

Synthesis and microbiological properties of novel bis-quaternary ammonium compounds based on 4,4'-oxydiphenol spacer

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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⁻¹ 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 per ml measured by OD₆₀₀), then added to each well of the compound containing plates, giving a cell density of 5 x 10⁵ CFU per ml 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 (OD₆₀₀), 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 $\geq 80\%$. In addition, the maximal percentage of growth inhibition is reported as DMax, indicating any compounds with partial activity.

Hits were classified by MIC $\leq 16 \mu\text{g ml}^{-1}$ or MIC $\leq 10 \mu\text{M}$ 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 1×10^6 to 5×10^6 CFU per ml (as determined by OD₅₃₀) 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 2.5×10^3 CFU per ml 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 (OD₆₃₀), while the growth inhibition of *C. neoformans* was determined measuring the difference in absorbance between 600 and 570 nm (OD₆₀₀₋₅₇₀), 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 $\leq 16 \mu\text{g ml}^{-1}$ 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 μl . 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% CO₂.

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 μl 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% 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.* $\log C$ (C is 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 $\text{CC}_{50} \leq 32 \mu\text{g ml}^{-1}$ or $\text{CC}_{50} \leq 10 \mu\text{M}$ in either replicate ($n=2$ on different plates). In addition, samples were flagged as partial cytotoxic if $D_{\text{Max}} \geq 50\%$, even with $\text{CC}_{50} >$ 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 0.5×10^8 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 μl . 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 1000 g for 10 min to pellet cells and debris, 25 μl 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.* $\log C$ (C is 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).

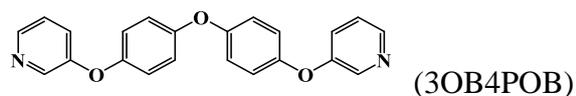
Haemolysis samples were classified by $HC_{10} \leq 32 \mu\text{g ml}^{-1}$ or $HC_{10} \leq 10 \mu\text{M}$ in either replicate ($n=2$ on different plates). In addition, samples were flagged as partial haemolytic if $D_{\text{Max}} \geq 50\%$, even with $HC_{10} >$ the maximum tested concentration.

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-d₆, MeOD and CDCl₃. Biologically tested compound (Product) purities were confirmed by HPLC on a Stayer 0892 series HPLC system with Luna® 5 μm C18 100 Å, LC column 250×4.6 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. 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.

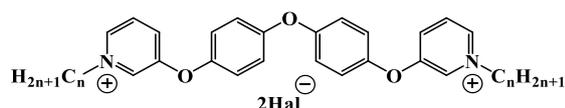
3. Synthesis and Characterization of Substrates and Products

Synthesis of 3,3'-[oxybis(4,1-phenyleneoxy)]dipyridine 2



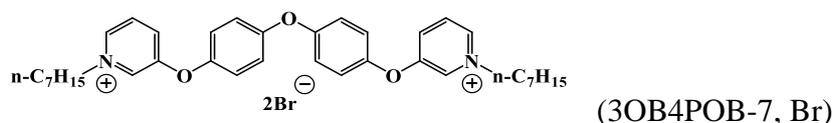
A mixture of bis(4-bromophenyl) ether **1** (3.28 g, 10 mmol), 3-hydroxypyridine (2.09 g, 22 mmol), potassium carbonate (6.07 g, 44 mmol) and copper powder (2.79 g, 44 mmol) in dry DMF (50 ml) was heated to reflux for 48 hours in argon atmosphere. The solvent was removed under reduced pressure, ethyl acetate (50 ml) was added to 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 flash chromatography (EtOAc/hexane, 1:1) to provide a white solid product (2.74 g, 7.7 mmol, 77% yield). C₂₂H₁₆N₂O₃; M. w. 356.4; White solid; M. p. 68-70 °C; ¹H NMR (300 MHz, CDCl₃): 7.01-7.08 (m, 8H, CH_{Ar}), 7.26-7.31 (m, 4H, CH_{Ar}), 8.35-8.44 (m, 4H, 4CH) ppm; ¹³C NMR (75 MHz, CDCl₃): δ 120.0 (4C), 120.5 (4C), 123.9 (2C), 124.5 (2C), 140.8 (2C), 144.0 (2C), 151.5 (2C), 153.6 (2C), 154.2 (2C) ppm

Quaternization of 3,3'-[oxybis(4,1-phenyleneoxy)]dipyridine 2 with alkyl halides:



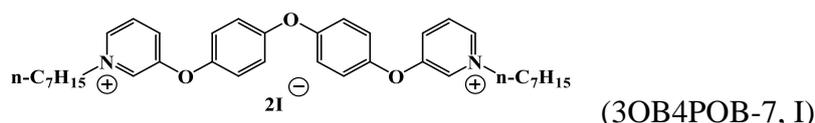
Alkyl halide (2.4 mmol) was added to a solution of 3,3'-[oxybis(4,1-phenyleneoxy)]dipyridine **2** (0.356 g, 1 mmol) in methyl isobutyl ketone (3 ml). The mixture was heated under reflux for 24 hours, then cooled and filtered. The filtered solid was washed with cold acetone (10 ml) and dried to give white solid products **3**. The yield is 77-95% depending on alkyl halide.

3,3'-[Oxybis(4,1-phenyleneoxy)]bis(1-heptylpyridinium) dibromide 3a



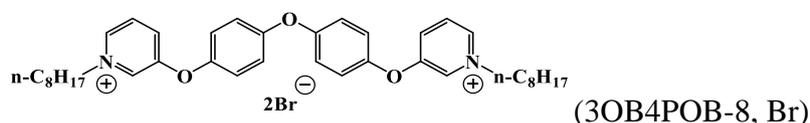
$C_{36}H_{46}Br_2N_2O_3$; M. w. 714.6; White solid (0.62 g, 0.87 mmol, 87% yield); M. p. 183-185 °C; 1H NMR (300 MHz, MeOD): δ 0.85 (t, J = 6.7 Hz, 6H, 2CH₃), 1.15-1.42 (m, 16H, 8CH₂), 1.91-2.05 (m, 4H, 2CH₂), 4.59 (t, J = 7.6 Hz, 4H, 2CH₂N⁺), 7.16 (d, J = 9.0 Hz, 4H, CH_{Ar}), 7.25 (d, J = 9.0 Hz, 4H, CH_{Ar}), 7.93-8.04 (m, 2H, 2CH), 8.12 (d, J = 8.7 Hz, 2H, 2CH), 8.68 (d, J = 5.7 Hz, 2H, 2CH), 8.84 (s, 2H, 2CH) ppm; ^{13}C NMR (75 MHz, MeOD): δ 14.4 (2C), 23.5 (2C), 27.1 (2C), 29.7 (2C), 32.5 (2C), 32.7 (2C), 63.4 (2C), 122.0 (4C), 123.1 (4C), 130.2 (2C), 133.6 (2C), 136.2 (2C), 139.9 (2C), 150.9 (2C), 156.6 (2C), 159.5 (2C) ppm; m/z (%): 540 (2), 455 (3), 356 (27), 335 (1), 279 (10), 202 (4), 157 (6), 135 (58), 57 (99), 41 (100); ν_{max} , (KBr): 3103, 2929, 2858, 1580, 1490, 1402, 1281, 1242, 1189, 752, cm^{-1}

3,3'-[Oxybis(4,1-phenyleneoxy)]bis(1-heptylpyridinium) diiodide 3'a



$C_{36}H_{46}I_2N_2O_3$; M. w. 808.6; Yellow solid (0.74 g, 0.91 mmol, 91% yield); M. p. 157-159 °C; 1H NMR (300 MHz, MeOD): δ 0.85 (t, J = 6.7 Hz, 6H, 2CH₃), 1.15-1.42 (m, 16H, 8CH₂), 1.91-2.05 (m, 4H, 2CH₂), 4.60 (t, J = 7.6 Hz, 4H, 2CH₂N⁺), 7.16 (d, J = 9.0 Hz, 4H, CH_{Ar}), 7.27 (d, J = 9.0 Hz, 4H, CH_{Ar}), 7.93-8.04 (m, 2H, 2CH), 8.12 (d, J = 8.7 Hz, 2H, 2CH), 8.68 (d, J = 5.7 Hz, 2H, 2CH), 8.84 (s, 2H, 2CH) ppm; ^{13}C NMR (75 MHz, MeOD): δ 14.4 (2C), 23.5 (2C), 27.1 (2C), 29.7 (2C), 32.5 (2C), 32.7 (2C), 63.4 (2C), 122.1 (4C), 123.2 (4C), 130.2 (2C), 133.7 (2C), 136.2 (2C), 139.8 (2C), 150.8 (2C), 156.5 (2C), 159.4 (2C) ppm; m/z (%): 540 (2), 455 (3), 356 (27), 335 (1), 279 (10), 202 (4), 157 (6), 135 (58), 57 (99), 41 (100); ν_{max} , (KBr): 3103, 2929, 2858, 1580, 1490, 1402, 1281, 1242, 1189, 752, cm^{-1}

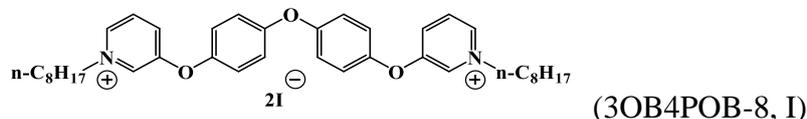
3,3'-[Oxybis(4,1-phenyleneoxy)]bis(1-octylpyridinium) dibromide 3b



$C_{38}H_{50}Br_2N_2O_3$; M. w. 742.6; White solid (0.63 g, 0.85 mmol, 85% yield); M. p. 200-202 °C; 1H NMR (300 MHz, MeOD): δ 0.84 (t, J = 6.5 Hz, 6H, 2CH₃), 1.11-1.47 (m, 20H, 10CH₂), 1.86-2.07 (m, 4H, 2CH₂), 4.59 (t, J = 7.4 Hz, 4H, 2CH₂N⁺), 7.16 (d, J = 8.50 Hz, 4H, CH_{Ar}), 7.25 (d, J = 8.5 Hz, 4H, CH_{Ar}), 7.92-8.03 (m, 2H, 2CH), 8.01 (d, J = 8.5 Hz, 2H, 2CH), 8.68 (d, J = 5.1 Hz, 2H, 2CH), 8.85 (s, 2H, 2CH) ppm; ^{13}C NMR (75 MHz, MeOD): δ 14.4 (2C), 23.6 (2C), 27.2 (2C), 30.0 (2C), 30.1 (2C), 32.5 (2C), 32.8 (2C), 63.5 (2C), 122.1 (4C), 123.1 (4C), 130.2 (2C), 133.7 (2C), 136.2 (2C), 139.9 (2C), 150.9 (2C), 156.6 (2C), 159.5 (2C) ppm;

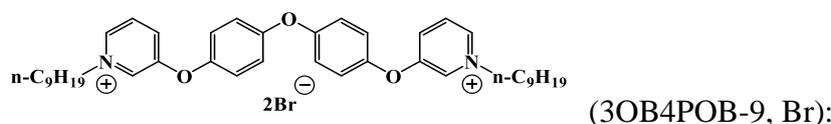
m/z (%): 580 (2), 469 (2), 436 (1), 356 (100), 327 (1), 278 (4), 194 (2), 137 (23), 71 (12), 41 (15); ν_{\max} , (KBr): 2927, 2855, 1490, 1281, 1240, 1189, 1151, 850, 809, 684 cm^{-1}

3,3'-[Oxybis(4,1-phenyleneoxy)]bis(1-octylpyridinium) diiodide 3'b



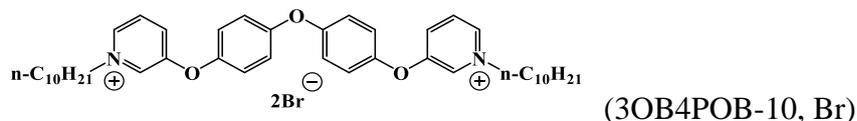
$\text{C}_{38}\text{H}_{50}\text{I}_2\text{N}_2\text{O}_3$; M. w. 836.6; Yellow solid (0.75 g, 0.9 mmol, 90% yield); M. p. 159-160 $^{\circ}\text{C}$; ^1H NMR (300 MHz, MeOD): δ 0.85 (t, $J = 6.5$ Hz, 6H, 2 CH_3), 1.11-1.47 (m, 20H, 10 CH_2), 1.86-2.07 (m, 4H, 2 CH_2), 4.60 (t, $J = 7.4$ Hz, 4H, 2 CH_2N^+), 7.17 (d, $J = 8.50$ Hz, 4H, CH_{Ar}), 7.27 (d, $J = 8.5$ Hz, 4H, CH_{Ar}), 7.91-8.03 (m, 2H, 2CH), 8.08 (d, $J = 8.5$ Hz, 2H, 2CH), 8.68 (d, $J = 5.1$ Hz, 2H, 2CH) ppm; ^{13}C NMR (75 MHz, MeOD): δ 14.4 (2C), 23.6 (2C), 27.1 (2C), 30.0 (2C), 30.1 (2C), 32.5 (2C), 32.8 (2C), 63.4 (2C), 122.1 (4C), 123.2 (4C), 130.3 (2C), 133.7 (2C), 136.2 (2C), 139.8 (2C), 150.8 (2C), 156.5 (2C), 159.4 (2C) ppm; m/z (%): 580 (2), 469 (2), 436 (1), 356 (100), 327 (1), 278 (4), 194 (2), 137 (23), 71 (12), 41 (15); ν_{\max} , (KBr): 2927, 2855, 1490, 1281, 1240, 1189, 1151, 850, 809, 684 cm^{-1}

3,3'-[Oxybis(4,1-phenyleneoxy)]bis(1-nonylpyridinium) dibromide 3c



$\text{C}_{40}\text{H}_{54}\text{Br}_2\text{N}_2\text{O}_3$; M. w. 770.7; White solid (0.65 g, 0.84 mmol, 84% yield); M. p. 207-208 $^{\circ}\text{C}$; ^1H NMR (300 MHz, MeOD): δ 0.84 (t, $J = 6.8$ Hz, 6H, 2 CH_3), 1.15-1.42 (m, 24H, 12 CH_2), 1.91-2.05 (m, 4H, 2 CH_2), 4.59 (t, $J = 7.6$ Hz, 4H, 2 CH_2N^+), 7.15 (d, $J = 8.5$ Hz, 4H, CH_{Ar}), 7.25 (d, $J = 8.5$ Hz, 4H, CH_{Ar}), 7.95-8.03 (m, 2H, 2CH), 8.08 (d, $J = 8.7$ Hz, 2H, 2CH), 8.68 (d, $J = 5.6$ Hz, 2H, 2CH) ppm; ^{13}C NMR (75 MHz, MeOD): δ 14.4 (2C), 23.7 (2C), 27.1 (2C), 30.1 (2C), 30.2 (2C), 30.4 (2C), 32.5 (2C), 32.9 (2C), 63.4 (2C), 122.0 (4C), 123.1 (4C), 130.2 (2C), 133.6 (2C), 136.2 (2C), 139.9 (2C), 150.9 (2C), 156.6 (2C), 159.5 (2C) ppm; m/z (%): 483 (3), 406 (3), 356 (8), 279 (7), 171 (6), 135 (76), 85 (24), 71 (34), 55 (53), 41 (100); ν_{\max} , (KBr): 2955, 2927, 2855, 1580, 1490, 1280, 1242, 1189, 850, 684 cm^{-1}

