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Regioselective Synthesis of Water-Soluble Monophosphate Derivatives of Combretastatin A-1

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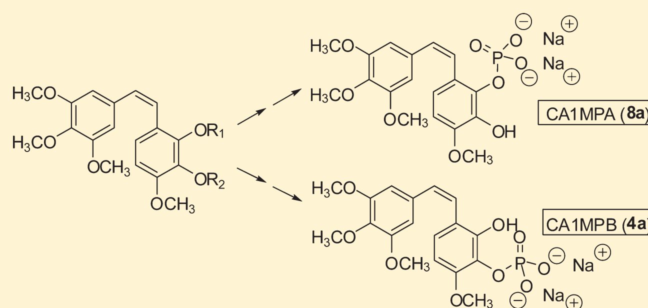
Supporting Information

ABSTRACT: The natural products combretastatin A-4 (CA4) and combretastatin A-1 (CA1) are potent cancer vascular disrupting agents and inhibitors of tubulin assembly (IC_{50} = 1−2 μM). The phosphorylated prodrugs CA4P and CA1P are undergoing human clinical trials against cancer. CA1 is unique due to its incorporation of a vicinal phenol, which has afforded the opportunity to prepare both diphosphate and regioisomeric monophosphate derivatives. Here, we describe the first synthetic routes suitable for the regiospecific preparation of the CA1-monophosphates CA1MPA (**8a**/**b**) and CA1MPB (**4a**/**b**). The essential regiochemistry necessary to distinguish between the two vicinal phenolic groups was accomplished with a tosyl protecting group strategy. Each of the four monophosphate analogues (including *Z* and *E* isomers) demonstrated in vitro cytotoxicity against selected human cancer cell lines comparable to their corresponding diphosphate congeners. Furthermore, *Z*-CA1MPA (**8a**) and *Z*-CA1MPB (**4a**) were inactive as inhibitors of tubulin assembly (IC_{50} > 40 μM), as anticipated in this pure protein assay.



Combretastatin A-1 (CA1)[1] and combretastatin A-4 (CA4)[2−4] are both *Z*-stilbenoid natural products originally isolated from the African bush willow tree, *Combretum caffrum Kuntze* (Combretaceae). CA1 and CA4 are remarkably potent against human cancer cell lines (in vitro)[5] and are strongly inhibitory against the polymerization of tubulin into microtubules.[6,7] Formulated as phosphate prodrugs [(CA1P, OXi4503)[8,9] and (CA4P, Zybrestat, fosbretabulin)[10]] to increase aqueous solubility, these compounds are currently under investigation in human clinical trials as anticancer drugs.[11−21] CA1P and CA4P fall into a relatively new grouping of compounds collectively referred to as vascular disrupting agents (VDAs).[14,22−24] Mechanistically distinct from antiangiogenic agents, VDAs are characterized by their ability to selectively damage existing microvasculature[25] feeding a tumor, thus starving that tumor of oxygen and required nutrients.[26−30] Interestingly, while CA4 (*Z*) is generally more potent than CA1 (*Z*) against human cancer cell lines (in vitro), the corresponding prodrug CA4P (*Z*) is somewhat less active than CA1P (*Z*) in certain in vivo preclinical tumor growth delay studies carried out in severe combined immunodeficiency (SCID) mice.[31,32] CA1 (*Z*) showed more consistent results against murine P388 leukemia in vitro, and CA1 (*Z*), in preclinical development, showed higher vascular disruption and antitumor activity than CA4 (*Z*).[31] The increased effectiveness of CA1 (*Z*), compared to CA4 (*Z*), may be attributed to the presence of the second hydroxy substituent, which facilitates formation of the highly reactive *ortho* quinone analogue, obtainable through biological oxidation of the 1,2-diol functionality present in CA1 (*Z*).[33,34]

Recent studies by Kirwan et al. have shown that the additional phosphate group present in CA1P, as compared to CA4P, results in the formation of numerous metabolites of CA1, several of which have been identified as monophosphates, monoglucuronides, and a bis-glucuronide.[35] Partial enzymatic dephosphorylation of CA1P may lead to two regioisomeric CA1-monophosphates [combretastatin A-1 monophosphate A (CA1MPA) and combretastatin A-1 monophosphate B (CA1MPB), Figure 1] in addition to formation of the active drug

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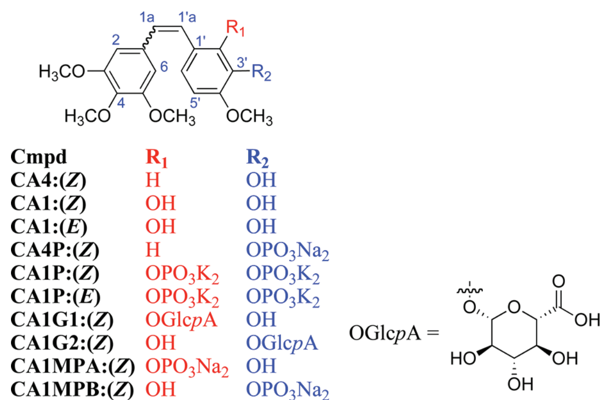

Figure 1. Combretastatin A-1 (CA1) and combretastatin A-4 (CA4) derivatives.

CA1.[35] Each of the monophosphates (CA1MPA **8a**(*Z*), **8b**(*E*) and CA1MPB **4a**(*Z*), **4b**(*E*)) has distinct chemical properties.[36] Since the regioisomeric monophosphate salts are structurally distinct from both CA1P and CA1, it is anticipated that they may have different activity profiles in biological systems.

RESULTS AND DISCUSSION

The synthesis of the stilbenoid core of CA4 and CA1, along with numerous derivatives, has been efficiently achieved using the Wittig approach.[4,32,33] The regiospecificity necessary for the synthesis of the CA1-monophosphates (Scheme 1) was accomplished by distinguishing between the two vicinal phenolic functional groups at C-2′ and C-3′ of stilbenes **1a**/**b** and **5a**/**b** with a selective tosyl protecting group strategy.[37] The phosphorylation of monophenols **1a**/**b** and **5a**/**b** was achieved with dibenzylphosphite as the reagent of choice due to its high reactivity, which resulted in good yields. Deprotection of the resultant benzyloxy phosphate esters **2a**/**b** and **6a**/**b** with TMSBr followed by treatment with MeONa provided the corresponding CA1-disodium phosphate salts **3a**/**b** and **7a**/**b**. Removal of the tosyl group was achieved using NaOH (2 M) in a microwave reactor at a moderate temperature (50 °C) to afford CA1MPA **8a**(*Z*), CA1MPA **8b**(*E*), CA1MPB **4a**(*Z*), or CA1MPB **8b**(*E*). In addition to characterization of the intermediates and final compounds by ^{1}H and ^{13}C NMR and HRMS data, the phosphorus-containing compounds were further characterized by their ^{31}P NMR data. Compounds **4a**, **4b**, **8a**, and **8b** each showed a singlet in their respective ^{31}P NMR spectrum at ca. −6.2 ppm, a characteristic of phosphoric acid triesters. On conversion of these phosphoric acid triesters to their respective sodium salts, each of them showed a downfield shift of ∼9 ppm in their ^{31}P NMR spectrum. HPLC studies were carried out on each of the *Z*-monophosphates (**4a**, **8a**) to provide further confirmation of their chemical purity. Under these HPLC conditions neither dephosphorylation, *Z*/*E* isomerization, nor intramolecular phosphate migration was observed (see Supporting Information).

