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SYNTHESIS OF [^{11}C]-OHMEFENTANYL, A NOVEL, HIGHLY POTENT AND SELECTIVE AGONIST FOR OPIATE μ-RECEPTORS

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SUMMARY

The authors of the present paper reported the synthesis of [^{11}C]-ohmefentanyl in a symposium abstract in 1991(1). We here describe the results of synthesis and analysis in detail. Ohmefentanyl 1 is a novel, highly potent and selective agonist for opiate μ-receptors. In order to visualize the μ-receptor by Positron Emission Tomography (PET), this compound was labelled with carbon-11. The unlabelled cis-A-ohmefentanyl was prepared in a nine-step synthesis and two-step fractional crystallization, and the OH-precursor 11 for [^{11}C]-ohmefentanyl labelling was obtained by hydrolysis of the 4-N-propionyl group of cis-A-ohmefentanyl in 6 N hydrochloric acid. The [^{11}C]-propionyl chloride was prepared by carbonation of ethylmagnesium bromide with cyclotron-produced [^{11}C]-carbon dioxide followed by direct treatment of the intermediate complex with phthaloyl dichloride and 2,6-di-t-butylpyridine. Reaction of the OH-precursor 11 with [^{11}C]-propionyl chloride yields [^{11}C]-ohmefentanyl separated by HPLC, with a high specific activity of 11.1 - 14.8 GBq μmol^{-1} (300-400 mCi μmol^{-1}), 49 minutes after the end of bombardment.
The keto-precursor 12, prepared by hydrolysis of the 4-N-propionyl group of cis-10 in 8 N hydrochloric acid, was also used for [^{11}C]-ohmefentanyl labelling. Reaction of the [^{11}C]-propionyl chloride with keto-precursor 12, followed by addition of sodium borohydride, yields [^{11}C]-ohmefentanyl.
The [^{11}C]-labelled ohmefentanyl obtained using the OH-precursor 11 is a cis-A form, while that obtained using the keto-precursor is a mixture of cis-A and cis-B forms.

Key words : ohmefentanyl, opiate receptor, [^{11}C]-ohmefentanyl, [^{11}C]-propionyl chloride.

INTRODUCTION

With the discovery of specific opiate receptors and endogenous opioid peptides, considerable interest has been developed in the relationship of opiate receptor systems to pain control and to psychiatric disorders. The opiate receptors that mediate the diverse actions of endogenous opioid peptides as well as exogenous opiate drugs have been identified as three major types, the best documented of which are named μ, δ and k. Opioid ligands are among the best-known classes of physiologically active agent that interact with multiple subpopulations of opiate receptors and efforts to develop such ligands have been pursued as biochemical and pharmacological probes aimed at addressing the problem of receptor multiplicity and receptor isolation(2). Within recent years, a few opioid ligands have been reported to have very high affinities and selectivities for one particular type of binding site and have been used in numerous studies for investigation of the physiological role of opiate receptors.

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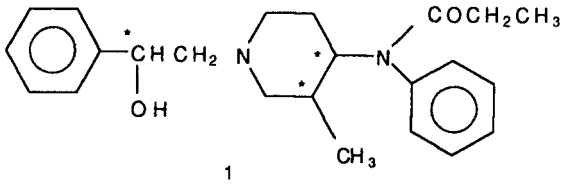
Interestingly, after modifications of fentanyl derivatives [3-5], ohmefentanyl 1, N-[1-(ß-hydroxy-ß-phenylethyl)-3-methyl-4-piperidyl]-N-phenylpropionamide, synthesized through introduction of a methyl group in 3-position and replacement of the phenylethyl by the hydroxy phenylethyl group in 1-position of the piperidine ring of fentanyl, was found to be a very potent agent with an analgesic activity 28 times greater than fentanyl and 6,300 times greater than morphine in the mouse (hot plate, i.p.). As compared under identical conditions with some well-known representative analgesics, such as etorphine, etonitazene,

carfentanil, sufentanil, 4-(p-bromophenyl)-4-dimethylamino-1-phenylethyl-cyclo-hexanol and 3-(ß-phenylethyl)-9ß-methoxy-9α-(m-hydroxy-phenyl)-3-azabicyclo-[3,3,1]-nonane(P-7521), ohmefentanyl is the most potent.



