Synthesis of [N-C^3H_3]-Racemic-*trans*-1-phenyl-3-dimethylamino-6-chloro-7-hydroxy-1,2,3,4-tetrahydronaphthalene (PAT-6)

Steven D. Wyrick, Raymond G. Booth, Andrew M. Myers, Nora S. Kula* and Ross J. Baldessarini,* Radiosynthesis Laboratory, Division of Medicinal Chemistry and Natural Products, School of Pharmacy, University of North Carolina, Chapel Hill, NC 27599-7360 and Harvard Medical School and Mailman Research Center, McLean Division of Massachusettes General Hospital, Belmont MA 02178.*

SUMMARY

The biological function of the sigma receptor in the central nervous system is not well understood at the present time. Once thought to be an opiate receptor, the sigma receptor has now been shown to have a neuromodulatory effect upon the synthesis of dopamine in the striatal nerve terminal. A novel sigma agonist, racemic-*trans*-1-phenyl-3-dimethylamino-6-chloro-7-hydroxy-1,2,3,4-tetrahydronaphthalene, PAT-6, has demonstrated the greatest potency of any compound tested to date as a sigma agonist in stimulating the synthesis of dopamine *in vitro* and may be functioning at a novel sigma receptor subtype. The synthesis of tritiated PAT-6 at high specific activity is described herein. This labeled ligand was prepared for use in radioreceptor binding studies in order to identify the putative sigma receptor subtype responsible for mediation of the stimulatory effect on *in vitro* dopamine synthesis.

Key Words: 1-phenyl-3-aminotetralins, tritium, sigma receptor, dopamine, PAT-6

INTRODUCTION

The sigma receptor, once thought to be an opiate receptor in the central nervous system, is no longer considered to be such since the behavioral effects produced by sigma ligands are not antagonized by opiate antagonists such as naloxone. Sigma binding sites are commonly referred to as pharmacological receptors although no endogenous neurohormone for sigma sites has been identified. There appears to be evidence for multiple sigma receptor subtypes. The biological function of sigma receptors in the CNS has not been identified, however, they are capable of binding ligands such as neuroleptics,[1,2] phencyclidine,[3] dextromethorphan[4] and progesterone.[5] There is evidence that ligands which affect sigma receptors may affect motor systems and protect from neuronal damage and therefore may have therapeutic utility in the treatment of motor disorders[6] and in protecting from the detrimental effects of ischemia and stroke.[7] Booth and Baldessarini[8] have demonstrated that both (+)-N-allylnormetazocine (NANM) and (+)-pentazocine stereospecifically stimulated dopamine synthesis in minces of rat corpus striatum by 15-23% over control values at concentrations of 0.1-1.0 μM. This effect was blocked by the reported sigma antagonist, BMY-

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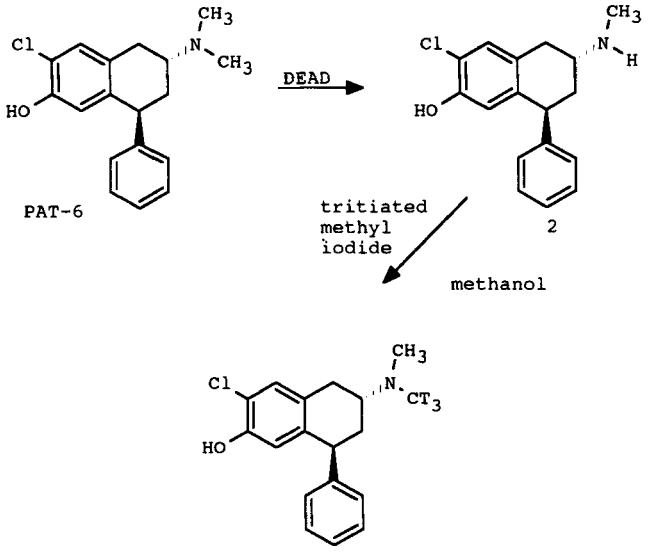
14802 but not by naloxone indicating that the stimulatory effect on dopamine synthesis *in vitro* may be modulated by putative sigma heteroreceptors on striatal nerve terminals or through some indirect mechanism.

We have shown that certain 1-phenyl-3-aminotetralins (PAT's) also produce this stimulatory effect upon dopamine synthesis *in vitro* but with a more potent pharmacological profile.[9] Racemic-*trans*-1-phenyl-3-dimethylamino-6-chloro-7-hydroxy-1,2,3,4-tetrahydronaphthalene (PAT-6) stimulates *in vitro* dopamine synthesis by 35% compared to basal levels and this effect is antagonized by BMY-14802. PAT-6 was shown to have little affinity for D_1 and D_2 dopamine receptors in striatum and marginal affinity for known sigma receptors leading to the hypothesis that PAT-6 may be producing its neuromodulatory effect by binding to a previously unidentified sigma subtype.[9] In order to investigate this possibility, radiolabeled PAT-6 at high specific activity was required for competitive radioreceptor binding studies using various known CNS receptor ligands.



