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The use of tetrabutylammonium fluoride to promote *N*- and *O*-^{11}C-methylation reactions with iodo[^{11}C]methane in dimethyl sulfoxide

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The *N*- or *O*-methylation reactions of compounds bearing amide, aniline, or phenol moieties using iodo[^{11}C]methane (1) with the aid of a base are frequently applied to the preparation of ^{11}C-labeled radiopharmaceuticals. Although sodium hydride and alkaline metal hydroxides are commonly employed as bases in these reactions, their poor solubility properties in organic solvents and hydrolytic activities have sometimes limited their application and made the associated ^{11}C-methylation reactions difficult. In contrast to these bases, tetrabutylammonium fluoride (TBAF) is moderately basic, highly soluble in organic solvents, and weakly nucleophilic. Although it was envisaged that TBAF could be used as the preferred base for ^{11}C-methylation reactions using 1, studies concerning the use of TBAF to promote ^{11}C-methylation reactions are scarce. Herein, we have evaluated the efficiency of the ^{11}C-methylation reactions of 13 model compounds using TBAF and 1. In most cases, the *N*-^{11}C-methylations were efficiently promoted by TBAF in dimethyl sulfoxide at ambient temperature, whereas the *O*-^{11}C-methylations required heating in some cases. Comparison studies revealed that the efficiencies of the ^{11}C-methylation reactions with TBAF were comparable or sometimes greater than those conducted with sodium hydride. Based on these results, TBAF should be considered as the preferred base for ^{11}C-methylation reactions using 1.

Keywords: isotopic labeling; carbon-11; iodomethane; methylation; tetrabutylammonium fluoride; amide; aniline; phenol

Introduction

A variety of convenient methods have been developed for the synthesis of ^{11}C-labeled compounds that facilitate the routine preparation of radiopharmaceuticals for use in positron emission tomography. The attachment of a ^{11}C-methyl group to a pendant amino, hydroxy, or sulfenyl moiety represents a common strategy in the preparation of the ^{11}C-labeled compounds. Iodo[^{11}C]methane (**1**) is commonly used as a methylating agent. This material is introduced through a nucleophilic substitution reaction, and methods for the automated preparation of **1** are well established. The nucleophilic substitution reactions of **1** with the NH moieties of amides and anilines, as well as the OH groups of phenols, require the addition of a base to generate the corresponding anions, because the parent functional groups are poorly reactive towards **1**. Suspensions of sodium hydride (NaH) and alkaline metal hydroxides are typically employed as the bases for these reactions. Unfortunately, however, these materials are poorly soluble in organic solvents, and their application to the automated preparation of radiopharmaceuticals can therefore be cumbersome. These poor solubility properties can also lead to the addition of ambiguous concentrations of the bases to the precursor solution because the bases are added as suspensions, and the amount of base present in solution might not therefore satisfy the requirements of current good manufacturing practices. In addition, the hydrolysis of **1** can occur as a considerable side reaction when alkaline metal hydroxides are used as the reaction base. A base that overcomes these technical and chemical issues is therefore desired for application with synthetic methods using **1**.

Tetrabutylammonium fluoride (TBAF) possesses good solubility properties in a range of organic solvents, as well as low hydrolytic activity and moderate basicity. Adam *et al*.[1] demonstrated that TBAF could be used as a base for the preparation of some radiopharmaceuticals using **1**. The labeling efficiencies achieved when TBAF was used as a base were reported to be comparable with or even higher than those achieved by sodium hydroxide (NaOH) in the same reactions. In their report, the TBAF-mediated *N*- and *O*-^{11}C-methylation reactions using **1** were performed at temperatures in the range of 80–100 °C both in the presence and in the absence of alkaline metal hydroxides.[1] In contrast, we reported a series of TBAF-promoted α-methylation reactions using **1** where different

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structural components, such as the α-carbons of phenyl acetates as well as Schiff bases bearing alanine analogs, were activated by TBAF, even at ambient temperature.[2,3] In general, however, the methylation did not occur as an exclusive reaction, and undesirable side reactions were also observed, including the radiolysis of the ^{11}C-labeled products. Furthermore, these side reactions became more pronounced when the reactions were conducted at higher temperatures. The development of a method allowing for the ^{11}C-methylation reactions to be conducted at lower temperatures could therefore provide greater radiochemical conversions (RCCs) and yields, if the desired reactions could occur at sufficiently fast rates.

In this context, we have evaluated the TBAF-promoted *N*- and *O*-^{11}C-methylation reaction of amides, anilines, and phenols using **1** with and without heating. The efficiencies of the ^{11}C-methylation reactions with TBAF have been compared with those observed with NaH, which is typically used in both organic and radio syntheses because of its low nucleophilicity. A series of model compounds have been selected and evaluated in the current study for their ^{11}C-methylation reactions, including the established radiopharmaceuticals, 8-[4-(4-fluorophenyl)-4-oxobutyl]-3-[^{11}C]methyl-1-phenyl-1,3,8-triazaspiro[4.5]decan-4-one (3-*N*-[^{11}C]methylspiperone, **2b**), ethyl 8-fluoro-5-[^{11}C]methyl-6-oxo-5,6-dihydro-4*H*-benzo[*f*]imidazo[1,5-*a*][1,4]diazepine-3-carboxylate ([^{11}C]flumazenil, **3b**), *N*-butan-2-yl-1-(2-chlorophenyl)-*N*-[^{11}C]methylisoquinoline-3-carboxamide ([^{11}C]PK11195, **4b**), and (*S*)-3,5-dichloro-*N*-((1-ethylpyrrolidin-2-yl)methyl)-2-hydroxy-6-[^{11}C]methoxybenzamide ([^{11}C]raclopride, **9b**) (Figure 1). With regard to the reaction solvent, dimethyl sulfoxide (DMSO) was used in the current study to evaluate the efficiency of the TBAF-promoted *N*- and *O*-^{11}C-methylation reactions, because use of DMSO was necessary for the TBAF-promoted methylation reactions of Schiff bases bearing alanine analogs with **1**, and these reactions did not occur in dimethylformamide (DMF), THF, or dichloromethane.[2,3] In addition, DMSO exhibited a protective effect against the radiolysis of the [^{11}C]ibuprofen methyl ester under high levels of radioactivity.[3]

