

**Synthesis and biological evaluation of novel bis-quaternary ammonium compounds with *p*-terphenyl spacer**

**Anatoly N. Vereshchagin, Nikita A. Frolov, Valeria Yu. Konyuhova, Evgeniya O. Dorofeeva, Karl A. Hansford and Mikhail P. Egorov**

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# 1. Biological Experiments

## 1.1 Sample Preparation

Samples were provided by the collaborator and stored frozen at -20 °C. Samples were prepared in DMSO and water to a final testing concentration of 32 µg ml<sup>-1</sup> or 20 µM (unless otherwise indicated in the data sheet) and serially diluted 1:2 fold for 8 times. Each sample concentration was prepared in 384-well plates, non-binding surface plate (NBS; Corning 3640) for each bacterial/fungal strain, tissue-culture treated (TC-treated; Corning 3712/3764) black for mammalian cell types and polypropylene 384-well (PP; Corning 3657) for haemolysis assays, all in duplicate (n=2), and keeping the final DMSO concentration to a maximum of 0.5%. All the sample preparation was done using liquid handling robots.

Compounds that showed notable solubility issues during stock solution preparation are detailed in the Data sheet for the individual Project.

## 1.2 Antibacterial Assay

### 1.2.1 Procedure

All bacteria were cultured in Cation-adjusted Mueller Hinton broth (CAMHB) at 37 °C overnight. A sample of each culture was then diluted 40-fold in fresh broth and incubated at 37 °C for 1.5-3 h. The resultant mid-log phase cultures were diluted (CFU/mL measured by OD<sub>600</sub>), then added to each well of the compound containing plates, giving a cell density of 5 x 10<sup>5</sup> CFU ml<sup>-1</sup> and a total volume of 50 µl. All the plates were covered and incubated at 37 °C for 18 h without shaking.

### 1.2.2 Analysis

Inhibition of bacterial growth was determined measuring absorbance at 600 nm (OD<sub>600</sub>), using a Tecan M1000 Pro monochromator plate reader. The percentage of growth inhibition was calculated for each well, using the negative control (media only) and positive control (bacteria without inhibitors) on the same plate as references.

The percentage of growth inhibition was calculated for each well, using the negative control (media only) and positive control (bacteria without inhibitors) on the same plate. The *MIC* was determined as the lowest concentration at which the growth was fully inhibited, defined by an inhibition ≥ 80%. In addition, the maximal percentage of growth inhibition is reported as *DMax*, indicating any compounds with partial activity.

Hits were classified by *MIC* ≤ 16 µg ml<sup>-1</sup> or *MIC* ≤ 10 µM in either replicate (n=2 on different plates).

## 1.3 Antifungal Assay

### 1.3.1 Procedure

Fungi strains were cultured for 3 days on Yeast Extract-Peptone Dextrose (YPD) agar at 30 °C. A yeast suspension of 1 x 10<sup>6</sup> to 5 x 10<sup>6</sup> CFU ml<sup>-1</sup> (as determined by OD<sub>530</sub>) was prepared from five colonies. The suspension was subsequently diluted and added to each well of the compound-containing plates giving a final cell density of fungi suspension of 2.5 x 10<sup>3</sup> CFU ml<sup>-1</sup> and a total volume of 50 µl. All plates were covered and incubated at 35 °C for 36 h without shaking.

### 1.3.2 Analysis

Growth inhibition of *C. albicans* was determined measuring absorbance at 630 nm ( $OD_{630}$ ), while the growth inhibition of *C. neoformans* was determined measuring the difference in absorbance between 600 and 570 nm ( $OD_{600-570}$ ), after the addition of resazurin (0.001% final concentration) and incubation at 35 °C for 2 h. The absorbance was measured using a Biotek Multiflo Synergy HTX plate reader.

In both cases, the percentage of growth inhibition was calculated for each well, using the negative control (media only) and positive control (fungi without inhibitors) on the same plate. The *MIC* was determined as the lowest concentration at which the growth was fully inhibited, defined by an inhibition  $\geq 80\%$  for *C. albicans* and an inhibition  $\geq 70\%$  for *C. neoformans*. Due to a higher variance in growth and inhibition, a lower threshold was applied to the data for *C. neoformans*. In addition, the maximal percentage of growth inhibition is reported as  $D_{Max}$ , indicating any compounds with marginal activity.

Hits were classified by  $MIC \leq 16 \mu\text{g/mL}$  or  $MIC \leq 10 \mu\text{M}$  in either replicate (n=2 on different plates).

## 1.4 Cytotoxicity Assay

### 1.4.1 Procedure

*HEK293* cells were counted manually in a Neubauer haemocytometer and then plated in the 384-well plates containing the compounds to give a density of 5000 cells per well in a final volume of 50  $\mu\text{l}$ . DMEM supplemented with 10% FBS was used as growth media and the cells were incubated together with the compounds for 20 h at 37 °C in 5%  $\text{CO}_2$ .

### 1.4.2 Analysis

Cytotoxicity (or cell viability) was measured by fluorescence, ex: 560/10 nm, em: 590/10 nm ( $F_{560/590}$ ), after addition of 5  $\mu\text{l}$  of 25  $\mu\text{g ml}^{-1}$  resazurin (2.3  $\mu\text{g ml}^{-1}$  final concentration) and after incubation for further 3 h at 37 °C in 5%  $\text{CO}_2$ . The fluorescence intensity was measured using a Tecan M1000 Pro monochromator plate reader, using automatic gain calculation.

$CC_{50}$  (concentration at 50% cytotoxicity) were calculated by curve fitting the inhibition values vs.  $\log(\text{concentration})$  using a sigmoidal dose-response function, with variable fitting values for bottom, top and slope. In addition, the maximal percentage of cytotoxicity is reported as  $D_{Max}$ , indicating any compounds with partial cytotoxicity.

The curve fitting was implemented using Pipeline Pilot's dose-response component, resulting in similar values to curve fitting tools such as GraphPad's Prism and IDBS's XIFit. Any value with  $>$  indicate sample with no activity (low  $D_{Max}$  value) or samples with  $CC_{50}$  values above the maximum tested concentration (higher  $D_{Max}$  value).

Cytotoxic samples were classified by  $CC_{50} \leq 32 \mu\text{g ml}^{-1}$  or  $CC_{50} \leq 10 \mu\text{M}$  in either replicate (n=2 on different plates). In addition, samples were flagged as partial cytotoxic if  $D_{Max} \geq 50\%$ , even with  $CC_{50} >$  the maximum tested concentration.

## 1.5 Haemolysis Assay

### 1.5.1 Procedure

Human whole blood was washed three times with 3 volumes of 0.9% NaCl and then resuspended in same to a concentration of  $0.5 \times 10^8$  cells per ml, as determined by manual cell count in a Neubauer haemocytometer. The washed cells were then added to the 384-well compound-containing plates for a final volume of 50  $\mu$ l. After a 10 min shake on a plate shaker the plates were then incubated for 1 h at 37 °C. After incubation, the plates were centrifuged at 1000g for 10 min to pellet cells and debris, 25  $\mu$ L of the supernatant was then transferred to a polystyrene 384-well assay plate.

### 1.5.2 Analysis

Haemolysis was determined by measuring the supernatant absorbance at 405 nm ( $OD_{405}$ ). The absorbance was measured using a Tecan M1000 Pro monochromator plate reader.

$HC_{10}$  and  $HC_{50}$  (concentration at 10% and 50% haemolysis, respectively) were calculated by curve fitting the inhibition values vs.  $\log(\text{concentration})$  using a sigmoidal dose-response function with variable fitting values for top, bottom and slope. In addition, the maximal percentage of haemolysis is reported as  $D_{Max}$ , indicating any compounds with partial haemolysis.

The curve fitting was implemented using Pipeline Pilot's dose-response component, resulting in similar values to curve fitting tools such as GraphPad's Prism and IDBS's XIFit. Any value with  $>$  indicate sample with no activity (low  $D_{Max}$  value) or samples with  $HC_{10}$  values above the maximum tested concentration (higher  $D_{Max}$  value).

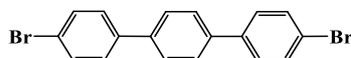
Haemolysis samples were classified by  $HC_{10} \leq 32 \mu\text{g ml}^{-1}$  or  $HC_{10} \leq 10 \mu\text{M}$  in either replicate ( $n=2$  on different plates). In addition, samples were flagged as partial haemolytic if  $D_{Max} \geq 50\%$ , even with  $HC_{10} >$  the maximum tested concentration.

## 2. Chemical Experimental Details

Reagents were purchased and used without further purification. 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 spectrometer at ambient temperature in DMSO- $d_6$ . Biologically tested compound (Product) purities were confirmed by HPLC on a Stayer 0892 series HPLC system with Luna® 5  $\mu\text{m}$  C18 100 Å, LC column 250 x 4.6 mm. Mobile phase: 85:15 MeCN/H<sub>2</sub>O (0.25 M NaClO<sub>4</sub>, 0.1% H<sub>3</sub>PO<sub>4</sub>). All melting points were determined on a Gallenkamp melting point apparatus in open capillaries and are uncorrected. Mass spectra were recorded on a Finnigan MAT INCOS 50 mass-spectrometer. 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.