BIOLOGICAL EVALUATION

The cytotoxicity of the four CA1-monophosphates CA1MPA (**8a**,**b**) and CA1MPB (**4a**,**b**) was evaluated using a panel of three human cancer cell lines, prostate (DU-145), ovarian (SK-OV-3), and lung (NCI-H460), with doxorubicin as a reference compound. This procedure was based on the standard sulforhodamine B (SRB) assay.[38,39] The GI_{50} values are shown in Table 1. The *Z*-series CA1-monophosphates (**4a** and **8a**) showed enhanced cytotoxicity compared to the corresponding *E*-series regioisomers (**4b** and **8b**). This reflects the increased cytotoxicity of *Z*-CA1 compared to *E*-CA1. The *Z*-series CA1-monophosphates (**4a** and **8a**) were evaluated for their ability to inhibit tubulin assembly and were found to be inactive (IC_{50} > 40 μM), which is consistent with the results obtained with CA1P.

EXPERIMENTAL SECTION

General Experimental Procedures. Dichloromethane, acetonitrile, and tetrahydrofuran (THF) were used in their anhydrous forms, as obtained from the chemical suppliers. Reactions were performed under an inert atmosphere using nitrogen gas, unless specified. Thin-layer chromatography (TLC) plates (precoated glass plates with silica gel 60 F_{254}, 0.25 mm thickness) were used to monitor reactions. Purification of intermediates and products was carried out with a flash purification system using silica gel (200−400 mesh, 60 Å) or RP-18 prepacked columns. Intermediates and products synthesized were characterized on the basis of their ^{1}H NMR (500 MHz), ^{13}C NMR (125 MHz), ^{31}P NMR (202 MHz), gHSQC, and gHMBC spectroscopic data. TMS was used as an internal standard for spectra recorded in $CDCl_3$. For spectra recorded in D_2O: δ ^{1}H 4.80. All the chemical shifts are expressed in ppm (δ), coupling constants (*J*) are presented in Hz, and peak patterns are reported as broad (br), singlet (s), doublet (d), triplet (t), quartet (q), septet (sept), and multiplet (m). HRESIMS were obtained using (+ve or −ve) electrospray ionization (ESI) techniques. Purity of the final compounds was further analyzed at 25 °C using an Agilent 1200 HPLC system with a diode-array detector (λ = 190−400 nm), a Zorbax XDB-C18 HPLC column (4.6 mm × 150 mm, 5 μm), and a Zorbax reliance cartridge guard-column; eluents, solvent A, 0.1% TFA in H_2, solvent B, 0.08% TFA in acetonitrile−H_2 (80:20 (v/v) ratio); gradient, 80% A/20% B over 0 to 5 min; 80% A/20% B → 5% A/95% B over 5 to 35 min; 5% A/95% B over 35 to 45 min; post-time 15 min; flow rate 1.0 mL/min; injection volume 20 μL; monitored at wavelengths of 254, 264, 280, and 300 nm.

(*Z*)-1-[3′,4′,5′-Trimethoxyphenyl]-2-[2″-[(*para*-toluenesulfonyl)oxy]-3″-[([bis[(benzyl)oxy]]phosphoryl)oxy]-4″-methoxyphenyl]ethene (2a). Phenol **1a**[37] (0.120 g, 0.247 mmol) was dissolved in acetonitrile (10 mL) and cooled to −10 °C. CCl_4 (0.20 mL, 2.1 mmol) was added, and the reaction mixture was stirred for 5 min. Diisopropylethylamine (0.40 mL, 2.3 mmol) and DMAP (0.065 g, 0.532 mmol) were added, and the reaction mixture was stirred for an additional 10 min. Dibenzylphosphite (0.30 mL, 1.4 mmol) was added slowly to the reaction mixture, which was then stirred for 45 min and monitored by TLC. On completion, KH_2PO_4 (25 mL) was added, and the reaction mixture was allowed to return to ambient temperature. H_2O (25 mL) was added to the reaction mixture, and the organic phase was separated. The aqueous phase was extracted with EtOAc (3 × 10 mL), and the combined organic phases were dried with Na_2SO_4, filtered, and concentrated under reduced pressure. Flash chromatography of the crude product using a prepacked 10 g silica column [eluents; solvent A, EtOAc, solvent B, hexanes; gradient, 40% A/60% B over 1.15 min (1 CV), 40% A/60% B → 10% A/90% B over 12.30 min (10 CV), 10% A/90% B over 3.07 min (2.5 CV); flow rate 12.0 mL/min; monitored at 254 and 280 nm] afforded **2a** (0.12 g, 0.59 mmol, 67%) as a yellow oil: ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.77 (2H, d, *J* = 8.4 Hz, H-2″, H-6″), 7.40−7.30 (10H, m, Ph), 7.16 (2H, d, *J* = 8.4 Hz, H-3″, H-5″), 7.00 (1H, d, *J* = 8.8 Hz, H-6′), 6.71 (1H, d, *J* = 8.8 Hz, H-5′), 6.47 (2H, s, H-2, H-6), 6.36 (1H, d, *J* = 16.2 Hz, H-1a), 6.30 (1H, d, *J* = 16.2 Hz, H-1′a), 5.12 (4H, m, OCH_2Ph), 3.81 (3H,

Scheme 1. Synthesis of *Z*/*E*-CA1-2′-monophosphates and *Z*/*E*-CA1-3′-monophosphates

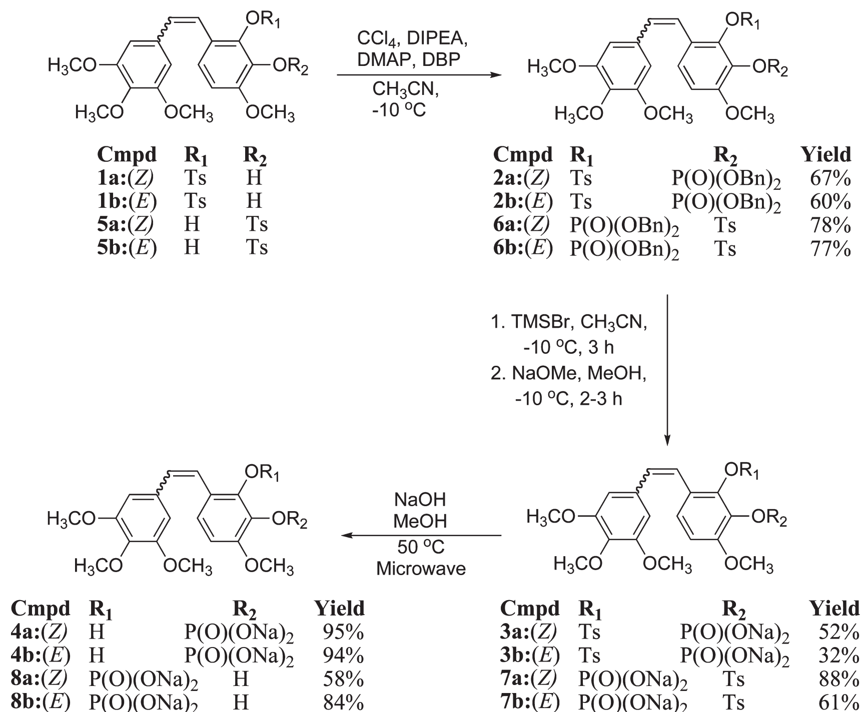

Table 1. Cytotoxicity of CA1-monophosphate Analogues and Related Compounds against Human Cancer Cell Lines SK-OV-3, NCI-H460, and DU-145 and Inhibition of Tubulin Polymerization

