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Inhibition of radical reactions for an improved potassium *tert*-butoxide-promoted ^{11}C-methylation strategy for the synthesis of α-^{11}C-methyl amino acids†,‡

Chie Suzuki,[a,b] Koichi Kato,[c,d]* Atsushi B. Tsuji,[a] Ming-Rong Zhang,[d] Yasushi Arano,[b] and Tsuneo Saga[a]

α-^{11}C-Methyl amino acids are useful tools for biological imaging studies. However, a robust procedure for the labeling of amino acids has not yet been established. In this study, the ^{11}C-methylation of Schiff-base-activated α-amino acid derivatives has been optimized for the radiosynthesis of various α-^{11}C-methyl amino acids. The benzophenone imine analog of methyl 2-amino butyrate was ^{11}C-methylated with [^{11}C]methyl iodide following its initial deprotonation with potassium *tert*-butoxide (KO*t*Bu). The use of an alternative base such as tetrabutylammonium fluoride, triethylamine, and 1,8-diazabicyclo[5.4.0]undec-7-ene did not result in the ^{11}C-methylated product. Furthermore, the KO*t*Bu-promoted ^{11}C-methylation of the Schiff-base-activated amino acid analog was enhanced by the addition of 1,2,4,5-tetramethoxybenzene or 2,2,6,6-tetramethylpiperidine-1-oxyl (TEMPO) and inhibited by the addition of 1,10-phenanthroline. These results suggest that inhibition of radical generation induced by KO*t*Bu improves the α-^{11}C-methylation of the Schiff-base-activated amino acids. The addition of a mixture of KO*t*Bu and TEMPO to a solution of Schiff-base-activated amino acid ester and [^{11}C]methyl iodide provided optimal results, and the *tert*-butyl ester and benzophenone imine groups could be readily hydrolyzed to give the desired α-^{11}C-methyl amino acids with a high radiochemical conversion. This strategy could be readily applied to the synthesis of other α-^{11}C-methyl amino acids.

Keywords: α-methylation; amino acid; Schiff base; potassium *tert*-butoxide; PET; electron transfer; radical

Introduction

Amino acids have been labeled with various isotopes, including positron emitting radionuclides.[1,2] Because amino acids are the building blocks of proteins and hormones and intermediates for neurotransmitters and energy metabolism,[1,2] amino acid analogs labeled with ^{11}C, ^{18}F, and ^{13}N have been explored extensively as tool compounds for the imaging of biological phenomenon by positron emission tomography (PET).

The incorporation of a methyl group at the α-position of amino acids is one of the strategies used for the design of amino acid-based PET tracers. Radiolabeled α-methyl amino acids are generally more stable toward metabolism than their desmethyl counterparts because their α,α-dialkylated structure makes them more resistant to transamination, the first step of amino acid catabolism.[3] The metabolic characteristics of radiolabeled α-methyl amino acids allow them to be used as noninvasive imaging probes, which can be used to simplify the kinetic analysis of amino acid transport activity[4–9] or investigate the metabolism of amino acid side chains during the synthesis of neurotransmitters.[10] However, the number of radiolabeled α-methyl amino acid tracer compounds available for imaging studies is limited, because the vast majority of these compounds are synthesized by the incorporation of a radiohalogen into their side chain.[4,5,11] In contrast, L-[^{11}C]-α-methyl tryptophan ([^{11}C]AMT)[12] and 2-amino-[3-^{11}C]isobutyric acid ([3-^{11}C]AIB)[13] were labeled with ^{11}C at their α-methyl group by the ^{11}C-methylation of the corresponding Schiff-base-activated amino acid derivatives. The radiolabeling of the core structure of α-methyl amino acids with ^{11}C could potentially be used as an effective strategy to

[a]*Diagnostic Imaging Program, Molecular Imaging Center, National Institute of Radiological Sciences, 4-9-1 Anagawa, Inage-ku, Chiba 263-8555, Japan*

[b]*Department of Molecular Imaging and Radiotherapy, Graduate School of Pharmaceutical Sciences, Chiba University, 1-8-1 Inohana, Chuo-ku, Chiba 260-8675, Japan*

[c]*Department of Integrative Brain Imaging, National Center of Neurology and Psychiatry, 4-1-1 Ogawahigashi-cho, Kodaira, Tokyo 187-5551, Japan*

[d]*Molecular Probe Group, Molecular Imaging Center, National Institute of Radiological Sciences, 4-9-1 Anagawa, Inage-ku, Chiba 263-8555, Japan*

**Correspondence to: Koichi Kato, Department of Integrative Brain Imaging, National Center of Neurology and Psychiatry, 4-1-1 Ogawahigashi-cho, Kodaira, Tokyo 187-5551, Japan.*
E-mail: katok@ncnp.go.jp

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synthesize a variety of ^{11}C-labeled α-amino acids and evaluate their biological properties by PET. In this study, we have developed an optimized procedure for the ^{11}C-methylation of Schiff-base-activated α-amino acid derivatives that can be used for the radiosynthesis of a wide variety of α-^{11}C-methyl amino acids.

