

SUPPORTING INFORMATION

Synthesis of a 3,4-Disubstituted 1,8-Naphthalimide Based DNA Intercalator for Direct Imaging of *Legionella pneumophila*

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¹H NMR of S-12

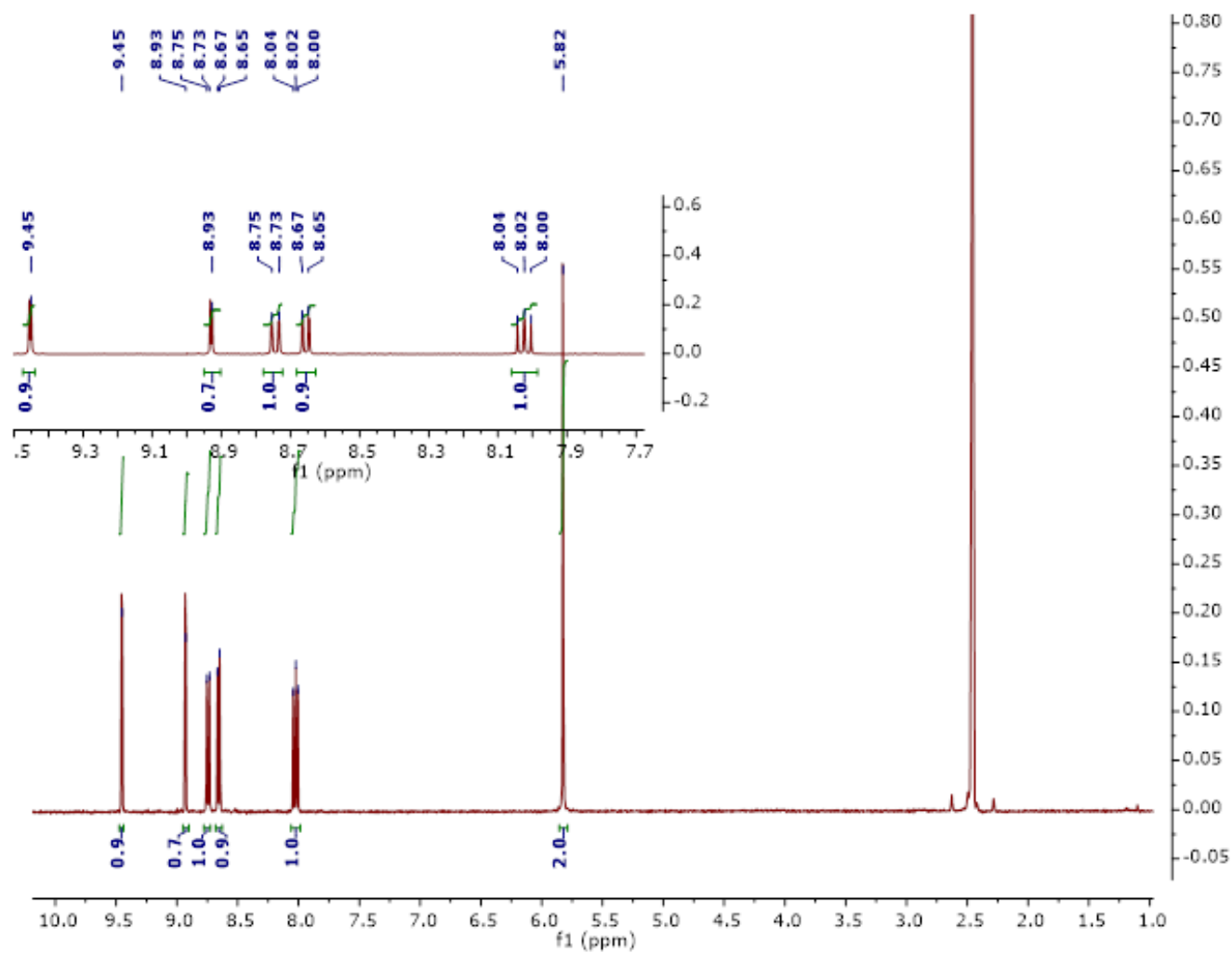
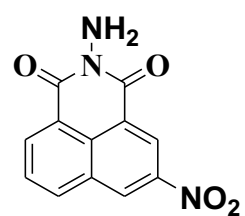


Figure S1. ¹H NMR spectra of S-12 in DMSO-*d*₆

^{13}C NMR of S-12

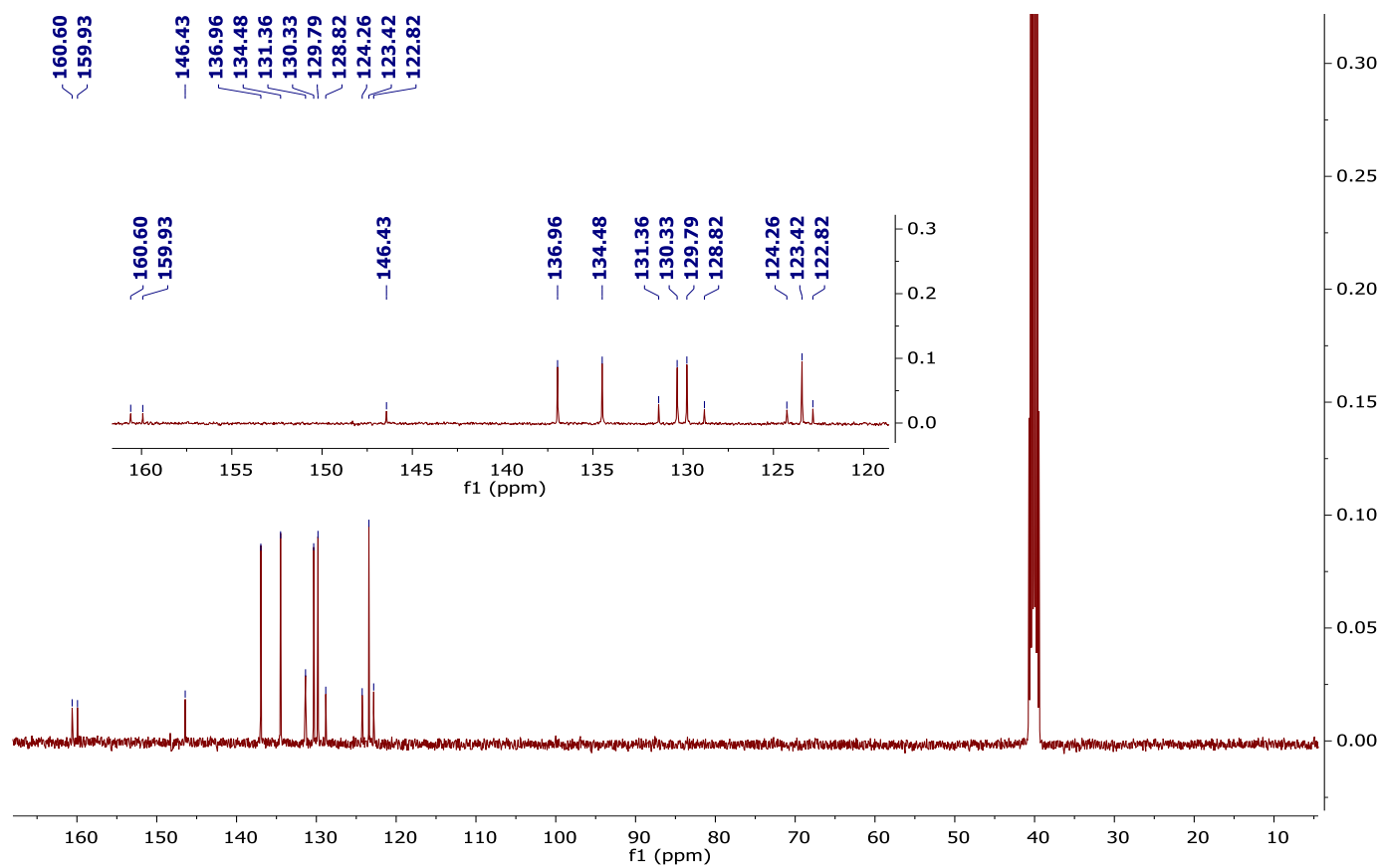
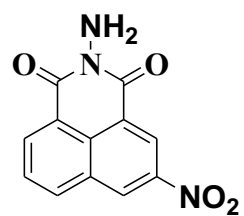


