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[^{11}C]-dimethylamine as a labeling agent for PET biomarkers

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Abstract

The dimethylamine functional group is a common component of the chemical structure of numerous drugs. The most commonly used synthetic route for carbon-11 labeled radiopharmaceuticals which contain the dimethylamine group is via C-11 methylation of the monomethyl amine precursors. Here we describe the radiosynthesis of [^{11}C]dimethylamine (**1**) and its application in the direct labeling of several positron emission tomography (PET) imaging agents by-passing the preparation of the monomethyl amine precursors.

Keywords: [^{11}C]-dimethylamine; PET

1. Introduction

Positron Emission Tomography (PET), a nuclear medicine imaging modality, allows for the four-dimensional, quantitative determination of the distribution of radioactivity within the human body (Ben-David et al., 2003). PET has been used for in vivo non-invasive biochemical investigations in several medicinal fields such as oncology, cardiology and neurology (Wang and Maurer, 2005; Shiue and Welch, 2004). The fundamental component of PET imaging is the acquisition of selective labeled biomarkers, which target various biochemical components in the human body to allow sensitive molecular imaging (Elsinga, 2002). However, the design and construction of such selective PET biomarkers is dependent on the availability of reactive radiochemical reagents that afford direct and rapid incorporation of the short-lived PET isotopes into a specific site of potential biomarkers. In addition, when aiming at in vivo molecular imaging of low-capacity systems such as receptors and enzymes, one should work with high-specific activity levels. Under such conditions, the reaction kinetics at the tracer levels significantly differs from those at the mass levels. Therefore, it is sometimes challenging to imitate chemical transformation from the mass to the tracer levels. This obstacle warrants the need for a broad spectrum of novel radiochemical reagents that will lead to a wide variety of synthetic approaches. One of the most commonly used PET isotopes is C-11 which has a half-life of 20.3 min (Hosoya et al., 2006; Långström et al., 1987). While its short half-life is an advantage for imaging, it becomes a challenge for labeling. Numerous C-11 labeling reagents have been developed with C-11 methyl iodide (MeI) being the most frequently used (Elsinga, 2002; Hosoya et al., 2006).

The dimethylamine functional group is a common component of the chemical structure of numerous drugs (Heal et al., 1998; Codling et al., 2005; Hughes et al., 2003) that target various low capacity systems such as the epidermal growth factor receptor (EGFR) (Mishani et al., 2004), the serotonin transporter (Jarkas et al., 2005) or the androgen receptor (AR) (Jacobson et al., 2006), and is an attractive candidate site for labeling with carbon-11. While labeling is usually done using C-11 MeI, in some cases, it requires the use of unstable precursors (Mishani et al., 2004; Jacobson et al., 2006).

Here we describe the radiosynthesis of [^{11}C]dimethylamine (**1**) and its application in the direct labeling of several PET imaging agents bypassing the preparation of the monomethyl amine precursors.

2. Results and discussion

C-11 MeI is frequently used for the construction of labeled drugs containing the C-11 dimethylamine functional

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group. This approach requires the preparation of monomethylamine derivatives as precursors. One way to produce these precursors is by the reaction of primary amines with methylation reagents, to form secondary methylamines. Since the secondary amines are more reactive towards methylation than the primary amines, this reaction involves the formation of several products and should therefore be carefully handled and monitored. Another possible approach requires the substitution of bromide ion with monomethylamine. In some cases, the secondary amine precursors are not stable and undergo intramolecular nucleophilic attack to form side products such as epoxide, lactam (Jacobson et al., 2006) or a stable aromatic methyl–pyrole group (Mishani et al., 2004). Furthermore, due to the instability of some precursors, the monomethylamine derivatives were used for labeling application as a crude mixture since they might decompose during the purification process by silica/alumina columns. This fact leads to low radiochemical yields and formation of several labeled by-products, which interfere with the purification process of the desired labeled product.

