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Rapid synthesis of ^{11}C-labeled FK506 for positron emission tomography

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Dedicated to Dr. Kazuo Sakane, former Director of Medicinal Chemistry, Fujisawa Pharmaceutical Co. Ltd., who died on October 1st, 2001

Abstract—The present study describes a rapid synthesis method for labeled [^{11}C]FK506 for positron emission tomography (PET). A one-pot reaction from [^{11}C]CH_3I, involving a Wittig reaction as the key carbon–carbon bond formation was developed. The chemical process was accomplished using a designed, fully automated synthetic apparatus, and an injectable solution of [^{11}C]FK506 was obtained in only 34 min from [^{11}C]CH_3I. The decay-corrected radiochemical yield based on [^{11}C]CH_3I was 11.9%, and the specific activity was 39.8 GBq/μmol.

Positron emission tomography (PET) is an advanced biofunctional imaging technology that can be used for clinical diagnosis, and uses very short half-life radioisotopes (ca. ^{11}C: 20.2 min, ^{15}O: 2 min, respectively). Since the distribution of radio-tracer in the human body can be detected by PET, we believe that PET studies can be useful for pharmacokinetic studies of new drug candidates using the corresponding labeled drug. In general, however, synthesis of the labeled drug has a number of technical issues which need to be addressed. First, a very rapid synthesis process from the organic reaction to the purification process is required. Second, the synthetic operation must be performed with an automated synthesis apparatus to avoid radiation exposure, and lastly, the reaction scale is very small, usually nano- or pico-mole scale, so that quality control is difficult. In this paper, we describe a rapid synthesis method for ^{11}C-labeled FK506[1–3] (tacrolimus; Fujisawa Pharmaceutical Co. Ltd., Osaka, Japan) using our in house developed automated apparatus.

The immunosuppressive agent FK506 is widely used for prevention of rejection in transplantation and for treatment of atopic dermatitis worldwide. Recently, the neuroprotective effects of FK506 were also found and demonstrated in a non-human primate model of stroke.[4–8] For development of FK506 as a new drug candidate for central nervous system disease, brain PET of FK506 could serve as a source of accurate pharmacokinetic data a key requirement, because FK506 must be regulated within a strict therapeutic range in a clinical setting.

On the other hand, FK506 is a good target for total synthesis and many research groups have attempted total synthesis because of its complicated chemical structure. Jones et al.[9] and Nakatsuka et al.[10] succeeded in 1989 and 1990, respectively, however, these chemical processes are not applicable for the synthesis of ^{11}C-labeled FK506 since they are multi-step and time consuming. As a result, we needed to develop a new and short chemical process that ideally would involve few synthetic operations.

As the first step, we investigated the appropriate labeling position for FK506. There are four possible positions for labeling by [^{11}C]CH_3I, which is a popular labeling reagent in PET, and is made from the ^{14}N(p, α)^{11}C nuclear reaction with a cyclotron, followed by reduction with $LiAlH_4$ and reaction with HI, as shown in Scheme 1. Methylation of the hydroxyl groups by CH_3I were examined but suitable reaction conditions

Keywords: PET; ^{11}C; FK506; Wittig reaction.

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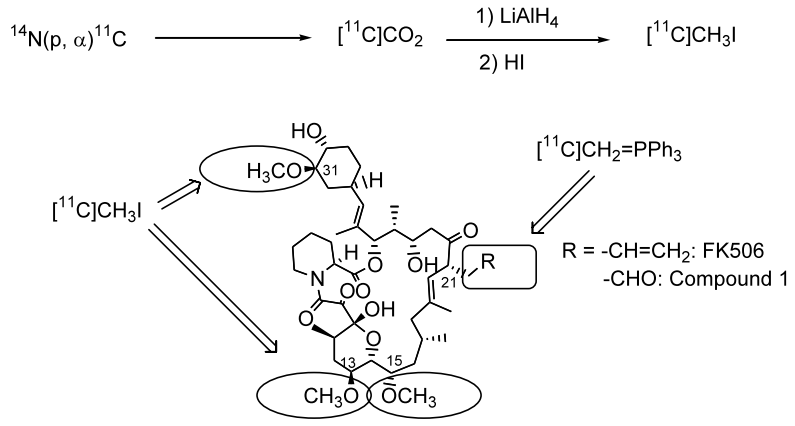


Scheme 1.

could not be obtained, thus our attention turned to the synthesis of ^{11}C-labeled alkenes from [^{11}C]CH_3I. ^{11}C-Labeled alkenes have been synthesized from [^{11}C]CH_3I yielding ^{11}C-labeled terminal alkenes,[11,12] hence, we decided to label the allyl position of FK506 using a Wittig reaction as summarized in Scheme 2.

