**Electronic supplementary materials** *Mendeleev Commun.*, 2021, **31**, 509–511

# Irregular cationic lipotetrapeptides for pharmaceutical multifunctional transport systems

**Zaret G. Denieva, Uliana A. Budanova and Yurii L. Sebyakin**

**General Materials and Procedure for Synthesis**

All reagents and organic solvents were purchased from Acros, Aldrich, or Reachim in reagent grade or better and used without further purification. Column chromatography was accomplished on silica gel 60 (60−200 mesh). Thin layer preparative chromatography (TLPC) was accomplished on silica gel 60 (2−25 mesh). NMR spectra were recorded on a Bruker 400 MHz spectrometer in DMSO-d6 solutions. MALDI mass spectrometry (MALDI-MS) was performed on a MALDI mass spectrometer Ultraflex (Bruker Daltonics GmbH).

To a solution of BocAsp (0.36 g, 1.55 mmol) in $CH_2Cl_2$ (10 ml) and DMF (350 μl) cooled to 0°C, DMAP (0.57 g, 4.65 mmol), DCC (1.28 g, 6.21 mmol) $CH_2Cl_2$ (10 ml) and compound **1a** (1.11 g, 3.88 mmol) in $CH_2Cl_2$ (20 ml) were added. The mixture was kept under vigorous stirring for 24 hours. The product was isolated by column chromatography in $CCl_4$ – MeOH (15:1) to afford 0.73g (61.3%) of compound **2a**.

To compound **2a** (0.56 g, 0.73 mmol) in $CH_2Cl_2$ (25 ml) cooled to 0 °C, a solution of TFA in $CH_2Cl_2$ (12 ml, 1:1 v / v) was added, and this was stirred at 0ºC for 2 h. After completion of the reaction, the solvent with TFA was distilled off *in vacuo*. The crude product was dissolved in $CHCl_3$ (15 ml) and sequentially washed with 10% $NaHCO_3$ (4 × 15 ml) and water to pH 7, dried over $Na_2SO_4$, the solvent was distilled off. The yield of product **3a** was 0.52 g (92%).

To a solution of $Boc_2$Lys (0.40 g, 1.16 mmol) in $CH_2Cl_2$ (15 ml) cooled to 0°C, DMAP (0.28 g, 2.32 mmol), a solution of DCC (0.48 g, 2.32 mmol) in $CH_2Cl_2$ (20 ml), and a solution of compound **4a** (0.51 g, 0.77 mmol) in $CH_2Cl_2$ (20 ml) were added. The mixture was kept under vigorous stirring at 0°C for 4 hours, then at room temperature for 24 hours. The product was isolated using column chromatography in system toluene – ethyl acetate 5:1. The yield of compound **4a** after Boc-removing was 0.46 g (60%). Compounds **4b-d** were synthesized similarly.

**Compound 2a**. $^1$H NMR ($CDCl_3$, δ): 0.88 (t, 6 H, $CH_2CH_3$); 1.26-1.32 (m, 44 H, $CH_2CH_3$); 1.38 - 1,40 (s, 6 H, $CHCH_3$); 1.46 (s, 9 H, $CCH_3$); 1.64 (m, 4 H, $C(O)OCH_2CH_2$); 2.59 (d.d., 1 H, $CH_2CONH$); 2.85 (d, 2 H, $CHCH_2$); 3.50 (t, 1 H, $CHCH_2$); 4.11 (t, 4 H, $C(O)OCH_2CH_2$); 4.52 (m, 2 H, $CHCH_3$); 6.40 (d, 1 H, NH (Asp)); 7.39 (d, 2 H, CHCONH).

**Compound 2b**. $^1$H NMR ($CDCl_3$, δ): 0.88 (t, 6 H, $CH_2CH_3$); 1.26 (m, 52 H, $CH_2CH_3$); 1.39 (s, 6 H, $CHCH_3$); 1.46 (s, 9 H, $CCH_3$); 1.63 (m, 4 H, $C(O)OCH_2CH_2$); 2.35 (d, 2 H, $CHCH_2$); 2.58 (d.d., 1 H, $CH_2CONH$); 3.48 (m, 1 H, $CHCH_2$); 4.12 (m, 4 H, $C(O)OCH_2CH_2$); 4.51 (m, 2 H, $CHCH_3$); 6.42 (d, 1 H, NH (Asp)); 7.16 (d, 2 H, CHCONH).

**Compound 2c**. $^1$H NMR ($CDCl_3$, δ): 0.87 (t, 6 H, $CH_2CH_3$); 1.25 (m, 44 H, $CH_2$); 1.44 (s, 9 H, $CCH_3$); 1.63 (q, 4 H, $C(O)OCH_2CH_2$); 1.91 (d, 1 H, $CHCH_2$); 2.65 (d.d., 1 H, $CHCH_2OH$); 3.01 (d.d., 1 H, $CHCH_2OH$); 3.91 (m, 4 H, $CH_2OH$); 4.14 (t, 4 H, $C(O)OCH_2CH_2$); 4.57 (d, 2 H, $CHCH_2$); 6.24 (d, 1 H, NH (Asp)); 7.18 (d, 1 H, CHCONH); 7.59 (d, 1 H, $CH_2CONH$).

**Compound 2d**. $^1$H NMR ($CDCl_3$, δ): 0.88 (t, 6 H, $CH_2CH_3$); 1.26 (m, 52 H, $CH_2$); 1.45 (s, 9 H, $CCH_3$); 1.64 (m, 4 H, $C(O)OCH_2CH_2$); 1.77 (d, 1 H, $CHCH_2$); 3.48 (s, 1 H, $CHCH_2OH$); 3.63 (s, 1 H, $CHCH_2OH$); 3.93 (m, 4 H, $CH_2OH$); 4.16 (t, 4 H, $C(O)OCH_2CH_2$); 4.55 (d, 2 H, $CHCH_2$); 6.22 (d, 1 H, NH (Asp)); 7.18 (d, 1 H, CHCONH); 7.49 (d, 1 H, $CH_2CONH$).

