Enantiospecific synthesis of antifungal dasyscyphin E from cupressic acid

Antonio Fernández [a], Ettahir Boulifa [b], Fermín Jiménez [a], Soumicha Mahdjour [a], Ahmed I. Mansour [b], Rachid Chahboun [a, *], Enrique Alvarez-Manzaneda [a, **]

[a] *Departamento de Química Orgánica, Facultad de Ciencias, Instituto de Biotecnología, Universidad de Granada, 18071 Granada, Spain*
[b] *Laboratoire de Chimie Organique Appliquée, Département de Chimie, Faculté des Sciences, Université Abdelmalek Essaâdi, 93000 Tetouan, Morocco*

A B S T R A C T

The first synthesis of the sesquiterpene quinol dasyscyphin E has been achieved starting from cupressicacid. Key steps of the synthetic sequence are the oxidative degradation of the diterpene side chain to givea methylketone, the diastereoselective α-methylation of a protected ketoaldehyde, the subsequent intramolecular aldol condensation and the further Diels-Alder cycloaddition of a dienol ester.

1. Introduction

Merosesquiterpenes, natural products of mixed biosynthetic origin containing a sesquiterpene unit joined to a phenolic or quinone moiety and bearing a bicyclic terpene fragment, are the most important compounds in this family of metabolites, with several interesting properties.[1] In the present century, a series of this type of compounds, with a tetracyclic 6/6/5/6 skeleton possessing significant biological activity, have been reported. Within this group of compounds two types of structures can be distinguished: those bearing a *trans* B/C union and those with a *cis* B/C junction. The first group of metabolites includes pelorol (1), which is isolated from the marine sponge *Dactylospongia* elegans[2] and exhibits the activating activity of the inositol 5-phosphatase (SHIP),[3] walsucochins A (2) and B (3), with significant protecting activity against H_2O_2-induced PC12 cell damage,[4] and walsucochinoids A (4) and B (5), which present remarkable neuroprotective activity.[5] Merosesquiterpenes with a *cis* B/C union include akaol A (6),[6] the cytotoxic dasyscyphin B (7),[7] the antileishmanial and antiviral dasyscyphin C (8),[7,8] and the antifungal dasyscyphins D (9) and E (10)[9] (Fig. 1).

Despite the interest of this type of metabolites, only a few syntheses can be found in the literature, probably due to the difficulty of obtaining them, and in particular terpenoids with a *cis* B/C junction, such as compounds 6-10.

The first synthesis of this type of compounds was that of pelorol (1), a *trans* B/C fused metabolite, reported by Andersen et al.[10] These authors utilized as the key intermediate an aryldrimanol[11] prepared after the condensation of an aryllithium with a drimane hydroxy aldehyde, derived from (-)-sclareolide. The construction of the cyclopentane C ring was achieved after a difficult intramolecular Friedel-Crafts alkylation. More recently a new strategy for synthesizing this type of compounds, including pelorol (1) has been reported.[12] She et al.[13] reported a total synthesis of dasyscyphin D (9), after successive acid-catalyzed Robinson annulations of an indanone obtained after a $PtCl_2$-catalyzed pentannulation. More recently, these authors described an enantioselective total synthesis of (–)-walsucochin B (5), through a cationic polyolefin cyclization initiated by chiral epoxide.[14] Very recently an interesting study concerning the biosynthesis and the chemoenzymatic preparation of this type of compounds has been published.[15]

* Corresponding author.
** Corresponding author.
E-mail addresses: rachid@ugr.es (R. Chahboun), eamr@ugr.es (E. Alvarez-Manzaneda).

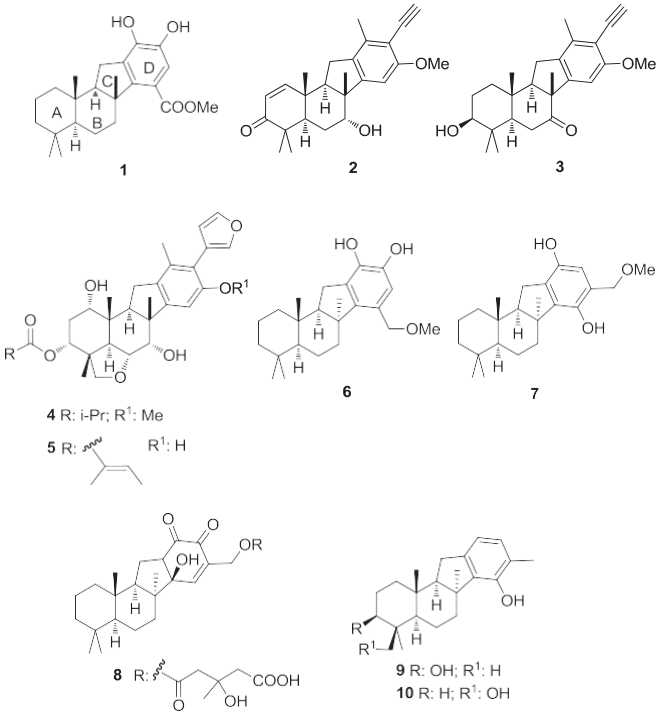


Fig. 1. Representative merosesquiterpenes with a 6/6/5/6 tetracyclic skeleton.

2. Results and discussion

Continuing our research into the synthesis of biologically active merosesquiterpenes, we examined the synthesis of sesquiterpene quinols such as compounds 6-10. Achieving this goal requires two major obstacles to be addressed: first, the construction of the cyclopentane C ring, and then obtaining the proper configuration at C-8. As observed above, the synthesis of merosesquiterpenes by means of an aryl drimane prepared by the two-synthon strategy, has been utilized extensively because in most cases this approach makes it possible to access the target compounds utilizing short synthetic sequences. Construction of the required tetracyclic skeleton usually involves electrophilic cyclization processes. When the C ring is a five-membered carbocyclic, the intramolecular Friedel-Crafts alkylation entails some difficulty, as noted by Andersen with respect to the synthesis of pelorol (1), which required a sufficiently activated aromatic moiety.[10] Similar problems were found during our initial attempts to synthesize the 6/5/6 taiwaniaquinoid skeleton *via* the electrophilic cyclization of arylcyclogeranyl derivatives; however, this problem was overcome by employing a highly reactive aryl allyl cation, which underwent fast cyclization even when non-activated aromatic substrates were used.[16] With respect to the second problem, that of achieving the appropriate configuration at C-8, probably more difficult than the previous one, the results reported by Andersen, and those of our own preliminary studies, suggested that electrophilic cyclization processes would inexorably lead to the obtention of compounds with a *R* configuration at C-8, as in the case of pelorol (1).

After successful synthesis of the construction of the 6/5/6 skeleton (fluorene skeleton) of taiwaniaquinoids, *via* the acid cyclization of a monoterpene aryldiene, we then considered extrapolating this strategy to synthesize the 6/6/5/6 skeleton (benzofluorene skeleton) of sesquiterpene quinols, such as compounds 6–10, through the electrophilic cyclization of a sesquiterpene aryl diene; however, as was to be expected, this reaction afforded a mixture of 8-epimers, in which the undesired 8*R* isomer was the major constituent.[17] Recently, Bisai et al. reported similar results in the synthesis of akaol A (6). These authors obtained, in the best conditions, a 1:1 mixture of 8-epimers, which after separation enabled the desired compound to be achieved.[18]

The undesired diastereoselectivity of the intramolecular Friedel-Crafts alkylation of aryl dienes led us to discard this strategy for synthesizing merosesquiterpene quinols such as compounds 6–10, and prompted us to address the synthesis of akaol A (6)[19] and dasyscyphin B (8),[20] utilizing as the key intermediate a tricyclic compound with the proper configuration at C-8.

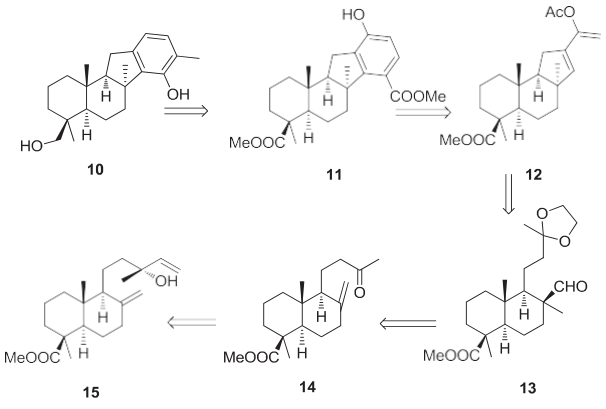
Continuing these studies, we address the synthesis of dasyscyphin E (10), not yet described. Scheme 1 shows the planned retrosynthesis starting from the methyl ester of cupressic acid (15).[21]

The aromatic ring of precursor 11 is formed after the Diels-Alder cycloaddition of dienol ester 12 with methyl propiolate. The cyclopentane C ring is obtained *via* an intramolecular aldol condensation of a ketoaldehyde. The suitable configuration at C-8 is attained through the diastereoselective α-methylation of a suitably protected ketoaldehyde. This, in turn, is obtained after the oxidative hydroboration of methylketone 14, easily derived from ester 15.

