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Novel naphthalene-based bis-pyridinium compounds with pronounced antibacterial activity

Nikita A. Frolov, Vladimir A. Smirnov, Elena V. Detusheva and Anatoly N. Vereshchagin

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1. Biological Experiments

1.1 Samples

Samples, quaternary ammonium compounds based on pyridine were obtained from the N. D. Zelinsky Institute of Organic Chemistry Russian Academy of Sciences (ZIOC RAS).

1.2 Antibacterial Assay

1.2.1 Strains of microorganisms

Reference strains of microorganisms *E. coli* ATCC 25922, *K. pneumoniae* ATCC 70060, *S. aureus* ATCC 43300, *P. aeruginosa* ATCC27853, and *A. baumannii* ATCC 15308 were obtained from the State Collection of Pathogenic Microorganisms (Obolensk, Russia). Clinical strains of *E. coli* B-3421/19, *K. pneumoniae* B-2523/18, *S. aureus* B-8648, *P. aeruginosa* B-2099/18 and *A. baumannii* B-2926/18 isolated in the Molecular Microbiology department of Federal Budget Institution of Science State Research Center for Applied Biotechnology and Microbiology (FBSI SRC PMB, Obolensk, Russia) from clinical samples in the investigation of infections cases in 2016-2018.

1.2.2 Species identification of microorganisms

The species identification of microorganisms was carried out on a MALDI-TOF Biotyper mass spectrometer (Bruker, Germany).

1.2.3 Cultivation of microorganisms

For the cultivation of microorganisms, agar and GRM broth (SRCAMB) were used. Cultivation of microorganisms was carried out for 20-24 hours at a temperature of 37 °C.

1.2.4 Evaluation of the antibacterial activity of new bis-QACs

The MIC was determined by the micromethod of serial dilutions in broth in the presence of various stepwise concentrations of antibacterial drugs in sterile 96-well plates. Mueller-Hinton broth, Mueller-Hinton agar produced by SRCAMB were used as a nutrient medium.

In the course of the study, two-fold serial dilutions of the studied drugs were used. The mother liquor was prepared in 10% DMSO. Working solutions of the drug and its two-fold dilutions (500-1 mg ml⁻¹) were prepared from the stock solution with the addition of the GRM broth.

Nutrient broth with the appropriate concentration of the test drug was introduced into 10 holes in 10 holes in horizontal rows of seven test bacterial strains. Broth was added to separate rows without a drug to control the growth of cultures.

From single colonies grown on the GRM medium at 37 °C for 18 hours, a suspension was prepared with an optical density of 0.5 according to McFarland's standard in sterile saline, which corresponds to approximately $1-2 \times 10^8$ CFU*ml⁻¹. Then the suspension was diluted 1:100 by adding 0.2 ml of the suspension to a flask containing 19.8 ml of Mueller-Hinton broth. The concentration of microorganisms in this case was 10⁶ CFU*ml⁻¹. The original suspension (0.1 ml) was introduced into the wells with the test drug and control wells with broth. The final concentration of microorganism in each well was 5x10⁵ CFU*ml⁻¹. The plates were covered with lids and placed in a thermostat (37 °C) for 20 hours. The presence of bacterial growth was taken into account visually (by the presence of turbidity in the well). The minimum inhibitory concentration (MIC) was taken as the minimum concentration of the preparation at which the growth of bacteria was absent after 20 h of incubation. The minimum bactericidal concentration (MBC) was determined based on the results of seeding on solid nutrient media. For this, from all the wells in which there was no visible growth (by the presence of turbidity), 10 µl were seeded on Mueller-Hinton nutrient agar. The results were taken into account by the presence of culture growth at the site of application after 24 hours of incubation at 37 °C. If there was no growth in the well, but at the same time, the growth of the studied culture was observed when sowing from this well on a dense nutrient medium, then this concentration was taken as bacteriostatic. The MBC

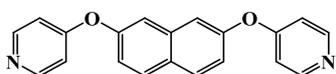
was taken as the lowest concentration at which cell growth was completely inhibited when plated on a solid nutrient medium.

2. Chemical Experimental Details

Reagents were purchased and used without further purification. The reactions were monitored by TLC on silica gel 60 F254 aluminum sheets under UV light. All products were analyzed by NMR spectroscopy on Bruker AM300, Bruker XWIND or Bruker AV600 spectrometers at ambient temperature in DMSO- d_6 or $CDCl_3$. Biologically tested compound (Product) purities were confirmed by HPLC on a Stayer 0892 series HPLC system with Luna® 5 μ m C18 100 Å, LC column 250 x 4.6 mm. Mobile phase: 85:15 MeCN/H₂O (0.25 M NaClO₄, 0.1% H₃PO₄). All melting points were determined on a Gallenkamp melting point apparatus in open capillaries and are uncorrected. IR spectra were recorded with a Bruker ALPHA-T FT-IR spectrometer in KBr pellets. The ESI contains detailed experimental protocols, summaries of spectral data, and spectra.

3. Synthesis and Characterization of Substrates and Products

Synthesis of 4,4'-[naphthalene-2,7-diylbis(oxy)]dipyridine (2):



A mixture of 2,7-dihydroxynaphthalene (1.60 g, 10 mmol), 4-chloropyridine hydrochloride (3.00 g, 20 mmol), copper(I) iodide (1.90 g, 10 mmol), potassium phosphate (16.96 g, 80 mmol) and picolinic acid (0.25 g, 2 mmol) in dry DMSO (50 ml) was heated to 140 °C for 72 hours in argon atmosphere. The solvent was removed under reduced pressure, ethyl acetate (50 ml) was added to the crude residue, and the mixture was heated to reflux for 1 hour. Then mixture was filtered through a filter paper. The solid remaining on the filter paper was washed with hot ethyl acetate (20 ml). The organic filtrate was concentrated under reduced pressure and the residue was purified by recrystallization in heptane to provide a white solid product (2.67 g, 8.5 mmol, 85% yield).

C₂₀H₁₄N₂O₂; M=314.34; White solid; M. p. 96-98°C; ¹H NMR (300 MHz, CDCl₃): δ 6.95 (d, J=5.3 Hz, 4H, 4CH_{Py}), 7.27 (dd, J=8.6, 2.1 Hz, 2H, 2CH_{Ar}), 7.48 (d, J - 2.1 Hz, 2H, 2CH_{Ar}), 7.96 (d, J=8.6 Hz, 2H, 2CH_{Ar}), 8.53 (d, J=5.3 Hz, 4H, 4CH_{Py}) ppm; ¹³C NMR (75 MHz, CDCl₃): δ 112.6, 116.7, 120.2, 128.8, 130.5, 135.3, 151.4, 152.8, 164.6 ppm; MS (EI, 70 eV): m/z=314 [M⁺].

Quaternization of 4,4'-[naphthalene-2,7-diylbis(oxy)]dipyridine (2) with alkyl halides (3a-g):



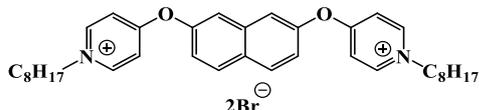
To a solution of 4,4'-[naphthalene-2,7-diylbis(oxy)]dipyridine (0.36 g, 1 mmol) in acetonitrile (3 ml) was added alkyl halide (4 mmol). The mixture was heated under reflux for 24 hours, then cooled and filtered. The filtered solid was washed with cold acetone (10 ml) and dried to give a white solid product. The yield is 68-90% depend on alkyl halide.

4,4'-[Naphthalene-2,7-diylbis(oxy)]bis(1-heptylpyridinium) dibromide (3a):



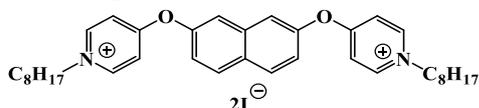
C₃₄H₄₄Br₂N₂O₂; M. w. 672.6; White solid (0.61 g, 0.91 mmol, 91% yield); M. p. 134-140°C; ¹H NMR (300 MHz, DMSO-*d*₆): δ 0.86 (t, J = 6.6 Hz, 6H, 2CH₃), 1.16-1.38 (m, 16H, 8CH₂), 1.79-1.94 (m, 4H, 2CH₂), 4.51 (t, J = 6.8 Hz, 4H, 2CH₂N⁺), 7.60 (dd, J = 8.9, 2.4 Hz, 2H, 2CH_{Ar}), 7.66 (d, J = 7.3 Hz, 4H, 4CH_{py}), 8.00 (d, J = 2.4 Hz, 2H, 2CH_{Ar}), 8.32 (d, J = 8.9 Hz, 2H, 2CH_{Ar}), 8.99 (d, J = 7.3 Hz, 4H, 4CH_{py}) ppm; ¹³C NMR (75 MHz, DMSO-*d*₆): δ 13.8, 21.9, 25.3, 28.0, 30.6, 31.0, 59.4, 114.9, 118.1, 120.5, 130.22, 131.6, 134.9, 146.9, 150.7, 169.0 ppm;

4,4'-[Naphthalene-2,7-diylbis(oxy)]bis(1-octylpyridinium) dibromide (3b):



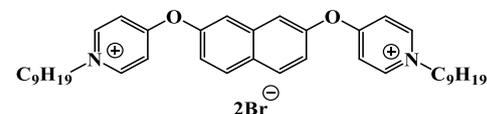
C₃₆H₄₈Br₂N₂O₂; M. w. 700.6; White solid (0.54 g, 0.77 mmol, 77% yield); M. p. 150-152°C; ¹H NMR (300 MHz, DMSO-*d*₆): δ 0.86 (t, J = 6.6 Hz, 6H, 2CH₃), 1.15-1.38 (m, 20H, 10CH₂), 1.79-1.92 (m, 4H, 2CH₂), 4.51 (t, J = 6.8 Hz, 4H, 2CH₂N⁺), 7.60 (dd, J = 8.9, 2.4 Hz, 2H, 2CH_{Ar}), 7.66 (d, J = 7.3 Hz, 4H, 4CH_{py}), 8.00 (d, J = 2.4 Hz, 2H, 2CH_{Ar}), 8.32 (d, J = 8.9 Hz, 2H, 2CH_{Ar}), 8.99 (d, J = 7.3 Hz, 4H, 4CH_{py}) ppm; ¹³C NMR (75 MHz, DMSO-*d*₆): δ 13.8, 21.9, 25.2, 28.3, 28.4, 30.5, 31.0, 59.1, 114.8, 118.1, 120.4, 130.1, 131.5, 134.8, 146.9, 150.6, 168.9 ppm;

