Supplementary Material

An Efficient and Practical Synthesis of [2-^{11}C]Indole via Superfast Nucleophilic [^{11}C]Cyanation and Raney Nickel Catalyzed Reductive Cyclization

So Jeong Lee[†, ‡], Joanna S. Fowler[†], David Alexoff[†], Michael Schueller[†], Dohyun Kim[†], Alexander Nauth[†, ¶], Carina Weber[†, ¶], Sung Won Kim[†], Jacob M. Hooker[†, §], Ling Ma[§], Wenchao Qu*[†]

[†]Biological, Environmental & Climate Sciences Department, Brookhaven National Laboratory, Upton, NY 11973, USA

[‡]Department of Chemistry, Stony Brook University, Stony Brook, NY 11794, USA

[§]Athinoula A. Martinos Center for Biomedical Imaging, Department of Radiology, Massachusetts General Hospital, Harvard Medical School, Charlestown, Massachusetts 02129, USA

[¶]Institut für Kernchemie, Johannes Gutenberg-Universität, D-55128, Mainz, Germany

Email: wqu@bnl.gov

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1. Detailed Experimental Procedures

1.1. General

All commercial chemical reagents, 2-nitrobenzyl bromide, 2-nitrophenylacetonitrile, 18-crown-6, potassium bicarbonate, potassium carbonate, Raney-nickel in water (60% slurry), hydrazine monohydrate, formic acid (98%) and solvents for synthesis and analysis were purchase from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA) with a minimum of ACS reagent grade and used without further purification. Solid phase extraction (SPE) cartridges (SepPak® C18 plus) manufactured by Waters (Waters® Association, MA, USA) were used. The reaction was carried out in a 10 cc microwave reaction vial sealed by a cap and septum to withstand high pressure (Biotage® INC., VA, USA).

Preliminary model reactions performed without radioisotopic labeling were characterized by ultra-high performance liquid chromatography (UHPLC) using an Agilent model 1200 system (Agilent Technologies Inc., Santa Clara, CA). MBq and KBq levels of radioactivity were measured using a Capintec CRC-712MV and a Capintec CRC-ultra radioisotope dose calibrator (Capintec Inc. NJ, USA), respectively. Semi-preparative high performance liquid chromatography (HPLC) was performed using a Knauer HPLC system equipped with a model K-1001 pump, a model 87 variable wavelength monitor, a NaI detector and a SRI peak simple integration system. Analytical HPLC was performed using a Knauer model K-1001 pump, a Knauer model K2501 UV detector (254 nm), a Geiger Muller ionization detector and a SRI Peak Simple integration system. Radiochemical yields (decay-corrected back to end of cyclotron bombardment (EOB)) were obtained based on the total radioactivity trapped in the reaction vessel at the start of the reaction. Specific activity values, decay corrected back to EOB and

recorded in GBq/μmol, were determined from the C-11 activity in the product peak in the HPLC and the mass of compound. Total synthesis times were calculated from EOB to the end of radiotracer HPLC purification.

1.2. Controlled production system for anhydrous [^{11}C]HCN

1.2.1. Production of [^{11}C]CO_2 and [^{11}C]HCN

Carbon-11 was generated as [^{11}C]CO_2 using 17.4 MeV proton irradiation of an N_2 gas target containing 100 ppm O_2 to induce the $^{14}N(p, \alpha)^{11}C$ nuclear reaction. Irradiations were carried out on the BNL EBCO TR-19 cyclotron. The [^{11}C]HCN was produced via an in-house built, automated gas phase synthesis system[1]. Briefly, [^{11}C]CO_2 produced from cyclotron bombardment process was collected over molecular sieves, catalytically reduced to [^{11}C]CH_4 by H_2 under the catalysis of Ni at 420 °C. The [^{11}C]CH_4 was converted the [^{11}C]HCN by adding gaseous ammonia and passage through a Pt furnace at 950 °C at a flow rate of 350~400mL/min. The amount of radioactive [^{11}C]HCN was measured by a Capintec CRC-712MV radioisotope dose calibrator (Capintec Inc., Ramsey, NJ).

1.2.2. Purification of [^{11}C]HCN

The purification procedure for ensuring anhydrous [^{11}C]HCN was based on a previously published method[2]. The helium stream containing [^{11}C]HCN radioactivity produced by the automated [^{11}C]HCN production system was bubbled through a sulfuric acid bath (50%, 5 mL), heated by a 65 °C oil bath and the stream was then passed though a drying tube (freshly packed P_2O_5, ~ 8 mL). This anhydrous helium stream was next bubbled into the reaction vessel, in

which was pre-loaded with reaction solvent (0.3 mL), cation source (15 μmol), 18-crown-6 (30 μmol) and a magnetic stirring bar, prior to trapping of [^{11}C]cyanide radioactivity.