To avoid using monomethylamine derivatives as a starting material for the labeling, we have developed a novel radio-chemical reagent [^{11}C]dimethylamine (**1**) which leads to a rapid and direct radio-synthetic route to produce C-11 labeled dimethylamine derivatives as potential PET biomarkers from bromide starting materials, which are easier to handle and purify.

The [^{11}C]dimethylamine was prepared by the reaction of [^{11}C]MeI (Crouzel et al., 1987) with a solution of monomethylamine. [^{11}C]MeI was distilled to a reactor containing monomethylamine (2 M in THF) in a mixture with DMSO at −10 °C. We have found a good correlation between an increasing concentration of monomethylamine and the radiochemical yield of [^{11}C]dimethylamine. In addition, upon investigating the relationship between temperature and yield, we found that the optimal temperature for this reaction was 45 °C. At a lower temperature, the formation rate of the [^{11}C]dimethylamine decreased and at a higher temperature, the radio-chemical yield was lower. After a 5 min reaction, [^{11}C]dimethylamine was formed and the crude mixture was injected into a HPLC system equipped with a C-18 column [Bischoff Nucleosil 100-7-C18 reversed phase preparative column], a radioactivity detector containing a NaI crystal and a UV detector (Fig. 1). The product was collected and analyzed by a co-injection with dimethylamine (2 M in THF, Sigma-Aldrich), on a cation exchange analytical column (Waters Corporation, Milford, MA) (Mishani et al., 2002). The HPLC co-injection revealed a 70% radiochemical yield of [^{11}C]dimethylamine. In addition to the formation of [^{11}C]dimethylamine, we also observed the existence of the remaining labeled intermediates, namely, [^{11}C]CH_3I and [^{11}C]CH_3OH. No other labeled byproducts were detected by the HPLC injection.

The radiochemical yield of the production of [^{11}C]dimethylamine (**1**) was 70% decay corrected, with a specific activity of 3.5–3.8 Ci/μmol end of bombardment (EOB).

The utilization of dimethylamine in organic synthetic chemistry is well known. In order to evaluate its potential as a labeling agent at the tracer level and under short reaction time, we tested its reaction with

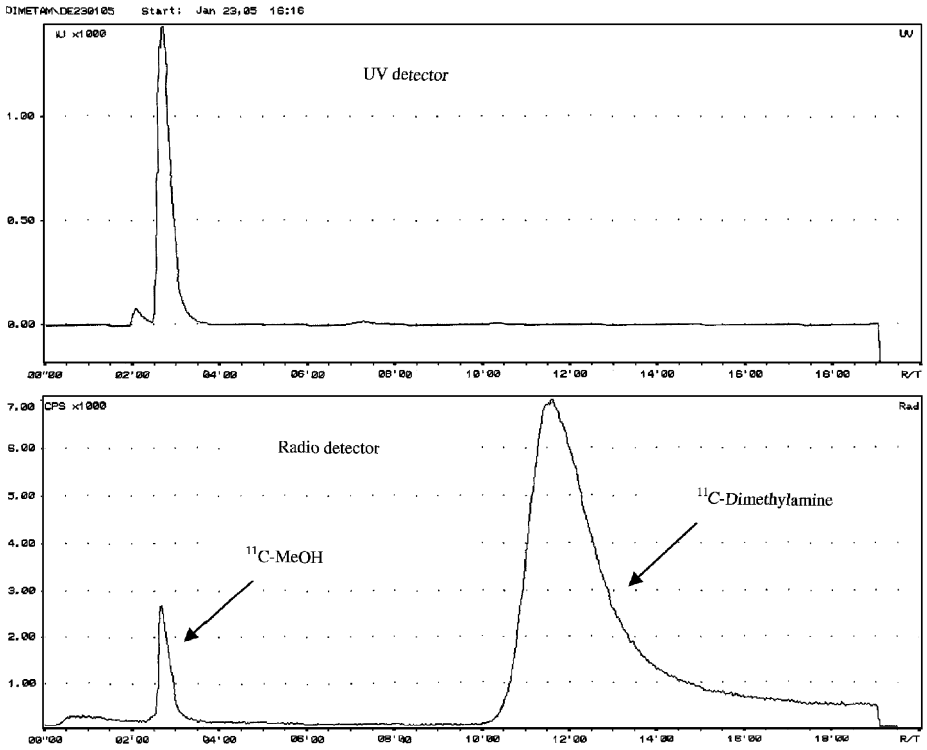


Fig. 1. Preparative HPLC purification of ^{11}C-dimethylamine.

