Supporting Information

Toward the development of the direct and selective detection of nitrates by a bio-inspired Mo-Cu system.

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Reagents and Methods.

All the reagents and solvents are commercially available and were used as purchased, without further purification. ^{1}H and ^{13}C NMR spectra were recorded on Bruker Avance 400 or 500 MHz spectrometers in $CDCl_3$, or CD_3CN. ^{1}H and ^{13}C NMR signals are reported in ppm. ^{1}H NMR signals are referenced to the residual proton (7.26 ppm for $CDCl_3$ and 1.94 ppm for CD_3CN) of a deuterated solvent and ^{13}C NMR spectra, the signal of $CDCl_3$ (77.0 ppm) or CD_3CN (1.79 and 118.26 ppm) was used as a reference. ^{13}C NMR spectra interpretations were supported by DEPT experiments. Mass spectra were obtained on a spectrometer equipped with CI and EI probes. HRMS results were obtained on MALDI-TOF and ESI mass spectrometers. Infrared (IR) spectra were recorded on a Bruker Vector 22 spectrophotometer. Melting points were determined with a Büchi B-540 apparatus and are uncorrected. The progress of reactions was monitored by TLC (SiO_2) and visualized by UV light. Flash chromatography was performed with UltraPure Silica Gel (40-63 μm) (Silicycle). UV-vis spectra were measured using a Varian's Cary 5000 spectrometer. Fluorescence spectra were recorded by Jobin Yvon's fluorospectrometer.

Experimental

6-Bromo-2-(2,6-diisopropylphenyl)-1H-benzo[de]isoquinoline-1,3(2H)-dione (2). 2,6-Diiso-propyl-aniline (0.55 mL, 2.9 mmol) was added to a 100 mL round-bottom flask containing **1** (0.4 g, 1.5 mmol) in acetic acid (50 mL). The reaction mixture was refluxed for 3 days and then poured into ice-water. The resulting precipitate was washed with water and dried. The crude product was purified by flash column chromatography (SiO_2; hexanes/CH_2Cl_2; 6:4) to give **23** as a yellowish solid (0.59 gr, 90%).

2-(2,6-Diisopropylphenyl)-6-(4-(methylthio)phenyl)-1H-benzo[de]isoquinoline-1,3(2H)-dione (3).
2 (0.5 g, 1.2 mmol), 4-(methylthio)benzeneboronic acid (0.2 g, 1.2 mmol), $Pd(PPh_3)_4$ (140 mg, 0.12 mmol), aqueous Na_2CO_3 (2.0 M, 10 mL), dry ethanol (10 mL) and dry toluene (20 mL) were mixed in a flask. The mixture was degassed and refluxed for 24 h under an argon atmosphere. After being cooled, the solvent was evaporated under vacuum and the product was extracted with dichloromethane. The CH_2Cl_2 solution was washed with water and dried with $MgSO_4$. The crude product was purified by column chromatography (SiO_2; hexanes/CH_2Cl_2; 4:6) to give the desired product as a yellow solid (175 mg, 87%).

2-(2,6-Diisopropylphenyl)-6-(2-(methylthio)phenyl)-1H-benzo[de]isoquinoline-1,3(2H)-dione (6). 6-Bromo-2-(2,6-diisopropylphenyl)-1H-benzo[de]isoquinoline-1,3(2H)-dione was prepared as described above.

6-Bromo-2-(2,6-diisopropylphenyl)-1H-benzo[de]isoquinoline-1,3(2H)-dione (0.5 g, 1.2 mmol), 4-(methylthio)benzeneboronic acid (0.2 g, 1.2 mmol), Pd $(PPh_3)_4$ (140 mg, 0.12 mmol), aqueous Na_2CO_3 (2.0 M, 10 mL), ethanol (10 mL) and toluene (20 mL) were mixed in a flask. The mixture was degassed and refluxed for 24 h under argon atmosphere. After being cooled, the solvent was evaporated under vacuum and the product was extracted with dichloromethane. The CH_2Cl_2 solution was washed with water and dried with $MgSO_4$. The crude product was purified by column chromatography (SiO_2; hexane/CH_2Cl_2; 4:6) to give the desired product as a yellow solid (160 mg, 80%).

Preparation of a stock of the catalytic mixture:

A) **$MoO_2Cl_2(OPPh_3)_2$** The complex was prepared as previously reported by us. MoO_2Cl_2 (10 mg, 0.0503 mmol) was dissolved in CH_3CN (3 mL). Then, H_2O_2 (9.234M, 10.9 µL, 2.0 equiv.) was added. The solution turned yellow and was stirred for 5 minutes at room temperature. Next, PPh_3 (26.5 mg, 2eq) was added and the mixture was left stirring for further 5 minutes at 60°C, which resulted in an almost colorless solution.

B) **Catalytic mixture** $CuCl_2 \cdot 2H_2O$ (8.49 mg, 1.0 equiv.) was added to the dissolved $MoO_2Cl_2(OPPh_3)_2$ solution, returning the solution's color to yellow. Within 5 minutes of stirring at 60°C, 4-methoxy thioanisole (7 µL, 1.0 equiv.) was also added and the mixture was left stirring for an additional 30 min.

General procedure for the detection of nitrate (using SA86 for example).

Reaction:

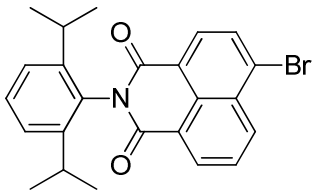
The Catalytic mixture was prepared as described above. SA86 (1 mg, 2.09 µm) was dissolved in 1 mL of HPLC grade CH_3CN and transferred into a 1.5 mL vial with a clamp-sealed corck (still unsealed), equipped with a small magnet. Tetra buthyl ammonium nitrate (TBAN, 2.1 mg, 3.3 eq) was added to the vial, followed by an exact amount of the catalytic mixture (67 µL, containing 0.33 eq of the Mo complex and additives) and the vial was clamp-sealed with a septumed-corck. The vial was placed in an oil bath at 60°C in order to start the reaction which was monitored in time.

Monitoring:

The reaction was monitored by the change in fluorescence. After sealing the vial a first sample (t = 0 min) of 10 µL was extracted through the septum by a 10 µL syringe. The sample was diluted in 3 mL of HPLC-grade CH_3CN and transferred to a 3 mL cuvette. Then a UV analysis was carried, followed by the fluorescence (emission) analysis. The same was repeated every 30 minutes until the completion of the reaction (5 h). Each sample was analyzed by UV-vis spectroscopy in order to determine the concentration remained constant. The fluoresence analysis was carried for a pre-determined concentrtion (1.5×10^5 M) corresponding to the maximal emission region.

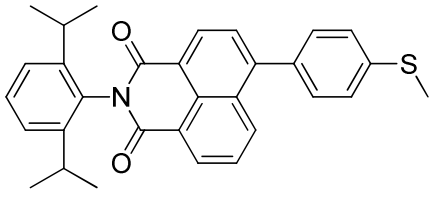
Analytical data for compounds

6-Bromo-2-(2,6-diisopropylphenyl)-1H-benzo[de]isoquinoline-1,3(2H)-dione (2).



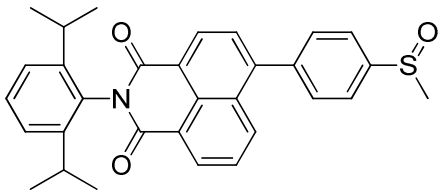
^{1}H NMR (400 MHz, $CDCl_3$) δ 1.16 (d, *J* = 6.8 Hz, 12H), 2.73 (sept, *J* = 8.0 Hz, 2H), 7.34 (d, *J* = 7.6 Hz, 2H), 7.51-7.47 (m, 1H), 7.93-7.89 (m, 1H), 8.10 (d, *J* = 7.6 Hz, 1H), 8.50 (d, *J* = 8.0 Hz, 1H), 8.67 (dd, *J* = 8.6 Hz, *J* = 1.2 Hz, 1H), 8.74 (dd, *J* = 7.2 Hz, *J* = 1.2 Hz, 1H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 23.9, 29.1, 122.2, 123.1, 124.0, 128.2, 129.6, 130.5, 130.6, 130.9, 131.2, 131.7, 132.6, 133.6, 145.6, 163.6. MS (CI^+): *m/z* 435.05 ($M^{+\cdot}$).

2-(2,6-Diisopropylphenyl)-6-(4-(methylthio)phenyl)-1H-benzo[de]isoquinoline-1,3(2H)-dione (3).



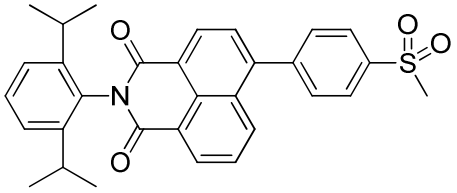
^{1}H NMR (200 MHz, $CDCl_3$) δ 1.16 (d, *J* = 6.0 Hz, 12H), 2.57 (s, 3H), 2.78 (sep, *J* = 6.0 Hz, 2H), 7.41-7.31 (m, 2H), 7.78-7.70 (m, 2H), 8.37 (dd, *J* = 8.0 Hz, *J* = 2.0 Hz, 1H), 8.70 (d, *J* = 8.0 Hz, 2H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 15.5, 23.9, 29.1, 121.7, 122.9, 124.0, 126.3, 126.9, 127.8, 127.0, 129.4, 130.2, 130.8, 131.3, 131.7, 132.8, 134.2, 135.2, 139.7, 145.6, 146.6, 164.1, 164.3. MS (CI^+): *m/z* 480.2 (MH^+). HRMS (CI^+): *m/z* calcd for $C_{31}H_{30}O_2NS$ (MH^+): 480.1998, Found: 480.1997. FT-IR (KBr): ν = 2961, 2868, 1704, 1667, 1586, 1493, 1460, 1384, 1358, 1237, 1190, 1133, 1098, 1082, 909, 827, 803, 785, 759, 744 cm^{-1}.

2-(2,6-diisopropylphenyl)-6-(4-(methylsulfinyl)phenyl)-1H-benzo[de]isoquinoline-1,3(2H)-dione (4).



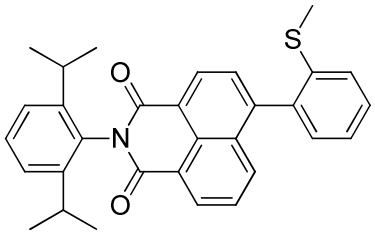
^{1}H NMR (200 MHz, $CDCl_3$) δ 1.16 (d, *J* = 6.8 Hz, 12H), 2.77 (sep, *J* = 6.8 Hz, 2H), 2.87 (s, 3H), 7.34 (d, *J* = 7.6 Hz, 2H), 7.48 (t, *J* = 7.6 Hz, 1H), 7.71-7.79 (m, 4H), 8.26 (d, *J* = 8.4 Hz, 1H), 8.73 (m, 2H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 24.7, 29.9, 44.8, 123.2, 123.8, 124.8, 128.1, 128.8, 130.1, 130.3, 130.9, 131.6, 132.0, 132.6, 133.0, 142.5, 146.2, 146.3, 147.1, 164.7, 164.9. MS (ES^+): m/z 518.2 (MNa^+). HRMS (ES^+): calcd. for $C_{31}H_{29}NO_3S$ (MNa+): 518.1761, found: 518.1759. FT-IR (KBr): ν = 2961, 2103, 1703, 1666, 1586, 1511, 1466, 1359, 1261, 1238, 1190, 1091, 1054, 956, 909, 804, 759, 744 cm^{-1}.

2-(2,6-Diisopropylphenyl)-6-(4-(methylsulfonyl)phenyl)-1H-benzo[de]isoquinoline-1,3(2H)-dione (5).



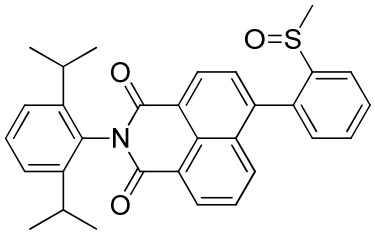
^{1}H NMR (400 MHz, $CDCl_3$) δ 1.16 (d, *J* = 8.0 Hz, 12H) 2.76 (sep, *J* = 8.0 Hz, 2H), 2.86 (s, 3H), 7.33 (d, *J* = 8.0 Hz, 2H), 7.47 (d, *J* = 4.0 Hz, 1H), 7.72 (d, *J* = 8.0 Hz, 2H), 7.77 (m, 2H), 7.87 (d, *J* = 8.0 Hz, 2H), 8.26 (d, *J* = 8.0 Hz, 1H), 8.72 (dd, *J* = 8.0 Hz, 2H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 29.1, 30.9, 44.0, 122.4, 123.0, 124.0, 127.3, 128.0, 128.4, 128.5, 129.3, 129.5, 130.1, 130.8, 131.2, 131.9, 132.0, 132.1, 132.3, 141.7, 145.4, 145.6, 146.3, 163.9, 164.1. MS (ES^+): m/z 534.2 (MNa^+). HRMS (ES^+): calcd. for $C_{31}H_{29}NO_4S$ (MNa^+): 534.1710, found: 534.1716

2-(2,6-Diisopropylphenyl)-6-(2-(methylthio)phenyl)-1H-benzo[de]isoquinoline-1,3(2H)-dione (6).



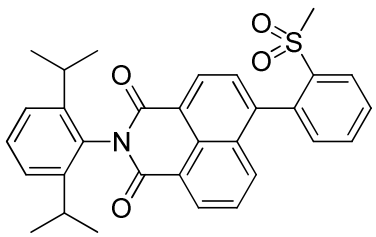
^{1}H NMR (200 MHz, $CDCl_3$) δ 1.16 (d, *J* = 6.0 Hz, 12H), 2.57 (s, 3H), 2.78 (sep, *J* = 6.0 Hz, 2H), 7.31-7.41 (m, 2H), 7.41-7.51 (m, 5H), 7.70-7.78 (m, 2H), 8.37 (dd, *J* = 8.0 Hz, *J* = 2.0 Hz, 1H), δ 8.70 (d, *J* = 8.0 Hz, 2H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 164.3, 164.1, 146.6, 145.6, 139.7, 135.2, 134.2, 132.8, 131.7, 131.3, 130.8, 130.2, 129.4, 127.8, 127.0, 126.9, 126.3, 124.0, 122.9, 121.7, 29.1, 23.9, 15.5. MS (CI^+): *m/z* 480.2 (MH^+). HRMS (CI^+): *m/z* calcd for $C_{31}H_{30}O_2NS$ (MH^+): 480.1998, Found: 480.1997.

2-(2,6-diisopropylphenyl)-6-(2-(methylsulfinyl)phenyl)-1H-benzo[de]isoquinoline-1,3(2H)-dione (7).



MS (ES^+): m/z 518.2 (MNa^+), 1013.4 ($2MNa^+$). HRMS (ES^+): calcd. for $C_{31}H_{29}NO_3S$ (MNa^+): 518.1760, found: 518.1743.

2-(2,6-diisopropylphenyl)-6-(2-(methylsulfonyl)phenyl)-1H-benzo[de]isoquinoline-1,3(2H)-dione (8).



^{1}H NMR (500 MHz, $CDCl_3$) δ 1.16 (d, *J* = 5.0 Hz, 12H), 2.75 (sep, *J* = 5.0 Hz, 2H), 2.85 (s, 3H), 7.36 (d, *J* = 10.0 Hz, 1H), 7.44 (d, *J* = 5.0 Hz, 1H), 7.49 (tr, *J* = 5.0 Hz, 1H), 7.70-7.82 (m, 5H), 8.36 (d, *J* = 5.0 Hz, 1H), 8.68 (d, *J* = 5.0 Hz, 1H), 8.71 (d, *J* = 5.0 Hz, 2H). ^{13}C NMR (125 MHz, $CDCl_3$) δ 24.1, 29.2, 44.7, 122.9, 123.1, 123.9, 124.1, 127.2, 128.2, 128.7, 129.5, 129.6, 130.3, 130.6, 131.5, 131.9, 132.7, 132.8, 133.5, 138.1, 139.7, 143.1, 145.5, 163.8, 164.0. MS (ES^+): m/z 512.2 (MH^+), 534.2 (MNa^+). HRMS (ES^+): calcd. for $C_{31}H_{30}NO_4S$ (MH^+): 512.1890, found: 512.1847.

NMR Spectra.