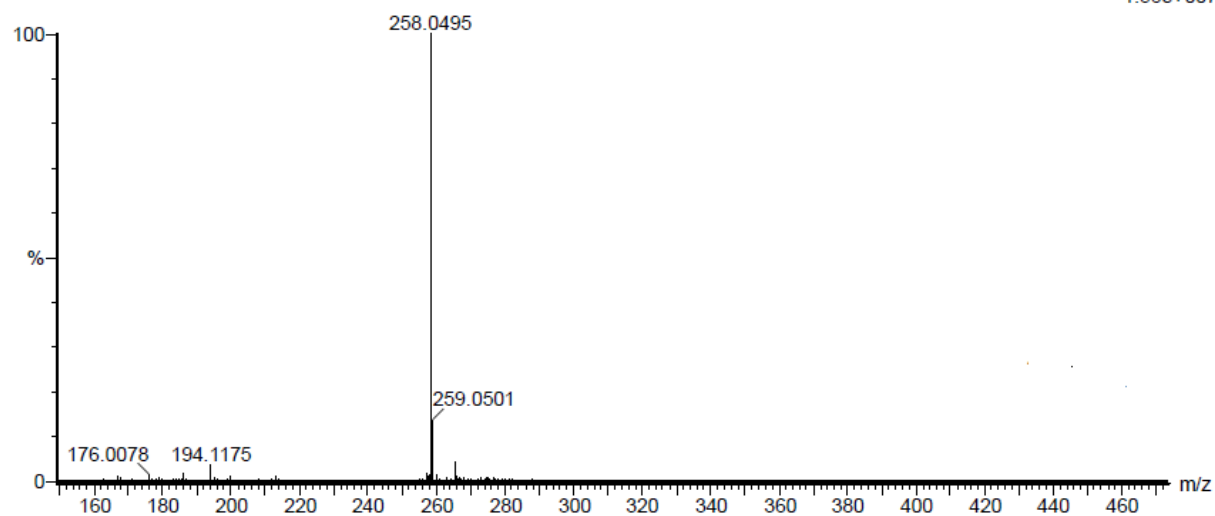
Figure S2. ^{13}C NMR spectra of S-12 in $\text{DMSO}-d_6$

Sample Name : S-12
 Test Name : HRMS-1
 280119-S-12- 11 (0.122) AM (Top,4, Ar,10000.0,0.00,0.00); Cm (6:17)

I.I.T.ROPAR

XEVO G2-XS QTOF

1: TOF MS ES+
 1.56e+007



Minimum: -1.5
 Maximum: 5.0 10.0 50.0

Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	Norm	Conf(%)	Formula
258.0495	258.0515	-2.0	-7.8	10.5	859.2	n/a	n/a	C12 H8 N3 O4

Figure S3. Mass spectra of **S-12**.

^1H NMR of S-13

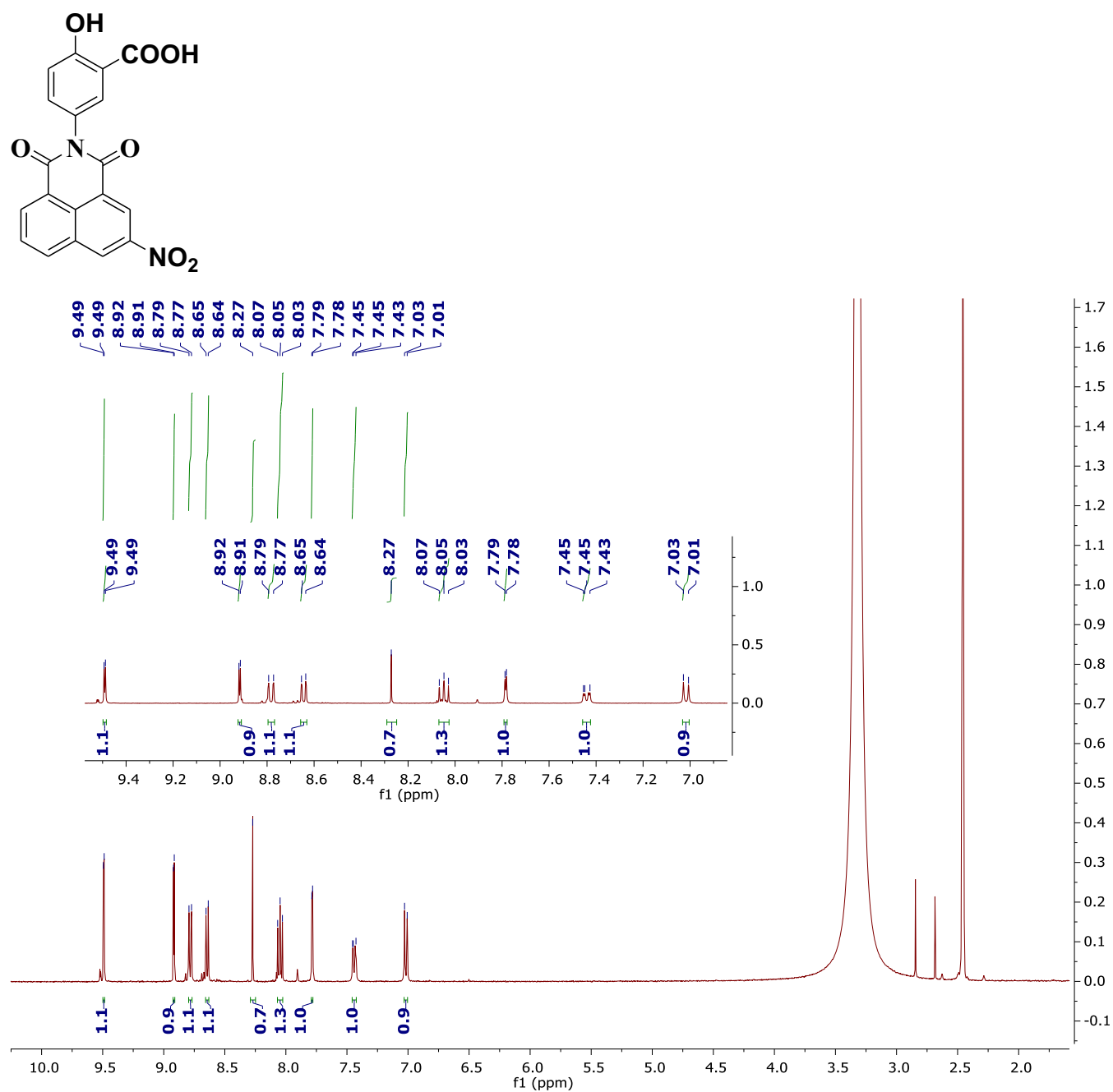


Figure S4. ^1H NMR spectra of S-13 in $\text{DMSO}-d_6$.

