Iodonium Ylide–Mediated Radiofluorination of -^{18}F-FPEB and Validation for Human Use

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Contents

Experimental Details

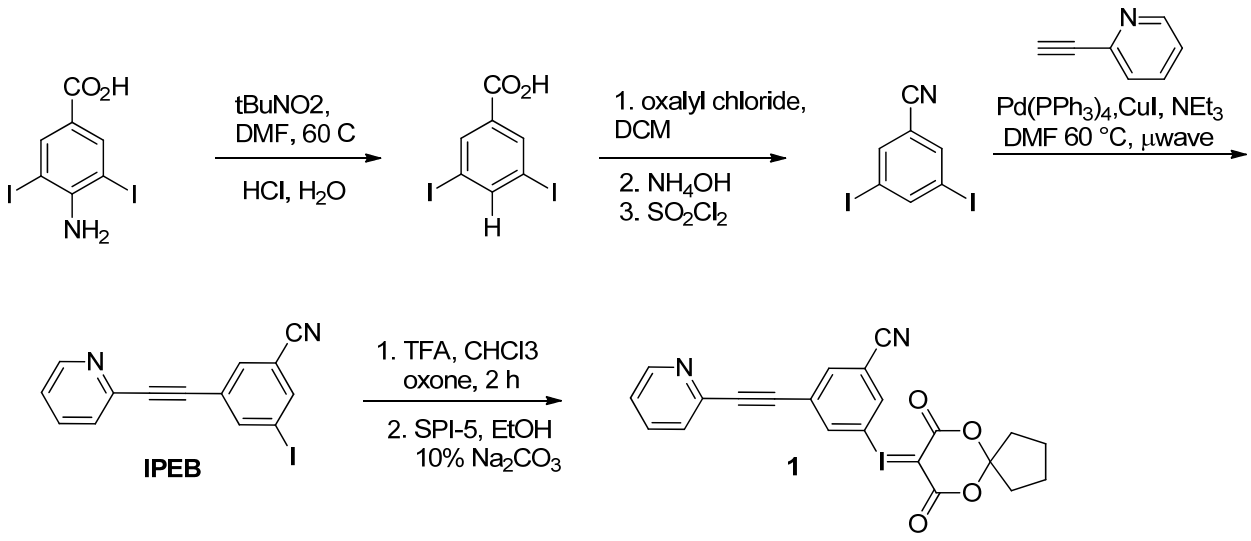
Chemistry

All commercial reagents were purchased from Sigma-Aldrich or Fisher Scientific and, unless otherwise stated, used as received. All solvents were of reagent or anhydrous grade quality and purchased from Sigma-Aldrich or Fisher Scientific. All deuterated solvents were purchased from Cambridge Isotopes. Analytical thin-layer chromatography (TLC) was performed on precoated glass-backed plates (EMD TLC Silica gel 60 F254) and visualized using a UV lamp (254 nm), potassium permanganate, and/or iodine stain. Flash column chromatography was performed using a Biotage Isolera One system and preloaded Biotage columns. Silica gel for flash chromatography was high purity grade 40 – 63 μm pore size and purchased from Sigma-Aldrich. Yields refer to purified and spectroscopically pure compounds. Melting points were determined using a Thomas Hoover model apparatus and are uncorrected.

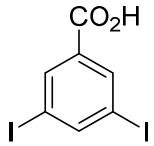
Spectroscopy: ^{1}H, and ^{13}CNMR spectra were recorded on a Bruker 300 MHz or a Varian Unity Inova 500 MHz spectrometer, and resonances given in parts per million (ppm) relative residual solvent IR spectra were recorded from neat compounds on a Bruker ALPHA FT-IR. Only select IR absorbances are reported. HRMS spectra were recorded on an Agilent 6220 ESI TOF mass spectrometer using flow injection analysis.

Synthetic Procedures and Characterization Data

Synthesis of Iodonium Ylide Precursor **2**; synthetic scheme

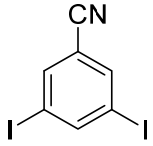


3,5-diiodobenzoic acid



The titled compound was prepared by a literature procedure (*1*) with slight modifications. 4-amino-3,5-diiodobenzoic acid (2.0 g, 5.4 mmol) was added portion-wise to a stirred solution of t-butyl nitrite (1.07 g, 10.4 mmol) in DMF (10 mL) heated at 50ºC in a 3-neck round bottom flask equipped with a reflux condenser. Additional DMF (10 mL) was added halfway through the addition. Gas evolution was observed after each addition of the benzoic acid. Upon completion of the addition the reaction mixture was heated at 60ºC for 30 min and then allowed to cool to room temperature. The brown solution was diluted with diethyl ether (60 mL) and poured over dilute HCl (100 mL, 3N). The ethereal layer was removed and washed with 3N HCl (2 × 20 mL), water (3 × 20 mL) and brine (1 × 20 mL) then dried over anhydrous $MgSO_4$. Removal of diethyl ether *in vacuo* and subsequent recrystallization in methanol afforded the desired compound in 77% yield (1.5 g, 4.0 mmol); mp 234ºC–236ºC ^{1}H-NMR (300 MHz, DMSO-d_6) δ (ppm): 13.49 (s, 1H), 8.31 (s, 1H), 8.17 (s, 2H) ^{13}C-NMR (75 MHz, DMSO-d_6) δ (ppm): 165.1, 148.6, 137.5, 134.7, 96.6 HRMS (m/z): $[M - H]^-$ calcd. for $C_7H_3I_2O_2$, 372.8222; found 372.8231.

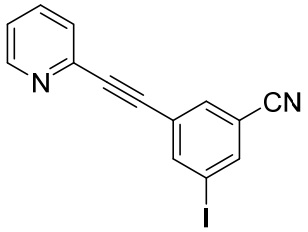
3,5-diiodobenzonitrile



To a stirred solution of 3,5-diiodobenzoic acid (1.7 g, 4.5 mmol) in dichloromethane (DCM; 10 mL) was added oxalyl chloride (2. 9 g, 23 mmol). After 5 hours, the volatile contents were removed under reduced pressure. The resulting residue was poured with caution into cold ammonium hydroxide (50 mL, 28%) and stirred for 2 h. The amide product was removed by filtration and the collected residue was dissolved in DCM and washed with 1 M HCl, 1 M NaOH, water, and brine. The organic layer was dried with $MgSO_4$. Removal of DCM *in vacuo* afforded 3,5-diiodobenzamide, which was used without further purification. Thionyl chloride (8.2 g, 69 mmol) was added to the collected amide and the mixture was heated under reflux for 18 h. The reaction mixture was allowed to cool and excess thionyl chloride removed under reduced pressure. The resulting residue was dissolved in EtOAc and washed with a saturated

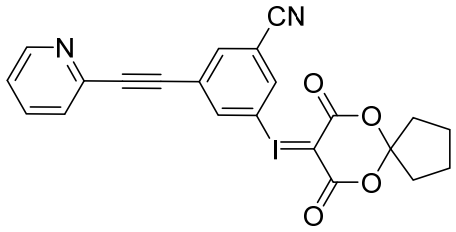
solution of $NaHCO_3$ (3 × 10 mL), water (2 × 10 mL), and brine and dried over anhydrous $MgSO_4$. Concentration of the organic solution followed by column chromatography purification yielded the titled compound as an off-white solid in 60% yield; mp 129ºC–131ºC; ^{1}H-NMR (300 MHz, $CDCl_3$) δ (ppm): δ 8.30 (t, J = 1.4 Hz, 1H), 7.94 (d, J = 1.5 Hz, 2H). ^{13}C-NMR (75 MHz, $CDCl_3$) δ (ppm): 149.7, 139.6, 115.6, 115.5, 94.5 HRMS (m/z): $[M + H]^+$ calcd. for $C_7H_4I_2N$, 355.8428; found 355.8439.

3-iodo-5-(pyridin-2-ylethynyl)benzonitrile (IPEB).



The titled compound was prepared by a literature procedure (*2*) to give a white solid in 50% yield;; mp 156°C–157°C; ^{1}H NMR: (300.1 MHz, $CDCl_3$) δ (ppm): 8.65 (d, J = 4.5 Hz, 1H), 8.15 (t, J = 1.5 Hz, 1H), 7.95 (t, J = 1.4 Hz, 1H), 7.81 (t, J = 1.4 Hz, 1H), 7.72 (td, J = 7.7, 1.8 Hz, 1H), 7.53 (dt, J = 7.8, 1.0 Hz, 1H), 7.31 (ddd, J = 7.7, 4.8, 1.2 Hz, 1H). ^{13}C NMR: (75.5 MHz, $CDCl_3$) δ (ppm) 150.4, 144.6, 142.2, 140.3, 136.4, 134.2, 127.5, 125.5, 123.7, 116.3, 114.4, 93.5, 91.9, 84.7, 77.5, 77.0, 76.6. HRMS (m/z): $[M + H]^+$ calcd. for $C_{14}H_8IN_2$, 330.9727; found 330.9742.