^{1}H NMR (200 MHz, $CDCl_3$):

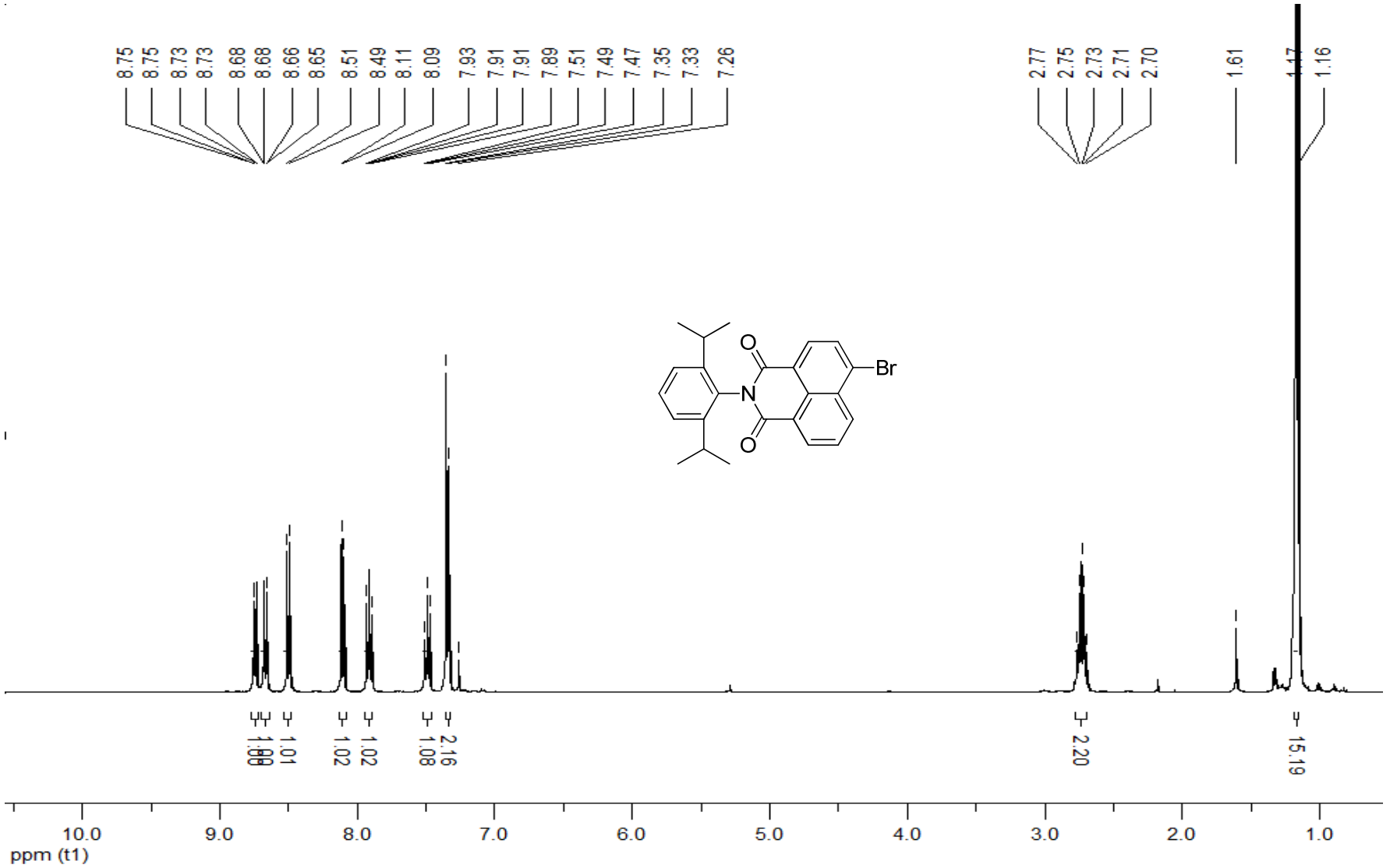


Figure S1. 6-Bromo-2-(2,6-diisopropylphenyl)-1H-benzo[de]isoquinoline-1,3(2H)-dione (compound **2**).

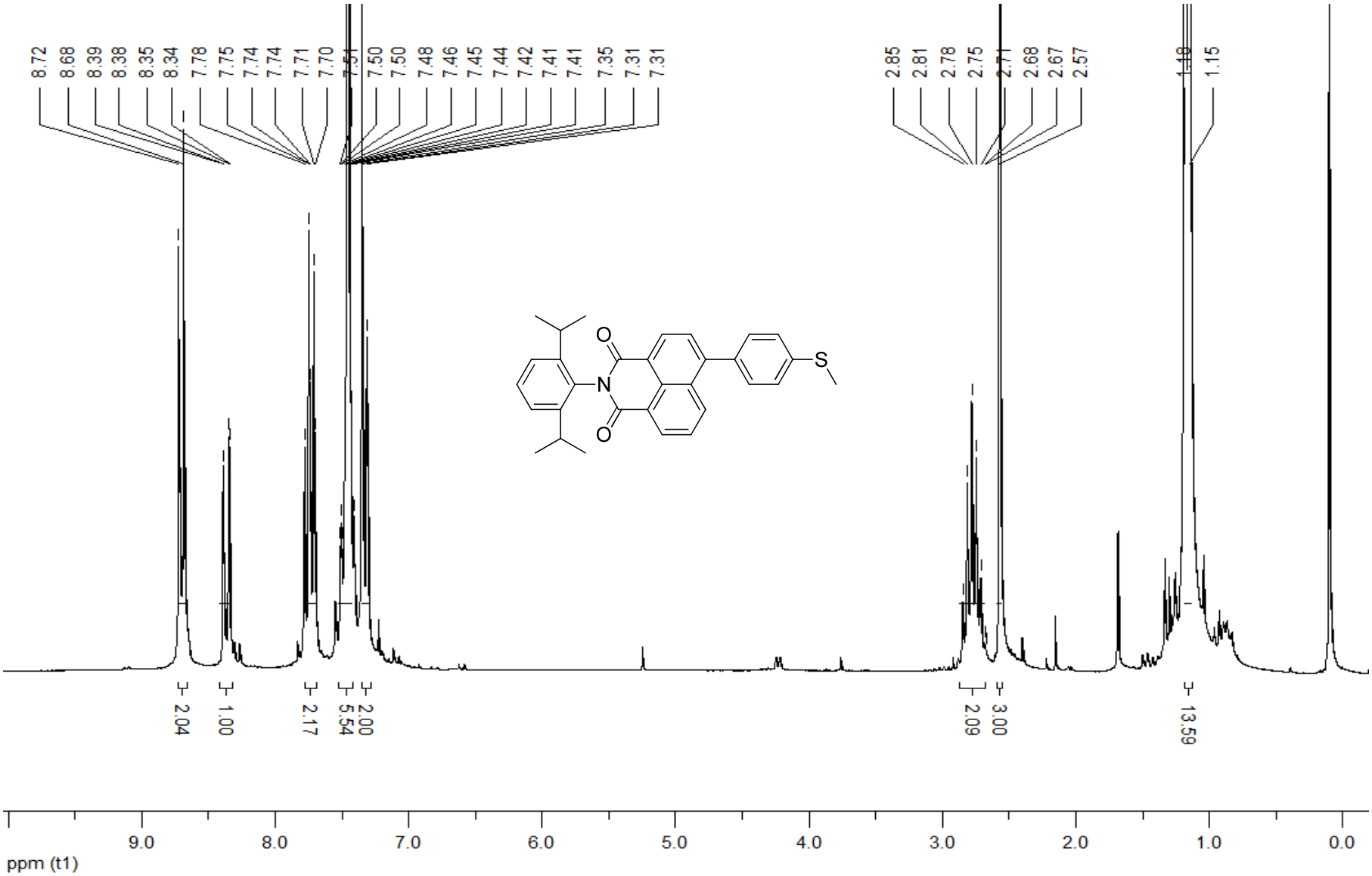


Figure S2. 2-(2,6-Diisopropylphenyl)-6-(4-(methylthio)phenyl)-1H-benzo[de]isoquinoline-1,3(2H)-dione (compound **3**).

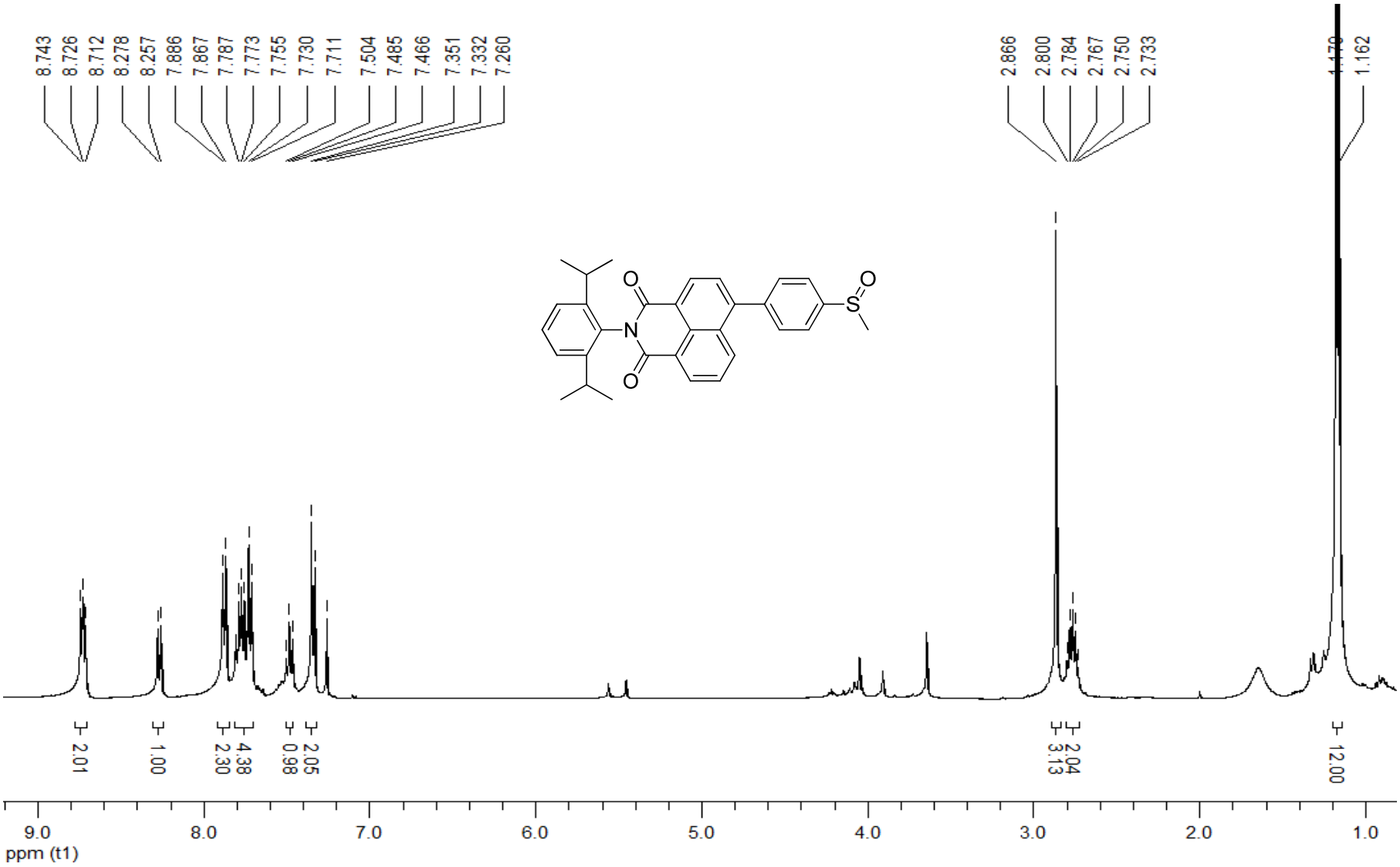


Figure S3. 2-(2,6-diisopropylphenyl)-6-(4-(methylsulfinyl)phenyl)-1H-benzo[de]isoquinoline-1,3(2H)-dione (compound **4**).

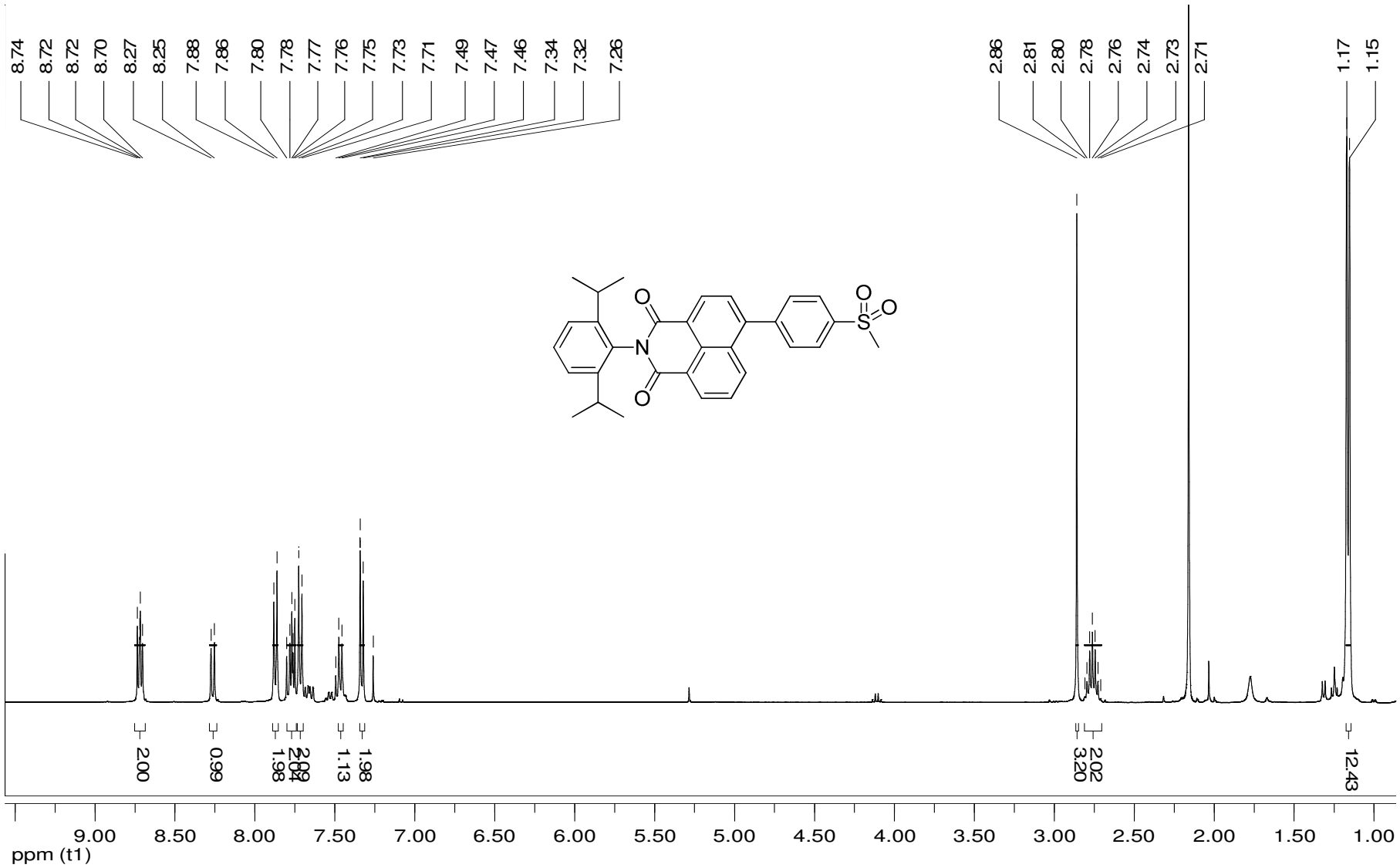


Figure S4. 2-(2,6-Diisopropylphenyl)-6-(4-(methylsulfonyl)phenyl)-1H-benzo[de]isoquinoline-1,3(2H)-dione (compound **5**). (400MHz, CDCl3)

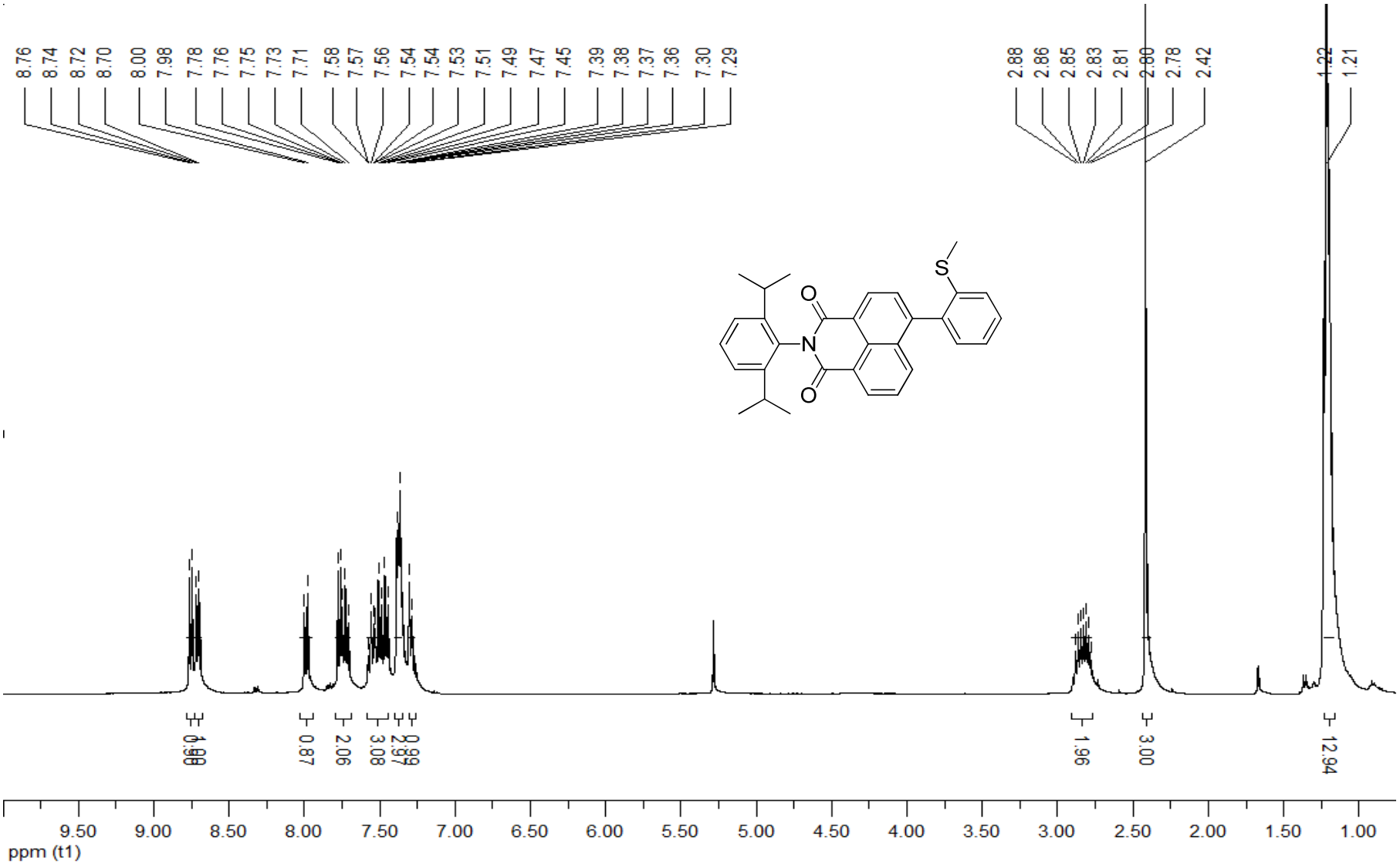


Figure S5. 2-(2,6-Diisopropylphenyl)-6-(2-(methylthio)phenyl)-1H-benzo[de]isoquinoline-1,3(2H)-dione (compound **6**).