4,4'-[Naphthalene-2,7-diylbis(oxy)]bis(1-octylpyridinium) diiodide (3c):



C₃₆H₄₈I₂N₂O₂; M. w. 794.6; White solid (0.58 g, 0.74 mmol, 74% yield); M. p. 104-107°C; ¹H NMR (300 MHz, DMSO-*d*₆): δ 0.86 (t, J = 6.6 Hz, 6H, 2CH₃), 1.18-1.32 (m, 20H, 10CH₂), 1.79-1.94 (m, 4H, 2CH₂), 4.50 (t, J = 6.8 Hz, 4H, 2CH₂N⁺), 7.60 (dd, J = 8.9, 2.4 Hz, 2H, 2CH_{Ar}), 7.65 (d, J = 7.3 Hz, 4H, 4CH_{py}), 7.99 (d, J = 2.4 Hz, 2H, 2CH_{Ar}), 8.32 (d, J = 8.9 Hz, 2H, 2CH_{Ar}), 8.95 (d, J = 7.3 Hz, 4H, 4CH_{py}) ppm; ¹³C NMR (75 MHz, DMSO-*d*₆): δ 14.4, 22.5, 25.8, 28.8, 28.9, 31.0, 31.6, 59.7, 115.4, 118.6, 121.0, 130.7, 132.0, 135.3, 147.4, 151.2, 169.4 ppm;

4,4'-[Naphthalene-2,7-diylbis(oxy)]bis(1-nonylpyridinium) dibromide (3d):



C₃₈H₅₂Br₂N₂O₂; M. w. 728.7; White solid (0.66 g, 0.9 mmol, 90% yield); M. p. 184-186°C; ¹H NMR (300 MHz, DMSO-*d*₆): δ 0.85 (t, J = 6.4 Hz, 6H, 2CH₃), 1.15-1.37 (m, 24H, 12CH₂), 1.78-1.94 (m, 4H, 2CH₂), 4.51 (t, J = 6.8 Hz, 4H, 2CH₂N⁺), 7.60 (dd, J = 8.9, 2.4 Hz, 2H, 2CH_{Ar}), 7.66 (d, J = 7.3 Hz, 4H, 4CH_{py}), 8.00 (d, J = 2.4 Hz, 2H, 2CH_{Ar}), 8.32 (d, J = 8.9 Hz, 2H, 2CH_{Ar}), 9.00 (d, J = 7.3 Hz, 4H, 4CH_{py}) ppm; ¹³C NMR (75 MHz, DMSO-*d*₆): δ 13.8, 22.0, 25.2, 28.3, 28.5, 28.7, 30.5, 31.1, 59.1, 114.8, 118.1, 120.4, 130.1, 131.5, 134.8, 146.9, 150.6, 168.9 ppm;

4,4'-[Naphthalene-2,7-diylbis(oxy)]bis(1-decylpyridinium) dibromide (3e):



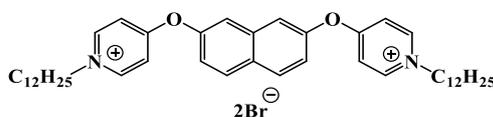
$C_{40}H_{56}Br_2N_2O_2$; M. w. 756.7; White solid (0.63 g, 0.83 mmol, 83% yield); M. p. 197-198°C; 1H NMR (300 MHz, $DMSO-d_6$): δ 0.85 (t, $J = 6.4$ Hz, 6H, 2CH₃), 1.14-1.33 (m, 28H, 14CH₂), 1.79-1.91 (m, 4H, 2CH₂), 4.49 (t, $J = 6.8$ Hz, 4H, 2CH₂N⁺), 7.59 (dd, $J = 8.9, 2.4$ Hz, 2H, 2CH_{Ar}), 7.65 (d, $J = 7.3$ Hz, 4H, 4CH_{py}), 7.98 (d, $J = 2.4$ Hz, 2H, 2CH_{Ar}), 8.31 (d, $J = 8.9$ Hz, 2H, 2CH_{Ar}), 8.96 (d, $J = 7.3$ Hz, 4H, 4CH_{py}) ppm; ^{13}C NMR (75 MHz, $DMSO-d_6$): δ 13.8, 22.0, 25.2, 28.3, 28.5, 28.7, 28.8, 30.5, 31.1, 59.1, 114.8, 118.1, 120.4, 130.1, 131.5, 134.8, 146.9, 150.6, 168.9 ppm;

4,4'-[Naphthalene-2,7-diylbis(oxy)]bis(1-undecylpyridinium) dibromide (3f):



$C_{42}H_{60}Br_2N_2O_2$; M. w. 784.8; White solid (0.67 g, 0.85 mmol, 85% yield); M. p. 200-204°C; 1H NMR (300 MHz, $DMSO-d_6$): δ 0.86 (t, $J = 6.4$ Hz, 6H, 2CH₃), 1.14-1.39 (m, 32H, 16CH₂), 1.79-1.91 (m, 4H, 2CH₂), 4.51 (t, $J = 6.8$ Hz, 4H, 2CH₂N⁺), 7.59 (dd, $J = 8.9, 2.4$ Hz, 2H, 2CH_{Ar}), 7.66 (d, $J = 7.3$ Hz, 4H, 4CH_{py}), 8.00 (d, $J = 2.4$ Hz, 2H, 2CH_{Ar}), 8.32 (d, $J = 8.9$ Hz, 2H, 2CH_{Ar}), 8.99 (d, $J = 7.3$ Hz, 4H, 4CH_{py}) ppm; ^{13}C NMR (75 MHz, $DMSO-d_6$): δ 13.8, 22.0, 25.2, 28.3, 28.6, 28.7, 28.8, 30.5, 31.2, 59.1, 114.8, 118.1, 120.4, 130.1, 131.5, 134.8, 146.9, 150.6, 168.9 ppm;

