First synthesis of antitumoral dasyscyphin B†

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The first synthesis of dasyscyphin B, an antitumoral metabolite obtained from the ascomycete Dasyscyphus niveus, has been achieved starting from commercial abietic acid. The key steps of the synthetic sequence are the diastereoselective α-methylation of a ketoaldehyde, followed by an intramolecular aldol condensation and the further Diels–Alder cycloaddition of a dienol ester. The procedure reported will allow the synthesis of related metabolites functionalized in the A ring.

Introduction

During the last few years, a new type of merosesquiterpene (a natural product of mixed biosynthetic origin) with a tetracyclic structure, including a cyclopentane ring, has been isolated from vegetable species, marine sponges and terrestrial fungi. Examples of this are pelorol (1), first isolated from Dactylospongia elegans,1 akaol A (2), found in a Micronesian sponge of the genus Aka,2 and dasyscyphins A–E (3–7), metabolites from the ascomycete Dasyscyphus niveus (Fig. 1).3,4 Even though the bioactivities of this family of compounds are yet to be examined comprehensively, preliminary studies have revealed that pelorol (1) is an activator of the inositol 5-phosphatase SHIP,5 whereas dasyscyphin B (4) and C (5) show potent cytotoxic activities in several human cell lines,3,6 and dasyscyphin D (6) and E (7) exhibit antifungal properties.4

Despite the significant biological activities and the interesting tetracyclic structure of the above mentioned compounds, only a few syntheses have been reported. A total synthesis of dasyscyphin D (6) was recently described by She et al., including a PtCl2-catalyzed pentannulation reaction and acid-catalyzed Robinson annulations as key steps.6 Andersen et al. previously reported the first synthesis of pelorol (1) starting from (+)-sclareolide, after condensation of an aryllithium with a dirimane hydroxy aldehyde and a further diastereomeric Friedel–Crafts alkylation to create the cyclopentane C ring; the success of the latter process required a sufficiently activated aromatic moiety.7 The results reported by Andersen’s group in their enantiospecific synthesis of pelorol (1), corroborated by our preliminary studies, revealed that the two-synthon strategy8 followed by intramolecular Friedel–Crafts alklylation led to the tetracyclic intermediate bearing a C8β methyl group as the major diastereoisomer, but this is not applicable for synthesizing sesquiterpene quinols such as compounds 2–7, bearing a B/C cis fused system. Considering the above arguments, we recently developed a new strategy to access this type of compound, based on the Diels–Alder cycloaddition of a tricyclic diene, having a cyclopentane C ring with a C8α methyl group. Utilizing this, the first enantiospecific synthesis of akaol A (2) from commercial (−)-sclareol was achieved.9

Results and discussion

Continuing our investigations of this new strategy to access dasyscyphins and related compounds, we explored the use of other terpenes, such as abietic acid (12), as the starting
material. This, as well as being a very cheap commercial compound, will make it possible to synthesize natural dasycyphins bearing a function in the A ring, such as dasycyphins A (3), D (6) or E (7), which could be difficult to prepare by alternative methods. The synthesis starting from acid 12 will also enable the preparation of unnatural analogues of these compounds functionalized in the A ring, in order to investigate the structure–activity relationship. Thus, we planned the preparation of dasycyphin B (4), which had not previously been synthesized, starting from abietic acid (12).

Scheme 1 shows the retrosynthesis of metabolite 4. The cyclopentane C ring of the target compound will be obtained through the intramolecular aldol condensation of a ketoaldehyde. The aromatic D ring will be elaborated after the Diels–Alder cycloaddition of dienol ester 9, obtained from the α,β-ene one resulting from the intramolecular aldol condensation of ketoaldehyde derived from ketal 10. The C8α methyl group of compound 4 will be introduced after the diastereoselective C-methylation of enol derived from the corresponding ketal aldehyde. This will be prepared from ketoaldehyde 11, after the chemoselective reduction of the enal group and the subsequent oxidative degradation of the (CH₃)₂CH–CO bond. Compound 11 will be synthesized after the regioselective oxidation of the C₁₃–C₁₄ bond of abietic acid.

Scheme 2 shows the preparation of tricyclic intermediate 20, which contains the cyclopentane C ring and the methyl group in the appropriate C8α arrangement. The regioselective oxidative rupture of the C₁₃–C₁₄ double bond of acid 12, affording ketoaldehyde 13, in good yield, was achieved after successive treatments with OsO₄ and NaIO₄, without isolating the intermediate diol. The α,β-unsaturated aldehyde 11 was chemoselectively reduced to ketoalcohol 14 by reaction with RANEY® Ni. The diol 15, obtained as a mixture of diastereoisomers, was regioselectively dehydrated after treatment with I₂ and PPh₃ to give the corresponding tetrasubstituted alkene 16, without iodination, which was transformed into the hydroxy ketone 17 by ozonolysis. In an improved procedure, the diol 15 was directly converted into the hydroxy ketone 17 without isolating the intermediate alkene 16, after the treatment of a solution of diol 15 in dichloromethane with I₂ and PPh₃ and further bubbling of this solution with an O₃/O₂ mixture. After protecting the ketone carbonyl group of compound 17 as ethylene ketal, the hydroxymethyl group was converted into the formyl group, thus obtaining the aldehyde 19. The diastereoselective α-methylation of the latter was efficiently achieved by treatment with MeI and t-BuOK in benzene, affording compound 10. The ketal aldehyde 10 after treatment with 1 M HCl in THF under reflux for 3 h underwent simultaneous ketone deprotection and intramolecular aldol condensation, leading to tricyclic α,β-ene one 20.