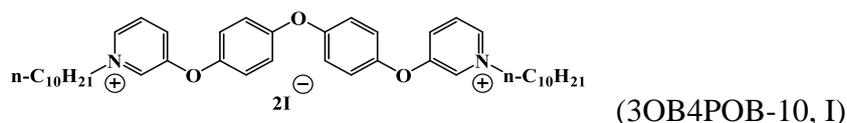
3,3'-[Oxybis(4,1-phenyleneoxy)]bis(1-decylpyridinium) dibromide 3d



$\text{C}_{42}\text{H}_{58}\text{Br}_2\text{N}_2\text{O}_3$; M. w. 798.8; White solid (0.72 g, 0.9 mmol, 90% yield); M. p. 207-208 $^{\circ}\text{C}$; ^1H NMR (300 MHz, $\text{DMSO}-d_6$): δ 0.87 (t, $J = 6.3$ Hz, 6H, 2 CH_3), 1.13-1.41 (m, 28H, 14 CH_2), 1.82-2.03 (m, 4H, 2 CH_2), 4.65 (t, $J = 7.3$ Hz, 4H, 2 CH_2N^+), 7.23 (d, $J = 8.8$ Hz, 4H,

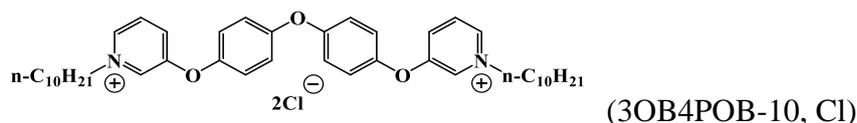
CH_{Ar}), 7.37 (d, J = 8.8 Hz, 4H, CH_{Ar}), 8.09-8.17 (m, 2H, 2CH), 8.20 (d, J = 8.5 Hz, 2H, 2CH), 8.94 (d, J = 5.1 Hz, 2H, 2CH), 9.18 (s, 2H, 2CH) ppm; ¹³C NMR (75 MHz, DMSO-*d*₆): δ 13.9 (2C), 22.0 (2C), 25.3 (2C), 28.3 (2C), 28.6 (2C), 28.7 (2C), 28.8 (2C), 30.7 (2C), 31.2 (2C), 60.9 (2C), 120.6 (4C), 121.6 (4C), 128.9 (2C), 132.6 (2C), 135.6 (2C), 139.1 (2C), 149.6 (2C), 154.1 (2C), 156.6 (2C) ppm; *m/z* (%): 636 (1), 581 (2), 497 (3), 436 (1), 356 (100), 278 (4), 222 (2), 149 (4), 135 (26), 43 (17); *v*_{max}, (KBr): 2956, 2924, 2853, 1497, 1474, 1282, 1244, 1190, 811, 680 cm⁻¹

3,3'-[Oxybis(4,1-phenyleneoxy)]bis(1-decylpyridinium) diiodide 3'd



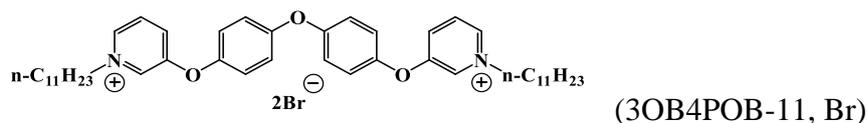
C₄₂H₅₈I₂N₂O₃; M. w. 892.8; Yellow solid (0.82 g, 0.92 mmol, 92% yield); M. p. 161-163 °C; ¹H NMR (300 MHz, DMSO-*d*₆): δ 0.85 (t, J = 6.3 Hz, 6H, 2CH₃), 1.13-1.41 (m, 28H, 14CH₂), 1.82-2.03 (m, 4H, 2CH₂), 4.60 (t, J = 7.3 Hz, 4H, 2CH₂N⁺), 7.21 (d, J = 8.8 Hz, 4H, CH_{Ar}), 7.35 (d, J = 8.8 Hz, 4H, CH_{Ar}), 8.09-8.17 (m, 2H, 2CH), 8.18 (d, J = 8.5 Hz, 2H, 2CH), 8.86 (d, J = 5.1 Hz, 2H, 2CH), 9.11 (s, 2H, 2CH) ppm; ¹³C NMR (75 MHz, DMSO-*d*₆): δ 13.9 (2C), 22.0 (2C), 25.3 (2C), 28.3 (2C), 28.6 (2C), 28.7 (2C), 28.8 (2C), 30.7 (2C), 31.2 (2C), 61.0 (2C), 120.6 (4C), 121.7 (4C), 128.9 (2C), 132.6 (2C), 135.5 (2C), 139.0 (2C), 149.5 (2C), 154.1 (2C), 156.7 (2C) ppm; *m/z* (%): 636 (1), 581 (2), 497 (3), 436 (1), 356 (100), 278 (4), 222 (2), 149 (4), 135 (26), 43 (17); *v*_{max}, (KBr): 2956, 2924, 2853, 1497, 1474, 1282, 1244, 1190, 811, 680 cm⁻¹

3,3'-[Oxybis(4,1-phenyleneoxy)]bis(1-decylpyridinium) dichloride 3''d



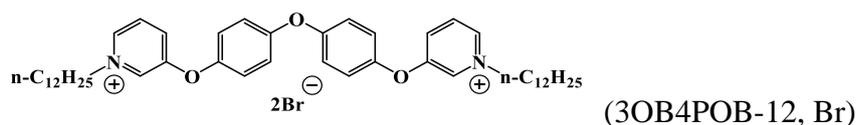
C₄₂H₅₈Cl₂N₂O; M. w. 709.8; White solid (0.55 g, 0.77 mmol, 77% yield); M. p. 229-230 °C; ¹H NMR (300 MHz, DMSO-*d*₆): δ 0.84 (t, J = 6.7 Hz, 6H, 2CH₃), 1.21-1.32 (m, 28H, 14CH₂), 1.83-2.00 (m, 4H, 2CH₂), 4.64 (t, J = 6.6 Hz, 4H, 2CH₂N⁺), 7.21 (d, J = 9.0 Hz, 4H, CH_{Ar}), 7.35 (d, J = 9.0 Hz, 4H, CH_{Ar}), 8.09-8.22 (m, 4H, 4CH), 8.94 (d, J = 5.4 Hz, 2H, 2CH), 9.20 (s, 2H, 2CH) ppm; ¹³C NMR (75 MHz, DMSO-*d*₆): δ 13.8 (2C), 22.0 (2C), 25.3 (2C), 28.3 (2C), 28.5 (2C), 28.7 (2C), 28.8 (2C), 30.8 (2C), 31.2 (2C), 60.7 (2C), 120.5 (4C), 121.6 (4C), 128.9 (2C), 132.6 (2C), 135.7 (2C), 139.2 (2C), 149.6 (2C), 154.1 (2C), 156.5 (2C) ppm; *m/z* (%): 639 (1), 497 (11), 420 (1), 356 (8), 279 (1), 263 (1), 157 (1), 91 (82), 43 (100), 27 (38); *v*_{max}, (KBr): 3018, 2926, 2855, 1581, 1491, 1402, 1280, 1242, 1189, 751 cm⁻¹

3,3'-[Oxybis(4,1-phenyleneoxy)]bis(1-undecylpyridinium) dibromide 3e



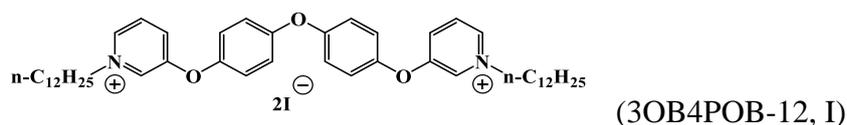
C₄₄H₆₂Br₂N₂O₃; M. w. 826.8; White solid (0.79 g, 0.95 mmol, 95% yield); M. p. 216-217 °C; ¹H NMR (300 MHz, MeOD): δ 0.84 (t, J = 6.3 Hz, 6H, 2CH₃), 1.15-1.46 (m, 32H, 16CH₂), 1.94-2.08 (m, 4H, 2CH₂), 4.59 (t, J = 7.6 Hz, 4H, 2CH₂N⁺), 7.16 (d, J = 8.8 Hz, 4H, CH_{Ar}), 7.25 (d, J = 8.8 Hz, 4H, CH_{Ar}), 7.95-8.03 (m, 2H, 2CH), 8.08 (d, J = 8.7 Hz, 2H, 2CH), 8.68 (d, J = 5.1 Hz, 2H, 2CH), 8.85 (s, 2H, 2CH) ppm; ¹³C NMR (75 MHz, MeOD): δ 14.4 (2C), 23.7 (2C), 27.2 (2C), 30.1 (2C), 30.4 (2C), 30.5 (2C), 30.6 (2C), 30.7 (2C), 32.6 (2C), 33.0 (2C), 63.4 (2C), 122.1 (4C), 123.1 (4C), 130.2 (2C), 133.6 (2C), 136.3 (2C), 139.9 (2C), 150.9 (2C), 156.6 (2C), 159.5 (2C) ppm; *m/z* (%): 511 (1), 356 (12), 279 (5), 234 (6), 178 (1), 137 (63), 109 (19), 57 (43), 43 (100), 28 (65); *v*_{max}, (KBr): 3013, 2926, 2855, 1580, 1490, 1401, 1280, 1242, 1189, 752 cm⁻¹

3,3'-[Oxybis(4,1-phenyleneoxy)]bis(1-dodecylpyridinium) dibromide 3f



C₄₆H₆₆Br₂N₂O₃; M. w. 854.9; White solid (0.81 g, 0.95 mmol, 95% yield); M. p. 212-213 °C; ¹H NMR (300 MHz, DMSO-*d*₆): δ 0.87 (t, J = 6.4 Hz, 6H, 2CH₃), 1.12-1.45 (m, 36H, 18CH₂), 1.94 (m, 4H, 2CH₂), 4.65 (t, J = 7.4 Hz, 4H, 2CH₂N⁺), 7.23 (d, J = 8.1 Hz, 4H, CH_{Ar}), 7.37 (d, J = 8.1 Hz, 4H, CH_{Ar}), 8.08-8.17 (m, 2H, 2CH), 8.20 (d, J = 7.9 Hz, 2H, 2CH), 8.93 (d, J = 5.1 Hz, 2H, 2CH), 9.17 (s, 2H, 2CH) ppm; ¹³C NMR (75 MHz, DMSO-*d*₆): δ 13.9 (2C), 22.0 (2C), 25.3 (2C), 28.3 (2C), 28.6 (2C), 28.7 (6C), 28.9 (2C), 30.7 (2C), 31.2 (2C), 60.9 (2C), 120.6 (4C), 121.6 (4C), 128.9 (2C), 132.6 (2C), 135.6 (2C), 139.1 (2C), 149.6 (2C), 154.1 (2C), 156.6 (2C) ppm; *m/z* (%): 525 (5), 432 (1), 383 (1), 356 (100), 278 (4), 250 (3), 178 (2), 137 (45), 69 (28), 43 (64); *v*_{max}, (KBr): 2956, 2923, 2852, 1497, 1473, 1282, 1244, 1191, 811, 680 cm⁻¹

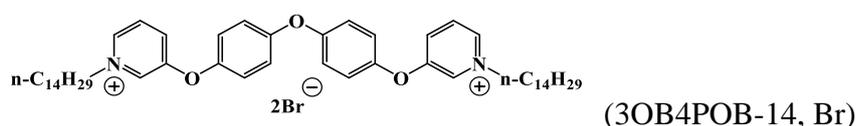
3,3'-[Oxybis(4,1-phenyleneoxy)]bis(1-dodecylpyridinium) diiodide 3'f



C₄₆H₆₆I₂N₂O₃; M. w. 948.9; Yellow solid (0.87 g, 0.92 mmol, 92% yield); M. p. 153-155 °C; ¹H NMR (300 MHz, DMSO-*d*₆): δ 0.85 (t, J = 6.4 Hz, 6H, 2CH₃), 1.12-1.45 (m, 36H, 18CH₂), 1.85-2.01 (m, 4H, 2CH₂), 4.60 (t, J = 7.4 Hz, 4H, 2CH₂N⁺), 7.21 (d, J = 8.1 Hz, 4H, CH_{Ar}), 7.35 (d, J = 8.1 Hz, 4H, CH_{Ar}), 8.08-8.16 (m, 2H, 2CH), 8.18 (d, J = 7.9 Hz, 2H, 2CH), 8.86 (d, J = 5.1 Hz, 2H, 2CH), 9.10 (s, 2H, 2CH) ppm; ¹³C NMR (75 MHz, MeOD): δ 14.5 (2C), 23.7 (2C), 27.1 (2C), 30.1 (2C), 30.4 (2C), 30.5 (4C), 30.6 (2C), 30.7 (2C), 32.5

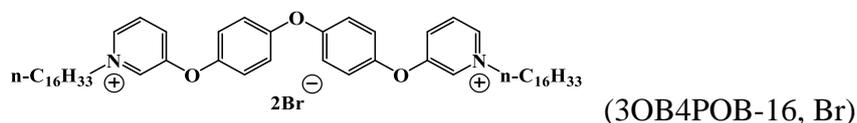
(2C), 33.0 (2C), 63.4 (2C), 122.1 (4C), 123.2 (4C), 130.3 (2C), 133.7 (2C), 136.2 (2C), 139.8 (2C), 150.8 (2C), 156.5 (2C), 159.4 (2C) ppm; m/z (%): 525 (5), 432 (1), 383 (1), 356 (100), 278 (4), 250 (3), 178 (2), 137 (45), 69 (28), 43 (64); ν_{\max} , (KBr): 2956, 2923, 2852, 1497, 1473, 1282, 1244, 1191, 811, 680 cm^{-1}

