Synthesis and Nicotinic Acetylcholine Receptor Binding Properties of *exo*-2-(2′-Fluoro-5′-pyridinyl)-7-azabicyclo-[2.2.1]heptane: A New Positron Emission Tomography Ligand for Nicotinic Receptors

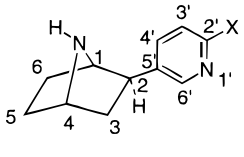
Feng Liang,[†] Hernán A. Navarro,[†] Philip Abraham,[†] Pravin Kotian,[†] Yu-Shin Ding,[‡] Joanna Fowler,[‡] Nora Volkow,[‡] Michael J. Kuhar,[§] and F. Ivy Carroll*,[†]

Chemistry and Life Sciences, Research Triangle Institute, P.O. Box 12194, Research Triangle Park, North Carolina 27709, Brookhaven National Laboratory, Upton, New York 11973, and National Institute on Drug Abuse, Addiction Research Center, P.O. Box 5180, Baltimore, Maryland 21224

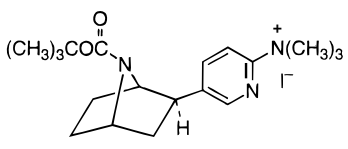
Received March 18, 1997

Epibatidine, a unique alkaloid, was originally isolated from the skin of the Ecuadoran poison frog, *Epipedobates tricolor*, by Daly and co-workers and has been shown to have the structure *exo*-2-(2′-chloro-5′-pyridinyl)-7-azabicyclo[2.2.1]heptane (**1a**).[1,2] Later reports showed that the natural alkaloid possessed the 1*R*,2*R*,4*S* stereochemistry.[3] Numerous biological studies have demonstrated that epibatidine (**1a**) is a potent analgesic agent.[4] Its effects appear to be mediated via neuronal nicotinic receptors (nAChRs); however, details of this action at the molecular level have not been fully elucidated. Nevertheless, epibatidine (**1a**) has been a very useful tool for gaining new information concerning the pharmacological properties of neuronal nAChRs.[4]

Recently, we synthesized and characterized the in vivo binding properties of (+)- and (−)-*exo*-2-(3-[6-^{3}H]-pyridinyl)-7-azabicyclo[2.2.1]heptane (**1c**, [^{3}H]norchloroepibatidine, [^{3}H]NCEPB) in rats.[5] Both (+)- and (−)-[^{3}H]NCEPB bind with high affinity but with higher levels of specific binding in vivo for nAChR as compared to epibatidine (**1a**).[5] Other investigators observed similar results with [^{3}H]epibatidine.[6] These studies suggested that *exo*-2-(2′-fluoro-5′-pyridinyl)-7-azabicyclo-[2.2.1]heptane (**1b**, norchlorofluoroepibatidine, NFEP) might also have favorable binding properties and that the fluorine-18 analog would be useful as a positron emission tomography (PET) ligand for further characterization of the nAChRs.



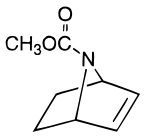
1a, X = Cl
b, X = F
c, X = ^{3}H ([^{3}H]NCEPB)
d, X = Br



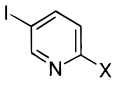
2

In this report, we present a synthesis of **1b** which can be adapted to provide a new synthesis of **1a** and compare the nAChR binding properties of **1a**,**b** to that of nicotine. We also report the synthesis of 7-(*tert*-butyloxycarbonyl)-2-*exo*-[2′-(*N*,*N*,*N*-trimethylammoniumyl)-5′-pyridinyl]-7-azabicyclo[2.2.1]heptane iodide (**2**), which is a more efficient precursor[7,8] for the synthesis of [^{18}F]-**1b** (70% radiochemical yield)[7] than *exo*-2-(2′-bromo-5′-pyridinyl)-7-azabicyclo[2.2.1]heptane (**1d**) or N-protected analogs.[9−11]

A number of different chemical syntheses[3,12−23] of epibatidine (**1a**) have been reported, including one from our laboratory.[15] A review of these methods suggested that **1b** might best be prepared by appropriate modification of the synthesis of epibatidine (**1a**) reported by Clayton and Regan.[13] Their synthesis involved subjection of the olefin (**3**) to a reductive Heck arylation using 2-chloro-5-iodopyridine (**4a**) followed by removal of the *N*-methyloxycarbonyl protecting group with hydrogen bromide in acetic acid. This is the shortest synthesis of epibatidine (**1a**) thus far reported and gives only the desired *exo* product (**1a**).



