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Radiosynthesis of three [^{11}C]ureido-substituted benzenesulfonamides as PET probes for carbonic anhydrase IX in tumors

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ABSTRACT

Three ureido-substituted benzenesulfonamides **1a–c** have been developed as potent inhibitors for carbonic anhydrase IX, which is overexpressed in hypoxic tumors. In this study, we labeled these unsymmetrical ureas **1a–c** using [^{11}C]phosgene ([^{11}C]$COCl_2$) as a labeling agent with the expectation that [^{11}C]**1a–c** could become promising positron tomography probes for imaging carbonic anhydrase IX in tumors. The strategy for radiosynthesis of [^{11}C]**1a–c** was to react hydrochloride of anilines **2a–c** with [^{11}C]$COCl_2$ to give isocyanate [^{11}C]**4a–c**, followed by a reaction with 4-aminobenzenesulfonamide (**3**).

Carbonic anhydrase IX (CA IX) is a multidomain protein with the CA subdomain located outside the cell and is one of the most overexpressed genes in hypoxic conditions.[1] CA IX has recently been shown to be a target for drug development and imaging of hypoxic tumors, which have high expression of this protein.[2–4] It has been found that CA IX expression was significantly increased in many types of solid tumors, including glioma/ependymomas, mesotheliomas, and papillary/follicular carcinomas, carcinomas of the bladder, uterine cervix, kidneys, esophagus, lungs, head and neck, breast, brain, and vulva; and squamous/basal cell carcinomas.[5,6] CA IX has high CO_2 hydrase catalytic activity, which causes CA IX to play a key role in the regulation of pH in tumors.[7] Because hypoxic tumors do not respond to the general chemo- and radiotherapy, inhibitors of CA IX in the hypoxic tumors may become promising anticancer drugs with a novel action mechanism.[2–5]

Recently, dozens of ureido-substituted benzenesulfonamide analogs have been developed and show potent inhibition for several human CA proteins.[8] Of these CA inhibitors, compounds **1a–c** (Scheme 1) had high inhibitory affinity for human CA IX, with K_i in the range of 0.3–5.4 nM. Moreover, these compounds showed selectivity for other CA subtypes, such as CA I (K_i: 9–38 nM), CA II (2.4–1060 nM), CA XII (4.6–50 nM). These data indicate that these sulfonamides showed more potent inhibition for tumor growth as CA IX-specific inhibitors. In particular, 4-{[(3-nitrophenyl)carbamoyl]amino}benzenesulfonamide (**1a**) was shown to significantly inhibit the formation of metastases in a model of breast cancer metastases at a pharmacologic dose of 45 mg/kg, suggesting that **1a** is a candidate for the development of novel antimetastatic drugs. This finding motivated us to label **1a–c** (Scheme 1) with positron-emitting carbon-11 (^{11}C; half life: 20.4 min) as new positron emission tomography (PET) probes for imaging CA IX in hypoxic tumors.

PET is a potential and quantitative molecular imaging technique with high functional sensitivity, which permits repeatable, noninvasive assessment and understanding of biological and pharmacological processes.[9] Development of PET probes for imaging receptors, enzymes, and transporters has enabled PET to quantitatively evaluate the expression and pharmacological action of receptors, etc. in humans, which contributes to disease diagnosis and provides information about the most effective doses of drug therapy. We have synthesized several anticancer drugs which target specific enzymes overexpressed in tumors, such as [^{11}C]gefitinib for epidemic growth factor receptor and tyrosine kinase,[10,11] [^{11}C]sorafenib for vascular endothelial growth factor receptor and Raf kinase,[12] [^{11}C]topotecan for topoisomerase I.[13] Using these PET probes, we measured in vitro cellular uptake, determined in vivo pharmacokinetics in animals and performed imaging of tumor-bearing rodents.

