Potential Cerebral Perfusion Agents. Synthesis and Evaluation of a 1,4-Disubstituted Dihydropyridine Analogue

Prem C. Srivastava*, Furn F. Knapp, Jr., and Carol D. Pruitt†

Nuclear Medicine Group, Health and Safety Research Division, Oak Ridge National Laboratory, Oak Ridge, Tennessee 37831

Received September 24, 1987

The synthesis of a 1,4-disubstituted dihydropyridine, 1-(*E*-1[^{125}I]iodo-1-penten-5-yl)-4-(β-*N*-acetylaminoethyl)-1,4-dihydropyridine ([^{125}I]**10**), is described. Acetylation of 4-(β-aminoethylpyridine) with acetic anhydride followed by condensation with *E*-1-borono-5-iodo-1-pentene (**7**) gave 1-(*E*-1-borono-1-penten-5-yl)-4-(β-*N*-acetylaminoethyl)pyridinium iodide (**8**). Chloramine-T and sodium iodide iodination of **8** gave the corresponding *E*-1-iodo compound **9** which was reduced with sodium borohydride to furnish 1-(*E*-1-iodo-1-penten-5-yl)-4-(β-*N*-acetylaminoethyl)-1,4-dihydropyridine (**10**). The corresponding radioiodinated compound was prepared similarly using Na[^{125}I]. The tissue distribution studies in rats indicate that [^{125}I]**10** crosses the blood brain barrier (0.49% dose/g in the brain) but gradually washes out from the brain.

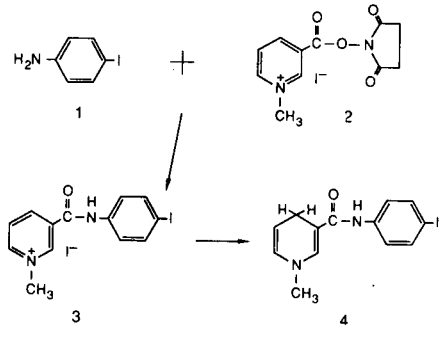
J. Heterocyclic Chem., **25**, 667 (1988).

Introduction.

Many radiolabeled lipophilic agents cross the intact blood brain barrier and distribute in the brain as a function of regional cerebral blood flow [1]. If such agents are modified and appropriately radiolabeled to show high brain uptake with high brain:blood ratios and prolonged retention with minimal redistribution in the brain, they can potentially be used for single photon emission computerized tomographic (SPECT) imaging studies [2].

Recently, we have described a new approach for the delivery of radiolabeled agents to the brain for high brain uptake and prolonged retention [3]. The approach involved the attachment of a radiolabeled agent, (*p*-[^{125}I]iodoaniline) (**1**), with a 1,3-disubstituted 1,4-dihydropyridine carrier moiety through a carboxamide linkage **4** (Scheme I). The carrier apparently helps carry the radiolabeled

Scheme I



agent to the brain [3]. The dihydropyridine moiety of the radiolabeled agent **4**, undergoes oxidation in the brain to regenerate non-lipophilic **3** which is then retained. The present study was directed toward the synthesis and evaluation of the brain uptake and retention in rats of a model 1,4-disubstituted dihydropyridine analogue.

Chemistry.

The precursor, 4-(β-aminoethyl)pyridine (**5**) was first acetylated with acetic anhydride to give 4-(β-*N*-acetylaminoethyl)pyridine (**6**) [4]. Condensation of **6** with *E*-1-borono-5-iodo-1-pentene (**7**) [5] in dimethylformamide gave 1-(*E*-1-borono-1-penten-5-yl)-4-(2-*N*-acetylaminoethyl)pyridinium iodide (**8**). Iodination of **8** in the presence of chloramine-T and sodium iodide gave 1-(*E*-1-iodo-1-penten-5-yl)-4-(2-*N*-acetylaminoethyl)pyridinium iodide (**9**). The

