A Simple One-Pot Synthesis of Cyclopropane [^{11}C]Carbonyl Chloride. Synthesis and Biodistribution of [^{11}C]Cyclorphan*

* (-)-3-Hydroxy-N-Cyclopropylmethyl-morphinan

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Summary

A rapid, one-pot, synthesis of cyclopropane [^{11}C]carbonyl chloride was developed. This synthesis proceeded in 80% radiochemical yield (EOB) in a synthesis time of 10 minutes. This acid chloride was then used to synthesize a model compound, [^{11}C]cyclorphan, by alkylation of norlevorphanol followed by reduction of the intermediate [^{11}C]amide in an overall synthesis time of 55 minutes and a radiochemical yield of 15% (EOB). The biodistribution of [^{11}C]cyclorphan in control and naloxone pretreated mice showed non-specific binding and rapid clearance from brain.

Key Words: Cyclopropane [^{11}C]carbonyl chloride, [^{11}C]cyclorphan, opiate receptor, carbon-11

Introduction

Since opiate receptor antagonists, in general, are less toxic and have a slower dissociation rate from the receptor site than the corresponding agonists and are therefore superior candidates for labeling for PET studies, we have sought to develop a general strategy for labeling these molecules. The approach undertaken has been to label the N-cyclopropylmethyl group, a common structural feature of a number of opiate receptor ligands (Fig. 1). It has been demonstrated that the cyclopropylmethyl group increases greatly the antagonistic property of morphine or morphine analogs (1). Such a strategy would use the corresponding nor-compounds of which many are readily available.

We report here the development of a rapid, one-pot synthesis of cyclopropane [^{11}C]carbonyl chloride and its use in the synthesis of a model compound, [^{11}C]cyclorphan, in high yield and high specific activity. The

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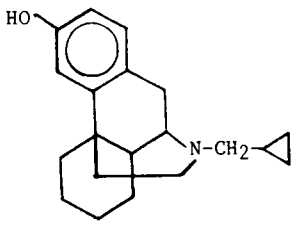
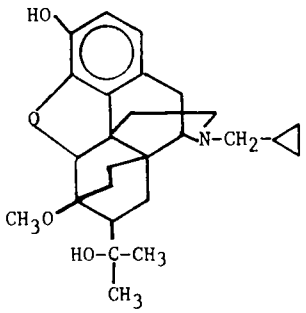
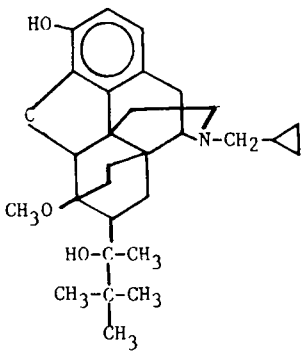


Cyclorphan



Diprenorphine



Buprenorphine

Figure 1. Structure of Opiate Antagonists Containing the N-Cyclopropylmethyl Group

biodistribution of [^{11}C]cyclorphan in mice (control and pretreated with naloxone) was also measured. While this manuscript was being prepared, the synthesis of [^{11}C]diprenorphine, another opiate antagonist having the N-cyclopropylmethyl group was reported (2).

Materials and Methods

Materials. Triethylamine was dried over anhydrous potassium carbonate and distilled prior to use. Tetrahydrofuran was dried over sodium/benzophenone and distilled prior to use.

Radiochemical Assay. Radiochemical and chemical purity for cyclorphan

was assayed by high performance liquid chromatography (HPLC) using an analytical silica column (IBM, 5 mm x 250 mm) with a mobile phase of methylene chloride:methanol (9.5:0.5) with 0.1% diisopropylamine added. The elution profile of the radioactivity was congruent with added carrier. The radiochemical purity was also assayed by thin layer chromatography (TLC) on a silica plate (Eastman) by spotting [^{11}C]cyclorphan with authentic carrier material and showing that the radioactivity was coincident with the spot corresponding to authentic compound. The mobile phase used was the same described above. Cyclorphan had an R_f value of 0.95 and was visualized with iodine.

Preparation of Cyclopropylmagnesium Bromide. A modification of the method of Nakatsuka and coworkers was used to prepare cyclopropylmagnesium bromide (3). The reaction and transfer of product was carried out under a dry nitrogen atmosphere. Magnesium turnings (0.3 g, 12.3 mmol) were flame dried and anhydrous tetrahydrofuran (10 ml) was added. The solution was heated to reflux and 1,2-dibromoethane (22 mg, 0.1 mmol) was added to initiate the reaction. The solution was refluxed for 5 minutes and a solution of cyclopropyl bromide (1.2 g, 10.0 mmol) (Aldrich Chemical Company) in anhydrous tetrahydrofuran (10 ml) was slowly added over a period of 15 minutes. The resulting solution was refluxed for an additional 40 minutes, allowed to cool and transferred to a storage vessel. The concentration of cyclopropylmagnesium bromide solution was ~ 0.4 M.