	GI_{50} (μM) SRB assay[a]			inhibition of tubulin polymerization IC_{50} (μM)
compd	SK-OV-3	NCI-H460	DU-145	
CA1 (*Z*)[b]	0.0384 ± 0.0242	0.0153 ± 0.0158	0.0326 ± 0.0173	1.9[c]
CA1 (*E*)[d]	2.27	1.32	2.14	11 ± 0.9
8a (*Z*)	0.00164 ± 0.000700	0.00356 ± 0.000267	0.00277 ± 0.000884	>40
8b (*E*)	0.465 ± 0.0597	1.55 ± 1.30	2.13 ± 1.37	nd[e]
4a (*Z*)	0.00260 ± 0.0000403	0.0334 ± 0.00206	0.0155 ± 0.00836	>40
4b (*E*)	0.681 ± 0.177	0.336 ± 0.0856	0.520 ± 0.302	nd[e]
CA1P (*Z*)[f]	0.00103	0.0133	0.00287	>40[g]

[a] Average of $n \geq 3$ independent determinations. [b] See ref 7 for additional data. This batch of CA1 was synthesized by the current authors using the method found in ref 1. [c] See ref 40. [d] See ref 41. [e] nd = not determined. [f] See ref 42. [g] See ref 43 for additional data.

s, OCH_3-4), 3.75 (3H, s, OCH_3-4′), 3.68 (6H, s, OCH_3-3, -5), 2.33 (3H, s, CH_3-4″); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 152.8 (C, C-3, C-5), 151.5 (C, C-4′), 145.2 (C, C-4″), 140.4 (C, C-2′), 137.2 (C, C-4), 135.9 (C, Ph), 134.2 (C, C-3′), 133.7 (C, C-1″), 132.0 (C, C-1), 131.5 (CH, C-1a), 129.6 (CH, C-3″, C-5″), 128.5 (CH, C-2″, C-6″), 128.4 (4CH, Ph), 128.3 (2CH, Ph), 127.7 (4CH, Ph), 127.0 (CH, C-6′), 126.0 (C, C-1′), 123.9 (CH, C-1′a), 110.8 (CH, C-5′), 106.1 (CH, C-2, C-6), 69.7 ($2CH_2$, OCH_2Ph), 60.9 (CH_3, OCH_3-4), 56.5 (CH_3, OCH_3-4′), 56.0 (CH_3, OCH_3-3, -5), 21.6 (CH_3, CH_3-4″); ^{31}P NMR ($CDCl_3$, 202 MHz) δ −6.16; HRESIMS *m*/*z* 746.2017 $[M + 1]^+$ (calcd for $C_{39}H_{40}O_{11}PS^+$, 746.2024).

(*Z*)-1-[3′,4′,5′-Trimethoxyphenyl]-2-[2″-[(*para*-toluenesulfonyl)oxy]-3″-[(disodium)phosphate]-4″-methoxyphenyl]ethene (3a). Dibenzylphosphate **2a** (0.31 g, 0.41 mmol) was dissolved in acetonitrile (25 mL) cooled to −10 °C. Freshly distilled TMSBr (0.27 mL, 2.1 mmol) was added, and the reaction mixture was stirred for 3 h at −10 °C. Next the reaction mixture was added dropwise to a suspension of NaOMe (0.111 g, 2.06 mmol) in MeOH (10 mL) cooled to −10 °C. The reaction mixture was stirred for 3 h and then allowed to slowly return to ambient temperature. On completion, MeOH was removed in vacuo. Flash chromatographic separation of the crude product using a prepacked 30 g RP-18 silica column [eluents, solvent A, H_2O, solvent B, acetonitrile; gradient, 100% A/0% B over 0 to 1.19 min (1 CV), 100% A/0% B → 60% A/40% B over 18.28 min (14 CV), 0% A/100% B over 3.57 min (3 CV); flow rate 25.0 mL/min; monitored at 254 and 280 nm] afforded sodium phosphate **3a** (0.13 g, 52%) as an off-white solid: ^{1}H NMR (CD_3OD, 500 MHz) δ 7.93 (2H, d, J = 8.1 Hz, H-2″, H-6″), 7.28 (2H, d, J = 8.1 Hz, H-3″, H-5″), 6.78 (1H, d, J = 8.6 Hz, H-6′), 6.75 (1H, dd, J = 8.6 Hz, H-5′), 6.53 (2H, s, H-2, H-6), 6.04 (1H, d, J = 12.2 Hz, H-1′a), 6.01 (1H, d, J = 12.2 Hz, H-1a), 3.86 (3H, s, OCH_3-4′), 3.74 (3H, s, OCH_3-4), 3.64 (6H, s, OCH_3-3, -5), 2.38 (3H, s, CH_3-4″); ^{13}C NMR (CD_3OD, 125 MHz) δ 154.9 (C, C-4′), 154.1 (C, C-3, C-5), 146.8 (C, C-4″), 143.4 (C, C-2′), 140.5 (C, C-3′), 138.4 (C, C-4), 135.7 (C, C-1″), 133.9 (C, C-1), 131.5 (CH, C-1a), 130.8 (CH, C-3″, C-5″), 129.8 (CH, C-2″, C-6″), 126.1 (CH, C-1′a), 124.8 (CH, C-6′), 124.5 (C, C-1′), 112.2 (CH, C-5′), 107.9 (CH, C-2, C-6), 61.3 (CH_3, OCH_3-4), 57.2 (CH_3, OCH_3-4′), 56.6 (CH_3, OCH_3-3, -5), 21.8 (CH_3, CH_3-4″); ^{31}P

NMR (CD_3OD, 122 MHz) δ 2.73; HRESIMS m/z 611.0723 [M + 1]$^+$ (calcd for $C_{25}H_{26}Na_2O_{11}PS^+$, 611.0723).

(*Z*)-1-[3′,4′,5′-Trimethoxyphenyl]-2-[2″-[hydroxy]-3″-[(disodium)phosphate]-4″-methoxyphenyl]ethene (4a). A solution of sulfonate ester **3a** (0.089 g, 0.146 mmol) and NaOH (3 mL, 2M) in MeOH (3 mL) in a 5 mL microwave safe sealed vial was heated to 50 °C for 30 min. Reversed-phase TLC (30:70 acetonitrile−H_2O) was used to monitor the reaction. On completion, aqueous solvents were evaporated under reduced pressure. The crude product was subjected to flash chromatography using a prepacked 30 g RP-18 silica column [eluents, solvent A, H_2O, solvent B, acetonitrile; gradient, 100% A/0% B over 1.19 min (1 CV), 100% A/0% B → 60% A/40% B over 13.12 min (10 CV), 0% A/100% B over 3.57 min (2 CV); flow rate 25.0 mL/min; monitored at 254 and 280 nm], affording sodium phosphate **4a** (0.063 g, 0.14 mmol, 95%) as an off-white solid: ^{1}H NMR (D_2O, 500 MHz) δ 6.82 (1H, d, J = 8.6 Hz, H-6′), 6.69 (1H, d, J = 12 Hz, H-1′a), 6.65 (2H, s, H-2, H-6), 6.60 (1H, d, J = 12 Hz, H-1a), 6.53 (1H, d, J = 8.6 Hz, H-5′), 3.83 (3H, s, OCH_3-4′), 3.75 (3H, s, OCH_3-4), 3.69 (6H, s, OCH_3-3, -5); ^{13}C NMR (D_2O, 125 MHz) δ 152.0 (C, C-3, C-5), 151.6 (C, C-4′), 147.3 (C, C-2′), 135.6 (C, C-4), 133.7 (C, C-1), 131.3 (C, C-3′), 129.5 (CH, C-1a), 126.3 (CH, C-1′a), 123.8 (CH, C-6′), 119.7 (C, C-1′), 106.4 (CH, C-2, C-6), 104.4 (CH, C-5′), 60.8 (CH_3, OCH_3-4), 56.0 (CH_3, OCH_3-4′), 55.8 (CH_3, OCH_3-3, -5); ^{31}P NMR (D_2O, 122 MHz) δ 3.68; HRESIMS m/z 457.0633 [M + H]$^+$ (calcd for $C_{18}H_{20}Na_2O_9P^+$, 457.0635).