3



4a, X = Cl
b, X = NH_2
c, X = F

We found that **1b** could be prepared by the route shown in Scheme 1. Since we knew that the 2-fluoropyridinyl group of **1b** would be more reactive than the 2-chloropyridinyl group of epibatidine, we chose to use an *N*-*tert*-butyloxycarbonyl protecting group which could be removed under milder conditions in place of the *N*-methyloxycarbonyl group used by Clayton and Regan.[13] Thus, heating a solution of (*p*-tolylsulfonyl)-acetylene[13] (**5**) and *N*-(*tert*-butoxycarbonyl)pyrrole[24] (**6**) provided 65% of the diene **7**. Nickel boride reduction of **7** gives 86% of the olefin **8**. Desulfonation of **8** using 2.5% sodium amalgam in a 1:1 mixture of ethyl acetate and *tert*-butyl alcohol yielded 55% of the desired 7-(*tert*-butoxycarbonyl)-7-azabicyclo[2.2.1]hept-2-ene (**9**). Coupling of **9** with 2-amino-5-iodopyridine (**4b**) using palladium acetate as catalyst in dimethylformamide containing tetrabutylammonium chloride and potassium formate provided 68% of 7-(*tert*-butoxycarbonyl)-*exo*-2-(2′-amino-5′-pyridinyl)-7-azabicyclo[2.2.1]heptane (**10**). The 2-*exo* stereochemistry assigned to **10** was based on an analysis of the ^{1}H NMR ($CDCl_3$) spectrum. The spectrum showed a doublet of doublets at 2.74 ppm for the H-2 proton with $J_{2\alpha,3\beta} = 5.0$ Hz, $J_{2\alpha,3\alpha} = 8.8$ Hz, and $J_{2\alpha,1} = 0$ Hz, which is characteristic of the 2-*exo* stereochemistry.[15] Diazotization[25] of **10** in pyridine containing 70% hydrogen fluoride effected conversion of the 2-amino group to a fluoro group and deprotection of the *N*-Boc group to give 46% of **1b**. Compound **1b** could also be obtained by direct reductive Heck coupling of 2-fluoro-5-iodopyridine (**4c**, obtained by diazotization of 2-amino-5-iodopyridine (**4b**)[13] in pyridine·HF) with **9** to give **11** which on removal of the *N*-Boc protecting group with trifluoroacetic acid gave the desired **1b**. However, this route was less desirable since the overall yield was 39% and the coupling of 2-fluoro-5-iodopyri-

[†] Research Triangle Institute.
[‡] Brookhaven National Laboratory.
[§] National Institute on Drug Abuse.