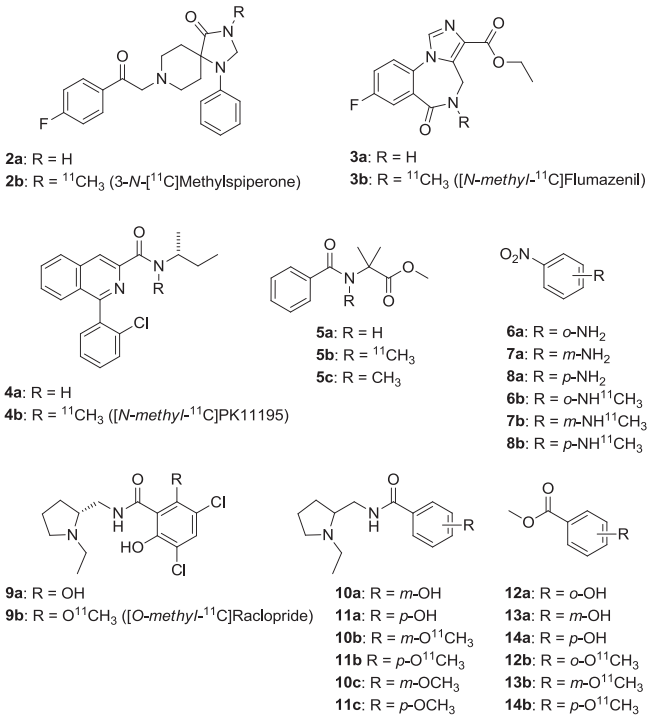

Figure 1. Chemical structures of the model compounds.

Experimental

Chemicals

Aqueous 57% hydrogen iodide (HI) solution was purchased from Wako Pure Chemical Industries Ltd. (Osaka, Japan). A 0.05-M solution of lithium aluminum hydride (LAH) in THF was prepared by diluting a 1.0-M solution of the same in material in THF, which was purchased from Sigma Aldrich (Tokyo, Japan). THF was purchased as the anhydrous grade from Wako Pure Chemical Industries Ltd. An approximately 1.0-M solution of TBAF in THF and NaH (60%, dispersion in paraffin liquid) was purchased from Tokyo Chemical Industry Co., Ltd. (Tokyo, Japan). The reaction solvents used in the current study, including DMF and DMSO, were purchased from Wako Pure Chemical Industries Ltd. as the anhydrous grades. The acetonitrile (MeCN) and ammonium acetate ($AcONH_4$) (aqueous solution) used for HPLC analysis in the current study were purchased from Wako Pure Chemical Industries Ltd. as the HPLC grade. Precursor **2a** and nonlabeled **2b** (hydrochloride salt), and **4b** and **6b–8b** were purchased from Sigma Aldrich. Precursors **3a** and **4a**, and nonlabeled **3b** and **9b** were purchased from ABX GmbH (Radeberg, Germany). The precursors **6a–8a** and **12a–14a**, and nonlabeled **12b–14b** were purchased from Wako Pure Chemical Industries Ltd. Precursor **9a** was purchased from Pharma Synth AS (Tallinn, Estonia). The benzyloxy and methoxy benzoyl chlorides, 2-aminomethyl-1-ethylpyrrolidine, 10% Pd/C, and triethylsilane used for the preparation of compounds **10a**, **11a**, **10c**, and **11c** were purchased from Sigma Aldrich, Tokyo Chemical Industry Co., Ltd., Wako Pure Chemical Industries Ltd., and Shin-Etsu Chemical Co., Ltd. (Tokyo, Japan), respectively. The benzoyl chloride, methyl 2-amino-2-methylpropanoate hydrochloride and methyl 2-methyl-2-(methylamino)propanoate used for the preparation of **5a** and **5c** were purchased from Tokyo Chemical Industry Co., Ltd., Sigma Aldrich Japan, and Enamine Ltd. (Kiev, Ukraine), respectively. All of the other chemicals used in the current study were purchased as the highest grades commercially available. All of the reagents in the current study were used without further purification.