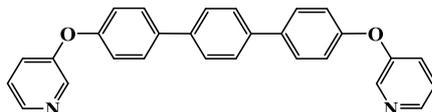
### 2.1. Synthesis and Characterization of Substrates and Products

#### Synthesis of 4,4''-dibromo-*p*-terphenyl (**5**)



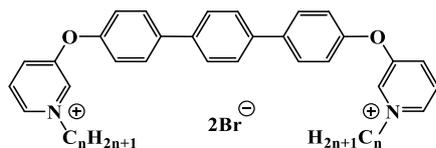
To a mixture of *p*-terphenyl **4** (2.30 g, 10 mmol), hydrogen peroxide 20% (3.6 ml), water (3.9 ml) and dichloromethane (50 ml), bromine (1.29 ml, 25 mmol) was slowly added. The mixture was refluxed for 4 h, then cooled and filtered. Solvent was removed under reduced pressure, ethyl acetate (200 ml) was added to crude residue, and the mixture was refluxed for 1 hour. Then solid was filtered, washed with water (50 ml), petroleum ether (50 ml) and dried to give a pure product (3.80 g, 9.8 mmol, 98% yield). C<sub>18</sub>H<sub>12</sub>Br<sub>2</sub>; M. w. 388.1; White solid; M. p. 312-314°C; <sup>1</sup>H NMR (300 MHz, DMSO- $d_6$ ):  $\delta$  7.62-7.75 (m, 8H, 8CH<sub>Ar</sub>), 7.76-7.83 (m, 4H, 4CH<sub>Ar</sub>) ppm.

#### Synthesis of 3,3'-[*p*-terphenyl-4,4''-diylbis(oxy)]dipyridine (3TP4BO, **6**):



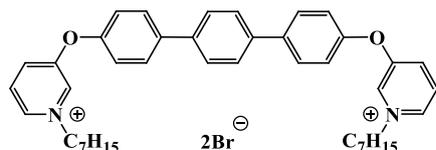
A mixture of 4,4''-dibromo-*p*-terphenyl **5** (3.88 g, 10 mmol), 3-hydroxypyridine (2.09 g, 22 mmol), potassium carbonate (6.07 g, 44 mmol), and copper (2.81 g, 44 mmol) in dry DMA (100 ml) was refluxed for 3 days in argon atmosphere. Solvent was removed under reduced pressure, ethyl acetate (200 ml) was added to crude residue, and the mixture was refluxed for 1 h. Then mixture was filtered, washed with 50 ml of hot ethyl acetate. The organic filtrate was concentrated under reduced pressure and the residue was purified by recrystallization in ethyl acetate to provide a white solid product **6** (2.7 g, 6.5 mmol, 65% yield). C<sub>28</sub>H<sub>20</sub>N<sub>2</sub>O<sub>2</sub>; M. w. 416.5; White solid; M. p. 187-189°C; <sup>1</sup>H NMR (300 MHz, DMSO- $d_6$ ):  $\delta$  7.17 (d, J = 8.6 Hz, 4H, 4CH<sub>Ar</sub>), 7.45 (dd, J = 8.3, 4.5 Hz, 2H, 2CH<sub>py</sub>), 7.51 (d, J = 8.3, 2.4 Hz, 2H, 2CH<sub>py</sub>), 7.76 (s, 4H, 4CH<sub>Ar</sub>), 7.77 (d, J = 8.6 Hz, 4H, 4CH<sub>Ar</sub>), 8.40 (d, J = 4.5 Hz, 2H, 2CH<sub>py</sub>), 8.44 (d, J = 2.4 Hz, 2H, 2CH<sub>py</sub>) ppm; <sup>13</sup>C NMR (150 MHz, CDCl<sub>3</sub>):  $\delta$  118.6, 124.3, 125.5, 126.6, 127.9, 135.0, 137.8, 140.7, 144.3, 152.8, 155.5 ppm.

### Synthesis of 3,3'-[*p*-terphenyl-4,4''-diylbis(oxy)]bis(1-alkylpyridinium) dibromides **7** (3TP4BO-*n*,Br)



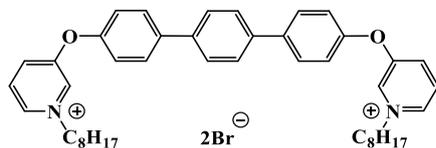
Alkyl bromide (2.4 mmol) was added to a solution of 3,3'-[terphenyl-4,4''-diylbis(oxy)]dipyridine **6** (0.42 g, 1 mmol) in DMF (3 ml). The mixture was heated to 120°C for 4 days, then cooled and filtered. The filtered solid was washed with 5 ml of cold acetone and dried to give a pure product. The yields are 34-53% depend on alkyl bromide.

#### 3,3'-[*p*-Terphenyl-4,4''-diylbis(oxy)]bis(1-octylpyridinium) dibromide (**7a**, 3TP4BO-7,Br):



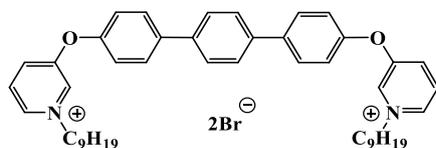
C<sub>42</sub>H<sub>50</sub>Br<sub>2</sub>N<sub>2</sub>O<sub>2</sub>; M. w. 784.5; White solid (0.39 g, 50% yield); M. p. 280-283-°C; <sup>1</sup>H NMR (300 MHz, DMSO-*d*<sub>6</sub>): δ 0.86 (t, J = 6.6 Hz, 6H, 2CH<sub>3</sub>), 1.19-1.39 (m, 16H, 8CH<sub>2</sub>), 1.84-2.04 (m, 4H, 2CH<sub>2</sub>), 4.63 (t, J = 6.2 Hz, 4H, 2CH<sub>2</sub>N<sup>+</sup>), 7.42 (d, J = 8.6 Hz, 4H, 4CH<sub>Ar</sub>), 7.84 (s, 4H, 4CH<sub>Ar</sub>), 7.90 (d, J = 8.6 Hz, 2H, 2CH<sub>py</sub>), 8.15 (dd, J = 8.7, 5.9 Hz, 2H, 2CH<sub>py</sub>), 8.28 (dd, J = 8.7, 2.0 Hz, 2H, 2CH<sub>py</sub>), 8.93 (d, J = 5.9 Hz, 2H, 2CH<sub>py</sub>), 9.20 (d, J = 2.0 Hz, 2H, 2CH<sub>py</sub>) ppm; <sup>13</sup>C NMR (75 MHz, DMSO-*d*<sub>6</sub>): δ 13.8, 21.9, 25.3, 28.4, 30.7, 31.0, 60.9, 120.0, 127.1, 128.7, 129.0, 133.5, 136.1, 136.9, 138.0, 139.5, 153.9, 156.0 ppm; *m/z* (%): 43 (27), 51 (16), 78 (21), 135 (17), 177 (6), 215 (11), 228 (4), 310 (7), 338 (34), 416 (100); *v*<sub>max</sub>, (KBr): 2981, 2924, 2851, 1582, 1494, 1268, 1207, 823, 677, 503 cm<sup>-1</sup>

#### 3,3'-[*p*-Terphenyl-4,4''-diylbis(oxy)]bis(1-octylpyridinium) dibromide (**7b**, 3TP4BO-8,Br):



C<sub>44</sub>H<sub>54</sub>Br<sub>2</sub>N<sub>2</sub>O<sub>2</sub>; M. w. 802.7; White solid (0.37 g, 0.46 mmol, 46% yield); M. p. 284-287-°C; <sup>1</sup>H NMR (300 MHz, DMSO-*d*<sub>6</sub>): δ 0.86 (t, J = 6.6 Hz, 6H, 2CH<sub>3</sub>), 1.19-1.39 (m, 20H, 10CH<sub>2</sub>), 1.84-2.04 (m, 4H, 2CH<sub>2</sub>), 4.63 (t, J = 6.2 Hz, 4H, 2CH<sub>2</sub>N<sup>+</sup>), 7.40 (d, J = 8.6 Hz, 4H, 4CH<sub>Ar</sub>), 7.84 (s, 4H, 4CH<sub>Ar</sub>), 7.90 (d, J = 8.6 Hz, 2H, 2CH<sub>py</sub>), 8.15 (dd, J = 8.7, 5.9 Hz, 2H, 2CH<sub>py</sub>), 8.28 (dd, J = 8.7, 2.0 Hz, 2H, 2CH<sub>py</sub>), 8.93 (d, J = 5.9 Hz, 2H, 2CH<sub>py</sub>), 9.20 (d, J = 2.0 Hz, 2H, 2CH<sub>py</sub>) ppm; <sup>13</sup>C NMR (75 MHz, DMSO-*d*<sub>6</sub>): δ 13.8, 21.9, 25.3, 28.3, 28.4, 30.7, 31.0, 60.9, 120.0, 127.1, 128.7, 129.0, 133.5, 136.1, 136.9, 138.0, 139.5, 153.9, 156.0 ppm; *m/z* (%): 43 (13), 51 (10), 78 (25), 177 (4), 202 (6), 215 (11), 228 (6), 310 (7), 338 (32), 416 (100); *v*<sub>max</sub>, (KBr): 2983, 2924, 2855, 1582, 1494, 1267, 1206, 824, 677, 503 cm<sup>-1</sup>

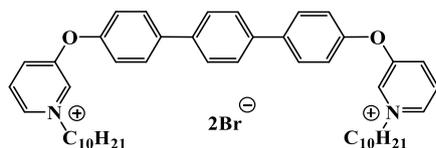
#### 3,3'-[*p*-Terphenyl-4,4''-diylbis(oxy)]bis(1-nonylpyridinium) dibromide (**7c**, 3TP4BO-9,Br):



C<sub>46</sub>H<sub>58</sub>Br<sub>2</sub>N<sub>2</sub>O<sub>2</sub>; M. w. 830.9; White solid (0.39 g, 0.48 mmol, 48% yield); M. p. 281-283°C; <sup>1</sup>H NMR (300 MHz, DMSO-*d*<sub>6</sub>): δ 0.86 (t, J = 6.6 Hz, 6H, 2CH<sub>3</sub>), 1.14-1.41 (m, 24H, 12CH<sub>2</sub>), 1.84-2.05 (m,

