AMINAL STUDIES

At the present time, animals are being maintained i
Facility at the University of New Mexico. These are ra
following tumor lines: 2M2N and 13762E mammary adenoca

CONTRACT NUMBER DE-AC04-81EV10596

DEVELOPMENT OF BROMINE-77 FROM THE LAMPF FACILITY

FRED A. METTLER, JR., M.D., M.P.H., PRINCIPAL INVESTIGATOR

DOE/EV/10596--T3

DE91 000114

OBJECTIVE

The objective of the work is to conduct the necessary studies required to evaluate the efficacy, potential benefit and role of Bromine-77 labelled steroids in the detection and evaluation of treatment for hormone-dependent tumors.

TASKS TO BE PERFORMED BY THE CONTRACTOR

1) An initial investigation will concentrate on the radiobromination at Carbon 6 in selected simple steroids utilizing the nuclides of Bromine-82 nad Bromine-77.

2) Conduct analytical spectroscopy of radiolabelled compounds.

3) Investigate the biodistribution, toxicology and tumor affinity of labelled agents.

PROGRESS

The progress for tasks (above) 1 and 2 are outlined as follows.

This work has been performed by Dr. Mark Hylarides who is the synthetic biochemist hired under the terms of the Contract. His work has been conducted predominantly at the University of New Mexico, although there have been cooperative efforts with Dr. Scott Wilbur at the Los Alamos Radiochemistry Division (CNC-3).

PROGRESS REPORT

RECENT DEVELOPMENTS IN THE STUDY OF C-RING RADIOLABELED ESTRADIOLS AND SYNTHETIC APPROACHES TO A-RING RADIOLABELED ESTRADIOLS

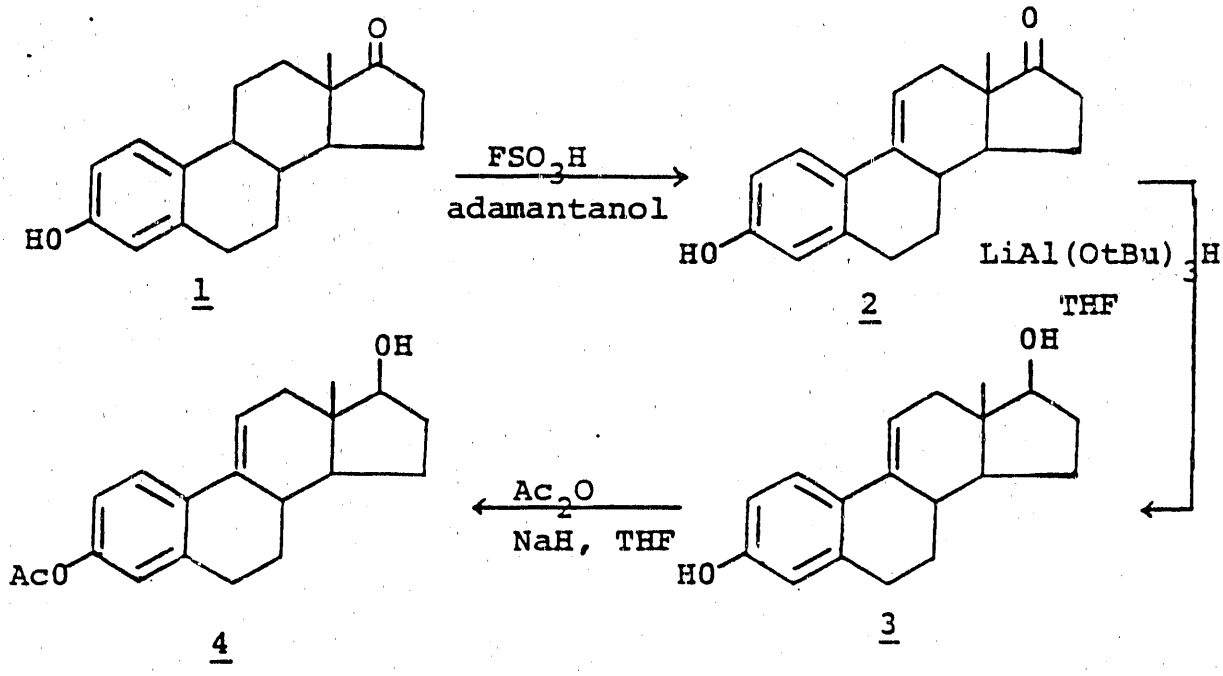
ABSTRACT

The synthetic goals of 1982-3 included the synthesis estradiol derivatives which were radiohalogenated in the A- or C-ring with bromine-77 or bromine-82. Estradiol derivatives in which the radiohalogen was incorporated into the C-ring were prepared and purified with high specific activity. Biodistribution studies of the resultant compounds will be performed on rats in the near future. Various synthetic approaches toward estradiol which is radiohalogenated in the 1-position are discussed.