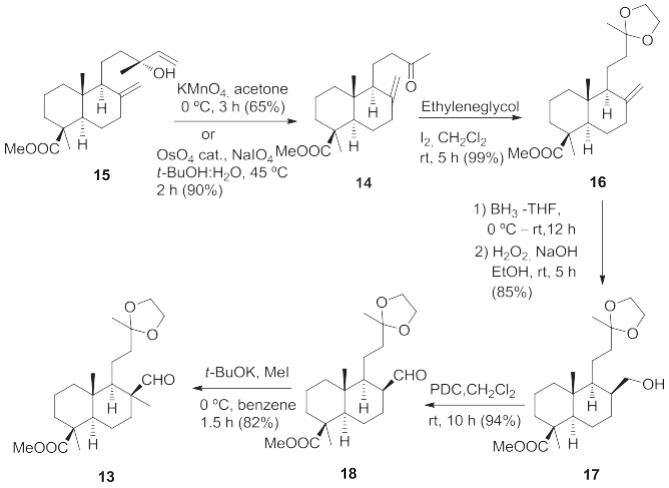
Scheme 2 shows the transformation of methyl ester of cupressic acid (15) into aldehyde 13 containing the C_8-Me$_\alpha$ of the target compound, as it was confirmed by nOe experiments. Treatment of ester 15 with $KMnO_4$ in acetone at 0 °C provoked side chain oxidative degradation and afforded methylketone 14.[22] Oxidation with $NaIO_4$ and catalytic OsO_4 gave the same ketone in higher yield. Oxidative hydroboration of ethyleneketal 16 gave alcohol 17, which was then converted into aldehyde 18. Methylation of its enolate took place with high stereoselectivity affording the corresponding α-methyl aldehyde 13 in high yield.

Next, the construction of the tetracyclic carbon skeleton of target compounds was undertaken (Scheme 3). Refluxing aldehyde 13 with 1 M HCl in THF gave the tricyclic α,β–enone 19, resulting from the intramolecular aldol condensation of the corresponding ketoaldehyde. The treatment of ketone 19 with catalytic *p*-toluenesulfonic acid in isopropenyl acetate under reflus afforded dienol acetate 12, which after heating with methyl propiolate in xylene at 180 °C for 4 days gave a mixture of epimer cycloadducts 20 and 21, which were separated after column chromatography. The 1H NMR spectra of these showed significative differences (Figs. 2 and 3). Thus, the Me-18 of compound 21 is deshielded by the C_{16}-COOMe group. The α disposition of the C_{17}-H for this epimer was confirmed in the 1D noesy spectrum of this diastereoisomer (Fig. 4).

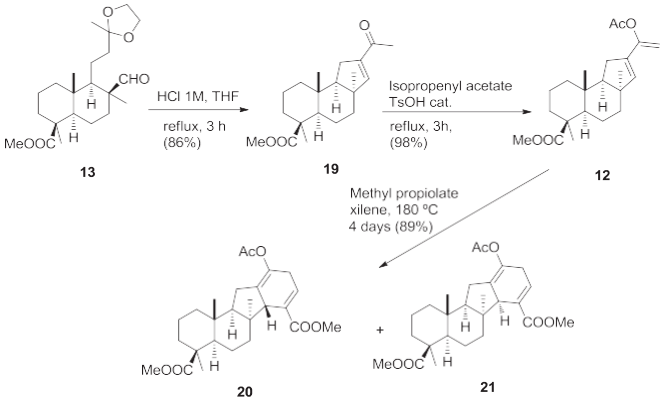
Then, the aromatization and functionalization of the D ring was tackled. Scheme 4 shows the transformation of cycloadducts 20-21



Scheme 1. Retrosynthesis of dasyscyphin E (10).



Scheme 2. Synthesis of aldehyde 13 from ester 15.



Scheme 3. Construction of the tetracyclic carbon skeleton of target compound.

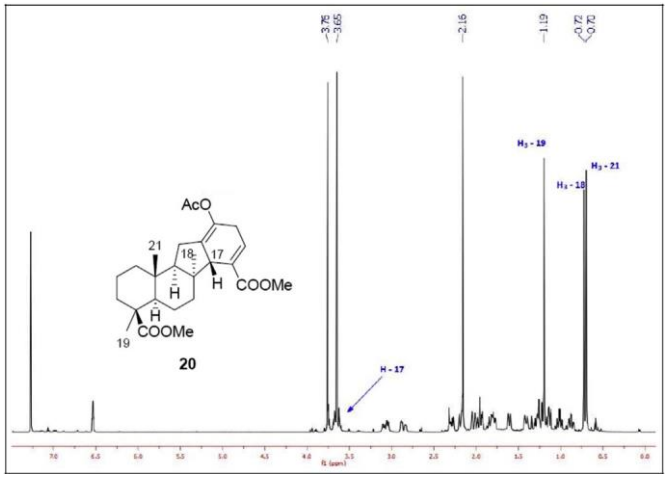


Fig. 2. ^{1}H NMR spectrum of cycloadduct 20.

into diol 25. When the mixture of epimers 20e21 was refluxed with DDQ in dioxane for 3 h, acetoxyester 22 was obtained, which after refluxing with concentrated HCl in methanol gave phenol 11. The treatment of triflate 23 with Raney nickel led to diester 24, which was finally reduced to diol 25.

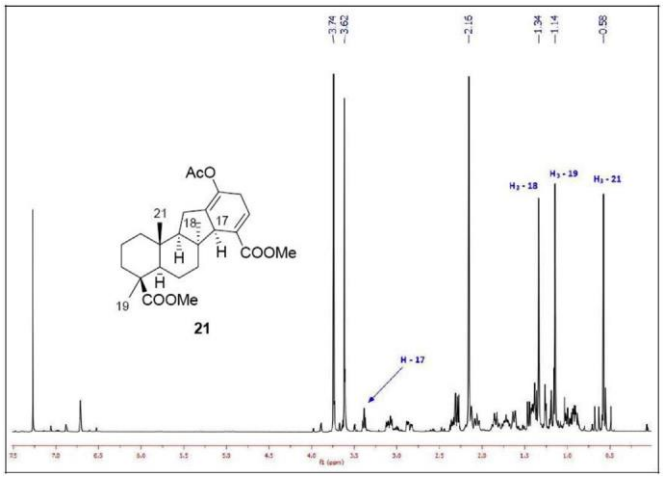


Fig. 3. ^{1}H NMR spectrum of cycloadduct 21.

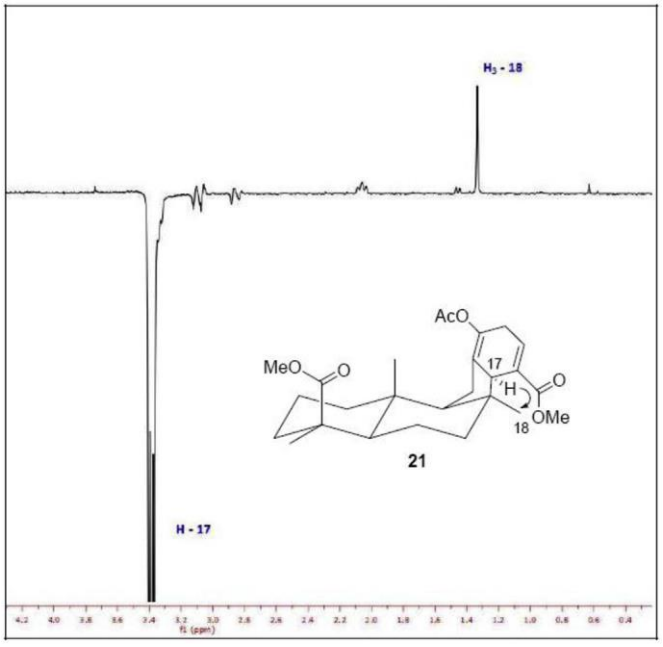
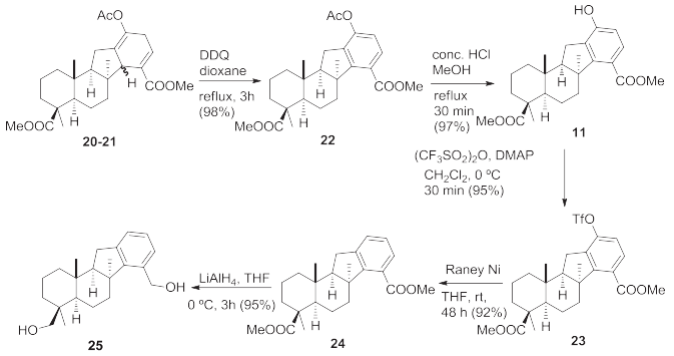
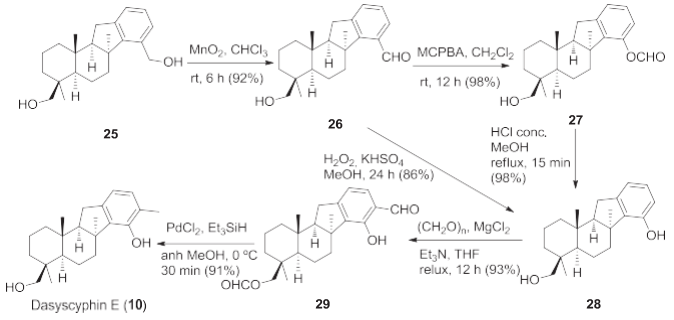


Fig. 4. 1D NOESY spectrum of cycloadduct 21.