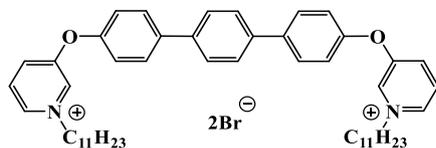
4H, 2CH<sub>2</sub>), 4.63 (t, J = 6.2 Hz, 4H, 2CH<sub>2</sub>N<sup>+</sup>), 7.40 (d, J = 8.6 Hz, 4H, 4CH<sub>Ar</sub>), 7.84 (s, 4H, 4CH<sub>Ar</sub>), 7.90 (d, J = 8.6 Hz, 2H, 2CH<sub>py</sub>), 8.17 (dd, J = 8.7, 5.9 Hz, 2H, 2CH<sub>py</sub>), 8.28 (dd, J = 8.7, 2.0 Hz, 2H, 2CH<sub>py</sub>), 8.93 (d, J = 5.9 Hz, 2H, 2CH<sub>py</sub>), 9.20 (d, J = 2.0 Hz, 2H, 2CH<sub>py</sub>) ppm; <sup>13</sup>C NMR (75 MHz, DMSO-*d*<sub>6</sub>): δ 13.9, 22.0, 25.3, 28.3, 28.5, 28.7, 30.7, 31.2, 61.0, 120.0, 127.2, 128.7, 129.0, 133.6, 136.1, 136.9, 138.1, 139.5, 154.0, 156.1 ppm; *m/z* (%): 43 (2), 55 (1), 71 (1), 85 (5), 135 (2), 215 (8), 228 (4), 310 (1), 338 (5), 416 (100);  $\nu_{\max}$ , (KBr): 2981, 2924, 2853, 1582, 1494, 1268, 1207, 824, 677, 503 cm<sup>-1</sup>

**3,3'-[*p*-Terphenyl-4,4''-diylbis(oxy)]bis(1-decylpyridinium) dibromide (7d, 3TP4BO-10,Br):**



C<sub>48</sub>H<sub>62</sub>Br<sub>2</sub>N<sub>2</sub>O<sub>2</sub>; M. w. 859.1; White solid (0.29 g, 0.34 mmol, 34% yield); M. p. 290-292°C; <sup>1</sup>H NMR (300 MHz, DMSO-*d*<sub>6</sub>): δ 0.86 (t, J = 6.6 Hz, 6H, 2CH<sub>3</sub>), 1.17-1.38 (m, 28H, 14CH<sub>2</sub>), 1.86-2.02 (m, 4H, 2CH<sub>2</sub>), 4.63 (t, J = 6.2 Hz, 4H, 2CH<sub>2</sub>N<sup>+</sup>), 7.40 (d, J = 8.6 Hz, 4H, 4CH<sub>Ar</sub>), 7.83 (s, 4H, 4CH<sub>Ar</sub>), 7.89 (d, J = 8.6 Hz, 2H, 2CH<sub>py</sub>), 8.15 (dd, J = 8.7, 5.9 Hz, 2H, 2CH<sub>py</sub>), 8.28 (dd, J = 8.7, 2.0 Hz, 2H, 2CH<sub>py</sub>), 8.93 (d, J = 5.9 Hz, 2H, 2CH<sub>py</sub>), 9.19 (d, J = 2.0 Hz, 2H, 2CH<sub>py</sub>) ppm; <sup>13</sup>C NMR (75 MHz, DMSO-*d*<sub>6</sub>): δ 13.8, 22.0, 25.3, 28.3, 28.6, 28.7, 28.8, 30.7, 31.2, 60.9, 120.0, 127.1, 128.7, 129.0, 133.5, 136.1, 136.9, 138.0, 139.5, 153.9, 156.0 ppm; *m/z* (%): 43 (54), 57 (44), 71 (29), 85 (22), 135 (89), 149 (11), 215 (7), 310 (11), 338 (30), 416 (100);  $\nu_{\max}$ , (KBr): 2983, 2924, 2853, 1581, 1493, 1267, 1206, 823, 677, 503 cm<sup>-1</sup>

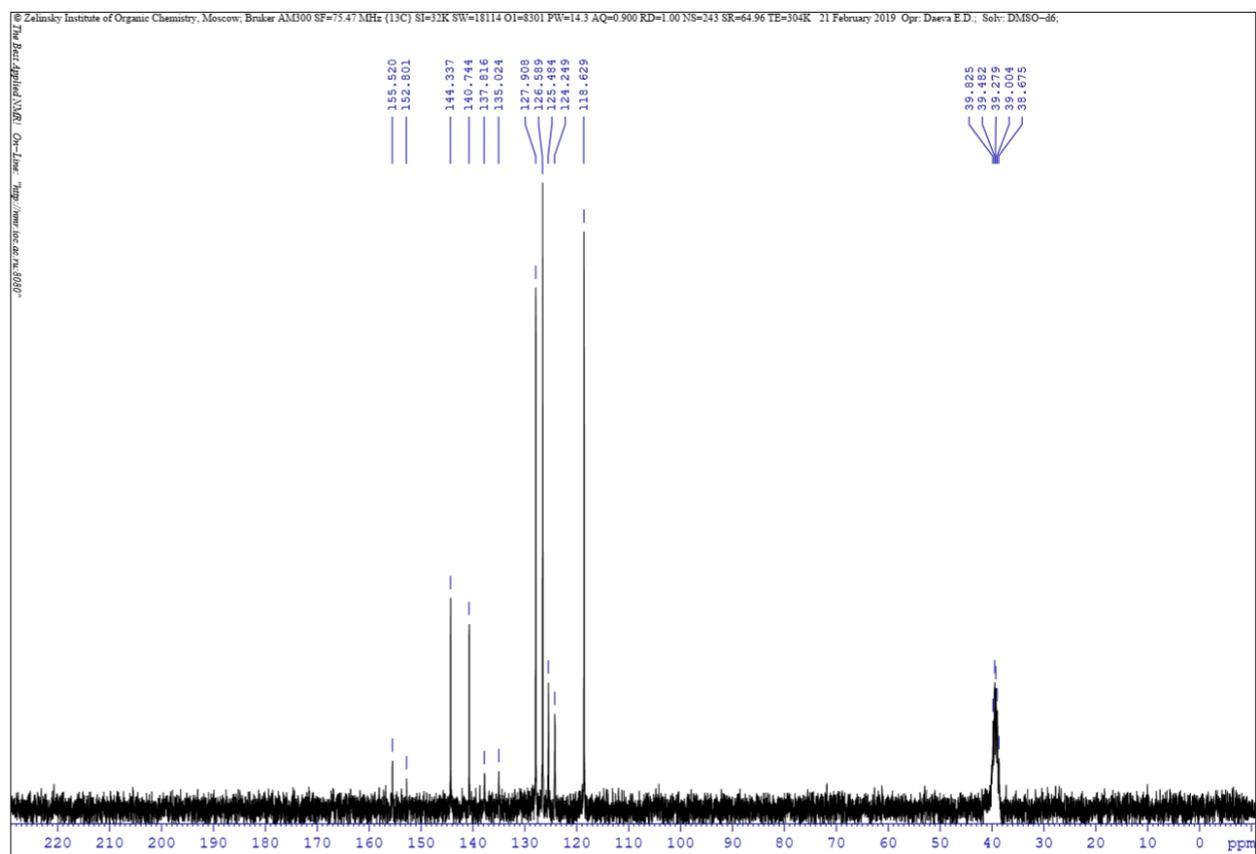
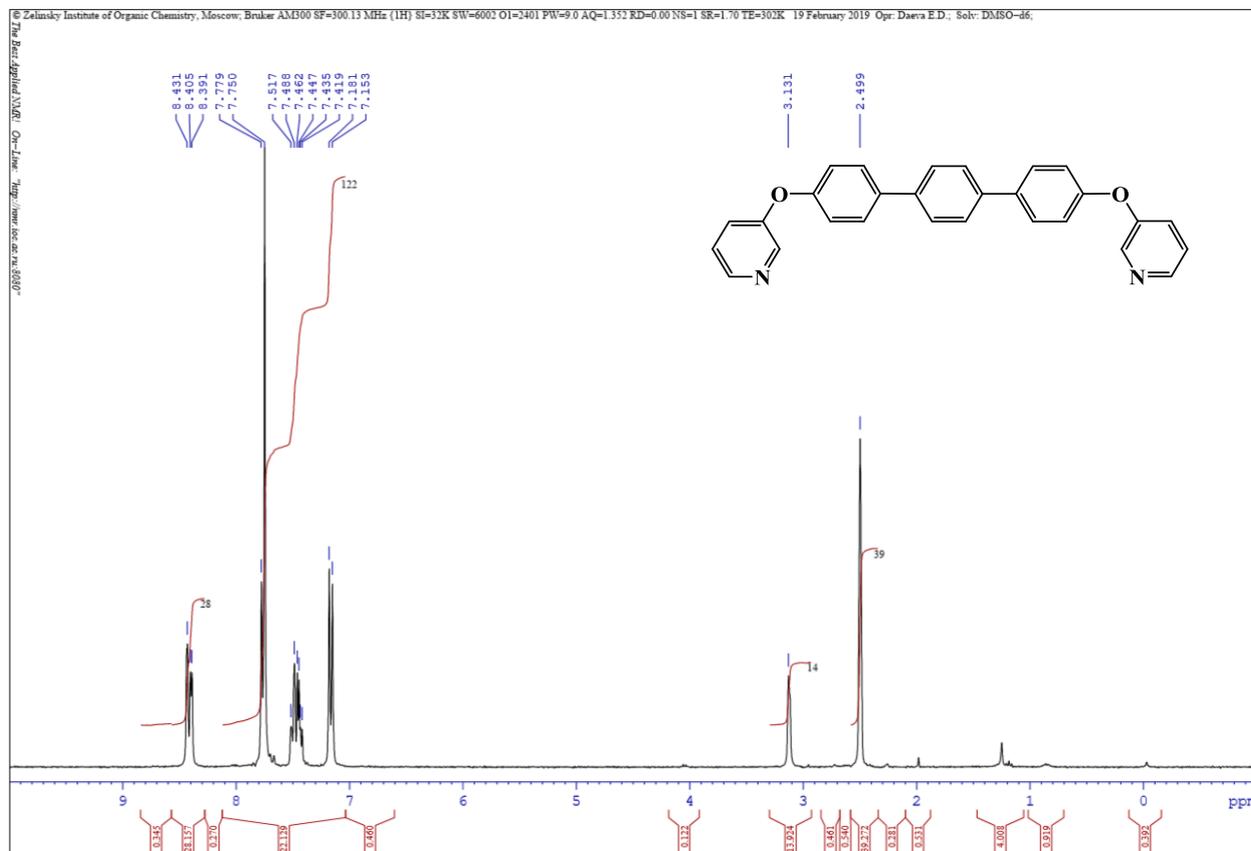
**3,3'-[*p*-Terphenyl-4,4''-diylbis(oxy)]bis(1-undecylpyridinium) dibromide (7e, 3TP4BO-11,Br):**