3,3'-[Oxybis(4,1-phenyleneoxy)]bis(1-tetradecylpyridinium) dibromide 3g



$\text{C}_{50}\text{H}_{74}\text{Br}_2\text{N}_2\text{O}_3$; M. w. 911.0; White solid (0.85 g, 0.93 mmol, 93% yield); M. p. 221-222 °C; ^1H NMR (300 MHz, DMSO- d_6): δ 0.87 (t, J = 6.6 Hz, 6H, 2CH₃), 1.27 (m, 44H, 22CH₂), 1.82-2.04 (m, 4H, 2CH₂), 4.63 (t, J = 7.2 Hz, 4H, 2CH₂N⁺), 7.23 (d, J = 8.8 Hz, 4H, CH_{Ar}), 7.37 (d, J = 8.8 Hz, 4H, CH_{Ar}), 8.08-8.17 (m, 2H, 2CH), 8.20 (d, J = 8.3 Hz, 2H, 2CH), 8.91 (d, J = 5.3 Hz, 2H, 2CH) ppm; ^{13}C NMR (75 MHz, DMSO- d_6): δ 13.8 (2C), 22.0 (2C), 25.3 (2C), 28.3 (2C), 28.6 (2C), 28.7 (4C), 28.9 (6C), 30.0 (2C), 30.7 (2C), 31.2 (2C), 60.9 (2C), 120.5 (4C), 121.6 (4C), 128.9 (2C), 132.6 (2C), 135.6 (2C), 139.1 (2C), 149.6 (2C), 154.1 (2C), 156.6 (2C) ppm; m/z (%): 553 (3), 472 (1), 436 (1), 356 (100), 327 (1), 278 (4), 178 (2), 135 (29), 78 (10), 57 (17); ν_{\max} , (KBr): 2956, 2922, 2851, 1498, 1470, 1282, 1244, 1200, 810, 679 cm^{-1}

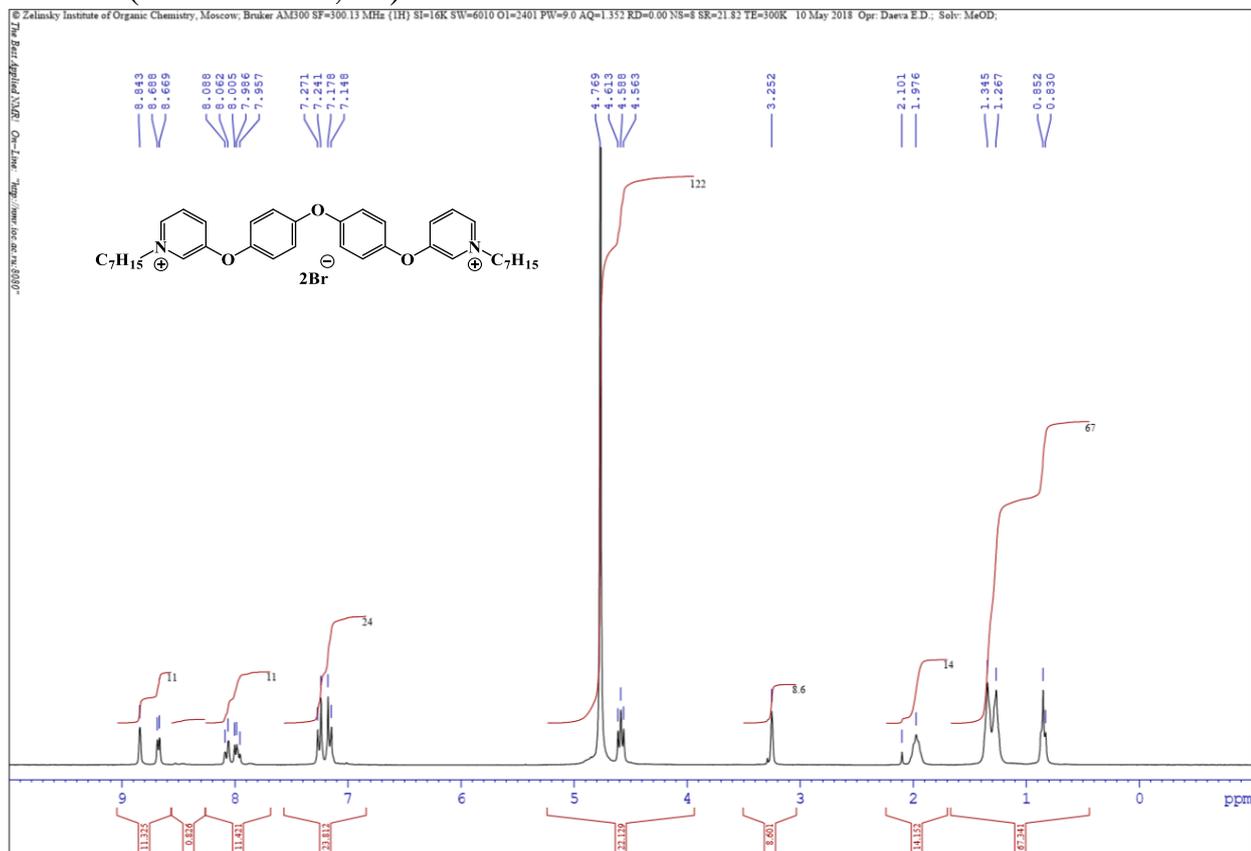
3,3'-[Oxybis(4,1-phenyleneoxy)]bis(1-hexadecylpyridinium) dibromide 3h

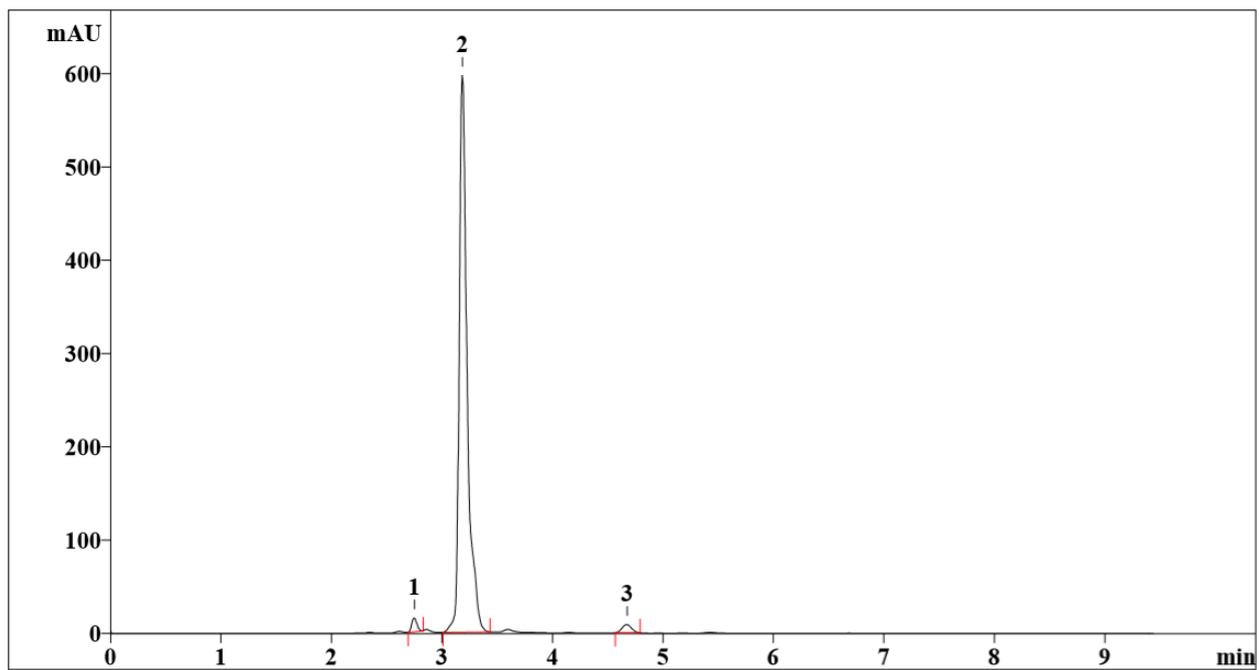
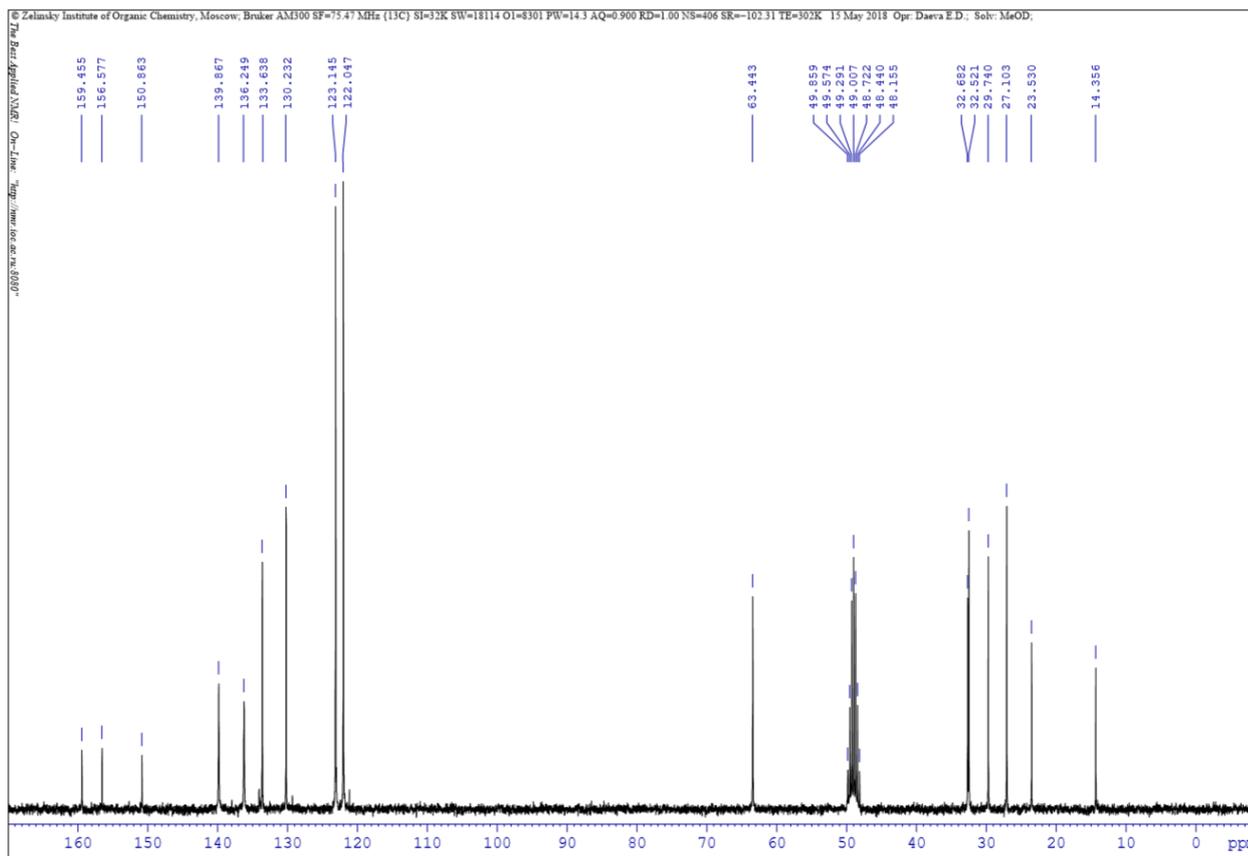


$\text{C}_{54}\text{H}_{82}\text{Br}_2\text{N}_2\text{O}_3$; M. w. 967.1; White solid (0.83 g, 0.86 mmol, 86% yield); M. p. 215-216 °C; ^1H NMR (300 MHz, MeOD): δ 0.84 (t, J = 6.6 Hz, 6H, 2CH₃), 1.11-1.43 (m, 52H, 26CH₂), 1.86-2.05 (m, 4H, 2CH₂), 4.59 (t, J = 7.3 Hz, 4H, 2CH₂N⁺), 7.16 (d, J = 8.8 Hz, 4H, CH_{Ar}), 7.26 (d, J = 8.0 Hz, 4H, CH_{Ar}), 7.94-8.02 (m, 2H, 2CH), 8.08 (d, J = 8.8 Hz, 2H, 2CH), 8.68 (d, J = 5.7 Hz, 2H, 2CH), 8.84 (s, 2H, 2CH) ppm; ^{13}C NMR (75 MHz, MeOD): δ 14.4 (2C), 23.6 (2C), 27.1 (2C), 30.0 (2C), 30.1 (2C), 30.4 (2C), 30.5 (2C), 30.6 (4C), 30.7 (8C), 32.5 (2C), 33.0 (2C), 63.5 (2C), 122.0 (4C), 123.1 (4C), 130.2 (2C), 133.7 (2C), 136.2 (2C), 139.9 (2C), 150.9 (2C), 156.6 (2C), 159.5 (2C) ppm; m/z (%): 581 (3), 540 (1), 436 (1), 371 (1), 356 (100), 278 (3), 225 (2), 135 (26), 85 (8), 57 (17); ν_{\max} , (KBr): 2988, 2920, 2851, 1498, 1470, 1282, 1244, 1199, 811, 679 cm^{-1}

