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An efficient and practical synthesis of [2-^{11}C]indole *via* superfast nucleophilic [^{11}C]cyanation and RANEY® Nickel catalyzed reductive cyclization†

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A rapid method for the synthesis of carbon-11 radiolabeled indole was developed using a sub-nanomolar quantity of no-carrier-added [^{11}C]cyanide as radio-precursor. Based upon a reported synthesis of 2-(2-nitrophenyl)acetonitrile (**2**), a highly reactive substrate 2-nitrobenzyl bromide (**1**) was evaluated for nucleophilic [^{11}C]cyanation. Additionally, related reaction conditions were explored with the goal of obtaining of highly reactive 2-(2-nitrophenyl)-[1-^{11}C]acetonitrile (**[^{11}C]-2**) while inhibiting its rapid conversion to 2,3-bis(2-nitrophenyl)-[1-^{11}C]propanenitrile (**[^{11}C]-3**). Next, a RANEY® Nickel catalyzed reductive cyclization method was utilized for synthesizing the desired [2-^{11}C]indole with hydrazinium monoformate as the active reducing agent. Extensive and iterative screening of basicity, temperature and stoichiometry was required to overcome the large stoichiometry bias that favored 2-nitrobenzylbromide (**1**) over [^{11}C] cyanide, which both caused further alkylation of the desired nitrile and poisoned the RANEY® Nickel catalyst. The result is an efficient two-step, streamlined method to reliably synthesize [2-^{11}C]indole with an entire radiochemical yield of 21 ± 2.2% (n = 5, ranging from 18–24%). The radiochemical purity of the final product was >98% and specific activity was 176 ± 24.8 GBq μmol^{-1} (n = 5, ranging from 141–204 GBq μmol^{-1}). The total radiosynthesis time including product purification by semi-preparative HPLC was 50–55 min from end of cyclotron bombardment.

The synthesis of indole and its derivatives has played a prominent role in organic chemistry because of the importance of natural products containing the indole ring, such as alkaloids and other biologically active molecules.[1] One of the most important plant hormones, indole-3-acetic acid (auxin or IAA), is such an example and it plays a pivotal role in plant growth and development.[2] Indole is known to be the core precursor for all five known auxin biosynthetic pathways including tryptophan-dependent and tryptophan-independent pathways.[3–6] Tracking the downstream flow of indole could help us to answer some core questions, such as the relative importance of different auxin biosynthesis pathways in various plant tissues as well as the role of auxin biosynthesis in plant–microbe interactions. In this regard, we set out to synthesize carbon-11 (half life: 20.4 min) radiolabeled indole as a tool for improved mapping and understanding of both biosynthetic and metabolic pathways of auxin in whole plants *in vivo* using positron emission tomography (PET) at tracer levels.

Although the synthesis of molecules containing an indole ring as a core structure has been extensively investigated,[7,8] there are only limited reports related to the incorporation of stable and radioactive isotopes into the indole ring. In 2002, Czeskis and colleagues reported an elegant method for synthesis of 4-hydroxy [2-^{14}C]indole.[9] Starting from Na^{14}CN, they first synthesized *p*-chlorophenoxy [1-^{14}C]acetonitrile which was reacted with benzyl-protected 3-nitrophenol *via* a vicarious nucleophilic substitution reaction to afford a 2-(2-nitrophenyl) acetonitrile derivative, which was reduced and cyclized to form ^{14}C-labeled 4-hydroxyindole. Four years later, Pedras and Okinyo reported a synthesis of stable isotope deuterium labeled [5,6,7,8-^{2}H$_4$]indole using chloroacetonitrile direct alkylation and reductive cyclization reactions.[10] More recently,

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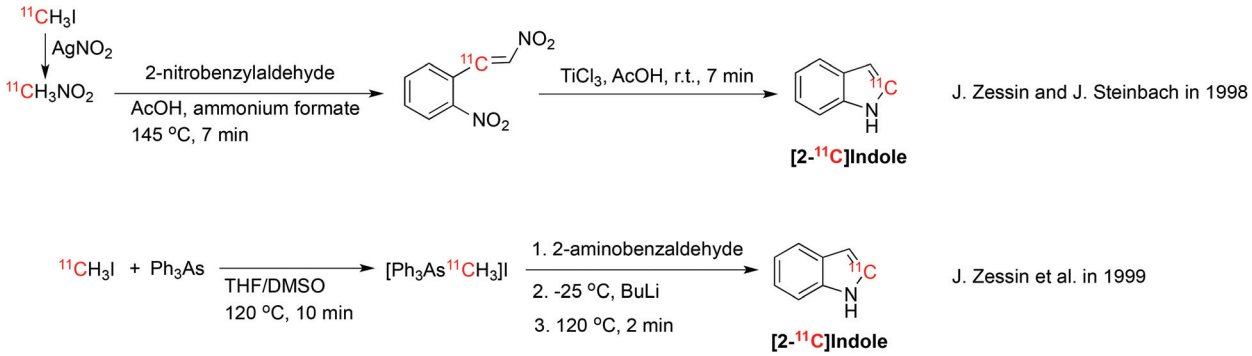
†Electronic supplementary information (ESI) available: Diagrams of [^{11}C]HCN production procedure, detailed experimental procedure, representative HPLC analysis results of intermediate and product for measuring radiochemical purities as well as semi-prep HPLC profile for purification of final product, *etc.* See DOI: 10.1039/c5ob01654a



Scheme 1 Previous reports for synthesis of ^{11}C-labeled indole.

