C-Terminal-Modified Oligourea Foldamers as a Result of Terminal Methyl Ester Reactions under Alkaline Conditions

Supporting Information

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Table of contents

1. **Building blocks and final compounds** **2**
2. **NMR spectra of building blocks BB1-BB4** **3**
3. **NMR spectra of oligourea-esters of type 5** **13**
4. **Nmr spectra of oligourea-acids 1.** **18**
5. **NMR spectra of oligourea-hydantoin/dihydrouracil 2.** **21**
6. **Signal assignments of oligourea-acids 1 and oligourea-hydantoin/dihydrouracil 2 based on 2D NMR spectra.** **24**
7. **Additional 1D and 2D NMR spectra** **29**
8. **RP-HPLC chromatograms of reaction mixtures of oligourea-esters 5 under alkaline conditions** **30**
9. **HRMS of building blocks, oligourea esters and final compounds** **33**
10. **CD spectra of selected oligourea-esters 5, oligourea-acids 1 and oligourea-hydantoin/dihydrouracil 2** **44**
11. **Additional HPLC chromatograms** **45**
12. **Crystallography** **45**
13. **References** **49**

1. Building blocks and final compounds

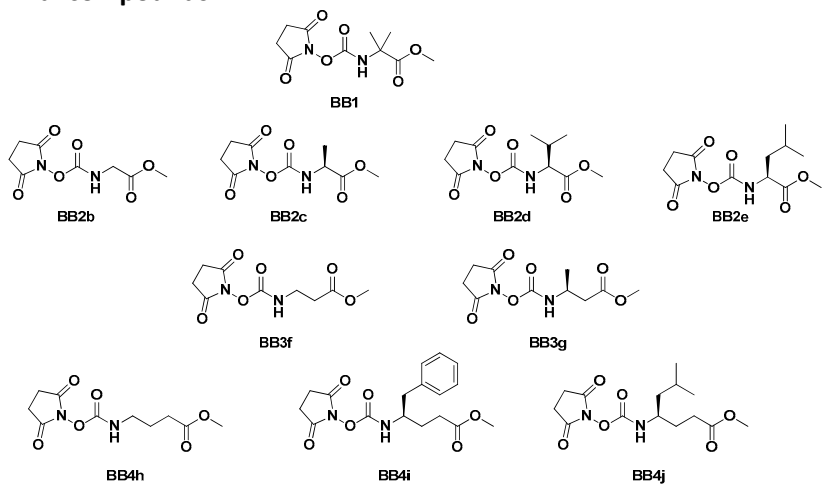


Figure S 1. Succinimidyl carbamate derivatives of methyl esters of α-, β-, γ-amino acids

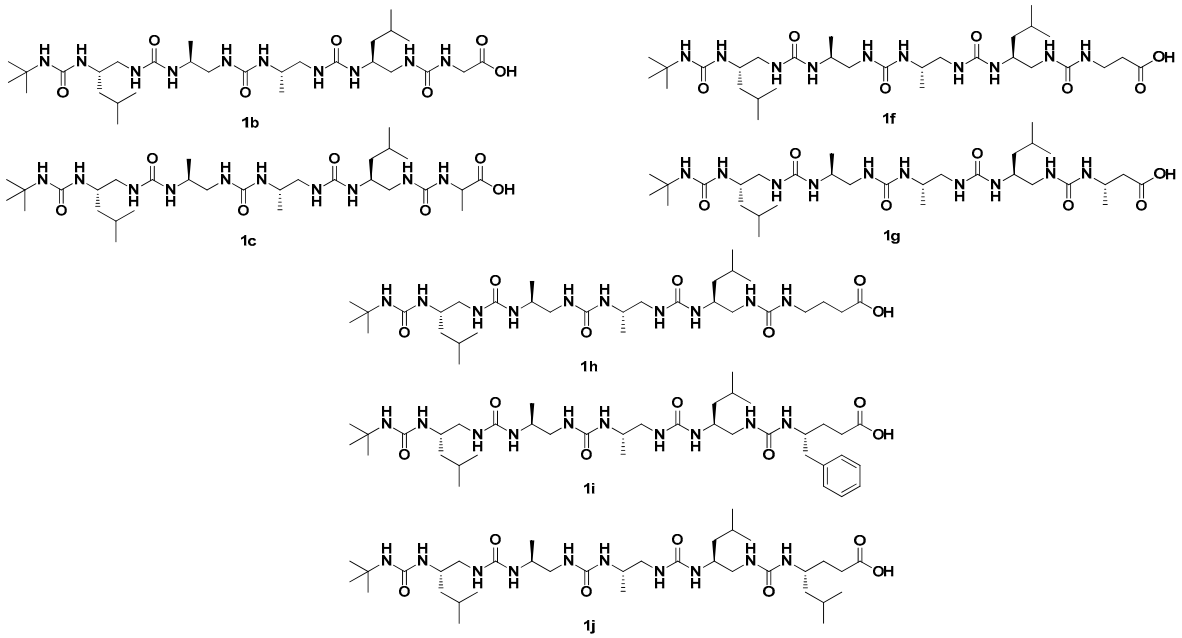


Figure S 2. Structures of oligourea-acids **1**

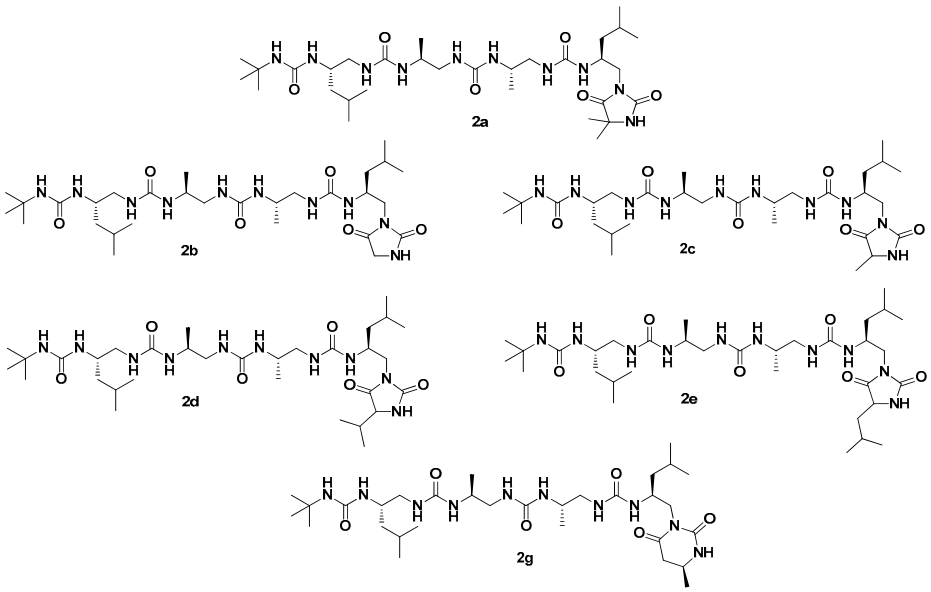


Figure S 3. Structures of oligourea-hydantoins and oligourea-dihydrouracil **2**

2. NMR spectra of building blocks BB1-BB4

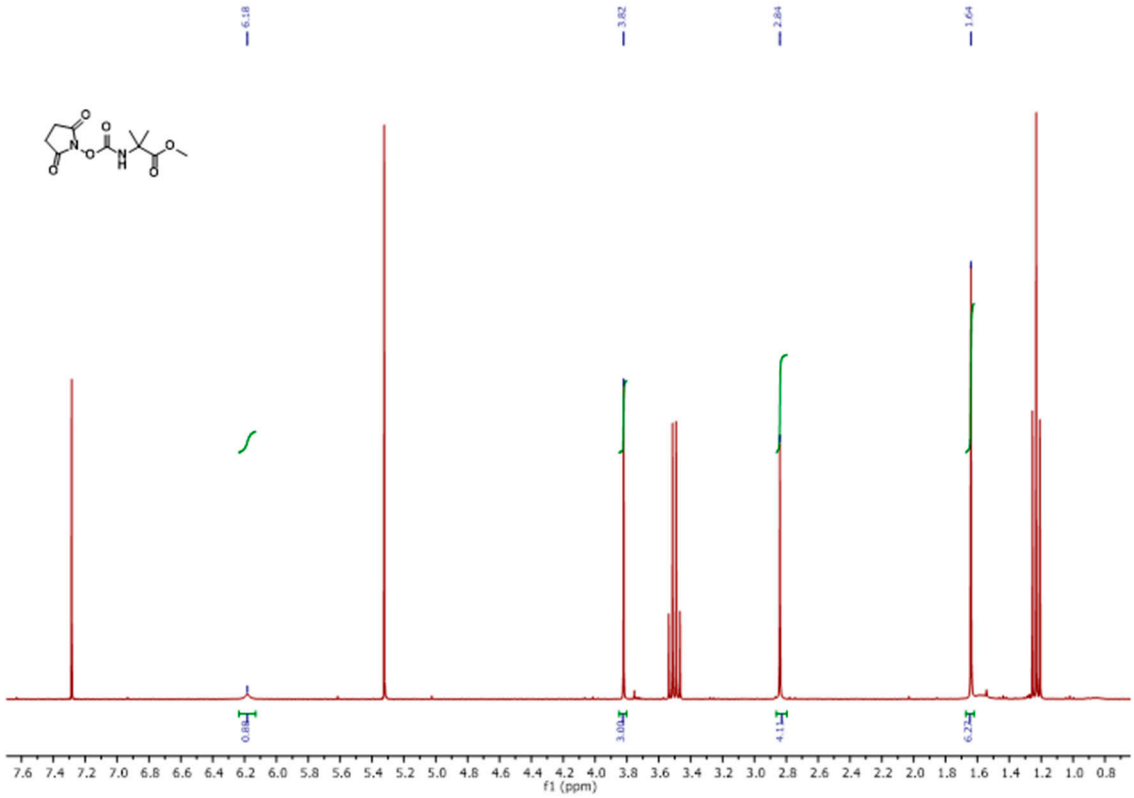


Figure S 4. ^{1}H NMR spectrum of **BB1** in $CDCl_3$ (300 MHz)

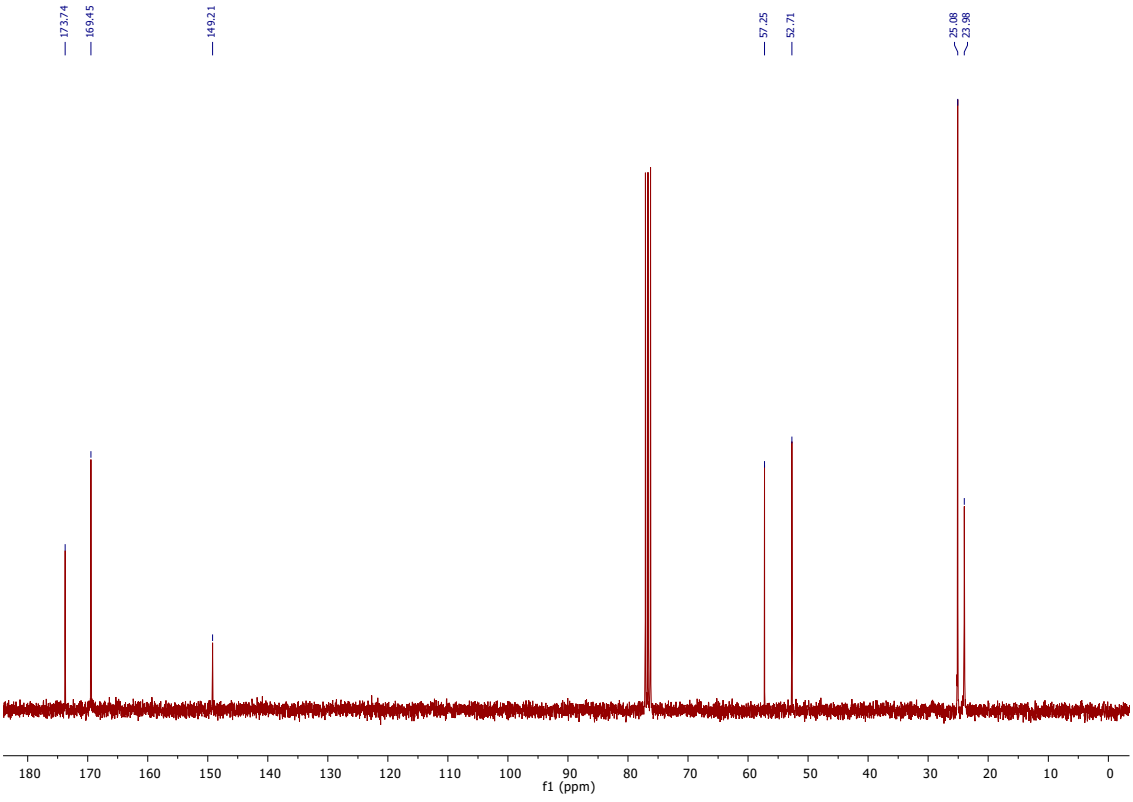


Figure S 5. ^{13}C NMR spectrum of **BB1** in $CDCl_3$ (300 MHz)

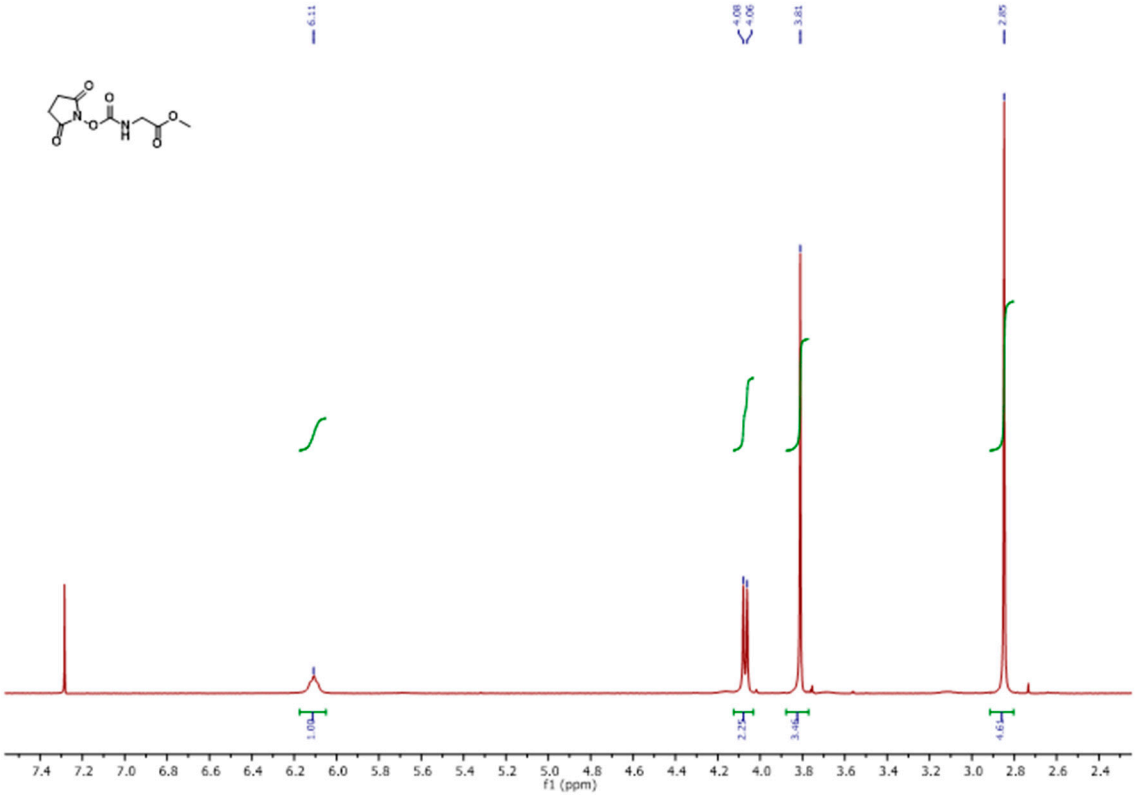


Figure S 6. ^{1}H NMR spectrum of **BB2b** in $CDCl_3$ (300 MHz)

