

Electronic Supplementary Information

A Dual Colorimetric-Ratiometric Fluorescent Probe NAP-3 for Selective Detection and Imaging of Endogenous Labile Iron (III) Pools in *C. elegans*

Atul Goel,^{*,a} Shahida Umar,^a Pankaj Nag,^a Ashutosh Sharma,^a Lalit Kumar,^b

Shamsuzzama,^b Zakir Hossain,^c Jiaur R. Gayen,^c Aamir Nazir^b

^a*Department of Medicinal and Process Chemistry, Central Drug Research Institute, Lucknow 226031, India.*

^b*Department of Toxicology, Central Drug Research Institute, Lucknow 226031, India.*

^c*Department of Pharmacokinetics and Metabolism, Central Drug Research Institute, Lucknow 226031, India.*

**Correspondence should be addressed to A.G. (atul_goel@cdri.res.in).*

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References

Cartesian coordinates (\AA) of **NAP-3** optimized at the B3LYP/6-31G(d,p)

General Information

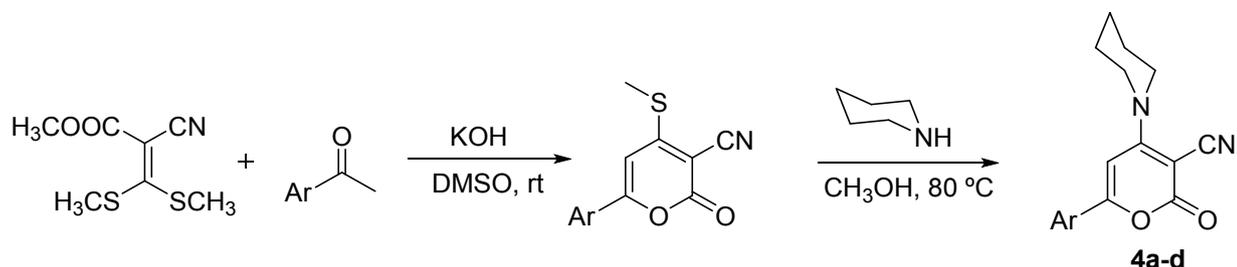
^1H NMR spectra were recorded at 300 MHz or 400 MHz and ^{13}C NMR spectra were recorded at 100.6 MHz. Chemical shifts are reported in parts per million shift (δ -value) from Me_4Si (δ 0 ppm for ^1H) or based on the middle peak of the solvent (CDCl_3) (δ 7.26 ppm for ^1H NMR and δ 77.00 ppm for ^{13}C NMR) as an internal standard. Signal pattern are indicated as s, singlet; d, doublet; t, triplet; m, multiplet. Coupling constant (J) is given in Hertz. Infrared (IR) spectra were recorded in KBr disc and reported in wave number (cm^{-1}). The ESI-MS were recorded on MICROMASS Quadro-II LCMS system. The HRMS spectra were recorded as EI-HRMS on a mass analyzer system. All the reactions were monitored by TLC and visualization was done with UV light (254 nm).

Photophysical (absorption and emission) studies

Triple Distilled Water (TDW) was used for preparing all analytical samples. The stock solutions (2.5 mM) of 1, 10-Phenanthroline, naphtho[2,1-*b*][1,10]-phenanthrolines, cysteine (Cys), glutathione (GSH), Deferoxamine (DFO) were prepared in analytical grade DMSO and subsequent dilutions were made using TDW. Stock solutions (2.5 mM) of the perchlorate salts of all metal ions were prepared in analytical grade DMSO. Stock solutions (2.5 mM) of hydroxylamine hydrochloride and hydrogen peroxide (30% aqueous solution) were prepared in TDW. The absorption and emission studies of **9a-d** and **10a-d** were carried out in TDW:DMSO, 9:1 (v/v) having concentration of $\sim 10^{-5}$ M at neutral pH. The absorption and fluorescence spectra for metal sensing experiments were obtained after 1 hour of analyte addition at 25 °C at 365 nm excitation (or 561nm excitation for the **NAP-3-Fe³⁺** complex) in TDW-DMSO, 7:3 (v/v). During the titration studies, pH of the solutions were maintained to 7.0-7.4 using 0.01M NaOH solution.

Experimental Details of Synthesized Compounds

General procedure for the synthesis of 1a-d



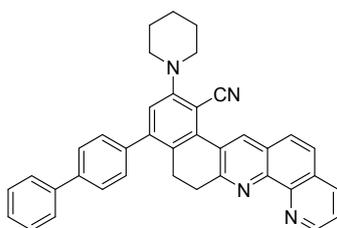
Compounds **4a-d** were synthesized following previously described synthetic procedures.¹

General procedure for the synthesis of 10,11-dihydrobenzo[*b*][1,10]phenanthrolin-8(9*H*)-one (**8**)

A mixture of 8-aminoquinoline (144mg, 1mmol) and paraformaldehyde (30mg, 1mmol) was heated in ethanol (2ml) at 70-80°C for 2-3 minutes. Then added NaOH (2mg, 0.05mmol) and stirred at the same temperature for 5min till the solution became clear. Further a solution of cyclohexane-1,3-dione (112mg, 1mmol) in 1ml ethanol was added and the reaction mixture was heated at the same temperature till the solid began to separate. The progress of the reaction was monitored by TLC and on completion solvent was evaporated and the reaction mixture was poured onto crushed ice with vigorous stirring. The precipitate obtained was filtered and purified on a silica gel column with 90% chloroform in hexane as the eluent to afford 200 mg (0.8mmol, 80%) of 10,11-dihydrobenzo[*b*][1,10]phenanthrolin-8(9*H*)-one **2** as a yellow solid. $R_f = 0.51$ (chloroform); mp (chloroform/hexane) 238-240 °C; MS (ESI) 249 [M + H⁺]; IR (KBr) $\nu = 1689$ (CO); ¹H NMR (300 MHz, CDCl₃): δ 9.24 (s, 1H), 8.89 (s, 1H), 8.15-8.38 (m, 1H), 7.56-7.98 (m, 3H), 3.46-3.70 (m, 2H), 2.72-2.96 (m, 2H), 2.20-2.44 (m, 2H) ppm; ¹³C NMR (100.6 MHz, CDCl₃): δ 198.0, 162.9, 150.6, 148.0, 145.4, 136.1, 130.1, 127.5, 127.2, 126.5, 123.8, 38.9, 33.6, 21.9 HRMS calculated for C₁₆H₁₃N₂O [M + H]⁺ 249.1028, found: 249.1020.

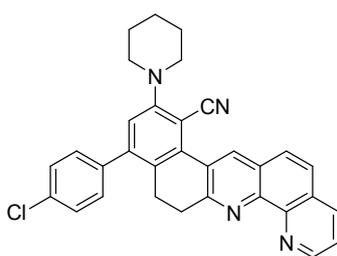
Experimental Data of Synthesized donor acceptor based phenanthrolines (**9a-d** & **10a-d**)

11-(biphenyl-4-yl)-9-(piperidin-1-yl)-12,13-dihydronaphtho[2,1-*b*][1,10]phenanthroline-8-carbonitrile (**9a**)



A mixture of 6-(biphenyl-4-yl)-2-oxo-4-(piperidin-1-yl)-2H-pyran-3-carbonitrile (356 mg, 1 mmol, 1 equiv.), 10,11-dihydrobenzo[*b*][1,10]phenanthroline-8(9*H*)-one (248 mg, 1 mmol, 1 equiv.), NaH(60% dispersion in oil, 1.5 mmol, 1.5 equiv.) in dry DMF (5 mL) was stirred 27°C for 20 min. On completion the reaction mixture was poured onto crushed ice with vigorous stirring and finally neutralized with 10% HCl. The precipitate obtained was filtered and purified on a silica gel column with 0.5% methanol in chloroform as the eluent to afford 450 mg (83%) as a yellow solid; $R_f = 0.55$ (chloroform/methanol, 20:1, v/v); mp (chloroform/methanol) 176-178 °C; MS (ESI) 543 [M + H⁺]; IR (KBr) $\nu = 2212\text{ cm}^{-1}$; ¹H NMR (400 MHz, CDCl₃): δ 9.17-9.31 (m, 1H), 9.15 (s, 1H), 8.28 (dd, $J_1 = 8.0\text{ Hz}$, $J_2 = 1.6\text{ Hz}$, 1H), 7.95 (d, $J = 8.8\text{ Hz}$, 1H), 7.81 (d, $J = 8.8\text{ Hz}$, 1H), 7.54-7.74 (m, 5H), 7.28-7.52 (m, 5H), 7.06 (s, 1H), 3.32-3.49 (m, 2H), 3.16-3.31 (m, 4H), 2.73-3.06 (m, 2H), 1.74-1.99 (m, 4H), 1.56-1.70 (m, 2H) ppm; ¹³C NMR (100.6 MHz, CDCl₃): $\delta = 160.3, 157.7, 150.4, 145.9, 145.6, 144.9, 140.9, 140.4, 138.9, 138.5, 136.1, 134.4, 130.9, 129.4, 129.2, 128.9, 127.7, 127.6, 127.5, 127.2, 127.1, 126.7, 126.1, 122.9, 120.4, 119.2, 102.9, 53.9, 33.4, 26.2, 24.0$ ppm; HRMS calculated for C₃₈H₃₁N₄ [M + H]⁺ 543.2549, found: 543.2559.

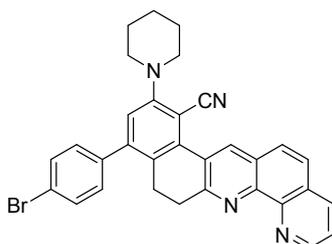
11-(4-chlorophenyl)-9-(piperidin-1-yl)-12,13-dihydrobenzo[2,1-*b*][1,10]phenanthroline-8-carbonitrile (9b)



A mixture of 6-(4-chlorophenyl)-2-oxo-4-(piperidin-1-yl)-2H-pyran-3-carbonitrile (315 mg, 1mmol, 1equiv.), 10,11-dihydrobenzo[*b*][1,10]phenanthroline-8(9*H*)-one (248 mg, 1 mmol, 1 equiv.), and NaH(60% dispersion in oil, 1.5 mmol, 1.5 equiv.) in dry DMF (5 mL) was stirred at 25°C for 15 min. On completion the reaction mixture was poured onto crushed ice with vigorous stirring and finally neutralized with 10% HCl. The precipitate obtained was filtered and purified on a silica gel column with 1% methanol in chloroform as the eluent to afford 395 mg (79%) as an yellow solid; $R_f = 0.46$ (chloroform/methanol, 20:1, v/v); mp (chloroform/methanol) >290 °C; MS (ESI) 501 [M + H⁺]; IR (KBr)

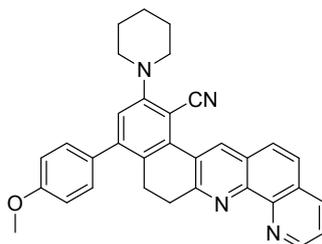
$\nu = 2216 \text{ cm}^{-1}$; $^1\text{H NMR}$ (400 MHz, CDCl_3): δ 8.99-9.27 (m, 2H), 8.22 (d, $J = 8.2 \text{ Hz}$, 1H), 7.89 (d, $J = 8.6 \text{ Hz}$, 1H), 7.76 (d, $J = 8.6 \text{ Hz}$, 1H), 7.59 (dd, $J_1 = 7.6 \text{ Hz}$, $J_2 = 4.2 \text{ Hz}$, 1H), 7.36-7.46 (m, 2H), 7.12-7.35 (m, 2H), 6.91 (s, 1H), 3.10-3.42 (m, 6H), 2.68-2.91 (m, 2H), 1.70-1.94 (m, 4H), 1.48-1.68 (m, 2H) ppm; $^{13}\text{C NMR}$ (100.6 MHz, CDCl_3): $\delta = 160.1, 157.7, 150.4, 145.6, 145.1, 138.6, 138.5, 136.1, 134.4, 134.2, 130.7, 130.2, 129.2, 128.7, 127.5, 127.0, 126.1, 123.0, 120.2, 119.1, 103.2, 53.9, 33.3, 26.2, 26.1, 23.9$ ppm; HRMS calculated for $\text{C}_{32}\text{H}_{26}\text{ClN}_4$ $[\text{M} + \text{H}]^+$ 501.1846, found: 501.1836

11-(4-bromophenyl)-9-(piperidin-1-yl)-12,13-dihydronaphtho[2,1-*b*][1,10]phenanthroline-8-carbonitrile (9c)



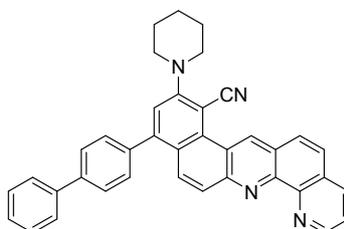
A mixture of 6-(4-bromophenyl)-2-oxo-4-(piperidin-1-yl)-2*H*-pyran-3-carbonitrile (359 mg, 1 mmol, 1 equiv.), 10,11-dihydrobenzo[*b*][1,10]phenanthroline-8(9*H*)-one (248 mg, 1 mmol, 1 equiv.), and NaH(60% dispersion in oil, 1.5 mmol, 1.5 equiv.) in dry DMF (5 mL) was stirred at 25°C for 10 min. On completion the reaction mixture was poured onto crushed ice with vigorous stirring and finally neutralized with 10% HCl. The precipitate obtained was filtered and purified on a silica gel column 1% methanol in chloroform as the eluent to afford 410 mg (75%) as a yellow solid; $R_f = 0.45$ (chloroform/methanol, 20:1, v/v); mp (chloroform/methanol) >290 °C; MS (ESI) 545 $[\text{M} + \text{H}]^+$; IR (KBr) $\nu = 2211 \text{ cm}^{-1}$; $^1\text{H NMR}$ (400 MHz, CDCl_3): δ 9.16-9.30 (m, 1H) 9.13 (s, 1H), 8.28 (dd, $J_1 = 8.1 \text{ Hz}$, $J_2 = 1.7 \text{ Hz}$, 1H), 7.94 (d, $J = 8.8 \text{ Hz}$, 1H), 7.81 (d, $J = 8.8 \text{ Hz}$, 1H), 7.49-7.72 (m, 4H), 7.12-7.36 (m, 1H), 6.96 (s, 1H), 3.32-3.46 (m, 2H), 3.14-3.30 (m, 4H), 2.73-2.96 (m, 2H), 1.76-1.98 (m, 4H), 1.56-1.75 (m, 2H) ppm; $^{13}\text{C NMR}$ (100.6 MHz, CDCl_3): $\delta = 160.1, 157.7, 150.4, 145.1, 144.9, 138.9, 138.6, 136.1, 134.4, 132.3, 131.7, 130.6, 130.5, 129.2, 127.6, 126.9, 126.1, 123.0, 122.4, 120.1, 119.1, 103.1, 53.9, 33.2, 26.1, 26.0, 23.9$ ppm; HRMS calculated for $\text{C}_{32}\text{H}_{26}\text{BrN}_4$ $[\text{M} + \text{H}]^+$ 545.1341, found: 545.1330, $[\text{M}+2+\text{H}]^+$ 547.1320, found: 547.1310.

