

The Supplementary Data

Novel potent uracil-based inhibitors of acetylcholinesterase for the treatment of memory impairment in animal model of Alzheimer's disease

Vyacheslav E. Semenov ^{1,*}, Irina V. Zueva ¹, Sofya V. Lushchekina ², Eduard G. Suleimanov ¹, Lilya M. Gubaidullina ¹, Marina M. Schulaeva ¹, Oksana A. Lenina ¹, Konstantin A. Petrov ^{1,3,*}

¹ Arbuzov Institute of Organic and Physical Chemistry, FRC Kazan Scientific Center of RAS, Arbuzov str. 8, Kazan 420088, Russia

² Emanuel Institute of Biochemical Physics, Kosygina st. 4, Moscow, 119334, Russia

³ Kazan Federal University, Kremlyovskaya str., 18, Kazan, 420008, Russia

* Correspondence: sve@iopc.ru (V.E.S.); kpetrov2005@mail.ru (K.A.P.)

Table of contents

	Pages
NMR study of the compounds synthesized	S2
NMR experiments details	S2
MS experiments details	S2
Figure S1. 1D ¹ H NMR spectrum of 2a in CDCl ₃ at T = 303	S4
Figure S2. 1D ¹³ C NMR spectrum of 2a in CDCl ₃ at T = 303.....	S4
Figure S3. MALDI-TOF mass spectrum of 2a	S5
Figure S4. MALDI-TOF mass spectrum of 2b	S5
Figure S5. 1D ¹ H NMR spectrum of 2b in CDCl ₃ at T = 303	S6
Figure S6. 1D ¹³ C NMR spectrum of 2b in CDCl ₃ at T = 303.....	S6
Figure S7. 1D ¹ H NMR spectrum of 2c in CDCl ₃ at T = 303.....	S7
Figure S8. 1D ¹³ C NMR spectrum of 2c in CDCl ₃ at T = 303.....	S7
Figure S9. MALDI-TOF mass spectrum of 2c	S8
Figure S10. MALDI-TOF mass spectrum of 2d	S8
Figure S11. 1D ¹ H NMR spectrum of 2d in CDCl ₃ at T = 303.....	S9
Figure S12. 1D ¹³ C NMR spectrum of 2d in CDCl ₃ at T = 303.....	S9
Figure S13. 1D ¹ H NMR spectrum of 3a in CDCl ₃ at T = 303.....	S10
Figure S14. 1D ¹³ C NMR spectrum of 3a in CDCl ₃ at T = 303.....	S10
Figure S15. MALDI-TOF mass spectrum of 3a	S11
Figure S16. MALDI-TOF mass spectrum of 3b	S11
Figure S17. 1D ¹ H NMR spectrum of 3b in CDCl ₃ at T = 303.....	S12
Figure S18. 1D ¹³ C NMR spectrum of 3b in CDCl ₃ at T = 303.....	S12
Figure S19. 1D ¹ H NMR spectrum of 3c in CDCl ₃ at T = 303.....	S13

Figure S20. 1D ^{13}C NMR spectrum of 3c in CDCl_3 at $T = 303$	S13
Figure S21. MALDI-TOF mass spectrum of 3c	S14
Figure S22. MALDI-TOF mass spectrum of 4a	S14
Figure S23. 1D ^1H NMR spectrum of 4a in CDCl_3 at $T = 303$	S15
Figure S24. 1D ^{13}C NMR spectrum of 4a in CDCl_3 at $T = 303$	S15
Figure S25. 1D ^1H NMR spectrum of 4b in CDCl_3 at $T = 303$	S16
Figure S26. 1D ^{13}C NMR spectrum of 4b in CDCl_3 at $T = 303$	S16
Figure S27. MALDI-TOF mass spectrum of 4b	S17
Figure S28. MALDI-TOF mass spectrum of 2e	S17
Figure S29. 1D ^1H NMR spectrum of 2e in DMSO-d_6 at $T = 303$	S18
Figure S30. 1D ^1H NMR spectrum of 2e in D_2O at $T = 303$	S18
Figure S31. 1D ^{13}C NMR spectrum of 2e in D_2O at $T = 303$	S19
Figure S32. 1D ^1H NMR spectrum of 3d in D_2O at $T = 303$	S19
Figure S32. 1D ^1H NMR spectrum of 3d in DMSO-d_6 at $T = 303$	S20
Figure S34. 1D ^{13}C NMR spectrum of 3d in D_2O at $T = 303$	S20
Figure S35. MALDI-TOF mass spectrum of 3d	S21
Figure S36. MALDI-TOF mass spectrum of 2f	S21
Figure S37. 1D ^1H NMR spectrum of 2f in DMSO-d_6 at $T = 303$	S22
Figure S38. 1D ^{13}C NMR spectrum of 2f in DMSO-d_6 at $T = 303$	S22
Figure S39. 1D ^1H NMR spectrum of 2f in D_2O at $T = 303$	S23
Figure S40. 1D ^{13}C NMR spectrum of 2f in D_2O at $T = 303$	S23
NMR study of the stability of compound 2c	S24

NMR and MS study of the compounds synthesized

NMR experiments details

All NMR experiments were performed with 400.1 MHz and 600.1 MHz for ^1H NMR, and 100.6 and 150.6 MHz for ^{13}C NMR spectrometer equipped with 5 mm diameter gradient inverse broad band probehead and a pulsed gradient unit capable of producing magnetic field pulse gradients in the z -direction of $53.5 \text{ G}\cdot\text{cm}^{-1}$. NMR experiments were carried out at 303 K. Chemical shifts (δ in ppm) were referenced to the solvents DMSO-d_6 ($\delta = 2.50 \text{ ppm}$ for ^1H and 40.0 ppm for ^{13}C NMR), CDCl_3 ($\delta = 7.26 \text{ ppm}$ for ^1H and 77.0 ppm for ^{13}C NMR) and D_2O ($\delta = 4.70 \text{ ppm}$ for ^1H).

MS experiments details

MALDI-TOF mass spectra were recorded in a positive and negative ion mode on a Bruker ULTRAFLEX III mass spectrometer (Bruker Daltonic GmbH, Bremen, Germany) using *p*-nitroaniline as a matrix for 10^{-3} mg/ml solutions in MeOH. A Nd:YAG laser ($\lambda = 355 \text{ nm}$, repetition rate 100 Hz) was used. The mass spectrum was obtained with an

accelerating voltage of 25 kV and an ion extraction delay time of 30 ns. The resulting mass spectrum was formed due to multiple laser irradiation of the crystal (50 shots). The metal target MTP AnchorChipTM was used. Portions (0.5 µl) of a 1% matrix solution in acetonitrile and sample solution were consecutively applied onto the target and evaporated. The polyethylene glycol was used to calibrate the mass scale of the device. The data was obtained using the FlexControl program and processed using the FlexAnalysis 3.0 program (Bruker Daltonik GmbH, Germany).

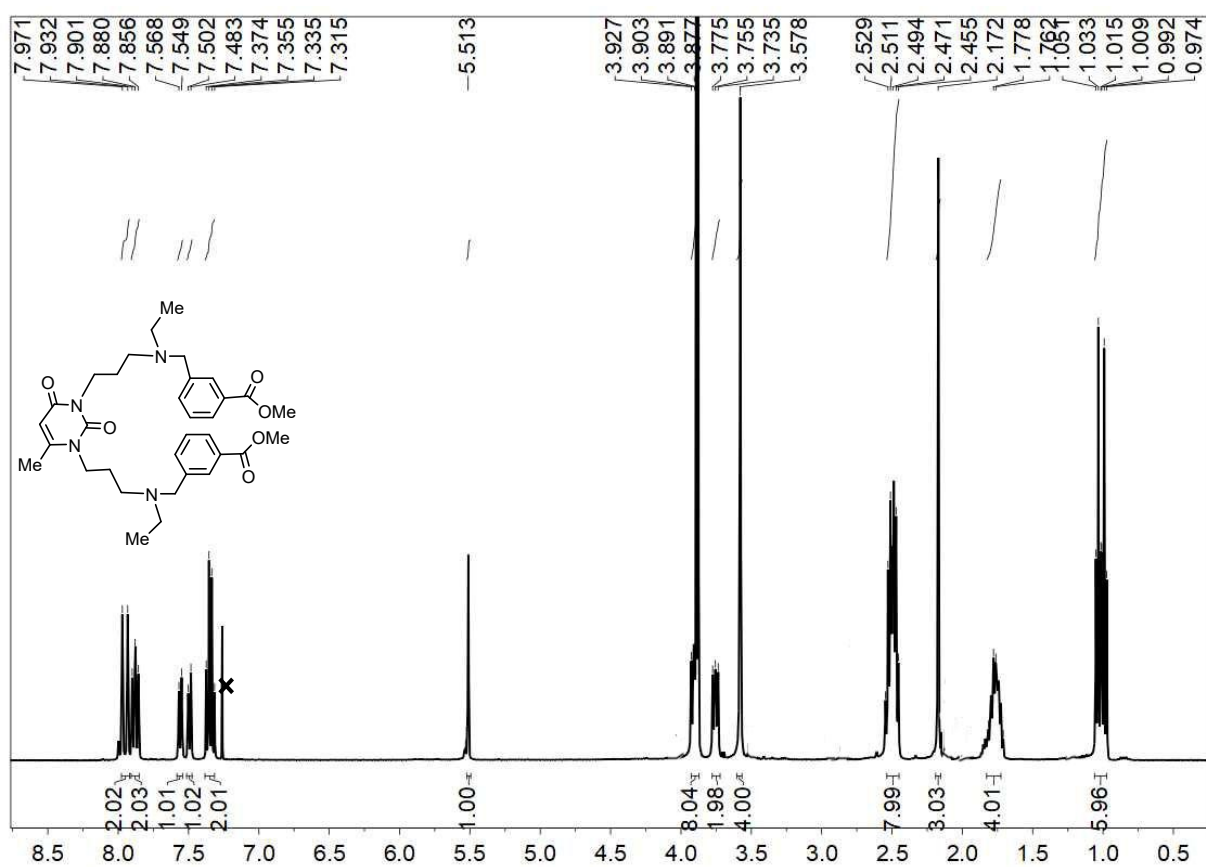


Figure S1. 1D ¹H NMR spectrum of **2a** in CDCl₃ (400 MHz) at T = 303 K. x - residual solvent peak.

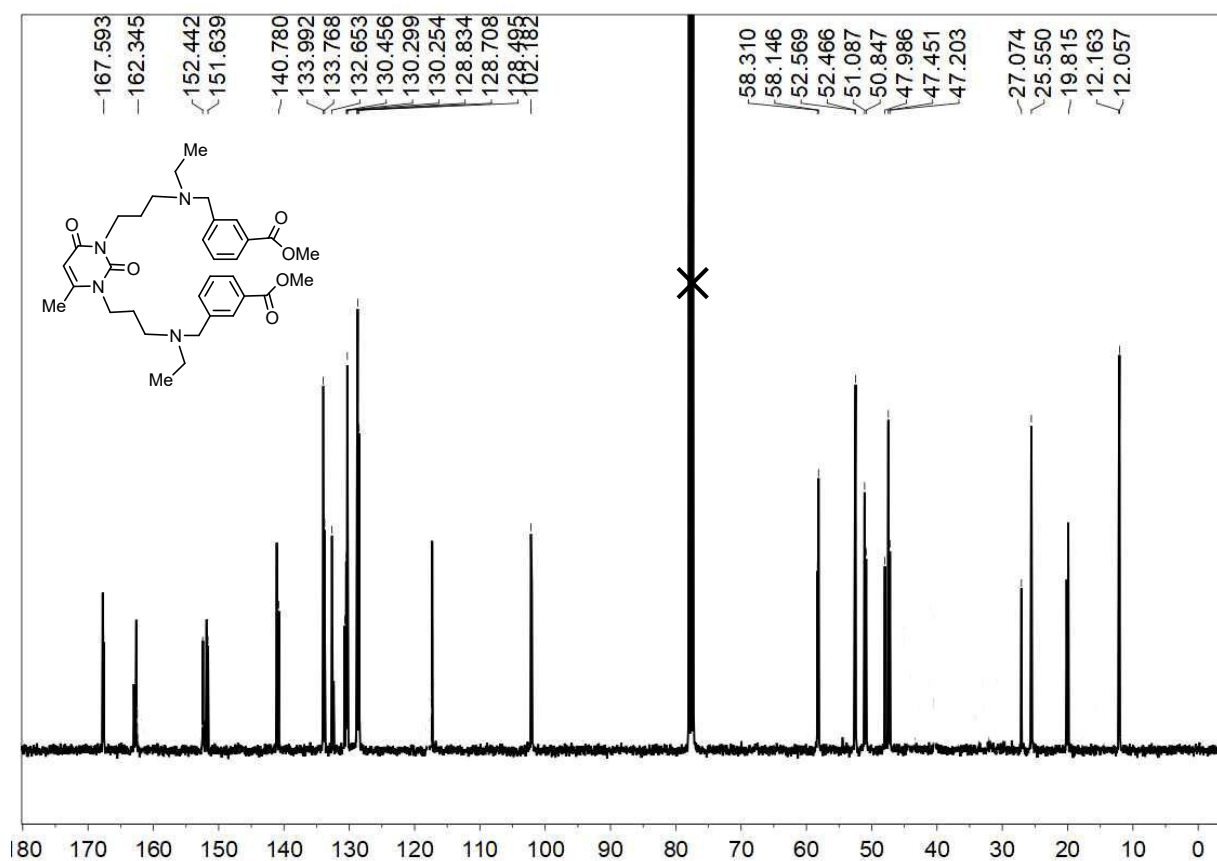


Figure S2. 1D ^{13}C NMR spectrum of **2a** in CDCl_3 at $T = 303$. x - residual solvent peak.

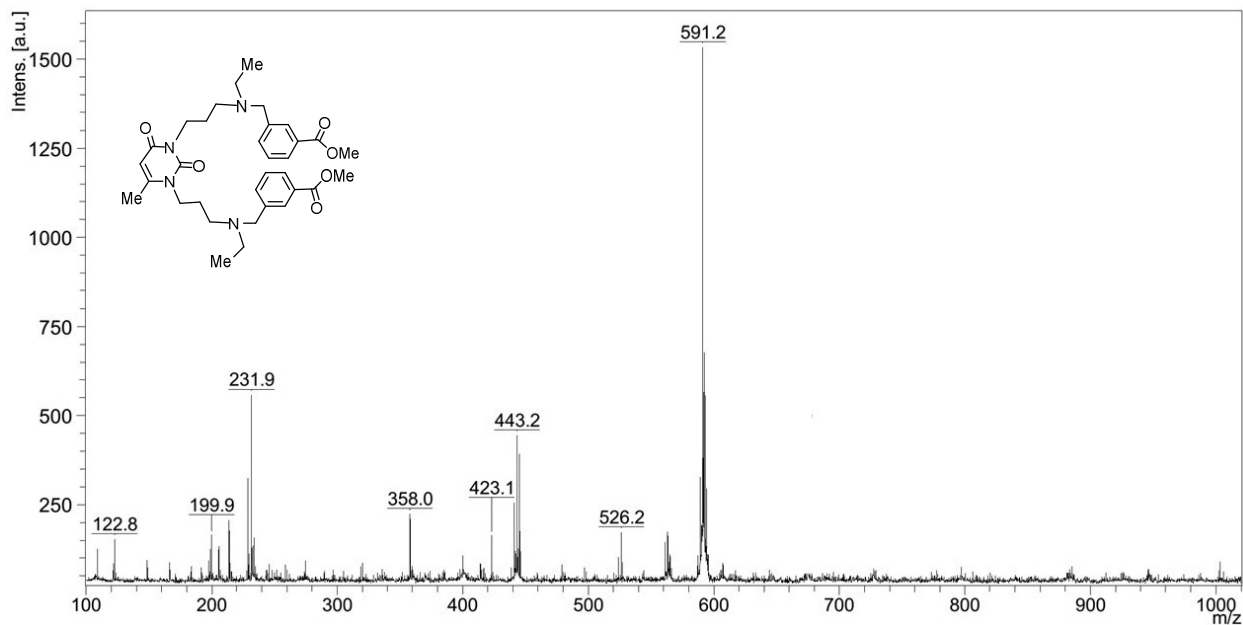


Figure S3. MALDI-TOF mass spectrum of **2a**.

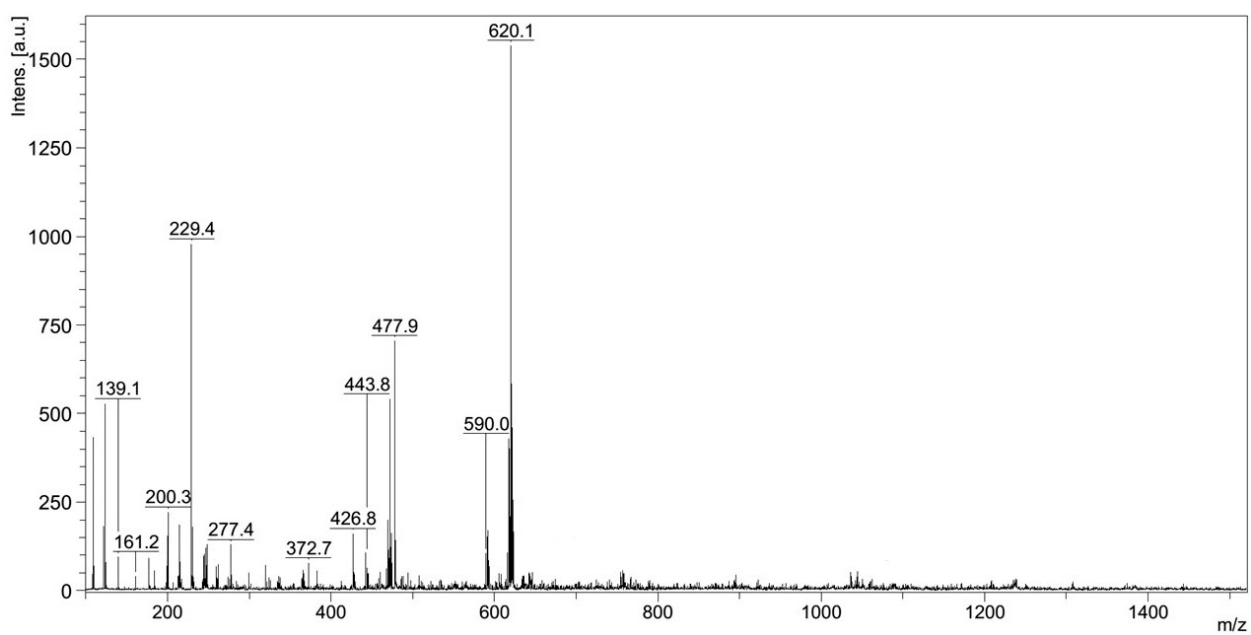


Figure S4. MALDI-TOF mass spectrum of **2b**.