The next step was to address the construction of the aromatic D ring of the target compound (Scheme 3). Heating of dienol ester 9 with methyl propiolate in xylene in a sealed tube, and further oxidation with DDQ in dioxane at reflux,
Conclusions

In conclusion, the first synthesis of antitumoral dasyscyphin B (4) starting from commercial abietic acid (12) is reported. The optical rotation of the synthetic product, similar to that of the natural compound, confirms its absolute stereochemistry. The C ring of the starting material was efficiently transformed, utilizing conventional reactions realizable at a multigram scale, providing the intermediate alcohol 17. The appropriate configuration on C-8 was obtained after the diastereoselective α-methylation of aldehyde 19. The cyclopentane C ring of the target compound was obtained after intramolecular aldol condensation, and the aromatic D ring was constructed via a Diels–Alder cycloaddition involving dienol ester 9. The procedure reported here could also allow us to achieve the enantiospecific synthesis of dasyscyphins functionalized at the A ring, such as dasyscyphins A (3), D (6) or E (7).

Experimental section

General methods

Dichloromethane (DCM) was dried over calcium hydride. Benzene and tetrahydrofuran were dried over sodium–benzophenone. Chromatography separations were carried out by flash column chromatography on silica gel 60 (230–400 mesh), utilizing hexane–methyl tert-butyl ether (H–E) mixtures as an eluent.

Instrumentation: Infrared (IR) spectra were obtained with samples between sodium chloride plates. Data are presented as the frequency of absorption (cm$^{-1}$). Only selected absorbances ($\nu_{\text{max}}$) are reported. Proton and carbon-13 nuclear magnetic resonance (1H NMR or 13C NMR) spectra were recorded at 400 or 500 MHz, for 1H, and at 100 or 125 MHz, for 13C, as indicated in each case; chemical shifts are expressed in parts per million (δ scale) downfield from tetramethylsilane. Data are presented as follows: chemical shift, multiplicity (s = singlet, br = broad singlet, d = doublet, t = triplet, m = multiplet), J = coupling constant in hertz (Hz) and the signals of the 13C NMR were assigned utilizing DEPT experiments and on the basis of heteronuclear correlations. High resolution mass spectra (HRMS) were recorded using a Q-TOF spectrometer, utilizing the APcl ionization technique.

(1R,4aR,5R,8aR)-6-Formyl-1,4a-dimethyl-5-(4-methyl-3-oxopentyl)-1,2,3,4,4a,5,8,8a-octahydronaphthalene-1-carboxylic acid (13).10

To a solution of 12 (13 g, 43 mmol) in strictly deoxygenated t-BuOH (150 mL) were added trimethylamine N-oxide dihydrate (6.2 g, 51.6 mmol) and pyridine (1 mL) under an argon atmosphere. The solution was stirred for 10 min at room temperature and 2%aq. OsO4 (20 mL, 0.2%, 1.5 mmol) was added and the reaction mixture was further stirred under an argon atmosphere at reflux for 5 days, at which time TLC indicated no remaining starting material. Then NaO4 (12 g, 56.1 mmol) was added, and the mixture was stirred for 1 h at room temperature. After filtering and removing the solvent, the crude product was directly purified by flash chromatography on silica gel (20% ether–hexanes) to yield pure 13 (11.8 g, 82%) as a colorless syrup. $\delta_{\text{ppm}}$ (CDCl3, 300 MHz): δ = +42.1 (c 1.1, CHCl3). 1H NMR (CDCl3, 300 MHz): δ: 9.35 (s, 1H), 6.76 (t, J = 2.7 Hz, 1H), 3.10 (dd, J = 17.2, 10.8, 4.7 Hz, 1H), 2.62 (h, J = 6.9 Hz, 1H), 2.43 (dd, J = 17.0, 10.8, 5.7 Hz, 1H), 2.35–2.07 (m, 2H), 2.05–1.81 (m, 4H), 1.79–1.45 (m, 6H), 1.26 (s, 3H), 1.09 (d, J = 6.9 Hz, 6H), 0.82 (s, 3H). 13C NMR (CDCl3, 75 MHz): δ: 215.6 (C), 195.0 (CH), 184.4 (C), 152.3 (CH), 144.4 (C), 50.0 (CH), 46.1 (C), 44.0 (CH), 42.6 (CH2), 40.8 (CH), 37.8 (CH2), 37.1 (CH3), 36.4 (C), 26.8 (CH3), 20.9 (CH3), 18.5 (CH3), 18.4 (CH3), 17.7 (CH3), 16.9 (CH3), 14.3 (CH3). HRMS (APcl) m/z: caled for C26H34O3Na (M + Na+): 357.2042, found: 357.2037.
(1R,4aR,5R,8aR)-Methyl 6-formyl-1,4a-dimethyl-5-(4-methyl-3-oxopentyl)-1,2,3,4,4a,5,8a-octahydropyridin-1-carboxylate (10).11 Potassium carbonate (538 mg, 3.89 mmol) and methyl iodide were added to a stirred solution of 13 (1 g, 2.99 mmol) in acetone (30 mL) and the reaction mixture was stirred at reflux for 12 h, at which time TLC showed no starting material. The mixture was concentrated in vacuo to give a crude product, which was diluted with ether–water (50–20 mL) and the phases were separated and washed. The organic phase was washed with water and brine and dried over anhydrous Na2SO4. Removal of the solvent under vacuum afforded a crude product which was purified by flash chromatography on silica gel (10% ether–hexanes) affording pure (957 mg, 92%) as a colorless syrup. [α]D 25 = +9.0 (c = 12.5, CHCl3). 1H NMR (CDCl3, 500 MHz): δ 0.83 (s, 3H), 0.88 (m, 1H), 1.09 (m, 1H), 1.10 (d, J = 6.9 Hz, 3H), 1.10 (d, J = 6.9 Hz, 3H), 1.26 (s, 3H), 1.47–1.77 (m, 5H), 1.88 (m, 1H), 1.93–2.10 (m, 3H), 2.29 (m, 1H), 2.43 (dd, J = 17.0, 10.6, 5.7 Hz, 1H), 2.62 (h, J = 6.9 Hz, 1H), 3.11 (dd, J = 17.1, 10.9, 4.7 Hz, 1H), 3.65 (s, 3H), 6.75 (br s, 1H), 9.36 (s, 1H). 13C NMR (CDCl3, 125 MHz): δ 14.0 (CH3), 214.9 (C), 194.5 (CH), 178.2 (C), 152.0 (CH), 144.0 (C), 51.8 (CH3), 49.6 (CH), 45.9 (C), 43.9 (CH), 42.2 (CH2), 40.4 (CH), 37.5 (CH), 36.8 (CH3), 36.1 (CH1), 26.5 (CH2), 20.5 (CH3), 18.1 (CH1), 18.0 (CH2), 17.4 (CH2), 16.8 (CH3), 14.0 (CH). IR (film): 1724, 1688, 1461, 1245, 1187, 1144, 1008, 727, 671 cm–1. HRMS (Apel) m/z: calced for C22H36O4Na (M + Na+) 371.2198, found: 371.2206.

