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Carbon-11 radiolabeling of an oligopeptide containing tryptophan hydrochloride via a Pictet-Spengler reaction using carbon-11 formaldehyde

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A procedure for the synthesis of a ^{11}C-labeled oligopeptide containing [1-^{11}C]1,2,3,4-tetrahydro-β-carboline-3-carboxylic acid ([1-^{11}C]Tpi) from the corresponding Trp•HCl-containing peptides has been developed involving a Pictet-Spengler reaction with [^{11}C]formaldehyde. The synthesis of [1-^{11}C]Tpi from Trp and [^{11}C]formaldehyde was examined as a model reaction with the aim of developing a facile and effective method for the labeling of peptides with carbon-11. The Pictet-Spengler reaction of Trp and [^{11}C]formaldehyde in acidic media (TsOH or HCl) afforded the desired [1-^{11}C]Tpi in a moderate radiochemical yield. Herein, the application of a Pictet-Spengler reaction to an aqueous solution of Trp•HCl gave the desired product with a radiochemical yield of 45.2%. The RGD peptide cyclo[Arg-Gly-Asp-D-Tyr-Lys] was then selected as a substrate for the labeling reaction with [^{11}C]formaldehyde. The radiolabeling of a Trp•HCl-containing RGD peptide using the Pictet-Spengler reaction was successful. Furthermore, the remote-controlled synthesis of a [1-^{11}C]Tpi-containing RGD peptide was attempted by using an automatic production system to generate [^{11}C]CH_3I. The radiochemical yield of the [1-^{11}C]Tpi-containing RGD at the end of synthesis (EOS) was 5.9 ± 1.9% (n = 4), for a total synthesis time of about 35 min. The specific activity was 85.7 ± 9.4 GBq/μmol at the EOS. Copyright © 2013 European Peptide Society and John Wiley & Sons, Ltd.

Keywords: Positron emission tomography (PET); ^{11}C-labeled oligopeptides; [1-^{11}C]-1,2,3,4-Tetrahydro-β-carboline-3-carboxylic acid ([1-^{11}C]Tpi); Pictet-Spengler reaction; [^{11}C]Formaldehyde; Remote-controlled synthesis

Introduction

Molecular imaging is an emerging technology that allows for the visualization of the interactions between molecular probes and biological targets. Positron emission tomography (PET), in particular, is a useful modality that enables *in vivo* biological information to be obtained in a noninvasive manner using a variety of PET radiopharmaceuticals [1]. Short half-life positron emitters, such as ^{11}C ($T_{1/2}$ = 20 min), ^{13}N ($T_{1/2}$ = 10 min), ^{15}O ($T_{1/2}$ = 2 min), and ^{18}F ($T_{1/2}$ = 110 min), have generally been used for PET molecular imaging studies [2]. It is possible to synthesize PET probes that possess the same chemical structures as the parent unlabeled molecules without altering their biological activity because, with the exception of ^{18}F, the positron-emitting radionuclides described earlier can replace their stable analogues in biomolecules. However, ^{15}O and ^{13}N have only ever been used in simple compounds such as [^{15}O]O_2 and [^{13}N]NH_3, respectively, because of the limitations imposed by their short half-lives. Carbon is present in all organic molecules to such an extent that the introduction of carbon-11 to a molecule does not modify its properties. In addition, low β^+energy of carbon-11, 0.96 MeV, promises a short positron range in tissue, contributing to high spatial resolution PET imaging [1,2]. Furthermore, the short half-life and rapid decay of carbon-11 out of radioactivity allow for same-day imaging and repeated investigations with either the same or different radio tracers within a few hours. All of these properties represent desirable options for longitudinal therapy monitoring.

Some low molecular weight oligopeptides have been considered as potential imaging agents because they possessed good permeability properties that could permit rapid access to the target tissues. Radiolabeled peptides are becoming increasingly important in nuclear oncology, where they are used in the diagnosis and treatment of several different cancers [3–5]. One of the main challenges of PET for radiochemists is the development of rapid synthetic methods for the introduction of short-lived positron-emitting radionuclides into the peptide of interest. Several methods have been developed for the synthesis of ^{11}C-labeled oligopeptides using [^{11}C]CH_3I. The ^{11}C-labeled peptides reported to date include four enkephaline-peptides prepared by the reaction of [^{11}C]CH_3I with the corresponding sodium-generated sulfide-anion in liquid ammonia [6], as well as ^{11}C-labeled peptides derived from a chemoselective radiolabeling strategy involving the formation of oximes via the reaction of *p*-[^{11}C]methoxy-benzaldehyde with aminooxy-functionalized

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peptides [7]. Unfortunately, however, these complex synthetic procedures invariably occur over long periods relative to the short half-life of carbon-11, and this has severely limited their application. The development of procedures amenable to the synthesis of novel carbon-11 labeled agents for use as tracers in biomedical research is important for moving the PET imaging technique forward into the future.