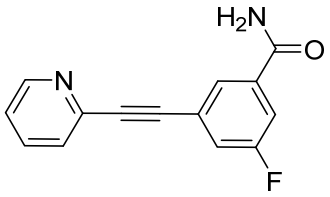
3-((7,9-dioxo-6,10-dioxaspiro[4.5]decan-8-ylidene)-λ3-iodanyl)-5-(pyridin-2-ylethynyl)benzonitrile (1)



Trifluoroacetic acid (0.9 mL) was added to a solution of IPEB (120 mg, 0.36 mmol) in chloroform (0.12 mL). Oxone (179 mg, 0.58 mmol) was added and the reaction mixture was stirred for 5 h, until full conversion of starting materials was determined by TLC. Volatile contents were then removed by rotary evaporation. The dried residue was suspended in ethanol (1.5 mL) and 6,10-dioxaspiro[4.5]decane-7,9-dione (67 mg, 0.54 mmol) was added followed by

10% Na_2CO_3(aq) (w/v, 1.5 mL, 0.33 M solution). The pH of the reaction mixture was tested and adjusted with Na_2CO_3 until the reaction pH > 10. The reaction mixture was stirred for 5 h until full conversion to the iodoinium ylide was determined by TLC. The reaction mixture was then diluted with water, and extracted with chloroform. The chloroform extracts were combined and washed with water (4 × 10 mL) and brine (1 × 10). The organic layer was dried with anhydrous MgSO4, filtered, and concentrated. To the residue was added ethyl acetate and hexanes to induce precipitation (at room temperature or −25°C). Solids were collected by filtration and purified by flash chromatography using 10% EtOH in EtOAc as the eluent. Compound **1** (56 mg, 0.11 mmol) was isolated as a white powder in 41% yield; mp 145ºC–150ºC (decomposed) ^{1}H-NMR (500 MHz, DMSO-d6) δ (ppm): δ 8.64 (d, J = 4.6 Hz, 1H), 8.32 (m, 1H), 8.27 (m, 1H), 8.20 (m, 1H), 7.89 (t, J = 8.0 Hz, 1H), 7.70 (d, J = 7.7 Hz, 1H), 7.47 (m, 1H), 2.01 (m, 4H), 1.68 (m, 4H) ppm. ^{13}C-NMR (128.5 MHz, DMSO-d6) δ (ppm): 164.0, 150.9, 141.6, 139.2, 137.5, 137.5, 136.3, 128.3, 125.0, 124.9, 117.0, 116.9, 114.4, 112.9, 92.6, 84.9, 60.0, 37.3, 23.2. HRMS (m/z): $[M + Na]^+$ calcd. for $C_{22}H_{15}IN_2O_4Na$, 520.9974; found 520.9967.

3-fluoro-5-(pyridin-2-ylethynyl)benzamide



A DMF (1 mL) solution of 3-fluoro-5-(pyridin-2-ylethynyl)benzonitrile (50 mg, 0.22 mmol) and tetraethylammonium bicarbonate (0.44 mmol) was heated at 120ºC for 10 min. The yellow reaction mixture was cooled and diluted with DCM (10 mL) and washed with aqueous LiCl (5% w/v, 3 × 5 mL), water (3 × 5 mL), and brine and dried over anhydrous $MgSO_4$. Removal of DCM under reduced pressure yielded quantitative benzamide as a white solid; mp 175ºC–176ºC ^{1}H-NMR (300 MHz, DMSO-d6) δ (ppm): δ 8.63 (d, J = 4.9 Hz, 1H), 8.17 (s, 1H), 7.97 (s, 1H), 7.88 (td, J = 7.8, 1.7 Hz, 1H), 7.76 - 1.52 (m, 4H), 7.44 (m, 1H) ppm ^{13}C-NMR (75 MHz, DMSO-d6) δ (ppm): 166.0, 162.0 (d, J_{CF} = 246.6 Hz), 150.78, 142.17, 137.68 (d, J_{CF} = 8.0 Hz), 137.41, 128.06, 127.47, 124.45, 123.85 (d, J_{CF} = 9.1 Hz), 121.34 (d, J_{CF} = 22.7 Hz), 116.17 (d, J_{CF} = 22.7 Hz), 90.56, 86.83 ppm HRMS (m/z): $[M + H]^+$ calcd. for $C_{14}H_{10}F_2O$, 241.0772; found 241.0783.

General Methods for Radioisotope Production and Preparation

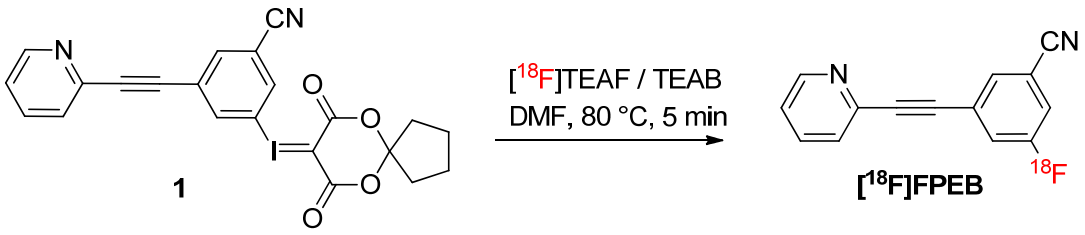
A GE PETtrace 16.5 MeV cyclotron was used for [^{18}F]fluoride production by the ^{18}O(p,n)^{18}F nuclear reaction to irradiate ^{18}O-enriched water. A GE high yield niobium target containing > 97% enriched O-18 water (Isotec, Taiyo Nippon Sanso or Rotem) was bombarded with protons at integrated currents up to 65 μA to produce [^{18}F]fluoride. [^{18}F]Fluoride was delivered to a lead-shielded hot cell in ^{18}O-enriched water by nitrogen gas pressure. [^{18}F]Fluoride was prepared for

radiofluorination as follows: A solution of base (*e.g.*, tetraethylammonium bicarbonate, 3 mg) in acetonitrile and water (1 mL, v/v 7:3) was added to an aliquot of target water (≤ 1 mL) containing the appropriate amount of [^{18}F]fluoride in a V-shaped glass vial sealed with a Teflon-lined septum. The vial was heated to 110°C while nitrogen gas was passed through a P_2O_5-Drierite column followed by the vented vial. When no liquid was visible in the vial, it was removed from heat, anhydrous acetonitrile (1 mL) was added, and the heating was resumed until dryness. This azeotropic drying step was repeated an additional three times. The vial was then cooled at room temperature under nitrogen pressure.

General methods for analysis of radiofluorination reactions

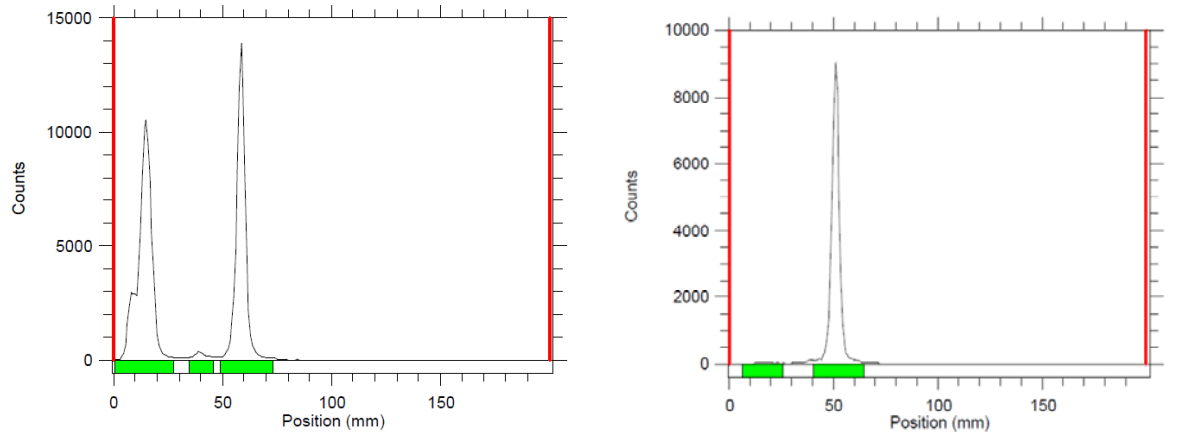
Radioactivity was measured using a Capintec Radioisotope Calibrator (CRC-712M) ion chamber. Radiochemical conversion was determined by radioTLC. EMD TLC silica gel 60 plates (10 × 2 cm) were spotted with an aliquot (1–5 μL) of crude reaction mixture approximately 1.5 cm from the bottom of the plate (baseline). TLC plates were developed in a chamber containing ethyl acetate and ethanol (5%), until within 2 cm of the top of the plate (front). Analysis was performed using a Bioscan AR-2000 radio-TLC imaging scanner and WinScan software. Radiochemical identity and purity were determined by radioHPLC. A Phenomenex Luna C18 (250 × 4.6 mm, 5 μm) or a XSELECT HSS T3 (4.6 × 150 mm, 5 μm) HPLC column was used with a Waters 1515 Isocratic HPLC Pump equipped with a Waters 2487 Dual λ Absorbance Detector, a Bioscan Flow-Count equipped with a NaI crystal, and Breeze software.

[^{18}F]FPEB manual radiosynthesis



Precursor **1**, (4 mg) was dissolved in DMF (400 μL) and added to a V-vial containing azeotropically dried [^{18}F]Et_4NF (typically 1–3 mCi). The reaction was heated at 80°C for 5 min. The reaction mixture was cooled for 3 min and then quenched with HPLC buffer (60:40 CH_3CN:H_2O + 0.1N ammonium formate, 2 mL). The reaction was further diluted with water (16 mL) and passed through a Waters C18 Sep-Pak, which had been activated by flushing sequentially with ethanol (1 mL) and water (5 mL). The Sep-Pak was flushed with water (2 mL) and the desired product was eluted with ethanol (1 mL). Product identity and purity were determined by radioHPLC (60:40 CH_3CN:H_2O + 0.1N ammonium formate, Phenomenex Luna C-18 column), and radioTLC (EtOAc + 5% EtOH). The product was >99% radiochemically

pure. Radiochemical yield was determined as the percentage of radioactivity that was isolated as the final product from the amount of activity present in the V-vial before addition of **1** to dried [^{18}F]Et_4NF and is not decay-corrected.



Run	1	2	3	mean	standard deviation
rTLC yield (%)	56	46	44	49	6
isolated yield (%)	41	34	35	34	4

Figure S1. RadioTLC traces of crude reaction mixture (left) and [^{18}F]FPEB after elution from a C18 SPE. Radiochemical conversions are provided in the table below (% RCC).

