RADIOBROMINATION OF THE 1-POSITION OF ESTRADIOL USING NO-CARRIER-ADDED BROMINE-77

Mark D. Hylarides, Patricia L. Buksa, Fred A. Mettler,
Department of Radiology, University of New Mexico, Albuquerque,
NM 87131

D. Scott Wilbur, Medical Radioisotope Research (INC-3)
Los Alamos National Laboratory, Los Alamos, NM 87545

SUMMARY

The target compound, 1-([^{77}Br]bromo)estradiol (1a), was prepared in three steps from 4-aminoestradiol 3-methyl ether (2). Electrophilic bromination of 2 with *in situ* generated ^{77}BrCl followed by deamination in the same reaction mixture gave 1-([^{77}Br]bromo)estradiol 3-methyl ether (4a) in 59% radiochemical efficiency. Subsequent demethylation of 4a gave 1a in 92% radiochemical efficiency with an estimated specific activity of 300-600 Ci/mmol. An overall radiochemical yield of approximately 20% was obtained. Stability studies of 1a showed no decomposition over a three-day period.

Key words: bromine-77, 1-([^{77}Br]bromo)estradiol, electrophilic bromination, radiobromination.

INTRODUCTION

The synthesis of radiolabeled estrogen derivatives for potential use as diagnostic imaging agents is an area of great interest (1-4). Consequently, there has been a large effort in the design of radiohalogenated steroidal derivatives to achieve molecules which demonstrate high *in vivo* radiochemical stability and high receptor binding affinities. Since stability is a key consideration, direct radiohalogenation of the aromatic A-ring is attractive due to the increased stability of aryl-halogen bonds over aliphatic bonds. Bromine-77 is available in high specific activity (2000-5000 Ci/mmol) which is necessary for an efficient receptor-binding radiopharmaceutical. Bromine-77

0362-4803/85/050437-05$01.00

Received April 23, 1984
Revised October 5, 1984

has been used for imaging (5); however, its imaging properties are not optimal. The nuclide also represents a working model for the ultimate use of bromine-75.

Previous studies have shown that radiobromination in the 2- and 4-positions of the A-ring of estradiol led to very stable compounds (6); however, their binding affinity to estrogen receptor sites *in vitro* (7) was greatly reduced. Since it is believed that the 3-hydroxyl moiety is involved in receptor binding, the close proximity of the halogen to the hydroxyl group appears to have decreased the binding affinity. Due to the increased distance between the halogen and the 3-hydroxyl and the anticipated chemical stability, the synthesis of high specific activity 1-([^{77}Br]bromo)estradiol (1a) was undertaken.

DISCUSSION

The synthesis of 1-bromoestradiol (1) has been accomplished by our group (8) and the same sequence of reactions was used for incorporation of radiobromine. Electrophilic aromatic substitution to incorporate the radiohalogen was attractive (9) due to reaction simplicity; however, prior to the electrophilic radiobromination, it was necessary to activate the 1-position towards electrophilic substitution by preparation of 4-aminoestradiol 3-methyl ether (2). Radiobromination of 2 was carried out by reaction of ^{77}BrCl, prepared from Na^{77}Br and N-chlorosuccinimide (NCS), in 9:1 dioxane/HOAc. After completion of the radiobromination reaction, the adduct 3a was readily deaminated in the same reaction mixture by preparation of the diazonium salt in the presence of added hydrogen peroxide (11). The two steps, radiobromination and deamination to prepare 4a, could be carried out as a "one-flask" reaction in a 59% overall radiochemical efficiency (12). The product 4a was isolated after a simple workup procedure and purified by high performance liquid chromatography (HPLC) (13). Demethylation of 4a with BBr_3/CH_2Cl_2 (14) generated 1a in 92% radiochemical efficiency. The HPLC retention time of the radiobrominated adduct 1a coincided with that of 1. The stability of isolated 1a in CH_3CN/H_2O was monitored by radio-HPLC and no detectable radiochemical decomposition was observed over a 3-day period.

EXPERIMENTAL

Materials and Methods. N-Chlorosuccinimide was obtained from Aldrich as AR grade and was used as obtained. Sodium bromide was obtained from Mallinckrodt and was finely pulverized prior to use. Dioxane was freshly purified prior to use by a previously published procedure (15). All other reagents were used as obtained.

High performance liquid chromatography was performed using a Spectra Physics 8700 equipped with a BioRad variable wavelength UV detector set at 254 nm and Waters Z-Module fitted with a Waters C_{18}-reverse phase radial compression column. The radioactivity was measured using a Canberra NIM BIN module equipped with a nuclear counter and a 2 in sodium iodide crystal in effluent line following the UV detector. This system allowed the UV and radiation responses to be measured concurrently. Acetonitrile/water mixtures were used as the mobile phase.

1-([^{77}Br]bromo)estradiol 3-methyl Ether (4a).

To a vial containing 13 mCi of dry Na^{77}Br was added 50 μL of an NCS solution (1 mg/mL in 9:1 dioxane-HOAc). After 2 min 1.0 mg of 4-aminoestradiol-3-methyl ether (2) was added as a solid to the reaction mixture and the resultant suspension was allowed to stir at room temperature for 2.5 h. Following this period, 30 μL of 9:1 dioxane-HOAc and 33 μL of 1.1 M HCl were added. The solution was cooled to 0°C in an ice/H_2O bath and 2 μL of 3% H_2O_2 and 24 μL of an aqueous solution of $NaNO_2$ (10 mg/mL) was added. The evolution of nitrogen was observed immediately and the solution was allowed to stir for an additional 15 min at 0°C and 10 min at 25°C.