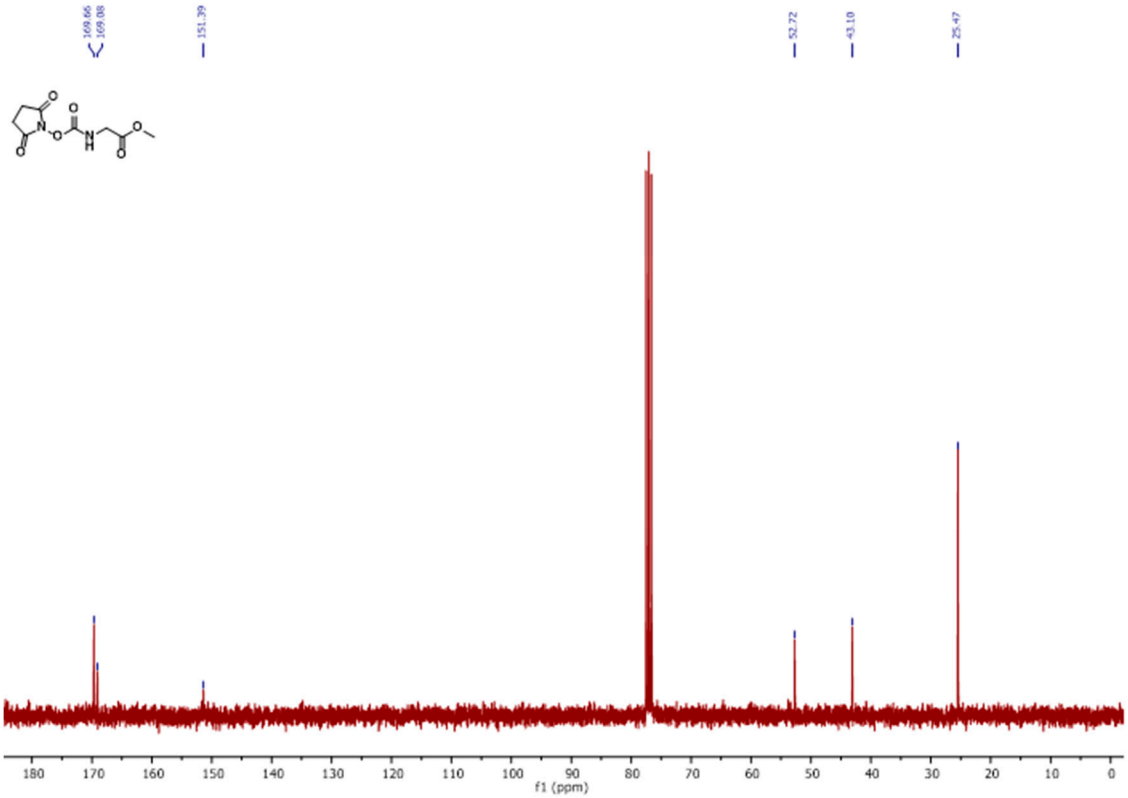


Figure S 7. ^{13}C NMR spectrum of **BB2b** in $CDCl_3$ (300 MHz)

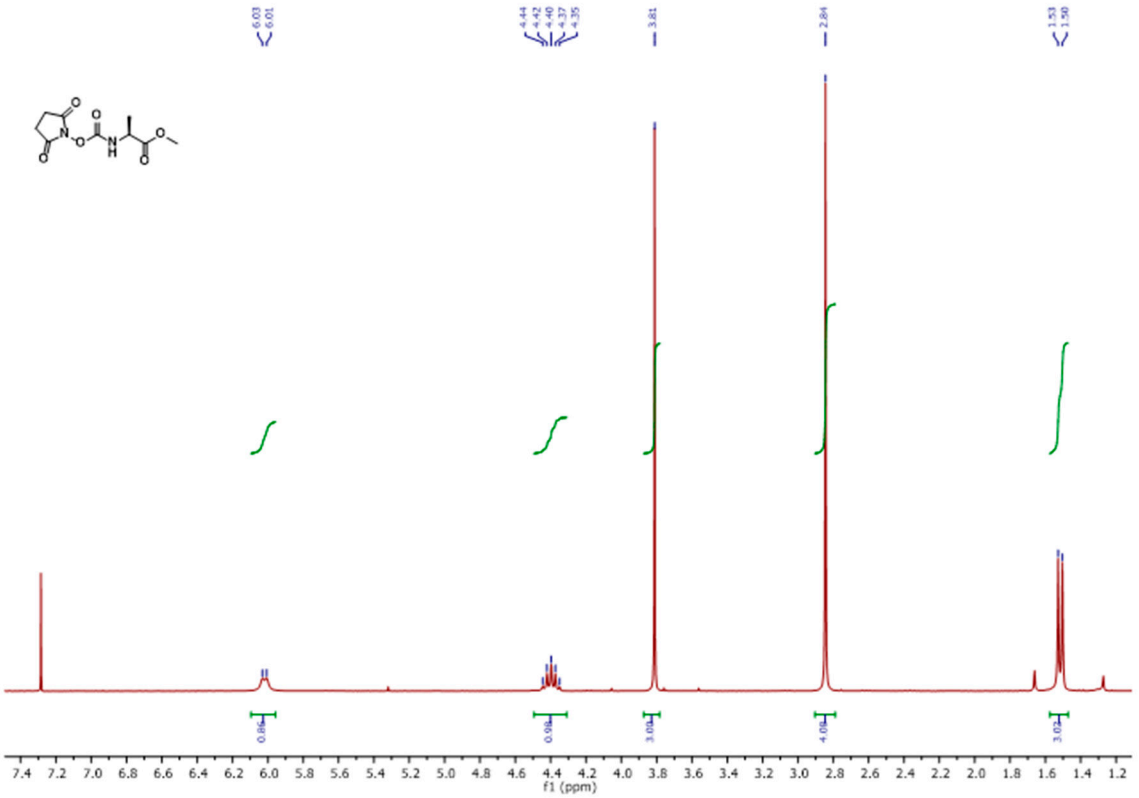


Figure S 8. ^{1}H NMR spectrum of **BB2c** in $CDCl_3$ (300 MHz)

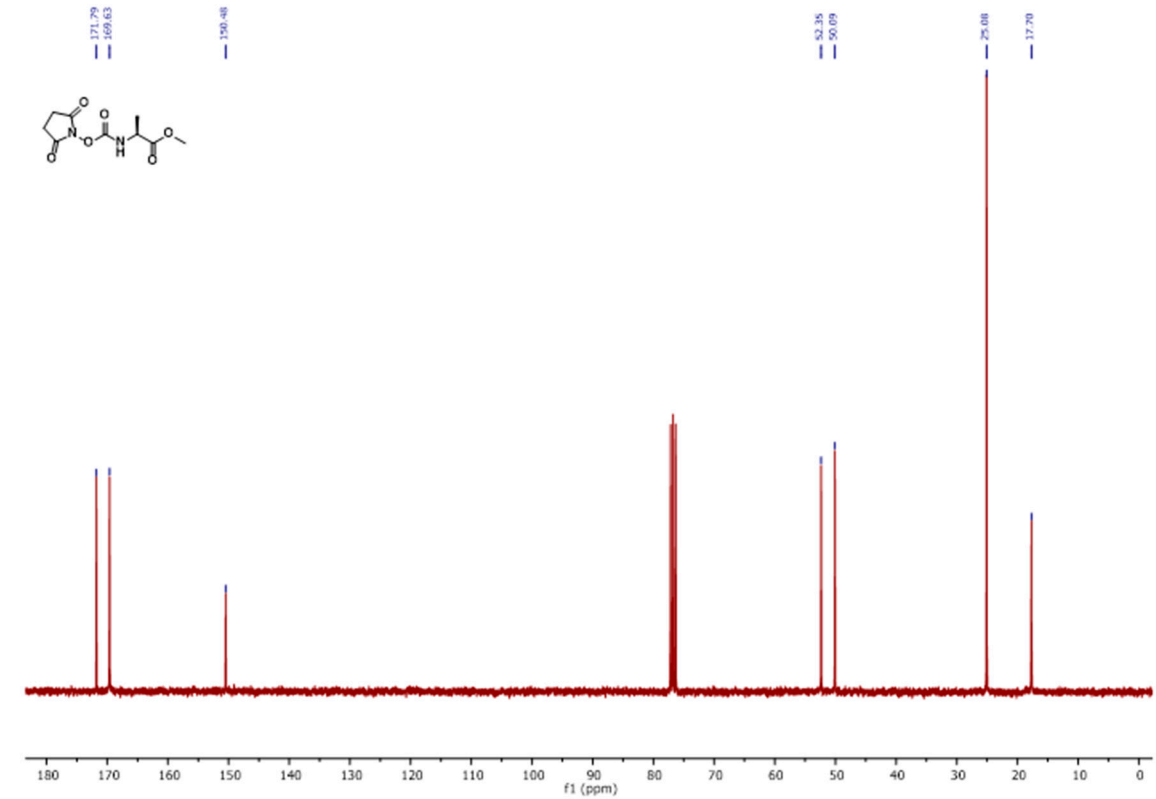


Figure S 9. ^{13}C NMR spectrum of **BB2c** in $CDCl_3$ (300 MHz)

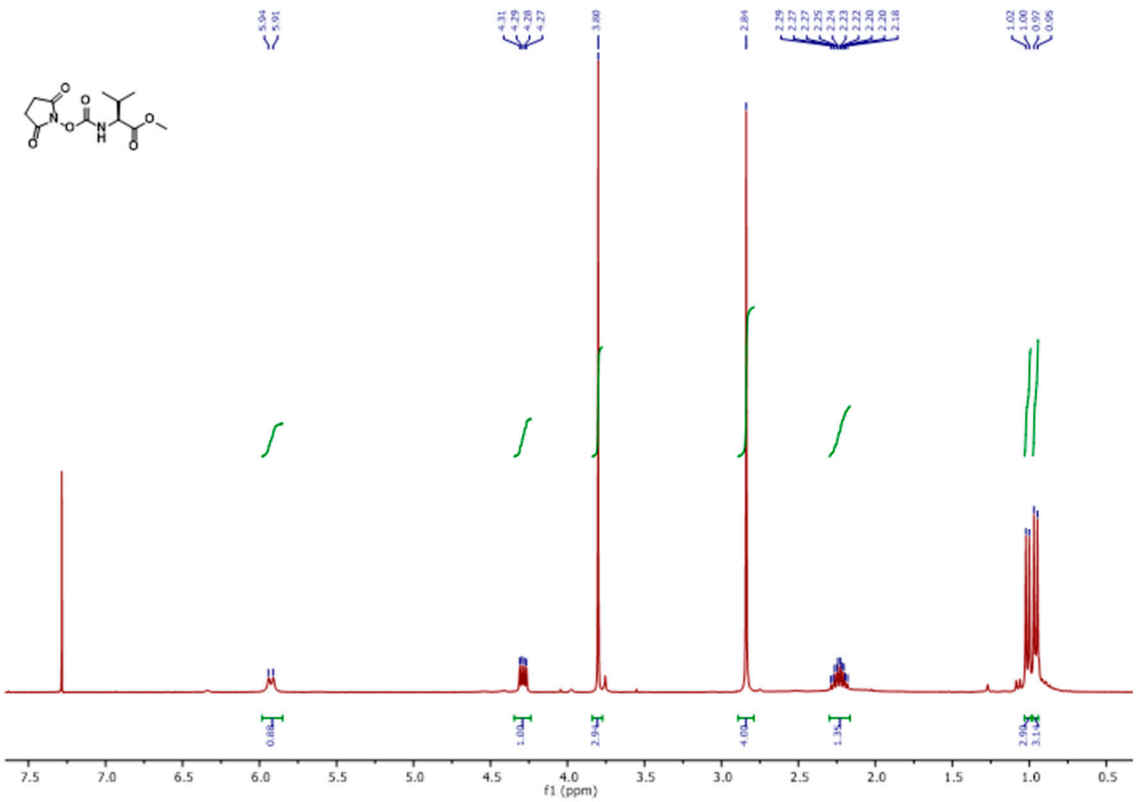


Figure S 10. ^{1}H NMR spectrum of **BB2d** in $CDCl_3$ (300 MHz)

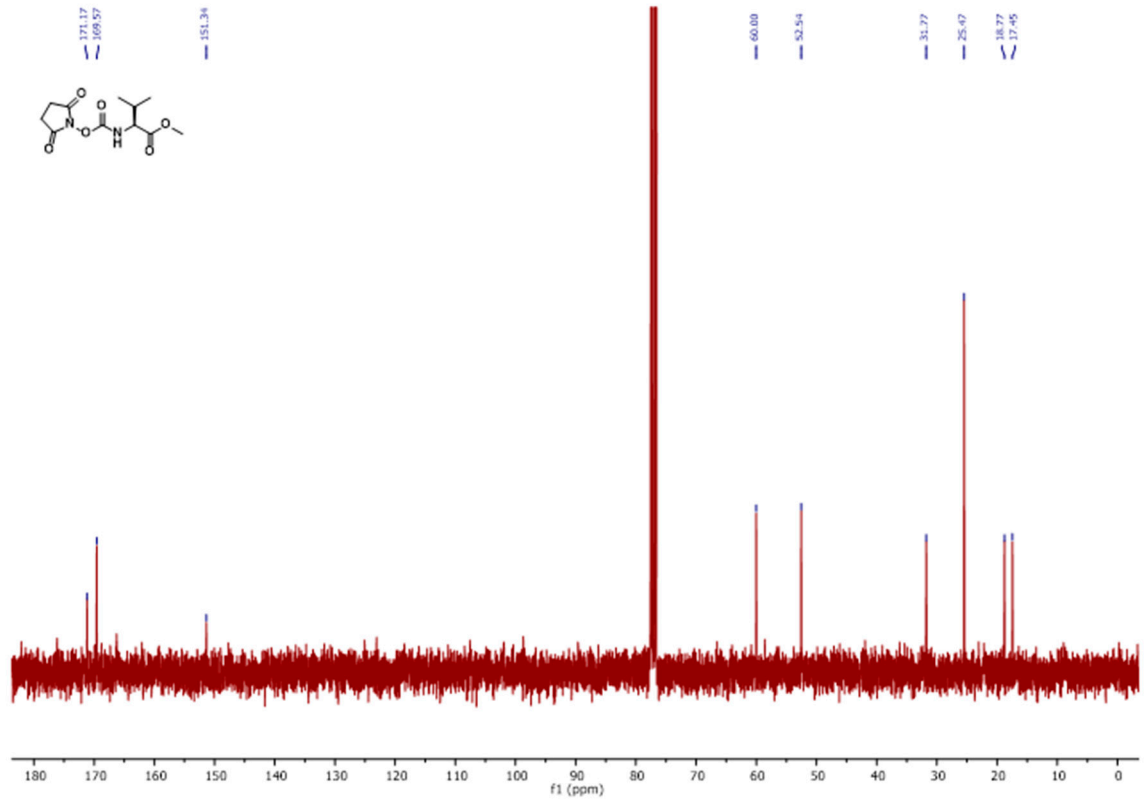


Figure S 11. ^{13}C NMR spectrum of **BB2d** in $CDCl_3$ (300 MHz)

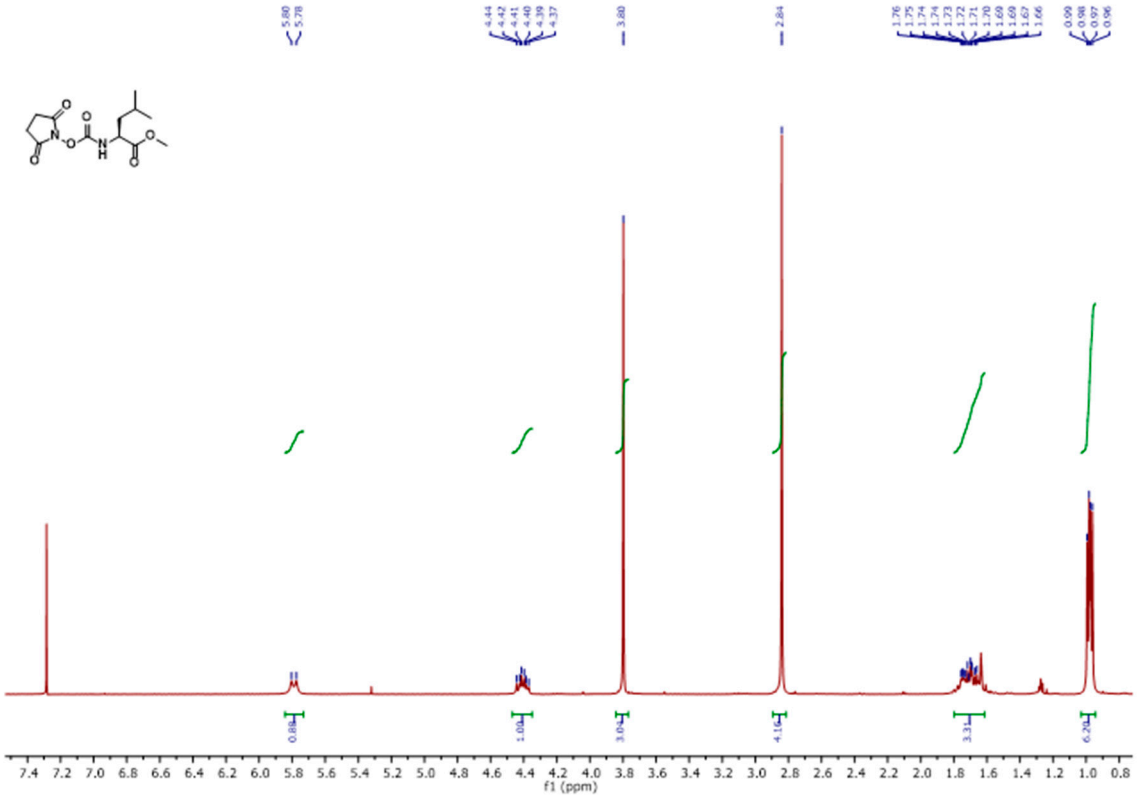


Figure S 12. ^{1}H NMR spectrum of **BB2e** in $CDCl_3$ (300 MHz)

