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Research Article

New preparation of [^{123}I]PE2I: investigation of the oxidation and purification steps

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Summary

In order to simplify preparation of [^{123}I]PE2I, iodogen and hydrogen peroxide were examined as oxidants for the preparation of radioiodinated PE2I, e.g. (*E*)-*N*-(3-iodoprop-2-enyl)-2β-carbomethoxy-3β-(4′-tolyl) nortropane). Among the oxidizing compounds assayed, iodogen appears to afford the best results for this purpose (high radiochemical yield, high chemical purity and a reasonable reaction time). A simplified and efficient method is described here for the preparation of [^{123}I]PE2I based on the exchange of a ^{123}I tributyl tin analogue in oxidative conditions followed by purification by solid phase extraction (SPE). Using this method, [^{123}I]PE2I was obtained with a radiopharmaceutical yield over 60%, with chemical and radiochemical purities higher than 95% without the addition of a carrier (Na^{127}I) (2G Bq/nmol) or with a specific activity adjusted to 85–90 MBq/nmol in the presence of the carrier. Copyright © 2002 John Wiley & Sons, Ltd.

Key Words: [^{123}I]; PE2I; cocaine analogue; radiolabelling; iodogen; hydrogen peroxide; SPECT

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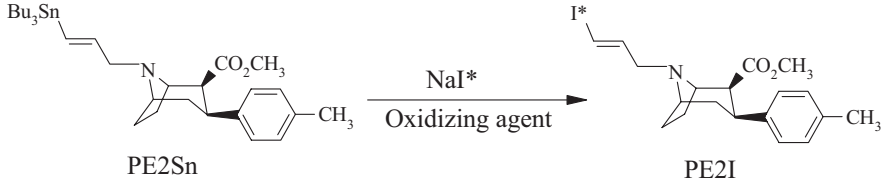
Introduction

Dopamine transporters (DAT) have been extensively studied *in vivo*, with positron emission tomography (PET) using ligands labelled with $\beta+$ emitting isotopes such as [^{11}C] β-CIT-FE[1] or [^{76}Br]PE2Br.[2,3] The measurement of the density evolution of DAT in the living human brain by single photon emission computed tomography (SPECT) offers tremendous clinical potential in several neuro degenerative disorders such as the Parkinson's disease.[4–7] Research efforts on the development of specific DAT radioligands have shown the potential of new analogues of cocaine usable in SPECT, such as, [^{123}I]β-CIT[8] and [^{123}I]β-CIT-FE.[9]

PE2I: (*E*)-*N*-(3-iodoprop-2-enyl)-2β-carbomethoxy-3β-(4′-tolyl) nortropane (Scheme 1) belongs to the group of structurally related tropanes, which presents *in vitro* a high affinity and selectivity for DAT over serotonin (5-HTT) and norepinephrine (NET) transporters[10]. Moreover PE2I, when labelled with ^{125}I or ^{123}I, presents *in vivo* a high specific binding at the DAT in monkey and in human with a kinetic allowing visualization of the DAT one hour after injection of the radioligand.[11,12]



PE2Sn PE2I

Scheme 1. Radiolabelling scheme

The desirable characteristics for the preparation of [^{123}I]PE2I with and without carrier are a high yield, a short reaction time and a simple purification process. To fulfil these requirements, various oxidants (iodogen and hydrogen peroxide) and two different purification processes (HPLC and solid phase extraction SPE) were evaluated for preparing [^{123}I]PE2I using the demetallation of a tri-butyl stannyl precursor leading to radioiodination.

Experimental

Reagents

N-[3-(Tri-*n*-butylstannyl) prop-(2*E*)-enyl]-2β-carbomethoxy-3β-(4′-tolyl) nortropane (PE2Sn) was synthesized as previously described.[10]

Carrier-free added ^{123}I-iodide was diluted in 0.02 M sodium hydroxide solution to reach a radioactivity concentration of 3.7 GBq/ml. Iodogen and hydrogen peroxide (30%) were purchased from Aldrich Chemicals (St. Quentin Fallavier, France). The hydrogen peroxide was diluted to 3% with distilled water before use. Methanol, ethyl acetate and water were of HPLC grade and purchased from Prolabo. Sep-Pak C18 cartridges Vac 3cc were purchased from Waters Chromatography Division (Milford, MA, USA). All other chemicals were of reagent grade and used as received.

