SYNTHESIS OF [^{3}H]NALTRINDOLE [1]

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SUMMARY

[^{3}H]Naltrindole ([^{3}H]**1**) was prepared for use as a selective radiolabeled ligand for delta opioid receptors. The Fischer indole synthesis from naltrexone and 2,4-dibromophenylhydrazine produced dibromide precursor **2**, which was catalytically dehalogenated with carrier free tritium gas to afford [^{3}H]**1** in 45.8% yield at a specific activity of 39.5 Ci/mmol.

KEY WORDS: Tritium, Naltrindole, Catalytic tritiolysis, Delta opioid receptor ligand

INTRODUCTION

Naltrindole, 6,7-dehydro-4,5α-epoxy-3,14-dihydroxy-6,7,2′,3′-indolo-17-cyclopropylmethylmorphinan (**1**), has been characterized as a highly selective antagonist ligand for the delta opioid receptor [2,3,4], which may interact preferentially with one type of delta subtype of receptor [5]. High specific activity tritiated naltrindole was needed to identify and characterize sub-types of delta opioid receptors in the rat by autoradiography. Incorporation of the label in naltrindole (NTI) by catalytic dehalogenation of dibromide precursor **2** with carrier free tritium gas was expected to provide tritiated **1** suitable for the above study.

Preparation of unlabeled **1** and substituted phenyl analogs by the Fischer indole synthesis of naltrexone and the appropriate phenylhydrazine has been described [2,3]. This method was used to prepare dibromide (**2**), and monobromides **3** and **4**, potentially arising from incomplete dehalogenation of **2**. Conditions for hydrogenolysis of **2** to unlabeled NTI were developed and used for the synthesis of tritiated naltrindole.

Autoradiographic analysis and biochemical characterization of [^{3}H]NTI in the rat will be described elsewhere.

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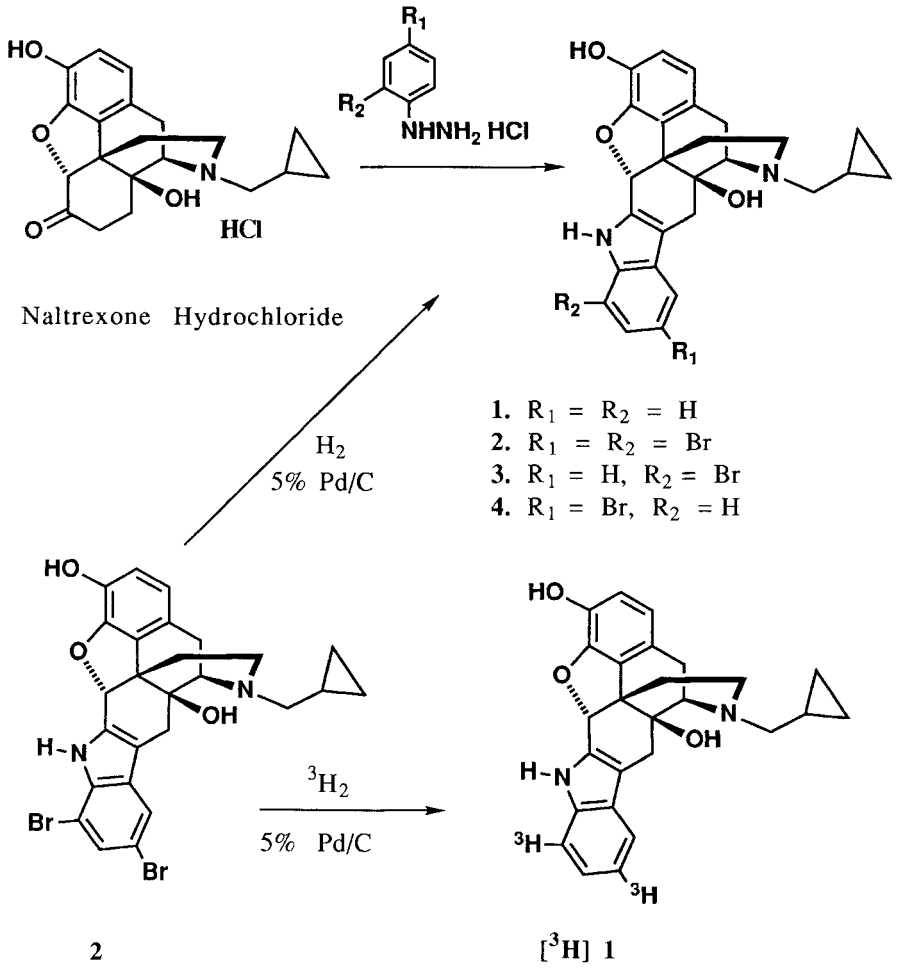
SCHEME 1



