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Synthesis of a Radiotracer for Studying Serotonin Uptake Sites with Positron Emission Tomography: [^{11}C]McN-5652-Z

Makiko Suehiro, Hayden T. Ravert, Robert F. Dannals*, Ursula Scheffel, and Henry N. Wagner, Jr.

Divisions of Nuclear Medicine and Radiation Health Sciences
The Johns Hopkins Medical Institutions
615 North Wolfe Street
Baltimore, Maryland 21205-2179, U.S.A.

Summary

The highly potent serotonin (5-HT) uptake blocker, McN-5652-Z (*trans*-1,2,3,5,6,10b - hexahydro - 6 - [4 - (methylthio)phenyl] pyrrolo - [2,1-a]-isoquinoline) was labeled with ^{11}C for studying serotonin uptake sites using positron emission tomography (PET). [^{11}C]McN-5652-Z was synthesized by S-methylation of the normethyl precursor with [^{11}C]iodomethane in DMF at 30 - 35° C. The radiosyntheses including purification by HPLC and formulation for injection were completed in an average of 16 minutes following the end of bombardment (E.O.B.) with an overall radiochemical yield of 12 %. The average specific activity determined at the end of synthesis (E.O.S.) was approximately 4250 mCi/μmole; this corresponds to approximately 7350 mCi/μmole at E.O.B. [^{11}C]McN-5655-Z, a less potent blocker, was also prepared by the same procedure.

Key Words: radiotracer, synthesis, serotonin uptake, carbon-11, positron emission tomography

Introduction

A selective PET radiotracer for the presynaptic element of serotonin (5-HT) neurons has yet to be developed. Once available, such a radiotracer should greatly facilitate the study of 5-HT function in the living human brain. It should also permit detection of subclinical 5-HT neural damage in human populations considered at risk, e.g. individuals exposed to neurotoxic drugs such as 3,4-

* Author for correspondence

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methylenedioxymethamphetamine (MDMA) or fenfluramine (1 - 4). Although a number of 5-HT uptake blockers, such as imipramine, cyanoimipramine, citalopram or fluoxetine, have been labeled with carbon-11 or fluorine-18 (5 - 14), none of these ligands studied to date has been suitable for imaging the 5-HT transporter in human brain by PET.

Recently, McN-5652-Z (*trans* - 1,2,3,5,6,10b - hexahydro - 6 - [4-(methylthio)phenyl]pyrrolo[2,1-a]isoquinoline) has been described as a highly potent 5-HT uptake inhibitor *in vitro* and *ex vivo* (15, 16). This compound displayed an *in vitro* K_i value of 0.60 nM for [^{3}H]5-HT uptake inhibition in rat cerebral cortex. Pharmacological data demonstrate that McN-5652-Z is a faster potentiator of L-5-hydroxytryptophan-induced syndromes than other potent 5-HT blockers such as paroxetine, sertraline, or citalopram. These data indicate that McN-5652-Z labeled with ^{11}C may be a good radioligand for studying serotonin uptake sites by PET.

This paper describes the radiosynthesis, purification, and quality control analysis of [^{11}C]McN-5652-Z from its normethyl precursor.

Results and Discussion

[^{11}C]McN-5652-Z was synthesized by S-methylation of the normethyl precursor with [^{11}C]iodomethane (Figure 1).

The precursor synthesis by demethylation of the parent compound, McN-5652-Z, was successfully carried out with sodium thiomethoxide (17) (Figure 1). The reaction was fast and efficient. With a 5 fold excess of sodium thiomethoxide, 95 - 99 % demethylation was observed in 1 hour.