Finally, diol 25 was converted into dasyscyphin E (10) (Scheme 5). Oxidation of compound 25 with MnO_2 gave hydroxyaldehyde 26, which after treating with MCPBA in dichloromethane at room temperature for 12 h gave formate 27 in high yield. The acid hydrolysis of this afforded phenol 28 in almost quantitative yield. This phenol was also obtained directly from aldehyde 26, by treating it with H_2O_2 and $KHSO_4$ in MeOH. Treatment of phenol 28 with $(CH_2O)_n$ and $MgCl_2$ in Et_3N and THF under reflux allowed the expected introduction of formyl group in the aromatic ring and the simultaneous formation of formate, affording compound 29, which was finally transformed into dasyscyphin E (10) by treating it with $PdCl_2$ and Et_3SiH. The optical rotation of synthetic dasyscyphin E (10) ($[\alpha]_D^{25}$: +2.1; c 0.5, $CHCl_3$) was similar to that reported for the natural product ($[\alpha]_D^{25}$: +1.07; c 0.33, $CHCl_3$); its spectroscopic properties closely matched with those of the natural compound (Table 1).[9] The sign of the optical rotation of the synthetic compound was confirmed after repeated measurements. This fact,



Scheme 4. Synthesis of diol 25 from adducts 20e21.



Scheme 5. Synthesis of dasyscyphin E (10) from diol 25.

together with the absolute stereochemistry, well established for other previously synthesized dasyscyphins and related compounds, confirm the absolute stereochemistry of the natural product.

3. Conclusion

In summary, we report the first synthesis of antifungal dasyscyphin E (10) starting from the methyl ester of cupressic acid (15). The physical properties of the synthetic product were similar to those reported for the natural compound, allowing to establish the absolute stereochemistry.

4. Experimental section

4.1. General information

Unless stated otherwise, reactions were performed in oven-dried glassware under an argon atmosphere using dry solvents. Solvents were dried as follows: Tetrahydrofuran (THF) and benzene over Naebenzophenone; dichloromethane (DCM) and methanol (MeOH) over CaH_2. Thin-layer chromatography (TLC) was performed using F254 precoated plates (0.25 mm) and visualized by UV fluorescence quenching and phosphomolybdic acid solution in ethanol staining. Flash chromatography was performed on silica gel (230e400 mesh). Chromatography separations were carried out by conventional column on silica gel 60 (230e400 Mesh), using Hexanes-*tert*-Butyl methyl ether (ether) mixtures of increasing polarity. ^1H and ^{13}C NMR spectra were recorded at 500, 400 MHz and 125, 100 MHz, respectively. $CDCl_3$ was treated with K_2CO_3. Chemical shifts (d H) are quoted in parts per million (ppm) referenced to the appropriate residual solvent peak and tetramethylsilane. Data for ^1H NMR spectra are reported as follows: chemical shift (d ppm) (multiplicity, coupling constant (Hz), integration), with the abbreviations s, br s, d, br d, t, q, and m denoting singlet, broad singlet, doublet, broad doublet, triplet, quartet and multiplet respectively. $J =$ coupling constant in Hertz (Hz). Data for ^{13}C NMR spectra are reported in terms of chemical shift relative to Me_4Si (d 0.0) and the signals were assigned utilizing DEPT experiments and on the basis of heteronuclear correlations. Infrared spectra (IR) were recorded as thin films or as solids on a FTIR spectrophotometer with samples between sodium chloride plates or as potassium bromide pellets and are reported in frequency of absorption (cm^{-1}). Only selected absorbances (n_{max}) are reported. ([a]^D) measurements were carried out in a polarimeter; utilizing a 1 dm length cell and $CHCl_3$ as a solvent. Concentration is expressed in mg/mL. HRMS were recorded on a spectrometer, using FAB with thioglicerol or glycerol matrix doped in NaI 1%, with a trisector EBE analyzer, or ApcI, or ESþ, with a TOF analyzer, as indicated.

Table 1
1H (400 MHz) and 13C NMR (100 MHz) data in CDCl3 for the synthetic and natural dasyscyphin E (10).

Synthetic dasyscyphin E		Natural dasyscyphin E (ref. 9)	
6.86 (1H, d, J = 7.4 Hz)	150.3 (C)	6.86 (1H, d, J = 7.5 Hz)	150.3 (C)
6.61 (1H, d, J = 7.4 Hz)	144.1 (C)	6.62 (1H, d, J = 7.5 Hz)	144.1 (C)
4.53 (1H, br s)	135.7 (C)	4.50 (1H, s)	135.6 (C)
3.73 (1H, d, J = 10.9 Hz)	129.1 (CH)	3.73 (1H, br d, J = 10.9 Hz)	129.1 (CH)
3.43 (1H, d, J = 10.9 Hz)	120.9 (C)	3.42 (1H, d, J = 10.9 Hz)	120.8 (C)
3.00 (1H, dd, J = 16.5, 7.7 Hz)	116.5 (CH)	2.99 (1H, dd, J = 16.5, 7.8 Hz)	116.5 (CH)
2.79 (1H, m)	65.4 (CH_2)	2.79 (1H, m)	65.3 (CH_2)
2.64 (1H, d, J = 16.6 Hz)	62.6 (CH)	2.63 (1H, d, J = 16.5 Hz)	62.5 (CH)
2.19 (3H, s)	53.4 (CH)	2.20 (3H, s)	53.4 (CH)
1.83 (1H, br d, J = 13.6 Hz)	47.8 (C)	1.83 (1H, ddd, J = 13.7, 4.7, 3.1 Hz)	47.7 (C)
1.77e1.55 (m, 4H)	41.5 (CH_2)	1.76e1.55 (m, 4H)	41.4 (CH_2)
1.48 (1H, br d, J = 13.6 Hz)	38.6 (C)	1.48 (1H, m)	38.6 (C)
1.45e1.25 (2H, m)	37.3 (C)	1.41 (1H, m), 1.34 (1H, m)	37.3 (C)
1.23 (3H, s)	35.8 (CH_2)	1.23 (3H, s)	35.7 (CH_2)
1.16 (1H, dd, J = 11.3, 3.7 Hz)	34.8 (CH_2)	1.15 (1H, dd, J = 11.6, 3.7 Hz)	34.8 (CH_2)
1.00 (3H, s)	32.5 (CH_2)	0.99 (3H, br s)	32.5 (CH_2)
0.99e0.83 (3H, m)	31.0 (CH_3)	1.04 (1H, br s), 0.95 (1H, m), 0.93 (1H, m)	31.0 (CH_3)
0.46 (3H, s)	26.7 (CH_3)	0.45 (3H, s)	26.7 (CH_3)
	19.7 (CH_2)		19.7 (CH_2)
	18.3 (CH_2)		18.3 (CH_2)
	16.7 (CH_3)		16.7 (CH_3)
	15.5 (CH_3)		15.5 (CH_3)

4.1. Obtention of cupressic acid methyl ester (15)

1 Kg of cypress fruits, dried and crushed, were extracted with hexane (6 L) in a soxhlet apparatus for 12 h. After evaporating to a fifth of the volume, the resulting solution was allowed to stand for 12 h at –18 °C, and then filtered. Evaporation of the filtrate gave 40.8 g of a solid residue. This was dissolved in diethyl ether (250 mL) and subjected to esterification by passing a diazomethane stream. After the reaction was completed, the ether was evaporated to afford 42.2 g of a residue, which was subjected to silica gel column chromatography, yielding *trans*-communic acid methyl ester (18.6 g) (eluting with 5% AcOEt-hexanes) and cupressic acid methyl ester (15) (12.2 g) (eluting with 10% AcOEt-hexanes).

4.2. Synthetic procedures

4.2.1. (1S,4aR,5S,8aR)-Methyl 1,4a-dimethyl-6-methylene-5-(3-oxobutyl)decahydronaphthalene-1-carboxylate (14)

4.2.1.1. Treatment of compound 15 with $KMnO_4$. To a solution of 15 (10 g, 29.9 mmol) in acetone (250 mL), cooled at 0 °C, potassium permanganate (19 g, 119.6 mmol) was added in several portions for 30 min and the mixture was stirred at 0 °C for 3 h. $NaHSO_3$ (5 g) in water (20 mL) was added and the stirring was continued for 15 min. The mixture was filtered and washed with acetone (100 mL). The acetone was evaporated and the resulting crude was diluted with ether (100 mL), washed with water (2 × 30 mL) and brine (30 mL), dried over anhydrous Na_2SO_4 and evaporated to give a crude product, which was purified by flash chromatography on silica gel (20% ether/hexanes), affording 5.9 g of methylketone 14 (65%), as a colourless syrup. Compound 14 had identical spectroscopic properties to those reported in the literature.[20]

4.2.1.2. Treatment of compound 15 with OsO_4/$NaIO_4$. To a stirred solution of 15 (4.35 g, 13 mmol) in *tert*-butanol (60 mL) and water (11 mL) was added sodium periodate (13.91 g, 65 mmol) and then 0.2% aq. osmium tetroxide (16.4 mL, 0.13 mmol) was added over 5 min. The mixture was further stirred for 2 h at 45 °C. After filtering and removing the solvent, the residue was diluted in ether and evaporated to afford a crude product, which was purified by flash chromatography on silica gel (15% ether/hexanes), affording 3.58 g of 14 (90%), as a colourless syrup.

