2.28 (t, J = 7 Hz, 4 H, CH_2CO), 1.22–1.80 (m, 48 H, CH_2). The multiplet at δ 1.22–1.80 contained an intense singlet at 1.28. Trilactone was not isolated in pure form, but its presence was indicated by an $M^{+\cdot}$ signal at m/e 720 in its mass spectrum.

Isolation of Pentadecanolide from a Large-Scale Run with Carbodiimide 4 in Benzene. A large-scale run similar to run 14 was performed with 73.6 mg (0.285 mmol) of 1, 0.721 g (1.70 mmol) of 4, and 150 mL of C_6H_6 by using the same procedure. The reaction mixture was added to a 3 × 30 cm column of silica gel packed in hexane and eluted with 400 mL of 1:1 (v/v) ether–hexane. The eluate was rotary evaporated to give an oil which was dissolved in 1 mL of HPLC-grade THF. Preparative GLC (180 °C) yielded 2 [retention time 10.8 min, mp 34–34.5 °C (lit.[2] mp 32 °C)], whose ^1H NMR spectrum was identical with that of authentic lactone 2: δ 4.04 (t, J = 6 Hz, 2 H, CH_2O), 2.27 (t, J = 6 Hz, 2 H, CH_2CO), 1.18–1.80 (m, 24 H, CH_2). The multiplet at δ 1.18–1.80 contained an intense singlet at δ 1.30. Likewise, the IR (neat film on NaCl) and mass spectra of the GLC-collected 2 were identical with those of authentic material, including a strong band at 1735 cm^{-1} (C=O) and a signal for $M^{+\cdot}$ at m/e 240, respectively.

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Registry No. 1, 4617-33-8; 2, 106-02-5; 3 (n = 1), 78651-85-1; 3 (n = 2), 79134-82-0; 4, 2491-17-0; 5, 25952-53-8; *N,N'*-dicyclohexylcarbodiimide, 538-75-0; didodecyldimethylammonium bromide, 3282-73-3; bis(2-ethylhexyl)sodium sulfosuccinate, 20542-42-1; lauroyl chloride, 112-16-3; hexyl alcohol, 111-27-3; hexyl laurate, 34316-64-8; 1,17-dioxacyclodotriacontane-2,18-dione, 659-76-7; 1,17,33-trioxacyclooctatetracontane-2,18,34-trione, 79134-83-1.

Reduction of the *N*-Propargyl Group with Tritium. General Procedure for the Preparation of *N*-[2,3-^{3}H]Allyl Opiate Ligands at High Specific Activity[1]

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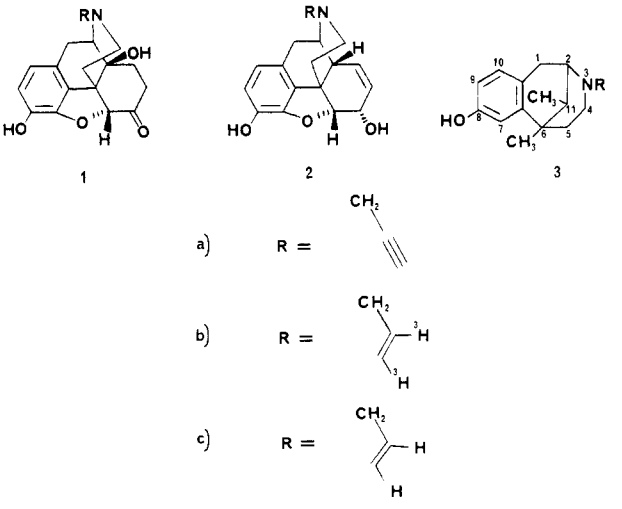
Radiolabeled neurotransmitter receptor ligands are exceedingly valuable substances for obtaining information about their respective receptors, and a number of them possess the *N*-allyl group. A method is outlined to prepare (−)-*N*-([2,3-^{3}H]allyl)naloxone (**1b**), (−)-*N*-([2,3-^{3}H]allyl)nalorphine (**2b**), and (±)-*N*-([2,3-^{3}H]allyl)normetazocine (**3b**) from their respective *N*-propargyl precursors. Triton nuclear magentic resonance studies confirm labeling specificity. This labeling strategy affords the highest specific activities for such ligands reported to date and possesses a number of other advantages over previous methods. Utilization of such tritiated ligands for receptor binding assay will undoubtedly lead to a more comprehensive mapping of and increased information about their respective receptors.