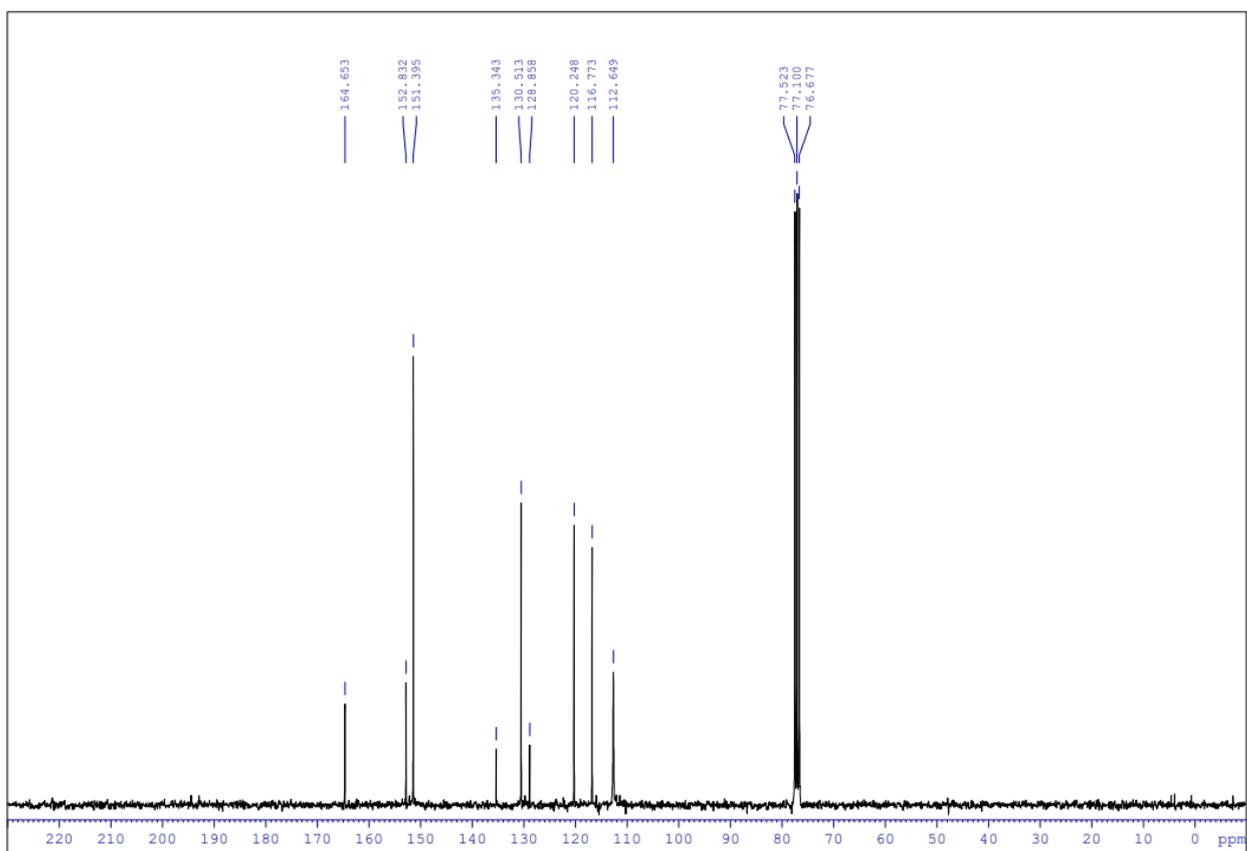
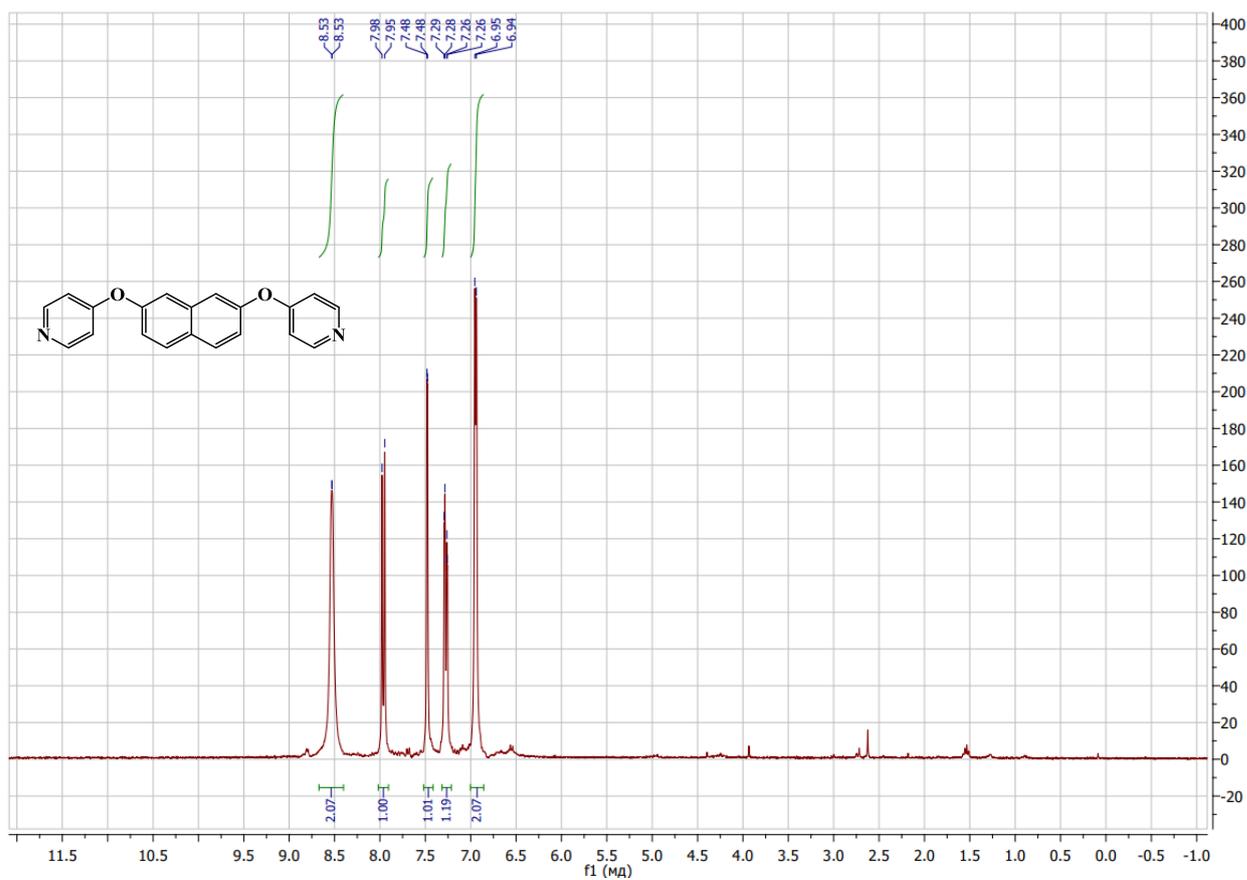
4,4'-[Naphthalene-2,7-diylbis(oxy)]bis(1-dodecylpyridinium) dibromide (3g):



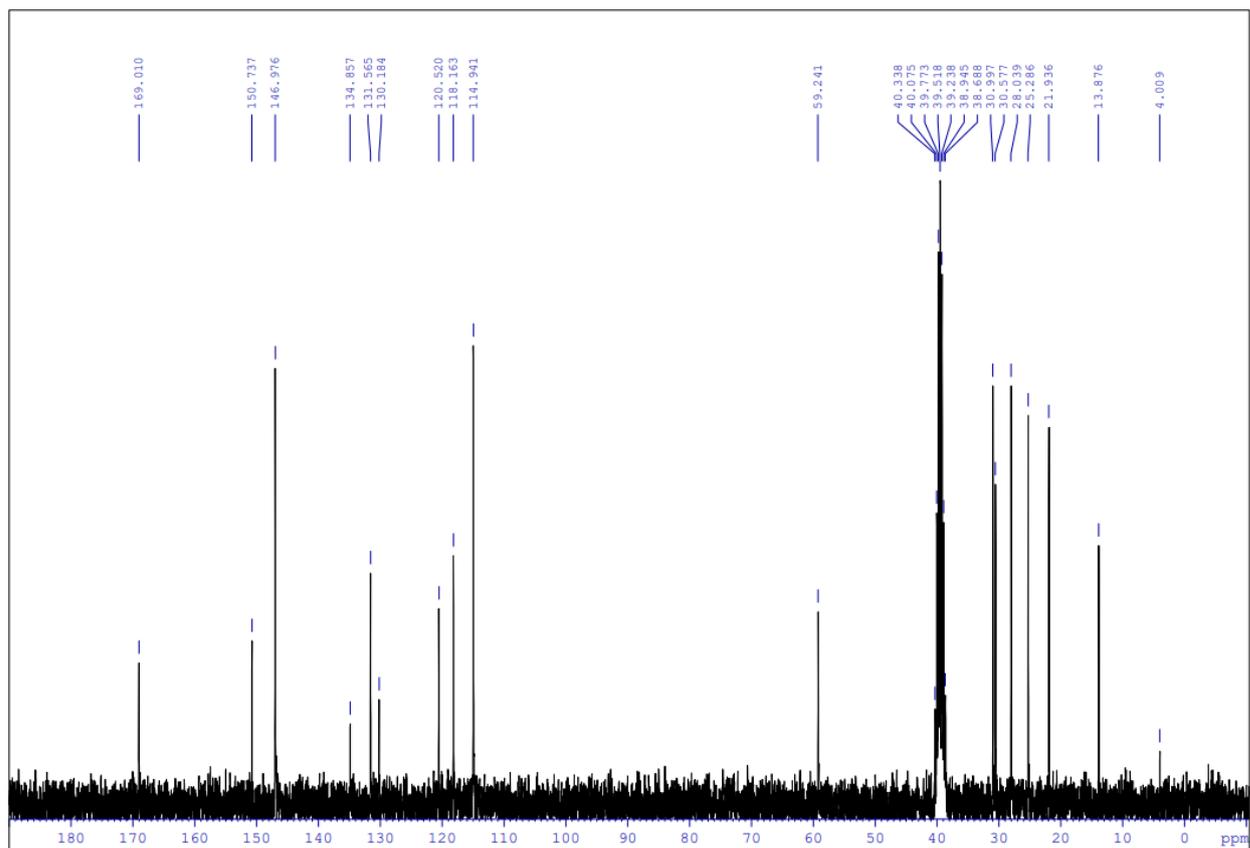
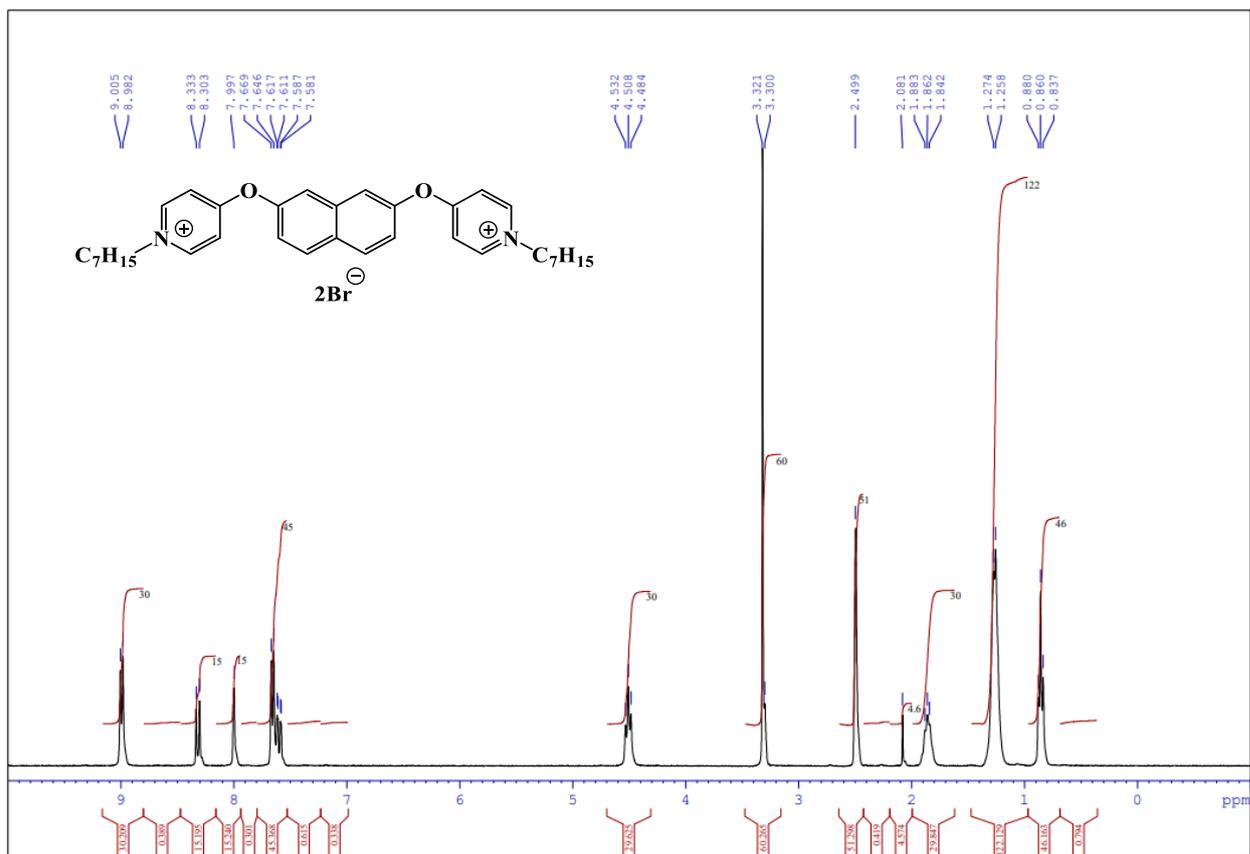
$C_{44}H_{64}Br_2N_2O_2$; M. w. 812.8; White solid (0.55 g, 0.68 mmol, 68% yield); M. p. 213-216°C; 1H NMR (300 MHz, $DMSO-d_6$): δ 0.85 (t, $J = 6.4$ Hz, 6H, 2CH₃), 1.12-1.40 (m, 36H, 18CH₂), 1.72-1.92 (m, 4H, 2CH₂), 4.50 (t, $J = 6.8$ Hz, 4H, 2CH₂N⁺), 7.60 (dd, $J = 8.9, 2.4$ Hz, 2H, 2CH_{Ar}), 7.65 (d, $J = 7.3$ Hz, 4H, 4CH_{py}), 7.99 (d, $J = 2.4$ Hz, 2H, 2CH_{Ar}), 8.32 (d, $J = 8.9$ Hz, 2H, 2CH_{Ar}), 8.98 (d, $J = 7.3$ Hz, 4H, 4CH_{py}) ppm; ^{13}C NMR (75 MHz, $DMSO-d_6$): δ 13.8, 22.0, 25.2, 28.3, 28.6, 28.7, 28.8, 30.5, 31.2, 59.1, 114.8, 118.1, 120.4, 130.1, 131.5, 134.8, 146.9, 150.6, 168.9 ppm;