4.3.2. (1S,4aR,5S,8aR)-Methyl 1,4a-dimethyl-5-(2-(2-methyl-1,3-dioxolan-2-yl)ethyl)-6-methylene-decahydronaphthalene-1-carboxylate (16)

To a stirred solution of ketone 14 (1 g, 3.26 mmol) in dry CH_2Cl_2 (5 mL), was added successively ethylene glycol (5 mL) and iodine (812 mg, 0.98 mmol) and the mixture was stirred at room temperature under an argon atmosphere for 5 h. Then the reaction was worked up by the addition of 5% aqueous sodium bisulfite solution (10 mL), and it was extracted with ether (2 × 30 mL). The organic phase was washed with sat. $NaHCO_3$ solution (10 mL), brine (10 mL), dried over anhydrous Na_2SO_4, and the solvent was evaporated to give 1.13 g of pure 16 (99%) as a colourless syrup. $[\alpha]_D^{25}$ ¼ þ35.0 (c 0.15, $CHCl_3$). ^{1}H NMR (500 MHz, $CDCl_3$) d: 4.83 (s, 1H), 4.54 (s, 1H), 4.00e3.81 (m, 4H), 3.59 (s, 3H), 2.38 (ddd, *J* ¼ 12.2, 3.4, 3.4, 1H), 2.15 (dd, *J* ¼ 13.0, 1.5 Hz, 1H), 1.96 (ddd, *J* ¼ 9.9, 6.5, 2.8 Hz, 1H), 1.91e1.70 (m, 4H), 1.66e1.45 (m, 3H), 1.44e1.32 (m, 3H), 1.29 (s, 3H), 1.24 (m, 1H), 1.16 (s, 3H), 1.11e0.79 (m, 2H), 0.49 (s, 3H). ^{13}C NMR (125 MHz, $CDCl_3$) d: 177.9 (C), 148.1 (C), 110.5 (C), 106.7 (CH_2), 64.7 ($2CH_2$), 56.5 (CH), 56.3 (CH), 51.2 (CH_3), 44.4 (C), 40.4 (C), 39.3 (CH_2), 38.8 (CH_2), 38.4 (CH_2), 38.1 (CH_2), 28.9 (CH_3), 26.4 (CH_2), 23.9 (CH_3), 20.1 (CH_2), 18.2 (CH_2), 12.6 (CH_3). IR (film): 1724, 1449, 1377, 1226, 1153, 1064, 890, 756 cm^{-1}. HRMS (APcI) *m/z*: calcd for $C_{21}H_{34}O_4Na$ (M þ $Na^þ$) 373.2355, found: 373.2357.

4.3.3. (1S,4aR,5S,6S,8aR)-Methyl 6-(hydroxymethyl)-1,4a-dimethyl-5-(2-(2-methyl-1,3-dioxolan-2-yl)ethyl)-decahydronaphthalene-1-carboxylate (17)

1 M Borane tetrahydrofuran complex (4.0 mL, 4.0 mmol) was added to a stirred solution of 16 (1 g, 2.85 mmol) in dry THF (25 mL) cooled to 0 °C. After 1 h, the reaction was allowed to warm to room temperature and stirred for 12 h, at which time TLC showed no starting material. Then the reaction mixture was cooled to 0 °C and ethanol (2 mL) was added dropwise, after 5 min, H_2O_2 (4 mL, 30% aq. solution) and NaOH (4 mL, 4 N ethanol solution) were added sequentially. The reaction mixture was allowed to warm to room temperature and stirred for an additional 5 h. The mixture was concentrated in vacuo, ether - water (50:15 mL) was added, and the mixture was washed with brine (3 × 10 mL). The organic phase was dried over anhydrous Na_2SO_4 and the solvent was evaporated to give a crude product, which was purified by flash chromatography on silica gel (50% ether/hexanes), affording 894 mg of 17 (85%), as a colourless syrup. $[\alpha]_D^{25}$ = þ32.0 (c 0.11, $CHCl_3$). ^{1}H NMR (500 MHz, $CDCl_3$) d: 4.01e3.86 (m, 4H), 3.64 (d, *J* ¼ 10.9 Hz, 1H), 3.62 (s, 3H), 3.59 (d, *J* ¼ 10.3 Hz, 1H), 2.13 (t, *J* ¼ 6.4 Hz, 1H), 2.05 (m, 1H), 1.90e1.70 (m, 6H), 1.63e1.39 (m, 4H), 1.30 (s, 3H), 1.28e1.21 (m, 2H), 1.16 (s, 3H), 1.09 (dd, *J* ¼ 8.6, 6.4 Hz, 1H), 1.00 (td, *J* ¼ 13.4, 4.3 Hz, 1H), 0.89 (m, 1H), 0.53 (s, 3H). ^{13}C NMR (125 MHz, $CDCl_3$) d: 178.1 (C), 110.4 (C), 64.8 ($2CH_2$), 61.2 (CH_2), 57.3 (CH), 52.7 (CH), 51.3 (CH_3), 44.0 (C), 39.5 (CH_2), 39.4 (CH), 38.5 (C), 38.2 (CH_2), 37.8 (CH_2), 29.6 (CH_2), 28.9 (CH_3), 23.9 (CH_3), 19.7 (CH_2), 19.3 (CH_2), 19.2 (CH_2), 13.9 (CH_3). IR (film): 3444, 1723, 1459, 1376, 1228, 1151, 1066, 1029, 859 cm^{-1}. HRMS (ESþ/TOF) *m/z*: calcd for $C_{21}H_{36}O_5Na$ (M þ $Na^þ$) 3912460, found: 391.2455.

4.3.4. (1S,4aR,5S,6S,8aR)-Methyl 6-formyl-1,4a-dimethyl-5-(2-(2-methyl-1,3-dioxolan-2-yl)ethyl)-decahydronaphthalene-1-carboxylate (18)

Pyridinium dichromate (PDC) (4.69 g, 12.47 mmol) was added to a stirred solution of 17 (2.30 g, 6.24 mmol) in dry CH_2Cl_2 (40 mL) and the mixture was stirred at room temperature under an argon atmosphere for 10 h, at which time TLC showed no remaining starting material. Then, the reaction mixture was worked up by the addition of ether (40 mL), and the resulting mixture was filtered through a silica gel pad and washed with ether (20 mL). The filtrate was washed with 1 N HCl solution (20 mL), brine and dried over anhydrous Na_2SO_4. The solvent was evaporated to yield 2.13 g of aldehyde 18 (94%), as a colourless syrup. $[\alpha]_D^{25}$ ¼ þ182.4 (c 0.20, $CHCl_3$). ^{1}H NMR (500 MHz, $CDCl_3$) d: 9.98 (s, 1H), 4.02e3.86 (m, 4H), 3.60 (s, 3H), 2.47 (t, *J* ¼ 4.3 Hz, 1H), 2.37 (m, 1H), 2.16 (dd, *J* ¼ 9.3, 4.4 Hz, 1H), 1.95e1.86 (m, 2H), 1.85e1.71 (m, 4H), 1.70e1.40 (m, 5H), 1.33 (s, 3H), 1.16 (s, 3H), 1.20e1.09 (m, 2H), 1.00 (ddd, *J* ¼ 28.2, 14.1, 4.7 Hz, 1H), 0.57 (s, 3H). ^{13}C NMR (125 MHz, $CDCl_3$) d: 205.1 (CH), 177.8 (C), 110.2 (C), 64.8 ($2CH_2$), 56.5 (CH), 53.2 (CH), 51.4 (CH_3), 47.2 (CH), 44.0 (C), 39.1 (CH_2), 39.0 (C), 38.3 (CH_2), 38.1 (CH_2), 28.9 (CH_3), 27.1 (CH_2), 23.9 (CH_3), 20.5 (CH_2), 19.7 (CH_2), 19.4 (CH_2), 13.3 (CH_3). IR (film): 1721, 1459, 1376, 1220, 1153, 1065, 773 cm^{-1}. HRMS (ESþ/TOF) *m/z*: calcd for $C_{21}H_{35}O_5$ (M þ $H^þ$) 367.2484, found: 367.2480.

4.3.5. (1S,4aR,5R,6S,8aR)-Methyl 6-formyl-1,4a,6-trimethyl-5-(2-(2-methyl-1,3-dioxolan-2-yl)ethyl)-decahydronaphthalene-1-carboxylate (13)

Potassium *tert*-butoxide (276 mg, 2.46 mmol) was added to a stirred solution of 18 (750 mg, 2.05 mmol) in dry benzene (40 mL) under an argon atmosphere. After 5 min, methyl iodide (0.4 mL, 6.42 mmol) was added and the reaction mixture was stirred at room temperature for 1.5 h, at which time TLC showed no starting material. The mixture was concentrated in vacuo to give a crude product, which was dissolved in ether (40 mL) and washed with brine (2 × 10 mL). The organic phase was dried over anhydrous

Na_2SO_4 and the solvent was evaporated to give a crude product, which was purified by flash chromatography on silica gel (15% ether/hexanes), affording 638 mg of 13 (82%) as a colourless syrup. $[\alpha]_D^{25}$ ¼ þ 17.1 (c 0.13, $CHCl_3$). ^1H NMR (500 MHz, $CDCl_3$) d: 9.81 (s, 1H), 4.03e3.85 (m, 4H), 3.58 (s, 3H), 2.21 (dt, *J* ¼ 13.5, 3.3 Hz, 1H), 2.15 (br d, *J* ¼ 12.9 Hz, 1H), 1.89e1.63 (m, 7H), 1.55e1.41 (m, 4H), 1.31 (s, 3H), 1.14 (s, 3H), 1.11e1.02 (m, 2H), 1.01 (s, 3H), 0.97 (m, 1H), 0.54 (s, 3H). ^{13}C NMR (125 MHz, $CDCl_3$) d: 206.5 (CH), 177.7 (C), 109.8 (C), 64.8 ($2CH_2$), 60.0 (CH), 56.6 (CH), 51.3 (CH_3), 49.8 (C), 44.0 (C), 43.4 (CH_2), 39.8 (C), 39.0 (CH_2), 38.2 (CH_2), 36.2 (CH_2), 28.7 (CH_3), 24.5 (CH_3), 23.8 (CH_3), 20.8 (CH_2), 19.4 (CH_2), 19.2 (CH_2), 13.0 (CH_3). IR (film): 1738, 1469, 1256, 1120, 1003, 988, 770 cm^{-1}. HRMS (APcI) *m/z*: calcd for $C_{22}H_{37}O_5$ (M þ H^+) 381.2641, found: 381.2638.