Iodogen coating

In a conical vial, 100–400 μg of iodogen in 1 ml of dichloromethane was slowly evaporated to dryness under a slow stream of nitrogen. The vial was stoppered under nitrogen and stored for 24 h at room temperature or up to 10 weeks at −20°C in the dark.

HPLC systems

The radiolabelling yield, radiochemical purity and specific activity were measured using reverse phase analytical HPLC (system 1). The later associates a Varian solvent delivery pump, a radioactivity detector, a Varian UV/VIS detector ($\lambda = 254$ nm) and a reverse phase column (Kromasil RP 18-5 μm, $4.6 \times 250\ mm^2$ Merck, Darmstadt, Germany). Elution was performed at 1 ml/min with MeOH/H_2O (80/20 v/v).

The specific radioactivity was calculated from the HPLC radiochromatogram. The area of UV absorbance of the radioiodinated PE2I was measured and compared to a calibration curve relating the mass to UV data by integration (D2500, Merck)

The chemical purity was assessed by HPLC (system 2) using a Varian solvent delivery pump, a Varian UV/VIS detector ($\lambda = 210$ nm) and a reverse phase column (symmetry C18-5 μm, $4.6 \times 250\ mm^2$ Waters Corporation, Milford, MA, USA). The column was eluted at 1 ml/min with methanol.

Radiolabelling

Iodogen. To PE2Sn (54 μg, 94 nmol in 150 μl of absolute ethanol), without carrier, Na^{123}I (1480 MBq, 50 μL) and 70 μl of sodium

phosphate buffer (0.1 M pH 7.4) were added to the previously described conical vial containing the iodogen (100–400 μg).

For the preparation in the presence of the carrier [^{123}I]PE2I, Na^{127}I (20.8 nmol diluted in 20 μl of 0.1 M $NaHCO_3$ solution) were added before the labelling reaction.

The reaction was allowed to proceed at room temperature. Removing the reaction mixture from the vial stopped the oxidation. The radiolabelling yield and specific activity were checked using HPLC (system 1) and the chemical purity was analysed by HPLC (system 2).

Hydrogen peroxide. This oxidation process was already described.[11] Briefly, 50 μl of 0.1 M HCl, 50 μl of 3% w/v hydrogen peroxide and 1480 MBq of Na^{123}I (in 50 μl of 0.02 M NaOH) were added to a vial containing 50 μg (87 nmol) of PE2Sn in 100μl of ethanol. For assays containing the carrier, Na^{127}I (20.8 nmol diluted in 20 μl of a 0.1 M $NaHCO_3$ solution,) was also added. After 20 min at room temperature, the addition of $Na_2S_2O_5$ (1 mg, 100 μl) stopped the reaction. The radiolabelling yield, specific activity and chemical purity were analysed as described previously.

Radiopharmaceutical preparation

HPLC. [^{123}I]PE2I and the precursor for labelling, PE2Sn, were extracted from the reaction mixture with 2 ml of ethyl acetate. Ionic compounds and unreacted ^{123}I remained in the aqueous phase. The organic phase was evaporated to dryness and purified by HPLC (system 1). The radioactivity corresponding to the PE2I peak was collected in a vial, diluted with 10 ml of water, and passed through a Sep Pak$^{@}$ C18 cartridge. The later was washed with 50 ml of water to eliminate methanol. Then, the labelled radioiodinated compound was eluted from the cartridge using 1.5 ml of ethanol.