**Compound 4a** with Boc. $^1$H NMR ($CDCl_3$, δ): 0.88 (t, 6 H, $CH_2C\underline{H}_3$); 1.26 (m, 44 H, $C\underline{H}_2CH_3$); 1.38 (s, 6 H, $CHC\underline{H}_3$); 1.45 (s, 18 H, $CC\underline{H}_3$); 1.55 (m, 4 H, $CHCH_2C\underline{H}_2C\underline{H}_2CH_2$); 1.71 (m, 4 H, $C(O)OCH_2C\underline{H}_2$); 1.90 (d, 2 H, $CHC\underline{H}_2CH_2CH_2CH_2$); 2.03 (s, 1 H, $CH_2CON\underline{H}$); 3.10 (d, 2 H, $CHCH_2CH_2CH_2C\underline{H}_2$); 3.48 (s, 1 H, $C\underline{H}CH_2CH_2CH_2CH_2$); 4.10 (s, 1 H, CHCON<u>H</u> (Ala)); 4.20 (m, 4 H, $C(O)OC\underline{H}_2CH_2$); 4.50 (m, 2 H, $C\underline{H}CH_3$); 4.75 (d, 2 H, $CHC\underline{H}_2$); 5.10 (d, 1 H, CHCON<u>H</u> (Asp)); 6.61 (t, 1 H, $C\underline{H}CH_2$); 7.45 (s, 1 H, ε-NH); 7.80 (s, 1 H, α-NH).

**Compound 4a** without Boc. MS (MALDI), m/z: 834.609 $[M+K]^+$

**Compound 4b** with Boc. $^1$H NMR ($CDCl_3$, δ): 0.87 (t, 6 H, $CH_2C\underline{H}_3$); 1.27 (m, 52 H, $C\underline{H}_2CH_3$); 1.34 (d, 6 H, $CHC\underline{H}_3$); 1.44 (s, 18 H, $CC\underline{H}_3$); 1.60 (m, 4 H, $CHCH_2C\underline{H}_2C\underline{H}_2CH_2$); 1.69 (dt, 4 H, $C(O)OCH_2C\underline{H}_2$); 1.92 (d, 2 H, $CHC\underline{H}_2CH_2CH_2CH_2$); 2.03 (s, 1 H, $CH_2CON\underline{H}$); 3.10 (d, 2 H, $CHCH_2CH_2CH_2C\underline{H}_2$); 3.47 (s, 1 H, $C\underline{H}CH_2CH_2CH_2CH_2$); 4.11 (s, 1 H, CHCON<u>H</u> (Ala)); 4.19 (d, 4 H, $C(O)OC\underline{H}_2CH_2$); 4.54 (d, 2 H, $C\underline{H}CH_3$); 4.74 (d, 2 H, $CHC\underline{H}_2$); 5.07 (s, 1 H, CHCON<u>H</u> (Asp)); 6.62 (m, 1 H, $C\underline{H}CH_2$); 7.50 (d, 1 H, ε-NH); 7.81 (s, 1 H, α-NH).

**Compound 4b** without Boc. MS (MALDI), m/z: 888.043 $[M+K]^+$

**Compound 4c** with Boc. $^1$H NMR ($CDCl_3$, δ): 0.87 (t, 6 H, $CH_2C\underline{H}_3$); 1.25 (m, 44 H, $C\underline{H}_2CH_3$); 1.41-1.44 (m, 4 H, $CHCH_2C\underline{H}_2C\underline{H}_2CH_2$); 1.43 (s, 18 H, $CC\underline{H}_3$); 1.68 (m, 4 H, $C(O)OCH_2C\underline{H}_2$); 2.54 (d, 2 H, $CHC\underline{H}_2$); 2.95 (d, 2 H, $CHC\underline{H}_2CH_2CH_2CH_2$); 3.10 (d, 2 H, $CHCH_2CH_2CH_2C\underline{H}_2$); 3.91-3.95 (m, 4 H, $C\underline{H}_2OH$); 4.14 (s, 1 H, $C\underline{H}CH_2CH_2CH_2CH_2$); 4.17 (m, 4 H, $C(O)OC\underline{H}_2CH_2$); 4.27 (m, 2 H, $C\underline{H}CH_2OH$); 4.53 (s, 1 H, $C\underline{H}CH_2$); 5.09 (t, 1 H, ε-N<u>H</u>); 5.54 (d, 1 H, α-N<u>H</u>); 7.13 (d, 1 H, COCHN<u>H</u> (Ser)); 7.56 (d, 1 H, COCHN<u>H</u> (Asp)); 8.36 (d, 1 H, $CH_2CON\underline{H}$).

**Compound 4c** without Boc. MS (MALDI), m/z: 866.601 $[M+K]^+$.

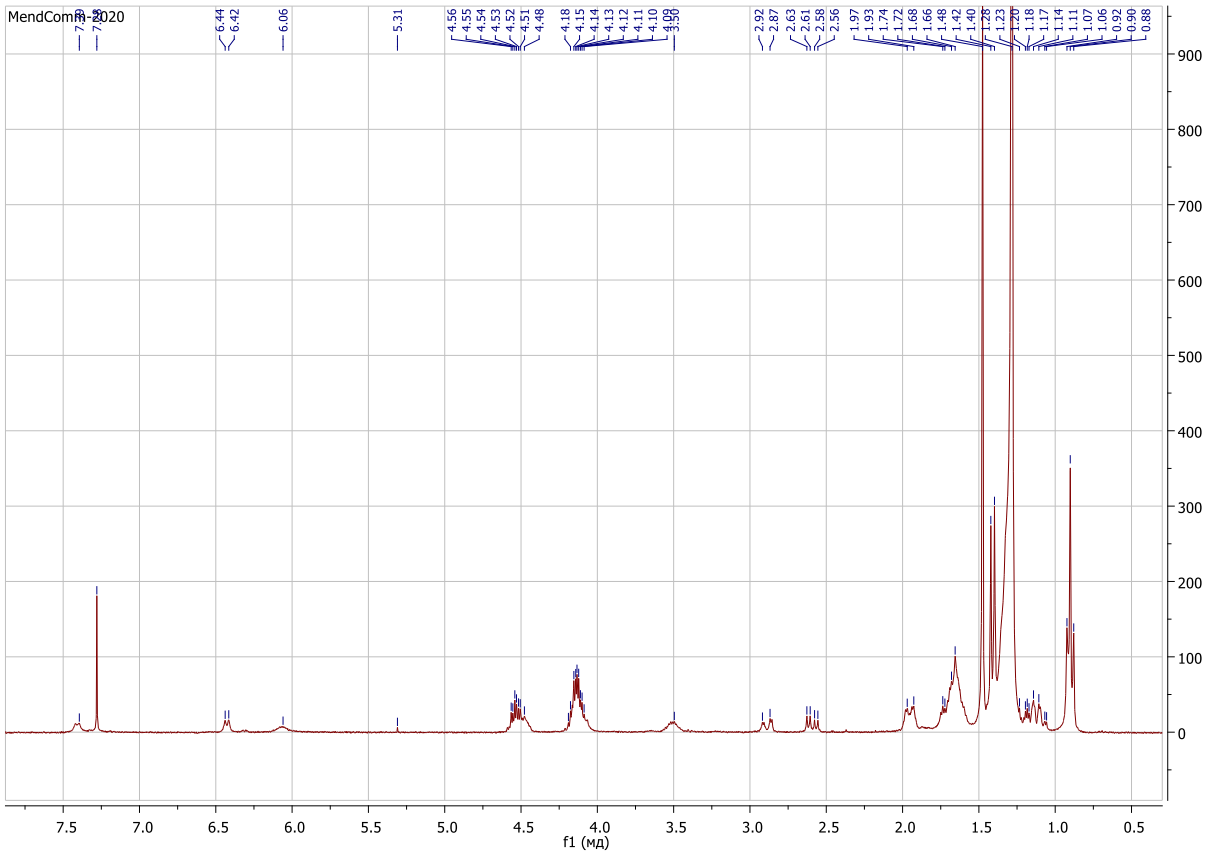
**Compound 4d** with Boc. $^1$H NMR ($CDCl_3$, δ): 0.86 (t, 6 H, $CH_2C\underline{H}_3$); 1.25 (m, 52 H, $C\underline{H}_2CH_3$); 1.42 (m, 4 H, $CHCH_2C\underline{H}_2C\underline{H}_2CH_2$); 1.43 (s, 18 H, $CC\underline{H}_3$); 1.91 (m, 4 H, $C(O)OCH_2C\underline{H}_2$); 2.62 (d, 2 H, $CHC\underline{H}_2$); 3.11 (d, 4 H, $CHC\underline{H}_2CH_2CH_2C\underline{H}_2$); 3.48 (m, 4 H, $C\underline{H}_2OH$); 3.64 (t, 1 H, $C\underline{H}CH_2CH_2CH_2CH_2$); 4.15 (m, 4 H, $C(O)OC\underline{H}_2CH_2$); 4.50 (m, 2 H, $C\underline{H}CH_2OH$); 4.78 (s, 1 H, $C\underline{H}CH_2$); 5.04 (s, 1 H, ε-N<u>H</u>); 5.55 (d, 1 H, α-N<u>H</u>); 6.52 (d, 1 H, COCHN<u>H</u> (Ser)); 7.67 (d, 1 H, COCHN<u>H</u> (Asp)); 7.94 (d, 1 H, $CH_2CON\underline{H}$).

