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SYNTHESIS OF N1′-([^{11}C]METHYL)NALTRINDOLE ([^{11}C]MeNTI): A RADIOLIGAND FOR POSITRON EMISSION TOMOGRAPHIC STUDIES OF DELTA OPIOID RECEPTORS

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Summary

A delta opioid receptor antagonist, N1′-methylnaltrindole (MeNTI), has been labeled with carbon-11. The precursor for radiolabeling was prepared in 71% yield by benzylation of the phenolic moiety of naltrindole. Alkylation of the indole nitrogen using [^{11}C]iodomethane and aqueous tetra(*n*-butyl)ammonium hydroxide at 80 °C in dimethylformamide followed by hydrogenolysis (H_2, 10% Pd-C) of the benzyl protecting group gave [^{11}C]MeNTI. The average (n = 10) time for radiosynthesis, HPLC purification and formulation was 24 min from end-of-bombardment. [^{11}C]MeNTI of high radiochemical purity was obtained at end-of-synthesis with an average specific activity of 2050 mCi/μmol and radiochemical yield, based on [^{11}C]iodomethane, of 6%.

Key Words: naltrindole, carbon-11, radiotracer, positron emission tomography, delta opioid receptor

Introduction

Multiple receptor types, broadly classed as μ, κ and δ, mediate the diverse actions of the endogenous opioid system (1). Within the last ten years, several radioligands have been identified for imaging central opioid sites in human beings by positron emission tomography (PET). These include the μ-selective ligand [^{11}C]carfentanil (2,3), as well as the non-selective agents [^{11}C]diprenorphine (4-7) and [^{18}F]cyclofoxy (8,9). Radioligands for direct assessment of κ and δ sites by

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PET would allow complementary studies, and would facilitate efforts to understand the differential regulation of each type of opioid receptor in health and disease. Potential avenues of investigation include the pathophysiology of certain neurologic and psychiatric disorders (10) as well as the neurochemistry of drug abuse (11-14).

For PET studies of δ opioid receptors, we chose to evaluate N1′-([^{11}C]methyl)naltrindole ([^{11}C]MeNTI, Figure 1). Selection of this target was based on the finding by Portoghese and colleagues (15) that MeNTI displays high affinity (K_i 20 pM) for δ sites *in vitro*, exhibits excellent overall selectivity (> 700-fold) for δ over either μ or κ sites, and behaves as an opioid receptor antagonist in functional assays. In a brief communication, we reported that [^{11}C]MeNTI labels δ sites *in vivo* in mouse brain (16). Recent studies indicate that [^{11}C]MeNTI and PET can be used for localization (17) and quantification (18) of δ opioid receptors in human brain, and for detection of alterations in δ sites associated with temporal lobe epilepsy (19). Here we provide the details of the synthetic route to [^{11}C]MeNTI.

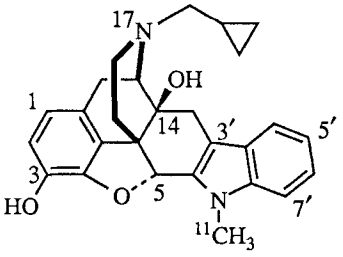


Figure 1. Structure of [^{11}C]MeNTI.

