ESIPT-Driven Imaging: A Thiazole Probe for Lipid Droplets and Plasma Membranes

José L. Belmonte-Vázquez,[a] Juan L. Cortes-Muñoz,[b] Adriana Romo-Pérez,[b] Braulio Rodríguez-Molina,*[b] Arturo Jiménez-Sánchez*[b]

[a] Departamento de Química Orgánica, Facultad de Química (FQ), Universidad Nacional Autónoma de México (UNAM), Ciudad Universitaria, Ciudad de México 04510, México.

[b] Instituto de Química, Universidad Nacional Autónoma de México, Circuito Exterior, Ciudad Universitaria, Ciudad de México, 04510, México.

Acknowledgments

J. L. B.-V. acknowledges financial support from DGAPA-PAPIIT (grant IA201524) and FQ-PAIP (grant 5000-9219). A. J.-S. thanks Conahcyt for grant PCC-319214, and J. L. C.-M. acknowledges support from Conahcyt (CVU: 740981). We appreciate the assistance of Ruth Rincón Heredia, Ph.D. (Unidad de Imagenología, IFC-UNAM) in imaging microscopy, Teresa Ramírez Apán, M.Sc., in tissue culture, and Everardo Tapia Mendoza, Ph.D., along with M. del Carmen García González and J. Pérez Flores, for HRMS studies. We also acknowledge R. A. Toscano for XRD studies, and Elizabeth Huerta Salazar, M.Sc., Beatriz Quiróz García, and Martha E. García-Aguilera, Ph.D., for their support in NMR analysis at LURMN, IQ-UNAM. The NMR facility is funded by CONACYT Mexico (0224747) and UNAM.

Experimental details

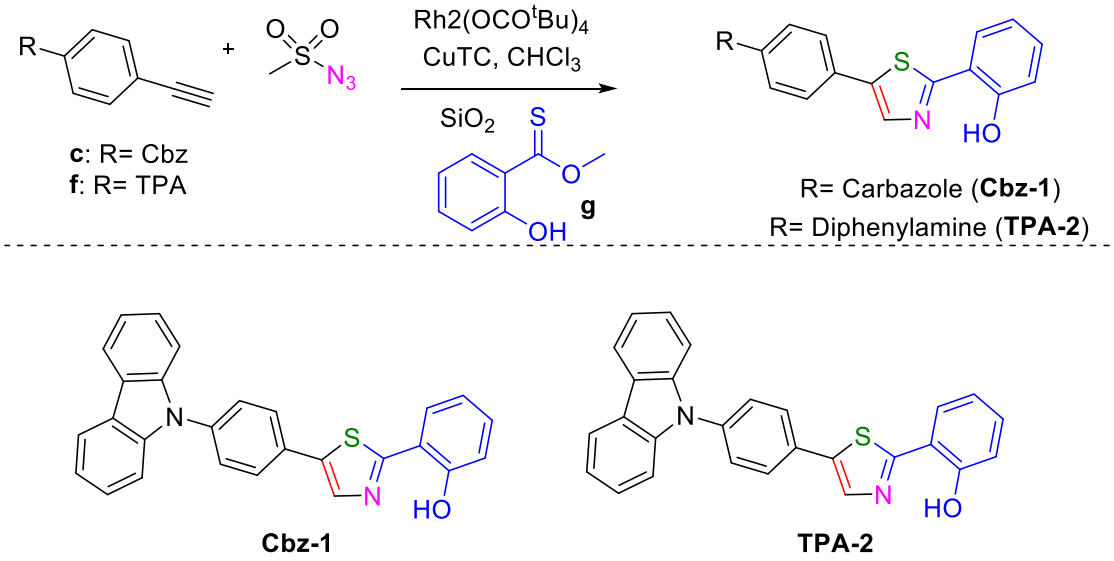
Materials and Methods

All reagents were purchased from Sigma Aldrich, J. T. Baker, and were used without further purification. Solvents were purchased locally and distilled before use. All experiments were carried out under nitrogen unless otherwise mentioned. NMR spectra were measured on a Bruker Avance III 400 MHz using $CDCl_3$ (residual peak: ^1H at 7.26 ppm, ^{13}C at 77.0 ppm) and DMSO-d_6 (residual peak: ^1H at 2.50 ppm, ^{13}C at 39.5 ppm). Data are reported in parts per million (ppm) reported as follows: singlet (s), doublet (d), triplet (t), and multiplet (m). Reaction progress was monitored using thin-layer chromatography (TLC) with EtOAc:hexanes or THF:hexanes mixtures as

eluent. High-Resolution Mass Spectra (HRMS) were recorded on a JEOL JMS-T100LC AccuTOF mass spectrometer via Direct Analysis in Real Time (DART). X-ray crystallographic analysis was acquired using a Bruker Apex-Duo and Bruker Smart Apex II. Absorption and emission spectra were measured using an FS5 Edinburgh Instruments spectrophotometers, for fluorescence emission spectra the excitation and emission slits were maintained constant at 1.5 nm using 1 cm quartz cells and regulated at 20 °C temperature. Melting points were determined using a Fischer-Johns apparatus and are uncorrected.

For the synthesis of starting materials, such as mesyl azide, compounds **a**, **b**, and **c** were prepared under the same conditions as described in our previous work. Compounds **g**, and **d** were synthesized according to the reported literature procedures and the experimental data obtained were consistent with the reported values.

In Scheme S1 it is shown the general reaction sequence for the synthesis of compounds **Cbz-1** and **TPA-2**.



Scheme S1. Schematic representation for the synthesis of compounds **Cbz-1** and **TPA-2**.

Synthetic procedures

General procedure for the Ullmann coupling reaction (GP1).

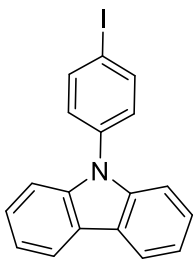
In a dry round-bottom flask under a nitrogen atmosphere and equipped with a magnetic stirrer R_2NH (1.0 equiv.), 1,4-diiodobenzene (2.0 equiv.), CuI (0.05 equiv.), 18-crown-6 (0.02 equiv.), K_2CO_3 (2.0 equiv.), and DMPU were added. The reaction mixture was stirred at reflux for the specified time. After completion of the reaction, a saturated NH_4Cl solution was added, and the mixture was extracted with DCM. The organic phase was dried over anhydrous Na_2SO_4, and the excess of solvent was removed under vacuum. The crude product was absorbed onto silica gel and purified by column chromatography using the appropriate eluent.

General procedure for the Sonogashira cross-coupling reaction (GP2).

In a dry round-bottom flask under a nitrogen atmosphere and equipped with a magnetic stirrer, an aryl halide (1.0 equiv.), $PdCl_2(PPh_3)_2$ (0.10 equiv.), CuI (0.05 equiv.), Et_3N, and ethynyltrimethylsilane (1.0 equiv.) were added. The reaction mixture was stirred at room temperature for the specified time. Then, a saturated NH_4Cl solution was added, and extractions were carried out with DCM (3 X 20 mL). The organic phase was dried over anhydrous Na_2SO_4, and the excess of solvent was evaporated under vacuum. The crude product was adsorbed onto silica gel and purified by column chromatography using the appropriate eluent.

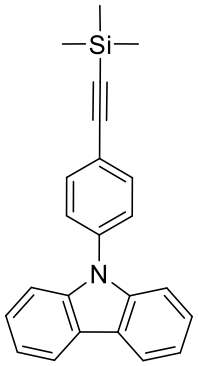
General procedure for the desilylation reaction (GP3).

A round-bottom flask was charged with R-$SiMe_3$ (1.0 equiv.), K_2CO_3 (0.2 equiv.), and MeOH. The reaction mixture was stirred overnight at room temperature. Then, water was added, and the mixture was extracted with EtOAc. The organic phase was dried over anhydrous Na_2SO_4, filtered, and the excess of solvent was evaporated under vacuum. The crude product was adsorbed onto silica gel and purified by column chromatography using hexanes as the eluent.

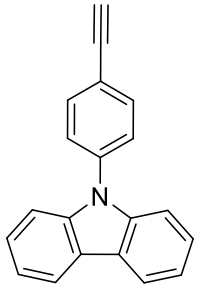


9-(4-iodophenyl)-9H-carbazole (**a**) (According to GP1). Carbazole (1.000 g, 6.0 mmol, 1.0 equiv.), 1,4-diiodobenzene (3.946 g, 12.0 mmol, 2.0 equiv.), CuI (0.057 g, 0.3 mmol, 0.05 equiv.), 18-crown-6 (0.025 g, 0.1 mmol, 0.02 equiv.), K_2CO_3 (1.653 g, 12.0 mmol, 2.0 equiv.), and DMPU (12.0 mL). The reaction mixture is stirred at reflux for 15 h. Then, 80 mL of a saturated NH_4Cl solution was added, and extractions were

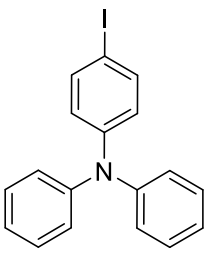
carried out with DCM (3 X 60 mL). The product was purified using hexanes as eluent. The product was obtained as a white solid (1.5 g, 4.1 mmol, 68%). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.15 (d, *J* = 7.8 Hz, 2H), 7.93 (d, *J* = 9.0 Hz, 2H), 7.45 – 7.38 (m, 4H), 7.36 – 7.29 (m, 4H).



9-(4-((trimethylsilyl)ethynyl)phenyl)-9*H*-carbazole (**b**) (According to GP2). **a** (0.500 g, 1.4 mmol, 1.0 equiv.), $PdCl_2(PPh_3)_2$ (0.095 g, 0.14 mmol, 0.10 equiv.), CuI (0.013 g, 0.07 mmol, 0.05 equiv.), THF (6.0 mL), Et_3N (1.0 mL), and ethynyl trimethylsilane (0.133 g, 1.4 mmol, 1.0 equiv.). The reaction mixture was stirred at room temperature for 2 h. Then, 10 mL of a saturated NH_4Cl solution was added, and extractions were carried out with DCM (3 X 20). The product was purified using hexanes as eluent. The product was obtained as a white solid (0.436 g, 1.3 mmol, 95%). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.14 (d, *J* = 7.8 Hz, 2H), 7.70 (d, *J* = 8.5 Hz, 2H), 7.52 (d, *J* = 8.5 Hz, 2H), 7.44 – 7.38 (m, 4H), 7.34 – 7.25 (m, 2H), 0.30 (s, 9H).

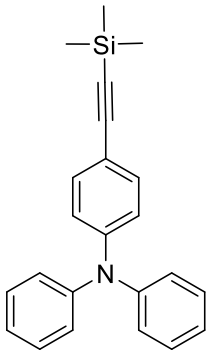


9-(4-ethynylphenyl)-9*H*-carbazole (**c**) (According to GP3). **b** (0.400 g, 1.2 mmol, 1.0 equiv.), K_2CO_3 (0.033 g, 0.2 mmol, 0.2 equiv.), and MeOH (8.0 mL). The reaction mixture was stirred overnight at room temperature. Then, 10 mL of water was added, and extractions were carried out with EtOAc (3 X 30 mL). The crude was purified using hexanes as eluent. The product was obtained as a yellow solid (0.299 g, 1.1 mmol, 95%). TLC (10 % EtOAc/hexanes, R_f = 0.66); m.p. 106-107 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 8.14 (d, *J* = 7.8 Hz, 2H), 7.73 (d, *J* = 8.5 Hz, 2H), 7.55 (d, *J* = 8.5 Hz, 2H), 7.44 – 7.39 (m, 4H), 7.35 – 7.26 (m, 2H), 3.18 (s, 1H).

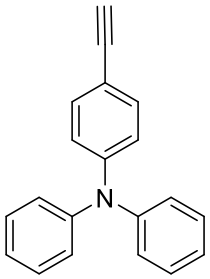


4-iodo-*N*,*N*-diphenylaniline (**d**). In a dry round-bottom flask under a nitrogen atmosphere and equipped with a magnetic stirrer, diphenylamine (0.338 g, 2.0 mmol, 1.0 equiv.), 1,4-diiodobenzene (1.317 g, 4.0 mmol, 2.0 equiv.), CuI (0.019 g, 0.10 mmol, 0.05 equiv.), 1,10-phenantroline (0.011 g, 0.04 mmol, 0.02 equiv.), KOH

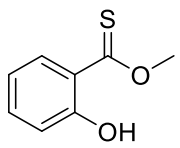
(0.552 g, 4.0 mmol, 2.0 equiv.), and *o*-dichlorobenzene (2.0 mL) were charged. The reaction mixture was stirred for 4 h at 180 °C. Then, 10 mL of water was added, and the mixture was extracted with DCM (3 X 10 mL). The organic phase was dried over anhydrous Na_2SO_4, and the excess of solvent was evaporated under vacuum. The crude was adsorbed onto silica gel for purification by column chromatography using hexanes as the eluent. The desired product was obtained as a pale pink solid (0.468 g, 1.3 mmol, 63%). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.49 (d, *J* = 7.6 Hz, 2H), 7.33 – 7.16 (m, 4H), 7.10 – 6.95 (m, 6H), 6.82 (d, *J* = 7.2 Hz, 2H).