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Receptor binding assays demonstrated that [^{3}H]-ohmefentanyl had high affinity and selectivity for opiate μ-receptors in mouse and rat brain membranes [6, 7]. It is noteworthy that, as compared with DAGO and sufentanil, ohmefentanyl could be superior to [^{3}H]-DAGO as a μ-selective radioligand and its high affinity and selectivity as compared with morphine might well make it a superior analgesic agent [8]. Very recently, Rothman et al[9] reported that RTI-4614-4, which is in fact cis-ohmefentanyl, had Ki values (nM) as follows : μ(0.0055), δ (148), k_1 (84.8), k_{2a} (2275) and k_{2b} (22.3). The selectivity of RTI-4614-4 for μ was 2,700 vs δ, 15,400 vs k_1, 413,700 vs k_{2a} and 4,054 vs k_{2b}. This study also corroborated the results described above. In addition, depending on its oil-water partition coefficient [10], this compound could be expected to cross the blood-brain barrier. Taken together, these characteristics encouraged us to label ohmefentanyl that could be suitable for in vivo labelling of opiate μ-receptors and visualizing the receptors by positron emission tomography, as was recently done for carfentanil [11].

This study was reported in a symposium abstract in 1991 [1] and we now describe the synthesis and analysis results in detail.

RESULTS AND DISCUSSION

The OH-precursor 11 for [^{11}C]-ohmefentanyl labelling was obtained by hydrolysis of the 4-N-propionyl group of cis-A-ohmefentanyl prepared by a nine-step synthesis starting from benzylamine and a two-step fractional crystallization. The procedure is outlined in Fig. 1.
There are three chiral centers in the ohmefentanyl molecule, so there are four diastereoisomeric pairs which have been separated and are designated cis-A, cis-B, trans-A and trans-B ohmefentanyl [12]. The cis-form implies a predominant equatorial 4-N-$COCH_2CH_3$ Ph group with an axial 3-CH_3 group and a trans-form, with an equatorial 3-CH_3 group in the chair conformation of the piperidine ring of ohmefentanyl. After treatment with a chemical shift reagent and double irradiation, the cis-form was measured on the basis of a 100-MHz NMR spectrum with (JH_3, H_4) = 5 cps [3]. Reduction of the keto-group of cis-10 by sodium borohydride to the hydroxy group (cis-1) produces another chiral center. After fractional crystallization, the first crop is called cis-A-ohmefentanyl, the second, obtained from the parent solution, as is called cis-B. The analgesic activity of the cis-form is more potent than that of the