^{13}C NMR of S-13

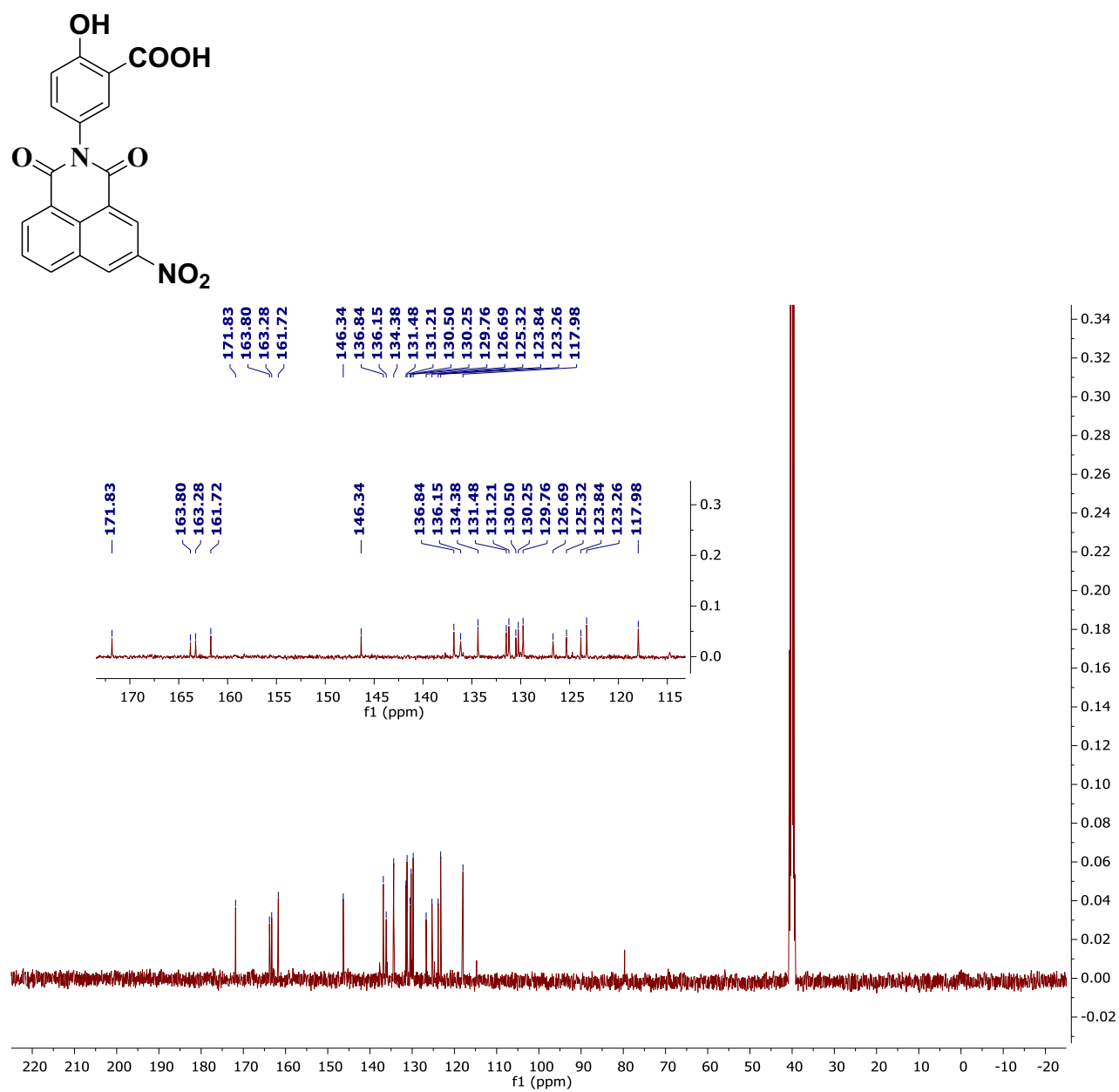
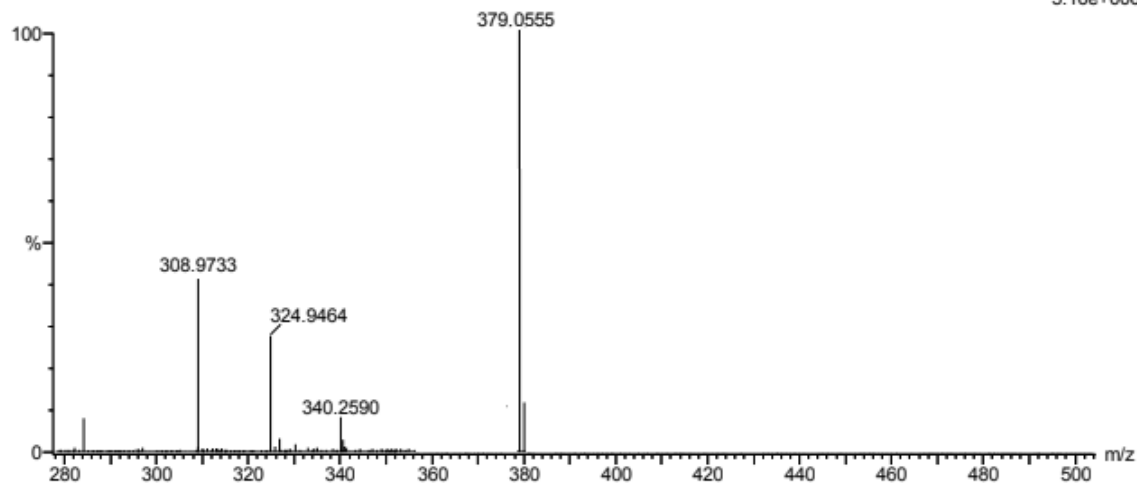


Figure S5. ^{13}C NMR spectra of S-13 in DMSO- d_6 .

Sample Name : S-13 I.I.T.ROPAR XEVO G2-XS QTOF
 Test Name : HRMS-1
 280119-S-13- 11 (0.123) AM2 (Ar,19000.0,0.00,0.00); Cm (7:18) 1: TOF MS ES+
 5.16e+006



Minimum: -1.5
 Maximum: 5.0 5.0 50.0

Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	Norm	Conf(%)	Formula
379.0555	379.0566	-1.1	-2.9	15.5	598.1	n/a	n/a	C19 H11 N2 O7

Figure S6. Mass spectra of **S-13**.

¹H NMR of S-21

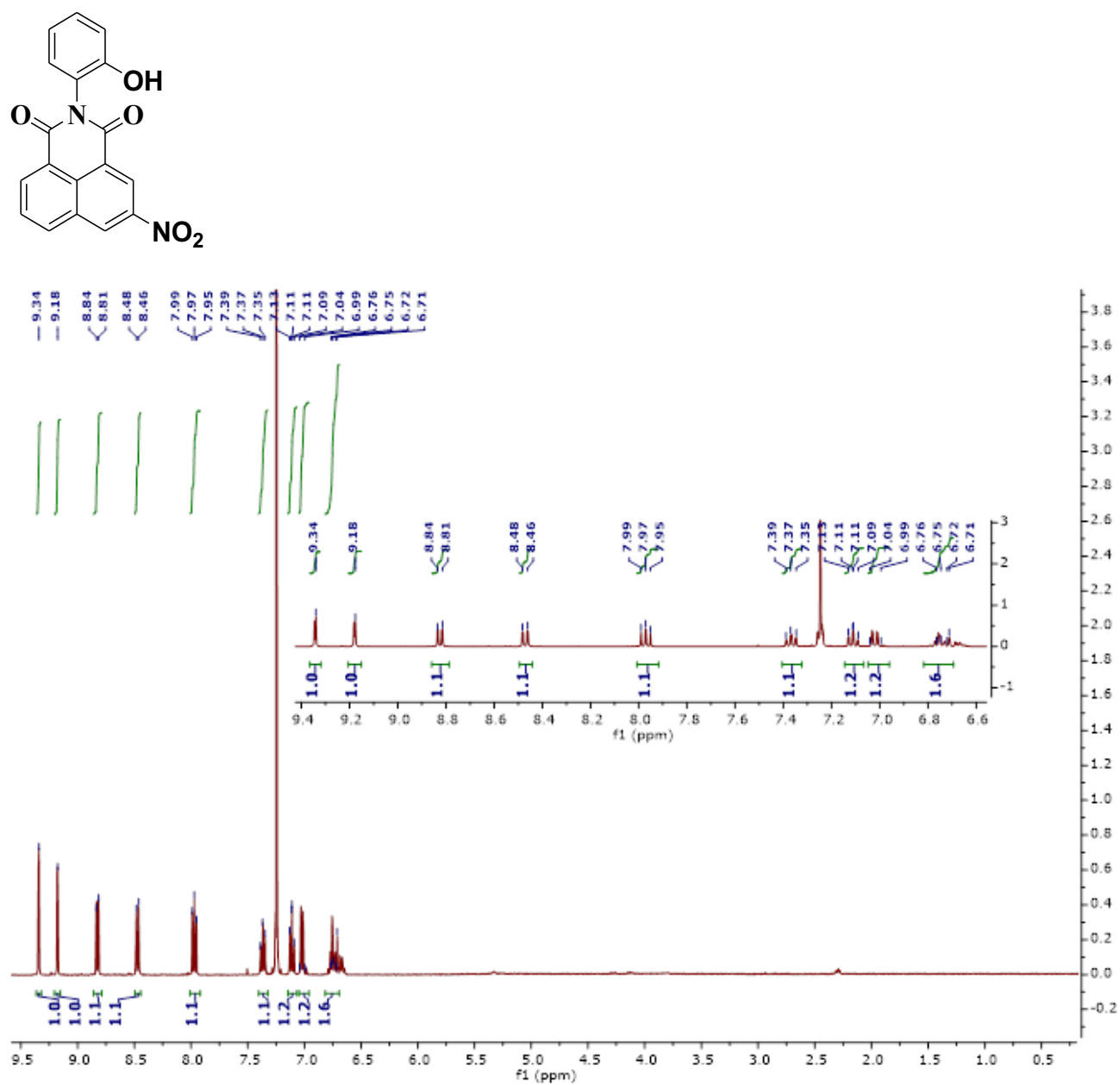


Figure S7. ¹H NMR spectra of S-21 in CDCl₃.

