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Synthesis and evaluation of ^{82}Br and ^{77}Br labeled (17α, 20E)-21-bromo-19-norpregna-1,3,5(10),20-tetraene-3,17β -diol

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SUMMARY

17α -E-Bromovinylestradiol and its radiobrominated analogs were prepared by halodestannylation. The synthesis was achieved by bromination of the tri-n-butylstannylvinyl intermediate to give a 90% isolated yield. The reaction of the intermediate with ammonium [^{82}Br] bromide or sodium [^{77}Br] bromide in the presence of an oxidant give the corresponding radiolabeled bromovinylestradiol in 80-90% yields after isolation by HPLC. The radiochemical purity was greater than 98% and no other UV-active compounds could be detected by HPLC. An *in vivo* comparison of this compound with the previously prepared 17α -E-[^{125}I]iodovinyl estradiol indicated that the two compounds had similar uterine uptake and specific receptor binding properties. The results suggest that radiobromodestannylation may be the method of choice for the preparation of this and other structurally similar compounds.

Key words: Bromine-77, Estrogens, Halodestannylation, synthesis, radiolabeling, biodistribution

INTRODUCTION

The quantitation of estrogen receptors in human mammary carcinoma is an important factor in the selection of an appropriate therapeutic regimen, and in the determination of the prognosis for

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long term survival (1-5). Because of the potential advantages associated with noninvasive in vivo detection and localization of estrogen receptor containing tumors several approaches have been undertaken to synthesize radiolabeled ligands that have a high affinity for the estrogen receptor protein (6). These ligands, labeled with gamma- or positron-emitting radionuclides, could potentially provide external visualization of the estrogen receptor containing tumors via scintigraphic imaging (7-17). This goal has been realized by the demonstration that 16α-[F-18]-estradiol can indeed image the estrogen receptor containing breast tumors in vivo. (18,19) In previous studies we have reported the preparation and evaluation of the radioiodinated 17α-E-iodovinylestradiol and its derivatives as potential tumor imaging agents (20-23) from which 17α-iodovinyl-11β-methoxyestradiol demonstrated that the best uterine uptake and selectivity (17,22,24,25).

Although most of the research related to radiolabeled estrogens has focused on the use of fluorine-18 and iodine-123, bromine which also has several radioisotopes has been discussed in several reviews (26,27). Bromine-75, a positron emitter (T1/2 = 101 minutes) has the greatest potential for radiodiagnostic applications, and bromine-80m, an Auger electron-emitter (T1/2 = 4.4 h.), has potential for radiotherapy. The other radioisotopes, bromine-77 (T1/2 = 56 hr.) and bromine-82 (t1/2 = 35.3 h) have poorer clinical characteristics but are more readily produced, can be used for chemical and biological studies. This has been done previously for synthesis of several radiobrominated estrogens (12,13,28-31). With the potential incorporation of ^{75}Br in mind, we report here our studies involving the bromination and radiobromination of 17α-tri-n-butylstannyl- vinylestradiol, 1, and a preliminary evaluation of 17α-[Br-77]bromovinylestradiol in vivo.

Chemistry

The 17α-E-bromovinylestradiol 2 was synthesized in high yield from the corresponding 17α-E-tri-n-butylstannylvinyl intermediate 1