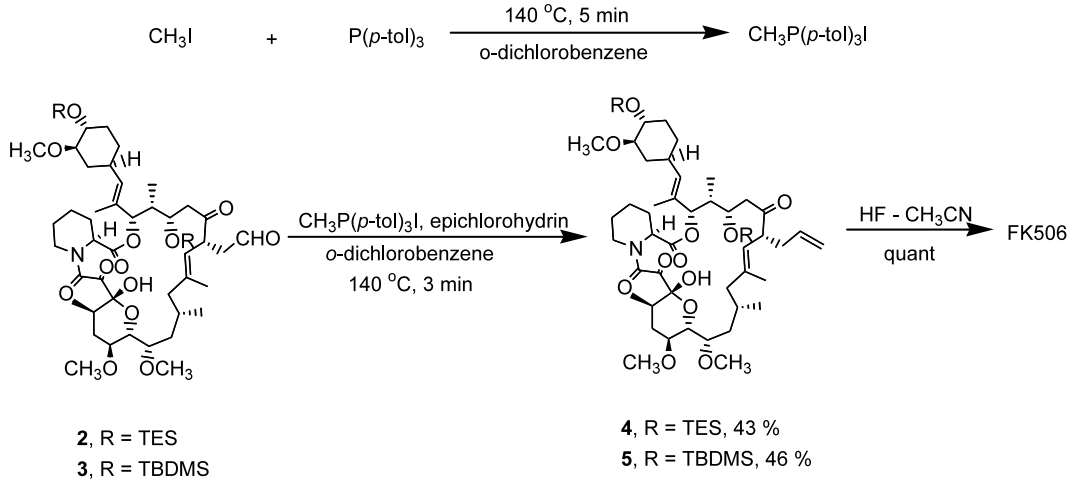
Strong bases are commonly used for proton abstraction of the phosphonium salt in the Wittig reaction, whereas FK506 is unstable under basic conditions. Therefore, neutral or acidic conditions used were seemed most suitable for the process.

Kihlberg et al.[11] used epichlorohydrin for rapid Wittig reaction, instead of using strong base. Thus, we tried Wittig reaction of aldehyde **1**[13] under such neutral conditions. However, these conditions were not suitable, and FK506 was not obtained. A trialkylsilyl group was then chosen as a protecting group for two hydroxy groups of the precursor,[14] because deprotection is known to occur under mild acidic conditions. In a preliminary cold experiment, Wittig reaction with the protected precursor proceeded in moderate yield. After optimization of this key chemical process by cold synthesis on a micro scale, the rapid synthetic method shown in Scheme 2 was discovered. The reaction of precursor **2**† with tri-*p*-tolylphosphine and epichlorohydrin in *o*-dichlorobenzene at 140°C gave compound **4** in only 3 min.

In our experiment, the tri-*p*-tolylphosphine reagent provided good yields in comparison with the corresponding triphenylphosphonium salt. A rapid synthesis method of the tri-*p*-tolylphosphonium reagent was also developed for thus PET study.

Concerning the final deprotection step, the previous report[14] described that the trialkylsilyloxy groups were cleaved by HF in CH_3CN at room temperature. However, we found that reaction of triethylsilyloxy compound **4** or *t*-butyldimethysilyloxy compound **5** in a

† Triethylsilyloxy compound **2** was prepared by the same procedure as the *t*-butyldimethylsilyloxy compound **3**.



Scheme 2. Synthetic route of ^{11}C labelled FK506 from CH_3I.

mixture of 47% aqueous HF and CH_3CN gave the final compound in 3 min at rt and 12 min at 50°C, respectively. In this way, a one-pot synthetic route of ^{11}C-labeled FK506 from CH_3I was completed.

