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Hydroxylation of eleuthoside synthetic intermediates by $P450_{BM3}$ (CYP102A1)

Leonidas-Dimitrios Syntrivanis,[a] Luet Lok Wong*[b] and Jeremy Robertson*[a]

Abstract: A seven-step synthesis of the first tricyclic intermediate in Danishefsky's total synthesis of eleutherobin is reported, with Wittig homologation and sequential additions of metallated furan derivatives to aldehydes as key steps. Aiming to prepare hydroxylated eleutherobin analogues for cytotoxicity screening, the furan diol epimers **6** and precursors **1** and **2** were exposed to a 48-member panel of engineered cytochrome $P450_{BM3}$ variants. Both furan-containing substrates reacted solely at the furan ring, one resulting in overall biocatalytic Achmatowicz reaction. Selective hydroxylation at four separate sp^3 C–H centres was achieved in the substrates lacking the furan component. $P450_{BM3}$-catalysed hydroxylation of racemic substrates can proceed with kinetic resolution; in the case reported here, the 2°-allylic alcohol product **10** was generated with an 82:18 enantiomeric ratio.

Introduction

The development of methods for selective C–H bond oxidation at unactivated aliphatic positions in complex molecules facilitates the implementation of late-stage oxidation strategies for natural product synthesis and drug discovery programmes.[1] Biocatalytic methods hold great promise in this regard, as protein engineering can lead to selective transformations that are inaccessible to the chemical reagents currently available. Among these, the cytochrome P450 monooxygenases, a family of heme proteins that play important roles in xenobiotic metabolism and in the biosynthesis of secondary metabolites, are pre-eminent.[2] The $P450_{BM3}$ (CYP102A1) from *Bacillus megaterium* has attracted considerable interest as it contains a reductase domain fused to the *C*-terminus of the monooxygenase domain, endowing it with catalytic self-sufficiency and some of the highest turnover activity of all the P450s. Furthermore, $P450_{BM3}$ has been expressed to high levels in *Escherichia coli*, and kilogram-scale reactions have been reported.[3] These attributes suit the needs of academic synthetic chemistry groups, and applications of $P450_{BM3}$ variants for late-stage oxidation are becoming more frequently reported.[4] Notable recent developments include the production of chiral metabolites from achiral precursors with, in some cases, impressive enantioselectivity, and inclusion of $P450_{BM3}$-mediated oxidation as a key step in natural product total synthesis.[5]

In this paper, we describe the application of a panel of engineered $P450_{BM3}$ variants[6,7] to the oxidation of terpenoid structures of relevance to the synthesis of hydroxylated analogues of eleutherobin (Fig. 1), a diterpene isolated from the soft coral *Eleutherobia* sp.[8] Eleutherobin is cytotoxic towards a variety of human cancer cell lines with a potency comparable to that of taxol and a similar mechanism of action: induction of tubulin polymerisation and microtubule stabilisation.[9] Two total syntheses of eleutherobin have been accomplished to date[10] but the overall quantities obtained have not permitted an in-depth investigation of its therapeutic potential,[11] and medicinal chemistry studies on the compound have focused primarily on substitution of the side-chain substituents.[12] Investigation of analogues in which the tricyclic core has been modified *via*, for example, hydroxylation, has been limited. Such an endeavour is also of interest for developing strategies for the synthesis of eleuthoside natural products that bear additional oxidised sites (Fig. 1).[13] Conceptually, such analogues could be made readily-available by (biocatalytic) hydroxylation at a late stage of a fully-formed eleuthoside core, or by hydroxylation of a mid-stage intermediate followed by parallel synthesis from the so-formed alcohol regioisomers.

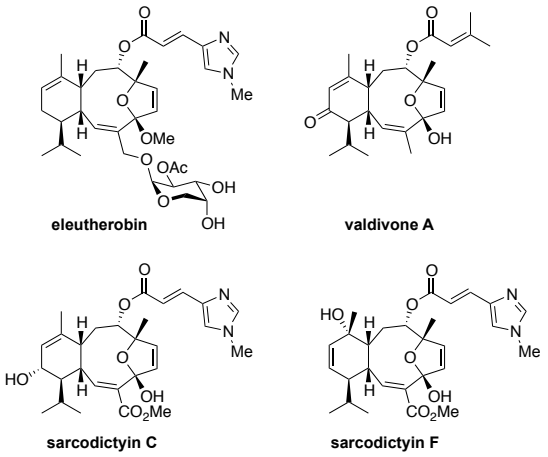

Figure 1. Eleutherobin, and related natural products bearing additional sites of oxidation in the cyclohexene ring.

Results and Discussion

A short route was developed to furanoid eleuthoside **6** (Scheme 1), since Danishefsky's group, as part of their total synthesis of eleutherobin,[10b,d] had demonstrated that the furan ring was a suitable precursor for the dihydrofuran via an Achmatowicz

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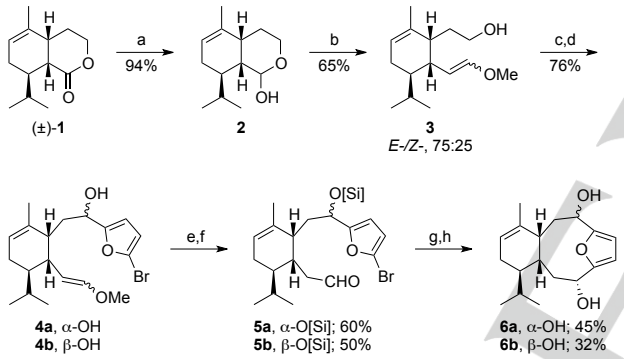
reaction / nucleophilic addition / isomerisation sequence. This route is strategically similar to Danishefsky's but differs tactically in two main respects: (1) the *cis*-,*trans*-stereochemistry around the cyclohexene ring is established in lactone **1** by a Diels–Alder cycloaddition as described by us recently[14] rather than by dichloroketene addition to α-phellandrene; and (2) one-carbon homologation was effected at an earlier stage, in our sequence, and the method differs from the four-step sequence employed in Danishefsky's route. The route began with reduction of racemic lactone **1** to lactol **2** then homologation to enol ether **3**. Swern oxidation of the primary alcohol and addition of 2-bromo-5-lithiofuran afforded an approximately equimolar mixture of epimers **4a** and **4b** which were separated and protected as the corresponding TBDPS ethers (not shown). The enol ether functionality was then hydrolysed to give aldehydes **5a** and **5b** prior to Nozaki–Hiyama–Kishi coupling, following Danishefsky's precedent, to give tricyclic epimeric diols **6a** and **6b** after deprotection. These compounds, containing all but two of the carbon atoms present in the eleuthoside tricyclic core, were chosen as the initial hydroxylation substrates. The preparation of the immediate precursor to epimer **6a** (i.e. the TBDPS-protected intermediate) constitutes a formal synthesis of eleutherobin, linking with Danishefsky's enantiospecific synthesis from (*R*)-(–)-phellandrene.



Scheme 1. Synthetic route to furanoid eleuthosides **6a,b**. a) DIBAL, CH_2Cl_2, –78 °C; b) $Ph_3P^+CH_2OMe\ Cl^-$, *t*-BuOK, THF, –78 °C to RT; c) DMSO, $(COCl)_2$, CH_2Cl_2, –78 °C then Et_3N, –78 °C to RT; d) 2,5-dibromofuran/BuLi, THF, –78 °C to RT; e) *t*-$BuPh_2SiCl$, imidazole, CH_2Cl_2; f) $Hg(OCOCF_3)_2$, THF/H_2O (10:1), 0 °C then aq KCl; g) $CrCl_2$, $NiCl_2$, DMF, –20 °C to RT; h) Bu_4NF, THF. [Si]: *t*-$BuPh_2Si$; DIBAL: *i*-Bu_2AlH.

The library of $P450_{BM3}$ variants used in this study is based on four variants known from our previous work to oxidise a wide range of organic compounds.[6,7] Additional mutations were introduced at two or more active site residues to generate variants with diverse substrate binding pocket topology. Screening of the two eleuthoside precursors **6a** and **6b** against a 24-member subset of this $P450_{BM3}$ library gave no evidence of hydroxylation of the alicyclic region of the molecules, with reaction being confined to the strained and electron-rich furan ring. The two epimers behaved differently towards the P450 panel, the *cis*-diol undergoing overall (redox-neutral) hydrolysis to diketone **7** (Fig. 2), the *trans*-diol being converted into hydroxypyrone **8** in an overall Achmatowicz reaction.[15] These primary products were characterised following scale-up using mutant GVQ/IG[16] for **7** (61%, from 0.018 mmol **6a**) and mutant RP/IA/EV for **8** (46%, from 0.012 mmol **6b**).

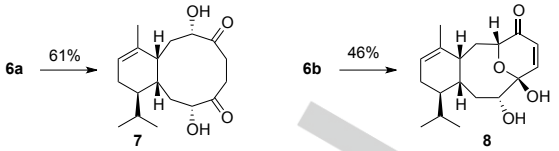


Figure 2. Products isolated from the reactions of diols **6a** and **6b** with $P450_{BM3}$ mutants GVQ/IG and RP/IA/EV, respectively.

This study revealed an unappreciated reactivity of the furan ring towards the $P450_{BM3}$ mutants and that access to hydroxylated eleuthoside analogues would require the oxidation to be carried out either following transformation of the furan into the substituted dihydrofuran or prior to installation of the furan. Following-up on the latter strategy, racemic lactone **1** was screened against a 48-member panel of $P450_{BM3}$ mutants which furnished five oxidised metabolites, **9**–**13** (selected results, Table 1; full results, Table S2), whose structures were established after preparative scale reactions with selected mutants. Small quantities of an unidentified sixth compound were formed with some mutants.

Primary alcohol **9** was the major product with many variants, likely as a consequence of steric accessibility of the methyl group even in the presence of relatively activated secondary and tertiary allylic C–H bonds. The mutant RP/HL/IG displayed complete selectivity for this metabolite (entry 1). Variants with the F87A and F87V mutations (e.g. entries 3, 5, and 11) promoted further oxidation of this metabolite to aldehyde **13** over the timeframe of the screening experiment (18 h) while retaining high regioselectivity. The effect of mutations at I263 is indicated by comparison with the selectivity of the precursor mutants; i.e. RP/HL (entry 13, 22% of **9**), RLYF/KSK19 (entry 19, 33%), GVQ (entry 20, 32%), and RLYF/KSK19/AI (entry 9, 64%); the A184I mutation also promoted the formation of **9** (e.g. entries 4 and 7).

