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Rapid in situ synthesis of [^{11}C]methyl azide and its application in ^{11}C click-chemistry

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ABSTRACT

We synthesized [^{11}C]methyl azide ([^{11}C]MeA) by reacting [^{11}C]methyl iodide ([^{11}C]MeI) in situ with an azide-donor and used it in the synthesis of ^{11}C-labeled 1,2,3-triazoles. A one-pot click approach comprised the infusion of gaseous [^{11}C]MeI into a mixture of NaN_3, ethynylbenzene, and CuI in water at a temperature of 100 °C yielding the ^{11}C-triazole in radiochemical yields (RCY) of 25%. In a two-step labeling protocol, we synthesized the [^{11}C]MeA in acetonitrile in advance to the click step. Using the more soluble Na^+/18-crown-6/N_3^- complex as source of N_3^-, a much higher trapping efficiency of [^{11}C]MeI in this solvent ensured an almost quantitative conversion of [^{11}C]MeI to [^{11}C]MeA within 5–10 min at room temperature. The [^{11}C]MeA was thereafter reacted with ethynylbenzene at 100 °C yielding 1-[^{11}C]methyl-4-phenyl-1*H*-1,2,3-triazole in preparative RCY of 60%. As a final proof of applicability, we used ^{11}C-click-chemistry for the labeling of N-terminal 4-ethynylbenzene derivatized D-Glu–D-Tyr–[Cys–Tyr–Trp–Lys–Thr–Cys]–Thr, a cyclic water-soluble Tyr^3-octreotate derivative.

1. Introduction

It has recently been demonstrated that click-chemistry has great potential for the preparation of ^{18}F-PET radiopharmaceuticals and might become a valuable tool in labeling chemistry.[1–3] The term 'click-chemistry' was coined by Sharpless and denotes chemical reactions characterized by high selectivity providing high yields of products from easily accessible building blocks.[4] The most commonly used click-reaction is the Cu(I)-catalyzed Huisgen reaction, a 1,3-dipolar cyclo-addition of terminal alkynes with azides, yielding 1,4 disubstituted 1,2,3-triazoles under mild conditions. This reaction owns its usefulness to the relative ease with which both necessary functional moieties, azide and alkyne, can be introduced into various molecules.[5] Both groups are relatively stable to the majority of common reaction conditions in organic synthesis so that they can be introduced into target molecules whenever convenient.[6] The discovery that Cu(I) catalysis leads to 1,4-regioisomers only while drastically enhancing reaction rates has led to its application in ^{18}F-radiochemistry.[7] In the case of ^{18}F-click-chemistry either the azide or the alkyne was ^{18}F-labeled and used for the triazole formation. To our best knowledge click-chemistry has so far not been applied to the synthesis of carbon-11 labeled compounds. In comparison to carbon-11 with a very short half-life (^{11}C, $t_{1/2}$ = 20 min), fluorine-18 has a far more convenient physical half-life of 110 min rendering synthesis times of up to 2 h possible. Such a long synthesis time is not applicable to the preparation of ^{11}C-radiopharmaceuticals and it is important to find easy protocols for labeling. Another important difference between these two isotopes is the available specific activity (SA, in MBq/μmol) which defines the degree of dilution by non-radioactive fluorine-19 or carbon-12 of the radioactive isotope. The SA of a radio-isotope is a function of time, demanding rapid and efficient methods for its introduction into bio-molecules. This is especially true for the synthesis of ^{11}C-labeled compounds, where the SA is decreasing rapidly during synthesis. The most frequently applied labeling precursor for the labeling of small compounds is [^{11}C]MeI,[8–10] which can be routinely prepared in fully automated synthesis modules. This precursor cannot be used for the labeling of multifunctional compounds such as peptides as a result of its low selectivity regarding different nucleophiles. In this Letter, we report the fast reaction of the click-reactant [^{11}C]methyl azide ([^{11}C]MeA) with a simple model compound ethynylbenzene (**1**) yielding 1-[^{11}C]methyl-4-phenyl-1*H*-1,2,3-triazole (**2**) using two different approaches (one-step and a two-step approach, Scheme 1) and the transferal of the latter method to the ^{11}C-labeling of a peptide as proof of feasibility.

