Phenazine *N*,*N*'-dioxide scaffold as selective hypoxic cytotoxin pharmacophore. Structural modifications looking for further DNA topoisomerase II-inhibition activity

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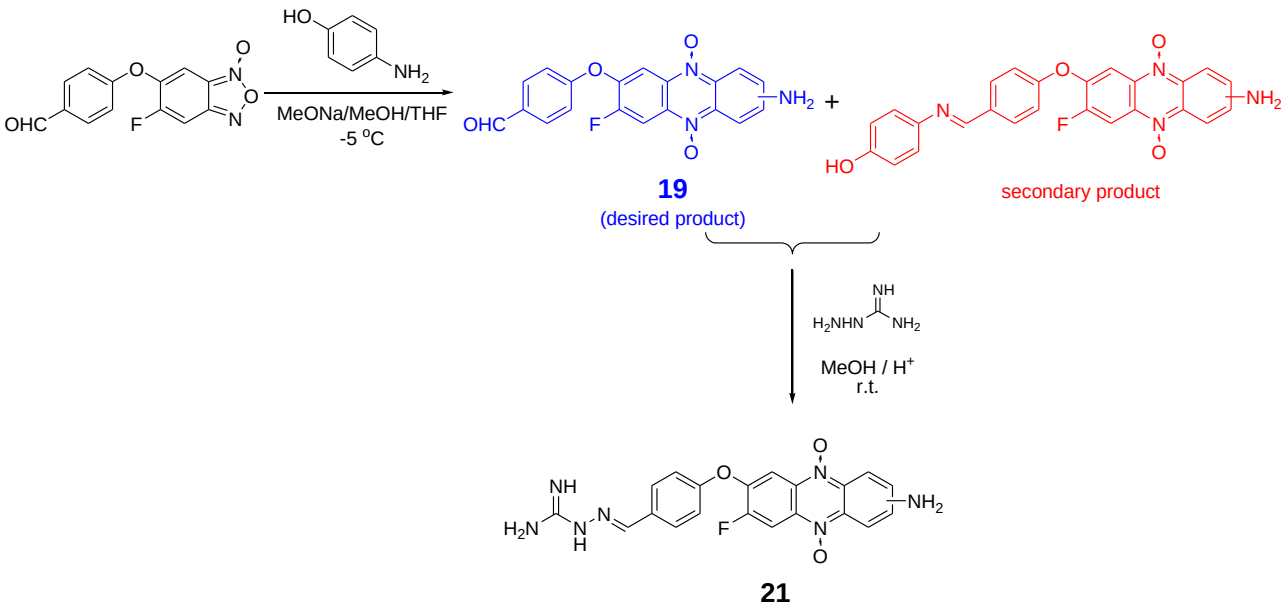


Figure S1. Synthetic scheme and results in the preparation of PDO **19**. This compound was obtained mixed with the corresponding imine (secondary product). This mixture treated with aminoguanidine yield PDO **21**.

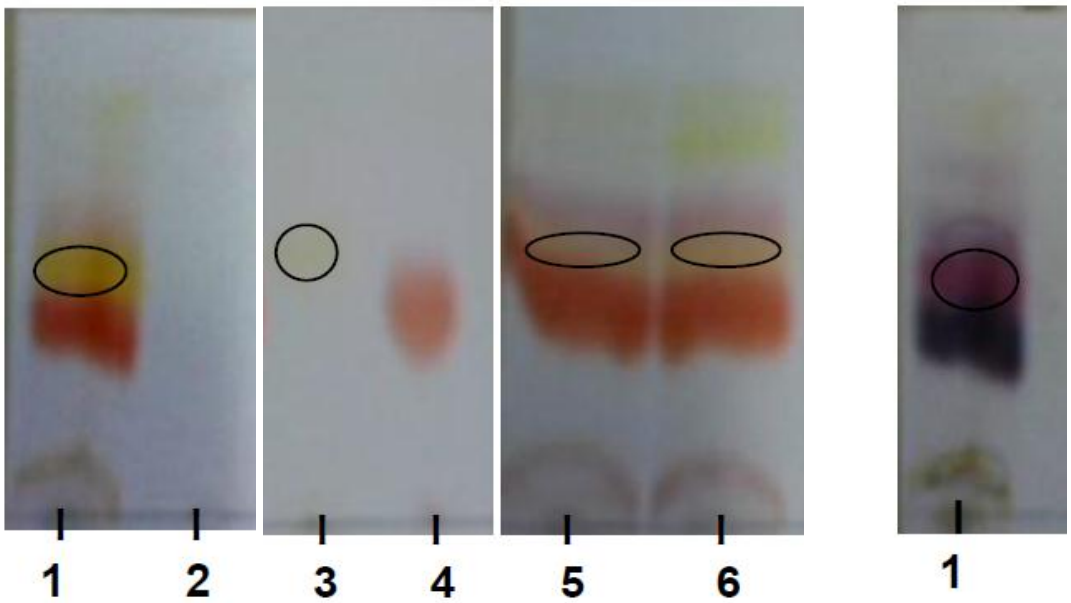
Table S1. Proportions of 7- and 8-Isomers of Studied Compounds.

compound	7:8 isomers ratio[a]
6	50 : 50
7	55 : 45
8	50 : 50
9	53 : 47
10	51 : 49
11	44 : 56
12	52 : 48
13	65 : 35
14	59 : 41
15	56 : 44
16	55: 45
17	58 : 42
18	60 : 40
19	60 : 40[b]
20	65 : 35[b]
21	58 : 42[b]
22	60 : 40[b]
23	56 : 44
25	50 : 50[b]

[a] Determined by ^{1}H-NMR from the isolated products. [b] 7-fluoro- and 8-fluoro-isomers.

Simulated hypoxia

a) **b)**



Simulated normoxia

c) **d)**

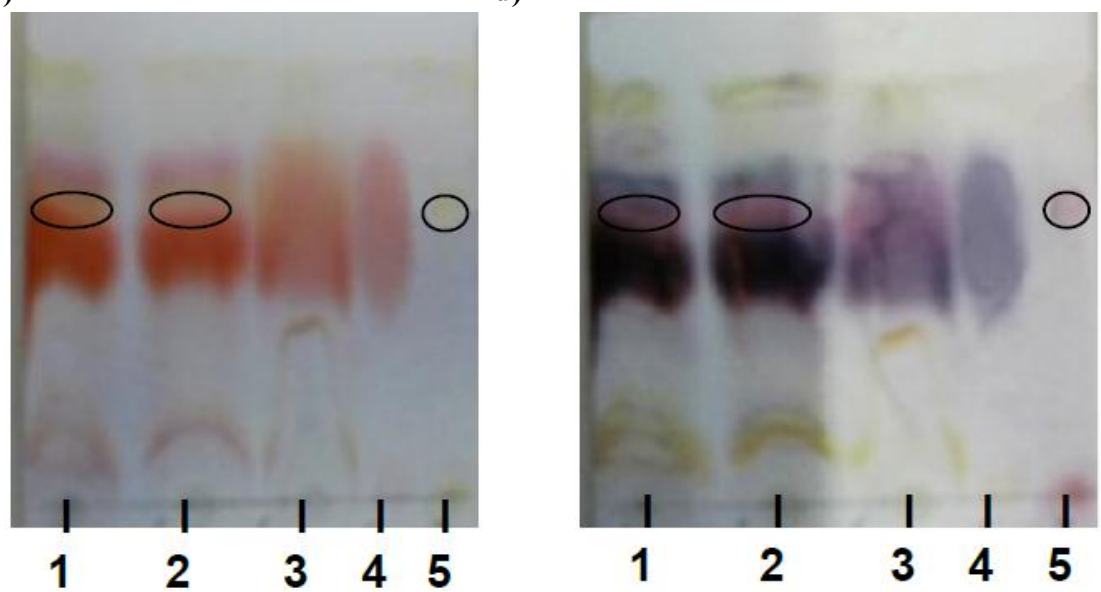


Figure S2. TLC chromatograms (see Material and methods for experimental conditions) taken after 30 min of incubation of PDO **12** with different protein fractions and in different gasification conditions. **Simulated hypoxia: a)** Spots without revealed (PDO, orange; phenazine monoxides, yellow); **b)** Run 1 spots visualised by spraying with a solution of *p*-anisaldehyde:H_2SO_4(c):EtOH (95:4:1) followed by heating. Runs: **1.** Incubation with S9 fraction; **2.** Control of enzymatic fractions; **3.** PDO **23**; **4.** PDO **12**; **5.** Incubation with cytosolic fraction; **6.** Incubation with microsomal fraction. **Simulated normoxia: c)** Spots without revealed (PDO, orange; phenazine monoxides, yellow); **d)** Spots visualised by spraying with a solution of *p*-anisaldehyde:H_2SO_4(c):EtOH (95:4:1) followed by heating. Runs: **1-3.** Incubations with cytosolic, microsomal, and S9 fractions; **4.** PDO **12**; **5.** PDO **23**.

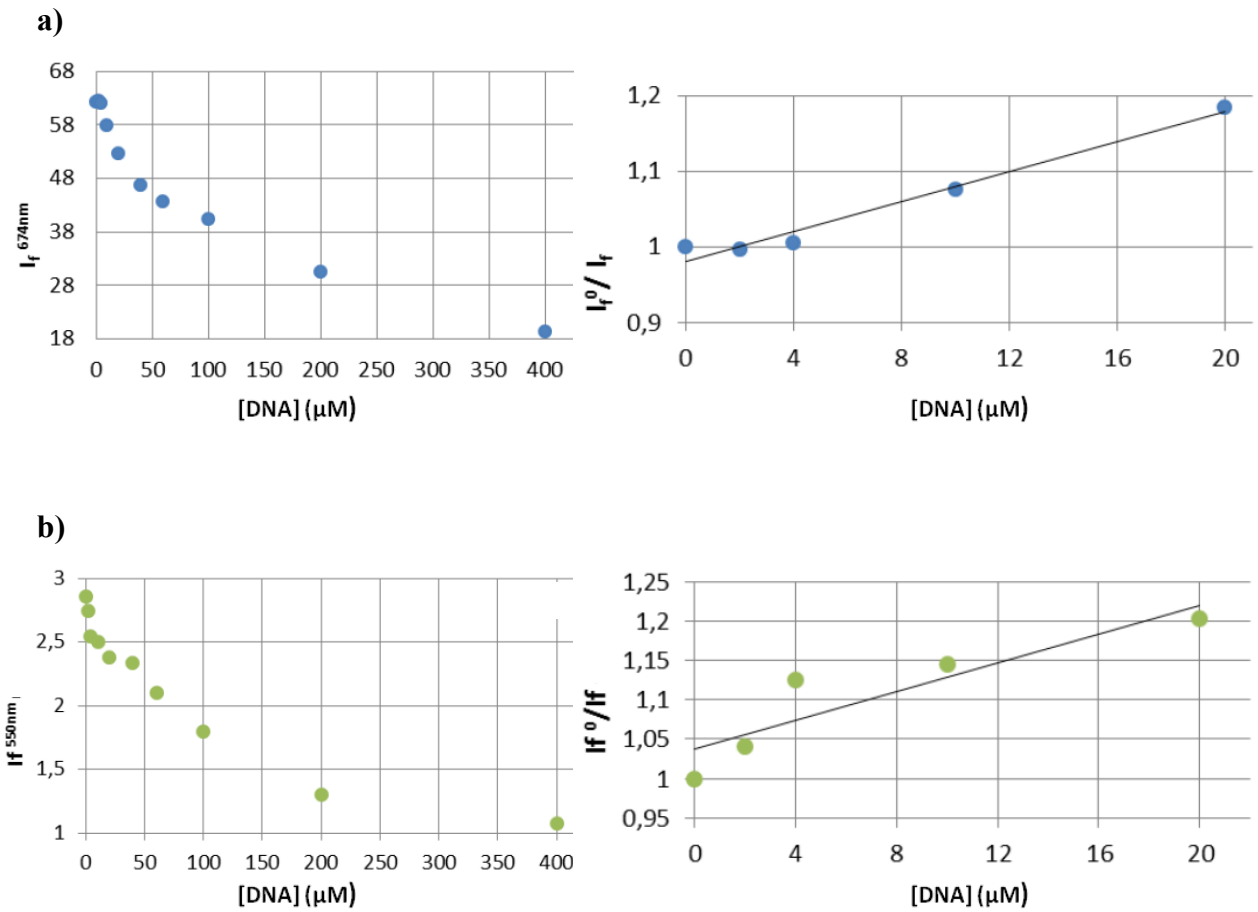


Figure S3. Stern–Volmer quenching plot (right) from the fluorescence data with increasing concentrations of DNA in PBS (left). **a)** For toluene blue (reference compound). **b)** For PDO **7**.

