Research Article

Journal of Labelled Compounds and Radiopharmaceuticals

Received 16 June 2008, Revised 15 December 2008, Accepted 23 December 2008 Published online 26 January 2009 in Wiley Interscience

(www.interscience.wiley.com) DOI: 10.1002/jlcr.1584

One-pot radiosynthesis of [^{13}N]urea and [^{13}N]carbamate using no-carrier-added [^{13}N]NH_3

Katsushi Kumata,[a] Makoto Takei,[a,b] Masanao Ogawa,[a,c] Koichi Kato,[a] Kazutoshi Suzuki,[a] and Ming-Rong Zhang[a]*

The aim of this study was to develop a practical labeling method of [^{13}N]ligands using no-carrier-added [^{13}N]NH_3 with high specific activity. [^{13}N]urea analogues [^{13}N]1a and [^{13}N]2a or [^{13}N]carbamate [^{13}N]3a were synthesized by reacting isocyanate 5a, carbamoyl chloride 6a or chloroformate 7a with [^{13}N]NH_3. The precursors 5a–7a were prepared by treating amines 8a and 9a and alcohol 10a with triphosgene *in situ*. These reaction mixtures were not purified and were used directly for [^{13}N]ammonolysis, respectively. Using the one-pot method, we synthesized [^{13}N]carbamazepine ([^{13}N]4), a putative positron emission tomography ligand for brain imaging.

Keywords: [^{13}N]NH_3; [^{13}N]urea; [^{13}N]carbamate; [^{13}N]carbamazepine; positron emission tomography

Introduction

Many pharmaceuticals and biological substances developed for clinical use and pharmacological test contain nitrogen atoms. Labeling these drugs and bioactive compounds with a radioactive nitrogen isotope and using the radioligands for *in vitro* and *in vivo* studies is a powerful tool to examine their behavior, therapeutic and toxic properties without changing their chemical structures and pharmacological profiles. Of several nitrogen isotopes, nitrogen-13 (^{13}N; half-life: 9.965 min; 100% β^+ decay) is an important positron-emitting radioisotope for positron emission tomography (PET) study. In PET study, ^{13}N has been used mostly in the chemical form [^{13}N]NH_3.[1–3] The short half-life of ^{13}N has the advantage that repeated studies may be performed on the same individual within a short period of time. Compared with other positron emitters such as ^{11}C and ^{18}F, PET scan using [^{13}N]ligand gives relatively low radiation damage on the subject. However, no useful [^{13}N]ligands except [^{13}N]NH_3 have been used for clinical investigation until now.

The main reason is that practical labeling technique using no-carrier-added (nca) [^{13}N]NH_3 has not been established. Radiosynthesis for PET ligands involving short-lived positron emitters, such as ^{13}N, requires practical methods and techniques under radiation protection. Another characteristic of radiosynthesis is the efficient production and application of labeling agents such as [^{13}N]NH_3. There had been a number of reports about the syntheses of [^{13}N]ligands using low-specific-activity [^{13}N]NH_3 or carrier-added [^{13}N]NH_3.[4–8] Owing to the short half-life of ^{13}N and easy contamination by the N carrier, it is difficult to synthesize [^{13}N]ligands with high specific activity and sufficient radioactivity to carry out receptor studies with PET. However, to image a receptor with low density in the brain, a PET ligand with high specific activity is required.[9] Recently, Suzuki and Yoshida developed an automated synthetic system to produce [^{13}N]NH_3 with high specific activity (1850 GBq/μmol).[10] Using this system, they have prepared *p*-nitrophenyl [^{13}N]carbamate by reacting the corresponding chloroformate with nca [^{13}N]NH_3.[11]