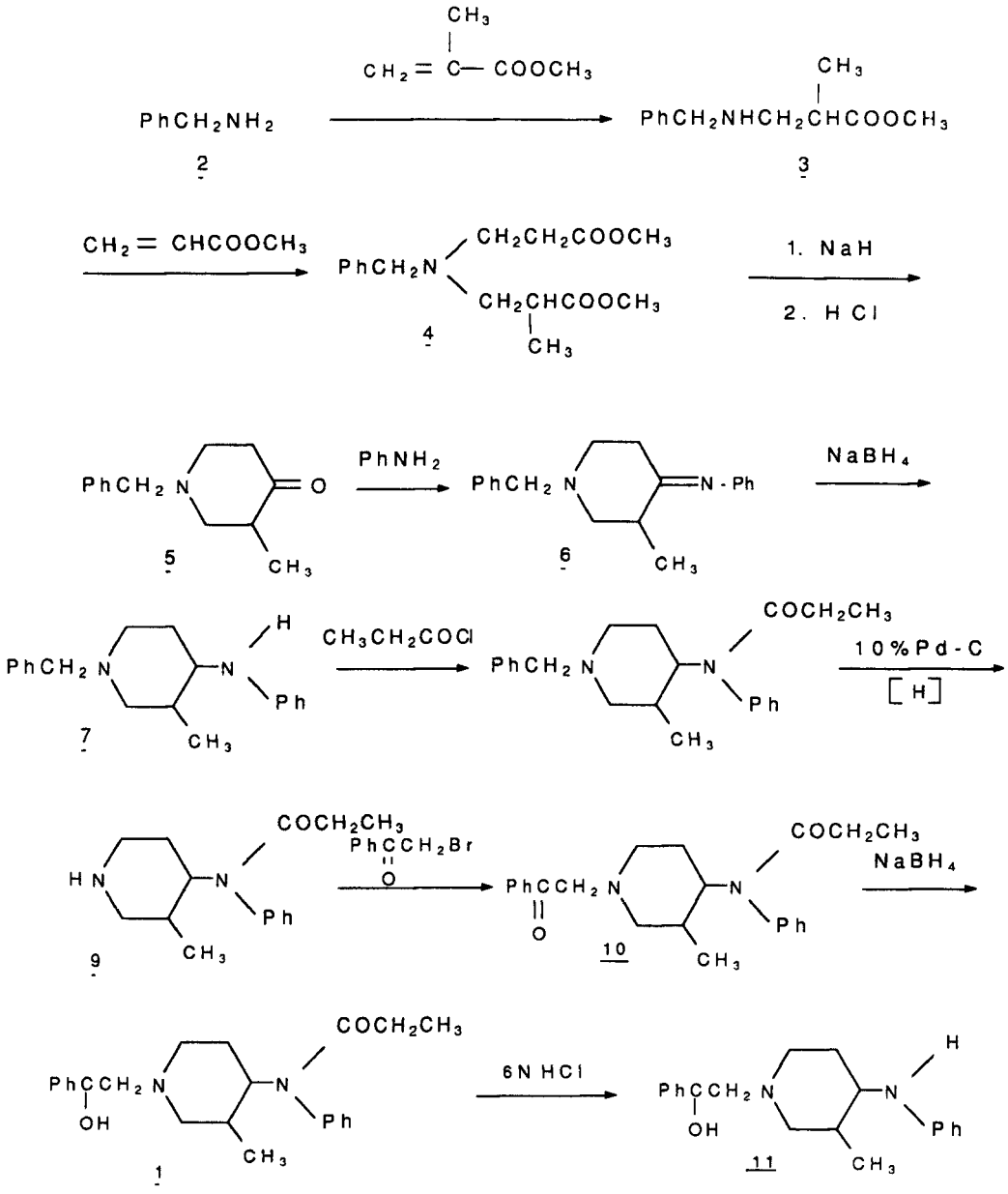

Fig. 1 Synthetic route of ohmefentanyl and OH-precursor 11 for [^{11}C]-ohmefentanyl labelling

trans-form and cis-A-ohmefentanyl, which was most widely used in reported biochemical and pharmacological assays, is the most potent among the four diastereoisomers. [^{11}C]-propionyl chloride was synthesized as described in Fig. 2, from ethylmagnesium bromide and cyclotron-produced [^{11}C]-carbon dioxide followed by direct treatment of the intermediate complex with phthaloyl dichloride and 2,6-di-t-butyl-pyridine [13]. Reaction of [^{11}C]-propionyl chloride with an excess of the OH-precursor 11 yielded the desired [^{11}C]-cis-A-ohmefentanyl.

The keto-precursor 12 prepared by hydrolysis of the 4-N-propionyl group of the cis-10 in 8 N hydrochloric acid (Fig. 3) was also used for [^{11}C]-ohmefentanyl labelling. Reaction of [^{11}C]-propionyl chloride with the keto-precursor 12 followed by addition of sodium borohydride yielded the [^{11}C]-ohmefentanyl, which was a mixture of the cis-A and cis-B forms (Fig. 3).

[^{11}C]-Propionanilide was prepared in order to check the active reagent [^{11}C]-propionyl chloride on HPLC (ODS column, partisil 10, M9, Whatman, 25 cm. R_t-propionanilide : 9.5 min, R_t-aniline : 2.5 min, 5 ml/min, U.V. 224 nm, H_2O : EtOH : phosphated buffer (pH 2.3) = 70 : 30 : 1 (V/V).

