Atropisomeric 1-phenylbenzimidazoles affecting microtubules organization: influence of axial chirality

Jana Pospíšilová[1], Tomáš Heger[2], Ondřej Kurka[3], Marie Kvasnicová[2,4,] Anna Chládková[1], Ivan Nemec[5], Lucie Rárová[2,4,#], Petr Cankař[1,#]

[1]Department of Organic Chemistry, Faculty of Science, Palacký University Olomouc, 17. listopadu 1192/12, 779 00 Olomouc. Czech Republic
[2]Department of Experimental Biology, Faculty of Science, Palacký University, Šlechtitelů 27, 779 00 Olomouc, Czech Republic
[3]Department of Analytical Chemistry, Faculty of Science, Palacký University, 17. Listopadu 1192/12, 779 00 Olomouc, Czech Republic
[4]Laboratory of Growth Regulators, Institute of Experimental Botany of the Czech Academy of Sciences, and Faculty of Science, Palacký University, Slechtitelu 27, 779 00 Olomouc, Czech Republic
[5]Department of Inorganic Chemistry, Faculty of Science, Palacký University Olomouc, 17. listopadu 1192/12, 779 00 Olomouc. Czech Republic

Supporting information

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General

All used chemicals were purchased from VWR, Sigma-Aldrich, Fluorochem, or Acros Organics. All reactions were carried out under regular conditions without any specific precautions to exclude moisture or air from the reaction mixture. The reaction workup and column chromatography were performed with commercial solvents. ^{1}H NMR and ^{13}C NMR spectra were measured on a Jeol ECA400II (400 MHz) in $CDCl_3$ or DMSO-d_6 as a solvent, and referred to the residual nondeuterated solvent peak (7.26 and 77.16 ppm for $CDCl_3$ and 2.50 and 39.52 ppm for DMSO-d_6).

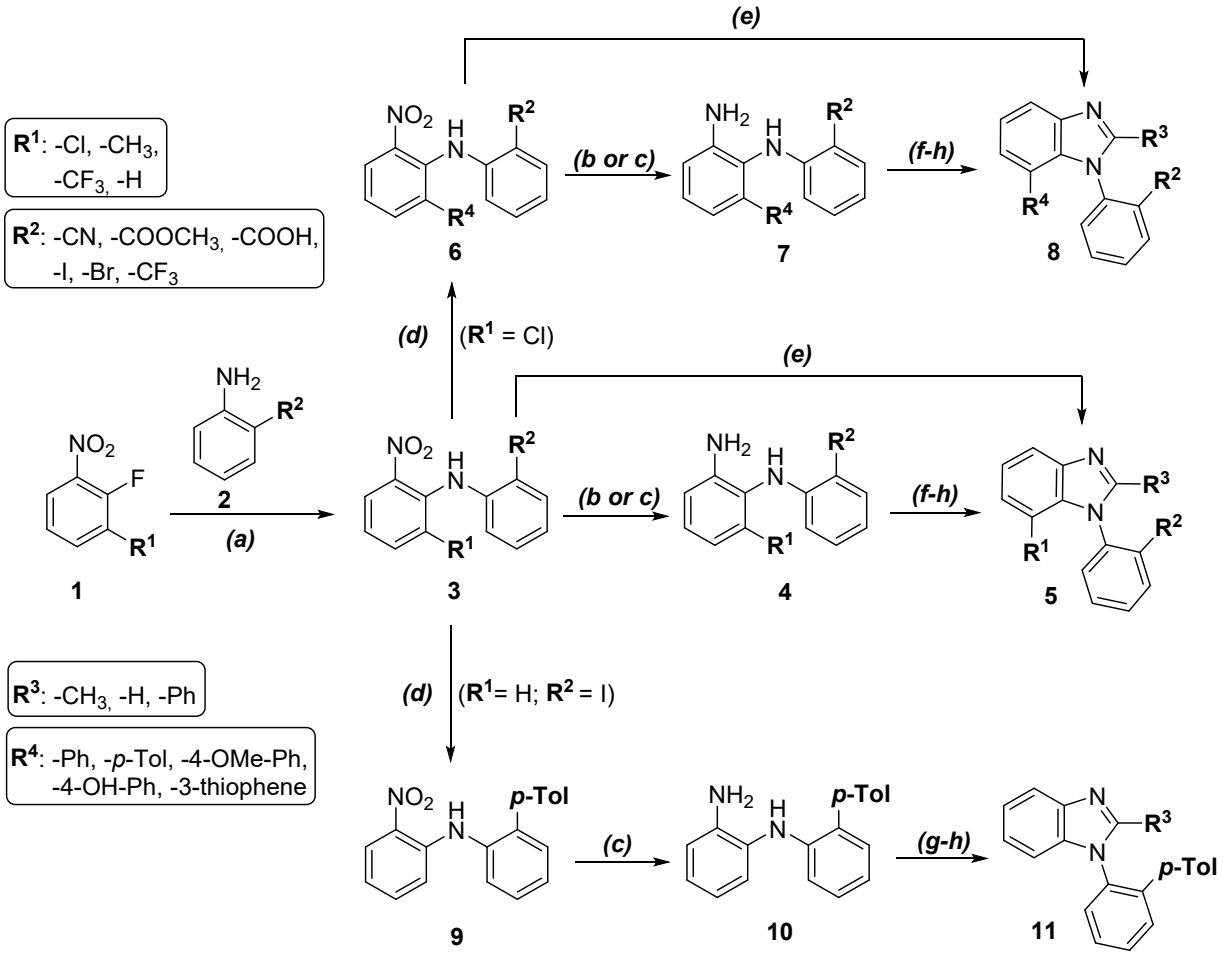
The reactions were monitored with analytical thin layer chromatography (TLC), performed on Kieselgel 60 F254 plates (Merck) and visualized by UV light (254 nm). Flash chromatography was performed using silica gel (35 – 70 µm partical size) column chromatography.

HRMS analysis was performed using an LC-MS Orbitrap Elite high-resolution mass spectrometer with electrospray ionization (Dionex Ultimate 3000, Thermo Exactive Plus). The samples were dissolved in MeOH or acetonitrile and injected into the mass spectrometer over the autosampler after HPLC separation; the precolumn was a Phenomenex Gemini (C18, 50 × 2 mm, 2.6 µm) and the isocratic mobile phase MeOH/water/HCOOH 95:5:0.1.

Resolution of selected axially chiral benzimidazoles and racemization stability was carried out on HPLC Agilent 1100 MWD with column Chiralpak IA-3, 3µm, 4.6mm x 100mm; mobil phase *n*-heptan/ethanol 90:10, flow 0.5-1 mL/min or column Lux i-Amylose-3, 5µm, 4.6mm x 250mm; mobil phase *n*-hexan/IPA 80:20.

Semipreparative isolation of each atropoisomer (for compounds **5n** and **5l**) was performed using Smartline HPLC (Knauer) on a Lux Cellulose-1 (Phenomenex) column (250 × 10 mm, 5 µm particle size) with mobile phases in isocratic mode: hexane/ethanol 9:1 (v/v), 15 min analysis time for **5l** and hexane/methanol/ethanol 40:1:1 (v/v/v), 35 min analysis time for **5n**.

General synthetic methods and characterization of intermediates



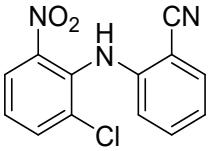
Scheme 1: General Synthetic Scheme

General procedure:

Method ***a)***

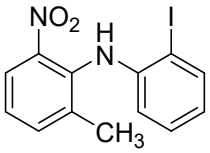
Ortho-substituted aniline (5.7 mmol) was dissolved in DMSO (11 mL), then substituted 1-fluoro-2-nitrobenzene (5.7 mmol) and potassium hydroxide (22.8 mmol) were added. The reaction mixture was stirred for 4 – 12 h at room temperature, then added dropwise to ice water (80 mL), and neutralized with hydrochloric acid at pH 7. The desired nitroaniline was filtered off to give a crude product with sufficient purity for the next step.

2-((2-Chloro-6-nitrophenyl)amino)benzonitrile 3a



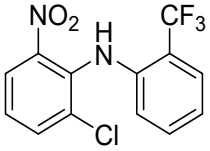
A yellow solid (1.371 g, 88%, m.p. 135 – 137 °C). ^{1}H NMR (400 MHz, DMSO-d_6) δ = 8.66 (s, 1H), 8.01 (dd, *J* = 1.6, 8.0 Hz, 1H), 7.93 (dd, *J* = 1.6, 8.0 Hz, 1H), 7.64 (dd, *J* = 1.4, 7.8 Hz, 1H), 7.51 (dt, *J* = 2.6, 8.3 Hz, 1H), 7.45 (ddd, *J* = 1.4, 7.6, 8.6 Hz, 1H), 6.95 (dt, *J* = 1.4, 7.6 Hz, 1H), 6.59 (d, *J* = 8.2 Hz, 1H) ppm. ^{13}C NMR (101 MHz, DMSO-d_6) δ = 148.1, 147.8, 135.4, 134.7, 134.1, 133.8, 132.4, 127.8, 124.9, 120.6, 117.8, 116.7, 98.6 ppm. HRMS (ESI): m/z calcd $C_{13}H_8ClN_3O_2$ for $[M-H]^-$ 272.0221, found 272.0225.

_N_-(2-Iodophenyl)-2-methyl-6-nitroaniline 3b



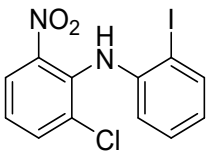
An orange solid (2.75 g, 78%, m.p. 75 °C). ^{1}H NMR (500 MHz, DMSO-d_6) δ = 7.90 (dd, *J*=8.6, 1.4 Hz, 1H), 7.80 (dd, *J*=8.0, 1.4 Hz, 1H), 7.64 (d, *J*=7.2 Hz, 1H), 7.51 (s, 1H), 7.33 (t, *J*=7.9 Hz, 1H), 7.17 (ddd, *J*=8.0, 7.6, 1.4 Hz, 1H), 6.65 (ddd, *J*=7.6, 1.4 Hz, 1H), 6.28 (dd, *J*=8.0, 1.4 Hz, 1H), 2.05 (s, 3H) ppm. ^{13}C NMR (126 MHz, DMSO-d_6) δ = 145.1, 144.4, 139.2, 136.5, 136.3, 134.6, 129.0, 124.8, 123.2, 122.2, 115.5, 88.0, 18.4 ppm. HRMS (ESI): m/z calcd $C_{13}H_{11}IN_2O_2$ for $[M+H]^+$ 354.9938, found 354.9938.

2-Chloro-6-nitro-_N_-(2-(trifluoromethyl)phenyl)aniline 3c



A yellow solid (157 mg, 50%, m.p. 70 – 72 °C). ^{1}H NMR (400 MHz, $CDCl_3$) δ = 8.42 (br. s., 1H), 8.07 (dd, *J*=8.4, 1.7 Hz, 1H), 7.66 (td, *J*=7.9, 1.1 Hz, 2H), 7.33 – 7.40 (m, 1H), 7.08 – 7.18 (m, 2H), 6.70 (d, *J*=8.2 Hz, 1H) ppm. ^{13}C NMR (101 MHz, $CDCl_3$) δ = 139.7, 136.5, 135.3, 132.3, 129.4, 126.8, 125.6, 124.9, 122.9, 122.7, 122.5, 119.9, 119.4 ppm. HRMS (ESI): m/z calcd $C_{13}H_8ClF_3N_2O_2$ for $[M+H]^+$ 317.0299, found 317.0300.

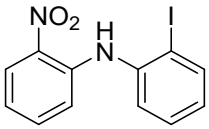
2-Chloro-_N_-(2-iodophenyl)-6-nitroaniline 3d



An orange solid (6.38 g, 85%, m.p. 90 – 91 °C). ^{1}H NMR (500 MHz, DMSO-d_6) δ = 8.03 (dd, *J*=8.3, 1.4 Hz, 1H), 7.89 (dd, *J*=8.0, 1.4 Hz, 1H), 7.80 (dd, *J*=7.7, 1.4 Hz, 1H), 7.68 (s, 1H), 7.39 (t, *J*=8.1 Hz, 1H), 7.19 (ddd, *J*=7.6, 1.4 Hz, 1H), 6.70 (ddd, *J*=7.6, 1.4 Hz, 1H), 6.46 (dd, *J*=8.0, 1.4 Hz, 1H) ppm. ^{13}C NMR (126

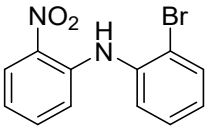
MHz, DMSO-d_6) δ = 145.2, 143.6, 139.1, 135.4, 134.1, 130.9, 128.8, 125.0, 124.7, 123.0, 116.9, 88.7 ppm. HRMS (ESI): m/z calcd $C_{12}H_8ClIN_2O_2$ for $[M-H]^-$ 372.9235, found 372.9246.

2-Iodo-*N*-(2-nitrophenyl)aniline 3e



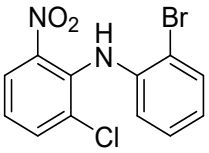
A yellow solid (1.35 g, 79%, m.p. 105 – 108 °C). ^{1}H NMR (400 MHz, $CDCl_3$) δ = 9.40 (br. s., 1H), 8.24 (dd, *J*=8.6, 1.5 Hz, 1H), 7.96 (dd, *J*=7.6, 0.9 Hz, 1H), 7.37 – 7.42 (m, 3H), 7.04 (dd, *J*=8.6, 1.2 Hz, 1H), 6.95 – 7.00 (m, 1H), 6.85 (ddd, *J*=8.5, 7.1, 1.2 Hz, 1H) ppm. ^{13}C NMR (101 MHz, $CDCl_3$) δ =142.0, 140.7, 140.2, 135.6, 133.8, 129.3, 127.2, 126.7, 125.0, 118.2, 116.2, 96.4 ppm. HRMS (ESI): m/z calcd $C_{12}H_9IN_2O_2$ for $[M+H]^+$ 340,9781, found 340.9782.

2-Bromo-*N*-(2-nitrophenyl)aniline 3f



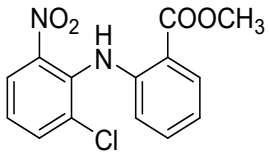
A yellow solid (445 mg, 76%, m.p. 104 – 105 °C). ^{1}H NMR (400 MHz, $CDCl_3$) δ = 9.46 (br. s., 1H), 8.23 (dd, *J*=8.6, 1.2 Hz, 1H), 7.70 (dd, *J*=8.1, 1.4 Hz, 1H), 7.39 – 7.47 (m, 2H), 7.35 (td, *J*=7.8, 1.2 Hz, 1H), 7.16 (dd, *J*=8.7, 1.4 Hz, 1H), 7.10 (td, *J*=7.6, 1.5 Hz, 1H), 6.86 (ddd, *J*=8.6, 7.0, 1.5 Hz, 1H) ppm. ^{13}C NMR (101 MHz, $CDCl_3$) δ =141.6, 137.6, 135.5, 133.8, 128.2, 126.7, 126.5, 124.6, 119.3, 118.4, 116.3, 100.1 ppm. HRMS (ESI): m/z calcd $C_{12}H_9BrN_2O_2$ for $[M+H]^+$ 292.9920, found 292.9920.

***N*-(2-Bromophenyl)-2-chloro-6-nitroaniline 3g**



An orange solid (463 mg, 71%, m.p. 91 – 95 °C). ^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.99 – 8.05 (m, 2H), 7.68 (dd, *J*=7.9, 1.5 Hz, 1H), 7.59 (dd, *J*=8.1, 1.4 Hz, 1H), 7.13 – 7.19 (m, 2H), 6.89 (td, *J*=7.7, 1.4 Hz, 1H), 6.59 (dd, *J*=8.1, 1.4 Hz, 1H) ppm. ^{13}C NMR (101 MHz, $CDCl_3$) δ = 139.3, 136.1, 135.0, 133.0, 130.0, 127.6, 124.8, 123.4, 122.8, 120.1, 117.9, 99.9 ppm. HRMS (ESI): m/z calcd $C_{12}H_8BrClN_2O_2$ for $[M-H]^-$ 324.9374, found 324.9387.

Methyl 2-((2-Chloro-6-nitrophenyl)amino)benzoate 3h



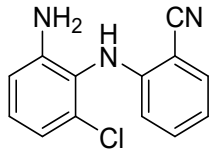
A yellow solid (725 mg, 47%, m.p. 250 – 255 °C). ^{1}H NMR (500 MHz, $CDCl_3$) δ = 9.72 (s, 1H), 8.07 (dd, *J*=8.3, 1.4 Hz, 1H), 7.96 (dd, *J*=8.0, 1.4 Hz, 1H), 7.92 (dd, *J*=8.0, 1.4 Hz, 1H), 7.50 (t, *J*=8.2 Hz, 1H), 7.38 (ddd, *J*=8.5, 7.2, 1.4 Hz, 1H), 6.89 (ddd, *J*=8.0, 7.2, 1.2 Hz, 1H), 6.42 (dd, *J*=8.6, 0.9 Hz, 1H), 3.90 (s, 3H) ppm. ^{13}C NMR (126 MHz, $CDCl_3$) δ =167.6, 146.6, 145.2, 135.1, 134.1, 132.2, 131.3, 130.9, 126.5, 124.3, 118.8, 114.2, 112.5, 52.0 ppm. HRMS (ESI): m/z calcd $C_{14}H_{11}ClN_2O_4$ for $[M+H]^+$ 307.0480, found 307.0480.

Other compounds prepared according to this method: *N*-(2-Iodophenyl)-2-nitro-6-(trifluoromethyl)aniline **3i** and 2-chloro-6-nitro-*N*-phenylaniline **3j** were isolated by extraction with DCM and used directly as a crude product without characterization in the next reaction step.

Method ***b)***

Compound **3** or **6** (10 mmol) was dissolved in glacial acetic acid (450 mL). 500 mg of 10% palladium on activated charcoal were weighed into a three-necked flask in a hydrogen reduction apparatus and, then, a solution of the starting material in acetic acid was added. The reaction mixture was stirred at room temperature in hydrogen atmosphere for 1.5 h. After completion of the reaction, palladium on activated charcoal was removed by filtration. The filtrate was diluted with water (200 mL) and extracted twice with dichloromethane (2×100 mL). Then, the dichloromethane solution was three times washed with a 5% aqueous solution of sodium bicarbonate (50 mL) and water (50 mL). Organic phase was evaporated on RVO to give a crude product.

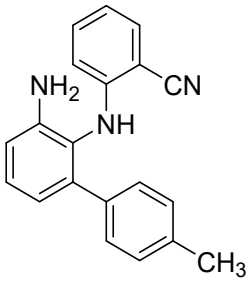
2-((2-Amino-6-chlorophenyl)amino)benzonitrile 4a



A white solid (706 mg, 73%, m.p. 102 – 105 °C). After completion of the reaction, palladium on activated charcoal was removed by filtration. The filtrate was diluted with water (80 mL). Then, an 5% aqueous solution of sodium bicarbonate was added dropwise until pH 7 was reached. A solution was extracted 3 × with CH_2Cl_2, 1 × with brine, dried under $MgSO_4$ and evaporated on rotavap.

^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.52 (dd, *J*=7.6, 1.5 Hz, 1H), 7.34 (td, *J*=7.9, 1.2 Hz, 1H), 7.05 – 7.11 (m, 1H), 6.82 – 6.89 (m, 2H), 6.73 (dd, *J*=7.9, 1.2 Hz, 1H), 6.40 (d, *J*=8.6 Hz, 1H), 5.93 (br. s., 1H), 4.02 (br. s., 2H) ppm. ^{13}C NMR (101 MHz, $CDCl_3$) δ = 148.0, 146.0, 134.3, 133.8, 132.7, 128.8, 121.7, 119.1, 117.4, 114.0, 113.2, 99.9, 97.6 ppm. HRMS (ESI): m/z calcd $C_{13}H_{10}ClN_3$ for $[M+H]^+$ 244.0636, found 244.0635.

2-((3-Amino-4'-methyl-[1,1'-biphenyl]-2-yl)amino)benzonitrile 7a

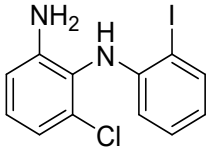


A light brown solid (2.63 g, 88%, m.p. 124 – 125 °C). ^{1}H NMR (400 MHz, DMSO-d_6) δ = 7.35 (dd, *J* = 1.6, 7.6 Hz, 1H), 7.30 (s, 1H), 7.24 (d, *J* = 7.8 Hz, 2H), 7.17 (dt, *J* = 1.4, 7.8 Hz, 1H), 7.13 – 7.02 (m, 3H), 6.79 (dd, *J* = 1.4, 7.8 Hz, 1H), 6.61 – 6.53 (m, 2H), 6.16 (d, *J* = 8.7 Hz, 1H), 5.01 (s, 2H), 2.23 (s, 3H) ppm. ^{13}C NMR (101 MHz, DMSO-d_6) δ = 149.4, 146.5, 141.0, 137.0, 135.7, 133.7, 133.0, 128.4, 127.8, 120.9, 117.91, 117.89, 116.5, 114.2, 113.0, 99.5, 94.7, 20.7 ppm. HRMS (ESI): m/z calcd $C_{20}H_{17}N_3$ for $[M-H]^-$ 298.1339, found 298.1335.

Method ***c)***

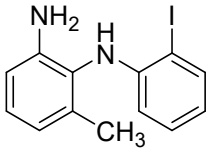
Compound **3**, **6** or **9** (12.5 mmol) was dissolved in methanol (50 mL) and glacial acetic acid (12.5 mL). Then, zinc (50 mmol) was added and the reaction mixture was stirred for 0.5 – 2 h. The reaction mixture was filtered and the filtrate was poured into water (150 mL). Then, an 5% aqueous solution of sodium bicarbonate was added dropwise to reach pH 7. A solution was extracted 3 × with CH_2Cl_2, 1 × with brine, and organic phase was dried over $MgSO_4$ and evaporated on a rotovap. The crude product was purified by column chromatography (DCM:hexane).

6-Chloro-*N*^{1}-(2-iodophenyl)benzene-1,2-diamine 4b



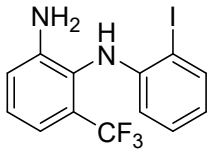
The crude product was purified by column chromatography (CH_2Cl_2:hexane 2:3). A white solid (2.55 g, 59%, m.p. 93 – 95 °C). ^{1}H NMR (500 MHz, DMSO-d_6) δ = 7.70 (dd, *J*=7.7, 1.4 Hz, 1H), 7.09 (td, *J*=7.9, 1.5 Hz, 1H), 7.01 (t, *J*=8.0 Hz, 1H), 6.75 (dd, *J*=8.2, 1.3 Hz, 1H), 6.69 (dd, *J*=7.9, 1.3 Hz, 1H), 6.50 (td, *J*=7.5, 1.6 Hz, 1H), 6.12 (s, 1H), 6.08 (dd, *J*=8.3, 1.4 Hz, 1H), 5.16 (br. s., 2H) ppm. ^{13}C NMR (126 MHz, DMSO-d_6) δ = 147.7, 145.3, 138.8, 132.7, 129.0, 127.9, 122.7, 120.0, 116.4, 113.5, 112.7, 85.2 ppm. HRMS (ESI): m/z calcd $C_{12}H_{10}ClIN_2$ for $[M+H]^+$ 344.9650, found 344.9652.

***N*^{1}-(2-Iodophenyl)-6-methylbenzene-1,2-diamine 4c**



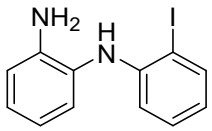
The crude product was purified by column chromatography (CH_2Cl_2:hexane 1:2). A brown solid (1.21 g, 72%, m.p. 60 °C). ^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.73 (dd, *J*=7.9, 1.5 Hz, 1H), 7.10 (td, *J*=7.8, 1.5 Hz, 1H), 7.05 (t, *J*=7.8 Hz, 1H), 6.69 (dd, *J*=7.8, 2.0 Hz, 2H), 6.52 (td, *J*=7.6, 1.5 Hz, 1H), 6.27 (dd, *J*=8.1, 1.4 Hz, 1H), 5.43 (s, 1H), 3.86 (br. s., 2H), 2.15 (s, 3H) ppm. ^{13}C NMR (101 MHz; $CDCl_3$) δ = 145.3, 144.8, 139.0, 137.3, 129.5, 127.7, 125.1, 120.3, 120.0, 113.4, 112.3, 84.9, 18.0 ppm. HRMS (ESI): m/z calcd $C_{13}H_{13}IN_2$ for $[M+H]^+$ 325.0196, found 325.0196.

N^1-(2-Iodophenyl)-6-(trifluoromethyl)benzene-1,2-diamine 4d



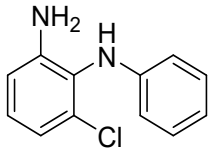
The crude product was purified by column chromatography (CH_2Cl_2:hexane 1:2). A light brown solid (908 mg, 48%, m.p. 75 – 78 °C). ^{1}H NMR (500 MHz, DMSO-d_6) δ = 7.70 (dd, *J*=7.7, 1.4 Hz, 1H), 7.22 (t, *J*=7.6 Hz, 1H), 7.07 – 7.11 (m, 2H), 6.92 (dd, *J*=7.7, 1.2 Hz, 1H), 6.49 (td, *J*=7.6, 1.4 Hz, 1H), 6.05 (s, 1H), 6.02 (dd, *J*=8.3, 1.4 Hz, 1H), 5.19 (s, 2H) ppm. ^{13}C NMR (126 MHz, DMSO-d_6) δ = 147.5, 145.0, 138.7, 129.0, 127.9, 127.6, 121.7, 119.8, 119.1, 113.2, 113.2, 112.4, 84.7 ppm. HRMS (ESI): m/z calcd $C_{13}H_{10}F_3IN_2$ for $[M+H]^+$ 378.9914, found 378.9913.

N^1-(2-Iodophenyl)benzene-1,2-diamine 4e



The crude product was purified by column chromatography (CH_2Cl_2). A brown solid (691 mg, 74%, m.p. 135 – 138 °C). ^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.74 (dd, *J*=7.6, 1.5 Hz, 1H), 7.08 – 7.16 (m, 3H), 6.84 (dd, *J*=8.6, 1.5 Hz, 1H), 6.78 (td, *J*=7.5, 1.5 Hz, 1H), 6.51 – 6.57 (m, 2H), 5.58 (br. s., 1H), 3.81 (br. s., 2H) ppm. ^{13}C NMR (101 MHz, $CDCl_3$) δ = 145.7, 143.4, 139.4, 129.6, 127.4, 127.3, 127.3, 120.7, 119.3, 116.4, 114.0, 86.2 ppm. HRMS (ESI): m/z calcd $C_{12}H_{11}IN_2$ for $[M+H]^+$ 311.0040, found 311.0037.

6-Chloro-N^1-phenylbenzene-1,2-diamine 4f



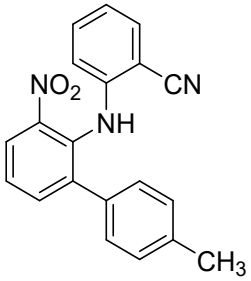
The crude product was purified by column chromatography (CH_2Cl_2:hexane 2:3). A light brown solid (50 mg, 56%, m.p. 130 – 133 °C). ^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.19 – 7.25 (m, 2H), 6.98 – 7.03 (m, 1H), 6.83 – 6.88 (m, 2H), 6.70 (dd, *J*=8.1, 1.4 Hz, 1H), 6.64 – 6.68 (m, 2H), 5.38 (br. s., 1H), 3.98 (br. s., 2H) ppm. ^{13}C NMR (101 MHz, $CDCl_3$) δ = 144.6, 132.6, 129.3, 127.1, 124.4, 119.8, 118.9, 114.6, 113.9, 99.9 ppm. HRMS (ESI): m/z calcd $C_{12}H_{11}ClN_2$ for $[M+H]^+$ 219.0684, found 219.0686.

Other compounds prepared according to this method: 2-((3-amino-4'-hydroxy-[1,1'-biphenyl]-2-yl)amino)benzonitrile **7b**, 2-((3-amino-4'-methoxy-[1,1'-biphenyl]-2-yl)amino)benzonitrile **7c**, 2-((3-amino-[1,1'-biphenyl]-2-yl)amino)benzonitrile **7d**, 2-((2-amino-6-(thiophen-3-yl)phenyl)amino)benzonitrile **7e**, 4'-methyl-N2-(2-(trifluoromethyl)phenyl)-[1,1'-biphenyl]-2,3-diamine **7f** and N^1-(4'-methyl-[1,1'-biphenyl]-2-yl)benzene-1,2-diamine **10** were extracted with dichloromethane and then with an 5% aqueous solution of sodium bicarbonate. The organic phase was evaporated on a rotovap. The crude product was used directly without characterization in the next reaction step.

Method ***d)***

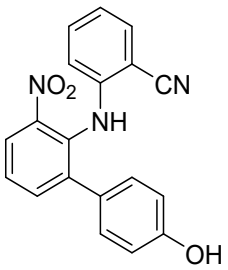
Nitroaniline **3** (3.7 mmol), boronic acid (4.4 mmol), and tripotassium phosphate (7.4 mmol) were dissolved in dioxane (14.5 mL) and water (3.5 mL). After all components were dissolved, XPhos Pd G2 (0.02 mmol, 0.5 mol%) was added and the reaction mixture was heated at 100 °C for 2.5 h. Upon completion, the reaction mixture was acidified with diluted hydrochloric acid (50 mL, 1:10). The product was extracted with dichloromethane (3×30 mL), the organic phase was dried over $MgSO_4$ and evaporated on a rotovap. A crude product was purified on column chromatography.

2-((4'-Methyl-3-nitro-[1,1'-biphenyl]-2-yl)amino)benzonitrile 6a



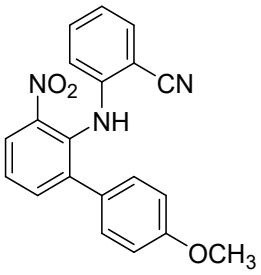
The crude product was purified by column chromatography (CH_2Cl_2:heptane 1:3). A yellow solid (1.09 g, 90%, m.p. 156 – 158 °C). ^{1}H NMR (400 MHz, DMSO-d_6) δ = 8.37 (s, 1H), 7.96 (dd, *J* = 1.6, 8.0 Hz, 1H), 7.63 (dd, *J* = 1.4, 7.6 Hz, 1H), 7.51 (t, *J* = 8.0 Hz, 1H), 7.32 (dd, *J* = 1.4, 7.8 Hz, 1H), 7.29 (d, *J* = 8.2 Hz, 2H), 7.13 (dt, *J* = 1.4, 7.8 Hz, 1H), 7.05 (d, *J* = 7.8 Hz, 2H), 6.62 (dt, *J* = 1.4, 7.6 Hz, 1H), 6.34 (d, *J* = 8.2 Hz, 1H), 2.17 (s, 3H) ppm. ^{13}C NMR (101 MHz, DMSO-d_6) δ = 147.49, 147.47, 141.6, 137.5, 136.3, 135.1, 134.1, 133.6, 131.9, 129.3, 129.1, 127.4, 124.6, 119.4, 117.9, 116.2, 97.6, 21.2 ppm. HRMS (ESI): m/z calcd $C_{20}H_{15}N_3O_2$ for $[M+H]^+$ 330.1237, found 330.1237.

2-((4'-Hydroxy-3-nitro-[1,1'-biphenyl]-2-yl)amino)benzonitrile 6b



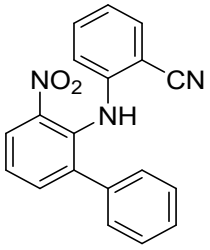
The crude product was purified by column chromatography (EtOAc:heptane 1:2). A light yellow oil (171 mg, 35%). ^{1}H NMR (400 MHz, $CDCl_3$) δ = 8.41 (s, 1H), 8.11 (dd, *J*=8.1, 1.6 Hz, 1H), 7.55 (d, *J*=7.6 Hz, 1H), 7.21 – 7.35 (m, 4H), 7.06 (t, *J*=7.8 Hz, 1H), 6.77 (t, *J*=7.6 Hz, 1H), 6.68 (dd, *J*=8.4, 1.4 Hz, 2H), 6.46 (d, *J*=8.5 Hz, 1H), 4.93 (s, 1H) ppm. ^{13}C NMR (101 MHz, $CDCl_3$) δ = 154.9, 144.5, 137.1, 136.8, 134.0, 132.5, 132.2, 130.1, 129.7, 129.6, 124.6, 123.4, 121.3, 117.9, 116.6, 115.1, 102.0 ppm.

2-((4'-Methoxy-3-nitro-[1,1'-biphenyl]-2-yl)amino)benzonitrile 6c



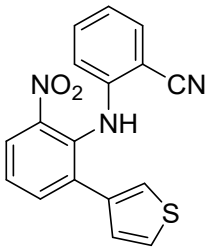
The crude product was purified by column chromatography (EtOAc:hexane 1:4).An orange solid (461 mg, 73%, m.p. 119 – 122 °C). ^{1}H NMR (400 MHz, DMSO-d_6) δ = 8.39 (s, 1H), 7.98 (dd, *J*=7.9, 1.5 Hz, 1H), 7.66 (dd, *J*=7.9, 1.5 Hz, 1H), 7.54 (t, *J*=8.1 Hz, 1H), 7.35 – 7.39 (m, 3H), 7.17 (ddd, *J*=8.5, 7.2, 1.7 Hz, 1H), 6.81 – 6.86 (m, 2H), 6.67 (td, *J*=7.5, 0.9 Hz, 1H), 6.39 (d, *J*=8.2 Hz, 1H), 3.68 (s, 3H) ppm. ^{13}C NMR (101 MHz, DMSO-d_6) δ = 158.7, 147.5, 147.4, 140.8, 135.6, 133.5, 133.0, 131.3, 129.9, 129.6, 126.8, 123.8, 118.9, 117.3, 115.8, 113.6, 97.0, 55.0 ppm. HRMS (ESI): m/z calcd $C_{20}H_{15}N_3O_3$ for $[M+H]^+$ 330.1237, found 330.1237.

2-((3-Nitro-[1,1'-biphenyl]-2-yl)amino)benzonitrile 6d



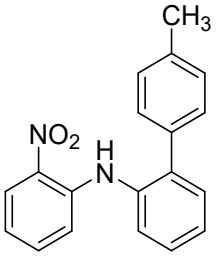
The crude product was purified by column chromatography (EtOAc:heptane 1:2).A yellow solid (327 mg, 69%, m.p. 109 – 111 °C). ^{1}H NMR (400 MHz, $CDCl_3$) δ = 8.47 (s, 1H), 8.16 (dd, J=8.2, 1.5 Hz, 1H), 7.59 (dd, J=7.6, 1.5 Hz, 1H), 7.31 – 7.37 (m, 3H), 7.28 – 7.30 (m, 1H), 7.18 – 7.23 (m, 2H), 7.09 – 7.14 (m, 1H), 7.04 (ddd, J=8.6, 7.5, 1.4 Hz, 1H), 6.74 (td, J=7.5, 0.9 Hz, 1H), 6.48 (dd, J=8.1, 0.9 Hz, 1H) ppm. ^{13}C NMR (101 MHz, $CDCl_3$) δ = 144.7, 142.9, 138.0, 137.7, 137.2, 134.6, 132.7, 132.5, 128.5, 128.4, 127.8, 125.3, 123.6, 121.6, 118.5, 116.8, 111.6 ppm. HRMS (ESI): m/z calcd $C_{19}H_{13}N_3O_2$ for $[M+H]^+$ 316.1081, found 316.1078.

2-((2-Nitro-6-(thiophen-3-yl)phenyl)amino)benzonitrile 6e



The crude product was purified by column chromatography (CH_2Cl_2:hexane 1:1).A yellow solid (484 mg, 82%, m.p. 96 – 99 °C). ^{1}H NMR (500 MHz, $CDCl_3$) δ = 8.52 (s, 1H), 8.11 (dd, *J*=8.3, 1.7 Hz, 1H), 7.64 (dd, *J*=7.6, 1.6 Hz, 1H), 7.35 (dd, *J*=7.7, 1.4 Hz, 1H), 7.24 – 7.30 (m, 2H), 7.10 (dd, *J*=2.1, 1.0 Hz, 2H), 7.02 – 7.07 (m, 1H), 6.78 (td, *J*=7.6, 0.9 Hz, 1H), 6.43 (d, *J*=8.3 Hz, 1H) ppm. ^{13}C NMR (126 MHz, $CDCl_3$) δ = 144.6, 138.2, 137.0, 136.2, 134.3, 133.4, 133.2, 132.7, 132.6, 132.5, 127.4, 126.1, 125.2, 124.0, 123.7, 121.5, 117.5 ppm. HRMS (ESI): m/z calcd $C_{17}H_{11}N_3O_2S$ for $[M+H]^+$ 322.0645, found 322.0646.

4'-Methyl-*N*-(2-nitrophenyl)-[1,1'-biphenyl]-2-amine 9



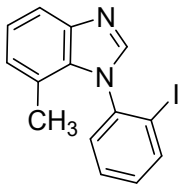
The crude product was purified by column chromatography (CH_2Cl_2:hexane 2:3).An orange solid (98 mg, 97%, m.p. 98 – 100 °C). ^{1}H NMR (400 MHz, $CDCl_3$) δ = 9.35 (s, 1H), 8.14 (dd, *J*=8.6, 1.2 Hz, 1H), 7.42 – 7.46 (m, 2H), 7.39 (td, *J*=7.6, 1.7 Hz, 1H), 7.30 – 7.36 (m, 2H), 7.25 – 7.28 (m, 2H), 7.16 – 7.20 (m, 3H), 6.72 (ddd, *J*=8.5, 7.1, 1.2 Hz, 1H), 2.36 (s, 3H) ppm. ^{13}C NMR (101 MHz, $CDCl_3$) δ = 143.1, 137.6, 137.4, 136.0, 135.5, 135.4, 133.3, 131.4, 129.3, 128.6, 128.1, 126.5, 126.0, 125.0, 117.3, 116.2, 21.1 ppm. HRMS (ESI): m/z calcd $C_{19}H_{16}N_2O_2$ for $[M+H]^+$ 305.1285, found 305.1285.

General synthetic methods and characterization of final compounds

Method ***e)***

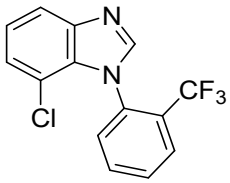
After reduction (Method **b** or **c**), trimethyl orthoformate (10-20 eq.) was added to the reaction mixture, which was stirred at room temperature overnight. After completion of the cyclization, the palladium on activated charcoal or zinc salts were filtered-off. The solvent was evaporated on a rotovap and the residue was dissolved in dichloromethane, washed with an 5% aqueous solution of sodium bicarbonate and water. The organic phase was dried over $MgSO_4$ and a solvent was evaporated on a rotovap. The crude product was purified by column chromatography.

1-(2-Iodophenyl)-7-methyl-1*H*-benzo[*d*]imidazole 5a



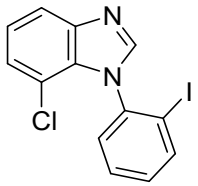
The crude product was purified by column chromatography (CH_2Cl_2:MeOH 40:1). A light brown solid (100 mg, 86%, m.p. 140 – 142 °C). ^{1}H NMR (400 MHz, $CDCl_3$) δ = 8.02 (dd, *J*=7.9, 1.2 Hz, 1H), 7.84 (s, 1H), 7.75 (dd, *J*=7.9, 1.2 Hz, 1H), 7.47 – 7.55 (m, 2H), 7.27 – 7.30 (m, 1H), 7.22 – 7.27 (m, 1H), 7.04 (dd, *J*=7.3, 0.9 Hz, 1H), 1.99 (s, 3H) ppm. ^{13}C NMR (101 MHz, $CDCl_3$) δ =143.5, 143.1, 140.4, 139.4, 132.8, 131.1, 129.7, 129.0, 125.6, 122.8, 121.8, 118.4, 99.5, 17.6 ppm. HRMS (ESI): m/z calcd $C_{14}H_{11}IN_2$ for $[M+H]^+$ 335.0040, found 335.0037.