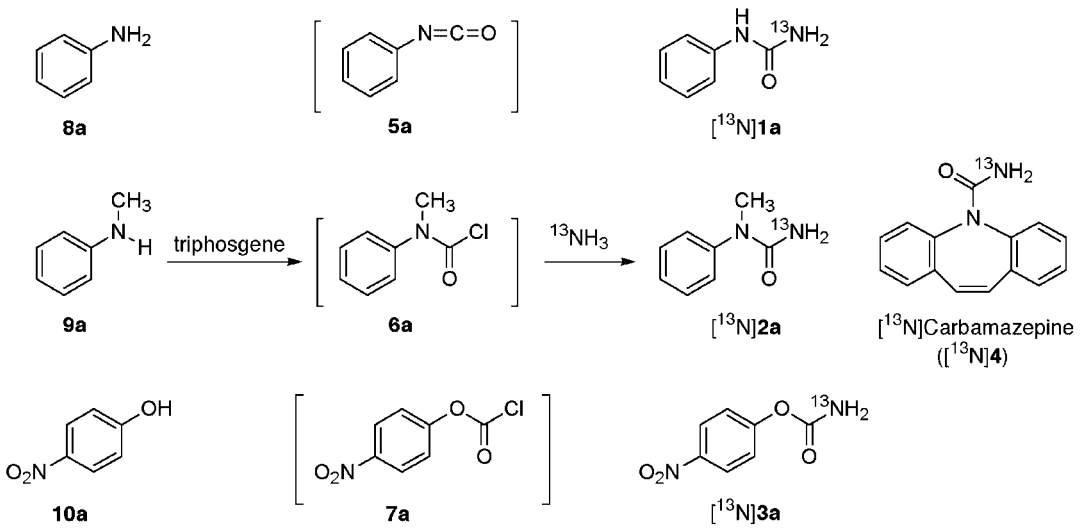
Here, we aimed to determine a practical labeling technique using nca [^{13}N]NH_3 with high specific activity. We wanted to develop a one-pot method for preparing [^{13}N]urea analogues [^{13}N]**1a** and [^{13}N]**2a** and [^{13}N]carbamate [^{13}N]**3a** (Scheme 1) starting from amine and alcohol. [^{13}N]urea and [^{13}N]carbamate analogues, such as [^{13}N]carbamazepine ([^{13}N]**4**, Scheme 1), often have abundant pharmacological profiles and biological activities.[12–14] Although ^{11}C-labeled urea and carbamate analogues have been synthesized using [^{11}C]CO,[15] radiosynthesis of [^{13}N]urea analogues with nca [^{13}N]NH_3 starting from amine remained a challenging task. In this study, ligands [^{13}N]**1a**–**3a** were prepared by reacting nca [^{13}N]NH_3 with isocyanate **5a**, carbamoyl chloride **6a** and chloroformate **7a**, respectively. Owing to their putative unstability, **5a**–**7a** were prepared by treating the corresponding substrates **8a**–**10a** with triphosgene[16–18] *in situ*, and these reaction mixtures were used directly for [^{13}N]ammonolysis. Using the new labeling method, we

[a]*Department of Molecular Probes, Molecular Imaging Center, National Institute of Radiological Sciences, 4-9-1 Anagawa, Inage-ku, Chiba 263-8555, Japan*

[b]*Tokyo Nuclear Service Co. Ltd., 7-2-7 Ueno, Taito-ku, Tokyo 110-0005, Japan*

[c]*SHI Accelerator Service Co. Ltd., 1-17-6 Osaki, Shinagawa-ku, Tokyo 141-8686, Japan*

**Correspondence to: Ming-Rong Zhang, Department of Molecular Probes, Molecular Imaging Center, National Institute of Radiological Sciences, 4-9-1 Anagawa, Inage-Ku, Chiba 263-8555, Japan.*
E-mail: zhang@nirs.go.jp



Scheme 1. One-pot synthesis of [^{13}N]urea analogues [^{13}N]**1a** and [^{13}N]**2a** and [^{13}N]carbamate [^{13}N]**3a** starting from amines **8a** and **9a** and alcohol **10a** using nca [^{13}N]NH_3.

synthesized [^{13}N]**4**, a promising PET ligand for brain imaging study.

Results and discussion

Anhydrous [^{13}N]NH_3 gas used for [^{13}N]ammonolysis was produced by the nuclear ^{16}O(p, α)^{13}N reaction.[10,11] A target containing 10 mM ethanol in water was irradiated by a cyclotron beam. The irradiated solution was quickly passed through a pre-conditioned cation exchange column and the [^{13}N]NH_3 in the irradiated water was concentrated on this column. The [^{13}N]NH_3 was eluted with aqueous KOH and dried through a small column filled with CaO, and introduced into a cooled vessel containing various anhydrous organic solvents. The trapping efficiency of [^{13}N]NH_3 was 85% ($n = 3$ in 1 mL DMF) and 72% ($n = 3$ in 1 mL DME), respectively. The specific activity of [^{13}N]NH_3 was about 37–370 GBq/μmol ($n > 20$) at the end of [^{13}N]NH_3 production. Radiochemical yields were measured by HPLC equipped with a detector for monitoring radioactivity. All the yields presented in this article are decay-corrected.

