to each well. Plates were sonicated for approximately 10 s and the absorbance of each well was determined on a Titertek plate reader (Multiscan, MCC, Flow). The culture conditions for Manca human lymphoma cells were similar to those described for SW480 and MCF-7 cells.[11]

Transport Inhibition. Materials. ^{3}H-MTX was purchased from Amersham (specific activity 20 Ci/mmol) and purified by thin layer chromatography on Brinkmann Instruments, Inc., precoated cellulose TLC glass plates COL 300-25 a with 3% ammonium chloride mobile phase; R_f = 0.75. The purified MTX was extracted from the plate with water, dried under reduced pressure, and stored dry at -70 °C until use. Maximum storage time was 1 month. The procedure removed contaminants accounting for approximately 5% of the total radioactivity. Unlabeled MTX was a product of Sigma and was used without further purification.

Assay for Binding to the Reduced Folate Transporter. MOLT-4 human T-cell leukemia cells were grown in folate-free RPMI-1640 medium containing folate with 10% dialyzed fetal calf serum and 20 nM leucovorin to a density of ≤1 million cells per mL. The cells were harvested and 20 million cells were resuspended in 2.5 mL of assay buffer [107 mM NaCl, 20 mM Tris-HCl, 26.2 mM $NaHCO_3$, 5.3 mM KCl, 1.9 mM $CaCl_2$, 1 mM $MgCl_2$, and 7 mM glucose, pH 7.4 (Sirotnak et al.[19]) plus 10 mM dithiothreitol] at 37 °C.[16] To this was added 100 μL of MTX containing 1 μCi of purified ^{3}H-MTX. Aliquots of 0.45 mL were removed at specified times, layered on top of 0.5 mL of 1-bromododecane[19,20] in a 1.5-mL snap top disposable centrifuge tube, and centrifuged 30 s at 10000 rpm in a microcentrifuge to separate cells from medium and stop the reaction. The aqueous layer and most of the organic layer were then carefully removed, and tube bottom was cut off into a scintillation vial, and the pellet was extracted with 2 mL of 0.5 M NaOH at 37 °C overnight; 2 mL of 1 M HCl was then added, and the samples were counted in a liquid scintillation cocktail. Rates of MTX accumulation were linear for 8 to 10 min; all rates were calculated from uptake plots with four or five linear time points at ≤10 min. For determination of inhibition, test compounds at specified concentrations were added to the MTX solution at t = 0. Since the MTX concentration in the assay was well below K_m (K_m = 1 μM; G. K. Smith, unpublished), the calculated IC_{50} approximately equals K_i for a competitive inhibitor.

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Registry No. 3, 112887-62-4; 4, 133933-13-8; 5a, 133933-14-9; 5b, 133933-12-7; 6, 133933-15-0; 7, 133933-16-1; 8, 133933-17-2; 9, 133933-18-3; 11, 24188-72-5; 12, 26829-47-0; 13, 133933-19-4; 14, 133933-20-7; 15, 76858-72-5; 16, 133933-21-8; 17, 42473-28-9; 18, 133933-22-9; 19, 133933-23-0; 20, 133933-24-1; 21, 133933-25-2; 22, 15644-82-3; 23, 133933-26-3; 24, 133933-27-4; 25, 133933-28-5; 27, 18731-19-6; 28, 133933-29-6; 29, 133933-30-9; 30, 133933-31-0; 31, 133933-32-1; 32, 6232-11-7; 33, 133933-33-2; 34, 58677-07-9; 35, 112888-43-4; 36, 133933-34-3; 37, 133933-35-4; DDH, 77-48-5; TS, 9031-61-2; FPGS, 63363-84-8; 3-MeC_6H_4COCl, 1711-06-4; $H_2NCH_2CH(OMe)_2$, 22483-09-6; $Me_3CCOOCH_2Cl$, 18997-19-8; 4-$MeC_6H_4NH_2$, 106-49-0; MeCH=CHCHO, 4170-30-3; HC≡C-CH_2Br, 106-96-7; H-Glu(OEt)-OEt, 16450-41-2.

