Synthesis of [N-C^3H_3]-*trans*-(1*R*,3*S*)-(−)-1-Phenyl-3-N,N-dimethylamino-1,2,3,4-tetrahydronaphthalene (H_2-PAT)

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SUMMARY

Subsequent to the discovery that the (+)-benzomorphan sigma receptor ligands, (+)-pentazocine and (+)-N-allylnormetazocine, stimulated tyrosine hydroxylase activity and dopamine synthesis in rat striatum *in vitro*, we reported a similar effect on a structurally similar series of 1-phenyl-3-aminotetrahydronaphthalenes (phenylaminotetralins, PAT's). Both racemic 1-phenyl-3-dimethylamino-6-chloro-7-hydroxytetralin (Cl,OH-PAT) and racemic 1-phenyl-3-dimethylaminotetralin (H_2-PAT) stimulated tyrosine hydroxylase with an EC_{50} of approximately 0.1 μM. The former was also found to have a non-specific dopamine releasing effect while the latter was devoid of such activity affording it the less complicated pharmacological profile of the two analogs. We previously reported the synthesis of tritium labeled Cl,OH-PAT to be used in radioreceptor and autoradiography studies and found that it labeled a sigma-like site in guinea pig brain with an apparent Kd of ~50 pM and with a pharmacological profile unique from other known CNS receptors. Here we report the synthesis of high specific activity tritium labeled *trans*-(1*R*,3*S*)-(−)-H_2-PAT as this enantiomer was found to be more active in the tyrosine hydroxylase assay and possessed approximately 45 fold greater affinity for the novel neuromodulatory sigma-like receptor.

Key Words: 1-phenyl-3-aminotetralins, tritium, sigma-like receptor, tyrosine hydroxylase, dopamine, H_2-PAT

INTRODUCTION

Although sigma sites in the CNS were originally considered to be an opioid receptor subtype, it was subsequently shown that these receptors displayed non-opioid pharmacology. The specific function of sigma sites is currently unknown and no endogenous ligand has been reported, however, these sites bind a variety of ligands from diverse structural classes.[1-5] The (+)-benzomorphans, (+)-pentazocine and (+)-N-allylnormethazocine, have been shown to bind to especially the sigma 2 site and to stimulate tyrosine

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hydroxylase activity and the synthesis of dopamine (EC_{50} ~ 0.1 μM) *in vitro* in rat striatum.[6] This effect was blocked by the sigma receptor antagonist BMY14802, but not by naloxone, suggesting that the the functional effect was mediated by sigma receptors.

In order to investigate the possibility that this neuromodulatory effect may occur among other structurally similar classes, we synthesized and tested a series of N-substituted 1-phenyl-3-amino-1,2,3,4-tetrahydronaphthalenes (1-phenyl-3-aminotetralins, PAT's).[7] This structure-activity study revealed that both the (±)-*trans*-1-phenyl-3-N,N-dimethylamino-6-chloro-7-hydroxytetralin (Cl,OH-PAT) and (±)-1-phenyl-3-N,N-dimethylaminotetralin (H_2-PAT) stimulated tyrosine hydroxylase *in vitro* (EC_{50} ~ 0.1 μM) in rat striatum. Cl,OH-PAT also induced a non-specific release of dopamine at higher concentrations to inhibit tyrosine hydroxylase activity.[8] Subsequent tritium labeling of (±)-Cl,OH-PAT[9] (formerly referred to as PAT-6) and its use in radioreceptor and autoradiography studies in guinea pig brain revealed that this analog possessed very high affinity (apparent Kd ~ 50 pM) for a novel sigma-like binding site in the CNS[8] and that such sites were found in highest concentration in the lateral septal nucleus and hippocampus.[10] Since H_2-PAT was devoid of this releasing effect and appeared to possess a less complicated pharmacological profile, the *trans* enantiomers were resolved and tested,[7] revealing that it was the (1*R*,3*S*)-(−)-H_2-PAT isomer which was responsible for the functional effect on dopamine synthesis and which possessed approximately 45 fold greater affinity for the novel sigma-like neuromodulatory site labeled by (±)-[^{3}H]-Cl,OH-PAT. Accordingly, we report herein the preparation of high specific activity tritium labeled (1*R*,3*S*)-(−)-H_2-PAT for use in further radioreceptor and autoradiography studies.