**Compound 4d** without Boc. MS (MALDI), m/z: 887.933$[M+H]^+$, 925.891 $[M+K]^+$.

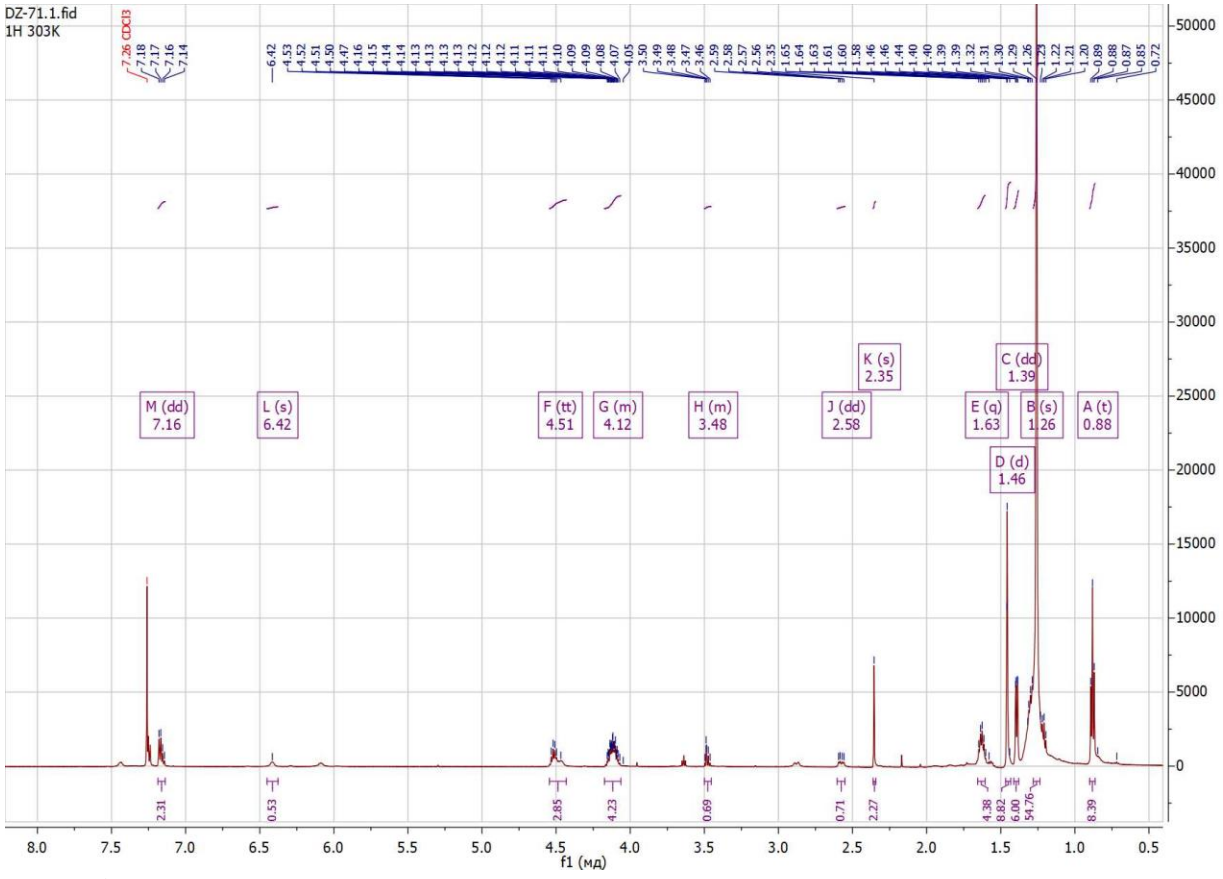
*The critical packing parameter (CPP) describes the geometric dimensions of lipid molecules*. $CPP = \frac{v}{(l * S_0)}$, *where* *v/l* – cross-sectional area of the hydrocarbon region of the molecule (*v*– molecular volume of the hydrocarbon region, *l* – maximum hydrocarbon tail length), $S_0$ – surface area to accommodate the lipid polar head

