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Synthesis of the F11334's from *o*-prenylated phenols: μM inhibitors of neutral sphingomyelinase (N-SMase)

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Abstract—F11334A_1, F11334A_2, F11334A_3, and their corresponding resorcinol analogs were synthesized along with F11334B_1, and F11263. In the course of these synthetic studies, several conditions for manipulating (2,3-propanediol) and (2,3-epoxypropyl) *o*-substituted phenols were developed as well as a variety of conditions for cleavage of aryl O-*t*-butyl carbonates. From enzyme assays it appears that the hydroquinone nucleus is the essential structural necessary for N-SMase inhibition. Furthermore, it appears that this family of compounds is comprised of irreversible inhibitors. © 2002 Published by Elsevier Science Ltd.

Recently, small molecules that serve as inhibitors for sphingomyelinase have become of interest from a therapeutic standpoint.[1] Sphingomyelinases are a subclass of phospholipase C enzymes responsible for the cleavage of sphingomyelin to ceramide and phosphocholine; an event believed essential for triggering cell apoptosis. Two mammalian sphingomyelinases are known. Acidic sphingomyelinase (A-SMase), located in lysosomes, is stimulated by cytostatic levels of FADD and interleukin **1**.[2] Neutral sphingomyelinase (N-SMase), found in the plasma membrane, is stimulated by TNFR1/p55TNF activation of the FAN protein and requires Mg^{2+} for activity.[3] It is thought that inhibitors of sphingomyelinase may prove useful for the treatment of a variety of maladies[4] including herpes,[5] stroke,[6] and myocardial infarction.[7] One potentially useful application for N-SMase inhibitors is stopping the progression of HIV to AIDS. Apoptotic cell death, regarded as the principal mechanism for depletion of T-cells, is believed to be distinct from the mechanism of HIV infection and replication.[8] Some suspect that the HIV proteins, gp120, gp160, and HIV-tat interact with the Fas/FasL, TRAIL, and TNFR1/p55TNF receptors to accelerate apoptotic cell death of T-lymphocytes via activation of neutral sphingomyelinase (N-SMase).[9] Activation of N-SMase, in turn, stimulates caspase production leading to T-cell apoptosis.[10] Interestingly, long-term survivors of HIV who have refused all treatment and remained healthy and immunocompetent for 10 or more years, have been shown to maintain lower than normal ceramide levels.[11] This has led some to deduce that therapies directed at down-modulating ceramide should slow the progression of HIV by reducing lymphocyte apoptosis even if viral replication is unaffected by this course of action.[12]

By far the simplest inhibitors reported to be specific for N-SMase are the F11334's (**1a**–**3a**, **4**, **5**),[1e] and F11263 (**6**)[1f] (Fig. 1). These compounds are produced by the fungus *Acremonium murorum*. For compounds **1a**–**3a** and **4**–**6** Ogita reported IC_{50} values ranging from 0.8–200 μg/mL (15–942 μM) against N-SMase that was harvested from rat brain microsomes. The increased potency of hydroquinone derivative **1a** relative to the cyclic ethers **2a** and **3a** was ascribed to free hydroxyl groups. In view of the activity of F11263 (**6**), we concluded that the bio-activity of **1a**–**3a** and **4**, **5** resulted from the ability of these entities to oxidize to the corresponding *p*-quinone. In order to confirm this suspicion, we set out to construct **1a**–**3a**, **4**–**6**, and their corresponding resorcinol analogs **1b**–**3b** using a common synthetic sequence and then compare the inhibitory activity between the resorcinol and hydroquinone derivatives.

Recently, we reported a very efficient three-pot construction of *o*-prenylated phenols from the corresponding salicylaldehydes.[13a,b] This procedure was used to construct **7a** and **7b** in 63% and 60% overall yield from the respective salicylaldehydes. Upon oxidation with 1,1-dimethyldioxirane (DMDO) (2.0 equiv.), these respective *o*-prenylated phenols **7a** and **7b** produce the corresponding epoxides **8a** and **8b** in excellent yield (Scheme 1). In principle, this epoxidation is amenable to enantioselectivity incorporating Shi's method,[14] however, it is believed that racemic compounds would prove adequate for this survey.