Variant RT2/FW/AI (entry 12) formed secondary allylic alcohol **10** as the major product; RP/HL and RT2/SW/AW also displayed good selectivity for this metabolite (entries 13 and 14). The A330P mutation diverts reactivity towards formation of tertiary allylic alcohol **12**. Thus, this alcohol was formed as the secondary product (of two, with alcohol **9** being major) with RT2/AP (entry 6), RT2/AP/AI (entry 7), RT2/AP/IM (entry 8), and RT2/AP/AM (entry 10). In contrast, the RT2 precursor mutant gave none of this product.

Tertiary alcohol **11** was the major product, formed with low to moderate selectivity, in entries 18–21 in which the variants all bear the F87A or F87V mutations that lead to an increase in the space available around the heme cofactor; potentially, this favours access to conformations in which the isopropyl group is placed closer to the iron centre.

For the isolation and subsequent structure elucidation of these metabolites, small-scale preparative reactions were carried out (0.04–0.11 mmol), employing $P450_{BM3}$ variants that express well in *E. coli*. Reaction with KSK19/AI/AI gave alcohol **9** with complete conversion and in 47% isolated yield. Secondary alcohol **10** was isolated from reaction with RT2/IP in 36% yield (formed along with primary alcohol **9**, not isolated). Similar results were obtained in a reaction with RT2/FW, which provided secondary alcohol **10** in 36% yield and primary alcohol **9** in 8% yield. The stereochemistry of the newly-installed hydroxyl group in **10** was inferred on the basis of a lack of nOe

correlations between the C*H*OH proton with the protons on the *exo*- face of the molecule, as shown in Fig. 3. Reaction with KSK19/IG afforded samples of aldehyde **13** and tertiary alcohol **11** (both in 10% yield), as well as primary alcohol **9** (30% yield). Lastly, variant RT2/AP/AM required a prolonged reaction time to provide an appreciable amount of metabolite **12**, likely due to problems in enzyme quality encountered during its large-scale expression. After 10 d (at ~50% overall conversion) alcohol **12** was isolated in 14% yield. The nOe correlations shown support a *cis*-decalin arrangement in this compound, arising from oxidation on the *exo*- face of lactone **1**. The potential to scale up these reactions to produce adequate quantities of hydroxylated metabolites for subsequent synthetic elaboration was addressed next. In the oxidation of a 104 mg sample (0.5 mmol) of lactone **1** using variant KSK19/AI/AI, the conversion (92%) and yield (52%) were comparable to those of the smaller-scale reactions.

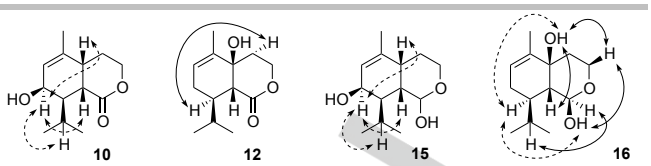
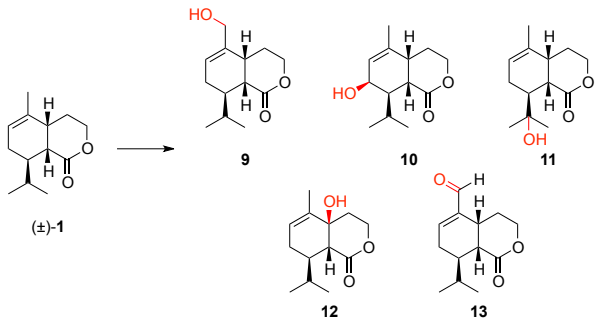


Figure 3. Selected nOe correlations for the determination of relative stereochemistry (dashed arrows indicate a lack of nOe correlations, supporting the assigned structure).

Table 1. Selected results from the screening of lactone **1** against members of the $P450_{BM3}$ library.



	Mutant	Conv	9	10	11	12	13	Unk
1	RP/HL/IG	100%	**100%**	–	–	–	–	–
2	KSK19/AI/FV	95%	**85%**	10%	–	–	5%	–
3	KSK19/AI/IA	93%	**84%**	–	–	–	16%	–
4	KSK19/AI/AI	90%	**83%**	–	–	6%	11%	–
5	GVQ/IG	94%	**75%**	–	6%	–	19%	–
6	RT2/AP	69%	**72%**	–	–	28%	–	–
7	RT2/AP/AI	100%	**71%**	–	–	29%	–	–
8	RT2/AP/IM	87%	**66%**	–	–	34%	–	–
9	RL/YF/KSK19/AI	89%	**64%**	–	28%	–	7%	–
10	RT2/AP/AM	100%	**64%**	–	–	36%	–	–
11	KSK19/IG	100%	**63%**	–	11%	–	26%	–
12	RT2/FW/AI	100%	19%	**70%**	–	–	–	11%
13	RP/HL	100%	22%	**64%**	–	–	–	14%
14	RT2/SW/AW	100%	25%	**61%**	–	–	–	14%
15	RT2/FW	100%	38%	**53%**	–	–	–	9%
16	RT2/IP	100%	39%	**52%**	–	–	–	9%
17	RT2	95%	41%	**50%**	–	–	–	9%
18	FA	98%	27%	14%	**48%**	–	11%	–
19	RL/YF/KSK19	98%	33%	6%	**48%**	–	13%	–
20	GVQ	90%	32%	14%	**47%**	–	7%	–
21	KSK19/QP/FV	100%	39%	8%	**41%**	–	11%	–

Preliminary work indicated that the $P450_{BM3}$ variants are able to discriminate between enantiomers when presented with racemic substrates, resulting in kinetic resolution. In this study, lactone **1** was used as a racemic mixture and it was recognised that each enantiomer could undergo oxidation at a different position or to a differing extent within the chiral environment of the active site, furnishing enantioenriched products and unreacted starting material. The preparative-scale oxidation of lactone **1** with variant RT2/IP provided alcohol **10** in ~64% ee (36% yield) as determined via Mosher ester analysis; more detailed further study of this aspect is underway.

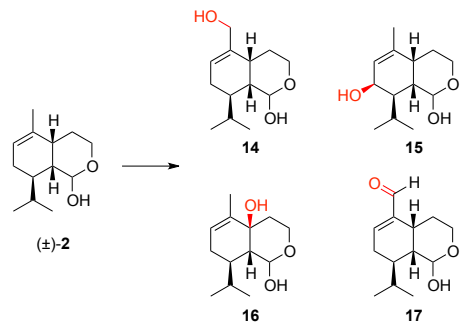
It was envisaged that a study of the oxidation of lactol **2** would provide information on the influence of the carbonyl group on preferred binding conformations in the enzymes' active site, potentially leading to complementary outcomes. In the event, screening of the lactol against a 48-mutant library provided four metabolites, **14**–**17** (selected results, Table 2; full results, Table S2), analogous to those formed in the lactone oxidation study. Overall, oxidations in the lactol series were less selective, and the product of hydroxylation of the isopropyl group was not detected, perhaps as a consequence of the dual H-bond donor/acceptor capacity of the lactol (**2**), with the lactone functionality in **1** acting as H-bond acceptor only. A preliminary computational study has provided little insight into this aspect and further work is needed.

Primary allylic alcohol **14** was the major product for many variants, with KSK19/AI/YL providing complete selectivity for this product (entry 1). Again, a number of variants catalysed further oxidation to aldehyde **17**, notably the mutants in entries 6 (29% of **17**), 5 (27%), 4 (25%), and 2 (20%). The RP/FV mutant (entry 15) provided a higher conversion to the aldehyde (30%) but displayed limited selectivity between primary alcohol **14** and secondary alcohol **15**. The F87I mutation in the R19/FI variant biased oxidation towards the alkenyl methyl group completely to give 73% of **14** and 27% of **17** (entry 5).

Variants such as RT2 (entry 12), RP (entry 10), and RP/HL (entry 13), that do not contain substrate binding pocket mutations and thus are expected to have WT-like selectivity, showed ~50% selectivity for secondary alcohol **15**, with a roughly 50:50 ratio of primary alcohol **14** and tertiary alcohol **16** accounting for the remaining products. RL/YF/KSK19 (entry 9), with the F87A mutation, proved the most effective in forming secondary allylic alcohol **15**; RT2/FW/AI (entry 11), that demonstrated high selectivity for the corresponding alcohol in

the lactone series, was also effective in producing alcohol **15** from the lactol. As in the lactone series, the A330P mutation promoted the formation of tertiary alcohol **16** (e.g. entry 18, *cf.* entry 12).

Table 2. Selected results from screening of lactol **2** against members of the P450 library.



	Mutant	Conv	14	15	16	17
1	KSK19/AI/YL	85%	**100%**	–	–	–
2	RL/YF/KSK19/AI	100%	**80%**	–	–	20%
3	RP/EV	93%	**76%**	5%	10%	9%
4	RP/FV/EV	100%	**75%**	–	–	25%
5	R19/FI	79%	**73%**	–	–	27%
6	RP/FV/EV/LQ	98%	**66%**	5%	1%	29%
7	RP/HL/IA/EV	92%	**44%**	13%	40%	3%
8	RT2/AP/AM	92%	**39%**	25%	35%	1%
9	RL/YF/KSK19	90%	21%	**63%**	12%	4%
10	RP	100%	20%	**54%**	26%	–
11	RT2/FW/AI	100%	19%	**54%**	24%	3%
12	RT2	42%	24%	**50%**	26%	–
13	RP/HL	99%	21%	**50%**	27%	2%
14	RT2/IP	96%	25%	**45%**	30%	–
15	RT2/AP/AI	100%	13%	**43%**	42%	2%
16	RP/FV	100%	34%	**37%**	–	30%
17	RT2/AP/VI/AI	64%	11%	41%	**48%**	–
18	RT2/AP	68%	22%	32%	**46%**	–
19	RT2/AP/IM	82%	32%	28%	**40%**	–

Preparative scale reactions (0.14 mmol) were carried out in order to isolate and characterise metabolites **14**–**17**. Reaction with RT2/IP gave a mixture of secondary alcohol **15** and tertiary alcohol **16** which were partially resolved by column chromatography (1.9 : 1 ratio of **15** : **16**, 32% combined yield). For secondary alcohol **15**, the relative stereochemistry was assigned based on nOe observations (Fig. 3) analogous to those described for lactone metabolite **10**. In the case of tertiary alcohol **16**, the nOe correlations shown indicate that, as expected, the product arises from oxidation on the *exo*- face of the substrate; these correlations, and the coupling patterns in the ^{1}H NMR spectrum, indicate a predominantly chairlike conformation of the lactol ring with both hydroxyl groups occupying axial sites. Reaction with RP/FV provided a separable mixture of aldehyde **17** (10%), secondary alcohol **15** (8%), and primary alcohol **14** (18%).