(*Z*)-1-[3′,4′,5′-Trimethoxy]-2-[2″-[(benzyl)oxy]]phosphoryl)-oxy]-3″-[(*para*-toluenesulfonyl)oxy]-4″-methoxyphenyl]ethene (6a). Phenol **5a**[37] (0.77 g, 1.8 mmol) was dissolved in acetonitrile (15 mL) cooled to −10 °C. CCl_4 (2.00 mL, 20.7 mmol) was added, and the reaction mixture was stirred for 5 min. Diisopropylethylamine (0.7 mL, 4 mmol) and DMAP (0.151 g, 1.23 mmol) were added, and the reaction mixture was stirred for an additional 10 min. Dibenzylphosphite (0.50 mL, 2.3 mmol) was added slowly to the reaction mixture, which was then stirred for 1 h and monitored by TLC. On completion, saturated KH_2PO_4 solution (25 mL) was added, and the reaction mixture was allowed to return to ambient temperature. H_2O (25 mL) was added to the reaction mixture, and the organic phase was separated. The aqueous phase was extracted with EtOAc (2 × 20 mL), and the combined organic phases were dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The crude product was subjected to flash chromatography using a prepacked 100 g silica column [eluents, solvent A, EtOAc, solvent B, hexanes; gradient, 30% A/70% B over 3.18 min (1 CV), 30% A/70% B → 80% A/20% B over 33.00 min (10 CV), 80% A/20% B over 6.36 min (2 CV); flow rate 40.0 mL/min; monitored at 254 and 280 nm], affording **6a** (0.91 g, 1.2 mmol, 78%) as a yellow oil: ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.75 (2H, d, J = 8.4 Hz, H-2″, H-6″), 7.35−7.25 (10H, m, Ph), 7.21 (2H, d, J = 8.4 Hz, H-3″, H-5″), 7.04 (1H, d, J = 8.8 Hz, H-6′), 6.63 (1H, d, J = 11.9 Hz, H-1′a), 6.62 (1H, d, J = 8.8 Hz, H-5′), 6.51 (1H, d, J = 11.9 Hz, H-1a), 6.42 (2H, s, H-2, H-6), 5.06 (4H, m, OCH_2Ph), 3.82 (3H, s, OCH_3-4), 3.64 (6H, s, OCH_3-3, -5), 3.60 (3H, s, OCH_3-4′), 2.36 (3H, s, CH_3-4″); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 152.8 (C, C-3, C-5), 152.7 (C, C-4′), 144.9 (C, C-4″), 142.8 (C, C-2′), 137.2 (C, C-4), 135.7 (2C, Ph-C1), 134.3 (C, C-1″), 132.1 (C, C-1a), 132.0 (C, C-1), 131.6 (C, C-3′), 129.3 (CH, C-3″, -5″), 129.0 (CH, C-6′), 128.5 (CH, C-2″, C-6″), 128.43 (4CH, Ph), 128.36 (2CH, Ph), 127.8 (4CH, Ph), 124.7 (C, C-1′), 124.3 (CH, C-1′a), 109.1 (CH, C-5′), 106.2 (CH, C-2, C-6), 69.9 (2CH_2, OCH_2Ph), 60.9 (CH_3, OCH_3-4), 56.1 (CH_3, OCH_3-4′), 55.9 (CH_3, OCH_3-3, -5), 21.6 (CH_3, CH_3-4″); ^{31}P NMR ($CDCl_3$, 202 MHz) δ −6.16; HRESIMS m/z 747.2024 [M]$^+$ (calcd for $C_{39}H_{40}O_{11}PS^+$, 747.2023); *anal.* C 62.92, H 5.29%, calcd for $C_{39}H_{39}O_{11}PS$, C 62.73, H 5.26%.

(*Z*)-1-[3′,4′,5′-Trimethoxy]-2-[2″-[(disodium)phosphate]-3″-[(*para*-toluenesulfonyl)oxy]-4″-methoxyphenyl]ethene (7a). Compound **6a** (0.60 g, 0.80 mmol) was dissolved in acetonitrile (12 mL) cooled to −10 °C. Freshly distilled TMSBr (0.3 mL, 2.3 mmol) was added dropwise, and the mixture was stirred for 1 h at −10 °C. At that point the mixture was added to a suspension of NaOMe (0.45 g, 8.3 mmol) in MeOH (25 mL) cooled to −10 °C. The mixture was then stirred for 2 h and allowed to slowly return to ambient temperature. On completion, MeOH was evaporated at 50 °C in vacuo, and flash chromatographic separation of the crude product using a prepacked 12 g RP-18 silica column [eluents, solvent A, H_2O, solvent B, acetonitrile; gradient, 100% A/0% B over 1.15 min (1 CV), 100% A/0% B → 45% A/55% B over 17.30 min (14 CV), 0% A/100% B over 3.45 min (3 CV); flow rate 12.0 mL/min; monitored at 254 and 280 nm] led to sodium phosphate **7a** (0.43 g, 0.71 mmol, 88%): ^{1}H NMR (D_2O, 500 MHz) δ 7.71 (2H, d, J = 8.4 Hz, H-2″, -6″), 7.39 (2H, d, J = 8.1 Hz, H-3″, -5″), 7.01 (1H, d, J = 8.7 Hz, H-6′), 7.00 (1H, d, J = 12.1 Hz, H-1′a), 6.60 (1H, d, J = 12.1 Hz, H-1a), 6.59 (1H, d, J = 8.8 Hz, H-2, -6), 6.49 (2H, s, H-5′), 3.73 (3H, s, OCH_3-4), 3.70 (6H, s, OCH_3-3, -5), 3.29 (3H, s, OCH_3-4′), 2.41 (3H, s, CH_3-4″); ^{13}C NMR (D_2O, 125 MHz) δ 151.9 (C, C-3, C-5), 151.3 (C, C-4′), 146.6 (C, C-2′), 146.5 (C, C-4″), 135.6 (C, C-4), 133.7 (C, C-1), 131.8 (C, C-1″), 131.5 (C, C-3′), 129.5 (CH, C-1a), 129.5 (CH, C-3″, C-5″), 128.8 (CH, C-6′), 128.2 (CH, C-2″, C-6″), 127.2 (CH, C-1′a), 125.3 (C, C-1′), 106.9 (CH, C-5′), 106.6 (CH, C-2, C-6), 60.8 (CH_3, OCH_3-4), 55.8 (CH_3, OCH_3-3, -5), 55.5 (CH_3, OCH_3-4′), 20.7 (CH_3, CH_3-4″); ^{31}P NMR (D_2O, 202 MHz) δ −3.19; HRESIMS m/z 611.0716 [M + 1]$^+$ (calcd for $C_{25}H_{26}Na_2O_{11}PS^+$, 611.0723).

