SYNTHESIS OF TRITIATED N1′-ALKYL DERIVATIVES OF THE DELTA OPIOID RECEPTOR LIGAND NALTRINDOLE

John R. Lever* and Suzanne M. Johnson

Department of Environmental Health Sciences
The Johns Hopkins University School of Hygiene and Public Health
615 North Wolfe Street, Baltimore, MD 21205-2179, USA

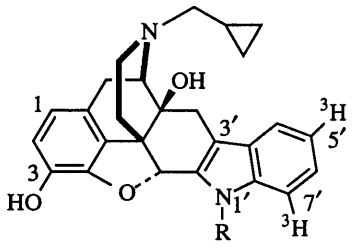
Summary

Tritiated N1′-methyl and N1′-ethyl analogues of naltrindole (NTI) have been synthesized for evaluation as radioligands for studies of delta opioid receptors. The two N1′-alkyl-5′,7′-dibromoNTI precursors for radiolabeling were prepared by base-promoted alkylation of 2,4-dibromophenylhydrazine with either iodomethane or iodoethane followed by condensation with naltrexone using the Fischer indole synthesis. Catalytic debromotritiation followed by HPLC purification afforded [^{3}H]MeNTI (17.3 Ci/mmol) and [^{3}H]EtNTI (22.5 Ci/mmol) with high chemical and radiochemical purities (≥ 99.8%).

Key Words: naltrindole, tritium, delta opioid receptor

Introduction

We have identified N1′-([^{11}C]methyl)naltrindole ([^{11}C]MeNTI) as a radioligand that allows selective localization of δ opioid receptors *in vivo* in mouse brain (1), and permits positron emission tomographic (PET) studies of δ opioid receptors in human brain (2-6). Radioligands labeled with positron emitters, however, are not appropriate for routine use in basic science investigations due to constraints imposed by the radionuclidic decay scheme and the expense of production. Accordingly, we now report the synthesis of [^{3}H]MeNTI (Figure 1) for



R = H, [^{3}H]NTI
R = Me, [^{3}H]MeNTI
R = Et, [^{3}H]EtNTI

Figure 1.