Synthesis and studies of C-ring radiohalogenated estradiols:

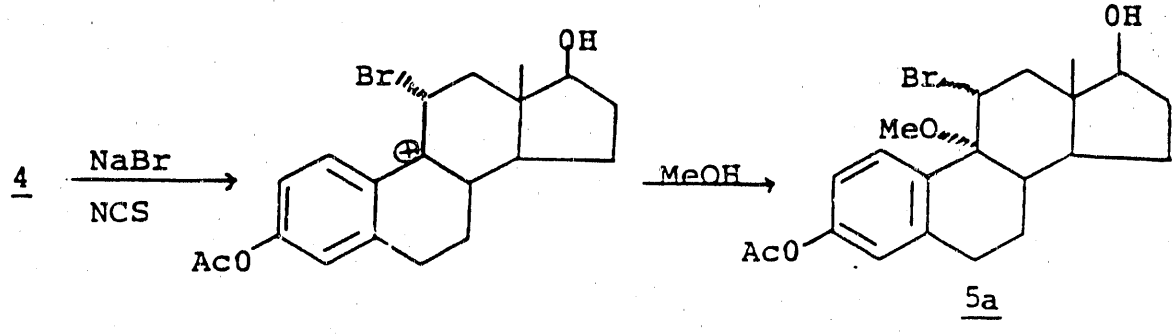
The preparation of C-ring radiolabeled estradiol derivatives is a collaborative effort involving Dr. Ray Counsil from the University of Michigan, LANL, and the Department of Radiology at the University of New Mexico. The desired precursor, $\Delta^{9,11}$-estrone 2 was donated to our group by Dr. Counsil. From a synthetic procedure worked out in his laboratory, we are able to prepare gram quantities of the material. The precursor 2 was selectively reduced with $LiAl(OtBu)_3H$ to the 17β-OH 3 which was directly acylated to generate $\Delta^{9,11}$-estradiol-3-acetate 4 in high yield (Scheme I). It is necessary to protect the phenol 3 as an acetate in order to eliminate A-ring bromination in the 2- and 4-positions. After incorporation of the radiohalogen the acetate protecting group can be removed by treatment with mild base. The valuble precursor 4 allows us access to the "bromomethoxy" addition product 5a and possibly the vinyl bromide 6.

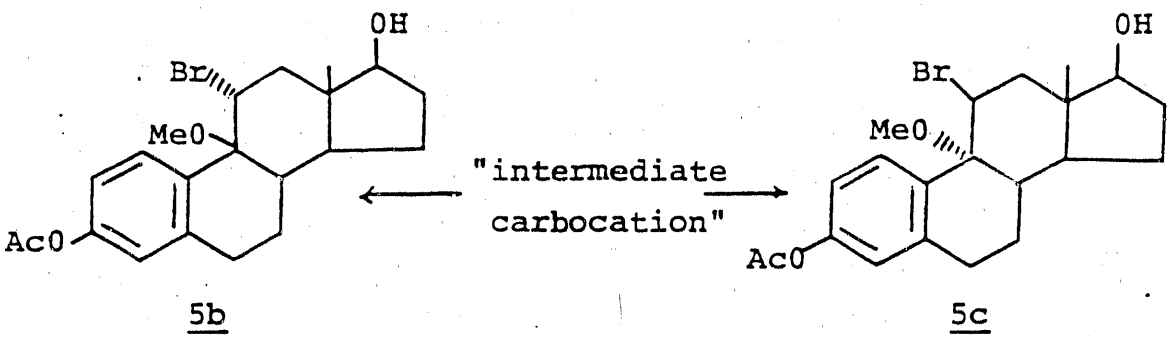
SCHEME I



The reaction mixture resulting from treatment of 4 with NCS/NaBr in methanol was analyzed by high field NMR. The two separate methoxy hydrogen resonances in the ratio of 3:1 suggested the presence of two bromo-methoxy adducts. Further structural analysis suggested that the major component was probably the α-bromo-α-methoxy adduct 5a. Also, it is likely that the minor product is the α-bromo-β-methoxy adduct 5b or the β-bromo-α-methoxy adduct 5c. These products arise from initial formation of a bromonium ion, opening to a tertiary carbocation followed by solvent capture (Scheme II).