Analysis of radiochemical conversions

Radiochemical conversions were analyzed on an HPLC system consisting of a JASCO PU-2080 pump (JASCO Corporation, Tokyo, Japan), JASCO UV-2075 UV detector, Rheodyne manual injector (IDEX Health & Science LLC, Tokyo, Japan) with a 20-μL loop), and a NaI(Tl) scintillation detector with an ACE Mate Amplifier and BIAS supply (925-SCINT, ORTEC, Oak Ridge, TN, USA) for radioactivity detection. Data acquisition and interpretation were performed with ChromNAV (ver. 1.5.2.0, JASCO Corporation). The RCC values were calculated following the correction of the radiochromatograms for decay. XBridge C18 (150 × 4.6 mm, 3.5 μm, Waters Corporation, Milford, MA, USA) and J'sphere ODS-H80 (150 × 4.6 mm, 4 μm, YMC Co. Ltd., Kyoto Japan) reversed phase analytical columns were used for the RCC analyses with a mobile phase consisting of a mixture of MeCN and $AcONH_4$ buffer (pH 4.7, 20 mM). The peaks corresponding to the desired ^{11}C-labeled compounds in the radiochromatogram were confirmed on the basis of the UV absorptions of the corresponding nonradioactive compounds, which were coinjected into the HPLC.

The retention times (t_R) of **2b** and **6b–8b** (XBridge C18 column; mobile phase of MeCN/$AcONH_4$ buffer = 30:70; flow rate of 1 mL/min) were 9.9 (**2b**), 14.7 (**6b**), 13.8 (**7b**), and 8.5 min (**8b**), respectively, whereas the t_R of iodomethane under these conditions was 7.3 min. The t_R values of **3b**, **5b**, and **9b** (J'sphere ODS-H80 column; mobile phase of MeCN/$AcONH_4$ buffer = 30:70; flow rate of 1 mL/min) were 8.3, 4.8, and 10.5 min, respectively, whereas the t_R value of iodomethane under the same conditions was 12.9 min. The t_R of **4b** (XBridge C18 column; mobile phase of MeCN/$AcONH_4$ buffer = 60:40; flow rate of 1 mL/min) was 6.4 min, whereas that of iodomethane under these conditions was 3.1 min. The t_R

values of **10b** and **11b** (J'sphere ODS-H80 column; mobile phase of MeCN/$AcONH_4$ buffer = 15:85; flow rate of 1 mL/min) were 6.7 and 6.3 min, respectively, whereas the t_R value of iodomethane with the same conditions was 12.5 min. The t_R values of **12b–14b** (J'sphere ODS-H80 column; mobile phase of MeCN/$AcONH_4$ buffer = 30:70; flow rate of 1.5 mL/min) were 7.4 (**12b**), 17.7 (**13b**), and 16.0 min (**14b**), respectively, whereas that of iodomethane under these conditions was 8.4 min. The radiochromatograms of the materials were monitored until the radioactivity in the columns could no longer be detected using a Geiger–Müller counter (TGS-146B, Hitachi Aloka Medical, Ltd., Tokyo, Japan).

Production of iodo[^{11}C]methane (1)

[^{11}C]Carbon dioxide was produced by the $^{14}N(p,\alpha)^{11}C$ nuclear reaction in an atmosphere of nitrogen gas containing 0.01% oxygen with 18-MeV protons using the CYPRIS HM-18 cyclotron (Sumitomo Heavy Industry, Tokyo, Japan). Following the bombardment process, the [^{11}C]carbon dioxide was transferred to a reaction vessel containing a 0.05-M solution of LAH in THF (500 μL) at 0 °C. The THF solvent was then evaporated, and an aqueous 57% HI solution (400 μL) was added to the vessel. The resulting mixture was then heated to 150 °C to produce **1**. Gaseous **1** was transferred by a N_2 gas stream with a flow rate of 30 mL/min and collected in DMSO or DMF (0.5–1 mL) in a glass vessel at ambient temperature. The preparation time of **1** was around 7 min following the end of the bombardment process.

General condition for ^{11}C-methylation

The DMSO was used as the reaction solvent for the ^{11}C-methylation reactions using TBAF, whereas DMF was used for the ^{11}C-methylation reactions using NaH. TBAF (1 eq, 1.0 M in THF, stored in plastic bottle) or NaH (dispersion in paraffin liquid, 5 eq, 1.0 M in DMF) was added to a solution of the precursor (1 μmol in 200 μL) 30 or 5 min prior to the addition of **1**, respectively. A DMSO or DMF solution of **1** (~111 MBq, 100 μL) was then added to the reaction mixture, and the resulting mixture was held at ambient temperature or 80 °C for 3 min. At the end of reaction, the reaction mixture was cooled on ice and quenched by the addition of 200 μL of the $AcONH_4$ buffer solution (pH 4.3, 200 mM). The resulting mixture was then analyzed by HPLC.

Synthesis of methyl 2-benzamido-2-methylpropanoate (5a, precursors for 5b) and methyl 2-methyl-2-(*N*-methylbenzamido) propanoate (5c)

A solution of benzoyl chloride (2.13 mmol) in dioxane (20 mL) was added to a stirred solution of K_2CO_3 (5.86 mmol) and methyl 2-amino-2-methylpropanoate hydrochloride (1.95 mmol) in water (15 mL), and the resulting mixture was stirred for 1 h at ambient temperature. EtOAc was then added to the reaction resulting in the formation of a biphasic mixture. The organic phase was collected and washed sequentially with saturated $NaHCO_3$ and water. The organic layer was dried over anhydrous $MgSO_4$, and the solvent was removed *in vacuo* to give a crude product as a residue, which was purified by column chromatography over silica gel (Wakogel C-200, Wako Pure Chemical Ltd.) using hexane/EtOAc (3:2, v/v) as the eluent to give **5a** (88%).

