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Utilization of [^{11}C]phosgene for radiosynthesis of *N*-(2-{3-[3,5-bis(trifluoromethyl)]phenyl[^{11}C]ure2;ido}ethyl)glycyrrhetinamide, an inhibitory agent for proteasome and kinase in tumors

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ABSTRACT

N-(2-{3-[3,5-Bis(trifluoromethyl)]phenylureido}ethyl)glycyrrhetinamide (**2**), an ureido-substituted derivative of glycyrrhetinic acid (**1**), has been reported to display potent inhibitory activity for proteasome and kinase, which are overexpressed in tumors. In this study, we labeled this unsymmetrical urea **2** using [^{11}C]phosgene ([^{11}C]$COCl_2$) as a labeling agent with the expectation that [^{11}C]**2** could become a positron emission tomography ligand for the imaging of proteasome and kinase in tumors. The strategy for the radiosynthesis of [^{11}C]**2** was to react hydrochloride of 3,5-bis(trifluoromethyl)aniline (**4**·HCl) with [^{11}C]$COCl_2$ to possibly give isocyanate [^{11}C]**6**, followed by the reaction of [^{11}C]**6** with *N*-(2-aminoethyl)glycyrrhetinamide (**3**).

18-β-Glycyrrhetinic acid (GA, **1**, Scheme 1) is the main constituent of *Glycyrrhiza glabra*, which has antitussive, antiulcer, antiallergic, anti-inflammatory, immunotrophic, and hypolipidemic activity.[1] In vitro inhibitory activity of **1** for animal and human tumor cells was found 30 years ago.[2] In vivo antitumor activity induced by **1** was also reported for various tumors-bearing animals.[3,4] The recent development of **1** derivatives has mainly focused on compounds having both cytotoxic and cytostatic properties, which can overcome multidrug resistance for chemotherapy.[5–8]

N-(2-{3-[3,5-Bis(trifluoromethyl)phenyl]ureido}ethyl)glycyrrhetinamide (**2**) is a new derivative of **1** with antitumor activity that enables drug resistance to be overcome when administrated with chemotherapeutic drugs.[9] Compound **2** showed cytostatic properties and high inhibitory activity (IC_{50}: 4–12 μM) for 8 tumor cell lines, including A549, SKMEL, T98G, HS683, U373, PC3, MGF7 and B16F10. This compound at 1 μM further inhibited the enzymatic activity of 12 kinases, such as anaplastic lymphoma, B-tyrosine, p-21 associated kinases family, enriched in tumors by >50%. In addition, **2** at 7 μM bound with 3 catalytic units of proteasome and significantly inhibited the activation of proteasome. Therefore, **2** was considered as a potent inhibitor of proteasome and kinases which are overexpressed in many types of tumors. The promising and unique pharmacological profiles of **2** as an antitumor agent promoted us to label this compound with positron-emitting carbon-11 (^{11}C; half life: 20.4 min) (Scheme 1), with the expectation that [^{11}C]**2** may become the first positron emission tomography (PET) ligand for the double imaging of kinase and proteasome in tumors.

PET is a useful molecular imaging modality with high functional sensitivity, which permits repeatable and non-invasive assessment of biological and pharmacological processes.[10,11] PET studies with radioligands have been used to quantitatively measure and elucidate the distribution and pharmacological action of receptors, enzymes, and transporters etc., which contributes to disease diagnosis and determination of the most effective doses of therapeutic drugs.[10–12] We have already performed a number of ^{11}C-labeling of antitumor agents[13–17] and used them as PET ligands for in vitro and in vivo evaluations.

In the present study, we synthesized [^{11}C]**2** (Scheme 1) for the first time. Regarding the chemical structure of **2** with hydroxy, amide, α,β-unsaturated ketone and urea functional groups, it is difficult to envisage the labelling of this compound using the conventional [^{11}C]methyl iodide without changing its structure and pharmacological activity. Fortunately, the presence of an unsymmetrical urea moiety in **2** prompted us to label the molecule using [^{11}C]phosgene ([^{11}C]$COCl_2$) as the labeling agent[18,19] (Scheme 1). The labeling approach included: (1) routine production of

