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Synthesis of a Radiotracer for Studying σ Receptors *In Vivo* Using PET: (+)-*N*-[^{11}C]-benzyl-*N*-normetazocine (1*S*,5*S*,9*S*-(+)-*cis*-2-[^{11}C]-benzyl-2'-hydroxy-5,9-dimethyl-6,7-benzomorphan)

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Summary

(+)-*N*-[^{11}C]-Benzyl-*N*-normetazocine (1*S*,5*S*,9*S*-(+)-*cis*-2-[^{11}C]-benzyl-2'-hydroxy-5,9-dimethyl-6,7-benzomorphan), a potent and selective ligand for the σ receptor, was prepared by N-benzylation of (+)-*cis*-*N*-normetazocine with [α-^{11}C]-benzyl iodide in ethanol using sodium hydrogen carbonate as the proton acceptor. The radiotracer was purified by semi-preparative reverse-phase HPLC. The average specific activity was 746 mCi/μmol calculated at end-of-synthesis (EOS). The average time of synthesis including formulation was 35 minutes.

Introduction

Sigma (σ) receptors have been the focus of numerous recent studies since these high affinity, saturable, and stereospecific binding sites have been implicated in psychoses (1-3), movement disorders (4), and neuroprotection (5). Although the functional role of σ receptors is not yet clarified, it is known that atypical antipyschotic drugs from a variety of chemical classes display high affinity for σ

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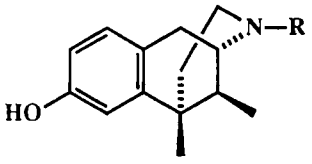
receptors (6). Moreover, these compounds lack many of the side-effects associated with dopamine D_2 neuroleptics. These findings have heightened interest to develop new, highly specific and selective ligands for central σ receptors (7-12).

With the advent of positron emission tomography (PET) and single photon emission computed tomography (SPECT), it is now possible to study neuroreceptors in the living human brain. Such studies have proven useful in the localization and quantification of neuroreceptors and offer insight into the relationship of these receptors in normal and disease states (13-15). PET and SPECT studies of central σ receptors have been limited due to a lack of positron emitting or single-photon emitting radioligands that display high affinity and selectivity for σ receptors (16). Only recently, radioiodinated 1-(*p*-iodophenyl)-3-(1-adamantyl)guanidine (17), iodoallyl tetralins (18), and *N*-(arylalkyl)ethylenediamine derivatives (19) have been reported as possible σ SPECT agents. Additionally, [^{11}C]- and [^{18}F]-labeled guanidines (20), [^{18}F]-haloperidol (21), and [^{18}F]-BMY 14802 (22) have been synthesized and offer potential to study σ receptors via PET.



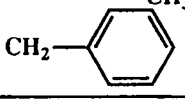
Compound	R
1	H
2	$CH_2-CH=CH_2$
3	$CH_2-CH=C(CH_3)_2$
4	

Figure 1. N-substituted (+)-*cis*-*N*-normetazocine analogs

Our efforts to develop a PET radioligand to study σ receptors *in vivo* focused on the dextrorotatory benzomorphan series (23, 24), of which (+)-*cis*-*N*-normetazocine (1, Figure 1) is a lead member. These tricyclic compounds have aided greatly in the characterization of σ receptors. For example, Martin first proposed the existence of σ receptors (25) using the N-allyl analog of (+)-*cis*-*N*-normetazocine, (+)-SKF10,047 or (+)-NANM (2, Figure 1). The 3,3-dimethyl-allyl substituted derivative of (+)-*cis*-*N*-normetazocine, (+)-pentazocine (3, Figure 1), exhibits even greater selectivity for σ receptors in comparison to 2 (26, 27), and [^{3}H]-(+)-pentazocine has been used extensively for *in vitro* characterization of these receptors

(28). Recently, a large number of N-substituted (+)-*cis*-*N*-normetazocine analogs were prepared and their apparent binding affinities for σ, phencyclidine (PCP), and μ opiate receptors measured (29). From this study, (+)-*cis*-*N*-benzyl-*N*-normetazocine (4) was identified as the ligand that exhibited highest affinity for σ receptors (K_i = 0.67 nM vs [^{3}H]-3) and excellent selectivity over PCP and μ opiate receptors.

Based on these *in vitro* results and the stated need for a positron emitting (+)-*cis*-*N*-normetazocine derivative (30, 31), we chose to radiolabel (+)-*cis*-*N*-benzyl-*N*-normetazocine (4) with [^{11}C] using [α-^{11}C]benzyl iodide. Here, we report the radiosynthesis, purification, and quality control of this σ receptor ligand for PET.