Methyl 2-benzamido-2-methylpropanoate (**5a**): ^{1}H-NMR (300 MHz, $CDCl_3$) δ (ppm): 1.69 (6H, s), 3.79 (3H, s), 6.79 (1H, broad s), 7.40–7.50 (3H, m) and 7.77–7.80 (2H, m); ^{13}C-NMR (75 MHz, $CDCl_3$) δ (ppm): 24.73, 52.78, 56.91, 126.92, 128.53, 131.54, 134.50, 166.52 and 175.30. HRMS (*m/z*) calcd for $C_{12}H_{15}NO_3$ $[M+H]^+$: 222.1130; Found: 222.1125.

Compound **5c** was synthesized according to the procedure described earlier using methyl 2-methyl-2-(methylamino)propanoate as the starting material (55% yield).

Methyl 2-methyl-2-(*N*-methylbenzamido)propanoate (**5c**): ^{1}H-NMR (300 MHz, $CDCl_3$) δ (ppm): 1.55 (6H, s), 2.97 (3H, s), 3.73 (3H, s) and 7.38–7.47 (5H, m); ^{13}C-NMR (75 MHz, $CDCl_3$) δ (ppm): 22.95, 33.44, 52.31, 60.70, 127.28, 128.35, 129.83, 136.44, 171.83 and 174.57. HRMS (*m/z*) calcd for $C_{13}H_{17}NO_3$ $[M+H]^+$: 236.1287; Found: 236.1292.

Synthesis of *N*-(1-ethylpyrrolidin-2-ylmethyl)-hydroxybenzamide (10a and 11a, precursors for 10b and 11b)

A solution of 3- or 4-benzyloxybenzoyl chloride (1.74 mmol) in dioxane (15 mL) was added to a stirred solution of K_2CO_3 (2.89 mmol) and 2-aminomethyl-1-ethylpyrrolidine (1.56 mmol) in dioxane (15 mL), and the resulting mixture was stirred for 3 h at ambient temperature. EtOAc was then added to the reaction, resulting in the formation of a biphasic solution. The organic phase was collected and washed sequentially with saturated $NaHCO_3$ and water before being dried over anhydrous $MgSO_4$. The solvent was then removed *in vacuo*, and the resulting residue was purified by column chromatography over Chromatorex NH silica gel (Fuji Silysia Chemical Ltd., Aichi, Japan) using hexane/EtOAc (2:1, v/v) as the eluent to give the desired product *N*-(1-ethylpyrrolidin-2-ylmethyl)-benzyloxybenzamide (>90%). The benzyl group was removed according to the following procedure. Triethylsilane (15 mmol) was added to a mixture of 10% Pd/C (100 mg) and *N*-(1-ethylpyrrolidin-2-ylmethyl)-benzyloxybenzamide (1.5 mmol) in ethanol (100 mL), and the resulting mixture was stirred for 3 h at ambient temperature. The reaction mixture was then filtered to remove the Pd/C, and the filtrate was concentrated under vacuum to give the crude product as a residue, which was purified by column chromatography over silica gel (Wakogel C-200, Wako Pure Chemical Ltd.) using EtOAc/2-propanol/28% aqueous NH_3 (3:1:0.5, v/v/v) as the eluent to give the desired product *N*-(1-ethylpyrrolidin-2-ylmethyl)-hydroxybenzamide (>86%).

N-(1-Ethylpyrrolidin-2-ylmethyl)-3-hydroxybenzamide (**10a**): ^{1}H-NMR (300 MHz, $CDCl_3$) δ (ppm): 1.21 (3H, t), 1.71–1.95 (4H, m), 2.27–2.42 (2H, m), 2.72–2.82 (1H, broad m), 2.98–3.05 (1H, m), 3.32–3.43 (2H, m), 3.95–4.04 (1H, m), 6.82–6.85 (1H, dd), 7.25–7.30 (1H, m), 7.45 (1H, d), 7.57 (1H, m) and 7.68 (1H, broad d); ^{13}C-NMR (75 MHz, $CDCl_3$) δ (ppm): 13.04, 22.37, 26.52, 39.25, 48.61, 53.53, 64.23, 114.33, 118.06, 119.21, 129.69, 135.91, 156.60 and 167.70. HRMS (*m/z*) calcd for $C_{14}H_{20}N_2O_2$ $[M+H]^+$: 249.1603; Found: 249.1569.

N-(1-Ethylpyrrolidin-2-ylmethyl)-4-hydroxybenzamide (**11a**): ^{1}H-NMR (300 MHz, $CDCl_3$) δ (ppm): 1.15 (3H, t), 1.66–1.81 (3H, m), 1.88–1.95 (1H, m), 2.26–2.39 (2H, m), 2.79–2.81 (1H, m), 2.86–2.95 (1H, m), 3.23–3.27 (1H, m), 3.36–3.40 (1H, m), 3.74–3.83 (1H, m), 6.84 (2H, d) 7.26 (1H, broad, overlapped with $CDCl_3$) and 7.72 (2H, d); ^{13}C-NMR (75 MHz, $CDCl_3$) δ (ppm): 13.32, 22.78, 27.55, 40.33, 48.57, 53.51, 63.43, 115.83, 125.16, 129.07, 160.61 and 168.03. HRMS (*m/z*) calcd for $C_{14}H_{20}N_2O_2$ $[M+H]^+$: 249.1603; Found: 249.1557.

Synthesis of the *N*-(1-ethylpyrrolidin-2-ylmethyl)-methoxybenzamides (10c and 11c, analogs of raclopride)

The *N*-(1-ethylpyrrolidin-2-ylmethyl)-methoxybenzamides were prepared from the corresponding 3- and 4-methoxybenzoyl chlorides using the procedure described earlier (95%).