Structure-Activity Relationships of Estrogenic Ligands: Synthesis and Evaluation of (17α,20*E*)- and (17α,20*Z*)-21-Halo-19-norpregna-1,3,5(10),20-tetraene-3,17β-diols

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As part our program to probe the molecular requirement for estrogen-receptor binding we undertook the synthesis and evaluation of the 17α,*E* and 17α,*Z* halovinyl estradiols. By use of an improved variation of the existing synthetic strategy, the targeted compounds were prepared stereospecifically and in 92-98% yields from the corresponding 17α,*E* or 17α,*Z* [(tri-*n*-butylstannyl)vinyl]estradiol 3-acetates. The novel estradiol derivatives were evaluated for their relative binding affinity (RBA) for the estrogen receptor with use of a rat uterine preparation. The results demonstrated a marked difference between the *E* and *Z* isomers and among the halogen employed. The *Z* isomers possessed significantly higher RBA values and the larger halogens (I, Br) were more effective than the smaller Cl substituent. These results modify the previous interpretations of estrogen-receptor binding for steroidal ligands. As a result, our design of (radio)halogenated ligands will incorporate this concern for *Z* vs *E* stereochemistry.

Our research laboratories have had a long standing interest in the development of a radiolabeled estrogen derivatives as potential in vitro/in vivo radiodiagnostic agents.[1-6] Among the criteria for such compounds are the need for high specific activity, appropriate radioemission properties, rapid labeling conditions, and high receptor affinity.[7-12] While our group, as well as others,[13-20] has made substantial progress on the first three criteria, the systematic evaluation of the receptor-binding characteristics of the ligands, as a prospective method for the development of the 19-norpregna-1,3,5(10),20-tetraene-3,17β-diol derivatives, has been generally deficient. From the beginning of our program we had an interest in the effect of stereoisomerism at the 21-position; however, the recent report of the synthesis and tissue distribution of the (17α,20*Z*)-21-iodo-19-norpregna-1,3,5(10),20-tetraene-3,17β-diol ((*Z*)-(iodovinyl)estradiol) (**21**) in which a com-

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†On leave from the University of Pisa (R.F.) and from Scuola Normale Superiore (E.N.).

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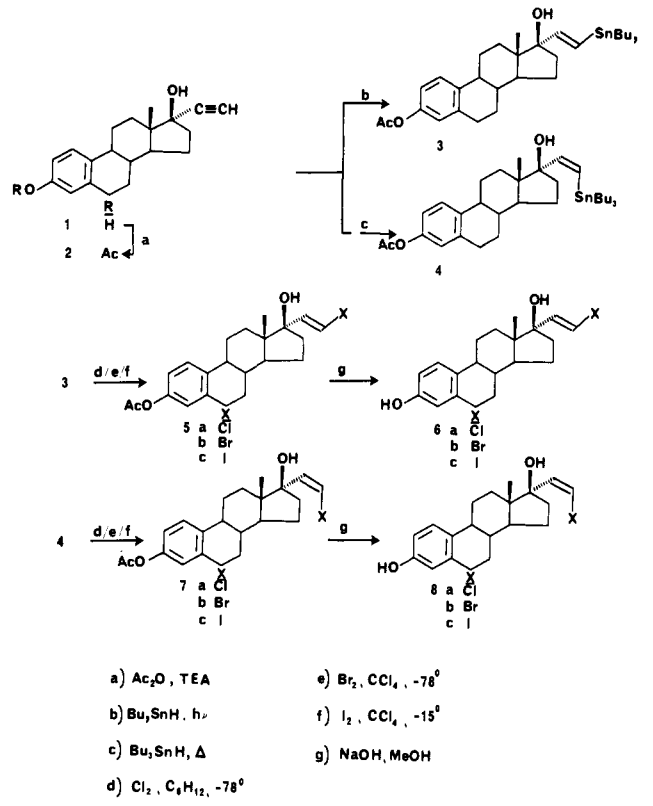
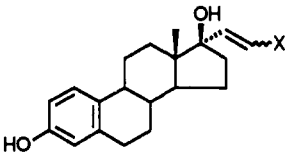
Scheme I



parison of the relative binding affinities (RBA) of the 20*E* and 20*Z* isomers was presented stimulated us to conduct a more complete evaluation of the 20-stereochemistry (*E* or *Z*) and the effect of the 21-substituent. In this paper we describe a significantly improved general synthesis of the (*E*)- and (*Z*)-substituted-vinyl estradiols and the estrogen receptor binding affinities under nonequilibrium and equilibrium conditions. The results, which are at variance with those previously reported, illustrate both a marked effect of 20-stereochemistry upon binding (*Z* > *E*) and a substantial, positive tolerance (I > Br > Cl) for the (*Z*)-halovinyl compounds at the receptor. The interpretation of the results provides additional information concerning the interaction between the estrogenic ligand and the receptor molecule.

