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THIS IS TO CERTIFY THAT THE THESIS PREPARED UNDER MY SUPERVISION BY

Andrea Marie Thieme

ENTITLED Development of Fluorine-18 Labeled Estrogens, Progestins, and Corticosteroids as Potential Receptor-Based Imaging Agents for Positron Emission Tomography

IS APPROVED BY ME AS FULFILLING THIS PART OF THE REQUIREMENTS FOR THE

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Development of Fluorine-18 Labeled Estrogens, Progestins, and Corticosteroids as Potential Receptor-Based Imaging Agents for Positron Emission Tomography

By

Andrea Marie Thieme

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Introduction

Steroid hormones and receptors:

Steroid hormones are an important class of regulatory ligands which involve intracellular receptor sites that interact with the genome. There are three main types of steroid hormones: the sex steroids, which are further subdivided into androgens, progestins, and estrogens, adrenal steroids, which consist of the glucocorticoids and the mineralocorticoids, and vitamin D_3.[1] Metabolic transformations of these steroids, especially of the androgens, are important modes of regulation as these transformations produce either more or less active metabolites. Vitamin D_3's main role is calcium metabolism and bone development and its major transformation to an active metabolite occurs in the kidney and the liver. The sex steroids, as the name implies, regulate the development of secondary sex characteristics, and are also capable, with metabolic transformation in the nervous system, of governing neuronal excitability and dopamine release in the brain.[2] Corticosteroids too play a very significant role in the central nervous system. Corticosteroid receptors are divided into three main types, the mineralocorticoid and corticosterone-preferring (Type I) and the glucocorticoid receptors (Type II). The importance of the adrenal steroids lies in their ability to mediate the stress response and maintain mineral balance (see Figure I). The mechanism of steroid hormone action is not definitive at this time. When circulating in the blood, steroids are generally attached to steroid binding proteins. The steroid then diffuses through the target cell membrane either passively or by facilitated transport. It is not known[3] whether the location of the steroid receptors is nuclear or cytoplasmic. There are several theories regarding the effects of the steroid binding to the receptor, one of which is that the binding induces a conformational change in the DNA strand to allow transcription to commence.[4]

Figure I.

CORTICOSTEROID RECEPTORS IN RAT BRAIN

Type I

MINERALOCORTICOID (MR)

Affinity: Aldosterone (K_d~0.5-3 n*M*), ~corticosterone (CORT)
Localization: Circumventricular organs
Function: Control of salt appetite

CORTICOSTERONE - PREFERRING (CR)

Affinity: Corticosterone (K_d~0.5 n*M*), aldosterone > dexamethasone
Localization: Hippocampus CA_1 > dentate gyrus > CA_3 > lat. septum
Function: Mediates tonic influences of CORT, index of brain aging

Type II

GLUCOCORTICOID (GR)

Affinity: Dexamethasone > corticosterone (K_d~5.0 n*M*) > aldosterone
Localization: Hippocampal, thalamic, hypothalamic, cortical regions, glia
Function: Mediation of stress response

Steroid hormones and breast cancer:

For breast cancer, the receptors which have the most clinical significance are the progestin (PgR) and the estrogen receptors (ER). The presence or absence of these steroid receptors in a malignant breast tumor may be used as a prognostic tool and a guide to therapy.[5] Currently, ER presence or absence is what is usually assessed and the typical method of detection used is the DCC (dextran-coated charcoal) method, which involves incubating a cell homogenate from biopsied tissue with radiolabelled (with tritium or I-125) hormone.[6] Theoretically, the presence of ER indicates that the patient will respond to endocrine therapy, such as treatment with an antiestrogen like TAM (tamoxifen), however, the actual response rate of patients with ER^+ tumors is only 50-55%.[7,8] ER-negativity is a better indicator, as only 10% of ER^- tumors respond favorably.[9] The commonly accepted reasons for the lack of

correlation are heterogeniety of receptor distribution in the cells of the tumor[10] and the uncertainty of the souce of the receptor in the biopsy tissue, i.e. whether it is the tumor or surrounding epithelium. The DCC method can be improved by testing for PgR-positivity as PgR content is a better prognostic test than ER content, and when both test are used the correlation between PgR and ER-positivity and endocrine therapy responsiveness becomes more acceptable. However, the DCC method also suffers from other disadvantages, for example small tumors may not provide enough tissue for an accurate assay and the analyzed tissue is consumed in the assay. Breast tumors may evolve from ER^+ to ER^- during the course of the illness, and ER content can vary with the menstrual cycle, which often requires many assays to be performed.[11] The need to execute the assay repeatedly makes the relative invasiveness (need for a biopsy) of the DCC method even less desirable.

Positron emission tomography and breast cancer:

PET is a noninvasive, *in vivo* imaging method which employs radiotracers that are injected into the patient's bloodstream and the distribution of the tracer is measured by external detectors. A positron-emitting nuclide (usually ^{11}C, ^{13}N, ^{15}O, or ^{18}F) is introduced into the desired ligand by radiochemical synthesis. The short half-lives of these isotopes requires a cyclotron to produce the radionuclides on demand and a brief synthesis of the tracer; however, the advantage of the short half-life is that the patient receives a limited exposure to the radiation. Positron emission occurs when there is a conversion of a proton to a neutron in a neutron-deficient nucleus. The positron possesses the properties of an electron except that it is positively charged, and when it is combined with an electron, a matter-antimatter annihilation occurs with the release of energy in the form of two high energy photons. The high energy photons are detected by coincidence circuits placed around the patient. The nature of the positron decay, which produces two photons which leave the annihilation site in opposite directions, allows for signal processing to generate good resolution.

Many radiopharmaceuticals have been developed for use in PET, but most are either nonspecific ligands, such as $H_2{}^{15}O$ for the evaluation of flow-related phenomena, or target brain receptor systems such as the dopaminergic, muscarinic, and opiate systems.[12] Another approach is to use radiolabelled steroid hormones as the tracers, which utilizes the selective binding of these hormones to receptors to direct the ligand to a specific target tissue. For instance, ^{18}F-labelled estrogens in conjunction with PET can be used for assessment of steroid receptors in human breast carcinoma.[13] A method using estrogens and progestins is superior to the aforementioned DCC assay in that it is a noninvasive and performed *in vivo*. Ideally, all the steroid hormone receptors systems could be exploited for various clinical diagnoses with PET by the use of the appropriate radiolabelled steroid hormone. For example, a radiolabelled androgen could be used to assess prostatic carcinoma, or a corticosteroid could aid in the diagnosis of pituitary disorders.

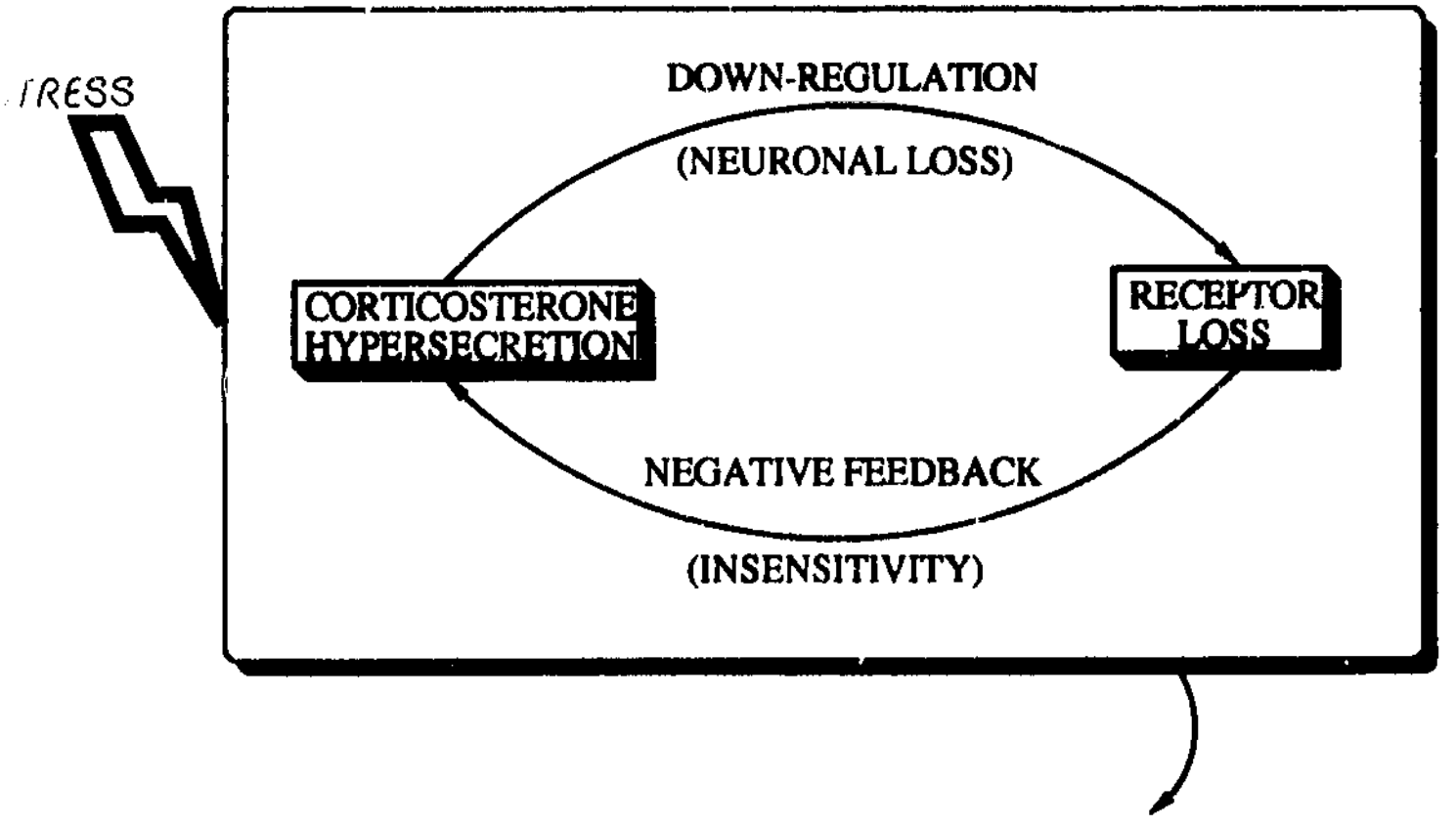
Alzheimer's dementia (AD) and steroid hormones:

AD is the most common form of dementia in late life as it exists in approximately 7% of the population over age 65, and accounts for two-thirds of the cases of senile dementia.[14] Outwardly, the signs of AD are loss of cognitive functions, most markedly in memory, language, and orientation functions, although in the later stages of the disease personality, judgment, problem-solving, calculation, and motor abilities may be affected. A general observation of the AD brain shows marked atrophy, particularly in the hippocampus and the cortex. Microscopic examination reveals the histopathological hallmarks of the disease: the neuritic plaques, the neurofibrillary tangles, and granulovacular degeneration. These lesions are found in distinct areas of the brain, primarily in the cortex or limbic system, and secondarily in the hippocampus, amygdala, and the nuclei providing the cholinergic interaction between the cortex and the hippocampus (such as the basal nucleus of Meynert).[15]

Many theories as to the cause(s) of AD have been advanced (a brief discussion of which are described elsewhere)[16] such as viral injection,[17] aluminosilicate toxicity,[18] lack of trophic factors,[19] and excessive production of excitotoxic amino acid neurotransmitters such as glutamate.[20] The mechanism of the latter would involve the action of these amino acids on neurons weakened by continuous exposure to hypersecretion of endogenous glucocorticoids.[21,22] If this were the case, the areas most affected by the neurotoxicity of elevated glucocorticoid levels are those containing glucocorticoid receptors, i.e. primarily in the hippocampus.[23] The hippocampus also utilizes glutamate and is responsible for feedback inhibition of the production of corticosteroids. The result of the loss of hippocampal neurons is thus a disinhibition of glucocorticoid receptors by unabated production of corticosteroid leading to even higher concentrations of glucocorticoids, and leading to a "feed-forward cascade"[21] of neuronal death (see Figure II). Because AD destroys these corticosteroid receptors, if appropriate ^{18}F-labelled mineralocorticoids and glucocorticoids are synthesized, the amounts of Type I and Type II corticosteroid binding proteins destroyed could be determined using PET, providing a diagnostic test for AD. Analogous to the PET scan for a breast tumors, PET with corticosteroids would provide a non-invasive, *in vivo* test for the disease, and because the brain is the area affected by AD, the non-invasiveness becomes imperative. The area of the brain visualized would depend on the type of corticosteroid used, primarily the hippocampus with the mineralocorticoids, and a more general image with the glucocorticoids. The technique of using receptor binding sights in the brain to binding an imaging agent might also be useful in other dementia that affect a brain receptor system, such dopamine receptors in Parkinson's dementia.[24]

Figure 1

THE GLUCOCORTICOID CASCADE HYPOTHESIS



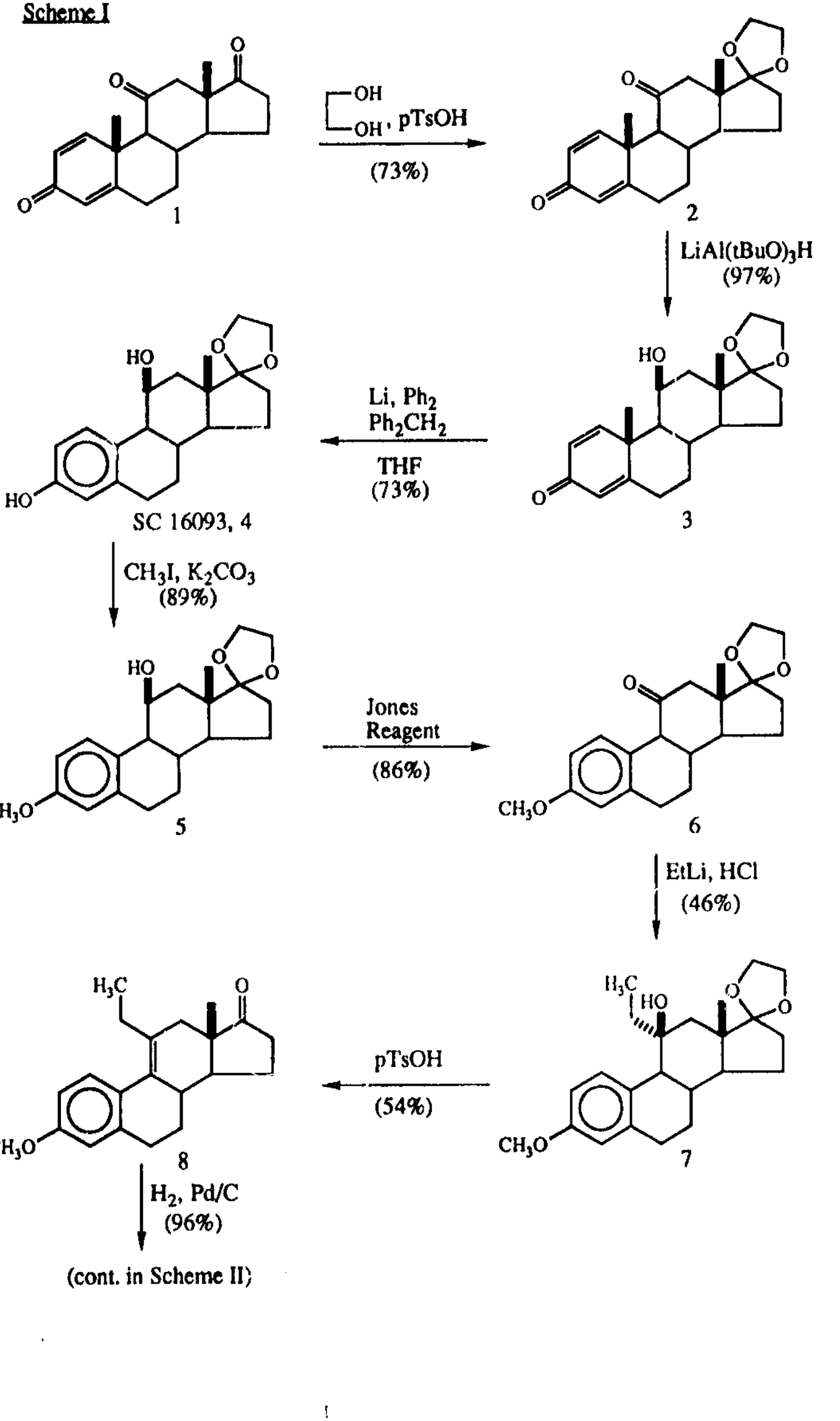
CONCOMITANTS OF HYPERADRENOCORTISM

Immunosuppression
Muscle atrophy
Osteoporosis/Hypercalcemia
Hyperglycemia/Hyperlipidemia
Arteriosclerosis
Steroid diabetes