Results and Discussion

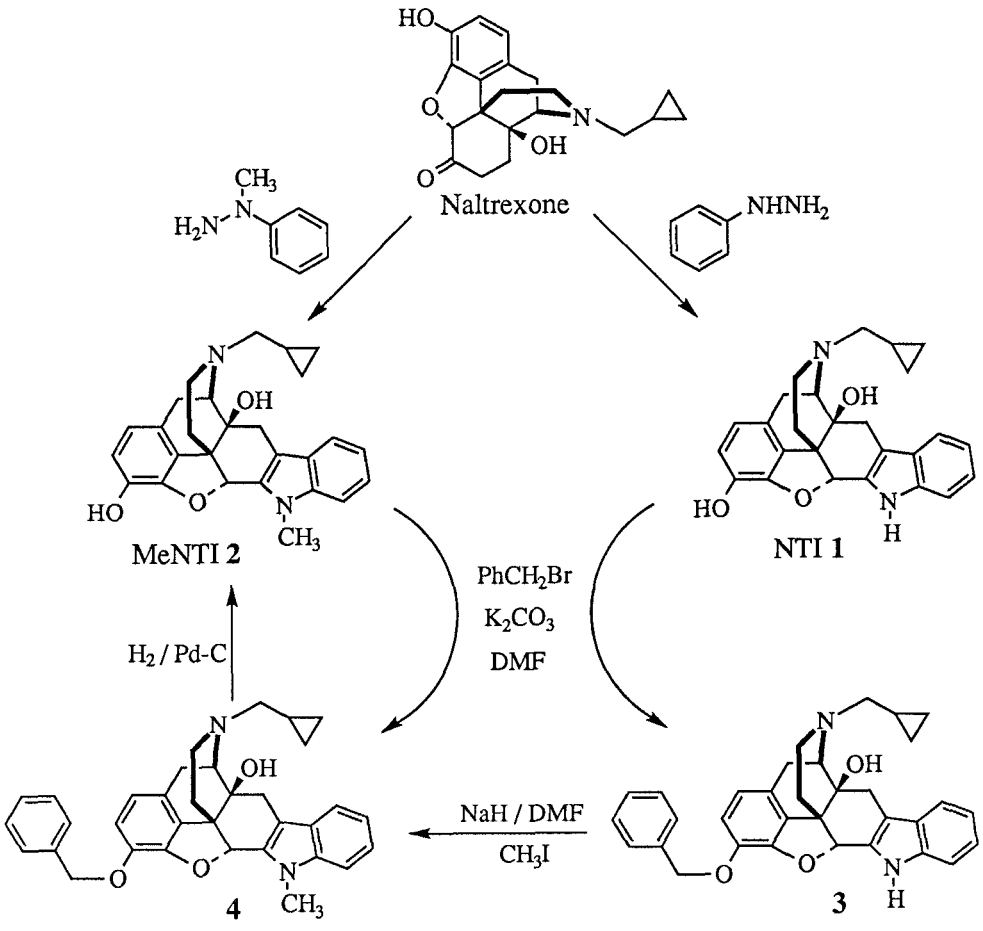
The strategy for production of [^{11}C]MeNTI calls for selective [^{11}C]-methylation of the indole nitrogen in preference to the phenolic and tertiary hydroxyl groups (*cf.* Figure 1). Since the sterically hindered tertiary hydroxyl at the C-14 position of such 4,5α-epoxymorphinans is resistant to alkylation by iodomethane (20), we anticipated that a sequence involving protection of the phenol, followed by N1′-methylation and deprotection would suffice. The synthetic chemistry conducted as a prelude to radiolabeling is shown in Scheme 1.

Using the Fischer indole synthesis, NTI (**1**) and MeNTI (**2**) were prepared by condensation of naltrexone with the appropriate phenylhydrazine as described by Portoghese (15). The phenolic moiety of NTI was then protected as a benzyl ether to provide **3** for use as a precursor in the methylation step. This conversion was achieved in 71% yield by treatment of NTI with potassium carbonate and benzyl bromide in DMF. In similar fashion, an authentic sample of the intermediate N1′-methylation product **4** was prepared in 72% yield by benzylation of MeNTI.

Treatment of **3** with an excess of either powdered NaH or aqueous tetra(*n*-butyl)ammonium hydroxide (TBAH) and 0.2 equivalents of iodomethane in DMF at

90 °C for 10 min gave nearly quantitative conversion, based upon alkylating agent, to material having the reverse-phase HPLC chromatographic properties of **4**. When 1.2 equivalents of iodomethane were used, the product was isolated in 54% yield by normal-phase TLC. Indicative of selective N1′- *vs.* O-alkylation, no resonance (*ca.* 3.30 ppm) appropriate for a C-14 methoxy group (20) was observed by ^{1}H NMR. In fact, the ^{1}H NMR spectral characteristics of material synthesized by base-promoted methylation of **3** were identical to those of **4** prepared as described above.

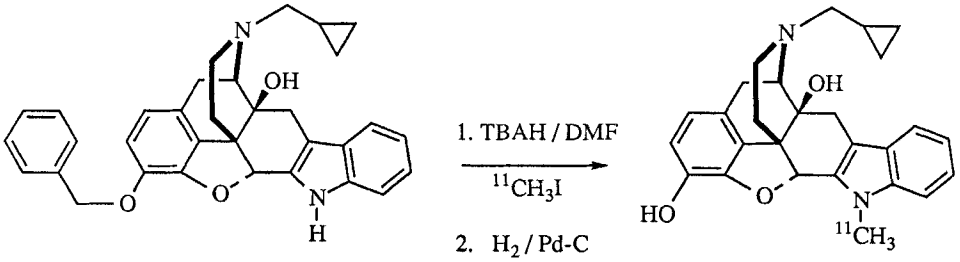
The benzyl protecting group of **4** was removed within 10 min by hydrogenolysis using 10% Pd-C and hydrogen gas at atmospheric pressure, or by catalytic transfer hydrogenation using methanolic ammonium formate and 10% Pd-C (21). Isolated yields of **2** were on the order of 30%.