[^{11}C]Formaldehyde ([^{11}C]CH_2O) has been used as a carbon-11 labeling agent for compounds required in PET studies [8–10]. Several methods have been reported for the preparation of [^{11}C]CH_2O from [^{11}C]CH_3OH by metal catalysis [11,12]. There have also been several reports describing the enzyme-mediated synthesis of [^{11}C]CH_2O [13]. Nader *et al*. [14] reported the use of [^{11}C]CH_2O for the synthesis of [1-^{11}C]1,2,3,4-tetrahydro-β-carboline-3-carboxylic acid ([1-^{11}C]Tpi) (Figure 1) via a Pictet-Spengler reaction [15,16]. The methods currently available for the synthesis of [^{11}C]CH_2O used in the construction of PET compounds, however, require particularly low temperatures (−52°C) under rigidity control using lithium aluminum hydride [14]. For these reasons, the use of [^{11}C]CH_2O has not been developed to any great extent because the current labeling approaches using [^{11}C]CH_2O are generally inaccessible. Hooker *et al*. [17] recently reported the development of a facile method for the preparation of [^{11}C]CH_2O. Furthermore, the treatment of tryptamine with [^{11}C]CH_2O under acidic conditions provided [^{11}C]2,3,4,9-tetrahydro-1H-β-carboline in a good radiochemical yield. We envisaged that the treatment of Trp with [^{11}C]CH_2O under acidic conditions would provide [1-^{11}C]Tpi as well as several related analogues. Herein, we report the synthesis of [1-^{11}C]Tpi-containing oligopeptides via a Pictet-Spengler reaction using [^{11}C]CH_2O.

Materials and Methods

All of the commercial reagents and solvents used in this work were used without further purification. 1,2,3,4-Tetrahydro-β-carboline-3-carboxylic acid (Tpi), anhydrous *N*,*N*-dimethylformamide (DMF), 1 mol/l NaOH, high performance liquid chromatography (HPLC) grade MeCN and HPLC grade water were purchased from Wako Pure Chemical Industries (Osaka, Japan). Boc-Tpi-OH was purchased from Watanabe Chemical Industries, Ltd (Hiroshima, Japan). Boc-Trp-OSu and 1 mol/l HCl in AcOH were purchased from Kokusan Chemical Co., Ltd (Tokyo, Japan). Cyclo[Arg-Gly-Asp-D-Tyr-Lys] was purchased from Peptides International (Louisville, KY, USA). Trp hydrochloride was purchased from Tokyo Chemical Institute Co., Ltd (Tokyo, Japan). Sodium phosphate Corrective Injection 0.5 mol/l was purchased from Otsuka Pharmaceutical Factory, Inc. (Tokushima, Japan). Fetal bovine serum was purchased from NICHIREI BIOSCIENCES INC. (Tokyo, Japan). A dose calibrator (IGC-3R Curiemeter; Aloka, Tokyo, Japan) was used for all of the radioactivity measurements unless otherwise stated. HPLC analyses were performed using a JASCO HPLC system (Tokyo, Japan). The effluent radioactivity was measured using a NaI(Tl) scintillation detector system (Ohyo Koken Kogyo Co., ltd, Tokyo, Japan). Electrospray ionization mass spectroscopy (ESI-MS) analyses were conducted on an Applied Biosystems MDS SCIEX Q-TRAP 4000 (Tokyo, Japan).

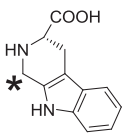

Figure 1. Structure of [1-^{11}C]1,2,3,4-tetrahydro-β-carboline-3-carboxylic acid ([1-^{11}C]Tpi).

