The Preparation of Carbon-11 Labelled Diprenorphine: a New Radioligand for the Study of the Opiate Receptor System *In vivo*

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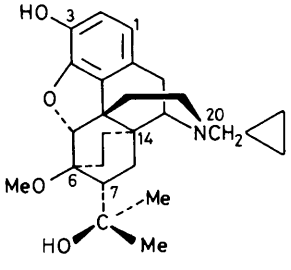
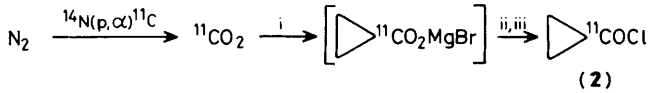
Reaction of *N*-(de-cyclopropylmethyl)diprenorphine (**3**) with [1-^{11}C]cyclopropanecarbonyl chloride (**2**), itself prepared from cyclotron-produced [^{11}C]carbon dioxide, followed by reduction with $LiAlH_4$, provides a fast and efficient route to carbon-11 labelled diprenorphine (**7**), a new radioligand for the study of the opiate receptor system *in vivo*.

With the discovery of specific opiate receptors[1] and subsequently of endogenous opioid peptides[2] there has developed considerable interest in the relationship of opiate receptor systems to pain control and to psychiatric disorders. The developing technique of positron emission tomography (P.E.T.)[3] has potential for the examination of the opiate receptor system *in vivo*, provided that an appropriate high affinity ligand for the opiate receptor can be labelled in adequate specific activity with a suitable positron-emitting radionuclide, such as carbon-11 ($t_{\frac{1}{2}}$ 20.4 min). For this reason we now report the first fast and efficient method for labelling diprenorphine (**1**), a high affinity antagonist of the opiate receptor,[4] with carbon-11. The method is based on a novel radiosynthesis of [1-^{11}C]cyclopropanecarbonyl chloride (**2**) from cyclotron-produced [^{11}C]carbon dioxide, involving the [^{11}C]carbonation of cyclopropylmagnesium bromide followed by direct treatment with phthaloyl dichloride (Scheme 1). Labelling is then achieved by treatment of *N*-(de-cyclopropylmethyl)diprenorphine (**3**) with the [^{11}C]acid chloride (**2**) followed by reduction with $LiAlH_4$ (Scheme 2), a route that is analogous to the original macroscale synthesis of diprenorphine (**1**).[5,6]

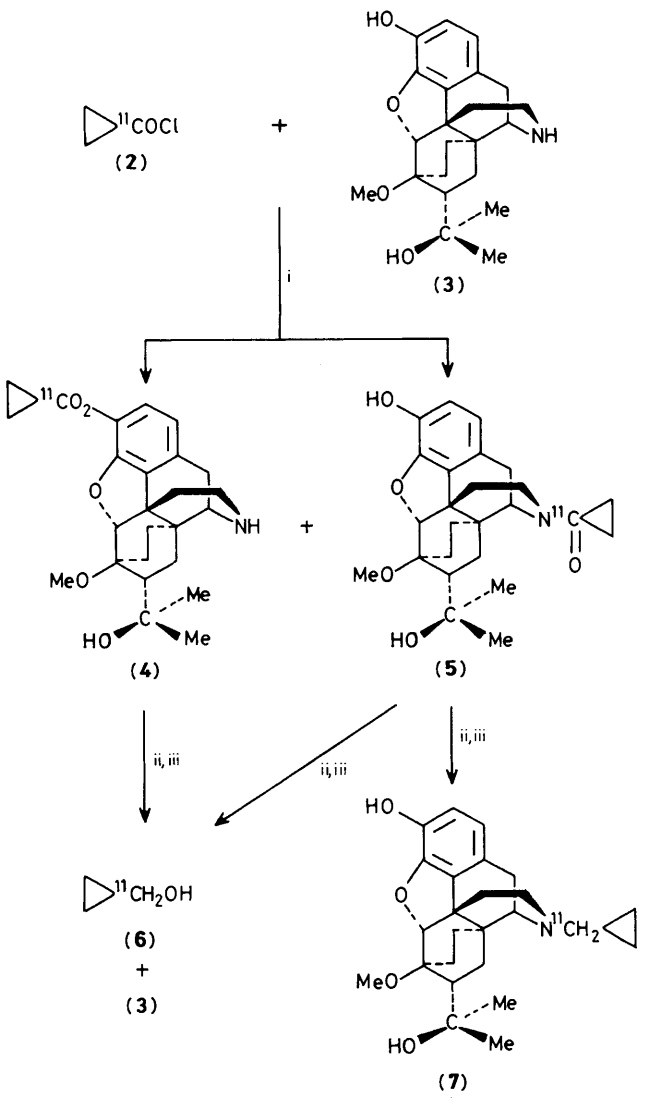


(**1**)



(**2**)

Scheme 1. *Reagents and conditions:* i, cyclopropylmagnesium bromide–Et_2O, room temperature, 2 min; ii, phthaloyl dichloride; iii, 2,6-di-t-butylpyridine, 120—140 °C.



Scheme 2. *Reagents and conditions:* i, THF, −78 °C to room temperature, 2 min; ii, $LiAlH_4$–THF, reflux, 5 min; iii, MeOH.