*Author for Correspondence: jlever@welchlink.welch.jhu.edu

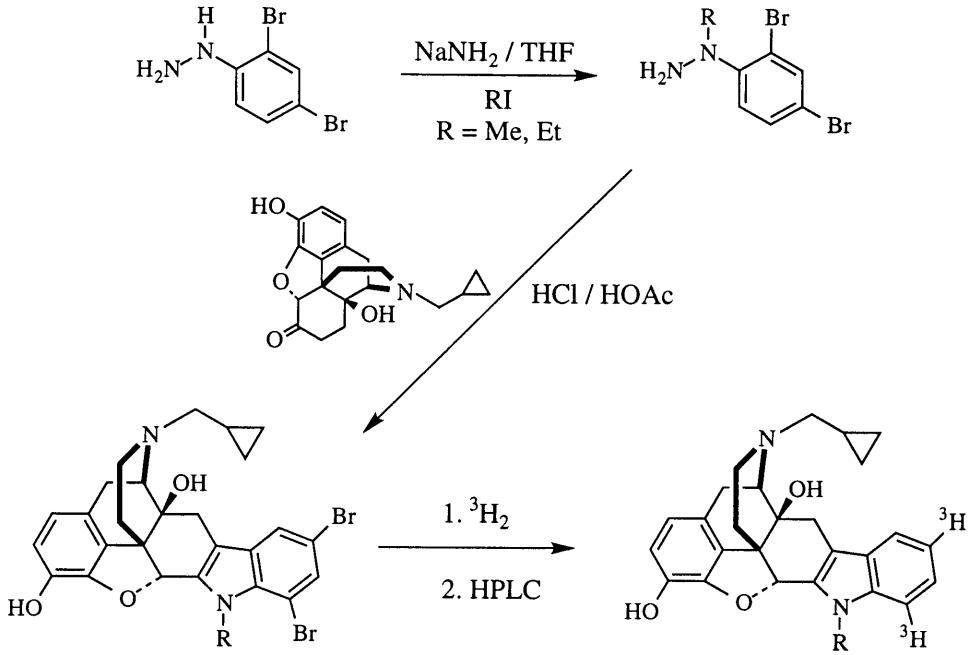
CCC 0362–4803/97/020115–08

Received 25 July 1996
Revised 26 August 1996

in vivo work in rodents, and for detailed *in vitro* binding studies. We also prepared the ethyl homologue, [^{3}H]EtNTI, for radiopharmacological evaluation. The synthetic route to both ligands culminated in catalytic dehalotritiation of a 5′,7′-dibrominated precursor, and mirrored that previously employed for the preparation of high specific activity [^{3}H]NTI (7,8).

Results and Discussion

[^{3}H]MeNTI and [^{3}H]EtNTI were prepared as shown in Scheme 1. Selective direct alkylation of 2,4-dibromophenylhydrazine at the 1-position was accomplished using sodium amide in tetrahydrofuran according to the general method of Lerch and König (9). These 1-alkyl-1-(2′,4′-dibromophenyl)hydrazines, obtained in approximately 50% yield, were then used in the Fischer indole synthesis. Condensation with naltrexone in a mixture of concentrated HCl and glacial HOAc at 85 - 120 °C provided the N1′-alkyl-5′,7′-dibromoNTI derivatives in 13 - 21% yields. Nonradioactive MeNTI was synthesized from naltrexone and 1-methyl-1-phenylhydrazine in methanol saturated with HCl (g) as described by Portoghese *et al.* (10). A sample of EtNTI was prepared (10%) in similar fashion.



Scheme 1. Synthesis of [^{3}H]MeNTI and [^{3}H]EtNTI.

Model hydrogenolysis reactions were conducted to lay the groundwork for radiolabeling. Samples of the dibromoindolomorphinans were treated with 5% Pd/C in DMF containing Et_3N under approximately 5 psi H_2 at room temperature for 2 h. Reverse phase HPLC indicated *ca.* 95% conversions to MeNTI and EtNTI. Minor components, < 4% of the reaction mixtures, were also observed with retention times between those of the products and the starting materials. These were tentatively assigned as 5′- and 7′-monobromides. In fact, one of the two side products observed along with MeNTI matched the retention time of a characterized sample of 7′-Br-MeNTI prepared during the course of our work on radioiodinated NTI analogues

(11). The findings are consistent with those of Dorn *et al.* (7), who previously reported trace levels of monobromides as side products of the [^{3}H]NTI synthesis.

The N1′-alkyl-5′,7′-dibromoNTI derivatives were then used as substrates for catalyzed halogen displacement with tritium gas by the Custom Synthesis group of DuPont / New England Nuclear. The catalyst and labile tritium were removed, and the radiolabeled products were returned in 92 - 96% purity. Portions (10 - 20 mCi) were then refined to homogeneity by HPLC, and isolated by means of solid phase extraction as solutions (*ca.* 1 mCi/mL) in buffered ethanol (1% *v/v* 5 mM TRIS, pH 7.4). No chemical or radiochemical impurities were detected by analytical HPLC in the final formulations of [^{3}H]MeNTI or [^{3}H]EtNTI (*cf.* Figure 2).