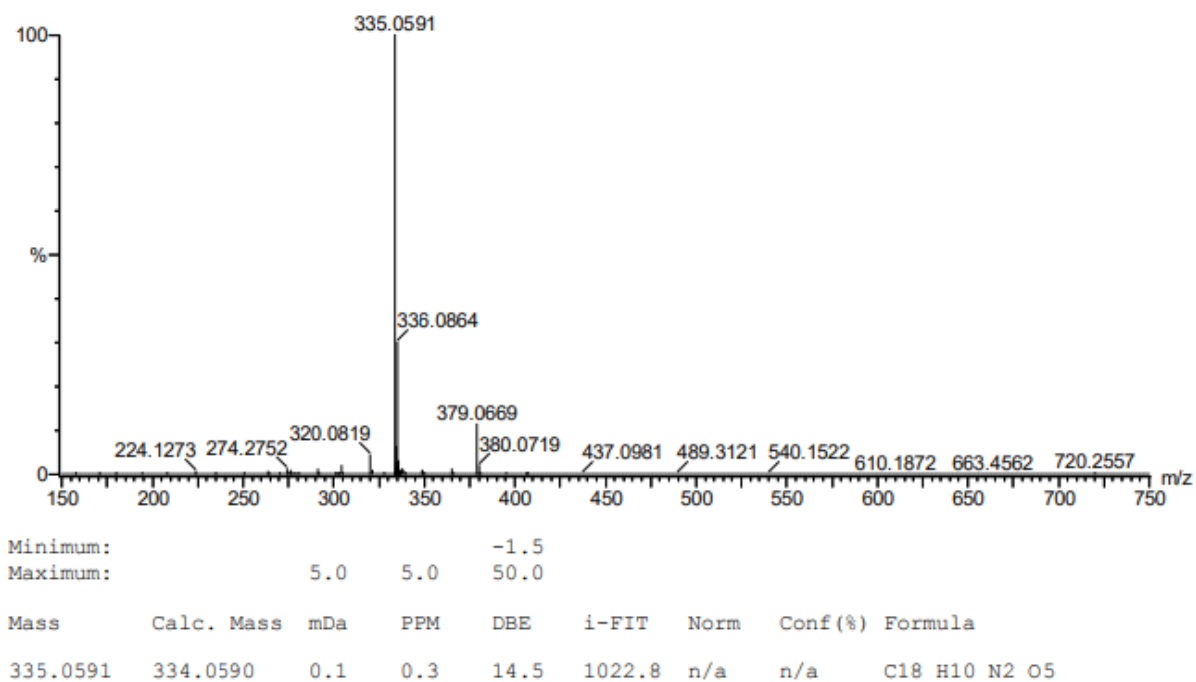


Figure S8. Mass spectra of S-21.

¹H NMR of 3

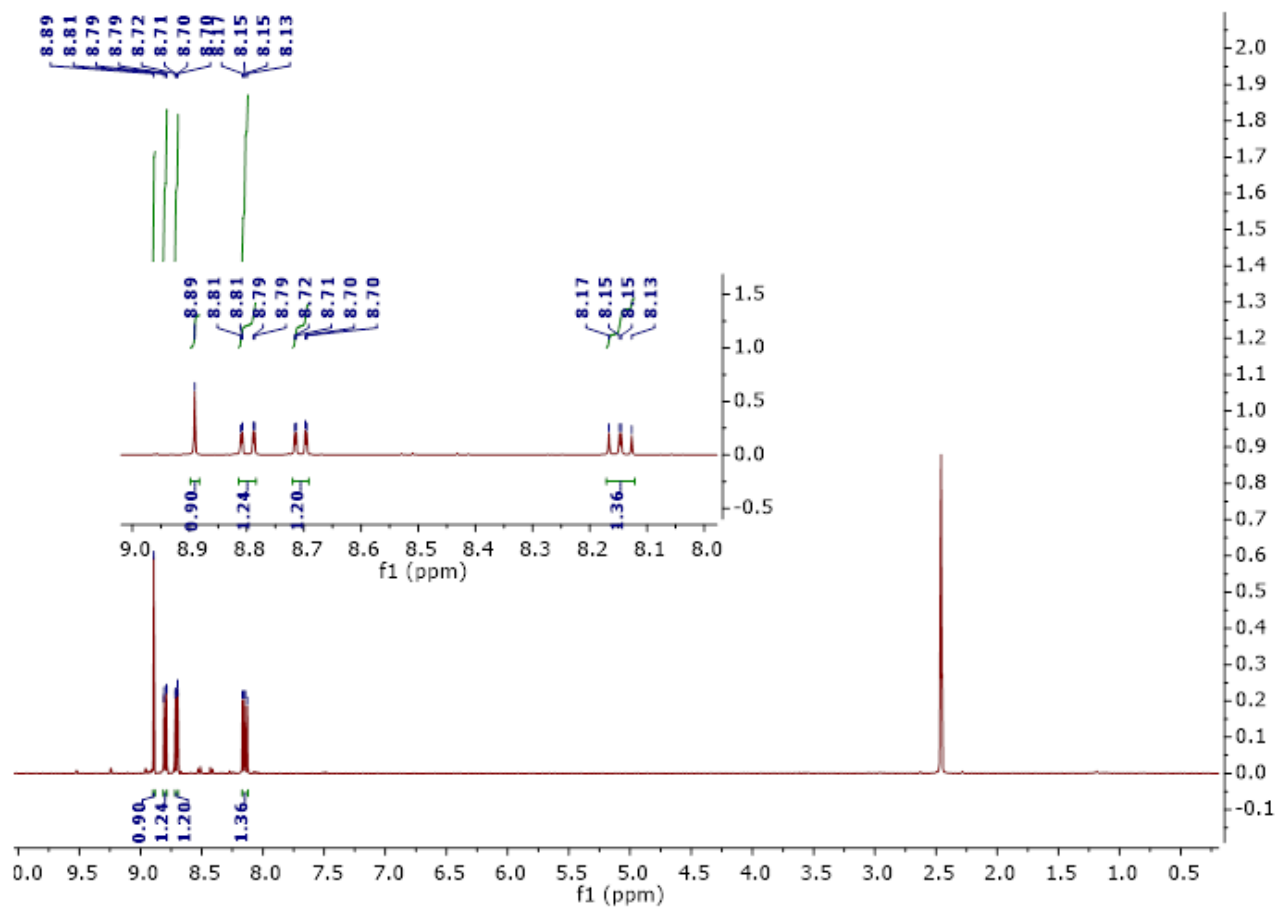
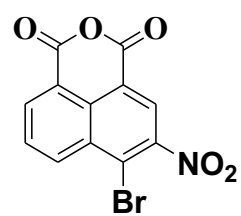


Figure S9. ¹H NMR spectra of **3** in DMSO-*d*₆.