Solid phase extraction. [^{123}I]PE2I was purified from the stannyl precursor and by-products by passing the reaction mixture directly through a Sep Pak$^{@}$ C18 column. Radioactivity was eluted from the column by 1 ml fractions of an ethanol–water mixture (70/30: v/v). [^{123}I]PE2I was collected in the fourth fraction (Figure 1).

Following HPLC and SPE purification, [^{123}I]PE2I (5 mCi -185 MBq) was formulated in sterile saline containing no more than 10% ethanol.

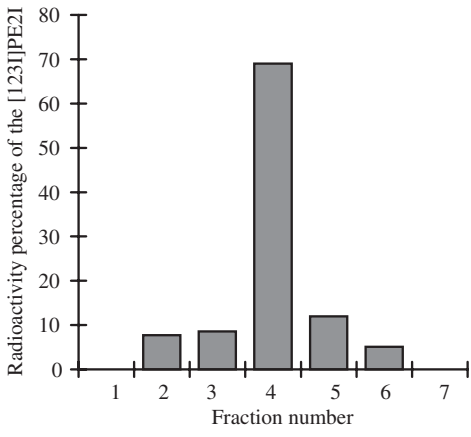


Figure 1. Elution profile in solid phase extraction purification. Percentage [^{123}I]PE2I in function of elution fractions (1 ml)

Results and discussion

The radiochemical yield (RCY) was measured on the HPLC (system 1) radiochromatogram of system and the radiochemical and chemical purity of [^{123}I]PE2I particularly in respect to PE2Sn was checked by HPLC analysis (system 2). The retention volume of the main radioactive peak corresponded to that of authentic PE2I in the HPLC system 1 (19–20 ml).

Radiolabelling of PE2I by ^{123}I was studied using various oxidizing agents. RCY results are presented in Table 1. It appeared that an influence on the specific activity was observed on RCY when iodogen was used as oxidant. The radiochemical yield (RCY) was higher at no carrier-added level 81 ± 1 versus 60 ± 2. This value (60 ± 2) could be explained by a too small amount of iodogen (94 nmol) in comparison to the amount of iodide in the reaction mixture (≈ 20 nmol) The ratio iodogen/iodide is 100/5.

On the contrary, when iodogen was used, the RCY was higher without carrier (81 versus 60%).

Statistically, with hydrogen peroxide and in the conditions described before, the RCY were not dependent of the specific activity: 71 ± 4 (without carrier) versus 75 ± 2 (with carrier).

In HPLC system 1 and using simultaneous gamma and UV detection, it was proved that the main radioactive peak observed in the crude mixture of iodogen reaction (Figure 2) is radioiodinated PE2I, which

Table 1. Radiochemical yields obtained at high and low specific activities using various oxidizing agents (mean ± SD)

Oxidizing agent	*n*	Iodine	Buffer	Time (min)	Radiolabelling yield (%)
Iodogen	5	No carrier added	Na-phosphate pH 7.4 0.1 M	15	81 ± 1
Iodogen	5	With carrier	Na-phosphate pH 7.4 0.1 M	15	60 ± 1
Hydrogen peroxide	5	No carrier added	0.1 M HCl solution	20	70 ± 4
Hydrogen Peroxide	5	With carrier	0.1 M HCl solution	20	75 ± 2