(*Z*)-1-[3′,4′,5′-Trimethoxyphenyl]-2-[2″-[(disodium)phosphate]-3″-[hydroxy]-4″-methoxyphenyl]ethene (8a). Sulfonate ester **7a** (0.107 g, 0.175 mmol) was dissolved in MeOH (3 mL) in a 5 mL microwave safe vial with a stir bar. To this solution NaOH (2 mL, 2 M) was added, the vial was capped, and the reaction mixture was prestirred for 5 min. The reaction mixture was heated at 50 °C in a microwave reactor for 30 min. Temperatures higher than 50 °C may lead to isomerization of the compound. On completion, the solvents were evaporated in vacuo and the crude product was subjected to flash chromatographic separation using a prepacked 30 g RP-18 silica column [eluents, solvent A, H_2O, solvent B, acetonitrile; gradient, 100% A/0% B over 0 to 1.19 min (1 CV), 100% A/0% B → 60% A/40% B over 13.12 min (10 CV), 60% A/40% B over 3.57 min (3 CV); flow rate 25.0 mL/min; monitored at 254 and 280 nm], affording **8a** (0.046 g, 0.100 mmol, 58%): ^{1}H NMR (500 MHz, D_2O) δ 6.82 (1H, d, J = 12 Hz, H-1′a), 6.67 (1H, d, J = 8.7 Hz, H-6′), 6.66 (2H, s, H-2, -6), 6.64 (1H, d, J = 8.7 Hz, H-5′), 6.62 (1H, d, J = 12 Hz, H-1a), 3.83 (3H, s, OCH_3-4′), 3.76 (3H, s, OCH_3-4), 3.69 (6H, s, OCH_3-3, -5); ^{13}C NMR (125 MHz, D_2O) δ 151.9 (C, C-3, C-5), 148.6 (C, C-4′), 140.4 (C, C-2′), 138.5 (C, C-3′), 135.5 (C, C-4), 133.9 (C, C-1), 129.1 (CH, C-1a), 127.2 (CH, C-1′a), 124.2 (CH, C-1′), 120.2 (C, C-6′), 107.3 (CH, C-5′), 106.5 (CH, C-2, C-6), 60.9 (CH_3, OCH_3-4), 56.0 (CH_3, OCH_3-4′), 55.8 (CH_3, OCH_3-3, -5); ^{31}P NMR (D_2O, 202 MHz) δ 3.21. HRESIMS m/z 457.0632 [M + H]$^+$ (calcd for $C_{18}H_{20}Na_2O_9P^+$, 457.0635).

(*E*)-1-[3′,4′,5′-Trimethoxy]-2-[2″-[(benzyl)oxy]]phosphoryl)-oxy]-3″-[(*para*-toluenesulfonyl)oxy]-4″-methoxyphenyl]ethene (6b). Phenol **5b**[37] (0.47 g, 0.96 mmol) was dissolved in acetonitrile (10 mL) cooled to −10 °C, CCl_4 (1 mL, 10 mmol) was added, and the reaction mixture was stirred for 5 min. Diisopropylethylamine (0.3 mL, 1.7 mmol) and DMAP (0.1 g, 0.8 mmol) were added, and the reaction mixture was stirred for an additional 10 min. Dibenzylphosphite (0.25 mL, 1.1 mmol) was added slowly to the mixture, and stirring continued for 45 min (monitored by TLC). On completion, saturated KH_2PO_4 solution (50 mL) was added, and the mixture was allowed to return to ambient temperature. H_2O (50 mL) was added, and the organic phase was separated. The aqueous phase was extracted with EtOAc (3 × 10 mL), and the combined organic phase was dried over Na_2SO_4. The solution was filtered and concentrated under reduced pressure. Flash chromatographic separation of the crude product was performed using a prepacked 100 g silica column [eluents; solvent A, EtOAc, solvent B, hexanes; gradient, 40% A/60% B over 1.15 min (1 CV), 40% A/60% B → 54% A/46% B over

3.22 min (2.7 CV), 54% A/46% B → 100% A/0% B over 11.15 min (9 CV), 100% A/0% B over 3.07 min (2.5 CV); flow rate 12.0 mL/min; monitored at 254 and 280 nm] and yielded a pure yellow oil, **6b** (0.55 g, 0.74 mmol, 77%): ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.84 (2H, d, J = 8.4 Hz, H-2″, H-6″), 7.50 (1H, d, J = 9.0 Hz, H-6′), 7.34 (1H, d, J = 16.3 Hz, H-1′a), 7.28 (2H, d, J = 8.4 Hz, H-3″, H-5″), 7.26−7.22 (10H, m, Ph), 6.88 (1H, d, J = 16.3 Hz, H-1a), 6.76 (1H, dd, J = 9.0, 0.8 Hz, H-5′), 6.67 (2H, s, H-2, H-6), 5.08 (4H, m, OCH_2Ph), 3.86 (3H, s, OCH_3-4), 3.76 (6H, s, OCH_3-3, -5), 3.58 (3H, s, OCH_3-4′), 2.41 (3H, s, CH_3-4″); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 153.3 (C, C-3, C-5), 152.6 (C, C-4′), 144.9 (C, C-4″), 142.4 (C, C-2′), 137.9 (C, C-4), 135.5 (2C, Ph), 134.2 (C, C-1″), 133.0 (C, C-1), 131.5 (C, C-3′), 129.5 (CH, C-1a), 129.3 (CH, C-3″, C-5″), 128.6 (CH, C-2″, C-6″), 128.42 (4CH, Ph), 128.40 (2CH, Ph), 127.9 (4CH, Ph), 124.5 (C, C-1′), 124.2 (CH, C-6′), 121.5 (CH, C-1′a), 109.6 (CH, C-5′), 103.6 (CH, C-2, C-6), 70.0 (2CH_2, OCH_2Ph), 60.9 (CH_3, OCH_3-4), 56.0 (CH_3, OCH_3-3, -5), 55.9 (CH_3, OCH_3-4′), 21.6 (CH_3, CH_3-4″); ^{31}P NMR ($CDCl_3$, 202 MHz) δ −5.84; HRESIMS m/z 747.2019 $[M]^+$ (calcd for $C_{39}H_{40}O_{11}PS^+$, 747. 2023); *anal.* C 62.46, H 5.23%, calcd for $C_{39}H_{39}O_{11}PS$, C 62.73, H 5.26%.

(*E*)-1-[3′,4′,5′-Trimethoxy]-2-[2″-[(disodium)phosphate]-3″-[(*para*-toluenesulfonyl)oxy]-4″-methoxyphenyl]ethene (7b). Dibenzylphosphate **6b** (0.32 g, 0.43 mmol) was dissolved in acetonitrile (5 mL) cooled to −10 °C. Freshly distilled TMSBr (0.25 mL, 1.9 mmol) was added, and the reaction mixture was stirred for 3 h at −10 °C. The initial mixture was added dropwise to a suspension of NaOMe (0.22 g, 4.1 mmol) in MeOH (15 mL) cooled to −10 °C. The reaction was stirred for 3 h and allowed to return to ambient temperature. On completion, solvents were evaporated in vacuo (<50 °C to prevent isomerization), and the crude product was subjected to flash chromatographic separation using a prepacked 30 g RP-18 silica column [eluents, solvent A, H_2O, solvent B, acetonitrile; gradient, 100% A/0% B over 1.19 min (1 CV), 100% A/0% B → 61% A/39% B over 12.52 min (9.7 CV), 61% A/39% B → 45% A/55% B over 5.16 min (4 CV), 0% A/100% B over 3.57 min (3 CV); flow rate 25.0 mL/min; monitored at 254 and 280 nm], affording **7b** (0.16 g, 2.6 mmol, 61%) as an off-white solid: ^{1}H NMR (D_2O, 500 MHz) δ 7.59 (2H, d, J = 8.4 Hz, H-2″, H-6″), 7.36 (1H, d, J = 16.3 Hz, H-1′a), 7.28 (1H, d, J = 9.5 Hz, H-6′), 7.22 (2H, d, J = 8.4 Hz, H-3″, H-5″), 6.69 (2H, s, H-2, H-6), 6.63 (1H, d, J = 16.5 Hz, H-1a), 6.58 (1H, dd, J = 8.5 Hz, H-5′), 3.82 (6H, s, OCH_3-3, -5), 3.70 (3H, s, OCH_3-4), 3.33 (3H, s, OCH_3-4′), 2.28 (3H, s, CH_3-4″); ^{13}C NMR (D_2O, 125 MHz) δ 152.3 (C, C-3, C-5), 151.6 (C, C-4′), 146.4 (C, C-4″), 144.5 (C, C-2′), 136.1 (C, C-4), 134.0 (C, C-1), 131.6 (C, C-1″), 131.0 (C, C-3′), 129.5 (CH, C-3″, C-5″), 128.3 (CH, C-2″, C-6″), 128.0 (CH, C-1a), 124.5 (C, C-1′), 124.3 (CH, C-6′), 122.2 (CH, C-1′a), 108.7 (CH, C-5′), 103.7 (CH, C-2, C-6), 60.8 (CH_3, OCH_3-4), 55.9 (CH_3, OCH_3-3, -5), 55.6 (CH_3, OCH_3-4′), 20.8 (CH_3, CH_3-4″); ^{31}P NMR ($CDCl_3$, 202 MHz) δ −4.25; HRESIMS m/z 611.0716 $[M + H]^+$ (calcd for $C_{25}H_{26}Na_2O_{11}PS^+$, 611.0723); *anal.* C 48.51, H 4.62%, calcd for $C_{25}H_{25}Na_2O_{11}PS \cdot 0.5H_2O$, C 48.47, H 4.23%.