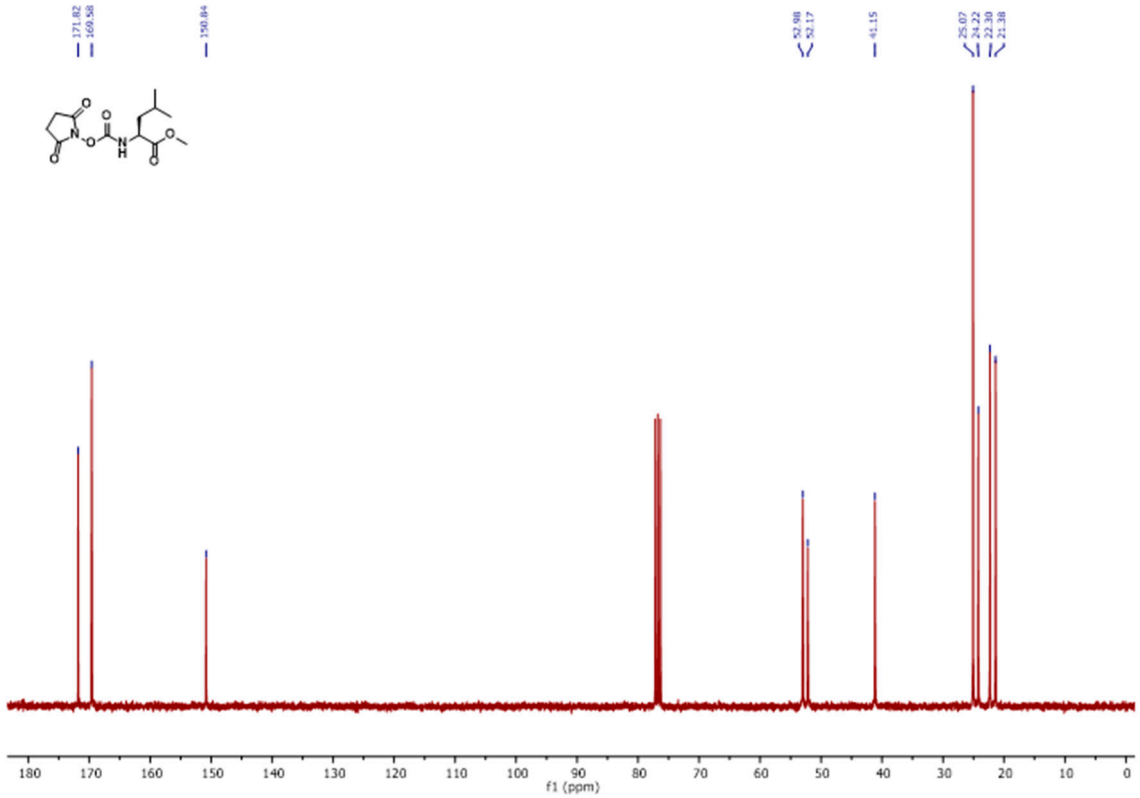


Figure S 13. ^{13}C NMR spectrum of **BB2e** in $CDCl_3$ (300 MHz)

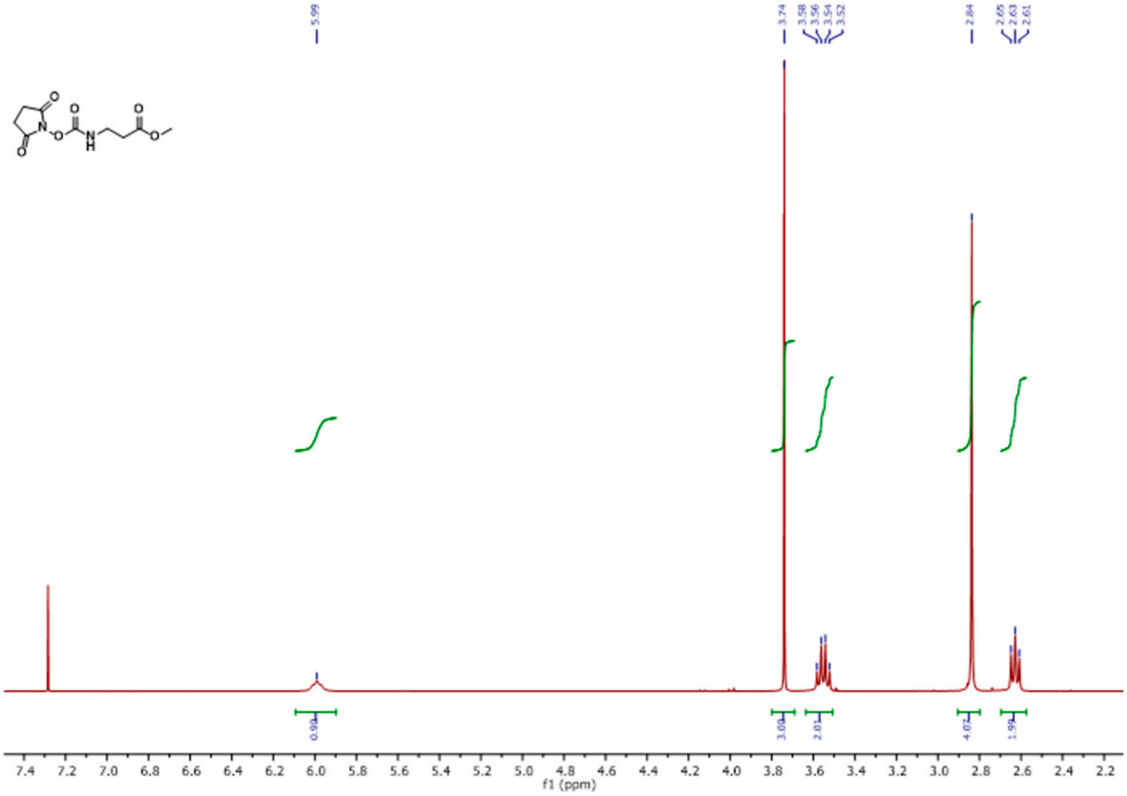


Figure S 14. ^{1}H NMR spectrum of **BB3f** in $CDCl_3$ (300 MHz)

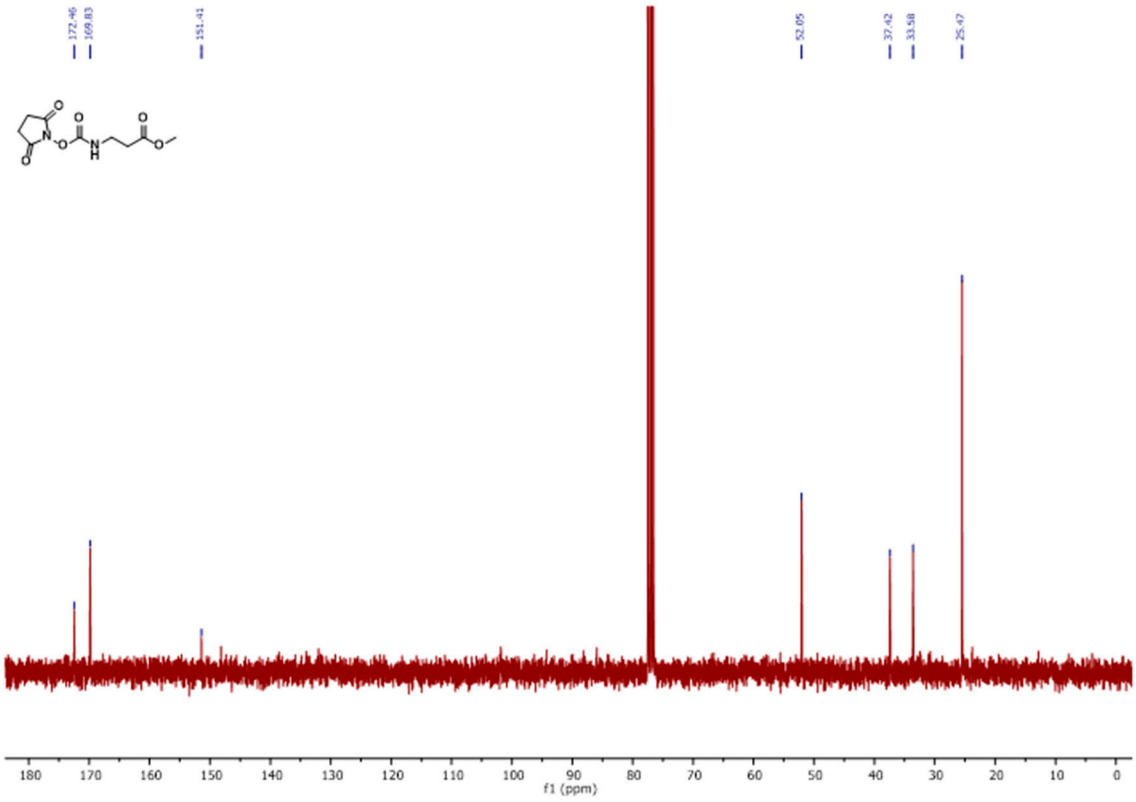


Figure S 15. ^{13}C NMR spectrum of **BB3f** in $CDCl_3$ (300 MHz)

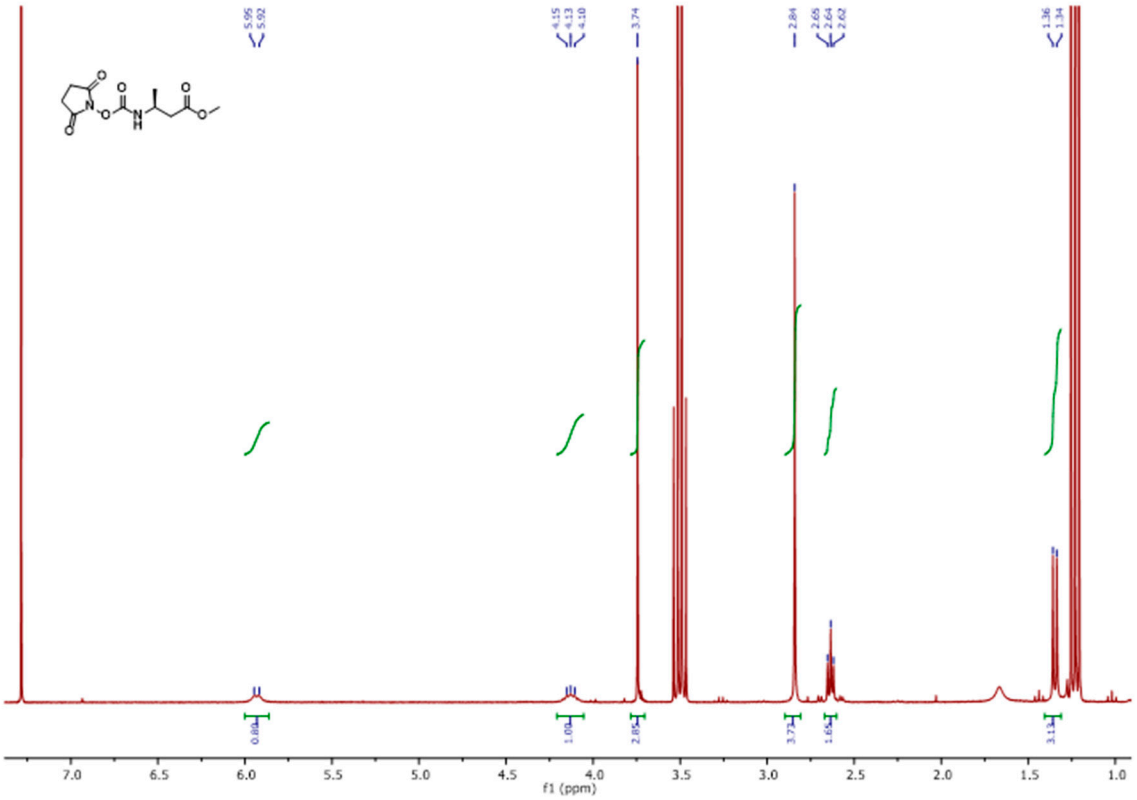


Figure S 16. ^{1}H NMR spectrum of **BB3g** in $CDCl_3$ (300 MHz)

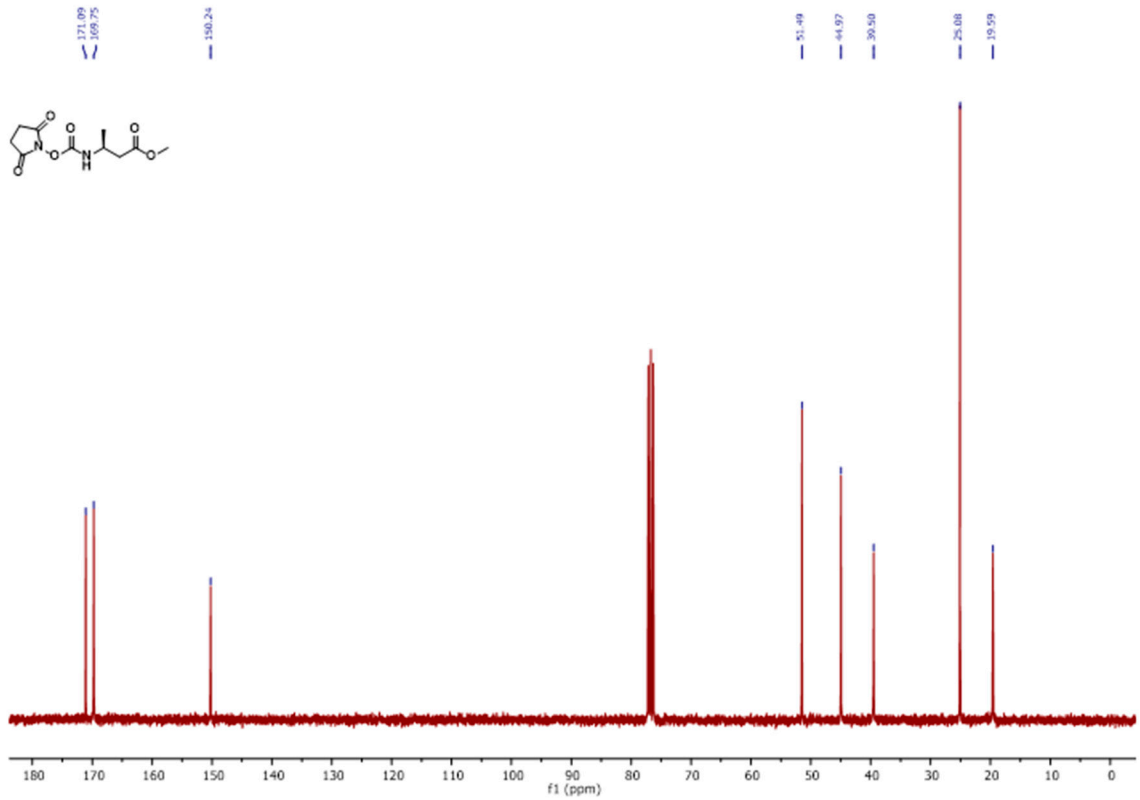


Figure S 17. ^{13}C NMR spectrum of **BB3g** in $CDCl_3$ (300 MHz)

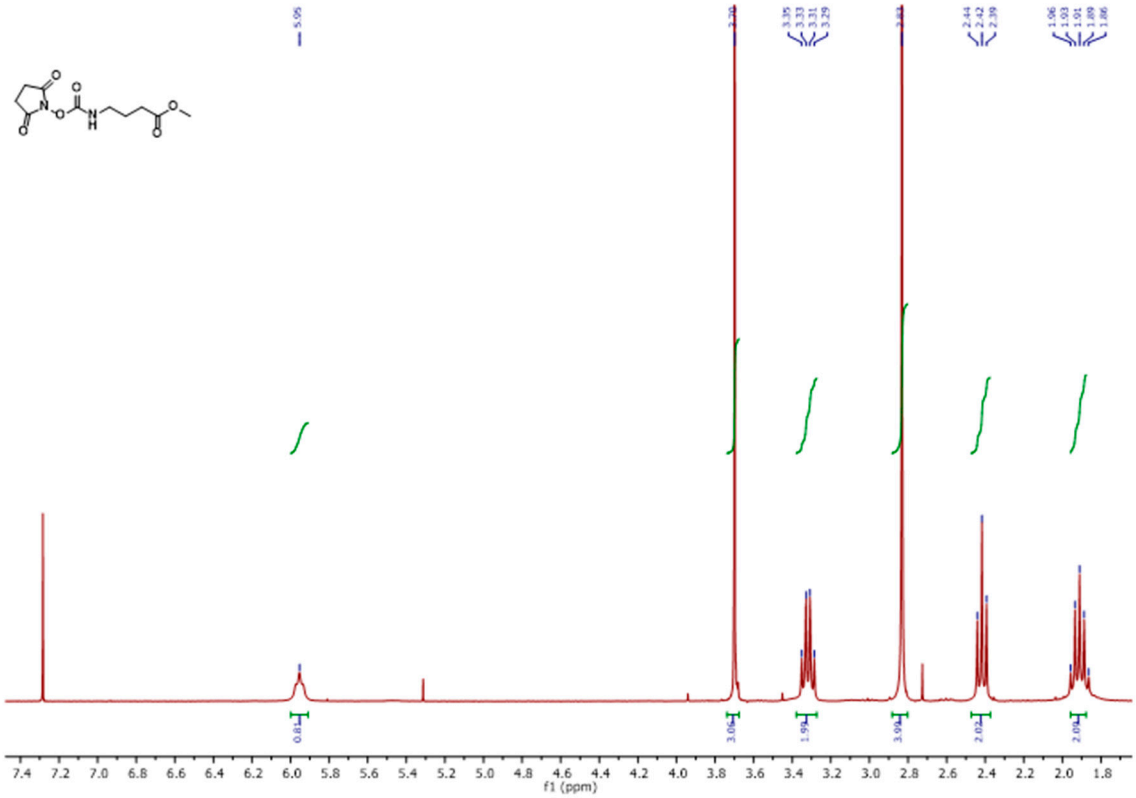


Figure S 18. ^{1}H NMR spectrum of **BB4h** in $CDCl_3$ (300 MHz)