Table I. Yields of 17-X-Vinylestradiols (Electrophilic Destannylation[a] and Saponification[b])



X	reagent	*E*: % yield (*J*, Hz)	*Z*: % yield (*J*, Hz)
Cl	Cl_2	92 (14.0)*[c]	94 (8.1)
Br	Br_2	95 (13.3)*[c]	98 (8.1)*[c]

[a] Reaction performed in CCl_4 at −15 °C. [b] Reaction performed in MeOH with 4-fold excess of 10 M aqueous NaOH, followed by acidification with AcOH, dilution with water extraction, and chromatography. [c] Determined on the acetate.

Chemistry

Synthesis of (17α,20*E*/*Z*)-21-Halo-19-norpregna-1,3,5(10),20-tetraene-3,17β-diols (Substituted Vinylestradiols). The synthesis of the target substituted (*E*)- and (*Z*)-vinylestradiols is illustrated in Scheme I. Previous syntheses that we and others had employed utilized the 17α-ethynylestradiols unprotected at the 3-phenolic hydroxyl.[1-8,20,21] While this was satisfactory for the preparation of small quantities of the intermediate (*E*)- and (*Z*)-[(tri-*n*-butylstannyl)vinyl]estradiols and their subsequent conversion to the radiohalogenated halovinyl estradiols, it was unsuitable for the preparation of the quantities of materials required in this study. The presence of the phenolic hydroxyl tended to cause protiodestannylation of the highly reactive (*Z*)-[(tri-*n*-butylstannyl)vinyl]estradiol when the isolated material was stored either as an oil or in solution. This in part influenced our initial decision to examine the labeled (*E*)-halovinyl estrogens rather than the (*Z*)-halovinyl analogues since contamination of the product would reduce the effective specific activity. Selective hydrostannation of **2** to **3** or **4** was accomplished according to the modified reaction conditions developed in our laboratory. Specifically, compound **3** was obtained as the major component of the reaction of **2** with a 3-fold excess of tributyltin hydride at 60 °C in tetrahydrofuran under UV irradiation, whereas **4** was the major product of the thermal reaction (60 °C) with tributyltin hydride in tetrahydrofuran when the reaction was stopped at 50% conversion. Separation of the (17α,*E*)- and (17α,*Z*)-[(tri-*n*-butylstannyl)vinyl]estradiol 3-acetate was readily achieved by column chromatography. In addition to providing ready access to either the *E* or the *Z* isomers, the acetates exhibited greater long-term stability than the corresponding phenolic intermediates.

The electrophilic destannylation reaction using the 17α-[(tri-*n*-butylstannyl)vinyl]estradiol acetates proceeded in very high yields with the predicted retention of configuration. The addition of the electrophilic species to the vinylstannanes in hexane or CCl_4 at temperatures from −78 to 15 °C gave a virtually instantaneous conversion to the (*E*)- and (*Z*)-[substituted-vinyl]estradiol 3-acetates **5a–c** and **7a–c**. In some cases the intermediate product precipitated from the CCl_4 and was characterized prior to saponification. Otherwise the crude reaction product was

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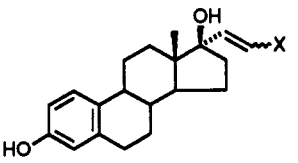
Table II. Relative Binding Affinities (RBA) for (*E*)- and (*Z*)-X-Vinylestradiols with Lamb and Rat Uterine Estrogen Receptors



	lamb, 0 °C		rat, 0 °C		rat, 25 °C	
X	*E*	*Z*	*E*	*Z*	*E*	*Z*
Cl	115	74	102	126	89	199
Br	71	117	78	195	56	661
I			77	202	62	776

subjected to saponification with methanol and sodium hydroxide, isolated, and purified by column chromatography to give **6a–c** and **8a–c**. There was no evidence of A-ring halogenation, and the overall yields for the two steps were in the 85–98% range (Table I). The stereochemistry of the product was determined by ^{1}H NMR spectrometric analysis of the coupling constants for the vinylic protons. In all cases, the coupling constants were greater for the *E* isomer than for the *Z* isomer (13–14 Hz vs 8 Hz), consistent with predicted values. The *Z* isomers were also consistently less polar, with R_f values slightly greater than the corresponding *E* isomers on normal-phase TLC and lower R_f values by reverse-phase TLC.