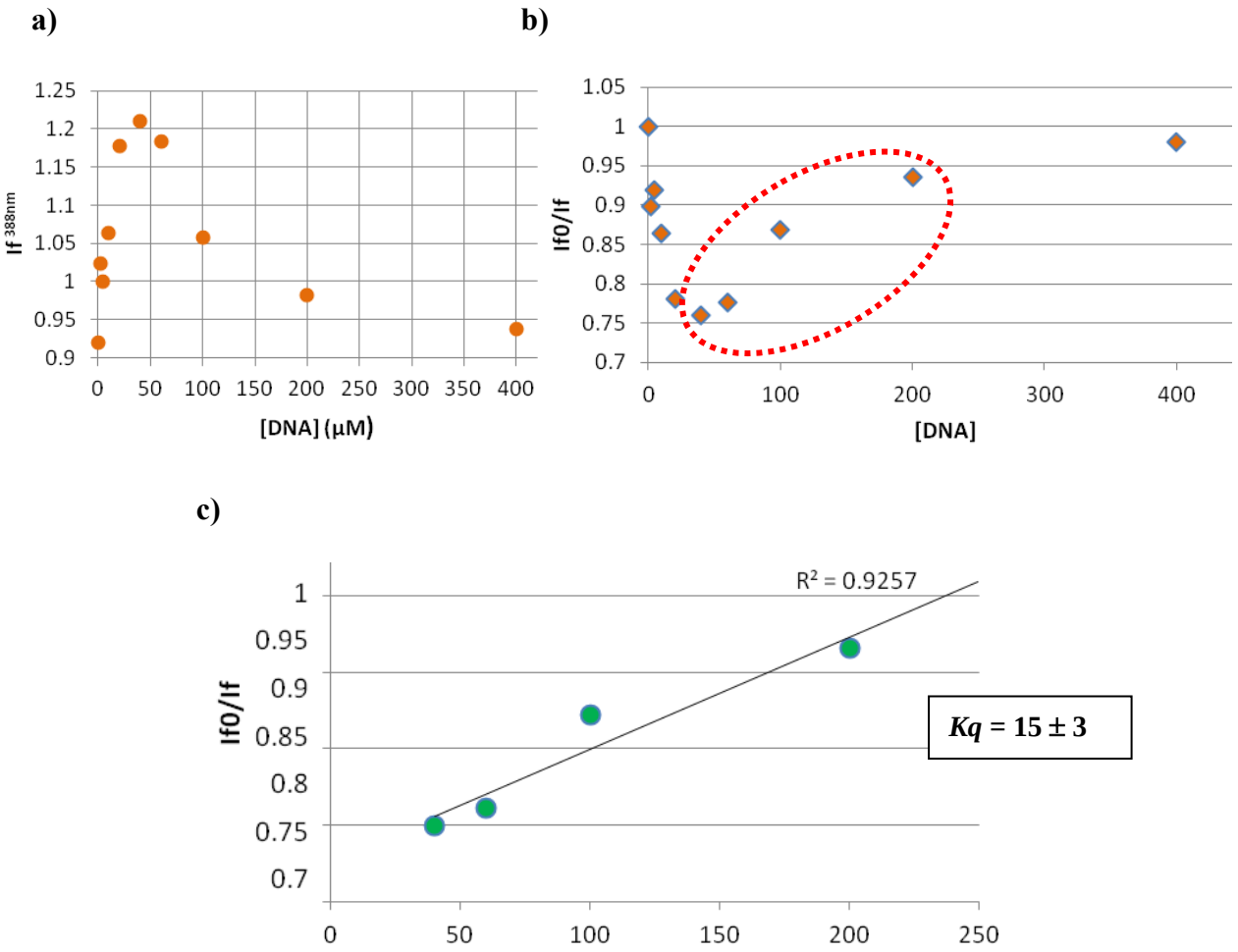
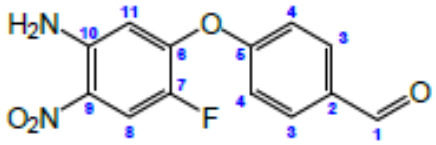
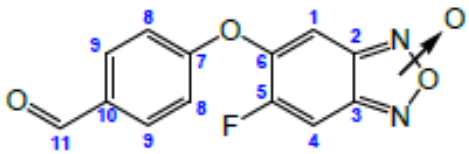


Figure S4. **a)** Variation of fluorescence of PDO **6** with increasing concentrations of DNA. **b)** Stern–Volmer quenching plot from the fluorescence data with increasing concentrations of DNA in PBS for PDO **6**. The red circle point to the region used to determine the *Kp* (**c)**). **c)** *Kp* determination for PDO **6** in the DNA concentrations range 40-200 μM.

Detailed experimental procedures and spectroscopic characterization of benzofuroxan (IV)



Synthesis of 4-(5-amino-2-fluoro-4-nitrophenoxy)benzaldehyde. Dried molecular sieves (3 Å) were loaded into the main chamber of a Soxhlet extractor equipment. Then the extractor was placed onto a flask containing a mixture of 4,5-difluoro-2-nitroaniline (4.5 mmol), *p*-hydroxybenzaldehyde (4.1 mmol), anhydrous potassium carbonate (4.1 mmol), 18-crown-6 (4.1 mmol) and dried toluene (70 mL). The mixture was heated at reflux during 2.5 h. After that, the toluene was evaporated *in vacuo* and the residue was dissolved in EtOAc (50 mL) and washed with an aqueous solution of sodium hydroxide (10 %) (3 × 20 mL). The organic phase was dried over anhydrous Na_2SO_4 and evaporated *in vacuo*. The formed solid corresponded to the desired product. Green solid (91 %). **^{1}H-NMR** ($CDCl_3$+D_2O, 400 MHz) δ (ppm): 10.01 (1H, s, H_1), 8.04 (1H, d, J= 10.8Hz, H_8), 7.94 (2H, d, J= 8.6 Hz, H_3), 7.21 (2H, d, J= 8.6 Hz, H_4), 6.41 (1H, d, J= 6.8 Hz, H_{11}). **^{13}C-NMR** ($CDCl_3$, 100 MHz) δ (ppm): 191.8, 160.7, 151.0, 146.5, 137.1, 132.6, 130.1, 127.5, 119.1, 114.5, 108.8. MS, *m/z* (%): 276 ($M^{\cdot+}$, 100), 260 ($M^{\cdot+}$ - 16, 2), 246 ($M^{\cdot+}$ - 30, 10), 230 ($M^{\cdot+}$ - [NO_2], 10).

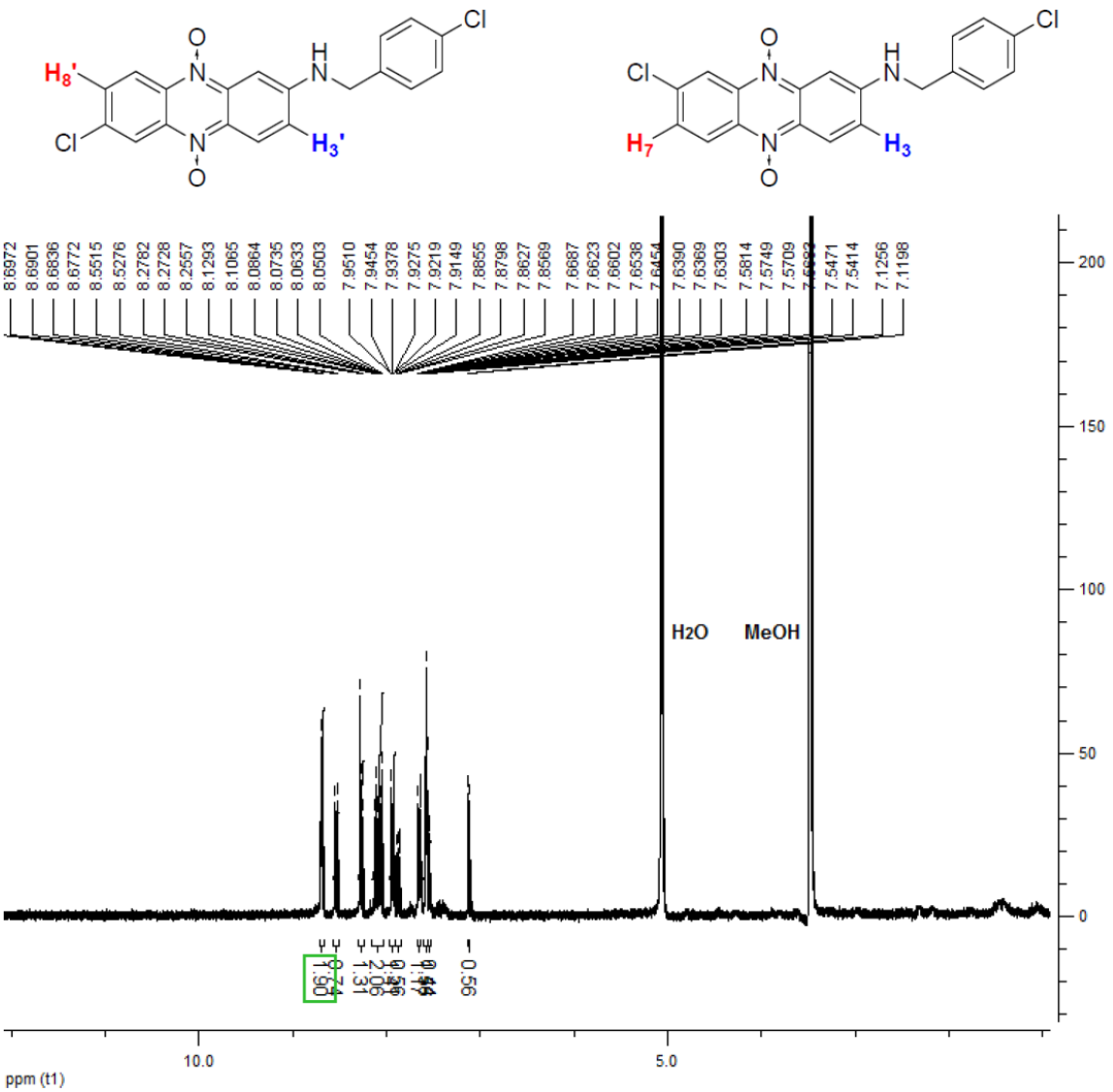


Synthesis of 5-fluoro-6-(4-formylphenoxy)benzo[1,2-c][1,2,5]oxadiazole (IV). A solution of 4-(5-Amino-2-fluoro-4-nitrophenoxy)benzaldehyde (4.3 mmol) in acetone (19 mL) and glacial acetic acid (12 mL) was cooled at 0 ^{o}C and a solution of sodium nitrite (4.3 mmol) in concentrated hydrochloric acid (1.2 mL) and water (3.3 mL) was added dropwise. Then the reaction mixture was stirred during 30 min at 0 ^{o}C. After that, a solution of sodium azide (4.3 mmol) and sodium acetate (4.3 mmol) in water (1.1 mL) was added dropwise and the reaction mixture was raised to room temperature and stirred for 2 h. The acetone was evaporated *in vacuo* and the residue was dissolved in EtOAc (50 mL) and washed with an aqueous solution of sodium hydroxide (10 %) (3 × 20 mL). The organic phase was dried over anhydrous Na_2SO_4 and evaporated *in vacuo*. The residue was dissolved in toluene (75 mL) and the solution was heated at reflux for 2 h. The toluene was evaporated *in vacuo*. The residue was purified by chromatography (SiO_2, petroleum ether:EtOAc, 8:2) yielding the desired product as a yellow solid (71 %). **^{1}H-NMR** ($CDCl_3$, 400 MHz) δ (ppm): 10.05 (1H, s, H_{11}), 8.01 (2H, d, J= 9.1 Hz, H_9), 7.30 (2H, d, J= 9.0 Hz, H_8), 7.45-7.20 (1H, bs, H_1), 7.25-7.05 (1H, bs, H_4). **^{13}C-NMR** ($CDCl_3$, 100 MHz) δ (ppm): 190.7, 159.4, 159.3, 149.0, 134.3, 132.7, 118.9, 118.4, 113.1. MS, *m/z* (%):274 ($M^{\cdot+}$, 100), 258 ($M^{\cdot+}$ - [O], 15), 228 ($M^{\cdot+}$ - [NO_2], 2), 213 ($M^{\cdot+}$ - [N_2O_2] - [H], 85).