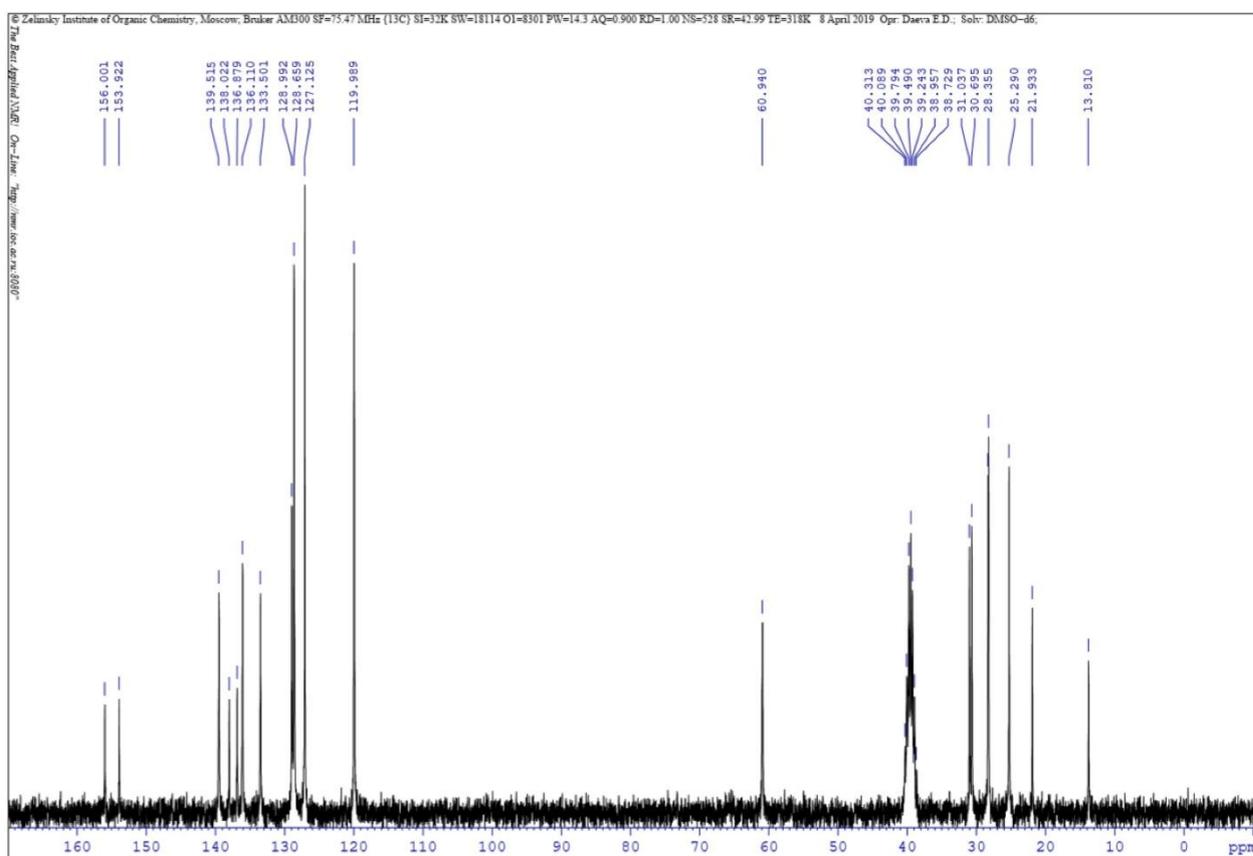
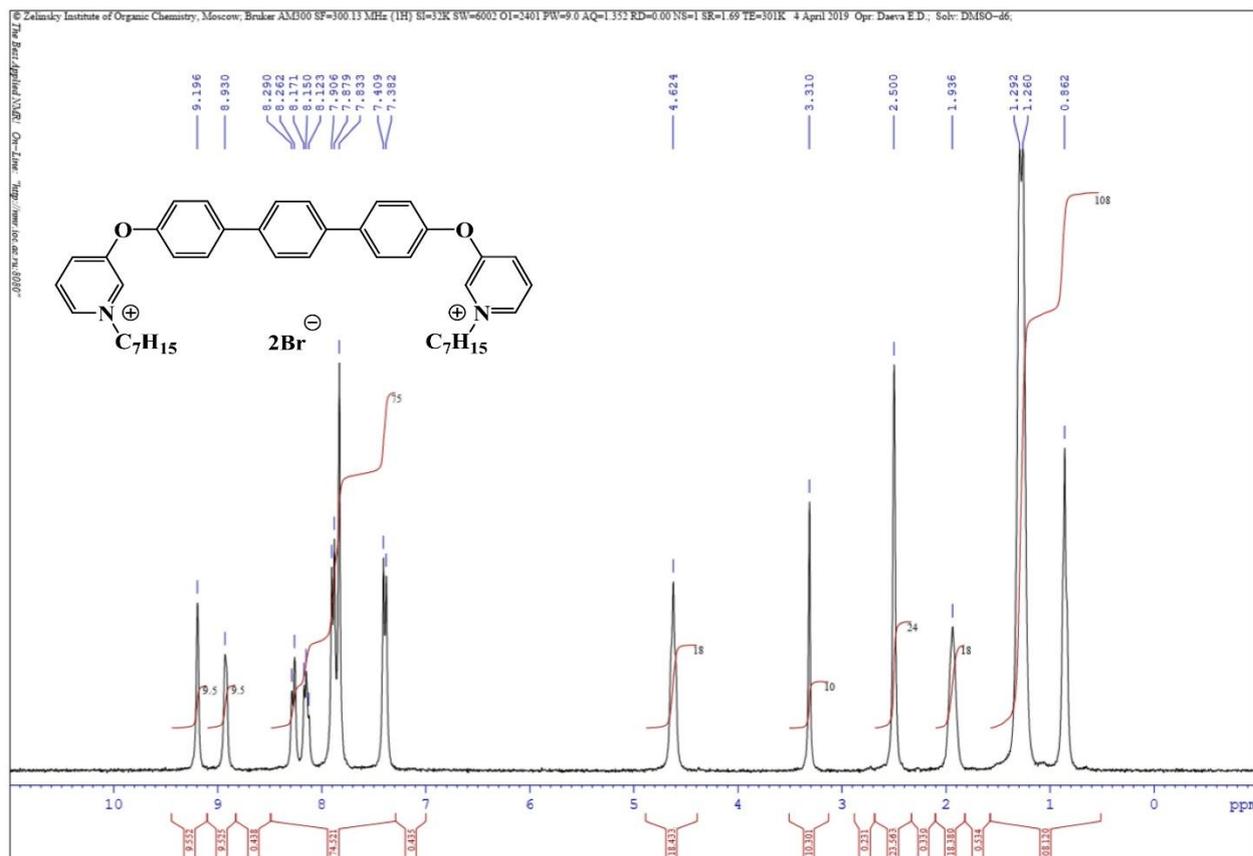
C<sub>50</sub>H<sub>66</sub>Br<sub>2</sub>N<sub>2</sub>O<sub>2</sub>; M. w. 887.3; White solid (0.47 g, 0.53 mmol, 53% yield); M. p. 292-294°C; <sup>1</sup>H NMR (300 MHz, DMSO-*d*<sub>6</sub>): δ 0.85 (t, J = 6.6 Hz, 6H, 2CH<sub>3</sub>), 1.16-1.40 (m, 32H, 16CH<sub>2</sub>), 1.86-2.02 (m, 4H, 2CH<sub>2</sub>), 4.63 (t, J = 6.2 Hz, 4H, 2CH<sub>2</sub>N<sup>+</sup>), 7.40 (d, J = 8.6 Hz, 4H, 4CH<sub>Ar</sub>), 7.84 (s, 4H, 4CH<sub>Ar</sub>), 7.89 (d, J = 8.6 Hz, 2H, 2CH<sub>py</sub>), 8.15 (dd, J = 8.7, 5.9 Hz, 2H, 2CH<sub>py</sub>), 8.28 (dd, J = 8.7, 2.0 Hz, 2H, 2CH<sub>py</sub>), 8.94 (d, J = 5.9 Hz, 2H, 2CH<sub>py</sub>), 9.20 (d, J = 2.0 Hz, 2H, 2CH<sub>py</sub>) ppm; <sup>13</sup>C NMR (75 MHz, DMSO-*d*<sub>6</sub>): δ 13.9, 22.1, 25.4, 28.4, 28.7, 28.7, 28.8, 28.9, 30.7, 31.3, 61.1, 120.1, 127.2, 128.7, 129.1, 133.6, 136.2, 137.0, 138.1, 139.5, 154.0, 156.1 ppm; *m/z* (%): 43 (40), 51 (33), 69 (7), 78 (67), 97 (5), 135 (17), 215 (11), 310 (13), 338 (16), 416 (100);  $\nu_{\max}$ , (KBr): 2983, 2923, 2852, 1582, 1494, 1267, 1206, 822, 677, 503 cm<sup>-1</sup>

