Some Adenine and Adenosine Methylene-Bridged Estrogens[1a]

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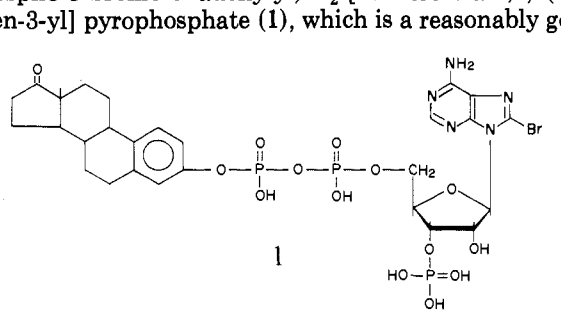
A distinguishing characteristic of human mammary cancer, vis a vis the normal gland, is its capacity to sulfoconjugate estrogens. The title compounds (4 and 12), which are multisubstrate analogues of a putative enzyme-bound transition state of estrogen sulfation (sulfurylation), were synthesized in search of specific chemotherapeutic agents for the treatment of human mammary cancer. 4-Nitroestrone 3-*O*-(ω-alkyl) ethers (**3a**), obtained in 69–73% yield on treatment of 4-nitroestrone (**2a**) with an α,ω-dibromoalkane [$-(CH_2)_n-$, n = 2–4] and NaH in Me_2SO, reacted with adenine in THF containing TBAF to give the corresponding 9-alkyladenine (**4a–c**) in 55–60% yield. Reaction of 9-(3-chloropropyl)adenine (**5**) and estrone (**2b**) yielded a compound (**4d**) identical with the major product of the interaction of estrone 3-*O*-(3-bromopropyl) ether (**3d**) and adenine. The structures of **4a–d** are thereby established. The preparation of the adenosine methylene-bridged estrogens (**12a,b**) first required the 5-*O*-alkylation of methyl 2,3-*O*-isopropylidene-5-*O*-triflyl-β-D-ribofuranoside (**7**) with the 4-nitroestrone 3-*O*-(ω-hydroxyalkyl) ethers, **6a,b**. The 5-substituted ribofuranosides (**8a,b**) were converted to the corresponding ribofuranose derivatives (**9a,b**) in 50% yield in refluxing aqueous acetic acid. Treatment of **9a,b** with acetic anhydride in pyridine gave the corresponding 1,2,3-tri-*O*-acetate derivatives (**10a,b**), which reacted with adenine in acetonitrile and $SnCl_4$ to give the β-nucleoside 2′,3′-di-*O*-acetates, **11a,b**, respectively. Saponification of the latter with NH_3/CH_3OH gave **12a,b**. Compounds **4a–d** and **12a,b** showed weak to modest inhibition of estrone sulfurylation as mediated by purified bovine adrenal estrogen sulfotransferase. However, **4a,c** and **12a,b** exhibited significant growth inhibition of the breast cancer cell line MCF-7 and P388 murine leukemia cells.

The mechanism of sulfoconjugation (sulfurylation[2]) of steroidal estrogens by 3′-phosphoadenosine 5′-phosphosulfate (PAPS), as mediated by bovine adrenal estrogen sulfotransferase (BAES, EC 2.8.2.4), has been the subject of extensive study in our laboratory over the last several years.[3–7] Characterization of BAES with respect to both substrates, i.e., steroid[3] and PAPS,[4] together with inhibition studies that utilized representative analogues of the cosubstrates,[5,6] as well as the coproducts (estrone 3-sulfate and adenosine 3′,5′-diphosphate),[7] led to an hypothesis of an enzyme-bound transition state[6] that includes the concept of an association (stacking) of the adenine moiety of active sulfate and the aromatic ring of the steroid.

Efforts were then directed toward the synthesis of multisubstrate analogues of the putative transition state as chemotherapeutic agents to effect a block of estrogen sulfurylation, which is regarded as a distinguishing characteristic of the human mammary tumor.[8,9] The preparation of a candidate multisubstrate analogue, P_1-(3′-phospho-8-bromo-5′-adenylyl)-P_2-[17-oxoestra-1,3,5(10)-trien-3-yl] pyrophosphate (**1**), which is a reasonably good



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inhibitor of the BAES reaction (see Table I), has been reported.[1a,b] However, the unlikely possibility of an ionized form of **1** penetrating intact cells prompted a study of feasible synthetic routes to more lypophilic analogues. The present paper describes the development of methods to interpose methylene chains of varying lengths between a steroidal estrogen at 0–3 (ring A) and adenine at N-9, as well at 0-C-5′ of adenosine. The synthetic effort centers principally on the modified estrogen, 4-nitroestrone, on the basis of observations that its 3-methyl ether is an effective

Table I. Inhibition of Estrogen Sulfotransferase by Some Estrogen-Bridged Adenines and Adenosines

compd	$-(CH_2)-$ n	estrone 4-substituent, X	K_I,[b] μM
1		H	0.25[b,c]
4-nitroestrone 3-*O*-methyl ether		NO_2	4.3[d]
4a	2	NO_2	1000
4b	3	NO_2	350
4c	4	NO_2	140
4d	3	H	1000
12a	2	NO_2	32
12b	3	NO_2	84

[a] The procedure of enzyme assay is that of Rozhin et al.,[3] which uses 5 μg of enzyme, [S^{35}]PAPS, estrone ([S] = 13 μM, = K_m) and a fixed concentration of inhibitor in 90% aqueous Me_2SO. [b] Reference 1. [c] Mathematical relationships reported previously[6] for the derivative of K_I's for competitive inhibitors were employed in the present work (see text). The K_I's of **1** were determined at 50 μM and represent a single determination. The poorer solubility of **4** restricted measurements to 11 μM, but the K_I's represent an average of five determinations. [d] Reference 6.

