35.3 (t, C-10), 215.0 (s, C-11), 47.1 (d, C-12), 31.8 (t, C-13), 30.4 (t, C-14), 147.5 (s, C-15), 20.8 (q, C-16), 110.5 (t, C-17), 13.9 (q, C-18), 19.6 (q, C-19), 16.8 (q, C-20), 170.9 (s, C-21), 20.9 (q, C-22); HRESIMS m/z 387.2508 [M+Na]$^+$ (Calcd for $C_{22}H_{36}O_4Na$, 387.2506).

The NMR data signals for compound **35** at δ_C 170.9, s, for C-21 and δ_C 20.9, q, δ_H 2.00 (s, 3H) for C-22 corroborated the monoacetylation.

4.3.13.2. (+)-1*R*,3*R*,4*R*,8*S*,11*R*,12*R*-3,11-Diacetoxy-dihydroplexaurolone 36. White crystals; $[\alpha]_D^{25}$ +19.8 (c 0.12, $CHCl_3$); IR (KBr), ν_{max} 3425, 1710, 1636, 891 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz), δ 1.89 (m, 1H, H-1), 1.64 (m, 1H, H-2a), 1.40 (m, 1H, H-2a), 4.67 (dd, J = 8.7, 4.1 Hz, 1H, H-3), 1.62 (m, 1H, H-4), 1.89 (m, 1H, H-5a), 1.45 (m, 1H, H-5b), 2.50 (m, 1H, H-7a), 2.23 (m, 1H, H-7b), 1.90 (m, 1H, H-8), 1.45 (m, 1H, H-9a), 1.18 (m, 1H, H-9b), 1.71 (m, 1H, H-10a), 1.46 (m, 1H, H-10b), 4.73 (dd, J = 9.8,

4.8 Hz, 1H, H-11), 2.32 (m, 1H, H-12), 2.63 (m, 1H, H-13a), 2.14 (m, 1H, H-13b), 1.71 (m, 1H, H-14a), 1.45 (m, 1H, H-14b), 1.64 (s, 3H, H-16), 4.63 (br s, 1H, H-17a), 4.58 (br s, 1H, H-17b), 0.80 (d, J = 6.5 Hz, 3H, H-18), 1.00 (d, J = 6.7 Hz, 3H, H-19), 0.88 (d, J = 7.1 Hz, 3H, H-20), 2.01 (s, 3H, H-22), 1.99 (s, 3H, H-24); ^{13}C NMR ($CDCl_3$, 100 MHz), δ 43.6 (d, C-1), 36.0 (t, C-2), 76.0 (d, C-3), 35.3 (d, C-4), 49.7 (t, C-5), 209.7 (s, C-6), 47.3 (t, C-7), 33.7 (d, C-8), 30.3 (t, C-9), 29.2 (t, C-10), 77.2 (d, C-11), 29.9 (d, C-12), 28.7 (t, C-13), 28.2 (t, C-14), 148.8 (s, C-15), 21.1 (q, C-16), 109.4 (t, C-17), 15.6 (q, C-18), 20.2 (q, C-19), 16.1 (q, C-20), 171.0 (s, C-21), 20.9 (q, C-22), 170.7 (s, C-23), 20.8 (q, C-24); HRESIMS m/z 431.2773 $[M+Na]^+$ (Calcd for $C_{24}H_{40}O_5Na$, 431.2768).

4.3.13.3. (−)-1*R*,3*R*,4*R*,8*R*,11*R*,12*R*-3,11-Diacetoxy-dihydroplexaurolone 37. White crystals; $[\alpha]_D^{25}$ −11.4 (c 0.58, $CHCl_3$); IR (KBr), ν_{max} 3425, 1710, 1636, 891 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz), δ 1.90 (m, 1H, H-1), 1.64 (m, 1H, H-2a), 1.40 (m, 1H, H-2a), 4.66 (dd, J = 8.7, 4.1 Hz, 1H, H-3), 1.62 (m, 1H, H-4), 1.86 (m, 1H, H-5a), 1.47 (m, 1H, H-5b), 2.44 (m, 1H, H-7a), 2.16 (m, 1H, H-7b), 1.97 (m, 1H, H-8), 1.45 (m, 1H, H-9a), 1.18 (m, 1H, H-9b), 1.71 (m, 1H, H-10a), 1.46 (m, 1H, H-10b), 4.72 (dd, J = 8.7, 5.1 Hz, 1H, H-11), 2.32 (m, 1H, H-12), 2.63 (m, 1H, H-13a), 2.14 (m, 1H, H-13b), 1.71 (m, 1H, H-14a), 1.45 (m, 1H, H-14b), 1.64 (s, 3H, H-16), 4.63 (br s, 1H, H-17a), 4.58 (br s, 1H, H-17b), 0.80 (d, J = 6.6 Hz, 3H, H-18), 1.00 (d, J = 6.7 Hz, 3H, H-19), 0.87 (d, J = 7.1 Hz, 3H, H-20), 2.00 (s, 3H, H-22), 1.98 (s, 3H, H-24); ^{13}C NMR ($CDCl_3$, 100 MHz), δ 43.7 (d, C-1), 36.1 (t, C-2), 76.0 (d, C-3), 35.4 (d, C-4), 49.8 (t, C-5), 209.7 (s, C-6), 47.4 (t, C-7), 33.8 (d, C-8), 30.4 (t, C-9), 29.3 (t, C-10), 77.2 (d, C-11), 30.0 (d, C-12), 28.8 (t, C-13), 28.3 (t, C-14), 148.8 (s, C-15), 21.2 (q, C-16), 109.4 (t, C-17), 15.7 (q, C-18), 20.2 (q, C-19), 16.1 (q, C-20), 171.0 (s, C-21), 21.0 (q, C-22), 170.7 (s, C-23), 20.9 (q, C-22); HRESIMS m/z 431.2768 $[M+Na]^+$ (Calcd for $C_{24}H_{40}O_5Na$, 431.2768).