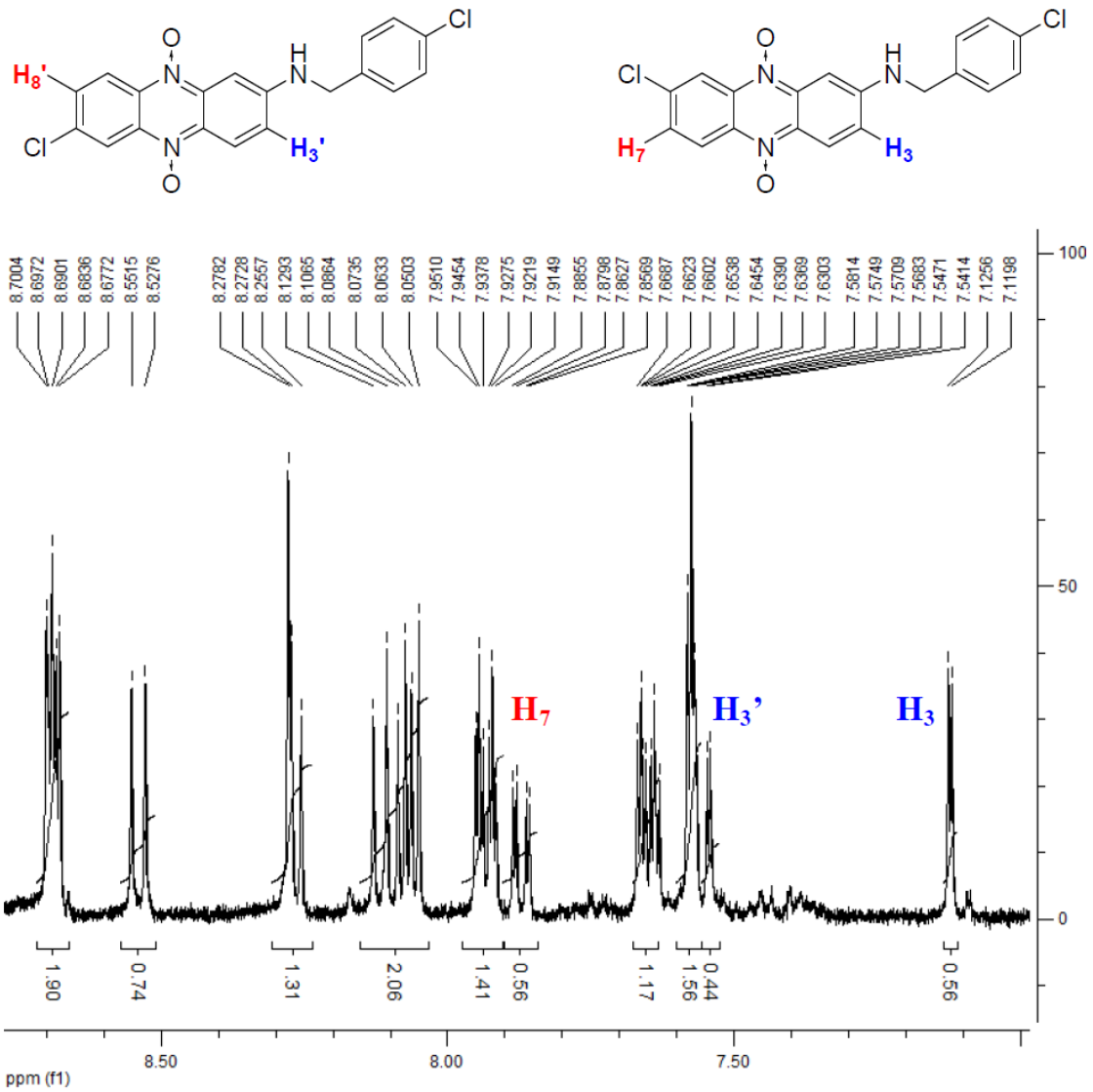
Selected NMR spectra

7(8)-Chloro-2-(4-chlorobenzylamino)phenazine 5,10-dioxide (11)

(7:8 isomers ratio, 44:56)



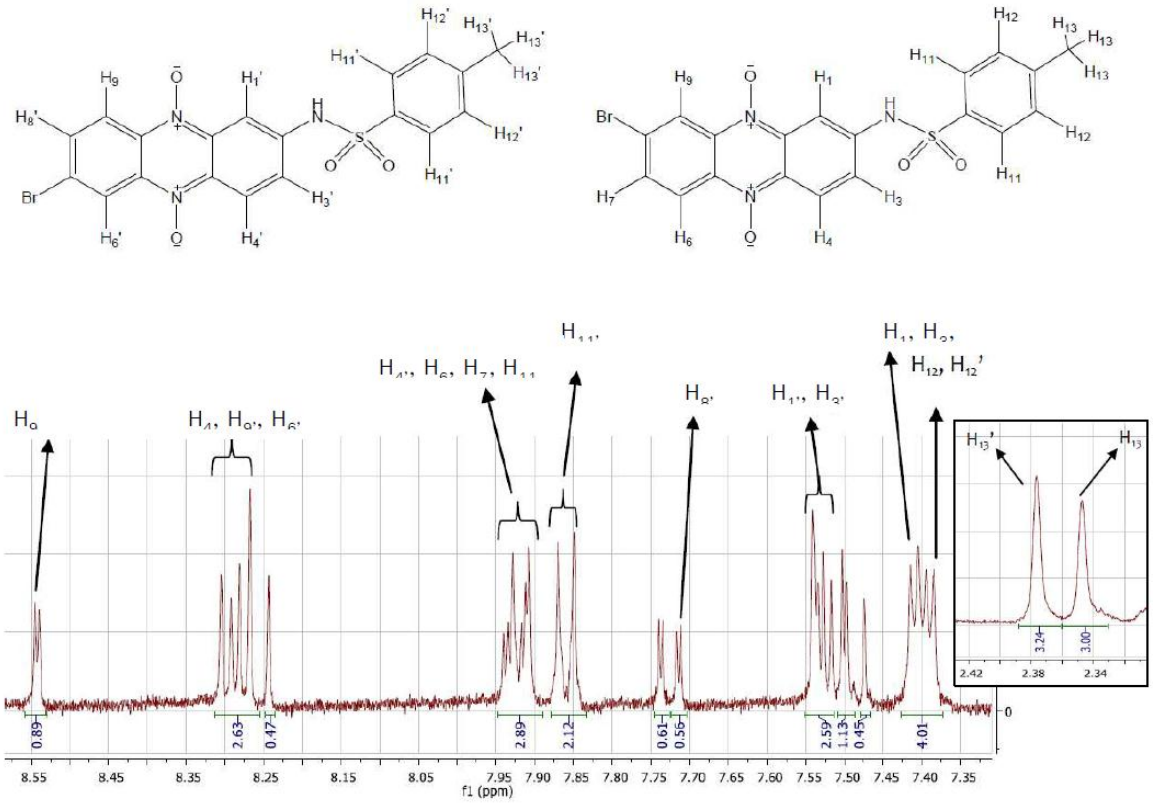
^{1}H NMR spectrum recorded on a Bruker DPX-400 spectrometer at 298 K and using CD_3OD:D_2O (9:1) as solvent.



Selected region, aromatics, of the proton NMR spectrum recorded on a Bruker DPX-400 spectrometer at 298 K and using CD_3OD:D_2O (9:1) as solvent.

7(8)-Bromo-2-(4-methylphenylsulfonylamino)phenazine 5,10-dioxide (12)

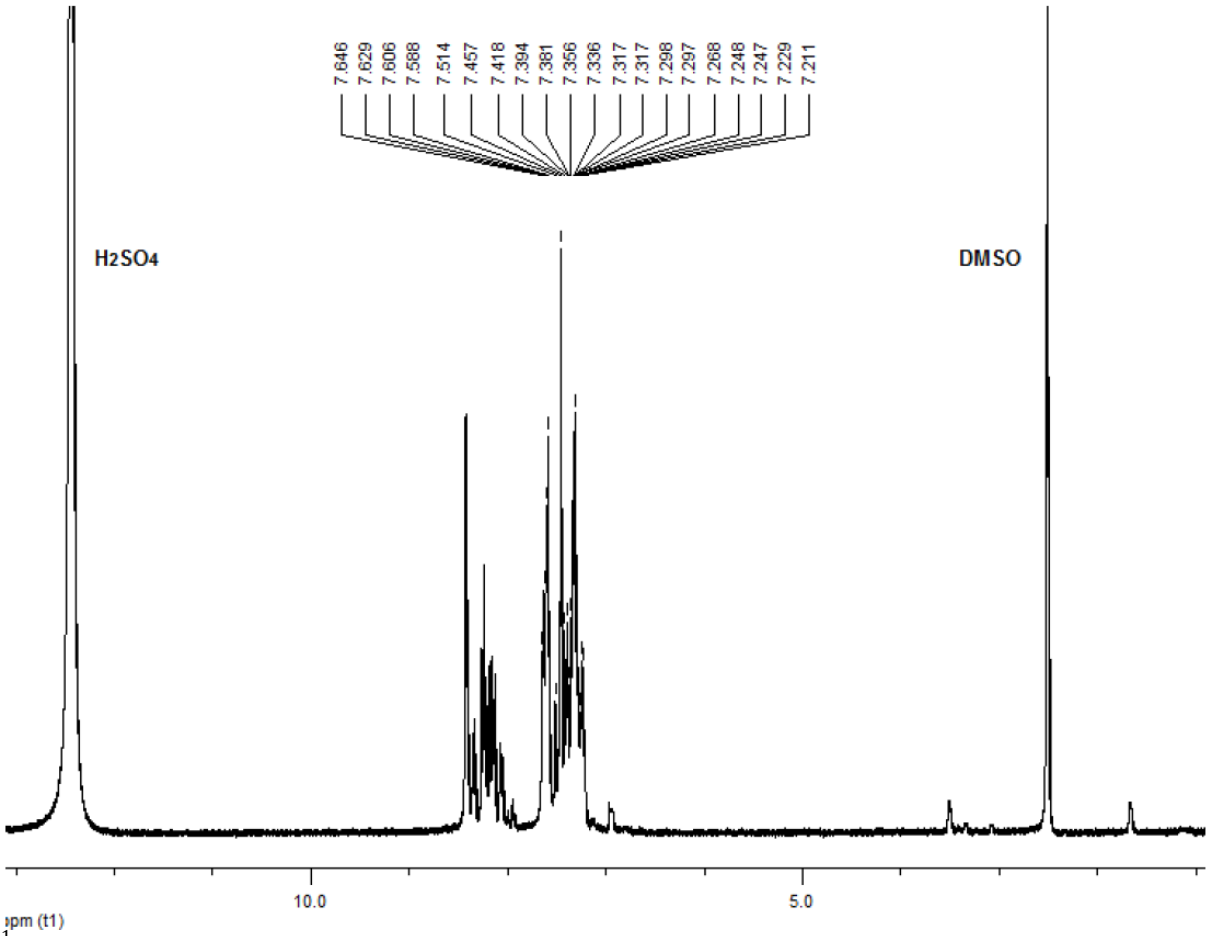
(7:8 isomers ratio, 52:48)