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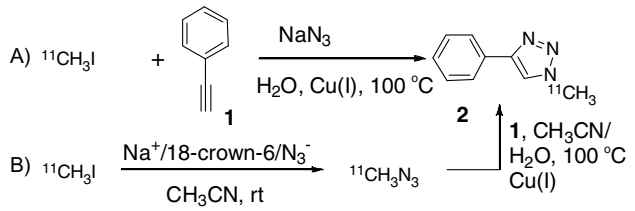


Scheme 1. (A) One-pot ^{11}C-labeling of alkynylbenzene (1, 10 mg, 0.09 mmol) via in situ generation of [^{11}C]MeA from [^{11}C]MeI in H_2O (1 mL) at 100 °C within 10 min under CuI (10 mg, 0.052 mmol) catalysis; (B) conversion of [^{11}C]MeI to [^{11}C]MeA in CH_3CN (0.5 mL) using Na^+/18-crown-6/N_3^- (10–15 mg, 30.4–45.6 mmol) at rt for 5–10 min. An aliquot of [^{11}C]MeA in acetonitrile (0.3–0.5 mL) was subsequently reacted with 1 (10 mg, 0.09 mmol) in water (0.5 mL) at 100 °C for 10 min.

2. Results and discussion

One original requirement for click-chemistry is the use of non-toxic solvents such as water and we therefore started our investigation in this solvent. The one-pot labeling approach in water is based on the in situ conversion of routinely available [^{11}C]MeI to [^{11}C]MeA which has been proposed by Sreedhar and Surendra.[11] By simply bubbling [^{11}C]MeI[12] using a sweep flow of N_2 (10 mL/min) into a mixture consisting of NaN_3, CuI, and **1** in water at room temperature and subsequently heating to 100 °C, **2** was formed to 55–60% according to the radio-HPLC chromatogram (Scheme 1, Fig. 1). At temperatures below 100 °C the efficiency of triazole formation was constantly decreasing. The non-radioactive standard compound **2** as a reference compound for HPLC was synthesized as described.[11] Although the one-pot reaction yielding **2** proceeded with acceptable radiochemical yields, (based on HPLC, Fig. 1) the initial amount of trapped [^{11}C]MeI in water was unreliable and varied between 60% and 75%. This loss in ^{11}C-radioactivity decreased preparative yields considerably and additional radioactivity was also lost during the reaction possibly in form of volatile [^{11}C]MeI and [^{11}C]MeA accumulating in the small gas phase of the reaction vessel (15–20% loss). The overall RCY of **2** after solid phase C18 cartridge purification was 25% (decay corrected) after 12–14 min.[13] In the absence of **1** and CuI, the reaction mixture showed a new radioactive peak (HPLC) at 15 min, which we attributed to the [^{11}C]MeA. It has been described in the literature that various ligands such as L-proline and more complex ligands such as tris((1*H*-benzoimidazol-2-yl)methyl)amine can accelerate the cycloaddition considerably.[14] However, in radiochemistry the ultimate aim is the production of a radiotracer suitable for iv injection into humans, so we decided not to add any compounds which would result in a longer, more complex work-up procedure. In order to overcome the insufficient trapping yield of [^{11}C]MeI in water, to reduce the loss of radioactivity at higher temperatures and to get a higher conversion of [^{11}C]MeI to [^{11}C]MeA, we bubbled the [^{11}C]MeI into a solution of Na^+/18-crown-6/N_3^-[15] in CH_3CN using a sweep flow of nitrogen (10 mL/min). Trapping yields were 85–90%. According to the HPLC chromatogram (not shown), the conversion of [^{11}C]MeI into [^{11}C]MeA was 97% after 5–10 min at room temperature. To remove Na^+/18-crown-6/N_3^- which might negatively affect the triazole formation and could become a toxic contaminant in possible in vivo studies, the solution was passed through a silica-gel cartridge (Waters) to yield [^{11}C]MeA in a preparative yield of 70% (decay corrected) after 12 min. An aliquot of [^{11}C]MeA in acetonitrile was taken and transferred to a solution of **1** in water and heated to 100 °C for 10 min (Scheme 1). The RCYs of the triazole formation were 85–90% according to the radio-HPLC chromatogram (not shown) and preparative yields were determined to be 60% (decay corrected) after solid phase extraction.