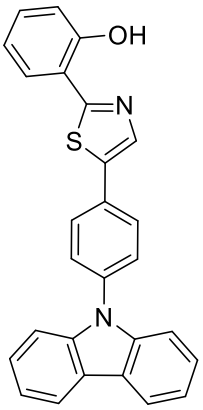
***N,N*-diphenyl-4-((trimethylsilyl)ethynyl)aniline** (**e**). According to GP2. **d** (1.000 g, 2.7 mmol, 1.0 equiv.), $PdCl_2(PPh_3)_2$ (0.189 g, 0.27 mmol, 0.10 equiv.), CuI (0.026 g, 0.13 mmol, 0.05 equiv.), THF (13.0 mL), Et_3N (2.0 mL), and ethynyl trimethylsilane (0.265 g, 2.7 mmol, 1.0 equiv.). The reaction mixture was stirred at room temperature for 2 h. Then, 10 mL of a saturated NH_4Cl solution was added, and the mixture was extracted with DCM (3 X 20 mL). The product was purified using hexanes as eluent. The product was obtained as a yellow oil (0.423 g, 1.2 mmol, 68%). TLC (hexanes, R_f = 0.47). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.34 (d, *J* = 8.7 Hz, 2H), 7.32 – 7.26 (m, 4H), 7.15 – 7.04 (m, 6H), 6.98 (d, *J* = 8.7 Hz, 2H), 0.27 (s, 9H). ^{13}C NMR (101 MHz, $CDCl_3$) δ: 148.2, 147.3, 133.1, 129.5, 125.1, 123.7, 122.3, 116.1, 105.5, 93.2, 0.2. HRMS (DART): calculated for $C_{23}H_{24}NSi$ $[M+H]^+$ 342.16780; found 342.16689 (Fig. S23).



4-ethynyl-*N,N*-diphenylaniline (**f**). According to GP3. **e** (0.915 g, 2.7 mmol, 1.0 equiv.), K_2CO_3 (0.074 g, 0.5 mmol, 0.2 equiv.), and MeOH (16.0 mL). The product was purified using hexanes as the eluent. The product was obtained as a pale yellow solid (0.522 g, 1.9 mmol, 72%). TLC (10 % EtOAc/hexanes, R_f = 0.68); m.p. 106-107 °C. ^{1}H NMR (400 MHz, $CDCl_3$) δ: 7.33 (d, J = 8.8 Hz, 2H), 7.30 – 7.22 (m, 4H), 7.13 – 7.02 (m, 6H), 6.96 (d, *J* = 8.7 Hz, 2H), 3.02 (s, 1H). ^{13}C NMR (101 MHz, $CDCl_3$) δ: 148.5, 147.3, 133.2, 129.6, 125.2, 123.8, 122.2, 114.9, 84.1, 76.3. HRMS (DART): calculated for $C_{20}H_{16}N$ $[M+H]^+$ 269.1204; found 269.1205 (Fig. S24).

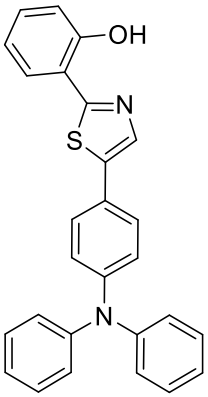


***O*-methyl 2-hydroxybenzothioate** (**g**). In a round-bottom flask methyl-2-hydroxybenzoate (2.000 g, 13.1 mmol, 1.0 equiv.), Lawesson reagent (5.317 g, 13.1 mmol, 2.0 equiv.), and *p*-xylene (60 mL). The reaction mixture was stirred for 24 h at 130 °C. Then, 100 mL of hexane was added, and the reaction mixture was passed through a silica gel pad. The excess of solvent was evaporated under vacuum, and the crude was adsorbed onto silica gel for purification by column chromatography using hexanes as the eluent. The product was obtained as a yellow liquid (0.586 g, 3.5 mmol, 27%). TLC (10 % EtOAc/hexanes, R_f = 0.71). ^{1}H NMR (400 MHz, $CDCl_3$) δ: 12.00 (s, 1H), 7.97 (d, *J* = 10.0 Hz, 1H), 7.44 (t, *J* = 8.6 Hz, 1H), 7.05 (d, *J* = 9.6 Hz, 1H), 6.86 (t, *J* = 8.3 Hz, 1H), 4.27 (s, 3H). ^{13}C NMR (101 MHz, $CDCl_3$) δ: 209.8, 161.9, 135.8, 127.0, 121.1, 119.4, 118.9, 58.1. HRMS (DART): calculated for $C_8H_9O_2S$ $[M+H]^+$ 168.0245; found 168.0252 (Fig. S25).



Compound **Cbz-1**. To an over-dried thick-walled reaction tube equipped with a stirrer bar the 9-(4-ethynylphenyl)-9*H*-carbazole (0.050 g, 0.2 mmol, 1.0 equiv.), mesyl azide (0.023 g, 0.2 mmol, 1.0 equiv.), CuTC (0.004 g, 0.02 mmol, 0.10 equiv.), molecular sieves 4Å (20 mg), and $CHCl_3$ (3.0 mL) were added. The tube was sealed, and the reaction was stirred at room temperature for 24h. Then, $Rh_2(OCO^tBu)_4$ (0.002 g, 0.004 mmol, 0.02 equiv.), and the O-methyl 2-hydroxybenzothioate (0.063 mg, 0.4 mmol, 2.0 equiv.) were added to the reaction mixture. The reaction mixture was heated at 70 ºC for 2 hours, and then cooled to room temperature, silica gel (Aldrich, 0.4 gr) was added. The reaction mixture was stirred at room temperature for 4 h. Then, the crude material was passed through a short pad of Na_2SO_4 (Anhydride) and eluted with ethyl acetate. The filtrate was concentrated under reduced pressure, adsorbed in SiO_2, and purified by column chromatography using 30% of DCM:hexanes. The desired product was obtained as a green solid (0.010 g, 0.02 mmol, 13%). TLC (10 % EtOAc/hexanes, R_f = 0.48); m.p. 228-230 °C. ^{1}H NMR (400 MHz, DMSO-d_6) δ: 11.34 (s, 1H), 8.45 (s, 1H), 8.27 (d, *J* = 7.8 Hz, 2H), 8.16 (d, *J* = 7.9 Hz, 1H), 8.03 (d, *J* = 8.5 Hz, 2H), 7.73 (d, *J* = 8.6 Hz, 2H), 7.53 – 7.42 (m, 4H), 7.39 – 7.24 (m, 3H),

7.08 (d, J = 7.1 Hz, 1H), 7.00 (t, *J* = 7.5 Hz, 1H). ^{13}C NMR (101 MHz, DMSO-d_6) δ: 162.8, 155.0, 140.0, 138.5, 137.3, 136.5, 131.2, 130.3, 127.9, 127.4, 127.2, 126.3, 122.9, 120.6, 120.2, 119.6, 119.1, 116.6, 109.7. HRMS (DART): calculated for $C_{27}H_{19}N_2OS$ $[M+H]^+$ 419.12181; found 419.12065 (Fig. S26).



Compound **TPA-2**. To an over-dried thick-walled reaction tube equipped with a stirrer bar the corresponding 4-ethynyl-*N*,*N*-diphenylaniline (0.200 g, 0.7 mmol, 1.0 equiv.), mesy lazide (0.090 g, 0.7 mmol, 1.0 equiv.), CuTC (0.014 g, 0.07 mmol, 0.10 equiv.), molecular sieves 4Å (80 mg), and $CHCl_3$ (12.0 mL) were added. The tube was sealed, and the reaction was stirred at room temperature for 24h. Then, $Rh_2(OCO^tBu)_4$ (0.009 g, 0.01 mmol, 0.02 equiv.), and the O-methyl 2-hydroxybenzothioate (0.250 mg, 1.5 mmol, 2.0 equiv.) were added to the reaction mixture. Then, the reaction mixture was heated at 70 ºC for 2 h, and then cooled to room temperature, silica gel (Aldrich, 3.2 gr) was added. The reaction mixture was stirred at room temperature for 4 h. Then, the crude material was passed through a short pad of Na_2SO_4 (anhydride), and eluted with ethyl acetate. The filtrate was concentrated under reduced pressure, adsorbed in SiO_2, and purified by column chromatography using 30% of DCM:hexanes. The desired product was obtained as a green solid (0.037 g, 0.1 mmol, 12%). TLC (10 % EtOAc/hexanes, R_f = 0.62); m.p. 140-143 °C. ^{1}H NMR (400 MHz, Acetone-d_6) δ: 12.01 (s, 1H), 8.15 (s, 1H), 7.74 (d, *J* = 7.9 Hz, 1H), 7.64 (d, *J* = 9.0 Hz, 2H), 7.40 – 732 (m, 5H), 7.17 – 7.05 (m, 8H), 7.03 – 6.94 (m, 2H). ^{13}C NMR (101 MHz, Acetone-d_6) δ: 167.8, 157.9, 149.5, 148.2, 138.7, 137.0, 132.6, 130.5, 128.5, 128.1, 125.9, 124.9, 124.7, 123.7, 120.5, 118.3, 117.8. HRMS (DART): calculated for $C_{27}H_{21}N_2OS$ $[M+H]^+$ 421.13746; found 421.13735 (Fig. S27).

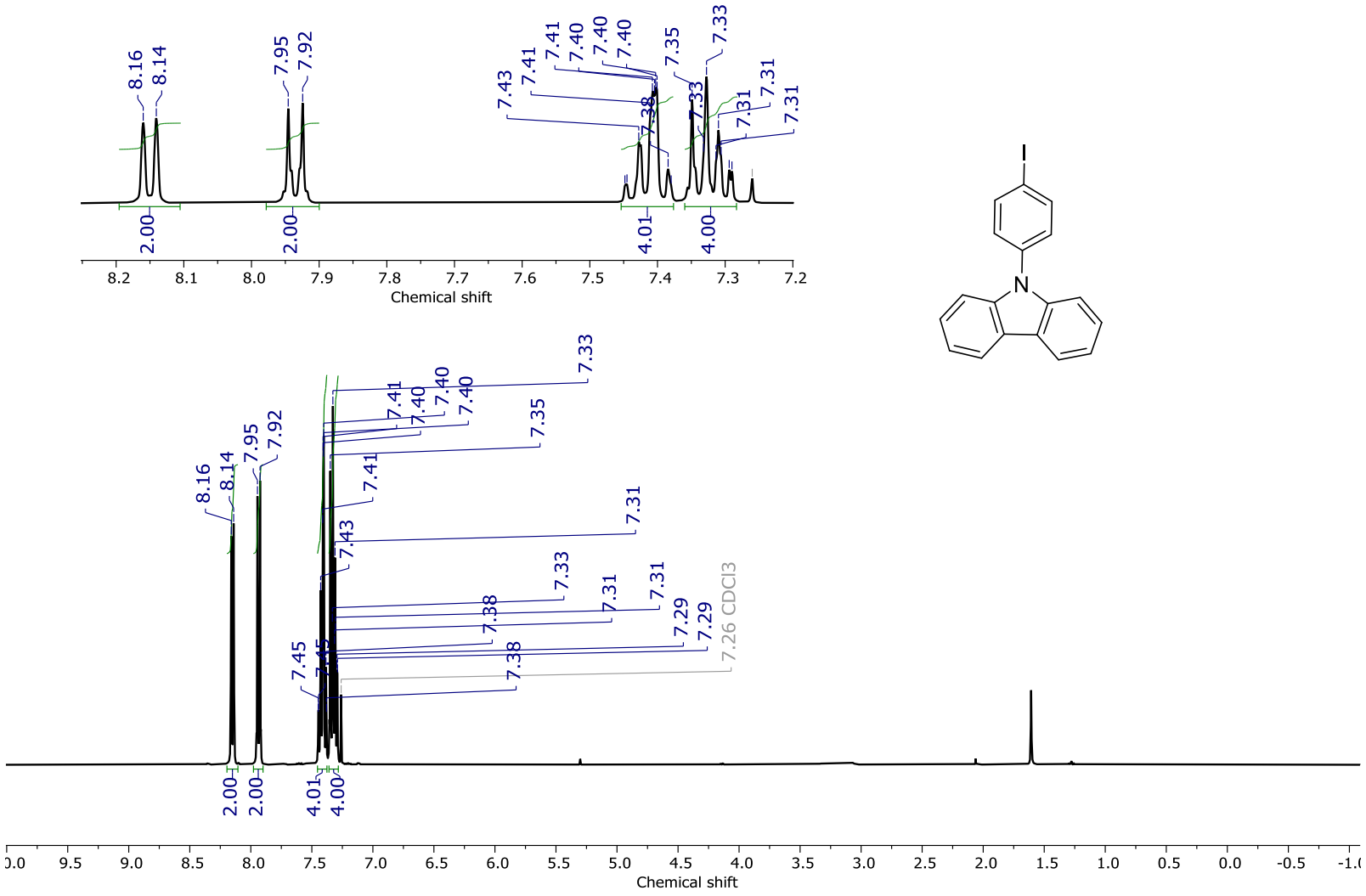


Figure S1. ^{1}H NMR of compound **a** (400 MHz, $CDCl_3$).