By means of generally labeled (−)-[^{3}H]naloxone (6.1 Ci/mmol), a potent opiate receptor antagonist, the existence of a specific opiate receptor was first demonstrated by Snyder and Pert[2] in 1973. Since then, a number of investigators have tried to improve the utility of this valuable tritiated ligand for receptor binding assay by preparing it specifically labeled and at higher (greater than 30 Ci/mmol) specific activity. A synthesis of (−)-[7,8-^{3}H]naloxone was reported[3] but suffered from the disadvantage of introducing tritium into a chemically labile position. Subsequently, a route to (−)-[15-^{3}H]naloxone was described[4] but yielded material of very low (4 Ci/mmol) specific activity. The lack of a satisfactory preparation of specifically tritiated (−)-naloxone at high specific activity prompted our interest in this compound.

A structural feature present in (−)-naloxone (**1c**) and common to a number of other useful receptor ligands is the *N*-allyl group. It seemed altogether reasonable to expect that the reduction of an *N*-propargyl group (A, Scheme I) with tritium gas to an *N*-[2,3-^{3}H]allyl group (B, Scheme I) would occur at high specific activity and thereby

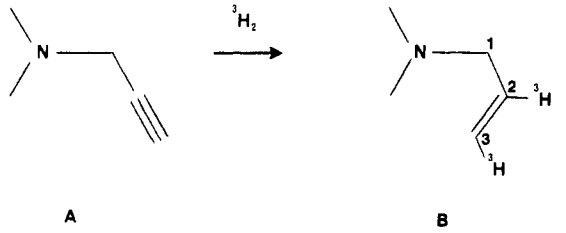


constitute a useful strategy to prepare specifically tritiated (−)-naloxone and other *N*-allyl ligands. To demonstrate the utility of this methodology, we now describe the preparation of (−)-*N*-([2,3-^{3}H]allyl)naloxone (**1b**), (−)-*N*-([2,3-^{3}H]allyl)nalorphine (**2b**), a mixed opiate receptor agonist–antagonist,[5] and (±)-*N*-([2,3-^{3}H]allyl)nor-

(1) Presented in part at the 11th Northeast Regional Meeting of the American Chemical Society, Rochester, NY, Oct, 1981.
(2) Snyder, S. H.; Pert, C. B. *Science* **1973**, *179*, 1011.
(3) Fishman, J.; Cotter, M. L.; Norton, B. I. *J. Med. Chem.* **1973**, *16*, 556.
(4) Brine, G. A.; Kepler, J. A. *J. Labelled Compd.* **1976**, *12*, 401.
(5) Snyder, S. H. *Sci. Am.* **1977**, *236*, 44.

0022-3263/81/1946-4968$01.25/0

Scheme I



A B

metazocine (SKF 10047, **3b**), a σ opiate receptor agonist,[6] at high specific activity from appropriate *N*-propargyl precursors **1a**, **2a**, and **3a**, respectively.

N-Propargyl precursors **1a**, **2a**, and **3a** were conveniently prepared by alkylation of the appropriate nor compounds with propargyl bromide.[7] Reduction of **1a**, **2a**, and **3a** with tritium gas over Lindlar catalyst smoothly afforded tritiated ligands **1b**, **2b**, and **3b**, respectively. Purification of the latter compounds was accomplished by TLC with solvent systems that clearly separated the desired N-[2,3-^{3}H]allyl ligands from both unreacted starting material and potentially overreduced byproducts. However, overreduced byproducts comprised only a small fraction of the crude product mixture (see Experimental Section). The low radiochemical yield (based on the nor precursor) of **2b** (2%) in contrast to the reasonable radiochemical yields of **1b** (47%) and **3b** (37%) appeared to be due at least in part to the poorer recovery of **2b** during each preparative TLC. In this way, N-[2,3-^{3}H]allyl ligands **1b**, **2b**, and **3b** were prepared in greater than 97% radiochemical purity (TLC, HPLC) and at 30–50 Ci/mmol.

