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Synthesis of a Radiotracer for Studying κ-Subtype Opiate Receptors: N-[^{11}C-methyl]-N-(*trans*-2-pyrrolidinyl-cyclohexyl)-3,4-dichlorophenylacetamide ([^{11}C](±)U-50488H)

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Summary

The radiochemical synthesis of a κ-subtype opiate receptor ligand, [^{11}C](±)U-50488H, was accomplished either in two steps or by direct methylation with [^{11}C]iodomethane. The radiotracer was purified using reversed phase semipreparative HPLC. The average specific activity was 1830 mCi/μmole (calculated at the end-of-synthesis; EOS) for the direct methylation and 2140 mCi/μmole EOS for the two-step one-pot synthesis. The average time of synthesis including formulation was approximately 22 minutes for the direct methylation and 27 minutes for the two-step one-pot synthesis.

Key Words: radiotracer, synthesis, kappa opiate receptor, carbon-11, positron emission tomography

Introduction

Over the past decade, major advances in positron emission tomographic (PET) imaging of receptors within the central nervous system have been preceded by the syntheses of appropriately chosen ligands, radiolabeled at high specific radioactivity. In choosing an appropriate ligand, selectivity and affinity of the ligand for a particular receptor are important when studying receptors that have multiple subtypes such as the opiate receptor originally classified as μ, κ, and δ (1). The

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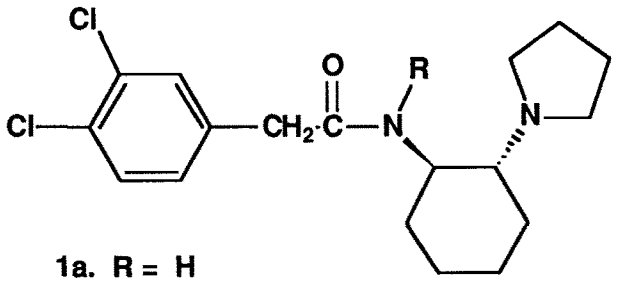
continued development of more selective ligands for each opiate receptor subtype will greatly enhance the study of the opiate receptor using PET.

Several selective ligands for the μ-subtype opiate receptor have already been radiolabeled. [^{11}C]Carfentanil was the first radiotracer successfully developed to study the opiate receptor using PET (2, 3). Experiments have shown that radiolabeled carfentanil binds to the μ-subtype opiate receptor *in vivo* (3). More recently two μ-selective radiotracers, [^{18}F]cyclofoxy (4) and [^{11}C]ohmefentanyl (5), have been synthesized for PET studies. [^{11}C]Diprenorphine has been radiolabeled in several different positions by several investigators (6 - 9). Both *in vitro* binding studies (10 - 12) and recent PET studies (13 - 15) have shown that [^{11}C]diprenorphine binds with high affinity to both the μ- and δ-subtype opiate receptors. [^{11}C]Buprenorphine, an analog of diprenorphine that is also non-selective, has also been synthesized (16, 17) and used in PET studies of the opiate receptor (18, 19).

In recent years, several groups have described the development of a novel series of N-[(2-aminocyclohexyl)aryl]acetamide derivatives (20 - 25) as κ-subtype selective opiate receptor ligands. The first and perhaps the simplest member of this series of κ-selective ligands is N-methyl-N-(*trans*-2-pyrrolidinyl-cyclohexyl)-3,4-dichlorophenylacetamide, **1b**, also known as U-50488H (26 - 28). Recently, a radiofluoroalkylated derivative of U-50488H was synthesized and characterized *in vitro* and *in vivo* (29); but fluoroalkylation proved detrimental to the ligand affinity. In an isotopically labeled form, U-50488H may be useful for studying κ-subtype opiate receptors using PET.



1a. R = H
1b. R = CH_3
1c. R = $^{11}CH_3$

This paper describes two alternative methods for the synthesis, purification, characterization, and formulation of [^{11}C](±)U-50488H, **1c**, for *in vivo* studies with PET.