several bromide derivatives exhibiting wide reactivity ranges toward nucleophilic reaction: propargyl bromide (**2**), 4-nitrobenzyl bromide (**3**), 4-methylbenzyl bromide (**4**), (2E)-4-bromo-*N*-{4-[(3,4-dichloro-6-fluoro-phenyl)amino]-quinazolin-6-yl}-2-butenamide (**5**) and (R)-3-bromo-2-hydroxy-2-*N*-(4-fluoro-3-(trifluoromethyl)phenyl) propanamide (**6**).

After the formation of the [^{11}C]dimethylamine, the reactor was cooled to 0 °C, the bromide starting materials (**2–6**) were added in a solution of dimethylacetamide (DMA) with diisopropylethylamine to the crude mixture of [^{11}C]dimethylamine, and then the reactor was sealed and heated to 45 °C for 10 min. The crude reaction mixtures were purified and separated from the labeling agent, [^{11}C]dimethylamine (see Fig. 2 as an example) by reversed phase preparative HPLC to yield the final labeled products. It should be noted that the monomethylamine present in the reaction mixture could compete with the [^{11}C]dimethylamine; however, since satisfactory radiochemical yields and purification conditions for the desired final labeled products were obtained, and as half-life of carbon-11 is short, [^{11}C]dimethylamine was used without any further purification. The desired products were collected and analyzed by a co-injection with standards on an analytical C-18 column (Scheme 1).

As expected and similar to the kinetics at the mass level, the reaction of the [^{11}C]dimethylamine at the tracer level with propargyl bromide (**2**), gave the highest yield (25% EOB), due to the presence of a triple bond which makes the propynyl carbon electron-deficient.

The benzyl bromides (**3** and **4**) gave moderate yields (20–22% EOB). According to our radiosynthesis results, there was no significant difference between these two groups in the degree of reactivity towards [^{11}C]dimethylamine at the tracer level. Although compound **3** contains an electron-withdrawing group (NO_2) on the aromatic ring, it did not lead to an increased radiochemical yield relative to its methyl analogue (compound **4**). We hypothesize that the reactivity differences between compounds **3** and **4** were not evident at the tracer levels toward the reaction of [^{11}C]dimethylamine under the prevailing temperature and reaction time conditions.

A reaction of [^{11}C]dimethylamine with the less reactive allyl bromide (compound **5**) gave a lower radiochemical yield of 10–15% EOB under similar conditions as described above.

The described procedure enabled us to reliably and reproducibly ($n = 4$) obtain [^{11}C]-**7a-d** in moderate radiochemical yields (10–25%, 2.3–3.8 Ci/μmol EOB).

While reaction of aliphatic bromides such as compound **6** with dimethylamine solution at the mass level produced the desired products after 2 h, with a good yield (52–59%) at a temperature of 45 °C, no reaction was observed between compound **6** and [^{11}C]dimethylamine at the tracer level. The incorporation of [^{11}C]-dimethylamine did not succeed using a wide range of temperatures (45–90 °C) and different solvents (dimethylacetamide, DMSO, CH_3CN, and THF). When the crude mixture was injected into the preparative column, the major radioactive peak observed was of the [^{11}C]-dimethylamine that did not react with the aliphatic bromide compound. It should be noted that other aliphatic bromides were also tested and yielded the same results as **6,** reflecting on the lower reactivity of aliphatic

