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Synthesis of a Radiotracer for Studying Nicotinic Acetylcholine Receptors: 2-[^{18}F]fluoro-3-(2(*S*)-azetidinylmethoxy)pyridine (2-[^{18}F]A-85380)

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Summary

The radiochemical synthesis of 2-[^{18}F]fluoro-3-(2(*S*)-azetidinylmethoxy)pyridine (2-[^{18}F]A-85380, [^{18}F]**1**) was accomplished by Kryptofix® 222 assisted nucleophilic no-carrier-added [^{18}F]fluorination of 2-iodo-3-((1-*tert*-butoxycarbonyl-2(*S*)-azetidinyl)methoxy)pyridine, **2** followed by acidic deprotection. The average radiochemical yield was 10% and the average specific radioactivity was 1050 mCi/μmol, calculated at end-of-synthesis (EOS).

Key Words: radiotracer, A-85380, ^{18}F, fluorination, halogen-exchange, nicotinic receptors, acetylcholine, positron emission tomography.

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Introduction

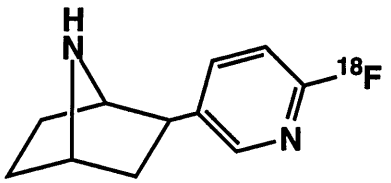
Significant interest has been displayed in non-invasive imaging of central nicotinic acetylcholine receptors (nAChRs) in recent years. Mediation of excitatory neurotransmission by nAChRs promotes a variety of central processes such as learning and memory (1-4). Post mortem autoradiographic studies in Alzheimer's disease have shown reduced concentration of nAChRs in the neocortex and hippocampus (5,6). These receptors also mediate the action of nicotine, the active ingredient that promotes dependence on tobacco (7).

To date much of the mapping and quantification of central nAChRs by PET has been done with ^{11}C-nicotine, a radiotracer which shows high non-specific binding, rapid clearance from the brain, as well as rapid metabolism (8,9).

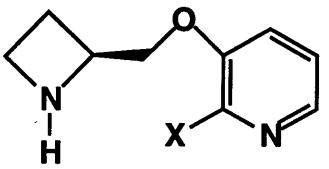
In 1996, the (+/-)-exo-2-(2-[^{18}F]fluoro-5-pyridyl-7-azabiocyclo[2.2.1]heptane ([^{18}F]FPH, Figure 1), an analog of the powerful nAChR agonist, epibatidine, was synthesized (10). [^{18}F]FPH has shown promise as a ligand with favorable brain uptake and kinetics for nAChRs by PET (11-15). However, FPH exhibits a profound toxic effect (16,17). Recently studies have concentrated on the synthesis of less toxic epibatidine-based tracers (16, 18). However, most of the analogs studied to date demonstrate a substantial portion of the parent ligand's toxic effect and this remains an impediment to the widespread use of epibatidine analogs in PET studies.

A recent series of 3-pyridyl ethers with subnanomolar affinity for the major $\alpha_4\beta_2$ subtype of central nAChRs were synthesized by Abbott Laboratories (19). The most potent compounds of this published series, A-85380 and A-84543, have been labeled with ^{3}H and ^{11}C, respectively (20, 21). [^{3}H]A-85380 was shown to be an excellent nAChR probe for *in vitro* studies (20) while [^{11}C]A-84543 has recently been studied in mice (22). A-85380 (Figure 1) was equipotent to epibatidine in binding assays but only 1/40 as potent as epibatidine in activating the $\alpha_4\beta_2$ subtype of nAChRs (19) suggesting a relatively low toxic effect of A-85380-based ligands while retaining a high potency toward nAChRs.

In this paper we describe the synthesis of 2-fluoro-3-(2(*S*)-azetidinylmethoxy)pyridine, 2-fluoro-A-85380 ([^{19}F]**1**, Figure 1), and the radiosynthesis of 2-[^{18}F]fluoro-3-(2(*S*)-azetidinylmethoxy)pyridine (2-[^{18}F]A-85380, [^{18}F]**1**, Figure 1) as a potential nAChR tracer for use with PET.



[18F]FPH



X=H, A-85380
X=^{19}F, 2-[^{19}F]fluoro-A-85380, [^{19}F]1
X=^{18}F, 2-[^{18}F]fluoro-A-85380, [^{18}F]1

Figure 1. nAChR ligands

Results and Discussion

Compounds **2** and **3** were prepared via a Mitsunobu coupling of the corresponding 2-halo-3-pyridinols with 2(*S*)-azetidinylmethanol. The target ligand [^{19}F]**1** was obtained by subsequent acidic deprotection of **3** using trifluoroacetic acid (Figure 2). In preliminary binding assays (23), [^{19}F]**1** shows high binding affinity for nAChRs (K_i = 80 pM vs. [^{3}H]epibatidine in rat brain tissue).

