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Synthesis and *in vivo* evaluation of ^{11}C-labeled (1,7-dicarba-*closo*-dodecaboran-1-yl)-*N*-{[(2*S*)-1-ethylpyrrolidin-2-yl]methyl}amide†

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Boron clusters, and especially dicarba-*closo*-dodecaboranes, can be used as hydrophobic pharmacophores in the design of new drugs and radiotracers because of their hydrophobic character, spherical structure, and excellent chemical and photochemical stability. In the present paper, the synthesis and *in vivo* evaluation of ^{11}C-labeled (1,7-dicarba-*closo*-dodecaboran-1-yl)-*N*-{[(2*S*)-1-ethylpyrrolidin-2-yl]methyl}amide, an analog of the D_2 receptor ligand [^{11}C]raclopride, is described. The radiosynthesis was approached by reaction of the demethylated precursor with [^{11}C]CH_3I in basic media; moderate radiochemical yields (18.2 ± 2.8%, decay corrected), and excellent radiochemical purities (>98%) were obtained in overall synthesis time of ~50 min. *In vivo* assays showed a biodistribution pattern with significant uptake in liver, kidneys and lungs at short times (*t* = 4 min) after administration and increasing accumulation in bladder at longer times (t ≥ 14.5 min). Although brain positron emission tomography scans showed good blood brain barrier penetration, the high unspecific uptake observed in different brain regions impedes its applicability as D_2 receptor ligand. Copyright © 2013 John Wiley & Sons, Ltd.

Keywords: carbon-11; raclopride; PET; dopamine; receptor; dicarba-*closo*-dodecaboranes

Introduction

Positron emission tomography (PET) is a non-invasive *in vivo* imaging technique, which produces three-dimensional images of functional processes in living organisms after administration of a radiotracer. Among all positron emitters, carbon-11 (half-life of 20.4 min and maximum positron energy of 960.5 KeV) is one of the most interesting ones because it can be produced in relatively high yields in commercially available cyclotrons. It can be easily introduced in biomolecules; its decay mode is close to 100% positron emission, and its stable isotope is present in all organic molecules. PET has been used for the early diagnosis,[1] evaluation of response to treatment,[2] determination of mechanistic aspects of biological and pathological processes,[3] and assessment of pharmacokinetic and pharmacodynamic properties of new drugs.[4] In this context, new radiotracers with improved properties, for example, higher affinity and specificity for a selected target, slower metabolism, or good penetration of blood brain barrier are becoming more and more demanded by the scientific community.

During the last decades, there has been an increasing interest in the development of radioligands for central D_2 receptors, which are G-protein coupled receptors implicated in many neurological processes, including motivation,[5] cognition,[6] memory,[7] learning,[8] and fine motor control.[9] Some of them [e.g., [^{11}C]raclopride, Figure 1(a)] have been widely applied both in preclinical[10] and in clinical studies.[11]

Endo and co-workers have reported that 1,2- and 1,7-dicarba-*closo*-dodecaborane (*o*-carborane and *m*-carborane) can mimic the hydrophobic structure of various biologically active molecules, because both their spherical structure and their hydrophobic surface could interact with hydrophobic residues of the ligand binding site of receptors.[12,13] Moreover, carboranes have excellent thermal and photochemical stability in the ultraviolet (UV)-visible range and are chemically versatile molecules. On the basis of the approach of Endo *et al.*, we have recently reported new analogs of raclopride by introducing different carborane clusters acting as hydrophobic pharmacophores using two different synthetic pathways.[14,15] In the present paper, we report the radiosynthesis of an analog of [^{11}C]raclopride incorporating a 1,7-dicarba-*closo*-dodecaborane unit [[^{11}C]-**1**, Figure 1(b)] and its evaluation as a potential D_2 receptor marker *in vivo*.