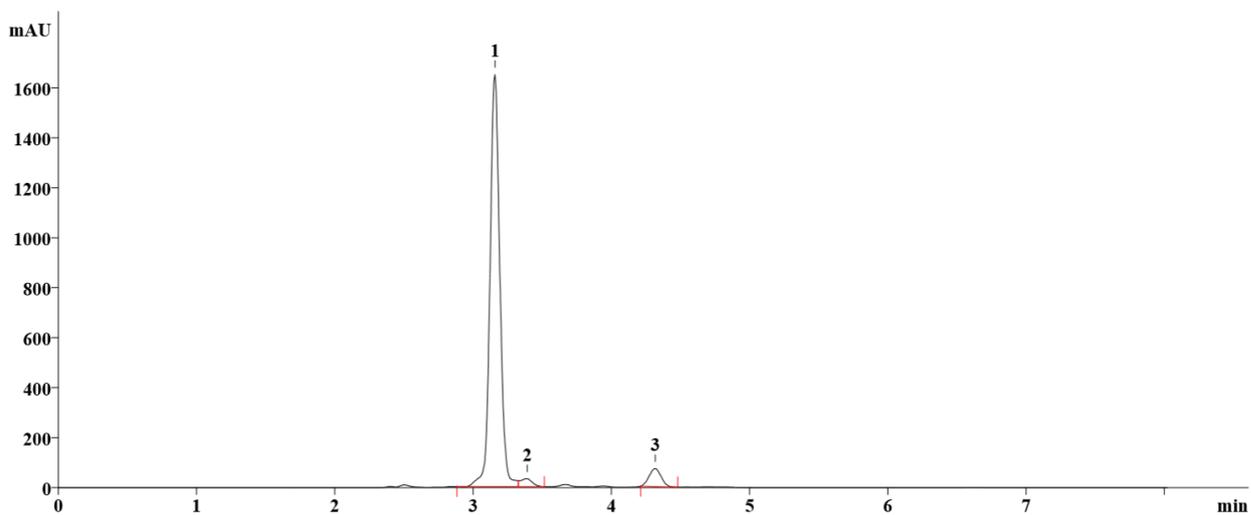
4. ^1H NMR, ^{13}C NMR and HPLC data

2



3a – 7, Br

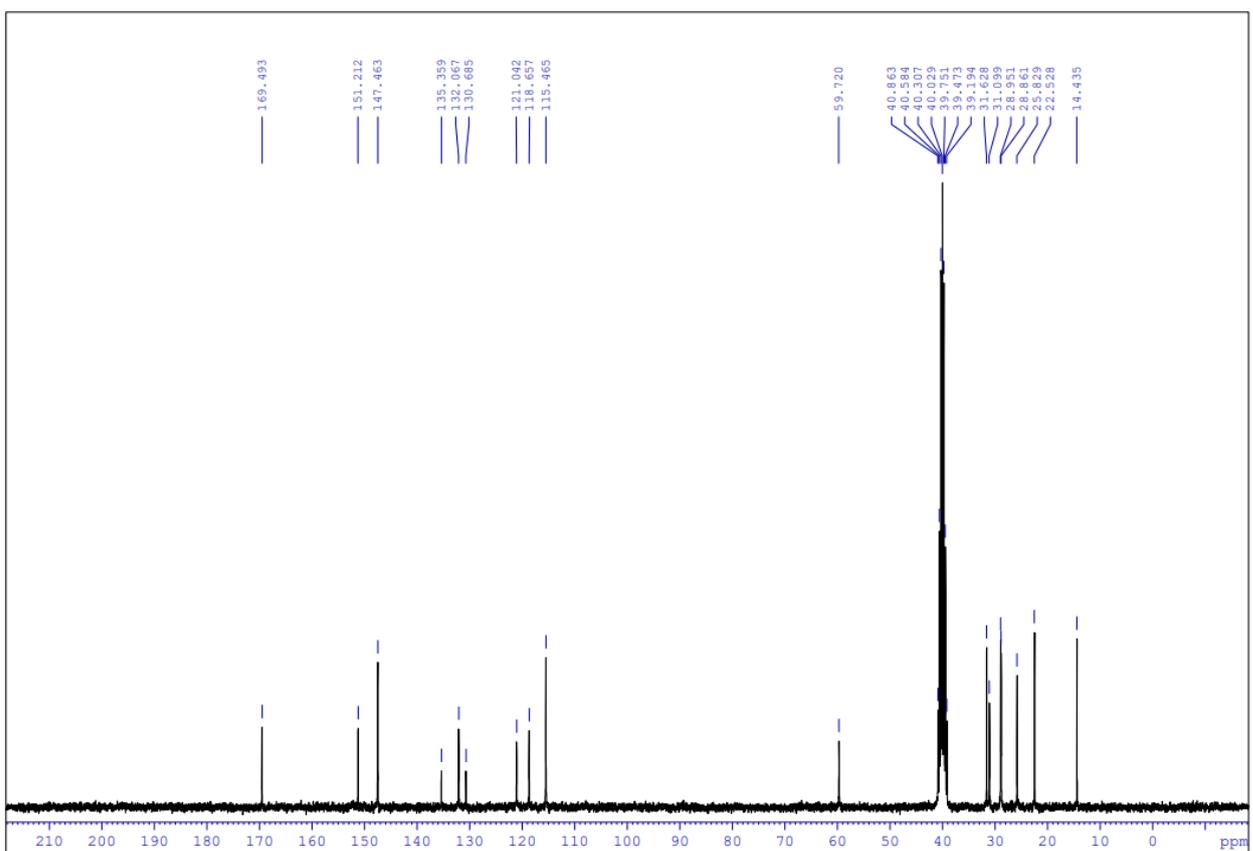
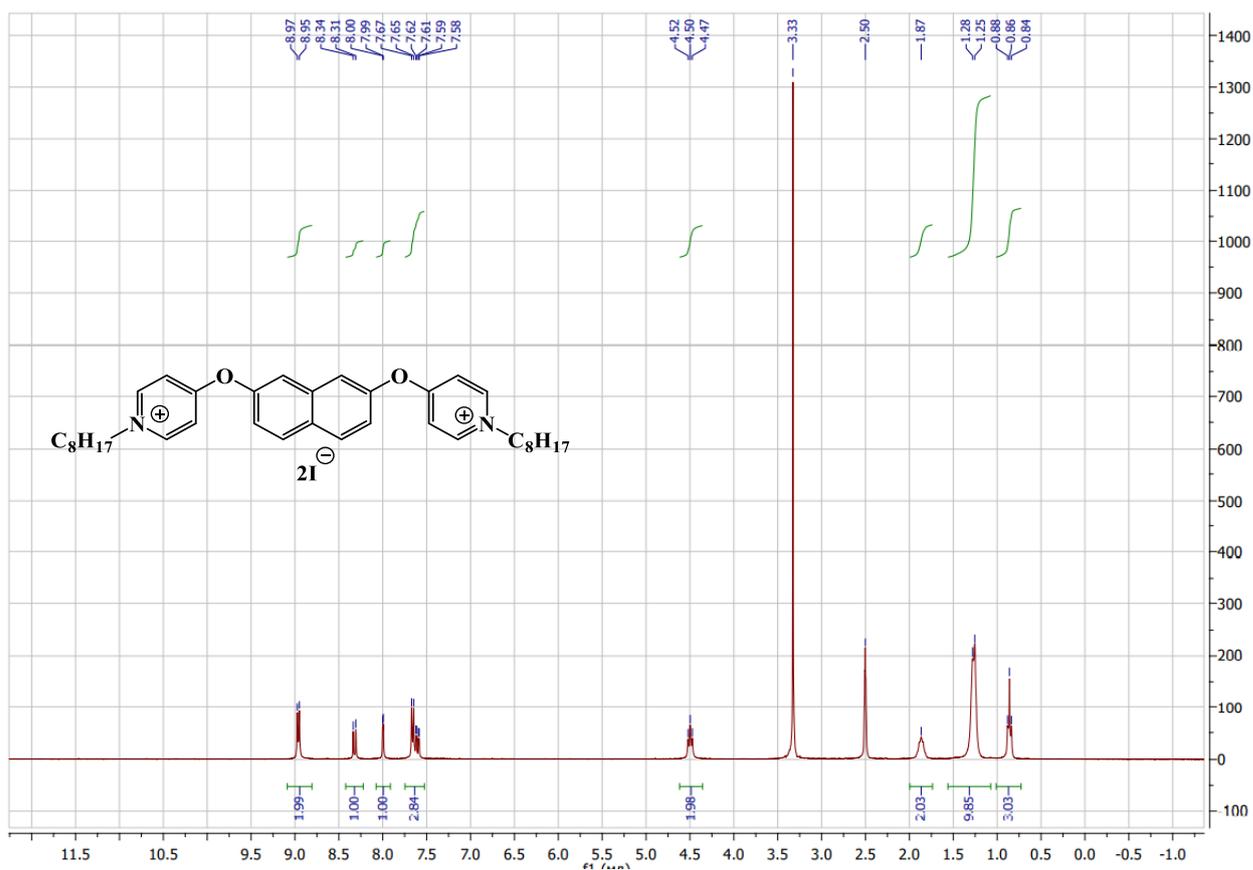




