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Article

Hydroxylated Rotenoids Selectively Inhibit the Proliferation of Prostate Cancer Cells

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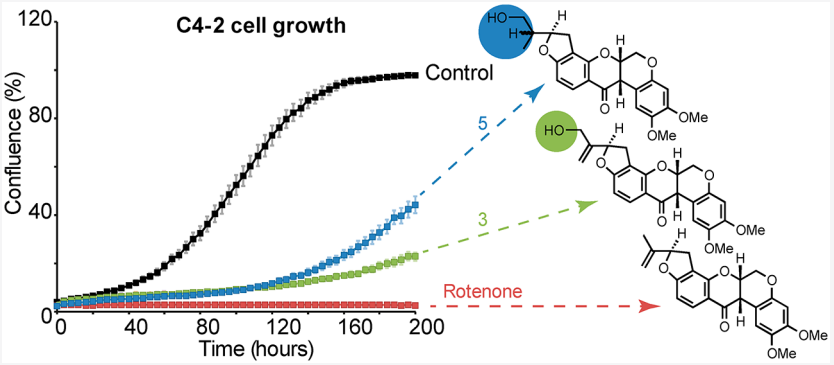

ABSTRACT: Prostate cancer is one of the leading causes of cancer-related death in men. The identification of new therapeutics to selectively target prostate cancer cells is therefore vital. Recently, the rotenoids rotenone (**1**) and deguelin (**2**) were reported to selectively kill prostate cancer cells, and the inhibition of mitochondrial complex I was established as essential to their mechanism of action. However, these hydrophobic rotenoids readily cross the blood−brain barrier and induce symptoms characteristic of Parkinson's disease in animals. Since hydroxylated derivatives of **1** and **2** are more hydrophilic and less likely to readily cross the blood−brain barrier, 29 natural and unnatural hydroxylated derivatives of **1** and **2** were synthesized for evaluation. The inhibitory potency (IC_{50}) of each derivative against complex I was measured, and its hydrophobicity ($Slog_{10}P$) predicted. Amorphigenin (**3**), dalpanol (**4**), dihydroamorphigenin (**5**), and amorphigenol (**6**) were selected and evaluated in cell-based assays using C4-2 and C4-2B prostate cancer cells alongside control PNT2 prostate cells. These rotenoids inhibit complex I in cells, decrease oxygen consumption, and selectively inhibit the proliferation of prostate cancer cells, leaving control cells unaffected. The greatest selectivity and antiproliferative effects were observed with **3** and **5**. The data highlight these molecules as promising therapeutic candidates for further evaluation in prostate cancer models.

Prostate cancer is one of the leading causes of cancer-related death in men.[1,2] Prostate tumors generally respond well to androgen deprivation therapy and shrink, but then almost invariably become androgen insensitive, regrow autonomously, and metastasize, often to bone.[3,4] The identification of new therapeutic agents to selectively target prostate cancer cells is therefore vital. Recently, the natural rotenoids rotenone (**1**) and especially deguelin (**2**) (Figure 1) were shown to selectively kill phosphatase and tensin homologue deleted on chromosome 10 (PTEN)-null prostate cancer cells.[5] The inhibition of mitochondrial complex I (NADH:ubiquinone oxidoreductase) was established as essential to their mechanism of action and selective toxicity.[5] This finding further highlights the therapeutic potential of **1** and **2**[6−11] and is consistent with their being canonical ubiquinone-binding site inhibitors of complex I.[12−15] Complex I is a major entry point for electrons into the mitochondrial respiratory chain and an integral contributor to oxidative phosphorylation (OxPhos),[16] which is a target for other potential anticancer compounds, such as metformin[17,18] and IACS-010759[19] from the MD Anderson Cancer Center. It oxidizes the NADH produced by the citric acid cycle and other metabolic pathways, transfers the electrons to ubiquinone to sustain the reduction of molecular oxygen through complexes III and IV, and transports protons across the mitochondrial inner membrane to maintain the proton motive force that drives ATP synthesis and metabolite transport.[16] In cells, **1** and **2** inhibit complex I and disrupt OxPhos, leading to a decrease

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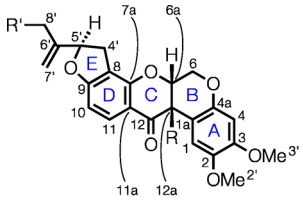
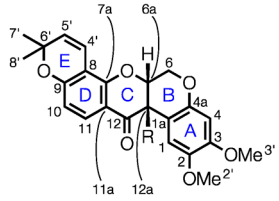
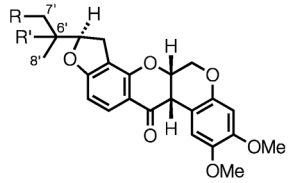


R = H R' = H **1** rotenone
R = H R' = OH **3*** amorphigenin
R = OH R' = H **7*** rotenolone



R = H **2** deguelin
R = OH **8*** tephrosin



R = H R' = OH **4** dalpanol
R = OH R' = H **5** dihydroamorphigenin
R = OH R' = OH **6*** amorphigenol

Figure 1. Structures of rotenoids **1**–**8** with ring labels and atom numbers. Molecules marked * are both plant secondary metabolites and products of detoxifying metabolism in mammals, fish, and insects.[31,32]

in mitochondrial ATP production, hydrolysis of ATP to maintain the mitochondrial membrane potential, and ultimately cell death by apoptosis.[5] Selective toxicity toward prostate cancer cells arises because these cells require greater than normal amounts of ATP to sustain unchecked growth, following loss of the tumor suppressor PTEN early in tumorigenesis, and this ATP is produced most efficiently by OxPhos.[5] PTEN-null prostate cancer cells are therefore highly dependent on OxPhos and especially vulnerable to inhibitors of complex I, while PTEN-positive normal prostate cells remain relatively tolerant of them.[5]

While rotenone (**1**) and particularly deguelin (**2**) thus appear as promising therapeutic agents, their neurotoxicity must be properly considered. Both **1** and **2** cross the blood–brain barrier (BBB) in rats and mice, cause neuronal damage when administered in doses of 3 and 6 mg kg^{-1} day^{-1}, respectively, and induce symptoms characteristic of Parkinson's disease.[20] It is clear that **2** is less neurotoxic than **1** because it is more susceptible to oxidation and so metabolized and cleared more rapidly,[20] but it is not clear whether **2** is suitable for long-term systemic administration.[21] Importantly, recent work[5] suggests that rotenoids related to **1** and **2** that inhibit complex I should also selectively kill prostate cancer cells. The pool of potential rotenoid therapeutic agents can therefore be expanded to include those with potentially lower neurotoxicity profiles.

To a first approximation, rotenone (**1**) and deguelin (**2**) readily cross the BBB because they are hydrophobic.[22,23] Accordingly, hydroxylated rotenoids such as amorphigenin (**3**),[24,25] dalpanol (**4**),[26,27] dihydroamorphigenin (**5**),[28] and amorphigenol (**6**) (Figure 1),[29−32] which by comparison are more hydrophilic than **1** and **2**, would not be expected to readily cross the BBB. Hydroxylated metabolites of **1** and **2** were analyzed in the rat model of Parkinson's disease, but only rotenolone (**7**) and tephrosin (**8**) (Figure 1) were detected in the brains of treated animals.[20] Hydroxylated rotenoids that are more hydrophilic than **7** and **8**, such as **3** and **6**, could thus constitute potentially promising therapeutic agents for the treatment of prostate cancer.

However, although hydrophobicity is an important factor in determining whether a molecule can cross the BBB,[22,23] the model outlined above is simplistic. Many additional factors can influence whether a molecule can cross the BBB, including molecular weight, volume, topological molecular polar surface area, solvent-accessible surface area, and the number of hydrogen bond donors and acceptors.[33,34] Molecules can also be actively expelled from the brain by various efflux transporters, such as P-glycoprotein, and their accumulation thereby prevented.[34] Moreover, mice administered with 100–900 mg kg^{-1} of plant extracts rich in dalpanol (**4**) were reported to suffer severe neurological impairments,[35] although histopathological analysis of the brain tissue of treated mice did not show any differences relative to those of control mice. Given these complicating factors, it is therefore important that the neurotoxicity profile of any rotenoid of therapeutic interest is established through carefully designed animal studies. A recent example of a detailed pharmacological evaluation of rotenone (**1**) provides a framework that can be applied to the analysis of related rotenoids.[36]

No comprehensive study has been made of the inhibitory properties of hydroxylated rotenoids against complex I or their ability to selectively target prostate cancer cells, though a number of reports have been made on the cytotoxicity of certain hydroxylated rotenoids.[37−44] Thus, a library of 29 hydroxylated rotenoids was prepared from rotenone (**1**) and deguelin (**2**) to identify inhibitors of complex I that might be better suited to development as therapeutics than the parent compounds. The inhibitory effect of each hydroxylated rotenoid on complex I was determined and its hydrophobicity predicted. Amorphigenin (**3**), dalpanol (**4**), dihydroamorphigenin (**5**), and amorphigenol (**6**) (Figure 1) were selected for evaluation in cell-based assays using C4-2 and C4-2B bone-metastasized prostate cancer cells and control PNT2 prostate cells. The ability of each compound to reach and inhibit complex I in cells, its antiproliferative activity, and its selective toxicity toward prostate cancer cells over healthy prostate cells were then established. Each compound reaches and inhibits complex I in cells, selectively inhibits the proliferation of prostate cancer cells, and leaves healthy prostate cells unaffected. Further, the antiproliferative activities and inhibitory potencies are directly correlated. Finally, **3** and **5** are highlighted as highly active potential therapeutic candidates worthy of detailed evaluation in more sophisticated cell-based assays or animal models of prostate cancer.

RESULTS AND DISCUSSION

Preparation of a Library of Hydroxylated Rotenoids. To prepare a library of hydroxylated derivatives of rotenone (**1**) and deguelin (**2**), redox reactions were applied to the C-12

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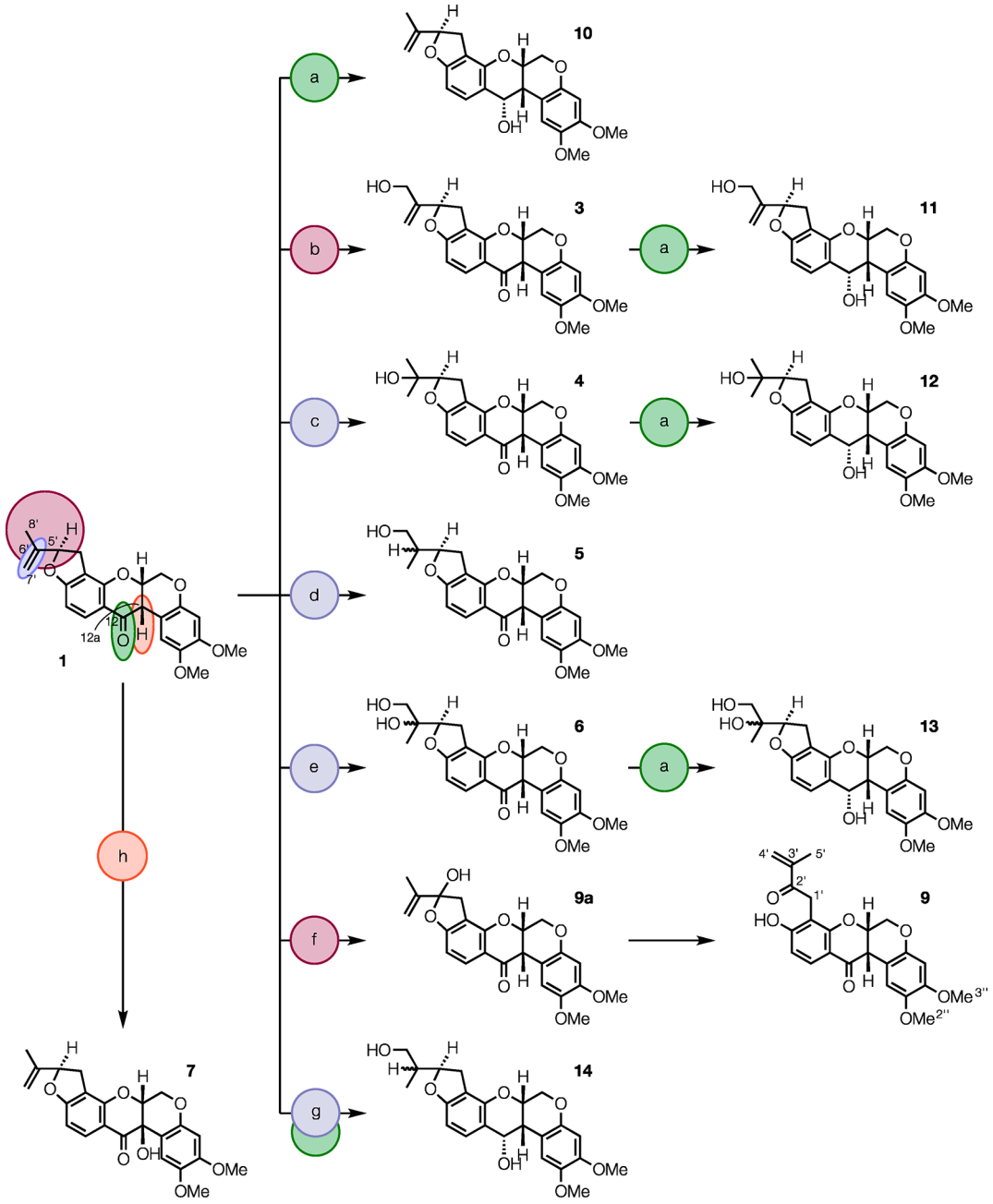
Scheme 1. Synthesis of Derivatives of Rotenone (1)[a]



[a]Reagents and conditions: (a) $NaBH_4$, MeOH, 0 °C to rt, 2 h, 88−92%; (b) i. *N*-PSP, H_2O, CSA, CH_2Cl_2, rt, 72 h; ii. H_2O_2, pyridine 0 °C to rt, 2 h, 33%; (c) i. $Hg(OAc)_2$, THF, H_2O, rt, 18 h; ii. NaCl then $NaHCO_3$, $NaBH_4$, THF, H_2O, rt, 0.5 min, 48%; (d) i. $[Ir(COD)Cl]_2$, DPPE, HBPin, CH_2Cl_2, rt, 20 h; ii. H_2O_2, $NaHCO_3$, THF, H_2O, rt, 20 h, 14%; (e) OsO_4, NMO, citric acid, acetone, H_2O (see ref 48); (f) SeO_2, 4 Å MS, 1,4-dioxane, 80 °C, 3 h, 21%; (g) i. $BH_3{\cdot}SMe_2$, THF, 0 °C to rt, 1.5 h; ii. H_2O_2, NaOH, THF, H_2O, 0 °C to rt, 18 h, 80%; (h) $K_2Cr_2O_7$, AcOH, H_2O, 60 °C, 0.5 h then rt, 18 h, 82%. Abbreviations: MS, molecular sieves; *N*-PSP, *N*-(phenylseleno)phthalimide; CSA, (+)-*β*-camphorsulfonic acid; COD, 1,5-cyclooctadiene; DPPE, 1,2-bis(diphenylphosphino)ethane; HBPin, pinacolborane; NMO, *N*-methylmorpholine *N*-oxide.

carbonyl groups and the C-12a benzylic sites of **1** and **2**, the Δ6′(7′) olefinic unit and the C-5′ and C-8′ allylic sites of **1**, and the Δ4′(5′) olefinic unit of **2** (Schemes 1−4). Allylic oxidation of **1** at C-5′ with excess SeO_2 in dry 1,4-dioxane gave keto-phenol **9** via hemiacetal **9a** (Scheme 1).[45] Allylic oxidation of **1** at C-8′, using *N*-(phenylseleno)phthalimide and H_2O in the presence of catalytic camphorsulfonic acid, followed by oxidation of the resulting *β*-hydroxyselenide with H_2O_2 in pyridine and elimination of the tertiary selenoxide, afforded amorphigenin (**3**).[46] Markovnikov hydration of the Δ6′(7′) double bond, using an oxymercuration−demercuration sequence in which **1** was treated with $Hg(OAc)_2$ in aqueous THF followed by workup with saturated NaCl solution and rapid demurcuration with $NaBH_4$ under weakly basic conditions, gave dalpanol (**4**).[27] Anti-Markovnikov hydration, using an iridium-catalyzed hydroboration−oxidation sequence in which **1** was treated with pinacolborane in the presence of catalytic $[Ir(COD)Cl]_2$ and bis-(diphenylphosphino)ethane followed by oxidation with H_2O_2 under weakly basic conditions, provided dihydroamorphigenin

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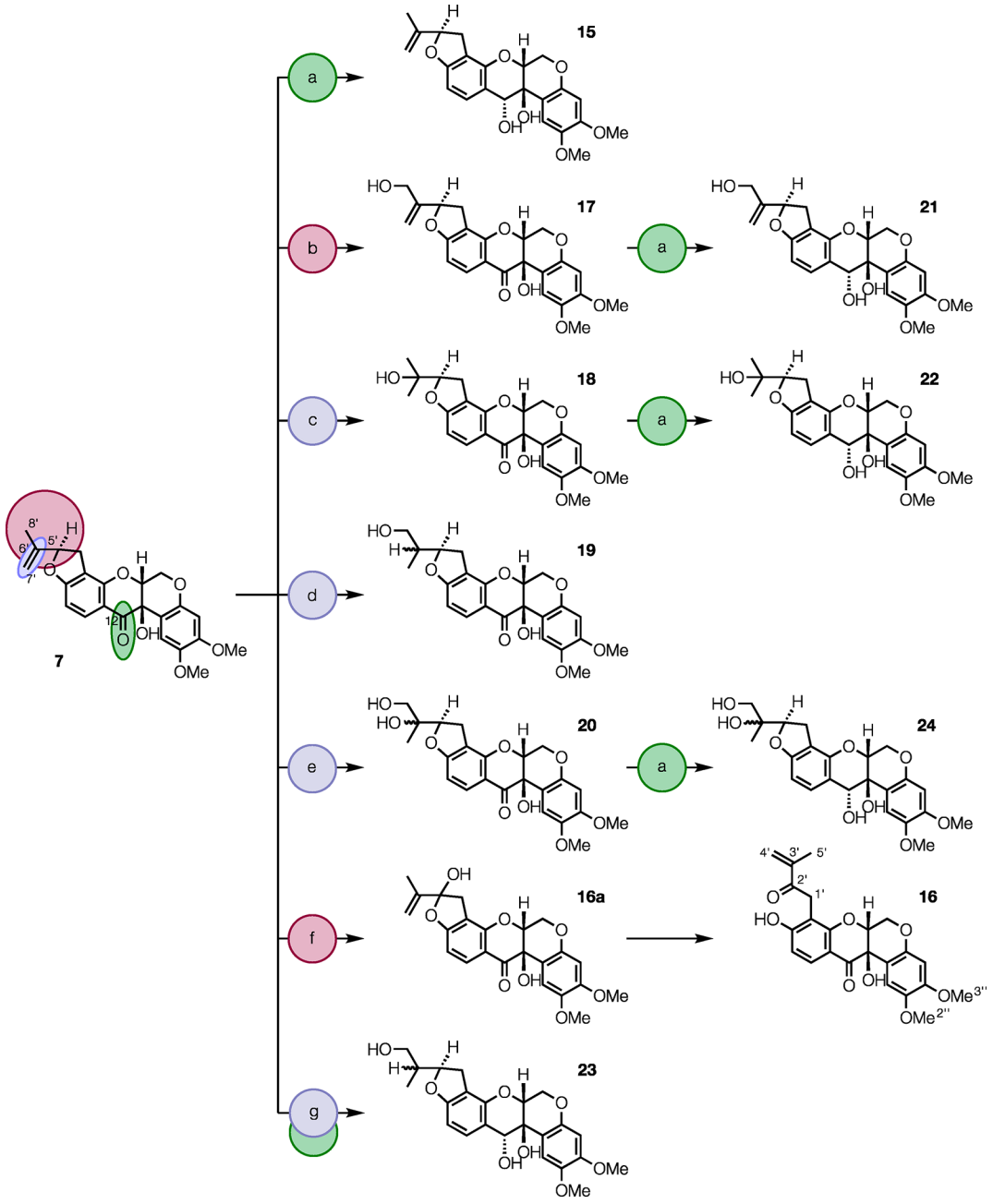
Scheme 2. Synthesis of Derivatives of Rotenolone (7)[a]



[a]Reagents and conditions: (a) $NaBH_4$, MeOH, 0 °C to rt, 2 h, 82−90%; (b) i. *N*-PSP, H_2O, CSA, CH_2Cl_2, rt, 72 h; ii. H_2O_2, Al_2O_3, THF, 0 °C to rt, 6.5 h, 21%; (c) i. $Hg(OAc)_2$, THF, H_2O, rt, 18 h; ii. NaOH, $NaBH_4$, THF, H_2O, rt, 0.5 min, 51%; (d) i. $[Ir(COD)Cl]_2$, DPPE, HBPin, CH_2Cl_2, rt, 20 h; ii. H_2O_2, $NaHCO_3$, THF, H_2O, 0 °C to rt, 20 h, 12%; (e) OsO_4, NMO, pyridine, acetone, H_2O, rt, 42 h, 49%; (f) SeO_2, 4 Å MS, 1,4-dioxane, 80 °C, 3 h, 20%; (g) i. $BH_3{\cdot}SMe_2$, THF, 0 °C to rt, 1.5 h; ii. H_2O_2, NaOH, THF, H_2O, 0 °C to rt, 18 h, 87%. Abbreviations: MS, molecular sieves; *N*-PSP, *N*-(phenylseleno)phthalimide; CSA, (+)-*β*-camphorsulfonic acid; COD, 1,5-cyclooctadiene; DPPE, 1,2-bis-(diphenylphosphino)ethane; HBPin, pinacolborane; NMO, *N*-methylmorpholine *N*-oxide.