(1) This paper has been presented in part, see "Abstracts of Papers", 182nd National Meeting of the American Chemical Society, New York, Aug 23–28, 1981; American Chemical Society: Washington, DC, 1981; Abstr MEDI 74. (b) To be published.
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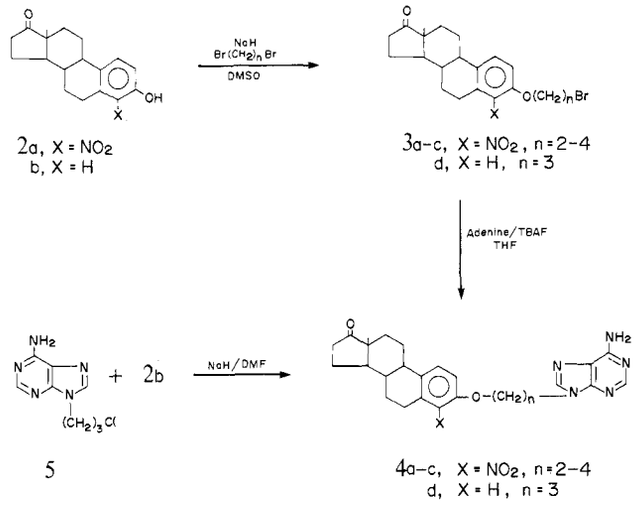
Scheme I

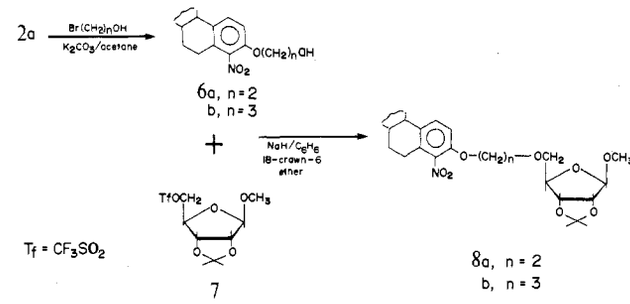


inhibitor of the sulfurylation of 17β-estradiol[6] and is, in addition, a potent growth inhibitor of the DMBA-induced rat mammary tumor.[10] Moreover, the assumption of stacking (vide supra) also includes the possibility of hydrogen bonding between the adenine N-6 group and a 4-nitro substituent in estrogen derivatives.[6]

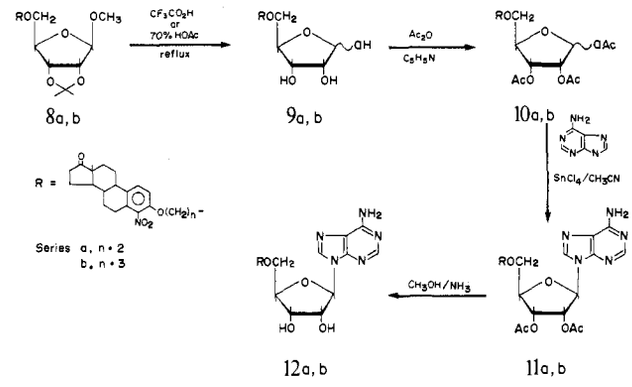
Chemistry. The etherification of 4-nitroestrone (**2a**) with α,ω-dibromoalkanes (Scheme I, n = 2–4) was achieved in reasonably good yields (69–73%) in Me_2SO containing a twofold molar excess of NaH. The 4-nitroestrone 3-*O*-(ω-bromoalkyl) ethers (**3**) reacted with adenine in THF and in the presence of tetra-*n*-butylammonium fluoride (TBAF)[11] to give the corresponding 9-alkyladenine derivative (**4**) in yields of 55–60%. The reaction mixtures are presumed (vide infra) to include in each case, as byproducts, some of the isomeric 7-alkylated adenine derivative, which was readily separated from **4** by chromatography but not identified. Assignment of 9-substituted adenine structures to the principal products of alkylation, i.e., **4**, was made initially on the basis of UV spectral data (λ_{max} 264–266 nm).[12] The fact that the reaction of 9-(3-chloropropyl)adenine (**5**)[13] and estrone (**2b**) yielded a solid (**4d**) identical in all respects with the principal product of the interaction of estrone 3-*O*-(3-bromopropyl) ether (**3d**) and adenine in TBAF/THF lends credence to the structure (**4**) assigned to the principal product.