Optimizing reaction conditions of [^{13}N]ammonolysis

Prior to one-pot synthesis, we firstly optimized the reaction conditions of isocyanate **5a**, carbamoyl chloride **6a** and chloroformate **7a** with nca [^{13}N]NH_3. Table 1 shows the radiochemical yields of [^{13}N]**1a**–**3a** based on [^{13}N]NH_3. The reactions of **5a**–**7a** with anhydrous [^{13}N]NH_3 and various bases (K_2CO_3, pyridine, Et_3N, DMAP, *i*-Pr_2NEt or lutidine) were performed in THF, dichloroethane (DCE), CH_3CN and DMF, respectively. The precursors **5a**–**7a** in DCE displayed high reactivity with [^{13}N]NH_3 using *i*-Pr_2NEt as a base at 75°C for 3 min to give [^{13}N]**1a**–**3a** with radiochemical yields of 84–96%. Without using base, the [^{13}N]ammonolysis of **5a** gave [^{13}N]**1a** in a radiochemical yield of 85%, but **6a** and **7a** did not afford high yields.

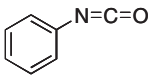
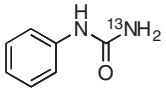
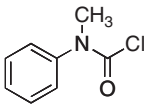
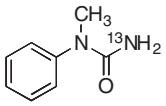
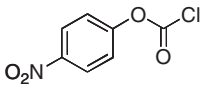
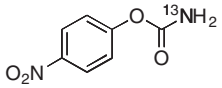
One-pot synthesis

Since many isocyanate, carbamoyl chloride and chloroformate precursors are not very stable and some are not commercially available, we attempted a practical one-pot synthesis starting from more conventional and stable amine and alcohol. We used **8a**–**10a** as model compounds to prepare **5a**–**7a**, which were not purified and were used directly for *in situ* [^{13}N]ammonolysis, respectively. The substrates **8a**–**10a** (10 μmol) were first reacted with triphosgene (20 μmol) to form **5a**–**7a**, which were identified by gas chromatography. After acylation, excess $COCl_2$ was removed by flowing N_2 into the mixture. Then, an anhydrous [^{13}N]NH_3 solution was added into each reaction mixture to perform [^{13}N]ammonolysis.

Table 2 shows the [^{13}N]ammonolysis efficiency by the above one-pot radiosynthesis. In contrast to the direct reaction of **5a**–**7a** with [^{13}N]NH_3, [^{13}N]ammonolysis followed by acylation of **8a**–**10a** did not give [^{13}N]**1a**–**3a** after a 3-min reaction. Addition of 100 nmol of NH_3 to the reaction mixture afforded [^{13}N]ammonolysis products with low yields (21–25%). Addition of 10^4 nmol of NH_3 increased the yields even to 68–80%, which were similar to the yields obtained by the direct reaction of **5a**–**7a** with [^{13}N]NH_3. The different results between the addition of NH_3 and no addition of NH_3 could be explained by the reaction scale of PET chemistry. Since the carrier contained in the [^{13}N]NH_3 for the present radiosynthesis was only about 1 nmol, [^{13}N]NH_3 was easily exhausted by trace $COCl_2$ and HCl, which were yielded throughout the synthesis process. Although the unreacted $COCl_2$ was removed as much as possible, the trace agent left in the reaction mixture was enough to trap [^{13}N]NH_3, which stopped the subsequent [^{13}N]ammonolysis. The addition of the NH_3 carrier to the [^{13}N]NH_3 solution improved the reaction efficiency; however, the yielded [^{13}N]ligand had too low specific activity and could not be used for PET study, especially for brain imaging.

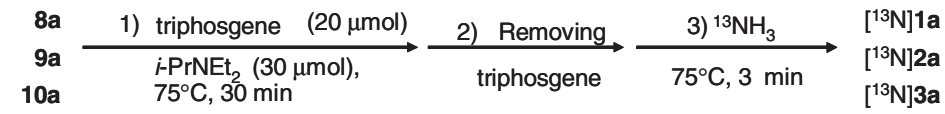
The difference between radiosynthesis in PET chemistry and conventional chemical synthesis prompted us to make more efforts to achieve the one-pot method using nca [^{13}N]NH_3. Our strategy was to control the amount of triphosgene relative to substrates **8a**–**10a** for guaranteeing the perfect consumption of $COCl_2$ and improving the labeling efficiency. Table 3 shows the one-pot synthesis results starting from **8a** to **10a** with [^{13}N]NH_3 according to the reaction conditions listed. When 3.3 μmol of triphosgene (10 μmol of $COCl_2$) was treated with 10 μmol of **8a**–**10a**, [^{13}N]**1a**–**3a** were obtained but their yields were not reproducible. This result was attributed to the trace amount of