DISCUSSION

The tritium labeling of PAT-6 was accomplished by methylation of the corresponding N-normethyl analog **2** with high specific activity tritiated methyl iodide (85 Ci/mmol, Amersham) as shown in the scheme above. The preparation of PAT-6 is reported elsewhere.[10] The starting material **2** was prepared by N-demethylation of the PAT-6 using diethyl azodicarboxylate (DEAD)[11] and was assigned the *trans*

stereochemistry based upon ^{1}H-NMR spectra correlated with molecular mechanics-generated low energy conformations. The tritiated methyl iodide (provided in 1.0 mL of toluene) was reacted in methanol with a 30 fold excess of the secondary amine **2** at room temperature for 72 hours. After column chromatography, a low yield of labeled PAT-6 was obtained. This yield is likely due to the extremely dilute concentration of the reaction mixture and may be improved in the future by reaction under positive pressure in a bomb.

EXPERIMENTAL PROCEDURES

All chemicals and reagents were used as received from the manufacturers. Tritiated methyl iodide (85 Ci/mmol) was obtained from Amersham, Arlington Heights, Illinois. Column chromatography was performed using silica gel 60 and thin layer chromatograpy was performed using silica gel 60 glass plates with fluorescent indicator, Fisher Scientific. ^{1}H-NMR spectra were obtained on a Bruker 300 MHz NMR spectrometer. Elemental analyses were performed by MHW Laboratories, Phoenix AZ and are correct within $\pm$0.4% of theoretical values. Radiochemical purity was assessed using a Bioscan BID-100 image analyzer and tritium was counted using a Packard Tricarb 4000 liquid scintillation spectrometer using Scintiverse BD counting solution.

Racemic-*trans*-1-phenyl-3-methylamino-6-chloro-7-hydroxy-1,2,3,4-tetrahydronaphthalene (2). PAT-6 (71.0 mg, 0.24 mmol) was dissolved in 3.0 mL of toluene and to this solution was added a solution of 45 mg (0.26 mmol) of diethyl azodicarboxylate (DEAD) in 2.0 mL of toluene. After stirring at 50° C for 16 h, the toluene was removed *in vacuo* and 2.5 mL each of absolute ethanol and saturated NH_4Cl was added followed by reflux for 3 h. The volatiles were removed *in vacuo*, 10 mL of water was added to the residue and the solution was extracted with 10 mL of $CHCl_3$. The aqueous phase was adjusted to pH=8-9 with NH_4OH and again extracted with $CHCl_3$. The combined organic extracts were dried (Na_2SO_4) and evaporated *in vacuo* to afford 57 mg of crude product which was chromatographed on 3.0 g of silica gel 60 eluting with $CHCl_3$-THF-NH_4OH (70:30:1) to afford 25 mg (38%) of product as a colorless solid; mp=208-210°C. ^{1}H-NMR ($CDCl_3$--DMSO-d_6,TMS) δ 7.25-6.90 (m, 6H, Ar$\underline{H}$5+Ar$\underline{H}$5), 6.45(s, 1H, Ar$\underline{H}$8), 4.10 (t, 1H, $\phi C\underline{H}\phi$), 3.10-2.80 (m, 2H, $\phi C\underline{H}_2$), 2.51 (m, 1H, C$\underline{H}$N), 1.95 (m, 2H, $\phi CH\phi C\underline{H}_2$) and 2.35 (s, 3H, N$C\underline{H}_3$). Analysis: Calc. C=70.95 , H=6.30; Found C=70.76, H=6.47.

[N-C^{3}H$_3$]-Racemic-*trans*-1-phenyl-3-dimethylamino-6-chloro-7-hydroxy-1,2,3,4-tetrahydronaphthalene (Tritiated PAT-6). Tritiated methyl iodide (85 Ci/mmol, 10 mCi, 0.12 μmol) in 1.0 mL of toluene was added to a solution of 1.0 mg (3.5 μmol) of **2** in 1.0 mL of methanol and the reaction was stirred in a closed vessel at room temperature for 72 h. The volatiles were removed under a stream of nitrogen and the residue was chromatographed on 1.0 g of silica gel 60 (70-230 mesh) eluting with

CH_2Cl_2-methanol-NH_4OH (95:5:1) to afford 0.6 mCi (6% radiochemical yield) of >98% radiochemically pure (by TLC-radioscan) labeled PAT-6 with a specific activity of 85 Ci/mmol (281 mCi/mg). The product was stored in 2.0 mL of absolute ethanol.

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