The identity of these ligands was conclusively demonstrated by cochromatography (TLC, HPLC) with authentic cold ligands **1c**, **2c**, and **3c** and by superimposable UV spectra with those of these ligands. Also the ^{1}H-decoupled ^{3}H NMR (CD_3OD) spectra of the free bases of products **1b**, **2b**, and **3b** (Figure 1) indicate exclusive tritium incorporation in the *N*-allyl group of each. The chemical shift values for the downfield and upfield tritium resonances of **1b**, **2b**, and **3b** are consistent with the assignment of them to the 2- and 3-position vinyl tritons, respectively. Also, these spectra confirm the presence of a ditritiated species (characterized by two coupled doublets) and two monotritiated species (characterized by a singlet superimposed on each doublet) for **1b**, **2b**, and **3b**.

Several distinct advantages recommend the reduction of the *N*-propargyl group with tritium as being the method of choice to prepare (–)-[^{3}H]naloxone and other ^{3}H-substituted ligands bearing the *N*-allyl group: (1) the accessibility of *N*-propargyl precursors from readily available nor compounds, (2) the mildness of the method, (3) the chemical stability of the N-[2,3-^{3}H]allyl labeling position, (4) the postponement of tritium incorporation until the very end of the synthetic sequence.

Utilization of **1b**, **2b**, and **3b** along with other potential *N*-[2,3-^{3}H]allyl ligands for receptor binding assay will undoubtedly lead to a more comprehensive mapping of and increased information about their respective receptors.

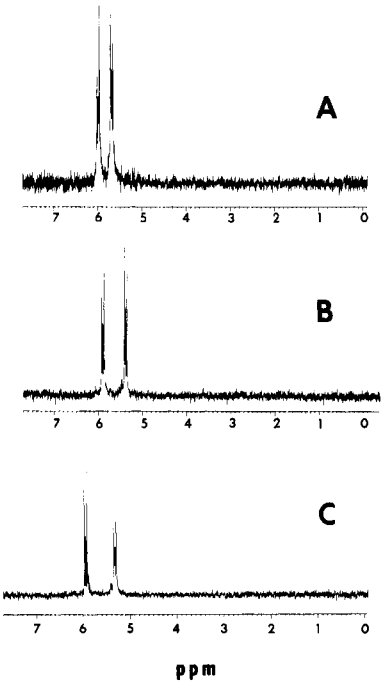


Figure 1. ^{3}H NMR (CD_3OD) spectra of (A) (–)-*N*-([2,3-^{3}H]allyl)naloxone (**1b**), (B) (–)-*N*-([2,3-^{3}H]allyl)nalorphine (**2b**), and (C) (±)-*N*-([2,3-^{3}H]allyl)normetazocine (**3b**). Chemical shift values are given in parts per million downfield from internal $(CH_3)_4Si$.