7-Chloro-1-(2-(trifluoromethyl)phenyl)-1*H*-benzo[*d*]imidazole 5b



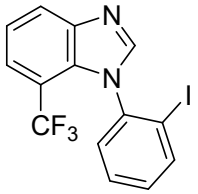
The crude product was purified by column chromatography (EtOAc: hexane 2:3). A white solid (74 mg, 50%, m.p. 73 – 75 °C). ^{1}H NMR (400 MHz, DMSO-d_6) δ = 8.46 (s, 1H), 8.00 (dd, *J*=7.6, 1.8 Hz, 1H), 7.82 – 7.92 (m, 2H), 7.80 (dd, *J*=7.8, 1.4 Hz, 1H), 7.77 (dd, *J*=6.7, 2.4 Hz, 1H), 7.26 – 7.32 (m, 2H) ppm. ^{13}C NMR (101 MHz, $CDCl_3$) δ = 144.8, 144.7, 134.2 (q, *J*=1.5 Hz) 132.4, 131.9, 131.6, 130.2, 129.1 (q, *J*=30.8 Hz) 126.8 (q, *J*=4.6 Hz) 124.9, 123.5, 122.8 (q, *J*=273.8 Hz) 119.4, 116.7 ppm. HRMS (ESI): m/z calcd $C_{14}H_8ClF_3N_2$ for $[M+H]^+$ 297.0401, found 297.0398.

7-Chloro-1-(2-iodophenyl)-1*H*-benzo[*d*]imidazole 5c



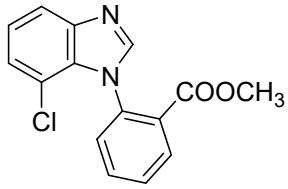
The crude product was purified by column chromatography (CH_2Cl_2). A white solid (35 mg, 86%, m.p. 156 – 159 °C). ^{1}H NMR (400 MHz, $CDCl_3$) δ = 8.00 (dd, *J*=8.1, 1.4 Hz, 1H), 7.89 (s, 1H), 7.79 – 7.84 (m, 1H), 7.46 – 7.54 (m, 2H), 7.25 – 7.29 (m, 3H) ppm. ^{13}C NMR (101 MHz, $CDCl_3$) δ = 145.1, 144.0, 139.3, 139.2, 131.2, 129.7, 128.8, 124.8, 123.4, 119.4, 116.9, 99.9, 99.4 ppm. HRMS (ESI): m/z calcd $C_{13}H_8ClIN_2$ for $[M+H]^+$ 354.9493 found 354.9492.

1-(2-Iodophenyl)-7-(trifluoromethyl)-1*H*-benzo[*d*]imidazole 5d



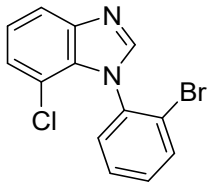
The crude product was purified by column chromatography (CH_2Cl_2:MeOH 80:1). A white solid (177 mg, 87%, m.p. 95 – 98 °C). ^{1}H NMR (400 MHz, $CDCl_3$) δ = 8.11 (d, *J*=8.2 Hz, 1H), 8.01 (dd, *J*=7.9, 1.2 Hz, 1H), 7.91 (s, 1H), 7.65 (d, *J*=7.6 Hz, 1H), 7.47 – 7.56 (m, 2H), 7.40 – 7.46 (m, 1H), 7.28 (ddd, *J*=7.9, 7.0, 2.1 Hz, 1H) ppm. ^{13}C NMR (101 MHz, $CDCl_3$) δ = 145.5, 145.4, 139.5, 139.2, 131.4, 130.2, 129.8 (q, *J*=1.0 Hz) 128.9, 124.9, 123.0 (q, *J*=272.3 Hz), 122.2 (q, *J*=5.5 Hz), 114.6 (q, *J*=35.2 Hz) 99.2, 91.4 ppm. HRMS (ESI): m/z calcd $C_{14}H_8F_3IN_2$ for $[M+H]^+$ 388.9757, found 388.9756.

Methyl 2-(7-Chloro-1*H*-benzo[*d*]imidazol-1-yl)benzoate 5e



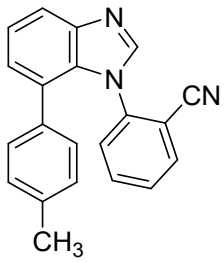
The crude product was purified by column chromatography (EtOAc:hexane 4:1). A light brown solid (47 mg, 49%, m.p. 300 – 304 °C). ^{1}H NMR (400 MHz, $CDCl_3$) δ = 8.95 (s, 1H), 7.99 (dd, *J*=7.9, 1.2 Hz, 1H), 7.27 – 7.31 (m, 1H), 7.02 – 7.07 (m, 1H), 6.87 (dd, *J*=8.1, 1.4 Hz, 1H), 6.70 – 6.77 (m, 2H), 6.39 (dd, *J*=8.4, 0.8 Hz, 1H), 3.95 (s, 3H) ppm. ^{13}C NMR (101 MHz, $CDCl_3$) δ = 169.0, 148.7, 146.0, 134.5, 134.0, 131.4, 128.0, 123.0, 119.1, 117.1, 113.7, 113.4, 111.5, 93.1, 51.8 ppm. HRMS (ESI): m/z calcd $C_{15}H_{11}ClN_2O_2$ for $[M+H]^+$ 287.0582, found 287.0574.

1-(2-Bromophenyl)-7-chloro-1*H*-benzo[*d*]imidazole 5f



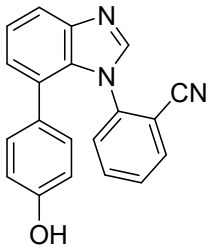
The crude product was purified by column chromatography (CH_2Cl_2:hexane 1:1).A light brown solid (74 mg, 72%, m.p. 110 – 113 °C). ^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.90 (s, 1H), 7.78 – 7.82 (m, 1H), 7.74 – 7.77 (m, 1H), 7.47 – 7.50 (m, 2H), 7.42 – 7.45 (m, 1H), 7.26 (m, 2H) ppm. ^{13}C NMR (101 MHz, $CDCl_3$) δ = 144.2, 135.7, 133.1, 132.7, 131.1, 130.4, 127.9, 124.8, 123.8, 123.4, 119.4, 116.8, 95.2 ppm. HRMS (ESI): m/z calcd $C_{13}H_8BrClN_2$ for $[M+H]^+$ 306.9632, found 306.9633.

2-(7-(*p*-Tolyl)-1*H*-benzo[*d*]imidazol-1-yl)benzonitrile 8a



The crude product was purified by column chromatography (CH_2Cl_2:hexane 1:1).A white solid (263 mg, 85%, m.p. 175 °C). ^{1}H NMR (400 MHz, DMSO-d_6) δ = 8.50 (s, 1H), 7.82 (dd, *J*=8.0, 1.1 Hz, 1H), 7.63 (dd, *J*=7.6, 1.1 Hz, 1H), 7.53 – 7.58 (m, 1H), 7.38 – 7.48 (m, 3H), 7.19 (dd, *J*=7.3, 0.9 Hz, 1H), 6.80 – 6.88 (m, 4H), 2.17 (s, 3H) ppm. ^{13}C NMR (101 MHz, DMSO-d_6) δ = 145.0, 143.9, 138.9, 136.0, 133.7, 133.6, 132.8, 131.3, 128.8, 128.7, 128.7, 127.9, 127.1, 125.1, 122.8, 119.1, 115.7, 110.5, 20.6 ppm. HRMS (ESI): m/z calcd $C_{21}H_{15}N_3$ for $[M+H]^+$ 310.1339, found 310.1340.

2-(7-(4-Hydroxyphenyl)-1*H*-benzo[*d*]imidazol-1-yl)benzonitrile 8b

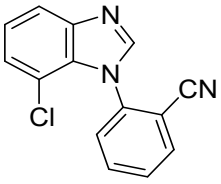


The crude product was purified by column chromatography (EtOAc:heptane 1:3).A colourless oil (46 mg, 52%). ^{1}H NMR (400 MHz, DMSO-d_6) δ = 9.31 (s, 1H), 8.47 (s, 1H), 7.78 (dd, *J*=8.0, 1.1 Hz, 1H), 7.68 (dd, *J*=7.8, 1.4 Hz, 1H), 7.57 – 7.62 (m, 1H), 7.47 (td, *J*=7.7, 1.1 Hz, 1H), 7.43 (dd, *J*=7.8, 0.9 Hz, 1H), 7.37 (td, *J*=7.8, 0.9 Hz, 1H), 7.15 (dd, *J*=7.3, 0.9 Hz, 1H), 6.78 (d, *J*=8.7 Hz, 2H), 6.40 (d, *J*=8.7 Hz, 2H) ppm. ^{13}C NMR (101 MHz, DMSO-d_6) δ =156.2, 144.9, 143.9, 138.9, 133.6, 132.8, 131.3, 129.8, 128.9, 128.7, 127.2, 127.1, 125.2, 122.7, 118.6, 115.6, 114.1, 110.5 ppm. HRMS (ESI): m/z calcd $C_{20}H_{13}N_3O$ for $[M+H]^+$ 312.1131, found 312.1131.

Method ***f)***

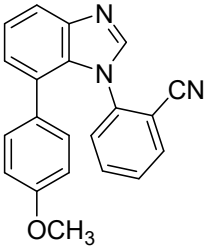
Phenylendiamine **4** or **7** (0.33 mmol) was dissolved in dichloromethane (2 mL). Then, trimethyl orthoformate (3.33 mmol) and *p*-toluenesulfonic acid (0.17 mmol) were added. The reaction mixture was stirred at room temperature overnight. After that, the solution was diluted with dichloromethane (10 mL) and extracted twice with water (10 mL). The organic phase was evaporated and the crude product was purified by column chromatography.

2-(7-Chloro-1*H*-benzo[*d*]imidazol-1-yl)benzonitrile 5g



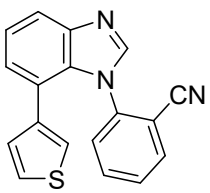
The crude product was purified by column chromatography (CH_2Cl_2:MeOH 40:1). A white solid (57 mg, 68%, m.p. 104 – 106 °C). ^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.95 (s, 1H), 7.81 – 7.84 (m, 1H), 7.79 (t, *J*=4.58 Hz, 1H), 7.73 (dd, *J*=7.78, 1.68 Hz, 1H), 7.64 (dd, *J*=7.63, 1.22 Hz, 1H), 7.53 (dd, *J*=7.93, 1.22 Hz, 1H), 7.25 – 7.27 (m, 2H) ppm. ^{13}C NMR (101 MHz, $CDCl_3$) δ = 145.3, 143.8, 138.5, 133.3, 130.7, 129.9, 129.8, 125.4, 124.0, 119.8, 116.5, 115.1, 113.1, 101.2 ppm. HRMS (ESI): m/z calcd $C_{14}H_8ClN_3$ for $[M+H]^+$ 254.0480, found 254.0479.

2-(7-(4-Methoxyphenyl)-1*H*-benzo[*d*]imidazol-1-yl)benzonitrile 8c



The crude product was purified by column chromatography (EtOAc:heptane 2:3). A white solid (15 mg, 16%, m.p. 217 – 220 °C). ^{1}H NMR (400 MHz, $CDCl_3$) δ = 8.05 (s, 1H), 7.91 (dd, *J*=7.9, 1.0 Hz, 1H), 7.50 – 7.53 (m, 1H), 7.43 (td, *J*=7.6, 0.9 Hz, 1H), 7.31 – 7.35 (m, 2H), 7.26 (dd, *J*=7.4, 1.2 Hz, 1H), 7.02 – 7.05 (m, 1H), 6.95 – 6.98 (m, 2H), 6.55 – 6.58 (m, 2H), 3.73 (s, 3H) ppm. ^{13}C NMR (101 MHz, $CDCl_3$) δ = 158.7, 144.0, 143.9, 138.9, 133.01, 132.95, 130.2, 129.2, 128.6, 128.4, 127.1, 126.4, 123.9, 119.4, 115.2, 113.1, 111.0, 55.3 ppm. HRMS (ESI): m/z calcd $C_{21}H_{15}N_3O$ for $[M+H]^+$ 326.1288, found 326.1284.

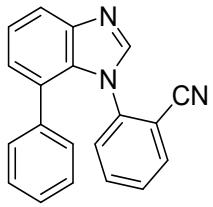
2-(7-(Thiophen-3-yl)-1*H*-benzo[*d*]imidazol-1-yl)benzonitrile 8d



The crude product was purified by column chromatography (CH_2Cl_2:MeOH 40:1). A white solid (22 mg, 24%, m.p. 155 – 157 °C). ^{1}H NMR (500 MHz, $CDCl_3$) δ = 8.07 (s, 1H), 7.93 (dd, *J*=8.0, 0.9 Hz, 1H), 7.57 – 7.59 (m, 1H), 7.37 – 7.45 (m, 3H), 7.31 (dd, *J*=7.3, 0.7 Hz, 1H), 7.08 – 7.11 (m, 1H), 6.90 – 6.95 (m, 2H), 6.67 (dd, *J*=4.9, 1.2 Hz, 1H) ppm. ^{13}C NMR (126 MHz, $CDCl_3$) δ = 143.6, 139.0, 137.7, 133.2, 133.0, 129.9,

129.8, 128.5, 128.5, 127.9, 126.1, 125.4, 124.6, 123.3, 121.9, 120.3, 115.4, 110.7 ppm. HRMS (ESI): m/z calcd $C_{18}H_{11}N_3S$ for $[M+H]^+$ 302.0746, found 302.0746.

2-(7-Phenyl-1*H*-benzo[*d*]imidazol-1-yl)benzonitrile 8e

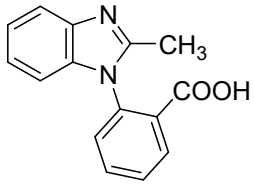


The crude product was purified by column chromatography (CH_2Cl_2:MeOH 40:1). A white solid (83 mg, 80%, m.p. 214 – 216 °C). ^{1}H NMR (400 MHz, $CDCl_3$) δ = 8.04 (s, 1H), 7.93 (dd, *J*=8.1, 1.1 Hz, 1H), 7.42 – 7.50 (m, 2H), 7.26 – 7.31 (m, 3H), 6.98 – 7.06 (m, 6H) ppm. ^{13}C NMR (126 MHz, $CDCl_3$) δ = 144.4, 143.6, 139.1, 137.2, 133.0, 132.9, 131.4, 129.0, 128.4, 128.2, 127.6, 127.1, 126.9, 126.0, 123.4, 120.0, 115.4, 110.8 ppm. HRMS (ESI): m/z calcd $C_{20}H_{13}N_3$ for $[M+H]^+$ 296.1182, found 296.1181.

Method ***g)***

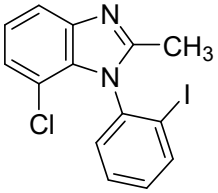
Phenylendiamine **4**, **7**, or **10** (0.33 mmol) was dissolved in dichloromethane (2 mL). Then, trimethyl orthoacetate (3.33 mmol) and *p*-toluenesulfonic acid (0.17 mmol) were added. The reaction mixture was stirred at room temperature overnight. After that, the solution was diluted with dichloromethane (10 mL) and extracted twice with water (10 mL). The organic phase was evaporated and the crude product was purified by column chromatography.

2-(2-Methyl-1*H*-benzo[*d*]imidazol-1-yl)benzoic acid 5h



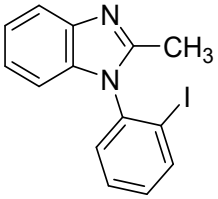
The pure product was filtrated directly from the reaction mixture and washed with MeOH. A white solid (94 mg, 75%, m.p. 240 – 242 °C). ^{1}H NMR (400 MHz, DMSO-d_6) δ = 8.08 (dd, *J*=7.9, 1.5 Hz, 1H), 7.83 (td, *J*=7.6, 1.2 Hz, 1H), 7.72 (td, *J*=7.6, 1.2 Hz, 1H), 7.58 (ddd, *J*=8.1, 2.6, 0.9 Hz, 2H), 7.09 – 7.20 (m, 2H), 6.85 (dd, *J*=7.8, 1.4 Hz, 1H), 2.29 (s, 3H) ppm. ^{13}C NMR (101 MHz, DMSO-d_6) δ = 166.2, 151.8, 142.3, 136.8, 134.5, 133.5, 131.4, 130.3, 129.9, 129.7, 122.1, 121.5, 118.2, 109.3, 13.8 ppm. HRMS (ESI): m/z calcd $C_{15}H_{12}N_2O_2$ for $[M+H]^+$ 253.0972, found 253.0971.

7-Chloro-1-(2-iodophenyl)-2-methyl-1*H*-benzo[*d*]imidazole 5i



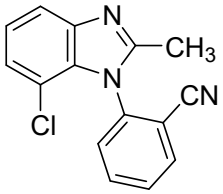
The crude product was purified by column chromatography (EtOAc:hexane 2:1).A light brown solid (80 mg, 81%, m.p. 152 – 153 °C). ^{1}H NMR (400 MHz, $CDCl_3$) δ = 8.01 (dd, *J*=7.9, 1.2 Hz, 1H), 7.68 (dd, *J*=7.6, 1.2 Hz, 1H), 7.53 (td, *J*=7.6, 1.2 Hz, 1H), 7.43 (dd, *J*=7.9, 1.4 Hz, 1H), 7.25 – 7.30 (m, 1H), 7.15 – 7.23 (m, 2H), 2.37 (s, 3H) ppm. ^{13}C NMR (101 MHz, $CDCl_3$) δ = 152.6, 144.4, 139.4, 131.4, 131.1, 130.0, 129.1, 124.0, 123.0, 118.5, 118.0, 116.1, 100.1, 14.3 ppm. HRMS (ESI): m/z calcd $C_{14}H_{10}ClIN_2$ for $[M+H]^+$ 368.9650, found 368.9648.

1-(2-Iodophenyl)-2-methyl-1*H*-benzo[*d*]imidazole 5j



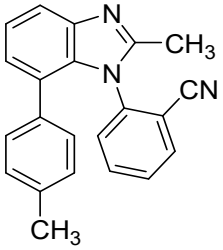
The crude product was purified by column chromatography (EtOAc:hexane 2:1). A light brown solid (85 mg, 77%, m.p. 127 – 130 °C). ^{1}H NMR (400 MHz, $CDCl_3$) δ = 8.08 (dd, *J*=8.1, 1.4 Hz, 1H), 7.78 (d, *J*=7.9 Hz, 1H), 7.57 (td, *J*=7.6, 1.2 Hz, 1H), 7.38 (dd, *J*=7.6, 1.5 Hz, 1H), 7.26 – 7.32 (m, 2H), 7.21 (ddd, *J*=8.1, 7.1, 1.2 Hz, 1H), 6.89 – 6.92 (m, 1H), 2.44 (s, 3H) ppm. ^{13}C NMR (101 MHz, $CDCl_3$) δ = 151.3, 140.4, 138.9, 135.9, 131.2, 131.1, 129.8, 129.4, 122.7, 122.5, 119.1, 110.0, 98.6, 14.4 ppm. HRMS (ESI): m/z calcd $C_{14}H_{11}IN_2$ for $[M+H]^+$ 335.0040, found 335.0025.

2-(7-Chloro-2-methyl-1*H*-benzo[*d*]imidazol-1-yl)benzonitrile 5k



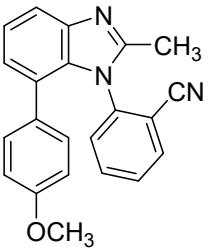
The crude product was purified by column chromatography (CH_2Cl_2). A white solid (71 mg, 80%, m.p. 103 – 106 °C). ^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.88 (dd, *J*=7.6, 1.5 Hz, 1H), 7.79 (td, *J*=7.6, 1.5 Hz, 1H), 7.66 – 7.71 (m, 2H), 7.50 (dd, *J*=7.9, 1.2 Hz, 1H), 7.17 – 7.25 (m, 2H), 2.42 (s, 3H) ppm. ^{13}C NMR (101 MHz, $CDCl_3$) δ =152.5, 144.6, 139.1, 133.5, 133.4, 132.0, 130.5, 130.2, 124.3, 123.5, 118.4, 115.7, 115.0, 114.0, 14.1 ppm. HRMS (ESI): m/z calcd $C_{15}H_{10}ClN_3$ for $[M+H]^+$ 268.0636, found 268.0635.

2-(2-Methyl-7-(*p*-tolyl)-1*H*-benzo[*d*]imidazol-1-yl)benzonitrile 8f



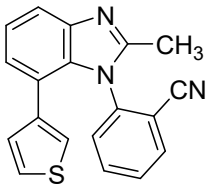
The crude product was purified by column chromatography (CH_2Cl_2:MeOH 40:1). A white solid (259 mg, 80%, m.p. 198 – 200 °C). ^{1}H NMR (400 MHz, DMSO-d_6) δ = 7.68 (dd, *J*=8.2, 0.9 Hz, 1H), 7.58 – 7.63 (m, 2H), 7.51 – 7.54 (m, 1H), 7.43 – 7.48 (m, 1H), 7.29 – 7.34 (m, 1H), 7.05 (dd, *J*=7.6, 1.2 Hz, 1H), 6.76 – 6.83 (m, 4H), 2.31 (s, 3H), 2.16 (s, 3H) ppm. ^{13}C NMR (101 MHz, DMSO-d_6) δ = 151.9, 143.0, 139.0, 135.8, 134.0, 133.4, 133.1, 132.8, 130.0, 129.2, 128.7, 127.8, 126.5, 124.5, 122.2, 117.9, 115.6, 111.5, 20.6, 14.0 ppm. HRMS (ESI): m/z calcd $C_{22}H_{17}N_3$ for $[M+H]^+$ 324.1495, found 324.1492.

2-(7-(4-Methoxyphenyl)-2-methyl-1*H*-benzo[*d*]imidazol-1-yl)benzonitrile 8g



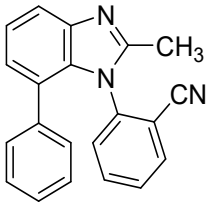
The crude product was purified by column chromatography (CH_2Cl_2:MeOH 40:1). A white solid (61 mg, 71%, m.p. 210 – 213 °C). ^{1}H NMR (500 MHz, $CDCl_3$) δ = 7.79 (dd, *J*=8.2, 1.0 Hz, 1H), 7.51 (dd, *J*=7.5, 1.7 Hz, 1H), 7.31 – 7.39 (m, 3H), 7.12 (dd, *J*=7.3, 1.0 Hz, 1H), 7.01 (dd, *J*=8.0, 1.2 Hz, 1H), 6.91 (d, *J*=8.6 Hz, 2H), 6.53 (d, *J*=8.9 Hz, 2H), 3.72 (s, 3H), 2.45 (s, 3H) ppm. ^{13}C NMR (126 MHz, $CDCl_3$) δ = 158.3, 152.0, 143.4, 139.8, 133.4, 133.1, 133.1, 130.2, 129.7, 129.5, 128.7, 126.3, 125.3, 122.7, 118.5, 115.4, 112.9, 112.8, 55.3, 14.3 ppm. HRMS (ESI): m/z calcd $C_{22}H_{17}N_3O$ for $[M+H]^+$ 340.1444, found 340.1446.

2-(2-Methyl-7-(thiophen-3-yl)-1*H*-benzo[*d*]imidazol-1-yl)benzonitrile 8h



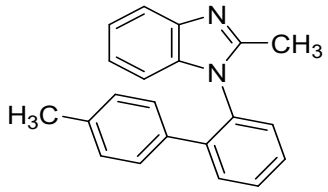
The crude product was purified by column chromatography (CH_2Cl_2:MeOH 40:1). A white solid (65 mg, 67%, m.p. 196 – 199 °C). ^{1}H NMR (500 MHz, $CDCl_3$) δ = 7.80 (dd, *J*=8.2, 1.0 Hz, 1H), 7.57 (dd, *J*=7.7, 1.7 Hz, 1H), 7.37 – 7.46 (m, 2H), 7.34 (t, *J*=7.7 Hz, 1H), 7.16 (dd, *J*=7.3, 1.0 Hz, 1H), 7.07 (dd, *J*=7.9, 1.0 Hz, 1H), 6.86 – 6.90 (m, 2H), 6.61 (dd, *J*=4.7, 1.3 Hz, 1H), 2.45 (s, 3H) ppm. ^{13}C NMR (126 MHz, $CDCl_3$) δ = 152.1, 143.4, 139.5, 137.5, 133.4, 133.2, 130.1, 129.3, 128.9, 128.6, 125.4, 124.3, 123.2, 122.6, 121.2, 119.0, 115.4, 112.6, 14.3 ppm. HRMS (ESI): m/z calcd $C_{19}H_{13}N_3S$ for $[M+H]^+$ 316.0903, found 316.0901.

2-(2-Methyl-7-phenyl-1*H*-benzo[*d*]imidazol-1-yl)benzonitrile 8i



The crude product was purified by column chromatography (CH_2Cl_2:MeOH 40:1). A white solid (79 mg, 73%, m.p. 189 – 192 °C). ^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.86 (dd, *J*=8.1, 1.1 Hz, 1H), 7.49 (dd, *J*=7.6, 1.5 Hz, 1H), 7.34 – 7.46 (m, 3H), 7.22 (dd, *J*=7.3, 1.2 Hz, 1H), 7.18 (dd, *J*=7.8, 1.1 Hz, 1H), 6.98 – 7.06 (m, 5H), 2.58 (s, 3H) ppm. ^{13}C NMR (101 MHz, $CDCl_3$) δ = 152.0, 139.5, 138.3, 136.2, 133.4, 133.2, 132.0, 129.6, 129.5, 129.1, 127.5, 127.2, 127.0, 126.3, 123.9, 117.5, 115.0, 112.6, 13.7 ppm. HRMS (ESI): m/z calcd $C_{21}H_{15}N_3$ for $[M+H]^+$ 310.1339, found 310.1339 ppm.

2-Methyl-1-(4'-methyl-[1,1'-biphenyl]-2-yl)-1*H*-benzo[*d*]imidazole 11a

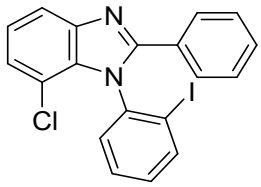


The crude product was purified by column chromatography (CH_2Cl_2). A light brown solid (22 mg, 44%, m.p. 142 – 145 °C). ^{1}H NMR (400 MHz, $CDCl_3$) δ =7.70 (dd, *J*=7.3, 0.9 Hz, 1H), 7.57 – 7.65 (m, 2H), 7.52 (td, *J*=7.6, 1.9 Hz, 1H), 7.39 (dd, *J*=7.9, 1.2 Hz, 1H), 7.26 (td, *J*=7.6, 1.4 Hz, 1H), 7.21 (td, *J*=7.6, 1.4 Hz, 1H), 7.11 – 7.15 (m, 1H), 6.96 (dd, *J*=7.6, 1.0 Hz, 2H), 6.86 (d, *J*=7.6 Hz, 2H), 2.24 (s, 3H), 2.07 (s, 3H) ppm. ^{13}C NMR (101 MHz, $CDCl_3$) δ = 151.8, 142.7, 140.5, 137.6, 137.2, 134.8, 133.3, 131.5, 129.6, 129.4, 128.8, 128.4, 127.8, 122.4, 122.1, 118.9, 110.0, 21.0, 14.0 ppm. HRMS (ESI): m/z calcd $C_{21}H_{18}N_2$ for $[M+H]^+$ 299.1543, found 299.1540.

Method **h)**

Phenylendiamine **4**, **7**, or **10** (0.33 mmol) was dissolved in dichloromethane (2 mL). Then, trimethyl orthobenzoate (3.33 mmol) and *p*-toluenesulfonic acid (0.17 mmol) were added. The reaction mixture was stirred at room temperature overnight. After that, the solution was diluted with dichloromethane (10 mL) and washed twice with water (10 mL). The organic phase was evaporated and the crude product was purified by column chromatography.

7-Chloro-1-(2-iodophenyl)-2-phenyl-1*H*-benzo[*d*]imidazole 5l



The crude product was purified by column chromatography (CH_2Cl_2). A white solid (148 mg, 57%, m.p. 143 – 145 °C). ^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.94 (dd, *J*=7.9, 1.2 Hz, 1H), 7.83 (dd, *J*=7.5, 1.4 Hz, 1H),

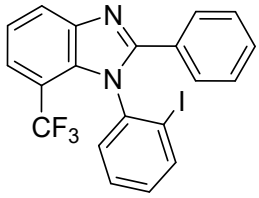
7.55 – 7.62 (m, 2H), 7.42 – 7.49 (m, 2H), 7.20 – 7.37 (m, 6H) ppm. ^{13}C NMR (101 MHz, $CDCl_3$) δ = 153.2, 144.7, 140.0, 139.3, 131.9, 131.1, 131.0, 129.8, 129.52, 129.48, 128.8, 128.3, 124.9, 123.5, 118.9, 116.7, 101.3 ppm. HRMS (ESI): m/z calcd $C_{19}H_{12}ClIN_2$ for $[M+H]^+$ 430.9806, found 430.9803.

For the separation method see the chapter Chiral separation.

(*S*$_a$)-(-)-7-chloro-1-(2-iodophenyl)-2-phenyl-1*H*-benzo[*d*]imidazole (*S*$_a$)-(-)5l: $[\alpha]_D^{22}$ -49.5° (*c* = 0.20 g/100 mL, DCM)

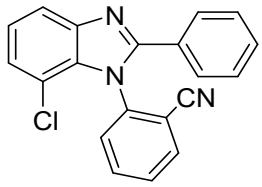
(*R*$_a$)- (+)-7-chloro-1-(2-iodophenyl)-2-phenyl-1*H*-benzo[*d*]imidazole (*R*$_a$)-(+)5l: $[\alpha]_D^{22}$ +49.5° (*c* = 0.20 g/100 mL, DCM)

1-(2-Iodophenyl)-2-phenyl-7-(trifluoromethyl)-1*H*-benzo[*d*]imidazole 5m



The crude product was purified by column chromatography (CH_2Cl_2). A white solid (72 mg, 62%, m.p. 192 – 195 °C). ^{1}H NMR (400 MHz, $CDCl_3$) δ = 8.12 (d, *J*=7.9 Hz, 1H), 7.85 (dd, *J*=8.1, 1.4 Hz, 1H), 7.67 – 7.71 (m, 1H), 7.64 (d, *J*=7.6 Hz, 1H), 7.54 – 7.58 (m, 2H), 7.52 (td, *J*=7.6, 1.2 Hz, 1H), 7.41 – 7.46 (m, 1H), 7.33 – 7.38 (m, 1H), 7.27 – 7.32 (m, 2H), 7.19 (td, *J*=7.8, 1.5 Hz, 1H) ppm. ^{13}C NMR (101 MHz, $CDCl_3$) δ = 154.7, 144.9, 139.7, 132.1, 131.5, 131.1, 129.8, 129.7, 128.5, 128.1, 127.0, 124.5, 123.0 (q, *J*=272.3 Hz), 122.2, 122.0, 118.9, 114.6 (q, *J*=34.6 Hz) 100.6 (q, *J*=1.4 Hz) ppm. HRMS (ESI): m/z calcd $C_{20}H_{12}F_3IN_2$ for $[M+H]^+$ 465.0070, found 465.0075.

2-(7-Chloro-2-phenyl-1*H*-benzo[*d*]imidazol-1-yl)benzonitrile 5n



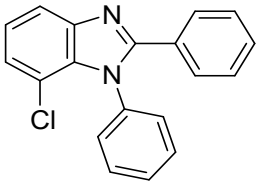
The crude product was purified by column chromatography (CH_2Cl_2:MeOH 80:1). A white solid (82 mg, 75%, m.p. 180 – 182 °C). ^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.84 (dd, *J*=7.6, 1.5 Hz, 1H) 7.77 (dd, *J*=7.6, 1.5 Hz, 1H) 7.70 (td, *J*=7.9, 1.5 Hz, 1H) 7.61 (td, *J*=7.6, 1.5 Hz, 1H) 7.49 – 7.55 (m, 3H) 7.36 – 7.41 (m, 1H) 7.26 – 7.34 (m, 4H) ppm. ^{13}C NMR (101 MHz, $CDCl_3$) δ =153.9, 145.0, 139.8, 133.1, 133.1, 132.5, 131.4, 130.0, 129.9, 129.5, 129.0, 128.5, 125.2, 123.9, 119.3, 116.2, 115.1, 114.7 ppm. HRMS (ESI): m/z calcd $C_{20}H_{12}ClN_3$ for $[M+H]^+$ 330.0793, found 330.0787.

For the separation method and crystallography see the chapter Chiral separation.

(*S*$_a$)-(-)-2-(7-chloro-2-phenyl-1*H*-benzo[*d*]imidazol-1-yl)benzonitrile (*S*$_a$)-(-)5n: $[\alpha]_D^{22}$ -12.5° (*c* = 0.15 g/100 mL, DCM)

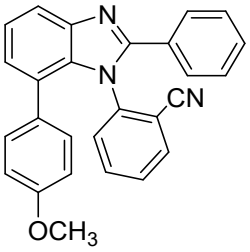
(*R*$_a$)-(+)-2-(7-chloro-2-phenyl-1*H*-benzo[*d*]imidazol-1-yl)benzonitrile (*R*$_a$)-(+)5n: $[\alpha]_D^{22}$ +12.5° (*c* = 0.15 g/100 mL, DCM)

7-Chloro-1,2-diphenyl-1*H*-benzo[*d*]imidazole 5o



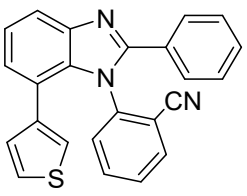
The crude product was purified by column chromatography (CH_2Cl_2). A light brown solid (35 mg, 40%, m.p. 142 – 146 °C). ^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.81 (dd, *J*=7.3, 2.4 Hz, 1H), 7.42 – 7.54 (m, 5H), 7.31 – 7.41 (m, 3H), 7.23 – 7.30 (m, 4H) ppm. ^{13}C NMR (101 MHz, $CDCl_3$) δ = 154.0, 144.8, 137.0, 132.8, 129.8, 129.7, 129.6, 129.3, 128.8, 128.2, 124.9, 123.2, 118.7, 116.7, 91.9 ppm. HRMS (ESI): m/z calcd $C_{19}H_{13}ClN_2$ for $[M+H]^+$ 305.0840, found 305.0837.

2-(7-(4-Methoxyphenyl)-2-phenyl-1*H*-benzo[*d*]imidazol-1-yl)benzonitrile 8j



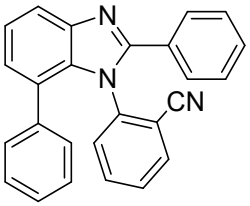
The crude product was purified by column chromatography (CH_2Cl_2:MeOH 40:1). A white solid (66 mg, 58%, m.p. 233 – 235 °C). ^{1}H NMR (500 MHz, $CDCl_3$) δ = 7.94 (dd, *J*=8.3, 1.2 Hz, 1H), 7.39 – 7.43 (m, 3H), 7.33 – 7.37 (m, 2H), 7.27 – 7.29 (m, 3H), 7.25 – 7.27 (m, 1H), 7.18 (dd, *J*=7.3, 1.0 Hz, 1H), 7.01 – 7.04 (m, 1H), 6.89 (d, *J*=6.9 Hz, 2H), 6.51 – 6.54 (m, 2H), 3.74 (s, 3H) ppm. ^{13}C NMR (126 MHz, $CDCl_3$) δ =158.3, 153.2, 143.7, 140.5, 133.7, 132.84, 132.78, 130.39, 130.30, 130.2, 129.7, 129.61, 129.58, 128.5, 128.3, 126.9, 126.3, 123.1, 119.4, 115.3, 113.3, 112.9, 55.3 ppm. HRMS (ESI): m/z calcd $C_{27}H_{19}N_3O$ for $[M+H]^+$ 402.1601, found 402.1600.

2-(2-Phenyl-7-(thiophen-3-yl)-1*H*-benzo[*d*]imidazol-1-yl)benzonitrile 8k



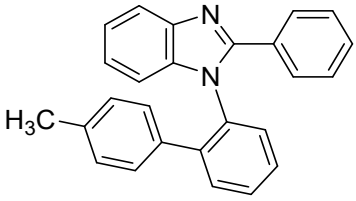
The crude product was purified by column chromatography (CH_2Cl_2:MeOH 80:1). A white solid (75 mg, 64%, m.p. 206 – 209 °C). ^{1}H NMR (500 MHz, $CDCl_3$) δ = 7.95 (dd, *J*=8.0, 1.2 Hz, 1H), 7.39 – 7.44 (m, 4H), 7.32 – 7.37 (m, 3H), 7.27 – 7.30 (m, 2H), 7.22 (dd, *J*=7.5, 1.2 Hz, 1H), 7.08 (dd, *J*=7.9, 1.3 Hz, 1H), 6.86 – 6.90 (m, 2H), 6.55 – 6.57 (m, 1H) ppm. ^{13}C NMR (126 MHz, $CDCl_3$) δ = 153.3, 143.7, 140.2, 137.5, 133.7, 133.0, 132.9, 129.8, 129.7, 129.6, 129.5, 128.7, 128.3, 126.4, 125.2, 124.1, 123.4, 123.0, 121.8, 119.9, 115.3, 113.0 ppm. HRMS (ESI): m/z calcd $C_{24}H_{15}N_3S$ for $[M+H]^+$ 378.1059, found 378.1059.

2-(2,7-Diphenyl-1*H*-benzo[*d*]imidazol-1-yl)benzonitrile 8l



The crude product was purified by column chromatography (CH_2Cl_2:MeOH 40:1). A white solid (75 mg, 57%, m.p. 188 – 190 °C). ^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.96 (dd, *J*=8.1, 1.1 Hz, 1H), 7.40 – 7.45 (m, 3H), 7.27 – 7.37 (m, 3H), 7.22 – 7.27 (m, 3H), 7.20 (dd, *J*=7.5, 1.1 Hz, 1H), 6.96 – 7.08 (m, 6H) ppm. ^{13}C NMR (126 MHz, $CDCl_3$) δ = 153.3, 143.8, 140.3, 137.2, 133.4, 132.9, 132.8, 130.1, 129.7, 129.6, 129.5, 129.3, 128.6, 128.3, 127.4, 127.2, 126.6, 126.1, 123.1, 119.6, 115.3, 113.2 ppm. HRMS (ESI): m/z calcd $C_{26}H_{17}N_3$ for $[M+H]^+$ 372.1495, found 372.1494.