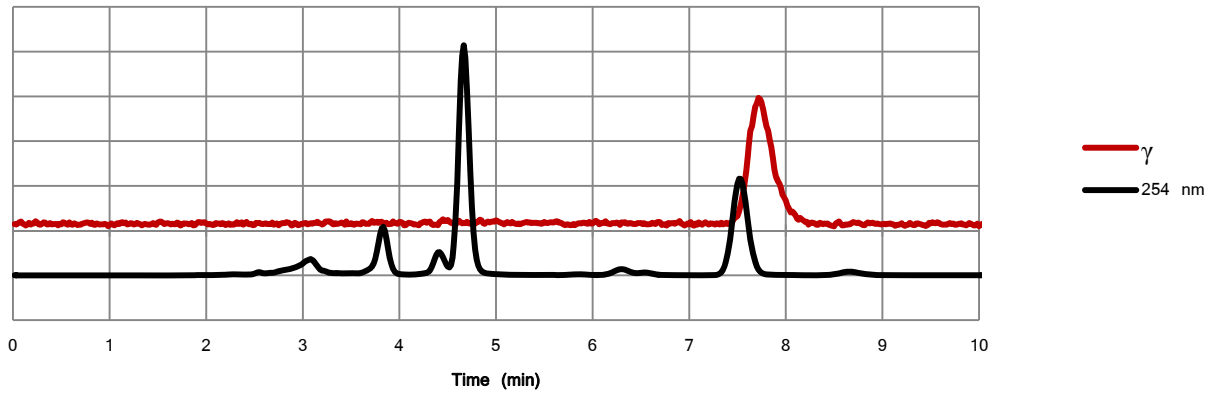


Figure S2. HPLC trace of [^{18}F]FPEB after elution from C18 SPE with coinjection of cold standard.

Automated synthesis of [^{18}F]FPEB by GE TracerLab FX_{FN} method

Following completion of bombardment, the [^{18}F]fluoride was transferred to the GE TRACERlab FX_{FN} radiosynthesis module via helium gas overpressure. A schematic diagram of the GE medical systems commercial TRACERlab FX_{FN} radiosynthesis module used for the synthesis of [^{18}F]FPEB is shown in the Figure S3. Automated synthesis involves the following: (1) azeotropic drying of [^{18}F]fluoride; (2) [^{18}F]fluorination; and (3) HPLC purification, followed by solid-phase formulation of the final product. The synthesis module was operated in the following sequences with numerical references to the figure below.

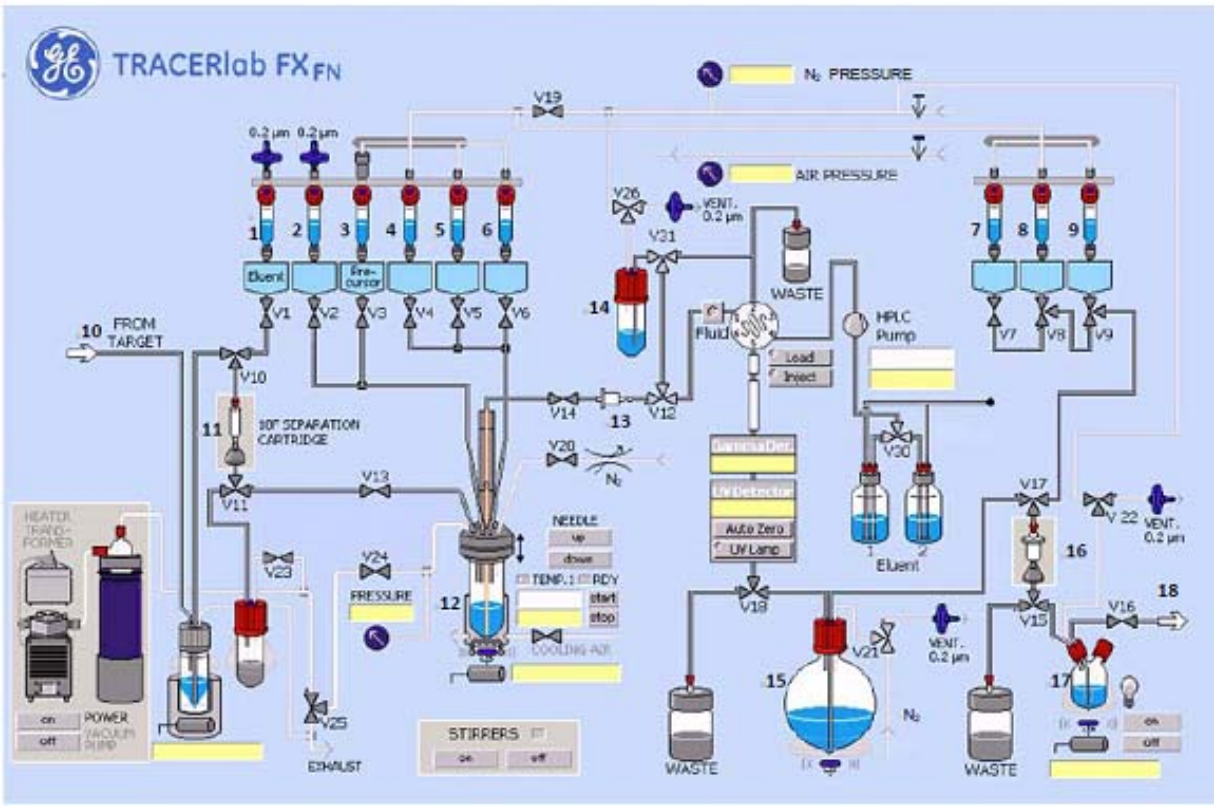


Figure S3: Schematic of the GE TRACERlab™ FX_{FN} radiosynthesis module automated synthesis manifold for [^{18}F]FPEB.

1. [^{18}F]Fluoride was produced by the ^{18}O(p,n)^{18}F nuclear reaction using a GE cyclotron and delivered to the radiosynthesis module via 10. The [^{18}F]fluoride was quantitatively trapped on a QMA carbonate ion exchange solid phase extraction (SPE) light cartridge (Waters; activated with 6 mL of trace grade H_2O).
2. Automated synthesis began with the elution of resin-bound [^{18}F]fluoride using a solution (0.02 M, 0.8 mL) of tetraethylammonium hydrogen carbonate, preloaded into 1 and delivered to the reactor (12).
3. The reaction mixture (12) was dried azeotropically by addition of 1 mL anhydrous CH_3CN, preloaded into 5, at 85°C under N_2 flow and vacuum over 8 min, then at 110°C under N_2 flow and vacuum for 4 min.
4. After cooling to 40°C, ylide precursor (4 mg in 0.5 mL DMF) preloaded into 3 was added to 12. The reactor was sealed via the closure of valve V13, V20, and V24 and the reaction mixture was heated to 80ºC and this temperature was maintained for 4.5 min.
5. The reaction mixture was then cooled to 40ºC, vented via valves V24 and V25, and diluted with 20:80 CH_3CN/20 mM ammonium acetate (2 mL), preloaded into 6.
6. The crude reaction mixture was eluted into 14 and the contents of 14 were transferred to the HPLC loop via N_2 pressure via a fluid detector, injected onto a semipreparative column (X-Select HSS T3, 250 × 10.00 mm, 5μ), and eluted with 45:55 CH_3CN/20 mM ammonium acetate by volume (pH 6) at a flow rate of 4 mL/min. The eluent was monitored by UV (λ = 254 nm)
7. and radiochemical detectors connected in series.
8. A typical semipreparative HPLC chromatogram is shown in Figure S4. The fraction containing the major radiochemical product (t_R = 19 min) was collected, via valve 18, into a large dilution vessel (15), which was preloaded with 20 mL of sterile water for injection (United States Pharmacopeia (USP); Hospira).
9. The diluted HPLC fraction was then loaded onto a C18 light SPE cartridge (16) (Waters; preactivated with 5 mL EtOH followed by 10 mL H_2O).
10. Cartridge 16 was washed with 10 mL sterile water for injection, USP, preloaded into 7, to remove traces of salts, CH_3CN, and [^{18}F]fluoride.
11. Then 16 was eluted with 1 mL dehydrated alcohol for injection, USP (ethanol) preloaded into 8, into collection vial 17 followed by 10 mL 0.9% sodium chloride for injection, USP preloaded into 9.
12. The solution was transferred and passed through a 0.22 μm Millipore GV sterilizing filter (EMD Millipore) into a vented sterile 30 mL dose vial (Hospira).

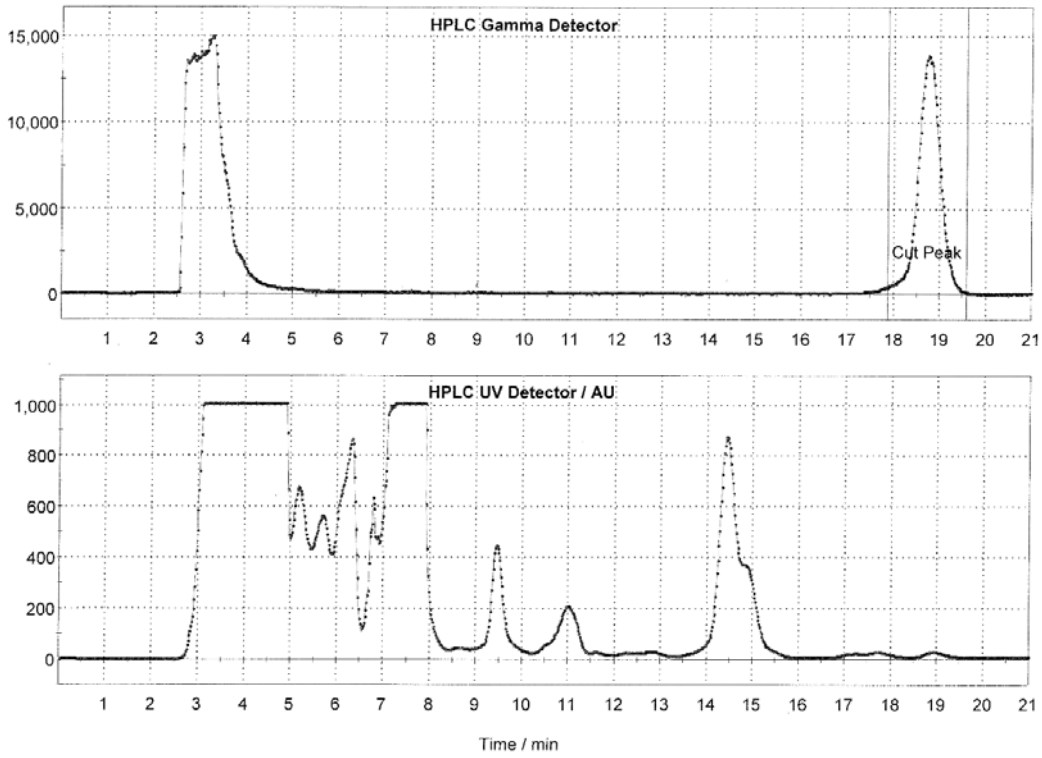


Figure S4: Semipreparative HPLC trace of a typical radiosynthesis of [^{18}F]FPEB.