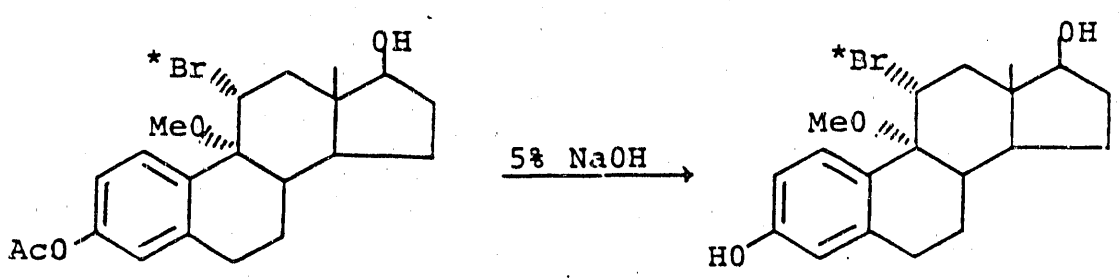
Scheme II





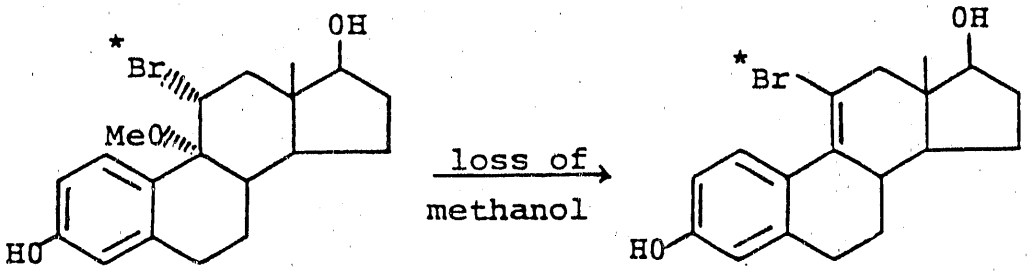
5b 5c

As predicted from studies with "cold" NaBr, the reaction of $\Delta^{9,11}$-estradiol-3-acetate 4 with Na^{77}Br generated two radiolabeled products in the ratio of 85:15. The major peak, which comprised about 85% of the total activity, was isolated and showed good chemical stability. Subsequent treatment of this major component with 5% NaOH for 15 min facilitated the removal of the acetate protecting group. Upon generation of the ^{77}Br-labeled estradiol derivative 5, little or no activity was lost from the molecule. The radiohalogenated compound 5 showed excellent chemical stability and the specific activity was approximated at greater than 1,000 Ci per mmol.



5a 5

We speculate that radiolabeled vinyl bromide 6 can be prepared from the bromo-methoxy adduct 5. Further investigative efforts are necessary, however, in order to determine the availability of this compound.



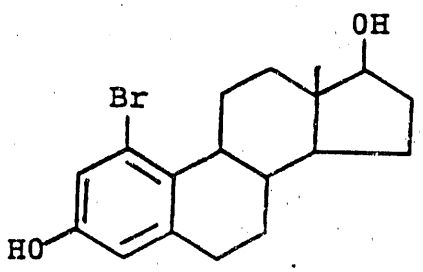
5 6

Since the bromo-methoxy adduct 5 is readily available in high purity and specific activity a complete biodistribution study will be performed on rats in December 1982. If the compound shows a high uterus to blood ratio, further work involving the optimization of the imaging techniques will follow. Additionally, in order to determine the precise molecular conformation of the compound, an X-ray structure will be obtained from the facility in the Department of Chemistry at UNM.

Synthetic Approaches to A-Ring Radiolabeled Estradiols:

Based on present literature estradiol has been labeled with bromine-77 in the 2- and 4- positions of the A-ring (1). These labeled materials showed fair to poor uterus to blood ratios presumably due to their close proximity to the phenolic function. Due to obvious synthetic difficulties, radiohalogenation of the 1- position has not been achieved; however, one would expect such a derivative to show high uterus to blood ratios. The incorporated halogen would be far enough removed from the phenol as not to drastically effect the binding characteristics of that functionality. Also, the halogen would be enclosed in the "bay" region of the steroid

thus eliminating most steric and conformational perturbations. In summary, 1-bromoestradiol 7 should exhibit binding affinities similar to that of estradiol.