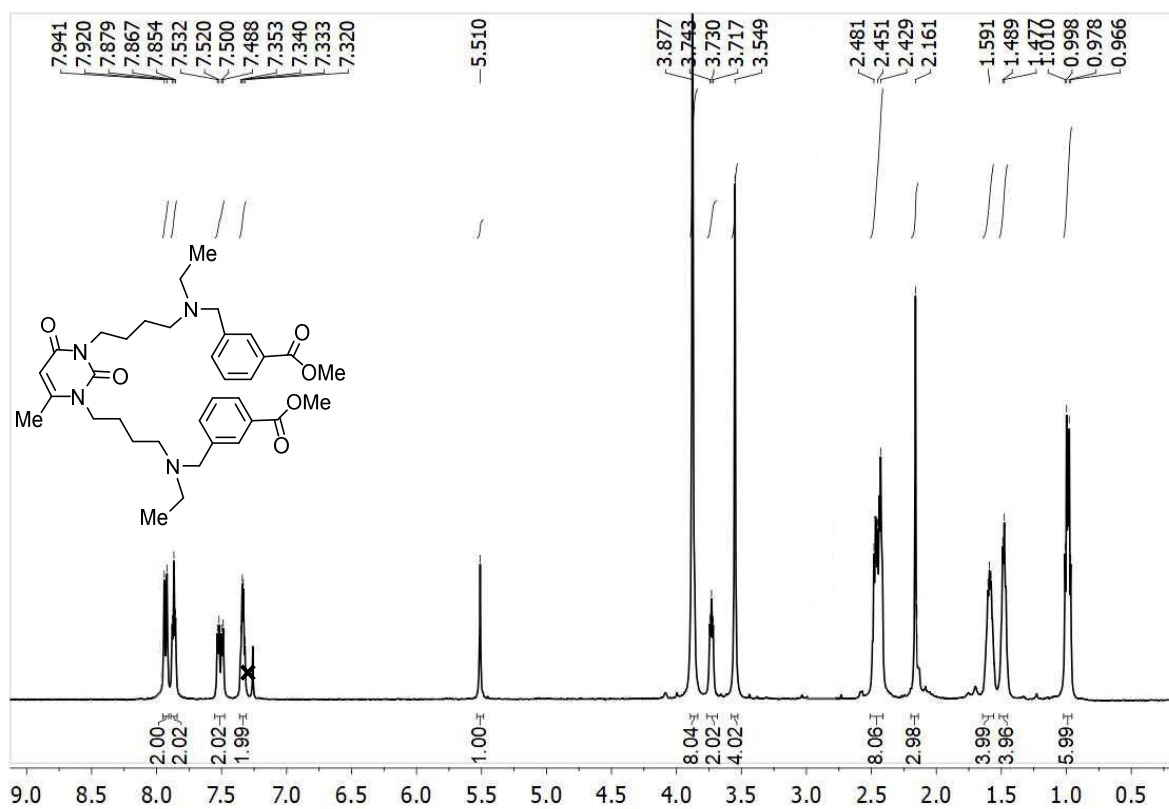


Figure S5. 1D ^1H NMR spectrum of **2b** in CDCl_3 (600 MHz) at $T = 303$ K. x - residual solvent peak.

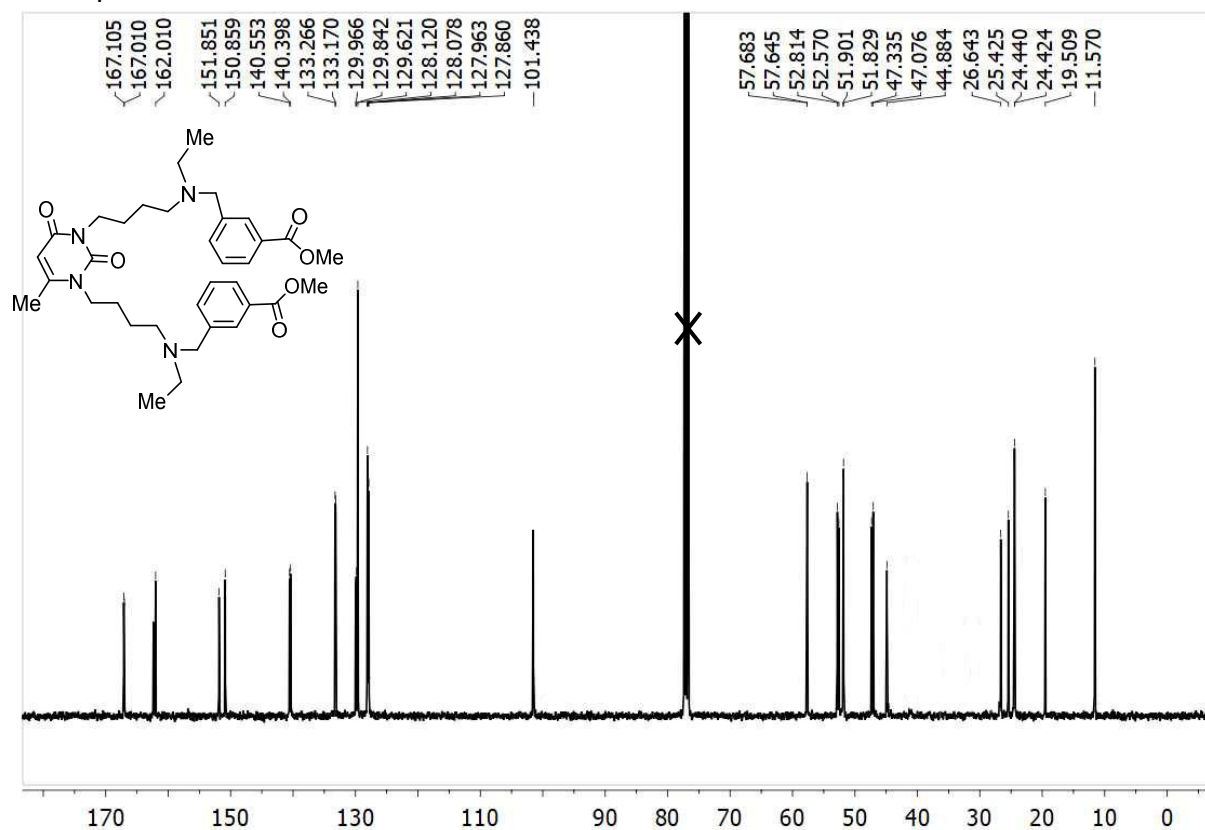


Figure S6. 1D ^{13}C NMR spectrum of **2b** in CDCl_3 (150 MHz) at $T = 303$. x - residual solvent peak.

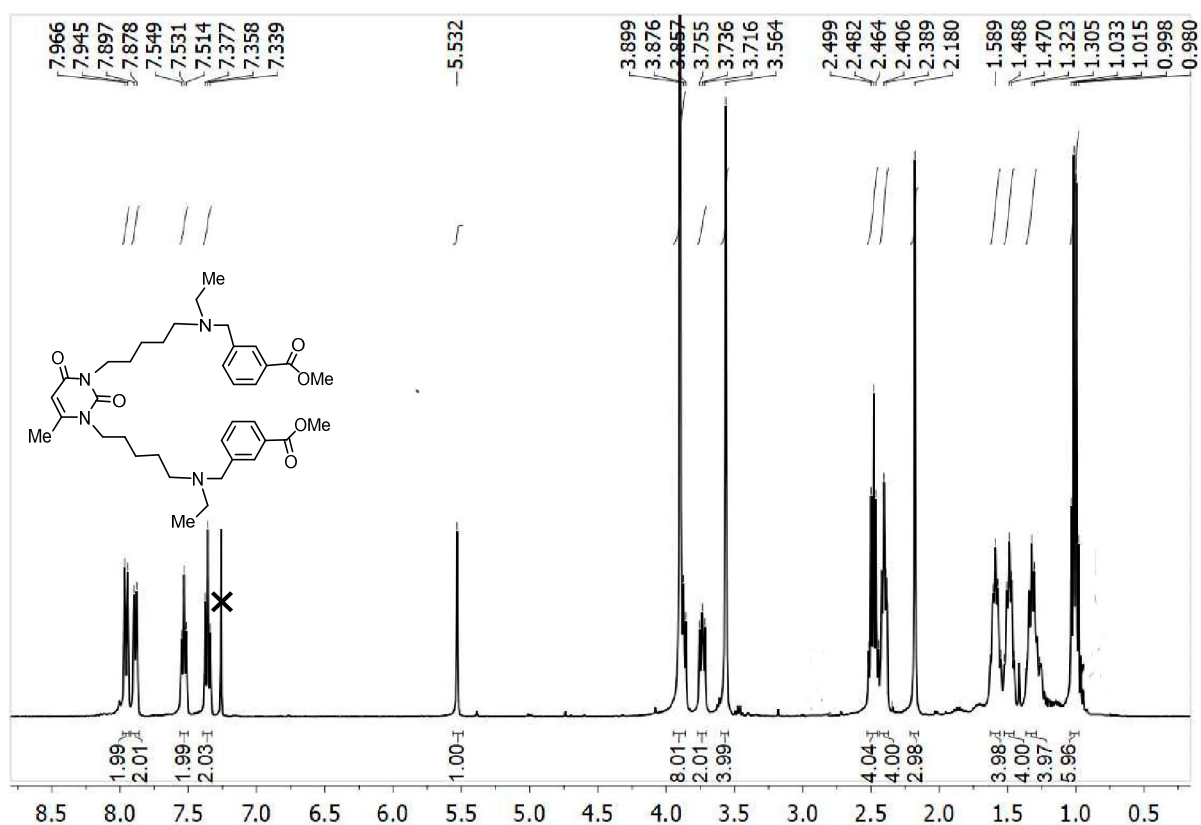


Figure S6. 1D ^1H NMR spectrum of **2c** in CDCl_3 (600 MHz) at $T = 303$ K. x - residual solvent peak.

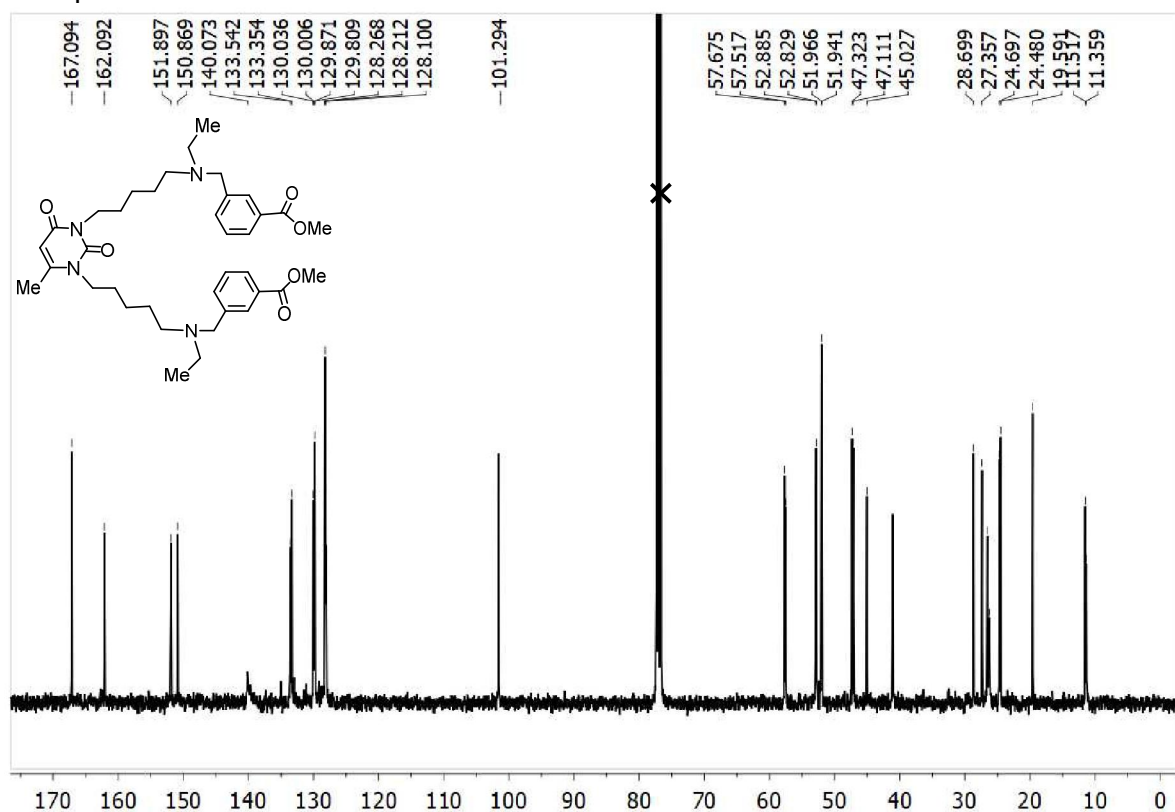


Figure S8. 1D ^{13}C NMR spectrum of **2c** in CDCl_3 (150 MHz) at $T = 303$. x - residual solvent peak.

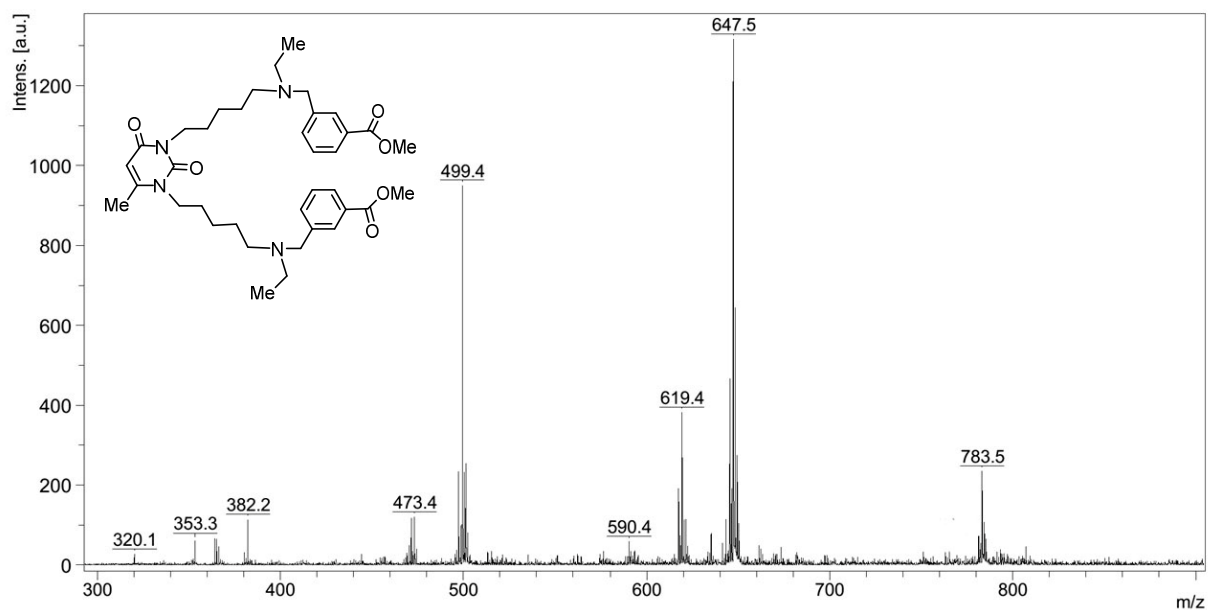


Figure S9. MALDI-TOF mass spectrum of **2c**.

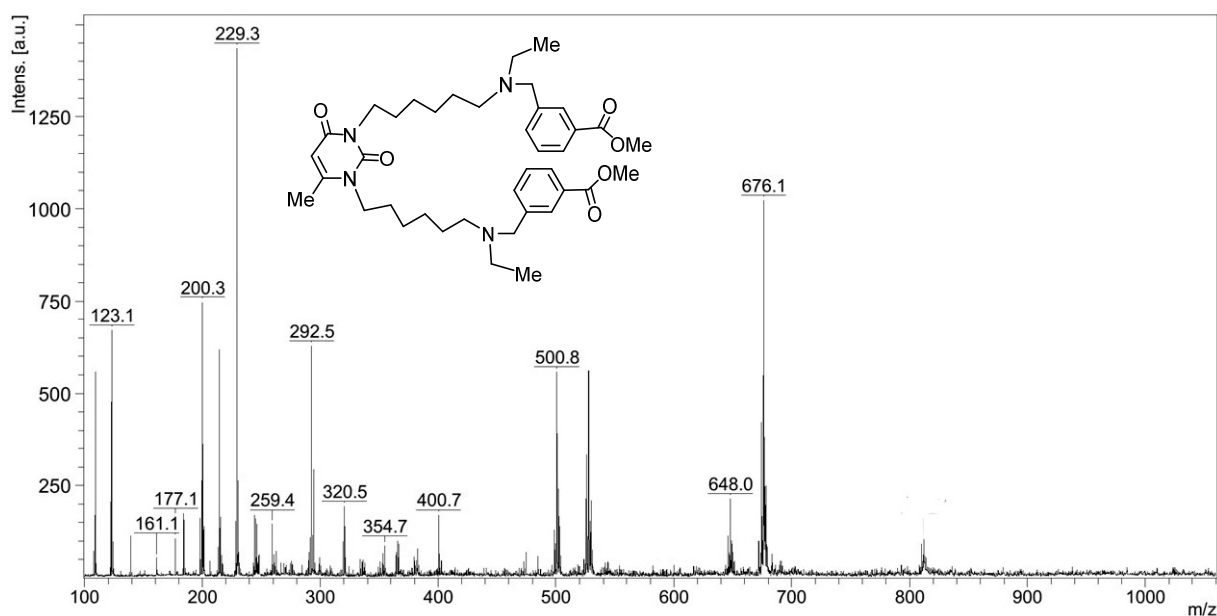


Figure S10. MALDI-TOF mass spectrum of **2d**.