Synthesis of the precursor for the radiosynthesis of cyclo [Arg-Gly-Asp-D-Tyr-Lys([1-^{11}C]Tpi)] ([^{11}C]2) Cyclo[Arg-Gly-Asp-D-Tyr-Lys(Boc-Trp)] (4)

A 1 mol/l NaOH solution (100 μl) was added to a solution of cyclo [Arg-Gly-Asp-D-Tyr-Lys] (12.3 mg, 20 μmol) and Boc-Trp-OSu (10.1 mg, 25 μmol) in a mixture of water (250 μl) and DMF (300 μl) at room temperature, and the resulting mixture was stirred at room temperature for 6 h. The resulting crude products were purified on a semi-preparative reversed-phase HPLC system. Purification was performed on a YMC-Triart C18 column (10 mm i.d. × 250 mm, 5 μm) (YMC, Kyoto, Japan). A flow rate of 5 ml/min was used with a linear mobile phase gradient starting from 95% solvent A (0.1% TFA in water) and 5% solvent B (0.1% TFA in MeCN) to 0% solvent A and 100% solvent B over a 40-min run time. The absorbance was monitored at a wavelength of 220 nm. The retention time for **4** was found to be 18.2 min, whereas the retention time for cyclo[Arg-Gly-Asp-D-Tyr-Lys] was for 8.63 min. The fractions containing the product were collected and concentrated *in vacuo* to give 12.3 mg (67.9 %) of the pure product. ESI-MS *m/z* found to be 906.3; calculated for $[C_{43}H_{59}N_{11}O_{11}+H]^+$ requires 906.4.

Cyclo[Arg-Gly-Asp-D-Tyr-Lys(Trp)] hydrochloride (3•HCl)

Compound **4** (9.5 mg, 10 μmol) was added to a solution of 1 mol/l HCl in AcOH (6 ml) and the resulting solution was allowed to stand for 3 h at room temperature. The solution was then concentrated *in vacuo* and the crude product purified by semi-preparative HPLC. Purification was performed on a YMC-Triart C18 column (10 mm i.d. × 250 mm, 5 μm). A flow rate of 5 ml/min was used with a linear mobile phase gradient starting from 95% solvent A (0.1% TFA in water) and 5% solvent B (0.1% TFA in MeCN) to 0% solvent A and 100% solvent B over a 40-min run time. The absorbance was monitored at a wavelength of 220 nm. The retention time for **3** was found to be 11.3 min. The fractions containing **3** were collected and concentrated *in vacuo* to give a residue that was added to a 1 mol/l HCl solution (3 ml). Removal of the solution gave the product as the corresponding HCl salt (7.1 mg, 88.0%). ESI-MS *m/z* was found to be 806.6; calculated for $[C_{38}H_{51}N_{11}O_9+H]^+$ requires 806.3.

Reference compound of cyclo[Arg-Gly-Asp-D-Tyr-Lys(Tpi)] (2) Cyclo[Arg-Gly-Asp-D-Tyr-Lys(Boc-Tpi)] (5)

To a solution of Boc-Tpi-OH (5.5 mg, 17.4 μmol) and *N*-hydroxy succinimide (2.2 mg, 19.1 μmol) in DMF (1.0 ml) was added *N*-(3-dimethylaminopropyl)-*N*′-ethylcarbodiimide hydrochloride (3.6 mg, 19.1 μmol), and the resulting mixture was allowed to stand for 1 h at room temperature. A solution of cyclo [Arg-Gly-Asp-D-Tyr-Lys] (7.2 mg, 11.6 μmol) in 1 mol/l NaOH (100 μl) was then added to the reaction, and the resulting mixture was allowed to proceed for 6 h at ambient temperature. The crude product was then purified on a semi-preparative reversed-phase HPLC system. Purification was performed on a YMC-Triart C18 column (10 mm i.d. × 250 mm, 5 μm). A flow rate of 5 ml/min was used with a linear mobile phase gradient

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starting from 95% solvent A (0.1% TFA in water) and 5% solvent B (0.1% TFA in MeCN) to 0% solvent A and 100% solvent B over a 40-min period. The absorbance was monitored at a wavelength of 220 nm. The retention time for **5** was found to be 20.5 min, whereas the retention time for cyclo[Arg-Gly-Asp-D-Tyr-Lys] was 8.63 min. The fractions containing the product were concentrated *in vacuo* to give 6.9 mg (65.3%) of the desired product as an oil. ESI-MS *m/z* found to be 918.5; calculated for $[C_{44}H_{59}N_{11}O_{11}+H]^+$ requires 918.4.