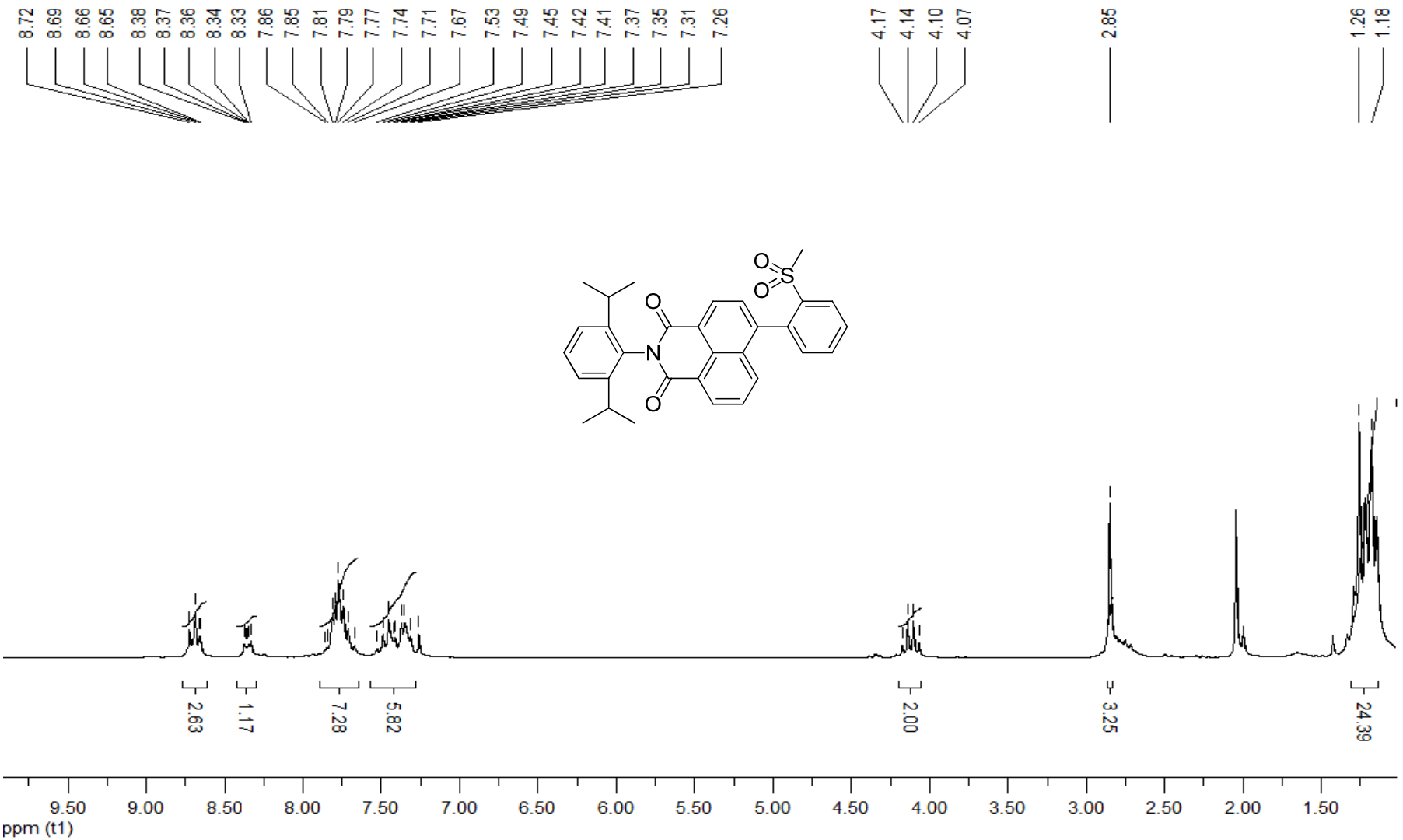


Figure S6. 2-(2,6-diisopropylphenyl)-6-(2-(methylsulfonyl)phenyl)-1H-benzo[de]isoquinoline-1,3(2H)-dione (compound **8**).

^{13}C NMR (100 MHz, $CDCl_3$):

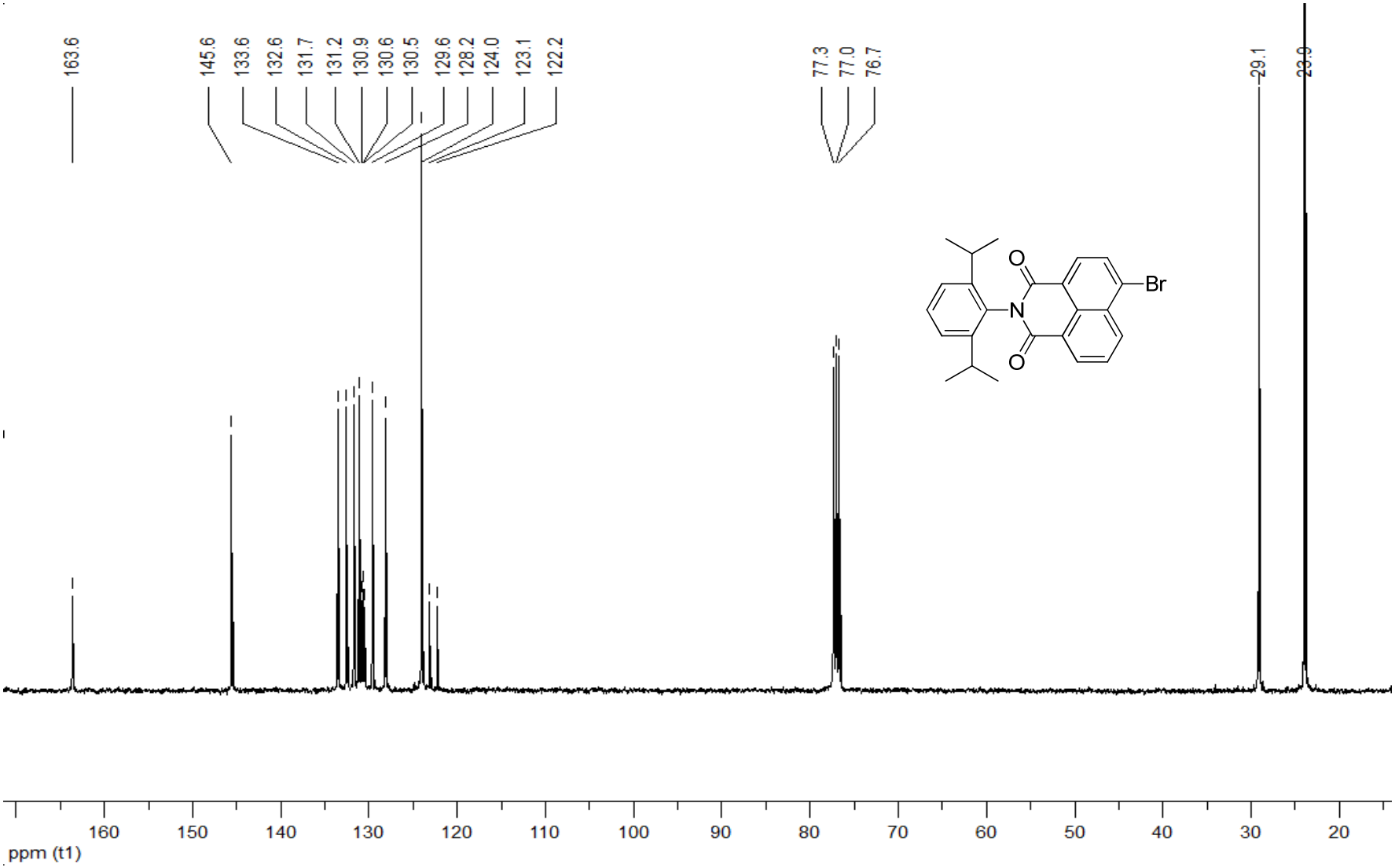


Figure S7. 6-Bromo-2-(2,6-diisopropylphenyl)-1H-benzo[de]isoquinoline-1,3(2H)-dione (compound **2**).

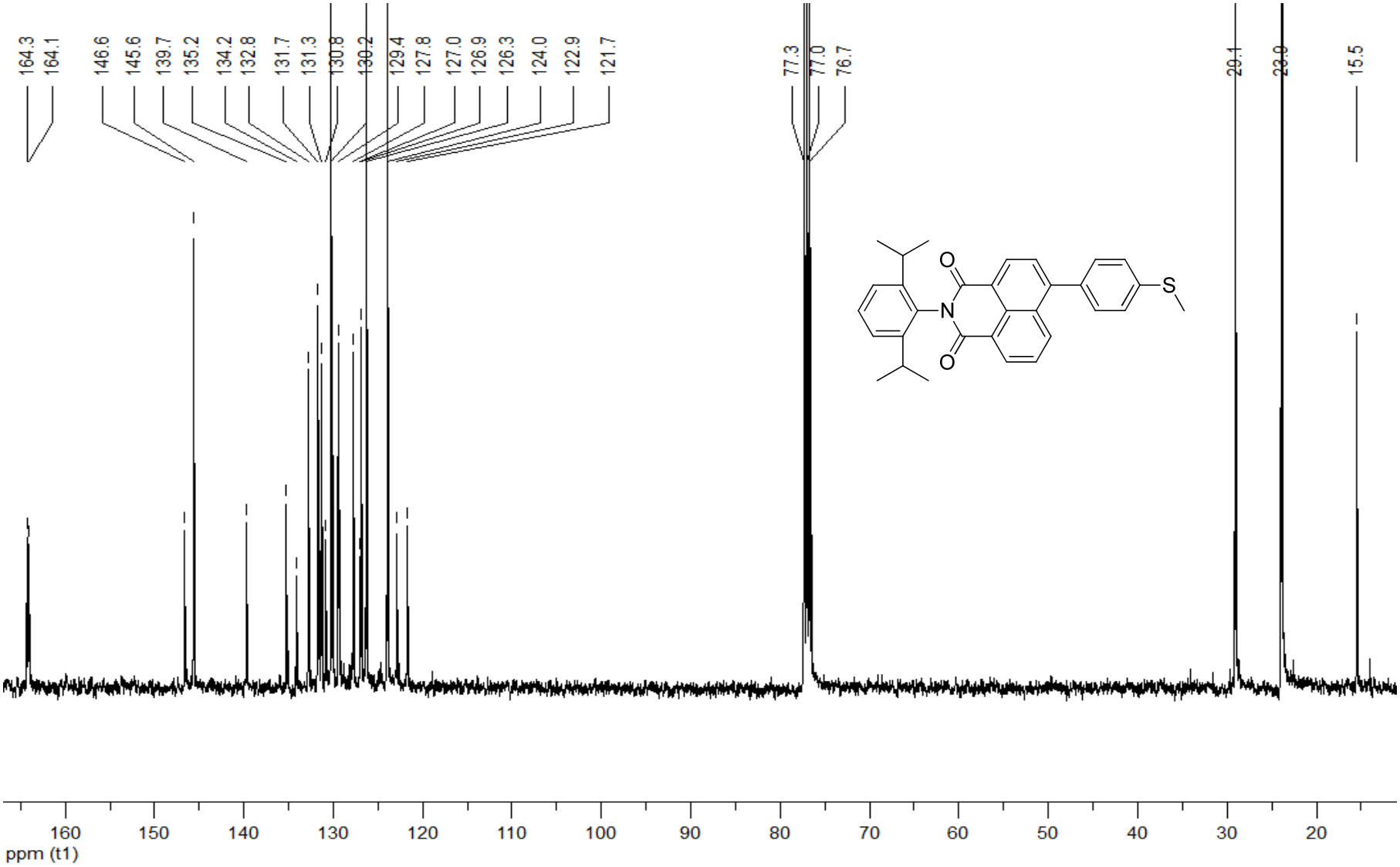


Figure S8. 2-(2,6-Diisopropylphenyl)-6-(4-(methylthio)phenyl)-1H-benzo[de]isoquinoline-1,3(2H)-dione (compound **3**).

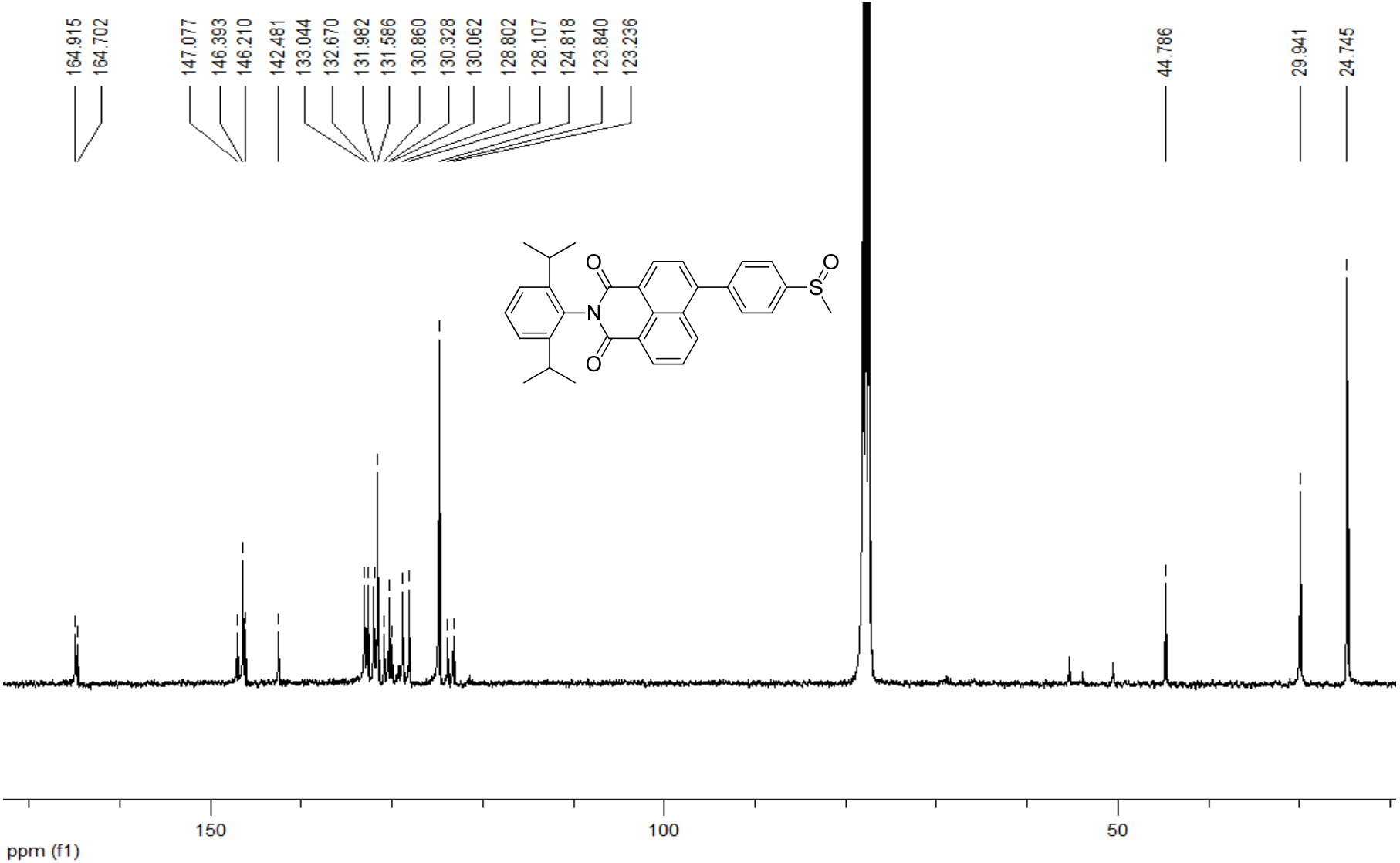


Figure S9. 2-(2,6-diisopropylphenyl)-6-(4-(methylsulfinyl)phenyl)-1H-benzo[de]isoquinoline-1,3(2H)-dione (compound **4**).