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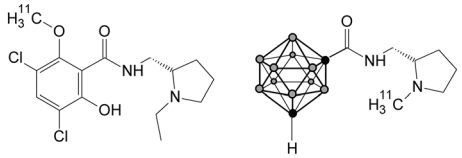


Figure 1. Chemical structure of (a) [^{11}C]raclopride and (b) ^{11}C-labeled (1,7-dicarba-*closo*-dodecaboran-1-yl)-*N*-{[(2*S*)-1-ethylpyrrolidin-2-yl]methyl}amide ([^{11}C]-**1**). ○ stands for BH, and ● stands for C.

Experimental section

General

Water and acetonitrile (MeCN, HPLC grade) were obtained from Panreac Química (Madrid, Spain). C-18 Light Sep-Pak® cartridges were obtained from Waters and were pre-conditioned, sequentially, with ethanol (5 mL) and water (5 mL). All other chemicals and solvents were Analytical Grade purity and purchased from Sigma-Aldrich, unless otherwise specified.

Radiochemistry

The synthesis of [^{11}C]CH_3I was carried out using a TRACERlab FX$_C$ Pro synthesis module (GE Healthcare). [^{11}C]CH_4 was directly generated in an IBA Cyclone 18/9 cyclotron by irradiation (target current = 22 μA, integrated current = 2 μAh) of a N_2/H_2 gas mixture with 18 MeV protons. The radioactive gas was trapped in Carbosphere 60/80 (Alltech Associates, Inc.) at −140°C, desorbed by heating at 80°C and allowed to react with iodine at 720°C to form [^{11}C]CH_3I in a gas circulating process. [^{11}C]CH_3I was selectively retained in a trap containing Porapak™ Q (50–80 mesh, Waters Corporation) at room temperature, while unreacted [^{11}C]CH_4 was recirculated. After 8–9 cycles, the Porapak™ Q trap was heated at 190°C, and [^{11}C]CH_3I was distilled under continuous helium flow at 20 mL/min for 2.5 min. The gas stream was passed through a trap containing phosphorous pentoxide and Ascarite II® (20–30 mesh) before being introduced in the reaction loop (AutoLoop™ system, Bioscan Inc.) pre-charged with a solution of (1,7-dicarba-*closo*-dodecaboran-1-yl)-*N*-{[(2*S*)-pyrrolidin-2-yl]methyl}amide (trifluoroacetate salt) in dimethylformamide (DMF, 100 μL) and triethylamine (10 μL). After complete trapping of [^{11}C]CH_3I, the reaction was allowed to occur for 6 min at room temperature. For those experiments performed with [^{11}C]CH_3OTf, [^{11}C]CH_3I synthesized as described previously was passed, before entering the reaction loop, through a trap filled with 150–200 mg of silver triflate fixed on Carbowax® 1500 80/100 (Grace Davison Discovery Science) in an online flowthrough process at 180°C under continuous helium flow (20 mL/min).[16] After reaction in the loop, the crude was purified by HPLC (stationary phase: Supelcosil™ LC-ABZ + C18 column, 250 × 10 mm, 5 μm; mobile phase: water/MeCN 10/90; flow rate = 5 mL/min), and the desired fraction (retention time = 20–21 min, radiometric and UV detection) was collected and reformulated using the TRACERlab FX$_C$ Pro synthesis module by diluting with water (20 mL), trapping in a C-18 cartridge, rinsing with water, elution with ethanol (1 mL), further elution with physiological saline solution (2 mL), and final filtration through 0.22 μm filter (Millex®-GS, Millipore).

The amount of radioactivity of the final radiotracer was measured in a dose calibrator (PETDOSE HC, Comecer), and a sample was submitted to quality control. The radiochemical purity was determined by HPLC, using an Agilent 1200 Series HPLC system with a multiple wavelength UV detector (λ = 254 nm) and a radiometric detector (Raytest). A RP-C18 column (Eclipse XDB C18, 4.6 × 150 mm, 5 μm particle size) was used as stationary phase and water/MeCN 25/75 as a mobile phase (retention time = 11 min). Specific activity was estimated based on historical specific activity values obtained using the same synthetic configuration, with the corresponding decay correction according to total preparation time. For those experiments devoted to the optimization of synthetic conditions, the crude reaction mixture was collected in a vial, and radiochemical conversion (RCC) values were calculated from chromatographic profiles obtained under the same conditions.