Cleavage of thioethers occurs much less readily than ethers. In fact, methyl aryl sulfides, such as thioanisole or McN-5652-Z, are stable under the conditions that cleave dialkyl sulfides or benzyl, triphenylmethyl, and diphenylmethyl aryl sulfides (18). Gilman et al. studied 14 conditions with 9 agents for cleavage of thioanisole and obtained yields of only 2 - 23% (19). Testaferri showed an alternative route to cleave alkyl-sulfur bonds in alkyl aryl sulfides using sodium in N,N-dimethylacetamide (DMA) heated to 100° C. They reported 85 - 90% dealkylation in 15 hours (17). The sodium-DMA reaction was attempted with McN-5652-Z. However, the thiophenol product was not stable (unpublished observation). By contrast, the cleavage with sodium thiomethoxide was fast enough to obtain the active thiophenol. Further derivatization such as S-benzylation or S-triphenylmethylation was unnecessary. Although the precursor for [^{11}C]McN-5652-Z was active and fairly unstable at room temperature, it could be stored at 4° C under nitrogen for several days. An advantage of starting with a thiophenol as precursor of methylation such as the case of synthesis of [^{11}C]McN-5652-Z is that thiophenols readily methylate with reagents such as iodomethane.

During the demethylation reaction in DMF at 160° C, a conformational change took place and an equilibrium was reached between McN-5652-Z and McN-5655-Z, the cis isomer, in a ratio of approximately 40:60. Consequently, normethyl