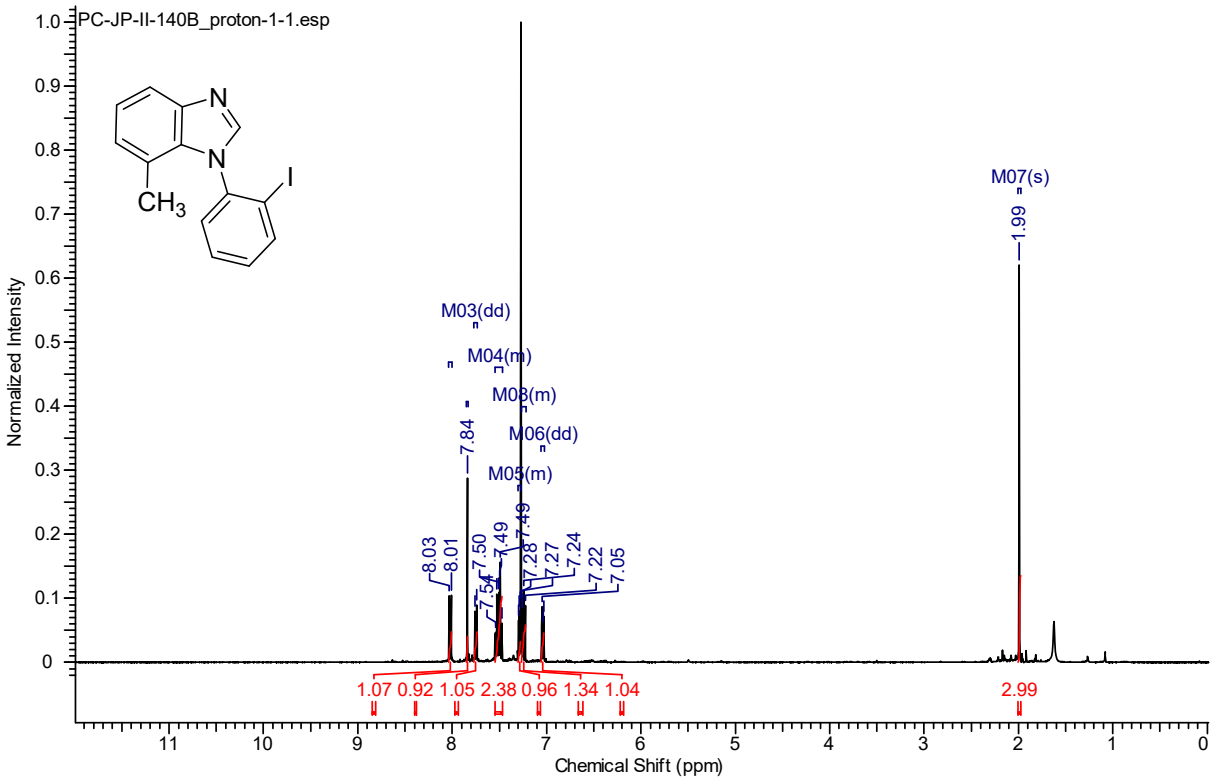
1-(4'-Methyl-[1,1'-biphenyl]-2-yl)-2-phenyl-1*H*-benzo[*d*]imidazole 11b

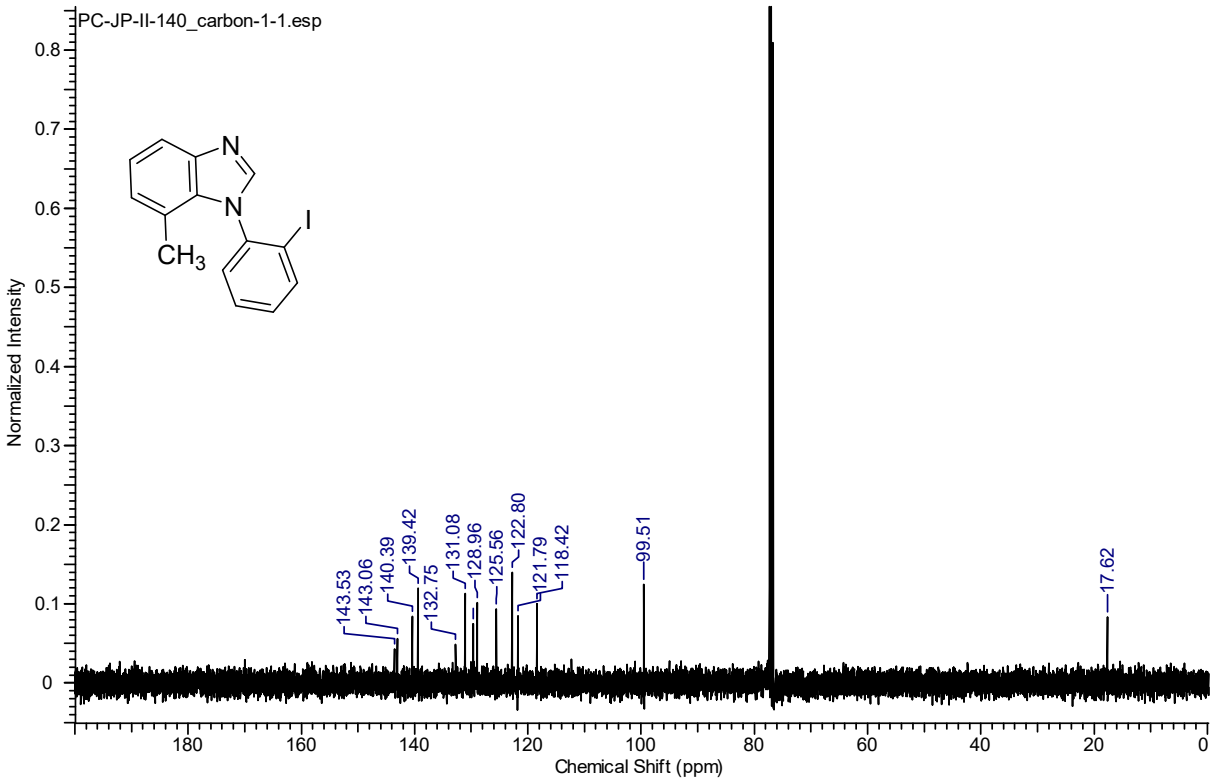


The crude product was purified by column chromatography (CH_2Cl_2). A light brown solid (37 mg, 64%, m.p. 131 – 134 °C). ^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.84 – 7.87 (m, 1H), 7.62 – 7.65 (m, 1H), 7.53 – 7.60 (m, 2H), 7.41 – 7.45 (m, 1H), 7.32 – 7.37 (m, 1H), 7.28 – 7.32 (m, 2H), 7.22 – 7.27 (m, 1H), 7.11 – 7.14 (m, 4H), 6.75 (dd, *J*=7.9, 0.6 Hz, 2H), 6.32 (d, *J*=8.2 Hz, 2H), 2.21 (s, 3H) ppm. ^{13}C NMR (101 MHz, $CDCl_3$) δ = 152.4, 142.6, 139.9, 137.2, 136.6, 134.3, 134.0, 131.3, 129.7, 129.0, 128.6, 128.5, 128.14, 128.10, 128.08, 127.4, 127.1, 122.9, 122.4, 119.4, 110.2, 20.6 ppm. HRMS (ESI): m/z calcd $C_{26}H_{20}N_2$ for $[M+H]^+$ 361.1699, found 361.1700.

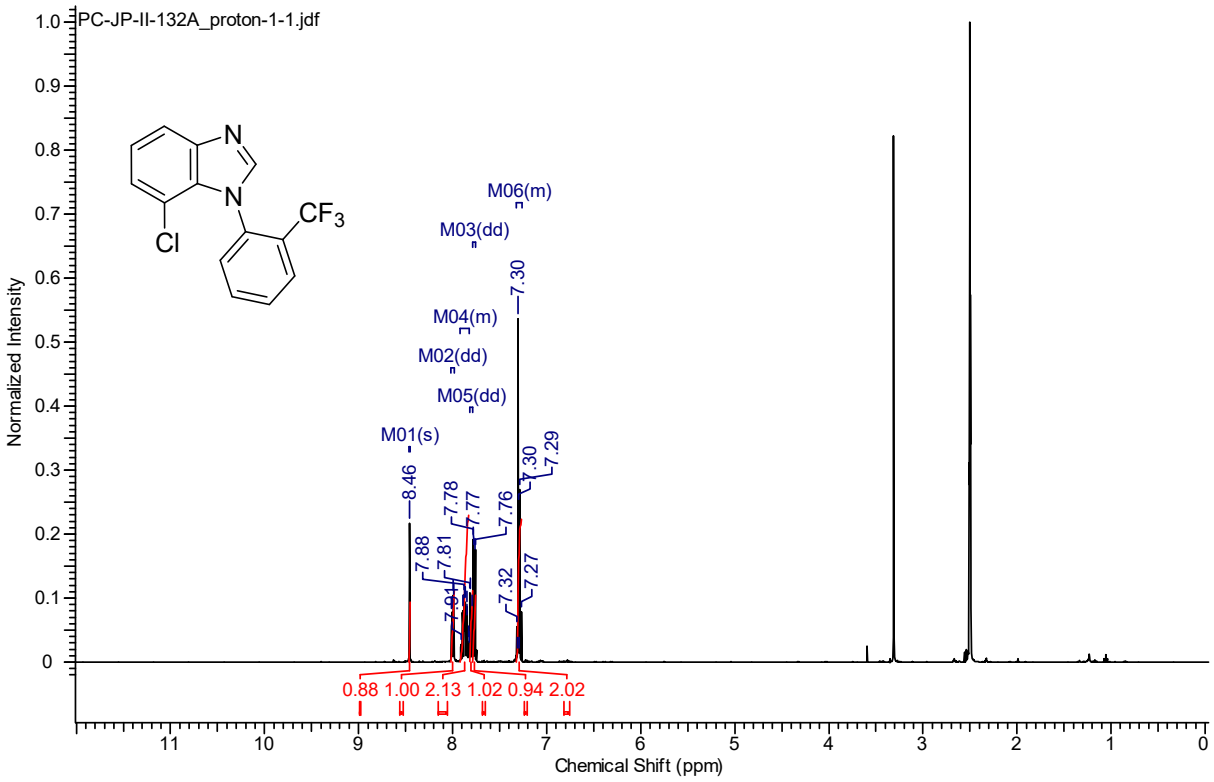
Copies of NMR spectra of the final compounds

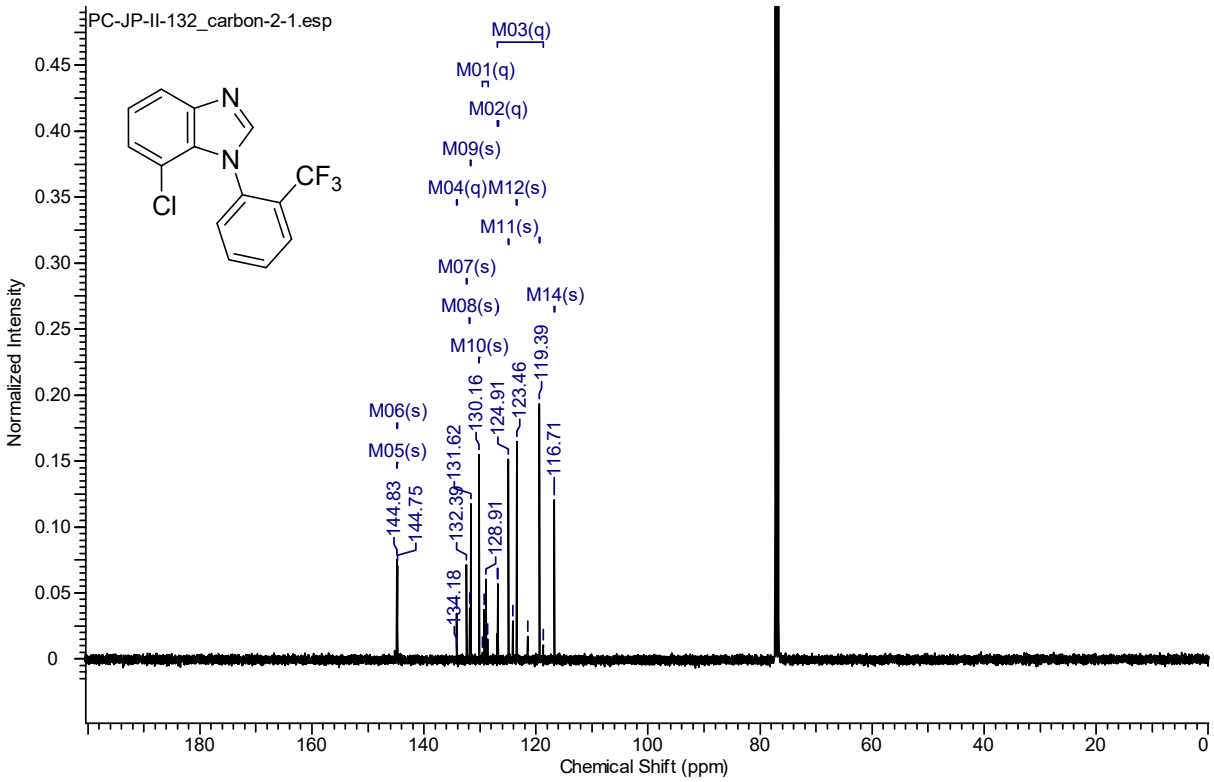
^{1}H and ^{13}C NMR of 5a ($CDCl_3$):



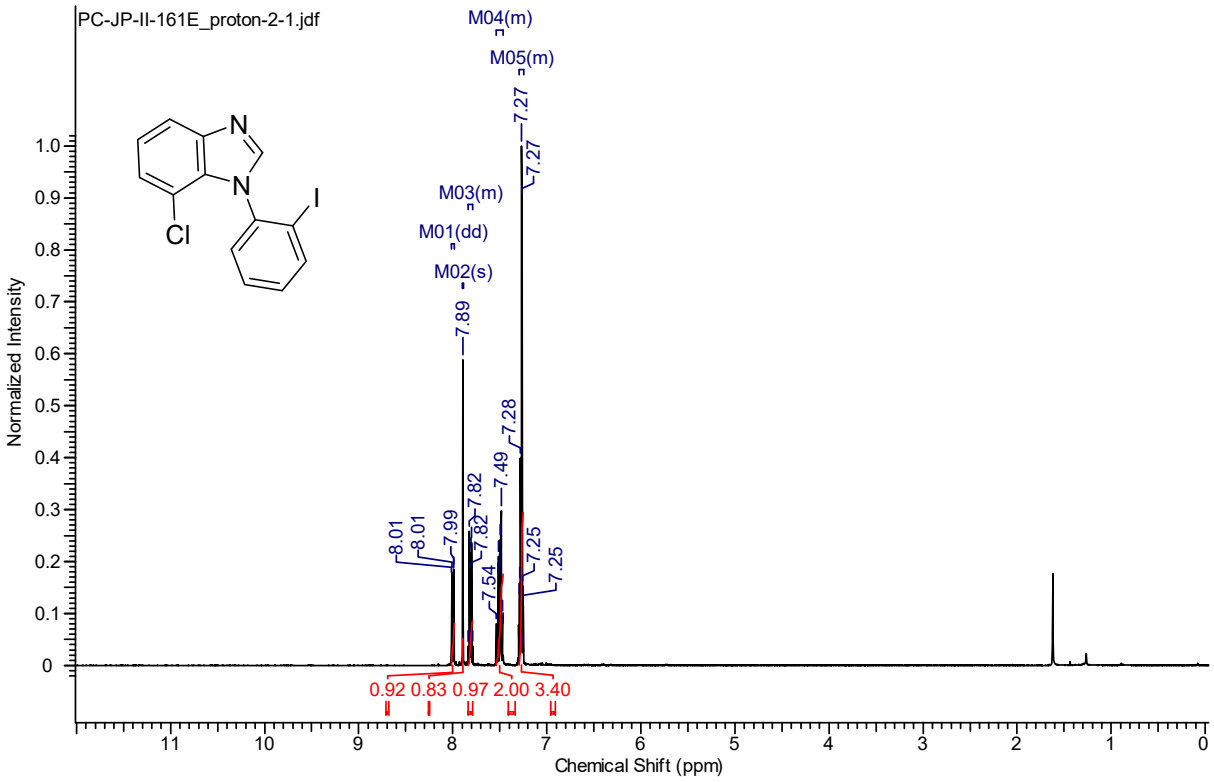


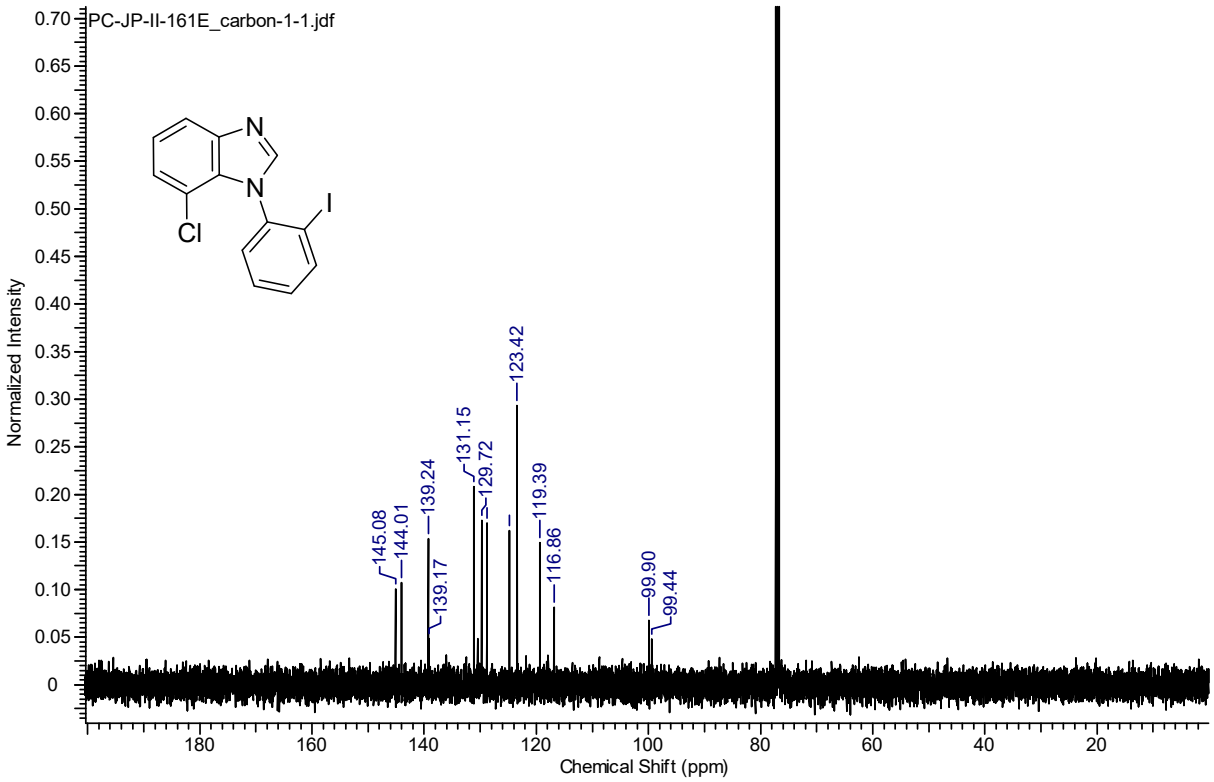
^{1}H (DMSO-d_6) and ^{13}C NMR ($CDCl_3$) of 5b:



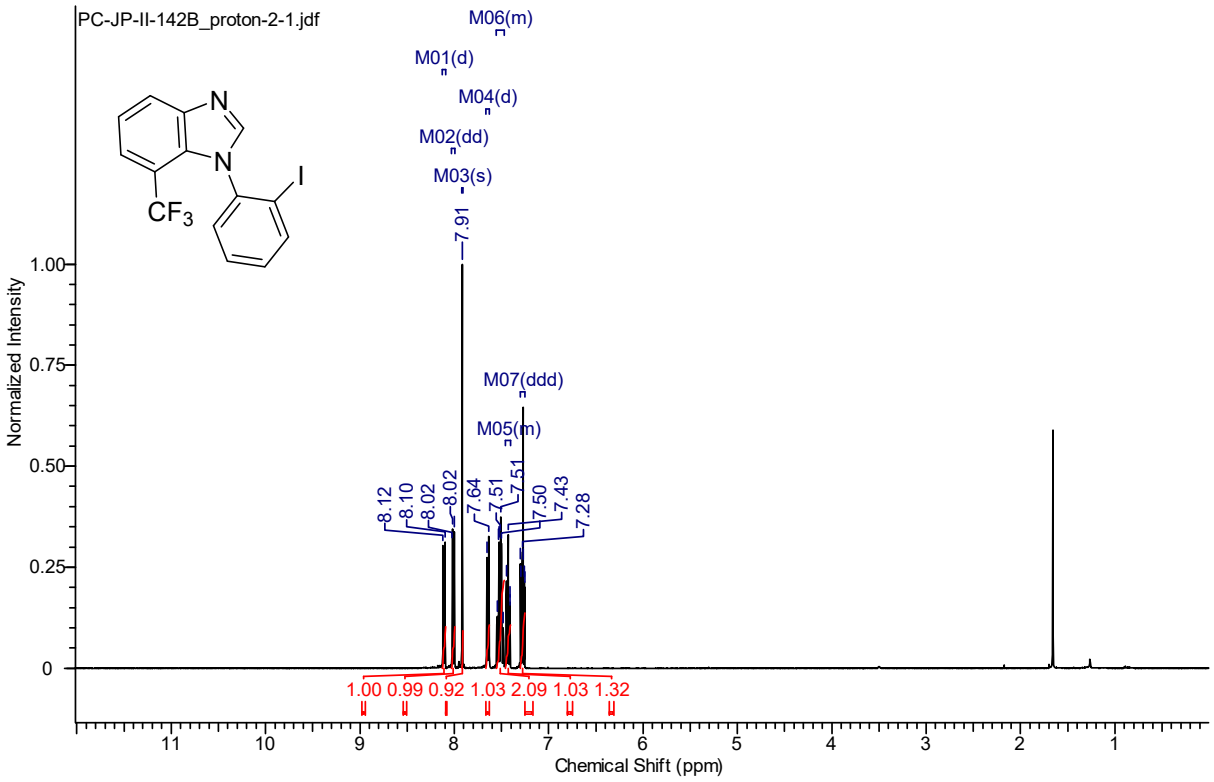


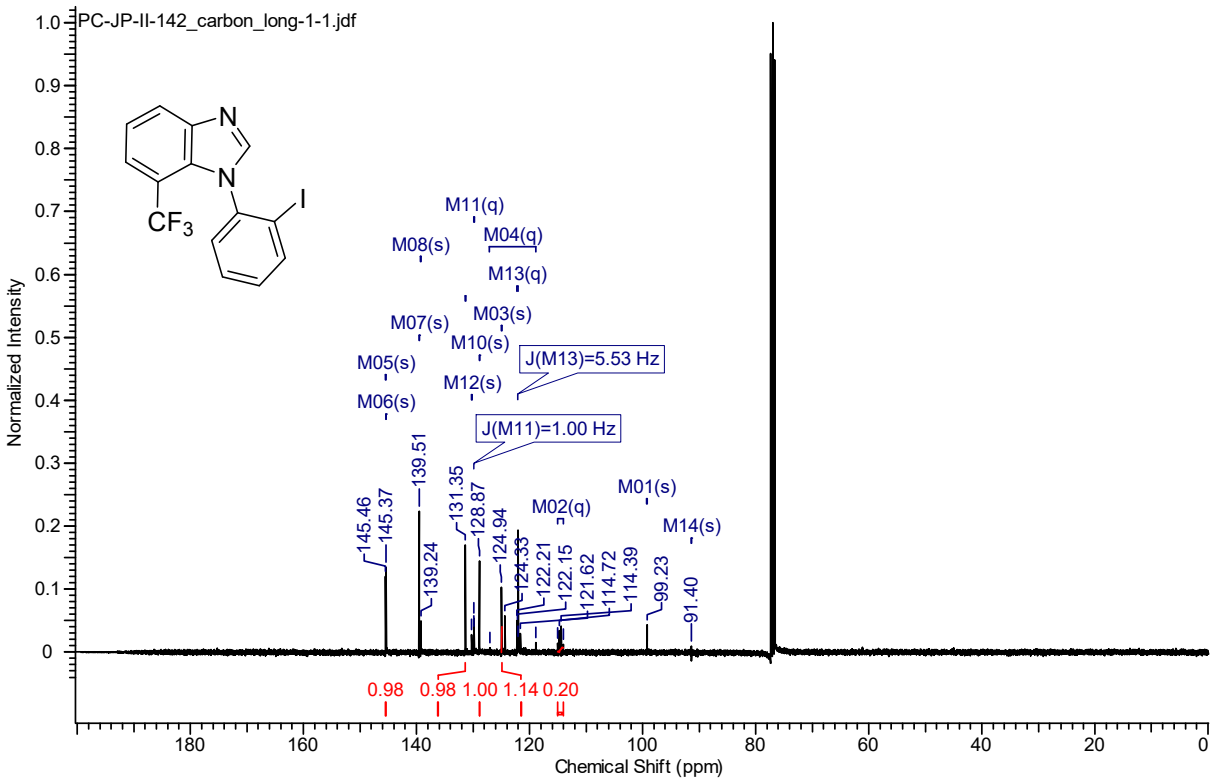
^{1}H and ^{13}C NMR of 5c ($CDCl_3$):



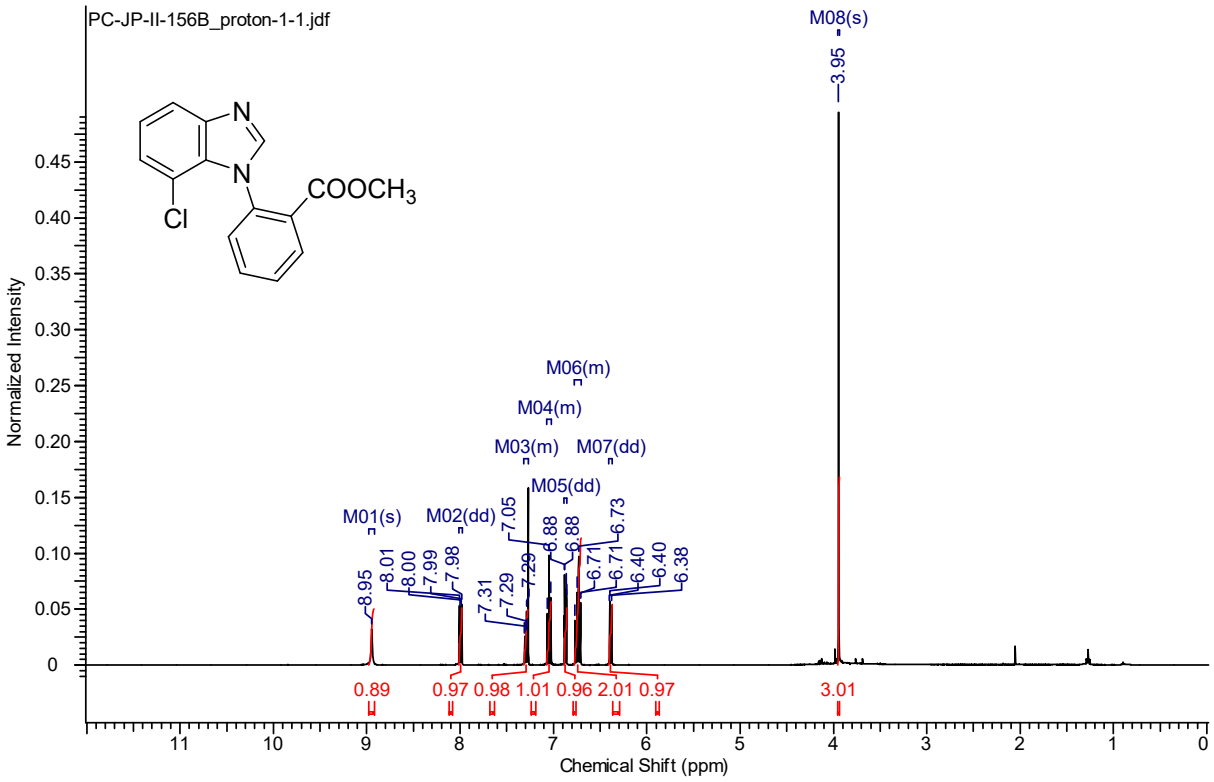


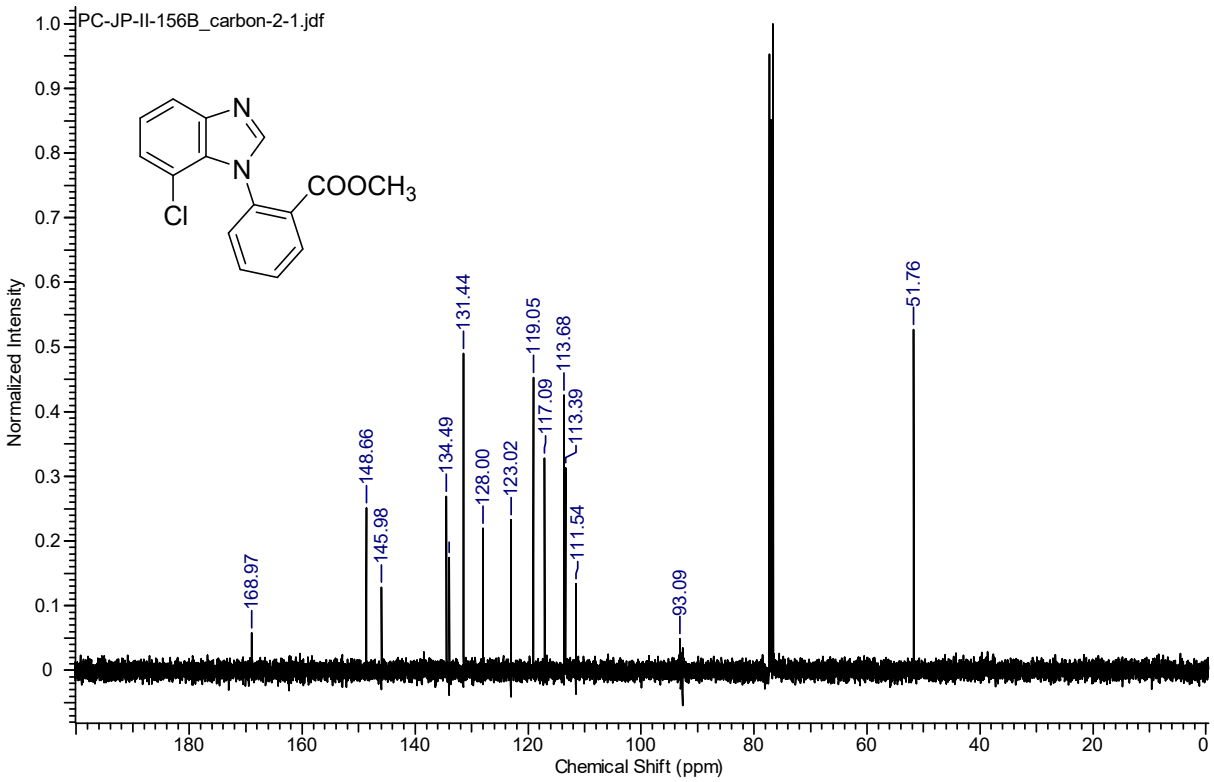
^{1}H and ^{13}C NMR of 5d ($CDCl_3$):



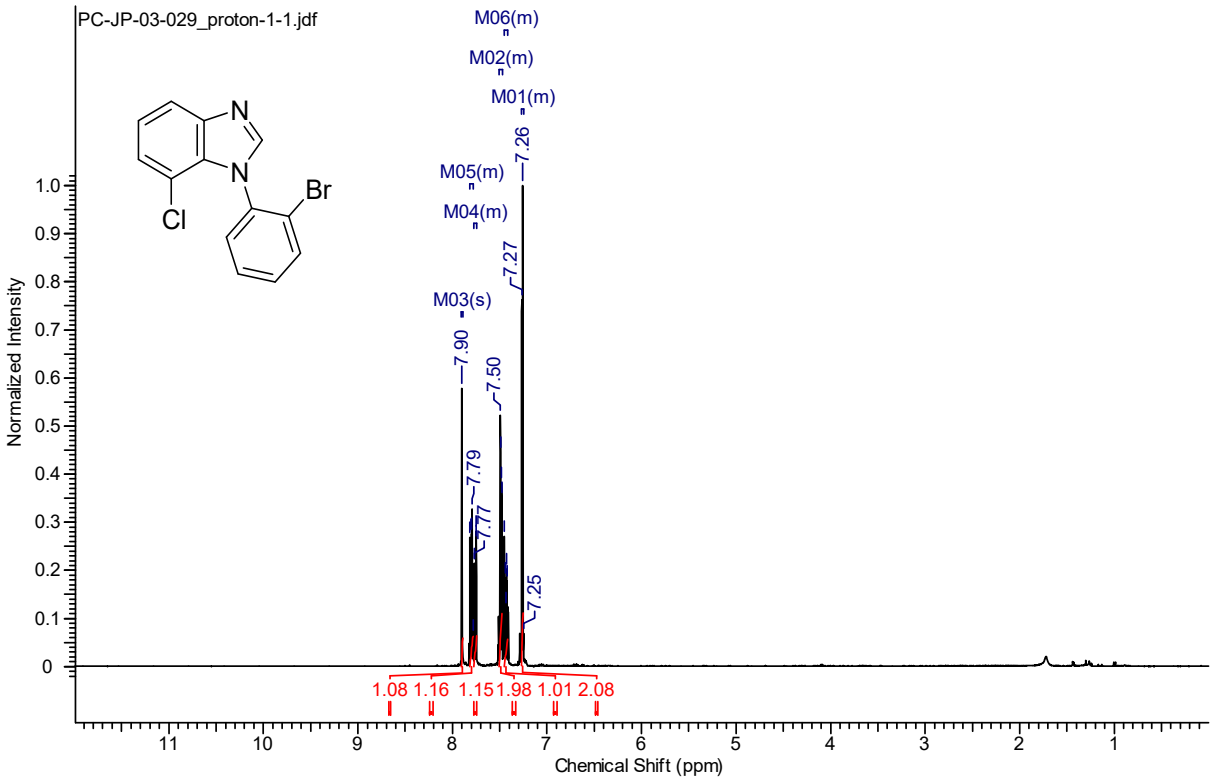


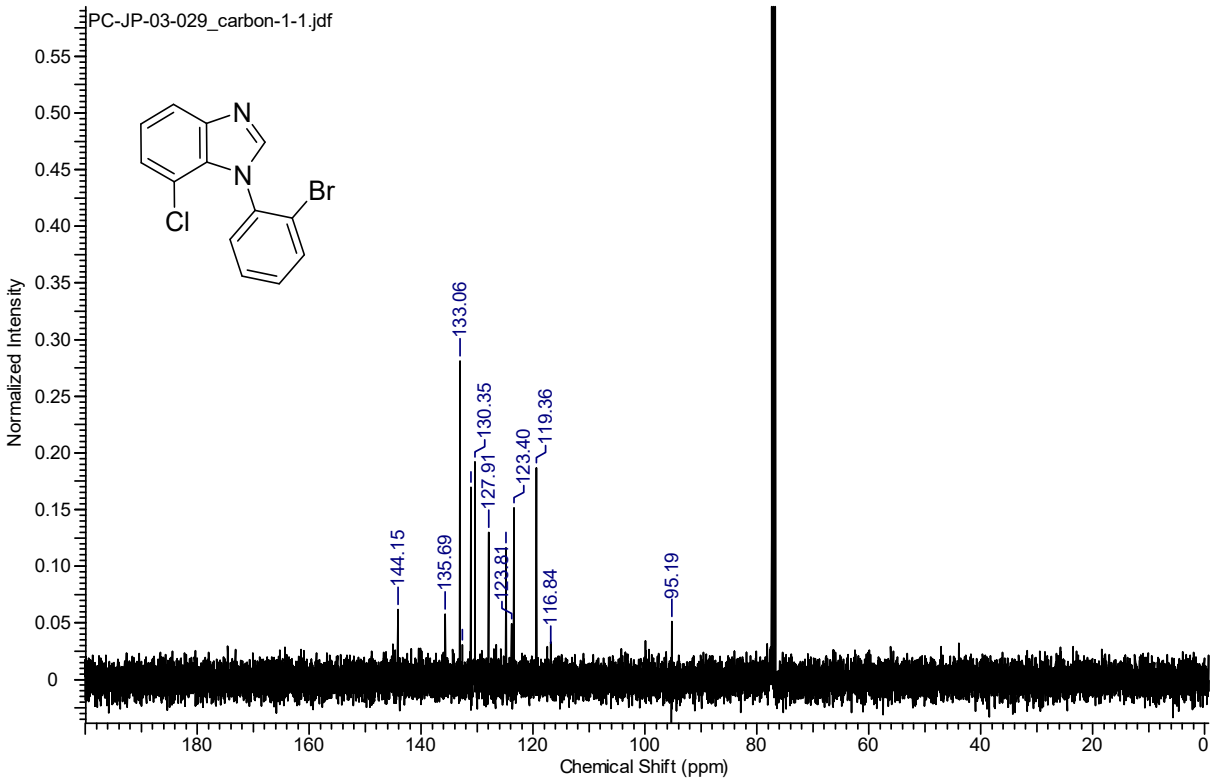
^{1}H and ^{13}C NMR of 5e ($CDCl_3$):



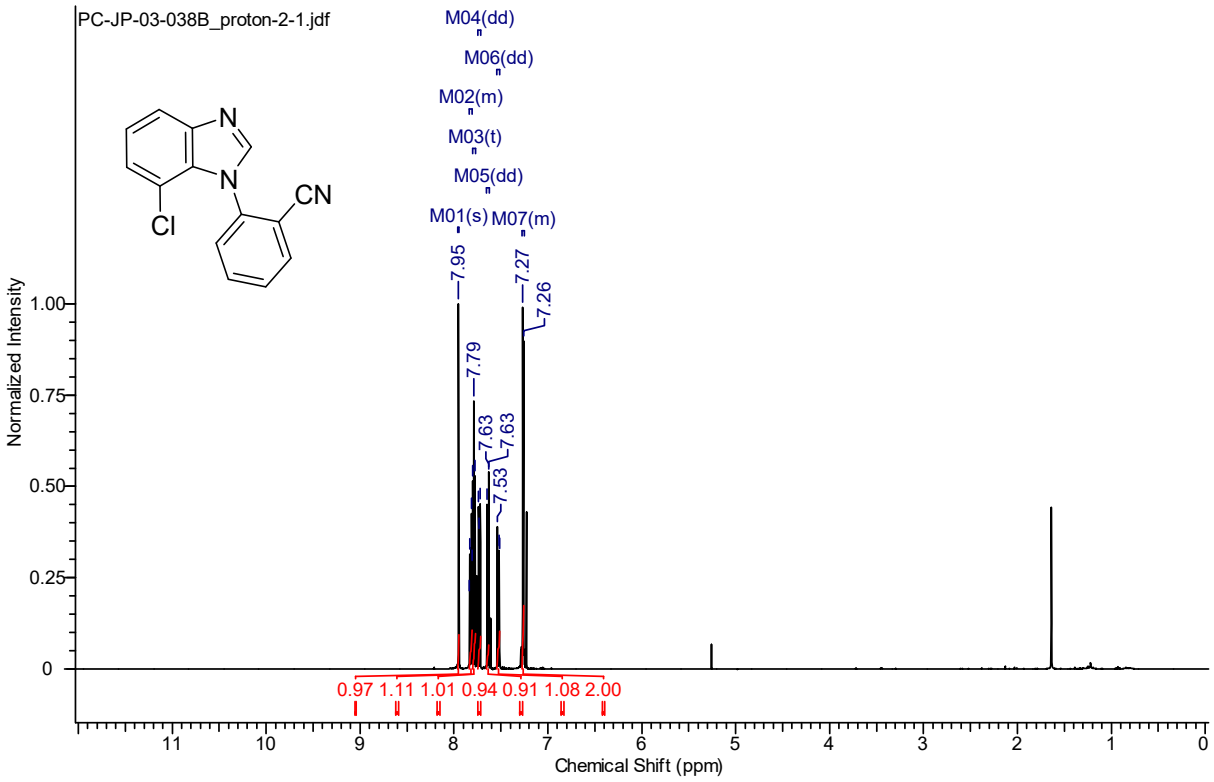


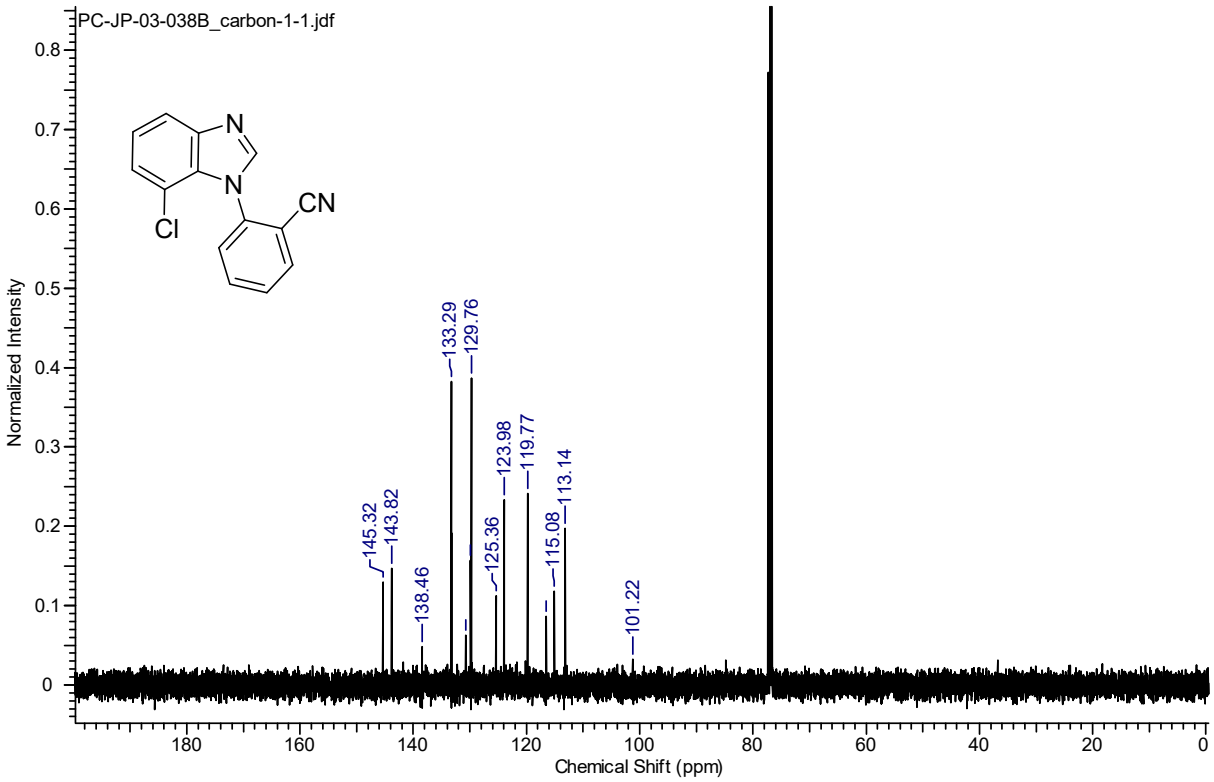
^{1}H and ^{13}C NMR of 5f ($CDCl_3$):