Table 1. Reaction of isocyanate **5a**, carbamoyl chloride **6a** and chloroformate **7a** with nca [^{13}N]NH3 for 3 min at 75°C

Precursor	Solvent	Base	Product	Radiochemical yields (%)[a] based on [^{13}N]NH_3
 **5a**	THF	*i*-Pr_2NEt	 **1a**	68
	CH_3CN	*i*-Pr_2NEt		64
	DMF	*i*-Pr_2NEt		32
	DCE	*i*-Pr_2NEt		87
	DCE	None		85
 **6a**	THF	*i*-Pr_2NEt	 **2a**	75
	DCE	*i*-Pr_2NEt		84
	DCE	Et_3N		70
	DCE	Pyridine		45
	DCE	Lutidine		31
	DCE	DMAP		41
	DCE	K_2CO_3		82
	DCE	None		22
 **7a**	THF	*i*-Pr_2NEt	 **3a**	81
	DCE	*i*-Pr_2NEt		96
	DCE	Et_3N		47
	DCE	K_2CO_3		98
	DCE	None		17

[a]Radiochemical yield (decay-corrected) was determined by analytical HPLC. Radioactive products were identified using authentic non-radioactive samples.

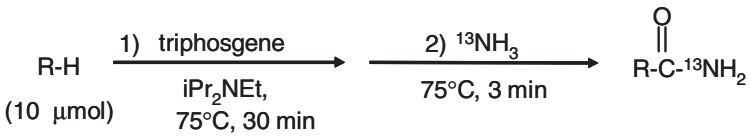
Table 2. One-pot synthesis of **8a–10a** with triphosgene, followed by [^{13}N]ammonolysis

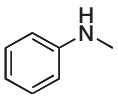
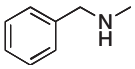
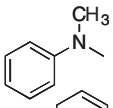
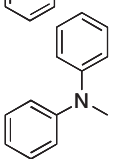
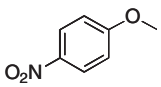
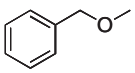


Substrate	[^{13}N]NH_3 (nmol)	Addition of NH_3 (nmol)	Radiochemical yields (%)[a] based on [^{13}N]NH_3
8a	~1	None	No reaction
	~1	100	25
	~1	10 000	80
9a	~1	None	No reaction
	~1	100	24
	~1	10 000	76
10a	~1	None	No reaction
	~1	100	21
	~1	10 000	68

[a]Radiochemical yield (decay-corrected) was determined by analytical HPLC. All results are the mean ($n = 3$) with a maximum range of ±5%. Radioactive products were identified using authentic non-radioactive samples.

Table 3. Radiosynthesis of [^{13}N]urea analogues [^{13}N]**1** and [^{13}N]**2** and [^{13}N]carbamate [^{13}N]**3** using one-pot synthesis



Number	R	Amount of triphosgene (μmol)	Radiochemical yields (%)[a] based on [^{13}N]NH_3
8a		3.3	0–70 ($n = 5$)
		2.5	78
8b		2.5	85
9a		3.3	0–36 ($n = 5$)
		2.5	52
9b		2.5	34
10a		3.3	0–62 ($n = 5$)
		2.5	84
10b		2.5	90

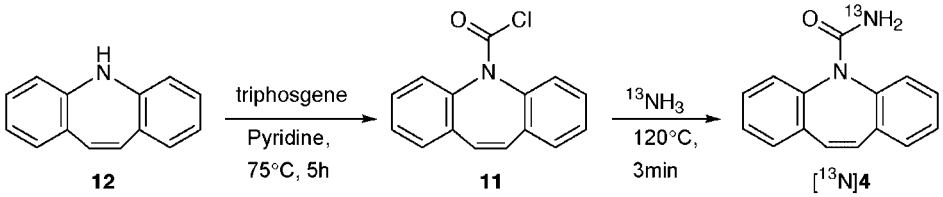
[a]Radiochemical yield (decay-corrected) was determined by analytical HPLC. All results are the mean ($n = 3$) with a maximum range of $\pm$10%. Radioactive products were identified using authentic non-radioactive samples.