Experimental

General

All of the chemicals used in the current study were purchased as the analytical grade and used without further purification. H-Abu(2)-O*t*Bu-HCl (**2b**), H-Ala-OMe (**2c**), H-Nva-O*t*Bu-HCl (**2d**), and H-Phe-O*t*Bu-HCl (**2e**) were purchased from Watanabe Chemical Industries, Ltd. (Hiroshima, Japan). (*R*)-α-Ethylalanine monohydrate[5] and (*R*)-α-methylphenylalanine monohydrate[7] were purchased from Nagase & Co., Ltd. (Kobe, Japan). Compounds **3c** and **4c** were prepared as described previously.[13] Solutions of potassium *tert*-butoxide (KO*t*Bu) (1 M solution in THF) and tetrabutylammonium fluoride (TBAF) (1 M solution in THF) were purchased from Sigma Aldrich (Milwaukee, WI, USA) and Tokyo Chemical Industry Co., Ltd (Tokyo, Japan), respectively. 1,2,4,5-Tetramethoxy benzene (TMB) was synthesized according to a previously published procedure.[14] 2,2,6,6-Tetramethylpiperidine-1-oxyl (TEMPO) and 1,10-phenanthroline (*o*-phen) were purchased from Sigma Aldrich and Tokyo Chemical Industry Co., Ltd, respectively. ^{1}H and ^{13}C NMR spectra were obtained on a JEOL-AL-300 spectrometer (JEOL, Tokyo, Japan). Chemical shifts (δ) have been reported in units of parts per million (ppm) relative to tetramethylsilane (0.00 ppm for ^{1}H and ^{13}C), which was used as an internal reference. Fast atom bombardment-mass spectrometry (FAB-MS) was conducted on a JEOL JMS-SX 102A spectrometer (JEOL). Carbon-11 was produced by an ^{14}N (p, α) ^{11}C nuclear reaction with a CYPRIS HM18 cyclotron (Sumitomo Heavy Industry, Tokyo, Japan). HPLC was conducted on a JASCO HPLC system (JASCO, Tokyo, Japan). Effluent radioactivity was determined with a NaI (Tl) scintillation detector system (ORTEC, Oak Ridge, TN, USA). Analytical reversed-phase (RP)-HPLC was performed over a COSMOSIL 5C18-AR-II column (150 × 4.6 mm, i. d.; Nacalai Tesque, Kyoto, Japan) at a flow rate of 1 mL/min with a mobile phase consisting of acetonitrile/H_2O (70/30 – v/v) for the methyl ester (system 1) or acetonitrile/H_2O (85/15 – v/v) for the *tert*-butyl ester (system 2). Analytical hydrophilic interaction chromatography (HILIC)-HPLC was performed over a Cosmosil HILIC column (150 × 4.6 mm, i. d.; Nacalai Tesque) at a flow rate of 1 mL/min with a mobile phase consisting of acetonitrile/30 mM aqueous ammonium acetate solution (80/20 – v/v). Semipreparative HPLC was performed over a Cosmosil HILIC column (250 × 10 mm, i. d.; Nacalai Tesque) at a flow rate of 5 mL/min with a mobile phase consisting of acetonitrile/30 mM aqueous ammonium acetate (80/20 – v/v).

Chemistry

*Methyl 2-aminobutanoate (**2a**)*

Thionyl chloride (21.1 mL, 291 mmol) was added in a dropwise manner to a suspension of DL-2-aminobutyric acid (**1a**, 3.0 g, 29.1 mmol) in methanol (30 mL) at −30 °C under an atmosphere of N_2, and the resulting mixture was stirred for 3 h at −30 °C. The mixture was then warmed to room temperature and stirred for 18 h. The solvent was removed *in vacuo* to give a residue, which was codistilled from toluene to remove any residual thionyl chloride. The resulting residue was then crystallized from a mixture of methanol/diethyl ether to provide **2a** as a white solid (4.01 g, 89.7%). ^{1}H NMR ($CDCl_3$): δ 8.82 (2H, s, NH_2), 4.09–4.14 (1H, t, J = 5.7 Hz, C*H*), 3.83 (3H, s, COOCH_3), 2.08–2.16 (2H, quin, J = 7.7 Hz, CH_2CH$_3$), 1.10–1.15 (3H, t, J = 7.3 Hz, CH$_2$CH_3); FAB-MS $[M+H]^+$: *m/z* 118, found: 118.

*Methyl 2-((diphenylmethylene)amino)butyrate (**3a**)*

Benzophenone imine (819 µL, 4.88 mmol) and compound **2a** (500 mg, 3.25 mmol) were added to dichloromethane (10 mL), and the resulting mixture was stirred at room temperature for 24 h. The reaction mixture was then filtered, and the filtrate was collected and concentrated *in vacuo* to give a residue, which was dissolved in diethyl ether (20 mL). The resulting solution was washed with brine (3 × 20 mL) and dried over anhydrous magnesium sulfate. The solvent was removed *in vacuo* to give a residue, which was purified by column chromatography over silica gel eluting with a mixture of ethyl acetate/hexane (1:50 – v/v) to provide **3a** as a colorless oil (765.8 mg, 83.6%). ^{1}H NMR ($CDCl_3$): δ 7.15–7.66 (10H, m, *aromatic*), 3.99–4.03 (1H, dd, J = 7.7, 5.5 Hz, C*H*), 3.72 (3H, s, COOCH_3), 1.89–1.97 (2H, m, CH_2CH$_3$), 0.83–0.88 (3H, t, J = 7.3 Hz, CH$_2$CH_3); ^{13}C NMR ($CDCl_3$): δ 173.1, 170.6, 139.7, 136.6, 130.2, 129.0, 128.7, 128.4, 128.2, 128.0, 66.9, 52.2, 27.2, 10.7; FAB-MS $[M+H]^+$: *m/z* 282, found: 282.