Results and Discussion

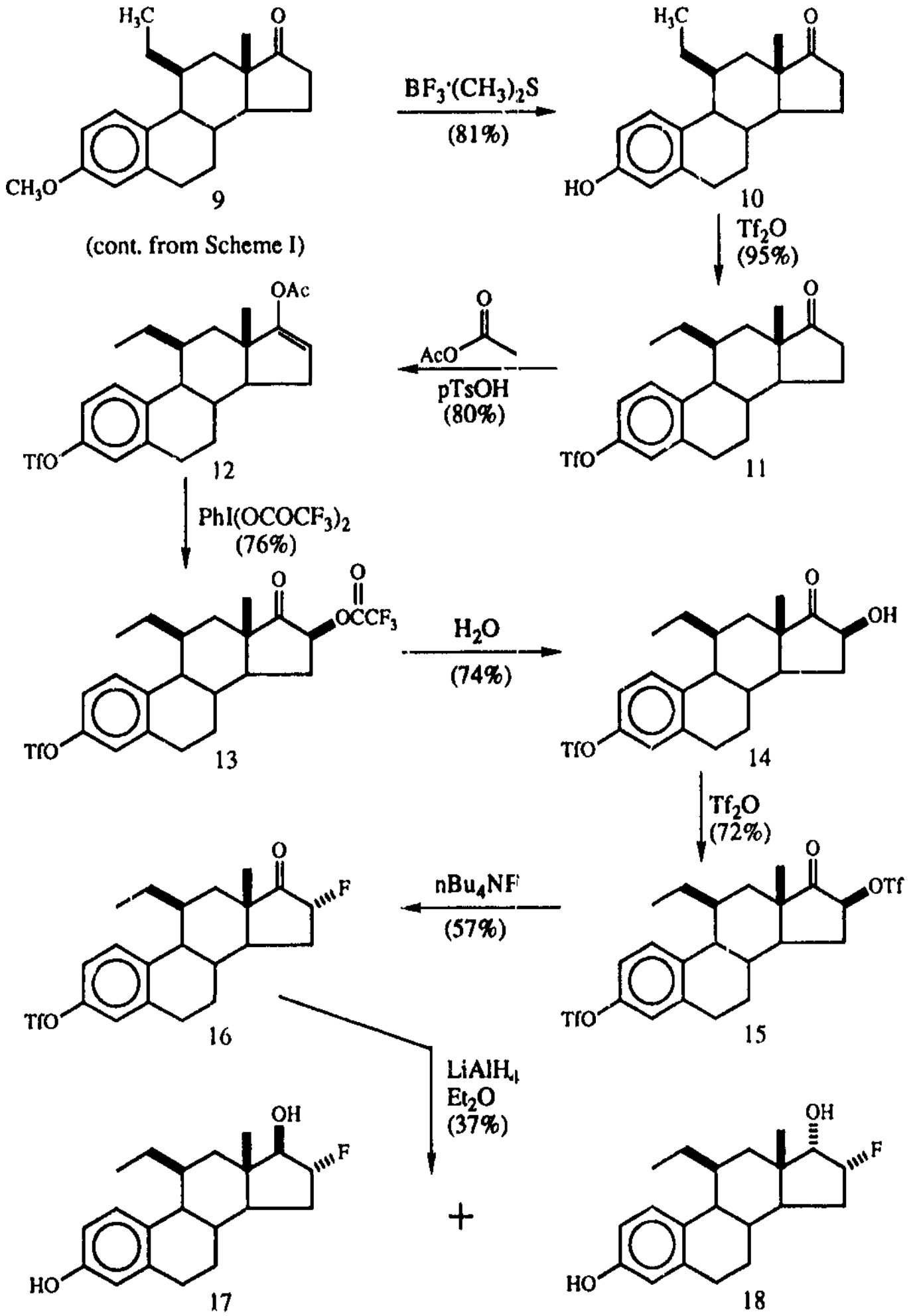
Estrogen. The 11β-ethyl-16α-fluoroestradiol (17) was prepared from 1-dehydroadrenosterone (1) in 16 steps as shown in Schemes I and II. The ketone at C-17 was selectively protected with ethylene glycol in refluxing benzene using a catalytic amount of *p*-toluene sulfonic acid monohydrate. The bulky lithium tri-*t*-butoxyaluminum hydride was employed to reduce the ketone at C-11 diastereoselectively. Reductive aromatization using lithium, biphenyl and diphenylmethane (to scavenge the methylithium formed) gave the aromatic A-ring. The newly-formed hydroxyl at C-3 was then protected as the methyl ether using methyl iodide with potassium carbonate as the base. Next the hydroxyl at C-11 was

Scheme I



(cont. in Scheme II)

Scheme II


H3C
O
CH3O
9
(cont. from Scheme I)
BF3·(CH3)2S
(81%)
HO
10
Tf2O
(95%)
TfO
11
AcO
pTsOH
(80%)
OAc
12
PhI(OCOCF3)2
(76%)
OCCF3
13
H2O
(74%)
OH
14
Tf2O
(72%)
OTf
15
nBu4NF
(57%)
F
16
LiAlH4
Et2O
(37%)
17
18
+

oxidized with Jones reagent and the product of this reaction was used without further purification due to its instability. Addition of EtLi at -78°C to the 11-ketone and addition of HCl upon warming gave the 11α-ethyl (7) compound by EtLi attack from the less hindered side and effected deprotection at C-17. The steroid was then dehydrated by treatment with refluxing benzene and tosic acid for 10 min. to give the 9,11-dehydro adduct (8). Hydrogenation of the double bond with H_2 over Pd/C gave a 2.6:1 of 11β- (9) to 11α-ethyl epimers, whose absolute stereochemistry was confirmed by single crystal x-ray analysis. These epimers proved difficult to separate; however, after deprotection of the methyl ether with boron trifluoride-methyl sulfide complex, they could be separated by fractional recrystallization. 11β-Ethyl estrone (10) could be recrystallized from acetone/hexane while 11α-ethyl estrone remained soluble. The remainder of the steps in the sequence were carried out by another worker in this laboratory.[16]

A summary of the data for the binding assays of 11β-ethyl-16α-flurorestradiol (17) is shown in Table I. The RBA's (relative binding affinities) were ascertained *in vitro* through a competitive

Table I. Binding Data for 11β-ethyl estradiols

	RBA*[1] 25°C (0°C)	NSB*[2]	BSI*[3]
16α-protio	1120 (117)	2.63	429
16α-fluoro	891 (128)	1.85	482

*[1]Estradiol = 100
*[2]Estradiol = 1
*[3]Estradiol = 100

radiometric binding assay, using rat uterine cytosol as a source of ER, and [^{3}H]estradiol as the tracer.[25] NSB is an acronym for non-specific binding and BSI (binding selectivity index) is the quotient RBA/NSB. In preparing the 11β–ethyl derivative of fluoro-estradiol, it was hoped

that it would have a greater BSI than the parent compound. As can be seen from the data, that result was achieved.

Next the tissue distribution of the radiolabelled [^{18}F]-containing compound was determined *in vivo*. The studies were performed at the Mallinckrodt Institute of Radiology of Washington University Medical School, St. Louis, MO. The ^{18}F was produced in a cyclotron by bombardment of ^{18}O-enriched water *via* the ^{18}O(p, n)^{18}F reaction. The hot fluoride was dried by azeotropic removal of water (CH_3CN) to render it more nucleophilic during subsequent reaction in an appropriate solvent. TBAF was used as the phase transfer catalyst in the resolubilization. The triflate precursor (15) was chosen so displacement would occur rapidly, necessitated by the short ^{18}F half-life. After displacement, the 17-ketone was reduced by treatment with lithium aluminum hydride. After workup, the product was purified by HPLC employing both a radiation and a UV detector. Ideally, the radioactivity peak corresponding to the desired product should not correspond to any mass peak, as limiting amounts of fluoride are being used (see HPLC trace). After purification, the uptake studies were performed by the St. Louis group employing a method similar to that used for 16α-[^{18}F]fluoroestradiol.[26] The results of the biodistribution study are summarized in Table II. To verify that the uptake was mediated by a high-affinity, saturable system, one set of animals was given a high dose of the unlabeled compound together with the labeled estrogen. The dose given was enough to saturate the estrogen receptors of the animal and should then block the uptake of the labeled compound (1h blocked). The ascertain that target tissue uptake of 11β-ethyl-16α-[^{18}F]fluoroestradiol was not being limited by undetected ER-binding impurities, one set of animals was injected with a smaller amount of the radioactive ligand (1h low dose).

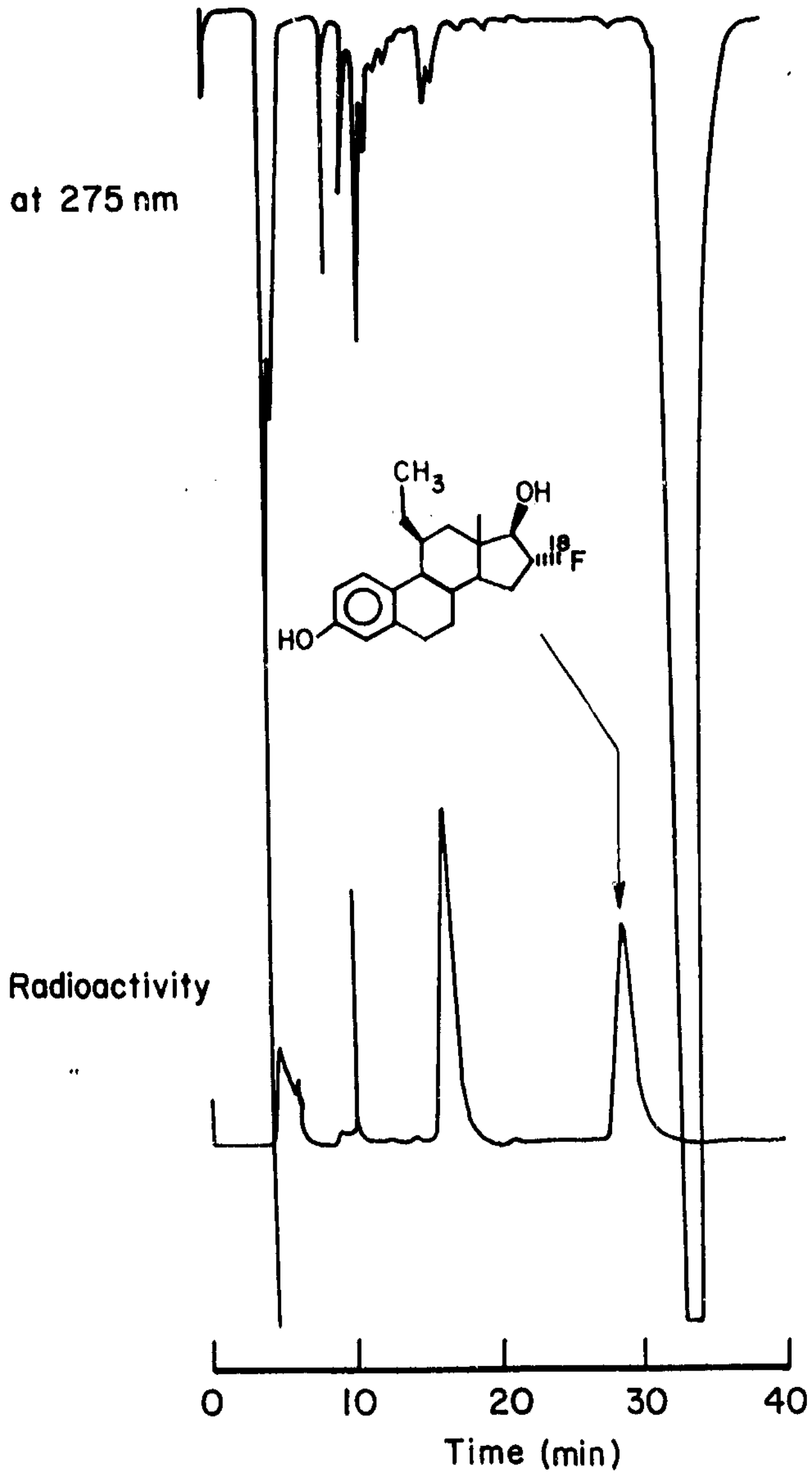

UV at 275 nm
CH3
OH
18F
HO
Radioactivity
0
10
20
30
40
Time (min)