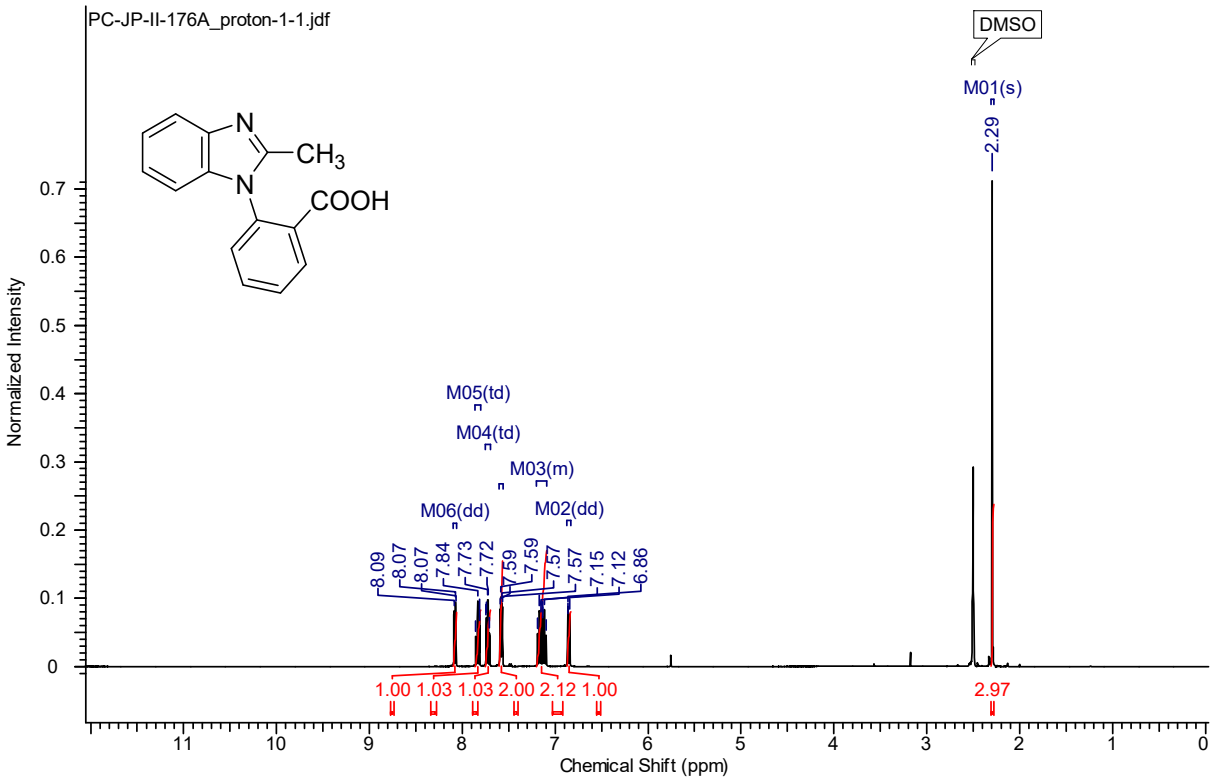


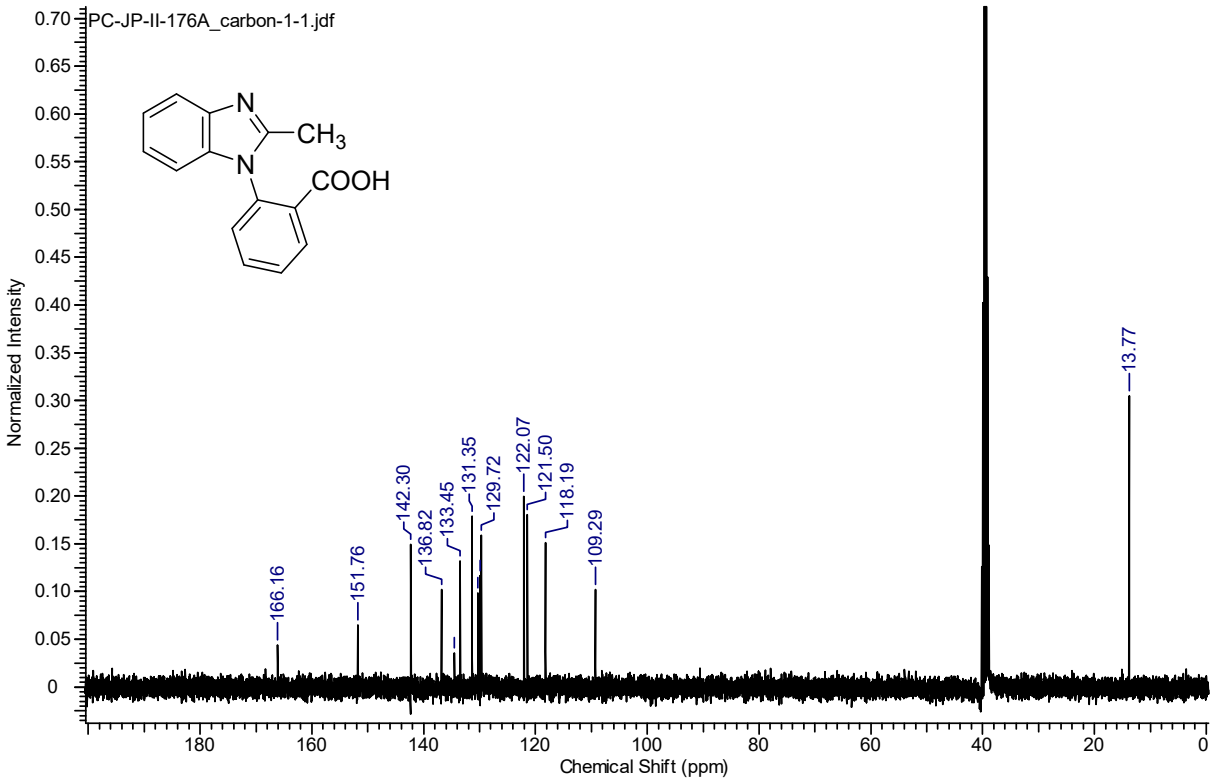
^{1}H and ^{13}C NMR of 5g ($CDCl_3$):



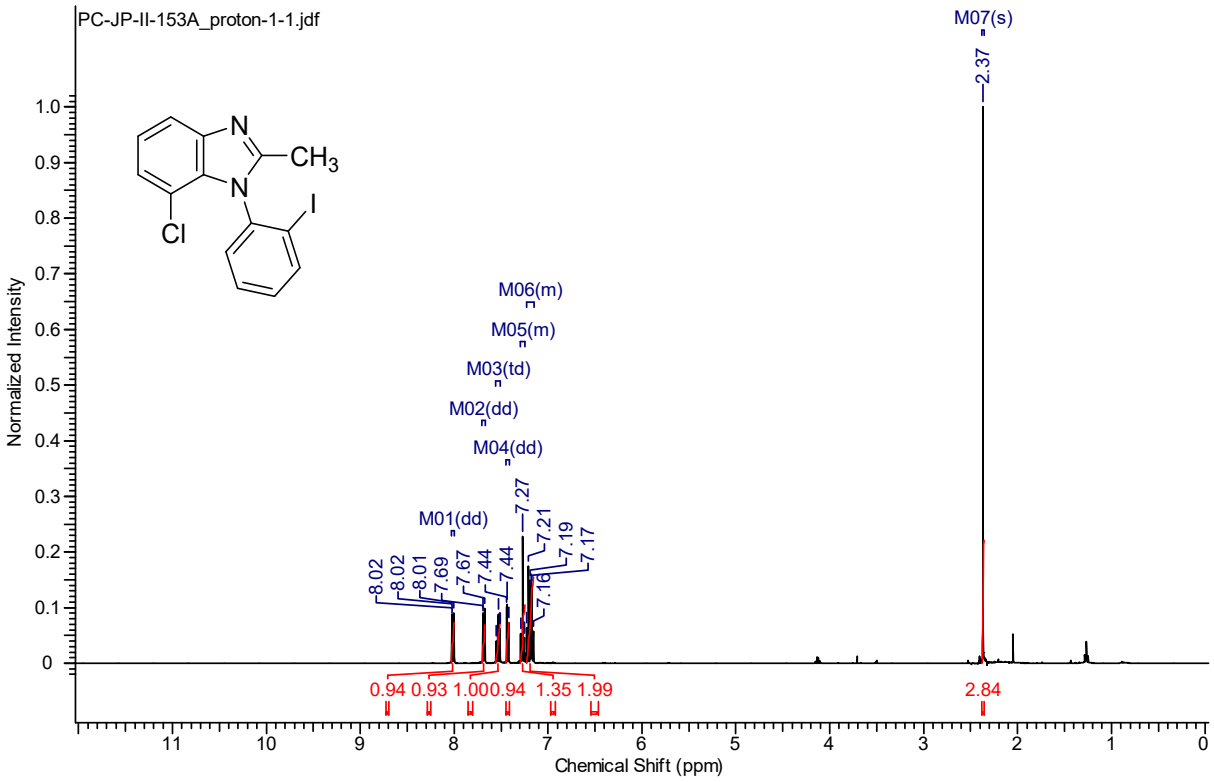


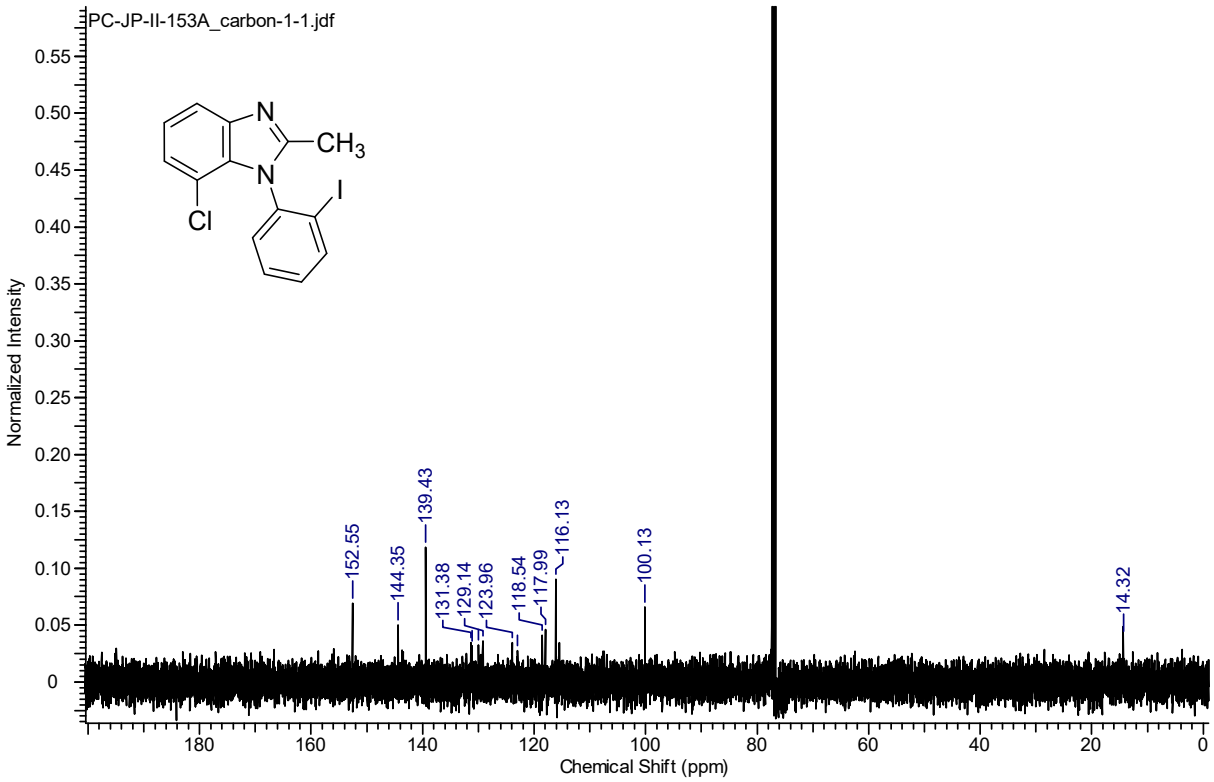
^{1}H and ^{13}C NMR of 5h (DMSO-d_6):



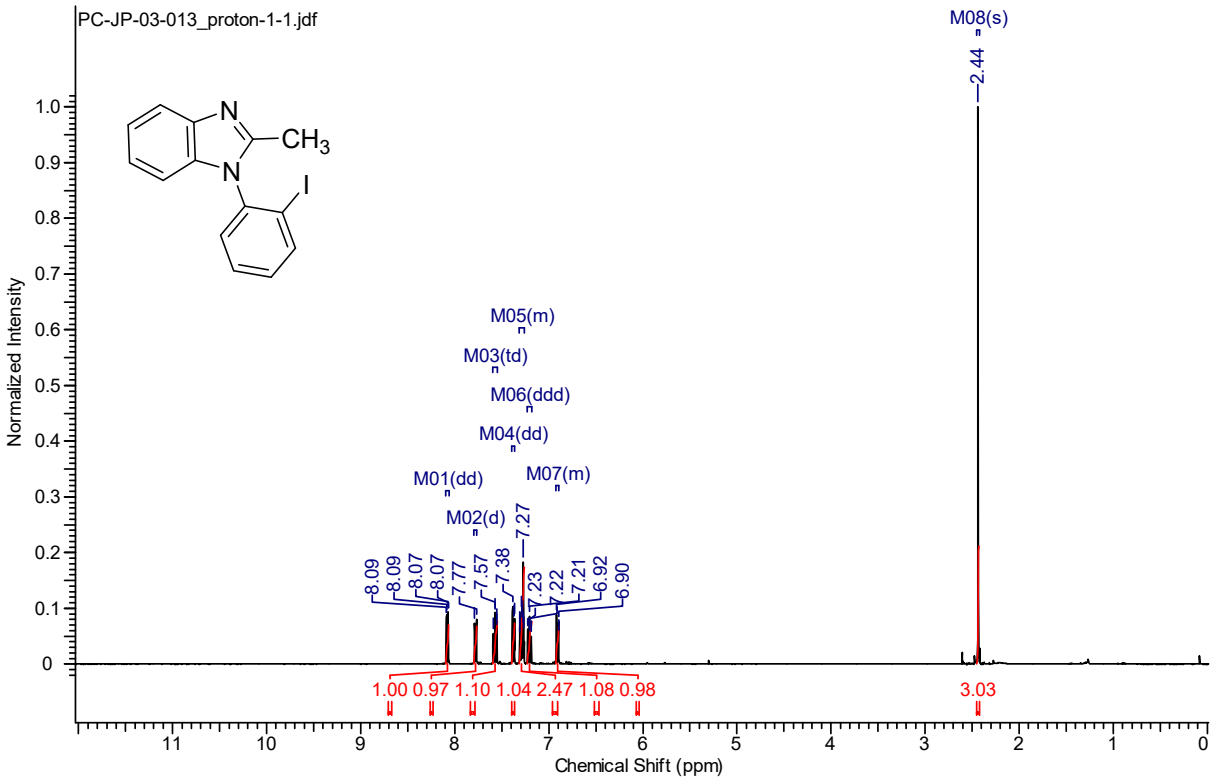


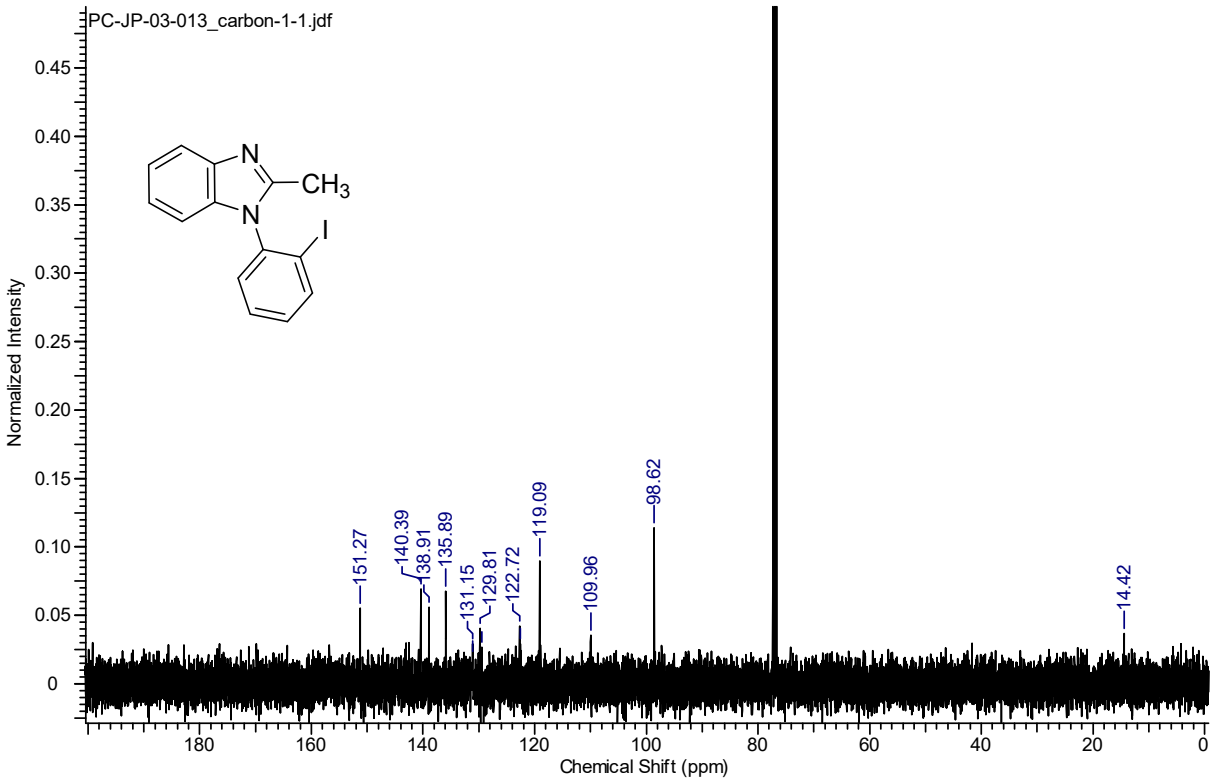
^{1}H and ^{13}C NMR of 5i ($CDCl_3$):



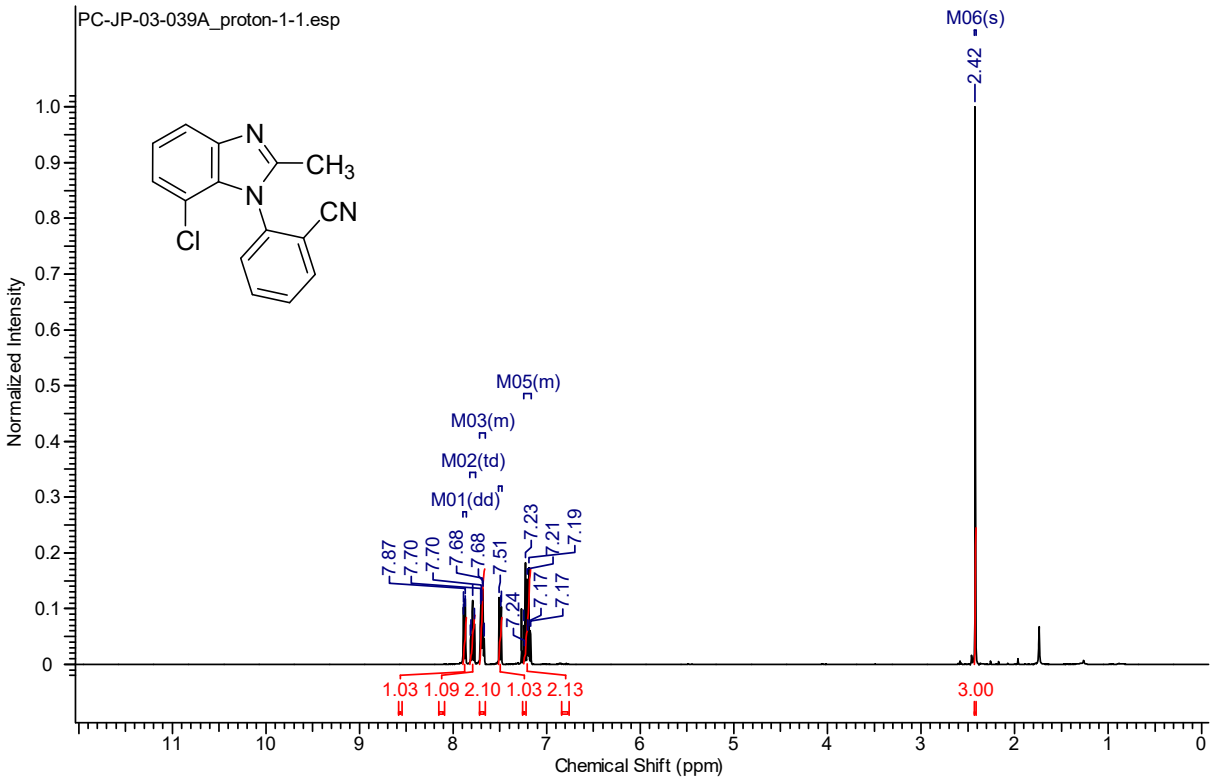


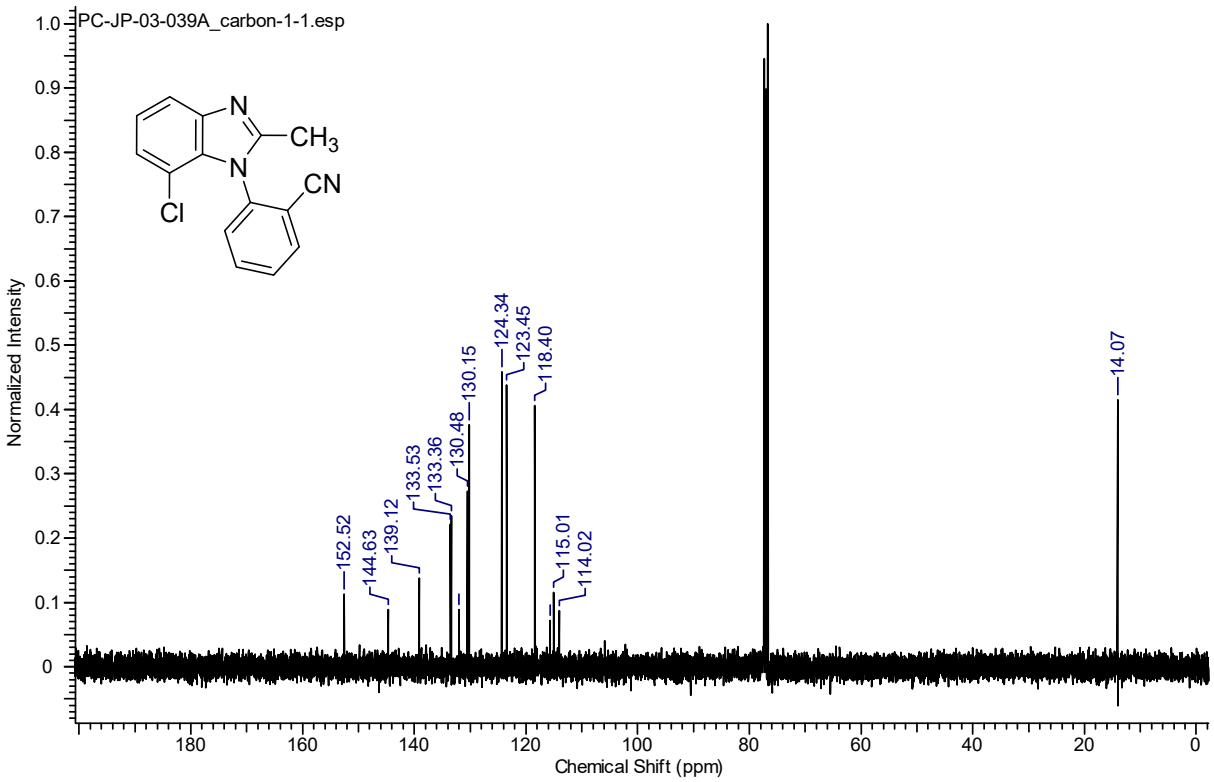
^{1}H and ^{13}C NMR of 5j ($CDCl_3$):



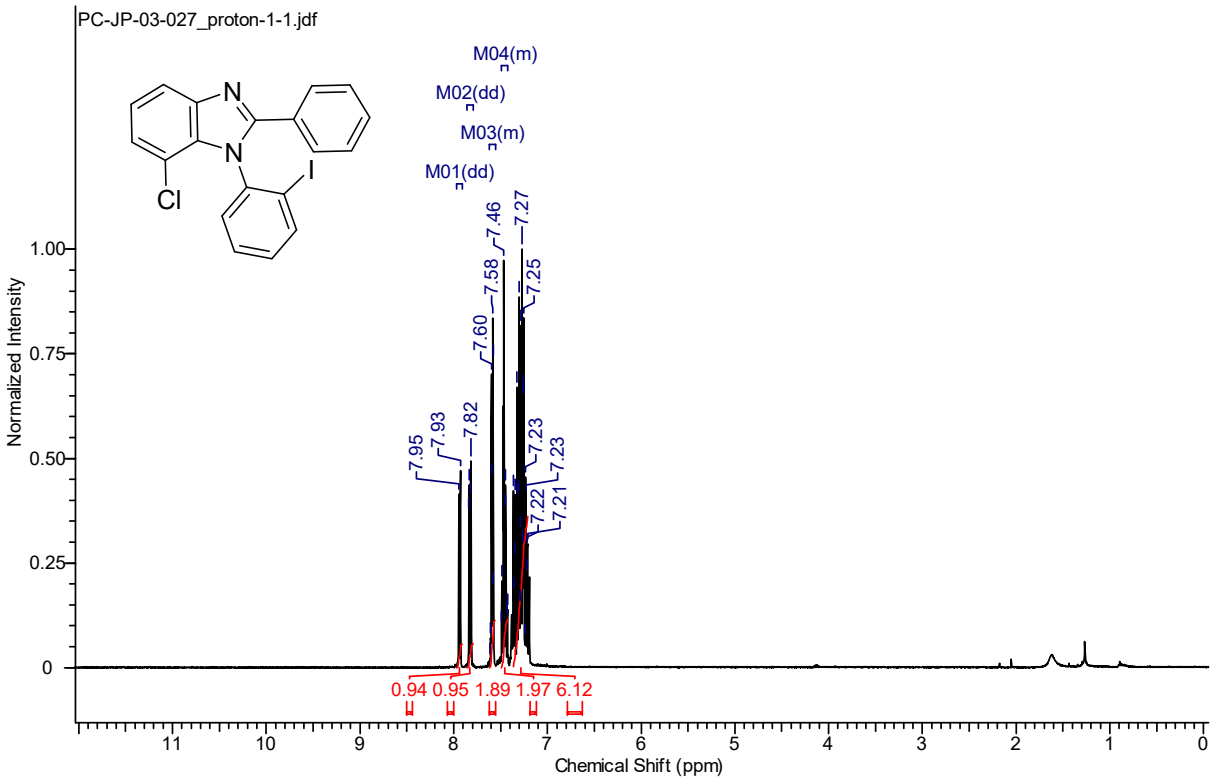


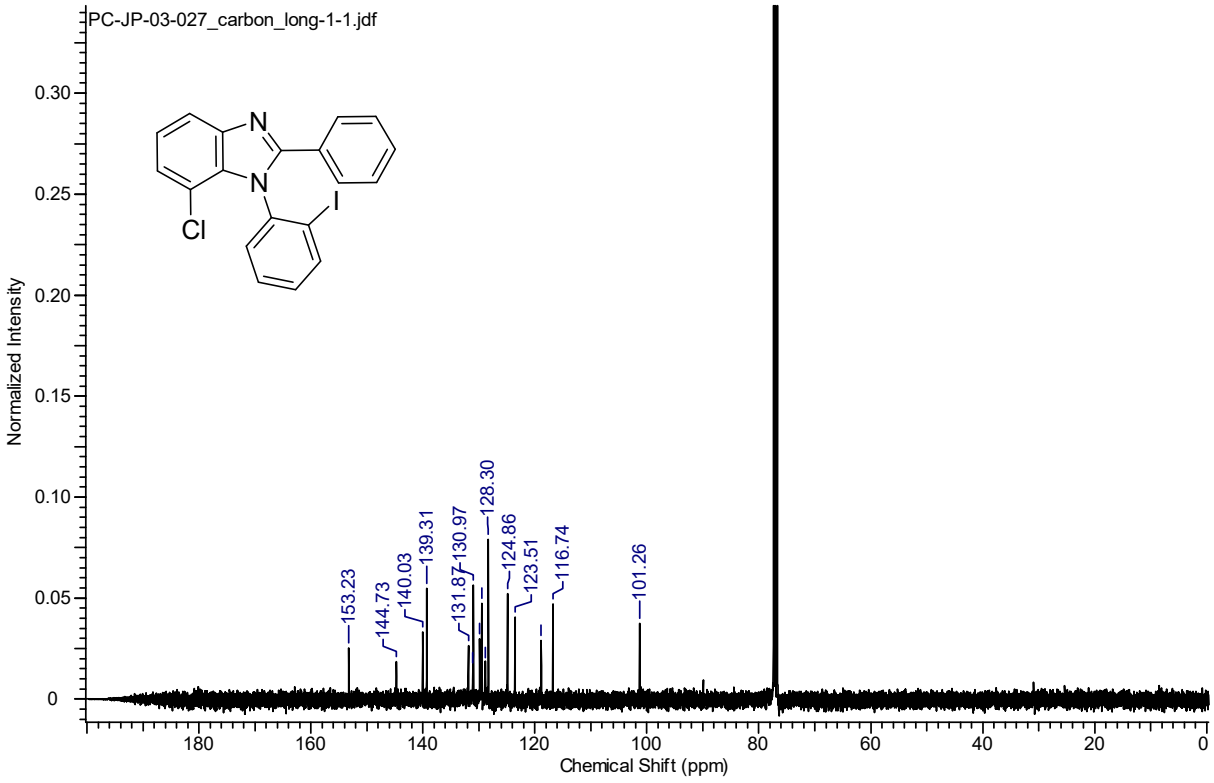
^{1}H and ^{13}C NMR of 5k ($CDCl_3$):



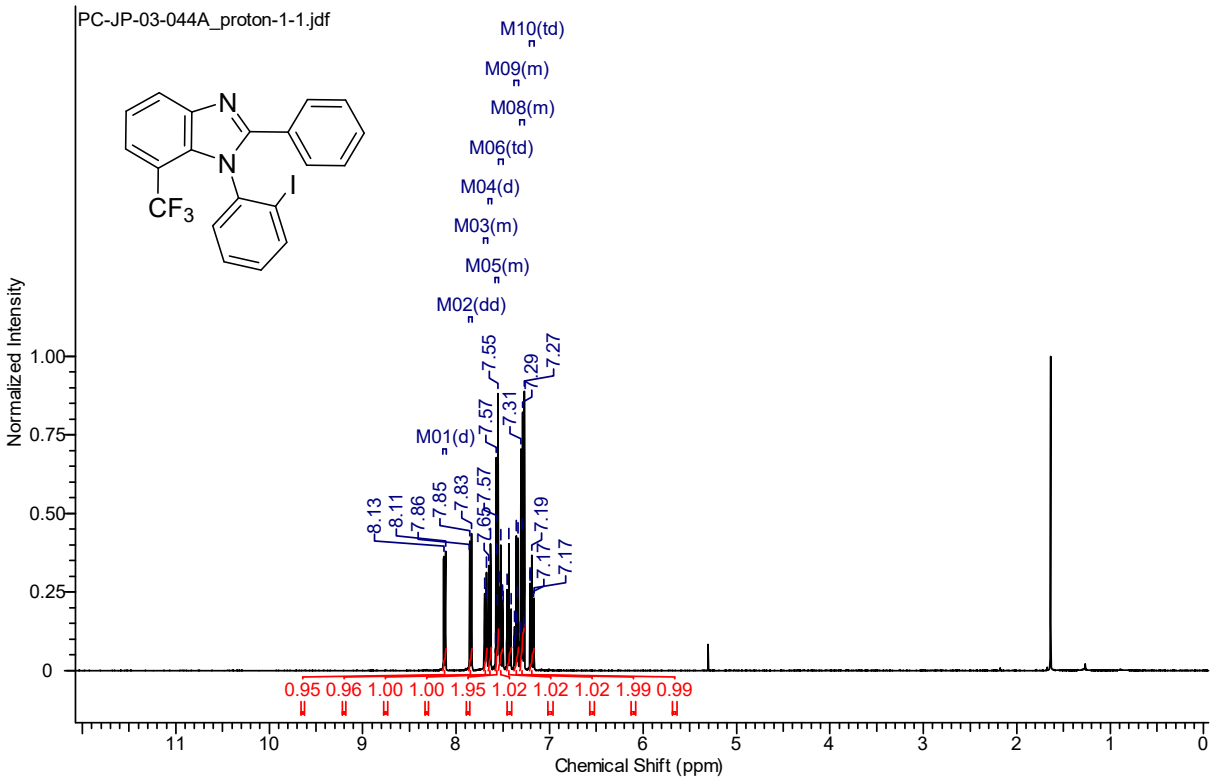


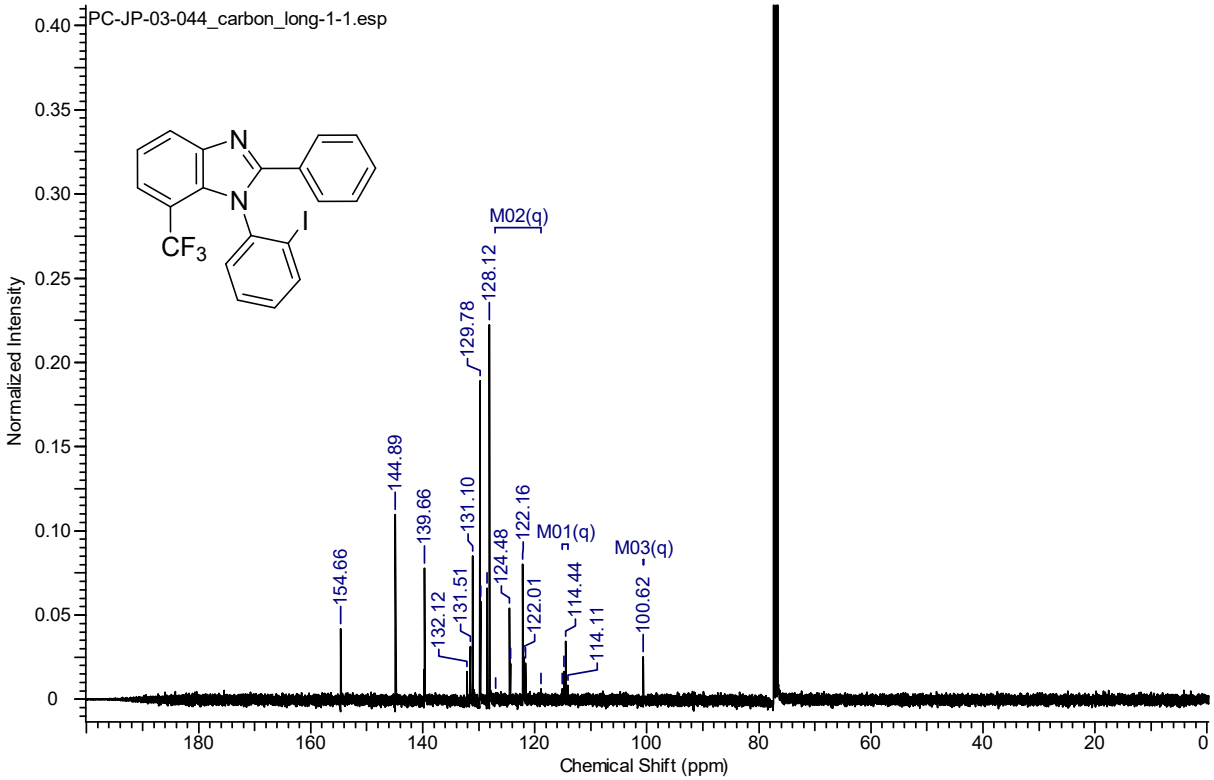
^{1}H and ^{13}C NMR of 5l ($CDCl_3$):