Estrogen-Receptor Binding

The ligands were incubated with rat uterine cytosol prepared from immature female rats or with lamb uterine cytosol to determine the relative binding affinities (RBA) compared to estradiol as the standard ligand.[22,23] Incubations were performed both at 0–4 °C for 1–2 h and at 25 °C for 18 h. The results of the competitive binding assays for the *E* and *Z* isomers with the rat cytosol preparations are shown in Figure 1A,B. In general, the results obtained with the lamb uterine cytosol at 0 °C were virtually identical with those observed with the rat uterine cytosol under the same incubation conditions. The two rat assays were used because it had been previously observed that a number of estrogenic substances reach equilibrium with the receptor much more slowly than estradiol.[24,25] In these cases, the RBA at 25 °C exceeds that determined at 0–4 °C. The two values therefore provided an indication of receptor affinity and the rates of interaction with the receptor.

The results of the competitive binding assay experiments are shown in Table II. The results clearly demonstrated that the 21-position, whether of *E* or *Z* configuration, tolerated ample substitution because all of the compounds demonstrated significant receptor binding. The RBA values at 0 °C, which are primarily an indication of the association rates, ranged from 77 to 202% while the RBA values at 25 °C, which more closely reflect equilibrium, ranged from 56 to >776%.

The data also indicated a significant difference in receptor binding for the 20*E* and 20*Z* isomers, both in the RBA values at 0 °C and at 25 °C. For this series of 17α-(halovinyl)estradiols, the affinity of the *Z* isomer was somewhat greater than the corresponding *E* isomer at 0 °C. This difference proceeds in the order Cl < Br < I. There was less of a difference in RBA values in the *E* series where Cl > Br ≈ I. The substituent effect was highlighted when the affinity was determined at 25 °C. The RBA values for the *Z* isomers increased by a factor of 50–250%, but the rank order of affinities remained the same, Cl < Br < I. For the *E* isomers, no increase in affinity was apparent when the incubation temperature was raised, and in fact, a slight decrease was observed. The same rank order was maintained, Cl > Br ≈ I.

Discussion

The binding to the intracellular receptor consitutes the final process in the localization of the steroid hormone radioligand. Its effectiveness as an imaging agent, in addition to such factors as specific activity and ease of synthesis, is related to its affinity for, and retention by, the receptor. We had previously demonstrated that a 17α-substituted-vinyl moiety constituted an effective group for the placement of a potential radionuclide; however, few studies had focused on the influence of *E*/*Z* stereoisomerism upon receptor binding. A single study using (17α,*E*)- and (17α,*Z*)-(iodovinyl)estradiol reported that the *Z* isomer possessed higher receptor binding affinity and that this constituted the basis for the enhanced localization and selectivity in vivo demonstrated by the *Z* isomer.[21] The only binding data consisted of an assay conducted at 0–4 °C and the actual reported difference in receptor affinities was small (47% vs 33%). It is improbable that this could account for the significant, observed difference in tissue distribution.

The data obtained in this study indicated substantially different properties for the (17α,*E*)- and (17α,*Z*)-(iodovinyl)estradiols in which both the magnitude of the receptor affinities and the effect under equilibration conditions are at variance. The *Z*/*E* ratio at 0–4 °C in our system was almost 3:1 (202% vs 77%), and this ratio increased to almost 13:1 when the incubation was conducted at 25 °C. The dissociation of the *E* isomer was sufficiently rapid to ensure that the equilibrium was established at 0–4 °C, whereas the *Z* isomer was much more slowly dissociative. It is this property that may account for the observed difference in clearance of the (*E*)- and (*Z*)-([^{125}I]iodovinyl)estradiols reported by Ali et al.[21] However, it does not explain differences in the magnitudes of uterine uptake that is at variance with previously reported (*E*)-([^{125}I]-iodovinyl)estradiol.[1,27]

The present study examined a greater number of vinylic substituents (X = Cl, Br, I) and evaluated the ligands under two different conditions to determine structure–activity relationships. Other groups have reported the effects of substituents at the 16α- and 17α-positions.[26–31] The general conclusions derived from these studies were

