20% methylamine in diisopropyl ether (30 mL) was then added, and the temperature was allowed to rise to room temperature. After being stirred overnight, the reaction mixture was diluted with water, extracted with $CHCl_3$, and evaporated, and the compound was purified by flash chromatography (increasing the polarity of the eluant from ethyl acetate–cyclohexane, 1:1, to pure ethyl acetate) to give 1.08 g (68%) of IIb, mp 158 °C dec: ^{1}H NMR (DMSO-d_6) δ 2.62 (s, 3 H), 2.55–3.10 (m, 4 H), 4.79 (s, 1 H), 6.47 (dd, 1 H), 6.60 (dd, 1 H), 6.90–7.40 (m, 3 H), 8.17 (dd, 1 H), 10.07 (s, 1 H); MS, m/z 253 (M^+) 252 (100), 210. Anal. ($C_{16}H_{15}NO_2$) C, H, N.

6-Methyl-4,5,6,6a-tetrahydro[1]benzopyrano[4,3,2-*ij*]isoquinolin-10-ol (IIc). Analogously to compound IIb, with **14** (1.8 g, 5.64 mmol) as starting material, 0.86 g (60%) of IIc was obtained as pale yellow crystals, mp 200–210 °C dec: ^{1}H NMR (DMSO-d_6) δ 2.60 (s, 3 H), 2.45–3.10 (m, 4 H), 4.78 (s, 1 H), 6.62–7.54 (m, 6 H); MS, m/z 253 (M^+). Anal. ($C_{16}H_{15}NO_2$) C, H, N.

7-Methyl-6a,7,8,9-tetrahydro-1,3-dioxolo[5,6][1]benzopyrano[4,3,2-*ij*]isoquinoline (IId). Analogously to compound IIb, with **15** (1.39 g, 4 mmol) as starting material, 0.83 g (74%) of IId was obtained as a colorless oil: ^{1}H NMR ($CDCl_3$) δ 2.70 (s, 3 H), 2.70–3.30 (m, 4 H), 4.91 (s, 1 H), 5.98 (d, 1 H, J = 2 Hz), 6.05 (d, 1 H), 6.50 (d, 1 H, J = 8.5 Hz), 6.68 (d, 1 H), 7.00–7.40 (m, 2 H), 7.81 (dd, 1 H); MS, m/z 281 (M^+). Anal. ($C_{17}H_{15}NO_3$) C, H, N.

6-Propyl-4,5,6,6a-tetrahydro[1]benzopyrano[4,3,2-*ij*]isoquinolin-11-ol (IIf). Analogously to compound IIb, with **13** (3 g, 9.4 mmol) and excess *n*-propylamine as starting materials, 2 g (77%) of IIf was obtained as a solid, mp 150 °C dec: ^{1}H NMR ($CDCl_3$) δ 0.95 (t, 3 H), 1.64 (m, 2 H), 2.95 (m, 6 H), 5.13 (s, 1 H), 6.54 (d, 2 H), 6.90–7.40 (m, 3 H), 8.02 (dd, 1 H), 10.10 (br, 1 H); MS, m/z 281 (M^+), 280 (100). Anal. ($C_{18}H_{19}NO_2$) C, H, N.

6-Propyl-4,5,6,6a-tetrahydro[1]benzopyrano[4,3,2-*ij*]isoquinolin-10-ol (IIg). Analogously to compound IIb, with **14** (1.3 g, 4.1 mmol) and excess *n*-propylamine as starting materials, 0.7 g (64%) of IIg, mp 164–166 °C, was obtained: ^{1}H NMR ($CDCl_3$/DMSO-d_6) δ 0.90 (t, 3 H), 1.62 (m, 2 H), 2.70–3.20 (m, 6 H), 4.96 (s, 1 H), 6.66 (dd, 1 H), 6.76 (d, 1 H), 7.10–7.50 (m, 4 H), 8.91 (s, 1 H); MS, m/z 281 (M^+), 280, 251. Anal. ($C_{18}H_{19}NO_2$) C, H, N.

7-Propyl-6a,7,8,9-tetrahydro-1,3-dioxolo[5,6][1]benzopyrano[4,3,2-*ij*]isoquinoline (IIh). Analogously to compound IIb, with **15** (1.4 g, 4 mmol) and excess *n*-propylamine as starting materials, 0.84 (68%) of IIh was obtained as a colorless oil: ^{1}H NMR ($CDCl_3$) δ 0.93 (t, 3 H), 1.67 (m, 2 H), 2.70–3.20 (m, 6 H), 5.14 (s, 1 H), 6.02 (d, 1 H, J = 1.5 Hz), 6.11 (d, 1 H), 6.53 (d, 1 H), 6.70 (d, 1 H), 7.13 (d, 1 H), 7.30 (t, 1 H), 7.83 (d, 1 H); MS, m/z 309. Anal. ($C_{19}H_{19}NO_3$) C, H, N.