The radiosynthesis of [^{18}F]**1** was accomplished by the no-carrier-added nucleophilic halogen-exchange radiofluorination of the N-BOC-protected 2-iodo-3-(2(*S*)-azetidinylmethoxy)pyridine, **2** , followed by deprotection (Figure 3).

Attempts to perform a one step radiofluorination based on the preparation of another [^{18}F]fluoropyridine derivative, [^{18}F]FPH (10), using Kryptofix 222 and elevated temperature on 2-iodo-3-(2(*S*)-azetidinylmethoxy)pyridine failed due to thermal unstability of [^{18}F]**1** (data not presented). However Kryptofix 222 assisted radiofluorination of the BOC protected azetidine derivative, 2-iodo-3-(1-*tert*-butoxycarbonyl-2(*S*)-azetidinylmethoxy)pyridine **2**, in DMSO at 190° C yielded the corresponding 2-[^{18}F]fluoro-3-(1-*tert*-butoxycarbonyl-2(*S*)-azetidinylmethoxy) pyridine [^{18}F]**3** in 40% radiochemical yield after HPLC purification (Figure 2). Acidic deprotection of the intermediate **3** in a solution of trifluoroacetic acid gave the desired radiotracer [^{18}F]**1** with a radiochemical yield of 10% (calculated at EOS). The average time of synthesis was 120 minutes and has not been optimized in this study.

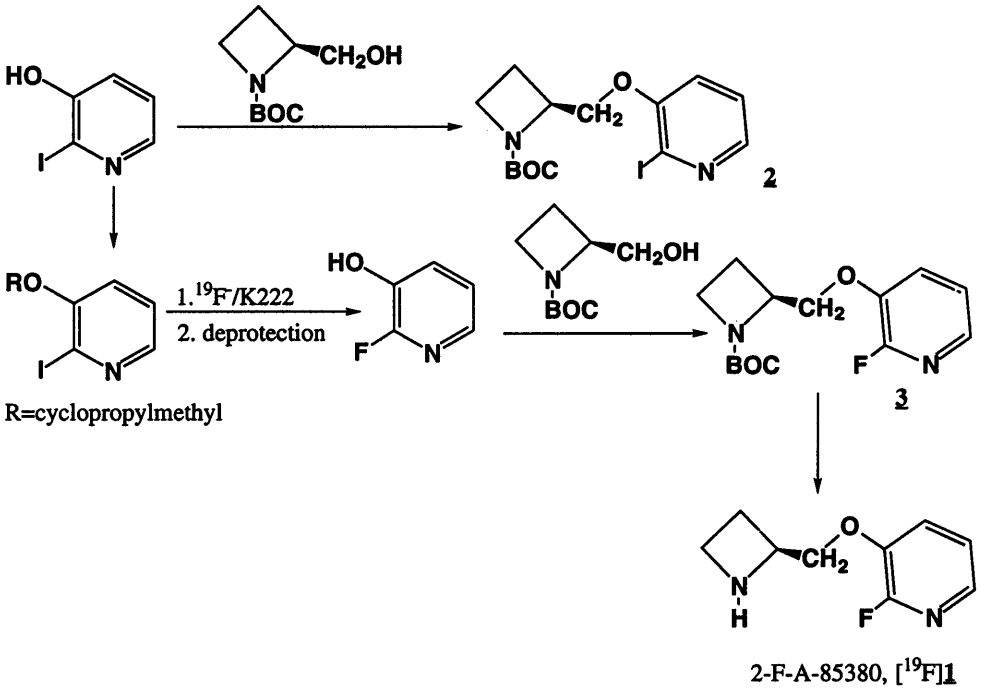


Figure 2 Synthesis of 2-fluoro-A-85380, [^{19}F]1 and 2-iodo-3-(1-*tert*-butoxycarbonyl-2(*S*)-azetidinylmethoxy)pyridine, 2

In a one-pot reaction, when the intermediate product [^{18}F]3 was deprotected without its isolation, the final radiolabeled compound [^{18}F]1 was contaminated with coeluting non-radioactive, unidentified by-products. However, when the

