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A new convenient method for the synthesis of [2-^{11}C]thymine utilizing [^{11}C]phosgene

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Abstract—β-(*N*-Benzoylamino)methacrylamide, a key intermediate for the preparation of [2-^{11}C]thymine, was synthesized in three steps from ethyl α-formylpropionate and NH_3. Reaction of the alkali metal salts of β-(*N*-benzoylamino)methacrylamide with [^{11}C]phosgene gave [2-^{11}C]thymine. The yield of [2-^{11}C]thymine was 362 ± 53 MBq at EOS ($n = 3$) (18 MeV proton beam; 10 μA, 10 min). The total synthesis was accomplished in just 16 min from the end of bombardment.

Uracil derivatives are of considerable interest because of their wide array of pharmacological properties, and many pyrimidine-based radiopharmaceuticals have been developed for clinical diagnosis in the field of single photon or positron emission computed tomography.[1] The search for new or improved synthetic routes leading to labeled thymidine for evaluation of cellular proliferation by positron emission tomography (PET) has attracted much attention in recent years. Because of the short-lived positron emitting radionuclide (ca. ^{11}C: 20.4 min, ^{13}N: 10.0 min, ^{15}O: 2.0 min, respectively) and the radioactive level, a very rapid and simple labeling process with an efficient organic reaction and an automated synthesis apparatus to avoid radiation exposure are essential conditions for PET radiotracer synthesis.

In 1991, Vander Borght et al. synthesized [2-^{11}C]thymidine from [2-^{11}C]thymine formed via cyclocondensation of diethyl β-methyl malate with [^{11}C]urea.[2] Although other investigators have attempted to improve this method or develop related methodologies, the key ring closure reactions have traditionally relied on the condensation of a malate intermediate with the labeling agent [^{11}C]urea derived from phosgene,[2,3] cyanide,[4] or carbon dioxide.[5] Typically the reactions are carried out under as drastic conditions as those employed for ^{14}C labeled thymine synthesis.[6] The complexity of the currently available synthetic routes along with the extended length of preparation time has limited the extensive applicability of ^{11}C labeled nucleosides in PET studies.

Recently, we have developed a simplified and highly efficient synthesis of [^{11}C]$COCl_2$ with high specific activity.[7] ^{11}C labeled phosgene is a high-potency agent for the introduction of a ^{11}C carbonyl group to form versatile heterocyclic compounds as well as a variety of ureas. We have explored the utility of [^{11}C]$COCl_2$ for producing high-specific activity [^{11}C]CGP-12177, a radioligand for β-adrenoreceptors in the field of clinical PET.[8] This work has now prompted us to apply [^{11}C]$COCl_2$ in a ring closure step that would provide an efficient synthesis of [2-^{11}C]thymine.

We have designed alternative precursors as sources for the carbon skeleton that can be cyclocondensed with [^{11}C]$COCl_2$ to form the desired [2-^{11}C]thymine. Our strategy for the synthesis of [2-^{11}C]thymine is depicted in Scheme 1, wherein the key intermediate is β-aminomethacrylamide derivative (**1**). We now report herein a facile synthesis of [2-^{11}C]thymine by the direct condensation of [^{11}C]$COCl_2$ with the novel intermediate (**1**).

Keywords: [^{11}C]phosgene; [2-^{11}C]thymine; Positron emission tomography; β-(*N*-Benzoylamino)methacrylamide.
Abbreviations: EOS, end of synthesis; EOB, end of bombardment; PET, positron emission tomography; DME, 1,2-dimethoxyethane.
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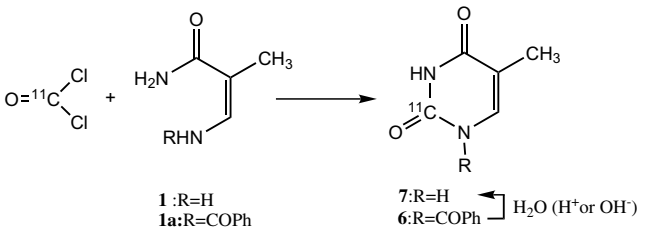


Scheme 1. Synthetic route of [2-^{11}C]thymine.