Scheme 1.

The results suggested that the radiosynthesis of [^{11}C]MeNTI might be possible through sequential reactions without discrete isolation of [^{11}C]-labeled **4**. This would be advantageous due to constraints imposed by the short half-life (20.4 min) of carbon-11. In exploratory radiochemistry, treatment of **3** with [^{11}C]iodomethane and either NaH or TBAH for 2 min at 80 °C in DMF gave 70 - 80% conversion to one major radioproduct which corresponded to [^{11}C]-labeled **4** by analytical HPLC. Subsequent treatment of these reaction mixtures for 4 min at 80 °C with 10% Pd-C and either methanolic ammonium formate or hydrogen gas gave approximately 20 - 30% conversion of [^{11}C]-labeled **4** to material which co-eluted with an authentic sample of non-radioactive MeNTI under several different HPLC conditions. Attempts to improve the conversion by HPLC purification of [^{11}C]-labeled **4** prior to hydrogenolysis, or by performing the debenzylation step twice, were not successful. In addition, it was noted that a wash of the charcoal with acidic DMF was required to ensure efficient recovery of the radioactive materials.

Various combinations of reaction parameters and the use of HPLC for purification gave [^{11}C]MeNTI in radiochemical yields up to 10% at end-of-synthesis based on [^{11}C]iodomethane. Operationally, the tandem use of TBAH as base along with a single debenzylation by hydrogen gas and 10% Pd-C proved an expedient method to implement for routine production (Scheme 2).



Scheme 2. Radiosynthesis of [^{11}C]MeNTI.

Under the semi-preparative reverse-phase HPLC conditions employed for purification, [^{11}C]MeNTI (t_R = 8.1 min, k' = 8.9) was well separated from NTI (t_R = 5.4 min, k' = 5.6), the predominant non-radioactive material (Figure 2). The radioactive material corresponding to [^{11}C]MeNTI was collected, evaporated to dryness under reduced pressure, and reconstituted under sterile conditions with 0.9% saline / 8.4% sodium bicarbonate (70/30 *v/v*) to provide a solution of approximately pH 7.4. For ten runs, the average time for radiosynthesis, HPLC purification and formulation was 24 min from end-of-bombardment.

Chemical and radiochemical purity, as well as specific activity, were determined by analytical HPLC. Specific activities were calculated by relating the area of the UV absorbance peak of carrier MeNTI in an aliquot of known radioactivity to the area of a standard sample of MeNTI. [^{11}C]MeNTI of > 99% radiochemical purity was obtained at end-of-synthesis with an average (n = 10)

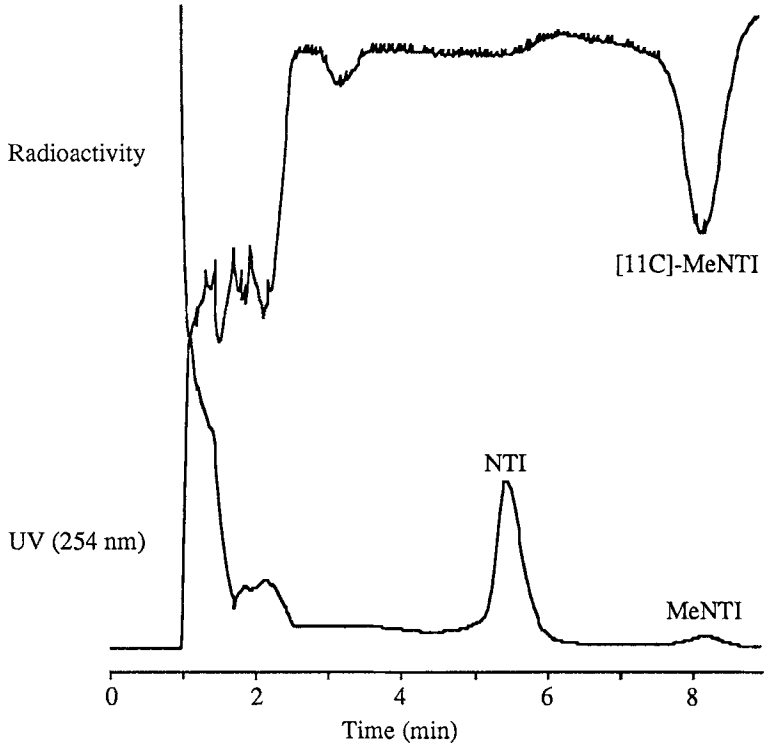


Figure 2. Semi-preparative HPLC chromatograms for [^{11}C]MeNTI radiosynthesis.