(**5**).[47] Amorphigenol (**6**) was prepared from **1** as described previously.[48] Stereocontrolled reduction of the C-12-carbonyl group of **1** with $NaBH_4$ in MeOH afforded alcohol **10**,[49] while Étard-like hydroxylation at C-12a of **1** using $K_2Cr_2O_7$ in aqueous AcOH provided rotenolone (**7**).[50] The diastereoselectivity of the last two reactions probably results from attack on the butterfly wing conformer of **1**,[49,51] with hydride delivery (as in the conversion of **1** into **10**) and the pericyclic Étard-like reaction (as in the conversion of **1** into **7**) taking place on the more accessible convex face of the molecule. Reduction of the C-12 carbonyl groups of **3**, **4**, and **6** with $NaBH_4$ gave diols **11** and **12** and triol **13**, respectively (Scheme 1). Finally, diol **14** was obtained by concomitant hydroboration of the Δ6′(7′) double bond and reduction of the C-12 carbonyl group of **1**, using $BH_3{\cdot}SMe_2$, followed by oxidation of the intermediate borane with H_2O_2 under strongly basic conditions.

The library of compounds was expanded by derivatization of rotenolone (**7**) (Scheme 2) using the methods described above. Stereocontrolled reduction of the C-12 carbonyl group of **7** with $NaBH_4$ gave diol **15**.[50] Regioselective allylic oxidation

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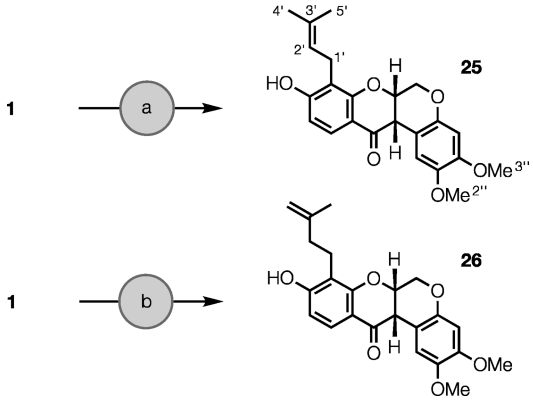
Scheme 3. Synthesis of Isomeric Rotenonic Acids[a]



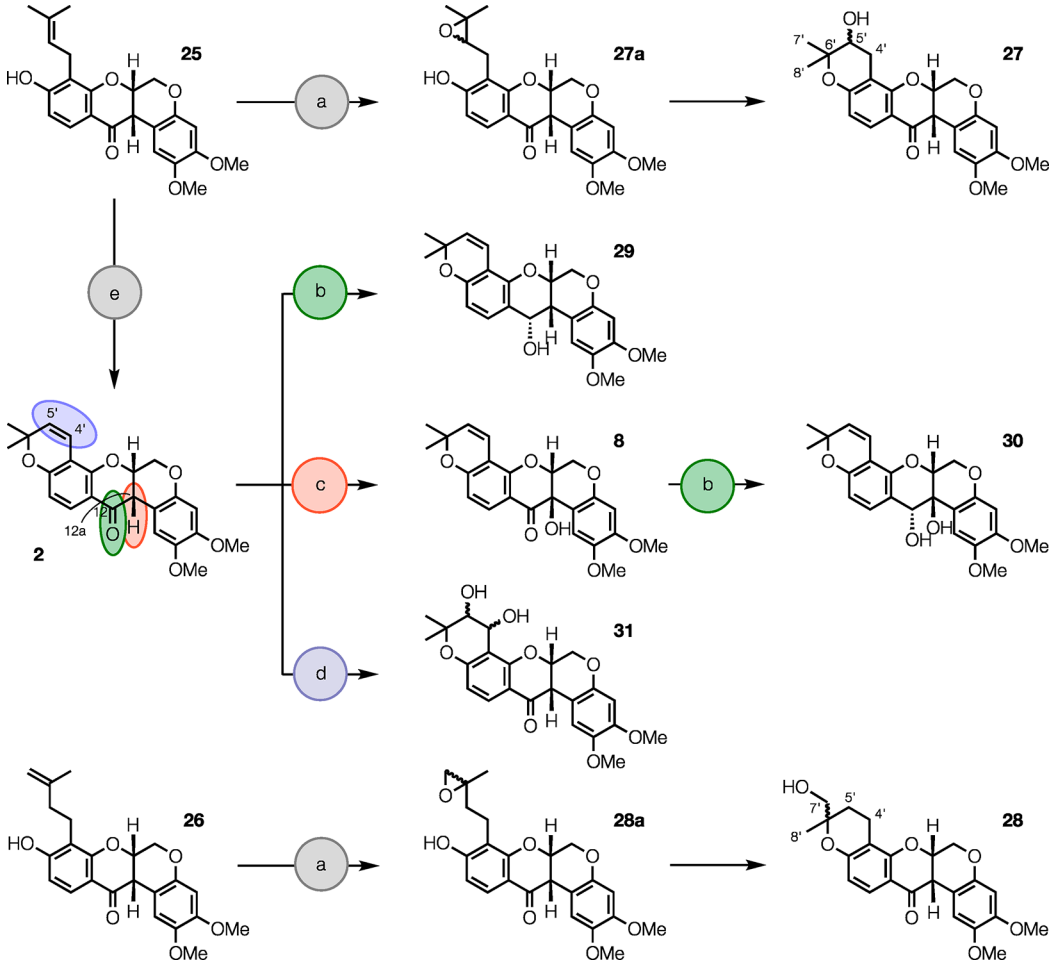
[a]Reagents and conditions: (a) i. HBr, AcOH; ii. Zn, NH_4Cl, THF, H_2O (see ref 54); (b) BBr_3, CH_2Cl_2, −20 °C, 0.5 h, 60%; ii. Zn, NH_4Cl, THF, H_2O, rt, 0.5 h, 79%.

at C-5′ and C-8′ of **7** gave keto-phenol **16**, via hemiacetal **16a**, and 12a-hydroxyamorphigenin (**17**), respectively. Markovnikov and anti-Markovnikov hydration of the Δ6′(7′) double bond of **7** afforded 12a-hydroxydalpanol (**18**) and 12a-hydroxydihydroamorphigenin (**19**), respectively. 12a-Hydroxyamorphigenin (**20**) was obtained by dihydroxylation of **7** using catalytic OsO_4 and stoichiometric NMO in the presence of pyridine.[45] Reduction of **17**, **18**, and **20** with $NaBH_4$ gave triols **21** and **22** and tetraol **24**, respectively. Finally, concomitant hydroboration of the Δ6′(7′) double bond and reduction of the C-12 carbonyl group of **7** followed by oxidation of the intermediate borane afforded triol **23**. Reduction of keto-phenols **9** (Scheme 1) and **16** (Scheme 2) was not attempted, as this would have led to further rotenonic acid-like molecules, which would likely be inactive.[52,53]

Next, rot-2′-enonic acid (**25**) was prepared from rotenone (**1**) as described previously (Scheme 3).[54] The isomericrot-3′-enonic acid (**26**) was also prepared from **1** (Scheme 3) by ring opening with BBr_3,[55,56] to give 4′-bromorot-2′-enonic acid (see Experimental Section), and reaction with activated zinc powder and NH_4Cl in aqueous THF.

Finally, hydroxylated derivatives of deguelin (**2**), which was synthesized from **25** as described previously,[54] were prepared (Scheme 4). Epoxidation and acid-catalyzed cyclization reactions of the isomeric rotenonic acids **25** and **26**, using *m*-CPBA and tosic acid,[57] yielded alcohols **27** and **28**, via

Scheme 4. Synthesis of Derivatives of Deguelin (2)[a]



[a]Reagents and conditions: (a) *m*-CPBA, TsOH, $CHCl_3$, 0 °C, 2 h, 62−69%; (b) $NaBH_4$, MeOH, 0 °C to rt, 2 h, 90−92%; (c) $K_2Cr_2O_7$, AcOH, H_2O (see ref 54); (d) OsO_4, NMO, citric acid, acetone, H_2O, rt, 28 h, 78%; (e) i. PhSeCl, CH_2Cl_2; ii. H_2O_2, THF (see ref 54). Abbreviations: *m*-CPBA, *meta*-chloroperoxybenzoic acid; TsOH, tosic acid, NMO, *N*-methylmorpholine *N*-oxide.

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epoxides **27a** and **28a**, respectively. Reduction of the C-12 carbonyl group of **2** with $NaBH_4$ gave alcohol **29** and hydroxylation at C-12a of **2** with $K_2Cr_2O_7$ afforded tephrosin (**8**), as described previously.[54] Reduction of the C-12 carbonyl group of **8** with $NaBH_4$ afforded diol **30**, while dihydroxylation of the Δ4′(5′) double bond of **2** using catalytic OsO_4 and stoichiometric NMO in the presence of citric acid gave diol **31**.[58]

In summary, by applying chemo-, regio-, and stereoselective redox reactions to rotenone (**1**), deguelin (**2**), and their derivatives, a library of 29 hydroxylated rotenoids (23 derivatives of **1** and six derivatives of **2**) was assembled without the use of protecting groups.

Measurement of IC_{50} Values and Calculation of $Slog_{10}P$ Values for Each Derivative. Each derivative was tested for its inhibitory effect on complex I activity by using a bovine heart mitochondrial membrane assay for NADH:O_2 oxidoreduction through complexes I, III, and IV. The established bovine model for the human enzyme was used due to ease of access to material. Half-maximal inhibitory concentrations (IC_{50} values) were calculated for all inhibitors with IC_{50} < 10 μM (Table 1). Rotenone (**1**) and deguelin (**2**) were included as positive controls and were the most potent, with IC_{50} values of 6.9 and 10.6 nM, respectively. Importantly, 23 out of the 29 hydroxylated derivatives (compounds **3**−**15**, **17**, **19**, **23**, and **25**−**31**) were found to inhibit complex I with IC_{50} < 10 μM. Further, where direct comparisons can be made, for compounds **1**, **2**, **7**, and **8** for example, the IC_{50} values agree well with those determined previously.[39] It should be noted that compounds **5**, **6**, **13**, **14**, **19**, **20**, **23**, **24**, **27**, and **28** were tested as mixtures of two C-5′- or C-6′-diastereoisomers (epimers) due to difficulties encountered in their separation, while compound **31** was tested as a mixture of two *cis*-4′,5′-diastereoisomers. IC_{50} values reported for these compounds are therefore reflective of their diastereoisomeric composition (see Experimental Section). It is well-established that the activity of rotenoids on complex I is strongly dependent on the stereochemistry of the *cis*-6a,12a BC ring junction (Figure 1),[53,59,60] which is conserved within our library, and less dependent on the stereochemistry of the (remote) C-5′ substituent in the E-ring.[59,60] Individual C-5′- and, by extension, C-6′-diastereoisomers are therefore expected to have similar inhibitory activities on complex I.

Next, the octanol−water partition coefficient ($Slog_{10}P$) of each derivative was predicted using the Molecular Operating Environment (MOE) software package (version 2012.10, Chemical Computing Group) (Table 1). This parameter describes the hydrophobicity of a molecule and indicates the probability of it crossing the BBB by passive diffusion.[22,23] The high $Slog_{10}P$ values for rotenone (**1**) and deguelin (**2**) of 3.70 and 4.01, respectively, were calculated for comparison, showing that they are among the most hydrophobic molecules in the library. Comparison of the IC_{50} and $Slog_{10}P$ data revealed a clear correlation between the hydrophobicity and inhibitory activity of the rotenoids tested, and the plot of IC_{50} vs $Slog_{10}P$ (Figure 2) shows that the more hydrophobic rotenoids are better complex I inhibitors. This is consistent with a binding model in which rotenoids bind in (or close to) the ubiquinone headgroup-binding site.[61,62] This binding site is accessed through a channel that leads out into the membrane,[63−66] and since rotenoids, as ubiquinone-binding site inhibitors, must partition into the lipid membrane to enter this channel, it

Table 1. Experimentally Measured IC_{50}Values and Computationally Predicted $Slog_{10}P$ Values for the Rotenoids Tested

compound	IC_{50} (nM)[a]	95% CI	$Slog_{10}P$
1	6.9	6.5−7.4	3.70
2	10.6	10.0−11.1	4.01
3	130	124−136	2.68
4	652	598−711	2.90
5[b]	228	220−237	2.76
6[b]	1800	1620−2000	1.87
7	137	127−148	3.12
8	77.5	71.6−83.8	3.42
9	2570	2460−2690	3.02
10	11.4	10.3−12.6	3.65
11	458	437−480	2.62
12	750	700−802	2.84
13[b]	4570	4400−4730	1.82
14[b]	460	425−498	2.70
15	125	119−131	3.06
16	>10 000	n/a	2.44
17	3810	3520−4120	2.09
18	>10 000	n/a	2.31
19[b]	3580	2620−5000	2.17
20[b]	>10 000	n/a	1.29
21	>10 000	n/a	2.04
22	>10000	n/a	2.26
23[b]	2930	2770−3090	2.12
24[b]	>10 000	n/a	1.23
25	1090	1050−1120	4.04
26	572	540−607	4.04
27[c]	850	787−922	2.90
28[b]	195	179−213	2.90
29	14.6	13.8−15.4	3.95
30	135	128−142	3.37
31[d]	5310	4970−5680	2.48

[a]The error for each IC_{50} value is reported as a 95% confidence interval (CI). [b]Tested as a mixture of two C-6′-diastereoisomers (epimers). [c]Tested as a mixture of two C-5′-diastereoisomers (epimers). [d]Tested as a mixture of two *cis*-4′,5′-diastereoisomers.

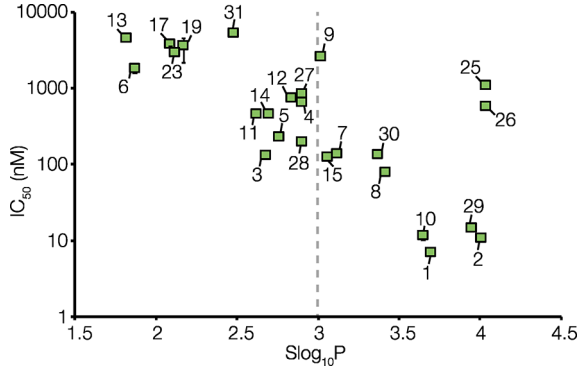

Figure 2. Dependence of IC_{50} values for complex I in bovine mitochondrial membranes on $Slog_{10}P$ for each rotenoid tested. IC_{50} values were determined from dose−response curves measured in triplicate. The best-fit values are shown alongside error bars showing 95% confidence intervals. The dotted line at $Slog_{10}P$ = 3 represents the cutoff value used to select rotenoids for evaluation in cell-based assays.

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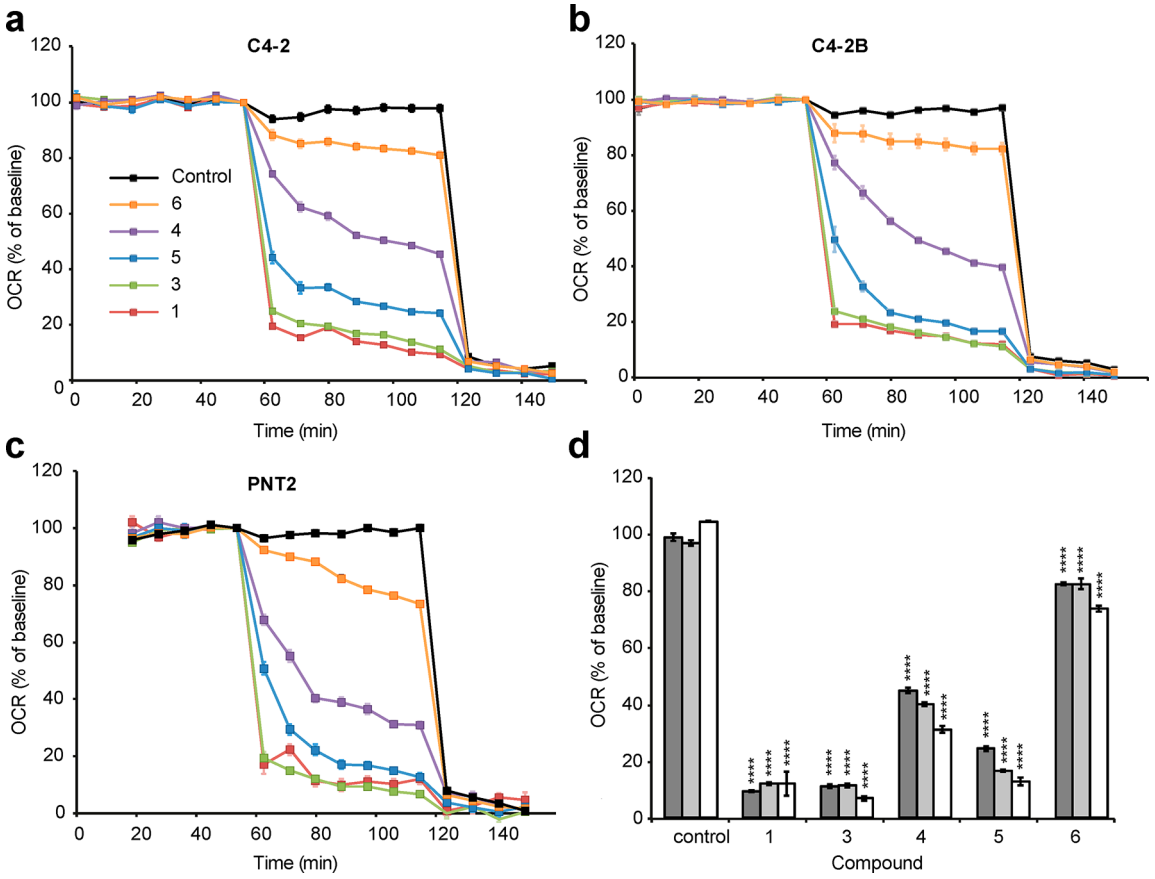

Figure 3. Antimycin-sensitive baselined oxygen consumption rates of (a) C4-2, (b) C4-2B, and (c) PNT2 cells measured in a Seahorse XF96 instrument. Rotenone (**1**), amorphigenin (**3**), dalpanol (**4**), dihydroamorphigenin (**5**), and amorphigenol (**6**) were added at 1 μM after 1 h of basal rate measurement followed by the addition of 2 μM rotenone and 2 μM antimycin after 2 h. Black, control; orange, **6**; purple, **4**; blue, **5**; green, **3**; red, **1**. (d) Antimycin-sentitive baselined oxygen consumption rates after 1 h of treatment. Dark gray, C4-2; light gray, C4-2B; white, PNT2. ****$P \leq 0.0001$. Error bars show SEM, $n = 6$ for treated groups, $n = 16$ for controls.

follows that the more hydrophobic rotenoids are also the more potent inhibitors.