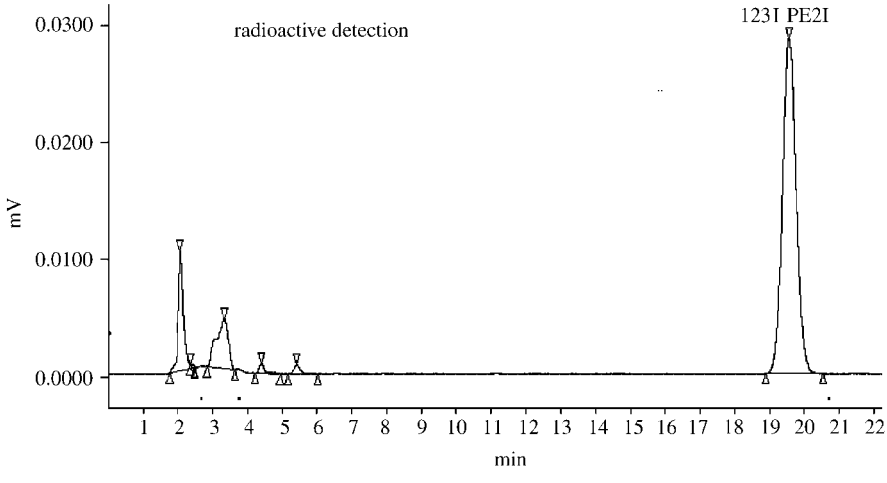


Figure 2. HPLC radiochromatogram (system 1) of the crude mixture reaction using iodogen as oxidant. The radiolabelling yield is 80%

displayed the same retention time (~20 min) as the cold PE2I detected by UV tracing. Under the same chromatographic conditions, the stannyl precursor, PE2Sn, showed a retention time longer than 60 min. Using HPLC 2 system, the PE2Sn precursor was observed at 14 min retention time (Figure 3).

With the hydrogen peroxide, in acidic conditions, undesired by-products were formed. UV profile displayed other impurities that eluted before the major peak. This acidic pH was not favourable because of a competition between hydrogen and iodine atoms and the main impurity (probably hydrogen precursor) can be damaging for further purification.[13]

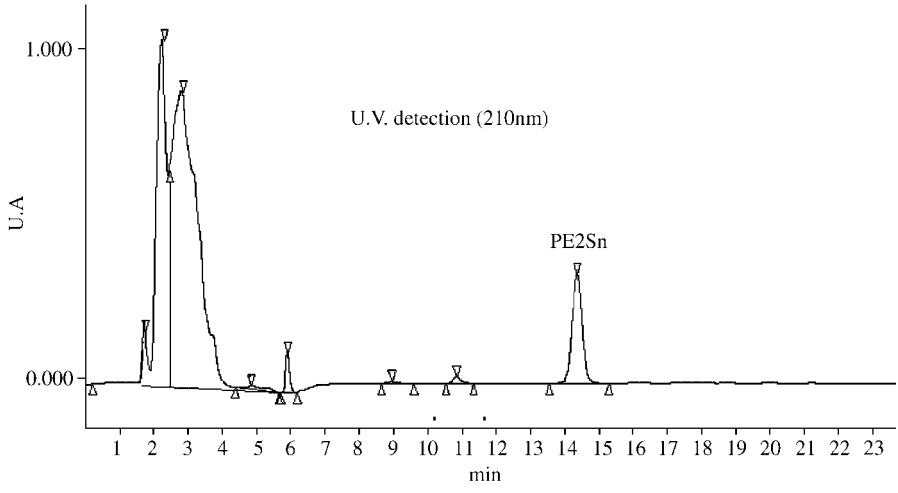


Figure 3. UV HPLC chromatogram (system 2–210 nm) of the crude mixture reaction before SPE purification. The remaining PE2Sn is observed at 14 min retention time

Among the two oxidants studied, as it appeared that the use of iodogen gave the highest radiolabelling yield for [^{123}IPE2I preparation without carrier and allowed also a simple and efficient purification process, labelling conditions with this oxidant were investigated more extensively. The oxidation with iodogen was achieved at pH 7.4. This neutral value avoided a neutralization step before purification.

The radiolabelling reaction from the PE2Sn precursor with iodogen was rapid at room temperature. A 81% labelling yield was achieved in 3 min and it remained constant for 15 min (Figure 4).

The influence of the molar ratio of the oxidant (iodogen) on the precursor was studied. The RCY was analysed for a constant mass of PE2Sn precursor (94 nmol), which represented at least 50 times more than the mass of iodide and a mass of iodogen ranging from 115 to 460 nmol. Table 2 summarizes the RCY versus the molar ratio of oxidant to precursor.

One of the main advantages of the iodogen over hydrogen peroxide was its insolubility in water. Therefore, that remained coated on the reaction vial. After removal of the soluble part, the oxidant was therefore removed from the reaction solution. The second advantage of this method is the low quantity of the precursor used. Consequently, the purification process was simplified.