Analyses of radioactive mixtures were performed by HPLC with an in-line UV (λ = 254 nm) detector in series with a CsI PIN diode radioactivity detector. To determine the identity of [^{18}F]FPEB, aliquots of the formulated product were injected onto an analytical HPLC system using a Novapak C18 column, 150 × 4.6 mm, 4 μm, and eluted with 45:55 EtOH/water at a flow rate of 1 mL/min, monitored at λ = 254 nm. The major radiochemical product was identified as [^{18}F]FPEB (t_R = 4.7 min; Figure S5). Uncorrected radiochemical yields of [^{18}F]FPEB were 20.0 ± 5% relative to starting [^{18}F]fluoride, and high specific activities were obtained in the final formulation (18 ± 1.4 Ci/μmol). Further characterization and validation of [^{18}F]FPEB was carried out as described in Quality Control section.

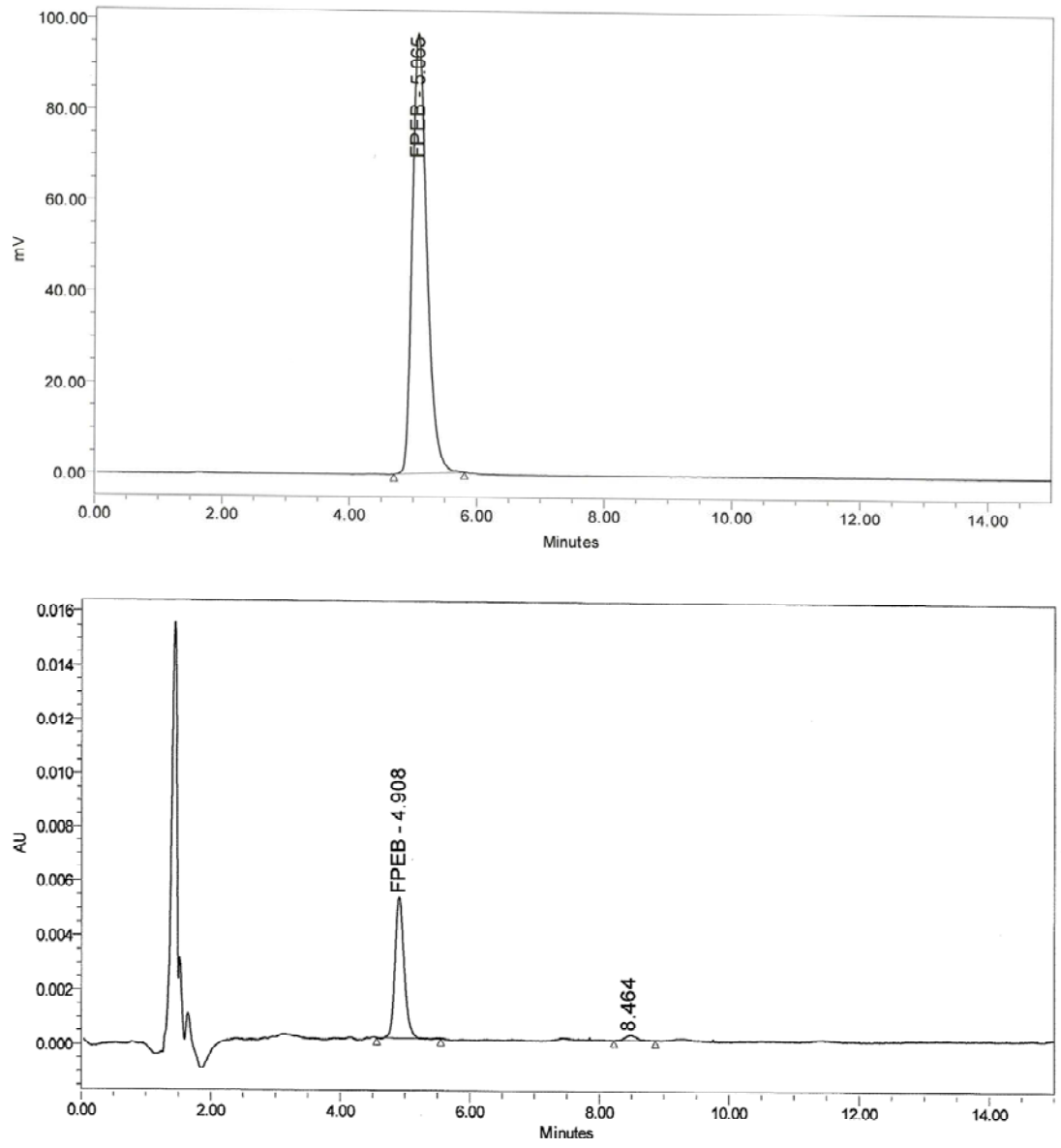


Figure S5: Analytical radioactive (top) and UV (bottom) HPLC traces for [^{18}F]FPEB.

Quality control for [^{18}F]FPEB

The following tests were carried out in accordance with an MGH approved protocol, as per ICH and USP guidelines.

Visual Inspection: The [^{18}F]FPEB dose was clear, colorless, and free of particulate matter.

Radiochemical Identity, Radiochemical Purity, Injectable Mass, and Specific Activity: To determine the identity of [^{18}F]FPEB, aliquots of the formulated product were injected onto an analytical HPLC system using a Novapak C18 column, 150 × 4.6 mm, 4 μm and eluted with 45:55 EtOH/water at a flow rate of 1 mL/min, monitored at λ = 254 nm. After completion of the chromatograph, peaks on UV and radioactivity detector were integrated and the radiochemical and chemical purity were determined by the area of integration.

The major radiochemical product was identified as [^{18}F]FPEB (t_R ~4.9 min; Figure S5), followed by coinjection with the reference standard FPEB. The retention time of [^{18}F]FPEB was compared to that of the standard [^{19}F]FPEB and was within ±10% error. The radiochemical purity was >99% and chemical purity was >98%. Allowed injectable masses are as follows: ≤3.6 μg and ≤0.36 μg of unknown chemical impurities. Specific activity was determined using standard FPEB specific activity calibration curve. Specific activity must be ≥800 mCi per micromole at time of administration.

Residual Solvent Analysis: Residual solvent assay was performed to verify that residual solvents from the synthesis and maintenance of the synthesis units were within acceptable limits. Gas chromatography (GC) was used to determine the solvent residue, and the results met the following specifications.

DMF (class II) <0.88 mg/mL; acetone (class III) <5 mg/mL; acetonitrile (class II) <0.4 mg/mL; ethanol (class III) <10% v/v ± 10% (formulation agent)

pH Assay: The pH of [^{18}F]FPEB was determined by applying a few drops of the dose to pH indicator paper. Match the reference color and the pH value conformed to our release specifications (4.5–8.5).

Sterile Filter Integrity Test: Sterile filter integrity test was performed as per manufacturer specification and the pressure and was ≥ 50psi for the Millipore Millex GV 0.22 μm sterilizing filter.

Radionuclidic ID – Photopeak and Half-Life: Measure the radioactivity of the formulated product at two separated time points. The half-life consistently met our release specifications (105–115 min).

Photopeak Was Determined Based on the Following Protocol:

Introduce small amount of radioactivity of formulated product into gamma spectrometer. Record the spectrum and integrate the areas under the signals of the spectrum. The result was >99.5% emission at 511 keV, 1.022 MeV.

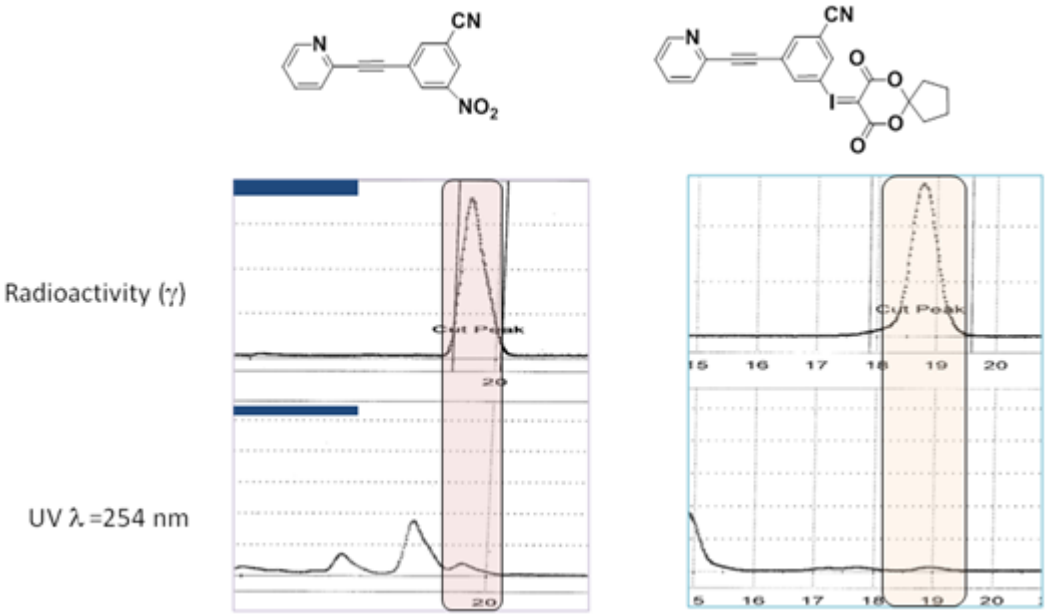
Endotoxin Analysis: Endotoxin analysis was performed on a Charles River Laboratories Endosafe PTS system using a 1:100 dilution. Doses contained ≤ 5 EU/mL per injected dose.

Sterility Testing: Sterility testing was performed after release and must be started within 30 hours from end of synthesis. [^{18}F]FPEB sample was inoculated into Trypitcase Soy Broth (TSB) and Fluid Thioglycollate Medium (FTM) media tubes. TSB tubes were incubated at 20°C–25°C and FTM tubes were incubated at 30°C–35°C for 14 days and must be free of culture growth after 14 days.

