Synthesis and Biological Evaluation of Highly Functionalized Analogues of Ingenol

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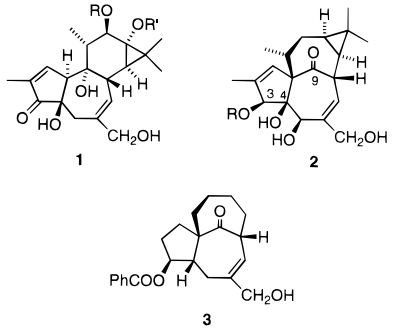
Abstract: The synthesis and preliminary biological evaluation of the first analogues of ingenol, a potent activator of protein kinase C, containing all of the oxygen functionality and unsaturation present in the natural product, is described.

Introduction

The identification of cellular signaling systems and the design and synthesis of small molecules that regulate these systems is at the forefront of modern drug design.[1] Protein kinase C is a central mediator of cellular signal transduction for a large class of hormones and cellular effectors that generate the lipophilic secondary messenger *sn*-1,2-diacylglycerol, e.g., through activation of phosphatidylinositol 4,5-bis(phosphate) turnover.[2] Several structurally diverse, naturally occurring compounds including bryostatin, teleocidin, aplysiatoxin, and esters of phorbol, **1**, and ingenol, **2** (R = H; Chart 1), mimic the function of diacylglycerol, the endogenous activator of protein kinase C (PKC), but possess much greater potency.[3] The synthesis and study of specifically modified derivatives of these natural product leads should establish the structural requirements for the activation of PKC that are common to these dissimilar substances and ultimately lead to the development of new therapeutic agents for the treatment of inflammatory and proliferative diseases.[4,5] We describe herein the synthesis and preliminary biological evaluation of the most highly functionalized analogues of ingenol prepared to date, containing all of the oxygen functionality and unsaturation present in the natural product.[6,7] These new compounds are significantly more potent than our previously described analogue **3**[7j] and point to the significance of hydrophobic effects in the interaction of ingenol with the PKC receptor.

Chart 1



Results and Discussion

The conversion of the previously described photoaddition−fragmentation product **4**[7d] to **18**, the first ingenol analogue containing all of the unsaturation and oxygen functionality in the natural product, is outlined in Scheme 1. The first key transformation involves the regioselective introduction of the $\Delta^{5,6}$ unsaturation in **6**. While we had previously reported that selenation/oxidation of **4** led to the selective formation of the $\Delta^{6,7}$-unsaturated ester **5**, we have discovered that reaction of **4** with NBS/AIBN in refluxing carbon tetrachloride, followed by treatment of the derived mixture of α-bromoesters with excess

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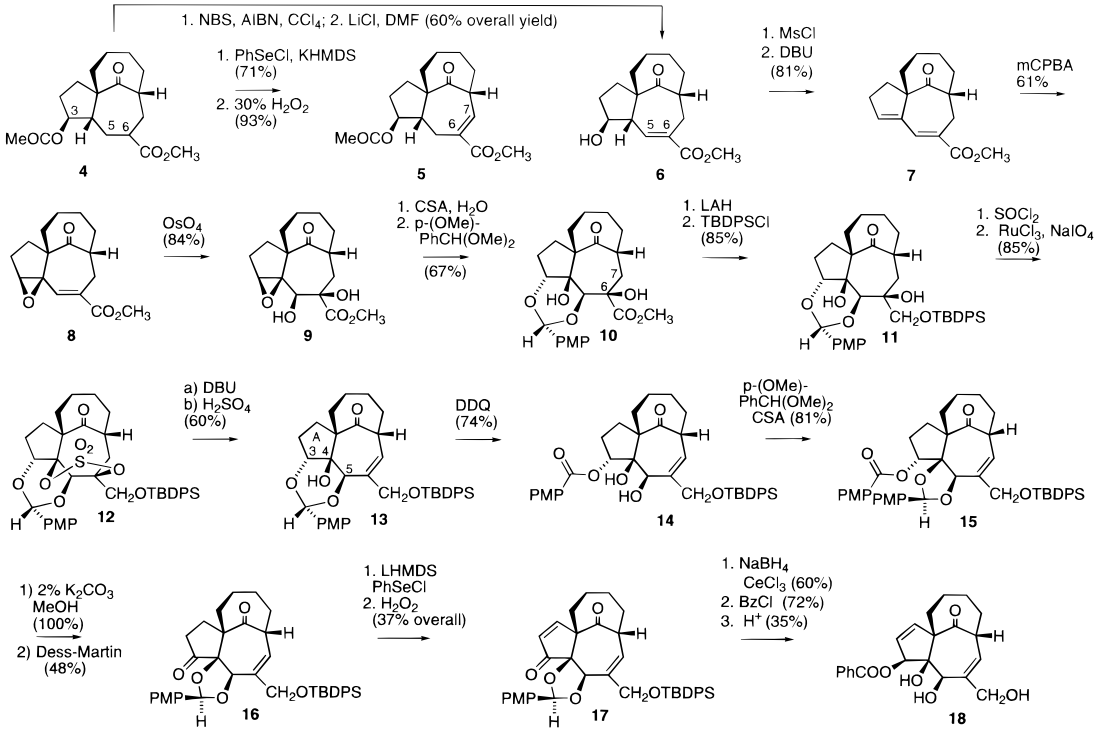
Scheme 1



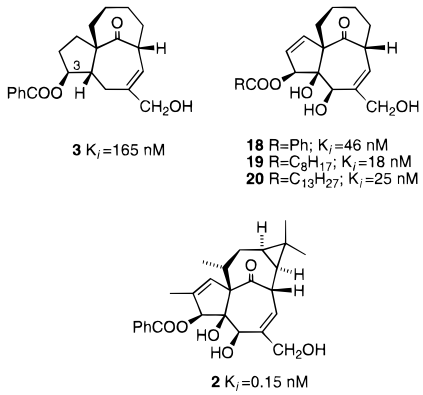
lithium chloride in refluxing DMF, leads to the exclusive formation of the $\Delta^{5,6}$-unsaturated ester **6** (with concomitant removal of the methyl carbonate).