We have attempted to prepare the novel precursor β-aminomethacrylamide derivative (**1**), which leads to thymine or 1-acylated thymines by condensation reaction with phosgene. Previously, it had been reported that 1-acetylated or benzoylated thymine derivatives are rapidly hydrolyzed in ca. 20 s to produce thymine (Scheme 1).[9]

The key intermediate (**1**) was synthesized from β-aminomethacrylate (**3**) formed by the reaction of ethyl α-formylpropionate (**2**)[10] with NH_3. With the intent to activate the ester for producing amide, **3** was benzoylated prior to treatment with NH_3.

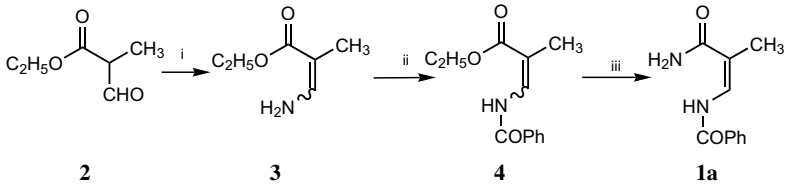
Treatment of the resulting compound (**4**) with NH_3 exclusively afforded β-(*N*-benzoylamino)methacrylamide (**1a**) without any detectable formation of the hydrolyzed product β-aminomethacrylamide (**1**, R = H) (Scheme 2). Attempts at the hydrolysis of benzoylated compound (**1a**) did not give **1**, hence, we examined the ring closure reaction using β-(*N*-benzoylamino)methacrylamide (**1a**).[11]

Synthesis of cold thymine was examined with **1a** by using triphosgene, which can be easily handled as a safe and stable replacement for phosgene. In order to accomplish the desired cyclocondensation, **1a** was activated as its alkali-metal salt. Addition of triphosgene to the sodium salt in DMF followed by hydrolysis gave thymine quantitatively.[12]

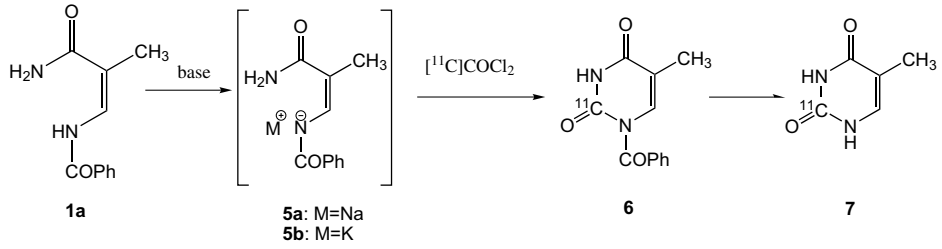
^{11}C labeled thymine production was achieved by using the same automated synthesis system as used for [^{11}C]CGP production from [^{11}C]$COCl_2$.[8] [^{11}C]$COCl_2$ was synthesized from [^{11}C]methane via [^{11}C]CCl_4 according to our previously reported method.[7] [^{11}C]methane was produced using an ultracompact cyclotron by the ^{14}N (p, α) ^{11}C nuclear reaction on nitrogen containing hydrogen (5%) in an aluminum target. Bombardment was carried out with a 10 μA beam of 18 MeV protons for 10 min.

The direct ring closure reaction of [^{11}C]$COCl_2$ possessing superior reactivity with non-activated precursor (**1a**) was first examined, but this approach failed to give the target product. Thymine precursor (**1a**), which was activated as the alkali metal salt (**5a**,**b**) reacted with [^{11}C]$COCl_2$ as well as triphosgene to afford [2-^{11}C]thymine (Scheme 3). The best result was obtained when the reaction was performed with potassium salt (**5b**) in DME as shown in Table 1.[13] The yield of [2-^{11}C]thymine under these conditions was 362 ± 53 MBq at EOS ($n = 3$). The radiochemical yield of [2-^{11}C]thymine was ca. 27% from [^{11}C]$COCl_2$.[14] The [2-^{11}C]thymine produced was identified by co-chromatography with authentic thymine and found to be radiochemically homogeneous by HPLC. Additionally, it was confirmed by subsequent enzymatic conversion to [2-^{11}C]thymidine.[3,15] Thus our method, which utilizes the reaction of [^{11}C]$COCl_2$ with the appropriate activated precursor is a viable approach to providing an adequate supply of [2-^{11}C]thymine, reliably and reproducibly, for clinical PET tracer studies with [2-^{11}C]thymidine.