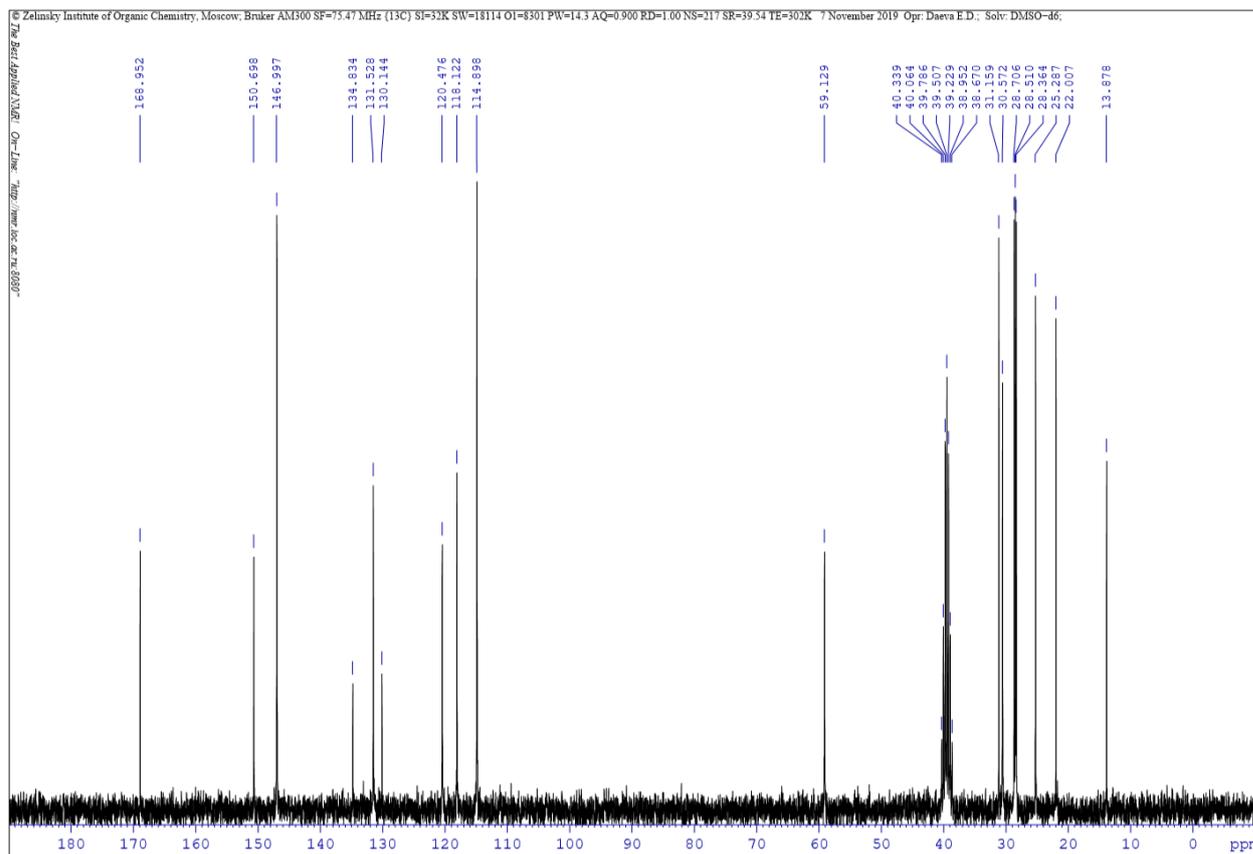
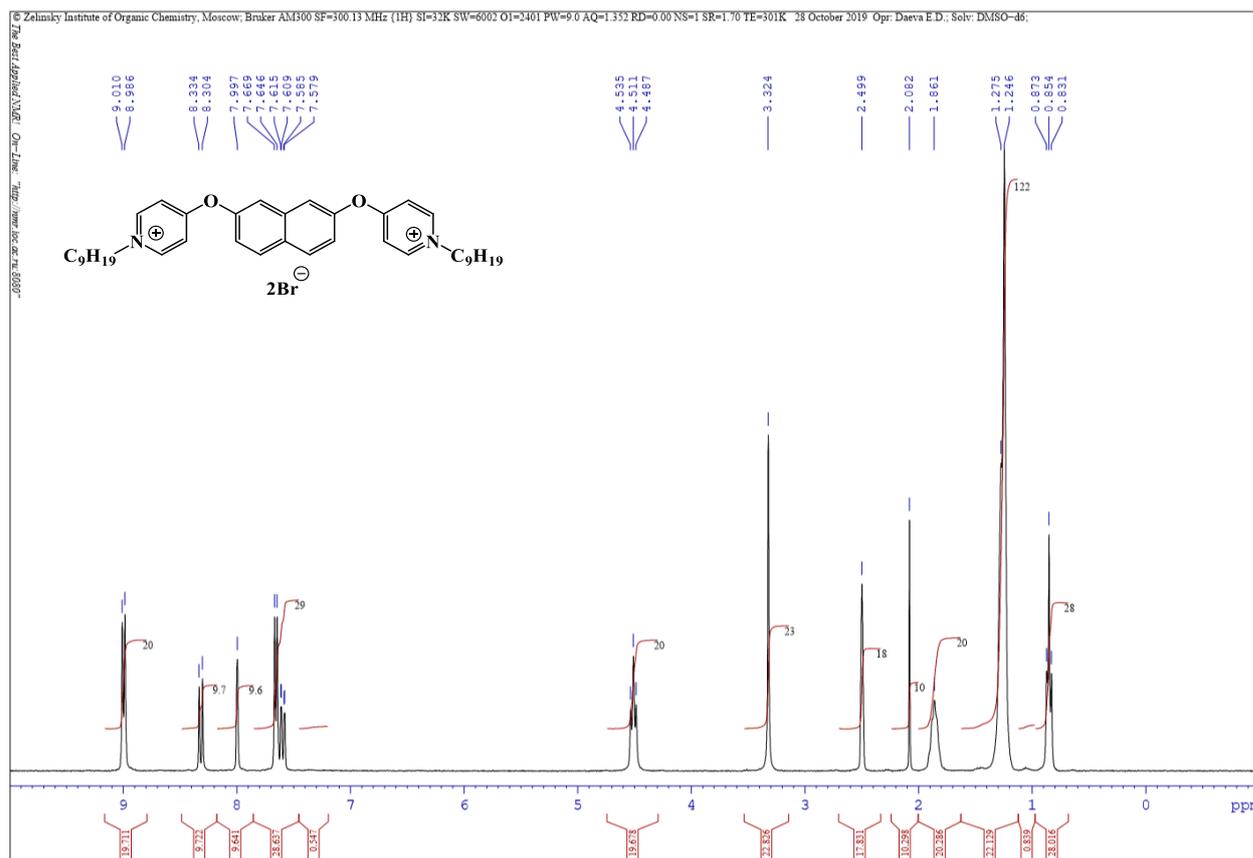
Peak table

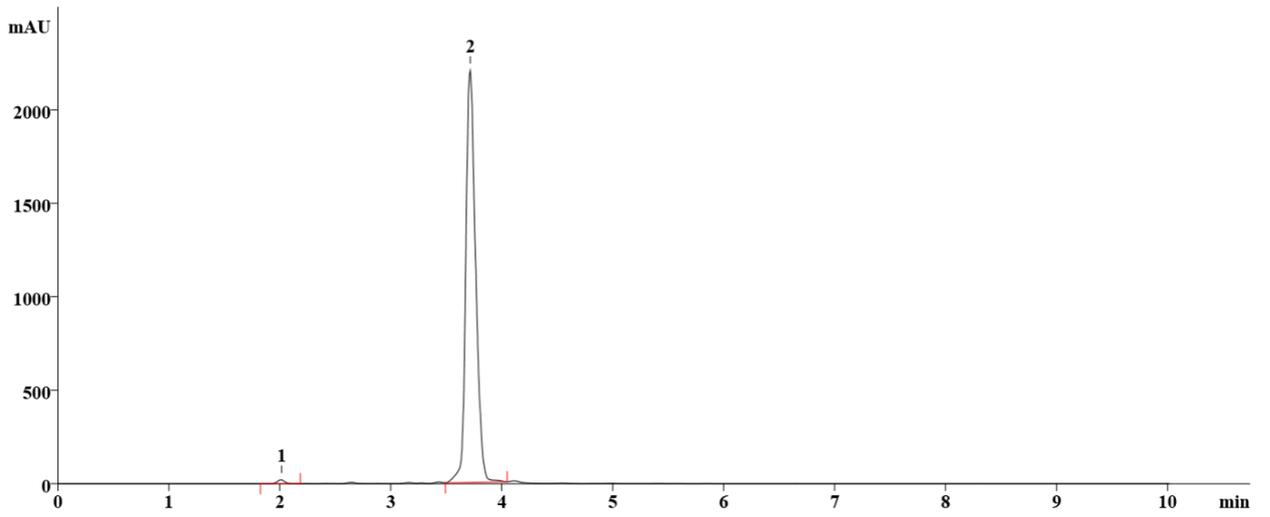
Peak	Retention min	Area	Height	Width h/2	Area%	Type
1	3,16	8139,973	1650,055	0,0732	92,925	BD :
2	3,39	188,335	31,489	0,103	2,150	DB :
3	4,32	431,413	71,699	0,0970	4,925	BB :