Identification of the desired radiotracer was carried out by HPLC-MS performed on the purified fraction after complete decay, using an AQUITY UPLC separation module coupled to a LCT TOF Premier XE mass spectrometer (Waters, Manchester, UK). An Acquity BEH C18 column (1.7 μm, 5 mm, 2.1 mm) was used as stationary phase. The elution buffers were MEOH (A) and 0.1% formic acid aqueous solution (B). The column was eluted with a linear gradient: t = 0 min, 95% B; t = 3 min, 1% B; t = 9 min, 1% B; and t = 10 min, 95%B. Total run was 10 min; injection volume was 5 μL, and the flow rate was 600 μL/min. The detection was carried out in positive ion mode, monitoring the most abundant isotope peaks from the mass spectra. Compound [^{11}C]-**1** was detected as a protonated species (m/z = 286.3) with retention time = 4.35 min.

In vivo studies

Male rats weighting 275–300 g (Sprague–Dawley, 9 weeks, Harlan, Udine, Italy) were used to perform PET studies. The animals were maintained and handled in accordance with the Guidelines for Accommodation and Care of Animals (European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes) and internal guidelines. PET studies were performed using an eXploreVista-CT small animal PET-CT system (GE Healthcare). During the PET studies, rats were kept normothermic using a heating blanket (Homeothermic Blanket Control Unit; Bruker). Three animals were submitted to brain scans, and three animals were subjected to whole body (WB) scans to assess the biodistribution pattern. In all cases, anesthesia was induced with 3% isoflurane and maintained by 1.5–2% of isoflurane in 100% O_2. The tail vein was catheterized with a 24-gauge catheter for intravenous administration of [^{11}C]-**1** (12 ± 2.4 MBq, 300 μL), which was injected concomitantly with the start of a PET dynamic acquisition.

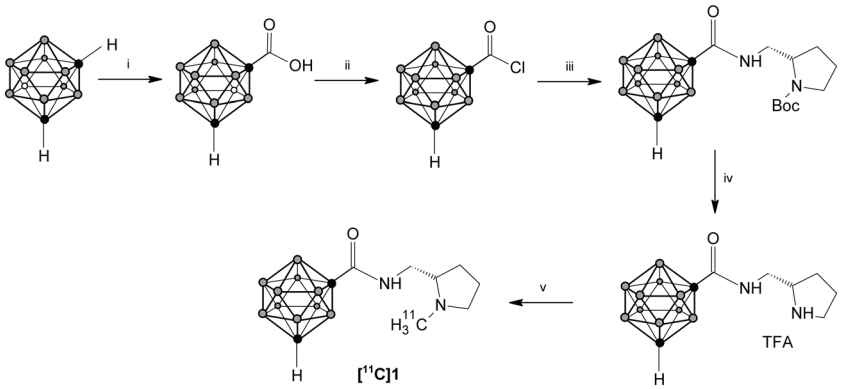
For brain scans, dynamic images were acquired (14 frames: 6 × 100 s, 4 × 4.5 min, and 4 × 8 min) in the 400–700 keV energetic window, with a total acquisition time of 1 h; for WB scans, the same frames were defined, but four beds were acquired at each frame. After each PET scan, CT acquisitions were also performed (140 μA intensity and 40 kV voltage), providing anatomical information of each animal as well as the attenuation map for the later image reconstruction. Dynamic acquisitions were reconstructed, decay and CT-based attenuation corrected, with filtered back projection using a Ramp filter with a cut off frequency of 1 Hz.