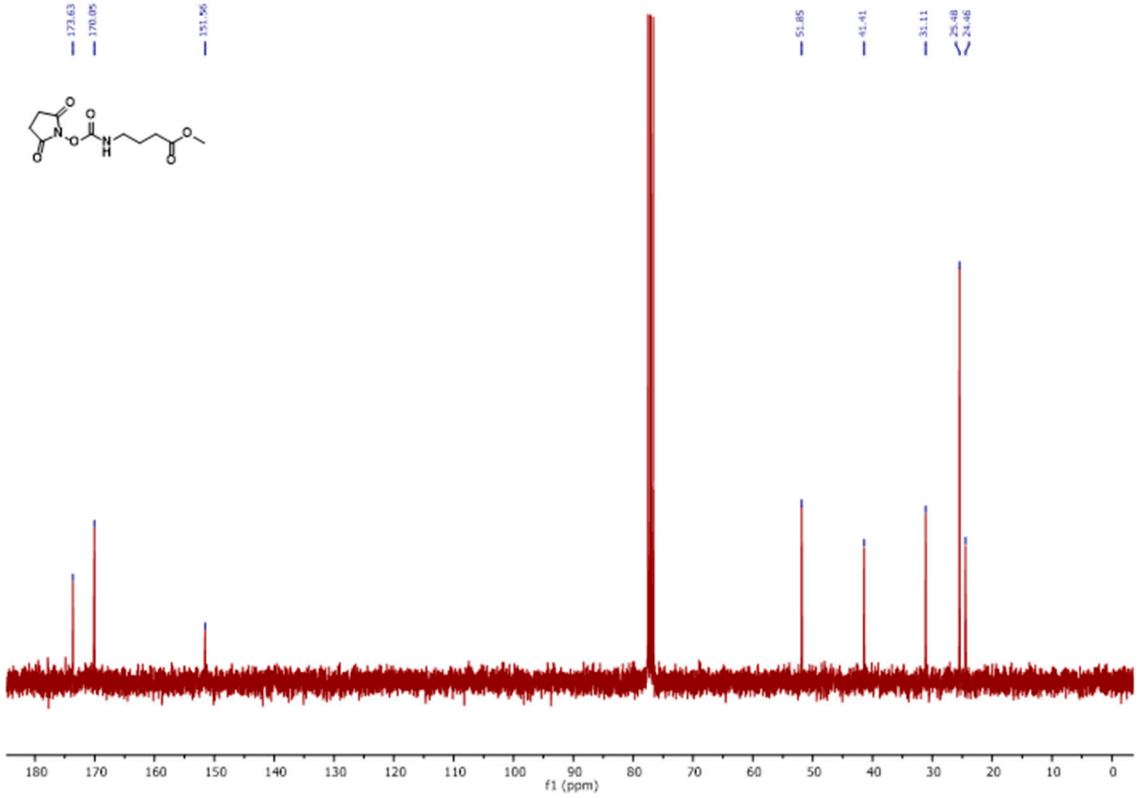


Figure S 19. ^{13}C NMR spectrum of **BB4h** in $CDCl_3$ (300 MHz)

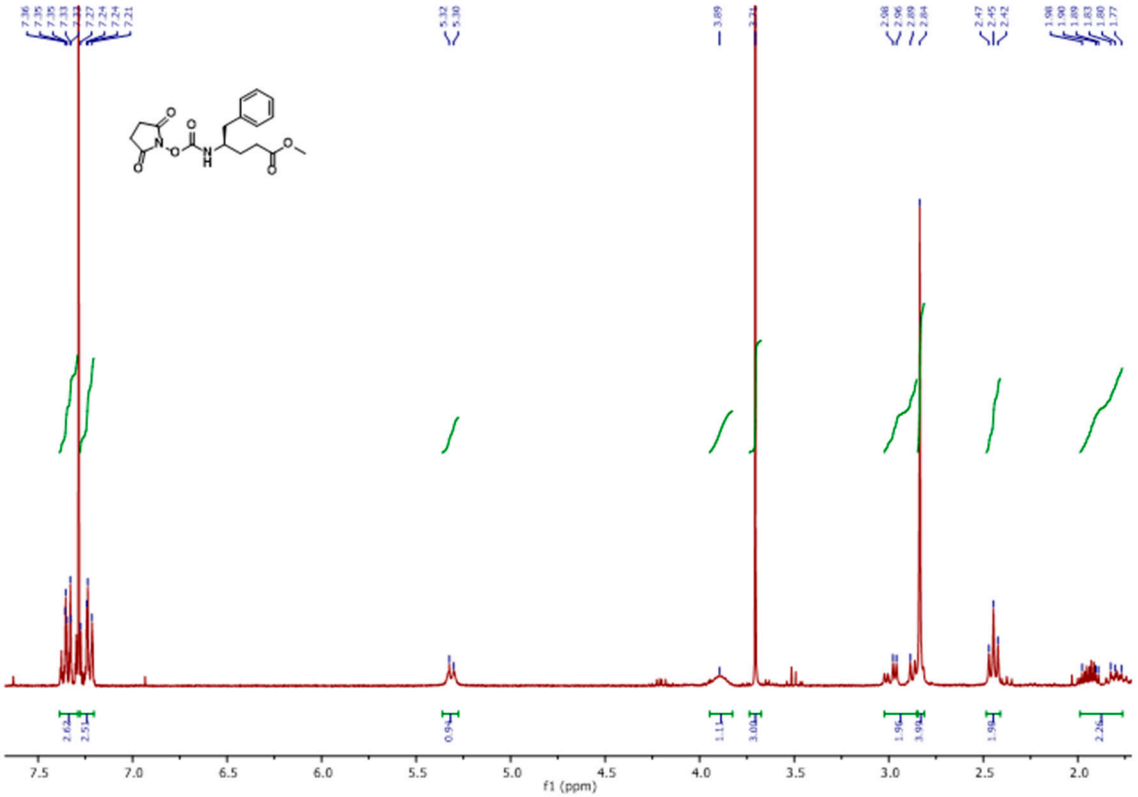


Figure S 20. ^{1}H NMR spectrum of **BB4i** in $CDCl_3$ (300 MHz)

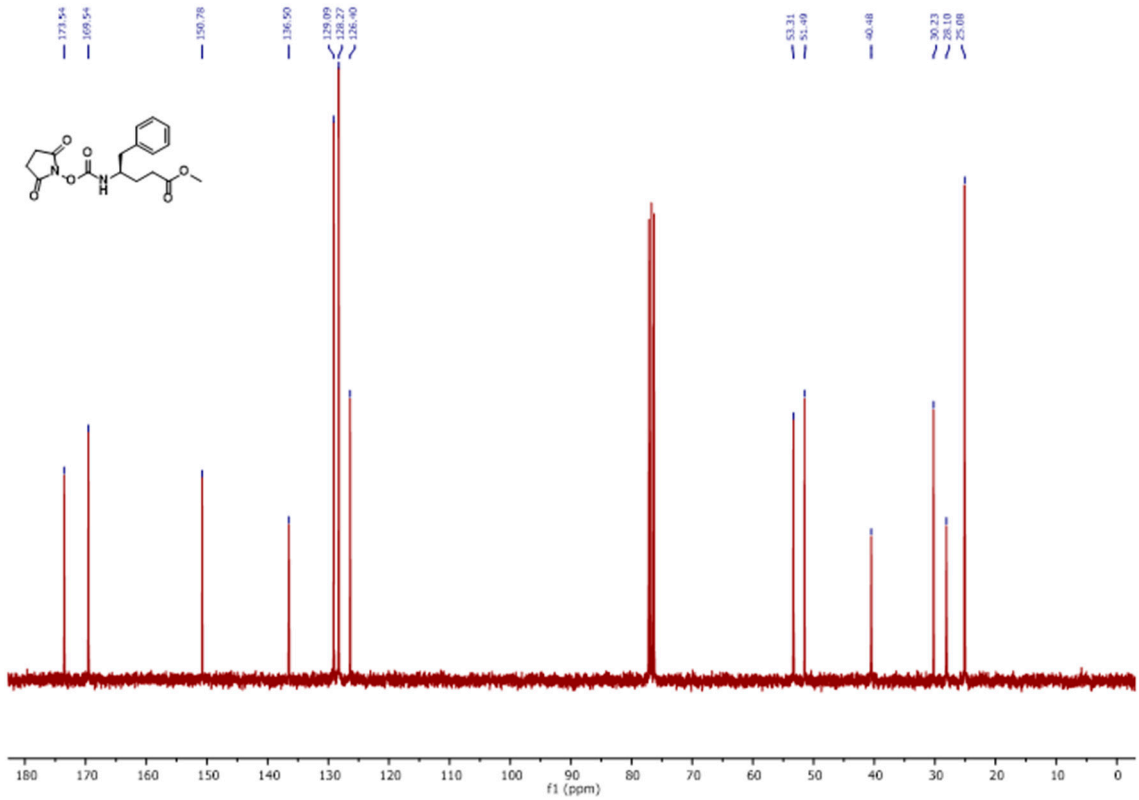


Figure S 21. ^{13}C NMR spectrum of **BB4i** in $CDCl_3$ (300 MHz)

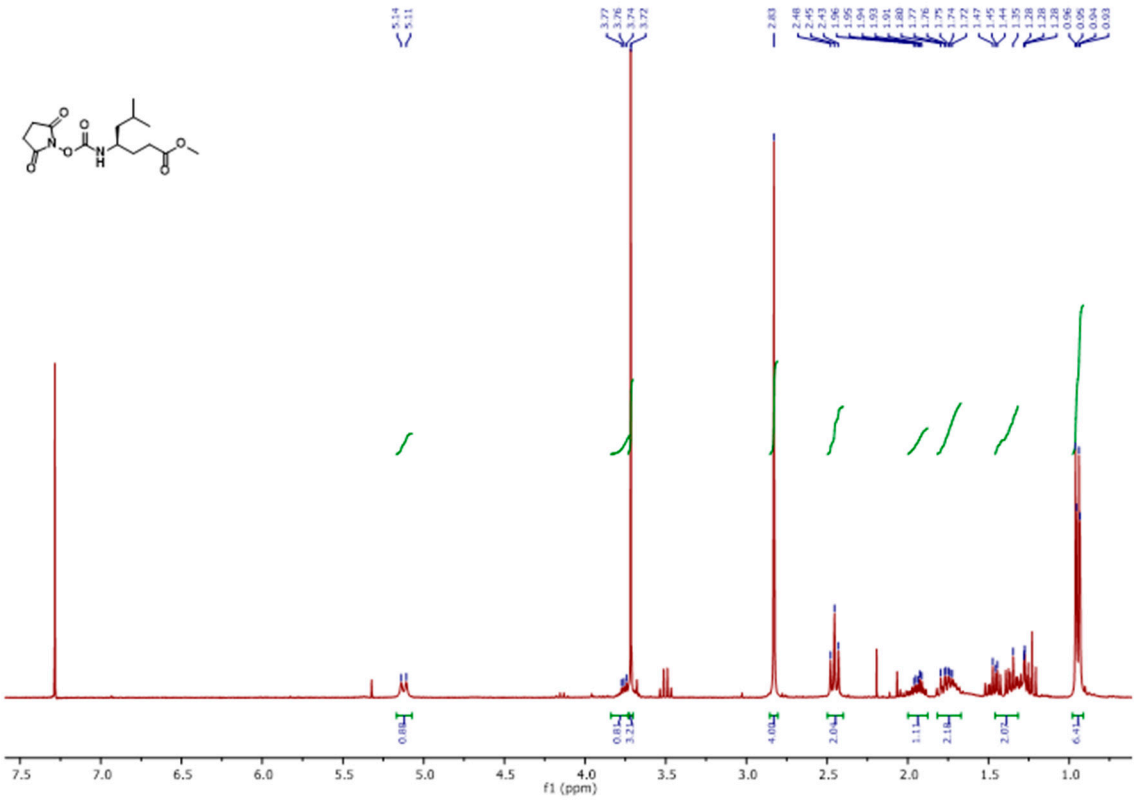


Figure S 22. ^{1}H NMR spectrum of **BB4j** in $CDCl_3$ (300 MHz)

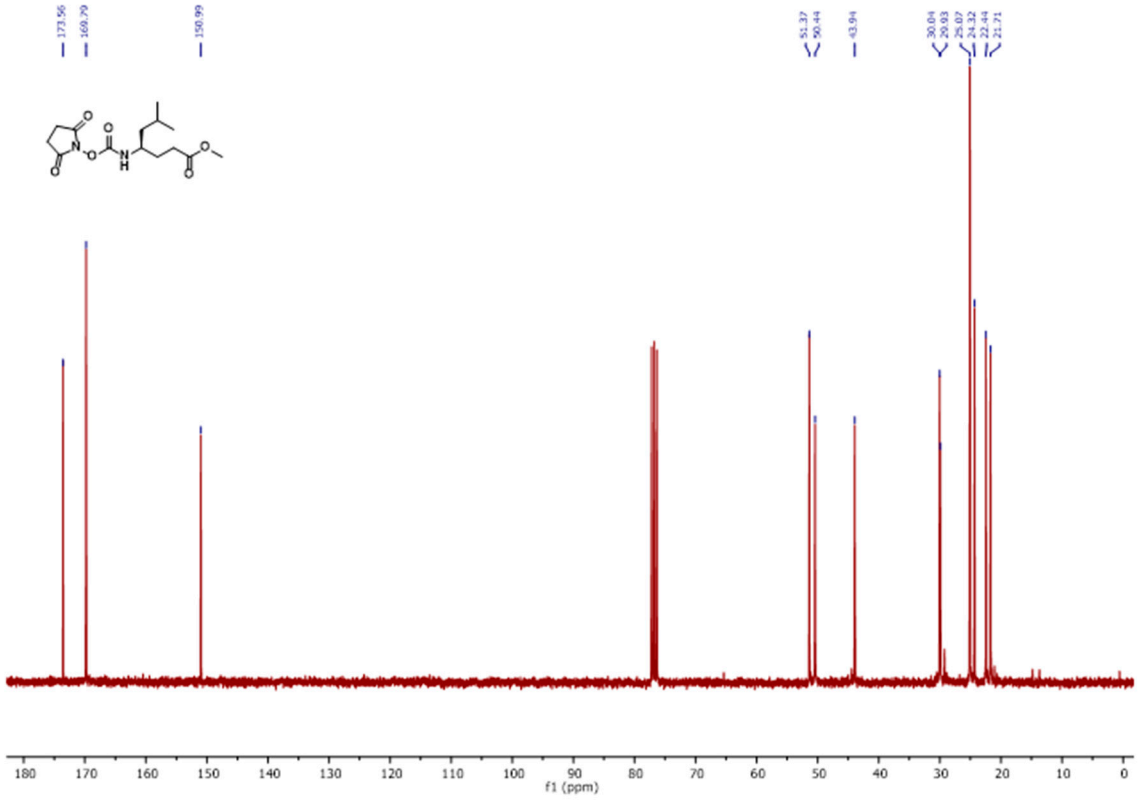


Figure S 23. ^{13}C NMR spectrum of **BB4j** in $CDCl_3$ (300 MHz)

3. NMR spectra of oligourea-esters of type 5

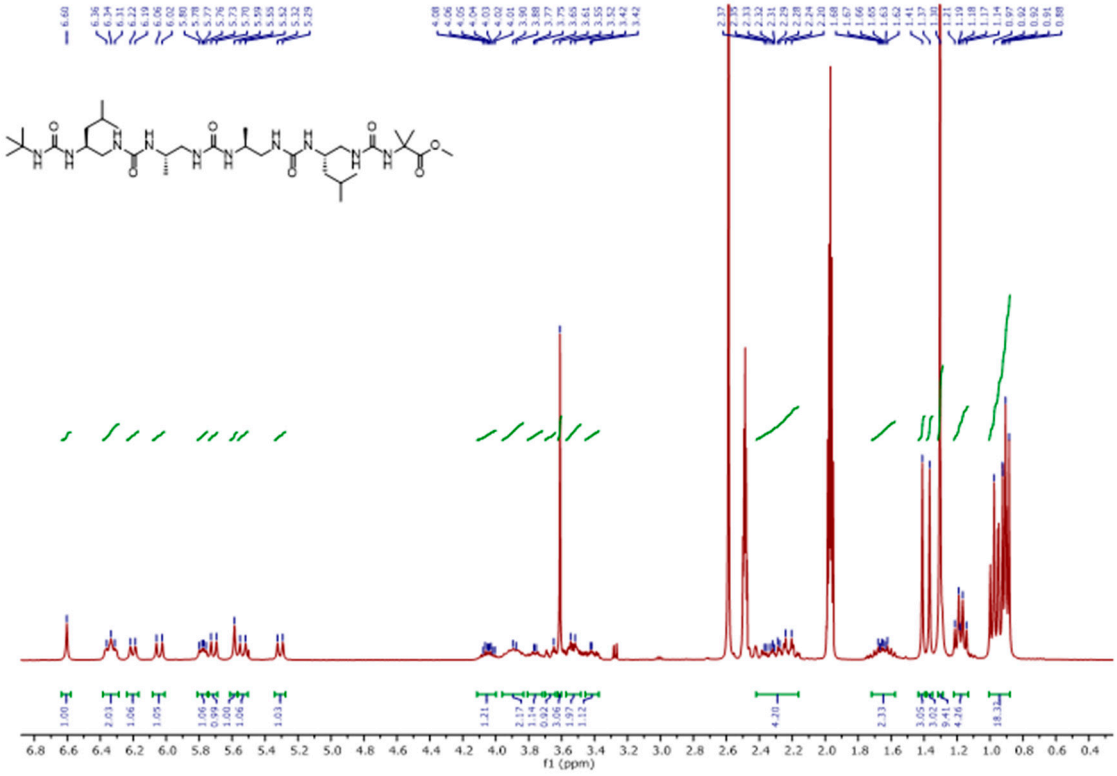


Figure S 24. ^{1}H NMR spectrum of **5a** in CD_3CN + 10% DMSO-d6 (300 MHz)