Selection of Hydroxylated Rotenoids for Evaluation in Cell-Based Assays of Prostate Cancer. Of the hydroxylated metabolites of rotenone (**1**) and deguelin (**2**), only rotenolone (**7**) and tephrosin (**8**), which have $Slog_{10}P$ values of 3.12 and 3.42, respectively, are known to cross the BBB in mice and rats.[20] Thus, only molecules with $Slog_{10}P < 3$ were considered for further evaluation, as they are expected to cross the BBB less readily than **7** and **8** based on a simple hydrophobicity model (see above for a discussion of additional determinants of BBB penetration). Fourteen of the 23 inhibitors with $IC_{50} < 10$ μM (**3**–**6**, **11**–**14**, **17**, **18**, **23**, **27**, **28**, and **31**) have $Slog_{10}P < 3$. From these 14 compounds, the natural hydroxylated rotenoids amorphigenin (**3**), dalpanol (**4**), dihydroamorphigenin (**5**), and amorphigenol (**6**) were selected for evaluation in cell-based assays using PTEN-null prostate cancer cells and PTEN-positive control prostate cells. Compounds **3**–**6** have inhibitory potencies ranging from 130 to 1800 nM (Table 1) and were chosen to establish whether a correlation exists between potency and antiproliferative effect. In addition, compounds **3**–**6** share a conserved core structure (the ABCD ring system shown in Figure 1) and differ only in their E-ring substituents, so differences in their activities must rest on these structural differences alone.

Evaluation of Hydroxylated Rotenoids in Cell-Based Assays of Prostate Cancer. To establish whether amorphigenin (**3**), dalpanol (**4**), dihydroamorphigenin (**5**), and amorphigenol (**6**) reach and inhibit complex I in cells, oxygen consumption rate (OCR) assays were performed using a Seahorse XF96 instrument in which C4-2, C4-2B, and PNT2 cells were treated with each compound (1 μM). C4-2 and C4-2B cells were chosen as PTEN-null prostate cancer cells representative of advanced metastatic disease (C4-2B cells derive from C4-2 cells and therefore represent an even more invasive form of cancer). PTEN-positive PNT2 cells were selected as cells representative of normal prostate cells. All cell lines were also treated with rotenone (**1**) (1 μM) as a positive control. The results show that **3**–**6** all reach and inhibit complex I and that the reduction in the oxygen consumption rates of all cells, independent of type, correlated with the inhibitory strength of the compound added (Figure 3a–d). Thus, cells were most affected (and most rapidly affected) by **3** (IC_{50} 130 nM), followed by **5** (IC_{50} 228 nM), **4** (IC_{50} 652 nM), and then **6** (IC_{50} 1800 nM). Interestingly, **3** was almost as effective as **1** at inhibiting the oxygen consumption of all cells, even though it is a 19-fold weaker inhibitor of complex I in membranes (Table 1), while **5** also exerted a powerful effect.

To determine whether the inhibition of complex I by amorphigenin (**3**), dalpanol (**4**), dihydroamorphigenin (**5**), and amorphigenol (**6**) leads to selective antiproliferative activity in prostate cancer cells, C4-2, C4-2B, and PNT2 cells were treated with each compound (1 μM) and with rotenone (**1**) (1 μM) as a positive control. The results (Figure

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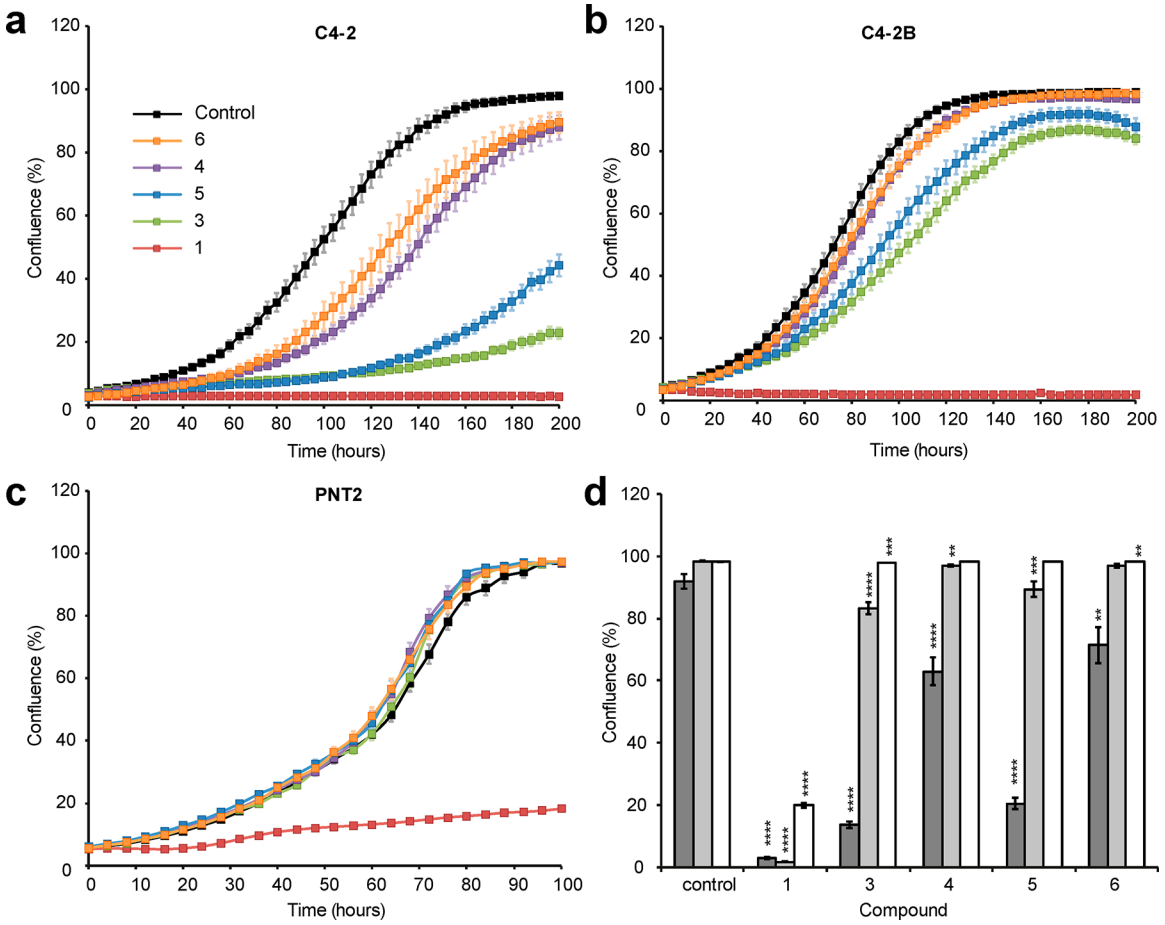

Figure 4. Cell growth curves for (a) C4-2, (b) C4-2B, and (c) PNT2 cells in the presence of 1 μM rotenone (**1**), amorphigenin (**3**), dalpanol (**4**), dihydroamorphigenin (**5**), and amorphigenol (**6**). Black, control; orange, **6**; purple, **4**; blue, **5**; green, **3**; red, **1**. (d) Confluence (the percentage surface area of each well covered by adherent cells) after 150 h of rotenoid treatment. Error bars show SEM, $n = 8$ for all groups. Dark gray, C4-2; light gray, C4-2B; white, PNT2. $^{**}P \leq 0.01$, $^{***}P \leq 0.001$, $^{****}P \leq 0.0001$.

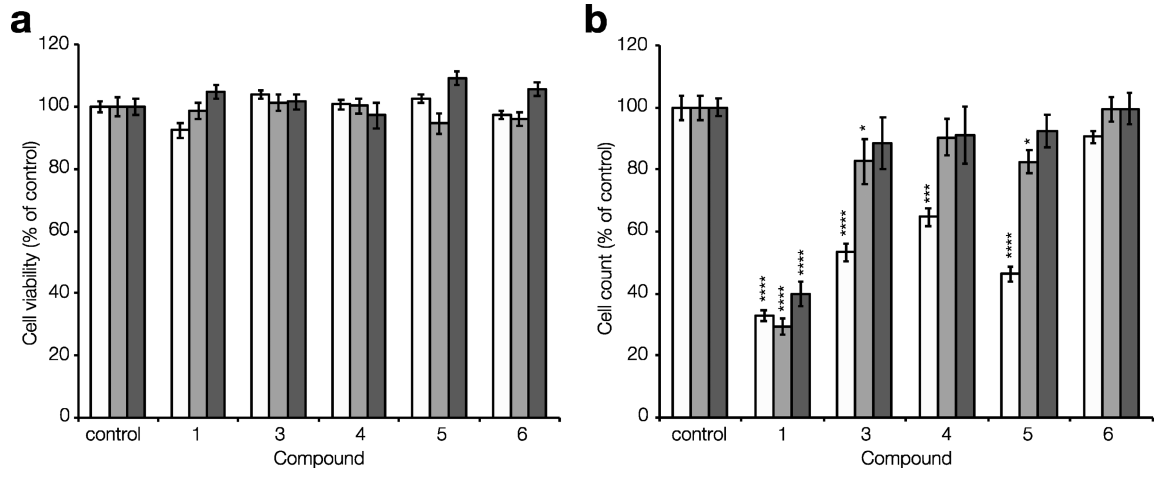

Figure 5. (a) Cell viability and (b) cell count following 48 h of treatment with 1 μM rotenone (**1**), amorphigenin (**3**), dalpanol (**4**), dihydroamorphigenin (**5**), and amorphigenol (**6**). White, C4-2; light gray, C4-2B; dark gray, PNT2. Error bars show SEM, $n = 4$ for treated groups, $n = 10$ for control. $^{*}P \leq 0.1$, $^{***}P \leq 0.001$, $^{****}P \leq 0.0001$.

4a–d) show that **3**–**6** inhibit the proliferation of the C4-2 and C4-2B prostate cancer cells, but leave the healthy PNT2 prostate cells relatively unaffected. The antiproliferative activity of each derivative against C4-2 and C4-2B was found to correlate directly with its inhibitory activity against complex I. Thus, C4-2 and C4-2B cells treated with **3** (IC_{50} 130 nM) proliferated the slowest, followed by those treated with **5** (IC_{50} 228 nM), **4** (IC_{50} 652 nM), and finally **6** (IC_{50} 1800 nM). C4-2 cells were particularly affected by treatment with **3** and **5** (Figure 4a), while, encouragingly, C4-2B cells also responded to **3** and **5**, but not **4** and **6** (Figure 4b). Importantly, PNT2 cells treated with **3**–**6** proliferated at the same rate as

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untreated cells (Figure 4c). C4-2 and C4-2B cells treated with **1** did not proliferate (Figure 4a,b); however, treated PNT2 cells retained their ability to proliferate, albeit at a substantially reduced rate (Figure 4c).

Finally, to confirm that the poor cell growth in the presence of rotenoids **3**–**6** was caused by inhibited cell proliferation rather than loss of cell viability, cells were treated for 48 h with each compound (1 μM) before their viability was tested and cell counts were measured (Figure 5) using the acridine orange assay and a Chemometec NC-3000. The data show that while rotenone (**1**) affected cell counts in all cells, and the cell count was lower for all compounds with C4-2 cells, no effect was seen on cell viability with any of the compounds tested. In addition, amorphigenin (**3**) and dihydroamorphigenin (**5**) had marginal effects on cell count for C4-2B cells. Therefore, under the growth conditions used (RPMI 1640 containing 11 mM glucose), inhibition of complex I leads primarily to the inhibition of cell proliferation. In contrast, a recent study reports ~70% loss of cell viability of PTEN-null cells in an MTT assay after 24 h with deguelin (**2**) (0.5 μM) at the same glucose concentration.[5] Inhibitors of the respiratory chain, including **1**, have been shown to slow down the rate of the MTT to formazan reaction.[67] Interestingly, the complex I inhibitor IACS-01079 has been reported to inhibit both cancer cell proliferation and viability as assayed using annexin V flow cytometry.[19]

A previous study revealed that **3** and **4** are nonselectively cytotoxic toward a variety of human cancer cell types, including A-549 lung, HCT-8 intestinal, RPMI-7951 skin, TE671 brain, and KB cervical cancer cells.[37] Half-maximal effective concentrations (ED_{50} values) determined for all cell types were consistently lower for **3** (0.01–0.05 $\mu g\ mL^{-1}$) than for **4** (0.48–4.83 $\mu g\ mL^{-1}$),[37] in agreement with our data showing that in every assay **3** is more active than **4** (Figures 3–5). The cytotoxicity of several hydroxylated rotenoids toward mouse lymphoma L5178Y cells has also been reported, with IC_{50} values ranging from 0.2 to 0.9 μM, as measured using the MTT assay,[43] suggesting that these cells are as sensitive to treatment with rotenoids as the PTEN-null prostate cancer cells used in our study. Most interestingly, a number of hydroxylated rotenoids, including rotenolone (**7**) and tephrosin (**8**) (Figure 1), have been shown to be selectively cytotoxic toward LNCaP prostate cancer cells, with ED_{50} values ranging from 0.3 to 0.7 μM.[40] The mechanism of action of these compounds was not investigated, but references were made to studies on the involvement of complex I. Taken together, our results show that the inhibition of complex I is essential to the mechanism of action of **3**–**6**, that prostate cancer cells display a greater dependence on OxPhos than healthy prostate cells, and that this dependence makes them vulnerable to inhibitors of complex I. The most significant effect of the inhibition of complex I by rotenoids **3**–**6** on prostate cancer cells occurs by inhibiting proliferation.

In summary, successive redox reactions were applied to rotenone (**1**), deguelin (**2**), and their derivatives to produce a library of 29 hydroxylated rotenoids. The inhibitory potency (IC_{50}) of each hydroxylated rotenoid against complex I was measured using a mitochondrial membrane assay, and its hydrophobicity ($Slog_{10}P$) was predicted computationally. From this library, amorphigenin (**3**), dalpanol (**4**), dihydroamorphigenin (**5**), and amorphigenol (**6**) were selected for evaluation in cell-based assays using C4-2 and C4-2B prostate cancer cells and control PNT2 prostate cells. Compounds **3**–**6** reach and inhibit complex I in cells, selectively inhibit the proliferation of prostate cancer cells, and leave control prostate cells unaffected. Further, the clear correlation between the inhibitory potency against complex I and the antiproliferative activity of **3**–**6** suggests that complex I inhibition is essential to their mechanism of action. Since the strongest antiproliferative effects were obtained with **3** and **5**, these hydroxylated rotenoids, which can be synthesized as described above or extracted from natural sources,[25,28,43] are proposed as candidates for detailed evaluation in more sophisticated cell-based assays of prostate cancer and animal models. Finally, the results highlight that prostate cancer cells are more dependent on OxPhos than healthy prostate cells and that they are vulnerable to a range of rotenoid inhibitors of complex I. It is anticipated that this work will stimulate further research into the therapeutic potential of carefully selected rotenoids and related metabolism-based approaches toward the treatment of prostate cancer.

EXPERIMENTAL SECTION

General Experimental Procedures. Melting points were determined using a Büchi B-545 melting point apparatus and are uncorrected. Optical rotations were measured using an Anton-Paar MCP 100 polarimeter. $[\alpha]^{20}_{D}$ values are reported in 10^{-1} deg $cm^2\ g^{-1}$ at 598 nm; concentration (c) is given in g (100 mL)$^{-1}$. Infrared spectra were recorded on a PerkinElmer Spectrum One spectrometer with internal referencing as neat films. Absorption maxima (ν_{max}) are reported in wavenumbers (cm^{-1}). ^{1}H NMR spectra were recorded on a Bruker Avance 500 Cryo Ultrashield spectrometer, operating at 500 MHz. Chemical shifts (δ) are quoted in parts per million (ppm) to the nearest 0.01 ppm and are referenced to residual solvent signals, $CHCl_3$ (δ 7.26) or acetone (δ 2.05). Coupling constants (J) are reported in hertz (Hz) to the nearest 0.5 Hz. Data are reported as follows: chemical shift, integration, multiplicity (br, broad; s, singlet; d, doublet; t, triplet; q, quartet; quint, quintet; m, multiplet; app, apparent; obsc, obscured; or as combinations or these (e.g., dd)), coupling constant(s), and assignment. ^{13}C NMR spectra were recorded on a Bruker Avance 500 Cryo Ultrashield spectrometer, operating at 125 MHz, with broadband proton spin decoupling. Chemical shifts (δ) are quoted in ppm to the nearest 0.1 ppm and are referenced to the solvent signals, $CDCl_3$ (δ 77.16) or acetone-d_6 (δ 29.84). High-resolution mass spectra were recorded using a Micromass LCT Premier spectrometer, and reported mass values are within the error limits of ±5 ppm. Analytical TLC was performed using glass plates coated with Sigma-Aldrich 60 (F_{254}) silica gel, and compounds were visualized by ultraviolet irradiation followed by staining with ceric ammonium molybdate solution and heating. Flash chromatography was performed with Sigma-Aldrich 60 (230–400 mesh) silica gel. Rotenone (**1**) (Molekula Fine Chemicals, Darlington, UK, 90–95%) was crystallized from EtOH three times. Deguelin (**2**), amorphigenol (**6**), tephrosin (**8**), and rot-2′-enonic acid (**25**) were prepared from **1** as described previously.[48,54] *N*-(Phenylseleno)-phthalimide (Sigma-Aldrich, technical grade) was crystallized from dry CH_2Cl_2/*n*-hexane under N_2. *m*-CPBA (Sigma-Aldrich, Gillingham, UK, <77%) was crystallized from CH_2Cl_2. Zinc powder was activated by washing with 2.0 M HCl for 0.5 h, and the coagulated metal was collected by filtration, washed in sequence with H_2O, EtOH, and Et_2O, dried *in vacuo*, and finely ground using a pestle and mortar. Powdered 4 Å molecular sieves were activated by heating *in vacuo* at 150 °C overnight. CH_2Cl_2, *n*-hexane, and MeOH were distilled from CaH_2 under N_2. THF was distilled from a mixture of CaH_2 and $LiAlH_4$ in the presence of triphenylmethane under N_2. All other solvents and reagents were used as obtained from commercial suppliers. Moisture-sensitive reactions were carried out with freshly distilled dry solvents under N_2 in glassware that was oven-dried.

General Procedure 1 (GP1). $NaBH_4$ (10 molar equiv) was added in small portions to a 0.02 M solution/suspension of the relevant

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rotenoid (1 molar equiv) in dry MeOH under N_2 at 0 °C. The mixture was warmed to rt and stirred for a further 2 h or until the reaction had gone to completion as determined by TLC. H_2O was added, and the mixture was extracted with Et_2O. The organic extracts were combined, washed with brine, dried ($MgSO_4$), filtered, and concentrated to afford the product. Products obtained by this method were sufficiently pure such that no further purification was required.