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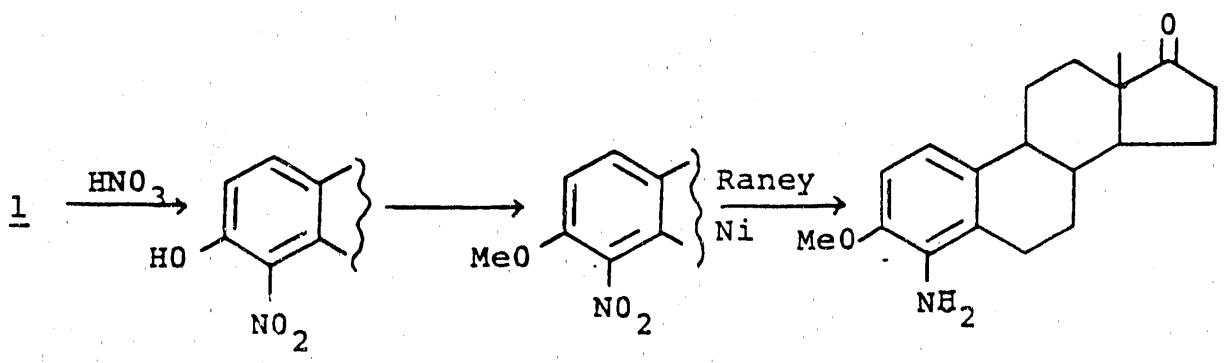
The preparation of 1-bromoestradiol was undertaken in our laboratory in August 1982. The synthetic strategy ultimately involved two different major routes with 4-amino-3-methoxyestrone 10 as the key synthetic intermediate. The intermediate 10 was prepared by a previously published procedure (2). Using the procedure, treatment of estrone 1 with nitric acid in acetic acid generated 4-nitroestrone 8. Subsequent preparation of the 3-methyl ether 9 followed by reduction of the nitro group gave the desired 4-amino-3-methoxyestrone 10 (Scheme III).