This result encouraged us to carry out the hot synthetic experiment on a pico mole scale, however it was found that purification of [^{11}C]FK506 from the reaction mixture by preparative HPLC was difficult because of tailing of the chromatographic peak of P(*p*-tol)$_3$ and *o*-dichlorobenzene, which overlapped the peak for FK506. Thus, two additional processes were added to the synthesis as follows: (1) *o*-dichlorobenzene in the reaction mixture was distilled away under reduced pressure before the deprotection step: (2) in the deprotection step, excess P(*p*-tol)$_3$ in the reaction mixture was oxidized with CBr_4 to tris-4-methylphenylphosphine oxide which has a very different HPLC retention time in comparison to that of P(*p*-tol)$_3$.

On the basis of these results, a fully automated synthetic apparatus for ^{11}C-labeled FK506 was developed as shown in Figure 1.

This apparatus was constructed based on previous reports,[15,16] and was compact to minimize the loss in solution transfer and synthesis time. The optimization of the each reaction was accomplished by many cold test runs using this apparatus. As a result, these investigations brought to the shorter synthesis time of about only 30 min. The automated labeled synthesis using above synthesis apparatus was examined as shown in Scheme 3.

Every chemical step proceeded smoothly and [^{11}C]FK506 was obtained, however, the yield was only 3–5% from [^{11}C]CH_3I, whereas HPLC analysis of the reaction mixture indicated 20–30% yield. It was found that adsorption of [^{11}C]FK506 occurred in the preparative HPLC silica gel column because the amount was very small. Thus, two low adsorptive columns (Shodex Rspak DE-LG (10×50 mm and SUPELCO TPR-100 (10×250 mm, 5 μm) were connected instead of the silica gel column and were eluted with H_2O (CH_3CN:THF = 6:1) = 35:65 (flow rate: 3.8 ml/min, column temperature: 60°C) to give a reasonable yield of the fraction containing [^{11}C]FK506 which was collected and solvent removed under reduced pressure. The residue was dissolved in 33% ethanol–saline solution and passed through a sterile filter (Millipore GV) to give [^{11}C]FK506 as an injectable solution for PET study. The total synthesis time was 44 min after ^{11}C-labeled carbon dioxide formation. The radioactivity was 0.99 GBq (end of synthesis), radiochemical purity was higher than 99%, the specific activity was 39.8 GBq/μmol.