*Determination of phase transition temperature*

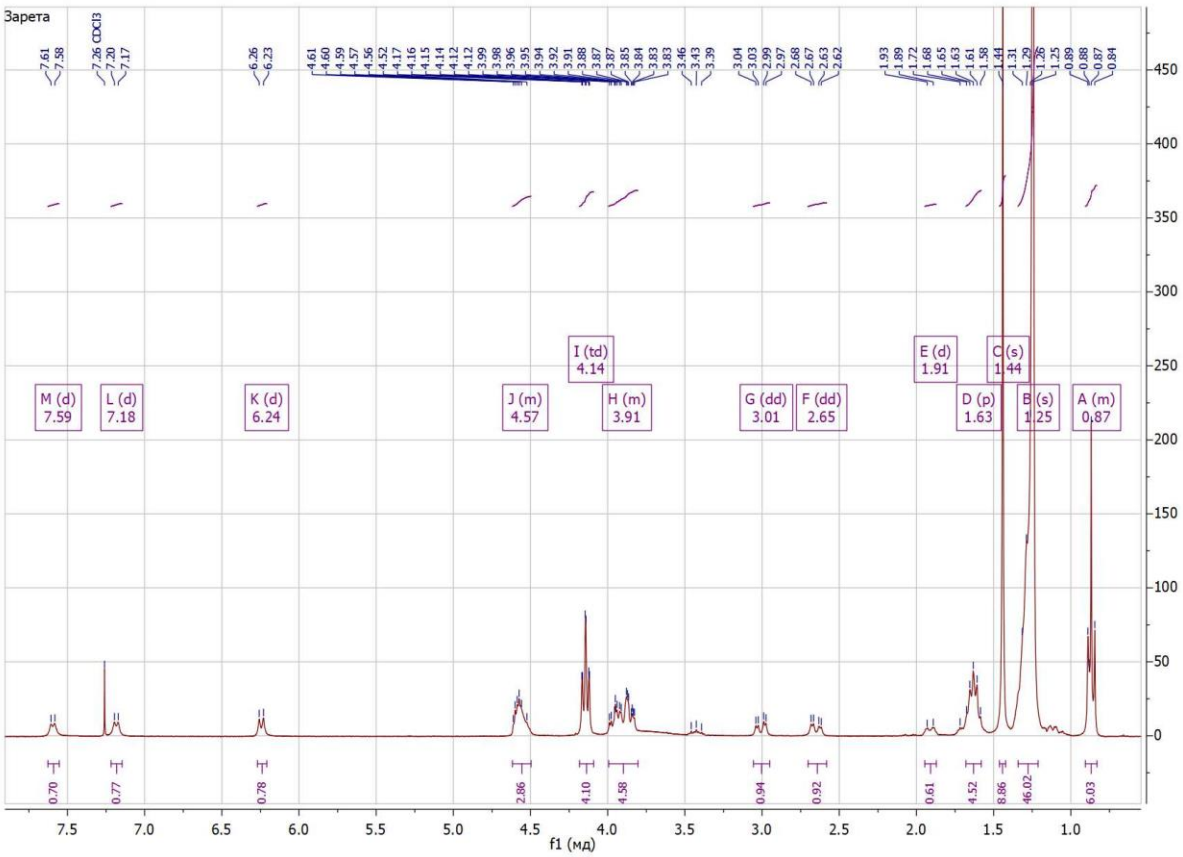
All mixtures with eosin were prepared by adding the necessary amount of dye to the liposome dispersion - [eosin] / [liposome] = 1:20. Samples were placed for 10 min in a certain temperature water bath. The optical density of aggregates was measured in the visible region of the absorption spectrum of eosin (λdimer = 400 nm, λmonomer = 525 nm). The phase transition temperature was determined from the D / T°C plot.