4.3.6. (3aR,5aR,6S,9aR,9bS)-Methyl 2-acetyl-3a,6,9a-trimethyl-3a,4,5,5a,6,7,8,9,9a,9b-decahydro-1H-cyclopenta[a]naphthalene-6-carboxylate (19)

HCl (5 mL, 1 M) was added to a stirred solution of 13 (600 mg, 1.58 mmol) in THF (30 mL). The reaction mixture was heated under reflux for 3 h, at which time TLC showed no 13. The reaction was allowed to cool to room temperature and the solvent was evaporated in vacuo. Then, the residue was dissolved in ether (40 mL) and washed with water (2 × 10 mL) and brine (2 × 10 mL). The organic phase was dried over anhydrous Na_2SO_4 and concentrated under vacuum. The crude product was purified by flash chromatography (15% ether/hexanes) to give 432 mg (86%) of methyl ketone 19 as a colourless syrup. $[\alpha]_D^{25}$ = þ46.1 (c 0.16, $CHCl_3$). ^1H NMR (500 MHz, $CDCl_3$) d: 6.51 (d, *J* ¼ 2.3 Hz, 1H), 3.59 (s, 3H), 2.58 (ddd, *J* ¼ 16.7, 6.8, 2.5 Hz, 1H), 2.45 (d, *J* ¼ 16.7 Hz, 1H), 2.26 (s, 3H), 2.17 (dd, *J* ¼ 13.3, 1.5 Hz, 1H), 2.05 (dt, *J* ¼ 13.9, 4.2 Hz, 1H), 1.85 (m, 1H), 1.77e1.59 (m, 3H), 1.57 (d, *J* ¼ 6.7 Hz, 1H), 1.44e1.32 (m, 2H), 1.18 (s, 3H), 1.09e0.97 (m, 3H), 1.03e1.01 (m, 2H), 0.91 (td, *J* ¼ 13.3, 3.8 Hz, 1H), 0.48 (s, 3H). ^{13}C NMR (125 MHz, $CDCl_3$) d: 197.0 (C), 178.0 (C), 154.9 (CH), 144.3 (C), 59.0 (CH), 53.6 (CH), 51.4 (CH_3), 48.7 (C), 43.95 (C), 41.3 (CH_2), 38.1 (CH_2), 37.2 (C), 36.4 (CH_2), 31.3 (CH_2), 30.0 (CH_3), 28.9 (CH_3), 26.6 (CH_3), 21.6 (CH_2), 19.2 (CH_2), 14.2 (CH_3). HRMS (ESþ/TOF) *m/z*: calcd for $C_{20}H_{31}O_3$ (M þ H^+) 319.2273, found: 319.2289.

4.3.7. (3aR,5aR,6S,9aR,9bS)-Methyl 2-(1-acetoxyvinyl)-3a,6,9a-trimethyl-3a,4,5,5a,6,7,8,9,9a,9b-decahydro-1H-cyclopenta[a] naphthalene-6-carboxylate (12)

p-Toluenesulfonic acid (15 mg, 0.08 mmol) was added to a stirred solution of 19 (600 mg, 1.88 mmol) in isopropenyl acetate (15 mL). The reaction mixture was heated under reflux for 3 h, at which time TLC showed no 19. The reaction was allowed to cool to room temperature and ether (40 mL) was added. Then, the solution was washed with saturated aqueous $NaHCO_3$ (3 × 10 mL). The organic phase was dried over anhydrous Na_2SO_4 and the solvent was evaporated to give a crude product, which was purified by flash chromatography on silica gel (5% ether/hexanes), affording 664 mg of 12 (98%) as a colourless syrup. $[\alpha]_D^{25}$ = þ52.2 (c 0.11, $CHCl_3$). ^1H NMR (500 MHz, $CDCl_3$) d: 5.58 (d, *J* = 2.2 Hz, 1H), 4.91 (d, *J* = 1.5 Hz, 1H), 4.78 (d, *J* = 1.6 Hz, 1H), 3.59 (s, 3H), 2.65 (ddd, *J* = 15.8, 6.9, 2.5 Hz, 1H), 2.20 (s, 3H), 1.97 (dt, *J* ¼ 13.9, 4.4 Hz, 1H), 1.85e1.75 (m, 2H), 1.70 (br d, *J* ¼ 14.5 Hz, 1H), 1.67e1.52 (m, 3H), 1.45e1.23 (m, 3H), 1.18 (s, 3H), 1.04 (ddd, *J* ¼ 17.4, 10.5, 3.6 Hz, 2H), 0.98 (s, 3H), 0.92 (m, 1H), 0.57 (s, 3H). ^{13}C NMR (125 MHz, $CDCl_3$) d: 178.1 (C), 169.0 (C), 150.7 (C), 140.1 (CH), 135.9 (C), 102.1 (CH_2), 59.5 (CH), 53.8 (CH), 51.4 (CH_3), 47.8 (C), 44.0 (C), 41.6 (CH_2), 38.2 (CH_2), 37.2 (C), 36.6 (CH_2), 33.0 (CH_2), 30.7 (CH_3), 28.9 (CH_3), 21.4 (CH_2), 21.1 (CH_3), 19.3 (CH_2), 13.9 (CH_3). IR (film): 1763, 1723, 1459, 1439, 1204, 1151, 847 cm^{-1}. HRMS (APcI) *m/z*: calcd for $C_{22}H_{32}O_4Na$ (M þ Na^+) 383.2198, found: 383.2100.

4.3.8. (4S,4aR,6aS,6bR,11aR,11bR)-Dimethyl10-acetoxy-4,6a,11b-trimethyl-2,3,4,4a,5,6,6a,6b,9,11,11a,11b-dodecahydro-1H-benzo[a] fluorene-4,7-dicarboxylate (20) and (4S,4aR,6aS,6bS,11aR,11bR)-dimethyl 10-acetoxy-4,6a,11b-trimethyl-2,3,4,4a,5,6,6a,6b,9,11,11a,11b-dodecahydro-1H-benzo[a]fluorene-4,7-dicarboxylate (21)

Methyl propiolate (0.23 mL, 2.58 mmol) was added to a solution of diene 12 (620 mg, 1.72 mmol) in xylene (10 mL), and the mixture was heated at 180 °C for 4 days in a sealed tube. At this time, TLC showed no remaining starting material. The reaction was allowed to cool to room temperature and then concentrated in vacuo to give a resolvable mixture of adducts (1:1), which was chromatographed on silica gel (10% ether/hexanes) to give 342 mg of pure 20 (45%) and 337 mg of pure 21 (44%).

Compound 20: $[\alpha]_D^{25}$ = þ20.1 (c 0.11, $CHCl_3$). ^1H NMR (500 MHz, $CDCl_3$) d: 6.52 (dd, *J* = 5.7, 3.6 Hz, 1H), 3.75 (s, 3H), 3.67 (dd, *J* = 7.8 Hz, 1H), 3.64 (s, 3H), 3.06 (ddd, *J* = 22.6, 10.4, 2.9 Hz, 1H), 2.85 (ddd, *J* = 22.6, 7.9, 3.8 Hz, 1H), 2.35e2.23 (m, 2H), 2.15 (s, 3H), 2.06e1.72 (m, 4H), 1.60 (d, *J* = 13.0 Hz, 1H), 1.45e1.23 (m, 3H), 1.18 (s, 3H), 1.12 (dd, *J* = 11.3, 3.0 Hz, 1H), 1.00 (td, *J* = 13.4, 4.2 Hz, 2H), 0.87 (td, *J* = 13.4, 3.6 Hz, 1H), 0.71 (s, 3H), 0.69 (s, 3H). ^{13}C NMR (125 MHz, $CDCl_3$) d: 178.1 (C), 169.1 (C), 168.8 (C), 137.2 (C), 134.7 (CH), 131.7 (C), 128.1 (C), 58.5 (CH), 55.6 (CH), 51.6 (CH_3), 51.3 (CH_3), 45.4 (CH), 45.0 (C), 44.0 (C), 41.4 (CH_2), 38.3 (CH_2), 37.8 (C), 36.3 (CH_2), 30.2 (CH_2), 28.6 (CH_3), 27.4 (CH_3), 25.0 (CH_2), 20.9 (CH_3), 20.0 (CH_2), 19.0 (CH_2), 14.0 (CH_3). IR (film): 1782, 1720, 1436, 1387, 1266, 1023, 790 cm^{-1}. HRMS (ESþ/TOF) *m/z*: calcd for $C_{26}H_{37}O_6$ (M þ H^+) 445.2590, found: 445.2591.