1.3. Nucleophilic [^{11}C]cyanation for synthesis of 2-(2-nitrophenyl)-[1-^{11}C]acetonitrile (**[^{11}C]-2**)

1.3.1. Radio-HPLC analysis procedure for monitoring the nucleophilic [^{11}C]cyanation reaction

At the appropriate time point, a micro syringe was used to take a sample of the reaction mixture (~ 50 μL) which was diluted with aqueous formic acid solution (0.1%, 0.2 mL). The diluted sample (~ 10 μL) was injected onto the HPLC for the analysis (column: Agilent, Eclipse XDB-C8, 4.6 × 150 mm, 5 μm; mobile phase: aqueous formic acid (0.1%)/methanol=50/50; flow rate: 1 mL/min). Once the of 2-(2-nitrophenyl)-[1-^{11}C]acetonitrile (**[^{11}C]-2**) peak eluted (3 – 4 min), the HPLC solvent was switched to 100% MeOH for flushing out radioactive by-product 2,3-bis(2-nitrophenyl)-[1-^{11}C]propanenitrile (**[^{11}C]-3**) (8 – 9 min).

1.3.2. Optimized radiosynthesis of 2-(2-nitrophenyl)-[1-^{11}C]acetonitrile (**[^{11}C]-2**) from 2-nitrobenzyl bromide (**bromide, 1**)

Into a microwave use U-shape reaction vessel, a stock solution containing K_2CO_3/$KHCO_3$/18-crown-6 (2.5 μmol/10 μmol/30 μmol) in aqueous acetonitrile and a stir bar were placed. This mixture was evaporated by heating at 120 °C under a mild argon stream. After most solvent had evaporated, the reaction tube was further dried azeotropically with CH_3CN (2 × 1 mL) to remove all moisture. Once the drying process was complete, the reaction vessel was cooled to room temperature, dimethylacetamide (DMA, 0.3 mL) was added, and the tube was immersed into a 40 °C oil bath and ready for trapping [^{11}C]HCN radioactivtiy.

Once the [^{11}C]HCN radioactivity was ready, a helium stream containing [^{11}C]HCN radioactivity was bubbled into above reaction vessel and trapped. After 3 min, the helium flow was stopped and starting material, 2-nitrobenzyl bromide (**bromide**, **1**, 0.2 mg, 0.926 μmol, dissolved in 0.2 mL of DMA) was immediately added into reaction vessel. The reaction solution was maintained at 40 °C for 35 seconds. An aqueous formic acid solution (0.1% v/v, 1 mL) was quickly added to quench the reaction. The resulting mixture was further diluted with aqueous formic acid solution (0.1% v/v, 9 mL). The diluted mixture was passed through a C18Plus cartridge. Following a wash with H_2O (1 × 5 mL), the radioactivity (**[^{11}C]-2** and **[^{11}C]dimer**, **[^{11}C]-3**) trapped on the cartridge were eluted into second U-shape reaction vessel with EtOH (1.3 mL). A sample of the radioactivity was submitted to radio-HPLC for quality analysis prior to proceeding to the next step reductive cyclization for synthesizing **[^{11}C]indole** (see Fig. 3.1 for a representative analytic HPLC profile).

1.4. Reductive cyclization of **[^{11}C]-2** to **[2-^{11}C]indole**

To the second reaction vessel (pre-equipped with a stir bar) containing the radioactivity from the above [^{11}C]cyanation reaction dissolved in aqueous EtOH (1.3 mL EtOH 1.3 mL mixed with ~ 0.2 mL water which came from the dead volume of the C18Plus cartridge), Raney-nickel (38 mg, 50% slurry in water) and hydrazinium monoformate (0.5 mmol, N_2H_4/HCO_2H = 1 : 1 mol ratio) were added and the reaction vessel was immediately capped. The mixture was maintained at 40 °C with fast stirring for 10 min to allow the reductive cyclization. The resulting mixture was diluted with water (1 mL) and filtered through a Celite pad for removal of solids. The filtrate was injected onto a semi-preparative HPLC for purification (Column: Phenomenex Luna C18, 10 × 250 mm, 5 μm; mobile phase: 0.1% aqueous formic acid solution/methanol = 45/55;

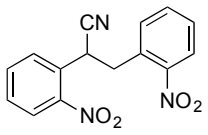
flow rate: 5 mL/min). The radioactivity that eluted at 12 – 13 min was collected as final product **[2-^{11}C]indole** (see Fig. 3.2 for a representative semi-prep HPLC profile). This time point was defined as the end of synthesis (EOS). The total synthesis time from EOB to EOS was 50 – 55 min. The overall radiochemical yield of **[2-^{11}C]indole** based on total [^{11}C]HCN trapped was 21 ± 2.2% (n = 5, ranging from 18 – 24%).