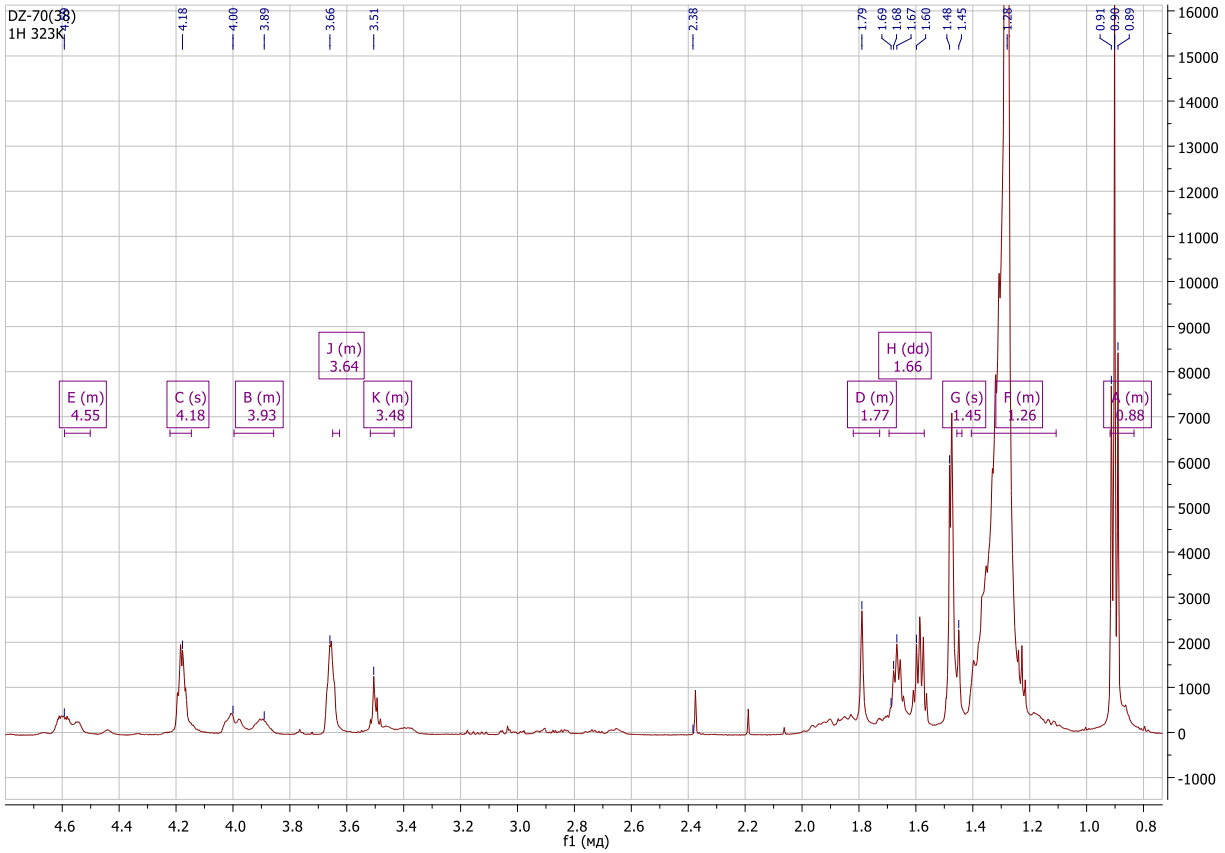

$^{1}$H NMR **2a**



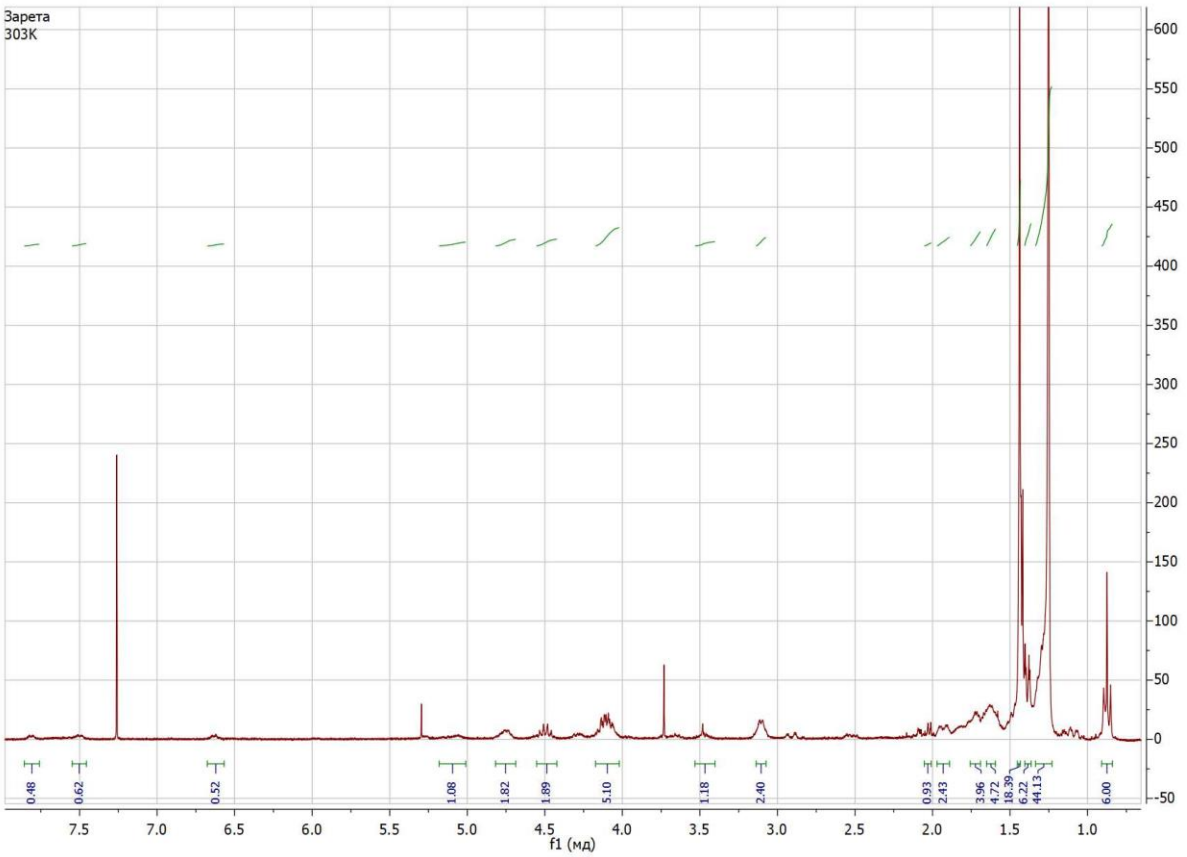

$^{1}$H NMR **2b**



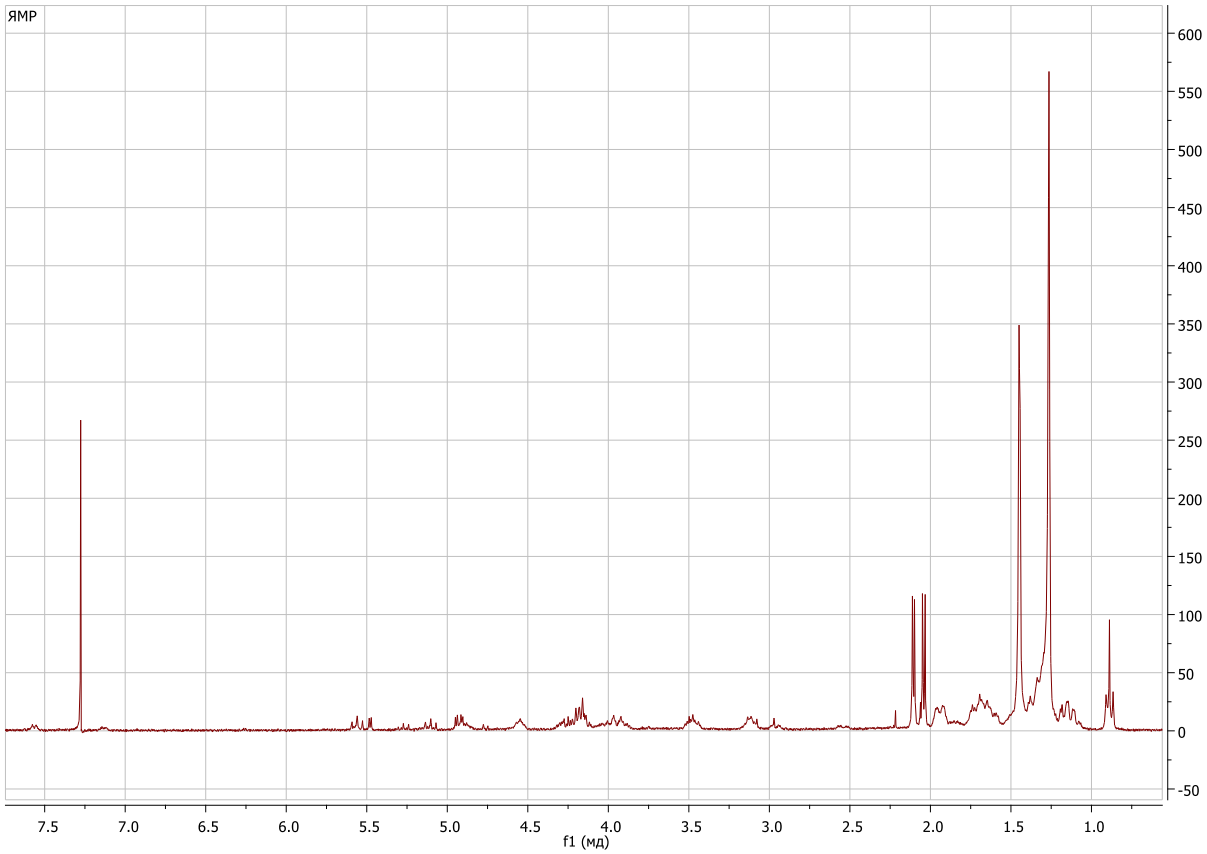