S0022-2623(97)00187-8 CCC: $14.00

Scheme 1

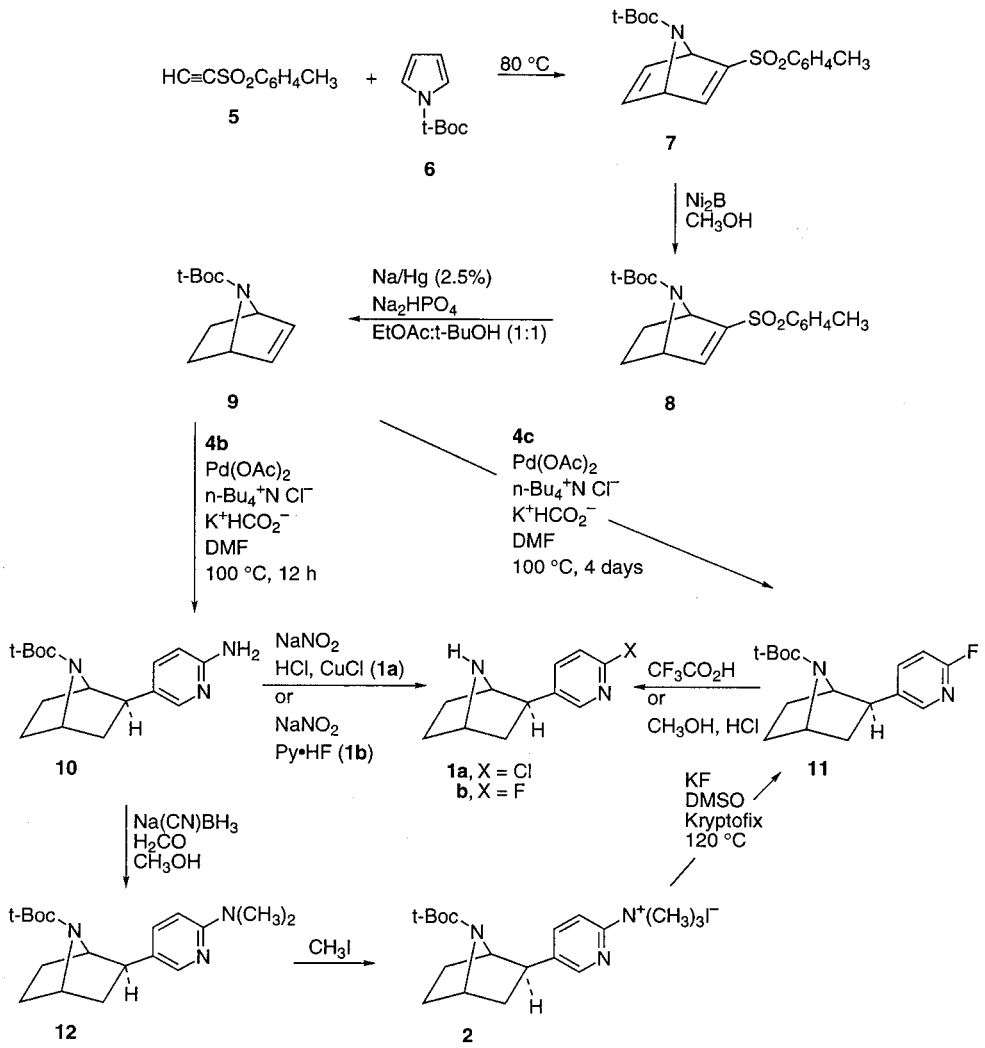
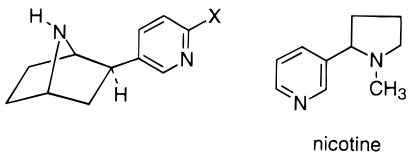


Table 1. K_{app} Values for **1a**,**b** and Nicotine at the nAChR[a,b]



compd	X	K_{app} (nM) (SEM)
1a	Cl	0.024 ± 0.001
1b	F	0.020 ± 0.002
nicotine		6.2 ± 0.5

[a] The K_{app} values are reported as mean ± SEM and were calculated from 3−4 separate competition binding experiments. [b] Compound **1b** was characterized as its hydrochloride salt. C, H, and N analyses were within 0.4% of theoretical values.