Czeskis incorporated carbon-14 into the 2-position on the indole ring through the reaction of 2-nitrobenzyl bromide with $Na^{14}CN$ to form 2-(2-nitrophenyl)-[1-^{14}C]acetonitrile followed by reductive cyclization.[11]

Additionally, two methods for synthesis of ^{11}C-labeled indole, **[2-^{11}C]indole**, were reported in 1998 and 1999 (Scheme 1):[12,13] in the first report, the radioprecursor [^{11}C]CH_3NO_2 (synthesized from [^{11}C]CH_3I) was condensed with 2-nitrobenzaldehyde to form β,2-dinitro-[β-^{11}C]styrene which when treated with titanium(III) chloride underwent reductive cyclization to form desired **[2-^{11}C]indole**. In the second report [^{11}C]CH_3I was reacted with triphenylarsine followed by butyl lithium to form triphenylarsonium [^{11}C]methylide which was then reacted with 2-aminobenzaldehyde to give **[2-^{11}C]indole**. Although both reports showed the feasibility of synthesizing **[2-^{11}C]indole**, they only provided the analytical HPLC data for the reaction mixture and there were no details on either the purification method or isolated yield of **[2-^{11}C]indole**.

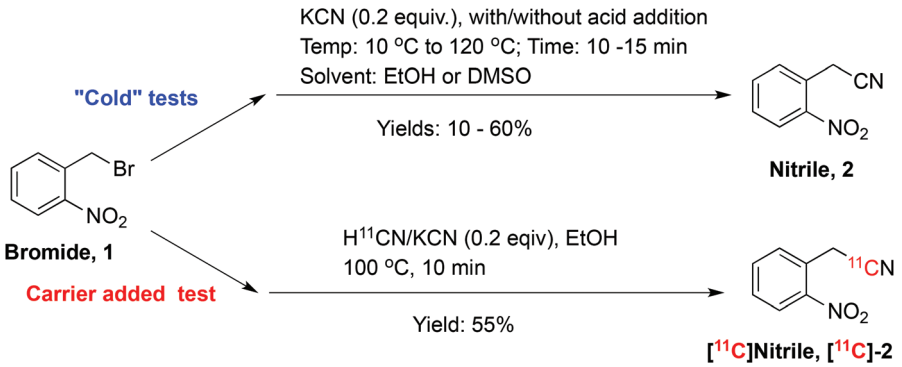
Given the availability of [^{11}C]HCN and a long history of utilizing [^{11}C]HCN as a radiolabeling precursor to synthesize various ^{11}C-labeled bioactive radiotracers,[14–17] as well as above-mentioned successful examples of cyanide based methods for synthesizing various isotopically labeled indole derivatives,[9–11] we envisioned the synthesis of **[2-^{11}C]indole** using [^{11}C]HCN as the radiolabeling precursor (Scheme 2): starting from 2-nitrobenzyl bromide (referred to hereafter as **bromide**, **1**), nucleophilic cyanation with [^{11}C]cyanide ([^{11}C]CN^-) could provide us with 2-(2-nitrophenyl)-[1-^{11}C]acetonitrile (**[^{11}C]nitrile**, **2**). Next, reductive cyclization, if successful, would give us desired radiotracer, **[2-^{11}C]indole**. Herein, we describe our recent explorations in developing a rapid method for synthesis of **[2-^{11}C]indole** based upon a [^{11}C]CN^- nucleophilic cyanation followed by reductive cyclization.

Results and discussion

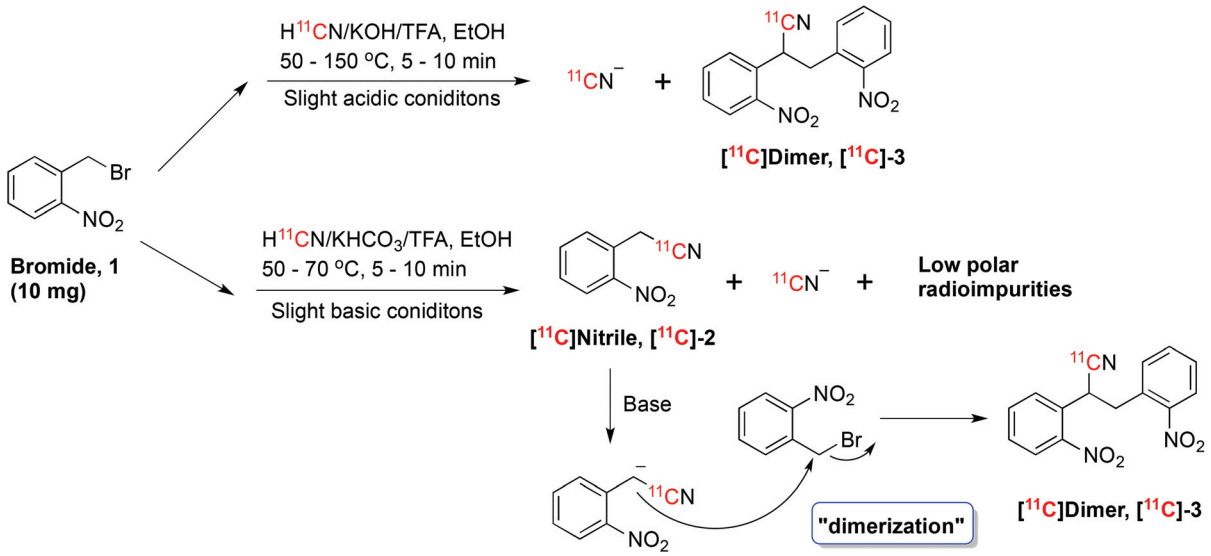
We started by testing the feasibility of synthesizing the intermediate **nitrile**, **2**. One report by Kalir and Mualem in 1987 [18] disclosed a reliable method using NaCN plus HCN (for pH adjustment) using **bromide**, **1** as starting material, which provided the **nitrile**, **2** with yields up to 90%. A later report showed that this compound was also synthesized in 52% yield in aqueous DMSO at near neutral conditions for 1 h at 0–5 °C.[11] Following these reports, we initiated the "cold" test for synthesis of **nitrile**, **2** intermediate and we found, with slightly modified conditions (Scheme 3), that a 10–60% yield of **nitrile**, **2** could be synthesized by reacting **bromide**, **1** with either KCN or a mixture of KCN/HCN.