General Procedure 2 (GP2). $BH_3{\cdot}SMe_2$ (10 molar equiv) was added dropwise to a 0.04 M solution of the relevant rotenoid (1 molar equiv) in dry THF under N_2 at 0 °C. The mixture was stirred at 0 °C for 0.5 h, warmed to rt, and stirred for a further 1 h. The mixture was cooled to 0 °C, and 3.0 M aqueous NaOH solution (30 molar equiv) was added. H_2O_2 (30% aqueous solution, 30 molar equiv) was cautiously added dropwise, and the mixture was stirred for 18 h while slowly warming to rt. H_2O was added, and the mixture extracted with Et_2O. The organic extracts were combined, washed with brine, dried ($MgSO_4$), filtered, and concentrated to afford the product. Products obtained by this method were sufficiently pure such that no further purification was required.

General Procedure 3 (GP3). Using a modified version of the procedure described for the synthesis of **9**,[45] SeO_2 (4 molar equiv) was added to a 0.04 M solution/suspension of the relevant rotenoid **1** (1 molar equiv) and powdered 4 Å molecular sieves (equivalent mass to that of the rotenoid) in dry 1,4-dioxane under N_2. The mixture was heated at 80 °C for 3 h, cooled to rt, filtered through a pad of Celite, and concentrated. The residue was subjected to flash chromatography to afford the product.

General Procedure 4 (GP4). Using the procedure described for the synthesis of **27**,[57] a 0.2 M solution of *m*-CPBA (2 molar equiv) in dry $CHCl_3$ was added to a 0.04 M solution of the relevant rotenonic acid (1 molar equiv) in dry $CHCl_3$ under N_2 at 0 °C. Tosic acid monohydrate (0.5 molar equiv) was then added, and the mixture stirred at 0 °C for 2 h. H_2O was added and the mixture extracted with $CHCl_3$. The organic phases were combined, washed with saturated aqueous $NaHCO_3$ solution and brine, dried ($MgSO_4$), filtered, and concentrated. The residue was subjected to flash chromatography to afford the product.

*(6aS,12aS,5′R)-Amorphigenin (**3**).* **3** was prepared by modifying a literature procedure.[46] *N*-(Phenylseleno)phthalimide (153 mg, 0.508 mmol) was added to a solution of **1** (200 mg, 0.508 mmol), (+)-*β*-camphorsulfonic acid (12.0 mg, 0.051 mmol), and H_2O (183 μL, 10.1 mmol) in CH_2Cl_2 (10 mL) under N_2 in a flask wrapped in aluminum foil. The mixture was stirred at rt for 72 h, then concentrated. The yellow solid obtained was cooled to 0 °C and dissolved in pyridine (4.0 mL) before H_2O_2 (0.5 mL, 30% aqueous solution) was added dropwise over 10 min. The mixture was stirred at 0 °C for 0.5 h, warmed to rt, and stirred for 1.5 h. Saturated aqueous $NaHCO_3$ solution (20 mL) was added, and the mixture was extracted with $CHCl_3$ (3 × 20 mL). The organic extracts were combined, washed with 3.0 M HCl (3 × 20 mL), H_2O (3 × 20 mL), and brine (20 mL), dried ($MgSO_4$), filtered, and concentrated. The residue was subjected to flash chromatography (SiO_2, 1:1 hexanes/EtOAc) to give **3** as a pale yellow solid that crystallized from $CHCl_3$/MeOH as colorless needles (68 mg, 33%): mp 196−198 °C (lit.[46] mp 196−197 °C); $[\alpha]^{20}_D$ −127 (*c* 0.1, $CHCl_3$) [lit.[46] $[\alpha]^{24}_D$ −124 (*c* 0.2, $CHCl_3$)]; IR ν_{max} 3500−3100, 1671, 1599, 1517, 1455, 1349, 1303, 1234, 1212, 1195, 1087, 817 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 3.08 (1H, dd, *J* 9.0, 15.5 Hz, H_a-4′), 3.39 (1H, dd, *J* 9.0, 15.5 Hz, H_b-4′), 3.76 (3H, s, H-2′), 3.81 (3H, s, H-3′), 3.85 (1H, d, *J* 4.0 Hz, H-12a), 4.18 (1H, d, *J* 12.0 Hz, H_a-6), 4.23 (1H, d, 13.5 Hz, H_a-8′), 4.28 (1H, d, 13.5 Hz, H_b-8′), 4.61 (1H, dd, *J* 3.0, 12.0 Hz, H_b-6), 4.93 (1H, dd, *J* 3.0, 4.0 Hz, H-6a), 5.26 (1H, s, H_a-7′), 5.28 (1H, s, H_b-7′), 5.39 (1H, t, *J* 9.0 Hz, H-5′), 6.45 (1H, s, H-4), 6.51 (1H, d, *J* 9.0 Hz, H-10), 6.76 (1H, s, H-1), 7.84 (1H, d, *J* 9.0 Hz, H-11); ^{13}C NMR(125 MHz, $CDCl_3$) δ 32.0 (C-4′), 44.8 (C-12a), 56.0 (C-3′), 56.5 (C-2′), 63.1 (C-8′), 66.4 (C-6), 77.4 (C-6a), 85.7 (C-5′), 101.1 (C-4), 104.9 (C-1a), 105.1 (C-10), 110.5 (C-1), 112.9 (C-7′), 113.0 (C-8), 113.7 (C-11a), 130.2 (C-11), 144.0 (C-2), 146.8 (C-6′), 147.5 (C-3), 149.7 (C-4a), 158.1 (C-7a), 167.0 (C-9), 189.1 (C-12); HRESIMS *m*/*z* 411.1440 $[M + H]^+$ (calcd for $C_{23}H_{23}O_7$, *m*/*z* 411.1444).

*(6aS,12aS,5′R)-Dalpanol (**4**).* **4** was prepared by modifying a literature procedure.[27] [***Caution**: Hg(OAc)$_2$ and elemental Hg are highly toxic and must be handled with extreme care; all operations must be carried out in a fume-hood and all Hg-containing waste retained for proper waste disposal*]. H_2O (2.0 mL) was added dropwise to a suspension of $Hg(OAc)_2$ (178 mg, 0.558 mmol) in THF (2.0 mL), and the bright yellow mixture was stirred at rt for 10 min. **1** (200 mg, 0.508 mmol) was added, and the mixture was stirred at rt for 18 h. Brine (20 mL) was added followed by $CHCl_3$ (20 mL), and the two phases were thoroughly mixed. The organic layer was separated, dried ($MgSO_4$), filtered, and concentrated. The solid obtained was dissolved in THF (2.0 mL) and H_2O (1.5 mL). Saturated aqueous $NaHCO_3$ solution (0.5 mL) was added followed by $NaBH_4$ (19.0 mg, 0.508 mmol), and the mixture was stirred vigorously for 0.5 min as elemental Hg precipitated and coated the walls of the flask. H_2O (20 mL) was added followed by $CHCl_3$ (20 mL), and the two phases were thoroughly mixed. The biphasic mixture was carefully decanted, and the organic layer was separated. The aqueous phase was extracted with $CHCl_3$ (2 × 20 mL), and the organic phases were combined, washed with brine (20 mL), dried ($MgSO_4$), filtered, and concentrated. The residue was subjected to flash chromatography (SiO_2, 1:1 hexanes/EtOAc) to give **4** as a white solid that crystallized from MeOH/H_2O as very fine colorless needles (128 mg, 48%): mp 208−210 °C (lit.[27] mp 189−191 °C, benzene); $[\alpha]^{20}_D$ −102 (*c* 0.1, $CHCl_3$) [lit.[27] $[\alpha]^{24}_D$ −122 (*c* 2.0, $CHCl_3$)]; IR ν_{max} 3500−3100, 1678, 1641, 1608, 1514, 1462, 1440, 1348, 1269, 1235, 1212, 1198, 1182, 1095, 1081, 817 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 1.22 (3H, s, H-7′), 1.35 (3H, s, H-8′), 1.76 (1H, br s, OH-6′), 3.09 (1H, dd, *J* 9.0, 15.0 Hz, H_a-4′), 3.14 (1H, dd, *J* 9.0, 15.0 Hz, H_b-4′), 3.76 (3H, s, H-2′), 3.81 (3H, s, H-3′), 3.84 (1H, d, *J* 4.0 Hz, H-12a), 4.18 (1H, d, *J* 12.0 Hz, H_a-6), 4.62 (1H, dd, *J* 3.0, 12.0 Hz, H_b-6), 4.67 (1H, t, *J* 9.0 Hz, H-5′), 4.93 (1H, dd, *J* 3.0, 4.0 Hz, H-6a), 6.45 (1H, s, H-4), 6.49 (1H, d, *J* 8.5 Hz, H-10), 6.76 (1H, s, H-1), 7.82 (1H, d, *J* 8.5 Hz, H-11); ^{13}C NMR (125 MHz, $CDCl_3$) δ 24.1 (C-7′), 26.4 (C-8′), 27.5 (C-4′), 44.8 (C-12a), 56.0 (C-3′), 56.5 (C-2′), 66.4 (C-6), 71.8 (C-6a), 72.3 (C-6′), 91.6 (C-5′), 101.1 (C-4), 104.9 (C-1a), 104.9 (C-10), 110.4 (C-1), 113.6 (C-8), 113.8 (C-11a), 130.0 (C-11), 144.0 (C-2), 147.5 (C-3), 149.6 (C-4a), 158.1 (C-7a), 167.3 (C-9), 189.2 (C-12); HRESIMS *m*/*z* 413.1584 $[M + H]^+$ (calcd for $C_{23}H_{25}O_7$, *m*/*z* 413.1595).

*(6aS,12aS,5′R,6′R)- and (6aS,12aS,5′R,6′S)-6′,7′-Dihydroamorphigenin (**5**).* **1** (200 mg, 0.508 mmol) was added to a solution of $[Ir(COD)Cl]_2$ (3.4 mg, 0.005 mmol), 1,2-bis(diphenylphosphino)-ethane (4.0 mg, 0.010 mmol), and pinacol borane (295 μL, 2.030 mmol) in dry CH_2Cl_2 (6.0 mL) under N_2. The mixture was stirred at rt for 20 h. MeOH (1 mL) was then added, and the mixture was concentrated. The resulting yellow oil was dissolved in THF (6.0 mL), and $NaHCO_3$ (21 mg, 0.254 mmol) was added followed by H_2O_2 (1.73 mL, 30% aqueous solution, 15.2 mmol), which was cautiously added dropwise. The mixture stirred at rt for 20 h. H_2O (20 mL) was added, and the mixture was extracted with $CHCl_3$ (3 × 20 mL). The organic extracts were combined, washed with brine (3 × 20 mL), dried ($MgSO_4$), filtered, and concentrated. The oily residue was subjected to flash chromatography (SiO_2, 1:1 hexanes/EtOAc) to give the mixture of diastereoisomers **5** as a white solid (28 mg, 14%, dr 74:26 unassigned): IR ν_{max} 3500−3200, 1682, 1609, 1514, 1454, 1438, 1348, 1308, 1238, 1212, 1190, 1090, 1074, 1005, 974, 829, 818 cm^{-1}; NMR data for the major diastereoisomer, δ_H (500 MHz, $CDCl_3$) 0.99 (3H, d, *J* 7.0 Hz, H-8′), 2.00−2.18 (1H, m obsc, H-6′), 2.95 (1H, dd, *J* 8.5, 16.0 Hz, H_a-4′), 3.24 (1H, dd, *J* 8.5, 16.0 Hz, H_b-4′), 3.66−3.74 (2H, m obsc, H_a-7′ and H_b-7′), 3.76 (3H, s, H-2′), 3.81 (3H, s, H-3′), 3.84 (1H, d, *J* 4.0 Hz, H-12a), 4.18 (1H, d, *J* 12.0 Hz, H_a-6), 4.62 (1H, dd, *J* 3.0, 12.0 Hz, H_b-6), 5.00 (dd, *J* 4.5, 8.5 Hz, H-5′), 4.93 (1H, ddd, *J* 1.0, 3.0, 4.0 Hz, H-6a), 6.45 (1H, s, H-4), 6.46 (1H, d, *J* 8.5 Hz, H-10), 6.77 (1H, s, H-1), 7.82 (1H, d, *J* 8.5 Hz, H-11); δ_C (125 MHz, $CDCl_3$) 10.9 (C-8′), 29.8 (C-4′), 40.6 (C-6′), 44.8 (C-12a), 56.0 (C-3′), 56.5 (C-2′), 65.2 (C-7′), 66.4 (C-6), 72.4 (C-6a), 86.7 (C-5′), 101.1 (C-4), 104.9 (C-1a), 105.0 (C-10), 110.5 (C-1), 113.2 (C-11a), 113.4 (C-8), 130.1 (C-11), 144.0 (C-2), 147.5 (C-4a), 149.6 (C-3), 158.1 (C-7a), 167.6 (C-9), 189.1 (C-12); NMR

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data for the minor diastereoisomer, δ_H (500 MHz, $CDCl_3$) 0.99 (3H, d, *J* 7.0 Hz, H-8′), 2.00−2.18 (1H, m obsc, H-6′), 2.91 (1H, dd, *J* 8.5, 16.0 Hz, H_a-4′), 3.27 (1H, dd, *J* 8.5, 16.0 Hz, H_b-4′), 3.66−3.74 (2H, m obsc, H_a-7′ and H_b-7′), 3.76 (3H, s, H-2′), 3.81 (3H, s, H-3′), 3.84 (1H, d, *J* 4.0 Hz, H-12a), 4.18 (1H, d, *J* 12.0 Hz, H_a-6), 4.62 (1H, dd, *J* 3.0, 12.0 Hz, H_b-6), 4.78 (dd, *J* 4.5, 8.5 Hz, H-5′), 4.93 (1H, ddd, *J* 1.0, 3.0, 4.0 Hz, H-6a), 6.45 (1H, s, H-4), 6.47 (1H, d, *J* 8.5 Hz, H-10), 6.77 (1H, s, H-1), 7.83 (1H, d, *J* 8.5 Hz, H-11); δ_C (125 MHz, $CDCl_3$) 12.7 (C-8′), 30.7 (C-4′), 41.1 (C-6′), 44.8 (C-12a), 56.0 (C-3′), 56.5 (C-2′), 65.2 (C-7′), 65.9 (C-6), 72.4 (C-6a), 88.9 (C-5′), 101.1 (C-4), 104.9 (C-1a), 105.1 (C-10), 110.5 (C-1), 113.2 (C-11a), 113.5 (C-8), 130.1 (C-11), 144.0 (C-2), 147.5 (C-4a), 149.6 (C-3), 158.1 (C-7a), 167.1 (C-9), 189.1 (C-12); HRESIMS *m*/*z* 435.1403 $[M + Na]^+$ (calcd for $C_{23}H_{24}O_7Na$, *m*/*z* 435.1414).

*(6aR,12aR,5′R)-Rotenolone (**7**).* **7** was prepared by modifying a literature procedure.[50] A solution of $K_2Cr_2O_7$ (2.8 g, 9.45 mmol) in H_2O (40 mL) was added dropwise over 10 min to a solution of **1** (4.0 g, 10.15 mmol) in AcOH (80 mL) at 60 °C. The mixture was stirred for 0.5 h, then cooled to rt and stirred for a further 18 h. The dark green mixture was poured onto crushed ice (400 mL), and the resulting suspension was stirred for 1 h while an off-white precipitate formed. The precipitate was collected by filtration, washed thoroughly with H_2O (200 mL), dried *in vacuo*, and purified by flash chromatography (SiO_2, 2:1 hexanes/EtOAc) to give **7** as a white solid (3.42 g, 82%): $[\alpha]^{20}_D$ −176 (*c* 0.1, $CHCl_3$) [lit.[50] $[\alpha]^{16}_D$ −189 (*c* 2.0, $CHCl_3$)]; IR ν_{max} 3600−3300, 1673, 1607, 1508, 1455, 1331, 1258, 1216, 1154, 1085, 1023, 905, 816 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 1.76 (3H, s, H-8′), 2.93 (1H, dd, *J* 8.0, 16.0 Hz, H_a-4′), 3.29 (1H, dd, *J* 8.0, 16.0 Hz, H_b-4′), 3.72 (3H, s, H-2′), 3.82 (3H, s, H-3′), 4.47 (1H, s, OH-12a), 4.49 (1H, d, *J* 11.5 Hz, H_a-6), 4.58 (1H, dd, *J* 1.0, 2.5 Hz, H-6a), 4.59 (1H, dd, *J* 2.5, 11.5 Hz, H_b-6), 4.93 (1H, s, H_a-7′), 5.06 (1H, s, H_b-7′), 5.23 (1H, app t, *J* 8.0 Hz, H-5′), 6.48 (1H, s, H-4), 6.53 (1H, d, *J* 8.5 Hz, H-10), 6.55 (1H, s, H-1), 7.82 (1H, d, *J* 8.5 Hz, H-11); ^{13}C NMR (125 MHz, $CDCl_3$) δ 17.2 (C-8′), 31.3 (C-4′), 56.0 (C-3′), 56.5 (C-2′), 64.0 (C-6), 67.7 (C-12a), 76.2 (C-6a), 88.1 (C-5′), 101.2 (C-4), 105.5 (C-10), 108.9 (C-1a), 109.4 (C-1), 111.9 (C-11a), 112.9 (C-7′), 113.3 (C-8), 130.2 (C-11), 143.0 (C-6′), 144.1 (C-2), 148.5 (C-4a), 151.2 (C-3), 157.8 (C-7a), 168.2 (C-9), 191.2 (C-12); HRESIMS *m*/*z* 433.1241 $[M + Na]^+$ (calcd for $C_{23}H_{22}O_7Na$, *m*/*z* 433.1258).

*(6aS,12aS)-2′-Oxorot-3′-enonic Acid (**9**).*[45] **9** was prepared from **1** (200 mg, 0.508 mmol) by GP3 and obtained as a yellow solid (44 mg, 21%): $[\alpha]^{20}_D$ +34 (*c* 0.1, $CHCl_3$) [lit.[45] $[\alpha]^{25}_D$ +38 (*c* 0.1, $CHCl_3$)]; IR ν_{max} 3400−3100, 1675, 1579, 1547, 1513, 1444, 1347, 1286, 1259, 1216, 1196, 1051, 817 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 1.86 (3H, s, H-5′), 3.75 (3H, s, H-2″), 3.81 (3H, s, H-3″), 3.84 (1H, d, *J* 4.0 Hz, H-12a), 3.87 (1H, d, *J* 13.5 Hz, H_a-1′), 4.22 (1H, d, *J* 12.0 Hz, H_a-6), 4.23 (1H, d, *J* 13.5 Hz, H_b-1′), 4.64 (1H, dd, *J* 3.5, 12.0 Hz, H_b-6), 4.95 (1H, ddd, *J* 1.0, 3.5, 4.0 Hz, H-6a), 6.00 (1H, s, H_a-4′), 6.42 (1H, s, H-4), 6.59 (1H, s, H_b-4′), 6.63 (1H, d, *J* 8.5 Hz, H-10), 6.74 (1H, s, H-1), 7.81 (1H, d, *J* 8.5 Hz, H-11), 8.97 (1H, s, OH-9); ^{13}C NMR(125 MHz, $CDCl_3$) δ 17.5 (C-5′), 32.3 (C-1′), 44.4 (C-12a), 56.1 (C-3″), 56.4 (C-2″), 66.5 (C-6), 72.6 (C-6a), 100.9 (C-4), 104.7 (C-1a), 108.5 (C-8), 110.4 (C-1), 112.6 (C-10), 112.8 (C-11a), 128.7 (C-11), 130.3 (C-4′), 143.7 (C-3′), 144.1 (C-2), 147.5 (C-4a), 149.7 (C-3), 159.1 (C-7a), 163.9 (C-9), 189.4 (C-12), 203.7 (C-2′); HRESIMS *m*/*z* 433.1239 $[M + Na]^+$ (calcd for $C_{23}H_{22}O_7Na$, *m*/*z* 433.1258).