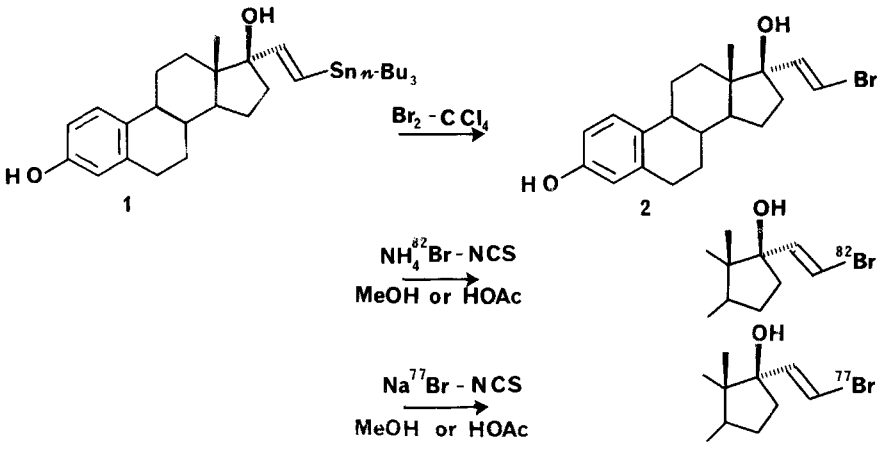

Figure 1. Synthesis of unlabeled 17α-bromovinylestradiol ($BrVE_2$) and the ^{82}Br and ^{77}Br analogs.

The brominated steroid 2 had the same E-stereochemistry about the vinyl group as the stannyl intermediate. This is evident from the vinyl proton coupling constant for C_{20}- and C_{21}-protons which is consistant with E- rather than Z-stereochemistry (J=14 Hz vs. J=8 Hz) (32-34). The reaction with bromine was virtually instantaneous as a immediate disappearance of the bromine color upon its addition to the reaction solution at -40° to 0°C was observed. The reaction using a 1:1 stoichiometry did not produce any significant A-ring bromination as no A-ring brominated products could be detected by NMR or HPLC. As the ratio of bromine to stannyl precursor was increased to greater than 1 or the temperature was elevated, other products began to appear in the reaction mixture. Therefore, in the bromodestannylation reaction, deactivation of the phenolic ring by esterification or methylation was unnecessary to produce the bromovinyl compounds.

This finding is in contrast to previously described synthesis of halovinyl estrogens via deboronation reactions (17,35,36), or similar desilylation reactions (37).

The enhanced reactivity towards electrophilic substitution reactions provided tri-n-butylstannylvinyl moiety resulted in only one major product being formed. This factor along with the highly

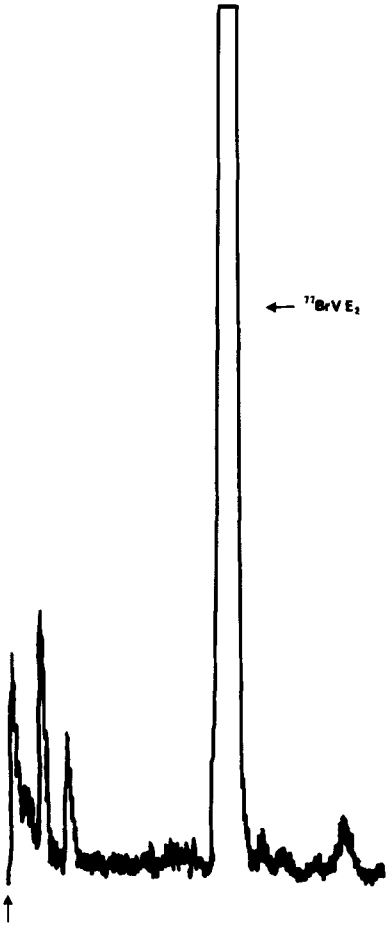

Figure 2A. HPLC trace of the reaction of the 17α-tri-n-butyl-stannyl-vinyl-estradiol with NA^{77}Br and N-Chlorosuccinimide in methanol at room temperature for 3 minutes.