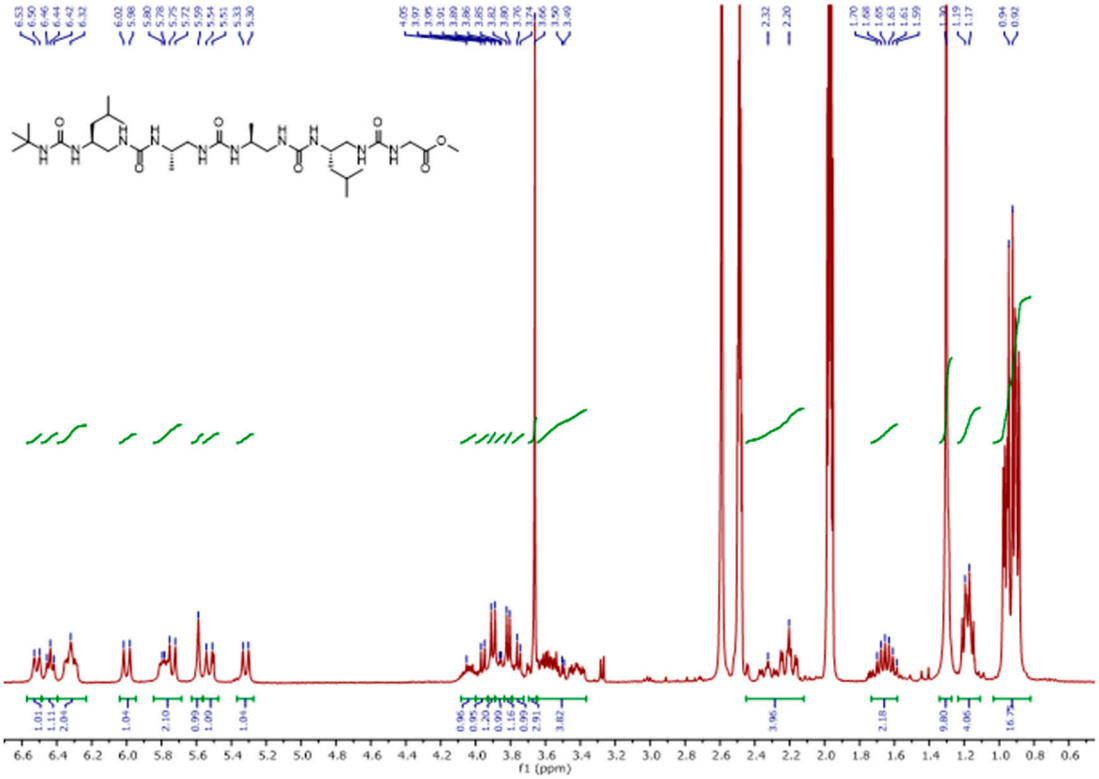


Figure S 25. ^{1}H NMR spectrum of **5b** in CD_3CN + 10% DMOS-d6 (300 MHz)

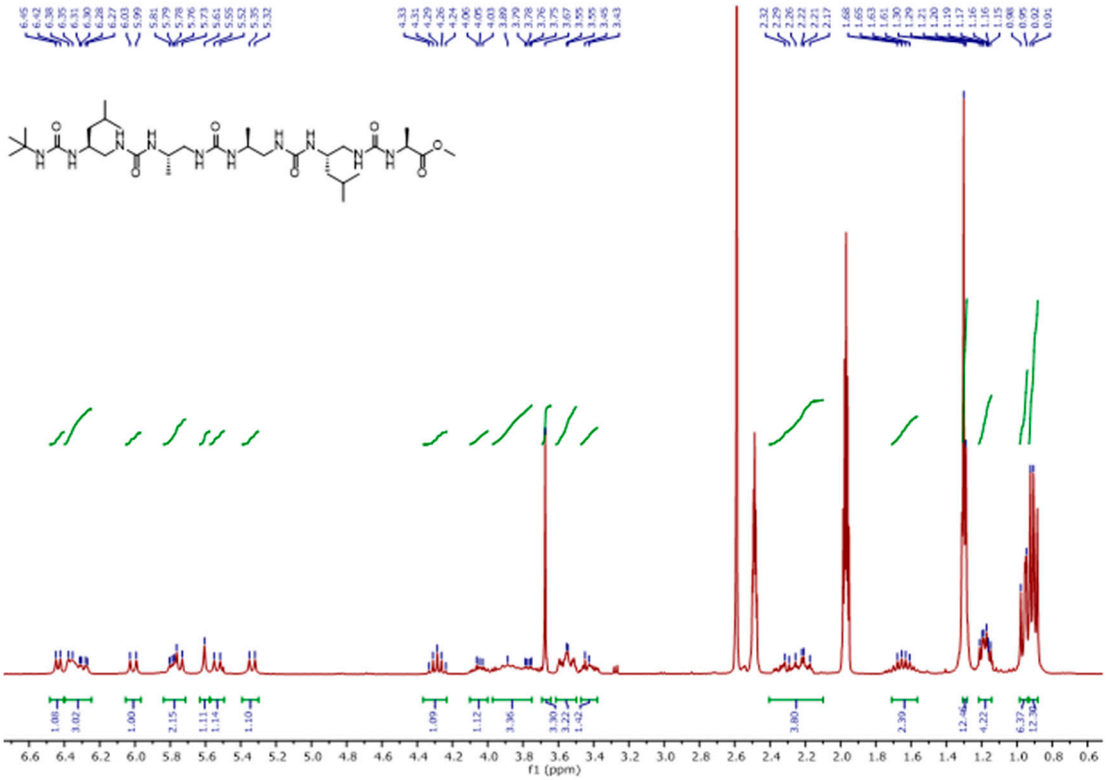


Figure S 26. ^{1}H NMR spectrum of **5c** in CD_3CN + 10% DMSO-d6 (300 MHz)

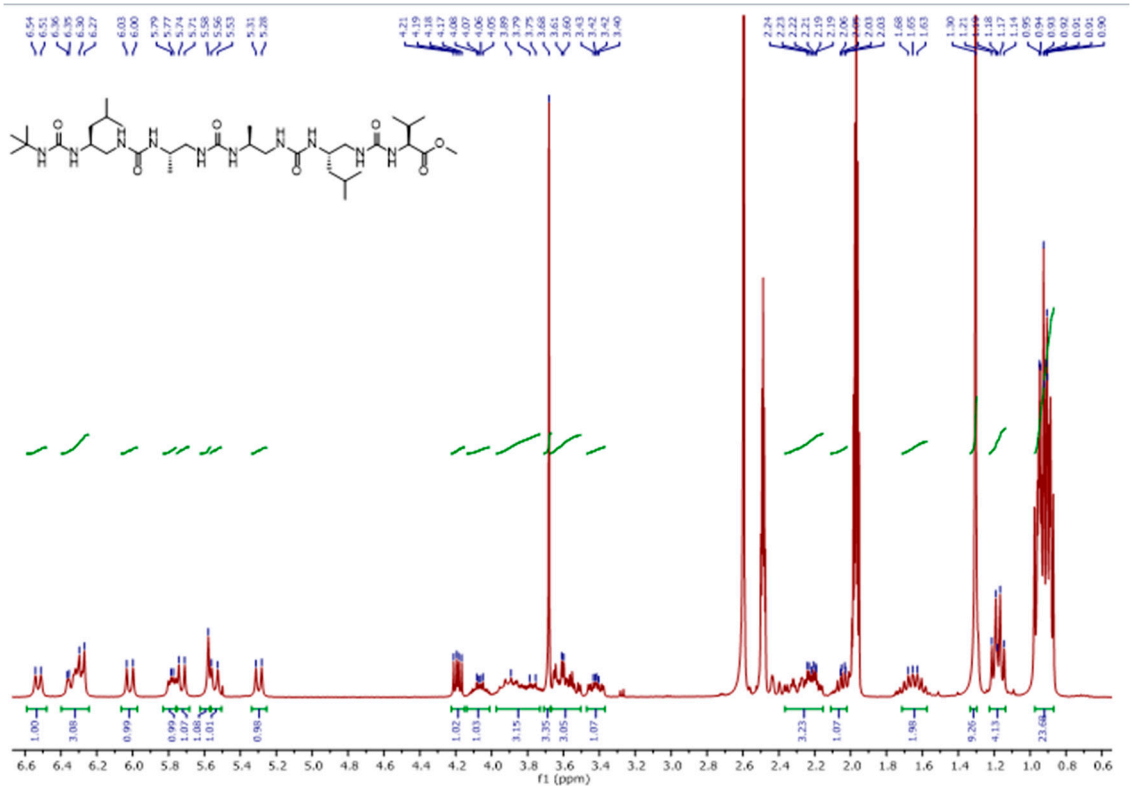


Figure S 27. ^{1}H NMR spectrum of **5d** in CD_3CN + DMSO-d6 (300 MHz)

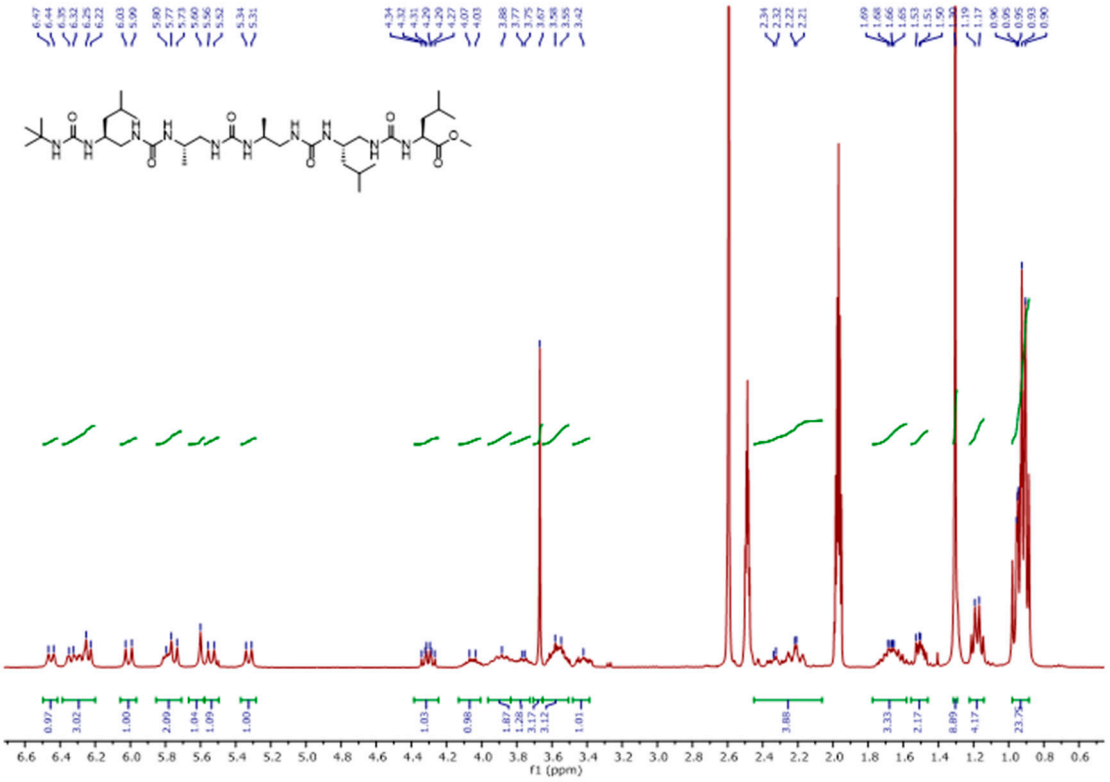


Figure S 28. ^{1}H NMR spectrum of **5e** in CD_3CN + 10% DMSO-d6 (300 MHz)

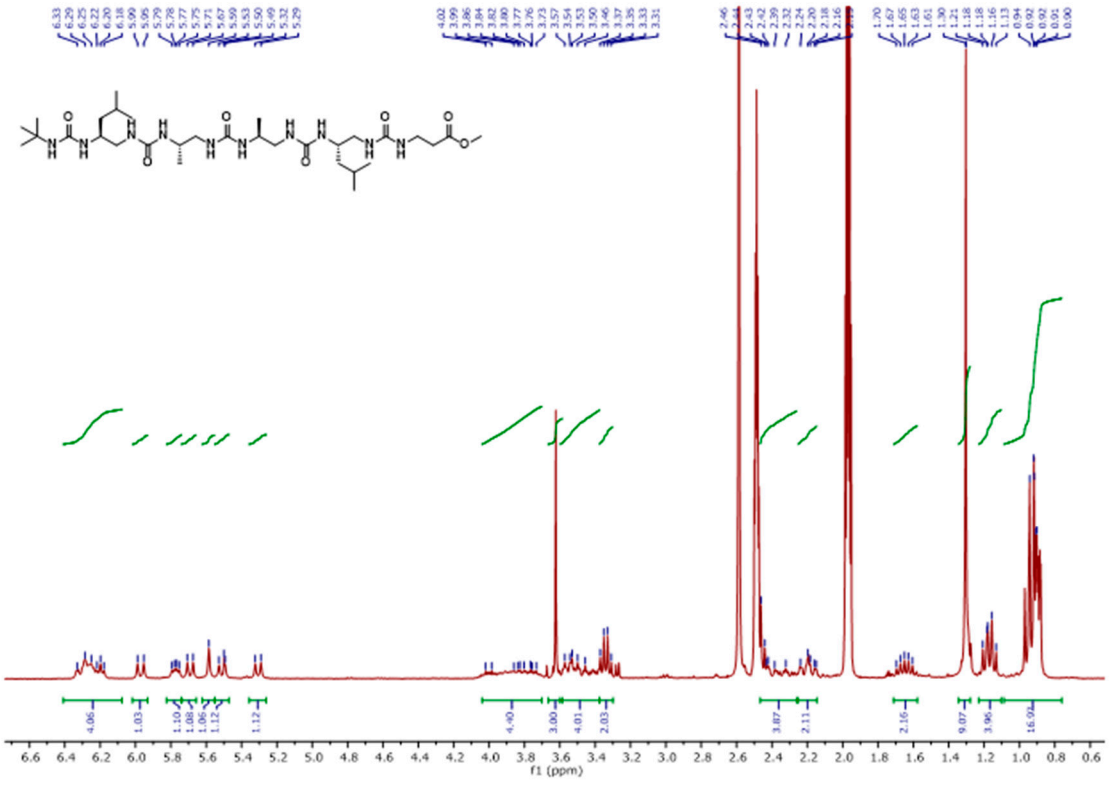


Figure S 29. ^{1}H NMR spectrum of **5f** in CD_3CN + 10% DMSO-d6 (300 MHz)