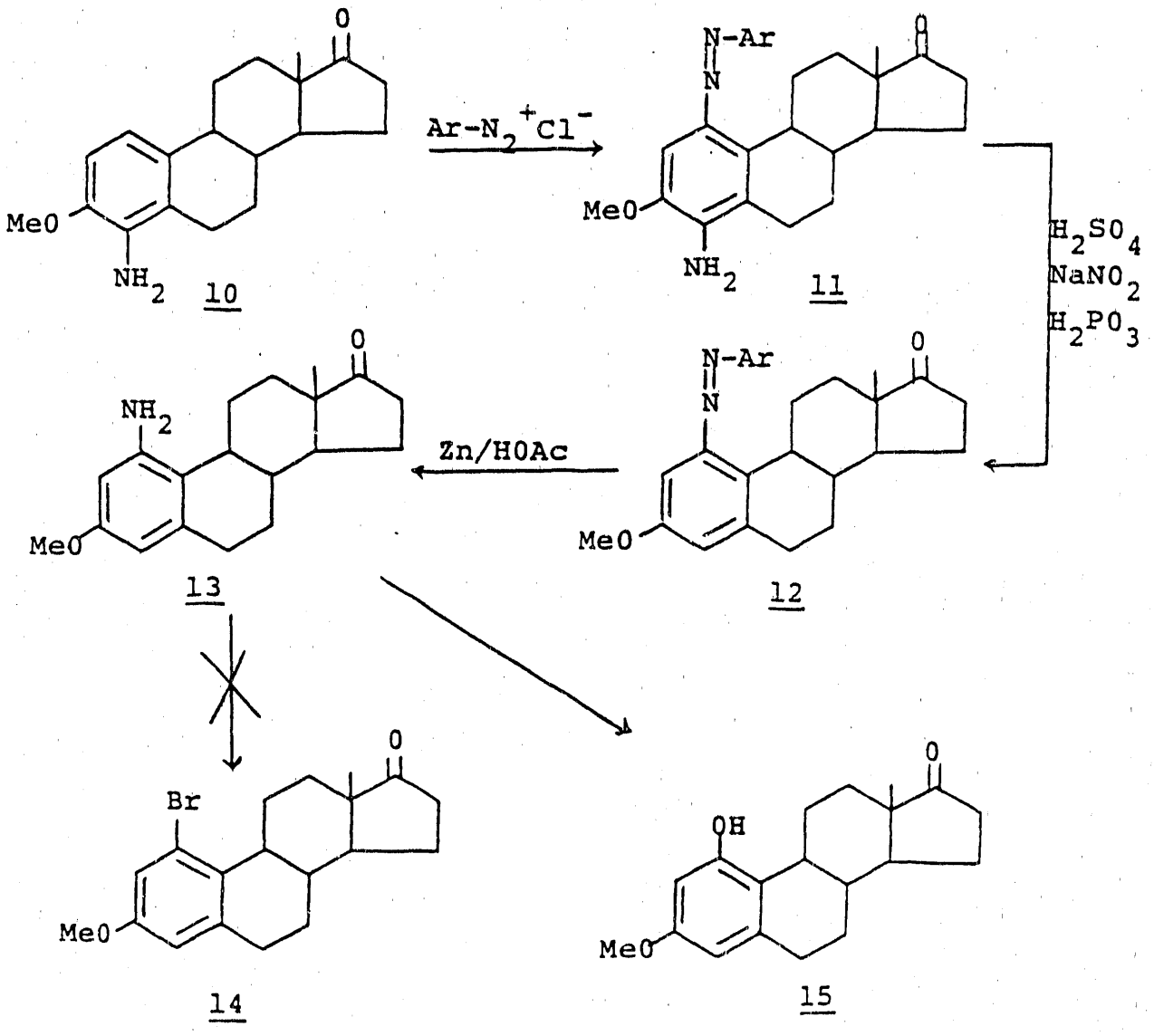
Scheme III



8 9 10

The first route to the target compound involved the preparation of 1-amino-3-methoxyestrone 13 from 10 using previously published procedures (3) (Scheme IV). Treatment of 10 with p-nitro-benzenediazonium chloride generated the desired 4-amino-1-azo intermediate 11 which was directly deaminated to yield the 1-azo compound 12. Reductive cleavage of 12 with zinc and acetic acid generated 1-amino-3-methoxyestrone in reasonable yield.

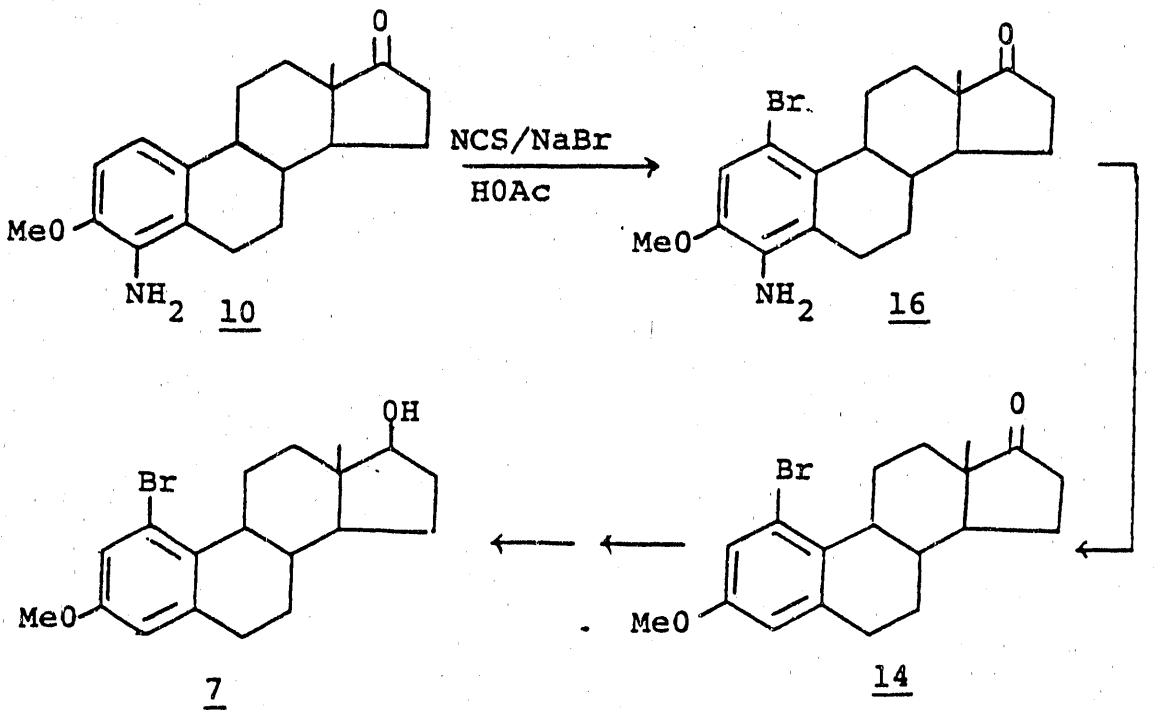
Scheme IV



All attempts to prepare 1-bromo-3-methoxyestrone 14 from 13 via the diazonium salt intermediate failed presumably due to the instability of the diazonium salt. In all cases 1-hydroxy-3-methoxyesterone 15 was obtained as a major product.

An alternate and more efficient route to 14 was investigated. The direct electrophilic bromination (NCS/NaBr) of 4-amino-3-methoxyestrone 10 in acetic acid gave 4-amino-1-bromo-3-methoxyestrone 16 in quantitative yield. Furthermore, deamination of 16 under standard conditions gave 1-bromo-3-methoxyestrone 14 in high yields (Scheme V).