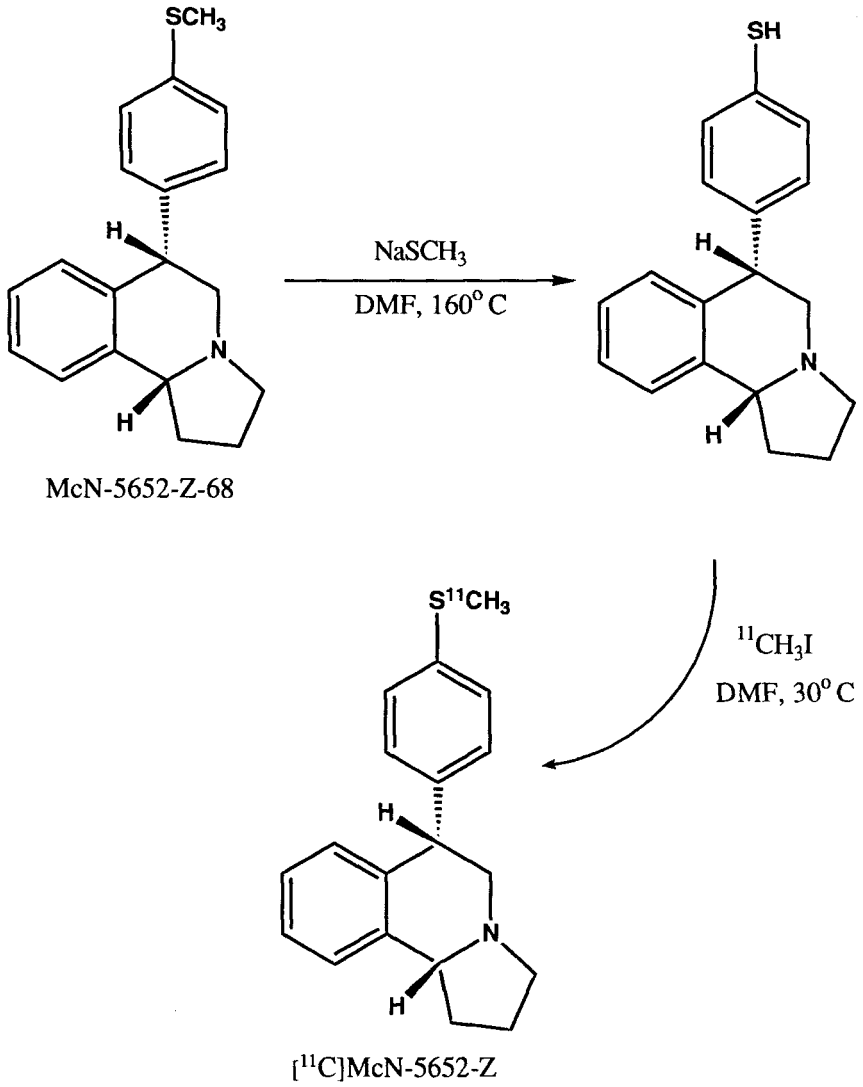

Figure 1. Synthesis of [^{11}C]McN-5652-Z

precursors with trans and cis conformation resulted (Figure 2). The conformational isomers eluted from the C-18 HPLC column at different retention times; 4.9 minutes (k' = 4.4) for the trans isomer and 5.7 minutes (k' = 5.3) for the cis isomer. The precursors were also separable from the parent compounds, McN-5652-Z and McN-5655-Z eluting at 7.7 (k' = 7.3) and 9.5 (k' = 9.4) minutes, respectively (Figure 2). The conformationally mixed precursors were readily methylated with iodomethane yielding McN-5652-Z and McN-5655-Z, respectively. After methylation of the precursors with iodomethane, the products were characterized. According to the N.M.R. and mass spectral data, the products were determined to be identical to the authentic McN-5652-Z and McN-5655-Z.

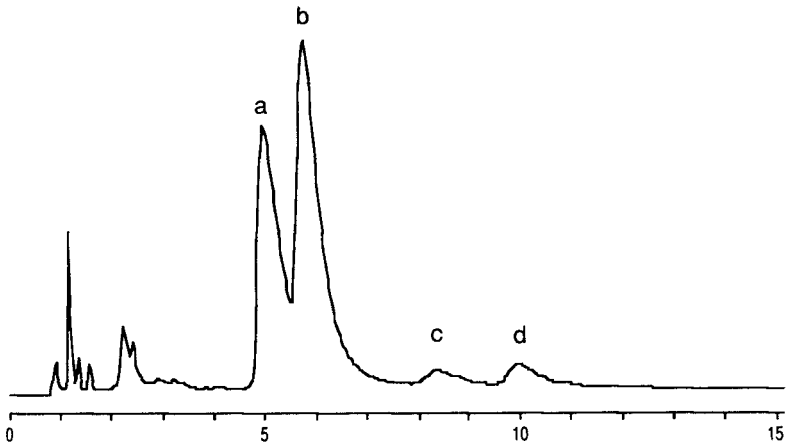

Figure 2. HPLC purification of normethyl McN-5652-Z (a: normethyl McN-5652-Z, b: normethyl McN-5655-Z, c: McN-5652-Z, and d: McN-5655-Z)

The thermodynamic equilibration between cis and trans conformation of 6-pyrrolo[2,1-a]isoquinoline derivatives has been well studied by Maryanoff et al (20). The equilibration occurs under hot, basic conditions. For example, one of the amines tested, 1,2,3,5,6,10b-hexahydro-6-phenylpyrrolo[2,1-a]isoquinoline, has a similar chemical structure of McN-5652-Z. It was reported that this amine reached an equilibrium with a cis to trans ratio of 64:36 after heating at 120° C in DMSO containing sodium hydroxide.

The equilibration was reached from either diastereomer. Thus, starting with McN-5655-Z, a similar conformational change took place during demethylation at 160° C, resulting in a mixture of normethyl McN-5655-Z and normethyl McN-5652-Z conformation in a ratio of approximately 60:40 (Figure 2). A similar equilibration between McN-5655-Z and McN-5652-Z was observed in a N,N-dimethylacetamide solution containing sodium, heated at 100° C (unpublished observation). Taking advantage of this thermodynamic equilibrium between cis and trans isomers, the normethyl precursors for the synthesis of both [^{11}C]McN-5655-Z and [^{11}C]McN-5652-Z were available after demethylation of either McN-5655 or McN-5652-Z.

In the radiosynthesis of [^{11}C]McN-5652-Z, the normethyl precursor reacted quickly with [^{11}C]iodomethane at 30 - 35° C, yielding [^{11}C]McN-5652-Z in an overall yield of 12% (decay corrected to E.O.B.). At higher temperatures, such as 80° C, the yield decreased. Presumably the methylation also occurs competitively at the nitrogen of McN-5652 at high temperature. The purification of [^{11}C]McN-5652-Z was successfully performed on a semipreparative C-18 HPLC column (Figure 3). [^{11}C]McN-5652-Z eluted at approximately 6.6 minutes (k'= 4.5) while the precursor and [^{11}C]iodomethane eluted at 4.9 (k' = 3.1) and 3.5 (k' = 2.0) minutes, respectively. Since the normethyl precursor with cis conformation had been separated previously, no production of [^{11}C]McN-5655-Z occurred. The

radiosynthesis including HPLC purification and formulation were completed in an average of 16 minutes following E.O.B. The specific activity determined at E.O.S. was approximately 4250 mCi/μmole; this corresponds to approximately 7350 mCi/μmole at E.O.B.