4. NMR (¹H, ¹³C) spectra and HPLC images

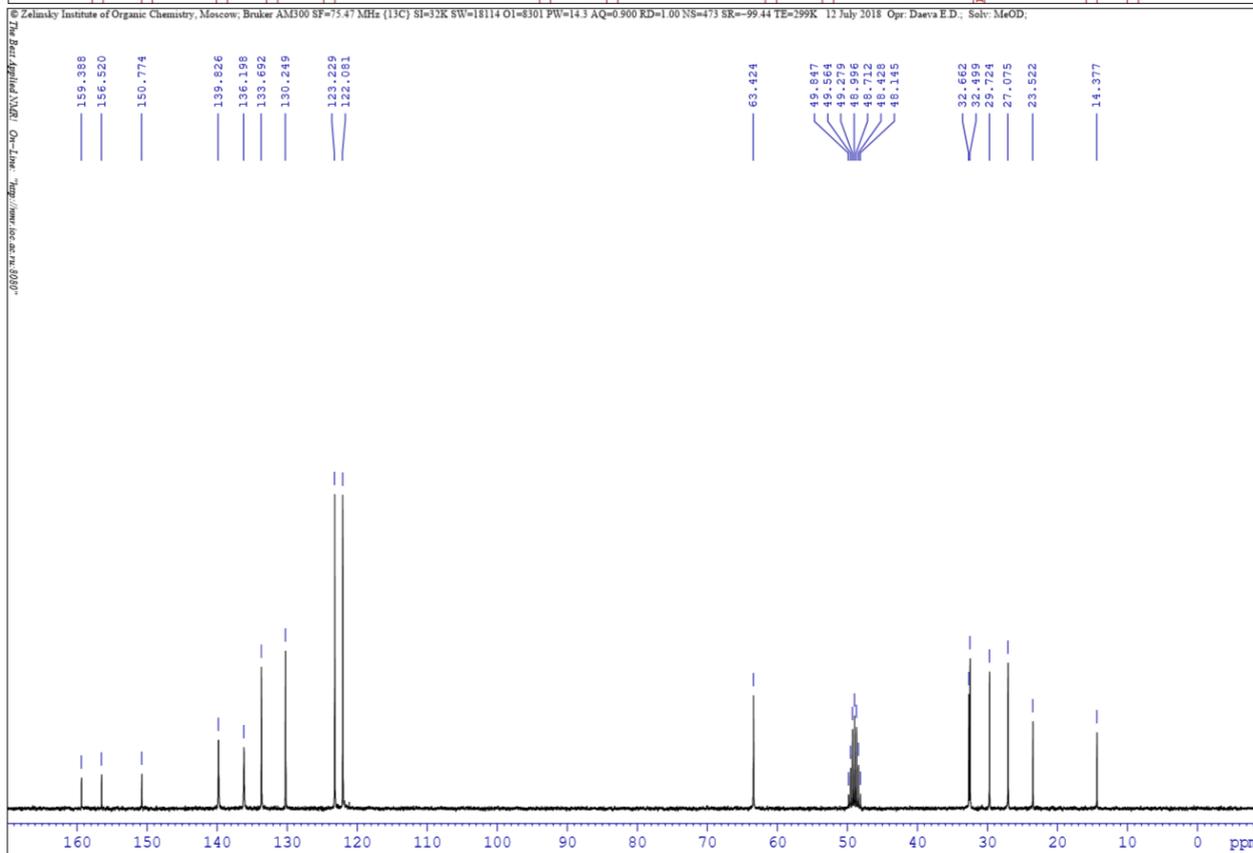
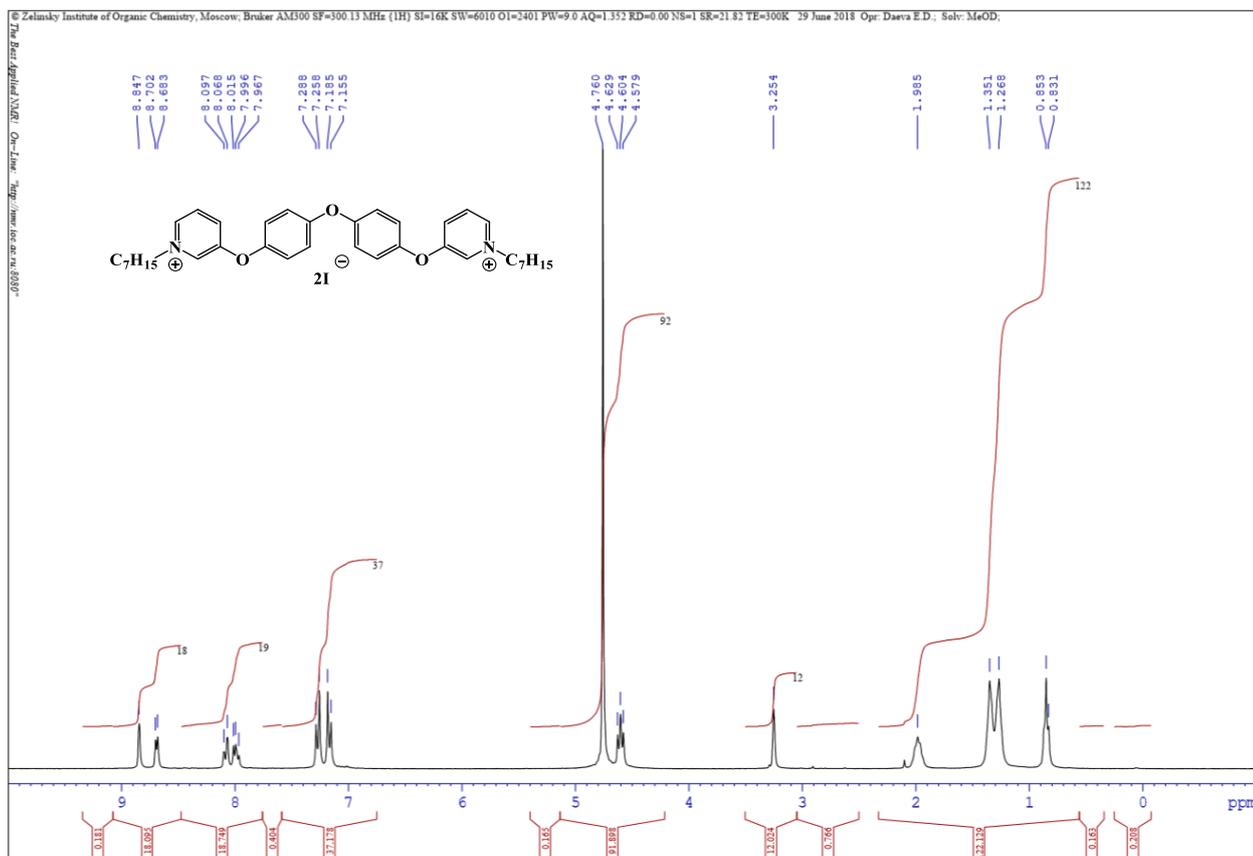
3a (3OB4POB-7, Br)

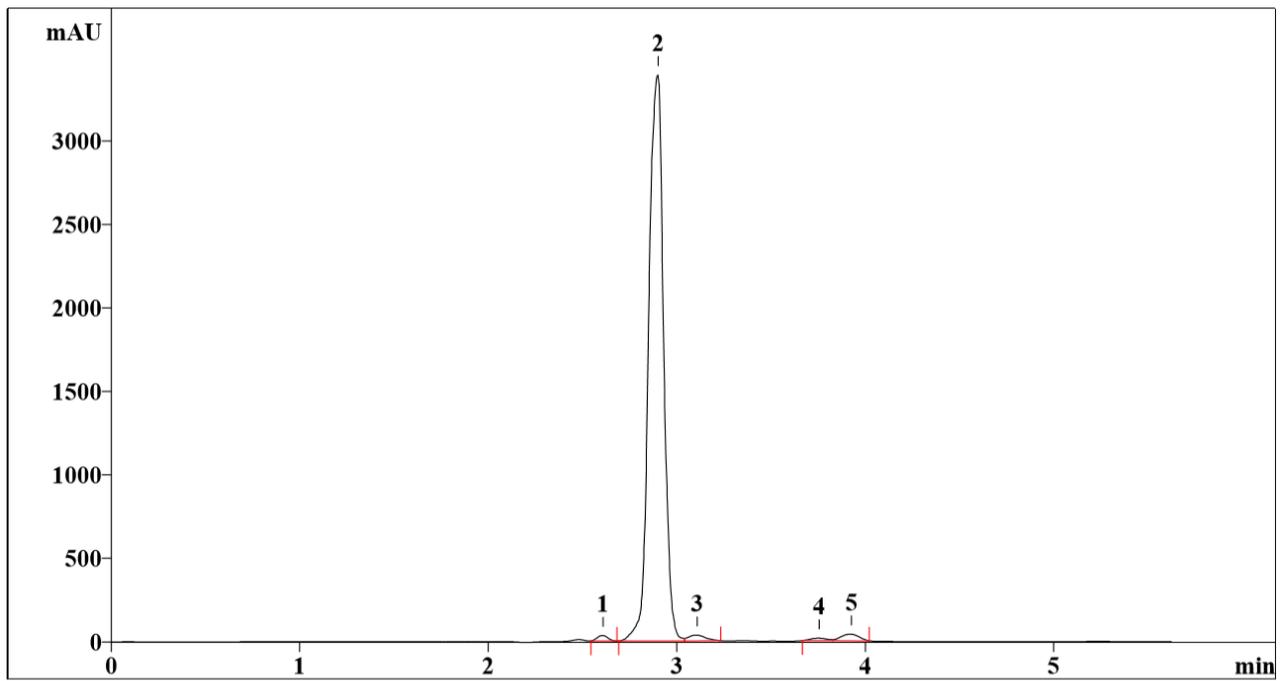




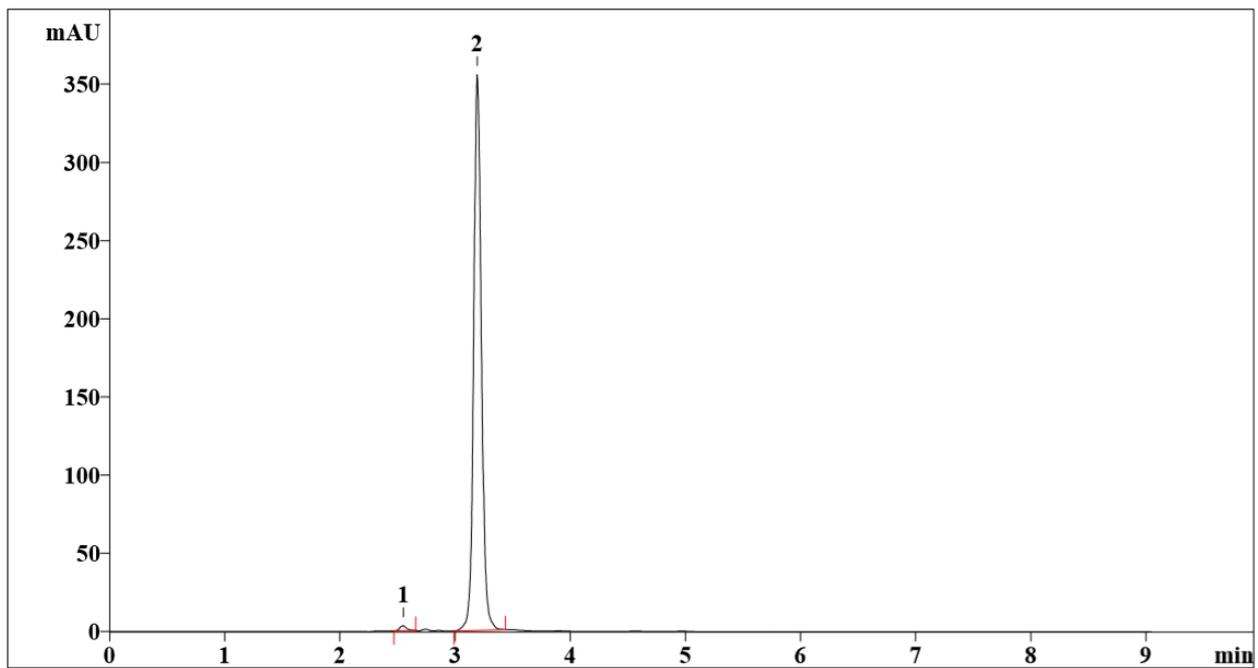
Peak	Retention min	Width h/2 min	Height mAU	Area mAU*sec	Area %	Type
1	2.75	0.054	14.50	47.245	1.53	BB
2	3.18	0.070	595.84	2995.303	96.77	BB
3	4.67	0.093	8.89	52.766	1.70	BB

3'a (3OB4POB-7, I)



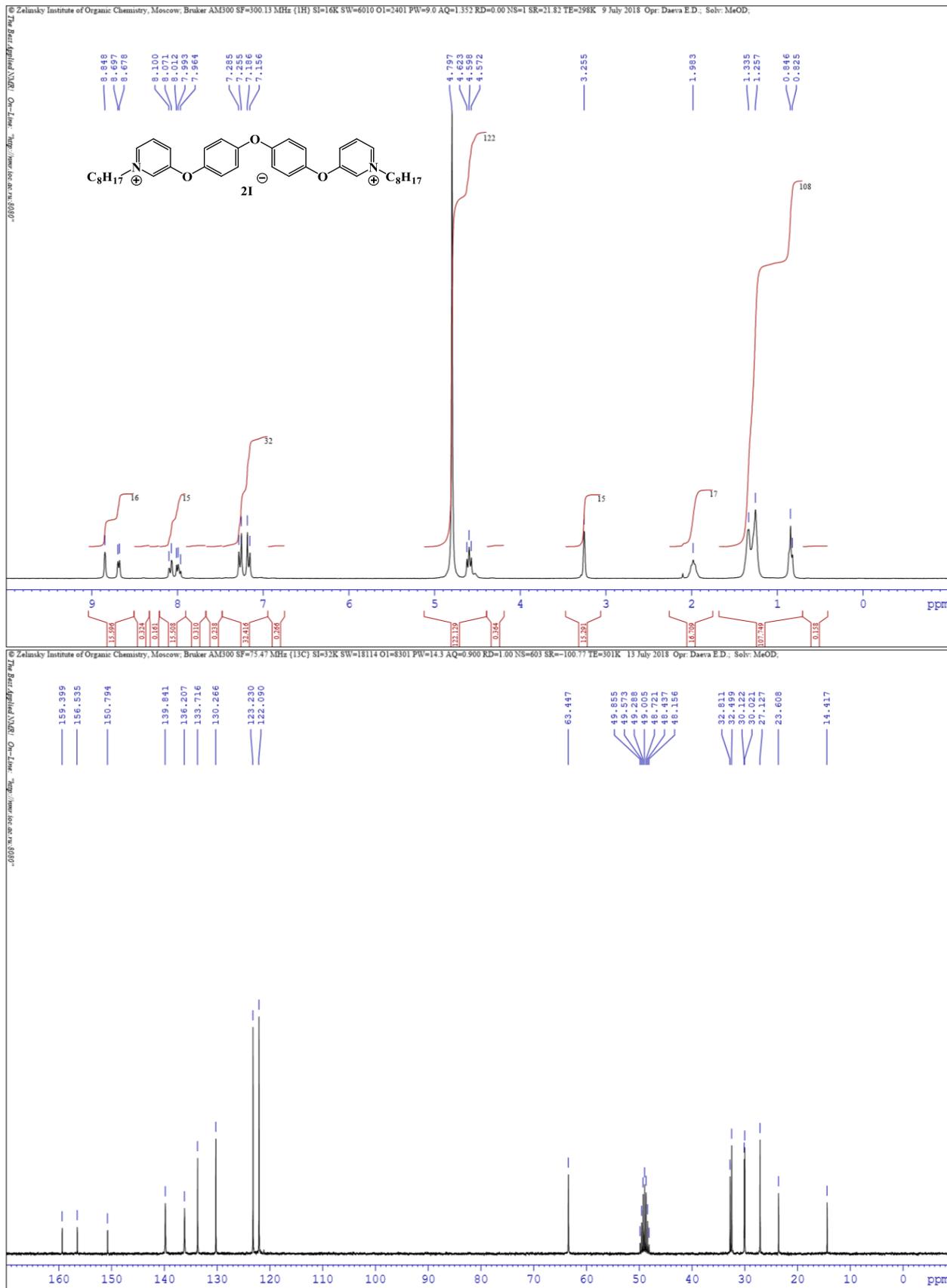


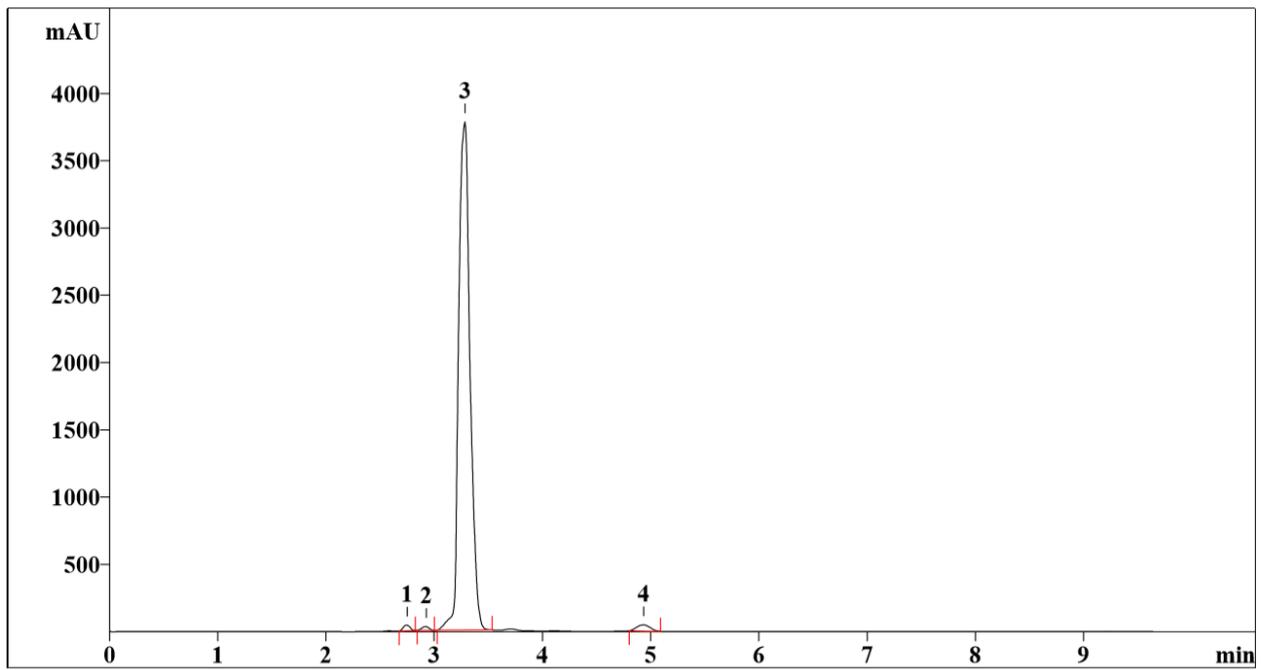
Peak	Retention min	Width h/2 min	Height mAU	Area mAU*sec	Area %	Type
1	2.61	0.063	32.94	130.138	0.68	BB
2	2.90	0.085	3389.46	18570.304	96.38	BD
3	3.11	0.105	33.53	212.876	1.10	DB
4	3.75	0.109	14.51	90.939	0.47	BD
5	3.92	0.110	39.64	263.547	1.37	DB