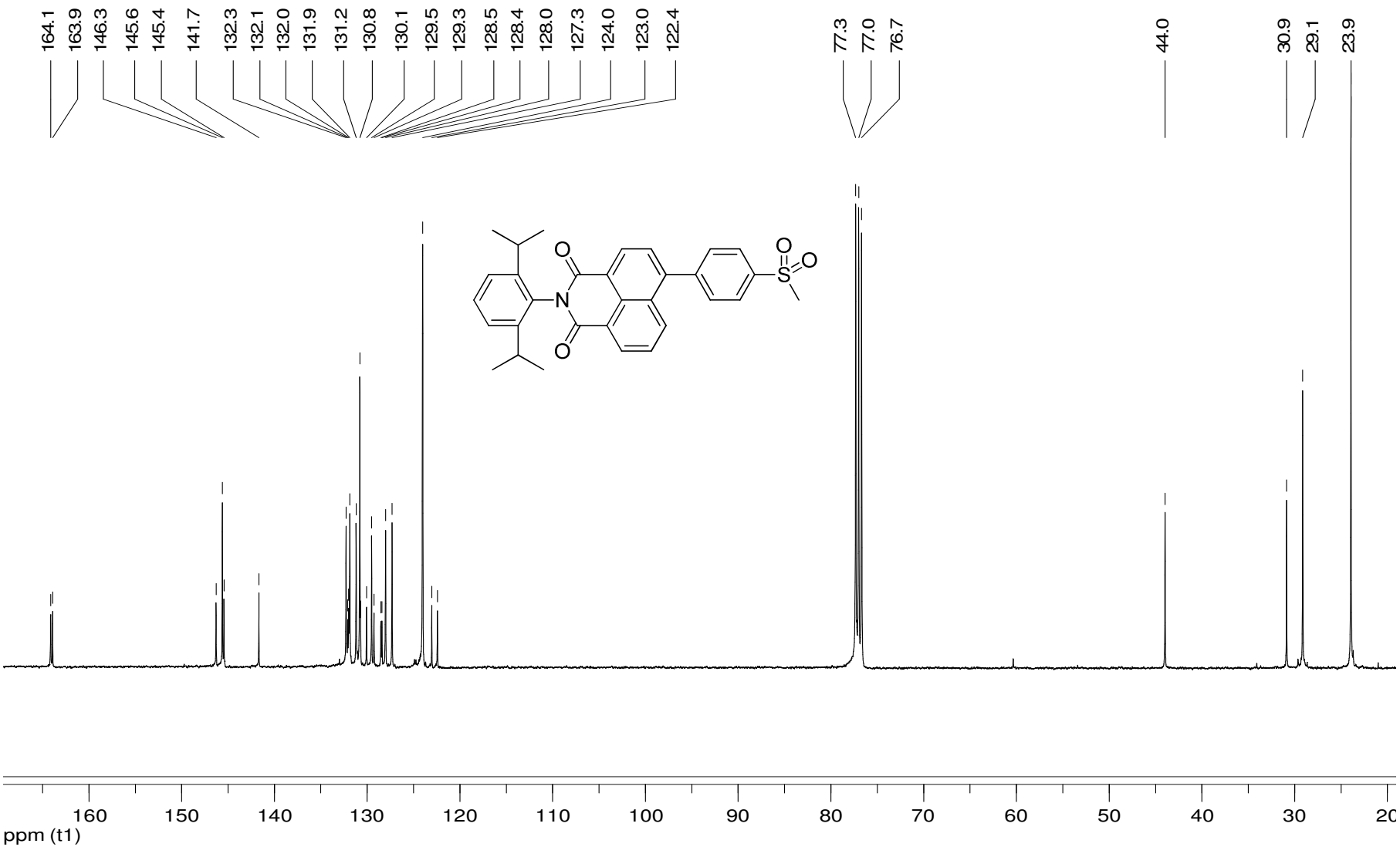


Figure S10. 2-(2,6-Diisopropylphenyl)-6-(4-(methylsulfonyl)phenyl)-1H-benzo[de]isoquinoline-1,3(2H)-dione (compound **5**).

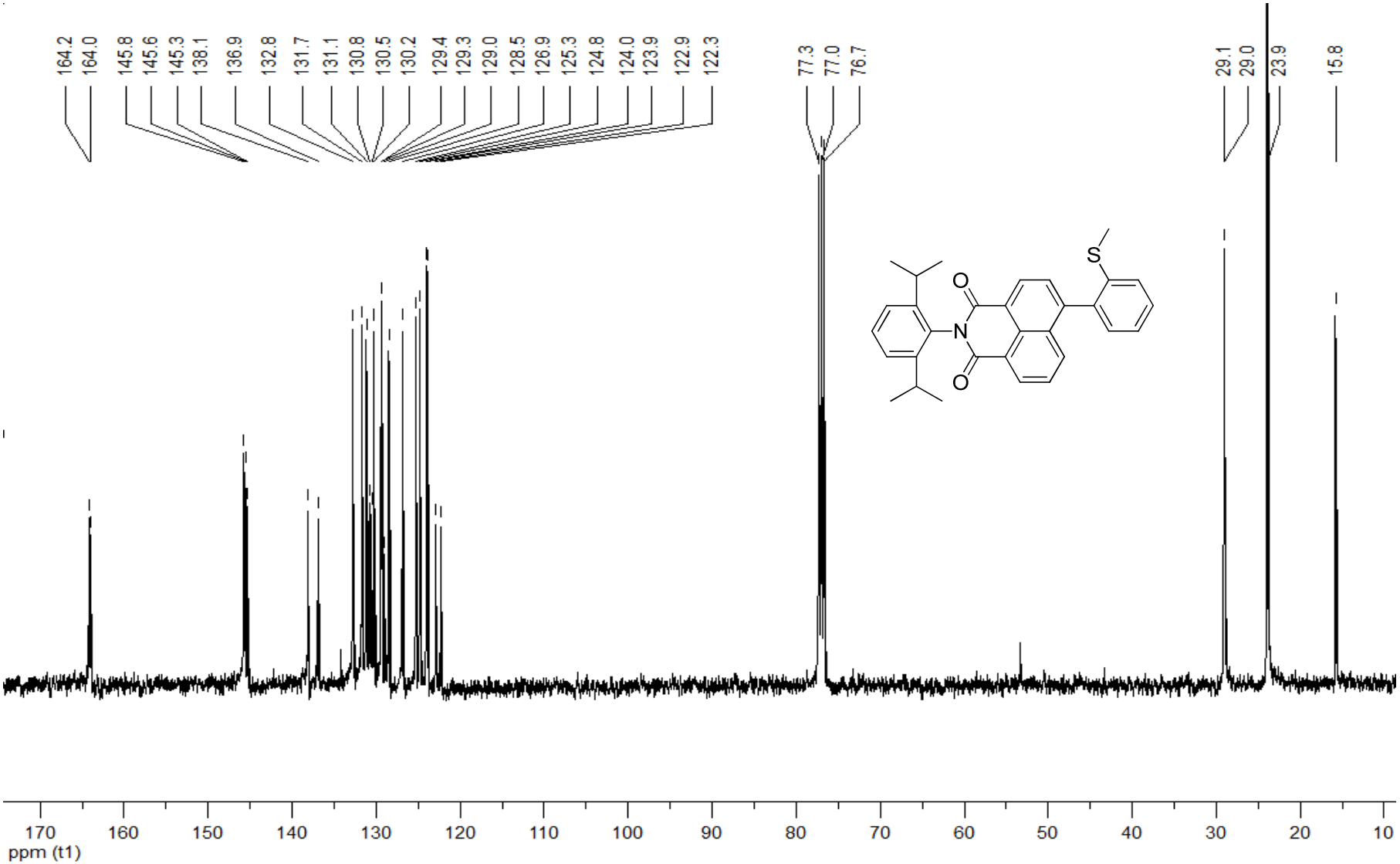


Figure S11. 2-(2,6-Diisopropylphenyl)-6-(2-(methylthio)phenyl)-1H-benzo[de]isoquinoline-1,3(2H)-dione (compound **6**).

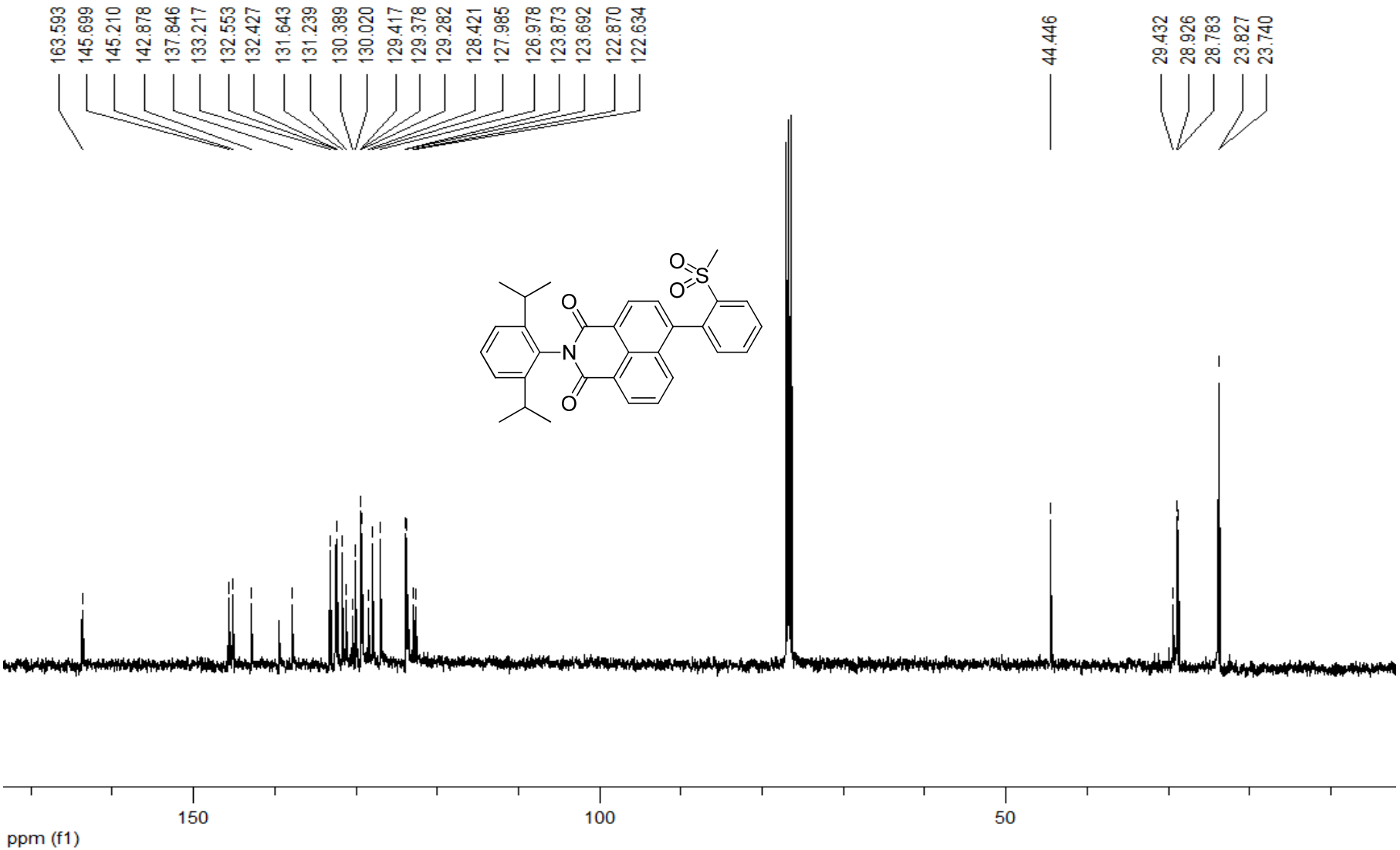


Figure S12. 2-(2,6-diisopropylphenyl)-6-(2-(methylsulfonyl)phenyl)-1H-benzo[de]isoquinoline-1,3(2H)-dione (compound **8**).

MS Spectra.

MS (CI^+):

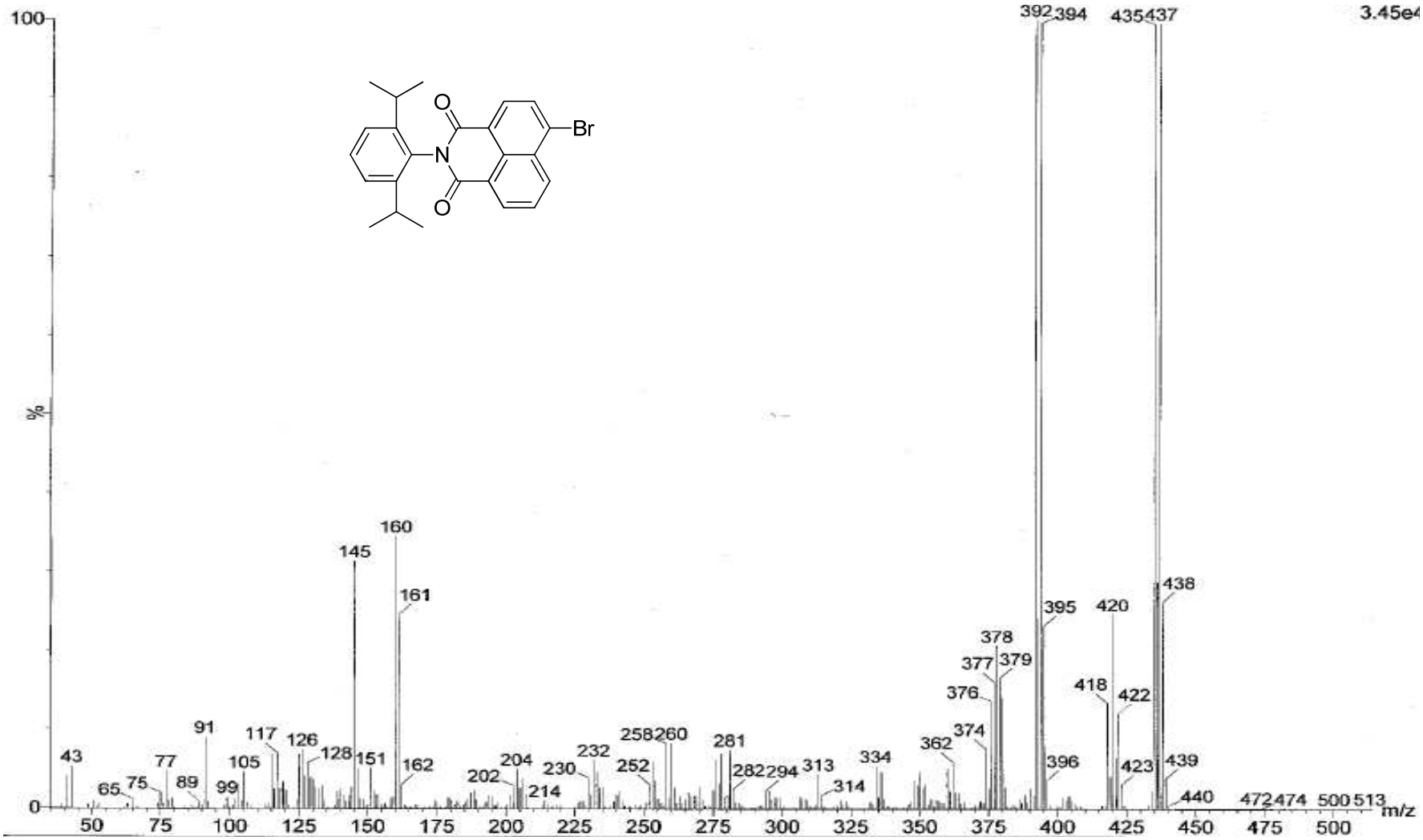


Figure S13. 6-Bromo-2-(2,6-diisopropylphenyl)-1H-benzo[de]isoquinoline-1,3(2H)-dione (compound **2**).

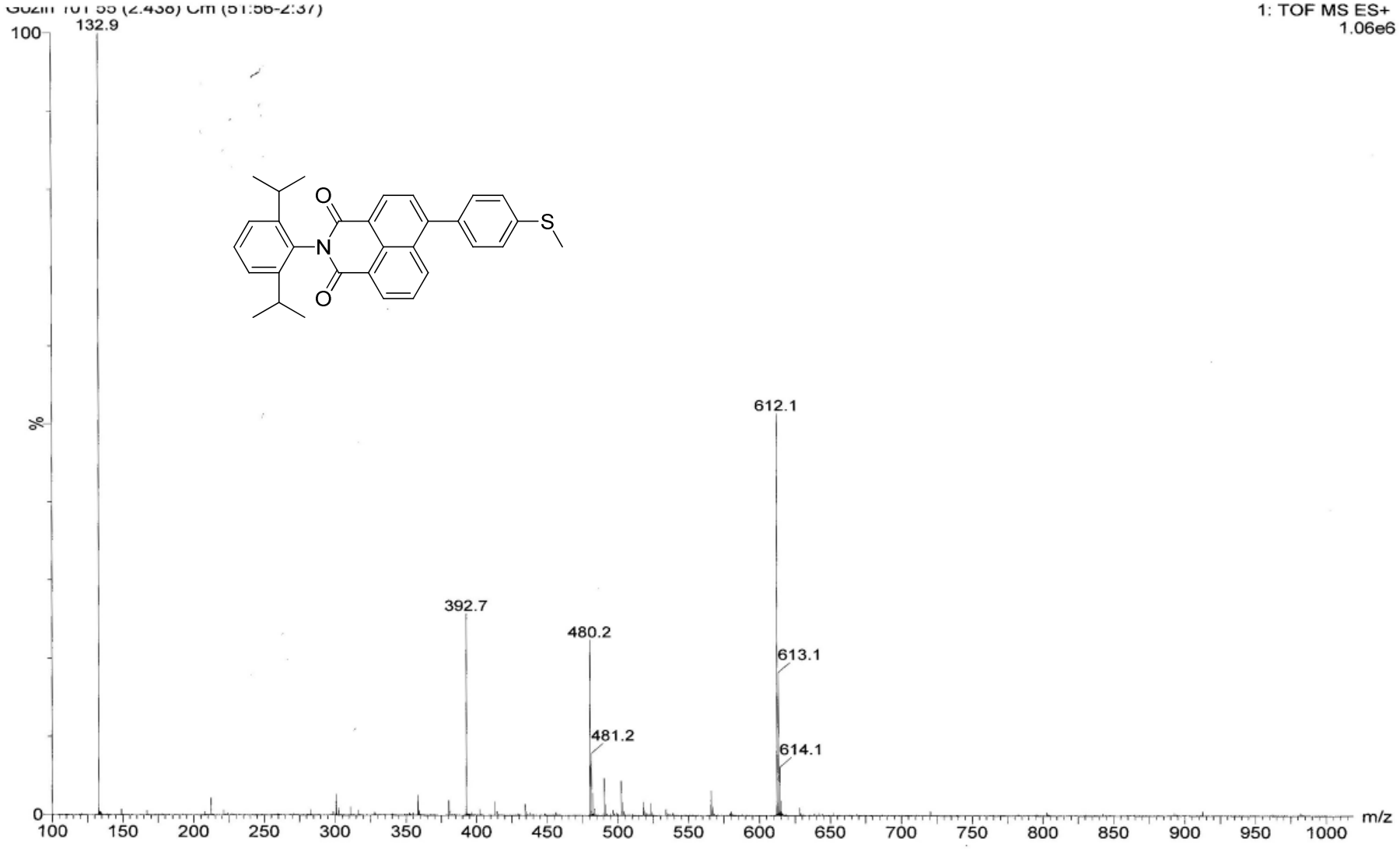


Figure S14. 2-(2,6-Diisopropylphenyl)-6-(4-(methylthio)phenyl)-1H-benzo[de]isoquinoline-1,3(2H)-dione (compound **3**).