1.5. Quality control and specific activity determination

Both the radiochemical purity and specific activity of final product **[2-^{11}C]indole** were determined by an analytical radio-HPLC system (Column: Agilent Eclipse XDB-C8, 4.6 × 150 mm, 5 μm; mobile phase: 0.1% aqueous formic acid/methanol = 50/50; flow rate: 1 mL/min). The radioactivity peak at 6 min corresponds to desired **[2-^{11}C]indole**. A UV standard calibration curve was first measured using an authentic sample of reagent indole for the mass determination. The injected radioactivity was measured in the range of MBq using a low dose Capintec radioisotope dose calibrator. The specific activity was determined as the ratio of the injected radioactivity (GBq, decay-corrected to EOB) and mass (μmol). The radiochemical purity (%) was determined by integrating radio peaks on the analytical radio-HPLC profile. The chemical identity of the **[2-^{11}C]indole** was determined by co-injection with a standard indole sample (see Fig. 3.3 for a representative analytic HPLC profile).

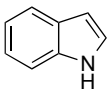
1.6. "Cold" chemistry

1.6.1. Synthesis of 2,3-bis(2-nitrophenyl)propanenitrile (**dimer, 3**)



The sample of 2,3-bis(2-nitrophenyl)propanenitrile was synthesized following a literature reported method[3]. 2-nitrobenzyl bromide (0.05 g, 0.23 mmol) was added to a mixture of potassium cyanide (0.015 g, 0.23 mmol) in DMSO (2 mL). This mixture was stirred for 1 h at r.t. and then 1 h at 40 °C. This reaction mixture was poured into water (10 mL) and extracted with ethyl acetate (3 × 10 mL). The organic phases were combined, dried over anhydrous Na_2SO_4 and concentrated by rota-vapo evaporation. The left residue was purified by flash chromatography (gradient eluent solvent system: ethyl acetate/hexane from 10/90 to 30/70) to give desired sample **dimer, 3** as a white solid (0.03 g, yield: 88 %). ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.05 (dt, J = 8, 1.2 Hz, 2H), 7.76 – 7.70 (m, 2H), 7.65-7.44 (m, 4H), 5.19 (dd, J = 8.8, 6 Hz, 1H), 3.84 (dd, J = 13.6, 8.8 Hz, 1H), 3.51 (dd, J = 13.6, 6 Hz, 1H) (^{1}H NMR is correspondant to the literature[3]); ^{13}C NMR (100 MHZ, $CDCl_3$): δ 134.53, 133.92, 133.14, 130.93, 130.87, 130.15, 129.95, 129.40, 125.97, 125.76, 119.01, 37.8, 35.1; MS (ESI): m/z [M + NH_4^+] calcd for $C_{15}H_{11}N_3O_4$ 297.1 found 315.0.

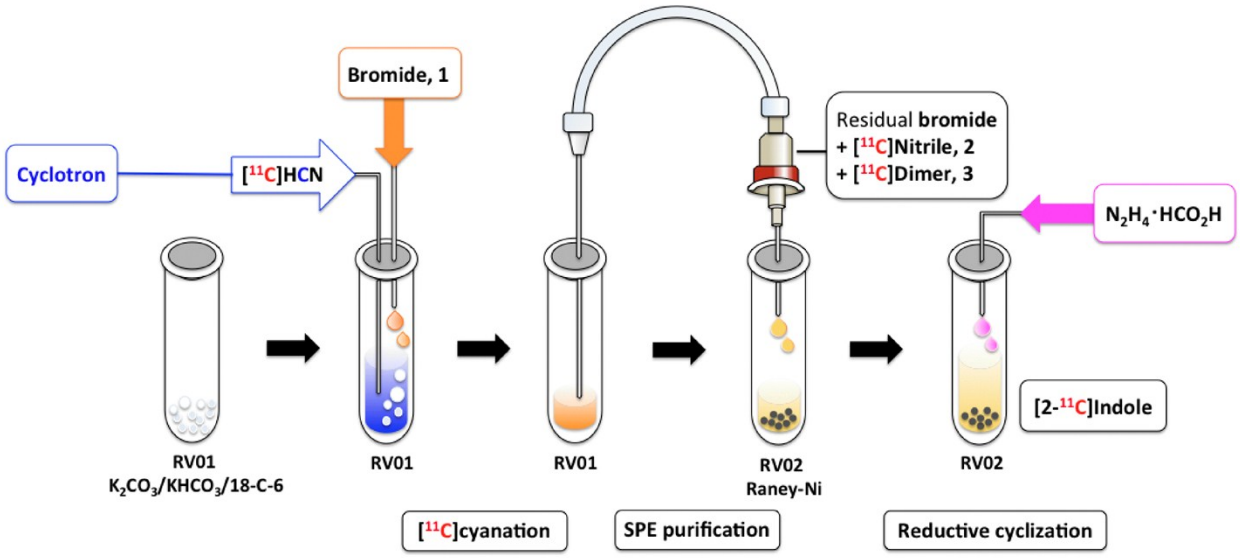
1.6.2. Preparation of indole from the nitrile without the benzyl bromide