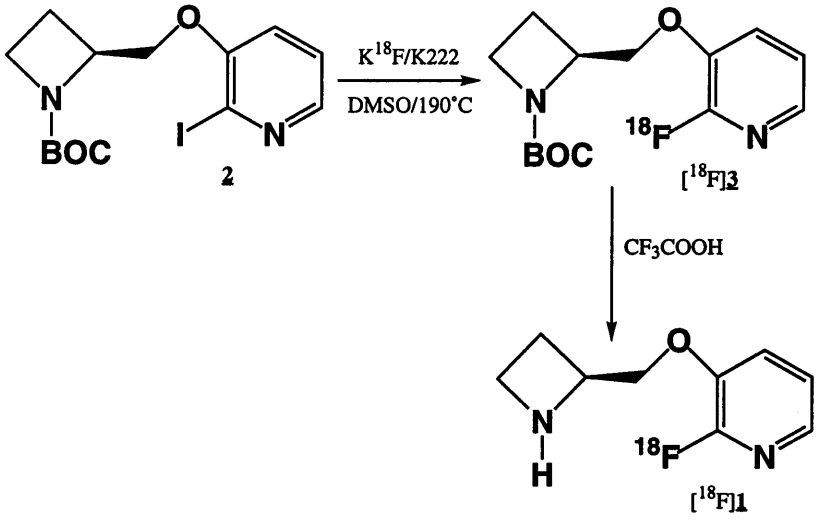


Figure 3. Radiochemical synthesis of 2-[^{18}F]fluoro-A-85380, [^{18}F]1

intermediate product [^{18}F]**3** was purified by semipreparative HPLC followed by deprotection, [^{18}F]**1** was obtained free from the non-labeled contaminants. [^{18}F]**1** prepared by this method was shown to be radiochemically pure (>99% by radio HPLC), and coeluted with the authentic standard. The only detectable HPLC peak on the UV chromatogram corresponded to the carrier [^{19}F]**1**. The final product was free of the precursor, **2** and 2-iodo-3-(2(*S*)-azetidinylmethoxy)pyridine. The average specific radioactivity of the final product prepared from 120 - 150 mCi of starting [^{18}F]fluoride was 1050 mCi/μmol (EOS).

Conclusion

In summary, a simple radiosynthesis of a high specific activity radiofluorinated ligand for studying nAChRs has been developed. The synthesis yields sufficient quantities of chemically and radiochemically pure ligand for use in *in vivo* PET studies.

Experimental

All reagents used were ACS or HPLC grade and were purchased from Aldrich. ^{1}H-NMR spectra were recorded on a Bruker AM 300 (300 MHz); chemical shifts (δ) were recorded in parts per million (ppm) downfield from TMS. High resolution mass-spectra was recorded on an AEI MS-30 (University of Minnesota Mass Spectra Laboratory). High performance liquid chromatographic analysis and purification were performed with two Waters 590EF HPLC pumps, an in-line fixed wavelength (254 nm) detector (Waters Associates), and a single two inch NaI crystal radioactive detector (Ortec). HPLC chromatograms were recorded by a Rainin Dynamax dual channel control/interface module connected to a Macintosh computer with appropriate program software (Dynamax - version 1.3). HPLC semipreparative purifications and all HPLC analyses were completed on a Hamilton PRP-1 columns, 10 μm (7 x 305 mm). Flash chromatography was performed on silica gel (Merck, grade 9385, 230-400 mesh). TLC analysis was performed on Macherey-Nagel Sil G/UV254 plates (Alltech). Melting point was determined in an open glass capillary by Mel-Temp apparatus. A dose calibrator (Capintec CRC-12R) was used for all radioactivity measurements.