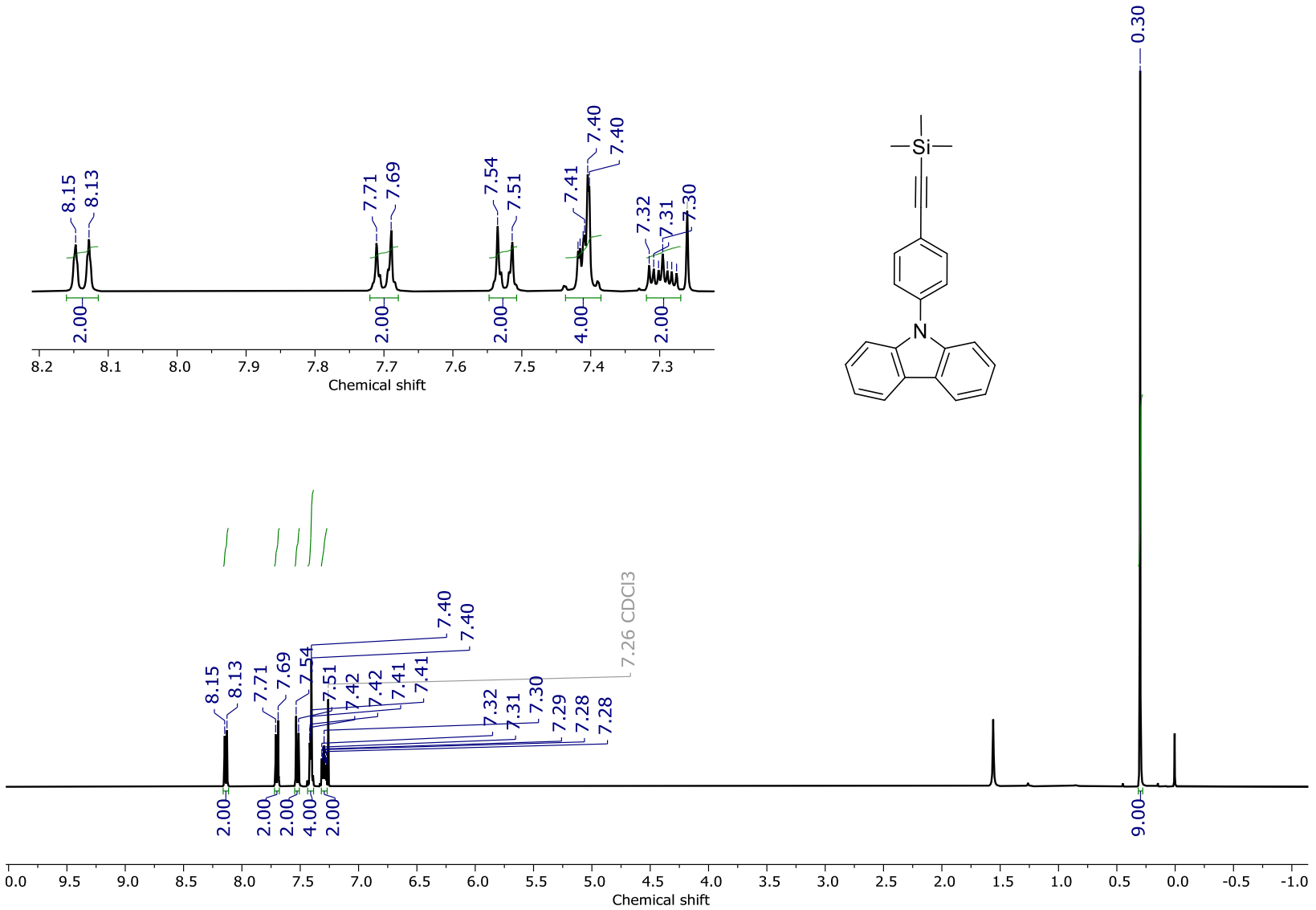


Figure S2. ^{1}H NMR of compound **b** (400 MHz, $CDCl_3$).

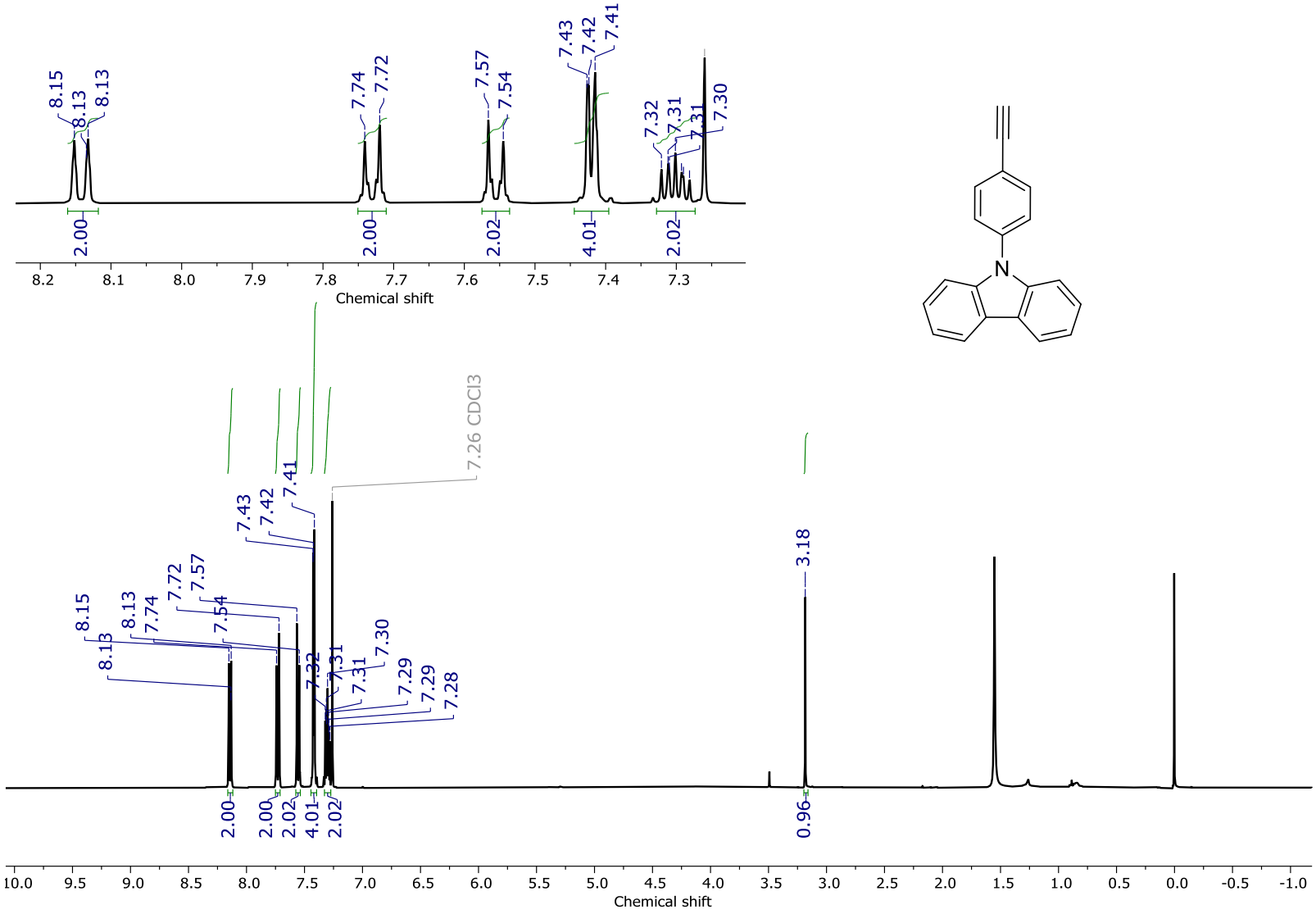


Figure S3. ^{1}H NMR of compound **c** (400 MHz, $CDCl_3$).

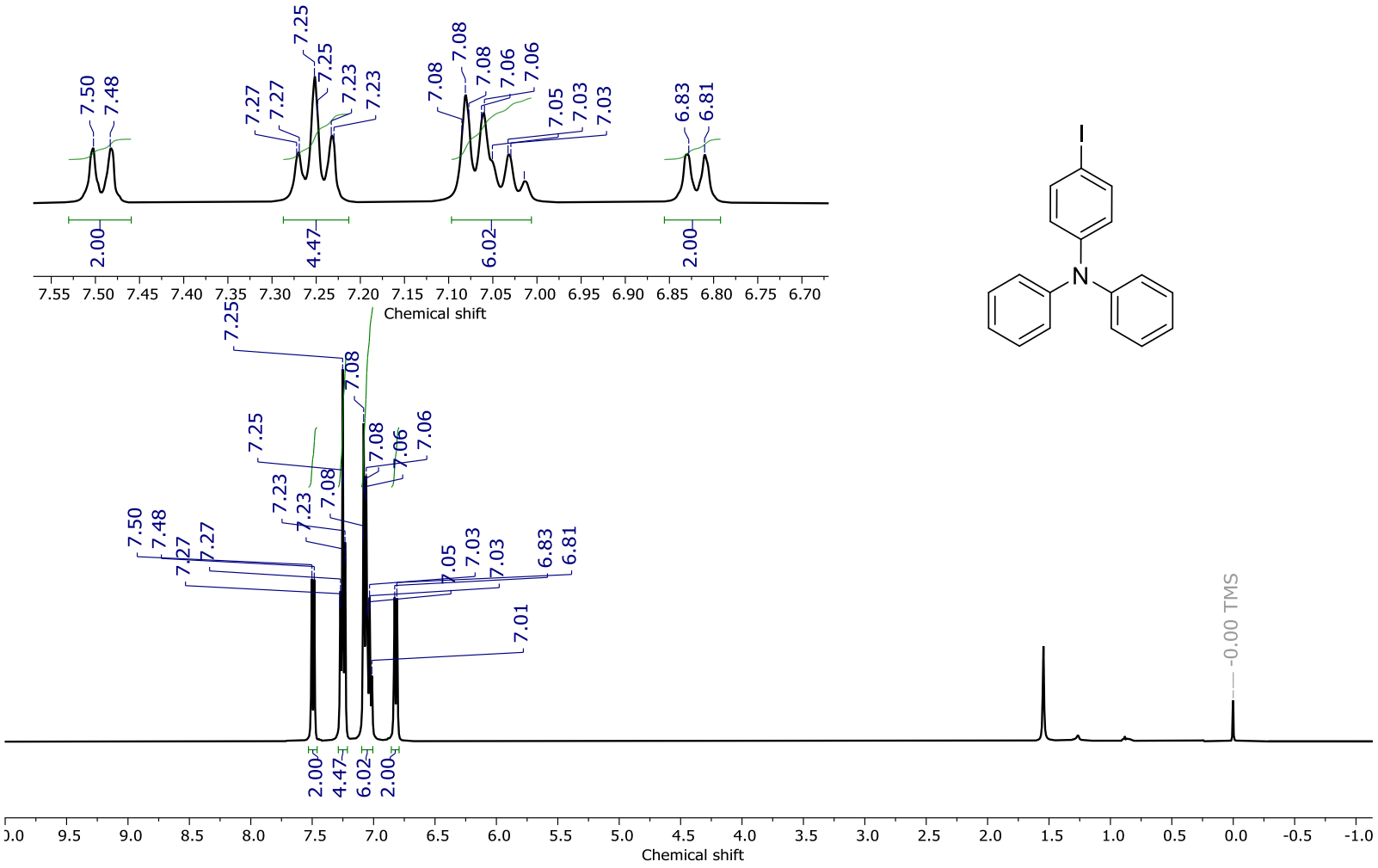


Figure S4. ^{1}H NMR of compound **d** (400 MHz, $CDCl_3$).

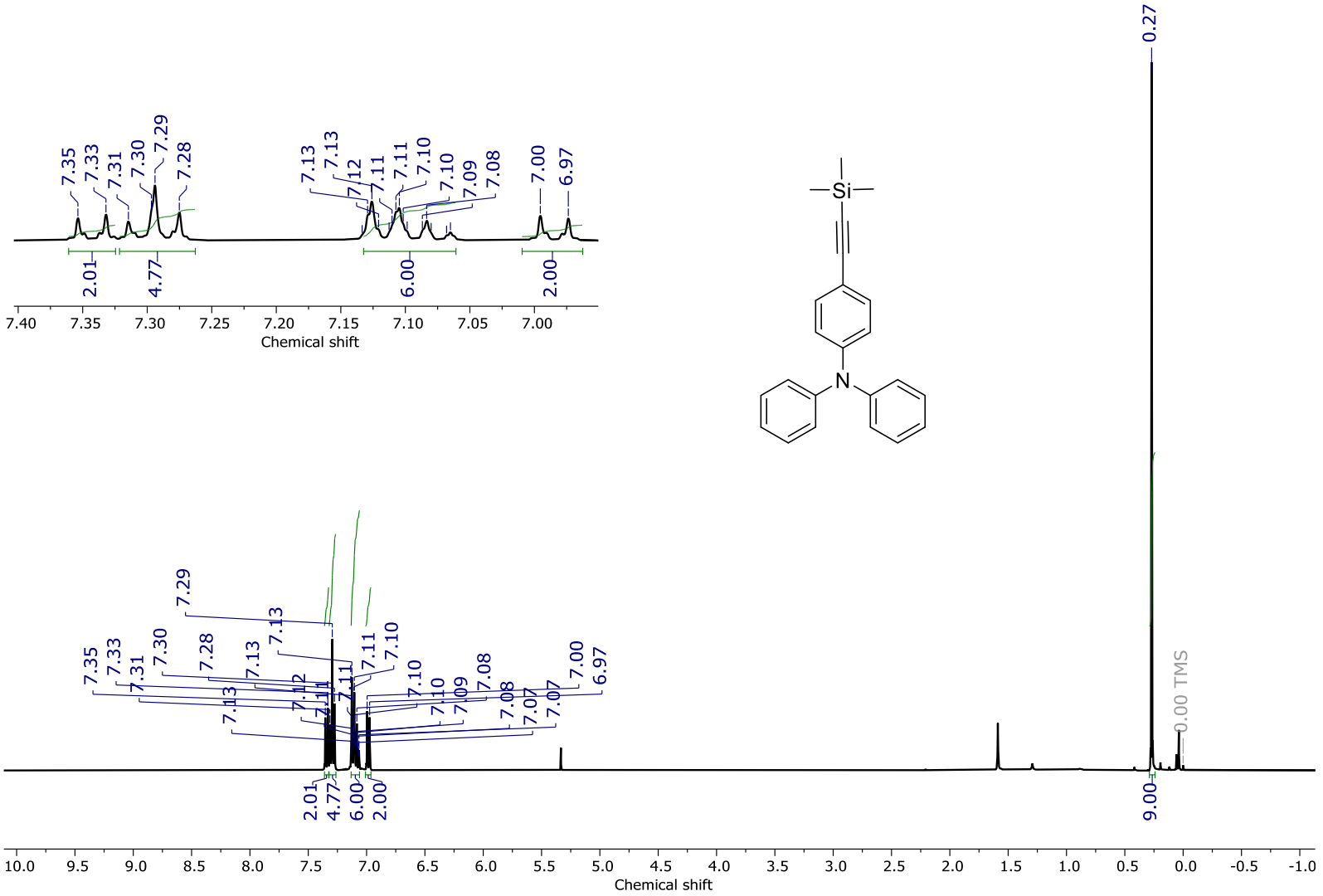


Figure S5. ^{1}H NMR of compound **e** (400 MHz, $CDCl_3$).