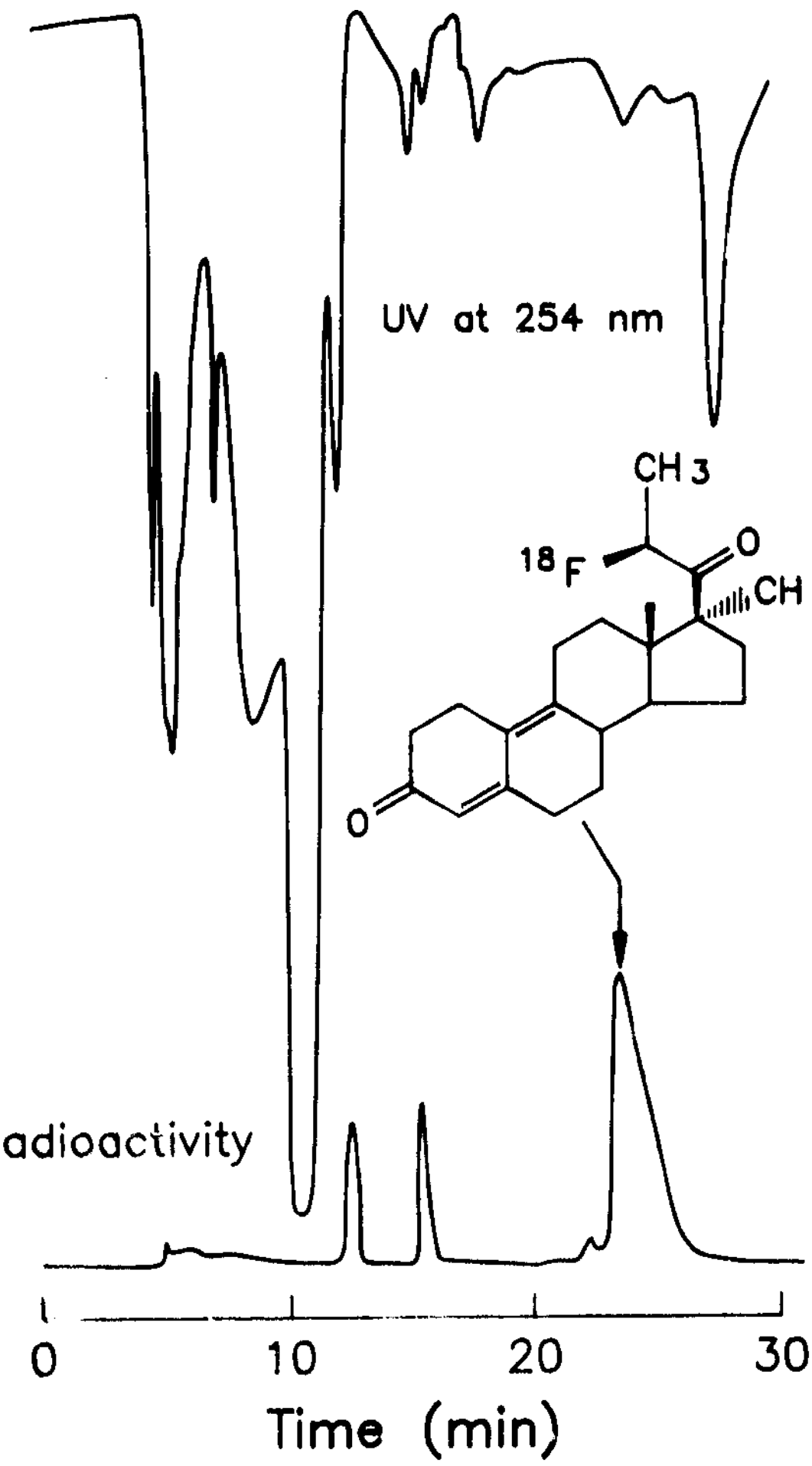
Table II. Tissue Uptake of 11β-ethyl-16α-[^{18}F]fluoroestradiol

Tissue	%ID'/g ± SEM* (n = 5)			
	1h (high dose)	1h (low dose)	1h (blocked)	3h (high dose)
Uterus	12.59 ± 3.40	13.50 ± 3.26	0.48 ± 0.15	11.49 ± 2.11
Ovaries	5.62 ± 1.41	5.08 ± 0.66	0.76 ± 0.27	4.61 ± 0.70
Blood	1.09 ± 0.46	0.82 ± 0.11	1.10 ± 0.23	0.84 ± 0.55
Muscle	0.75 ± 0.15	0.82 ± 0.15	0.24 ± 0.09	0.39 ± 0.03
Lung	0.99 ± 0.23	1.35 ± 0.37	0.92 ± 0.18	0.71 ± 0.07
Brain	0.17 ± 0.04	------	0.13 ± 0.04	------
Liver	3.31 ± 2.12	1.94 ± 0.57	3.35 ± 1.50	2.47 ± 0.90
Kidney	4.05 ± 2.40	3.29 ± 0.65	3.06 ± 1.16	2.52 ± 0.87
Fat	2.27 ± 0.88	1.42 ± 0.04	1.86 ± 0.43	0.98 ± 0.16
Bone	0.44 ± 0.10	0.51 ± 0.18	0.38 ± 0.13	0.35 ± 0.17
Uterus/Blood	12.99 ± 2.11	16.60 ± 3.40	0.52 ± 0.19	13.70 ± 2.80
Uterus/Muscle	16.97 ± 4.50	17.20 ± 6.30	2.12 ± 0.59	32.50 ± 5.70

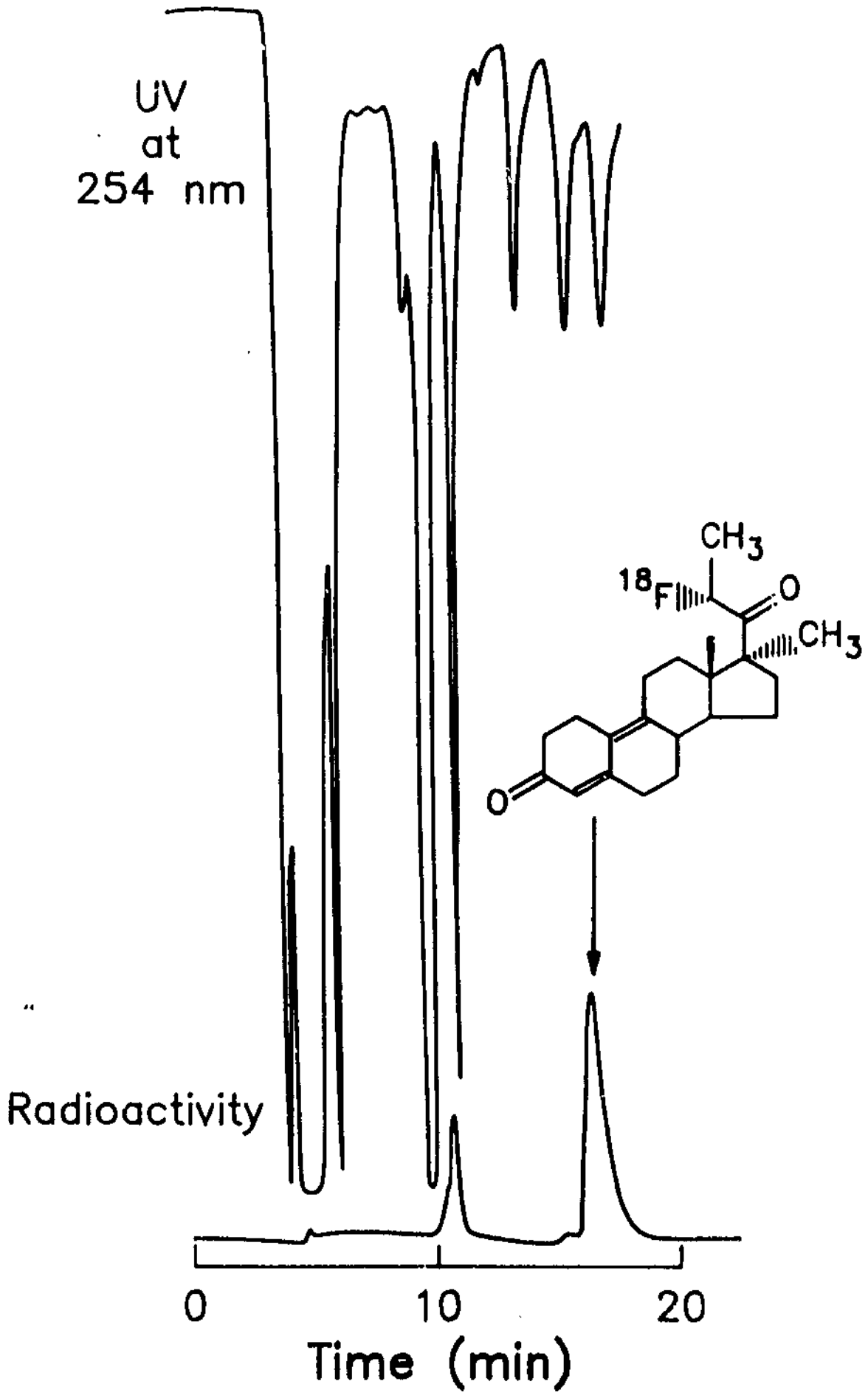
*SEM is standard error of the mean.

Progestins. The 21-fluoro derivatives of R5020 were easily prepared in four steps as shown in Scheme III from the corresponding optically pure starting material (21*R*-fluoro (27) from RU27987 (19) and 21*S*-fluoro (28) from RU27988 (20)). The cyclic ketal was formed at C-3 with ethylene glycol employing a catalytic amount of tosic acid and triethylorthoformate as a dehydrating agent. The 21-trifluoromethanesulfonate was then prepared by addition of triflic anhydride and 2,6-lutidine to a methylene chloride solution of the steroid (21 or 22). Fluoride displacement of the triflates (23 and 24) was effected with TBAF and the ketal protecting group was subsequently removed with concentrated sulfuric acid in acetone.

The RBA's of compounds 27 and 28 were measured relative to R5020 utilizing E_2-primed rat uterine cytosol. The radiochemical synthesis of the ^{18}F-labelled compounds was performed in a method similar to that of 11β-ethyl-16α-[^{18}F]fluoroestradiol, except after fluoride displacement, the ketal at C-3 was deprotected with concentrated sulfuric acid. After purification by HPLC (see trace) only the 21*S*-^{18}F epimer was subjected to the



SCS 0043-2



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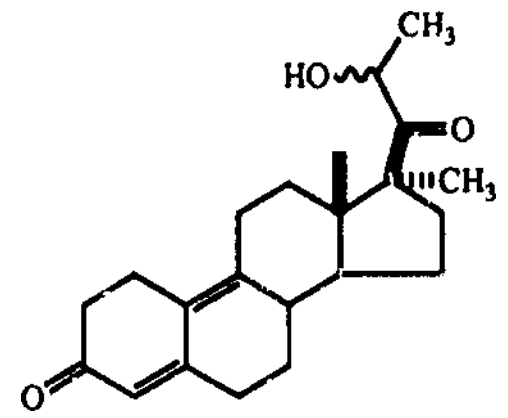
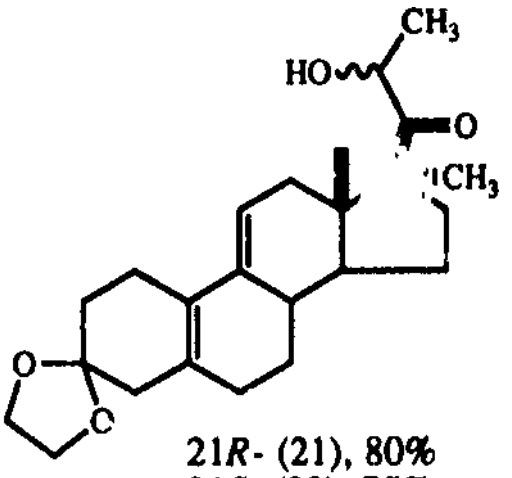
Scheme III. Synthesis of 21-Fluoro R5020



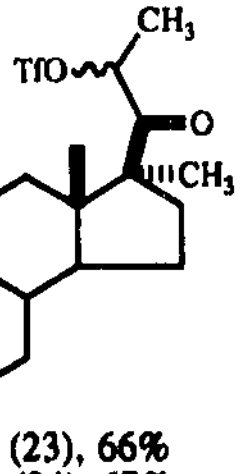
21*R*- RU27987 (19)
21*S*- RU27988 (20)

HO—CH₂—CH₂—OH
pTsOH, $CH(OEt)_3$



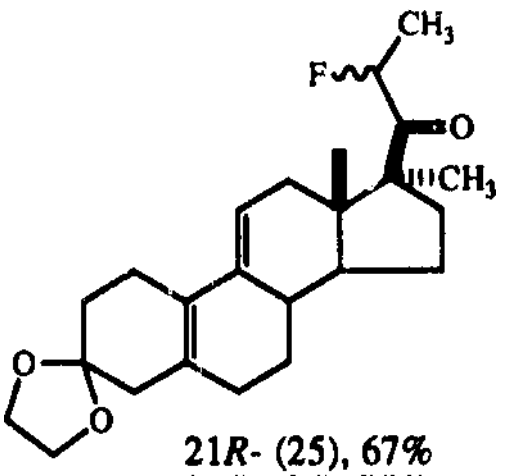
21*R*- (21), 80%
21*S*- (22), 75%

Tf_2O, 2,6-lutidine
CH_2Cl_2



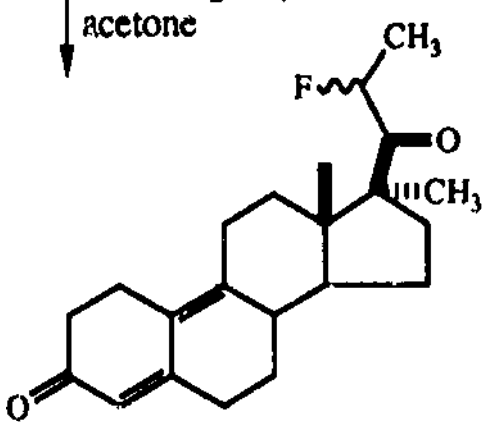
21*S*- (23), 66%
21*R*- (24), 67%

nBu_4NF
THF



21*R*- (25), 67%
21*S*- (26), 75%

conc. H_2SO_4
acetone



21*R*- (27), 63%
21*S*- (28), 63%

	RBA
21*R*-F	11
21*S*-F	50
21-H	100

Table III. Tissue Uptake of 21S-[F^{18}]fluoroR5020 (n = 5)