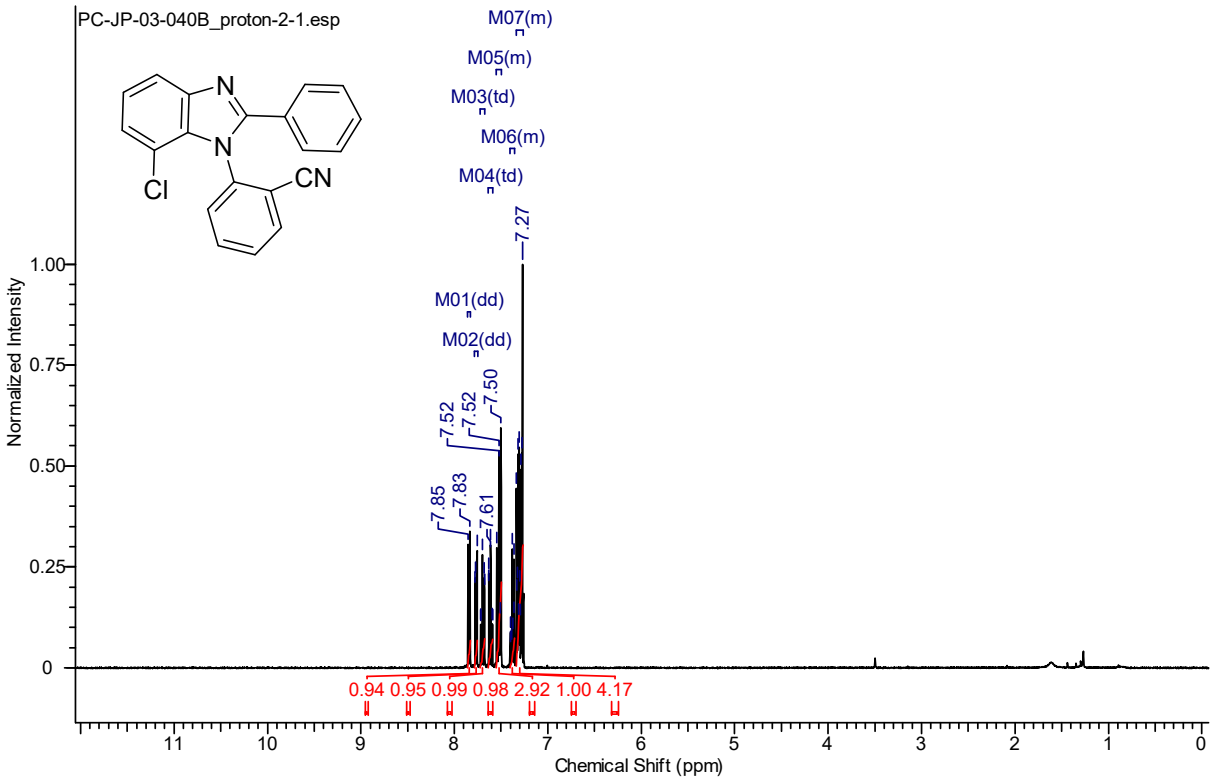


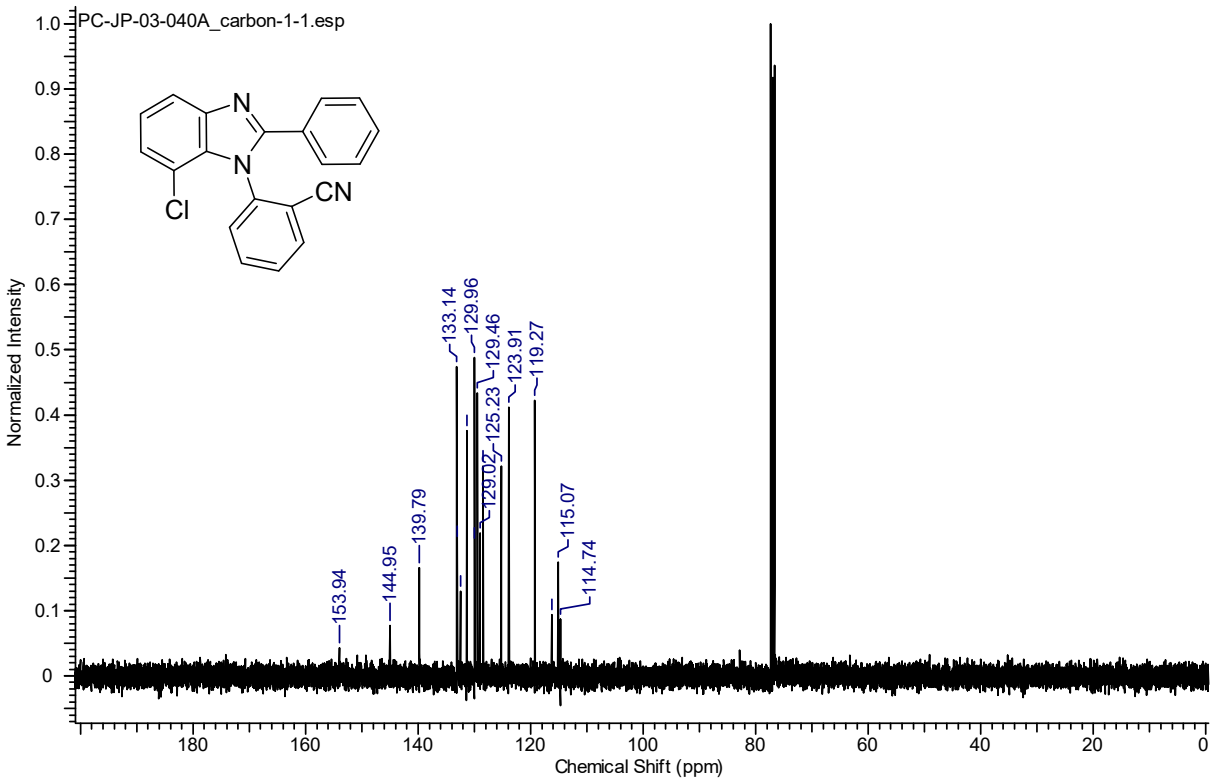
^{1}H and ^{13}C NMR of 5m ($CDCl_3$):



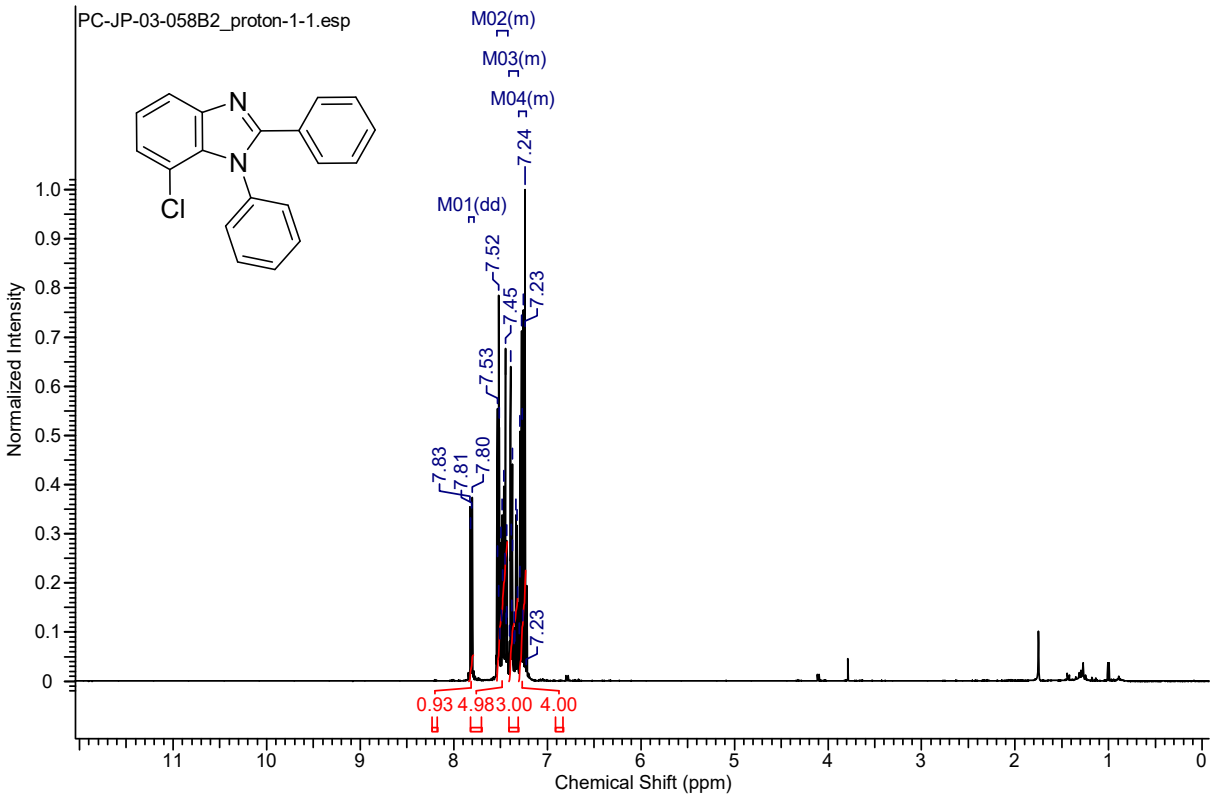


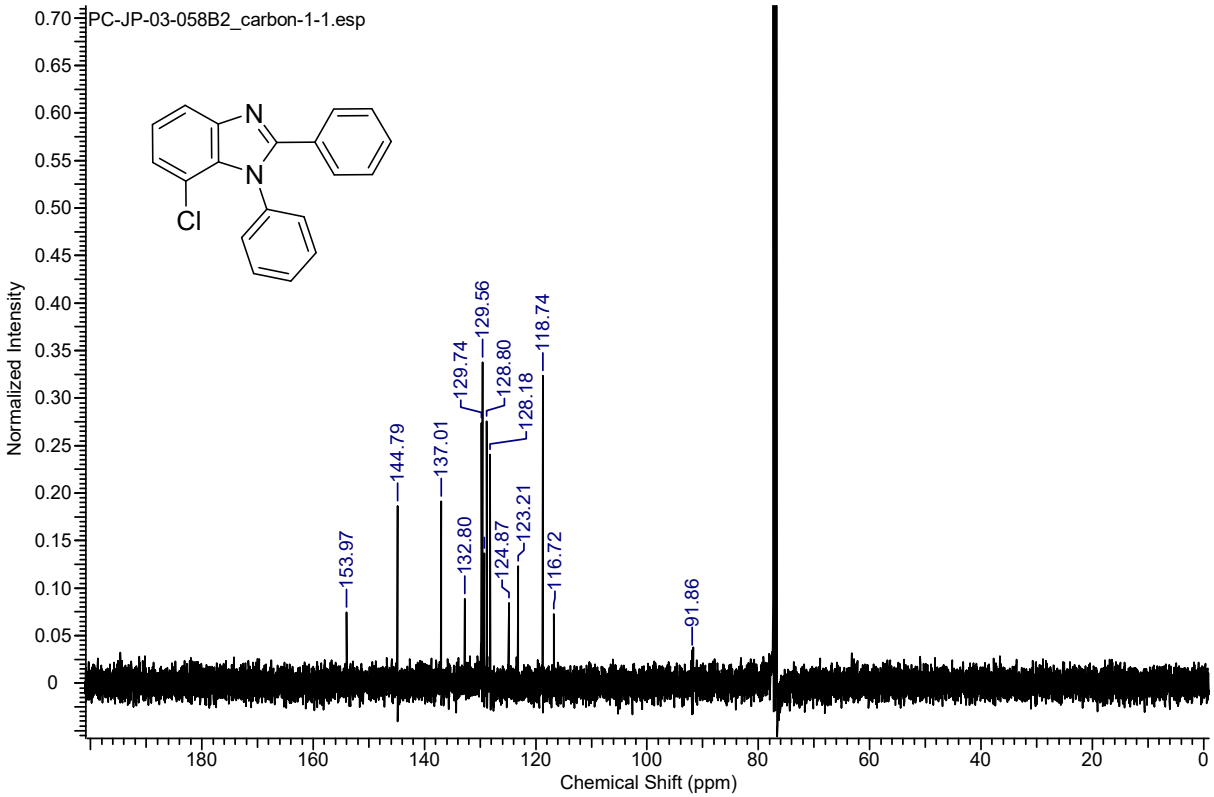
^{1}H and ^{13}C NMR of 5n ($CDCl_3$):



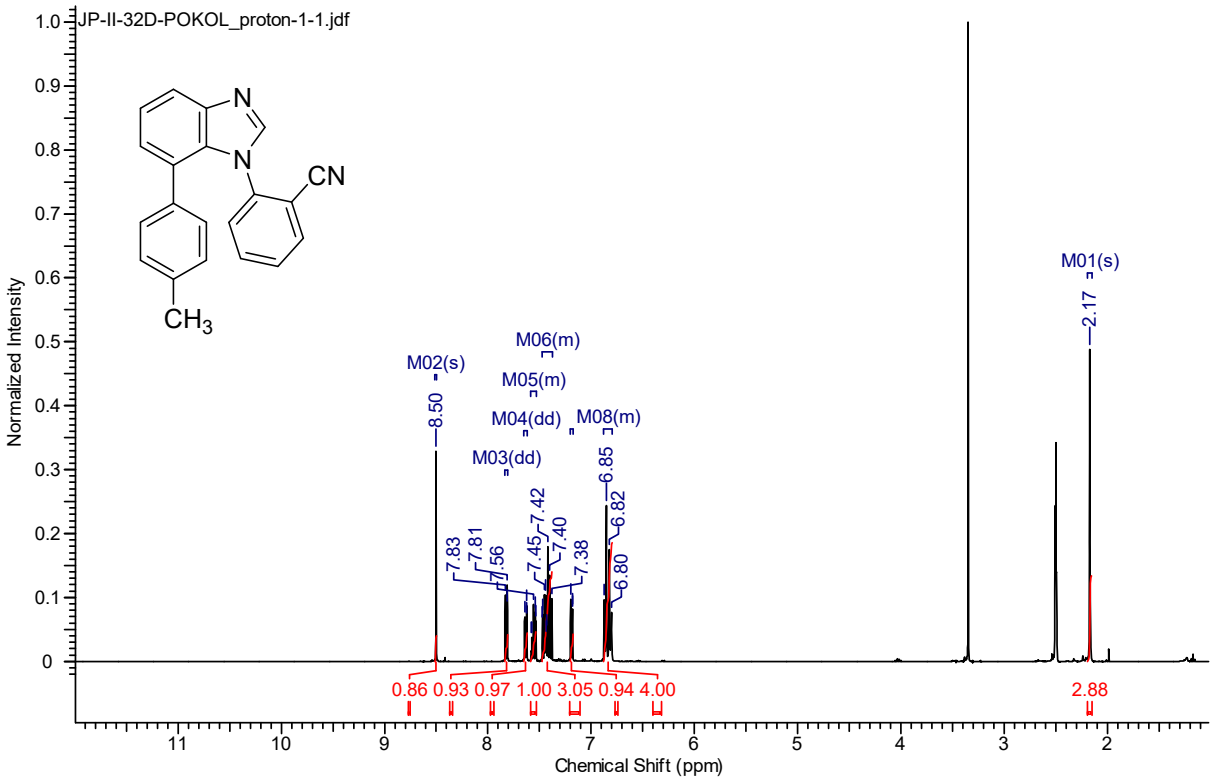


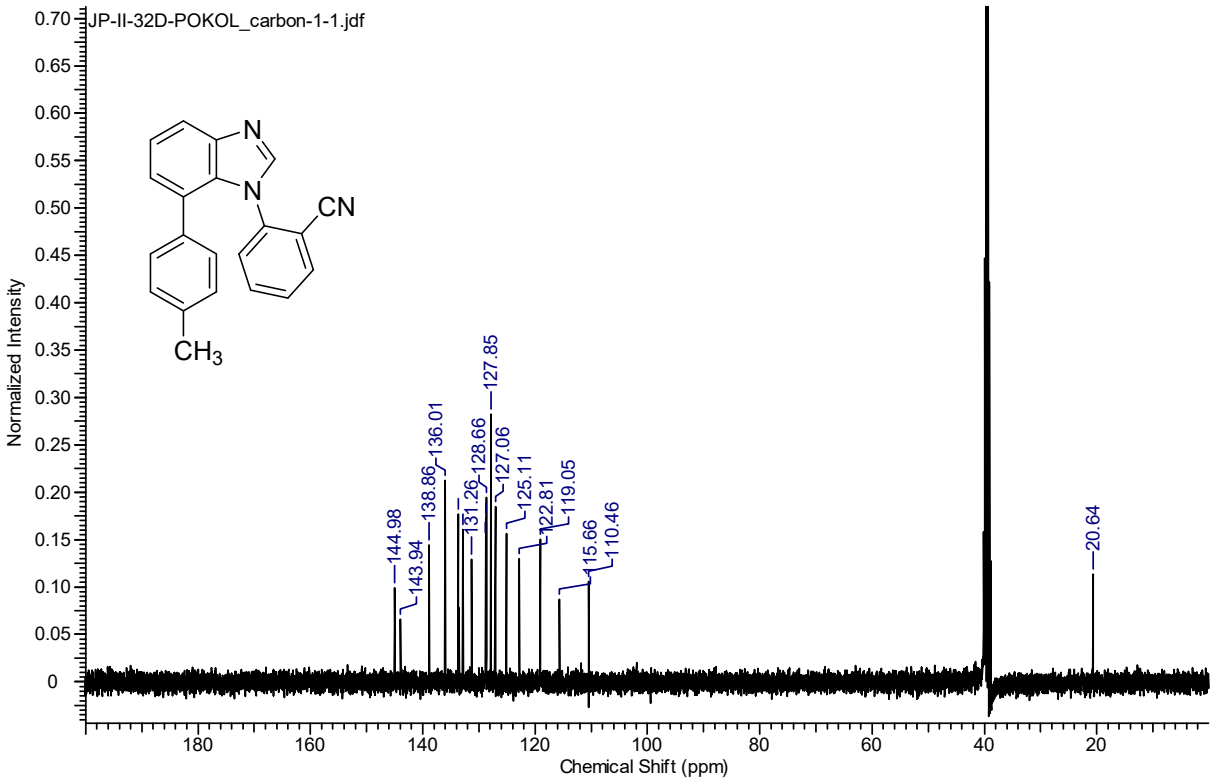
^{1}H and ^{13}C NMR of 5o ($CDCl_3$):



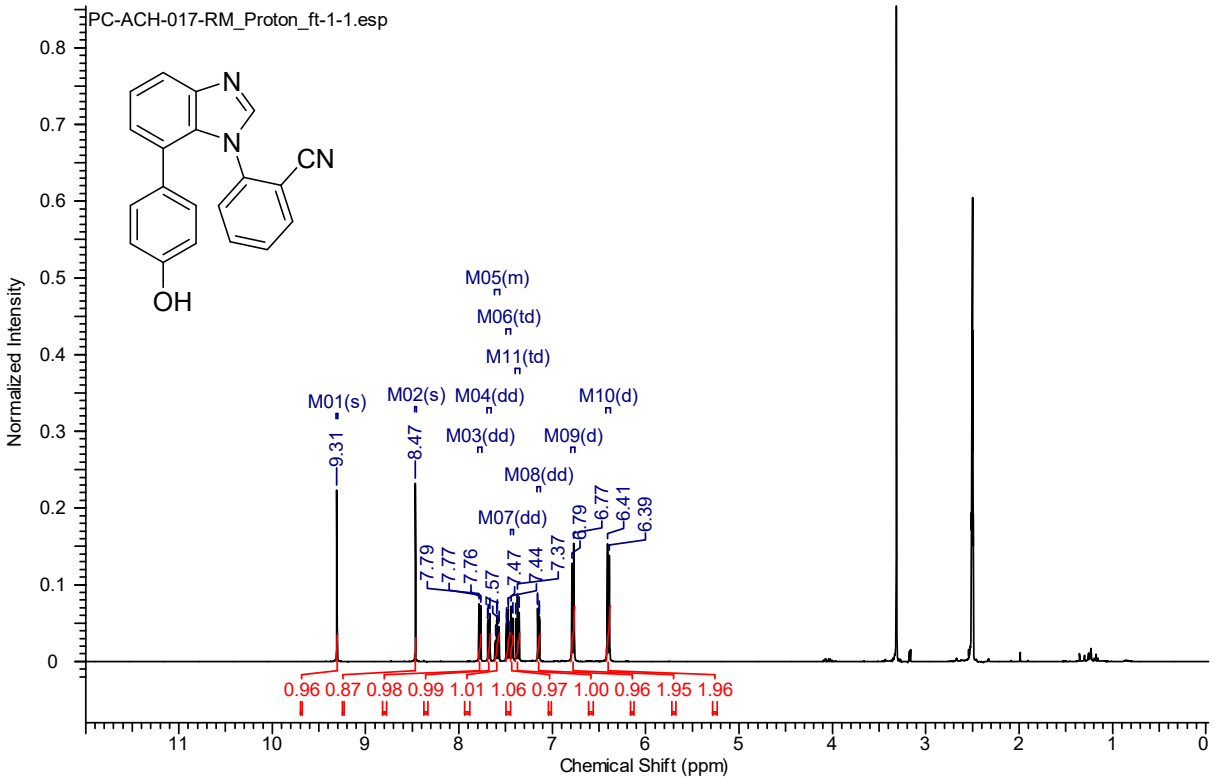


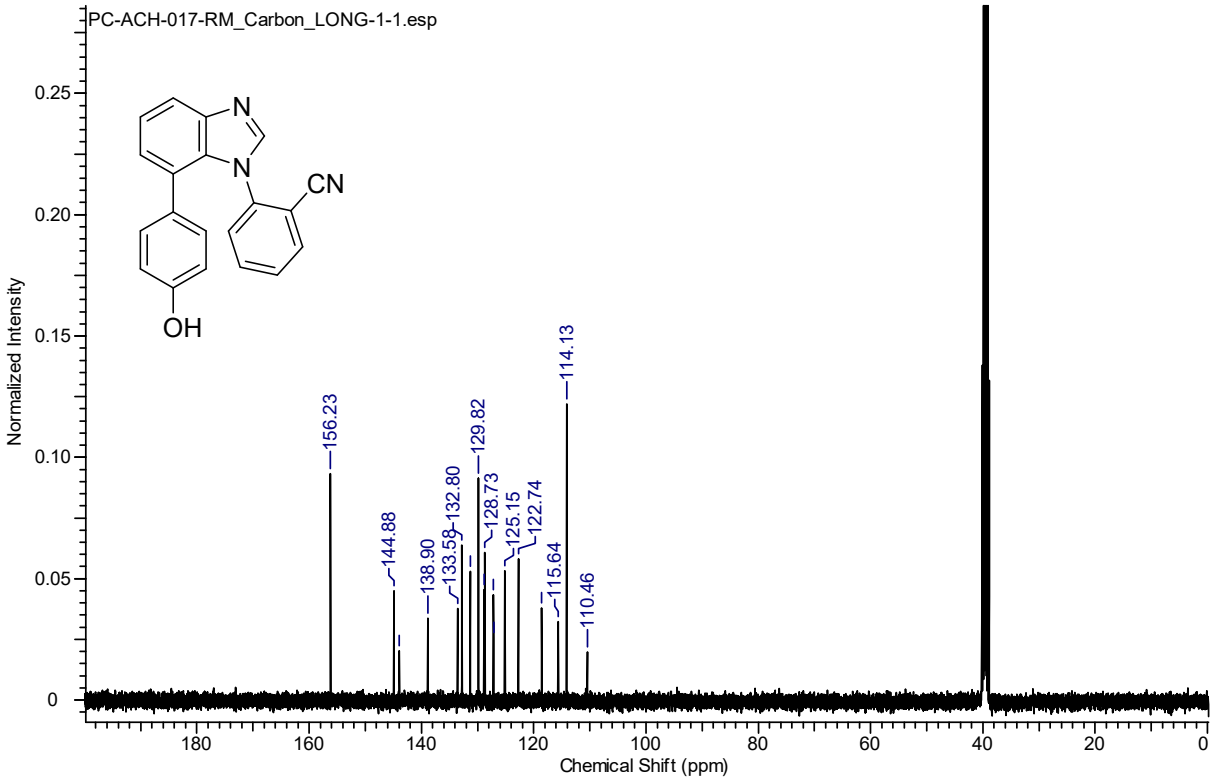
^{1}H and ^{13}C NMR of 8a (DMSO-d_6):



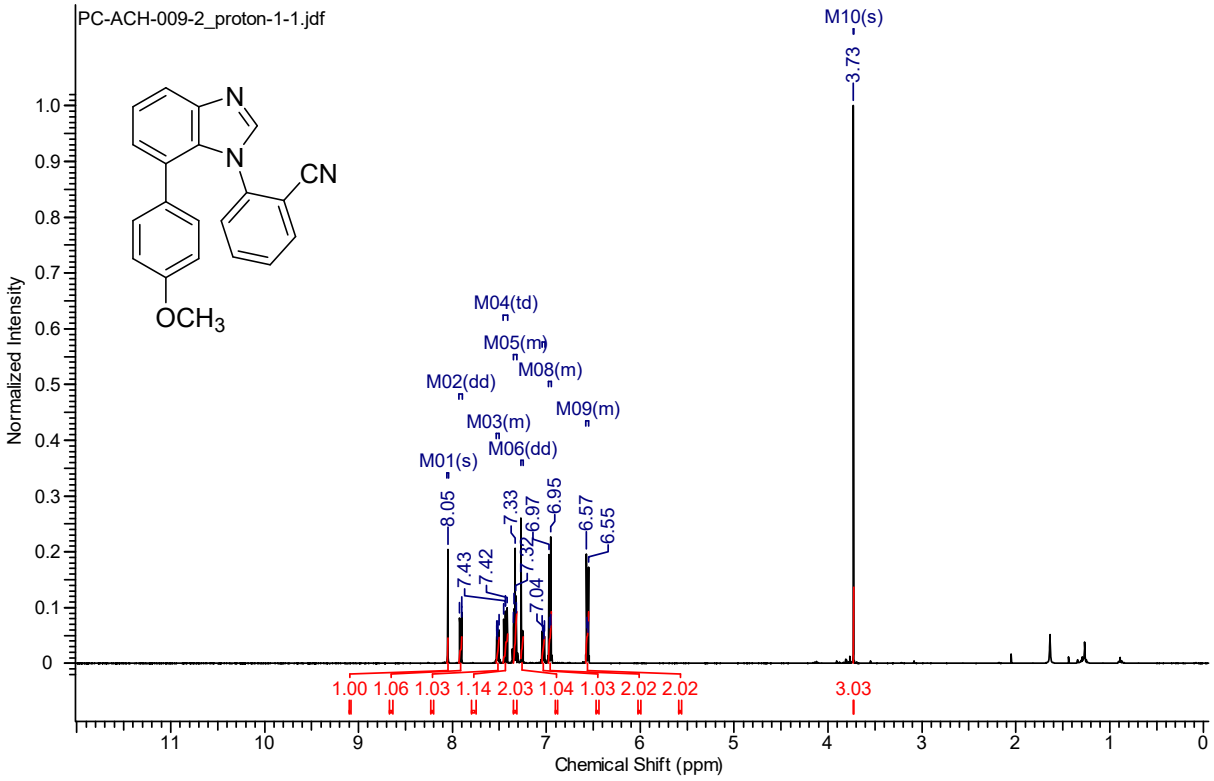


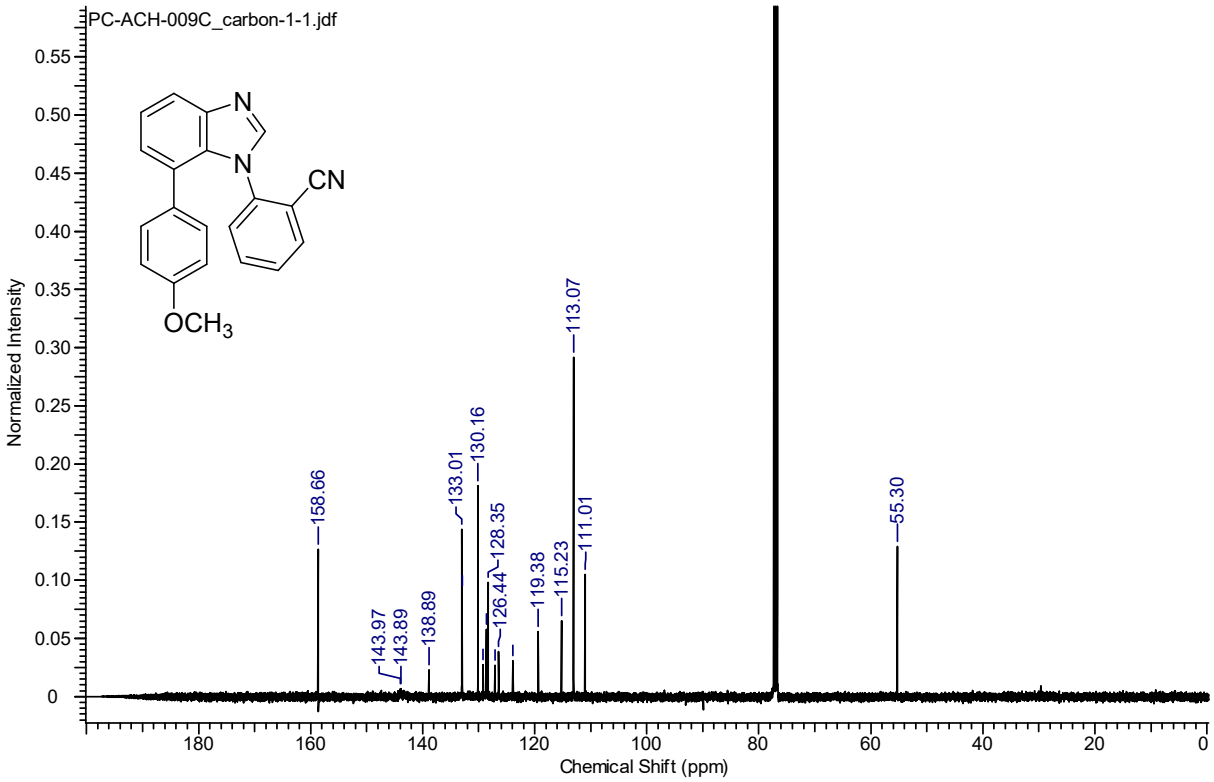
^{1}H and ^{13}C NMR of 8b ($CDCl_3$):



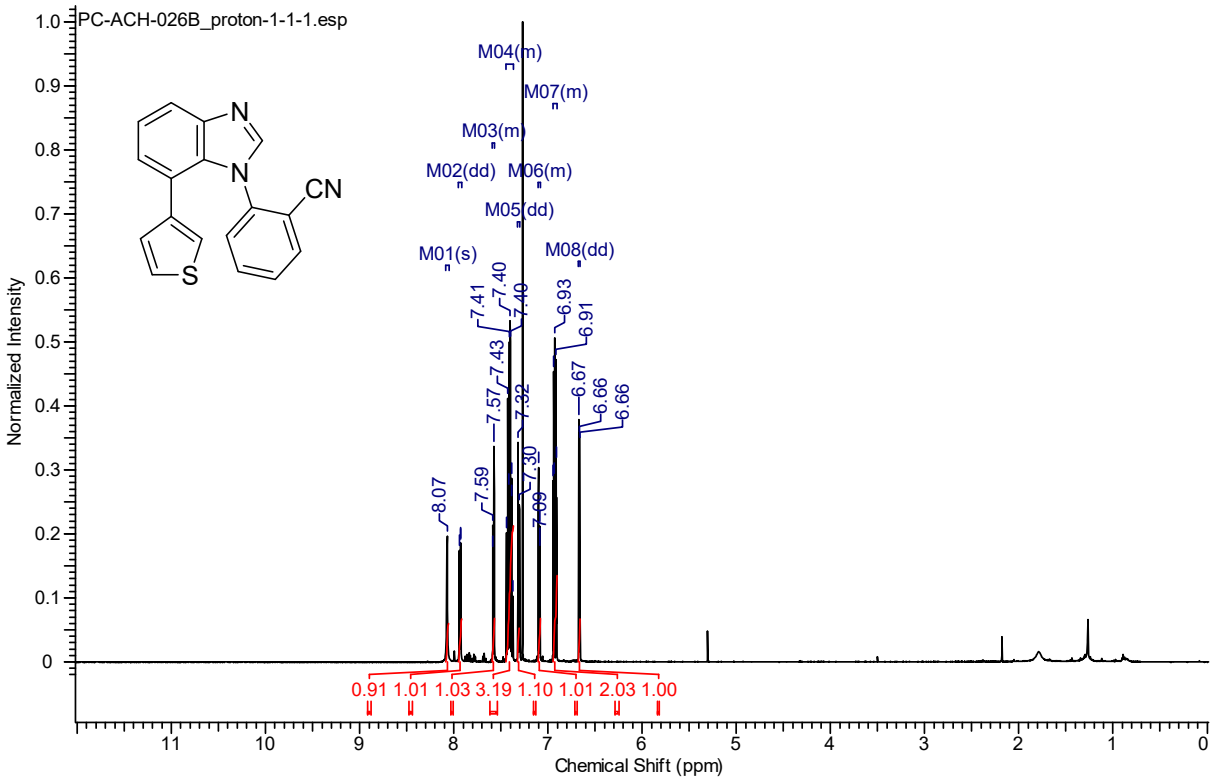


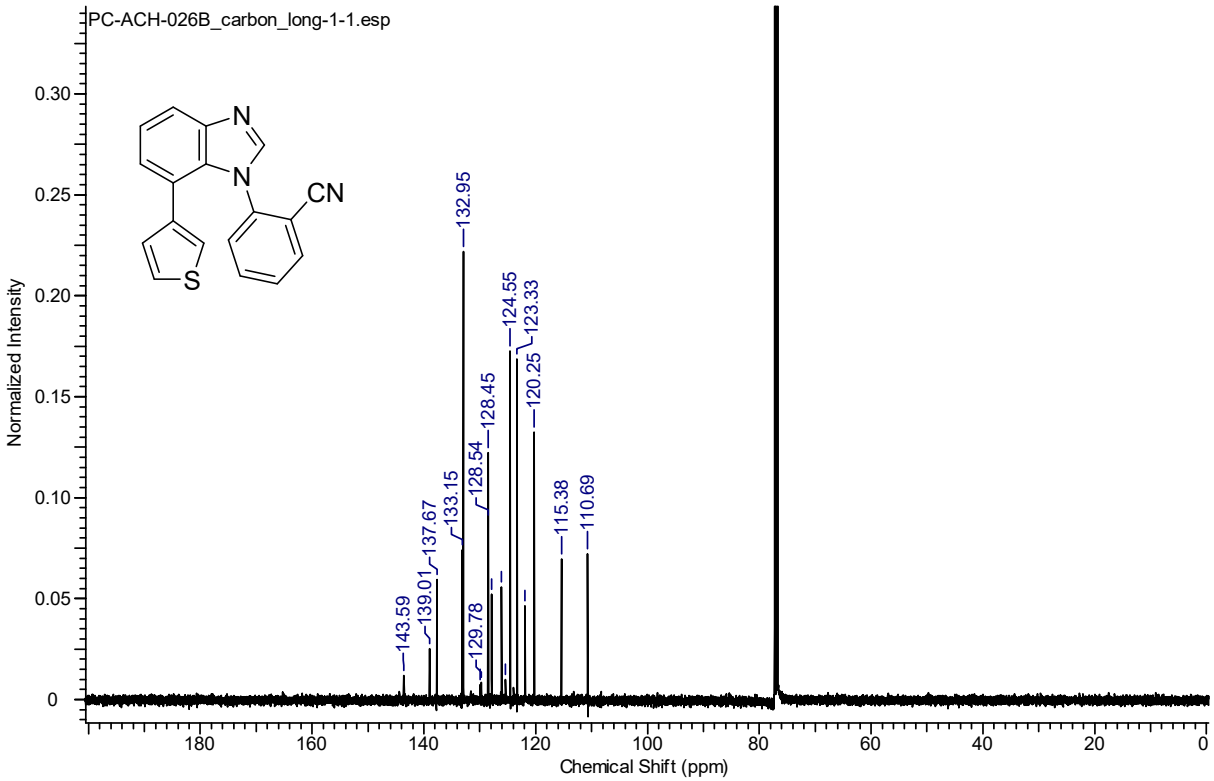
^{1}H and ^{13}C NMR of 8c ($CDCl_3$):


PC-ACH-009-2_proton-1-1.jdf
N
N
CN
OCH3
M10(s)
3.73
M04(td)
M05(m)
M08(m)
M02(dd)
M09(m)
M03(m)
M06(dd)
M01(s)
8.05
7.43
7.42
7.33
7.32
6.97
7.04
6.95
6.57
6.55
1.00 1.06 1.03 1.14 2.03 1.04 1.03 2.02 2.02
3.03
Normalized Intensity
Chemical Shift (ppm)

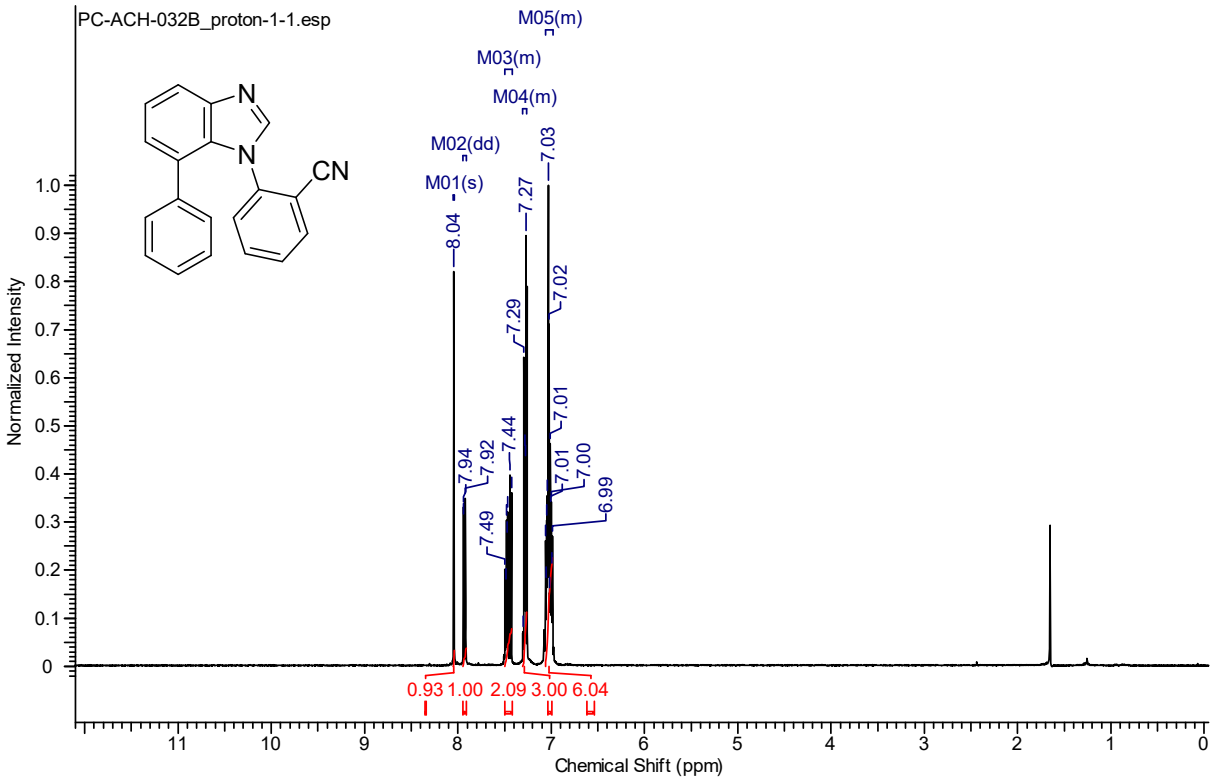

PC-ACH-009C_carbon-1-1.jdf
N
N
CN
OCH3
158.66
143.97
143.89
138.89
133.01
130.16
128.35
126.44
119.38
115.23
113.07
111.01
55.30
Normalized Intensity
Chemical Shift (ppm)

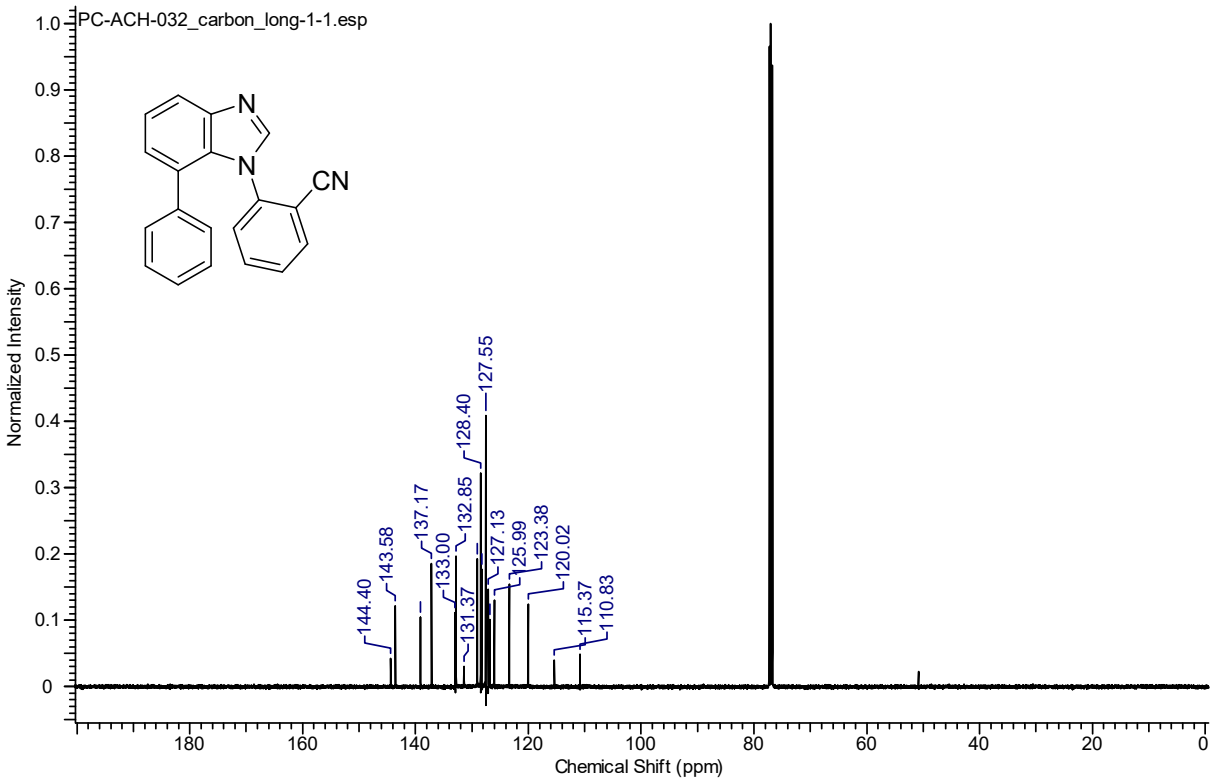
^{1}H and ^{13}C NMR of 8d ($CDCl_3$):