Attempts to effect the etherification of N^6-(dimethylamino)-2′,3′-*O*-isopropylideneadenosine[14,15] with **4a** or **4b** in either dipolar aprotic solvents, which included DMF, Me_2SO, and HMPTA in the presence of 2 equiv NaH, or benzene/18-crown-6-ether containing (2 equiv) KH were uniformly unsuccessful. Similar results have been reported in connection with efforts to promote 5′-*O*-alkylation of 2′,3′-*O*-isopropylideneuridine with 1-halogeno-3,3-diethoxypropanes in both aqueous alkali and NaH/Me_2SO.[16]

Scheme II



Scheme III



The possibility of utilizing an adenosine derivative with a good C-5′ leaving group in order to achieve 5′-*O*-alkylation requires, in addition, that the molecule be stabilized against N^3,5′-cyclonucleoside formation.[17] However, only limited success has been realized in efforts to effect efficient inhibition of the intramolecular reactions where the displacement of, for example, iodine at C-5′ of an N^6,N^6-dibenzoyladenosine derivative has involved oxygen nucleophiles.[17a,c]

These considerations prompted a change in synthetic strategy to the less direct approach of first interposing a methylene chain between a ribofuranoside at O-C-5 and the phenolic OH of the estrogen. Condensation of the 5-substituted sugar with adenine in the presence of an appropriate Friedel–Crafts catalyst should then generate the nucleoside.

5-*O*-Alkylation of methyl 2,3-*O*-isopropylidene-5-*O*-triflyl-β-D-ribofuranoside (**7**)[18] with the 4-nitroestrone 3-*O*-(ω-hydroxyalkyl) ethers **6a,b**[19] (Scheme II) was readily effected in benzene containing 18-crown-6 ether and 2 equiv of NaH to give **8a,b**, respectively, in 61–65% yields. Brief treatment (10–20 min) of the 5-substituted methyl β-D-ribofuranosides, **8a,b** with 9:1 trifluoroacetic acid–water at ambient temperatures to remove the 2,3-*O*-isopropylidene group produced, in each case, a mixture of products. Longer reaction times increased the complexity of these mixtures, which may have included the desired

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Table II. Growth Inhibition of MCF-7 and P388 Cells

compd	n	X	MCF-7[a] ID_{30},[c] μM	MCF-7[a] ID_{50},[c] μM	P388[b] ID_{50},[c] μM
4a	2	NO_2	7.8 ± 0.4	10	30 ± 3
4b	3	NO_2	(−20)[e]		nt[d]
4c	4	NO_2	2.1 ± 0.5	4.0 ± 0.9	5 ± 0.5
4d	3	H	(−20)[e]		nt
12a	2	NO_2	11 ± 4	>10	70 ± 7
12b	3	NO_2	3.6 ± 0.4	6.4 ± 0.8	15 ± 1.5
5-fluorouracil			0.2[f]	0.3[f]	nt
4-nitroestrone 3-*O*-methyl ether			>10	>10	nt

[a] The human breast cancer cell line[21] was grown in sterile glass scintillation vials as previously described[23] in medium containing 5% calf serum. Experiments were carried out on cells in the log phase of growth. Compounds were dissolved in Me_2SO and diluted in culture medium so that the final concentration of solvent did not exceed 0.2%. Growth was measured over 2–3 days, and the results represent an average of two or more experiments. [b] Exponentially growing P388 murine leukemia cells, maintained with 10% horse serum + 1 μM mercaptoethanol, were treated with graded levels of drugs, and total cell numbers were determined 24 h later. [c] IC_{30} and IC_{50} are the concentrations required to inhibit growth during the test period by 30 and 50%, respectively. [d] Not tested. [e] A minus sign means that no inhibition of growth was observed at the concentration shown in parentheses. [f] Growth inhibition was measured over a period of 5 days.

deprotected methyl 5-substituted ribofuranosides, but the presence of the latter could not be established. In 70% refluxing acetic acid, **8a** afforded a product (50% yield) to which the ribofuranose structure (**9a**, Scheme III) was assigned based upon the disappearance of the CH_3 ^1H NMR peaks, which were assigned to the methyl glycoside and isopropylidene bonds, respectively of **7a**. It is noteworthy that the migration of **9a**, on TLC, corresponded to the most polar component (~15%) of the mixture derived from the attempted selective deblocking of **8a** in trifluoroacetic acid.

Treatment of **9a** with acetic anhydride in pyridine gave the 1,2,3-tri-*O*-acetate (**10a**, 78% yield), which, on reaction with adenine in acetonitrile catalyzed by $SnCl_4$, gave a product in 64% yield with (UV and ^1H NMR) spectral properties consistent with a 9-β-ribonucleoside 2′,3′-di-*O*-acetate structure (**11a**). These findings are consistent with the report of Saneyosh and Satoh[20] that unprotected 6-substituted purines in $SnCl_4$-catalyzed ribosylation reactions give the 9-β anomer in good yield with no evidence of the formation of either the corresponding α anomer or a 1-, 3-, or 7-riboside. Saponification of **11a** with NH_3/CH_3OH generated the corresponding deprotected estrogen dimethylene-bridged nucleoside, **12a** (68% yield). The same series of reactions applied to **8b** led to the corresponding intermediates, i.e., **9b–11b**, and final product, **12b**, in comparable yields.