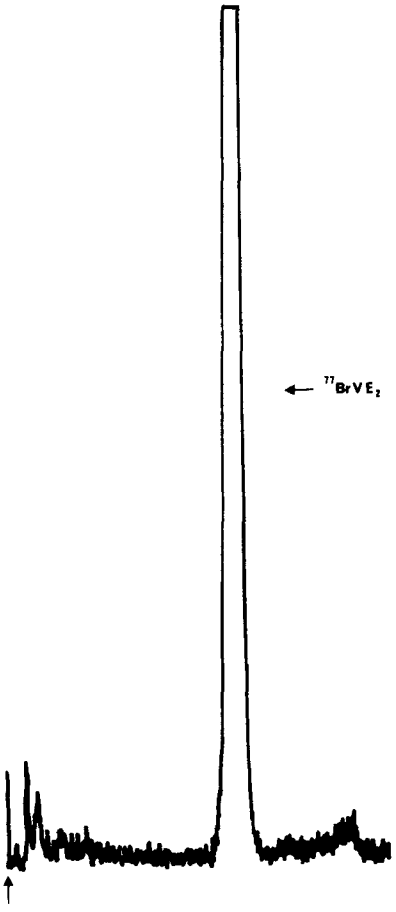

Figure 2B. HPLC trace of the reaction of the 17α-tri-n-butyl-stannyl-vinylestradiol with NA^{77}Br and N-Chlorosuccinimide in acetic acid at room temperature for 30 minutes followed by the addition of sodium acetate for 10 minutes.

lipophilic character of the stannyl intermediate permitted the rapid preparation and facile purification of the 17 E-[^{77}Br] and [^{82}Br]bromovinylestradiols. The bromodestannylation reaction proceeded very rapidly (>3 min.) in methanol or acetic acid using N-chlorosuccinimide (NCS) as the oxidant (Figures 2A, 2B).
The desired radiochemical 2 was produced in an 80-90% yield and could be readily separated from bromide ion and starting material by reversed-phase HPLC. In the HPLC separation, the brominated product eluted first, followed significantly later by both the minor radiolabeled contaminants and the starting material. The solvent for the reaction had only a slight effect on either yield or purity. The specific activity of the bromine-77 labeled material was not determined, but the quantity of steroid was sufficiently low to prevent its detection with the UV monitor on its most sensitive setting (0.001 AUFS).

The results of the distribution study of the bromine-77 labeled 2 in immature female rats are shown in Table 1. The data for 17α-E-[^{125}I]iodovinylestradiol which were reported previously (20).

Table 1. Distribution of (^{77}Br) and (^{125}I)VE_2 in Immature Female Rats at 2 hr. (Percent Injected Dose x Mass (kg)/Gram of Tissue

Compound	N	Treatment	Uterus*	Liver	Kidney	Muscle	Blood	Thyroid
(^{77}Br)VE_2	10	--	0.320 ±.21	0.109 ±.011	0.035 ±.009	0.028 ±.007	0.029 ±.003	0.03 ±.01
	5	+E_2	0.087 ±.015	0.105 ±.011	0.039 ±.003	0.021 ±.002	0.027 ±.003	0.03 ±.01
(^{125}I)VE_2+		--	0.364 ±.016	0.155 ±.014	0.049 ±.004	0.035 ±.004	0.031 ±.002	0.09 ±.01
	6	+E_2	0.129 ±.010	0.157 ±.010	0.052 ±.005	0.034 ±.004	0.037 ±.002	0.10 ±.01

*mean (s.e.m.) for N animals

percent of injected dose

+data from reference **20**

are included for comparison. The uterus is the tissue with the highest concentration of activity, followed by the liver and kidney which are involved in the metabolism and excretion of estrogens. The nontarget tissues, such as heart and muscle, do not demonstrate high concentration of activity. The data show that at 2 hours the distribution of labeled 2 is very similar to that observed for the iodovinyl derivative. The present of a high dose of estradiol coadministered with the radiochemical selectivity depresses the uterine level of radioactivity without significantly affecting the other tissues and organs. The magnitude of this effect, 73%, is slightly greater than that previously reported for the iodine-125 labeled comound (60%). This may be due to a slight reduction in lipophilicity which in turn reduces nonspecific interactions. Thyroid uptake is also reduced compared to the iodovinyl analog. Since bromide ion is not sequestered by the thyroid as avidly as iodide, metabolism of the radioligand which releases the halide from the vinyl moiety will not produce thyroid uptake in the case of bromine-77 labeled 2. In all other aspects, the distribution of the bromovinyl compound is similar to that of the iodovinyl analog.