Compound 21: $[\alpha]_D^{25}$ = þ12.5 (c 0.13, $CHCl_3$). ^1H NMR (500 MHz, $CDCl_3$) d: 6.70 (td, *J* ¼ 3.6, 1.6 Hz, 1H), 3.73 (s, 3H), 3.61 (s, 3H), 3.37 (dd, *J* ¼ 8.6 Hz, 1H), 3.08 (m, 1H), 2.84 (d br, *J* ¼ 23.0 Hz, 1H), 2.39e2.24 (m, 2H), 2.15 (s, 3H), 2.10e2.00 (m, 2H), 1.90e1.67 (m, 3H), 1.62 (d, *J* ¼ 12.9 Hz, 1H), 1.48e1.34 (m, 2H), 1.33 (s, 3H), 1.19 (m, 1H), 1.13 (s, 3H), 1.06e0.84 (m, 3H), 0.57 (s, 3H). ^{13}C NMR (125 MHz, $CDCl_3$) d: 178.0 (C), 169.3 (C), 168.4 (C), 135.7 (CH), 133.9 (C), 130.6 (C), 128.5 (C), 58.9 (CH), 51.6 (CH_3), 51.6 (CH), 51.4 (CH_3), 48.4 (CH), 44.7 (C), 43.9 (CH_2), 43.7 (C), 38.7 (CH_2), 37.4 (C), 31.4 (CH_3), 29.5 (CH_2), 28.0 (CH_3), 27.3 (CH_2), 26.6 (CH_2), 21.0 (CH_3), 19.7 (CH_2), 17.7 (CH_2), 14.2 (CH_3). IR (film): 1771, 1713, 1411, 1337, 1245, 1004, 812 cm^{-1}. HRMS (ESþ/TOF) *m/z*: calcd for $C_{26}H_{37}O_6$ (M þ H^+) 445.2590, found: 445.2591.

4.3.9. (4S,4aR,6aS,11aR,11bR)-Dimethyl 10-acetoxy-4,6a,11b-trimethyl-2,3,4,4a,5,6,6a,11,11a,11b-decahydro-1H-benzo[a] fluorene-4,7-dicarboxylate (22)

To a solution of the diasteroisomers 20 and 21 (550 mg, 1.24 mmol) in 1,4-dioxane (15 mL) was added 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (314 mg, 1.4 mmol), and the reaction mixture was stirred at 100 °C for 3 h. Then, the solvent was evaporated in vacuo, and the residue was dissolved in ether (50 mL), washed with water (5 × 15 mL) and brine. The organic phase was dried over anhydrous Na_2SO_4 and concentrated to give a crude product which was chromatographed on silica gel (10% ether/hexanes) to give 537 mg of pure 22 (98%) as a yellow syrup. $[\alpha]_D^{25}$ = þ80.4 (c 0.14, $CHCl_3$). ^1H NMR (500 MHz, $CDCl_3$) d: 7.39 (d, *J* ¼ 8.3 Hz, 1H), 6.87 (d, *J* ¼ 8.3 Hz, 1H), 3.88 (s, 3H), 3.49 (s, 3H), 2.86 (dd, *J* ¼ 16.5, 6.9 Hz, 1H), 2.60 (d, *J* ¼ 5.0 Hz, 1H), 2.56 (d, *J* ¼ 16.5 Hz, 1H), 2.30 (s, 3H), 2.16 (br d, *J* ¼ 12.0 Hz, 1H), 1.84e1.66 (m, 4H), 1.46e1.35 (m, 3H), 1.33 (s, 3H), 1.16 (s, 3H), 1.11 (m, 1H), 1.05e0.85 (m, 2H), 0.05 (s, 3H). ^{13}C NMR (125 MHz, $CDCl_3$) d: 177.9 (C), 169.2 (C), 168.6 (C), 151.6 (C), 148.7 (C), 137.8 (C), 128.8 (CH), 125.78 (C), 119.4 (CH), 62.4 (CH), 55.3 (CH), 52.2 (CH_3), 51.4 (CH_3), 49.6 (C), 43.7 (C), 40.9 (CH_2), 38.1 (CH_2), 37.6 (C), 34.9 (CH_2), 31.2 (CH_3), 28.9 (CH_3), 28.7 (CH_2), 21.3 (CH_2), 21.0 (CH_3), 19.1 (CH_2), 14.0 (CH_3). IR (film): 1766, 1724, 1434, 1280, 1206, 1166, 1126, 755 cm^{-1}. HRMS

(APcI) *m/z*: calcd for $C_{26}H_{34}O_6Na$ (M þ $Na^{þ}$) 465.2253, found: 465.2256.

4.3.10. (4S,4aR,6aS,11aR,11bR)-Dimethyl 10-hydroxy-4,6a,11b-trimethyl-2,3,4,4a,5,6,6a,11,11a,11b-decahydro-1H-benzo[a] fluorene-4,7-dicarboxylate (11)

Conc. hydrochloric acid (1 mL) was added to a stirred solution of 22 (480 mg, 1.08 mmol) in MeOH (10 mL) and the reaction mixture was refluxed for 30 min, at which time TLC showed no starting material remaining. Then, the solvent was removed in vacuum and ether - water (30: 10 mL) was added. The phases were shaken and separated. The organic phase was washed with water and brine, and dried over anhydrous Na_2SO_4. Removal of the solvent under vacuum afforded a crude product which was directly purified by flash chromatography (35% ether/hexanes) to yield 421 mg of 11 (97%) as a white solid. $[a]_D^{25}$ = þ113.2 (c 0.13, $CHCl_3$). ^1H NMR (500 MHz, $CDCl_3$) d: 7.38 (d, *J* ¼ 8.3 Hz, 1H), 6.58 (d, *J* ¼ 8.3 Hz, 1H), 5.30 (s, 1H), 3.87 (s, 3H), 3.50 (s, 3H), 2.86 (dd, *J* ¼ 15.9, 6.8 Hz, 1H), 2.66 (d, *J* ¼ 16.1 Hz, 1H), 2.16 (d, *J* ¼ 13.8 Hz, 1H), 1.83e1.65 (m, 4H), 1.49e1.37 (m, 2H), 1.35 (s, 3H), 1.28e1.24 (m, 2H), 1.17 (s, 3H), 1.21e1.12 (m, 2H), 1.00 (m, 1H), 0.06 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) d: 178.2 (C), 169.4 (C), 154.0 (C), 152.3 (C), 130.9 (C), 130.1 (CH), 120.7 (C), 112.9 (CH), 62.8 (CH), 55.4 (CH), 51.9 (CH_3), 51.4 (CH_3), 49.7 (C), 43.8 (C), 41.0 (CH_2), 38.2 (CH_2), 37.8 (C), 34.9 (CH_2), 31.0 (CH_3), 28.9 (CH_3), 27.6 (CH_2), 21.4 (CH_2), 19.2 (CH_2), 14.0 (CH_3). IR (KBr): 3407, 1721, 1696, 1582, 1434, 1278, 1255, 1128, 755 cm^{-1}. HRMS (ESþ/TOF) *m/z*: calcd for $C_{24}H_{33}O_5$ (M þ $H^{þ}$) 401.2328, found: 401.2340.

4.3.11. (4S,4aR,6aS,11aR,11bR)-Dimethyl 4,6a,11b-trimethyl-10-(trifluoromethylsulfonyloxy)-2,3,4,4a,5,6,6a,11,11a,11b-decahydro-1H-benzo[a]fluorene-4,7-dicarboxylate (23)

Trifluoromethanesulfonic anhydride (0.35 mL, 2.06 mmol) was added at 0 °C dropwise to a solution of 11 (410 mg, 1.02 mmol) and 4-dimethylaminopyridine (377 mg, 3.09 mmol) in dry CH_2Cl_2 (15 mL) and the reaction mixture was stirred for 30 min, at which time TLC showed no 11. Then, sat. aqueous $NaHCO_3$ (5 mL) was added to quench the reaction, and the mixture was extracted with ether (2 × 25 mL), and the combined organic layer was washed with 0.5 M solution HCl (3 × 10 mL), water (3 × 10 mL), brine (3 × 10 mL) and dried over anhydrous Na_2SO_4 and evaporated in vacuo. The crude product was purified by flash column chromatography on silica gel (10% ether/hexanes), to give 23 (518 mg, 95%) as a colourless oil. $[a]_D^{25}$ = þ73.1 (c 0.16, $CHCl_3$). ^1H NMR (400 MHz, $CDCl_3$) d: 7.41 (d, *J* ¼ 8.5 Hz, 1H), 7.05 (d, *J* ¼ 8.5 Hz, 1H), 3.91 (s, 3H), 3.51 (s, 3H), 3.07 (dd, *J* ¼ 16.8, 6.9 Hz, 1H), 2.85 (d, *J* ¼ 16.8 Hz, 1H), 2.54 (dd, *J* ¼ 11.5, 3.4 Hz, 1H), 2.18 (d, *J* ¼ 13.3 Hz, 1H), 1.84e1.69 (m, 4H), 1.52e1.38 (m, 4H), 1.34 (s, 3H), 1.17 (s, 3H), 1.16 (m, 1H), 0.99 (m, 1H), 0.04 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) d: 177.7 (C), 168.5 (C), 153.0 (C), 147.2 (C), 138.5 (C), 129.4 (CH), 128.2 (C), 118.8 (CH), 118.8(q, *J* = 320 Hz), 62.4 (CH), 55.1 (CH), 52.4 (CH_3), 51.4 (CH_3), 49.9 (C), 43.7 (C), 40.8 (CH_2), 38.0 (C), 37.6 (CH_2), 34.8 (CH_2), 31.2 (CH_3), 29.0 (CH_2), 28.9 (CH_3), 21.2 (CH_2), 19.1 (CH_2), 14.1 (CH_3). IR (film): 1727, 1424, 1250, 1216, 1143, 941, 880, 762, 641, 608 cm^{-1}. HRMS (APcI) *m/z*: calcd for $C_{25}H_{31}O_7F_3SNa$ (M þ $Na^{þ}$) 555.1640, found: 555.1644.

