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# Synthesis of (*R*,*S*)-[4-$^{11}$C]baclofen via Michael addition of nitromethane labeled with short-lived $^{11}$C

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ABSTRACT

The synthesis of (*R*,*S*)-[4-$^{11}$C]baclofen, the first $^{11}$C-labeled $GABA_B$ agonist, was demonstrated via Michael addition of nitro[$^{11}$C]methane as a key step. A tetrabutylammonium fluoride promoted Michael addition of nitro[$^{11}$C]methane to methyl *p*-chlorocinnamate, followed by the nitro-group reduction in the presence of $NiCl_2$ and $NaBH_4$ in aqueous MeOH and alkaline hydrolysis yielded (*R*,*S*)-[4-$^{11}$C]baclofen in 36.4 ± 1.8% radiochemical conversion in three steps within 20 min.

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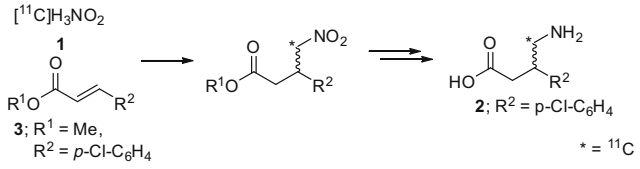
Positron emission tomography (PET) is a non-invasive in vivo imaging technology using molecules labeled with short-lived positron-emitting radionuclides such as $^{11}$C and $^{18}$F ($T_{1/2}$ = 20.3 and 109.7 min). There is an ongoing need for the development of new versatile probe molecules for a variety of applications. Its success as a tool for diagnosis and drug research and development depends on employing a molecular probe with adequate pharmacokinetics or high and specific uptake to target tissues.[1] Many factors must be considered when identifying potentially new PET tracers. Labeling position and type of isotope are important for the design of tracers. However, the number of labeling reactions and the overall time of the reaction sequence need to be limited because of the short half-life of the radionuclide.[2] Therefore, labeling methodologies and synthetic methods become vital to the success of the probe.

We focused on the $^{11}$C-labeling synthesis of baclofen by the Michael addition reaction of nitro[$^{11}$C]methane (**1**) that leads to the construction of the γ-amino acid framework as a key step (Scheme 1, $R^2$ = *p*-Cl–$C_6H_4$). Baclofen is a γ-amino butyric acid (GABA) analog and is considered to be the only agonist of the $GABA_B$ receptors. GABA is the major inhibitory transmitter in the central nervous system and exerts its effect through three different receptor subtypes: $GABA_A$, $GABA_B$, and $GABA_C$.[3] $GABA_A$ and $GABA_C$ are ligand-gated ion channels permeable to anions and they convey fast synaptic transmissions. $GABA_B$ is a G-protein coupled receptor; it is involved in the presynaptic inhibition of transmitter release and mediates the slow synaptic inhibition. GABA receptors are important targets of brain PET studies; however, almost all PET studies of GABA receptors are focused on $GABA_A$.[4] There has been only one report about the $^{11}$C-labeling synthesis of CGP62349, a $GABA_B$ antagonist; however, significant results were not obtained because of the negligible uptake into the brain.[5] The importance of $GABA_B$ PET for understanding seizures and epilepsy is suggested[6] and recently the tumor suppressive effect of a $GABA_B$ agonist for cancers in peripheral organs has been reported.[7] Therefore, the $^{11}$C-labeling synthesis of baclofen should prove useful for PET studies of the $GABA_B$ receptor.[8] Moreover, development of the Michael addition reaction of **1** can assist in the $^{11}$C-labeling synthesis of GABA derivatives beyond baclofen, because it yields a common framework for those compounds.

As outlined in Scheme 1, we planned the Michael addition mediated $^{11}$C-labeling synthesis of (*R*,*S*)-[4-$^{11}$C]baclofen (**2**) by reaction of **1** to methyl *p*-chlorocinnamate (**3**) followed by nitro-group reduction and ester hydrolysis. Although Michael addition of nitroalkanes is one of the most important synthetic processes devoted to C–C bond formation and many reports have been published on the topic, there are no applications using **1**.[9] Requirements of rapid and appropriate conditions for a Michael addition reaction




**Scheme 1.** Synthetic plan for $^{11}$C-labeled γ-amino acids via Michael addition.

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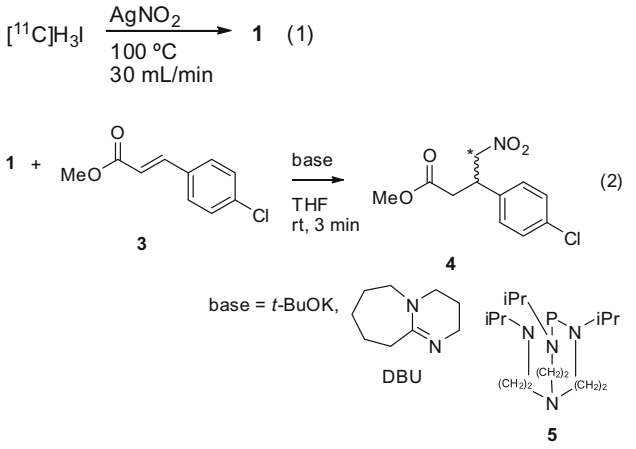
followed by a step such as nitro-group reduction hamper the development of $^{11}$C-labeling synthesis. In this context, the Michael addition of **1** to **3** was of interest to us.