[^{13}N]NH_3 (about 1 nmol of carrier), which made the amount of triphosgene and the [^{13}N]ammonolysis difficult to control. When 2.5 μmol of triphosgene (7.5 μmol of $COCl_2$) was used, a high efficiency of [^{13}N]ammonolysis was achieved. This result suggested that no $COCl_2$ was left in the reaction mixture after acylation. We found that this amount of triphosgene was suitable for the efficient production of **5a**–**7a** without disturbing the following [^{13}N]ammonolysis. As shown in Table 3, both aromatic – **8a** and aliphatic – **8b** amines gave high radiochemical yields in the presence of *i*-Pr_2NEt. Compared with primary amines **8**, secondary amines **9** provided lower reaction efficiency after the 3-min reaction. In particular, diphenylamine (**9b**) only gave a yield of 34%, which was due to the low reactivity of diphenyl carbamoyl chloride itself with [^{13}N]NH_3. Phenol (**10a**) and benzyl alcohol (**10b**) afforded high yields of [^{13}N]carbamates, respectively. In addition to *i*-PrNEt_2, other bases such as Et_3N, K_2CO_3 and pyridine could also give the [^{13}N]ammonolysis product (data not shown).

Radiosynthesis of [^{13}N]carbamazepine ([^{13}N]4)

We have determined a practical labeling method starting from amine and alcohol to synthesize [^{13}N]ligands. Next, we used this method to prepare [^{13}N]**4**, a putative PET ligand for brain imaging study (Scheme 2). Carbamazepine (**4**) is a useful drug for treating epilepsy[12–14] and [^{13}N]**4** could be used to image the state of several neurodegenerative diseases, such as brain damage and inflammation. Although [^{13}N]**4** preparation could be planned by [^{13}N]ammonolysis of carbamoyl chloride **11** with [^{13}N]NH_3, the unstability of **11** prevented its storage and delivery to other facilities for clinical routine production. Thus, radiosynthesis of [^{13}N]**4** using the present one-pot method is a promising task.

Compared with acyl chloride **6a, 11** displayed lower reactivity with [^{13}N]NH_3. The usefulness of Et_3N, *i*-Pr_2NEt or Na_2CO_3 as a base at 75°C did not afford high yield within 3 min. When pyridine was used, the reaction of **11** with [^{13}N]NH_3 in DMF progressed at 120°C for 3 min with a radiochemical yield of 77%. Acylation of amine **12** (5 μmol) with triphosgene (1.6 μmol) and pyridine in DMF gave **11**, which was identified in the reaction mixture by gas chromatography. This mixture was used directly for the following [^{13}N]ammonolysis. An anhydrous [^{13}N]NH_3 solution (1.1–2.0 GBq in DMF) was added to the reaction mixture and the mixture was heated at 120°C for 3 min. The one-pot synthesis gave [^{13}N]**4** with 56–74% of radiochemical yields based on [^{13}N]NH_3 ($n = 3$). At the end of synthesis, 185–296 MBq of [^{13}N]**4** was obtained with a specific activity of 22–33 GBq/μmol. The synthesis time was about 17 min from the end of bombardment. Next, we will perform



Scheme 2. Radiosynthesis of [^{13}N]carbamazepine ([^{13}N]**4**).

automatic synthesis of [^{13}N]**4** using an automatic synthesis system developed in our institute.[11]

Experimental

Materials and general methods

Melting points (mp) were uncorrected. Nuclear magnetic resonance (^{1}H-NMR) spectra were recorded on a JNM-GX-300 spectrometer (JEOL, Tokyo) with tetramethylsilane as an internal standard. All chemical shifts (δ) were reported in parts per million downfield from the standard. Fast atom bombardment mass spectra (FAB-MS) were obtained on a JEOL NMS-SX102 spectrometer (JEOL). Column chromatography was performed on Merck Kieselgel gel 60 F_{254} (70–230 mesh). Nitrogen-13 (^{13}N) was produced by the ^{16}O(p, α)^{13}N nuclear reaction using a CYPRIS HM-18 cyclotron (Sumitomo Heavy Industry, Tokyo). Radioactivity was determined with a dose calibrator (IGC-3R Curiemeter, Aloka, Tokyo). HPLC was performed using a JASCO HPLC system (JASCO, Tokyo): effluent radioactivity was monitored using an NaI (Tl) scintillation detector system. As standards, *N*-phenylurea (**1a**), *N*-benzylurea (**1b**), *p*-nitrophenyl carbamate (**3a**), benzyl carbamate (**3b**) and carbamazepine (**4**) were purchased from Aldrich Chemical (Milwaukee, WI). If not otherwise stated, chemicals were purchased from Aldrich Chemical and Wako Pure Industries (Osaka) with the highest grade commercially available.