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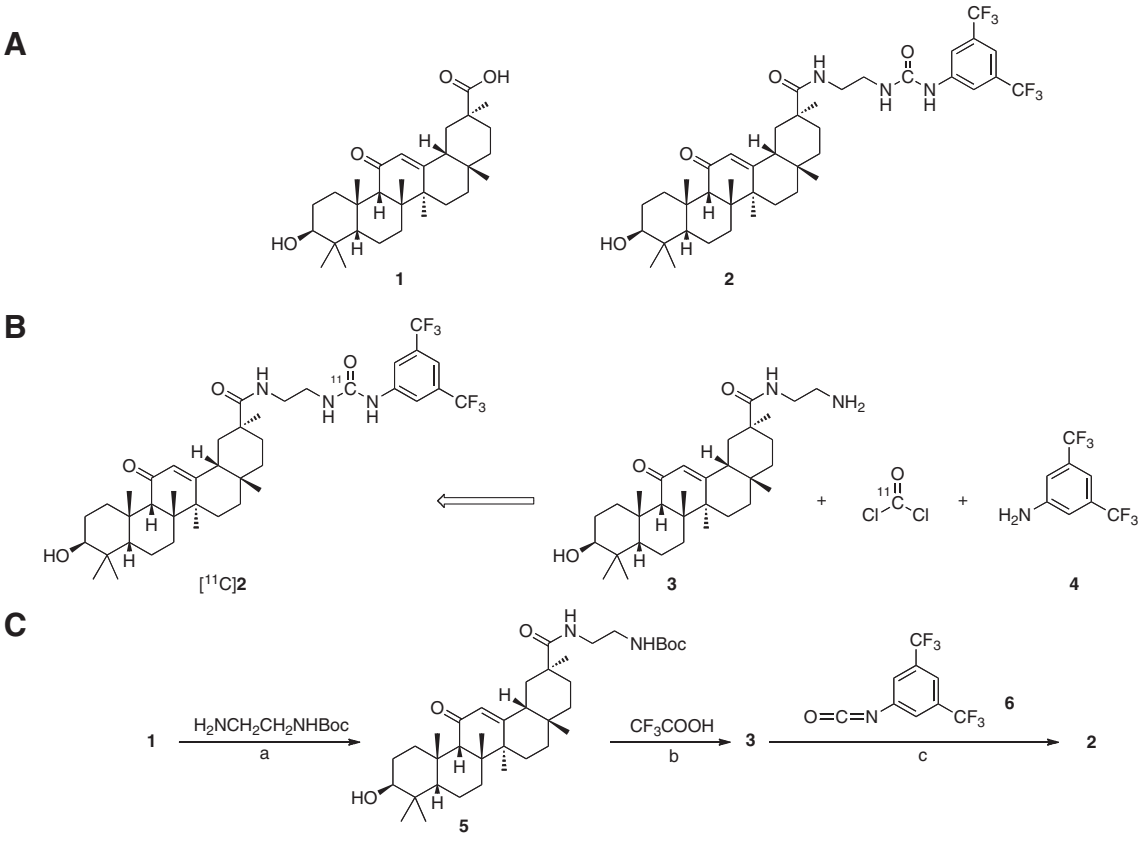


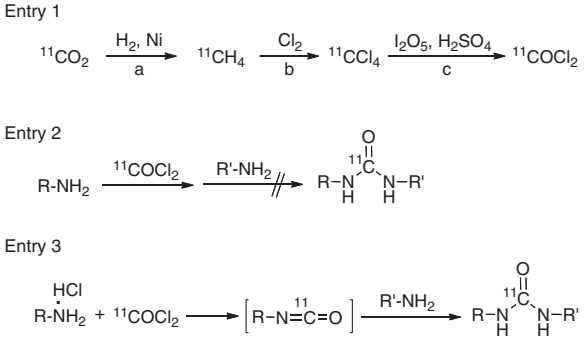
Scheme 1. Reagents and conditions: (A) Chemical structures of **1** and **2**; (B) retrosynthesis of [^{11}C]**2**; (C) syntheses of **2** and **3**: (a) *N*,*N*′-dicyclohexylcarbodiimide, hydroxybenzotriazole, DMF, room temperature, 10 h, 90%; (b)CH_2Cl_2, room temperature, 2 h, 65%; (c) THF, room temperature, 10 h, 76%.