The epoxides **8a** and **8b**, both of which were unstable

Keywords: dihydroxylation; quinines; phenols; epoxidation; aryl O-*t*-butyl carbonate deprotection; neutral sphingomyelinase.
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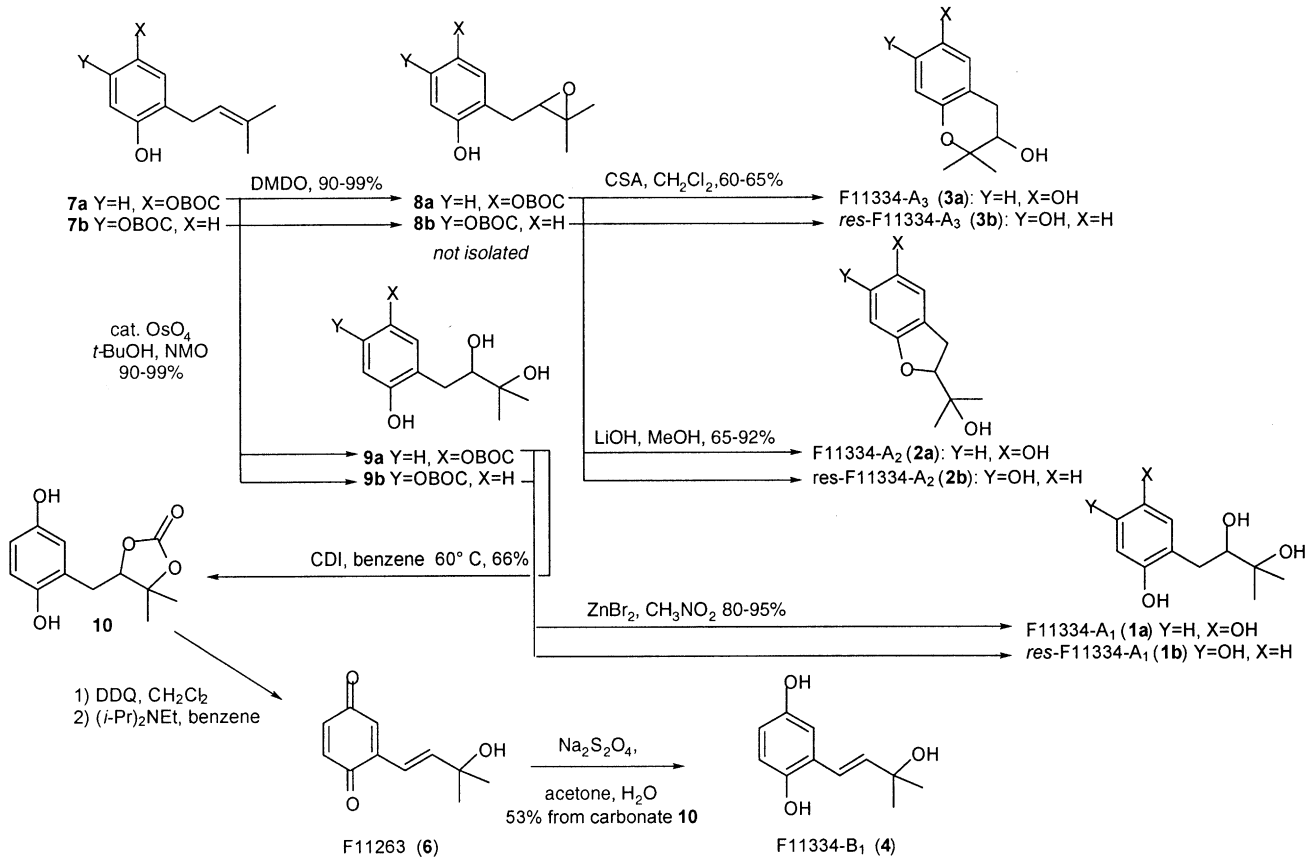
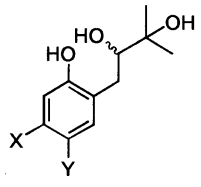
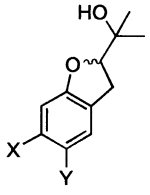


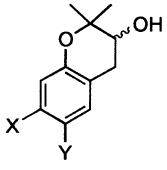
Scheme 1. Synthesis of F11334A_1 (**1**), F11334A_2 (**2**), F11334A_3 (**3**), F11334B_1 (**4**) and F11263 (**6**).



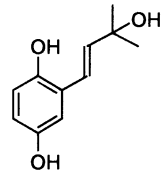
1a: X=H; Y=OH [F11334A_1] IC_{50} = 7.5 μg/mL
1b: X=OH; Y=H [*res*-F11334A_1]



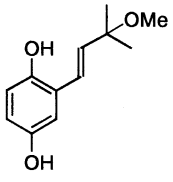
2a: X=H; Y=OH [F11334A_2] IC_{50} = 200.0 μg/mL
2b: X=OH; Y=H [*res*-F11334A_2]



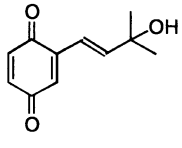
3a: X=H; Y=OH [F11334A_3] IC_{50} = 200.0 μg/mL
3b: X=OH; Y=H [*res*-F11334A_3]



4: [F11334B_1] IC_{50} = 3.6 μg/mL



5: [F11334B_2] IC_{50} = 3.2 μg/mL



6: [F11263] IC_{50} = 0.8 μg/mL

res- = resorcinol nucleus

Figure 1. F11334's with respective inhibition of N-Smase. The IC_{50}'s for **1a**–**3a** and **4**–**6** were reported by Ogita.[1e,f]

Table 1. Calculated IC_{50}'s for **1a** and **1b** regarding N-Smase from microsomes of rat brain

Compound	Inhibition without pre-incubation (μg/mL)	Inhibition with pre-incubation (μg/mL)
1a	46.6	34.4
1b	>300.0	>300.0

Increased inhibition with pre-incubation suggests the compound acts in an irreversible reaction with the enzyme.