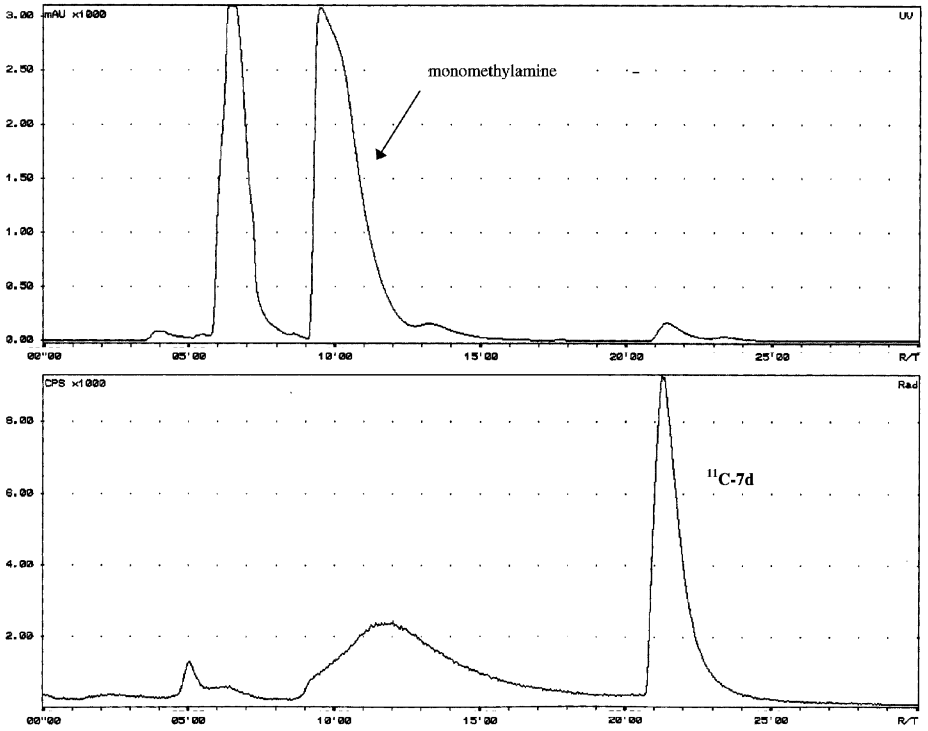
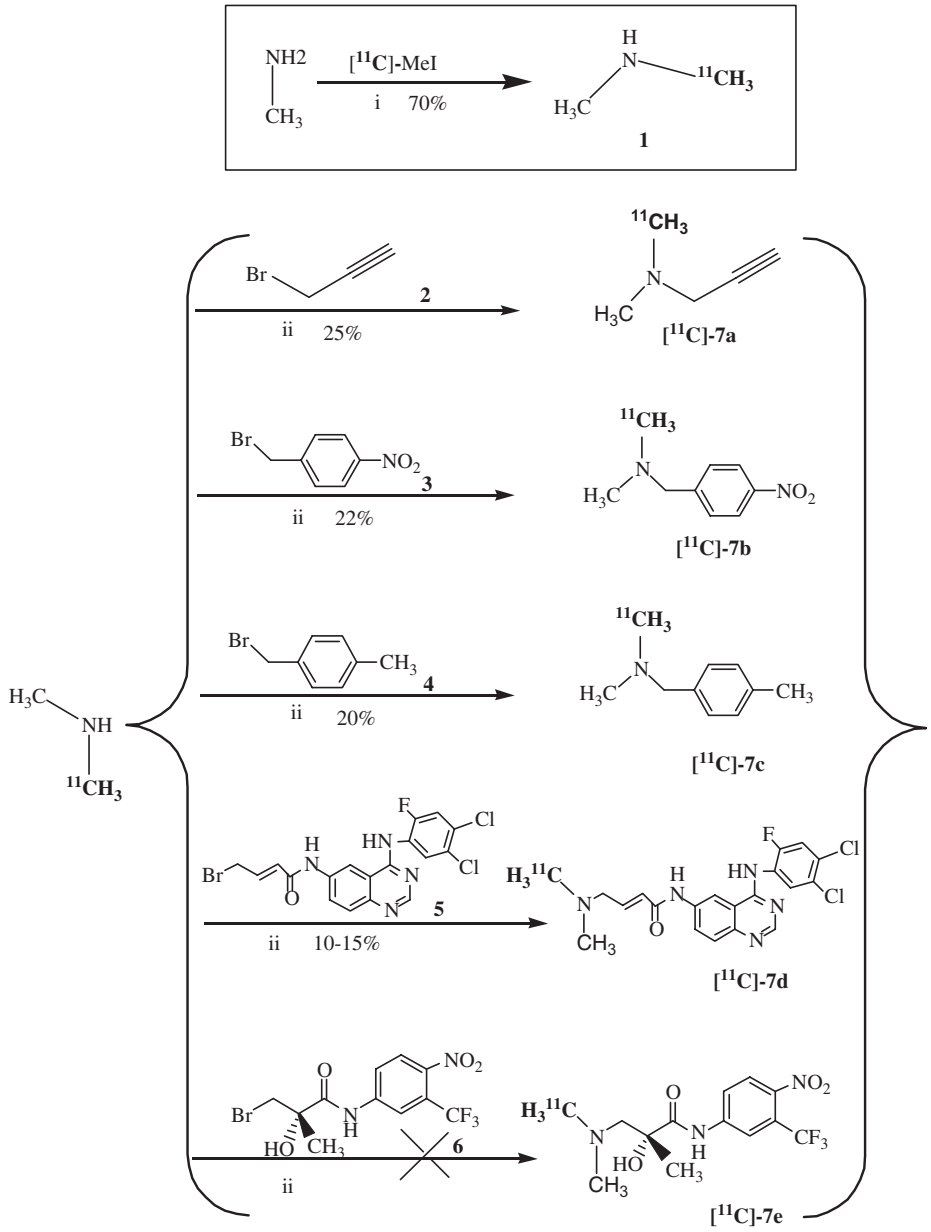