2-iodo-3-(1-tert-butoxycarbonyl-2(S)-azetidinylmethoxy)pyridine, 2

Neat diethyl azodicarboxylate (1.219 g, 7 mmol) was added dropwise to a solution of triphenylphosphine (1.836 g, 7 mmol) in anhydrous THF (20 mL) at 0 °C, and the mixture was stirred at the same temperature for 1 h. A solution of (1-*tert*-butoxycarbonyl-2(*S*)-azetidinyl)methanol (19) (0.873 g, 4.67 mmol) in anhydrous THF (5 mL) was added dropwise at 0 °C. 2-Iodo-3-hydroxypyridine (1.445 g, 6.54 mmol) was added in one portion. The reaction mixture was allowed to warm gradually to room temperature and left to stand for 60 h. The solvent was removed under reduced pressure, the residue was dissolved in CH_2Cl_2 (50 mL) and washed with saturated $NaHCO_3$ (25 mL) and water (3 × 15 mL). The organic solution was dried over anhydrous $MgSO_4$ and evaporated to yield a light yellow oil. The crude compound was purified by flash chromatography (hexane-ethyl acetate, 60:40) to afford 1.6 g (88%) of the desired product as a colorless oil. TLC R_f = 0.23 (hexane-ethyl acetate, 67:33); MS, *m/z* (rel. intensity), $[M+H]^+$ 391.0537 (1%), calcd. for $C_{14}H_{19}IN_2O_3$: M 390.0440; ^1H NMR ($CDCl_3$) δ 1.41 (s, 9H), 2.35-2.52 (m, 2H), 4.07 (m, 2H), 4.17 m (1H), 4.54 (m, 2H), 7.06 (dd, J = 1.4, 8.1 Hz, 1H), 7.18 (dd, J = 4.6, 8.1 Hz, 1H), 8.01 (dd, J = 1.4, 4.6 Hz, 1H).

2-Fluoro-3-((1-tert-butoxycarbonyl-2(S)-azetidinyl)methoxy)pyridine, 3

A mixture of 1,3-dicyclohexylcarbodiimide (2.06 g, 10 mmol), copper (I) chloride (20 mg) and cyclopropanemethanol (0.79 g, 11 mmol) was stirred under argon at 60° C for 6 h. The reaction mixture was then diluted with anhydrous benzene (20 mL) and 2-iodo-3-hydroxypyridine (2.21 g, 10 mmol) was added. This mixture was heated to reflux while stirring for 24 h. After the formed precipitate had been filtered off, the solvent was removed under reduced pressure, and the residue was extracted with hexane-ethyl acetate (80:20) (4 x 20 mL). The combined extracts were washed with 20 mL portions of saturated $NaHCO_3$, 10% $Na_2S_2O_3$, and water. The organic layer was then dried over anhydrous $MgSO_4$, and the solvent was removed. The resulting crude material was purified by flash chromatography (hexane-ethyl acetate, 75:25) to give 1.13 g (41%) of 2-iodo-3-cyclopropylmethoxypyridine as a colorless liquid. The liquid was dissolved in anhydrous DMSO (80 mL) and Kryptofix® 222 (1.506 g, 4 mmol) and anhydrous KF (11.6 g, 200 mmol) were added. The resulting mixture was stirred under argon at 160° C for 24 h. The reaction mixture was diluted with water (160 mL) and

extracted with CCl_4 (3 x 40 mL). The combined extracts were washed with water (3 x 20 mL), dried over anhydrous $MgSO_4$ overnight, and the solvent was removed under reduced pressure at 30° C. The resulting crude material was purified by flash chromatography (hexane-ether, 70:30) to give 0.31 g (45%) of 2-fluoro-3-cyclopropylmethoxypyridine as a colorless liquid which was used without further purification in the subsequent steps.

2-Fluoro-3-cyclopropylmethoxypyridine (0.31 g) was mixed with a 45% w/v solution of HBr in acetic acid (5 mL). The resulting solution was stirred at room temperature for 3 h, neutralized by dropwise addition of 10 M NaOH (5 mL) while cooling with ice water, and extracted with ether (4 x 10 mL). The combined extracts were washed with water (10 mL), dried over anhydrous Na_2SO_4 overnight, and the solvent was removed under reduced pressure. The solid residue was purified by flash chromatography (hexane-ether, 30:70), and the resultant solid was sublimed under vacuum to afford 128 mg (61%) of 2-fluoro-3-hydroxypyridine as a white solid (m.p. 125.5-126° C) which was used without further purification in the subsequent steps.