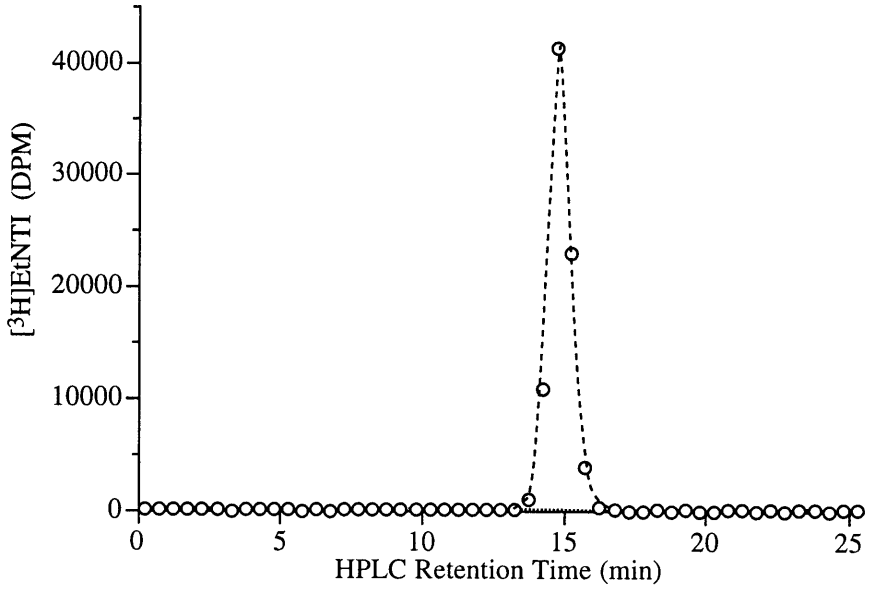


Figure 2. Analytical HPLC (β-trace) chromatogram for purified [^{3}H]EtNTI.

For specific activity determinations, the mass in samples of known radioactivity was determined by relating the UV absorbance (λ actual: 254 and 288 nm) peak height from HPLC to linear, seven-point standard curves ($r^2 \geq 0.999$). These standard curves were established with the non-radioactive materials over ranges that bracketed the regions of interest ([^{3}H]MeNTI: 280 μCi samples, 1.5 - 20.0 μg MeNTI standards; [^{3}H]EtNTI: 40 μCi samples, 0.25 - 5.0 μg EtNTI standards). Calculated values (mean ± SD; n = 3) were 17.3 ± 0.1 Ci/mmol for [^{3}H]MeNTI, and 22.5 ± 0.4 Ci/mmol for [^{3}H]EtNTI.

Catalytic tritiodehalogenation typically provides radiotracers specifically labeled with tritium in the positions that had been occupied by the halogen (7,12). In the case of [^{3}H]EtNTI, tritium NMR in CD_3OD was performed at New England Nuclear on the material of 92% initial purity. This revealed that the label was located exclusively and in equal proportions at two sites, δ 7.06 and 7.44, that correspond to the 5′- and 7′-positions. In confirmation, the proton NMR spectrum showed decreased signal integrations for $H_{5'}$ and $H_{7'}$.

Summary

[^{3}H]MeNTI and [^{3}H]EtNTI have been prepared with a high degree of chemical and radiochemical purity. The specific activities are high enough to permit their evaluation as radioligands to probe δ opioid receptors. Initial studies indicate that [^{3}H]MeNTI selectively labels δ sites in mouse brain *in vivo*, and binds to δ sites in mouse brain membranes with Kd = 75 pM, Bmax = 115 fmol/mg protein, and 82% specific binding when used at the Kd concentration (Lever *et al.*, in preparation).

Experimental

Melting points (uncorrected) were determined with a Thomas-Hoover capillary apparatus. ^{1}H NMR spectra were obtained with a Bruker WM-300 (300.13 MHz) instrument. Chemical shifts are reported in ppm (δ) relative to internal Me_4Si. High resolution electron impact mass spectroscopy (HREIMS) was performed at the University of Minnesota Mass Spectroscopy Facility, and elemental analyses were determined by Atlantic Microlab, Inc. (Norcross, GA). Tetrahydrofuran (THF) was distilled under N_2 from sodium benzophenone ketyl. Dimethylformamide (DMF) was distilled under reduced pressure from CaH_2. Iodomethane was distilled from CaH_2, and stored over copper shot and molecular sieves under argon. Other chemicals and solvents were reagent grade, and were used as received from commercial sources. Nonradioactive MeNTI was obtained from naltrexone•HCl (Mallinckrodt, Inc.) as previously reported (5,10). 1-Ethyl-1-phenylhydrazine was synthesized as described by Lerch and König (9). 2,4-Dibromophenylhydrazine was prepared by the general method of Hunsberger (13). Column chromatography was conducted with E. Merck 7729 silica gel (< 230 mesh) under N_2 pressure. Preparative thin layer chromatography (TLC) was performed with Analtech silica gel 60 F-254 plates (1000 μm) having a pre-adsorbent zone. The HPLC equipment consisted of a Rheodyne Model 7125 injector, Waters Model 510 EF pumps and Model 490 ultraviolet (UV) absorbance detector. Waters C-18 Nova-Pak columns were used for analytical (radial compression module, 8 x 100 mm, 4 μm) and preparative (7.8 x 300 mm, 6 μm) HPLC. Ternary mobile phases consisted of an organic phase (MeOH / CH_3CN; 50:50, *v/v*) mixed in various proportions with an aqueous solution of Et_3N (2.1% *v/v*) and HOAc (2.8% *v/v*). Activated Waters SEP-PAK *t*-C-18 Plus cartridges were used for solid-phase extraction. For radioactivity determinations, aliquots of eluent fractions from HPLC analyses or samples of radioligand formulations were diluted with cocktail (10 mL; Formula 963, DuPont NEN Research Products, Boston, MA), allowed to dark adapt, and counted by liquid scintillation spectrometry (Packard Instrument Co., Downers Grove, IL) at an efficiency of 47%. Counts were corrected for quenching, and had <3% error.