### 3. NMR ( $^1\text{H}$ , $^{13}\text{C}$ )

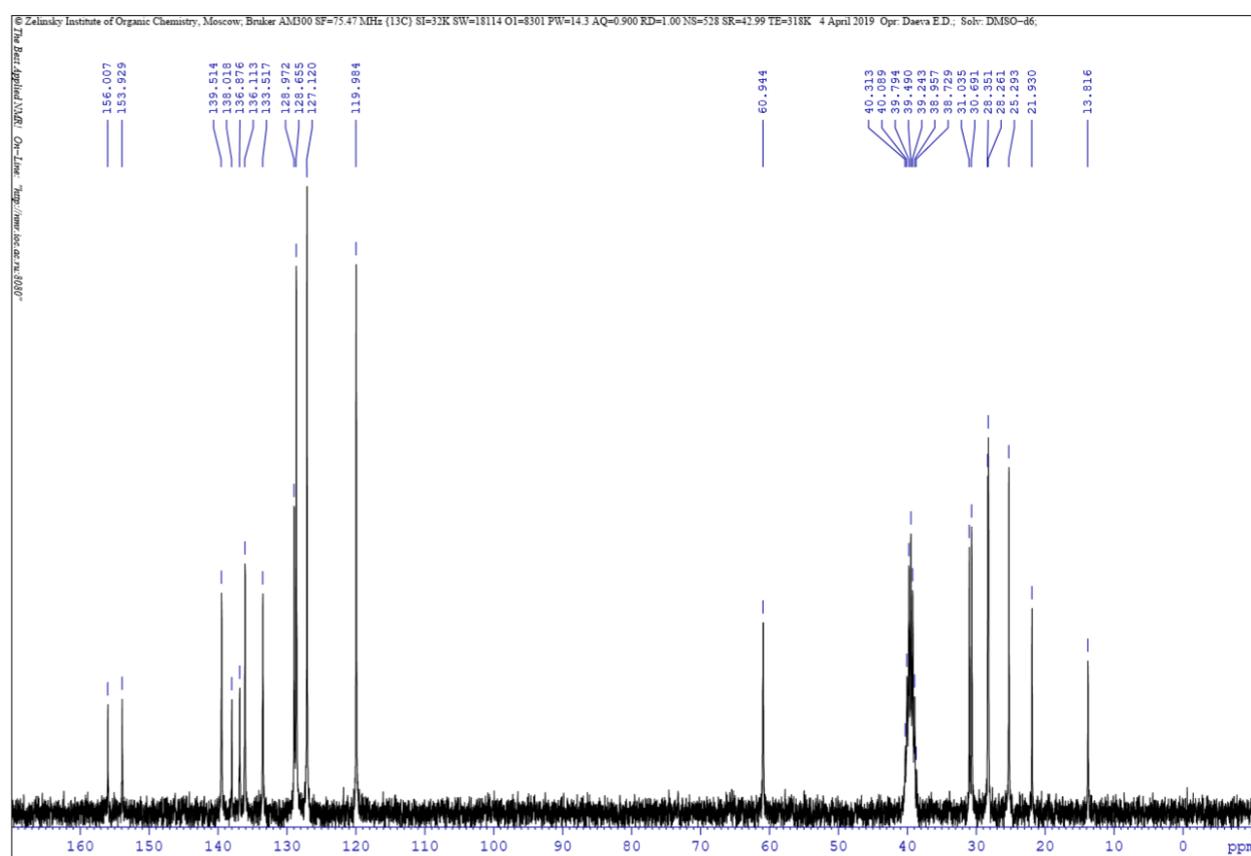
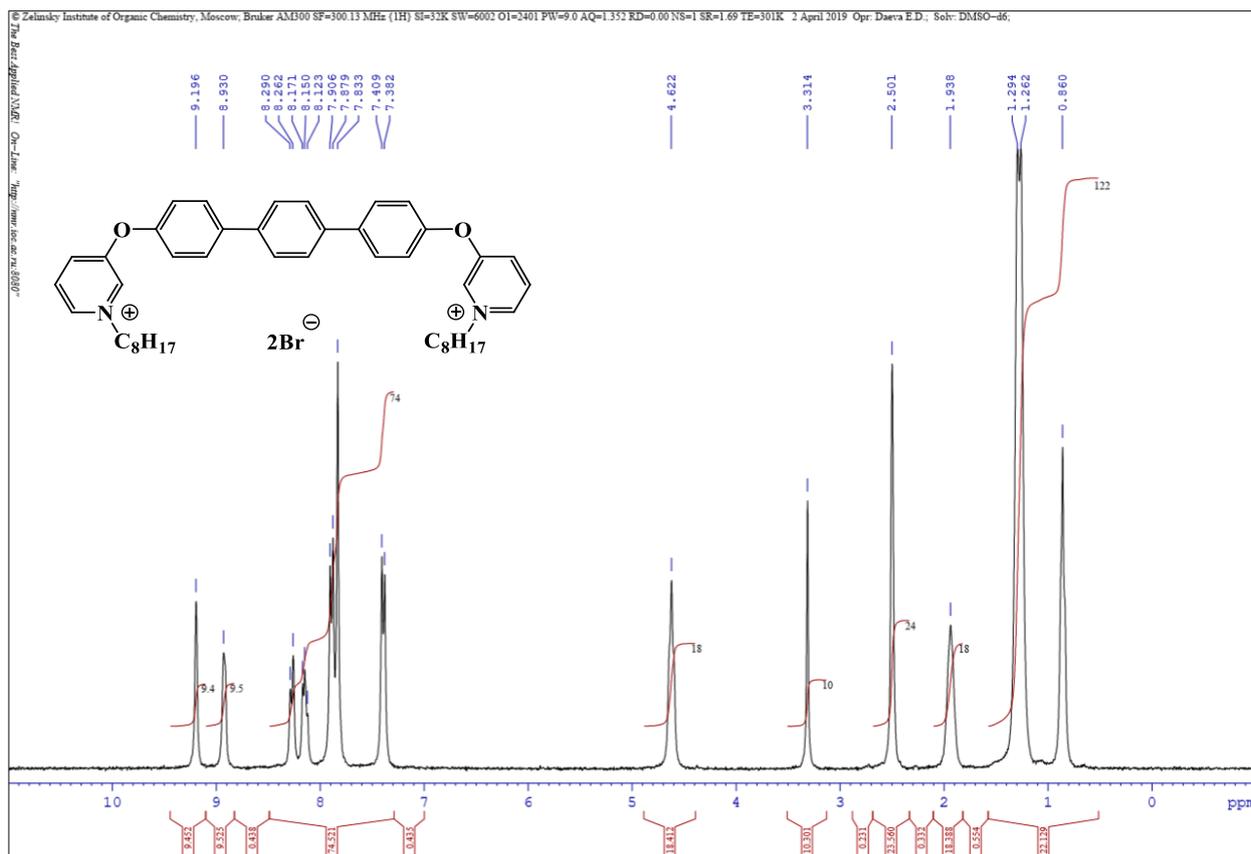
#### 6 (3TP4BO)