Materials and Methods

With the exception of (+)-*cis*-*N*-normetazocine, all chemicals were available from commercial sources and used as received. (+)-*cis*-*N*-Normetazocine ($[\alpha]_D$ = + 73.5° (c 1, EtOH) was obtained as a gift from the Research Triangle Institute. An authentic sample of (+)-*cis*-*N*-benzyl-*N*-normetazocine was prepared by N-alkylation of (+)-*cis*-*N*-normetazocine with benzyl bromide following the described literature procedure (29). Radioactivity measurements were made using a Capintec CRC-12 dose calibrator.

Synthesis of [α-^{11}C]benzyl iodide

[α-^{11}C]-Benzyl iodide was prepared with minor modifications of a published method (32). Briefly, [^{11}C]-carbon dioxide was produced by 16 MeV proton bombardment of a nitrogen gas target using a Scanditronix RNP-16 biomedical cyclotron. At the end-of-bombardment (EOB), the [^{11}C]-CO_2 was trapped in a stainless steel coil cooled in liquid nitrogen and evacuated by an oilless vacuum pump. The cooling bath was removed and the [^{11}C]-CO_2 transferred to the reaction vessel via a nitrogen sweep gas (approximately 15-25 mL/min). The reaction vessel consisted of a teflon septum sealed 5 mL glass v-vial that was purged with argon and charged with 0.5 mL of 1.0 M phenylmagnesium chloride (Aldrich) in tetrahydrofuran (THF). To measure [^{11}C]-CO_2 not trapped in the reaction vessel (usually less than 20%), an ascarite column (approx. 50 g) connected to the vessel via 1/8 inch teflon tubing was placed in the dose calibrator. After obtaining maximum radioactivity in the v-vial, the sweep gas was turned off and the vessel was kept at room temperature for 1 minute. Lithium aluminum hydride (Fluka, 10 mg) in 0.5 mL THF was added, and the mixture was immediately evaporated to dryness at 120 °C under an argon stream. Hydriodic acid (0.5 mL, 57% in water) was slowly added. A new septum was placed on the vessel, and the solution was heated at 140 °C for 2 minutes.

Isolation of [α-^{11}C]-benzyl iodide by solid-phase extraction

To the acid solution was added 4 mL of 20:80 acetonitrile/water, and the contents of the v-vial were loaded onto an activated C-18 Sep-Pak Plus cartridge. The

Sep-Pak was washed with successive 5 mL portions of saturated aqueous NaS_2O_3, 8.4% aqueous $NaHCO_3$, and H_2O. [α-^{11}C]-Benzyl iodide was then eluted off the cartridge with 1.5 mL CH_2Cl_2, passed through a column of anhydrous Na_2SO_4 (0.2 g) and collected in a 5 mL v-vial containing the (+)-*cis*-*N*-normetazocine precursor.

Synthesis and purification of (+)-cis-[^{11}C]-N-benzyl-N-normetazocine ([^{11}C]-4)

(+)-*cis*-*N*-Normetazocine (1 - 2 mg, 4.6-9.2 μmol) dissolved in 0.4 mL absolute ethanol was mixed with [α-^{11}C]-benzyl iodide and 40 mg (476 μmol) of sodium hydrogen carbonate. The reaction volume was reduced by venting solvent through two 20 gauge needles while heating the mixture for 5 minutes at 120 °C. The remaining residue was dissolved in 0.5 mL of 20:80 acetonitrile/water and injected onto a C-18 Waters Associates Nova-Pak HPLC column (7.8 mm x 30 cm) eluted with a mobile phase of acetonitrile:water (0.1 M ammonium formate with 0.5% glacial acetic acid) (20:80). The effluent from the column was monitored with a UV detector (254 nm, Waters module 440) and an in-line radioactivity detector (Ortec 449 ratemeter, 575 amplifier, 550 single channel analyzer, with a NaI(Tl) crystal). The radioactive peak corresponding to (+)-*cis*-[^{11}C]-*N*-benzyl-*N*-normetazocine (t_R = 8.2 minutes, k' = 3.8) was collected in a rotary evaporator and the solvent evaporated to dryness under reduced pressure. The residue was dissolved in sterile, normal saline (7 mL) and filtered through a sterile, 0.22 μm filter (Gelman Acrodisc) into a sterile, pyrogen-free evacuated vial. Sterile aqueous sodium bicarbonate (3 mL, 8.4%) was then added and the radioactivity was measured.