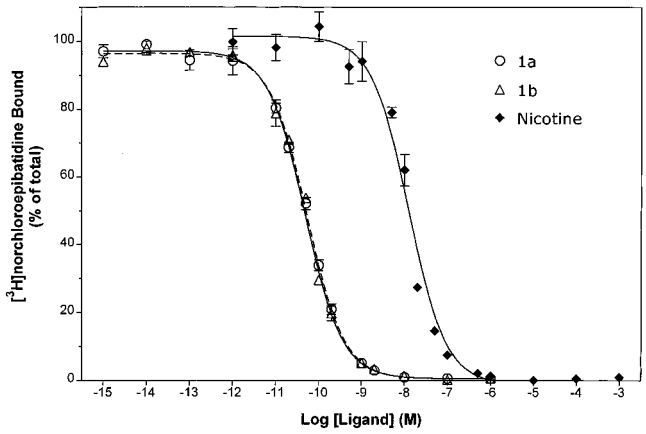


Figure 1. [^{3}H]Norchloroepibatidine competition binding experiments. The data represent the mean ± SEM from 3−4 binding experiments using 11−14 concentrations of test compound run in triplicate with different rat forebrain homogenate preparations for each experiment. The final assay concentration of [^{3}H]norchloroepibatidine (K_d = 0.026 ± 0.002 nM; B_{max} = 5.7 ± 0.05 fmol/mg of tissue) was 30 pM.

dine (**4c**) with **9** took 4 days, whereas the coupling of 2-amino-5-iodopyridine (**4b**) with **9** was complete in 12 h. We also found that diazotization of **10** in hydrochloric acid in the presence or absence of cuprous chloride gave a 76% yield of epibatidine (**1a**) which was identical to an authentic sample.[15] This provided additional support for the 2-*exo* structural assignment of **1a**,**b**.

K_{app} values for inhibition of [^{3}H]NCEPB binding for **1a**,**b**, and nicotine were obtained using a modification of previously reported procedures.[2,26] The data are presented in Table 1 and Figure 1. For each compound, the IC_{50} values obtained from three to four independent competition binding experiments were used to calculate K_{app} values.[27,28] The 20 pM K_{app} value for **1b** makes this compound more than 100 times more potent than nicotine at the nAChR. A recent report has shown that [^{18}F]-**1b** is an excellent PET ligand for mapping nAChR in vivo, and it provides the first evidence for using this

class of ligand to visualize nAChR in vivo with PET.[7] The studies demonstrated a high brain uptake of [^{18}F]-**1b** in both baboon (12−15%) and mouse and high specificity of its binding for nAChR in vivo. The high thalamus to cerebellum ratio (4.0−4.5 in baboon at the end of a 2-h study) used as the index for specific to nonspecific binding provided a high signal-to-noise ratio suggesting a new approach to investigate the nAChR system and its role in neurodegeneration and addiction.

The synthesis of **2** and its conversion to **1b** are shown in Scheme 1. Reductive methylation of **10** using formaldehyde and sodium cyanoborohydride gives the 2-dimethylamino compounds **12**. Alkylation of **12** with methyl iodide affords 7-(*tert*-butyloxycarbonyl)-*exo*-2-[2′-(*N*,*N*,*N*-trimethylammoniumyl)-5′-pyridinyl]-7-azabicyclo-[2.2.1]heptane iodide (**2**). Treatment of **2** with potassium fluoride in dimethyl sulfoxide containing Kryptofix gave a 75% yield of **11** which when treated with hydrochloric acid provided 55% of **1b**·HCl. Details for the synthesis of [^{18}F]NFEP will be published elsewhere.

In summary, an efficient synthesis of *exo*-2-(2′-fluoro-5′-pyridinyl)-7-azabicyclo[2.2.1]heptane (**1b**) and epibatidine (**1a**) was developed. 7-(*tert*-Butyloxycarbonyl)-2-*exo*-[2′-(*N*,*N*,*N*-trimethylammoniumyl)-5′-pyridinyl]-7-azabicyclo[2.2.1]heptane (**2**) was synthesized as an excellent precursor for the synthesis of [^{18}F]-**1b**.

Acknowledgment. This research was supported in part by a grant from Guilford Pharmaceuticals, Inc. (Baltimore, MD), Fisons Pharmaceuticals (now Astra Research Corp., Rochester, NY), Department of Energy, Office of Health and Environmental Research, and National Institutes of Health NS15380 and Grant CTR402 from the Council of Tobacco Research.