(*E*)-1-[3′,4′,5′-Trimethoxyphenyl]-2-[2″-[(disodium)phosphate]-3″-[hydroxy]-4″-methoxyphenyl]ethene (8b). Sulfonate ester **7b** (0.100 g, 0.164 mmol) was dissolved in MeOH (17 mL) in a 20 mL microwave safe vial. To this solution was added NaOH (3 mL, 2 M), the vial was capped, and the reaction was prestirred for 2 min. The reaction mixture was heated at 50 °C in a microwave reactor for 30 min. As noted above, temperatures higher than 50 °C can lead to isomerization. On completion, the solvents were evaporated in vacuo, and the crude product was subjected to flash chromatographic separation using a prepacked 30 g RP-18 silica column [eluents, solvent A, H_2O, solvent B, acetonitrile; gradient, 100% A/0% B over 1.19 min (1 CV), 100% A/0% B → 60% A/40% B over 13.12 min (10 CV), 60% A/40% B over 3.57 min (3 CV); flow rate 25.0 mL/min; monitored at 254 and 280 nm], providing sodium phosphate **8b** (0.063 g, 0.138 mmol, 84%): ^{1}H NMR (D_2O, 500 MHz) δ 7.46 (1H, d, J = 16.55 Hz, H-1′a), 7.25 (1H, d, J = 8.75 Hz, H-6′), 7.07 (1H, d, J = 16.55 Hz, H-1a), 7.03 (2H, s, H-2, H-6), 6.86 (1H, d, J = 8.75 Hz, H-5′), 3.94 (6H, s, OCH_3-3, -5), 3.89 (3H, s, OCH_3-4′), 3.81 (3H, s, OCH_3-4); ^{13}C NMR (D_2O, 125 MHz) δ 152.6 (C, C-3, C-5), 148.9 (C, C-4′), 140.2 (C, C-2′), 138.3 (C, C-3′), 136.1 (C, C-4), 134.6 (C, C-1), 127.5 (CH, C-1a), 124.2 (CH, C-1′), 123.9 (C, C-1′a), 116.5 (CH, C-6′), 107.9 (CH, C-5′), 104.1 (CH, C-2, C-6), 60.9 (CH_3, OCH_3-4), 56.1 (CH_3, OCH_3-3, -5), 56.0 (CH_3, OCH_3-4′); ^{31}P NMR (D_2O, 202 MHz) δ 3.54; HRESIMS m/z 457.0632 $[M + H]^+$ (calcd for $C_{18}H_{20}Na_2O_9P^+$, 457.0635).

(*E*)-1-[3′,4′,5′-Trimethoxyphenyl]-2-[2″-[(*para*-toluenesulfonyl)oxy]-3″-[([bis[(benzyl)oxy]]phosphoryl)oxy]-4″-methoxyphenyl]ethene (2b). To a solution of phenol **1b**[37] (0.499 g, 1.03 mmol) in acetonitrile (10 mL) cooled to −10 °C was added CCl_4 (0.20 mL, 2.07 mmol), and the reaction mixture was stirred for 5 min. Diisopropylethylamine (0.7 mL, 4.0 mmol) and DMAP (0.06 g, 0.49 mmol) were added, and the mixture was stirred for an additional 10 min. Next, dibenzylphosphite (0.5 mL, 2.26 mmol) was slowly added to the mixture. After stirring for 45 min (monitored by TLC), KH_2PO_4 (10 mL) was added, and the mixture was allowed to return to ambient temperature. H_2O (20 mL) was then added, and the organic phase was separated. The aqueous phase was extracted with EtOAc (3 × 10 mL), the combined organic phase was dried (Na_2SO_4), and the solution was filtered and concentrated in vacuo. The crude product was separated by flash chromatography using a prepacked 25 g silica column [eluents; solvent A, EtOAc, solvent B, hexanes; gradient, 20% A/80% B over 1.19 min (1 CV), 20% A/80% B → 70% A/30% B over 13.51 min (10.5 CV), 70% A/30% B over 1.27 min (1.1 CV), 100% A/0% B over 3.57 min (3 CV); flow rate 25.0 mL/min; monitored at 254 and 280 nm] to yield **2b** (0.46 g, 0.61 mmol, 60%) as a yellow oil: ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.73 (2H, d, J = 8.1 Hz, H-2″, H-6″), 7.40 (1H, d, J = 9.0 Hz, H-6′), 7.38−7.32 (10H, m, Ph), 7.06 (2H, d, J = 8.1 Hz, H-3″, H-5″), 6.90 (1H, d, J = 9.0 Hz, H-5′), 6.80 (1H, d, J = 16.2 Hz, H-1′a), 6.71 (1H, d, J = 16.2 Hz, H-1a), 6.53 (2H, s, H-2, H-6), 5.21 (4H, m, OCH_2Ph), 3.89 (6H, s, OCH_3-3, -5), 3.88 (3H, s, OCH_3-4), 3.80 (3H, s, OCH_3-4′), 2.16 (3H, s, CH_3-4″); ^{13}C NMR ($CDCl_3$, 125 MHz): δ 153.2 (C, C-3, C-5), 151.7 (C, C-4′), 145.6 (C, C-4″), 140.1 (C, C-2′), 137.9 (C, C-4), 136.0 (C, Ph), 134.5 (C, C-3′), 133.2 (C, C-1″), 132.8 (C, C-1), 129.6 (CH, C-3″, C-5″), 129.0 (CH, C-1a), 128.6 (CH, C-2″, C-6″), 128.4 (4CH, Ph), 128.3 (2CH, Ph), 127.8 (4CH, Ph), 125.6 (C, C-1′), 122.1 (CH, C-6′), 121.4 (CH, C-1′a), 111.5 (CH, C-5′), 103.7 (CH, C-2, C-6), 69.8 (2CH_2, OCH_2Ph), 61.0 (CH_3, OCH_3-4), 56.4 (CH_3, OCH_3-4′), 56.1 (CH_3, OCH_3-3, -5), 21.5 (CH_3, CH_3-4″); ^{31}P NMR ($CDCl_3$, 202 MHz) δ −6.23; HRESIMS m/z 747.2020 $[M + 1]^+$ (calcd for $C_{39}H_{40}O_{11}PS^+$, 747.2023); *anal.* C 62.71, H 5.31%, calcd for $C_{39}H_{39}O_{11}PS$, C 62.73, H 5.26%.