Table S1. Summary of [^{18}F]FPEB Quality Control Data

Parameter	Results (n = 3)
Synthesis time	60 min (ready for injection)
Isolated product	203 ±64 mCi at end of synthesis (EOS)
Visual inspection	Clear, absence of particulates
Radiochemical identity	3.3% ± 0.23% of FPEB reference standard retention time
Radiochemical purity	≥ 99%
Chemical purity	≥ 98%
Specific activity	18 ±1.4 Ci/μmol at EOS
Residual solvent analysis	DMF < 0.88 mg/mL
	Acetone < 5 mg/mL
	Acetonitrile < 0.4 mg/mL
	Ethanol 10% v/v ± 10%
pH assay	5–5.5
Sterile filter integrity test	≥50 psi
Radionuclide ID: photopeak	≥99.5% emission at 511 keV, 1.022 MeV, or Compton scatter peaks
Radionuclide ID: half-life	105–115 min
Endotoxin analysis	≤ 5 EU/mL
Sterility testing	No evidence of growth at 14 days after inoculation

Comparison of ^{18}F-FPEB synthesis using the nitro-precursor and 1



Semipreparative column: X-select HSS T3, 250 × 10.00 mm, 5 μ; mobile phase: 45:55 CH_3CN/20 mM ammonium acetate at pH 6; flow rates: 4 and 5 mL min^{-1}; respectively.

References

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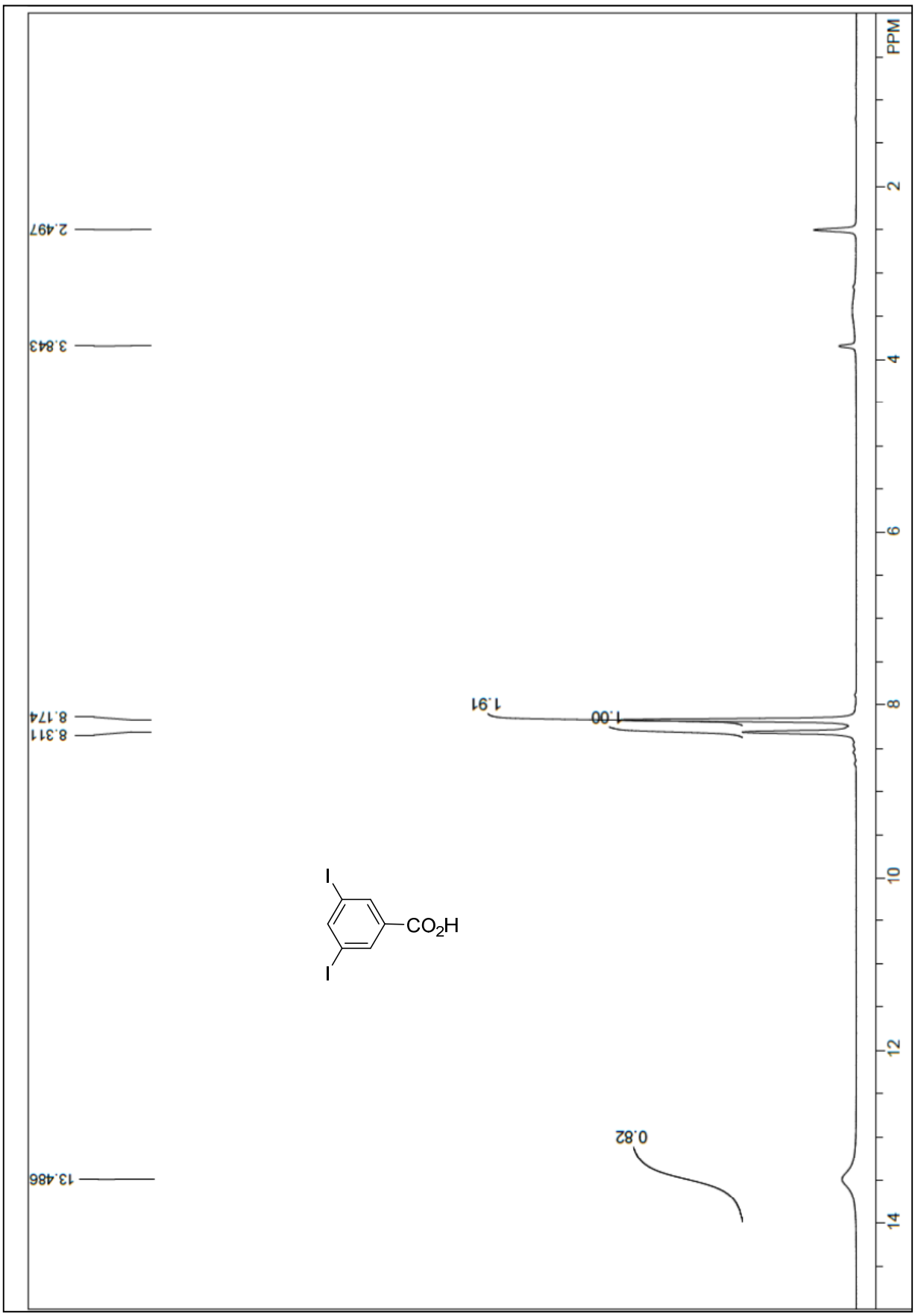
2. Alagille D, DaCosta H, Chen Y, et al. Potent mGluR5 antagonists: pyridyl and thiazolyl-ethynyl-3,5-disubstituted-phenyl series. *Bioorg Med Chem Lett.* 2011;21:3243–3247.

