

Supplementary materials

Synthesis of a new diarylhydrazone derivative and an evaluation of its *in vitro* biofilm inhibition and quorum sensing disruption along with a molecular docking study

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Abstract

Molecules that target quorum-sensing and biofilm inhibition are useful antimicrobials. In this regard, a new diarylhydrazone was synthesized and characterized using infrared, high-resolution mass spectrometry and nuclear magnetic resonance experiments as *N*-[(*E*)-4-bromo-2,5-diheptyloxybenzylideneamino]-2,4-dinitroaniline (BHBANA). Minimal inhibitory concentrations (MICs) vary from 0.625 to 2.5 mg/mL. This compound was screened *in vitro* for its inhibition of quorum-sensing-mediated violacein production by *C. violaceum* CV12472 at MIC and sub-MIC and showed percentage inhibition varying from 100% at MIC to 5.7 ± 0.2% at MIC/32. Against *C. violaceum* CV026, BHBANA exhibited anti-quorum-sensing zone diameters of 10.5 ± 0.3 mm and 7.0 ± 0.1 mm at MIC and MIC/2 respectively. BHBANA shows concentration-dependent inhibition of swarming motility on flagellated *P. aeruginosa* PA01 with the highest % inhibition of 28.30 ± 0.50 % µg/mL at MIC. The product inhibits biofilm formation with the best biofilm inhibition being observed against *S. aureus* varying from 72.24 ± 0.86% (MIC) to 09.82 ± 0.10% (MIC/8). Molecular docking studies carried out utilizing the Schrodinger software identified interactions between BHBANA and different receptor compartments of *C. violaceum*, which can block pathogenic gene expression. The results suggest the potential of BHBANA in reducing microbial virulence.

Keywords: Hydrazone; *N*-[(*E*)-4-bromo-2,5-diheptyloxybenzylideneamino]-2,4-dinitroaniline; quorum-sensing; anti-biofilm; swarming motility; molecular docking

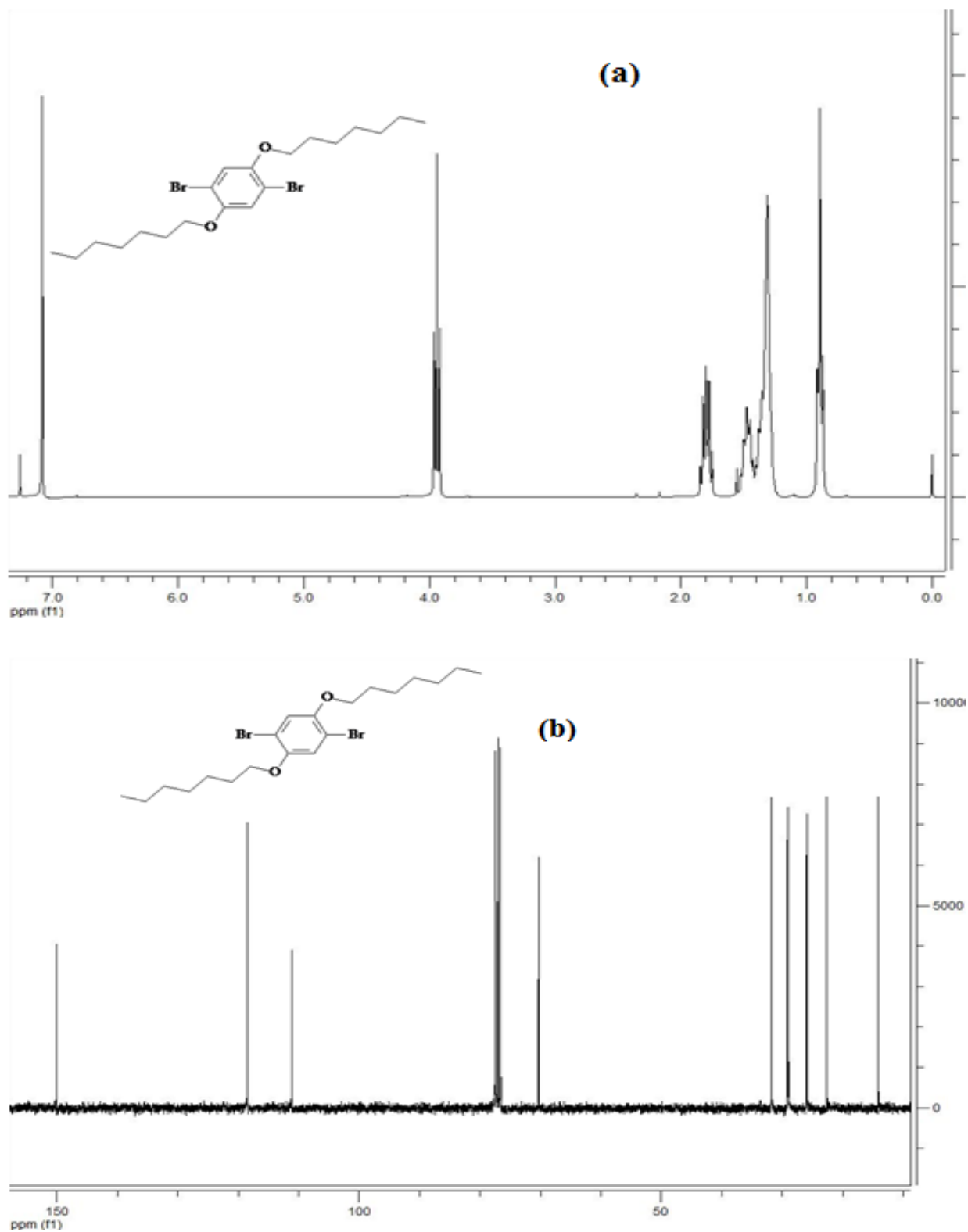


Figure S1: (a) ¹H NMR and (b) ¹³C NMR, spectra of the synthesized 1,4-dibromo-2,5-di(heptyloxy)benzene (3) recorded in CDCl₃