11-(4-methoxyphenyl)-9-(piperidin-1-yl)-12,13-dihydronaphtho[2,1-*b*][1,10]phenanthroline-8-carbonitrile (9d)



A mixture of 6-(4-methoxyphenyl)-2-oxo-4-(piperidin-1-yl)-2*H*-pyran-3-carbonitrile (310 mg, 1mmol, 1equiv.), 10,11-dihydrobenzo[*b*][1,10]phenanthroline-8(9*H*)-one (248 mg, 1 mmol, 1 equiv.), and NaH (60% dispersion in oil, 1.5 mmol, 1.5 equiv.) in dry DMF (5 mL) was stirred at 25°C for 15 min. On completion the reaction mixture was poured onto crushed ice with vigorous stirring and finally neutralized with 10% HCl. The precipitate obtained was filtered and purified on a silica gel column with 1% methanol in chloroform as the eluent to afford 400 mg (80%) as a yellow solid; $R_f = 0.47$ (chloroform/methanol, 20:1, v/v); mp (chloroform/methanol) 180 °C; MS (ESI) 497 [M + H⁺]; IR (KBr) $\nu = 2213 \text{ cm}^{-1}$; ¹H NMR (400 MHz, CDCl₃): δ 9.12 (s, 1H), 9.14-9.30 (m, 1H) 8.26 (dd, $J_1 = 8.0 \text{ Hz}$, $J_2 = 1.7 \text{ Hz}$, 1H), 7.93 (d, $J = 8.8 \text{ Hz}$, 1H), 7.79 (d, $J = 8.8 \text{ Hz}$, 1H), 7.63 (dd, $J_1 = 8.0 \text{ Hz}$, $J_2 = 4.3 \text{ Hz}$, 1H), 7.16-7.39 (m, 2H), 6.88-7.11 (m, 3H), 3.87 (s, 3H), 3.13-3.45 (m, 6H), 2.77-3.00 (m, 2H), 1.78-1.97 (m, 4H), 1.55-1.76 (m, 2H) ppm; ¹³C NMR (100.6 MHz, CDCl₃): δ 160.3, 159.5, 157.7, 150.4, 146.1, 145.7, 144.9, 138.4, 136.1, 134.3, 132.4, 130.9, 130.1, 129.2, 127.8, 127.6, 127.0, 126.0, 122.9, 120.5, 119.3, 113.9, 102.6, 55.4, 53.9, 33.4, 26.2, 24.0 ppm; HRMS calculated for C₃₃H₂₉N₄O [M + H]⁺ 497.2341, found: 497.2350.

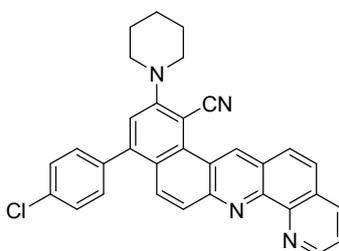
11-(biphenyl-4-yl)-9-(piperidin-1-yl)naphtho[2,1-*b*][1,10]phenanthroline-8-carbonitrile (10a)



Compound 11-(biphenyl-4-yl)-9-(piperidin-1-yl)-12,13-dihydronaphtho[2,1-*b*][1,10]phenanthroline-8-carbonitrile (543mg, 1 mmol, 1equiv.) was refluxed with DDQ (2 mmol, 2 equiv.) in 1,4-dioxane (5 ml) for 1 hr. On completion the reaction mixture was cooled to room temperature and then was filtered. The

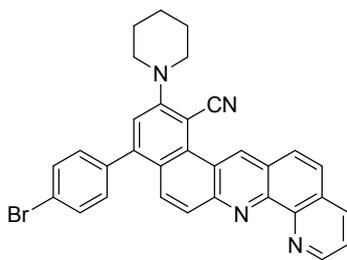
filtrate was collected and solvent was evaporated. Finally the residue was purified by silica gel column chromatography using 0.5% methanol in chloroform as the eluent to afford 200 mg (37%) as orange solid; $R_f = 0.58$ (chloroform/methanol, 20:1, v/v); mp (chloroform/methanol) >290 °C; MS (ESI) 541 [M + H⁺]; IR (KBr) $\nu = 2208$ cm⁻¹; ¹H NMR (300 MHz, CDCl₃): δ 10.7 (m, 1H), 9.28 (s, 1H), 8.25-8.48 (m, 2H), 7.95-8.23 (m, 2H), 7.29-7.93 (m, 12H), 3.38-3.61 (m, 4H), 1.86-2.05 (m, 4H), 1.60-1.84 (m, 2H) ppm; ¹³C NMR (100.6 MHz, CDCl₃): $\delta = 159.4, 150.2, 149.2, 146.7, 146.2, 141.3, 140.4, 138.6, 136.1, 134.2, 132.8, 130.2, 129.7, 129.1, 128.9, 128.5, 127.7, 127.3, 127.2, 126.2, 126.1, 125.1, 123.7, 123.6, 121.1, 120.6, 114.0, 98.9, 54.1, 26.2, 24.1$ ppm; HRMS calculated for C₃₈H₂₉N₄ [M + H]⁺ 541.2392, found: 541.2393.

11-(4-chlorophenyl)-9-(piperidin-1-yl)naphtho[2,1-b][1,10]phenanthroline-8-carbonitrile (10b)



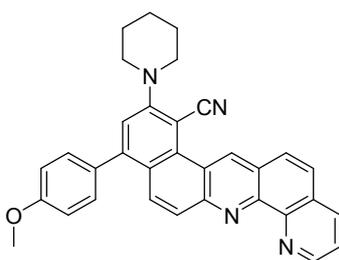
11-(4-chlorophenyl)-9-(piperidin-1-yl)-12,13-dihydronaphtho[2,1-b][1,10]phenanthroline-8-carbonitrile (501 mg, 1 mmol, 1 equiv.) was refluxed with DDQ (2 mmol, 2 equiv.) in 1,4-dioxane (5 mL) for 1 hr.. On completion the reaction mixture was cooled to room temperature and then was filtered. The filtrate was collected and solvent was evaporated. Finally the residue was purified by silica gel column chromatography using 1% methanol in chloroform as the eluent to afford 180 mg (36%) as an orange solid; $R_f = 0.50$ (chloroform/methanol, 20:1, v/v); mp (chloroform/methanol) >290 °C; MS (ESI) 499 [M + H⁺]; IR (KBr) $\nu = 2209$ cm⁻¹; ¹H NMR (300 MHz, CDCl₃): δ 10.64 (s, 1H), 9.09-9.31 (m, 1H), 8.12-8.39 (m, 2H), 8.04 (d, $J = 8.8$ Hz, 1H), 7.84 (d, $J = 9.4$ Hz, 1H), 7.76 (d, $J = 8.8$ Hz, 1H), 7.52-7.75 (m, 1H), 7.47 (d, $J = 8.3$ Hz, 2H), 7.36 (d, $J = 8.3$ Hz, 2H), 7.23 (s, 1H), 3.29-3.52 (m, 4H), 1.75-1.98 (m, 4H), 1.51-1.72 (m, 2H) ppm; ¹³C NMR (100.6 MHz, CDCl₃): δ 159.3, 150.3, 149.2, 146.8, 146.2, 145.7, 138.1, 136.1, 134.6, 134.2, 132.8, 131.1, 129.8, 128.9, 128.7, 128.6, 127.7, 126.3, 124.9, 123.7, 123.6, 121.0, 120.5, 114.0, 99.1, 54.1, 26.2, 24.0 ppm; HRMS calculated for C₃₂H₂₄CIN₄ [M + H]⁺ 499.1689, found: 499.1682.

11-(4-bromophenyl)-9-(piperidin-1-yl)naphtho[2,1-b][1,10]phenanthroline-8-carbonitrile 10c (NAP-3)



11-(4-bromophenyl)-9-(piperidin-1-yl)-12,13-dihydro-1,10-phenanthroline-8-carbonitrile (545 mg, 1 mmol, 1 equiv.) was refluxed with DDQ (2 mmol, 2equiv.) in 1,4-dioxane (5 mL) for 1 hr. On completion the reaction mixture was cooled to room temperature and then was filtered. The filtrate was collected and solvent was evaporated. Finally the residue was purified by silica gel column chromatography using 1% methanol in chloroform as the eluent to afford 230 mg (42%) as an orange solid. $R_f = 0.52$ (chloroform/methanol, 20:1, v/v); mp (chloroform/methanol) >290 °C; MS (ESI) 543 [M + H⁺]; IR (KBr) $\nu = 2208$ cm⁻¹; ¹H NMR (400 MHz, CDCl₃): δ 10.64 (s, 1H), 9.10-9.31 (m, 1H), 8.13-8.39 (m, 2H), 8.04 (d, $J = 8.9$ Hz, 1H), 7.84 (d, $J = 9.5$ Hz, 1H), 7.77 (d, $J = 8.9$ Hz, 1H), 7.52-7.70 (m, 3H), 7.09-7.41 (m, 3H), 3.30-3.52 (m, 4H), 1.79-1.98 (m, 4H), 1.53-1.78 (m, 2H) ppm; ¹³C NMR (100.6 MHz, CDCl₃): $\delta = 159.3, 150.2, 149.1, 146.7, 146.1, 145.7, 138.6, 136.1, 134.2, 132.8, 131.8, 131.7, 131.3, 130.5, 129.7, 128.7, 128.6, 127.7, 126.3, 124.8, 123.7, 122.7, 120.9, 120.4, 99.1, 54.1, 26.2, 24.0$ ppm; HRMS calculated for C₃₂H₂₄BrN₄ [M + H]⁺ 543.1184, found: 543.1178, [M + 2 + H]⁺ 545.1164, found: 545.1170.

11-(4-methoxyphenyl)-9-(piperidin-1-yl)naphtho[2,1-b][1,10]phenanthroline-8-carbonitrile (10d)



11-(4-methoxyphenyl)-9-(piperidin-1-yl)-12,13-dihydro-1,10-phenanthroline-8-carbonitrile (497 mg, 1 mmol, 1 equiv.) was refluxed with DDQ (2 mmol, 2 equiv.) in 1,4-dioxane (5 mL) for 1 hr. On completion the reaction mixture was cooled to room temperature and then was filtered. The filtrate was collected and solvent was evaporated. Finally the residue was purified by silica gel column chromatography using 1% methanol in chloroform as the eluent to afford 180 mg (36%) as an orange solid $R_f = 0.53$ (chloroform/methanol, 20:1, v/v); mp (chloroform/methanol) >290 °C; MS (ESI) 495 [M + H⁺]; IR (KBr) $\nu = 2207$ cm⁻¹; ¹H NMR (400 MHz, CDCl₃): $\delta = 10.62$ (m, 1H), 9.08-9.29 (m, 1H), 8.13-

8.37 (m, 2H), 7.84-8.12 (m, 2H), 7.80 (d, $J = 8.8$ Hz, 1H), 7.62 (dd, $J_1 = 7.6$ Hz, $J_2 = 4.3$ Hz, 1H), 7.15-7.46 (m, 3H), 6.90-7.13 (m, 2H), 3.85 (s, 3H), 3.28-3.51 (m, 4H), 1.76-1.97 (m, 4H), 1.52-1.74 (m, 2H) ppm; ^{13}C NMR (100.6 MHz, CDCl_3): δ 159.7, 159.4, 150.1, 149.2, 146.8, 146.6, 146.2, 136.0, 134.2, 132.7, 131.9, 130.9, 129.7, 129.2, 128.3, 127.7, 126.2, 126.1, 125.2, 123.7, 123.6, 121.2, 120.7, 114.1, 98.6, 55.4, 54.1, 26.2, 24.0 ppm; HRMS calculated for $\text{C}_{33}\text{H}_{27}\text{N}_4\text{O}$ $[\text{M} + \text{H}]^+$ 495.2185, found: 495.2177.

Photophysical Properties of Synthesized Phenanthroline Dyes

Table S1 Photophysical Data of **9a–d** and **10a–d** in TDW:DMSO, 9:1 (v/v) having concentration of 10^{-5} M, $\lambda_{\text{ex}} = 365\text{nm}$.

entry	$\phi_{\text{f}}(\%)$	
	Water ^a	DMSO ^b
9a	9.0	66.0
9b	2.6	50.0
9c	2.74	43.0
9d	8.0	48.0
10a	2.9	14.6
10b	2.6	18.0
10c (NAP-3)	1.4	11.0
10d	2.5	12.0

Fluorescence quantum yield in ^aTDW:DMSO, 9:1 (v/v), ^bDMSO relative to harmine in 0.1M H_2SO_4 as a standard ($\phi = 45\%$).

Absorption and Emission spectra of 9a-d and 10a-d

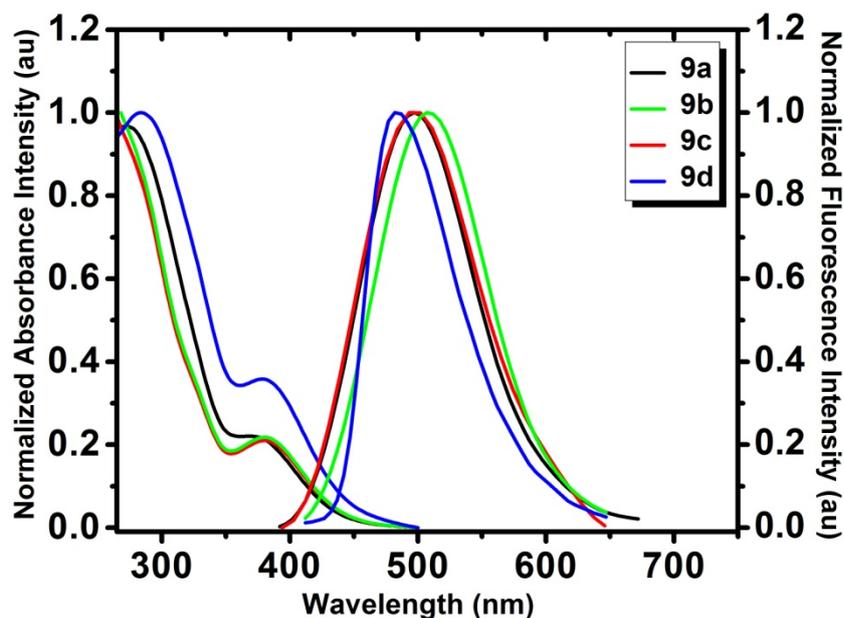


Figure S1: Absorption and Emission Spectra of Phenanthrolines 9a-d

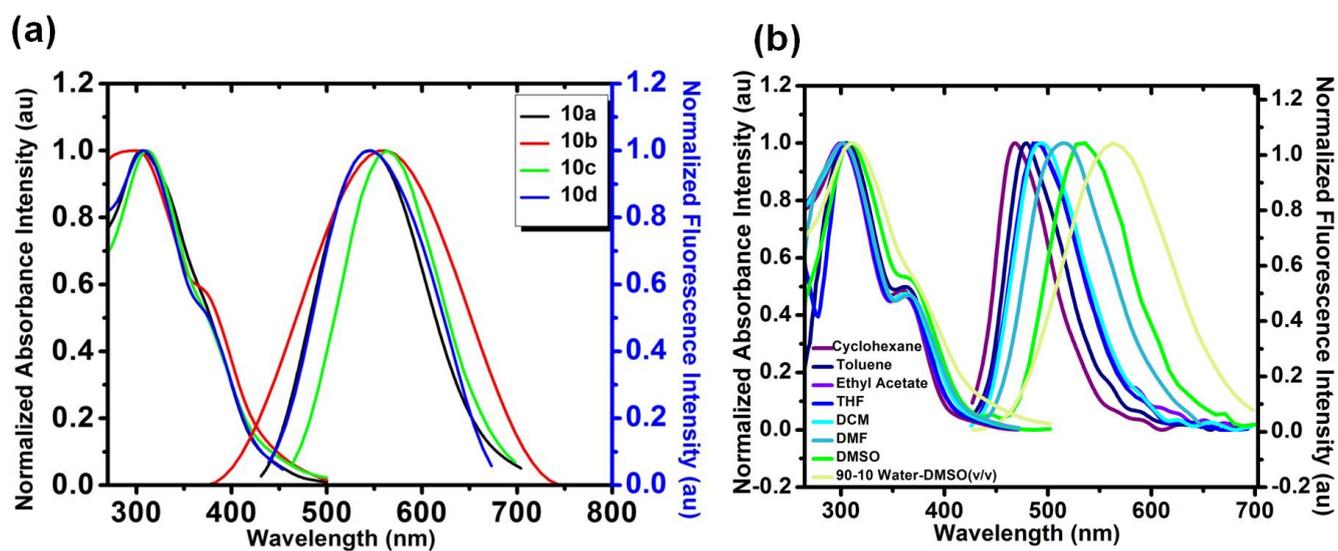


Figure S2: (a) Absorption and Emission Spectra of NAPs 10a-d. (b) Solvatochromism study of 10c (NAP-3) (2.5×10^{-6} M in each solvent)

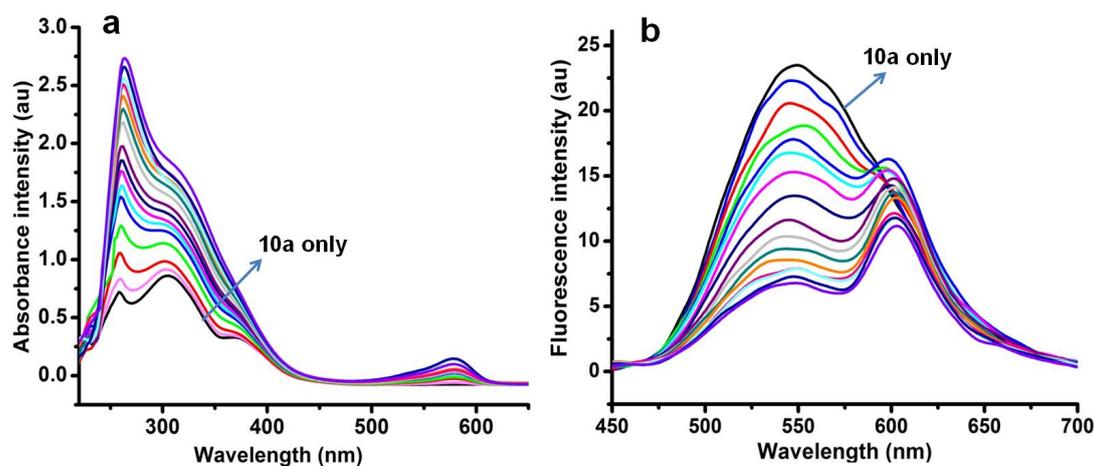


Figure S3: Response of 10a towards Fe^{3+} ions. a) Absorption and b) Emission spectra of 10a (2.5×10^{-5} M) upon gradual addition of Fe^{3+} ($0-6.25 \times 10^{-4}$ M), $\lambda_{\text{ex}} = 365$ nm.