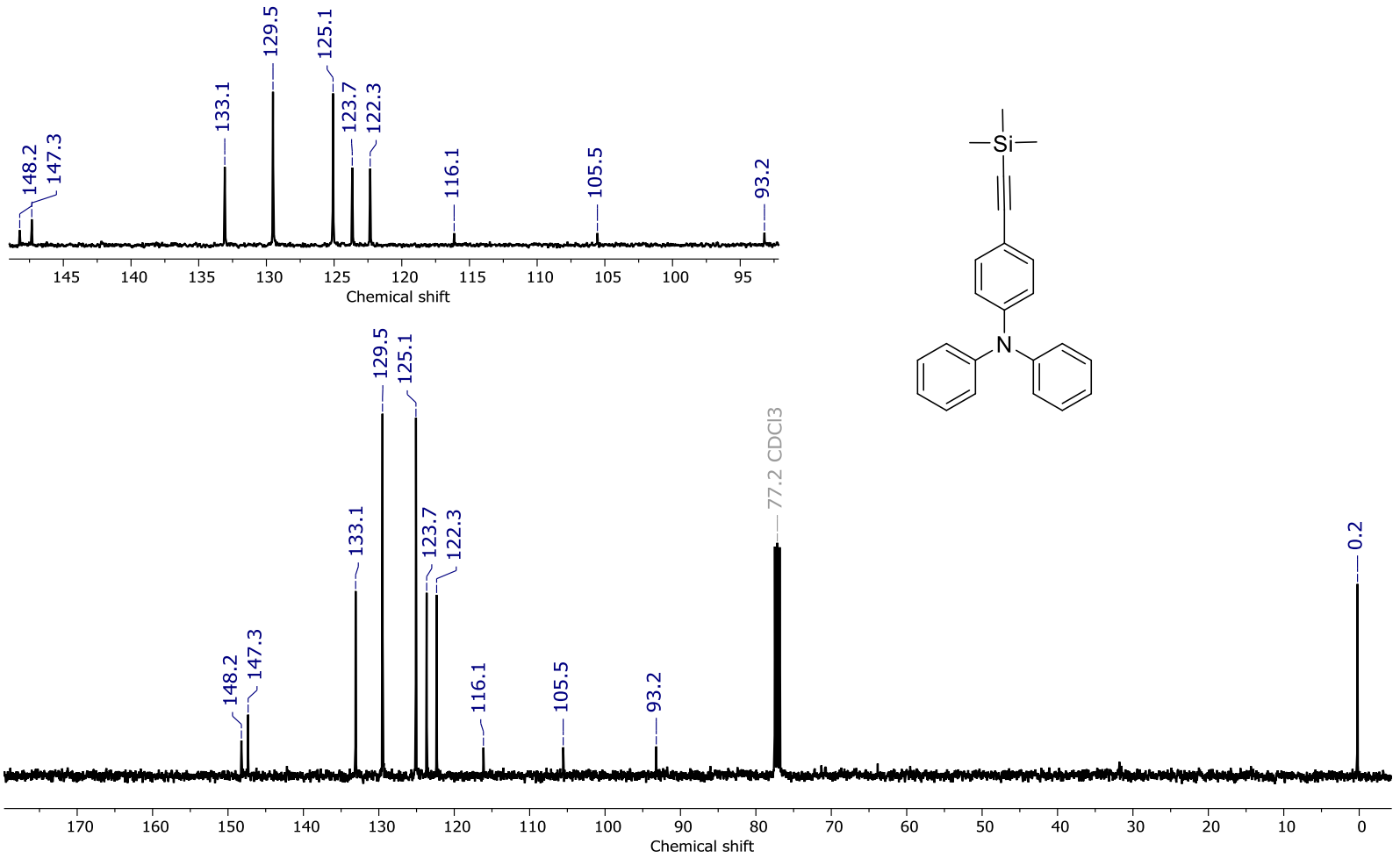


Figure S6. ^{13}C NMR of compound **e** (400 MHz, $CDCl_3$).

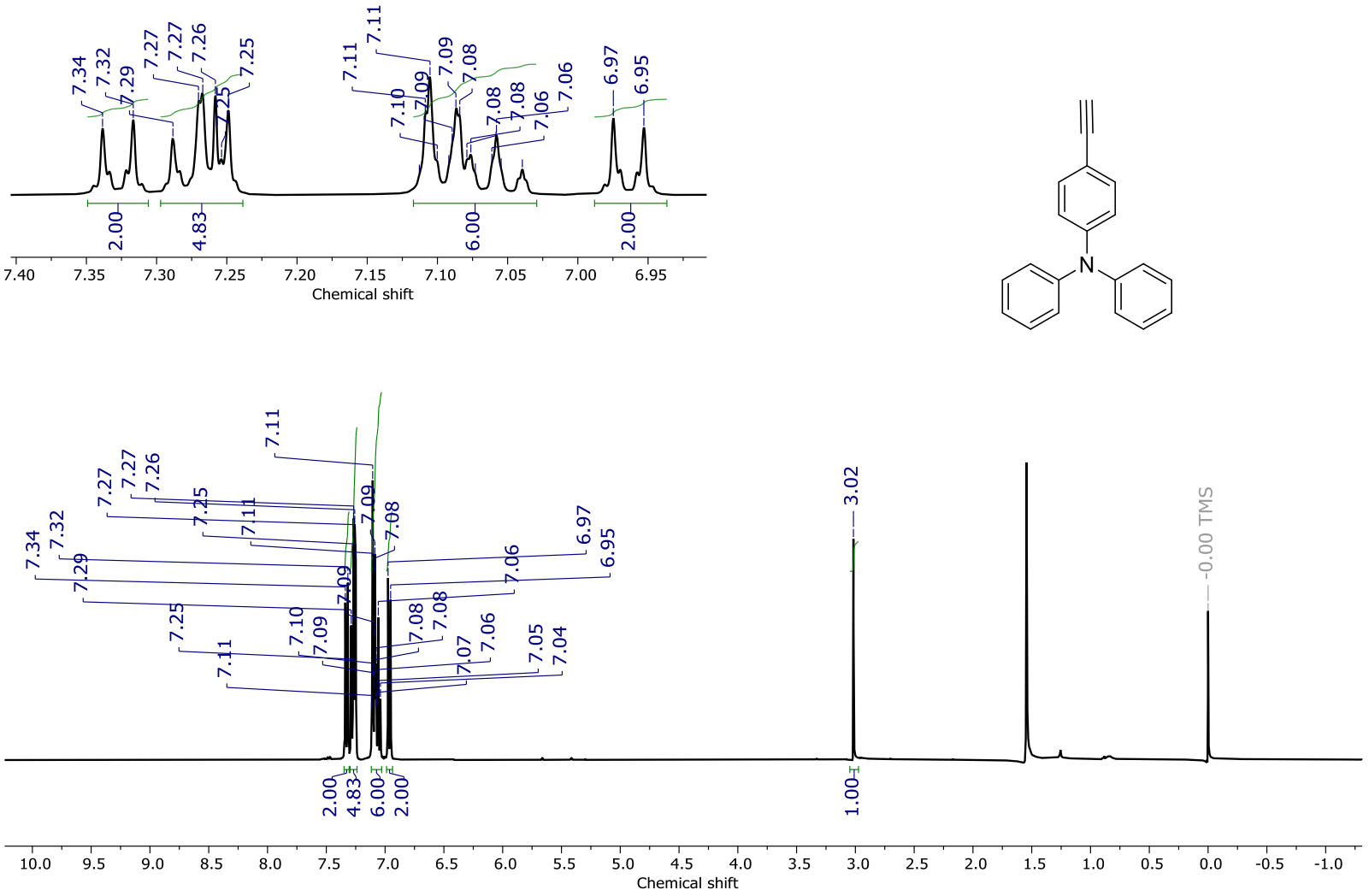


Figure S7. ^{1}H NMR of compound **f** (400 MHz, $CDCl_3$).

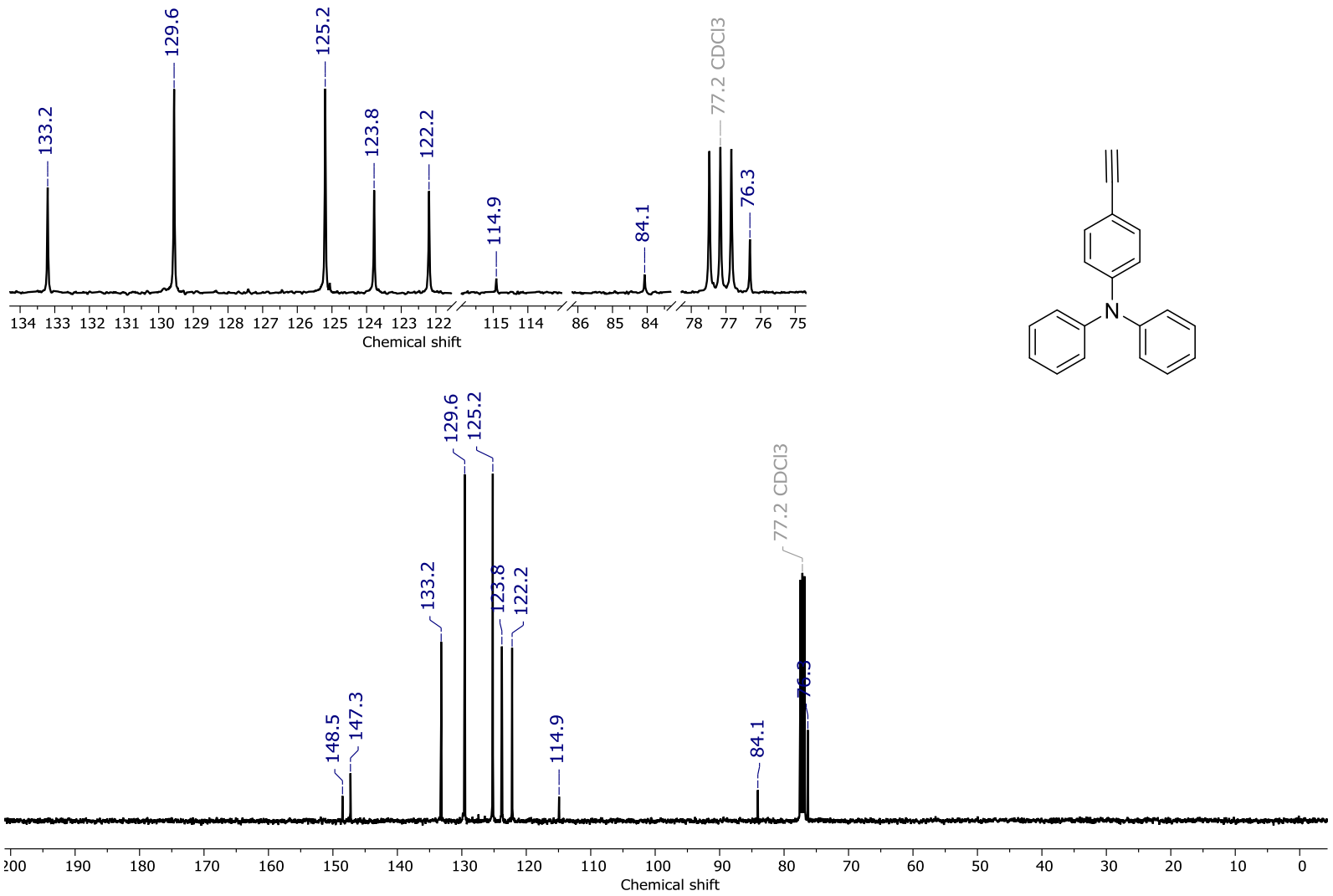


Figure S8. *^{13}C NMR of compound **f** (400 MHz, $CDCl_3$).*

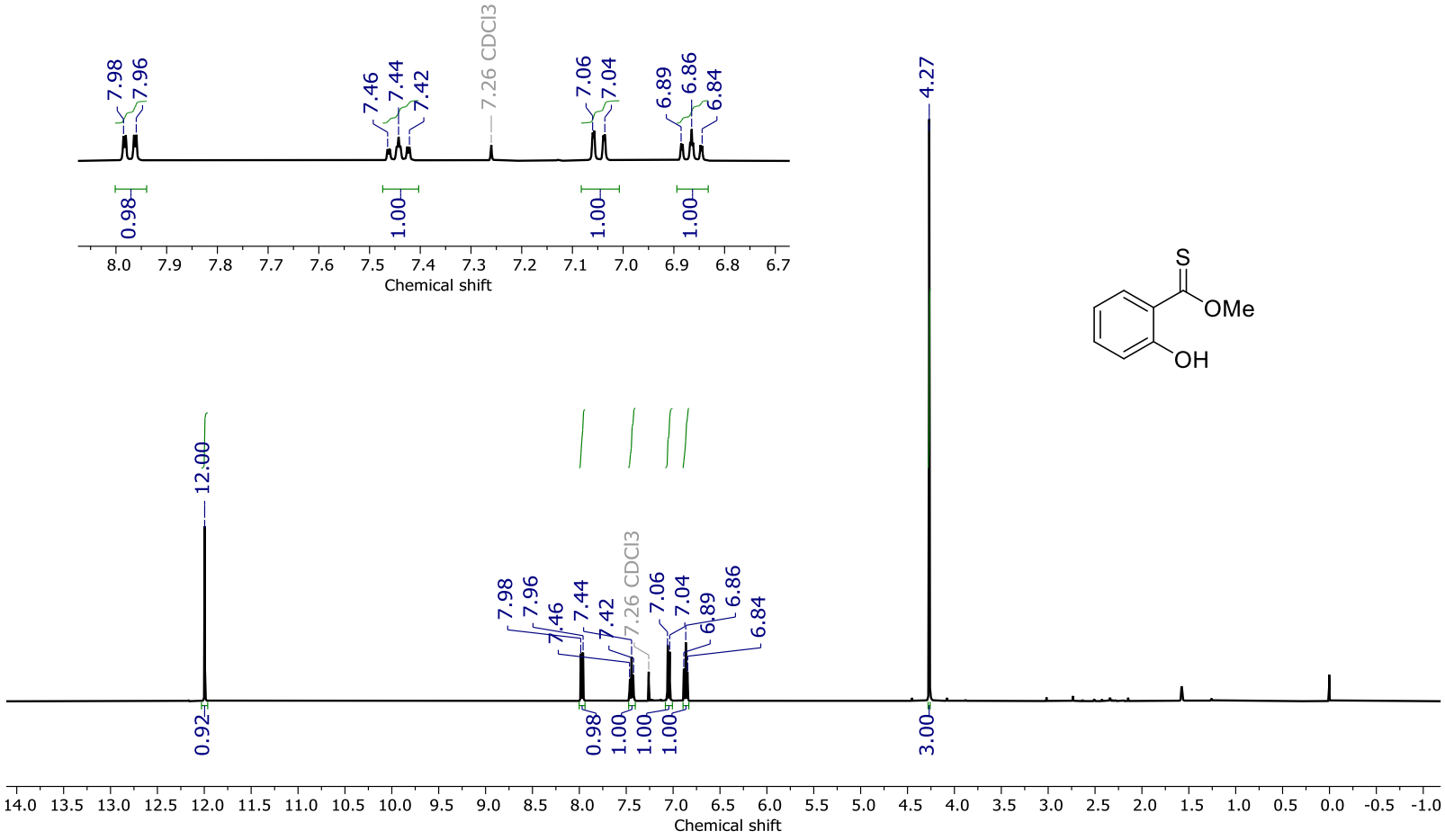


Figure S9. ^{1}H NMR of compound **g** (400 MHz, $CDCl_3$).