Biochemical and Biological Evaluations. The inhibition of the estrogen sulfotransferase reaction was determined with each of the estrogen-bridged adenines (**4**) and adenosines (**12**) with enzyme isolated from bovine adrenals that was purified[3] by successive ammonium sulfate fractionation and column chromatography on DEAE-Sephadex A-50. Modest inhibition of the BAES reaction, relative to **1**, was observed with only those adenine derivatives linked to 4-nitroestrone by either a tri- (**4b**) or tetramethylene chain (**4c**). The importance of the nitro substituent and the need of a chain length in excess of two carbon atoms were indicated by the lack of activity of structures **4d** and **4a**, respectively.

The estrogen-bridged adenosines (**12a,b**) both show a greater capacity to inhibit estrone sulfurylation than **4a–d**. The comparison, however, may be tenuous, as it remains to be established that the same loci of binding accommodates the two classes of inhibitors. Nevertheless, the calculation of K_i values (Table I), in each case, assumes a process of competitive inhibition based upon an analogy with **1**.[1a,7] Accordingly, these constants must, for the present, be regarded as first approximations.

Compounds **4a–d** and **12a,b** were evaluated relative to 5-fluorouracil (5-FU) for in vitro inhibition of growth (ID_{30} and ID_{50}) of the human breast cancer cell line, MCF-7,[21] and the results are given in Table II. The most active of these (**4a,c** and **12a,b**) gave similar ID_{50} values for the inhibition of growth of P388 murine leukemia cells. However, the apparent inactivity of **4b,d** may be due to a problem of solubility. Studies carried out with P388 cells by the soft agar technique for measurement of colony-forming units confirmed the data obtained from growth curves (data not shown). However, inhibition of cell growth by either **4c** or **12b** is approximately one-order of magnitude less than that observed with 5-FU but significantly greater than 4-nitroestrone 3-*O*-methyl ether.

There appears, at this time, no reason to believe that the marked inhibition of growth of both MCF-7 and P388 cells by the two series of compounds involves estrogen sulfotransferase. Whereas the latter has been implicated in human mammary cancer, there is no evidence of its presence in P388 cells. Moreover, the relatively large K_i's (low inhibition) of the BAES reaction by **4** and **12** are not consistent with the effect on growth inhibition of the human mammary cancer cell line. Lastly, the pyrophosphate compound (**1**), a relatively potent inhibitor of the BAES reaction (see Table I), failed to inhibit the growth of MCF-7 cells at 10^{-6} M levels. Studies designed to elaborate the mechanism(s) by which **4** and **12** inhibit cell growth are currently in progress. In this connection, it is perhaps of interest that the structures comprising **4** are analogous to some of the purine acyclic nucleosides that inhibit the deoxythymidine kinase of HSV.[22]

Experimental Section

^1H and ^{13}C NMR spectra were obtained with a JEOL FX 100 Fourier transform spectrometer in the indicated solvent with $(CH_3)_4Si$ as an internal standard. Electron-impact mass spectra were determined with a JEOL JMS 01SG2 with an ionization

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voltage of 70 eV and a source temperature of 250 °C. UV spectra were measured in 95% EtOH with a Beckman Model 25 spectrophotometer.

Elemental analyses were performed by M-H-W Laboratories, Phoenix, AZ. Melting points were obtained on a Thomas-Hoover capillary melting point apparatus and were uncorrected. All solvent evaporations were carried out under reduced pressure in a Büchi rotoevaporator. TLC was carried out with a precoated silica gel F-254 aluminum foil in the following solvents: S_1, toluene/ethyl acetate, 60:40; S_2, MeOH/CH_2Cl_2, 10:90; S_3, MeOH/$CHCl_3$, 10:90; S_4, MeOH/$CHCl_3$, 20:80.

THF, CH_2Cl_2, and C_6H_6 were distilled from $LiAlH_4$, P_2O_5, and benzophenone ketyl, respectively. Me_2SO was distilled from CaH_2 under high vacuum and stored over 4Å molecular sieves. Pyridine was distilled successively from KOH, P_2O_5, and CaH_2. The triply distilled solvent was stored over molecular sieves (4Å).