[^{11}C]$COCl_2$[20–23] (2) synthesis and usefulness of two different amines: *N*-(2-aminoethyl)glycyrrhetinamide (**3**) and 3,5-bis(trifluoromethyl)aniline (**4**), and (3) construction of an unsymmetrical [^{11}C]urea moiety, as shown in the retro-synthetic route.

Before radiolabeling, we synthesized the non-radioactive compound **2**[9] and **3** as a precursor of the present radiosynthesis, according to the synthetic route delineated in Scheme 1C. The coupling reaction of **1** with *N*-(*tert*-butoxycarbonyl)-1,2-diaminoethane was prepared in the presence of *N*,*N*′-dicylclohexylcarbodiimide and hydroxybenzotriazole to give **5** in 90% yield. Removal of the Boc group of **5** with trifluoroacetic acid gave **3** in 65% yield. Reaction of **3** with commercially available isocyanate **6** in anhydrous THF at room temperature for 10 h afforded **2** as an authentic sample in 76% yield. Compounds **5**, **3** and **2** were identified by NMR and high-resolution mass spectra.[24]

The synthetic route for the preparation of of [^{11}C]$COCl_2$ as a labeling agent is shown in entry 1 of Scheme 2. [^{11}C]CH_4 was produced by the reduction of cyclotron-produced [^{11}C]CO_2 with H_2 using nickel metal as a catalyst. Chlorination of [^{11}C]CH_4 with Cl_2, followed by oxidation of [^{11}C]CCl_4, gave [^{11}C]$COCl_2$.[20–23]

In our facility, we have developed a convenient and reliable system to achieve the automated production of [^{11}C]$COCl_2$ for routine radiosynthesis.[21,22] After irradiation by a cyclotron beam on the target, [^{11}C]CO_2 was recovered from the target and concentrated in the inner space of a stainless steel tube, which was cooled under liquid N_2 in advance. [^{11}C]CO_2 was released from the steel tube and mixed with H_2 gas. Passing this mixture with H_2 of 10 mL/min through a methanizer, which was coated with nickel catalyst and heated at 400 °C, gave [^{11}C]CH_4. After mixing [^{11}C]CH_4 with Cl_2 gas carefully, the gaseous mixture was flowed through a quartz tube heated at 560 °C with N_2 of 50 mL/min to produce [^{11}C]CCl_4.



Scheme 2. Reagents and conditions: Production of [^{11}C]$COCl_2$ and strategy for constructing an unsymmetrical [^{11}C]urea moiety: (a) 400 °C, 2 min; (b) 560 °C, 2 min; (c) room temperature, 2 min, 60–85% from [^{11}C]CO_2 (*n* >50, decay-corrected).

Continuous passage of [^{11}C]CCl_4 through a glass tube filled with oxidizing agents at room temperature produced [^{11}C]$COCl_2$. The multi-step reaction afforded [^{11}C]$COCl_2$ with 60–85% radiochemical yield (*n* >50, decay-corrected) based on the total [^{11}C]CO_2, which required about 10 min from the end of bombardment under this online system. The routine production of [^{11}C]$COCl_2$ has enabled efficient synthesis of PET ligands containing [^{11}C]urea and [^{11}C]carbamate moieties.[15,17,25]

Reliable construction of an unsymmetrical [^{11}C]urea moiety had been a challenging task using the reaction of [^{11}C]$COCl_2$ with two different amines.[26–30] As shown in entry 2 of Scheme 2, formation of symmetrical [^{11}C]urea due to the reaction of [^{11}C]$COCl_2$ with two molecules of the same amine was the main problem. To

prevent the formation of symmetrical [^{11}C]urea, we have set up a reliable and convenient technique (entry 3). In place of the free amine (RNH_2), hydrochloride of the amine (RNH_2·HCl) was used as the starting material for the first step-reaction with [^{11}C]$COCl_2$. It was found that RNH_2·HCl had enough nucleophilicity to react with [^{11}C]$COCl_2$, producing a [^{11}C]isocyante as a possible intermediate.[15] Due to decreased nucleophilicity, the excess RNH_2·HCl could not further react with the [^{11}C]isocyante to yield the undesired symmetrical [^{11}C]urea. After the first reaction step, another amine $R'NH_2$ was added to the reaction mixture and reacted with the [^{11}C]isocyante to produce the desired unsymmetrical [^{11}C]urea. In this present study, we thus applied this technique to synthesize [^{11}C]**2** using hydrochloride of **3** or **4** (**3**·HCl or **4**·HCl) in place of free amine **3** or **4**.