$^{1}$H NMR **2c**



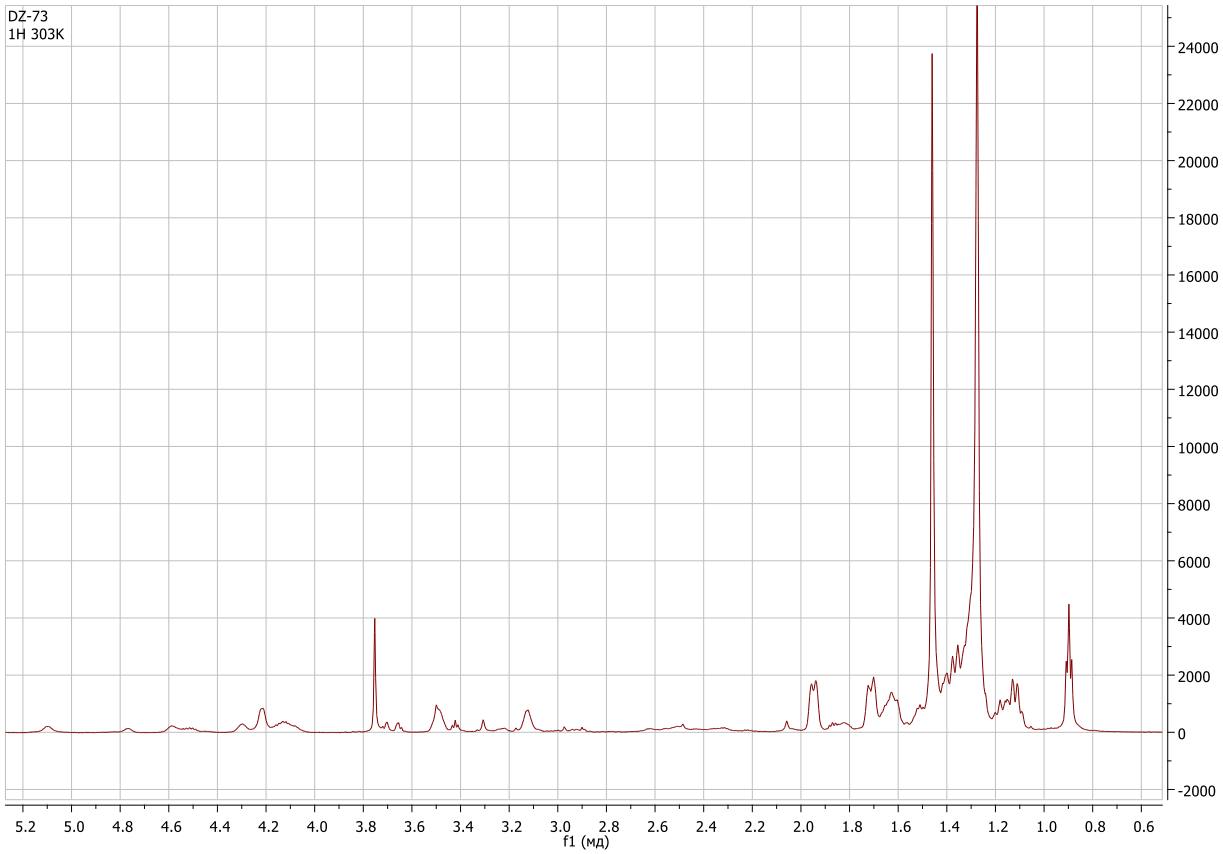

$^{1}$H NMR **2d**



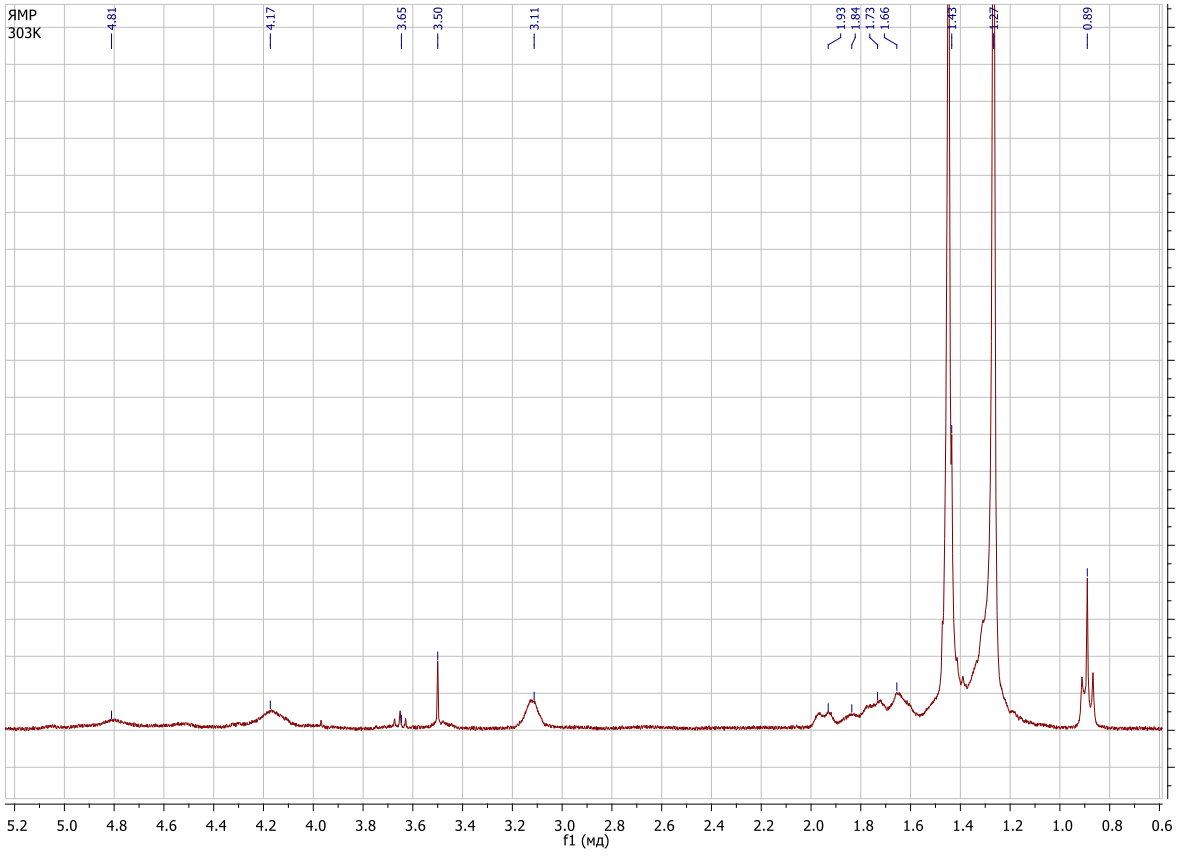

$^{1}$H NMR **4a** with Boc