4-Nitroestrone 3-*O*-(2-Bromoethyl) Ether (3a). To a solution of **2a** (630 mg, 2 mmol) in Me_2SO (15 mL) was added, in portions with stirring, NaH (190 mg, 8 mmol) in the form of a 50% oil dispersion. After 15 min, 1,2-dibromoethane (1.38 mL, 16 mmol) was introduced, and the reaction mixture was stirred at room temperature for 18 h. Excess NaH was destroyed by the addition of MeOH, and the reaction mixture was evaporated at 10^{-3} mm. The oily residue was partitioned between CH_2Cl_2 (100 mL) and H_2O (20 mL), and the dried (Na_2SO_4) organic fraction was evaporated. The product crystallized in the form of yellow needles (600 mg, 71% yield) from benzene–methanol: mp 197–199 °C; ^1H NMR ($CDCl_3$) δ 7.35 and 6.86 (dd, 2, aromatic H_1, H_2), 4.34 (t, 2, ArOCH_2), 3.57 (t, 2 CH_2Br), 2.8–1.5 (m, 15), 0.91 (s, 3, C_{18} CH_3). Anal. ($C_{20}H_{24}BrNO_4$) C, H, Br, N.

4-Nitroestrone 3-*O*-(3-Bromopropyl) Ether (3b). The reaction of **2a** (630 mg, 2 mmol) and 1,3-dibromopropane (1.62 mL) in Me_2SO (15 mL) containing NaH (190 mg, 8 mmol, 50% oil dispersion) was carried out as described above to give a product that crystallized from C_6H_6–MeOH as yellow needles (640 mg, 73% yield): mp 178–180 °C; ^1H NMR ($CDCl_3$) δ 7.32 and 6.84 (dd, 2, aromatic H_1, H_2), 4.04 (t, 2, ArOCH_2), 3.44 (t, 2, CH_2Br), 2.78–1.50 (m, 17), 0.91 (s, 3, C_{18} CH_3). Anal. ($C_{21}H_{26}BrNO_4$) C, H, Br, N.

4-Nitroestrone 3-*O*-(4-Bromobutyl) Ether (3c). The reaction of **2a** (630 mg, 2 mmol) and 1,4-dibromobutane (1.9 mL, 16 mmol) in 15 mL of Me_2SO containing NaH (190 mg, 8 mmol, 50% oil dispersion) was performed as described for **3a**. The product crystallized from C_6H_6–MeOH as needles (620 mg, 69% yield): mp 183–186 °C dec; ^1H NMR ($CDCl_3$) δ 7.35 and 6.86 (dd, 2, aromatic H_1, H_2), 4.34 (t, 2, ArOCH_2), 3.57 (t, 2, CH_2Br), 2.8–1.5 (m, 19), 0.91 (s, 3, C_{18} CH_3). Anal. ($C_{22}H_{28}BrNO_4$) C, H, Br, N.

Estrone 3-*O*-(3-Bromopropyl) Ether (3d). The reaction of **2b** (540 mg, 2 mmol), 1,3-dibromopropane (1.62 mL), and NaH (190 mg, 8 mmol, 50% oil dispersion) in 15 mL Me_2SO was carried out as described for **2a**. The product crystallized from methanol as colorless neeldes (440 mg, 63% yield): mp 98–99 °C; ^1H NMR ($CDCl_3$) δ 7.21–6.66 (m, 3, aromatic H_1, H_2, H_4), 4.08 (t, 2, ArOCH_2), 3.60 (t, 2, CH_2Br), 2.92–1.56 (m, 17), 0.91 (s, 3, C_{18} CH_3). Anal. ($C_{21}H_{27}BrO_2$) C, H, Br.

9-[β-[(17-Oxo-4-nitroestra-1,3,5(10)-trien-3-yl)oxy]ethyl]-adenine (4a). To a suspension of adenine (405 mg, 3 mmol) in THF (10 mL) containing TBAF (5 mmol) was added **3a** (420 mg, 1 mmol), and the mixture was stirred at room temperature for 6 h, at which time TLC (S_3) indicated the complete disappearance of **3a**. The solvent was evaporated, and the residue was partitioned between $CHCl_3$ (200 mL) and H_2O (20 mL). The organic extract was washed with H_2O (3 × 20 mL), dried (Na_2SO_4), and evaporated. The product was obtained as an amorphous powder of indefinite melting point following preparative TLC (S_3). Occasionally, it was necessary to redevelop the preparative plate in the same solvent system to remove the last traces of impurities, which then afforded a product that crystallized from C_6H_6–MeOH as needles (270 mg, 57% yield): mp 246–248 °C dec; UV λ_{max} 262 nm (ε 15960); ^1H NMR (Me_2SO-d_6) δ 8.11 (s, 1, H_8), 8.01 (s, 1, H_2), 7.35, 6.86, and 7.10 (br, 2 NH_2, dd, 2, aromatic, H_1, H_2), 4.29 (t, 2, CH_2N), 4.08 (t, 2, OCH_2), 2.92–1.60 (m, 15), 0.91 (s, 3, C_{18} CH_3). Anal. ($C_{25}H_{28}N_6O_4 \cdot C_6H_6$) C, H, N.