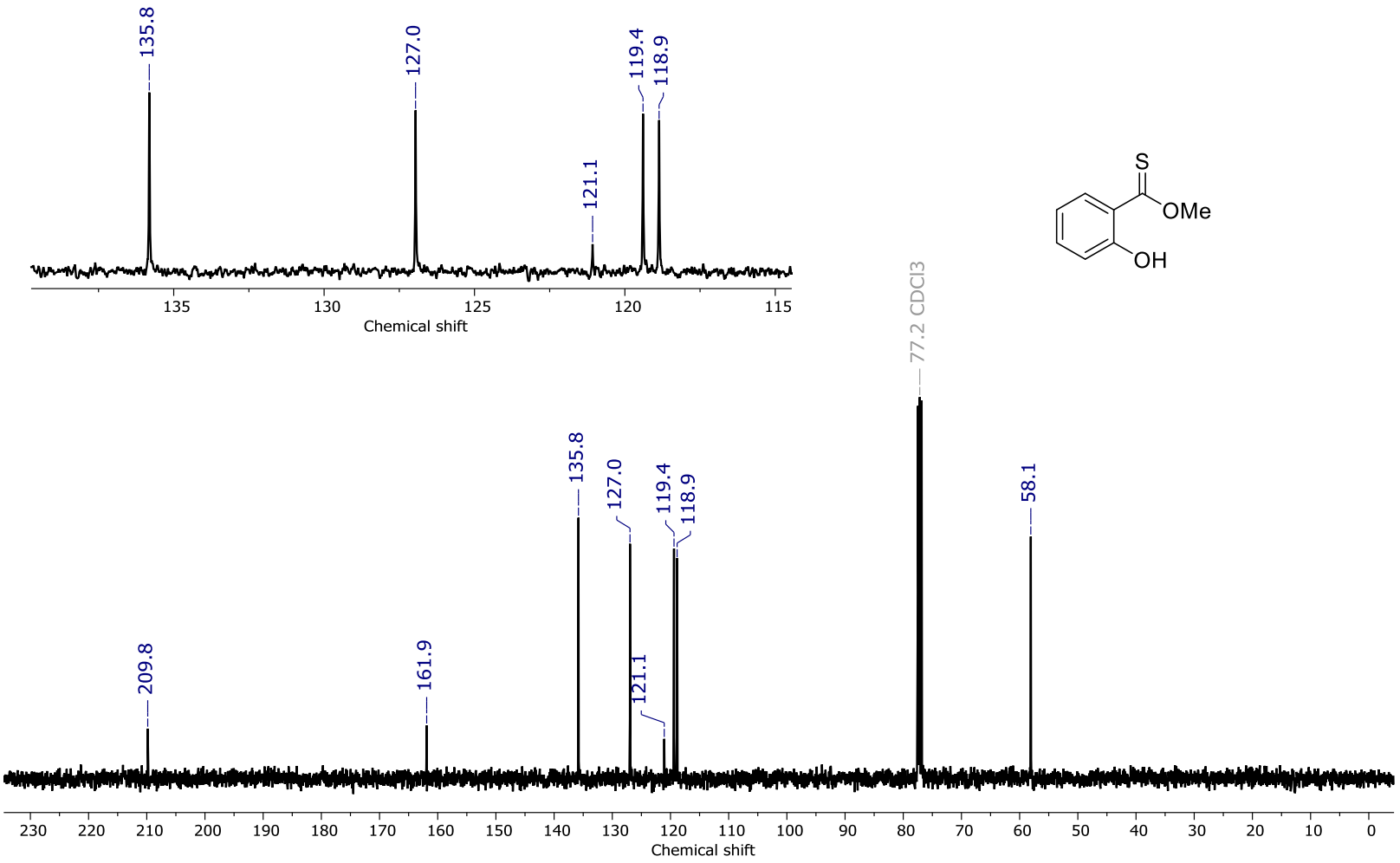


Figure S10. ^{13}C NMR of compound **g** (400 MHz, $CDCl_3$).

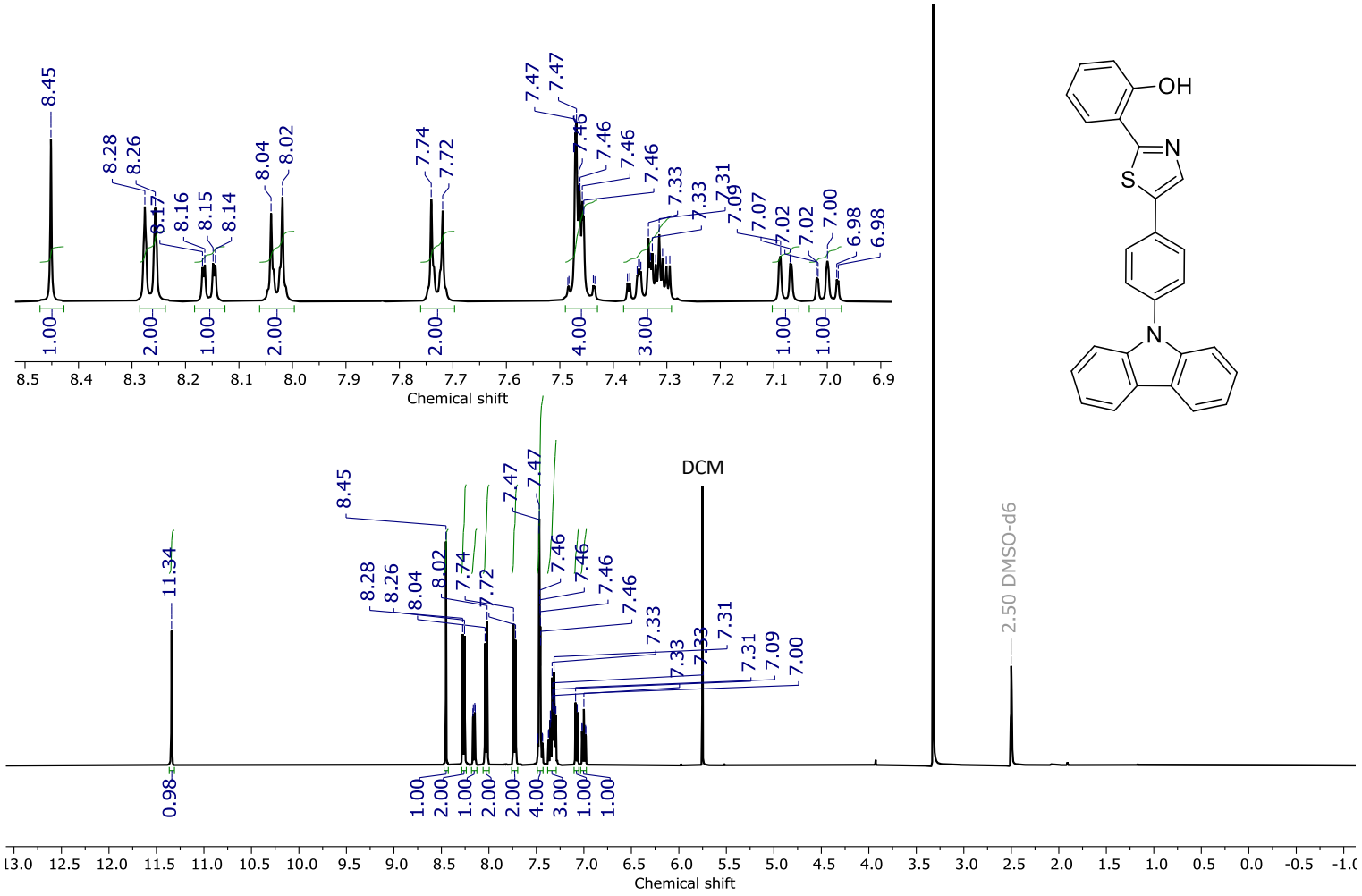


Figure S11. ^{1}H NMR of compound **Cbz-1** (400 MHz, DMSO-d_6).

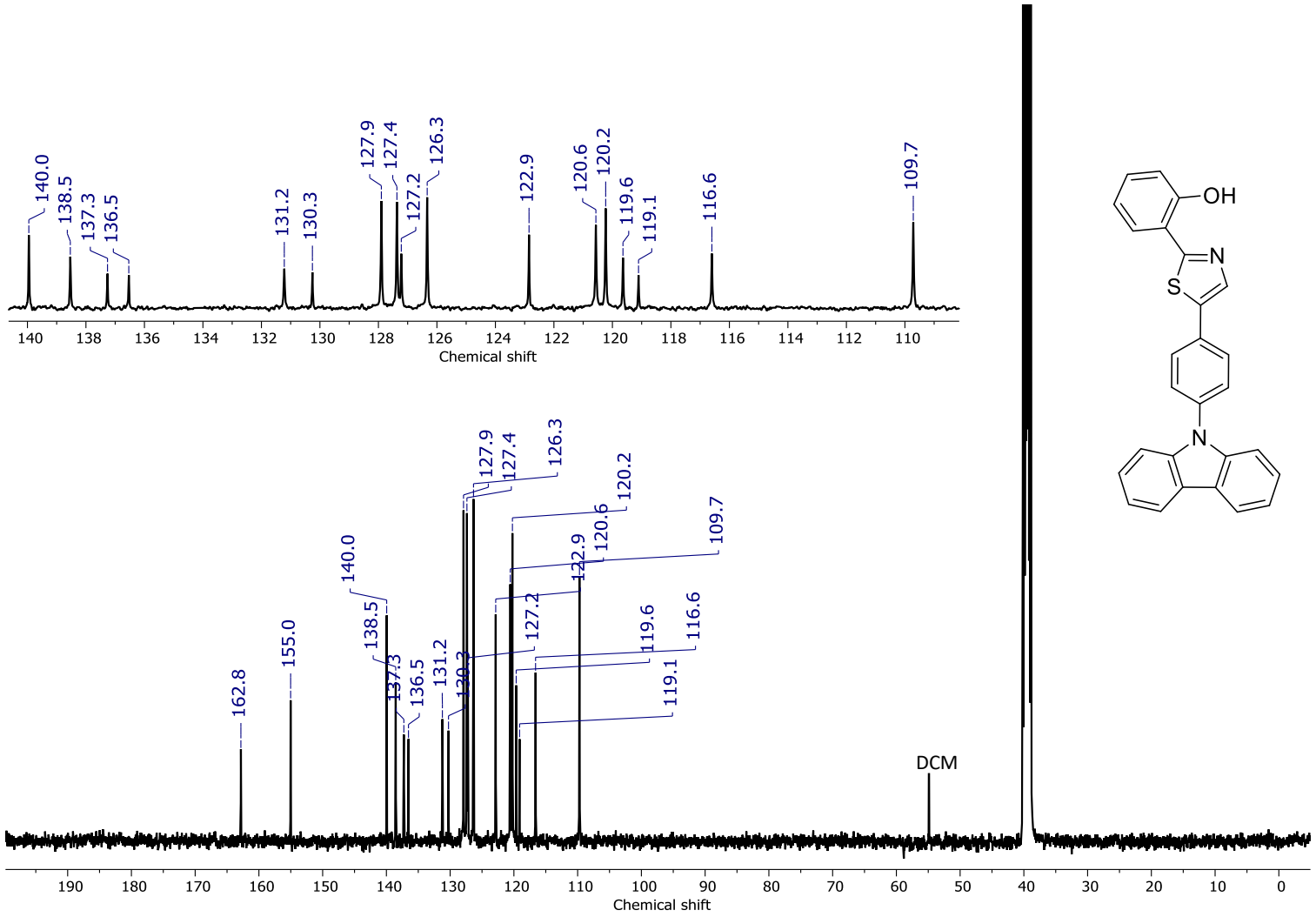


Figure S12. ^{13}C NMR of compound **Cbz-1** (400 MHz, DMSO-d_6).