*tert-Butyl 2-((diphenylmethylene)amino)butyrate (**3b**)*

Compound **3b** was prepared from benzophenone imine and H-Abu(2)-O*t*Bu-HCl (**2b**) according to the procedure described earlier for **3a**. Compound **3b** was isolated as a colorless oil (824.2 mg, 99.7%). ^{1}H NMR ($CDCl_3$): δ 7.16–7.83 (10H, m, *aromatic*), 3.83–3.87 (1H, dd, J = 7.3, 5.1 Hz, C*H*), 1.83–1.98 (2H, m, CH_2CH$_3$), 1.44 (9H, s, C(CH_3)$_3$), 0.85–0.89 (3H, t, J = 7.3 Hz, CH$_2$CH_3); ^{13}C NMR ($CDCl_3$): δ 171.7, 170.1, 139.9, 136.9, 130.2, 128.9, 128.6, 128.5, 128.1, 128.0, 80.9, 67.5, 28.2, 27.0, 10.7; FAB-MS $[M+H]^+$: *m/z* 324, found: 324.

*tert-Butyl 2-((diphenylmethylene)amino)pentanoate (**3d**)*

Compound **3d** was prepared from benzophenone imine and H-Nva-O-*t*Bu-HCl (**2d**) according to the procedure described earlier for **3a**. Compound **3d** was isolated as a colorless oil (774.6 mg, 96.4%). ^{1}H NMR ($CDCl_3$): δ 7.16–7.82 (10H, m, *aromatic*), 3.89–3.93 (1H, t, J = 6.2 Hz, C*H*), 1.83–1.90 (2H, q, J = 6.6 Hz, CH_2CH$_2$CH$_3$), 1.44 (9H, s, C(CH_3)$_3$), 1.21–1.37 (2H, m, CH$_2$CH_2CH$_3$), 0.82–0.86 (3H, t, J = 7.3 Hz, CH$_2$CH$_2$CH_3); ^{13}C NMR ($CDCl_3$): δ 171.8, 170.0, 139.9, 136.9, 130.2, 128.9, 128.6, 128.5, 128.1, 128.0; FAB-MS $[M+H]^+$: *m/z* 338, found: 338.

*tert-Butyl 2-((diphenylmethylene)amino)-3-phenylpropanoate (**3e**)*

Compound **3e** was prepared from benzophenone imine and H-Phe-O*t*Bu-HCl (**2e**) according to the procedure described earlier for **3a**. Compound **3e** was isolated as a colorless oil (824.2 mg, 99.7%). ^{1}H NMR ($CDCl_3$): δ 7.60–7.80 (15H, m, *aromatic*), 4.08–4.12 (1H, dd, J = 8.8, 4.4 Hz, C*H*), 3.12–3.30 (2H, m, CH_2Ph), 1.44 (9H, s, C(CH_3)$_3$); ^{13}C NMR ($CDCl_3$): δ 171.0, 170.5, 139.7, 138.5, 136.5, 130.2, 130.0, 128.9, 128.3, 128.2, 128.1, 128.0, 127.8, 126.3, 81.3, 68.1, 39.7, 28.2; FAB-MS $[M+H]^+$: *m/z* 386, found: 386.

*Methyl 2-((diphenylmethylene)amino)-2-methylbutanoate (**4a**)*

A solution of KO*t*Bu in THF (1.0 M, 1.42 mL, 1.42 mmol) was added to a solution of **3a** (80 mg, 0.28 mmol) in THF (3 mL) in a dropwise manner at 0 °C. Methyl iodide (89.0 µL, 1.42 mmol) was then added to the mixture in a dropwise manner at 0 °C, and the resulting mixture was stirred for 16 h at room temperature. The solvent was removed *in vacuo* to give a residue, which was purified by column chromatography over silica gel eluting with a mixture of ethyl acetate/hexane (1/50 – v/v) to give unlabeled **4a** as a yellow oil (55.4 mg, 66.0%). ^{1}H NMR ($CDCl_3$): δ 7.15–7.82 (10H, m, *aromatic*), 3.29 (3H, s, COOCH_3), 1.97–2.02 (2H, m, CH_2CH$_3$), 1.39 (3H, s, CCH_3), 0.86–1.00 (3H, t, J = 7.7 Hz, CH$_2$CH_3); ^{13}C NMR ($CDCl_3$): δ 175.4, 166.7, 141.3, 137.7, 130.2, 128.7, 128.6, 128.4, 128.1, 127.8, 66.8, 51.4, 33.2, 23.7, 8.6; FAB-MS $[M+H]^+$: *m/z* 296, found: 296.