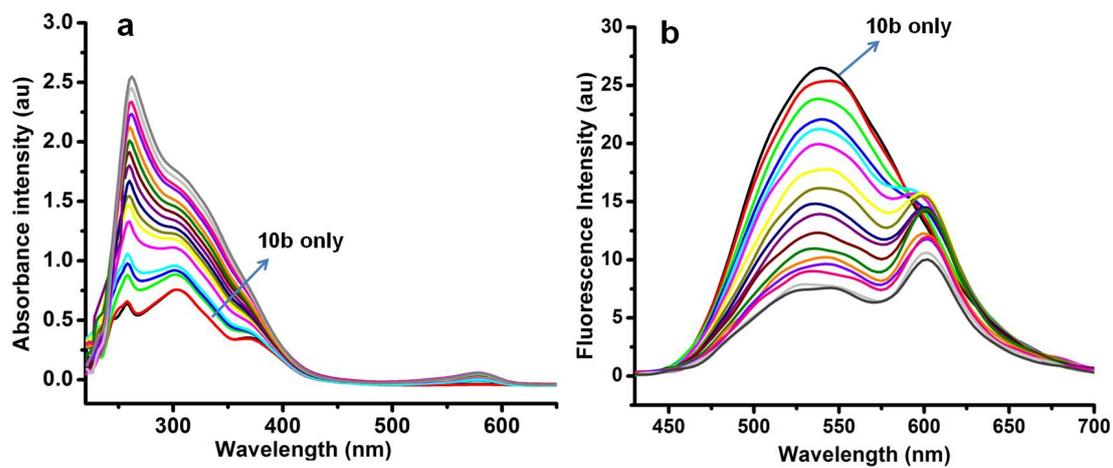


Figure S4 Response of 10b towards Fe^{3+} ions. a) Absorption and b) Emission spectra of 10b (2.5×10^{-5} M) upon gradual addition of Fe^{3+} ($0-6.25 \times 10^{-4}$ M), $\lambda_{\text{ex}} = 365$ nm.

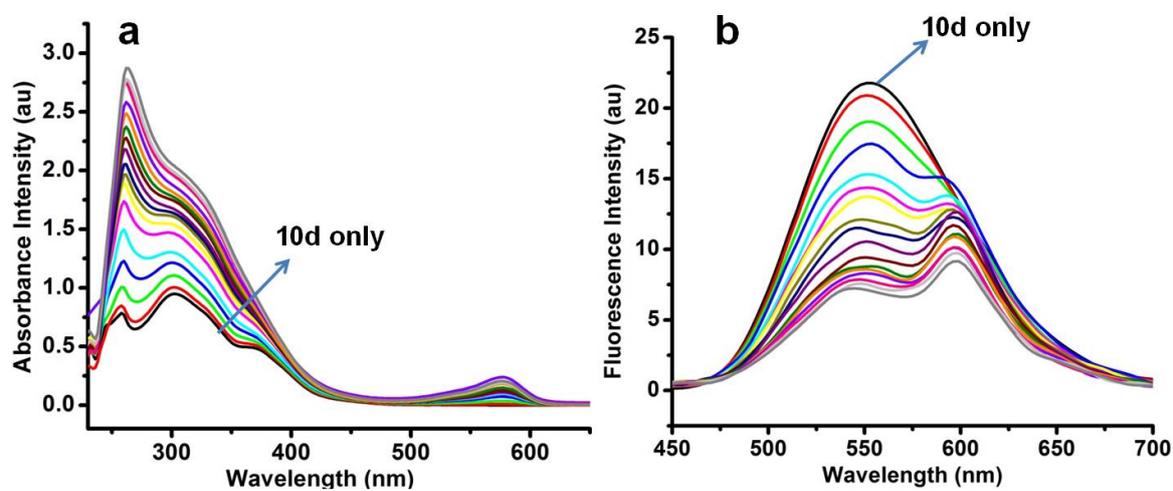


Figure S5 Response of 10d towards Fe^{3+} ions. a) Absorption and b) Emission spectra of 10d (2.5×10^{-5} M) upon gradual addition of Fe^{3+} ($0-6.25 \times 10^{-4}$ M), $\lambda_{\text{ex}}=365$ nm.

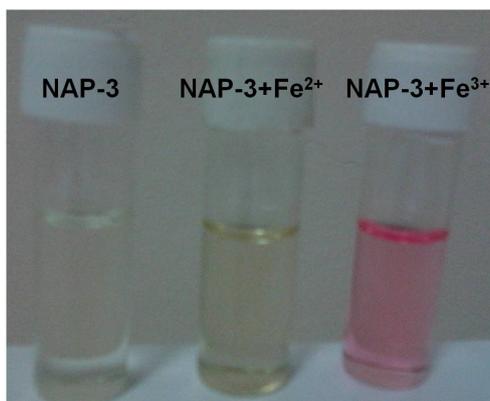


Figure S6 Selective colorimetric response of NAP-3 towards Fe^{3+} in visible light.

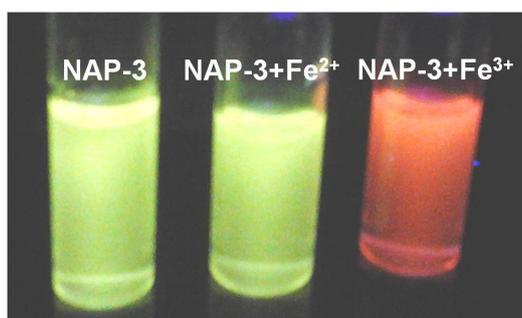


Figure S7 Selective fluorescence response of NAP-3 towards Fe^{3+} in UV light (254nm).

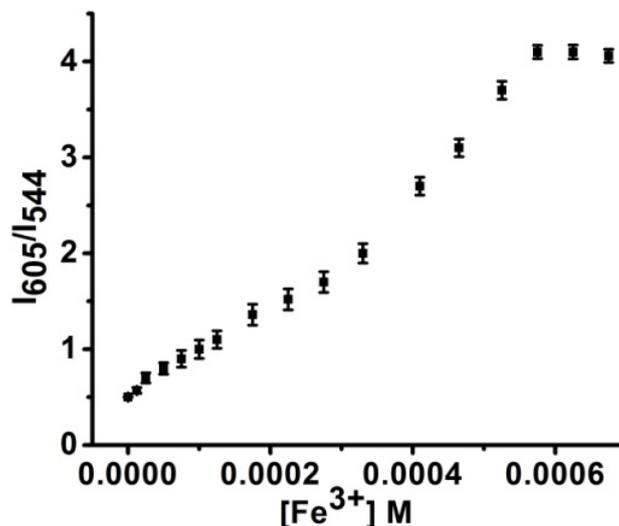


Figure S8 Variation of fluorescence intensity ratio (I_{605}/I_{544}) of **NAP-3** (2.5×10^{-5} M) with increasing Fe^{3+} (0 – 6.8×10^{-4} M). Error bars are \pm s.e.m. ($n = 2$).

Determination of detection limit

The detection limit was calculated based on the fluorescence titration.² The fluorescence emission spectrum of **NAP-3** was measured by seven times and the standard deviation of blank measurement was achieved. To gain the slop, fluorescence titration was carried out between **NAP-3** (7.5×10^{-7} M) and Fe^{3+} the ratio of the fluorescence intensity at 605 nm to the fluorescence intensity at 544 nm (I_{605}/F_{544}) was plotted against the concentration of Fe^{3+} . So the detection limit was calculated with the following equation:

$$\text{Detection limit} = 3\sigma/k$$

Where σ is the standard deviation of blank measurement, k is the slop between the fluorescence intensity ratio versus Fe^{3+} concentration.

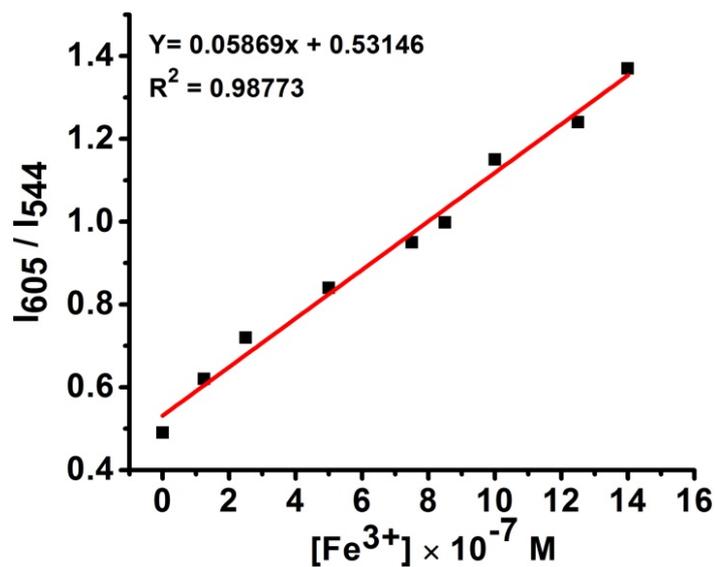


Figure S9 Variation of intensity ratio (I_{605}/I_{544}) of of **NAP-3** ($7.5 \times 10^{-7} M$) on increasing concentration of Fe^{3+} for the determination of detection limit, $\lambda_{ex} = 365 nm$, Ex/Em slits = 2.5/5 nm.

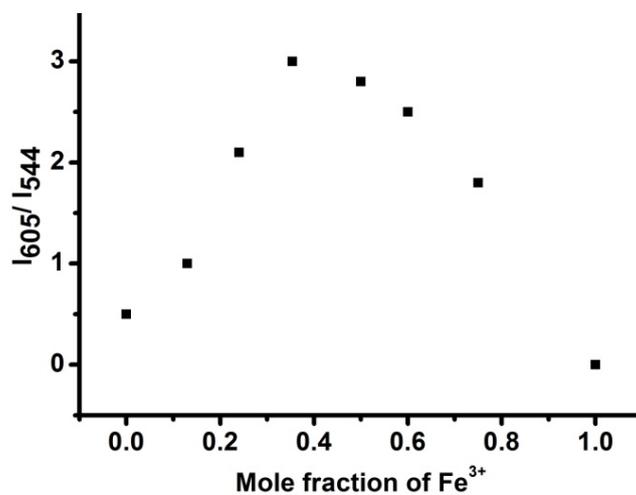


Figure S10 Job's plot³ showing 2:1 stoichiometry between **NAP-3** and Fe^{3+} . $[NAP-3] + [Fe^{3+}] = 8.25 \times 10^{-7} M$.

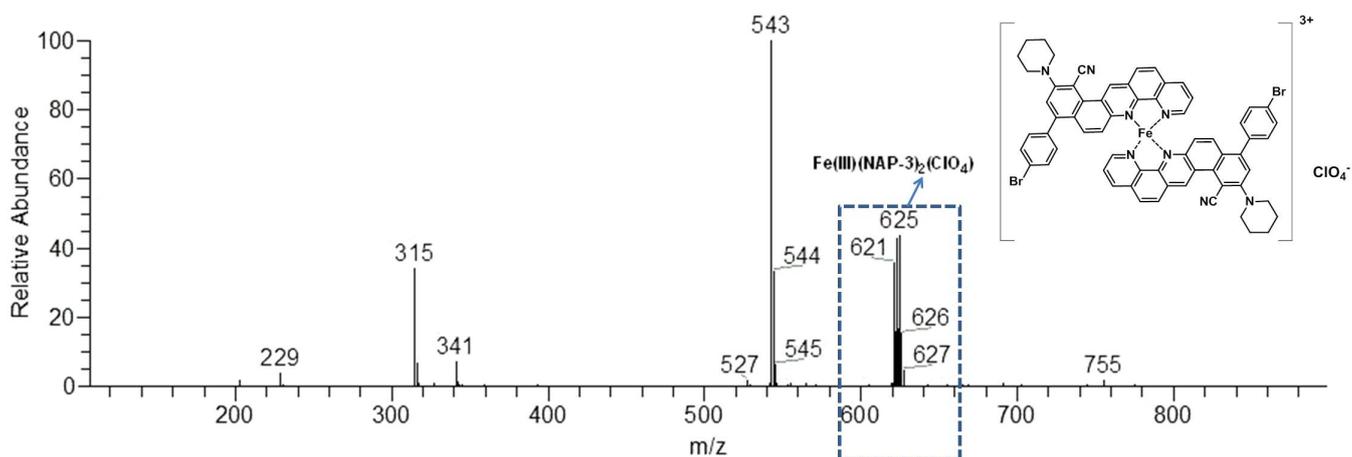


Figure S11 Mass spectrum of the complex between **NAP-3** and Fe^{3+} with the probable complex structure. The sample was prepared using ferric perchlorate (10 eq) and **NAP-3** (2.5×10^{-3} M) in TDW-DMSO. The solvent was removed and analyzed by mass spectrometry.

Determination of association constant

The association constant for 2:1 complex was calculated based on the titration curve of **NAP-3** (2.5×10^{-5} M) with Fe^{3+} (0.68×10^{-4} M) ions. Association constant was determined by a nonlinear least squares fitting⁴ of the fluorescence titration data of **NAP-3** with the following equation

$$y = \frac{x}{[2 \times a \times b \times (1 - x)^2]} + \frac{(x \times b)}{2}$$

Where x is $I_t - I_0 / I_f - I_0$, (I_0 , I_t and I_f are intensities at 544 nm when metal ion concentration is zero, at each metal ion concentration tested during titration and final intensity respectively), y is the concentration of metal ions, a is the association constant, and b is the concentration of **NAP-3**.

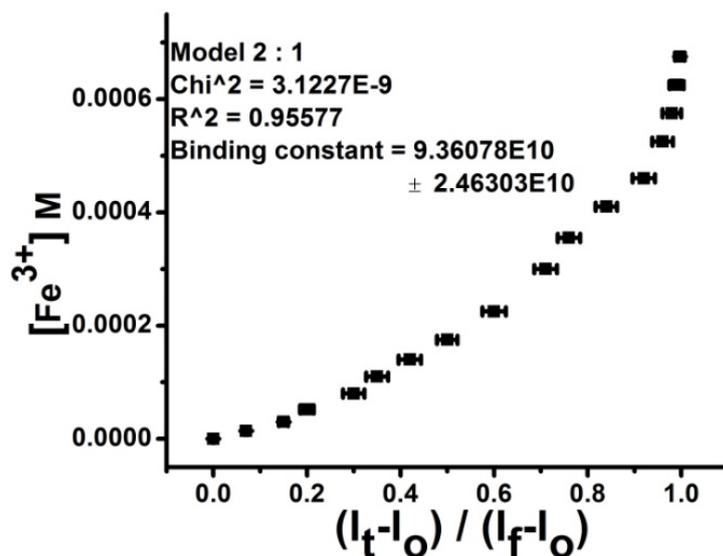


Figure S12 Plot of fluorescence intensity change at 544 nm of NAP-3 (2.5×10^{-5} M) with increasing Fe^{3+} ($0-6.8 \times 10^{-4}$ M). Error bars are \pm s.e.m. ($n = 2$).

