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Synthesis of a Bisubstrate Analogue Targeting Estrogen Sulfotransferase

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Received June 11, 2002

Abstract: Sulfotransferases catalyze the transfer of a sulfuryl group from the eukaryotic sulfate donor 3′-phosphoadenosine 5′-phosphosulfate to an acceptor biomolecule. Sulfotransferases have been linked with several disease states, prompting our investigation of specific sulfotransferase inhibitors. Presented herein is the synthesis and evaluation of a bisubstrate analogue designed to inhibit estrogen sulfotransferase. The synthesis utilizes a novel, orthogonally protected 3′-phosphoadenosine 5′-phosphate (PAP) derivative allowing the selective functionalization of the 5′-phosphate with a sulfate acceptor mimic. Kinetic studies revealed significant inhibitory activity and provide guidance for improved inhibitor design.

Sulfotransferases modulate the biological activity of biomolecules via the regioselective installation of a sulfate ester (Figure 1).[1] Carbohydrate sulfotransferases participate in the biosynthesis of the pro-inflammatory epitope 6-sulfo sialyl Lewis x,[2] glycans of pituitary hormones,[3] and numerous proteoglycans in the extracellular matrix.[4] The tyrosylprotein sulfotransferases (TPSTs)[5] modify several chemokine receptors including CXCR4 and CCR5,[6] as well as the P-selectin glycoprotein ligand-1 (PSGL-1)[7,8] expressed on leukocytes. The cytosolic sulfotransferases mediate sulfuryl group transfer to both endogenous steroids and phenolic xenobiotics.[9] The discovery that sulfotransferases participate in several disease states has positioned the enzymes as important therapeutic targets.[10] Initial success has been achieved in identifying inhibitors of individual sulfotransferase enzymes, including the bacterial carbohydrate sulfotransferase, NodST,[11] the cytosolic enzyme estrogen sulfotransferase (EST),[12,13] and TPST-2.[14] As part of our continuing effort to develop selective sulfotransferase inhibitors, we report the synthesis and evaluation of a bisubstrate analogue targeting EST.

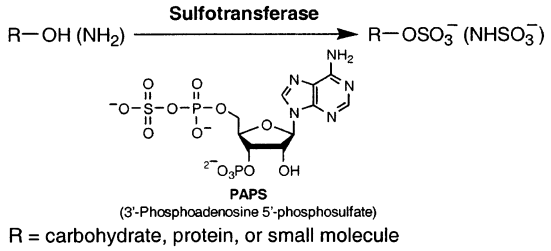

FIGURE 1. Sulfotransferases catalyze sulfuryl group transfer from 3′-phosphoadenosine 5′-phosphosulfate (PAPS) to biomolecules.

Bisubstrate analogue inhibitors benefit from interactions within both substrate binding sites.[15] They are applicable to enzymes that catalyze the direct transfer of a group from one substrate to the other without the intermediacy of a modified enzyme. Group transfer enzymes such as glycosyltransferases[16] and kinases[17−19] have been successfully targeted with bisubstrate analogues. Their enhanced binding energy compared to individual substrate analogues derives primarily from entropic advantages, although the enthalpic contribution of the intersubstrate analogue linker can be significant.[20] Bisubstrate analogues can overcome selectivity problems often encountered in the design of inhibitors against enzymes from large, highly homologous families. Such enzymes share one common substrate but often have dissimilar second substrates. Bisubstrate analogues can exploit interactions within the binding pocket for that unique substrate, thereby gaining selectivity.

EST has been characterized at both the structural and mechanistic levels and was therefore an ideal target for our initial studies. Crystallographic analysis revealed the simultaneous binding of both substrates in distinct binding pockets, and suggested that the mechanism

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(1) Bowman, K. G.; Bertozzi, C. R. *Chem. Biol.* **1999**, *6*, R9.

(2) Bistrup, A.; Bhakta, S.; Lee, J. K.; Belov, Y. Y.; Gunn, M. D.; Zuo, F. R.; Huang, C. C.; Kannagi, R.; Rosen, S. D.; Hemmerich, S. *J. Cell Biol.* **1999**, *145*, 899.

(3) Manzella, S. M.; Dharmesh, S. M.; Beranek, M. C.; Swanson, P.; Baenziger, J. U. *J. Biol. Chem.* **1995**, *270*, 21665.

(4) Habuchi, O. *Biochim. Biophys. Acta* **2000**, *1474*, 115.

(5) Kehoe, J. W.; Bertozzi, C. R. *Chem. Biol.* **2000**, *7*, R57.