1-Methyl-1-(2′,4′-dibromophenyl)hydrazine Hydrochloride (1). Powdered $NaNH_2$ (0.48 g, 12 mmol) was added over 105 min to a stirred solution of 2,4-dibromophenylhydrazine (2.8 g, 10 mmol) in THF (15 mL) maintained at 4 °C. The ice bath was removed, and ammonia was eliminated by a N_2 purge for 20 min. The ice bath was replaced, and iodomethane (2.0 g, 13 mmol; neat) was added over 100 min such that the temperature did not exceed 10 °C. The mixture was allowed to warm to room temperature with stirring over a 2 h period, the reaction was quenched with H_2O (2 mL), and the THF was removed under reduced pressure. The residue was extracted with diethyl ether, dried (K_2CO_3) and filtered. The ethereal

solution was cooled at ice bath temperature, and treated with HCl (g). The solid was collected, and washed with a mixture of THF:pentane (1:1) to give 1.88 g of material with a broad mp (145 – 172 °C). ^{1}H NMR (DMSO-d_6) showed signals for **1** [δ 2.99 (s, 3H), 7.74 (s, 1H), 7.75 (d, J = 7.0 Hz, 1H), 7.97 (d, J = 7.0 Hz, 1H), 10.38 (br s, 3H)] and 2,4-dibromophenylhydrazine in an 80:20 ratio. HREIMS for **1** (free base: $C_7H_8Br_2N_2$): *m/z* calcd 277.9054, 279.9034, 281.9014; found 277.9057 (M+, 1.5%), 279.9026 (M+, 2.7%), 281.9017 (M+, 1.5%). Analytical HPLC (60% organic / 40% aqueous) at 3.5 mL/min showed 2,4-dibromophenylhydrazine (20%; t_R = 5.7 min) and **1** (80%; t_R = 17.8 min) without evidence for other components, and the mixture was carried on without further purification.

1-Ethyl-1-(2′,4′-dibromophenyl)hydrazine Hydrochloride (2). In a fashion similar to that described for **1**, treatment of 2,4-dibromophenylhydrazine (4.0 g, 15 mmol) with $NaNH_2$ (0.7 g, 18 mmol) and iodoethane (2.9 g, 19 mmol) in THF (25 mL) gave **2**•HCl contaminated with 2,4-dibromophenylhydrazine•HCl. The free base was liberated, and then purified by short-path chromatography (40 g silica gel; hexane:EtOAc:Et_3N, 91:5:4) to give a white solid that was dissolved in CH_2Cl_2 and treated with HCl (g) to provide **2**•HCl (2.6 g, 53%): mp 185 – 187 °C. ^{1}H NMR (DMSO-d_6): δ 1.18 (t, J = 7.2 Hz, 3H), 3.40 (q, J = 7.2 Hz, 2H), 7.84 (s, 1H), 7.85 (d, J = 2.1 Hz, 1H), 8.08 (d, J = 2.1 Hz), 10.46 (br s, 3H). Anal. Calcd. for $C_8H_{11}Br_2N_2Cl$: C, 29.08; H, 3.36; N, 8.48. Found: C, 29.19; H, 3.38; N, 8.38. HREIMS (free base: $C_8H_{10}Br_2N_2$): *m/z* calcd 291.9210, 293.9190, 295.9170; found 291.9211 (M+, 1.2%), 293.9201 (M+, 2.5%), 295.9158 (M+, 1.2%).