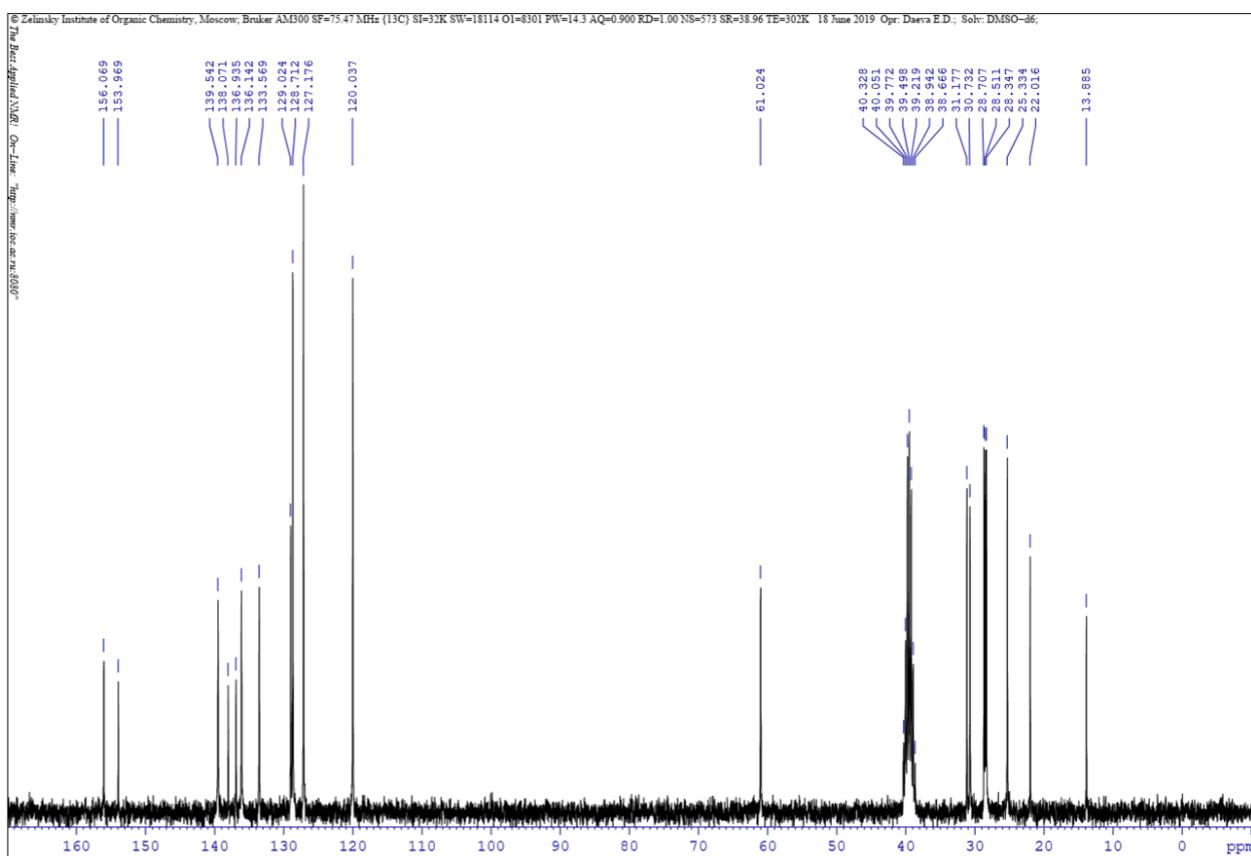
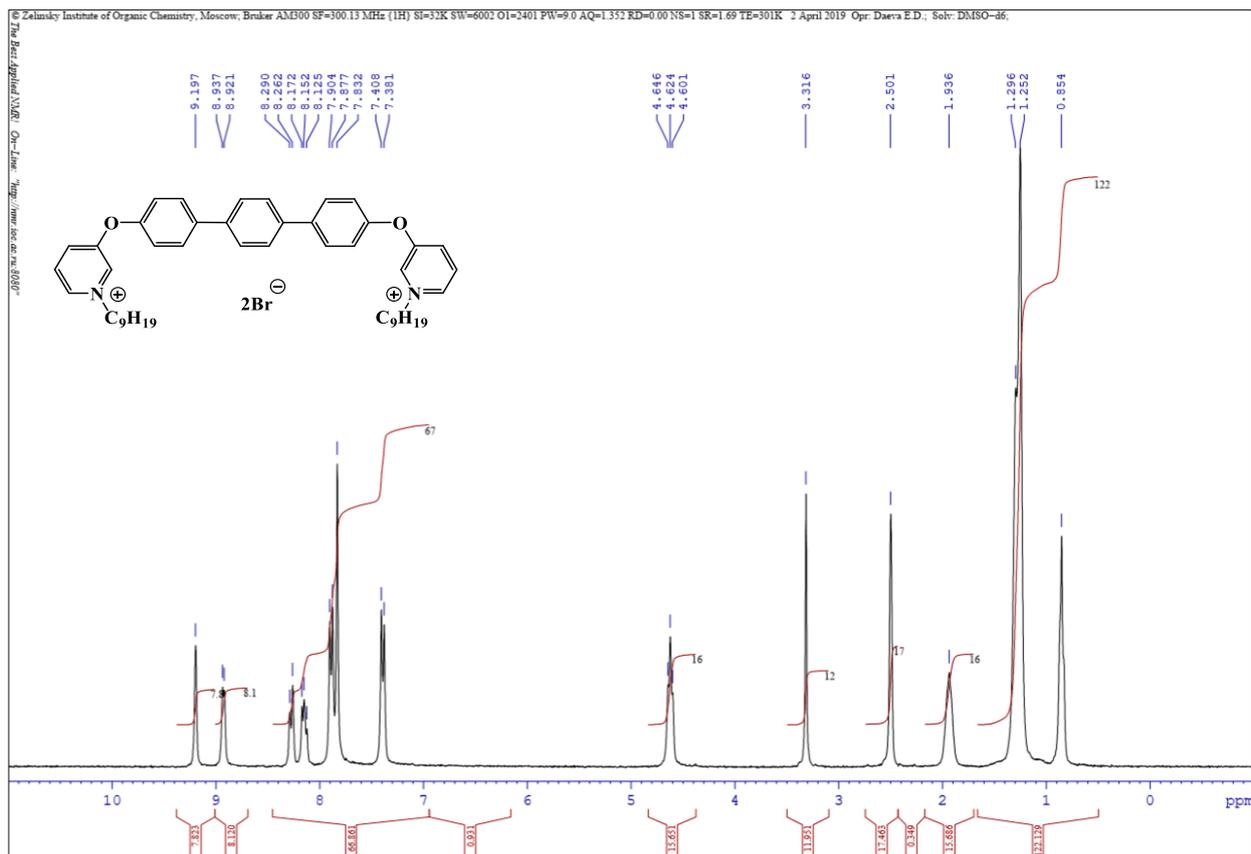
**7a (3TP4BO-7,Br)**



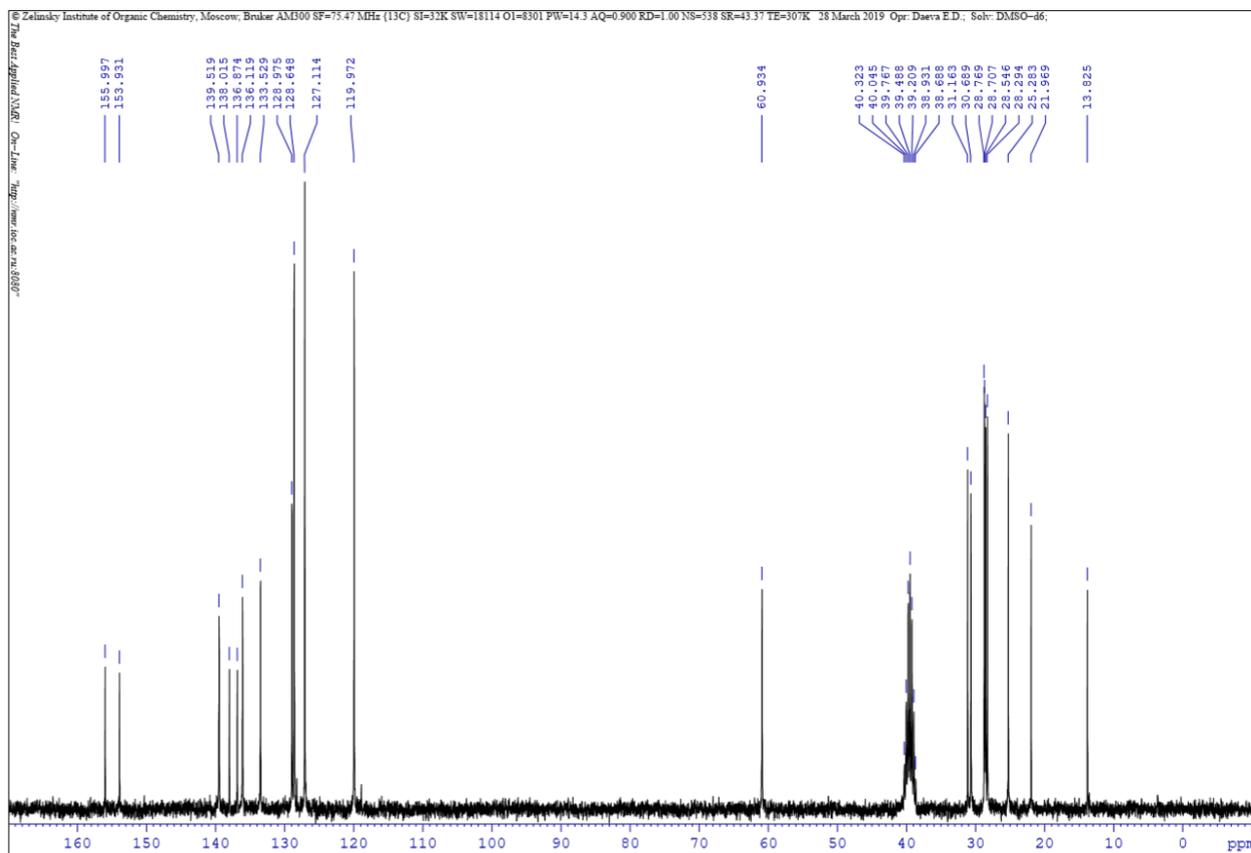
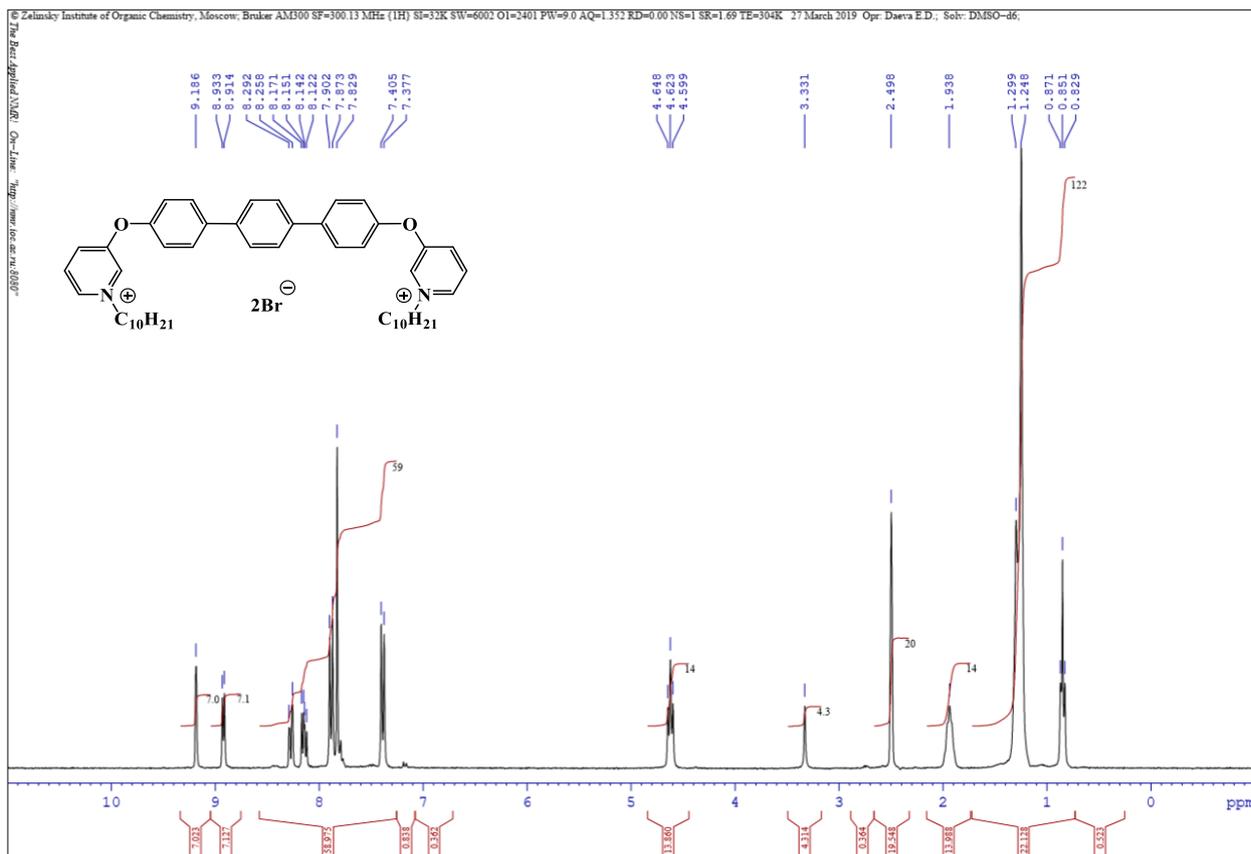
**7b (3TP4BO-8,Br)**