In the present study, we synthesized [^{11}C]ureido-substituted benzenesulfonamides ([^{11}C]**1a–c**, Scheme 1) with the expectation

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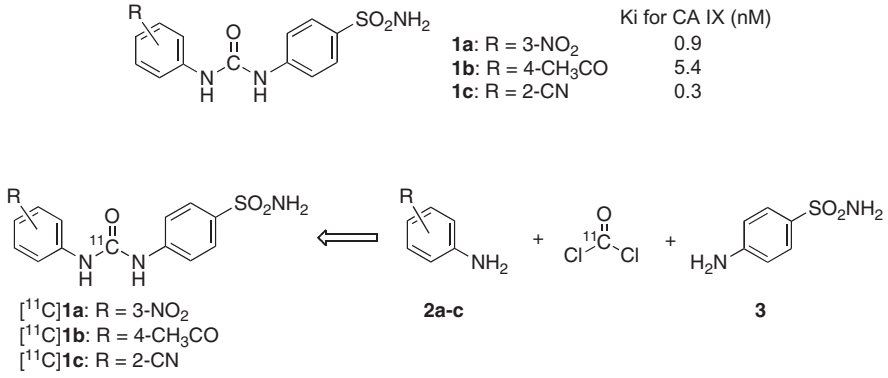


Scheme 1. Chemical structure of **1a–c** and retrosynthesis of [^{11}C]**1a–c**.

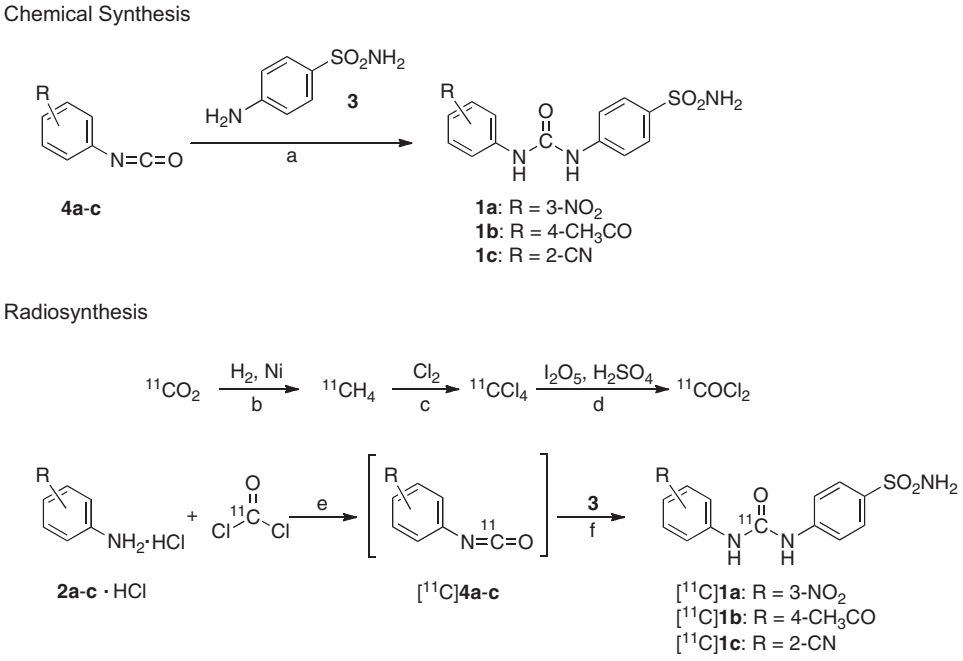
that these radioligands may become promising PET probes for imaging CA IX in tumors. Previously, an imaging study of CA IX with an ^{177}Lu-labeled human monoclonal antibody has been performed on nude mice which bear adenocarcinoma xenografts expressing rich CA IX.[14,15] However, to our knowledge, there was no report about the development and application of PET probes for imaging CA IX in hypoxic tumors.