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Registry No. **1**, 100527-36-4; **2**, 113793-78-5; **2** (diazoketone), 113794-02-8; **3**, 113793-79-6; **4**, 113793-80-9; **5**, 113793-81-0; **6**, 16932-44-8; **7**, 25245-35-6; **8**, 113793-82-1; **9**, 113793-83-2; **10**, 113793-84-3; **11**, 113793-85-4; **12**, 113793-86-5; **13**, 113793-87-6; **14**, 113793-88-7; **15**, 113793-89-8; **16**, 113579-20-7; **17**, 113793-90-1; **18**, 113810-69-8; **19**, 113793-91-2; **20**, 113793-92-3; **21**, 113793-93-4; IIa, 113793-94-5; IIb, 113793-95-6; IIc, 113793-96-7; IId, 113793-97-8; IIe, 113793-98-9; IIf, 113793-99-0; IIg, 113794-00-6; IIh, 113794-01-7; 1,3-$(OCH_3)_2C_6H_4$, 151-10-0; 1,4-$(OCH_3)_2C_6H_4$, 150-78-7; 1,2,3-CO_2H,$(COOCH_3)$,$NO_2C_6H_3$, 6744-85-0; 5-(benzyloxy)-1,3-benzodioxole, 66177-24-0.

Synthesis and Estrogen Receptor Selectivity of 1,1-Bis(4-hydroxyphenyl)-2-(*p*-halophenyl)ethylenes

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A series of triarylethylenes (**1a–e**) were synthesized and evaluated for their ability to compete with [^{3}H]estradiol for high-affinity estrogen receptors (ER) in immature rat uterine cytosol. All compounds showed affinity comparable to that of estradiol, with **1c** having the highest affinity and the lowest calculated nonspecific binding of the para-halogenated members. Compound **1a** had a higher affinity than did its chlorovinyl counterpart **1b**, indicating that a vinyl hydrogen was suitable for high ER affinity in this series. Compound **1c** was labeled with ^{3}H ortho to one or both of its hydroxyls. Its ratio of specific to nonspecific binding in rat uterine cytosol, 3.2, was 140% of that of a related triarylethylene, 4-hydroxytamoxifen, and was 24% that of estradiol. Administration of [^{3}H]-**1c** to immature female rats resulted in accumulation of ^{3}H in uterine tissue which was decreased 39% when [^{3}H]-**1c** was coadministered with estradiol. The major site of accumulation 1, 4, and 8 h after administration was in the intestinal tract. Chromatographic analysis showed that levels of **1c** were less than those of **1c** glucuronide in blood plasma, liver, and intestinal contents of rats 1 h after administration of **1c**. Uterine ^{3}H was comprised of 85% of **1c** and 11% of **1c** glucuronide. These results indicate that **1c** undergoes ER-mediated uptake in the immature female rat, but selectivity is reduced due to nonspecific accumulation of free and conjugated **1c** in uterine tissue.

The presence of estrogen receptors (ER) has become a determining factor in the choice of therapy for breast cancer.[1] Such cancers, which have significant concentrations of ER, can often be suppressed by use of hormones and antihormones.[2]

For in vivo detection of ER, a variety of steroidal and nonsteroidal compounds known to interact strongly with ER in vitro, and capable of bearing short-lived radioisotopes of fluorine, bromine, or iodine, have been evaluated for their ability to locallize in ER-containing normal and malignant tissue.[3] The aim of such studies is to identify