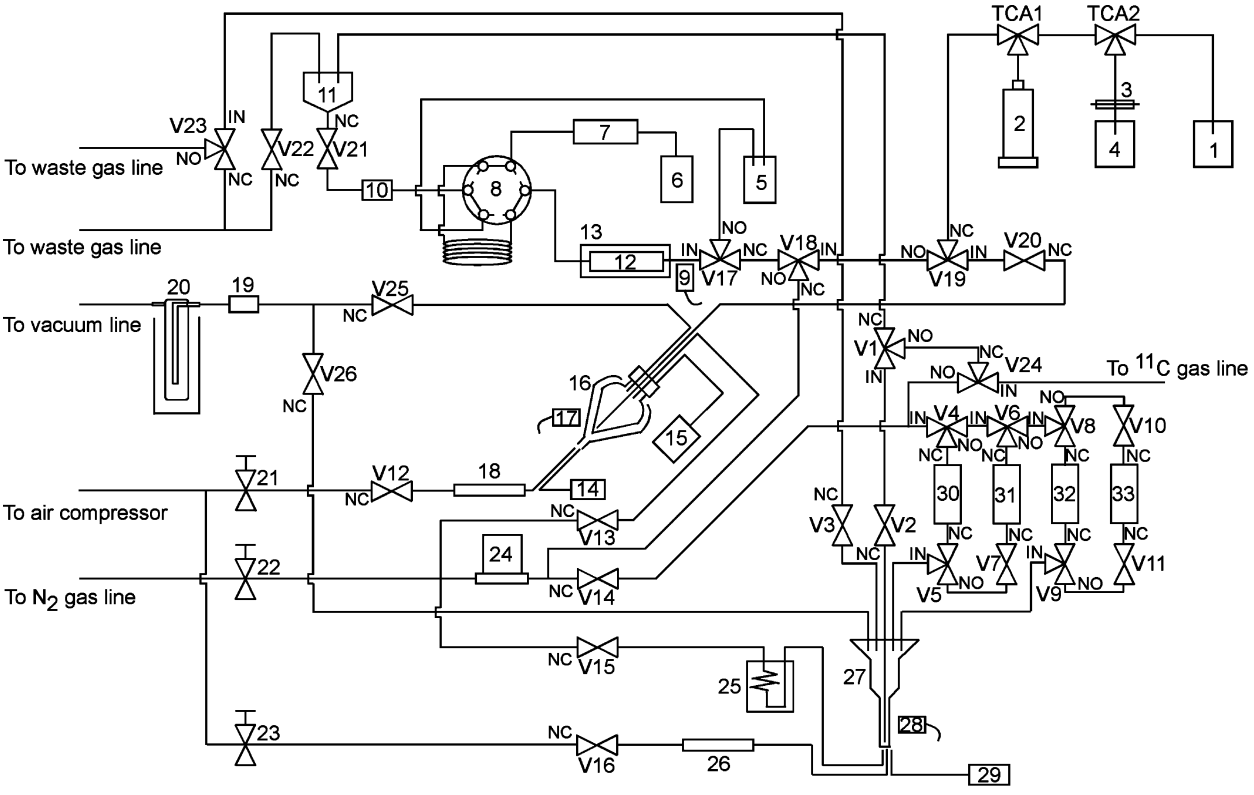
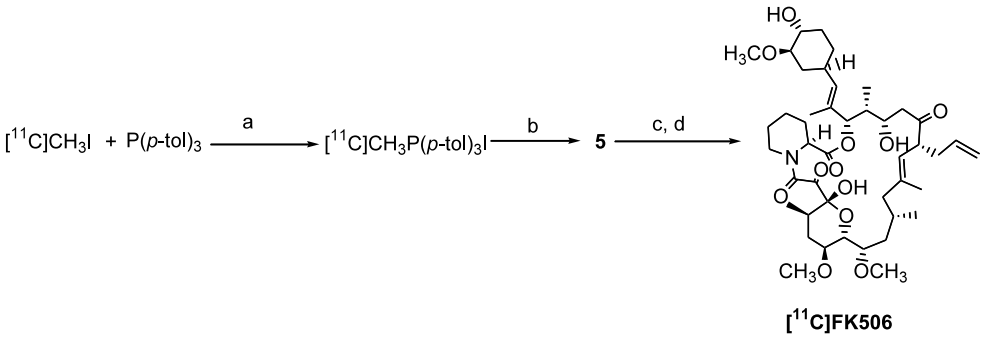


Figure 1. Diagram of the synthesis system; V1–26 = solenoid valves; TCA1–2 = 3-way cock actuator; 1 and 30–33 = reservoirs; 2 = syringe pump; 3 = filter; 4 = vial; 5 = waste bottle; 6 = solvent bottle; 7 = HPLC pump; 8 = 6-way valve; 9, 17 and 28 = radio detectors; 10 = photosensor; 11 = glass vessel; 12 = HPLC column; 13 = column oven; 14, 15 and 29 = thermosensors; 16 = evaporator; 18 and 26 = heaters.



Scheme 3. Rapid synthesis of ^{11}C-labeled FK506: (a) *o*-dichlorobenzene, 150°C, 3 min; (b) 2, *o*-dichlorobenzene, epichlorohydrin, 150°C, 3 min; (c) concentration of reaction mixture; (d) 47% aq. HF, CH_3CN, CBr_4, rt, 3 min.

In conclusion, we have succeeded in developing a rapid automated synthesis method of [^{11}C]FK506 using Wittig reaction as a key reaction and a good quality injectable solution of [^{11}C]FK506 for PET study was obtained our automated synthetic apparatus. This work represents one of the most complicated ^{11}C-labeled tracer syntheses reported to date. We are hopeful that the PET study using [^{11}C]FK506 will provide useful pharmacokinetic data for new drug research and development.

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