towards chromatography, could be opened to exclusively yield either the benzohydropyran or benzohydrofuran nucleus. For example, exposure of either the resorcinol or hydroquinone epoxide (0.1 M in methylene chloride) to camphorsulphonic acid (CSA) (2.0 equiv.) results in a [6-*endo*-trig]-closure affording the respective benzohydropyran. Presumably treatment with acid leads to a cation intermediate which undergoes cyclization. Prolonged contact with CSA results in subsequent cleavage of the aryl O-*t*-butyl carbonate (aryl-OBOC) to afford **3a** or **3b**, respectively. Alternatively, an exclusive [5-*exo*-tet]-cyclization leading to the corresponding benzohydrofuran is executed on either of these epoxides by exposure of the respective substrate (0.1 M in MeOH) to LiOH (2.0 equiv). Presumably treatment with base leads to the corresponding *o*-(2,3-epoxypropyl)phenoxide, which undergoes a nucleophilic cyclization.[15] Prolonged contact with LiOH results in subsequent cleavage of the aryl O-*t*-butyl carbonate and affords **2a** and **2b**, respectively. Our attempts to open type **8** epoxides to their corresponding diols failed. Efforts to convert various *o*-(2,3-epoxypropyl)phenols to their corresponding *o*-(3-hydroxyprop-1-ene)phenols by eliminatory opening were also unsuccessful. We therefore devised a different sequence to address the synthesis of **1a**, **1b**, **4**, and **6**.

Independent dihydroxylation of the *o*-prenylated phenols **7a** and **7b** is accomplished in *t*-BuOH (0.26 M) with osmium tetroxide (OsO_4) (0.05 equiv.), and *N*-methylmorpholine *N*-oxide (NMO) (1.1 equiv). Yields of the respective *o*-(2,3-propanediol)-phenols **9a** and **9b** are essentially quantitative. Exposing either the hydroquinone or resorcinol diol (0.05 M in CH_3NO_2) to $ZnBr_2$ (5 equiv.) cleaves the remaining aryl O-*t*-butyl carbonate furnishing the respective tetrol **1a** and **1b** in yields ranging from 80 to 95%. On the other hand refluxing **9b** (0.1 M in benzene) with *N*,*N*′-carbonyldiimidazole (CDI) (4 equiv.) affords a cyclic carbonate, which undergoes BOC cleavage most likely as a result of the imidazole that is liberated during the course of the reaction.[16] Oxidation of the resulting hydroquinone **10** (0.1 M in CH_2Cl_2) with 2,3-dichloro-5,6-dicyanobenzoquinone (DDQ) (1.1 equiv.) and subsequent base promoted opening of the cyclic carbonate (0.05 M in benzene) triggered by refluxing with *N*,*N*-diisopropylethylamine (DIPEA) (12.0 equiv.) provides the quinone **6**. Reduction of F11263 (**6**) (0.5 M in 1/1 acetone and H_2O) with excess of a 0.5 M aqueous solution of sodium dithionite produces F11334B_1 (**4**) in 53% from the cyclic carbonate **10**.

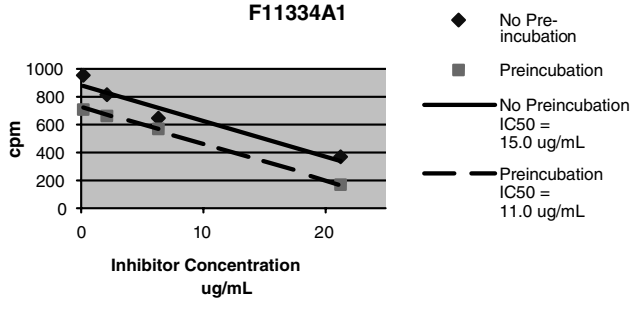


Figure 2. Inhibition of PM N-SMase with **1a** at concentrations of 0.2, 2.1, 6.4, 21.2 μg/mL. A negative slope indicates increasing inhibition at higher concentrations of the inhibitor. Counts per minute (CPM). 488.0 and 419.0 cpm equals 50% inhibition without and with pre-incubation, respectively.

1. Neutral-sphingomyelinase inhibition

We suspected that the biological activity of **1a**, **4**, and **5** results from their ability to easily oxidize to the corresponding *para*-quinones. This conclusion is suggested by the potency of F11263 (**6**) and the lack thereof for **2a** and **3a**.[1f] However, it is surprising that these small molecules display even moderate amounts of activity, since all other inhibitors with comparable activity such as CoQ_{10},[17] scyphostatin,[18] and manumycin[1h] are substantially more complex with large lipophyllic regions.

Inhibitory assays were conducted on **1a** and **1b** with N-SMase isolated from the plasma membranes (PM) of rat livers as well as the microsomes of rats brain. Assays with **1a** and **1b** were carried out with and without pre-incubation. Activity without pre-incubation suggests the ability of the inhibitor to compete with the sphingomyelin substrate at the active site of the enzyme, whereas increased inhibition with pre-incubation suggests irreversible inhibition.

The IC_{50} for **1a** and **1b** regarding N-SMase isolated from brain microsomes are shown in Table 1. Our studies confirm that F11334A_1 (**1a**) is an inhibitor of N-SMase [68% inhibition at 63.67 μg/mL]. This is within the experimental error of the activity observed with scyphostatin and manumycin.[1h] Furthermore, the 25% increased activity of **1a** [93% inhibition at 63.67 μg/mL] upon pre-incubation, suggests that **1a** is an irreversible inhibitor. The resorcinol derivative **1b**, on the other hand, shows very little if any inhibition of N-SMase. This observation therefore supports the idea that the activity of **1a** requires oxidation to the corresponding *p*-quinone, a difficult proposition in the case of the resorcinol compound **1b**.