3c – 8, I



3d – 9, Br

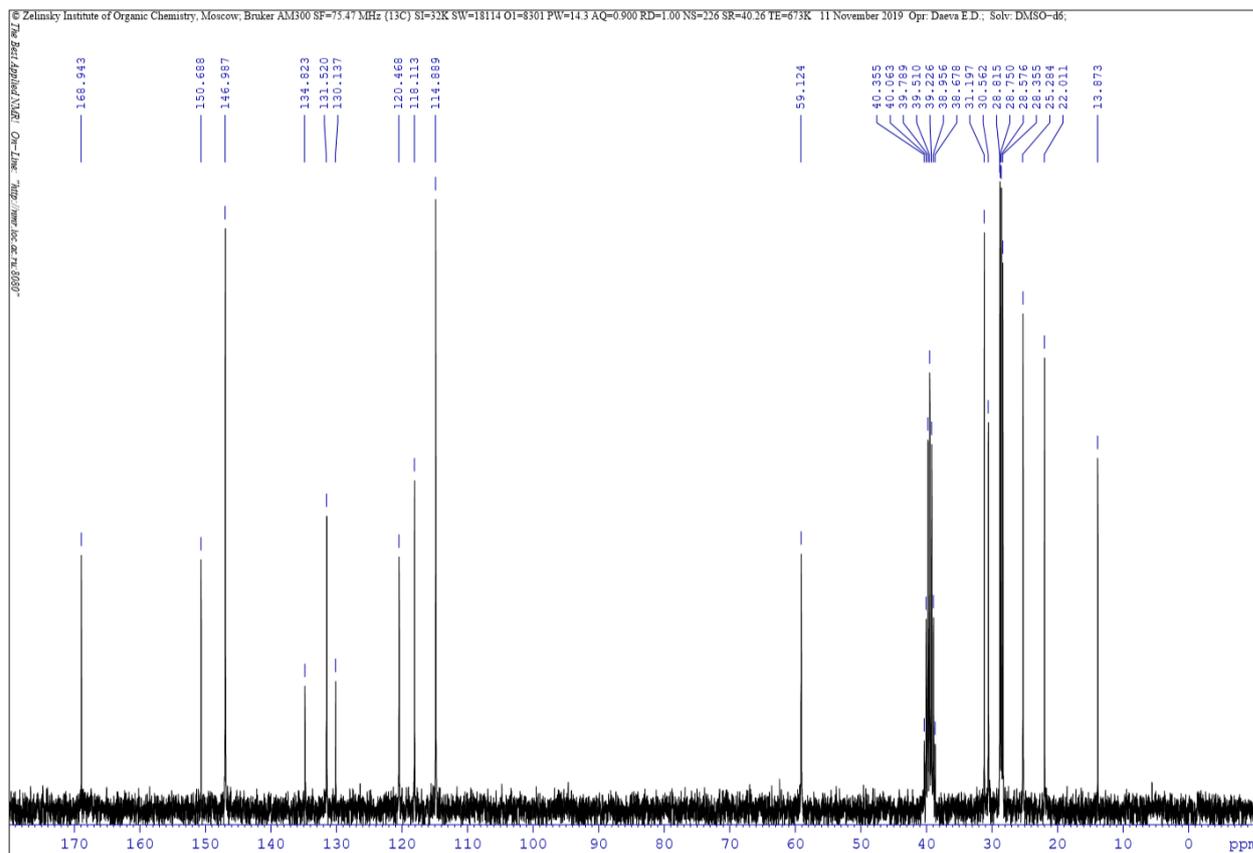
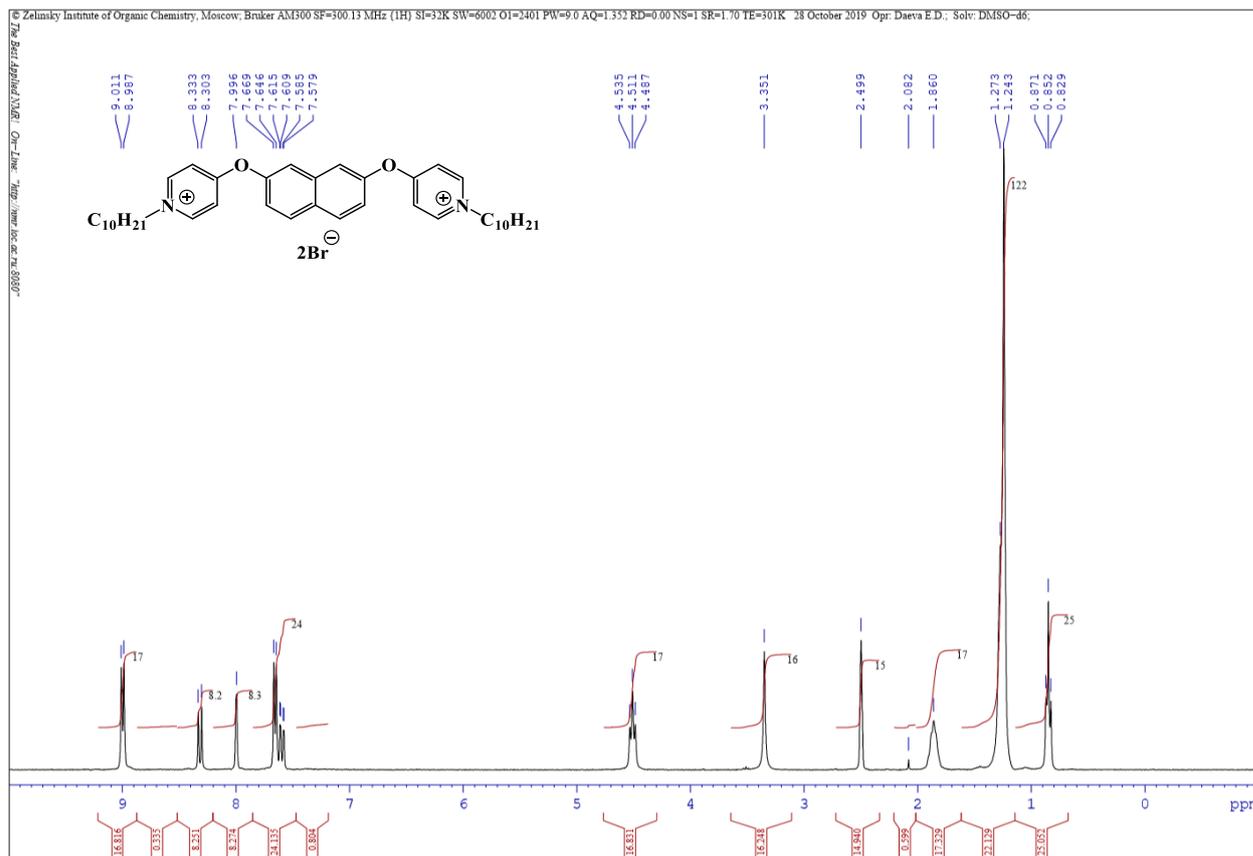


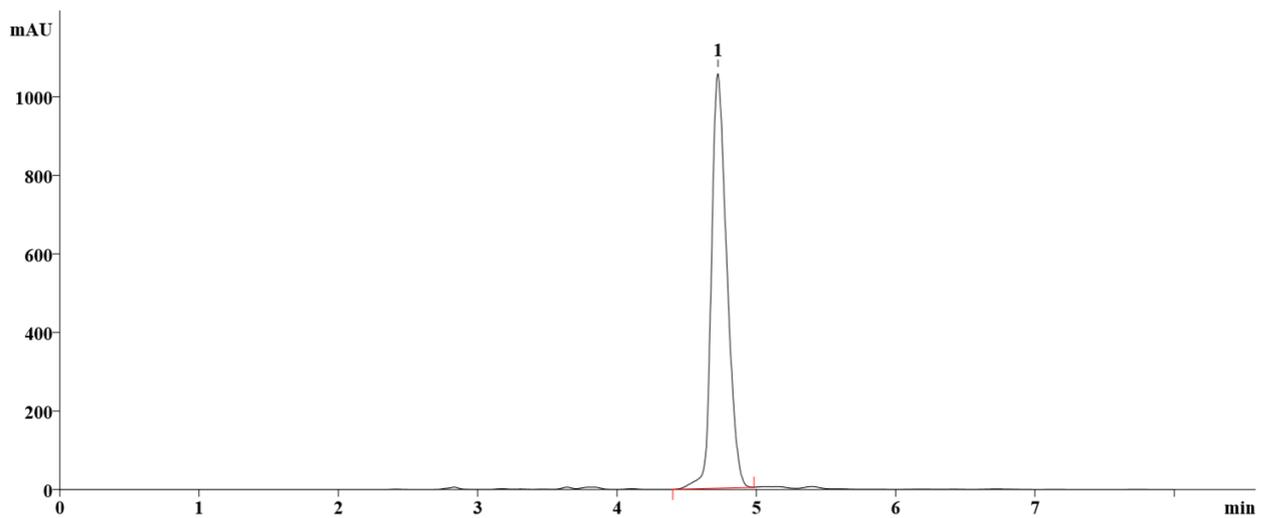


Peak table

Peak	Retention min	Area	Height	Width h/2	Area%	Type
1	2,01	96,386	20,446	0,0712	0,703	BB :
2	3,72	13611,056	2204,894	0,0948	99,297	BB :