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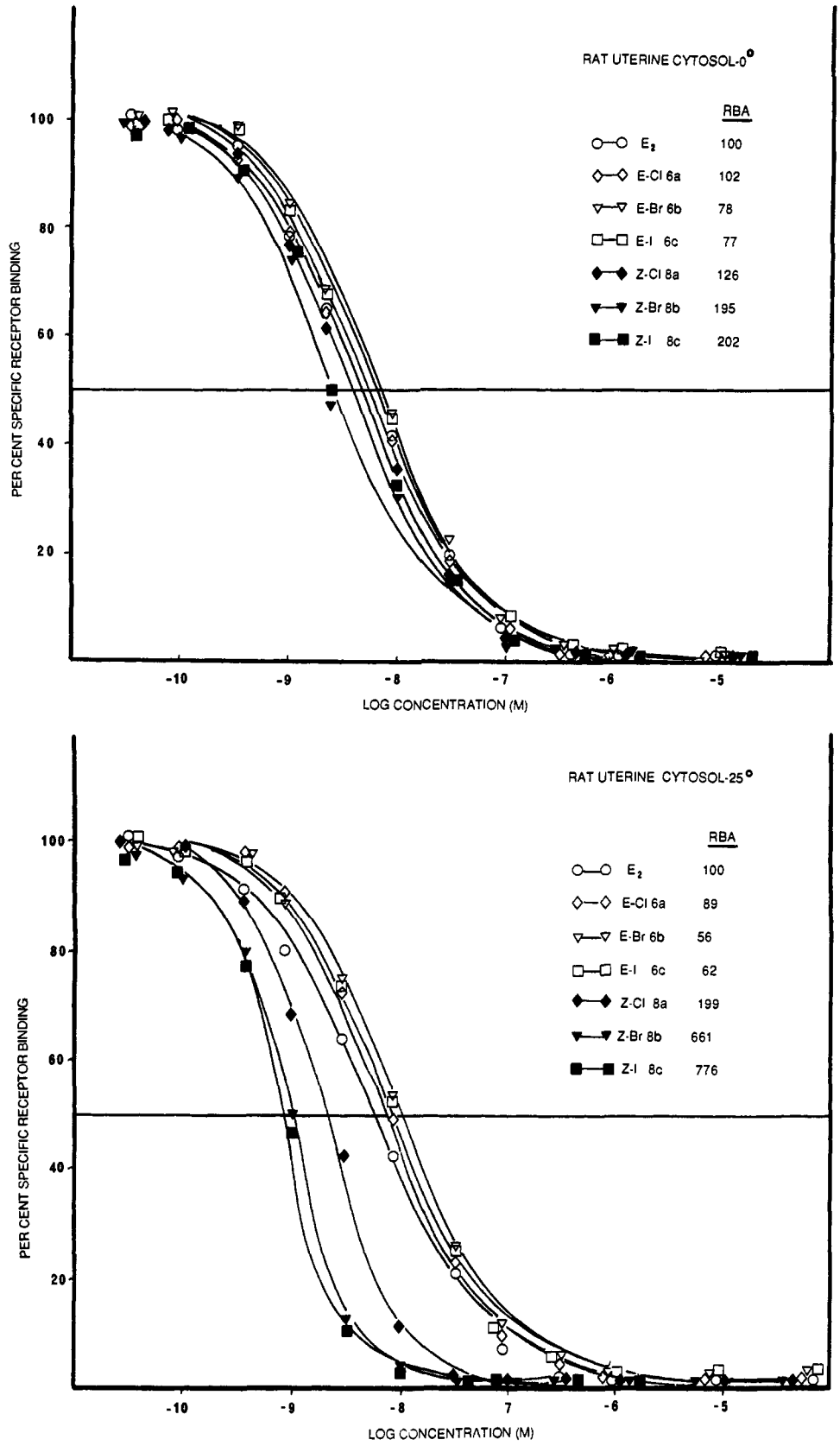

Figure 1. Relative binding affinity assay. Various concentrations of the unlabeled competitor, E_2-estradiol, **6a–c**, **8a–c**, were incubated with [^{3}H]estradiol in rat uterine cytosol at 0 °C (A) or 25 °C (B). Textran-coated charcoal was used to absorb the unbound steroid. Relative binding affinity (RBA) values were determined relative to estradiol (RBA = 100).

that only small, nonpolar groups could be accommodated on the D ring without a substantial loss of receptor binding, i.e., RBA ≥ 20%. The limitation in size at the 17α-position appeared to be an unbranched three-carbon unit (propyl, allyl) and that the order of affinities was ethynyl > propynyl ≥ allyl > propyl. Similar restrictions in size were observed at the 16-position. The results of our study suggest that the interaction between the 17α moiety and the receptor requires a degree of reinterpretation. The orientation of the substituent on the vinyl group differs

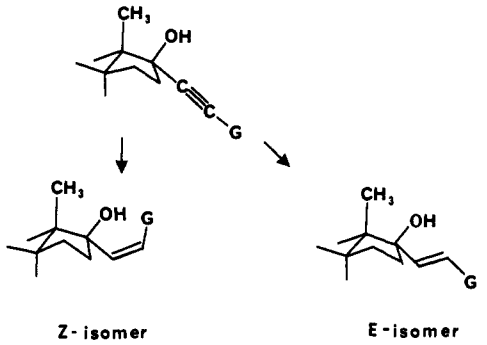

Figure 2. Preferred D-ring conformations for the 17α-alkynyl- and 17α-(G-vinyl)estradiols.

