Synthesis of 1-Bromoestradiol

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The total synthesis of 1-bromoestradiol (**6**) is described. The starting material, 4-aminoestradiol 3-methyl ether (**2**), was obtained from commercially available estrone (**1**) in four steps following literature procedures. Reaction of **2** with BrCl, generated in situ from NCS/NaBr in 9:1 dioxane–acetic acid, afforded 4-amino-1-bromoestradiol 3-methyl ether (**4**). Compound **4** could be isolated or directly converted (with no isolation) to 1-bromoestradiol 3-methyl ether (**5**) in 50% yield from **3**. This was accomplished by a reductive deamination sequence that utilized $NaNO_2$/HCl followed by addition of H_2O_2. Demethylation of **5** using BBr_3/CH_2Cl_2 afforded 1-bromoestradiol (**6**) in 44% yield.

The thesis that radiohalogen-labeled estrogens might be used to detect and/or determine the course of therapy of hormone-dependent tumors[1] has led to a large effort in the synthesis of these compounds.[2-4] Several factors must be considered in the design of efficient radiolabeled estrogens, including specific activity, in vivo stability, and target specificity of the synthesized compound.

While it is difficult to predict in vivo stability of a radiolabeled estrogen, it might be assumed that a high in vitro stability would be a necessary prerequisite. Since aryl–halogen bonds are generally stable, direct halogenation of the aromatic A ring of estradiol (**1a**) was attractive.

Previous studies have shown that radiobrominations of the 2- and 4-positions of estrogens lead to very stable compounds in vivo.[5] Evaluation of the unlabeled compounds by in vitro binding assays has shown that the protein receptor binding is greatly reduced compared to estradiol.[6] Additionally, in vivo biodistribution studies have shown that the radiolabeled analogues are not appreciably concentrated in tissues containing estrogen receptors.[7] Since it is believed that the 3-hydroxyl moiety is involved in receptor binding, the close proximity of a bulky halogen, such as bromine, to the hydroxyl group may be a factor in the observed decrease of the binding affinity.

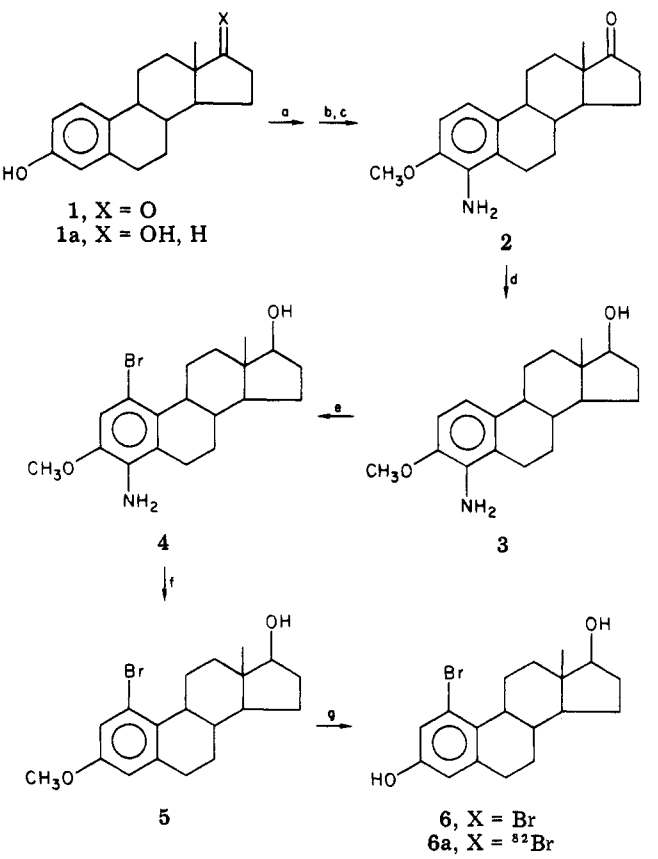
Due to the increased distance between the halogen and the 3-hydroxyl moiety and the anticipated chemical stability, the synthesis of 1-bromoestradiol (**6**) was undertaken.

