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A two-step one-pot radiosynthesis of the potent dopamine D_2/D_3 agonist PET radioligand [^{11}C]MNPA

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(*R*)-(−)-2-[^{11}C]Methoxy-*N*-*n*-propylnorapomorphine ([^{11}C]MNPA ([^{11}C]2)) is an agonist radioligand of interest for imaging D_2/D_3 receptors *in vivo*. Here we sought to develop an improved radiosynthesis of this radioligand. Reference 2 was synthesized in nine steps with an overall yield of about 5%, starting from codeine. Trimethylsilyldiazomethane proved to be a practical improvement in comparison to diazomethane in the penultimate methylation step. A protected precursor for radiolabeling ((*R*)-(−)-2-hydroxy-10,11-acetonide-*N*-*n*-propylnoraporphine, 4) was prepared from (*R*)-(−)-2-hydroxy-*N*-*n*-propylnorapomorphine (1) in 30% yield. [^{11}C]2 was prepared from 4 via a two-step one-pot radiosynthesis. The first step, methylation of 4 with [^{11}C]methyl triflate, occurred in quantitative radiochemical yield. The second step, deprotection of the catechol moiety with HCl and heat, yielded 60–90% of [^{11}C]2 giving an overall incorporation yield from [^{11}C]methyl triflate of 60–90%. In a typical run more than 1 GBq of [^{11}C]2, was produced from carbon-11 generated from a 10-min proton irradiation (16 MeV; 35 μA) of nitrogen–hydrogen target gas. The radiochemical purity of [^{11}C]2 was $>$ 99% and specific radioactivity at the time of injection was 901 ± 342 GBq/μmol (n = 10). The total synthesis time was 35–38 min from the end of radionuclide production. The identity of [^{11}C]2 was confirmed by comparing its LC-MS/MS spectrum with those of reference 2 and (*R*)-(−)-10-methoxy-2,11-dihydroxy-*N*-*n*-propylnoraporphine.

Keywords: PET; MNPA; dopamine D_2/D_3 agonist; radiosynthesis; LC-MS/MS analysis

Introduction

The binding of agonist radioligands to dopamine D_2/D_3-receptors has been found to be more sensitive to altered endogenous dopamine levels, than that of antagonist radioligands.[1–3] [^{11}C]MNPA ((*R*)-(−)-2-[^{11}C]methoxy-*N*-*n*-propylnorapomorphine, [^{11}C]**2**, Scheme 1) is an agonist radioligand that has been used to visualize dopamine D_2/D_3-receptors in living non-human primate brain.[4] [^{11}C]**2** was initially prepared by direct alkylation of (*R*)-(−)-2-hydroxy-*N*-*n*-propylnorapomorphine (TNPA, **1**, Scheme 2) with [^{11}C]methyl iodide.[4] The selectivity for *O*-alkylation at the 2-hydroxy position was determined by post-derivatization of the radioactive product. However, recent attempts to produce [^{3}H]MNPA following this direct methylation strategy, produced a mixture of both [^{3}H]MNPA and (*R*)-(−)-10-[^{3}H]methoxy-2,11-dihydroxy-*N*-*n*-propylnoraporphine.[5] Furthermore, our subsequent evaluation of the direct methylation of **1** with [^{13}C]methyl iodide by LC-MS/MS indicated that this alkylation was not completely selective for the 2-hydroxy position, as the product ion spectrum of the labeled product differed from that of the reference standard **2**. For future use of [^{11}C]**2** in human studies, we considered it necessary to modify the radiosynthesis in such a way that [^{11}C]**2** would be formed selectively. Protection of the catechol moiety would ensure selective labeling at the 2-hydroxy position. Such protection would need to be stable under the methylation conditions but easily removable afterwards. The previously reported methylenedioxy-protecting group needs harsh reaction with BCl_3 to be removed.[6–9] The conditions of this deprotection reaction would very likely hydrolyse some of the product, as indicated by tritiation attempts.[5]

The aim of this work was (i) to develop a two-step one-pot radiosynthesis that selectively produces [^{11}C]**2** and (ii) to synthesize the acetonide precursor **4** (Scheme 3) and reference standard **2** (Scheme 4), each from codeine.