Regarding the chemical structures of **1a–c** with an unsymmetrical urea moiety, we decided to label these compounds using [^{11}C]phosgene ([^{11}C]$COCl_2$) as a labeling agent[16,17] (Scheme 1). This approach involves convenient and reliable production of [^{11}C]$COCl_2$,[18–20] the usefulness of two different amines: 3-nitro (**2a**), 4-acetyl (**2b**) or 2-cyano (**2c**) anilines and 4-aminobenzenesulfonamide (**3**), and construction of a [^{11}C]urea site, as shown in the retro-synthetic route.

Prior to radiosynthesis, the non-radioactive benzenesulfonamides **1a–c** were prepared by heating commercially available isocyanates **4a–c** with **3** in anhydrous CH_3CN at 50 °C for 2–8 h with 26–50% chemical yields (Scheme 2). Compounds **1a–c** were analyzed by mp, NMR and high resolution MS, which were identical to those reported previously[8] (also see Supplementary data).

The preparation of [^{11}C]$COCl_2$ for the present radiosynthesis is routinely performed using a home-made synthetic unit (>2 times/week).[19,20] As shown in Scheme 2, [^{11}C]$COCl_2$ was produced starting from cyclotron-produced [^{11}C]CO_2 via [^{11}C]CH_4 and then [^{11}C]CCl_4 as two intermediates.[18,19] After irradiation, [^{11}C]CO_2 was recovered from the cyclotron target and concentrated on the inner space of a stainless steel tube under liquid N_2. Release of [^{11}C]CO_2 from the tube and continuous passage of [^{11}C]CO_2 with H_2 of 10 mL/min through a heated methanizer at 400 °C gave [^{11}C]CH_4.[21] A mixture of [^{11}C]CH_4 and Cl_2 gas was then passed through a heated quartz tube at 560 °C with N_2 of 50 mL/min to afford [^{11}C]CCl_4. [^{11}C]CCl_4 was passed through a glass tube[22] coated with oxidizing agents at room temperature to produce [^{11}C]$COCl_2$. This multi-step process gave [^{11}C]$COCl_2$ at an average of 75% decay-corrected radiochemical yield ($n > 50$) based on the total [^{11}C]CO_2, which took about 10 min from the end of bombardment. The convenient and reliable production of [^{11}C]$COCl_2$ paved the way for radiolabeling with this agent.

Constructing a moiety of unsymmetrical [^{11}C]urea by the reaction of [^{11}C]$COCl_2$ with two different amines has been a challenging task.[23–26] The formation of symmetrical [^{11}C]urea due to the



Scheme 2. Chemical synthesis and radiosynthesis. Reagents and conditions: (a) CH_3CN, 50 °C, 2–8 h, 26–50%; (b) 400 °C, 2 min; (c) 560 °C, 2 min; (d) room temperature, 2 min, 70–80% from [^{11}C]CO_2; (e) THF, −15 °C, 1 min; (f) THF, 60 °C, 3 min, 21–70% (incorporation of total radioactivity in the final reaction mixture).