N-(1-Ethylpyrrolidin-2-ylmethyl)-3-methoxybenzamide (**10c**): ^{1}H-NMR (300 MHz, $CDCl_3$) δ (ppm): 1.12 (3H, t), 1.63–1.77 (3H, m), 1.86–1.95 (1H, m), 2.15–2.29 (2H, m), 2.67 (1H, broad m), 2.81–2.87 (1H, m), 3.17–3.33 (2H, m), 3.66–3.74 (1H, m), 3.85 (3H, s), 6.85 (1H, broad s), 7.01–7.05 (1H, m) and 7.27–7.39 (3H, m); ^{13}C-NMR (75 MHz, $CDCl_3$) δ (ppm): 14.06, 22.93, 28.19, 40.75, 48.01, 53.55, 55.39, 62.19, 112.34, 117.37, 118.61, 129.46, 136.32, 159.74 and 167.49. HRMS (*m/z*) calcd for $C_{15}H_{22}N_2O_2$ $[M+H]^+$: 263.1760; Found: 263.1711.

N-(1-Ethylpyrrolidin-2-ylmethyl)-4-methoxybenzamide (**11c**): ^{1}H-NMR (300 MHz, $CDCl_3$) δ (ppm): 1.12 (3H, t), 1.60–1.77 (3H, m), 1.84–1.93 (1H, m), 2.14–2.30 (2H, m), 2.65 (1H, broad m), 2.78–2.89 (1H, m), 3.17–3.31 (2H, m), 3.65–3.73 (1H, m), 3.85 (3H, s), 6.77 (1H, broad s), 6.93 (2H, d) and 7.75 (2H, d); ^{13}C-NMR (75 MHz, $CDCl_3$) δ (ppm): 14.10, 22.91, 28.19, 40.67, 47.96, 53.56, 55.34, 62.21, 113.64, 127.14, 128.61, 161.94 and 167.16. HRMS (*m/z*) calcd for $C_{15}H_{22}N_2O_2$ $[M+H]^+$: 263.1760; Found: 263.1801.

Results and discussion

The effect of the timing of the TBAF addition to a solution containing **2a** was evaluated because the duration of the reaction of TBAF with the precursors prior to the addition of **1** had a significant impact on the success of the methylation reaction in THF. As shown in Table 1, the RCC was found to be independent of the timing of the TBAF addition (Table 1, entries 1–3). These results suggested that the anions generated by TBAF were relatively stable in DMSO. Adam *et al*. reported that TBAF can be added at least 20 min prior to the addition of **1**,[1] whereas it would not be necessary to consider the timing of TBAF addition to a DMSO solution containing a precursor. Furthermore, when TBAF was added to the reaction immediately after the trapping of **1** in the precursor solution, the RCCs in the reaction following 3 min at ambient temperature of **2b**, **3b**, **5b**, **8b**, and **13b** were 68 ± 4.9, 89 ± 0.34, 46 ± 2.9, 56 ± 1.4, and 74 ± 6.2%, respectively ($n = 3$ each), and were comparable with the values shown in Tables 1 (entries 1–3, 7, 15, and 27) and 2 (entry 20). These results implied that this reaction could be usefully applied to precursors that are labile to ionic fluoride. The addition of TBAF to a reaction immediately after the trapping of **1** in a precursor solution could be performed using an automatic synthesis module because of the soluble nature of TBAF to organic solvents. We believe that the amenability of TBAF to automated approaches represents a significant advantage to the current approach over the use of traditional bases, such as NaH.

Using **2b** as a model compound, we also performed a preliminary evaluation of time-dependent changes in the activity of TBAF solutions in storage towards the ^{11}C-methylation reaction. Once they had been opened, the commercially available TBAF solutions, which were bottled in plastic and glass, were stored at ambient temperature without any special treatment. These TBAF solutions provided a consistent level of labeling efficiency for the labeling of **2b** over a 1-month period (Figure 2). In the current study, we used a TBAF solution contained in a plastic bottle as a precautionary measure. This step was taken on the basis of our previous experience, where a significant reduction in the fluoride activity of a TBAF solution