(*E*)-1-[3′,4′,5′-Trimethoxyphenyl]-2-[2″-[(*para*-toluenesulfonyl)oxy]-3″-[(disodium)phosphate]-4″-methoxyphenyl]ethene (3b). To a solution of dibenzylphosphate **2b** (0.16 g, 0.21 mmol) in acetonitrile (12 mL cooled to −10 °C) was added freshly distilled TMSBr (0.15 mL, 1.14 mmol), and the mixture was stirred for 3 h at −10 °C. The solution was added dropwise to a suspension of NaOMe (0.15 g, 2.78 mmol) in MeOH (10 mL) cooled to −10 °C. The mixture was stirred for 3 h and allowed to slowly return to ambient temperature, and the solvent was evaporated in vacuo. The crude product was separated by flash chromatography using a prepacked 30 g RP-18 silica column [eluents, solvent A, H_2O, solvent B, acetonitrile; gradient, 100% A/0% B over 1.19 min (1 CV), 100% A/0% B → 45% A/55% B over 18.28 min (14 CV), 0% A/100% B over 3.57 min (3 CV); flow rate 25.0 mL/min; monitored at 254 and 280 nm] to afford **3b** (0.042 g, 32%): ^{1}H NMR (D_2O, 500 MHz) δ 7.52 (2H, d, J = 8.0 Hz, H-2″, H-6″), 7.08 (1H, d, J = 9.0 Hz, H-6′), 6.91 (2H, d, J = 8.0 Hz, H-3″, H-5″), 6.84 (1H, d, J = 9.0 Hz, H-5′), 6.42 (1H, d, J = 16.5 Hz, H-1a), 6.36 (1H, d, J = 16.5 Hz, H-1′a), 6.34 (2H, s, H-2, H-6), 3.82 (3H, s, OCH_3-4′), 3.73 (6H, s, OCH_3-3, -5), 3.71 (3H, s, OCH_3-4), 1.90 (3H, s, CH_3-4″); ^{13}C NMR (D_2O, 125 MHz)

δ 152.3 (C, C-4′), 152.1 (C, C-3, C-5), 146.5 (C, C-4″), 140.2 (C, C-2′), 136.1 (C, C-4), 135.7 (C, C-3′), 133.5 (C, C-1), 131.8 (C, C-1″), 129.8 (CH, C-2″, C-6″), 128.3 (CH, C-1a), 127.9 (CH, C-3″, C-5″), 124.2 (C, C-1′), 121.4 (CH, C-6′), 121.2 (CH, C-1′a), 111.8 (CH, C-5′), 103.7 (CH, C-2, C-6), 60.7 (CH_3, OCH_3-4), 56.1 (CH_3, OCH_3-4′), 55.8 (CH_3, OCH_3-3, -5), 20.5 (CH_3, CH_3-4″); ^{31}P NMR (D_2O, 202 MHz) δ −3.99; HRESIMS m/z 611.0713 $[M + H]^+$ (calcd for $C_{25}H_{26}Na_2O_{11}PS^+$, 611.0723).

(*E*)-1-[3′,4′,5′-Trimethoxyphenyl]-2-[2″-[hydroxy]-3″-[(disodium)phosphate]-4″-methoxyphenyl]ethene (4b). A solution of NaOH (3 mL, 2 M) was added to sulfonate ester **3b** (0.050 g, 0.082 mmol) in MeOH (17 mL, in a 20 mL microwave safe vial with a stir bar). The vial was capped and placed in the microwave. The solution was prestirred for 2 min and then heated at 50 °C in a microwave reactor for 30 min. (Caution: temperatures higher than 50 °C may lead to isomerization.) Reversed-phase TLC (30:70 acetonitrile−H_2O) was used to monitor the reaction course. After the reaction was complete, the solvent was evaporated in vacuo. The crude product was subjected to flash chromatographic separation using a prepacked 30 g RP-18 silica column [eluents, solvent A, H_2O, solvent B, acetonitrile; gradient, 100% A/0% B over 1.19 min (1 CV), 100% A/0% B → 60% A/40% B over 13.12 min (10 CV), 0% A/100% B over 3.57 min (3 CV); flow rate 25.0 mL/min; monitored at 254 and 280 nm] to yield **4b** (0.035 g, 0.077 mmol, 94%): ^{1}H NMR (D_2O, 500 MHz) δ 7.36 (1H, d, J = 16.35 Hz, H-1′a), 7.29 (1H, d, J = 8.75 Hz, H-6′), 6.98 (1H, d, J = 16.35 Hz, H-1a), 6.86 (2H, s, H-2, H-6), 6.67 (1H, d, J = 8.65 Hz, H-5′), 3.88 (3H, s, OCH_3-4′), 3.86 (6H, s, OCH_3-3, -5), 3.77 (3H, s, OCH_3-4); ^{13}C NMR (D_2O, 125 MHz) δ 152.4 (C, C-3, C-5), 151.9 (C, C-4′), 147.6 (C, C-2′), 135.8 (C, C-4), 134.7 (C, C-1), 131.3 (C, C-3′), 126.5 (C, C-1a), 123.3 (CH, C-1′a), 120.6 (CH, C-6′), 119.6 (CH, C-1′), 104.7 (CH, C-5′), 103.4 (CH, C-2, C-6), 60.8 (CH_3, OCH_3-4), 55.9 (CH_3, OCH_3-4′), 55.8 (CH_3, OCH_3-3, -5); ^{31}P NMR (D_2O, 202 MHz) δ −3.74; HRESIMS m/z 457.0631 $[M + 1]^+$ (calcd for $C_{18}H_{20}Na_2O_9P^+$, 457.0635).

ASSOCIATED CONTENT

Supporting Information. General experimental details regarding tubulin and cytotoxicity assays, ^{1}H NMR, ^{13}C NMR, gHSQC, gHMBC, HRESIMS, and HPLC. This material is available free of charge via the Internet at http://pubs.acs.org.

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REFERENCES

(1) Pettit, G. R.; Singh, S. B.; Niven, M. L.; Hamel, E.; Schmidt, J. M. *J. Nat. Prod.* **1987**, *50*, 119–131.

(2) Pettit, G. R.; Singh, S. B.; Hamel, E.; Lin, C. M.; Alberts, D. S.; Garcia-Kendall, D. *Experientia* **1989**, *45*, 209–211.

(3) Lin, C. M.; Singh, S. B.; Chu, P. S.; Dempcy, R. O.; Schmidt, J. M.; Pettit, G. R.; Hamel, E. *Mol. Pharmacol.* **1988**, *34*, 200–208.

(4) Lin, C. M.; Ho, H. H.; Pettit, G. R.; Hamel, E. *Biochemistry* **1989**, *28*, 6984–6991.

(5) NCI, Cancer Screening Data, DTP, 2010.

(6) Pettit, G. R.; Toki, B.; Herald, D. L.; Verdier-Pinard, P.; Boyd, M. R.; Hamel, E.; Pettit, R. K. *J. Med. Chem.* **1998**, *41*, 1688–1695.

(7) Pettit, G. R.; Grealish, M. P.; Herald, D. L.; Boyd, M. R.; Hamel, E.; Pettit, R. K. *J. Med. Chem.* **2000**, *43*, 2731–2737.

(8) Pettit, G. R.; Lippert, J. W., III. *Anti-Cancer Drug Des.* **2000**, *15*, 203–216.

(9) Pettit, G. R.; Rhodes, M. R. *Anti-Cancer Drug Des.* **1998**, *13*, 183–191.