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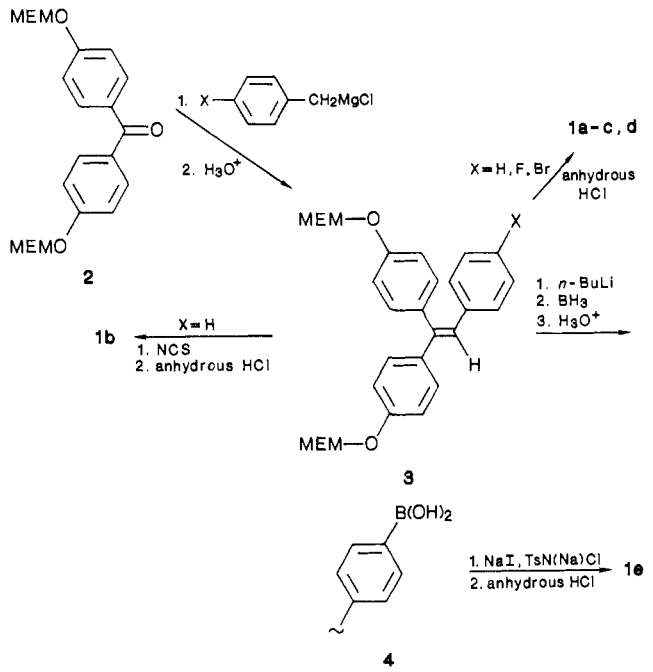
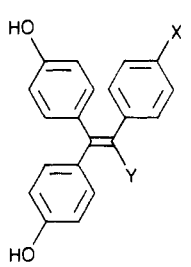
0022-2623/88/1831-1471$01.50/0

Scheme I



ligands with noninvasive diagnostic and radiotherapeutic applications in breast cancer.

ER affinity of compounds of general structure **1** was initially suggested by the finding that **1a** was a potent estrogen in the ovariectomized mouse.[4] Later studies indicated that such ligands, in which X = H and Y = CN, Et, or Br, had ER affinity at least equal to that of estradiol and accumulated in ER-containing tissue.[5] We wanted to find out if similar high ER affinity was retained in analogues of **1** in which Y = H. Also, we wanted to find out the degree to which such compounds, bearing para halogens, underwent ER-mediated uptake and retention in the rat.



	X	Y
1a:	H;	H
b:	H;	Cl
c:	F;	H
d:	Br;	H
e:	I;	H

Results and Discussion

Chemistry. Compounds **1a,c,d** were prepared by reaction of protected benzophenone **2** with the appropriately substituted benzylmagnesium chloride, prepared in diethyl ether,[6] followed by deprotection (Scheme I). Elemental analysis of **1d** indicated it to contain ca. 5% of the product of debromination (**1a**). Arylboronic acid **4** was prepared by lithiation of **3** (X = Br) with *n*-butyllithium in tetrahydrofuran, followed by addition of borane and selective acid hydrolysis of the resulting arylborane.[7] Compound **1e** was prepared by iododeboronation of **4**[8] followed by deprotection.

[^{3}H]-**1c** with specific activity of 44 Ci/mmol was prepared by iodination of **1c** followed by palladium-catalyzed hydrogenolysis with tritium gas, with conditions shown in preliminary experiments to minimize reduction of the ethylenic bond. Similar iodination–deiodination procedures have been used to prepare other ortho-tritiated ER ligands.[9]

In Vitro Studies. Relative abilities of **1a–e** to displace specifically bound estradiol in rat uterine cytosol are summarized in Table I. Comparison of RBA values of **1a** and **1b** indicated that a vinyl hydrogen substituent would be suitable instead of larger substituents necessary in other

Table I. Estrogen Receptor Affinities of **1a–e**[a]

compd	EC_{50}, nM[b]	RBA[c]
1a	3.2	178
1b	4.5	127
1c	2.9	197
1d	10.4	55
1e	3.3	173
estradiol	5.7	100

[a] The concentrations required to displace, by 50%, specifically bound [^{3}H]estradiol in cytosol from immature rat uterine homogenates (EC_{50}) were determined. Each incubation mixture contained 0.2 mL uterine cytosol, 10 μL of an ethanol solution of [^{3}H]estradiol (final concentration, 3.5 nM), and 10 μL of a solution of the competing ligand in *N,N*-dimethylacetamide. Mixtures were kept at 4 °C for 4 h and specifically bound ^{3}H was determined as described.[17] Values shown are averages of two determinations. [b] With use of 3.0 nM [^{3}H]-4-hydroxytamoxifen, EC_{50} values for **1c**, **1e**, and 4-hydroxytamoxifen were 170, 9.5, and 6 nM, respectively. [c] Relative binding affinity.

Table II. Comparative Binding of ER Ligands[a]

^{3}H-labeled ligand	specific binding, *S*, fmol/uterus	nonspecific binding, *N*, fmol/uterus	*S*/*N*
1c	971 ± 20	305 ± 5	3.2
4-hydroxytamoxifen	1082 ± 6	475 ± 33	2.3
estradiol	501 ± 13	37 ± 5	13.5

[a] Data are averages ± SD of three to six determinations. Each radiolabeled ligand (2–4 nM) was incubated with 0.2-mL aliquots of cytosol from 1:2 homogenates of uteri from immature rats, as described in Table I. Incubations containing a ca. 300-fold molar excess of respective unlabeled ligands were analyzed to determine nonspecific binding.