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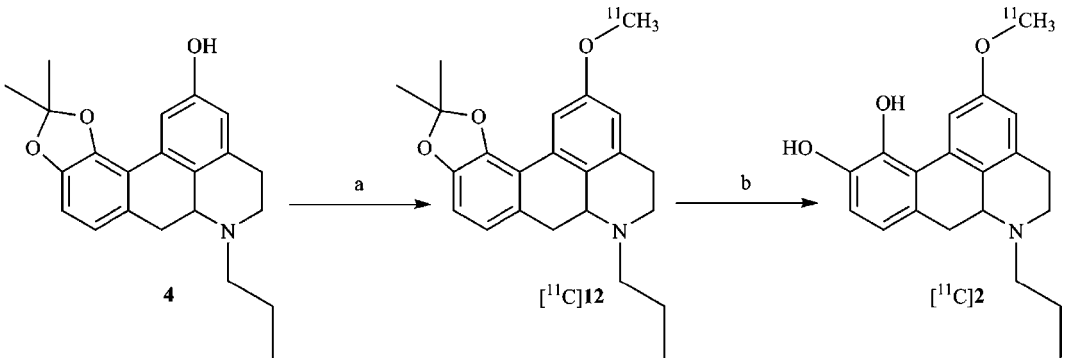
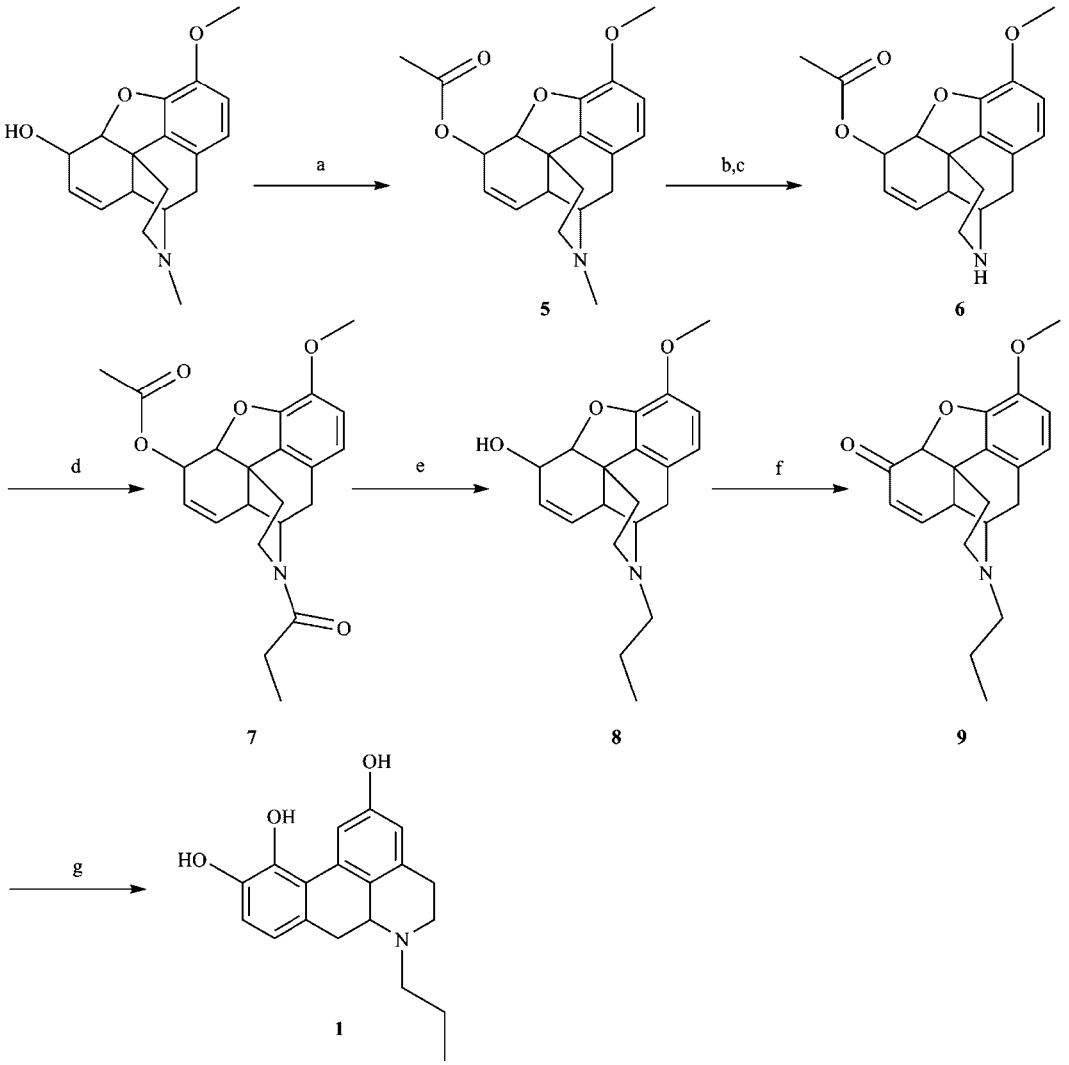


Scheme 1. Radiosynthesis of [^{11}C]**2**. Reagents: (a) [^{11}C]methyl triflate, 0.5 M NaOH, MeCN and (b) 6 M HCl, 150°C, 8 min.

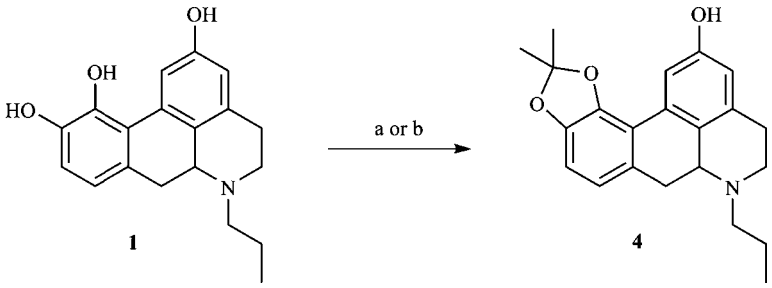


Scheme 2. Synthesis of **1**. Reagents: (a) acetic anhydride, reflux; (b) 1-chloroethyl chloroformate, proton sponge®, 1,2-dichloroethane, reflux; (c) MeOH, reflux; (d) propionyl chloride, CH_2Cl_2, 2.5 M NaOH; (e) $LiAlH_4$, THF, 0°C; (f) MnO_2, toluene; (g) 48% HBr, reflux.