Synthesis of Cyclopropane [^{11}C]Carbonyl Chloride. The apparatus was flushed with anhydrous nitrogen for 30 minutes prior to the reaction. All additions and transfers were performed under anhydrous nitrogen. A cyclopropylmagnesium bromide-tetrahydrofuran solution (300 µl) was placed in the reaction vessel and [^{11}C]carbon dioxide, produced according to the published method (4), was bubbled into the stirring solution with a flow rate of ~ 20 ml/min. After the transfer was complete the solution was stirred for an additional minute. To the vessel was then added dimethylformamide (500 µl) and phthaloyl chloride (500 µl) and the solution was heated to 140°C for 5 min. Cyclopropane [^{11}C]carbonyl chloride produced was removed from the

vessel during the reaction by a stream of nitrogen with a flow of ~ 150 ml/min. The product was passed through a water cooled condensor and an air cooled glass loop (8 mm x 60 mm) to remove any reactants which are in the nitrogen flow.

Synthesis of [^{11}C]Cyclorphan. Cyclopropane [^{11}C]carbonyl chloride was carried by the nitrogen flow into a cooled solution (-78°C) of norlevorphanol (5-10 mg) dissolved in methanol (200 µl), chloroform (1.0 ml) and triethylamine (300 µl). The solution was stirred at 50°C for 8 minutes and then evaporated to dryness under vacuum. Lithium aluminum hydride (1.5 ml of a 1 M solution in tetrahydrofuran) (Aldrich Chemical Company) was added to the dried residue and the solution was stirred and refluxed at 140°C for 10 minutes. The solution was then allowed to cool for a minute and a sodium hydroxide solution (2.7%, 0.5 ml) was added. The resulting solution was evaporated to dryness under vacuum. The product was taken up in 1 N HCl (3 ml + 2 ml), filtered and passed through a reversed phase C_{18} Sep Pak cartridge (Waters Associate). The vessel, filter and Sep Pak cartridge were rinsed with water (2.0 ml) and the Sep Pak cartridge was rinsed with methylene chloride (2.0 ml). The product was removed with methanol (0.5 ml) followed by a methylene chloride:methanol (9:1) solution (1.0 ml). The solution was then injected onto a preparative HPLC silica column (10 mm x 250 mm, IBM) with a mobile phase of methylene chloride:methanol (9:1) with 0.1% diisopropyl amine. [^{11}C]Cyclorphan had a retention time of ~ 8.5 minutes, and norlevorphanol had a retention time of ~ 20 minutes with a flow rate of 3.0 ml/min. The product was collected over a period of ~ 2.0 minutes and was transferred to a rotary evaporator and the solvent removed under vacuum. The product was dissolved in saline (3 ml) and filtered (sterile 0.22 µ millipore) into a multi-injection vial. [^{11}C]Cyclorphan was then ready for injection ~ 55 minutes from the end of bombardment (EOB) with a yield of ~ 15% based on the amount of [^{11}C]carbon dioxide trapped at EOB. In a typical experiment using a 25 µA beam for 1 minute, 40.0 mCi of [^{11}C]carbon dioxide was trapped and 1.2 mCi of [^{11}C]cyclorphan was obtained

at the end of synthesis. Radiochemical purity was assayed by TLC and found to be greater than 98.0%.

Specific activity was determined to be greater than 0.72 Ci/μmol by analysis with HPLC. The analysis was performed using a calibration curve of peak area versus normal concentration of six standard cyclorphan solutions of 185.0 to 4.6 nmol and no mass for cyclorphan was observed. There were, however, two unidentified, UV absorbing peaks, one immediately preceding and one closely following the elution of [^{11}C]cyclorphan. The similarity of retention times precluded complete separation and hence the product was contaminated by approximately 1 mg of these two unlabeled contaminants. That neither of these compounds was cyclorphan itself was demonstrated by adding unlabeled cyclorphan to the product and observing a new peak between the two unidentified unlabeled compounds. The retention time of the authentic cyclorphan was 9.0 minutes with a flow rate of 1.0 ml/min.