Finally, a comparison was made with a chemical reagent commonly employed to effect allylic oxidation, SeO_2. Treatment of lactone **1** with 2.0 equiv. of SeO_2 in dichloromethane resulted in a sluggish reaction to provide solely tertiary alcohol **12**; stopping the reaction after 48 h gave a 22% isolated yield of this product. No variant within the $P450_{BM3}$ library displayed good selectivity for this allylic alcohol over the other two, demonstrating that there exists some complementarity between the chemical and biocatalytic approaches. In contrast, SeO_2-mediated oxidation of lactol **2** resulted in a complex reaction mixture from which no hydroxylated products were identified.

Conclusions

In conclusion, the $P450_{BM3}$-mediated hydroxylation of eleuthoside precursors was studied. Tricyclic furanoid variants (e.g. **6a** and **6b**) proved to be unsuitable substrates in this regard, but biocatalytic oxidation of intermediates **1** and **2**, corresponding to the cyclohexene substructure within the natural products, provided a range of oxidised metabolites. The capacity to fine-tune the selectivity for specific metabolites by employing different variants of the enzyme was established. Furthermore, this work has demonstrated the scalability of the approach to provide quantities relevant to synthetic chemistry, and has hinted at its capacity to furnish enantiomerically enriched products from racemic substrates. The metabolites obtained can serve as starting points for the synthesis of hydroxylated and further derivatised eleuthoside analogues, structures of interest in the search for novel cytotoxic compounds.

Experimental Section

General Information: All reactions were carried out under an argon atmosphere in dried glassware unless otherwise noted. “Petrol” refers to the fraction of petroleum ether boiling in the range 30–40 °C. Dichloromethane and DMF were obtained from an MBraun 5 solvent purification system. Tetrahydrofuran (THF) was freshly distilled from sodium and benzophenone under a nitrogen atmosphere. Reagents were used as obtained from the supplier. Flash column chromatography was performed using Merck Geduran® silica gel (40–63 μm). Analytical thin-layer chromatography (TLC) was performed on aluminium-backed plates pre-coated with silica gel (0.2 mm, Merck 60 F254), which were developed using $KMnO_4$. IR spectra were recorded as a thin film on a Bruker Tensor 27 FT-IR spectrometer. Proton (^{1}H) and carbon (^{13}C) NMR spectra were recorded on Bruker AVII-500 (500/125 MHz), Bruker AVIII-400 (400/100 MHz), or Bruker AV-400 (400/100 MHz) spectrometers. Chemical shifts (δ_H and δ_C) are quoted in parts per million (ppm), referenced to the appropriate residual solvent peak; ^{1}H–^{1}H coupling constants (*J*) are rounded to the nearest 0.5 Hz. High-resolution mass spectra (HRMS) were recorded by the staff of the Chemistry Research Laboratory, Oxford on a Bruker Daltonics MicroTOF spectrometer; mass to charge ratios (*m/z*) are reported in Daltons. Compounds whose assigned numbers are of the form S# are not illustrated in the Schemes. The Supporting Information contains a key to the $P450_{BM3}$ mutants used

in this work, general methods and full results for the enzyme screening runs, and supporting spectra.

(4a*R,8*R**,8a*R**)-8-Isopropyl-5-methyl-3,4,4a,7,8,8a-hexahydro-1*H*-isochromen-1-ol (2):** To a cooled (–78 °C) solution of lactone **1**[14] (2.38 g, 11.4 mmol) in dichloromethane (150 mL) was added DIBAL (1.0 M solution in cyclohexane, 13.7 mL, 13.7 mmol) dropwise. The reaction mixture was stirred for 3 h then satd. aq. Rochelle salt solution (150 mL) was added and the mixture allowed to warm up to RT; the layers were separated and the aqueous layer was extracted with dichloromethane (3 × 150 mL). The combined organic layers were washed with brine (300 mL), dried ($MgSO_4$), and the solvent removed *in vacuo* to give the *title compound* (2.25 g, 94%) as a yellow oil, that was of sufficient purity to be used directly in the next step. R_f 0.33 (petrol / ether, 1:1); ν_{max} (thin film)/cm^{-1} 3379br, 2958s, 1464m, 1438m, 1387m, 1368m, 1114m, 1071m, 1030s, 1000m; ^{1}H NMR δ_H (400 MHz, C_6D_6) 0.73 (3 H, d, *J* = 6.5 Hz), 0.84 (3 H, d, *J* = 6.5 Hz), 1.38–1.52 (2 H, m), 1.56 (3 H, d, *J* = 2.0 Hz), 1.62–1.80 (3 H, m), 1.80–1.90 (1 H, m), 1.91–2.02 (2 H, m), 2.46–2.57 (1 H, m), 3.48 (1 H, ddd, *J* = 11.0, 4.5, 3.5 Hz), 3.99 (1 H, app. td, *J* = 11.0, 3.5 Hz), 5.23 (1 H, d, *J* = 2.0 Hz), 5.32 (1 H, br s); ^{13}C NMR δ_C (100 MHz, C_6D_6) 15.4, 20.8, 21.1, 24.3, 26.3, 27.5, 33.4, 34.1, 40.3, 59.1, 92.0, 121.4, 135.7; HRMS (ESI$^+$) found 233.1514, $C_{13}H_{22}O_2Na$ [MNa]$^+$ requires 233.1512.

(3*R,4*R**,5*R**)-3-(2-Hydroxyethyl)-5-isopropyl-4-[(*E*)-2-methoxyvinyl]-2-methylcyclohexene (3):** To a suspension of (methoxymethyl)triphenylphosphonium chloride (18.1 g, 52.4 mmol) in THF (200 mL) at –78 °C was added potassium *tert*-butoxide (1.0 M solution in THF, 47.6 mL, 47.6 mmol). The mixture was stirred for 30 min then a solution of lactol **2** (5.0 g, 23.8 mmol) in THF (20 mL) was added and the mixture was allowed to warm to RT then stirred for 18 h. Brine (200 mL) was added, the layers were separated, and the aqueous layer was extracted with ether (3 × 200 mL). The combined organic layers were washed with brine (200 mL), dried ($MgSO_4$), and the solvent removed *in vacuo*. The crude residue was purified by flash chromatography (petrol / ether, 9:1 → 4:1) to give the *title compound* along with the *Z*- isomer as a colourless oil (3:1 *E*-/*Z*-, 3.66 g, 65%; the isomer ratio was determined by integration of the -C*H*=CHOMe signal in the ^{1}H NMR spectrum). R_f 0.25 (petrol / ether, 1:1); ν_{max} (thin film)/cm^{-1} 3351br, 2957s, 2931s, 2891s, 1651s, 1466m, 1450m, 1208s, 1145m, 1115m, 1033m, 1020m, 939m, 773m; ^{1}H NMR δ_H (400 MHz, C_6D_6, major isomer) 0.73 (3 H, d, *J* = 7.0 Hz), 0.91 (3 H, d, *J* = 7.0 Hz), 1.36–1.46 (1 H, m), 1.60–1.66 (2 H, m), 1.68 (3 H, s), 1.75–1.89 (2 H, m), 1.90–2.00 (2 H, m), 2.10 (1 H, app. td, *J* = 10.0, 4.5 Hz), 3.18 (3 H, s), 3.45–3.57 (2 H, m), 4.67 (1 H, dd, *J* = 12.5, 10.0 Hz), 5.35 (1 H, br s), 6.25 (1 H, d, *J* = 12.5 Hz) [*Diagnostic signals for Z- enol ether isomer*: 4.33 (1 H, dd, *J* = 10.0, 6.5 Hz, 5.65 (1 H, d, *J* = 6.5 Hz)]; ^{13}C NMR δ_C (100 MHz, C_6D_6) 16.5, 21.3, 22.8, 24.6, 28.1, 33.7, 39.8, 40.3, 41.3, 55.5, 62.4, 104.5, 121.4, 136.9, 147.8; HRMS (ESI$^+$) found 239.2007, $C_{15}H_{27}O_2$ [MH]$^+$ requires 239.2006.