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Table III. Distribution of [^{3}H]-1c in the Immature Rat[a]

time after ip dosing, h	fmol equivalents per 100 mg			
	uterus[b]	plasma[c]	liver	intestinal contents
1	496	122	251	2360
1[d]	302	198	373	880
1[e]	429	144	318	3269
4	196	56	97	834
8	87	46	61	665

[a] Each value is the average of three to four determinations of ^{3}H in extracts from pooled biological specimens, in which individual values varied by less than 5%. [b] Average uterine wet weight was 43 mg. [c] Preliminary studies indicated only minor differences in recoveries of ^{3}H from whole blood and plasma. [d] A 100-fold molar excess (8 μg) of estradiol was also given ip at a separate injection site. [e] The radioligand was administered iv via the tail vein.

hydroxytriarylethylenes for high ER affinity[10] and potent estrogenic activity.[11]

Besides ER affinity, another criterion for prospective assessment of ligands for in vivo applications is degree of nonspecific binding, a property linearly related to ligand liposolubility. On the basis of the use of fragmental constants,[3a] the extent of nonspecific binding of the halogenated triarylethylenes was calculated to be lowest in **1c**. Since this analogue also had the highest ER affinity among these compounds, it was chosen for further study.

The extent of specific binding of **1c** was nearly twice that of estradiol in rat uterine cytosol and was comparable to that of 4-hydroxytamoxifen, a related hydroxytriarylethylene with high ER affinity (Table II).[12] The extent of nonspecific binding of **1c** was 8.2 times greater than that of estradiol.

In Vivo Studies. Previously [^{125}I]-**1e** was found *not* to accumulate in ER-containing tissue,[13] even though the present data indicate **1e** to have high ER affinity. Excessive nonspecific binding or metabolic loss of ^{125}I may have accounted for this. Aryl iodides have been shown to undergo deiodination in the presence of liver enzymes.[14]

In contrast, uterine tissue was an important site of distribution of [^{3}H]-**1c** (Table III). Relative levels of ^{3}H in uterine and liver tissue, and the degree of estradiol-reversible accumulation, were similar to those observed in rats given [^{3}H]-4-hydroxytamoxifen.[15] The ratios of uterus/plasma levels (4.1, 3.5, and 1.9 at 1, 4, and 8 h after dosing, respectively) were less than those seen after administration of [^{3}H]estradiol, as was the degree of observable estradiol-reversible accumulation.[15,16] These results may be a consequence of the lower S/N of **1c** compared to estradiol (Table II). Also, since estradiol coadministration increased the systemic availability of ^{3}H in liver and blood plasma by 49% and 62%, respectively, it may have done so as well in uterine tissue, an effect that would lower the observable estradiol-reversible accumulation.

Table IV. Effect of β-Glucuronidase on Relative Recoveries of ^{3}H Associated with 1c and Polar Components in Biologic Extract Concentrates[a]

extract from	treatment with	% of total ^{3}H as	
		1c	polar components[b]
uterus	-	84.5	15.5
	G	95.9	4.1
plasma	-	28.7	71.3
	G	92.1	7.9
liver	-	15.7	84.3
	G	75.9	24.1
intestinal contents	-	5.3	94.7
	G	95.4	4.6

[a] Results are averages of duplicate determinations. Reconstituted extracts, from biological specimens obtained 1 h after administration of [^{3}H]-1c, were incubated without (-) and with (G) β-glucuronidase. Recoveries shown were 95–100% of total ^{3}H in chromatograms. [b] The ^{3}H remaining at the origin in developed chromatograms.

Another factor known to influence efficacy and tissue selectivity of ER ligands is drug metabolism. In particular, such ligands can undergo conjugation at phenolic hydroxyls critical for high ER affinity. To assess this, we subjected tissue, plasma, and intestinal content extracts obtained 1 h after administration of [^{3}H]-**1c** to chromatographic analysis. In each extract the sum of polar components and unchanged **1c** accounted for nearly all of the ^{3}H present. Treatment of extracts with β-glucuronidase prior to chromatography resulted in decreased recoveries of polar material and increased respective recoveries of **1c** (Table IV). This was particularly significant in plasma, liver, and intestinal content extracts, where **1c** glucuronide was calculated to account in turn for 63%, 60%, and 90% of total ^{3}H. These results indicate that **1c** was readily excreted into the intestine as its glucuronide conjugate, presumably after this is formed in the liver.