4.3.12. (4S,4aR,6aS,11aR,11bR)-Dimethyl 4,6a,11b-trimethyl-2,3,4,4a,5,6,6a,11,11a,11b-decahydro-1H-benzo[a]fluorene-4,7-dicarboxylate (24)

60-70% Ni Raney dispersion in water (1 mL) was added to a stirred solution of 23 (120 mg, 0.22 mmol) in THF (10 mL), with an H_2 atmosphere provided by a balloon, and the mixture was stirred at room temperature for 48 h, at which time TLC showed no remaining starting material. Then, the resulting mixture was filtered through a silica gel-anhydrous Na_2SO_4 pad and washed with acetone (50 mL). The solvent was evaporated to yield 80 mg of pure 24 (92%), as a colourless oil. $[a]_D^{25}$ = þ165.6 (c 0.11, $CHCl_3$). ^1H NMR (400 MHz, $CDCl_3$) d: 7.31 (d, *J* ¼ 7.6 Hz, 1H), 7.22 (d, *J* ¼ 7.2 Hz, 1H), 7.12 (t, *J* ¼ 7.3 Hz, 1H), 3.90 (s, 3H), 3.50 (s, 3H), 3.08 (dd, *J* ¼ 16.2, 6.6 Hz, 1H), 2.68 (d, *J* ¼ 16.2 Hz, 1H), 2.56 (m, 1H), 2.16 (d, *J* ¼ 12.7 Hz, 1H), 1.83e1.57 (m, 5H), 1.51e1.37 (m, 4H), 1.32 (s, 3H), 1.17 (s, 3H), 1.02 (m, 1H), 0.05 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) d: 178.0 (C), 170.1 (C), 148.3 (C), 146.3 (C), 128.1 (C), 127.1 (CH), 126.8 (CH), 126.2 (CH), 62.6 (CH), 55.5 (CH), 52.1 (CH_3), 51.3 (CH_3), 48.8 (C), 43.8 (C), 41.2 (CH_2), 38.2 (CH_2), 37.8 (C), 35.3 (CH_2), 32.3 (CH_2), 31.4 (CH_3), 28.9 (CH_3), 21.4 (CH_2), 19.2 (CH_2), 14.5 (CH_3). IR (film): 1725, 1464, 1435, 1262, 1236, 1194, 1151, 1119, 760 cm^{-1}. HRMS (ESþ/TOF) *m/z*: calcd for $C_{24}H_{33}O_4$ (M þ $H^{þ}$) 385.2379, found: 385.2375.

4.3.13. ((4S,4aR,6aS,11aR,11bR)-4,6a,11b-Trimethyl-2,3,4,4a,5,6,6a,11,11a,11b-decahydro-1H-benzo[a]fluorene-4,7-diyl) dimethanol (25)

$LiAlH_4$ (46 mg, 1.20 mmol) was added to a stirred solution of 24 (230 mg, 0.60 mmol) in dry THF (15 mL) cooled to 0 °C and the reaction mixture was kept stirred under an argon atmosphere for 3 h, at which time TLC showed no remaining starting material. Then 2 N HCl (1 mL) was added slowly at 0 °C, and the mixture was extracted with ether (2 × 20 mL). The organic phase was washed with brine, dried over anhydrous Na_2SO_4, and the solvent was evaporated to give 187 mg of pure 25 (95%) as a white solid. $[a]_D^{25}$ ¼ þ9.2 (c 0.10, $CHCl_3$). ^1H NMR (500 MHz, $CDCl_3$) d: 7.13e7.06 (m, 3H), 4.78 (d, *J* ¼ 12.4 Hz, 1H), 4.74 (d, *J* ¼ 12.4 Hz, 1H), 3.66 (d, *J* ¼ 11.0 Hz, 1H), 3.34 (d, *J* ¼ 11.0 Hz, 1H), 3.04 (dd, *J* ¼ 16.5, 7.3 Hz, 1H), 2.66 (d, *J* ¼ 16.5 Hz, 1H), 1.85e1.73 (m, 2H), 1.73e1.59 (m, 2H), 1.53e1.35 (m, 2H), 1.34e1.24 (m, 4H), 1.21 (s, 3H), 1.14 (dd, *J* ¼ 11.8, 2.8 Hz, 1H), 0.99 (s, 3H), 0.94 (m, 1H), 0.30 (s, 3H). ^{13}C NMR (125 MHz, $CDCl_3$) d: 148.3 (C), 145.1 (C), 135.1 (C), 127.0 (CH), 126.6 (CH), 124.3 (CH), 65.4 (CH_2), 63.3 (CH_2), 62.7 (CH), 54.3 (CH), 49.2 (C), 40.8 (CH_2), 38.5 (C), 37.4 (C), 36.3 (CH_2), 35.6 (CH_2), 32.6 (CH_3), 32.2 (CH_2), 27.0 (CH_3), 20.0 (CH_2), 18.3 (CH_2), 16.7 (CH_3). IR (KBr): 3351, 1591, 1466, 1365, 1216, 1014, 757 cm^{-1}. HRMS (ESþ/TOF) *m/z*: calcd for $C_{22}H_{31}O_2$ (M þ $H^{þ}$) 327.2324, found: 327.2314.

4.3.14. (4S,4aR,6aS,11aR,11bR)-4-(Hydroxymethyl)-4,6a,11b-trimethyl-2,3,4,4a,5,6,6a,11,11a,11b-decahydro-1H-benzo[a] fluorene-7-carbaldehyde (26)

To a solution of 25 (360 mg, 1.10 mmol) in chloroform (25 mL) was added manganese (IV) oxide (574 mg, 6.60 mmol) and the reaction mixture was stirred at room temperature for 6 h. The inorganic solid was removed by filtration of the mixture through silica gel pad (15 g) and washed with ether (20 mL). The combined filtrates were evaporated to yield 329 mg (92%) of compound 26 as a white solid. $[a]_D^{25}$ = þ26.0 (c 0.12, $CHCl_3$). ^1H NMR (400 MHz, $CDCl_3$) d: 10.49 (s, 1H), 7.64 (d, *J* ¼ 7.5 Hz, 1H), 7.34 (d, *J* ¼ 7.3 Hz, 1H), 7.23 (t, *J* ¼ 7.5 Hz, 1H), 3.69 (d, *J* ¼ 10.9 Hz, 1H), 3.40 (d, *J* ¼ 10.9 Hz, 1H), 3.11 (dd, *J* ¼ 16.8, 8.1 Hz, 1H), 2.79 (d, *J* ¼ 16.8 Hz, 1H), 2.55 (dt, *J* ¼ 11.7, 5.6 Hz, 1H), 1.95e1.88 (m, 1H), 1.84 (br d, *J* ¼ 13.6 Hz, 1H), 1.75 (br d, *J* ¼ 8.2 Hz, 2H), 1.50e1.40 (m, 3H), 1.39 (s, 3H), 1.33e1.19 (m, 3H), 0.99 (s, 3H), 0.95 (m, 1H), 0.39 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) d: 191.9 (CH), 153.4 (C), 145.7 (C), 133.0 (C), 129.9 (CH), 128.0 (CH), 126.9 (CH), 65.1 (CH_2), 62.8 (CH), 53.0 (CH), 49.5 (C), 41.2 (CH_2), 38.5 (C), 37.4 (C), 36.8 (CH_2), 35.7 (CH_2), 32.9 (CH_3), 32.4 (CH_2), 26.6 (CH_3), 19.4 (CH_2), 18.3 (CH_2), 16.8 (CH_3). IR (KBr): 3415, 1686, 1593, 1461, 1235, 1016, 756 cm^{-1}. HRMS (ESþ/TOF) *m/z*: calcd for $C_{22}H_{31}O_2$ (M þ $H^{þ}$) 327.2324, found: 327.2331.