17-(Cyclopropylmethyl)-6,7-dehydro-4,5α-epoxy-3,14-dihydroxy-5′,7′-dibromo-6,7-2′,3′-(1′-methyl)–indolomorphinan (3). To naltrexone•HCl (1.5 g, 4.1 mmol) in a mixture of concentrated HCl (40 mL) and glacial HOAc (160 mL) at ambient temperature was added **1**•HCl (2.0 g, 6.3 mmol; 80% purity). The mixture was refluxed for 1 h, and then stirred overnight at 85 °C. After cooling to room temperature and concentration under reduced pressure, the residue was dissolved in MeOH/H_2O (1:1, 150 mL) and the pH adjusted to 10 with NaOH pellets. The mixture was extracted with CH_2Cl_2 (3 x 50 mL), and the combined extracts were dried (K_2CO_3), filtered and concentrated under reduced pressure to give a brown residue. Short-path column chromatography (120 g silica gel; hexane:EtOAc:MeOH:Et_3N, 78:15:5:2) gave 0.50 g (21%) of **3**. Final purification (5 mg samples) was achieved by preparative HPLC (40% organic / 60% aqueous; 12.0 mL/min). Eluent fractions containing product (t_R = 52 min, k' = 34) were extracted with CH_2Cl_2, passed through a column of W-200B alumina, and concentrated to give **3**, >99% purity by analytical HPLC, as a white solid: ^{1}H NMR ($CDCl_3$): δ 0.15 (d, J = 4.6 Hz, 2H), 0.54 (d, J = 6.9 Hz, 2H), 0.84 (m, 1H), 1.79 (d, J = 11.5 Hz, 1H), 2.37 (m, 3H), 2.52 (d, J = 18.0 Hz, 1H), 2.73 (m, 3H), 3.12 (d, J = 20.0 Hz, 1H), 3.32 (d, J = 6.0 Hz, 1H), 4.15 (s, 3H), 4.6 (br s, 1H), 5.68 (s, 1H), 6.54 (d, J = 8.2 Hz, 1H), 6.58 (d, J = 8.2 Hz, 1H), 7.42 (dd J = 2.2, 13.5 Hz, 1H). HREIMS ($C_{27}H_{26}Br_2N_2O_3$): *m/z* calcd 584.0310, 586.0290, 588.0270; found 584.0303 (M+, 3.4%), 586.0340 (M+, 6.7%), 588.0324 (M+, 3.3%).

17-(Cyclopropylmethyl)-6,7-dehydro-4,5α-epoxy-3,14-dihydroxy-5′,7′-dibromo-6,7-2′,3′-(1′-ethyl)–indolomorphinan (4). As described for **3**, Fischer indole synthesis using naltrexone•HCl (0.53 g, 1.4 mmol) and **2**•HCl (0.74 g, 2.2 mmol) gave a brown residue that was purified by short path column

chromatography (50 g silica gel; hexane:EtOAc:MeOH:Et_3N, 78:15:5:2) to give **4** as a white solid. This material was dissolved in $CHCl_3$ and treated with HCl (g) to generate the salt. Subsequently, the free base was liberated by dissolution in aqueous base (pH 10). Extraction with CH_2Cl_2, drying (K_2CO_3), and concentration under reduced pressure gave **4** (0.10 g, 13%) of >99% purity by analytical HPLC. ^1H NMR ($CDCl_3$): δ 0.16 (d, J = 5.0 Hz, 2H), 0.56 (d, J = 6.0 Hz, 2H), 0.87 (m, 1H), 1.48 (t, J = 7.5 Hz, 3H), 1.80 (d, J = 15.0 Hz, 1H), 2.38 (m, 5H), 2.54 (d, J = 22.0 Hz, 1H), 2.78 (m, 3H), 3.12 (d, J = 22.0 Hz, 1H), 3.32 (d, J = 5.0 Hz, 1H), 4.60 (m, 1H), 4.73 (m, 1H), 5.69 (s, 1H), 6.54 (d, J = 6.4 Hz, 1H) 6.62 (d, J = 6.4 Hz, 1H), 7.45 (d, J = 6.4 Hz, 1H). HREIMS ($C_{28}H_{28}Br_2N_2O_3$): *m/z* calcd 598.0466, 600.0446, 602.0426; found 598.0455 (M+, 1.2%), 600.0489 (M+, 1.9%), 602.0467 (M+, 1.0%).