*tert-Butyl 2-((diphenylmethylene)amino)-2-methylbutanoate (**4b**)*

Compound **4b** was prepared from **3b** (100 mg, 0.31 mmol) using methyl iodide (96.7 µL, 1.55 mmol) and a solution of KO*t*Bu in THF (1.0 M, 1.55 mL, 1.55 mmol) according to the procedure described earlier for **4a**. Compound **4b** was isolated as a yellow oil (58.6 mg, 56.1%). ^{1}H NMR ($CDCl_3$): δ 7.20–7.83 (10H, m, *aromatic*), 1.86–1.94 (2H, m, CH_2CH$_3$), 1.31 (9H, s, C(CH_3)$_3$), 1.21 (3H, s, CCH_3), 1.00–1.05 (3H, t, J = 7.3 Hz, CH$_2$CH_3); ^{13}C NMR ($CDCl_3$): δ 174.3, 166.9, 141.9, 138.9, 129.8, 128.9, 128.6, 128.3, 128.0, 127.9, 80.6, 67.1, 36.6, 28.1, 24.0, 8.8; FAB-MS $[M+H]^+$: *m/z* 338, found: 338.

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*tert-Butyl 2-((diphenylmethylene)amino)-2-methylpentanoate (**4d**)*

Compound **4d** was prepared from **3d** (100 mg, 0.30 mmol) using methyl iodide (92.6 μL, 1.48 mmol) and a solution of KO*t*Bu in THF (1.0 M, 1.48 mL, 1.48 mmol) according to the procedure described earlier for **4a**. Compound **4d** was isolated as a yellow oil (26.5 mg, 25.4%). ^{1}H NMR ($CDCl_3$): δ 7.19–7.82 (10H, m, *aromatic*), 1.75–1.88 (2H, m, $CH_2CH_2CH_3$), 1.38–1.56 (2H, m, $CH_2CH_2CH_3C$), 1.32 (9H, s, $C(CH_3)_3$), 1.23 (3H, s, CH_3), 0.91–0.96 (3H, t, $J = 7.3$ Hz, $CH_2CH_2CH_3$); ^{13}C NMR ($CDCl_3$): δ 174.2, 166.6, 141.7, 138.7, 129.6, 128.7, 128.5, 128.2, 127.8, 127.7, 80.4, 66.7, 45.6, 27.9, 24.4, 17.4, 14.6; FAB-MS $[M+H]^+$: *m/z* 352, found: 352.

*tert-Butyl 2-((diphenylmethylene)amino)-2-methylbutanoate (**4e**)*

Compound **4e** was prepared from **3e** (100 mg, 0.26 mmol) using methyl iodide (81.1 μL, 1.30 mmol) and a solution of KO*t*Bu in THF (1.0 M, 1.30 mL, 1.30 mmol) according to the procedure described earlier for **4a**. Compound **4e** was isolated as a yellow oil (31.7 mg, 30.6%). ^{1}H NMR ($CDCl_3$): δ 6.95–7.82 (15H, m, *aromatic*), 3.08–3.37 (2H, m, CH_2CH_3), 1.56 (3H, s, CCH_3), 1.28 (9H, s, $C(CH_3)_3$); ^{13}C NMR ($CDCl_3$): δ 173.8, 166.7, 141.8, 138.8, 137.7, 131.4, 130.2, 128.8, 128.7, 128.2, 128.1, 127.9, 127.8, 126.5, 81.0, 68.0, 49.1, 28.1, 24.0; FAB-MS $[M+H]^+$: *m/z* 400, found: 400.

*2-Amino-2-methylpentanoic acid (**6**)*

A 4 M solution of HCl in EtOAc (2 mL) was added in a dropwise manner to a solution of **4d** (47.0 mg, 0.13 mmol) in EtOAc (2 mL) at 0 °C, and the resulting mixture was stirred for 4 h at room temperature. The organic phase was extracted with water (3 × 5 mL), and the combined aqueous extracts were distilled to dryness under vacuum to give a residue. The residue codistilled from acetonitrile under reduced pressure to provide **6** as a white solid (9.7 mg, 41.2%). ^{1}H NMR (CD_3OD): δ 1.71–1.96 (2H, m, $CH_2CH_2CH_3$), 1.54 (3H, s, CCH_3), 1.50–1.26 (2H, m, $CH_2CH_2CH_3C$), 1.01–0.96 (3H, t, $J = 7.3$ Hz, $CH_2CH_2CH_3$); ^{13}C NMR (CD_3OD): δ 173.8, 61.0, 40.6, 22.9, 18.0, 14.3; FAB-MS $[M+H]^+$: *m/z* 132, found: 132.

Radiochemistry

[^{11}C]methyl iodide

[^{11}C]Methyl iodide ([^{11}C]H_3I) was prepared by wet method[15] as described later. After the end of bombardment (EOB), [^{11}C]carbon dioxide was bubbled into a solution of lithium aluminum hydride in THF (0.05 M, 500 μL) at 0 °C for ~3 min. After the solvent was removed at 150 °C, an aqueous solution of hydriodic acid (HI, 57%, 400 μL) was added to the residue at 0 °C. The resulting [^{11}C]H_3I was transferred through ascarite and phosphorus pentoxide columns under a stream of N_2 gas into THF or dimethyl sulfoxide (DMSO) (1 mL). The radiochemical purity (RCP) was determined to be >98% by analytical RP-HPLC using system 1.