(6) Farzan, M.; Mirzabekov, T.; Kolchinsky, P.; Wyatt, R.; Cayabyab, M.; Gerard, N. P.; Gerard, C.; Sodroski, J.; Choe, H. *Cell* **1999**, *96*, 667.

(7) Pouyani, T.; Seed, B. *Cell* **1995**, *83*, 333.

(8) Sako, D.; Comess, K. M.; Barone, K. M.; Camphausen, R. T.; Cumming, D. A.; Shaw, G. D. *Cell* **1995**, *83*, 323.

(9) Falany, C. N. *FASEB J.* **1997**, *11*, 206.

(10) Armstrong, J. I.; Bertozzi, C. R. *Curr. Opin. Drug Discovery Dev.* **2000**, *3*, 502.

(11) Armstrong, J. I.; Portley, A. R.; Chang, Y. T.; Nierengarten, D. M.; Cook, B. N.; Bowman, K. G.; Bishop, A.; Gray, N. S.; Shokat, K. M.; Schultz, P. G.; Bertozzi, C. R. *Angew. Chem.* **2000**, *39*, 1303.

(12) Verdugo, D. E.; Cancilla, M. T.; Ge, X.; Gray, N. S.; Chang, Y. T.; Schultz, P. G.; Negishi, M.; Leary, J. A.; Bertozzi, C. R. *J. Med. Chem.* **2001**, *44*, 2683.

(13) Armstrong, J. I.; Ge, X.; Verdugo, D. E.; Winans, K. A.; Leary, J. A.; Bertozzi, C. R. *Org. Lett.* **2001**, *3*, 2657.

(14) Kehoe, J. W.; Maly, D. J.; Verdugo, D. E.; Armstrong, J. I.; Cook, B. N.; Ouyang, Y. B.; Moore, K. L.; Ellman, J. A.; Bertozzi, C. R. *Bioorg. Med. Chem. Lett.* **2002**, *12*, 329.

(15) Radzicka, A.; Wolfenden, R. *Methods Enzymol.* **1995**, *249*, 284.

(16) Wong, C. H. *Acc. Chem. Res.* **1999**, *32*, 376.

(17) Traxler, P. M.; Wacker, O.; Bach, H. L.; Geissler, J. F.; Kump, W.; Meyer, T.; Regenass, U.; Roesel, H. L.; Lydon, N. *J. Med. Chem.* **1991**, *34*, 2328.

(18) Parang, K.; Till, J. H.; Ablooglu, A. J.; Kohanski, R. A.; Hubbard, S. R.; Cole, P. A. *Nat. Struct. Biol.* **2001**, *V8*, 37.

(19) Yan, X. W.; Lawrence, D. S.; Corbin, J. D.; Francis, S. H. *J. Am. Chem. Soc.* **1996**, *118*, 6321.

(20) Olejniczak, E. T.; Hajduk, P. J.; Marcotte, P. A.; Nettesheim, D. G.; Meadows, R. P.; Edalji, R.; Holzman, T. F.; Fesik, S. W. *J. Am. Chem. Soc.* **1997**, *119*, 5828.

10.1021/jo0260443 CCC: $25.00
Published on Web 10/18/2002

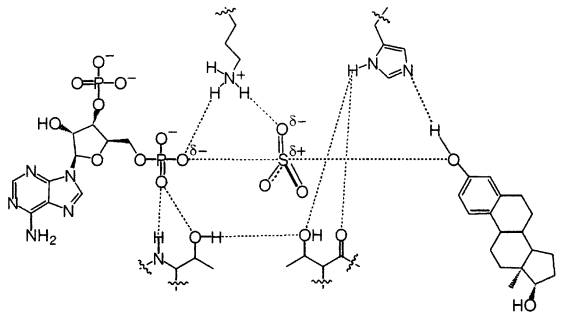


FIGURE 2. A schematic representation of sulfuryl group transfer from PAPS to the acceptor substrate based on EST structural studies (adapted from ref 10).