3e – 10, Br

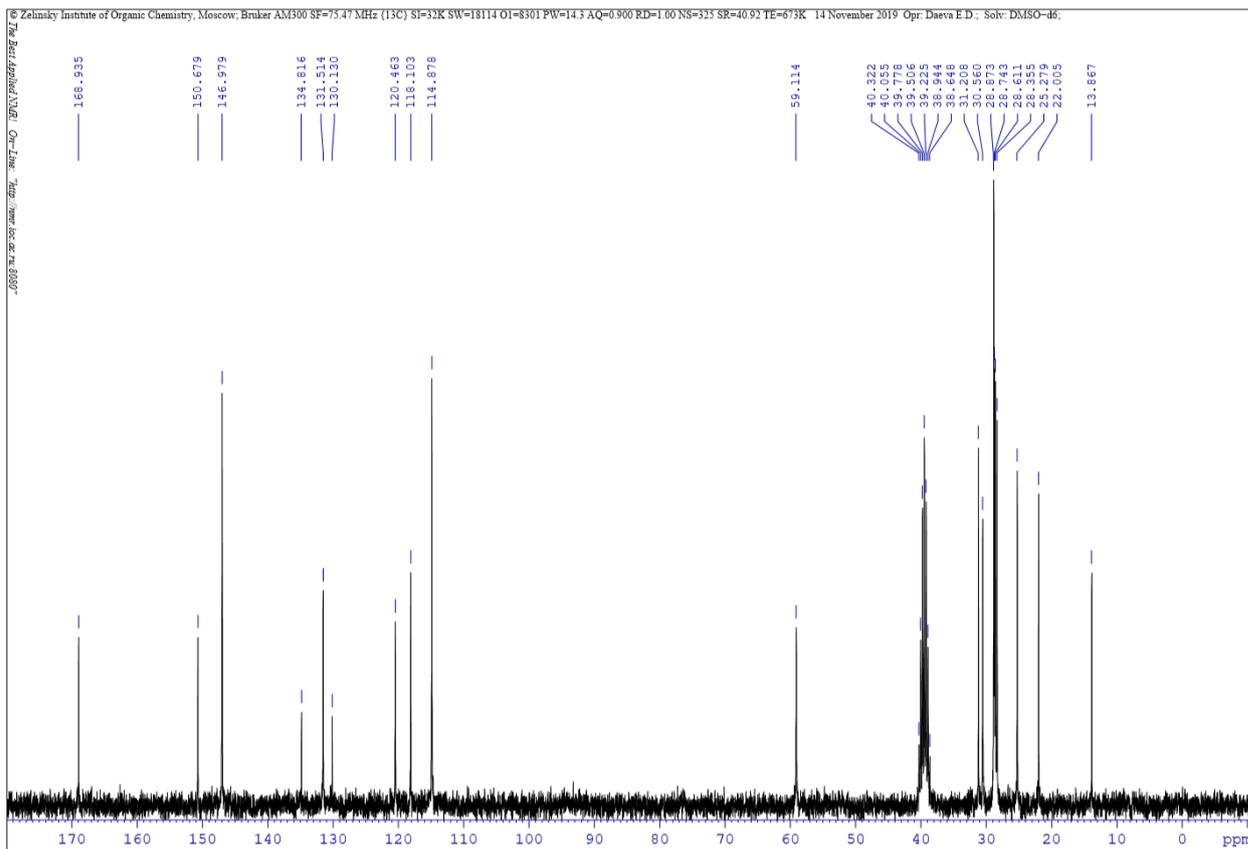
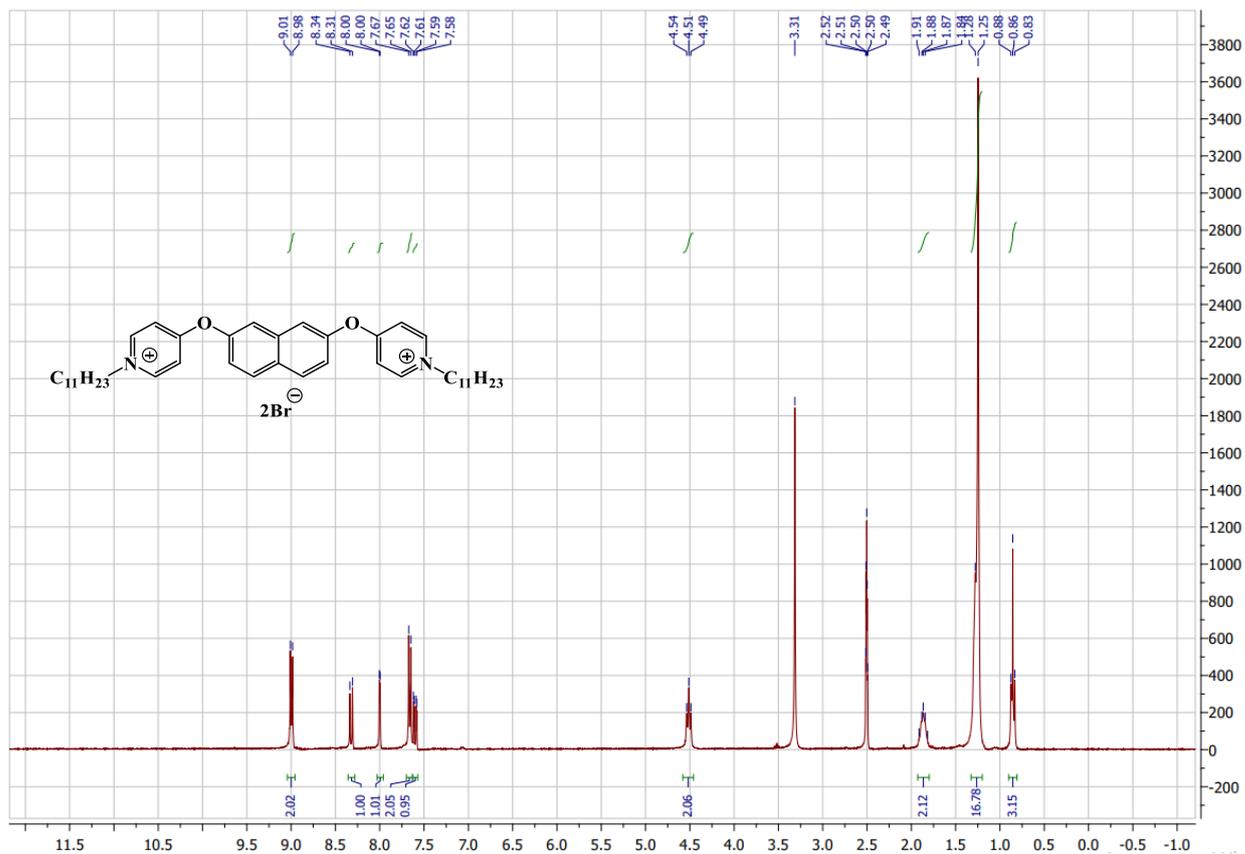


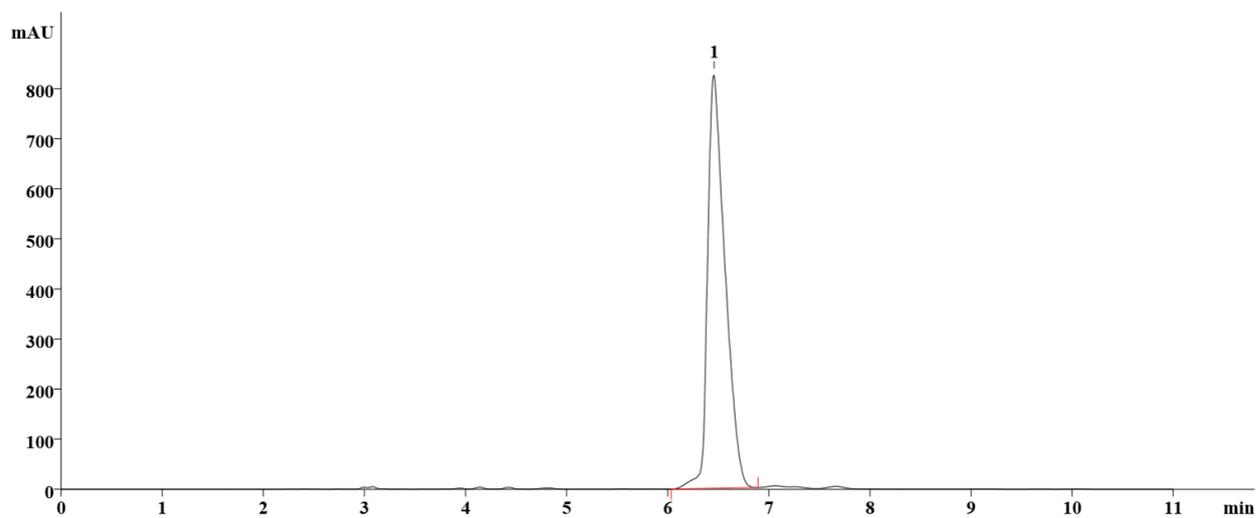


Peak table

<i>Peak</i>	<i>Retention min</i>	<i>Area</i>	<i>Height</i>	<i>Width h/2</i>	<i>Area%</i>	<i>Type</i>
1	4,72	8146,471	1054,421	0,118	100,00	BB :