Cyclo[Arg-Gly-Asp-D-Tyr-Lys(Tpi)] hydrochloride (2•HCl)

Compound **5** (4.9 mg, 5.3 μmol) was added to a 1 mol/l HCl/AcOH (6 ml) and allowed to stand for 3 h at room temperature. The resulting solution was subsequently concentrated *in vacuo* to give the crude product, which was purified by semi-preparative HPLC. The purification was performed on a YMC-Triart C18 column (10 mm i.d. × 250 mm, 5 μm). A flow rate of 5 ml/min was used with a linear mobile phase gradient starting from 95% solvent A (0.1% TFA in water) and 5% solvent B (0.1% TFA in MeCN) to 0% solvent A and 100% solvent B over a 40-min period. The absorbance was monitored at a wavelength of 220 nm. The retention time for **2** was found to be 13.2 min. The fractions containing **2** were collected and concentrated *in vacuo* to give a solid residue, which was added to 1 mol/l HCl solution (3 ml). Subsequent concentration of the solution *in vacuo* gave the desired product as the corresponding HCl salt (3.9 mg, 90.0%). ESI-MS *m/z* was found to be 818.7; calculated for $[C_{39}H_{51}N_{11}O_9+H]^+$ requires 818.9.

Preparation of $[^{11}C]CH_2O$

The $[^{11}C]CH_2O$ for the radiosynthesis was prepared from $[^{11}C]CH_3I$ as previously described [17]. $[^{11}C]CO_2$ was produced by using the $^{14}N(p,\alpha)^{11}C$ nuclear reaction in a 0.01% oxygen-containing nitrogen gas with 18 MeV proton beams. Following the bombardment process, $[^{11}C]CO_2$ was transferred to a vessel containing a 50 mmol/l solution of lithium aluminum hydride in THF (500 μl) at a temperature in the range −10 to −15°C. The resulting solution was concentrated to dryness and a 57% solution of HI (400 μl) was added to the vessel. The mixture was then heated to 150°C to produce $[^{11}C]CH_3I$. The $[^{11}C]CH_3I$ was purified by passing it through a column composed of Ascarite II and P_2O_5 and collecting the eluent into a reaction vial containing a solution of trimethylamine *N*-oxide (4.0 mg) in DMF (300 μl) at room temperature with N_2 gas (30 ml/min). When the radioactivity in the reaction vial reached a plateau, the gas stream was suspended and the resulting solution was heated to 70°C for 2 min to give $[^{11}C]CH_2O$.

Radiolabeling of [1-^{11}C]Tpi ([^{11}C]1) by manual synthesis

The $[^{11}C]CH_2O$/DMF solution formed earlier was added to a reaction vial containing a Trp (Trp•HCl) solution (200 μl). The reaction vial was left open to the environment and the reaction mixture was heated at 100°C for 5 min. The reaction mixture was then quenched by the addition of water (500 μl). Aliquots of this mixture were analyzed by reverse-phase HPLC by using a SunFire C18 column (4.6 mm i.d. × 150 mm, 5 μm) and a mobile phase consisting of MeCN and 50 mM $HCOONH_4$ (1 : 4). A flow rate of 1.0 ml/min was used and the absorption was monitored at a wavelength of 254 nm. The retention time for $[^{11}C]$**1** was found to be approximately 5.6 min.

Radiolabeling of $[^{11}C]$2 by Manual Synthesis

The $[^{11}C]CH_2O$/DMF solution formed earlier was added to a reaction vial containing **3**•HCl in water (200 μl). The reaction vial was left open to the environment and the reaction mixture was heated at 100°C for 5 min. The reaction mixture was then quenched by the addition of water (500 μl). Aliquots of this mixture were analyzed by reverse-phase HPLC by using a YMC-Triart C18 column (4.6 mm i.d. × 150 mm, 5 μm). A flow rate of 1 ml/min was used with a linear mobile phase gradient starting from 95% solvent A (0.1% TFA in water) and 5% solvent B (0.1% TFA in MeCN) to 0% solvent A and 100% solvent B over a 40-min period. The absorbance was monitored at a wavelength of 280 nm.