^1H NMR of S-15

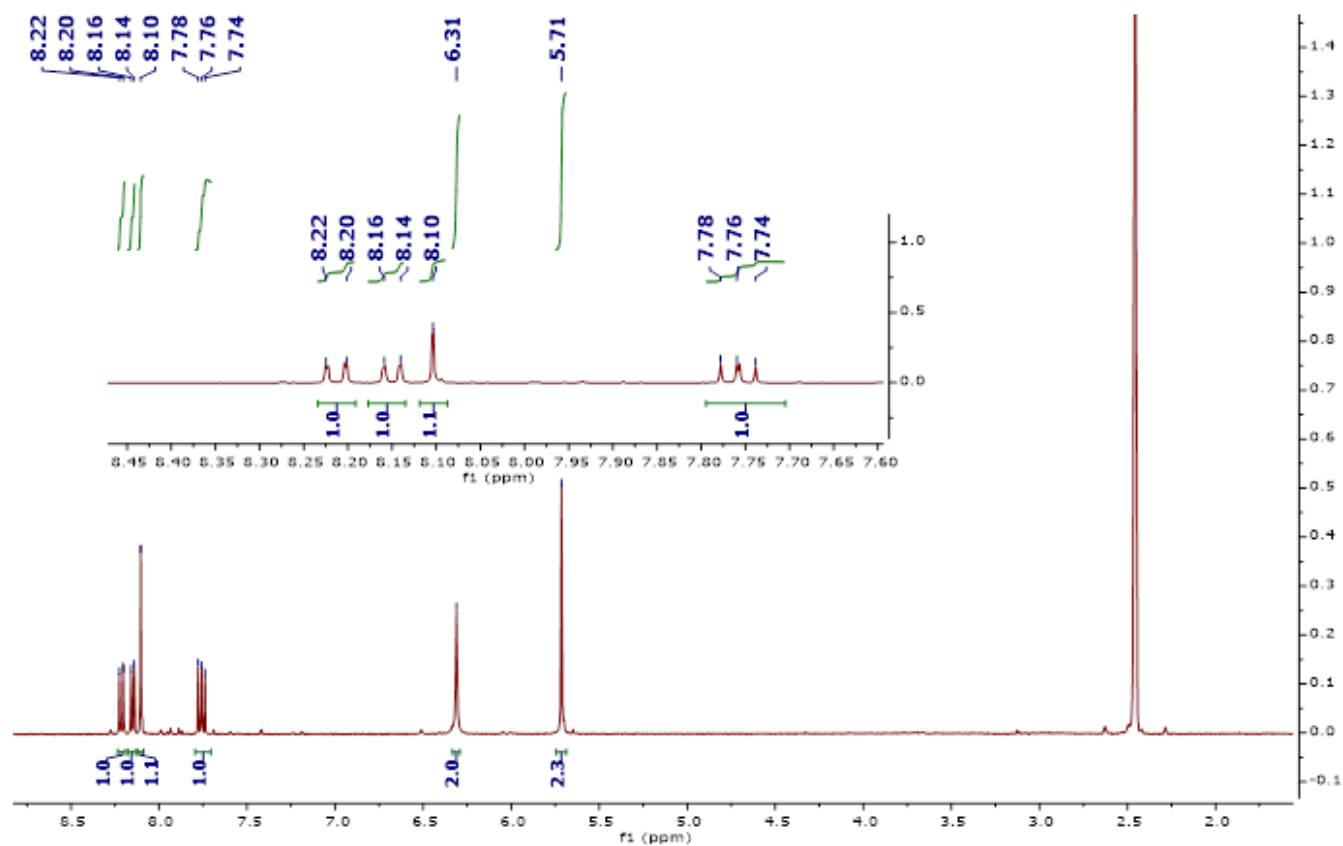
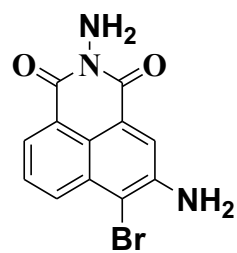


Figure S10. ^1H NMR spectra of S-15 in $\text{DMSO}-d_6$.

^{13}C NMR of S-15

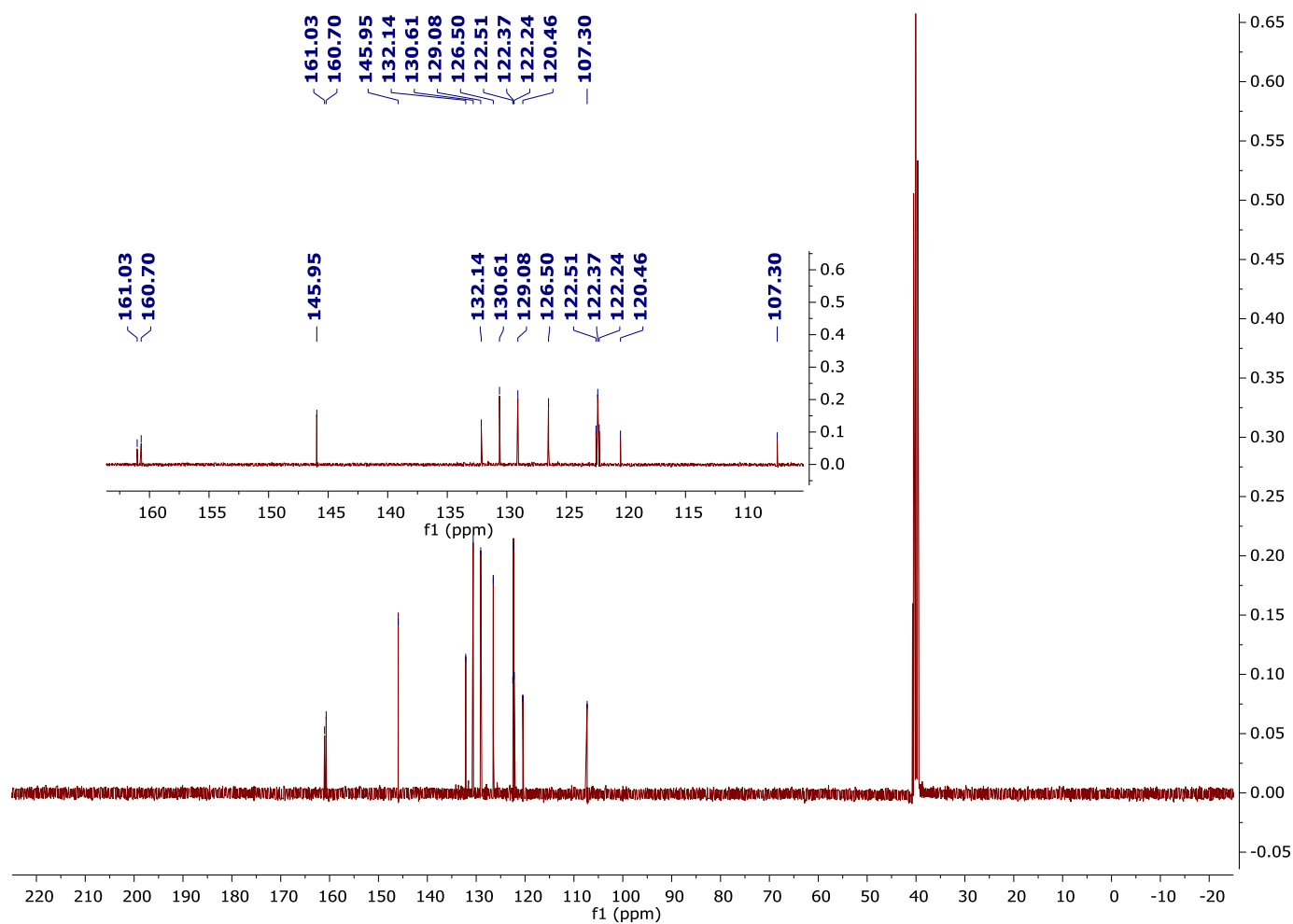
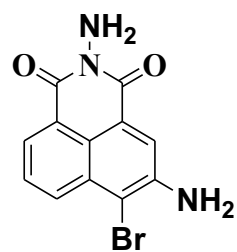


Figure S11. ^{13}C NMR spectra of S-15 in $\text{DMSO-}d_6$.

