JOURNAL OF LABELLED COMPOUNDS AND RADIOPHARMACEUTICALS
J Label Compd Radiopharm 2002; **45**: 1199–1207.
Published online in Wiley InterScience (www.interscience.wiley.com). DOI: 10.1002/jlcr.632

Research Article

Fully automated high yield synthesis of (*R*)- and (*S*)-[^{11}C]verapamil for measuring P-glycoprotein function with positron emission tomography

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Summary

Racemic ($\pm$) verapamil is a well characterized substrate for P-glycoprotein (P-gp). However, the *in vivo* pharmacokinetics and pharmacodynamics of both enantiomers are reported to be different. In the preparation of evaluation studies of both enantiomers in animals and humans, the purpose of the present study was to optimize and automate the synthesis of (*R*)- and (*S*)-[^{11}C]verapamil.

(*R*)- and (*S*)-[^{11}C]verapamil were prepared from (*R*)- and (*S*)-desmethyl-verapamil, respectively, by methylation with no-carrier added [^{11}C]methyliodide or [^{11}C]methyltriflate. Different conditions of the methylation reaction were studied: reaction time, temperature, base and solvent, and chemical form of the precursor using either the hydrochloric acid salt or the free base of the starting material. After optimization, the synthesis was fully automated using home-made modules and performed according to GMP

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Received 4 February 2002
Revised 6 May 2002
Accepted 31 May 2002

guidelines. Optimal yields of 60–70% for the methylation reaction were obtained using 1.5 mg of the free base of (*R*)- or (*S*)-desmethyl-verapamil in 0.5 ml of acetonitrile at 50°C for 5 min with [^{11}C]methyltriflate as methylating agent. Under the same reaction conditions, but with a reaction temperature of 100°C, the radiochemical yield starting with [^{11}C]methyliodide as methylation reagent was 40%. The specific activity of (*R*)- and (*S*)-[^{11}C]verapamil was >20 GBq/μmol and the radiochemical purity was >99% for both methods. The total synthesis time was 45 min. The automated high yield synthesis of (*R*)- and (*S*)-[^{11}C]verapamil provides the means for evaluating both enantiomers as *in vivo* tracers of P-gp function. Copyright © 2002 John Wiley & Sons, Ltd.

Key Words: [^{11}C]methyliodide; [^{11}C]methyltriflate; enantiomers; P-gp; verapamil

Introduction

Positron emission tomography (PET) is a powerful technique for the quantification of biochemical processes *in vivo*. Several PET-radiopharmaceuticals have been developed for P-glycoprotein (P-gp) imaging. Amongst others these are [^{11}C]colchicine, [^{94m}Tc]-complexes, [^{64}Cu]–complexes, [^{68}Ga]-complexes, [^{11}C]carvedilol and [^{11}C]verapamil.[1–6] P-gp is a multi-drug transporter and is expressed at high levels in a variety of tissues such as the endothelial cells of the blood–brain barrier (BBB) capillaries. P-gp acts as an ATP dependent efflux pump for a large range of hydrophobic drugs and natural compounds such as neurotransmitters and peptides. The P-gp pump plays an important role in multi-drug resistance of tumours[7] and in drug transport[8] and there is evidence that P-gp is an important factor in several neurological diseases such as Alzheimer's disease.[9]

Verapamil is a specific high affinity substrate for the P-gp pump. The compound is well characterized both pharmacologically and with regard to its metabolic pathway. Therefore, verapamil seems to be a suitable PET tracer for measuring P-gp function *in vivo*. Recently, a method was developed using racemic [^{11}C]verapamil.[10] For the quantification of P-gp function *in vivo*, a mathematical model to describe the tracer kinetic data is required. As the kinetics of the two isomers might be different, for accurate quantification, an optically pure compound is needed. This is also true for verapamil as it has been shown that the two enantiomers have different pharmacokinetic profiles.[11–13]

For the evaluation of these enantiomers and to select the most appropriate candidate for clinical studies, a reliable high yield, fully automated, GMP compliant synthesis of (*R*) or (*S*)-[^{11}C]verapamil is needed.