Experimental Section

General Methods. Evaporations were carried out on a Büchi rotary evaporator in vacuo at bath temperatures below 40 °C. TLC was performed either on Analtech 5 × 15 cm, 250-μm (analytical), and 20 × 20 cm, 1000-μm (preparative), silica gel GF coated glass plates or on Whatman 5 × 15 cm, 200-μm (preparative), KC18F coated glass plates. Common solvent combinations were S_1 ($CHCl_3$–CH_3OH, 12:1), S_2 (EtOH–HOAc–H_2O, 6:3:1), S_3 (CH_3OH–1% Et_2NH in H_2O, 4:1), S_4 ($CHCl_3$–EtOH–NH_4OH, 90:10:1), and S_5 ($CHCl_3$–CH_3OH, 9:1). Autoradiography was performed at 0 °C after spraying TLC plates with PPO (New England Nuclear) and exposure to Eastman Kodak SB-5 film. TLC plates were also scanned for activity by using a Packard 7201 scanner. UV spectra were measured on a Beckman Model 25 spectrophotometer, and optical rotations were obtained on a Perkin-Elmer 141 polarimeter. The IR spectra were measured on a Perkin-Elmer Model 700 spectrophotometer. The proton and triton magnetic resonance spectra were obtained on a Bruker WP 200-MHz NMR spectrometer. Chemical shift values are expressed in parts per million downfield from internal $(CH_3)_4Si$. The high-resolution mass spectrum was performed by Shrader Analytical Laboratories. Analytical HPLC determinations were run on a Waters instrument using μ-Porasil and μ-C_{18} columns. Common solvent combinations were S_6 (CH_3CN–0.01 KH_2PO_4 (pH 3), 15:85), S_7 (CH_2Cl_2–CH_3OH–0.2% Et_2NH(aq), 90:10:1), and S_8 (CH_3OH–0.2% Et_2NH(aq), 70:30). Peak detection was performed simultaneously by a Waters 440 UV detector at 280 nm and a liquid scintillation flow monitor.

(–)-*N*-([2,3-^{3}H]allyl)naloxone (1b). (–)-*N*-propargylnoroxymorphone[8] (**1a**; 10 mg, 0.03 mmol) was exposed to tritium gas (60 Ci) in 2 mL of ethyl acetate with 10 mg of prereduced Lindlar catalyst for 45 min at 24 °C with rapid stirring. Following catalyst removal, excess solvent was evaporated off, and the residue was dissolved in 10 mL of CH_3OH (total radioactivity 1306 mCi). The crude reduction product was first purified by preparative TLC with one 1000-μm silica gel plate (S_1) followed by preparative TLC on a second 1000-μm silica gel plate (S_2). Authentic (–)-naloxone (**1c**) was allowed to elute each time side by side with crude product **1b** to facilitate its location on the TLC plates. Each time, the main radioactive band corresponding to **1b** was visualized by UV, scraped off, and eluted with EtOH. The first TLC system (silica

(6) Martin, W. R.; Eades, C. G.; Thompson, J. A.; Huppler, R. E.; Gilbert, P. E. *J. Pharmacol. Exp. Ther.* **1976**, *197*, 517.

(7) The preparation of such nor compounds from their parent alkaloids has been greatly facilitated by the use of vinyl chloroformate (Olofson, R. A.; Schnur, R. C.; Bunes, L. A. U. S. Patent 3 905 981, 1975; *Chem. Abstr.* **1976**, *84*, 42634). *N*-Propargyl precursors **1a** and **2a** have previously been described (see ref 8 and 9), but **3a** is apparently a novel compound.

(8) Precursor **1a** was prepared as previously described from (–)-noroxymorphone (Sankyo Co. Ltd., Belgian Patent 615 009, 1962; *Chem. Abstr.* **1962**, *57*, 15171*d*) and afforded spectra (IR, ^{1}H and ^{13}C NMR ($CDCl_3$), UV, and high-resolution mass spectra) and an optical rotation ($[\alpha]^{25}_D$ –93.0° (*c* 6.43, EtOH)) in harmony with its structure.

gel, S_1) nicely separated unreduced **1a** (R_f 0.84) from product **1b** (R_f 0.77, comprising about 80% of the radiochemical product mixture) and overreduced (−)-[^{3}H]-*N*-propylnoroxymorphone (R_f 0.70, comprising about 20% of the radiochemical product mixture). The total radioactivity of purified **1b** in the final EtOH eluant was 705 mCi (a 47% radiochemical yield based on **1a**). Compound **1b** was found to be 98% radiochemically pure and to coelute with **1c** on TLC (silica gel, S_1, S_2) and HPLC (μ-C_{18}, S_6). The UV (EtOH) spectrum of **1b** was completely superimposable on that of **1c**, and the specific activity of **1b** was determined to be 50 Ci/mmol by UV spectroscopy [282 nm (ϵ 1200) for **1c**]. For the ^{3}H NMR (CD_3OD) of **1b** (free base), see Figure 1.