The experimental procedure for reductive cyclization to synthesize indole from **nitrile, 2** was modified and optimized based on a previously published method[4]. Hydrazinium monoformate was prepared by carefully mixing equal moles of hydrazine monohydrate and 98% formic acid in an ice water bath. The hydrazinium monoformate prepared was used directly for the reductive cyclization reaction. A mixture of 2-nitrophenylacetonitrile (2 mg, 12.3 μmol) and Raney-nickel (0.4 mg) in EtOH/H_2O (1.5 mL, v/v = 4/1) was stirred with hydrazinium monoformate (0.5 μmol, 41 μL), for 15 min at r.t. The reaction mixture was filtered through a celite pad and a

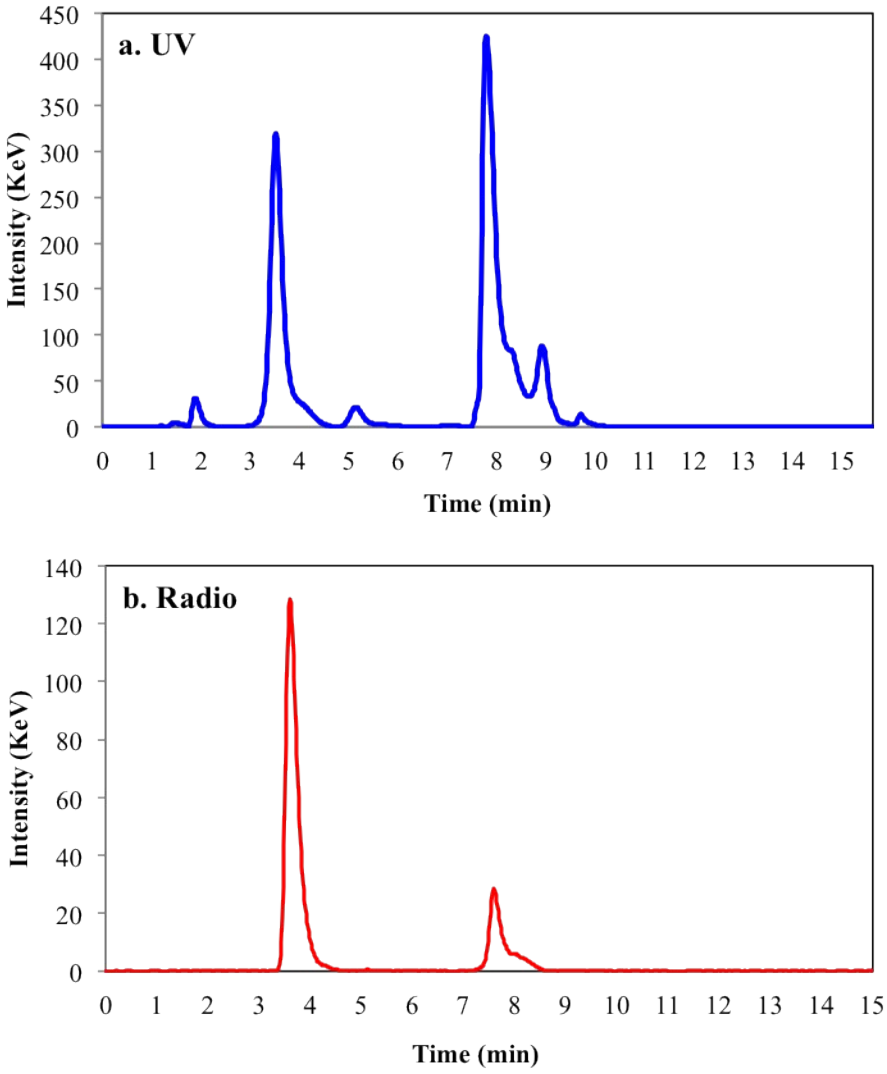
sample of filtrate was injected onto an analytical HPLC (column: Waters, XSelect HSS PEP, 4.6 × 30 mm, 2.5 μm; mobile phase: 0.1% aqueous formic acid solution/methanol = 50/50, V/V; flow rate: 1 mL/min). An unidentified by-product eluted at 1 min. Unreacted **nitrile**, **2** eluted at 1.2 min and product **indole** eluted at 1.7 min. The yield of indole (60 %) was estimated by integrating peaks on the analytical UV-UHPLC profile.