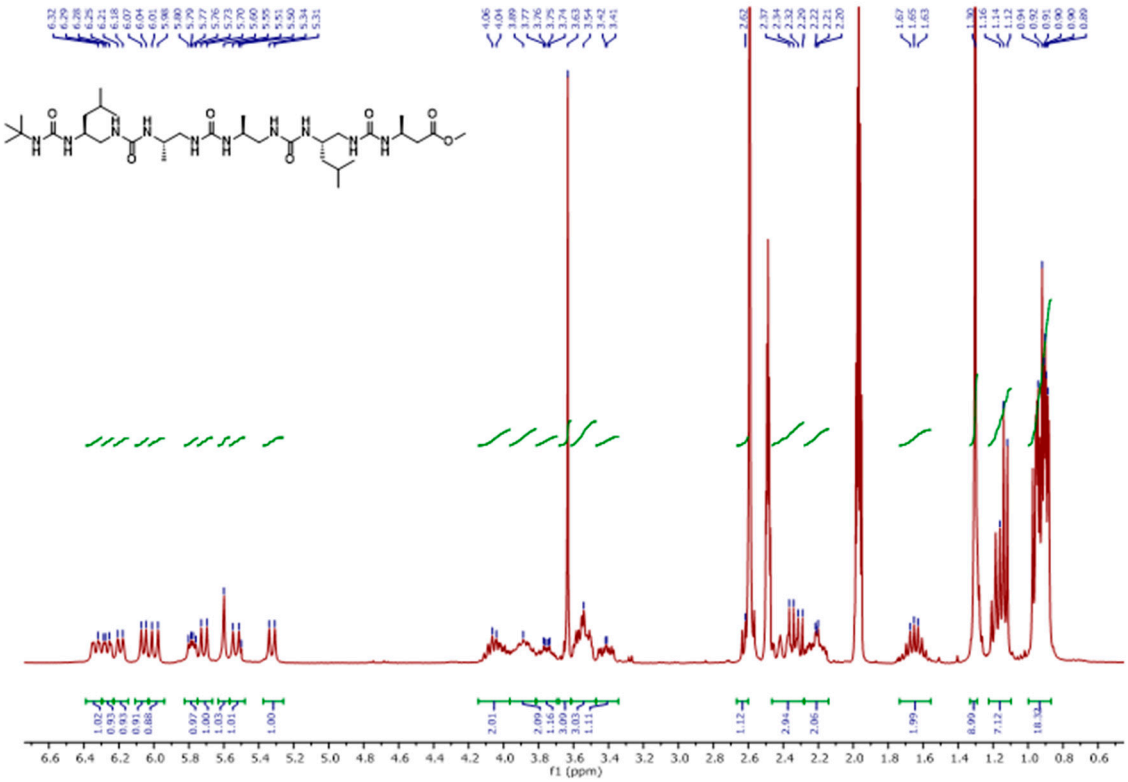


Figure S 30. ^{1}H NMR spectrum of **5g** in CD_3CN + 10% DMSO-d6 (300 MHz)

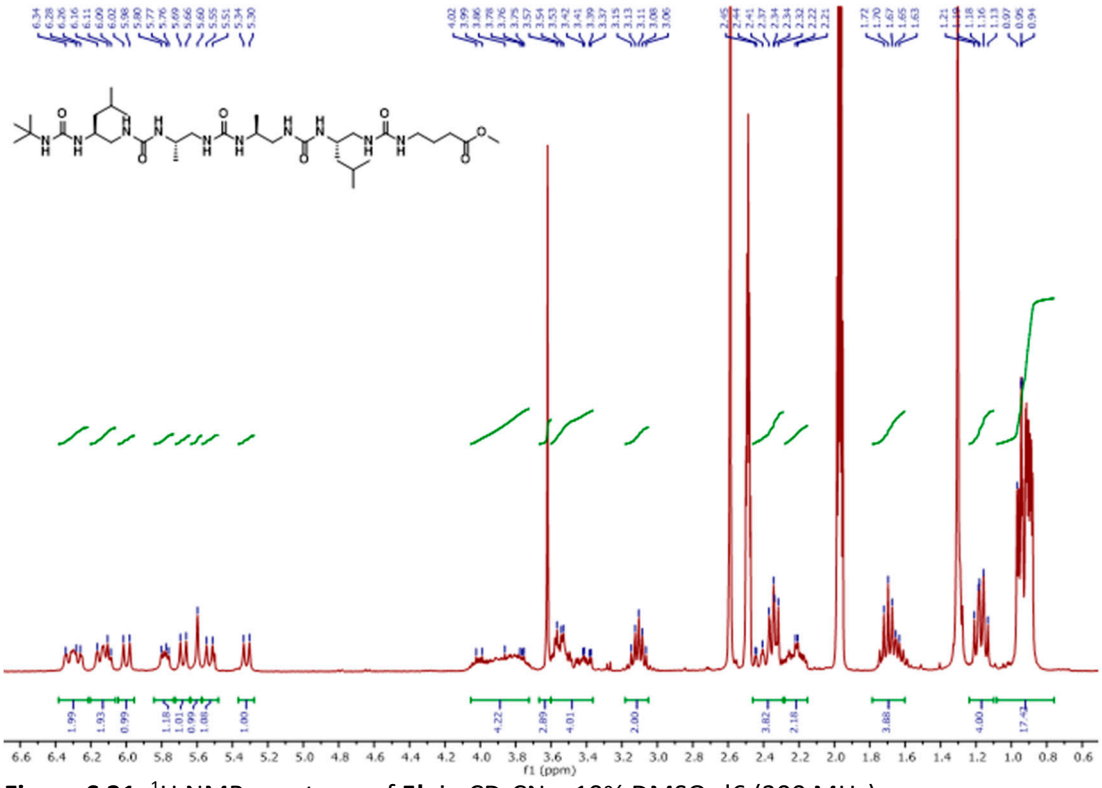


Figure S 31. ^{1}H NMR spectrum of **5h** in CD_3CN + 10% DMSO-d6 (300 MHz)

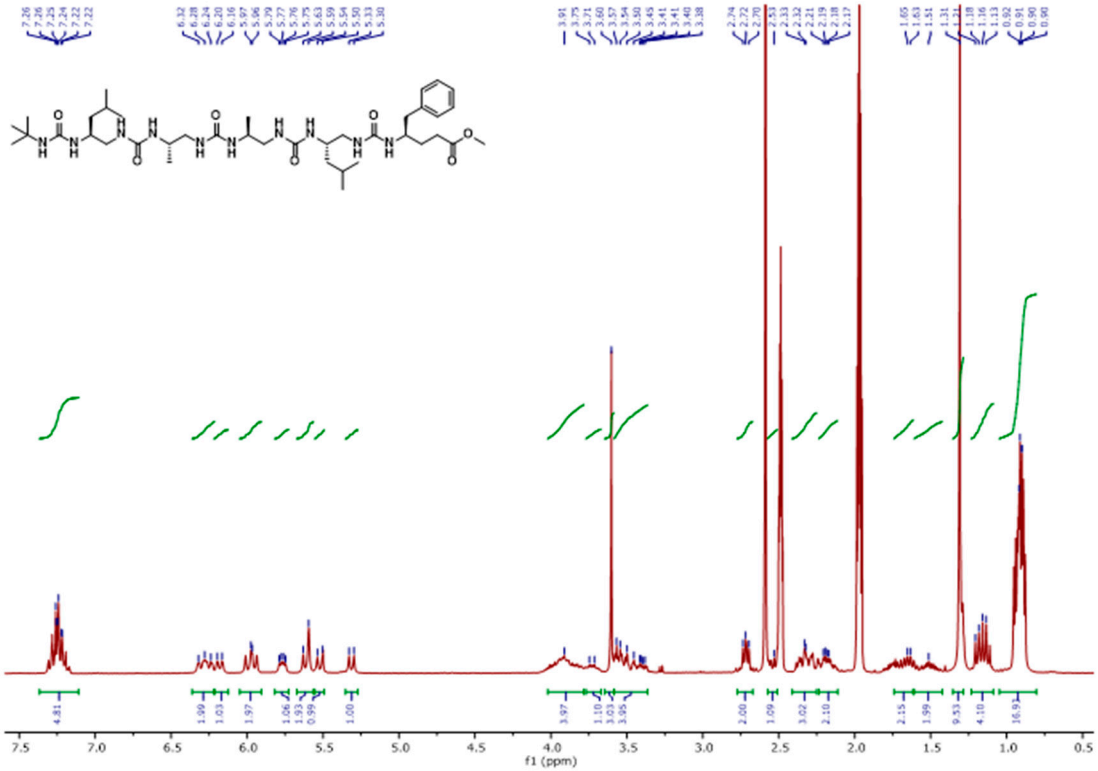


Figure S 32. ^{1}H NMR spectrum of **5i** in CD_3CN + 10% DMSO-d6 (300 MHz)

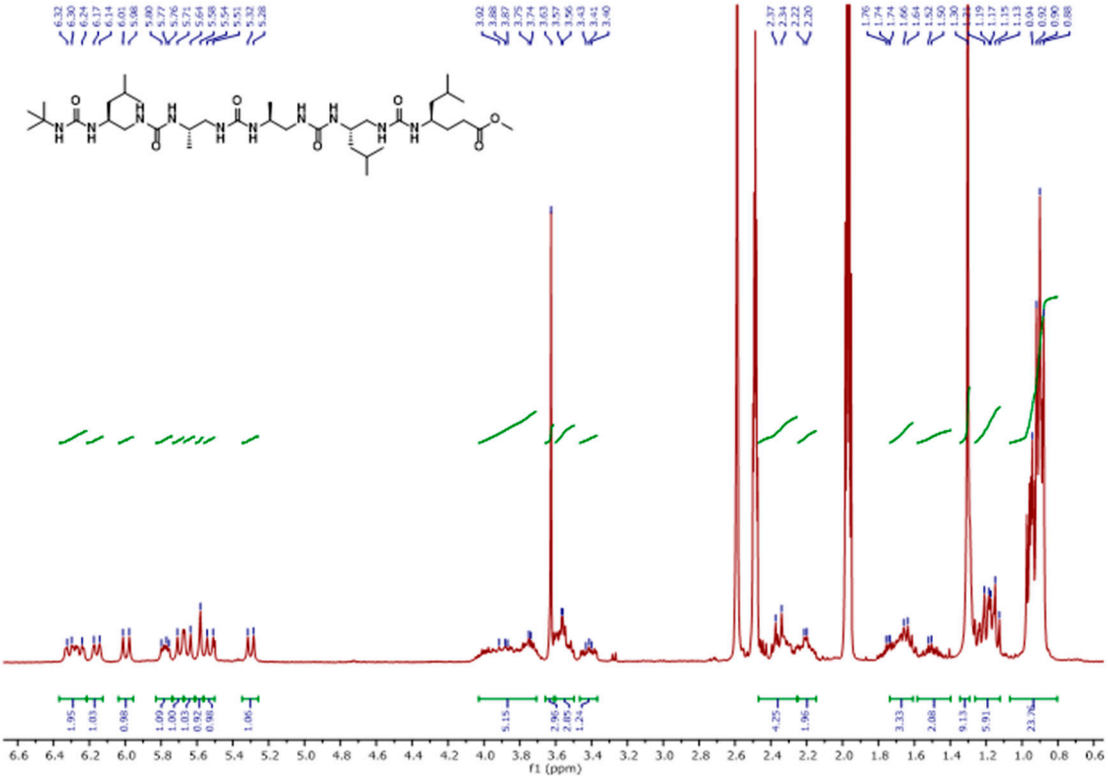


Figure S 33. ^{1}H NMR spectrum of **5j** in CD_3CN + 10% DMSO-d6 (300 MHz)

4. NMR spectra of oligourea-acids 1.

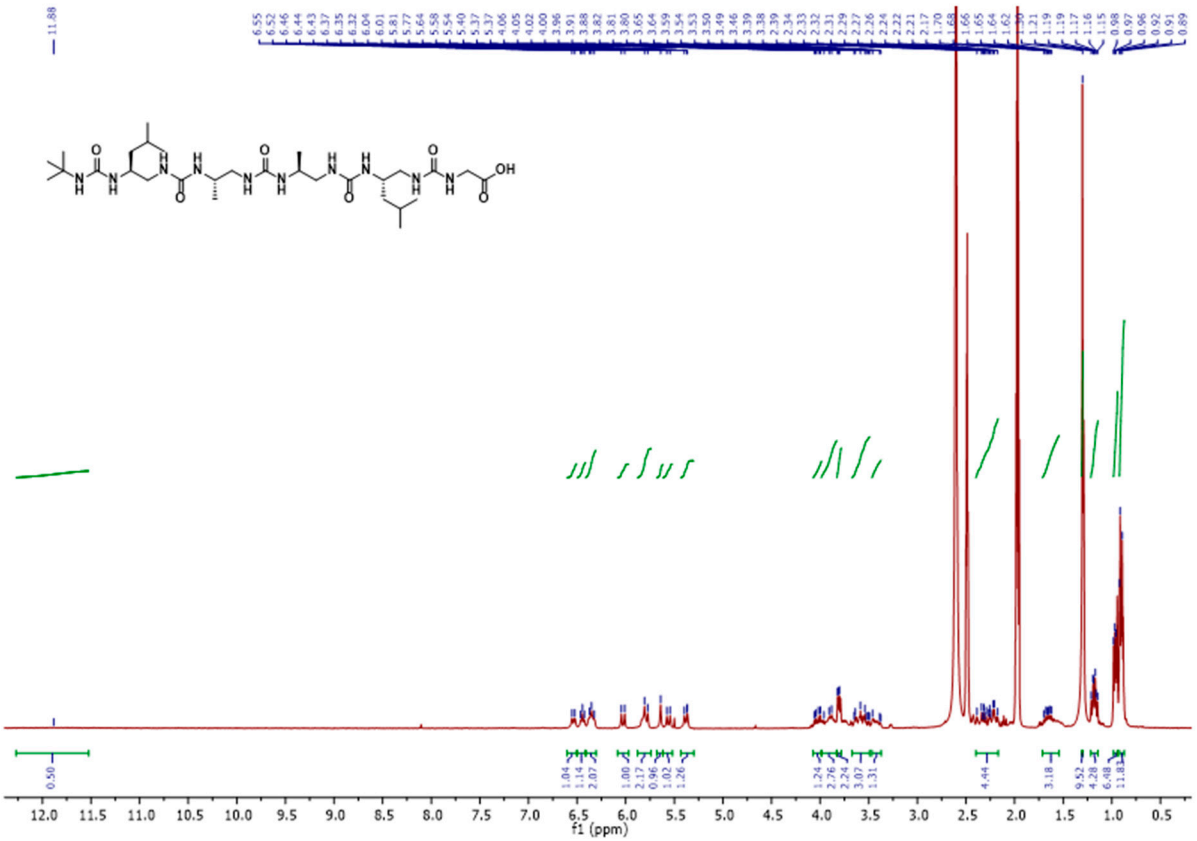


Figure S 34. ^{1}H NMR spectrum of **1b** in CD_3CN + 10% DMSO-d6 (300 MHz)

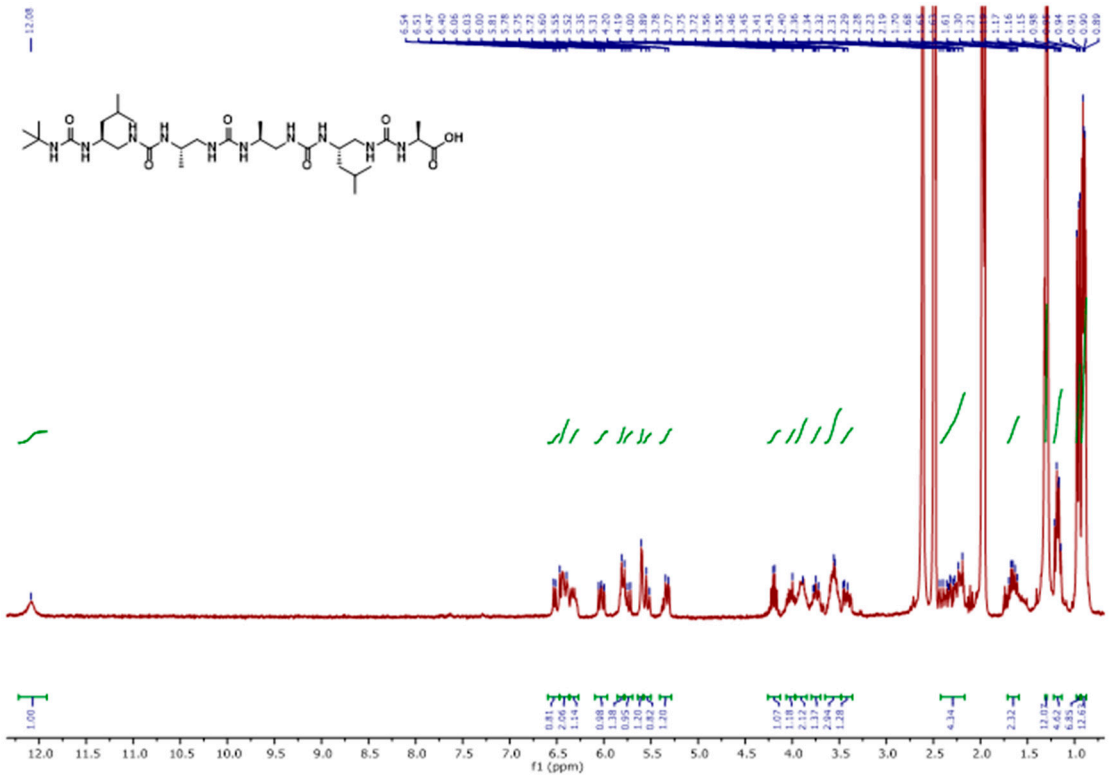


Figure S 35. ^{1}H NMR spectrum of **1c** in CD_3CN + 10% DMSO-d6 (300 MHz)