(−)-*N*-([2,3-^{3}H]Allyl)nalorphine (2b). (−)-*N*-Propargylnormorphine[9] (**2a**; 20 mg, 0.064 mmol) was exposed to tritium gas (85 Ci) in 2 mL of ethyl acetate with 5 mg of prereduced Lindlar catalyst and 5 μL of synthetic quinoline for 30 min at 24 °C with rapid stirring. Following catalyst removal, excess solvent was evaporated off, and the residue was dissolved in 10 mL of CH_3OH (total radioactivity 541 mCi). The crude reduction was first purified by preparative TLC with one 200-μm KC18F plate (S_3) followed by preparative TLC on a second 1000-μm silica gel plate (S_4). Authentic (−)-nalorphine (**2c**) was allowed to elute each time side by side with crude product **2b** to facilitate its location on the TLC plates. Each time, the main radioactive band correspondingly to **2b** was visualized by UV, scraped off, and eluted with EtOH. The first TLC system (KC18F, S_3) nicely separated unreduced **2a** (R_f 0.74) from product **2b** (R_f 0.56) and the potentially overreduced sice products (−)-[^{3}H]-*N*-propylnormorphine (R_f 0.41) and (−)-[^{3}H]-*N*-propylnordihydromorphine (R_f 0.28). However, none of the latter overreduced products were observed. The total radioactivity of purified **2b** in the final EtOH eluant was 55 mCi (a 2% radiochemical yield based on **2a**). Compound **2b** was found to be 98% radiochemically pure and to coelute with **2c** on TLC (KC18F, S_3; silica gel, S_4) and HPLC (μ-Porasil, S_7). The UV (EtOH) spectrum of **2b** was completely superimposable on that of **2c**, and the specific activity of **2b** was determined to be 37 Ci/mmol by UV spectroscopy [285 nm (ϵ 1696) for **2c**]. For the ^{3}H NMR (CD_3OD) of **2b** (free base), see Figure 1.

(±)-*N*-Propargylnormetazocine (3a). To a solution of (±)-normetazocine (217, mg, 1 mmol) with K_2CO_3 (270 mg, 3 mmol) in 3 mL of DMF was added propargyl bromide (133 mg, 1.1 mmol) over a 1-min period with rapid stirring. The reaction was allowed to stir at 24 °C under nitrogen for 72 h. It was then filtered and diluted with 10 mL of H_2O, causing crude **3a** to precipitate out of solution. Crude **3a** was purified by preparative TLC on two 1000-μm silica gel plates (S_5). The main band (R_f 0.38) was visualized by UV, scraped off, and eluted with $CHCl_3$. Solvent evaporation yielded 101 mg (39%) of (±)-*N*-propargylnormetazocine (**3a**) as a white solid: mp 169–170 °C; homogeneous on TLC (silica gel, S_5; KC18F, S_3) and HPLC (μ-C_{18} S_8); ^{1}H NMR ($CDCl_3$) δ 6.93 (d, 1, J = 8.06 Hz, C_{10} H), 6.73 (d, 1, J = 2.69 Hz, C_7 H), 6.60 (dd, 1, J = 2.69, 8.06 Hz, C_9H), 3.40 (d, 2, J = 2.20 Hz, $NCH_2{\equiv}CH$), 3.20–1.75 (m, 8), 2.23 (t, 1, J = 2.20 Hz, $NCH_2C{\equiv}\mathbf{C}H$), 1.32 (s, 3, C_6 methyl), 0.85 (d, 3, J = 2.30 Hz, C_{11} methyl); ^{13}C NMR ($CDCl_3$) δ 154.20 (s), 143.28 (s), 128.25 (d), 113.08 (d), 112.40 (d), 80.63 (d), 72.47 (d), 57.18 (d), 45.64 (t), 43.98 (t), 41.94 (t), 41.56 (d), 36.32 (s), 25.38 (q), 23.37 (t), 14.11 (q); IR (KBr) 3250, 2930, 2120, 1620, 1590, 1470, 1370, 1350, 1310 cm^{-1}; UV (EtOH) λ_{max} 284 nm (ϵ 2410); mass spectrum Calcd for $C_{17}H_{21}NO$ (molecular ion) m/e 255.1622, found m/e 255.1631.