(1R,4aR,5S,6S,8aR)-Methyl 6-(hydroxymethyl)-1,4a-dimethyl-5-(4-methyl-3-oxopentyl)-decahydronaphthalene-1-carboxylate (11).11 To a solution of 11 (1.00 g, 2.87 mmol) in THF (20 mL) was added a 50% aqueous solution of Raney® Nickel (4 mL), and the mixture was stirred at room temperature for 2 days, at which time TLC showed no 11. Then, the reaction mixture was filtered through silica gel–Na2SO4 (15:3 g), washed with acetone (20 mL) and concentrated to give pure 11 (998 g, 97%) as a colorless syrup. [α]D 25 = +33.8 (c = 71.5, CHCl3). 1H NMR (CDCl3, 500 MHz): δ 0.73 (s, 3H), 0.93–1.05 (m, 2H), 1.08 (d, J = 6.9 Hz, 3H), 1.08 (d, J = 6.9 Hz, 3H), 1.13 (s, 3H), 1.29 (m, 1H), 1.39–1.62 (m, 7H), 1.67–1.85 (m, 5H), 1.96 (m, 1H), 2.40 (dd, J = 17.1, 9.1, 6.1 Hz, 1H), 2.53 (dd, J = 15.2, 10.0, 6.1 Hz, 1H), 2.59 (h, J = 6.9 Hz, 1H), 3.54 (dd, J = 10.1, 10.1 Hz, 1H), 3.64 (s, 3H). 13C NMR (CDCl3, 125 MHz): δ 215.1 (C), 179.3 (C), 61.3 (CH3), 52.7 (CH), 51.9 (CH2), 50.8 (CH), 47.7 (C), 41.0 (CH), 39.7 (CH), 38.9 (CH3), 38.2 (CH2), 37.6 (C), 36.8 (CH2), 28.8 (CH2), 20.6 (CH3), 19.2 (CH2), 19.2 (CH3), 18.4 (CH3), 18.3 (CH3), 17.8 (CH2), 16.4 (CH3), 15.9 (CH3). IR (film): 3472, 1712, 1459, 1386, 1248, 1141, 1023, 752 cm–1. HRMS (Apel) m/z: calced for C22H36O4Na (M + Na+) 375.2511, found: 375.2504.

(1R,4aR,5S,6S,8aR)-Methyl 5-(3-hydroxy-3,4-dimethylpent-3-enyl)-6-(hydroxymethyl)-1,4a-dimethyl-decahydronaphthalene-1-carboxylate (15). To a solution of 14 (10 g, 28.37 mmol) in anhydrous THF (70 mL) was added dropwise a solution of methylimagnesium bromide (50.7 mL of a 1.4 M solution in toluene–THF, 70.92 mmol) at 0 °C. The mixture was stirred under an argon atmosphere at room temperature for 3 h, at which time TLC showed no starting material. Then, 5% aqueous NH4Cl (15 mL) was added slowly at 0 °C and the mixture was extracted with ether (3 × 100 mL). The combined organic layers were washed with water and brine, and dried over anhydrous Na2SO4 and concentrated in vacuum. Purification by flash column chromatography on silica gel (15% ether–hexanes) gave 9.72 g of 15 (93%) (a 1:1 mixture of diastereomers) as a colourless oil. 1H NMR (CDCl3, 400 MHz): δ 3.74–3.66 (m, 2H), 3.65 (s, 6H), 3.55–3.47 (m, 2H), 2.00–1.85 (m, 4H), 1.79–1.64 (m, 8H), 1.61–1.39 (m, 12H), 1.36–1.22 (m, 8H), 1.14 (s, 6H), 1.07 (s, 6H), 0.99–0.92 (m, 4H), 0.91 (d, J = 6.7 Hz, 6H), 0.88 (d, J = 6.7 Hz, 6H), 0.74 (s, 6H). 13C NMR (CDCl3, 100 MHz): δ 179.3 (2C), 77.2 (C), 74.9 (C), 61.7 (CH3), 61.6 (CH3), 53.8 (CH), 53.7 (CH), 51.8 (2CH2), 50.8 (2CH2), 47.7 (2C), 39.7 (2CH2), 38.5 (CH2), 38.2 (CH3), 38.0 (CH3), 37.6 (2CH2), 37.6 (CH), 36.9 (CH2), 36.1 (CH), 29.7 (CH2), 29.2 (2CH2), 29.0 (CH2), 23.2 (CH3), 23.7 (CH2), 20.8 (CH2), 20.7 (CH2), 18.4 (CH3), 18.3 (CH3), 17.8 (CH2), 17.6 (2CH2), 16.9 (2CH2), 16.4 (2CH2), 16.1 (2CH2). IR (film): 3370, 1726, 1457, 1386, 1250, 1195, 1145, 1018, 914, 733 cm–1. HRMS (Apel) m/z: calcd for C22H28O4Na (M + H+) 369.3005, found: 369.3012.
temperature over 15 min and further stirred for 4 h. The solvent was removed, and the crude product was purified by flash column chromatography on silica gel (20% ether–hexanes) to give 1.72 g of 17 (93%) as a colourless oil. $\delta_{13}^{13} = +24.3$ (c 23.1, CHCl$_3$). $^1$H NMR (CDCl$_3$, 500 MHz) $\delta$: 3.64 (s, 3H), 3.63 (d, $J = 10.1$ Hz, 1H), 3.52 (dd, $J = 10.1, 10.1$ Hz, 1H), 2.55 (dd, $J = 10.7, 6.3$ Hz, 1H), 2.51 (dd, $J = 10.0, 5.6$ Hz, 1H), 2.38 (dd, $J = 9.5, 6.0$ Hz, 1H), 2.34 (dd, $J = 10.3, 6.7$ Hz, 1H), 2.13 (s, 3H), 1.95 (dd, $J = 9.0, 3.2$ Hz, 1H), 1.83–1.66 (m, 4H), 1.58–1.38 (m, 4H), 1.31–1.22 (m, 2H), 1.12 (s, 3H), 1.01–0.93 (m, 2H), 0.72 (s, 3H). $^{13}$C NMR (CDCl$_3$, 125 MHz) $\delta$: 209.45 (C), 179.39 (C), 61.34 (CH$_3$), 52.71 (CH), 50.21 (CH$_2$), 50.87 (CH), 47.83 (C), 42.45 (CH$_3$), 39.76 (CH$_2$), 38.33 (CH), 37.63 (C), 36.94 (CH$_2$), 30.19 (CH$_3$), 28.89 (CH$_2$), 20.75 (CH$_2$), 19.35 (CH$_2$), 17.93 (CH$_2$), 16.50 (CH$_3$), 16.05 (CH$_3$). IR (film): 3477, 1720, 1672, 1582, 1517 cm$^{-1}$. HRMS (APcI)$\text{m/z}$: calcd for C$_{19}$H$_{33}$O$_4$ (M + H$^+$) 325.2379, found: 325.2371.