Table 1. Labeling efficiency of the *N*-^{11}C-methylation reaction using **1**

Entry	Product	Base (eq)	Timing of base addition (min)[a]	Reaction temperature (°C)	RCC (%)[b]	Remained **1** (%)[c]
1	**2b**	TBAF (1)	15	rt	67 ± 2.8	nd.
2	**2b**	TBAF (1)	30	rt	63 ± 1.9	nd.
3	**2b**	TBAF (1)	60	rt	64 ± 6.6	nd.
4	**2b**	TBAF (1)	30	80	60 ± 4.9	nd.
5	**2b**	NaH (5)	5	rt	82 ± 2.1	nd.
6	**2b**	NaH (5)	5	80	55 ± 4.1	nd.
7	**3b**	TBAF (1)	30	rt	80 ± 5.7	nd.
8	**3b**	TBAF (1)	30	80	74 ± 1.9	nd.
9	**3b**	NaH (5)	5	rt	97 ± 0.62	nd.
10	**3b**	NaH (5)	5	80	96 ± 1.2	nd.
11	**4b**	TBAF (1)	30	rt	9.4 ± 0.90	nd.
12	**4b**	TBAF (1)	30	80	8.6 ± 0.70	nd.
13	**4b**	NaH (5)	5	rt	3.6 ± 0.020	nd.
14	**4b**	NaH (5)	5	80	2.6 ± 0.86	nd.
15	**5b**	TBAF (1)	30	rt	48 ± 2.9	nd.
16	**5b**	TBAF (1)	30	80	42 ± 3.9	nd.
17	**5b**	NaH (5)	5	rt	45 ± 0.77	nd.
18	**5b**	NaH (5)	5	80	48 ± 1.1	nd.
19	**6b**	TBAF (1)	30	rt	65 ± 7.2	nd.
20	**6b**	TBAF (1)	30	80	66 ± 1.6	nd.
21	**6b**	NaH (5)	5	rt	35 ± 7.9	nd.
22	**6b**	NaH (5)	5	80	29 ± 5.6	nd.
23	**7b**	TBAF (1)	30	rt	4.5 ± 1.7	nd.
24	**7b**	TBAF (1)	30	80	5.0 ± 2.4	nd.
25	**7b**	NaH (5)	5	rt	3.1 ± 2.5	nd.
26	**7b**	NaH (5)	5	80	5.7 ± 2.7	nd.
27	**8b**	TBAF (1)	30	rt	59 ± 3.1	1.3 ± 0.89
28	**8b**	TBAF (1)	30	80	63 ± 4.1	nd.
29	**8b**	NaH (5)	5	rt	27 ± 4.9	nd.
30	**8b**	NaH (5)	5	80	29 ± 7.0	nd.

rt, room temperature; nd., not detected.
[a]The time after the addition of the base to a precursor solution prior to the start of the reaction when **1** was added.
[b]The radiochemical conversion (RCC) values were determined using the radiochromatograms from the analytical HPLC following a decay correction.
[c]These reactions were conducted in three independent experiments. The values are expressed as mean ± standard deviation.

Table 2. Labeling efficiency of the *O*-methylation reaction using **1**

Entry	Product	Base (eq)	Timing of base addition (min)[a]	Reaction temperature (°C)	RCC (%)[b]	Remained **1** (%)[c]
1	**9b**	TBAF (1)	30	rt	nd.	72 ± 6.3
2	**10b**	TBAF (1)	30	rt	84 ± 4.1	nd.
3	**10b**	TBAF (1)	30	80	73 ± 12	nd.
4	**10b**	NaH (5)	5	rt	13 ± 0.51	58 ± 19
5	**10b**	NaH (5)	5	80	76 ± 0.70	nd.
6	**11b**	TBAF (1)	30	rt	78 ± 12	nd.
7	**11b**	TBAF (1)	30	80	75 ± 12	nd.
8	**11b**	NaH (5)	5	rt	8.9 ± 0.87	67 ± 11
9	**11b**	NaH (5)	5	80	77 ± 2.8	nd.
10	**12b**	TBAF (1)	30	rt	51 ± 7.7	23 ± 9.4
11	**12b**	TBAF (1)	30	80	67 ± 2.2	nd.
12	**12b**	NaH (5)	5	rt	7.1 ± 3.7	nd.
13	**12b**	NaH (5)	5	80	5.8 ± 5.7	nd.
14	**12b**	NaH (2.5) (degreased)	5	rt	23 ± 10	nd.
15	**12b**	NaH (2.5) (degreased)	5	80	40 ± 31	nd.
16	**12b**	NaH (5) (degreased)	5	rt	2.6 ± 1.1	nd.
17	**12b**	NaH (5) (degreased)	5	80	4.2 ± 6.0	nd.
18	**12b**	NaH (10) (degreased)	5	rt	1.2 ± 1.1	nd.
19	**12b**	NaH (10) (degreased)	5	80	0.56 ± 0.55	nd.
20	**13b**	TBAF (1)	30	rt	75 ± 7.1	2.8 ± 2.7
21	**13b**	TBAF (1)	30	80	74 ± 1.6	nd.
22	**13b**	NaH (5)	5	rt	93 ± 3.6	nd.
23	**13b**	NaH (5)	5	80	92 ± 1.6	nd.
24	**14b**	TBAF (1)	30	rt	38 ± 3.1	27 ± 11
25	**14b**	TBAF (1)	30	80	58 ± 4.7	nd.
26	**14b**	NaH (5)	5	rt	74 ± 0.61	12 ± 7.7
27	**14b**	NaH (5)	5	80	91 ± 2.6	nd.

rt, room temperature; nd., not detected.
[a]The time after the addition of the base to a precursor solution prior to the start of the reaction when **1** was added.
[b]The radiochemical conversion (RCC) values were determined using the radiochromatograms from the analytical HPLC following a decay correction.
[c]These reactions were conducted in three independent experiments. The values are expressed as mean ± standard deviation.

contained in a glass bottle was observed within 1 month of opening the bottle for the ^{11}C-methylation of phenylacetate analogs (data not shown). In the previous study, poorly reactive polyfluorosilicates could be formed on the silicon atoms of the glass in the presence of water, and this could lead to a reduction in the fluoride activity of the solution. In contrast, similar losses in activity have not been observed for TBAF solutions bottled in plastic, even after several months of storage.