The results shown in Fig. 2 depict a more precise picture of this family of compounds as inhibitors of apoptotic signaling. These tests were performed using the liver plasma membrane rather than brain microsomes.[19] In our experience, purified plasma membranes prove more reliable as assays for apoptotic signal transduction because the presence of the A-SMase is minimal.[19] Moreover, the participation of N-SMases obtained from brain homogenates rich in the *Golgi apparatus* and *Endoplasmic Reticulum* has been questioned in regards to their role in apoptotic signaling and the sphingomyelin cycle.[20] Compound **1a** displayed greater potency towards N-SMase isolated from this source. The IC_{50} for **1a** was calculated to be 15.6 μg/mL. With pre-incubation the IC_{50} value fell by approximately 25% (more active) to 11.9 μg/mL,

suggesting again that **1a** acts as an irreversible competitive inhibitor.

In conclusion, a common prenylated intermediate is converted into the constituents of the F11334 family of natural products as well as some of their resorcinol analogs. A key feature is the application of basic, acidic, and neutral conditions for cleaving aryl O-*t*-butyl carbonates concurrently with other synthetic operations. F11334A_1 (**1a**) is confirmed to be a micromolar inhibitor of N-SMase, while its resorcinol counterpart **1b** shows no activity. The increased activity of **1a** upon pre-incubation suggests that it is an irreversible inhibitor. The plasma membrane appears to provide N-SMase that is more readily inhibited by **1a** than N-SMase isolated from brain microsomes. From these experiments we conclude that the F11334 family of natural products offer greater structural simplicity and comparable biological activity to significantly more complex molecules such as scyphostatin and manumycin A, thereby demonstrating that a large lipophyllic region is not necessary for N-SMase inhibition.

2. Experimental

2.1. General information

All the reagents were newly purchased or freshly prepared as stipulated. All column chromatography was conducted using 60 microns silica gel with the indicated solvent systems.

2.2. Preparation of biological samples

The preparation of membranes used as a source for N-SMase assays was carried out analogous to the method described by Hannun[21] and modified with respect to his more recent publications.[22]

Preparation of liver plasma membrane N-SMase. A crude membrane fraction was obtained from rat liver homogenates by differential centrifugation. Highly enriched plasma membranes were then purified from crude fractions by the two-phase partition method with a phase system composed of 6.4% Dextran T 500, 6.4% PEG 3350, 0.25 M sucrose and 5 mM potassium phosphate buffer, pH 7.8.[23] Membranes were then washed by centrifugation, resuspended in 50 mM Tris–HCl, pH 7.6 containing 1 mM phenylmethylsulfonylfluoride and 20 μg/mL each of chymostatin, leupeptin, antipain and pepstatin A, and stored at −80°C until needed. Plasma membrane purity was checked by marker enzymes as described.[24]

Preparation of brain membranes. A membranous fraction was obtained from rat brains as described by Liu et al.[21] Frozen brains were briefly thawed and the cerebella and brain stem removed. The remainder of the brain tissue was homogenized with the aid of a motor-driven Teflon pestle glass homogenizer in five volumes of 50 mM Tris–HCl, pH 7.4 containing 2 mM EDTA, 5 mM EGTA, 1 mM sodium orthovanadate, 10 mM B-glycerolphosphate, 1 mM sodium fluoride, 1 mM sodium molybdate, 1 mM phenylmethylsulfonylfluoride and 20 μg/mL each of chymostatin, leupeptin, antipain and pepstatin A. Crude homogenates were centrifuged for 15 min at 1000*g* to remove nuclei and cell debris. Membranous material contained in supernatants was recovered by ultracentrifugation at 105,000*g* for 90 min and resuspended in 50 mM Tris–HCl, pH 7.6 containing 1 mM phenylmethylsulfonylfluoride and 20 μg/mL each of chymostatin, leupeptin, antipain and pepstatin A. Brain membranes were stored at −80°C until needed.

2.3. N-SMase assay

For the determination of the N-SMase activity, the potential inhibitors were dissolved in ethanol and aliquots containing the required amounts for the assays dried under a nitrogen stream in the dark, redissolved in 40 μL of assay buffer (75 mM Tris–HCl, pH 7.4 containing 0.05% Triton X-100 and 5 mM $MgCl_2$) and mixed with 10 μL of membrane sample (30–35 μg protein). Together with controls, the probes were pre-incubated for 0 and 30 min at 37°C. After addition of 10 nmol [^{14}C]sphingomyelin (ca. 50,000 cpm) in 50 μL of assay buffer, the reaction was proceeded for another 30 min. The reaction was stopped by adding 750 μL chloroform/methanol (1:1, v/v). After addition of 200 μL water, lipids were extracted and the radioactivity of the polar upper phase, containing [^{14}C]phosphorylcholine, was determined by scintillation counting. As **1a** showed a loss of activity in the presence of a thiol, the assays were carried out in a buffer free of dithiothreitol and β-mercaptoethanol.