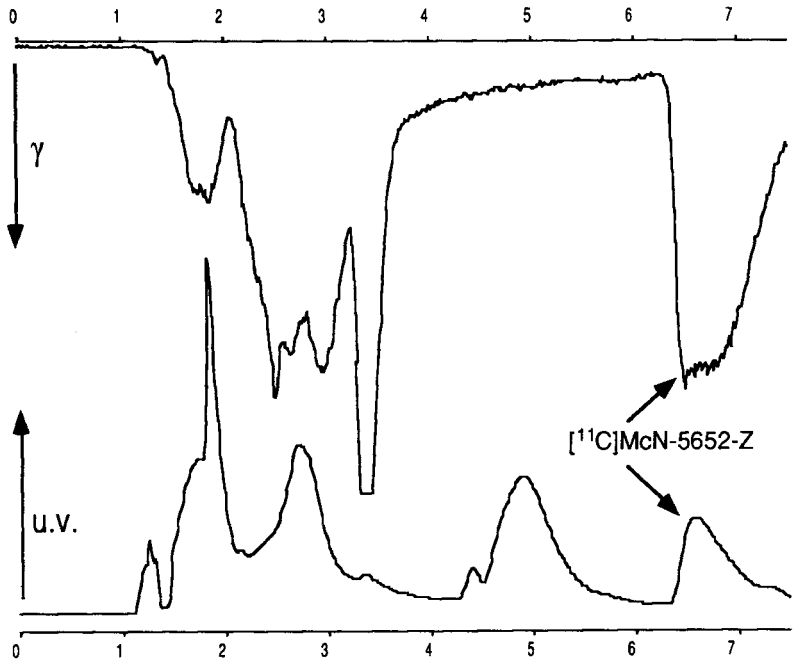

Figure 3. Semipreparative HPLC purification of [^{11}C]McN-5652-Z

[^{11}C]McN-5655-Z was also synthesized by the same procedure with a precursor of cis conformation. The average radiochemical yield was 11 % (decay corrected to E.O.B.) and the average specific activity was approximately 4460 mCi/μmole at E.O.S.

In initial biodistribution studies in rodents, [^{11}C]McN-5652-Z showed promising properties as a radiotracer for *in vivo* labeling of 5-HT uptake sites in mouse brain (21). The target-to-non-target ratio of 4.6:1 reached with this radiotracer at 90 minutes in the hypothalamus, a region of high density of 5-HT uptake sites, is the highest ratio reported to date.

Experimental

Authentic McN-5652-Z (trans isomer) and McN-5655-Z (cis isomer) were gratefully provided as gifts from Dr. Bruce E. Maryanoff of The R. W. Johnson Pharmaceutical Research Institute, Spring House, PA, USA. ^{1}H-N.M.R. spectra were obtained on a Bruker WN 300/wb using $(CH_3)_4Si$ as an internal standard. Mass spectrometric analyses were performed by Mass Spectrometry Service Laboratory, University of Minnesota.

