ChemPubSoc Europe

DOI: 10.1002/cmdc.201300167

Synthesis of an ^{11}C-Labeled Antiprion GN8 Derivative and Evaluation of Its Brain Uptake by Positron Emission Tomography

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N,*N*′-(Methylenedi-4,1-phenylene)bis[2-(1-pyrrolidinyl) acetamide] (GN8) is a promising candidate for the treatment of prion diseases. The purpose of this study was to synthesize a GN8 derivative labeled with a positron emitting radionuclide and to clarify the blood–brain barrier (BBB) permeability of the resultant derivative by positron emission tomography (PET). As a key synthetic intermediate, a GN8 derivative bearing a tributylstannyl group was prepared from commercially available materials in four steps. Palladium(0)-mediated rapid C-methylation of the aryltributylstannane using [^{11}C]methyl iodide yielded a [^{11}C]methyl-substituted GN8 derivative ([^{11}C]-**1**) with sufficient radioactivity (0.5–2.0 GBq) and specific radioactivity in the region of 60–126 GBq μmol^{-1}. [^{11}C]-**1** was injected into the tail vein of rats, and its biodistribution was determined by PET; the results unambiguously demonstrated the brain penetration of [^{11}C]-**1** in rat brain.

Prion diseases, also referred to as transmissible spongiform encephalopathies (TSEs), are a family of fatal neurodegenerative disorders that affect both humans and animals.[1,2] Human prion diseases include Creutzfeldt–Jakob disease (CJD), Gerstmann–Sträussler–Scheinker (GSS) syndrome, fatal familial insomnia, and kuru. The representatives of animal prion diseases are scrapie in sheep and goats, bovine spongiform encephalopathy (BSE) in cattle, and chronic wasting disease in deer and elk. These disorders are characterized by vacuolar degeneration of the central nervous system (CNS). The causative agent of the diseases is thought to be an infectious isoform of the prion protein, designated PrP^{Sc}, and an accumulation of PrP^{Sc} in the CNS gives rise to prion diseases.

Since the epidemic of BSE and the appearance of a new variant of CJD, which seems to be caused by PrP^{Sc}-contaminated beef consumption, much effort has been devoted to developing a therapeutic agent for prion diseases. A wide range of compounds have been identified as having antiprion activity in TSE-infected cells.[3,4] However, current therapeutic agents directed at prion diseases remain unsatisfactory[5,6] due to a lack of blood–brain barrier (BBB) permeability. Although an intraventricular injection of BBB-impermeable antiprion compounds is an alternative route for drug administration, it is invasive and harmful to the patients, and poses a risk for iatrogenic prion infections. Therefore, it is essential to develop antiprion agents that can cross the BBB.

During the course of our antiprion drug discovery and development studies,[7,8] GN8 was identified as a novel antiprion compound (Figure 1 a).[9–15] Subcutaneous administration of GN8 offered a survival benefit to TSE-infected mice.[9] Furthermore, nonclinical safety assessment of GN8 in rats and dogs revealed that GN8 could be used safely at the concentration nec-

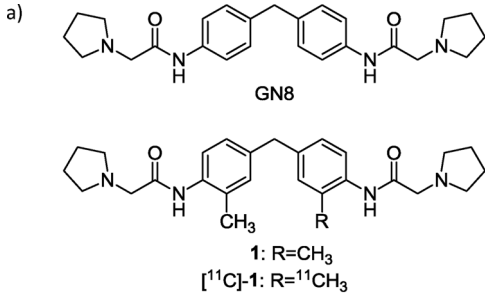

Figure 1. a) Chemical structures of GN8, and the unlabeled and ^{11}C-labeled methylated derivative of GN8 (**1**). b) Western blotting of proteinase K-resistant prion protein in GT+FK cells after treatment of the cells with GN8 and **1** at 10 μM.

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Supporting information for this article is available on the WWW under http://dx.doi.org/10.1002/cmdc.201300167.