*(6aS,12S,12aR,5′R)-12-Deoxo-12-hydroxyrotenone (**10**).*[49] **10** was prepared from **1** (1.0 g, 2.54 mmol) by GP1 and obtained as a white solid (921 mg, 92%): $[\alpha]^{20}_D$ −150 (*c* 0.1, acetone); IR ν_{max} 3600−3300, 1619, 1512, 1479, 1463, 1217, 1194, 1131, 1091, 1037, 799 cm^{-1}; ^1H NMR (500 MHz, acetone-d_6) δ 1.76 (3H, s, H-8′), 2.88 (1H, dd, *J* 9.0, 15.5 Hz, H_a-4′), 3.22 (1H, dd, *J* 9.0, 15.5 Hz, H_b-4′), 3.44 (1H, app t, *J* 4.5 Hz, H-12a), 3.70 (3H, s, H-2′), 3.74 (3H, s, H-3′), 4.17 (1H, dd, *J* 3.0, 10.0 Hz, H_a-6), 4.48 (1H, d, *J* 4.0 Hz, OH-12), 4.61 (1H, app t, *J* 10.0 Hz, H_b-6), 4.81 (1H, ddd, *J* 3.0, 4.5, 10.0 Hz, H-6a), 4.88 (1H, s, H_a-7′), 5.06 (1H, s, H_b-7′), 5.12 (1H, dd, *J* 4.0, 4.5 Hz, H-12), 5.16 (1H, app t, *J* 9.0 Hz, H-5′), 6.32 (1H, d, *J* 8.0 Hz, H-10), 6.37 (1H, s, H-4), 7.10 (1H, d, *J* 8.0 Hz, H-11), 7.17 (1H, s, H-1); ^{13}C NMR (125 MHz, acetone-d_6) δ 17.4 (C-8′), 32.7 (C-4′), 38.5 (C-12a), 55.9 (C-3′), 56.8 (C-2′), 66.5 (C-6), 67.6 (C-12), 71.2 (C-6a), 86.8 (C-5′), 101.3 (C-4), 102.3 (C-10), 111.5 (C-7′), 111.6 (C-1a), 112.8 (C-8), 114.6 (C-1), 117.6 (C-11a), 129.6 (C-11), 144.4 (C-2), 145.5 (C-6′), 150.2 (C-3), 150.3 (C-4a), 150.6 (C-7a), 161.9 (C-9); HRESIMS *m*/*z* 419.1449 $[M + Na]^+$ (calcd for $C_{20}H_{24}O_6Na$, *m*/*z* 419.1465).

*(6aS,12S,12aR,5′R)-12-Deoxo-12-hydroxyamorphigenin (**11**).* **11** was prepared from **3** (20 mg, 0.048 mmol) by GP1 and obtained as a white solid (17.7 mg, 88%): $[\alpha]^{20}_D$ −164 (*c* 0.1, acetone); IR ν_{max} 3600−3300, 1620, 1515, 1465, 1220, 1194, 1131, 1093, 1039, 984, 787 cm^{-1}; ^1H NMR (500 MHz, acetone-d_6) δ 3.00 (1H, dd, *J* 8.5, 15.5 Hz, H_a-4′), 3.28 (1H, dd, *J* 8.5, 15.5 Hz, H_b-4′), 3.44 (1H, app t, *J* 4.5 Hz, H-12a), 3.70 (3H, s, H-2′), 3.74 (3H, s, H-3′), 4.17 (1H, *J* 3.0, 9.5 Hz, H_a-6), 4.18 (1H, d, *J* 14.0 Hz, H_a-8′), 4.19 (1H, d, *J* 14.0 Hz, H_b-8′), 4.50 (1H, d, *J* 4.0 Hz, OH-12), 4.59 (1H, app t, *J* 9.5 Hz, H_b-6), 4.80 (1H, ddd, *J* 3.0, 4.5, 9.5 Hz, H-6a), 5.16 (1H, dd, *J* 4.0, 4.5 Hz, H-12), 5.17 (1H, s, H_a-7′), 5.19 (1H, s, H_b-7′), 5.30 (1H, app t, *J* 8.5 Hz, H-5′), 6.33 (1H, d, *J* 8.0 Hz, H-10), 6.37 (1H, s, H-4), 7.10 (1H, d, *J* 8.0 Hz, H-11), 7.17 (1H, s, H-1); ^{13}C NMR (125 MHz, acetone-d_6) δ 33.3 (C-4′), 38.5 (C-12a), 55.9 (C-3′), 56.8 (C-2′), 62.3 (C-8′), 66.5 (C-6), 67.6 (C-12), 71.2 (C-6a), 84.6 (C-5′), 101.3 (C-4), 102.3 (C-10), 109.6 (C-7′), 111.6 (C-1a), 112.9 (C-8), 114.6 (C-1), 117.7 (C-11a), 129.6 (C-11), 144.4 (C-2), 150.2 (C-6′), 150.2 (C-3), 150.3 (C-4a), 150.6 (C-7a), 161.8 (C-9); HRESIMS *m*/*z* 435.1402 $[M + Na]^+$ (calcd for $C_{23}H_{24}O_7Na$, *m*/*z* 435.1414).

*(6aS,12S,12aR,5′R)-12-Deoxo-12-hydroxydalpanol (**12**).*[27] **12** was prepared from **4** (20 mg, 0.049 mmol) by GP1 and obtained as a white solid (18.5 mg, 92%): $[\alpha]^{20}_D$ −120 (*c* 0.1, acetone); IR ν_{max} 3600−3300, 1617, 1518, 1505, 1483, 1467, 1240, 1220, 1192, 1131, 1078, 1039, 956, 789 cm^{-1}; ^1H NMR (500 MHz, acetone-d_6) δ 1.20 (3H, s, H-7′), 1.24 (3H, s, H-8′), 2.98 (1H, dd, *J* 8.5, 13.5 Hz, H_a-4′), 3.10 (1H, dd, *J* 8.5, 13.5 Hz, H_b-4′), 3.43 (1H, app t, *J* 5.0 Hz, H-12a), 3.70 (3H, s, H-2′), 3.74 (3H, s, H-3′), 4.17 (1H, dd, *J* 4.0, 10.0 Hz, H_a-6), 4.44 (1H, s, OH-12), 4.59 (1H, app t, *J* 8.5 Hz, H-5′), 4.60 (1H, app t, *J* 8.5 Hz, H_b-6), 4.80 (1H, ddd, *J* 4.0, 5.0, 10.0 Hz, H-6a), 5.11 (1H, dd, *J* 4.0, 5.0 Hz, H-12), 6.26 (1H, d, *J* 8.0 Hz, H-10), 6.37 (1H, s, H-4), 7.05 (1H, d, *J* 8.0 Hz, H-11), 7.16 (1H, s, H-1); ^{13}C NMR (125 MHz, acetone-d_6) δ 25.6 (C-7′), 26.0 (C-8′), 28.5 (C-4′), 38.6 (C-12a), 55.9 (C-3′), 56.8 (C-2′), 66.5 (C-6), 67.6 (C-12), 71.1 (C-6a), 71.5 (C-6′), 90.9 (C-5′), 101.3 (C-4), 102.2 (C-10), 111.7 (C-1a), 113.7 (C-8), 114.6 (C-1), 117.3 (C-11a), 129.3 (C-11), 144.4 (C-2), 150.2 (C-4a), 150.3 (C-7a), 150.5 (C-3), 162.2 (C-9); HRESIMS *m*/*z* 437.1557 $[M + Na]^+$ (calcd for $C_{23}H_{26}O_7Na$, *m*/*z* 437.1571).

*(6aS,12S,12aR,5′R,6′R)- and (6aS,12S,12aR,5′R,6′S)-12-Deoxo-12-hydroxyamorphigenol (**13**).* **13** was prepared from **6** (20.0 mg, 0.047 mmol) by GP1, and the mixture of diastereoisomers **13** was obtained as a white solid (17.9 mg, 89%, dr 63:37 unassigned): IR ν_{max} 3600−3200, 1621, 1513, 1464, 1261, 1218, 1195, 1132, 1094, 1038, 987, 787 cm^{-1}; NMR data for the major diastereoisomer, δ_H (500 MHz, acetone-d_6) 1.15 (3H, s, H-8′), 2.98 (1H, dd, *J* 6.5, 15.5 Hz, H_a-4′), 3.17 (1H, dd, *J* 6.5, 15.5 Hz, H_b-4′), 3.43 (1H, app t, *J* 6.5 Hz, H-12a), 3.57 (1H, dd, *J* 5.5, 11.0 Hz, H_a-7′), 3.68 (1H, dd, *J* 5.5, 11.0 Hz, H_b-7′), 3.70 (3H, s, H-2′), 3.74 (3H, s, H-3′), 4.16 (1H, dd, *J* 3.0, 10.0 Hz, H_a-6), 4.44 (1H, d, *J* 4.0 Hz, OH-12), 4.60 (1H, app t, *J* 10.0 Hz, H_b-6), 4.81 (1H, m obsc, H-6a), 4.83 (1H, app t, *J* 6.0 Hz, H-5′), 5.12 (1H, dd, *J* 4.0, 6.5 Hz, H-12), 6.27 (1H, d, *J* 8.0 Hz, H-10), 6.37 (1H, s, H-4), 7.06 (1H, d, *J* 8.0 Hz, H-11), 7.16 (1H, s, H-1); δ_C (125 MHz, acetone-d_6) 19.7 (C-8′), 28.0 (C-4′), 38.6 (C-12a), 55.9 (C-3′), 56.8 (C-2′), 66.5 (C-6), 67.6 (C-12), 68.1 (C-7′), 71.1 (C-6a), 73.8 (C-6′), 86.7 (C-5′), 101.4 (C-4), 102.3 (C-10), 111.7 (C-1a), 113.7 (C-8), 114.6 (C-1), 117.3 (C-11a), 129.3 (C-11), 144.4 (C-2), 150.2 (C-3), 150.3 (C-4a), 150.5 (C-7a), 162.1 (C-9); NMR data for the minor diastereoisomer, δ_H (500 MHz, acetone-d_6) 1.16 (3H, s, H-8′), 2.98 (1H, dd, *J* 6.5, 15.5 Hz, H_a-4′), 3.18 (1H, dd, *J* 6.5, 15.5 Hz, H_b-4′), 3.43 (1H, app t, *J* 6.5 Hz, H-12a), 3.49 (1H, dd, *J* 5.5, 11.0 Hz, H_a-7′), 3.65 (1H, dd, *J* 5.5, 11.0 Hz, H_b-7′), 3.70 (3H, s, H-2′), 3.74 (3H, s, H-3′), 4.16 (1H, dd, *J* 3.0, 10.0 Hz, H_a-6), 4.42 (1H, d, *J* 4.0 Hz, OH-12), 4.60 (1H, app t, *J* 10.0 Hz, H_b-6), 4.80

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(1H, m obsc, H-6a), 4.83 (1H, app t, *J* 6.0 Hz, H-5′), 5.12 (1H, dd, *J* 4.0, 7.0 Hz, H-12), 6.25 (1H, d, *J* 8.0 Hz, H-10), 6.37 (1H, s, H-4), 7.04 (1H, d, *J* 8.0 Hz, H-11), 7.15 (1H, s, H-1); δ_C (125 MHz, acetone-d_6) 20.8 (C-8′), 28.0 (C-4′), 38.6 (C-12a), 55.9 (C-3′), 56.8 (C-2′), 66.5 (C-6), 67.6 (C-12), 67.9 (C-7′), 71.1 (C-6a), 74.0 (C-6′), 87.8 (C-5′), 101.4 (C-4), 102.3 (C-10), 111.7 (C-1a), 113.7 (C-8), 114.6 (C-1), 117.3 (C-11a), 129.3 (C-11), 144.4 (C-2), 150.2 (C-3), 150.3 (C-4a), 150.5 (C-7a), 162.1 (C-9); HRESIMS *m/z* 453.1505 $[M + Na]^+$ (calcd for $C_{23}H_{26}O_8Na$, *m/z* 453.1520).

*(6aS,12S,12aR,5′R,6′R)- and (6aS,12S,12aR,5′R,6′S)-6′,7′-Dihydro-12-deoxo-12-hydroxyamorphigenin (**14**).* **14** was prepared from **1** (200 mg, 0.508 mmol) by GP2, and the mixture of diastereoisomers **14** was obtained as a white solid (168 mg, 80%, dr 53:47 unassigned): IR ν_{max} 3600−3300, 1620, 1508, 1463, 1261, 1218, 1194, 1130, 1089, 1037, 985, 786 cm^{-1}; NMR data for the major diastereoisomer, δ_H (500 MHz, acetone-d_6) 0.98 (3H, d, *J* 8.0 Hz, H-8′), 1.90 (1H, app quint, *J* 6.5, H-6′), 2.91 (1H, dd, *J* 9.0, 15.5 Hz, H_a-4′), 3.12 (1H, dd, *J* 9.0, 15.5 Hz, H_b-4′), 3.43 (1H, app t, *J* 6.0 Hz, H-12a), 3.52−3.57 (1H, m, H_a-7′), 3.59−3.64 (1H, m obsc, H_b-7′), 3.70 (3H, s, H-2′), 3.74 (3H, s, H-3′), 4.17 (1H, dd, *J* 3.0, 9.5 Hz, H_a-6), 4.42 (1H, d, *J* 4.5 Hz, OH-12), 4.60 (1H, app t, *J* 9.5 Hz, H_b-6), 4.79 (1H, m obsc, H-5′), 4.82 (1H, m obsc, H-6a), 5.11 (1H, dd, *J* 4.5, 5.0 Hz, H-12), 6.27 (1H, d, *J* 8.0 Hz, H-10), 6.37 (1H, s, H-4), 7.06 (1H, d, *J* 8.0 Hz, H-11), 7.15 (1H, s, H-1); δ_C (125 MHz, acetone-d_6) 12.0 (C-8′), 31.3 (C-4′), 38.6 (C-12a), 42.2 (C-6′), 55.9 (C-3′), 56.8 (C-2′), 64.8 (C-7′), 66.5 (C-6), 67.6 (C-12), 71.1 (C-6a), 85.8 (C-5′), 101.3 (C-4), 102.2 (C-10), 111.7 (C-1a), 113.5 (C-8), 114.6 (C-1), 117.3 (C-11a), 129.5 (C-11), 144.4 (C-2), 150.2 (C-3), 150.3 (C-4a), 150.6 (C-7a), 162.2 (C-9); NMR data for the minor diastereoisomer, δ_H (500 MHz, acetone-d_6) 0.97 (3H, d, *J* 8.0 Hz, H-8′), 2.04 (1H, app quint, *J* 6.5, H-6′), 2.86 (1H, dd, *J* 9.0, 15.5 Hz, H_a-4′), 3.08 (1H, dd, *J* 9.0, 15.5 Hz, H_b-4′), 3.43 (1H, app t, *J* 6.0 Hz, H-12a), 3.52−3.57 (1H, m, H_a-7′), 3.59−3.64 (1H, m obsc, H_b-7′), 3.70 (3H, s, H-2′), 3.74 (3H, s, H-3′), 4.17 (1H, dd, *J* 3.0, 9.5 Hz, H_a-6), 4.43 (1H, d, *J* 4.5 Hz, OH-12), 4.60 (1H, app t, *J* 9.5 Hz, H_b-6), 4.72 (1H, dd, *J* 8.0, 9.0 Hz, H-5′), 4.82 (1H, ddd, *J* 3.0, 5.0, 9.5 Hz, H-6a), 5.11 (1H, dd, *J* 4.5, 5.0 Hz, H-12), 6.29 (1H, d, *J* 8.0 Hz, H-10), 6.37 (1H, s, H-4), 7.06 (1H, d, *J* 8.0 Hz, H-11), 7.16 (1H, s, H-1); δ_C (125 MHz, acetone-d_6) 12.4 (C-8′), 30.5 (C-4′), 38.6 (C-12a), 41.8 (C-6′), 55.9 (C-3′), 56.8 (C-2′), 64.8 (C-7′), 66.5 (C-6), 67.5 (C-12), 71.1 (C-6a), 86.5 (C-5′), 101.3 (C-4), 102.3 (C-10), 111.7 (C-1a), 113.3 (C-8), 114.6 (C-1), 117.4 (C-11a), 129.5 (C-11), 144.4 (C-2), 150.2 (C-3), 150.5 (C-4a), 150.6 (C-7a), 162.0 (C-9); HRESIMS *m/z* 437.1554 $[M + Na]^+$ (calcd for $C_{23}H_{26}O_7Na$, *m/z* 437.1571).

*(6aR,12R,12aS,5′R)-12-Deoxo-12-hydroxyrotenolone (**15**).*[50] **15** was prepared from **7** (200 mg, 0.489 mmol) by GP1 and obtained as a white solid that crystallized from MeOH/H_2O as colorless needles (173 mg, 86%): mp 126−127 °C (lit.[50] mp 125−126 °C); $[\alpha]^{20}_D$ −143 (*c* 0.1, $CHCl_3$) [lit.[50] $[\alpha]^{20}_D$ −152 (*c* 2.0, $CHCl_3$)]; IR ν_{max} 3600−3300, 1620, 1508, 1479, 1464, 1265, 1219, 1195, 1137, 1099, 1061, 1040, 849 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 1.78 (3H, s, H-8′), 2.14 (1H, d, *J* 2.5 Hz, OH-12), 2.35 (1H, s, OH-12a), 2.95 (1H, dd, *J* 8.5, 16.0 Hz, H_a-4′), 3.26 (1H, dd, *J* 8.5, 16.0 Hz, H_b-4′), 3.84 (6H, s, H-2′ and H-3′), 4.35 (1H, dd, *J* 4.0, 10.5 Hz, H_a-6), 4.61 (1H, dd, *J* 4.0, 9.5 Hz, H-6a), 4.68 (1H, dd, *J* 9.5, 10.5 Hz, H_b-6), 4.84 (1H, app s, H-12), 4.91 (1H, s, H_a-7′), 5.08 (1H, s, H_b-7′), 5.18 (1H, app t, *J* 8.5 Hz, H-5′), 6.41 (1H, s, H-4), 6.47 (1H, d, *J* 8.0 Hz, H-10), 7.13 (1H, d, *J* 8.0 Hz, H-11), 7.24 (1H, s, H-1); ^{13}C NMR (125 MHz, $CDCl_3$) δ 17.4 (C-8′), 32.1 (C-4′), 56.0 (C-2′), 56.6 (C-3′), 65.0 (C-6), 68.6 (C-12a), 72.0 (C-12), 75.2 (C-6a), 86.8 (C-5′), 100.4 (C-4), 103.3 (C-10), 108.9 (C-1), 112.2 (C-7′), 112.3 (C-1a), 112.8 (C-8), 113.1 (C-11a), 129.7 (C-11), 143.9 (C-3), 144.1 (C-6′), 148.7 (C-7a), 149.4 (C-4a), 150.7 (C-2), 161.9 (C-9); HRESIMS *m/z* 435.1404 $[M + Na]^+$ (calcd for $C_{23}H_{24}O_7Na$, *m/z* 435.1414).