While the direct functionalization of the $\Delta^{5,6}$ alkene by either epoxidation or dihydroxylation could not be realized, reaction of **6** with methanesulfonyl chloride followed by treatment with base led to the formation of the diene ester **7** in excellent yield. Epoxidation of **7** with *m*CPBA occurred with complete regio- and stereochemical control to give **8**. Treatment of the epoxy-unsaturated ester **8** with osmium tetroxide led to the exclusive formation of epoxy diol **9**. The difference in reactivity between **6** and **8** can be attributed to the change in conformation effected by the introduction of the $\Delta^{3,4}$ epoxide, reducing the steric hindrance about C-5 and leading to the formation of **9**. Reaction of epoxy diol **9** with camphorsulfonic acid in wet dichloromethane gave the corresponding tetraol, which on exposure to *p*-anisaldehyde dimethyl acetal produced **10** as a single isomer.

The selective elimination of the C-6 hydroxyl group to generate the requisite $\Delta^{6,7}$ unsaturation could not be achieved under a variety of reaction conditions on either the ester **10** or the derived silyl ether **11**. However, we found that reaction of the derived cyclic sulfate **12** with DBU led, after acidic workup, to the formation of the desired allylic alcohol **13**, thereby completing the functionalization of the B ring of ingenol. Oxidative cleavage of the PMP acetal of **13** led to the regioselective formation of the C-3 ester **14**, which on treatment with *p*-anisaldehyde dimethyl acetal gave the acetal **15**. Ester hydrolysis followed by oxidation gave the C-3 ketone **16**. Selenation and oxidation of **16** afforded enone **17**. Luche reduction of **17** proceeded stereoselectively to generate exclusively the C-3β alcohol, which upon benzoylation and sequential deprotection of the silyl ether and PMP acetal gave **18**.

The C-3 monobenzoate **18** was evaluated for its ability to interact with the regulatory site on protein kinase C, as quantitated by inhibition of [^{3}H]PDBU binding to protein kinase C-α reconstituted in the presence of 100 μg/mL phosphatidylserine and 0.1 mM Ca^{2+}, and incubated for 5 min at 37 °C.[7j] The curves for inhibition of [^{3}H]PDBU binding, obtained using a large excess of ligand, were consistent with a competitive mechanism. Under these conditions, ingenol 3-monobenzoate, **2** (R = PhCO), had yielded an apparent K_i of 0.15 ± 0.03 nM (mean ± SEM, n = 4) for protein kinase C-α, **3** had a K_i of 165 ± 21 nM (mean ± SEM, n = 3), and the more highly functionalized analogue **18** had a K_i of 46 ± 8 nM (mean ± SEM, n = 3).

Chart 2



These data establish the significance of the A ring unsaturation and the B ring oxygen functionalities, all of which are present in **18** but not in the previously described analogue **3** (Chart 2), on the binding affinity of ingenol to PKC. These compounds have been prepared as racemates, so the biological activity of the corresponding scalemic analogues is almost certainly greater than that reported here. While these synthetic efforts have resulted in a ca. 3-fold increase in binding affinity (46 nM for **18** vs 165 nM for **3**), there remains a ca. 10^2-fold difference between **18** and ingenol 3-monobenzoate, **2** (R = PhCO), indicating that the C-2 and C-11 methyl groups and the dimethylcyclopropane play an important role in the binding of ingenol to the regulatory domain of PKC.[8,9] These data do

not, however, distinguish between the role of the increased hydrophobicity and the increased rigidity imparted by these three substituents.

To probe the role of hydrophobicity on the binding affinity of ingenol to PKC, we have examined **19** and **20**, both of which contain more hydrophobic C-3 esters than **18**. We have found that the C-3 myristate **20** has a K_i of 25 ± 8 nM (mean ± SEM, $n = 3$) and that the C-3 nonanoate **19** has a K_i of 18 ± 3 nM (mean ± SEM, $n = 3$). These more hydrophobic esters are ca. 2−3 times more active than the C-3 benzoate **18** and, therefore, a full order of magnitude more potent than the previously described analogue **3**. Incremental introduction of the remaining functionalities of ingenol should lead to the establishment of the relative importance of conformational vs hydrophobic effects on the binding affinity of these ligands to PKC. The synthesis of such analogues is currently underway, and our results will be reported in due course.

Experimental Section

Unsaturated Ester 6. To a solution of ester **4** (919.8 mg, 2.720 mmol) in dry CCl_4 were added NBS (726 mg, 4.08 mmol) and AIBN (10 mg) at 25 °C. The mixture was heated to reflux under an Ar atmosphere. After 20 h, additional NBS (242 mg, 1.36 mmol) and AIBN (5 mg) were added to ensure complete consumption of the starting ester. After refluxing for a total of 24 h, the reaction mixture was cooled to 0 °C. The precipitated solid was removed by filtration, and the filtrate was concentrated to give 1.13 g of a mixture of α-bromo-esters (1/1.3 ratio by proton NMR). A mixture of the resulting crude α-bromo-esters (1.13 g) and LiCl (576 mg, 13.6 mmol) in dry DMF (27 mL) was heated to reflux for 2 h. The resulting solution was diluted with EtOAc (200 mL) and washed with brine (100 mL × 2). The organic layer was dried ($MgSO_4$), concentrated, and chromatographed (hexane/EtOAc = 2/1) to afford unsaturated ester **6** (452 mg, 60% for two steps) and **5** (49 mg, 5% for two steps) as an oil.