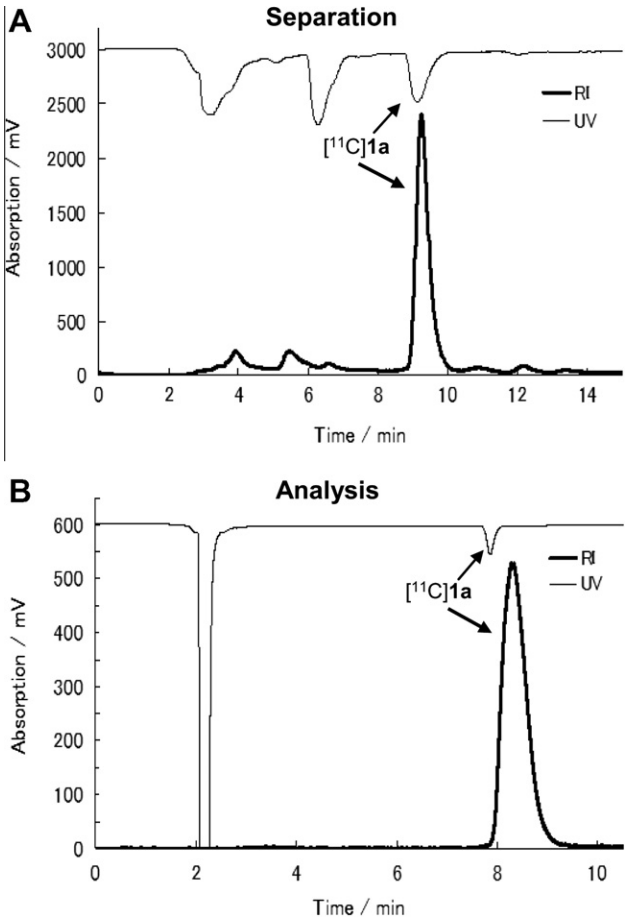


Figure 1. Typical chromatograms from the HPLC separation (A) and analysis (B) used in the radiosynthesis of [^{11}C]**1a**.

reaction of [^{11}C]$COCl_2$ with two molecules of the same amine is a main problem, which significantly decreases the labeling efficiency of unsymmetrical [^{11}C]urea. In the present experiment, after [^{11}C]$COCl_2$ gas had been bubbled into THF solution containing a mixture of 3-nitroaniline (**2a**; 0.28 mg, 2.03 μmol) and **3** (0.35 mg, 2.03 μmol) at −15 °C for 1 min, no radioactive peak corresponding to [^{11}C]**1a** was found in the reaction mixture. Two symmetrical [^{11}C]ureas of **2a** and **3** were found in the mixture, respectively. On the other hand, after [^{11}C]$COCl_2$ gas had been trapped into THF solution of **2a** at −15 °C for 1 min, subsequent treatment with **3** did not afford [^{11}C]**1a**. Only the symmetrical [^{11}C]urea of **2a** was formed in the reaction mixture. These results indicate that isocyante [^{11}C]**4a** (Scheme 2), which was yielded as an intermediate by reaction of [^{11}C]$COCl_2$ with one molecule of **2a**, was difficult to retain in the reaction mixture containing excess **2a** within the trapping of [^{11}C]$COCl_2$. Because the mass of **2a** largely exceeded that of [^{11}C]$COCl_2$, [^{11}C]**4a** could easily react with **2a** to give the symmetrical [^{11}C]urea.

To prevent formation of the symmetrical [^{11}C]urea, we used hydrochloride of **2a** (**2a**·HCl) in place of the free **2a** as a starting material (Scheme 2). Although the nucleophilicity of **2a**·HCl was decreased by this treatment, its reactivity remained enough to react with [^{11}C]$COCl_2$ perfectly to give [^{11}C]**4a**, even at −15 °C. On the other hand, due to the decreased reactivity of **2a**·HCl, [^{11}C]**4a** did not further react with excess **2a**·HCl at −15 °C. After 1-min trapping of [^{11}C]$COCl_2$, subsequent treatment with **3** gave the desired [^{11}C]**1a** with 70% incorporation yield of the total radioactivity in the reaction mixture. Moreover, no symmetrical [^{11}C]urea of **2a** or **3** was confirmed in the reaction mixture.

According to the reaction conditions examined here, we carried out automated synthesis of [^{11}C]**1a** using a multi-step synthesis system, which was previously developed in our institute.[19,20,27] [^{11}C]$COCl_2$ was bubbled into a solution of **2a**·HCl (0.18 mg, 1.02 μmol in 300 mL THF) at −15 °C for 1 min, followed by reaction of **3** (0.35 mg, 2.03 μmol in 300 mL THF) at 60 °C for 3 min. After the two-step reactions, THF was evaporated by N_2 flow. The reaction mixture was diluted and loaded onto a reversed-phase HPLC system.[28] Figure 1A shows a representative HPLC chromatogram for separation, which shows that [^{11}C]**1a** was produced as the main radioactive peak in the reaction mixture. Starting from 18–21 GBq of [^{11}C]CO_2, 0.74–0.88 GBq of [^{11}C]**1a** was obtained at the end of synthesis ($n = 5$). The total synthesis time was averaged as 38 min from the end of bombardment.