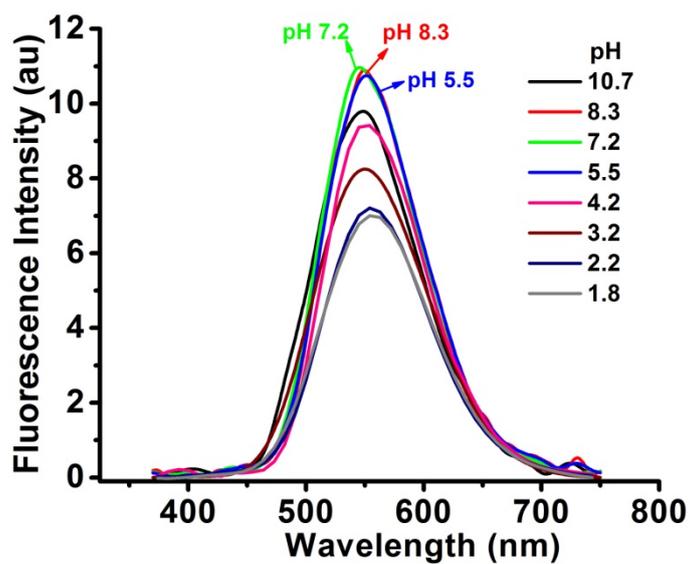


Figure S13 pH study (pH range 1.8-10.7) of NAP-3 (2.5×10^{-5} M), $\lambda_{\text{ex}} = 365 \text{ nm}$.

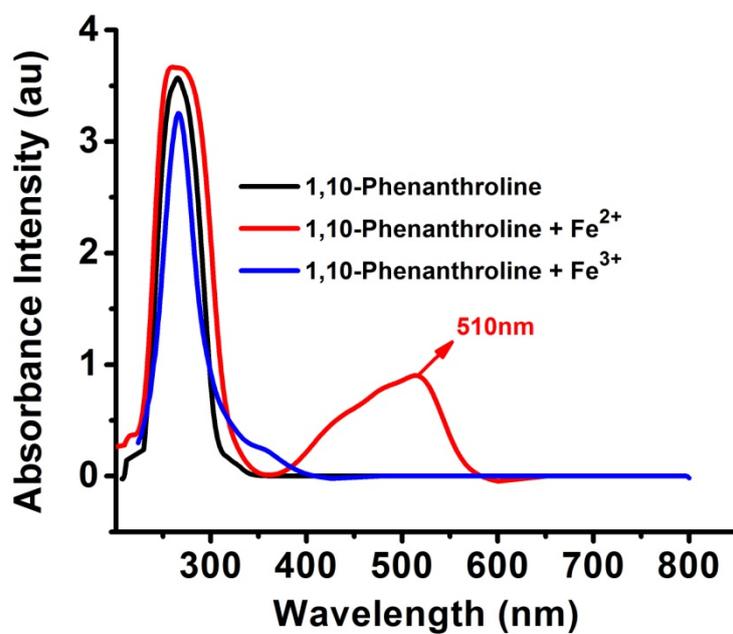


Figure S14 Changes in the UV spectra of 1,10-Phenanthroline (2.5×10^{-5} M) in the presence of Fe²⁺ (2.5×10^{-4} M) and Fe³⁺ (2.5×10^{-4} M)

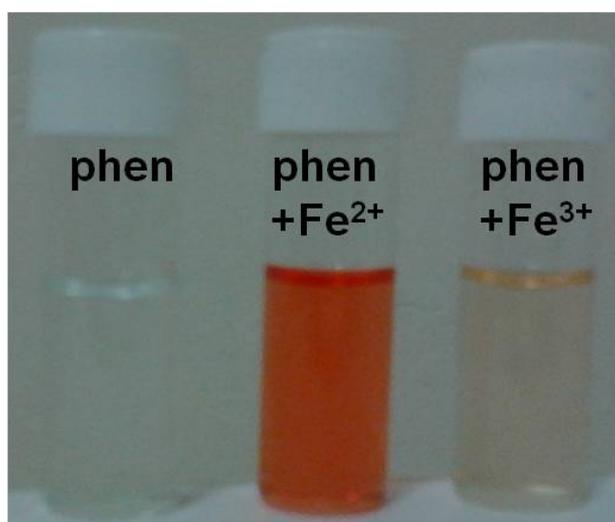


Figure S15 Selective colorimetric response of 1,10-Phenanthroline towards Fe²⁺ in visible light.

TDDFT study of probe NAP-3

To study the electronic behaviour of **NAP-3** time-dependent density functional theory (TDDFT) calculations were performed with a Gaussian 09 package.⁵ The geometries were optimized at DFT/B3LYP level using a 6-31G(d,p) basis set. TDDFT calculations were performed using a B3LYP/6-311++G(d,p) method.

Table S2. Computed values of vertical excitations, oscillator strength (f), assignment, HOMO, LUMO and energy bandgap.

λ_{\max} (nm)	f	Assignment	HOMO (eV)	LUMO (eV)	E_g (eV)
383.8	0.0370	HOMO \rightarrow LUMO (72 %)	-6.34	-2.55	3.79
305.7	0.3877	HOMO-2 \rightarrow LUMO+1 (63 %)			

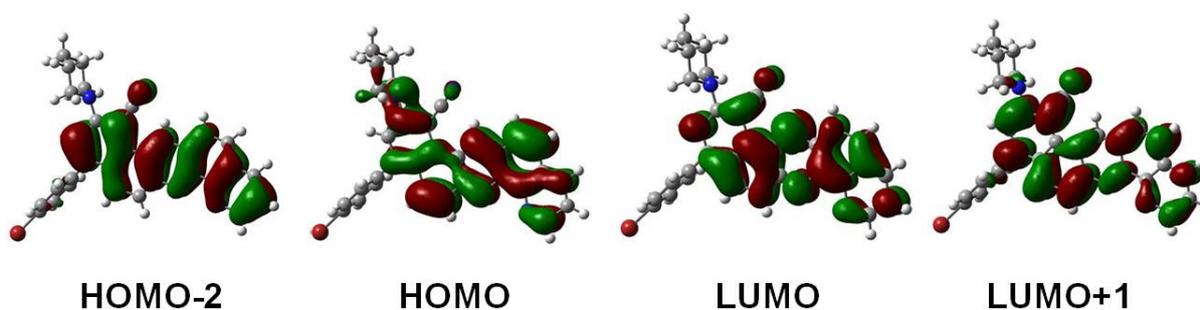


Figure S16 Computed molecular orbital energy diagrams and isodensity surface plots of NAP-3 as obtained from TDDFT calculations.

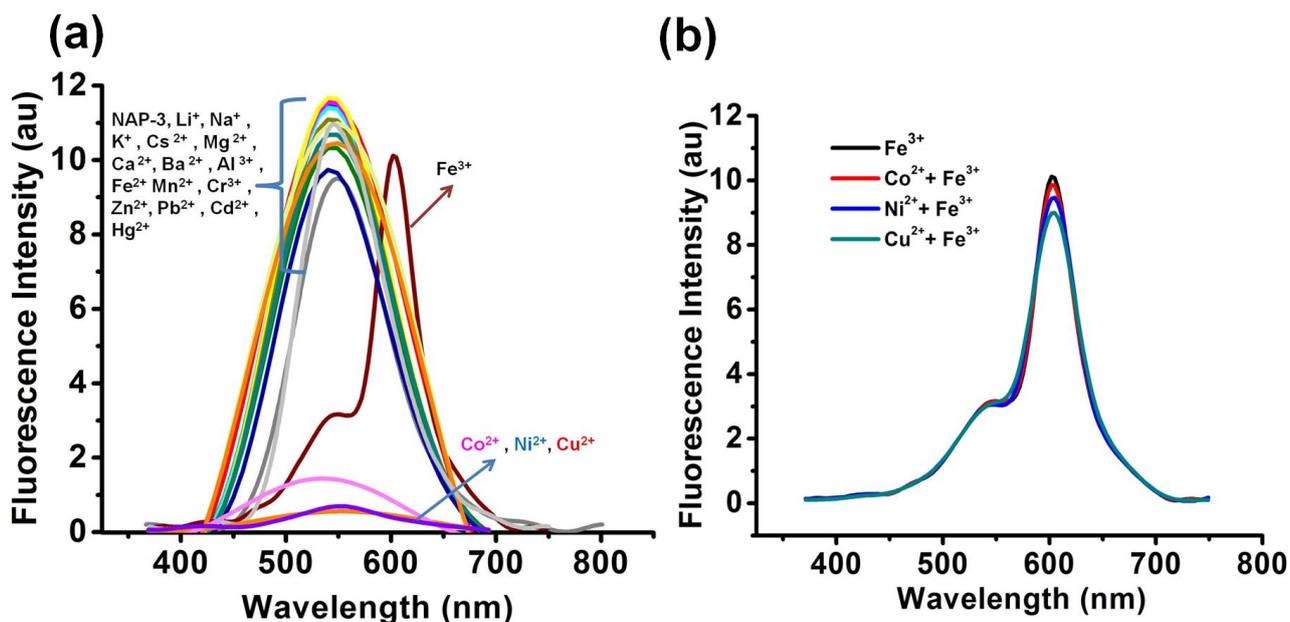


Figure S17 (a) Selectivity studies of NAP-3 (2.5×10^{-5} M) in the absence or presence of a series of metal ions (5×10^{-4} M). (b) Experiment of NAP-3 with Fe^{3+} (5×10^{-4} M) and metal ions Co^{2+} (5×10^{-4} M), Ni^{2+} (5×10^{-4} M), Cu^{2+} (5×10^{-4} M) revealing no significant interference of these metal ions on the ratiometric response of NAP-3 towards Fe^{3+} . $\lambda_{\text{ex}} = 365\text{nm}$

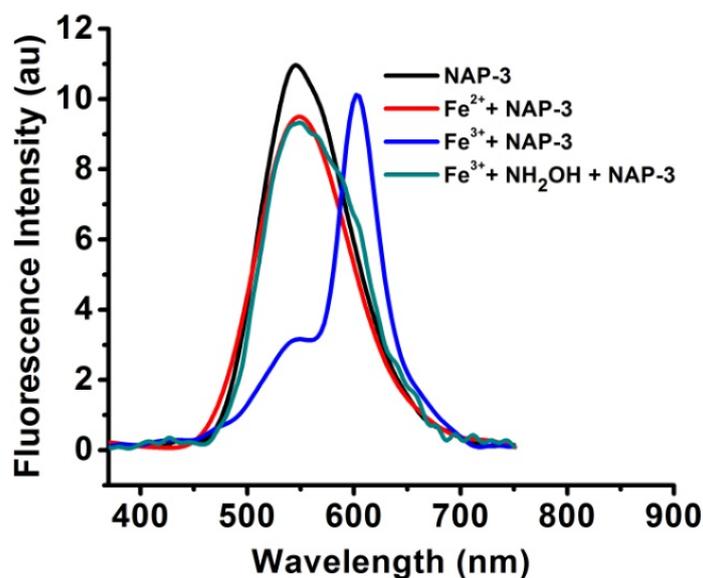


Figure S18 Changes in emission intensity of NAP-3 (2.5×10^{-5} M) in the presence of Fe^{3+} (5×10^{-4} M) and hydroxylamine hydrochloride (5×10^{-4} M, sodium acetate (6.0×10^{-4} M)) demonstrating the selectivity between $\text{Fe}^{2+}/\text{Fe}^{3+}$ under reducing environment, $\lambda_{\text{ex}} = 365\text{nm}$. $[\text{Fe}^{3+}] = 5 \times 10^{-4}$ M, $[\text{Fe}^{2+}] = 5 \times 10^{-4}$ M. NAP-3 was added after 10 min. of mixing of hydroxylamine hydrochloride and Fe^{3+} .

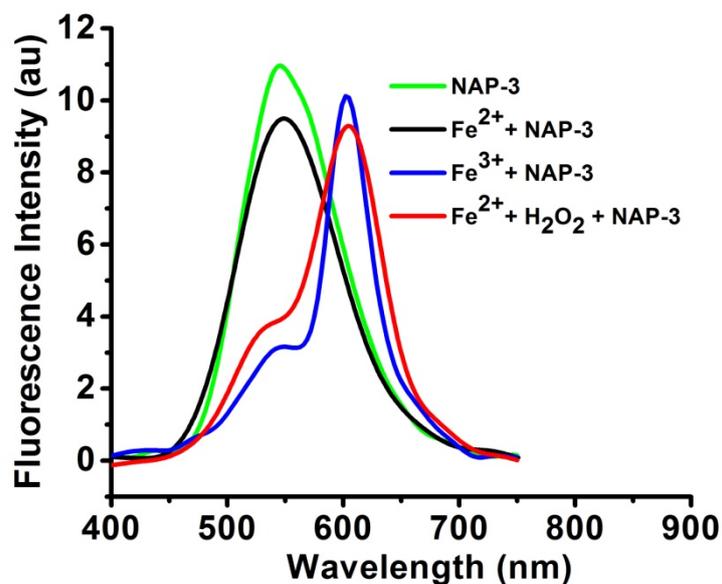


Figure S19 Changes in emission intensity of NAP-3 (2.5×10^{-5} M) in the presence of Fe²⁺ (5×10^{-4} M) and hydrogen peroxide (5×10^{-4} M) demonstrating the selectivity between Fe²⁺/Fe³⁺ under oxidizing environment, $\lambda_{\text{ex}} = 365$ nm. $[\text{Fe}^{3+}] = 5 \times 10^{-4}$ M, $[\text{Fe}^{2+}] = 5 \times 10^{-4}$ M. NAP-3 was added after 10 min. of mixing of hydrogen peroxide and Fe²⁺.

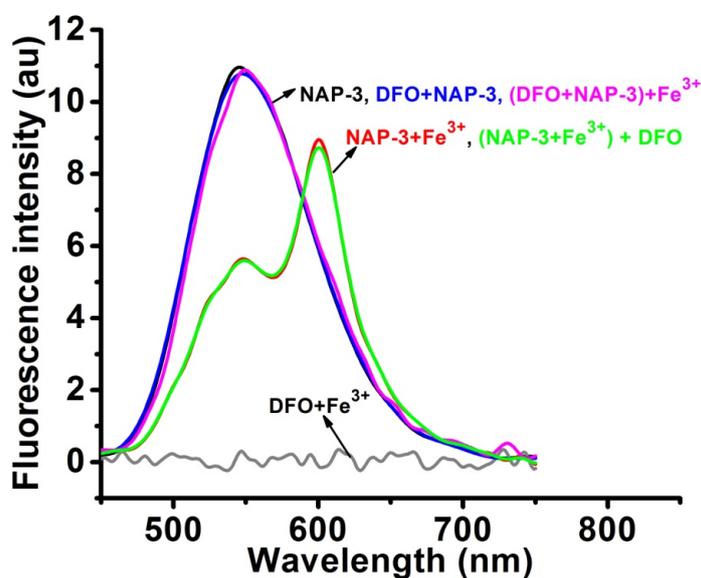


Figure S20 Competitive experiment between NAP-3 and DFO. Changes in emission intensity of NAP-3 (2.5×10^{-5} M) in the presence of Fe³⁺ (2.5×10^{-4} M) and DFO (2.5×10^{-4} M), $\lambda_{\text{ex}} = 365$ nm. $[\text{Fe}^{3+}] = 5 \times 10^{-4}$ M, $[\text{Fe}^{2+}] = 5 \times 10^{-4}$ M.