Supporting Information Available: Experimental data for the synthesis of **1a**, **2**, **7**−**11**, and **1b** from **10**, **11**, or **2** and the radioligand binding (7 pages). Ordering information is found on any current masthead page.

References

(1) Spande, T. F.; Garraffo, H. M.; Edwards, M. W.; Yeh, H. J. C.; Pannell, L.; Daly, J. W. Epibatidine: A novel (chloropyridyl)-azabicycloheptane with potent analgesic activity from an Ecuadoran poison frog. *J. Am. Chem. Soc.* **1992**, *114*, 3475−3478.

(2) Badio, B.; Daly, J. W. Epibatidine, a potent analgetic and nicotinic agonist. *Mol. Pharmacol.* **1994**, *45*, 563−569.

(3) Fletcher, S. R.; Baker, R.; Chambers, M. S.; Herbert, R. H.; Hobbs, S. C.; Thomas, S. R.; Verrier, H. M.; Watt, A. P.; Ball, R. G. Total synthesis and determination of the absolute configuration of epibatidine. *J. Org. Chem.* **1994**, *59*, 1771−1778.

(4) Sullivan, J. P.; Bannon, A. W. Epibatidine: Pharmacological properties of a novel nicotinic acetylcholine receptor aginist and analgesic agent. *CNS Drug Rev.* **1996**, *2* (1), 21−39.

(5) Scheffel, U.; Taylor, G. F.; Kepler, J. A.; Carroll, F. I.; Kuhar, M. J. *In vivo* labeling of neuronal nicotinic acetylcholine receptors with radiolabeled isomers of norchloroepibatidine. *NeuroReport* **1995**, *6* (18), 2483−2488.

(6) London, E. D.; Scheffel, U.; Kimes, A. S.; Kellar, K. J. In vivo labeling of nicotinic acetylcholine receptors in bain with [^{3}H]-epibatidine. *Eur. J. Pharmacol.* **1995**, *278*, R1−R2.

(7) 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. Mapping nicotinic acetylcholine receptors with PET. *Synapse* **1996**, *24*, 403−407.

(8) 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. Mapping nicotinic acetylcholine receptors with PET. Society for Neuroscience, Washington, DC, Nov. 16−21, 1996; Abstract 22, 269.

(9) Horti, A.; Ravert, H. T.; London, E. D.; Dannals, R. F. Synthesis of a radiotracer for studying nicotinic acetylcholine receptors: (+/-)-*exo*-2-(2-[^{18}F]fluoro-5-pyridyl)-7-azabicyclo[2.2.1]heptane. *J. Labelled Compd. Radiopharm.* **1996**, *38* (4), 355−365.

(10) Horti, A.; Scheffel, U.; Dannals, R. F.; Stathis, M.; Finley, P. A.; Ravert, H. T.; London, E. D. [^{18}F] (±)-*exo*-2-(2-Fluoro-5-pyridyl)-7-azabicyclo[2.2.1]heptane. A radioligand for in vivo labeling and imaging of central nicotinic actylcholine receptors. *J. Nucl. Med.* **1996**, *37*, 11P (abstract).

(11) Villemagne, V. L.; Horti, A.; Scheffel, U.; Ravert, H. T.; Finley, P. A.; London, E. D.; Dannals, R. F. Imaging nicotinic acetylcholine receptors in baboon brain by PET *J. Nucl. Med.* **1996**, *37*, 11P (abstract).

(12) Huang, D. F.; Shen, T. Y. A versatile total synthesis of epibatidine and analogs. *Tetrahedron Lett.* **1993**, *34* (28), 4477−4480.

(13) Clayton, S. C.; Regan, A. C. A total synthesis of (±)-epibatidine. *Tetrahedron Lett.* **1993**, *34*, 7493−7496.