*(6aR,12aR)-2′-Oxorot-3′-enolonic Acid (**16**).*[45] **16** was prepared from **7** (200 mg, 0.488 mmol) by GP3 and obtained as a yellow solid (42 mg, 20%): $[\alpha]^{20}_D$ +24 (*c* 0.1, $CHCl_3$) [lit.[45] $[\alpha]^{25}_D$ +33 (*c* 0.1, $CHCl_3$)]; IR ν_{max} 3500−3100, 1672, 1598, 1508, 1446, 1332, 1272, 1200, 1083, 1049, 816 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 1.85 (3H, s, H-5′), 3.71 (3H, s, H-2″), 3.78 (1H, d, *J* 14.0 Hz, H_a-1′), 3.81 (3H, s, H-3″), 4.24 (1H, d, *J* 14.0 Hz, H_b-1′), 4.44 (1H, s, OH-12a), 4.53 (1H, d, *J* 12.0 Hz, H_a-6), 4.60 (1H, dd, *J* 1.0, 2.5 Hz, H-6a), 4.64 (1H, dd, *J* 2.5, 12.0 Hz, H_b-6), 6.00 (1H, s, H_a-4′), 6.44 (1H, s, H-4), 6.51 (1H, s, H-1), 6.55 (1H, s, H_b-4′), 6.65 (1H, d, *J* 8.5 Hz, H-10), 7.79 (1H, d, *J* 8.5 Hz, H-11) 9.13 (1H, s, OH); ^{13}C NMR (125 MHz, $CDCl_3$) δ 17.4 (C-5′), 32.1 (C-1′), 56.1 (C-3″), 56.4 (C-2″), 64.0 (C-6), 67.4 (C-12a), 76.3 (C-6a), 101.0 (C-4), 108.5 (C-1a), 108.7 (C-8), 109.3 (C-1), 111.1 (C-11a), 113.1 (C-10), 128.7 (C-11), 130.5 (C-4′), 143.6 (C-3′), 144.2 (C-2), 148.4 (C-4a), 151.3 (C-3), 158.8 (C-7a), 164.7 (C-9), 191.4 (C-12), 203.7 (C-2′); HRESIMS *m/z* 449.1213 $[M + Na]^+$ (calcd for $C_{23}H_{22}O_8Na$, *m/z* 449.1207).

*(6aR,12aR,5′R)-12a-Hydroxyamorphigenin (**17**).* *N*-(Phenylseleno)phthalimide (147 mg, 0.488 mmol) was added to a solution of **7** (200 mg, 0.488 mmol), (+)-β-camphorsulfonic acid (11.0 mg, 0.049 mmol), and H_2O (176 μL, 9.76 mmol) in CH_2Cl_2 (10 mL) under N_2 in a flask wrapped in aluminum foil. The mixture was stirred at rt for 72 h and was then concentrated. The yellow solid obtained was cooled to 0 °C and dissolved in THF (4 mL) before H_2O_2 (0.5 mL, 30% aqueous solution) was added dropwise over 10 min, followed by basic Al_2O_3 (400 mg). The suspension was stirred at 0 °C for a further 0.5 h, then warmed to rt and stirred for an additional 6 h. Saturated aqueous $NaHCO_3$ solution (20 mL) was added followed by Et_2O (20 mL), and the two phases were mixed vigorously for 10 min. The organic layer was separated, washed with H_2O (3 × 20 mL) and brine (20 mL), dried ($MgSO_4$), filtered, and concentrated. The residue was purified by flash chromatography (SiO_2, 1:1 hexanes/EtOAc) to give **17** as a yellow solid. The solid was dissolved in $CHCl_3$, and activated charcoal was added. The suspension was then filtered and concentrated to give a white solid that crystallized from MeOH/H_2O as colorless needles (44 mg, 21%): mp 89−91 °C (lit.[68] mp 92−95 °C); $[\alpha]^{20}_D$ −173 (*c* 0.1, $CHCl_3$) [lit.[68] $[\alpha]_D{}^{20}$ −175 (*c* 1.8, $CHCl_3$)]; IR ν_{max} 3500−3200, 2924, 1672, 1607, 1509, 1455, 1258, 1216, 1196, 1154, 1085, 1025, 816 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 3.05 (1H, dd, *J* 9.0, 16.0 Hz, H_a-4′), 3.36 (1H, dd, *J* 9.0, 16.0 Hz, H_b-4′), 3.72 (3H, s, H-2′), 3.82 (3H, s, H-3′), 4.24 (1H, d, *J* 14.0 Hz, H_a-8′), 4.28 (1H, d, *J* 14.0 Hz, H_b-4′), 4.46 (1H, s, OH-12a), 4.48 (1H, dd, *J* 1.0, 11.5 Hz, H_a-6), 4.58 (1H, dd, *J* 1.0, 2.5 Hz, H-6a), 4.59 (1H, dd, *J* 2.5, 11.5 Hz, H_b-6), 5.25 (1H, s, H_a-7′), 5.28 (1H, s, H_b-7′), 5.39 (1H, app t, *J* 9.0 Hz, H-5′), 6.48 (1H, s, H-4), 6.53 (1H, d, *J* 8.5 Hz, H-10), 6.54 (1H, s, H-1), 7.83 (1H, d, *J* 8.5 Hz, H-11); ^{13}C NMR (125 MHz, $CDCl_3$) δ 31.8 (C-4′), 56.0 (C-3′), 56.5 (C-2′), 63.1 (C-8′), 64.0 (C-6), 67.7 (C-12a), 76.2 (C-6a), 85.8 (C-5′), 101.2 (C-4), 105.5 (C-10), 108.8 (C-1a), 109.4 (C-1), 112.1 (C-11a), 113.0 (C-7′), 113.3 (C-8), 130.3 (C-11), 144.1 (C-2), 146.6 (C-6′), 148.5 (C-4a), 151.3 (C-3), 157.8 (C-7a), 167.7 (C-9), 191.2 (C-12); HRESIMS *m/z* 449.1210 $[M + Na]^+$ (calcd for $C_{23}H_{22}O_8Na$, *m/z* 449.1207).

*(6aR,12aR,5′R)-12a-Hydroxydalpanol (**18**).* [***Caution**: Hg(OAc)$_2$and elemental Hg are highly toxic and must be handled with extreme care; all operations must be carried out in a fume-hood and all Hg-containing waste retained for proper waste disposal*]. H_2O (2.0 mL) was added to a suspension of $Hg(OAc)_2$ (171 mg, 0.537 mmol) in THF (2.0 mL), and the bright yellow solution was stirred at rt for 10 min. **7** (200 mg, 0.488 mmol) was added, and the mixture was stirred at rt for 18 h. The mixture was diluted with THF (2.0 mL), and a 3.0 M NaOH solution (2.0 mL) was added followed by $NaBH_4$ (18.5 mg, 0.488 mmol). The mixture was stirred vigorously for 0.5 min as elemental Hg rapidly precipitated and coated the walls of the flask. The reaction was worked up as described for **4**, and the residue was subjected to flash chromatography (SiO_2, 1:1 hexanes/EtOAc) to give **18** as a white solid that crystallized from MeOH/H_2O as colorless needles (106 mg, 51%): mp 202−204 °C (lit.[37] mp 206.5−207 °C); $[\alpha]^{20}_D$ −174 (*c* 0.1, $CHCl_3$); IR ν_{max} 3600−3300, 2924, 1666, 1611, 1509, 1456, 1337, 1247, 1220, 1192, 1085, 1027, 951, 856, 808 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 1.21 (3H, s, H-7′), 1.34 (3H, s, H-8′), 1.76 (1H, s, OH-6′), 3.07 (1H, dd, *J* 9.0, 14.0 Hz, H_a-4′), 3.10 (1H, dd, *J* 9.0, 14.0 Hz, H_b-4′), 3.72 (3H, s, H-2′), 3.81 (3H, s, H-3′), 4.47 (1H, s, OH-12a), 4.49 (1H, dd, *J* 1.0, 12.0 Hz, H_a-6), 4.57 (1H, dd, *J* 1.0, 2.5 Hz, H-6a), 4.60 (1H, dd, *J* 2.5, 12.0 Hz, H_b-6), 4.68 (1H, app t, *J* 9.0 Hz, H-5′), 6.48 (1H, s, H-4), 6.51 (1H, d, *J* 8.5 Hz, H-10),

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6.54 (1H, s, H-1), 7.81 (1H, d, *J* 8.5 Hz, H-11); ^{13}C NMR (125 MHz, $CDCl_3$) δ 24.2 (C-7′), 26.3 (C-8′), 27.4 (C-4′), 56.0 (C-3′), 56.5 (C-2′), 64.0 (C-6), 67.7 (C-12a), 71.8 (C-6′), 76.1 (C-6a), 91.8 (C-5′), 101.2 (C-4), 105.3 (C-10), 108.8 (C-1a), 109.4 (C-1), 112.0 (C-11a), 114.0 (C-8), 130.1 (C-11), 144.1 (C-2), 148.5 (C-4a), 151.2 (C-3), 157.8 (C-7a), 167.9 (C-9), 191.2 (C-12); HRESIMS *m*/*z* 451.1367 $[M + Na]^+$ (calcd for $C_{23}H_{24}O_8Na$, *m*/*z* 451.1363).

*(6aR,12aR,5′R,6′R)- and (6aR,12aR,5′R,6′S)-6′,7′-Dihydro-12a-hydroxyamorphigenin (**19**).* **7** (200 mg, 0.488 mmol) was added to a solution of $[Ir(COD)Cl]_2$ (6.5 mg, 0.010 mmol), 1,2-bis(diphenylphosphino)ethane (7.8 mg, 0.020 mmol), and pinacol borane (283 μL, 1.951 mmol) in dry CH_2Cl_2 (6.0 mL) under N_2. The mixture was stirred at rt for 20 h. MeOH (1.0 mL) was then added and the mixture was concentrated. The yellow oil obtained was dissolved in THF (6.0 mL), and $NaHCO_3$ (20 mg, 0.254 mmol) was added followed by H_2O_2 (1.7 mL, 30% aqueous solution, 15.2 mmol), which was added dropwise over 10 min. The mixture was stirred vigorously at rt for an additional 20 h. The reaction was worked up as described for **5**, and the residue was subjected to flash chromatography (SiO_2, 1:2 hexanes/EtOAc) to give the mixture of diastereoisomers **19** as a white solid (24 mg, 12%, dr 59:41 unassigned): IR ν_{max} 3600−3300, 2920, 1672, 1607, 1509, 1455, 1259, 1215, 1200, 1085, 1026, 817 cm^{-1}; NMR data for the major diastereoisomer, δ_H (500 MHz, $CDCl_3$) 0.98 (3H, d, *J* 7.0 Hz, H-8′), 2.00 (1H, dq, *J* 7.0, 9.0 Hz, H-6′), 2.93 (1H, dd, *J* 8.5, 16.0 Hz, H_a-4′), 3.22 (1H, dd, *J* 8.5, 16.0 Hz, H_b-4′), 3.67−3.74 (2H, m obsc, H_a-7′ and H_b-7′), 3.73 (3H, s, H-2′), 3.81 (3H, s, H-3′), 4.47 (1H, s, OH-12a), 4.49 (1H, d, *J* 11.5 Hz, H_a-6), 4.58 (1H, dd, *J* 1.5, 2.5 Hz, H-6a), 4.60 (1H, dd, *J* 2.5, 11.5 Hz, H_b-6), 5.00 (1H, app td, *J* 4.5, 9.0 Hz, H-5′), 6.48 (1H, s, H-4), 6.49 (1H, d, *J* 8.5 Hz, H-10), 6.54 (1H, s, H-1), 7.80 (1H, d, *J* 8.5 Hz, H-11); δ_C (125 MHz, $CDCl_3$) 10.8 (C-8′), 29.7 (C-4′), 40.6 (C-6′), 56.0 (C-3′), 56.5 (C-2′), 64.0 (C-6), 65.1 (C-7′), 67.7 (C-12a), 76.2 (C-6a), 86.7 (C-5′), 101.2 (C-4), 105.4 (C-10), 108.8 (C-1a), 109.4 (C-1), 111.7 (C-11a), 113.6 (C-8), 130.2 (C-11), 144.1 (C-2), 148.5 (C-4a), 151.3 (C-3), 157.8 (C-7a), 168.3 (C-9), 191.2 (C-12); NMR data for the minor diastereoisomer, δ_H (500 MHz, $CDCl_3$) 0.98 (3H, d, *J* 7.0 Hz, H-8′), 2.08 (1H, dq, *J* 7.0, 9.0 Hz, H-6′), 2.91 (1H, dd, *J* 8.5, 16.0 Hz, H_a-4′), 3.21 (1H, dd, *J* 8.5, 16.0 Hz, H_b-4′), 3.67−3.74 (2H, m obsc, H_a-7′ and H_b-7′), 3.73 (3H, s, H-2′), 3.81 (3H, s, H-3′), 4.47 (1H, s, OH-12a), 4.49 (1H, d, *J* 11.5 Hz, H_a-6), 4.58 (1H, dd, *J* 1.5, 2.5 Hz, H-6a), 4.60 (1H, dd, *J* 2.5, 11.5 Hz, H_b-6), 4.76 (1H, dd, *J* 4.5, 9.0 Hz, H-5′), 6.48 (1H, s, H-4), 6.49 (1H, d, *J* 8.5 Hz, H-10), 6.54 (1H, s, H-1), 7.80 (1H, d, *J* 8.5 Hz, H-11); δ_C (125 MHz, $CDCl_3$) 12.6 (C-8′), 30.5 (C-4′), 41.0 (C-6′), 56.0 (C-3′), 56.5 (C-2′), 64.0 (C-6), 65.7 (C-7′), 67.7 (C-12a), 76.2 (C-6a), 88.9 (C-5′), 101.2 (C-4), 105.5 (C-10), 108.8 (C-1a), 109.4 (C-1), 111.8 (C-11a), 113.4 (C-8), 130.2 (C-11), 144.1 (C-2), 148.5 (C-4a), 151.3 (C-3), 157.8 (C-7a), 167.8 (C-9), 191.2 (C-12); HRESIMS *m*/*z* 451.1350 $[M + Na]^+$ (calcd for $C_{23}H_{24}O_8Na$, *m*/*z* 451.1363).

*(6aR,12aR,5′R,6′R)- and (6aR,12aR,5′R,6′S)-12a-Hydroxyamorphigenol (**20**).* These were prepared by modifying a literature procedure.[45] OsO_4 (99 μL of a 2.5 wt % solution in *t*-BuOH, 0.010 mmol) was added to a solution of **7** (200 mg, 0.488 mmol), *N*-methylmorpholine *N*-oxide (71 mg, 0.976 mmol), and pyridine (78 μL, 0.976 mmol) in acetone (8.0 mL) and H_2O (0.8 mL). The mixture was stirred at rt for 42 h. EtOAc (20 mL) was added followed by saturated aqueous Na_2SO_3 solution (20 mL), and the two phases were mixed vigorously for 10 min. The organic layer was separated, washed with 3.0 M HCl (3 × 20 mL), H_2O (20 mL), and brine (20 mL), dried ($MgSO_4$), filtered, and concentrated. The residue was subjected to flash chromatography (SiO_2, 1:1 hexanes/EtOAc) to give the mixture of diastereoisomers **20** as a white solid (106 mg, 49%, dr 62:38 unassigned): IR ν_{max} 3600−3300, 1672, 1607, 1509, 1455, 1338, 1248, 1216, 1199, 1155, 1085, 1048, 1025, 817, 746 cm^{-1}; NMR data for the major diastereoisomer, δ_H (500 MHz, $CDCl_3$) 1.21 (3H, s, H-8′), 3.17 (1H, dd, *J* 11.0, 17.5 Hz, H_a-4′), 3.19 (1H, dd, *J* 11.0, 17.5 Hz, H_b-4′), 3.53 (1H, d, *J* 11.0 Hz, H_a-7′), 3.71 (3H, s, H-2′), 3.74 (1H, d, *J* 11.0 Hz, H_b-7′), 3.82 (3H, s, H-3′), 4.46 (1H, s, OH-12a), 4.49 (1H, d, *J* 12.0 Hz, H_a-6), 4.59 (1H, dd, 1.0, 2.5 Hz, H-6a), 4.60 (1H, dd, *J* 2.5, 12.0 Hz, H_b-6), 4.86 (1H, app t, *J* 11.0 Hz, H-5′), 6.49 (1H, s, H-4), 6.50 (1H, d, *J* 8.5 Hz, H-10), 6.53 (1H, s, H-1), 7.80 (1H, d, *J* 8.5 Hz, H-11); δ_C (125 MHz, $CDCl_3$) 19.4 (C-8′), 27.0 (C-4′), 56.0 (C-3′), 56.5 (C-2′), 63.9 (C-6), 67.0 (C-7′), 67.8 (C-12a), 73.5 (C-6′), 76.2 (C-6a), 87.5 (C-5′), 101.2 (C-4), 105.4 (C-10), 108.8 (C-1a), 109.4 (C-1), 112.1 (C-11a), 113.9 (C-8), 130.1 (C-11), 144.1 (C-2), 148.6 (C-4a), 151.3 (C-3), 157.8 (C-7a), 167.7 (C-9), 191.3 (C-12); NMR data for the minor diastereoisomer, δ_H (500 MHz, $CDCl_3$) 1.16 (3H, s, H-8′), 3.16 (1H, dd, *J* 11.0, 17.5 Hz, H_a-4′), 3.20 (1H, dd, *J* 11.0, 17.5 Hz, H_b-4′), 3.57 (1H, d, *J* 11.0 Hz, H_a-7′), 3.71 (3H, s, H-2′), 3.79 (1H, d, *J* 11.0 Hz, H_b-7′), 3.82 (3H, s, H-3′), 4.45 (1H, s, OH-12a), 4.49 (1H, d, *J* 12.0 Hz, H_a-6), 4.59 (1H, dd, 1.0, 2.5 Hz, H-6a), 4.60 (1H, dd, *J* 2.5, 12.0 Hz, H_b-6), 4.86 (1H, app t, *J* 11.0 Hz, H-5′), 6.48 (1H, s, H-4), 6.51 (1H, d, *J* 8.5 Hz, H-10), 6.53 (1H, s, H-1), 7.81 (1H, d, *J* 8.5 Hz, H-11); δ_C (125 MHz, $CDCl_3$) 19.8 (C-8′), 27.3 (C-4′), 56.0 (C-3′), 56.5 (C-2′), 63.9 (C-6), 67.7 (C-12a), 68.6 (C-7′), 73.1 (C-6′), 76.2 (C-6a), 90.2 (C-5′), 101.2 (C-4), 105.3 (C-10), 108.8 (C-1a), 109.4 (C-1), 112.2 (C-11a), 113.7 (C-8), 130.1 (C-11), 144.1 (C-2), 148.6 (C-4a), 151.3 (C-3), 157.8 (C-7a), 167.4 (C-9), 191.3 (C-12); HRESIMS *m*/*z* 467.1316 $[M + Na]^+$ (calcd for $C_{23}H_{24}O_9Na$, *m*/*z* 467.1313).

*(6aR,12R,12aS,5′R)-12-Deoxo-12,12a-dihydroxyamorphigenin (**21**).*[69] **21** was prepared from **17** (18.0 mg, 0.042 mmol) by GP1 and obtained as a white solid (14.8 mg, 82%): $[\alpha]^{20}_D$ −86 (*c* 0.1, $CHCl_3$); IR ν_{max} 3600−3300, 1620, 1509, 1466, 1265, 1221, 1194, 1137, 1099, 1036, 734 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$) δ 2.25 (1H, d, *J* 2.5 Hz, OH-12), 2.51 (1H, s, OH-12a), 3.04 (1H, dd, *J* 9.0, 15.5 Hz, H_a-4′), 3.32 (1H, dd, *J* 9.0, 15.5 Hz, H_b-4′), 3.83 (6H, s, H-2′ and H-3′), 4.22 (1H, d, *J* 14.0 Hz, H_a-8′), 4.27 (1H, d, *J* 14.0 Hz, H_b-8′), 4.32 (1H, dd, *J* 4.0, 10.5 Hz, H_a-6), 4.59 (1H, dd, *J* 4.0, 8.0 Hz, H-6a), 4.66 (1H, dd, *J* 8.0, 10.5 Hz, H_b-6), 4.84 (1H, app s, H-12), 5.24 (1H, s, H_a-7′), 5.25 (1H, s, H_b-7′), 5.33 (1H, app t, *J* 9.0 Hz, H-5′), 6.40 (1H, s, H-4), 6.46 (1H, d, *J* 8.5 Hz, H-10), 7.14 (1H, d, *J* 8.5 Hz, H-11), 7.24 (1H, s, H-1); ^{13}C NMR (125 MHz, $CDCl_3$) δ 32.6 (C-4′), 56.0 (C-2′), 56.6 (C-3′), 63.2 (C-8′), 64.9 (C-6), 68.5 (C-12a), 72.0 (C-12), 75.2 (C-6a), 84.8 (C-5′), 100.4 (C-4), 103.3 (C-10), 109.0 (C-1), 112.3 (C-1a), 112.6 (C-8), 112.7 (C-7′), 113.6 (C-11a), 129.7 (C-11), 144.1 (C-3), 147.4 (C-6′), 148.7 (C-7a), 149.4 (C-4a), 150.7 (C-2), 161.3 (C-9); HRESIMS *m*/*z* 451.1373 $[M + Na]^+$ (calcd for $C_{23}H_{24}O_8Na$, *m*/*z* 451.1369.

