source and the hydroxylapatite assay to separate bound steroid from free.[15]

log P Determinations. log P values were estimated from the log k'_w values determined by HPLC chromatography following the recommendations of Minick.[21] The solvents were analytical grade methanol (Fisher), 1-octanol (Aldrich), and n-decylamine and 3-morpholinopropanesulfonic acid (MOPS) (Sigma). The standards were obtained from Aldrich and used without further purification. A Spectra Physics SP8700 chromatographic system equipped with a 7125 Rheodyne injector, a Beckman 254-nm fixed wavelength UV detector and a Hewlett Packard 3390A integrator was used. The stationary phase was a 15 cm × 4.6 mm column packed with 5 μm, 60 Å, Chromegabond C8 silica (ES Industries). The organic portion of the mobile phase was composed of methanol containing 0.25% (v/v) 1-octanol. The aqueous portion was prepared from octanol-saturated water containing 0.02 M MOPS buffer, 0.15% (v/v) n-decylamine and adjusted to pH 7.4. A silica saturator column was included in the line, as well as a guard column packed with C8 silica. The flow was 1 mL/min.

The steroids were dissolved at 1 mg/mL in methanol and ~4 μg was injected onto the column. The standards were also dissolved in methanol, but only ~0.04–0.4 μg were used per injection. Column void volume was estimated from the retention time of uracil, which was included as a nonretained internal reference with each injection.[23] The log k'_w was determined by linear extrapolation of log'ϕ vs ϕ methanol data acquired in the region $0.55 \leq \phi$ methanol ≤ 0.85.

In Vivo Uptake Studies. In vivo uptake studies were performed as previously reported.[4c] Immature, female Sprague-Dawley rats (22–25 day, 50–60 g) were injected under ether anesthesia, via the femoral vein, with ~0.1 mL of the radiopharmaceutical. At the indicated times, the animals were killed by decapitation and samples of blood and tissue were excised, weighed, and immediately assayed for radioactivity in a gamma counter with 60% efficiency. The ^{18}F radiopharmaceutical was prepared for injection by dissolving in ethanol and diluting to the proper dosage with physiological saline, so that the injection dose contained <20% ethanol. In the blocked experiments, 15 μg of estradiol was coinjected with the isotope.

Metabolism Studies. The metabolism of the ^{18}F-estradiols was evaluated by analysis of target and nontarget tissues and blood samples at various times after injection.[25] The ^{18}F compounds were extracted from the homogenized tissues with ethanol and assayed by silica gel thin-layer chromatography, by comparing them to an authentic unlabeled sample of the substituted estrogen. No attempt was made to identify the metabolites.

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Registry No. 1, 129000-37-9; [^{18}F]-1, 129000-38-0; 2, 129000-39-1; [^{18}F]-2, 129000-40-4; [^{18}F]-3, 92817-14-6; 5a, 21375-11-1; 5b, 64109-55-3; 6, 67020-41-1; 7, 17253-50-8; 8, 129098-85-7; 9, 129098-86-8; 10, 129000-41-5; 11a, 129000-42-6; 11b, 129000-43-7; 12a, 129000-44-8; 12b, 129000-45-9; 13a, 129000-46-0; 13b, 129000-47-1; 14a, 129000-48-2; 14b, 129000-49-3; 15a, 129000-50-6; 15b, 129000-51-7; 16a, 129000-52-8; 16b, 129000-53-9; 17a, 129000-54-0; [^{18}F]-17a, 129000-55-1; 17b, 129000-56-2; [^{18}F]-17b, 129000-57-3; 18, 92817-08-8; [^{18}F]-19, 129000-58-4.

Supplementary Material Available: Atomic numbering schemes and tables of atomic coordinates, thermal parameters, bond lengths, and bond angles for compounds 5b and 10 (8 pages). Ordering information is given on any current masthead page.

Synthesis and Estrogen Receptor Binding of Novel 11β-Substituted Estra-1,3,5(10)-triene-3,17β-diols

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As part of our program to develop estrogenic radioligands for use in nuclear medicine, a study was undertaken to investigate the effect of substituents on the receptor affinity of putative radiochemicals. In the study a synthetic strategy directed toward the introduction of an 11β-(fluoroethyl) substituent was devised. The target compound **9** was prepared via a five-step procedure starting from 11β-vinylestrone 3-acetate (**4**) in an overall 43% yield. The stereochemistry of the 11β-vinyl moiety was established by X-ray crystallography. The final product and several analogues, 11β-ethyl-, -vinyl-, and (hydroxyethyl)estradiols (**11**, **5**, and **12**), were evaluated for their estrogen receptor binding affinity. The results indicate that the target compound and several 11β-substituted analogues possess relative binding affinities greater than of estradiol and its 16α-fluorinated derivatives. The manner in which the target compound **9** was prepared is amenable to use with ^{18}F incorporation.

As part of our program for the development of radiopharmaceuticals for the external visualization of estrogen receptor containing human breast cancer, we have previously focused on radioiodinated and brominated derivatives.[1-6] Because of the desirable properties associated with the positron-emitting radionuclide fluorine-18[7,8] we have sought to include in our program ligands capable of incorporating this radionuclide. The clinical potential for agents labeled with this radionuclide was recently demonstrated by the successful noninvasive imaging of human breast cancer with 16α-[^{18}F]-fluoroestradiol.[9,10] Although the images obtained provide a measure of diagnostic in-

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† On leave from the University of Pisa.