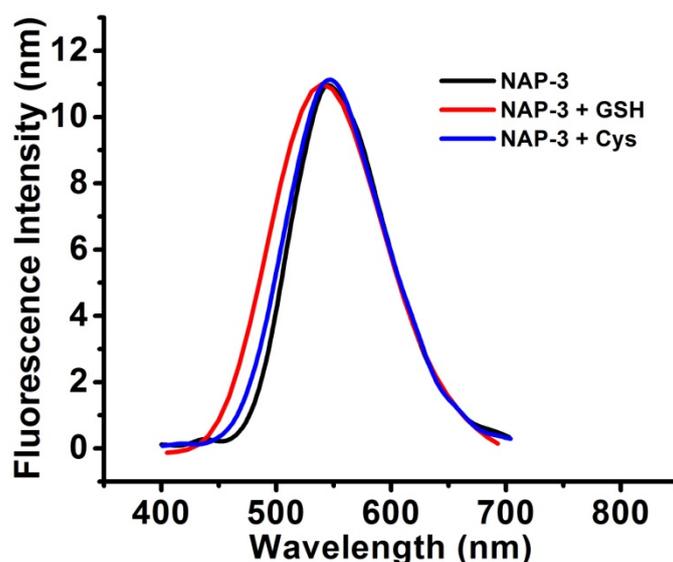


Figure S21 Fluorescence spectra of NAP-3 (2.5×10^{-5} M) in the presence of Glutathione (GSH, 5×10^{-4} M) and Cysteine (Cys, 5×10^{-4} M), $\lambda_{\text{ex}} = 365$ nm.

Materials and Methods for *in vitro* studies conducted in HepG2 cells

Cell culture

Human HepG2 cells (Origin- derived from the liver tissue of a 15-year-old Caucasian American male with a well-differentiated hepatocellular carcinoma, Source- ATCC, USA) were cultured in Phenol Red free Low Glucose Dulbecco's Modified Eagle Medium (LG DMEM, Invitrogen) supplemented with 10% fetal bovine serum, 100 units/ml penicillin, 100 $\mu\text{g}/\text{ml}$ streptomycin, 2mM l-glutamine (Sigma, USA), and incubated at 37°C in a humidified atmosphere of 5% CO_2 in air. For experiments, cells were seeded and treated with compounds after 24 hours or when 70% confluence is reached.

Confocal Microscopy Analysis

The cells were seeded in a 24-well plate at 1×10^5 cells/ml and incubated under 5% CO_2 and 95% humidity at 37°C . When 70% confluence was reached, it was treated with Ferric citrate dissolved in Phenol Red free LG DMEM media.⁶ After 24 h, the medium was removed and the cells were briefly washed with phosphate buffered saline (PBS) and 3 μM / 1 μM of NAP-3 (NAP-3 was dissolved in DMSO to obtain a stock solution of 3 mM and further diluted in Phenol Red free LG DMEM to obtain

final concentration of 3 μ M) was added to the wells for 24h and the images of the phase contrast and fluorescence were obtained under a laser scanning microscope LSM 510 META (Carl Zeiss, Jena, Germany). Images were acquired with a 63x Plan Apochromat Oil Phase II 1.4 objective. Lasers used were Diode 405 nm and DPSS 561 nm. The control group containing **NAP-3** was excited at 405 nm and emissions collected within band pass 505-550 nm. The iron loaded group containing **NAP-3** + Iron were excited at 561 nm and emissions collected with a long pass of 575 nm. Further, fluorescence intensity was quantified using image J software (Image J, National Institute of Health, Bethesda, MD).

Statistical Analysis

Graph pad software package was used for statistical analysis employing student's t test to analyze statistical significance in various groups. All results are given as mean \pm standard error of the mean.

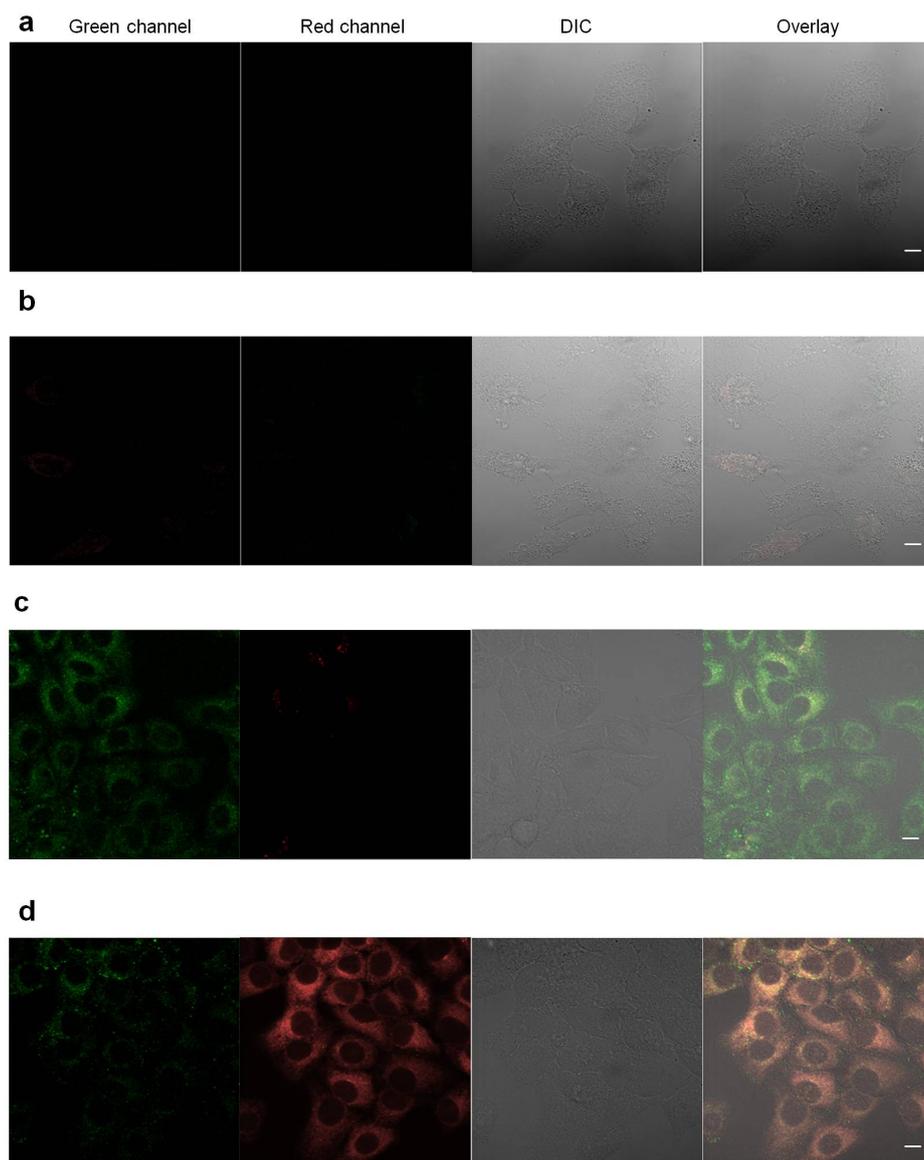


Figure S22 Representative confocal images of HepG2 cells with **NAP-3** tested at 1 μM concentration. (a) HepG2 cells only. (b) HepG2 cells incubated with Fe^{3+} ($5 \times 10^{-4} \text{M}$, 24 h) only. (c) HepG2 cells incubated with **NAP-3** (1 μM , 24 h) only. (d) HepG2 cells preincubated with Fe^{3+} ($5 \times 10^{-4} \text{M}$, 24 h) followed by incubation with **NAP-3** (1 μM , 24 h). Green channel, $\lambda_{\text{ex}} = 405 \text{ nm}$, $\lambda_{\text{em}} = 505\text{-}550 \text{ nm}$. Red channel, $\lambda_{\text{ex}} = 561 \text{ nm}$, $\lambda_{\text{em}} = 575 \text{ nm}$ Long Pass. Scale bar: 10 μm .

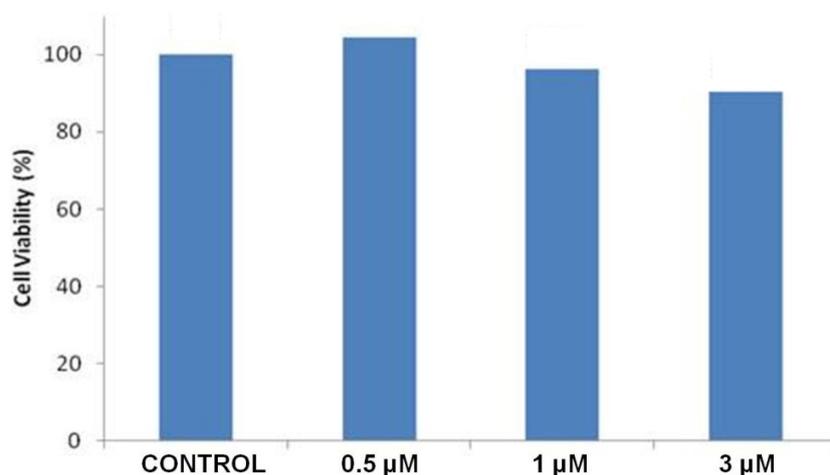


Figure S23 Cell viability assessment of **NAP-3** in HepG2 cells. Cells were seeded into 96-well plates at a density of 2×10^3 cells/well and cultured for 24 h. After incubation of the cells with compounds at different concentrations for 48 h, the cyto-toxicity of the isolated compounds was determined by the MTT assay as previously described.⁷ Percent cell viability was calculated based on the absorbance measured relative to the absorbance of control cells exposed to the vehicle alone.

Culture and maintenance for *in vivo* studies conducted in *C. elegans*.

The studies were carried out using wild type strain of *C. elegans* (N2 var Bristol), obtained from Caenorhabditis Genetics Center (University of Minnesota, MN, USA), maintained and propagated at 22°C on nematode growth media (NGM), seeded with the uracil auxotroph strain, OP50, of *E. coli* as described earlier.⁸ Plates were maintained at 22 °C and each experiment was carried out in synchronized nematode populations for nullifying any effects caused as a result of difference in age/developmental stages of the nematodes. Age synchronized worms were obtained by axenization of gravid worms using standard hypochlorite bleaching method.⁹

Treatment of worms with compound NAP-3 and/or Ferric Citrate (FC)

NAP-3 or Ferric citrate (FC) was mixed with OP50 strain of *E. coli* to obtain a final concentration of 3 μM and 25 mM respectively, followed by seeding onto NGM plates. NGM plates seeded with *E. coli* strain OP50 served as culture plates for the control group. The seeded plates were incubated overnight at 22°C for culturing of bacteria into a fine lawn. Isolated embryos were added onto these plates and the

nematodes were raised on these plates for 48 h in order to obtain an age synchronized population of early-adult worms for further analysis.

RNAi induced silencing of gene *ftn-1*

Silencing of *ftn-1* was achieved by employing RNAi induced gene silencing approach via standard feeding protocol as described previously.¹⁰ We used bacterial clone, specific for *ftn-1*, from the Ahringer RNAi library that was purchased from SA Biosciences. These clones expressing *ftn1* specific dsRNA were cultured overnight at 37°C, in Luria Bertani Broth (Himedia, Cat. No. M1245) containing 50ug/ml ampicillin(Sigma, Cat. No. A0166). The freshly cultured clones were seeded onto NGM plates containing 1mM isopropyl β-D- 1-thiogalactopyranoside (IPTG; Sigma, Cat. No. I6758) and 25mg/L carbenicillin (Sigma; Cat. No. C1389) and incubated overnight at 22°C for successful induction.¹¹

Confocal Microscopy Analysis

Age synchronized worms under study were washed 2 to 3 times using M9 buffer, to remove adhering bacteria and transferred onto 2% agarose padded slides carrying a drop of mounting medium and sealed with a cover slip. The mounting medium was pre-mixed with 100 mM sodium azide (Sigma, Cat no. 71289) which aids in immobilization of the worms without killing them.¹² Imaging of these live immobilized worms was performed using confocal microscope LSM 510 META (Carl Zeiss, Jena, Germany). Images were acquired with a 63x Plan Apochromat Oil Phase II 1.4 objective. The analysis of unbound/ free compound **NAP-3** was carried out with an excitation of 405 nm and emissions collected within band pass 505-550 nm. Further the analysis of compound bound/complexed to iron was carried out with an excitation of 561 nm and emissions collected with a long pass of 575 nm. Further, fluorescence intensity was quantified using image J software (Image J, National Institute of Health, Bethesda, MD).

Studies on expression of iron response gene *ftn-1* using quantitative real-time PCR (qPCR)

Age synchronized N2 worms of various groups were washed twice with 0.2% DEPC (Sigma, Cat. No.-D5758) treated water to remove adhering bacteria, following which total RNA was isolated using RNAzol® RT method (Sigma, Cat. No. R4533), quantified through NanoDrop (Thermo, Quawell, UV-Vis Spectrophotometer, Q5000). About 5µg of total RNA was used for the synthesis of cDNA using RevertAid First Strand cDNA synthesis kit (Thermo Scientific, Cat. No. #K1622). Quantification of mRNA levels was carried out using SYBR Green (Thermo Scientific Cat. No. #K0251) chemistry. In brief cDNA equivalent to 125ng was amplified in 25ul maxima using Stratagene MX3005P detection system (Agilent Technologies). The program for amplification was, 50°C for 2 minutes 95°C for 10 minutes (1 cycle), followed by 95°C for 30 seconds, 55°C for 30 seconds and 60°C for 30 seconds (40 cycle) and melting curve detection (95°C for 5 sec, 65°C for 1 min).Experiment of each sample was

carried out in duplicate sets. Fold change of all samples were analyzed using comparative $2^{-\Delta\Delta CT}$. Integrated DNA Technologies (IDT) software was used for designing of primers of desired genes. act-1 mRNA was used as endogenous control for normalization. Primers sequences of targets genes used, are as follows:

act- 1 forward: TTA CTC TTT CAC CAC CAC CGC TGA

act- 1 reverse: TCG TTT CCG ACG GTG ATG ACT TGT

ftn-1 forward: CTC TAC GCC TCC TAT GTC TAT CT

ftn-1 reverse: CTC ATC CGA TTG CTC CTT GAA

Statistical Analysis

Graph pad software package was used for statistical analysis employing student's t test to analyze statistical significance in various groups. All results are given as mean \pm standard error of the mean.