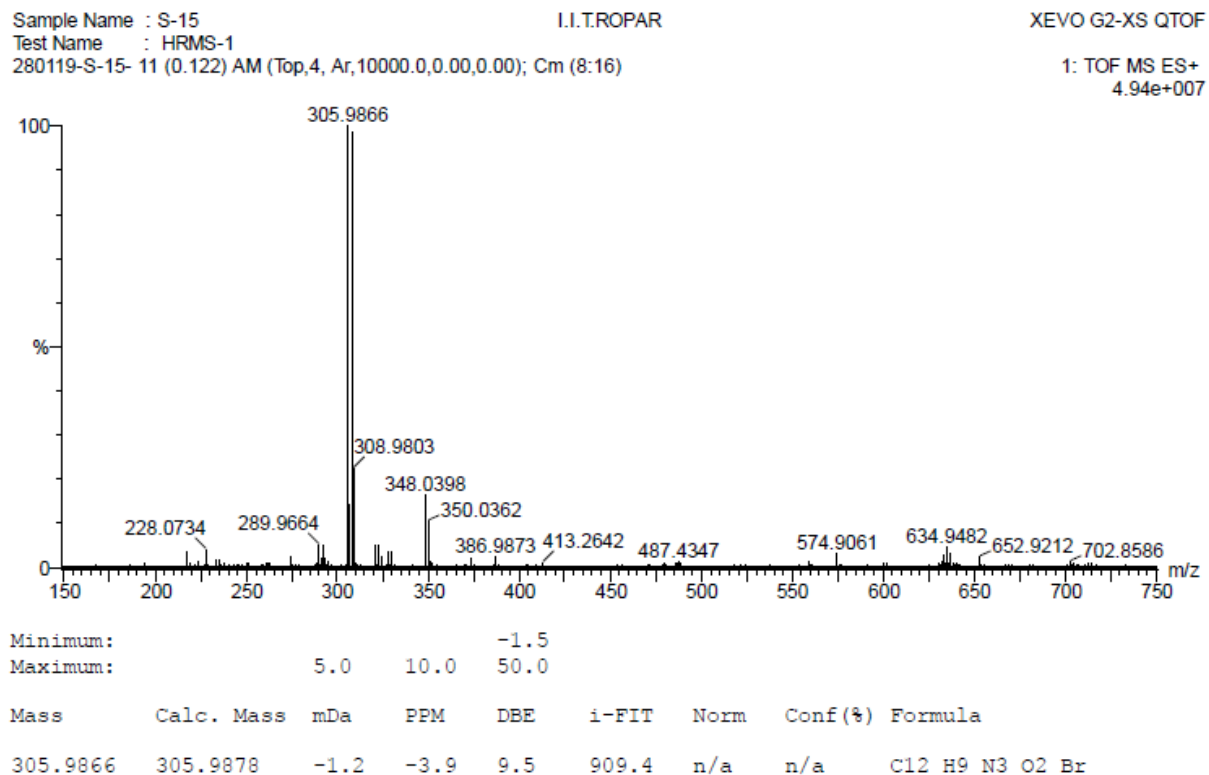


Figure S12. Mass spectra of S-15.

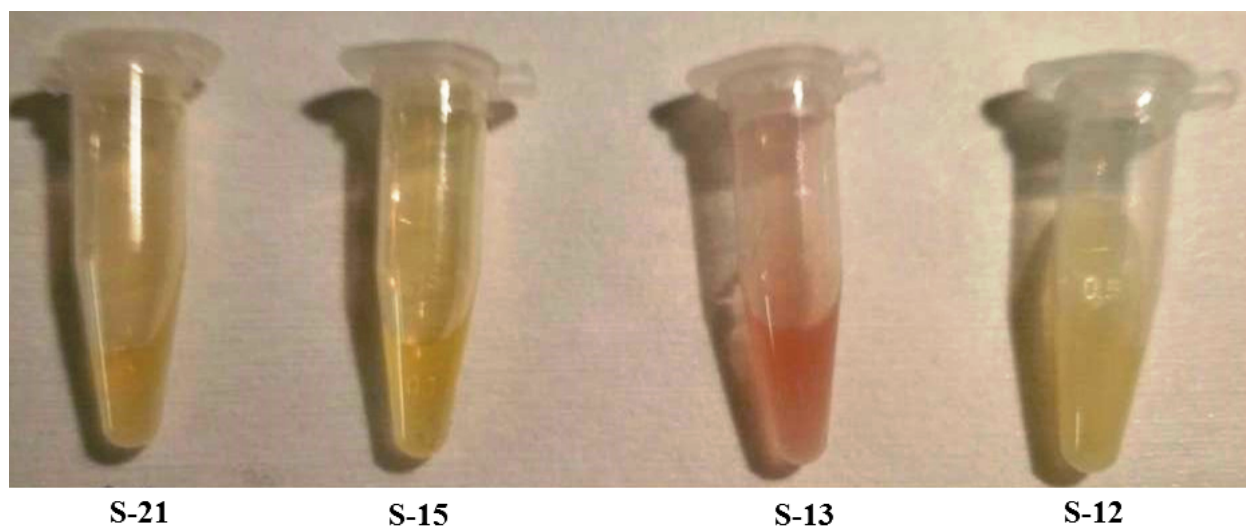


Figure S13. The picture shows the formation of precipitates and bleaching of solutions S-21, S-13 and S-12 except S-15 in DMSO/H₂O (4:6, v/v).

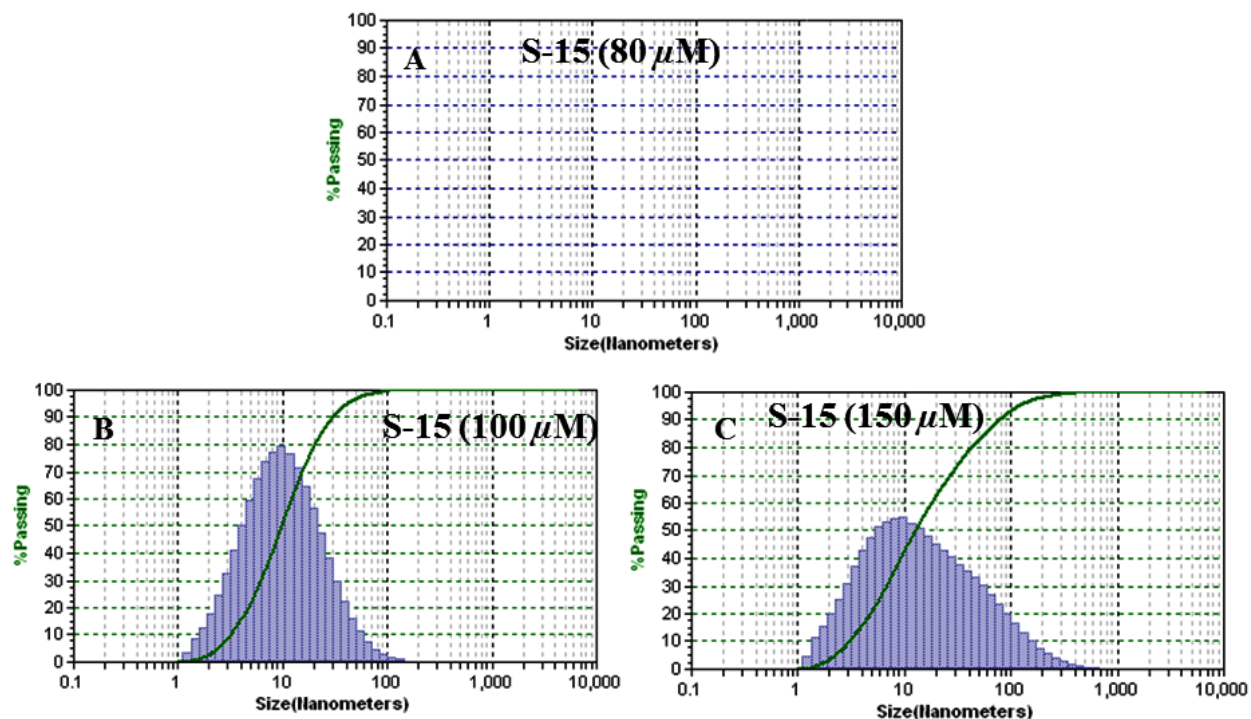


Figure S14. Dynamic light scattering (DLS) spectra of **S-15** prepared in DMSO/H₂O (4:6, v/v) at different concentrations: (A) 80 μM , (B) 100 μM and (C) 150 μM .