DISCUSSION

Catalytic dehalogenation of dibromide **2** without disturbance of the cyclopropyl group was tested by treatment of a solution of **2** in THF/Et_3N in the presence of 5% Pd/C under 5.5 psi of hydrogen for 4 h at room temperature. These conditions gave a less than 10% yield of naltrindole (**1**), by HPLC; the main product was a polar unknown accompanied by monobromides **3** and **4** and dibromide **2**. Changing the hydrogenolysis solvent from THF to DMF resulted in a viable synthesis of naltrindole. The product was isolated in 99% yield and corresponded to authentic **1** by HPLC and NMR.

A low specific activity preparation from dibromide **2** and a mixture of hydrogen and tritium gas (9 mCi/mmol) yielded labeled NTI having a radiochemical purity of 93.6% by HPLRC along with radiolabeled polar unknowns (5.49%) and tritium labeled monobromides **3** and **4** (0.59% and 0.34%, respectively). The specific activity of [^{3}H]NTI was determined as 4.39 mCi/mmol based on the HPLC response of NTI hydrochloride at 225 nm. Isolation of [^{3}H]NTI was tested by Bond Elut extraction in anticipation of removal of TEAP from the mobile phase to be used for the purification of high specific activity [^{3}H]naltrindole by preparative HPLC.

Tritiolysis of 0.022 mmol of **2** with carrier free tritium gas [6] yielded 600 mCi of [^{3}H]NTI after HPLC purification and Bond Elut extraction. Both ^{3}H and ^{1}H NMR indicated labeling at the sites of the displaced bromines. Tritium NMR located the tritium atoms in equal amounts at the 5′ and 7′ aromatic positions. Proton NMR suggested a specific activity of 40.5 Ci/mmol based on integration of the decreased signals for H-5′ and H-7′. Comparison of the HPLC response of tritiated **1** with unlabeled **1** at 225 nm determined a specific activity of 39.5 Ci/mmol. The radiochemical purity of [^{3}H]NTI by HPLRC was 96.5 ± 0.2% with 99.7 ± 0.5% recovery of injected radioactivity.

EXPERIMENTAL

Materials and Methods

Naltrindole hydrochloride was prepared according to the method of Portoghese et al. [2,3]. Naltrexone hydrochloride was purchased from Mallinckrodt.

HPLC was performed on a Supelcosil LC-18 column (250 x 4.6 mm) with a mobile phase of triethylammonium phosphate, pH 3.0/MeOH, 50/50 (v/v) at a flow rate of 1.0 mL/min. Ultraviolet absorbance at 225 nm was monitored with a multiwavelength detector. Radiochemical detection employed a Radiomatic FLO-ONE Beta A-250 instrument. Typical Retention times are listed in Table 1.

Table 1. Retention Times of NTI and NTI Bromides

Compound	Retention (min)
NTI (**1**)	8.2
5′,7′-Dibromo NTI (**2**)	56.0
7′-Bromo NTI (**3**)	22.4
5′-Bromo NTI (**4**)	20.6

Proton NMR spectra were recorded on a Varian XL-400 spectrophotometer. Tritium NMR were obtained on an IBM Instruments AF-300 spectrophotometer at 320 Hz.