Radiolabeling of $[^{11}C]$2 by Remote-Controlled Synthesis

For the remote-controlled synthesis of $[^{11}C]$**2**, a versatile synthesis system was used for multiple PET radiopharmaceuticals [18], which was developed at the National Institute of Radiological Sciences. The use of this system led to the formation of a sequence program in accordance with the results from the manual synthesis. The $[^{11}C]CH_3I$ was bubbled into a reaction vial containing a solution of trimethylamine *N*-oxide (2.0 mg) in DMF (150 μl) at a flow rate of 30 ml/min at a temperature in the range of −10 to −15°C. When the radioactivity reached a plateau, the reaction vial was heated to 70°C with an air heater and held at that temperature for 2 min. The valve for the exhaust line was then turned on. Following the addition of a **3**•HCl solution (16 μmol/200 μl), the valve for the exhaust line was left open and the solution was heated at 100°C for 5 min. A 0.1% H_3PO_4 solution (1 ml) was then added to the reaction vial and the resulting mixture was passed through a 0.20 μm filter (Millex-LG, 13 mm) before being placed into a 2 ml sample loop on an injector valve. Purification was performed on an X-Bridge C18 column (10 mm i.d. × 250 mm, 5 μm). A flow rate of 5 ml/min was used with a linear mobile phase gradient starting from 95% solvent A (0.1% H_3PO_4 in water) and 5% solvent B (0.1% H_3PO_4 in MeCN) to 0% solvent A and 100% solvent B over a 40-min period. The eluent was monitored for its ultraviolet absorbance (280 nm) and radioactivity. The peak corresponding to $[^{11}C]$**2** was collected into a rotary evaporator flask containing a 25% ascorbic acid injection (100 μl). The solvent was removed *in vacuo* at 150°C, and the resulting residue was dissolved in a sodium phosphate buffer (3 ml, pH 6.5).

Radiochemical Purity and Specific Activity Determinations

The radiochemical purity was assayed by analytical HPLC. Aliquots of the final products were analyzed by reverse-phase HPLC onto an X-Bridge C18 column (4.6 mm i.d. × 150 mm, 5 μm). A flow rate of 1 ml/min was used with a linear mobile phase gradient starting from 95% solvent A (0.1% TFA in water) and 5% solvent B (0.1% TFA in MeCN) to 0% solvent A and 100% solvent B over a 40-min period. The absorbance was monitored at a wavelength of 220 nm. The retention time for $[^{11}C]$**2** was found to be approximately 12 min. The identity was confirmed by co-injection with the **2**. The peak lag on the chromatogram corresponds to line length between ultraviolet detector and NaI (Tl) scintillation detector. The radiochemical purity for $[^{11}C]$**2** was found to be >98%, and the average specific activity was determined to be 85.7 ± 9.4 GBq/μmol.

Stability of [^{11}C]2

The approximately 20 MBq of [^{11}C]**2** was added to a reaction vial containing a fetal bovine serum (100 μl). The reaction mixture was incubated at 37°C. After 60 min, the mixture was then quenched by the addition of MeCN (200 μl), and then centrifuged at 12 000 revolutions per minute for 4 min. Stability was analyzed with reverse-phase HPLC system using a X-Bridge C18 column (4.6 mm i.d. × 150 mm, 5 μm). A flow rate of 1 ml/min was used with a linear mobile phase gradient starting from 95% solvent A (0.1% TFA in water) and 5% solvent B (0.1% TFA in MeCN) to 0% solvent A and 100% solvent B over a 40-min period.

Results and Discussion

The synthesis of [1-^{11}C]Tpi ([^{11}C]**1**) was initially examined under the traditional acid catalyzed Pictet-Spengler conditions using Trp and a solution of [^{11}C]CH_2O in DMF. The radiosynthesis of [^{11}C]**1** was conducted by mixing a solution of [^{11}C]CH_2O in DMF with a solution TsOH in DMF, and using Trp instead of tryptamine according to the previously described method [17]. The desired product was obtained with a moderate radiochemical yield (Table 1, entry 1). The reaction was then conducted by using an aqueous solution of TsOH, because the Trp was poorly soluble in the TsOH/DMF solution at room temperature. The Trp dissolved well in the aqueous solution of TsOH at room temperature. These reaction conditions provided a similar result (Table 1, entry 2) to the initial conditions, indicating that the reaction between [^{11}C]CH_2O and Trp in the DMF/water solution did not have any discernible impact on the radiolabeling of [^{11}C]**1**. When the reaction was performed in the presence of the acid catalyst Yb$(OTf)_3$ [20], similar results were achieved (Table 1, entry 3). There was no discernible improvement in the radiochemical conversion of ([^{11}C]**1**) when Yb$(OTf)_3$ was used as an acid catalyst.