Next, we introduced radioactive [^{11}C]HCN into the reaction system and tested a carrier-added (CA) [^{11}C]cyanation reaction (Scheme 3). The radio-HPLC analysis was very encouraging. It showed that 55% of the radioactivity converted to the desired **[^{11}C]nitrile**, **[^{11}C]-2** in the presence of KCN in EtOH for 10 min at 100 °C. We then eliminated the carrier KCN from the reaction to test the conditions for the synthesis of the no-carrier-added (NCA) **[^{11}C]-2** (Scheme 4). Under slightly acidic conditions, there was no reaction. Increasing the reaction temperature (from 50 °C to 150 °C) initiated the [^{11}C]cyanation reaction but gave only 2,3-bis(2-nitrophenyl)-[1-^{11}C]propanenitrile (**[^{11}C]dimer**, **[^{11}C]-3**), which was formed by the further alkylation (here called "dimerization") of **[^{11}C]-2** with starting material **bromide**, **1**. When $KHCO_3$ was used to trap the [^{11}C]HCN and the reaction mixture was maintained at slightly basic pH, radio-HPLC analysis showed that the NCA **[^{11}C]-2** was sometimes formed, but these results were not reproducible and, in most cases, only a small amount of **[^{11}C]-2** could be detected and the majority of radioactivity in the reaction

Scheme 2 Our design of synthesis **[2-^{11}C]indole** using [^{11}C]HCN as radiolabeling precursor.



Scheme 3 Results for the synthesis of **nitrile, 2** and carrier added [^{11}C]-**2**.



Scheme 4 Preliminary results for the radiosynthesis of [^{11}C]-**2**.

mixture was [^{11}C]CN^-, [^{11}C]-**3** and an unidentified low polarity radioactive compound(s) (Scheme 4).

These initial results were not surprising nor were they unprecedented. Kalir and Mualem also reported the formation of **dimer**, **3** from the reaction of **bromide**, **1** with cyanide. They minimized its formation by carefully controlling the reaction stoichiometry with excess NaCN to obtain a ratio of 3 : 1 (NaCN : **bromide**, **1**) and conducting the reaction under acidic conditions.[18] Czeskis also reported **dimer**, **3** formation and minimized it by using aqueous DMSO and quenching the reaction after a 1 h reaction at 0–5 °C (Scheme 4).[11] Adapting these conditions to [^{11}C]cyanation with no-carrier-added [^{11}C] cyanide was challenging because the chemical quantity of cyclotron-produced [^{11}C]cyanide is fixed and very small (0.03 μmol–0.06 μmol) whereas milligram quantities (10 mg, 46.3 μmol) of the **bromide**, **1** is typically used to drive the nucleophilic substitution reaction. This results in a ratio of [^{11}C] cyanide : **bromide**, **1** of ~1 : 700–1500 in a typical radiosynthesis. Though this low ratio of radioprecursor to substrate greatly favors the nucleophilic cyanation reaction, it also favors further alkylation once a certain amount of [^{11}C]-**2** has accumulated in the reaction mixture (Scheme 4). At this stage, we speculated that it was necessary to carefully examine the NCA [^{11}C]cyanation conditions in order to out-compete the very rapid further alkylation and provide a sufficient amount of [^{11}C]-**2** for the next reductive cyclization step.

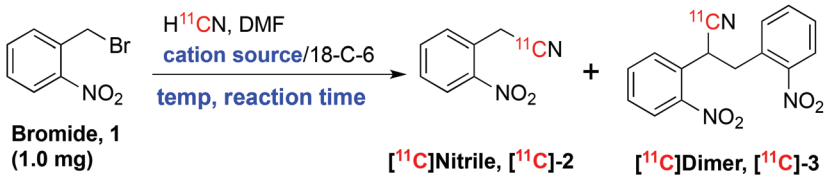
Because we anticipated that careful pH adjustment would be necessary to avoid further alkylation of the [^{11}C]-**2**, we envisioned that it was necessary to remove ammonia and water which were carried in the gas phase production of our radioprecursor [^{11}C]HCN.[19] For this purpose, a [^{11}C]HCN purification method, first introduced by Långström,[20] was adapted for our [^{11}C]HCN delivery system: the helium gas stream containing radioactivity was first purged through a 50% H_2SO_4 acid bath heated at 65 °C to remove NH_3, and then passed through a freshly packed P_2O_5 drying tube for further removal of trace H_2O. With this purified [^{11}C]HCN in hand, we started to screen nucleophilic [^{11}C]cyanation conditions for the syn-

thesis of NCA [^{11}C]-**2** using $CsHCO_3$/18-crown-6/DMF which was first introduced in our recent paper on the optimized synthesis of carbon-11 labeled L-glutamine.[21] Results are reported in Table 1.