5′,7′-Dibromo-6,7-dehydro-4,5α-epoxy-3,14-dihydroxy-6,7,2′,3′-indolo-17-cyclopropylmethylmorphinan Hydrochloride (2·HCl). A mixture of naltrexone hydrochloride (1.7 g, 4.60 mmol) and 2,4-dibromophenylhydrazine hydrochloride (2.78 g, 9.19 mmol) was slurried in glacial acetic acid (25 mL) and heated to reflux for 6 h. The mixture was cooled to room temperature and the resulting precipitate was collected by filtration and washed with ether. The solid was taken up in 1N aqueous NH_4OH (35 mL) and extracted with EtOAc (2 x 50 mL). The organic extracts were dried (Na_2SO_4) and evaporated in vacuo to give a residue which was purified by chromatography (silica gel; CH_2Cl_2/MeOH, 9/1) to yield the dibromoindole 2 (0.63 g, 23%). HPLC at 225 nm indicated 96.7% purity. A portion of the free base was converted to the hydrochloride salt by dissolution in MeOH and addition of MeOH saturated with HCl gas. ^1H NMR (DMSO-d_6) δ 7.57 (d, 1H, H-4′), 7.51 (d, 1H, H-6′), 6.65 (dd, 2H, H-1, H-2), 5.67 (s, 1H, H-5). Anal. calcd for $C_{26}H_{24}Br_2N_2O_3 \cdot HCl \cdot H_2O$: C, 49.82; H, 4.47; N, 4.30. Found: C, 49.68; H, 4.47; N, 4.30.

7′-Bromo-6,7-dehydro-4,5α-epoxy-3,14-dihydroxy-6,7,2′,3′-indolo-17-cyclopropylmethylmorphinan Hydrochloride (3·HCl). A mixture of naltrexone hydrochloride (8.44 g, 22.4 mmol), 2-bromophenylhydrazine hydrochloride (10.0 g, 44.7 mmol), and glacial acetic acid (100 mL) was heated to reflux for 6 h and then stirred at room temperature for 60 h. The resulting solid was collected by filtration. The supernatant was diluted with an equal volume of ether, and the resulting solid was collected. The two solids were combined, dissolved in MeOH, treated with Darco G-60 charcoal, filtered, and concentrated to a solid. The solid was purified by chromatography (silica gel; CH_2Cl_2/EtOH/NH_4OH, 97/3/0.5) to provide 3. The purified material was converted to its hydrochloride salt by dissolution in ether and addition of 6N HCl in dioxane. ^1H NMR (CD_3OD) δ 7.40 (d, 1H, H-4′), 7.30 (d, 1H, H-6′), 6.91 (t, 1H, H-5′), 6.69 (dd, 2H, H-1, H-2). Anal. Calcd for $C_{26}H_{25}BrN_2O_3 \cdot HCl \cdot 0.75\ H_2O$: C, 57.47; H, 5.10; N, 5.16; Br, 14.71; Cl, 6.52. Found: C, 57.42; H, 4.92; N, 5.03; Br, 15.06; Cl, 6.39.

5′-Bromo-6,7-dehydro-4,5α-epoxy-3,14-dihydroxy-6,7,2′,3′-indolo-17-cyclopropylmethylmorphinan Hydrochloride (4·HCl). A stirred mixture of naltrexone hydrochloride (8.44 g, 22.4 mmol), 4-bromophenylhydrazine hydrochloride

(10.0 g, 22.4 mmol), and glacial acetic acid (100 mL) was heated to reflux for 6 h and then stirred at room temperature for 60 h. The resulting solid was collected by filtration and suspended in boiling water (100 mL). EtOH (3A, 50 mL) was added, and the resulting solution was filtered and concentrated to dryness. The solid was purified by preparative HPLC on a Partisil ODS-3 column using a linear gradient of H_2O/HOAc, 99.5/0.5 to MeOH/HOAc, 99.5/0.5. The purified material was dissolved in MeOH and precipitated with ether. Trituration of the resulting gum with ether yielded the hydrochloride salt. ^1H NMR (CD_3OD) δ 7.54 (s, 1H, H-4′), 7.27 (d, 1H, H-6′), 7.20 (d, 1H, H-7′), 6.69 (dd, 2H, H-1, H-2). Anal. Calcd for $C_{26}H_{25}BrN_2O_3 \cdot HCl \cdot 1.375H_2O$: C, 56.30; H, 5.23; N, 5.05; Br, 14.41; Cl, 6.39. Found: C, 56.52; H, 5.36; N, 4.93; Br, 14.40; Cl, 5.98.