3f – 11, Br

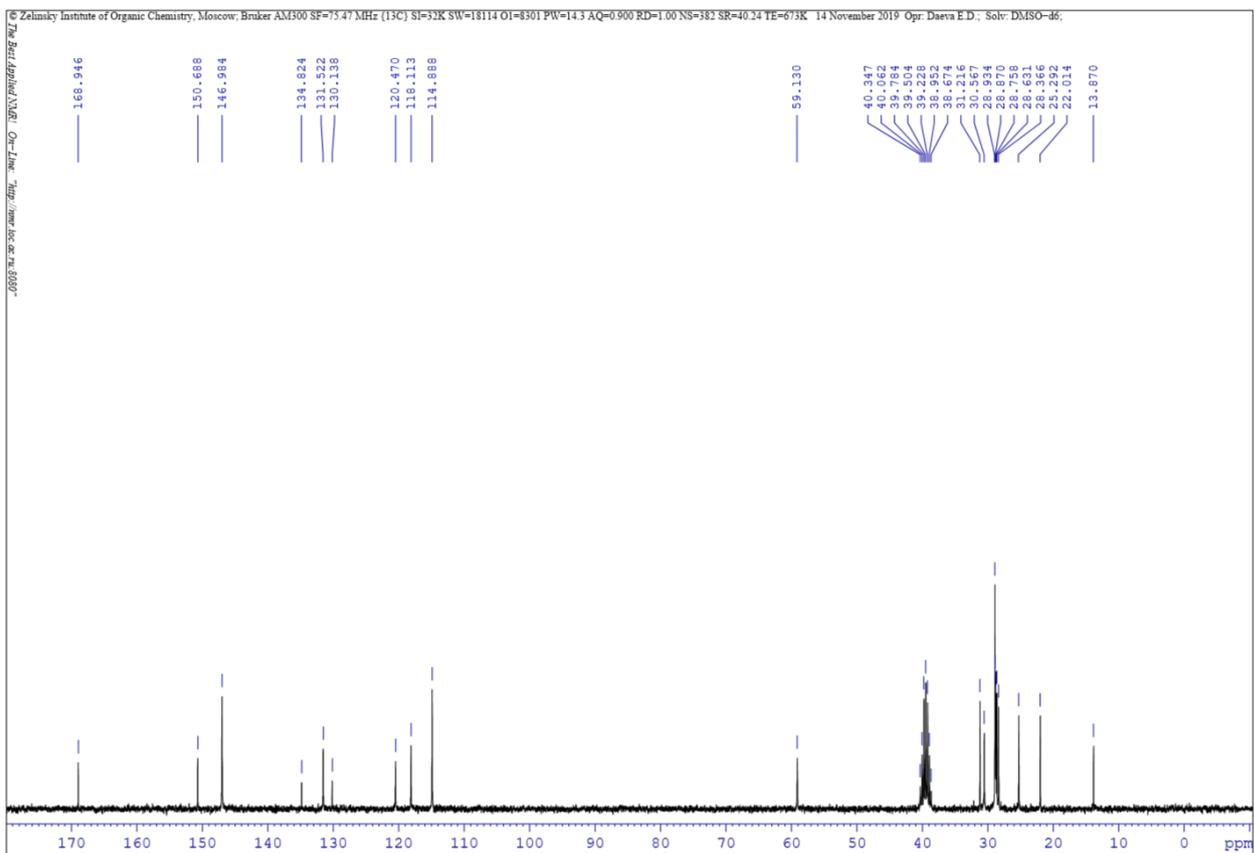
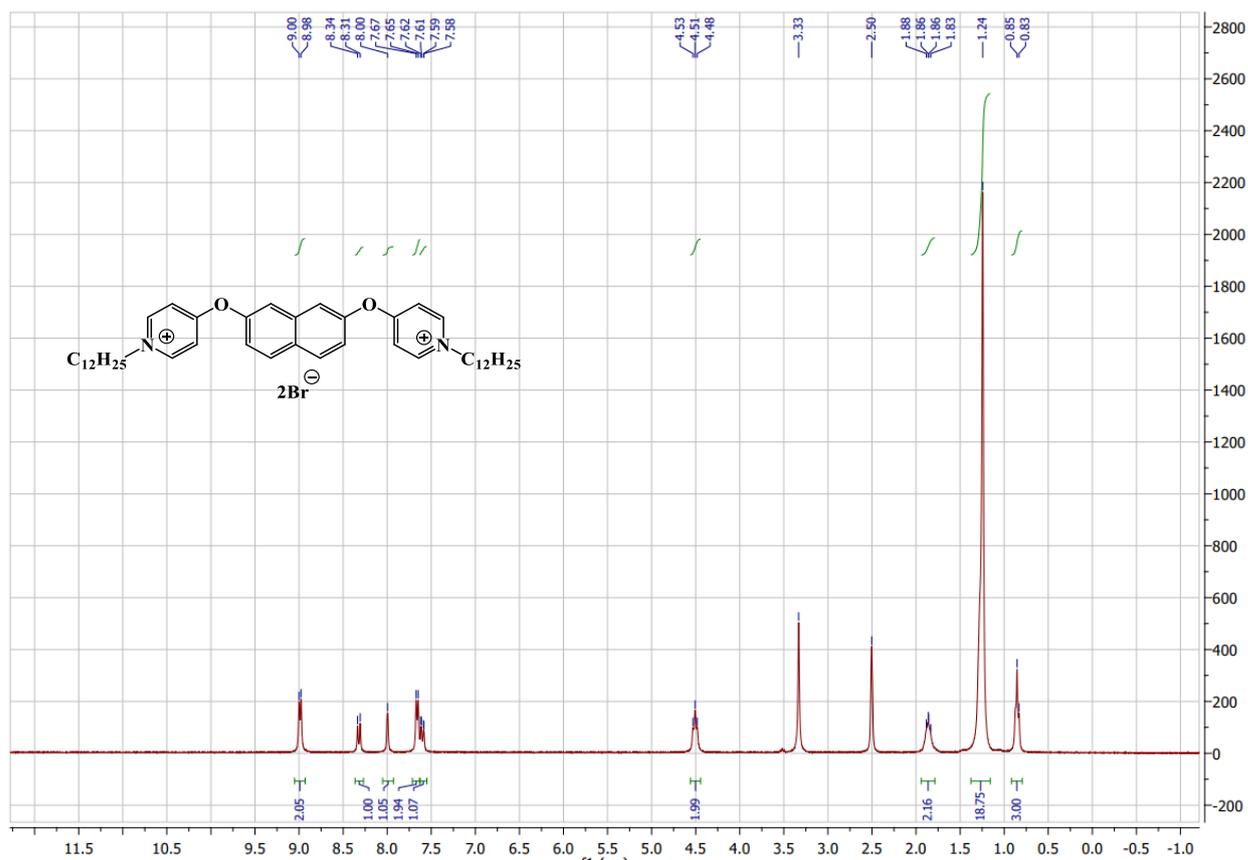


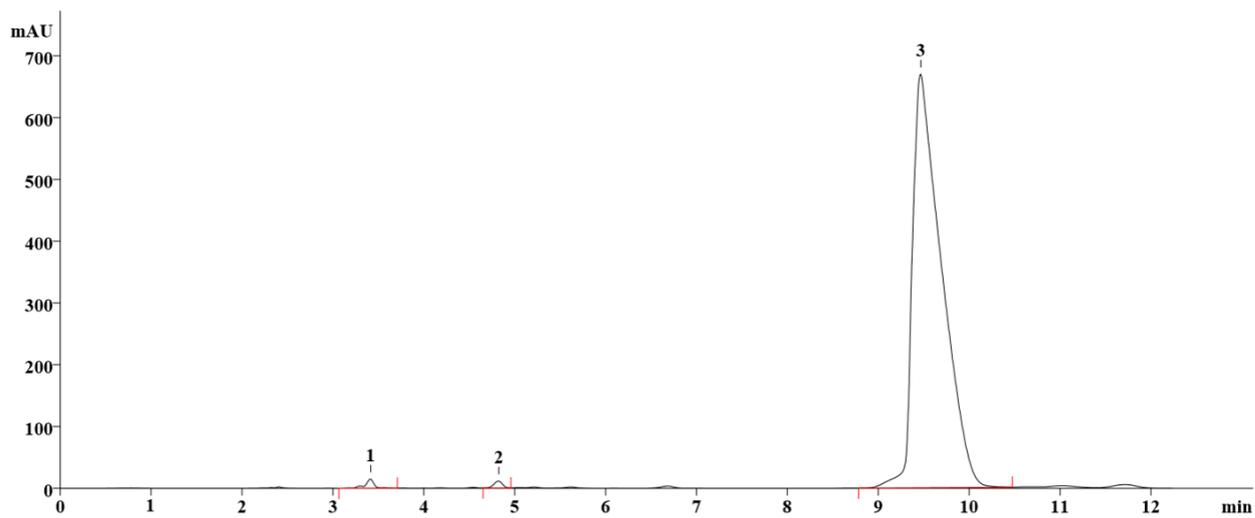


Peak table

<i>Peak</i>	<i>Retention min</i>	<i>Area</i>	<i>Height</i>	<i>Width h/2</i>	<i>Area%</i>	<i>Type</i>
1	6,46	9896,183	824,172	0,186	100,00	BB :

3g – 12, Br





Peak table

<i>Peak</i>	<i>Retention min</i>	<i>Area</i>	<i>Height</i>	<i>Width h/2</i>	<i>Area%</i>	<i>Type</i>
1	3,41	95,894	14,590	0,0817	0,636	BB :
2	4,82	72,374	11,026	0,102	0,480	BB :
3	9,46	14902,646	668,986	0,351	98,883	BB :