(3*R,4*R**,5*R**)-3-[2-(*S**)-Hydroxy-2-(5-bromofuran-2-yl)ethyl]-5-isopropyl-4-[(*E*)-2-methoxyvinyl]-2-methylcyclohexene (4a) and (3*R**,4*R**,5*R**)-3-[2-(*R**)-hydroxy-2-(5-bromofuran-2-yl)ethyl]-5-isopropyl-4-[(*E*)-2-methoxyvinyl]-2-methylcyclohexene (4b):** To a solution of alcohol **3** (3.66 g, 15.4 mmol) and DMSO (1.4 mL, 20.0 mmol) in THF (120 mL) at –78 °C was added oxalyl chloride dropwise (1.6 mL, 18.5 mmol). The mixture was stirred for 20 min then triethylamine (6.4 mL, 46.1 mmol) was added. The reaction mixture was allowed to warm to RT and, after 30 min, ether (100 mL) was added and the mixture was filtered through Celite®. The solvent was removed *in vacuo* to give the aldehyde, all of which was used immediately in the following reaction. BuLi (2.5 M solution in hexanes, 18.8 mL, 47.0 mmol) was added to a solution of 2,5-dibromofuran[17] (5.0 mL, 50.0 mmol) in THF (40 mL) at –78 °C. The mixture was stirred for 10 min then added to a cooled (–78 °C) solution of the previously-prepared aldehyde in THF (100 mL). The reaction mixture was allowed to warm to RT and stirred for 18 h, then brine (100 mL) was added, the layers were separated, and the aqueous layer was extracted with ether (2 × 100 mL). The combined organic layers were washed with brine (200 mL), dried ($MgSO_4$), the solvent removed *in vacuo*, and the crude residue purified by flash chromatography (petrol / ether, 9:1 → 4:1) to give the epimers **4a** (3:1 *E*-/*Z*-, 2.30 g, 39% from alcohol **3**) and **4b** (3:1 *E*-/*Z*-, 2.20 g, 37% from alcohol **3**), both as colourless oils; the *E*-/*Z*- isomer ratio was determined in both cases by integration of the -C*H*=CHOMe signal in the ^{1}H NMR spectrum. Data for **4a**: R_f 0.44 (petrol / ether, 7:3); ν_{max} (thin film)/cm^{-1} 3421br, 2955s, 1652s, 1505m, 1450m, 1207s, 1125s, 1010m, 939m, 784s; ^{1}H NMR δ_H (400 MHz, C_6D_6) 0.75 (3 H, d, *J* = 7.0 Hz), 0.92 (3 H, d, *J* = 7.0 Hz), 1.30–1.40 (1 H, m), 1.71 (3 H, d, *J* = 2.0 Hz,), 1.74–1.89 (4 H, m), 1.91–2.03 (1 H, m), 2.15–2.27 (2 H, m), 3.18 (3 H, s), 4.57 (1 H, dd, *J* = 9.0, 4.5 Hz), 4.66 (1 H, dd, *J* = 12.5, 10.0 Hz), 5.35 (1 H, br s), 5.82 (1 H, d, *J* = 3.5 Hz), 5.90 (1 H, d, *J* = 3.5 Hz), 6.27 (1 H, d, *J* = 12.5 Hz) [*Diagnostic signals for Z- enol ether isomer*: 4.32 (1 H, dd, *J* = 10.5, 6.5 Hz), 5.61 (1 H, d, *J* = 6.5 Hz)]; ^{13}C NMR δ_C (100 MHz, C_6D_6) 17.0, 21.2, 22.4, 24.5, 28.0, 36.4, 39.3, 39.9, 40.7, 55.5, 66.9, 104.1, 108.2, 112.1, 121.0, 121.6, 136.5, 147.9, 160.6; HRMS (CI, NH_3) found 400.1476, 402.1440, $C_{19}H_{31}{}^{79}BrNO_3$ [MNH$_4$]$^+$ requires 400.1482, $C_{19}H_{31}{}^{81}BrNO_3$ [MNH$_4$]$^+$ requires 402.1462. Data for **4b**: R_f 0.23 (petrol / ether, 7:3); ν_{max} (thin film)/cm^{-1} 3397br, 2955s, 1651s, 1504m, 1451m, 1208s, 1124s, 1055s, 940s, 785s; ^{1}H NMR δ_H (400 MHz, C_6D_6) 0.68 (3 H, d, *J* = 6.5 Hz), 0.84 (3 H, d, *J* = 7.0 Hz), 1.32–1.41 (1 H, m), 1.63 (3 H, d, *J* = 1.5 Hz), 1.68–1.80 (2 H, m), 1.83–1.99 (4 H, m), 2.00–2.09 (1 H, m), 3.17 (3 H, s), 4.57–4.75 (2 H, m), 5.31 (1 H, br s), 5.86 (1 H, d, *J* = 3.5 Hz), 5.91 (1 H, d, *J* = 3.5 Hz), 6.23 (1 H, d, *J* = 12.5 Hz) [*Diagnostic signals for Z- enol ether isomer*: 4.31 (1 H, dd, *J* = 10.0, 6.5 Hz), 5.61 (1 H, d, *J* = 6.5 Hz)]; ^{13}C NMR δ_C (100 MHz, C_6D_6) 16.1, 21.2, 22.7, 24.4, 28.0, 37.1, 39.6, 40.1, 41.4, 55.6, 67.7, 104.3, 109.3, 112.1, 121.1, 121.9, 136.7, 148.0, 159.8; HRMS (ESI$^+$) found 405.1036, 407.1015, $C_{19}H_{27}{}^{79}BrO_3Na$ [MNa]$^+$ requires 405.1036, $C_{19}H_{27}{}^{81}BrO_3Na$ [MNa]$^+$ requires 407.1016.

(3*R,4*R**,5*R**)-3-[2-(*S**)-(*tert*-Butyldiphenylsilyl)oxy-2-(5-bromofuran-2-yl)ethyl]-5-isopropyl-4-[(*E*)-2-methoxyvinyl]-2-methylcyclohexene (S1a):** A solution of enol ether **4a** (56 mg, 0.15 mmol), *tert*-butyldiphenylsilyl chloride (156 µL, 0.60 mmol), and imidazole (82 mg, 1.20 mmol) in dichloromethane (7 mL) was stirred for 18 h at RT. The reaction mixture was then diluted with dichloromethane (10 mL), washed with water (10 mL) then brine (10mL), dried ($MgSO_4$), and the solvent removed *in vacuo*. The crude residue was purified by flash chromatography (petrol / ether, 19:1) to give the *title compound* as a colourless oil (3:1 *E*-/*Z*-, 79 mg, 85%; the *E*-/*Z*- isomer ratio was determined by integration of the -C*H*=CHOMe signal in the ^{1}H NMR spectrum). R_f 0.89 (petrol / ether, 9:1); ν_{max} (thin film)/cm^{-1} 2955m, 2931m, 1651m, 1463m, 1111s, 701s; ^{1}H NMR δ_H (400 MHz, C_6D_6) 0.67 (3 H, d, *J* = 6.5 Hz), 0.84 (3 H, d, *J* = 7.0 Hz), 1.16 (9 H, s), 1.30–1.40 (1 H, m), 1.66–1.81 (3 H, m), 1.83 (3 H, s), 1.98–2.07 (2 H, m), 2.17–2.31 (2 H, m), 3.05 (3 H, s), 4.41 (1 H, dd, *J* = 12.5, 10.5 Hz), 4.95 (1 H, dd, *J* = 8.5, 4.0 Hz), 5.36 (1 H, br s), 5.41 (1 H, d, *J* = 3.0 Hz), 5.65 (1 H, d, *J* = 3.0 Hz), 6.17 (1 H, d, *J* = 12.5 Hz), 7.17–7.31 (6 H, m), 7.64–7.68 (2 H, m), 7.78–7.87 (2 H, m) [*Diagnostic signals for Z- enol ether isomer*: 4.19 (1 H, dd, *J* = 10.5, 6.5 Hz), 5.60 (1 H, d, *J* = 6.5 Hz)]; ^{13}C NMR δ_C (100 MHz, C_6D_6) 15.4, 19.7, 21.4, 22.8, 24.3, 27.1, 27.8, 38.0, 38.7, 40.6, 41.7, 55.1, 69.3, 103.7, 110.1, 111.8, 121.1, 121.5, 129.7, 130.0, 133.7, 133.9, 135.9, 136.4, 136.5, 147.5, 158.6 (two signals are obscured by the solvent peak); HRMS (ESI$^+$) found 643.2212, 645.2190, $C_{35}H_{45}O_3{}^{79}BrNaSi$ [MNa]$^+$ requires 643.2214, $C_{35}H_{45}O_3{}^{81}BrNaSi$ [MNa]$^+$ requires 645.2194.

(3*R,4*R**,5*R**)-3-[2-(*R**)-(*tert*-Butyldiphenylsilyl)oxy-2-(5-bromofuran-2-yl)ethyl]-5-isopropyl-4-[(*E*)-2-methoxyvinyl]-2-methylcyclohexene (S1b):** A solution of enol ether **4b** (62 mg, 0.16 mmol), *tert*-butyldiphenylsilyl chloride (167 µL, 0.64 mmol), and imidazole (87 mg, 1.28 mmol) in dichloromethane (7 mL) was stirred for 18 h at RT. The reaction mixture was then diluted with dichloromethane (10 mL), washed with water (10 mL) then brine (10 mL), dried ($MgSO_4$), and the solvent removed *in vacuo*. The crude residue was purified by flash chromatography (petrol / ether, 19:1) to give the *title compound* as a colourless oil (3:1 *E*-/*Z*-, 86 mg, 87%; the *E*-/*Z*- isomer ratio was determined by integration of the -C*H*=CHOMe signal in the ^{1}H NMR

spectrum); R_f 0.89 (petrol / ether, 9:1); ν_{max} (thin film)/cm^{-1} 2956m, 2932m, 1650m, 1428m, 1112s, 703s; ^{1}H NMR δ_H (500 MHz, C_6D_6) 0.66 (3 H, d, *J* = 6.5 Hz), 0.78 (3 H, d, *J* = 7.0 Hz), 1.14 (9 H, s), 1.22–1.29 (1 H, m), 1.54–1.60 (1 H, m), 1.62 (3 H, s), 1.68–1.76 (2 H, m), 1.87–2.00 (2 H, m), 2.10–2.21 (1 H, m), 2.30–2.39 (1 H, m), 3.04 (3 H, s), 4.45 (1 H, dd, *J* = 12.5, 10.5 Hz), 4.99 (1 H, dd, *J* = 10.5, 5.5 Hz), 5.28 (1 H, br s), 5.66 (1 H, d, *J* = 3.0 Hz), 5.80 (1 H, d, *J* = 3.0 Hz), 6.11 (1 H, d, *J* = 12.5 Hz), 7.17–7.27 (6 H, m), 7.63–7.70 (2 H, m), 7.77–7.85 (2 H, m) [*Diagnostic signal for Z- enol ether isomer*: 5.41 (1 H, d, *J* = 6.5 Hz]; ^{13}C NMR δ_C (125 MHz, C_6D_6) 16.9, 19.6, 21.1, 22.2, 24.4, 27.1, 27.8, 37.6, 39.5, 40.5, 55.4, 69.0, 104.2, 111.0, 111.8, 121.4, 121.6, 129.9, 130.1, 134.0, 134.3, 136.2, 136.4, 147.9, 158.2 (the =*C*(CH_3)- resonance was not observed, and three further signals are obscured by the solvent peak); HRMS (ESI$^+$) found 643.2214, 645.2192, $C_{35}H_{45}{}^{79}BrNaO_3Si$ [MNa]$^+$ requires 643.2214, $C_{35}H_{45}{}^{81}BrNaO_3Si$ [MNa]$^+$ requires 645.2193.