Scheme II

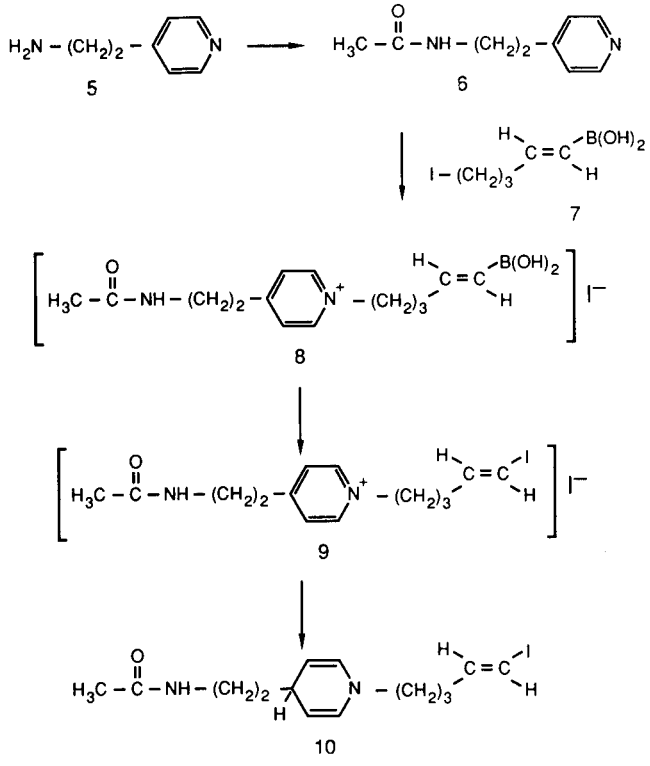


Table 1

Distribution of Radioactivity in Tissues of Fisher-344 Rats Following Intravenous Administration of 1-(*E*-[^{125}I]Iodopenten-5-yl)-4-(2-*N*-acetylaminoethyl)-1,4-dihydropyridine ([^{125}I]**10**) [a]

Time After Injection	Mean Percent Injection Dose/g (range) Tissue					
	Brain	Blood	Liver	Kidneys	Heart	Lungs
5 minutes	0.49 (0.42-0.58)	0.38 (0.34-0.42)	2.95 (2.31-3.50)	5.45 (4.19-6.53)	0.88 (0.84-0.92)	1.80 (1.52-2.02)
30 minutes	0.20 (0.16-0.23)	0.46 (0.42-0.50)	2.17 (1.95-2.32)	2.37 (2.04-2.85)	0.52 (0.43-0.59)	1.21 (1.05-1.29)
60 minutes	0.12 (0.10-0.13)	0.44 (0.39-0.48)	1.72 (1.62-1.84)	1.48 (1.35-1.60)	0.39 (0.38-0.41)	0.91 (0.81-1.05)

[a] Each animal (5 animal per time point) received 5.34 μCi of [^{125}I]**10** by tail vein injection.

1,4-disubstituted pyridinium compound **9** was found to be resistent to sodium dithionite reduction as compared to 1,3-disubstituted pyridinium compounds [3]. However, **9** was reduced smoothly using sodium borohydride under mild conditions (ethanol and argon atmosphere, room temp). The reduction of **9** under mild conditions would be expected to provide the kinetically and thermodynamically stable compound, 1-(*E*-1-iodo-1-penten-5-yl)-4-(*β*-*N*-acetylaminoethyl)-1,4-dihydropyridine (**10**) as a major product. The signals for the dihydropyridine protons of **10** in the proton nuclear magnetic resonance (nmr) (deuteriochloroform) appearing at δ 5.5 and 5.7 (m and m, H-2 and H-6), 4.0 and 4.2 (m and m, H-3 and H-5) and 3.2-3.6 (dm, H-4) were consistent with the assigned structure. In addition, as expected, the reduced compound **10** showed greater mobility (Rf value) than **9** on tlc. The radioiodinated compounds **9** and **10** were synthesized similarly using Na[^{125}I] instead of NaI during iodination (Scheme II).

Biological Studies.

The data from Table 1 show moderate (0.49% dose/g after 5 minutes) brain uptake of [^{125}I]**10** which gradually washes out from the brain. This may indicate the inability of 1,4-disubstituted reduced pyridines to be easily oxidized in the brain. In the absence of such oxidation mechanism (or in a slow oxidation process), [^{125}I]**10** will not be transformed to the non-lipophilic quaternary species (such as **9**), resulting in washout of **10**. The data from this study also suggest that the presence of a 1,4-dihydronicotinamide carrier moiety in a radiopharmaceutical (such as in **4**) may be necessary for cerebral transport and retention of such agents.

EXPERIMENTAL

General.

The iodine-125 was purchased from New England Nuclear, Inc (North Billierica, MA). The melting points (mp) were determined in capillary tubes using a Buchi SP apparatus and are uncorrected. The thin-layer chromatographic analyses (tlc) were performed using 250 μm thick layers of silica gel G PF-254 coated on glass plates (Analtech, Inc.). The proton nuclear magnetic resonance spectra (nmr) were obtained at 60 MHz with a Varian 360-L instrument. Samples (30-40 mg) were dissolved in the solvents indicated, and the resonances (ppm) are reported downfield (δ) from the internal tetramethylsilane standard. The presence of exchangeable protons was confirmed by the addition of deuterium oxide and reintegration.

Animal Tissue Distribution Studies.