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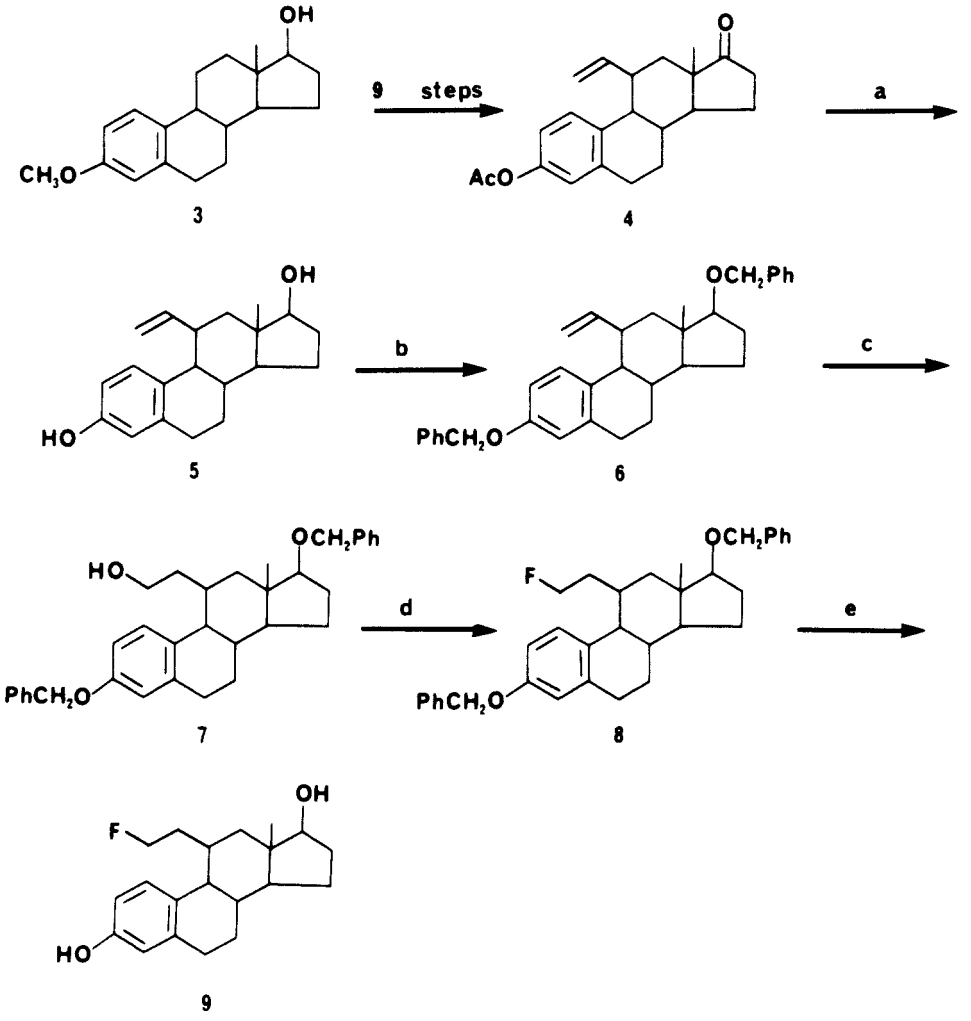
Scheme I



formation, greater clinical utilization will require agents with greater receptor affinity, retention, and metabolic stability. It is with these factors in mind that the current study was undertaken.

Recent investigations by our laboratory[11] and by Katzenellenbogen[12] have illustrated that the introduction of an 11β-ethyl substituent improved the receptor binding properties of the estrogenic ligands 11β-ethyl-17α-(iodovinyl)estradiol and 11β-ethyl-16α-fluoroestradiol compared to the corresponding 17α-(iodovinyl)estradiol and 16α-fluoroestradiol (Figure 1). These results are consistent with the structure–activity relationships previously described[13-16] that demonstrated the marked influence of the

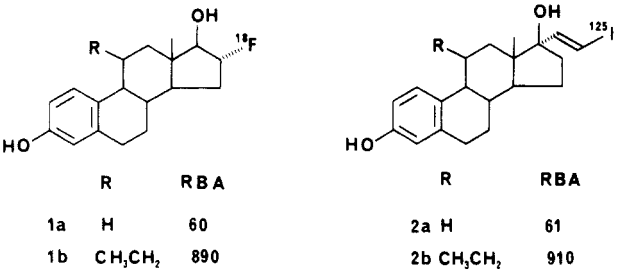


Figure 1.

11β-substituent on estrogen receptor binding.