(1R,4aR,5S,6S,8aR)-Methyl 6-(hydroxymethyl)-1,4a-dimethyl-5-(3-oxobutyl)-decahydrophthalalene-1-carboxylate (17). Iodine (2.10 g, 8.26 mmol) was added to a solution of triphenylphosphine (2.2 g, 8.39 mmol) was added. Following the same work-up used for 17 from 16, 2.35 g of 17 (89%) was obtained as a colourless oil.

(1R,4aR,5S,8aR)-Methyl 6-formyl-1,4a-dimethyl-5-(2-(2-methyl-1,3-dioxolan-2-yl)-ethyl)-decahydrophthalalene-1-carboxylate (19). Pyridinium dichromate (PDC; 5.33 g, 14.16 mmol) was added to a stirred solution of 18 (2.61 g, 7.08 mmol) in dry CH$_2$Cl$_2$ (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 a mixture of ether (20 mL). The filtrate was washed with a 1 N HCl solution (20 mL) and brine and dried over anhydrous Na$_2$SO$_4$. The solvent was evaporated to yield 2.39 g of aldehyde 19 (92%) as a colourless syrup. $\delta_{13}^{13} = +23.7$ (c 39.4, CHCl$_3$). $^1$H NMR (CDCl$_3$, 500 MHz) $\delta$: 9.97 (s, 1H), 3.99–3.86 (m, 4H), 3.65 (s, 3H), 2.51 (t, $J = 4.9$ Hz, 1H), 2.31 (br d, $J = 14.6$ Hz, 1H), 1.97–1.86 (m, 1H), 1.81–1.68 (m, 5H), 1.65–1.51 (m, 5H), 1.46 (dd, $J = 12.9, 12.9$, 3.6 Hz, 1H), 1.34 (d, $J = 3.2$ Hz, 1H), 1.18 (m, 1H), 1.12 (s, 3H), 1.05 (m, 1H), 0.76 (s, 3H). $^{13}$C NMR (CDCl$_3$, 125 MHz) $\delta$: 204.8 (CH), 179.3 (C), 110.2 (C), 64.8 (2CH$_2$), 53.9 (CH), 52.1 (CH$_3$), 50.2 (CH), 47.7 (C), 47.6 (CH$_3$), 38.3 (C), 38.0 (2CH$_2$), 37.1 (CH$_2$), 26.0 (CH$_3$), 23.9 (CH$_3$), 21.8 (CH$_3$), 19.3 (CH$_3$), 18.0 (CH$_2$), 16.5 (CH$_3$), 15.5 (CH$_3$). IR (film): 1724, 1448, 1388, 1248, 1061, 754 cm$^{-1}$. HRMS (APcI)$\text{m/z}$: calcd for C$_{25}$H$_{33}$O$_6$Na (M + Na$^+$) 389.2354, found: 389.2306.

(3aR,5aR,5S,6S,8aR)-Methyl 2-acetyl-3a,6,9a-trimethyl-1,3-dioxolan-2-yl)-ethyl-decahydrophthalalene-1-carboxylate (20). Potassium tert-butoxide (252 mg, 2.249 mmol) was added to a stirred solution of 19 (633 mg, 1.73 mmol) in dry benzene (30 mL) under an argon atmosphere. After 5 min methyl iodide (0.4 mL, 5.19 mmol) was added and the reaction mixture was stirred at room temperature for 2 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 (MgSO$_4$) and concentrated under reduced pressure to give the title compound 20 (11.80 g, 98%) as a colourless oil. $\delta_{13}^{13} = +16.8$ (c 17.5, CHCl$_3$). $^1$H NMR (CDCl$_3$, 500 MHz) $\delta$: 3.73–3.87 (m, 4H), 3.67 (dd, $J = 10.3, 10.3$ Hz, 1H), 3.64 (s, 3H), 3.52 (dd, $J = 10.3, 10.3$ Hz, 1H), 2.15 (d, $J = 9.5$ Hz, 1H), 1.96 (d, $J = 7.4$ Hz, 1H), 1.87 (d, $J = 9.4$ Hz, 1H), 1.80–1.67 (m, 4H), 1.60–1.47 (m, 4H), 1.46–1.38 (m, 2H), 1.31 (s, 3H), 1.30–1.22 (m, 2H), 1.13 (s, 3H), 1.03–0.93 (m, 2H), 0.73 (s, 3H). $^{13}$C NMR (CDCl$_3$, 125 MHz) $\delta$: 197.4 (C), 110.4 (C), 64.8 (2CH$_2$), 61.5 (CH$_2$), 53.3 (CH$_2$), 52.0 (CH$_2$), 51.0 (CH), 47.9 (C), 39.9 (CH), 38.4 (CH$_2$), 37.7 (CH), 37.7 (C), 37.0 (CH$_2$), 28.9 (CH$_3$), 27.1 (CH$_3$), 23.9 (CH$_2$), 20.8 (CH$_3$), 19.5 (CH$_2$), 18.0 (CH$_3$), 16.6 (CH$_3$), 16.2 (CH$_3$). IR (film): 3498, 1783, 1724, 1580, 1451, 1389, 1247, 1187 cm$^{-1}$. HRMS (APcI)$\text{m/z}$: calcd for C$_{21}$H$_{27}$O$_3$ (M + H$^+$) 369.2641, found: 369.2653.
temperature and the solvent was evaporated in vacuo. Then, the residue was dissolved in ether (30 mL) and washed with water (2 × 10 mL) and brine (2 × 10 mL). The organic phase was dried over anhydrous Na2SO4 and concentrated under vacuum. The organic phase was dried over anhydrous Na2SO4 and concentrated to give a crude product which was chromatographed on silica gel (10% ether–hexanes) to give 552 mg of the 6:1 mixture of compound 21 and its regioisomer (92%) as a colourless syrup. A further chromatography of this mixture on silica gel (5% ether–hexanes) provided 420 mg of the pure regioisomer 21 (70%). [α]D20 = +21.0 (c 13.5, CHCl3).