4.3.15. (4S,4aR,6aS,11aR,11bR)-4-(Hydroxymethyl)-4,6a,11b-trimethyl-2,3,4,4a,5,6,6a,11,11a,11b-decahydro-1H-benzo[a]fluoren-7-yl formate (27)

m-Chloroperoxybenzoic acid (75%; 239 mg, 1.04 mmol) was added at 0 °C to a stirred solution of 26 (226 mg, 0.69 mmol) in CH_2Cl_2 (15 mL) and the reaction was stirred for 12 h, at which time TLC indicated no starting material remaining. The reaction was quenched with sat. aq Na_2SO_3 (0.5 mL) and stirred for an additional 10 min. Then, it was poured into ether/water (20: 7 mL), and the organic phase was washed with sat. aq $NaHCO_3$ (5 × 8 mL) and brine, dried over Na_2SO_4 and concentrated to give 27 (234 mg, 98%) as a yellow oil. $[\alpha]_D^{25}$ = þ18.9 (c 0.12, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$) d: 8.31 (s, 1H), 7.12 (t, *J* = 7.6 Hz, 1H), 7.04 (d, *J* = 7.3 Hz, 1H), 6.78 (d, *J* = 7.9 Hz, 1H), 3.69 (d, *J* = 10.9 Hz, 1H), 3.50 (d, *J* = 7.4 Hz, 1H), 3.36 (d, *J* = 10.9 Hz, 1H), 3.06 (dd, *J* = 16.7, 7.5 Hz, 1H), 2.72 (d, *J* = 16.7 Hz, 1H), 2.60 (m, 1H), 1.83 (br d, *J* = 13.6 Hz, 1H), 1.74 (br d, *J* ¼ 14.2 Hz, 1H), 1.72e1.51 (m, 3H), 1.51e1.21 (m, 3H), 1.18 (s, 3H), 1.11 (m, 1H), 0.98 (s, 3H), 0.93 (dd, *J* ¼ 13.0, 4.1 Hz, 1H), 0.38 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) d: 159.9 (CH), 147.3 (C), 146.0 (C), 141.2 (C), 127.8 (CH), 122.7 (CH), 120.2 (CH), 65.2 (CH_2), 62.5 (CH), 53.7 (CH), 48.1 (C), 41.1 (CH_2), 38.5 (C), 37.3 (C), 35.6 (CH_2), 35.0 (CH_2), 32.5 (CH_2), 31.8 (CH_3), 26.9 (CH_3), 19.5 (CH_2), 18.2 (CH_2), 16.6 (CH_3). IR (film): 3444, 1740, 1581, 1466, 1215, 1124, 1014, 757 cm^{-1}. HRMS (APcI) *m/z*: calcd for $C_{22}H_{30}O_3Na$ (M þ Na$^+$) 365.2093, found: 365.2098.

4.3.16. (4S,4aR,6aS,11aR,11bR)-4-(Hydroxymethyl)-4,6a,11b-trimethyl-2,3,4,4a,5,6,6a,11,11a,11b-decahydro-1H-benzo[a]fluoren-7-ol (28)

4.3.16.1. Treatment of formate 27 with conc. HCl in MeOH. Conc. hydrochloric acid (1.5 mL) was added to a stirred solution of 27 (410 mg, 1.20 mmol) in MeOH (15 mL) and the reaction mixture was refluxed for 15 min, at which time TLC showed no starting material remaining. Then, the solvent was removed in vacuum and ether - water (30: 10 mL) was added. Phases were shaken and separated. The organic phase was washed with water and brine, and dried over anhydrous Na_2SO_4. Removal of the solvent under vacuum afforded a crude product which was directly purified by flash chromatography (35% ether/hexanes) to yield 369 mg of 28 (98%) as a yellow solid. $[\alpha]_D^{25}$ = þ4.5 (c 0.12, $CHCl_3$). ^{1}H NMR (500 MHz, $CDCl_3$) d: 6.95 (t, *J* ¼ 7.6 Hz, 1H), 6.70 (d, *J* ¼ 7.4 Hz, 1H), 6.45 (d, *J* ¼ 7.9 Hz, 1H), 3.74 (d, *J* ¼ 11.0 Hz, 1H), 3.42 (dd, *J* ¼ 11.0, 1.0 Hz, 1H), 3.02 (dd, *J* ¼ 16.6, 7.7 Hz, 1H), 2.83 (m, 1H), 2.67 (d, *J* ¼ 16.6 Hz, 1H), 1.83 (ddd, *J* ¼ 13.6, 4.6, 3.0 Hz, 1H), 1.74 (ddd, *J* ¼ 14.8, 5.6, 3.9 Hz, 1H), 1.72e1.60 (m, 4H), 1.51e1.25 (m, 3H), 1.22 (s, 3H), 1.15 (dd, *J* ¼ 11.7, 3.5 Hz, 1H), 1.00 (s, 3H), 0.93 (m, 1H), 0.43 (s, 3H). ^{13}C NMR (125 MHz, $CDCl_3$) d: 151.6 (C), 146.1 (C), 135.55 (C), 127.2 (CH), 116.6 (CH), 113.6 (CH), 65.0 (CH_2), 61.9 (CH), 53.1 (CH), 47.3 (C), 41.0 (CH_2), 38.1 (C), 36.9 (C), 35.3 (CH_2), 34.4 (CH_2), 32.5 (CH_2), 30.6 (CH_3), 26.4 (CH_3), 19.3 (CH_2), 17.9 (CH_2), 16.2 (CH_3). IR (KBr): 3374, 1590, 1460, 1294, 1010, 755 cm^{-1}. HRMS (APcI) *m/z*: calcd for $C_{21}H_{30}O_2Na$ (M þ Na$^+$) 337.2143, found: 337.2144.

4.3.16.2. Treatment of aldehyde 26 with H_2O_2-$KHSO_4$. To a solution of aldehyde 26 (370 mg, 1.13 mmol) in methanol were added hydrogen peroxide (30%, 2 mL) and $KHSO_4$ (100 mg, 0.73 mmol) and the mixture was stirred at room temperature for 24 h, at which time TLC showed no starting material remaining. Then, the solvent was removed in vacuum and ether - water (30: 10 mL) was added. Phases were shaken and separated. The organic phase was washed with water and brine, and dried over anhydrous Na_2SO_4. Removal of the solvent under vacuum afforded a crude product which was directly purified by flash chromatography (45% ether/hexanes) to yield 306 mg of 28 (86%) as a yellow solid.

4.3.17. ((4S,4aR,6aS,11aR,11bR)-8-Formyl-7-hydroxy-4,6a,11b-trimethyl-2,3,4,4a,5,6,6a,11,11a,11b-decahydro-1H-benzo[a]fluoren-4-yl)methyl formate (29)

To a stirred solution of phenol 28 (310 mg, 0.99 mmol) in freshly distilled THF (15 mL) was added anhydrous magnesium chloride (142 mg, 1.49 mmol), paraformaldehyde (400 mg) and triethylamine (401 mg, 3.96 mmol) at room temperature. The reaction mixture was stirred at reflux for 12 h. After completion of the reaction (monitored by TLC), the reaction mixture was cooled to room temperature and quenched with 1 M aqueous HCl solution. The solvent was removed in vacuum and ether e water (20: 10 mL) was added. The organic phase was washed with water and brine, dried over anhydrous Na_2SO_4. Removal of the solvent under vacuum afforded a crude product which was directly purified by flash chromatography (20% ether/hexanes) to yield 339 mg of 29 (93%) as a colourless syrup. $[\alpha]_D^{25}$ = þ66.4 (c 0.15, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$) d: 11.21 (s, 1H), 9.79 (s, 1H), 8.05 (s, 1H), 7.30 (d, *J* ¼ 7.7 Hz, 1H), 6.81 (d, *J* ¼ 7.7 Hz, 1H), 4.33 (d, *J* ¼ 11.0 Hz, 1H), 3.92 (d, *J* ¼ 11.0 Hz, 1H), 3.51 (d, *J* ¼ 8.8 Hz, 1H), 3.06 (dd, *J* ¼ 17.7, 7.6 Hz, 1H), 3.01e2.93 (m, 1H), 2.72 (d, *J* ¼ 17.7 Hz, 1H), 1.80e1.70 (m, 4H), 1.66 (m, 1H), 1.52e1.30 (m, 2H), 1.24 (s, 3H), 1.21e1.13 (m, 2H), 1.02 (m, 1H), 1.01 (s, 3H), 0.47 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) d: 196.3 (CH), 161.4 (CH), 158.4 (C), 155.6 (C), 137.3 (C), 133.6 (CH), 120.1 (C), 116.4 (CH), 66.5 (CH_2), 62.1 (CH), 53.2 (CH), 47.6 (C), 41.1 (CH_2), 37.2 (C), 36.9 (C), 36.2 (CH_2), 33.8 (CH_2), 33.7 (CH_2), 30.5 (CH_3), 27.3 (CH_3), 19.7 (CH_2), 18.1 (CH_2), 16.7 (CH_3). IR (film): 1723, 1643, 1461, 1314, 1218, 1181, 757 cm^{-1}. HRMS (ESþ/TOF) *m/z*: calcd for $C_{23}H_{31}O_4$ (M þ H$^+$) 371.2222, found: 371.2227.

4.3.18. (þ)-dasyscyphin E (10)

To a solution of compound 29 (80 mg, 0.21 mmol) and triethylsilane (0.14 mL, 0.86 mmol) in dry methanol (5 mL) was added a catalytic amount of palladium (II) chloride (10 mg, 0.057 mmol) under an argon atmosphere. The resulting mixture was kept under stirring for 30 min at 0 °C. Then, the inorganic solid was removed by filtration of the mixture through silica gel pad (15 g) and washed with ether (20 mL). The combined filtrates were evaporated to give a crude product which was directly purified by flash chromatography (40% ether/hexanes) to yield 64 mg of 10 (91%) as a colourless syrup. $[\alpha]_D^{25}$ = þ2.1 (c 0.11, $CHCl_3$). IR (film): 3401, 1585, 1472, 1366, 1262, 1215, 1011, 797, 757 cm^{-1}. HRMS (ESþ/TOF) *m/z*: calcd for $C_{22}H_{33}O_2$ (M þ H$^+$) 329.2481, found: 329.2488.

Acknowledgements

The authors thank the Spanish Ministry of Economy and Competitiveness (Project CTQ2014-56611-R/BQU) for financial support and for the predoctoral fellowship granted to F. Jiménez and the Regional Government of Andalucia (Project P11-CTS-7651) for financial support and assistance provided to the FQM-348 group, and the European Commission for the Erasmus Mundus e Al Idrisi predoctoral fellowship granted to S. Mahdjour.

Appendix A. Supplementary data

Supplementary data related to this article can be found at.

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