Results and Discussion

Although 1-halogenated steroid derivatives have been reported,[8] no methods for the preparation of 1-halogenated estradiol have been described. Since our ultimate goal is to prepare radiobrominated 1-bromoestradiol (**6a**), the incorporation of bromine via electrophilic aromatic substitution was most attractive. To activate the 1-position of the steroid nucleus toward this type of reaction it was necessary to place a highly activating substituent, such as an amino group, at the 4-position. The 3-hydroxyl was protected as a methyl ether in order to minimize bromination in the 2- and 4-positions.[5]

The desired precursor, 4-aminoestrone 3-methyl ether (**2**) was prepared from estrone (**1**) by nitration, methylation of the 3-hydroxy, and reduction of the nitro group as previously described (Scheme I).[9,10] Reduction of the

Scheme I[a]



[a] (a) HNO_3, HOAC. (b) $(CH_3)_2SO_4$, NaOH. (c) $Na_2S_2O_4$, base. (d) $LiAl(O\text{-}t\text{-}Bu)_3H$, THF. (e) NCS/NaBr, dioxane-HOAC. (f) HCl, $NaNO_2$, H_2O_2. (g) BBr_3, CH_2Cl_2.

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17-ketone of 2 was accomplished with $LiAl(O\text{-}t\text{-}Bu)_3H$ in THF which gave almost exclusively the 17β-ol epimer 3 in 95% yield. Bromination of 3 with BrCl, generated from bromide ion and *N*-chlorosuccinimide (NCS)[11] in 9:1 dioxane–acetic acid, afforded 4-amino-1-bromoestradiol 3-methyl ether (4) in 60% isolated yield. The isolated product 4 was shown to undergo a facile deamination in the same solvent system by preparation of its diazonium salt in the presence of hydrogen peroxide ($NaNO_2$, HCl, H_2O_2).[12] The isolation of intermediate 4 became unnecessary since the bromination and deamination steps could be carried out as a "one pot" two-step reaction sequence. The deaminated product, 1-bromoestradiol 3-methyl ether (5), was obtained in 50% overall yield from 3. Removal of the methyl protecting group with BBr_3 in methylene chloride afforded 1-bromoestradiol (6) in 44% yield from 5.[13]

The stability of radiobromine in the 1-position was addressed by preparation of 1-[^{82}Br]bromoestradiol (6a) using the described sequence of reactions. No detectable decomposition or loss of radioactivity over a 6-day period was observed.

Experimental Section

Melting points were obtained on an Electrothermal melting point apparatus and are uncorrected. Elemental analyses were performed by Ruby Ju of the Department of Chemistry at the University of New Mexico. IR measurements were obtained on a Beckman Model IR-33 Spectrophotometer. Proton NMR spectra were recorded at 60 MHz on a Hitachi Perkin Elmer R-24B instrument and are referenced to tetramethylsilane as an internal standard. Carbon NMR spectra were obtained on a pulse Fourier Transform Varian FT-80 spectrometer and chemical shifts obtained are referenced to the center peak of the deuterated solvent used. Mass spectra (MS) were obtained on a Finnigan Model 4510 using a solid probe insert.

Product purity and reaction progress were detected by analytical thin-layer chromatography using Baker Plates coated with silica gel GF or by high-performance liquid chromatography (HPLC). The Spectra Physics 8700 HPLC is equipped with a UV detector at 254 nm and an Alltech reverse-phase C_{18} column using 65:35 acetonitrile/water as the mobile phase at 1.5 mL/min. Medium-pressure liquid chromatography (MPLC) was performed at 80 psi by using a Fluid Metering pump, 9 mm × 1000 mm glass column, and Woelm 32-64 micron silica gel as the stationary phase. Ethyl acetate–toluene mixtures were used as the eluting solvents.

Tetrahydrofuran (THF) was distilled from lithium aluminum hydride (LAH) prior to use.

Dioxane was purified by washing with sulfuric acid followed by distillation from sodium metal.[14] Methylene chloride was dried over 4-Å molecular sieves 24 h prior to use. All other reagents were used as obtained.