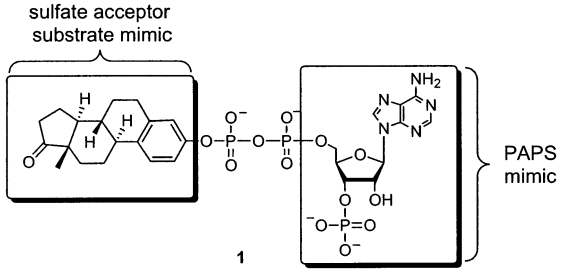


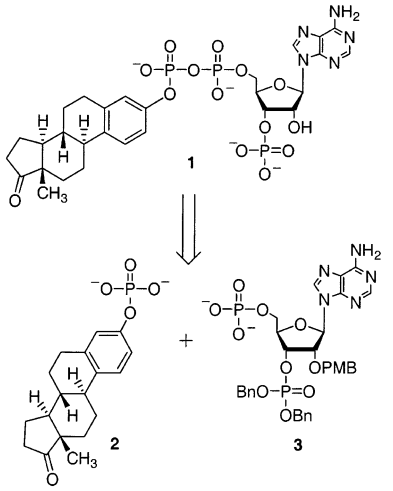
FIGURE 3.

proceeds by direct transfer of the sulfuryl group from the sulfate donor 3′-phosphoadenosine 5′-phosphosulfate (PAPS) to the acceptor hydroxyl group of estradiol (Figure 2).[21,22] Coupled with kinetic studies,[23] these data exclude the possibility of a ping-pong mechanism for EST and suggest that bisubstrate mimicry is a viable mechanism to achieve selective inhibition. If direct sulfuryl group transfer is revealed as a general mechanism for the sulfotransferases, bisubstrate mimicry could prove a valuable approach to inhibiting these enzymes.

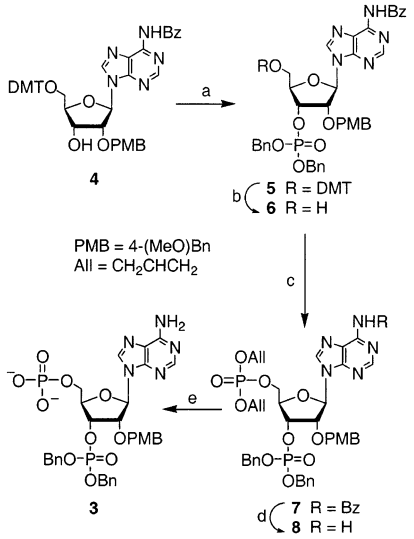
We designed compound **1** (Figure 3) as a bisubstrate analogue for EST. A phosphoanhydride linker was chosen to enable both electrostatic and polar interactions with residues lining the space between binding pockets. Crystallographic studies have underscored the importance of a 3′-phosphate group for PAPS binding.[21,22] To incorporate this element into our bisubstrate analogue, we required a synthetic strategy to differentiate the 5′- and 3′-phosphate groups on the adenosine moiety of **1**. A variety of steroids in addition to estradiol can be efficiently sulfated by EST. We selected estrone as the sulfate-acceptor mimic to avoid the synthetic difficulties associated with differentiating the 3- and 17-hydroxyl groups of estradiol and to prevent 17-*O*-sulfation from interfering with our kinetic assay.

We envisioned compound **1** arising from the coupling of selectively protected 3′-phosphoadenosine 5′-phosphate (PAP) derivative **3** and phosphorylated estrone **2**. Compound **3** was prepared in eight steps from adenosine and relied on a selective alkylation of the adenosine 2′-

SCHEME 1



SCHEME 2



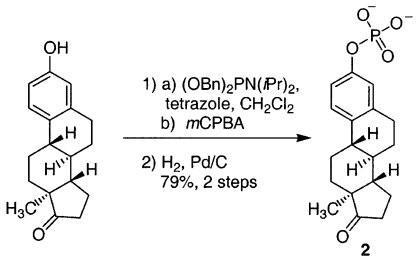
a) i) $(BnO)_2PN(iPr)_2$, tetrazole, CH_2Cl_2; ii) *m*-CPBA, 87%; b) 80% AcOH, 74%; c) $(AllO)_2PN(i\text{-}Pr)_2$, tetrazole, CH_2Cl_2; ii) *m*-CPBA, 84%; d) *n*-$PrNH_2$, THF, 68%; e) $Pd(PPh_3)_4$, *n*-$BuNH_2$, HCOOH, THF, 65%.

hydroxyl group with *p*-methoxylbenzyl bromide (PMBBr) following literature methods.[24] A subsequent perbenzoylation/de-*O*-benzoylation sequence followed by protection of the 5′-hydroxyl group with 4,4′-dimethoxytrityl chloride (DMTCl) provided compound **4**[24] with only the 3′-hydroxyl group unmasked. Phosphoramidite chemistry was utilized to install a 3′-dibenzyl phosphite group that was oxidized in situ with *m*-CPBA to yield the phosphate **5** (Scheme 2). The DMT group was removed under mild conditions using AcOH to yield compound **6**. Phosphitylation with diallyl diisopropylphosphoramidite followed by oxidation with *m*-CPBA provided compound **7** in which the 3′- and 5′-phosphate groups are orthogonally protected. After purine debenzoylation, the 5′-phosphate was deprotected with $Pd(PPh_3)_4$ in a *n*-$BuNH_2$/formic acid buffered solution to provide the coupling partner **3**. Compound **3** is a versatile intermediate that can be elaborated at the 5′-position with a variety of phosphate