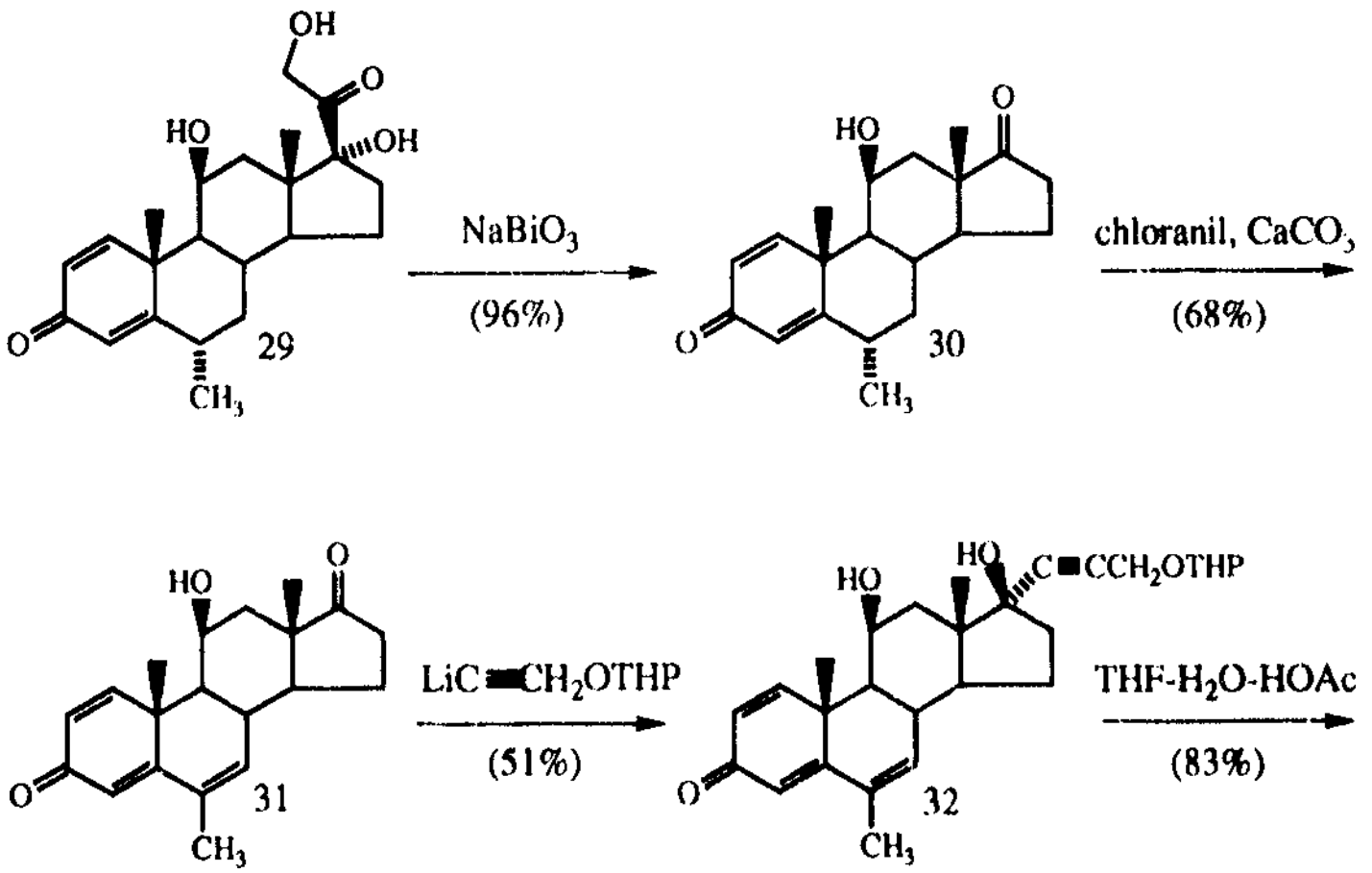
%ID/gram	1 hour* low	1 hour high	1 hour blocked	3 hour high
Blood	0.338 ± 0.072	0.481 ± 0.071	0.329 ± 0.013	0.119 ± 0.026
Liver	1.79 ± 0.344	2.46 ± 0.449	1.86 ± 0.204	0.905 ± 0.239
Spleen	0.337 ± 0.047	0.423 ± 0.099	0.300 ± 0.025	0.103 ± 0.015
Kidney	0.700 ± 0.118	0.976 ± 0.186	0.684 ± 0.057	0.247 ± 0.047
Muscle	0.366 ± 0.189	0.508 ± 0.147	0.389 ± 0.162	0.117 ± 0.034
Brain	0.278 ± 0.059	0.398 ± 0.115	0.261 ± 0.024	0.088 ± 0.021
Bone	7.14 ± 1.67	10.24 ± 2.64	6.24 ± 0.901	13.68 ± 2.25
Uterus	1.47 ± 0.484	2.07 ± 0.276	0.283 ± 0.027	0.387 ± 0.091
Ovaries	1.35 ± 0.371	2.01 ± 0.549	1.04 ± 0.144	0.511 ± 0.133
%ID/organ				
Blood	1.36 ± 0.378	1.94 ± 0.311	1.50 ± 0.104	0.528 ± 0.119
Liver	5.07 ± 0.956	7.09 ± 0.410	5.84 ± 0.896	2.65 ± 0.651
Spleen	0.083 ± 0.023	0.089 ± 0.019	0.081 ± 0.015	0.029 ± 0.010
Kidney	0.239 ± 0.049	0.317 ± 0.057	0.250 ± 0.036	0.090 ± 0.016
Muscle	2.95 ± 1.59	4.14 ± 1.32	3.52 ± 1.38	1.04 ± 0.319
Brain	0.375 ± 0.086	0.525 ± 0.174	0.367 ± 0.050	0.122 ± 0.032
Bone	44.60 ± 11.83	63.26 ± 10.21	44.37 ± 6.67	93.55 ± 5.53
Uterus	0.145 ± 0.052	0.199 ± 0.050	0.031 ± 0.003	0.043 ± 0.019
Ovaries	0.039 ± 0.012	0.062 ± 0.022	0.034 ± 0.006	0.015 ± 0.004
Uterus/blood	4.275 ± 0.544*	4.328 ± 0.340	0.860 ± 0.073	3.294 ± 0.523
Uterus/muscle	4.476 ± 1.664	4.359 ± 1.373	0.857 ± 0.394	3.386 ± 0.507
Ovaries/blood	4.009 ± 0.953	4.150 ± 0.716	3.153 ± 0.447	4.363 ± 0.778
Ovaries/muscle	4.075 ± 1.391	4.083 ± 1.13	3.314 ± 2.052	4.519 ± 0.980

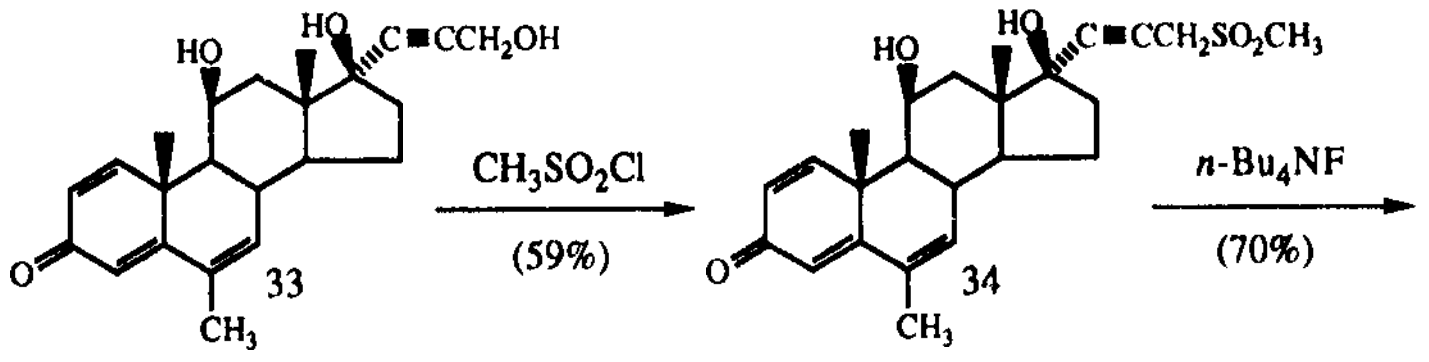
*n = 6

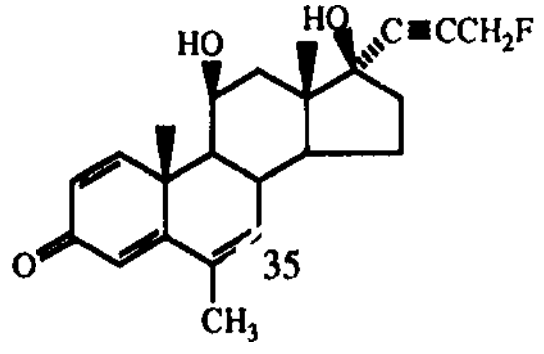
biodistribution assay, as the 21*R*-^{18}F epimer had a prohibitively low RBA. The results of that study are summarized in Table III.

Glucocorticoid. The fluoro analog of RU28362 (35) was conveniently prepared from 6α-methylpredinsolone (29) in 6 steps as depicted in Scheme IV. Cleavage at C-17 with sodium bismuthate gave the 17-ketone, which was followed by chloranil desaturation to give the Δ6-7 olefin. Next, propargyltetrahydropyranyl ether was added at C-11 and the THP ether was cleaved with HOAc in water and THF to give a 3' hydroxyl. The hydroxyl was then converted

Scheme IV. SYNTHESIS OF FLUORO - RU28362

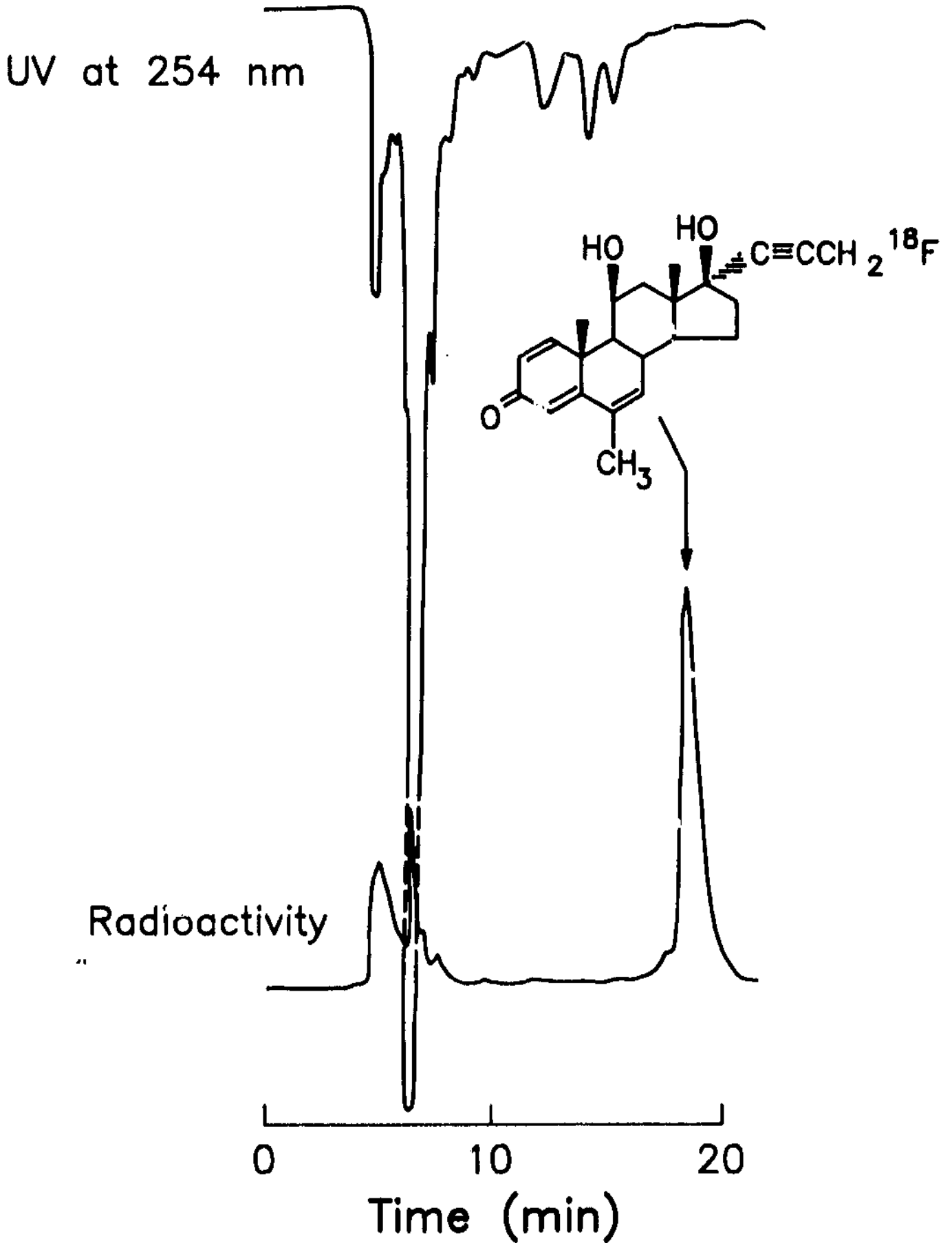






	RBA	K_d
RU28362	100	11 nM
F-RU28362	29	38 nM

SCS 0043-1


UV at 254 nm
HO
HO
C≡CCH 2 18F
O
CH3
Radioactivity
0
10
20
Time (min)

to the mesylate with mesyl chloride and displacement of the mesylate with TBAF gave fluoro-RU28362 (35).

The RBA of com pound 32 was measured relative to RU28362 utilizing liver cytosol from adrenalectomized rats as the receptor source. The radiochemical synthesis of the ^{18}F-labelled compounds was performed in a method similar to that of 11β-ethyl-16α-[^{18}F]fluoroestradiol, except a mesylate was used as the leaving group rather than a triflate. After purification by HPLC (see trace) the biodistribution assay was performed. The results of that study are summarized in Table IV.

Table IV. TIssue Uptake of [^{18}F]fluoroRU28362 (n = 4)

%ID/gram	15 min	1 hour*	1 hour blocked	2 hour
Blood	0.394 ± 0.049	0.309 ± 0.026	0.216 ± 0.033	0.308 ± 0.05
Liver	2.314 ± 0.257	1.65 ± 0.242	1.035 ± 0.195	1.487 ± 0.21
Kidney	1.287 ± 0.187	0.964 ± 0.08	0.68 ± 0.216	0.869 ± 0.110
Muscle	0.403 ± 0.049	0.323 ± 0.015	0.209 ± 0.04	0.300 ± 0.037
Fat	0.892 ± 0.332	0.658 ± 0.083	0.382 ± 0.062	0.634 ± 0.112
Striatum	0.139 ± 0.016	0.128 ± 0.01	0.077 ± 0.009	0.139 ± 0.024
Cerebellum	0.151 ± 0.014	0.132 ± 0.014	0.086 ± 0.008	0.141 ± 0.024
Cortex	0.143 ± 0.010	0.125 ± 0.012	0.075 ± 0.007	0.131 ± 0.02
Hippocampus	0.120 ± 0.014	0.115 ± 0.013	0.078 ± 0.003	0.125 ± 0.021
Hypothalamus	0.134 ± 0.022	0.114 ± 0.023	0.095 ± 0.019	0.166 ± 0.04
Rest of brain	0.135 ± 0.011	0.124 ± 0.011	0.08 ± 0.008	0.137 ± 0.023
Total Brain	0.138 ± 0.012	0.125 ± 0.012	0.08 ± 0.007	0.137 ± 0.023
Bone	0.438 ± 0.053	1.27 ± 0.279	2.73 ± 0.287	1.51 ± 0.137
%ID/organ				
Blood	5.41 ± 0.256	4.10 ± 0.239	2.865 ± 0.201	4.13 ± 0.321
Liver	18.67 ± 1.81	12.54 ± 0.622	7.70 ± 0.493	13.03 ± 2.19
Kidney	1.095 ± 0.038	0.766 ± 0.045	0.591 ± 0.168	0.73 ± 0.05
Muscle	11.15 ± 1.276	8.59 ± 0.442	5.55 ± 0.679	8.09 ± 0.375
Fat	23.75 ± 7.54	17.27 ± 2.54	10.07 ± 1.65	16.80 ± 1.74
Rest of brain	0.179 ± 0.019	0.172 ± 0.021	0.111 ± 0.013	0.186 ± 0.028
Total brain	0.244 ± 0.032	0.231 ± 0.029	0.153 ± 0.015	0.255 ± 0.042
Bone	9.39 ± 0.537	26.26 ± 5.28	56.65 ± 2.02	32.05 ± 4.66