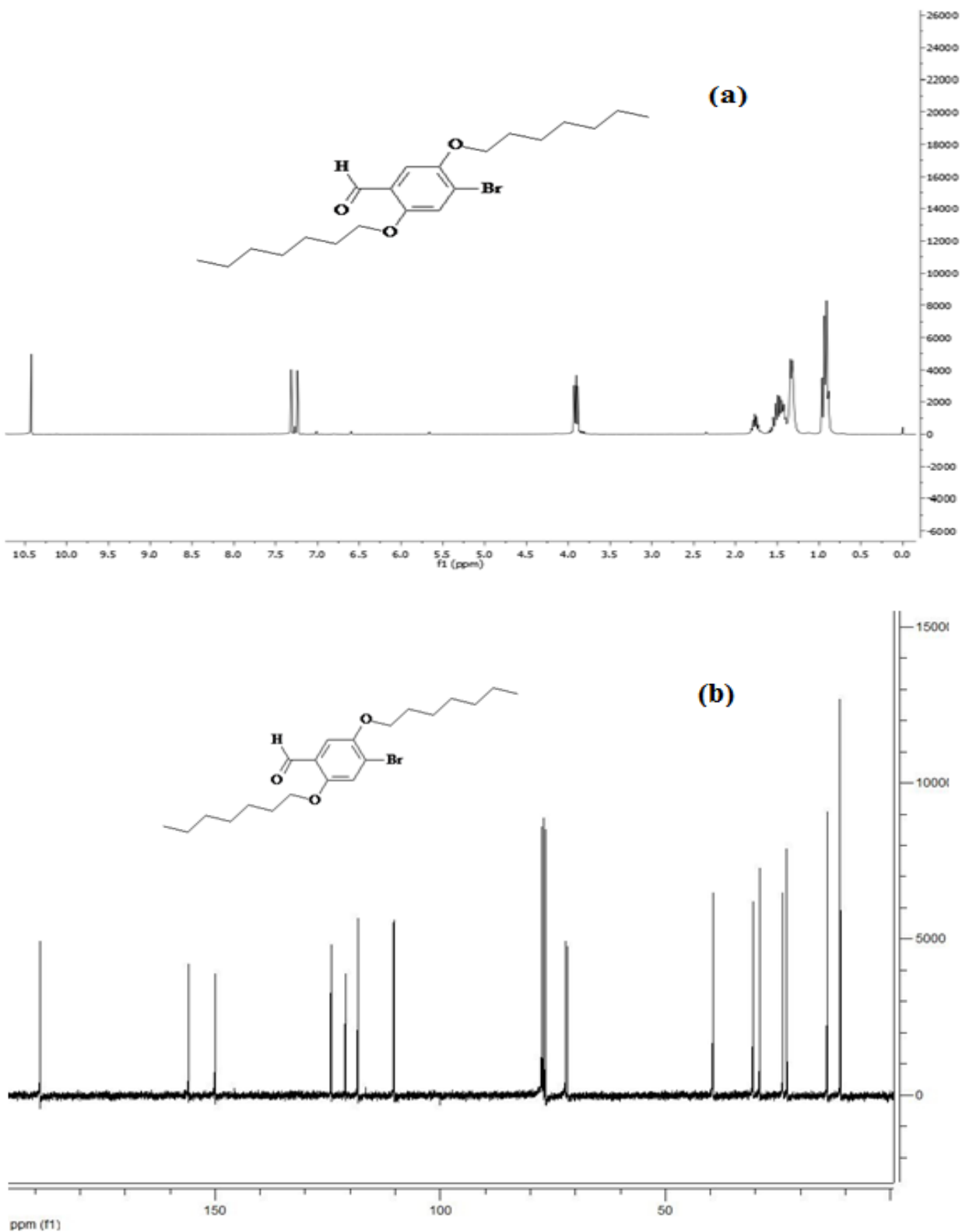


Figure S2: (a) ^1H NMR and (b) ^{13}C NMR, spectra of the synthesized 4-bromo-2,5-bis(heptyloxy)benzaldehyde (4) recorded in CDCl_3