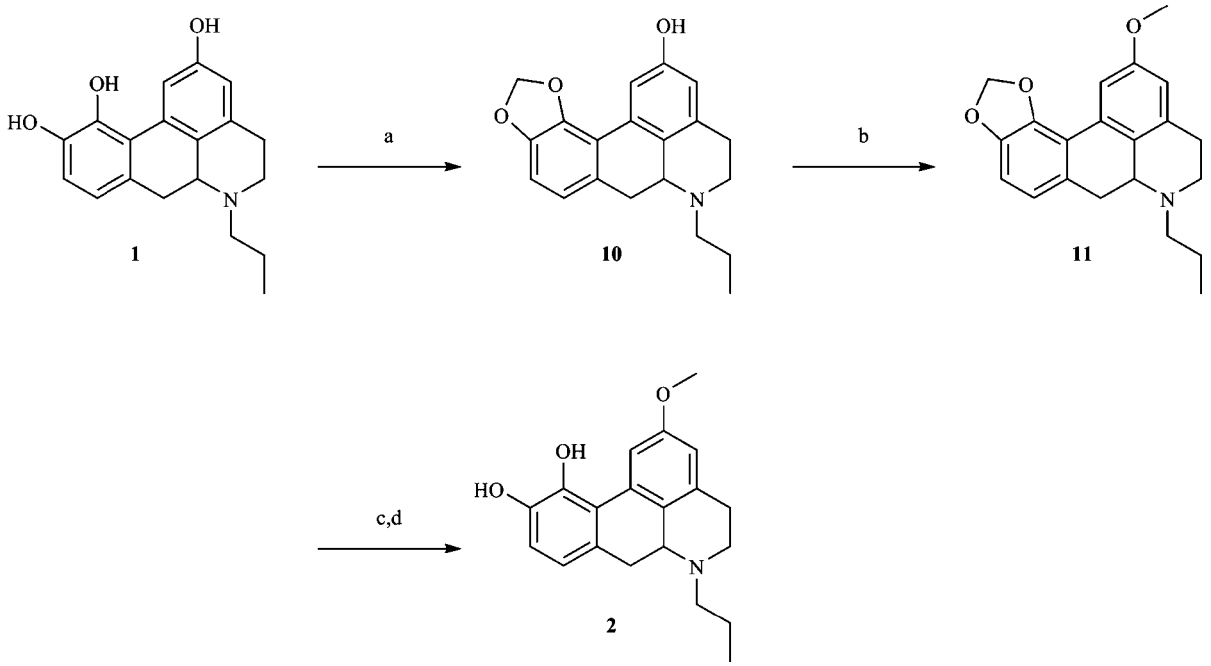
Results and discussion

Chemistry

Both reference compound **2** and precursor **4** were synthesized from **1** (Schemes 3 and 4). For the synthesis of **1**, we followed the general strategy of Gao *et al*. and Neumeyer *et al*.,[7,10] but started from codeine phosphate hemihydrate instead of thebaine. The synthesis of **1** is depicted in Scheme 2. Refluxing codeine in acetic anhydride gave **5** in high yield (98%). Reaction of **5** with 1-chloroethyl chloroformate in the presence of proton sponge® and subsequent reflux in methanol yielded the



Scheme 3. Synthesis of **4**. Reagents: (a) TFA, 2,2-dimethoxypropane, 0°C and (b) acetone (excess), 10 eq. P_2O_5, THF, reflux.



Scheme 4. Synthesis of reference **2**. Reagents: (a) DMSO, NaOH (solid), dibromomethane, 80°C, 4 h; (b) MeCN/MeOH (50/50), DIPEA, 2 M TMS-diazomethane in hexane, 36 h; (c) CH_2Cl_2, 1 M BCl_3 in hexane, 20 h; (d) MeOH, reflux.

secondary amine **6**. Compound **6** was treated with propionyl chloride to yield propionamide **7**. Reduction of **7** with $LiAlH_4$ gave **8**. Oxidation of **8** with MnO_2 yielded ketone **9**. Compound **9** was rearranged by reflux in freshly distilled 48% HBr solution to give **1**. The overall yield of the synthesis of **1** was approximately 22% starting from codeine phosphate hemihydrate.

All chemical reactions in this sequence were quite convenient and gave high yields. The only practical problem was purification of the ketone **9**, which was later found to be unnecessary. Using the crude compound **9** in the rearrangement reaction caused no further problems since we were able to purify **1** by precipitation using cold trifluoroacetic acid in the next step.

The synthesis of **4** is depicted in Scheme 3. Two methods of synthesizing **4** are presented of which the latter one is currently in use, due to its more consistent and higher yields. *Method 1*: Solid **1** was cooled on an ice-bath. TFA (5 ml) was added with stirring, followed by addition of the same volume of 2,2-dimethoxypropane. This procedure was repeated until TLC showed no more increase in **4**. The best result with this method was a 10% yield after work-up procedures. *Method 2*:[11] **1** was dissolved in THF. After addition of P_2O_5 and acetone, both in large excess, the mixture was refluxed for 5 h, yielding **4** in moderate yields (25–30%) after work-up and purification.

The synthesis of reference compound **2** is outlined in Scheme 4. Reaction of **1** with CH_2Br_2 gave **10**.[6–8,10] Methylation of **10** with trimethylsilyldiazomethane (TMS-diazomethane) gave **11**, which was purified by column chromatography on SiO_2.[12] Finally, deprotection of **11** with BCl_3 gave **2**, which was purified by HPLC and subsequent recrystallization.[7–10] The overall yield of reference compound **2** starting from **1** was approximately 20%. With codeine phosphate hemihydrate as starting point the overall yield of **2** was about 5%. The use of TMS-diazomethane instead of diazomethane was a great improvement. No special glassware or any other special precautions were needed. Stirring the TMS-diazomethane mixture at room temperature, in a regular round-bottomed flask for 1.5 days yielded 88% **11**.

Radiochemistry

The two-step one-pot radiosynthesis of [^{11}C]**2** is shown in Scheme 1. [^{11}C]methyl triflate was trapped at room temperature in a vessel containing **4**, acetonitrile and sodium hydroxide. Methylation was instantaneous and quantitative. Subsequent addition of hydrochloric acid and heating of the mixture, yielded the crude [^{11}C]**2**. A sodium acetate solution was added to the crude reaction mixture after which the mixture was injected