substantially depending upon the configuration of the double bond *E* and Z. Such an interaction in comparison with the 17α-alkynyl derivatives is depicted in Figure 2. For the *E* isomers and alkynyl derivatives, the G substituents are oriented in a similar fashion and qualitatively similar receptor affinities are observed. The RBA value for the 17α-(bromo- and iodovinyl)estradiols are relatively similar to the ratio of K_A values reported for the 17α-(bromoethynyl)estradiol (77–78% vs 63%).[28] The corresponding K_A value for the 17α-(iodoethynyl)estradiol could not be determined because of the instability of the compound[29] and the value for the 17α-chloroethynyl derivative was not reported.[30] The *Z* isomers have the G substituent directed at a different portion of the receptor and the binding that is observed is of a different order. Receptor affinities are relatively greater at both 0 and 25 °C, and increased lipophilicity enhances the effect within the halogen series. This can be rationalized by permitting the steroid binding domain of the estrogen receptor to possess an additional hydrophobic pocket that can accommodate small to moderately sized lipophilic moieties in that region.

The elucidation of this interaction has had the effect of stimulating further interest in our laboratory to explore both the extent of this hydrophilic pocket and whether there is a synergistic effect between 11β-binding and 17α,*Z*-substituted-vinyl groups. As some of our recent papers on the 11β-substituted estradiols indicate, appropriate substituents such as the fluoroethyl group at the site also dramatically improve the estrogen receptor binding properties of the steroid.[32-34] The presence of such synergism would lead to the generation of ultrahigh-affinity estrogenic ligands. The results of such studies will be the subject of subsequent publications.

Experimental Section

Melting points were determined with a Meltemp apparatus using open capillaries and are uncorrected. Flash chromatography was performed with silic gel according to the procedure of Still.[35] ^{1}H NMR spectra were obtained with a Varian XL 300 in $CDCl_3$ and chemical shifts are reported in ppm downfield from tetramethylsilane as an internal standard. THF was distilled from sodium benzophenone prior to each reaction; otherwise solvents were obtained as reagent grade and used without purification.

3-Acetoxy-17α-ethynylestra-1,3,5(10)-trien-17β-ol (2). The treatment of 17α-ethynylestradiol (1) with a 50% excess of acetic anhydride and triethylamine in CH_2Cl_2 (8 h, room temperature) gave the 3-acetyl derivative quantitatively; mp 152–154 °C.

3-Acetoxy-17α-(*E*)-[2-(tri-*n*-butylstannyl)ethenyl]estra-1,3,5(10)-trien-17β-ol (3). A solution of **2** (100 mg, 0.31 mmol), tri-*n*-butyltin hydride (0.27 g, 0.93 mmol), and THF (1.5 mL) was prepared under nitrogen in a sealed quartz tube. The lower portion of the tube containing the reaction solution was immersed just below the surface of a water bath (50–55 °C). The reaction solution was magnetically stirred and irradiated (15 cm) with a UV sunlamp (GE, 275 W). The reaction was monitored by TLC (silica gel, hexane–EtOAc 4:1), which indicated the gradual disappearance of **2**. There was the initial and temporary appearance of the *Z* isomer (R_f = 0.51) which was transformed almost completely to the product (R_f = 0.41) within 1.5–2 h. The solvent was removed by evaporation and the residue was purified (170 mg, 90%) by flash chromatography to give the product **3**, which crystallized upon standing: mp 65–67 °C; ^{1}H NMR δ 0.90–2.32 (m, 43 H), 2.27 (s, 3 H, CH_3CO), 2.85 (m, 3 H), 6.06, 6.21 (dd, *J* = Hz, 2 H, SnCH=CH), 6.78 (d, *J* = 2.5 Hz, 1 H, C(4)H), 6.83 (dd, *J* = 2.5, 8.5 Hz, 1 H, C(2)H), 7.27 (d, *J* = 8.5 Hz, 1 H, C(1)H).