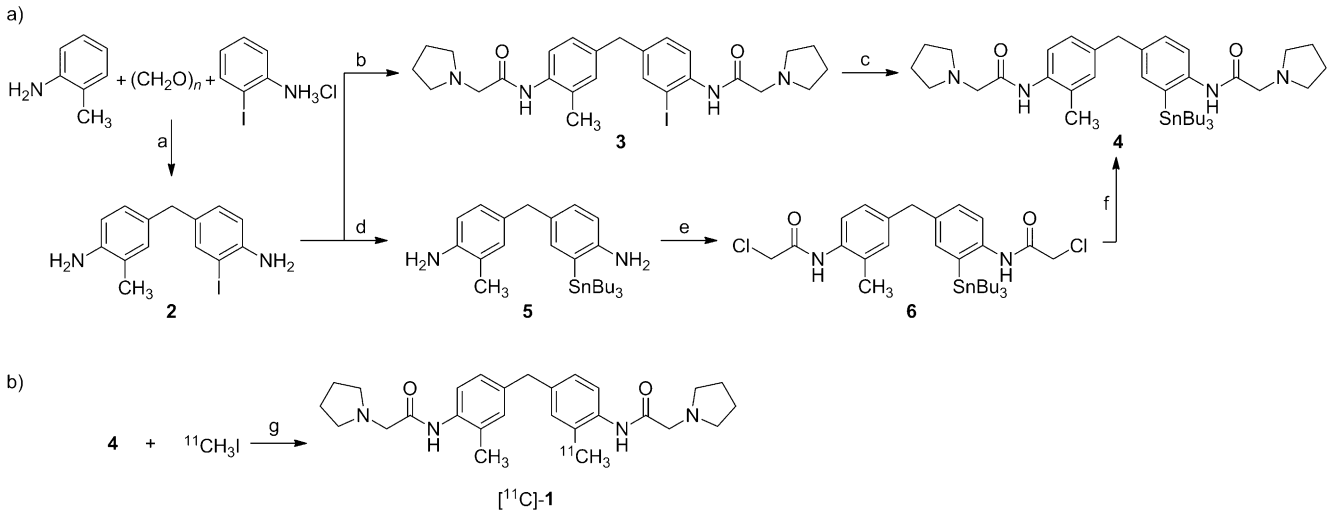
essary to exert antiprion activity.[15] PET is a powerful technique to assess the clinical dose, regimen selection, as well as brain penetration of the emitter-labeled compounds in prion disease patients. PET analysis of a GN8 derivative labeled with a positron-emitting radionuclide would give us important clues for antiprion drug development.[16] Herein, we report the synthesis of a ^{11}C-labeled GN8 derivative ([^{11}C]-**1**) by palladium-catalyzed rapid methylation of aryltributylstannane, and its subsequent assessment for BBB permeability and biodistribution in rat by PET.

As a positron emitting radionuclide-labeled compound, we designed [^{11}C]-**1** in which two methyl groups are linked to the diphenylmethane unit of GN8 at the 2,2′-positions via carbon–carbon bonds (Figure 1 a). To confirm the antiprion activity of the dimethyl-modified GN8 derivative, cold GN8 derivative **1** was prepared by bromoacetylation of 4,4′-diamino-3,3′-dimethyldiphenylmethane and subsequent substitution of the bromo groups with pyrrolidine (see Supporting Information).[14] The antiprion activity of **1** was tested in GT+FK cells, which are mouse neuronal cells (GT1–7) persistently infected with a mouse-adapted GSS agent (Fukuoka-1 strain) (Figure 1 b).[7,17,18] GN8 derivative **1** exhibited a similar order of activity to that of GN8 with an IC_{50} value of 2.35±0.12 μM. Thus, the modification of GN8 by the introduction of two methyl groups did not substantially affect its antiprion activity.[9]

^{11}C is a short-lived positron emitting radionuclide ($t_{1/2}$= 20.4 min). A ^{11}C-labeled methyl group can be incorporated into an aromatic framework via a carbon–carbon bond in a short synthesis time using palladium(0)-mediated rapid cross-coupling of aryltributylstannane with [^{11}C]methyl iodide.[19–23] Aryltributylstannane **4** is a key synthetic intermediate for the synthesis of [^{11}C]-**1** by rapid C-[^{11}C]methylation (Scheme 1 a). Initially, synthesis of **4** was examined via palladium-catalyzed tributylstannylation of aryl iodide **3**.[24] Unsymmetrically substituted 4,4′-diaminodiphenylmethane **2** was prepared from *ortho*-toluidine, 2-iodoaniline hydrochloride, and paraformaldehyde.[14,25] Bromoacetylation of diamine **2** and subsequent substitution reaction of the resulting bis(2-bromoacetamide) with pyrrolidine yielded compound **3**.[14] Unfortunately, several attempts to convert **3** to **4** by palladium-catalyzed tributylstannylation with bis(tributylstannane) were unsuccessful, presumably because of the substituent effect of the neighboring acylamino group. Alternatively, we examined the synthesis of **4** via tributylstannylation at an earlier stage. Aryl iodide **2** underwent a palladium-catalyzed coupling reaction with bis(tributylstannane) to afford aryltributylstannane **5** at a yield of 67%. Chloroacetylation of **5** gave bis(2-chloroacetamide) **6**, and the reaction of **6** with pyrrolidine in the presence of K_2CO_3 provided the desired aryltributylstannane **4**.