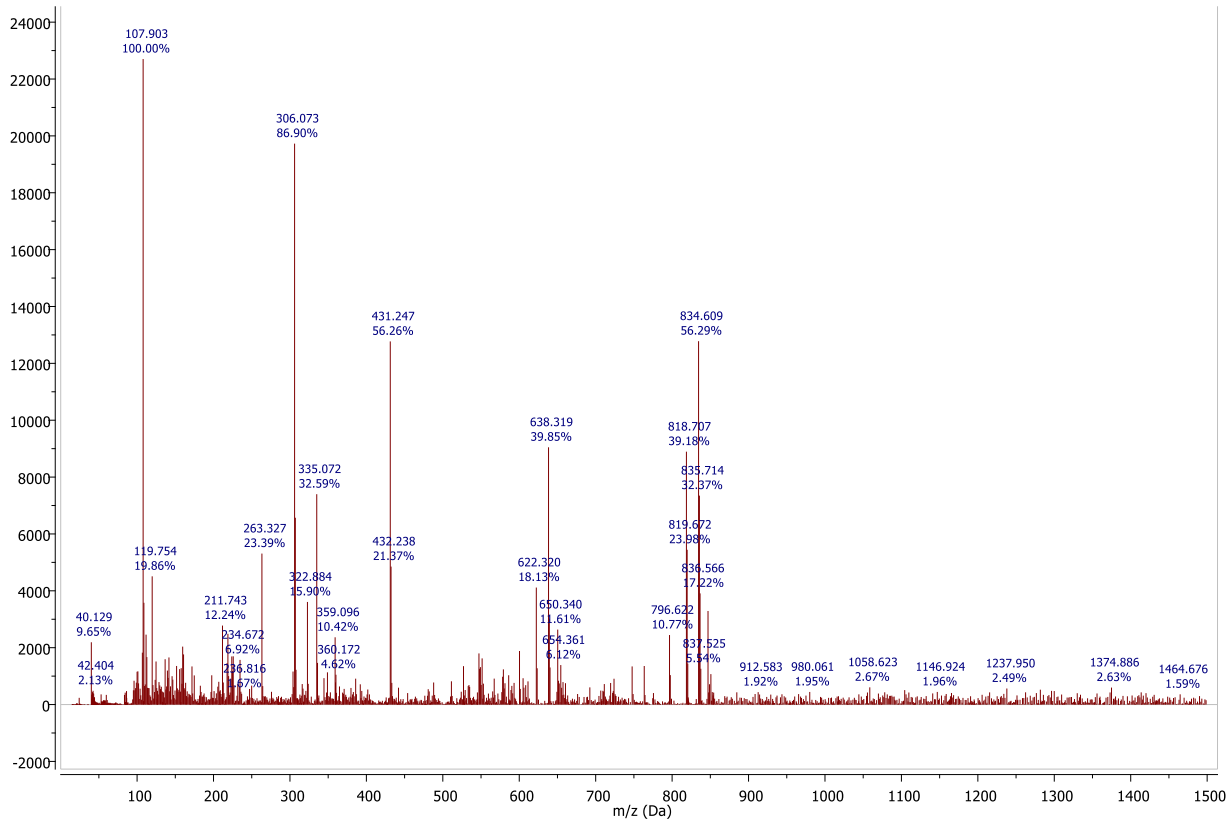

$^{1}$H NMR **4b** with Boc



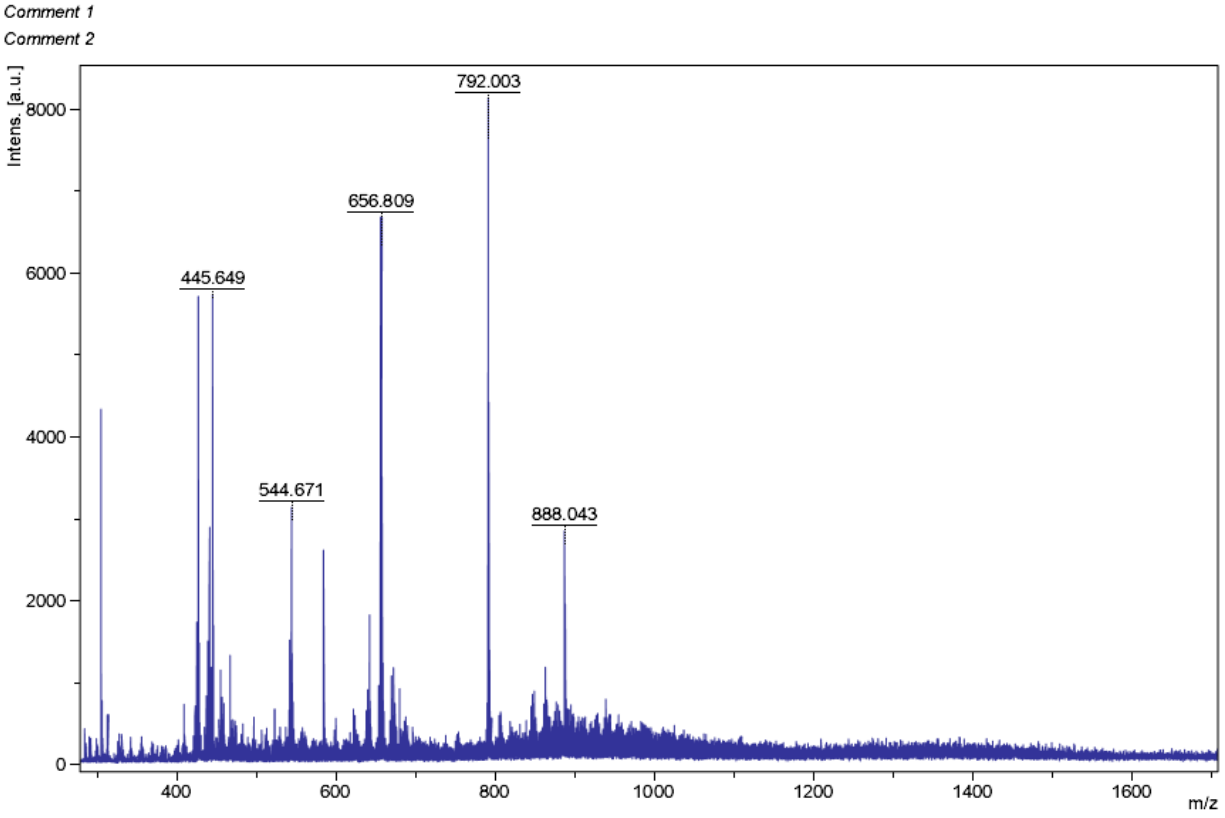

$^{1}$H NMR **4c** with Boc



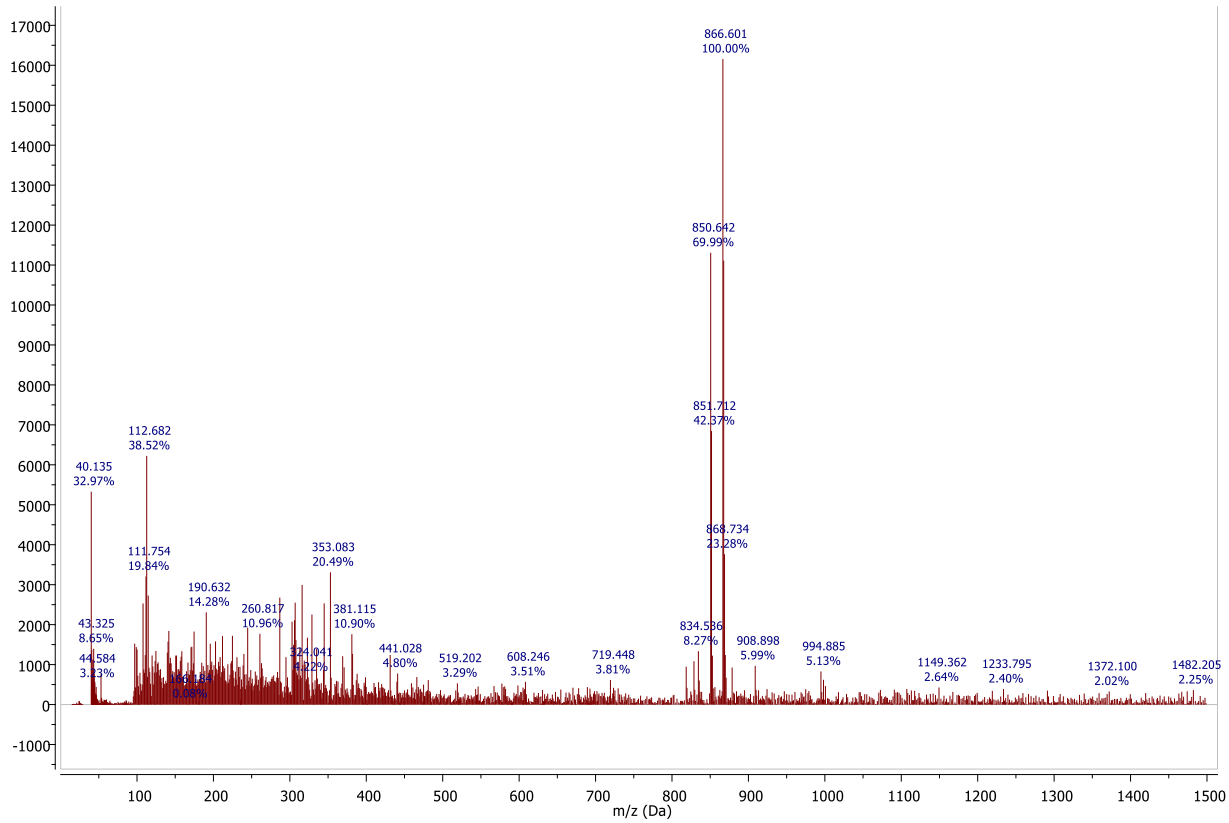