(3*R,4*R**,5*R**)-3-[2-(*S**)-(*tert*-Butyldiphenylsilyl)oxy-2-(5-bromofuran-2-yl)ethyl]-5-isopropyl-4-(2-oxoethyl)-2-methylcyclohexene (5a):**[10d,18] $Hg(OCOCF_3)_2$ (25 mg, 0.06 mmol) was added to a cooled (0 °C) solution of enol ether **S1a** (36 mg, 0.06 mmol) in THF / H_2O (10:1, 3 mL). The reaction mixture was stirred for 30 min, then satd. aq. KCl solution (3 mL) was added and the mixture was stirred at RT for 18 h. The layers were separated and the aqueous layer was extracted with ether (3 × 5 mL). The combined organic layers were washed with brine (15 mL), dried ($MgSO_4$), and the solvent removed *in vacuo*. The crude residue was purified *via* flash chromatography (petrol / ether, 19:1) to give the title compound as an orange oil (25 mg, 70%). R_f 0.65 (petrol / ether, 4:1); ν_{max} (thin film)/cm^{-1} 2959s, 1724s, 1427m, 1111s; ^{1}H NMR δ_H (500 MHz, $CDCl_3$) 0.71 (3 H, d, *J* = 6.5 Hz), 0.80 (3 H, d, *J* = 6.5 Hz), 1.03 (9 H, s), 1.21–1.29 (1 H, m), 1.44 (3 H, s), 1.53–1.61 (1 H, m), 1.75–2.02 (5 H, m), 2.12–2.18 (1 H, m), 2.24 (1 H, ddd, *J* = 16.5, 7.0, 1.5 Hz), 2.32 (1 H, ddd, *J* = 16.5, 7.0, 2.5 Hz), 4.62 (1 H, dd, *J* = 9.0, 5.5 Hz), 5.21 (1 H, s), 5.74 (1 H, d, *J* = 3.0 Hz), 6.04 (1 H, d, *J* = 3.0 Hz), 7.29–7.34 (2 H, m), 7.36–7.50 (6 H, m), 7.65–7.70 (2 H, m), 9.56 (1 H, app. t, *J* = 2.0 Hz); ^{13}C NMR δ_C (125 MHz, $CDCl_3$) 17.4, 19.4, 21.0, 22.8, 24.1, 27.0, 27.5, 34.6, 36.5, 37.7, 39.1, 43.9, 68.8, 110.8, 111.8, 121.0, 121.2, 127.5, 127.8, 129.6, 129.9, 133.5 (two peaks), 135.8, 136.1, 136.5, 157.4, 202.9; HRMS (ESI$^+$) found 629.2055, $C_{34}H_{43}O_3{}^{79}BrNaSi$ [MNa]$^+$ requires 629.2058 (the peak for the ^{81}Br isotope was not reported).

(3*R,4*R**,5*R**)-3-[2-(*R**)-(*tert*-Butyldiphenylsilyl)oxy-2-(5-bromofuran-2-yl)ethyl]-5-isopropyl-4-(2-oxoethyl)-2-methylcyclohexene (5b):** $Hg(OCOCF_3)_2$ (232 mg, 0.54 mmol) was added to a cooled (0 °C) solution of enol ether **S1b** (338 mg, 0.54 mmol) in THF / H_2O (10:1, 24 mL). The reaction mixture was stirred for 30 min then satd. aq. KCl solution was added (3 mL) and the mixture was stirred at RT for 18 h. The layers were separated and the aqueous layer was extracted with ether (3 × 5 mL). The combined organic layers were washed with brine (15 mL), dried ($MgSO_4$), and the solvent removed *in vacuo*. The crude residue was purified *via* flash chromatography (petrol / ether, 19:1) to give the *title compound* as an orange oil (191 mg, 58%); R_f 0.69 (petrol / ether, 4:1); ν_{max} (thin film)/cm^{-1} 2959s, 1725s, 1112s, 1057s, 703s; ^{1}H NMR δ_H (500 MHz, C_6D_6) 0.59 (3 H, d, *J* = 6.5 Hz), 0.67 (3 H, d, *J* = 6.5 Hz), 0.99–1.07 (1 H, m), 1.13 (9 H, s), 1.27–1.35 (1 H, m), 1.50 (3 H, s), 1.51–1.56 (1 H, m), 1.59–1.67 (1 H, m), 1.74–1.94 (4 H, m), 2.01–2.11 (1 H, m), 2.21–2.31 (1 H, m), 4.75 (1 H, dd, *J* = 10.0, 5.5 Hz), 5.13 (1 H, br s), 5.79 (1 H, d, *J* = 3.0 Hz), 5.82 (1 H, d, *J* = 3.0 Hz), 7.16–7.27 (6 H, m), 7.59–7.66 (2 H, m), 7.74–7.84 (2 H, m), 9.20 (1 H, dd, *J* = 3.0, 2.0 Hz); ^{13}C NMR δ_C (125 MHz, C_6D_6) 18.6, 19.5, 20.8, 22.2, 24.0, 27.2, 27.4, 32.6, 35.7, 37.1, 39.7, 43.0, 68.1, 111.1, 112.0, 121.4, 121.5, 130.1, 130.3, 133.7, 134.0, 135.4, 136.2, 136.4, 157.9, 201.2 (two resonances were not observed separately); HRMS (ESI$^+$) found 629.2056, 631.2035, $C_{34}H_{43}O_3{}^{79}BrNaSi$ [MNa]$^+$ requires 629.2058, $C_{34}H_{43}O_3{}^{81}BrNaSi$ [MNa]$^+$ requires 631.2037.

(4*R,4a*R**,6*R**,11*S**,12a*R**)-11-[(*tert*-Butyldiphenylsilyl)oxy]-4-isopropyl-1-methyl-3,4,4a,5,6,11,12,12a-octahydro-7,10-epoxybenzo[10]annulen-6-ol (S2a):**[10d,18] A solution of aldehyde **5a** (660 mg, 1.08 mmol) in DMF (75 mL) was degassed by three cycles of a freeze-pump-thaw sequence, then $CrCl_2$ (1.34 g, 10.8 mmol) and $NiCl_2$ (141 mg, 1.08 mmol) were added in one portion at –20 °C. The reaction mixture was stirred for 18 h at RT, then water (75 mL) was added, the layers were separated, and the aqueous layer was extracted with ether (3 × 75 mL). The combined organic layers were washed with water (2 × 50 mL) then brine (75 mL), dried ($MgSO_4$), and the solvent removed *in vacuo*. The crude residue was purified *via* flash chromatography (petrol / ether, 19:1 → 4:1) to give the title compound as a white solid (334 mg, 58%); M.p. 45 °C; R_f 0.17 (petrol / ether, 4:1); ν_{max} (thin film)/cm^{-1} 3334br, 2958s, 1428m, 1065s, 702s; ^{1}H NMR δ_H (500 MHz, $CDCl_3$) –0.55 (1 H, br s), 0.73 (3 H, d, *J* = 7.0 Hz), 0.90 (3 H, d, *J* = 7.0 Hz), 1.10 (12 H, s), 1.20–1.29 (1 H, m), 1.33–1.42 (1 H, m), 1.43–1.50 (1 H, m), 1.61–1.73 (1 H, m), 1.81–1.89 (1 H, m), 1.92–1.95 (1 H, m), 2.01–2.07 (1 H, m), 2.12 (1 H, dt, *J* = 13.0, 3.5 Hz), 2.41 (1 H, app. q, *J* = 13.0 Hz), 2.58 (1 H, ddd, *J* = 12.5, 10.0, 2.5 Hz), 4.48 (1 H, ddd, *J* = 10.0, 6.0, 3.5 Hz), 4.62 (1 H, dd, *J* = 10.0, 5.5 Hz), 5.04 (1 H, br s), 5.98 (1 H, d, *J* = 3.0 Hz), 6.07 (1 H, d, *J* = 3.0 Hz), 7.35–7.46 (6 H, m), 7.68–7.80 (4 H, m); ^{13}C NMR δ_C (125 MHz, $CDCl_3$) 15.2, 19.4, 21.6, 22.3, 25.1, 27.1, 27.2, 37.0, 37.6, 38.6, 40.0, 43.6, 68.4, 70.8, 110.9, 116.7, 118.7, 127.6, 127.8, 129.8, 129.9, 134.0 (two peaks), 135.9, 136.1, 139.2, 159.8, 160.8; HRMS (ESI$^+$) found 551.2951, $C_{34}H_{44}O_3NaSi$ [MNa]$^+$ requires 551.2952.

(4*R,4a*R**,6*R**,11*R**,12a*R**)-11-[(*tert*-Butyldiphenylsilyl)oxy]-4-isopropyl-1-methyl-3,4,4a,5,6,11,12,12a-octahydro-7,10-epoxybenzo[10]annulen-6-ol (S2b):** A solution of aldehyde **5b** (660 mg, 1.08 mmol) in DMF (75 mL) was degassed *via* three cycles of a freeze-pump-thaw sequence, then $CrCl_2$ (1.34 g, 10.8 mmol) and $NiCl_2$ (141 mg, 1.08 mmol) were added in one portion at –20 °C. The reaction mixture was stirred for 18 h at RT, then water (75 mL) was added, the layers were separated, and the aqueous layer was extracted with ether (3 × 75 mL). The combined organic layers were washed with water (2 × 50 mL) then brine (50 mL), dried ($MgSO_4$), and the solvent removed *in vacuo*. The crude residue was purified *via* flash chromatography (petrol / ether, 19:1 → 4:1) to give the *title compound* as a white solid (316 mg, 55%); M.p. 55 °C; R_f 0.08 (petrol / ether, 4:1); ν_{max} (thin film)/cm^{-1} 3335br, 2959m, 1107s, 701s; ^{1}H NMR δ_H (400 MHz, C_6D_6) 0.52–0.57 (1 H, m), 0.68 (3 H, d, *J* = 7.0 Hz), 0.80 (3 H, d, *J* = 7.0 Hz), 0.94–1.03 (1 H, m), 1.14–1.20 (1 H, m), 1.22 (9 H, s), 1.53 (1 H, ddd, *J* = 14.5, 4.0, 1.5 Hz), 1.63–1.74 (1 H, m), 1.75–1.82 (1 H, m), 1.85 (3 H, s), 1.87–1.92 (2 H, m), 2.06 (1 H, dt, *J* = 13.0, 10.5 Hz), 2.19–2.27 (1 H, m), 4.19 (1 H, dd, *J* = 10.5, 4.5 Hz), 5.24 (1 H, br s), 5.32 (1 H, dt, *J* = 5.0, 1.5 Hz), 5.89 (1 H, d, *J* = 3.0 Hz), 6.43 (1 H, dd, *J* = 3.0, 1.5 Hz), 7.17–7.24 (6 H, m), 7.73–7.80 (2 H, m), 7.80–7.88 (2 H, m); ^{13}C NMR δ_C (125 MHz, C_6D_6) 16.6, 19.6, 21.5, 23.3, 25.3, 27.3, 27.9, 36.0, 38.5, 40.2, 43.7, 67.5, 73.2, 112.3, 113.0, 119.9, 125.7, 125.9, 128.6, 129.3, 130.3, 133.9, 136.3, 139.6, 159.8, 163.3 (two signals are obscured by the solvent peak); HRMS (ESI$^+$) found 551.2953, $C_{34}H_{44}O_3NaSi$ [MNa]$^+$ requires 551.2952.