In this study, we have described the stereospecific synthesis of 17α-E-bromovinylestradiol and its bromine-77 and bromine-82 labeled derivatives. The radiosynthesis proceeds rapidly and in high yield at both the millimolar and no-carrier-added levels. An advantage for this method is that the reaction proceeds without the protection of the phenolic A-ring and the substitution of a bromo moiety for a more lipophilic tri-n-butyl group facilitates the isolation and purification of the product. The ease of the sequence suggests that this would be the preferred method for preparing other radiobrominated vinyl compounds. Importantly, the data indicate that the substitution of a bromine for an iodine in

17α-vinylestrogens produces little or no change in the biological properties. Therefore, the results generated with the more readily available 17α-iodovinylestradiols might subsequently be extended to the brominated derivatives as well. Recent presentations by M.J. Adam and J. Balatoni from the TRIUMF facility in Vancouver, British Columbia (38) and R.C. Mease and co-workers at the Argonne National Laboratory and U. of Chicago (39-40) have confirmed this is a highly sufficient method for the preparation of the 17α-bromovinyl estrogens. Their reported biological results are essentially identical to those described here.

Experimental Section

General

All solvents were purchased as reagent grade and used without purification. 17α-tri-n-butylstannylestradiol 1 was prepared as previously described (20,21). The solvents used in NCA radiobrominations with bromine-77 were HPLC grade. All reagents were obtained from Aldrich Chemical Company and were used as obtained. Thin layer chromatography was performed using precoated silica gel plates, 100 um thick with fluorescent indicator (Whatman) and precoated reversed phase RP-18 plates with fluorescent indicator (Whatman).

NMR spectroscopy was performed in a Varian 300 MHz instrument with a multinuclear probe or a Bruker 300 MHz instrument (Los Alamos). Mass spectroscopy was performed by Dr. Paul Vouros, Northeastern University.

HPLC analyses at Los Alamos were performed on a Waters Associates HPLC system consisting of two 6000A pumps, U6K injector, Model 450

UV detector (at 254 nm), a Data Module, and a System Controller. Separations were carried out using a Waters Radial Compression Module with a Radial Pak C_{18} cartridge with a solvent mixture of 50% CH_3CN and 50% H_2O at 2 mL/minute.

Analyses of the radiolabeled compounds were accomplished by the above described HPLC using a 2-inch NaI crystal adjacent to the effluent line from the HPLC. The NaI crystal was coupled with an Ortec power bin, high voltage supply, ratemeter and amplifier. Evaluations of the peak areas were accomplished via an Ortec counter and timer and line printer using manual counter and corrected for background activity.

HPLC analyses at Northeastern University were performed on an LDC HPLC system consisting of two Constametric III pumps, a Rheodyne injector, and a Spectromonitor III UV detector (at 280 nm). Separations were carried out on a Rainin Microsorb (5um, C_{18}, 15cm bed) with a solvent mixture of 60% acetonitrile 40% water at 1mL/min.

Radionuclides

Bromine-82 was produced by neutron irradiation (n,γ) of 100 mg NH_4Br (analytical grade) for 1-2 h in a sealed quartz container at a flux of 9.7×10^{12} neutrons/cm^2/sec. The irradiated sample was allowed to cool for 24 hours from end of bombardment to allow the short-lived nuclides to decay. This resulted in 5-10 mCi of activity (0.5-1.0 Ci/g). The bromine-82 sample was dissolved in 5.0 mL MeOH. Aliquots of this solution were used directly. Bromine-77 was produced by 800 MeV proton irradiation of a molybdenum target as previously described (41). The bromine-77 samples were purified by ion chromatography to yield a 0.003 M Na_2CO_3 aqueous solution of sodium [^{77}Br] bromide. The aqueous

solutions were diluted with EtOH or MeOH to aid in transfer of small quantities of activity.

Synthesis of 17α-E-bromovinylestradiol, 2.