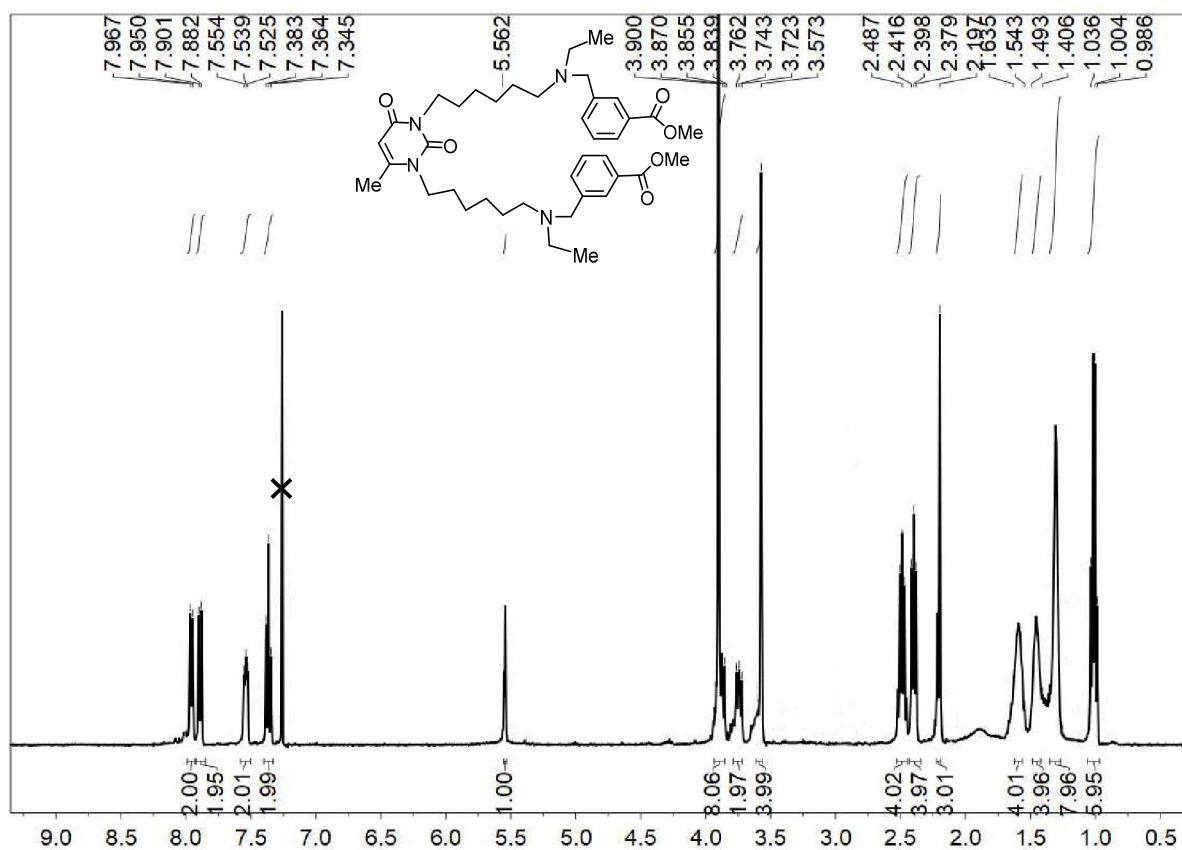


Figure S11. 1D ^1H NMR spectrum of **2d** in CDCl_3 (400 MHz) at $T = 303$ K. x - residual solvent peak.

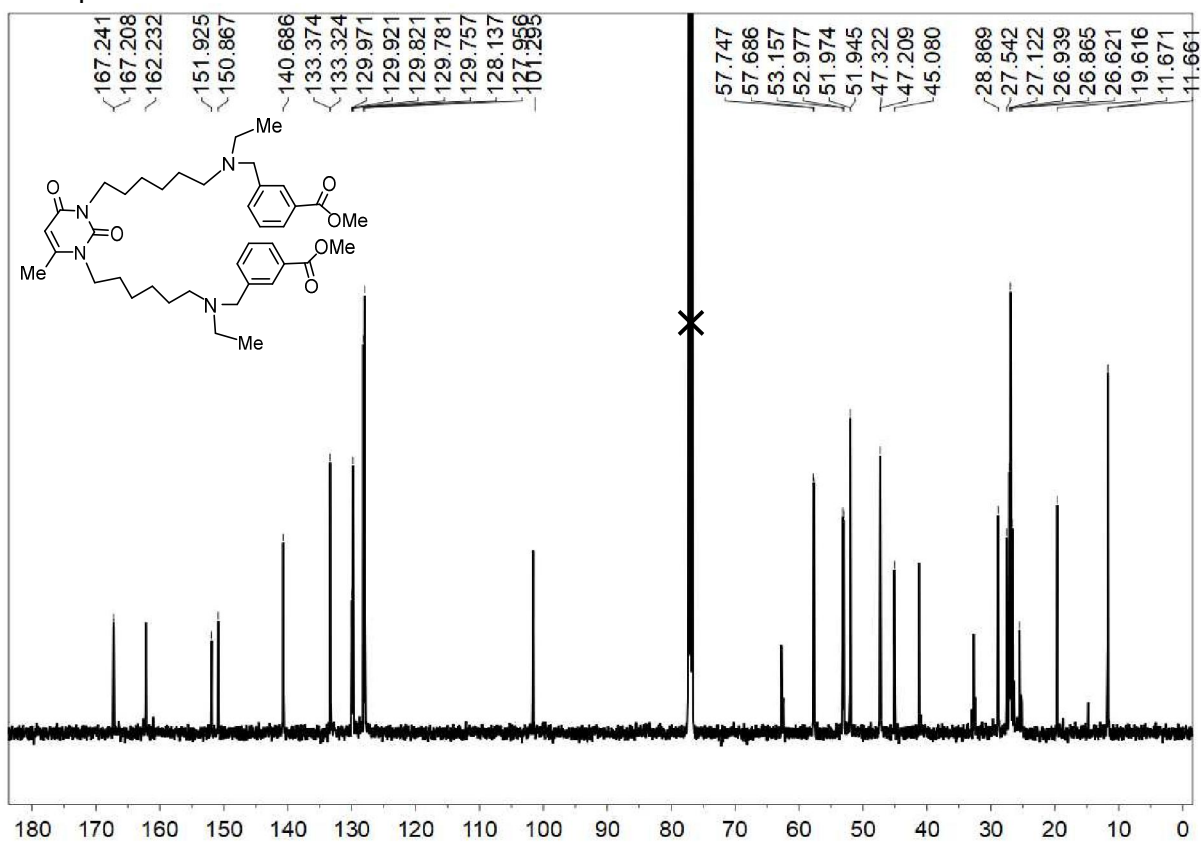


Figure S12. 1D ^{13}C NMR spectrum of **2d** in CDCl_3 at $T = 303$. x - residual solvent peak.

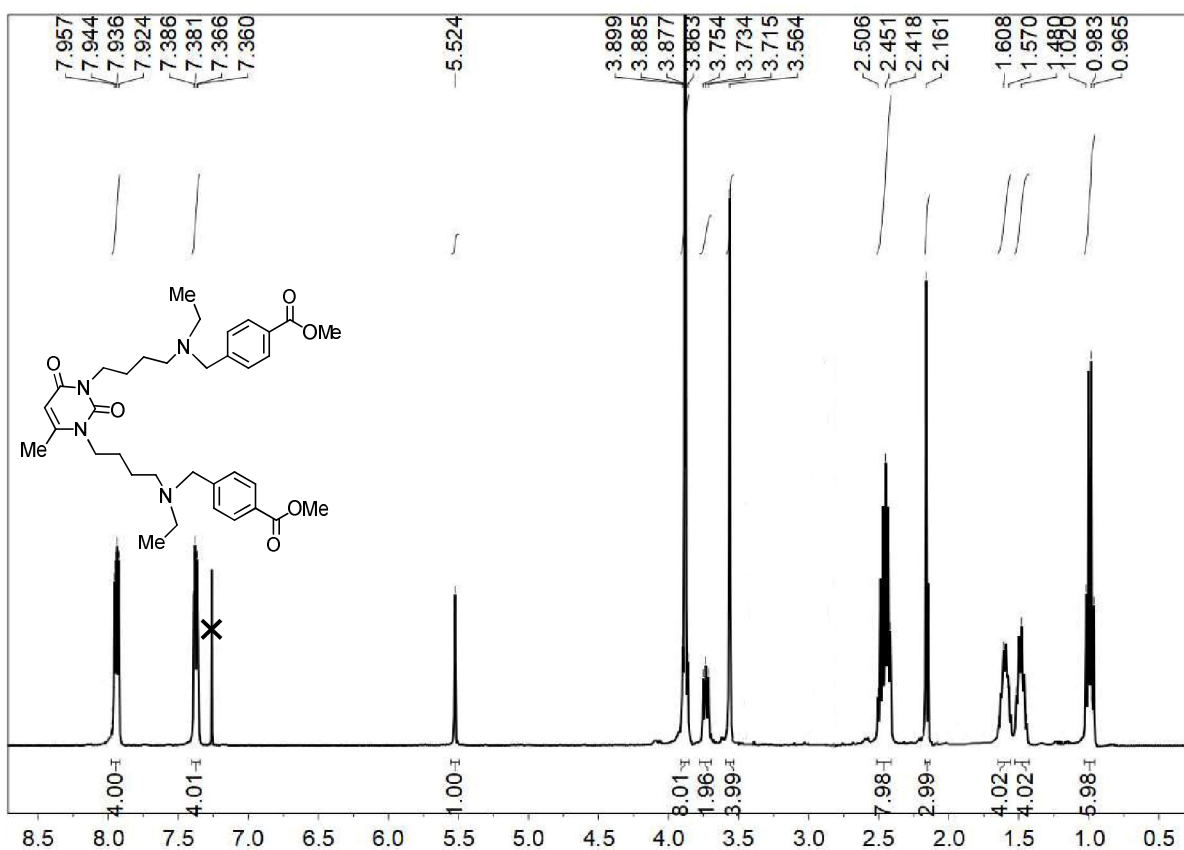


Figure S13. 1D ¹H NMR spectrum of **3a** in CDCl₃ (400 MHz) at T = 303 K. x - residual solvent peak.

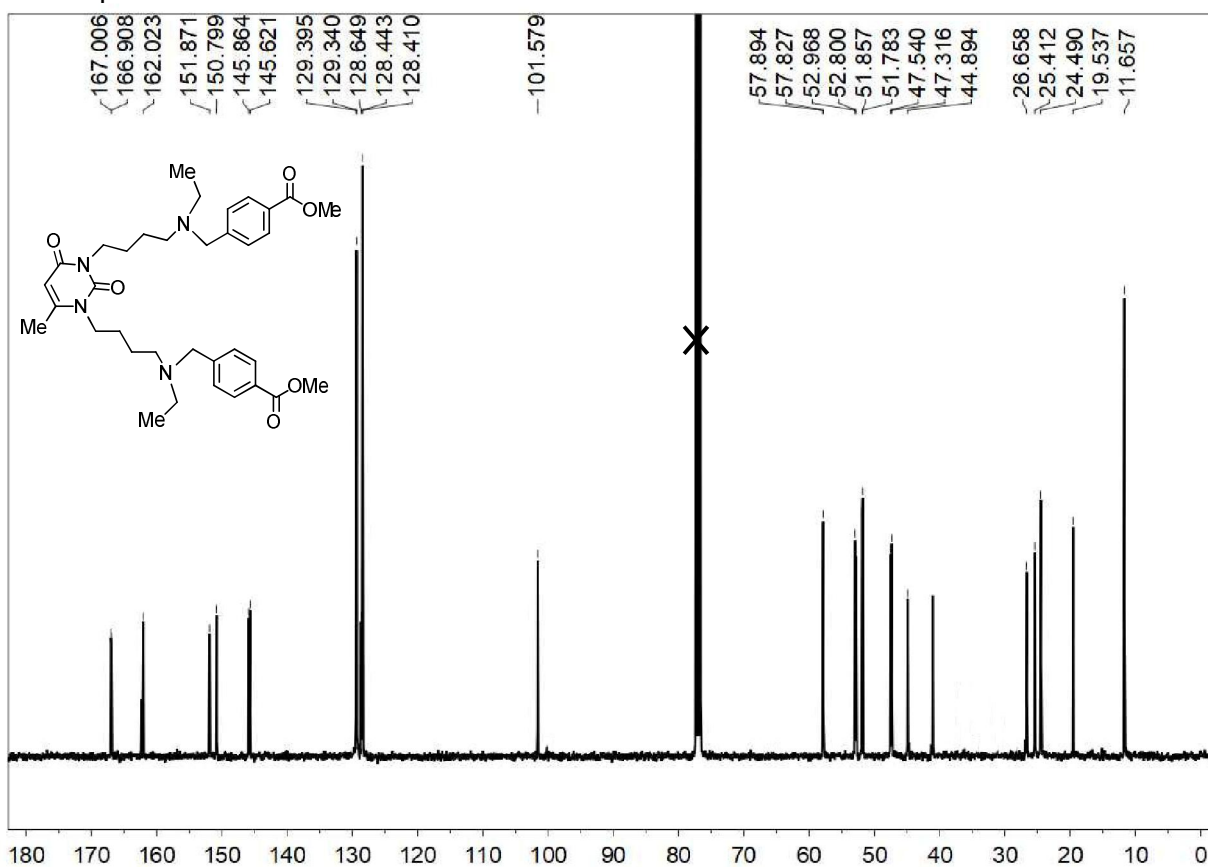


Figure S14. 1D ¹³C NMR spectrum of **3a** in CDCl₃ at T = 303. x - residual solvent peak.

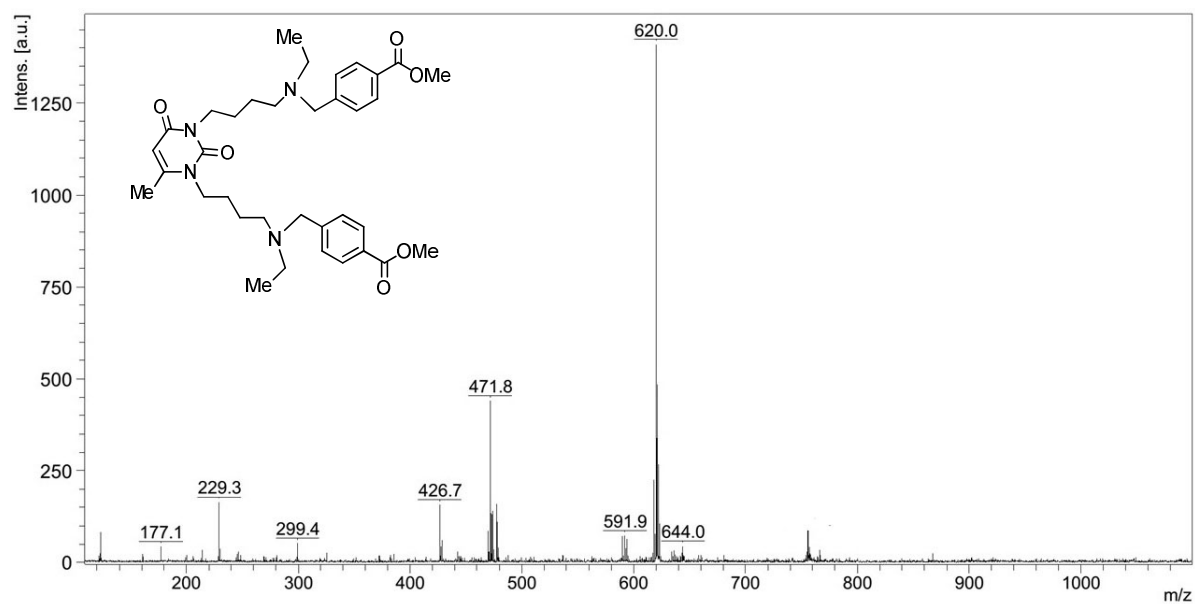


Figure S15. MALDI-TOF mass spectrum of **3a**.

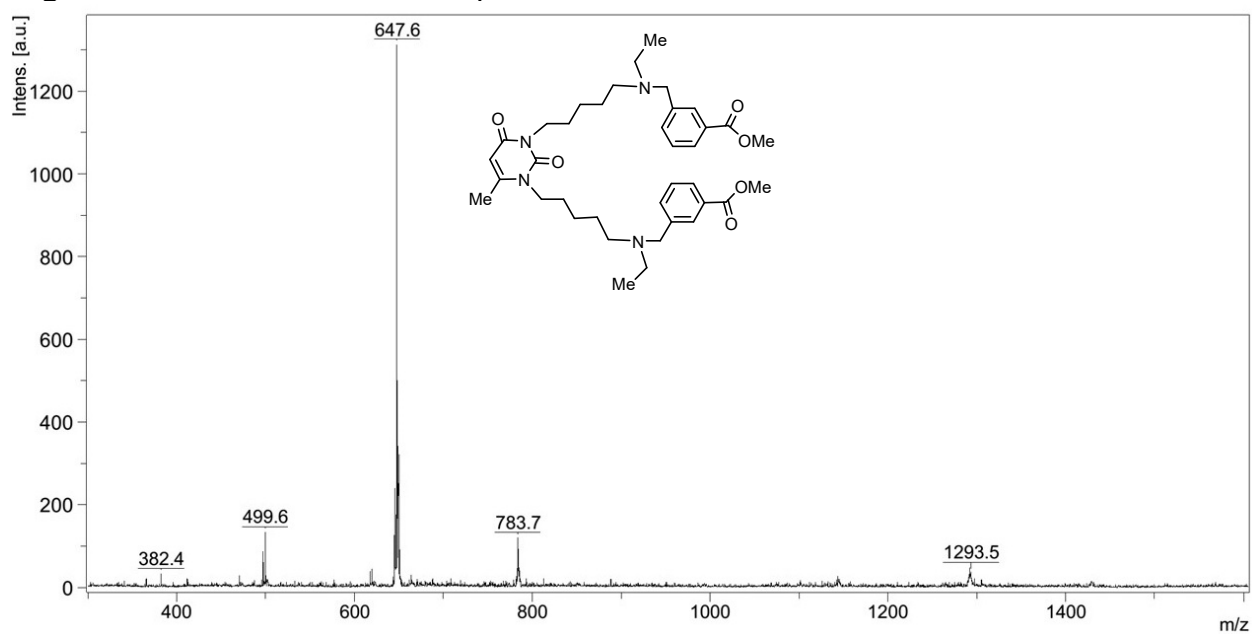


Figure S16. MALDI-TOF mass spectrum of **3b**.