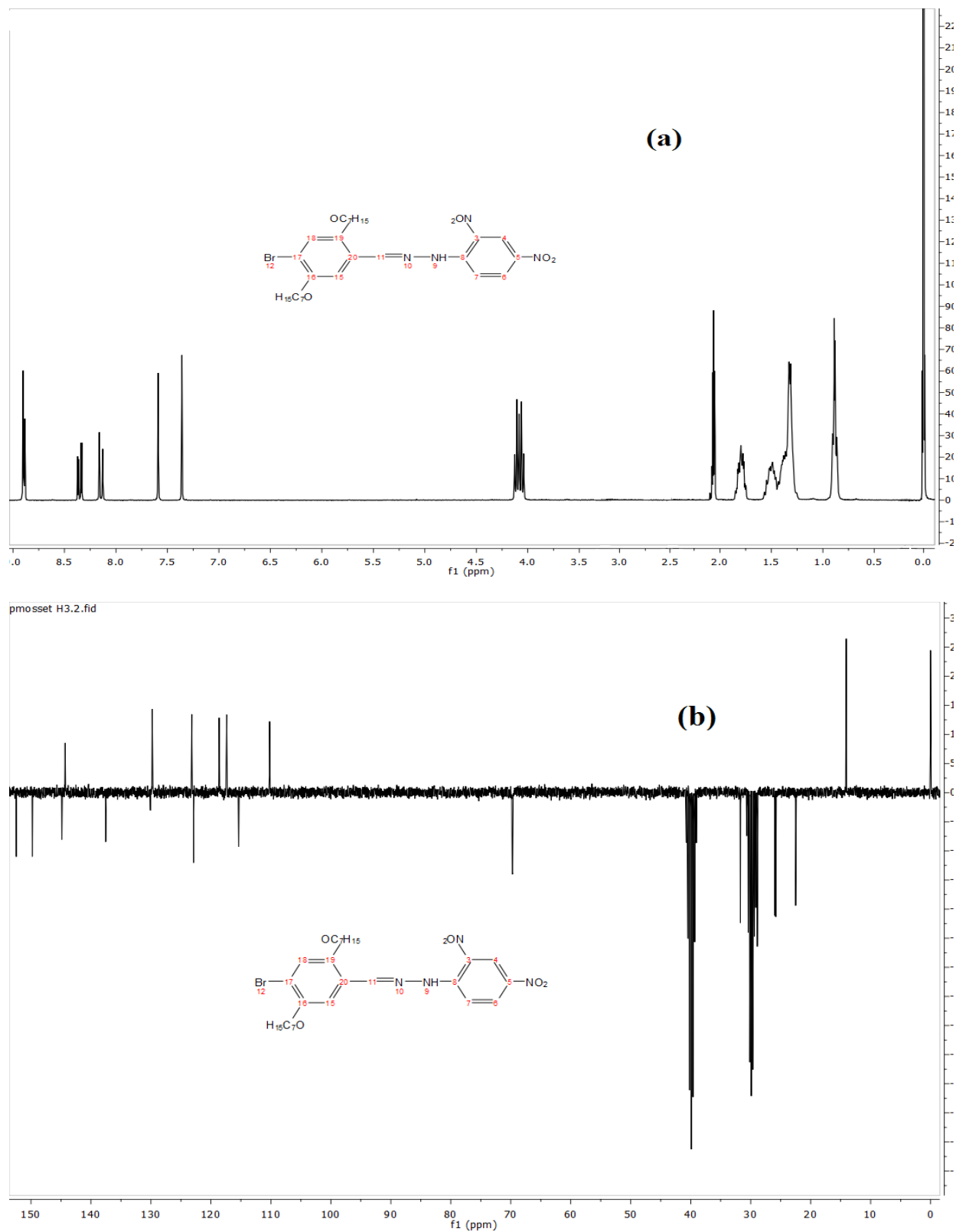
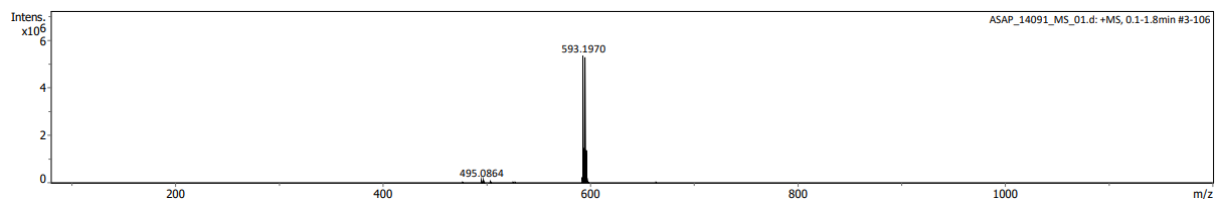
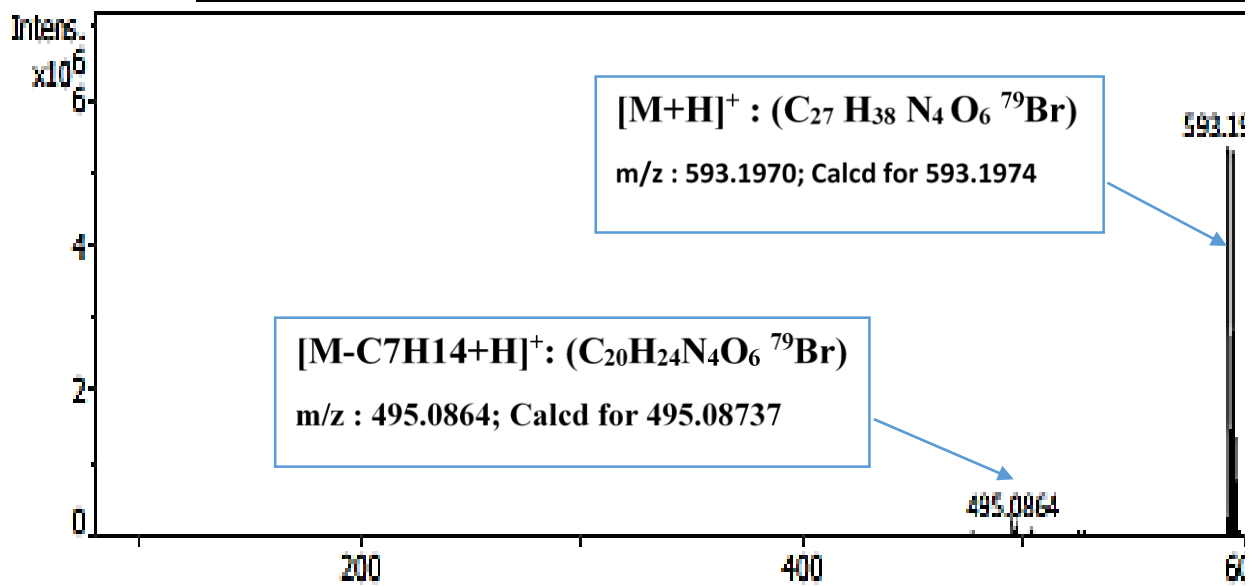
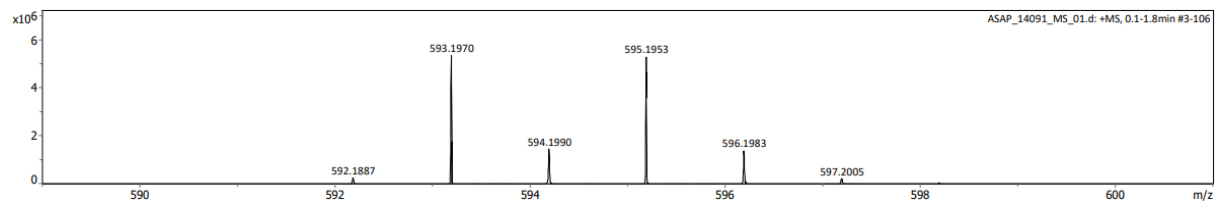