General conditions for the ^{11}C-methylation process

A portion of the [^{11}C]H_3I solution (100 μL) containing [^{11}C]H_3I (50 MBq) was added to 50 μL solutions of compounds **3a–e** (10 μmol). The individual mixtures were then treated with a solution of base (i.e., TBAF, triethylamine (TEA), 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU), or KO*t*Bu; 5, 10, or 20 μmol) in THF (150 μL) both in the presence and absence of an additive (i.e., TMB, TEMPO, or *o*-phen; 10 μmol). The resulting mixtures were held at room temperature for 90 s, and a portion of each mixture was collected for analysis by RP-HPLC. The radiochemical identity of the ^{11}C-labeled compounds was verified by comparing their HPLC profiles with those of the corresponding unlabeled compounds, and the efficiency of each reaction was evaluated according to the radiochemical conversion (RCC), which was calculated from the radiochromatogram after a decay correction.

*Deprotection of compound **4a** in THF*

The products of the ^{11}C-methylation process were treated with a 1.0 M solution of NaOH (150 μL) at 100 °C for 90 s. The reaction mixtures were then treated with a 2.0 M solution of aqueous HCl (150 μL) and held at room temperature for 90 s. A portion of each mixture was then collected and analyzed by HILIC-HPLC.

*Deprotection of compound **4a** in DMSO*

Following the ^{11}C-methylation process, the solvent was evaporated under a stream of N_2 gas to give a residue, which was dissolved in DMSO (150 μL) and treated with a 1.0 M solution of NaOH (150 μL). The resulting mixture was heated at 100 °C for 90 s and then treated with a 2.0 M aqueous solution of HCl (150 μL). The resulting mixture was held at room temperature for 90 s before being sampled for analysis by HILIC-HPLC.

*Deprotection of compounds **4b**, **4d**, and **4e***

The product of the ^{11}C-methylation process was treated with a 2.0 M aqueous solution of HCl (150 μL), and the resulting mixture was heated at 100 °C for 5 min. The mixture was then cooled to room temperature before being sampled for analysis by HILIC-HPLC.

*Procedure for the remote-controlled synthesis of compound **5***

After EOB, the [^{11}C]carbon dioxide was transferred to a reaction vessel containing a 0.05 M solution of lithium aluminum hydride in THF (300 μL) and trapped until the solution reached a radioactivity plateau at 0 °C. The radioactive solution was then heated at 150 °C to remove THF through evaporation. The resulting residue was then cooled to 0 °C before being treated with an aqueous solution of HI (57%, 400 μL). The mixture of radioactive compounds and HI was then heated at 150 °C to generate [^{11}C]H_3I. Gaseous [^{11}C]H_3I was distilled from the mixture using a stream of N_2 gas at a flow rate of 30 mL/min and transferred through ascarite and phosphorus pentoxide columns into the reaction vessel containing 10 μmol of **3b** in THF (150 μL) at −10 °C. After the solution reached its radioactivity plateau, it was treated with a solution of 10 μmol KO*t*Bu and 10 μmol TEMPO in THF, and the resulting solution was heated for 3 min at 20 °C. The reaction mixture was then treated with a 2.0 M aqueous solution of HCl (150 μL), and the resulting mixture was heated for 5 min at 100 °C. The mixture was then cooled to room temperature and purified by semipreparative HILIC-HPLC. The fraction containing **5** (retention time: 10–11 min) was evaporated to dryness to give a radioactive residue, which was dissolved in water (2 mL). The RCP of the resulting solution of **5** was determined to be 99.6 ± 0.4% by analytical HILIC-HPLC.

Statistical analysis

All of the experiments described in this study were performed in triplicate, and the resulting data have been presented as the mean ± standard deviation. The effects of the additives were evaluated using ANOVA with Dunnett's multiple comparison test (Microsoft Excel software; Microsoft, Seattle, WA, USA).

Results and discussion

The ^{11}C-methylation of Schiff-base-activated α-amino acids represents a desirable strategy for the generation of radiolabeled amino acids because the enhanced acidity[13,16] of the α-protons of these compounds facilitates their alkylation. Aldimine analogs are usually employed as precursors for the synthesis of α,α-dialkylated amino acids under nonlabeling conditions. Unfortunately, however, most aldimine derivatives show a low level of chemical stability, and this represents a significant issue for PET chemistry, which requires compounds to be stable to the routine handling conditions required of conventional and remote-controlled syntheses. Benzophenone imine analogs are more stable than benzaldimine analogs,[17] and benzophenone imine-type labeling precursors are therefore more suitable for the radiosynthesis of α-^{11}C-methyl amino acids.

We initially explored the ^{11}C-methylation of the benzophenone imine analog of methyl 2-amino butyrate **3a** under various

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Table 1. Radiochemical conversions for the α-^{11}C-methylation of **3a**

Entry	Precursor	Base (μmol)	Solvent	RCC[a] (%)
1	**3a**	TBAF (20)	DMSO	nd
2	**3a**	TBAF (20)	THF	nd
3	**3a**	TEA (10)	DMSO	nd
4	**3a**	DBU (10)	DMSO	nd
5	**3a**	KO*t*Bu (5)	DMSO	21.2 ± 3.0
6	**3a**	KO*t*Bu (10)	DMSO	15.3 ± 4.9
7	**3a**	KO*t*Bu (20)	DMSO	0.9 ± 0.2
8	**3a**	KO*t*Bu (5)	THF	14.1 ± 0.6
9	**3a**	KO*t*Bu (10)	THF	54.3 ± 6.9
10	**3a**	KO*t*Bu (20)	THF	58.8 ± 2.7