NMR Spectral Data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4
6
8
10
12
14
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8.174
8.311
1.91
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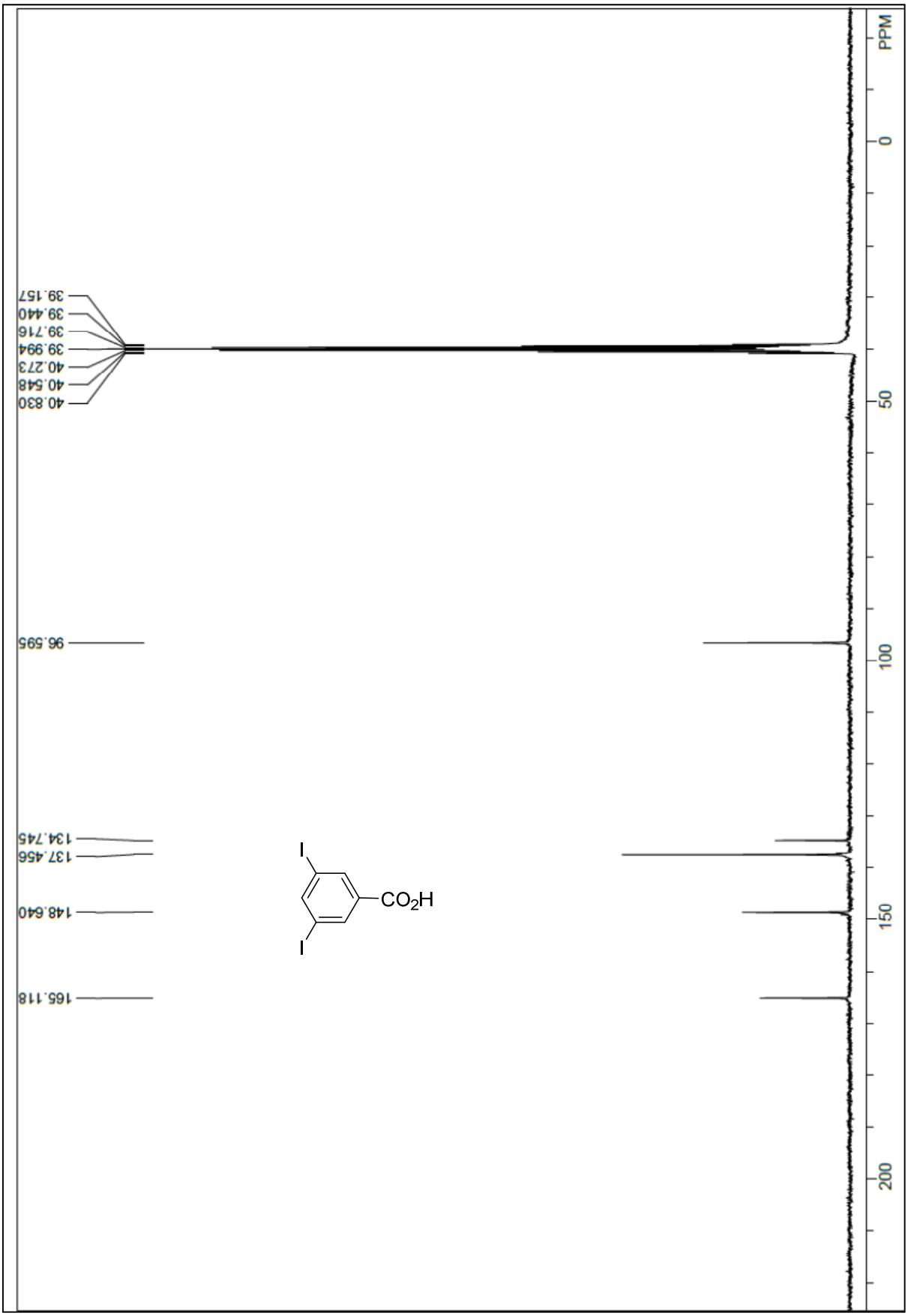
0.82
13.486
I
I
CO2H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100
150
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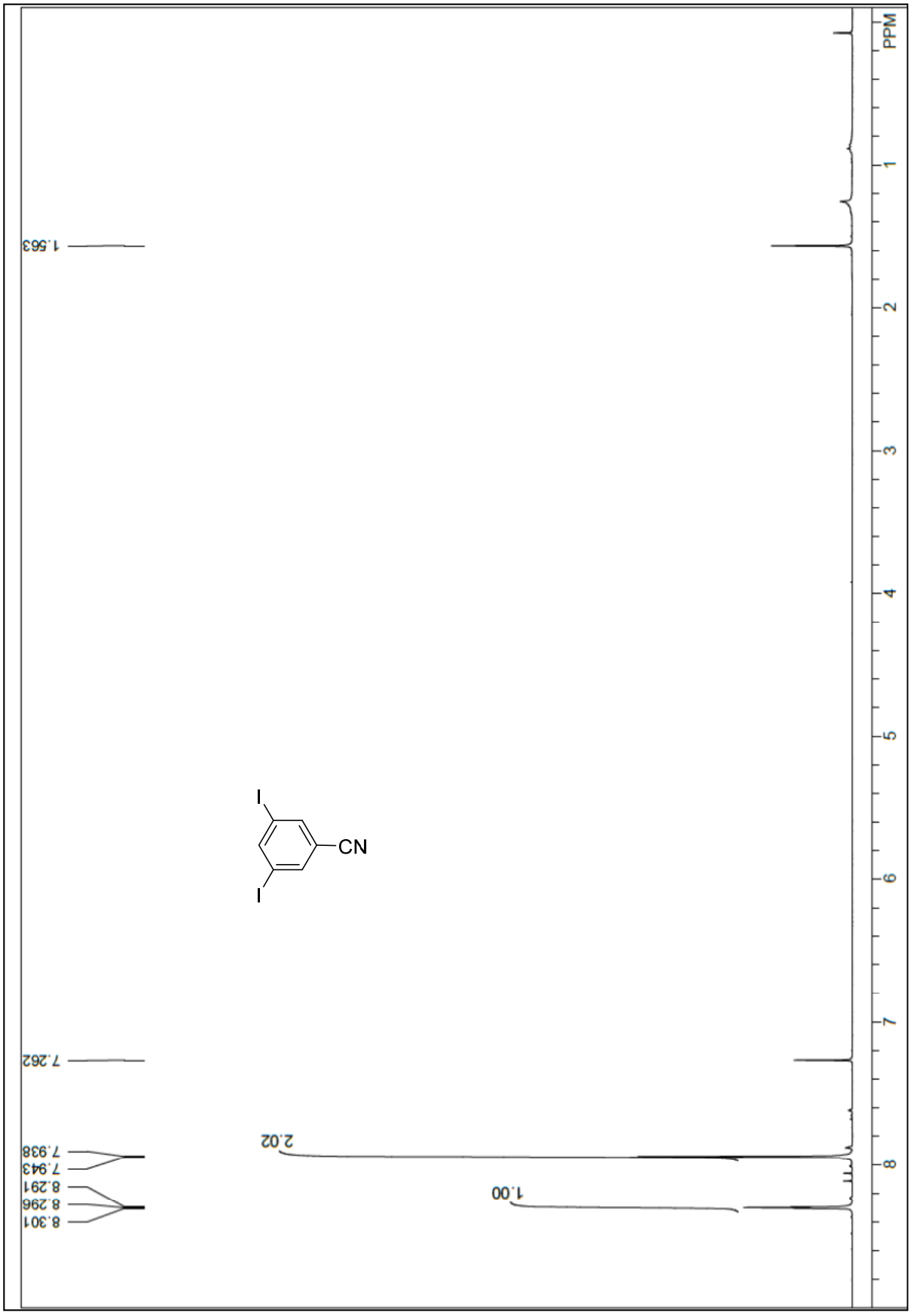
CO2H
I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CN
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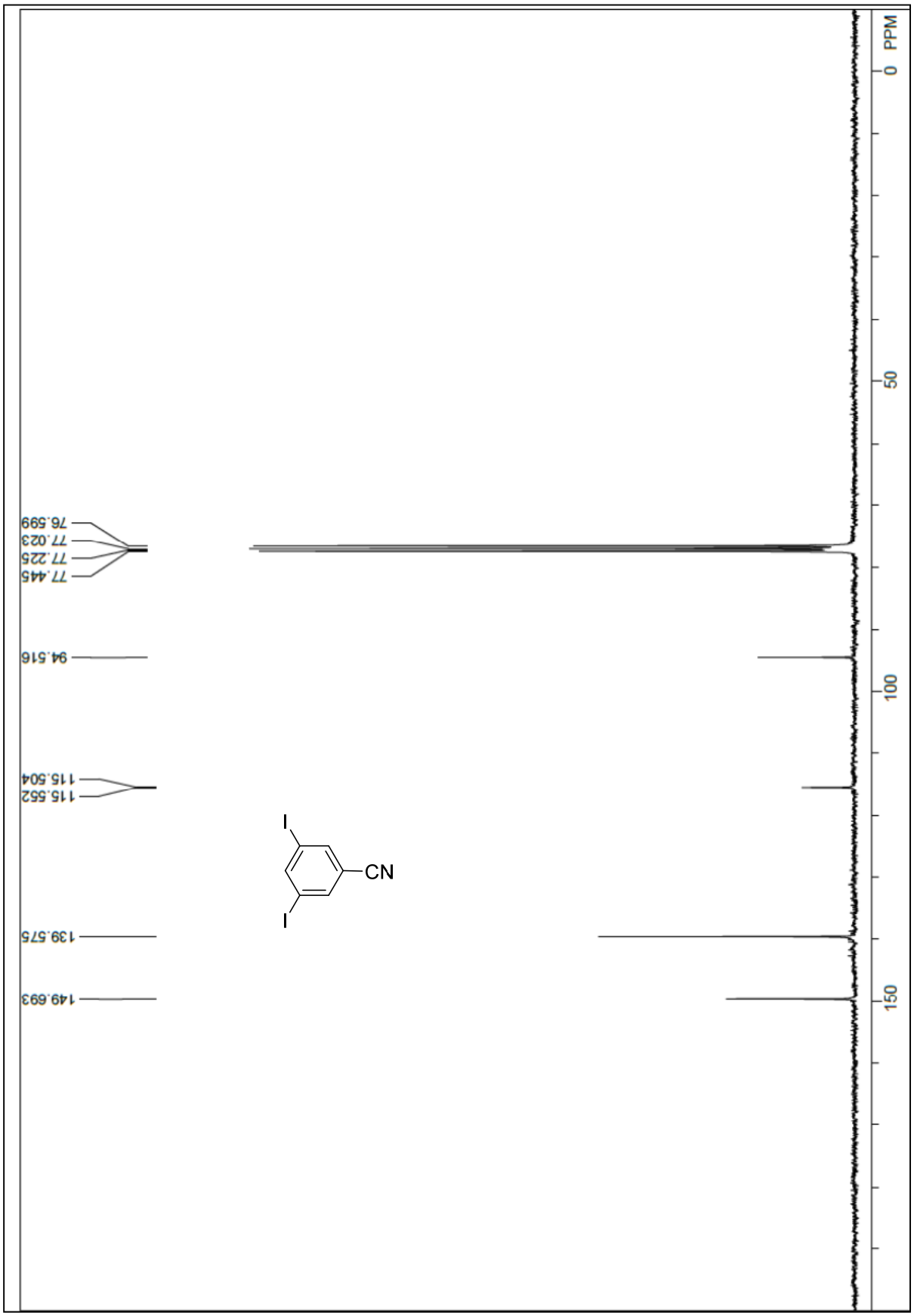
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I
CN
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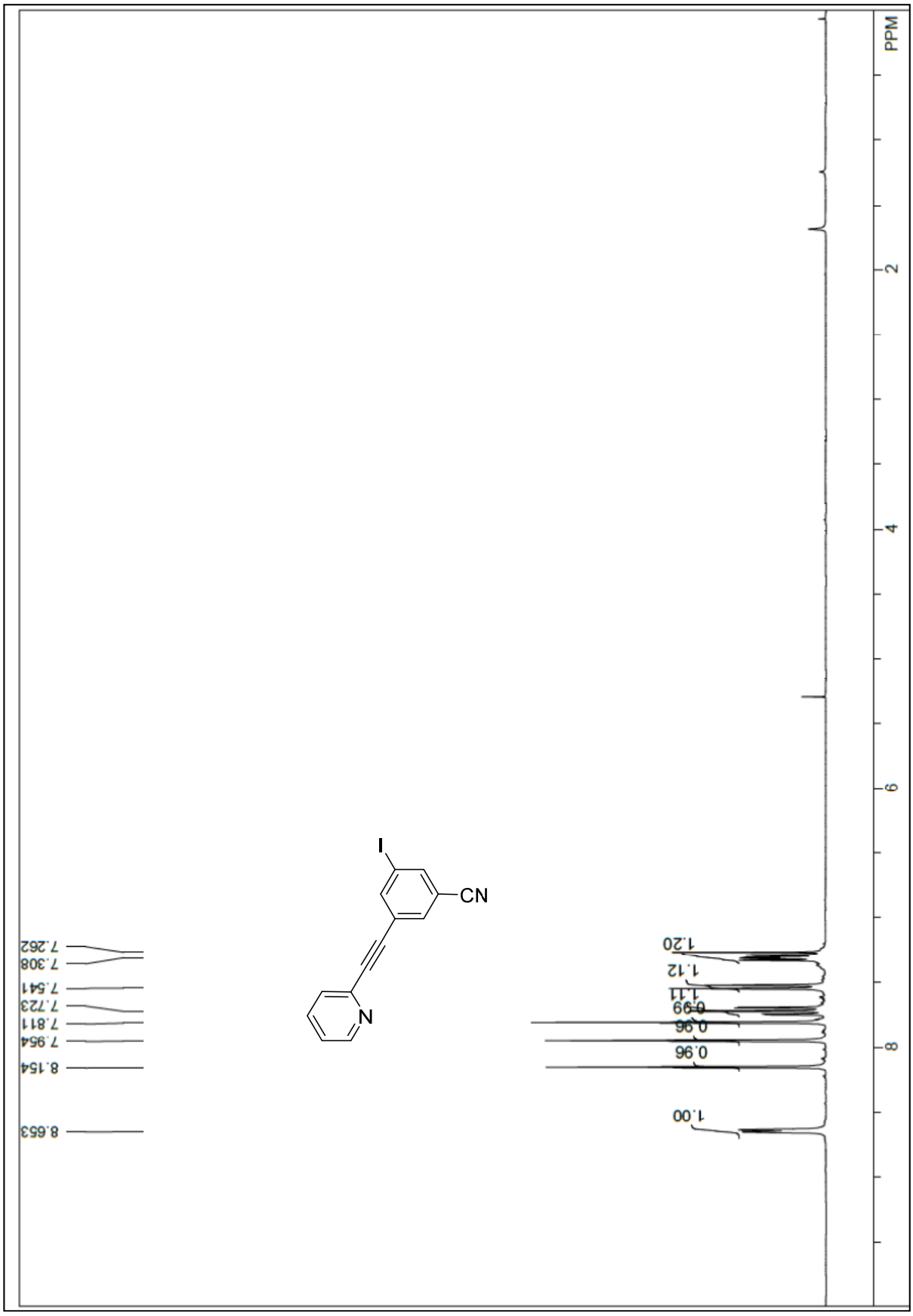
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4
6
8
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1.20
1.12
1.11