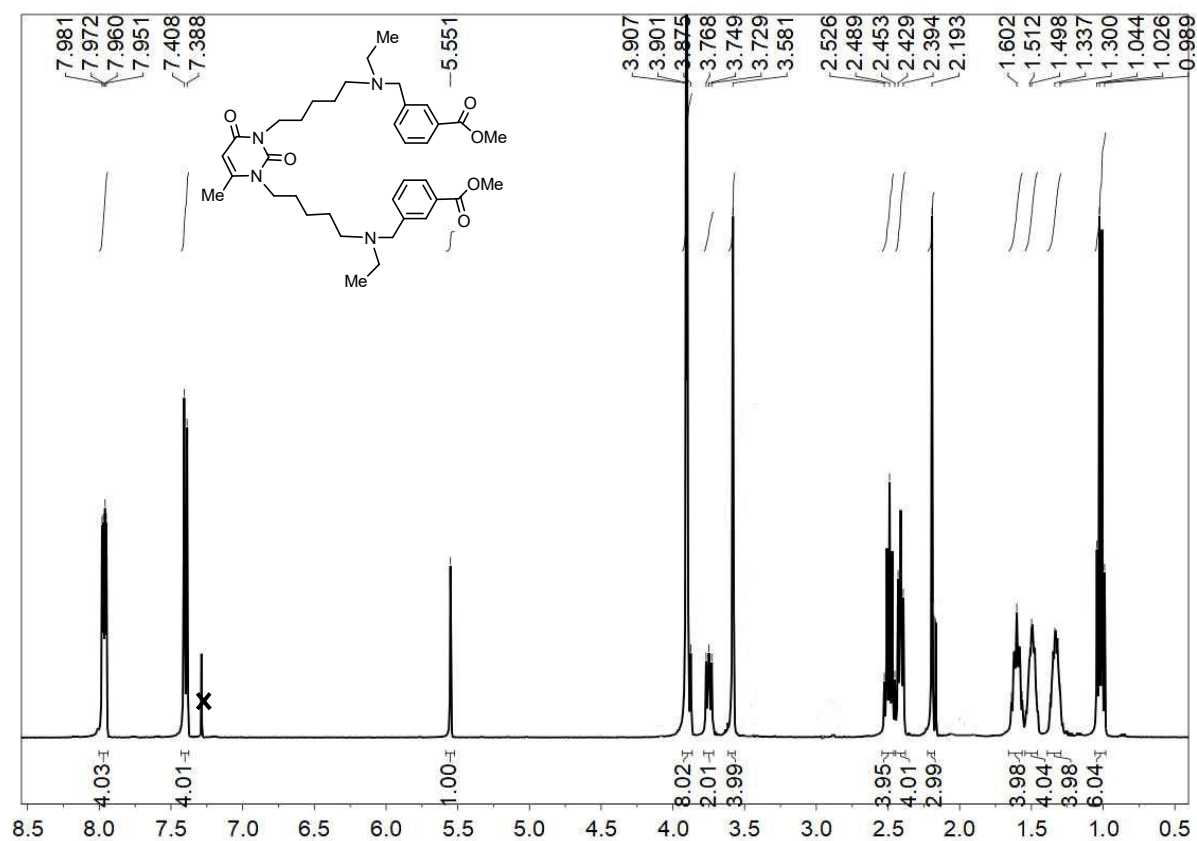


Figure S17. 1D ^1H NMR spectrum of **3b** in CDCl_3 (400 MHz) at $T = 303$ K. x - residual solvent peak.

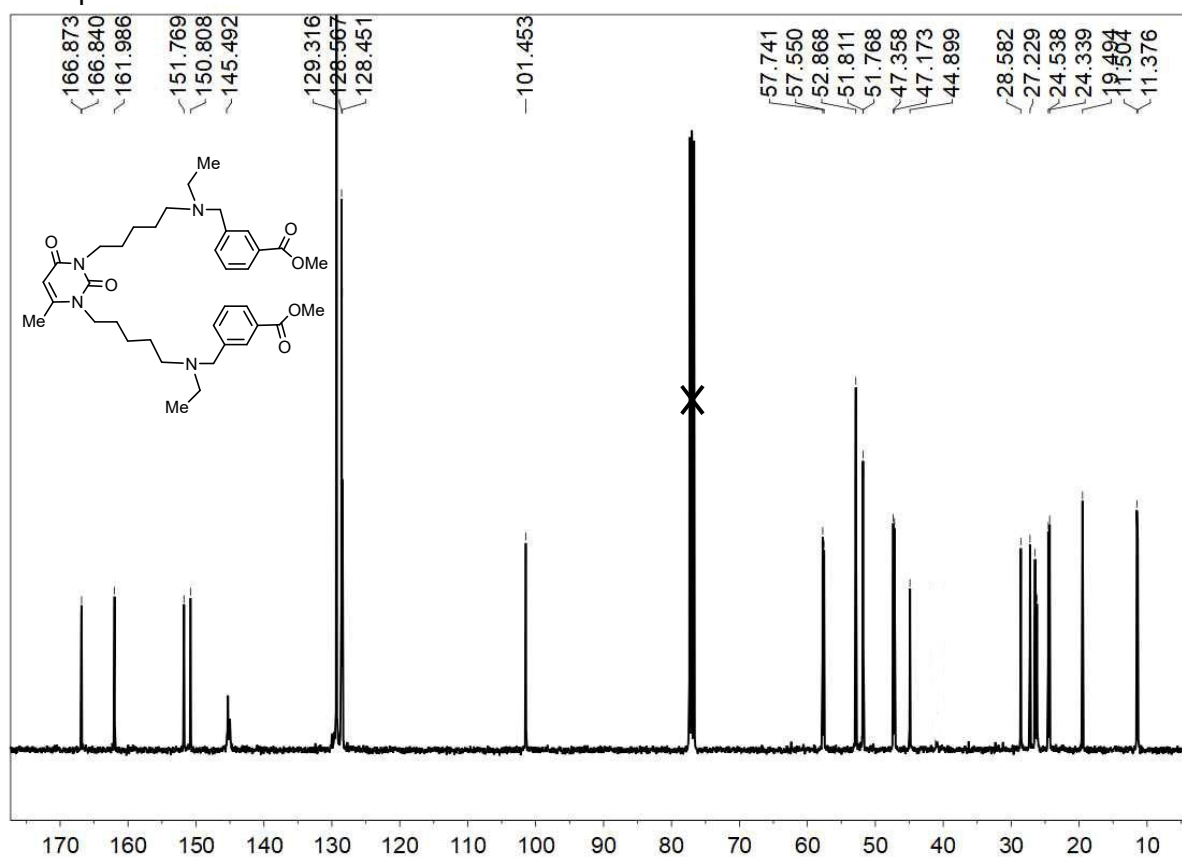


Figure S18. 1D ^{13}C NMR spectrum of **3b** in CDCl_3 at $T = 303$ K. x - residual solvent peak.

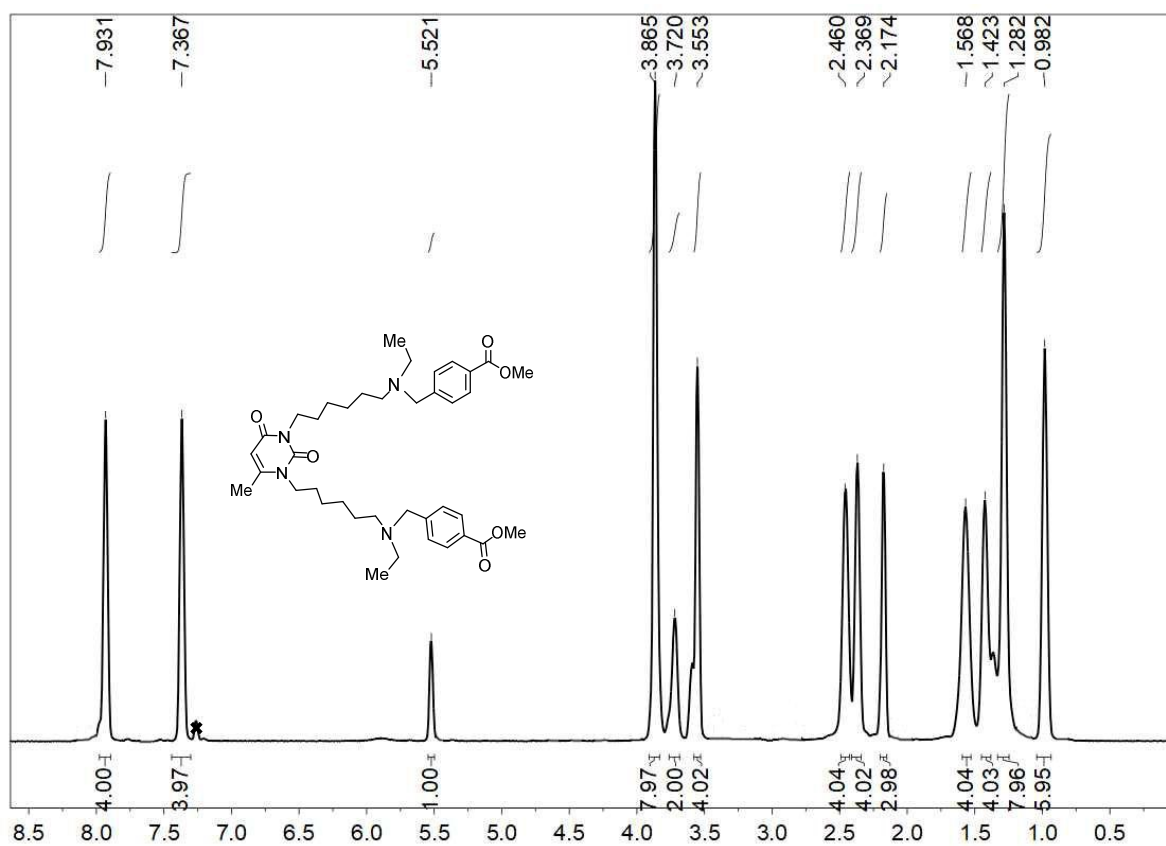


Figure S19. 1D ^1H NMR spectrum of **3c** in CDCl_3 (400 MHz) at $T = 303$ K. x - residual solvent peak.

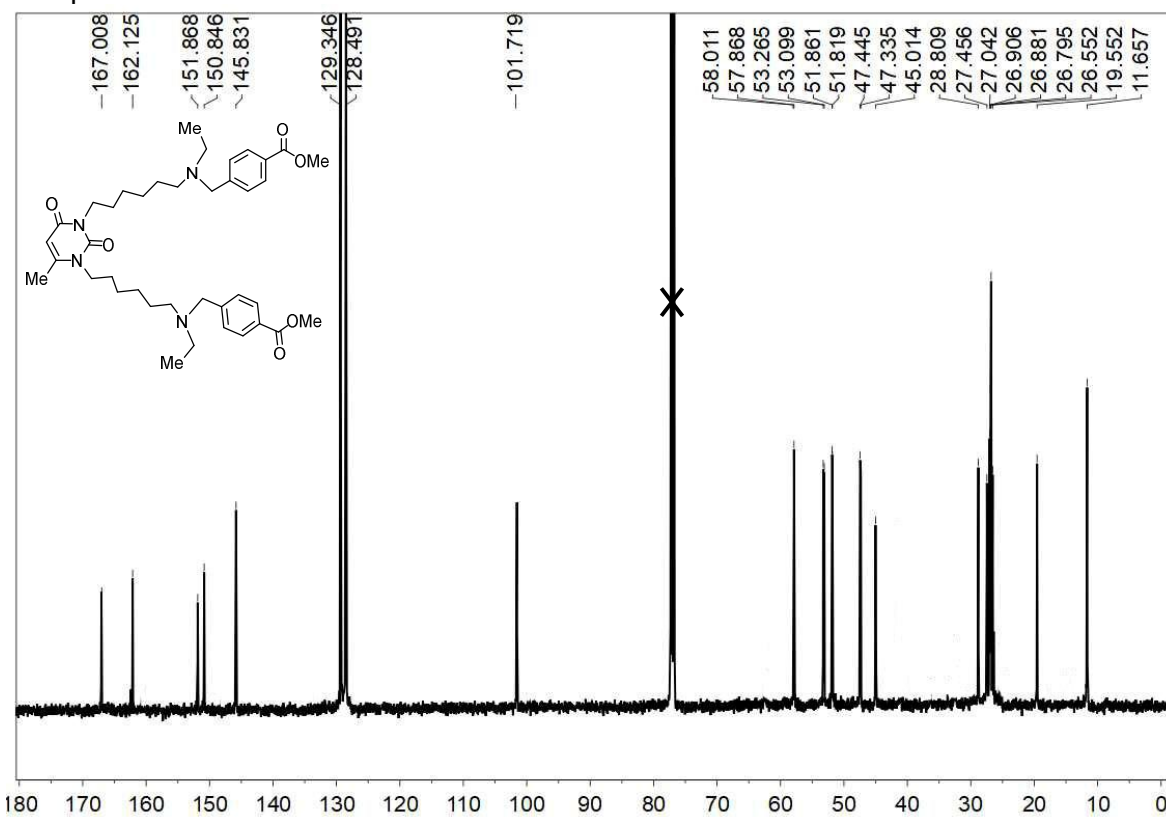


Figure S20. 1D ^{13}C NMR spectrum of **3c** in CDCl_3 at $T = 303$. x - residual solvent peak.

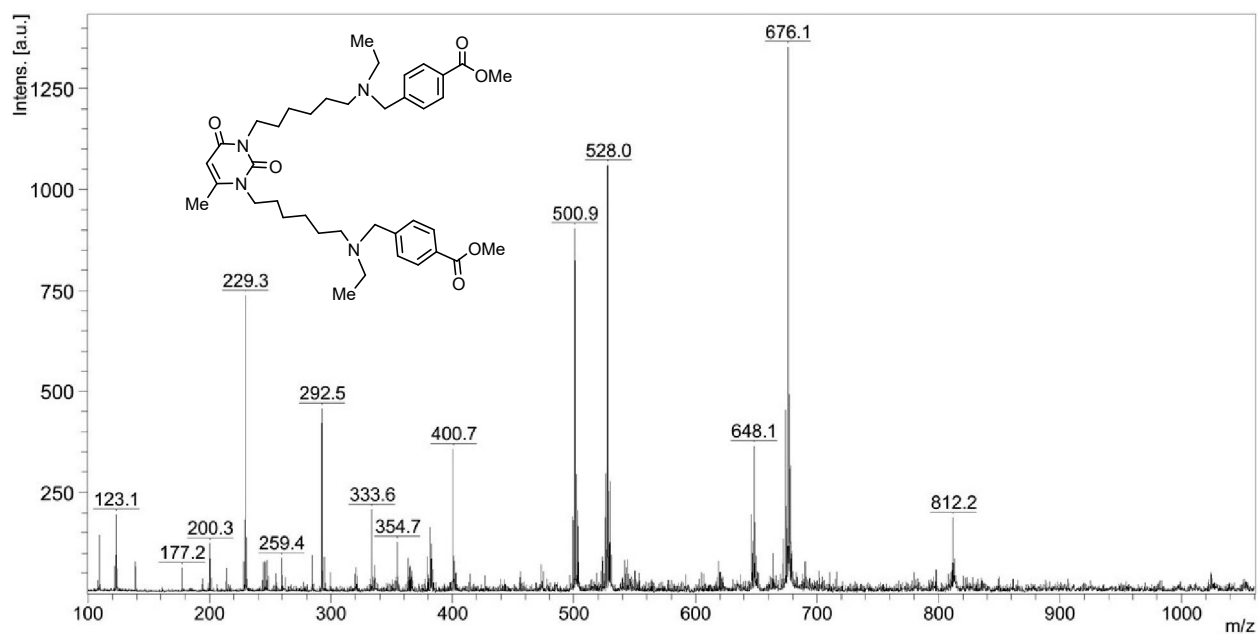


Figure S21. MALDI-TOF mass spectrum of **3c**.

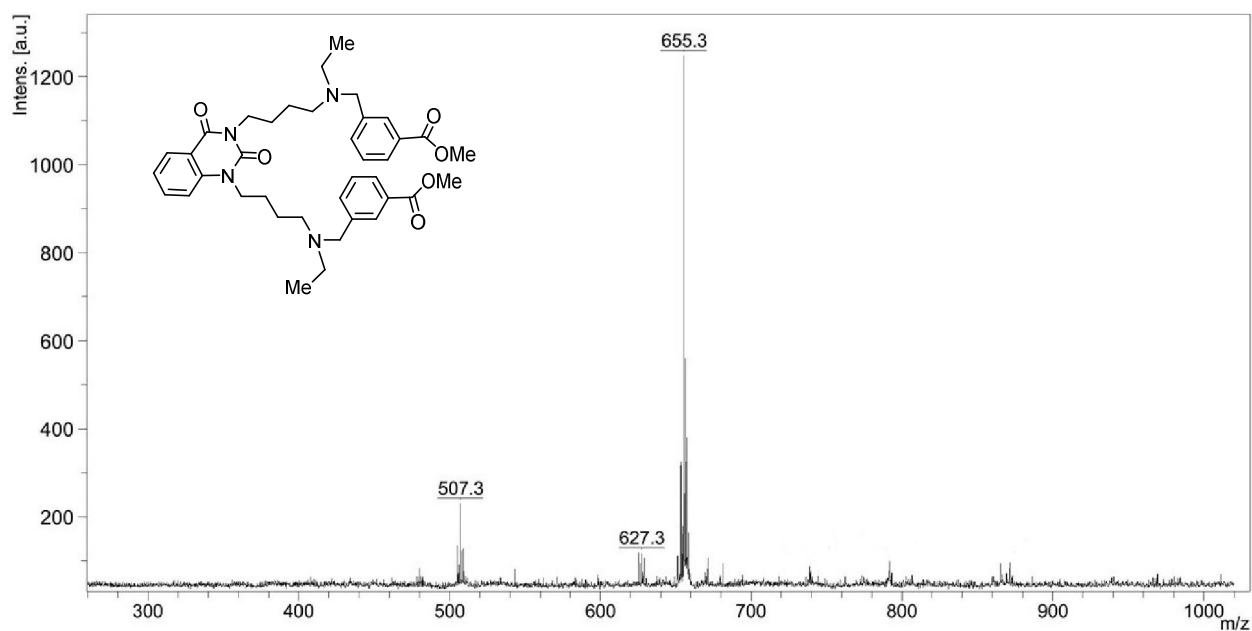


Figure S22. MALDI-TOF mass spectrum of **4a**.

