Preparation of 1,2-Dimethoxy-4-(bis-diethylaminoethyl-[^{14}C]-amino)-5-bromobenzene

Theodore S.T. Wang, Rashid A. Fawwaz, and Ronald H. Van Heertum

Division of Nuclear Medicine, Department of Radiology, College of Physicians and Surgeons, Columbia University, New York, NY 10032

Correspondence and reprint requests should be addressed to:

Theodore S.T. Wang, Ph.D. Division of Nuclear Medicine, Department of Radiology, Columbia Presbyterian Medical Center, 622 West 168th Street, New York, NY 10032

SUMMARY

1,2-Dimethoxy-4-(bis-diethylaminoethyl-[^{14}C] -amino)-5-bromobenzene (**III**) was synthesized for *in vivo* pharmacokinetic and pharmacodynamic evaluation. The synthesis of (**III**) was easily obtained by the direct alkylation of 1,2-dimethoxy-5-bromo-aniline with beta-diethylaminoethyl chloride-ethylene-[^{14}C], using CH_3MgCl as the proton abstractor. Compound **III** was obtained with a 22.3% yield. The specific activity was 2.2mCi/mmol, with a radiochemical purity of >99%.

Key words: Antimalarial agent, RC-12-[^{14}C], chlorination, Grignard reagent, bis-N-alkylation.

INTRODUCTION

Pyrocatechol, 1,2-Benzendiol, a topical antiseptic agent, is one of the products of *gum catechu* obtained from certain Asiatic tropical plants. The 1,2-dimethyl ether derivative of pyrocatechol,

0362–4803/93/111007–06$08.00

Received 5 March, 1993
Revised 24 May, 1993

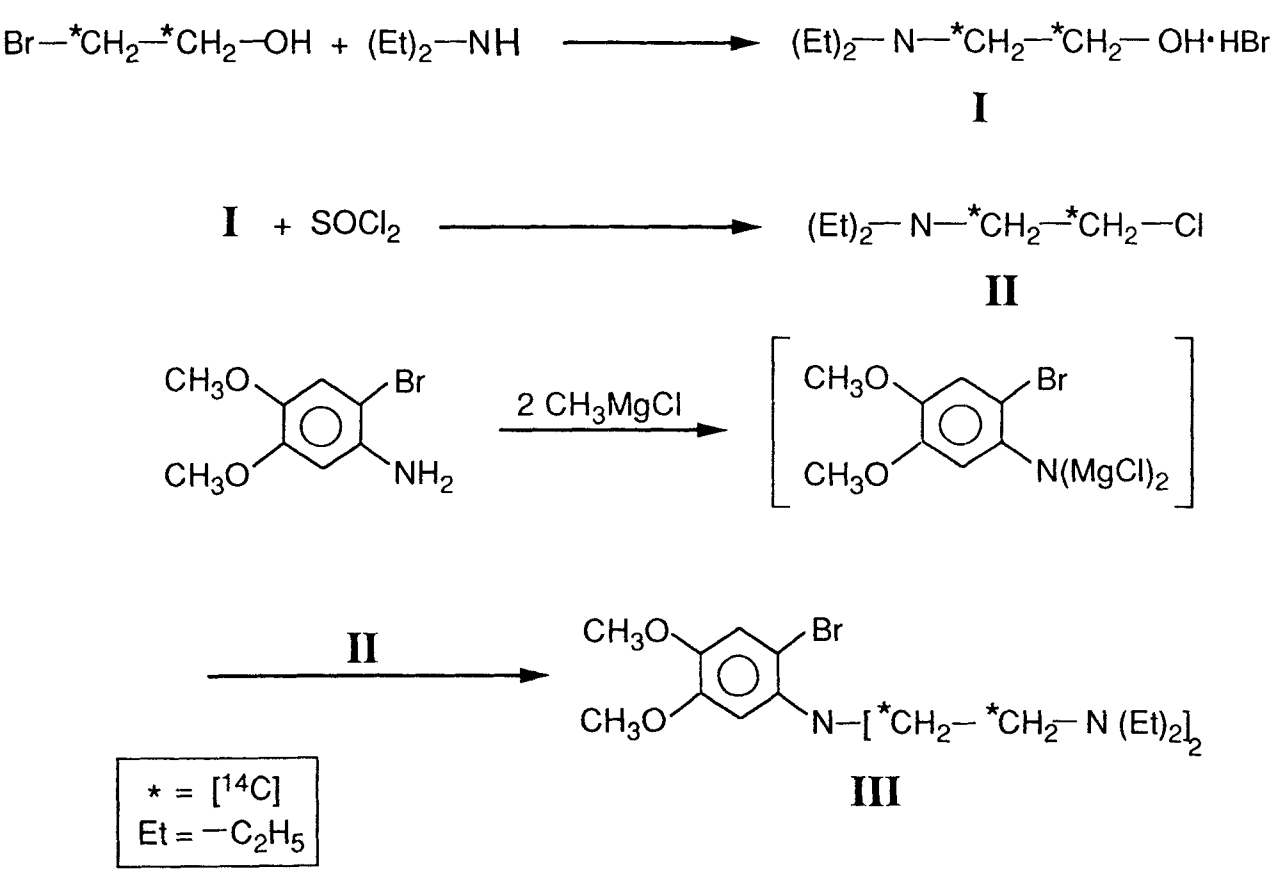
Guaiacol carbonate, is used as an expectorant in cough remedies. The 4-amino substituted derivatives of 1,2-dimethyl ether interestingly have been found to possess antimalarial properties (1). Among them, 1,2-dimethoxy-4-(bis-diethylaminoethylamino)-5-bromobenzene, RC-12 (2,3), the most promising compound, has recently attracted the attention of investigators because RC-12 has remarkable potential as a prophylactic and radical curative agent to protect rhesus monkeys against infection with sporozoites of the B strain of *plasmodium cynomolgi*. RC-12 was shown to cure established infections and control parasitemia at dose levels that were tolerated by the monkeys (4,5,6,7). RC-12 was even tested as a potential antimalarial drug in human volunteers infected with the Chesson strain of *plasmodium vivax*. (8,9).