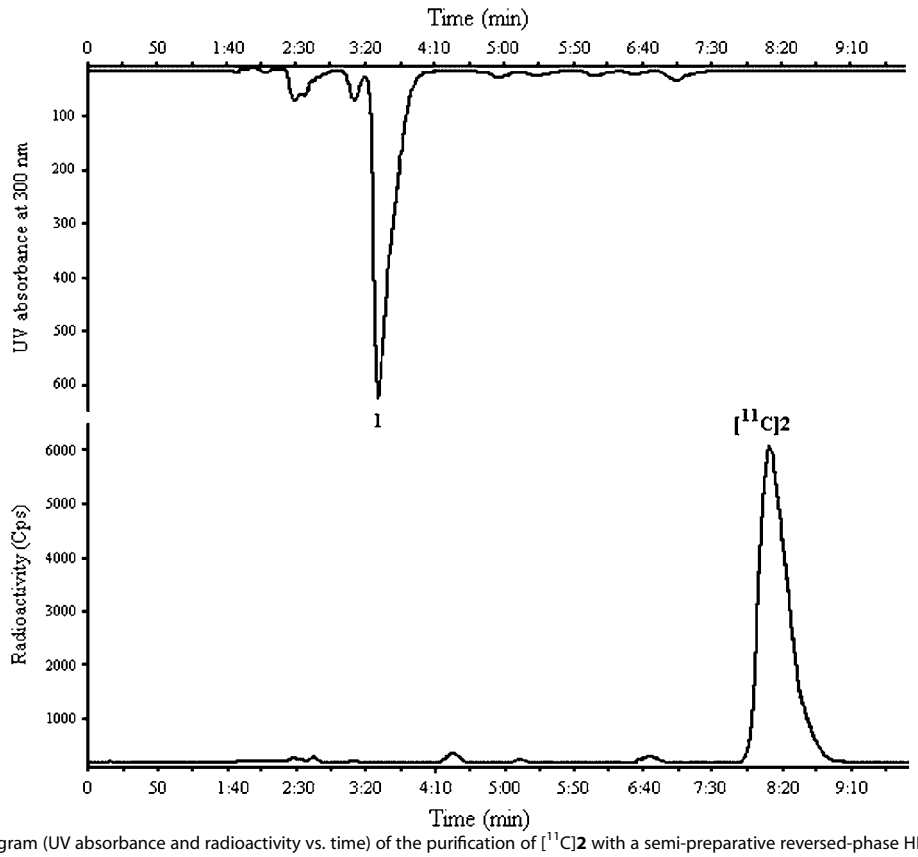


Figure 1. HPLC chromatogram (UV absorbance and radioactivity vs. time) of the purification of [^{11}C]**2** with a semi-preparative reversed-phase HPLC column. (**1**) TNPA and ([^{11}C]**2**) [^{11}C]MNPA.

onto the semi-preparative HPLC column. [^{11}C]**2** eluted after 8–9 min (Figure 1). The total synthesis time was 35–38 min from the end of radionuclide production. The yield of the deprotection step varied between 60 and 90% giving an overall incorporation yield from [^{11}C]methyl triflate of 60–90%. From a 10-min bombardment more than 1 GBq of [^{11}C]**2** was collected at the end of synthesis. The radiochemical purity was better than 99%. The specific radioactivity of the product was 901 ± 342 GBq/μmol ($n = 10$) at the time of injection into the subject for PET study.

Identification of carrier associated with [^{11}C]MNPA by LC-MS/MS

For identification of the carrier associated with [^{11}C]**2** by LC-MS/MS, a sample of the purified and filtered product was taken after radioactive decay without further dilution. For analysis of reference standard **2** and (*R*)-(−)-10-methoxy-2,11-dihydroxy-*N*-*n*-propylnoraporphine, solutions of 1 μg/ml were prepared. The samples were analysed and the ion spectrum of the carrier of [^{11}C]**2** was compared to that of the reference compound **2** (Figure 2) and (*R*)-(−)-10-methoxy-2,11-dihydroxy-*N*-*n*-propylnoraporphine (Figure 3) to identify the product. The ion spectrum of the product was identical to that of the reference compound **2**. All the major fragments present in the ion spectrum of the decayed product were also present in the ion spectrum of reference compound **2**. Furthermore, the ion spectrum of (*R*)-(−)-10-methoxy-2,11-dihydroxy-*N*-*n*-propylnoraporphine (Figure 3) showed two ions *m/z* 235 and 207, which are absent in the ion spectrum of the carrier of [^{11}C]**2**. The spectrum of the carrier of [^{11}C]**2** was somewhat noisier, because of the lower concentration of the sample.

Experimental

Chemistry

All chemicals, except codeine phosphate hemihydrate, were purchased from commercial companies such as Sigma Aldrich and Fluka Chemicals and were used without further purification. Codeine phosphate hemihydrate was purchased from the Karolinska Hospital Pharmacy. All NMR spectra were recorded on a Bruker Avance 500 spectrometer (500.1 MHz for [^{1}H]) with tetramethylsilane as an internal standard. Chemical shifts (δ) are given in ppm. The electrospray ionization (ESI) mass spectra of the intermediates and final compounds in the synthesis of reference compound **2** and precursor **4** were obtained on an LCQ ion trap mass spectrometer equipped with an ESI source (Finnigan LTQ, San Jose, CA, USA) using 90% acetonitrile plus 10% 10 mM-ammonium acetate as eluent.

*6-Acetoxy-7,8-didehydro-4,5-epoxy-3-methoxy-17-methylmorphinan (**5**)*

Codeine phosphate hemihydrate (40.0 g; 98 mmol) was suspended in acetic anhydride (200 ml). The reaction mixture was heated to reflux for 3 h. During heating the suspension became a solution and turned red. The reaction mixture was cooled to room temperature and the solvent was evaporated off under reduced pressure. The residue was dissolved in CH_2Cl_2 and washed twice with saturated $NaHCO_3$ and twice with water. The