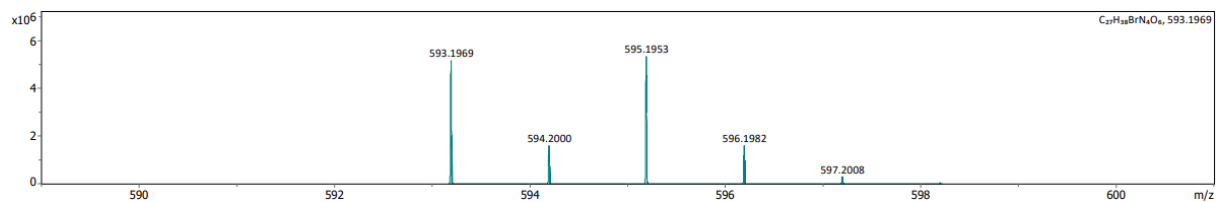
Figure S3: (a) ^1H NMR and (b) ^{13}C NMR (DEPT), spectra of the synthesized N-[(E)-4-bromo-2,5-diheptyloxybenzylideneamino]-2,4-dinitroaniline (BHBANA) (6) recorded in solution DMSO- d_6 : acetone- d_6 , 2:1



a) **Full view of the mass spectrum:** Expansion of the molecular peak (the two nearly equally most intense peaks highlights the presence of one atom of bromine)



b) **Isotopic cluster calculation for the formula $C_{27}H_{38}BrN_4O_6$ corresponding to the $[M + H]^+$ peak**



A perfect agreement between the expansion of the molecular peak and the isotopic cluster calculation can be seen.

Figure S4: Source: ASAP (Atmospheric Solid Analysis Probe) at 160°C (this compound exhibited poor electrospray ionization) Instrument: Bruker MaXis 4G

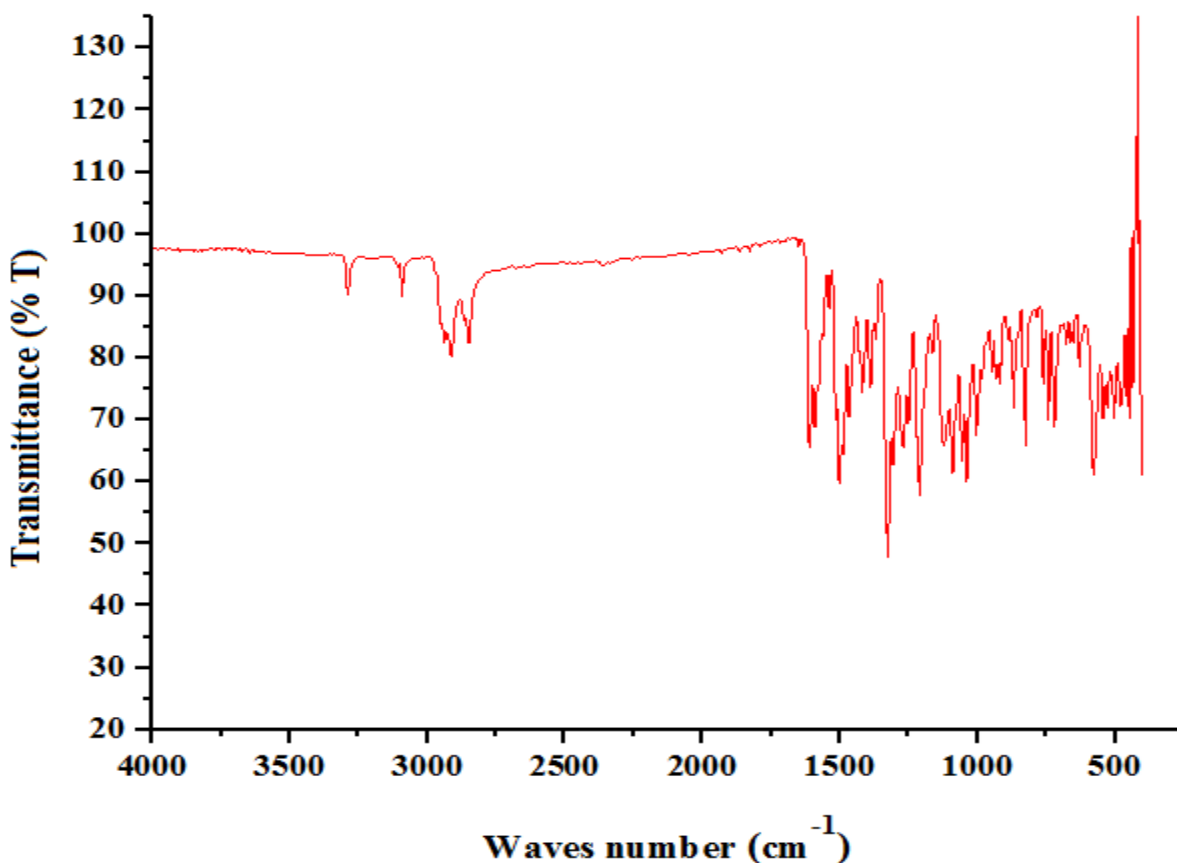


Figure S5: FTIR spectra of the synthesized N-[(E)-4-bromo-2,5-diheptyloxybenzylideneamino]-2,4-dinitroaniline (BHBANA)