[^{11}C]$COCl_2$ gas was firstly trapped in a THF solution containing **3**·HCl (0.25 mg, 0.46 μmol) at −15 °C for 1 min, possibly producing [^{11}C]isocyanate [^{11}C]**7**. Then, **4** (0.21 mg, 0.92 μmol) was added to this mixture and the reaction mixture was heated at 60 °C for 3 min (Fig. 1A). After the two-step reactions, the reaction mixture was analyzed and purified using a reversed-phase HPLC system.[31] Figure 1B shows the HPLC chromatogram of this reaction mixture, in which the desired [^{11}C]**2** was produced with 32.7% incorporation yield of the total radioactivity.

In place of **3**·HCl, **4**·HCl was also used as the precursor for radiosynthesis (Fig. 2). [^{11}C]$COCl_2$ gas was trapped in a THF solution containing **4**·HCl (0.25 mg, 0.94 μmol) at −15 °C for 1 min. After the 1-min [^{11}C]$COCl_2$ trapping step giving the possible [^{11}C]isocyanate [^{11}C]**6** , **3** (0.96 mg, 1.88 μmol) was added to the mixture and this reaction mixture was heated at 60 °C for 3 min (Fig. 2A). Figure 2B shows the HPLC chromatogram of the reaction mixture, in which the desired [^{11}C]**2** was produced with 69.1% incorporation yield of the total radioactivity.

By comparing the two synthetic routes of [^{11}C]**2**, we found that utilization of **4**·HCl gave a higher incorporation yield of radioactivity than utilization of **3**·HCl (Fig. 1B vs Fig. 2B). The difference between the two results might be caused by different nucleophilicity of **3**·HCl and **4**·HCl. Compared to the reactivity of alkylamine **3**·HCl with [^{11}C]**7**, aniline **4**·HCl might be less reactive towards [^{11}C]**6**. Subsequent addition of free **3** to the reaction mixture thus gave [^{11}C]**2** with more efficiency.

After optimizing the reaction conditions, such as the precursor amount, reaction temperature and time, we carried out automated synthesis of [^{11}C]**2** using a home-made system[32] to obtain reliable and reproducible amount of radioactivity. Starting from 16.7–22.2 GBq of [^{11}C]CO_2, 0.70–1.01 GBq of [^{11}C]**2** was obtained at the end of synthesis ($n = 7$) with a synthesis time of 34–37 min from the end of bombardment. The identity of [^{11}C]**2** was confirmed by co-injection with non-radioactive **2** on analytic HPLC. In the solution of finally-formulated product, the radiochemical purity of [^{11}C]**2** was higher than 97% and specific activity was 32–48 GBq/μmol. No significant UV peaks corresponding to **3**, **4** and other chemical impurities were observed on the HPLC chart of the product solution. Moreover, the radiochemical purity of [^{11}C]**2** remained >95% after standing for 90 min at room temperature, showing that this product was radiochemically stable within the period of at least one PET scan. The analytical results were in compliance with quality control/assurance specifications in our institute.

In conclusion, [^{11}C]**2** was synthesized using **4**·HCl as a precursor and [^{11}C]$COCl_2$ as a labeling agent, followed by the reaction with **3**. The labeling route was reliable and reproducible, which produced enough radioactivity of [^{11}C]**2** for evaluation experiment. We will use several tumor cell lines to perform an in vitro cellular uptake experiment and PET imaging of tumor-bearing rodents with [^{11}C]**2**. We hope that the results obtained by this study may be useful for elucidating the relationship between kinase/proteasome and chemotherapeutic resistance and studying the antitumor effects of **2** and its analogs.