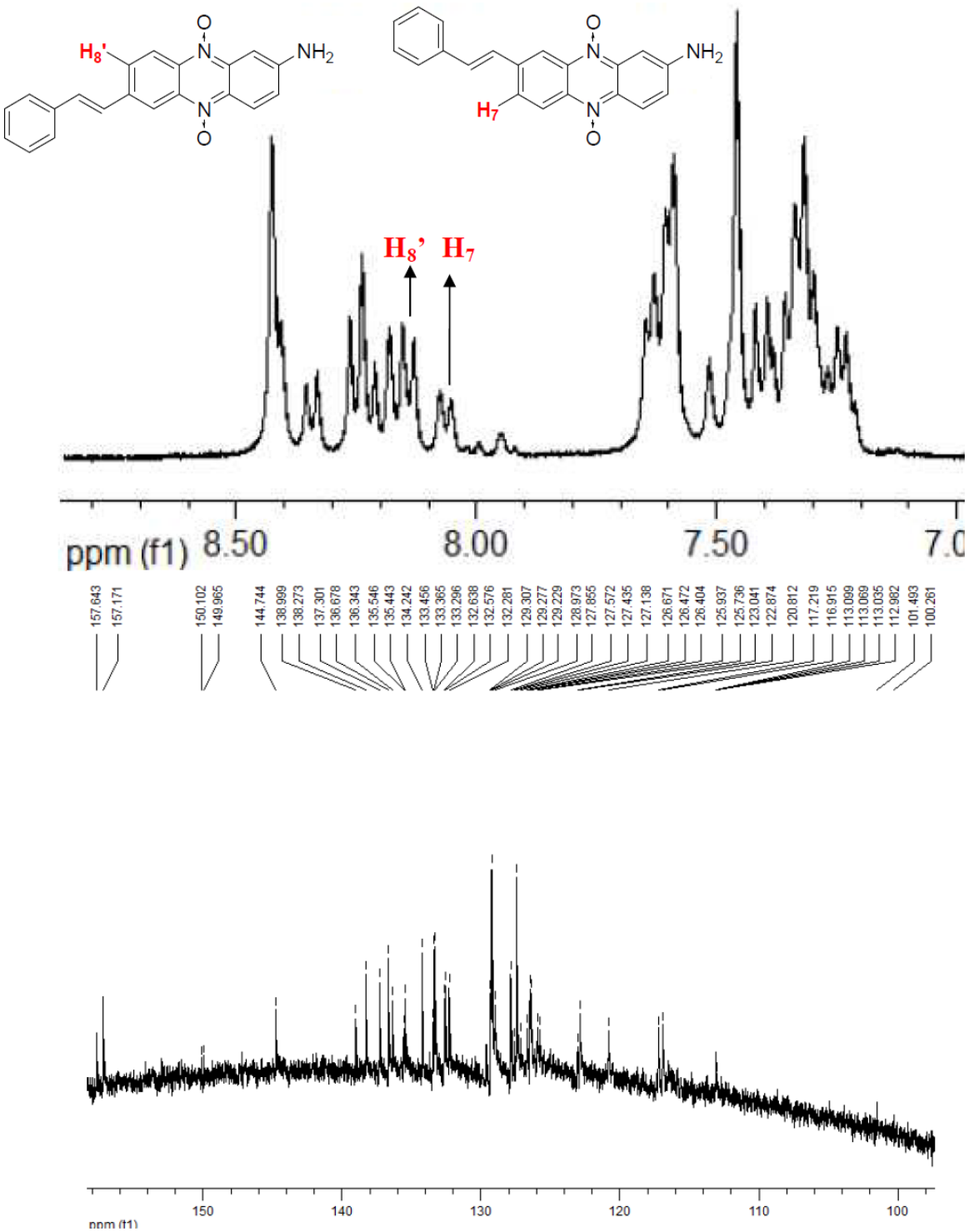
Selected region, aromatics, of the proton NMR spectrum recorded on a Bruker DPX-400 spectrometer at 298 K and using DMSO-d_6:D_2O (1:1) as solvent. Inset: region of the methyl-protons.

2-Amino-7(8)-(*E*-2-phenylethenyl)phenazine 5,10-dioxide (13)

(7:8 isomers ratio, 65:35)



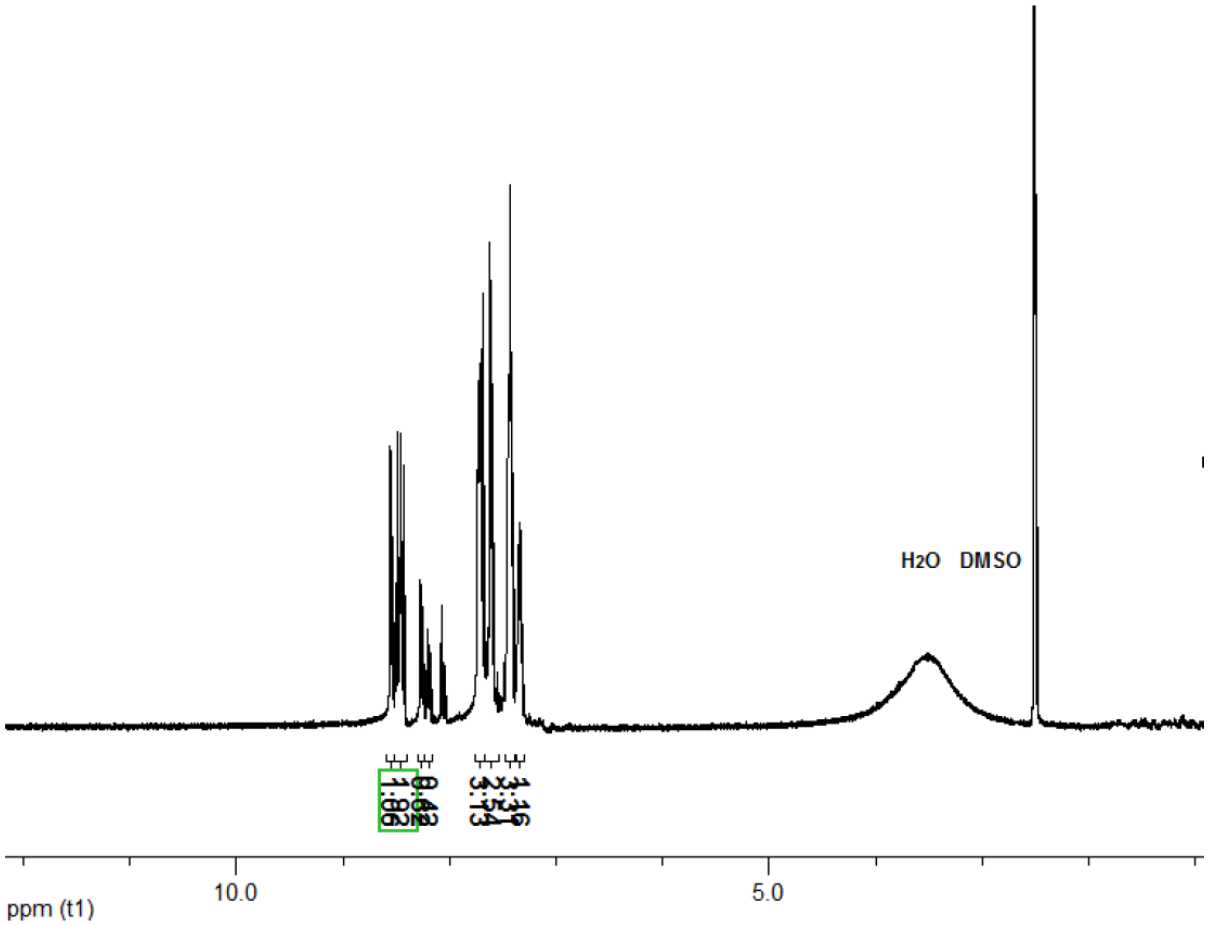
^{1}H NMR spectrum recorded on a Bruker DPX-400 spectrometer at 298 K and using DMSO-d_6:D_2SO_4 (9.5:0.5) as solvent.



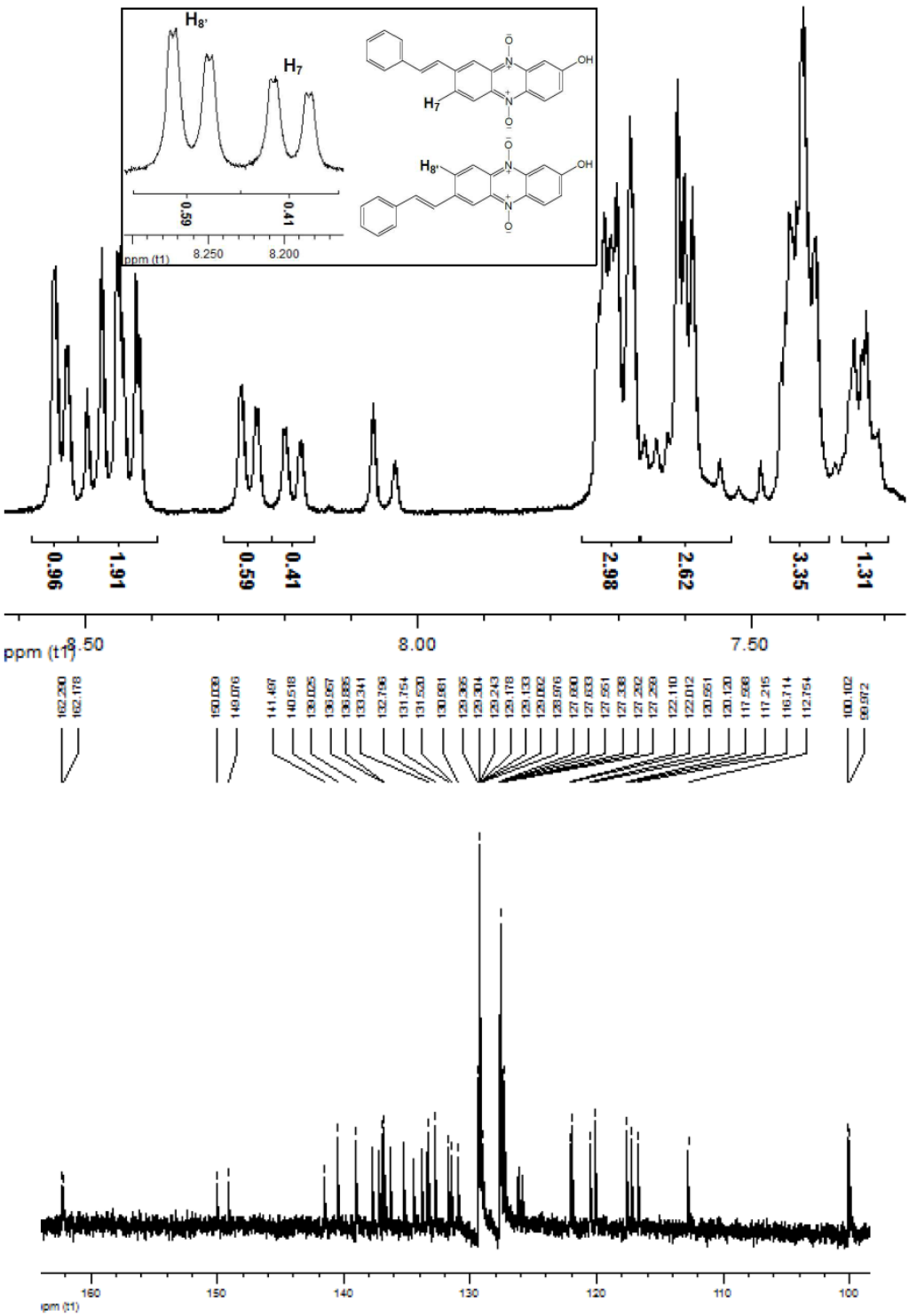
Selected regions, aromatics, of the proton and carbon NMR spectra recorded on a Bruker DPX-400 spectrometer at 298 K and using DMSO-d_6:D_2SO_4 (9.5:0.5) as solvent.