^{1}H NMR ($CDCl_3$, 500 MHz): δ 6.78 (dd, 1H, $J = 4.2$, 2.0 Hz), 3.96 (dd, 1H, $J = 10.9$, 5.3 Hz), 3.67 (s, 3H), 3.35 (dddd, 1H, $J = 9.4$, 9.4, 2.5, 2.5 Hz), 2.87 (d, 1H, $J = 3.6$ Hz), 2.61−2.53 (m, 1H), 2.40−2.30 (m, 2H), 2.12 (bs, 1H), 2.07−1.98 (m, 2H), 1.86−1.45 (m, 7H), 1.32 (ddd, 1H, $J = 12.7$, 6.1, 6.1 Hz), 1.18−1.10 (m, 1H). ^{13}C NMR ($CDCl_3$, 125 MHz): δ 213.89, 168.05, 142.17, 132.38, 80.55, 63.66, 58.48, 52.04, 48.80, 43.08, 33.36, 32.67, 29.94, 28.62, 27.94, 25.75. IR (neat, cm^{-1}): 3500, 2950, 2900, 1720. Exact mass calculated for $C_{16}H_{22}O_4$ (M^+), 278.1518; found, 278.1523.

Diene Ester 7. To a solution of the alcohol **6** (410 mg, 1.473 mmol) and triethylamine (0.62 mL, 4.42 mmol) in dry CH_2Cl_2 (7.4 mL) was added dropwise MsCl (0.17 mL, 2.21 mmol) at 0 °C under N_2 atmosphere. After stirring for 20 min at 25 °C, the reaction mixture was treated with saturated aqueous NH_4Cl and then diluted with EtOAc (100 mL), and the organic layer was washed with brine (2 × 20 mL). The resulting organic layer was dried ($MgSO_4$) and concentrated to give a quantitative yield of mesylate (525 mg) which was used in the next step without purification. A solution of mesylate (525 mg, 1.473 mmol) and DBU (0.45 mL, 2.95 mmol) in dry benzene (7.4 mL) was heated to reflux for 2 h. After cooling to ambient temperature, the reaction mixture was diluted with ether (70 mL) and washed with 5% HCl (30 mL), saturated aqueous $NaHCO_3$ (20 mL), and brine (20 mL). The resulting organic layer was dried ($MgSO_4$) and concentrated, and the resulting oil was chromatographed (hexane/EtOAc = 4/1) to afford diene ester **7** (312 mg, 81%) as an oil.

^{1}H NMR ($CDCl_3$, 500 MHz): δ 7.44 (s, 1H), 6.26 (bs, 1H) 3.71 (s, 3H), 3.47 (t, 1H, $J = 12.1$ Hz), 2.64−2.41 (m, 4H), 2.33 (ddd, 1H, $J = 17.7$, 8.0, 3.7 Hz), 2.12 (dd, 1H, $J = 14.3$, 7.7 Hz), 1.95−1.65 (m, 5H), 1.57 (dd, 1H, $J = 12.0$, 5.9 Hz), 1.49−1.42 (m, 1H), 1.12 (q, 1H, $J = 12.1$ Hz). ^{13}C NMR ($CDCl_3$, 125 MHz): δ 215.5, 168.75, 144.88, 143.70, 135.04, 129.19, 67.16, 52.05, 49.56, 34.79, 33.21, 32.31, 30.19, 30.03, 29.91, 25.85. IR (neat, cm^{-1}): 2973, 1736, 1708. Exact mass calculated for $C_{16}H_{20}O_3$ (M^+), 260.1412; found, 260.1407.

Epoxide 8. To a solution of the diene ester **7** (312 mg, 1.199 mmol) in dry CH_2Cl_2 (24 mL) was added *m*CPBA (70%, 443 mg, 1.80 mmol) and $NaHCO_3$ (302 mg, 3.6 mmol) at 25 °C. The resulting suspension was allowed to stir for 3 h at 25 °C and then treated with saturated aqueous $NaHSO_3$ (10 mL). The resulting mixture was diluted with ether (100 mL) and washed with saturated aqueous $NaHCO_3$ (20 mL) and brine (20 mL). The resulting organic layer was dried ($MgSO_4$), concentrated, and chromatographed (hexane/EtOAc = 4/1) to give epoxide **8** (168.1 mg, 61%) as a white solid.

^{1}H NMR ($CDCl_3$, 500 MHz): δ 6.29 (d, 1H, $J = 1.2$ Hz), 3.70 (s, 3H), 3.47 (d, 1H, $J = 3.3$ Hz), 3.42 (dddd, 1H, $J = 11.9$, 11.9, 2.6, 2.6 Hz), 2.86 (ddd, 1H, $J = 13.1$, 9.9, 9.9 Hz), 2.63−2.50 (m, 2H), 2.20 (dd, 1H, $J = 14.5$, 12.0 Hz), 2.04−1.30 (m, 9H), 1.01 (q, 1H, $J = 12.9$ Hz). ^{13}C NMR ($CDCl_3$, 125 MHz): δ 214.37, 167.31, 140.38, 136.89, 70.96, 70.70, 62.86, 52.25, 49.86, 34.99, 34.81, 32.77, 30.11, 29.69, 25.93, 25.41. IR (neat, cm^{-1}): 2930, 2853, 1716, 1614. Exact mass calculated for $C_{16}H_{20}O_3$ (M^+), 276.1362; found, 276.1350. Mp 115−116 °C.

Epoxydiol 9. To a stirred solution of the epoxy ester **8** (97 mg, 0.351 mmol) in 8 mL of THF, t-BuOH, and water (2:1:1) was added OsO_4 (0.45 mL, 4 wt % in H_2O, 0.07 mmol) followed by NMO (82 mg, 0.7 mmol) at 25 °C. The resulting mixture was allowed to stir at 25 °C for 18 h and was then washed with saturated aqueous $NaHSO_3$ (2 mL) and brine (25 mL) and then extracted with EtOAc (4 × 20 mL). The combined organic layers were dried ($MgSO_4$), concenterated, and chromatographed (hexane/EtOAc = 2/3) to give epoxy diol **9** (91 mg, 84%) as a white solid.