In all previous reports, labeling of the 2-position of thymine was accomplished by condensation of [^{11}C]urea and malate at 130 °C in fuming sulfuric acid. Recently, Steel et al. reported an improved method for the preparation of [2-^{11}C]thymine via a multi-step process using [^{11}C]urea derived from [^{11}C]$COCl_2$. This radiosynthesis of [2-^{11}C]thymine took approximately 30 min from EOB and the yield was 38.5% from [^{11}C]$COCl_2$.[3]



Scheme 2. Synthesis of β-(*N*-benzoylamino)methacrylamide (**1a**). Reagents and conditions: (i) NH_3, CH_3OH, reflux, 2 h; (ii) PhCOCl, C_5H_5N, $CHCl_3$; (iii) NH_3, CH_3OH.



Scheme 3. Synthesis of [2-^{11}C]thymine (**7**).

Table 1. Yields of [2-^{11}C]thymine (**7**)

Solvent	Base	Mol equiv	Yield (MBq, EOS)
DME	—	—	ND
Toluene	NaH	5	41 ($n = 2$)
DME	NaH	2	68 ± 65 ($n = 3$)
DME	$(CH_3)_3COK$	2	137 ($n = 2$)
DME	$(CH_3)_3COK$	1	362 ± 53 ($n = 3$)

EOS: end of synthesis. ND: not detected. DME: 1,2-dimethoxyethane.

Although previous synthesis of [2-^{11}C]thymine via [^{11}C]urea is more efficient, our strategy involving the cyclocondensation with [^{11}C]$COCl_2$ for the direct production of [2-^{11}C]thymine is operationally simple, and offers fewer reaction steps at lower temperature. The total synthesis described herein takes 16 min from EOB to isolation of **7**, thus significantly shortening the reaction time, which is a crucial consideration for the preparation of short half-life radiopharmaceuticals.

In conclusion, we have provided a substantially useful method for the synthesis of [2-^{11}C]thymine. Having several merits over hitherto known methods, that is fewer reaction steps, mild reaction conditions, reliability of product yield, and simplified operations and synthetic instruments, the present methodology should find wide application in the preparation of many ^{11}C labeled radiopharmaceuticals. Extension of this method to the synthesis of other uracils and nucleosides is currently under investigation in our laboratory.

Acknowledgments

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11. Selected data **1a**: (*Z*)-β-(*N*-Benzoylamino)methacrylamide: mp 182–184 °C (recrystallized from 50% AcOEt–hexane). ^{1}H NMR ($CDCl_3$): δ 1.95 (3 H, d, $J = 1.2$ Hz), 5.40–5.80 (2H, br d, D_2O exchangeable, NH_2), 7.46 (2H, t, $J = 7.2$ Hz), 7.54 (1H, t, $J = 7.2$ Hz), 7.54 (1H, d, $J = 7.5$ Hz), 7.93 (2H, d, $J = 7.2$ Hz), 12.3 (1H, br s, D_2O exchangeable, NH). Anal. Calcd for $C_{11}H_{12}N_2O_2$: C, 64.69; H, 5.92; N, 13.72. Found: C, 64.50; H, 6.05; N, 13.60.
12. The chemical purity of the product isolated by HPLC was 99%. Spectroscopic data (NMR, IR, and MS) of the product were completely superimposable on those of authentic thymine.
13. [^{11}C]$COCl_2$ was bubbled with helium flow into a reaction vial containing a solution of **5b** (0.2 mg) in DME (0.5 mL) in the presence of the base at 30 °C for 1 min. After removal of the solvent, treatment of the residual [2-^{11}C]*N*-benzoylthymine (**6**) with 1.5 M ammonia–methanol for 1 min at room temperature resulted in debenzoylation to yield **7**. The reaction mixture was subjected to reverse-phase HPLC equipped with UV monitor and γ counter (μ-Bondapak C_{18}, 25 cm × 0.39 cm i.d., 3% EtOH–Saline, flow rate 0.5 mL/min at 40 °C). The radioactive peak at 11 min was the desired [2-^{11}C]thymine. The product was observed to be 99% radiochemically pure by HPLC.
14. The yield of [2-^{11}C]thymine was determined based on produced [^{11}C]$COCl_2$. We estimated the yield of [^{11}C]$COCl_2$ to be about 1500 MBq based on the yield of diphenylurea.
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