2-Hydroxy-7(8)-(*E*-2-phenylethenyl)phenazine 5,10-dioxide (14)

(7:8 isomers ratio, 59:41)



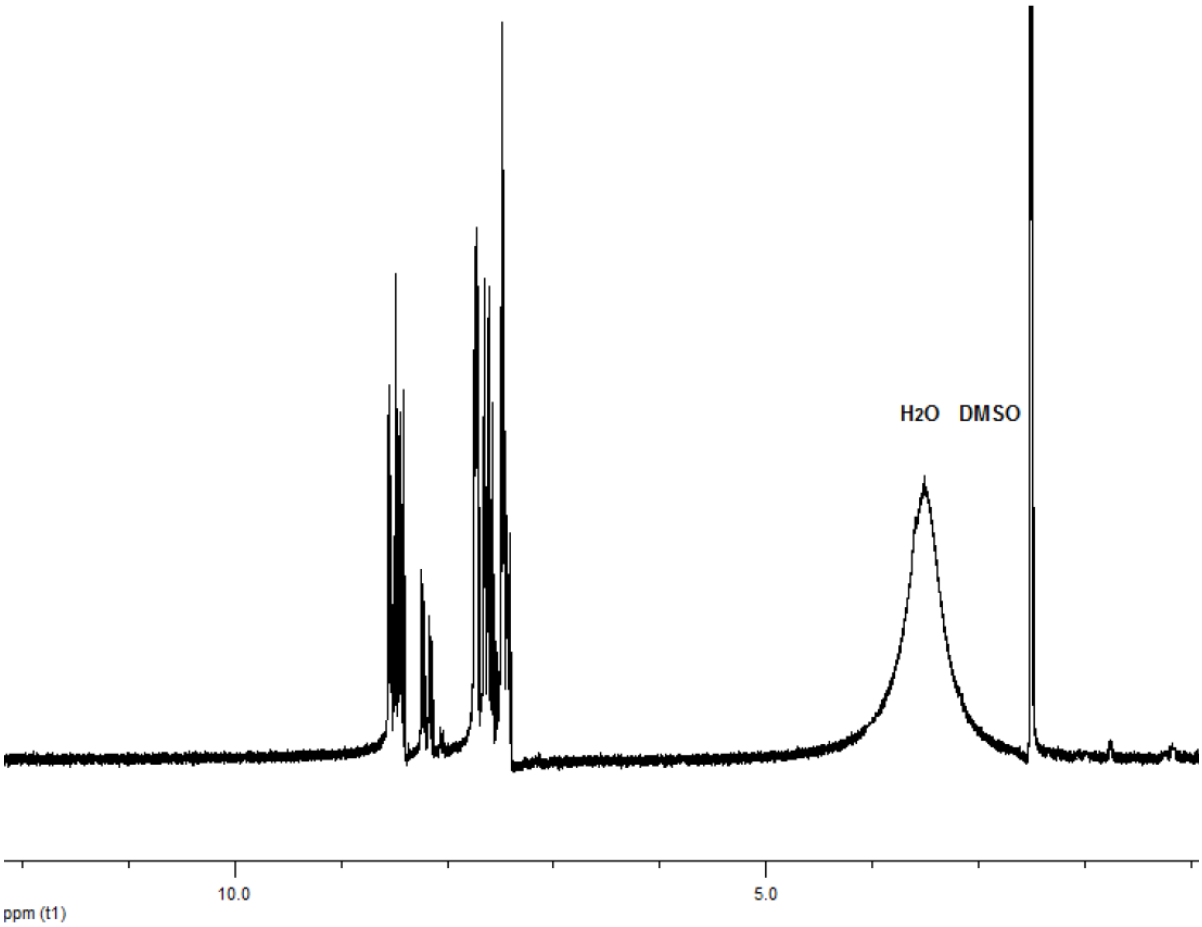
^{1}H NMR spectrum recorded on a Bruker DPX-400 spectrometer at 298 K and using DMSO-d_6:D_2O (9.5:0.5) as solvent.



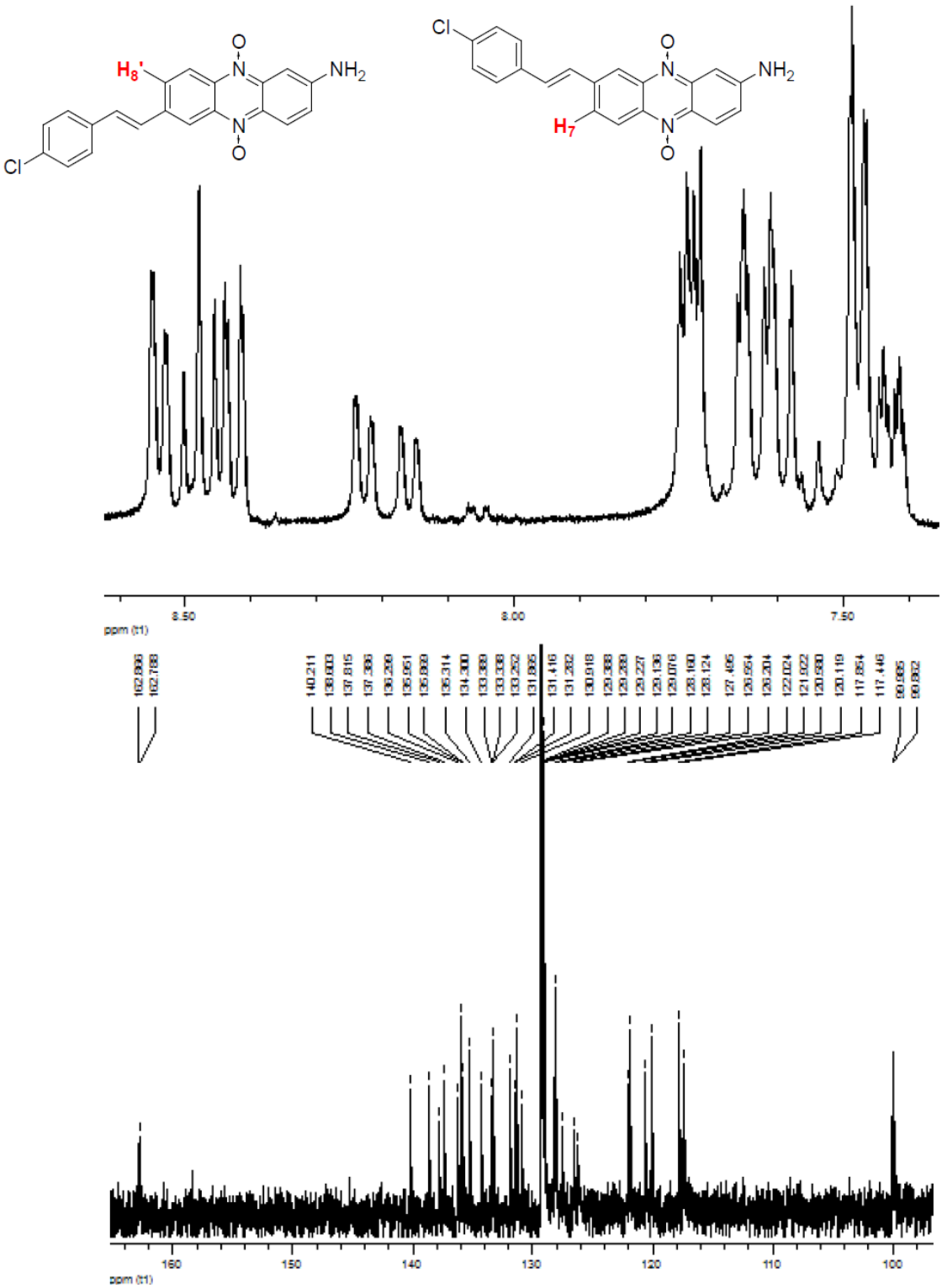
Selected regions, aromatics, of the proton and carbon NMR spectra recorded on a Bruker DPX-400 spectrometer at 298 K and using DMSO-d_6:D_2O (9.5:0.5) as solvent.. Inset: protons that allowed to determine the ratio of isomers.

2-Amino-7(8)-[*E*-2-(4-chlorophenyl)ethenyl)phenazine 5,10-dioxide (15)

(7:8 isomers ratio, 56:44)



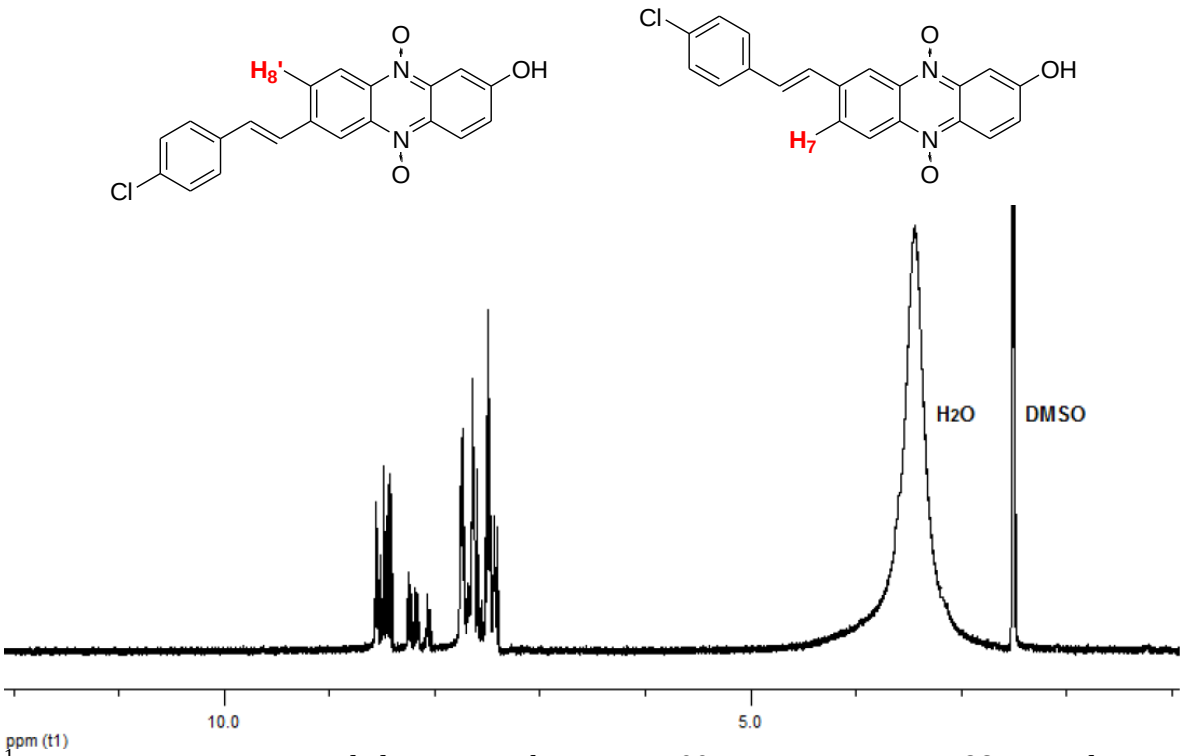
^{1}H NMR spectrum recorded on a Bruker DPX-400 spectrometer at 298 K and using DMSO-d_6:D_2O (9.5:0.5) as solvent.



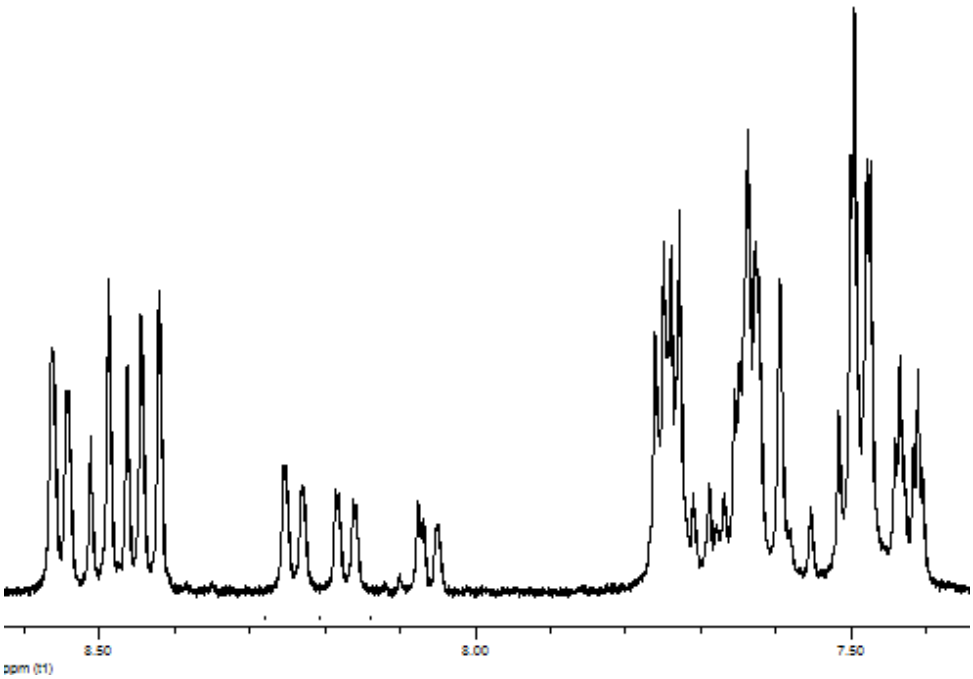
Selected regions, aromatics, of the proton and carbon NMR spectra recorded on a Bruker DPX-400 spectrometer at 298 K and using DMSO-d_6:D_2O (9.5:0.5) as solvent.