(1*R,4a*R**,6*S**,11*R**,12a*R**)-1-Isopropyl-4-methyl-1,2,4a,5,6,11,12,12a-octahydro-7,10-epoxybenzo[10]annulene-6,11-diol (6a):** To a solution of alcohol **S2a** (50 mg, 0.094 mmol) in THF (3 mL) was added TBAF (1.0 M solution in THF, 1.0 mL, 1.0 mmol) and the reaction mixture was stirred for 18 h at RT. Phosphate buffer (pH 7, 5 mL) and ether (5 mL) were added, the layers were separated, and the aqueous layer was extracted with ether (2 × 5 mL). The combined organic layers were washed with brine (15 mL), dried ($MgSO_4$), and the solvent removed *in vacuo*. The crude residue was purified *via* flash chromatography (petrol / ether, 4:1 → 1:1) to give the *title compound* as a white solid (21 mg, 77%). M.p. 120 °C; R_f 0.09 (petrol / ether, 1:1); ν_{max} (thin film)/cm^{-1} 3324br, 2959s, 1456m, 1026s, 734m; ^{1}H NMR δ_H (500 MHz, $CDCl_3$) –0.31 (1 H, br s), 0.74 (3 H, d, *J* = 7.0 Hz), 0.88 (3 H, d, *J* = 7.0 Hz), 1.24–1.31 (1 H, m), 1.31–1.40 (1 H, m), 1.45 (3 H, s), 1.65–1.75 (2 H, m), 1.85–1.93 (1 H, m), 1.96–2.04 (1 H, m), 2.09–2.15 (1 H, m), 2.29–2.44 (2 H, m), 4.54 (1 H, dd, *J* = 10.0, 3.5 Hz), 4.75 (1 H, dd, *J* = 10.0, 5.5 Hz), 5.14 (1 H, br s), 6.20 (1 H, dd, *J* = 3.0, 1.0 Hz), 6.47 (1 H, d, *J* = 3.0 Hz); ^{13}C NMR δ_C (125 MHz, $CDCl_3$) 15.4, 21.6, 22.6, 25.1, 27.3, 37.3, 37.7, 38.9, 39.7, 43.5, 68.3, 69.8, 111.3, 117.0, 119.2, 138.9, 160.4, 160.5; HRMS (ESI$^+$) found 313.1775, $C_{18}H_{26}O_3Na$ [MNa]$^+$ requires 313.1775.

(1*R,4a*R**,6*R**,11*R**,12a*R**)-1-Isopropyl-4-methyl-1,2,4a,5,6,11,12,12a-octahydro-7,10-epoxybenzo[10]annulene-6,11-diol (6b):** To a solution of alcohol **S2b** (25 mg, 0.047 mmol) in THF (1.5 mL) was added TBAF (1.0 M solution in THF, 500 μL, 0.5 mmol) and the reaction mixture was stirred for 18 h at RT. Phosphate buffer (pH 7, 3 mL) and ether (3 mL) were added, the layers were separated, and the aqueous layer was extracted with ether (2 × 3 mL). The combined organic layers were washed with brine (10 mL), dried ($MgSO_4$), and the solvent removed *in vacuo*. The crude residue was purified *via* flash chromatography (petrol / ether, 4:1 → 1:1) to give the *title compound* as a white solid (11 mg, 81%). M.p. 85 °C; R_f 0.33 (petrol / ether, 1:1); ν_{max} (thin film)/cm^{-1} 3364br, 2958s, 1196m, 1094s, 1069s, 1006s, 787s; ^1H NMR δ_H (500 MHz, C_6D_6) 0.22 (1 H, s), 0.67 (3 H, d, *J* = 6.5 Hz), 0.81 (3 H, d, *J* = 6.5 Hz), 1.04–1.12 (1 H, m), 1.20–1.33 (2 H, m), 1.65–1.68 (1 H, m), 1.70 (3 H, s), 1.79–1.92 (2 H, m), 1.95 (1 H, app. dt, *J* = 13.5, 4.0 Hz), 2.13–2.24 (1 H, m), 2.32 (1 H, ddd, *J* = 14.5, 5.0, 4.0 Hz), 4.19 (1 H, dd, *J* = 10.5, 4.0 Hz), 4.85 (1 H, d, *J* = 5.0 Hz), 5.22 (1 H, br s), 5.87 (1 H, d, *J* = 3.0 Hz), 6.31 (1 H, dd, *J* = 3.0, 1.5 Hz); ^{13}C NMR δ_C (125 MHz, C_6D_6) 16.0, 21.6, 23.1, 25.3, 27.8, 36.2, 38.3, 39.1, 39.8, 44.0, 67.5, 71.4, 111.8, 112.4, 119.8, 139.8, 159.7, 163.5; HRMS (CI, NH_3) found 308.2220, $C_{18}H_{30}NO_3$ $[MNH_4]^+$ requires 308.2221.

(1*R,4a*R**,6*S**,11*R**,12a*R**)-6,11-Dihydroxy-1-isopropyl-4-methyl-1,2,4a,5,6,8,9,11,12,12a-decahydrobenzo[10]annulene-7,10-dione (7):** To a solution comprising furan **6a** (0.26 mL of a 70 mM solution in methanol, 0.018 mmol), glucose (1.30 mL of a 1.0 M solution in water, 1.30 mmol), GDH (130 μL of a 2.0 U/μL solution in pH 7.5 phosphate buffer), and $NADP^+$ monosodium salt (130 μL of a 4.0 mM solution in water, 0.52 μmol) in phosphate buffer (200 mM, pH 7.5, 9.88 mL) was added $P450_{BM3}$ mutant GVQ/IG (1.3 mL of a 20 μM solution in phosphate buffer, pH 7.5, 0.026 μmol). The solution was stirred open to air for 24 h and was then centrifuged at 9,500 g, then extracted with ethyl acetate (4 × 10 mL). The combined organic layers were washed with brine (40 mL), dried ($MgSO_4$), and concentrated *in vacuo*. The crude product was purified by flash chromatography (petrol / ether, 1:4) to afford the *title compound* as a colourless oil (3.4 mg, 61%). R_f 0.24 (petrol / ether, 1:4); ν_{max} (thin film)/cm^{-1} 3414br, 2958m, 1707m, 1067m; ^1H NMR δ_H (500 MHz, $CDCl_3$) 0.78 (3 H, d, *J* = 7.0 Hz), 0.89 (3 H, d, *J* = 7.0 Hz), 1.20–1.25 (1 H, m), 1.32–1.40 (1 H, m), 1.45–1.49 (1 H, m), 1.73–1.76 (2 H, m), 1.77 (3 H, s), 1.79–1.85 (2 H, m), 1.86–1.98 (2 H, m), 2.03–2.11 (1 H, m), 2.86–3.03 (2 H, m), 3.04–3.15 (2 H, m), 4.23 (1 H, app. t, *J* = 7.5 Hz), 4.29 (1 H, app. t, *J* = 8.5 Hz), 5.31 (1 H, br s); ^{13}C NMR δ_C (125 MHz, $CDCl_3$) 14.8, 21.1, 22.6, 24.7, 26.9, 29.9, 30.5, 32.8, 34.0, 34.1, 36.4, 38.3, 73.0, 78.6, 120.7, 137.2, 210.8, 212.4; HRMS (ESI^+) found 331.1879, $C_{18}H_{28}O_4Na$ $[MNa]^+$ requires 331.1880.

(1*R,4a*R**,6*R**,10*R**,11*R**,12a*R**)-10,11-Dihydroxy-1-isopropyl-4-methyl-2,4a,5,6,10,11,12,12a-octahydro-6,10-epoxybenzo[10]annulen-7(1*H*)-one (8):** To a solution comprising furan **6b** (0.12 mL of a 100 mM solution in methanol, 0.012 mmol), glucose (0.6 mL of a 1.0 M solution in water, 0.60 mmol), GDH (60 μL of a 2.0 U/μL solution in phosphate buffer, pH 7.5), $NADP^+$ monosodium salt (60 μL of a 4.0 mM solution in water, 0.24 μmol), and methyl-β-cyclodextrin (300 μL of a 200 mM solution in water, 60 μmol) in phosphate buffer (200 mM, pH 7.5, 4.26 mL) was added $P450_{BM3}$ mutant RP/IA/EV (600 μL of a 20 μM solution in phosphate buffer, pH 7.5, 0.012 μmol). The solution was stirred open to air for 72 h and was then centrifuged at 9,500 g, then extracted with ethyl acetate (4 × 10 mL). The combined organic layers were washed with brine (40 mL), dried ($MgSO_4$), and concentrated *in vacuo*. The crude product was purified by flash chromatography (petrol / ether, 4:1 → 3:2) to afford the *title compound* as a colourless oil (1.7 mg, 46%). R_f 0.11 (petrol / ether, 1:1); ν_{max} (thin film)/cm^{-1} 3403br, 2958s, 1690s, 1087s; ^1H NMR δ_H (500 MHz, C_6D_6) 0.61 (3 H, d, *J* = 7.0 Hz), 0.74 (3 H, d, *J* = 7.0 Hz), 0.97–1.04 (1 H, m), 1.19 (1 H, ddd, *J* = 15.5, 12.0, 9.5 Hz), 1.39–1.44 (1 H, m), 1.51 (3 H, s), 1.52–1.65 (3 H, m), 1.65–1.72 (1 H, m), 1.72–1.78 (1 H, m), 1.93 (1 H, dd, *J* = 15.5, 4.5 Hz), 2.47 (1 H, br s), 3.58 (1 H, d, *J* = 9.5 Hz), 4.28 (1 H, dd, *J* = 11.5, 8.0 Hz), 5.24 (1 H, br s), 5.86 (1 H, d, *J* = 10.5 Hz), 6.47 (1 H, d, *J* = 10.5 Hz); ^{13}C NMR δ_C (125 MHz, C_6D_6) 14.7, 21.5, 22.0, 24.1, 26.3, 32.3, 32.4, 34.7, 37.9, 38.5, 76.0, 77.8, 96.8, 121.3, 127.5, 135.7, 144.8, 194.3; HRMS (ESI^-) found 305.1757, $C_{18}H_{25}O_4$ $[M–H]^-$ requires 305.1758.