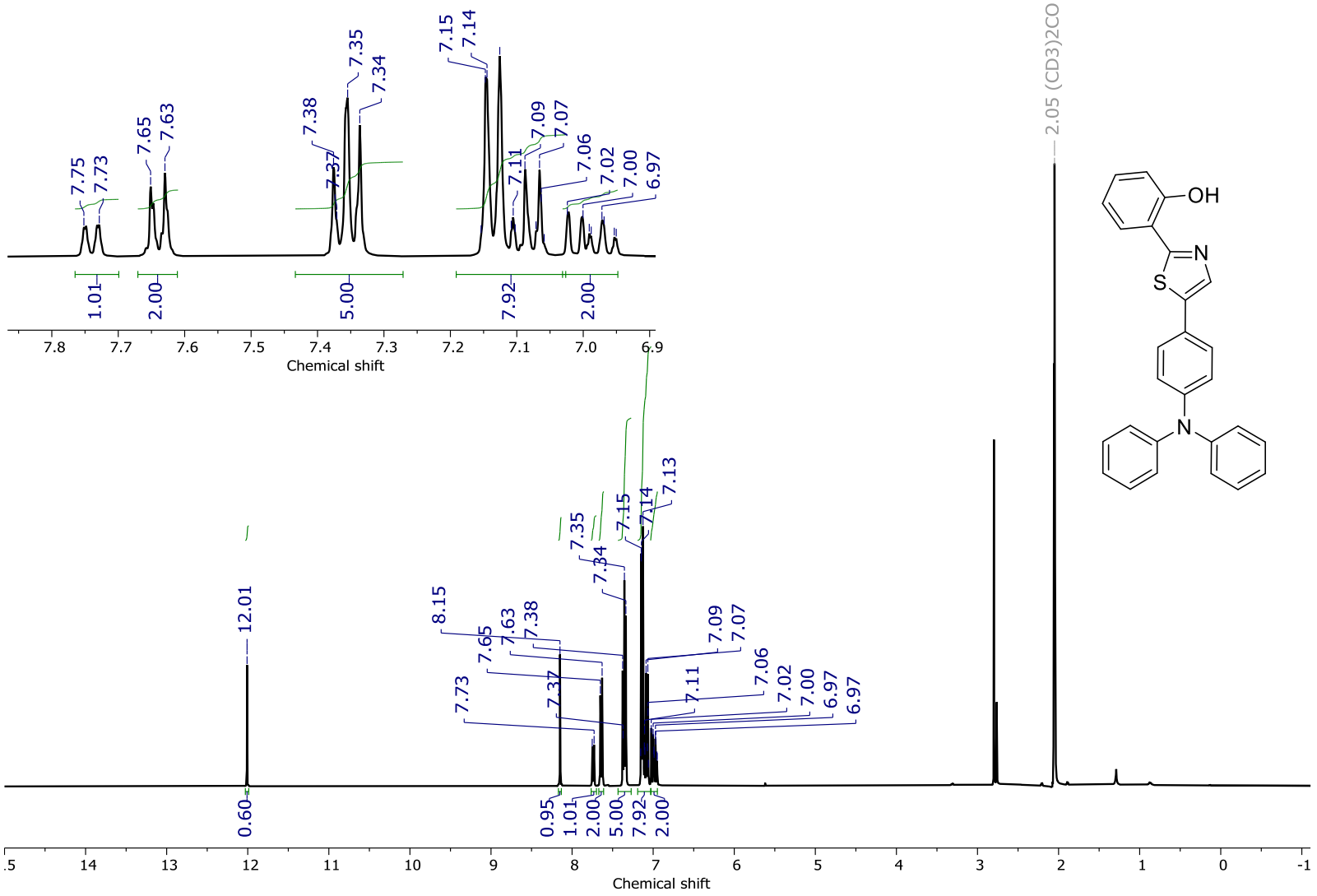


Figure S13. ^{1}H NMR of compound **TPA-2** (400 MHz, Acetone-d_6).

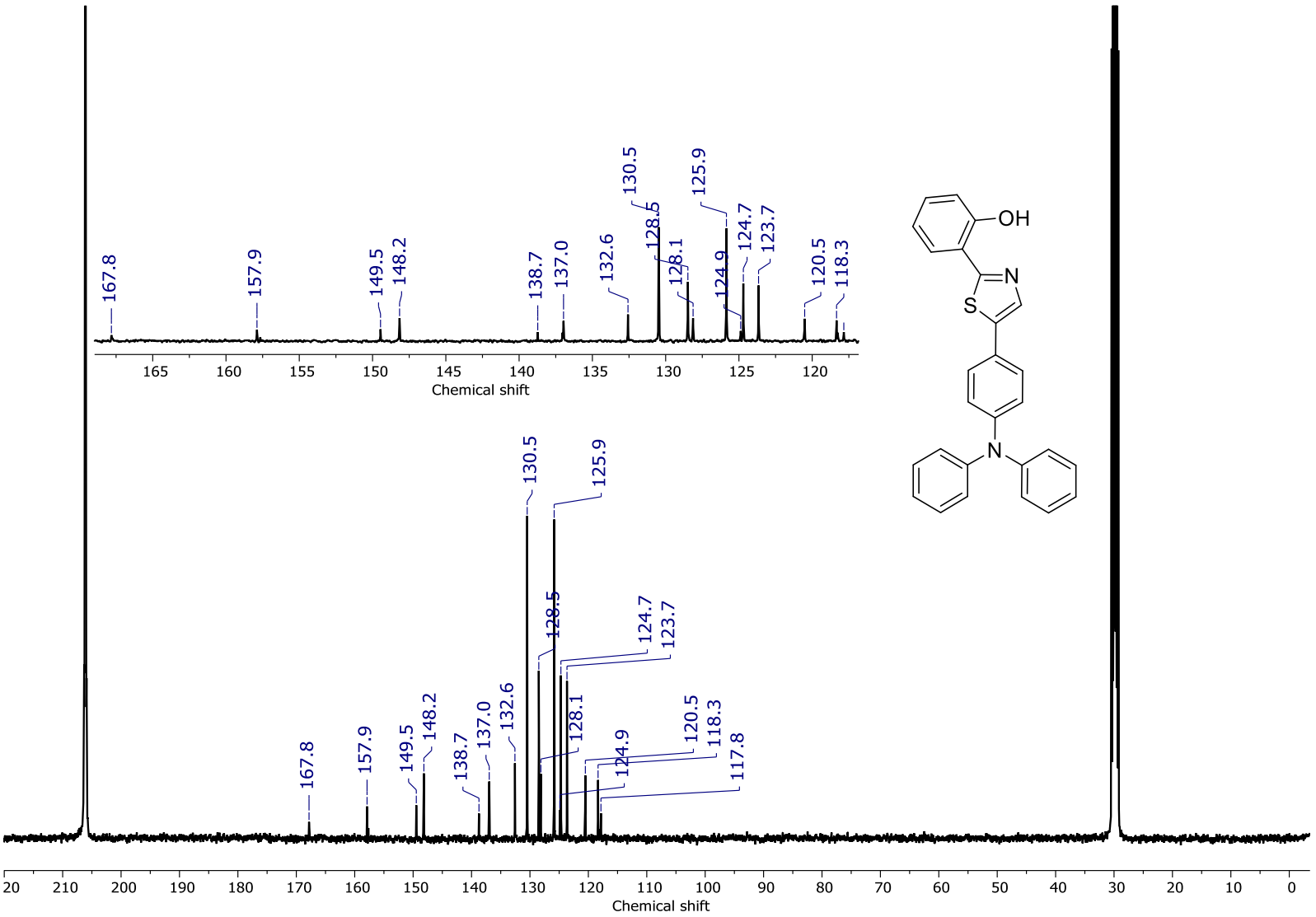


Figure S14. ^{13}C NMR of compound **TPA-2** (400 MHz, Acetone-d_6).

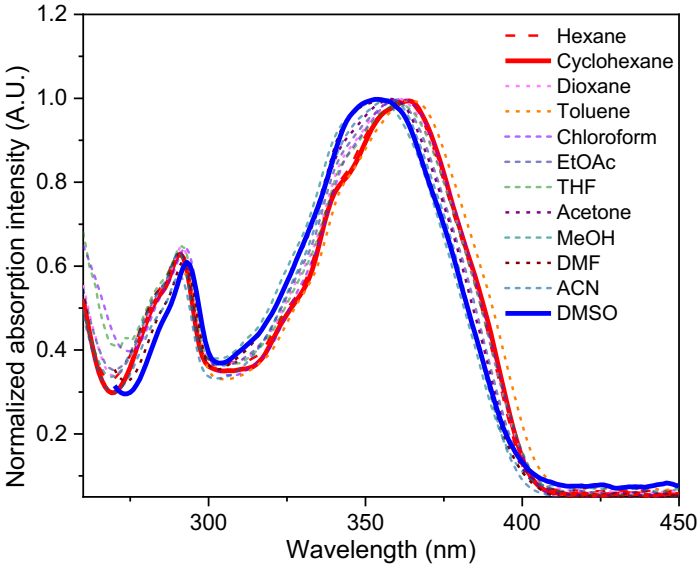


Figure S15. Absorption spectra of 40 μM **Cbz-1** in twelve different solvents.

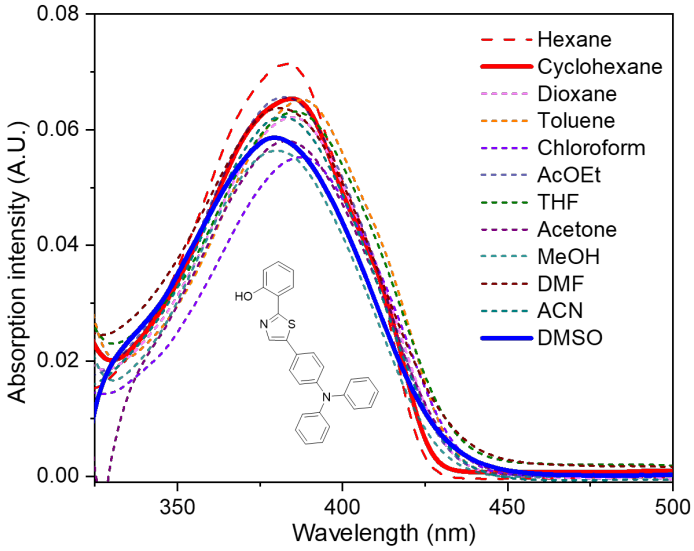


Figure S16. Absorption spectra of 40 μM **TPA-2** in different solvents from the less polar (hexane) to the most polar (DMSO).

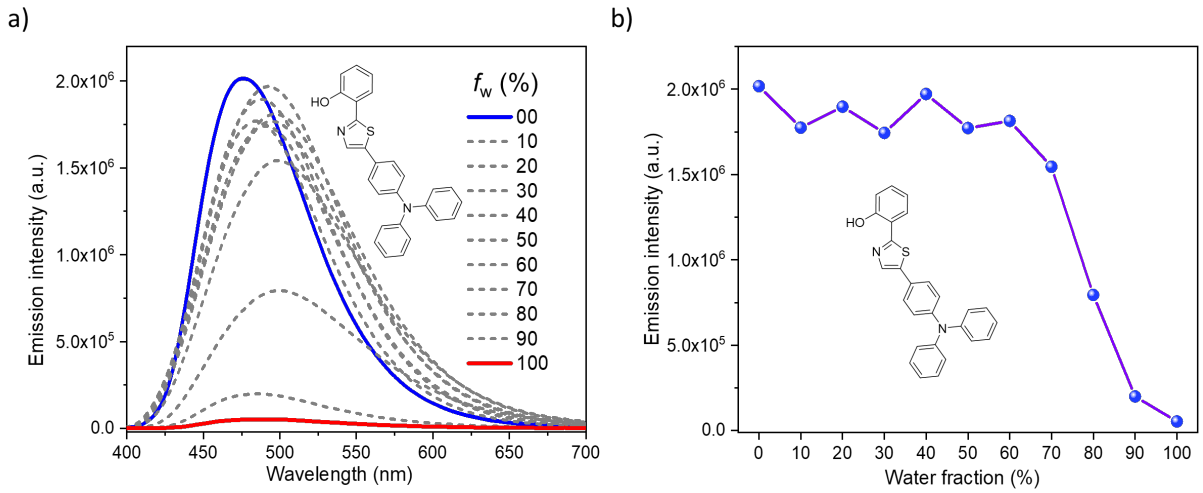


Figure S17. a) Aggregation-induced Emission experiment for 40 μM **TPA-2** and b) Plot of water fraction (f_w) against emission intensity of compound **TPA-2**.

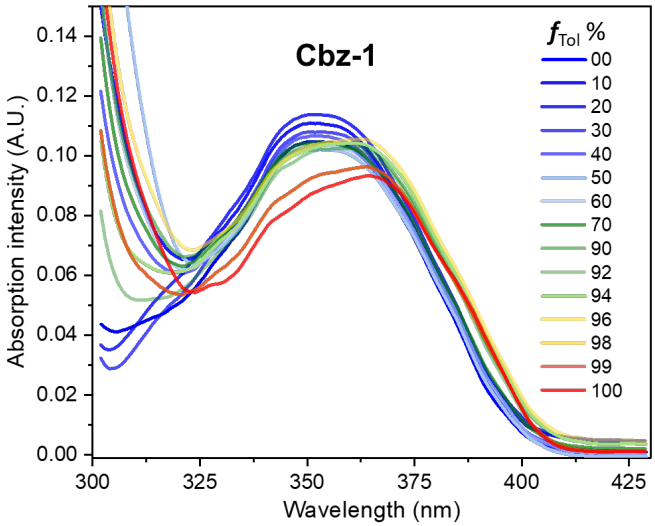


Figure S18. Absorption of the modulation of the keto-enol ratio of 40 μM **Cbz-1** by using a binary mixture of solvents (DMSO-toluene).

Cell line and culture conditions

The central nervous system carcinoma (U251) cell line was obtained from the American Type Culture Collection (Manassas, VA, USA) and supplied by M in C. M. Teresa Ramirez-Apan from IQ-UNAM. Cells were cultured at 37°C in a humidified atmosphere containing 5% CO_2 with RPMI-1640 medium supplemented with 10%

fetal bovine serum (both Gibco BRL, Gaithersburg, MD) and 1% antibiotic-antimycotic solution (10,000 U/ml penicillin, 10.000 µg/ml streptomycin and 25 µg/ml de amphotericin B, Corning).