Synthesis of the precursor of [^{11}C]McN-5652-Z

The normethyl precursor was synthesized by demethylation of McN-5652-Z with sodium thiomethoxide based on the procedure described by Testaterri et al. (17) (Figure 1). To a 100 µL dry DMF solution containing a 5 fold excess of sodium thiomethoxide, 10 to 15 mg of McN-5652-Z (free base) dissolved in 100 µL DMF was added. The reaction mixture was heated to 160° C for 1 hour, cooled, and 100 µL of 1N HCl was added. The reaction mixture was diluted with 200 µL HPLC solvent of acetonitrile and water (50:50) containing 0.1 M ammonium formate, and applied to a semipreparative reversed phase C-18 HPLC column (Alltech, 25 cm x 10 mm i.d.). Elution was performed with the above-mentioned solvent at a flow rate of 10 mL/min (Waters Associates Model 590 EF pump). The effluent was monitored with a u.v. detector at 254 nm. The precursor was collected (4.9 minutes; k' = 4.4) and extracted from the HPLC solvent with dichloromethane. After evaporation of the solvent, the precursor was stored under nitrogen at 4° C until needed in the radiosynthesis. The identification of the precursor was confirmed by N.M.R. spectroscopy and mass spectrometry. The identification of the precursor was also confirmed by converting it back into McN-5652-Z with cold iodomethane using the procedure described below for the radiosynthesis. The methylated product was identical by comparison of its N.M.R. spectrum with that of the authentic McN-5652-Z and by mass spectrometry.

trans-1,2,3,5,6,10b-hexahydro-6-[4-thiophenolyl]pyrrolo[2,1-a]isoquinoline: ^{1}H N.M.R. ($CDCl_3$) 0.85-3.4 (m, 8H; aliph), 1.29 (s, 1H, SH), 3.0 (dd, H_6), 4.1 (dd, H_{10b}), 6.9-7.3 (m,8H; arom); m/e 281

cis-1,2,3,5,6,10b-hexahydro-6-[4-thiophenolyl]pyrrolo[2,1-a]isoquinoline: ^{1}H N.M.R. ($CDCl_3$) 0.85-3.3 (m 8H; aliph), 1.26 (s, 1H, SH), 3.1 (dd, H_6), 4.3 (dd, H_{10b}), 6.8-7.4 (m, 8H; arom); m/e 281

Radiosynthesis of [^{11}C]McN-5652-Z

[^{11}C]McN-5652-Z was synthesized by S-methylation of the normethyl precursor with [^{11}C]iodomethane (Figure 1). [^{11}C]Iodomethane was synthesized by a procedure previously described (22). The [^{11}C]iodomethane was introduced in a stream of argon into a cooled 200 µL DMF solution containing 2 - 3 mg of the precursor sealed in a 1 mL glass vial. When the level of radioactivity reached a maximum, the carrier gas flow stopped and the S-methylation reaction was started by submerging the reaction vial in a 30 - 35° C water bath. After 1 minute, the reaction mixture was diluted with 200 µL HPLC solvent of acetonitrile and water (60:40) containing 0.1 M ammonium formate, and applied to a semipreparative C-18 column (Alltech, 25 cm x 10 mm i.d.).

HPLC separation of the product was carried out using the above-mentioned mobile phase at a flow of 10 mL/min. The effluent was monitored with a u.v. detector at 254 nm and a radioactivity detector (Ortec 449 ratemeter, 575 amplifier, 550 single channel analyzer, with a NaI(Tl) crystal). The eluant with a ^{11}C radioactivity peak corresponding to the retention time (6.6 minutes; k'= 4.5) of the

authentic McN-5652-Z was collected in a flask and the solvent was evaporated by a rotary evaporator. The residue was dissolved in sterile normal saline (7 mL) and filtered through a sterile 0.22 μm filter (Gelman Acrodisc) into a sterile evacuated vial containing sterile sodium bicarbonate solution (3 mL, 8.4%).

After formulation of the [^{11}C]McN-5652-Z solution, the specific activity was determined according to Dannals et al. (12). An aliquot of the final solution of known volume was applied to an analytical reversed phase HPLC column (Alltech C-18, 25 cm x 4.6 mm i.d.) eluted with a mobile phase of acetonitrile and water (60:40) containing 0.1 M ammonium formate. The mass of the [^{11}C]McN-5652-Z was determined by comparing the u.v. absorption of the [^{11}C]McN-5652-Z solution to that of a standard McN-5652-Z solution of a known concentration. The specific activity at the end of synthesis (E.O.S.) was calculated by dividing the radioactivity of the final solution by the mass of [^{11}C]McN-5652-Z. The specific activity determined at E.O.S. was approximately 4250 mCi/μmole. At the same time, the radiochemical and chemical purity was also determined.