Fig. 2. Preparative HPLC purification of ^{11}C-7d. Upper chromatogram—UV detector. Lower chromatogram—radio detector.



Scheme 1. Radiosynthesis of [^{11}C]-dimethylamine and its reaction with various compounds. Reaction conditions: (i) 0.4 ml of monomethylamine 2 M in THF in a mixture with 50 μl of DMSO, 45 °C, 5 min. (ii) 600 μl of dimethylacetamide in a mixture with 5–10 μl of diisopropylethylamine, 40 °C, 10 min.

bromide toward the nucleophilic reaction with dimethylamine, as clearly observed at the tracer level.

The described radiochemical transformation to produce [^{11}C]dimethylamine and its reaction with the various compounds were successfully translated into an automated procedure using the GE carbon-11 module (Munster, Germany).

3. Conclusion

The dimethylamine functional group is a common component of the chemical structure of numerous drugs, thus representing an attractive moiety for carbon-11 labeling. We succeeded in developing a new radiochemical reagent, [^{11}C]dimethylamine (**1**) and applied it to the direct labeling of various bromide-starting materials to furnish the carbon-11 labeled dimethylamine derivatives. This new and direct radiosynthetic route avoids the use of unstable monomethylamine precursors and furnishes the [^{11}C]dimethylamine desired products with minimal labeled by-products as compared with the traditional labeling procedures using [^{11}C]MeI.

4. Experimental

4.1. General

All solvents were of analytical grade. THF was distilled over sodium/benzophenone. Other solvents were purchased from Sigma-Aldrich (Tel Aviv, Israel), Fisher

Scientific (Pittsburgh, PA, USA), Merck (Darmstadt, Germany) or J.T. Baker (New Jersey, USA). Propargyl bromide and 3-dimethylamino-1-propyne were purchased from Sigma-Aldrich (Tel Aviv, Israel).