2.4. Epoxidation

To a flask containing the neat *o*-prenylated phenol, **7a** or **7b**[13] (280.0 mg, 1.00 mmol) was added a solution of DMDO (20.0 mL, 0.1 M in acetone, 2.00 mmol, 2.0 equiv.) in a dropwise fashion. After the reaction was stirred for 10 min at room temperature, the solution was subsequently dried with sodium sulfate and concentrated to give **8a** or **8b**. Analytical pure sample could not be obtained; however the corresponding NMR results for **8a** and **8b** are described below.

2.4.1. Compound 8a. ^{1}H NMR [$CDCl_3$, 400 MHz] δ 6.97–6.88 (m, 3H), 3.07 (dd, 1H, J_1=9.7 Hz, J_2=2.7 Hz), 2.91 (dd, 1H, J_1=14.8 Hz, J_2=2.9 Hz), 2.81 (dd, 1H, J_1=14.8 Hz, J_2=9.7 Hz), 1.56 (s, 9H), 1.50 (s, 3H), 1.37 (s, 3H); ^{13}C NMR [$CDCl_3$, 100.6 MHz] δ 153.5, 152.6, 144.4, 125.3, 123.2, 121.3, 117.8, 83.2, 65.3, 61.4, 32.0, 27.9, 24.9, 19.0.

2.4.2. Compound 8b. ^{1}H NMR [$CDCl_3$, 400 MHz] δ 7.08 (d, 1H, J=8.2 Hz), 6.75 (d, 1H, J=2.4 Hz), 6.68 (dd, 1H, J_1=8.2 Hz, J_2=2.4 Hz), 3.04 (dd, 1H, J_1=9.9 Hz, J_2=2.7 Hz), 2.94 (dd, 1H, J_1=14.8 Hz, J_2=2.7 Hz), 2.78 (dd, 1H, J_1=14.8 Hz, J_2=9.9 Hz), 1.56 (s, 9H), 1.49 (s, 3H), 1.37 (s, 3H); ^{13}C NMR [$CDCl_3$, 100.6 MHz] δ 156.6, 152.1, 151.3, 131.0, 122.1, 113.4, 110.7, 83.7, 65.5, 61.5, 31.7, 27.9, 24.9, 19.0.

2.5. Benzohydropyran formation/acidic cleavage of aryl *O-t*-butyl carbonate

To a solution of the epoxide **8a** or **8b**, (63.6 mg, 0.229 mmol,

0.1 M in CH_2Cl_2) was added the camphorsulfonic acid (0.106 g, 0.458 mmol, 2.0 equiv.) and the resulting mixture was allowed to stir for 2 days at room temperature. The mixture was concentrated, re-dissolved in ethyl acetate, washed with water and brine, dried with sodium sulfate and condensed. Chromatography with silica gel (85:15 EtOAc/pet. ether) furnished **3a** or **3b**.

2.5.1. Compound F11334A$_3$ (3a). All spectra was found to be identical to those previously reported. A yield of 65% was deduced using DMF as an internal comparing the methine proton signal at 3.70 ppm with the methyl signal at 2.90 ppm using ^{1}H NMR. White solid. Mp=154–155°C; ^{1}H NMR [CD_3OD, 400 MHz] δ 6.62 (s, 1H), 6.56 (d, 1H, J= 8.5 Hz), 6.52 (dd, 1H, J_1=8.5 Hz, J_2=2.5 Hz), 3.70 (dd, 1H, J_1=7.5, Hz, J_2=5.5 Hz), 2.93 (dd, 1H, J_1=16.5 Hz, J_2=5.5 Hz), 1.29 (3H, s), 1.20 (3H, s); ^{13}C NMR [$CDCl_3$, 100.6 MHz] δ 152.2, 147.8, 122.4, 118.9, 116.9, 116.1, 78.0, 71.2, 32.9, 26.3, 21.4; IR [CH_2Cl_2, ν_{max} cm^{-1}] 3586, 3000, 2932, 2857, 1703, 1497, 1278, 1256; MS (EI) *m/z* 194 (70.05), 161 (27.42), 136 (21.61), 123 (100.00), 71 (46.14); HRMS (EI) *m/z* calcd for $C_{11}H_{14}O_3$ 194.094294, found 194.094078.

2.5.2. *res*-F11334A$_3$ (3b). A yield of 60% was deduced using DMF as an internal comparing the methine proton signal at 3.70 ppm with the aldehyde proton at 7.96 ppm using ^{1}H NMR. White solid. Mp=149–150°C; ^{1}H NMR [CD_3OD, 400 MHz] δ 6.84 (d, 1H, J=8.2 Hz), 6.30 (dd, 1H, J_1=8.2 Hz, J_2=2.4 Hz), 6.18 (d, 1H, J=2.4 Hz), 3.70 (dd, 1H, J_1=7.7 Hz, J_2=5.5 Hz), 2.89 (dd, 1H, J_1=16.1 Hz, J_2=5.3 Hz), 2.61 (dd, 1H, J_1=16.1 Hz, J_2=7.7 Hz), 1.31 (s, 3H), 1.22 (s, 3H); ^{13}C NMR [$CDCl_3$, 100.6 MHz] δ 155.7, 153.7, 131.0 110.8, 108.8, 104.0, 77.1, 70.0, 30.0, 24.9, 22.6; IR [CH_2Cl_2, ν_{max} cm^{-1}] 3583, 2978, 2935, 1734, 1624, 1598, 1508, 1452, 1371; MS (EI) *m/z* 136 (7.53), 124 (15.19), 123 (100.0), 43 (16.61); HRMS (EI) *m/z* calcd for $C_{11}H_{14}O_3$ 195.102120, found 195.101500.