The $^{11}$C-labeling agent **1** can be prepared by nitration of iodo[$^{11}$C]methane (Eq. 1),[10] a routinely produced $^{11}$C-labeling agent, and used for $^{11}$C-labeling reactions by us and other groups.[11,12] We carried out initial studies of the Michael addition reaction yielding **4** in THF at room temperature in 3 min (Eq. 2). The $^{11}$C-labeling agent **1** was prepared from iodo[$^{11}$C]methane via on-line synthesis by heating a plug of $AgNO_2$.[10b] Our first attempts of the reaction **1** and **3** using *t*-BuOK or DBU yielded no desired product **4**.[13] Under non-labeling conditions, the reaction can be carried out with an excess of nitroalkanes and a longer reaction time to compensate for the slow reaction. In contrast, the $^{11}$C-labeling reaction should be carried out under a sub-micromole scale to realize the high specific activity and be terminated within a few minutes due to competing decay of both **1** and **4**. These big differences between labeling and non-labeling reaction conditions have been encountered frequently in other $^{11}$C-labeling syntheses. Moreover, the proazaphosphatranes, $P(RNCH_2CH_2)_3N$, developed by Verkade, promote the Michael addition reaction efficiently using a one-to-one ratio of nitroalkanes to α,β-unsaturated compounds under non-labeling conditions.[14] However, under $^{11}$C-labeling conditions, the desired **4** was not obtained by proazaphosphatrane **5** (R = *i*-Pr), although the $^{11}$C-labeling agent **1** was converted completely by **5**. Side reactions arising from the presence of a large excess of **3** might complicate the reaction under the $^{11}$C-labeling conditions.

Ionic fluoride can act as the base because of strong H–F bond energy, and the fact that its basicity against carbon acids like nitroalkanes is comparable to that of alkyl metals.[15] Therefore, we investigated the fluoride-assisted Michael addition reaction of **1** to **3** and the results are summarized in Table 1. Potassium fluoride is usually used as the base and alcohols are chosen as solvents for increased solubility.[16] However, treatment of **1** to **3** with KF did not afford **4** in *i*-PrOH under $^{11}$C-labeling conditions. Further attempts at the reaction with soluble fluoride led us to use TBAF in THF, and it was found that TBAF promotes the Michael addition reaction of **1** to **3** efficiently in THF in 3 min.[17] Thus, treatment of **1** and **3** with 5 μmol of TBAF in THF yielded the desired adduct **4** in 67.9 ± 1.2% radiochemical conversion.[18] When the TBAF was increased up to 20 μmol, conversion similarly increased to 77.1 ± 1.1%. Although a higher reaction temperature is frequently introduced for $^{11}$C-labeling syntheses to increase radiochemical conversion, when the reaction was carried out at 60 °C a decreased percent conversion was obtained (entry 4). Thus, we decided to use the reaction conditions shown in entry 3 for the synthesis of **2**.

**Table 1**
Fluoride-assisted Michael addition reaction of **1**–**3**[a,b]

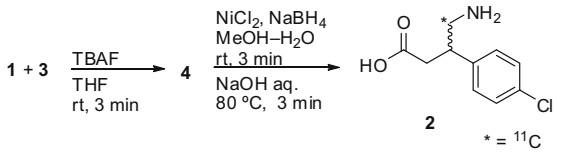
| Entry | Base (μmol) | Solvent | Temp | Radiochemical conversion of 4[c] (%) |
|---|---|---|---|---|
| 1 | KF (10) | *i*-PrOH | rt | 0 |
| 2 | TBAF (5) | THF | rt | 67.9 ± 1.2 |
| 3 | TBAF (20) | THF | rt | 77.1 ± 1.1 |
| 4 | TBAF (20) | THF | 60 °C | <47[d] |

[a] Reaction conditions: **1** (37–370 MBq); **3** (20 μmol); THF or *i*-PrOH (300 μL); reaction time (3 min).
[b] Each reaction was carried out more than three times.
[c] Determined by radiochromatogram of analytical HPLC after decay correction.
[d] Roughly determined because of noise and peak arising from side reactions.