We elected to situate the fluorine on the terminal carbon of the 11β-ethyl group in order to eliminate problems associated with 17-stereochemistry. For example, 16α-fluoroestra-3,17β-diol possesses reduced binding affinity

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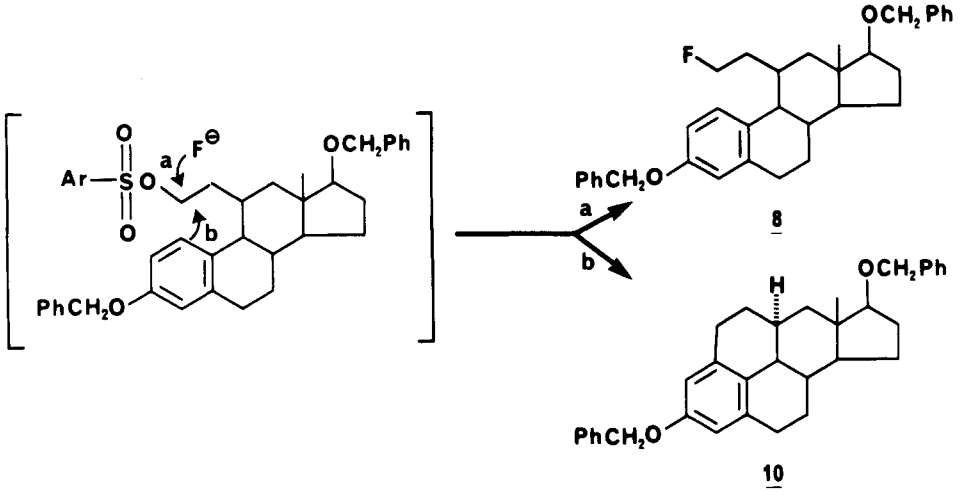
Scheme II



compared to that of the nonfluorinated estradiol and a marked susceptibility toward metabolism at the 17-position. The major concerns in our proposed synthesis involved the efficient preparation of the requisite 11β-vinylestradiol and the subsequent conversion of the intermediate to the desired 11β-(fluoroethyl)estradiol. Previous syntheses of the 11β-ethyl estrogens were long, multistep procedures that gave the target compounds in low overall yields.[20,21] The syntheseis described in this study utilizes the improved preparation of the 11β-vinyl estrogens which we have recently reported.[22] The readily synthesized resultant 11β-(fluoroethyl)estradiol demonstrates receptor binding properties that suggest its potential utility for imaging estrogen receptor positive mammary tumors.

Results and Discussion

Synthesis of 11β-(2-Fluoroethyl)estradiol. The synthesis of 11β-(2-fluoroethyl)estradiol from estradiol 3-methyl ether is illustrated in Scheme I. The nine-step synthesis of the 11β-vinylestrone 3-acetate (overall yield 37%), which can be rapidly performed on a multigram scale, has been reported in a separate communication.[22]

The β-stereochemistry of the 11-vinyl group has been established unabiguously by X-ray crystallography (Figure 2). The 1,3-diaxial substituents are slightly splayed from one another. The mean of the C12–C13–C18 and C14–C13–C18 angles is 113.2° while the C17–C13–C18 angle is 104.1° and the mean of the C_{vinyl}–C11–C angles is 113.1°. However, the C···C transannular distance is still only 3.19 Å. The C_{vinyl}–C11···C13–C18 virtual torsion angle is only 4.1°, indicating no twisting of this portion of the steroidal skeleton. The β-orientation of the 11-vinyl group is oriented sufficiently away from the 18-methyl and 17-carbonyl substituents to exert only minor effects on the reduction of the 17-carbonyl. As a result, the conversion to the 3,17β-diol with sodium borohydride was essentially quantitative. The product crystallized from the diluted reaction solution in a form suitable for the subsequent step. The free 3- and 17β-hydroxyls were protected as the *O*-benzyl ethers with sodium hydride and benzyl chloride in dimethylformamide. The isolated yields of the pure product were >95% (99% crude). The 11β-(2-hydroxyethyl)estradiol was obtained by hydroboration of the alkene using the borane–dimethyl sulfide complex. Oxidation of the intermediate with hydrogen peroxide (30%) in a basic medium gave, upon workup, a 57% yield of the desired product. A reexamination of the reaction suggested that other oxidants, such as sodium perborate,[23a] or sodium percarbonate,[23b] may improve the yield in this step.

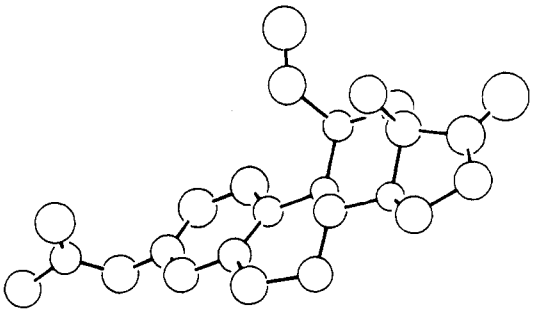


Figure 2.

The incorporation of the fluoride presented two challenges. The first involved avoidence of an electropositive center on the terminal carbon because this would lead to intramolecular cyclization. The use of diethylamidosulfur trifluoride (DAST), a common reagent used in the conversion of alcohols to alkyl fluorides, gave a high yield of the cyclized material **10**[24a,b] and little of the fluoroethyl product. The second challenge involved establishment of conditions which could be ultimately translated to a radiochemical synthesis involving high specific activity ^{18}F.