17-(Cyclopropylmethyl)-6,7-dehydro-4,5α-epoxy-3,14-dihydroxy-6,7-2′,3′-(1′-ethyl)–indolomorphinan (EtNTI, 5). A mixture of naltrexone•HCl (0.36 g, 0.96 mmol) and 1-ethyl-1-phenylhydrazine•HCl (0.25 g, 1.5 mmol) in MeOH (20 mL) saturated with HCl (g) was refluxed for 36 h, cooled to room temperature, and then concentrated under reduced pressure. The residue was partitioned between CH_2Cl_2 and aqueous base (pH 10), and the organic extracts were dried over K_2CO_3, filtered and concentrated to give a brown solid. Preparative TLC (hexane:EtOAc:MeOH:Et_3N, 59:29:5:2) gave a yellow solid that was crystallized from MeOH/H_2O to yield **5** as a white powder (40 mg, 10%): ^1H NMR ($CDCl_3$): δ 0.14 (d, J = 4.5 Hz, 2 H), 0.55 (d, J = 7.5 Hz, 2H), 0.88 (m, 1H), 1.42 (t, J = 7.5 Hz, 3H), 1.79 (d, J = 13.5 Hz, 1H), 2.35 (m, 3H), 2.62 (d, J = 15.5 Hz, 1H), 2.76 (m, 3H), 2.88 (d, J = 6.8 Hz, 1H), 3.10 (d, J = 18.4 Hz, 1H), 3.34 (d, J = 6.8 Hz, 1H), 4.25 (m, 2H), 4.82 (br s, 1H), 5.75 (s, 1H), 6.48 (d, J = 8.3 Hz. 1H), 6.55 (d, J = 8.3 Hz, 1H), 7.01 (t, J = 8.3 Hz, 1H), 7.16 (t, J = 7.5, 1H; $H_{5'}$), 7.25 (d, J = 8.3 Hz, 1H), 7.41 (d, J = 8.3 Hz, 1H; $H_{7'}$). HREIMS ($C_{28}H_{30}N_2O_3$): *m/z* calcd 442.2258; found 442.2260 (M+, 6.2%).

Catalytic hydrogenolysis reactions. Samples of **3** and **4** (1.7 - 4.1 mg, 2.7 - 7.0 μmol) were treated with 5% Pd/C (3 - 7 mg) in DMF (0.5 - 1.0 mL) containing Et_3N (25 - 50 μL) in a septum-sealed vial under a balloon (*ca.* 5 psi) of hydrogen gas at room temperature. After 2 h, the mixtures were passed through a PTFE filter (13 mm, 0.45 μm) and examined by analytical HPLC. Retention profiles at 40% organic / 60% aqueous (3.5 mL/min) showed that EtNTI (t_R = 5.8 min, k' = 2.2; 95%) was readily resolved from the dibrominated precursor (t_R = 76.0 min, k' = 41.0; 1.5%) and several minor unknowns (t_R 's 12 - 22 min; *ca.* 3.5%). Under these HPLC conditions, MeNTI (t_R = 3.1 min; 96%) was resolved from two minor components (t_R = 5.5 and 7.9 min; 4%). The material at 7.9 min matched the t_R of an authentic sample of 7′-Br-MeNTI (11).