specific activity of 2050 mCi/μmol and radiochemical yield, based upon [^{11}C]iodomethane, of 6%. Removal of > 99.8% of NTI was achieved during the HPLC purification. Thus, the ratio of carrier MeNTI to residual NTI in final formulations was typically > 9 to 1 although the proportions varied according to the specific activity. Final formulations of [^{11}C]MeNTI were determined to be sterile and pyrogen-free using standard tests.

Conclusion

We have developed synthetic procedures which provide [^{11}C]MeNTI with high specific activity and a high degree of radiochemical purity. Although radiochemical yields are modest, sufficient radioligand can be produced to allow PET investigations of central δ opioid receptors.

Experimental

Chemicals and solvents were reagent grade, and were used as received unless otherwise stated. DMF was prepared by sequential distillation under reduced pressure from CaH_2 and BaO. Iodomethane was distilled from CaH_2, and stored

over copper shot and molecular sieves under argon. Uncorrected melting points were determined with a Thomas-Hoover capillary apparatus. ^{1}H NMR spectra were obtained with a Bruker WM-300 (300.13 MHz) instrument. Characteristic chemical shifts are reported in ppm (δ) relative to internal Me_4Si in $CDCl_3$. High resolution fast atom bombardment mass spectroscopy (HRFABMS) was done at the University of Minnesota Mass Spectroscopy Facility. Elemental analyses were determined by Atlantic Microlab, Inc. Short-path column chromatography was conducted with E. Merck 7729 (< 230 mesh) silica gel under N_2 pressure. Analytical TLC was conducted on Macherey-Nagel silica gel 60 UV-254 plates (250 μm) and preparative TLC on Analtech silica gel 60 F-254 plates (1000 μm or 250 μm). The HPLC equipment consisted of Rheodyne 7126 injectors, Waters 590EF pumps, Waters 440 UV absorbance detector (254 nm), and a NaI(Tl) crystal (2") scintillation detector. Hewlett-Packard 3390A integrators and a Rainin Dynamax system were used to record and analyze HPLC chromatograms. Analytical (4.6 x 250 mm) and semi-preparative (10 x 250 mm) reverse-phase HPLC columns (Econosil C-18, 10 μm) were from Alltech Applied Sciences. Radioactivity was measured with a Capintec CRC-12R radioisotope dose calibrator. NTI and MeNTI were prepared from naltrexone (Mallinckrodt, Inc.) as previously described (14), and gave appropriate ^{1}H NMR and HRFABMS data. [^{11}C]Carbon dioxide, produced with a biomedical cyclotron (Instrument AB Scanditronix MC-16F) using the $^{14}N(p, \alpha)^{11}C$ reaction, was converted to [^{11}C]iodomethane as previously described (2). Conventional 4,5α-epoxymorphinan nomenclature is used in the text, while Chemical Abstracts Service (CAS) nomenclature for MeNTI is given in reference 22.