The identity of [^{11}C]**1a** was confirmed by co-injection with non-radioactive **1a** on analytic HPLC.[27] In the final product solution, the radiochemical purity of [^{11}C]**1a** was higher than 99% (Fig. 1B) and specific activity was 30 GBq/μmol. No significant UV peaks corresponding to **2a**, **3** and other chemical impurities were observed on the HPLC chart of the finally-formulated product. Moreover, the radiochemical purity of [^{11}C]**1a** remained >98% after 90 min at room temperature, and this product was radiochemically stable within the period of one PET scan. This analytical result was in compliance with our in-house quality control/assurance specifications.

Table 1 shows the synthetic results of [^{11}C]**1a–c**. In addition to [^{11}C]**1a**, the acetyl analog [^{11}C]**1b** was also obtained with enough radioactivity by reacting [^{11}C]$COCl_2$ with hydrochloride of 4′-aminoacetophenone (**2b**·HCl) in a moderate incorporation yield of radioactivity (Scheme 2). However, the radioactivity amount of the cyano analog [^{11}C]**1c**, which was obtained by reacting [^{11}C]$COCl_2$ with hydrochloride of 2-aminobenzonitrile (**2c**·HCl), was lower than those of [^{11}C]**1a** and [^{11}C]**1b**. By analyzing the reaction mixture, we found that [^{11}C]**1c** gradually decomposed within the reaction and purification processes. Although several agents (ascorbic acid, EtOH, etc.) for preventing radiolysis had been added to the reaction mixtures, decomposition of [^{11}C]**1c** over time could not be stopped.

Because it has been reported that **1a** had an observatory in vivo inhibitory effect on breast cancer metastases,[8] we selected [^{11}C]**1a** for preliminary in vitro evaluation. Cellular uptake of [^{11}C]**1a** was measured using HT-29 human colorectal adenocarcinoma cells,[15]

Table 1
Radiosynthesis results of [^{11}C]**1a–c**

Radioligand	R	Amount of radioactivity[a] (MBq)	Radiochemical purity (%)	Specific activity[c] (GBq/μmol)	Synthesis time from[d] (min)
[^{11}C]**1a**	3-NO_2	810 ± 32	99	31 ± 10	38 ± 2
[^{11}C]**1b**	4-CH_3CO	750 ± 81	97	34 ± 11	37 ± 5
[^{11}C]**1c**	2-CN	155 ± 20[b]	95	39 ± 8	38 ± 3

[a] Using 18.5–22.2 GBq of cyclotron-produced [^{11}C]CO2.
[b] The low yield was due to radiolysis of [^{11}C]**1c** within the reaction and purification processes.
[c] $n \geqslant 3$ at the end of synthesis.
[d] $n \geqslant 3$ at the end of bombardment.

which have high CA IX expression and MCF-7 breast tumor cells,[15] which have low CA IX expression, respectively. At 15 min and 60 min after incubation with [^{11}C]**1a**, the uptake level of radioactivity in HT-29 cells was 1.2-fold and 1.6-fold higher than that in MCF-7 cells, respectively. This result suggests that [^{11}C]**1a** might specifically accumulate in CA IX-rich tumor cells, although the difference in the uptake between HT-29 and MCF-7 cells was not as high as we anticipated. Because of the short half-life of ^{11}C, we could not measure the uptake of [^{11}C]**1a** in both tumor cell lines until after a longer time. To achieve a greater difference in the cellular uptake between the two cell lines, we will try to label **1a** with longer half-life positron emitters, such as ^{18}F (109 min) and ^{124}I (4.15 d).