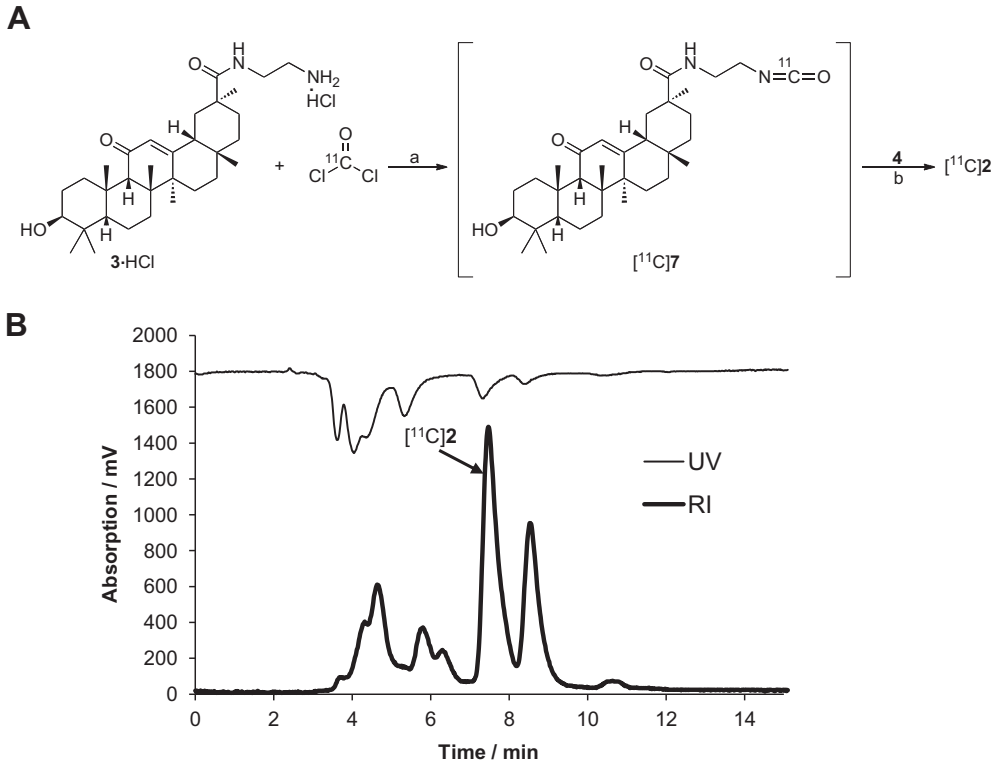


Figure 1. (A). Radiosynthesis from **3**·HCl: (a) THF, −15 °C, 1 min; (b) THF, 60 °C, 3 min, 32.7% (incorporation of total radioactivity in the final reaction mixture). (B) HPLC chromatogram for the reaction mixture.

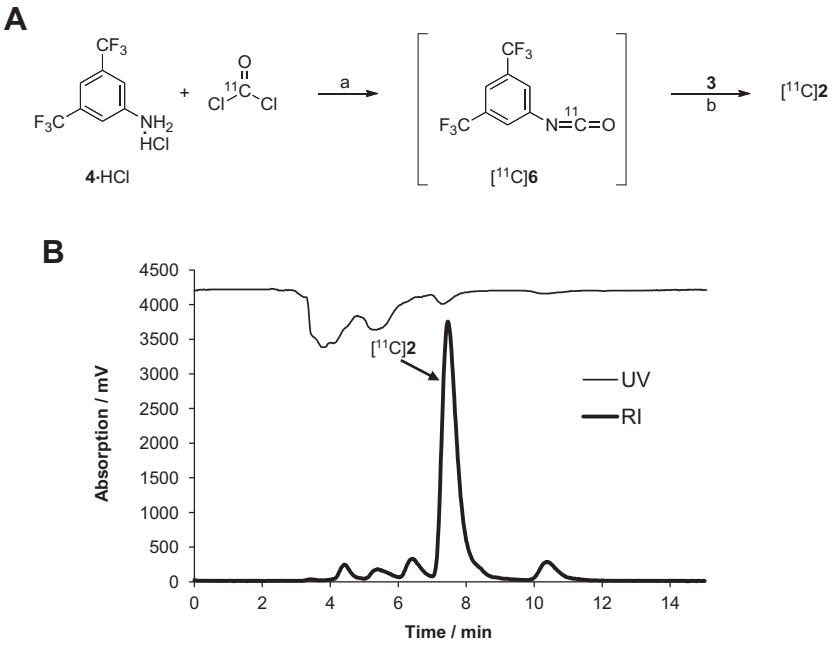


Figure 2. (A). Radiosynthesis from **4**·HCl: (a) THF, −15 °C, 1 min; (b) THF, 60 °C, 3 min, 69.1% (incorporation of total radioactivity in the final reaction mixture). (B) HPLC chromatogram for the reaction mixture.