The choice of solvent used in this reaction was found to be particularly important because the synthesis of [^{11}C]**1** from [^{11}C]CH_3I occurred over two steps but was conducted in one-pot. Saha *et al.* [19] reported the preparation of a tetrahydro-β-carboline derivative via a Pictet-Spengler reaction using a 10% aqueous TFA solution as a reaction solvent. Based on this report, we proceeded to evaluate the Pictet-Spengler reaction using 1 mol/l HCl as the reaction solvent (Table 1, entry 4). Under these reaction conditions, the radiochemical yield of [^{11}C]**1** was found to be similar to those reported earlier (Table 1, entries 1–3). Interestingly, the Pictet-Spengler reaction was found to proceed smoothly when an aqueous solution of Trp•HCl was used without the addition of an acid catalyst to give the desired product in a 45.2% radiochemical yield (Table 1, entry 5). In contrast, the reaction between [^{11}C]CH_2O and Trp in a mixture of DMF and water was unsuccessful (Table 1, entry 6). Thus, when Trp•HCl was used in the Pictet-Spengler reaction, it effectively provided the acid catalyst under trace amounts of [^{11}C]CH_2O. The effects of the temperature and the reaction time on the outcome of the reaction were also evaluated, and found to be important (Table 1, entries 7 and 8). In the current study, the Pictet-Spengler reaction between [^{11}C]CH_2O and Trp•HCl was found to proceed in the absence of an additional acid catalyst when the materials were heated in aqueous DMF. This procedure represents an effective radiolabeling method because it requires particularly mild conditions. The radiolabeling of bioactive oligopeptides containing Trp•HCl with [^{11}C]CH_2O via a Pictet-Spengler reaction could therefore be a useful procedure.

Cyclic RGD peptides, such as cyclo[Arg-Gly-Asp-D-Tyr-Lys], are potent antagonists for the $\alpha v\beta 3$ integrin receptor [21]. A variety of different cyclic RGD peptides conjugated to a radioactive tracer have been reported for the PET imaging of tumors that overexpress the $\alpha v\beta 3$ integrin receptor [22,23]. With this in mind and to establish further potential uses for our new labeling method, we investigated the application of our direct labeling method using [^{11}C]CH_2O to the model cyclic RGD peptide cyclo [Arg-Gly-Asp-D-Tyr-Lys(Trp)] hydrochloride (**3**•HCl). The substrate of **3**•HCl was prepared via the conjugation of Boc-Trp-OH with the model cyclic RGD peptide according to the activated ester method under slightly basic conditions, followed by the deprotection of the Boc group with a solution of 1 mol/l HCl/AcOH. Compound **3**•HCl was labeled with [^{11}C]CH_2O according to the same procedure used for the synthesis of [^{11}C]**1**. The reaction of compound **3**•HCl with [^{11}C]CH_2O under the conventional manual synthetic procedure proceeded smoothly to give the desired product cyclo[Arg-Gly-Asp-D-Tyr-Lys(1-[^{11}C]Tpi)] ([^{11}C]**2**) with a radiochemical yield of 22.3 ± 4.3% (not decay-corrected), as shown in Figures 2 and 3. Interestingly, the guanidino, phenolic hydroxy, carboxylic acid and amide groups of cyclic RGD peptide remained intact under the reaction conditions. Although this method needs an oligopeptide having Trp residue on the terminal site except for the C-terminal side, a Trp residue can be easily introduced to the epsilon amino group of Lys residue or *N*-terminal amino acid residue. Based on this result, it is therefore clear that this procedure could be particularly effective for the direct formation of cyclic C-C bonds for the radiolabeling of oligopeptides without the need for protecting groups.

Table 1. Survey conditions for the preparation of [1-^{11}C]Tpi [a].