2.6. Benzohydrofuran formation/basic cleavage of aryl *O*-*t*-butyl carbonate

To a solution of the epoxide **8a** or **8b** (79.1 mg, 0.269 mmol, 0.05 M in MeOH) was added LiOH·H_2O (0.0401 g, 0.957 mmol, 3.56 equiv.) at room temperature and the resulting mixture was allowed to stir for 2 days. The reaction was diluted with ethyl acetate and washed with aqueous 0.1N HCl. The aqueous layer was then extracted three times with ethyl acetate. The combined organic layers were then washed with brine, dried with sodium sulfate, and concentrated. Chromatography with silica gel (85:15 EtOAc/pet. ether) furnished **2a** or **2b**.

2.6.1. F11334A$_2$ (2a). A yield of 65% was deduced using DMF as an internal comparing the methine proton signal at 4.50 ppm with the aldehyde proton at 7.96 ppm using ^{1}H NMR. White solid. Mp=137–138°C; ^{1}H NMR [CD_3OD, 400 MHz] δ 6.62 (br s, 1H), 6.52 (br d, 1H, J=8.5 Hz), 6.48 (dd, 1H, J_1=8.5 Hz, J_2=2.5 Hz), 4.50 (dd, 1H, J_1=9.5 Hz, J_2=8.5 Hz), 3.12 (dd, J_1=15.5 Hz, J_2= 8.5 Hz) 3.06 (dd, 1H, J_1=15.5 Hz, J_2=9.5 Hz), 1.22 (s, 3H), 1.20 (s, 3H); ^{13}C NMR [CD_3OD, 100.6 MHz] δ 155.0, 152.6, 129.7, 115.3, 113.5, 110.2, 90.7, 73.0, 32.5, 25.9, 25.6; IR [CH_2Cl_2, ν_{max} cm^{-1}] 3584, 2978, 2935, 2854, 1701, 1493, 1260; MS (EI) *m/z* 491 (49.58), 136 (81.10), 123 (38.22), 107 (25.47), 59 (58.62), 43 (100.00); HRMS (EI) *m/z* calcd for $C_{11}H_{14}O_3$ 194.094294, found 194.093577.[1e]

2.6.2. *res*-F11334A$_2$ (2b). (85:15 EtOAc/pet. ether). A yield of 92% was deduced using DMF as an internal comparing the methine proton signal at 6.81 ppm with the aldehyde proton at 7.96 ppm using ^{1}H NMR. White solid. Mp=139–141°C; ^{1}H NMR [CD_3OD, 400 MHz] δ 6.81 (d, 1H, J=8.1 Hz), 6.22 (dd, 1H, J_1=8.1 Hz, J_2=2.2 Hz), 6.17 (d, 1H, J=1.8 Hz), 4.52 (t, 1H, J=8.8 Hz), 3.01 (d, 2H, J=8.8 Hz), 1.20 (s, 3H), 1.17 (s, 3H); ^{13}C NMR [CD_3OD, 100.6 MHz] δ 162.5, 158.8, 125.9, 119.1, 108.2, 98.1, 91.2, 72.7, 31.1, 25.4, 25.2; IR [CH_2Cl_2, ν_{max} cm^{-1}] 2583, 3043, 2977, 1620, 1499, 1460; MS (EI) *m/z* 136 (17.29), 101 (29.47), 85 (100.00); HRMS (EI) *m/z* calcd for $C_{11}H_{14}O_3$ 194.094294, found 194.094058.

2.7. Dihydroxylation

A solution of the respective *o*-prenylated phenol **7a** or **7b**, (0.0379 g, 0.138 mmol, 0.258 M in *t*-butanol, 1.9 M in water) was combined with NMO (0.0205 g., 0.158 mmol, 1.14 equiv.). A catalytic amount of OsO_4 (0.036 mL, 0.076 M in *t*-BuOH, 0.02 equiv.) was added and the solution was allowed to stir for 12 h at room temperature. Sodium sulfite (0.034 g, 0.28 mmol, 2.0 equiv.) was then added to the solution and allowed to stir for 0.5 h. This solution was then extracted with ethyl acetate (three times), washed with brine, dried with sodium sulfate, and concentrated. Chromatography (75:25 EtOAc/pet. ether) with silica gel furnished **9a** or **9b**.

2.7.1. Compound 9a. A yield of 93% was deduced using DMF as an internal comparing the methine proton signal at 3.46 ppm with the methyl proton signal at 2.99 ppm using ^{1}H NMR. White solid. Mp=40–42°C; ^{1}H NMR [$CDCl_3$, 400 MHz] δ 8.22 (bs, 1O*H*), 6.88 (dd, 1H, J_1=8.6 Hz, J_2=2.7 Hz), 6.85 (d, 1H, J_1=2.7 Hz), 6.75 (d, 1H, J=8.6 Hz), 4.06 (bs, 1O*H*), 3.46 (d, 1H, J=9.7 Hz), 2.73 (dd, 1H, J_1=14.3 Hz, J_2=10.3 Hz), 2.59 (dd, 1H, J_1=14.3 Hz, J_2=1.5 Hz), 1.71 (bs, O*H*), 1.55 (s, 9H), 1.24 (s, 3H), 1.20 (s, 3H); ^{13}C NMR [$CDCl_3$, 100.6 MHz] δ 153.6, 153.1, 144.1, 127.3, 123.7, 120.9, 117.5, 83.9, 80.5, 73.2, 34.1, 27.9, 26.0, 23.5; IR [CH_2Cl_2, ν_{max} cm^{-1}] 3313, 3043, 2977, 1755, 1598, 1496, 1372, 1241; MS (EI) *m/z* 123 (12.85), 85 (14.77), 71 (15.28), 59 (27.82), 57 (100.00), 42 (39.27); HRMS (EI) *m/z* $C_{16}H_{24}O_6$ 312.155946, found 312.156532.