With the key precursor in hand, rapid methylation of aryltributylstannane **4** with [^{11}C]methyl iodide was carried out in the presence of palladium catalyst (Scheme 1 b).[19–23] After several experiments, we found that *N*-methylpyrrolidone (NMP) was an effective solvent for the rapid methylation of aryltributylstannane **4**. The optimized conditions for the reaction of aryltributylstannane **4** with [^{11}C]methyl iodide were NMP for five minutes to give the ^{11}C-labeled GN8 derivative [^{11}C]-**1** with sufficient radioactivity (0.5–2.0 GBq) for PET and a specific radioactivity of 60–126 GBq μmol^{-1}. The chemical purity analyzed at 254 nm was 82–92%, and the radiochemical purity was determined to be greater than 95%. The decay-corrected radiochemical yield, which was calculated from the radioactivity of [^{11}C]methyl iodide trapped in the palladium catalyst-containing reaction mixture, was approximately 20%. The total synthesis time, including HPLC purification and radiopharmaceutical formulation for intravenous administration, was 43 min.

To investigate the BBB permeability and the biodistribution of [^{11}C]-**1**, consecutive PET scans of the brain and whole body of Sprague–Dawley rats (n=4) were conducted. A 90 minute emission scan of the brain revealed that, after intravenous ad-



Scheme 1. Synthesis of aryltributylstannane **4** and radiosynthesis of ^{11}C-labeled GN8 derivative [^{11}C]-**1** by palladium-catalyzed rapid methylation with [^{11}C]-CH_3I. *Reagents and conditions*: a) CH_3OH, reflux, 18 h, 22%; b) 1. $BrCH_2C(O)Br$, pyridine, DMAP, CH_2Cl_2, 25 °C, 3 h; 2. pyrrolidine, K_2CO_3, THF, 60 °C, 12 h, 69% (two steps); c) $(Bu_3Sn)_2$, $Pd(PPh_3)_4$ (10 mol%), toluene, reflux, 48 h, 10%; d) $(Bu_3Sn)_2$, $Pd(PPh_3)_4$ (5 mol%), toluene, 100 °C, 24 h, 67%; e) $ClCH_2C(O)Cl$, Et_3N, CH_2Cl_2, 0 °C, 10 min, 83%; f) pyrrolidine, K_2CO_3, THF, 60 °C, 12 h, 92%; g) $Pd_2(dba)_3$, (*o*-tolyl)$_3$P, CuCl, K_2CO_3, NMP, 80 °C, 5 min.

ministration of [^{11}C]-**1**, radioactivity was observed in the pituitary gland, pineal body, cerebral cortex, and choroid plexus (Figure 2a). Around the head and neck, accumulation of [^{11}C]-**1** was observed in the thyroid gland, submandibular gland, and Harderian gland (Figure 2a). Whole-body PET images indicated that [^{11}C]-**1** uptake was scanned in the lung, liver, kidney, intestine, and spleen (Figure 2b). After the PET scan, the

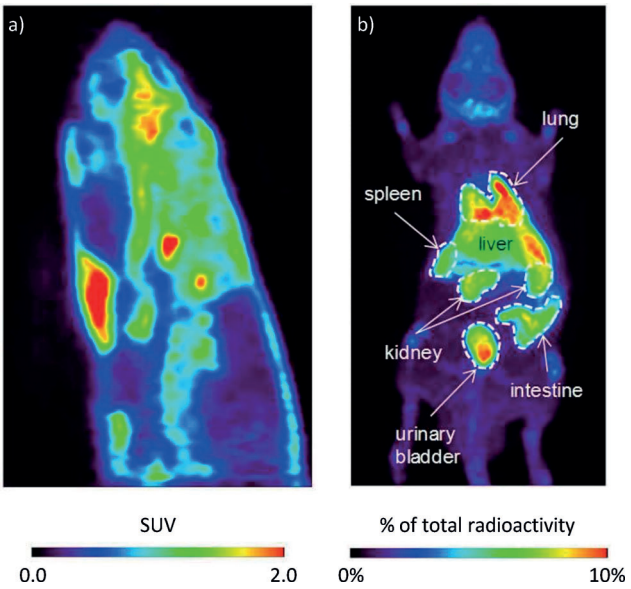


Figure 2. Positron emission tomography (PET) images of the brain and whole body. a) Maximum a posteriori probability (MAP) algorithm reconstructed static image taken 5–90 min after [^{11}C]-**1** injection (sagittal). b) Maximum intensity projection (MIP) image of 90–120 min after [^{11}C]-**1** injection. Standardized uptake values (SUV) are a semi-quantitative measure, derived from determination of tissue activity obtained from a single static image.