3-Acetoxy-17α-(*Z*)-[2-(tri-*n*-butylstannyl)ethenyl]estra-1,3,5(10)-trien-17β-ol (4). A solution of **2** (100 mg, 0.31 mmol) and tri-*n*-butyltin hydride (0.27 g, 0.93 mmol) in THF (1.5 mL) was sealed in an ampule and heated with stirring at 60 °C for 2 h. The solvent was removed by evaporation and the residue triturated with hexane to yield **2** (35 mg), which was removed by filtration. The filtrate was applied to a chromatographic column (silic gel) and eluted with ethyl acetate–hexane (1:9) to afford **4** (87 mg, 46%) and **3** (13 mg, 5%). Further elution with ethyl acetate hexane (1:1) gave additional starting material (12 mg). The overall yield of **4** based upon recovered starting material was 90%: mp 84–86 °C; ^{1}H NMR δ 0.75–2.05 (m, 38 H), 2.15–2.35 (m, 5 H), 2.28 (s, 3 H, CH_3CO), 2.82–2.90 (m, 3 H), 5.87 (d, *J* = 13.0 Hz, 1 H, SnCH=CH), 6.77 (d, *J* = 13.0 Hz, 1 H, SnCH=CH), 6.79 (d, *J* = 2.5 Hz, 1 H, C(4)H), 6.83 (dd, *J* = 2.5 Hz, 1 H, C(4)H), 6.83 (dd, *J* = 2.5, 8.5 Hz, 1 H, C(2)H), 7.28 (d, *J* = 8.5 Hz, C(1)H).

Conditions for the Preparation of 6a/8a. A solution of **3/4** (0.2 mmol) in hexane (10 mL) was cooled to −78 °C. Chlorine gas (4.5 mL, 0.2 mmol) was slowly added via a syringe. A few drops of cyclohexane were added, and the reaction was slowly warmed to ambient temperature. The solvent was removed by evaporation to yield a residue that was dissolved in 10 mL of methanol containing 0.2 mL of 10 N aqueous NaOH. After stirring at ambient temperature for 5 min, the solution was acidified by the dropwise addition of acetic acid. The solution was partitioned between ethyl acetate and water. The organic phase was washed with 10% $NaHCO_3$, dried over $MgSO_4$ (anhydrous), filtered, and evaporated to dryness. The residue was purified by column chromatography to afford pure **6a/8a** (92%/94%).

6a: mp 100 °C dec; $[\alpha]^{25}_D$ = +30.303° (*c* = 0.86; ethyl acetate); ^{1}H NMR ($CDCl_3$, DMSO-d_6) δ 0.92 (s, 3 H, C(18)H), 1.22–2.38 (m, 12 H), 2.68 (s, 1 H), 2.80 (m, 3 H), 6.20 (s, 2 H, ClCH=CH), 6.58 (d, *J* = 2.5 Hz, 1 H, C(H)H), 6.65 (dd, *J* = 2.5, 8.5 Hz, 1 H, C(1)H), 7.09 (d, *J* = 8.5 Hz, 1 H, C(1)H), 7.86 (s, 1 H); R_f = 0.32 (hexane–ethyl acetate 2:1). Anal. ($C_{20}H_{25}O_4Cl\cdot H_2O$) C, H.

8a: mp 97.99 °C dec; $[\alpha]^{25}_D$ = +35.168° (*c* = 0.65, ethyl acetate); ^{1}H NMR ($CDCl_3$) δ 0.95 (s, 3 H, C(18)H), 1.25–2.35 (m, 13 H), 2.80 (m, 3 H), 2.86 (s, 1 H), 4.80 (s, 1 H), 6.01, 6.04, 6.16, 6.19 (AB q, H, ClCH=CH), 6.56 (d, *J* = 2.5 Hz, 1 H, C(4)H), 6.63 (dd, *J* = 2.6, 8.5 Hz, 1 H, C(2)H), 7.15 (d, *J* = 8.5 Hz, 1 H, C(1)H); R_f = 0.32 (hexane–ethyl acetate 2:1). Anal. ($C_{20}H_{25}O_4ClH_2O$) C, H.

Conditions for the Preparation of 6b/8b. To a solution of **3/4** (0.2 mmol) in hexane (10 mL), stirred at −78 °C, was added dropwise 2 mL of a 0.1 M solution of Br_2 in CCl_4. A few drops of cyclohexane were added, and the reaction was warmed to ambient temperature. The solvent was removed by evaporation to yield a residue, which was worked up as described for **6a/8a**. Purification by column chromatography gave pure **6b/8b** (95%/98%).