Tissue Distribution Studies. Female Swiss albino mice (BNL strain, 22-28 g) were used in this study. The mice pretreated with naloxone were given an intraperitoneal injection of 2.5 mg/kg of naloxone HCl (Endo Pharmaceuticals, Inc., Manati, P. R.,) 50 minutes prior to the injection of [^{11}C]cyclorphan. [^{11}C]Cyclorphan (84-213 μCi/mouse) of specific activity > 0.72 Ci/μmol (EOB) was injected by tail vein and the animals killed by cervical dislocation at the desired time interval (5, 30 and 60 minutes). The various organs were rapidly removed, blotted free of blood and placed in preweighed counting vials and the vials were sealed. Tissue samples as well as injection standards were counted in a Packard automated sodium iodide well counter. Both percent injected dose per gram of tissue and percent injected dose per organ were determined from the decay corrected activity.

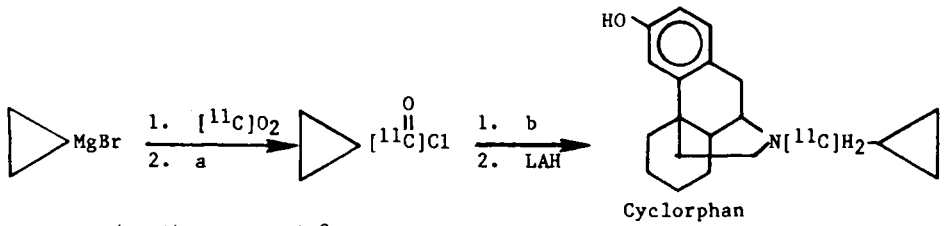
Results and Discussion

There are two approaches that can be envisioned for the introduction of the N-cyclopropylmethyl group to various compounds. The first approach involves the preparation of cyclopropyl [^{11}C]methyl halide based on the procedure used to produce [^{11}C]methyl iodide (5). However, since, even under mild reaction conditions, these halides have been shown to undergo rearrange-

ment to the allyl and cyclobutyl groups (6), this method would be predicted to result in a mixture of ^{11}C-products of similar structure and therefore to present difficulties in purification.

The second approach involves the preparation of cyclopropane [^{11}C]carbonyl acid halide. Nakatsuka and coworkers (3) reported the preparation of cyclopropane [^{14}C]carbonyl chloride by the addition of [^{14}C]carbon dioxide to a solution of cyclopropylmagnesium bromide followed by the reaction of the isolated cyclopropane [^{14}C]carbonyl acid with thionyl chloride to produce the desired [^{14}C]acyl chloride. Jeffrey and Vogel also reported the formation of unlabeled cyclopropane carbonyl acid chloride by a similar procedure (7). Our attempt at the synthesis of cyclopropane [^{11}C]carbonyl acid chloride by this method, using [^{11}C]carbon dioxide as the labeled precursor, was not successful. In addition to low yields and a long reaction time (> 20 minutes) there was difficulty in removing unreacted thionyl chloride from the [^{11}C]acyl chloride produced due to the low boiling point of thionyl chloride.

This led us to investigate the use of a higher boiling acid chloride as the chlorinating agent thereby taking advantage of a large difference in boiling points to facilitate separation (8). We therefore developed a one-pot synthesis of cyclopropane [^{11}C]carbonyl chloride utilizing phthaloyl chloride (bp 269-271°C) as the chlorinating agent. [^{11}C]Carbon dioxide reacted in greater than 97.0% yield with cyclopropylmagnesium bromide at room temperature. Dimethylformamide and phthaloyl chloride were then added to the reaction vessel and the solution was heated to 140°C. The cyclopropane [^{11}C] carbonyl acid chloride was removed under a stream of nitrogen and passed through a water cooled condensor and an air cooled condensor to remove reactants from the [^{11}C]acyl chloride. Cyclopropane [^{11}C]carbonyl chloride was produced in ~ 80.0% yield (EOB) in a synthesis time of 10 minutes after EOB. Optimum reaction conditions were achieved with a reaction temperature of 140°C, a nitrogen flow of 150 ml/min, and a reaction time of 5 minutes. At a lower nitrogen flow the [^{11}C]acyl chloride was not removed from the reaction solution, and at higher temperatures the yield was lowered due to



a. $\underline{o}$-$C_6H_4(COCl)_2$, DMF, 140°

b. Norlevorphanol, $CHCl_3$, CH_3OH, TEA, 50°

Fig. 2 Synthesis of ^{11}C-Labeled Opiate Antagonists

loss on the walls of the glass transfer tube.

The target opiate receptor ligand for this approach is cyclorphan, a mixed agonist-antagonist which is about 60 times more potent as an analgesic than morphine in man (9) and has a high affinity for μ and κ binding sites (10). Cyclorphan was chosen due to its simple morphinan structure which contains only the 3 hydroxyl group that may interfere in the labeling of this compound.