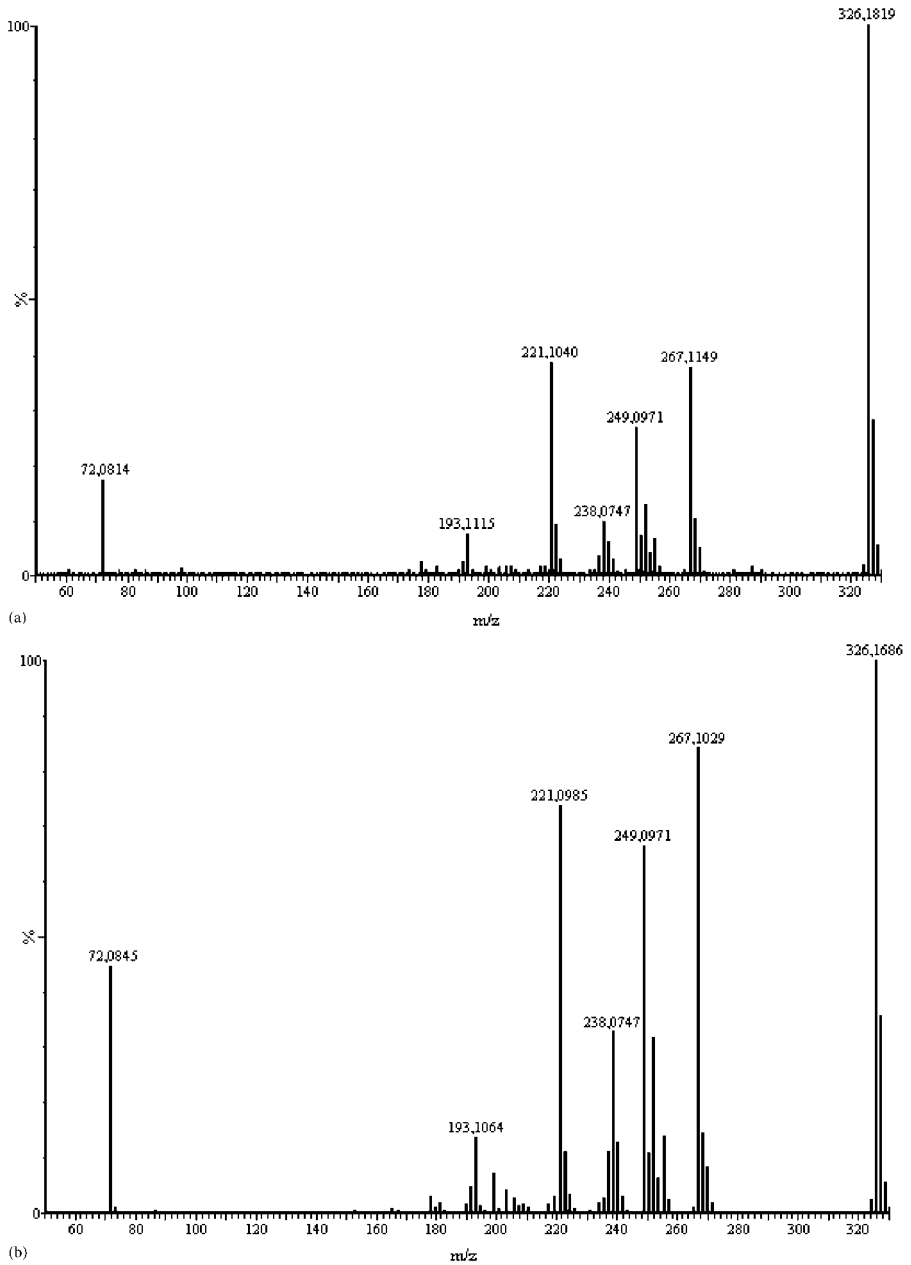


Figure 2. LC-MS/MS fragmentation spectrum of the carrier associated with [^{11}C]**2** (a) and reference compound **2** (b).

organic layer was dried on $MgSO_4$, filtered and evaporated to dryness, yielding **5** (33.0 g, 96.7 mmol, 98%) as a pinkish solid. A sample was recrystallized using CH_2Cl_2 and hexane, yielding off-white needles. ^{1}H-NMR ($CDCl_3$): δ: 1.85–1.88 (m, 1H); 2.01–2.07 (m, 1H); 2.16 (s, 3H); 2.27–2.40 (m, 2H); 2.44 (s, 3H); 2.56–2.60 (m, 1H); 2.73 (t, 1H); 3.04 (d, 1H); 3.34–3.36 (m, 1H); 3.85 (s, 3H); 5.07 (d, 1H); 5.17–5.20 (m, 1H); 5.42–5.45 (m, 1H); 5.63 (d, 1H); 6.54 (d, 1H); 6.66 (d, 1H). MS: ESI [MH^+] Calcd: 342.17; found: 342.18.

*6-Acetoxy-7,8-didehydro-4,5-epoxy-3-methoxy-17-normorphinan (**6**)*

A solution of **5** (33 g; 96.7 mmol), proton sponge® (21 g; 96.7 mmol) and 1-chloroethyl chloroformate (13.2 ml; 115 mmol) in 1,2-dichloroethane (200 ml) was heated to reflux under nitrogen for 90 min. After cooling to room temperature, the solvent was evaporated off under reduced pressure. Methanol (200 ml) was added to the residue. The solution was heated to reflux for 2 h. The solution was then cooled down to about 30°C and diethyl ether (100 ml) was added while stirring. The mixture was cooled further to room temperature. A precipitate formed, which was filtered off yielding **6** (26.3 g; 80 mmol, 83%) as an off-white powder. ^{1}H-NMR (CD_3OD): δ: 2.07 (m, 1H); 2.13 (s, 3H); 2.25 (m, 1H); 2.97–3.09 (m, 4H); 3.31 (m, 2H); 3.83 (s, 3H); 4.26 (t, 1H); 5.16 (d, 1H); 5.22 (m, 1H); 5.50 (m, 1H); 5.75 (m, 1H); 6.67 (d, 1H); 6.79 (d, 1H). MS: ESI [MH^+] Calcd: 328.16; found: 328.12.