Entry	Acid	Solvents	Reaction temperature (°C)	Reaction time	Substrate	Radiochemical yield [b] ($n = 4$)
1	TsOH (0.10 mmol)	DMF	100	5 min	Trp	42.3 ± 3.2 %
2	TsOH (0.10 mmol)	H_2O	100	5 min	Trp	45.3 ± 2.1 %
3	Yb$(OTf)_3$ (0.02 mmol)	H_2O	100	5 min	Trp	34.7 ± 8.5 %
4	—	1 mol/l HCl	100	5 min	Trp	44.8 ± 2.9 %
5	—	H_2O	100	5 min	Trp•HCl	45.2 ± 3.6 %
6	—	H_2O	100	5 min	Trp	—
7	—	H_2O	room temperature	5 min	Trp•HCl	—
8	—	H_2O	100	1 min	Trp•HCl	24.3 ± 3.3 %

[a] Reaction condition: [^{11}C]CH_2O/DMF (37-370 MBq) 200 μl; substrate (15 μmol); solvents 200 μl.
[b] Determined by a radiochromatogram of the analytical high performance liquid chromatography.

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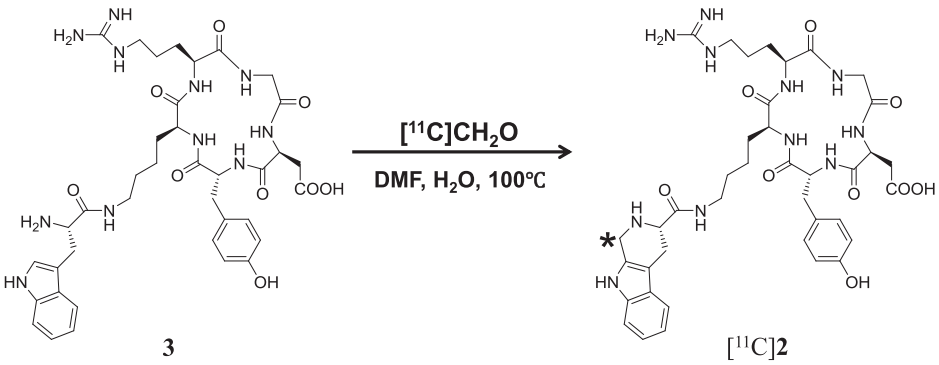

Figure 2. Radiolabeling method via the Pictet-Spengler reaction using [^{11}C]CH_2O.

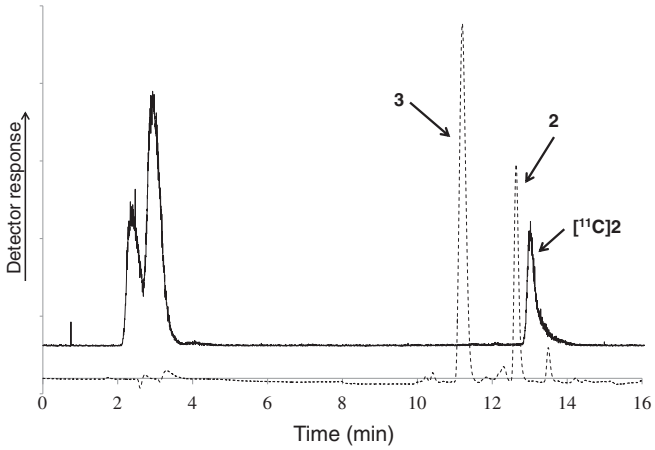

Figure 3. High performance liquid chromatography chromatogram: co-injection of an authentic sample of **2** with the reaction mixture. The black line represents the radioactive peak, and the black dotted line shows the ultraviolet peak at 280 nm.

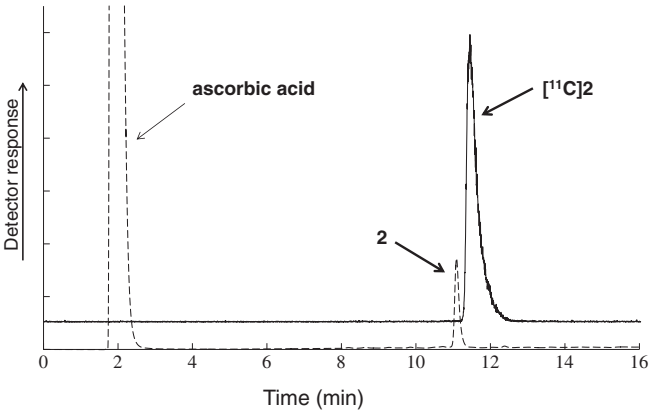

Figure 5. Analytical high performance liquid chromatography chromatogram of co-injection of authentic sample with the purified [^{11}C]**2**. The black line represents the radioactive peak, and the black dotted line shows the ultraviolet peak at 220 nm.