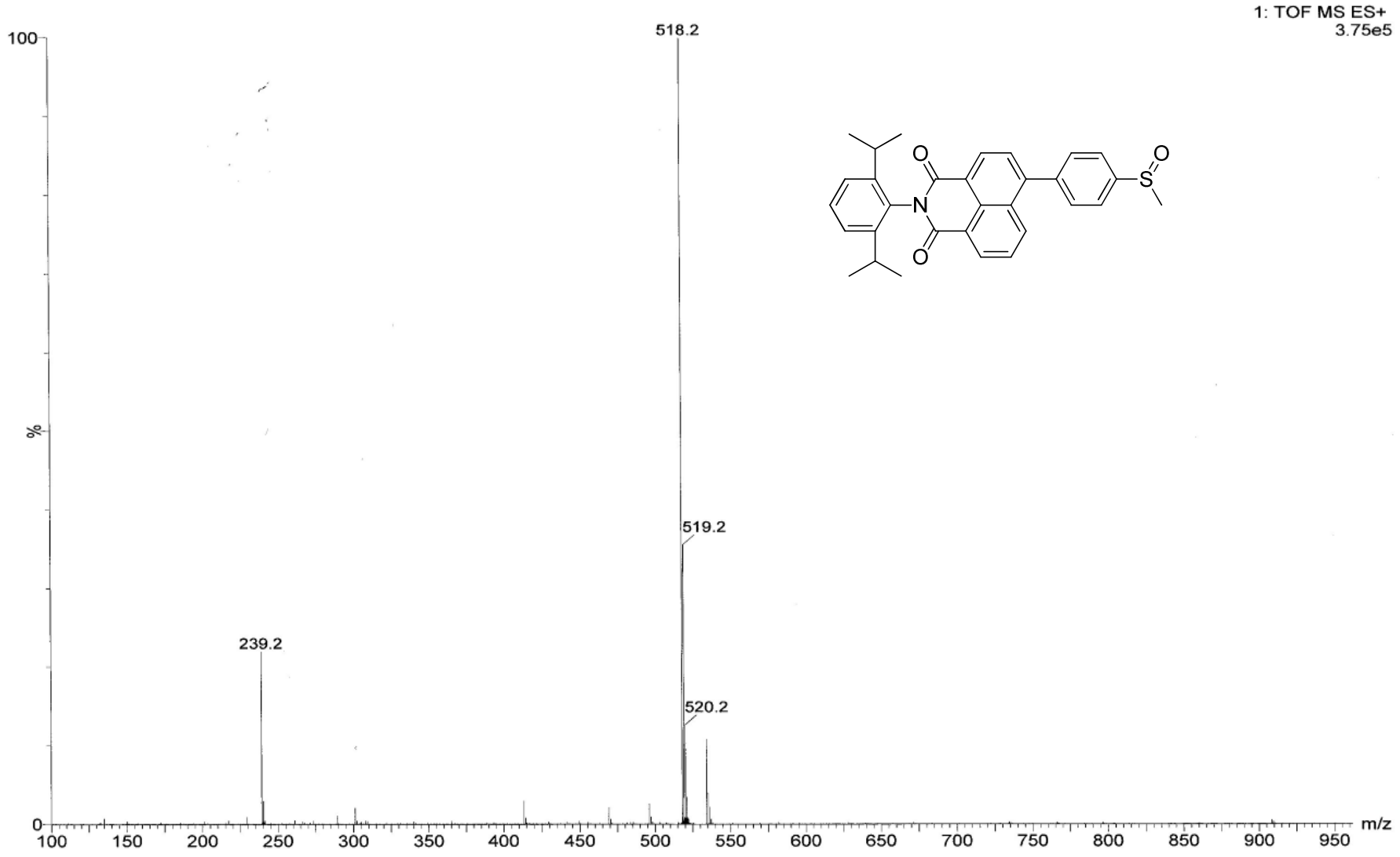


Figure S15. 2-(2,6-diisopropylphenyl)-6-(4-(methylsulfinyl)phenyl)-1H-benzo[de]isoquinoline-1,3(2H)-dione (compound **4**).

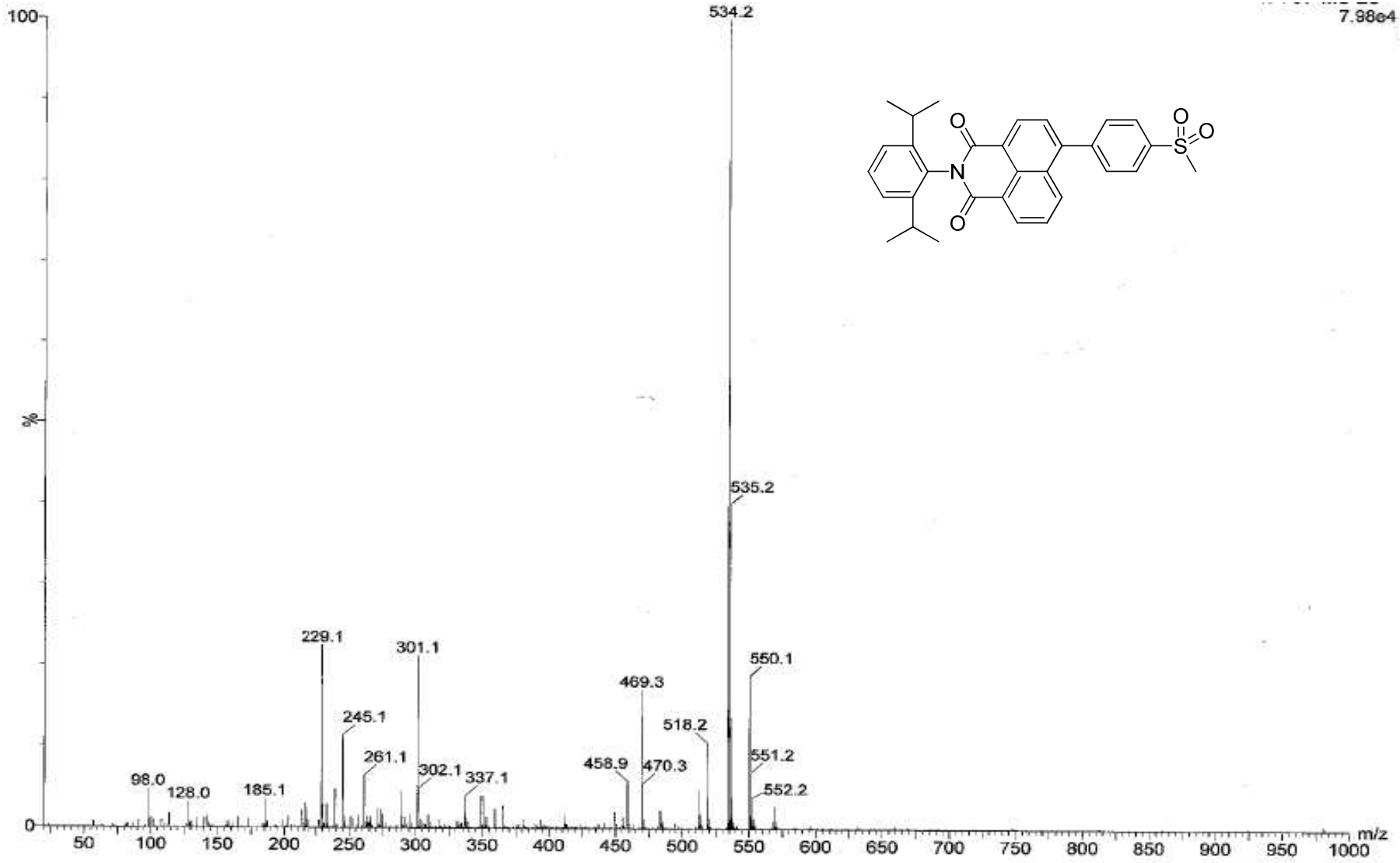


Figure S16. 2-(2,6-Diisopropylphenyl)-6-(4-(methylsulfonyl)phenyl)-1H-benzo[de]isoquinoline-1,3(2H)-dione (compound **5**).

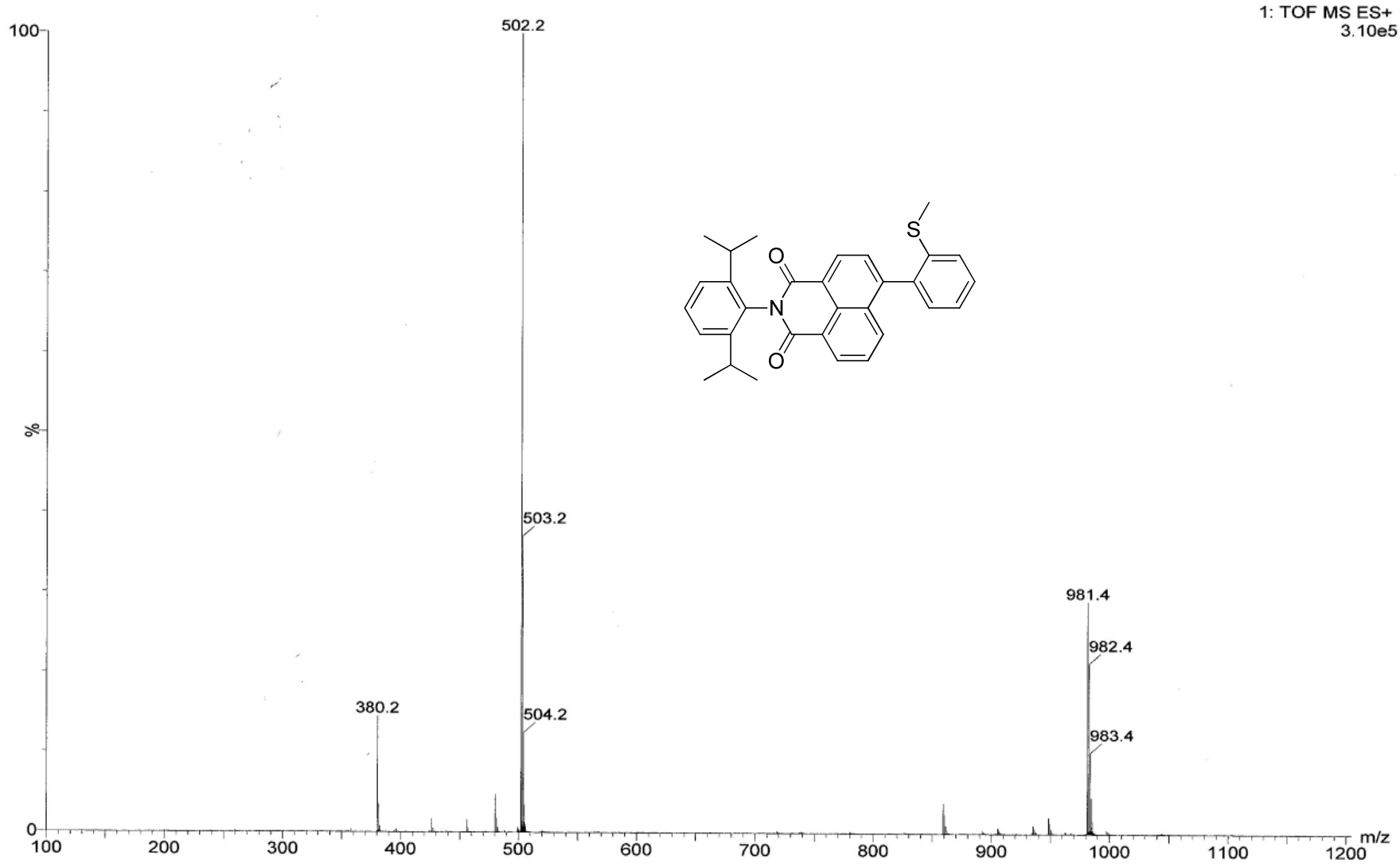


Figure S17. 2-(2,6-Diisopropylphenyl)-6-(2-(methylthio)phenyl)-1H-benzo[de]isoquinoline-1,3(2H)-dione (compound **6**).

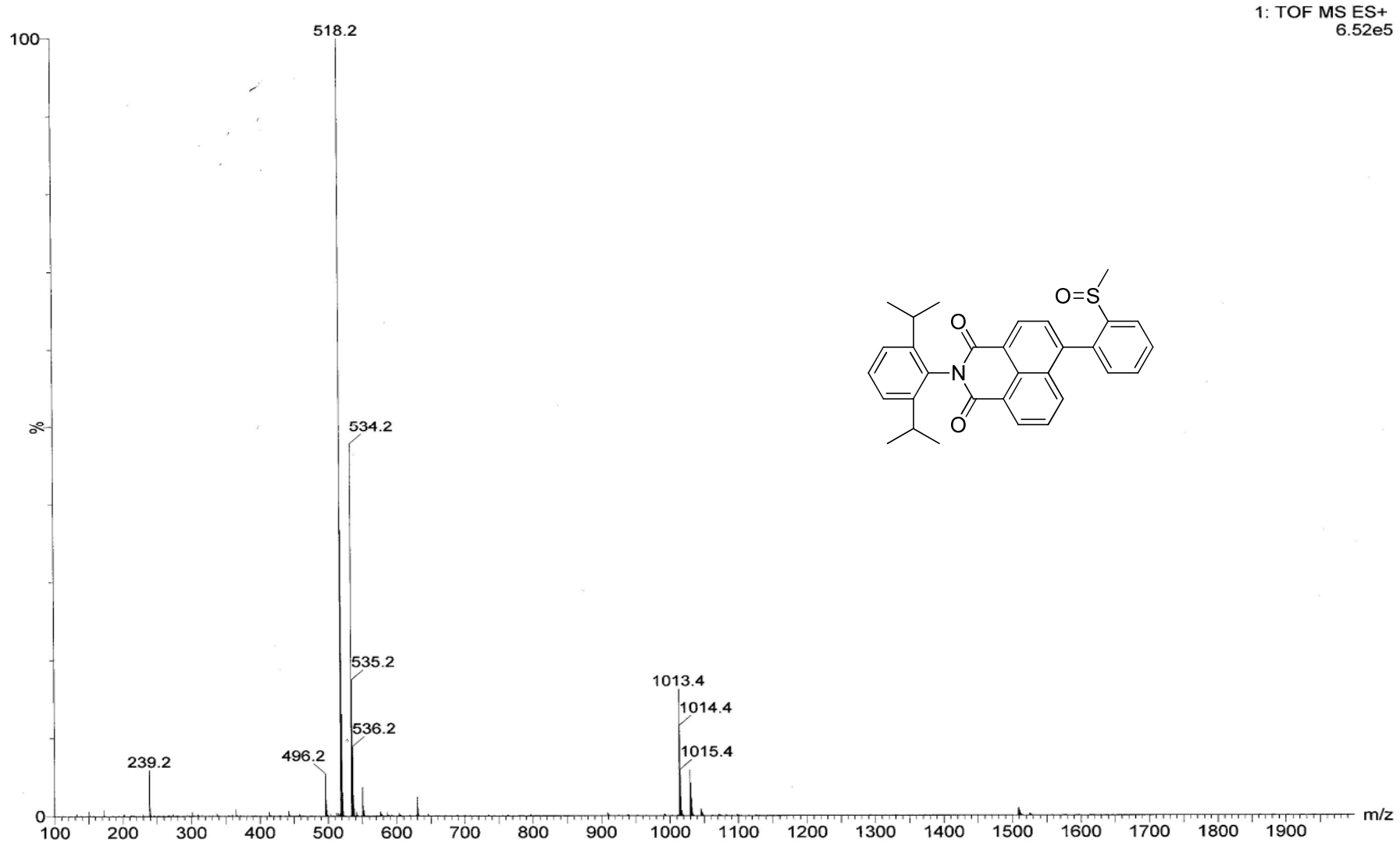


Figure S18. 2-(2,6-diisopropylphenyl)-6-(2-(methylsulfinyl)phenyl)-1H-benzo[de]isoquinoline-1,3(2H)-dione (compound **7**).