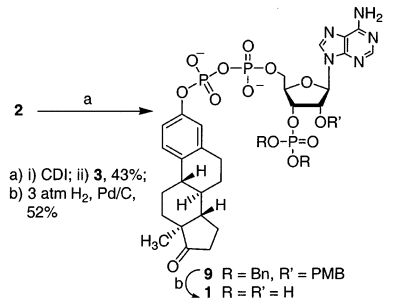
(21) Kakuta, Y.; Petrotchenko, E. V.; Pedersen, L. C.; Negishi, M. *J. Biol. Chem.* **1998**, *273*, 27325.
(22) Kakuta, Y.; Pedersen, L. G.; Carter, C. W.; Negishi, M.; Pedersen, L. C. *Nat. Struct. Biol.* **1997**, *4*, 904.
(23) Zhang, H. P.; Varmalova, O.; Vargas, F. M.; Falany, C. N.; Leyh, T. S. *J. Biol. Chem.* **1998**, *273*, 10888.

(24) Takaku, H.; Kamaike, K. *Chem. Lett.* **1982**, 189.

SCHEME 3



SCHEME 4



esters suited for different members of the sulfotransferase family.

Estrone 3-phosphate **2** was generated via a two-step procedure (Scheme 3). A dibenzyl phosphate group was installed via phosphoramidite chemistry and subsequent Pd-mediated hydrogenolysis provided the desired target. Literature methods were adopted for the condensation of **2** and **3** (Scheme 4).[25] Compound **3** was first treated with an excess of CDI to provide the activated phosphoimidazolium intermediate. After the remaining CDI was quenched, **2** was treated with **3** to form **9**. Finally, hydrogenolysis provided the target compound **1**.

EST catalysis is known to proceed via a random BiBi mechanism where either substrate can bind first but both substrates must be present prior to sulfuryl group transfer.[23] An EST bisubstrate analogue inhibitor should simultaneously occupy both binding pockets and demonstrate competitive behavior toward both substrates. Compound **1** was tested against EST and was found to display moderately potent inhibitory activity. Competitive behavior was observed against PAPS with a $K_{i(\mathrm{comp})}$ of 2.9(±0.9) nM. Noncompetitive behavior was observed against the substrate estrone with a $K_{i(\mathrm{noncomp})}$ of 4.0(±0.5) nM. The calculated K_i values are similar to the K_m values for the two substrates ($K_{m(\mathrm{PAPS})} = 2.5$ nM, and $K_{m(\mathrm{estrone})} = 50$ nM) and significantly lower than the K_i value for the product of sulfate transfer, 3′-phosphoadenosine 5′-phosphate ($K_{i(\mathrm{PAP})} = 286$ nM). Therefore, although compound **1** displays good potency, it does not fully capitalize on the binding potential of a true bisubstrate analogue. Both the potency and the noncompetitive behavior toward estrone indicate that **1** cannot optimally bind in both the PAPS and estrone binding pockets, reflecting either conformational constraints or a linker that is too short to span both substrate binding sites. Future studies will address the role of linker length and functionality on sulfotransferase bisubstrate analogue inhibitory potency with the ultimate goal of developing inhibitors containing more hydrophobic and cell-permeable functionality. Compound **3** should prove to be a useful intermediate in the preparation of future bisubstrate analogue inhibitors of the sulfotransferases.

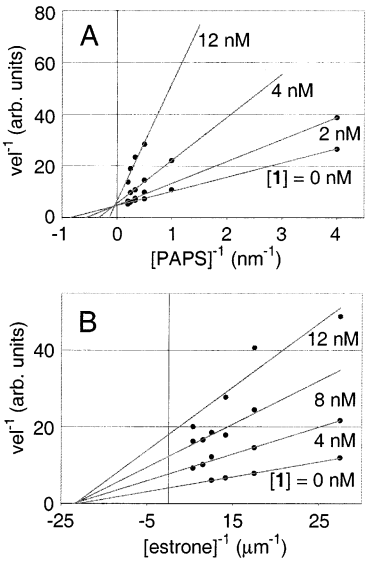


FIGURE 4. Kinetic analysis of **1**. (A) Double reciprocal plot with variable [PAPS] and constant [estrone]. (B) Double reciprocal plot with variable [estrone] and constant [PAPS]. Inhibitor concentrations are marked. Lines represent a best fit for each data set with an invariable [**1**]. K_i values were determined from the linear, transformed data.