^{1}H NMR was recorded on 300 MHz spectrometers in $CDCl_3$ or DMSO–d_6. ^{1}H NMR signals are reported in parts per million. ^{1}H NMR signals are referenced to the residual proton (7.26 ppm for $CDCl_3$ or 2.40 ppm for DMSO–d_6) of deuterated solvent. Mass spectra were obtained on a spectrometer equipped with ESI, CI, EI and FAB probes. Radiosynthesis was carried out on an automated module (GE, Munster Germany). Specific radioactivities were determined by HPLC, using cold mass calibration curves. [^{11}C]-carbon dioxide was produced by the $^{14}N(p,\alpha)^{11}C$ nuclear reaction on nitrogen containing 0.5% oxygen, using an 18/9 IBA cyclotron. At EOB, the target gas was delivered and trapped by a cryogenic trap in the [^{11}C]CH_3I module. HPLC was performed on a system with a variable wavelength-detector operating at 254 nm and with a radioactivity-detector with a NaI crystal. Three systems were used: (A) a reverse-phase system employing C-18 column [Bischoff Nucleosil 100-7-C18 reverse phase preparative column (7 μm, 250 × 16 mm), flow rate of 8 ml/min of 53% ammonium formate 0.1 M, 45% CH_3CN/2% THF as eluent. (B) a reverse-phase system using a C-18 column (10 μm, 300 × 3.9 mm) and 40% CH_3CN/60% acetate buffer 0.1 M as eluent with a flow rate of 1 ml/min for analysis of the formulated radiotracer, (C) a cation exchange column IC-PAK^{TM} Cation M/D 150 × 3.9 mm (Waters Corporation, Milford, MA) and 100% 5 mM HCl as eluent, with flow rate of 1 ml/min for analysis of the [^{11}C]-dimethylamine.

4.2. Chemistry

4.2.1. Dimethyl-(4-nitro-benzyl)-amine (7b) (Gupta and Lechner, 1998; Ben-David et al., 2003; Hosoya et al., 2006)

Dimethylamine (2 M in THF, 5 ml) was stirred and cooled in an ice bath. A solution of 4-nitrobenzyl bromide (110 mg, 0.5 mmol) in dimethylacetamide (2 ml) was added dropwise followed by the addition of diisopropylethylamine (0.25 mmol). The reaction temperature was raised to 40 °C, and the mixture was stirred for 2 h. Then, ethyl acetate (15 ml) and saturated $NaHCO_3$ (15 ml) were added. The layers were separated, and the organic layer was washed with brine, dried ($MgSO_4$) and evaporated. The residual oil was purified by flash chromatography on silica gel (MeOH–CH_2Cl_2, 5:95) to give 50 mg (56%) of compound **7b** as yellow oil.

MS (m/z) 181.42 (MH^+). ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.17(d, 2H, $J = 8.7$), 7.48(d, 2H, $J = 8.7$), 3.51(s, 2H), 2.28(s, 6H).

4.2.2. Dimethyl-(4-methyl-benzyl)-amine (7c)

Compound **7c** was prepared under similar conditions as **7b**. Briefly, 4-methylbenzyl bromide (303 mg, 1.641 mmol) was added to a cooled solution of dimethylamine (2M) to give 124 mg (50.7%) of Compound **7c** as yellow oil.

MS (m/z) 150 (MH^+). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.43(d, 2H, $J = 7.8$), 7.25(d, 2H, $J = 7.8$), 4.0(s, 2H), 2.69(s, 6H).

4.2.3. 4-dimethylamino-but-2-enoic acid [4-(3,4-dichloro-6-fluoro-phenylamino)-quinazolin-6-yl]-amide (7d) (Mishani et al., 2004)

Dimethylamine (2 M in THF, 38 ml) was added dropwise to a solution of (2E)-4-bromo-*N*-{4-[(3,4-dichloro-6-fluoro-phenyl)amino]-quinazolin-6-yl}-2-butenamide (564 mg, 1.21 mmol) in dry THF. The reaction was heated to 80 °C for 15 min, and cooled. EtOAc (50 ml) and saturated $NaHCO_3$ (50 ml) were added. The layers were separated, and the organic layer was washed with brine, dried with $MgSO_4$, and evaporated. The crude material was purified by flash chromatography on silica gel (MeOH: CH_2Cl_2, 5%: 95%) to yield 317 mg (73%) of 4-dimethylamino-but-2-enoic acid [4-(3,4-dichloro-6-fluoro-phenylamino)-quinazolin-6-yl]-amide (**7d**).