We also reduced the mole ratio of [^{11}C]cyanide : bromide to ~1 : 150 (by reducing the amount of **bromide**, **1** from 10 mg to 1 mg for this set of experiments) to minimize the further alkylation to form [^{11}C]-**3**. Although directly duplicating the L-[5-^{11}C]-glutamine synthesis conditions gave a 79% overall radiochemical yield (ORCY, includes total radioactivity of [^{11}C]-**2** and [^{11}C]-**3**, decay corrected), only undesired [^{11}C]-**3** was detected using radio-HPLC analysis (Table 1, entry 1). Clearly, milder reaction conditions were needed to avoid consumption of radioactive intermediate [^{11}C]-**2**. Reducing the reaction temperature from 60 °C to 30–35 °C gave similar results (entry 2). Further decreasing the temperature to 18–20 °C not only provided us with 90% ORCY but also 4% of the desired [^{11}C]-**2** (entry 3). For the next step, the reaction mixture was cooled with an ice bath and the [^{11}C]cyanation reaction rate was clearly reduced. Although the ORCY was only 22% with an 8 min reaction time at 0 °C (entry 4), the results were quite encouraging since all detected radioactivity was the desired [^{11}C]-**2** intermediate. Though the longer reaction time (from 8 min to 25 min) provided higher ORCY (58%), further alkylation occurred at the expense of [^{11}C]-**2**. Only 9% of the total radioactivity was [^{11}C]-**2** and the rest was [^{11}C]-**3**. Based upon these results (Table 1, entries 1 to 4), it was clear that **bromide**, **1** is a highly reactive substrate, and the ORCY reached 90% after an 8 min reaction time even at room temperature. It was also evident that conditions that favored a high ratio of [^{11}C]-**2** to [^{11}C]-**3** also dramatically reduced the ORCY. It was definitely necessary to adjust other reaction parameters to obtain a high ORCY while avoiding consumption of the intermediate [^{11}C]-**2**.

We next set out to investigate the effect of basicity on ORCY and radiolabeled product distribution. The results listed in Table 1, entries 5 and 6 showed that no [^{11}C]cyanation reaction occurred when the pH was less than 8. Neither the direct use of NCA [^{11}C]HCN (pH = 5) nor the addition of near neutral form salt KBr (pH = 7.7) initiated the [^{11}C]cyanation reaction. With the introduction of more basic K_2CO_3 into the reaction (entries 7 and 8 compared with entry 6), the [^{11}C]cyanation reaction rate was dramatically improved. Adjusting the pH to 8.4 resulted in a 26% ORCY with a mixture of [^{11}C]-**2** and [^{11}C]-**3** (ratio at 23 : 77) favoring the formation of [^{11}C]-**3** (entry 7).

Table 1 Exploratory results of [^{11}C]cyanation based upon conditions reported in the recent update of the synthesis of L-[5-^{11}C]-glutamine[21]



Entry	Cation source[a]	pH[b]	Time (min)	Temp (°C)	ORCY[c]	Radioactivity ratio ([^{11}C]-**2** : [^{11}C]-**3**[d])
1	$CsHCO_3$	8.5	8	60	79	0 : 100
2	$CsHCO_3$	8.5	8	30–35	73	0 : 100
3	$CsHCO_3$	8.5	8	18–20	90	4 : 96
4	$CsHCO_3$	8.5	8	0	22	100 : 0
			25		58	9 : 91
5[e]	None	5	8	60	0	nd
6	KBr	7.7	10	18	0	nd
7	K_2CO_3/KBr (1/98)	8.4	10	18	26	23 : 77
8	K_2CO_3/KBr (5/90)	8.7	10	18	67	0 : 100
9	K_2CO_3/$KHCO_3$ (1/4)	9.6	0.2	0	73	92 : 8
			0.6		72	71 : 29
			2		76	21 : 79
			5		77	16 : 84
			15		69	7 : 93
10	K_2CO_3	11.2	0.6	0	68	85 : 15
			2		81	49 : 51
			10		70	28 : 72
11[f]	**K_2CO_3/$KHCO_3$ (1/4)**	**9.6**	0.2	0	76	78 : 22
			0.6		77	**78 : 22**
			2		77	39 : 61
			5		74	28 : 72
			15		64	22 : 28

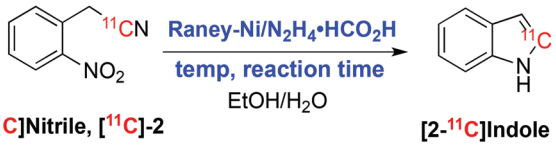
[a] All the experiments were performed with 2-nitrobenzyl bromide (1 mg, 4.63 μmol) with different cation sources (15 μmol) in presence of 18-C-6 (30 μmol) dissolved in 0.5 mL of DMF; if two different cation sources were used, the numbers listed in parenthesis were the mole ratios. [b] pH was measured with the cation source dissolved in aqueous solution (give the volume of water because pH is concentration dependent). [c] Overall radiochemical yield (%, ORCY, decay-corrected) of [^{11}C]cyanation was determined by analytical radio-HPLC and included both [^{11}C]-**2** and [^{11}C]-**3** (entries 1–11). [d] This number is the radioactivity ratio of [^{11}C]-**2** : [^{11}C]-**3** (decay-corrected), nd: not detected. [e] A solution of H^{11}CN in DMF was weakly acidic without addition of a cation source and 18-C-6. [f] Reaction solvent was changed to *N*,*N*-dimethylacetamide (DMA).