^{1}H NMR ($CDCl_3$, 500 MHz): δ 4.17 (t, 1H, $J = 12.0$ Hz), 3.75 (d, 1H, $J = 6.8$ Hz), 3.71 (s, 3H), 3.58 (s, 1H, OH), 3.18 (d, 1H, $J = 6.4$ Hz, OH), 3.15 (d, 1H, $J = 1.6$ Hz), 2.58 (ddd, 1H, $J = 15.6$, 8.6, 7.0 Hz), 2.43 (t, 1H, $J = 13.8$ Hz), 2.38 (t, 1H, $J = 13.3$ Hz), 2.00−1.44 (m, 9H), 1.23−1.18 (m, 1H), 0.94 (dq, 1H, $J = 14.7$, 2.1 Hz). ^{13}C NMR ($CDCl_3$, 125 MHz): δ 214.25, 174.93, 79.83, 78.14, 69.99, 63.63, 63.07, 53.14, 45.49, 38.44, 34.34, 33.30, 29.96, 29.77, 25.63, 25.34. IR (neat, cm^{-1}): 3468, 2938, 2856, 1733. Exact mass calculated for $C_{16}H_{23}O_6$ ($M^+ + H$), 311.1494; found, 311.1492. Mp 149−150 °C.

Acetal 10. A solution of epoxy diol **9** (830 mg, 2.70 mmol), water (90 μL, 5 mmol), and camphorsulfonic acid (46 mg, 0.2 mmol) in CH_2Cl_2 (50 mL) was allowed to stand at 25 °C for 1 h and was then treated with *p*-anisaldehyde dimethyl acetal (10 mL, 1.7 mL). The resulting solution was allowed to stand for 16 h at 25 °C and was then neutralized with triethylamine (0.2 mL). Concentration in vacuo followed by flash chromatography of the residue (hexane/EtOAc = 2/1) provided acetal **10** (810 mg, 67%) as a white solid.

^{1}H NMR ($CDCl_3$, 500 MHz): δ 7.26 (d, 2H, $J = 8.9$ Hz), 6.84 (d, 2H, $J = 8.7$ Hz), 5.85 (s, 1H), 3.78 (s, 1H), 4.23 (s, 1H), 3.95 (d, 1H, $J = 5.4$ Hz), 3.77 (s, 3H), 3.74 (s, 1H), 3.27 (dd, 1H, $J = 12.4$, 12.6 Hz), 3.10 (s, 3H), 2.51−2.41 (m, 2H), 2.20 (dd, 1H, $J = 13.1$, 13.1 Hz), 2.03−1.85 (m, 4H), 1.65−1.40 (m, 6H), 1.12 (dd, 1H, $J = 25.6$, 13.8 Hz). ^{13}C NMR ($CDCl_3$, 125 MHz): δ 215.67, 174.54, 161.50, 131.64, 127.73, 113.96, 98.42, 89.03, 86.49, 82.40, 70.58, 66.45, 55.73, 53.22, 46.50, 40.14, 31.25, 30.91, 30.51, 30.20, 29.59, 25.23. IR (neat, cm^{-1}): 3427, 2930, 1731. Exact mass calculated for $C_{24}H_{31}O_8$ ($M^+ + H$), 447.2018; found, 447.2025. Mp 82−84 °C.

Silyl ether 11. To a stirred solution of the ester **10** (728 mg, 1.63 mmol) in dry THF (7 mL) was added $LiAlH_4$ (124 mg, 3.26 mmol) in two portions over 0.5 h at 25 °C. After being stirred for an additional 0.5 h under Ar atmosphere, the reaction was quenched with water (0.5 mL), and the resulting mixture was treated with 0.5 mL of 1 N NaOH followed by water (1.5 mL). The resulting mixture was stirred for 1 h at 25 °C and then filtered through a Celite pad (EtOAc eluent). The filtrate was concentrated to afford the intermediate diol which was used without purification in the next step.

To a solution of the crude triol, triethylamine (2.1 mL, 15 mmol), and DMAP (12 mg, 0.1 mmol) in dry CH_2Cl_2 (20 mL) was added

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TBDPSCl (1.3 mL, 5 mmol) in three portions over 24 h at 25 °C. After standing for 24 h, the reaction was concentrated in vacuo, and the residue was treated with hexane and EtOAc (1/1). The resulting suspension was filtered through a small silica gel pad. Concentration of the filtrate and silica gel chromatography of the residue (hexane/EtOAc = 2/1) gave **11** (910 mg, 85%) as an oil.

^{1}H NMR ($CDCl_3$, 500 MHz): δ 7.52 (dd, 2 H, J = 1.4, 6.0 Hz), 7.51−7.30 (m, 8H), 7.09 (d, 2H, J = 8.6 Hz), 5.82 (s, 1H), 5.75 (bs, 1H), 3.96 (d, 1H, J = 5.3 Hz), 3.71 (s, 3H), 3.22 (s, 1H), 2.92 (d, 1H, J = 10.1 Hz), 2.48 (dd, 1H, J = 13.1 Hz), 2.40 (ddd, 1H, J = 6.0, 14.1, 14.1 Hz), 1.99−1.91 (m, 1H), 1.87−1.76 (m, 5H), 1.67−1.59 (m, 1H), 1.50−1.36 (m, 5H), 1.13−1.03 (m, 1H), 1.01 (s, 9H). ^{13}C NMR ($CDCl_3$, 125 MHz): δ 215.90, 159.73, 135.62, 135.46, 132.78, 132.73, 130.84, 129.90, 129.84, 128.78, 127.76, 127.05, 113.53, 97.51, 88.04, 87.47, 81.03, 70.24, 69.54, 65.96, 55.18, 45.78, 40.19, 31.36, 30.55, 30.26, 30.10, 28.97, 26.94, 24.88, 19.30. IR (neat, cm^{-1}): 3400, 2930, 1720.

Cyclic sulfate 12. To a solution of diol **11** (118 mg, 0.222 mmol) in dry pyridine (0.1 mL) and CH_2Cl_2 was added thionyl chloride (16 μL, 0.222 mmol). The resulting solution was then allowed to stir for 10 min at 25 °C under Ar atmosphere. The solution was diluted with EtOAc (25 mL) and was then washed with saturated aqueous $CuSO_4$ (15 × 2 mL) and water (15 mL). The resulting organic layer was dried ($MgSO_4$) and concentrated to give the crude cyclic sulfite which was submitted to the following oxidation conditions without further purification.