17-(Cyclopropylmethyl)-6,7-dehydro-4,5α-epoxy-3-benzyloxy-14-hydroxy-6,7-2′,3′-indolomorphinan (3-O-BenzylNTI, 3). A solution of NTI (100 mg, 0.242 mmol) in DMF (1.0 mL) containing K_2CO_3 (154 mg, 1.11 mmol) and benzyl bromide (57 μl, 0.48 mmol) was heated at 90 °C for 2 h. The mixture was partitioned between saturated $NaHCO_3$ and CH_2Cl_2. The organic extracts were dried (Na_2SO_4), filtered, and concentrated under reduced pressure. Short-path column chromatography using a gradient of hexane/ethyl acetate (75/25 to 50/50, *v/v*) containing 2% triethylamine gave 86 mg (71%) of **3** as an off-white solid which was recrystallized from CCl_4/hexane: mp 130 – 134 °C (dec.). ^{1}H NMR: δ 8.18 (s, 1H, NH), 7.42 (d, J = 9.0 Hz, 1H, $H_{7'}$), 7.33 – 7.20 (m, 6H, Ph), 7.15 (t, J = 7.0 Hz, 1H, $H_{6'}$), 7.04 (t, J = 8.0 Hz, 1H, $H_{5'}$), 6.65 (d, J = 8.2 Hz, 1H, H_2), 6.54 (d, J = 8.2 Hz, 1H, H_1), 5.68 (s, 1H, $H_{5\alpha}$), 5.06 (d, J = 17.8 Hz, 1H, $-OCH_2Ph$), 5.02 (d, J = 17.8 Hz, 1H, $-OCH_2Ph$), 3.39 (d, J = 6.4 Hz, 1H), 3.14 (d, J = 15.8 Hz, 1H), 2.91 (d, J = 15.8 Hz, 1H), 2.85 – 2.70 (m, 2H), 2.63 (d, J = 15.7 Hz, 1H), 2.50 – 2.20 (m, 4H), 1.81 (d, J = 13.0 Hz, 1H), 1.50 (bs, 1H, OH), 0.92 (m, 1H), 0.58 (br d, J = 8.4 Hz, 2H), 0.17 (br d, J = 4.5 Hz, 2H). HRFABMS: Calcd. for $C_{33}H_{33}N_2O_3$ (M+1): 505.2491, Found: 505.2477. Anal. Calcd. for $C_{33}H_{33}N_2O_3 \cdot 0.75\ H_2O$: C, 76.50; H, 6.51; N, 5.41. Found: C, 76.76; H, 6.48; N, 5.40.

17-(Cyclopropylmethyl)-6,7-dehydro-4,5α-epoxy-3-benzyloxy-14-hydroxy-6,7-2′,3′-(1′-methyl)–indolomorphinan (3-O-BenzylMeNTI, 4). Method A. A solution of MeNTI (33 mg, 77 μmol) in DMF (0.5 mL) containing K_2CO_3 (49 mg, 350 μmol) and benzyl bromide (18 μl, 150 μmol) was heated at 80 °C for 3 h. An additional aliquot of benzyl bromide (10 μl, 83 μmol) was added, and heating continued for 30 min. The mixture was partitioned between saturated $NaHCO_3$ and CH_2Cl_2. The organic extracts were dried (Na_2SO_4), filtered, and concentrated under reduced pressure. Short-path column chromatography using hexane/ethyl acetate (80/20, *v/v*) containing 2% triethylamine gave 29 mg (72%) of the product as a wax: ^{1}H NMR: δ 7.44 (d, J = 7.8 Hz, 1H, $H_{7'}$), 7.30 – 7.15 (m, 7H), 7.05 (t, J = 7.9 Hz, 1H, $H_{5'}$), 6.65 (d, J = 8.1 Hz, 1H, H_2), 6.54 (d, J = 8.1 Hz, 1H, H_1), 5.80 (s, 1H, $H_{5\alpha}$), 5.06 (m, 2H, –OCH_2Ph), 3.86 (s, 3H, NCH_3), 3.39 (d, J = 6.3 Hz, 1H), 3.14 (d, J = 18.5 Hz, 1H), 2.91 (d, J = 15.6 Hz, 1H), 2.85 – 2.73 (m, 2H), 2.67 (d, J = 15.6 Hz, 1H), 2.55 – 2.20 (m, 4H), 1.85 (d, J = 12.9 Hz, 1H), 1.5 (bs, 1H, OH), 0.91 (m, 1H), 0.58 (br d, J = 7.6 Hz, 2H), 0.17 (br d, J = 4.9 Hz, 2H). HRFABMS: Calcd. for $C_{34}H_{35}N_2O_3$ (M+1): 519.2649, Found: 519.2646. Anal. Calcd. for $C_{34}H_{35}N_2O_3 \cdot 1.0\ H_2O$: C, 75.95; H, 6.93; N, 5.21. Found: C, 76.20; H, 6.69; N, 5.30. **Method B.** A solution of **3** (22 mg, 44 μmol) in DMF (0.25 mL) containing NaH (5 mg, 208 μmol) and iodomethane (3.3 μL, 7.5 mg, 53 μmol) was heated at 90 °C for 10 min. The mixture was partitioned between saturated $NaHCO_3$ and CH_2Cl_2. The organic extracts were dried (Na_2SO_4), filtered, and concentrated under reduced pressure. Preparative TLC (ethyl acetate/hexane, 33/67; 2% triethylamine) gave 12 mg (54%) of a waxy solid with spectral characteristics identical to those described for the material prepared by Method A.