(4a*R,8*R**,8a*R**)-5-(Hydroxymethyl)-8-isopropyl-3,4,4a,7,8,8a-hexahydro-1*H*-isochromen-1-one (9):** To a solution comprising lactone **1** (0.60 mL of a 70 mM solution in methanol, 0.042 mmol), glucose (3.0 mL of a 1.0 M solution in water, 3.0 mmol), GDH (300 μL of a 2.0 U/μL solution in phosphate buffer, pH 7.5), and $NADP^+$ monosodium salt (300 μL of a 4.0 mM solution in water, 1.20 μmol) in phosphate buffer (200 mM, pH 7.5, 24.6 mL) was added $P450_{BM3}$ mutant KSK19/AI/AI (870 μL of a 68.9 μM solution in phosphate buffer, pH 7.5, 0.060 μmol). The solution was stirred open to air for 24 h and was then centrifuged at 9,500 g, then extracted with ethyl acetate (4 × 25 mL). The combined organic layers were washed with brine (100 mL), dried ($MgSO_4$), and concentrated *in vacuo*. The crude product was purified by flash chromatography (petrol / ether, 1:1 → 1:4) to afford the *title compound* as a colourless oil (4.5 mg, 47%). R_f 0.11 (petrol / ether, 1:4); ν_{max} (thin film)/cm^{-1} 3410br, 2959m, 1720s, 1284m, 1154m; ^1H NMR δ_H (500 MHz, C_6D_6) 0.74 (3 H, d, *J* = 6.5 Hz), 0.86 (3 H, d, *J* = 6.5 Hz), 1.28–1.38 (2 H, m), 1.46–1.55 (1 H, m), 1.74–1.87 (1 H, m), 2.05–2.13 (1 H, m), 2.14–2.23 (1 H, m), 2.30–2.36 (1 H, m), 2.49 (1 H, ddd, *J* = 6.5, 5.0, 1.5 Hz), 3.52 (1 H, d, *J* = 12.5 Hz), 3.56–3.65 (2 H, m), 3.70 (1 H, ddd, *J* = 11.0, 8.0, 4.5 Hz), 5.38 (1 H, br s); ^{13}C NMR δ_C (125 MHz, C_6D_6) 20.2, 21.0, 24.3, 26.1, 27.2, 30.0, 39.3, 42.7, 64.9, 66.0, 125.0, 137.1, 172.3; HRMS (ESI^-) found 223.1338, $C_{13}H_{19}O_3$ $[M–H]^-$ requires 223.1339.

(4a*R,7*R**,8*R**,8a*S**)-7-Hydroxy-8-isopropyl-5-methyl-3,4,4a,7,8,8a-hexahydro-1*H*-isochromen-1-one (10):** To a solution comprising lactone **1** (0.60 mL of a 70 mM solution in methanol, 0.042 mmol), glucose (3.0 mL of a 1.0 M solution in water, 3.00 mmol), GDH (300 μL of a 2.0 U/μL solution in phosphate buffer, pH 7.5), and $NADP^+$ monosodium salt (300 μL of a 4.0 mM solution in water, 1.20 μmol) in phosphate buffer (200 mM, pH 7.5, 23.7 mL) was added $P450_{BM3}$ mutant RT2/IP (2.1 mL of a 28.2 μM solution in phosphate buffer, pH 7.5, 0.059 μmol). The solution was stirred open to air for 24 h and was then centrifuged at 9,500 g, then extracted with ethyl acetate (4 × 25 mL). The combined organic layers were washed with brine (100 mL), dried ($MgSO_4$), and concentrated *in vacuo*. The crude product was purified by flash chromatography (petrol / ether, 1:1 → 2:3) to afford the *title compound* as a colourless oil (3.5 mg, 36%). R_f 0.29 (petrol / ether, 1:4); ν_{max} (thin film)/cm^{-1} 3438br, 2917m, 1721s, 1388m, 1255m, 1077s; ^1H NMR δ_H (500 MHz, C_6D_6) 0.91 (3 H, d, *J* = 6.5 Hz), 0.96–1.04 (1 H, m), 1.10–1.18 (1 H, m), 1.20 (3 H, d, *J* = 6.5 Hz), 1.25 (3 H, s), 1.81–1.90 (1 H, m), 1.91–2.01 (1 H, m), 2.01–2.07 (1 H, m), 2.69 (1 H, app. t, *J* = 6.5 Hz), 3.50 (1 H, ddd, *J* = 11.5, 7.5, 4.0 Hz), 3.66 (1 H, ddd, *J* = 11.5, 7.5, 4.0 Hz), 4.26 (1 H, br s), 5.34 (1 H, br s); ^{13}C NMR δ_C (125 MHz, C_6D_6) 20.4, 22.1, 22.2, 25.8, 27.2, 35.2, 41.5, 44.6, 65.7, 66.4, 135.3, 172.5 (one signal is obscured by the solvent peak); HRMS (ESI^+) found 247.1304, $C_{13}H_{20}O_3Na$ $[MNa]^+$ requires 247.1305.

(4a*R,8*S**,8a*S**)-8-(2-Hydroxypropan-2-yl)-5-methyl-3,4,4a,7,8,8a-hexahydro-1*H*-isochromen-1-one (11) and (4a*R**,8*R**,8a*R**)-8-isopropyl-1-oxo-3,4,4a,7,8,8a-hexahydro-1*H*-isochromene-5-carboxaldehyde (13):** To a solution comprising lactone **1** (1.40 mL of a 70 mM solution in methanol, 0.098 mmol), glucose (7.0 mL of a 1.0 M solution in water, 7.0 mmol), GDH (700 μL of a 2.0 U/μL solution in phosphate buffer, pH 7.5), and $NADP^+$ monosodium salt (700 μL of a 4.0 mM solution in water, 2.80 μmol) in phosphate buffer (200 mM, pH 7.5, 57.6 mL) was added $P450_{BM3}$ mutant KSK19/IG (2.60 mL of a 54.1 μM solution in phosphate buffer, pH 7.5, 0.141 μmol). The solution was stirred open to air for 24 h and was then centrifuged at 9,500 g, then extracted with ethyl acetate (4 × 50 mL). The combined organic layers were washed with brine (200 mL), dried ($MgSO_4$), and concentrated *in vacuo*. The crude residue was purified by flash chromatography (petrol / ether, 3:2 → 1:4) to afford *alcohol* **11** as a colourless oil (2.3 mg, 10%) and *aldehyde* **13** (2.2 mg, 10%), also a colourless oil; alcohol **9** (6.7 mg, 30%) was also obtained. Data for **11**: R_f 0.46 (petrol / ether, 1:4); ν_{max} (thin film)/cm^{-1} 3434br, 2916m, 1760s, 1269m, 1073m, 1042m; ^1H NMR δ_H (500 MHz, C_6D_6) 0.74 (3 H, s), 1.05 (3 H, s), 1.28–1.55 (4 H, m), 1.56

(3 H, s), 1.75 (1 H, ddd, *J* = 14.0, 11.5, 5.0 Hz), 2.05 (1 H, dd, *J* = 14.0, 5.0 Hz), 2.16 (1 H, br s), 2.40–2.49 (1 H, m), 3.59–3.80 (2 H, m), 5.02 (1 H, br s); ^{13}C NMR δ_C (125 MHz, C_6D_6) 21.1, 22.1, 25.9, 27.6, 34.5, 35.4, 42.3, 45.8, 61.7, 84.6, 120.2, 138.4, 176.0; HRMS (ESI$^+$) found 225.1486, $C_{13}H_{21}O_3$ [MH]$^+$ requires 225.1486. Data for **13**: R_f 0.59 (petrol / ether, 1:4); ν_{max} (thin film)/cm^{-1} 2960m, 1728s, 1682s, 1181m, 1159m; ^{1}H NMR δ_H (500 MHz, C_6D_6) 0.58 (3 H, d, *J* = 6.5 Hz), 0.69 (3 H, d, *J* = 6.5 Hz), 1.09–1.20 (1 H, m), 1.51–1.62 (1 H, m), 1.71–1.80 (2 H, m), 1.90–1.98 (1 H, m), 2.13–2.22 (1 H, m), 2.32 (1 H, ddd, *J* = 7.0, 5.0, 1.5 Hz), 2.55–2.64 (1 H, m), 3.50 (1 H, ddd, *J* = 11.5, 8.0, 4.0 Hz), 3.57 (1 H, ddd, *J* = 11.5, 6.5, 4.0 Hz), 5.88 (1 H, br s), 9.02 (1 H, s); ^{13}C NMR δ_C (125 MHz, C_6D_6) 19.9, 20.8, 25.8, 26.7, 27.1, 27.9, 38.6, 41.2, 65.8, 141.6, 151.1, 171.7, 192.3; HRMS (EI$^+$) found 222.1256, $C_{13}H_{18}O_3$ [M]$^+$ requires 222.1251.

(4a*S, 8*R**, 8a*R**)-4a-Hydroxy-8-isopropyl-5-methyl-3,4,4a,7,8,8a-hexahydro-1*H*-isochromen-1-one (12):** To a solution comprising lactone **1** (0.80 mL of a 70 mM solution in methanol, 0.056 mmol), glucose (4.0 mL of a 1.0 M solution in water, 4.0 mmol), GDH (400 μL of a 2.0 U/μL solution in phosphate buffer, pH 7.5), and NADP$^+$ monosodium salt (400 μL of a 4.0 mM solution in water, 1.6 μmol) in phosphate buffer (200 mM, pH 7.5, 28.9 mL) was added $P450_{BM3}$ mutant RT2/AP/AM (5.50 mL of a 14.6 μM solution in phosphate buffer, pH 7.5, 0.080 μmol). The solution was stirred open to air for 10 d and was then centrifuged at 9,500 g, then extracted with ethyl acetate (4 × 30 mL). The combined organic layers were washed with brine (100 mL), dried ($MgSO_4$), and concentrated *in vacuo*. The crude residue was purified by flash chromatography (petrol / ether, 1:1 → 1:4) to afford the *title compound* as a colourless oil (1.8 mg, 14%); alcohol **9** (4.5 mg, 35%) was also obtained. R_f 0.48 (petrol / ether, 1:4); ν_{max} (thin film)/cm^{-1} 3420br, 2959m, 1714s, 1265m, 1120m; ^{1}H NMR δ_H (500 MHz, C_6D_6) 0.79 (3 H, d, *J* = 6.5 Hz), 1.00 (3 H, d, *J* = 6.5 Hz), 1.28–1.36 (1 H, m), 1.40–1.42 (1 H, m), 1.43 (3 H, s), 1.62–1.72 (2 H, m), 1.73–1.81 (1 H, m), 1.84–1.93 (1 H, m), 2.35 (1 H, dd, *J* = 10.0, 1.5 Hz), 3.72 (1 H, ddd, *J* = 11.0, 5.5, 3.5 Hz), 4.12 (1 H, app. td, *J* = 11.0, 4.5 Hz), 5.10 (1 H, br s); ^{13}C NMR δ_C (125 MHz, C_6D_6) 16.5, 17.1, 21.2, 24.6, 28.7, 31.5, 41.3, 53.7, 64.9, 72.4, 124.0, 136.2, 171.2; HRMS (ESI$^+$) found 225.1487, $C_{13}H_{21}O_3$ [MH]$^+$ requires 225.1486.