(10) Pettit, G. R.; Temple, C., Jr.; Narayanan, V. L.; Varma, R.; Simpson, M. J.; Boyd, M. R.; Rener, G. A.; Bansal, N. *Anti-Cancer Drug Des.* **1995**, *10*, 299–309.

(11) Lippert, J. W., III. *Bioorg. Med. Chem.* **2007**, *15*, 605–615.

(12) Patterson, D. M.; Rustin, G. J. *Clin. Oncol. (R. Coll. Radiol.)* **2007**, *19*, 443–456.

(13) Patterson, D. M.; Ross, P.; Koetz, B.; Saleem, A.; Stratford, M.; Stirling, J.; Padhani, A.; Asselin, M.; Price, P.; Rustin, G. J. *J. Clin. Oncol. (Meet. Abstr.)* **2007**, *25*, 14146.

(14) Siemann, D. W.; Chaplin, D. J.; Walicke, P. A. *Expert Opin. Investig. Drugs* **2009**, *18*, 189–197.

(15) Chaplin, D. J.; Pettit, G. R.; Hill, S. A. *Anticancer Res.* **1999**, *19*, 189–195.

(16) Mooney, C. J.; Nagaiah, G.; Fu, P.; Wasman, J. K.; Cooney, M. M.; Savvides, P. S.; Bokar, J. A.; Dowlati, A.; Wang, D.; Agarwala, S. S.; Flick, S. M.; Hartman, P. H.; Ortiz, J. D.; Lavertu, P. N.; Remick, S. C. *Thyroid* **2009**, *19*, 233–240.

(17) Akerley, W. L.; Schabel, M.; Morrell, G.; Horvath, E.; Yu, M.; Johnsson, B.; Arbogast, K. *J. Clin. Oncol. (Meet. Abstr.)* **2007**, *25*, 14060.

(18) Nathan, P. D.; Judson, I.; Padhani, A.; Harris, A.; Carden, C. P.; Smythe, J.; Collins, D.; Leach, M.; Walicke, P.; Rustin, G. J. *J. Clin. Oncol. (Meet. Abstr.)* **2008**, *26*, 3550.

(19) www.Oxigene.com (accessed Aug 4, 2010).

(20) Dark, G. G.; Hill, S. A.; Prise, V. E.; Tozer, G. M.; Pettit, G. R.; Chaplin, D. J. *Cancer Res.* **1997**, *57*, 1829–1834.

(21) Grosios, K.; Holwell, S. E.; McGown, A. T.; Pettit, G. R.; Bibby, M. C. *Br. J. Cancer* **1999**, *81*, 1318–1327.

(22) Pinney, K. G. Molecular Recognition of the Colchicine Binding Site as a Design Paradigm for the Discovery and Development of Vascular Disrupting Agents; In *Vascular-Targeted Therapies in Oncology*; John Wiley & Sons, Ltd: New York, 2006; pp 95−121.

(23) Pinney, K. G.; Jelinek, C.; Edvardsen, K.; Chaplin, D. J.; Pettit, G. R. The Discovery and Development of the Combretastatins; Cragg, G. R., Kingston, D. G. I., Newman, D. J., Eds.; In *Anticancer Agents from Natural Products*; CRC Press/Taylor & Francis: Boca Raton, FL, 2005; pp 23−46.

(24) Siemann, D. W.; Bibby, M. C.; Dark, G. G.; Dicker, A. P.; Eskens, F. A.; Horsman, M. R.; Marme, D.; Lorusso, P. M. *Clin. Cancer Res.* **2005**, *11*, 416–420.
(25) Vincent, L.; Kermani, P.; Young, L. M.; Cheng, J.; Zhang, F.; Shido, K.; Lam, G.; Bompais-Vincent, H.; Zhu, Z.; Hicklin, D. J.; Bohlen, P.; Chaplin, D. J.; May, C.; Rafii, S. *J. Clin. Invest.* **2005**, *115*, 2992–3006.
(26) Jordan, M. A.; Wilson, L. *Nat. Rev. Cancer* **2004**, *4*, 253–265.
(27) Tozer, G. M.; Kanthou, C.; Baguley, B. C. *Nat. Rev. Cancer* **2005**, *5*, 423–435.
(28) Monk, K. A.; Siles, R.; Hadimani, M. B.; Mugabe, B. E.; Ackley, J. F.; Studerus, S. W.; Edvardsen, K.; Trawick, M. L.; Garner, C. M.; Rhodes, M. R.; Pettit, G. R.; Pinney, K. G. *Bioorg. Med. Chem.* **2006**, *14*, 3231–3244.
(29) Kanthou, C.; Tozer, G. M. *Expert Opin. Ther. Targets* **2007**, *11*, 1443–1457.
(30) Tozer, G. M.; Akerman, S.; Cross, N. A.; Barber, P. R.; Björndahl, M. A.; Greco, O.; Harris, S.; Hill, S. A.; Honess, D. J.; Ireson, C. R.; Pettyjohn, K. L.; Prise, V. E.; ReyesAldasoro, C. C.; Ruhrberg, C.; Shima, D. T.; Kanthou, C. *Cancer Res.* **2008**, *68*, 2301–2311.
(31) Hill, S. A.; Lonergan, S. J.; Denekamp, J.; Chaplin, D. J. *Eur. J. Cancer* **1993**, *29*, 1320–1324.
(32) Hua, J.; Sheng, Y.; Pinney, K. G.; Garner, C. M.; Kane, R. R.; Prezioso, J. A.; Pettit, G. R.; Chaplin, D. J.; Edvardsen, K. *Anticancer Res.* **2003**, *23*, 1433–1440.
(33) Folkes, L. K.; Christlieb, M.; Madej, E.; Stratford, M. R. L.; Wardman, P. *Chem. Res. Toxicol.* **2007**, *20*, 1885–1894.
(34) Pettit, G. R.; Thornhill, A. J.; Moser, B. R.; Hogan, F. *J. Nat. Prod.* **2008**, *71*, 1561–1563.
(35) Kirwan, I. G.; Loadman, P. M.; Swaine, D. J.; Anthoney, D. A.; Pettit, G. R.; Lippert, J. W.; Shnyder, S. D.; Cooper, P. A.; Bibby, M. C. *Clin. Cancer Res.* **2004**, *10*, 1446–1453.
(36) Stratford, M. R. L. *J. Chromatogr. A* **2008**, *1181*, 162–165.
(37) Tanpure, R. P.; Strecker, T. E.; Chaplin, D. J.; Siim, B. G.; Trawick, M. L.; Pinney, K. G. *J. Nat. Prod.* **2010**, *73*, 1093–1101.
(38) Monks, A.; Scudiero, D.; Skehan, P.; Shoemaker, R.; Paull, K.; Vistica, D.; Hose, C.; Langley, J.; Cronise, P.; Vaigro-Wolff, A.; Gray-Goodrich, M.; Campbell, H.; Mayo, J.; Boyd, M. *J. Natl. Cancer Inst.* **1991**, *83*, 757–766.
(39) Vichai, V.; Kirtikara, K. *Nat. Protoc.* **2006**, *1*, 1112–1116.
(40) Monk, K. A.; Siles, R.; Hadimani, M. B.; Mugabe, B. E.; Ackley, J. F.; Studerus, S. W.; Edvardsen, K.; Trawick, M. L.; Garner, C. M.; Rhodes, M. R.; Pettit, G. R.; Pinney, K. G. *Bioorg. Med. Chem.* **2006**, *14*, 3231–3244.
(41) Batch prepared by Dr. Ming Zhou (Baylor University).
(42) Gift from OXiGENE, Inc.
(43) Chaplin, D. J.; Garner, C. M.; Kane, R. R.; Pinney, K. G.; Prezioso, J. A.; Edvardsen, K. United States Patent 7384925, 2005.