Conclusion

The highly potent 5-HT uptake inhibitor, McN-5652-Z, can be labeled efficiently with ^{11}C by S-methylation of its normethyl precursor with high specific activity. The radiotracer may be useful as an *in vivo* ligand for studying 5-HT neurons in living human brain with PET.

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References

1. Battaglia G., Yeh S.Y., O'Hearn E., Molliver M.E., Kuhar M.J., and De Souza E.B. - J. Pharmacol. Exp. Ther. 241: 911-916 (1987).
2. Ricaurte G.A., DeLanney L.E., Irwin I., and Langston J.W. - Brain Res. 446: 165-168 (1988).
3. Ricaurte G.A., Molliver M.E., Martello M.B., Katz J.L., Wilson M.A., Martello A.L. - Lancet 338: 1487-1488 (1991).
4. Scheffel U. and Ricaurte G.A. - Brain Res. 527: 89-95 (1990).
5. Mazière M., Berger G., and Comar D. - J. Radioanal. Chem. 45: 453-457 (1978).

6. Berger G., Mazière M., Knipper R., Prenant C., and Comar D. - Int. J. Appl. Radiat. Isot. 30: 393-400 (1979).
7. Hashimoto K., Inoue O., Suzuki K., Yamasaki T., and Kojima M. - Nucl. Med. Biol. 14: 587-592 (1987).
8. Kilbourn M.R., Haka M.S., Mulholland G.K., Jewett D.M., and Kuhl D.E. - J. Label. Compd. Radiopharm. 26: 412-414 (1989).
9. Scheffel U., Dannals R.F., Suehiro M., Wilson A.A., Ravert H.T., Stathis M., and Wagner H.N. Jr. - J. Nucl. Med. 31: 883-884 (1990).
10. Hume S.P., Pascali C., Pike V.W., Turton D.R., Ashier R.G., Meyer R., Bateman D.M., Cremer J.E., Manjil L.G., and Dolan R. - Nucl. Med. Biol. 18: 339-351 (1991).
11. Lasne M.C., Pike V.W., and Turton D.R. - Appl. Radiat. Isot. 40: 147-152 (1989).
12. Dannals R.F., Ravert H.T., Wilson A.A., and Wagner H.N. Jr. - Int. J. Radiat. Appl. Instrum. Part A 31: 541-543 (1990).
13. Suehiro M., Wilson A.A., Scheffel U., Dannals R.F., Ravert H.T., and Wagner H.N. Jr. - Nucl. Med. Biol. 18: 791-796 (1991).
14. Suehiro M., Scheffel U., Dannals R.F., Wilson A.A., Ravert H.T., and Wagner H.N. Jr. - Nucl. Med. Biol. 19: in press (1992).
15. Maryanoff B.E., McComsey D.F., Gardocki J.F., Shank R.P., Costanzo M.J., Nortey S.O., Schneider C.R., and Setler P.E. - J. Med. Chem. 30: 1433-1454 (1987).
16. Shank R.P., Vaught J.L., Pelley K.A., Setler P.E., McComsey D.F., and Maryanoff B.E. - J. Pharm. Exp. Ther. 247: 1032-1038 (1987).
17. Testaterri L., Tiecco M., Tingoli M., Chianelli D., and Montanuui M. - Synthesis 1983: 751-755 (1983).
18. Tarbell D.S. and Harnish D.P. - J. Am. Chem. Soc. 74: 1862-1863 (1952).
19. Gilman H. and Webb F.J. - J. Am. Chem. Soc. 71: 4062-4066 (1949).
20. Maryanoff B.E., McComsey D.F., and Duhl-Emswiler B.A. - J. Org. Chem. 48: 5062-5074 (1983).
21. Suehiro M., Scheffel U., Dannals R.F., Ravert H.T., and Wagner H.N. Jr. - 39th Annual Meeting of the Society of Nuclear Medicine, Los Angeles, CA. J. Nucl. Med. 33: in press (1992).
22. Dannals R.F., Ravert H.T., Wilson A.A., and Wagner H.N. Jr. - Int. J. Appl. Radiat. Isot. 37: 433-434 (1986).