Positron emission tomography images were analyzed using PMOD image analysis software (PMOD Technologies Ltd, Zürich, Switzerland). For brain scans, Regions of Interests (ROIs) were automatically generated by using a magnetic resonance imaging rat brain atlas provided by the software. Subsequently, PET images were co-registered to the anatomical data of the atlas[17] by using the skull orientation provided by the CT image of the same animal, and ROIs were automatically applied. Specific brain regions such as the striatum and cerebellum were considered. For WB scans, ROIs were manually drawn using the CT images as anatomical reference. In both cases, time activity curves were derived for each ROI as percent of injected dose per gram of tissue.

Results and discussion

Radiochemistry

The radiosynthesis of ^{11}C-labeled (1,7-dicarba-*closo*-dodecaboran-1-yl)-*N*-{[(2*S*)-1-ethylpyrrolidin-2-yl]methyl}amide ([^{11}C]-**1**) was approached by *N*-methylation of the corresponding desmethyl precursor, which was prepared following our previously reported method (Scheme 1)[14]; briefly: *m*-carborane was converted into its

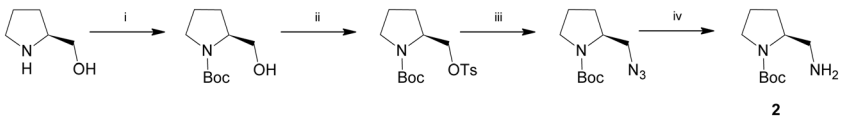


Scheme 1. Radiosynthesis of ^{11}C-labeled (1,7-dicarba-*closo*-dodecaboran-1-yl)-*N*-{[(2*S*)-1-ethylpyrrolidin-2-yl]methyl}amide and its desmethyl precursor. Reagents: (i) *n*-BuLi, ether, CO_2, then H_2O; (ii) PCl_5, distillation; (iii) (*S*)-(−)-2-Aminomethyl-1-Boc-pyrrolidine; (iv) trifluoroacetic acid; and (v) [^{11}C]CH_3I, dimethylformamide/triethylamine, room temperature, 6 min., grey circles stand for BH, and black circles stand for C.

corresponding acid by treatment with *n*-BuLi in THF followed by reaction with dry ice. After hydrolysis, the acid was reacted with phosphorous pentachloride in dry toluene and distilled to yield the acid chloride, which was reacted with (*S*)-(−)-2-aminomethyl-1-Boc-pyrrolidine (prepared according to Scheme 2) to yield the amide which gave the desired precursor (as trifluoroacetate salt) after treatment with TFA.

The synthesis of [^{11}C]CH_3I was carried out using the gas phase method, which was selected because the resulting radiotracers have higher specific activity than those prepared using the 'wet method'.[18] A methodology is well established in our laboratory. [^{11}C]CH_4 was directly generated in an IBA Cyclone 18/9 cyclotron by irradiation of a gas N_2/H_2 mixture with 18 MeV protons. The radioactive gas was allowed to react with iodine at 720°C to form [^{11}C]methyl iodide, which was first trapped, distilled under continuous helium flow and finally introduced in an HPLC loop pre-charged with a solution of the precursor and a base. During optimization runs, the reaction crude was analyzed by HPLC to determine RCC.

In our first attempts to synthesize [^{11}C]-**1**, DMSO was used as a solvent, and triethylamine was used as a base, while the amount of precursor was maintained at 1 mg (2.6 μmol). The reaction time was initially fixed to 6 min. None of the different base/precursor molar ratios used yielded the desired radiotracer. When DMF was used as solvent, RCC values in the range 6.5 ± 2.1–31.4 ± 4.8% were obtained for different precursor/base molar ratios (entries #1–#5, Table 1). Interestingly, an increase in the amount of base above 72 μmol did not lead to higher RCCs (30.3 ± 4.4% when 144 μmol of base were added). The same experimental conditions were



Scheme 2. (i) Synthesis of (*S*)-(−)-2-aminomethyl-1-Boc-pyrrolidine. Reagents: (i) Boc_2O, $NaHCO_3$(s), H_2O/THF; (ii) TsCl, pyridine; (iii) NaN_3, DMSO, microwave; and (iv) PPh_3, dimethylformamide and then H_2O.