1H NMR (CDCl3, 500 MHz) δ: 7.35 (d, J = 8.3 Hz, 1H), 6.88 (d, J = 8.3 Hz, 1H), 3.86 (s, 3H), 3.66 (s, 3H), 2.87 (dd, J = 16.7, 7.1 Hz, 1H), 2.57 (d, J = 16.6 Hz, 1H), 2.40 (dt, J = 14.9, 4.0 Hz, 1H), 2.31 (s, 3H), 1.82 (d, J = 7.1 Hz, 1H), 1.78 (dd, J = 11.7, 2.8 Hz, 1H), 1.75–1.66 (m, 3H), 1.66–1.51 (m, 3H), 1.48 (dt, J = 17.5, 6.5 Hz, 1H), 1.34 (s, 3H), 1.24 (m, 1H), 1.08 (s, 3H), 1.00 (dd, J = 12.5, 12.5, 4.9, 1H), 0.29 (s, 3H). 13C NMR (CDCl3, 125 MHz) δ: 179.5 (C), 169.5 (C), 168.5 (C), 151.8 (C), 148.6 (C), 137.4 (C), 128.7 (C), 125.9 (C), 119.5 (CH), 52.2 (CH2), 52.0 (CH3), 49.5 (C), 48.0 (CH), 47.3 (C), 39.7 (CH2), 37.0 (CH2), 36.8 (C), 33.7 (CH2), 31.8 (CH3), 28.4 (CH2), 22.1 (CH2), 21.0 (CH3), 17.7 (CH2), 17.1 (CH3), 15.6 (CH3). IR (film): 1768, 1725, 1448, 1252, 1207, 1162, 986, 755 cm⁻¹. HRMS (Apel) m/z: calcd for C23H28O2Na (M + Na⁺) 465.2253, found: 465.2246.

(4R,4aR,6aS,11aR,11bR)-Dimethyl 10-hydroxy-4,6a,11-tri-methyl-7,3a,4,5,6,7,8,9,9a,9b-decahydro-1H-benzo[a]fluorene-4,7-dicarboxylate (22). Conc. hydrochloric acid (1 mL) was added to a stirred solution of 21 (440 mg, 0.99 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 under 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 Na2SO4. Removal of the solvent under vacuum afforded a crude product which was directly purified by flash chromatography (35% ether–hexanes) to yield 386 mg of 22 (97%) as a colourless syrup. [α]D20 = +17.8 (c 11.5, CHCl3).

1H NMR (CDCl3, 500 MHz) δ: 7.35 (d, J = 8.3 Hz, 1H), 6.60 (d, J = 8.3 Hz, 1H), 5.31 (s, 1H), 3.84 (s, 3H), 3.66 (s, 3H), 2.87 (dd, J = 16.1, 7.1 Hz, 1H), 2.67 (d, J = 16.1 Hz, 1H), 2.45 (dt, J = 14.8, 4.3 Hz, 1H), 1.84 (d, J = 7.1 Hz, 1H), 1.79 (dd, J = 11.5, 3.1 Hz, 1H), 1.77–1.69 (m, 3H), 1.68–1.60 (m, 2H), 1.55 (m, 1H), 1.49 (dt, J = 10.1, 3.1 Hz, 1H), 1.37 (s, 3H), 1.24 (m, 1H), 1.08 (s, 3H), 1.02 (dd, J = 12.7, 12.7, 5.2 Hz, 1H), 0.31 (s, 3H). 13C NMR (CDCl3, 125 MHz) δ: 179.6 (C), 169.6 (C), 153.9 (C), 152.6 (C), 130.4 (C), 130.1 (CH), 120.8 (C), 120.9 (CH3), 63.5 (CH), 52.1 (CH2), 51.9 (CH2), 49.6 (C), 48.0 (CH), 47.3 (C), 39.9 (CH2), 37.0 (CH2), 36.9 (C), 33.4 (CH2), 31.5 (CH3), 27.4 (CH2), 22.2 (CH2), 17.8 (CH2), 17.1 (CH3), 15.7 (CH). IR (film): 3413, 1712, 1700, 1583, 1434, 1238, 1134, 757 cm⁻¹. HRMS (Apel) m/z: calcd for C24H21O3 (M + H⁺) 401.2328, found: 401.2331.
was added to a stirred suspension of 22 (350 mg, 0.87 mmol) and K$_2$CO$_3$ (180 mg, 1.30 mmol) in acetone (10 mL) under an argon atmosphere. The mixture was heated under reflux overnight. Then, the solvent was evaporated in vacuo, ether (40 mL) was added and the mixture was washed with water (2 x 10 mL) and brine (1 x 10 mL). The organic phase was dried over anhydrous Na$_2$SO$_4$ and concentrated to give a crude product which was purified by flash chromatography (10% ether–hexanes) to give 353 mg (98%) of 23 as a colourless syrup. [α]$_D^{25}$ = +21.1 (c 13.1, CHCl$_3$). $^1$H NMR (CDCl$_3$, 400 MHz) δ: 7.45 (d, J = 8.5 Hz, 1H), 6.55 (d, J = 8.5 Hz, 1H), 3.85 (s, 3H), 3.84 (s, 3H), 3.66 (s, 3H), 2.84 (dd, J = 17.0, 6.8 Hz, 1H), 2.72 (d, J = 16.7 Hz, 1H), 2.43 (dt, J = 14.8, 4.4 Hz, 1H), 1.83–1.76 (m, 9H), 1.68 (ddd, J = 14.0, 13.5, 8.2 Hz, 2H), 1.57–1.44 (m, 3H), 1.37 (s, 3H), 1.30–1.21 (m, 2H), 1.12 (m, 1H), 1.08 (s, 3H), 1.02 (m, 1H), 0.29 (s, 3H). $^{13}$C NMR (CDCl$_3$, 100 MHz) δ: 179.6 (C), 169.6 (C), 157.6 (C), 151.9 (C), 132.8 (C), 130.1 (CH), 120.5 (C), 107.7 (CH), 63.4 (CH), 55.4 (CH$_2$), 52.0 (CH$_3$), 51.9 (CH$_3$), 49.6 (C), 47.9 (CH), 47.3 (C), 39.8 (CH$_2$), 37.0 (C), 36.9 (CH$_3$), 33.3 (CH$_3$), 31.4 (CH$_2$), 28.0 (CH$_2$), 22.1 (CH$_3$), 17.8 (CH$_3$), 17.1 (CH), 15.7 (CH). IR (film): 3481, 1675, 1451, 1259, 1129, 1050, 757 cm$^{-1}$. HRMS (Apel) m/z: cale for C$_{27}$H$_{38}$O$_3$ (M + Na$^+$) 437.2304, found: 437.2297.