Experimental Section

***N*⁶-Benzoyl-5′-*O*-dimethoxytrityl-2′-*O*-(4-methoxybenzyl)-3′-[[bis(benzyloxy)phosphinyl]oxy]adenosine (5).** A suspension of **4**[24] (3.0 g, 3.8 mmol) and tetrazole (1.1 g, 15 mmol) in CH_2Cl_2 (50 mL) was chilled to 0 °C and dibenzyl diisopropylphosphoramidite (5.1 mL, 15 mmol) was added dropwise via syringe with vigorous stirring. After 1 h, *m*CPBA (3.3 g, 15 mmol) was added in several portions and the solution was allowed to stir for 1 h before it was quenched by pouring into sat. $NaHCO_3$ (100 mL). The layers were separated and the organic phase was washed with sat. $NaHCO_3$ followed by brine. The organic phase was then dried and concentrated. Purification by silica gel chromatography (1:1 EtOAc:Hex) provided 3.5 g (87%) of an amorphous solid. ^{1}H NMR (500 MHz, $CDCl_3$) δ 3.34 (dd, 1H, J = 4.1, 10.6), 3.49 (dd, 1H, J = 4.7, 10.6), 3.67 (s, 3H), 3.74 (s, 6H), 4.36−4.41 (m, 2H), 5.00−5.11 (m, 5H), 5.19−5.24 (m, 1H), 6.05 (d, 1H, J = 6.9), 6.60 (app d, 2H, J = 8.7), 6.79 (app d, 4H, J = 8.9), 6.97 (app d, 2H, J = 8.6), 7.19−7.36 (m, 18H), 7.41−7.59 (m, 5H), 7.98 (s, 1H), 8.05 (d, 1H, J = 7.1), 8.57 (s, 1H), 9.44 (br s, 1H) ppm. ^{13}C NMR (125 MHz, $CDCl_3$) δ 55.2, 62.7, 67.2 (d, J_{C-P} = 5), 69.5 (d, J_{C-P} = 13), 69.7 (d, J_{C-P} = 13), 72.4, 75.5 (d, J_{C-P} = 6), 76.8, 83.5 (d, J_{C-P} = 5), 86.6, 86.8, 123.6, 127.0, 127.4, 127.6, 127.9, 127.9, 128.0, 128.2, 128.4, 128.6, 128.6, 128.6, 128.8, 129.5, 130.1, 132.8, 133.7, 135.5, 135.5, 135.6, 135.7, 142.1, 144.4, 149.6, 151.6, 152.3, 158.6, 159.3, 164.8 ppm. ^{31}P NMR (164 MHz, $CDCl_3$) δ −4.0 ppm. HRMS calcd for $C_{60}H_{57}O_{11}N_5P$ $[M + H]^+$ 1054.3792, found 1054.3758.

***N*⁶-Benzoyl-3′-*O*-[[bis(benzyloxy)phosphinyl]oxy]-2′-*O*-(4-methoxybenzyl)adenosine (6).** Compound **5** (3.5 g, 3.3 mmol) was dissolved in 80% aqueous AcOH (50 mL) and stirred vigorously at room temperature. After 2 h, the orange solution was poured over 400 mL of ice-cold water and the aqueous solution was extracted with CH_2Cl_2. The combined organic layers were dried and concentrated to a yellow oil. Purification by silica gel chromatography (9:1:0.1 EtOAc:Hex:MeOH) provided 1.8 g (74%) of a clear glass. ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.50−3.58 (m, 4H), 3.77 (app d, 1H, J = 13.2), 4.09 (d, 1H, J = 11.9), 4.22 (m, 1H), 4.48 (d, 1H, J = 11.9), 4.76−4.81 (m, 1H), 5.06−5.18

(25) Zhao, Y. X.; Thorson, J. S. *J. Org. Chem.* **1998**, *63*, 7568.