Increasing the pH to 8.7 increased the ORCY to 60% but only [^{11}C]-**3** was formed (entry 8). These experiments showed that basic conditions were required to initiate the [^{11}C]cyanation reaction but confirmed that the formation of [^{11}C]-**3** was favored. Encouraged by the increase in ORCY, we further reduced the temperature to 0 °C, modified the cation source and increased the pH to 9.6, and then carefully monitored the reaction by analytical radio-HPLC (entry 9). The results again showed that this reaction was very fast. The ORCY reached to 73% after only 0.2 min with a product distribution favoring [^{11}C]-**2** (92 : 8). Longer reaction times reversed the ratio to favor the undesired [^{11}C]-**3**. When K_2CO_3 was chosen as a cation source and the pH was increased to 11.2 (entry 10), the radio-HPLC analysis results of samples taken at different time points showed a similar trend as the results listed in entry 9: the short reaction time (0.6 min) provided the higher [^{11}C]-**2** yield but that the longer reaction times favored further alkylation. Finally, the solvent *N*,*N*-dimethylacetamide (DMA) was tested and the results also showed that the ORCY plateaued at 76% even after 0.2 min with a ratio of [^{11}C]-**2** : [^{11}C]-**3** at 78 : 22 (entry 11). We noted that further alkylation is comparatively slower with DMA as the reaction solvent when comparing the results from entry 11 with the results listed in entry 9.

This series of experiments showed that it was difficult to avoid consumption of the [^{11}C]-**2** with the excess **bromide**, **1**. However, by carefully controlling several reaction parameters, *i.e.*, reaction temperature, basicity as well as reaction time, it is possible to obtain a good conversion of [^{11}C]cyanide to product [^{11}C]-**2**, while minimizing formation of side product [^{11}C]-**3**. Eventually, to achieve better reproducibility and to reach higher yields of [^{11}C]-**2**, the [^{11}C]cyanation reaction conditions listed in entry 11 (pH 9.6, **bromide**, **1** 1.0 mg, DMA as solvent, reaction at 0 °C for 0.6 min) were selected for the synthesis of intermediate [^{11}C]-**2**. We next turned our attention to the exploration of the reductive cyclization step.

We initially tested several reducing agents for the reductive cyclization of [^{11}C]-**2** to **[2-^{11}C]indole** including: Pd–C/H_2 in ethyl acetate[22,23] and in alcoholic solvents;[24,25] Pd–C/H_2 in ethanol/acetic acid;[9,26,27] Pd–C in the presence of *in situ* H_2;[28,29] RANEY® Nickel or Pd–C in the presence of NH_4CO_2H/HCO_2H;[30] Al–$NiCl_2$·$6H_2O$;[31] Zn/acetic acid.[32] Results were generally disappointing: although most worked under carrier-added conditions with or without carbon-11, they either provided only a trace of desired **[2-^{11}C]indole** or none at all under no-carrier-added (NCA) conditions. Later, a report by Gowda *et al.* using RANEY® Nickel based rapid reduction (both nitro and nitrile groups) caught our attention.[33] This method quickly and selectively reduced various compounds containing nitro or nitrile groups to the corresponding amines in high yield (70–94%) with hydrazinium monoformate as hydrogen donor and RANEY® Nickel as catalyst at r.t. in 2–6 min. Given the time limitations of carbon-11 radiochemistry, *i.e.* "Working Against Time",[34] this method was especially promising. We confirmed that this method rapidly converted the **nitrile**, **2** to indole under "cold" conditions and set out to adapt it to our NCA **[2-^{11}C]indole** synthesis process (Table 2).

Table 2 Exploration of reductive cyclization method to convert [^{11}C]-**2** to **[2-^{11}C]indole**



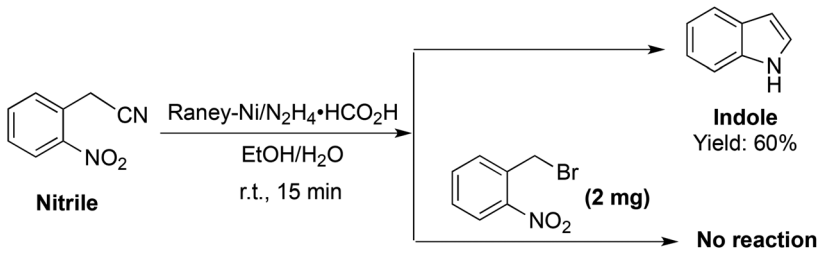
[^{11}C]Nitrile, [^{11}C]-**2** [2-^{11}C]Indole

Entry	Time (min)	N_2H_4·HCO_2H[a] (mmol)	RANEY® Ni[b] (mg)	Temp[c] (°C)	RCY[d] (%)
12	5	5	48	r.t	25
13	10	5	48	r.t	26
14	15	5	48	r.t	33
15	5	10	48	r.t	9
16	15	10	48	r.t	19
17	15	2.5	48	r.t	38
18	15	2.5	96	r.t	10
19	5	5	48	40	18
20	10	5	48	40	30
21	15	5	48	40	24
22	10	0.5	48	40	35
23	**10**	**0.5**	**38**	**40**	**41**
24[e]	10	0.5	48	40	nd

[a] The mole ratio of hydrazine monohydrate and formic acid was 1 : 1. [b] RANEY® Nickel slurry in water (50%) was used for reactions. [c] Room temperature indicates 18–20 °C. [d] RCY (decay-corrected) (%) was determined by radio-HPLC analysis of filtered crude mixture. [e] 2 mg of **bromide**, **1** was used for synthesis of [^{11}C]-**2**; after the Sep-Pak purification, the ORCY was 51% and 42% of radioactivity was [^{11}C]-**2**; nd: no **[2-^{11}C]indole** was detected.