Results and discussion

Automated radiosynthesis

The automated synthesis was performed using home-made units, which have been described in detail elsewhere.[14] (*R*)-[^{11}C]verapamil and (*S*)-[^{11}C]verapamil **2** were prepared from the corresponding des-methyl-starting materials **1** (free base or hydrochloric acid salt) by methylation with no-carrier added [^{11}C]methyliodide or [^{11}C]methyltriflate according to the following scheme (Figure 1):

The [^{11}C]methyliodide production was performed in a small volume trapping vessel. Therefore, less $LiAlH_4$ could be used for the reaction with [^{11}C]CO_2. This resulted in a higher specific activity, because of the reduced amount of atmospheric ^{12}C carbon in the $LiAlH_4$ solution. In 7 min after trapping [^{11}C]CO_2 in the liquid nitrogen cooled coil, 70–80% (corrected for decay) was converted into [^{11}C]methyliodide. The [^{11}C]methyltriflate production was performed according to literature procedures.[15,16] As previously reported, for optimal performance of the home-packed silvertriflate columns, it proved to be very important to impregnate the Graphpac^{TM} material with silvertriflate using a silvertriflate solution (1 g/10 ml) in acetonitrile instead of diethylether.[16,17] In addition, just prior to use, the packed silvertriflate column had to be pre-conditioned at 200°C with a helium flow of 100 ml/min for at least 10 min. The conversion of [^{11}C]methyliodide to [^{11}C]methyltriflate was higher than 80%. The conversion was tested on (nitrobenzyl)pyridine according to the method of Jewett.[15]

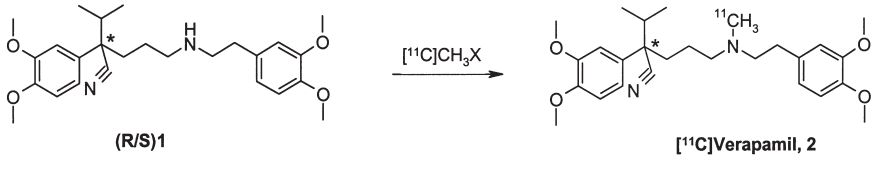

Figure 1. Synthesis of the enantiomers of [11C]verapamil (experimental details mentioned in Table 1)

The reactivity of the nucleophilic amine was studied by changing reaction parameters like solvent (DMF/ acetonitrile), base, reaction time, temperature and by using the hydrochloric acid salt or the free base as the starting material (Table 1). The overall radiochemical yields, including synthesis, purification and formulation, were calculated from the trapped amount of [^{11}C]methyliodide or [^{11}C]methyltriflate in the starting reaction mixture. The reaction conditions as previously reported[1] were 0.5 mg **1** (HCl), 25 mg Al_2O_3/KF as a base, 100°C and 10 min in 0.5 ml of acetonitrile. The radiochemical yield of the synthesis of the racemic [^{11}C]verapamil described in the literature was 16%. In the present study, these reaction conditions (except for the use of 1.5 mg rather than 0.5 mg (*R*/*S*) **1**) resulted in a comparable radiochemical yield of 17.2 ± 7.1% ($n = 6$). Initially, the methylation reaction was optimized by shortening the reaction time to 5 min and reducing the reaction volume to 250 µl. No significant effects were observed.[18] An overall radiochemical yield of 13.3 ± 3.4% ($n = 3$) was obtained. For practical reasons 1.5 mg (*R*/*S*) **1** in a volume of 500 µl of dry acetonitrile was maintained. The rationale of using solid Al_2O_3/KF was to absorb radiochemical impurities, which otherwise were difficult to remove from the final product. In the HPLC-system used, these radiochemical impurities did not disturb purification and therefore Al_2O_3/KF was omitted from the reaction mixture.