A solution to these two problems is demonstrated in synthetic Scheme II. Conversion of the 11β-(hydroxyethyl)estradiol to the 11β-(fluoroethyl)estradiol was achieved in 2 h with *p*-toluenesulfonyl fluoride and tetrabutylammonium fluoride in THF at reflux. The isolated yield for this step was 78%. The subsequent removal of the benzyl protecting group was accomplished essentially quantitatively within 15 min.

The conditions of the first step required *p*-toluenesulfonyl fluoride to give hydrogen fluoride as an initial byproduct. An excess of tetrabutylammonium fluoride ensured the conversion of the intermediate tosylate to the

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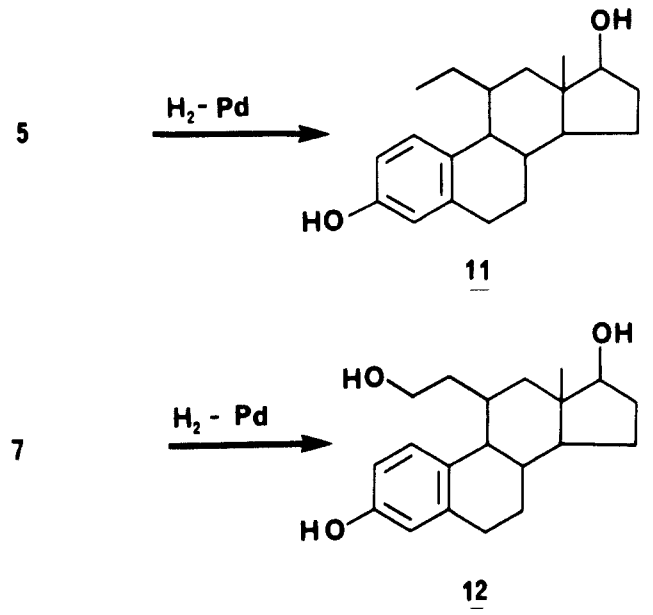
Scheme III



desired fluoroethyl product under conditions that favored substitution rather than intramolecular cyclizations as observed with DAST. Careful formation and isolation of the alkyl sulfonate intermediate would permit the use of high specific activity $Bu_4N[^{18}F]$ for the second half of the reaction sequence. The subsequent debenzylation step with hydrogen (atmospheric pressure) and colloidal palladium, formed in situ, would be compatible with a radiolabeling procedure with respect to time and facility constraints.

The 11β-ethyl and 11β-(hydroxyethyl)estradiols for the binding studies were prepared by hydrogenation of **5** and **7**, respectively (Scheme III).

Estrogen Receptor Binding of 11β-(Fluoroethyl)-estradiol and Analogues. The binding affinity of 11β-(fluoroethyl)estradiol, as well as 11β-vinyl-, 11β-ethyl-, and 11β-(hydroxyethyl)estradiols (**5**, **11**, **12**), was measured by a competitive radiometric binding assay using rat and lamb uterus as sources of the estrogen receptor, [^{3}H]estradiol as tracer (Figure 3, parts a and b), and dextran-treated charcoal as adsorbent for free ligand.[25,26] The relative binding affinities (RBA) for the rat uterine receptor were determined at 0 and 25 °C to observe whether the ligands were slow to reach equilibrium (RBA at 25 °C > RBA at 0 °C).[13] The binding affinities of the estrogens prepared in this study were determined from the competitive displacement curves and are shown in Table I. For comparison purposes, the relative binding affinities for 16α-fluoroestradiol and 11β-ethyl-16α-fluoroestradiol, previously reported by Pomper et al.,[12] are included.

As the results indicate, all of the 11β-substituted estrogens demonstrate moderate to high estrogen receptor affinity at 0 °C in both the lamb and rat receptor preparations, RBA = 14–110% in lamb and 13.5–123% in rat. The order of RBA values correlated directly with the relative lipophilicity of the 11β-substituent, CH_2CH_2OH (13.5–14%) < CH_2CH_2F (71–78%) < $CH{=}CH_2$, CH_2CH_3 (110–123%). Of perhaps greater significance, the RBA values for all the 11β-substituted estradiols increased dramatically when the receptor assay was conducted at 25 °C rather than 0 °C. Such observations are associated with estrogens that approach equilibrium binding more slowly than estradiol. Therefore, when the 25 °C values are considered, the compounds prepared in this study dem-