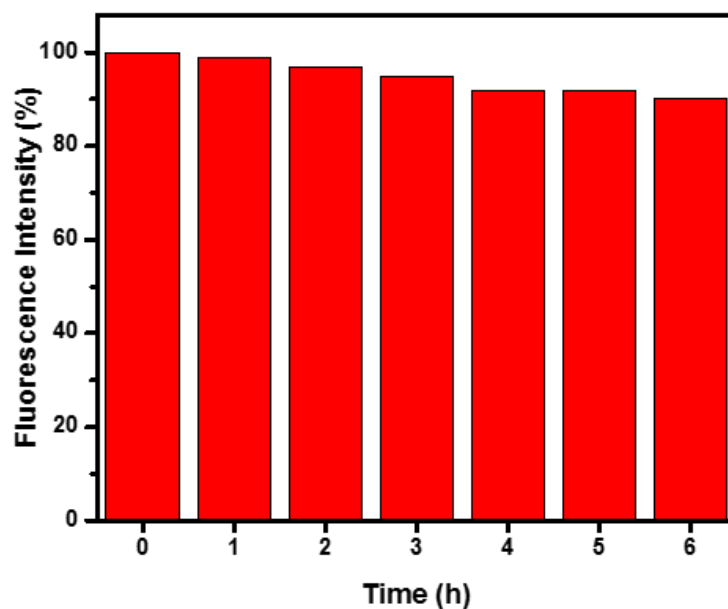


Figure S15. The change in emission intensity (at 552 nm) of **S-15** with time, $\lambda_{\text{ex}} = 430 \text{ nm}$; Ex. and Em. slit width 10 nm.

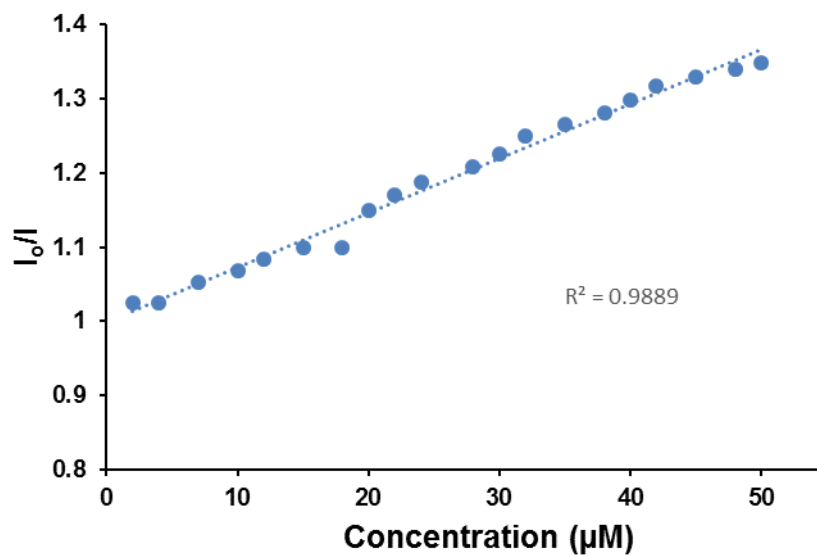


Figure S16. Stern-Volmer plot of **S-15** with increasing concentration of DNA, $\lambda_{\text{ex}} = 430 \text{ nm}$; Ex. and Em. slit width 10 nm.

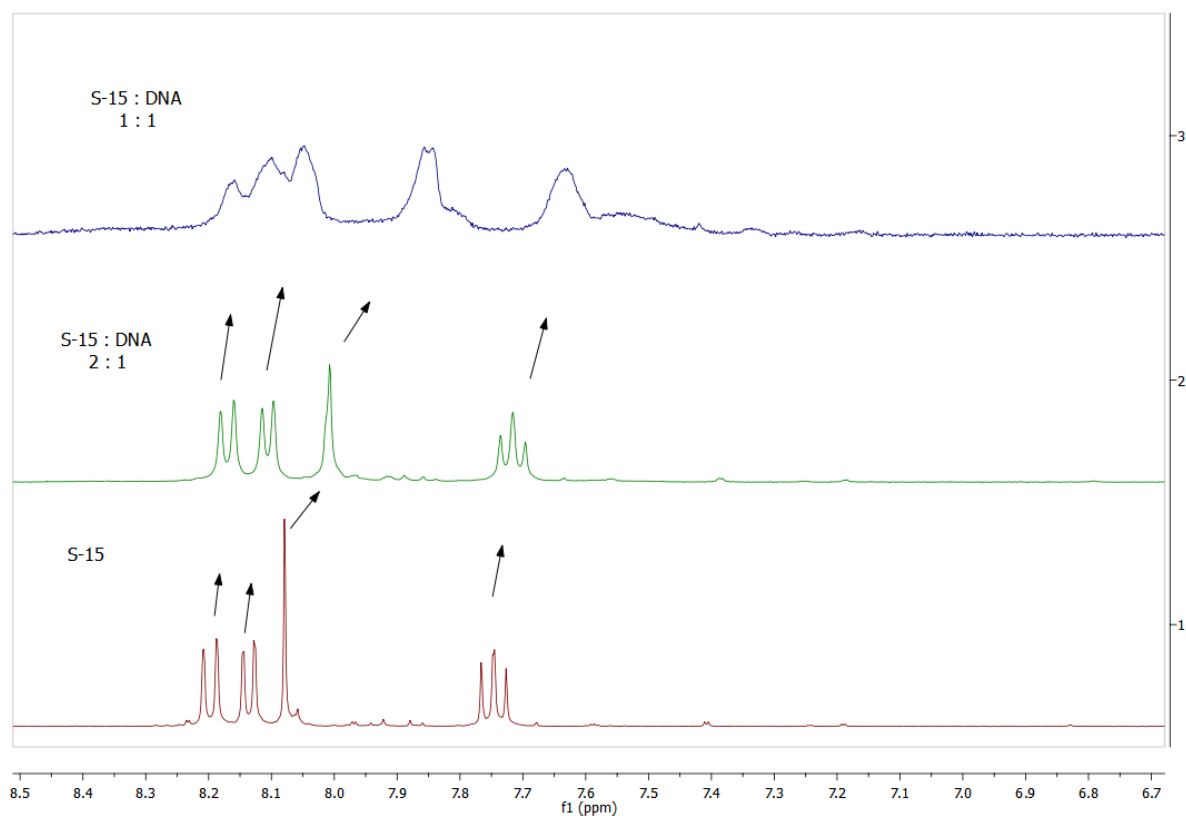


Figure S17. The partial ^1H NMR spectra of **S-15** in the presence of different amount of DNA.

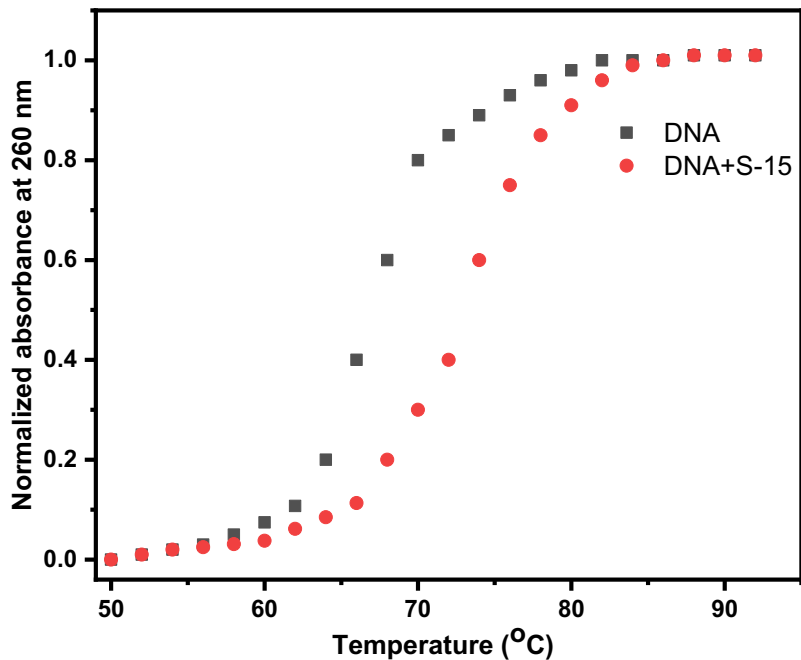


Figure S18. Temperature dependent the 260 nm absorbance of DNA both native and intercalated with S-15 (10 μ M).