9-[γ-[(17-Oxo-4-nitroestra-1,3,5(10)-trien-3-yl)oxy]-propyl]adenine (4b). The reaction of adenine (405 mg, 3 mmol) and **3b** (435 mg, 1 mmol) in THF (10 mL) containing TBAF (5 mmol) was carried out as described above to give the product as needles (270 mg, 55% yield): mp 257–258 °C dec; UV λ_{max} 262 nm (ε 16950); ^1H NMR (Me_2SO-d_6) δ 8.13 (s, 1, H_8), 8.04 (s, 1, H_2), 7.33 and 6.84 (dd, 2, aromatic H_1, H_2), 7.13 (s, 2, NH_2), 4.32 (t, 2, NCH_2), 4.08 (t, 2, ArOCH_2), 2.92–1.60 (m, 17), 0.92 (s, 3, C_{18} CH_3). Anal. ($C_{26}H_{30}N_6O_4$) C, H, N.

9-[δ-[(17-Oxo-4-nitroestra-1,3,5(10)-trien-3-yl)oxy]butyl]-adenine (4c). The reaction of adenine (405 mg, 1 mmol) in THF (10 mL) containing **3c** (450 mg, 1 mmol) and TBAF (5 mmol) was carried out as described for **4a** to give a product, which crystallized from C_6H_6–MeOH as yellow needles (280 mg, 56% yield): mp 216–219 °C dec; UV λ_{max} 262 nm (ε 15400); ^1H NMR (Me_2SO-d_6) δ 8.10 (s, 1, H_8), 8.03 (s, 1, H_2), 7.36 and 6.90 (dd, 2, aromatic H_1, H_2), 7.16 (s, 2, NH_2), 4.32 (t, 2, $CH_2N<$), 4.08 (t, 2, OCH_2), 2.95–1.60 (m, 19), 0.92 (s, 3, C_{18} CH_3). Anal. ($C_{27}H_{32}N_6O_4$) C, H, N.

9-[γ-[(Estra-1,3,5(10)-trien-3-yl)oxy]propyl]adenine (4d). The reaction of adenine (405 mg, 3 mmol) in THF (10 mL) containing **3d** (390 mg, 1 mmol) and TBAF (5 mmol) was carried out as described for **4a** to give a product of indefinite melting point: UV λ_{max} 264 nm (ε 13900); ^1H NMR (Me_2SO-d_6) 8.13 (s, 1, H_8), 8.02 (s, 1, H_2), 7.23–6.66 (m, 3, aromatic H_1, H_2, H_4), 4.32 (t, 2, $CH_2N<$), 7.10 (s, 2, NH_2), 4.08 (t, 2, OCH_2), 2.92–1.56 (m, 17), 0.92 (s, 3, C_{18} CH_3). Anal. ($C_{26}H_{31}N_5O_2$) C, H, N.

Estrone (270 mg, 1 mmol), dissolved in DMF (10 mL) containing NaH (100 mg, 4 mmol), as 50% oil dispersion was stirred for 30 min. 9-(3-Chloropropyl)adenine[13] (**5**; 250 mg, 1.18 mmol) was added, and the mixture was stirred at room temperature for 18 h. The solvent was removed at 10^{-3} mm, and crude material was chromatographed on a 20 × 2 cm column of silica gel with S_2 as eluent. The purified product (50 mg, 11% yield) gave spectral (UV and ^1H NMR) and TLC (S_2) data identical with that obtained with the analyzed sample of **4d**.

Methyl 2,3-*O*-Isopropylidene-5-*O*-triflyl-β-D-ribofuranoside (7). A solution of methyl 2,3-*O*-isopropylidene-β-D-ribofuranoside (400 mg, 2 mmol) in 20 mL of CH_2Cl_2 containing anhydrous pyridine (200 μL) was treated at −5 °C with triflic anhydride (720 mg, 2.52 mmol) as previously described.[19] After 45 min, the reaction mixture was washed successively with H_2O (2 × 5 mL) and 5% aqueous $NaHCO_3$ and then dried over Na_2SO_4. The solvent was removed, the oily residue, which showed a single spot on TLC (S_1), was dissolved in dry benzene (20 mL), and the solution was frozen for the subsequent reaction.

Methyl 2,3-*O*-Isopropylidene-5-*O*-[β-[(17-oxo-4-nitroestra-1,3,5(10)trien-3-yl)oxy]ethyl]-β-D-ribofuranoside (8a). To a solution of 4-nitroestrone 3-*O*-(2-hydroxyethyl) ether (**6a**;[19] 580 mg, 1.61 mmol) in dry benzene (150 mL) was added sodium hydride (200 mg, 4.2 mmol) in the form of a 50% oil dispersion. The mixture was protected from moisture and was stirred at room temperature for 20 min. The addition of 18-crown-6 ether (560 mg, 2.1 mmol) provided a clear solution to which the (thawed) solution of **7** was added. The reaction was allowed to reach room temperature gradually with stirring, which was maintained for 18 h. The turbid mixture was filtered through Celite, the filtrate was washed first with water (2 × 25 mL) and then with a 5% solution of KCl, and the dried (Na_2SO_4) solution was evaporated to dryness. The residue, on medium-pressure column chromatography (40 × 2 cm) with S_1 gave the product in the form of an off-white foam (0.57 g, 65% yield) after evaporation of the solvent mixture: mass spectrum, m/z 530 (M − CH_3); ^1H and ^{13}C NMR data have been reported previously.[19] Anal. ($C_{29}H_{39}NO_9$) C, H, N.