[^{3}H]MeNTI and [^{3}H]EtNTI. Tritiation reactions were carried out on **3** and **4** by the Custom Synthesis Group of DuPont / New England Nuclear (Boston, MA) under proprietary catalyzed halogen displacement conditions using tritium gas. Catalyst and labile tritium were removed, and the products were supplied in ethanol (1 mCi/mL) with quoted radiochemical purities of 92 - 96%. Portions of these solutions were taken to dryness under reduced pressure, and the residues were dissolved in HPLC mobile phase. Aliquots (10 - 20 mCi) were then purified by HPLC on a C-18 Nova-Pak column (8 x 100 mm) using the ternary mobile phase at

a flow rate of 3.5 mL/min with UV absorbance detection at 254 and 288 nm. The effluent fractions corresponding to [^{3}H]MeNTI (t_R = 19.2 min, k' = 23.0; 25% organic / 75% aqueous) and [^{3}H]EtNTI (t_R = 14.9 min, k' = 17.6; 30% organic / 70% aqueous) were collected and diluted with nine parts of distilled H_2O. These solutions were passed through activated solid-phase extraction cartridges that subsequently were flushed with water (10 mL) and purged with argon. Over 99% of the tritiated material was retained. Elution of the cartridges with EtOH (10 - 12 mL) containing 5 mM TRIS (pH 7.4, 1% *v/v*) gave solutions of the radioligands having >99.8% chemical and radiochemical purities by analytical HPLC. The mass in samples of known radioactivity (40 - 280 μCi) was assessed by comparing HPLC peak height to linear standard curves ($r^2 \geq 0.999$) for MeNTI (1.5 - 20.0 μg) and EtNTI (0.25 - 5.0 μg). Specific activities (mean ± SD; n = 3) were 17.3 ± 0.1 Ci/mmol for [^{3}H]MeNTI, and 22.5 ± 0.4 Ci/mmol for [^{3}H]EtNTI.

Acknowledgments

This research was supported by NIDA grants DA 08816 and DA 08870, and by a postdoctoral fellowship (SMJ) from training grant NCI CA 09199. The authors thank Dr. Chris M. Kinter for preliminary studies.

References

1. Lever J. R., Scheffel U., Kinter C. M., Ravert H. T., Dannals R. F., Wagner H. N., Jr., and Frost J. J. - Eur. J. Pharmacol. 216: 459 (1992).

2. Frost J. J., Lever J. R., Scheffel U., Dannals R. F., Kinter C. M., Ravert H. T. - J. Nucl. Med. 34S: 103P (1993).

3. Smith J. S., Zubieta J. K., Price J. C., Madar I., Lever J. R., Kinter C. M., Ravert H. T., Dannals R. F., and Frost J. J. - Soc. Neurosci. Abstr. 20: 1384 (1994).

4. Madar I., Lesser R., Krauss G., Lever J. R., Kinter C., Ravert H. T., Musachio J. L., Mathews W. B., Dannals R. F., and Frost J. J. - J. Nucl. Med. 35S: 31P (1994).

5. Lever J. R., Kinter C. M., Ravert H. T., Musachio J. L., Mathews W. B., and Dannals R. F. - J. Labelled Compds. Radiopharm. 36: 137 (1995).

6. Madar I., Lever J. R., Kinter C. M., Scheffel U., Ravert H. T., Musachio J. L., Mathews W. B., Dannals R. F., and Frost J. J. - Synapse, in press (1996).

7. Dorn C. R., Markos C. S., Dappen M. S., and Pitzele B. S. - J. Labelled Compds. Radiopharm. 31: 375 (1992).

8. Yamamura M. S., Horvath R., Toth G., Otvos F., Malatynska E., Knapp R. J., Porreca F., Hruby V. J., and Yamamura H. I. - Life Sci. 50: 24 (1992).

9. Lerch U. and König J. - Synthesis 157 (1983).

10. Portoghese P. S., Sultana M., and Takemori A. E. - J. Med. Chem. 33: 1714 (1990).

11. Kinter C. M. and Lever J. R. - Nucl. Med. Biol. 22: 599 (1995).

12. Evans E. A. *Tritium and its Compounds* (2nd Edition), Butterworths, London, (1974).

13. Hunsberger I. M., Shaw E. R., Fugger J., Ketcham R., and Lednicer D. - J. Org. Chem. 21: 394 (1956).