7(8)-[*E*-2-(4-Chlorophenyl)ethenyl)-2-hydroxyphenazine 5,10-dioxide (16)

(7:8 isomers ratio, 55:45)



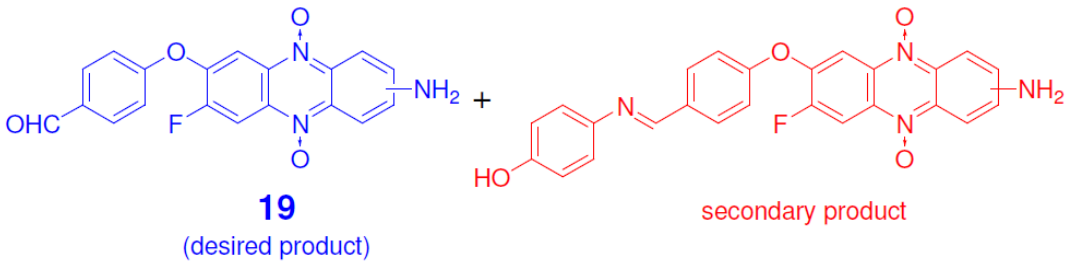
^{1}H NMR spectrum recorded on a Bruker DPX-400 spectrometer at 298 K and using DMSO-d_6:D_2O (9.5:0.5) as solvent.



Selected regions, aromatics, of the proton NMR spectrum recorded on a Bruker DPX-400 spectrometer at 298 K and using DMSO-d_6:D_2O (9.5:0.5) as solvent.

2-Amino-7(8)-fluoro-8(7)-(4-formylphenyloxy)phenazine 5,10-dioxide (19)

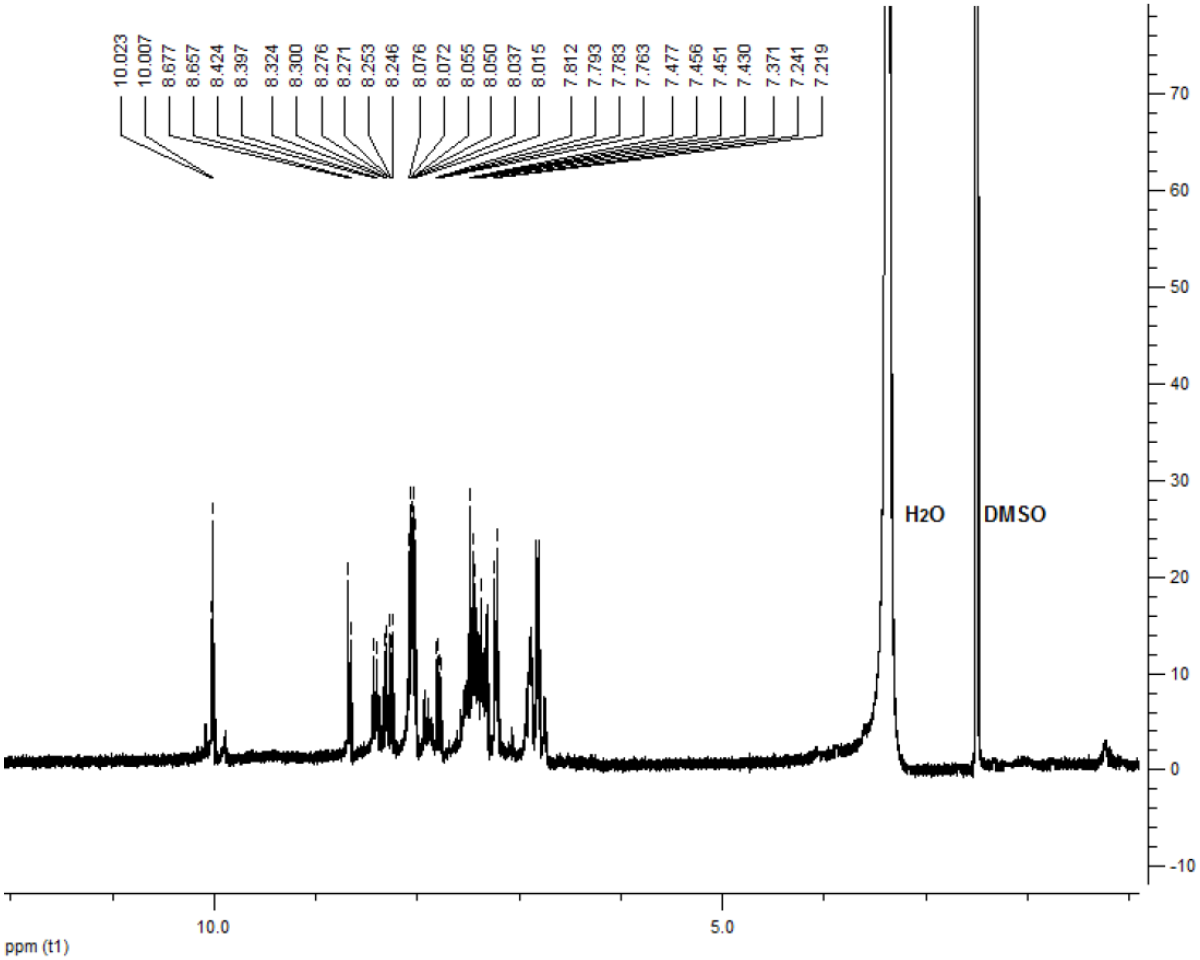
(as mixture of aldehyde and the corresponding imine)



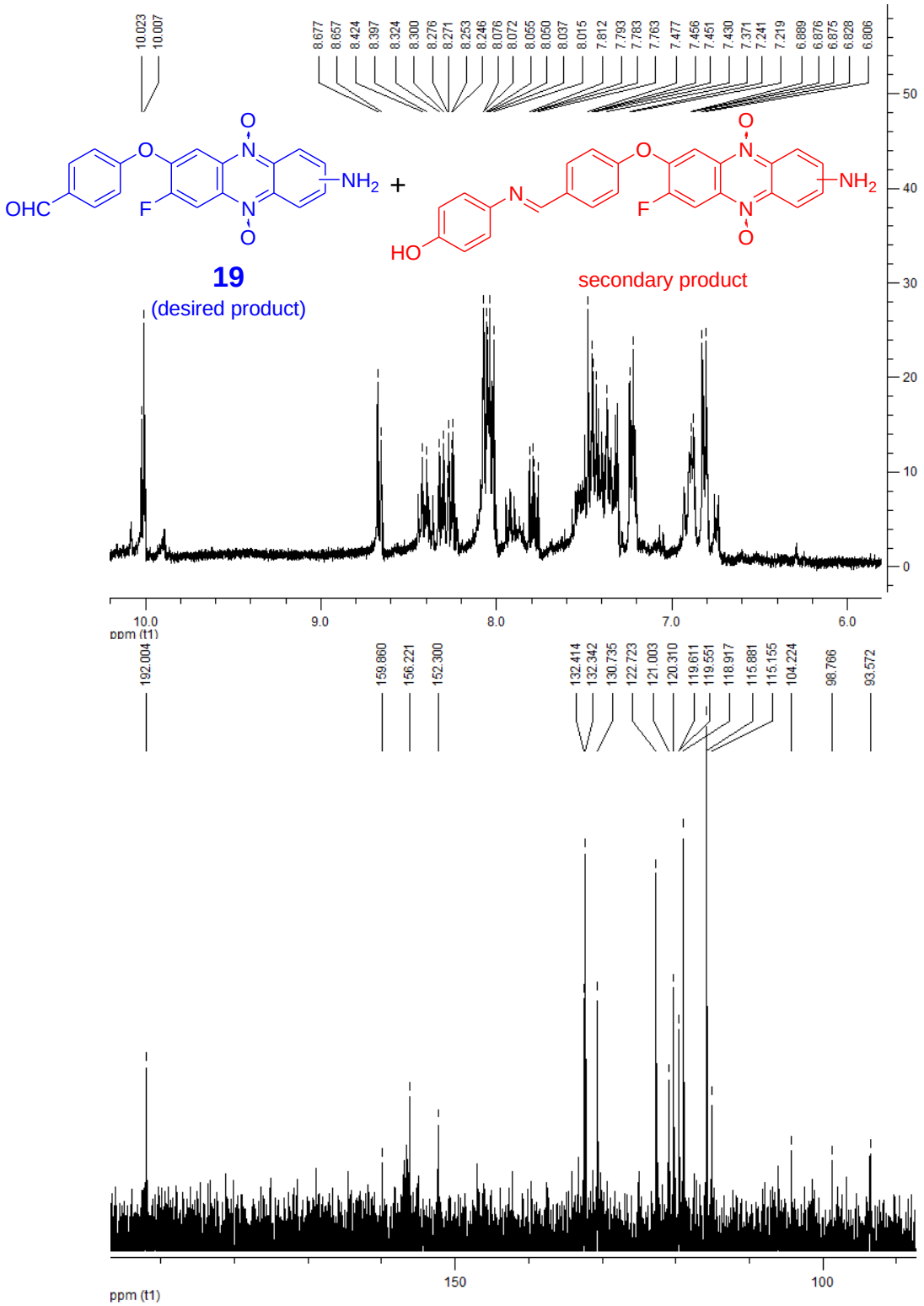
19

(desired product)

secondary product



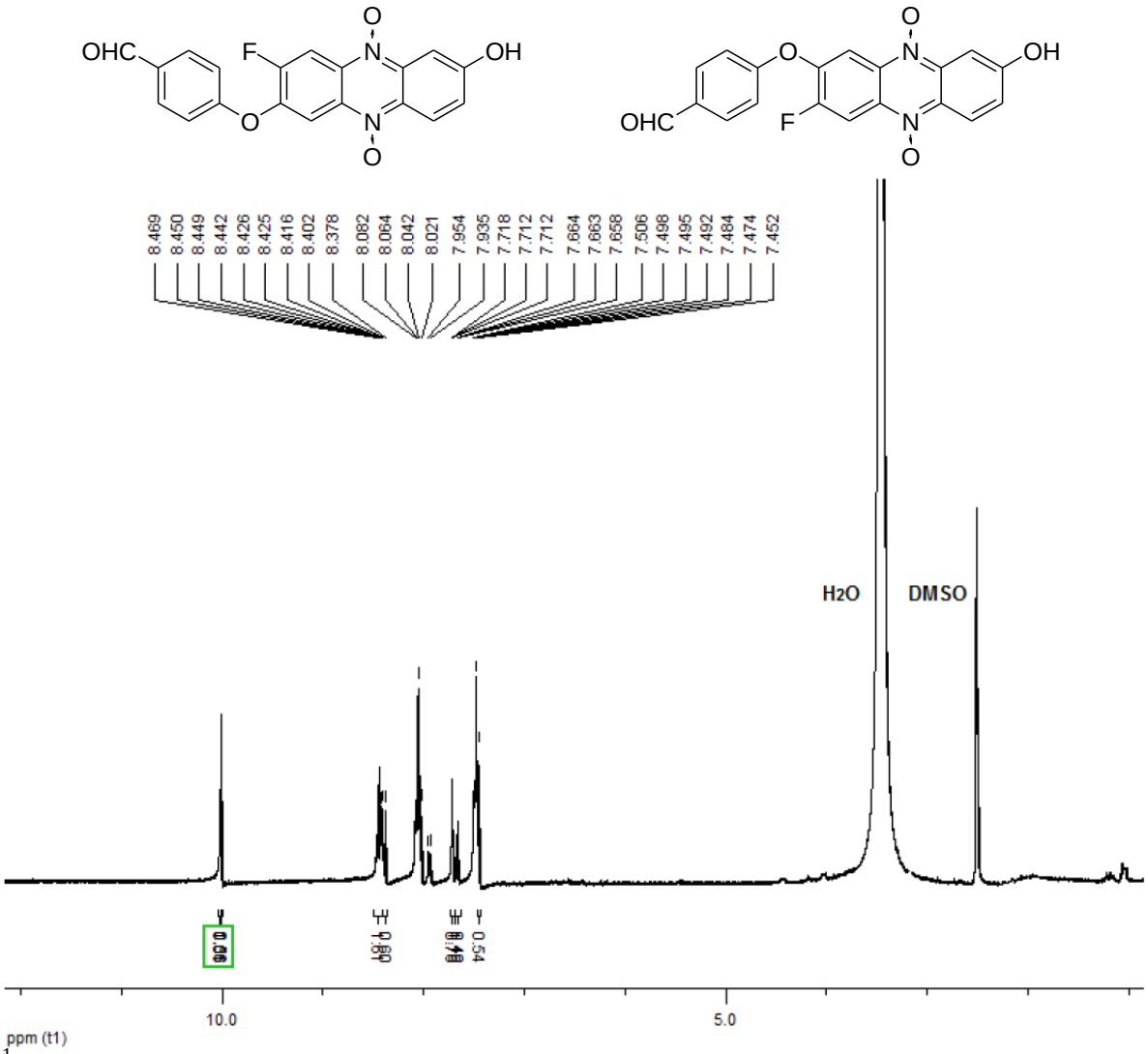
^{1}H NMR spectrum recorded on a Bruker DPX-400 spectrometer at 298 K and using DMSO-d_6:D_2O (9.5:0.5) as solvent.