4-Aminoestradiol 3-Methyl Ether (3). To an ice-cold solution of 4-aminoestrone 3-methyl ether (2) in 30 mL of freshly distilled THF was added 2.64 g (10.4 mmol) of $LiAl(O\text{-}t\text{-}Bu)_3H$. The resultant reaction mixture was allowed to warm to room temperature and then stirred for an additional 30 min. The resulting mixture was then cooled to 0 °C and hydrolyzed by the slow addition of water (10 mL), 40% KOH (10 mL), and 1.0 g of sodium tartrate. Ethyl ether (40 mL) was added and the organic phase was washed with water (2 × 25 mL) and dried over anhydrous $MgSO_4$. Removal of the solvents under reduced pressure gave 0.74 g (95% yield) of 3 which was recrystallized from methanol: mp 175–177 °C; TLC R_f 0.37 (25% ethyl acetate/toluene); IR (KBr) 3680–3200 cm^{-1} (N–H and O–H stretch); ^1H NMR ($CDCl_3$) δ 6.7 (s, 2 H), 3.8 (s, 3 H), 1.2–3.8 (m, 21 H), 0.75 (s, 3 H). ^{13}C NMR ($Me_2SO\text{-}d_6$) δ 144.15, 133.96, 132.75, 120.5, 112.84, 107.89, 80.06, 55.35, 49.65, 43.73, 42.59, 37.88, 36.60, 29.92, 26.70, 26.11, 24.21, 22.72, 11.05. MS, *m/e* (relative intensity) 302 (M^+ + 1, 20), 301 (M^+, 100). Anal. Calcd for $C_{19}H_{27}NO_2$: C, 75.74; H, 8.97. Found: C, 75.69; H, 9.10.

1-Bromoestradiol 3-Methyl Ether (5). A mixture of 89.1 mg (0.865 mmol) of NaBr and 115.1 mg (0.865 mmol) of NCS in 26 mL of 9:1 dioxane–acetic acid was allowed to stir at 25 °C for 10 min. After the addition of 0.26 g (0.865 mmol) of 3, as a solid, the reaction mixture was stirred for an additional 1 h.[15]

The pale-grey solution was then cooled to 0 °C and 10.59 mL of 0.67 M HCl was added, followed by 0.26 mL of 3% H_2O_2. Finally, a solution of 60 mg (0.870 mmol) of $NaNO_2$ in 4.2 mL of water was added slowly. The resultant pale-yellow solution was allowed to stir at 0 °C for 20 min. The reaction mixture was poured into 30 mL of 10% KOH and extracted with ethyl acetate (1 × 30 mL). The organic phase was then washed with water (2 × 15 mL) and dried over anhydrous $MgSO_4$, and the solvents were removed under reduced pressure to afford 0.21 g of crude product. The crude product was purified by chromatography in a MPLC system using silica gel and 15% ethyl acetate–toluene as the eluant and 159 mg (50% yield) of 5 as a white solid was obtained: mp 118.5–120 °C; TLC R_f 0.49 (25% ethyl acetate/toluene); IR (KBr) 3600–3200 cm^{-1} (O–H stretch); ^1H NMR ($CDCl_3$) δ 6.9 (d, 1 H, J = 4 Hz), 6.6 (d, 1 H, J = 4 Hz, 3.7 (s, 3 H), 1.2–3.7 (m, 17 H), 0.8 (s, 3 H); ^{13}C NMR ($CDCl_3$) 157.12, 141.37, 131.34, 124.14, 117.32, 113.75, 81.39, 55.12, 50.04, 47.54, 43.99, 41.63, 36.99, 32.66, 30.49, 27.31, 24.89, 22.91, 11.82; MS, *m/e*, (relative intensity) 366 (M^+ + 1, 9.80); 364 (M^+ − 1, 15.69). Anal. Calcd for $C_{19}H_{25}O_2Br$: C, 62.58; H, 6.82. Found: C, 62.46; H, 6.82.