2D NMR of BHBDAH

2D NMR spectra of N-[(E)-4-bromo-2,5-diheptyloxybenzylideneamino]-2,4-dinitroaniline (BHBANA) afforded the following correlations

COSY correlations: 8.89 ↔ 8.35, 8.35 ↔ 8.14, 4.11 and 4.06 ↔ 1.79, 1.30 ↔ 0.89 and 0.88.

HMQC correlations: 8.90 ↔ 144.30, 8.89 ↔ 123.18, 8.35 ↔ 129.77, 8.14 ↔ 117.36, 7.59 ↔ 110.20, 7.36 ↔ 118.63, 4.06 and 4.11 ↔ 69.70 and 69.75, 1.80 ↔ 1.80 ↔ 28.88 and 28.89, 1.49 ↔ 25.80 and 25.95, 1.33 ↔ 29.14 and 29.16, 1.32 ↔ 31.69 and 31.71, 1.31 ↔ 22.46 and 22.49, 0.88 and 0.89 ↔ 14.05.

HMBC correlations: 8.90 ↔ 152.45, 123.18, 110.20; 8.35 ↔ 144.85; 8.14 ↔ 137.53, 130.08; 7.59 ↔ 152.45, 149.77, 123.18 (weak), 115.36; 7.36 ↔ 152.45, 149.77, 144.30 (weak), 122.88, 115.36; 4.11 ↔ 149.77; 4.06 ↔ 152.45; 4.11 ↔ 25.80 and/or 25.95; 4.06 ↔ 28.89 and/or 29.14 and/or 29.16; 1.80 ↔ 69.70, 28.88 and/or 28.89; 1.49 ↔ 29.14 and/or 29.16; 1.30 ↔ 31.69 and/or 31.71; 0.88 and 0.89 ↔ 31.69 and/or 31.71, 22.46 and/or 22.49.

Noesy correlations: 7.59 ↔ 4.11, 7.36 ↔ 4.06, 4.11 and 4.06 ↔ 1.79.

¹ H NMR	¹³ C NMR
<p>Chemical structure showing ¹H NMR peaks: 4.06, 1.79, 7.36, 8.90, 7.59, 4.11, 1.79, 11.76, 8.14, 8.89, 8.35, and a nitro group.</p>	<p>Chemical structure showing ¹³C NMR peaks: 69.70 or 69.75, 28.88 or 28.89, 118.63, 152.45, 144.30, 115.36, 122.88, 149.77, 110.20, 144.85, 130.08, 117.36, 123.18, 129.77, 137.53, and a nitro group.</p>

NMR of the *n*-heptyloxy chain:

¹ H NMR	¹³ C NMR
<p>Chemical structure showing ¹H NMR peaks: 4.1, 1.49, 1.32, 0.88(9), 1.80, 1.33, 1.31.</p>	<p>Chemical structure showing ¹³C NMR peaks: 69.7, 25.9, 31.7, 14.0, 28.9, 29.1, 22.5.</p>

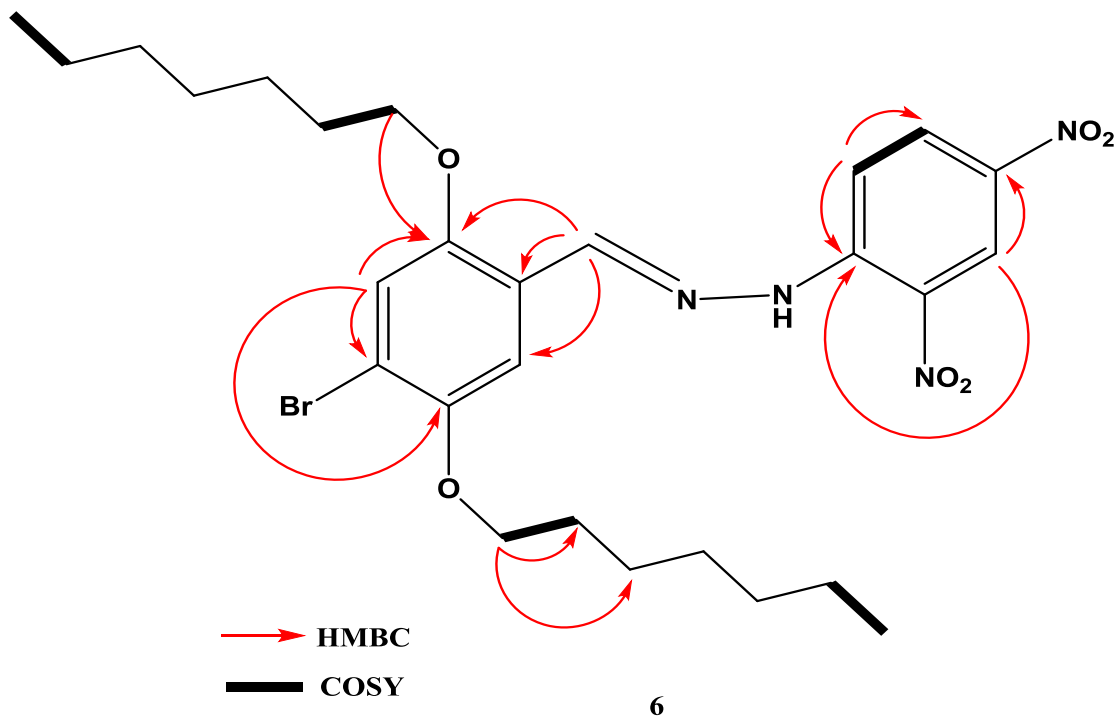


Figure S6: Structure of N-[(E)-4-bromo-2,5-diheptyloxybenzylideneamino]-2,4-dinitroaniline (BHBANA) (6) with key HMBC and COSY correlations