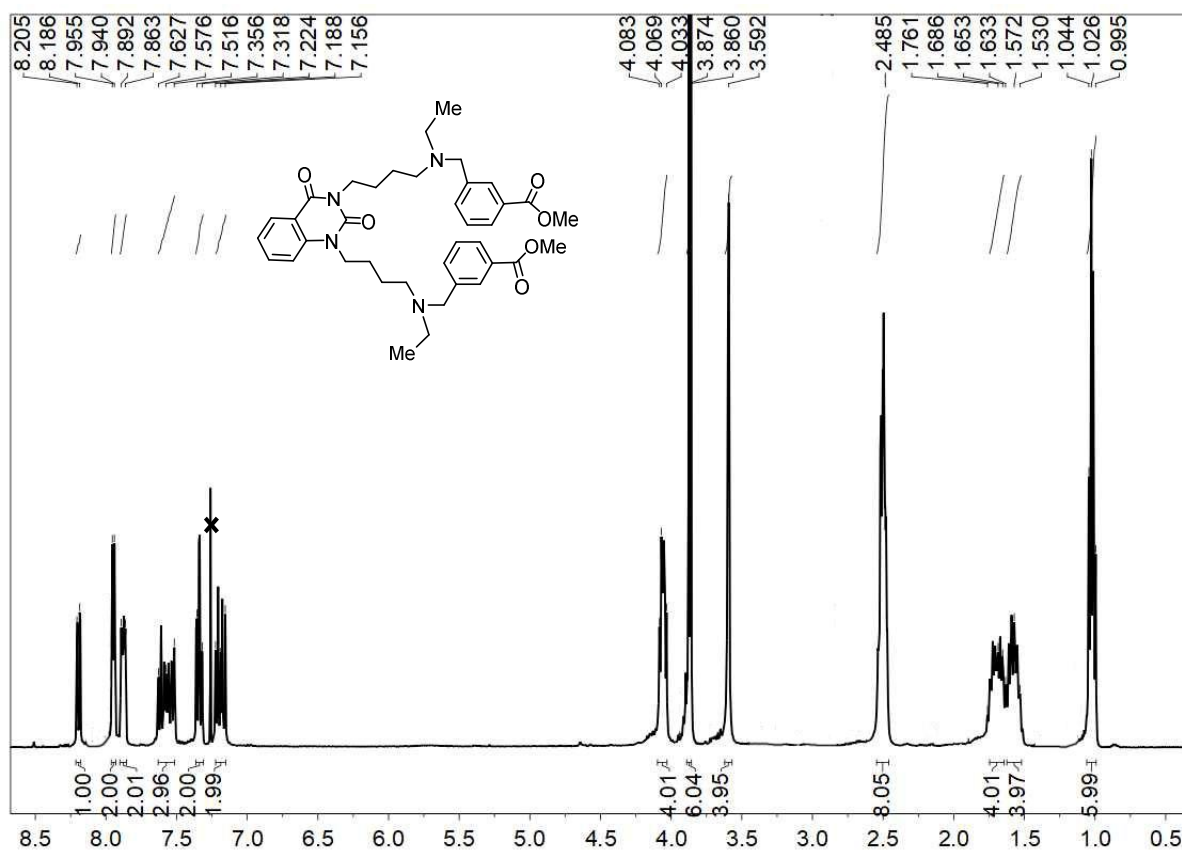


Figure S23. 1D ^{13}C NMR spectrum of **4a** in CDCl_3 at $T = 303$. x - residual solvent peak.

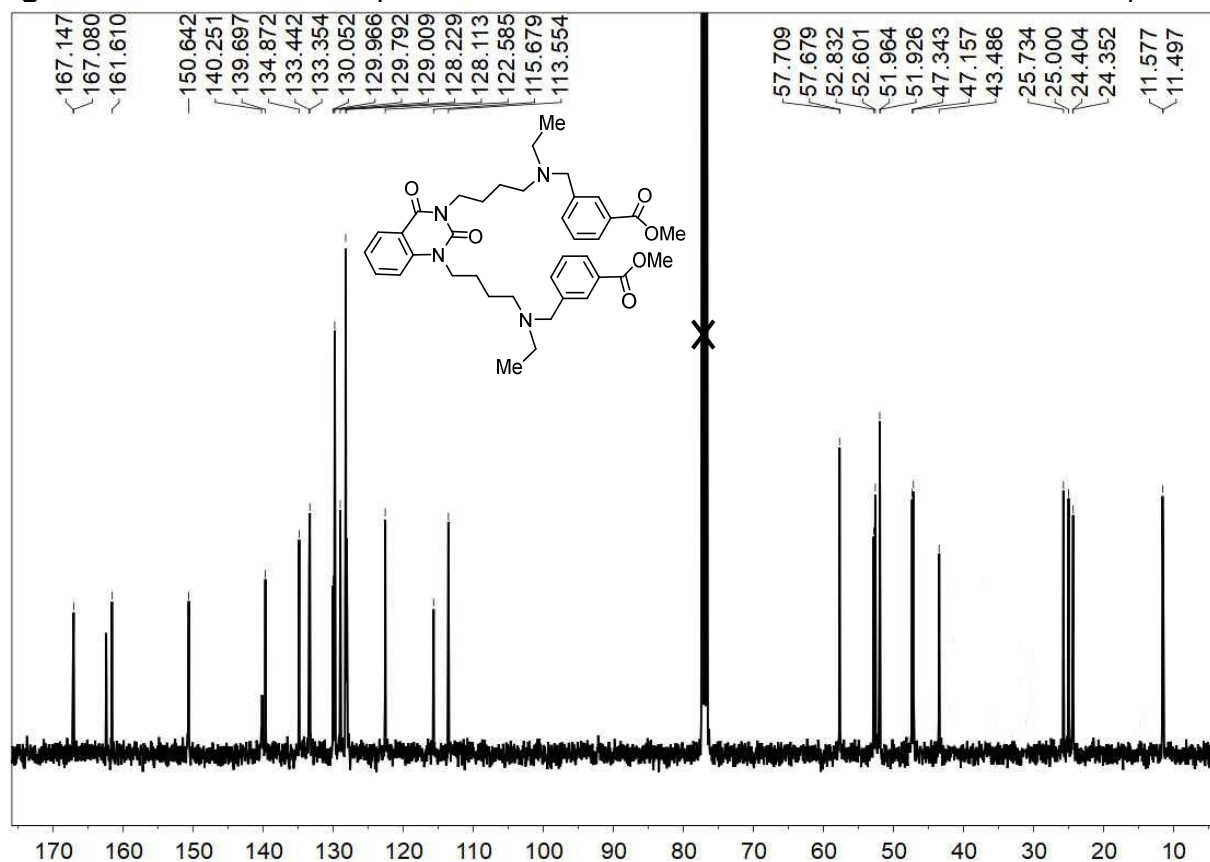


Figure S24. 1D ^{13}C NMR spectrum of **4a** in CDCl_3 at $T = 303$. x - residual solvent peak.

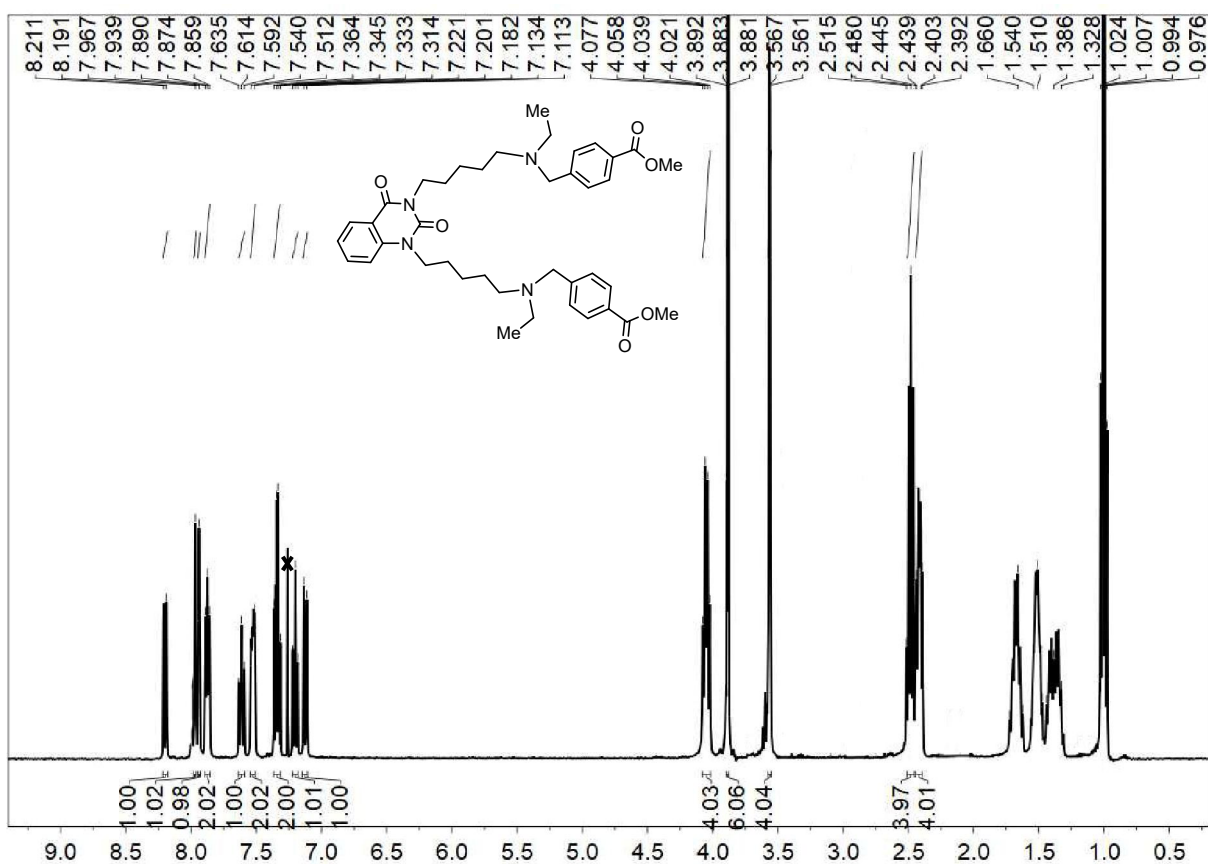


Figure S25. 1D ¹H NMR spectrum of **4b** in CDCl₃ (400 MHz) at T = 303 K. x - residual solvent peak.

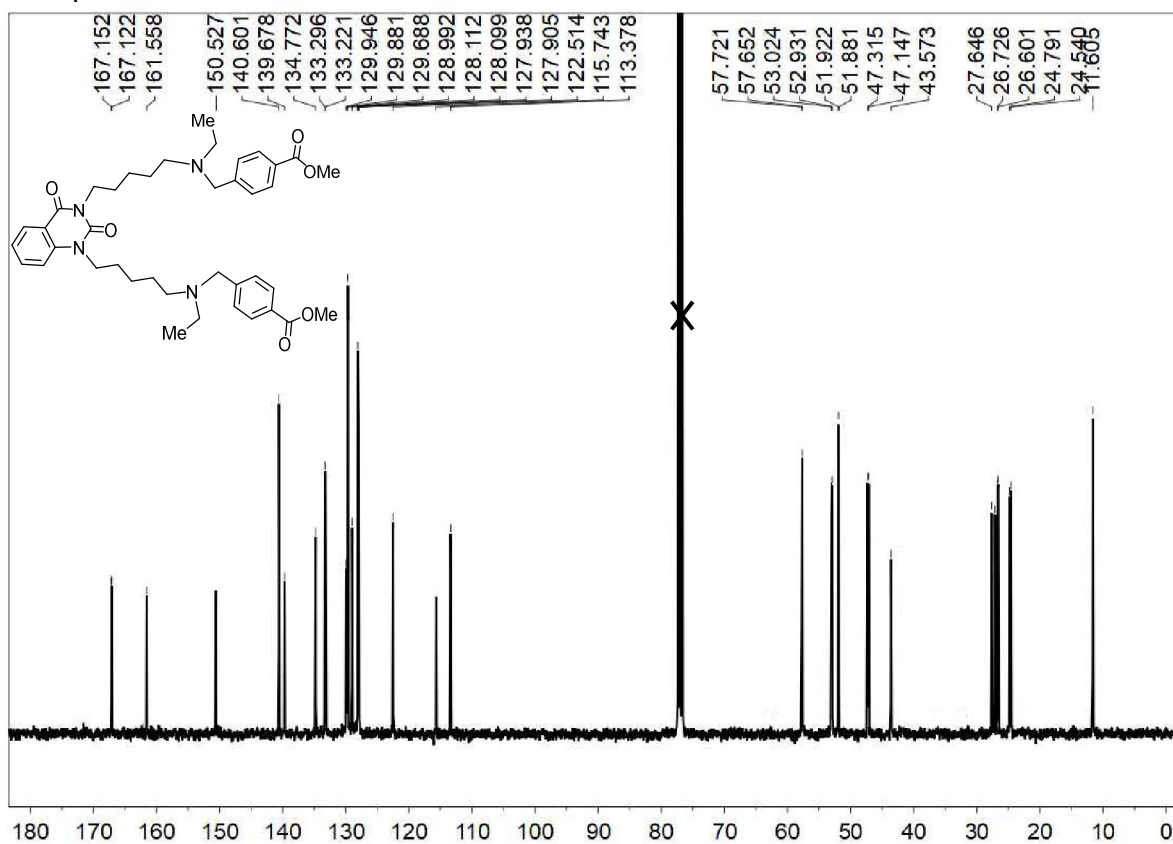


Figure S26. 1D ¹³C NMR spectrum of **4b** in CDCl₃ at T = 303. x - residual solvent peak.

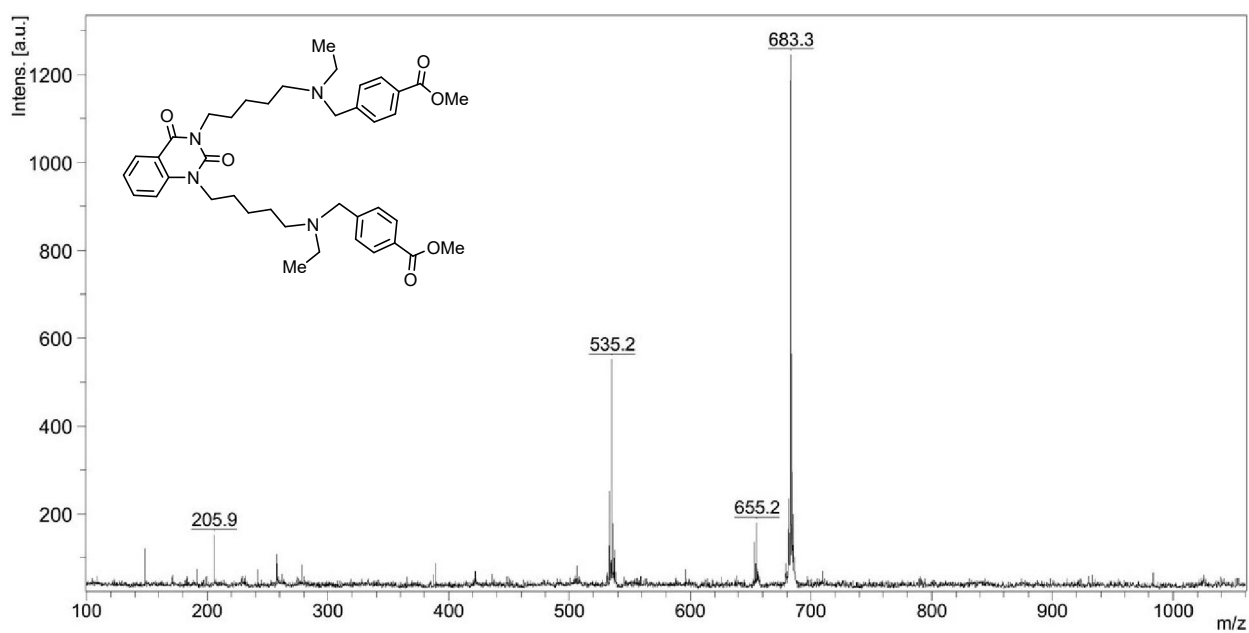


Figure S27. MALDI-TOF mass spectrum of **4b**.

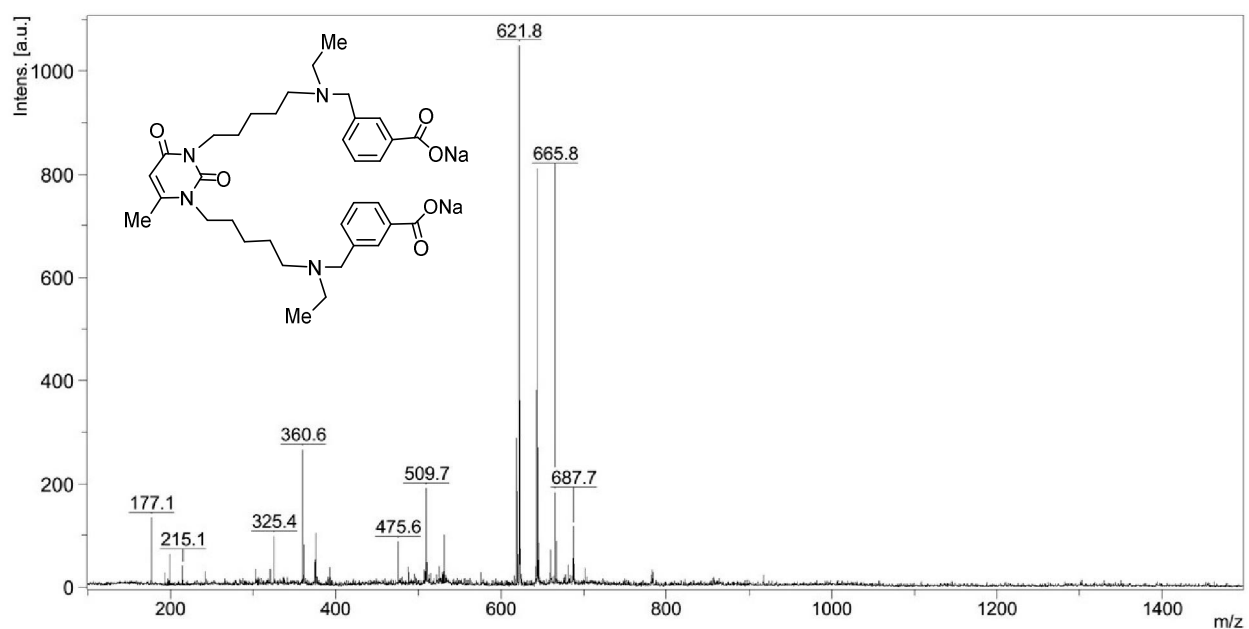


Figure S28. MALDI-TOF mass spectrum of **2e**.