1-Bromoestradiol (6). A solution of 1-bromoestradiol 3-methyl ether (5) (116 mg, 0.32 mmol) in 5 mL of CH_2Cl_2 was cooled to 0 °C under nitrogen, followed by dropwise addition of 0.64 mL (0.64 mmol) of 1 M BBr_3 in CH_2Cl_2. The cold bath was removed, and the mixture was allowed to stir at 25 °C for 1.25 h. After the addition of 10 mL of saturated NaCl solution and 20 mL of ethyl acetate, the organic phase was isolated, washed with H_2O (1 × 10 mL), and dried over anhydrous $MgSO_4$. Removal of the solvents under reduced pressure gave 100 mg of crude red material which was chromatographed on MPLC with 15% ethyl acetate/toluene. Collection of the appropriate fractions, followed by removal of the solvents gave 50 mg (44% yield) of a white crystalline solid, mp 239–241 °C. The analytical sample was obtained from recrystallization from methanol–H_2O: mp 242–244 °C; TLC R_f 0.54 (50% ethyl acetate–toluene); IR (KBr) 3620 cm^{-1} (ArOH stretch), 3560–3100 (alifatic -O–H stretch); ^1H NMR ($Me_2SO\text{-}d_6$) δ 8.3 (s, 1 H), 7.0 (d, 1 H, J = 4 Hz), 6.6 (d, 1 H, J = 4 Hz), 3.7 (t, 1 H 8 Hz), 1.2–3.0 (m, 16 H), 0.8 (s, 3 H); ^{13}C NMR ($Me_2SO\text{-}d_6$) 155.34, 141.59, 129.18, 123.46, 118.48, 115.38, 79.92, 49.81, 47.29, 43.80, 41.43, 37.18, 32.12, 30.16, 27.42, 24.84, 22.80, 12.16; MS, *m/e* (relative intensity) 352 (M^+ + 1, 97.55), 350 (M^+ − 1, 99.39). Anal. Calcd for $C_{18}H_{23}O_2Br$: C, 61.64; H, 6.60. Found: C, 61.60; H, 6.56.

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Registry No. 2, 13010-21-4; 3, 20810-75-7; 4, 90697-18-0; 5, 20146-91-2; 6, 90697-19-1.

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(12) This general deamination procedure was shown by our group to be widely appplicable to a variety of aromatic amines (work in progress).

(13) More labile phenolic protecting groups such as the tetrahydropyranyl (OTHP) and methoxyethoxymethyl (OMeM) were investigated. Attempts to brominate and deaminate these derivatives under similar conditions were unsuccessful.

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(15) If required the 4-amino-1-bromoestradiol 3-methyl ether (4) can be isolated at this point by following the following steps: the colorless solution was poured into 60 mL of 5% NaOH solution. The resulting mixture was then extracted with ethyl acetate (1 × 50 mL). The organic phase was washed with 5% NaOH (2 × 25 mL) and with H_2O (1 × 25 mL). After drying over anhydrous $MgSO_4$, the solvents were removed under reduced pressure. The crude material was purified by medium-pressure liquid chromatography (MPLC) using 15% ethyl acetate/toluene as the elutant. After isolation, 0.19 g (58% yield) of 4 as a pale yellow solid was obtained: mp 55–60 °C; TLC R_f 0.41 (25% ethyl acetate/toluene); IR (KBr) 3700–3100 cm^{-1} (N–H and O–H stretch); ^1H NMR ($CDCl_3$) δ 6.9 (s, 1 H), 3.8 (s, 3 H), 1.1–3.7 (m, 19 H), 0.8 (s, 3 H); ^{13}C NMR ($CDCl_3$) δ 144.76, 131.65, 125.14, 113.60, 111.12, 82.22, 55.48, 49.91, 47.59, 43.79, 40.77, 36.90, 30.32, 27.36, 26.80, 24.48, 22.80, 11.69. Anal. Calcd for $C_{19}H_{26}O_2NBr$: C, 60.27; H, 7.05. Found: C, 60.00; H, 6.89.