(4R,4aR,6aS,11aR,11bR)-4-(Hydroxymethyl)-10-methoxy-4,6a,11b-trimethyl-2,3,4,4a,5,6,6a,11,11a,11b-dodecahydro-1H-benzo[a]fluorene-7-yl formate (26). To a solution of triphenylphosphine (148 mg, 0.56 mmol) in dry CH$_2$Cl$_2$ (10 mL) was added slowly iodine (503 mg, 1.98 mmol) to a stirred solution of alcohol (148 mg, 97%) as a colourless syrup. [α]$_D^{25}$ = +4.2 (c 8.5, CHCl$_3$). $^1$H NMR (CDCl$_3$, 500 MHz) δ: 7.10 (d, J = 8.1 Hz, 1H), 6.64 (d, J = 8.3 Hz, 1H), 4.71 (s, 2H), 3.82 (s, 3H), 3.41 (d, J = 10.3 Hz, 1H), 3.13 (d, J = 10.6 Hz, 1H), 2.85 (dd, J = 16.9, 7.5 Hz, 1H), 2.73 (d, J = 17.0 Hz, 1H), 2.59 (d, J = 14.5 Hz, 1H), 1.81–1.67 (m, 3H), 1.66–1.35 (m, 5H), 1.25 (s, 3H), 1.03–0.80 (m, 3H), 0.73 (s, 3H). $^{13}$C NMR (CDCl$_3$, 100 MHz) δ: 155.4 (C), 151.0 (C), 132.0 (C), 129.3 (C), 127.9 (C), 108.0 (CH), 72.2 (CH), 63.1 (CH$_2$), 62.4 (CH$_3$), 55.3 (CH$_2$), 49.6 (C), 46.1 (CH), 40.4 (CH$_2$), 37.7 (C), 37.2 (C), 35.5 (CH$_2$), 34.8 (CH$_3$), 32.6 (CH$_3$), 28.3 (CH$_2$), 19.7 (CH$_2$), 17.9 (CH$_3$), 17.9 (CH$_3$). IR (film): 3357, 1727, 1457, 1434, 1259, 1188, 1138, 757 cm$^{-1}$. HRMS (Apel) m/z: cale for C$_{23}$H$_{33}$O$_4$ (M + H$^+$) 373.2379, found: 373.2386.

(4R,4aR,6aS,11aR,11bR)-4-(Iodomethyl)-10-methoxy-4,6a,11b-trimethyl-2,3,4,4a,5,6,6a,11,11a,11b-decachydro-1H-benzo[a]fluorene-7-yl formate (26). To a solution of triphenylphosphine (148 mg, 0.56 mmol) in dry CH$_2$Cl$_2$ (10 mL) was added slowly iodine (503 mg, 1.98 mmol) to a stirred solution of alcohol (148 mg, 97%) as a colourless syrup. [α]$_D^{25}$ = +4.2 (c 8.5, CHCl$_3$). $^1$H NMR (CDCl$_3$, 400 MHz) δ: 7.10 (d, J = 8.1 Hz, 1H), 6.64 (d, J = 8.3 Hz, 1H), 4.71 (s, 2H), 3.82 (s, 3H), 3.41 (d, J = 10.3 Hz, 1H), 3.13 (d, J = 10.6 Hz, 1H), 2.85 (dd, J = 16.9, 7.5 Hz, 1H), 2.73 (d, J = 17.0 Hz, 1H), 2.59 (d, J = 14.5 Hz, 1H), 1.81–1.67 (m, 3H), 1.66–1.35 (m, 5H), 1.25 (s, 3H), 1.03–0.80 (m, 3H), 0.73 (s, 3H). IR (film): 3357, 1727, 1457, 1434, 1138, 757 cm$^{-1}$. HRMS (Apel) m/z: cale for C$_{23}$H$_{33}$O$_4$ (M + H$^+$) 373.2379, found: 373.2386.
over anhydrous Na2SO4. Removal of the solvent under vacuum afforded a crude product which was directly purified by flash chromatography on silica gel (5% ether–hexanes) to give 27 (229 mg, 77%) as a colourless oil. [α]D25 = −32.8 (c 11.9, CHCl3).