2. Scheme of radiosynthesis process of [2-^{11}C]indole



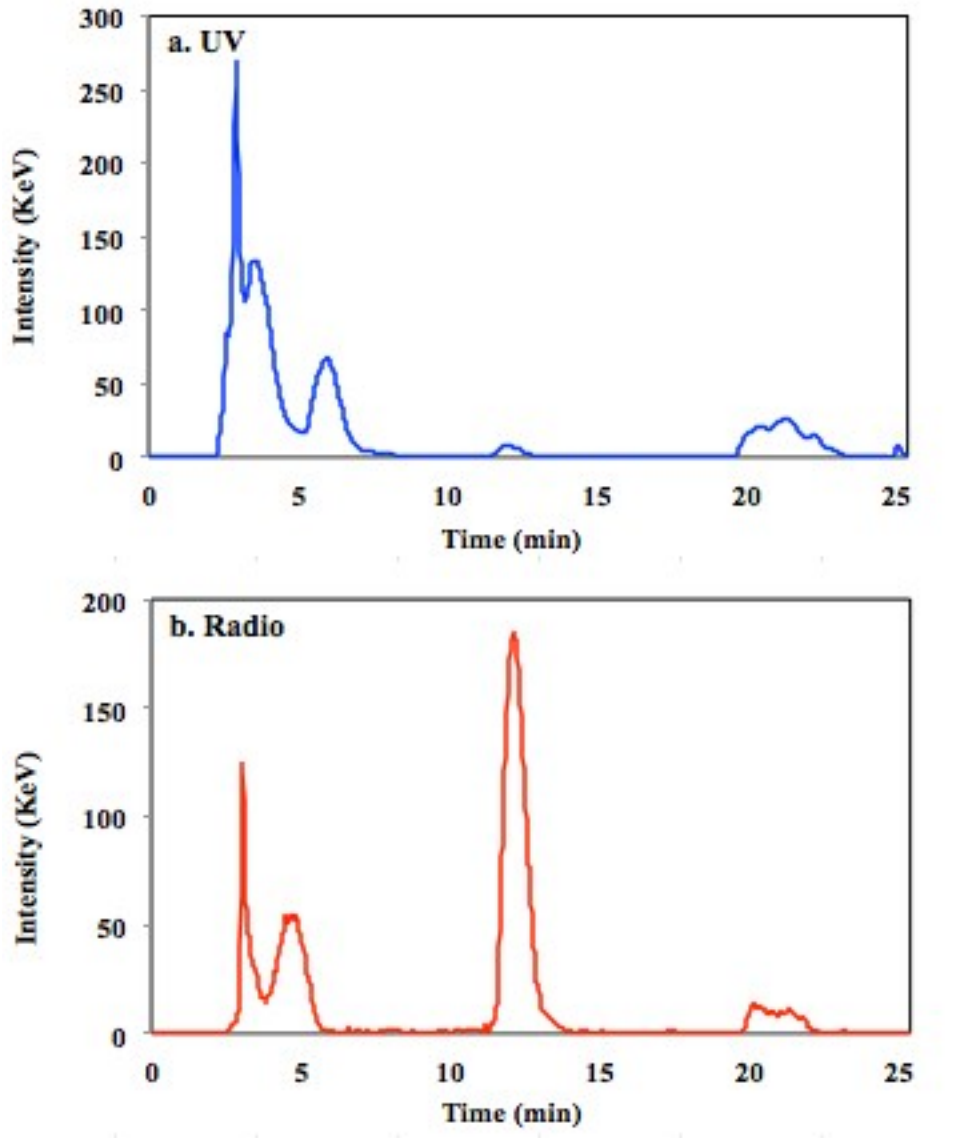
3. HPLC profiles

3.1. Analytical radio-HPLC profile of nucleophilic [^{11}C]cyanation reaction, analyzed sample was obtained from intermediate solution after C18plus cartridge purification, co-injected with **nitrile, 2** and **dimer,3** standards.