4.3.14. Reaction of 8*S*-plexaurolone (4) with acidified CH_2Cl_2

Compound **4** (50 mg, 0.15 mmol) was dissolved in acidified CH_2Cl_2 (3 mL of CH_2Cl_2 with 100 μL of acetic acid), stirred vigorously at room temperature for 2 h, treated with saturated $NaHCO_3$, and extracted with $CHCl_3$ (3 × 5 mL). The combined organic layers were dried over Na_2SO_4 and concentrated to leave a residue that was purified on silica gel (hexane/AcOEt 90:10 v/v) to obtain compound **38** (44 mg, 0.14 mmol, 93.3%) as a colorless oil.

4.3.14.1. (−)-1*R*,3*R*,4*R*,8*S*,12*R*-3,6-Oxa-cembra-15(17)-en-11-ceto-6-ol 38. White crystals; $[\alpha]_D^{25}$ −6.6 (c 0.23, $CHCl_3$); IR (KBr), ν_{max} 3425, 1710, 1636, 891 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz), δ 2.16 (m, 1H, H-1), 2.12 (m, 1H, H-2a), 1.22 (m, 1H, H-2a), 3.68 (dd, J = 14.1, 7.9 Hz, 1H, H-3), 2.02 (m, 1H, H-4), 2.04 (m, 1H, H-5a), 1.79 (m, 1H, H-5b), 1.65 (m, 1H, H-7a), 1.40 (m, 1H, H-7b), 1.65 (m, 1H, H-8), 1.63 (m, 1H, H-9a), 1.45 (m, 1H, H-9b), 2.45 (m, 1H, H-10a), 2.35 (m, 1H, H-10b), 2.48 (m, 1H, H-12), 1.50 (m, 1H, H-13a), 1.26 (m, 1H, H-13b), 1.47 (m, 1H, H-14a), 1.16 (m, 1H, H-14b), 1.63 (s, 3H, H-16), 4.73 (br s, 1H, H-17a), 4.66 (br s, 1H, H-17b), 0.94 (d, J = 6.8 Hz, 3H, H-18), 0.98 (d, J = 6.8 Hz, 3H, H-19), 1.01 (d, J = 6.8 Hz, 3H, H-20); ^{13}C NMR ($CDCl_3$, 100 MHz), δ 44.1 (d, C-1), 32.1 (t, C-2), 75.7 (d, C-3), 43.7 (d, C-4), 47.6 (t, C-5), 110.8 (s, C-6), 49.3 (t, C-7), 35.4 (d, C-8), 34.4 (t, C-9), 37.5 (t, C-10), 208.8 (s, C-11), 48.1 (d, C-12), 31.4 (t, C-13), 30.5 (t, C-14), 146.1 (s, C-15), 21.0 (q, C-16), 112.0 (t, C-17), 18.1 (q, C-18), 19.7 (q, C-19), 16.2 (q, C-20); HRESIMS m/z 345.2396 $[M+Na]^+$ (Calcd for $C_{20}H_{34}O_3Na$, 345.2400).

4.3.15. Reaction of 8*S*-dihydroplexaurolone (5) with acidified CH_2Cl_2

Compound **5** (50 mg, 0.15 mmol) was treated as described for **4**. After 2 h, the reaction mixture was dried and purified on a silica gel column, eluting with hexane/EtOAc (90:10 v/v) to yield **39** (42 mg, 0.13 mmol, 86.6%) as a colorless oil.