1H NMR (CDCl3, 500 MHz) δ: 8.30 (s, 1H), 6.79 (d, J = 8.6 Hz, 1H), 6.65 (d, J = 8.7 Hz, 1H), 3.91 (dd, J = 17.4, 8.2 Hz, 1H), 2.76 (dd, J = 17.3, 2.0 Hz, 1H), 2.32 (dt, J = 14.4, 6.2 Hz, 1H), 1.78 (dd, J = 8.3, 1.9 Hz, 1H), 1.75 (m, 1H), 1.70 (dt, J = 12.9, 3.0 Hz, 1H), 1.54–1.42 (m, 2H), 1.37–1.30 (m, 3H), 1.24 (s, 3H), 1.18 (s, J = 4.9 Hz, 1H), 1.11 (d, J = 12.3, 0.9 Hz, 3H), 0.73 (s, 3H), 0.93 (m, 1H), 0.53 (s, 3H). 13C NMR (CDCl3, 125 MHz) δ: 160.4 (CH), 153.7 (C), 143.5 (C), 139.6 (C), 133.1 (C), 121.1 (CH), 108.9 (CH), 62.1 (CH3), 55.6 (CH3), 48.6 (C), 48.1 (CH), 40.9 (CH2), 39.3 (CH2), 37.2 (C), 35.8 (C), 32.8 (C), 31.2 (CH3), 29.2 (CH3), 28.7 (CH3), 18.7 (CH3), 18.6 (CH3), 18.2 (CH2), 15.6 (CH3). 13C NMR (CDCl3, 125 MHz) δ: 152.0 (C), 147.4 (C), 145.7 (C), 132.6 (C), 114.9 (C), 113.6 (CH), 62.3 (CH), 61.6 (CH), 55.8 (CH3), 52.8 (CH2), 49.4 (C), 42.2 (CH2), 41.0 (CH2), 37.4 (C), 34.2 (CH3), 33.6 (CH3), 33.3 (C), 32.7 (CH3), 28.6 (CH2), 22.1 (CH3), 20.0 (CH2), 18.6 (CH), 15.5 (CH3). IR (film): 3525, 1474, 1451, 1268, 1224, 1175, 1061, 1013, 771, 669 cm−1. HRMS (APel) m/z: calculated for C22H18BrO2 (M + H+) 407.1586, found: 407.1586.

(4aS,6aS,11αR,11αs)-8-Bromo-7,10-dimethoxy-4,4,6a,11b-tetramethyl-2,3,4,4a,5,6,6a,11,11a,11b-decahydro-1H-benzo[a]fluorene (8). Methyl iodide (139 mg, 0.98 mmol) was added to a stirred suspension of 29 (305 mg, 0.75 mmol) and K2CO3 (155 mg, 1.13 mmol) in acetone (25 mL) under an argon atmosphere. The mixture was heated under reflux overnight. Then, the solvent was evaporated in vacuo, ether (40 mL) was added and the mixture was washed with water (2 × 10 mL) and brine (1 × 10 mL). The organic phase was dried over anhydrous Na2SO4 and concentrated to give a crude product which was purified by flash chromatography (5% ether–hexanes) to give 306 mg (97%) as a colorless syrup. [α]D25 = +0.2 (c 8.7, CHCl3). 1H NMR (CDCl3, 500 MHz) δ: 6.82 (s, 1H), 3.82 (s, 3H), 2.76 (ddd, J = 10.4, 8.3, 4.2 Hz, 1H), 2.65 (d, J = 16.8 Hz, 1H), 1.71 (br d, J = 11.3 Hz, 1H), 1.67–1.57 (m, 3H), 1.48 (m, 1H), 1.43–1.24 (m, 5H), 1.15 (ddd, J = 13.1, 13.1, 4.0 Hz, 1H), 0.95 (ddd, J = 11.5, 3.3 Hz, 1H), 1.22 (s, 3H), 0.88 (s, 3H), 0.78 (s, 3H), 0.41 (s, 3H). 13C NMR (CDCl3, 125 MHz) δ: 152.0 (C), 147.4 (C), 145.7 (C), 132.6 (C), 114.9 (C), 113.6 (CH), 62.3 (CH), 61.6 (CH), 55.8 (CH3), 52.8 (CH2), 49.4 (C), 42.2 (CH2), 41.0 (CH2), 37.4 (C), 34.2 (CH3), 33.6 (CH3), 33.3 (C), 32.7 (CH3), 28.6 (CH2), 22.1 (CH3), 20.0 (CH2), 18.6 (CH), 15.5 (CH3). IR (film): 1595, 1472, 1427, 1224, 1052, 966, 828, 770 cm−1. HRMS (APel) m/z: calculated for C22H18BrO2Na (M + Na+) 443.1562, found: 443.1557.
Na$_2$SO$_4$ and the solvent was evaporated to give a crude product (dd, 1H), 1.23 (s, 3H), 1.15 (ddd, 5H), 0.97 (dd, J = 11.5, 3.2 Hz, 1H), 0.95 (dd, J = 13.2, 13.2, 4.0 Hz, 1H), 0.95 (dd, J = 11.4, 3.1 Hz, 1H), 0.88 (s, 3H), 0.77 (s, 3H), 0.39 (s, 3H). $^{13}$C NMR (CDCl$_3$, 125 MHz): δ = 151.8 (C), 148.9 (C), 144.3 (C), 132.8 (C), 130.4 (C), 110.1 (CH), 69.9 (CH$_2$), 62.9 (CH), 62.6 (CH), 58.5 (CH$_2$), 55.6 (CH$_2$), 52.9 (CH$_3$), 48.9 (C), 42.3 (CH$_2$), 41.1 (CH$_2$), 37.4 (C), 34.4 (CH$_2$), 33.7 (CH), 33.3 (C), 32.8 (CH$_2$), 28.6 (CH$_2$), 22.1 (CH$_2$), 20.2 (CH$_2$), 18.6 (CH$_2$), 15.5 (CH$_3$). IR (film): 1595, 1464, 1378, 1319, 1218, 1191, 1054, 990 cm$^{-1}$. HRMS (Apel) m/z: calcd for C$_{23}$H$_{33}$O$_6$Na (M + Na$^+$) 409.2719, found: 409.2726.