To prove feasibility of this chemistry to the labeling of more complex molecules such as peptides which are used in nuclear medicine as imaging agents, we synthesized cyclic D-Glu–D-Tyr–[Cys–Tyr–Trp–Lys–Thr–Cys]–Thr (a water-soluble derivative of Tyr^3-octreotate,[16,17]TATE) used as a tumor imaging agent. The peptide was further derivatized at its N-terminus with aminooxy-acetic acid (AOAA) (compound **3**, Scheme 2) for the chemo selective coupling of 4-ethynylbenzaldehyde via oxime formation yielding compound **4** as a labeling precursor for the reaction with [^{11}C]MeA. This method of peptide derivatization has already proven to be useful in the synthesis of peptidic labeling precursors for ^{18}F-labeling.[18–20] The synthesis of the non-radioactive peptide **5** as a standard compound for HPLC (Scheme 2) was achieved by reacting **3** with 4-(1-methyl-1*H*-1,2,3-triazol-4-yl)benzalde-

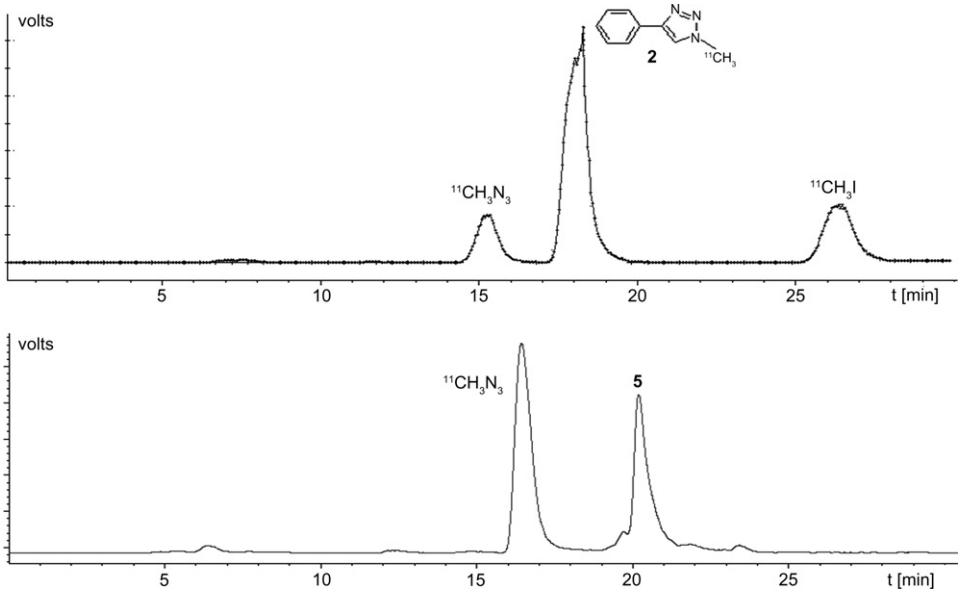
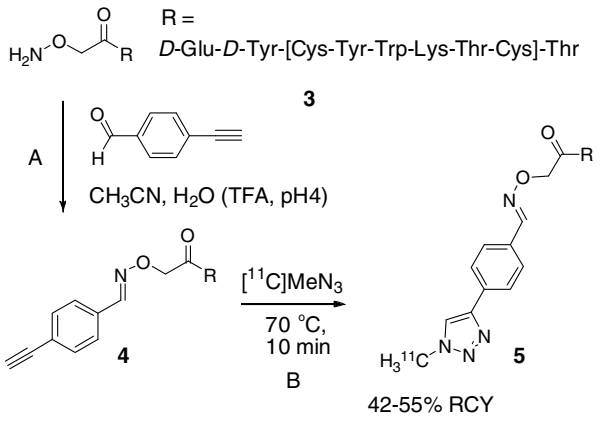


Figure 1. Top: radio-HPLC chromatogram of the one-pot synthesis of **2** [1:10 mg (0.09 mmol); NaN_3: 20–40 mg (0.3–0.6 mmol); CuI: 10 mg (0.052 mmol), water: 1 ml; t = 10 min, T = 100 °C]. Bottom: radio-HPLC chromatogram of the synthesis of 5 [4: 0.3 mg (0.22 μmol); CuI: 2.5 mg (0.013 mmol); Na-ascorbate: 25 mg (0.13 mmol); water (350 μL)/DMF (100 μL)/[^{11}C]MeA in CH_3CN (100 μL); t = 10 min; T = 70 °C].



