Journal of Labelled Compounds and Radiopharmaceuticals – Vol. XXIX, No. 9

NOTE

PREPARATION OF THE ANTIOESTROGENIC COMPOUND *N*-[METHYL-^{11}C]-TOREMIFENE FOR THE STUDY OF OESTROGEN-RECEPTOR POSITIVE TUMORS IN VIVO.

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Summary

The synthesis of the antioestrogenic compound *N*-[methyl-^{11}C]toremifene (*N*-[methyl-^{11}C]-(Z)-2-(4-(4-chloro-1,2-diphenyl-1-butenyl)-phenoxy)-*N*,*N*-dimethylethylamine) by alkylation with [^{11}C]methyl iodide of the desmethyl precursor, generated *in situ* from the corresponding citrate with 2,2,6,6-tetramethylpiperidine (TMP), is reported. The reaction product was purified with high performance liquid chromatography (HPLC) using either normal or reversed phase conditions. After solubilization by sonication in citric acid / propylene glycol / water the chemically and radiochemically pure product was obtained in 55-65 % radiochemical yield (decay corrected from [^{11}C]methyl iodide) in 40-45 min after end of bombardment (EOB).

Key words: [^{11}C]toremifene, synthesis, radioligand, oestrogen receptor, PET.

INTRODUCTION

Oestrogen receptor ligands labelled with ^{18}F or ^{11}C have a great potential for identification of oestrogen-receptor positive tumors *in vivo* with positron emission tomography (PET). A series of fluorine substituted oestrogens have been studied with regard to their affinity and selectivity for oestrogen receptors (1), and it is currently possible to quantify oestrogen receptors in human breast cancer *in vivo* with [^{18}F]-16α-18-fluoro-oestradiol-17β (2). ^{11}C-Labelling of tamoxifen, a drug commonly used in the treatment of breast cancer, has been reported by several research groups (3-5).

Toremifene is a tamoxifen analogue which has recently been introduced for clinical use. Preliminary results on the ^{11}C-labelling of toremifene and biodistribution studies in rats have been

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0362–4803/91/091085–05$05.00

Received 1 March, 1991
Revised 3 June, 1991

reported (6). In this paper, an evaluation of the preparation of ^{11}C-toremifene with regard to solvents for the methylation reaction, purification conditions and methods of solubilization is presented.

EXPERIMENTAL

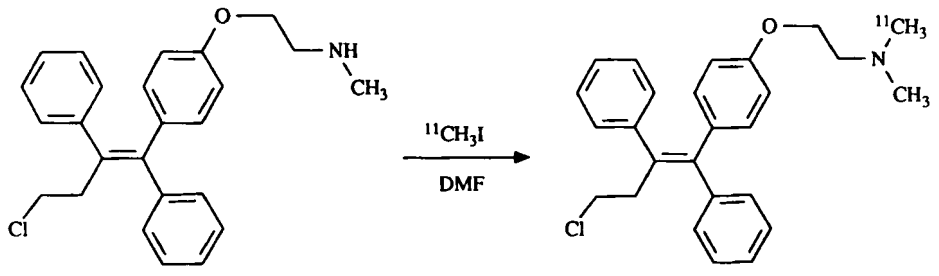
General

[^{11}C]Carbon dioxide, [^{11}C]CO_2, was produced with the ^{14}N(p,α)^{11}C reaction by the 103 cm isochronous cyclotron of Åbo Akademi. [^{11}C]Methyl iodide, [^{11}C]CH_3I, was produced by the standard procedure of our laboratory (7) using a one-pot synthesis (8). The *N*-desmethyl-precursor used in the ^{11}C-labelling syntheses, (Z)-2-(4-(4-chloro-1,2-diphenyl-1-butenyl)-phenoxy)--*N*-methylethylammonium citrate (Fc 1200), and toremifene were obtained from Lääkefarmos Ltd, Turku. Preparative HPLC was performed with a Kontron 414-T pump, Rheodyne 7010 injector and 7066 column selector on Waters semipreparative columns (μ-Bondapak C-18 or μ-Porasil, 7.8 x 300 mm) using eluents and flow rates specified below. UV-absorbance and radioactivity were monitored with a Pharmacia UV-1 detector at 254 nm and a coaxial-type air ionization chamber (9) connected in series. Sterile solutions used in the solubilization of the final product were obtained from the Pharmacy of the University Hospital of Turku.

Synthesis of N-[methyl-^{11}C]toremifene

[^{11}C]CH_3I was introduced to a solution of *N*-desmethyl-toremifene citrate (2 mg, 3.5 μmol) and TMP (0.7 μl, 4.0 μmol) in dimethylformamide (DMF, 300 μL), cooled at ice-water temperature, and the reaction solution was heated at 90 °C for 5 min. After addition of HPLC eluent (0.5 mL), the reaction solution was purified on a Waters μ-Bondapak C-18 column (7.8 x 300 mm) using an eluent of 0.002 % diethylaminoacetate in methanol at a flow of 5 mL/min. The purified product was evaporated to dryness and solubilized by addition of citric acid (0.04 % in water, 1 mL), propylene glycol (1 mL) and water (8 mL) during sonication at 50-60 °C for 2 min. After filtration through a Millex-GS sterile filter, the formulated product was ready for use in PET studies.