Table 1. Experimental conditions and radiochemical conversion for the preparation of [^{11}C]-**1**

Entry	Precursor (μmol)	Base (μmol)	Base/precursor molar ratio	Rt (min)	RCC (%)*
1	2.6	7.2	2.8	6	6.5 ± 2.1
2	2.6	14.4	5.5	6	8.2 ± 3.3
3	2.6	28.8	11	6	9.0 ± 1.8
4	2.6	72	28	6	31.4 ± 4.8
5	2.6	144	55	6	30.3 ± 4.4
6	5.2	28.8	11	6	33.1 ± 6.2
7	5.2	72	28	6	31.7 ± 2.3
8	2.6	28.8	11	1	4.3 ± 3.0
9	2.6	72	28	1	10.5 ± 2.4
10	2.6	72	28	10	32.5 ± 6.1

All experiments were performed using dimethylformamide as solvent.
Values are expressed as mean ± standard deviation ($n = 3$ except entries #8 and #9, $n = 2$).
*RCC: radiochemical conversion, calculated from chromatographic profiles.

employed using a higher amount of precursor (2 mg), but the RCCs did not improve significantly (entries #6 and #7, Table 1). Longer reaction times (up to 10 min) also did not enhance RCCs (entry #10), while shorter reaction times yielded lower RCCs (entries #8 and #9). [^{11}C]methyl triflate was also investigated as methylating agent under identical experimental conditions, but no desired radiotracer could be detected in any case, even when other bases (NaOH 5 M aqueous solution, 2,4,6-trimethylpyridine) were used (results not shown).

Although the RCCs are not high when compared with typical values obtained for single methylation reactions using [^{11}C]CH_3I, the capability to generate high amounts of radioactivity in the [^{11}C]CH_4 target (up to 37 GBq in 25 min irradiation time) should allow the preparation of enough [^{11}C]-**1** for further *in vivo* investigation. On the basis of this assumption, a new set of experiments were performed under the experimental conditions shown in entry #4; a purification step using HPLC (water/MeCN 10/90, Experimental Section) was used. Under these conditions, the desired product could be purified in 20–21 min. Attempts to shorten retention times for [^{11}C]-**1** were unsuccessful because of the presence of a non labeled and unidentified impurity with slightly shorter retention time (17–19 min, Figure 2) which co-eluted with [^{11}C]-**1** when higher MeCN/water ratios were used.

Three complete runs including purification were performed under optimal experimental conditions starting from 7.4 GBq of [^{11}C]CH_4. After collecting the purified fraction and reformulating via C-18 trapping, elution with ethanol and reconstitution, 245 ± 38 MBq of pure [^{11}C]-**1** were obtained in average production times of 50 min (decay corrected radiochemical yield of 18.2 ± 2.8%). Specific radioactivity values were estimated to be in the range 80–120 GBq/μmol (EOS) according to historical values obtained in our laboratory with the same automatic configuration. The identity of the desired radiotracer was confirmed by HPLC-MS on the collected fraction after complete decay. The molecule was detected as $[M+H]^+$ (m/z = 286.3, retention time = 4.35 min, Experimental Section). Radiochemical purity at the end of the synthesis was > 98% in all cases, and the radiotracer showed to be stable in ethanol/physiologic saline solution at least for 60 min after end of the synthesis (radiochemical purity >95% at t = 60 min). As expected, both the amount and the concentration of radioactivity (245 MBq and 81.7 MBq/mL, respectively) were sufficient to perform *in vivo* experiments.