Scheme 2. (A) Synthesis of **4** by reacting **3** with ethynylbenzaldehyd in acetonitrile/water (TFA, pH 4); (B) radio-synthesis of **5** [(**4** (0.3 mg, 0.22 μmol), [^{11}C]MeA in CH_3CN (50–100 μL), H_2O (350 μL), DMF (100 μL), DIEA (25 μL), CuI (2.5 mg, 0.013 mmol), Na-ascorbate (25 mg, 0.13 mmol), 70 °C, 10 min].

hyde[11,21] as described for **4**. Structural integrity for all non-radioactively synthesized peptides was proven by mass spectroscopy and purity was determined by HPLC.[22] It is noteworthy that unlike for the synthesis of ^{18}F-labeled peptides,[23] a simple labeling procedure with ^{11}C is not available. Henriksen et al. reported the use of 4-[^{11}C]methoxy-benzaldehyde which was reacted with a carbohydrate analogue of TATE in a reaction time of 1 h yielding the labeled peptide in an overall decay corrected yield of 21% (2.6% non-decay corrected after 3 half-lifes).[24] Two HPLC-purification steps were needed. It could be demonstrated by the investigators that their compound, despite the short half life of ^{11}C, shows suitable in vivo kinetics for PET imaging of somatostatin-receptor expressing tumors.

In a one-pot labeling approach, peptide **4**, NaN_3 and Cu(I)I were mixed in water and [^{11}C]MeI was bubbled through the reaction mixture as described for the synthesis of **2**.[25] The RCY for the formation of **5** was 5% (determined by HPLC, cf. Table 1, entry 1). Neither lower nor higher reaction temperatures had a positive effect on the RCYs. Using [^{11}C]MeA in acetonitrile, we applied the optimized conditions described by Marik and Sutcliffe[1] as well as Glaser and Årstad[2] for the formation of ^{18}F-labeled triazoles, but neither of those procedures yielded **5** in RCY of more than 10% (Table 1, entries 2 and 3). During the optimization of the two-step approach, we found that it is crucial to maintain a high water content while avoiding too much DMF (although in complete absence of DMF, the yields decreased considerably). Using a minimum amount of **4** (0.3 mg, 0.22 μmol) 42–55% RCY of **5** were obtained under optimized conditions after 10 min at 70 °C (Fig. 1, Table 1, entry 4).[26] It is noteworthy that if higher amounts of **4** (0.6–1 mg, 0.44–0.73 μmol) were used, the RCY of **5** increased considerably up to 85% under the same reaction conditions. As most peptides used in nuclear medicine are relatively costly we aimed at keeping the precursor amount as low as possible to make this approach commercially attractive. For final purification, the crude reaction mixture was diluted with water (0.5 mL) and passed through a C18 cartridge to trap the labeled peptide. The unreacted [^{11}C]MeA showed almost no retention (<5%). After elution with ethanol (1 mL), the overall preparative, decay corrected yield after a synthesis time of 30 min was determined to be 32–35% (11.3–12.4% non-decay corrected after 1.5 half-lifes). The radiochemical purity was 95% according to HPLC and specific activity was >25 GBq/μmol (UV-calibration curve).

3. Conclusions

In summary, we described the high yield in situ synthesis of [^{11}C]MeA and its applicability in the formation of ^{11}C-labeled triazoles. We successfully transferred the labeling of the simple model compound **2** by a two-step labeling approach to the preparation of a ^{11}C-labeled TATE derivative potentially useful in nuclear medicine. The reaction of [^{11}C]MeA with our alkyne-derivatized TATE demonstrates that reaction conditions for the click step are different from those which were already described in ^{18}F-click-chemistry.[1,2] Triazole formation in this particular case with [^{11}C]MeA required higher temperatures pointing at a lower reactivity of [^{11}C]MeA. Regardless of this, we demonstrated a simple and fast conversion of [^{11}C]MeI to [^{11}C]MeA and showed that click-chemistry can be applied to the synthesis of short-lived ^{11}C-compounds avoiding cumbersome workup procedures such as multiple HPLC purifications.