The initial test result was quite promising (Table 2, entry 12). We obtained 25% of desired **[2-^{11}C]indole** by using 5 mmol N_2H_4·HCO_2H and 48 mg RANEY® Ni at r.t. for 5 min. The results from next two experiments (entries 13 and 14) showed that a longer reaction time of 15 min resulted in a moderate increase in yield to 33%. Surprisingly, when the amount of N_2H_4·HCO_2H, was increased to 10 mmol, the yield was decreased (entries 15 and 16) and only afforded less than 20% of **[2-^{11}C]indole** even after 15 min. In contrast, reducing the N_2H_4·HCO_2H from 5 mmol to 2.5 mmol increased the reaction yield to 38% (entry 17). A two-fold increase in the RANEY® Ni catalyst negatively affected the yield (entry 18). Moreover, a large amount of radioactivity remained in the filtration solid cake after the filtering off the catalyst from the reaction mixture and only 10% of **[2-^{11}C]indole** was detected in the filtrate. When the reaction temperature was raised to 40 °C, we found that the reaction yield was reached a peak at 10 min (30%) and further extension of reaction time decreased the reaction yield to 24% (entries 19–21). Next, a sharp reduction of N_2H_4·HCO_2H from 5 mmol to 0.5 mmol clearly improved the reaction and gave a 35% yield (entry 22). A 20% reduction of RANEY® Ni catalyst also showed positive impact and increased reaction yield to 41% (entry 23) which was the highest reductive cyclization yield obtained for this series of experiments.

Since it appeared that we had reached a ceiling for the reductive cyclization reaction, we tried to further improve the [^{11}C]cyanation yield by simply increasing the amount of start-



Scheme 5 Reductive cyclization inhibition test.

ing material **bromide**, **1** thereby helping to increase overall yields for the two steps. We doubled the amount of **bromide**, **1** for the [^{11}C]cyanation and 42% of **[^{11}C]-2** was obtained after the Sep-Pak purification. However, when this reaction mixture was submitted to reductive cyclization, we were disappointed to find that no **[2-^{11}C]indole** was detected (entry 24). Using 2 mg of **bromide**, **1** for the [^{11}C]cyanation totally inhibited the following reductive cyclization reaction leading us to speculate that the presence of **bromide**, **1** in the reaction mixture may be responsible for the yield ceiling of ~40% which we observed on the reductive cyclization.

For all of our reactions, we incorporated a Sep-Pak solid phase extraction (SPE) method to quickly remove the reaction solvent DMA, unreacted [^{11}C]cyanide ion, inorganic salts, phase transfer catalyst (PTC) 18-crown-6 as well as H_2O after the [^{11}C]cyanation step. After that, the ethanol wash process not only eluted radioactive **[^{11}C]-2** and **[^{11}C]-3** but also a large amount of the unreacted **bromide**, **1** into the reduction vessel from this Sep-Pak cartridge. To test our hypothesis that the residual **bromide**, **1** would interfere or even totally inhibit the reductive cyclization, a "cold" test for reductive cyclization step was tested again with the addition of extra **bromide**, **1** (2 mg) and no indole was detected confirming that the presence of the residual **bromide**, **1** which was carried over to the second vessel poisoned the catalyst (Scheme 5).

To determine how much of the excess **bromide**, **1** starting material was carried over into the reductive cyclization reaction, we next carried out a series of "cold" cyanation reactions with different starting amount of **bromide**, **1**. After the following Sep-Pak SPE purification process, the ethanol elute was analyzed with analytical HPLC and the results showed that 55–78% of the **bromide**, **1** which was used for the cyanation reaction was carried over into the second reaction vessel after the Sep-Pak SPE purification (Fig. 1). For example, when 1.0 mg of **bromide**, **1** was used for cyanation reaction, 0.65 mg of it was eluted from the Sep-Pak and introduced into the next reaction vessel.

Based upon the high reactivity of the **bromide**, **1** to S_N2 [^{11}C]cyanation (Table 1), we predicted that it would be possible to get comparable RCY of **[^{11}C]-2** using less **bromide**, **1** by simply modifying the [^{11}C]cyanation reaction conditions. We next designed a series of experiments to measure RCY and product distribution when reducing the amount of **bromide**, **1** (Table 3). By decreasing the **bromide**, **1** from 1 mg to 0.1 mg, the reaction rate was clearly decreased (entry 25). After a 0.6 min reaction, only 25% of the radioactivity was converted to desired intermediate **[^{11}C]-2**. Longer times only decreased

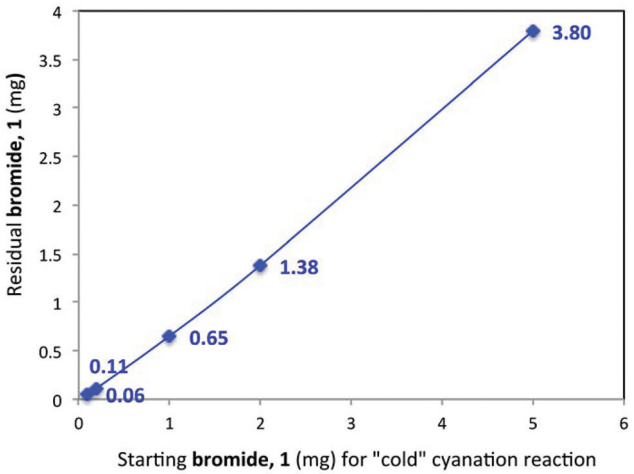


Fig. 1 The amount of residual **bromide**, **1** after purification of "cold" cyanation reaction mixture by SPE method.