In vivo studies

The WB biodistribution of [^{11}C]-**1** in rats was measured as the decay-corrected percentage of injected dose per gram of tissue (% ID/g). Figure 3 shows the data corresponding to the distribution of [^{11}C]-**1** in heart, bladder, kidneys, liver, brain, and lungs at different time points after administration of the radiotracer. The low amount of radioactivity detected in the heart suggests a fast clearance of the radiotracer from the bloodstream. High initial uptake of radioactivity was found in the kidneys, liver, and lungs, followed by a decrease of radioactivity in these organs thereafter. A significant uptake of the radiotracer was detected in the brain; the amount of radioactivity peaked at t = 2.5 min (%ID/g = 9.9 ± 1.2%) slowly decreasing to the end of the study (%ID/g = 4.7 ± 0.8% at t = 60 min). The accumulation of the tracer in the bladder confirms the elimination of the tracer by the urinary system, although significant accumulation was also detected in small intestine and stomach at t > 8 min (Figure 4).

When brain scans were performed, a good blood brain barrier penetration of the radiotracer was observed. However, slightly higher uptake was measured in the cerebellum when compared with striatum, suggesting a high unspecific uptake of the radiotracer (Figure 5). Although this difference was not statistically significant, the high uptake in a region with low density of D_2 receptors suggests that this tracer is not appropriate for the *in vivo* assessment of D_2 receptor density. The high uptake might be due to the lipophilic character of the radiotracer, resulting from the presence of the carborane

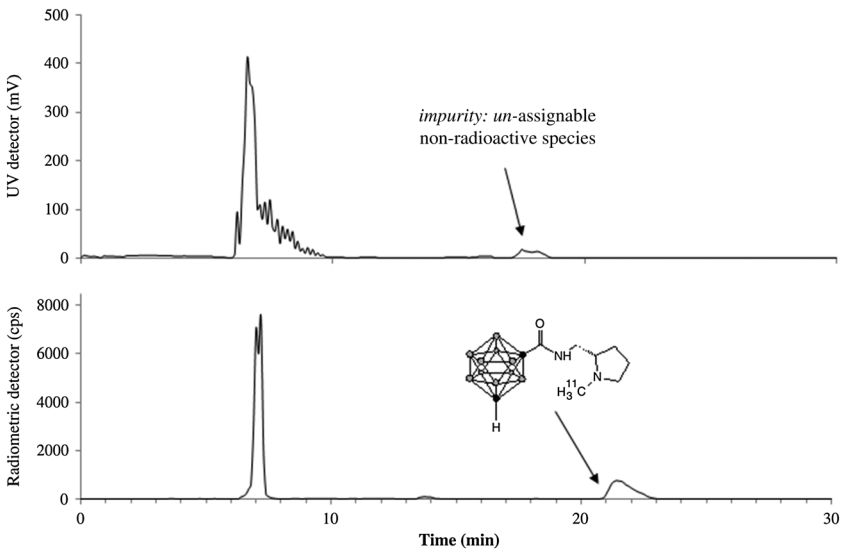


Figure 2. Chromatographic profiles corresponding to the purification of [^{11}C]-**1**. Radiometric detector (lower trace) and ultraviolet detector (upper trace) profiles are shown.

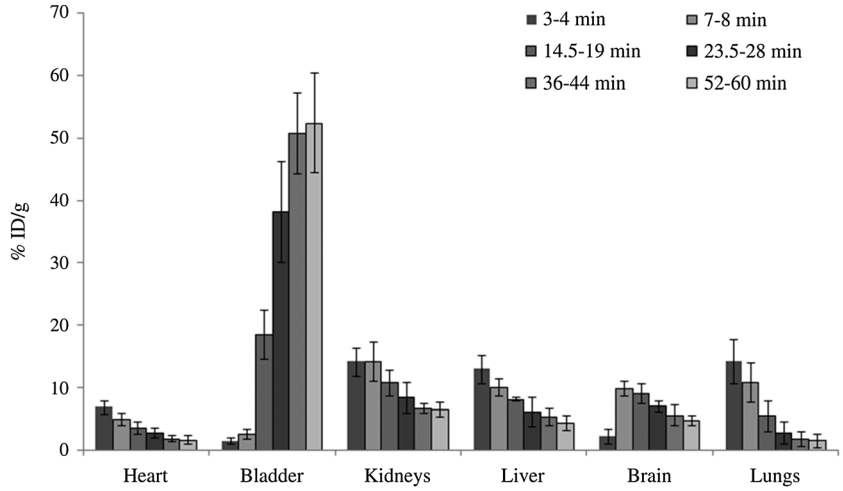


Figure 3. Biodistribution of [^{11}C]-**1** in rat tissues ($n = 3$) as determined by positron emission tomography. Radioactivity is expressed as the percentage of the injected dose per gram of tissue (% ID/g; mean ± SD).