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References and notes

1. Asl, M. N.; Hosseinzadeh, H. *Phytother. Res.* **2008**, *22*, 709.
2. Abe, H.; Ohya, N.; Yamamoto, K. F.; Shibuya, T.; Arichi, S.; Odashima, S. *Eur. J. Cancer Clin. Oncol.* **1987**, *23*, 1549.
3. Nishino, H.; Kitagawa, K.; Iwashima, A. *Carcinogenesis* **1984**, *5*, 1529.
4. O'Brian, C. A.; Ward, N. E.; Vogel, V. G. *Cancer Lett.* **1990**, *49*, 9.
5. Lee, C. S.; Kim, Y. J.; Lee, M. S.; Han, E. S.; Lee, S. J. *Life Sci.* **2008**, *83*, 481.
6. Gol'dberg, E. D.; Amosova, E. N.; Zueva, E. P.; Razina, T. G.; Krylova, S. G.; Zorikov, P. S. *Bull. Exp. Biol. Med.* **2008**, *145*, 252.
7. Logashenko, E. B.; Salomatina, O. V.; Markov, A. V.; Korchagina, D. V.; Salakhutdinov, N. F.; Tolstikov, G. A.; Vlassov, V. V.; Zenkova, M. A. *ChemBioChem* **2011**, *12*, 784.
8. Lallemand, B.; Gelbcke, M.; Dubois, J.; Prévost, M.; Jabin, I.; Kiss, R. *Mini Rev. Med. Chem.* **2011**, *11*, 881.
9. Lallemand, B.; Chaix, F.; Bury, M.; Bruyère, C.; Ghostin, J.; Becker, J. P.; Delporte, C.; Gelbcke, M.; Mathieu, V.; Dubois, J.; Prévost, M.; Jabin, I.; Kiss, R. *J. Med. Chem.* **2011**, *54*, 6501.
10. Lancelot, S.; Zimmer, L. *Trends. Pharmacol. Sci.* **2010**, *31*, 411.
11. Stephen, R. M.; Gillies, R. J. *Pharm. Res.* **2007**, *24*, 1172.
12. Wang, J.; Maurer, L. *Curr. Top. Med. Chem.* **2005**, *5*, 1053.
13. 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.
14. 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. Imaging Biol.* **2010**, *12*, 181.
15. Asakawa, 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.
16. Yamasaki, T.; Fujinaga, M.; Kumata, K.; Kawamura, K.; Yui, J.; Hatori, A.; Zhang, M.-R. *Nucl. Med. Biol.* **2011**, *38*, 707.
17. Asakawa, C.; Ogawa, M.; Kumata, K.; Fujinaga, M.; Yamasaki, T.; Xie, L.; Yui, J.; Kawamura, K.; Fukumura, T.; Zhang, M.-R. *Bioorg. Med. Chem. Lett.* **2011**, *21*, 7017.
18. Miller, P. M.; Long, N. J.; Vilar, R.; Gee, A. D. *Angew. Chem., Int. Ed.* **2008**, *47*, 8998.
19. Roeda, D.; Dollé, F. *Curr. Top. Med. Chem.* **2010**, *10*, 1680.
20. Nishijima, K.; Kuge, Y.; Seki, K.; Ohkura, K.; Motoki, N.; Nagatsu, K.; Tanaka, A.; Tsukamoto, E.; Tamaki, N. *Nucl. Med. Biol.* **2003**, *29*, 345.
21. Ogawa, M.; Takada, Y.; Suzuki, H.; Nemoto, K.; Fukumura, T. *Nucl. Med. Biol.* **2010**, *37*, 73.
22. Takada, Y.; Ogawa, M.; Suzuki, H.; Nemoto, K.; Fukumura, T. *Appl. Radiat. Isot.* **2010**, *68*, 1715.
23. Yashio, K.; Katayama, Y.; Takashima, T.; Ishiguro, N.; Doi, H.; Suzuki, M.; Wada, Y.; Tamai, I.; Watanabe, Y. *Bioorg. Med. Chem. Lett.* **2012**, *1*, 115.