In conclusion, [^{11}C]**1a**–**c** were synthesized using [^{11}C]$COCl_2$ as a labeling agent, via the intermediate preparation of isocyanate [^{11}C]**4a**–**c**. The labeling route was reliable and reproducible, which guaranteed enough radioactivity of [^{11}C]**1a** for in vitro and further in vivo evaluation. We are preparing animal models bearing several tumors and will perform PET imaging with [^{11}C]**1a** for the modeled animals. PET imaging with [^{11}C]**1a** may be a useful tool to study CA IX in the hypoxic tumors.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2011.09.102.

References and notes

1. Alterio, V.; Hilvo, M.; Di Fiore, A.; Supuran, C. T.; Pan, P.; Pakkila, S.; Scaloni, A.; Pastorek, J.; Pastorekova, S.; Pedone, C.; Scozzafava, A.; Monti, S. M.; De Simone, G. *Proc. Natl. Acad. Sci. U.S.A.* **2009**, *106*, 16233.
2. Supuran, C. T. *Nat. Rev. Drug Disc.* **2008**, *7*, 168–181.
3. Ozensoy, O.; De Simone, G.; Supuran, C. T. *Curr. Med. Chem.* **2010**, *17*, 1516.
4. De Simone, G.; Supuran, C. T. *Biochim. Biophys. Acta.* **2010**, *1804*, 404.
5. Wykoff, C. C.; Beasley, N. J.; Watson, P. H.; Turner, K. J.; Pastorek, J.; Sibtain, A.; Wilson, G. D.; Turley, H.; Talks, K. L.; Maxwell, P. H.; Pugh, C. W.; Ratcliffe, P. J.; Harris, A. L. *Cancer Res.* **2000**, *60*, 7075.
6. Said, H. M.; Supuran, C. T.; Hageman, C.; Staab, A.; Polat, B.; Katzer, A.; Scozzafava, A.; Anacker, J.; Flentje, M.; Vordemark, D. *Curr. Pharm. Des.* **2010**, *16*, 3288.
7. Hilvo, M.; Baranauskiene, L.; Salzano, A. M.; Scaloni, A.; Matulis, D.; Innocenti, S.; Scozzafava, A.; Monti, S. M.; Di Fiore, A.; De Simone, G.; Lindfors, M.; Janis, J.; Valjakka, J.; Pastorelova, S.; Pastorek, J.; Kulomaa, M. S.; Nordlund, H. R.; Supuran, C. T.; Parkkila, S. *J. Biol. Chem.* **2008**, *283*, 27799.
8. Pacchiano, F.; Carta, F.; McDonald, P. C.; Lou, Y.; Vullo, D.; Scozzafava, A.; Dedhar, S.; Supuran, C. T. *J. Med. Chem.* **2011**, *54*, 1896.
9. Fowler, J. S.; Volkow, N. D.; Wang, G.-J.; Ding, Y.-S.; Dewey, S. L. *J. Nucl. Med.* **1999**, *40*, 1154.
10. Kawamura, K.; Yamasaki, T.; Yui, J.; Hatori, A.; Konno, F.; Kumata, K.; Konno, F.; Irie, T.; Fukumura, T.; Suzuki, K.; Kanno, I.; Zhang, M.-R. *Nucl. Med. Biol.* **2009**, *36*, 239.
11. Zhang, M.-R.; Kumata, K.; Hatori, A.; Takai, N.; Toyohara, J.; Yanamoto, K.; Yamasaki, T.; Yui, J.; Kawamura, K.; Koike, S.; Ando, K.; Suzuki, K. *Mol. Imag. Biol.* **2010**, *12*, 181.