(m, 5H), 5.66 (d, 1H, J = 8.1), 6.06 (br s, 1H), 6.37 (d, 1H, J = 8.7), 6.76 (d, 1H, J = 8.7), 7.31−7.35 (m, 10H), 7.46−7.60 (m, 3H), 8.03 (s, 1H), 8.15−8.19 (m, 2H), 8.54 (s, 1H), 9.27 (br s, 1H) ppm. ^{13}C NMR (125 MHz, $CDCl_3$) δ 54.9, 60.2, 62.45, 69.5 (d, J_{C-P} = 6), 72.1, 76.3, 76.5, 86.8, 88.5, 113.0, 124.3, 127.8, 127.9, 128.0, 128.5, 128.6, 128.7, 129.4, 132.7, 133.5, 135.6, 142.7, 149.8, 150.1, 151.4, 159.0, 164.6 ppm. ^{31}P NMR (164 MHz, $CDCl_3$) δ −4.0 ppm. HRMS calcd for $C_{39}H_{39}O_9N_5P$ $[M + H]^+$ 752.2485, found 752.2490.

***N*^{6}-Benzoyl-5′-*O*-[[bis(allyloxy)phosphinyl]oxy]-3′-*O*-[[bis-(benzyloxy)phosphinyl]oxy]-2′-*O*-(4-methoxybenzyl)adenosine (7).** A suspension of **6** (1.7 g, 2.3 mmol) and tetrazole (640 mg, 9.1 mmol) in CH_2Cl_2 (30 mL) was chilled to 0 °C and diallyl diisopropylphosphoramidite (2.2 mL, 9.1 mmol) was added dropwise via syringe. The resulting cloudy solution was stirred for 2 h before the addition of *m*CPBA (1.8 g, 8.3 mmol) in portions. After an additional 1 h of stirring on ice, the reaction was poured into sat. $NaHCO_3$ (100 mL) and extracted with CH_2Cl_2. The combined organic layers were washed with sat. $NaHCO_3$ and brine, dried, and concentrated to an oil that was purified by silica gel chromatography (9:1 EtOAc:Hex) to provide a clear glass (1.8 g, 84%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.67 (s, 3H), 4.13−4.19 (m, 1H), 4.23−4.28 (m, 1H), 4.32−4.34 (m, 2H), 4.47−4.53 (m, 4H), 4.58 (d, 1H, J = 8.7), 4.76 (app t, 1H, J = 7.2), 5.03−5.13 (m, 5H), 5.22 (app dd, 2H, J = 7.0, 11.5), 5.29−5.31 (m, 1H), 5.33−5.35 (m, 1H), 5.83−5.92 (m, 2H), 6.03 (d, 1H, J = 8.0), 6.58 (d, 2H, J = 11.0), 6.93 (d, 2H, J = 11.0), 7.32−7.35 (m, 10H), 7.50 (app t, 2H, J = 9.5), 7.56 (app t, 1H, J = 9.0), 8.03 (s, 1H), 8.04 (d, 2H, J = 9.0), 8.70 (s, 1H) ppm. ^{13}C NMR (125 MHz, $CDCl_3$) δ 55.3, 65.9, 68.6, 69.9, 72.6, 74.6, 77.8, 82.3, 86.7, 113.7, 118.9, 123.2, 128.1, 128.1, 128.4, 128.7, 128.8, 128.8, 128.9, 129.0, 129.7, 132.2, 132.2, 133.0, 133.6, 135.7, 141.8, 149.5, 151.6, 152.2, 159.5, 164.8 ppm. ^{31}P NMR (164 MHz, $CDCl_3$) δ −4.2, −3.6 ppm. HRMS calcd for $C_{45}H_{48}O_{12}N_5P_2$ $[M + H]^+$ 912.2774, found 912.2757.