The reaction mixture was worked up by pouring the solution in a mixture of 2 mL of water and 5 mL of ethyl acetate. The organic phase was isolated, dried over anhydrous $MgSO_4$, filtered, and the solvents were removed under reduced pressure. Crude 4a was dissolved in 0.3 mL of methanol and 7.5 mCi was injected in HPLC (35/65 H_2O/CH_3CN, flow = 2.0 mL/min) and 4.4 mCi of radiochemically pure 4a was collected at 12.5 min. For the two synthetic steps from Na^{77}Br, an unoptimized 34% radiochemical yield was obtained.

1-([^{77}Br]bromo)estradiol (1a):

The solvents were removed from 4a under reduced pressure and the dry residue (4.4 mCi) was dissolved in 1.5 mL of dry CH_2Cl_2. The colorless solution was cooled to 0°C and 0.1 mL of 1.0 M BBr_3 in CH_2Cl_2 was added. The cooling bath was removed and after 0.5 h the CH_2Cl_2 and excess BBr_3 was removed under reduced pressure. The pale red residue was dissolved in 0.3 mL of methanol and 2.24 mCi of the solution was injected on HPLC (55/45 H_2O/CH_3CN, flow = 2.0 mL/min). The target compound 1a (2.08 mCi), collected at 14.2 min, had an identical HPLC retention time as 1 and was obtained in a 42% radiochemical yield (unoptimized) from 4a.

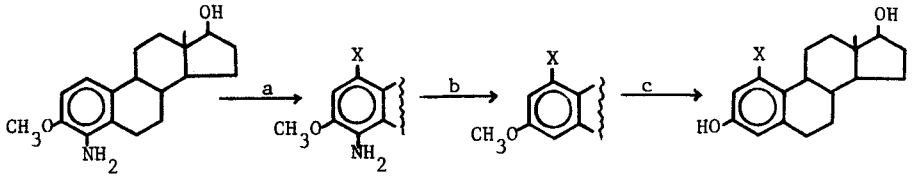
Calculation of specific activity:

Different concentrations of 1-bromoestradiol (1) were injected on HPLC and a calibration curve of mmol of 1 injected versus UV absorbance at 254 nm (ϵ_{254} = 763) was prepared. The UV response of the isolated 1a was experimentally obtained, thus, the amount (mmol) of 1a injected could be estimated from the calibration curve. The activity (mCi) associated with the UV response was also known experimentally; therefore, the specific activity (Ci/mmol) could be calculated. In the case of 1a, an estimated specific activity of 300-600 Ci/mmol was determined.

Stability studies of 1a:

The eluant collected from HPLC, which contained 1a, was reinjected on HPLC at 6, 12, 24, 48 and 72 h. No radiochemical decomposition was observed over the 3-day period.

Scheme I



2

3 X=Br
3a X=^{77}Br

4 X=Br
4a X=^{77}Br

1 X=Br
1a X=^{77}Br

a. NCS/Na^{77}Br, 9:1 dioxane-HOAc
b. $NaNO_2$, HCl, H_2O_2
c. BBr_3, CH_2Cl_2

ACKNOWLEDGEMENT

We wish to thank the Medical Radioisotope Research Group (INC-3) of Los Alamos National Laboratory for donating the bromine-77 that was used in this study. We are also grateful for the financial support of the Department of Energy (Contract #DE-AC04-81EV10596).

REFERENCES

1. Krohn, K. A., J. Nucl. Med., 21, 593 (1980).

2. Longcope, C., Arunachalam, T., Rafkind, I., and Caspi, E., Steroid Biochem., 14, 261 (1981).

3. Katzenellenbogen, J. A., Herman, D. F., Carlson, K. E., and Lloyd, J. E., "In "Receptor-Binding Radiotracers," Eckelman, W. C., Ed., Vol. 1, CRC Press, Boca Raton, FL, 1982, p. 93.

4. Njar, V.C.O., Arunachalam, J., and Caspi, E., J. Org. Chem., 48, 1007 (1983).

5. Katzenellenbogen, J. A., Senderoff, S. G., McElvany, K. D., O'Brien, H. A., Welch, M. J., Nucl. Med., 22, 42 (1981).

6. Wilbur, D. S., Bentley, G. E., and O'Brien, H. A., J. Label. Compds. Radiopharm., 18, 1693 (1981).

7. Heiman, D. F., Senderoff, S. G., Katzenellenbogen, J. A., and Neeley, R. J., J. Med. Chem., 23, 994 (1980).

8. Hylarides, M. D., Leon, A. A., Mettler, F. A., and Wilbur, D. S., J. Org. Chem., 49, 2744 (1984).

9. Wilbur, D. S. and O'Brien, H. A., J. Org. Chem., 47, 359 (1982).

10. Wilbur, D. S., and Anderson, K. W., J. Org. Chem., 47, 358 (1982).

11. This general deamination procedure was shown by our group to be widely applicable to a variety of aromatic amines.

12. Radiochemical efficiency was calculated based on the amount of activity injected in HPLC and the amount isolated from HPLC. The overall radiochemical yield does not represent an optimized yield.

13. It was necessary to isolate the purified radiobrominated compound 4a at this point. Demethylation of the crude reaction product mixture without prior HPLC purification led to excess UV contamination in the final product 1a.

14. Vickery, E. H., Pahler, L. F., and Eisenbraum, E. J., J. Org. Chem., 44, 4444 (1979).

15. Gordon, A. J. and Ford, R. A., "The Chemist Companion," John Wiley and Sons, N. Y., 1972, p. 433.