The synthetic scheme for [^{11}C]cyclorphan shown in Fig. 2, was carried out according to a modified procedure of Gates and Montzka (1). Cyclopropane[^{11}C]carbonyl acid chloride was trapped in a solution of norlevorphanol dissolved in chloroform, methanol and triethylamine at -78° with ~ 75% efficiency. The alkylation was carried out with a reaction time of 8 minutes at 50°C to afford 3-cyclopropyl[^{11}C]carbonyloxy-$\underline{N}$-cyclopropylcarbonylmorphinan in ~ 75% yield. Higher reaction temperatures gave unidentified carbon-11 labeled side products and thereby lowered the yield of the desired carbon-11 labeled amide. The reduction of the amide and hydrolysis of the ester was carried out in high yield (~ 75%) with lithium aluminum hydride at 140°C in 10 minutes. Therefore this method affords [^{11}C]cyclorphan in a four step synthesis after the production of [^{11}C]carbon dioxide in a yield of ~ 15% after HPLC purification and with a synthesis time of ~ 55 minutes.

Table 1 shows the results of the biodistribution of [^{11}C]cyclorphan in mice. Groups of six mice received intravenous injections of [^{11}C]cyclorphan and were killed at 5, 30 and 60 min after injection. In addition to the

Table 1. Tissue Distribution (% Dose/g ± S.D.) of NCA [^{11}C]Cyclorphan in Control and Naloxone-Pretreated Mice (n = 2-4)

Organ	Pretreatment	Time After Injection (Min)		
		5	30	60
Cerebellum	Control	1.1±0.0	0.82±0.58	0.17±0.00
	Naloxone	1.1±0.1	0.45±0.06	0.24,0.18
Thalamus	Control	0.98±0.18	0.92±0.10	0.37±0.11
	Naloxone	1.0±0.1	0.73±0.13	0.44,0.41
Rest of Brain	Control	1.1±0.0	0.90±0.14	0.41±0.03
	Naloxone	1.1±0.1	0.69±0.08	0.43,0.40
Blood	Control	1.4±0.2	0.86±0.15	0.72±0.55
	Naloxone	1.3±0.1	0.69±0.12	0.41,0.34
Heart	Control	4.1±0.2	1.3±0.3	0.52±0.03
	Naloxone	4.2±0.5	1.1±0.2	0.56,0.54
Lungs	Control	28 ● 9	8.6±3.0	2.7±0.3
	Naloxone	25 ± 1	6.9±0.5	3.6, 3.0
Liver	Control	9.6±1.6	11 ● 3	5.9 ● 0.4
	Naloxone	10 ±1	8.9±1.6	6.2, 5.6
Spleen	Control	7.0±1.0	3.4±0.8	1.2±0.3
	Naloxone	8.3±0.9	3.1±0.6	1.1, 1.0
Kidneys	Control	24 ± 2	15 ± 2	7.6±0.4
	Naloxone	24 ± 4	14 ● 2	9.8,6.6
Small Intestines	Control	5.5±1.1	11 ± 1	4.9±1.3
	Naloxone	7.1±0.3	11 ● 6	7.8, 10

other organs, the cerebellum, thalamus and rest of the brain were assayed for radioactivity. A matching group of six mice received the same dose of [^{11}C]cyclorphan but received an intraperitoneal injection of 2.5 mg/kg of naloxone 50 minutes prior to the injection of the carbon-11 labeled cyclorphan. In the mice receiving [^{11}C]cyclorphan alone there was no differentiation in the uptake of the carbon-11 label after 30 minutes between the cerebellum, which is low in concentration of opiate receptors, and the thalamus which is rich in opiate receptors (11). Furthermore, there was also only slightly less radioactivity associated with the thalamus and the rest of the brain in the naloxone-pretreated mice at 30 minutes. The washout of carbon-11 activity from the receptor site by 60 minutes was also observed. These results indicate that [^{11}C]cyclorphan is not an ideal candidate for use

as an opiate receptor binding ligand in PET experiments due to its nonspecific binding and rapid clearance from the brain.

In conclusion, we have developed a rapid and simple one-pot synthesis of cyclopropane [^{11}C]carbonyl chloride in high yield. We have also shown that the [^{11}C]acyl chloride can be used to synthesize [^{11}C]cyclorphan, a model structure for opiate receptor antagonists. Although [^{11}C]cyclorphan was found not to be a suitable tracer molecule for opiate receptor sites in vivo utilizing PET, this general synthetic strategy should be applicable to other opiate receptor antagonists containing the N-cyclopropylmethyl group.

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