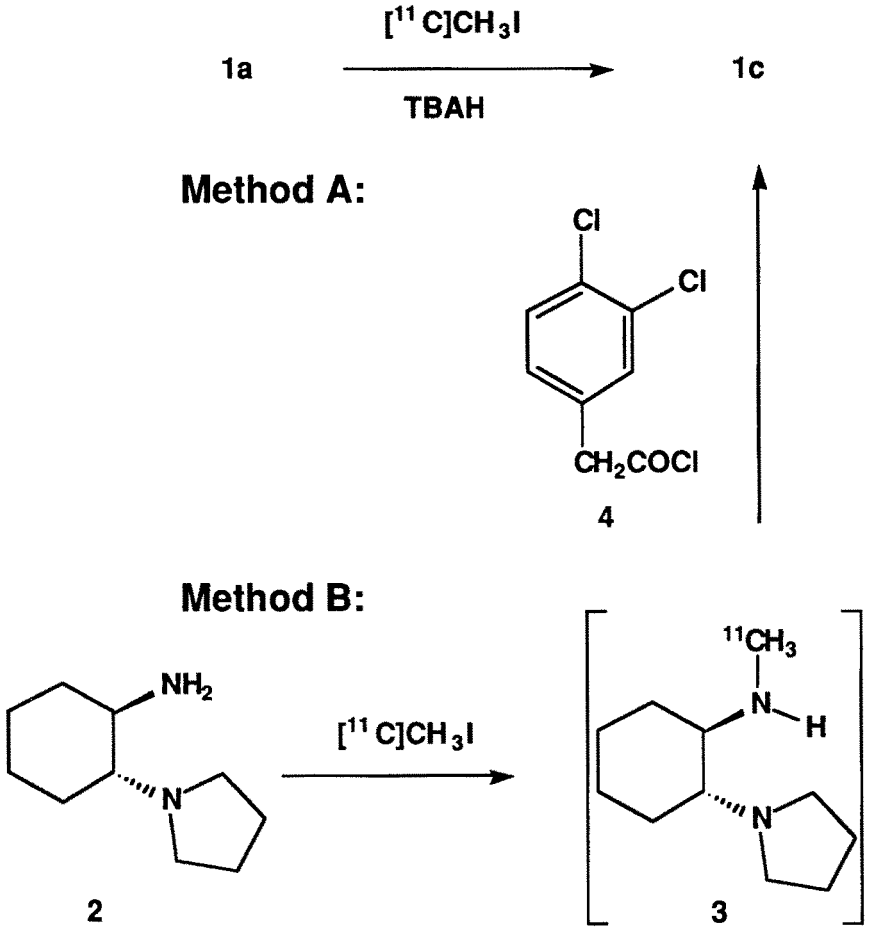
Discussion

Starting materials, *trans*-2-pyrrolidinylcyclohexylamine, **2** and 3,4-dichlorophenylacetyl chloride, **4**, were prepared by known literature procedures (29, 30). N-(*trans*-2-Pyrrolidinylcyclohexyl)-3,4-dichlorophenyl acetamide, **1a**, was prepared by acylation of **2** with **4** in dichloromethane at 0 °C.

The most straightforward approach to the synthesis of **1c** would involve direct methylation of the secondary amide nitrogen of **1a** with [^{11}C]iodomethane. This technique has been successful in the radiosynthesis of other tertiary amides, for example N-methyl-spiperone (31,32). Attempts to alkylate **1a** with 1-bromo-3-fluoropropane to produce a [^{18}F] labeled analog of U-50488H have been reported (29). It was observed that the secondary amide decomposed in the presence of the aqueous base, tetrabutylammonium hydroxide (TBAH), before alkylation could occur. Additional experiments with a model compound, N-isopropyl-3,4-dichlorophenylacetamide, and iodomethane with either LDA in THF or NaH in DMF eliminated the problem of decomposition of the precursor but only C-alkylation of the benzylic carbon was observed. Thus the poor nucleophilicity of the secondary amide nitrogen and the possible instability of **1a** were two problems faced in the direct methylation method.