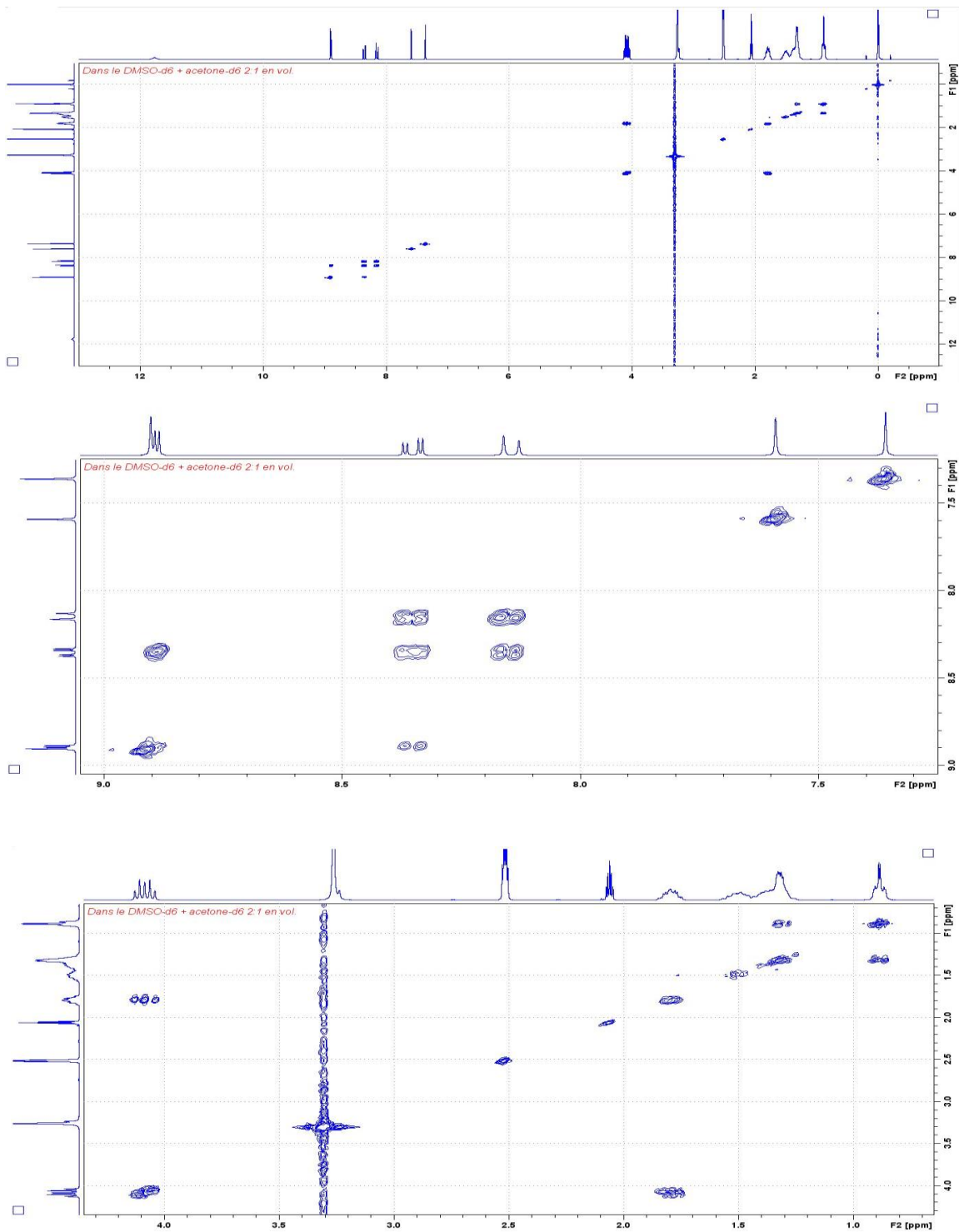


Figure S7: COSY spectrum of N-[(E)-4-bromo-2,5-diheptyloxybenzylideneamino]-2,4-dinitroaniline (BHBANA)