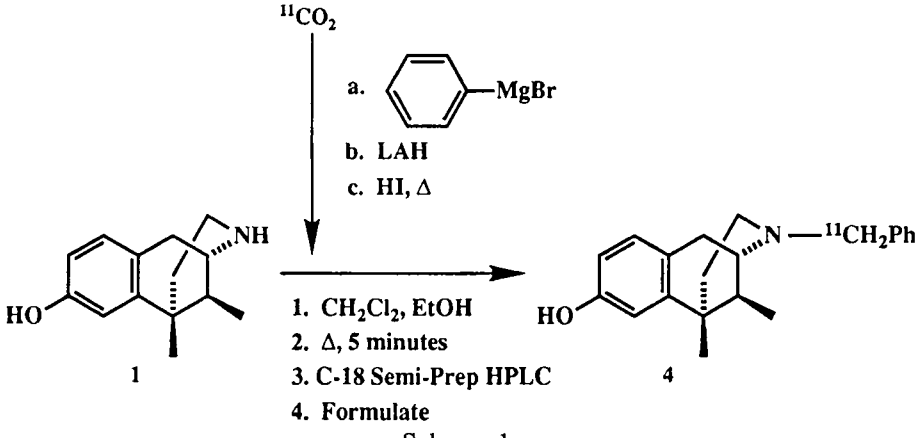
Determination of specific activity

An aliquot of the final solution of known volume and radioactivity was applied to an analytical reverse-phase HPLC cartridge (8 mm x 10 cm, Waters Associates Nova-Pak radial compression cartridge). A mobile phase of acetonitrile:water (0.1 M ammonium formate with 0.5 % glacial acetic acid, 4 ml/minute) (25:75) was used to elute the radioligand (t_R = 4.7 minutes, k' = 4.9). The area of the UV absorbance peak measured at 254 nm corresponding to carrier product was measured by an automated integrating recorder (Hewlett Packard 3390A) and compared to a standard curve relating mass to UV absorbance.

Results and Discussion

The synthesis of (+)-[^{11}C]-*N*-benzyl-normetazocine involved the N-alkylation of the nor-benzyl precursor, (+)-*cis*-*N*-normetazocine, with [α-^{11}C]-benzyl iodide (Scheme 1). The radiosynthetic procedure used in the preparation of [α-^{11}C]-benzyl iodide was a slight modification of that previously reported (32). Briefly, it involved the radiocarbonylation with [^{11}C]-CO_2 of a solution of phenylmagnesium chloride in THF with subsequent reduction of the resulting [^{11}C]-benzoic acid to [^{11}C]-benzyl alcohol with lithium aluminium hydride in THF. Following evaporation of the THF,

[^{11}C]-benzyl alcohol was converted to [^{11}C]-benzyl iodide in refluxing hydriodic acid. The minor modifications to the previously reported synthesis consisted of a simplified reaction vessel and a shorter carbonylation time.



Scheme 1.

Purification of [α-^{11}C]-benzyl iodide could be accomplished by either of two established procedures: solid-phase extraction or liquid-liquid extraction with methylene chloride. [α-^{11}C]-Benzyl iodide of high radiochemical purity (> 90%) was obtained 16 - 18 minutes after EOB by either method in non-decay corrected radiochemical yields of 20 - 30% based on radioactivity trapped in the Grignard solution. [α-^{11}C]-Benzyl iodide isolated by liquid-liquid extraction with methylene chloride did not couple with the (+)-*cis*-*N*-normetazocine precursor (unpublished results) although this purification method has proven useful previously (32). Thus, [α-^{11}C]-benzyl iodide purified by solid-phase extraction (C-18 Sep-Pak Plus) was used in preparation of [^{11}C]-**4**.

The [^{11}C]-alkylating agent eluted from the Sep-Pak in methylene chloride was reacted with (+)-*cis*-*N*-normetazocine and sodium hydrogen carbonate for 5 minutes at 120 °C. During the 5 minute heating period, the reaction volume was reduced by venting solvent. HPLC analysis of the reaction mixture revealed that the 5 minute reaction time was necessary for complete consumption of [α-^{11}C]-benzyl iodide. Shorter reaction times (3 and 4 minutes) resulted in incomplete coupling of [α-^{11}C]-benzyl iodide to (+)-*cis*-*N*-normetazocine. In order to verify that competing O-radioalkylation at the phenolic oxygen of (+)-*cis*-*N*-normetazocine did not occur, an attempt was made to O-alkylate an authentic sample (2 mg) of (+)-*cis*-*N*-benzyl-*N*-normetazocine with [α-^{11}C]-benzyl iodide using the same reaction conditions described above for the N-alkylation. No evidence for the formation of the radiolabeled O-benzylated product was found using analytical HPLC. All of the [α-^{11}C]-benzyl iodide remained unreacted suggesting that [^{11}C] O-benzylation was not a confounding factor in the preparation of [^{11}C]-**4** from phenolic **1**.