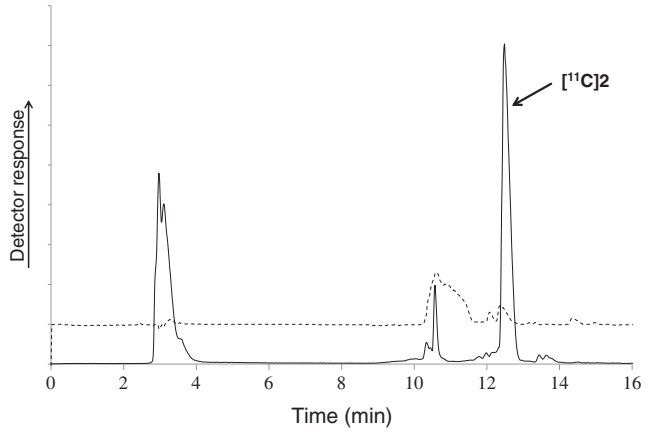

Figure 4. Preparative high performance liquid chromatography chromatogram of [^{11}C]**2**. The black line represents the radioactive peak, and the black dotted line shows the ultaviolet peak at 280 nm.

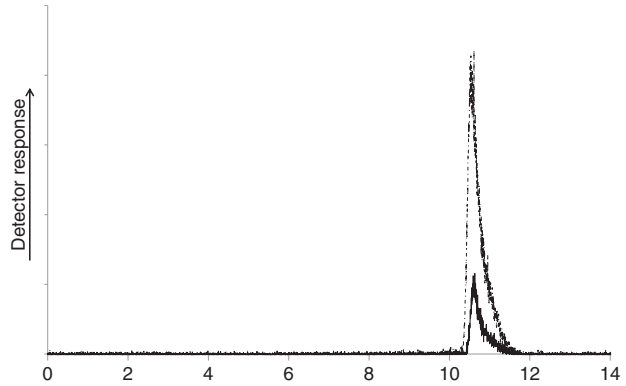

Figure 6. Analytical radio HPLC chromatogram of purified [^{11}C]**2** (the black dotted line) and [^{11}C]**2** after 60 min incubation with fetal bovine serum (the black line).

Based on the reaction conditions determined in the current study, we proceeded to investigate the remote-controlled radiosynthesis of [^{11}C]**2** by using an automatic production system to generate the [^{11}C]CH_3I. Figure 4 shows a representative HPLC chromatogram for the separation. This chromatogram shows that [^{11}C]**2** was produced as the main radioactive peak in the reaction mixture. The one-pot synthesis of [^{11}C]**2** from [^{11}C]CH_3I was succeeded using an automatic production system. From a starting point in the range of 21.0-22.2 GBq for the [^{11}C]CO_2, [^{11}C]**2** was obtained at the end of synthesis in the range of 0.8-1.4 GBq. The average time required for the synthesis was

found to be 35 min from the end of the bombardment. The identity of [^{11}C]**2** was confirmed by its co-injection with compound **2** on the analytical HPLC system, as shown in Figure 5. The radiochemical purity of [^{11}C]**2** was found to be greater than 98% and its specific activity was 85.7 ± 9.4 GBq/μmol. Stability of [^{11}C]**2** in fetal bovine serum was investigated by incubation at 37°C for 60 min. The radiochemical purity of [^{11}C]**2** remained >98%, as shown in Figure 6. The [^{11}C]**2** was radiochemically stable toward *in vitro* degradation for the time of one PET scan.

Conclusion

In conclusion, we have successfully achieved the preparation of [^{11}C]**2** via a Pictet-Spengler reaction. This labeling reaction was completed under mild reaction conditions over a short reaction time in only one step using the HCl salt of the precursor having Trp on the terminal site, except for C-terminal side, without the need for a protecting group. In addition, this labeling technique could be used to increase the overall utility of ^{11}C-labeled oligopeptides as PET probes because this method allows for the incorporation of carbon-11 into a cyclic C-C bond. This reaction could be readily applied to an automated radiolabeling platform using commercially available automated synthetic apparatus for [^{11}C]CH_3I. The results obtained in the current study can be extended to further studies aimed at the preparation of ^{11}C-labeled oligopeptides.

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