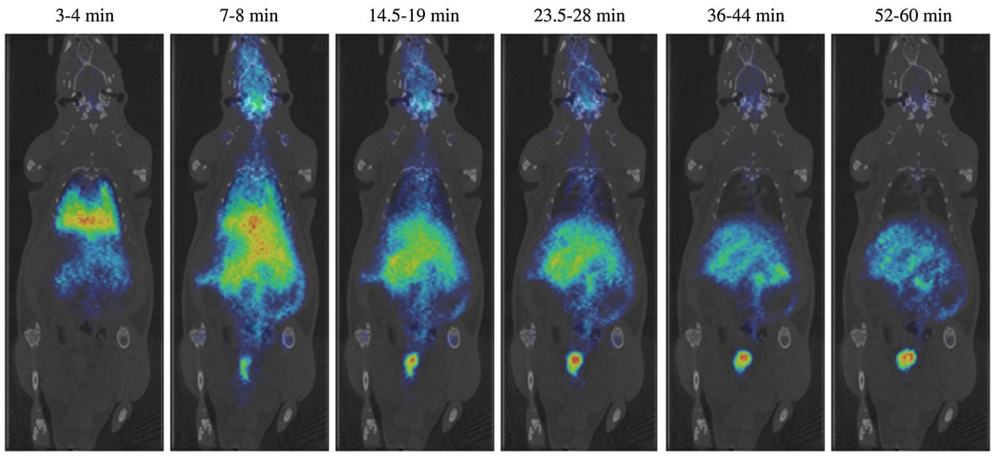


Figure 4. Positron emission tomography (PET) coronal projection images of [^{11}C]-**1** signal at different time points after tracer injection. The PET image is co-registered with the CT image to localize the PET signal anatomically. This figure is available in color online at wileyonlinelibrary.com/journal/jlcr

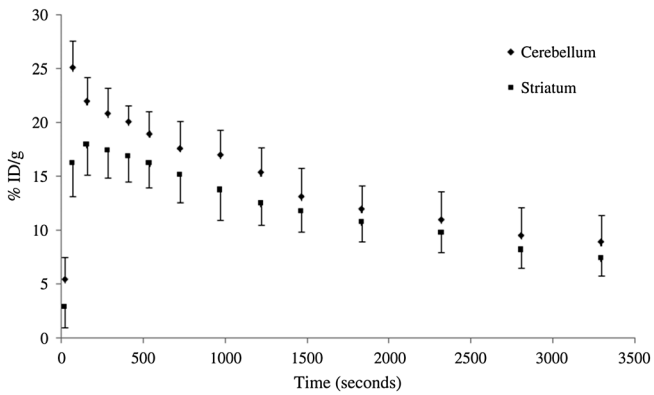


Figure 5. Time activity curves for cerebellum and striatum after administration of [^{11}C]-**1**. Radioactivity is expressed as the percentage of the injected dose per gram of tissue (% ID/g; mean ± SD).

unit. Future work will be focussed on appropriate substitution of the carborane cage and generation of the *nido* species to decrease hydrophobicity.