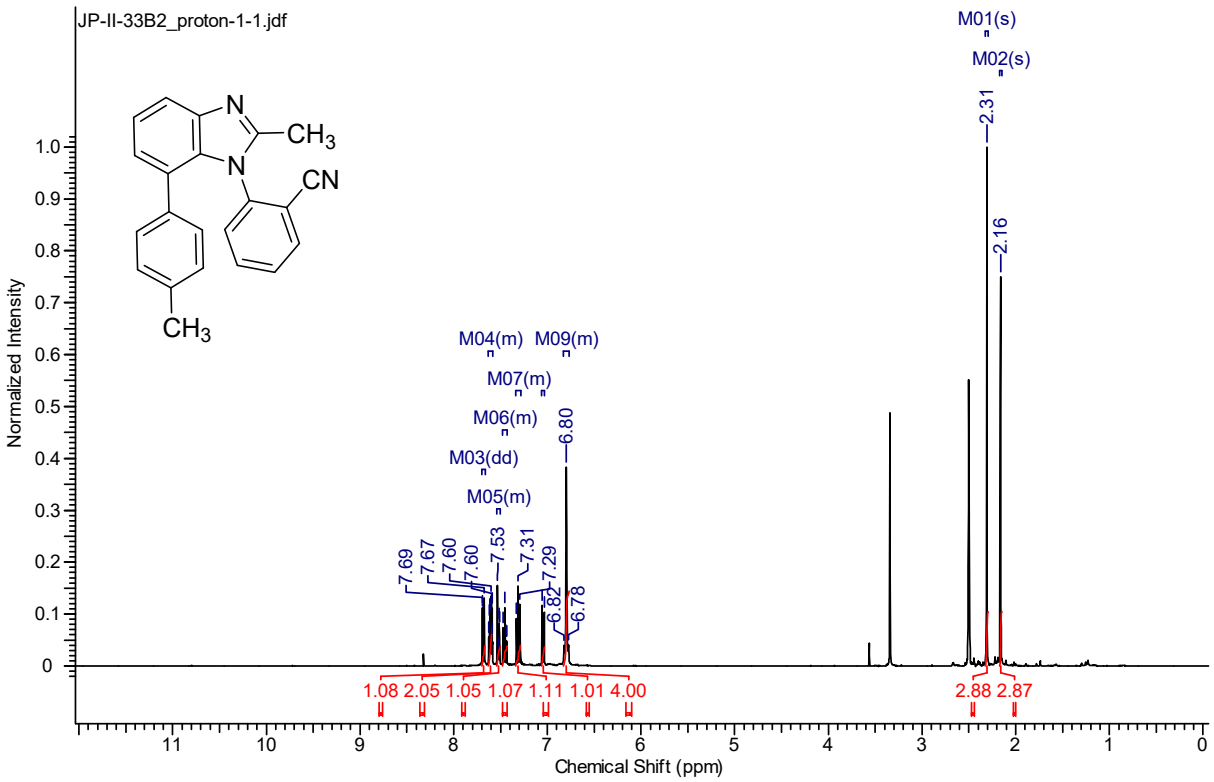


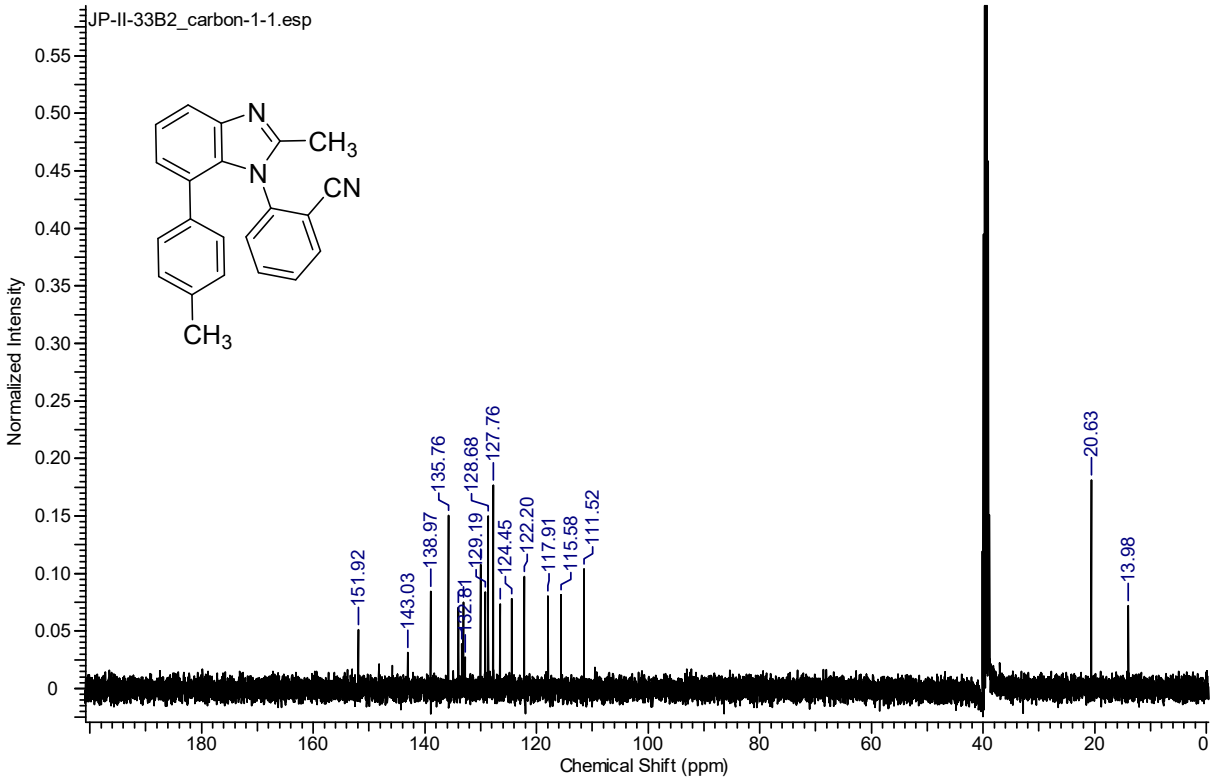
^{1}H and ^{13}C NMR of 8e ($CDCl_3$):



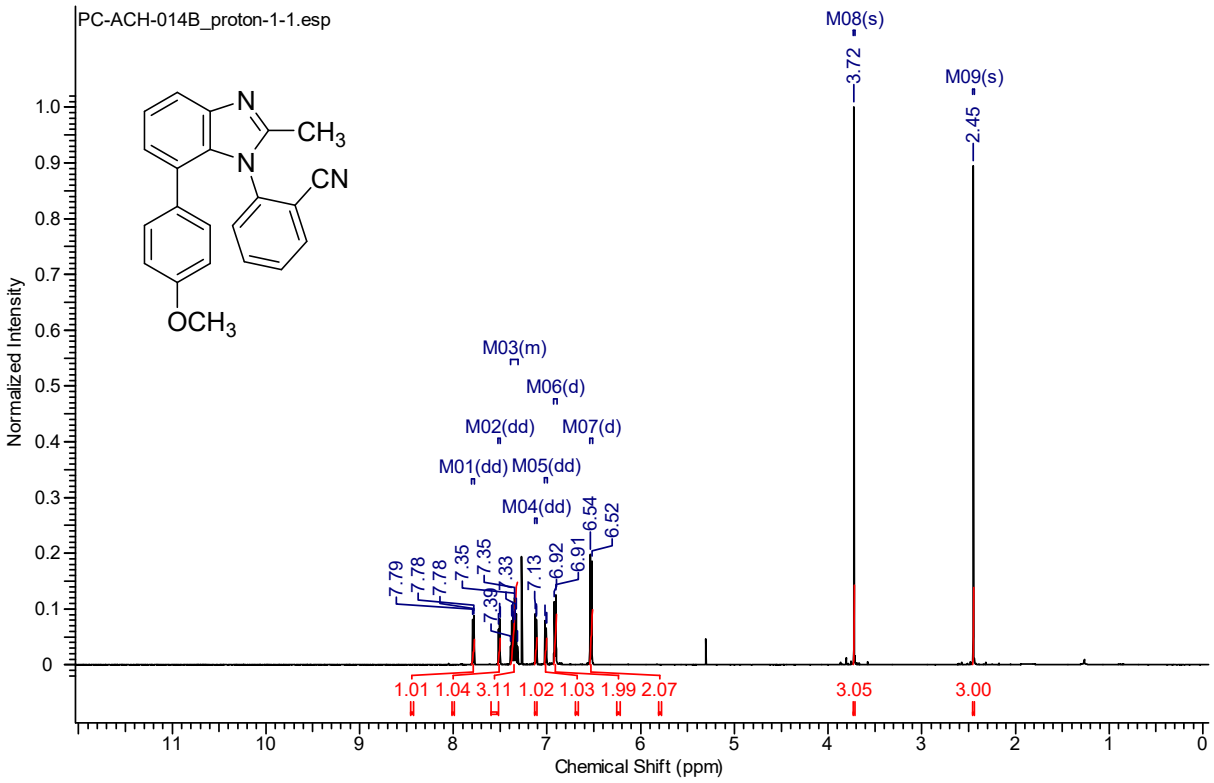


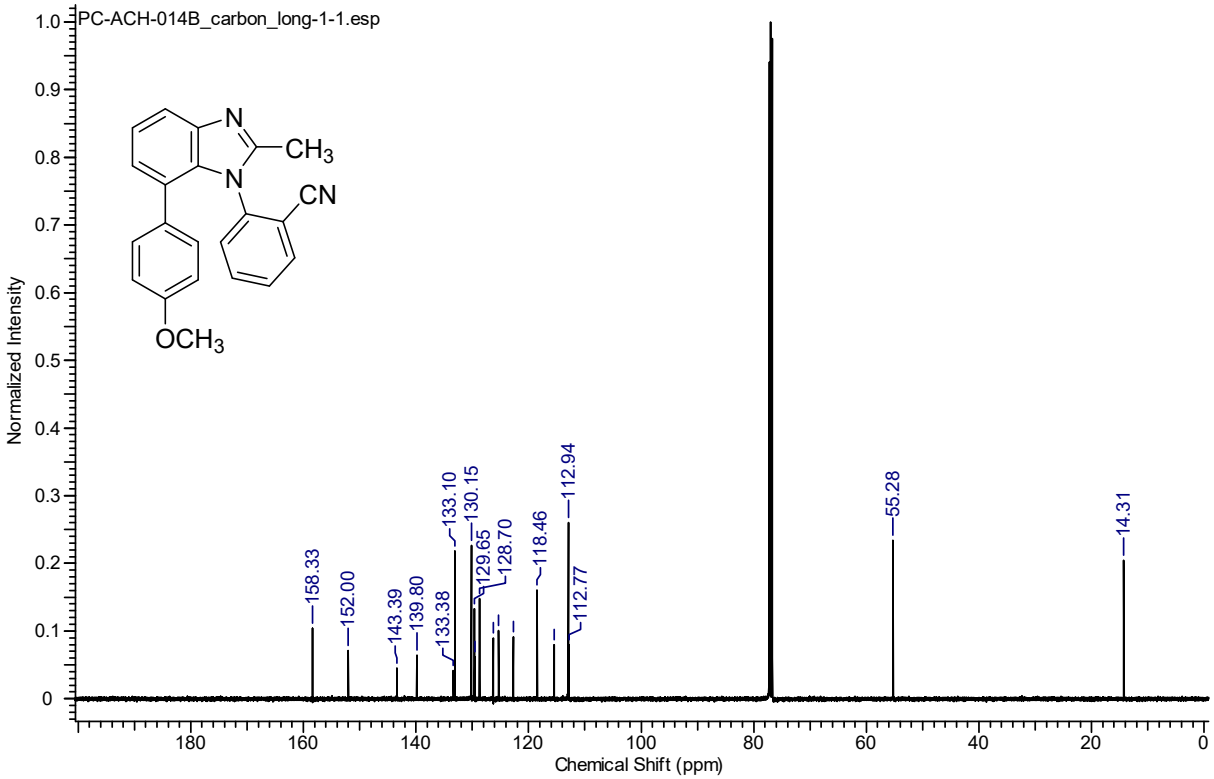
^{1}H and ^{13}C NMR of 8f (DMSO-d_6):



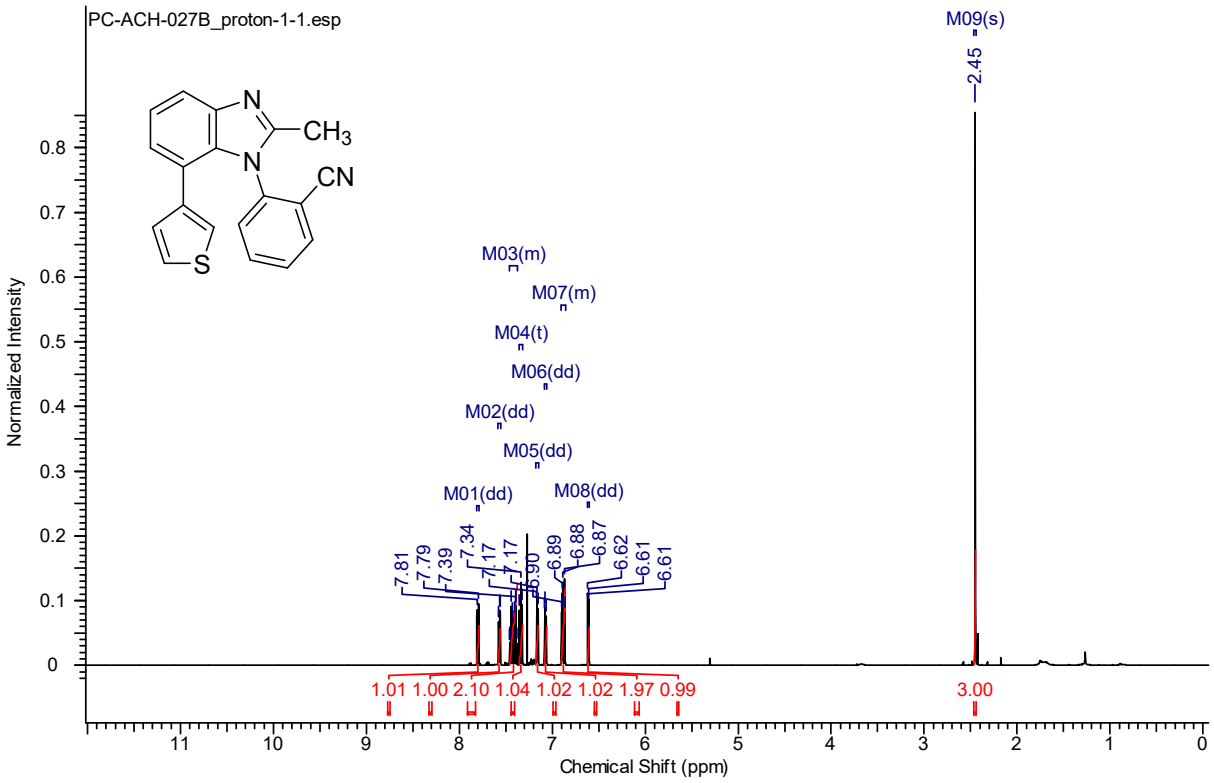


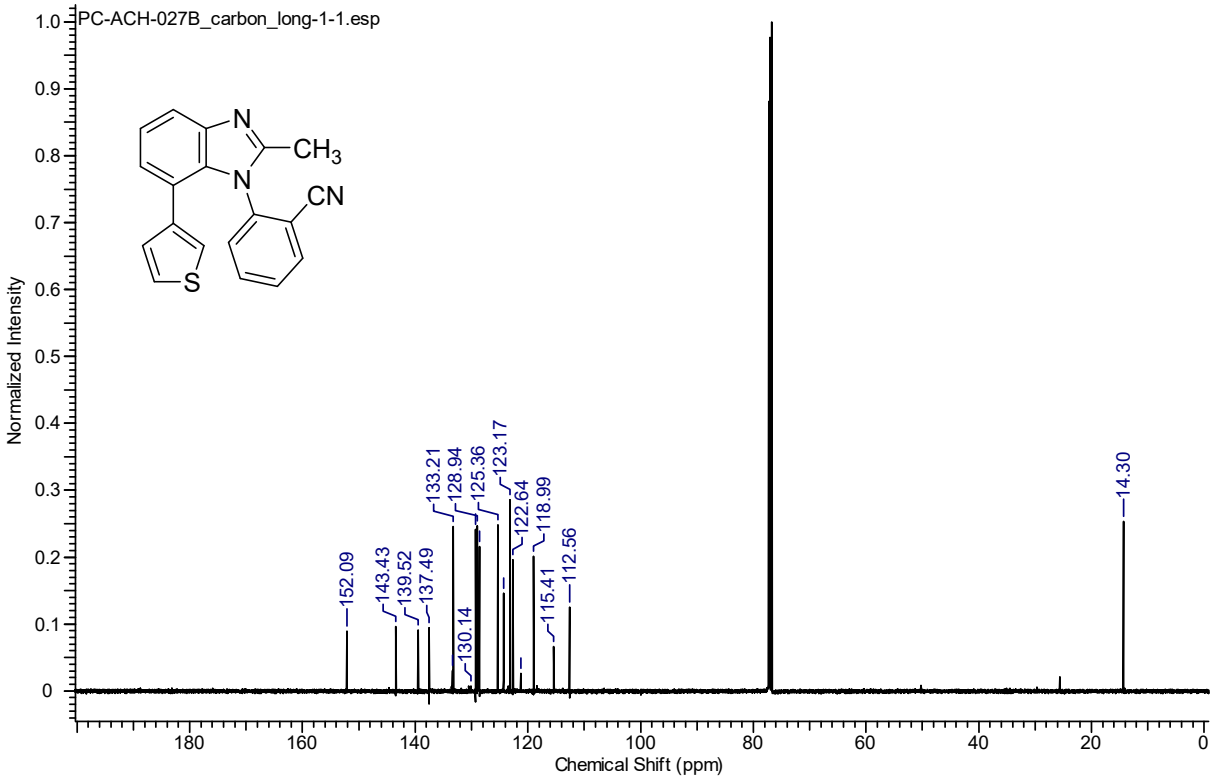
^{1}H and ^{13}C NMR of 8g ($CDCl_3$):



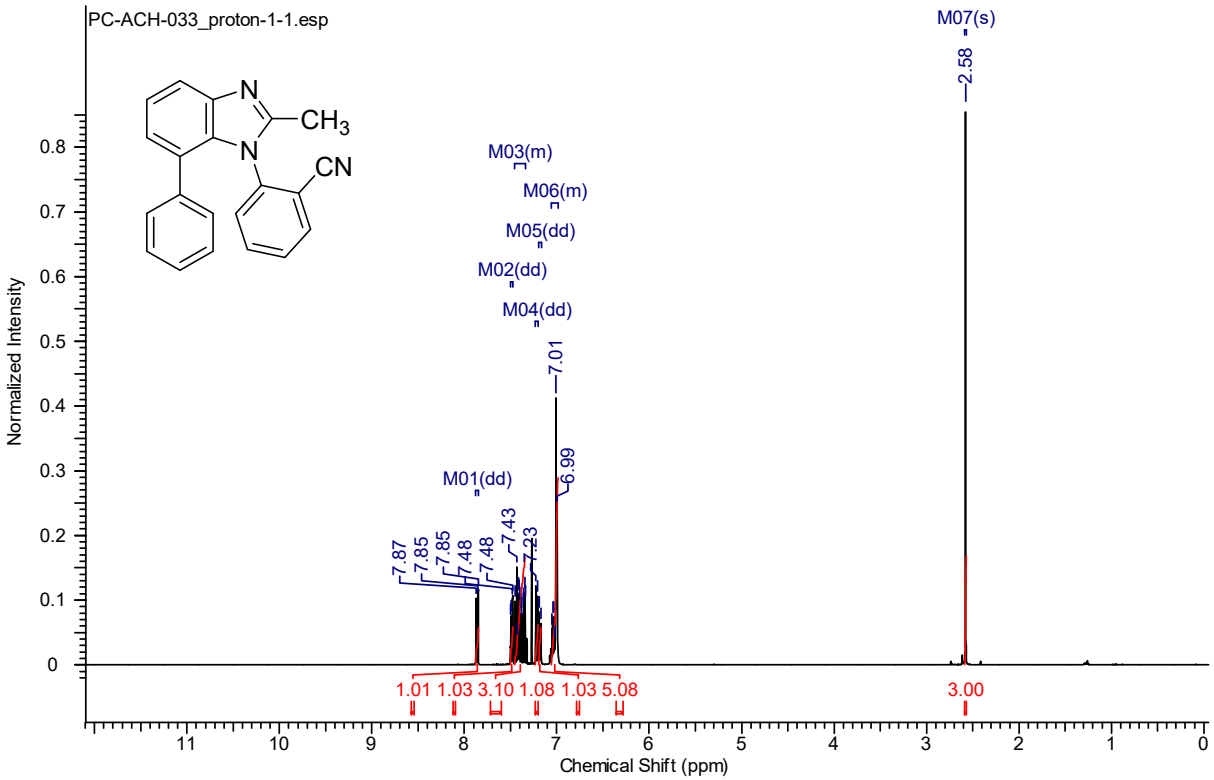


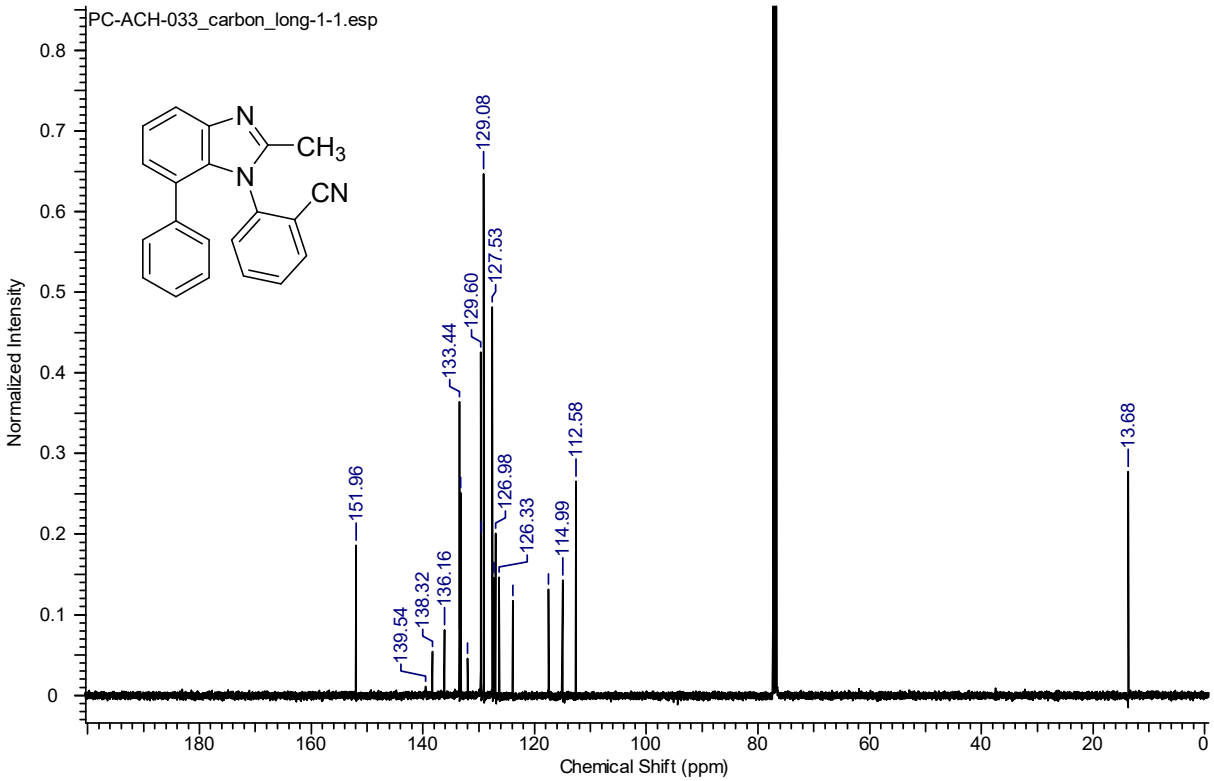
^{1}H and ^{13}C NMR of 8h ($CDCl_3$):



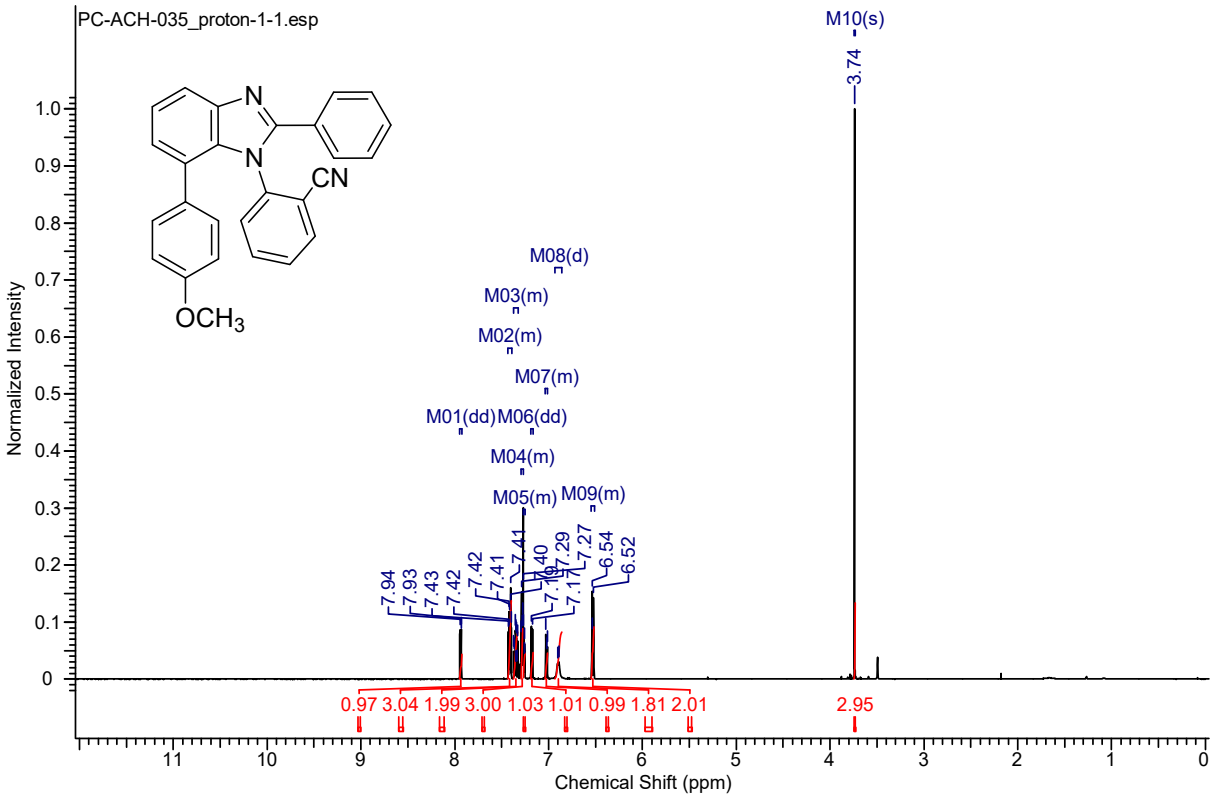


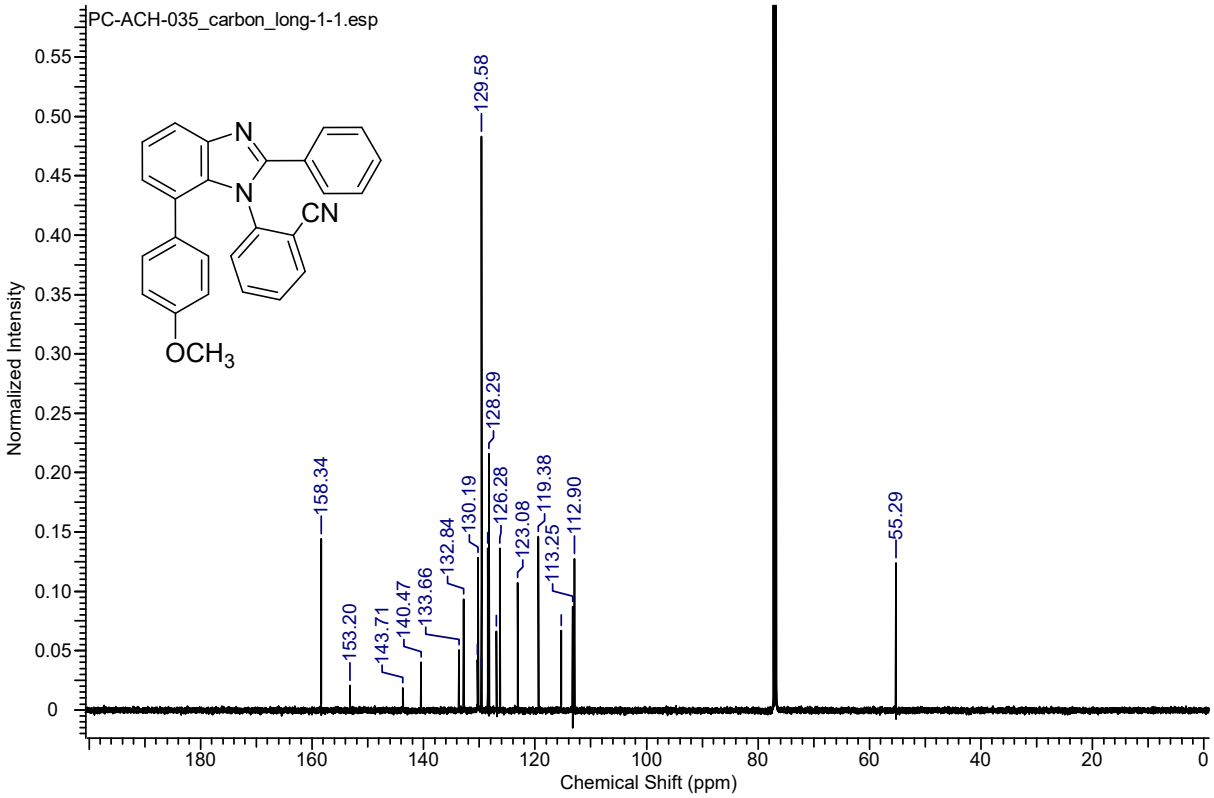
^{1}H and ^{13}C NMR of 8i ($CDCl_3$):



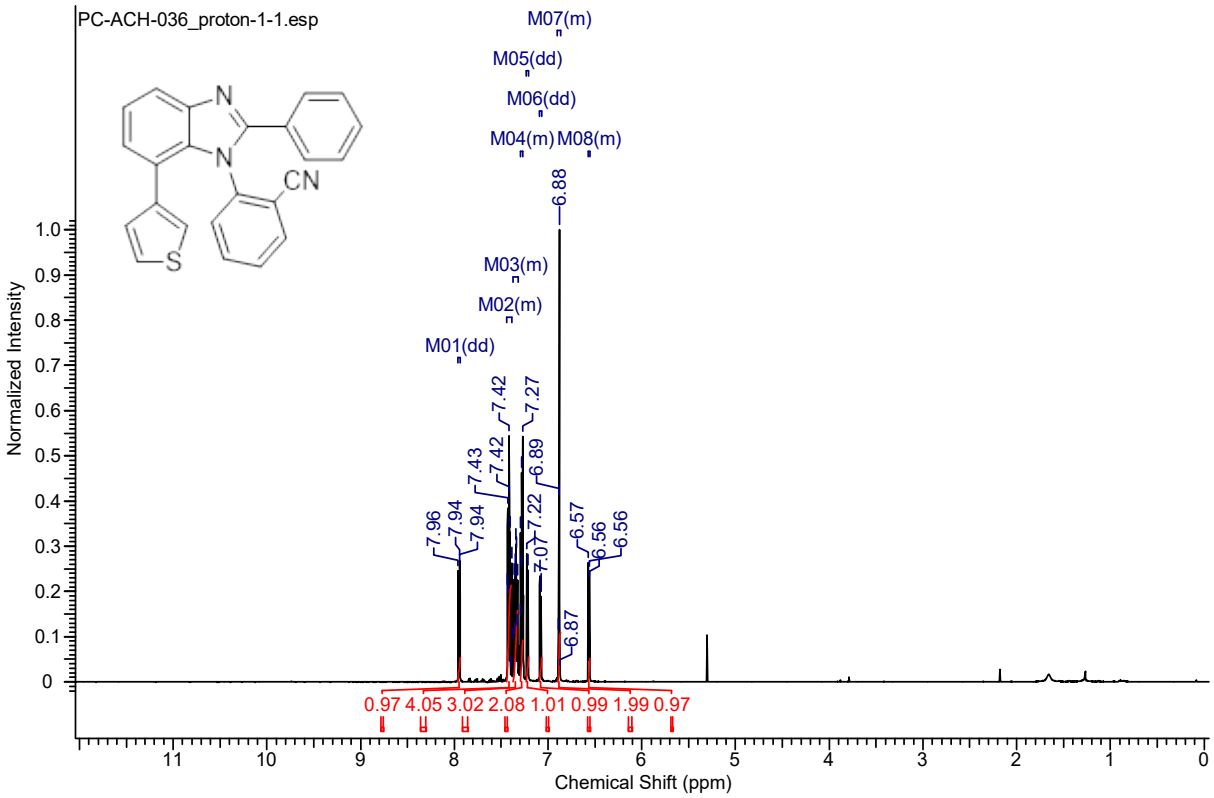


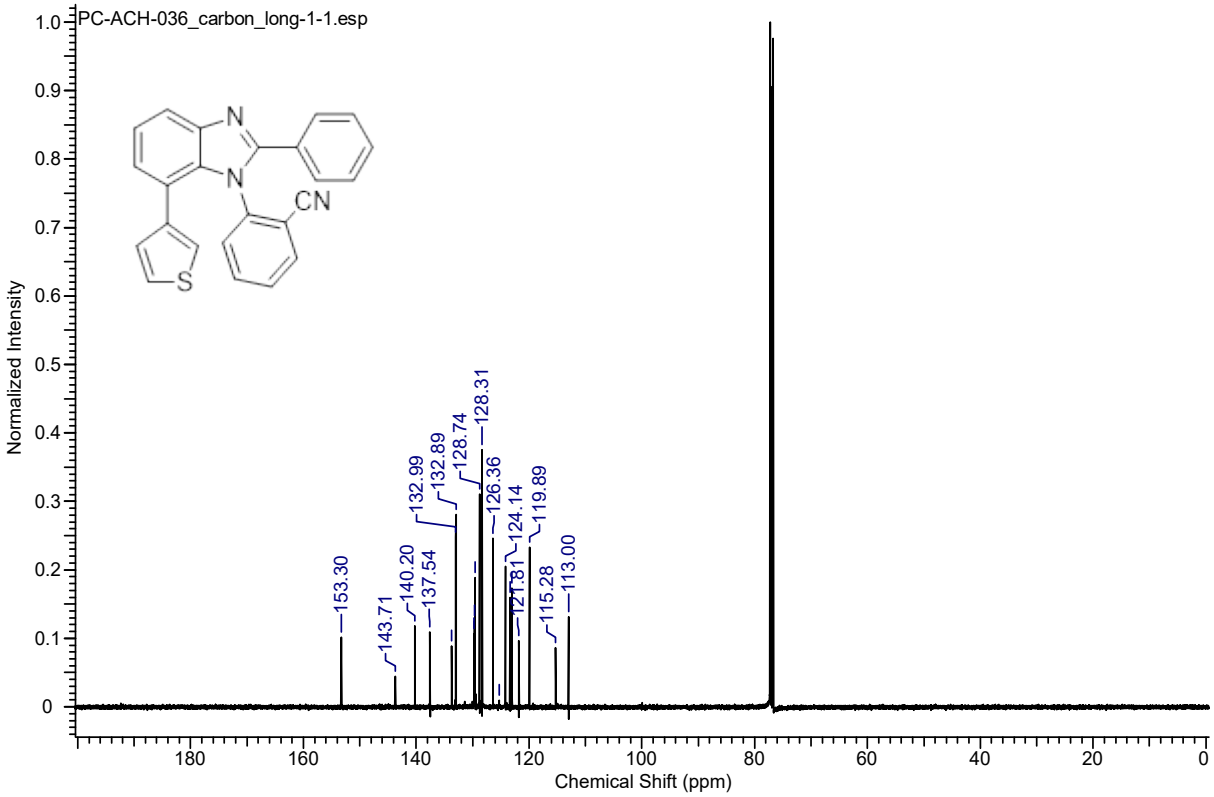
^{1}H and ^{13}C NMR of 8j ($CDCl_3$):