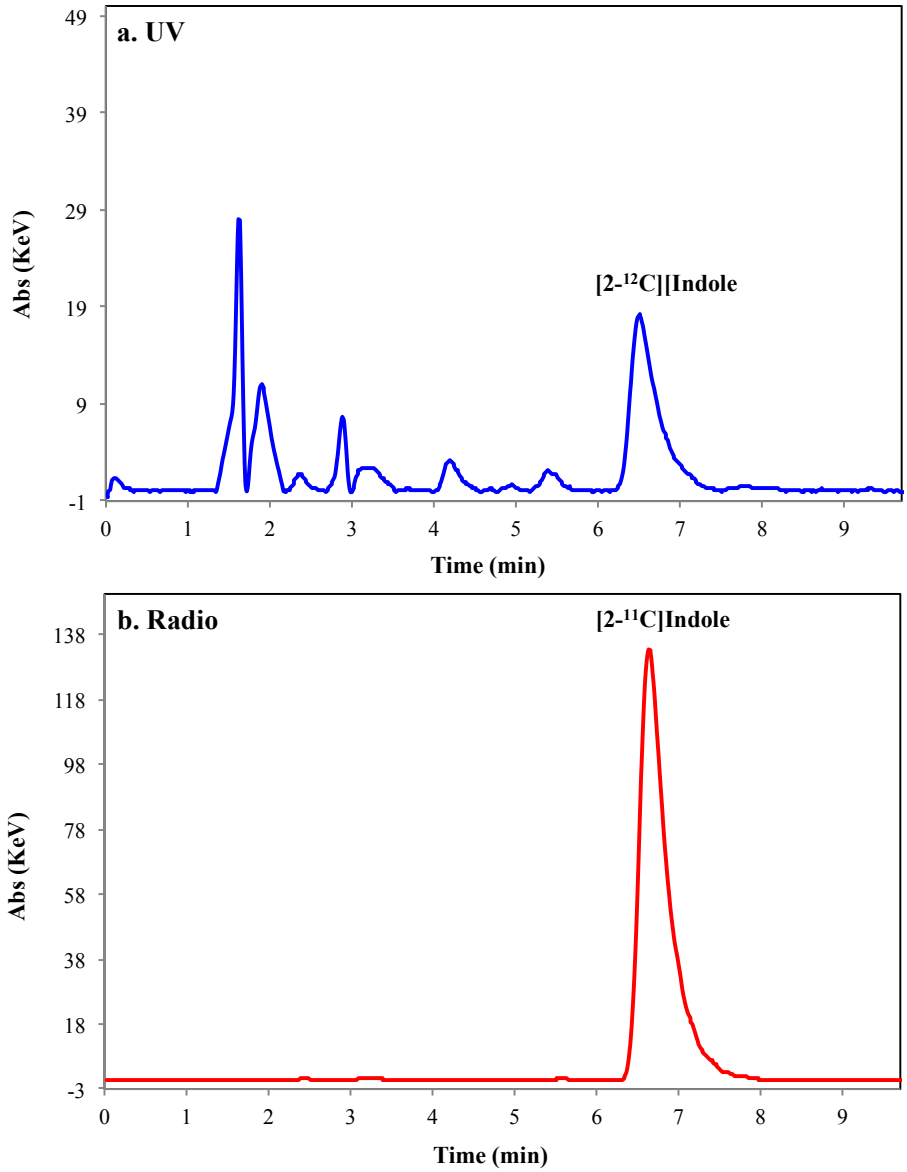
Peak at 3.5 min: **[^{11}C]nitrile, 2**; peak at 7.7 min: **[^{11}C]dimer, 3**.

3.2. Semi-Prep HPLC profile of purification of product **[2-^{11}C]indole**



Radio-Peak at 12 min: desired product **[2-^{11}C]indole**

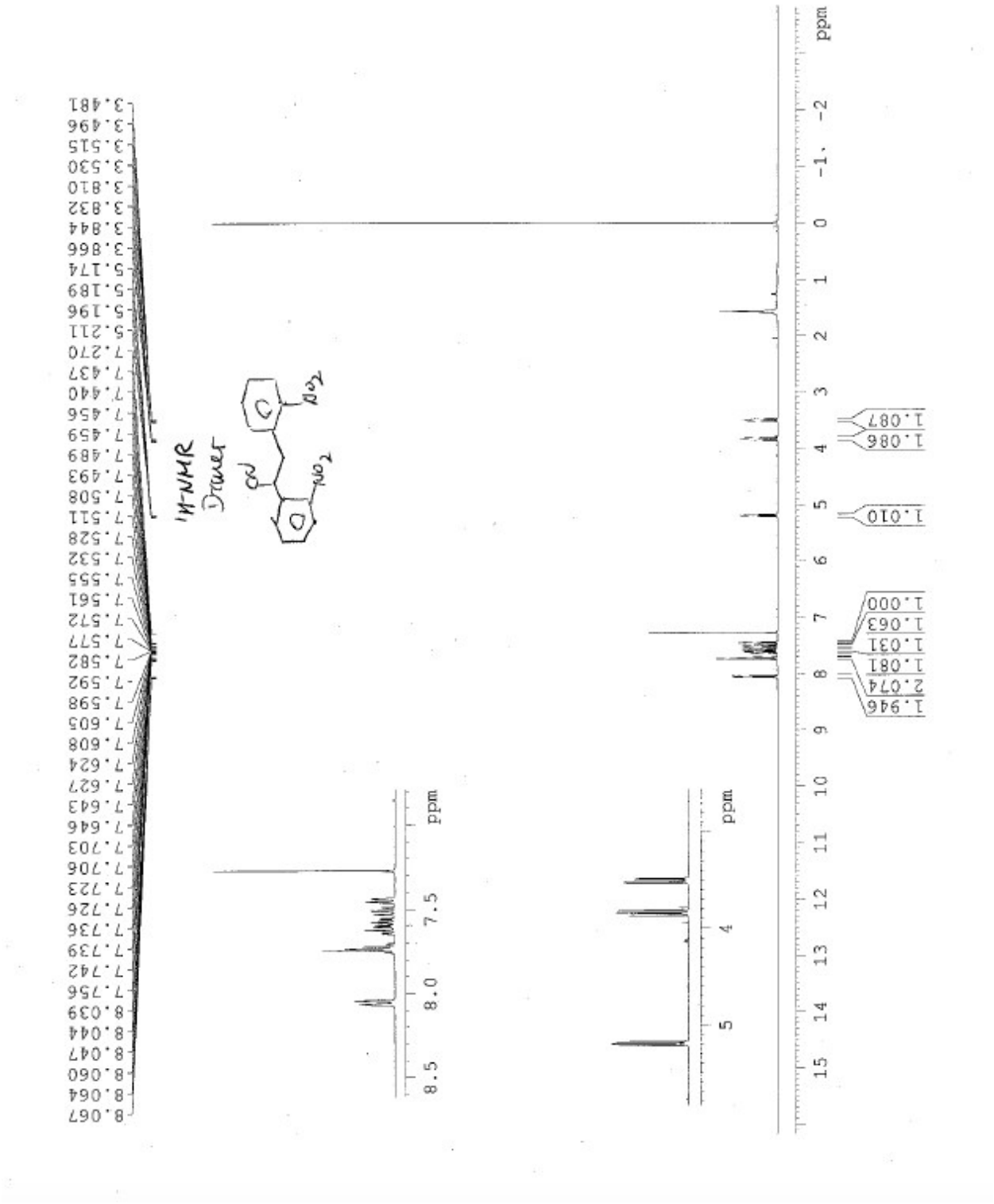
3.3. Product **[2-^{11}C]indole** quality control HPLC profile