RCC, radiochemical conversion; TBAF, tetrabutylammonium fluoride; TEA, triethylamine; DBU, 1,8-diazabicyclo[5.4.0] undec-7-ene; KO*t*Bu, potassium *tert*-butoxide; DMSO, dimethyl sulfoxide; nd, not detected.
[a]The RCC data were calculated using the radiochromatograms obtained by analytical reversed-phase HPLC following decay correction.

conditions. Several bases were screened against the reaction, including TBAF, TEA, and DBU, but all of these bases failed to provide the desired ^{11}C-methylated product (Table 1, entries 1–4). In our previous report concerning the synthesis of [3-^{11}C]AIB, treatment of a solution of Schiff-base-activated alanine analogs **3c** with TBAF resulted in the immediate formation of a yellow solution, which indicated that the substrate was rapidly deprotonated to give the corresponding anion.[13] In the current case, treatment of a solution of **3a** with TBAF, TEA, or DBU did not result in a color change after 90 s, which suggested that **3a** was not being deprotonated by these \bases. These results therefore demonstrated that increasing the length of the side-chain structure from methyl to ethyl had led to an increase in the pK_a of the α-protons in **3a**, making them resistant to deprotonation by TBAF. Based on this result, we proceeded to investigate the use of a stronger base for the deprotonation and subsequent ^{11}C-methylation of **3a**. When a solution of KO*t*Bu was added to a solution of **3a**, the reaction mixture immediately turned red, and the ^{11}C-methylated compound was obtained following the addition of [^{11}C]H_3I (Figure 1A and C; Table 1, entries 5–10). Having identified a suitable base for the reaction, we proceeded to screen different solvents and found that **4a** was obtained with a higher RCC when the reaction was conducted in THF compared with DMSO.

During the course of this work toward the ^{11}C-methylation of **3a**, we observed an unknown by-product peak that was eluted from the RP-HPLC column just before [^{11}C]H_3I (Figure 1C). This peak was also observed by RP-HPLC analysis when a mixture of [^{11}C]H_3I and KO*t*Bu was held at room temperature for 90 s (data not shown). KO*t*Bu has been reported to act not only as strong base but also as single-electron donor,[18,19] which could result in the formation of radicals and provide some explanation for the formation of the by-product observed by RP-HPLC. To evaluate the influence of the electron transfer reaction on the α-^{11}C-methylation of Schiff-base-activated α-amino acid and the formation of the observed by-products, we investigated the addition of three different additives to the reaction and compared the RCCs of the resulting products (Table 2). The addition of TMB,

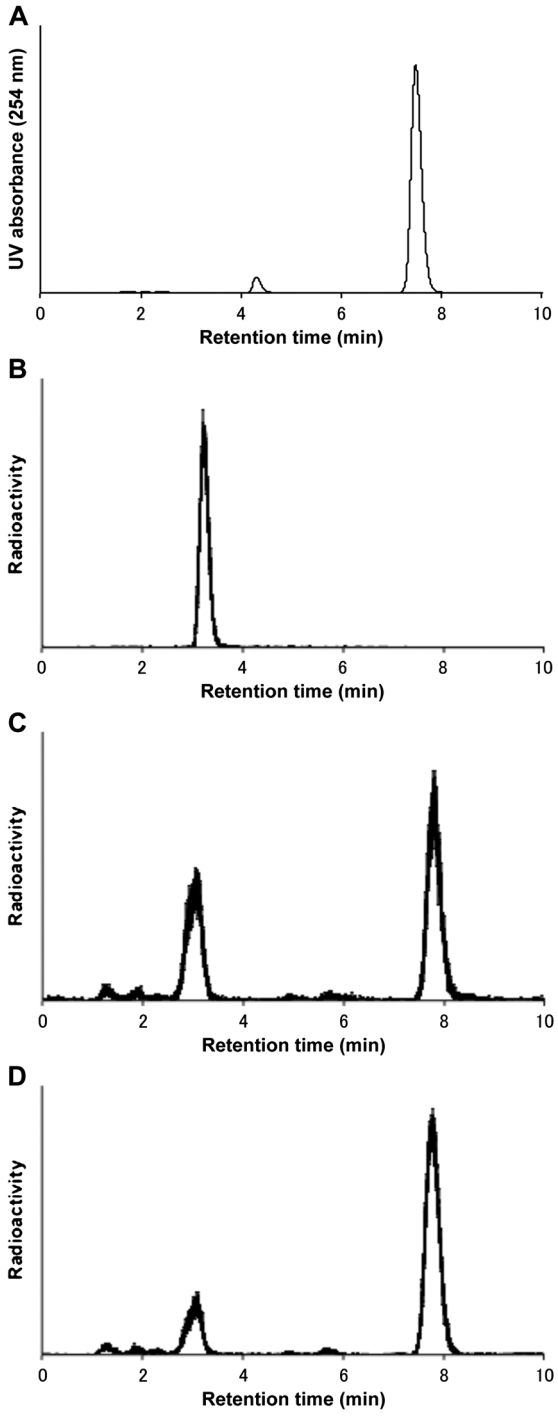

Figure 1. RP-HPLC profiles of (A) the ultraviolet absorbance of unlabeled **4a** at 254 nm, (B) radioactivity of a solution of [^{11}C]H_3I in THF, (C) radioactivity of the reaction mixture treated with entry 9 in Table 1 (entry 1 in Table 2), and (D) radioactivity of the reaction mixture treated with entry 3 in Table 2.