$^{1}$H NMR **4d** with Boc



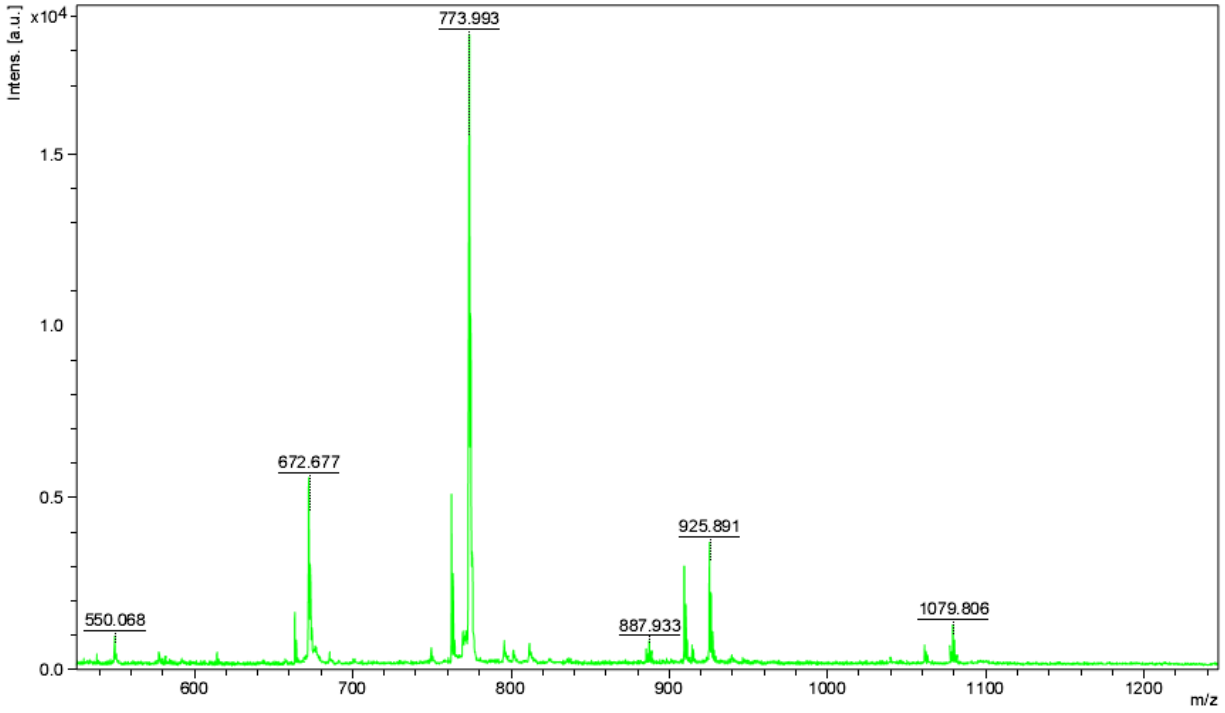

MS (MALDI) of **4a**




MS (MALDI) of **4b**




MS (MALDI) of **4c**




MS (MALDI) of **4d**