organs of the animal were dissected and the radioactivity of each tissue was measured with a gamma counter.

The tissue distribution data were in good agreement with the PET images, and the radioactivity of the brain was found to be higher than that of the blood or muscle (Table 1). These results demonstrated that [^{11}C]-**1** administered intravenously crosses the BBB and is retained in the brain.

To evaluate the degradation of **1** in vivo, we monitored its intravenous as well as intrabrain concentration in a time-dependent manner using a gas chromatography–mass spectrometry (GC/MS). The results showed that the elimination half-life of **1** is much longer than 24 hours, indicating that radioactivity is not originated from the degraded [^{11}C]-**1** (data not shown).

To confirm the distribution in brain, [^{11}C]-**1** was injected into rats, which were then sacrificed 40 minutes after administration. Auto-radiographic images of coronal sections of rat brain at 60 minutes post-injection are shown in Figure 3 along with their photographs. The auto-radiographic images clearly demonstrated that [11C]-**1** is primarily localized in the cerebral cortex, pineal body, pituitary gland, and choroid plexus.

Introduction of ^{11}C-lableled methyl group through a carbon–carbon bond is fascinating because it is resistant to in vivo metabolism in marked contrast to carbon–heteroatom bonds.

Table 1. Biodistribution data for [^{11}C]-**1**.

Tissue	ID [%][a]
Lung	4.98 ± 0.08
Kidney	2.17 ± 0.11
Spleen	2.11 ± 0.11
Liver	1.21 ± 0.06
Brain	0.36 ± 0.01
Heart	0.23 ± 0.03
Muscle	0.093 ± 0.005
Fat	0.04 ± 0.02
Blood	0.03 ± 0.002

[a] Data are the percent injected dose per gram of tissue ± standard error of the mean (SEM) of $n = 4$ independent experiments.

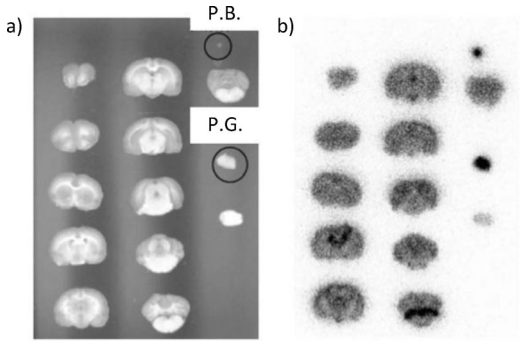


Figure 3. a) Photographic images of coronal sections of rat brain, and b) the corresponding auto-radiographic images at 60 min postinjection of [^{11}C]-**1**. Abbreviations: pineal body (P.B.) and pituitary gland (P.G.).

Transition-metal-catalyzed cross-coupling reactions are useful methods for constructing carbon–carbon bonds with excellent functional group tolerance.[26] We adopted palladium(0)-mediated rapid C-[^{11}C]-methylation using arylstannane **4** and [^{11}C]methyl iodide as a ^{11}C-labeling method,[19–23] since the conventional palladium-catalyzed cross-coupling reaction generally takes several hours. In addition, in practical terms, the ^{11}C-labeling must typically be carried out with small quantities of [^{11}C]methyl iodide (less than micromolar amounts) and excess amounts of stannyl precursor (greater than micromolar amounts). Thus, the palladium(0)-mediated rapid C-[^{11}C]methylation is an efficient method for ^{11}C-labeling that can meet the chemically difficult demands of radiolabeling conditions.[27,28]

As mentioned above, to date, a variety of compounds have already been identified as antiprion compounds in TSE-infected cells.[3,4] However, most of these compounds were ineffective in vivo, and only a limited number of compounds including amphotericin B and its derivative, pentosan polysulfate, and porphyrin derivatives have been reported to be effective in TSE-infected animals.[29–31] In clinical trials, pentosan polysulfate had to be administered intraventricularly since it did not penetrate the BBB, and no apparent improvement of clinical features was observed in the patients treated with this agent.[32] Treatment with quinacrine also failed to provide a therapeutic benefit, rather, it led to liver dysfunction.[33–35]