*n = 5

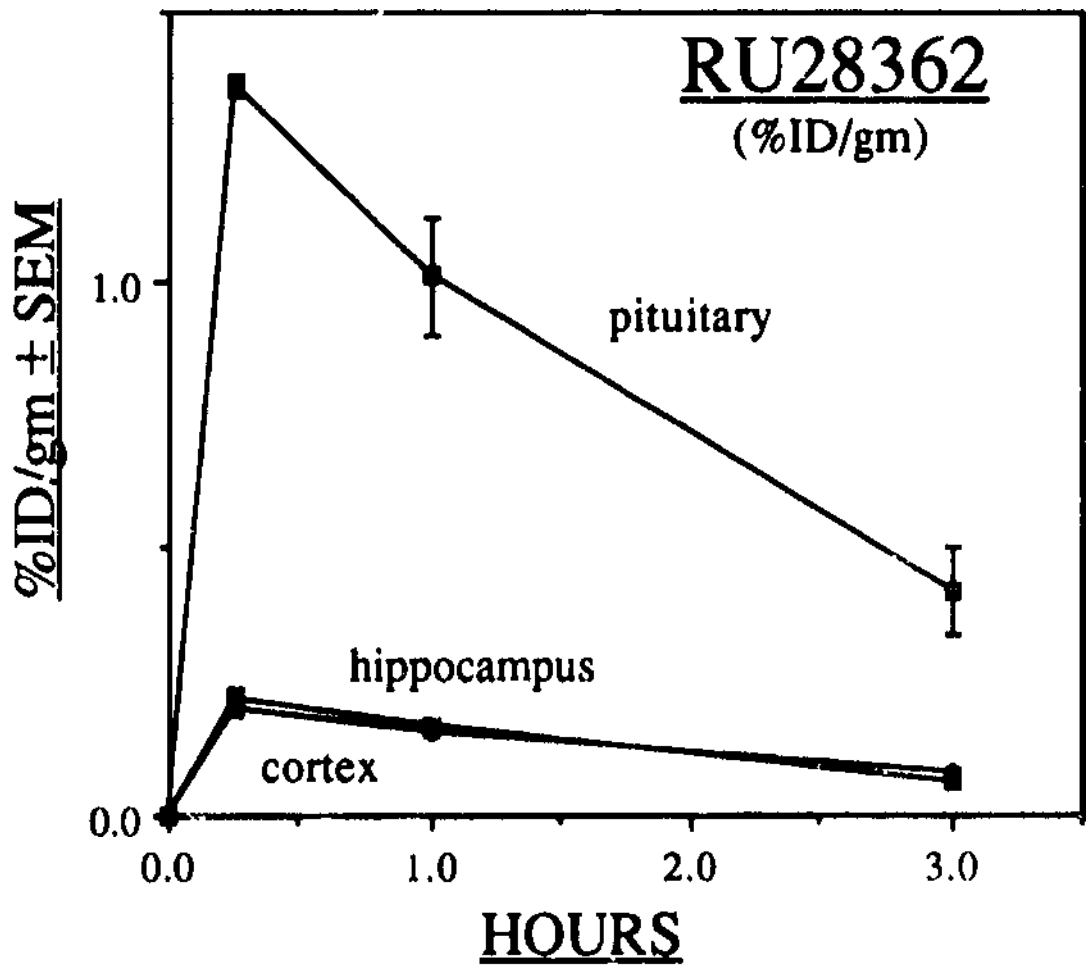
As can be seen from the table, the specific uptake of [F-18]RU28362 in the hippocampus was not demonstrated. In order to ascertain a reason for this low target tissue selectivity (i.e. *in vivo* defluorination) the study was repeated using [^{3}H]RU28362 as the radioactive ligand. The results of this study are summarized in Table V and Figure III.

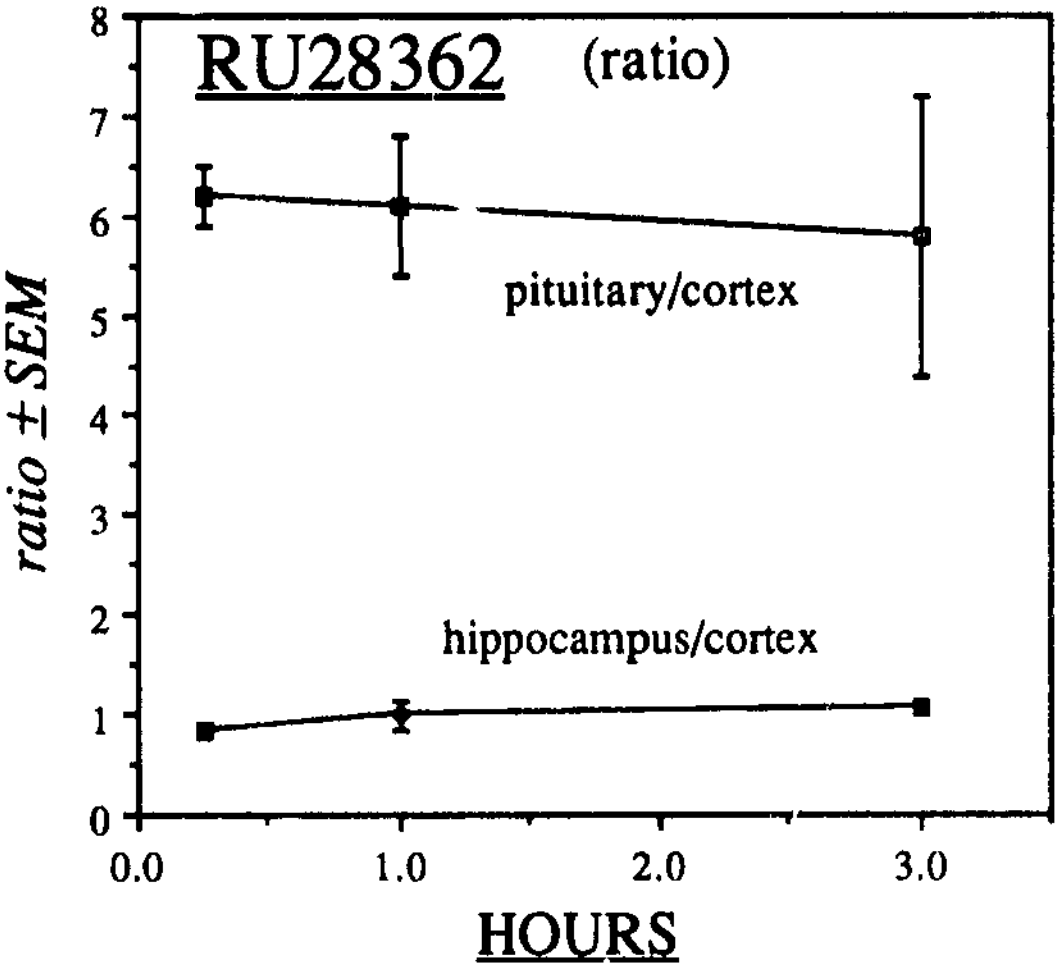
Table V. Tissue Uptake of [^{3}H]RU28362 (n = 4)

%ID/gram	15 min	1 hour	1 hour blocked	1 hour intact*	3 hour
Blood	0.189 ± 0.039	0.190 ± 0.011	0.189 ± 0.017	0.180 ± 0.065	0.142 ± 0.023
Fat	0.302 ± 0.083	0.593 ± 0.028	0.587 ± 0.074	0.604 ± 0.041	0.315 ± 0.032
Musc;e	0.251 ± 0.062	0.371 ± 0.026	0.362 ± 0.048	0.300 ± 0.036	0.171 ± 0.017
Kidney	0.847 ± 0.173	0.798 ± 0.062	0.683 ± 0.096	0.616 ± 0.054	0.422 ± 0.059
Spleen	0.429 ± 0.105	0.484 ± 0.076	0.414 ± 0.066	0.262 ± 0.048	0.143 ± 0.016
Liver	1.567 ± 0.358	1.541 ± 0.100	1.605 ± 0.077	1.030 ± 0.074	0.903 ± 0.090
Lung	0.772 ± 0.179	0.544 ± 0.088	0.436 ± 0.063	0.302 ± 0.022	0.099 ± 0.021
Thymus	0.419 ± 0.110	0.525 ± 0.067	0.414 ± 0.071	0.359 ± 0.015	0.186 ± 0.017
Pituitary	1.111 ± 0.246	1.008 ± 0.111	0.593 ± 0.042	0.684 ± 0.070	0.384 ± 0.054
Hippocampus	0.159 ± 0.040	0.162 ± 0.013	0.147 ± 0.009	0.139 ± 0.010	0.080 ± 0.014
Cortex	0.183 ± 0.042	0.168 ± 0.011	0.153 ± 0.016	0.146 ± 0.004	0.074 ± 0.009
Rest of brain	0.191 ± 0.064	0.184 ± 0.002	0.182 ± 0.012	0.158 ± 0.016	0.083 ± 0.018
pit/cortex	6.150 ± 0.328	6.055 ± 0.702	3.973 ± 0.374	4.692 ± 0.445	5.330 ± 0.923
hip/cortex	0.833 ± 0.083	0.987 ± 0.136	0.977 ± 0.056	0.958 ± 0.084	1.074 ± 0.081

*non-adrenalectomized rats

Figure III.


RU28362
(%ID/gm)
%ID/gm ± SEM
1.0
0.0
pituitary
hippocampus
cortex
0.0
1.0
2.0
3.0
HOURS


RU28362 (ratio)
ratio ± SEM
8
7
6
5
4
3
2
1
0
pituitary/cortex
hippocampus/cortex
0.0
1.0
2.0
3.0
HOURS

Conclusions:

Estrogen. The selectivity and affinity the 11β-ethyl-16α-[^{18}F]fluoroestradiol has for the ER is very encouraging. One current ligand used in PET imaging of breast tumors is 16α-[^{18}F]fluoroestradiol and the 11β-ethyl compound appears to be superior, in terms of the BSI, which would probably give greater image clarity. Clinical studies may be warranted on the basis of this data.

Progestins. Unfortunately, the results from the 21*S*-[^{18}F]fluoroR5020 study were not encouraging. The compound appears to undergo *in vivo* defluorination as evidenced by the high levels of radioactivity in the bone. The ligand might still be useful, but the ^{18}F will have to be substituted in a less metabolically labile position.

Glucocorticoids. The results of the biological studies of [^{18}F]fluoroRU28362 were rather discouraging. The hippocampus/cortex ratio was nearly 1:1 indicating no selective uptake. The pituitary/cortex ratio was somewhat higher and might provide enough contrast to use this compound as a pituitary imaging agent.

Experimental Section

General. Melting points (uncorrected) were determined on a Thomas-Hoover or a Fisher-Johns apparatus. Analytical thin-layer chromatography (TLC) was performed on Merck silica gel F-254 glass-backed plates. Visualization was achieved with short wave ultraviolet light and/or phosphomolybdic acid spray. Flash chromatography was performed according to Still,[27] using Woelm 32-63 μm silica gel.

^{1}H-NMR spectra were obtained on a Varian XL-200, a General Electric QE-300, a Nicolet NT-360, or a General Electric GN-500 spectrometer. Chemical shifts are reported in ppm downfield from a tetramethylsilane internal standard (δ scale). ^{19}F-NMR spectra were obtained on a Nicolet NT-360 spectrometer at 338.76 MHz and are reported downfield from

internal $CFCl_3$. Low resolution electron impact (LREI) mass spectra were acquired from a Finnigan MAT-CH5 spectrometer. High resolution electron impact (HREI) mass spectra were obtained on a MAT-731 instrument while a V. B. Instruments ZAB-HF mass spectrometer provided spectra *via* fast atom bombardment (FAB) employing a dithiothreitol matrix. For EI spectra, the reported data is for an electron energy of 70 eV and is in the form: m/e (intensity relative to base peak = 100). Infrared (IR) spectra were obtained on a Nicolet 7199 FT-IR or an IBM IR/32 FT instrument. Elemental analyses were performed by the Microanalytical Service Laboratory of the University of Illinois.

Analytical gas-liquid chromatography (GLC) was performed on a Hewlett-Packard 5793A instrument equipped with flame ionization detector. Analyses were performed on an Alltech RSL-150 capillary column (0.25 mm X 30 m) or a Hewlett-Packard Ultra 1 capillary column (0.20 mm X 12.5 m). High-performance liquid chromatography (HPLC) was performed isocratically with a Varian 5060 or a Spectra-Physics 8700 liquid chromatograph, using a 5 μm analytical silica gel column (4.6 mm X 30 cm, Varian Si-5 Micro Pak), a 10 μm preparative silica gel column (9 mm X 50 cm, Whatman Partisil M-9), or a C_{18} column (10 mm X 50 cm, Whatman Partisil M-9, ODS-2). HPLC eluent was monitored *via* UV absorbance (280 nm for estrogens, 254 nm for other steroids); for radiochemical purification, HPLC eluent was also monitored with a NaI(Tl) radioactivity detector. Radioactivity was determined in a dose calibrator.

X-ray crystallography involved diffraction experiments performed at room temperature with Mo radiation ($\lambda(K_\alpha^-) = 0.71073$ A).

Tetrahydrofuran (THF) was distilled from sodium benzophenone ketyl; all other solvents were distilled from CaH_2. The $BF_3 \cdot S(CH_3)_2$ complex was prepared by saturating $(CH_3)_2S$ with BF_3 gas.

Chemicals were obtained from the following sources and were used as received, unless otherwise noted: Baker, Fisher, Mallinckrodt, Aldrich, Sigma, Eastman or Alfa. 1-

Dehydroadrenosterone was obtained from Searle Laboratories, Skokie, IL. RU 27987 and RU 27988 were gifts of Roussel UCLAF of Paris, France. RU 28362 was purchased from DuPont NEN.

The following abbreviations are used: estradiol for 1, 3, 5 (10)-estratriene-3, 17β-diol; progesterone for pregn-4-ene-3, 20-dione; estrone for 1, 3, 5 (10)-estratriene-3-ol-17-one; SC 16093 for 17, 17-ethylenedioxy-estra-1, 3, 5 (10)-triene-3, 11β-diol; 1-dehydroadrenosterone for androsta-1, 4-diene-3, 11, 17-trione; RU 27987 for 17α, 21-dimethyl-21*S*-hydroxy-19-nor-pregna-4, 9-diene-3, 20-dione; RU 27988 for 17α, 21-dimethyl-21*S*-hydroxy-19-nor-pregna-4, 9-diene-3, 20-dione; RU 28362 for 11β, 17β-dihydroxy-6-methyl-17α-propionyl-androsta-1, 4, 6-triene-3-one.