Analytical HPLC was performed with a Kontron system consisting of pump 420, gradient former 425 and variable wavelength UV-detector 432 (at 239 nm) in series with a NaI crystal detector. The starting material and products were separated on a Waters μ-Bondapak C-18 column (3.9 x 300 mm) using an eluent of 0.05 M H_3PO_4/CH_3CN (55/45) at a flow rate of 2 mL/min. Retention times: *N*,2,2,6,6-pentamethylpiperidine (PMP, pempidin), 2.0 min; CH_3I, 3.2 min; Fc 1200, 7.1 min; toremifene, 8.1 min; *N*,*N*-dimethyl Fc 1200 (quartenary toremifene



Synthesis of N-[methyl-^{11}*C]toremifene*

Analysis of reaction mixtures and formulated product solutions

derivative): 8.9 min; N-ethyl Fc 1200, 9.7 min. The density of a 0.04 M citric acid/propylene glycol/water mixture (10/10/80), determined with a PAAR DMA 45 density meter, was 1.0061 g/mL at 20 °C. This value was used to determine the volume of formulated product solutions by weighing.

RESULTS AND DISCUSSION

Precursors of ^{11}C-labelled compounds may be successfully generated *in situ* by liberation of amines from their corresponding salts by using TMP (10). This approach was also applicable in the synthesis of *N*-[methyl-^{11}C]toremifene. The alkylation reaction can be performed in various polar organic solvents as examplified in Table 1. The amount of ^{11}C-labelled byproducts was low, *i.e.*, ^{11}C-PMP (^{11}C-pempidine) and the quaternary product obtained on reaction of [^{11}C]CH_3I with [^{11}C]toremifene were obtained in less than 6 and 2 %, respectively. However, it is important to use a molar ratio of TMP and Fc 1200 (nortoremifene) close to unity, as 17 % yield of the byproduct ^{11}C-PMP was obtained when two equivalents of TMP versus Fc 1200 was used. In some syntheses an unlabelled byproduct was found in the final product solution. It was identified as the *N*-ethyl derivative, formed on reaction of Fc 1200 with ethyl iodide. Ethyl iodide was formed from traces of ethanol, used in the washing procedure of the [^{11}C]CH_3I synthesis system. This byproduct was eliminated by final washing with hexane and careful drying of the [^{11}C]CH_3I synthesis system.

Purification was possible using either normal or reversed phase HPLC-systems, as shown in Table 2. Interestingly, neither of these systems separated the product and the *N*-ethyl derivative. Since the yield is high in DMF and since additional time is needed for evaporation of the solvent (acetone) for purification by normal phase HPLC, DMF was chosen as solvent and reversed phase HPLC as the purification method.

Table 1. Radiochemical yield of *N*-[methyl-^{11}C]toremifene*

Solvent	Temperature (°C)	Yield (%)
Acetonitrile	90	69
Acetone	100	74
Dimethylformamide	94	93

*Decay corrected radiochemical yield in the reaction of $^{11}CH_3I$ with 2 mg of Fc 1200 citrate and 0.7 µL of TMP in 300 µL of solvent for 5 min at specified temperature.

Citric acid/propylene glycol/water may be used for the solubilization of ^{11}C-tamoxifen (4). We found that this solvent mixture is superior to mixtures containing albumin or tween 20, and that a final concentration of 0.004 % of citric acid was sufficient for solubilization of ^{11}C-toremifene. This hundred-fold reduction in citric acid concentration, compared to conditions reported for ^{11}C-tamoxifen (4), can partly be attributed to differences in the solubility of toremifene and tamoxifen. The main reason is that ^{11}C-toremifene, prepared in our laboratory, is obtained in a lower concentration than previously reported for ^{11}C-tamoxifen by others (4). The specific radioactivity obtained in our laboratory for radiopharmaceuticals prepared from [^{11}C]methyl iodide is currently in the order of 30-74 GBq/µmol (0.8-2.0 Ci/µmol) decay corrected to the end of a 40 min bombardment with 12 MeV protons at 10 uA, which in the case of [^{11}C]toremifene gives a value of 7.4-18.5 GBq/µmol (200-500 mCi/µmol) at end of synthesis (EOS). This specific radioactivity corresponds to a total amount of 20-48 µg (50-120 nmol) of ^{11}C-toremifene.

Table 2. Purification of *N*-[methyl-^{11}C]toremifene by HPLC*

System	PMP	Toremifene	Fc 1200
µ-Bondapak C-18[1]	2.2	5.5	10.5
µ-Porasil[2]	3.0	6.8	12.0

*Retention times (min) of toremifene, its *N*-desmethyl derivative Fc 1200 and *N*,2,2,6,6-pentamethylpiperidine (PMP) using the following eluents and flow rates:

1: Diethylamino acetate (0.002 % in MeOH, w/v), 5mL/min.

2: CH_2Cl_2/MeOH, 68/3 (v/v), 3 mL/min.

ACKNOWLEDGEMENTS

We are grateful to Dr. Lauri Kangas, Lääkefarmos Ltd, Turku, for supplying Fc 1200 and toremifene, and to Kirsti Torniainen, Central Hospital Pharmacy, Turku, for supplying solutions for the solubilization of ^{11}C-toremifene.

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