Scheme V

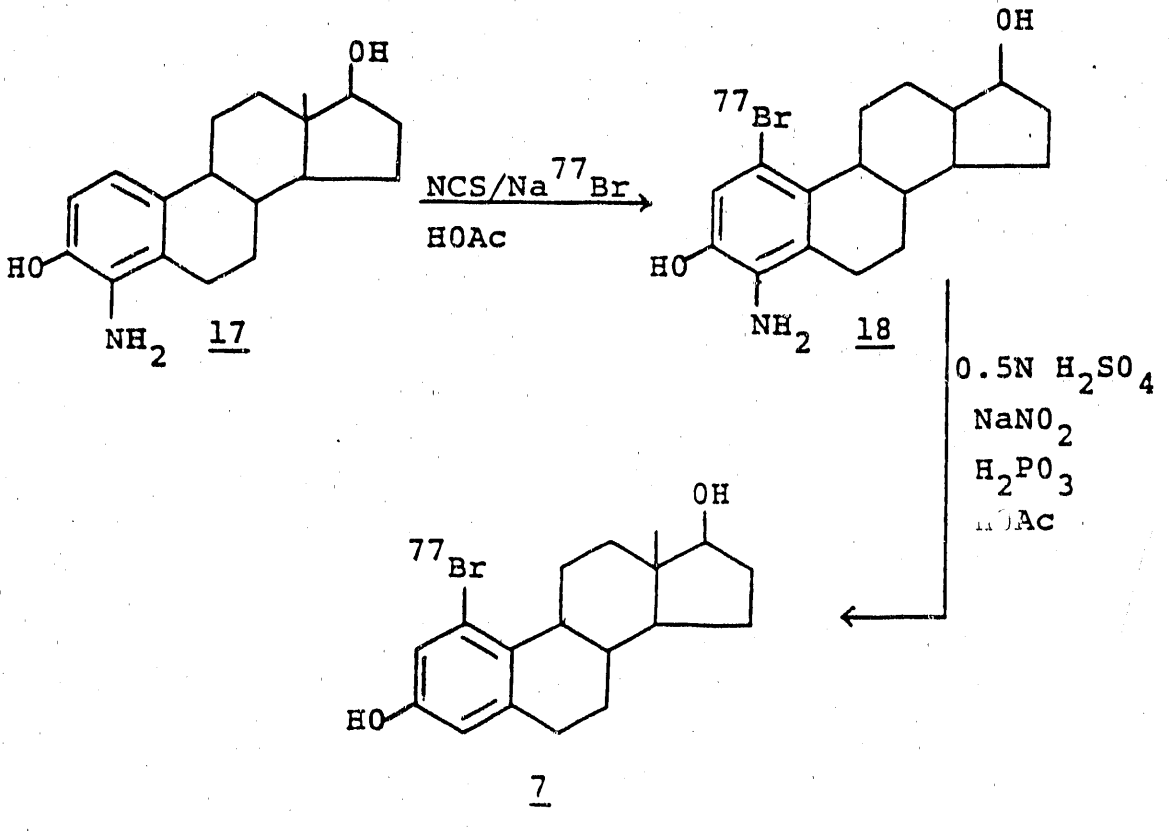


This particular sequence of reactions was appealing since 10 could be readily radiohalogenated with bromine-77 in the 1-position. The disadvantage is that three synthetic transformations must be employed after incorporation

of the radiohalogen in order to obtain 1-bromoestradiol 7. We searched for a procedure in which the radionuclide could be incorporated into the molecule during the last or second to the last step in the synthetic pathway.