In order to evaluate the pharmacokinetic, pharmacodynamic and toxicological properties of RC-12 in animals, especially the mechanisms involved in its action against the secondary or persisting tissue forms of the established sporozoite, and the effects of therapy, Carbon-14 labeled RC-12 (**III**) was prepared.

RESULTS

The synthesis of compound **III** was begun with the alkylation of 2-bromoethanol-ethylene-[^{14}C] with diethylamine to form beta-diethylaminoethyl alcohol-ethylene-[^{14}C] hydrobromide (**I**), which was then reacted with $SOCl_2$ to yield beta-diethylaminoethyl chloride-ethylene-[^{14}C] (**II**). Next, methyl Grignard reagent was used as the proton abstractor to carry out the bis-N alkylation of 1,2-dimethoxy-5-bromo-aniline with **II** to produce **III**, as in Scheme I. Separation of bis- and mono-N alkylated products was conveniently accomplished by a chromatographic method using a

Scheme I


Br—*CH2—*CH2—OH + (Et)2—NH
(Et)2— N—*CH2—*CH2— OH·HBr
I
I + SOCl2
(Et)2— N—*CH2—*CH2—Cl
II
CH3O
Br
CH3O
NH2
2 CH3MgCl
CH3O
Br
CH3O
N(MgCl)2
II
CH3O
Br
CH3O
N—[*CH2— *CH2— N (Et)2]2
III
* = [14C]
Et = —C2H5

preparative thick layer chromatograph plate (silica gel). The bis-N-alkylated product (**III**) was eluted from silica gel with methanol. The overall yield was 22.3%. The specific activity was 2.2 mCi/mmol.

EXPERIMENTAL

Beta-Diethylaminoethyl alcohol-ethylene-[^{14}C] hydrobromide (I)(10)

Diethylamine, 876 mg (12 mmol) in 5 mL of thiophene-free benzene was added dropwise to a solution of 760 mg (6.0 mmol, 30 mCi) 2-bromoethanol-ethylene-[^{14}C] (11) in 8 mL of thiophene-free benzene mL at 50^{o}C. The mixture was refluxed for 8 hrs. The reaction mixture was allowed to cool to room temperature and the solvent and excess diethylamine were evaporated *in vacuo*. The white crystal product was immediately used in the next reaction.

Beta-Diethylaminoethyl chloride-ethylene-[^{14}C] (II) (12)

119 mg (10 mmol) thionyl chloride in 5 mL thiophene-free benzene was added slowly to a mixture of (I) and 10 mL of thiophene-free benzene at 10^{o}C. The reaction mixture was then allowed to warm up to room temperature, and refluxed for 6 hrs. The mixture was cooled, and the solvent and excess thionyl chloride were evaporated *in vacuo* at 5-10^{o}C. The solid residue was treated with 10 mL of ice-cold water. The solution was then added with 20 mL of ether, and 5% of NaOH was added at 0^{o}C until the aqueous solution turned alkaline (phenolphthalein indicator). The ether layer was separated. The aqueous layer was extracted with 15 mL of ether twice. The extracts were combined, dried over K_2CO_3, and filtered. The ether was removed *in vacuo* at -5^{o}C. The free base was distilled and collected at 32-34^{o}C/5mm/Hg. The product was diluted with freshly distilled beta-diethylaminoethyl chloride (2,13) to yield 1.40 g (10 mmol, 24.6 mCi) and was immediately used for the next reaction.