5′-*O*-[[Bis(allyloxy)phosphinyl]oxy]-3′-*O*-[[bis(benzyloxy)-phosphinyl]oxy]-2′-*O*-(4-methoxybenzyl)adenosine (8). To a solution of **7** (1.0 g, 1.1 mmol) in dry THF (8 mL) was added *n*-$PrNH_2$ (1.2 mL, 11 mmol) slowly via syringe. The solution was stirred for 3 h and concentrated, and the resulting oil was purified by silica gel chromatography (5% MeOH in CH_2Cl_2) to provide 630 mg (68%) of an amorphous glass. ^{1}H NMR (500 MHz, $CDCl_3$) δ 3.68 (s, 3H), 4.14−4.18 (m, 1H), 4.24−4.35 (m, 2H), 4.39 (d, 1H, J = 11.6), 4.46−4.51 (m, 4H), 4.59 (d, 1H, J = 11.6), 4.76 (app t, 1H, J = 5.5), 5.03−5.08 (m, 4H), 5.12−5.13 (m, 1H), 5.19 (app t, 2H, J = 10.2), 5.30 (ddd, 2H, J = 1.4, 8.4, 9.8), 5.82−5.89 (m, 2H), 6.01 (d, 1H, J = 6.2), 6.61−6.63 (m, 4H), 6.98 (d, 2H, J = 8.6), 7.28−7.34 (m, 10H), 7.80 (s, 1H), 8.26 (s, 1H) ppm. ^{13}C NMR (125 MHz, $CDCl_3$) δ 55.1, 65.8 (d, J_{C-P} = 5), 68.4 (d, J_{C-P} = 5), 68.4 (d, J_{C-P} = 5), 69.7 (d, J_{C-P} = 9), 69.7 (d, J_{C-P} = 9), 72.4, 74.5 (d, J_{C-P} = 5), 77.7, 81.8, 86.5, 113.6, 118.6, 120.0, 127.9, 128.0, 128.5, 128.6, 128.6, 128.7, 129.6, 132.1 (d, J_{C-P} = 6), 132.1 (d, J_{C-P} = 6), 135.5 (d, J_{C-P} = 4), 135.5 (d, J_{C-P} = 4), 139.1, 149.5, 152.7, 155.6, 159.4 ppm. ^{31}P NMR (164 MHz, $CDCl_3$) δ −4.3, −3.8 ppm. HRMS calcd for $C_{38}H_{44}O_{11}N_5P_2$ $[M + H]^+$ 808.2512, found 808.2516.

3′-*O*-[[Bis(benzyloxy)phosphinyl]oxy]-2′-*O*-(4-methoxybenzyl)-5′-*O*-phosphate Adenosine (3). A solution of **8** (230 mg, 0.29 mmol) in dry THF (2.5 mL) was treated with *n*$BuNH_2$ (170 μL, 1.7 mmol), formic acid (260 μL, 6.9 mmol), PPh_3 (45 mg, 0.17 mmol), and $Pd(PPh_3)_4$ (31 mg, 0.027 mmol). The orange solution was heated to 40 °C for 4 h, then concentrated to an oil that was purified by silica gel chromatography (10:2:1 EtOAc:MeOH:H_2O) to provide 140 mg (65%) of a clear glass. ^{1}H NMR (500 MHz, $CDCl_3$) δ 3.35 (s, 6H), 3.66 (s, 3H), 4.06−4.07 (m, 2H), 4.31−4.33 (m, 2H), 4.55−4.58 (d, 1H, J = 11.4), 4.87−4.90 (m, 1H), 5.06−5.14 (m, 4H), 5.30 (dd, 1H, J = 4.9, 7.2), 6.13 (d, 1H, J = 7.6), 6.54 (d, 2H, J = 8.6), 6.93 (d, 2H, J = 8.6), 7.31−7.38 (m, 10H), 8.15 (s, 1H), 8.42 (s, 1H) ppm. ^{13}C NMR (125 MHz, $CDCl_3$) δ 55.5, 65.5, 71.0 (d, J_{C-P} = 5), 71.1 (d, J_{C-P} = 5), 73.7, 78.3, 81.7, 85.1, 86.7, 114.4, 120.0, 129.2, 129.6, 129.7, 130.4, 130.5, 137.0, 140.9, 150.6, 153.6, 157.0, 160.6 ppm. ^{31}P NMR (164 MHz, CDCl3) δ −1.2, 2.0 ppm. MS calcd for $C_{32}H_{36}O_{11}N_5P_2$ $[M + H]^+$ 728.2, found (ES (+)) m/z 728.3.