*6-Acetoxy-7,8-didehydro-4,5-epoxy-3-methoxy-17-propionylmorphinan (**7**)*

6 (26.3 g; 80 mmol) was dissolved in CH_2Cl_2 (180 ml). A 2.5 M NaOH solution (140 ml) was added. While the two-layer reaction

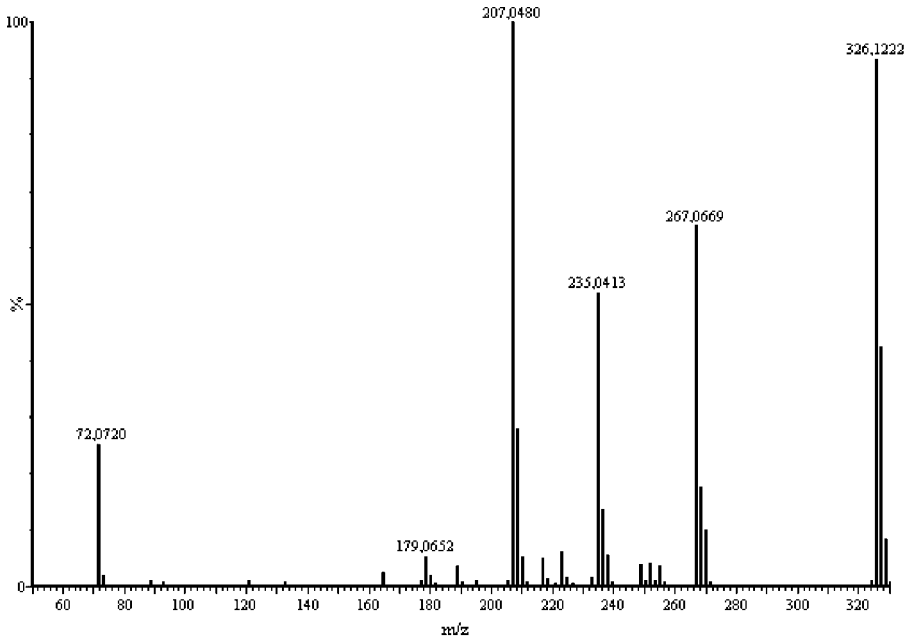


Figure 3. LC-MS/MS fragmentation spectrum of reference compound (*R*)-(−)-10-methoxy-2,11-dihydroxy-*N*-*n*-propylnoraporphine.

mixture was stirred vigorously, propionyl chloride (88 mmol, 8.0 ml) was added portion-wise to the organic layer. When the reaction was complete, the organic layer was separated from the basic layer and washed with 1 M HCl, dried on $MgSO_4$ and evaporated to dryness, yielding **7** (29.4 g; 77 mmol, 96%) as an orange foam. ^{1}H-NMR ($CDCl_3$): δ: 1.18 (t, 3H); 1.86–1.97 (m, 2H); 2.16 (s, 3H); 2.30–2.96 (m, 6H); 3.23 (m, 1H); 3.73 (m, 1H); 3.86 (s, 3H); 5.09 (d, 1H); 5.19 (m, 1H); 5.48 (m, 1H); 5.70 (m, 1H); 6.55 (d, 1H); 6.69 (d, 1H). MS: ESI [MH^+] Calcd: 384.18, found: 384.14.

*7,8-Didehydro-4,5-epoxy-3-methoxy-17-propylmorphinan-6-ol (**8**)*

7 (29.4 g; 77 mmol) was dissolved in THF (500 ml) on an ice-bath. Slowly 2.0 eq. of $LiAlH_4$ (154 mmol, 5.9 g) was added portion-wise to the reaction mixture. The reaction was quenched by cautious addition of H_2O (6.0 ml), aq. NaOH (2.5 M; 6.0 ml) and again H_2O (18.0 ml) (in that order!). The precipitate was filtered off over celite. The filtrate, a clear colourless solution, was dried on $MgSO_4$. Evaporation of the solvent under reduced pressure yielded **8** (23.8 g; 73 mmol, 95%) as an orange foam. ^{1}H-NMR (DMSO-d_6): δ: 1.02 (t, 3H); 1.77–1.92 (m, 3H); 2.42 (m, 1H); 2.76 (m, 1H); 3.08–3.40 (m, 7H); 3.75 (s, 3H); 4.10 (s, 1H); 4.21 (m, 1H); 4.82 (m,1H); 5.30 (d, 1H); 5.65 (d, 1H); 6.56 (d, 1H); 6.72 (d, 1H). MS: ESI [MH^+] Calcd: 328.19, found: 328.18.

*7,8-Didehydro-4,5-epoxy-3-methoxy-17-propylmorphinan-6-one (**9**)*

8 (23.8 g; 73 mmol) was dissolved in toluene (250 ml). MnO_2 (50 g) was added portion-wise to the reaction mixture (every 10 to 20 min). The MnO_2 was filtered off and the procedure was repeated once more. After filtering off the MnO_2 a second time, the solvent was removed under reduced pressure. The generated dark red oil (25 g) was further purified with column chromatography (SiO_2, eluent CH_2Cl_2 gradient up to 10% MeOH), yielding **9** (14.2 g; 44 mmol, 60%) as a crude, brown solid. This crude product was not analysed, but was used directly in the next reaction.

*(R)-(−)-2-Hydroxy-N-n-propylnorapomorphine (**1**)*

9 (14.2 g; 44 mmol) was suspended in freshly distilled HBr solution (48%, ∼250 ml). The reaction mixture was left to reflux for 3 h and then cooled to room temperature. Solvent was removed under reduced pressure. Cold (−5 to −10°C) trifluoroacetic acid (∼150 ml) was added to the residue. The suspension was stirred for 15 min after which the precipitate was filtered off, yielding **1** TFA-salt (9.0 g; 21 mol, 48%) as a pinkish grey powder. ^{1}H-NMR (DMSO-d_6): δ: 1.02 (t, 3H); 1.69–1.87 (m, 2H); 2.71 (t ,1H); 2.93 (m, 1H); 3.12–3.40 (m, 4H); 3.51 (m, 1H); 3.79 (m, 1H); 4.25 (m, 1H); 5.50–6.00 (br s 1H); 6.54 (s, 1H); 6.67 (d, 1H); 6.76 (d, 1H); 7.83 (s, 1H); 8.6–9.0 (br s, 1H); 9.81 (s, 1H). MS: ESI [MH^+] Calcd: 312.16, found: 312.12.