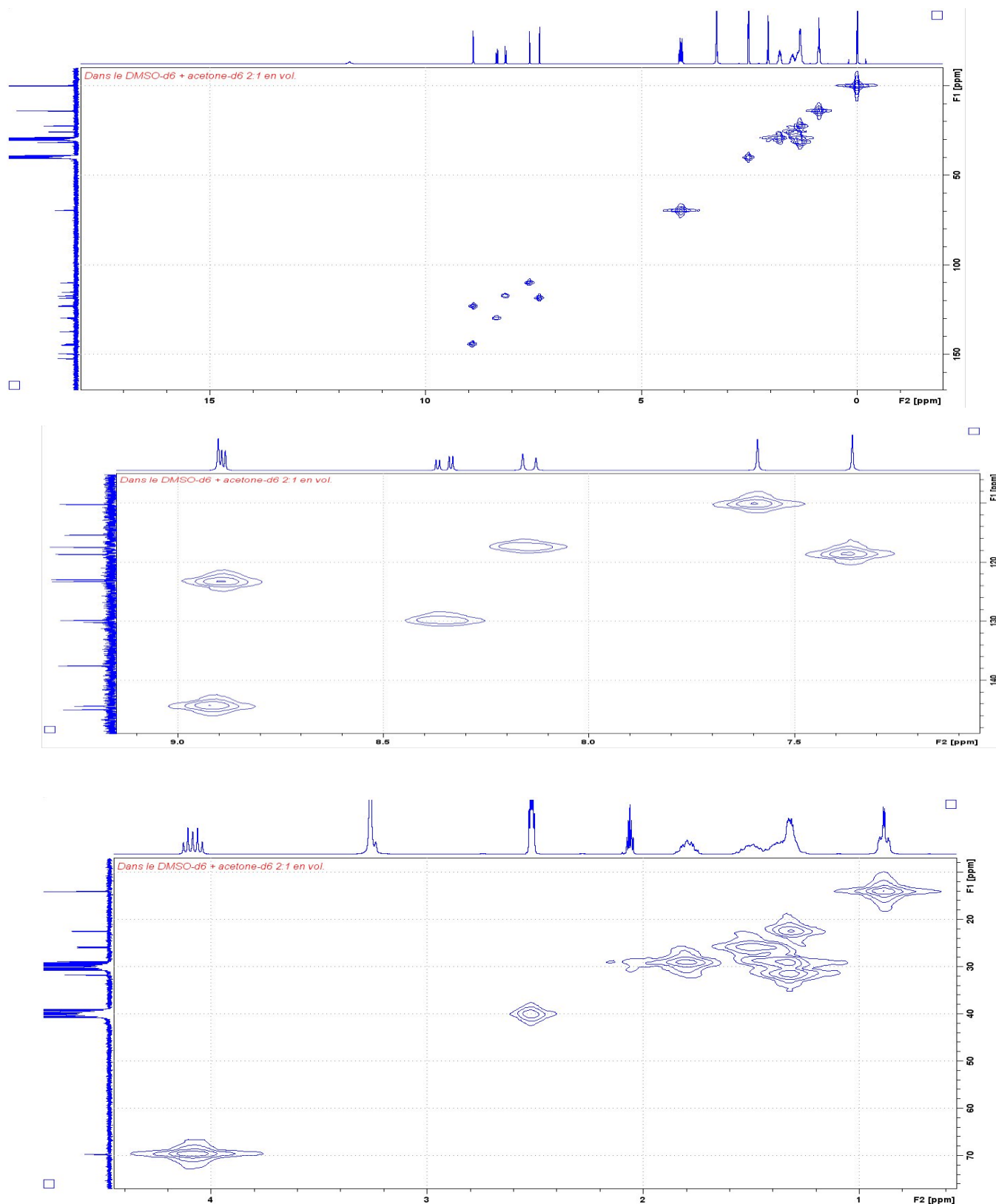


Figure S8: HMQC spectrum of N-[(E)-4-bromo-2,5-diheptyloxybenzylideneamino]-2,4-dinitroaniline (BHBANA)