*(6aR,12R,12aS,5′R)-12-Deoxo-12,12a-dihydroxydalpanol (**22**).* **22** was prepared from **18** (22.0 mg, 0.051 mmol) by GP1 and obtained as a colorless oil that solidified upon standing at rt to a white solid (19.9 mg, 90%): $[\alpha]^{20}_D$ −134 (*c* 0.1, $CHCl_3$); IR ν_{max} 3500−3200, 1620, 1508, 1481, 1464, 1266, 1220, 1194, 1138, 1098, 1063, 1038, 968 cm^{-1}; ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.20 (3H, s, H-7′), 1.33 (3H, s, H_a-8′), 1.94 (1H, s, OH-6′), 2.37 (1H, d, *J* 2.0 Hz, OH-12), 2.67 (1H, s, OH-12a), 3.03 (1H, dd, *J* 9.5, 17.0 Hz, H_a-4′), 3.06 (1H, dd, *J* 9.5, 17.0 Hz, H_b-4′), 3.81 (3H, s, H-2′), 3.82 (3H, s, H-3′), 4.34 (1H, dd, *J* 4.0, 10.5 Hz, H_a-6), 4.51 (1H, dd, *J* 4.0, 9.0 Hz, H-6a), 4.52 (1H, m obsc, H-5′), 4.65 (1H, dd, *J* 9.0, 10.5 Hz, H_b-6), 4.82 (1H, app s, H-12), 6.38 (1H, s, H-4), 6.42 (1H, d, *J* 8.0 Hz, H-10), 7.11 (1H, d, *J* 8.0 Hz, H-11), 7.24 (1H, s, H-1); ^{13}C NMR (125 MHz, $CDCl_3$) δ 24.0 (C-7′), 26.4 (C-8′), 28.1 (C-4′), 56.0 (C-2′), 56.6 (C-3′), 64.9 (C-6), 68.4 (C-12a), 72.0 (C-6′), 72.1 (C-12), 75.2 (C-6a), 90.4 (C-5′), 100.4 (C-4), 103.0 (C-10), 109.2 (C-1), 112.4 (C-1a), 113.3 (C-8), 113.5 (C-11a), 129.4 (C-11), 144.0 (C-3), 148.7 (C-7a), 149.4 (C-4a), 150.6 (C-2), 161.5 (C-9); HRESIMS *m*/*z* 435.1502 $[M + Na]^+$ (calcd for $C_{23}H_{26}O_8Na$, *m*/*z* 453.1520).

*(6aR,12R,12aS,5′R,6′R)- and (6aR,12R,12aS,5′R,6′S)-6′,7′-Dihydro-12-deoxo-12,12a-dihydroxyamorphigenin (**23**).* **23** was prepared from **7** (200 mg, 0.488 mmol) by GP2, and the mixture of diastereoisomers **23** was obtained as a white solid (182 mg, 87%, dr 57:43 unassigned): IR ν_{max} 3600−3300, 1619, 1512, 1464, 1281, 1218, 1193, 1130, 1090, 985, 870, 786 cm^{-1}; NMR data for the major diastereoisomer, δ_H (500 MHz, $CDCl_3$) 0.99 (3H, d, *J* 7.0 Hz, H-8′), 2.02 (1H, m, H-6′), 2.32 (1H, s, OH-12), 2.63 (1H, s, OH-12a), 2.92 (1H, dd, *J* 9.0, 15.0 Hz, H_a-4′), 3.16 (1H, dd, *J* 9.0, 15.0 Hz, H_b-4′), 3.64 (1H, dd, *J* 4.5, 11.0 Hz, H_a-7′), 3.74 (1H, dd, *J* 4.5, 11.0 Hz, H_b-7′), 3.82 (6H, s, H-2′ and H-3′), 4.34 (1H, dd, *J* 4.0, 10.5 Hz, H_a-6),

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4.60 (1H, dd, *J* 4.0, 9.0 Hz, H-6a), 4.68 (1H, dd, *J* 9.0, 10.5 Hz, H_b-6), 4.83 (1H, app s, H-12), 4.88 (1H, app td, *J* 4.5, 9.0 Hz, H-5′), 6.39 (1H, s, H-4), 6.41 (1H, d, *J* 8.0 Hz, H-10), 7.11 (1H, d, *J* 8.0 Hz, H-11), 7.23 (1H, s, H-1); δ_C (125 MHz, $CDCl_3$) 11.2 (C-8′), 30.1 (C-4′), 40.3 (C-6′), 56.0 (C-2′), 56.6 (C-3′), 64.9 (C-6), 65.4 (C-7′), 68.5 (C-12a), 72.0 (C-12), 75.2 (C-6a), 85.7 (C-5′), 100.3 (C-4), 103.1 (C-10), 109.0 (C-1), 112.4 (C-1a), 113.0 (C-8), 113.2 (C-11a), 129.6 (C-11), 144.0 (C-3), 148.7 (C-7a), 149.4 (C-4a), 150.6 (C-2), 161.7 (C-9); NMR data for the minor diastereoisomer, δ_H (500 MHz, $CDCl_3$) 0.95 (3H, d, *J* 7.0 Hz, H-8′), 2.02 (1H, m, H-6′), 2.32 (1H, s, OH-12), 2.63 (1H, s, OH-12a), 2.90 (1H, dd, *J* 9.0, 15.0 Hz, H_a-4′), 3.18 (1H, dd, *J* 9.0, 15.0 Hz, H_b-4′), 3.66 (1H, dd, *J* 4.5, 11.0 Hz, H_a-7′), 3.72 (1H, dd, *J* 4.5, 11.0 Hz, H_b-7′), 3.82 (6H, s, H-2′ and H-3′), 4.34 (1H, dd, *J* 4.0, 10.5 Hz, H_a-6), 4.60 (1H, dd, *J* 4.0, 9.0 Hz, H-6a), 4.63 (1H, dd, *J* 4.5, 9.0 Hz, H-5′), 4.69 (1H, dd, *J* 9.0, 10.5 Hz, H_b-6), 4.83 (1H, app s, H-12), 6.39 (1H, s, H-4), 6.41 (1H, d, *J* 8.0 Hz, H-10), 7.12 (1H, d, *J* 8.0 Hz, H-11), 7.23 (1H, s, H-1); δ_C (125 MHz, $CDCl_3$) 12.9 (C-8′), 31.6 (C-4′), 41.1 (C-6′), 56.0 (C-2′), 56.6 (C-3′), 64.9 (C-6), 65.4 (C-7′), 68.5 (C-12a), 72.0 (C-12), 75.2 (C-6a), 88.4 (C-5′), 100.3 (C-4), 103.2 (C-10), 109.0 (C-1), 112.4 (C-1a), 112.7 (C-8), 113.5 (C-11a), 129.6 (C-11), 144.0 (C-3), 148.7 (C-7a), 149.4 (C-4a), 150.6 (C-2), 161.2 (C-9); HRESIMS *m*/*z* 453.1524 $[M + Na]^+$ (calcd for $C_{23}H_{26}O_8Na$, *m*/*z* 453.1520).

*(6aR,12R,12aS,5′R,6′R)- and (6aR,12R,12aS,5′R,6′S)-12-Deoxo-12,12a-dihydroxyamorphigenol (**24**).* **24** was prepared from **20** (20.0 mg, 0.045 mmol) by GP1, and the mixture of diastereoisomers **24** was obtained as a white solid (18.1 mg, 90%, dr 62:38 unassigned): IR ν_{max} 3600−3300, 1621, 1515, 1479, 1219, 1195, 1132, 1094, 1039, 801 cm^{-1}; NMR data for the major diastereoisomer, δ_H (500 MHz, $CDCl_3$) 1.22 (3H, s, H-8′), 2.39 (1H, s, OH-12), 2.48 (1H, s, OH-12a), 3.13 (1H, dd, *J* 9.0, 15.5 Hz, H_a-4′), 3.15 (1H, dd, *J* 9.0, 15.5 Hz, H_b-4′), 3.52 (1H, d, *J* 13.0 Hz, H_a-7′), 3.75 (1H, d, *J* 13.0 Hz, H_b-7′), 3.83 (6H, s, H-2′ and H-3′), 4.36 (1H, dd, *J* 3.5, 10.5 Hz, H_a-6), 4.62 (1H, dd, *J* 3.5, 9.0 Hz, H-6a), 4.68 (1H, dd, *J* 9.0, 10.5 Hz, H_b-6), 4.77 (app t, *J* 9.0 Hz, H-5′), 4.84 (1H, app s, H-12), 6.41 (1H, s, H-4), 6.43 (1H, d, *J* 8.0 Hz, H-10), 7.12 (1H, d, *J* 8.0 Hz, H-11), 7.26 (1H, s, H-1); δ_C (125 MHz, $CDCl_3$) 19.8 (C-8′), 27.8 (C-4′), 56.0 (C-2′), 56.6 (C-3′), 64.9 (C-6), 67.0 (C-7′), 68.5 (C-12a), 72.0 (C-12), 73.7 (C-6′), 75.2 (C-6a), 87.0 (C-5′), 100.4 (C-4), 103.1 (C-10), 109.0 (C-1), 112.3 (C-1a), 113.1 (C-8), 113.6 (C-11a), 129.6 (C-11), 144.1 (C-3), 148.8 (C-7a), 149.4 (C-4a), 150.7 (C-2), 161.3 (C-9); NMR data for the minor diastereoisomer, δ_H (500 MHz, $CDCl_3$) 1.15 (3H, s, H-8′), 2.39 (1H, s, OH-12), 2.58 (1H, s, OH-12a), 3.12 (1H, dd, *J* 9.0, 15.5 Hz, H_a-4′), 3.22 (1H, dd, *J* 9.0, 15.5 Hz, H_b-4′), 3.54 (1H, d, *J* 13.0 Hz, H_a-7′), 3.77 (1H, d, *J* 13.0 Hz, H_b-7′), 3.83 (6H, s, H-2′ and H-3′), 4.36 (1H, dd, *J* 3.5, 10.5 Hz, H_a-6), 4.62 (1H, dd, *J* 3.5, 9.0 Hz, H-6a), 4.68 (1H, dd, *J* 9.0, 10.5 Hz, H_b-6), 4.78 (app t, *J* 9.0 Hz, H-5′), 4.84 (1H, app s, H-12), 6.41 (1H, s, H-4), 6.43 (1H, d, *J* 8.0 Hz, H-10), 7.12 (1H, d, *J* 8.0 Hz, H-11), 7.25 (1H, s, H-1); δ_C (125 MHz, $CDCl_3$) 19.8 (C-8′), 28.0 (C-4′), 56.0 (C-2′), 56.6 (C-3′), 64.9 (C-6), 68.4 (C-12a), 68.7 (C-7′), 72.0 (C-12), 73.1 (C-6′), 75.2 (C-6a), 89.1 (C-5′), 100.4 (C-4), 103.2 (C-10), 109.0 (C-1), 112.2 (C-1a), 112.9 (C-8), 113.9 (C-11a), 129.5 (C-11), 144.1 (C-3), 148.8 (C-7a), 149.4 (C-4a), 150.7 (C-2), 161.3 (C-9); HRESIMS *m*/*z* 469.1451 $[M + Na]^+$ (calcd for $C_{23}H_{26}O_9Na$, *m*/*z* 469.1469).

(6aS,12aS)-4′-Bromorot-2′-enonic Acid. This was prepared by adapting several literature procedures.[55,56] The following procedure gave consistently reproducible results: BBr_3 (2.54 mL of a 1.0 M solution in CH_2Cl_2, 2.54 mmol) was added dropwise over 10 min to a solution of **1** (1.00 g, 2.54 mmol) in dry CH_2Cl_2 (6.0 mL) under N_2 at −20 °C. The mixture was stirred at −20 °C for a further 20 min before the reaction was quenched with H_2O (20 mL), quickly warmed to rt, and extracted with EtOAc (20 mL). The organic layer was separated, washed with H_2O (3 × 20 mL) and brine (20 mL), dried ($MgSO_4$), filtered, and concentrated. The yellow oily residue was dissolved in MeOH (14 mL), and the solution set aside at 4 °C overnight to give 4′-bromorot-2′-enonic acid as a white powdery solid, which was collected by filtration and dried *in vacuo*. The solid crystallized from CH_2Cl_2/MeOH at 0 °C as large colorless needles (0.718 g, 60%): mp 152−154 °C (lit.[70] mp 152−154 °C); $[\alpha]^{20}_D$ +22 (*c* 0.1, $CHCl_3$) [lit.[70] $[\alpha]^{20}_D$ +27 (*c* 0.7, $CHCl_3$)]; IR ν_{max} 3200−3000, 1662, 1595, 1513, 1453, 1442, 1350, 1276, 1214, 1199, 1098, 882, 814 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ 1.93 (3H, s, H-5′), 3.37 (1H, dd, *J* 7.5, 15.0 Hz, H_a-1′), 3.42 (1H, dd *J* 7.5, 15.0 Hz, H_b-1′), 3.75 (3H, s, H-2″), 3.80 (3H, s, H-3″), 3.83 (1H, d, *J* 4.0 Hz, H-12a), 3.95 (2H, s, H-4′), 4.18 (1H, d, *J* 12.0 Hz, H_a-6), 4.62 (1H, dd, *J* 3.5, 12.0 Hz, H_b-6), 4.91 (1H, dd, *J* 3.5, 4.0 Hz, H-6a), 5.63 (1H, t, *J* 7.5 Hz, H-2′), 5.93 (1H, s, OH-9), 6.45 (1H, s, H-4), 6.52 (1H, d, *J* 8.5 Hz, H-10), 6.76 (1H, s, H-1), 7.74 (1H, d, *J* 8.5 Hz, H-11); ^{13}C NMR (125 MHz, $CDCl_3$) δ 14.9 (C-5′), 22.5 (C-1′), 41.5 (C-4′), 44.4 (C-12a), 56.0 (C-3″), 56.5 (C-2″), 66.4 (C-6), 72.4 (C-6a), 101.1 (C-4), 104.7 (C-1a), 110.6 (C-1), 110.8 (C-10), 113.3 (C-8), 113.9 (C-11a), 127.5 (C-11), 128.0 (C-2′), 133.4 (C-3′), 143.9 (C-2), 147.7 (C-3), 149.6 (C-4a), 160.4 (C-7a), 161.0 (C-9), 189.9 (C-12); HRESIMS *m*/*z* 475.0739 $[M + H]^+$ (calcd for $C_{23}H_{24}O_6{}^{79}Br$, *m*/*z* 475.0756) and *m*/*z* 477.039 $[M + H]^+$ (calcd for $C_{23}H_{24}O_6{}^{81}Br$, *m*/*z* 477.0736).

*(6aS,12aS)-Rot-3′-enonic Acid (**26**).* Zinc powder (219 mg, 3.368 mmol, activated as described above) was added to a solution of 4′-bromorot-2′-enonic acid (400 mg, 0.842 mmol) and NH_4Cl (180, mg, 3.368 mmol) in THF (12 mL) and H_2O (2.0 mL), and the suspension was stirred vigorously at rt for 0.5 h. The mixture was filtered through a pad of Celite, and the inorganic residues were washed with EtOAc (80 mL). The combined filtrates were washed with H_2O (3 × 80 mL) and brine (80 mL), dried ($MgSO_4$), filtered, and concentrated to afford **26** as a pale yellow solid that crystallized from MeOH/H_2O as small colorless prisms (264 mg, 79%): mp 177−178 °C (lit.[71] 176−178 °C); $[\alpha]^{20}_D$ +69 (*c* 0.1, $CHCl_3$); IR ν_{max} 3600−3300, 1657, 1604, 1594, 1515, 1447, 1439, 1350, 1296, 1273, 1216, 1192, 1077, 1006, 814 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) δ1.78 (3H, s, H-5′), 2.19 (2H, ddd, *J* 5.0, 7.0, 11.0 Hz, H-2′), 2.73 (1H, ddd, *J* 5.0, 7.0, 10.5 Hz, H_a-1′), 2.81 (1H, ddd, *J* 5.0, 7.0, 10.5 Hz, H_b-1′), 3.75 (3H, s, H-2″), 3.80 (3H, s, H-3″), 3.83 (1H, d, *J* 3.0 Hz, H-12a), 4.17 (1H, d, *J* 10.0 Hz, H_a-6), 4.60 (1H, d, *J* 1.0 Hz, H_a-4′), 4.62 (1H, dd, *J* 3.0, 10.0 Hz, H_b-6), 4.66 (1H, d, *J* 1.0 Hz, H_b-4′), 4.88 (1H, ddd, *J* 1.0, 2.5, 3.0 Hz, H-6a), 5.88 (1H, s, OH-9), 6.45 (1H, s, H-4), 6.47 (1H, d, *J* 7.0 Hz, H-10), 6.77 (1H, s, H-1), 7.72 (1H, d, *J* 7.0 Hz, H-11); ^{13}C NMR (125 MHz, $CDCl_3$) δ 21.6 (C-1′), 22.6 (C-5′), 36.9 (C-2′), 44.4 (C-12a), 56.0 (C-3″), 56.5 (C-2″), 66.5 (C-6), 72.3 (C-6a), 101.0 (C-4), 104.9 (C-1a), 110.5 (C-4′), 110.5 (C-10), 110.7 (C-1), 113.1 (C-8), 116.1 (C-11a), 127.0 (C-11), 143.9 (C-2), 145.9 (C-3′), 147.7 (C-3), 149.6 (C-4a), 160.7 (C-7a), 160.9 (C-9), 190.2 (C-12); HRESIMS *m*/*z* 397.1636 $[M + H]^+$ (calcd for $C_{20}H_{16}O_6$, *m*/*z* 397.1651).