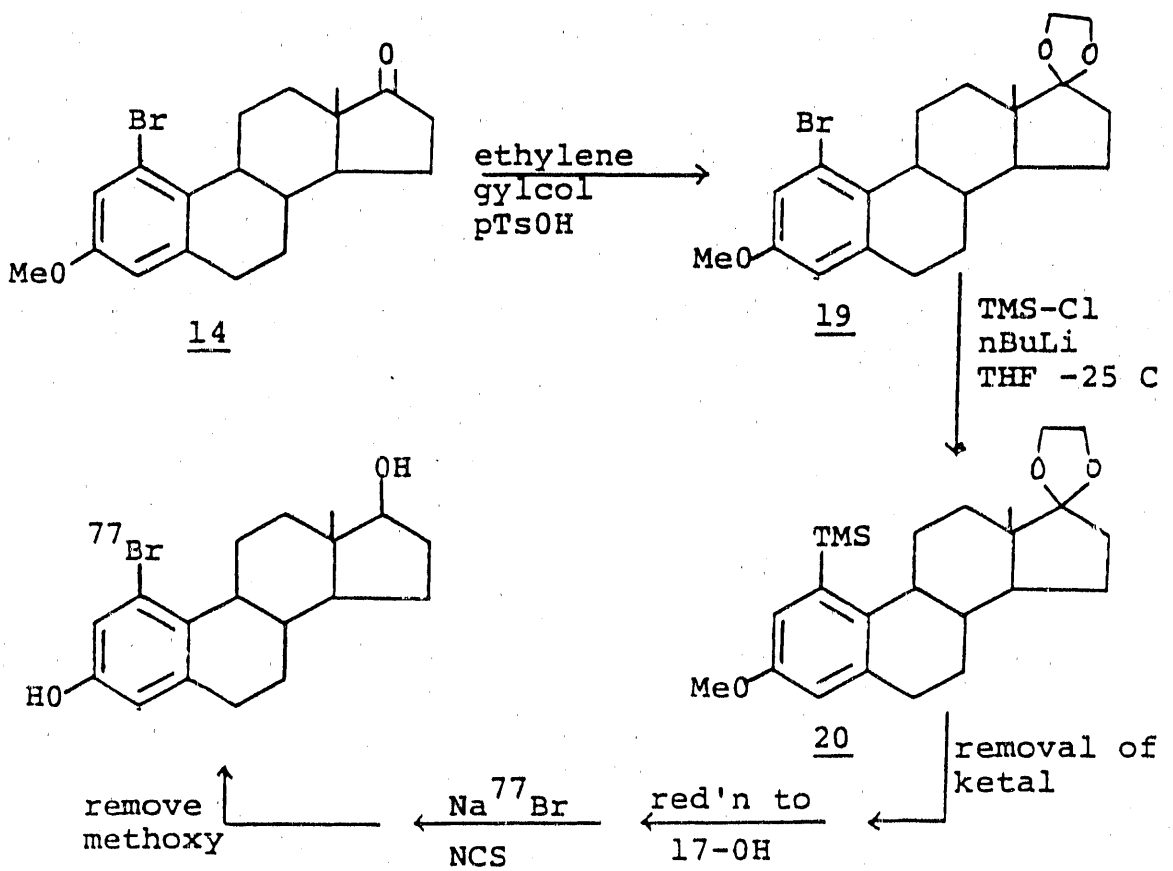
An alternate sequence for the preparation of 1-^{77}Br-estradiol 7 is presently under investigation. Electrophilic bromination of synthetically available 4-aminoestradiol 17 may generate the desired 4-amino-1-bromoestradiol 18. Only a deamination step is necessary after incorporation of the radiohalogen in order to obtain the 1-^{77}Br-estradiol (Scheme VI). This procedure may be a very effective method for the incorporation of a radiohalogen into the 1- position.