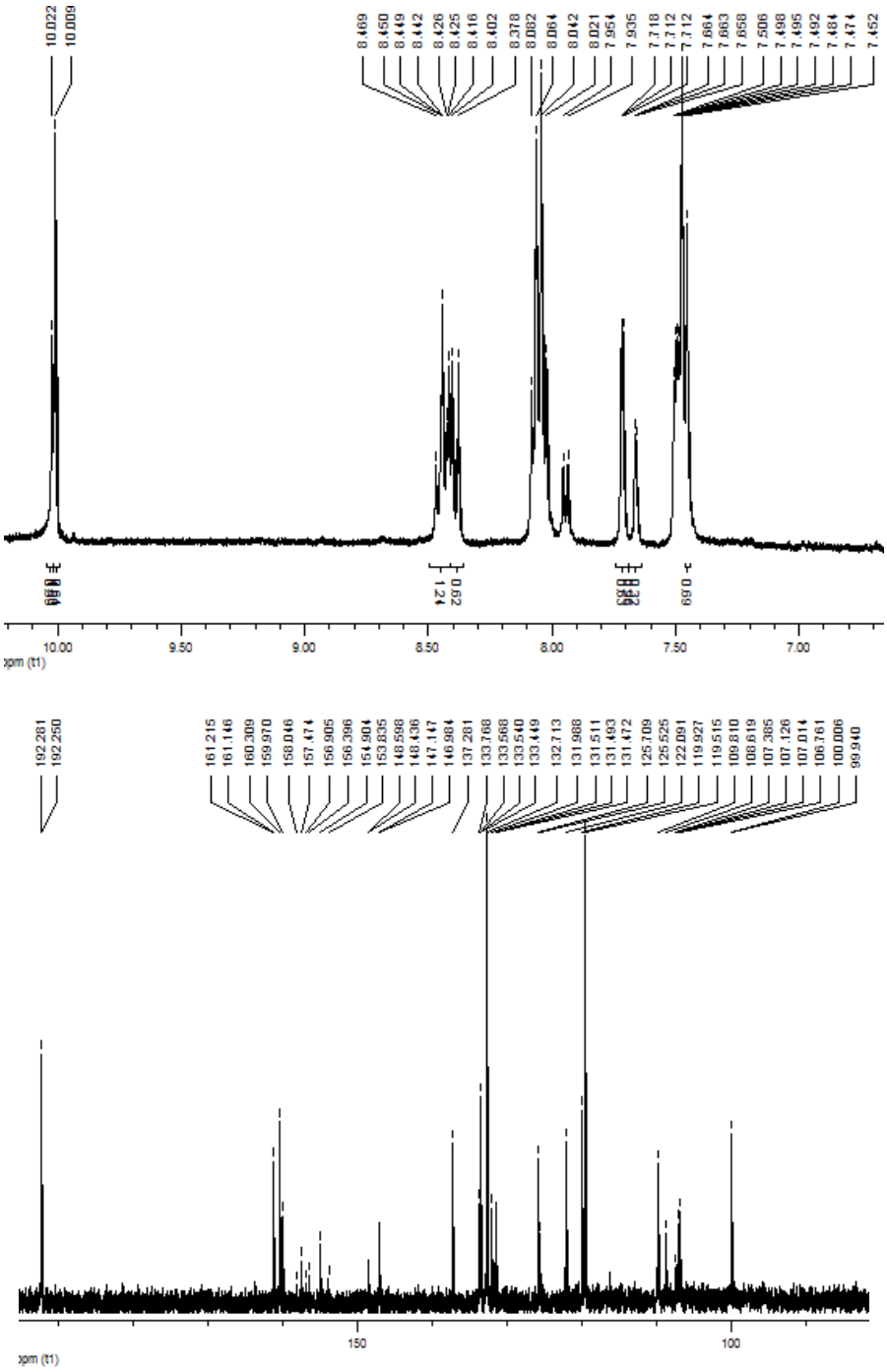
Selected regions, aromatics, of the proton and carbon NMR spectra recorded on a Bruker DPX-400 spectrometer at 298 K and using DMSO-d_6:D_2O (9.5:0.5) as solvent.

7(8)-Fluoro-8(7)-(4-formylphenyloxy)-2-hydroxyphenazine 5,10-dioxide (20)

(7:8 isomers ratio, 65:35)



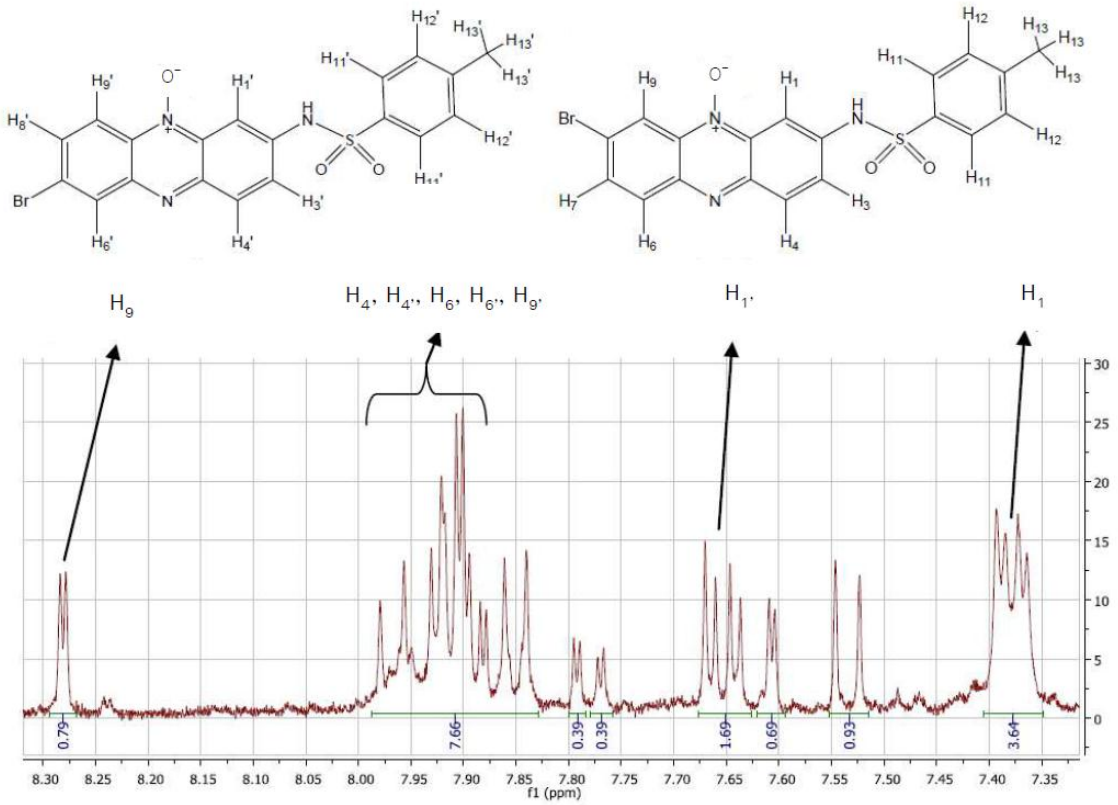
^{1}H NMR spectrum recorded on a Bruker DPX-400 spectrometer at 298 K and using DMSO-d_6:D_2O (9.5:0.5) as solvent.



Selected regions, aromatics, of the proton and carbon NMR spectra recorded on a Bruker DPX-400 spectrometer at 298 K and using DMSO-d_6:D_2O (9.5:0.5) as solvent.

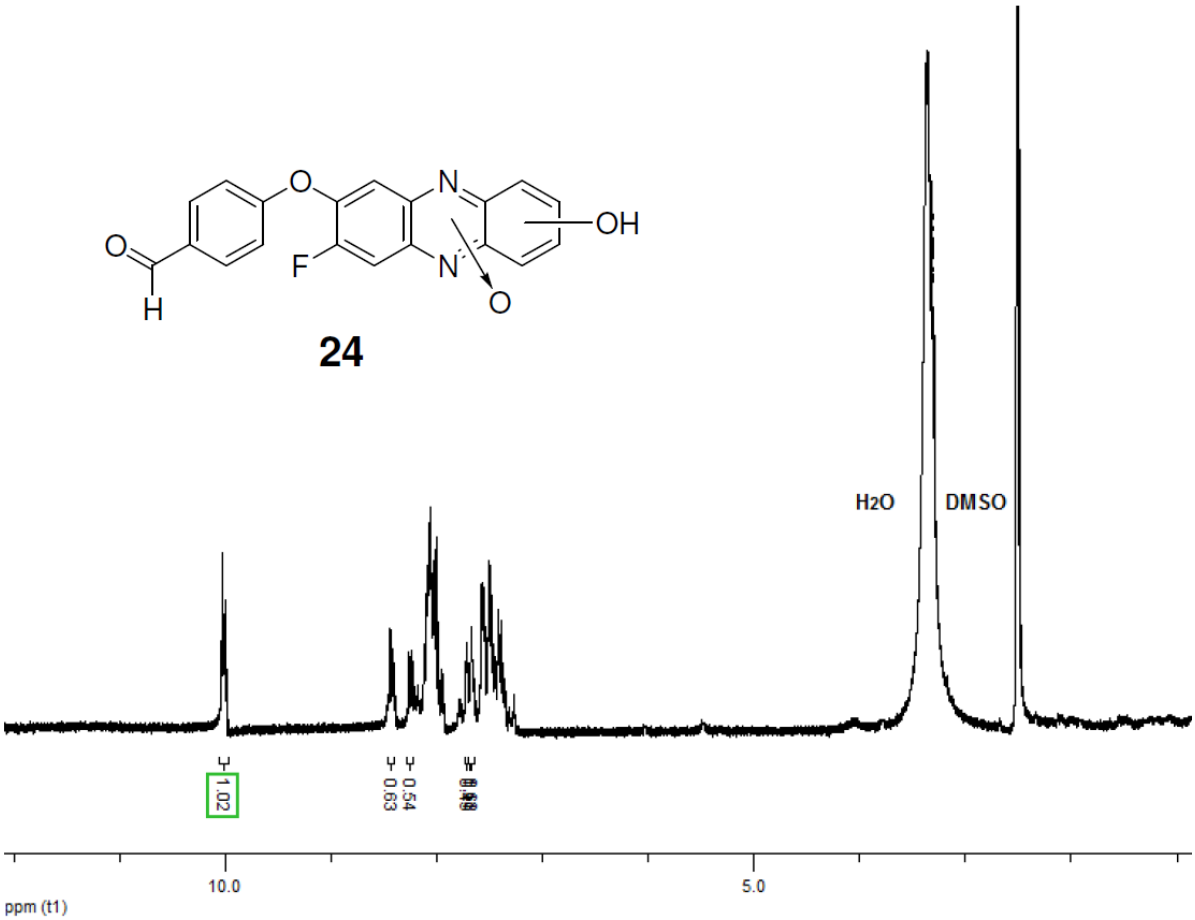
7(8)-Bromo-2-(4-methylphenylsulfonylamino)phenazine N^{10}-oxide (23)

(7:8 isomers ratio, 56:44)