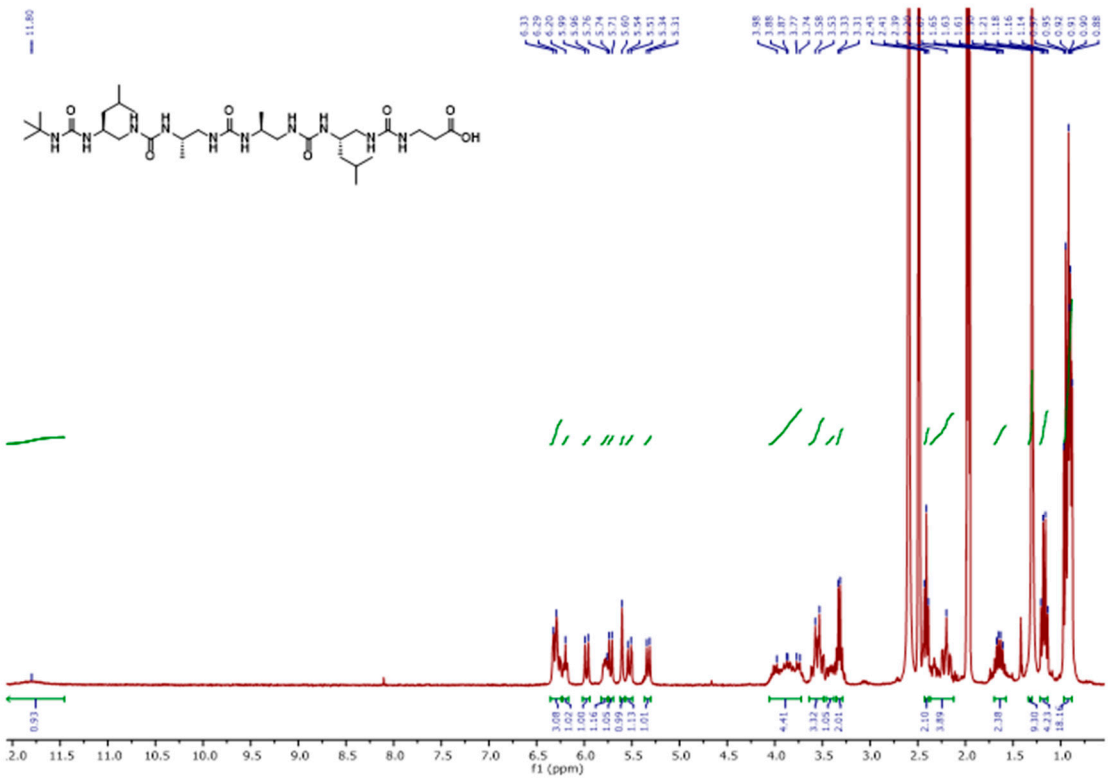


Figure S 36. ^{1}H NMR spectrum of **1f** in CD_3CN + 10% DMSO-d6 (300 MHz)

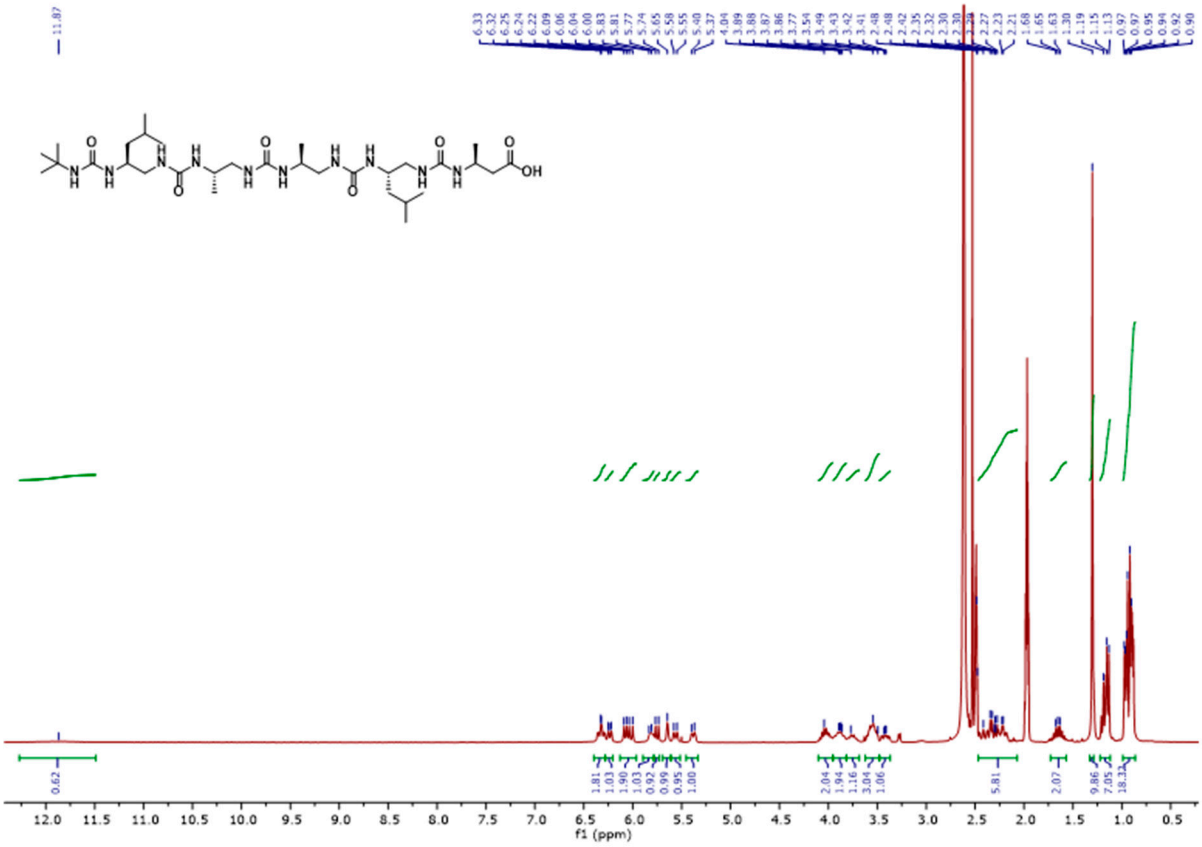


Figure S 37. ^{1}H NMR spectrum of **1g** in CD_3CN + 10% DMSO-d6 (300 MHz)

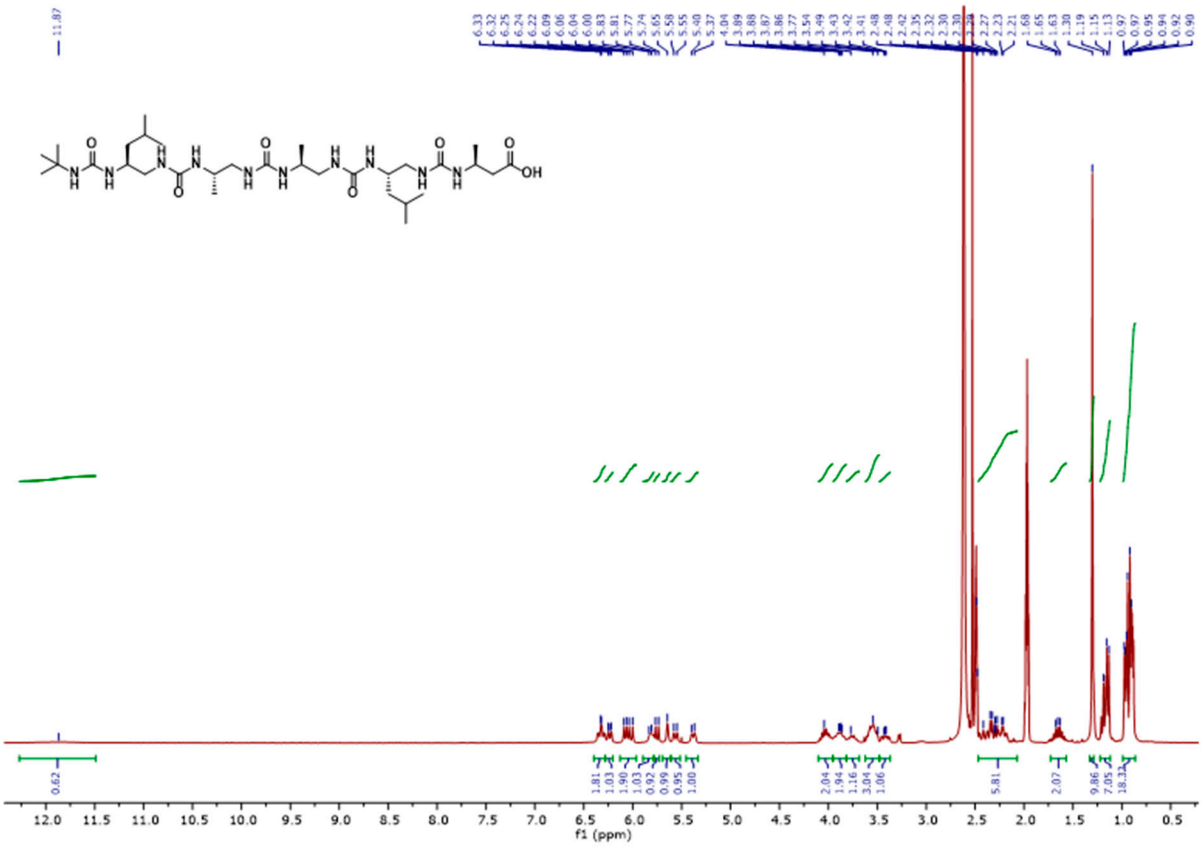


Figure S 38. ^{1}H NMR spectrum of **1h** in CD_3CN + 10% DMSO-d6 (300 MHz)

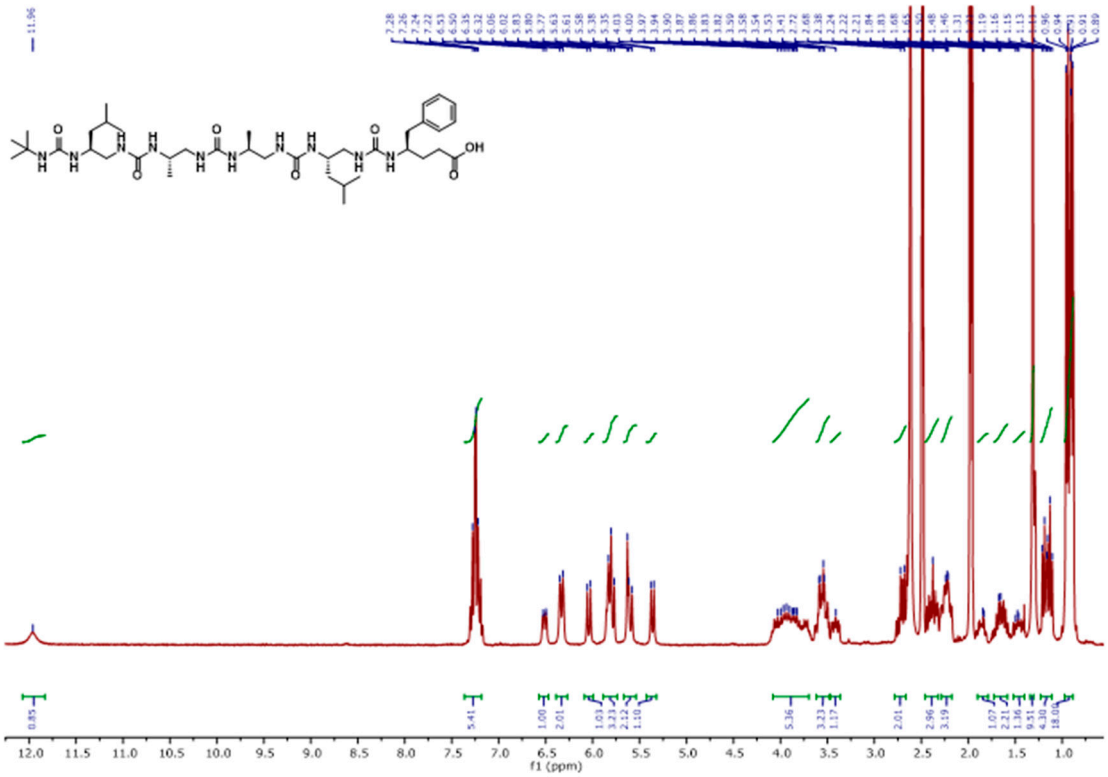


Figure S 39. ^{1}H NMR spectrum of **1i** in CD_3CN + 10% DMSO-d6 (300 MHz)

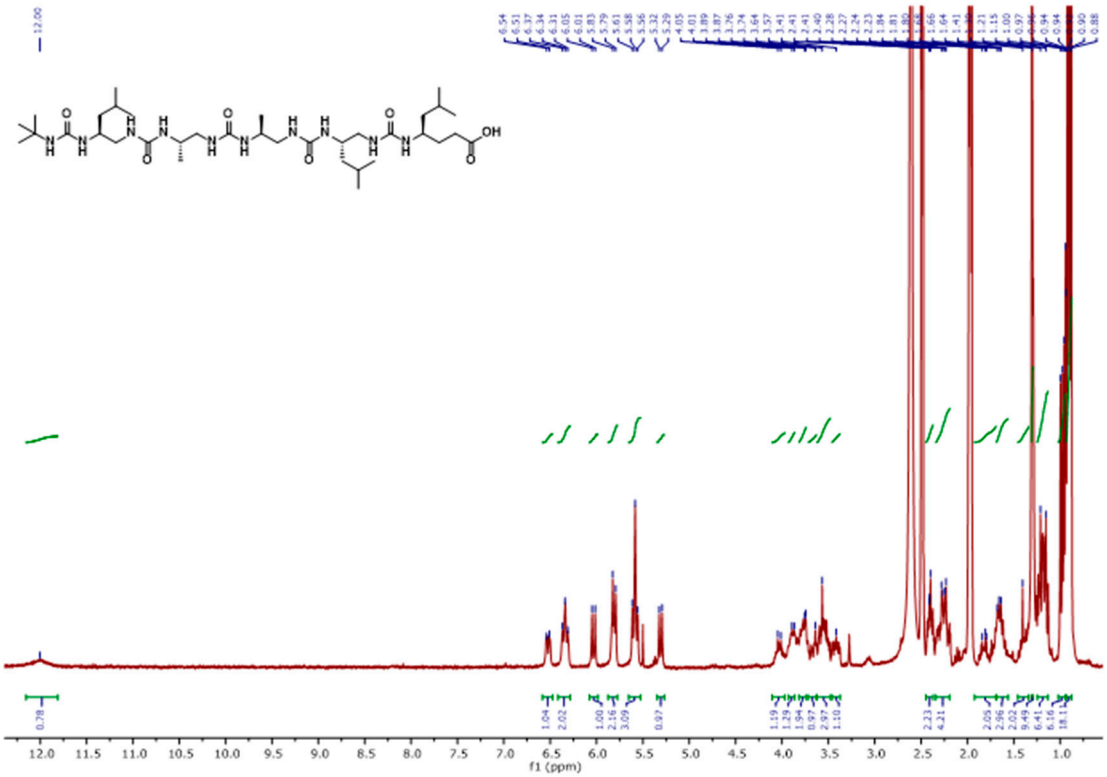


Figure S 40. ^{1}H NMR spectrum of **1j** in CD_3CN + 10% DMSO-d6 (300 MHz)

5. NMR spectra of oligourea-hydantoin/dihydrouracil 2.

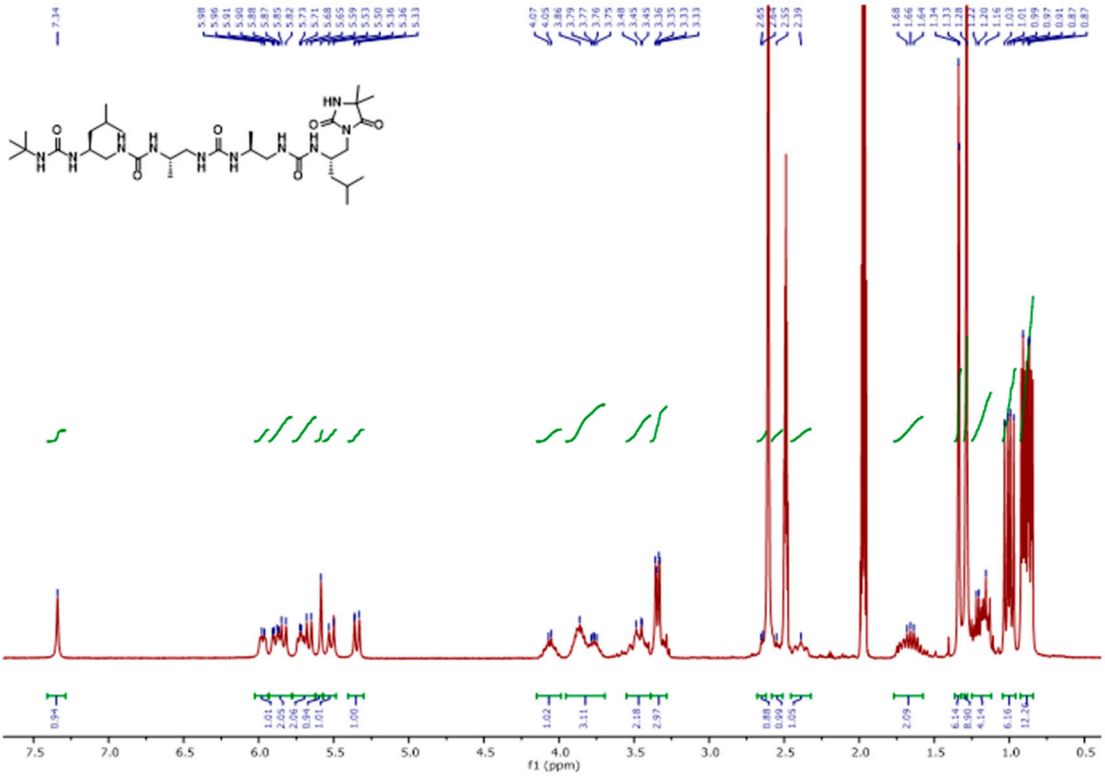


Figure S 41. ^{1}H NMR spectrum of **2a** in CD_3CN + 10% DMSO-d6 (300 MHz)