Compounds **2b** and **3b** are five- and seven-membered cyclic amides, respectively. Compounds of this particular type are usually prepared by the methylation of the parent amides using **1** in the presence of a metal hydroxide or tetraalkylammonium hydroxide at temperatures of 65–80 °C.[4–8] Suzuki *et al.*[9,10] reported the preparation of **2b** and **3b** using NaH and **1** under relatively mild conditions (<50 °C). In our hands, the ^{11}C-labeling of **2b** and **3b** using NaH as a base was highly efficient even at ambient temperature (Table 1, entries 5 and 9). Furthermore, compounds **2a** and **3a** were successfully ^{11}C-methylated on the presence of TBAF and **1** to give the desired products **2b** and **3b**, respectively (Table 1, entries 2 and 7). When the ^{11}C-methylation reactions of **2a** and **3a** were conducted at higher temperatures, the RCC values of the products **2b** and **3b** were similar to those obtained at ambient temperature (Table 1, entries 4 and 8). These results suggested that the methylation reactions of the cyclic amides **2b** and **3b** with **1** were sufficiently fast at ambient temperature when TBAF was used as a base. [^{11}C]Methyl trifluoromethanesulfonate ([^{11}C]methyl triflate) is a more reactive methylating agent than **1**. This material can be prepared from the substitution of **1** and has been used for the synthesis of **2b** and **3b** with the aid of NaOH.[11] The results of the current

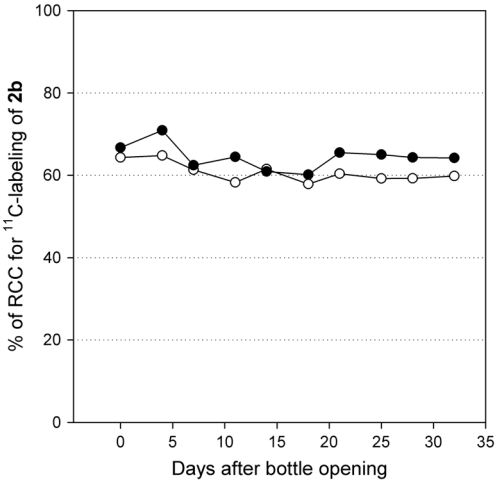

Figure 2. Time-dependent changes in the activity of the stored TBAF solution. The activity of the stored TBAF solution has been represented by the radiochemical conversion (RCC) of **2b**. Open circle, RCC with TBAF solution bottled in plastic bottle; closed circle, RCC with TBAF solution bottled in glass bottle.

study, however, suggested that it was not necessary to heat the reaction mixture or to transform **1** to [^{11}C]methyl triflate for the TBAF-promoted ^{11}C-methylation of cyclic amides.

To expand upon the scope of the current TBAF-promoted ^{11}C-methylation reaction, we proceeded to investigate the ^{11}C-methylation of acyclic amides. The treatment of **4a** with TBAF and **1** at ambient temperature led to the formation of the ^{11}C-methylated product **4b**, although the RCC of the reaction did not reach a practically useful level (Table 1, entry 11). The extent of the methylation reaction was improved by the use of a threefold increase in the amount of **4a** (3 μmol), which resulted in a moderate RCC for product **4b** (33%, $n = 2$). In these cases, **1** was completely consumed when the reaction was conducted at ambient temperature and could no longer be found in the reaction mixture. The use of a higher reaction temperature did not therefore lead to an improvement in the RCC value in the product (Table 1, entry 12). The results of the current study were comparable with those reported by Adam *et al.*[1] where the methylation reactions were performed at 100 °C. The ^{11}C-methylation of **4a** with NaH and **1** provided **4b** with a low RCC, likely because of the reaction solvent used in the current study. Although DMF was the solvent in this particular case, the ^{11}C-methylation of **4a** has also been reported using **1** and NaH in DMSO.[12] In contrast to the synthesis of **4b**, TBAF and NaH both promoted the ^{11}C-labeling of **5b** with moderate levels of efficiency (Table 1, entries 15–18). These results clearly indicated that TBAF could be applied to the ^{11}C-methylation of acyclic amides using **1**, although the efficiencies of these reactions were generally lower than those of the cyclic ones.

Anilines, especially those bearing electron-withdrawing groups, are weak nucleophiles[13–15] and require the aid of a base to be effectively methylated using **1**. Cai *et al.*[16] recently reported the ^{11}C-methylation reactions of the nitroanilines **6a–8a** at ambient temperature over a 10-min period using **1** and lithium nitride as a base under ultrasonic conditions. According to the report of Cai *et al.*, the *ortho*- and *para*-substituted nitroanilines (**6a** and **8a**) proceeded smoothly through the ^{11}C-methylation reaction in 62% and 37% yields, respectively, whereas the *meta*-substituted nitroaniline **7a** did not provide any of the desired methylated product.[16] In the current study, the ^{11}C-methylation reactions of **6a** and **8a** proceeded smoothly at ambient temperature using NaH or TBAF (Table 1, entries 19–22 and 27–30, respectively). TBAF in particular exhibited a much higher efficiency compared with the use of lithium nitride or NaH, even when it was used at ambient temperature for the ^{11}C-methylation of **8a**. In contrast, our attempts to achieve the ^{11}C-methylation of **7a** with NaH or TBAF were unsuccessful and therefore no better than the use of lithium nitride (Table 1, entries 23–26). The reason for the lower efficiency of ^{11}C-labeling of **7b** compared with those of **6b** and **8b** remains unclear, as mentioned by Cai *et al.*[16]

The use of **1** for the ^{11}C-methylation of other functional groups requiring the assistance of a base represents a significant target for the TBAF-promoted reaction. Phenolic hydroxy groups are more acidic than the NH moieties of amides and typically require the addition of base for the ^{11}C-methylation by **1**. A number of [^{11}C]methyl phenyl ethers have been observed in the structures of radiopharmaceuticals used for positron emission tomography. For this reason, the nucleophilic substitution of the phenolic hydroxy group with **1** was explored using TBAF (Table 2).