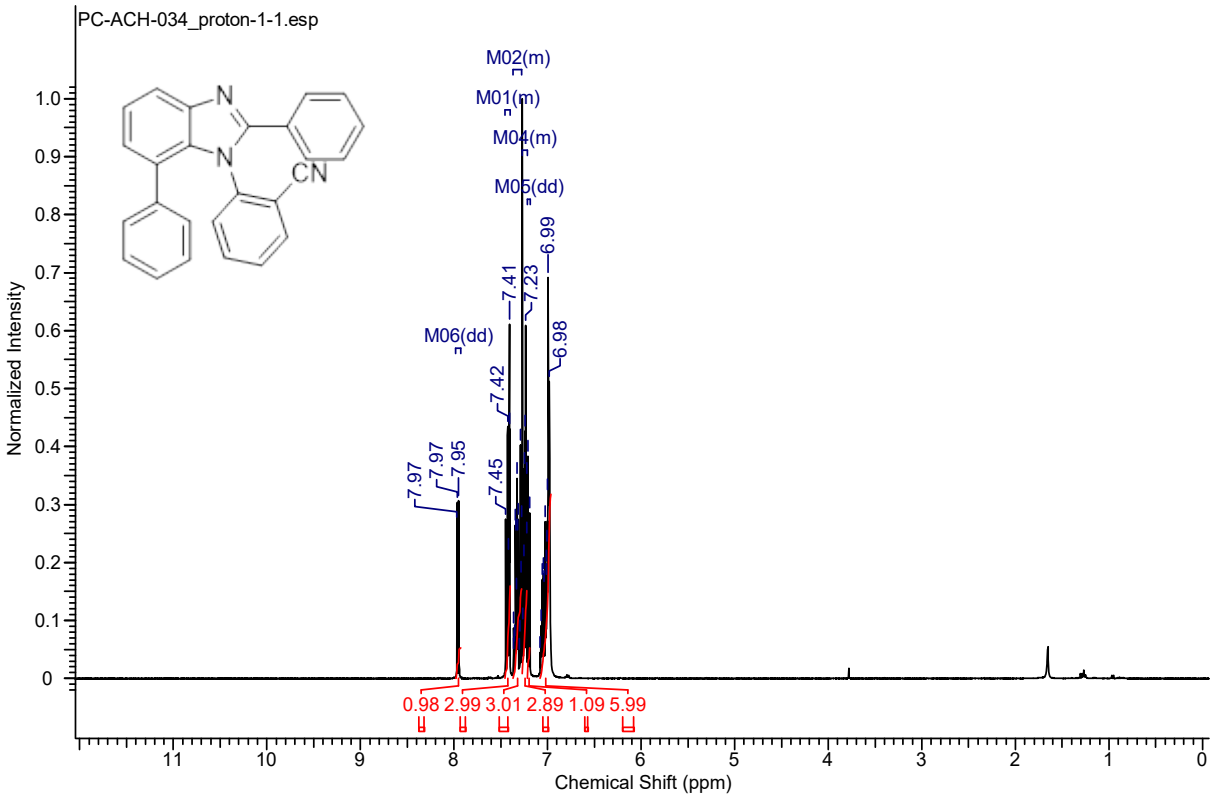


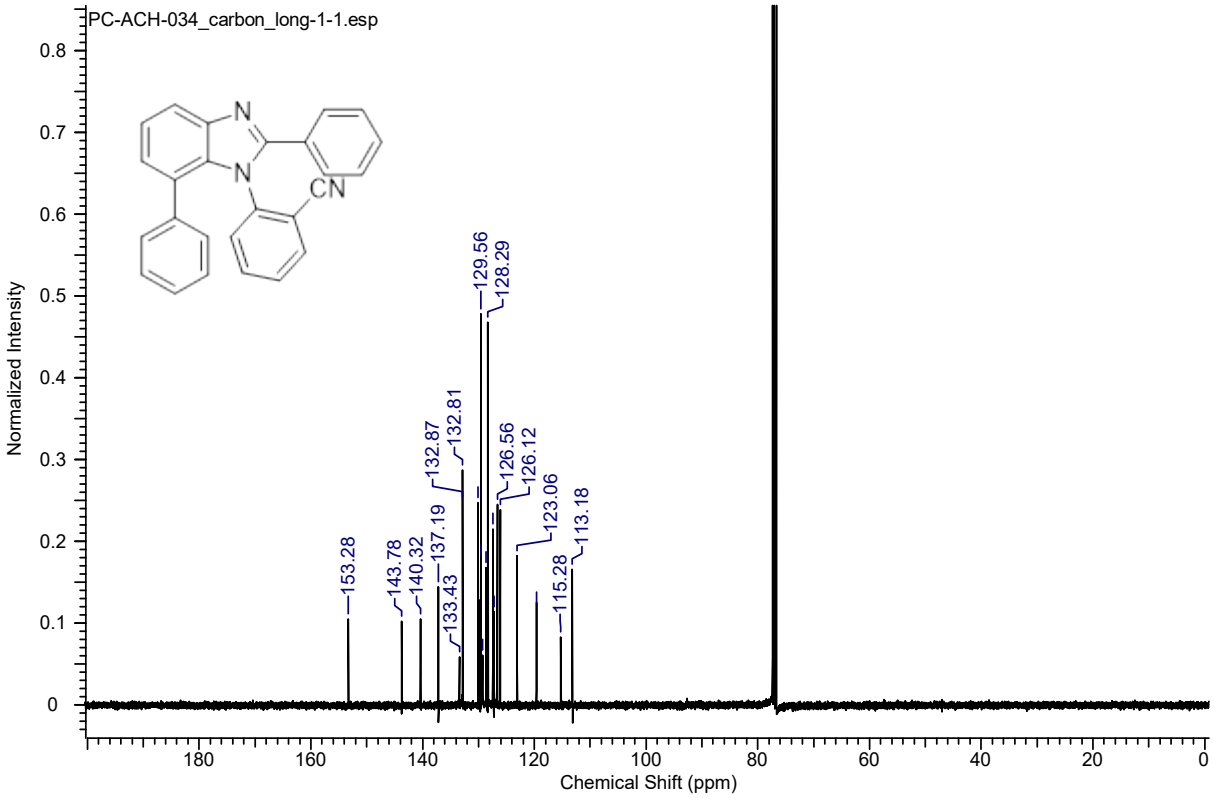
^{1}H and ^{13}C NMR of 8k ($CDCl_3$):



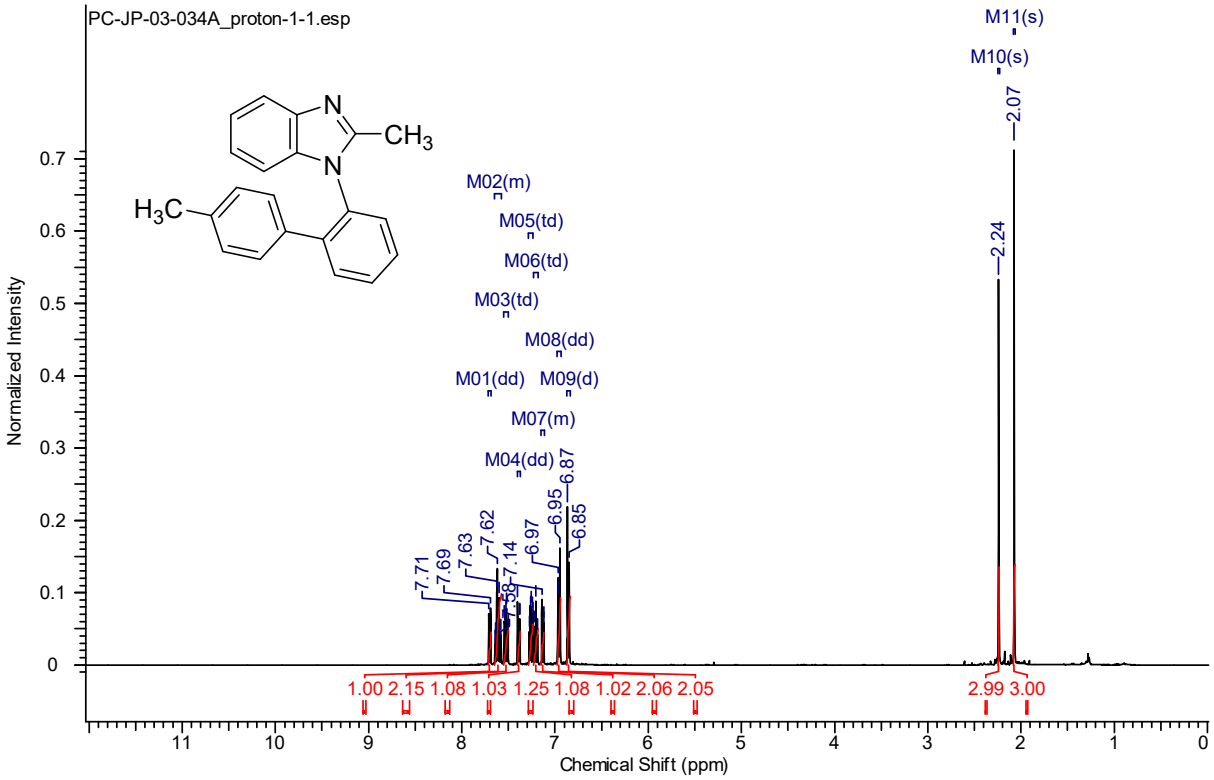


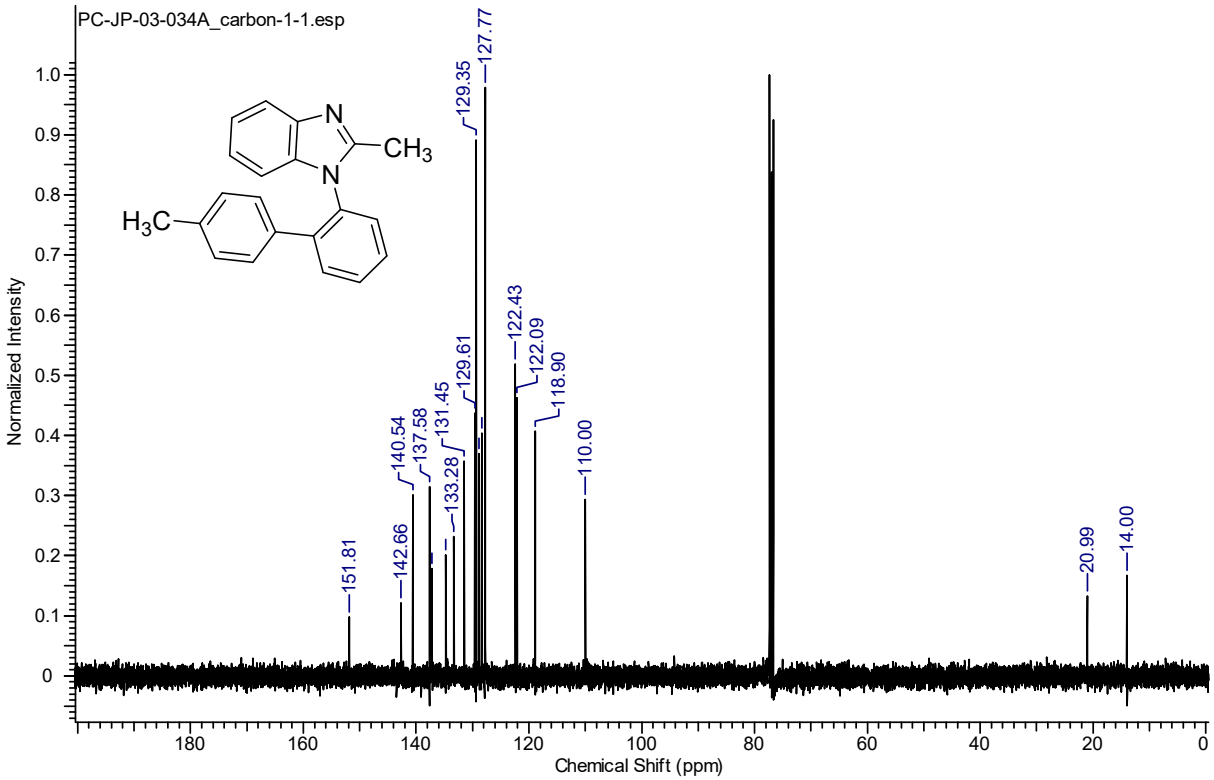
^{1}H and ^{13}C NMR of 8l ($CDCl_3$):



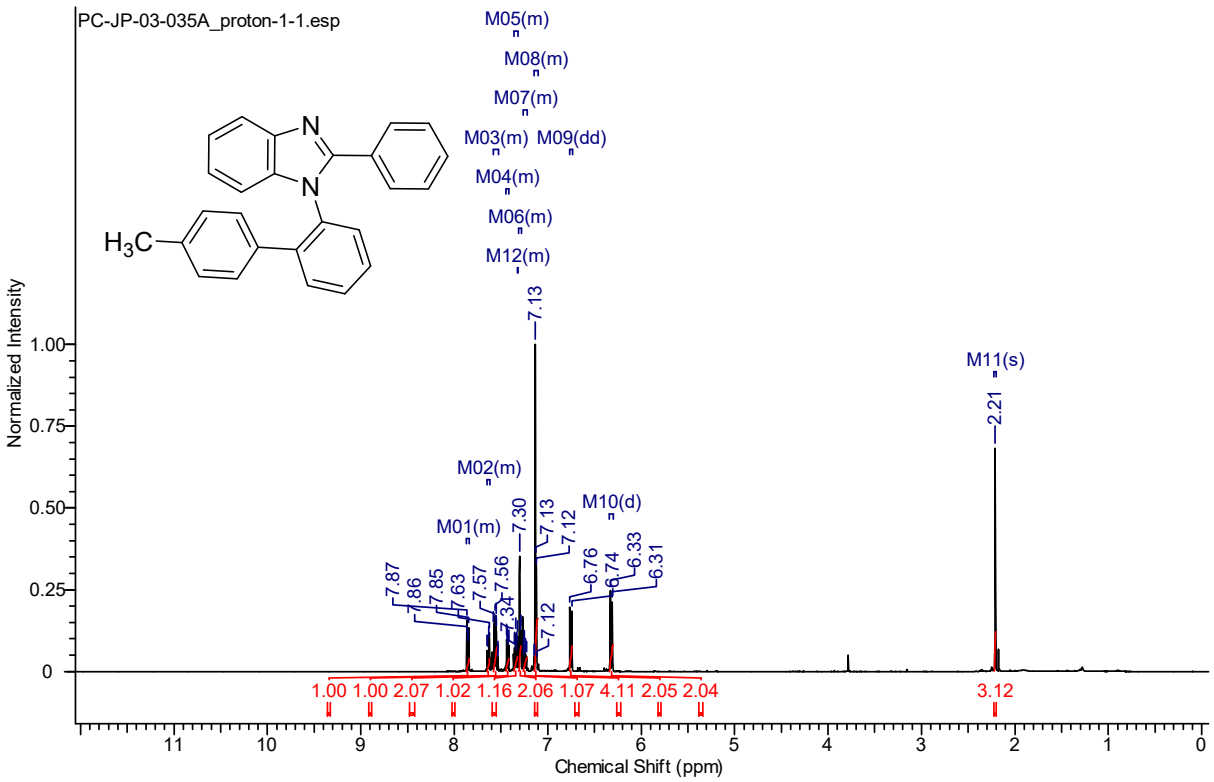


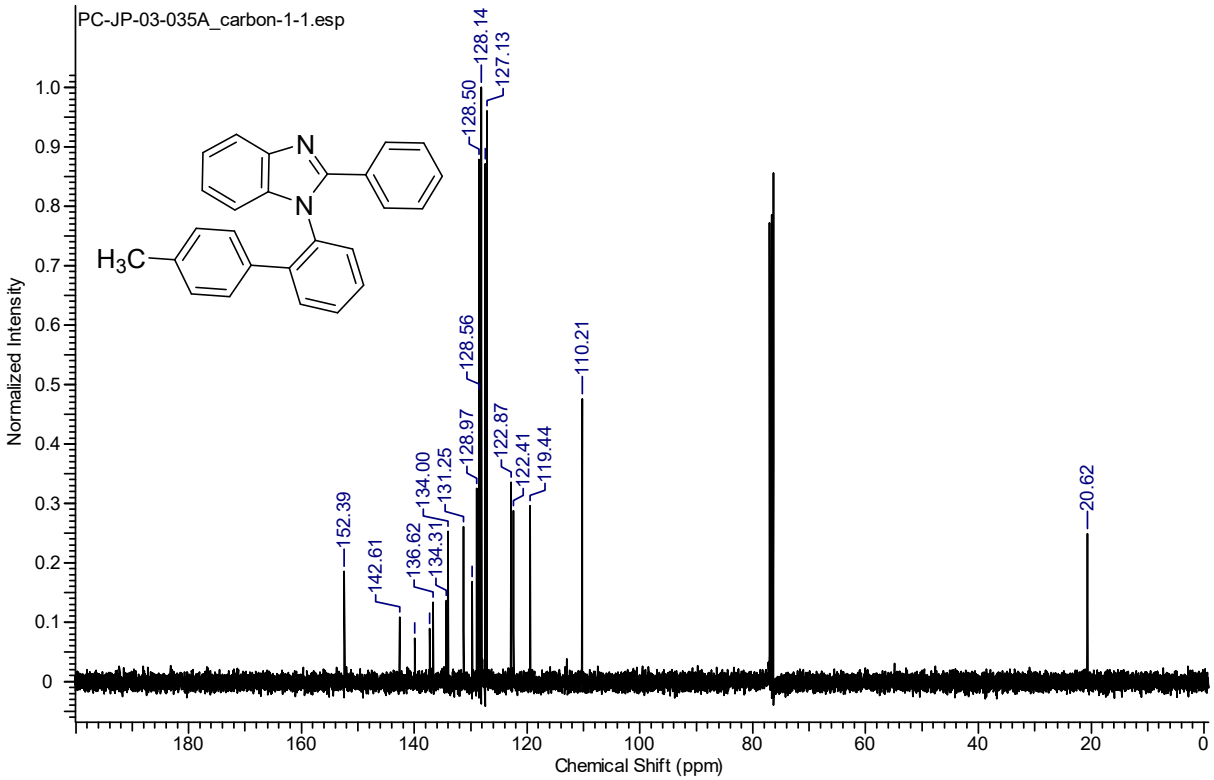
^{1}H and ^{13}C NMR of 11a ($CDCl_3$):





^{1}H and ^{13}C NMR of 11b ($CDCl_3$):

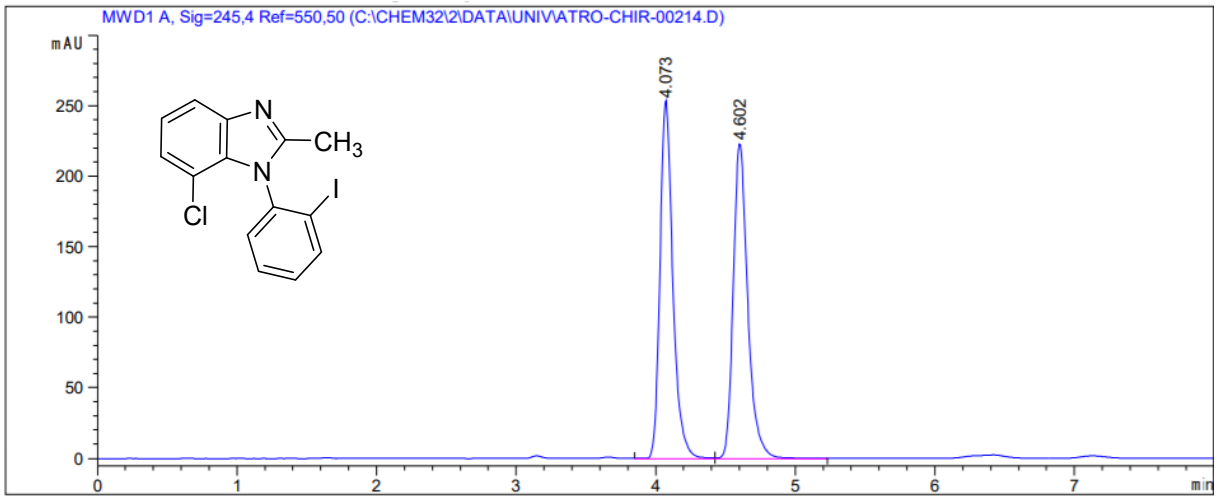




Verification of atropisomerism of selected compounds

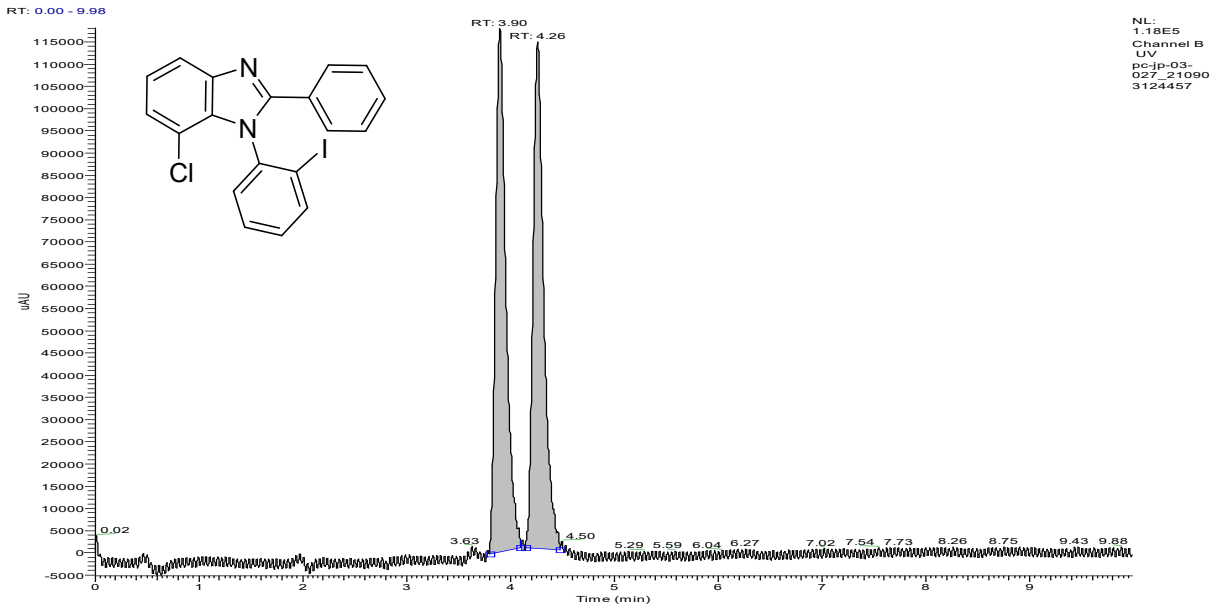
Compound 5i:

Column Chiralpak IA-3, 3µm, 4.6 mm x 100 mm; mobil phase *n*-heptan/ethanol 90:10, 0.75 mL/min, detection 245 nm, time of analysis 10 min



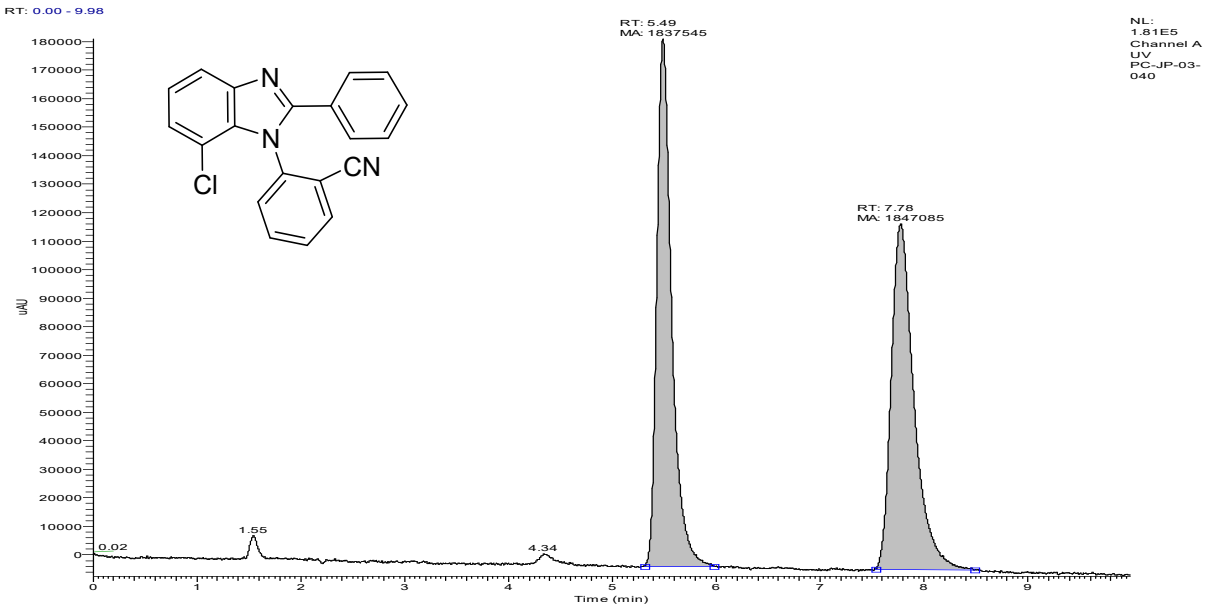
Compound 5l:

Column Chiralpak IA-3, 3 µm, 4.6 mm x 100 mm; mobil phase *n*-heptan/ethanol 90:10, 0.6 mL/min, detection 220 nm, time of analysis 10 min



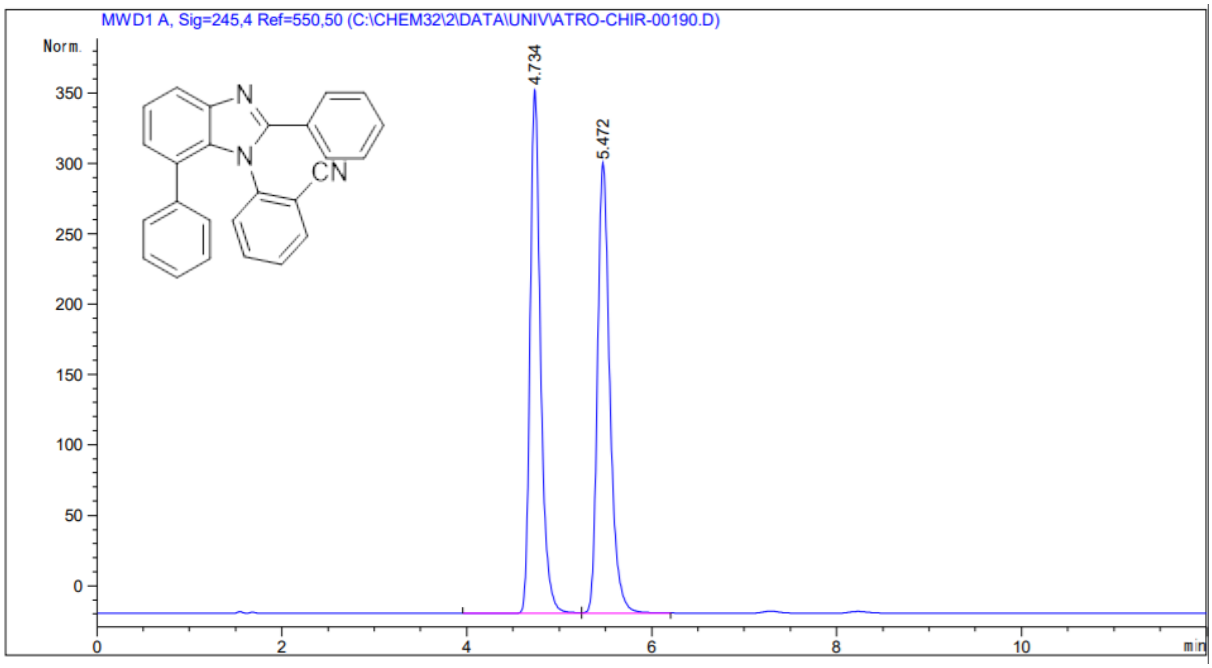
Compound 5n:

Column Chiralpak IA-3, 3µm, 4.6 mm x 100 mm; mobil phase *n*-heptan/ethanol 90:10, 0.8 mL/min, detection 220 nm, time of analysis 10 min



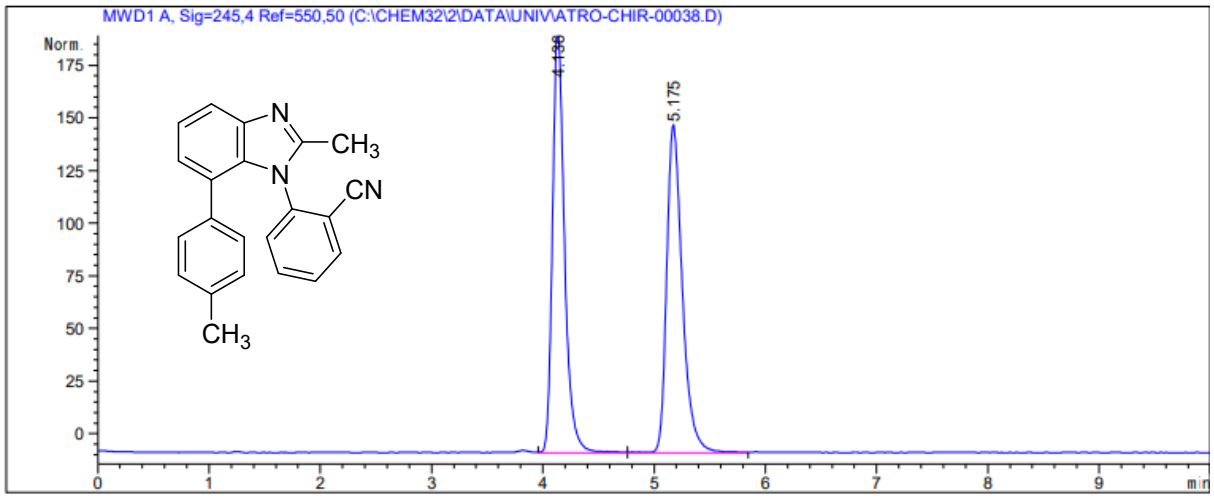
Compound 8e:

Column Chiralpak IA-3, 3µm, 4.6 mm x 100 mm; mobil phase *n*-heptan/ethanol 90:10, 0.75 mL/min, detection 245 nm, time of analysis 15 min



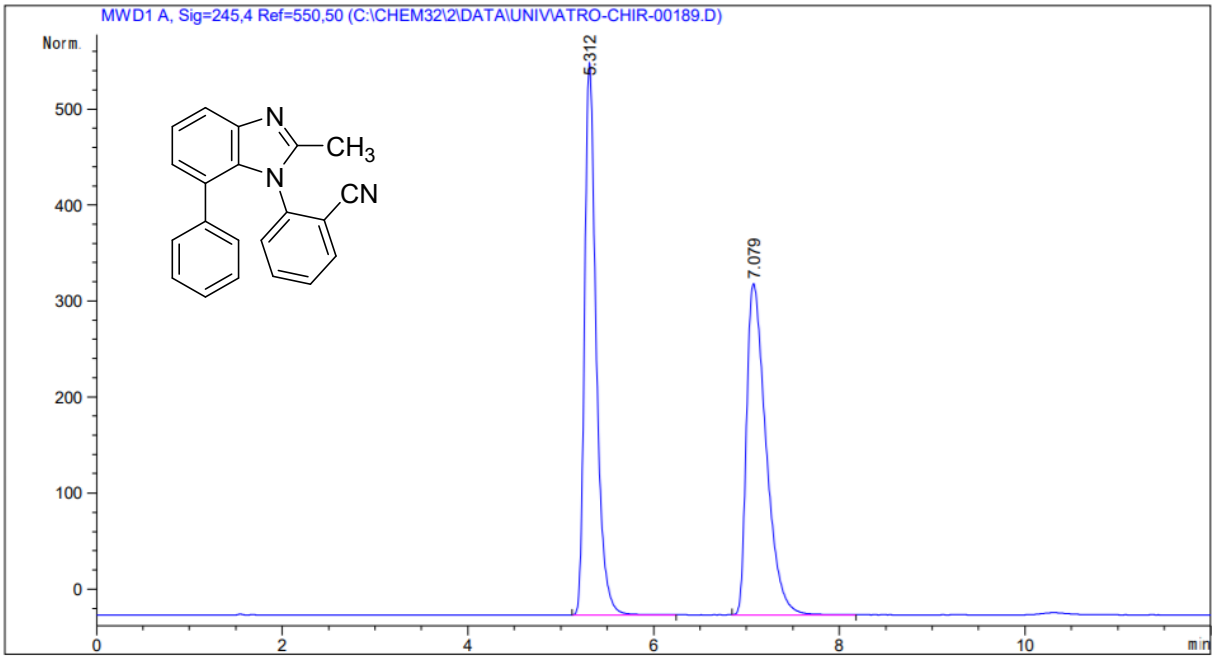
Compound 8f:

Column Chiralpak IA-3, 3µm, 4.6 mm x 100 mm; mobil phase *n*-heptan/ethanol 90:10, 1 mL/min, detection 245 nm, time of analysis 10 min



Compound 8i:

Column Chiralpak IA-3, 3µm, 4.6 mm x 100 mm; mobil phase *n*-heptan/ethanol 90:10, 0.75 mL/min, detection 245 nm, time of analysis 15 min

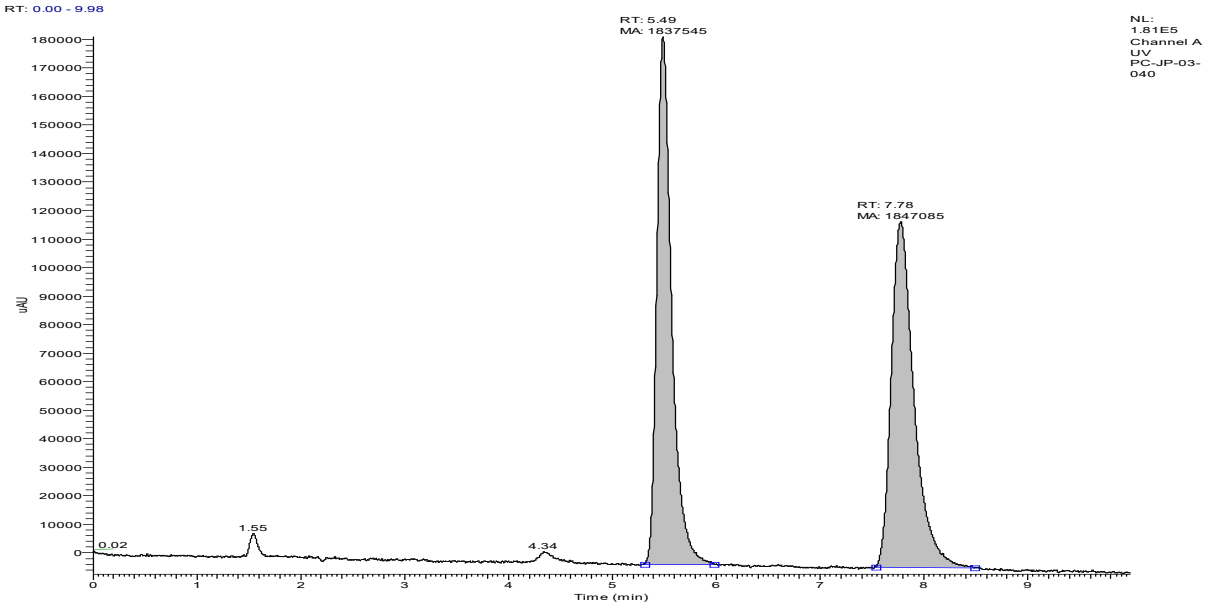


Racemization of selected final benzimidazoles

Stability of (+)-5n at 100°C

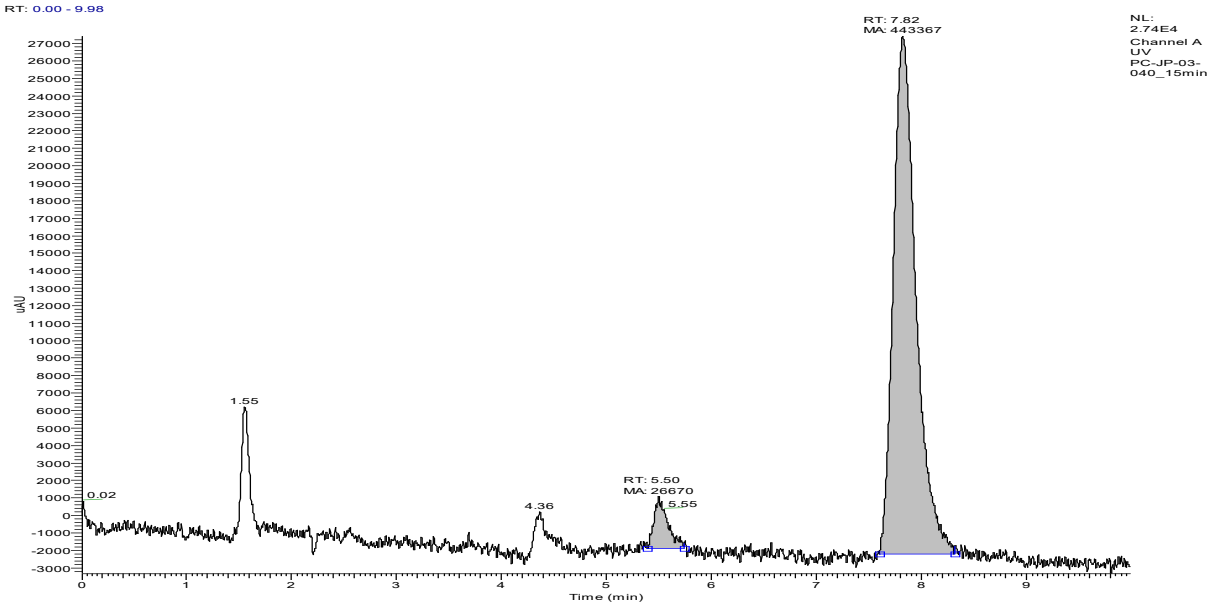
Column Chiralpak IA-3, 3 µm, 4.6 mm × 100 mm; mobil phase *n*-heptane/ethanol 90:10, 0.8 mL/min, detection at 220 nm, time of analysis 10 min

Racemate – ratio of atropisomers *50 : 50*

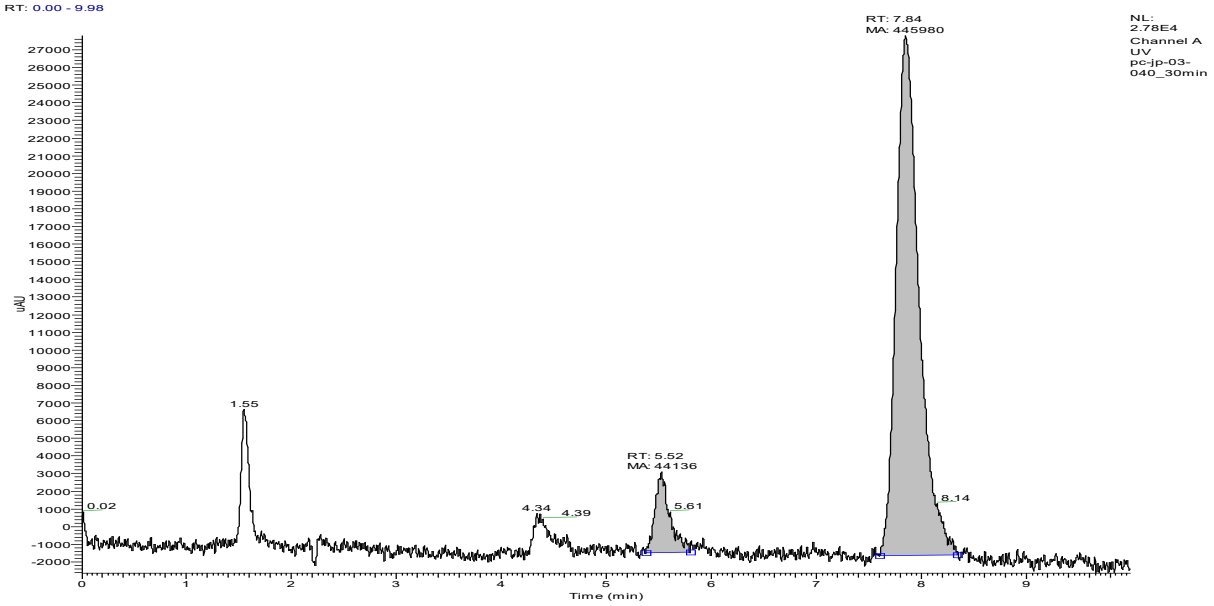


Racemization conditions: compound **(+)-5n** (15 mg) was dissolved in ethylene glycol (0.5 mL). The solution was stirred at 100 °C for 4 h. A sample of solution (50 µL) was taken after 15 min, 30 min, 45 min, 1 h, 1.5 h, 2 h, 2.5 h, 3 h, and 4 h for the analysis.