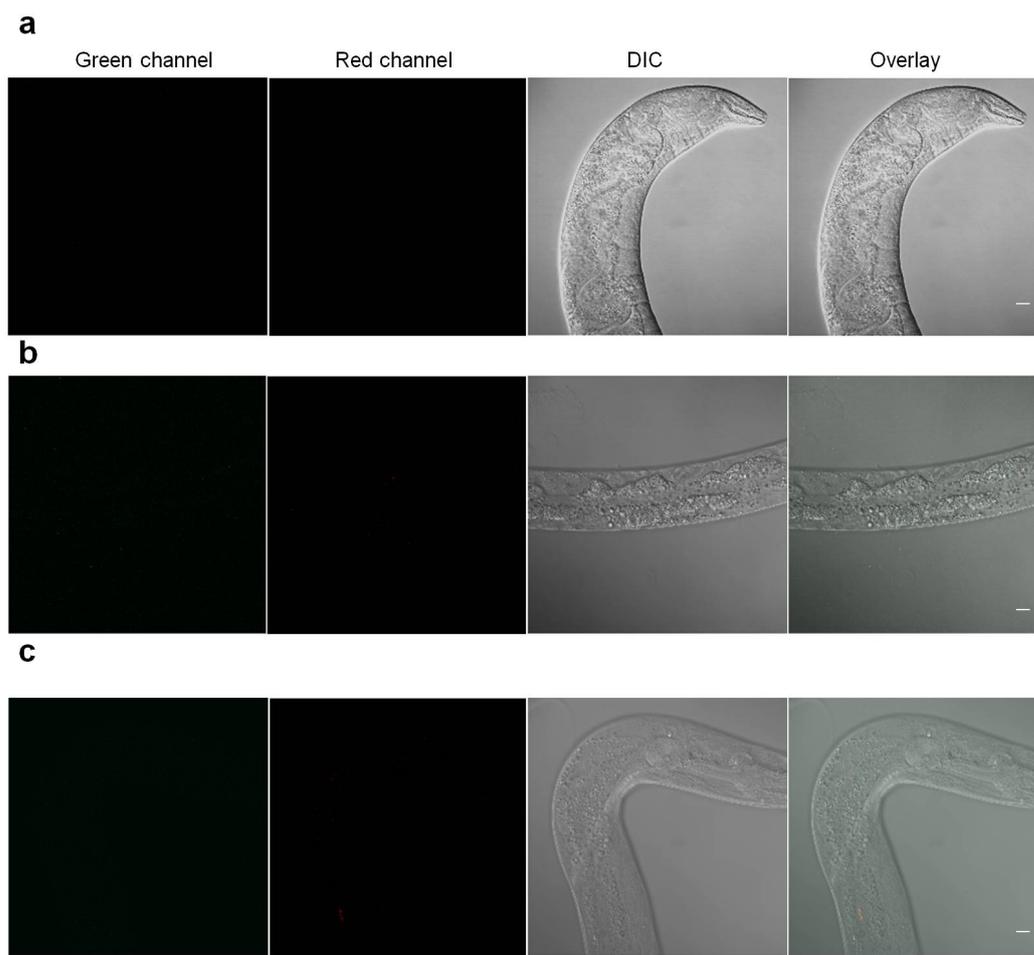


Figure S24 Representative confocal images of control groups for *C. elegans* studies. (a) *C. elegans* wild type (N2) only (b) *C. elegans* wild type (N2) incubated with aqueous solution of Ferric citrate (25mM, 24 h). (c) *ftn-1* silenced worm only. Green channel: $\lambda_{\text{ex}} = 405 \text{ nm}$, $\lambda_{\text{em}} = 505\text{-}550 \text{ nm}$. Red channel: $\lambda_{\text{ex}} = 561 \text{ nm}$, $\lambda_{\text{em}} = 575 \text{ nm}$ long pass. Scale bar: $10 \mu\text{m}$

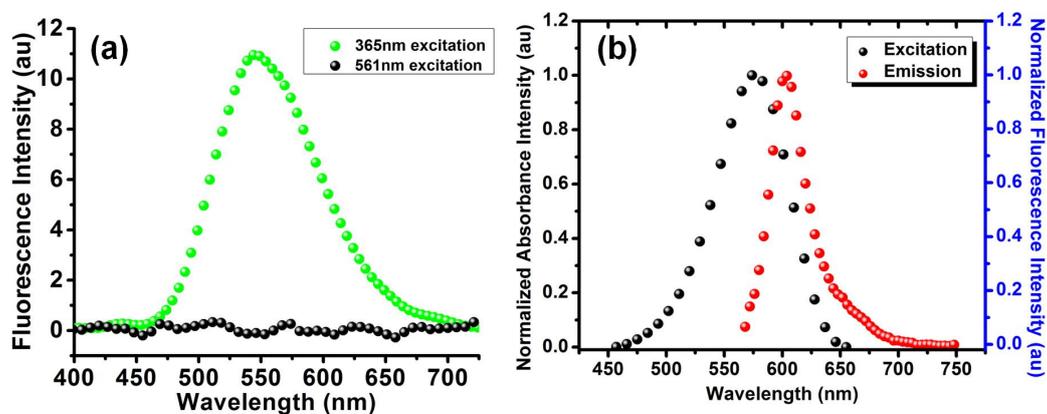
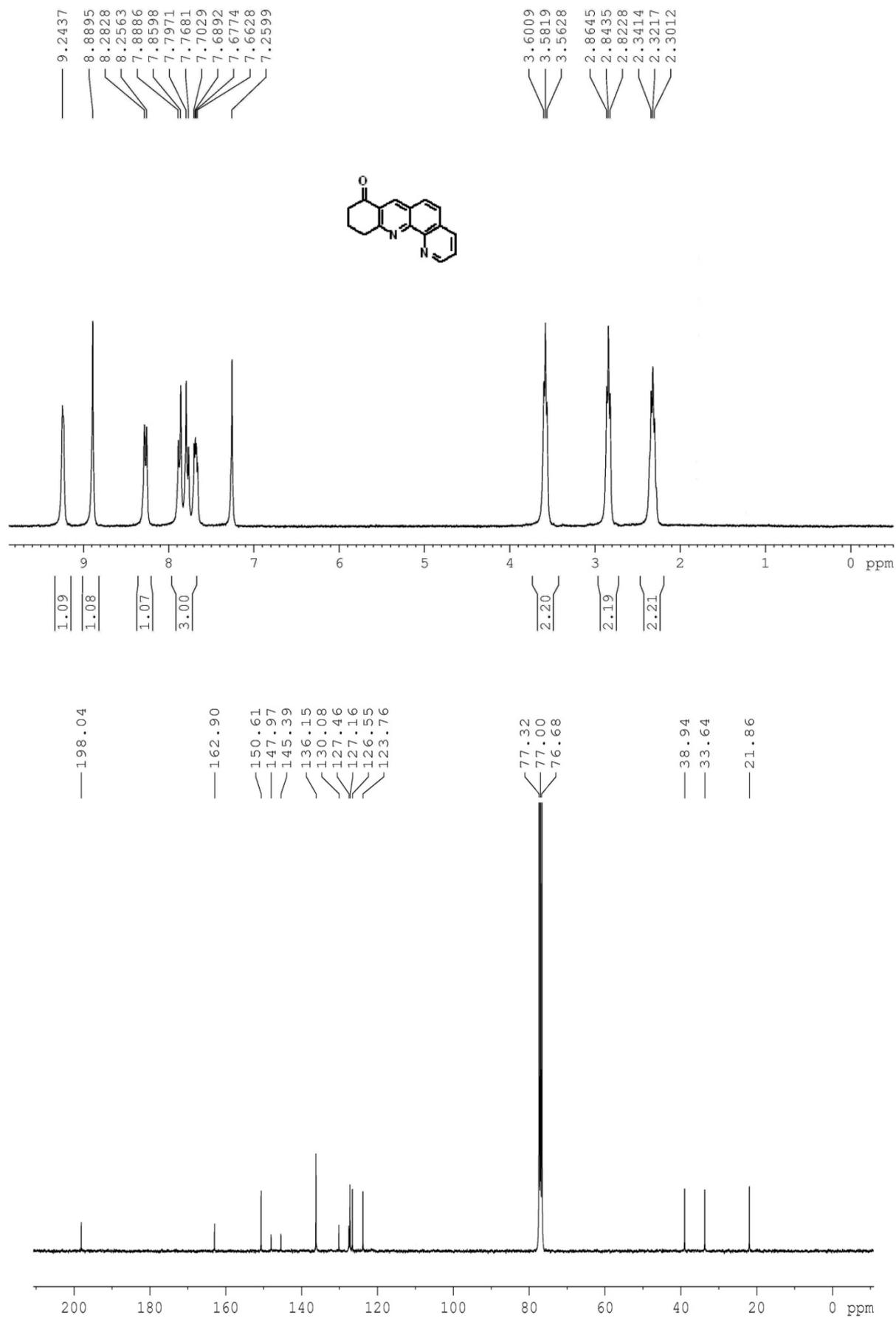
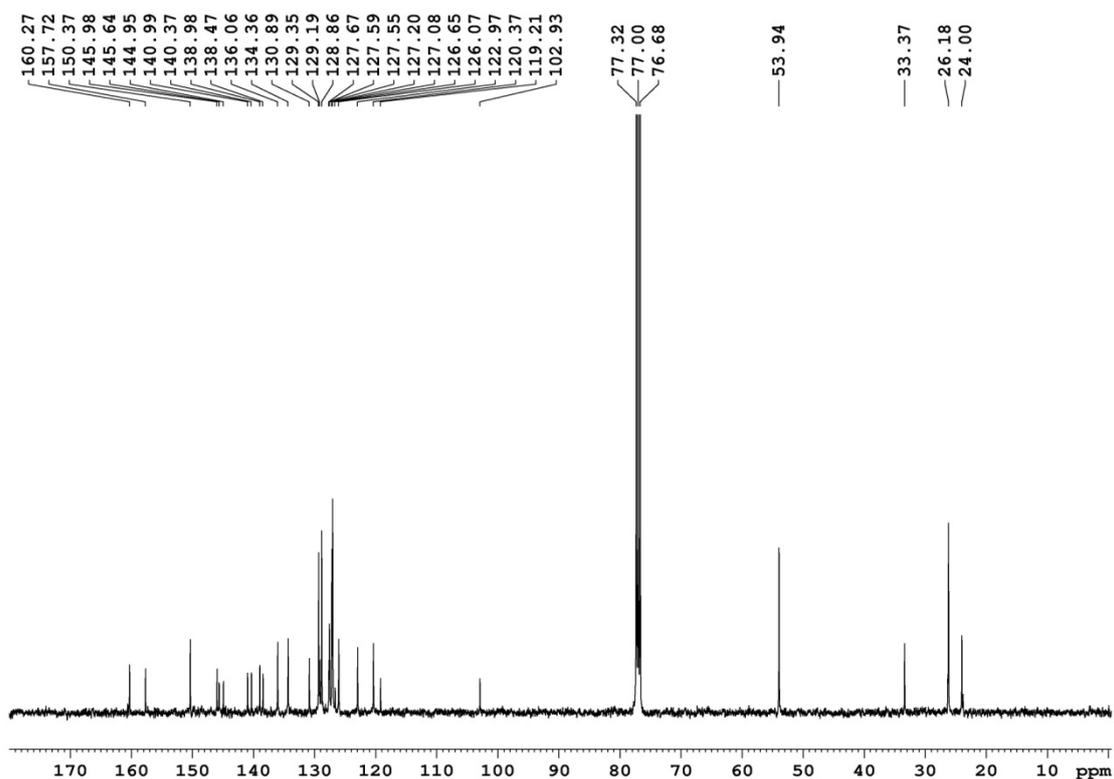
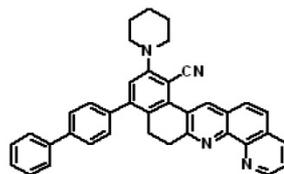
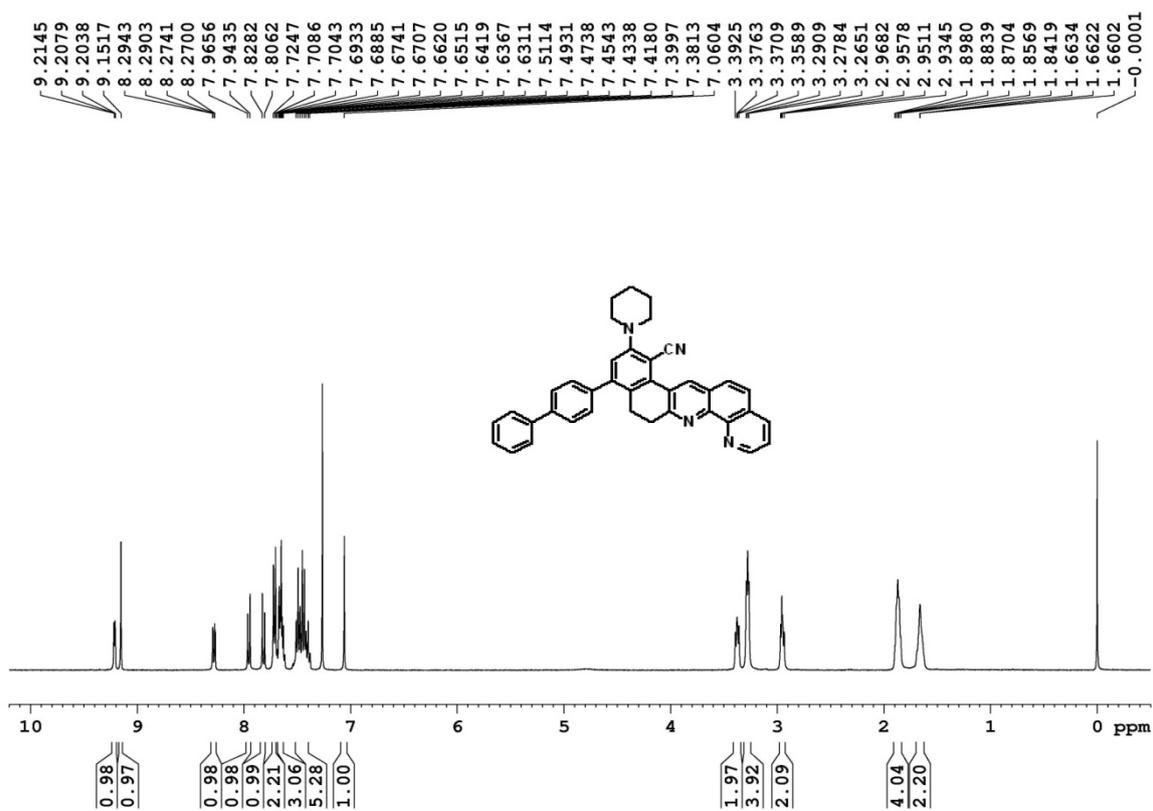


Figure S25 (a) Emission spectrum of NAP-3 ($2.5 \times 10^{-5} \text{ M}$) at excitation 365nm and 561nm. (b) Excitation and emission spectra (at 561 nm excitation) of NAP-3/ Fe^{3+} complex.

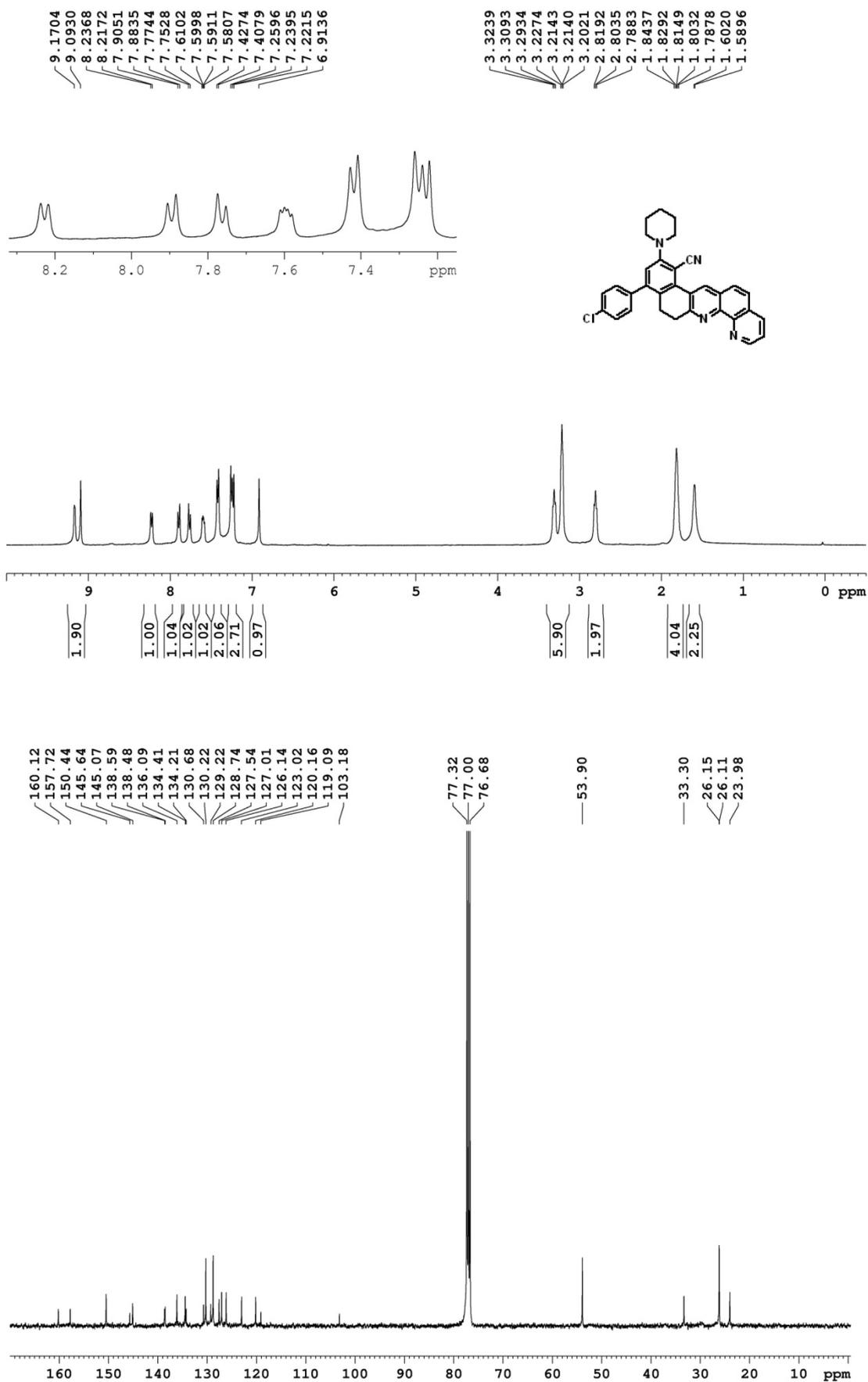
¹H NMR (CDCl₃, 300 MHz) and ¹³C NMR (CDCl₃, 100.6 MHz) of 8