which is an electron transfer inhibitor,[20] or TEMPO, which is a radical scavenger, prevented the formation of the by-product (Figure 1D), resulting in an improvement in the RCC of the KO*t*Bu-promoted ^{11}C-methylation of **3a** (Table 2, entries 2 and 3). In contrast, the addition of *o*-phen, which is an organocatalyst for electron transfer-related reactions,[19] had an adverse impact on the ^{11}C-methylation reaction of **3a** and gave a much lower yield of the desired product (Table 2, entries 4). Similar tendencies were also observed in the KO*t*Bu-promoted ^{11}C-methylation of **3c**

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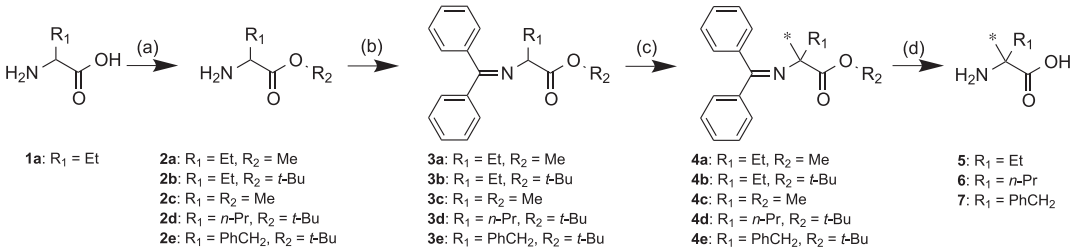
Table 2. The effect of additives on the radiochemical conversion of the α-^{11}C-methylation reaction

Entry	Precursor	Base (μmol)	Solvent	Additive	RCC[a] (%)
1	**3a**	KO*t*Bu (10)	THF	None	54.3 ± 6.9
2	**3a**	KO*t*Bu (10)	THF	TMB	80.6 ± 7.6*
3	**3a**	KO*t*Bu (10)	THF	TEMPO	79.4 ± 5.0*
4	**3a**	KO*t*Bu (10)	THF	*o*-phen	1.2 ± 1.0*
5	**3c**	KO*t*Bu (10)	THF	None	38.9 ± 7.8
6	**3c**	KO*t*Bu (10)	THF	TMB	45.9 ± 8.5
7	**3c**	KO*t*Bu (10)	THF	TEMPO	62.5 ± 3.9*
8	**3c**	KO*t*Bu (10)	THF	*o*-phen	8.3 ± 3.3*
9	**3c**	TBAF (20)	DMSO	None	78.0 ± 9.7
10	**3c**	TBAF (20)	DMSO	TMB	77.6 ± 8.4
11	**3c**	TBAF (20)	DMSO	TEMPO	76.7 ± 12.7
12	**3c**	TBAF (20)	DMSO	*o*-phen	84.1 ± 4.8

RCC, radiochemical conversion; TBAF, tetrabutylammonium fluoride; KO*t*Bu, potassium *tert*-butoxide; DMSO, dimethyl sulfoxide; TMB, 1,2,4,5-tetramethoxy benzene; TEMPO, 2,2,6,6-tetramethylpiperidine-1-oxyl; *o*-phen, 1,10-phenanthroline.
[a]The RCC data were calculated using the radiochromatograms obtained by analytical reversed-phase HPLC following decay correction.
*$P < 0.01$ compared with the RCC without additive analyzed by ANOVA with Dunnett's multiple comparison test.



Scheme 1. Synthesis of the α-^{11}C-methyl amino acids. (a) Methanol, thionyl chloride; (b) benzophenone imine, dichloromethane; (c) [^{11}C]H_3I, KO*t*Bu, DMSO, or THF; (d) (i) NaOH, H_2O; (ii) HCl, H_2O; or (i) HCl, H_2O.

(Table 2, entries 5–8). It is noteworthy, however, that the RCC of the TBAF-promoted ^{11}C-methylation of **3c** was not changed by the addition of TMB, TEMPO, or *o*-phen (Table 2, entries 9–12). These results suggest that KO*t*Bu, but not TBAF, would promote the generation of radicals by a single-electron transfer, which hampered the ^{11}C-methylation of Schiff-base-activated α-amino acids. The addition of 10 μmol TEMPO led to the highest RCC of all of the KO*t*Bu-promoted ^{11}C-methylation reactions.

Taken together, the results of these experiments indicated that the optimal reaction conditions involved the addition of a mixture of 10 μmol KO*t*Bu and 10 μmol TEMPO to the solution containing the Schiff-base-activated precursor and [^{11}C]H_3I. With the optimized conditions in hand, we proceeded to investigate the ^{11}C-methylation of several other benzophenone imine-amino acid analogs (Scheme 1). The benzophenone imine analog of *tert*-butyl 2-amino butyrate **3b** yielded the desired ^{11}C-methylated product **4b** with high RCC. The benzophenone imine analogs of amino acids bearing a longer alkyl side chain **3d** or aromatic ring **3e** also reacted smoothly under the optimized conditions to give the desired ^{11}C-methylated products with high levels of reproducibility and high RCCs (Table 3). These results therefore demonstrated that this new method involving the treatment of a mixture of Schiff-base-activated precursor and [^{11}C]H_3I with KO*t*Bu in the presence of TEMPO could be applied to the α-^{11}C-methylation of various α-amino acids.