These agents were originally developed for the treatment of interstitial cystitis and malaria, respectively, and would not be suitable for the treatment of CNS disorders. Therefore, the pharmacokinetic properties, especially the BBB permeability, of the compounds should be carefully considered in drug development for prion diseases.[36,37] Previously, we reported that the subcutaneous administration of GN8 prolonged the lifetime of prion-infected mice, indicating that GN8 can enter the brain across the BBB. In this study, we confirmed that [^{11}C]-**1** administered intravenously also reaches the brain.

In summary, an ^{11}C-labeled GN8 derivative [^{11}C]-**1**, in which an ^{11}C-labeled methyl group was connected to the aromatic ring via a carbon–carbon bond, was successfully synthesized by palladium-catalyzed rapid methylation of aryl-(tributyl)stannane with [^{11}C]methyl iodide. PET analysis using the ^{11}C-labeled compound unequivocally demonstrated that the GN8 derivative penetrated into the brain. These findings will facilitate further refinement of GN8 as a therapeutic agent for prion diseases. Further studies on the application of the ^{11}C-labeled GN8 derivative as a molecular imaging probe for detecting cellular prion protein[38–40] in vivo are currently ongoing and will be reported in due course.

Experimental Section

PET studies using rats: Male Sprague–Dawley rats (Japan SLC, Inc., Hamamatsu, Shizuoka, Japan) at 8–9 weeks old and weighing approximately 250 g each were used in the study. All PET scans were performed using microPET F220 (Siemens Co., Ltd, Knoxville, TN, USA). Rats ($n=4$) were anesthetized and maintained with a mixture of 1.5% isoflurane and nitrous oxide/oxygen (7:3) and positioned in the PET scanner gantry. After intravenous bolus injection of [^{11}C]-**1** via the tail vein (~100 MBq per animal), a 90 min emission scan of the brain was performed with 400–650 keV as the energy window and 6 ns as the coincidence time window. Emission data of the brain were acquired in the list mode and sorted into dynamic sinograms (6×10 s, 6×30 s, 11×60 s, 15×180 s, 3×600 s; a total of 41 frames). After scanning the brain, a 30 min whole-body PET scan was conducted. For whole-body scans, the scanner bed was moved continuously in a reciprocating motion to ensure the entire body was scanned, and the list-mode data were sorted into dynamic sinogram for every one-bed pass. The data were reconstructed by a statistical maximum a posteriori probability algorithm (MAP) of ten iterations with point spread function (PSF) effect. During the experiment, a thermo-sensing probe was inserted into the rectum to monitor body temperature, which was maintained at 37 °C with a temperature controller (CMA150, CMA/Microdialysis, Stockholm, Sweden). The radioactivity concentrations were normalized with cylinder phantom data and expressed as standardized uptake values (SUV). For the whole-body scans (continuous bed motion acquisition), the radioactivity in the region of interest was estimated by percent of total radioactivity at each frame. After PET scanning, rats were euthanized and perfused with saline. Radioactivity of each tissue type was measured by using a gamma counter (Wallac1470, PerkinElmer, Waltham, MA, USA).

All experimental protocols were approved by the RIKEN Ethics Committee on Animal Care and Use and were performed in accordance with the Principles of Laboratory Animal Care (NIH publication No. 85-23, revised 1985).

Supporting Information: Instrument details, synthetic protocols and characterization data including NMR spectra for compounds **1**–**6** and the radiosynthesis of [^{11}C]-**1** are given in the Supporting Information along with the ex vivo assay procedure for evaluation in GT + FK cells.

Acknowledgements

The authors thank Ms. Tomomi Saeki and Ms. Miku Yamada (Center for Emerging Infectious Diseases, Gifu University) for outstanding research assistance. The authors also thank Dr. Hiroko Koyama (United Graduate School of Drug Discovery and Medical Information Science, Gifu University) for helpful discussion. This work was supported by the Program for the Promotion of Fundamental Studies in Health Sciences of the National Institute of Biomedical Innovation and the Molecular Imaging Research Program of the Ministry of Education, Culture, Sports, Science and Technology of Japan.

Keywords: antiprion agents · brain uptake · positron emission tomography (PET) · prion diseases · radiolabeling

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Received: April 18, 2013
Published online on May 24, 2013