24. *Analytic data of compounds are listed as follows*: Compound **5**, white solid; mp: 220–222 °C (223 °C[9]). ^{1}H NMR (300 MHz, DMSO-d_6) δ: 0.69 (8H, d, J = 11.73 Hz), 1.01 (11H, d, J = 7.70 Hz), 1.16 (4H, t, J = 7.33 Hz), 1.33 (6H, s), 1.35 (9H, s), 1.49–1.84 (8H, m), 1.98 (2H, s), 2.30 (1H, s), 2.54 (1H, d, J = 9.16 Hz), 2.98–3.15 (6H, m), 4.29 (1H, d, J = 5.13 Hz), 5.49 (1H, s), 6.68 (1H, br), 7.57 (1H, br). HRMS (FAB) Calcd for $C_{37}H_{60}N_2O_5$, 612.450; Found: 613.455 ([M+H]$^+$).
Compound **3**, white solid; mp: 153–155 °C. ^{1}H NMR (300 MHz, DMSO-d_6) δ: 0.69 (8H, d, J = 9.90 Hz), 0.89 (5H, s), 1.01 (11H, d, J = 4.40 Hz), 1.10–1.15 (2H, m), 1.27 (4H, s), 1.33 (6H, s), 1.40–2.07 (8H, m), 2.30 (1H, s), 2.97–3.16 (4H, m), 4.30 (1H, d, J = 5.13 Hz), 5.47 (1H, s), 7.49 (1H, t, J = 5.13 Hz). HRMS (FAB) Calcd for $C_{32}H_{52}N_2O_3$, 512.783; Found: 513.406([M+H]$^+$).
Compound **2**, white solid; mp: 179–181 °C (201 °C[9]). ^{1}H NMR (300 MHz, DMSO-d_6) δ: 0.64 (9H, d, J = 14.66 Hz), 0.81 (4H, s), 0.88 (4H, s), 0.94 (4H, s), 0.99 (4H, s), 1.04–1.09 (2H, m), 1.29 (6H, s), 1.35–2.02 (8H, m), 2.23 (2H, s), 2.97–3.20 (4H, m), 4.28 (1H, d, J = 5.13 Hz), 5.49 (1H, s), 6.32 (1H, br), 7.46 (1H, s), 7.69 (1H, br), 8.04 (2H, s), 8.31 (1H, s), 9.44 (1H, s). HRMS(FAB) Calcd for $C_{41}H_{55}N_3O_4F_6$, 767.900; Found: ([M+H]$^+$).
25. Kawamura, K.; Ogawa, M.; Yui, J.; Yamasaki, T.; Wakizaka, H.; Fujinaga, M.; Asakawa, C.; Kumata, K.; Fukumura, T.; Zhang, M.-R. *J. Labelled Compd. Radiopharm.* **2011**, *54*, S91.
26. Link, J. M.; Krohn, A. *J. Labelled Compd. Radiopharm.* **1997**, *40*, 306.
27. Conway, T.; Diksic, M. *J. Nucl. Med.* **1988**, *29*, 1957.
28. Lidstrom, P.; Benasera, T. A.; Marquez-M, M.; Nilsson, S.; Bergstron, M.; Langstrom, B. *Steroids* **1998**, *63*, 228.
29. Lemoucheux, L.; Roudlen, J.; Ibezizene, M.; Sobrio, F.; Kasne, M. C. *J. Org. Chem.* **2003**, *68*, 7289.
30. Dollé, F.; Martarello, L.; Bramoulle, Y.; Bottlaendler, M.; Gee, A. D. *J. Labelled Compd. Radiopharm.* **2005**, *48*, 501.
31. HPLC was performed using a JASCO HPLC system (JASCO, Tokyo). Column: Capcell Pac C18 (Shiseido, Tokyo), 10 mm × 250 mm for purification, 4.5 mm × 250 mm for analysis; detector: UV 254 nm; eluent: CH_3OH/H_2O = 95/5 (0.1% trifluoroacetic acid). Purification: 4.0 mL/min, 7.8 min; analysis: 1.0 mL/min, 6.2 min.
32. Fukumura, T.; Suzuki, H.; Mukai, K.; Zhang, M.-R.; Yoshida, Y.; Nemoto, K.; Suzuki, K. *J. Labelled Compd. Radiopharm.* **2007**, *50*, S202.