Peak	Retention min	Width h/2 min	Height mAU	Area mAU*sec	Area %	Type
1	2.55	0.061	3.17	12.696	0.76	BB
2	3.19	0.070	353.87	1659.312	99.24	BB

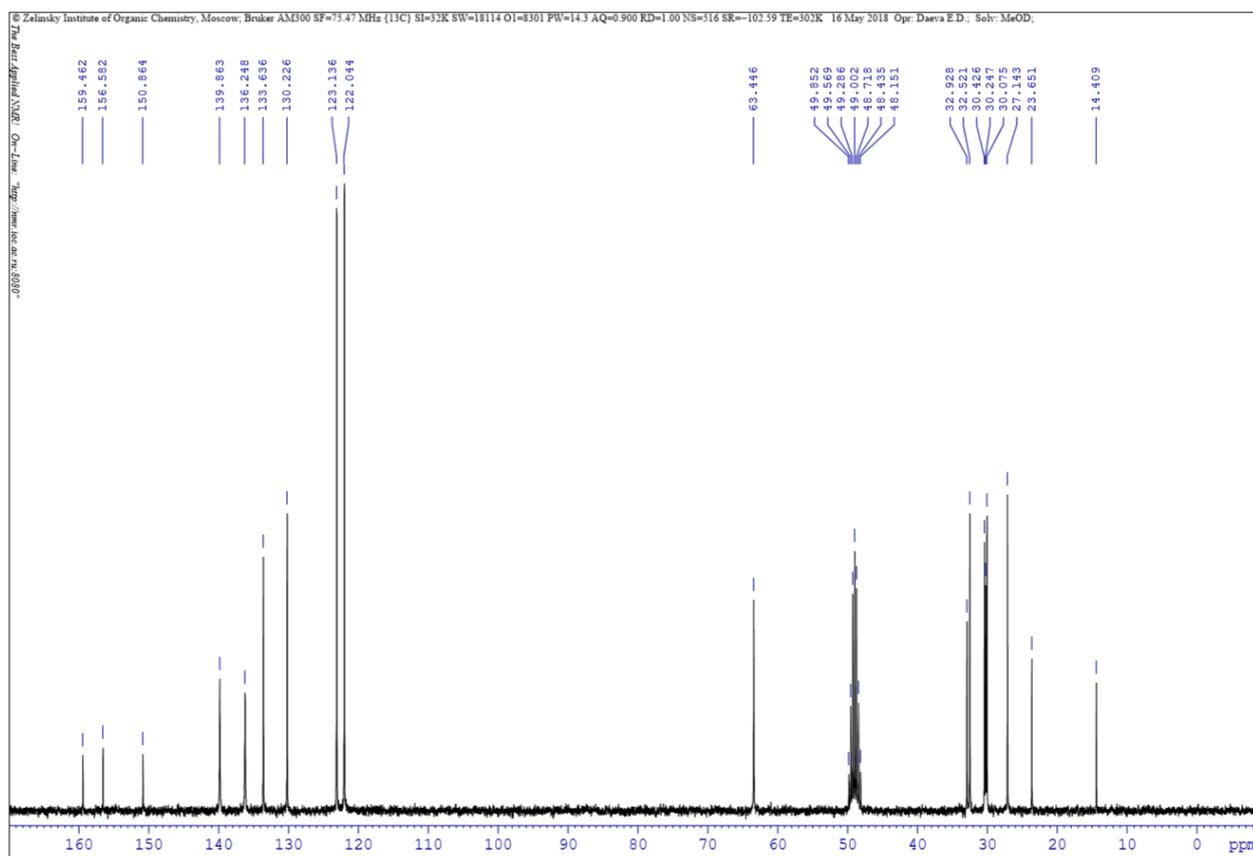
3'b (3OB4POB-8, I)

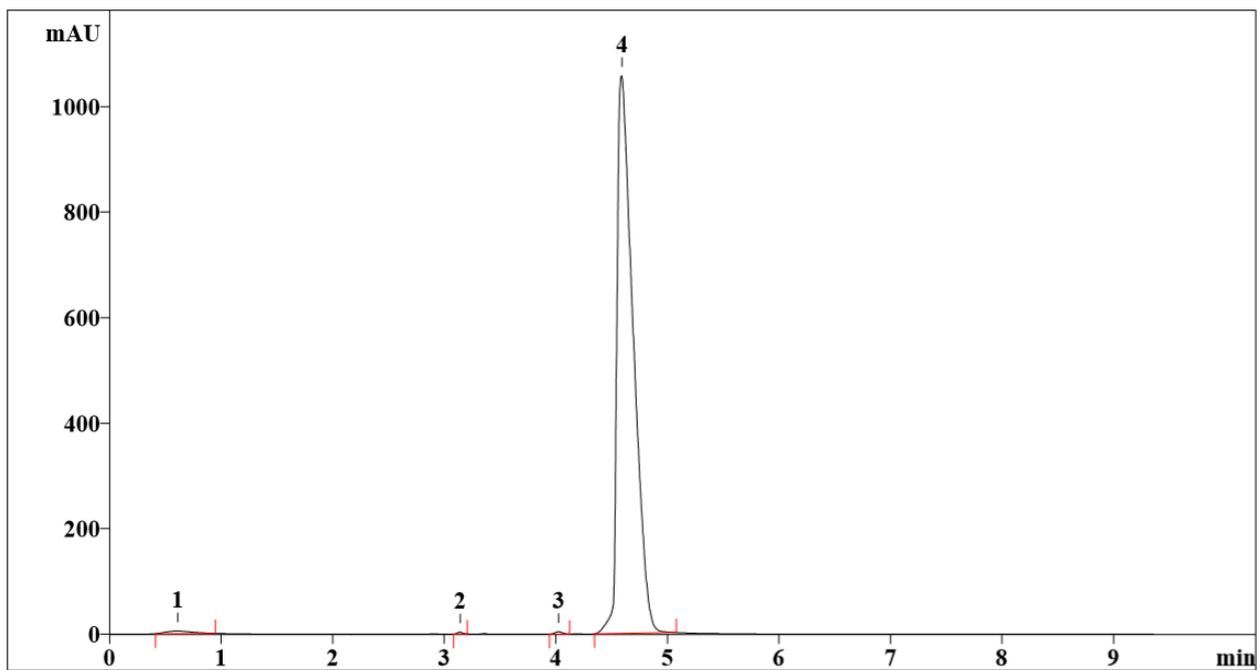




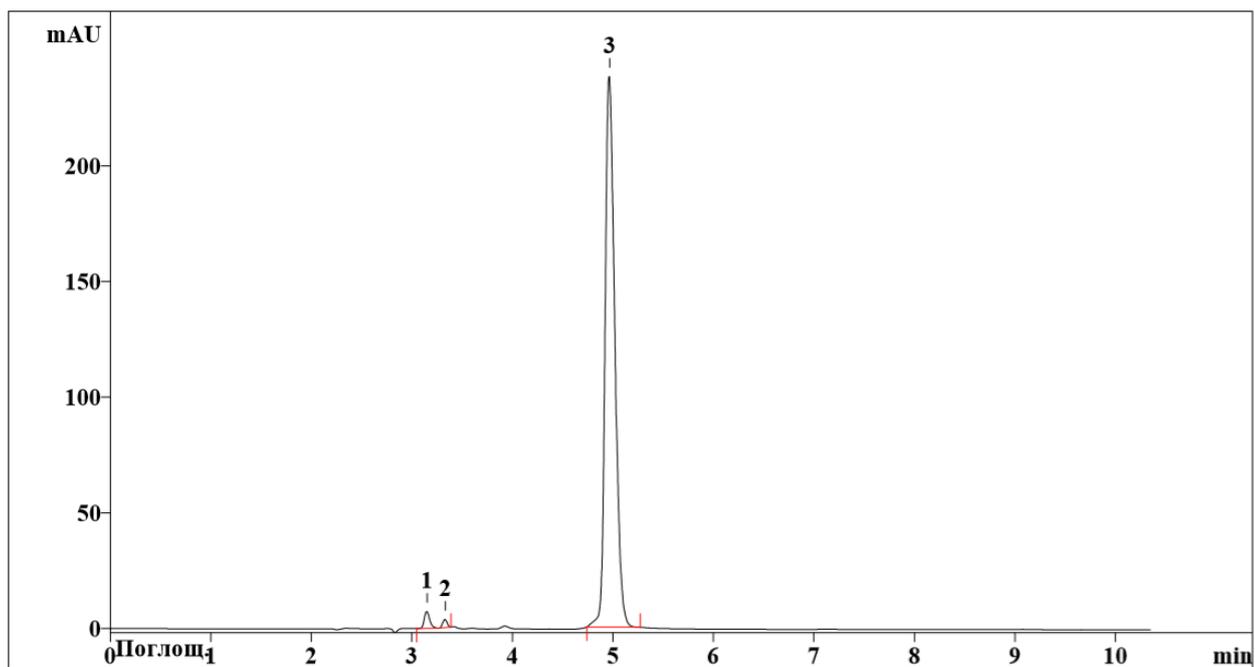
Peak	Retention min	Width min	h/2 min	Height mAU	Area mAU*sec	Area %	Type
1	2.75	0.072		41.98	184.309	0.65	BB
2	2.92	0.078		29.25	139.330	0.49	BB
3	3.28	0.114		3776.32	27670.271	97.46	BB
4	4.93	0.144		45.46	396.650	1.40	BB

3c (3OB4POB-9, Br)

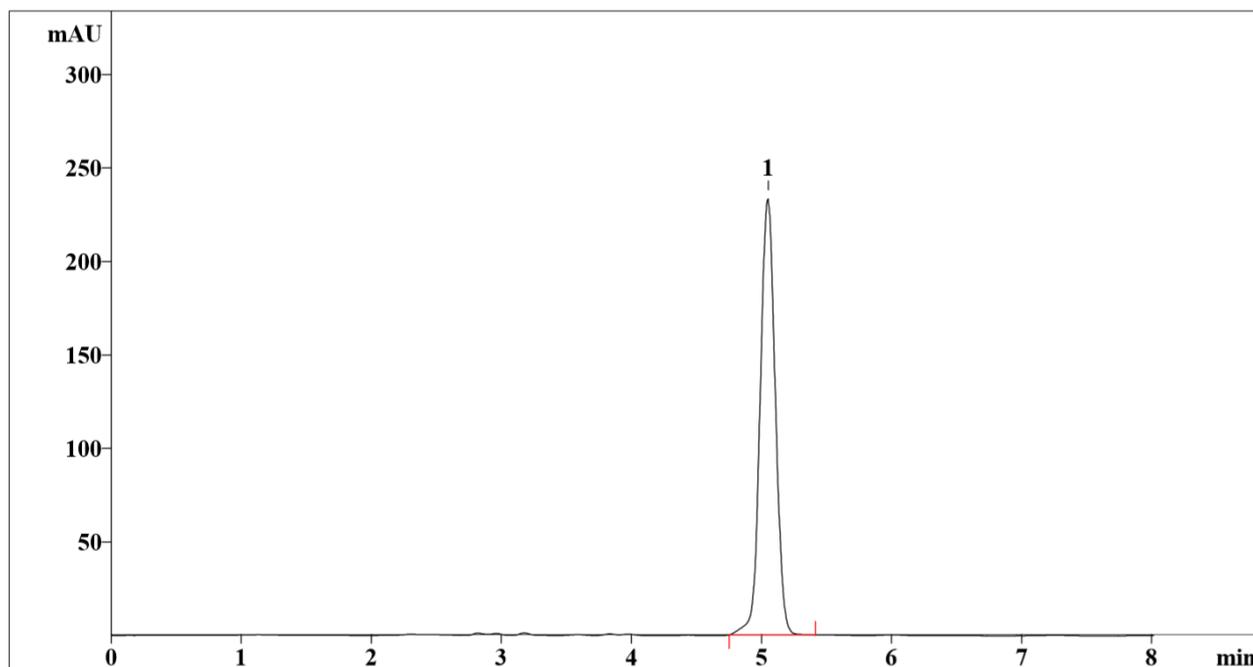




Peak	Retention min	Width h/2 min	Height mAU	Area mAU*sec	Area %	Type
1	0.61	0.261	4.78	77.485	0.72	BB
2	3.14	0.053	3.55	11.585	0.11	BB
3	4.03	0.073	4.23	19.590	0.18	BB
4	4.59	0.159	1057.24	10684.165	98.99	BB