In the reaction of [^{11}C]-propionyl chloride with an excess of keto-precursor 12 followed by addition of sodium borohydride, the remaining precursor had become OH-precursor 11 and two by-products, ohmefentanyl ester 13 and OH-precursor ester 15 could have been be formed. So the two reference compounds were specially prepared as described in Fig. 4-A and 4-B. We attempted to selectively hydrolyze the 4-N-propionyl group of ohmefentanyl ester 13 in 4N hydrochloric acid, but ohmefentanyl was first obtained (Fig. 4-A).

The physicochemical data on ohmefentanyl, the precursors and related reference compounds are shown in table 1. The elementary analysis of compounds 1, 11 and 13 (compound 15 is an oil) are consistent with the CHN theoretical composition.

Table 1
Physicochemical data on ohmefentanyl, the precursors and related reference compounds

Compounds					Anal		HPLC
N°	R_1	R_2	Formula	m p [a] °C	M S (M+1)	I R (cm^{-1})	R_t[b](min)
1	H	CH_3CH_2CO	$C_{23}H_{30}N_2O_2$	138-140	367	3300, 3000-2700, 1650, 1600	5
11	H	H	$C_{20}H_{26}N_2O$	132-134	311	3360, 3040-2800,1600	7.8, 8.5[c]
13	CH_3CH_2CO	CH_3CH_2CO	$C_{26}H_{34}N_2O_3$ HCl	194-196	423	1740, 1660, 1600	5.7
15	CH_3CH_2CO	H	$C_{23}H_{30}N_2O_2$	Oil bp 198°C (0.2 mm)	367	3450, 1740, 1600	11.5

a : uncorrected.
b : cation exchange column, Whatman partisil 10, M9, SCX, 25 cm, 5 ml/min. 214 nm, Saline : Ethanol : phosphated buffer (pH 2.3) = 72.5 : 27.5 : 1 (V/V).
c : two forms for OH-precursor.