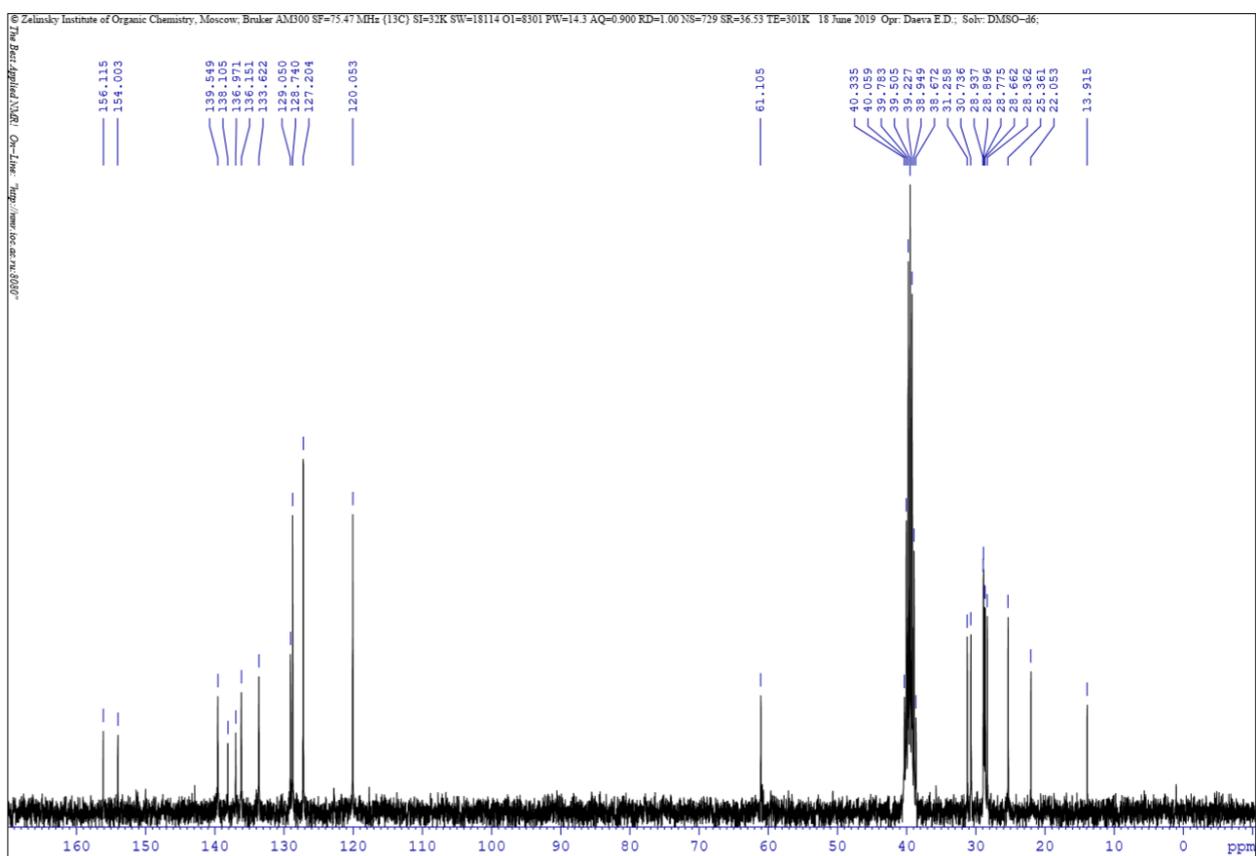
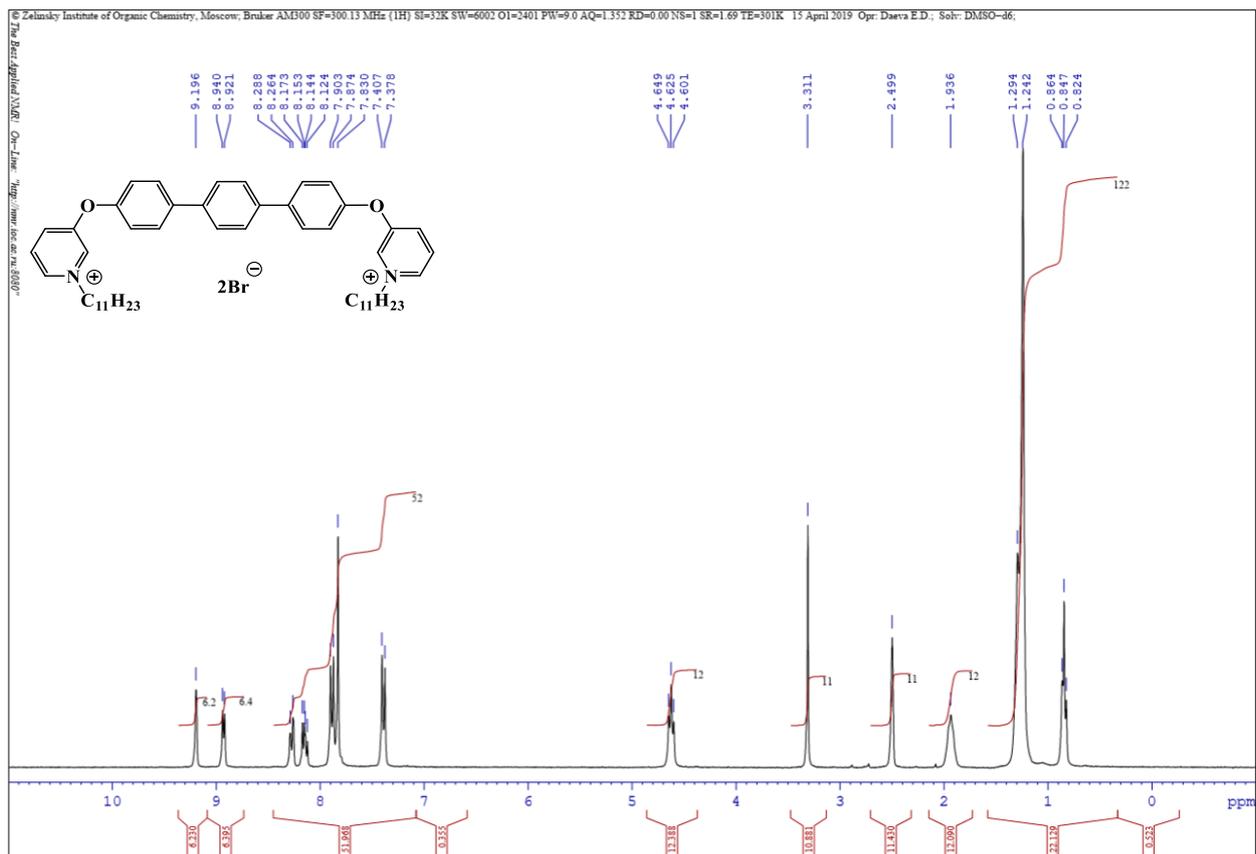
7c (3TP4BO-9,Br)



**7d (3TP4BO-10,Br)**

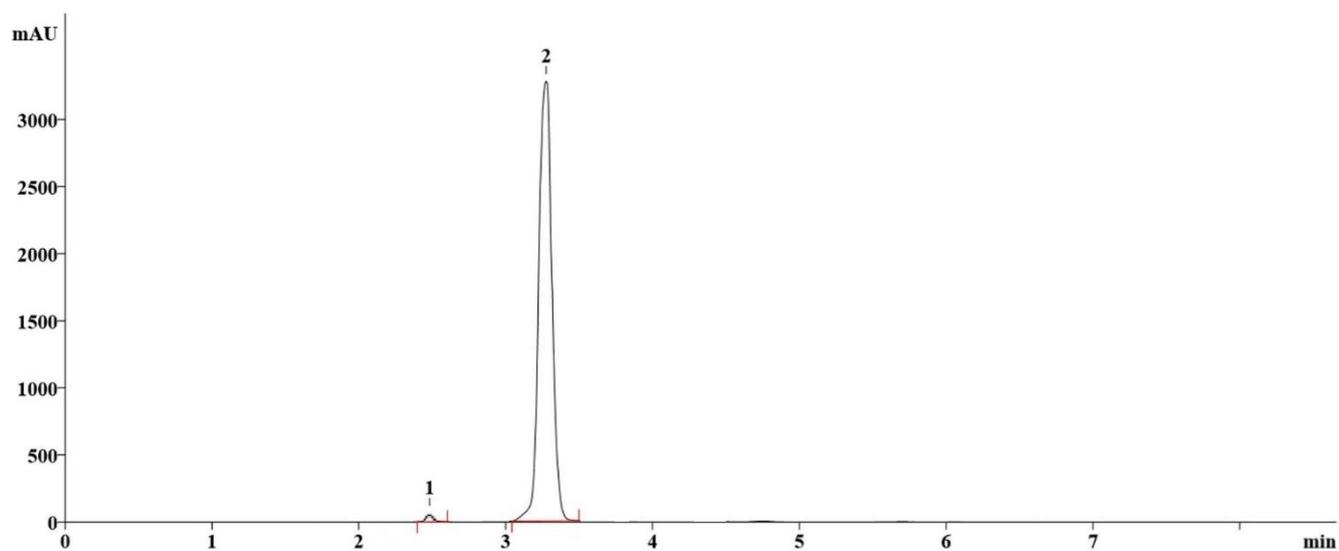


7e (3TP4BO-11,Br)