To a solution of the cyclic sulfite in CCl_4 (1 mL), acetonitrile (1 mL), and water (1 mL) was added $RuCl_3$ hydrate (2.1 mg, 0.01 mmol) followed by $NaIO_4$ (64.2 mg, 0.3 mmol) at 25 °C. The resulting biphasic soultion was allowed to stir for 3 h at 25 °C and then diluted with EtOAc (30 mL) and washed with brine (15 mL). The resulting organic layer was dried ($MgSO_4$), concentrated, and purified by chromatography (hexane/EtOAc = 4/1) to give cyclic sulfate **12** (117 mg, 85%).

^{1}H NMR ($CDCl_3$, 500 MHz): δ 7.58 (dd, 2H, J = 1.4, 8.0 Hz), 7.49 (dd, 2H, J = 1.4, 9.1 Hz), 7.42−7.40 (m, 2H), 7.10 (dd, 2H, J = 1.6, 6.8 Hz), 6.67 (d, 2H, J = 8.8 Hz), 5.86 (s, 1H), 4.32 (d, 1H, J = 2.2 Hz), 4.04 (d, 1H, J = 11.5 Hz), 3.73 (s, 3H), 3.67 (s, 1H), 3.55 (d, 1H, J = 11.6 Hz), 3.15 (dd, 1H, J = 12.4, 12.4 Hz), 2.50−2.43 (m, 1H), 2.33 (dd, 1H, J = 13.3, 13.3 Hz), 2.14 (dd, 1H, J = 1.7, 14.6 Hz), 2.06−2.00 (m, 2H), 1.98−1.90 (m, 2H), 1.84−1.81 (m, 1H), 1.77 (ddd, 1H, J = 5.3, 12.0, 17.2 Hz), 1.65−1.59 (m, 2H), 1.55−1.46 (m, 2H), 1.13−1.04 (m, 1H), 1.00 (s, 9H). ^{13}C NMR ($CDCl_3$, 125 MHz): δ 213.06, 160.12, 135.67, 135.52, 132.75, 132.24, 129.96, 129.81, 129.59, 127.83, 127.75, 127.09, 113.77, 99.70, 99.52, 97.14, 83.75, 67.35, 66.72, 65.31, 55.22, 45.77, 37.49, 32.54, 29.87, 29.83, 29.78, 29.67, 26.84, 24.86, 19.22. IR (neat, cm^{-1}): 2932, 2858, 1734. Exact mass calculated for $C_{39}H_{47}O_9SSi$ (M^+ + H), 719.2709; found, 719.2701. Mp 108−111 °C.

Alkene 13. A solution of cyclic sulfate **12** (730 mg, 0.197 mmol) and DBU (0.35 mL, 2.53 mmol) in toluene (20 mL) was heated to reflux for 2.5 h. After cooling to 25 °C, the resulting solution was treated with 1% aqueous H_2SO_4 in THF (5 mL, H_2O/THF = 1/9) and stirred for 1.5 h at 25 °C. The solution was diluted with EtOAc (150 mL) and washed with saturated aqueous $NaHCO_3$ (25 mL) and brine (10 mL). The organic layer was dried ($MgSO_4$), concentrated, and purified by column chromatography (hexane/EtOAc = 1/2) to give **13** (276 mg, 43%).

^{1}H NMR ($CDCl_3$, 500 MHz): δ 7.59 (dd, 2H, J = 1.3, 7.9 Hz), 7.55 (dd, 2H, J = 1.4, 8.0 Hz), 7.40−7.36 (m, 6H), 7.13 (dd, 2H, J = 1.8, 6.8 Hz), 6.64 (dd, 2H, J = 2.0, 8.7 Hz), 6.07 (bs, 1H), 5.64 (s, 1H), 4.25 (bs, 1H), 4.15 (d, 1H, J = 15.2 Hz), 4.08 (bd, 1H, J = 12.9 Hz), 3.92 (d, 1H, J = 6.3 Hz), 3.79 (d, 1H, J = 15.4 Hz), 3.73 (s, 3H), 3.12 (s, 1H), 2.42 (dd, 1H, J = 13.1, 13.1 Hz), 2.29 (ddd, 1H, J = 6.1, 6.1, 13.9 Hz), 2.06 (dddd, 1H, J = 6.2, 6.2, 14.0, 14.0 Hz), 1.92−1.77 (m, 4H), 1.31−1.44 (m, 3H), 1.14−1.05 (m, 1H), 1.00 (s, 9H). ^{13}C NMR ($CDCl_3$, 125 MHz): δ 209.42, 159.76, 135.53, 135.45, 135.31, 133.73, 133.58, 130.96, 129.51, 129.49, 127.60, 127.57, 126.97, 124.83, 113.51, 97.35, 88.24, 84.17, 71.74, 64.92, 64.22, 55.19, 48.15, 30.30, 29.84, 29.74, 29.19, 27.28, 26.86, 25.39, 19.22. IR (neat, cm^{-1}): 3454, 2931, 2856, 1731. Exact mass calculated for $C_{39}H_{46}O_6SiNa$ (M^+ + Na), 661.2961; found, 661.2954. Mp 151−153 °C.

PMB Ester 14. A solution of acetal **13** (265 mg, 0.414 mmol) and DDQ (141 mg, 0.621 mmol) in methylene chloride (4 mL) and water (0.2 mL) was stirred at 25 °C for 7 h. Concentration of the resulting mixture in vacuo followed by flash chromatography of the residue (hexane/EtOAc/CH_2Cl_2 = 1/1/0.1) provided 265 mg (98% combined yield) of anisate **14** contaminated with the corresponding C-5 ester **14a** in a 3:1 ratio.