Estrogen Synthesis: 16α-Fluoro-11β-ethylestra-1,3,5(10)-triene-3,17β-diol (17) and 16α-fluoro-11β-ethylestra-1,3,5(10)-triene-3,17αdiol (18)

17, 17-Ethylenedioxyandrosta-1, 4-diene-3, 11-dione (2). 1-Dehydro-adrenosterone (1) (11.45 mg, 38.4 mmol) was added to a one liter round-bottomed flask equipped with magnetic stirring, reflux condenser and a Dean-Stark trap. Benzene (500 mL) was added followed by 7.5 g (6.7 mL, 120.8 mmol) of ethylene glycol. *p*-Toluenesulfonic acid monohydrate (572 mg, 3.02 mmol) was added and the reaction mixture brought to reflux. The reaction was carried out under a nitrogen atmosphere. Analysis by TLC (40% EtOAc/hexane) indicated completion at 2 h. After cooling to room temperature, workup proceeded by extraction (2 X 50 mL, satd. aq. Na_2CO_3). The mixture was dried over $MgSO_4$ and concentrated *in vacuo* to give a pale yellow powder. Recrystallization from CH_3OH/CH_2Cl_2 afforded (2) as needles (9.66 g, 73 %): mp 217-219°C; ^{1}H-NMR (200 MHz, $CDCl_3$) δ 0.88 (s, 3 H, 18-CH_3), 1.45 (s, 3 H, 19-CH_3), 3.77-3.96 (m, 4 H, OCH_2CH_2O), 6.05 bs, 1 H, 4-H), 6.20 (dd, 1 H, J = 10.3, 2 Hz, 2-H), 7.68 (d, 1 H, J = 10.3 Hz, 1-H); IR (KBr) 1703 (C-11 ketone), 1658 cm^{-1} (C-3 ketone); EIMS, 342 (M^+, 7), 280 (7), 99 (100); Anal. (exact mass, HREIMS) Calcd for $C_{21}H_{26}O_4$ m/e 342.1836. Found, 342.1831.

17, 17-Ethylenedioxy-11β-hydroxyandrosta-1, 4-diene-3-one (3). A 500 mL round-bottomed flask equipped with a 150 mL addition funnel and magnetic stirring was evacuated with nitrogen after flame drying. The flask was charged with lithium tri-*tert*-butoxyaluminohydride (13.03 g, 51.24 mmol) in THF (156 mL). Steroid (2) (7.53 g, 21.99 mmol) was placed into the addition funnel and dissolved in THF (104 mL). The steroid in THF was added dropwise to the clear solution of reductant over a 65 min period. After stirring at room temperature for 24 h, TLC (40% EtOAc/hexane) indicated consumption of starting material. Workup was accomplished by dilution with water (50 mL) and extraction (150 mL, half-satd. sodium citrate). The aqueous layer was extracted (3 X 100 mL, EtOAc) and the organic layer was combined with the organic phase from the original half-satd. sodium citrate extraction. A brine wash of the combined organic layers preceded drying over $MgSO_4$. Evaporation of solvent *in vacuo* gave a yellow foam (7.63 g, 97%). An analytical sample was recrystallized from hexane/$CHCl_3$ to give a coarse white powder: mp 204-206°C; ^{1}H-NMR (200 MHz, $CDCl_3$) δ 1.16 (s, 3 H, 18-CH_3), 1.45 (s, 3 H, 19-CH_3), 3.75-3.93 (m, 4 H, OCH_2CH_2O), 4.44 (m, 1 H, 11α-H), 6.02 (bs, 1 H, 4-H), 6.26 (dd, 1 H, J = 10.3, 2 Hz, 2-H), 7.29 (d, 1 H, J = 10.3 Hz, 1-H); IR (KBr) 3380 (OH), 1660 cm^{-1} (ketone); EIMS, 344 (M^+, 5), 223 (8), 99 (74), 28 (100); Anal. Calcd for $C_{21}H_{28}O_4$: C, 73.21; H, 8.21. Found: C, 73.08; H, 5.96.

17, 17-Ethylenedioxyestra-1, 3, 5 (10)-triene-3, 11β-diol (4). The reaction was performed under a nitrogen atmosphere in a 50 mL round-bottomed flask with mechanical stirring. Hydroxydienone ketal (3) (800 mg, 2.32 mmol) in THF (6 mL) was added dropwise over a 60 min period to a dark green mixture containing biphenyl (1.43 g, 9.3 mmol), diphenylmethane (781 mg, 4.6 mmol) and 25% Li in oil (773 mg, 27.8 mmol) in refluxing THF (10 mL). At the completion of addition, the mixture was allowed to cool to room temperature and was subsequently quenched with CH_3OH (10 mL) followed by distilled water (15 mL). The resulting two-phase system was concentrated in vacuo and added to 60 mL of

25% hexane/benzene. This solution was extracted with 5% KOH (3 X 20 mL). The combined basic layers were washed with 20 mL of hexane and then placed on ice. Acetic acid was added (15 mL) dropwise until product fell out of solution. A white paste was recovered on vacuum filtration. The filtrate was back extracted with 3 X 15 mL of EtOAc. The white paste was redissolved in EtOAc and was combined with the organic layers from the back extraction. This was washed with 30 mL of water and 30 mL of brine. Drying over $MgSO_4$ was followed by removal of the solvent *in vacuo*. Flash chromatography (20 cm X 60 mm SiO_2, 20% hexane/EtOAc) gave a white solid (331.8 mg, 43%): mp 193-196°C; ^{1}H-NMR (200 MHz, $CDCl_3$) δ 1.14 (s, 3 H, 18-CH_3), 2.49 (bd, 1 H, J = 10.3 Hz, 9α-H), 3.88-4.00 (m, OCH_2CH_2O), 4.75 (m, 1 H, 11α-H), 6.61 (d, 1 H, J = 2.0 Hz, 4-H), 6.68 (dd, 1 H, J = 9.3, 2.0 Hz, 2-H), 7.18 (d, 1 H, J = 9.3 Hz, 1-H); IR (KBr) 3420 cm^{-1} (OH); EIMS, 330 (M^+, 8), 268 (25), 224 (19), 99 (100); Anal. (exact mass, HREIMS) Calcd for $C_{20}H_{26}O_4$ m/e 330.1831. Found, 330.1824.

17, 17-Ethylenedioxy-3-methoxyestra-1, 3, 5 (10)-triene-11β-ol (5). DMF was freshly distilled and stored over sieves under argon. Iodomethane (729 mg, 5.14 mmol) was added dropwise, as a 40% solution (v/v) in DMF, to a stirred heterogeneous mixture of K_2CO_3 (8.50 mg, 6.15 mmol) and diol (4) in DMF (6 mL) at room temperature under argon. TLC (20% hexane/EtOAc) indicated completion after 22 h. The reaction mixture was poured onto distilled water (25 mL) and extracted (3 X 30 mL, EtOAc). The combined organic phase was washed with brine and dried over Na_2SO_4. Concentration *in vacuo* gave a yellow oil which was subjected to flash chromatography (30% EtOAc/hexane, 15 cm X 35 mm SiO_2) to afford a white foam (307 mg, 89%): mp 120-122°C; ^{1}H-NMR (300 MHz, $CDCl_3$) δ 1.14 (s, 3 H, 18-CH_3), 2.49 (bd, 1 H, J = 10.6 Hz, 9α-H), 3.78 (s, 3 H, OCH_3), 3.87-3.99 (m, 4 H, OCH_2CH_2O), 4.75 (m, 1 H, 11α-H), 6.66 (d, 1 H, J = 3.3 Hz, 4-H), 6.75 (dd, 1 H, J = 8.9, 3.3 Hz, 2-H), 7.20 (d, 1 H, J = 8.9 Hz, 1-H); IR (KBr) 3420 cm^{-1} (OH); EIMS, 344

(M^+, 14), 282 (25), 238 (12), 211 (10), 99 (100); Anal. Calcd for $C_{21}H_{28}O_4$: C, 73.21; H, 8.21. Found: C, 73.20; H, 8.07.

17,17-Ethylenedioxy-3-methoxyestra-1,3,5(10)-triene-11-one (6). A 10 mL round-bottomed flask was charged with sterol (5) (240 mg, 0.70 mmol), in acetone (5 mL). The solution was brought to 0°C with an ice bath. Jones reagent was added (10 drops of 8 *N* CrO_3·25% aq. H_2SO_4), followed by 200 μL of CH_3OH one min later. After 5 min, the reaction mixture was diluted with distilled water (10 mL), and extracted (3 X 10 mL, EtOAc). the combined organic layers were washed (2 X 20 mL, Satd. aq. $NaHCO_3$). Drying over $MgSO_4$ and concentration *in vacuo* gave a white foam (204.1 mg, 86%) which was used without further purification. ^{1}H-NMR (200 MHz, $CDCl_3$) δ 0.88 (s, 3 H, 18-CH_3), 3.52 (d, 1 H, J = 13.4 Hz, 9α-H), 3.77 (s, 3 H, OCH_3), 3.85-3.98 (m, 4 H, OCH2CH2O), 6.62 (d, 1 H, J = 3.1 Hz, 4-H), 6.75 (dd, 1 H, J = 7.8, 3.1 Hz, 2-H), 7.26 (d, 1 H, J = 7.8 Hz, 1-H); IR (KBr) 1712 (ketone); EIMS, 342 (M^+, 42), 298 (8), 280 (11), 99 (100); Anal. (exact mass, HREIMS) Calcd for $C_{21}H_{26}O_4$ m/e 342.1826. Found 342.1827.

11α-Ethyl-3-methoxyestra-1,3,5(10)-triene-11β-ol-17-one (7). Ketone (6) (72 mg, 0.21 mmol) was added dropwise over a ten min period to a 5 mL round-bottomed flask containing 6.26 μL (0.88 mmol) of EtLi (1.4 *M* in benzene) in THF (1.2 mL) at -70°C. Magnetic stirring was employed, and the reaction was run under a blanket of argon. After 90 min, 3 *N* HCl (500 μL) was added to the brown reaction mixture. After warming to room temperature, workup was performed as follows: further addition of 3 *N* HCl (500 μL), extraction (EtOAc), washing of combined organic layers (1 X 10 mL, water), rinsing (2 X 15 mL satd. aq. $NaHCO_3$) and brine wash. Drying over $MgSO_4$ was followed by concentration *in vacuo* to give a yellow oil. This oil was dissolved in acetone (1 mL) and placed on ice. 3 *N* HCl (3 mL) was added and the mixture was allowed to stir overnight as it warmed to room temperature (12 h). Extraction (EtOAc) was followed by water (1 X 10 mL) then satd. aq.

$NaHCO_3$ (2 X 15 mL) rinses and brine wash. Drying over $MgSO_4$ followed by concentration *in vacuo* gave a purple oil which was subjected to flash chromatography (30% EtOAc/hexane). A purple solid was isolated (32 mg, 46%): mp 119-122°C; ^{1}H-NMR (300 MHz, $CDCl_3$) δ 1.01 (t, 3 H, J = 8.2 Hz, CH_2CH_3), 1.13 (s, 3 H, 18-CH_3), 2.33 (d, 1 H, J = 9.8 Hz, 9α-H), 3.81 (s, 3 H, OCH_3), 6.72 (m, 2 H, 4-H and 2-H), 7.69 (d, 1 H, J = 9.8 Hz, 1-H); IR (KBr) 3550 (OH), 1736 cm^{-1} (ketone); EIMS, 328 (M^+, 62), 256 (27), 160 (100), 134 (59), 97 (84): Anal. Calcd for $C_{21}H_{28}O_3$: C, 76.78; H, 8.61. Found C, 77.01; H, 8.54.

3-Methoxy-11-ethylestra-1,3,5(10),9(11)-tetraene-17-one (8). Steroid (7) (715 mg, 2.18 mmol) was dissolved in 25 mL of benzene in a 100 mL round-bottomed flask equipped with a reflux condenser and nitrogen inlet. *p*-Toluenesulfonic acid monohydrate (286 mg, 1.50 mmol) was added and the mixture was heated at reflux for 10 min, at which time GLC indicated consumption of starting material and formation of two products. Workup involved dilution (EtOAc, 20 mL) and rinsing (2 X 20mL, saturated aqueous $NaHCO_3$). The aqueous layer was washed (1 X 20 mL, EtOAc), the organic layers were combined, washed with brine, and dried (Na_2SO_4). Concentration *in vacuo* was followed by flash chromatography (5% EtOAc/hexane, 31 cm X 42 mm SiO_2) to provide the major product as a clear oil which solidified on standing (367 mg, 54%): mp 78-80°C; ^{1}H-NMR (200 MHz, $CDCl_3$) δ 0.92 (s, 3H, 18-CH_3), 1.10 (t, 3H, J = 7.5 Hz, CH_2CH_3), 3.81 (s, 3H, OCH_3), 6.70 (bs, 1H, 2-H), 7.24 (d, 1H, J = 8.2 Hz, 1-H); IR ($CHCl_3$) 1727 cm^{-1} (ketone); EIMS, 310 (M^+, 22), 219(100), 177(42), 173(38), 145(61); Anal. (exact mass, HREIMS) Calcd for $C_{21}H_{26}O_2$ m/e 310.1933. Found, 310.1933.

3-Methoxy-11β-ethylestra-1,3,5(10)-triene-17-one (9) and 3-Methoxy-11α-ethylestra-1,3,5(10)-triene-17-one. Tetraene (8) (938 mg, 3.03 mmol) was dissolved in 64 mL of 20% EtOAc/methanol in a 250 mL round-bottomed flask. 5% Pd/C (200 mg) was added and the mixture vigorously stirred under hydrogen at 1 atm for 31 h, at which time GLC indicated consumption of starting material and production of 9 and 9'(11α-ethyl) in a ratio of 2.6:1

respectively. The reaction mixture was filtered through neutral alumina (7 cm X 25 mm) then through filter paper. Concentration *in vacuo* gave a yellow foam (902 mg, 96%) which was used without further purification. Previous partial separation of the crude mixture *via* preparative TLC (5:3:2 $CHCl_3$, hexane, CH_2Cl_2, 7 developments) revealed two major products:

(9): GLC t_R, 6.52 min; ^{1}H-NMR (500 MHz, $CDCl_3$) δ 0.91 (t, 3H, J = 7.6 Hz, CH_2CH_3), 1.04 (s, 3H, 18-CH_3), 3.78 (s, 3H, OCH_3), 6.62 (d, 1H, J = 2.1 Hz, 4-H), 6.73 (dd, 1H, J = 9.5, 2.1 Hz, 2-H), 7.08 (d, 1H, J = 9.5 Hz, 1-H); EIMS, 312 (M^+, 100), 285 (6), 257 (7), 199 (84), 186 (41), 184 (25), 160 (46), 91 (16).

(9'(11α-ethyl)): GLC t_R, 5.54 min; ^{1}H-NMR (500 MHz, $CDCl_3$) δ 0.98 (t, 3H, J = 8.5 Hz, CH_2CH_3), 1.00 (s, 3H, 18-CH_3), 3.78 (s, 3H, OCH_3), 6.67 (dd, 1H, J = 8.4, 2.1 Hz, 2-H), 6.69 (d, 1H, J = 2.1 Hz, 4-H), 7.73 (d, 1H, J = 8.4 Hz, 1-H); EIMS, 312 (M^+, 100), 199 (72), 186 (31), 184 (16), 160 (43).