The hydrolysis of the ester moiety in the ^{11}C-methylated products was explored. Surprisingly, treatment of a solution of **4a** in THF with a 1.0 M aqueous solution of NaOH at 100 °C for 90 s did not give any of the desired hydrolysis product. We previously reported the hydrolysis of **4c** under the same conditions except that DMSO was used as the solvent instead of THF. DMSO has been reported to increase the rate of ester hydrolysis,[21] and we therefore proceeded to investigate the hydrolysis of **4a** in DMSO by removing the THF *in vacuo* following the ^{11}C-methylation reaction and replacing it with DMSO. The resulting DMSO solution of **4a** was then treated with a 1.0 M aqueous solution of NaOH at 100 °C for 90 s, which resulted in the complete hydrolysis of the methyl ester of **4a**.

Table 3. Radiochemical conversions of KO*t*Bu-promoted α-^{11}C-methylation reactions in the presence of 10 μmol 2,2,6,6-tetramethylpiperidine-1-oxyl

Entry	Precursor	RCC[a] (%)
1	**3a**	79.4 ± 5.0
2	**3b**	91.2 ± 5.1
3	**3c**	62.5 ± 3.9
4	**3d**	84.7 ± 2.4
5	**3e**	53.2 ± 0.7

[a]The radiochemical conversion (RCC) data were calculated using the radiochromatograms obtained by analytical reversed-phase HPLC following decay correction.

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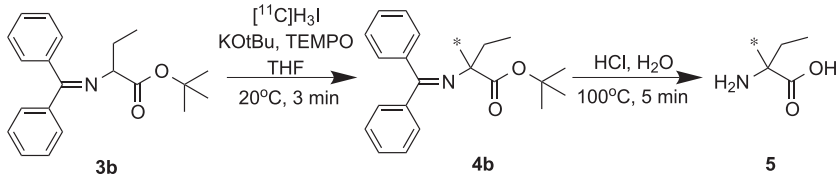

Scheme 2. Remote-controlled synthesis of compound **5**.

Subsequent removal of the benzophenone imine group by the treatment of the acid with a 2.0 M aqueous solution of HCl gave **5** with a conversion of 63.8 ± 12.2%. In contrast to the conditions described earlier for the methyl ester, the *tert*-butyl ester and benzophenone imine groups of compounds **4b**, **4d**, and **4e** were hydrolyzed simultaneously with high RCCs when they were heated at 100 °C for 5 min following the addition of a 2.0 M solution of aqueous HCl to the reaction mixture of the ^{11}C-methylation reaction. This strategy provided the desired hydrolyzed products **5**, **6**, and **7** with RCCs of 85.4 ± 3.9, 90.7 ± 1.7, and 63.5 ± 2.3%, respectively, following the two steps. Short reaction times and operationally simple procedures are preferred for remote-controlled ^{11}C-labeling syntheses, and the simultaneous hydrolyses of the ester and imine groups of the α-^{11}C-methylated amino acid analogs under acidic conditions therefore represent a better strategy for this approach. With this in mind, the use of amino acid analogs protected as the corresponding *tert*-butyl esters would be more appropriate for the remote-controlled radiosynthesis of α-^{11}C-methyl amino acids than the methyl ester.

Having optimized the ^{11}C-methylation and hydrolysis reactions, the synthesis of compound **5** was selected as a suitable example to evaluate the application of this strategy to the remote-controlled synthesis of radiolabeled α-^{11}C-methylated amino acids (Scheme 2). Each step in the synthesis of compound **5** was conducted under the conditions described earlier with only minor modifications. Thus, the reaction time for the ^{11}C-methylation reaction was extended from 90 s to 3 min to allow for an increase in the temperature of reaction mixture to 20 °C, because the [^{11}C]H_3I was dissolved at −10 °C. Upon completion of the reaction, the desired product **5** was purified by semipreparative HPLC. The total time required for the synthesis **5** from EOB to formulation, including the production of [^{11}C]H_3I and the purification of **5**, was approximately 40 min. The radioactivity of the final product was determined to be in the range of 1.88–2.55 GBq, and radiochemical yield was 10.3 ± 1.2% (decay uncorrected, relative to the calculated [^{11}C]carbon dioxide) when the synthesis was started from 18.5 to 22.2 GBq of [^{11}C]carbon dioxide at EOB. After purification, the RCP was determined to be 99.6 ± 0.4%. The ultraviolet absorption peak for **1a** at 210 nm was not observed in the chromatogram of purified **5**. The radioactivity of **5** is sufficient to allow future *in vitro* and *in vivo* animal studies.

Conclusions

A new method has been developed for the ^{11}C-methylation of benzophenone imine-bearing amino acid analogs using KO*t*Bu, with the corresponding α-^{11}C-methylated amino acid derivatives being isolated with high RCC. The occurrence of side reactions associated with radical species could be inhibited by the addition of TMB or TEMPO, which led to significant improvements in the KO*t*Bu-promoted ^{11}C-methylation reaction and allowed for the synthesis of the desired α-^{11}C-methyl amino acids in high RCCs. This new method for the synthesis of radiolabeled α-^{11}C-methyl amino acids could be extended to the synthesis of many other α-^{11}C-methyl amino acids.

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Conflict of Interest

The authors did not report any conflict of interest.

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