In common polar–aprotic solvents such as dimethylformamide (DMF), the nucleophile is less solvated and is more susceptible to nucleophilic reactions in comparison to protic solvents. The methylation with [^{11}C]methyliodide in DMF with sodium bis(trimethylsilyl) amide (TMSA) as a base resulted in a very low radiochemical yield of 2%. It can be assumed that methylation of the TMSA has occurred

Table 1. Optimizing the methylation reaction conditions of [^{11}C]-verapamil[a]

Nor-verapamil form	Radio-precursor	Temperature (°C)	Reaction Time (min)	Base	% radiochemical yield[a]	*N*
HCl	$^{11}CH_3I$	100	10	Al_2O_3/KF	17.2 ± 7.1	6
HCl	$^{11}CH_3I$	100	5	Al_2O_3/KF	13.3 ± 3.4	3
HCl	$^{11}CH_3I$	50	5	Al_2O_3/KF	14.2 ± 2.8	5
Free amine	$^{11}CH_3I$	100	5	—	45.8 ± 10.1	6
Free amine	$^{11}CH_3$triflate	50	5	—	70.5 ± 10.6	2

[a]The following standard reaction conditions were used: 1.5 mg starting material in 0.5 ml of dry acetonitrile; closed reaction vessel. The overall radiochemical yields were calculated from the starting amount of [^{11}C]methyliodide or [^{11}C]methyltriflate (corrected for decay).

under these conditions. Significantly higher yields were obtained with the free base of (*R*/*S*) **1** in acetonitrile. A radiochemical yield of 45.8 ± 10.1% ($n = 6$) of **2** was achieved under optimal conditions. About 55% of the [^{11}C]methyliodide activity, however, still had not reacted. For this reason, the more reactive [^{11}C]methyltriflate was used as an alternative methylation reagent. Under the same reaction conditions, a radiochemical yield of 70.5 ± 10.6% was achieved with [^{11}C]methyltriflate, independent of the reaction time. Increasing the reaction temperature to 100°C did not increase the radiochemical yield. Taking into account the 80% conversion yield of [^{11}C]methyltriflate from [^{11}C]methyliodide, the overall yields for both methods are not