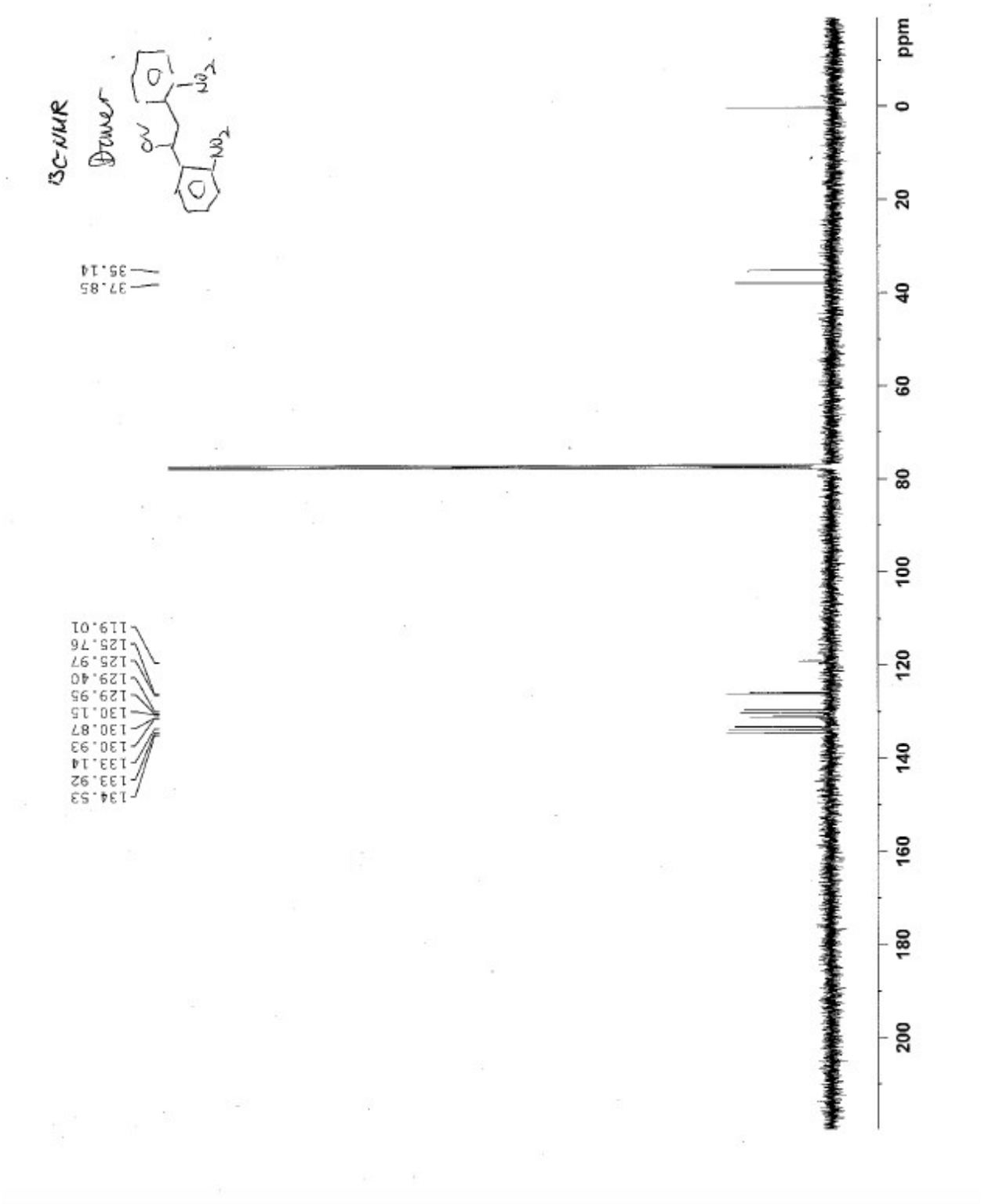


A. UV- trace: co-injected [2-^{12}C]indole, peak at 6.5 min;

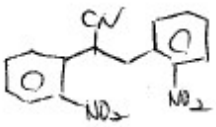
B. Radio- trace: desired product **[2-^{11}C]indole**, peak at 6.8 min.