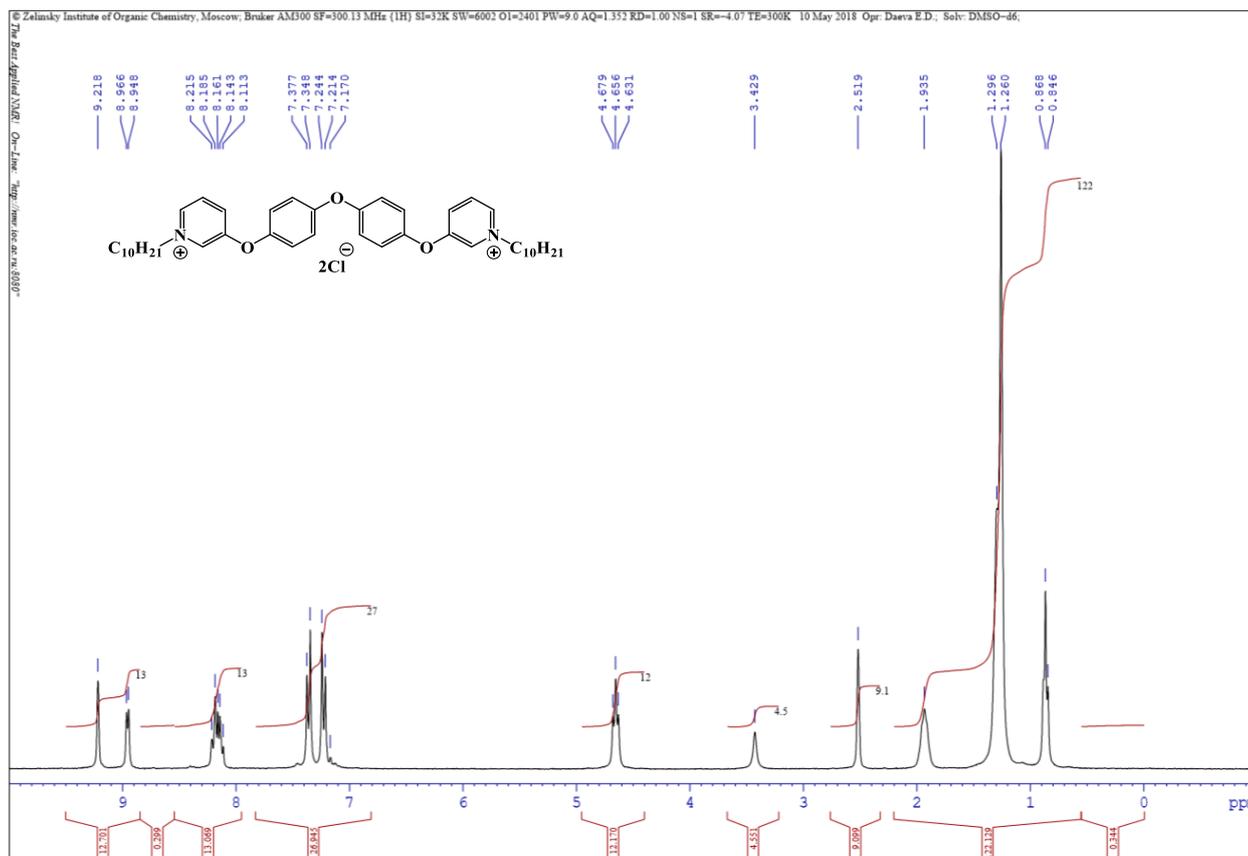
Peak	Retention min	Width h/2 min	Height mAU	Area mAU*sec	Area %	Type
1	3.15	0.058	7.23	27.011	1.64	BD
2	3.33	0.052	3.42	10.525	0.64	DB
3	4.96	0.103	238.14	1605.772	97.72	BB

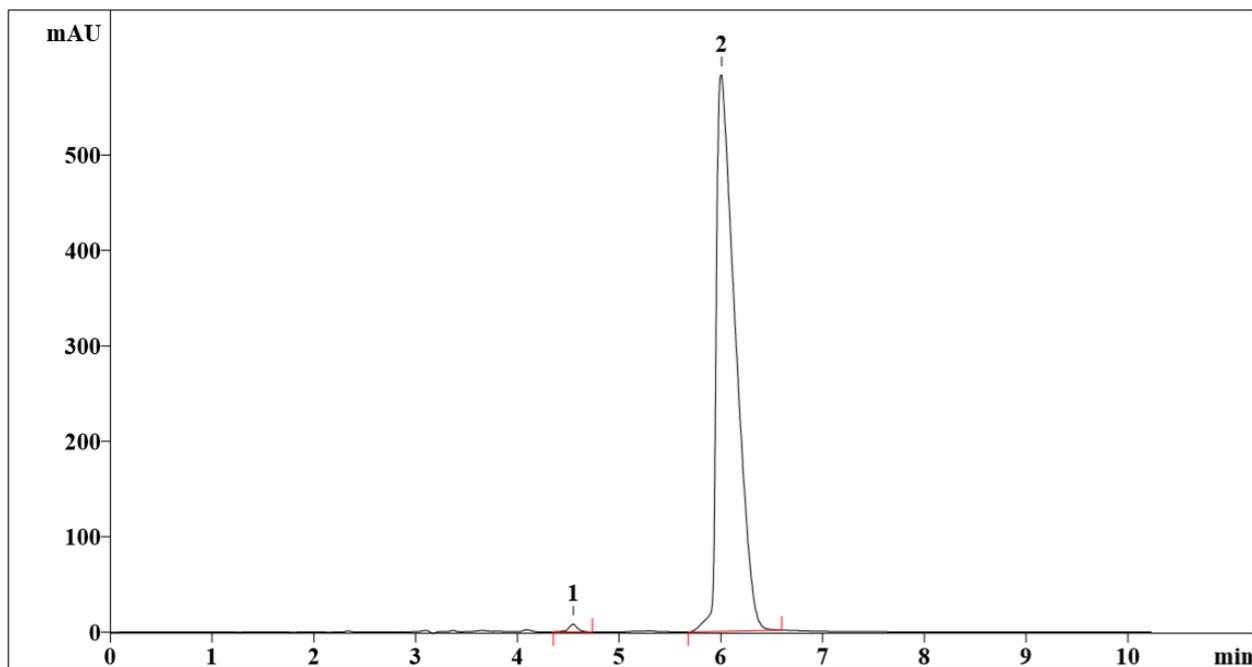
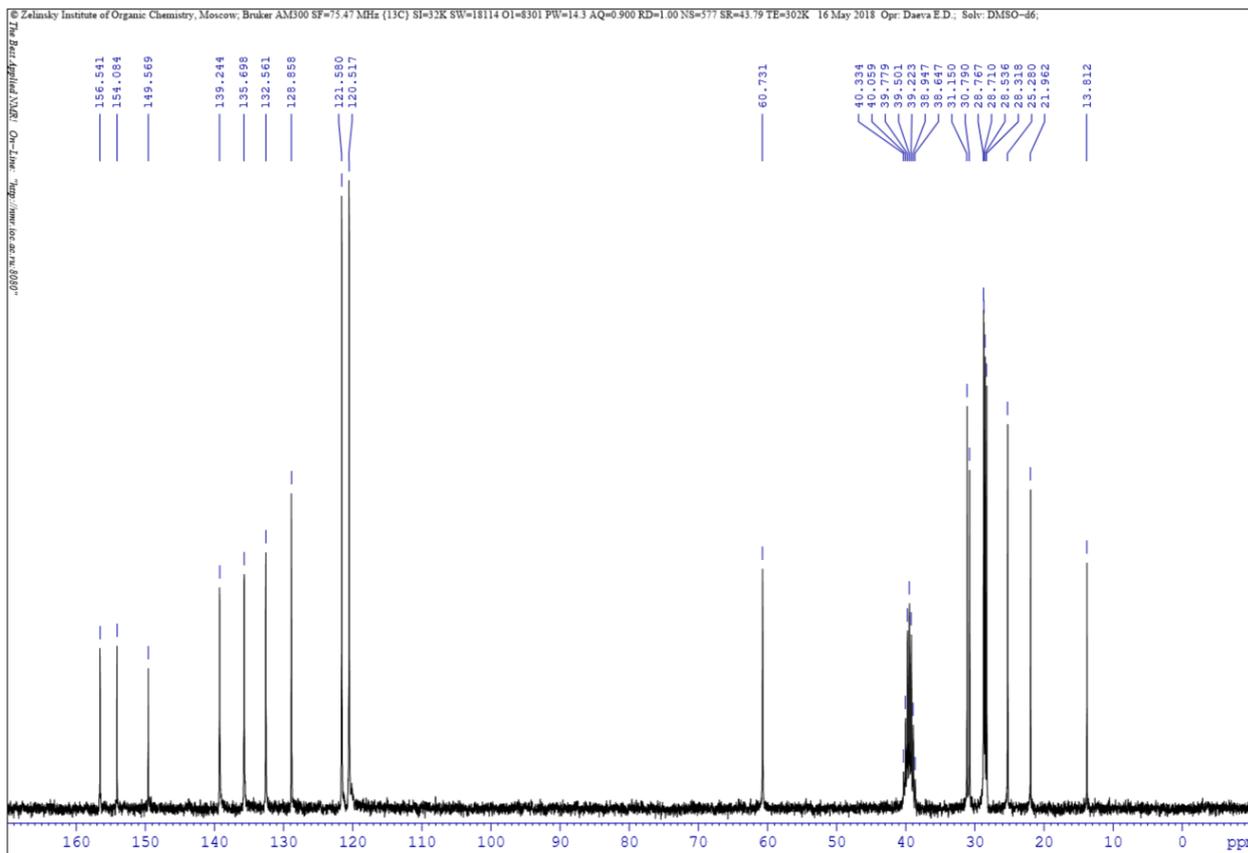


Peak	Retention min	Width h/2 min	Height mAU	Area mAU*sec	Area %	Type
1	5.05	0.127	232.88	1895.958	100.00	BB

(3'd)

3''d (3OB4POB-10, Cl)

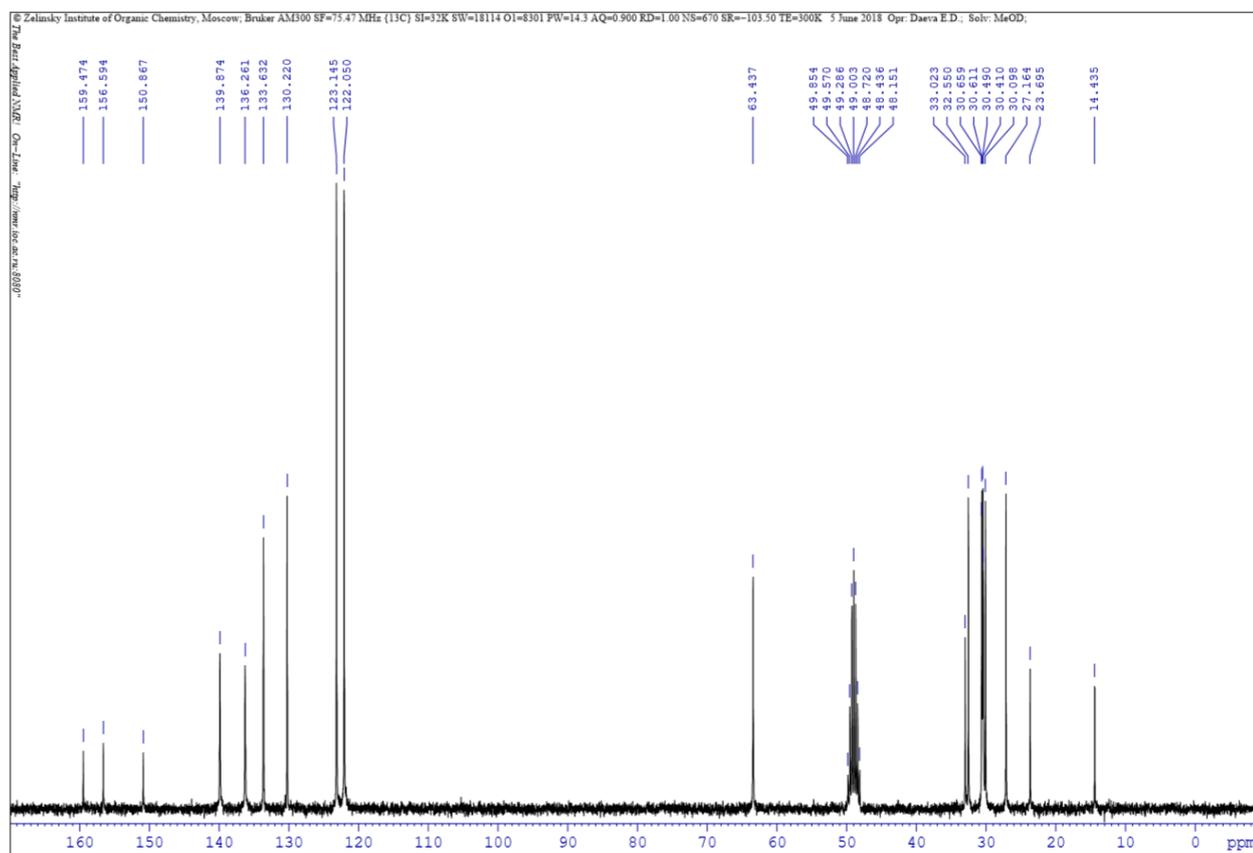
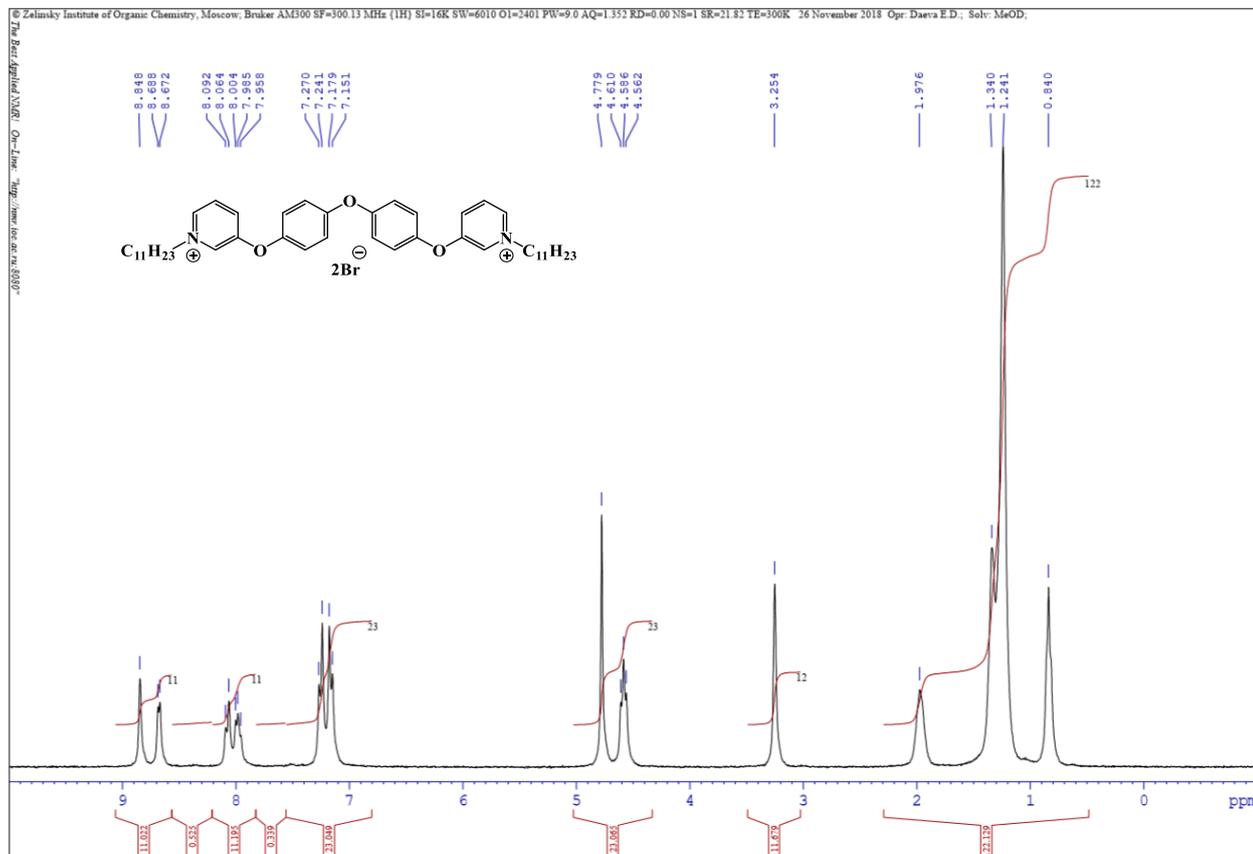