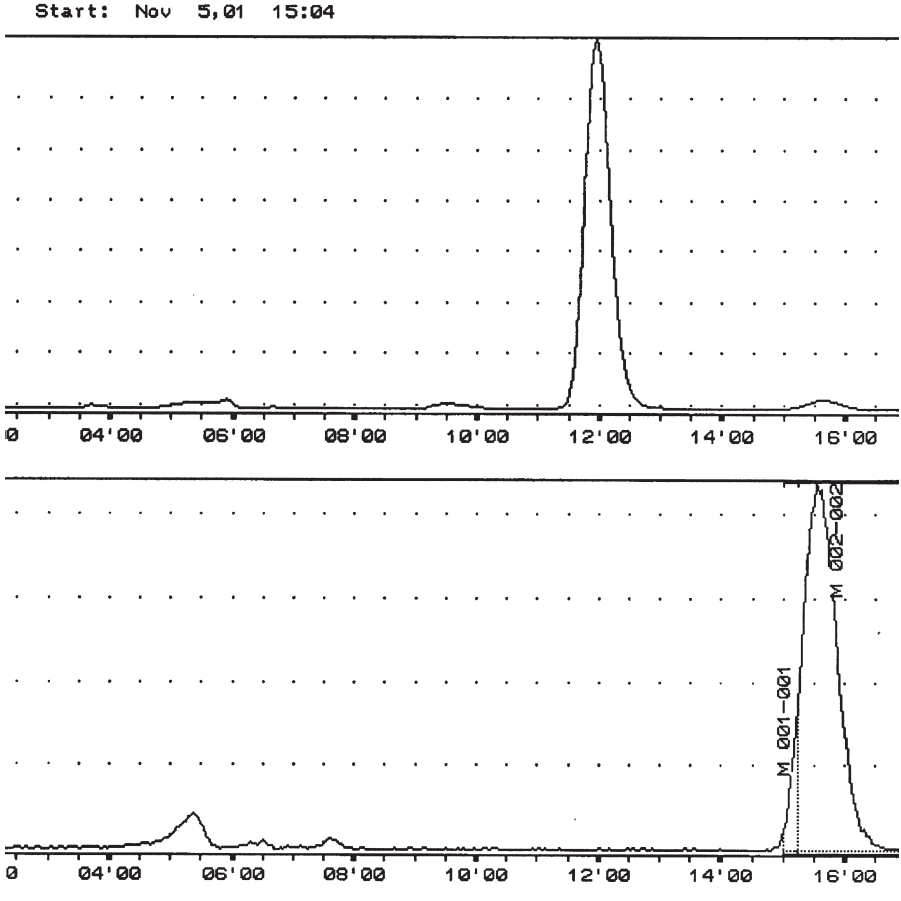

Figure 2. The purification of the reaction mixture. Semipreperative HPLC chromatograms purification of the reaction mixture

significantly different. From a chemical point of view there is no preference for one method or the other. For practical reasons, therefore, the methylation with [^{11}C]methyliodide instead of [^{11}C]methyltriflate is preferred.

Using the optimal reaction conditions, **2** was synthesized by methylation of the secondary amine with [^{11}C]methyltriflate in a solution of 1.5 mg of **1** in 500 µl of dry acetonitrile. The reaction was carried out at 50°C for 5 min in a closed reaction vial. Subsequently, 2 ml of HPLC eluent was added to the reaction mixture. The complete solution was transferred to the HPLC unit and subjected to a preparative HPLC purification equipped with a UV detector (230 nm) and a radioactivity detector (Figure 2). The collected fraction containing the product was diluted with 50 ml of sterile water in the formulation unit. The diluted product was passed through a Sep Pak cartridge, which was subsequently washed with sterile H_2O. The product was eluted from the Sep Pak with ethanol and saline and filtered. To check for possible occurrence of racemization during this procedure, a sample of the tracer was analysed using a Chiracel HPLC column. For enantiomeric identification a sample of the tracer was spiked with an optically pure reference of verapamil. The product was analysed by HPLC on a Symmetryshield C_{18} Column. A stereo and (radio)chemical purity of 99.5% was obtained. Characterization of **2** was performed using HPLC-MS/MS.

Conclusion

After optimization and automation of the [^{11}C]-synthesis of (*R*)- and (*S*)-[^{11}C]verapamil, a reproducible and high yield synthesis was developed. (*R*)- and (*S*)-[^{11}C]verapamil, synthesized according to this procedure, are now available for *in vivo* PET-studies to select the most appropriate candidate for quantitative PET-studies.

Experimental

Materials

The starting materials (*R*)- and (*S*)-desmethyl-verapamil as well as the reference compounds (*R*)- and (*S*)-verapamil were a gift from Knoll Pharmaceuticals, The Netherlands. DIPA, Al_2O_3/KF were obtained

from Aldrich; methanol, acetonitrile, $NaClO_4$ and 57% HI from Merck. All chemicals were of analytical grade.

Other laboratory supplies were obtained from Alltech; the HPLC-MS/MS from Perkin- Elmer API3000.

Production of [^{11}C]methyliodide

[^{11}C]CO_2 was produced by the $^{14}N(p,\alpha)^{11}C$ nuclear reaction with an IBA 18/9 cyclotron. An irradiation for 10 min with 30 μA of 18 MeV protons yielded 25–30 GBq of [^{11}C]CO_2 at EOB. After irradiation the [^{11}C]CO_2 was transferred through 190 m of 1/8″ stainless steel tubing with a helium flow of 1000 ml/min to the hot cell. The [^{11}C]CO_2 was trapped in a liquid nitrogen trap, subsequently transferred to the synthesis modules with a helium flow of 10 ml/min and reacted with 100 μl of 0.1 M $LiAlH_4$ in THF. After evaporation of the solvent, 200 μl of 57% HI was added and the [^{11}C]methyliodide obtained was distilled into a second reaction vessel containing the methylation reaction mixture. Following this procedure, a yield of 15–20 GBq of [^{11}C]methyliodide was produced in 12 min synthesis time after EOB (not decay corrected). The conversion yield was 70–80% (decay corrected).