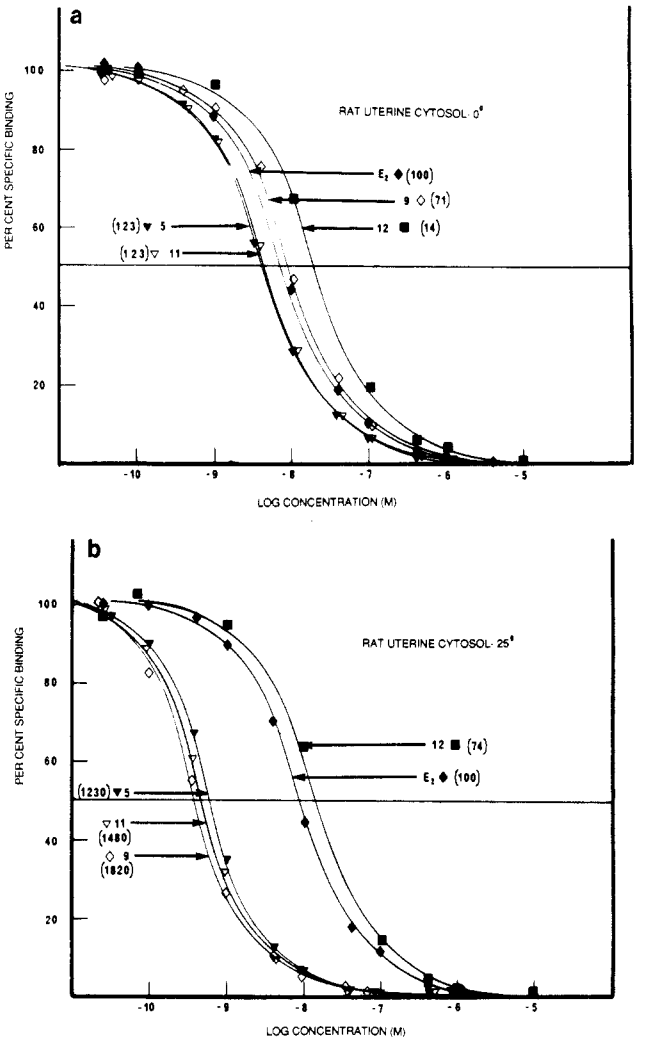
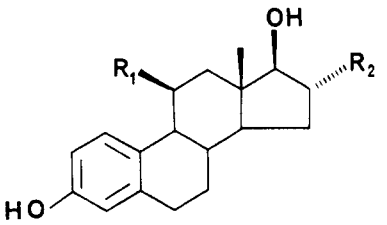


Figure 3.

Table I. Relative Binding Affinities (RBA) of Estrogenic Ligands for Estrogen Receptors from Lamb and Rat Uterine Cytosol



			RBA[a]		
				rat	
compd	R_1	R_2	lamb; 0 °C	0 °C	25 °C
estradiol	H	H	100	100	100
11	C_2H_5	H	110	123	1490 (1120)[b]
5	$CH_2{=}CH$	H	110	123	1230
12	$HOCH_2CH_2$	H	14	13.5	74.1
9	FCH_2CH_2	H	78	71	1820
1a	H	F			60[b]
1b	C_2H_5	F			890[b]

[a] The relative binding affinity (RBA) values are determined by competitive radiometric binding assays, according to previously described procedures (refs 25 and 26). [b] RBA values published in ref 12.

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onstrate relative binding affinities comparable to (for R = CH_2CH_2OH) or much greater than that of estradiol, the highest value being that of the target compound 11β-(fluoroethyl)estradiol.

The receptor binding effects of the 11β-(fluoroethyl) group can also be seen by comparison with the RBA values reported for 16α-fluoro-, 16α-fluoro-11β-ethyl-, and 11β-ethylestradiol.[12] The introduction of the 16-fluoro substituent reduced the RBA value for the parent estradiol, e.g., 100% → 60% for estradiol and 1120–1480% → 890% for 11β-ethylestradiol. Placement of the fluoro substituent on the 11β-ethyl moiety avoided this effect and instead provided a modest increase in receptor affinity, 1120–1480% → 1820%. Therefore, simply on the basis of receptor binding, the 11β-(fluoroethyl) group would appear to be the best of the potential receptor-directed ligands.

In preparing and evaluating radioligands for detecting estrogen-responsive tissues, other factors require consideration. Among these are the ease of radiosynthesis and purification, which this study has attempted to address prospectively, the binding to nonspecific proteins, which can be estimated by the calculation of agents lipophilicity relative to estradiol,[27] and in vivo metabolic stability of the radioligands.[27,28] The lipophilicity of compound **9** would be approximately equal to that of the previously reported 16α-fluoro-11β-ethylestradiol and therefore should share comparable nonspecific-binding properties. The effect of the placement of the fluorine on the 11β-ethyl group on in vivo metabolism is more problematic, both with respect to the metabolism of the fluoroethyl moiety and of the steroidal D ring. One may anticipate that the oxidation of the 17β-hydroxyl would be unaffected; however, this oxidation, which reduces the receptor affinity of the metabolite, would not result in the loss of fluoride (from the resultant fluoro ketone). These aspects must await the evaluation of studies employing the radiolabeling of the precursor with ^{18}F and the subsequent in vivo evaluation. This work is currently being undertaken.

Experimental Section

General Methods. Melting points were determined on a Meltemp apparatus using open capillaries and are uncorrected. Flash chromatography was performed according to Still.[28] Solvents are indicated parenthetically. Proton nuclear magnetic resonance (^1H NMR) spectra were obtained with a Varian XL 300 (300-MHz) spectrometer; chemical shifts are reported in ppm downfield from a tetramethylsilane internal standard. Optical rotations were obtained with a Perkin-Elmer Model 141 polarimeter.