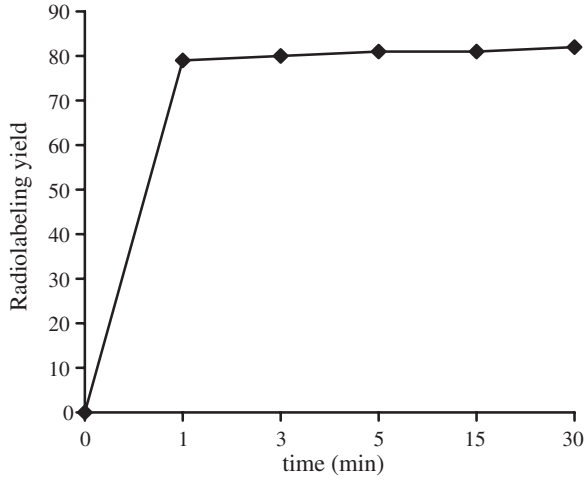


Figure 4. Radiolabelling yield of [^{123}I]PE2I as a function of the reaction time (amount of iodogen: 231 nmol; PE2Sn: 94 nmol; total volume 0.27 ml)

Table 2. Effect of the molar ratio of iodogen to PE2Sn on [^{123}I]PE2I radiolabelling yield (mean ± SD)

Molar ratio (iodogen/PE2Sn)	*n*	Radiolabelling yield %
1.23	5	48 ± 1
2.45	5	81 ± 1
4.9	5	76 ± 1

In conclusion, the radiolabelling best condition was the use of a molar ratio of 2.5 at pH 7.4 (phosphate buffer). Under these conditions, the radiolabelling yield reached a value of 80% within 3 min without carrier.

To simplify the purification step in the radiopharmaceutical preparation, we developed a simple and efficient method of [^{123}I]PE2I separation using solid phase extraction (SPE). We have tested the elution of 400 μg of precursor on this small column. The tributyl tin precursor was retained on the top of the SPE column. It was not detected after washing with 14 ml of a mixture of ethanol–water (v/v:70/30). The detection limit being < 1 μg means that the purification process allowed to eliminate 99.7% of the precursor from the crude reaction mixture. This very simple separation is due to the difference of lipophilicity of the stannyl derivative and [^{123}I]PE2I.

In labelling and purification process, the major part of the radioactivity (70%) is eluted in the fourth fraction (Figure 4). The

radiochemical and chemical purities of this collected fraction were checked by HPLC (systems 1 and 2). After SPE purification, no precursor peak was detected and using the radiochromatograms, the chemical and radiochemical purities of [^{123}I]PE2I were higher than 95%. As only 10% of the [^{123}I] PE2I is eluted in the fifth fraction. This fraction was not collected to avoid ethanol highest concentration.

Using iodogen as oxidant and SPE for purification, the overall yield for [^{123}I]PE2I radiopharmaceutical preparation was 60%.

The pharmacological properties of [^{123}I]PE2I obtained using this new method have been compared to those of the radioligand obtained via the chloramine-T method. The affinity and the densities of binding sites of these preparations were not meaningfully different whatever the purification method used (data not shown).

Conclusion

In conclusion, iodogen appears to be one competitive oxidizing agent for the preparation of [^{123}I]PE2I. The radiolabelling reaction carried out with $\approx 50\,\mu g$ of precursor in a iodogen to precursor ratio of 2:5, at room temperature and in sodium phosphate buffer (0.1 M, pH 7.4,), is rapid and efficient. The solid phase extraction purification allows to recover a pure radioligand possessing a high specific radioactivity (2 GBq/nmol) or easily modulate to about 85–90 MBq/nmol.

The solid phase extraction is a very convenient solution to separate the stannic precursor from the radioiodinated derivative. This method could be of interest for the preparation of this radiopharmaceutical in nuclear medical centres.

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