11β-ethylestra-1,3,5(10)-triene-3-ol-17-one (10) and 11α-ethylestra-1,3,5(10)-triene-3-ol-17-one. A mixture containing (9) and (9') (902 mg, 2.84 mmol) was dissolved in 42 mL of freshly distilled CH_2Cl_2 in a 100 mL round-bottomed flask. The solution was cooled to O°C and 5.3 mL of $BF_3{\cdot}S(CH_3)_2$ (*vide supra*) was added. The reaction mixture was allowed to warm to room temperature as it reacted and was quenched at 24 h with ice-water (40 mL). The heterogeneous mixture was washed with saturated aqueous $NaHCO_3$ (50 mL), the layers separated and the aqueous layer extracted (3 X 50 mL, CH_2Cl_2). The combined organic layers were washed with brine and dried (Na_2SO_4). Concentration *in vacuo*was followed by ether trituration to provide (10) as a white powder (490 mg, 57%), an analytical sample of which was recrystallized from acetone/hexane to afford white needles; (10'(11α-ethyl)) remained as the soluble component and produced a yellow foam (209 mg, 24%):

(10): mp 250-254°C; ^{1}H-NMR (500 MHz, $CDCl_3$) δ 0.89 (t, 3H, J = 7.4 Hz, CH_2CH_3), 1.05 (s, 3H, 18-CH_3), 4.60 (s, 1H, OH), 6.53 (d, 1H, J = 2.1 Hz, 4-H), 6.66

(dd, 1H, J = 10.6, 2.1 Hz, 2-H), 7.04 (d, 1H, J = 10.4 Hz, 1-H); IR (KBr) 3340 (OH), 1719 cm^{-1} (ketone); EIMS, 298 (M^+, 100), 296 (39), 185 (47), 172 (28), 170 (12), 146 (36); Anal. Calcd for $C_{20}H_{26}O_2$: C, 80.48; H, 8.80. Found C, 80.40; H, 8.72.

(10'): ^{1}H-NMR (300 MHz, $CDCl_3$) δ 0.96 (t, 3H, J = 7.36 Hz, CH_2CH_3), 1.00 (s, 3H, 18-CH_3), 4.60 (bs, 1H, OH), 6.61 (dd, 1H, J = 8.2, 5.1 Hz, 2-H), 6.62 (bs, 1H, 4-H), 7.27 (d, 1H, J = 8.2 Hz, 1-H); IR ($CHCl_3$) 3390 (OH), 1730 cm^{-1} (ketone); EIMS, 298 (M^+, 60), 185 (44), 172 (36), 146 (100), 97 (12); Anal. (exact mass, HREIMS) Calcd for $C_{20}H_{26}O_2$ m/e 298.1932. Found, 298.1936.

The remainder of the synthesis was elaborated upon by another worker in this laboratory.[16]

Progestin Syntheses: 21*R*-Fluoro-17α,21-dimethyl-19-nor-pregna-4,9-diene-3,20-dione (27) and 21*S*-Fluoro-17α,21-dimethyl-19-nor-pregna-4,9-diene-3,20-dione (28).

3,3-Ethylenedioxy-17α,21-dimethyl-21*R*-hydroxy-14-nor-pregna-5(10),9(11)-diene-20-one (21) and 3,3-ethylenedioxy-17α,21-dimethyl-21*S*-hydroxy-14-nor-pregna-5(10),9(11)-diene-20-one (22). RU27987 (19) (25 mg, 73 μmol for synthesis of (21)) or RU27988 (20) (25mg, 73 μmol for synthesis of (22)) was dissolved in a mixture of ethylene glycol (98 μL, 1.8 mmol) and triethyl orthoformate (97 μL, 0.58 mmol) After addition of p-toluenesulfonic acid monohydrate (1.4 mg, 7.3 μmol) the green reaction mixtures were allowed to stir at room temperature for 5 min at which time triethylamine (4.8 μL, 34 μmol) was added, discharging the green color. Each reaction mixture was then poured onto saturated $NaHCO_3$, extracted with ether and dried (Na_2SO_4). Flash chromatography (40% EtOAc/hexane, 16 cm x 25 mm SiO_2) provided white solids in each case, each able to be recrystallized from isopropyl ether:

(21) 22.5 mg, 80%, white solid. mp 132-133°C; ^{1}H-NMR (300 MHz, $CDCl_3$) δ 0.64 (s, 3H, 18-CH_3), 1.19 (s, 3H, 17α-CH_3), 1.32 (d, 3H, J = 6.4 Hz, 21-CH_3), 2.98 (d, 1H, J = 9.54, OH), 3.99 (s, 4H, OCH_2CH_2O), 4.41 (dq, 1H, J = 9.5, 6.5 Hz, 21*R*-H), 5.58 (m, 1H, 11-H); IR (KBr) 3410 (OH), 1700 (ketone), 1370 cm^{-1}; EIMS, 386(M^+, 21), 313 (26),

227 (14), 99 (18), 95 (100), 43 (15); Anal Calcd for $C_{24}H_{34}O_4$: C, 74.54; H, 8.88. Found: C, 74.5 · H, 8.86.

(22) 21.3 mg, 75%, white plates. mp 144-45°C; ^{1}H-NMR (300 MHz, $CDCl_3$) δ 0.63 (s, 3H, 18-CH_3), 1.15 (s, 3H, 17α-CH_3), 1.31 (d, 3H, J = 7.0 Hz, 21-CH_3), 3.71 (d, 1H, J = 6.5, OH), 3.98 (s, 4H, OCH_2CH_2O), 4.54 (quintet, 1H, J = 6.7 Hz, 21*S*-H), 5.57 (m, 1H, 11-H); IR (KBr) 3410 (OH), 1700 (ketone), 1370 cm^{-1}; EIMS, 386 (M+, 100), 313 (32), 227(17), 99(25), 95 (99), 45 (20); Anal Calcd for $C_{24}H_{34}O_4$: C, 74.54; H, 8.88. Found: C, 74.57; H, 8.93.

3,3-Ethylenedioxy-21*S*[[(trifluoromethyl)sulfonyl]oxy]-17α,21-dimethyl-19-norpregna-5(10),9(11)-dione-20-one (23) and 3,3-ethylenedioxy-21*R*[[(trifluoromethyl)sulfonyl]oxy]-17α,21-dimethyl-19-norpregna-5(10),9(11)-dione-20-one (24) Ketol (21) or (22) (49 mg, 0.13 mmol) was dissolved in freshly distilled CH_2CH_2 (1.5 mL) and cooled in a CO_2/iPrOH bath. Triflic anhydride (6.5 μL, 39 μmol) was added followed by 2,6-lutidine (33 μL, 0.19 mmol) and each reaction mixture was allowed to stir for 1 h at which time each was diluted ith cold CH_2Cl_2. Passage through a 2 cm plug of cold neutral alumina (CO_2 jacket) gave a brownish oil in each case which was subsequently purified by flash chromatography (25% EtOAc/hexane, 20 cm x 25 mm SiO_2) to give white foams.

(23) 44.2 mg, 66%; ^{1}H-NMR (300 MHz, $CDCl_3$) δ 0.67 (s, 3H, 18-CH_3), 1.20 (s, 3H, 17α-CH_3), 1.64(d, 3H, J = 6.6 Hz, 21-CH_3), 3.99 (s, 4H, OCH_2CH_2O), 5.53 (q, 1H, J = 6.6 Hz, 21*R*-H), 5.59 (m, 1H, 11-H); EIMS, 518 (M^+, 54), 195(38), 171 (50), 99 (83), 95 (100), 91 (45), 86 (66), 57 (56), 43 (64); Anal (exact mass, HREIMS) Calcd for $C_{25}H_{33}SO_6F_3$ m/e 518.1950. Found, 518.1963.

(24) 43.4 mg, 67%; ^{1}H-NMR (300 MHz, $CDCl_3$) δ 0.69 (s, 3H, 18-CH_3), 1.20 (s, 3H, 17α-CH_3), 1.66 (d, 3H, J = 6.9 Hz, 21-CH_3), 3.99 (s, 4H, OCH_2CH_2O), 5.52 (q, 1H, J = 7 Hz, 21*S*-H), 5.59 (m, 1H, 11-H); EIMS, 518 (M^+, 28), 195(31), 171 (40), 99 (68),

95 (100), 91 (40), 86 (50), 57 (54), 43 (28); Anal (exact mass, HREIMS) Calcd for $C_{25}H_{33}SO_6F_3$ m/e 518.1950. Found, 518.1948.

3,3-Ethylenedioxy-21*R*-fluoro-17α,21-dimethyl-19-norpregna-5(10),9(11)-diene-20-one (25) and 3,3-Ethylenedioxy-21*S*-fluoro-17α,21-dimethyl-19-norpregna-5(10),9(11)-diene-20-one (26). Triflates (23) and (24) (94 mg, 84 μmol) were dissolved in THF (2.5 mL) and nBu_4NF (1 *M* in THF, 76 μL, 76 μmol) was added. The reaction mixture was allowed to stir at room temperature for 15 min at which time solvent was removed *in vacuo*. The oils were then passed down a 2 cm column of neutral alumina and the residue subjected to flash chromatography (25% EtOAc/hexane, 24 cm x 25 mm SiO_2) producing a clear oil in each case.

(25) 22.1mg, 67%; ^{1}H-NMR (300 MHz, $CDCl_3$) δ 0.67 (s, 3H, 18-CH_3), 1.21 (s, 3H, 17α-CH_3), 1.50 (dd, 3H, J = 17, 6.9 Hz, 21-CH_3), 3.99 (s, 4H, OCH_2CH_2O), 5.09 (dq, 1H, J = 50, 6.8 Hz, 21*S*-H), 5.58 (m, 1H, 11-H); ^{19}F-NMR (338 MHz, $CDCl_3$) ϕ -180.64 (dq, J = 46, 24 Hz, 21*R*-F); EIMS, 388 (M^+, 100), 360 (49), 315 (46), 171 (54), 99 (50), 95 (96), 43 (34); Anal (exact mass, HREIMS) Calcd for $C_{24}H_{33}O_3F$ m/e 388.2414. Found, 388.2428.

(26) 24.2 mg, 75%; ^{1}H-NMR (360 MHz, $CDCl_3$) δ 0.67 (s, 3H, 18-CH_3), 1.18 (s, 3H, 17α-CH_3), 1.48 (dd, 3H, J = 18, 6.5 Hz, 21-CH_3), 3.98 (s, 4H, OCH_2CH_2O), 5.12 (dq, 1H, J = 48, 6.5 Hz, 21*R*-H), 5.58 (m, 1H, 11-H); ^{19}F-NMR (338 MHz, $CDCl_3$) ϕ -180.32 (dq, J = 46, 22 Hz, 21*S*-F); EIMS, 388 (M^+, 100), 360 (45), 315 (38), 171 (49), 99 (49), 95 (100), 43 (23); Anal (exact mass, HREIMS) Calcd for $C_{24}H_{33}O_3F$ m/e 388.2414. Found, 388.2428.

21*R*-Fluoro-17α,21-dimethyl-19-nor-pregna-4,9-diene-3,20-dione (27) and 21*S*-Fluoro-17α,21-dimethyl-19-nor-pregna-4,9-diene-3,20-dione (28). Fluoride (25) or (26) (20 mg, 51μmol) was dissolved in 700 μL of acetone. Concentrated sulfuric acid (two drops, ~20 μL) was added and each reaction mixture was allowed to stir at 60°C for 5 min. Dilution with

1 mL water was follwed by ether extraction, drying (Na_2SO_4) and passage through a 2 cm plug of neutral alumina. Flash chromatography (35% EtOAc/hexane, 20 cm x 25 mm SiO_2) gave (27) as a clear oil and (28) as a brownish solid, (11 mg, 63% in each case).

(27): ^{1}H-NMR (300 MHz, $CDCl_3$) δ 0.86 (s, 3H, 18-CH_3), 1.20 (s, 3H, 17α-CH_3), 1.50 (dd, 3H, J = 24, 6.9 Hz, 21-CH_3), 5.10 (dq, 1H, J = 50, 6.9 Hz, 21*S*-H), 5.67 (ms,1H, 4-H); ^{19}F-NMR (338 MHz, $CDCl_3$) ϕ -180.46 (dq, J = 49, 24 Hz, 21*R* F); EIMS, 344 (M^+, 87), 269(100), 213 (38), 159 (39), 107 (48), 95 (40), 91 (40); Anal (exact mass, HREIMS) Calcd for $C_{22}H_{29}O_2F$ m/e 344.2142. Found, 344.2141.

(28): mp 121-124°C; ^{1}H-NMR (300 MHz, $CDCl_3$) δ 0.85 (s, 3H, 18-CH_3), 1.27 (s, 3H, 17α-CH_3), 1.48 (dd, 3H, J = 24, 6.5 Hz, 21-CH_3), 5.06 (dq, 1H, J = 48, 6.6 Hz, 21*R*-H), 5.68 (brs,1H, 4-H); ^{19}F-NMR (338 MHz, $CDCl_3$) ϕ -179.98 (dq, J = 41, 21 Hz, 21*S*-F); EIMS, 344 (M^+, 48), 269(57), 213 (23), 159 (35), 107 (34), 95 (26), 91 (29); Anal (exact mass, HREIMS) Calcd for $C_{22}H_{29}O_2F$ m/e 344.2152. Found, 344.2158.

Glucocorticoid Synthesis: 17α-[3'-Fluoropropynyl)]-11β,17β-dihydroxy-6-methyl-androsta-1,4,6-triene-3-one (35).

6α-Methyl-11β-hydroxy-androsta-1,4-diene-3,17-dione (30). 6α-Methylpredinsolone (29) (1.5 g, 4.00 mmol) was dissolved in 50% aqueous acetic acid (144 mL) and placed under nitrogen. Sodium bismuthate (6.9 g, 24.8 mmol) was added and the mixture was stirred for 5 h at room temperature. The mixture was extracted (2 x 200 mL $CHCl_3$) and the organic layers were combined, washed with saturated aqueous $NaHCO_3$ (3 x 150 mL) and brine (150 mL), and dried (Na_2SO_4). Concentration *in vacuo* followed by flash chromatography (60% EtOAc/hexane, 20 cm x 40 mm SiO_2) gave a white solid (1.20 g, 96%): mp 247°C; ^{1}H-NMR (300 MHz, DMSO-d^6) δ 1.04 (s, 3H, 18-CH_3), 1.04 (d, 3H, J = 5.9 Hz, 6α-CH_3), 1.38 (s, 3H, 19-CH_3), 4.22 (m, 1H, 11α-H), 4.78 (d, J = 3.1 Hz, -OH), 5.79 (d, 1H, J = 1Hz, 4-H), 6.12 (dd, 1H, J = 10, 1.2 Hz, 2-H), 7.27 (d, 1H, J = 10 Hz, 1-H); IR (KBr) 3440 (OH),

1735, 1658 (C=O 17-ketone), 1620, 1600, 1412 cm^{-1}; EIMS, 314 (M^+, 3), 136(100), 121(17), 91(9); Anal Calcd for $C_{20}H_{25}O_3$: C, 76.40; H, 8.33. Found: C, 76.27; H, 8.25.