Cell viability assay

U-251 cells were seeded into 96-well plates at a density of 5×10^3 cells/well in 200 µL complete RPMI medium. Following 24 h pre-incubation, cells were treated for 24, 48 and 72 h with Cbz-1 and TPA-2 a several concentrations. Control cells treated with the same volume of the corresponding vehicle (dimethyl sulphoxide) was used to normalize each drug condition. After the treatment time, cells were washed with PBS and dyed with a violet crystal solution (50 µL) for 30 minutes, washed with tap H_2O, and air dried. After, the bound dye was solubilized by the addition of 1% SDS solution (200 µL). The plates were placed on a shaker for 5 min prior to analysis. Optical densities were determined with a *Cytation* 5 Imaging Reader (BIOTek Instruments, Inc.) at λ=570 nm. The cytotoxic effect of each compound was expressed as a percentage of cell viability relative to untreated control cells and is defined as treated cells/non-treated cells × 100 (Fig. S22).

Statistical analyses

Three independent experiments were carried out in triplicate, and data was expressed as the mean ± standard deviation. Data were statistically analyzed using GraphPad Prism V6 software (GraphPad Software Inc., La Jolla, CA, USA). Significant differences were determined using one-way analysis of variance (ANOVA) followed by Dunnett correction to determine significant differences between each experimental group against its respective control. $P<0.05$ was considered to be a statistically significant difference.

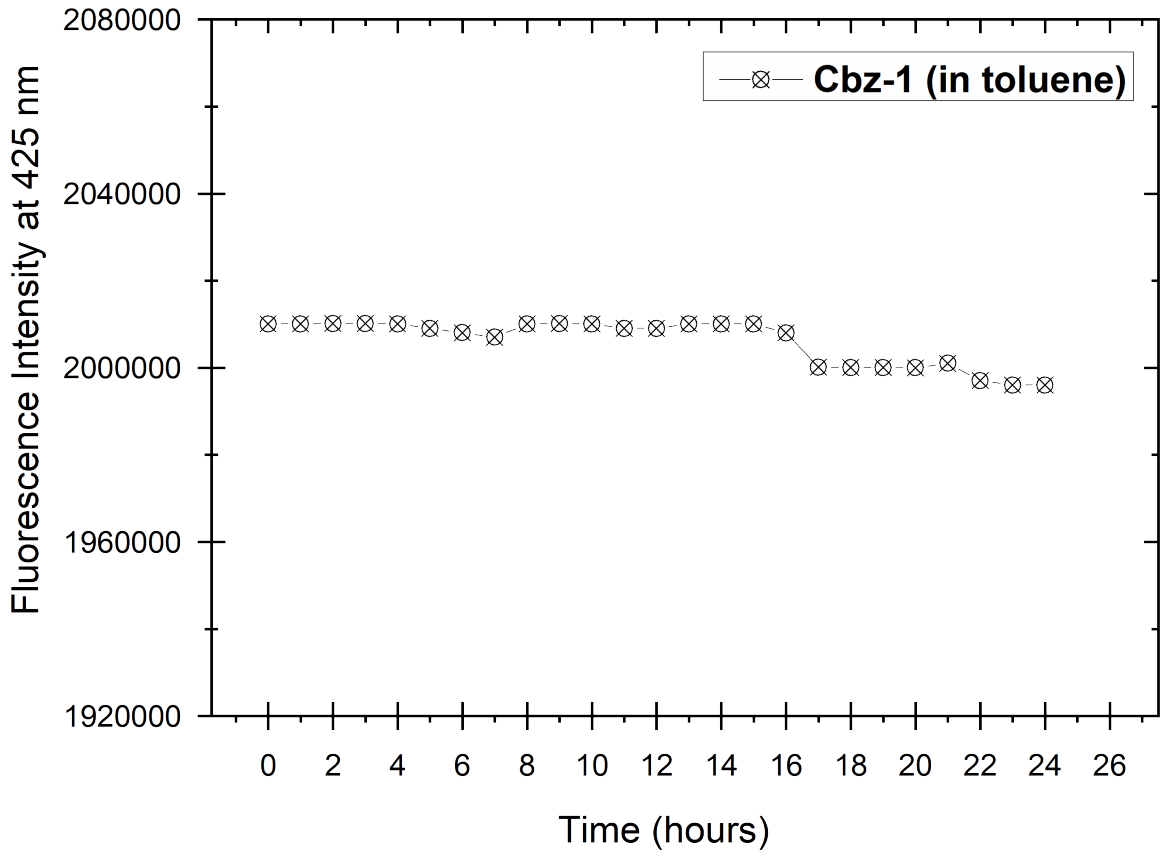


Figure S19. Photostability study of 40 μM **Cbz-1** under continuous excitation at 390 nm. Emission was detected at 425 nm, with both excitation and emission slits set to 2.5 nm. Fluorescence intensity was recorded over time to assess the stability of the probe under prolonged illumination.

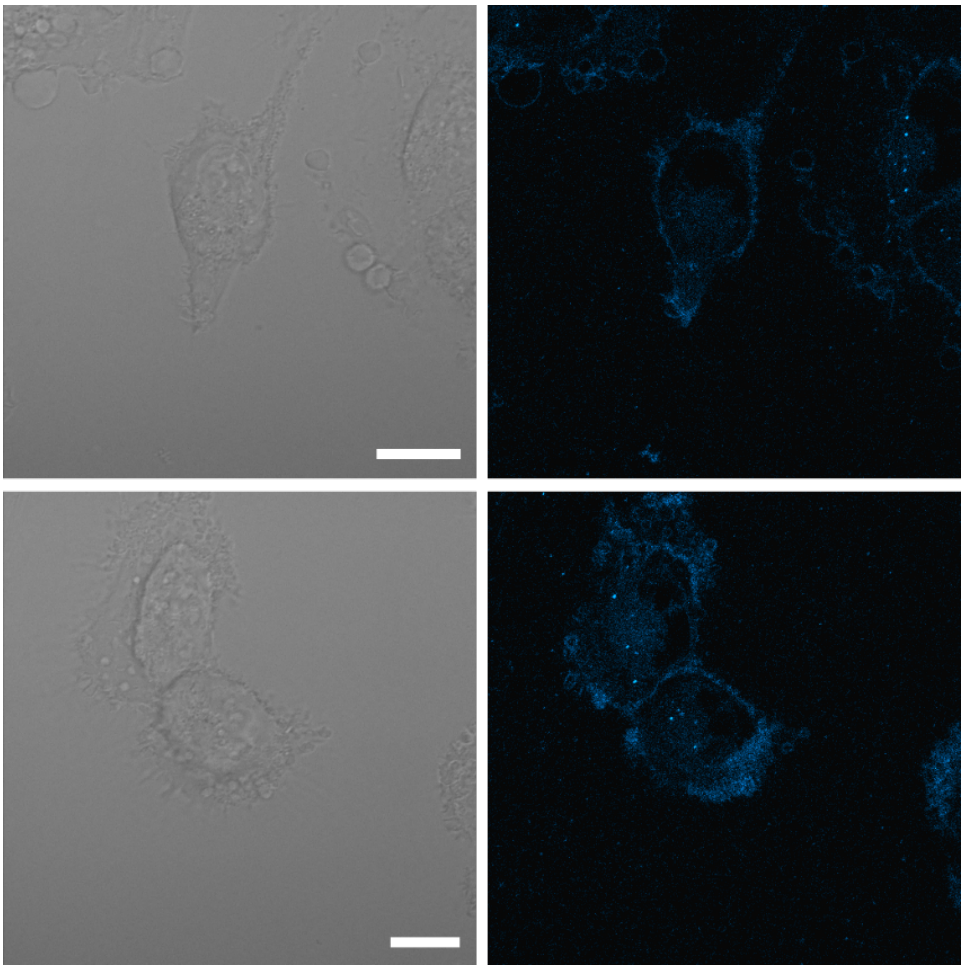


Figure S20. Imaging localization of 6 μM **TPA-2** in live U-251 cells observed in the bright field (left panels) and blue channel (right panels, DAPI, λ_{exc} = 400 nm; λ_{em} = 430-460 nm) after 30 min probe staining. Scale bar = 20 μm.

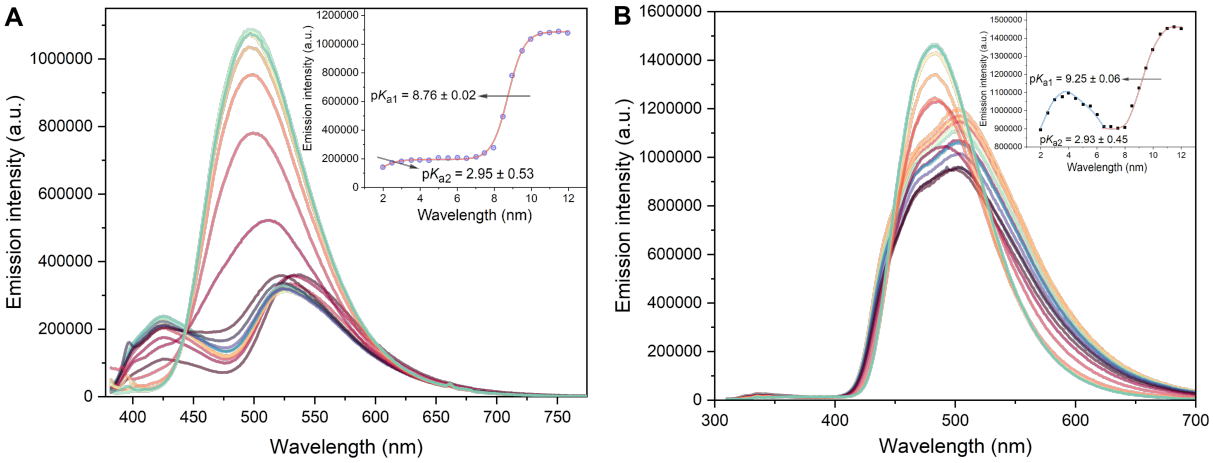


Figure S21. Fluorescence emission spectra of 40 μM A) **Cbz-1** and B) **TPA-2** under variable pH, excitation at 390 nm and 290 nm was used, respectively. The excitation and emission slits set to 2.5 nm. Insets show emission *vs.* pH profiles at selected wavelengths; solid lines are the theoretical pK_a fitting profiles.

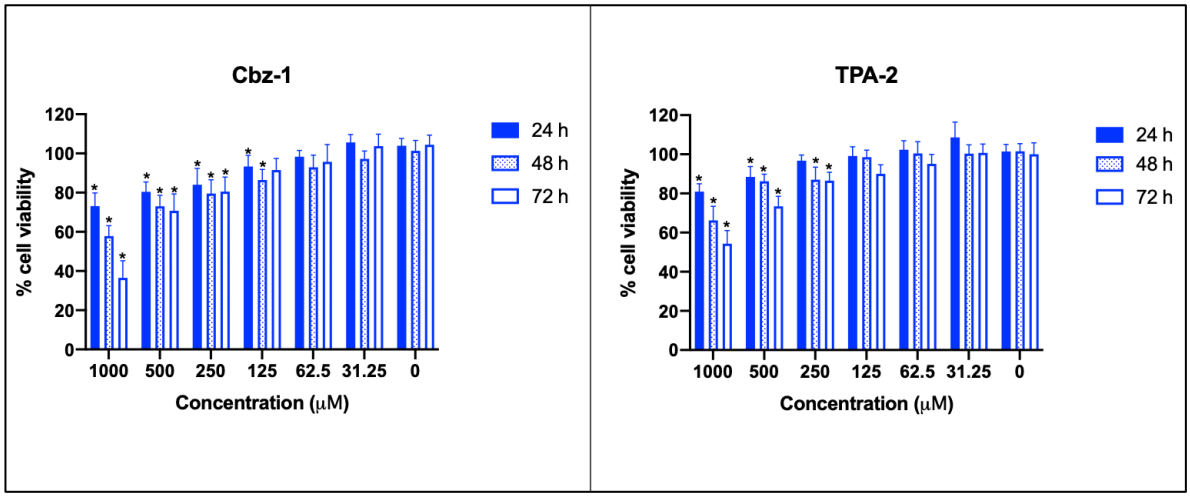
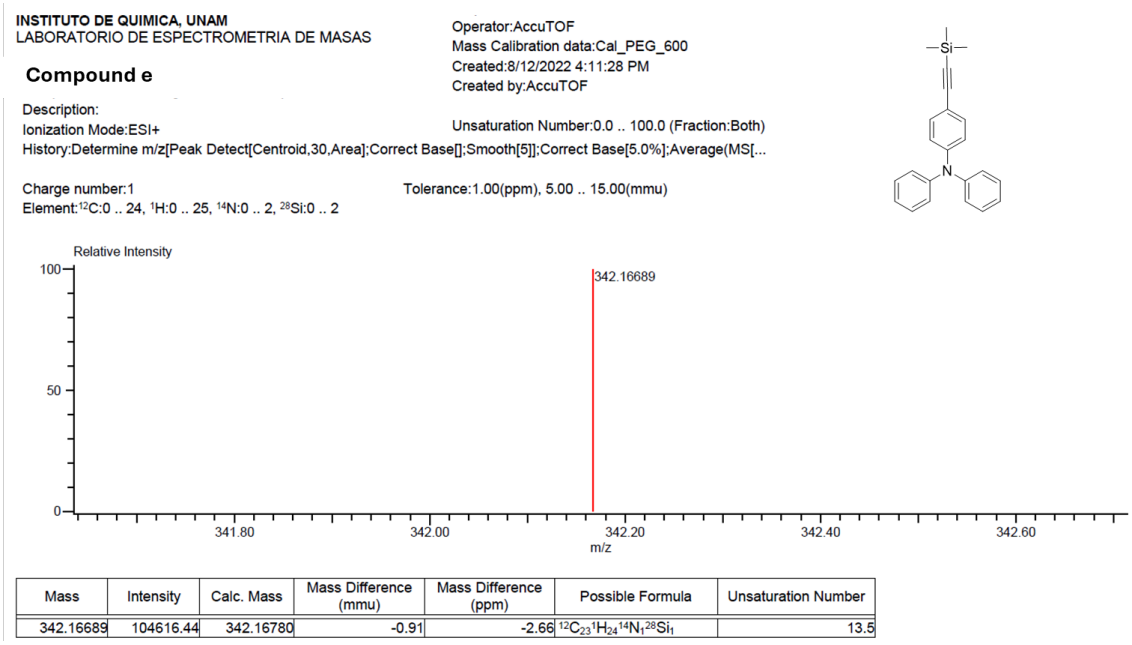


Fig. S22. Cell viability after treatment with Cbz-1 (left) and TPA-2 (right). Cell viability was evaluated in U251 cell line after 24, 48 and 72 h of treatment. Statistically significant difference in percentage of treated cells, compared against the respective control, were determined by ANOVA, with a significance of *P<0.0001.