The distribution of radioactivity was determined in tissues of 10-12 week old female Fischer 344 rats (170-200 g) after intravenous administration of [^{125}I]**10**. The animals were allowed food and water *ad libitum* prior to and during the course of the experiment. The radioiodinated agent was dissolved in a minimal volume of dimethyl sulfoxide (DMSO) and diluted with saline to a final concentration of 10% DMSO. The solution was filtered through a 0.22 μm Millipore and injected *via* a lateral tail vein into the ether-anesthetized animals. After the times indicated, the animals were killed by cervical fracture, and blood samples were obtained by cardiac puncture. The organs were then removed, rinsed with saline solution, and blotted dry to remove residual blood. The organs were weighed and counted in a NaI autogamma counter (Packard Instruments). Samples of the injected radioactive solutions were also assayed as standards to calculate the percent injected dose per gram of tissue values. The weight of the thyroid glands was not determined directly but was calculated in the usual manner by multiplying the animal weight by (7.5 mg/100 g).

1-(*E*-1-Borono-1-penten-5-yl)-4-(*β*-*N*-acetylaminoethyl)pyridinium iodide (**8**).

A commercial sample of **5** [4] was purified by column chromatography using silica gel (Davison) packed in chloroform. The column was eluted with methanol in chloroform (10 to 20%, v/v). The tlc was developed in 5% methanol/chloroform. The fractions containing the pure compound were collected and evaporated under vacuum. Compound **5** was first acetylated using conventional literature procedures by addition of acetic anhydride (1.008 g, 10 mmoles) to **5** (453 mg, 3.7 mmoles). The reaction mixture was stirred for 1 hour, and the progress of the reaction followed by the tlc of an aliquot, adjusted to *p*H 7 (sodium bicarbonate). The tlc indicated complete reaction with the appearance of a faster moving product. The solvent was evaporated under vacuum and the residue (syrup) was diluted with water (2 ml) and adjusted to *p*H 7 by adding aqueous sodium bicarbonate. The reaction solution was extracted with

chloroform (4 x 10 ml). The combined organic portion was dried (sodium sulfate). The material was concentrated by evaporation under vacuum and purified by column chromatography using Davison silica gel and methanol/chloroform as the eluent. The pure fractions (tlc, 10% methanol in chloroform) containing the product were collected and evaporated *in vacuo* to dryness to yield 58% of product **6** [4] as a syrup which was used for further reaction as described below.

Iodopentenylboronic acid (**7**, 500 mg, 2 mmoles) was added to a solution of **6** (358 mg, 2.2 mmoles) in dimethylformamide (5 ml) and the mixture stirred overnight in an oil bath at 50°. Acetone (10 ml) was added, and the reaction mixture stirred. The acetone layer was decanted and remaining material was triturated with fresh acetone to form a crystalline residue which was filtered, washed with acetone, and dried to give 500 mg of **8**. After concentration, the decanted acetone portion gave an additional 40 mg of **8**. Total yield was 540 mg (67%), mp 137°; nmr (DMSO-d_6): δ 8.1 and 9.1 (m and m, 2 and 2, pyridine), 7.6 (s, 2, HO-B-OH), 6.3 (s, br, 1, NH), 5.2 and 5.5 (m and m, 1 and 1, HC=CH), and other protons.

Anal. Calcd. for $C_{14}H_{22}N_2BO_3I$: C, 41.61; H, 5.49; N, 6.93; B, 2.68. Found: C, 41.45; H, 5.60; N, 6.71; B, 2.86.

1-(*E*-1-Iodo-1-penten-5-yl)-4-(β-*N*-acetylaminoethyl)pyridium Iodide (**9**).

A solution of sodium iodide (35 mg, 0.23 mmole) in water (0.5 ml) was stirred with **8** (90 mg, 0.2 mmole) at room temperature. The reaction vessel was protected from light and a solution of chloramine-T (46 mg, 0.2 mmole) in aqueous tetrahydrofuran 1:1, v/v, 1 ml) was added. The reaction mixture was stirred in the dark at room temperature for 30 minutes. The original deep orange colored reaction mixture turned nearly colorless (slightly yellow). Sodium iodide (30 mg, 0.2 mmole) and sodium metabisulfite (15 mg) were added, the solution stirred for 5 minutes and the solvent then evaporated to dryness. The residue was dissolved in methanol (2 ml) and loaded on a silica gel (Davison) column packed in chloroform. The column was eluted with chloroform followed by methanol in chloroform (20%, v/v) which gave **9** (90 mg, 97%) in the pure form, mp > 150° slow dec; nmr (perdeuteriomethanol): δ 8.3 and 9.2 (d and d, 2 and 2, J = 7 Hz, pyridine), 6.3-6.8 (m, 2, HC=CH) and other protons.