6α-Methyl-11ß-hydroxy-androsta-1,4,6-triene-3,17-dione (31). Dione (30) (2.70 g, 8.59 mmol) was dissolved in 2-pentanol (145 mL) in a 250 mL round-bottomed flask equipped with a reflux condenser, magnetic stirring, and a nitrogen inlet. Chloranil (12.71 g, 51.7 mmol) was added followed by approximately 4 g of $CaCO_3$ and the mixture was stirred as it was heated at reflux for 5 h. The mixture was allowed to cool to room temperature, filtered, and concentrated *in vacuo*. The reddish-orange residue was redissolved in ~200 mL of $CHCl_3$,washed with H_2O (4 x 200 mL), 5% aqueous NaOH(5 x 100 mL), H_2O (5 x 200 mL), and brine (200 mL), and dried (Na_2SO_4). Concentration *in vacuo was* followed by flash chromatography (50% EtOAc/hexane, 20 cm x 70 mm SiO_2) to give a yellow solid (1.83 g, 68%): mp 198-200°C; ^{1}H-NMR (300 MHz, $CDCl_3$) δ 1.23 (s, 3H, 18-CH_3), 1.43 (s, 3H, 19-CH_3), 1.92 (s, 3H, 6-CH_3), 4.51 (m, 1H, 11α-H), 5.97 (s, 1H, 4-H), 6.15 (s, 1H, 7-H), 6.32 (dd, J = 9.8, 1.2 Hz, 2-H), 7.31 (d, J = 9.8 Hz, 1-H); IR (KBr) 3410 (OH), 1730 (d), 1658 (C=O, 17-ketone), 1615 (C=O, 3-ketone), 1409 cm^{-1}; EIMS, 312 (M^+, 66), 294 (59), 279(43), 185(100), 159(41); Anal Calcd for $C_{20}H_{24}O_3$: C, 76.89; H, 7.74. Found: C, 76.55; H, 7.87.

17α-[3'-(2-Tetrahydropyranyl)]-11ß,17ß-dihydroxy-6-methyl-androsta-1,4,6-triene-3-one (32). Propargyltetrahydropyranyl ether (475 µL, 3.52 mmol) in pentane (18 mL) was placed in a flame-dried 50 mL 2-necked round-bottomed flask under nitrogen and was cooled to 0°C in an ice/salt bath. BuLi (2.27 mL, 3.52 mmol) was added and a white precipitate formed. Dione (31) (500 mg, 1.60 mmol) in THF (10 mL, freshly distilled) was added to the flask and the mixture was allowed to stir for 5 h. The reaction mixture was quenched with saturated aqueous NH_4Cl (50 mL) and extracted (3 x 50 mL EtOAc). The combined organic layers were washed with H_2O (50 mL), brine (50 mL), and dried (Na_2SO_4). Concentration *in vacuo* was followed by flash chromatography (60% EtOAc/hexane, 20 cm x 40 mm SiO_2) to

give a yellow oil (230 mg, 51%). ^{1}H-NMR (300 MHz, $CDCl_3$) δ 1.20 (s, 3H, 18-CH_3), 1.41 (s, 3H, 19-CH_3), 1.91 (s, 3H, 6-CH_3), 3.47 and 3.79 (m, 2H, -OCH_2 of THP), 4.26 (d, 2H, J = 4.6, C≡CCH_2O), 4.50 (brs, 1H, 11α-H), 4.73 (d, 1H, J = 1.6 Hz, -OCHO), 5.87 (s, 1H, 4-H), 6.13 (s, 1H, 7-H), 6.31 (dd, J = 10, 1.2 Hz, 2-H), 7.31 (d, J = 10 Hz, 1-H); IR (KBr) 3430 (OH), 2920, 2875, 1650 (C=O, ketone), 1600, 1020 cm^{-1}; FABMS, 453(M^++H, 100), 369(35), 309(33), 185(68), 155(100), 135(87), 119(100); Anal (exact mass, HRFABMS) Calcd for $C_{28}H_{36}O_5$: m/e (+H) 453.2641. Found: 453.2641.

17α-[3'-hydrooxypropynyl)]-11β,17β-dihydroxy-6-methyl-androsta-1,4,6-triene-3-one (33). Ether (32) (63.1 mg, 0.139 mmol) was dissolved in THF (1 mL, freshly distilled). Water (3 mL) was added followed by HOAc (3 mL). The reaction mixture was stirred at 50°C for 4 h. After cooling to room temperature, dilution with water (5 mL) was followed by pouring the reaction mixture onto saturated aqueous $NaHCO_3$ (10 mL), extraction (EtOAc, 2 x 15 mL), and drying (Na_2SO_4). Concentration *in vacuo* followed by flash chromatography (EtOAc, 20 cm x 30 mm SiO_2) gave a colorless oil (38.3 mg, 75%): ^{1}H-NMR (300 MHz, CD_3CN) δ 1.11 (s, 3H, 18-CH_3), 1.38 (s, 3H, 19-CH_3), 1.89 (d, 3H, J = 1.6 Hz, 6-CH_3), 4.10 (s, 2H, C≡CCH_2O), 4.39 (d, 1H, J = 2.1 Hz, 11α-H), 5.96 (s, 1H, 4-H), 6.01 (s, 1H, 7-H), 6.20 (dd, J = 9.9, 1.6 Hz, 2-H), 7.34 (d, J = 9.9 Hz, 1-H); IR (KBr) 3400, 2980-2870, 1645 (C=O, ketone), 1600, 1035 cm^{-1}; EIMS, 368(M^+, 8), 317(17), 185(47), 159(100), 135(43), 85(36); Anal (exact mass, HREIMS) Calcd for $C_{23}H_{28}O_4$: m/e 368.1988. Found: 368.1980.

17α-[3'-methanesulfonyloxypropynyl)]-11β,17β-dihydroxy-6-methyl-androsta-1,4,6-triene-3-one (34). Triol (33) (42.7 mg, 0.12 mmol) was dissolved in THF (1.3 mL, freshly distilled), and the solution was cooled to 0°C. Triethylamine (31 μL, 0.22 mmol) was added, followed by a solution of mesyl chloride in THF (0.21 mmol, 10%, v/v). After stirring at 0°C for 2 h, and additional 8 μL (57 μmol) of triethylamine was added followed by 4 μL (52 μmol) of MsCl. After 30 min further, the cloudy reaction mixture was diluted with ice-water and

extracted (EtOAc). Brine wash and drying (Na_2SO_4) were followed by concentration *in vacuo* then flash chromatography (80% EtOAc/hexane, 23 cm x 30 mm SiO_2) which afforded a brownish oil (32.1 mg, 62%); ^{1}H-NMR (300 MHz, $CDCl_3$) δ 1.22 (s, 3H, 18-CH_3), 1.41 (s, 3H, 19-CH_3), 1.91 (s, 3H, 6-CH_3), 3.06 (s, 3H, SO_2CH_3), 4.51 (brs, 1H, 11α-H), 4.84 (s, 2H, C≡CCH_2O), 5.86 (s, 1H, 4-H), 6.14 (s, 1H, 7-H), 6.31 (dd, J = 10.2, 1.3 Hz, 2-H), 7.31 (d, J = 10.2 Hz, 1-H); FABMS, 447(M^++H, 100), 309(90), 195(40), 155(100), 135(100), 119(100); Anal (exact mass, HRFABMS) Calcd for $C_{24}H_{30}SO_6$: m/e (+H) 447.1823. Found: 447.1832.

17α-[3'-Fluoropropynyl)]-11β-17β-dihydroxy-6-methyl-androsta-1,4,6-triene-3-one (35). Mesylate (34) (12.6 mg, 28.2 mmol) was dissolved in THF (0.540 mL, freshly distilled). *n*-Bu_4NF (1 *M* in THF, 102 μL, 0.102 mmol) was added and the reaction mixture was heated at 60°C for 20 min. After cooling to room temperature, the reaction mixture was passed through a 3 cm plug of neutral alumina. Concentration *in vacuo* was followed by flash chromatography (40% EtOAc/hexane, 16 cm x 10 mm SiO_2) to produce a light yellow oil (10.4 mg, 28.1 μmol, 100%); ^{1}H-NMR (300 MHz, $CDCl_3$) δ 1.22 (s, 3H, 18-CH_3), 1.41 (s, 3H, 19-CH_3), 1.91 (s, 3H, 6-CH_3), 4.52 (d, 1H, J = 2.7 Hz, 11α-H), 4.96 (d, 2H, J = 47.5 Hz, C≡CCH_2F), 5.87 (s, 1H, 4-H), 6.14 (s, 1H, 7-H), 6.31 (dd, J = 10.2, 1.8 Hz, 2-H), 7.32 (d, J = 10.2 Hz, 1-H); ^{19}F-NMR (338 MHz, $CDCl_3$) ϕ-214.62 (t, J = 47.8 Hz, CH_2F); EIMS, 370(M^+, 7), 252(10), 237(17), 203(46), 185(29), 159(100), 135(44), 91(24), 41(27); Anal (exact mass, HREIMS) Calcd for $C_{23}H_{27}O_3F$: m/e 370.1944. Found: 370.1951.

Radiochemical Synthesis

General. Fluorine-18 was prepared from [^{18}O] H_2O by the ^{18}O (p,n) ^{18}F reaction. Tetrabutylammoniumhydroxide (1 *M* in in water (unless otherwise indicated), ~3 μmol) was added *via* Hamilton syringe to the bottom of a Vacutainer®. Water containing ^{18}F-fluoride was added to the base followed by acetonitrile (200 μL). The solution was then heated in an oil

bath at 110°C while a gentle stream of nitrogen assisted in the azeotropic removal of water. When almost dry, heat was removed and an additional 200 μL of acetonitrile was added for a second azeotropic distillation. Once nearly dry, the Vacutainer® was removed from the oil bath and the final segment of the evaporation was performed without heat. The reaction solvent was then added to the Vacutainer® and the resolubilized fluoride was transferred to a borosilicate glass vial (15 mm x 45 mm, teflon-lined cap) containing 1-2 mg of substrate. The entire process requires ~10 min and ~80% of the initial activity is transferred. Yields given are only for reactions in which isolated activity was used for *in vivo* experiments; yield ranges for smaller activity scale reactions are shown parenthetically. Activity collected is uncorrected for decay while yield (and yield ranges) are corrected. All reactions are with no carrier added HPLC injection volume was 1 mL. Identity of the radiolabeled compounds was confirmed by coelution with authentic unlabeled standards on HPLC. Specific activities were determined by a competitive radiometric binding assay.[28]

21*R*-[^{18}F]Fluoro-17α,21-dimethyl-19-nor-pregn-4,9-diene-3,20-dione ([^{18}F] 27) and 21*S*-[^{18}F]Fluoro-17α,21-dimethyl-19-nor-pregn-4,9-diene-3,20-dione ([^{18}F] 28). The reaction employed 1.41 mg (2.8 μmol) of triflate (25) or (26) and 3.1 μL of nBu$_4$NOH. Activity (120 mCi) was transferred in THF (500 μL). After 10 min at room temperature, solvent was removed under a gentle stream of nitrogen, the residue dissolved in acetone (500 μL) and 20-25 μL of concentrated H_2SO_4 (2 drops) was added. The reaction mixture was stirred at 60°C for 7-8 min at which time it was diluted with water (1.5 mL) and extracted (2 x 1.5 mL ether). The ether layer was passed through a 3 cm plug of Na_2SO_4, blown down under nitrogen, and the residue was redissolved in 1,2-dichloroethane prior to injection on the HPLC [Whatman M-9, 87% hexane/13% (5% iPrOH/CH_2Cl_2), 5 mL/min].

([^{18}F] 27): 30.2 mCi, 34% (39-56%); t_R = 16 min; SA = 1060 Ci/mmol.

([^{18}F] 28): 8.8 mCi, 12% (12-50%); t_R = 16 min; SA = 740 Ci/mmol.

17α-[3'-[^{18}F]-Fluoropropyl]-11β,17β-dihydroxy-6-methyl-androsta-1,4,6-triene-3-one ([^{18}F] 35). The reaction employed 1.45 mg (3.25 μmol) of mesylate (?) and 3 μL of nBu$_4$NOH in the resolubilization. Activity (192 mCi) was transferred to substrate in THF (200 μL). Reaction conditions and workup were identical to those in the synthesis of [^{18}F]27 and 28 above (from mesylate (34)). Purification *via* HPLC [Whatman M-9, 90% (5% iPrOH/CH_2Cl_2/10% hexane), 5 mL/min], gave 5.34 mCi, 4.6% (3.4-19%), t_R = 17 min; SA = 68 Ci/mmol.

In Vivo Biodistribution Studies. The protocol used in uptake studies involving tritiated steroids has been detailed elsewhere,[29] and the technique used for tritiated RU28362 was similar. Briefly, the rats were adrenalectomized several days prior to the experiment. [^{3}H]RU28263 were prepared in 20% ethanol/saline and were injected *via* the carotid artery with the animals under ether anaesthesia. At the indicated times, animals were decapitated, blood and organs were removed, weighed and counted. Receptor-mediated uptake was demonstrated by co-injection with unlabeled blocking compound (~18 μg/animal).

After HPLC purification, the fluorine-18 labeled compounds were concentrated *in vacuo*, dissolved in a small volume of ethanol (~200 μL) and that solution was filtered through an ethanol-wetted Aerodisc-LC13 (Millipore) filter. The vessel containing the fluorine-18 labeled steroid was rinsed with 200 μL of saline and the rinsings passed through the filter to give a total volume of 400 μL of 1:1 ethanol/saline. Further dilution was necessary to achieve the desired dosage (~15 μCi or ~100 μCi/100 μL <20% ethanol/saline). Anaesthetised animals were injected intravenously *via* the femoral vein, were decapitated at the indicated times and blood and organs were removed, weighed and counted. Receptor-mediated uptake was ascertained as described above. In experiments in which the effect of tamoxifen pre-treatment was ascertained, rats were injected subcutaneously with 200 μg tamoxifen/animal on each of the three days preceding the experiment. Experiments involving progestins and estrogens

employed immature Sprague female rats (~24 days old). Assessment of corticosteroid uptake was determined in adrenalectomized (4-8 days prior to the experiment) male Sprague-Pauley rate (). Adrenalectomized animals were maintained on 0.9% NaCl.

In Vitro Studies. Receptor binding affinities were measured either directly, in the case of [$^3H_2$]-RU28362 which was available in radiolabeled form, or indirectly (all other cases). When measured directly, specific target receptor-mediated and non-specific binding were displayed in terms of a Scatchard plot[30] in order to facilitate calculation of the equilibrium association constants.[31] Indirect measurements of affinity were obtained through competitive binding assays and a relative binding affinity (RBA), e.g, relative to 100 for estradiol in the estrogen series, was calculated. In competitive binding assays, increasing concentrations of the non-radiolabeled test compound were added to a series of incubations containing receptor and a fixed, receptor-saturating concentration of the appropriate tritiated steroid. Both direct and indirect methods rely on a differential dissociation of ligand from low-affinity, non-specific and high-affinity, specific sites and therefore represent non-equilibrium (dynamic) systems.

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