Chemical synthesis

*N-methyl-N-phenylurea (**2a**)*

A solution of triphosgene (450 mg, 1.5 mmol) in 5 mL of DCE was added to a mixture of *N*-methylaniline (**9a**; 107 mg, 1 mmol) and *i*-Pr_2NEt (400 μL, 2.3 mmol) in DCE (3 mL). This mixture was stirred at room temperature under nitrogen gas. After flowing nitrogen gas into this mixture to remove excess phosgene for 10 min, a 27% aqueous NH_3 solution (2 mL) was added to the reaction and the mixture was vigorously stirred for 2 h. The reaction mixture was quenched with CH_2Cl_2, and washed with water and saturated NaCl solution. After the organic layer was dried over Na_2SO_4, these solvents were removed to give a residue. The residue was chromatographed on silica gel with $CHCl_3$/CH_3OH (95/5) to give **2a** (90 mg, 60%) as a colorless crystal. mp: 78–79°C (78°C).[19] ^{1}H-NMR ($CDCl_3$) δ: 7.39–7.46 (2H, m), 7.27–7.34 (3H, m), 4.50 (br, 2H), 3.30 (3H, s).

*N,N-diphenylurea (**2b**)*

A solution of triphosgene (450 mg, 1.5 mmol) in DCE (5 mL) was added to a mixture of diphenylamine (**9b**; 170 mg, 1 mmol) and *i*-Pr_2NEt (400 μL, 2.3 mmol) in DCE (6 mL). This mixture was stirred at room temperature under nitrogen. After flowing nitrogen gas into this mixture to remove excess phosgene, a 27% NH_3 solution (2 mL) was added to the reaction and the mixture was continuously stirred for 3 h. The reaction mixture was quenched with CH_2Cl_2, and washed with water and saturated NaCl solution. After the organic layer was dried over Na_2SO_4, these solvents were removed to give a residue. The residue was chromatographed on silica gel with CH_2Cl_2/hexane (100/1) to give **2b** (189 mg, 89%) as a colorless crystal. mp: 187–188°C (189°C).[20] ^{1}H-NMR ($CDCl_3$) δ: 7.21–7.39 (10H, m), 7.00 (2H, m).

*Dibenzazepinylcarbamoyl chloride (**11**)*

A solution of triphosgene (54 mg, 0.18 mmol) in anhydrous DCE (3 mL) was added to a mixture of dibenzazepine (**12**; 97 mg, 0.5 mmol) and anhydrous pyridine (120 μL, 1.5 mmol) in DCE (3 mL). This mixture was heated at 75°C under nitrogen gas for 3 h. The reaction mixture was quenched and extracted with $CHCl_3$. After the organic layer was dried over Na_2SO_4, the solvent was removed to give a residue. The residue was chromatographed on silica gel with CH_2Cl_2/hexane (1/1) to give **11** (96 mg, 75%) as a colorless crystal. mp: 148–149°C (149–153°C).[21] ^{1}H-NMR ($CDCl_3$) δ: 7.34–7.51 (8H, m), 7.00 (2H, s).

*Carbamazepine (**4**)*

A solution of triphosgene (60 mg, 0.6 mmol) in anhydrous DCE (1 mL) was added to a mixture of **12** (97 mg, 1 mmol) and anhydrous pyridine (240 μL, 3 mmol) in DCE (3 mL). This mixture was heated at 75°C under nitrogen gas for 3 h. To this mixture a solution of anhydrous NH_3 (ca 10%, 1 mL DCE) was added. The reaction mixture was continuously heated at 75°C for 30 min. After the reaction, the mixture was quenched and extracted with $CHCl_3$. After the organic layer was dried over Na_2SO_4, the solvent was removed to give a residue. The residue was chromatographed on silica gel with CH_2Cl_2 to give **4** (65 mg, 55%) as a colorless crystal. mp: 189–190°C (191–192°C).[13] ^{1}H-NMR ($CDCl_3$) δ: 7.28–7.47 (8H, m), 6.92 (2H, s), 4.81 (2H, br). FAB-MS (*m/z*): 237.2 ($M^+ + 1$).