(±)-*N*-([2,3-^{3}H]Allyl)normetazocine (3b). (±)-*N*-Propargylnormetazocine (**3a**; 20 mg, 0.078 mmol) was exposed to tritium gas (85 Ci) in 2 mL of ethyl acetate with 10 mg of prereduced Lindlar catalyst and 5 μL of synthetic quinoline for 30 min at 24 °C with rapid stirring. Following catalyst removal, excess solvent was evaporated off, and the residue was dissolved in 10 mL of CH_3OH (total radioactivity 3017 mCi). The crude reduction was purified by preparative TLC with one 1000-μm silica gel plate (S_5). Authentic (±)-*N*-allylnormetazocine (**3c**) was allowed to elute side by side with crude product **3b** to facilitate its location on the TLC plate. The main radioactive band corresponding to **3b** was visualized by UV, scraped off, and eluted with EtOH. In this preparative TLC system (silica gel, S_5) a nice separation of unreduced **3a** (R_f 0.66) from product **3b** (R_f 0.38, comprising about 90% of the radiochemical product mixture) and overreduced side product (±)-[^{3}H]-*N*-propylnormetazocine (R_f 0.34, comprising about 10% of the radiochemical mixture) was achieved. The total radioactivity of **3b** in the EtOH eluant was 1269 mCi (a 37% radiochemical yield based on **3a**). Compound **3b** was found to be 98% radiochemically pure and to coelute with **3c** on TLC (silica gel, S_5; KC18F, S_3) and HPLC (μ-C_{18}, S_8). The UV (EtOH) spectrum of **3b** was completely superimposable on that of **3c**, and the specific activity of **3b** was determined to be 44 Ci/mmol by UV spectroscopy [284 nm (ϵ 1702) for **3c**]. For the ^{3}H NMR (CD_3OD) of **3b** (free base), see Figure 1.

Acknowledgment. We gratefully acknowledge the technical assistance of K. Bradley (NEN) in the tritiation steps discussed herein and the help of Dr. P., Srinivasan (NEN) and L. Thomas (NEN) in obtaining the ^{3}H NMR data for **1b**, **2b**, and **3b**. We also thank M. Tutunjian (NEN) and R. Wellman (NEN) for performing HPLC experiments on **1b**, **2b**, and **3b**. Finally, we acknowledge Dr. G. Pasternak (Sloan-Kettering) for gifts of (−)-noroxymorphone and **1c**, Dr. J. Neumeyer (Northeastern University) for a gift of **2c**, and A. Soria (Sterling Winthrop) for a gift of (±)-normetazocine.

Registry No. **1a**, 73232-47-0; **1b**, 79121-18-9; **2a**, 20382-77-8; **2b**, 79121-19-0; **3a**, 79171-81-6; **3b**, 79121-20-3; (±)-normetazocine, 52079-30-8.

(9) Precursor **2a** was prepared as previously described from (−)-normorphine (Koch, M. V.; Cannon, J. G.; Burkman, A. M. *J. Med. Chem.* **1968**, *11*, 977) and afforded spectra (IR, ^{1}H and ^{13}C NMR ($CDCl_3$), UV, and high resolution mass spectra) and an optical rotation ([α]$^{25}_D$ −137.0° (*c* 1, EtOH)) in harmony with its structure.