Reverse-phase semi-preparative HPLC was used to purify [^{11}C]-(+)-*N*-benzyl-*N*-normetazocine (Figure 2). The principal by-product in the UV chromatogram of the radiolabeling was not the precursor **1** (t_R = 2.1, k' = 0.2), but an unidentified substance (t_R = 4.4 minutes, k' = 1.6). Preliminary ^{1}H NMR studies suggest this side-product may result from ring-opening of residual THF with hydriodic acid (33). With a retention time for [^{11}C]-**4** of 8.2 minutes (k' = 3.8), a satisfactory separation between the desired product and the UV absorbing contaminant was achieved. The radioactive peak corresponding to the product was collected remotely and the HPLC effluent removed via rotary evaporation under high vacuum. [^{11}C]-**4** was formulated in sterile saline, microfiltered into a sterile, evacuated dose-vial, and diluted with sterile sodium bicarbonate. Using this procedure, the formulated radiotracer proved to be sterile and pyrogen-free using normal methods.

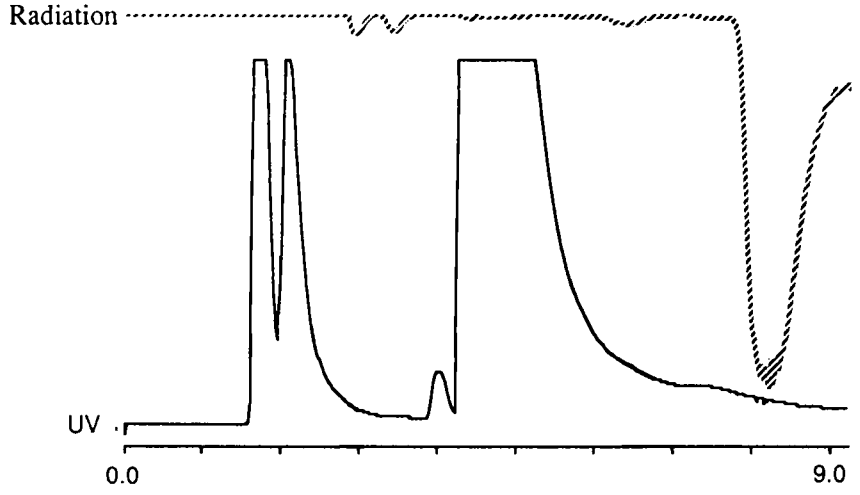


Figure 2. HPLC purification of [^{11}C]-**4** (t_R = 8.2 minutes)

The average time of synthesis of [^{11}C]-**4** from EOB was 35 minutes (n = 9). Non-decay corrected overall radiochemical yields (based on initial radioactivity trapped in the Grignard) ranged from 3 - 7% with an average radiochemical yield of 5%. To determine specific activity and final radiochemical purity of the radioligand, a known aliquot of radioactivity was injected onto an analytical reverse-phase HPLC cartridge. Comparison of the carrier peak associated with the radioactivity to that of a standard sample of (+)-*cis*-*N*-benzyl-*N*-normetazocine enabled calculation of the specific activity. The resulting chromatogram showed [^{11}C]-**4** to be of high radiochemical purity (>99%). The radioactive product co-eluted with an authentic sample of **4**. Specific activities calculated at the end of synthesis ranged from 500 to 1200 mCi/μmol with an average value of 746 mCi/μmol for 9 preparations. This corresponds to an average specific activity at EOB of approximately 2500 mCi/μmol. The specific activity for [^{11}C]-**4** was comparable to that obtained for the muscarinic receptor ligand [^{11}C]-dexetimide also synthesized from [α-^{11}C]-benzyl iodide (32).

Conclusions

[^{11}C]-(+)-*N*-benzyl-*N*-normetazocine of high specific activity can be synthesized in acceptable radiochemical yields from (+)-*cis*-*N*-normetazocine and [α-^{11}C]-benzyl iodide. A sufficient amount of the radioligand can be prepared to permit its use for localization and quantitation of σ receptors in the brain *in vivo* with PET.

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