It is critical that practical methods are developed and followed for all steps in multi-step $^{11}$C-labeling syntheses. In this context, we chose reduction by $Ni_2B$–$NaBH_4$ and ester hydrolysis by aqueous alkaline solution in a one-pot system. The preparation of $Ni_2B$–$NaBH_4$ by the treatment of $NiCl_2$ and $NaBH_4$ in situ in MeOH is simple enough for the synthesis of radio-labeled compounds.[19] Moreover, the agent $Ni_2B$–$NaBH_4$ does not seem to be affected by the contamination of THF. Therefore, we carried out the reduction reaction of **4** without any purification of the Michael addition mixture. However, we encountered a problem that might have arisen from a competing 1,4-reduction of **3**. Thus, a solution of $NiCl_2$ in MeOH was added to the mixture of the nitroaldol reaction and then the resulting solution was transferred to the next reaction vessel containing $NaBH_4$. After alkaline hydrolysis, however, the procedure did not yield **2**. In contrast, the desired **2** was obtained in about 15% conversion in three steps by adding MeOH to the reaction mixture of the Michael addition and then placing $NiCl_2$ and $NaBH_4$ in the next reaction vessel. In the first case, low or no yield of **2** resulted because of the incomplete nitro-group reduction of **4** as the competing 1,4-reduction of **3** and rapid decomposition of $NaBH_4$ forming $Ni_2B$ in MeOH retarded formation of the corresponding amine.[19b] In the second case, a measurable yield of **2** was obtained because of the gradual formation of $Ni_2B$ and the remaining $NaBH_4$. Further addition of $NaBH_4$ can assist amine formation under non-labeling conditions; however, because of issues related to protection from radiation, this cannot be done. Several attempts at a quick separation using a solid phase extraction cartridge were tried, but **3** and **4** were not separable.

The constraints of short-lived and radioactive $^{11}$C prompted us to investigate a milder condition for the decomposition of $NaBH_4$ to increase amine formation. Since decomposition of $NaBH_4$ is slower in $H_2O$ than in MeOH, we used aqueous MeOH instead of anhydrous MeOH. Thus, a 6:4 mixture of MeOH and $H_2O$ was added to the nitroaldol reaction mixture and the resulting solution was transferred to the next reaction vessel. After alkaline hydrolysis, this process yielded the desired **2** in 36.4 ± 1.8% radiochemical conversion in three steps (Scheme 2, Table 2).[20]

Thus, we developed an efficient method for the Michael addition reaction of **1** to **3** promoted by TBAF under $^{11}$C-labeling conditions. We also developed a practical method for the synthesis of **2** based on the Michael addition reaction. Currently, automated systems for the synthesis of **2** are under construction. Although the




**Scheme 2.** Synthesis of **2**.

**Table 2**
Summary of reaction conditions for the reduction of **4** and successive hydrolysis[a]

| Entry | Reagent 1[b] | Reagent 2[c] | Reagent 3[d] | Radio-chemical conversion of **2** (three steps)[e] (%) |
|---|---|---|---|---|
| 1 | $NiCl_2$ (10 μmol), MeOH (500 μL) | $NaBH_4$ (0.5 mmol) | 5 N NaOH (300 μL) | 0 |
| 2 | MeOH (500 μL) | $NiCl_2$ (10 μmol), $NaBH_4$ (0.5 mmol) | 5 N NaOH (300 μL) | <15 |
| 3 | MeOH/$H_2O$ (6/4, 500 μL) | $NiCl_2$ (10 μmol), $NaBH_4$ (0.5 mmol) | 5 N NaOH (300 μL) | 36.4 ± 1.8 |

[a] Other conditions: for reduction, at room temperature and in 3 min; for hydrolysis, at 80 °C and in 3 min.
[b] Reagents added to the mixture of Michael addition reaction.
[c] Reagents containing the second reaction vessel.
[d] Reagent added to the mixture of reduction reaction.
[e] Determined by radiochromatogram of analytical HPLC after decay correction.

synthetic method described here yields **2** as a racemic form, and a chiral center is sometimes important for the PET image analysis, $^{11}$C-labeled **2** is the first $GABA_B$ agonist labeled with a positron-emitting radionuclide. Moreover, the methods described in this Letter will allow syntheses of $^{11}$C-labeled γ-amino acids and hence promote the development of new PET tracers.

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20. Gaseous **1** was collected by a reaction vessel containing **3** (20 μmol) in THF (300 μL) with 30 mL/min flow rate at rt. To the mixture, a 1.0 M TBAF solution in THF (20 μmol) was added and stored at rt. After 3 min, a 60% MeOH aqueous solution (500 μL) was added and resulting mixture was transferred to the next reaction vessel containing $NiCl_2$ hexahydrate (2.4 mg) and $NaBH_4$ (19 mg) at rt. After 3 min, a 5 N NaOH aqueous solution (300 μL) was added and the resulting mixture was warmed to 80 °C. After 3 min at 80 °C, an aqueous buffer solution was added (500 μL) and the contents of the resulting mixture were analyzed by HPLC. HPLC conditions for **2**: column J'sphere ODS-H80 (4.6 × 150 mm, 4 μm, YMC Co. Ltd), flow rate 0.7 mL/min, eluent 30/70 (MeOH/50 mM phosphoric acid) detector NaI scintillation, retention time 5.1 min. The peak of radioactivity of **2** corresponded with the UV absorption (254 nm) of non-labeled baclofen purchased from Sigma–Aldrich Co. Ltd.