(4a*R,8*R**,8a*R**)-5-(Hydroxymethyl)-8-isopropyl-3,4,4a,7,8,8a-hexahydro-1*H*-isochromen-1-ol (14) and (4a*R**,8*R**,8a*R**)-1-hydroxy-8-isopropyl-3,4,4a,7,8,8a-hexahydro-1*H*-isochromene-5-carboxaldehyde (17):** To a solution comprising lactol **2** (2.0 mL of a 70 mM solution in methanol, 0.14 mmol), glucose (10.0 mL of a 1.0 M solution in water, 10.0 mmol), GDH (1.0 mL of a 2.0 U/μL solution in phosphate buffer, pH 7.5), and NADP$^+$ monosodium salt (1.0 mL of a 4.0 mM solution in water, 4.0 μmol) in phosphate buffer (200 mM, pH 7.5, 76.0 mL) was added $P450_{BM3}$ mutant RP/FV (10.0 mL of a 20.0 μM solution in phosphate buffer, pH 7.5, 0.20 μmol). The solution was stirred open to air for 24 h and was then centrifuged at 9,500 g, then extracted with ethyl acetate (4 × 75 mL). The combined organic layers were washed with brine (100 mL), dried ($MgSO_4$), and concentrated *in vacuo*. The crude product was purified by flash chromatography (petrol / ether, 1:1 → 1:4) to afford *alcohol* **14** as a colourless oil (5.7 mg, 18%) and *aldehyde* **17** (3.0 mg, 10%), also a colourless oil; alcohol **15** (2.4 mg, 8%) was also obtained (see below). Data for **14**: R_f 0.19 (petrol / ether, 1:4); ν_{max} (thin film)/cm^{-1} 3347br, 2956s, 1019s, 996s; ^{1}H NMR δ_H (500 MHz, $CDCl_3$) 0.80 (3 H, d, *J* = 6.5 Hz), 0.93 (3 H, d, *J* = 6.5 Hz), 1.65–1.72 (3 H, m), 1.83–1.89 (1 H, m), 1.89–2.01 (2 H, m), 2.04 (1 H, dtd, *J* = 17.0, 4.5, 1.5 Hz), 2.62 (1 H, d, *J* = 3.5 Hz), 2.72–2.81 (1 H, m), 3.59 (1 H, dt, *J* = 11.5, 4.0 Hz), 3.97–4.15 (2 H, m), 5.29 (1 H, s), 5.70 (1 H, t, *J* = 3.5 Hz); ^{13}C NMR δ_C (125 MHz, $CDCl_3$) 15.6, 21.1, 24.1, 26.5, 27.8, 29.6, 34.3, 39.8, 59.6, 65.4, 92.3, 123.9, 139.9; HRMS (ESI$^-$) found 225.1494, $C_{13}H_{21}O_3$ [M–H]$^-$ requires 225.1496. Data for **17**: R_f 0.60 (petrol / ether, 1:4); ν_{max} (thin film)/cm^{-1} 3388br, 2958s, 1683s, 1389m, 1182m; ^{1}H NMR δ_H (500 MHz, $CDCl_3$) 0.82 (3 H, d, *J* = 6.5 Hz), 0.98 (3 H, d, *J* = 6.5 Hz), 1.49–1.64 (2 H, m), 1.77 (1 H, d, *J* = 12.5 Hz), 2.02–2.16 (3 H, m), 2.32–2.38 (1 H, m), 2.39 (1 H, d, *J* = 4.0 Hz), 3.21 (1 H, dt, *J* = 12.0, 4.5 Hz), 3.57 (1 H, ddd, *J* = 11.5, 5.0, 2.0 Hz), 4.14 (1 H, ddd, *J* = 12.5, 11.5, 3.0 Hz), 5.37 (1 H, s), 6.79 (1 H, dd, *J* = 5.0, 2.5 Hz), 9.39 (1 H, s); ^{13}C NMR δ_C (125 MHz, $CDCl_3$) 14.7, 21.2, 26.0, 26.3, 26.5, 27.5, 34.0, 38.5, 59.0, 91.6, 144.4, 151.4, 193.5; HRMS (ESI$^+$) found 247.1305, $C_{13}H_{20}O_3Na$ [MNa]$^+$ requires 247.1305.

(4a*R,7*R**,8*R**,8a*S**)-8-Isopropyl-5-methyl-3,4,4a,7,8,8a-hexahydro-1*H*-isochromene-1,7-diol (15) and (4a*S**,8*R**,8a*R**)-8-isopropyl-5-methyl-1,3,4,7,8,8a-hexahydro-4a*H*-isochromene-1,4a-diol (16):** To a solution comprising lactol **2** (2.0 mL of a 70 mM solution in methanol, 0.14 mmol), glucose (10.0 mL of a 1.0 M solution in water, 10.0 mmol), GDH (1.0 mL of a 2.0 U/μL solution in phosphate buffer, pH 7.5), and NADP$^+$ monosodium salt (1.0 mL of a 4.0 mM solution in water, 4.0 μmol) in phosphate buffer (200 mM, pH 7.5, 79.0 mL) was added $P450_{BM3}$ mutant RT2/IP (7.0 mL of a 28.2 μM solution in phosphate buffer, pH 7.5, 0.20 μmol). The solution was stirred open to air for 24 h and was then centrifuged at 9,500 g, then extracted with ethyl acetate (4 × 75 mL). The combined organic layers were washed with brine (100 mL), dried ($MgSO_4$), and concentrated *in vacuo*. The crude product was purified by flash chromatography (petrol / ether, 1:1) to afford *alcohol* **15** and *alcohol* **16** (10.1 mg combined, 32%, 1.9:1 of 15:16) from which individual column fractions of the partially-separated products enabled acquisition of analytical data. Data for **15**: R_f 0.30 (petrol / ether, 2:3); ν_{max} (thin film)/cm^{-1} 3374br, 2956s, 1103m, 987s; ^{1}H NMR δ_H (500 MHz, $CDCl_3$) 1.07 (3 H, d, *J* = 7.0 Hz), 1.13 (3 H, d, *J* = 7.0 Hz), 1.51 (1 H, dd, *J* = 13.0, 5.0 Hz), 1.60–1.70 (1 H, m), 1.71 (3 H, s), 1.75 (1 H, ddd, *J* = 12.5, 5.0, 3.5 Hz), 1.99–2.12 (2 H, m), 2.38 (1 H, d, *J* = 3.0 Hz), 2.53 (1 H, dt, *J* = 12.5, 4.5 Hz), 3.62 (1 H, ddd, *J* = 11.0, 5.0, 1.5 Hz), 4.09 (1 H, ddd, *J* = 13.0, 11.0, 3.0 Hz), 4.23 (1 H, d, *J* = 5.5 Hz), 5.41 (1 H, d, *J* = 3.0 Hz), 5.56 (1 H, d, *J* = 5.0 Hz); ^{13}C NMR δ_C (125 MHz, $CDCl_3$) 18.4, 21.1, 21.3, 26.2, 27.0, 34.2, 34.7, 38.8, 58.9, 66.3, 92.0, 124.2, 141.4; HRMS (ESI$^+$) found 249.1462, $C_{13}H_{22}O_3Na$ [MNa]$^+$ requires 249.1462. Data for **16**: R_f 0.39 (petrol / ether, 2:3); ν_{max} (thin film)/cm^{-1} 3366br, 2958m, 1260m, 1103s, 1026s, 800s; ^{1}H NMR δ_H (500 MHz, C_6D_6) 0.62 (3 H, d, *J* = 7.0 Hz), 0.73 (3 H, d, *J* = 7.0 Hz), 1.35 (1 H, ddd, *J* = 13.5, 3.0, 1.5 Hz), 1.58 (3 H, s), 1.58–1.67 (3 H, m), 1.71 (1 H, td, *J* = 13.5, 5.5 Hz), 1.77–1.87 (1 H, m), 1.91–2.03 (1 H, m), 2.36 (1 H, s), 3.48 (1 H, dd, *J* = 11.5, 5.5 Hz), 4.15 (1 H, ddd, *J* = 13.5, 11.5, 3.0 Hz), 4.36 (1 H, d, *J* = 7.5 Hz), 5.19 (1 H, dd, *J* = 4.0, 1.0 Hz), 5.38 (1 H, d, *J* = 7.5 Hz); ^{13}C NMR δ_C (125 MHz, C_6D_6) 14.4, 15.9, 21.0, 24.1, 26.5, 33.0, 38.2, 46.1, 54.9, 72.9, 92.8, 122.7, 138.2; HRMS (ESI$^+$) found 249.1463, $C_{13}H_{22}O_3Na$ [MNa]$^+$ requires 249.1461.

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Keywords: P450 enzymes • eleutherobin • C–H activation • protein engineering • microtubule stabilisation

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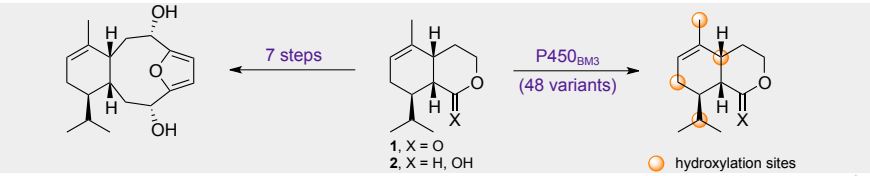

Leonidas-Dimitrios Syntrivanis, Luet Lok Wong, Jeremy Robertson**

1 – 9

Hydroxylation of eleuthoside synthetic intermediates by $P450_{BM3}$ (CYP102A1)

A short synthesis of an intermediate in Danishefsky's total synthesis of eleutherobin from bicyclic lactone **1** is reported. The furan diol shown undergoes biocatalytic Achmatowicz reaction upon treatment with engineered $P450_{BM3}$ variants, with no evidence of sp^3 C–H hydroxylation. In contrast, both lactone **1** and lactol **2** provide a variety of hydroxylated metabolites, which hold potential for the synthesis of hydroxylated eleutherobin analogues and other naturally-occurring eleuthosides.