^{1}H NMR ($CDCl_3$, 500 MHz, major isomer): δ 7.65 (d, 2 H, J = 7.0 Hz), 7.46−7.27 (m, 10H), 6.80 (d, 2H, J = 8.8 Hz), 5.88 (d, 1H, J = 6.4 Hz), 5.04 (d, 1H, J = 3.6 Hz), 4.77 (dd, 1H, J = 6.4, 12.3 Hz), 4.26 (d, 1H, J = 2.3 Hz), 3.95 (s, 1H), 3.86 (s, 1H), 3.76 (s, 3H), 3.70 (d, 1H, J = 12.8 Hz), 3.66 (d, 1H, J = 12.8 Hz), 2.90−2.85 (m, 1H), 2.50−2.39 (m, 2H), 1.99−1.57 (m, 6H), 1.51−1.37 (m, 2H), 1.18−1.106 (m, 1H), 0.92 (s, 9H). ^{13}C NMR (CDC_3, 125 MHz, mixture of isomers): δ 207.81, 165.33, 163.37, 141.58, 135.84, 135.57, 135.33, 135.29, 133.05, 132.66, 131.80, 131.68, 129.91, 129.87, 129.82, 129.73, 128.27, 127.81, 127.76, 127.70, 122.16, 114.01, 113.60, 85.56, 84.37, 77.15, 75.42, 68.03, 67.48, 55.53, 55.30, 47.06, 35.92, 30.21, 29.32, 28.54, 26.76, 26.67, 25.15, 19.09. IR (neat, cm^{-1}): 3449, 2933, 1714. Exact mass calculated for $C_{39}H_{46}O_7SNa$ (M^+ + Na), 667.2910; found, 667.2925.

PMP Acetal 15. To a solution the C-3 and C-5 anisates **14** and **14a** (260 mg, 0.397 mmol, 3:1 mixture) in methylene chloride under Ar was added *p*-anisaldehyde dimethyl acetal (0.270 mL, 289 mg, 1.59 mmol). Camphorsulfonic acid (10 mg, 0.040 mmol) was added, and the mixture was stirred at 25 °C under Ar for 12 h. The reaction was then neturalized by the addition of Et_3N (10 μL). The resulting mixture was concentrated under reduced pressure and purified by silica gel chromatography (hexane/EtOAc = 7/1) to yield the acetal **15** (178 mg, 81% based on 75% purity of starting material) as a white solid (mp 200−201 °C).

^{1}H NMR ($CDCl_3$, 500 MHz): δ 0.92 (s, 9H), 1.01−1.10 (m, 1H), 1.23−1.28 (m, 2H), 1.41−1.45 (m, 1H), 1.71 (dd, J = 7.5, 14.3 Hz, 1H), 1.82−1.87 (m, 2H) 1.91−2.05 (m, 3H), 2.37 (dd, J = 12.2, 14.0 Hz, 1H), 2.94 (ddd, J = 7.4, 13.2, 13.2 Hz, 1H), 3.74−3.78 (m, 4H), 3.92 (d, J = 14.5, 1H), 4.17 (s, 1H), 4.38 (dd, J = 6.9, 11.7, 1H), 5.13 (d, J = 2.4 Hz, 1H), 5.68 (s, 1H), 6.36 (d, J = 6.6 Hz, 1H), 6.81 (d, J = 8.8 Hz, 2H), 6.95 (d, J = 8.6 Hz, 2H), 7.18−7.26 (m, 4H), 7.31 (d, J = 5.5 Hz, 2H), 7.35 (d, J = 7.2 Hz, 2H), 7.41 (d, J = 7.0 Hz, 2H), 7.52 (d, J = 8.6 Hz, 2H), 7.73 (d, J = 8.8 Hz, 2H). ^{13}C NMR ($CDCl_3$, 125 MHz): δ 19.22, 24.75, 26.70, 27.18, 28.28, 29.83, 30.37 37.08, 48.31, 55.35, 65.58, 66.45, 83.24, 83.56, 92.11, 103.04, 113.88, 113.92, 121.80, 127.56, 127.64, 127.94, 128.22, 129.54, 129.60, 129.74, 131.90, 133.09, 133.28, 135.28, 135.32, 135.63, 160.65, 163.60, 165.19, 208.52. IR (neat, cm^{-1}): 2932, 2856, 1721. Exact mass calculated for $C_{47}H_{52}O_8SiNa$ (M^+ + Na), 795.3329; found, 795.3324.

Ketone 16. To a solution of the anisate **15** (170 mg, 0.232 mmol) in 4:1 methanol:THF (50 mL) was added K_2CO_3, and the resulting solution was allowed to stir at 25 °C for 12 h. The solution was then poured into $CHCl_3$ (200 mL) and diluted with water (100 mL), and the separated aqueous layer was washed with $CHCl_3$ (2 × 200 mL). The combined organic extracts were dried ($MgSO_4$), filtered, and concentrated under reduced pressure. The resulting yellow oil was purified by silica gel chromatography (EtOAc/THF/MeOH = 81/14/5/2) to yield the deprotected alcohol (153 mg, >100%).

^{1}H NMR ($CDCl_3$, 500 MHz): δ 1.04 (s, 9H), 1.22 (dd, J = 8.6, 13.6 Hz, 1H), 1.33−1.36 (m, 1H), 1.58−1.63 (m, 2H), 1.65−1.76 (m, 5H), 1.83−1.95 (m, 2H), 2.25 (dd, J = 11.9, 14.3 Hz, 1H), 2.92 (ddd, J = 7.2, 13.2, 13.2 Hz, 1H), 3.82 (s, 3H), 3.90 (d, J = 5.0 Hz, 1H), 4.16 (s, 2H), 4.33 (dd, J = 7.0, 12.5 Hz, 1H), 4.58 (s, 1H), 5.69 (s, 1H), 6.20 (d, J = 6.7 Hz, 1H), 6.94 (d, J = 8.7 Hz, 2H), 7.34−7.42 (m, 6H) 7.49 (d, J = 8.7 Hz, 2H), 7.63−7.65 (m, 4H). ^{13}C NMR ($CDCl_3$, 125 MHz): δ 19.20, 24.69, 26.74, 26.84, 29.60, 29.99, 30.29, 37.25, 48.34, 55.30, 64.93, 67.13, 81.41, 82.76, 93.24, 102.94, 113.85, 127.72, 127.74, 128.04, 128.37, 129.75, 129.78, 131.75, 133.10, 135.46, 135.61, 135.65, 160.48, 209.17. IR (neat, cm^{-1}): 3486, 2932, 1723.