In summary, our findings show **1c** to undergo ER-mediated accumulation in uterine tissue and to be efficiently metabolized by glucuronide conjugation. Structural modification of this ligand type will now be directed toward reducing nonspecific binding and susceptibility to conjugation, while retaining high ER affinity.

Experimental Section

Materials and Methods. Reactions involving air-sensitive materials were carried out under dry nitrogen. Tetrahydrofuran was dried immediately before use by distillation from lithium aluminum hydride. Triethylamine was purified by distillation over potassium hydroxide. All other solvents and reagents were used as purchased. Column chromatography was carried out with Baker silica gel (60–200 mesh). Thin-layer chromatography (TLC) was carried out on plastic-backed plates coated with 0.20-mm layers of silica gel 60 F_{254} (EM Reagents). Plates were developed in chloroform–methanol–28% aqueous NH_3 (90:10:0.5, v/v), unless indicated otherwise. [6,7-$^3H_2$]estradiol (58 Ci/mmol) and [*N*-*methyl*-^{3}H]4-hydroxytamoxifen (87 Ci/mmol) were obtained from Amersham Corp. Radiochemical purity was checked by TLC. Determination of ^{3}H in segments of TLC plates, and in organic extracts and aqueous supernatants, was carried out with use of Ecoscint liquid scintillation fluid (National Diagnostics). 2-Phenyl-1,1-bis(4-hydroxyphenyl)ethylene was prepared as reported,[18] mp 182 °C (lit.[18] mp 178 °C). 2-Chloro-2-phenyl-1,1-bis(4-hydroxyphenyl)ethylene was prepared by reaction of the MEM ether of the above compound with *N*-chlorosuccinimide

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under previously reported conditions,[19] followed by deprotection with HCl gas in chloroform. The product separated from benzene–hexane as light yellow crystals: mp 85–90 °C; EIMS, *m/e* 322 (M), 288 (B, MH–Cl). Anal. ($C_{20}H_{15}O_2Cl\cdot 3H_2O$) C, H, Cl.

4,4′-Bis[(2-methoxyethoxy)methoxy]benzophenone (2). Sodium hydride (1.6 g of 50% mineral oil suspension, 33 mmol) was deoiled with hexane (2 × 15 mL). To this was added dropwise with stirring a solution of 4,4′-dihydroxybenzophenone (3.0 g, 14 mmol) in tetrahydrofuran (30 mL). After hydrogen evolution ceased, the reaction flask was placed in an ice water bath, and a solution of methoxyethoxymethyl chloride in tetrahydrofuran (3.9 g, 31 mmol) was introduced over a period of 15 min. After completion of addition, the ice water bath was removed, and the reaction mixture was stirred for 75 min while a milky color was gradually imparted to the mixture with the copious precipitation of NaCl. The reaction mixture was filtered, and the filtrate was concentrated in vacuo. The residue was partitioned between chloroform (100 mL) and 5% NaOH (50 mL). The organic extract was washed with water (3 × 50 mL) and dried (Na_2SO_4). Evaporation of solvent afforded 5.5 g (100%) of **2**, which by TLC (benzene–triethylamine, 9:1, v/v) displayed a major spot (R_f 0.75) accompanied by trace impurities with R_f 0.71 and 0.78: IR (film) 1648 cm^{-1} (C=O); ^1H NMR ($CDCl_3$) δ 3.32 (s, 6 H, OCH_3), 3.36–3.94 (m, 8 H, OCH_2CH_2O), 5.30 (s, 4 H, OCH_2O), 7.05 (d, 4 H, Ar H ortho to methoxy, J = 9 Hz), 7.72 (d, 4 H, Ar H ortho to carbonyl, J = 9 Hz). Compound **2** was used directly in subsequent reactions without further analysis.