Mass	Intensity	Calc. Mass	Mass Difference (mmu)	Mass Difference (ppm)	Possible Formula	Unsaturation Number
342.16689	104616.44	342.16780	-0.91	-2.66	$^{12}C_{23}{}^{1}H_{24}{}^{14}N_{1}{}^{28}Si_{1}$	13.5

Figure S23. HRMS spectra of compound **e**.

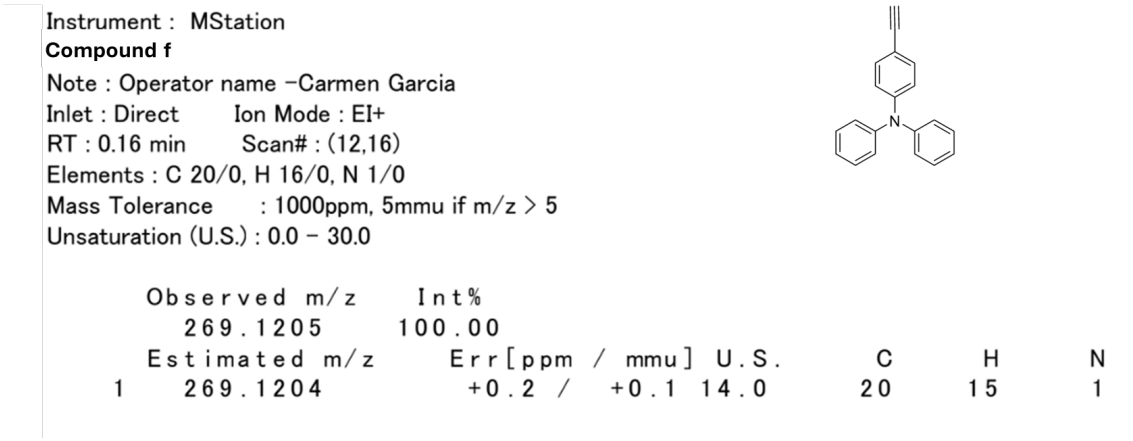


Figure S24. HRMS spectra of compound **f**.

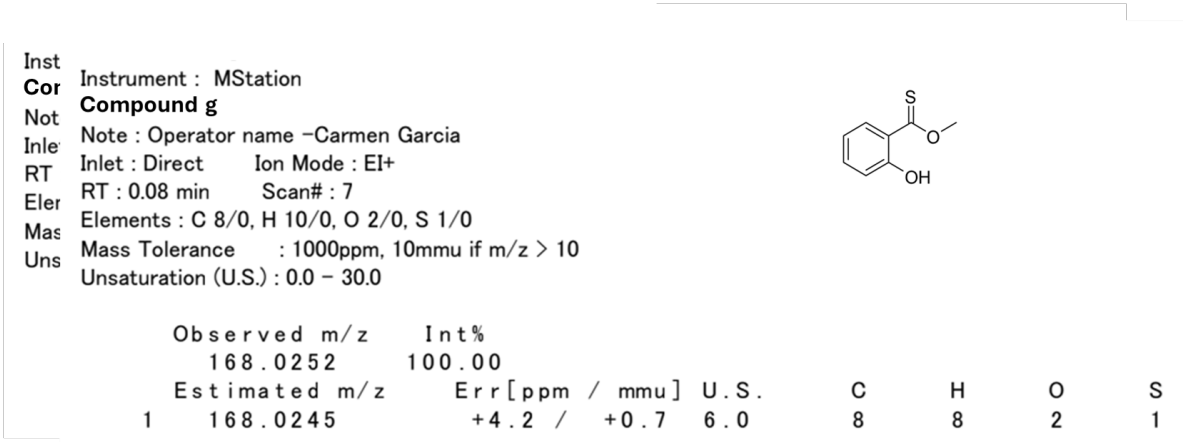
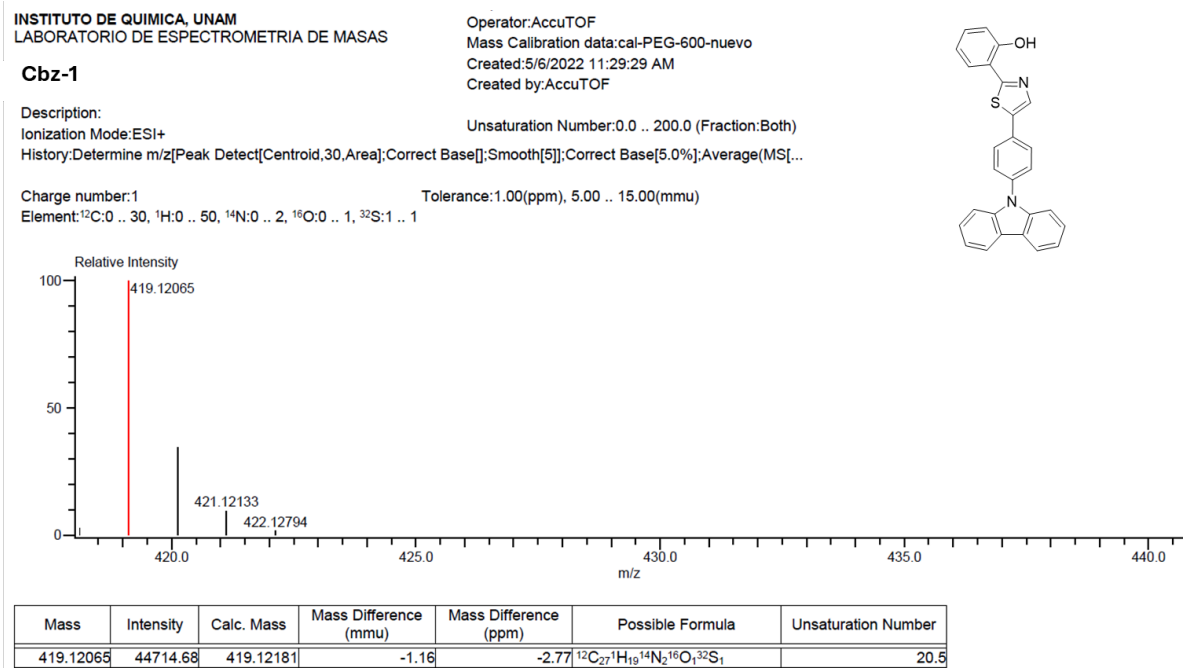
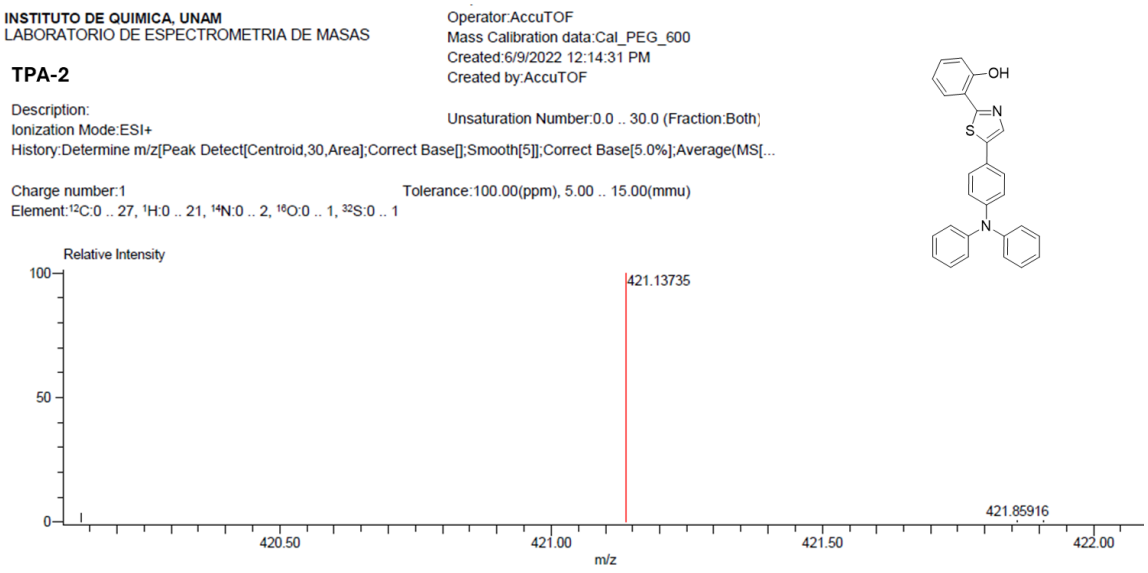


Figure S25. HRMS data of compound **g**.



Mass	Intensity	Calc. Mass	Mass Difference (mmu)	Mass Difference (ppm)	Possible Formula	Unsaturation Number
419.12065	44714.68	419.12181	-1.16	-2.77	$^{12}C_{27}{}^{1}H_{19}{}^{14}N_{2}{}^{16}O_{1}{}^{32}S_{1}$	20.5

Figure S26. HRMS spectra of compound **Cbz-1**.

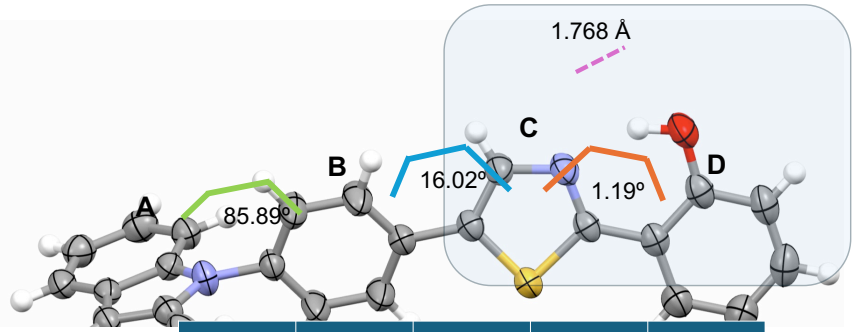


Mass	Intensity	Calc. Mass	Mass Difference (mmu)	Mass Difference (ppm)	Possible Formula	Unsaturation Number
421.13735	23840.91	421.13746	-0.11	-0.25	$^{12}C_{27}{}^{1}H_{21}{}^{14}N_{2}{}^{16}O_{1}{}^{32}S_{1}$	19.5

Figure S27. HRMS spectra of compound **TPA-2**.

X-ray single crystal analysis

After testing various crystallization conditions, single crystals suitable for X-ray diffraction were obtained by slow evaporation of a DCM/hexane solution. X-ray data, collected at room temperature, revealed the compound **Cbz-1** in a monoclinic P21/n space group (CCDC (Cbz-1): 2393894), with full crystallographic parameters in Table S1. The molecular structure analysis shows that the different rings (labelled A, B, C, and D) adopt distinct conformations with significant torsional angles between them. Specifically, the angle between rings A and B is 85.89°, driven by steric effects from the carbazole moiety, while rings B and C are separated by a smaller angle (16.02°). Also, rings C and D are nearly coplanar (1.19°). Additionally, the hydrogen atom from the phenol group is positioned toward the nitrogen acceptor, with a short H···O distance of 1.768 Å and a D-H···A angle of 148.13°, indicating a strong intramolecular hydrogen bond. This coplanarity and hydrogen bonding are critical for enhancing the ESIPT effect (highlighted in the blue box, Fig. S28).



	d D-H (Å)	*d* H···A (Å)	*d* D-A (Å)	D-H···A (º)
O-H···N	0.941	1.768	2.615	148.13

Figure S28. Molecular structure of **Cbz-1** with torsional angles among rings (labelled A, B, C, and D for clarity purposes) and the O-H···N intramolecular hydrogen bond with H···N distance of 1.768 Å.

Complete crystallographic data of compound **Cbz-1** are presented in Table S1.

Table S1. Crystallographic data for the single crystal refinement of probe **Cbz-1**.

Crystal	**Cbz-1**
Empirical Formula	$C_{27}H_{18}N_2OS$
Formula weight	418.51
Temperature (K)	298
System	Monoclinic
Space Group	$P\,2_1/n$
***a* (Å)**	8.8159(13)
***b* (Å)**	14.0135(17)
***c* (Å)**	18.159(3)
α (°)	90
β (°)	99.121(5)
γ (°)	90
ρ (Mg/cm^3)	1.382
V (Å^3)	2215.0(5)
Z	2
Absorption coefficient (mm^{-1})	0.291
Crystal size (mm)	0.210 x 0.366 x 0.390
Wavelength	0.71073
Collected reflections	61513

Independent reflections	5953
Data/rest/param	5953/73/337
GooF	1.004
Final R indexes [I_0>2σ(I_0)]	R1: 0.05050 wR2: 0.0990
Final R indexes [all data]	R1: 0.1420 wR2= 0.1257
Largest diff. peak/hole (eA^{-3})	0.216 and -2.66 eA^{-3}
CCDC number	2393894

[1] Belmonte-Vázquez, J. L.; Hernández-Morales, E. A.; Hernández, F.; García-González, M. C.; Miranda, L. D; Crespo-Otero, R.; Rodríguez-Molina, B. *Eur. J. Org. Chem*. **2022**, e202200372.

[2] a) Matsumoto, Y.; Tsuji, T.; Nakatake, D.; Yazaki, R.; Ohshima, T. *Asian J. Org. Chem.* **2019**, *8*, 1071 – 1074. b) Cho, D.; Ahn, J.; De Castro, K. A.; Ahn, H.; Rhee, H. *Tetrahedron* **2010**, *66*, 5583 – 5588.

[3] Wang, L.; Shi, Y.; Zhao, Y.; Liu, H.; Li, X.; Bai, M. *J. Mol. Struct*. **2014**, *1056-1057*, 339-346.