Anal. Calcd. for $C_{14}H_{20}N_2OI_2$: C, 34.59; H, 4.15; N, 5.76; I, 52.21. Found: C, 34.61; H, 4.30; N, 5.48; I, 51.94.

1-(*E*-1-Iodo-1-penten-5-yl)-4-(β-*N*-acetylaminoethyl)-1,4-dihydropyridine (**10**).

A solution of iodide **9** (96 mg, 0.2 mmole) in ethanol (2 ml) was added slowly to a stirred suspension of sodium bicarbonate (64 mg) followed by addition of sodium borohydride (10 mg). Argon was continuously bubbled through the reaction mixture. After 30 minutes of continued stirring under argon, chloroform (10 ml) was added followed by the addition of water (10 ml). The chloroform layer was separated, dried (sodium sulfate) and evaporated under argon to provide a syrup containing a major product and two minor impurities. The major product was separated by column chromatography. The column was packed with a silica gel slurry in chloroform and eluted with 5% methanol in chloroform. The fractions containing major product were evaporated to yield **10** (22 mg, 30%) as a syrup.

Anal. Calcd. for $C_{14}H_{21}N_2OI$: C, 46.67; H, 5.88; N, 7.78. Found: C, 46.86, H, 6.20; N, 7.53.

Radiochemistry.

Synthesis of 1-(*E*-[^{125}I]Iodopenten-5-yl)-4-(β-*N*-acetylaminoethyl)-1,4-dihydropyridine ([^{125}I]**10**).

A solution of chloramine-T (11 mg, 0.05 mmole) in methanol (1 ml) was added in the dark to a stirred solution of pyridinium boronic acid **8** (20 mg, 0.05 mmole), sodium iodide (7.5 mg, 0.05 mmole) and Na[^{125}I] (5 mCi). The solution was stirred continuously for an additional 30 minutes in the dark at room temperature. Sodium iodide (30 mg) and sodium metabisulfite (5 mg) were added with stirring. The solvent was reduced (argon) to approximately 1 ml and applied to a silica gel (Davison) column packed in chloroform. The column was eluted with chloroform (fractions 1 to 5, 20 ml each) 10% methanol in chloroform (fractions 6 to 10, 20 ml each) and 20% methanol in chloroform (fractions 11-19, 20 ml each). Fractions 12-16 on evaporation under argon gave 4.04 mCi (80% radiochemical yield) of pure [^{125}I]**9**. Under argon atmosphere, a solution of [^{125}I]**9** (1.0 mCi) in ethanol (1 ml) was added to a stirred suspension of sodium bicarbonate (32 mg) and sodium dithionite (39 mg) followed by the addition of sodium borohydride (5 mg) in ethanol (1 ml) through which argon was bubbled continuously. Water was added dropwise until a clear solution was obtained. After 15 minutes, chloroform (10 ml) was added, followed by the addition of water (10 ml) and a drop of vitamin E (antioxident). The chloroform layer was separated, dried (sodium sulfate) and evaporated under argon. Purification using column chromatography as described for the corresponding unlabeled standard **10** gave 250 μCi (25% radiochemical yield) of the radioactive compound [^{125}I]**10**. The tlc of radioactive compounds [^{125}I]**9** and [^{125}I]**10** exhibited single peaks on scanning and mobilities identical to respective unlabeled standards.

Acknowledgements.

This research was sponsored by the Office of Health and Environmental Research, U. S. Department of Energy under contract DE-AC05-840R21400 with Martin Marietta Energy Systems, Inc. The authors thank E. B. Cunningham and A. P. Callahan for technical assistance and L. S. Ailey for typing the manuscript.

REFERENCES AND NOTES

* To whom the correspondence should be addressed.

† Oak Ridge National Laboratory Teachers Summer Field Experience Participant 1985.

[1] W. H. Oldendorf, *Proc. Soc. Exp. Biol. Med.*, **19**, 1182 (1974).

[2] T. F. Budinger, *J. Nucl. Med.*, **21**, 279 (1980).

[3] M. L. Tedjamulia, P. C. Srivastava, and F. F. Knapp, Jr., *J. Med. Chem.*, **28**, 1574 (1985).

[4] G. Magnus and R. Levine, *J. Am. Chem. Soc.*, **78**, 2147 (1956). A sample of 4-(β-aminoethyl)pyridine for this research was kindly supplied by Reilly Tar and Chemical Corporation, Indianapolis, Indiana 46204.

[5] P. C. Srivastava, A. P. Callahan, E. B. Cunningham and F. F. Knapp, Jr., *J. Med. Chem.*, **26**, 742 (1983).