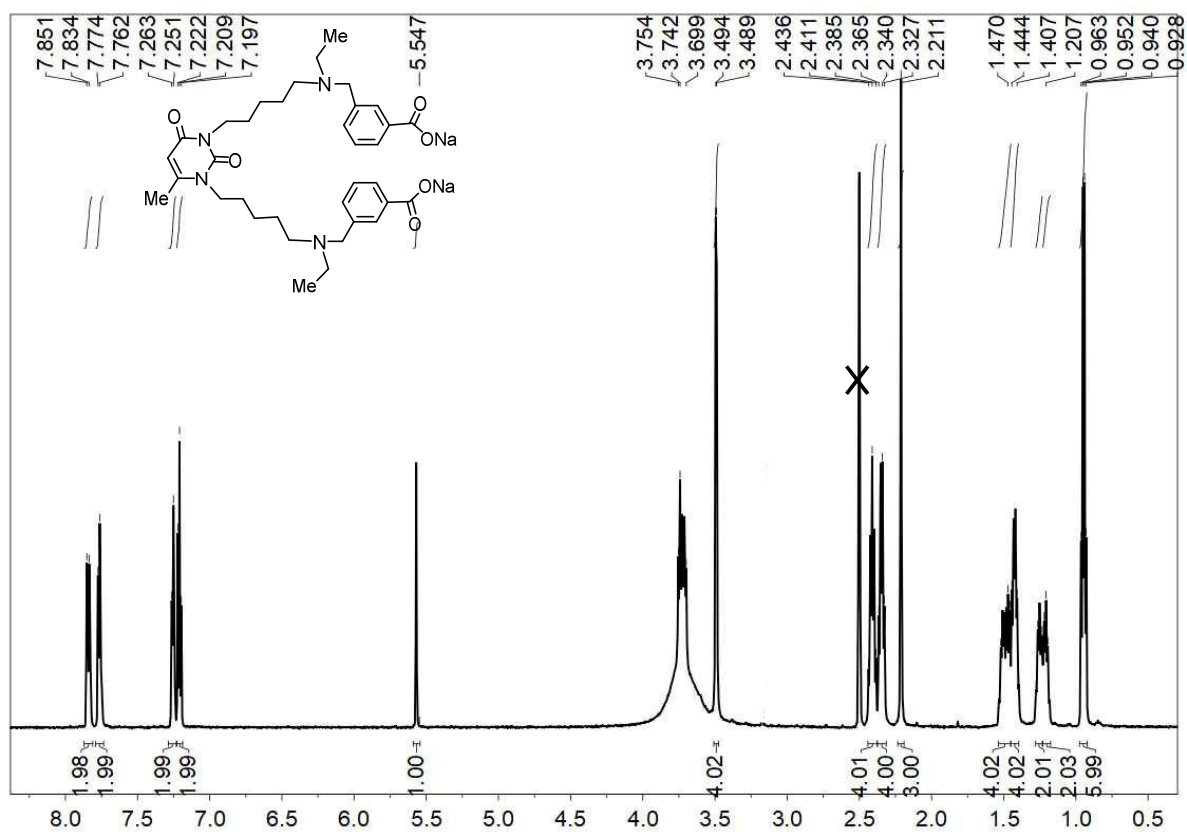


Figure S29. 1D ^1H NMR spectrum of **2e** in DMSO- d_6 (600 MHz) at T = 303 K. x - residual solvent peak.

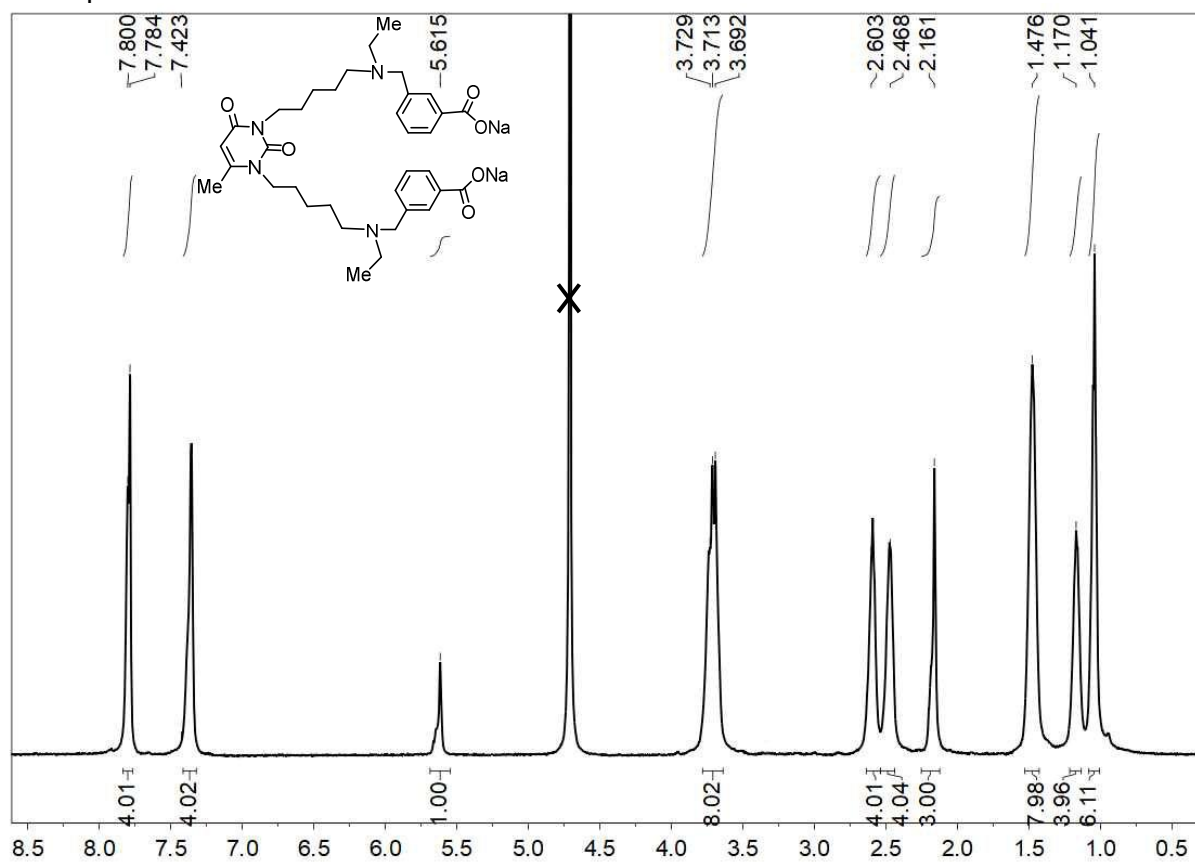


Figure S30. 1D ^1H NMR spectrum of **2e** in D_2O (600 MHz) at T = 303 K. x - residual solvent peak.

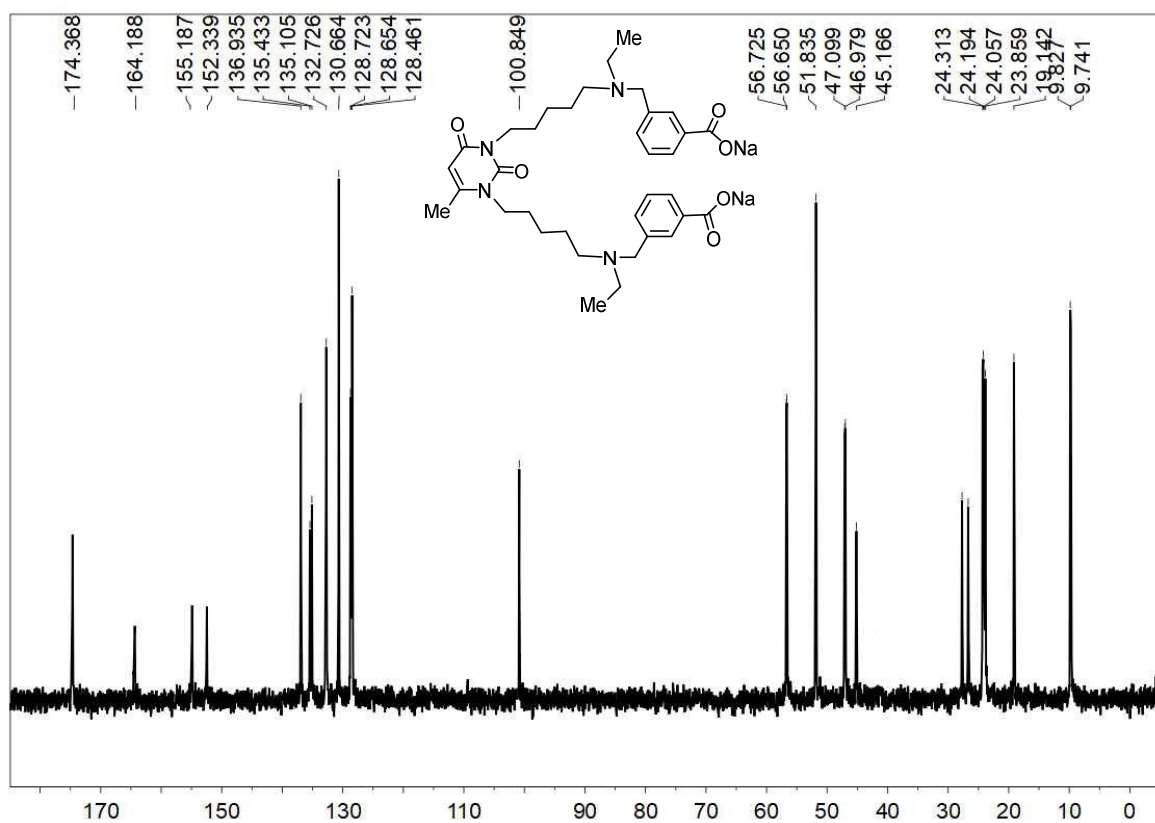


Figure S31. 1D ^{13}C NMR spectrum of **2e** in D_2O (150 MHz) at $T = 303\text{ K}$.

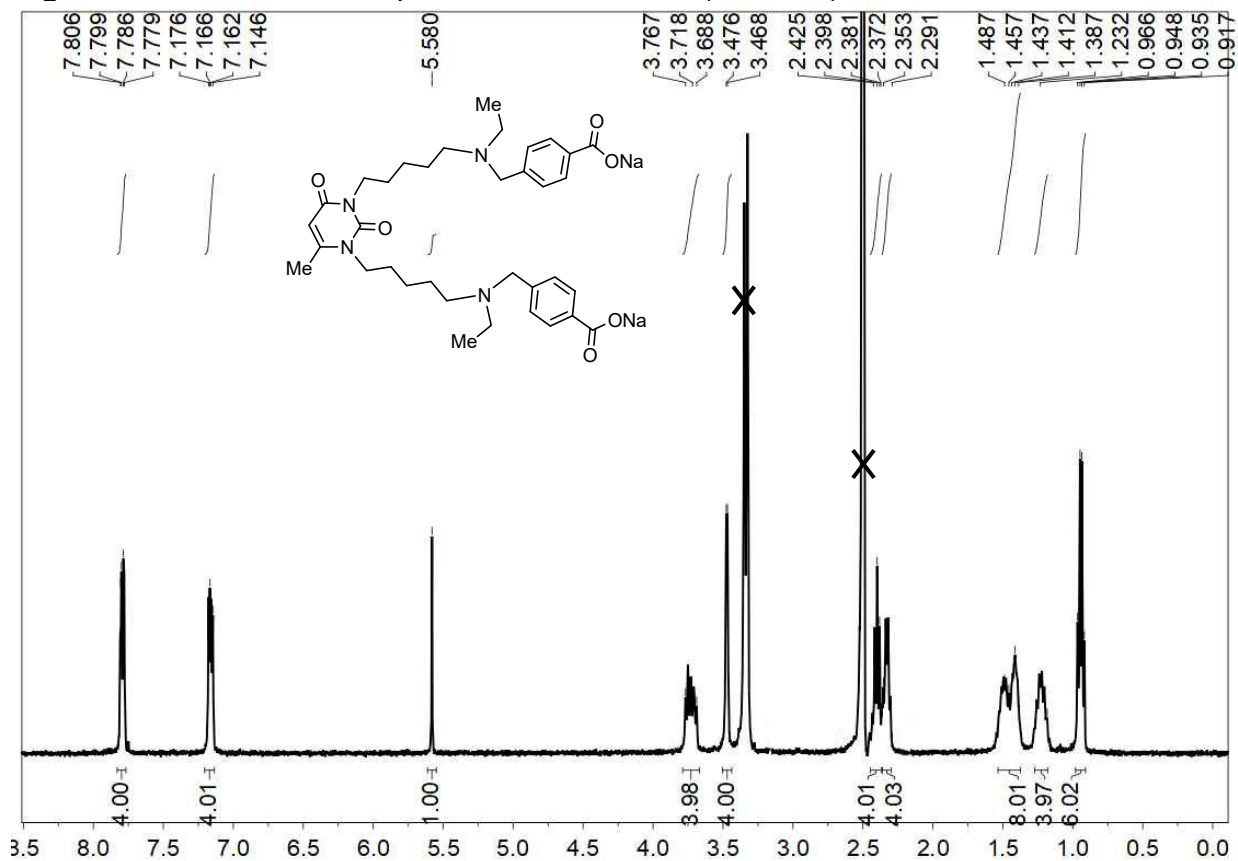


Figure S32. 1D ^1H NMR spectrum of **3d** in $\text{DMSO}-d_6$ (600 MHz) at $T = 303\text{ K}$. x - residual solvent peak.

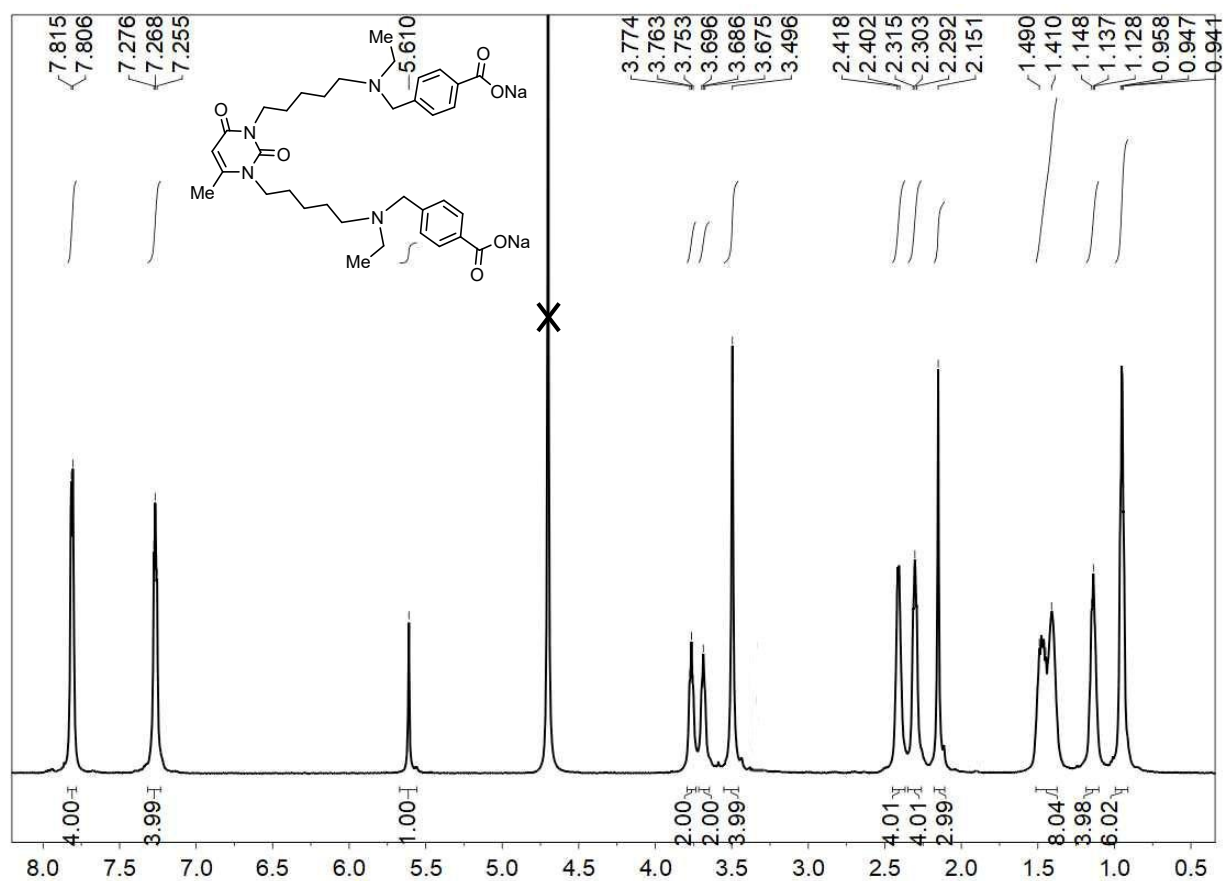


Figure S33. 1D ¹H NMR spectrum of **3d** in D₂O (600 MHz) at T = 303 K. x - residual solvent peak.

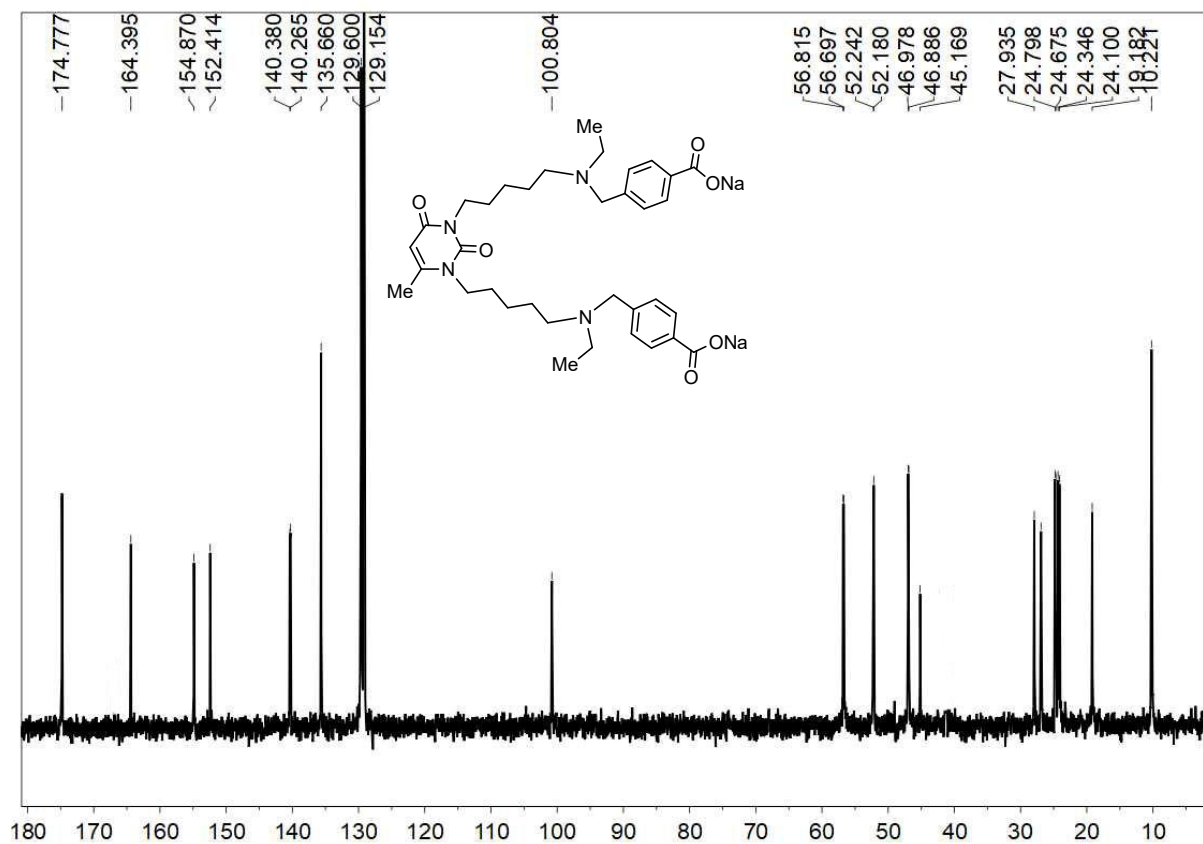


Figure S34. 1D ¹³C NMR spectrum of **3d** in D₂O (150 MHz) at T = 303 K.