Table 3 Further exploration of the [^{11}C]cyanation reaction with reduced amount of **bromide**, **1**

Entry	**Bromide**, **1** (mg)	Temp (°C)	Ratio of **[^{11}C]-2** : **[^{11}C]-3** (ORCY)[a] 0.6 min	2 min	5 min	15 min
25	0.1	0	100 : 0 (25)	100 : 0 (12)	100 : 0 (6)	100 : 0 (3)
26	0.1	20	97 : 3 (37)	96 : 4 (26)	93 : 7 (15)	83 : 17 (6)
27	0.1	40	96 : 4 (48)	91 : 9 (43)	95 : 5 (19)	91 : 9 (11)
28	0.1	60	96 : 4 (47)	98 : 2 (41)	97 : 3 (33)	94 : 6 (16)
29	**0.2**	**40**	**77 : 23 (78)**	72 : 28 (78)	42 : 58 (71)	27 : 73 (62)

[a] The ratio of **[^{11}C]-2** : **[^{11}C]-3** was calculated based upon radio-HPLC analysis of reaction solution samples taken at different time points (decay-corrected); ORCY (%, overall radiochemical yield, decay-corrected) included radioactive peaks of both **[^{11}C]-2** and **[^{11}C]-3**.

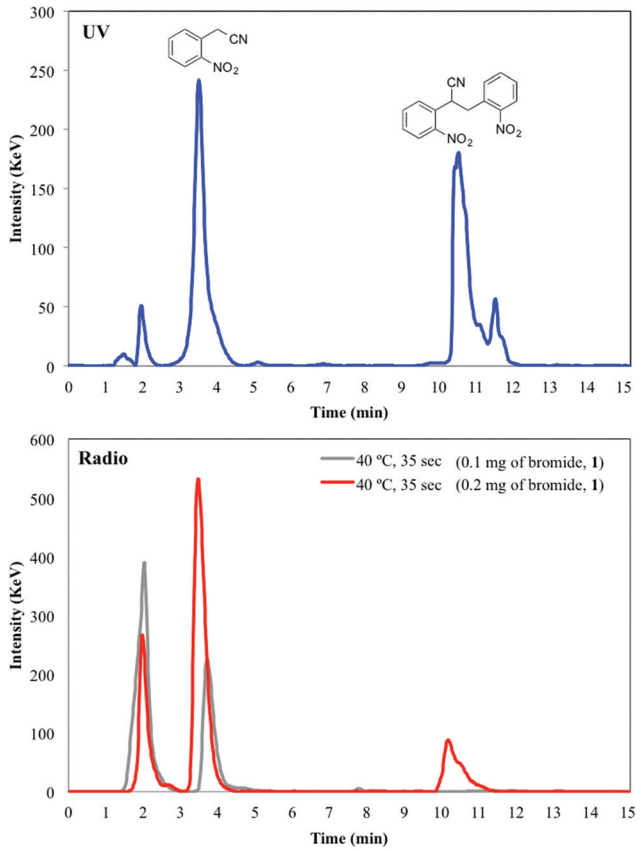


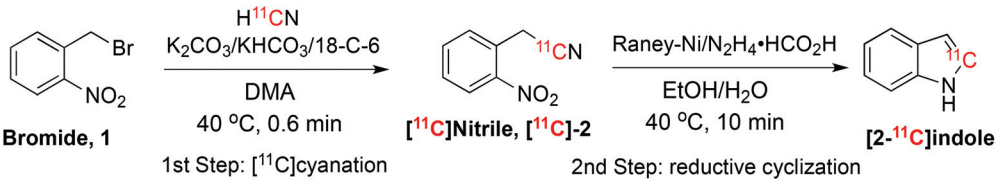
Fig. 2 Analytical radio-HPLC profiles of two reaction samples from the [^{11}C]cyanation reaction with 0.1 mg and 0.2 mg of **bromide**, **1**; top: UV-HPLC profile of sample co-injected with **nitrile**, **2** and **dimer**, **3**; bottom: radio-HPLC profile.

the amount of [^{11}C]-**2**. However, increasing the reaction temperature clearly improved the RCY (entries 26–28). The optimum conditions were found at 40 °C with 0.6 min reaction time (entry 27, Fig. 2). Further increasing the reaction temperature to 60 °C did not improve the reaction. One important finding was that only a negligible amount of [^{11}C]-**3** was formed in all experiments. Decreasing the amount of **bromide**, **1** from 1.0 mg to 0.1 mg, which is similar to the strategy that Kalir and Mualem used to increase the yield of **nitrile**, **2**,[18] clearly decreased the formation of undesired by-product [^{11}C]-**3**. To see if the yield of [^{11}C]-**2** could be further improved, the [^{11}C]cyanation reaction was further tested at 40 °C with a slight increase in the amount of **bromide**, **1** from 0.1 mg to 0.2 mg. The results were delightful: the ORCY reached 78% with ratio of [^{11}C]-**2** : [^{11}C]-**3** at 77 : 23 (entry 29, Fig. 2). Further extension of reaction time did not show any benefits, it not only reduced the ORCY but also decreased the ratio of [^{11}C]-**2** : [^{11}C]-**3**.

With the above [^{11}C]cyanation results in hand, we re-investigated the synthesis of [2-^{11}C]**indole** with a decreased amount of **bromide**, **1** starting material. Once a superfast (0.6 min) [^{11}C]cyanation was performed, the reaction mixture was quickly purified with a SPE Sep-Pak. Following elution with EtOH, the intermediate [^{11}C]-**2** was subjected to a reductive cyclization reaction using our optimized reaction conditions (Table 2, entry 23). When 0.1 mg of **bromide**, **1** was used for [^{11}C]cyanation reaction, only 21% of desired [^{11}C]-**2** was obtained (Table 4, entry 30). The subsequent reductive cyclization followed by semi-prep HPLC purification process yielded 57% of [2-^{11}C]**indole** and the entire two-step preparative RCY for this synthesis was 12% (entry 30). When the 0.2 mg of **bromide** was used, 48% of the desired [^{11}C]-**2** was obtained after the SPE purification. The next reductive cyclization step yielded 54% of [2-^{11}C]**indole** and the entire two-step preparative RCY was 26% (Table 4, entry 31). These two experiments showed that the reductive cyclization reaction was clearly improved by decreasing the amount of **bromide**, **1** and that the preparative (isolated) yield of the reductive cyclization step was over 50%. In addition, increasing the amount of **bromide**, **1** from 0.1 to 0.2 mg doubled the yield of [^{11}C]-**2** and also increased the entire radiochemical yield of [2-^{11}C]**indole** from 12% to 26% (Table 4, entry 31).