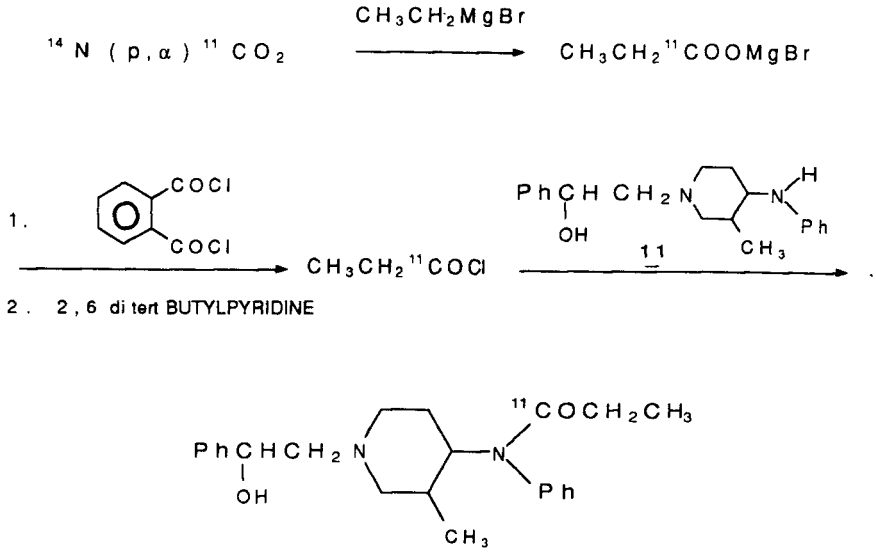

Fig. 2 Reaction scheme used in the synthesis of [^{11}C]-cis-A-ohmefentanyl

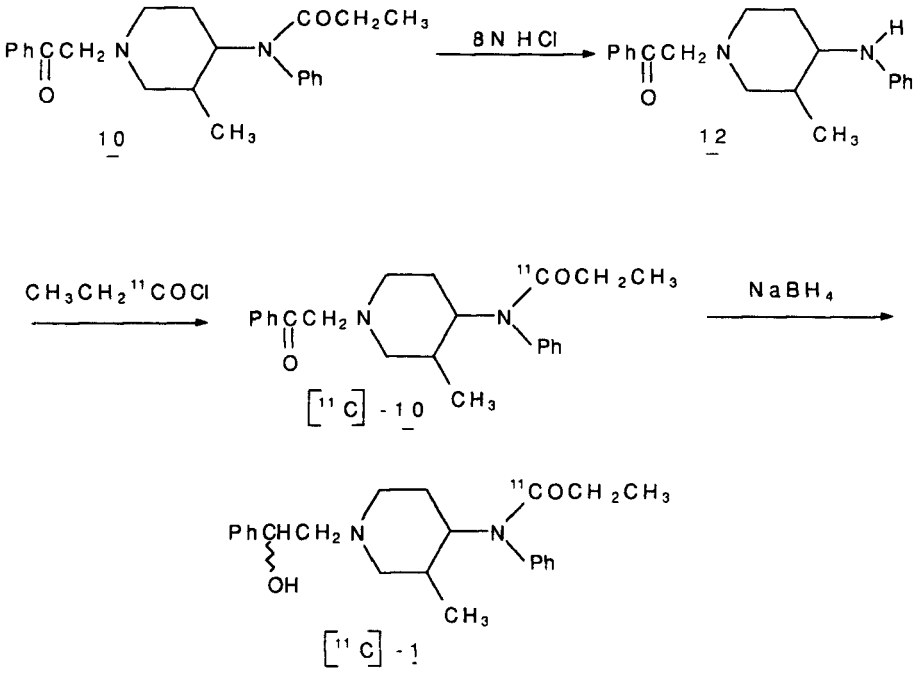

Fig. 3 Synthesis of the ketoprecursor and [^{11}C]-cis-A and [^{11}C]-cis-B ohmefentanyl labelling

Starting with OH-precursor 11 or keto-precursor 12, 30-50 mCi (1.11 - 1.85 GBq) were obtained 45-50 min after EOB with a specific radioactivity ranging from 500 to 700 mCi/μmol (18.5 - 25.9 GBq/μmol).

Radiosynthesis showed that the [^{11}C]-labelled product was identical to ohmefentanyl on analytical HPLC.