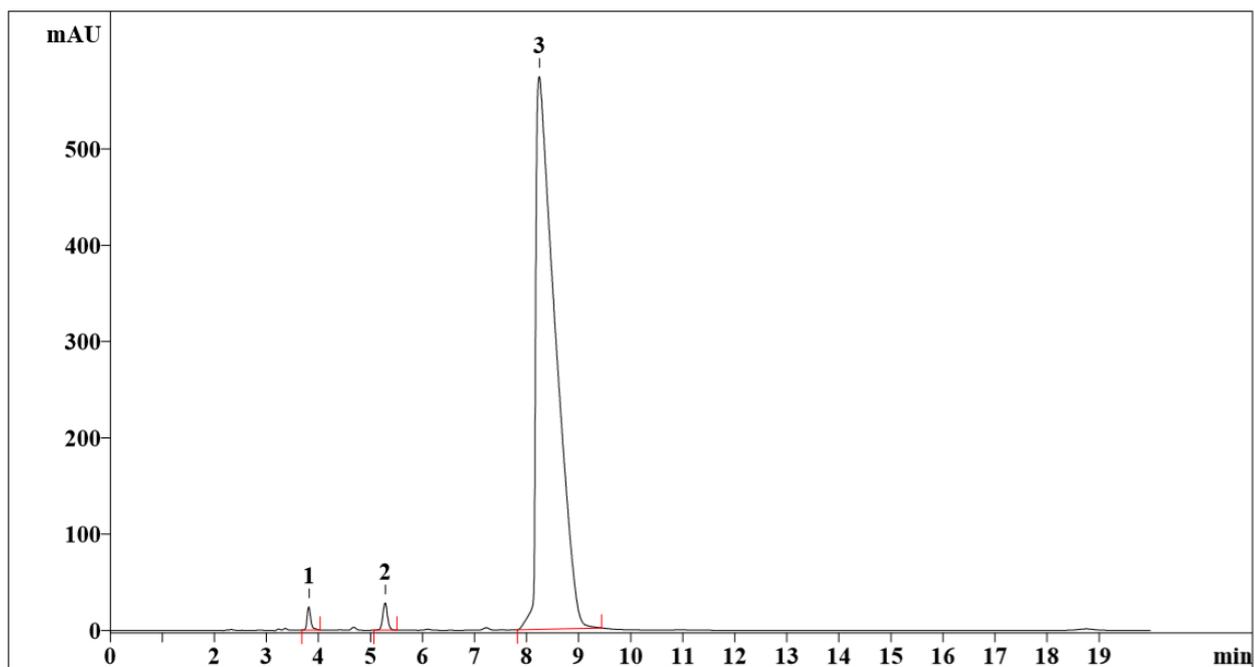


Peak	Retention min	Width h/2 min	Height mAU	Area mAU*sec	Area %	Type
1	4.55	0.090	7.85	47.859	0.63	BB
2	6.00	0.204	583.22	7572.236	99.37	BB

(3''d)

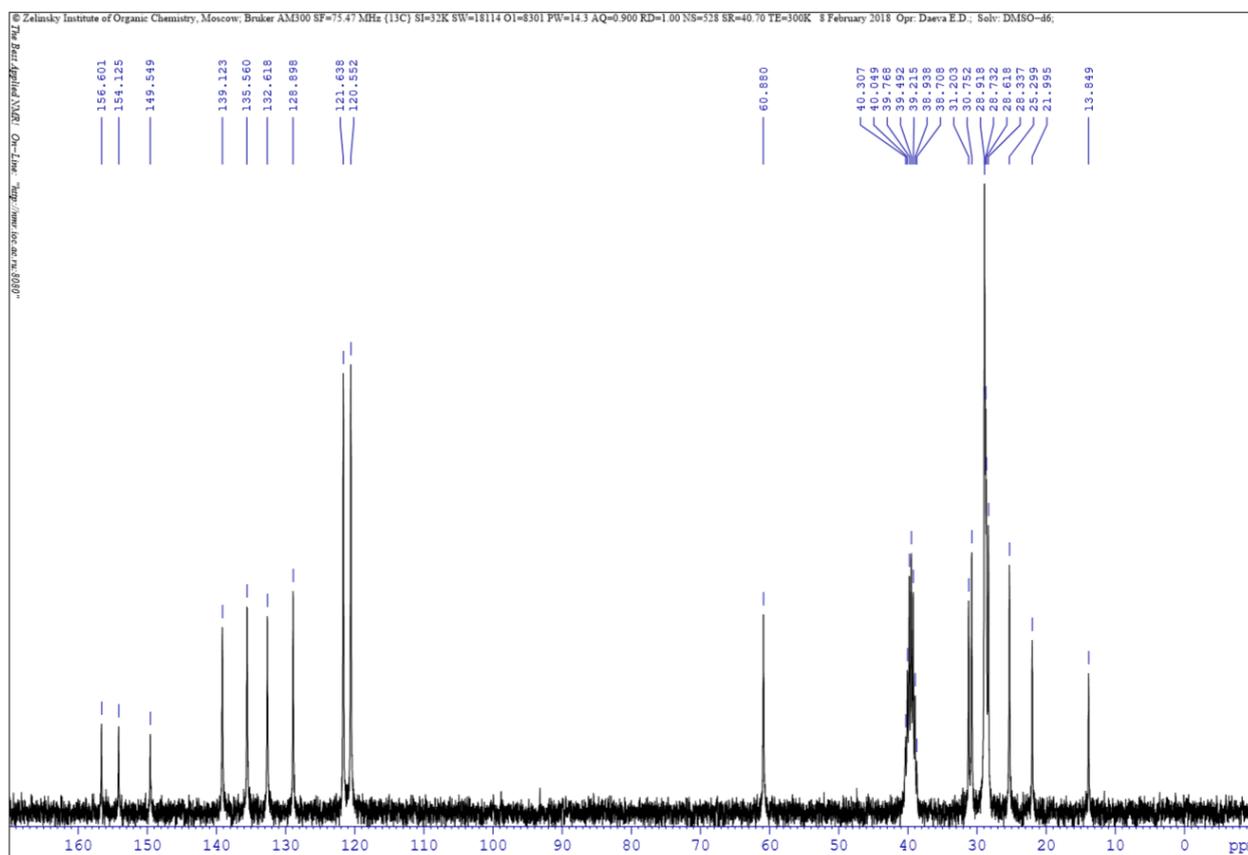
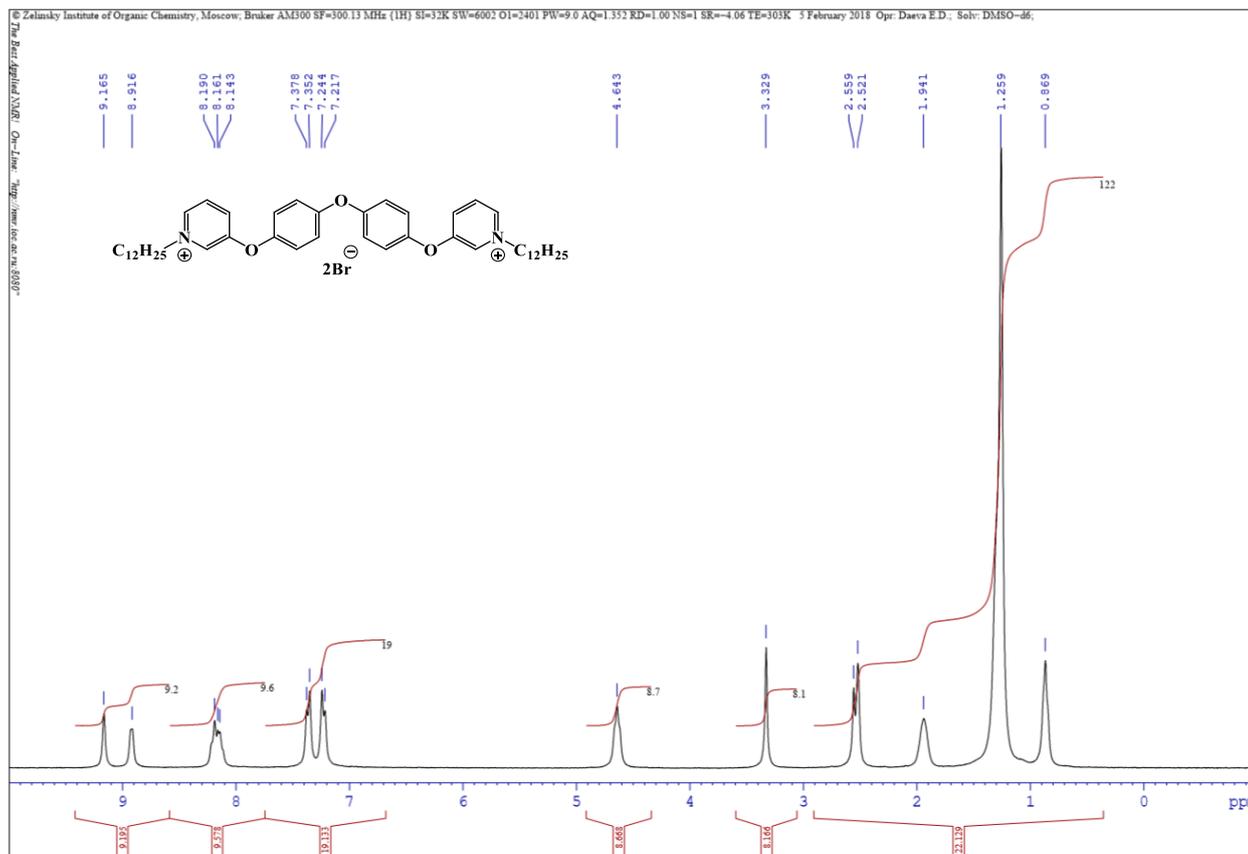
3e (3OB4POB-11, Br)

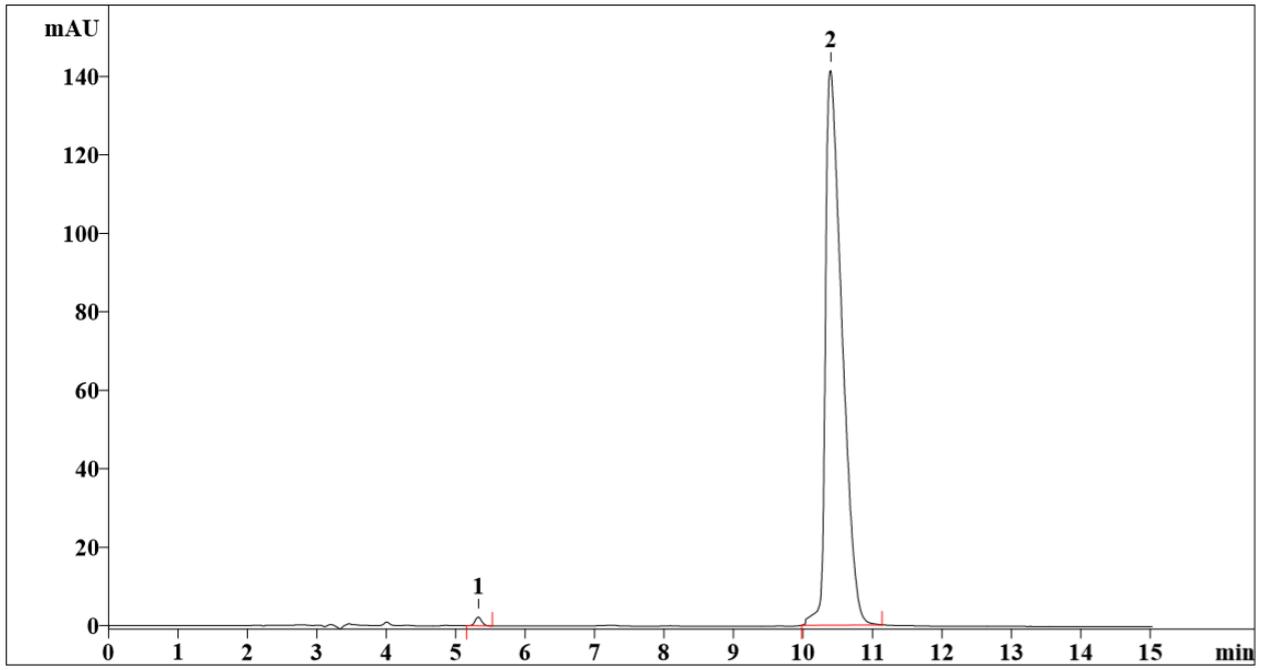




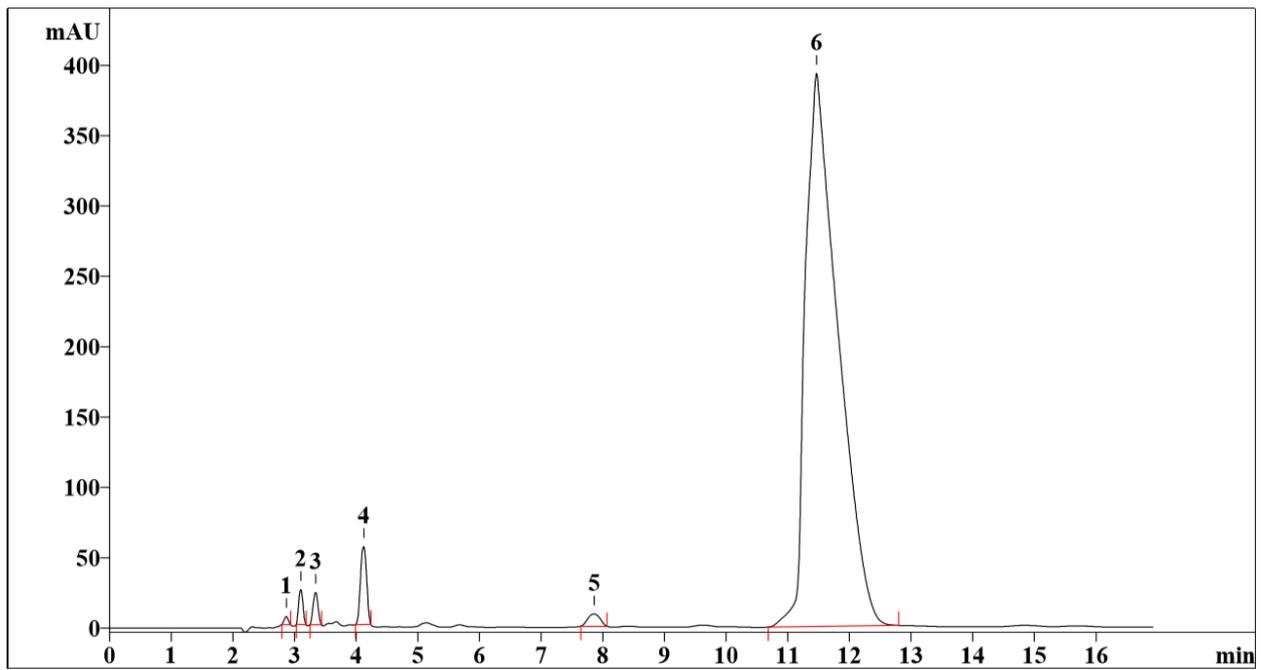
Peak	Retention min	Width h/2 min	Height mAU	Area mAU*sec	Area %	Type
1	3.82	0.071	24.02	118.895	0.78	BB
2	5.28	0.097	28.16	180.500	1.18	BB
3	8.24	0.410	573.98	14955.191	98.04	BB