1,1-Bis[4-[(2-methoxyethoxy)methoxy]phenyl]-2-(4-bromophenyl)ethene (3, X = Br). To magnesium turnings (0.28 g, 11.6 mmol) and an iodine crystal, was added a solution of *p*-bromobenzyl bromide (2.6 g, 10.4 mmol) in ether (25 mL). After being heated under reflux for 2 h, the Grignard reagent was cooled, and a solution of **2** (2.5 g, 6.4 mmol) in tetrahydrofuran (25 mL) was added in a rapid dropwise fashion. After this, reheating was initiated with a reflux period of 1 h required for consumption of **2** as indicated by TLC analysis (benzene–hexane–triethylamine, 9:9:1 v/v). The reaction mixture was cooled and quenched with saturated NH_4Cl (1.5 mL). The suspension was stirred for 30 min and filtered through Super-cel, and the filtrate was concentrated in vacuo. The residue was dissolved in 100 mL of ethanol–tetrahydrofuran (1:1), and 10 mL of 10% aqueous HCl was added. The solution was stirred at room temperature for 1 h, alkalinized with 10% Na_2CO_3, and concentrated in vacuo. The concentrate was partitioned between chloroform (100 mL) and water (50 mL). The organic extract was further washed with brine (2 × 50 mL) and dried (Na_2SO_4). Evaporation of solvent left an orange oil (5.7 g), which was chromatographed on 100 g of silica gel. Elution with methylene chloride was carried out until a major impurity was extracted from the column. Then the column was eluted with a linear gradient of benzene–hexane–triethylamine (18:9:1 to 9:9:1, v/v). This gave 2.0 g (57%) of an oil, which was homogeneous by TLC: ^1H NMR ($CDCl_3$) δ 3.30 (s, 6 H, OCH_3), 3.20–3.92 (m, 8 H, OCH_2CH_2O), 5.22 (s, 4 H, OCH_2O), 6.56–7.44 (m, 13 H, olefinic and aromatic H).

A solution of 0.5 g of **3** (X = Br) in cold chloroform (5 mL) was saturated with HCl gas and allowed to warm to room temperature. After evaporation of solvent, the residue was crystallized from benzene–hexane to afford 0.18 g of **1d** as light tan crystals, mp 85–90 °C. Anal. ($C_{20}H_{15}O_2Br$) C: calcd, 65.41; found, 66.26. H: calcd, 4.12; found, 4.85. Br: calcd, 21.76; found, 20.01.

1,1-Bis(4-hydroxyphenyl)-2-(4-fluorophenyl)ethene (1c). The precursor MEM ether (**3**, X = F) was prepared in the same way as was **3** (X = Br) by using similar amounts of reagents. Chromatography of the crude product on 70 g of silica gel with 5% petroleum ether in methylene chloride as solvent afforded 1.1 g (44%) of an oil, which was dissolved in 50 mL of dry chloroform. The solution was cooled in ice and saturated with HCl gas. After 1 h at 4 °C, the solution was washed with 25 mL of 5% aqueous $NaHCO_3$ and 50 mL of water. The organic extract was dried (Na_2SO_4) and concentrated to give 613 mg of a solid. This was chromatographed on 60 g of silica gel. Elution with chloroform–methanol–28% aqueous NH_3 (95:5:0.5, v/v) gave 400 mg (57%) of a solid, which was crystallized from benzene–ethanol–hexane to yield 283 mg, mp 196–197 °C. Anal. ($C_{20}H_{15}FO_2$) C, H.

1,1-Bis[4-[(2-methoxyethoxy)methoxy]phenyl]-2-(4-boronophenyl)ethene (4). A stirred solution of **3** (X = Br) (1.0 g, 1.84 mmol) in tetrahydrofuran (5 mL) was cooled to −78 °C, and a solution of *n*-butyllithium (2.50 mmol) in 5 mL of tetrahydrofuran plus 1 mL of hexane was added dropwise over a 10-min period. The mixture was stirred at the same temperature for 45 min. Borane–tetrahydrofuran complex (1.0 M, 2.5 mL) was added dropwise, and the resulting reaction mixture was allowed to warm to room temperature over a period of 1 h. This was quenched with water (1.0 mL) and then acidified with 10% HCl. Additional water (25 mL) was added followed by extraction with chloroform (2 × 50 mL). The combined chloroformic extracts were washed with water (2 × 50 mL) and dried (Na_2SO_4). The crude concentrate (0.8 g) left after evaporation of solvent was applied to a column of silica gel (40 g) with benzene–triethylamine (10:1, v/v), and enough of this solvent was passed through the column for collection of 0.33 g of starting material and a slower eluting impurity. Then the column was eluted with a linear gradient of chloroform–methanol (40:1 to 10:1, v/v). The fractions containing product (R_f 0.38, chloroform–methanol, 30:1, v/v) were combined, concentrated in vacuo, and redissolved in chloroform (30 mL). The solution, which was basic (pH >10) to moist litmus, was washed with 5% HCl (25 mL) and water (4 × 25 mL) and dried (Na_2SO_4). Evaporation of solvent left 0.3 g (48%) of **4**: IR (neat) cm^{-1} 3430 (O–H), 1340 (B–O); NMR ($CDCl_3$) δ 3.38 (s, 6 H, OCH_3), 3.48–3.68 (m, 4 H, CH_2OCH_3), 3.72–3.95 (m, 4 H, $OCH_2CH_2OCH_3$), 5.28 (s, 4 H, OCH_2O), 6.76–7.36 (m, 13 H, olefinic and aromatic H), 7.90, 7.98 (2 s, D_2O exchangeable, 2 H, OH). Anal. ($C_{28}H_{33}BO_8$) C, H.