11β-(1-Ethenyl)estra-1,3,5(10)-triene-3,17β-diol (5). A mixture of **4** (2.0 g, 5.9 mmol) and $NaBH_4$ (0.22 g, 5.3 mmol) in methanol (20 mL) was stirred at ambient temperature for 2 h. To the homogeneous solution was added 2 mL of 10 N NaOH and the resultant solution was stirred for 7 h. The reaction was acidified by the addition of acetic acid (4 mL) and then diluted with water (25 mL). The product precipitated from the solution and was collected as off-white needles (1.7 g, 100%): mp 196–198 °C; $[\alpha]^{25}_D$ +90.0° ($c = 6 \times 10^{-4}$, THF); ^1H NMR (DMSO-d_6) δ 0.69 (s, 3 H, C(18)H), 1.05–2.80 (m, 14 H, steroid nucleus), 3.50 (dt, J_d = 5.0, J_t = 7.5 Hz, 1 H, C(17)H), 4.45 (d, J = 5.0 Hz, 1 H, C(17)OH), 4.85 (d, J = 10.5 Hz, 1 H), 4.96 (d, J = 18.0 Hz, 1 H), 5.66 (ddd, J = 18.0, 10.5, 7.5 Hz, 1 H), 6.40 (d, J = 2.5 Hz, 1 H, C(4)H), 6.45 (dd, J = 2.5, 8.5 Hz, 1 H, C(2)H), 6.83 (d, J = 8.5 Hz, 1 H, C(1)H). Anal. ($C_{20}H_{26}O_2H_2O$) C, H.

11β-(1-Ethenyl)estra-1,3,5(10)-triene-3,17β-diol 3,17-Bis(benzyl ether) (6). Sodium hydride (1.1 g of 60% oil dispersion, 27.5 mmol) was washed free from oil with hexane under nitrogen. To the dry sodium hydride were added dimethyl formamide (8 mL), **5** (1.35 g, 4.6 mmol), and benzyl chloride (1.2 mL, 10.4 mmol) and the mixture was stirred under nitrogen at ambient temperature for 16 h. The excess sodium hydride was decomposed by the dropwise addition of water and the mixture was partitioned between ether and water. The organic phase was washed with water, brine, dried over $MgSO_4$ (anhydrous), filtered, and evaporated to give **6** (2.18 g, 98.5%) as an oil that solidifed on standing: mp 62–64 °C; $[\alpha]^{25}_D$ +74.7° ($c = 5.4 \times 10^{-3}$, EtOAc); ^1H NMR ($CDCl_3$) δ 0.90 (s, 3 H, C(18)H), 1.10–1.85 (m, 7 H), 1.90 (b d, J = 12.5 Hz, 1 H), 1.95–2.07 (m, 1 H), 2.29 (dd, J = 2.0, 13.0 Hz, 1 H), 2.46 (dd, J = 4.5, 10.5 Hz, 1 H), 2.70–2.92 (m, 2 H), 3.27 (m, 1 H), 3.46 (t, J = 8.0 Hz, 1 H, C(17)H), 4.55 (s, 2 H), C-(17)OCH_2), 4.96 (d, J = 10.5 Hz, 1 H), 4.98 (s, 2 H, C(3)OCH_2), 5.03 (d, J = 18.0 Hz, 1 H), 5.73 (ddd, J = 7.5, 10.5, 18.0 Hz, 1 H), 6.67 (d, J = 2.5 Hz, 1 H, C(4)H), 6.74 (dd, J = 2.5, 8.5 Hz, 1 H, C(2)H), 7.00 (d, J = 8.5 Hz, 1 H, C(1)H), 7.20–7.45 (m, 10 H). Anal. ($C_{34}H_{38}O_2$·0.5H_2O) C, H.

11β-(2-Hydroxyethyl)estra-1,3,5(10)-triene-3,17β-diol 3,17-Bis(benzyl ether) (7). To a solution of **6** (0.76 g, 1.59 mmol) in hexane–benzene (5.1, 5 mL) under nitrogen in an ice bath was added dropwise borane–dimethyl sulfide complex (0.3 mL, 3 mmol). The mixture was allowed to warm to ambient temperature and was stirred for 3 h. Ethanol (4 mL), 3 N NaOH (1.3 mL), and 30% H_2O_2 (1.5 mL) were added, and the mixture was heated at reflux for 1 h. The solution was cooled and partitioned between ether and water. The organic phase was dried over $MgSO_4$ (anhydrous), filtered, and evaporated to dryness. The residue was purified by column chromatography on silica gel using hexane–ethyl acetate (3:2) as the eluent to give pure **7** (0.45 g, 57%) as an oil: $[\alpha]^{25}_D$ +70.7° ($c = 2.0 \times 10^{-2}$, EtOAc); ^1H NMR ($CDCl_3$) δ 0.99 (s, 3 H, C(18)H), 1.05–1.75 (m, 7 H), 1.84 (b d, J = 12.5 Hz, 1 H), 1.90–2.05 (m, 1 H), 2.22 (d, J = 13.5 Hz, 1 H), 2.46–2.86 (m, 4 H), 3.44 (t, J = 8.0 Hz, C(17)H), 3.52–3.85 (m, 2 H, CH_2OH), 4.54 (s, 2 H, C(17)OCH_2), 4.96 (s, 2 H, C(3)OCH_2), 6.67 (d, J = 2.5 Hz, 1 H, C(4)H), 6.75 (dd, J = 2.5, 8.5 Hz, 1 H, C(2)H), 7.07 (d, J = 8.5 Hz, 1 H, C(1)H), 7.20–7.45 (m, 10 H). Anal. (C_{34}-$H_{40}O_3$·0.25H_2O) C, H.