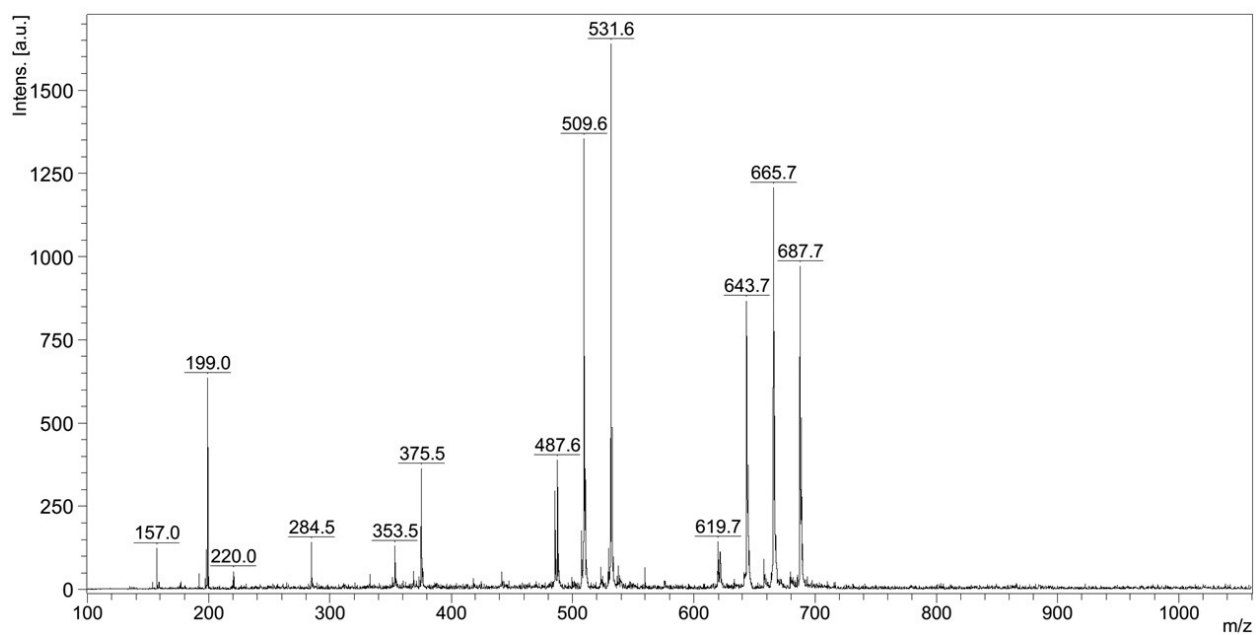


Figure S35. MALDI-TOF mass spectrum of **3d**.

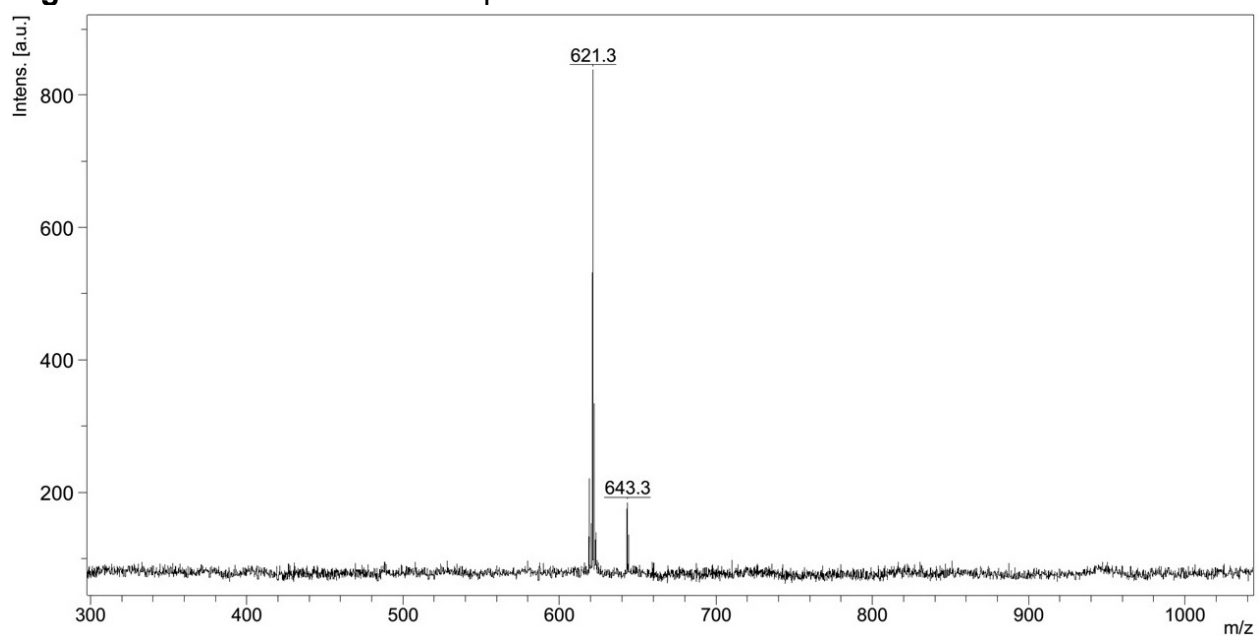


Figure S36. MALDI-TOF mass spectrum of **2f**.

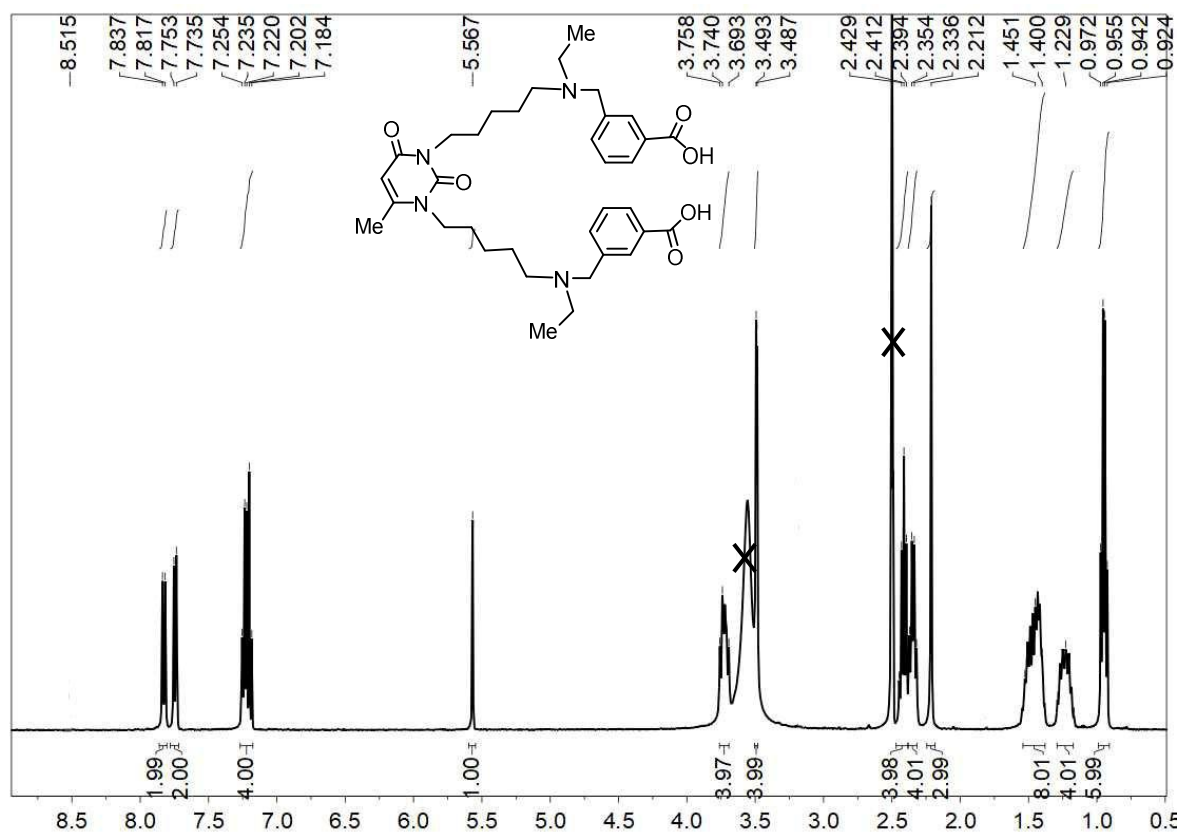


Figure S37. 1D ^1H NMR spectrum of **2f** in DMSO- d_6 (400 MHz) at T = 303 K. x - residual solvent peak.

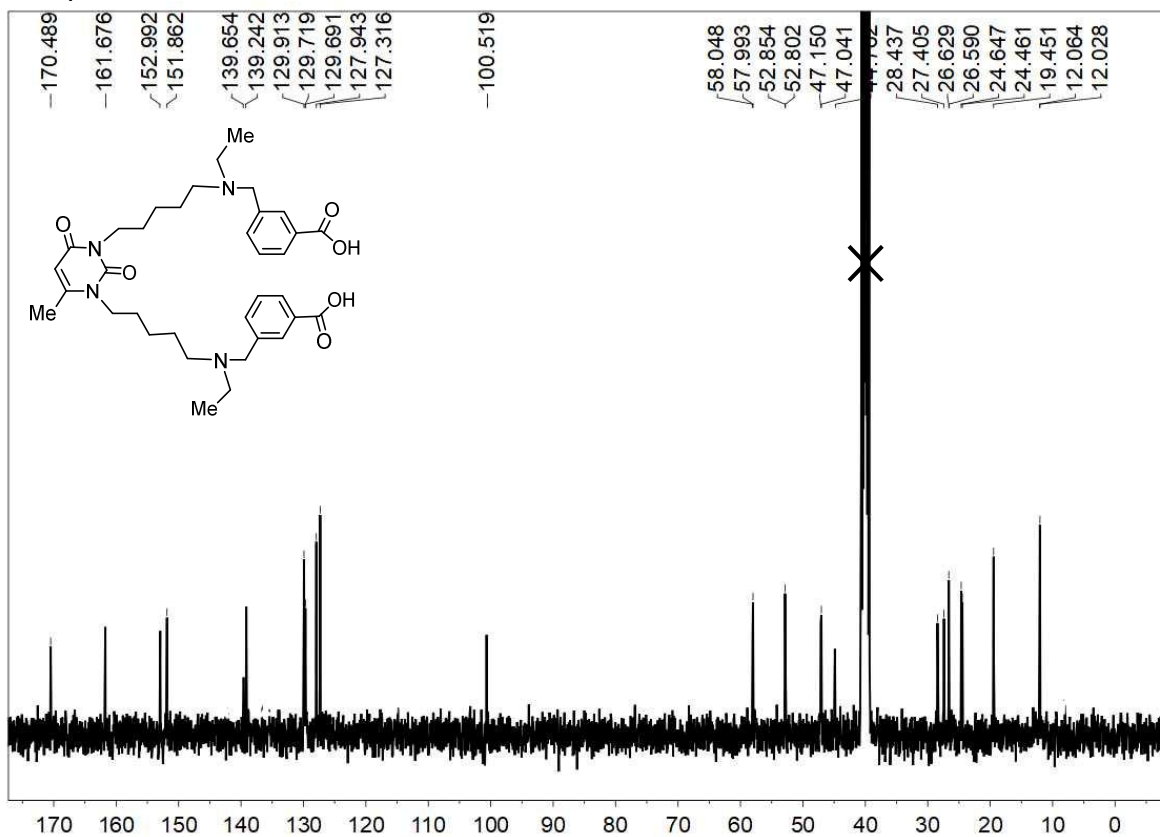


Figure S38. 1D ^{13}C NMR spectrum of **2f** in DMSO- d_6 (100 MHz) at T = 303 K.

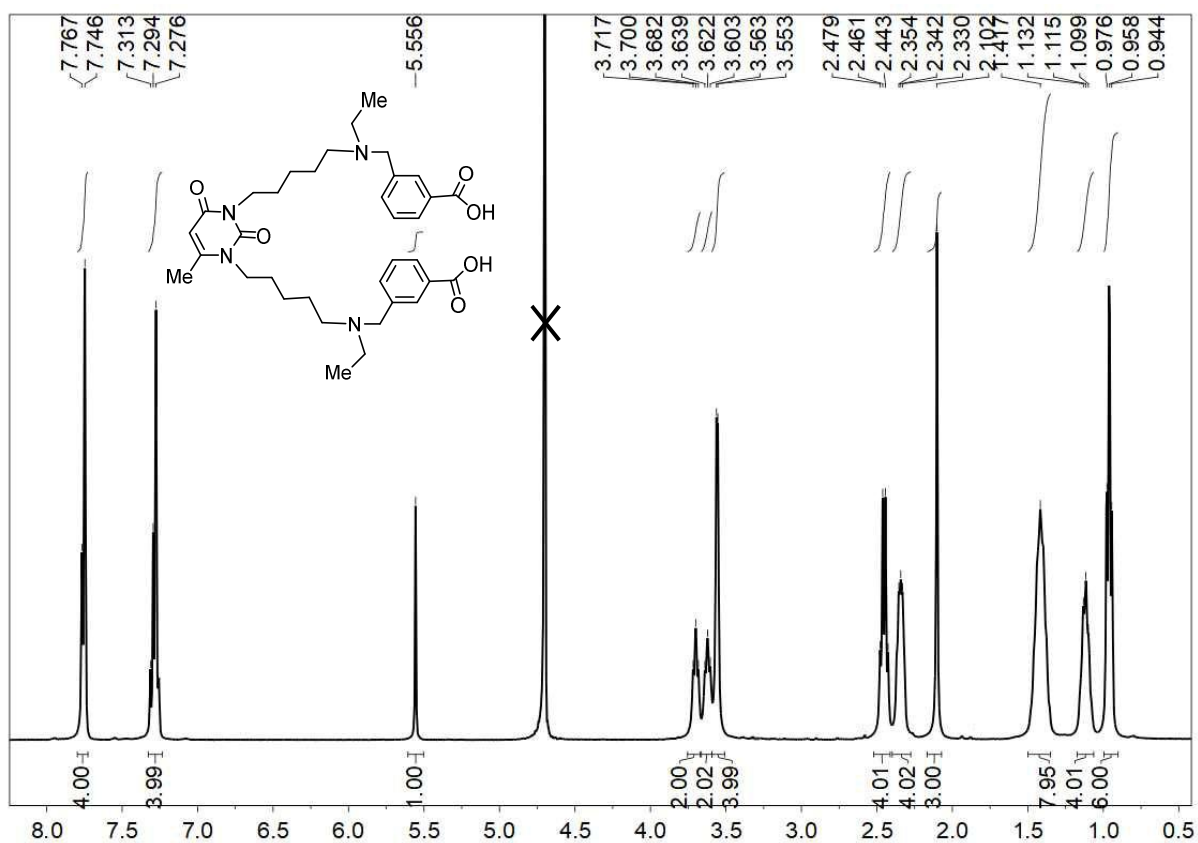


Figure S39. 1D ¹H NMR spectrum of **2f** in D₂O (600 MHz) at T = 303 K. x - residual solvent peak.

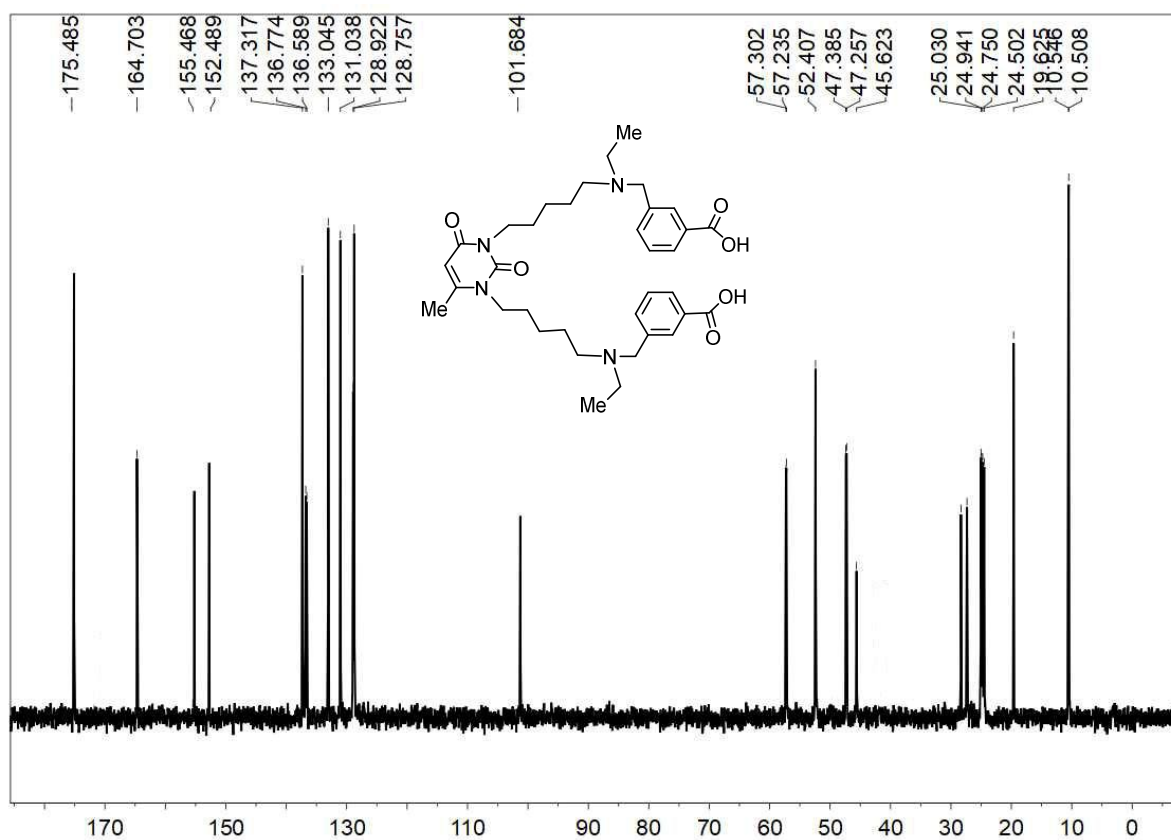


Figure S40. 1D ¹³C NMR spectrum of **2f** in D₂O (150 MHz) at T = 303 K.