Methyl 2,3-*O*-Isopropylidene-5-*O*-[γ-[(17-oxo-4-nitroestra-1,3,5(10)trien-3-yl)oxy]propyl]-β-D-ribofuranoside (8b). The reaction of **6b**[19] (600 mg, 1.61 mmol) and **7** (2 mmol) in benzene (150 mL) containing NaH (200 mg, 4.2 mmol) and 18-crown-6 ether (560 mg, 2.1 mmol) was carried out as described above (**8a**) to give the product as a foam (550 mg, 61% yield): mass spectrum, m/z 544 (M − CH_3); ^1H and ^{13}C NMR data have been reported previously.[19] Anal. ($C_{32}H_{45}NO_{10} \cdot 0.5C_4H_8O_2$) C, H, N.

1,2,3-Tri-*O*-acetyl-5-*O*-[β-(17-oxo-4-nitroestra-1,3,5(10)-trien-3-yl)oxy]ethyl]-D-ribofuranose (10a). A solution of **8a** (270 mg, 0.5 mmol) in 70% acetic acid (15 mL) was refluxed for 4 h, after which the reaction mixture was evaporated to dryness. The residue, on TLC (S_1), showed two major spots in addition

to minor impurities. The most polar major constituent (**9a**; 135 mg, 53% yield) was separated by preparative TLC in the same solvent system and, without further characterization, was utilized in the succeeding step. A solution of **9a** (250 mg, 0.5 mmol) in pyridine (10 mL) was stirred with excess acetic anhydride (5 mL) for 18 h. The residual acetic anhydride was destroyed by the dropwise addition of MeOH to the cooled reaction mixture, which was evaporated to near dryness and then coevaporated with toluene (2 × 10 mL) to remove traces of pyridine. TLC (S_2) indicated the residue (240 mg, 78% yield) to be homogeneous: ^{1}H NMR ($CDCl_3$) δ 7.34 and 6.83 (dd, 2, aromatic H_1, H_2), 6.33 (d, H_1, α anomer, ~25%), 6.14 (s, H_1, β anomer, ~75%), 2.09–2.06 (3 s, 9, CH_3CO), 0.91 (s, 3, C_{18} CH_3). Anal. ($C_{31}H_{39}NO_{12}$) C, H, N.

1,2,3-Tri-*O*-acetyl-5-*O*-[γ-[(17-oxo-4-nitroestra-1,3,5(10)-trien-3-yl)oxy]propyl]-D-ribofuranose (10b). The hydrolysis of **8b** (280 mg, 0.5 mmol) in 70% acetic acid (15 mL) was carried out as described above to give **9b** (130 mg, 50% yield). Acetylation of the latter (260 mg, 0.5 mmol) in pyridine (10 mL) with excess anhydride was performed as described for **10a** to give the product in the form of a foam (250 mg, 79% yield), which TLC (S_2) showed to be homogeneous: ^{1}H NMR ($CDCl_3$) δ 7.34 and 6.84 (dd, 2, aromatic, H_1, H_2), 6.39 (d, H_1, α anomer, ~25%), 6.15 (s, H_1, β anomer, ~75%), 2.11–2.05 (3 s, 9, CH_3CO), 0.91 (s, 3, C_{18} CH_3). Anal. ($C_{32}H_{41}NO_{12}\cdot 0.5CH_4O$) C, H, N.

5′-*O*-[β-[(17-Oxo-4-nitroestra-1,3,5(10)-trien-3-yl)oxy]ethyl]adenosine (12a). Adenine (80 mg, 0.4 mmol) was added to a solution of **10a** (200 mg, 0.32 mmol) in anhydrous acetonitrile (50 mL), and the stirred suspension, protected from moisture, was treated with $SnCl_4$ (80 μL, 0.72 mmol). The reaction mixture, which appeared to be homogeneous after 20 min, was stirred at room temperature for 18 h. The solution was concentrated to approximately 5 mL and then treated with an aqueous (1 mL) suspension of $NaHCO_3$ (800 mg). The solvents were then evaporated, and the residue was extracted with $CHCl_3$ (3 × 10 mL). The dried (Na_2SO_4) extract, which showed one major spot on TLC in S_2, was purified by preparative TLC in the same solvent system to give the 2′,3′-di-*O*-acetate derivative (**11a**) in the form of a foam (144 mg, 64% yield): UV λ_{max} 262 nm (ϵ 14 120); ^{1}H NMR (acetone-d_6) δ 8.34 (s, 1, H_8), 8.15 (s, 1, H_2), 7.31 and 6.89 (dd, 2, aromatic H_1, H_2), 6.30 (d, 1, $H_{1'}$, $J_{1',2'}$ = 6.34 Hz), 5.97 (s, 2, NH_2), 5.82 (t, 1, $H_{3'}$), 5.55 (m, 1, $H_{2'}$), 4.37 (t, 1, $H_{4'}$), 4.13 (t, 2, $ArOCH_2$), 3.70 (t, 2, $ArOCH_2CH_2O$), 3.48 (d, 2, $H_{5'}$, $H_{5''}$), 2.13 and 2.04 (2 s, 6 CH_3CO), 2.70–1.49 (m, 15), 0.91 (s, 3, C_{18} CH_3).