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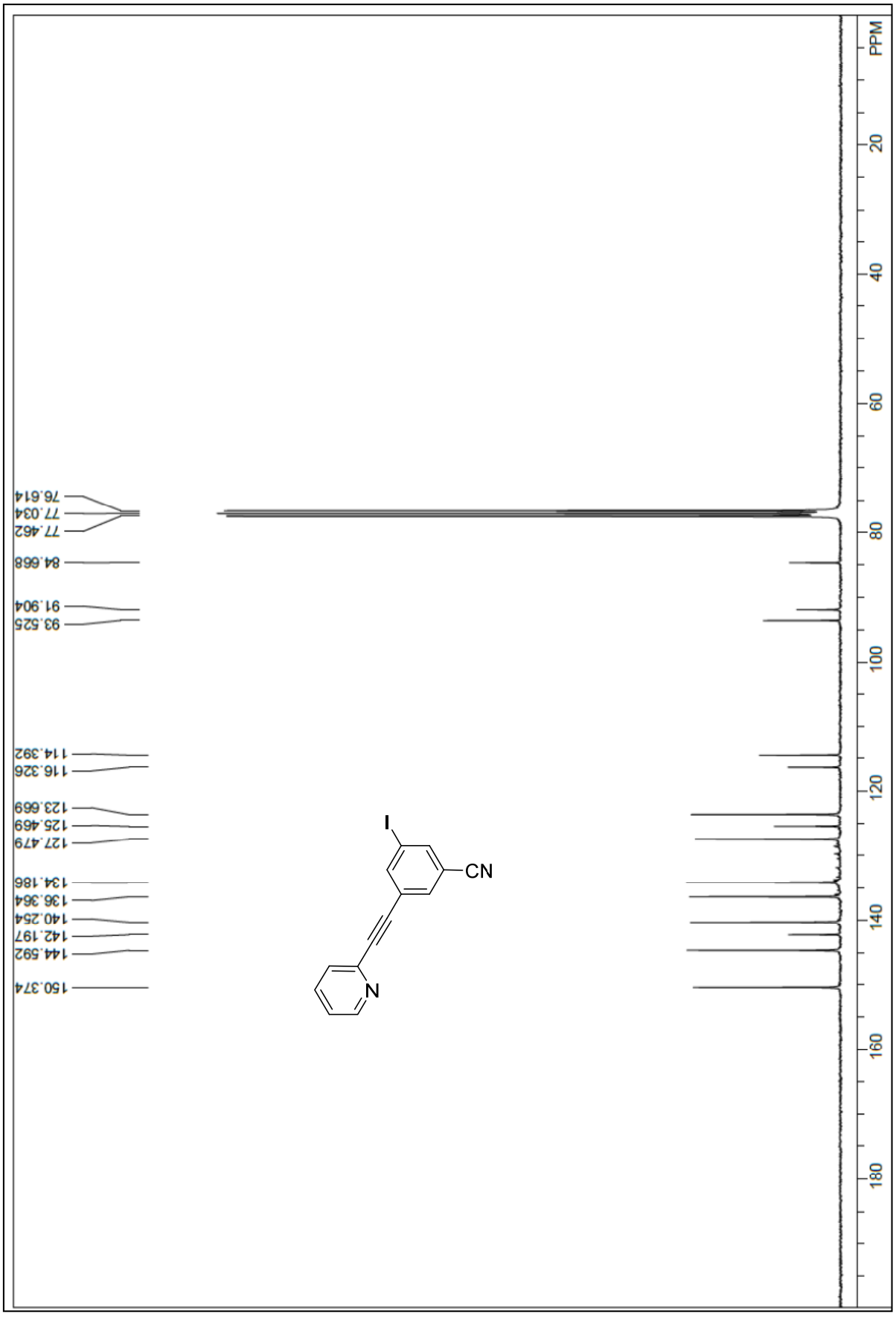
0.96
0.96
1.00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114.392
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136.364
140.254
142.197
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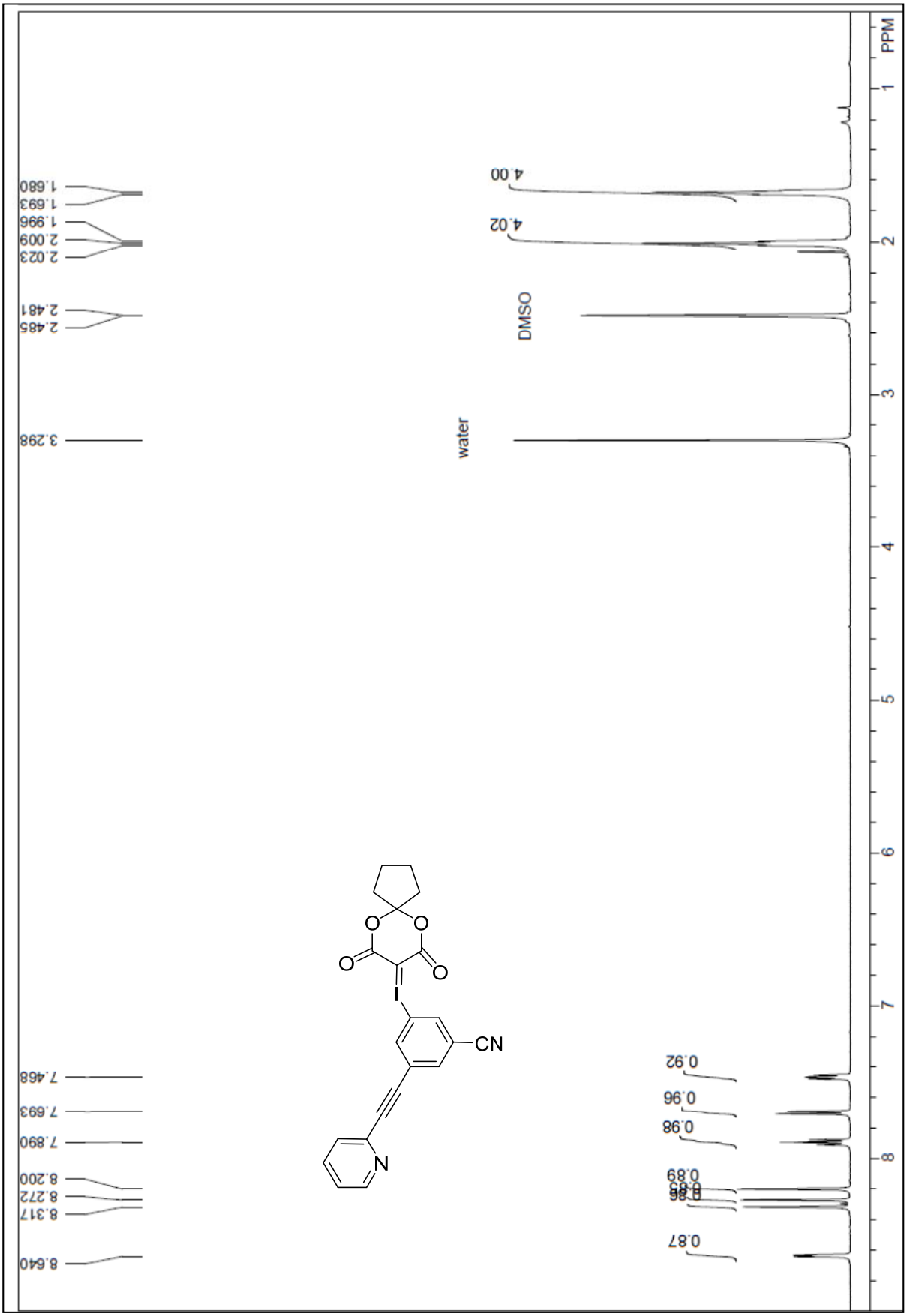
CN
N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8
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DMSO
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2.009
2.023
2.481
2.485
3.298
7.468
7.693
7.890
8.200
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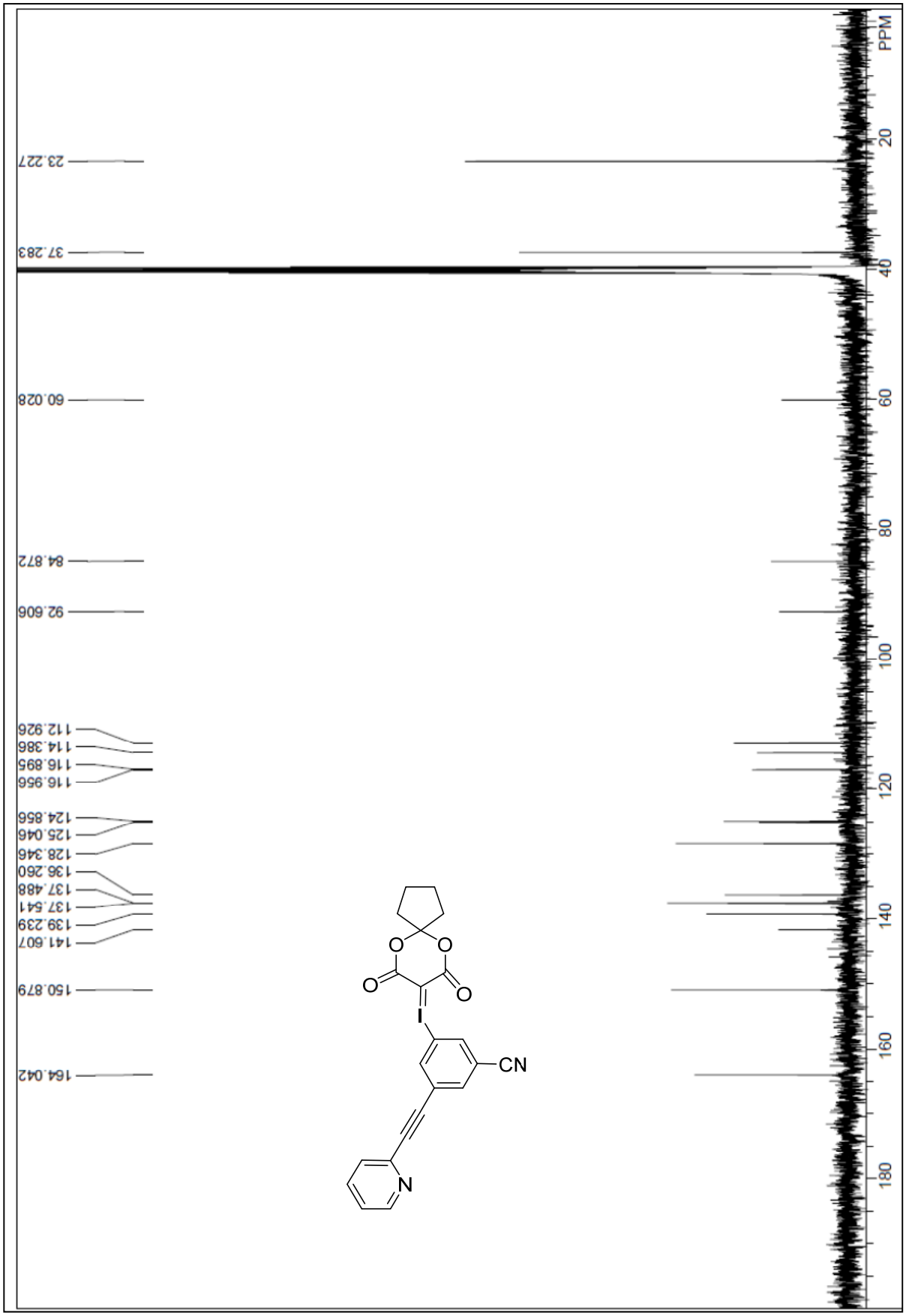
8.640
0.87
0.89
0.92
0.96
0.98
O
O
O
O
I
CN
N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140
160
180
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114.386
116.895
116.956
124.856
125.046
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137.541