Our approach to the direct methylation of **1a** with iodomethane was to use more mild conditions and thus limit the amount of decomposition of the precursor. In our hands direct methylation (Method A) of **1a** in DMF with [^{11}C]iodomethane and TBAH at 80 °C for 1 minute yielded the desired product, **1c**. Semipreparative HPLC was used to isolate **1c** and the specific activity was determined by analytical reversed phase HPLC to be approximately 1830 mCi/μmole (calculated at end of synthesis; EOS). The identity of the product was confirmed by co-injection of the product with an authentic sample of (±)U-50488H.

An alternative approach to the synthesis of **1c** was based on a reported method of alkylation of **2** with 1-bromo-3-fluoropropane followed by acylation (29). This two-step synthesis involving methylation of the primary amine of **2** with iodomethane followed by acylation of the resulting secondary amine, **3**, with **4** offered a convenient one-pot synthesis of **1c** (Method B). The methylation of **2** with [^{11}C]iodomethane at 80 °C in DMF was complete in 5 minutes. The acylation step of **3** with **4** was rapid at room temperature in the presence of triethylamine. Reaction times were determined by analysis on an analytical reversed phase HPLC column and were judged complete when no further change in product composition was observed. Semipreparative HPLC was used to isolate **1c**. After formulation and microfiltration, the average specific activity was determined by analytical reversed phase HPLC to be approximately 2140 mCi/μmole (calculated at EOS). Co-injection of the product with an authentic sample of (±)U-50488H confirmed its identity.



Both methods A and B are effective in producing **1c** with a synthesis and work-up time of less than 30 minutes from the end of bombardment. The radiochemical yields were 6% and 7%, calculated at EOS (not corrected for decay), based on starting $^{11}CO_2$ for methods A and B respectively. The advantage of method A is the potential flexibility in the choice of synthetic methods used in preparing the amide precursor since it is prepared in advance. Method A is simple and convenient but the potential decomposition of the amide could be a problem in obtaining consistent yields during routine production. Method B overcomes the problem of decomposition and may give more consistent yields, but the choice of synthetic methods for the acylation step is limited due to time constraints. These two alternative methods in the synthesis of **1c** offer the radiochemist several options not only in preparing **1c** but may be useful in the synthesis of other members of the series of N-[(2-aminocyclohexyl)aryl]acetamide derivatives reported as being κ-subtype selective opiate receptor ligands (33 - 35).

Experimental

^{1}H-NMR spectra were recorded on an IBM NR/80 spectrometer; chemical shifts (δ) were recorded in parts per million downfield from tetramethylsilane. IR spectra were recorded on a FT-IR Nicolet 205 spectrophotometer. Melting points are uncorrected. DMF was stirred overnight with BaO then distilled under reduced pressure from BaO. Chemicals were analytical grade and were obtained commercially from the Aldrich Chemical Co. An authentic sample of (±)U-50488H was obtained from The Upjohn Co. Purification and analyses of radioactive mixtures were performed with two Waters 590EF HPLC pumps, in-line uv detectors at two wavelengths (214 and 254 nm), and a single two inch NaI crystal radioactive detector. Semipreparative reversed phase HPLC was performed on an Alltech Econosil C_{18} (250 mm x 10 mm) column with CH_3CN/H_2O (40/60) containing 0.1 N NH_4HCO_2 at 10 mL/minute. Analytical HPLC was performed on an Alltech Econosil C_{18} (250 mm x 4.4 mm) column with 40/60 (CH_3CN/H_2O) containing 0.05 M NH_4Cl at 4 mL/minute. Peak areas were measured using two Hewlett-Packard 3390A recording integrators. Radioactivity measurements were made with a dose-calibrator (Capintec CRC-12R).

trans-2-Pyrrolidinylcyclohexylamine, 2.

The diamine, **2**, was prepared as described in the literature (29). ^{1}H-NMR ($CDCl_3$) δ 1.12-1.18 (m, 4H), 1.60-1.80 (m, 8H), 2.24-2.54 (m, 8H).

3,4-Dichlorophenylacetyl chloride, 4.

The acid chloride, **4**, was prepared from the action of thionyl chloride on 3,4-dichlorophenylacetic acid in benzene as described in the literature (30). ^{1}H-NMR ($CDCl_3$) δ 4.11 (s, 2H, CH_2), 7.40-7.60 (m, 3H, ArH). Compound **4** was used without further purification.