(14) Okabe, K.; Natsume, M. Total synthesis of a frog poison, (±)-epibatidine, a potent non-opioid analgesic. *Chem. Pharm. Bull.* **1994**, *42* (7), 1432−1436.

(15) Kotian, P. L.; Carroll, F. I. Synthesis of (+)- and (−)-epibatidine. *Synth. Commun.* **1995**, *25* (1), 63−71.

(16) Broka, C. A. Total synthesis of epibatidine. *Tetrahedron Lett.* **1993**, *34* (20), 3251−3254.

(17) Fletcher, S. R.; Baker, R.; Chambers, M. S.; Hobbs, S. C.; Mitchell, P. J. Synthesis of (+)- and (−)-epibatidine. *J. Chem. Soc., Chem. Commun.* **1993**, 1216−1218.

(18) Corey, E. J.; Loh, T.-P.; AchyuthaRao, S.; Daley, D. C.; Sarshar, S. Stereocontrolled total synthesis of (+)- and (−)-epibatidine. *J. Org. Chem.* **1993**, *58*, 5600−5602.

(19) Szantay, C.; Kardos-Balogh, Z.; Moldvai, I.; Szantay, C., Jr.; Major-Temesvary, E.; Blasko, G. A practical route to epibatidine. *Tetrahedron Lett.* **1994**, *35*, 3171−3174.

(20) Senokuchi, K.; Nakai, H.; Kawamura, M.; Katsube, N.; Nonaka, S.; Sawaragi, H.; Hamanaka, N. Synthesis and biological evaluation of (±)-epibatidine and the congeners. *Synlett* **1994**, 343−344.

(21) Sestanj, K.; Melenski, E.; Jirkovsky, I. Synthesis of epibatidine. *Tetrahedron Lett.* **1994**, *35* (30), 5417−5420.

(22) Ko, S. Y.; Lerpiniere, J.; Linney, I. D.; Wrigglesworth, R. The total synthesis of epibatidine. *J. Chem. Soc., Chem. Commun.* **1994**, 1775−1776.

(23) Albertini, E.; Barco, A.; Benetti, S.; De Risi, C.; Pollini, G. P.; Romagnoli, R.; Zanirato, V. Total synthesis of (±)-epibatidine. *Tetrahedron Lett.* **1994**, *35* (49), 9297−9300.

(24) Grehn, L.; Ragnarsson, U. A convenient method for the preparation of 1-(tert-butyloxycarbonyl)pyrroles. *Angew. Chem., Int. Ed. Engl.* **1984**, *23*, 296−297.

(25) Danso-Danquah, R.; Bai, X.; Zhang, X.; Mascarella, S. W.; Williams, W.; Sine, B.; Bowen, W. D.; Carroll, F. I. Synthesis and σ binding properties of 2′-substituted 5,9α-dimethyl-6,7-benzomorphans. *J. Med. Chem.* **1995**, *38*, 2978−2985.

(26) Houghtling, R. A.; Davila-Garcia, M. I.; Kellar, K. J. Characterization of (±)-[^{3}H]epibatidine binding to nicotinic cholinergic receptors in rat and human brain. *Mol. Pharmacol.* **1995**, *48*, 280−287.

(27) Cheng, Y. C.; Prusoff, W. H. Relationship between inhibition constants (K_i) and the concentration of inhibitor which causes 50% inhibition (IC_{50}) of an enzyme reaction. *Biochem. Pharmacol.* **1972**, *22*, 3099−3108.

(28) Under our experimental conditions, the concentration of free ligand was not much (>10×) greater than the number of receptors, a requirement if the binding data were to be analyzed as a pseudo-first-order bimolecular reaction. The result is the free ligand concentration and nonspecific binding will increase with increasing concentration of test compound leading to an underestimation of the K_i. However, for the purpose of this communication, a comparison of the K_{app} is sufficient to demonstrate the potency of **1b** relative to **1a** and nicotine. Thus, more sophisticated data analysis techniques designed to correct for changes in free ligand concentration were not employed.

JM970187D