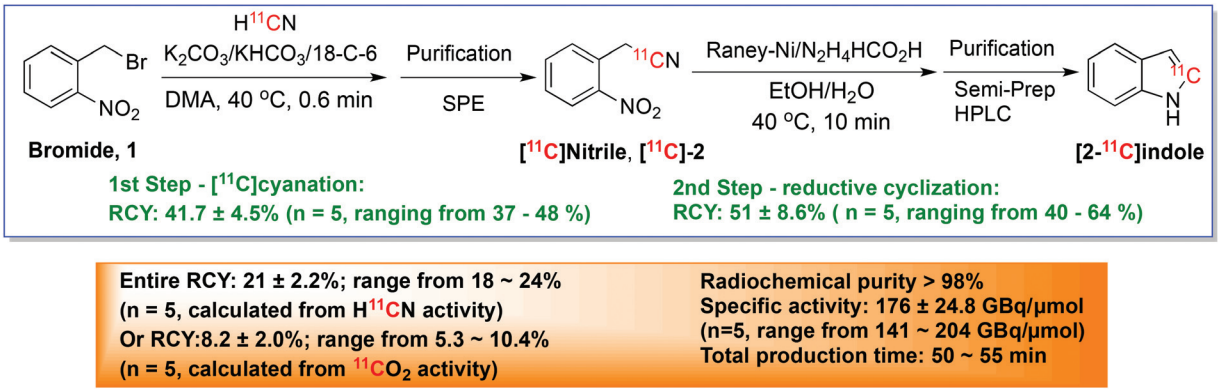
With the combination of a reduced amount of starting **bromide**, **1** (0.2 mg), strictly controlled and also superfast reaction time (0.6 min), mild temperature (40 °C) as well as a polar aprotic solvent DMA, the [^{11}C]cyanation reaction provided us a

Table 4 Further exploration of hydrogenation with reduced starting amount of **bromide**, **1** [a]



Entry	**Bromide**, **1** (mg)	1st step RCY (%)	2nd step RCY (%)	Entire RCY[b] (%)
30	0.1	21	57	12
31	**0.2**	**48**	**54**	**26**

[a] Both 1st step and 2nd step RCYs were preparative results (purified by SPE and semi-prep HPLC method, respectively). [b] Entire RCY (%), entire radiochemical yield (decay-corrected) for synthesis of [2-^{11}C]**indole**.



Scheme 6 Optimized reaction parameters for synthesis of [2-^{11}C]indole.

nearly 50% yield of [^{11}C]-2 intermediate. Next, the reductive cyclization of [^{11}C]-2 under selected conditions (0.5 mmol $N_2H_4{\cdot}HCO_2H$, 38 mg RANEY® Ni in EtOH/H_2O solution at 40 °C for 10 min) yielded over 50% of **[2-^{11}C]indole**. For the whole synthetic process, 26% of **[2-^{11}C]indole** (calculated from starting [^{11}C]HCN, decay-corrected) was obtained in 54 min, calculated from the time of end of bombardment (EOB) to the completion of the semi-prep HPLC purification.

To confirm the robustness of this newly developed synthetic method, we repeated the synthesis of **[2-^{11}C]indole** five times with the optimized reaction conditions described above. The results show the high reliability of this synthetic process (Scheme 6). With a six-min cyclotron beam time, which generates ~24.4 GBq (660 mCi) of [^{11}C]CO_2, 0.17–0.40 GBq (4.7–10.9 mCi) of **[2-^{11}C]indole** product was obtained at the end of synthesis (EOS). The radiochemical yield for both steps was 21 ± 2.2% (calculated from [^{11}C]HCN radioactivity collected in the first reaction vessel) and was 8.2 ± 2.0% (calculated from [^{11}C]CO_2 radioactivity generated from a six-min cyclotron beam). The radiochemical purity of final product was >98% and the specific activity of final product was 176 ± 24.8 GBq μmol^{-1} (4.8 ± 0.7 Ci μmol^{-1}). The complete processing time, calculated from EOB to the EOS, ranged from 50 to 55 min.

Conclusion

In summary, we successfully developed a practical and efficient method for synthesis of a biologically interesting PET tracer **[2-^{11}C]indole** from NCA [^{11}C]HCN with high specific activity. The overall process illustrates the challenges in determining optimum conditions for controlling a superfast [^{11}C] cyanation reaction that yielded a highly reactive intermediate **[^{11}C]nitrile**, [^{11}C]-2. More specifically, under NCA conditions, the amount of [$^{11/12}$C]HCN is very low (0.03–0.06 μmol) and fixed, biasing the stoichiometry in favor of the highly reactive **bromide**, **1**. In this case, the amount of **bromide**, **1** initially used in the formation of the intermediate [^{11}C]-2 both consumed the desired [^{11}C]-2 by further alkylation and also poisoned the reductive cyclization step. An iterative systematic investigation and optimization of each step, however, resulted in a robust, rapid and reproducible method for the radiosynthesis of **[2-^{11}C]indole**. Currently, we are working on plant and bacteria imaging studies using the **[2-^{11}C]indole** tracer and these results will be reported separately.

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