Triphenylphosphine (393 mg, 1.5 mmol) was added in one portion to a solution of 2-fluoro-3-hydroxypyridine (113 mg, 1 mmol) and (1-*tert*-butoxycarbonyl-2(*S*)-azetidinyl)methanol (280 mg, 1.5 mmol) in anhydrous THF (4 mL) stirred at 0 °C under argon followed by the dropwise addition of neat diethyl azodicarboxylate (261 mg, 1.5 mmol) over 30 min at the same temperature. The resulting solution was allowed to warm gradually to room temperature and left to stand for 90 h. The solvent was removed under reduced pressure, the residue was dissolved in CH_2Cl_2 (30 mL) and washed with saturated $NaHCO_3$ (10 mL) and water (2 × 10 mL). The organic layer was dried over anhydrous Na_2SO_4 and evaporated down to give a light yellow oil which was purified by flash chromatography (hexane-ethyl acetate, 50:50) to afford 200 mg (71%) of the desired compound **3** as a colorless oil. TLC Rf = 0.31 (hexane-ethyl acetate, 50:50); ^{1}H NMR (acetone-D_6) δ 1.37 (s, 9H), 2.24-2.45 (m, 2H), 3.84 (t, J = 7.7 Hz, 2H), 4.25 (dd, J = 2.4, 10.2 Hz, 1H), 4.46-4.57 (m, 2H), 7.25 (dd, J = 4.8, 7.8 Hz, 1H), 7.64 (ddd, J = 1.4, 7.8, 10.5 Hz, 1H), 7.73 (ddd, J = 1.4, 1.5, 4.8 Hz, 1H).

2-Fluoro-3-(2(S)-azetidinylmethoxy)pyridine·CF3COOH, [^{19}F]1.

To a solution of compound **3** (141 mg, 0.5 mmol) in CH_2Cl_2 (2 mL) was added TFA (2 mL). The resulting solution was stirred for 3 h at room temperature. After evaporation of the solvent, the residue was purified by HPLC (acetonitrile/methanol/water/TFA 6:4:90:0.2; flow rate of 7 mL/min). The desired compound has a retention time of 4.5 min. The fraction containing the desired product was evaporated in vacuo at 30 - 35° C to yield 110 mg (74%) of a colorless syrup. MS, *m/z* , M^+ 182.0858, calcd. for $C_9H_{11}FN_2O$: M 182.0855; ^{1}H NMR (CD_3CN) δ 2.59 (m, 2H), 4.04 (m, 2H), 4.39 (m, 2H), 4.85 (m, 2H), 7.24 (ddd, *J* = 1.0, 4.8, 7.8 Hz, 1H), 7.51 (ddd, *J* = 1.5, 7.8, 10.2 Hz, 1H), 7.77 (ddd, *J* = 1.5, 1.5, 4.8 Hz, 1H).

2-[^{18}F]fluoro-3-(2(S)-azetidinylmethoxy)pyridine, (2-[^{18}F]fluoro-A-85380), [^{18}F]1

In a 10 mL vessel an aqueous solution of the [^{18}F]fluoride (prepared by 16 MeV proton irradiation of 98% enriched $H_2{}^{18}O$), 25 mg of Kryptofix® 222, and 4.5 mg K_2CO_3 was heated in an oil bath at 120 °C under a stream of argon while water was azeotropically evaporated using repeated additions of dry CH_3CN. A solution of compound **2** (4 mg) in anhydrous DMSO (0.4 mL) was added into the reaction vessel. The reaction vessel was sealed with a Teflon cap and heated at 190±2° C for 15 min. The reaction mixture was cooled, diluted with 0.4 mL of preparative HPLC mobile phase (48:52 CH_3CN:H_2O), injected onto the HPLC column and eluted with mixture of CH_3CN:H_2O (48:52) at a flow rate of 7 mL/min. The radioactive peak with a retention time of 7.3 - 8.6 min corresponding to authentic [^{19}F]**3** was collected into a flask with 1 mL trifluoroacetic acid and the solvent was removed on a rotary-evaporator (temperature < 60°C). The residue was dissolved in 3 mL trifluoroacetic acid and allowed to stay for 15 min at 60° C. The solvent was evaporated on a rotary-evaporator (temperature < 60°C), the residue was redissolved in 0.6 mL of the mobile phase (CH_3CN:CH_3OH:0.2% CF_3COOH 6:4:90), injected onto the HPLC column and eluted with mixture of CH_3CN:CH_3OH:0.2% CF_3COOH 6:4:90 at a flow rate of 7 mL/min. The radioactive peak with a retention time of 4.8 - 5.6 min corresponding to authentic **1** was collected, and the solvent was removed on a rotary-evaporator. The product was redissolved in saline (5 mL).

An aliquot of the final solution of known volume and radioactivity was applied to HPLC column. A mobile phase of CH_3CN:CH_3OH:0.2% CF_3COOH 6:4:90 at a flow rate of 7·mL/min was used to elute the radioligand, which had a retention time of 4.8 min. The radiochemical purity was greater 99%. The area of the UV absorbance peak measured at 254 nm corresponding to carrier product was measured and compared to a standard curve relating mass to uv absorbance showing average specific radioactivity 1050 mCi/μmol. The radiochemical product also coeluted with a sample of authentic 2-[^{19}F]fluoro-A-85380, **1**.