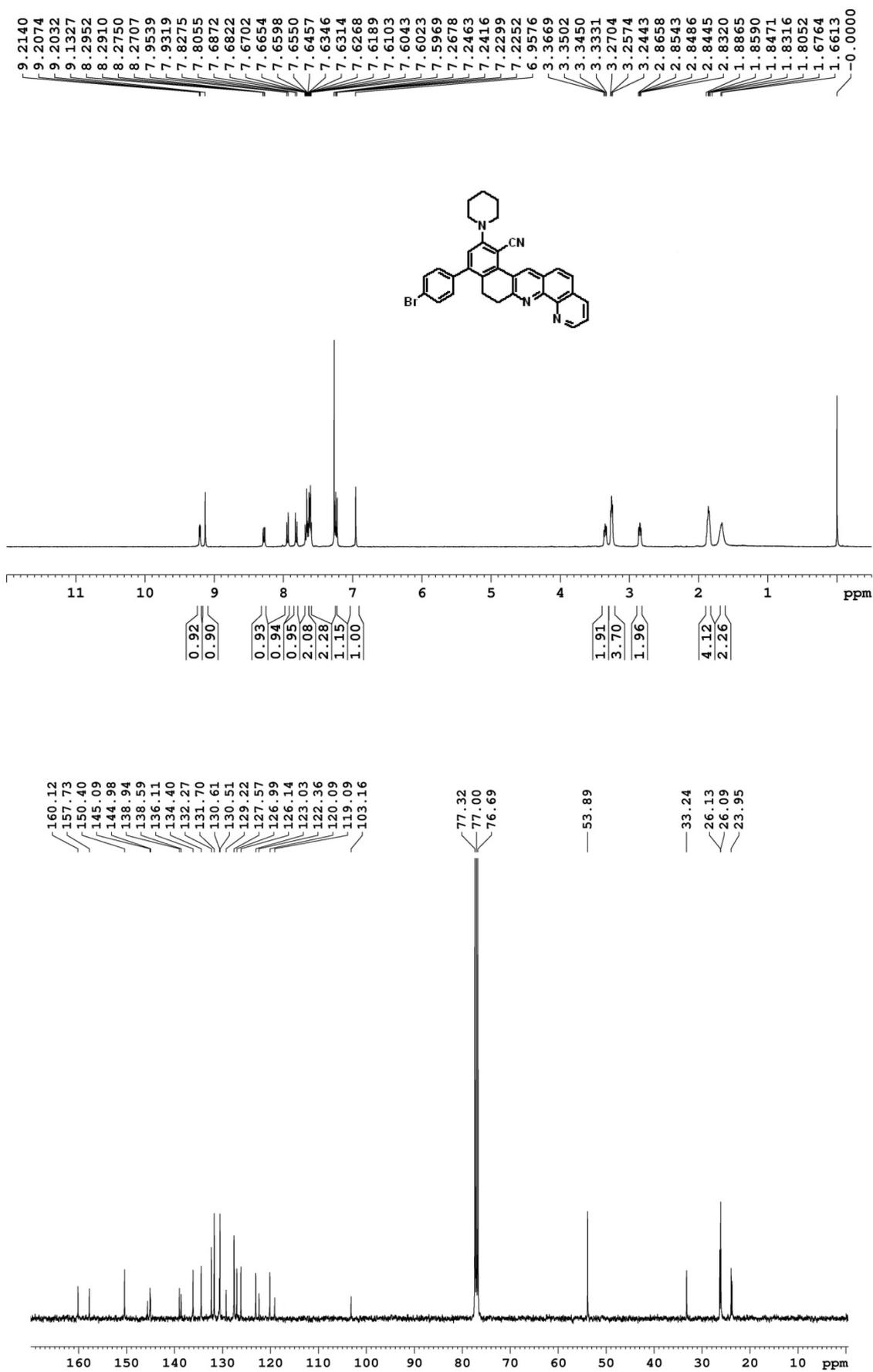
¹H NMR (CDCl₃, 400 MHz) and ¹³C NMR (CDCl₃, 100.6 MHz) of 9a



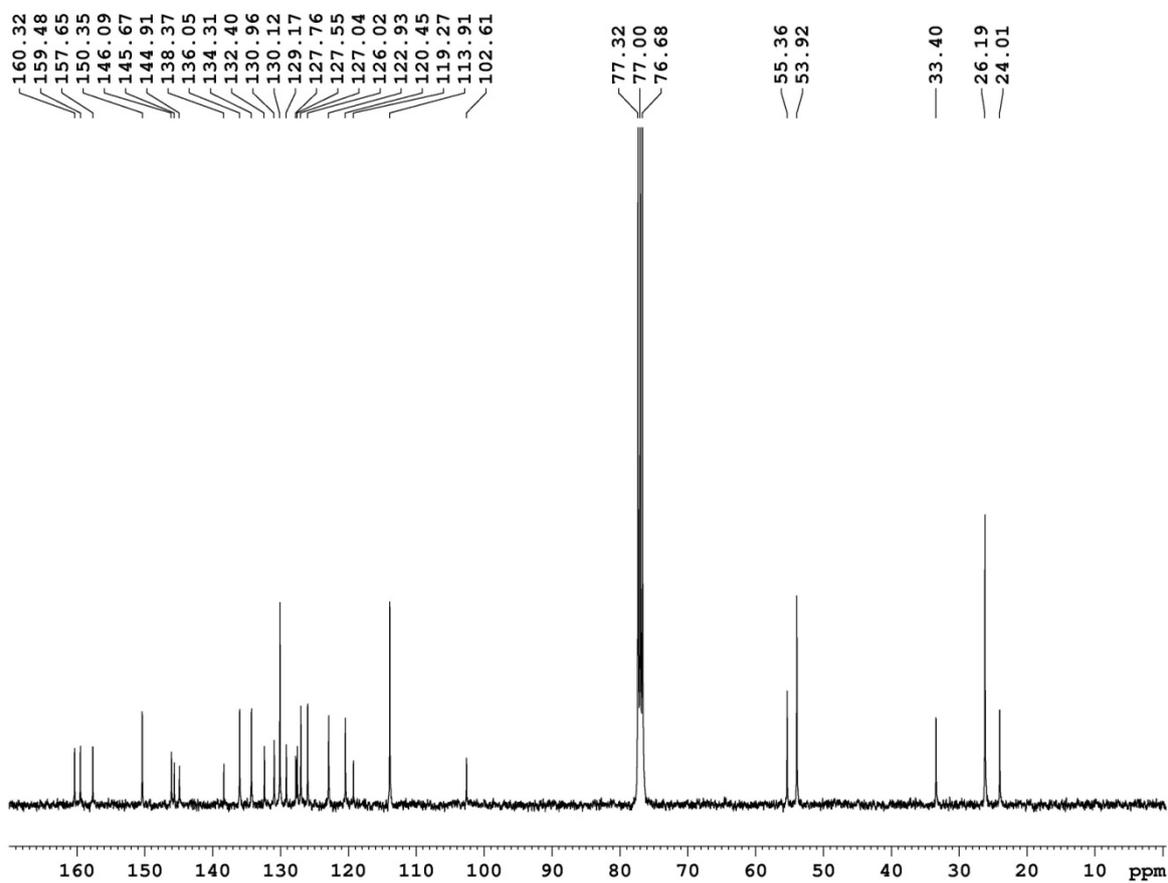
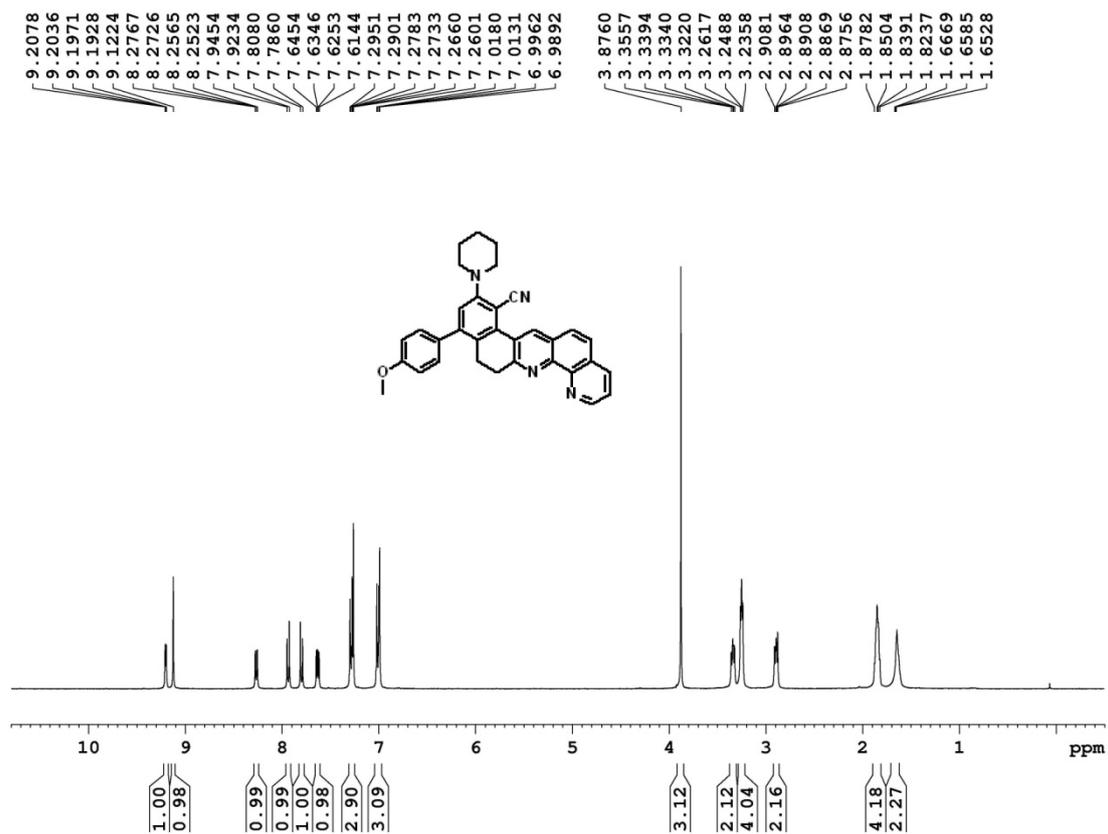
¹H NMR (CDCl₃, 400 MHz) and ¹³C NMR (CDCl₃, 100.6 MHz) of 9b



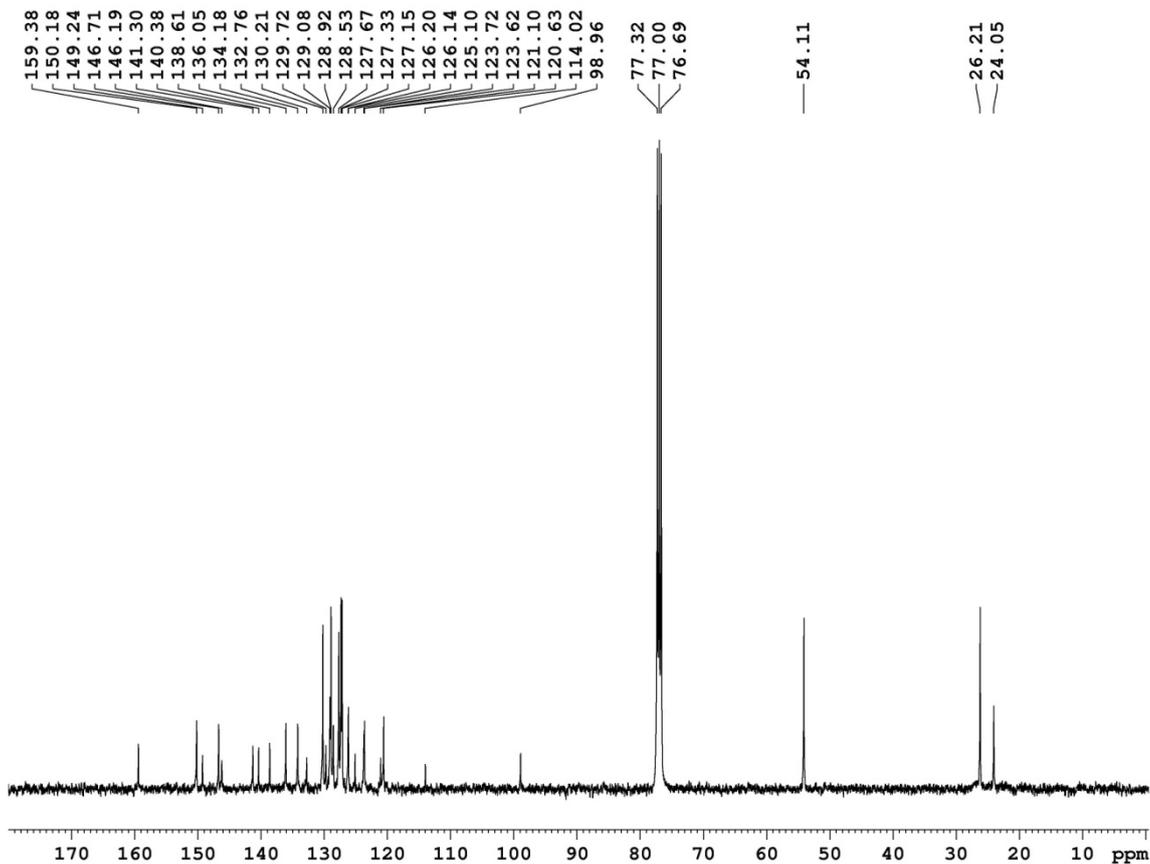
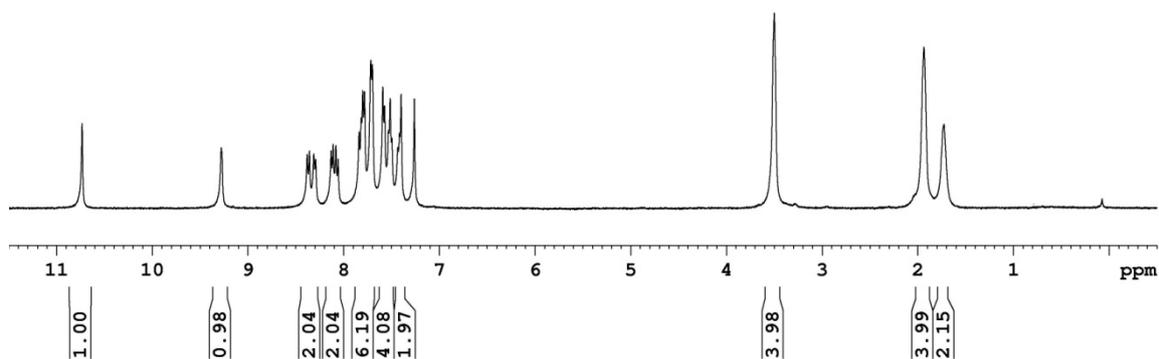
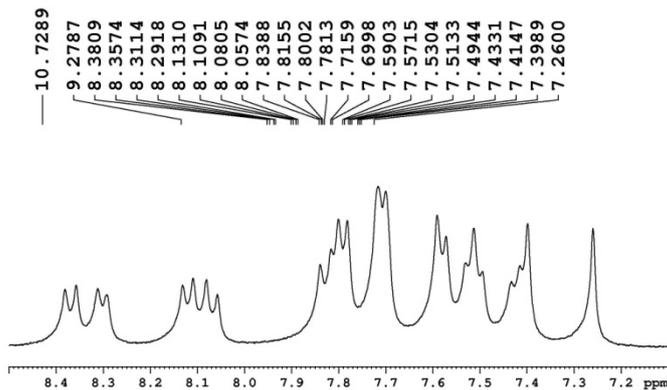
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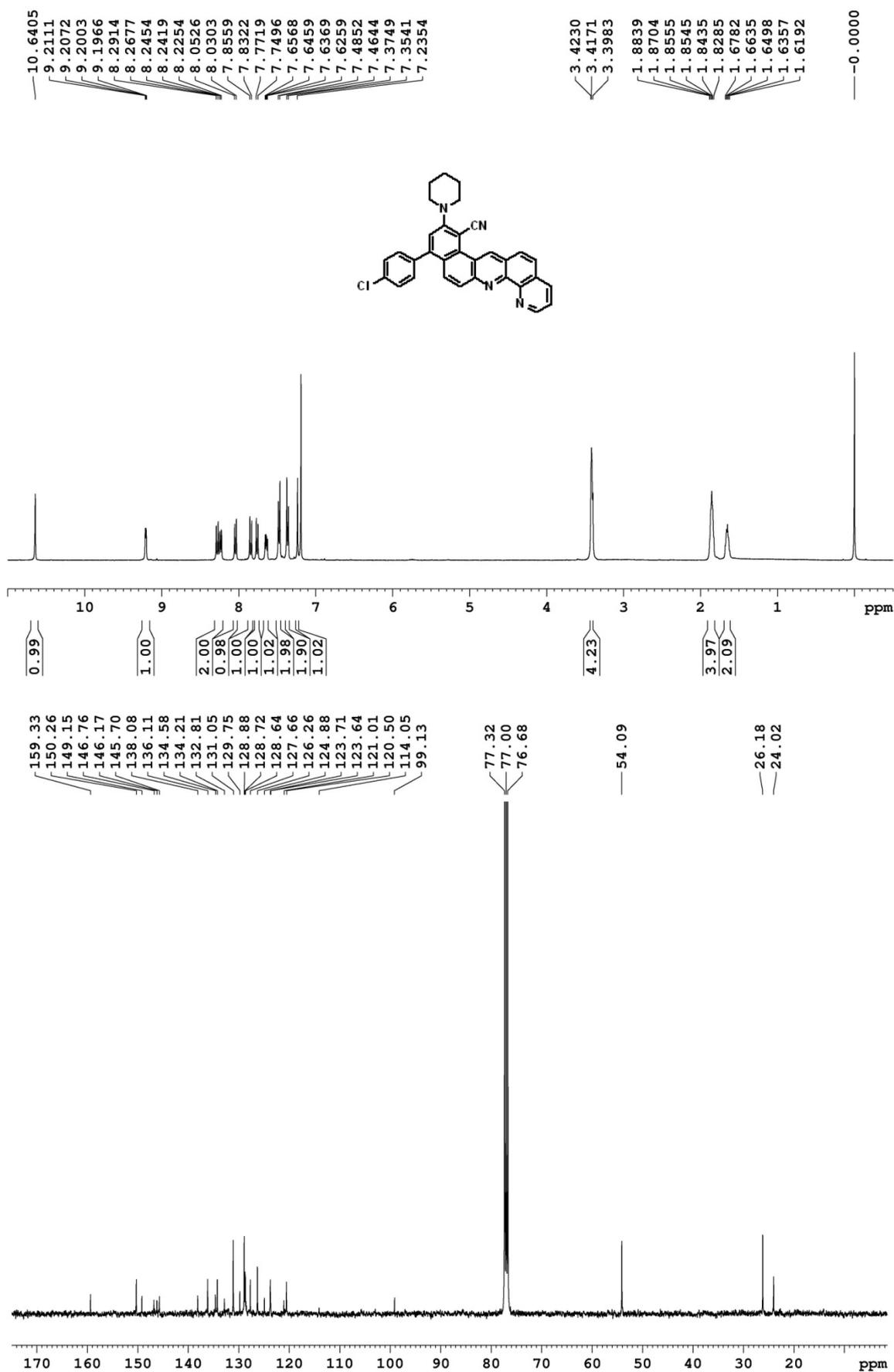
^1H NMR (CDCl_3 , 400 MHz) and ^{13}C NMR (CDCl_3 , 100.6 MHz) of 9d