6b: mp 100 °C dec; $[\alpha]^{25}_D$ (*c* = 0.65, ethyl acetate); ^{1}H NMR ($CDCl_3$, DMSO-d_6) δ 0.91 (s, 3 H, C(18)H), 1.20–2.50 (m, 16 H), 2.79 (m, 3 H), 6.25, 6.30, 6.45, 6.50 (AB q, 2 H, BrCH=CH), 6.57 (d, *J* = 2.5 Hz, 1 H, C(4)H), 6.64 (dd, *J* = 2.5, 8.5 Hz, 1 H, C(2)H), 7.10 (d, *J* = 8.5 Hz, 1 H, C(1)H). Anal. ($C_{20}H_{25}O_4Br\cdot 2H_2O$) C, H.

8b: mp 100 °C dec; $[\alpha]^{25}_D$ = +48.275° (*c* = 0.59, ethyl acetate); ^{1}H NMR ($CDCl_3$, DMSO-d_6) δ 0.96 (s, 3 H, C(18)H), 1.22–2.36

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(m, 13 H), 2.76–2.88 (m, 3 H), 4.70 (s, 1 H), 6.29, 6.31, 6.49, 6.51 (AB q, BrCH=CH), 6.56 (d, 2.5 Hz, 1 H, C(1)H); R_f = 0.32 (hexane–ethyl acetate 2:1). Anal. ($C_{20}H_{25}O_4Br \cdot 2.5H_2O$) C, H.

Conditions for the Preparation of 6c/8c. To a solution of **3/4** (0.2 mmol) in CCl_4 (10 mL), stirred at −18 °C, was added dropwise a solution of I_2 in CCl_4 (50 mg in 5 mL). The reaction was warmed to ambient temperature and the solvent was removed by evaporation. The reaction was worked up as previously described. Purification by column chromatography gave pure **6c/8c** (95%/96%).

6c: mp 112–115 °C (lit.[14] mp 113–115 °C).

8c: mp 124–126 °C (lit.[21] mp 123–125 °C).

Competitive Receptor Binding Assay. All cytosol for the estrogen receptor were prepared and stored in TEA buffer (0.01 M Tris·HCl, 0.0015 M EDTA, 0.02% sodium azide, pH 7.4 at 25 °C). Rat uterine cytosol was prepared from Holtman rats (21–25-day-old females) and stored in liquid nitrogen. The uterine cytosol was prepared and stored as previously described by Katzenellenbogen et al.[22] The competitive receptor binding assays were performed as previously described[22,23] and the results tabulated as relative binding affinities (RBA) relative to estradiol (RBA = 100).

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C-Glycosidic Analogues of Lipid A and Lipid X: Synthesis and Biological Activities

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The synthesis of a series of novel analogues of lipid A, the lipophilic terminal of lipopolysaccharides (LPS), and lipid X, the reducing monosaccharide unit in lipid A, is reported. In these compounds, the native 1-*O*-phosphate group has been replaced by a "bioisosteric" CH_2COOH substituent. The new N,O-acylated monosaccharide *C*-glycosides were obtained by Wittig reaction of suitably protected glucosamine derivatives. These lipid X analogues were recognized as substrates by the enzyme lipid A synthase and could be coupled with UDP-lipid X to afford the corresponding disaccharide analogues of the lipid A precursor on preparative scale. All compounds were characterized by NMR, MS, and elemental analysis, and were tested for their ability to enhance nonspecific resistance to infection in mice and also for endotoxicity. The results clearly show that the new compounds express biological activities similar to those of their O-phosphorylated natural counterparts. Furthermore, these compounds exhibit a better therapeutic index in mouse models than the standard LPS obtained from *Salmonella abortus equi*.

Introduction

Lipopolysaccharides (LPS), also called endotoxins, are constituents of the cell walls of Gram-negative bacteria. They cause a wide array of pathophysiological effects and are extremely potent immunostimulants.[1-9] Structurally, they consist of an essentially linear polysaccharide chain anchored to the cell wall by a lipophilic disaccharide, lipid A (1), which is a β-(1→6)-glucosamine disaccharide acylated with several fatty acids—mostly 3-hydroxy myristic acid—and bearing two phosphate groups in positions 1 and 4′ (see Chart I). While the composition of the polysaccharide chains of LPS varies widely among enterobacterial strains, their lipid A's differ only slightly. Lipid A is responsible for most of the immunopharmacological activities of LPS, including the induction of endotoxic shock.[9] Due to their toxicity, neither LPS, nor lipid A have found clinical applications.

Numerous compounds, including biosynthetic lipid A precursors and analogues related to lipid A partial structures have been isolated or synthesized with the aim of separating unwanted endotoxic properties from potentially beneficial immunostimulatory effects.[10-21] Compounds

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