1,1-Bis(4-hydroxyphenyl)-2-(4-iodophenyl)ethene (1e). The boronic acid **4** (300 mg, 0.59 mmol) was dissolved in 50% aqueous tetrahydrofuran (4 mL) in a round-bottomed flask, which was shielded from light. Aqueous NaI (88 mg, 0.59 mmol in 0.5 mL H_2O) was added followed by chloramine-T (268 mg, 1.18 mmol) in 50% aqueous tetrahydrofuran (2 mL). The reaction mixture was stirred at room temperature for 15 min at which time TLC analysis (benzene–triethylamine, 9:1, v/v) showed complete consumption of **4** and the emergence of a dark spot at R_f 0.53. Water (10 mL) was added, and the mixture was extracted with chloroform (2 × 25 mL). The combined organic extracts were dried (Na_2SO_4), filtered, chilled in an ice–water bath, and saturated with hydrogen chloride gas. After 30 min, TLC analysis, as described above, showed the presence of a sole product, R_f 0.21. Evaporation of solvent left 156 mg of a gum, which was eluted through silica gel (22 g) with the same solvent that was used for TLC. The concentrated residue was triturated in benzene–hexane to give **1e** (58 mg, 24%): mp 216–218 °C; NMR (pyridine-d_5) δ 6.73–7.57 (m, 13 H, olefinic and aromatic H), 8.61 (s, 2 H, OH). Anal. ($C_{20}H_{15}IO_2$) C, H, I.

[3H]-1,1-Bis(4-hydroxyphenyl)-2-(4-fluorophenyl)ethene ([^{3}H]-1c). To a solution of 3 mg (10 μmol) of **1c** in 0.2 mL of methanol were added 0.1 mL of 28% aqueous ammonia and a solution of 5 mg (20 μmol) of iodine in 0.1 mL of tetrahydrofuran. After being stirred for 24 h, the solution was concentrated, and the residue was partitioned between 0.5 mL of ether and 0.5 mL of 10% aqueous $Na_2S_2O_3$. The organic layer was washed with 0.5 mL of H_2O, dried (Na_2SO_4), and concentrated. The residue was triturated with heptane to give 5.1 mg (93%) of a yellow powder. Deiodination of this was carried out by Amersham Corp. as follows: To a solution of 2 mg of diiodo **1c** in 450 μL of ethanol and 50 μL of triethylamine was added 15 mg of 10% Pd on charcoal. The mixture was stirred under 1 atm of T_2 at room temperature for 1 h. The catalyst was filtered, and the filtrate was concentrated in vacuo. A solution of 20 mCi of the crude tritiated product was subjected to preparative TLC (5 × 20 cm Analtech silica gel GF_{254} 0.25 mm layer), with radioinert **1c** as external standard. The appropriate silica gel zone was removed and eluted for 12 h with 5 mL of methanol. The eluate was concentrated, rechromatographed, and eluted similarly except benzene–piperidine (5:2, v/v) was used as TLC solvent. The eluate was concentrated under a stream of dry N_2 gas, and the residue was redissolved in 5.0 mL of dry tetrahydrofuran and stored at

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-17 °C. The solution contained 9.9 mCi (50%) of a product that had an ultraviolet spectrum nearly identical with that of 1c. Radiochemical purity was 92–95% with use of either of the TLC solvents above. Specific activity of 44 Ci/mmol was calculated by dividing the amount of ^3H in an aliquot of the solution by the concentration of [^3H]-1c in the solution. Concentration was determined from the UV absorbance of an aliquot of the solution at 309 nm with ϵ 18.1 mM^{-1} cm^{-1} and from its EC_{50} determined with known amounts of unlabeled 1c in the presence of estrogen receptor.

Radioreceptor Assays. Affinities of 1a–e for estrogen receptor prepared from immature rat uteri were determined as described.[17] Incubation mixtures contained one of the following: [^3H]-1c (2.3 nM), [^3H]-4-hydroxytamoxifen (3.0 nM), or [^3H]estradiol (3.5 nM) and increasing concentrations (0, 1, 3, 10, 30, 100 nM) of competing ligand. Nonspecific binding was determined from incubations containing the ^3H-labeled ligand accompanied by an excess (1000 nM) of its radioinert counterpart.