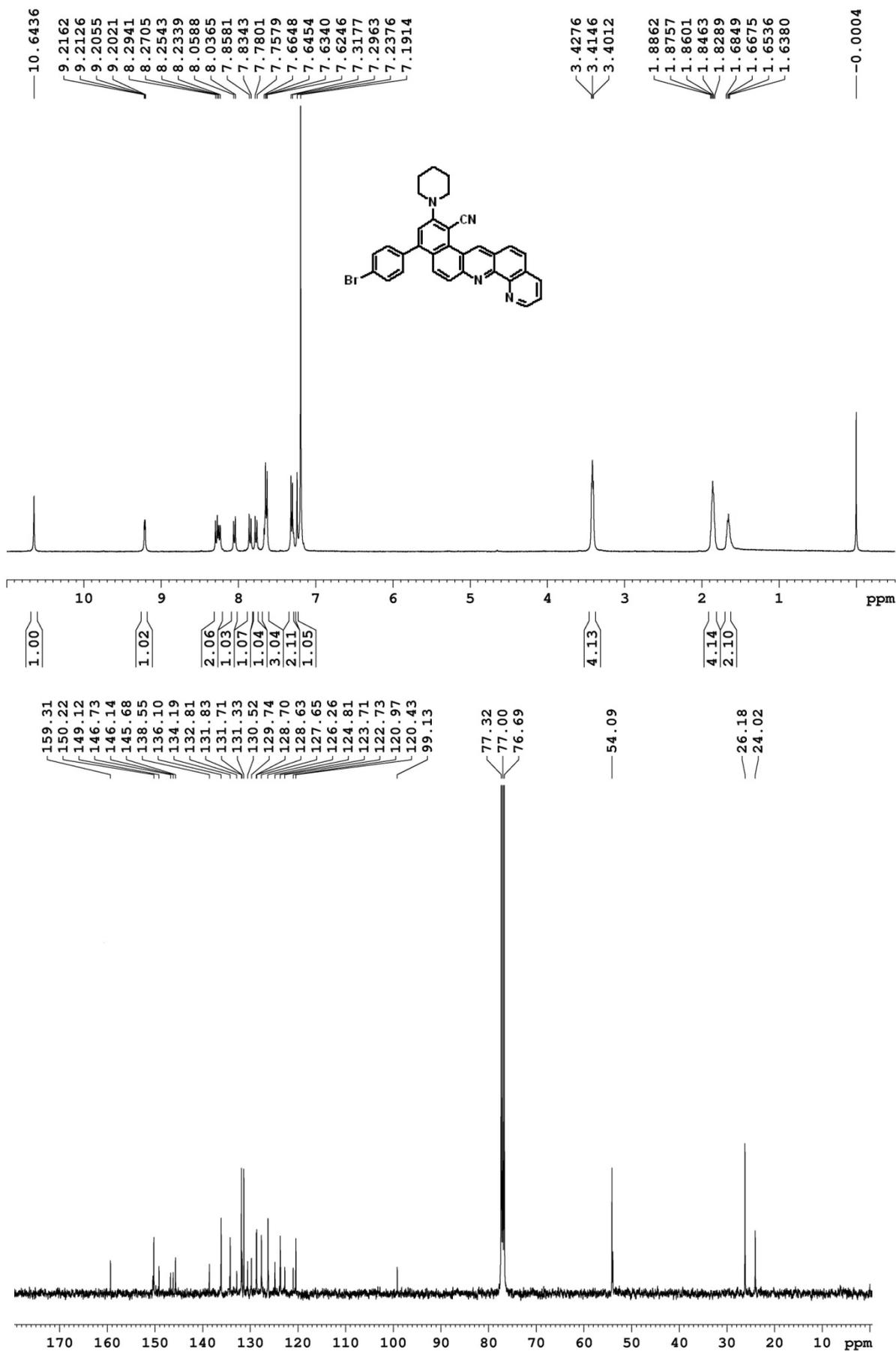
^1H NMR (CDCl₃, 300 MHz) and ^{13}C NMR (CDCl₃, 100.6 MHz) of 10a



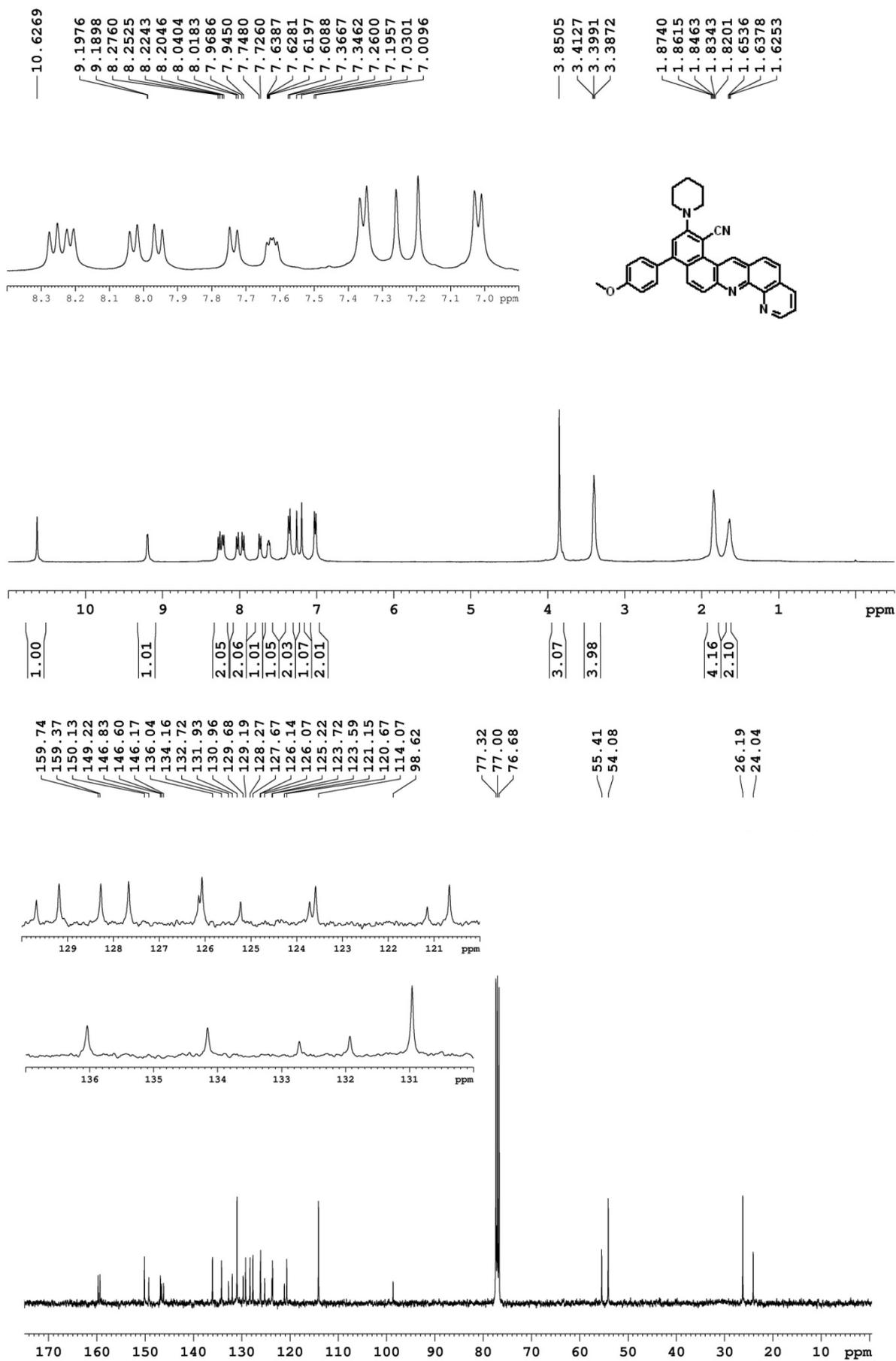
¹H NMR (CDCl₃, 300 MHz) and ¹³C NMR (CDCl₃, 100.6 MHz) of 10b



¹H NMR (CDCl₃, 400 MHz) and ¹³C NMR (CDCl₃, 100.6 MHz) of 10c (NAP-3)



¹H NMR (CDCl₃, 400 MHz) and ¹³C NMR (CDCl₃, 100.6 MHz) of 10d



References

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Cartesian coordinates (Å) of NAP-3 optimized at the B3LYP/6-31G(d,p)

Energy: -4026.6824265314 hartrees

Atom	X	Y	Z
C	-1.97181349	0.10276075	-0.00133261
C	-3.14537361	0.86335436	-0.00195873
C	-4.40450583	0.25796630	-0.00148527
C	-4.46982057	-1.16769325	-0.00028756
C	-2.16765900	-1.32339053	-0.00012656
C	-5.61145810	1.03810891	-0.00224131
C	-5.78219581	-1.81530366	0.00014167
C	-6.94458068	-0.99704966	-0.00067579
C	-6.82645562	0.43611683	-0.00186165
C	-8.19675265	-1.64289857	-0.00030552
H	-9.10475646	-1.04861484	-0.00091818
C	-8.24884507	-3.02015326	0.00082136
C	-7.03703536	-3.73368032	0.00156333
H	-5.52715053	2.11950027	-0.00315895
H	-3.11805650	1.94010538	-0.00290157
H	-7.73556716	1.02831024	-0.00246296
H	-9.19401109	-3.55016236	0.00113128
H	-7.04694182	-4.82091865	0.00245930
N	-3.36910709	-1.91516560	0.00038298
N	-5.84538381	-3.16114138	0.00124245
C	-0.60556304	0.64200794	-0.00200948
C	0.48138236	-0.28810472	-0.00159689
C	-0.26920495	2.03480795	-0.00358803
C	1.06505008	2.46657155	-0.00462654
C	2.09810838	1.52355332	-0.00406004

H	3.12674298	1.86416834	-0.00437647
C	-1.03526598	-2.19965429	0.00042820
C	0.22176226	-1.70381854	-0.00033565
C	2.97980005	-0.78835755	-0.00128091
C	3.53850966	-1.23103748	1.20229233
C	3.54114978	-1.23090883	-1.20365606
C	4.63157685	-2.09561023	1.21162523
H	3.11605165	-0.89952664	2.14426950
C	4.63421640	-2.09552438	-1.21065939
H	3.12073615	-0.89934386	-2.14652706
C	5.16900353	-2.52095579	0.00104780
H	5.05674731	-2.43154820	2.14862561
H	5.06139105	-2.43143912	-2.14675446
C	1.82804185	0.16824996	-0.00247579
Br	6.67301406	-3.70962675	0.00265008
C	1.95267569	4.36653194	1.23124476
C	1.99888346	4.35781891	-1.22097317
C	1.92855224	5.89660150	1.25977994
H	2.99710254	4.01433951	1.32677287
H	1.39494822	3.96162972	2.07922577
C	1.97470133	5.88757379	-1.26123450
H	3.04665543	4.00684505	-1.27460046
H	1.47396108	3.94615118	-2.08644975
C	2.59616610	6.48115752	0.00894731
H	2.43303908	6.24647873	2.16613882
H	0.88699090	6.22928820	1.31382926
H	2.51172587	6.23151768	-2.15102475
H	0.93559290	6.21901470	-1.35552906
H	2.50749730	7.57164922	0.00345283
H	3.67006729	6.25319209	0.02932790
N	1.32958157	3.87018907	-0.00531875

C	-1.22971005	3.09913943	-0.00418809
N	-1.99513997	3.96584506	-0.00434013
H	1.06843937	-2.37722640	-0.00005314
H	-1.23639320	-3.26364345	0.00141071