To a solution of 1 (300 mg; 0.51 mmol) in dry THF (3mL) was added bromine (82 mg; 0.52 mmol) in carbon tetrachloride (1mL), under nitrogen, at -78°C for 90 min. and then methanolic potassium fluoride (65mg in 2mL) was added. The solution was allowed to warm to room temperature and stirred for 30 min. The reaction mixture was filtered, diluted with ethyl acetate, then washed with sodium bisulfite solution (10%), brine and dried. Evaporation of the solvent afforded an oil which was recrystallized from methylene chloride to yield 17α-E-bromovinylestradiol (164mg) as colorless needles. The product was analysed by HPLC and indicated only one component. Retention time: 4.6 min.

^{1}H-NMR (CD_3OD-$CDCl_3$):0.96(s. 3H), 1.24-1.43(m, 11H, steroid nucleus), 2.75-2.78(m, 3H), 3.30(s, 1H), 6.24(d, J=14Hz, C_{21}-H), 6.45(d, J=14Hz, C_{20}-H), 6.51(d, J-3Hz, 1H), 6.58(dd, J=3Hz, 8Hz, 1H), 7.10(d, J-8Hz, 1H).

^{13}C-NMR (CD_3OD-$CDCl_3$):14.32, 23,69, 26,96, 28.10, 30.22, 33.07, 36.39, 40.30, 44.37, 47.77, 50.02, 85.61, 113.26, 115.69, 126.80, 130.80, 130.08, 138.39, 143.56, 155.08.

Mass spectrum (EI) m/e=376,374 (M+) for 1:1 bromine isotopes.
IR (KBr) :3491, 2911, 1606, 941 cm^{-1}
UV (MeOH):205(13,579), 220(6,804), 281(1,934) nm
$[\alpha]^{22}$ = +23.35 (c, 9.42 MeOH)

Radiobrominations with Bromine-82

I. To a vial containing 5.4 mg (9 μmole) 1 was added 47 μL (60

μCi) of a 19 mg/mL solutions of $NH_3{}^{82}Br$ (0.89 mg - 9 μmole) and 153 μL MeOH. To this solution was added 2.4 mg NCS (18 μmole). The reaction progress was followed by HPLC at 5 min., 18 hr.

II. A vial containing 25 μL of $NH_3{}^{82}Br$ solution (19 mg/mL - 4.8 μmole) was evaporated to dryness under vacuum. To the vial was added 200 mL HOAc, 3 mg 1 (5 μmole), and then 1.3 mg NCS (20 μmole). The reaction progress was observed at 2 min reaction time.

Radiobromination with NCA Bromine-77

I. To a vial containing 860 μCi $Na^{77}Br$ in 50 μL MeOH was added 50 μL of 1 as a 2 mg/mL MeOH (50 μg - 0.37 μmole). The reaction progress was checked at 3 minutes post addition of NCS and was found to be complete. After approximately 30 minutes reaction time 750 μCi of activity was removed from the reaction vessel. A total of 676 μCi was injected onto the HPLC column (74 μCi remained in syringe - no rinse) and 575 μCi was isolated (85%). This value was almost identical with the integration of the HPLC radioactivity trace (Figure 2A).

II. To a vial containing 30 μL $Na^{77}Br$ solution (680μCi) was added 1 mg 1 (1.8μmole), and then 100 μL of an NCS solution (3 mg/mL in HOAc - 300 μg, 2.2 μmole). The reaction progress was checked by HPLC at 3 minutes and was found to be complete (Figure 2B).

Tissue distribution

The 17α-E-[^{77}Br]bromovinylestradiol was diluted to a specific concentration of approximately 100 μCi/mL. The radiochemical was administered via the saphenous vein (0.1 mL) to a group of 15 immature female rats (21-24 days old, 45-55 g). Five of the rats were give a second injection of 5 μg of estradiol within 1-2 minutes to compete with the localization of the radiolabeled

estrogen. The rats were sacrificed at 2 hours after injection. The tissue and organs of interest were excised, rinsed free of blood, blotted dry, weighed and counted in a gamma well scintillation counter. The concentration of activity in the various tissues was calculated as percent of injected dose times the mass of the animal in kilograms per gram of tissue - %ID-kg/g. The specific receptor binding was determined as the difference between the tissue localization when the radiobrominated estradiol was injected alone and when the injected radiobrominated estradiol was followed by an injection of additional estradiol.

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