To a solution of the forementioned C-3α-alcohol (150 mg, 0.234 mmol) in methylene chloride (3 mL) at 25 °C was added the Dess−Martin periodinane reagent (130 mg, 0.304 mmol). After 10 min, an additional 130 mg of the Dess−Martin reagent was added. Over the next 4 h, an additional 550 mg of the Dess−Martin reagent was added

in portions. The resulting mixture was then treated with isopropyl alcohol (0.15 mL) followed by NaOH (1.5 M, 5 mL), and the resulting mixture was stirred for 10 min. The reaction mixture was diluted with EtOAc (15 mL) and washed with NaOH (1.5 M, 5 mL). The organic layer was dried ($MgSO_4$), filtered, and concentrated under reduced pressure. The crude product was purified by silica gel chromatography (hexane/EtOAc/MeOH = 5/1/0.1) to yield the pure ketone (72 mg, 48%).

^{1}H NMR ($CDCl_3$, 500 MHz): δ 1.01 (s, 9H) 1.03−1.10 (m, 1H), 1.32−1.43 (m, 1H), 1.50 (ddd, J = 7.7, 12.6, 12.6 Hz, 1H), 1.65 (dd, J = 7.7, 14.8 Hz, 1H), 1.79−1.88 (m, 4H), 2.24−2.35 (m, 2H), 2.42 (ddd, J = 2.2, 7.6, 15.7 Hz, 1H), 2.67 (ddd, J = 2.3, 8.9, 12.6 Hz, 1H), 3.82 (s, 3H), 4.06 (*AB*, J = 1.4, 13.5 Hz, 2H), 4.39 (dd, J = 6.7, 11.5 Hz, 1H), 4.68 (s, 1H), 6.01 (s, 1H), 6.19 (d, J = 6.02 Hz, 1H), 6.95 (d, J = 8.8 Hz, 2H), 7.34−7.39 (m, 6H), 7.49 (d, J = 8.7 Hz, 2H), 7.58−7.62 (m, 4H). ^{13}C NMR ($CDCl_3$, 125 MHz): δ 19.60, 25.06, 27.07, 27.14, 28.08, 30.50, 36.59, 37.42, 49.10, 55.73, 64.70, 67.08, 84.94, 85.62, 105.13, 114.32, 128.13, 128.14, 128.26, 128.41, 130.14, 130.15, 132.94, 133.55, 133.60, 135.25, 135.98, 161.02, 208.12, 216.35. IR (neat, cm^{-1}): 2931, 1749, 1724. Exact mass calculated for $C_{39}H_{44}O_6$-SiNa (M^+ + Na), 659.2805; found, 659.2827.

Enone 17. A solution of **16** (8.8 mg, 0.0138 mmol) in THF (1 mL) was cooled to −78 °C while stirring under Ar, treated with LHMDS (1.0 M in THF, 0.041 mL, 0.041 mmol), and stirred at −78 °C for 0.5 h. Phenylselenyl chloride (8 mg, 0.041 mmol) was added, and the mixture was stirred at −78 °C for 1.5 h. The reaction mixture was then treated with saturated NH_4Cl (0.5 mL) and allowed to warm to 25 °C. Brine (5 mL) was added, and the resulting mixture was extracted with EtOAc (15 + 5 mL). The organic extracts were dried ($MgSO_4$), filtered, and concentrated under reduced pressure to give the crude selenide, which was dissolved in methylene chloride (1 mL) and treated with an aqueous 30% H_2O_2 solution (100 μL). The resulting mixture was stirred at 25 °C for 0.75 h, and excess H_2O_2 was quenched with solid $NaHSO_3$ at 0 °C. The resulting mixture was diluted with brine (5 mL) and extracted with EtOAc (2 × 10 mL). The combined organic extracts were dried ($MgSO_4$), filtered, and concentrated, and the residue was purified by silica gel chromatography (hexane/EtOAc = 90/10) to yield the enone **17** (2.9 mg, 37% brsm) and recovered ketone **16** (1 mg).

^{1}H NMR ($CDCl_3$, 500 MHz): δ 0.99 (s, 9H), 1.30−1.34 (m, 1H), 1.68 (dd, J = 7.7, 14.8 Hz, 1H), 1.71−1.93 (m, 6H), 2.45 (dd, J = 11.1, 14.9 Hz, 1H), 3.81 (s, 3H), 4.05−4.11 (m, 2H), 4.11−4.18 (m, 1H), 4.91 (s, 1H), 6.06 (s, 1H), 6.16 (d, J = 6.7 Hz, 1H), 6.29 (d, J = 6.3 Hz, 1H), 6.93 (d, J = 8.7 Hz, 2H), 7.33−7.38 (m, 5H), 7.41−7.59 (m, 3H), 7.54−7.59 (m, 4H), 7.74 (d, J = 6.2 Hz, 1H). ^{13}C NMR ($CDCl_3$,125 MHz): δ 19.21, 24.84, 25.70, 26.72, 29.78, 31.87, 49.26, 55.33, 66.61, 67.08, 81.59, 82.49, 103.69, 127.52, 128.28, 129.70, 130.21, 130.59, 133.07, 135.27, 135.52, 135.58, 136.51, 160.20, 166.20, 205.65, 205.91. Exact mass calculated for $C_{39}H_{46}O_6SN$ ($M^+ + NH_4^+$), 652.3094; found, 652.3080.

Benzoate 18. A solution of **17** (2.7 mg, 0.0043 mmol) in methanol (1 mL) was cooled to 0 °C. Cerium(III) chloride heptahydrate (5 mg, 0.014 mmol) was added, and the mixture was stirred for 15 min. Sodium borohydride (0.5 mg, 0.013 mmol) was added, and the mixture was stirred for 15 min before an equal additional amount of $NaBH_4$ was added. After being stirred for an additional 30 min, the reaction mixture was treated with acetone (0.25 mL) and filtered through a silica gel pad with EtOAc. The eluent was concentrated and purified by silica gel chromatography (hexane/EtOAc = 4/1) to yield the allylic alcohol (1.8 mg, 60%).