N-(trans-2-Pyrrolidinylcyclohexyl)-3,4-dichlorophenylacetamide, 1a.

Compound **4** (0.19 g, 84 mmoles) in methylene chloride (2 mL) was added to **2** (0.14 g, 84 mmoles) in dichloromethane (2 mL) at 0° C. The solution was stirred for 20 minutes at 0 °C. The solution was concentrated under reduced pressure and a saturated solution of potassium carbonate (5 mL) was added to the residue. The mixture was extracted with ethyl ether (3 x 10 mL), dried (anhydrous sodium sulfate), and concentrated under reduced pressure to yield a yellow solid. The solid was recrystallized from ethyl acetate to yield **1a** as slightly yellow needles (0.1 g, 34%); mp: 110 - 111 °C (lit. mp: 118 °C) (29); ^{1}H-NMR ($CDCl_3$) δ 1.11-1.42 (m, 4H), 1.45-1.91 (m, 8H), 2.30-2.70 (m, 6H), 3.53 (s, 2H, CH_2), 6.50-6.70 (broad s,

1H, amide H), 7.11-7.52 (m, 3H, ArH); IR (CH_2Cl_2) cm^{-1} 1668 (carbonyl stretch); 1505, 1472 (aromatic stretch); Analytical HPLC Data: (retention time = 2.6 minutes; k' = 2.7)

Radiosynthesis of N-[^{11}C-methyl]-N-(trans-2-pyrrolidinyl-cyclo-hexyl)-3,4-dichlorophenylacetamide, [^{11}C](±)U-50488H, 1c.

Method A: [^{11}C]Iodomethane, produced as previously described (2), was swept by a stream of argon gas into a cooled (-78 °C) solution of **1a** (1.16 mg, 3.3 μmole) in DMF (200 μL). TBAH (10 μL, 15 μmole) was added and the mixture was heated at 80 °C for 1 minute. The reaction mixture was quenched with the addition of HPLC buffer (200 μL) and this mixture was applied to the semipreparative HPLC column. The fraction containing **1c** was collected (retention time = 7.9 minutes; k' = 6.1) and evaporated to dryness under reduced pressure. The residue was taken up in 7 mL of sterile saline. The resulting solution was passed through a 0.22 μm Millipore filter into a sterile, pyrogen free bottle and aqueous sodium bicarbonate (3 mL, 8.4%) was added. The radiochemical purity and specific activity of the final solution were determined by analytical HPLC. The product eluted with the same retention time as an authentic sample of (±)U-50488H (retention time = 3.6 minutes; k' = 4.6) and was verified by co-injection of the product and (±)U-50488H.

Method B: [^{11}C]Iodomethane, produced as previously described (2), was swept by a stream of argon gas into a cooled (-78 °C) solution of **2** (1.36 mg, 8.1 μmole) in DMF (200 μL). The solution was heated at 80 °C for 5 minutes. The mixture was briefly cooled to room temperature before the addition of triethylamine (5 μL, 36 μmole) and **4** (2 μL, 9 μmole). The reaction mixture was allowed to stand at room temperature for 1 minute before the addition of HPLC buffer (200 μL). This mixture was applied to the semipreparative HPLC column; the desired product (retention time = 7.9 minutes; k' = 6.1), **1c**, was collected and evaporated to dryness under reduced pressure. The residue was taken up in 7 mL of sterile saline. The resulting solution was passed through a 0.22 μm Millipore filter into a sterile, pyrogen free bottle and aqueous sodium bicarbonate (3 mL, 8.4%) was added. The radiochemical purity and specific activity of the final solution were determined by analytical HPLC. Co-injection of the product and an authentic sample of (±)U-50488H (retention time = 3.6 minutes; k' = 4.6) verified the identity of the product.

Conclusions

[^{11}C](±)U-50488H of high specific activity can be rapidly synthesized by methods A or B in reasonable radiochemical yields from the appropriate precursor and [^{11}C]iodomethane. A sufficient amount of the radiotracer can be prepared to

allow its use for investigating regional distributions and concentrations of κ-subtype opiate receptors in the brain *in vivo* with PET.

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