2.7.2. Compond 9b. A yield >99% was deduced employing DMF as an internal standard comparing the methine proton signal at 2.60 ppm with the methyl proton signal at 2.99 ppm using ^{1}H NMR. White solid. Mp=124–126°C; ^{1}H NMR [$CDCl_3$, 400 MHz] δ 8.39 (bs, O*H*), 7.023 (d, 1H, J=8.2 Hz), 6.76 (d, 1H, J=2.4 Hz), 6.68 (dd, 1H, J_1=8.2 Hz, J_2=2.4 Hz), 3.74 (bs, O*H*), 3.67 (dd, 1H, J_1=10.3 Hz, J_2=1.6 Hz), 2.82 (dd, 1H, J_1=10.1 Hz, J_2=14.6 Hz), 2.60 (dd, 1H, J_1=13.0 Hz, J_2=14.3 Hz), 1.70 (bs, O*H*), 1.56 (s, 9H), 1.31 (s, 3H), 1.29 (s, 3H); ^{13}C NMR [$CDCl_3$, 100.6 MHz] δ 153.7, 153.1, 144.3, 127.2,

123.7, 121.0, 117.8, 83.8, 80.8, 73.3, 34.3, 28.0, 26.4, 23.4; IR [CH_2Cl_2, ν_{max} cm^{-1}] 3270, 3022, 2977, 2934, 1755, 1623, 1595, 1507, 1460; MS (EI) *m/z* 124 (10.97), 123 (33.78), 59 (25.44), 57 (100.00), 43 (25.54); HRMS (EI) *m/z* calcd for $C_{16}H_{24}O_6$ 312.158063, found 312.157289.

2.8. Zinc bromide cleavage of aryl *O-t*-butyl carbonate

$ZnBr_2$ (36.0 mg, 0.16 mmol, 5.0 equiv.) was added to a solution of **9a** or **9b** (9.9 mg, 0.032 mmol, 0.05 M in nitromethane) and allowed to stir at room temperature overnight. The reaction was filtered through a plug of celite, diluted with ethyl acetate, washed with water and brine, dried with Na_2SO_4 and concentrated. Chromatography with silica gel (1:1 EtOAc/pet. ether) furnished **1a** or **1b**.

2.8.1. F11334A$_1$ (1a). The yield was computed to be >99% by ^{1}H NMR employing DMF as the internal standard comparing the methine proton signal at 2.60 ppm with the methyl proton signal at 2.99 ppm. Isolated yield, 57%. Red solid. Mp=29°C; ^{1}H NMR [CD_3OD, 400 MHz] δ 6.62 (d, 1H, J=3.0 Hz), 6.50 (dd, 1H, J_1=8.5 Hz, J_2=3.0 Hz), 6.63 (d, H, J=8.5 Hz), 3.60 (dd, 1H, J_1=10.0 Hz, J_2=2.0 Hz), 2.80 (dd, 1H, J_1=13.5 Hz, J_2=2.0 Hz), 2.60 (dd, 1H, J_1=13.5 Hz, J_2=10.0 Hz), 1.23 (s, 6H); ^{13}C NMR [CD_3OD, 100.6 MHz] δ 151.8, 150.1, 129.6, 119.3, 117.7, 115.2, 81.2, 74.3, 34.8, 26.2, 25.4; IR [CH_2Cl_2, ν_{max} cm^{-1}] 3577, 2971, 2927, 2854, 1701, 1497, 1271; MS (EI) *m/z* 152 (65.24), 124 (26.08), 123 (100.00), 122 (23.71), 59 (96.71), 43 (49.11); HRMS (EI) *m/z* calcd for $C_{11}H_{16}O_4$ 212.104859, found 212.105328.[1e]

2.8.2. *res*-F11334A$_1$ (1b). Isolated yield, 59%. Brown oil. ^{1}H NMR [CD_3OD, 400 MHz] δ 6.88 (d, 1H, J=8.1 Hz), 6.26 (d, 1H, J=2.4 Hz), 6.22 (dd, 1H, J_1=8.1 Hz, J_2=2.4 Hz), 3.35 (dd, 1H, J_1=11.4 Hz, J_2=2.0 Hz), 2.80 (dd, 1H, J_1=14.3 Hz, J_2=2.0 Hz), 2.50 (dd, 1H, J_1=14.3 Hz, J_2=10.3 Hz), 1.22 (s, 6H); ^{13}C NMR [CD_3OD, 100.6 MHz] δ 158.1, 157.7, 132.8, 119.3, 107.9, 104.1, 81.0, 74.0, 33.7, 25.8, 25.2; IR [CH_2Cl_2, ν_{max} cm^{-1}] 3627, 2949, 2839, 1730, 1595, 1511, 1296; MS (EI) *m/z* 212 (9.43), 123 (100), 107 (14.35), 59 (41.75); HRMS (EI) *m/z* mass calcd for $C_{11}H_{16}O_4$ 212.104859, found 212.105715.