4. NMR & MS spectra of 2,3-bis(2-nitrophenyl)propanenitrile (dimer, 3)




13C-NMR
Dimer
NO2
NO2
35.14
37.85
119.01
125.76
125.97
129.40
129.95
130.15
130.87
130.93
133.14
133.92
134.53
200
180
160
140
120
100
80
60
40
20
0
ppm

Print of window 80: MS Spectrum
Data File : D:\CHEM32\DATA\FOWLER\061215_DIMER.D
Sample Name : 061215_dimer.D



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Inj Volume : 1.0 µl
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Last changed : 6/12/2015 6:49:12 PM by So Jeong
(modified after loading)
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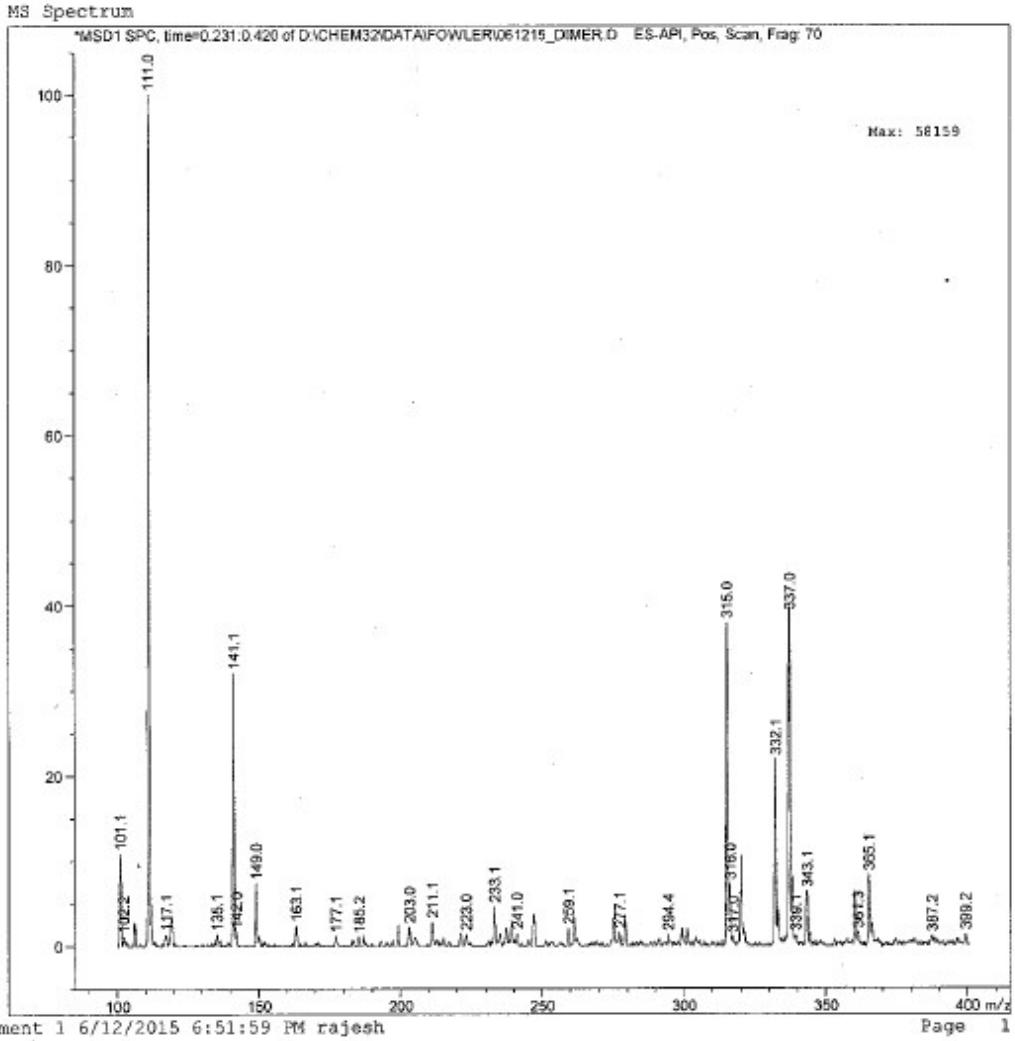
MS Spectrum



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5. References

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