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References and notes

1. Marik, J.; Sutcliffe, J. L. *Tetrahedron Lett.* **2006**, *47*, 6681–6684.
2. Glaser, M.; Årstad, E. *Bioconjugate Chem.* **2007**, *18*, 989–993.
3. Li, Z.-B.; Wu, Z.; Chen, K.; Chin, F. T.; Chen, X. *Bioconjugate Chem.* **2007**, *18*, 1987–1994.
4. Kolb, H. C.; Finn, M. G.; Sharpless, K. B. *Angew. Chem., Int. Ed.* **2001**, *40*, 2004–2021.
5. Bock, V.; Hiemstra, H.; von Maarseveen, J. H. Eur. *J. Org. Chem.* **2006**, 51–68.
6. Gothelf, K. V.; Jorgensen, K. A. *Chem. Rev.* **1998**, *98*, 863–909.
7. (a) Torne, C. W.; Christensen, C.; Meldal, M. *J. Org. Chem.* **2002**, *67*, 3057–3064; (b) Rostovtsev, V. V.; Green, L. G.; Fokin, V. V.; Sharpless, K. B. *Angew. Chem., Int. Ed.* **2002**, *41*, 2596–2599.
8. Långström, B.; Lundquist, H. *Int. J. Appl. Radiat. Isot.* **1976**, *27*, 357–363.
9. Larsen, P.; Ulin, J.; Dahlström, K.; Jensen, M. *Int. J. Appl. Radiat. Isot.* **1997**, *48*, 153–157.
10. For a review of ^{11}C-chemistry: Antoni, G.; Kihlberg, T.; Långström, B. Aspects on the synthesis of ^{11}C-labelled compounds. In Welch, M., Redvanly, C. S., Eds.; Handbook of Radiopharmaceuticals, Chemistry and Applications; Wiley: Chichester, 2003; pp 141–193.
11. Sreedhar, B.; Surendra, P. *Synth. Commun.* **2007**, *37*, 805–812; Compound **2** as a standard compound for HPLC and reactant for the synthesis of non-radioactive peptide **4** was synthesized according to this reference.
12. [^{11}C]MeI was synthesized using a commercially available synthesis unit MEI plus by Bioscan. [^{11}C]CO_2 (produced by the ^{14}N(p,α)^{11}C nuclear reaction using a cyclotron) is converted into [^{11}C]methanolate with $LiAlH_4$ in anhydrous THF and further treated with HI to yield [^{11}C]MeI.

Table 1
Synthesis of peptide **5** using different conditions

Entry	Catalytic system	Solvent/[^{11}C]MeA introduction/temperature (°C)	Time (min)	Yield[c] (%)
1	CuI	Water, NaN_3, [^{11}C]MeI was bubbled into solution, 100 °C	10	5
2	Cu^{II}/ascorbate	Water/acetonitrile, [^{11}C]MeA in acetonitrile, rt[a]	10	<5
3	CuI/ascorbate	Water/DMF/[^{11}C]MeA in acetonitrile/DIEA, rt[b]	10	5–10
4	CuI/ascorbate	Water/DMF/[^{11}C]MeA in acetonitrile/DIEA, 70 °C	10	42–55

[a] Conditions from Glaser and Årstad.[2]
[b] Conditions from Marik and Sutciffe.[1]
[c] Yields are based on HPLC.