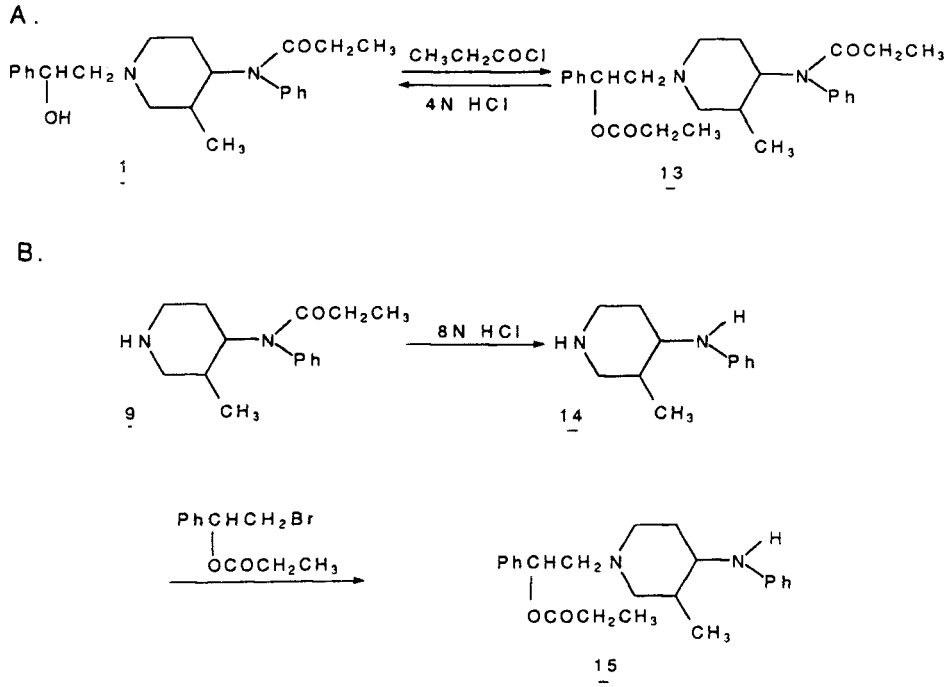

Fig. 4 Synthesis of the ester derivatives as reference compounds 13 and 15

EXPERIMENTAL

cis-A-N-[1-(ß-hydroxy-ß-phenylethyl)-3-methyl-4-piperidyl]-aniline 11.
A solution of cis-A-ohmefentanyl. HCl (1, 500 mg, 1.24 mmol) in 6N hydrochloric acid (30 ml) was refluxed with stirring under nitrogen for 6 h. After cooling in an ice-bath, the reaction mixture was diluted with water and then made basic with NH_4OH; the aqueous solution was extracted (three times) with chloroform. After drying the extracts ($MgSO_4$) and concentration, the residue was crystallized three times with petroleum ether (60-90°C) to obtain 11, yield 200 mg (51.9%).

cis-N-[1-(benzoyl methyl)-3-methyl-4-piperidyl]-aniline 12.
A solution of cis-N-[1-(benzoyl methyl)-3-methyl-4-piperidyl]-N-phenylpropionamide (10, 500 mg, 1.37 mmol) in 8N hydrochloric acid was refluxed under Nitrogen with stirring for 15 h. The resulting solution was concentrated in vacuo to dryness and 100 ml of isopropanol were added.

The crystals were isolated by filtration, dissolved in a small quantity of water and then treated with ethanol, processed in the same way three times yielding 250 mg of 12, 2HCl (48%) as colourless crystals, mp > 260°C. Anal ($C_{20}H_{24}N_2O$, 2HCl), CHN, IR (KBr), 3450, 1700, 1600 cm^{-1} ; m/e 309 (M+1).

cis-A-N-[1-(ß-propionyloxy-ß-phenylethyl)-3-methyl-4-piperidyl]-N-phenyl-propionamide 13.
A mixture of cis-A-ohmefentanyl HCl (1, 40 mg 0.1 mmol) and propionyl chloride was stirred at 120°C (oil bath) for 2 h. The excess propionyl chloride was removed and the residue was crystallized three times with methanol-ethyl acetate mixture solvent to obtain 13, yield 35 mg (76 %), mp 197-198°C. (Lit. 194-196°C[14]). Anal ($C_{26}H_{34}N_2O_3$, HCl) ; CHN ; IR (KBr) 1740, 1660, 1600 cm^{-1} ; m/e 423 (M+1).

cis-N-[1-(b-propionyloxy-b-phenylethyl)-3-methyl-4-piperidyl]- aniline 15.
A mixture of cis-N-(3-methyl-4-piperidyl)-aniline [3](14, 1.5 g, 7,9 mmol), ß-propionyloxy-ß-phenylethyl bromide (2.4 g, 9.3 mmol), triethylamine (1.4 ml, 10 mmol) in toluene (100 ml) was stirred at 80°C under nitrogen for 24 h. The white solid, triethylamine hydrobromide was precipitated and filtered off. After removal of the toluene, the residue was distilled in vacuo to yield 0.2 g of 15, bp 198°C/0.2 mm. Anal ($C_{23}H_{30}N_2O_2$), IR (film) 3450, 1740, 1600 cm^{-1} ; m/e 367 (M+1).