NMR study of the stability of compound **2c**

The compound **2c** was dissolved in ethanol and then in 2 ml of 0.1M sodium phosphate buffer (pH=8.0) at the final concentration of 0.1 mM. Then 2.0 units of hAChE was added. The final concentration of ethanol in the buffer was 0.1% vol. The compound **2c** was incubated with hAChE overnight at 25°C. The same volume of buffer containing compound **2c** at the concentration of 0.1 mM but without AChE was used as a control. After the end of incubation, the buffer was filtered out of AChE using a centricon-30 ultrafiltration micro-concentrator from Amicon (Millipore Corporation, Billerica, MA, USA). Samples of the phosphate buffer were evaporated in vacuum, 1 ml of deuterated chloroform was added to the residue, the mixture was filtered and transferred to a 5 mm ampoule to register NMR spectra.

A marker of the presence of an ester, in particular a methoxycarbonyl group is a signal of a methyl radical of the methoxycarbonyl fragment located in the region of 3.90 ppm as a singlet. In the case of complete hydrolysis of the ester group to a carboxylic one, this singlet will be absent in the spectrum of the hydrolysis product, as is the case, for example, in the ¹H NMR spectra of sodium salt **2e** and acid **2f**. These compounds were obtained as a result of five-hour boiling of ester **2c** in an alkali solution. In the case of partial hydrolysis of ester groups, a set of resonance signals of both the ester, *m*-methoxycarbonylbenzylethylamino fragment and the acid, *m*-carboxylbenzylethylamino fragment, would be observed in the spectrum of hydrolysis products.

Figure S41 shows the spectrum of ester **2c** and its description. Also, this spectrum is shown in the above figure S7.

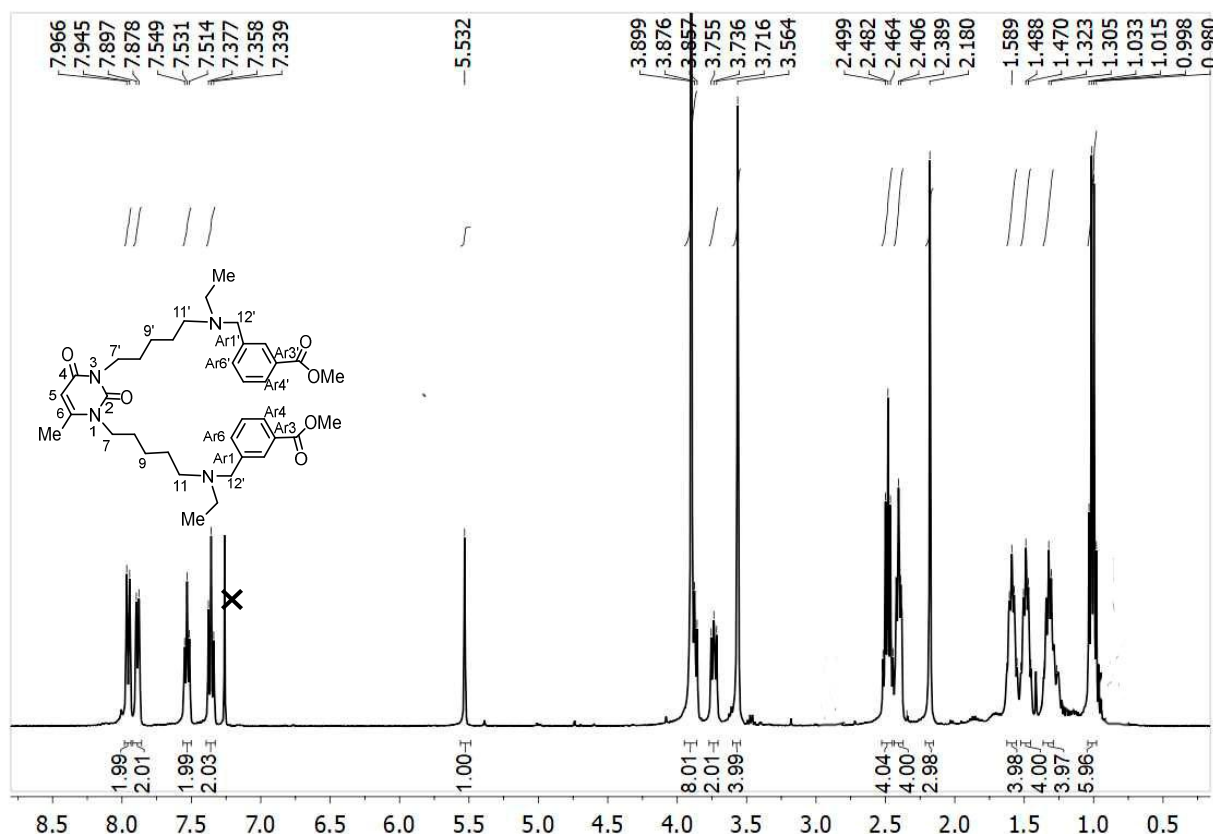


Figure S41. 1D ¹H NMR spectrum of **2c** in CDCl₃ (600 MHz) at T = 303 K. x - residual solvent peak.

1,3-Bis[5-(*m*-methoxycarbonylbenzylethylamino)pentyl]-6-methyluracil (2c**):** ¹H NMR (CDCl₃, 400 MHz) δ 7.97, 7.95 (both s, 1H each, 2H, Ar2H, Ar2'H), 7.90-7.88 (both m, 2H each, 4H, Ar5H, Ar5'H, Ar4H, Ar4'H), 7.38-7.34 (m, 2H, Ar6H, Ar6'H), 5.53 (s, 1H, C5H), 3.90 (br. s, 6H,

2COOCH₃), 3.88-(3.86 m, 2H, N3C7'H₂), 3.76-3.72 (m, 2H, N1C7'H₂), 3.56 (br. s, 4H, C12CH₂, C12'CH₂), 2.52-2.46, 2.45-2.39 (both m, 4H each, 8H, C11CH₂, C11'CH₂, 2NCH₂CH₃), 2.18 (s, 3H, C6CH₃), 1.63-1.55, 1.52-1.45, 1.36-1.27 (all m, 4H each, 12H, C8CH₂, C9CH₂, C10CH₂, C8'CH₂, C9'CH₂, C10'CH₂), 1.03-0.98 (m, 6H, 2NCH₂CH₃).

With the complete hydrolysis of ester **2c**, acid **2f** should be formed. Figure 42 shows ¹H NMR spectrum of acid **2f**, the same spectrum is shown above in Figure S22.

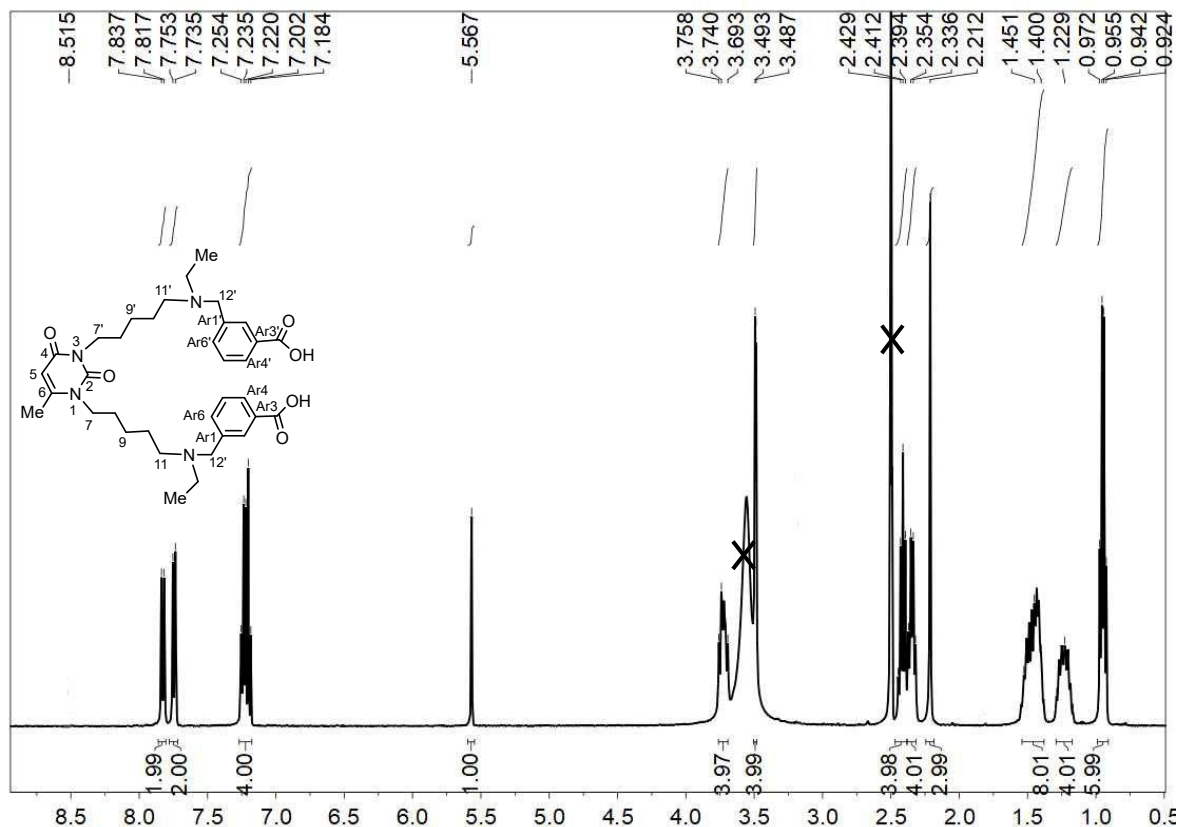


Figure S42. 1D ¹H NMR spectrum of **2f** in DMSO-d₆ (400 MHz) at T = 303 K. x - residual solvent peak.

1,3-Bis[5-(m-carboxybenzylethylamino)pentyl]-6-methyluracil (2f**):** ¹H NMR (DMSO-d₆, 400 MHz) δ 7.84, 7.82 (both s, 1H each, 2H, Ar2H, Ar2'H), 7.76-7.73 (m, 2H, Ar5H, Ar5'H), 7.25-7.18 (m, 4H, Ar4H, Ar4'H, Ar6H, Ar6'H), 5.57 (s, 1H, C5H), 3.76-3.69 (m, 4H, N3C7'H₂, N1C7'H₂), 3.49, 3.48 (both s, 2H each, 4H, C12CH₂, C12'CH₂), 2.45-2.39, 2.37-2.31 (both m, 4H each, 8H, C11CH₂, C11'CH₂, 2NCH₂CH₃), 2.21 (s, 3H, C6CH₃), 1.53-1.40 (m, 8H, C8CH₂, C10CH₂, C8'CH₂, C10'CH₂), 1.29-1.17 (m, 4H, C9CH₂, C9'CH₂), 0.97-0.92 (m, 6H, 2NCH₂CH₃). The figure S43 shows ¹H NMR spectrum of control, residue from sodium phosphate buffer without AChE in CDCl₃. The resonance signals on this spectrum correspond to the signals in the spectrum of compound **2c** (Figure S41). The treatment of compound **2c** with AChE did not cause any changes in the ¹H NMR spectrum of the residue (Figure S44). It is also almost identical to the spectrum of pure compound **2c**. Both in Figure S43 and Figure S44 there are no signals of acid forms that are obtained as a result of hydrolysis of ester groups (Figure S42).

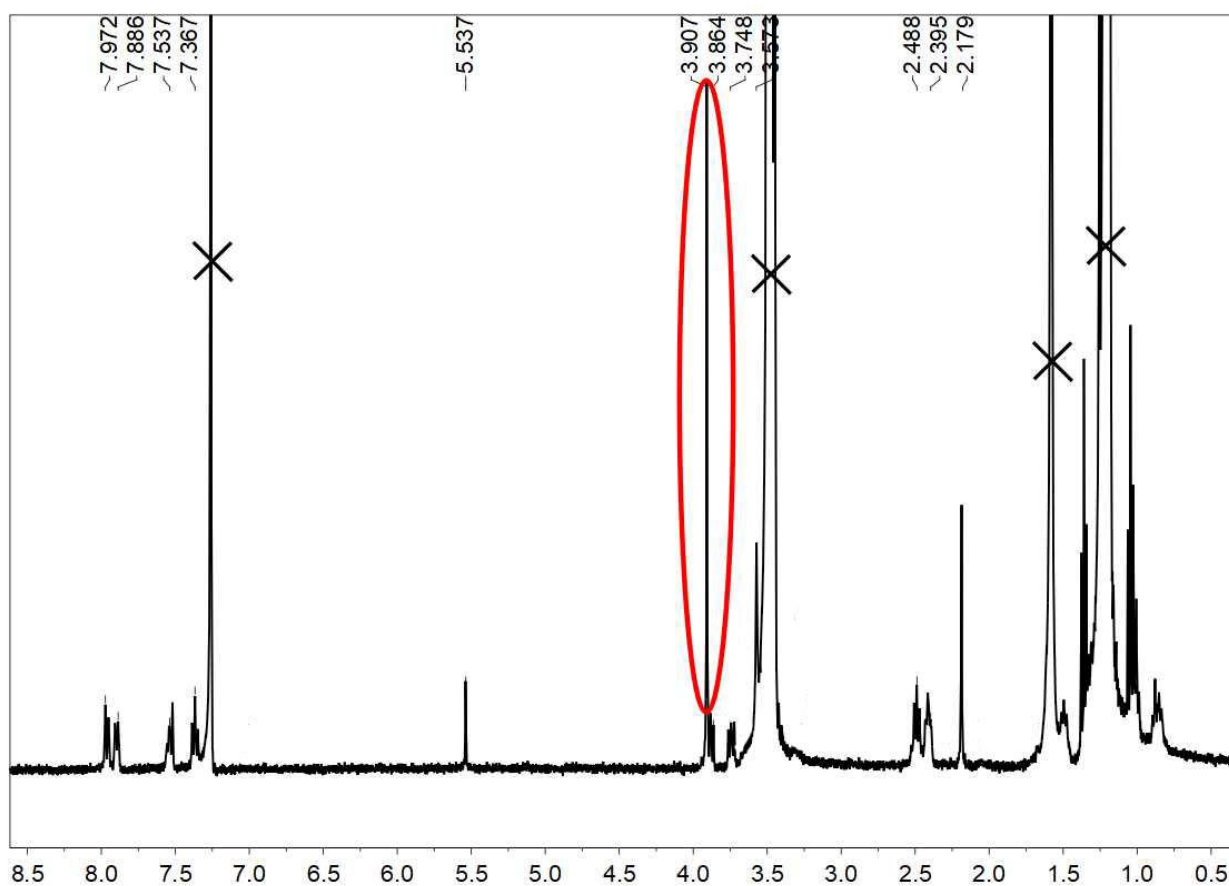


Figure S43. 1D ^1H NMR spectrum of residue from sodium phosphate buffer in CDCl_3 (600 MHz) at $T = 303$ K. x - residual solvent peaks. The signal of methyl radicals in ester groups is highlighted with a red ellipse.

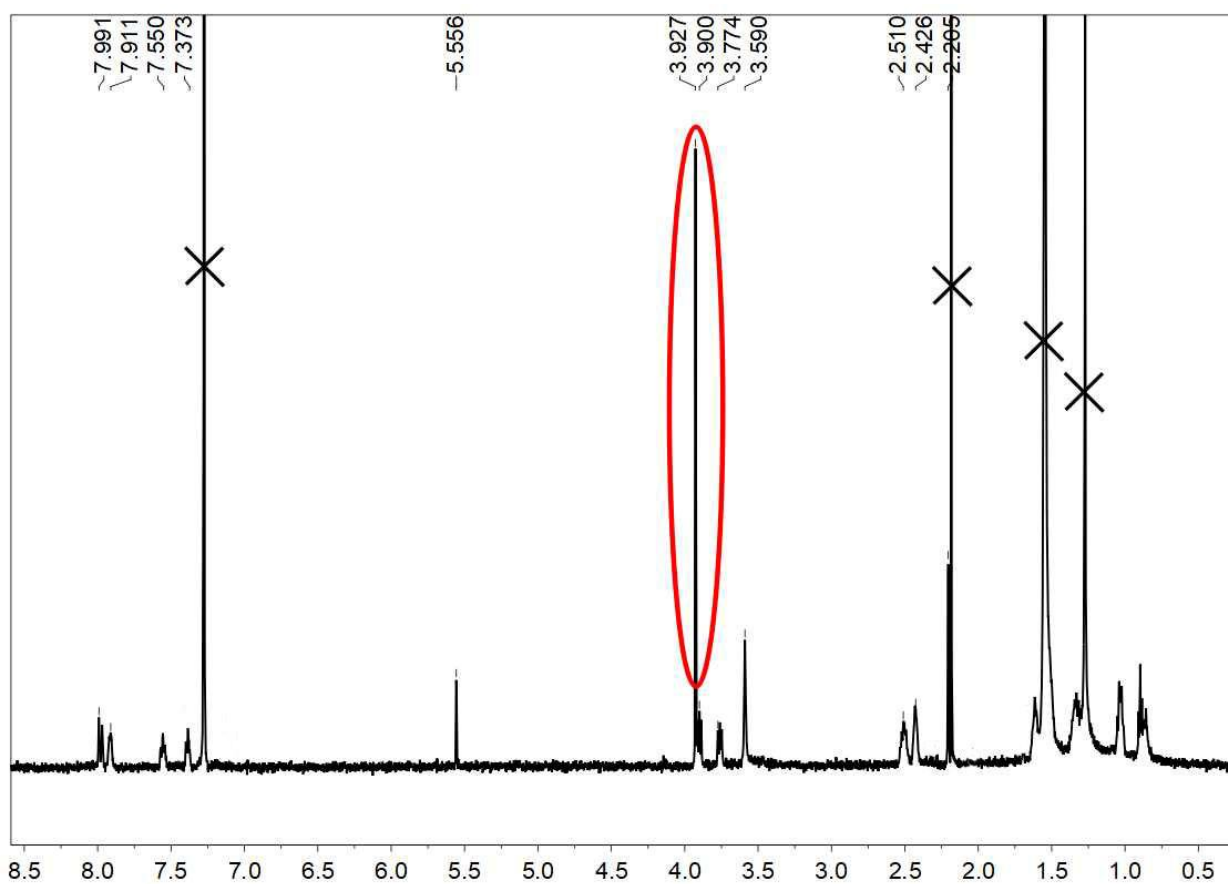


Figure S44. 1D ^1H NMR spectrum of residue from sodium phosphate buffer after AChE treatment in CDCl_3 (600 MHz) at $T = 303\text{ K}$. x - residual solvent peaks. The signal of methyl radicals in ester groups is highlighted with a red ellipse.