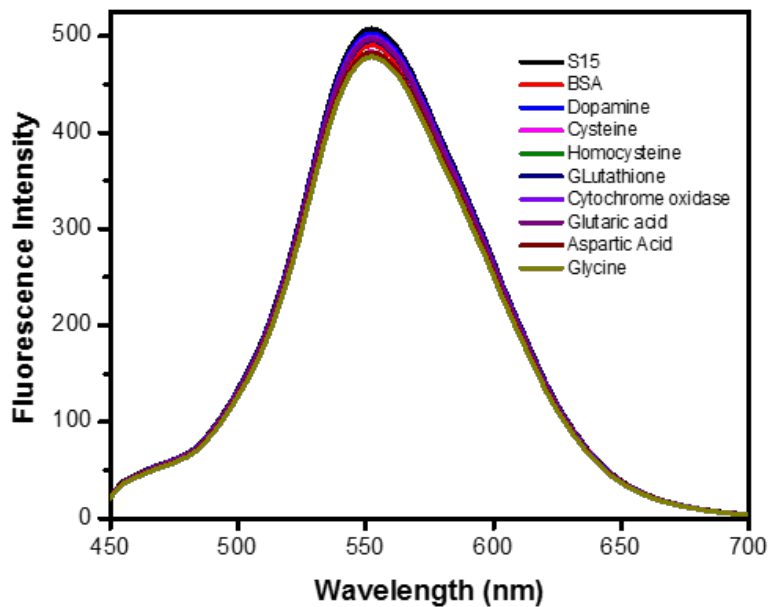


Figure S19. The change in the emission profile of S-15 in the presence of different amount of biological relevant analytes, $\lambda_{\text{ex}} = 430$ nm; Ex. and Em. slit width 10 nm.

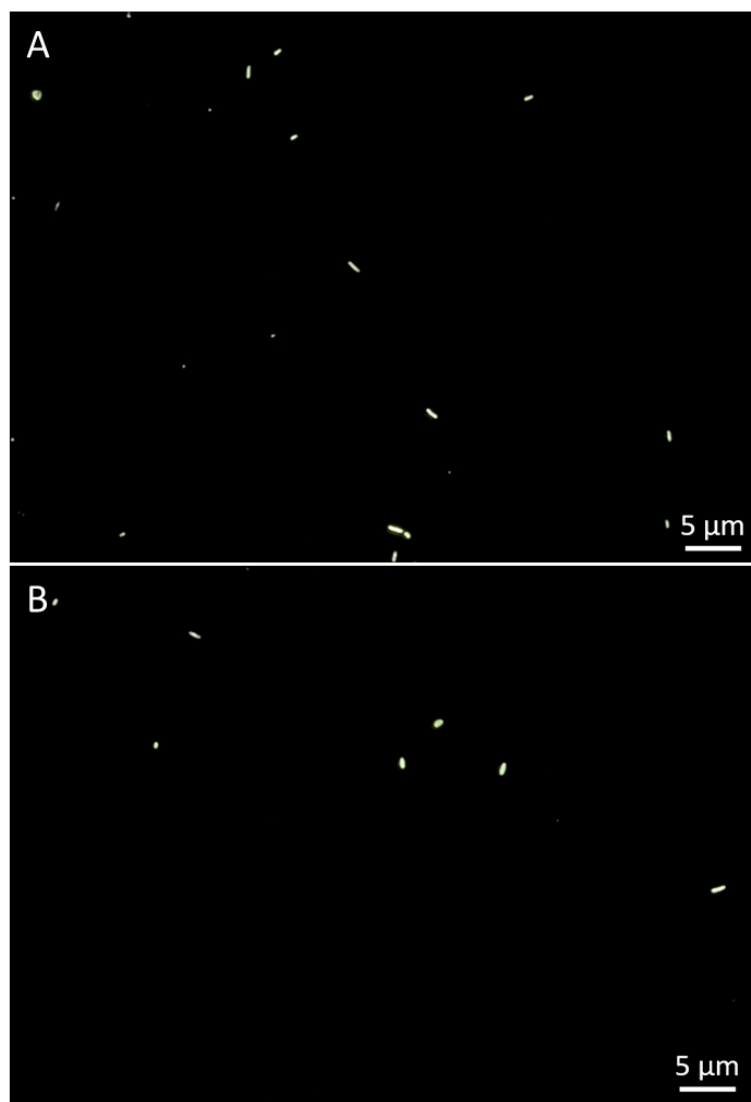


Figure S20. Optical image of *L. pneumophila* (10^5 CFU/ml) incubated for 1 hour in (a) 1X phosphate buffer (PBS), and (b) DMSO/phosphate buffer (40:60, v/v). The images were recorded at 50 X magnification.

Cytotoxicity of S-15

The cytotoxicity of **S-15** was investigated towards the HeLa cell line in MTT (MTT = (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay. The HeLa cells along with growth media were incubated at 37°C in 5% CO₂ in 96 well plate. The stock solution of **S-15** (100 μM)

was prepared in DMSO and different concentrations (10 μ M, 30 μ M, 50 μ M and 80 μ M) of **S-15** were added to well. To ensure the uniformity, solutions were thoroughly mixed. The plate was incubated for 24 h at 37°C in 5% CO₂. The MTT solution concentrated at 5 mg/mL in PBS was prepared and 20 μ l of this solution was added into each well. After carefully mixing the content of wells, the plate was further incubated for 2 h. Further, medium was removed and formazan (MTT metabolic product) was suspended in 200 μ l DMSO. The solution was mixed properly and optical density was measured at 560 nm. Each experiment was performed in triplicate. The dose-dependent studies results showed that 90-97% cell remained viable up to 50 μ M concentration of **S-15** as shown in Figure S21.

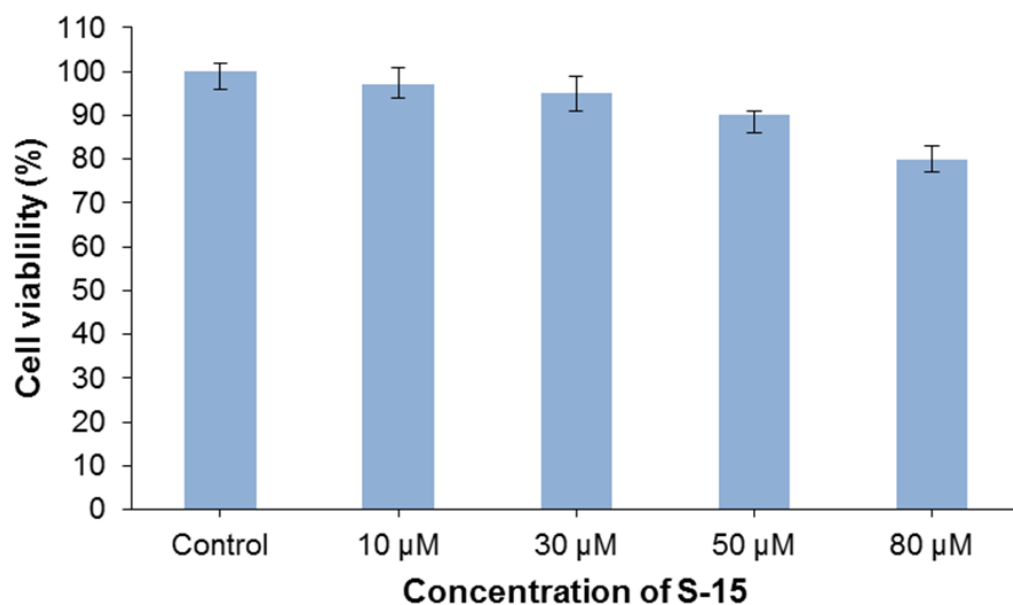


Figure S21. The dose-dependent MTT assay representing the percentage (%) of cell viability in different solutions of **S-15** diluted in DMSO.