Synthesis and purification of [^{11}C]-labelled ohmefentanyl.
Two vessels used for the preparation of [^{11}C]-ohmefentanyl were kept at 120°C and the apparatus was purged with dry nitrogen for 30 minutes prior to synthesis. One of the vessels (A) was filled with a solution of Grignard reagent ethylmagnesium bromide (7 µl of 3 M solution, 21 µmol, Aldrich) in dry diethyl ether (200 µl) and the other (B) with OH-precursor 11 (1.2 mg, 1 µmol) in dry chloroform (200 µl) or with keto-precursor 2HCl 12 (1.5 mg, 4 µmol) in 200 µl of dry chloroform with 12 µmol of triethylamine. (Fig. 5).
The carbon-11 was produced by the (p,α) reaction on pure nitrogen (N60, Air Liquide 99.9999 % purity). By irradiating with 20 MeV protons, 30 µA, for 30 minutes, about 55.5 GBq (1.5 Ci) of [^{11}C]-carbon dioxide [1] was obtained. After reaction of [^{11}C]-carbon dioxide with a Grignard reagent for 2 minutes, the radio-complex ($CH_3CH_2{}^{11}COOMgBr$) was quenched by the addition of phthaloyl dichloride (150 µl, 1 mmol) and 2, 6-Di-t-butylpyridine (200 µl, 0.9 mmol). The mixture was heated to 50°C under nitrogen to remove diethyl ether.
After evaporation of solvent from vessel A, the radioactive residue was heated to 105°C to distill the [^{11}C]-propionyl chloride with nitrogen flow into vessel B at 0°C. Then vessel B was allowed to heat to 75°C for 10 minutes to promote [^{11}C]-amide formation. The chloroform was removed at 105°C to dryness.

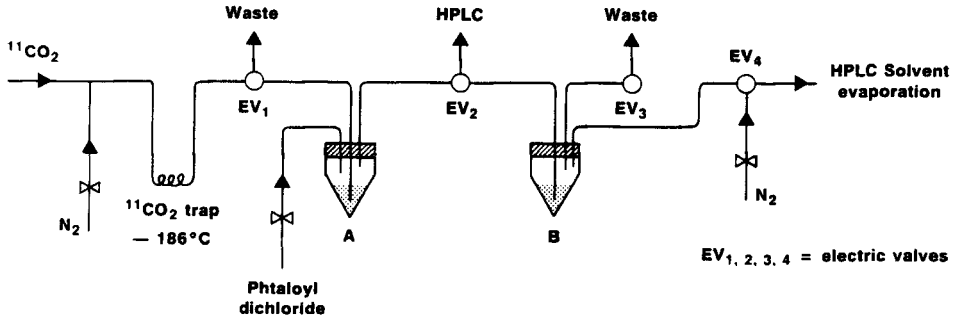

Fig.5 : Line for the [^{11}C]ohmefentanyl labelling

(A : from OH-precursor 11) : The radioactive residue was taken up into an HPLC solvent (1 ml) and injected onto an HPLC column (cation exchange column, Whatman, M9, Partisil 10, SCX, 50 cm), eluted at 7 ml/min, with saline, ethanol and phosphated buffer (pH 2.3) (72.5 : 27.5 : 1 v/v). The eluate was continuously monitored for both radioactivity and UV absorbance at 214 nm. The radioactive peak corresponding to the retention time of ohmefentanyl (11 min) was collected and gave about 1.11 GBq (30 mCi) of [^{11}C]-labelled ohmefentanyl. The excess precursor 11 was well separated (Rt = ~25 min) from the collected radioactive sample.

(B : from keto-precursor 12) : The radioactive residue was taken up into methanol (1 ml) with about 2 mg of sodium borohydride prior to HPLC purification (same HPLC system as in former paragraph) yielding 1.85 GBq (50 mCi) of [^{11}C]-ohmefentanyl at 45-50 min after EOB.

ANALYSIS OF [^{11}C]-OHMEFENTANYL

HPLC : The radioactive fraction corresponding to ohmefentanyl collected from the preparative HPLC column was injected onto an analytical HPLC column simultaneously with the reference compound (cation exchange column, Whatman, M9, partisil 10, SCX-25 cm), eluted at 6 ml/min with saline, ethanol and phosphated buffer (pH 2.3) (72.5 : 27.5 : 1 v/v). The eluate was monitored for radioactivity and optical density at 214 nm and a shoulder free peak was observed.

TLC : The fraction of [^{11}C]-ohmefentanyl was also analyzed on silica plates, developed in chloroform, methanol and conc. ammonium hydroxide (100 : 5 : 1 v/v). Radioactive scanning (chromelec 101) showed a single radioactive spot at Rf = 0.8, co-migrated with the reference ohmefentanyl and no other radiochemical or chemical impurity was detected.

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