The allylic alcohol (1.8 mg, 0.0028 mmol) was dissolved in methylene chloride (1 mL); benzoyl chloride (4 μL, 5 mg, 0.03 mmol) and 4-(dimethylamino)pyridine (15 mg, 0.123 mmol) were added. The reaction was stirred at room temperature for 0.5 h and was concentrated, filtered and purified by silica gel chromatography (hexane/EtOAc = 90/10) to yield the benzoylated product (1.5 mg, 72%).

^{1}H NMR ($CDCl_3$, 500 MHz): δ 0.95−1.01 (m, 9H), 1.28−1.37 (m, 1H), 1.45−1.50 (m, 1H), 1.72−1.78 (m, 4H), 1.83−1.92 (m, 3H), 2.48 (dd, J = 10.7, 14.7 Hz, 1H), 3.81 (s, 3H), 4.15 (s, 2H), 4.33−4.39 (m, 1H), 4.53 (s, 1H), 5.71 (s, 1H), 5.84 (s, 1H), 6.06 (dd, J = 2.0, 6.3 Hz, 1H) 6.11−6.13 (m, 2H), 6.88 (d, J = 8.7 Hz, 2H), 7.30−7.43 (m, 9H) 7.57−7.61 (m, 6H) 8.08 (d, J = 8.0 Hz, 1H). ^{13}C NMR ($CDCl_3$, 125 MHz): δ 19.23, 25.12, 25.80, 26.77, 29.44, 33.98, 47.91, 55.31, 66.53, 68.97, 80.96 82.10, 89.91, 103.85, 113.76, 127.69, 127.91, 128.50, 128.92, 129.38, 129.67, 129.79, 129.97, 133.26, 135.54, 135.60, 136.35, 138.16, 160.40, 165.92, 207.59. IR (neat, cm^{-1}): 2930, 1722. Exact mass calculated for $C_{46}H_{48}O_7SiNa$ (M^+ + Na), 763.3067; found, 763.3081.

A 3% HCl solution in methanol was prepared by the addition of 1 mL of acetyl chloride to 25 mL of dry methanol. To a solution of the aforementioned benzoate (1.5 mg, 0.0020 mmol) in diethyl ether (0.6 mL) was added the 3% HCl/methanol solution (0.3 mL). The mixture was stirred for 4.5 h, neutralized by the addition of solid $NaHCO_3$ (20 mg), and then diluted with 1 mL of EtOAc. The solid was removed by filtration through a silica pad, the eluent was concentrated, and the residue was purified by silica gel chromatography (hexane/EtOAc/MeOH = 1/1/0.1) to give the deprotected ingenane benzoate **18** (0.271 mg, 35%).

^{1}H NMR ($CDCl_3$, 500 MHz): δ 1.30−1.38 (m, 1H), 1.42−1.50 (m, 1H), 1.56−1.88 (m, 4H), 1.92−1.95 (m, 1H), 2.03 (dd, J = 6.0, 6.0 Hz, 1H), 2.48 (dd, J = 11.0, 14.3 Hz, 1H), 3.51 (s, 1H), 4.00 (d, J = 5.9 Hz, 1H), 4.13−4.22 (m, 4H), 5.95 (d, J = 4.0 Hz, 1H), 5.99−6.03 (2H), 6.45 (d, J = 6.2 Hz, 1H), 7.45 (dd, J = 7.8, 7.8 Hz, 2H), 7.58 (dd, J = 7.4, 7.4 Hz, 1H), 8.00 (d, J = 8.0 Hz, 2H). IR (thin film, cm^{-1}): 3443, 2929, 1716. Exact mass calculated for $C_{22}H_{24}O_6Na$ (M^+ + Na), 407.1471; found, 407.1486.

Nonanoate 19. ^{1}H NMR ($CDCl_3$, 500 MHz, mixture of isomers): δ 0.85−0.87 (m, 6H), 1.16−1.32 (m, 26H), 1.53−1.90 (m, 14H), 2.26−2.42 (m, 6H), 2.85 (d, J = 10.2 Hz, 1H), 3.41 (s, 1H), 3.78−3.81 (m, 2H), 4.04 (bs, 2H), 4.13−4.19 (m, 4H), 4.48 (d, J = 12.2 Hz, 1H), 4.71 (d, J = 12.2 Hz, 1H), 4.75 (dd, J = 2.6, 7.1 Hz, 1H), 5.69 (d, J = 2.8 Hz, 1H), 5.91−5.94 (m, 2H), 5.98 (dd, J = 2.6, 6.3 Hz, 1H), 6.02 (d, J = 4.7 Hz, 1H), 6.28 (d, J = 6.3 Hz, 1H), 6.36 (d, J = 6.3 Hz, 1H). IR (thin film, cm^{-1}): 3415, 2925, 2853, 1731. Exact mass calculated for $C_{24}H_{36}O_6Na$ (M^+ + Na), 443.2410; found, 443.2402.

Myristate 20. ^{1}H NMR ($CDCl_3$, 500 MHz): δ 0.85−0.88 (m, 3H), 1.13−1.31 (m, 22H), 1.57−2.00 (m, 7H), 2.32−2.38 (m, 3H), 3.41 (s, 1H), 3.75−3.80 (m, 1H), 4.03 (bs, 1H), 4.14−4.19 (m, 4H), 5.69 (d, J = 2.8 Hz, 1H), 5.94−5.91 (m, 2H), 6.36 (d, J = 6.3 Hz, 1H). IR (thin film, cm^{-1}): 3401, 2907, 2848, 1725. Exact mass calculated for $C_{29}H_{46}O_6Na$ (M^+ + Na), 513.3192; found: 513.3199.

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Supporting Information Available: Details of the analysis of the ligand binding studies (2 pages, print/PDF). See any current masthead page for ordering information and Web access instructions.

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