*(6aS,12aS,5′R)- and (6aS,12aS,5′S)-4′,5′-Dihydro-5′-hydroxydeguelin (**27**).*[57] These were prepared from **25** (180 mg, 0.455 mmol) by GP4, and the mixture of diastereoisomers **27** was obtained as a white solid (116 mg, 62%, dr 57:43 unassigned): IR ν_{max} 3600−3300, 1665, 1601, 1582, 1512, 1440, 1342, 1261, 1212, 1195, 1136, 1094, 1043, 1008, 817 cm^{-1}; NMR data for the major diastereoisomer, δ_H (500 MHz, $CDCl_3$) 1.32 (3H, s, H-7′), 1.34 (3H, s, H-8′), 1.65 (1H, s, OH-5′), 2.69 (1H, dd, *J* 5.5, 16.0 Hz, H_a-4′), 2.95 (1H, dd, *J* 5.5, 16.0 Hz, H_b-4′) 3.77 (3H, s, H-2′), 3.81 (3H, s, H-3′), 3.83−3.90 (2H, m obsc, H-12a and H-5′), 4.19 (1H, d, *J* 11.0 Hz, H_a-6), 4.63 (1H, dd, *J* 3.5, 11.0 Hz, H_b-6), 4.92 (1H, ddd, *J* 1.0, 3.5, 4.0 Hz, H-6a), 6.45 (1H, s, H-4) 6.50 (1H, d, *J* 9.0 Hz, H-10) 6.79 (1H, s, H-1), 7.75 (1H, d, *J* 9.0 Hz, H-11); δ_C (125 MHz, $CDCl_3$) 21.8 (C-7′), 25.1 (C-8′), 26.0 (C-4′), 44.4 (C-12a), 56.0 (C-3′), 56.5 (C-2′), 66.4 (C-6), 69.1 (C-5′), 72.6 (C-6a), 78.3 (C-6′), 101.1 (C-4), 104.9 (C-1a), 107.2 (C-8), 110.5 (C-1), 112.2 (C-10), 112.4 (C-11a), 127.1 (C-11), 144.0 (C-2), 147.6 (C-4a), 149.6 (C-3), 160.0 (C-9), 160.5 (C-7a), 189.5 (C-12); NMR data for the minor diastereoisomer, δ_H (500 MHz, $CDCl_3$) 1.27 (3H, s, H-7′) 1.39 (3H, s, H-8′) 1.72 (1H, s, OH-5′), 2.77 (1H, dd, *J* 5.5, 16.0 Hz, H_a-4′) 2.90 (1H, dd, *J* 5.5, 16.0 Hz, H_b-4′), 3.77 (3H, s, H-2′), 3.81 (3H, s, H-3′), 3.83−3.90 (2H, m obsc, H-12a and H-5′) 4.19 (1H, d, *J* 11.0 Hz, H_a-6), 4.64 (1H, dd, *J* 3.5, 11.0 Hz, H_b-6), 4.92 (1H, ddd, *J* 1.0, 3.5, 4.0 Hz, H-6a), 6.45 (1H, s, H-4), 6.51 (1H, d, *J* 9.0 Hz, H-10) 6.80 (1H, s, H-1), 7.76 (1H, d, *J* 9.0 Hz, H-11); δ_C (125 MHz, $CDCl_3$) 22.7 (C-7′), 24.8 (C-

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8′), 26.0 (C-4′), 44.4 (C-12a), 56.0 (C-3′), 56.5 (C-2′), 66.5 (C-6), 68.7 (C-5′), 72.6 (C-6a), 78.2 (C-6′), 101.1 (C-4), 105.0 (C-1a), 106.9 (C-8), 110.5 (C-1), 112.3 (C-10), 112.5 (C-11a), 127.1 (C-11), 144.0 (C-2), 147.6 (C-4a), 149.6 (C-3), 160.0 (C-9), 160.7 (C-7a), 189.5 (C-12); HRESIMS m/z 435.1400 $[M + Na]^+$ (calcd for $C_{23}H_{24}O_7Na$, m/z 435.1414).

*(6aS,12aS,6′R)- and (6aS,12aS,6′S)-4′,5′-Dihydro-7′-hydroxydeguelin (**28**).* These were prepared from **26** (180 mg, 0.455 mmol) by GP4, and the mixture of diastereoisomers **28** was obtained as a white solid (129 mg, 69%, dr 53:47 unassigned): IR ν_{max} 3600−3300, 1664, 1599, 1580, 1511, 1439, 1335, 1249, 1212, 1196, 1093, 1056, 1003, 817 cm^{-1}; NMR data for the major diastereoisomer, δ_H (500 MHz, $CDCl_3$) 1.22 (3H, s, H-8′), 1.70−1.75 (1H, m obsc, H_a-5′), 1.86 (1H, s, OH-7′), 1.93−1.97 (1H, m obsc, H_b-5′), 2.59−2.65 (1H, m obsc, H_a-4′), 2.77−2.81 (1H, m obsc, H_b-4′), 3.58−3.67 (2H, m obsc, H_a-7′ and H_b-7′), 3.77 (3H, s, H-2′), 3.81 (3H, s, H-3′), 3.84 (1H, d, J 4.5 Hz, H-12a), 4.20 (1H, d, J 12.0 Hz, H_a-6), 4.64 (1H, dd, J 3.0, 12.0 Hz, H_b-6), 4.91 (1H, ddd, J 1.0, 3.0, 4.0 Hz, H-6a), 6.46 (1H, s, H-4), 6.48 (1H, d, J 9.0 Hz, H-10), 6.81 (1H, s, H-1), 7.74 (1H, d, J 9.0 Hz, H-11); δ_C (125 MHz, $CDCl_3$) 16.0 (C-4′), 20.5 (C-8′), 26.5 (C-5′), 44.4 (C-12a), 56.0 (C-3′), 56.4 (C-2′), 66.5 (C-6), 69.3 (C-7′), 72.6 (C-6a), 78.2 (C-6′), 101.1 (C-4), 105.0 (C-1a), 109.4 (C-8), 110.5 (C-1), 112.1 (C-11a), 112.3 (C-10), 126.7 (C-11), 144.0 (C-2), 147.6 (C-4a), 149.6 (C-3), 160.1 (C-7a), 160.7 (C-9), 189.6 (C-12); NMR data for the minor diastereoisomer, δ_H (500 MHz, $CDCl_3$) 1.29 (3H, s, H-8′), 1.70−1.75 (1H, m obsc, H_a-5′), 1.80 (1H, s, OH-7′), 1.93−1.97 (1H, m obsc, H_b-5′), 2.59−2.65 (1H, m obsc, H_a-4′), 2.77−2.81 (1H, m obsc, H_b-4′), 3.58−3.67 (2H, m obsc, H_a-7′ and H_b-7′), 3.77 (3H, s, H-2′), 3.81 (3H, s, H-3′), 3.84 (1H, d, J 4.5 Hz, H-12a), 4.20 (1H, d, J 12.0 Hz, H_a-6), 4.63 (1H, dd, J 3.0, 12.0 Hz, H_b-6) 4.93 (1H, ddd, J 1.0, 3.0, 4.0 Hz, H-6a), 6.45 (1H, s, H-4), 6.48 (1H, d, J 9.0 Hz, H-10), 6.81 (1H, s, H-1), 7.73 (1H, d, J 9.0 Hz, H-11); δ_C (125 MHz, $CDCl_3$) 16.0 (C-4′), 20.8 (C-8′), 26.7 (C-5′), 44.4 (C-12a), 56.0 (C-3′), 56.5 (C-2′), 66.5 (C-6), 68.9 (C-7′), 72.4 (C-6a), 78.1 (C-6′), 101.0 (C-4), 105.0 (C-1a), 109.3 (C-8), 110.5 (C-1), 112.1 (C-11a), 112.2 (C-10), 126.7 (C-11), 144.0 (C-2), 147.6 (C-4a), 149.6 (C-3), 160.0 (C-7a), 160.6 (C-9), 189.6 (C-12); HRESIMS m/z 435.1398 $[M + Na]^+$ (calcd for $C_{23}H_{24}O_7Na$, m/z 435.1414).

*(6aS,12S,12aR)-12-Deoxo-12-hydroxydeguelin (**29**).*[58] **29** was prepared from **2** (120 mg, 0.305 mmol) by GP1 and obtained as a white solid (108 mg, 90%): $[\alpha]^{20}_D$ −79 (c 0.1, acetone); IR ν_{max} 3600−3300, 1612, 1586, 1509, 1462, 1444, 1216, 1191, 1152, 1094, 1083, 994, 808 cm^{-1}; ^1H NMR (500 MHz, acetone-d_6) δ 1.35 (3H, s, H-7′), 1.36 (3H, s, H-8′), 3.45 (1H, app t, J 5.0 Hz, H-12a) 3.70 (3H, s, H-2′), 3.73 (3H, s, H-3′), 4.18 (1H, dd, J 3.5, 10.0 Hz, H_a-6), 4.54 (1H, d, J 3.5 Hz, OH-12), 4.59 (1H, dd, J 8.5, 10.0 Hz, H_b-6), 4.84 (1H, ddd, J 1.0, 5.0, 10.0 Hz, H-6a), 5.12 (1H, d, J 3.5, 4.0 Hz, H-12), 5.63 (1H, d, J 10.0 Hz, H-4′), 6.30 (1H, d, J 8.0 Hz, H-10), 6.36 (1H, s, H-4), 6.63 (1H, d, J 10.0 Hz, H-5′), 7.08 (1H, d, J 8.0 Hz, H-11), 7.19 (1H, s, H-1); ^{13}C NMR (125 MHz, acetone-d_6) δ 27.9 (C-7′), 28.1 (C-8′), 38.3 (C-12a), 55.8 (C-3′), 56.8 (C-2′), 66.6 (C-6), 67.6 (C-12), 71.4 (C-6a), 76.3 (C-6′), 101.3 (C-4), 109.2 (C-10), 109.9 (C-8), 111.5 (C-1a), 114.6 (C-1), 117.5 (C-4′), 117.6 (C-11a), 129.4 (C-11), 129.6 (C-5′), 144.4 (C-2), 149.2 (C-3), 150.2 (C-4a), 150.3 (C-9), 154.2 (C-7a); HRESIMS m/z 419.1450 $[M + Na]^+$ (calcd for $C_{23}H_{24}O_6Na$, m/z 419.1465).

*(6aR,12R,12aS)-12-Deoxo-12-hydroxytephrosin (**30**).* **30** was prepared from **8** (20.0 mg, 0.049 mmol) by GP1 and obtained as a white solid (18.5 mg, 92%): $[\alpha]^{20}_D$ −87 (c 0.1, $CHCl_3$); IR ν_{max} 3600−3300, 2924, 1614, 1585, 1508, 1445, 1266, 1217, 1197, 1151, 1114, 1056, 732 cm^{-1}; ^1H NMR (500 MHz, $CDCl_3$) 1.40 (3H, s, H-7′) δ 1.41 (3H, s, H-8′) 2.22 (1H, d, J 2.5 Hz, OH-12), 2.46 (1H, s, OH-12a), 3.82 (3H, s, H-2′), 3.82 (3H, s, H-3′), 4.34 (1H, dd, J 4.0, 10.5 Hz, H_a-6), 4.62 (1H, dd, J 4.0, 9.5 Hz, H-6a), 4.67 (1H, dd, J 9.5, 10.5 Hz, H_b-6), 4.80 (1H, app s, H-12), 5.56 (1H, d, J 10.0 Hz, H-5′), 6.39 (1H, s, H-4), 6.44 (1H, d, J 8.5 Hz, H-10), 6.63 (1H, d, J 10.0 Hz, H-4′), 7.09 (1H, d, J 8.5 Hz, H-11), 7.23 (1H, s, H-1); ^{13}C NMR (125 MHz, $CDCl_3$) δ 28.0 (C-7′), 28.1 (C-8′), 56.0 (C-2′), 56.6 (C-3′), 64.9 (C-6), 68.5 (C-12a), 72.0 (C-12), 75.2 (C-6a), 76.2 (C-6′), 100.4 (C-4), 109.1 (C-1), 109.7 (C-8), 110.1 (C-10), 112.3 (C-1a), 113.0 (C-11a), 116.5 (C-4′), 129.3 (C-11), 129.3 (C-5′), 144.0 (C-3), 147.4 (C-7a), 149.4 (C-4a), 150.6 (C-2), 154.3 (C-9); HRESIMS m/z 435.1419 $[M + Na]^+$ (calcd for $C_{23}H_{24}O_7Na$, m/z 435.1414).

*(6aS,12aS,4′R,5′R)- and (6aS,12aS,4′S,5′S)-4′,5′-Dihydro-4′,5′-cis-dihydroxydeguelin (**31**).* These were prepared by modifying a literature procedure.[58] OsO_4 (20 μL of a 2.5 wt % solution in t-BuOH, 0.002 mmol) was added to a solution of **2** (40 mg, 0.102 mmol), N-methylmorpholine N-oxide (14 mg, 0.122 mmol), and citric acid (39 mg, 0.203 mmol) in acetone (4.0 mL) and H_2O (1.0 mL). The mixture was stirred at rt for 28 h. EtOAc (10 mL) was then added followed by saturated aqueous Na_2SO_3 solution (10 mL), and the two phases were mixed vigorously for 10 min. The organic layer was separated, washed with H_2O (10 mL) and brine (10 mL), dried ($MgSO_4$), filtered, and concentrated. The yellow oily residue was subjected to flash chromatography (SiO_2, 1:2 hexanes/EtOAc) to give the mixture of diastereoisomers **31** as a white solid (32 mg, 78%, dr 89:11): IR ν_{max} 3600−3100, 1662, 1601, 1582, 1512, 1441, 1259, 1213, 1195, 1096, 1042, 817 cm^{-1}; NMR data for the major diastereoisomer [tentatively assigned as (6aS,12aS,4′S,5′S)-4′,5′-dihydro-4′,5′-*cis*-dihydroxydeguelin, as the convex face of **2** is more accessible to reagents than the concave face[57]], δ_H (500 MHz, $CDCl_3$) 1.30 (3H, s, H-7′), 1.47 (3H, s, H-8′), 3.03 (1H, d, J 4.5 Hz, OH-4′), 3.67 (1H, d, J 2.0 Hz, OH-5′), 3.78 (3H, s, H-2′), 3.79 (1H, m obsc, H-5′), 3.81 (3H, s, H-3′), 3.90 (1H, d, J 4.5 Hz, H-12a), 4.23 (1H, d, J 12.5 Hz, H_a-6), 4.61 (1H, dd, J 3.5, 12.5 Hz, H_b-6), 5.04 (1H, dd, J 3.0, 4.5 Hz, H-4′), 5.07 (1H, ddd, J 1.0, 3.5, 4.5 Hz, H-6a), 6.45 (1H, s, H-4), 6.54 (1H, d, J 9.0 Hz, H-10), 6.78 (1H, s, H-1), 7.82 (1H, d, J 9.0 Hz, H-11); δ_C (125 MHz, $CDCl_3$) 22.7 (C-8′), 24.6 (C-7′), 44.4 (C-12a), 56.0 (C-3′), 56.5 (C-2′), 62.5 (C-4′), 66.4 (C-6), 70.6 (C-5′), 73.2 (C-6a), 79.1 (C-6′), 101.1 (C-4), 104.6 (C-1a), 109.6 (C-8), 110.5 (C-1), 112.6 (C-11a), 113.0 (C-10), 129.1 (C-11), 144.2 (C-2), 147.5 (C-4a), 149.7 (C-3), 160.1 (C-9), 161.2 (C-7a), 188.7 (C-12); NMR data for the minor diastereoisomer [tentatively assigned as (6aS,12aS,4′R,5′R)-4′,5′-dihydro-4′,5′-*cis*-dihydroxydeguelin], δ_H (500 MHz, $CDCl_3$) 1.35 (3H, s, H-7′), 1.43 (3H, s, H-8′), 3.10 (1H, d, J 4.5 Hz, OH-4′), 3.73 (1H, d, J 2.0 Hz, OH-5′), 3.78 (3H, s, H-2′), 3.79 (1H, m obsc, H-5′), 3.82 (3H, s, H-3′), 3.90 (1H, d, J 4.5 Hz, H-12a), 4.23 (1H, d, J 12.5 Hz, H_a-6), 4.61 (1H, dd, J 3.5, 12.5 Hz, H_b-6), 5.00 (1H, dd, J 3.0, 4.5 Hz, H-4′), 5.07 (1H, ddd, J 1.0, 3.5, 4.5 Hz, H-6a), 6.44 (1H, s, H-4), 6.54 (1H, d, J 9.0 Hz, H-10), 6.77 (1H, s, H-1), 7.82 (1H, d, J 9.0 Hz, H-11); δ_C (125 MHz, $CDCl_3$) 22.6 (C-8′), 24.7 (C-7′), 44.6 (C-12a), 56.0 (C-3′), 56.5 (C-2′), 62.3 (C-4′), 66.4 (C-6), 70.7 (C-5′), 73.1 (C-6a), 79.2 (C-6′), 101.2 (C-4), 104.7 (C-1a), 109.3 (C-8), 110.3 (C-1), 112.8 (C-11a), 113.4 (C-10), 129.1 (C-11), 144.2 (C-2), 147.5 (C-4a), 149.7 (C-3), 160.1 (C-9), 161.2 (C-7a), 188.6 (C-12); HRESIMS m/z 429.1539 $[M + H]^+$ (calcd for $C_{23}H_{25}O_8$, m/z 429.1549).

Preparation of and Kinetic Measurements on Mitochondrial Membranes. *Bos taurus* (bovine) heart mitochondrial membranes were prepared at 4 °C as described previously.[72] Assays were performed in a SpectraMax 96-well plate reader at 32 °C. NADH:O_2 oxidoreduction by complexes I, III, and IV in membranes was measured using 50 $\mu g\ mL^{-1}$ membranes in 10 mM Tris-HCl (pH 7.4) and 250 mM sucrose using 120 μM NADH and supplemented with 1.5 μM horse heart cytochrome c (Sigma-Aldrich). The reaction was monitored using $\epsilon_{340-380(NADH)} = 4.81\ mM^{-1}\ cm^{-1}$. Catalysis was initiated by addition of NADH, and maximal rates were determined by linear regression.

OCR Measurements on Cultured Human Cells. PNT2, C4-2, and C4-2B cells were obtained from the Cancer Research UK Cambridge Research Institute and tested for mycoplasma before use. Cells were grown in RPMI 1640 (Gibco) supplemented with 10% FBS (Themo Fisher Scientific), 25 mM HEPES, and 1 mM pyruvate. Seahorse cell plates were coated with poly-L-lysine hydrobromide (Sigma-Aldrich), washed with PBS, and dried prior to cell seeding. A total of 20 000 cells per well were plated into coated 96-well Seahorse plates and incubated for 16 h at 37 °C and 5% CO_2. The medium was exchanged for assay buffer containing RPMI without carbonate,

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supplemented with 4.5 g L^{-1} glucose, 1 mM pyruvate, 32 mM NaCl, 2 mM GlutaMAX, and 15 mg L^{-1} Phenol Red (pH 7.4), and the cells were placed in a CO_2-free incubator at 37 °C for 60 min. OCRs were measured in a Seahorse extracellular flux analyzer; after ∼1 h of baseline measurements, 1 μM rotenoids from ethanolic stocks diluted in media (as well as ethanol-only control wells) were added and incubated for a further hour. After this, a mixture of 1 μM rotenone and 1 μM antimycin was added to inhibit mitochondrial respiration. To calculate the normalized rotenone-sensitive OCRs, the rotenone-insensitive rates (determined at the end of the experiment) were subtracted and the traces baselined to 100% before addition of rotenoids. The measured basal OCR prior to baselining for PNT2, C4-2, and C42B cells were 45, 71, and 79 pM min^{-1}.

Cell Growth Curves. PNT2, C4-2, and C4-2B cells were each seeded to a density of 1000 cells per well in a 96-well plate in RPMI 1640 (Gibco) supplemented with 10% FBS (Themo Fisher Scientific), 25 mM HEPES, and 1 mM pyruvate. After 24 h, 1 μM rotenoid compounds from ethanolic stocks diluted in media (as well as ethanol-only controls) were added, and growth was monitored in an Essen Bioscience Incucyte until confluence was reached in the untreated control. Eight replicates were used per condition.

Cell Viability. Cell viability was tested with the acridine orange/DAPI method using a Chemometec NC-3000. Cells were seeded to a density of 150 000 cells per well in six-well plates and grown overnight. Rotenoids were added to 1 μM concentration from ethanolic stocks diluted in media, as well as ethanol control wells, and incubated for 48 h. Cells were trypsinized using TrypLe Express (Gibco) and quenched with complete media, and cells were pelleted by centrifugation at 400*g* for 5 min. Cells were resuspended in PBS and mixed with staining solution containing acridine orange and DAPI (Chemometec), then loaded onto slides and measured according to the manufacturer's instructions. Prior to trypsinization, no floating cells were visible in the wells and the viability of the control PNT2 and C4-2, and C4-2B cells were 89%, 87%, and 83%, respectively.

Statistical Methods. Data are presented as mean averages. IC_{50} values were calculated in Prism version 7.0e from normalized kinetic data using a log(inhibitor) vs normalized response fit with variable slope. Error is presented as either a 95% confidence interval or standard error of the mean, and statistical significance between samples was tested in Prism via an unpaired, two-tailed *t* test. $*P \leq 0.1$, $**P \leq 0.01$, $***P \leq 0.001$, $****P \leq 0.000.1$.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.jnatprod.9b01224.

^1H and ^{13}C NMR spectra for compounds **1**−**31** and 4′-bromorot-2′-enonic acid (PDF)

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Notes

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