*(R)-(−)-2-Hydroxy-10,11-acetonide-N-n-propylnoraporphine (**4**) (method 1)*

1 TFA-salt (600 mg; 1.4 mmol) was placed in a round-bottomed flask on an ice-bath. TFA (5 ml) was added to the solid, followed by 2,2-dimethoxypropane (5 ml). The reaction mixture was stirred for 10 min, after which the additions were repeated. This sequence was repeated 20 times, after which the reaction mixture was evaporated almost to dryness. The crude mixture was dissolved in dichloromethane. This solution was washed twice with saturated $NaHCO_3$ solution and subsequently dried on $MgSO_4$. The organic layer was evaporated to dryness. The residue was refluxed in hexane and left to cool to room temperature. The formed precipitate, which was a mixture of starting material and product, was filtered off. The product was purified with column chromatography (Al_2O_3, eluent CH_2Cl_2 gradient up to 20% MeOH) yielding **4** (49 mg; 0.14 mmol, 10%) as a brown solid. Mass analysis showed identical results to the product of method 2 (see below).

*(R)-(−)-2-Hydroxy-10,11-acetonide-N-n-propylnoraporphine (**4**) (method 2)*

1 TFA-salt (600 mg; 1.4 mmol) was dissolved in dry THF (8 ml). Acetone (20 ml) and P_2O_5 (∼10 eq.) was added and the mixture

was then heated to reflux. After 2.5 h more P_2O_5 (~5 eq.) was added. After 5 h the reaction mixture was left to cool to room temperature. Solvents were removed under reduced pressure. The residue was basified with saturated $NaHCO_3$ and extracted with diethyl ether. The organic layer was dried on $MgSO_4$ and evaporated to dryness. The residue was purified with flash chromatography (SiO_2, eluent CH_2Cl_2 gradient up to 4% MeOH). After evaporation of the solvents under reduced pressure, the residue was recrystallized using CH_2Cl_2, yielding **4** (150 mg; 0.4 mmol, 30%) as grey crystals. ^{1}H-NMR (DMSO-d_6): δ: 0.92 (t, 3H); 1.46–1.56 (m, 2H); 1.64 (s, 3H); 1.76 (s, 3H); 2.29–2.32 (m, 3H); 2.60 (d, 1H); 2.88 (m, 2H); 3.12 (m, 3H); 6.47 (s, 1H); 6.68 (d, 1H); 6.76 (d, 1H); 7.28 (s, 1H); 9.24 (s, 1H). MS: ESI [MH^+] Calcd: 352.19, found: 352.14.

*(R)-(−)-2-Hydroxy-10,11-methylenedioxy-N-n-propylnoraporphine (**10**)*

Compound **10** was synthesized according to the method of Ramsby *et al*. and Gao *et al*.[6,7] **1** TFA-salt (5.0 g; 11.7 mmol) was used as starting material. The product was converted into an HCl-salt with 2 M HCl in diethyl ether. Recrystallization from MeOH yielded **10** HCl-salt (2.2 g; 6.1 mmol, 52%) as grey crystals. ^{1}H-NMR and MS analysis yielded results that agreed with previously reported data. ^{1}H-NMR (DMSO-d_6 + CD_3OD): δ: 1.00 (t ,3H); 1.82 (m, 2H); 2.86–2.97 (m, 2H); 3.16 (m, 2H); 3.36 (m, 2H); 3.50 (m, 2H); 3.80 (d, 1H); 4.40 (d, 1H); 6.08 (s, 1H); 6.20 (s, 1H); 6.63 (s, 1H); 6.84–6.91 (m, 2H); 7.47 (s, 1H). MS: ESI [MH^+] Calcd: 324.16, found: 324.12.

*(R)-(−)-2-Methoxy-10,11-methylenedioxy-N-n-propylnoraporphine (**11**)*

10 HCl-salt (1.65 g; 4.6 mmol) was dissolved in acetonitrile/methanol (70 ml; 50:50 v/v). To the solution were added diisopropylethylamine (DIPEA; 3 ml) and 2 M trimethylsilyl-diazomethane in hexane (10 ml). The reaction mixture was stirred at room temperature. After 12 h, more 2 M trimethylsilyl-diazomethane in hexane (8 ml) was added to the reaction mixture, which was stirred at room temperature for another 24 h. The solvents were evaporated off under reduced pressure. The residue was purified by flash chromatography (SiO_2, eluent CH_2Cl_2 gradient up to 2% MeOH). Evaporation of the solvents under reduced pressure yielded yellow oil (1.5 g). This product was treated with 2 M HCl in diethyl ether, yielding **11** HCl-salt (1.5 g; 4.0 mmol, 88%) as an off-white solid. ^{1}H-NMR and MS analysis gave results that agreed with previously reported data.[7] ^{1}H-NMR (DMSO-d_6 + CD_3OD): δ: 1.02 (t, 3H); 1.85 (m, 2H); 2.90−3.20 (m, 3H); 3.40 (m, 2H); 3.52 (m, 2H); 3.80 (s, 3H); 3.83 (m, 1H); 4.46 (d, 1H); 6.07 (s, 1H); 6.20 (s, 1H); 6.84–6.92 (m, 3H); 7.54 (s, 1H). MS: ESI [MH^+] Calcd: 338.18, found: 338.14.