13. The reaction mixture was diluted with water (3 mL) and passed over a small C18 Sep-Pak cartridge (Waters), washed with water (2 mL) and eluted with CH_3CN (1 mL) to give compound **2** in a purity of 98.8% (as determined by HPLC). HPLC condition: HPLC column: Whatman Partisil 10 ODS2 9.5 × 250 mm; eluent: CH_3CN/0.01 M H_3PO_4 40/60; flow: 2 mL/min.
14. (a) Feldman, A. K.; Colasson, B.; Fokin, V. V. *Org. Lett.* **2004**, *6*, 3897–3899; (b) Liu, F.; Ma, D. *J. Org. Chem.* **2007**, *72*, 4844–4850; (c) Rodionov, V. O.; Presolski, S. I.; Diaz Diaz, D.; Fokin, V. V.; Finn, M. G. *J. Am. Chem. Soc.* **2007**, *129*, 12705–12712.
15. Na^+/18-crown-6/N_3^- was prepared by freeze-drying an equimolar solution made from NaN_3 and 18-crown-6 in water.
16. Wängler, B.; Beck, C.; Wagner-Utermann, U.; Schirrmacher, E.; Bauer, C.; Rosch, F.; Schirrmacher, R.; Eisenhut, M. *Tetrahedron Lett.* **2006**, *47*, 5985–5988.
17. Graham, K. A. N.; Wang, Q.; Eisenhut, M.; Haberkorn, U.; Mier, W. *Tetrahedron Lett.* **2002**, *43*, 5021–5024.
18. Thumshirn, G.; Hersel, U.; Goodmann, H.; Kessler, H. *Chem. Eur. J.* **2003**, *9*, 2717.
19. Poethko, T.; Schottelius, M.; Thumshirn, G.; Hersel, U.; Herz, M.; Henriksen, G.; Kessler, H.; Schwaiger, M.; Wester, H. J. *J. Nucl. Med.* **2004**, *45*, 892–902.
20. Schirrmacher, R.; Bradtmöller, G.; Schirrmacher, E.; Thews, O.; Tillmanns, J.; Siessmeier, T.; Buchholz, H. G.; Bartenstein, P.; Wängler, B.; Niemeyer, C. M.; Jurkschat, K. *Angew. Chem., Int. Ed.* **2006**, *45*, 6047–6051.
21. ^1H NMR (600 MHz, $CDCl_3$) δ 10 (s, 1H), 7.9–7.99 (m, 4H), 7.85 (s, 1H), 4.16 (s, 1H); ^{13}C (600 MHz, $CDCl_3$) δ 37.07, 122.05, 126.18, 130.56, 133.27, 135.95, 136.54, 191.87; MS (CI) *m*/*z* 188.13 (M+1).
22. **3, 4** (peptidic part + AOAA) and non-radioactive **5** (peptidic part + AOAA) were synthesized by Fmoc solid-phase peptide synthesis. Purification of all peptides was accomplished using the following conditions: Lichrosphere RPselectB C18 100 × 4.6 (Merck); gradient eluent: 100% H_2O + 0.1% TFA after 30 min 100% CH_3CN + 0.1% TFA; flow: 2 mL/min. Labeling precursor **4** and non-radioactive peptide **5** were synthesized coupling either ethynylbenzaldehyde or non-radioactive **2** to the N-terminal aminooxy moiety. MALDI-TOF molecular weight determination was carried out using a BRUKER DALTONICS/microflexLT apparatus. **3** *m*/*z* = 1266.3 $[M]^+$ (calcd 1266.5); **4** *m*/*z* = 1378.3 $[M]^+$ (calcd 1378.5); compound, **5** *m*/*z* = 1435.6 $[M]^+$ (calcd 1435.5).
23. for review Schirrmacher, R.; Wängler, C.; Schirrmacher, E. *Mini-Rev. Org. Chem.* **2007**, *4*, 317–329.
24. Henriksen, G.; Schottelius, M.; Poethko, T.; Hauser, A.; Wolf, I.; Schwaiger, M.; Wester, H.-J. *Eur. J. Nucl. Med.* **2004**, *31*, 1653–1657.
25. Peptide **4** (0.3 mg, 0.22 μmol), NaN_3 (40 mg, 0.61 mmol), Cu(I)I (10 mg, 0.052 mmol).
26. If 0.3 mg (0.22 μmol) of **4** were used, the optimized parameters were found to be as follows: water (350 μL)/DMF (100 μL)/[^{11}C]MeA in CH_3CN (100 μL), CuI (2.5 mg, 0.013 mmol), Na-ascorbate (25 mg, 0.13 mmol).