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141.607
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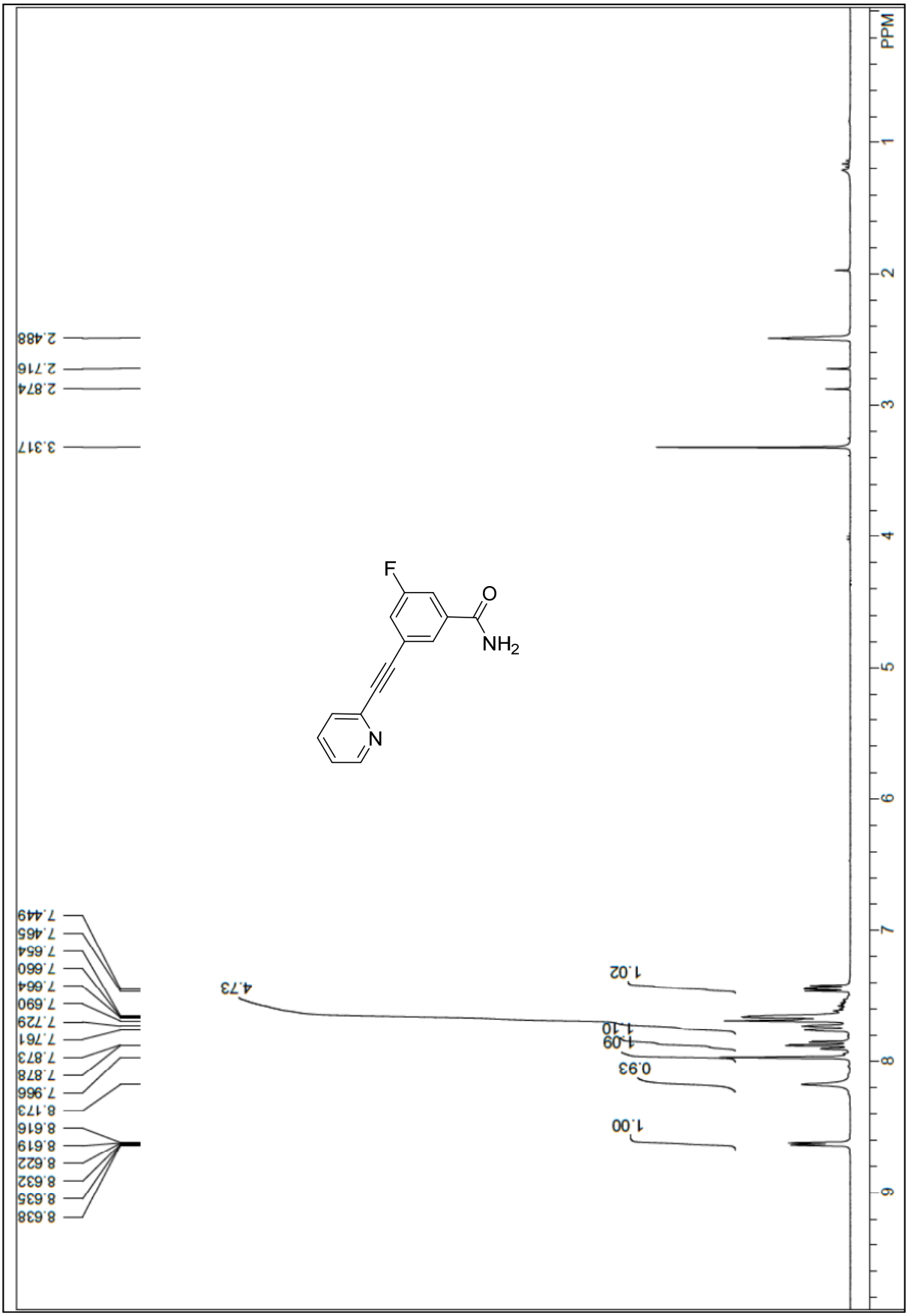
164.042
O
O
O
O
I
CN
N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3
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6
7
8
9
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2.716
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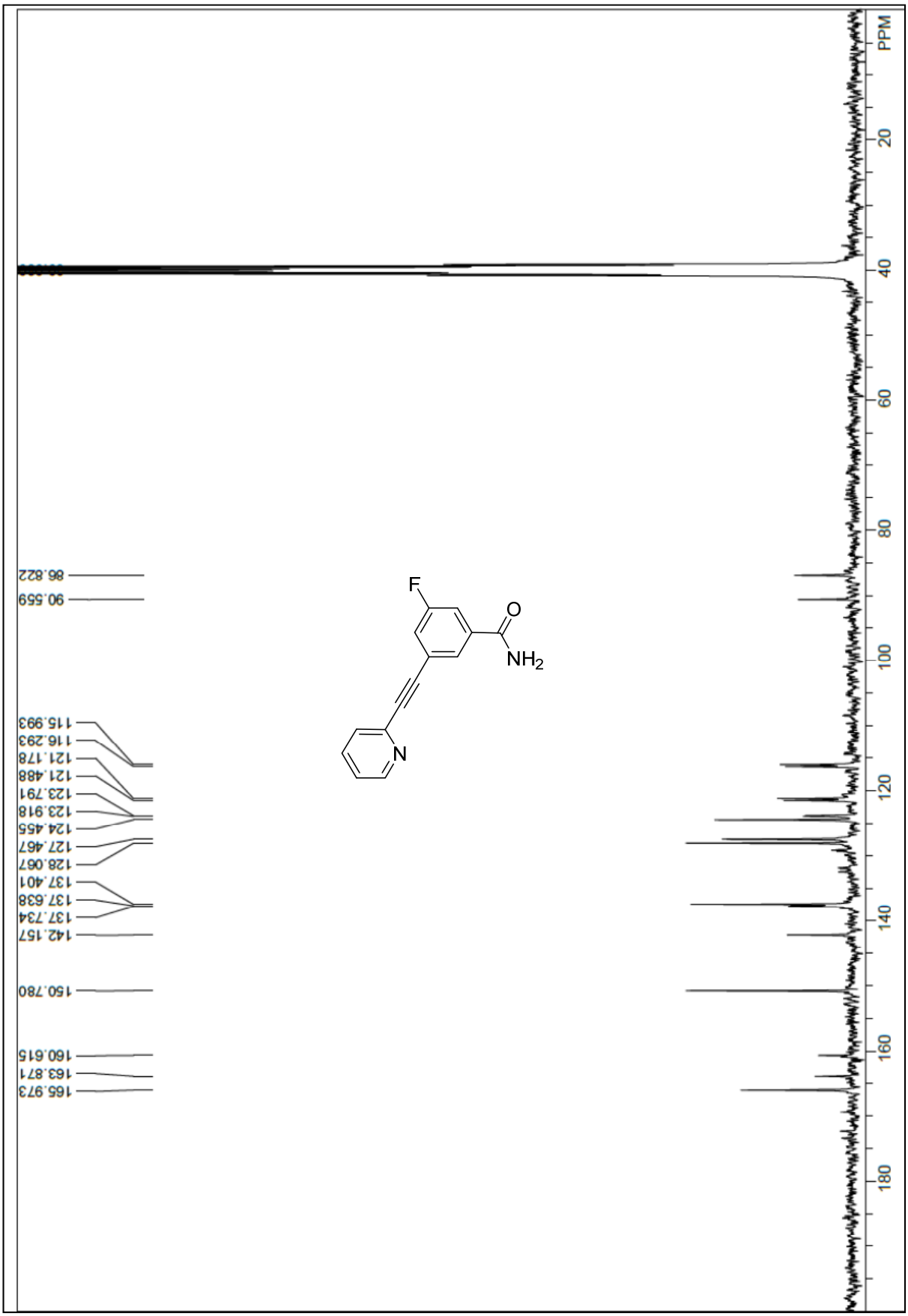
4.73
1.02
1.10
1.09
0.93
1.00
7.449
7.465
7.654
7.660
7.664
7.690
7.729
7.761
7.873
7.878
7.966
8.173
8.616
8.619
8.622
8.632
8.635
8.638
F
O
NH2
N


PPM
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100
120
140
160
180
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121.178
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123.791
123.918
124.455
127.467
128.067
137.401
137.638
137.734
142.157
150.780
160.615
163.871
165.973
F
O
NH2
N