*(R)-(−)-2-Methoxy-N-n-propylnorapomorphine (MNPA, **2**)*

Compound **2** was synthesized according to the method of Gao *et al*. and Teitel *et al*.[7,9] Crude **2** (900 mg) was purified with HPLC and converted into an HCl-salt with 2 M HCl in diethyl ether. Subsequent recrystallization using methanol and diethyl ether, yielded **2** (460 mg; 1.3 mmol) as off-white crystals. ^{1}H-NMR and MS analysis yielded results that agreed with previously reported data.[7] ^{1}H-NMR (DMSO-d^6): δ: 0.99 (t, 3H); 1.81 (m, 2H); 2.87 (t, 1H); 2.96 (m, 1H); 3.13 (m, 1H); 3.28–3.55 (m, 4H); 3.74 (m, 1H); 3.77 (s, 3H); 4.22 (m, 1H); 6.68 (d, 1H); 6.73 (s, 1H); 6.79 (d, 1H); 7.93 (d, 1H); 8.83 (s, 1H); 9.73 (s, 1H). MS: ESI [MH^+] Calcd: 326.18, found: 326.14. LC-MS/MS: *m/z*: 326, 267, 249, 238, 221, 193 and 72.

Radiochemistry

Acetonitrile and 0.5 M NaOH were obtained from VWR International and the Swedish Pharmacy, respectively. Thirty-seven percent HCl was purchased from Sigma Aldrich, which was diluted to a concentration of 6 M using sterile water obtained from Fresenius Kabi. Additional chemicals were obtained from various commercial sources and were, whenever possible, of analytical grade. Target product [^{11}C]methane was made using a GEMS PETtrace cyclotron at the Karolinska University Hospital, Stockholm, Sweden, using 16.4-MeV protons in the $^{14}N(p,\alpha)^{11}C$ reaction on nitrogen gas containing 10% H_2. The target gas was irradiated for 10 min with a beam intensity of 35 μA.

The radiosynthesis and purification of [^{11}C]**2** were performed in a fully automated system in which [^{11}C]methyl iodide was synthesized from [^{11}C]methane by gas-phase iodination.[13] Subsequently, the [^{11}C]methyl iodide was transformed on-line into [^{11}C]methyl triflate by means of a heated silver triflate column.[14] [^{11}C]**2** was purified with a semi-preparative reversed-phase HPLC system, containing a Waters XBridge® C-18 column (250 × 10 mm, 5 μm) and an absorbance detector (λ = 300 nm) in series with a GM tube for radiation detection. The mobile phase consisted of acetonitrile and sodium acetate buffer (100 mM, pH 4.7) containing 0.1% ascorbic acid (22:78 v/v), which was used at a flow rate of 5 ml/min. The radiochemical purity of [^{11}C]**2** was analysed by reversed-phase HPLC using a Waters μ-Bondapak C-18 column (300 × 3.9 mm, 10 μm) and an absorbance detector (λ = 270 nm) in series with a Beckman 170 β-flow radio detector for radiation detection. The mobile phase consisted of acetonitrile and phosphoric acid (10 mM) (25:75 v/v) used at a flow rate of 3 ml/min. [^{11}C]**2** was identified by coinjection with unlabeled reference standard **2**.

*(R)-(−)-2-[^{11}C]Methoxy-N-n-propylnorapomorphine ([^{11}C]**2**)*

[^{11}C]Methyl triflate was trapped at room temperature in a vessel containing **4** (0.3 mg), acetonitrile (100 μl) and sodium hydroxide (0.5 M, 4 μl). Subsequently, hydrochloric acid (6 M, 150 μl) was added to the reaction mixture. The vessel was heated to 150°C for 8 min, after which a sodium acetate solution (2 M, 1 ml) was added to the crude reaction mixture. This was followed by injection into the semi-preparative HPLC column. After [^{11}C]**2** eluted (8–9 min) and the fraction was collected, the mobile phase was evaporated off. The residue was dissolved in sterile phosphate-buffered saline (pH 7.4, 7 ml). The product was then filtered through a sterile millipore filter (0.22 μm), yielding a sterile solution free of pyrogens. Irradiation of the target gas for 10 min with a beam intensity of 35 μA yielded more than 1 GBq of >99% radiochemical pure [^{11}C]**2**, with a specific radioactivity of 901 ± 342 GBq/μmol (n = 10) at the time of injection. LC-MS/MS: *m/z*: 326, 267, 249, 238, 221, 193 and 72.

LC-MS/MS analysis

A Waters Acquity ultra performance LC™ system (Waters, Milford, MA, USA) was used for pumping the mobile phase, injection of the samples and heating of the column. The mobile phase consisted of 0.1% formic acid plus 5% acetonitrile in water (A) and 0.1% formic acid in acetonitrile (B). The pumps were

programmed to deliver a linear gradient running from 5 to 80% B in A, from 0 to 2.50 min, at a flow rate of 0.3 ml/min. The composition was returned to 5% B in A from 2.50 to 2.60 min. The end time of the program was set at 5 min. The column, a Waters Acquity UPLC™ BEH C18 (50 mm × 2.1 mm, 1.7 μm) was kept in a column heater at 30°C. A Micromass premier™ quadrupole time of flight mass spectrometer (Waters, Milford, MA, USA) was used for detection. It was operated in positive ESI mode, with the following settings: capillary voltage 3.0 kV; cone voltage 40 V; source temperature 80°C; desolvation temperature 180°C and collision energy ramp ranging from 5 to 25 V.

Conclusion

The required reference compound **2** and acetonide precursor **4** were prepared in good yields. [^{11}C]**2** was prepared in a two-step one-pot radiosynthesis, which consistently yielded sufficient amounts of [^{11}C]**2** for human application, with high radiochemical purity and high specific radioactivity. The overall incorporation yield from [^{11}C]methyl triflate is 60–90%. LC-MS/MS comparison of the ion fragmentation spectrum of the carrier associated with [^{11}C]**2** to the corresponding spectra of reference compounds **2** and (*R*)-(−)-10-methoxy-2,11-dihydroxy-*N*-*n*-propylnoraporphine confirmed the identity of [^{11}C]**2**.

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