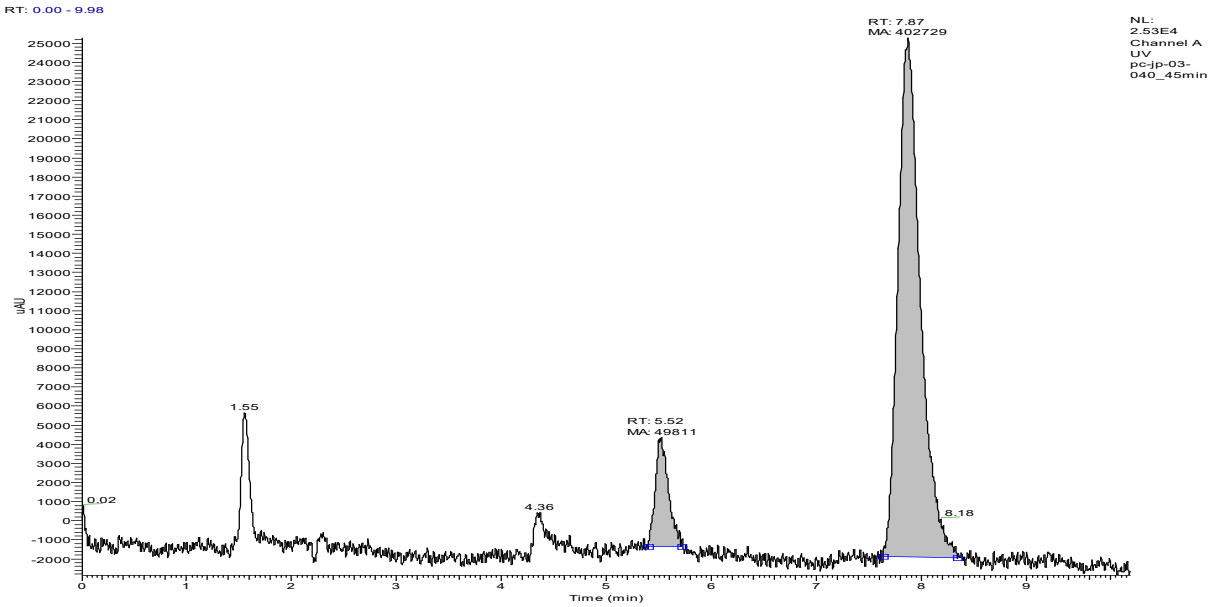
15 min - ratio of atropisomers *6 : 94*



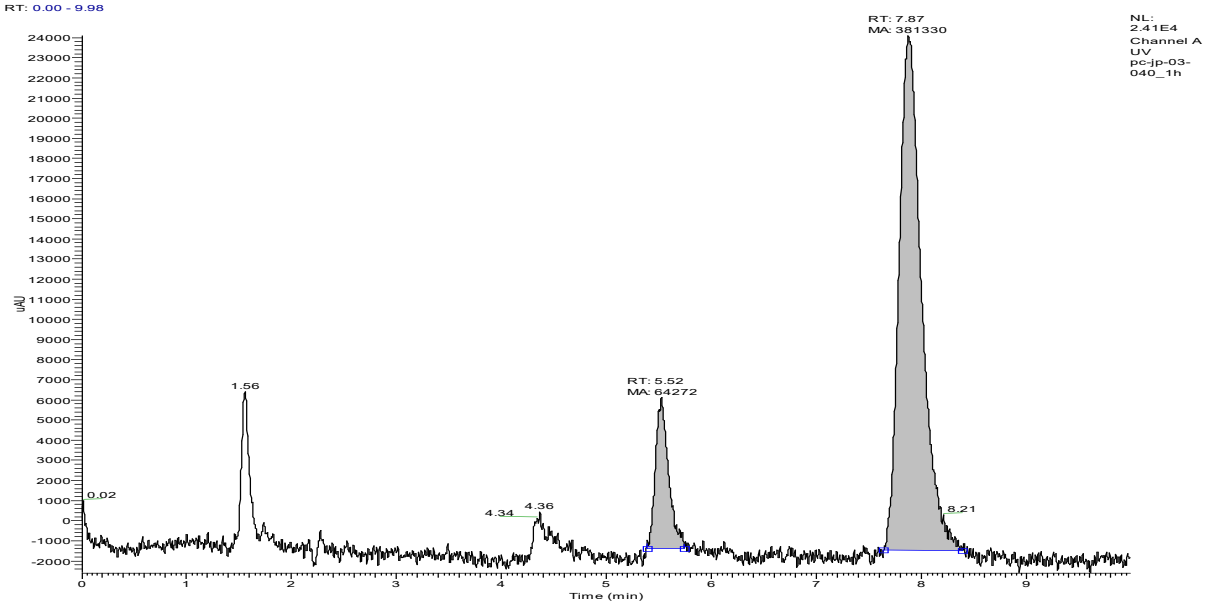
30 min - ratio of atropisomers *9 : 91*



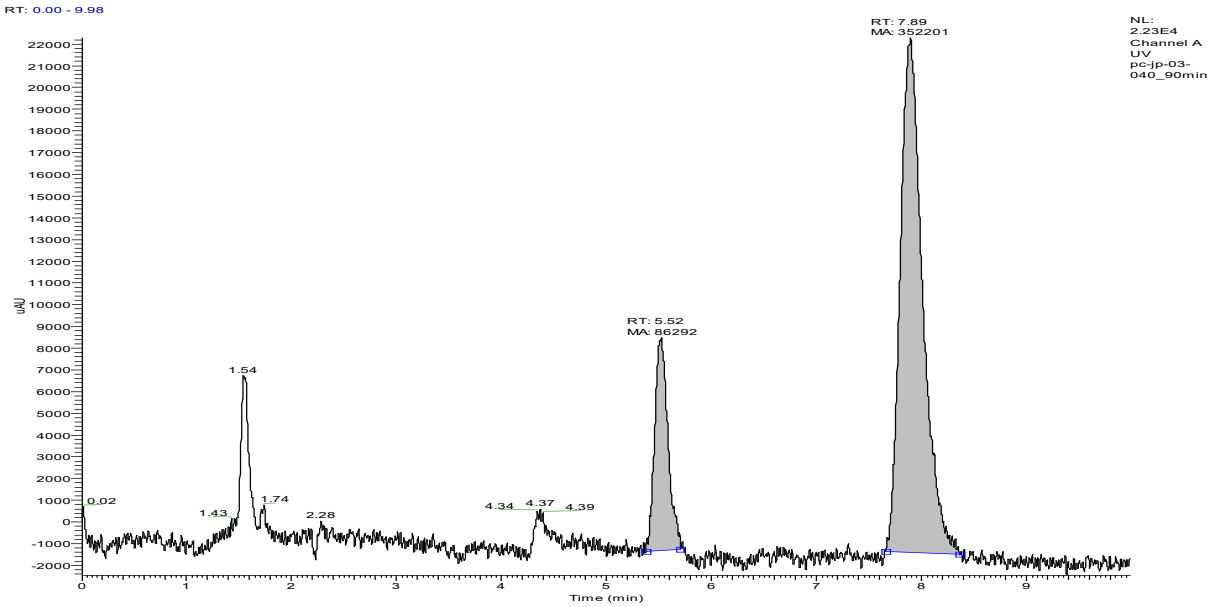
45 min - ratio of atropisomers *11 : 89*



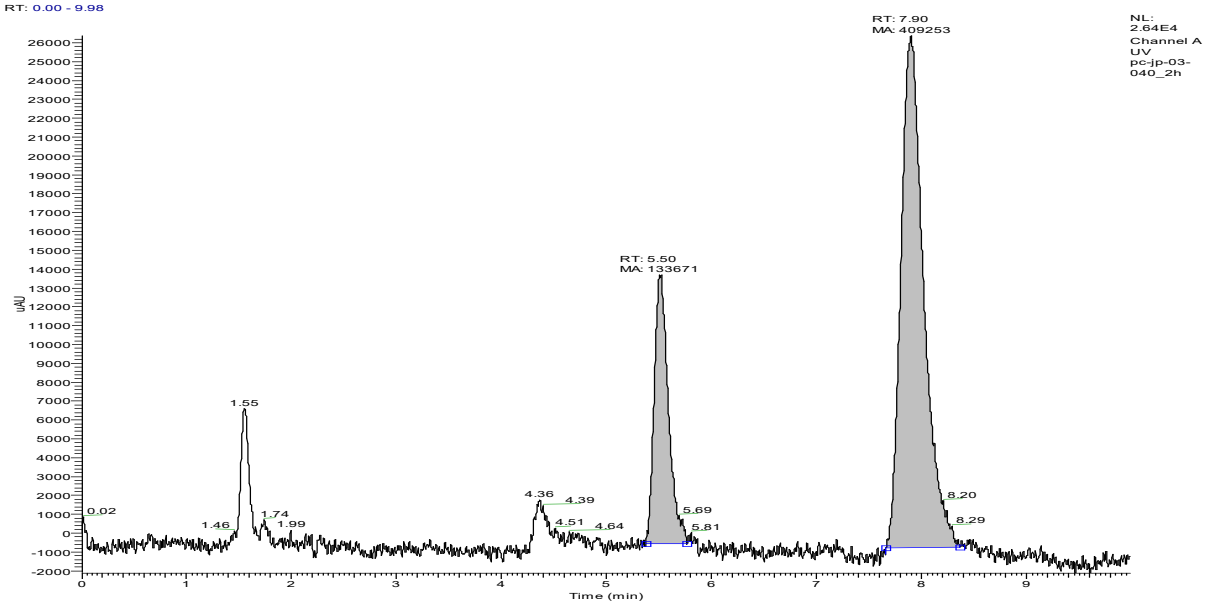
1 h - ratio of atropisomers *14 : 86*



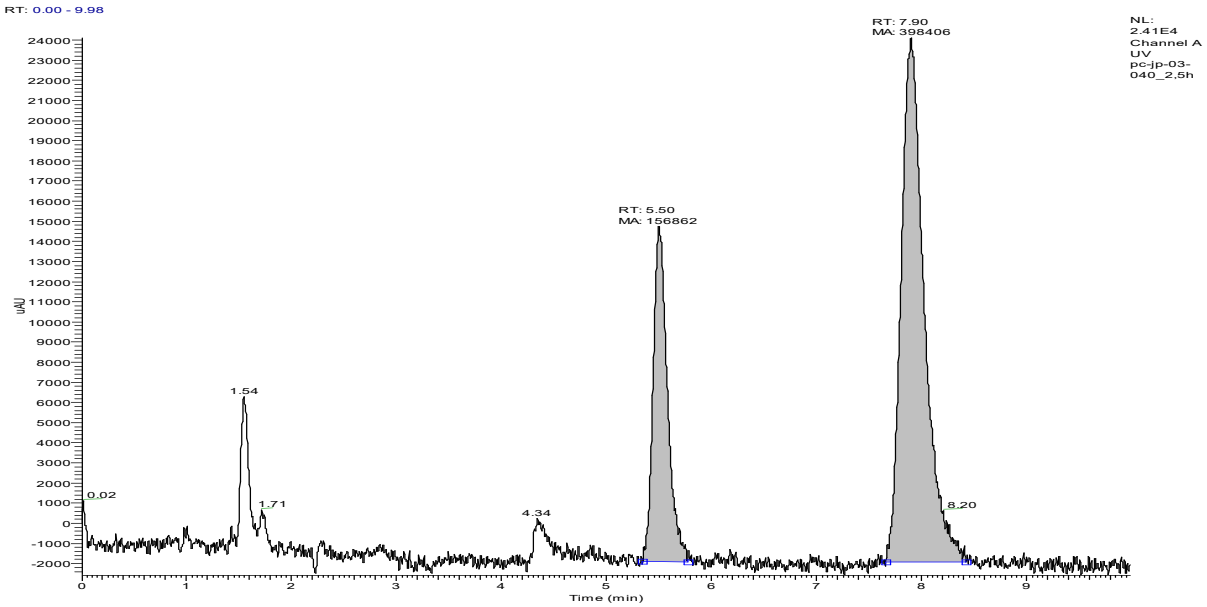
1.5 h - ratio of atropisomers *20 : 80*



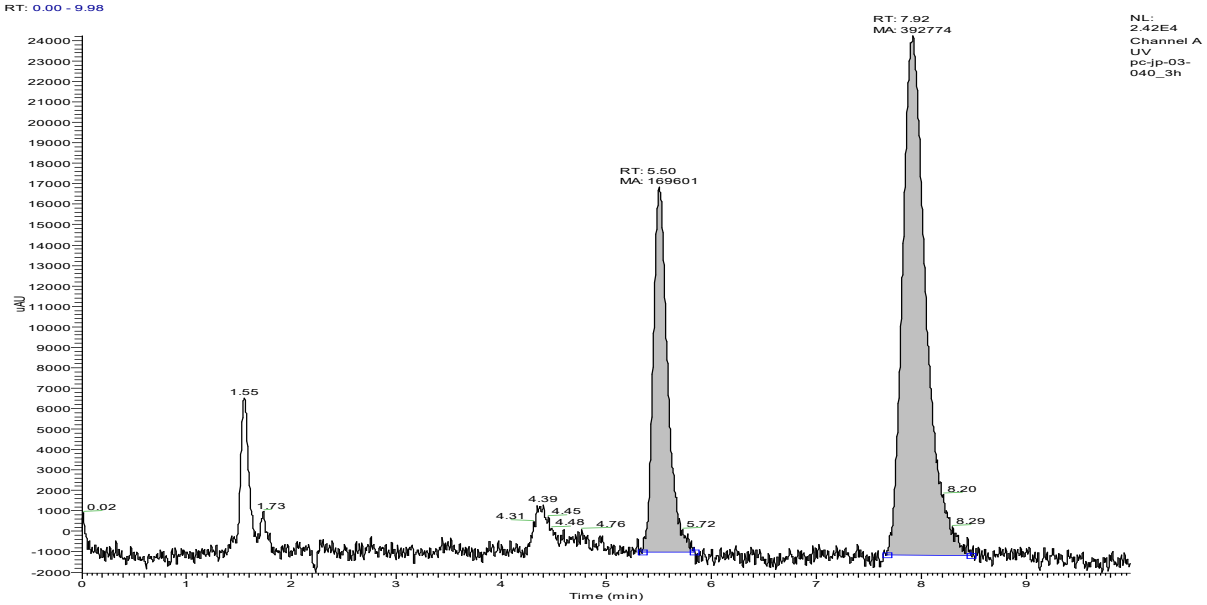
2 h - ratio of atropisomers *25 : 75*



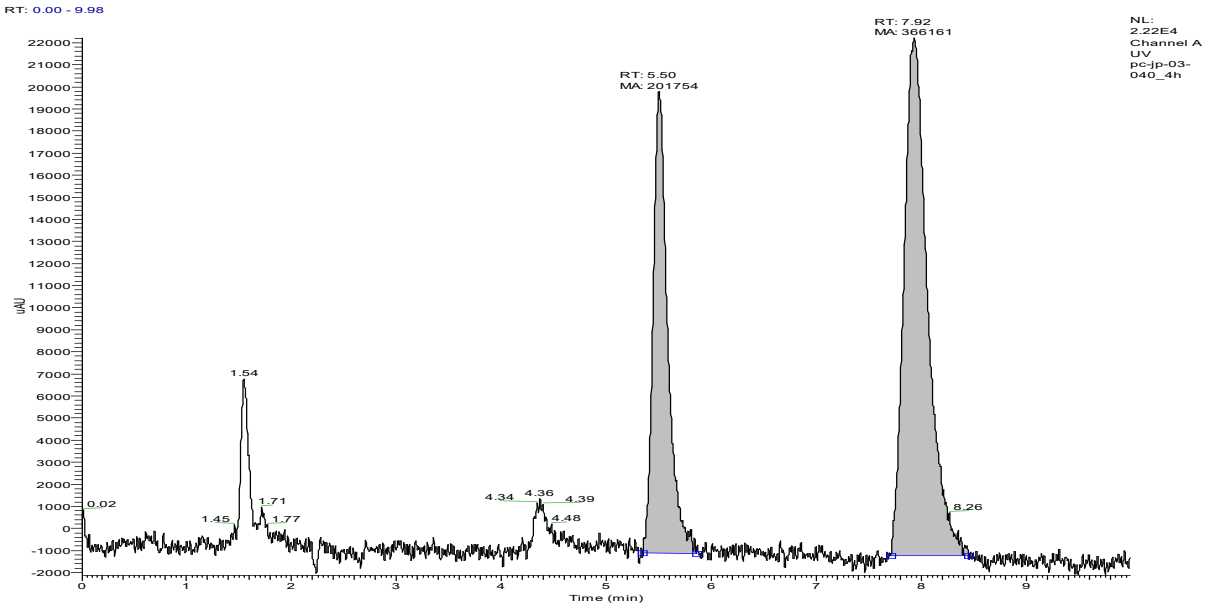
2.5 h - ratio of atropisomers *28 : 72*



3 h - ratio of atropisomers *30 : 70*



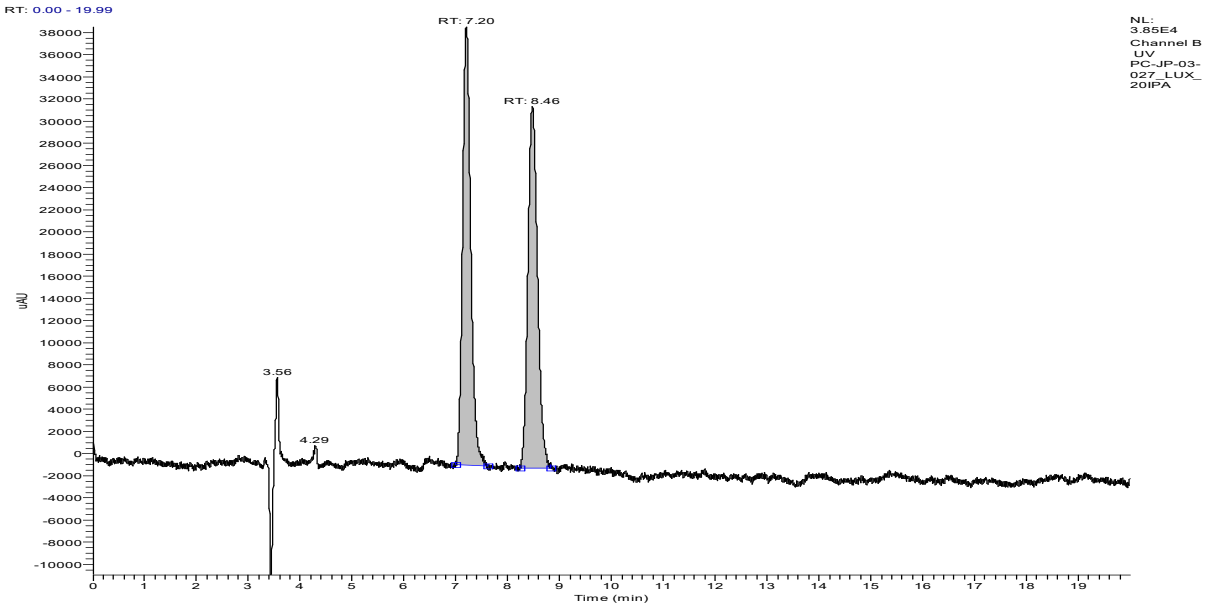
4 h - ratio of atropisomers *36 : 64*



Stability of (+)-5l at 100°C

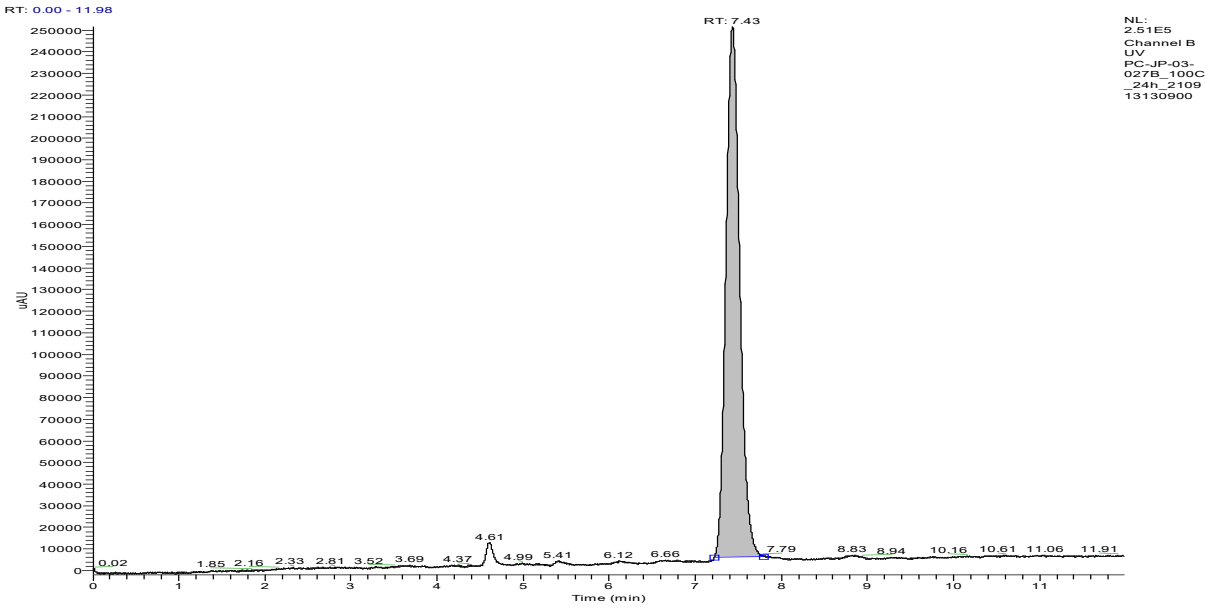
Column Lux i-Amylose-3, 5µm, 4.6mm x 250mm; mobil phase *n*-hexan/IPA 80:20, 0.8 mL/min, detection at 290 nm, time of analysis 12 min

Racemate:



Racemization conditions: compound **(+)-5n** (15 mg) was dissolved in ethylene glycol (0.5 mL). The solution was stirred at 100 °C for 4 h. A sample of solution (50 µL) was taken after 24 h for the analysis.

24 h – ratio of atropisomers *>99,9 :<0,1*



Semi-preparative chiral separation

For the chromatographic separation of both compounds, a modular HPLC system Smartline (Knauer, Berlin, Germany) consisting of Smartline Pump 1000, Smartline Manager 5000 and Smartline UV/VIS Detector 2600 was used. The detection wavelength was set to 210 nm. For analytical columns (4.6 mm i.d.), flow rate was set to 1 mL/min and injection volume was 20 µL (loop overfill mode was used). For semipreparative columns (10 mm i.d.), flow rate was set to 5 mL/min and injection volume was 1000 µL (also in loop overfill mode).

Analytical separation of **5l** was performed on a Lux Cellulose-1 column (Phenomenex, Torrance, California, USA) 250 × 4.6 mm, 5 µm particle size, using isocratic elution with hexane/ ethanol 1:1 (v/v). The stock solution of sample (2 mg/mL in ethanol) was diluted to 100 mg/L with the mobile phase before injection. For the semipreparative isolation of **5l**, a Lux Cellulose-1 (Phenomenex) 250 × 10 mm, 5 µm particle size column was used with a mixture of hexane/ethanol 9:1 (v/v) as the mobile phase in isocratic mode. The stock solution of sample (11.4 mg/mL in ethanol) was diluted to 4 mg/mL with the mobile phase before injection. The total analysis time was 6 min for the analytical mode and 15 min for the semipreparative mode.

Analytical separation of **5n** was performed on a Lux Cellulose-1 column (Phenomenex) 250 × 4.6 mm, 5 µm particle size, using isocratic elution with hexane/methanol/ethanol 14:1:1 (v/v/v). The flow rate was set to 1 mL/min and the injection volume was 20 µL. The stock solution of sample (2 mg/mL in ethanol) was diluted to 100 mg/L with the mobile phase before injection. For the semipreparative isolation of **5n**, a Lux Cellulose-1 (Phenomenex) 250 × 10 mm, 5 µm particle size column was used with a mixture of hexane/methanol/ethanol 40:1:1 (v/v/v) as the mobile phase in isocratic mode. The stock solution of sample (6.75 mg/mL in ethanol) was diluted to 2 mg/mL with the mobile phase before injection. The total analysis time was 12 min for the analytical mode and 35 min for the semipreparative mode.

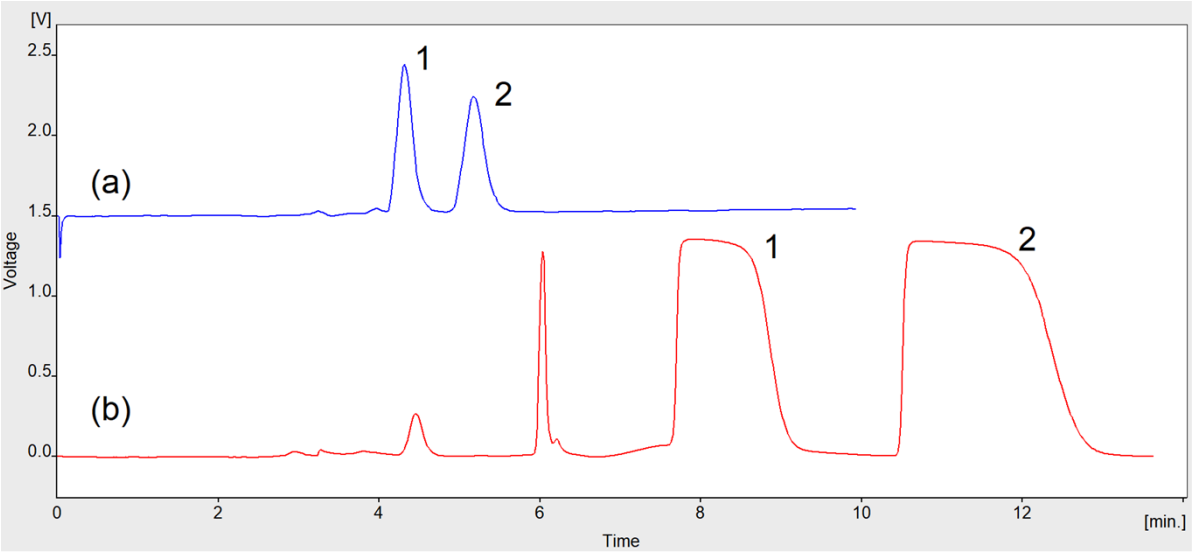


Fig. S1. Separation of **5l** in (a) analytical and (b) semipreparative mode (signal in analytical mode amplified 10× in Y axis).

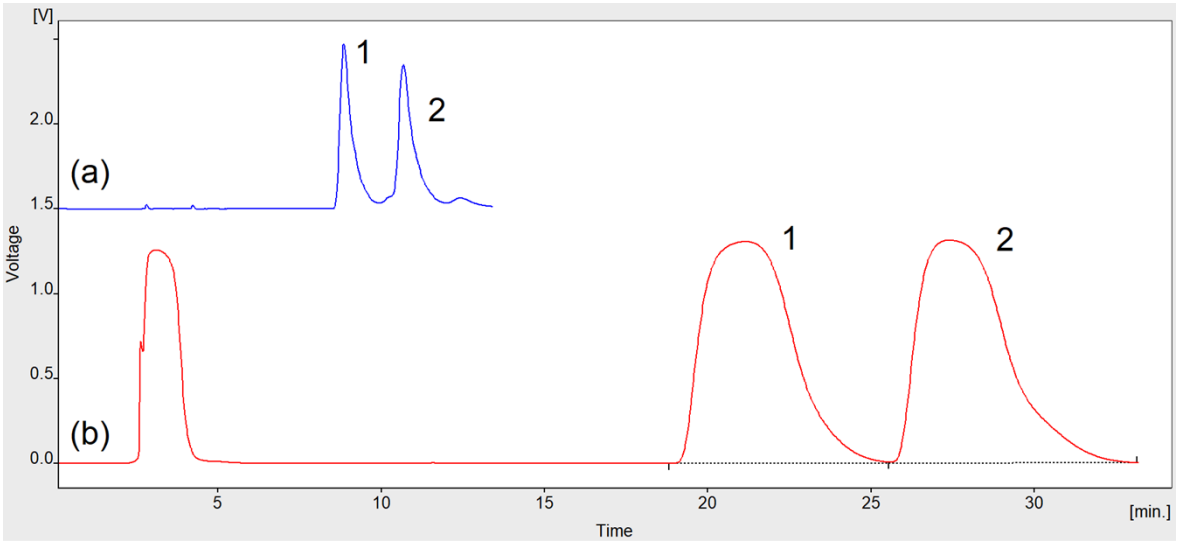
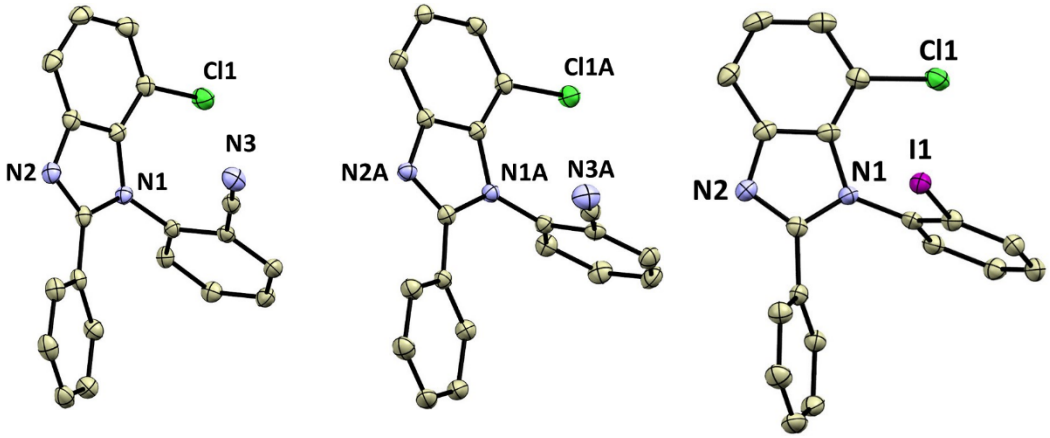


Fig. S2. Separation of **5n** in (a) analytical and (b) semipreparative (signal in analytical mode amplified 10× in Y axis).

Crystallography

The X-ray diffraction data for colorless crystals of ***(R$_a$)*-(+)-5n** and ***(R$_a$)*-(+)-5l** were collected using an XtaLAB Synergy-I diffractometer equipped with a HyPix3000 hybrid pixel array detector and a microfocused PhotonJet-I X-ray source (Cu Kα, 1.54184 Å). Absorption corrections were applied using the program CrysAlisPro 1.171.40.82a. The crystal structures were solved using SHELXT program and refined using the full matrix least-squares procedure with SHELXL in OLEX2 (version 1.3). All non-hydrogen atoms were refined anisotropically, while hydrogen atoms were located from the Fourier difference map and refined using the "riding" model. The crystal data and refinement details for ***(R$_a$)*-(+)-5n (CCDC 2285266)** and ***(R$_a$)*-(+)-5l (CCDC 2285267)** are presented in **Table S1**. Crystal structure of ***(R$_a$)*-(+)-5n** contains two molecules in its asymmetric unit. The structural visualizations were created using the Mercury 2020.2.0 software package (Cambridge Crystallography Data Centre, Cambridge, UK).



(*A*) (*B*)

Fig.S3 The crystal structures of ***(R$_a$)*-(+)-5n** (*A*) and ***(R$_a$)*-(+)-5l** (*B*). The hydrogen atoms were omitted for clarity. Colour code: carbon (light brown), chlorine (green), iodine (violet) and nitrogen (light blue). The thermal ellipsoids were drawn at a 50% probability level.

Table S1: Crystal data and structure refinement for ***(R$_a$)*-(+)-5n** and ***(R$_a$)*-(+)-5l**.

	***(R$_a$)*-(+)-5n**	***(R$_a$)*-(+)-5l**
Formula	$C_{20}H_{12}Cl_3N_3$	$C_{19}H_{12}ClIN_2$
M_r	329.78	430.66
Crystal system	monoclinic	orthorhombic
Space group	$P2_1$	$P2_12_12_1$
T/K	100.0(2)	100.0(2)
a (Å)	10.66903(6)	8.66885(4)
b (Å)	13.58537(6)	10.22838(6)
c (Å)	11.39283(5)	18.76755(10)
α (°)	90	90
β (°)	102.8862(5)	90
γ (°)	90	90
V (Å^3)	1609.720(13)	1664.085(15)
Z	4	4
λ (Å), Cu Kα	1.54184	1.54184

D_{calc} (g·cm^{-3})	1.361	1.719
μ (mm^{-1})	2.128	16.586
F (000)	680	840
Independent reflections	6013 [R_{int} = 0.0278, R_{sigma} = 0.0154]	3139 [R_{int} = 0.0483, R_{sigma} = 0.0144]
Data/restraints/parameters	6013/1/433	3139/0/208
Goodness-of-fit on F^2	1.034	1.116
Final R indices [$I > 2\sigma(I)$]	R_1 = 0.0242, wR_2 = 0.0620	R_1 = 0.0174, wR_2 = 0.0460
Final R indices (all data)	R_1 = 0.0243, wR_2 = 0.0621	R_1 = 0.0174, wR_2 = 0.0460
Flack parameter	0.003(6)	-0.006(2)
CCDC no.	2285266	2285267

Cell lines and cultivation media

Table S2: Cell lines and cultivation media

Human cell culture	Origin	Source	Catalogue number	Cultivation media	Cells per well seeded for 72h cytotoxicity test
K562	caucasian chronic myelogenous leukaemia	ECACC	89121407	DMEM + 10% FBS	5 000
HeLa	cervix epitheloid carcinoma	ECACC	93021013	DMEM + 10% FBS	5 000
MCF7	caucasian breast adenocarcinoma	ECACC	86012803	DMEM + 10% FBS	5 000
G-361	caucasian malignant melanoma	ECACC	88030401	DMEM + 10% FBS	5 000
hTERT RPE-1	immortalized human retinal pigment epithelial cells	ATCC	CRL-4000	DMEM + 10% FBS	5 000
MOLM-13	acute myeloid leukemia	DSMZ	ACC 554	RPMI 1640 + 10% FBS	20 000
MV-4-11	biphenotypic B-myelomonocytic leukaemia	ATCC	CRL-9591	RPMI 1640 + 10% FBS	20 000
A2780	human ovarian carcinoma	ECACC	93112519	RPMI 1640 + 10% FBS	5 000
THP-1	human monocytic leukaemia	ECACC	88081201	RPMI 1640 + 10% FBS	15 000
C4-2	prostate cancer	ATCC	CRL-3314	RPMI 1640 + 10% FBS	
CCRF-CEM	caucasian acute lymphoblastic leukaemia	ECACC	85112105	RPMI 1640 + 20% FBS	15 000

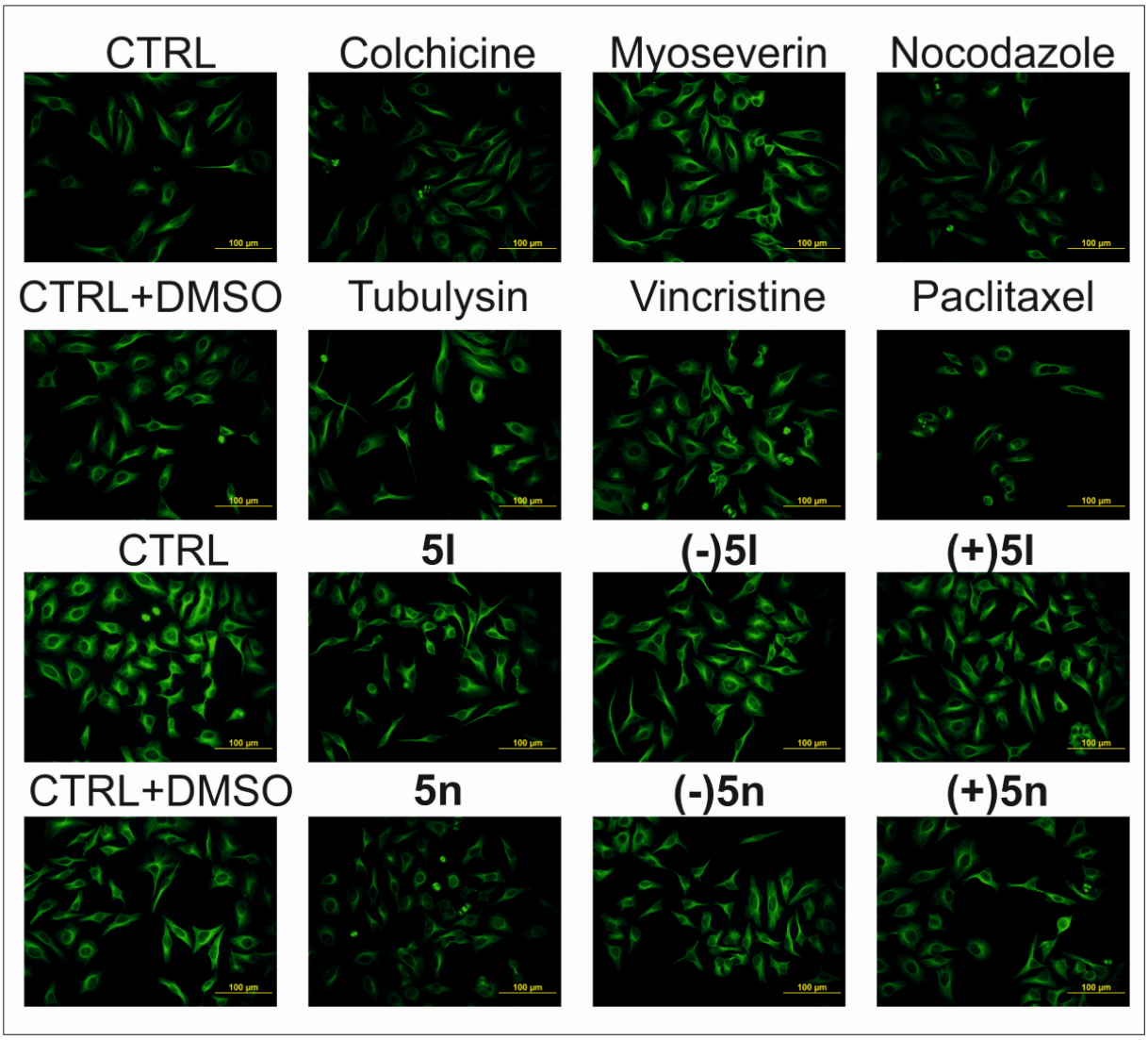


Fig. S4. Effects of racemic **5l** and **5n** and also their separated atropisomers on microtubules organisation of HeLa cells treated for 24 h with 10 nM **5l** or **5n**. Control means untreated cells, the CTRL+DMSO are control cells with DMSO in the same level as in treatment. Colchicine, myoseverin, nocodazole, paclitaxel, tubulysin or vincristine were used as positive controls.

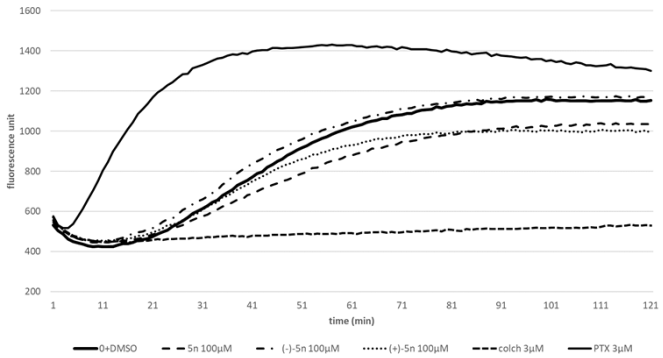


Fig. S5. Polymerization of tubulin in presence of paclitaxel (PTX), colchicine (colch), racemate **5n** and its atropisomers **(-)-5n** and **(+)-5n** during 120 min.

Further testing of the most interesting compounds in various cell lines

	R^1	R^2	R^3	K562	A-172	LNCaP C4-2	THP-1
5b	-Cl	$-CF_3$	-H	32.2 ± 1.0	60.1 ± 15.9	12.9±0.2	15.3 ± 5.2
5c	-Cl	-I	-H	27.7 ± 3.2	58.5 ± 4.3	12.6±0.6	16.7 ± 1.2
5f	-Cl	-Br	-H	22.4 ± 6.9	43.9 ± 11.5	14.9±1.9	9.6 ± 2.6
5i	-Cl	-I	$-CH_3$	29.4 ± 1.9	49.9 ± 1.0	23.4±6.3	14.7 ± 1.7
5l	-Cl	-I	-Ph	17.7 ± 3.9	18.6 ± 7.6	23.9±6.9	23.7 ± 0.2
5m	$-CF_3$	-I	-Ph	16.5	28.9	23.1±4.9	15.1
5n	-Cl	-CN	-Ph	9.3 ± 2.5	22.2 ± 1.1	12.4±3.8	16.4 ± 1.4

Table S3. Further testing of the most interesting compounds in various cell lines (IC_{50}; μM; 72 h*)*