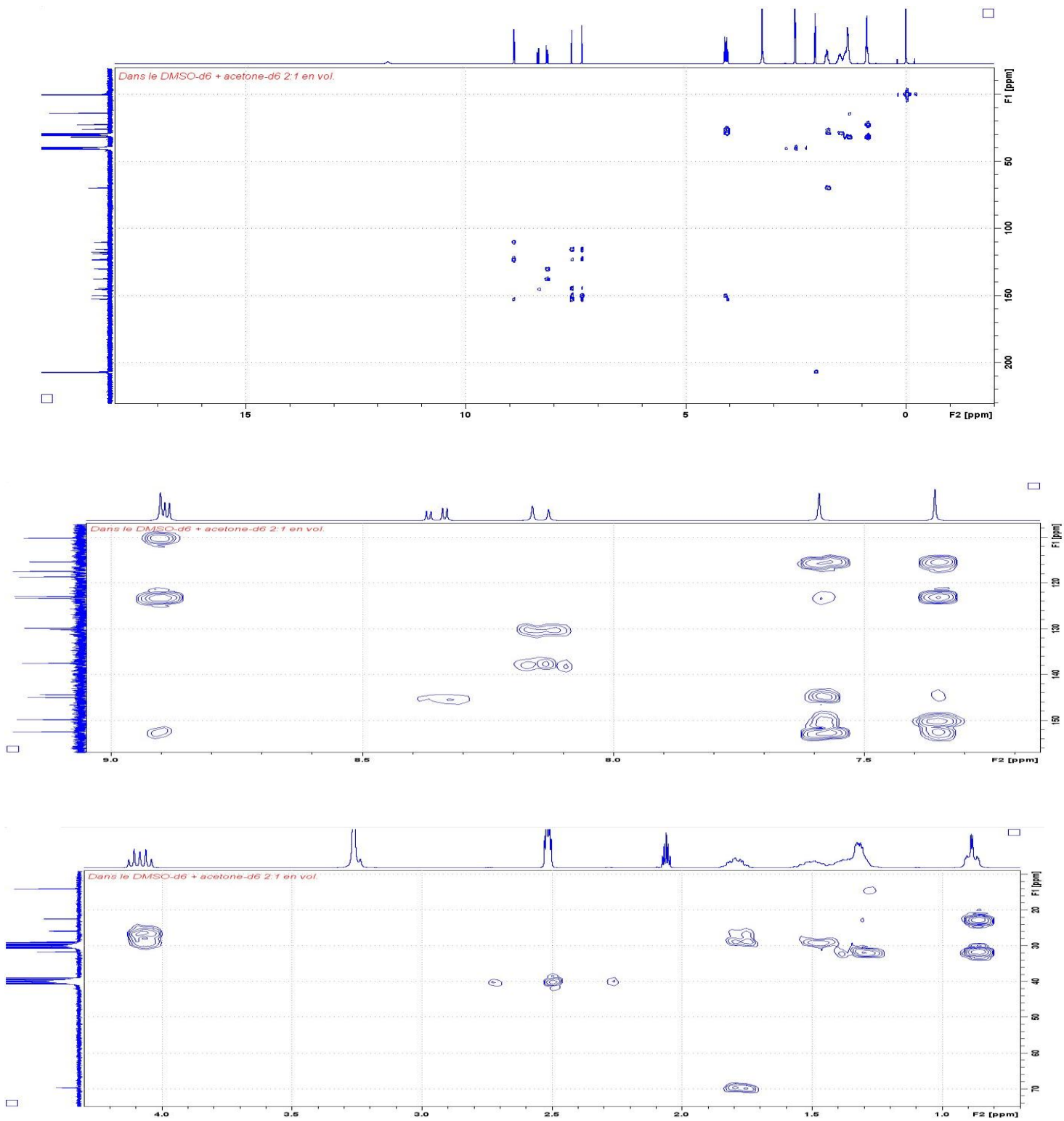


Figure S9: HMBC spectrum of N-[(E)-4-bromo-2,5-diheptyloxybenzylideneamino]-2,4-dinitroaniline (BHBANA)

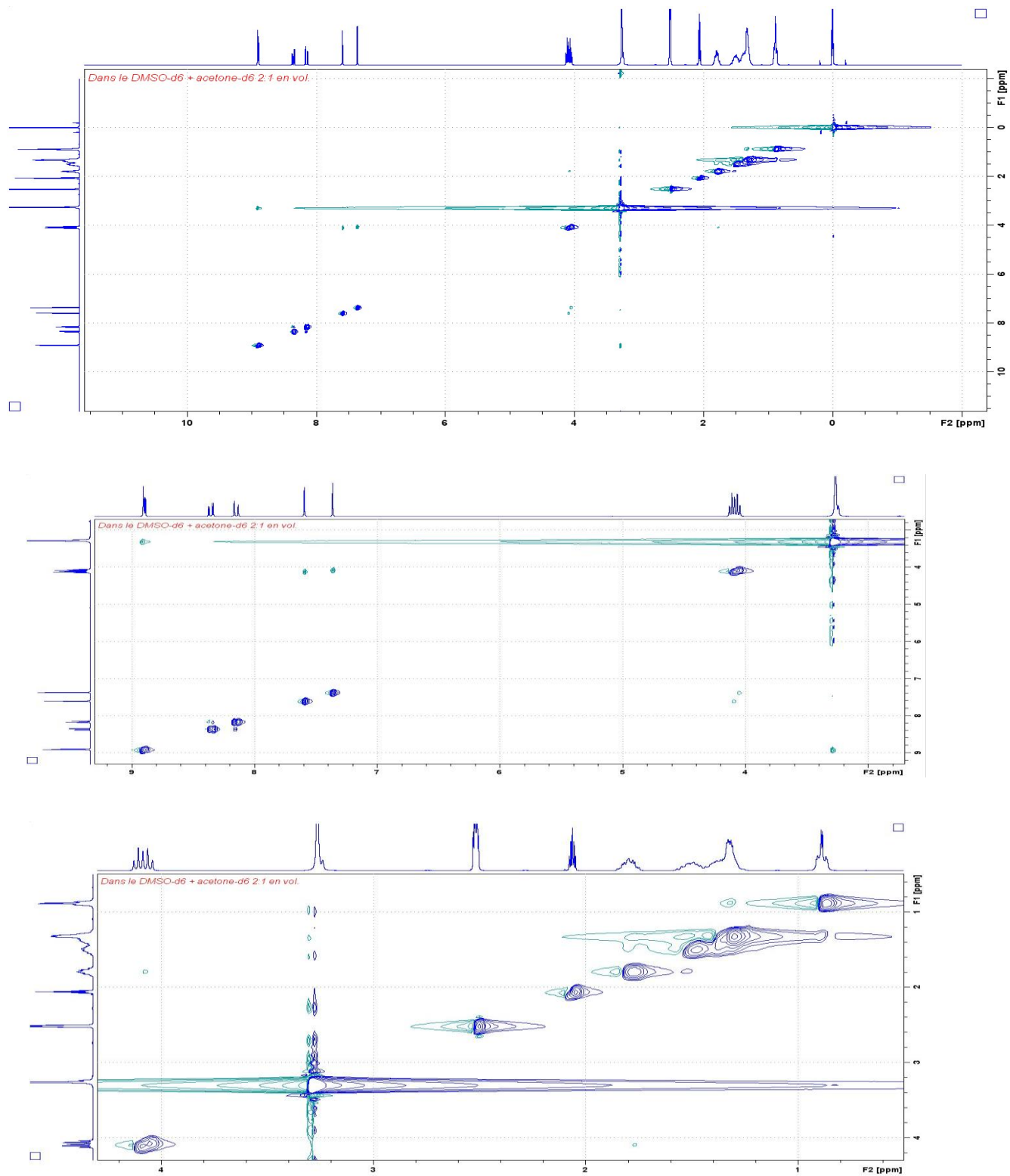


Figure S10: NOESY spectrum of N-[(E)-4-bromo-2,5-diheptyloxybenzylideneamino]-2,4-dinitroaniline (BHBANA)