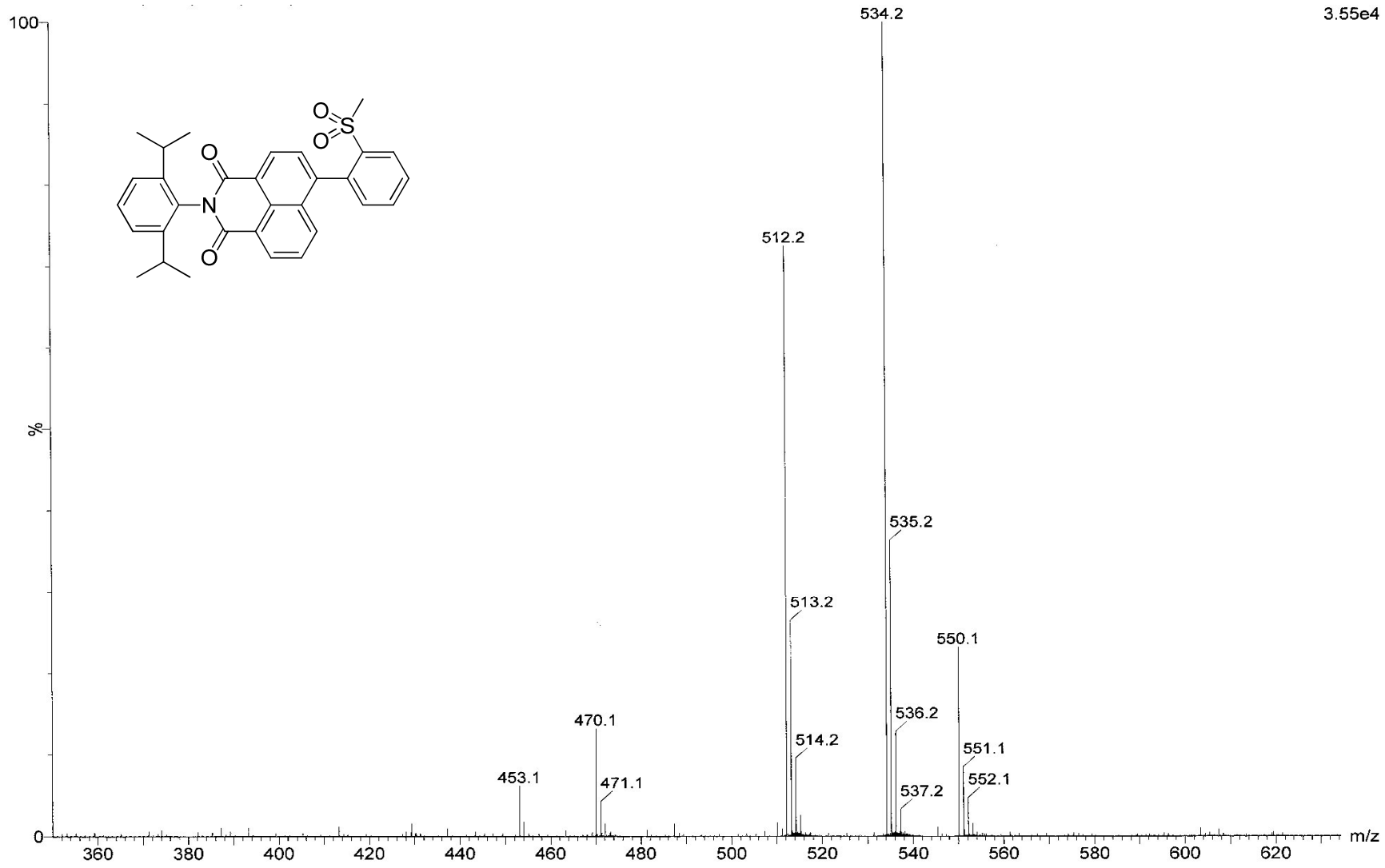


Figure S19. 2-(2,6-diisopropylphenyl)-6-(2-(methylsulfonyl)phenyl)-1H-benzo[de]isoquinoline-1,3(2H)-dione (compound **8**).

FT-IR (KBr).

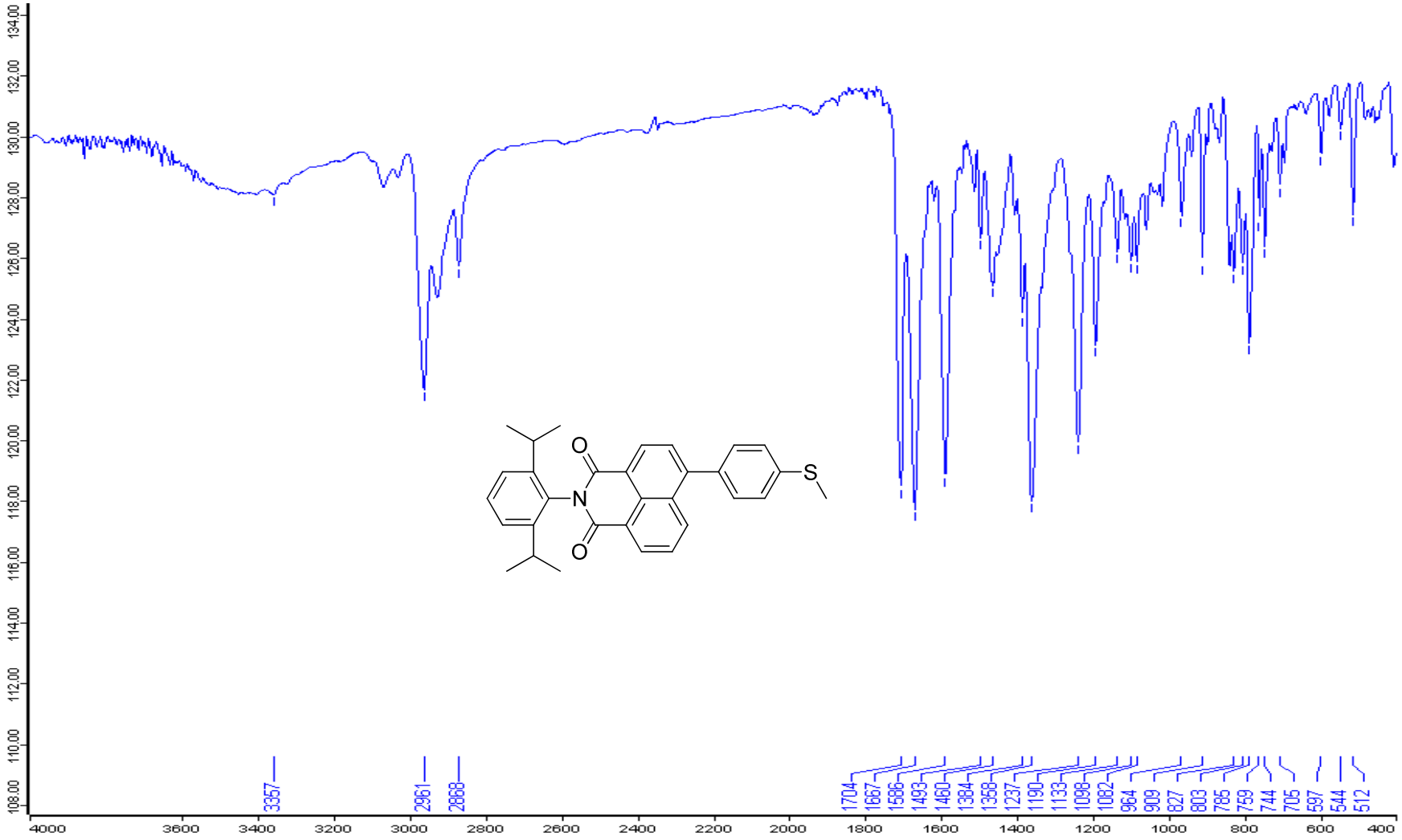


Figure S20. 2-(2,6-Diisopropylphenyl)-6-(4-(methylthio)phenyl)-1H-benzo[de]isoquinoline-1,3(2H)-dione (compound **3**).

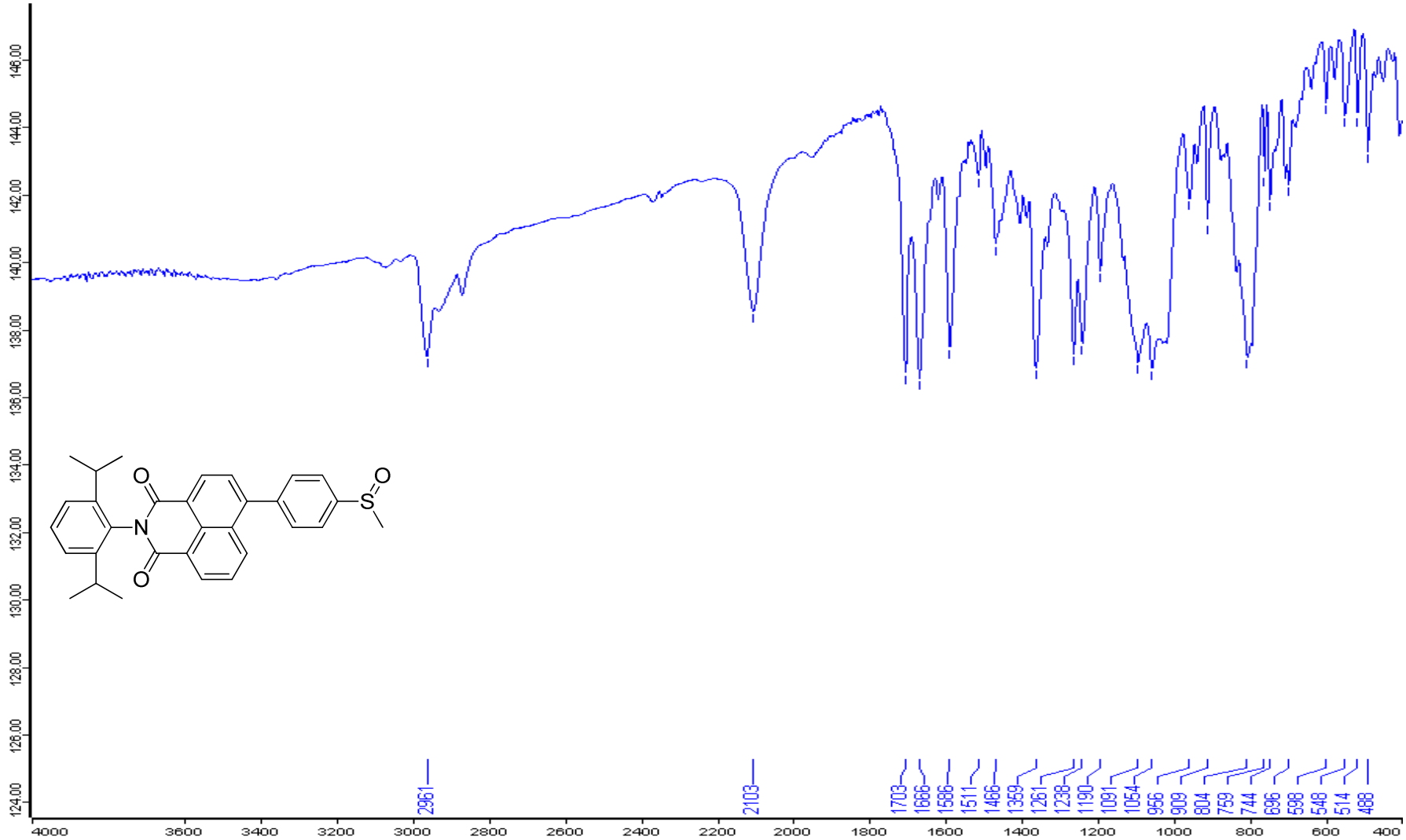


Figure S21. 2-(2,6-diisopropylphenyl)-6-(4-(methylsulfinyl)phenyl)-1H-benzo[de]isoquinoline-1,3(2H)-dione (compound **4**).

UV Spectra.

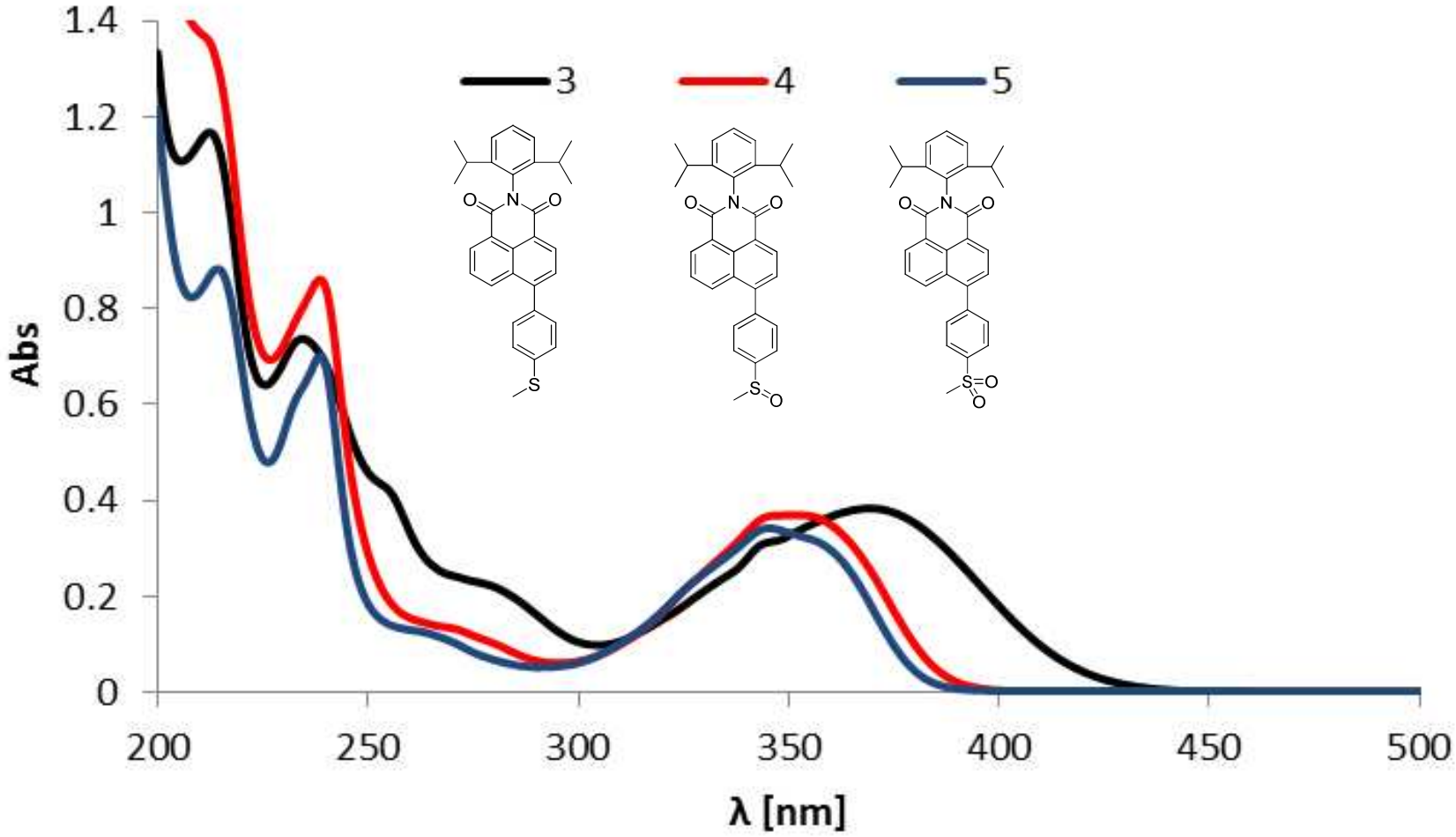


Figure S22. Overlay spectra of compounds **3**, **4** and **5**.