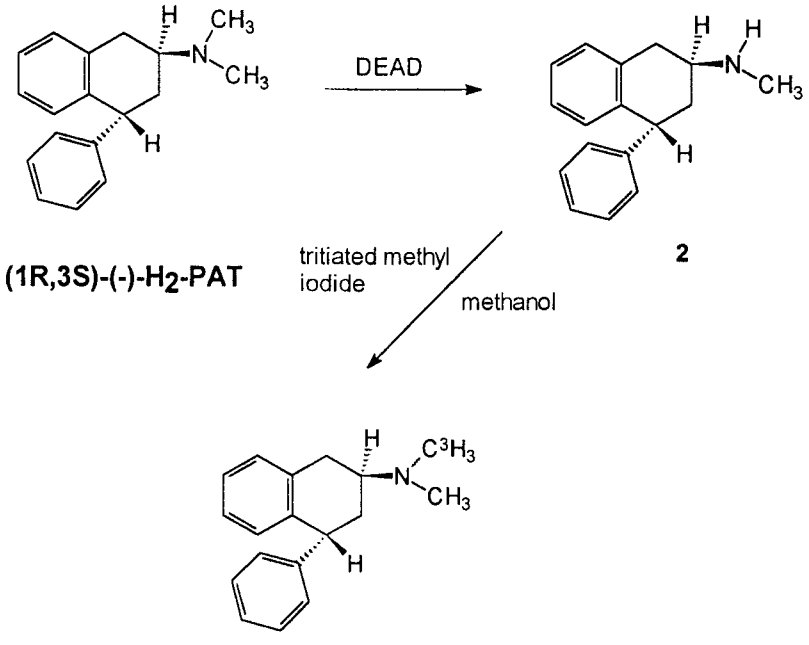
DISCUSSION

Trans-(±)-1-phenyl-3-N,N-dimethylaminotetralin was resolved as the camphor-10-sulfonic acid salts as previously described.[7] Diethyl azodicarboxylate (DEAD) was employed to N-demethylate the (1*R*,3*S*)-(−)-H_2-PAT isomer to afford **2** which would serve as the precursor to the tritiated product as described for the radiolabeling of Cl,OH-PAT.[9] Intermediate **2** was treated with tritiated methyl iodide (provided in 1.0 mL of toluene) in N,N-dimethylformamide at room temperature for 72 hours. The product was purified by column chromatography on silica gel.

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 chromatography was performed using silica gel 60 glass plates with fluorescent indicator, Fisher Scientific. ^{1}H-NMR spectra were obtained on a Bruker AC300 300 MHz NMR spectrometer. Optical rotations were performed in ethanol using a Rudolf Instruments Autopol optical

polarimeter. 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.



***trans*-(1*R*,3*S*)-(−)-1-Phenyl-3-N-methylamino-1,2,3,4-tetrahydronaphthalene (2).** *trans*-(1*R*,3*S*)-(−)-H_2-PAT (17 mg, 0.068 mmol) was dissolved in 2.0 mL of toluene and to this solution was added a solution of 16.5 mg (0.095 mmol) of diethyl azodicarboxylate in 1.0 mL of toluene. After stirring at 50°C for 16 h, the toluene was removed *in vacuo* and 1.0 mL each of ethanol and saturated NH_4Cl was added followed by reflux for 3 h. The volatiles were removed *in vacuo*, 8 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 35 mg of crude product which was column chromatographed on 1.0 g of silica gel 60 eluting with CH_2Cl_2-methanol (9:1) to afford 13.1 mg (81%) of product as a light yellow gum. ^{1}H-NMR ($CDCl_3$, TMS) δ 6.95-7.30 (m, 9H, ArH9), 4.10 (t, 1H, ϕCHϕ), 3.10-2.80 (m, 2H, ϕCH2), 2.51 (m, 1H, CHN), 1.95 (m, 2H, ϕCHϕCH2), 2.35 (s, 3H, NCH3). $[\alpha]^{25}_D$ = −34° (ethanol).

[N-C^3H_3]-*trans*-(1*R*,3*S*)-(−)-1-Phenyl-3-N,N-dimethylamino-1,2,3,4-tetrahydronaphthalene (tritiated (1*R*,3*S*)-(−)-H_2-PAT. 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 of **2** in 2 mL of DMF and the reaction was stirred in a closed vessel for 72 h at room temperature. The volatiles were removed under a stream of nitrogen and the residue was column chromatographed on 1.0 g of silica gel 60 (70-230 mesh) eluting with CH_2Cl_2-MeOH-NH_4OH (95:5:1) to afford 0.65 mCi (6.5% radiochemical yield) of > 98% radiochemically pure (by TLC radioscan) labeled (1*R*,3*S*)-(−)-H_2-PAT with a specific activity of 85 Ci/mmol (333 mCi/mg). The specific activity for the product was taken to be identical to that of the tritiated methyl iodide used since the secondary amine starting material was converted only to the tertiary amine product and the tritium on the methyl group is completely nonexchangeable. The product was stored in 10 mL of absolute ethanol.

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