3f (3OB4POB-12, Br)



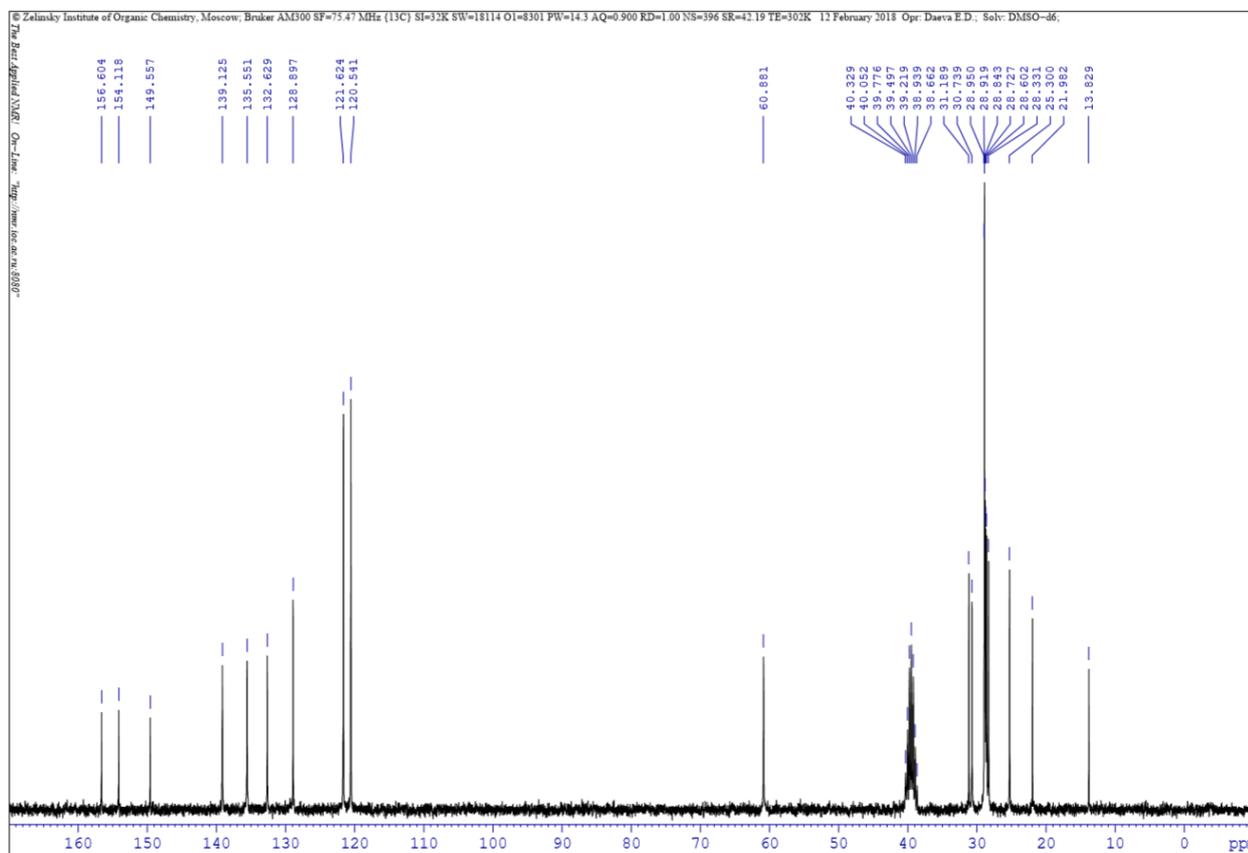


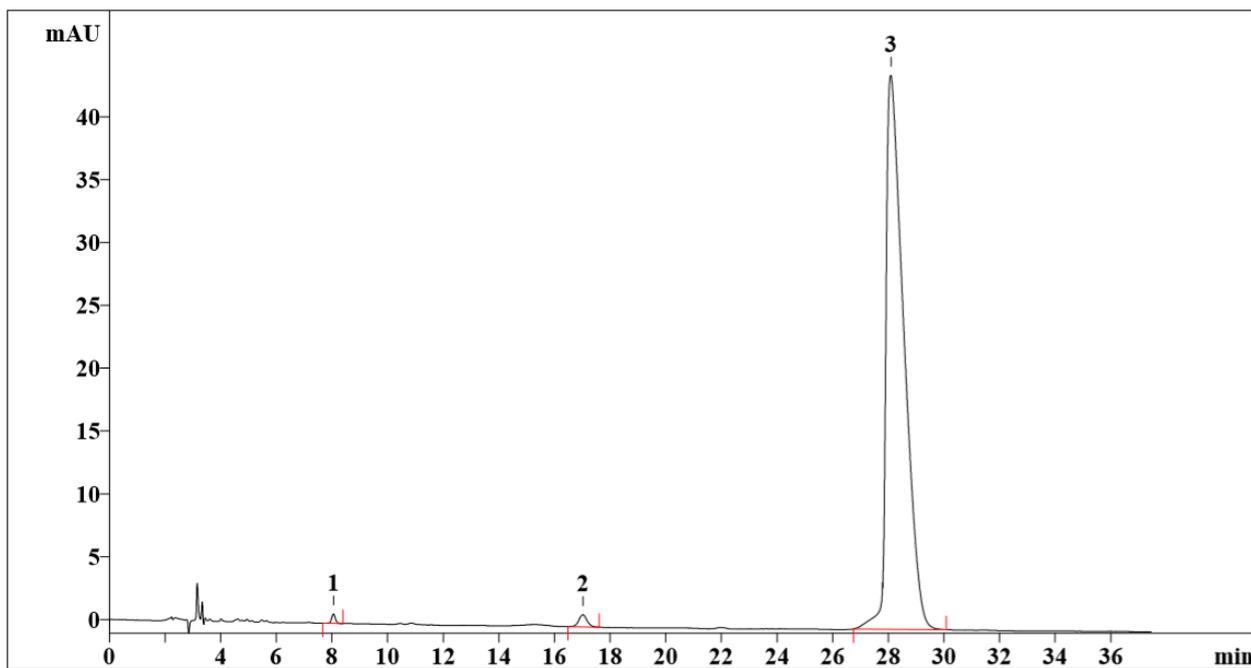
Peak	Retention min	Width h/2 min	Height mAU	Area mAU*sec	Area %	Type
1	5.33	0.102	2.23	14.993	0.63	BB
2	10.40	0.255	141.35	2347.675	99.37	BB



Peak	Retention min	Width h/2 min	Height mAU	Area mAU*sec	Area %	Type
1	2.87	0.079	5.71	26.955	0.18	BB
2	3.10	0.079	24.67	118.295	0.78	BB
3	3.34	0.091	22.88	127.650	0.84	BB
4	4.12	0.113	55.25	384.956	2.54	BB
5	7.86	0.231	8.70	120.491	0.79	BB
6	11.47	0.594	392.48	14399.367	94.87	BB

3g (3OB4POB-14, Br)

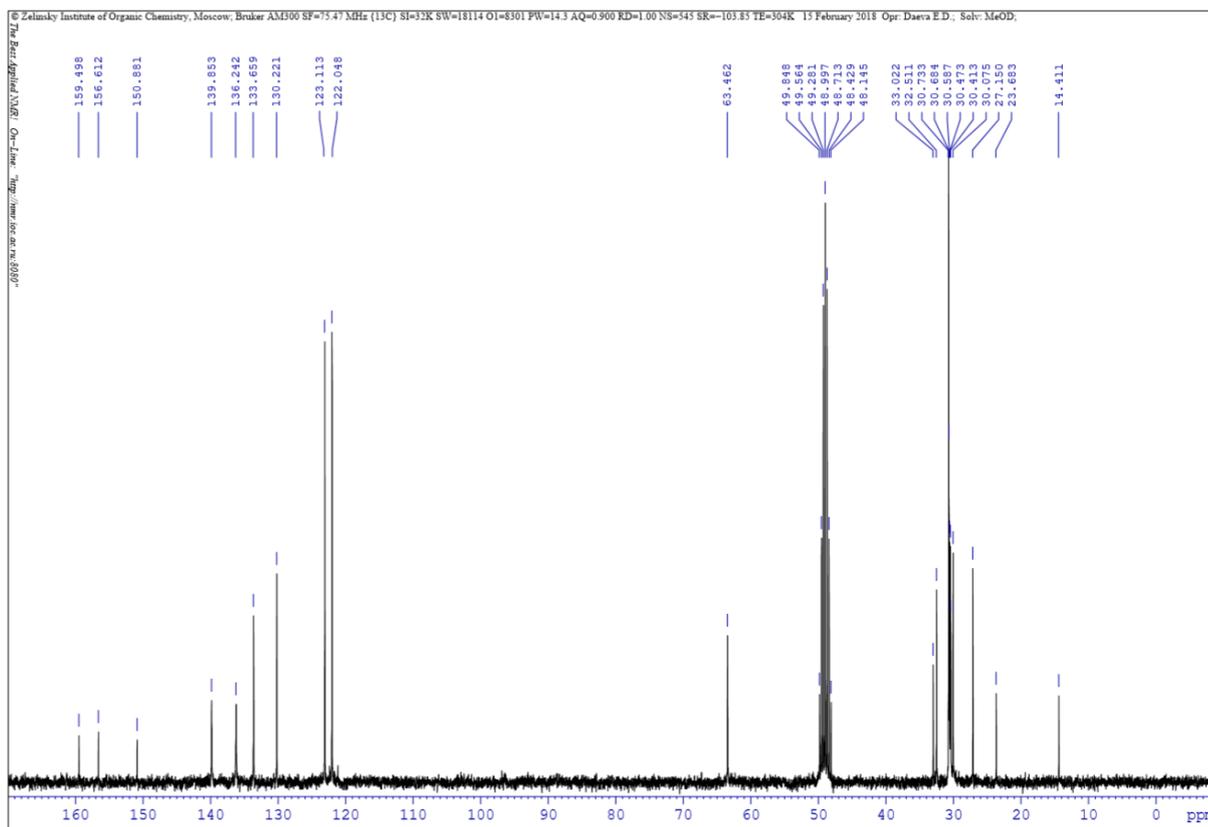




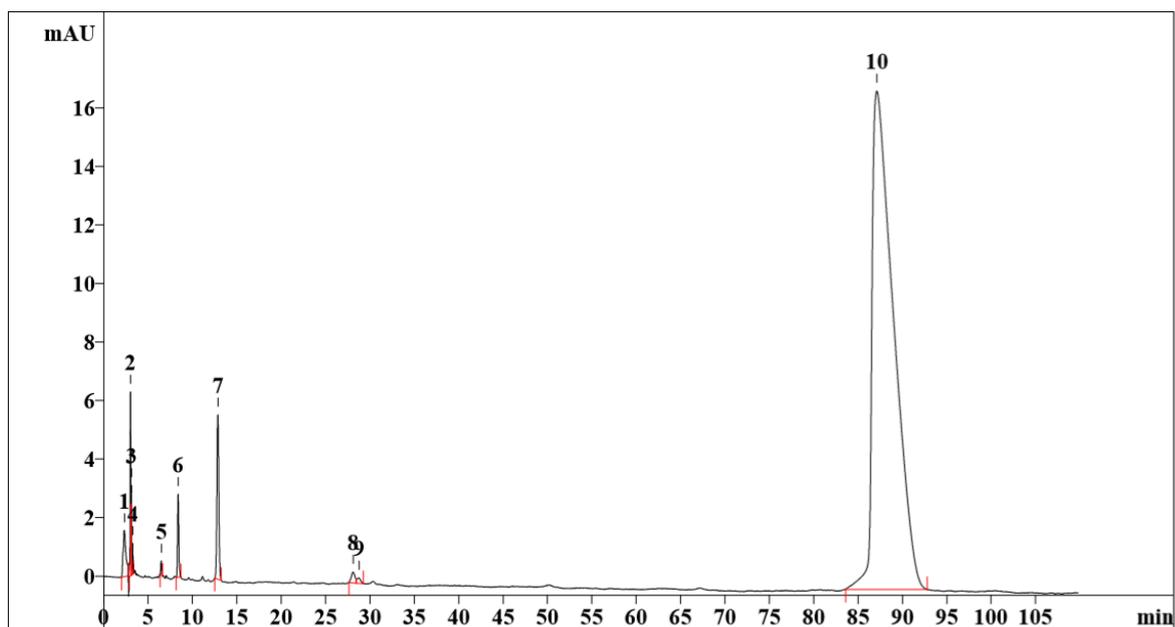
Peak	Retention min	Width h/2 min	Height mAU	Area mAU*sec	Area %	Type
1	8.06	0.147	0.73	6.988	0.35	BB
2	17.03	0.315	0.96	19.894	1.00	BB
3	28.09	0.689	44.08	1967.512	98.65	BB

3h (3OB4POB-16, Br)





(3h)



Peak	Retention min	Width h/2 min	Height mAU	Area mAU*sec	Area %	Type
1	2.32	0.336	1.57	34.477	1.13	BB
2	3.02	0.063	6.29	27.207	0.89	BD
3	3.10	0.066	3.07	13.262	0.43	DD
4	3.28	0.049	1.04	3.354	0.11	DB
5	6.49	0.119	0.48	3.551	0.12	BB
6	8.39	0.153	2.84	28.337	0.93	BB
7	12.87	0.227	5.62	82.899	2.71	BB
8	28.11	0.483	0.36	10.427	0.34	BD
9	28.74	0.525	0.18	5.489	0.18	DB
10	87.12	2.633	17.02	2853.793	93.18	BB