Production of [^{11}C]methyltriflate

For the conversion to [^{11}C]methyltriflate, [^{11}C]methyliodide was passed through a pre-conditioned column (7×0.5 cm) containing silvertriflate and Graphpac^{TM} (80–100 mesh) at a temperature of 200°C. The [^{11}C]methyltriflate was trapped at room temperature in the reaction vessel containing the mixture for the methylation reaction. The conversion yield was $>80\%$ (decay corrected).

Automated radiosynthesis

Compound **2** was synthesized by methylation of the secondary amine with [^{11}C]methyltriflate in a solution of 1.5 mg of (*R*/*S*)**1** (free base) in 500 μl of dry acetonitrile. The reaction was carried out at 50°C for 5 min in a closed reaction vial. The reaction mixture was diluted with 2 ml of HPLC eluent and subsequently transferred to the HPLC unit using a remote controlled operated HPLC injection system and subjected to a semi-preparative HPLC purification using a Varian Kromasil 250×22 mm (10 μm) with MeOH:H_2O:DIPA 82:18:0.2 (%v/v/v) as eluent and a flow of 10 ml/min. [^{11}C]verapamil had a retention time of

15 min, as detected by UV (230 nm) and radioactivity (Figure 2). The collected fraction containing the product was diluted with 50 ml of sterile water in the formulation unit.[14] The diluted product was passed through an activated tC_{18} Sep Pak cartridge using a helium overpressure, which was subsequently washed with 20 ml of sterile H_2O. Compound **2** was eluted from the Sep Pak with 1 ml of ethanol followed by 9 ml saline and filtered through a sterile 0.22 μm filter using a helium overpressure yielding a sterile and pyrogen free-ready for injection solution. The total synthesis time was 45 min including the purification and the formulation.

HPLC—analysis and identification

To check for racemization during this procedure, a sample of 20 μl of **2** was analysed using a Chiracel OD-R column 250 × 4.6 mm, with 1 M $NaClO_4$:CH_3CN 60:40 (%v/v) as eluent and a flow of 0.5 ml/min. The eluent was monitored using UV absorbance at a wavelength of 232 nm and the radioactivity using a sodium iodide scintillation detector. For identification, a sample of **2** was spiked with an optically pure reference of verapamil. Under these conditions the retention times of (*R*)- and (*S*)-verapamil were 15.5 and 18.5 min.

For the routine check on chemical and radiochemical purity a Waters Symmetryshield RP_{18}, 5 μm, 3.9 × 150 mm, with acetonitrile:methanol: 50 nM potassium phosphate pH = 7.0 33:18:49 (%v/v/v) as eluent and a flow of 1 ml/min, was used with detection as described above. The retention time for both enantiomers of **2** was 15 min with this HPLC-system. The characterization was performed using HPLC-MS/MS with a Varian Kromasil 250 × 4.6 mm with MeOH:H_2O:DIPA 72:28:0.05 (%v/v/v) as eluent and a flow of 1 ml/min. The batch was recorded in a range of *m*/*z* 200–600. The spectra showed a principal peak at *m*/*z* 455, with a retention time of 6 min. The mass spectrum corresponds with a reference of verapamil. MS: 455 (M + 1), 303, 260 ($C_{16}H_3NO_2^+$), 165 ($C_{10}H_{13}O_2^+$), 150 ($C_9H_{10}O_2^+$).

Acknowledgements

The authors thank Knoll Pharmaceuticals (Almere, the Netherlands) for their gift of (*R*)- and (*S*)-(desmethyl)-verapamil and Eduard Struys (VU Medical centre, Amsterdam, the Netherlands) for his expertise of

the LC-MS/MS. Peter van Leuffen and the personnel of the BV Cyclotron VU are gratefully acknowledged for the production of $[^{11}C]CO_2$.

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