Conclusions

The synthesis of ^{11}C-labeled (1,7-dicarba-*closo*-dodecaboran-1-yl)-*N*-{[(2*S*)-1-ethylpyrrolidin-2-yl]methyl}amide was achieved by reaction of the demethylated precursor with [^{11}C]CH_3I in DMF under basic conditions. Moderate radiochemical yields (18.2 ± 2.8%, decay corrected), and excellent radiochemical purities (>98%) were obtained in average synthesis times of ~ 50 min. The radiotracer showed a fast uptake in liver, kidneys, and lungs at short times after [^{11}C]-**1** administration followed by a significant increase in the bladder. Brain scans showed non-specific binding. These results suggest that [^{11}C]-**1** is not suitable to be used as D_2 receptor ligand.

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Conflict of Interest

The authors did not report any conflict of interest.

REFERENCES

[1] Z. Garami, Z. Hascsi, J. Varga, T. Dinya, M. Tanyi, I. Garai, L. Damjanovich, L. Galuska, *Eur. J. Surg. Oncol.* **2012**, *38*(1), 31.
[2] L. Kostakoglu, *PET Clinics* **2008**, *3*(1), 37.
[3] W. K. Schiffer, D. E. Lee, J. D. Brodie, S. L. Dewey, *Drug Discov. Today* **2005**, *10*(8), 547.
[4] C. C. Wagner, O. Langer, *Adv. Drug Deliver Rev.* **2011**, *63*(7), 539.
[5] Z. B. Bulwa, J. A. Sharlin, P. J. Clark, T. K. Bhattacharya, C. N. Kilby, Y. Wang, J. S. Rhodes, *Alcohol* **2011**, *45*(7), 631.
[6] B. Gelao, R. Romano, P. Taurisano, G. Caforio, A. Di Giorgio, A. Rampino, A. Porcelli, L. Fazio, L. Lo Bianco, M. Nardini, G. Blasi, A. Bertolino, *Schizophr. Res.* **2008**, *102*(1–3, Supplement 2), 181.
[7] I. S. Tarantino, R. F. Sharp, M. A. Geyer, J. M. Meves, J. W. Young, *Behav. Brain Res.* **2011**, *219*(2), 181.
[8] A. Stuchlik, L. Rehakova, P. Telensky, K. Vales, *Neurosci. Lett.* **2007**, *422*(3), 169.
[9] S. C. Fowler, T. J. Zarcone, E. Vorontsova, R. Chen, *Int. J. Dev. Neurosci.* **2002**, *20*(3–5), 309.
[10] A. Martín, V. Gómez-Vallejo, E. San Sebastián, D. Padró, I. Markuerkiaga, I. Llarena, J. Llop, *J. Cerebr. Blood F Met.* **2012**, *33*, 244.
[11] A. M. Catafau, M. Suarez, S. Bullich, J. Llop, G. Nucci, R. N. Gunn, C. Brittain, M. Laruelle, *Neuroimage* **2010**, *46*, 447.
[12] Y. Endo, T. Iijima, Y. Yamakoshi, M. Yamaguchi, H. Fukasawa, K. Shudo, *J. Med. Chem.* **1999**, *42*, 1501.
[13] T. Ogawa, K. Ohta, T. Yoshimi, H. Yamazaki, T. Suzuki, S. Ohta, Y. Endo, *Bioorg. Med. Chem. Lett.* **2006**, *16*, 3943.
[14] N. Vázquez, V. Gómez-Vallejo, J. Calvo, D. Padro, J. Llop, *Tetrahedron Lett.* **2011**, *52*(5), 615.
[15] K. B. Gona, V. Gómez-Vallejo, J. Llop, *Tetrahedron Lett.* **2013**, *54*(8), 941.
[16] D. M. Jewett, *Int. J. Rad. Appl. Instrum. Part A. Appl. Radiat. Isot.* **1992**, *43*(11), 1383.
[17] P. Schweinhardt, P. Fransson, L. Olson, C. Spenger, J. L. R. Andersson, *J Neurosci Meth* **2003**, *129*, 105.
[18] V. Gómez-Vallejo, V. Gaja, J. Koziorowski, J. Llop, in *Positron Emission Tomography-Current Clinical and Research Aspects*, (Ed.: C.-H. Hsieh), Intech, **2012**, 183.