2.8.3. Cyclic carbonate formation and OBOC cleavage. A dry flask charged with compound **9a** (0.02 g, 0.06 mmol, 0.1 M in benzene) and the carbonyldiimidazole (0.05 g, 0.3 mmol, 5.0 equiv.) was stirred at reflux for 12 h. The reaction was quenched with 0.1N HCl, extracted three times with ethyl acetate, washed once with brine, dried with sodium sulfate, and concentrated. Chromatography with silica gel (25:75 EtOAc/pet. ether) furnished **10** as a white solid. Isolated yield, 66%. Mp=159–160°C; ^{1}H NMR [CD_3OD, 400 MHz] δ 6.64–6.62 (m, 2H), 6.54 (dd, 1H, J_1=8.6 Hz, J_2=2.9 Hz), 4.75 (dd, 1H, J_1=8.8 Hz, J_2=4.9 Hz), 3.01 (dd, 1H, J_1=14.1 Hz, J_2=4.9 Hz), 2.83 (dd, 1H, J_1=14.1 Hz, J_2=8.8 Hz), 1.47 (s, 3H), 1.41 (s, 3H); ^{13}C NMR [CD_3OD, 100.6 MHz] δ 156.5, 151.3, 149.6, 124.4, 118.8, 116.9, 115.8, 86.3, 86.2, 31.4, 26.1, 21.4; IR [CH_2Cl_2, ν_{max} cm^{-1}] 3586, 3404, 3091, 3036, 2962, 2928, 2856, 1793, 1605, 1506, 1449, 1378, 1269, 1261, 1176, 1123, 1089; MS (EI) *m/z* 238 (12.90), 176 (14.99), 161 (100), 123 (88.38), 107 18), 77 (23), 68 (34); HRMS (EI) *m/z* mass calcd for $C_{12}H_{14}O_5$ 238.084124, found 238.084958.

2.8.4. Oxidation of hydroquinone and elimination of carbonate. To a solution of **10** (0.0946 g, 0.397 mmol, 0.1 M in methylene chloride) was added DDQ (0.0944 g, 0.416 mmol, 1.05 equiv.) at room temperature. After 10 min the reaction was quenched with water, extracted three times with methylene chloride, washed with brine, dried with sodium sulfate, and concentrated. Chromatography with silica gel (65:35 EtOAc/pet. ether) furnished a yellow solid. A solution of the resulting quinone (0.01629 g, 0.06896 mmol, 0.01 M in benzene) was stirred with diisopropylethylamine (0.134 g, 1.03 mmol, 15.0 equiv.) at reflux for 48 h. The reaction was quenched with 0.1N HCl, extracted three times with ethyl acetate, washed with brine once, dried with sodium sulfate, and concentrated. Analytically pure sample could not be obtained; however the corresponding NMR results are given. ^{1}H NMR [$(CD_3)_2CO$, 400 MHz] δ 6.91–6.86 (m, 2H), 6.69 (d, 1H, J=8.6 Hz), 6.66 (m, 1H) 6.33 (d, 1H, J=16.0 Hz), 1.34 (s, 6H).[1f]

2.8.5. Reduction of quinone. To a solution of F11263 (**6**) (0.0133 g, 0.0690 mmol, 0.1 M in acetone) was added sodium hydrosulfite (0.241 g, 1.38 mmol, 20.0 equiv., 0.5 M in water) and the mixture was allowed stir at room temperature for 5 min. The reaction was quenched with 0.1 M HCl, extracted three time with ethyl acetate, washed with brine, dried with sodium sulfate and concentrated. Chromatography using silica gel (25:75) EtOAc/pet. ether furnished F11334B$_1$ as a yellow oil. The yield was computed to be 53% by ^{1}H NMR employing DMF as the internal standard comparing the methine signal at 6.55 ppm with the methine proton signal of the aldehyde at 7.96 ppm. ^{1}H NMR [$(CD_3)_2CO$, 400 MHz] δ 6.90 (d, 1H, J=2.9 Hz), 6.88 (d, 1H, J=16.0 Hz), 6.69 (d, 1H, J=8.6 Hz), 6.55 (dd, 1H, J_1=8.4 Hz, J_2=2.9 Hz), 6.33 (d, 1H, J=16 Hz), 1.34 (s, 6H); ^{13}C NMR [CD_3OD, 100.6 MHz] δ 151.4, 149.2, 138.3, 126.4, 122.7, 117.7, 116.2, 113.5, 71.8, 30.1; IR [CH_2Cl_2, ν_{max} cm^{-1}] 3584, 3066, 2971, 2927, 2861, 1500, 1446, 1332, 1296, 1276, 1254; MS (EI) *m/z* 176 (17.81), 161 (100.0), 42 (12.15); HRMS (EI) *m/z* mass calcd for $C_{11}H_{13}O_3$ 194.094294, found 194.095008.[1e]

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