MS (m/z) 434.1 (MH^+). ^{1}H NMR (DMSO-d_6) δ 10.47(1H, s), 9.95(1H, s), 8.83(1H, s), 8.4(1H, s), 7.92(1H, dd, $J1 = 18$ Hz, $J2 = 3.8$ Hz), 7.78(1H, bd, $J = 20$ Hz), 7.22–7.43 (2H, m), 6.86(1H, dt, $J1 = 30$ Hz, $J2 = 11$ Hz), 6.36(1H, d, $J = 30$ Hz), 3.08(2H, d, $J = 11$ Hz), 2.18(6H, s).

4.3. Radiochemistry

4.3.1. Automated synthesis of [^{11}C]-dimethylamine

Carbon-11 MeI was prepared according to well-documented procedures (Crouzel et al., 1987). Briefly, [^{11}C]CO_2 (37 GBq, 1000 mCi) was trapped at −160 °C. The temperature of the cooling trap was increased to −50 °C, and the activity was transferred by a stream of argon (40 ml/min) into reactor 1 containing 300 μl of 0.25 N $LiAlH_4$ in THF at −50 °C. After 90 s, the solvent was removed under reduced pressure. In this manner, more than 80% of the activity was recovered. The reactor temperature was increased to 160 °C, HI was added and [^{11}C]MeI was distilled (argon flow of 15 ml/min) through a NaOH column to the second reactor, containing monomethylamine 2 M in THF (0.4 mL, 0.8 mmol) and 50 μL of DMSO. After 1 min of distillation, an average of 550 ± 30 mCi ($n = 4$) was trapped in the second reactor at −15 °C. The reactor was sealed and heated to 45 °C for 5 min. At the end of the 5 min reaction, the crude product was injected to the HPLC Bischoff Nucleosil 100-7-C18 reverse-phase preparative column (7 μm, 250 × 16 mm), flow rate of 8 ml/min and the product was collected at retention time (r.t.) of 7 min and injected for further analysis into a cation exchange column IC-PAK^{TM} Cation M/D 150 × 3.9 mm (Waters Corporation, Milford, Massachusetts) and 100% 5 mM HCl as eluent, with flow rate of 1 ml/min and r.t. of 5.6 min. The decay radiochemical yield for this synthesis was 70% (EOB).

4.3.2. Synthesis of compound [^{11}C]7a–7d

After formation of [^{11}C]-dimethylamine, the reactor was sealed and cooled to 0 °C. Solutions of **2** (6 μl, 0.06 mmol), **3** (13 mg, 0.06 mmol), **4** (11 mg, 0.06 mmol), **5** (28 mg, 0.06 mmol), were added from vial 2, in 600 μL of dimethylacetamide and 5–10 μL of diisopropylethylamine. The reactor was sealed again and heated to 40 °C for 10 min. Then, the solvent was removed under flow of argon at 80 °C for 1 min. The mixture was cooled to 40 °C; 0.6 ml of CH_3CN/H_2O (1:1) was added, and the crude product (average of 330 ± 20 mCi ($n = 3$–4) was automatically injected to the HPLC Bischoff Nucleosil 100-7-C18 reverse-phase preparative column (7 μm, 250 × 16 mm), flow rate of 8 ml/min at r.t of 11, 16, 18, 20–22 min for compounds [^{11}C]**7a**–**7d** respectively. The labeled products were collected in a flask and injected into reversed-phase system using a C-18 column (10 μm, 300 × 3.9 mm) and 40% CH_3CN/60% acetate Buffer 0.1M as eluent with a flow rate of 1 ml/min for analysis of the formulated radiotracer and calculation of the specific activity of 1.7–1.9 Ci/μmol and total radiochemical yield of 25, 22, 20 and 10–15% (decay corrected) for compounds [^{11}C]**7a**, **b**, **c** and **d** respectively.

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