12. Asagawa, C.; Ogawa, M.; Kumata, K.; Fujinaga, M.; Yamasaki, T.; Kato, K.; Yui, J.; Kawamura, K.; Hatori, A.; Fukumura, T.; Zhang, M.-R. *Bioorg. Med. Chem. Lett.* **2011**, *21*, 2220.
13. Yamasaki, T.; Fujinaga, M.; Kumata, K.; Kawamura, K.; Yui, J.; Hatori, A.; Zhang, M.-R. *Nucl. Med. Biol.* **2011**, *38*, 707.
14. Ahlskog, J. K. J.; Dumelin, C. E.; Trussel, S.; Marlind, J.; Neri, D. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 4851.
15. Ahlskog, J. K. J.; Schliemann, C.; Marlind, J.; Ammar, A.; Pedley, R. B.; Neri, D. *Br. J. Cancer.* **2009**, *101*, 645.
16. Miller, P. M.; Long, N. J.; Vilar, R.; Gee, A. D. *Angew. Chem., Int. Ed.* **2008**, *47*, 8998.
17. Roeda, D.; Dollé, F. *Curr. Top. Med. Chem.* **2010**, *10*, 1680.
18. Nishijima, K.; Kuge, Y.; Seki, K.; Ohkura, K.; Motoki, N.; Nagatsu, K.; Tanaka, A.; Tsukamoto, E.; Tamaki, N. *Nucl. Med. Biol.* **2003**, *29*, 345.
19. Ogawa, M.; Takada, Y.; Suzuki, H.; Nemoto, K.; Fukumura, T. *Nucl. Med. Biol.* **2010**, *37*, 73.
20. Takada, Y.; Ogawa, M.; Suzuki, H.; Nemoto, K.; Fukumura, T. *Appl. Radiat. Isot.* **2010**, *68*, 1715.
21. Link, J. M.; Krohn, A. *J. Labelled Compd. Radiopharm.* **1997**, *40*, 306.
22. This tube, which is produced by Komyo Rikagaku Kogyo (Tokyo, Japan), is called as a Kitagawa gas detection tube used to measure the CCl_4 concentration in working environment.
23. Conway, T.; Diksic, M. *J. Nucl. Med.* **1988**, *29*, 1957.
24. Lidstrom, P.; Benasera, T. A.; Marquez-M, M.; Nilsson, S.; Bergstron, M.; Langstrom, B. *Steroids* **1998**, *63*, 228.
25. Lemoucheux, L.; Roudlen, J.; Ibezizene, M.; Sobrio, F.; Kasne, M. C. *J. Org. Chem.* **2003**, *68*, 7289.
26. Dollé, F.; Martarello, L.; Bramoulle, Y.; Bottlaendler, M.; Gee, A. D. *J. Labelled Compd. Radiopharm.* **2005**, *48*, 501.
27. Fukumura, T.; Suzuki, H.; Mukai, K.; Zhang, M.-R.; Yoshida, Y.; Nemoto, K.; Suzuki, K. *J. Labellled Compd. Radiopharm.* **2007**, *50*, S202.
28. *HPLC was performed using a JASCO HPLC system (JASCO, Tokyo). Column*: Capcell Pac C_{18} (Shiseido, Tokyo), 10 mm × 250 mm for purification, 10 mm × 250 mm for analysis; detector: UV 254 nM.
[^{11}C] ***1a***, *purification*: CH_3CN/H_2O = 40/60 (0.1% trifluoroacetic acid (TFA)), 5.0 mL/min, 8.5 min; analysis: CH_3CN/H_2O = 40/60 (0.1% TFA), 1.0 mL/min, 8.2 min.
[^{11}C] ***1b***, *purification*: CH_3CN/H_2O = 35/65 (0.1% TFA), 4.0 mL/min, 9.7 min; analysis: CH_3CN/H_2O = 30/70 (0.1% TFA), 1.5 mL/min, 6.0 min.
[^{11}C] ***1c***, *purification*: CH_3CN/H_2O = 45/65 (0.1% TFA), 4.5 mL/min, 12.0 min; analysis: CH_3CN/H_2O = 45/65 (0.1% TFA), 1.2 mL/min, 7.3 min.