3′-[[Bis(benzyloxy)phosphinyl]oxy]adenosine 5′-Diphospho-(3-estrone) (9). To a solution of **2** (50 mg, 0.14 mmol) in DMF (400 μL) was added triethylamine (22 μL, 0.15 mmol) followed by carbonyldiimidazole (74 mg, 0.45 mmol). The resulting suspension was stirred at room temperature for 3 h and several drops of MeOH were added. After 5 min, the reaction mixture was concentrated and coevaporated with DMF. Compound **3** (30 mg, 0.09 mmol) was added along with DMF (400 μL) and the suspension was stirred at room temperature for 2 d. The reaction mixture was then diluted into 25 mM NH_4OAc (10 mL), washed with CH_2Cl_2 (2 mL), and purified by reversed-phase HPLC (10 to 100% CH_3CN in 25 mM NH_4OAc) to provide 33 mg (43%) of **9** as an amorphous solid. ^{1}H NMR (500 MHz, CD_3OD) δ 0.79 (s, 3H), 1.22−1.39 (m, 6H), 1.51−1.58 (m, 1H), 1.58−1.85 (m, 2H), 1.94−2.20 (m, 4H), 2.37−2.47 (m, 1H), 2.66−2.71 (m, 2H), 3.62 (s, 3H), 4.20−4.32 (m, 4H), 4.50 (d, 1H, J = 11.4), 5.06−5.13 (m, 4H), 5.35 (dd, 1H, J = 4.8, 7.1), 6.08 (d, 1H, J = 7.5), 6.50 (d, 2H, J = 8.7), 6.89 (d, 2H, J = 8.7), 6.92−7.06 (m, 3H), 7.32−7.48 (m, 10H), 8.12 (s, 1H), 8.60 (s, 1H) ppm. ^{13}C NMR (125 MHz, CD_3OD) δ 12.9, 21.0, 25.4, 26.0, 28.8, 31.2, 35.2, 38.0, 43.7, 48.4, 50.1, 54.2, 64.9, 69.6, 72.3, 77.0, 80.4, 83.8, 85.4, 113.0, 117.7, 118.4, 118.9, 120.3, 125.6, 127.8, 128.2, 128.3, 129.2, 134.0, 134.7, 135.7, 137.3, 140.8, 148.8, 150.5, 152.9, 159.2, 222.1 ppm. ^{31}P NMR (164 MHz, CD_3OD) δ −14.3 (d, J_{P-P} = 21), −10.2 (d, J_{P-P} = 21), −1.2 ppm. HRMS calcd for $C_{50}H_{55}N_5O_{15}P_3$ $[M]^-$ 1058.2913, found 1058.2904.

3′-Phosphoadenosine 5′-Diphospho-(3-estrone) (1). Compound **9** was dissolved in H_2O (5 mL), 10% Pd/C (25 mg) was added, and the suspension was shaken under 3 atm of H_2 for 6 h. The mixture was then filtered through Celite, concentrated, and purified by DEAE-sepharose anion exchange chromatography (0 to 1 M NH_4HCO_3 over 400 mL) followed by reversed-phase HPLC (0 to 30% CH_3CN in NH_4OAc). Lyophilization provided 12 mg (52%) of a white solid. ^{1}H NMR (500 MHz, D_2O) δ 0.74 (s, 3H), 1.07−1.12 (m, 3H), 1.27−1.32 (m, 2H), 1.49−1.52 (m, 1H), 1.68−1.74 (m, 3H), 1.94−2.03 (m, 8 H), 2.13−2.17 (m, 1H), 2.47−2.52 (m, 2H), 2.59−2.61 (m, 1H), 4.08−4.14 (m, 2H), 4.51 (app s, 1H), 4.72−4.47 (m, 2H), 4.85−4.89 (m, 2H), 6.04 (d, 1H, J = 6.5), 6.70 (app s, 1H), 6.76−6.83 (m, 2H), 8.15 (s, 1H), 8.25 (s, 1H) ppm. ^{13}C NMR (125 MHz, D_2O) δ 13.2, 20.9, 21.5, 24.8, 25.6, 28.4, 30.7, 35.9, 37.3, 42.9, 48.4, 49.6, 65.1, 73.8, 74.3, 83.3, 86.2, 117.2, 118.1, 120.0, 125.7, 135.2, 137.8, 139.9, 148.8, 149.3, 151.1, 154.1, 229.1 ppm. ^{31}P NMR (164 MHz, D_2O) δ −16.4 (d, J_{P-P} = 16), −11.8 (d, J_{P-P} = 16), −0.4 ppm. HRMS calcd for $C_{28}H_{35}N_5O_{14}P_3$ $[M]^-$ 758.1399, found 758.1384.

Acknowledgment. The Center for New Directions in Organic Synthesis is supported by Bristol-Myers Squibb as Sponsoring Member and Novartis as Supporting Member. This research was supported by grants from Dupont and the National Institutes of Health (GM59907). D.E.V. was supported by NIH Biotechnology Training Grant No. GM08352.

Supporting Information Available: ^{1}H and ^{13}C NMR spectra for all obtained compounds, procedures for the synthesis of compound **2** and for kinetic assays, and general experimental details. This material is available free of charge via the Internet at http://pubs.acs.org.

JO0260443

(26) Ehrhardt, D. W.; Atkinson, E. M.; Faull, K. F.; Freedberg, D. I.; Sutherlin, D. P.; Armstrong, R.; Long, S. R. *J. Bacteriol.* **1995**, *177*, 6237.

(27) Anderson, C.; Freeman, J.; Luca, L. H.; Farley, M.; Dalhoumi, H.; Widlanski, T. S. *Biochemistry* **1997**, *36*, 2586.