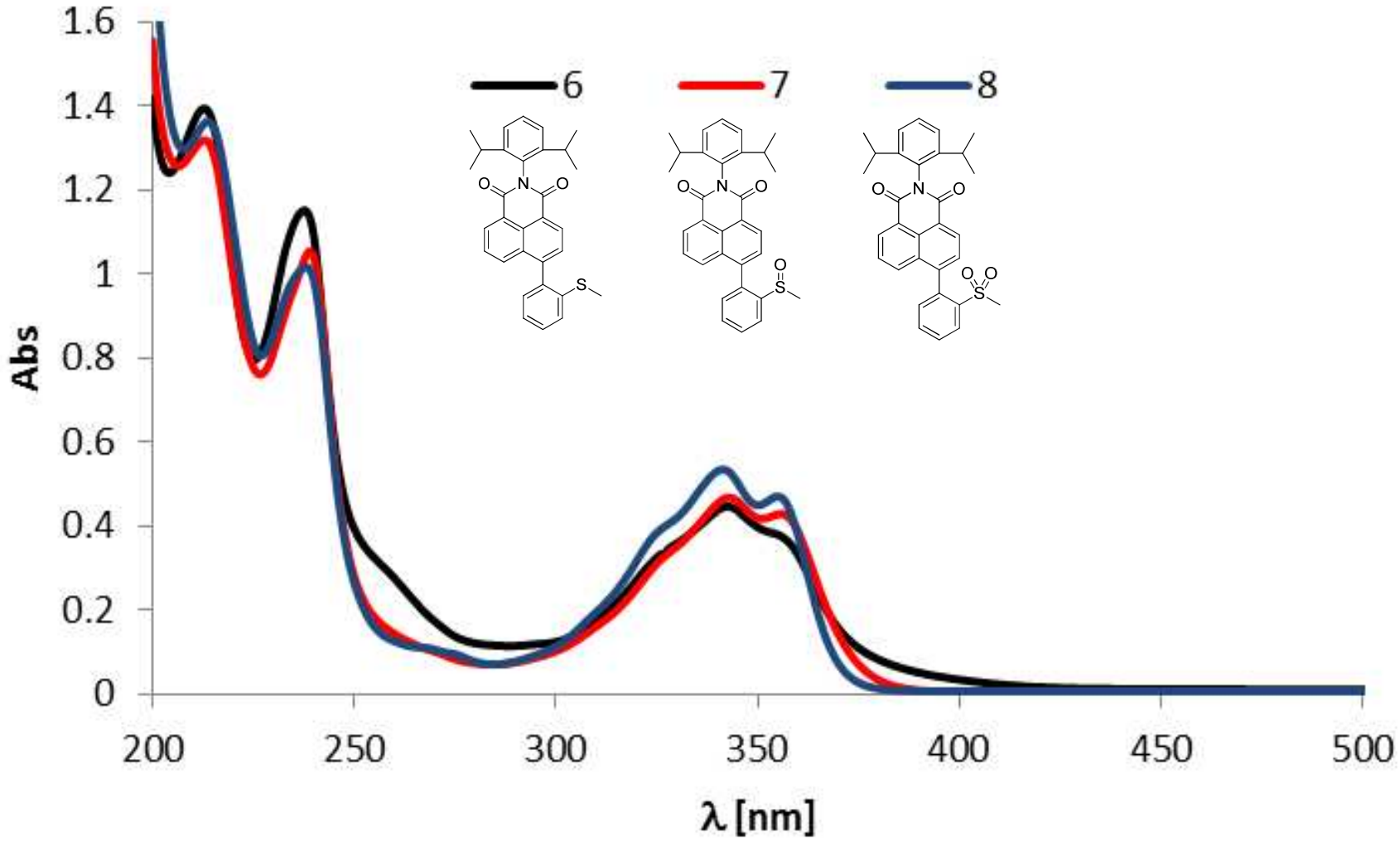


Figure S23. Overlay spectra of 6,7 and 8

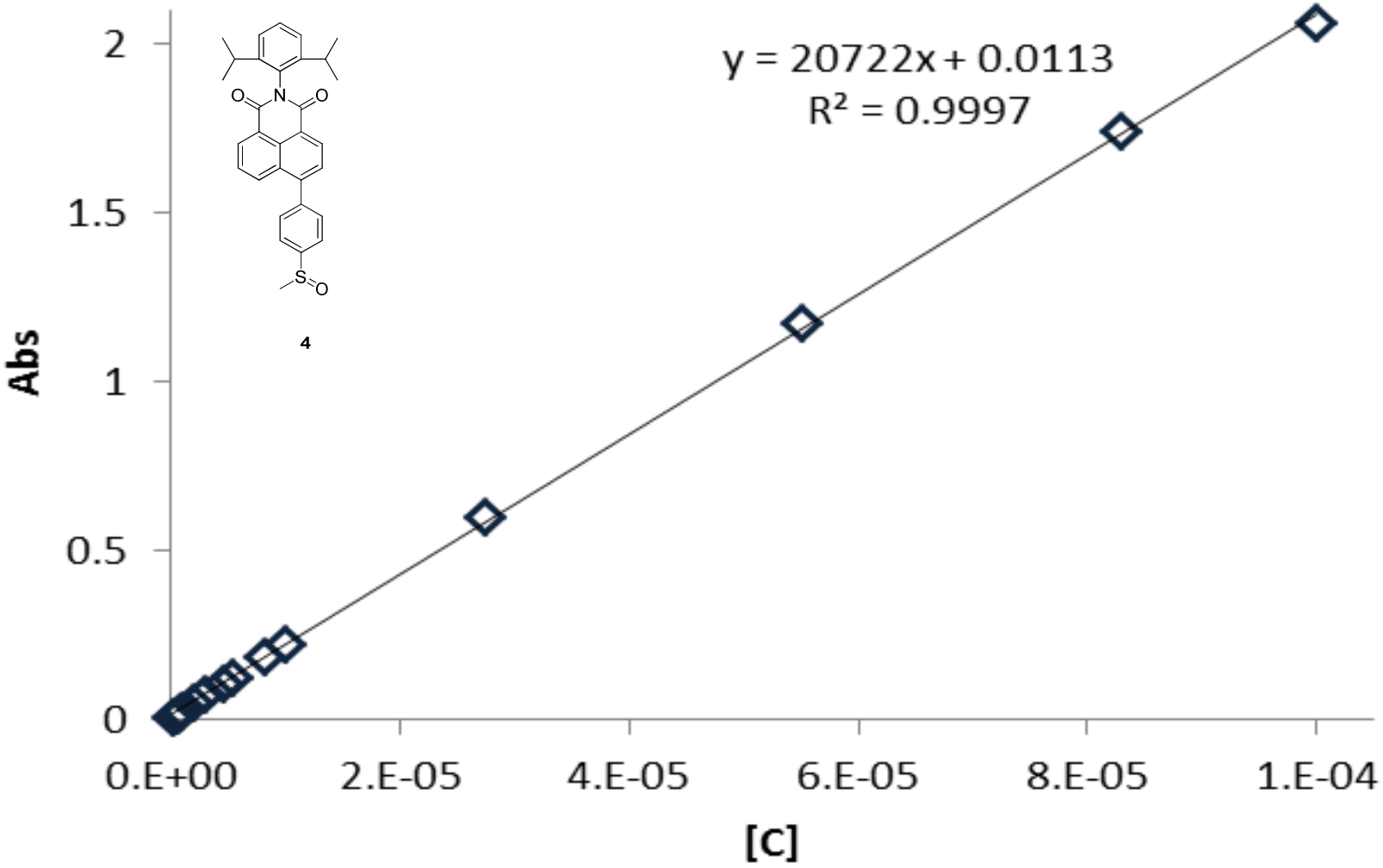


Figure S24. Correlation between the concentration of 4 and the intensity of the corresponding absorbance.

Fluorescence Spectra.

Concentration tests:

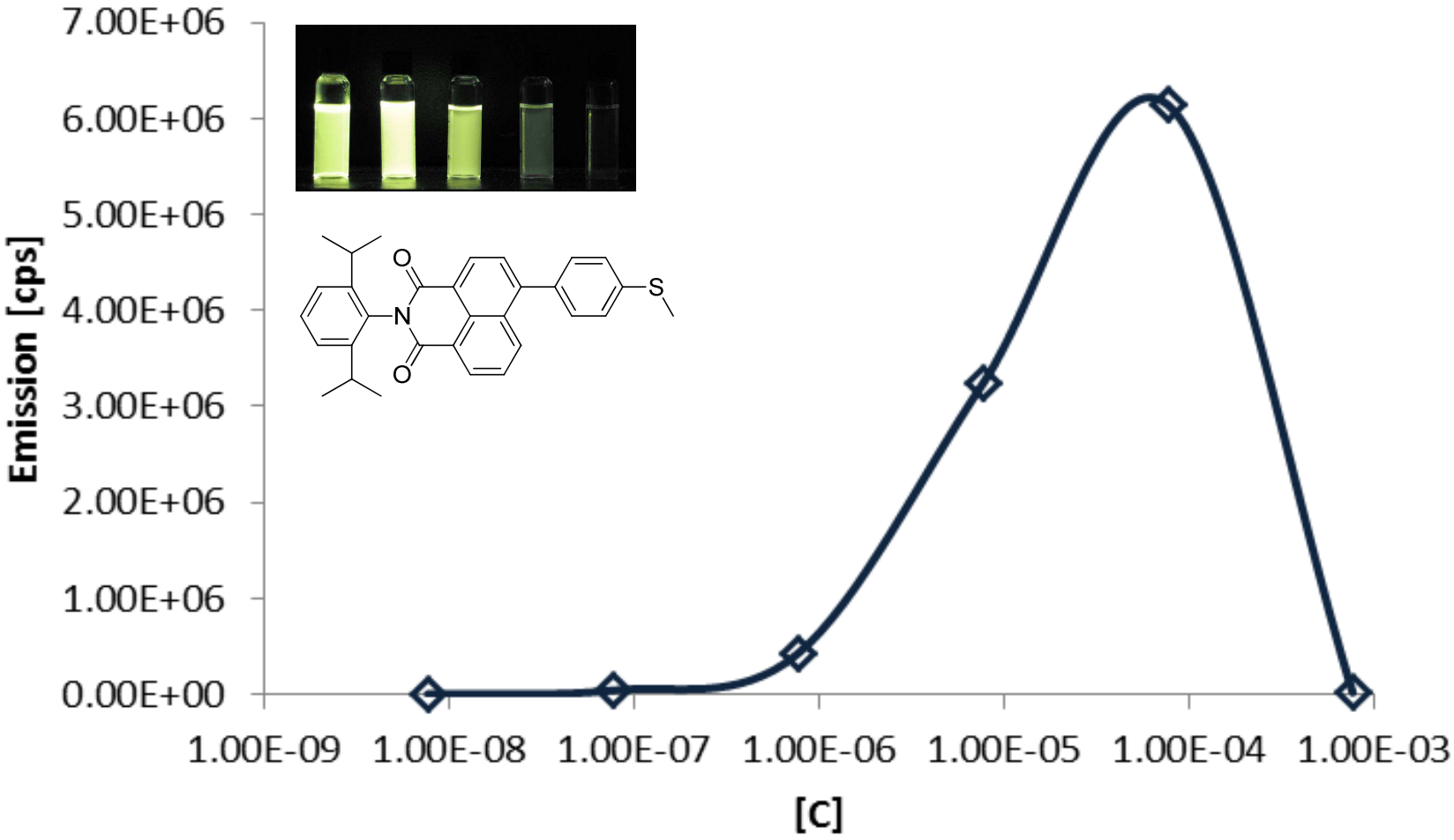


Figure S25. Concentration test for compound **3**, λ_{ex}= 370 [nm]

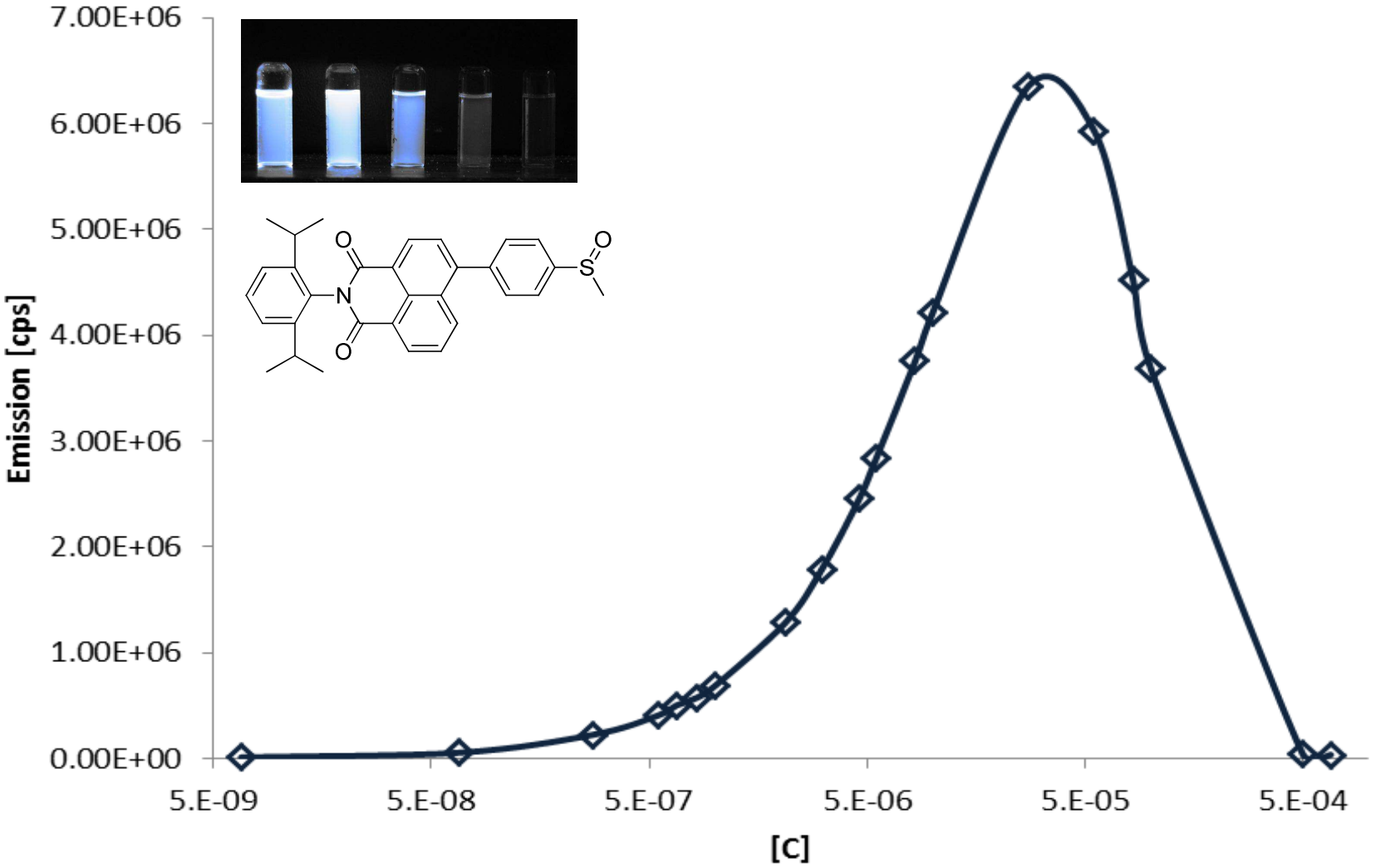


Figure S26. Concentration test for compound **4**, λ_{ex}= 350 [nm].

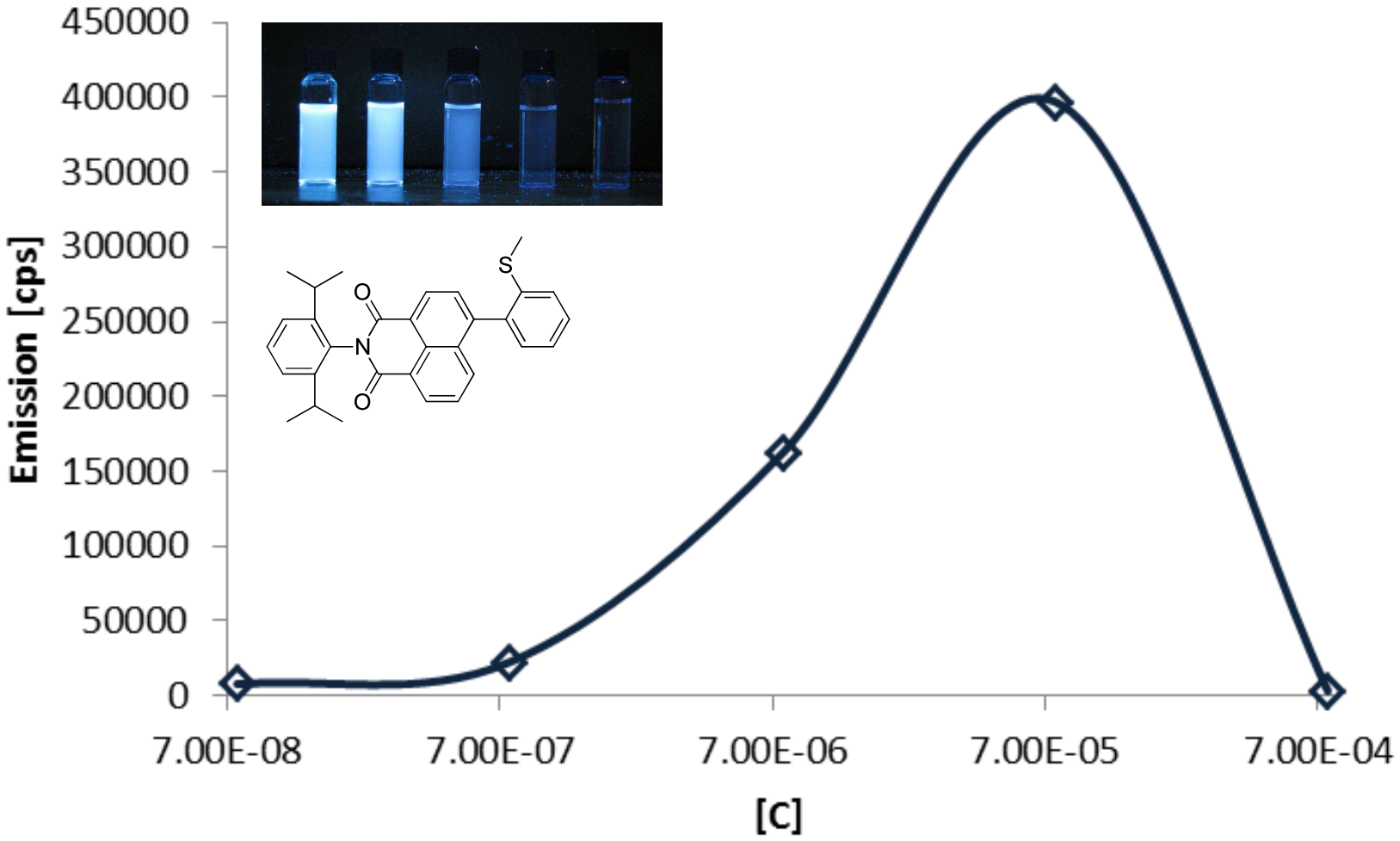


Figure S27. Concentration test for compound **6**, λ_{ex}= 340 [nm]

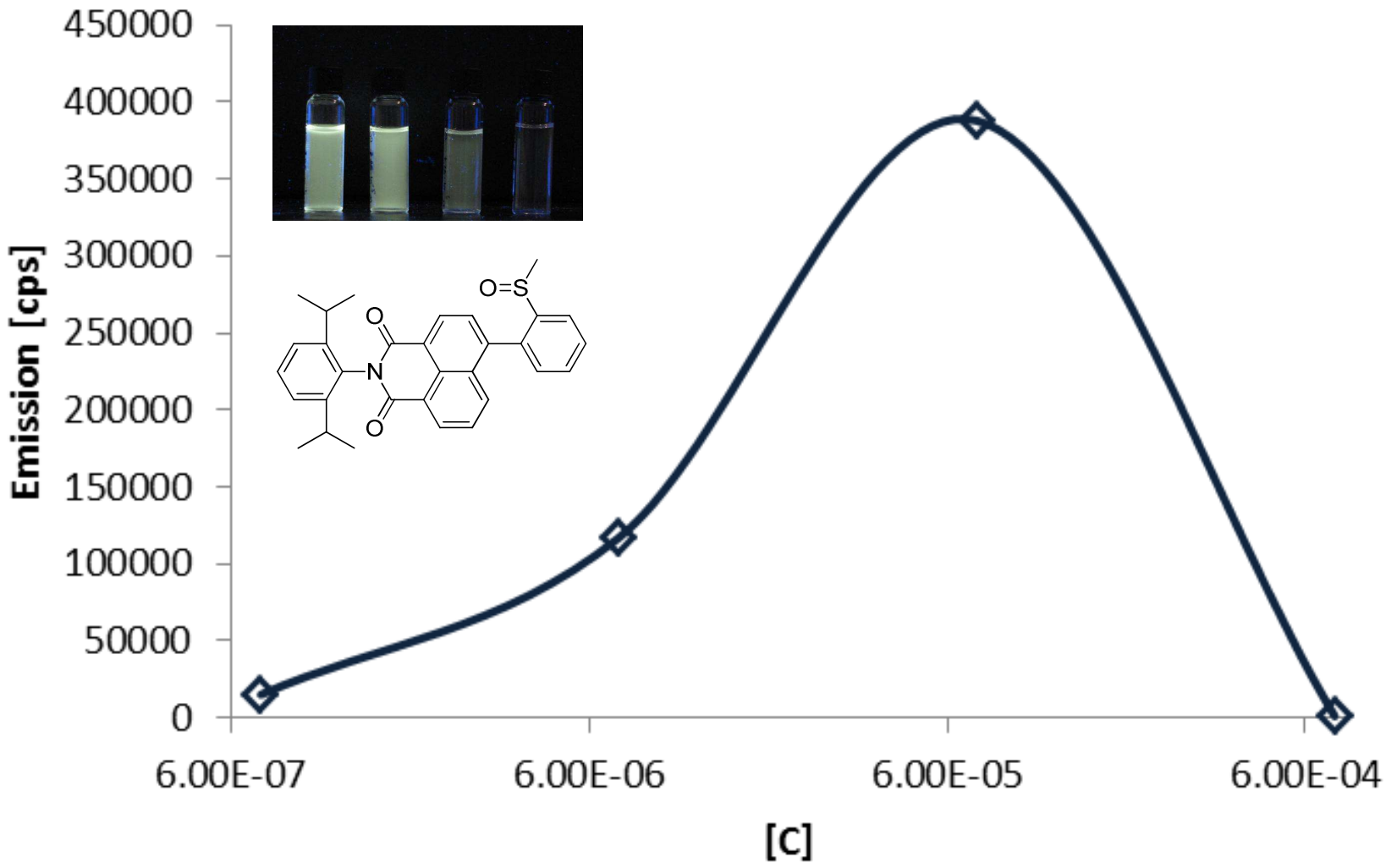


Figure S28. Concentration test for compound **7**, λ_{ex}= 340 [nm]