Radiosynthesis

Anhydrous [^{13}N]NH_3

Before irradiation, a 10 mM ethanol solution was saturated with pure O_2 and loaded into a target chamber. The target was irradiated at 15 μA for 15 min with 18 MeV protons (15.8 MeV on target). After bombardment, the irradiated solution was concentrated onto a cation exchange resin AG 50W-X8 (1 mm $\phi \times 40$ mm) for trapping [^{13}N]NH_3. Then, the [^{13}N]NH_3 in the column was eluted with 30 μL of 2 N KOH under an He gas flow

and desiccated through a small column filled with 250 mg of CaO (3 mm $\phi \times 30$ mm, kept at 150°C), and introduced into a vial (Pyrex, 5.0 mL) containing various anhydrous organic solvents (1 mL) cooled at −15°C. The production time of [^{13}N]NH_3 was about 4 min from the end of bombardment. The specific activity of [^{13}N]NH_3 was assumed from that of [^{13}N]**1a** determined by HPLC analysis. The analytical condition of [^{13}N]**1a** was as follows: J'sphore ODS H80 (4 mm $\phi \times 150$ mm), CH_3CN/H_2O (4/6), 1 mL/min and 254 nm (3.6 min). The effluent was monitored by a UV detector at 254 nm and a radioactivity detector and quantified using a calibration curve obtained with 0.5–10.0 mg/mL solutions of the authentic **1a**.

General analysis procedure for identification and radiochemical yield

After the addition of CH_3CN/H_2O (1/1, 200 μL) terminated the reaction, the radiochemical yield (decay-corrected) of [^{13}N]ammonolysis for [^{13}N]**1**, [^{13}N]**2** or [^{13}N]**3** was determined by analytic HPLC based on [^{13}N]NH_3. The analytical condition was as follows: J'sphore ODS H80 (4 mm $\phi \times 150$ mm), CH_3CN/H_2O (4/6–1/1), 1–2 mL/min and 254 nm. The confirmation of identity for each product was achieved by co-injection with the non-radioactive standard.

*[^{13}N]ammonolysis of isocyanate **5a**, carbamoyl chloride **6a** and chloroformate **7a** using nca [^{13}N]NH_3*

In a hot cell, a solution of [^{13}N]NH_3 (37–185 MBq) in 100 μL of DCE was added to a reaction vial (Pyrex, 5.0 mL) containing **5a**, **6a** or **7a** (10 μmol), 200 μL of anhydrous THF, CH_3CN, DMF or DCE and various bases (K_2CO_3, pyridine, Et_3N, DMAP, *i*-Pr_2NEt or lutidine: 30 μmol). The reaction mixture was sealed and heated at 75°C for 3 min in a hot oil bath. After the reaction, the reaction vial was cooled rapidly to determine the radiochemical yield of [^{13}N]ammonolysis based on [^{13}N]NH_3.

One-pot synthesis

(1) *Removing excess $COCl_2$*: A solution of triphosgene (20 μmol) in 100 μL of DCE was added to a reaction vial containing a mixture of **8a**, **9a** or **10a** (10 μmol) and *i*-Pr_2NEt (50 μmol) in 100 μL of DCE, respectively. The mixture was heated at 75°C under N_2 for 30 min. The excess $COCl_2$ was removed by flowing N_2 into the mixture. Then, an anhydrous [^{13}N]NH_3 solution (37–185 MBq) in 100–400 μL of DCE was added into the reaction mixture. The mixture was heated for further 3 min at 75°C.

(2) *Controlling the amount of triphosgene*: A solution of triphosgene (2.5 μmol) in 100 μL of DCE was added to a reaction vial containing a mixture of **8**, **9** or **10** (10 μmol) and *i*-Pr_2NEt (50 μmol) in 100 μL of DCE, respectively. The mixture was heated at 75°C under N_2 for 30 min. Then, an anhydrous [^{13}N]NH_3 solution (37–185 MBq) in 100–400 μL of DCE was added into the reaction vial. The reaction mixture was continuously heated for 3 min at 75°C.

*[^{13}N]carbamazepine ([^{13}N]**4**)*

A mixture of **12** (5 μmol), triphosgene (1.6 μmol) and pyridine (20 μL) in DMF (300 μL) was heated under N_2 at 75°C for 5 h to give **11**. This mixture was not purified and was used directly for the following [^{13}N]ammonolysis. An anhydrous [^{13}N]NH_3 solution (1.1 GBq) in 200 μL of DMF was added to the reaction mixture and the mixture was heated at 120°C for 3 min in a hot cell. After the reaction, the mixture was diluted with 500 μL of an HPLC mobile phase, and transferred onto a column (10 mm $\phi \times 250$ mm). Elution with CH_3CN/H_2O (4/6) (254 nm) at a flow rate of 4 mL/min gave a radioactive fraction corresponding to pure [^{13}N]**4**. The fraction was collected in a rotary evaporator flask and evaporated to dryness at about 90°C in a vacuum. The residue was dissolved in 10 μL of ethanol and 3 mL of sterile isotonic saline, and passed through a 0.22-μm Millipore filter. Total synthesis time was about 17 min from the EOB. At the end of the syntheses (EOS), 23 MBq of [^{13}N]**4** was obtained as an i.v. injectable solution. The radiochemical purity of [^{13}N]**4** was determined by analytical HPLC (J'sphore ODS H80 (4 mm $\phi \times 150$ mm), CH_3CN/H_2O (4/6), 1 mL/min and 254 nm). The identity of [^{13}N]**4** was confirmed by co-injection with the non-radioactive sample. The specific activity of [^{13}N]**4** was calculated by comparing the assayed radioactivity with the carrier at the UV peak of 254 nm.