6,7-Dehydro-4,5α-epoxy-3,14-dihydroxy-6,7,2′,3′-indolo-17-cyclopropylmethylmorphinan (1, Naltrindole, NTI). A mixture of free base **2** (13.0 mg, 0.028 mmol), 5% Pd/C (6.1 mg), Et_3N (0.1 mL), and DMF (6 mL) was rapidly stirred at room temperature for 1 h under 5.5 psi of hydrogen gas. The catalyst was removed by filtration through celite, and the celite was washed with MeOH (10 mL). The filtrate was concentrated to a crystalline residue which was triturated with EtOAc to give 10.2 mg of **1**. ^1H NMR (DMSO-d_6) δ 7.35 (d, 2H, H-4′, H-7′), 7.11 (t, 1H, H-6′), 6.95 (t, 1H, H-5′), 6.62 (dd, 2H, H-1, H-2), 5.70 (s, 1H, H-5), 0.8 to 0.4 (comp m, 4H, cyclopropyl protons).

Low Specific Activity Naltrindole ([^{3}H]1). A mixture of **2** (52 mg, 0.091 mmol), 5% Pd/C (25 mg), Et_3N (0.1 mL), and DMF (10 mL) was rapidly stirred under approximately 80 mCi of tritium gas (9 mCi/mmol) at 5.5 psi for 70 min at room temperature. The catalyst was removed by filtration through celite, and the cake was washed thoroughly with MeOH. Evaporation of the solvents under reduced pressure yielded a residue containing 397 μCi. A portion of the residue (40 μCi) in MeOH (1.0 mL) was evaporated to dryness and reconstituted in aqueous triethylammonium phosphate (TEAP), pH 3. The acidic solution was applied on a 1 cc Bond Elut C-18 cartridge previously washed with MeOH (20 mL) followed by H_2O (20 mL). The radioactive solution on the C-18 cartridge was washed with H_2O (20 mL) followed by MeOH (10 mL). The MeOH extract (32.3 μCI) was evaporated to a crystalline residue of [^{3}H]**1** having a radiochemical purity of 93.6% by HPLRC. The HPLC response at 225 nm of a 0.0323 μCi sample was equivalent to 0.00305 mg of authentic **1**.

High Specific Activity Naltrindole ([^{3}H]1). A stirred solution of free base 2 (12.5 mg, 0.0219 mmol), in DMF (2.0 mL) and Et_3N (0.1 mL) was placed under one atmosphere of carrier free tritium gas (60 Ci/mmol). The 5% Pd/C catalyst (6.6 mg) was added, and the mixture was stirred at room temperature for 2 h. The reaction flask was frozen and degassed under vacuum, then thawed and treated with MeOH, which was pumped out to remove the bulk of labile tritium. The catalyst was removed by passing the mixture through a glass fiber filter with a MeOH rinse. After overnight lyophilization of the filtrate, the residue was taken up in 2.0 mL of 50% aqueous methanol. Addition of 0.5 mL of TEAP, pH 3/MeOH, 50/50 gave a clear solution containing 1.55 Ci. Purification was done by HPLC injections (5 x 0.5 mL) on a Supelcosil LC-18 column (250 x 4.6 mm) with a mobile phase of MeOH/TEAP, pH 3, 50/50, at 1.0 mL/min. The effluent corresponding to [^{3}H]naltrindole at approximately 6.5 to 9.0 min was collected and lyophilized. The viscous residue was dissolved in 4.0 mL of H_2O for removal of TEAP by Bond Elut extraction. The radioactive solution was placed on a suitably conditioned C-18 cartridge, which was washed with H_2O (20 mL) followed by MeOH (20 mL). HPLRC of the MeOH extract (600 mCi) indicated a radiochemical purity of 96.5%. The HPLC response at 225 nm for a 600 μCi sample of tritiated 1 was equivalent to 0.00683 mg of NTI·HCl. ^{1}H NMR (DMSO-d_6) δ 7.35 (d, 1.38H, H-4′, H-7′), 7.07 (t, 1H, H-6′), 6.93 (t, 0.26H, H-5′), 6.52 (dd, 2H, H-1, H-2), 5.53 (s, 1H, H-5). ^{3}H NMR (DMSO-d_6) δ 7.365 (d, J=8.6 Hz, ^{13}H, ^{3}H-7′), 6.984 (t, J=7.8 Hz, ^{13}H, ^{3}H-5′).

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