Compound **11a** (50 mg, 0.072 mmol) was dissolved in methanolic NH_3, and the solution was maintained at 0 °C for 18 h. The solvent was evaporated, and the residue was dissolved in excess CH_2Cl_2. The solution was washed with water, and the dried (Na_2SO_4) layer was evaporated to dryness. The crude product was purified by preparative TLC with solvent system S_2 to give the product in the form of a foam (30 mg, 68% yield): UV λ_{max} 260 nm (ϵ 13 900); ^{1}H NMR (acetone-d_6) δ 8.23 (s, 1, H_8), 8.21 (s, 1, H_2), 7.38 and 7.03 (dd, 2, aromatic H_1, H_2), 6.06 (d, 1, $H_{1'}$, $J_{1,2}$ = 4.68 Hz), 5.90 (br, 2, NH_2), 4.73 (t, 2, $ArOCH_2$), 4.44 (t, 2, $ArOCH_2CH_2O$), 4.18 (m, 3, $H_{2'}$, $H_{3'}$, $H_{4'}$), 3.70 (m, H_5), 3.03–1.28 (m, 15), 0.91 (s, 3, C_{18} CH_3). Anal. ($C_{30}H_{40}N_6O_{10}\cdot 2H_2O$) C, H, N.

5′-*O*-[γ-[(17-Oxo-4-nitroestra-1,3,5(10)-trien-3-yl)oxy]propyl]adenosine (12b). The reaction of adenine (80 mg, 0.4 mmol), **11b** (200 mg, 0.32 mmol), and $SnCl_4$ (80 L, 0.72 mmol) in acetonitrile was carried out as described above (**11a**) to give **11b** in the form of a foam (140 mg, 62% yield): UV λ_{max} 262 nm (ϵ 13 725); ^{1}H NMR ($CDCl_3$) δ 8.32 (s, 1, H_8), 8.16 (s, 1, H_2), 7.31 and 6.81 (dd, 2, aromatic H_1, H_2), 6.29 (d, 1, $H_{1'}$, $J_{1',2'}$ = 6.30 Hz), 5.97 (s, 2, NH_2), 5.82 (t, 1, $H_{3'}$), 5.53 (m, 1, $H_{2'}$), 4.37 (t, 1, $H_{4'}$), 4.13 (t, 2, $ArOCH_2$), 3.70 (t, 2, $ArOCH_2CH_2CH_2O$), 3.48 (d, 2, $H_{5'}$, $H_{5''}$), 2.11 and 2.04 (2 s, 6, CH_3CO), 2.73–1.52 (m, 17), 0.91 (s, 3, C_{18} CH_3).

Deacetylation of **11b** (50 mg, 0.071 mmol) with CH_3OH–NH_3 was carried out as described above to give **12b** (30 mg, 70% yield) in the form of a foam. Attempts to crystallize this material from a number of solvents, which included ethyl acetate (see analysis), were all unsuccessful: UV λ_{max} 260 nm (ϵ 13 500); ^{1}H NMR (acetone-d_6) δ 8.23 (s, 1, H_8), 8.21 (s, 2, H_2) 7.38 and 7.03 (dd, 2, aromatic H_1, H_2), 6.06 (d, 1, $H_{1'}$, $J_{1',2'}$ = 4.68 Hz), 5.90 (br, 2, NH_2), 4.73 (t, 2, $ArOCH_2$), 4.44 (t, 2, $ArOCH_2CH_2CH_2O$), 4.18 (m, 3, $H_{2'}$, $H_{3'}$, $H_{4'}$), 3.05–1.26 (m, 17), 0.90 (s, 3, C_{18} CH_3). Anal. ($C_{35}H_{36}N_6O_{10}\cdot C_4H_8O_2$) C, H, N.

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Registry No. 2a, 5976-74-9; **2b**, 53-16-7; **3a**, 83876-65-7; **3b**, 83876-66-8; **3c**, 83876-67-9; **3d**, 975-65-5; **4a**, 83876-68-0; **4b**, 83876-69-1; **4c**, 83876-70-4; **4d**, 83876-71-5; **5**, 19255-49-3; **6a**, 10506-66-8; **6b**, 83876-72-6; **7**, 70209-11-9; **8a**, 80082-68-4; **8b**, 80082-69-5; **9a**, 83876-73-7; **9b**, 83876-74-8; **10a**, 83897-15-8; **10b**, 83876-75-9; **11a**, 83876-76-0; **11b**, 83876-77-1; **12a**, 83876-78-2; **12b**, 83876-79-3; 1,2-dibromoethane, 106-93-4; 1,3-dibromoethane, 109-64-8; 1,4-dibromoethane, 110-52-1; adenine, 73-24-5; methyl 2,3-*O*-isopropylidene-β-D-ribofuranoside, 4099-85-8; triflic anhydride, 358-23-6.