1,2-Dimethoxy-4-(bis-diethylaminoethyl-[^{14}C]-amino)-5-bromobenzene (III)(14)

Thirty mmol of CH_3MgCl in THF solution (10 mL of 3.0 mmol/mL) was added dropwise to a solution of 1.16 g (5 mmol) of 2-bromo-4,5-dimethyoxyaniline in 15 mL of dry THF. After the addition was complete, the mixture was stirred at room temperature for 30 min. Freshly prepared (II) 1.40 g (10 mmol, 24.6 mCi), in 8 mL of dry THF was added, and the mixture was stirred and refluxed overnight. The reaction mixture was cooled to room temperature, and 20 ml of 10% NH_4Cl solution was added. The mixture was then extracted twice with 30 mL of n-pentane each time. The extracts were combined, dried over $MgSO_4$, and filtered. The filtrate was passed into an acid alumina column and eluted with 100 mL of n-pentane, followed by 50 mL of n-pentane-ether (50:50,v/v). The combined eluates were concentrated and monitored by TLC (silica gel) with acetone and triethylamine (40:1,v/v). Two spots fluorescece under UV light with Rf values of 0.83 and 0.39. (authentic RC-12, Rf value=0.39). The crude product was separated and purified on thick layer chromatography (silica gel GF, 250 um) by elution with methanol. The yield was 1.30 g (29.6%, 2.96 mmol, 6.68 mCi). The specific activity was 2.2 mCi/mmol.

The NMR spectrum of authentic RC-12 and **III** in CCl_4 were found to be identical on a Varian A-60 using tetramethylsilane as an international standard. The peaks (p.p.m) intergrate for the following relative intensities: singlet 6.71(1H), singlet 6.91 (1H), singlet 3.75 (6H), multiplet 3.00 (4H), multiplet 2.43 (12H), triplet 0.93 (12H). The IR, UV absorptions, and Rf values by TLC (acetone-triethylamine, 40:1, v/v) and Beilstein tests of authentic RC-12 and the product were also found to be identical (14). The radiochemical purity was demonstrated by radiochromatogram and autoradiogram to be above 99%.

REFERENCES

1. Schonhofer F. "Chemotherapy", *Office Tech. Services Rept.*, PB-85033, 1948; FIAT, *Review of German Science*, 43-45.

2. Bruce-Chwatt L. J. *Trans. Roy. Soc. Trop. Med. Hyg.*, **59:**105-109 (1965)

3. Aviado D.M. Marco V. and Weed D. *Chemotherapy* **13:** 339-355 (1968)

4. Omar M. S. and Collins W. E. *Amer. J. Trop. Med. Hyg.* **23:** 339-349 (1974)

5. Schmidt L.H. Cramer D.V. Rossen N.R. and Harrison J. *Antimicrob. Agents Chemother.* **24:** 615-652 (1983)

6. Schmidt L.H. *Amer. J. Trop. Med. Hyg.* **32:** 231-257 (1983)

7 Schmidt L.H. Rossan R.N. Fradkin R. et al. *Antimicrob. Agents Chemother.* **28:** 612-625 (1985)

8. Clyde D.F. McCarthy V.C. and Miller R.M. *Trans. R. Soc Trop. Med. Hyg.* **68:** 167-168 (1974).

9. Rainer F. and Juergen S.W. *Quant. Struct-Act. Relat. Pharm. Chem. Biol.* **4:** 51-63 (1983)

10. Hartman A.H. "Synthesis of beta-diethylamnioethyl alcohol" in *Organic Synthesis Collective Vol. 2*, (Blatt A.H. ed.) 1961. John Weily Co., New York, N.Y. pp. 183-184.

11. 2-Bromoethanol-ethylene-[^{14}C] was supplied by New England Nuclear Coorporation-Du Pont, Boston, MA.

12 Breslow D.S. Yast R.S. and Hauser C.R. *J. Am. Chem. Soc.*, **66:** 1921-1924 (1944)

13. Nitrogen mustards undergo rearrangement or slow decomposition even at 0°C. The free base should be freshly prepared and immediately used.

14 Stogryn E.L. *J. Med. Chem.*, **13:** 864-866 (1970)