Thus, 'no-carrier added' [^{11}C]carbon dioxide (9.0—11 GBq) is produced[7] in high radionuclidic and radiochemical purity by the $^{14}N(p,\alpha)^{11}C$ nuclear reaction on nitrogen gas and dispensed into freshly prepared cyclopropylmagnesium bromide (0.6 mmol) in diethyl ether (4.0 ml) at room temperature under nitrogen. After 2 min the carbonation is quenched by the addition of phthaloyl dichloride (3.5 mmol) in diethyl ether (0.5 ml). 2,6-Di-t-butylpyridine (1.8 mmol) in diethyl ether (0.6 ml) is then added and the mixture heated gently under nitrogen to remove solvent. The radioactive residue is then heated more strongly and the released [^{11}C]acid chloride† (**2**) (b.p. 119 °C) carried by a slow stream of nitrogen into a solution of the amine (**3**) (5.4 μmol) in tetrahydrofuran (THF) (5 ml) under nitrogen at −78 °C. This solution is allowed to warm for 2 min to promote the formation of the [^{11}C]amide (**5**).‡ $LiAlH_4$ (1.0 M) in THF (0.7 ml) is then added. The resultant mixture is refluxed gently under nitrogen for min and then cooled to 0 °C. Methanol (1.0 ml) is then added slowly with stirring. The resultant solution is loaded onto a column of silica gel (2.25 g, 'Sep-pak,' Waters Associates) that has been pre-conditioned with THF (15 ml). This column is eluted at *ca.* 5 ml/min with THF (10 ml) and the radioactive eluate rotary evaorated to dryness. The radioactive residue is taken into solvent ($CHCl_3$–EtOH–conc. NH_4OH, 100 : 1 : 0.1 v/v; 1.4 ml) and injected onto a silica gel h.p.l.c. column (30 × 0.7 cm i.d., 'μ-Porasil,' Waters Associates) eluted at 4 ml/min with the same solvent. The radioactive fraction§ that elutes between 7.5 and 8.3 min is collected and rotary evaporated to dryness. Unchanged amine (**3**) is retained on the column.

Normal and reverse phase t.l.c. and normal phase h.p.l.c. demonstrate that the radioactive product from this procedure comigrates with authentic diprenorphine (**1**) and is both radiochemically and chemically pure. In one experiment ^{13}C-enriched carbon dioxide (90 atom %; 47 μmol) was co-included in the radiosynthesis and the purified product examined by broad-band proton-decoupled Fourier transform ^{13}C n.m.r. spectroscopy ($CDCl_3$, 22.5 MHz). The spectrum shows a single peak¶ at δ 59.2 in accord with the chemical shift assigned[8] to C-20 in (**1**). This result confirms that the only radioactive product from our procedure is diprenorphine labelled with carbon-11 at the *N*-cyclopropylmethyl carbon (**7**). The radiochemical yield of this new radioligand (**7**) is up to 35% from [^{11}C]carbon dioxide, corrected for radioactive decay.

H.p.l.c., monitored for both radioacivity and absorbance at 280 nm, reveals the presence of as low as 0.85 μg (2.0 nmol) of carrier (**1**) per % radiochemical yield of the radioligand (**7**). [In the worst case the value was 10 μg (24 nmol) per % radiochemical yield of (**7**).]

The radioligand (**7**) is formulated for human intravenous injection by solubilisation in acetic acid (0.1 ml; 2 M), dilution with sodium citrate solution for injection (3.8% w/v; 10 ml), and sterilisation by filtration (0.22 μm, pore size). All tested preparations have passed independent tests for apyrogenicity and sterility.

From the end of radionuclide production the preparation requires only 53 min and provides an injectable solution of radioligand (**7**) in 5—20% overall radiochemical yield (based on $^{11}CO_2$ used and corrected for radioactive decay). The speed and good efficiency of the radiosynthesis have enabled useful activities of radioligand (**7**) to be obtained in the presence of a quantity of carrier (**1**) that is acceptable for human intravenous injection (<80 μg; <190 nmol). Studies of the opiate receptor system in living human brain are now in progress using the new radioligand (**7**) with P.E.T. and preliminary results will be reported elsewhere.

† In separate experiments the [^{11}C]acid chloride (**2**) was identified by conversion into simple [^{11}C]amides and analysis by t.l.c. and h.p.l.c. The radiochemical yield of [^{11}C]acid chloride is 60—80% (from $^{11}CO_2$ and corrected for radioactive decay). Other volatile [1-^{11}C]acid chlorides have also been prepared by this procedure.

‡ H.p.l.c. and t.l.c. indicate that the [^{11}C]ester (**4**) is formed as a minor by-product.

§ Another radioactive product is the [^{11}C]alcohol (**6**) (elution time, 5.3—5.7 min). This is formed mainly by reductive cleavage of the [^{11}C]ester (**4**), and to a lesser extent by reductive cleavage of the [^{11}C]amide (**5**) as shown in Scheme 2. Any unchanged [^{11}C]acid chloride (**2**) is also reduced ultimately to the [^{11}C]alcohol (**6**).In some preparations a small proportion of the unchanged [^{11}C]amide (**5**) (elution time, 10.9—12.3 min) is observed.

¶ This peak was shown to be attributable to a CH_2 group with multiplicity established by the appropriate spin-echo gated decoupling technique.[9] In the same experiment a sample was taken before reduction and chromatographed, and the main radioactive peak collected. ^{13}C N.m.r. spectroscopy ($CDCl_3$) revealed a single peak at δ 172.0 attributable to an amido-carbon. Since the chemical shifts of amido-carbons in *N*-cyclopropylamides (*e.g.* δ 172.4 for *N*-cyclopropylcarbonyltetrahydroisoquinoline) are very similar, this is further evidence for the formation of the [^{11}C]amide (**5**) in the radiosynthesis.

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