Conclusions

We determined a practical method for preparing [^{13}N]urea and [^{13}N]carbamate analogues using nca [^{13}N]NH_3 with high specific activity. This method provides a labeling technique for synthesizing ^{13}N-labeled radiopharmaceuticals with abundant pharmacological profiles and biological activities. We are using this method to synthesize other [^{13}N]ligands for PET study on animal and human.

Acknowledgement

We thank the staff of the Cyclotron Operation Section and Radiochemistry Section, Department of Molecular Probes, National Institute of Radiological Sciences (NIRS) for their support in the operation of the cyclotron and production of radioisotopes. This study was partially supported by a consignment grant for Molecular Imaging Program on Research Base for PET Diagnosis from the Ministry of Education, Culture, Sports, Science and Technology (MEXT), Japanese Government.

References

[1] M. E. Pheles, E. J. Hoffman, C. Rayboud, *Stroke* **1977**, *8*, 694–702.
[2] H. R. Schelbert in *Positron Emission Tomography and Autoradiography: Principle and Application for the Heart and Brain* (Eds.: M. E. Phelps, J. C. Mazziota, H. R. Schelbert), Raven Press, New York, **1986**, pp. 581–662.
[3] W. Wijins, P. G. Camici, *Herz* **1997**, *2*, 87.
[4] A. J. Cooper, A. S. Gelbard, *Anal. Biochem.* **1981**, *111*, 42–48.
[5] K. Suzuki, K. Tamate, T. Nakayama, T. Yamazaki, Y. Kashida, K. Fukushi, Y. Maruyama, H. Maekawa, H. Nakaoka, *J. Labelled Compd. Radiopharm.* **1982**, *19*, 1374–1375.
[6] T. Tominaga, K. Suzuki, O. Inoue, T. Irie, T. Yamasaki, M. Hirobe, *Appl. Radiat. Isot.* **1987**, *38*, 437–1375.
[7] G. W. Kabalka, M. M. Goodman, J. F. Green, R. Marks, D. Longford, *J. Labelled Compd. Radiopharm.* **1993**, *32*, 165.
[8] P. Landais, P. Waltz, H. Tochon-Danguy, P. Goethals, K. Strijckmans, K. Rose, R. E. Offord, *J. Labelled Compd. Radiopharm.* **1993**, *32*, 171.
[9] J. Noguchi, M. R. Zhang, K. Yanamoto, R. Nakao, K. Suzuki, *Nucl. Med. Biol.* **2008**, *35*, 19–27.
[10] K. Suzuki, Y. Yoshida, *Appl. Radiat. Isot.* **1999**, *50*, 497–503.
[11] K. Suzuki, Y. Yoshida, N. Shikano, A. Kubodera, *Appl. Radiat. Isot.* **1999**, *50*, 1033–1038.
[12] W. Schindler, F. Hafliger, *Helv. Chim. Acta* **1954**, *37*, 472–483.

[13] H. Yoshikawa, T. Abe, *Brain Dev*. **2003**, *25*, 127–129.
[14] T. Okamura, A. Kishimoto, *Psychiatry Clin. Neurosci*. **1998**, *52*, 3–12.
[15] H . Doi, J. Barletta, M. Suzuki, R. Noyori, Y. Watanabe, B. Langstrom, *Org. Biomol. Chem*. **2004**, *2*, 3063–3066.
[16] D. Marotta, *Gazz. Chim. Ital.* **1929**, *59*, 955.
[17] H. Eckert, B. Forster, *Angew. Chem. Int. Ed. Engl.* **1987**, *26*, 894–895.
[18] W. H. Daly, D. Poche, *Tetrahedron Lett.* **1988**, *29*, 5859–5862.
[19] Sumitomon Chemical, JPS52-73801, **1977**.
[20] http://msds.chem.ox.ac.uk/DI/1,1-diphenylurea.html.
[21] W. Schindler, F. Hayliger, *Helv. Chim. Acta*. **1956**, *37*, 472–483..