## HPLC profiles

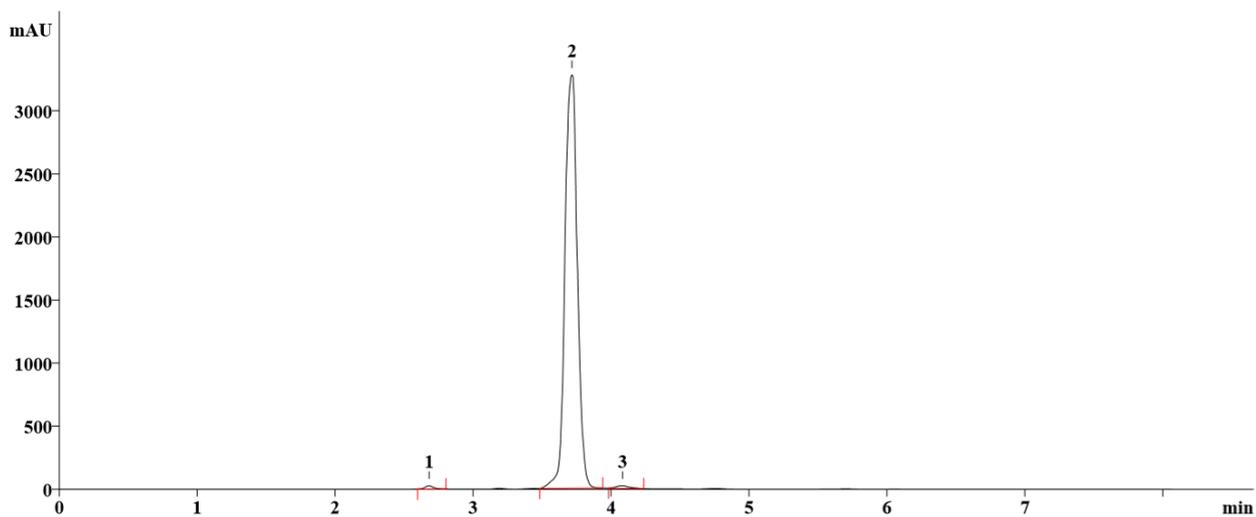
### 7a (3TP4BO-8,Br)



#### Peak table

Peak	Retention min	Area	Height	Width h/2	Area %	Type
1	2,48	118,795	29,707	0,0599	0,929	BB
2	3,27	12668,611	3209,957	0,0988	99,071	BB

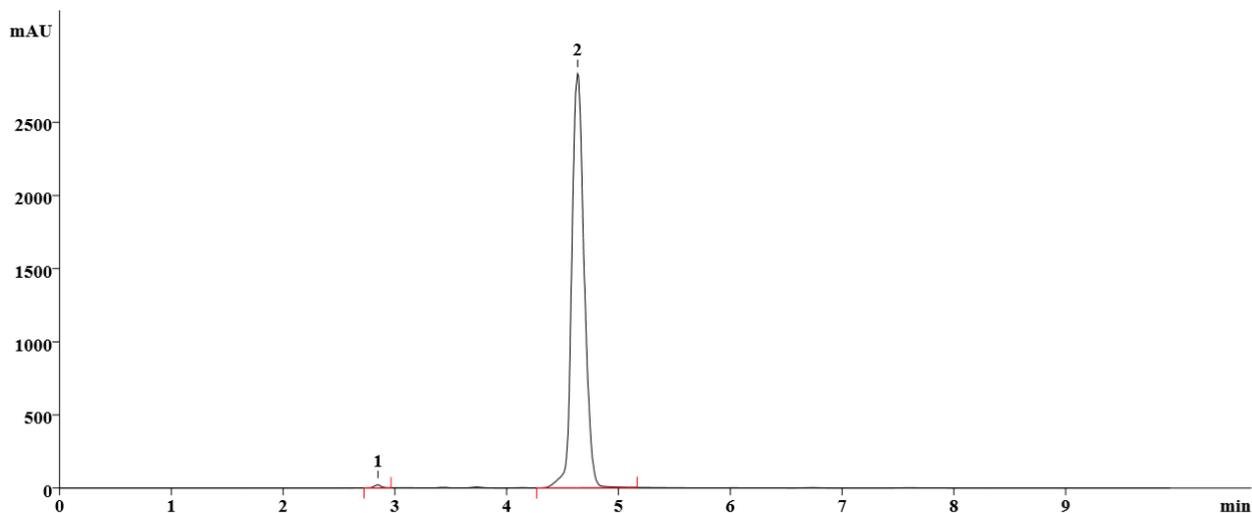
### 7b (3TP4BO-8,Br)



#### Peak table

Peak	Retention min	Area	Height	Width h/2	Area %	Type
1	2,68	108,457	25,790	0,0627	0,522	BB
2	3,72	20510,060	3277,153	0,0973	98,801	BB
3	4,08	140,515	21,186	0,0991	0,677	BB

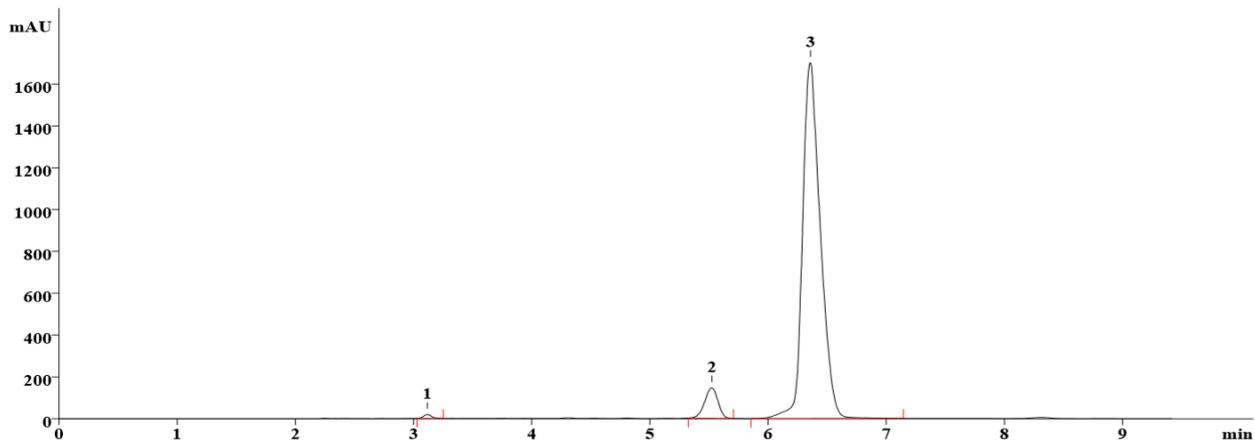
### 7c (3TP4BO-9,Br)



#### Peak table

Peak	Retention min	Area	Height	Width h/2	Area %	Type
1	2,85	81,835	19,457	0,0656	0,373	BB
2	4,63	21832,233	2829,567	0,117	99,627	BB

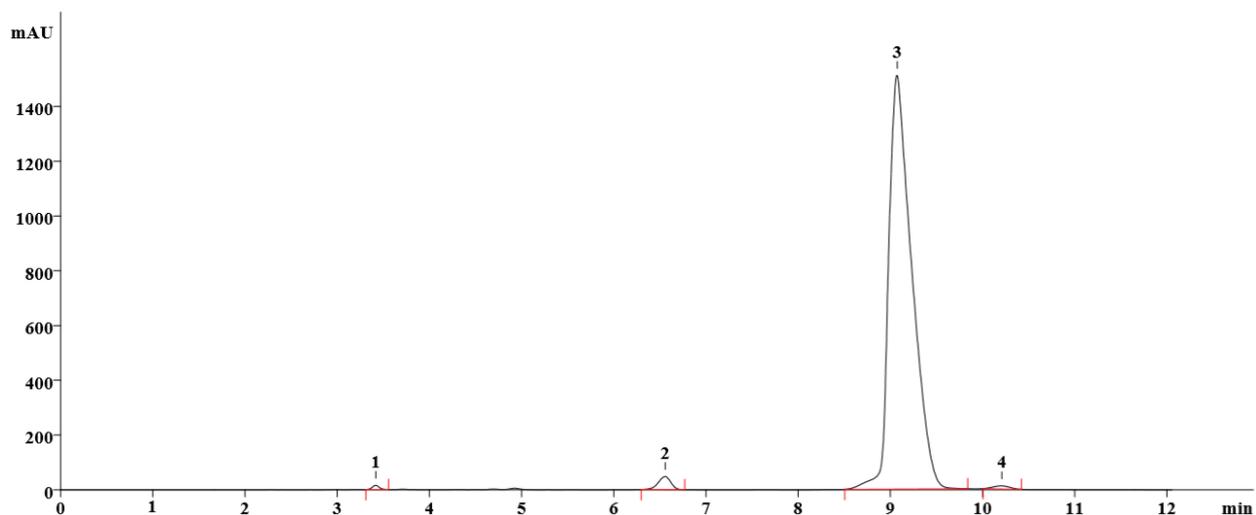
### 7d (3TP4BO-10,Br)



#### Peak table

Peak	Retention min	Area	Height	Width h/2	Area %	Type
1	3,12	86,565	17,977	0,0728	0,455	BB
2	5,52	1129,154	144,895	0,123	5,938	BB
3	6,36	17801,235	1700,933	0,159	93,607	BB

### 7e (3TP4BO-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	3,42	79,859	15,099	0,0813	0,297	BB
2	6,56	452,105	47,639	0,147	1,681	BB
3	9,08	26218,207	1510,252	0,271	97,494	BB
4	10,21	141,907	11,326	0,206	0,528	BB