11β-(2-Fluoroethyl)estra-1,3,5(10)-triene-3,17β-diol 3,17-Bis(benzyl ether) (8). A mixture composed of **7** (0.32 g, 0.65 mmol), tetrabutylammonium fluoride (2 mL of 1 M solution in THF), *p*-toluenesulfonyl fluoride (0.22 g, 1.26 mmol), molecular sieves (4 Å, 3 g), and THF (5 mL) was heated at reflux for 2 h. The molecular sieves were removed by filtration and washed with ethyl acetate. The organic phase was stirred for 2 h with a 10% $NaHCO_3$ solution containing a few drops of pyridine, washed with brine, dried over $MgSO_4$ (anhydrous), filtered, and evaporated to dryness. Purification of the residue by column chromatography on silica gel (hexane–ethyl acetate 10:1) gave pure **8** (0.25 g, 78%) as an oil: $[\alpha]^{25}_D$ = +63.0° ($c = 9.2 \times 10^{-3}$, EtOAc); ^1H NMR ($CDCl_3$) δ 0.99 (s, 3 H, C(18)H), 1.15–1.80 (m, 9 H), 1.87 (b d, J = 12.5 Hz, 1 H), 1.95–2.11 (m, 1 H), 2.28 (dd, J = 13.5, 1.5 Hz, 1 H), 2.52–2.88 (m, 4 H), 3.48 (t, J = 8.0 Hz, 1 H, C(17)H), 4.50 (b d, J = 47.5 Hz, 2 H, CH_2F), 4.57 (s, 2 H, C(17)OCH_2), 5.01 (s, 2 H, C(3)OCH_2), 6.69 (d, J = 2.5 Hz, 1 H, C(4)H), 6.79 (dd, J = 2.5, 8.5 Hz, 1 H, C(2)H), 7.07 (d, J = 8.5 Hz, 1 H, C(1)H), 7.20–7.50 (m, 10 H). Anal. ($C_{34}H_{39}FO_2$) C, H.

11β-(2-Fluoroethyl)estra-1,3,5(10)-triene-3,17β-diol (9). A solution composed of **8** (0.15 g), $PdCl_2(CH_3CN)_2$ (10 mg), ethanol (10 mL), and ethyl acetate (10 mL) was stirred in the presence of hydrogen. The solution rapidly changed from yellow to a gray suspension, and subsequently a black solid precipitated from the colorless solution (15 min). The mixture was filtered through Celite and the filtrate was evaporated to dryness. The residue was purified by column chromatography on silica gel (EtOAc–hexane 1:1) to give pure **9** (0.095 g, 100%): mp 228–232 °C; $[\alpha]^{25}_D$ = +136.2° ($c = 6.9 \times 10^{-4}$, THF); ^1H NMR (DMSO-d_6) δ 0.80 (s, 3 H, C(18)H), 1.05–1.65 (m, 9 H), 1.78 (b d, J = 12.5 Hz, 1 H), 1.86 (m, 1 H), 2.06 (d, J = 13.5 Hz, 1 H), 2.40–2.76 (m, 4 H), 3.50 (m, 1 H, C(17)H), 4.47 (dt, J_t = 6.5 Hz, J_d = 47.5 Hz, 2 H, CH_2F), 4.51 (b s, 1 H, C(17)OH), 6.43 (d, J = 2.5 Hz, 1 H, C(4)H), 6.54 (dd, J = 2.5, 8.5 Hz, 1 H, C(2)H), 6.93 (d, J = 8.5 Hz, 1 H, C(1)H), 9.00 (s, 1 H, C(3)OH). Anal. ($C_{20}H_{27}FO_2$) C, H.

11β-(2-Hydroxyethyl)estra-1,3,5(10)-triene-3,17β-diol (12). A solution composed of **7** (0.33 g, mmol), $PdCl_2(CH_3CN)_2$ (0.02

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g), ethanol (5 mL), and ethyl acetate (5 mL) was stirred under a hydrogen atmosphere until a black solid precipitated from the colorless solution (15 min). The mixture was filtered through Celite and the filtrate was evaporated to dryness. Trituration with ether gave pure **12** (0.15 g), 71%) as colorless crystals: mp 218-220 °C; $[\alpha]^{25}_D$ = +122.9° (c = 1.2 × 10^{-3}, THF); ^{1}H NMR (DMSO-d_6) δ 0.81 (s, 3 H, C(18)H), 1.09-1.62 (m, 8 H), 1.73-2.08 (m, 3 H), 2.36-2.76 (m, 5 H), 3.50 (m, 3 H, C(17)H, CH_2O), 4.26 (t, J = 5.5 Hz, CH_2OH), 4.48 (d, J = 5.0 Hz, CHOH), 6.41 (d, J = 2.5 Hz, 1 H, C(4)H), 6.52 (d, d, J = 2.5, 8.3 Hz, 1 H, C(2)H), 6.94 (d, J = 8.5 Hz, 1 H, C(1)H), 8.93 (s, 1 H, C(3)OH). Anal. ($C_{20}H_{28}O_3$·0.25H_2O) C, H.