17-(Cyclopropylmethyl)-6,7-dehydro-4,5α-epoxy-3,14-dihydroxy-6,7-2′,3′-(1′-methyl)–indolomorphinan (MeNTI, 2). To a solution of **4** (12 mg, 23 μmol) in DMF (0.2 mL) was added 10% Pd-C (5 mg) followed by methanolic NH_4HCO_2 (0.2 mL, 0.64 M). The mixture was heated at 85 °C for 10 min, cooled and filtered through Celite. After concentration under reduced pressure, preparative TLC (hexane/ethyl acetate, 70/30; 2% triethylamine) gave 3.1 mg (31%) of MeNTI which was spectroscopically identical to that prepared by the literature method (14). ^{1}H NMR: δ 7.41 (d, J = 7.4 Hz, 1H, $H_{7'}$), 7.19 (m, 2H), 7.03 (m, 1H), 6.83 (d, J = 8.4 Hz, 1H, H_2), 6.35 (d, J = 8.3 Hz, 1H, H_1), 5.78 (s, 1H, $H_{5\alpha}$), 3.73 (s, 3H, NCH_3), 3.12 (d, J = 18.4 Hz, 1H), 2.90 (d, J = 15.6 Hz, 1H), 2.85 – 2.70 (m, 3H), 2.66 (d, J = 15.6 Hz, 1H), 2.60 – 2.20 (m, 5H), 1.82 (d, J = 13.0 Hz, 1H), 0.88 (m, 1H), 0.57 (br d, J = 7.6 Hz, 2H), 0.17 (br d, J = 7.6 Hz, 2H).

Radiosynthesis of [^{11}C]MeNTI: [^{11}C]Iodomethane, carried by a stream of N_2, was trapped in a cooled (-78 °C) solution of **4** (1.0 mg, 2.0 μmol) in DMF (0.2 mL). Aqueous TBAH (20 μL, 0.5 M) was added, and the mixture was heated at 80 °C for 2 min. This solution, as well as an ethanol rinse (0.2 mL) of the vial, was transferred to a septum-sealed vessel previously charged with 10% Pd-C (5 mg) and

fitted with a H_2 filled balloon reservoir by means of a stopcock and needle assembly. After 4 min at 80 °C, the contents were passed through a PTFE filter (13 mm, 0.45 μm). The hydrogenolysis vessel was then rinsed with a mixture of DMF (0.2 mL) and HCl (0.05 mL, 1.0 N). This wash was passed through the PTFE filter, combined with the main batch, and applied to the semi-preparative HPLC column. Elution with CH_3CN/H_2O (30/70) containing NH_4HCO_2 (0.1 M) and HOAc (0.5% *v/v*) at 10 mL/min gave [^{11}C]MeNTI (t_R = 8.1 min, k' = 8.9) which was well separated from NTI (t_R = 5.4 min, k' = 5.6). After concentration to dryness under reduced pressure, the radioligand was reconstituted in sterile 0.9% saline (7.0 mL), and passed through a 0.2 μm sterile filter (Acrodisc®, Gelman) into a sterile, pyrogen-free multi-dose vial. Sterile $NaHCO_3$ (3.0 mL, 8.4%) was added to give a final formulation of pH 7.4. An aliquot was assayed for radioactivity, and checked by analytical HPLC using the mobile phase described above at 4.0 mL/min. The single radioactive product corresponded to MeNTI (t_R = 4.6 min, k' = 8.2), and was resolved from NTI (t_R = 3.0 min, k' = 5.0). Specific activity was calculated by relating radioactivity to the mass associated with the UV absorbance peak of carrier.

Acknowledgments

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22. MeNTI has CAS Registry Number [111555-57-8], and is listed as 4,8-Methanobenzofuro[2,3-*a*]pyrido[4,3-*b*]carbazole-1,8a(9*H*)-diol, 7-(cyclopropyl methyl)-5,6,7,8,14,14b-hexahydro-14-methyl-, [8*R*-(4b*S**,8α, 8aβ,14bβ)]-. Using this nomenclature, [^{11}C]MeNTI has carbon-11 at the 14-methyl position.