Compound **9a** is the desmethyl labeling precursor of the dopamine D2 ligand **9b** and has a phenolic hydroxy group on its aromatic ring. When the ^{11}C-methylation of **9a** was performed at ambient temperature with **1** and TBAF, the desired product **9b** was not obtained, despite the disappearance of the labeling agent **1** (Table 2, entry 1). On the basis of this result, the use of a higher reaction temperature for the same reaction was not investigated. Relevant to this, it has been reported in the literature that TBAF did not promote the ^{11}C-methylation of **9a** at 80 °C.[1] Furthermore, the use of NaH barely promoted the ^{11}C-methylation of **9a**, even at 120 °C.[17] To investigate the reason for the poor RCCs observed in these reactions, we explored the TBAF-promoted ^{11}C-methylation reactions of **10a** and **11a** that are both analogs of **9a** with the hydroxy groups at different positions. The reactions of **10a** and **11a** with TBAF and **1** at ambient temperature led to the efficient formation of **10b** and **11b**, respectively (Table 2, entries 2, 3, 6, and 7). The *meta*- and *para*-hydroxy moieties therefore represented acceptable functional groups for the TBAF-promoted ^{11}C-methylation using **1** in analogs of this type. The pK_a values of hydroxy groups usually differ by a few units, depending on the presence of other substituents on the ring, and *ortho*-substituted analogs bearing electron-withdrawing groups are usually the most acidic. TBAF would therefore be sufficiently basic to generate the phenolate anion of **9a**, although steric hindrance from the *ortho*-substituent, as well as the hydrogen bond between the phenolate anion and amide, may have led to the observed lower reactivity. In contrast to the completion of the ^{11}C-methylation reactions of **10a** and **11a** using TBAF at ambient temperature, **1** could still be detected at levels in excess of 50% of the original charge when NaH was used as the base at ambient temperature, and heating was required to drive the NaH-promoted ^{11}C-methylations of **10a** and **11a** towards completion (Table 2, entries 2–9).

The ^{11}C-methylation reactions of the hydroxybenzoate esters **12a–14a** were also explored using the current methodology. These compounds were particularly interesting because they possess a phenolic hydroxy group without a proximal hydrogen bonding donor group. In contrast to the ^{11}C-labeling reactions of the hydroxybenzamide analogs **10b** and **11b**, **1** could still be detected in the reaction mixture throughout the ^{11}C-labeling

reactions of **12b–14b** using TBAF at ambient temperature, and it was therefore necessary to heat the reaction to force the TBAF-promoted ^{11}C-methylation to completion (Table 2, entries 10, 11, 20, 21, 24, and 25). A reaction temperature of 80 °C was used in this particular case. It is noteworthy that the reaction temperature could be lowered, because the amount of **1** remaining in the reaction mixture when the reaction was conducted at ambient temperature was only small. The ^{11}C-methylation of 2-hydroxybenzoate (**12a**) gave **12b** with a good RCC when TBAF and **1** were used (Table 2, entries 10 and 11). These results indicated that the presence of the hydrogen bond led to a significant reduction in the rate of the ^{11}C-methylation of **9a**. In contrast, **13a** and **14a** both underwent the ^{11}C-methylation with high levels of efficiency at ambient temperature when NaH was used as a base (Table 2, entries 22, 23, 26, and 27). In this study, we found that ^{11}C-methylation reactions that were promoted by NaH were generally also promoted by TBAF and vice versa. Interestingly, however, the RCC of **12b** from the methylation reaction with NaH was lower than that of the same reaction performed with TBAF, even at high temperature (Table 2, entries 10–13). Further investigation of the reaction revealed that the amount of NaH used in the reaction was critical to the success of the ^{11}C-methylation of **12b** (Table 2, entries 14–19). The RCC of **12b** tended to decrease as the amount of NaH was increased. In this case, the paraffin contained in the NaH did not lead to the observed low RCC. In addition, the reproducibility of the RCC of **12b** was found to be poor when NaH was used as the base, because the concentration of NaH could not be accurately determined when it was used as a suspension.

Conclusion

Tetrabutylammonium fluoride performed as well as NaH as a base in the *N*- and *O*-^{11}C-methylation reactions of amides, anilines, and phenols using **1**. Furthermore, in some cases, TBAF exhibited higher levels of efficiency in its ^{11}C-methylation reactions compared with NaH. Based on our results, the *N*-^{11}C-methylations of different amides and anilines could be conducted at ambient temperature using TBAF as a base in DMSO with **1**, whereas the *O*-^{11}C-methylation reactions of phenols could be conducted under similar conditions with moderate heating required in some cases. Unfortunately, *meta*-substituted anilines bearing electron-withdrawing groups and 2-hydroxybenzamides were not good substrates for the current ^{11}C-methylation process. The soluble nature of TBAF in a range of organic solvents represents a significant advantage to this approach for the automated preparation of radiopharmaceuticals using microreactor systems. In addition, we found that commercially available THF solutions of TBAF could be used as supplied and stored for extended periods under ambient conditions without any special treatment after they had been opened. Based on these results, we believe that TBAF should be considered as the preferred base for ^{11}C-methylation reactions using **1** because of the practical advantages that it offers over the traditional bases.

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Conflict of Interest

The authors did not report any conflict of interest.

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