Scheme VI



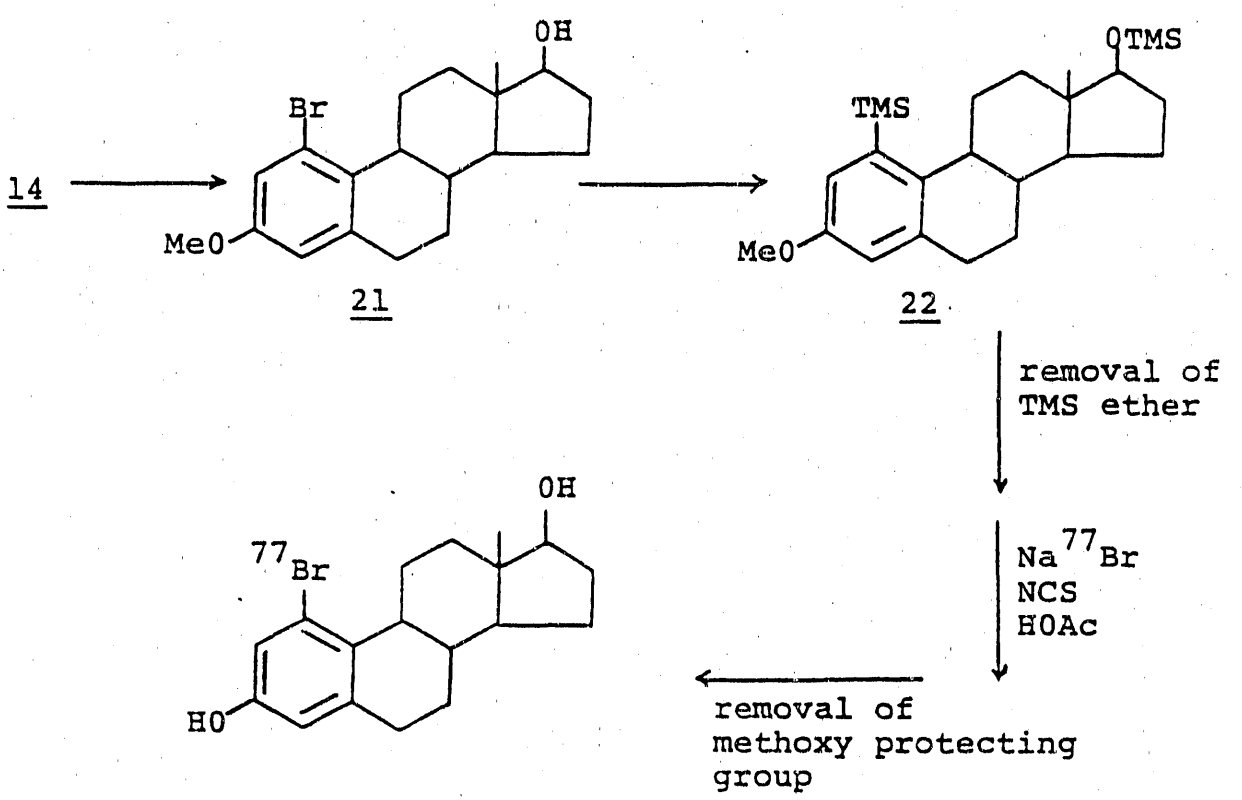
Another synthetic approach to the preparation of 1-^{77}Br-estradiol 7 involves the use of 1-bromo-3-methoxy-estrone 14 as a key intermediate. Subsequent replacement of the halogen by a trimethylsilyl (TMS) group would generate an aryl-trimethylsilyl intermediate. Introduction of radiohalogens via these types of intermediates has been previosly reported (4). The procedure that was attempted required initial protection of the 17-one of 14 with a ketal. Treatment of this intermediate 19 with n-butyl lithium at low temperatures followed by the addition of trimethylsilyl chloride should have given the aryl-trimethylsilane 20 (Scheme VII). Instead, the n-butyl lithium destroyed the ketal of 19 and no incorporation of TMS was observed.

Scheme VII



Since the ketal 19 was destroyed under the strong basic conditions, the ketone 14 was reduced to the 17β-OH 21. A similar reaction of 21 with TMS-Cl and n-butyl lithium should generate the aryl-TMS derivative 22 (Scheme VIII). If the TMS group can be incorporated into the 1- position of 21, it will be possible to incorporate a radiohalogen in the 1- position in high radiochemical yield. In this particular sequence of reactions only deprotection of the phenol is necessary after incorporation of the radiohalogen. This reaction scheme and those previously described are presently under investigation in our laboratory at UNM.

Scheme VIII



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3. E. W. Cantrall, R. Conrow and S. Bernstein, J. Org. Chem. (1964) 86, 2943.

4. D. S. Wilbur, W. E. Stone and K. W. Anderson, J. Org. Chem. submitted for publication.

AMINAL STUDIES

At the present time, animals are being maintained in the Animal Research Facility at the University of New Mexico. These are rats carrying the following tumor lines: 2M2N and 13762E mammary adenocarcinomas which have been maintained and transplanted with excellent success for over sixteen months and 3M2N and 13762E mammary adenocarcinomas recently acquired from EG&G Mason Research Institute to ensure the continued viability of the tumor lines. The original tumors have been tested for estrogen receptors with the following expected results: 3M2N negative; 13762E positive for estrogen receptors. Periodic testing of the tumors will be done.

IMAGING STUDIES

It has become apparent that imaging of Bromine-77 with the commercially available all-purpose collimators is going to be extremely difficult. Under the direction of Dr. James Christie and Dr. Charles Kelsey, work has begun to establish the modulation transfer function and observer performance of contrast and spatial resolution with differing collimators. A shipment of Bromine-77 will be received December 6 to investigate this matter.

COOPERATIVE STUDIES

Under cooperative projects in order to cooperate fully with other DOE funded facilities, Dr. Christie and the Division of Nuclear Medicine at this institution have agreed to participate in the evaluation of Ytterbium-169 citrate. This project is sponsored by Dr. Karl Hubner from Oak Ridge National Laboratories and we are co-investigators on this project with the isotope preparation occurring at Oak Ridge. This cooperative project is expected to have no financial impact on the current contract.

SUMMARY

The project currently is proceeding on or slightly behind schedule. Funding requested for year 3 is slightly above that initially requested. The reduction in operation of the Pi-Meson accelerator at Los Alamos at the present time has not had any significant impact on the schedule of this contract.

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