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References

1. Decker M.W., Brioni J.D., Bannon A.W., Arneric S.P. - *Life. Sci.* **56**: 545 (1995)
2. Flicker D.R.L., Watkins D.L., Fisher S.K., Bartus R.T. - *Pharmacol. Biochem. and Behav.* **18**: 973 (1983)
3. Irle E., Markowitsch H.J. - *Ann. Neurol.* **14**: 1025 (1985)
4. Levin E.D., Rose J.E. - *Ann. N.Y. Acad. Sci.* **757**: 245 (1995)
5. Whitehouse P.J., Martino A.M., Antuono P.G., Lowenstein P.R., Coyle J.T., Price D.L., Kellar K.J. - *Brain. Res.* **371**: 146 (1986)
6. London E.D., Ball M.J., Waller S.B. - *Neurochem. Res.* **14**: 745 (1989)
7. Henningfield J.E., Woodson P.P. - *J .Subst.Abuse* **1**: 301 (1989)
8. Maziere M., Berger G., Masse R., Phimmer D. and Comar D. - In *Electrophysiological Effects of Nicotine*. Remond, A.; Izard, C., Eds.; Elsevier: Amsterdam, pp 31-47(1979)
9. Nyback H., Nordberg A., Langstrom B., Halldin C., Ahlin A., Swahn C.G., Sedvall G. - *Progr. Brain Res.* **79**: 313 (1989)
10. Horti A., Ravert H.T., London E.D., Dannals R.F. - *J.Labelled.Compds.Radiopharm.* **38**: 355 (1996)

11. Horti A., Scheffel U., Ravert H.T., Finley P., London E.D., Dannals R.F. - *J. Nucl. Med.* **37**: 11P (1996)
12. Horti A., Scheffel U., Stathis M., Finley P., Ravert H.T., London E.D., Dannals R.F. - *J. Nucl. Med.* **38**: 1260 (1997)
13. Villemagne V.L., Horti A., Scheffel U., Ravert H.T., Finley P., London E.D., Dannals R.F. - *J. Nucl. Med.* **37**: 11P (1996)
14. Villemagne V.L., Horti A., Scheffel U., Ravert H.T., Finley P., Clough D.J., London E.D., Wagner H.N., Dannals R.F. - *J. Nucl. Med.* **38**:1737 (1997)
15. Ding Y-S., Gatley S.J., Fowler J.S., Volkow N.D., Aggarwal D., Logan J., Dewey S.L., Liang F., Carroll F.I., Kuhar M.J. - *Synapse* **24**: 403 (1996)
16. Horti A.G., Scheffel U., Kimes A.S., Musachio J.L., Ravert H.T., Mathews W.B., Zhan Y., Finley P.A., London E.D., Dannals R.F. - *J.Med.Chem.* submitted for publication
17. Molina P., Ding Y.-S., Carrol F.I., Liang F., Volkow N., Kuhar M., Pappas N., King P., Abumrad N., Fowler J.S. - *J. Nucl. Med.* **38**: 287P (1997)
18. Patt J.T., Spang J.E., Westera G., Schubiger P.A. - XIIth International Symposium on Radiopharmaceutical Chemistry, Uppsala, Sweden, June 15-19, 1997, pp.528-530
19. Abreo M.A., Lin N.-H., Garvey D.S., Gunn D.E., Hettinger A.-M., Wasicak J.T., Pavlik P.A., Martin Y.C., Donnelly-Roberts D.L., Anderson D.J., Sullivan J.P., Williams M., Arneric S.P., Holladay M.W. - *J.Med.Chem.* **39**: 817 (1996)
20. Pauly J.R., Anderson D.J., Sullivan J.P. - *Soc.Neurosci. Abstr.*, Vol.22, Part 2, p.1264 (1996)
21. Kassiou M, Ravert H.T., Mathews W.B., Musachio J.L., London E.D., Dannals R.F. - *J.Labelled Compds.Radiopharm.* **34**: 425 (1997)
22. Kassiou M., Scheffel U.A., Ravert H.T., Mathews W.B., Musachio J.L., London E.D., Dannals R.F. *J. Nucl. Med.* **38**: 65 P (1997)
23. Personal communication, Alexey Mukhin, M.D., PhD, Intramural Research Program, NIDA, Baltimore, MD.