Distribution of [^3H]-1c in Vivo. A. Animals and Dosing. Female Sprague–Dawley rats, 20–25 days old, were obtained from our breeding colony. Each treatment group had four animals. For each group, a solution of 48 μCi (1.1 nmol) of [^3H]-1c in 0.1 mL of ethanol was mixed with 0.8 mL of 1.15% aqueous KCl just prior to use. Each animal received 0.2 mL (0.26 nmol, 11.2 μCi) of this ip or iv (tail vein). Animals were sacrificed at 1, 4, or 8 h after dosing, and blood, uteri, and sections of liver and small intestine (contents) were removed and weighed. To study the effects of estradiol on distribution of [^3H]-1c, a solution of 8 μg (30 nmol/0.2 mL) was prepared as described above and given ip, in turn, to each of four animals. A second group received 0.2 mL of 1.15% aqueous KCl, ip. Each animal then received, ip, 0.26 nmol of [^3H]-1c as described above. After 1 h, all eight animals were sacrificed, and blood and organ samples were removed.

B. Tissue Processing. Pooled blood was centrifuged at 500g for 10 min, and 0.5-mL aliquots were counted. Uterine and liver tissues and intestinal contents were separately pooled and homogenized in 9 volumes of methanol. Homogenates were centrifuged at 500g for 10 min, and 1.0-mL aliquots were counted.

C. Treatment of Extracts with β-Glucuronidase. Freeze-dried plasma, or methanol extract concentrate, containing 150–200 nCi of ^3H, was redissolved in 1.0 mL of 50 mM phosphate buffer, pH 7.4. To 0.5 mL of the resulting solution was added 100 units of β-glucuronidase in 0.1 mL of water; to the remaining 0.5 mL was added 0.1 mL of water. The solutions were capped and allowed to stand at room temperature for 24 h. Then the solutions were freeze-dried. Each residue was extracted with two 0.8-mL portions of methanol. Respective extracts were combined and concentrated. Each residue was redissolved in 100 mL of methanol and subjected to TLC with 1c as an internal standard. Developed plates were cut into ca. 1 cm wide segments by using the position of 1c as a guide. ^3H in each segment was determined after elution in 8 mL of liquid scintillation fluid for at least 16 h.

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Registry No. 1c, 114221-52-2; [^3H]1C, 114221-53-3; diiodo 1c, 114221-54-4; 1d, 114221-55-5; 1e, 114221-56-6; 2, 114221-57-7; 3 (X = Br), 114221-58-8; 3 (X = F), 114221-59-9; 4, 114221-60-2; 2-phenyl-1,1-bis(4-hydroxyphenyl)ethylene, 66422-18-2; 2-chloro-2-phenyl-1,1-bis(4-hydroxyphenyl)ethylene, 106692-29-9; 4,4′-dihydroxybenzophenone, 611-99-4; *p*-bromobenzyl bromide, 589-15-1; *p*-fluorobenzyl bromide, 459-46-1.

1-(2,3-Anhydro-β-D-lyxofuranosyl)cytosine Derivatives as Potential Inhibitors of the Human Immunodeficiency Virus

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We report here that 1-(2,3-anhydro-β-D-lyxofuranosyl)cytosine has activity against the human immunodeficiency virus in vitro. A number of 2′,3′-anhydro-β-D-lyxofuranosyl nucleoside derivatives were prepared, but none had the activity of the title compound. New efficient procedures were developed for the synthesis of 3′-deoxy-3′-alkyl- and 3′-deoxy-β-D-arabinosylpyrimidine derivatives.

Of the agents currently being evaluated as potential therapeutic agents for the treatment of the acquired immunodeficiency syndrome[1] (AIDS), the best (in vitro) appear to be nucleoside analogues such as 3′-deoxy-3′-azidothymidine[2] (AZT) and other 2′,3′-dideoxynucleosides[3,4] (ddN). AZT and ddN appear to be converted into the corresponding 5′-triphosphates by cellular enzymes. These triphosphates selectively inhibit the synthesis of DNA by the human immunodeficiency virus (HIV) reverse transcriptase.[4,5] The precise mechanism of action has not been elucidated, but appears that compounds such as ddN-5′-triphosphate are better substrates for retroviral reverse transcriptase (RT) than for the cellular DNA polymerases such as polymerase α (Pol α).[6] When incorporated into a growing viral DNA chain, these analogues halt further DNA synthesis since they lack a 3′-hydroxyl group.

It has been shown, in the case of acyclovir, that the primer–template containing this nucleoside analogue at the 3′ terminus is itself a potent inhibitor of Pol α and herpes virus DNA polymerase.[7] Recently it has been demonstrated that (murine or avian) viral RT is not inhibited by synthetic oligodeoxynucleotide primer–template

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