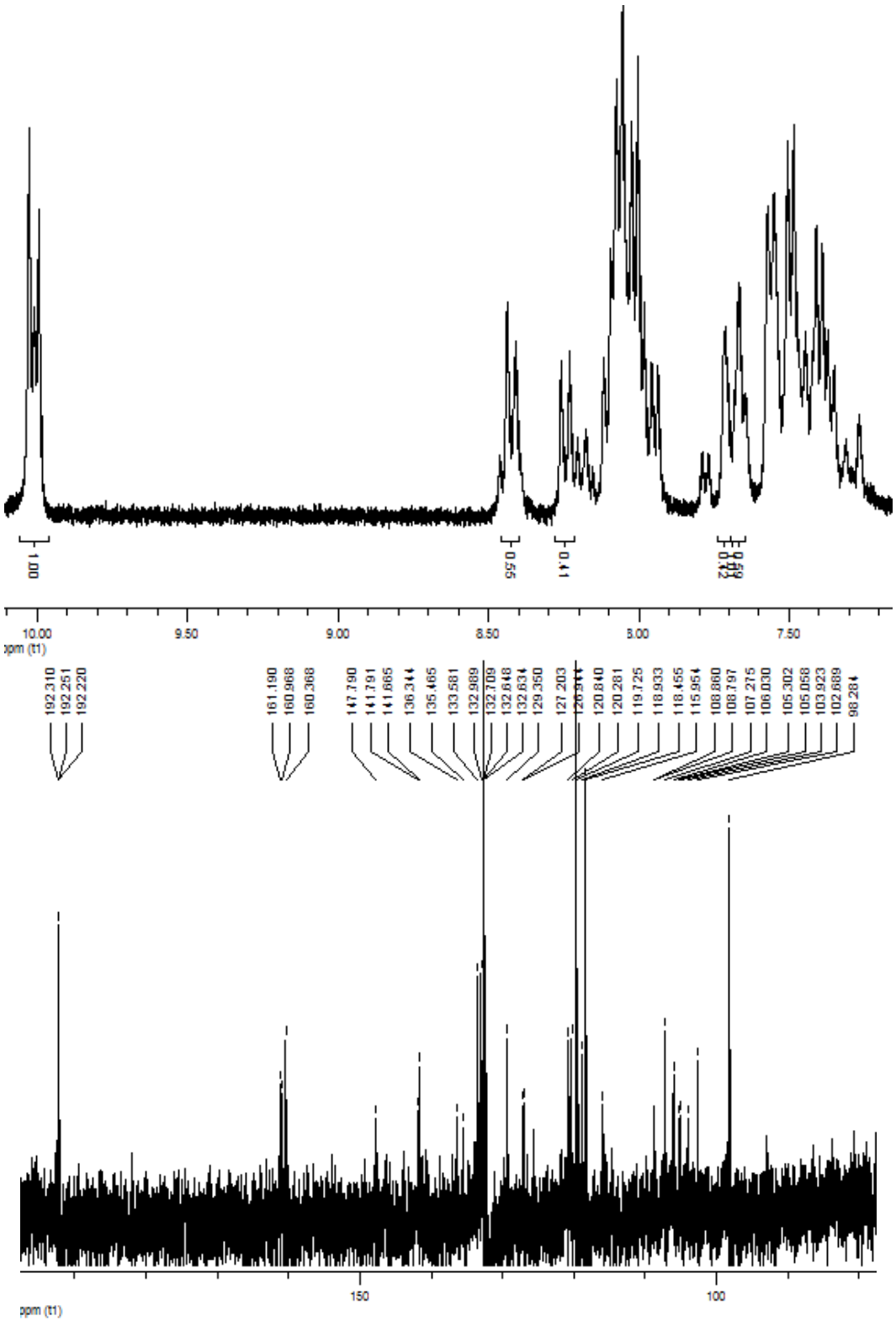


Selected regions, aromatics, of the proton NMR spectrum recorded on a Bruker DPX-400 spectrometer at 298 K and using DMSO-d_6:D_2O (1:1) as solvent.

7(8)-Fluoro-8(7)-(4-formylphenyloxy)-2-hydroxyphenazine *N*-oxide (24)



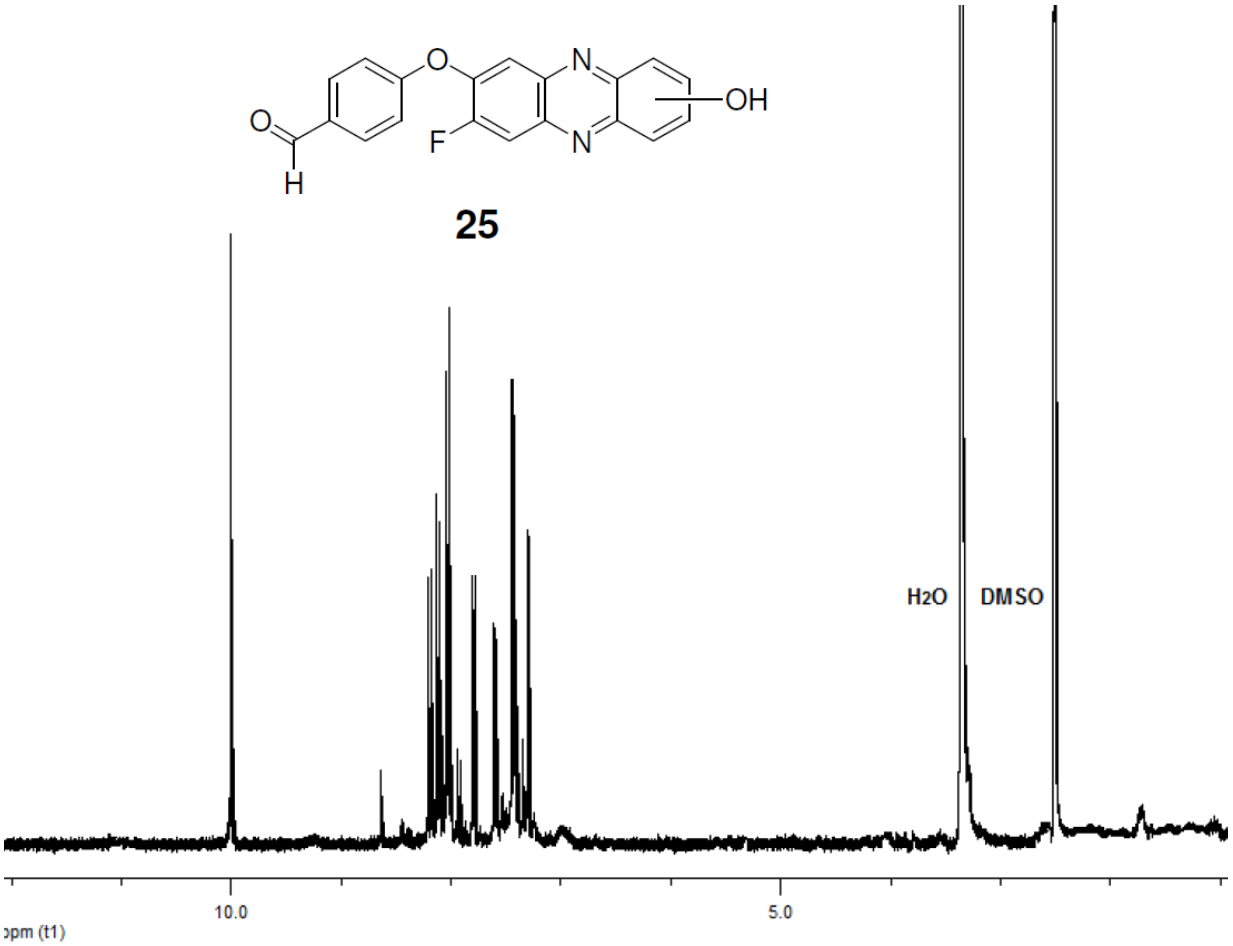
^{1}H NMR spectrum recorded on a Bruker DPX-400 spectrometer at 298 K and using DMSO-d_6:D_2O (9.5:0.5) as solvent.



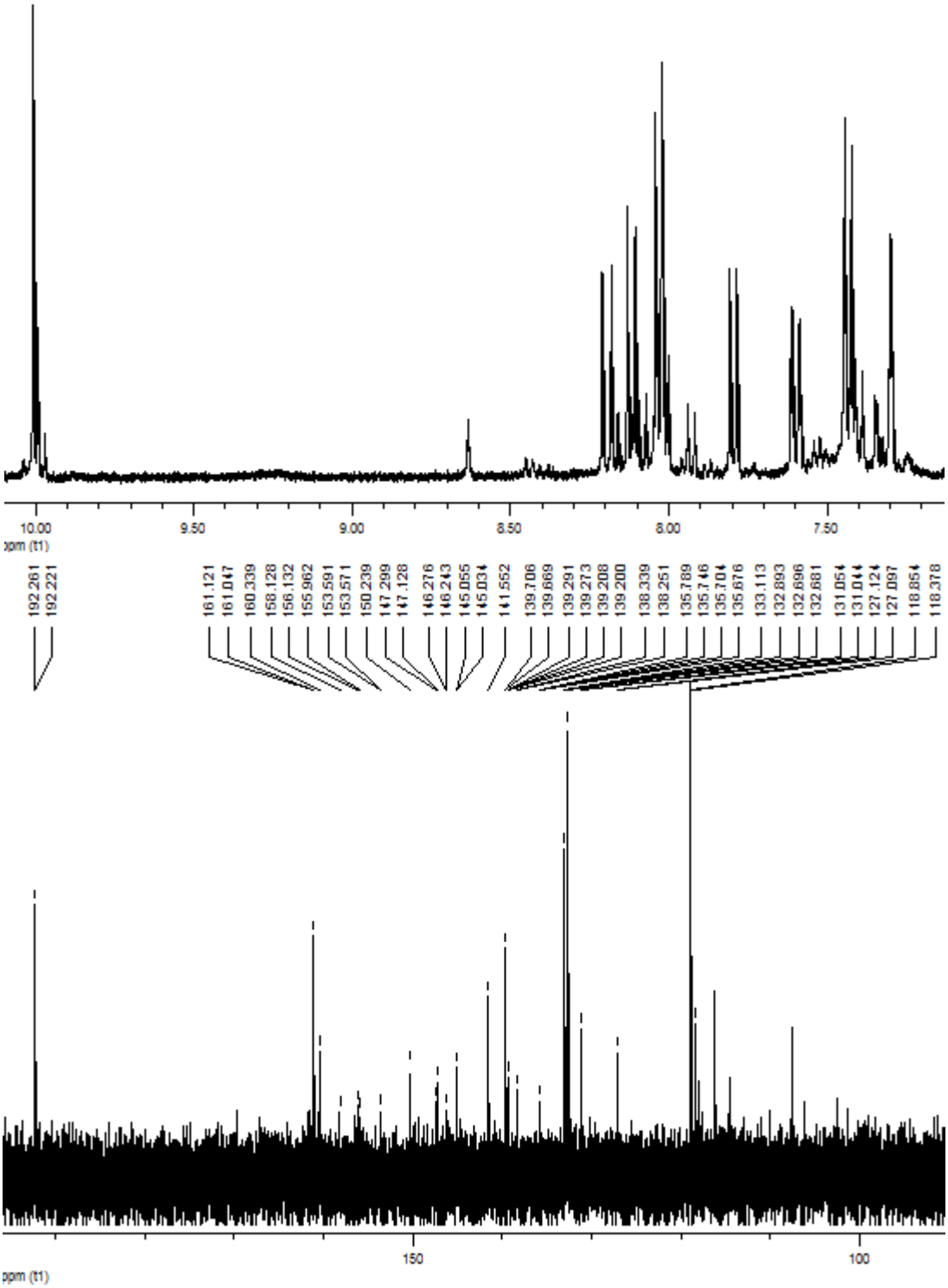
Selected regions, aromatics, of the proton and carbon NMR spectra recorded on a Bruker DPX-400 spectrometer at 298 K and using DMSO-d_6:D_2O (9.5:0.5) as solvent.

7(8)-fluoro-8(7)-(4-formylphenyloxy)-2-hydroxyphenazine (25)

(7:8 isomers ratio, 50:50)



^{1}H NMR spectrum recorded on a Bruker DPX-400 spectrometer at 298 K and using DMSO-d_6:D_2O (9.5:0.5) as solvent.



Selected regions, aromatics, of the proton and carbon NMR spectra recorded on a Bruker DPX-400 spectrometer at 298 K and using DMSO-d_6:D_2O (9.5:0.5) as solvent.