4.3.15.1. (+)-1*R*,3*R*,4*R*,8*S*,11*R*,12*R*-3,6-Oxa-cembra-15(17)-en-6,11-diol 39. White crystals; $[\alpha]_D^{25}$ +49.0 (c 0.25, $CHCl_3$); IR, NMR, and MS data are consistent with literature values.[17]

4.3.16. Reaction of 8*R*-dihydroplexaurolone (6) with acidified CH_2Cl_2

Compound **6** (20 mg, 0.06 mmol) was treated as described for **4**. After 2 h, the reaction mixture was dried and purified on a silica gel column, eluting with hexane/EtOAc (90:10 v/v) to yield **40** (15 mg, 0.04 mmol, 74.5%).

4.3.16.1. (+)-1*R*,3*R*,4*R*,8*R*,11*R*,12*R*-3,6-Oxa-cembra-15(17)-en-6,11-diol 40. White crystals; $[\alpha]_D^{25}$ +49.0 (c 0.25, $CHCl_3$); IR (KBr), ν_{max} 3428, 1687, 1648, 887 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz), δ 2.04 (m, 1H, H-1), 2.10 (m, 1H, H-2a), 1.25 (m, 1H, H-2a), 3.79 (ddd, J = 12.4, 9.6, 2.9 Hz, 1H, H-3), 2.27 (m, 1H, H-4), 2.04 (m, 1H, H-5a), 1.70 (m, 1H, H-5b), 1.75 (m, 1H, H-7a), 1.63 (m, 1H, H-7b), 1.54 (m, 1H, H-8), 1.78 (m, 1H, H-9a), 1.10 (m, 1H, H-9b), 1.53 (m, 1H, H-10a), 1.40 (m, 1H, H-10b), 4.03 (dd, J = 10.4, 4.1 Hz, 1H, H-11), 1.56 (m, 1H, H-12), 1.51 (m, 1H, H-13a), 1.33 (m, 1H, H-13b), 2.38 (m, 1H, H-14a), 1.35 (m, 1H, H-14b), 1.73 (s, 3H, H-16), 4.86 (br s, 1H, H-17a), 4.81 (br s, 1H, H-17b), 0.92 (d, J = 6.8 Hz, 3H, H-18), 0.86 (d, J = 6.8 Hz, 3H, H-19), 0.82 (d, J = 6.8 Hz, 3H, H-20); ^{13}C NMR ($CDCl_3$, 100 MHz), δ 43.4 (d, C-1), 24.4 (t, C-2), 79.0 (d, C-3), 37.9 (d, C-4), 47.6 (t, C-5), 109.5 (s, C-6), 51.1 (t, C-7), 36.7 (d, C-8), 39.2 (t, C-9), 33.8 (t, C-10), 70.4 (d, C-11), 29.1 (d, C-12), 31.1 (t, C-13), 24.1 (t, C-14), 148.5 (s, C-15), 23.2 (q, C-16), 110.4 (t, C-17), 13.5 (q, C-18), 24.4 (q, C-19), 15.5 (q, C-20); HRESIMS m/z 347.2546 $[M+Na]^+$ (Calcd for $C_{20}H_{36}O_3Na$, 347.2562).

4.4. QSI activity assay

A standard disk-diffusion assay was used to evaluate the QSI activity of the cembranoid compounds following the parameters of the NCCLS[21] using the biosensor strain *Chromobacterium violaceum* (ATCC 31532) grown in trypticase soy agar when required. Whatman filter paper disks (5.2 mm diameter) were initially sterilized at 10×10^4 Pa pressure for 15 min. The disks were then loaded with 30.0, 15.0, 7.5, 5.0, and 2.5 μg of every compound (**7**–**40**) predissolved in MeOH and allowed to dry at room temperature for 1 h. The disks were placed on agar dishes plated with 100 μL of *C. violaceum* culture grown in trypticase soy broth (10^6 cfu/mL, 0.5 Mac Farland) and finally, the agar plates were incubated for 48 h at 26 °C. This QSI assay is based on inhibition of QS pigment production (violet color) when the QS system is interrupted in *C. violaceum* and must take place without interfering with bacterial growth. Thus, the QSI activity was established by the appearance of a colorless, opaque but viable halo and was evaluated as the minimum quantity in μg per disk of compound required to inhibit violacein pigment and detected by the inhibition zones around the disks.[16,22] Kojic acid was used as a positive control, as it is a known inhibitor of quorum sensing systems.[1] The antifouling agent Cu_2O was also evaluated as a positive control.

Acknowledgements

This work was financially supported by grants from Colciencias. We thank Colciencias for a doctoral fellowship to support E.T. The authors deeply thank Professor Dr. S. Zea and Professor Dr. M. Puyana for the collection and morphological identification of the animal samples used in this study. The Ministerio de Ambiente,

Vivienda y Desarrollo Territorial granted permission (permission No. 4 of 10/02/2010, addendum resolution 0306 of 22/02/2011) for collecting samples and performing research on marine organisms collected in Santa Marta Bay.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmc.2012.10.022.

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