X-ray Crystal-Structure Analysis of 3-Acetoxy-11β-(1-ethenyl)estra-1,3,5(10)-trien-17-one (4). A crystal of **4** ($C_{22}H_{26}O_3$) suitable for X-ray diffraction studies (0.20 × 0.20 × 0.80 mm) was grown from an ether solution. The compound crystallized in the orthohombic space group $P2_12_12_1$ with cell dimension of a = 10.407 (3) Å, b = 11.155 (3) Å, c = 15.773 (4) Å and with Z = 4; the calculated density was 1.228 g cm^{-3}. Of the 1468 reflections collected ($1 \le 2\theta \le 45°$) on a Syntex $P2_1$ automated diffractometer (λ_{MoK} = 0.71069 Å) using a $\theta/2\theta$ scan, 849 were considered observed ($I > 2\sigma(I)$). The structure was solved with the direct methods package MITHRIL and refined by using full-matrix least-squares techniques.[29] Hydrogen atoms were assigned isotropic thermal factors of 6.5 Å^3. The function $w(|F_o| - |F_c|)^2$ was minimized with $w^{1/2}$ = 1 for $|F_o| \le 15$ and $15/|F_o|$ for $|F_o| > 15$; R = 0.062; w_R = 0.070; $(\Delta/\sigma)_{max}$ = 0.23; $(e/Å^3)_{max}$ = 0.3. No short intermolecular contacts were observed. Tables II-V, containing final atomic positional parameters, anisotropic thermal parameters for the non-hydrogen atoms, and bond lengths and angles, are available as supplementary material.

(29) The following crystallographic programs were used: MITHRIL (Gilmore, C. J., Department of Chemistry, University of Glasgow, Glasgow, Scotland), FMLS (Ganzel, P. L.; Sparks, R. A.; Trueblood, K. N., UCLA, Los Angeles, CA), and ORTEP (Johnson, C. K., Oak Ridge National Laboratory, Oak Ridge, TN, 1976).

Competitive Receptor Binding Assay. All cytosols for the estrogen receptor were prepared and stored in TEA buffer (0.01M Tris-Hcl-0.0015 M EDTA-0.02% sodium azide pH 7.4 at 25 °C). Rat uterine cytosol was prepared from Holtzman rats (21-25 day oil females) and stored in liquid nitrogen. Lamb uterine cytosol was prepared and stored as described by Katzenellenbogen et al.[25] The competitive receptor binding assays were performed as previously described[25,26] and the results were tabulated as relative binding affinities RBA relative to estradiol (RBA = 100).

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Supplementary Material Available: Tables of the atomic positional parameters for non-hydrogen atoms and for hydrogen atoms, anisotropic thermal parameters for the non-hydrogen atoms, and bond lengths and angles (6 pages). Ordering information is given on any current masthead page.

Arabinofuranosylpyrrolo[2,3-*d*]pyrimidines as Potential Agents for Human Cytomegalovirus Infections

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Protection of the 3′- and 5′-hydroxyl groups of the nucleoside antibiotic toyocamycin (1) with 1,3-dichloro-1,1,3,3-tetraisopropyldisiloxane was followed by (trifluoromethyl)sulfonylation of the 2′-hydroxyl group. A displacement of the resulting triflate ester moiety with lithium chloride, lithium bromide, sodium iodide, and lithium azide in hexamethylphosphoramide was followed by a removal of the disilyl moiety with tetra-*n*-butylammonium fluoride to afford the appropriate (2′-deoxy-2′-substituted-arabinofuranosyl)toyocamycin analogues **6a-d**. Hydrolysis of the carbonitrile moieties of **6a-d** with hydrogen peroxide gave the corresponding sangivamycin analogues (**7a-d**). A reduction of the azido moiety of **6a** and **7a** with 1,3-propanedithiol furnished the corresponding amino derivatives (**6e** and **7e**). The antiproliferative activity of **6a-e** and **7a-e** was evaluated in L1210 cell cultures. None of these compounds caused significant inhibition of cell growth. Evaluation of these compounds for antiviral activity showed that all the toyocamycin analogues were active against human CMV, but of the sangivamycin analogues, only (2′-deoxy-2′-azidoarabinosyl)sangivamycin (**7a**) was active against this virus. None of the compounds were active against HSV-1 or HSV-2. (2′-Deoxy-2′-aminoarabinofuranosyl)toyocamycin (**6e**) was studied more extensively and showed some separation between antiviral activity and cytotoxicity as measured by effects on DNA synthesis, cell growth, and cell-plating efficiency. Although **6e** also was active against murine CMV in vitro, it was not active against this virus in infected mice. We conclude that arabinosylpyrrolopyrimidines have potential as antivirals, but no members of the current series are potent enough to show significant activity in vivo.

The development of agents to control human cytomegalovirus (HCMV) has become increasingly important because of its significant contribution to the mortality of AIDS patients.[1-10] The potential of currently available

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