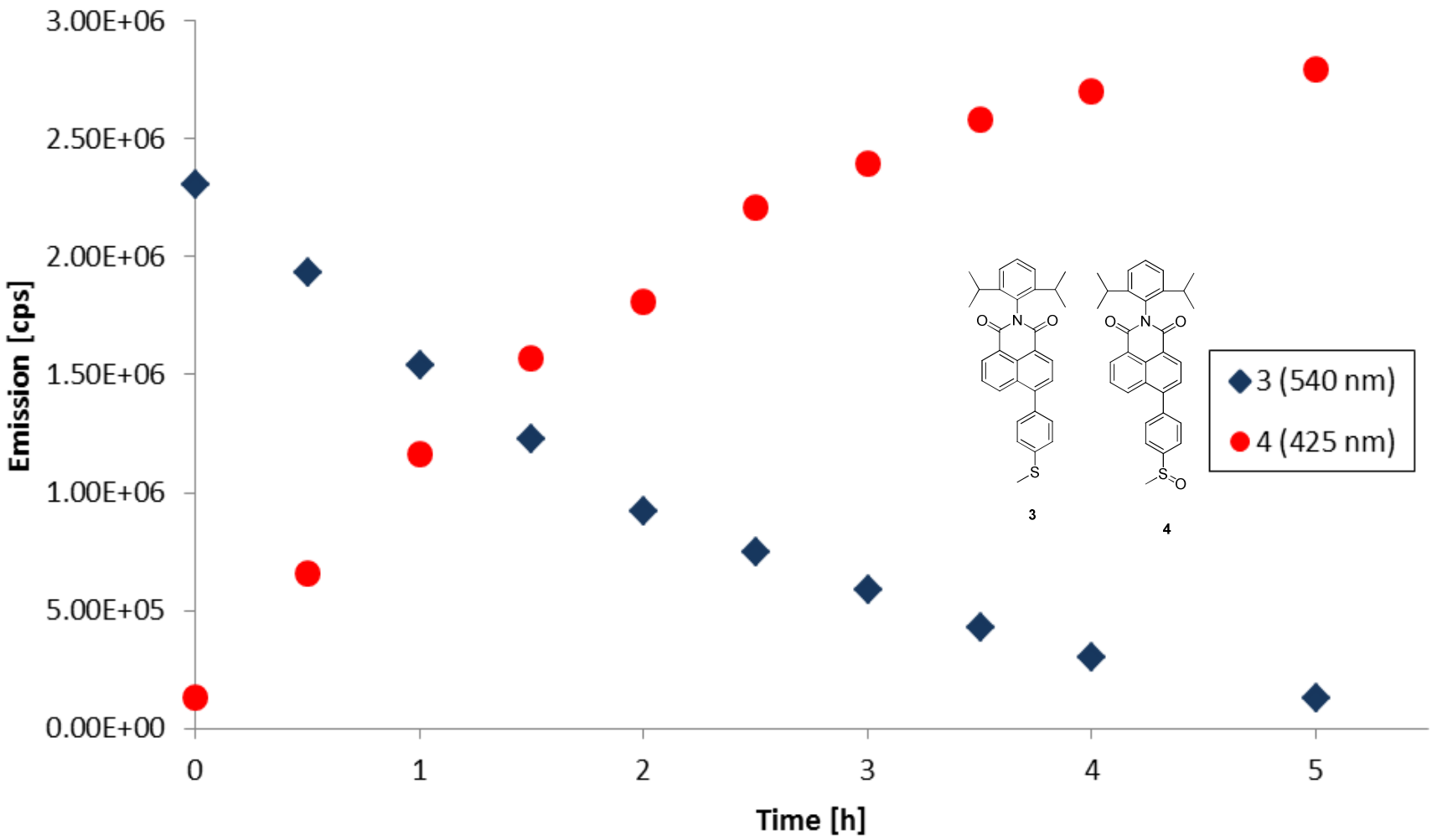


Figure S29. Chnages in fluorescence spectra during the oxidation of compound **3** to **4**.

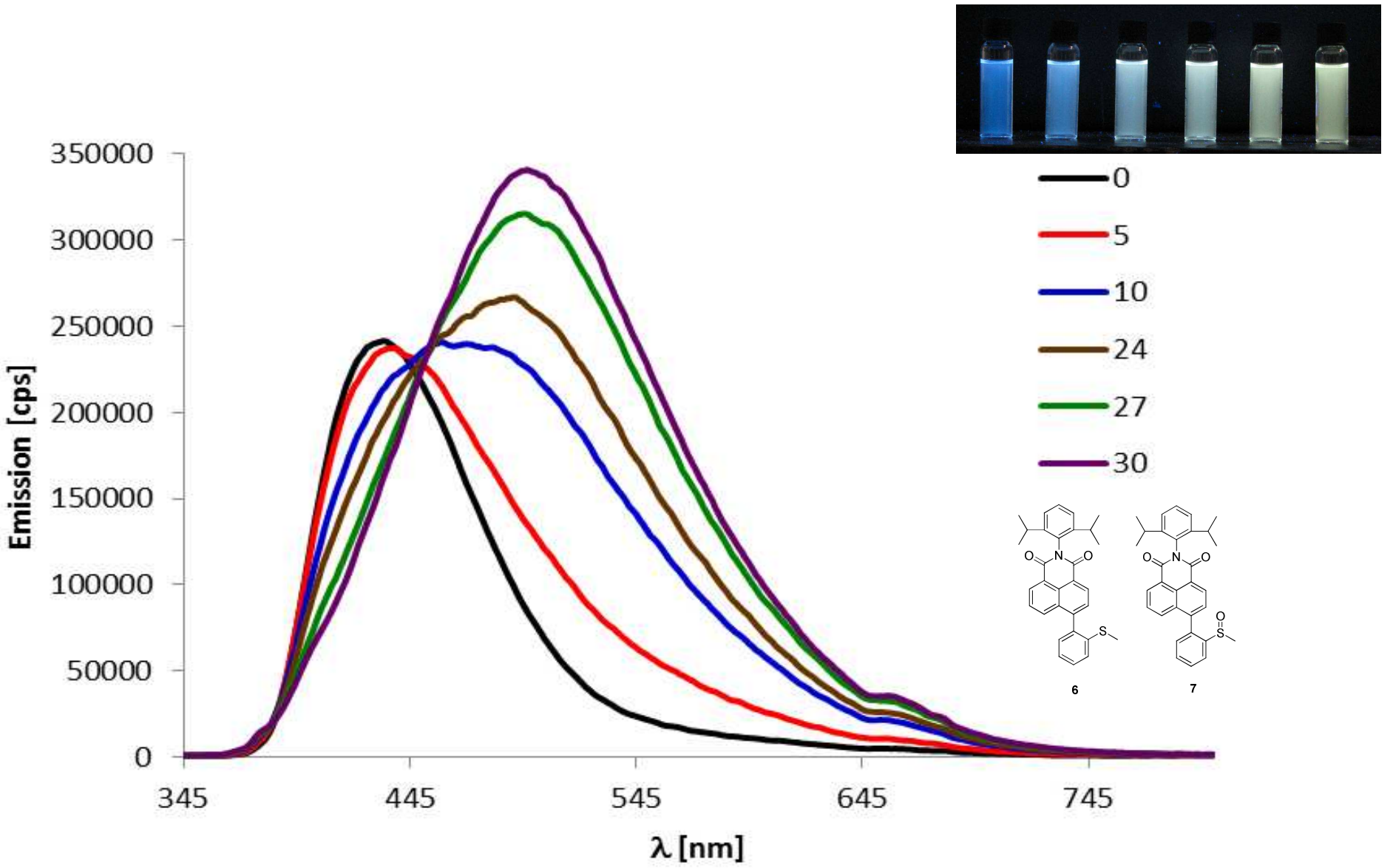


Figure S30. Chnages in fluorescence spectra during the oxidation of compound **6** to **7**.

Quantum Yields

Quantum yield values for **3** and **4** were calculated by ploting a graph of the integrated fluorescence intensity as a function of the analyt's concentration and refering to the following equation:

$$\Phi_{X;F} = \Phi_{ST;F}\left(\frac{A_X}{A_{ST}}\right)\left(\frac{\eta_X^2}{\eta_{ST}^2}\right)$$

Φ_F - quantum yield

A - slop of graph

η - refracting index of the solvent

X – analyte

ST – standard reference

Coumarin 102 (C102; CAS 41267-76-9) was used as a standard refrence (ST) with a known QY value (Φ_F = 0.76; Rurack, K.; Spieles, M. *Anal. Chem*. **2011**, *83*, 1232–1242) for the QY calculations. All measurment were carried for absorbances between 0.01 and 0.15, using a slit width of 2 nm. The fluoresence of the solvents (HPLC grade ethanol or acetonitrile) was subtracted from the analyt's integrated fluorescence values.

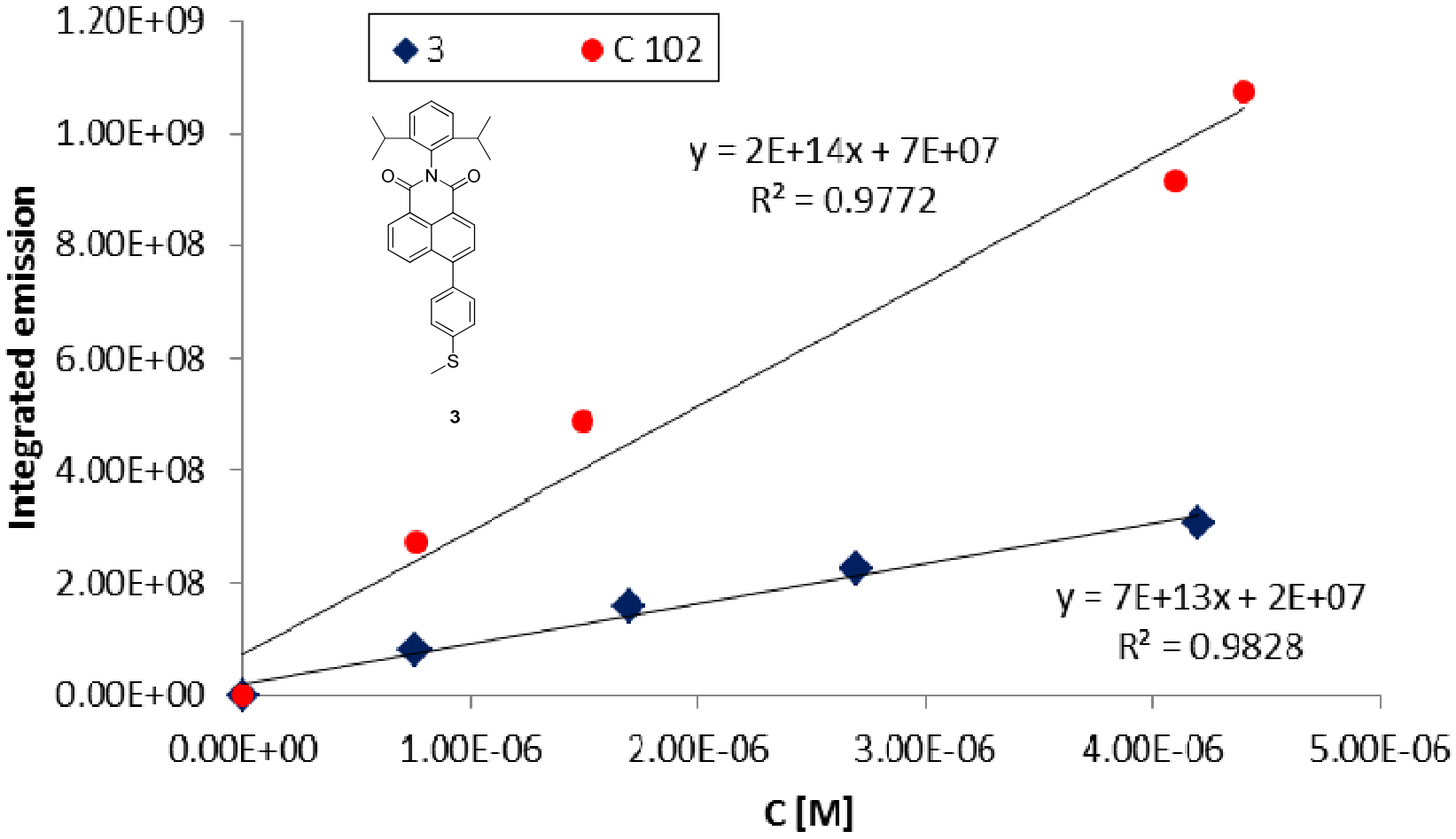


Figure S31. Quantum yield measurements of **3**.

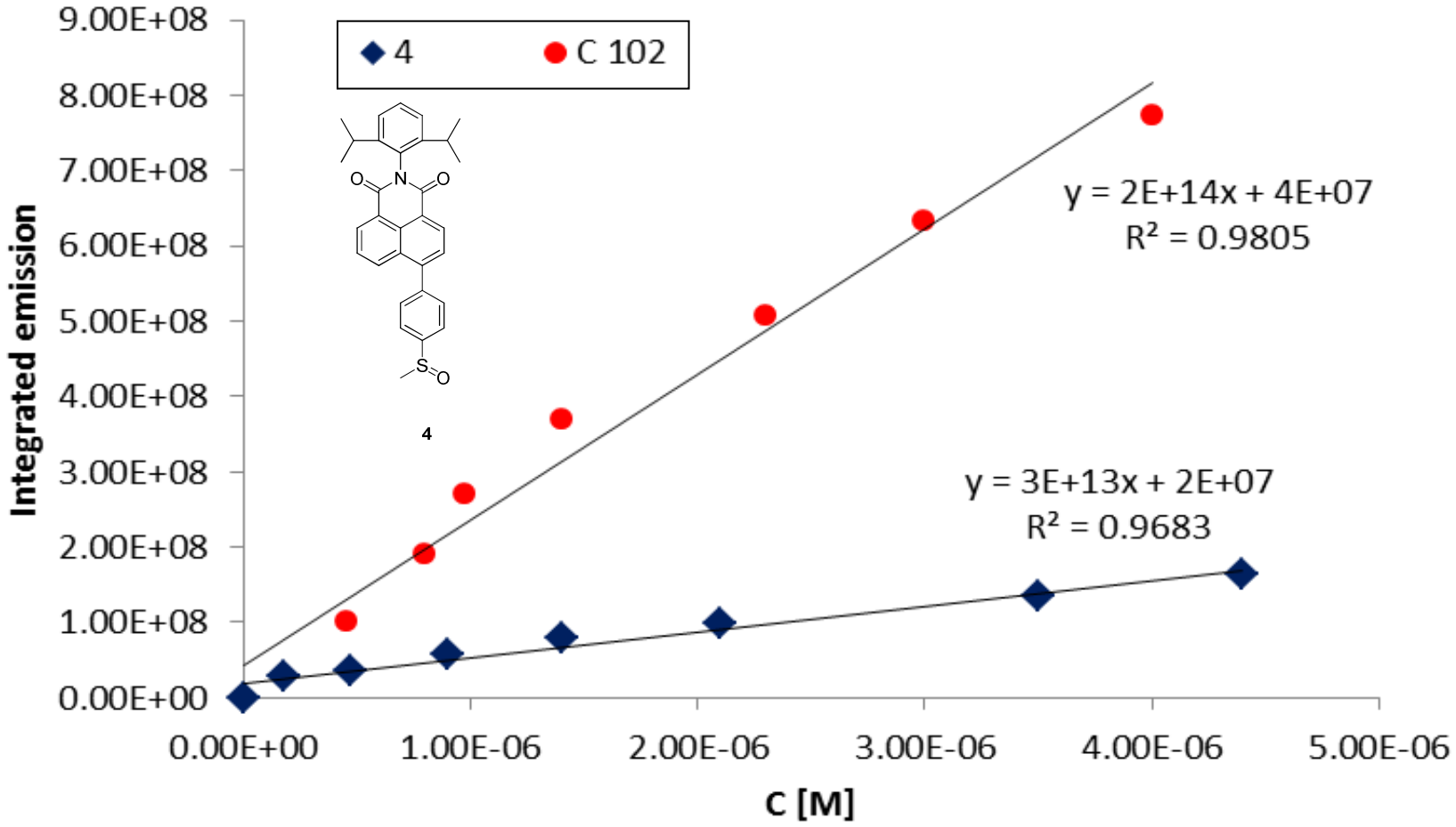


Figure S32. Quantum yield measurements of **4**.