$^{44}$As,6$^a$As,6$^a$Na,11$^a$R,11$^b$S)-8-(Methoxymethyl)-4,4,6,6a,11b-tetramethyl-2,3,4,4$^a$,5,6$^a$,6$^a$,11$^a$,11b-decahydro-1H-benzo[a]fluorene-7,10-dione (33). AgO (124 mg, 1.00 mmol) was added to a stirred solution of 32 (110 mg, 0.20 mmol) in 1,4-dioxane (6 mL) at 4°C. After 5 min, HNO$_3$ (4 N, 0.1 mL) was added dropwise and the mixture was stirred for a further 15 min, at which time TLC showed no starting material. Then, the reaction mixture was poured into H$_2$O (60 mL) and extracted with CH$_2$Cl$_2$ (3 × 50 mL). The combined organic layers were dried over anhydrous Na$_2$SO$_4$ and the solvent was evaporated to give a crude product which was purified by flash chromatography on silica gel (10% ether–hexanes) affording 90 mg of pure 33 (89%) as a yellow syrup. $[^{13}$C$_{[CD]}=+1.5$ (C, 3.3, CHCl$_3$). $^{1}$H NMR (CDCl$_3$, 600 MHz): δ = 6.63 (s, 1H), 4.28 (d, J = 2.2 Hz, 1H), 4.27 (d, J = 2.2 Hz, 1H), 3.45 (s, 3H), 2.73 (dd, J = 19.6, 8.9 Hz, 1H), 2.61 (dd, J = 19.6, 2.8 Hz, 1H), 2.17 (d, J = 14.3, 7.4 Hz, 1H), 1.80 (d, J = 14.5, 7.6 Hz, 1H), 1.73–1.63 (m, 2H), 1.60 (br d, J = 13.1 Hz, 1H), 1.46–1.30 (m, 3H), 1.25 (s, 3H), 1.15 (ddd, J = 13.2, 13.2, 3.3 Hz, 1H), 1.01 (dd, J = 10.9, 6.4 Hz, 1H), 0.97–0.87 (m, 2H), 0.86 (s, 3H), 0.85 (s, 3H), 0.71 (s, 3H). $^{13}$C NMR (CDCl$_3$, 150 MHz): δ = 187.3 (C), 186.1 (C), 153.6 (C), 147.5 (C), 146.2 (C), 131.0 (CH), 68.1 (CH$_2$), 59.9 (CH$_3$), 59.3 (CH), 49.9 (CH), 49.6 (C), 42.3 (CH$_2$), 41.8 (CH$_2$), 36.9 (C), 33.5 (C), 33.0 (CH$_2$), 29.8 (CH$_2$), 29.5 (CH$_2$), 21.6 (CH$_2$), 19.0 (CH$_2$), 18.5 (CH$_2$), 16.0 (CH$_2$). IR (film): 1732, 1651, 1456, 1107, 772, 669 cm$^{-1}$. HRMS (Apel) m/z: calcd for C$_{23}$H$_{32}$O$_6$Na (M + Na$^+$) 379.2249, found: 379.2244.

**Dasyacchin B (4).** Na$_2$SO$_4$ (157 mg, 0.90 mmol) was added to a suspension of quinone 33 (35 mg, 0.098 mmol) in 8 mL of CHCl$_3$–H$_2$O (1:1) and the mixture was stirred at room temperature for 4 h, at which time TLC showed no starting material. Then, CHCl$_3$ was removed under vacuum, and the mixture was diluted with ether (25 mL) and the phases were shaken and separated. The organic layer was washed with water and brine, and dried over Na$_2$SO$_4$. The solvent was evaporated to give a crude product which was purified by flash chromatography on silica gel (30% ether–hexanes) affording 22 mg of pure 4 (63%) as a colorless syrup. $[^{13}$C$_{[CD]}=-12$ (C, 0.2, CHCl$_3$). $^{1}$H NMR (CDCl$_3$, 600 MHz): δ = 6.31 (s, 1H), 4.59 (d, J = 11.8 Hz, 1H), 4.51 (d, J = 11.8 Hz, 1H), 3.42 (s, 3H), 2.70 (m, 1H), 2.62 (d, J = 16.4, 1H), 1.75–1.67 (m, 3H), 1.64–1.52 (m, 3H), 1.42–1.25 (m, 3H), 1.24 (s, 3H), 1.20–0.90 (m, 3H), 0.88 (s, 3H), 0.80 (s, 3H), 0.51 (s, 3H). $^{13}$C NMR (CDCl$_3$, 150 MHz): δ = 146.6 (C), 144.1 (C), 139.2 (C), 130.3 (C), 121.4 (C), 113.0 (CH), 74.4 (CH$_2$), 62.2 (CH), 58.2 (CH$_2$), 52.0 (CH), 48.5 (C), 42.2 (CH$_2$), 41.5 (CH$_2$), 37.3 (C), 33.5 (C), 33.3 (CH$_2$), 30.8 (CH$_3$), 28.3 (CH$_2$), 22.0 (CH$_3$),...
19.7 (CH$_3$)$_2$, 18.6 (CH$_2$)$_2$, 15.6 (CH$_3$)$_2$. HRMS (APCI) m/z: calcd for C$_{13}$H$_{15}$O$_3$ (M + H$^+$) 359.2586, found: 359.2590.

**Acknowledgements**

The authors thank the Spanish Ministry of Science and Innovation (project CTQ2009-09932) and the Regional Government of Andalucia (project P11-CTS-7651 and assistance to the FQM-348 group) for financial support. A.F. thanks the Spanish Ministry of Science and Innovation for the postdoctoral grant provided.

**Notes and references**