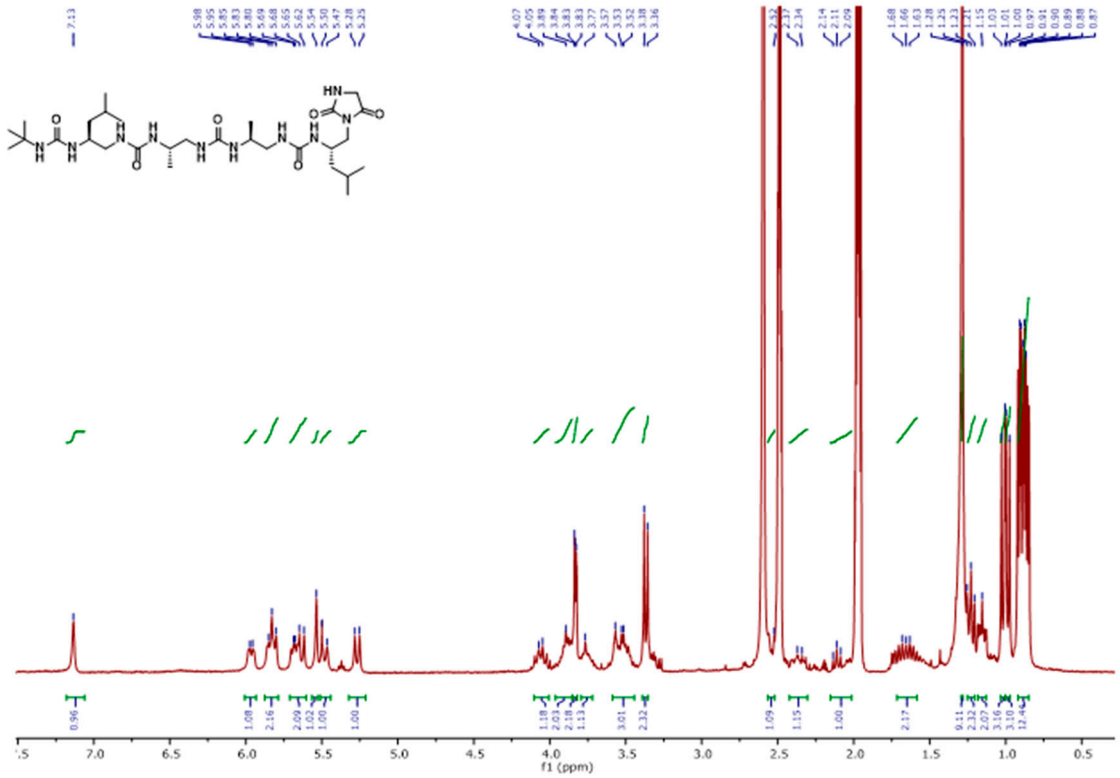


Figure S 42. ^{1}H NMR spectrum of **2b** in CD_3CN + 10% DMSO-d6 (300 MHz)

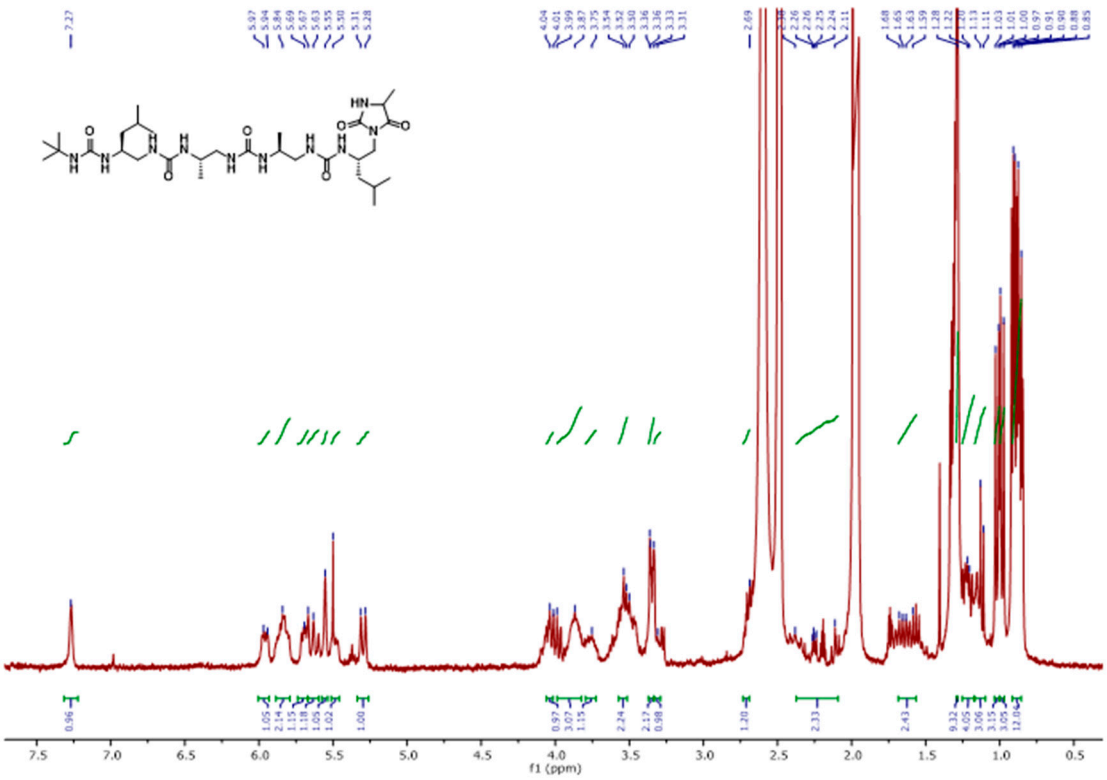


Figure S 43. ^{1}H NMR spectrum of **2c** in CD_3CN + 10% DMSO-d6 (300 MHz)

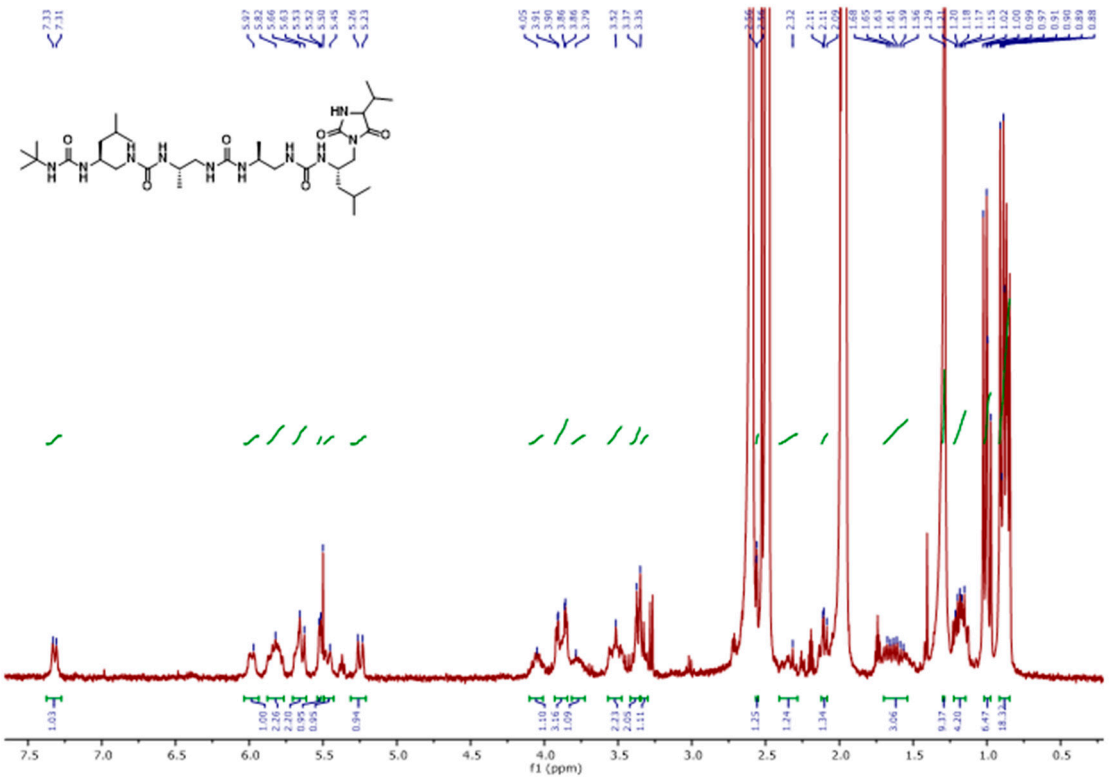


Figure S 44. ^{1}H NMR spectrum of **2d** in CD_3CN + 10% DMSO-d6 (300 MHz)

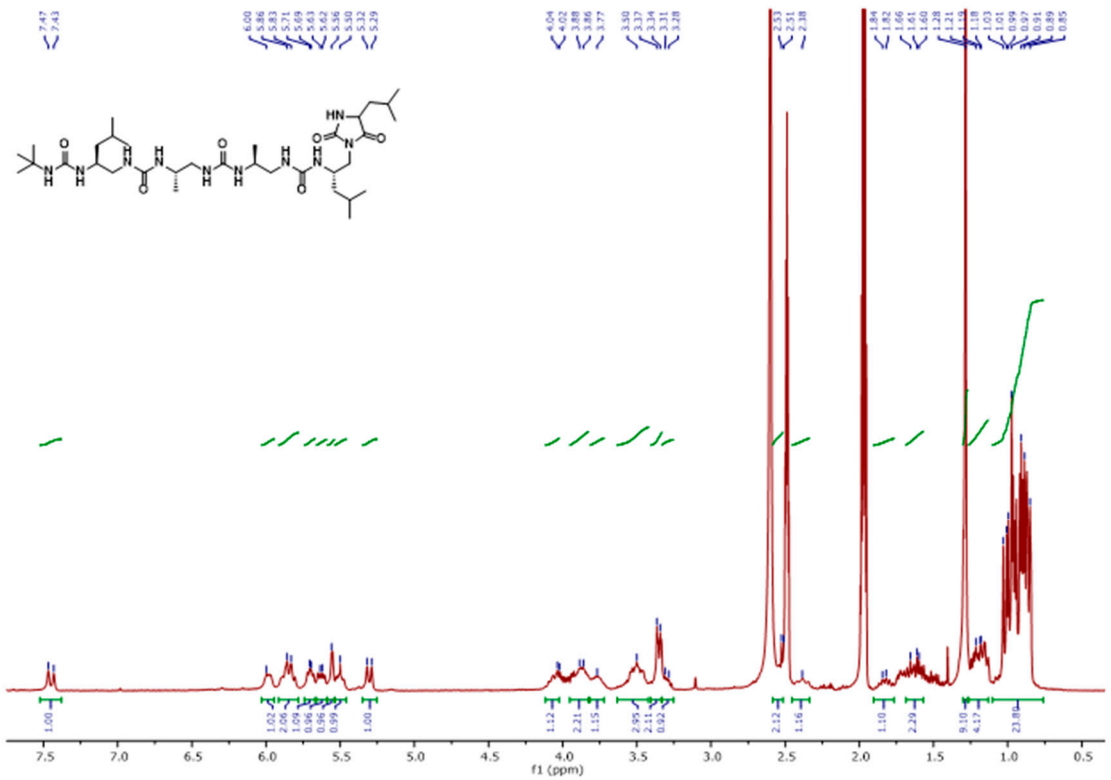


Figure S 45. ^{1}H NMR spectrum of **2e** in CD_3CN + 10% DMSO-d6 (300 MHz)

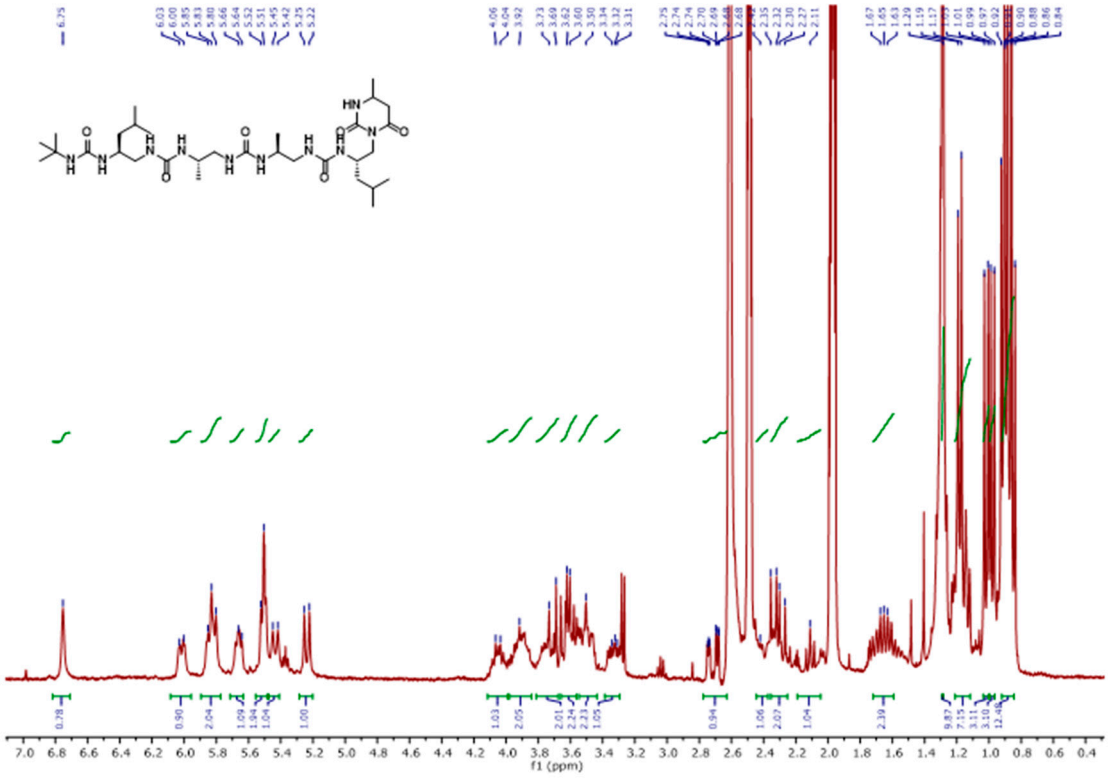
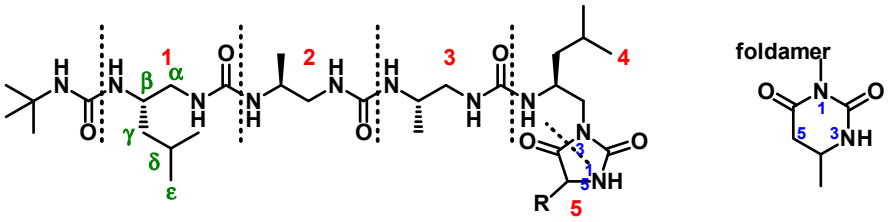


Figure S 46. ^{1}H NMR spectrum of **2g** in CD_3CN + 10% DMSO-d6 (300 MHz)

6. Signal assignments of oligourea-acids 1 and oligourea-hydantoin/dihydrouracil 2 based on 2D NMR spectra.

Experiments were recorded on a Bruker AVANCE 500 (500 MHz) spectrometer at 298K. Spin systems were assigned using COSY and TOCSY spectra. Sequential assignment was performed on the basis of N'H(*i*+1) / NH(*i*) ROESY correlations.

Table S 1. Signal assignments of oligoureas with hydantoin (**2a-e**) or dihydrouracil (**2g)** residues at C-terminus ased on 2D NMR spectra (doubling of signals resulting from epimerization was marked only for urea/hydantoin protons)



Res	Cmpd	HN	HN'	$^{\alpha}CH^{1}$	$^{\alpha}CH^{2}$	$^{\beta}CH$	$^{\gamma}CH$	$^{\delta}CH$	$^{\varepsilon}CH$	CH_3 (tBu)
tBuNH	**2a***	-	5.52 (s)	-	-	-	-	-	-	1.26
	2b	-	5.51 (s)	-	-	-	-	-	-	1.26
	2c	-	5.53 (s)	-	-	-	-	-	-	1.26
	2d	-	5.53 5.54 *(diastereomers)*	-	-	-	-	-	-	1.26

	2e[$]	-	5.46 5.47 *(diastereomers*)	-	-	-	-	-	-	1.26
	2g	-	5.48 (overlapped with NHLeu$_4$U)	-	-	-	-	-	-	1.26
Leu1u	**2a**	5.27 (d, *J* = 9.4 Hz)	5.66 (dd, *J* = 7.3, 4.7 Hz)	3.30	2.47	3.84	1.13	1.62	0.86	-
	2b	5.24 (d, *J* = 9.4 Hz)	5.65 (dd, *J* = 7.6, 4.5 Hz)	3.32	2.44	3.83	1.12	1.61	0.86	-
	2c	5.28 (d, *J* = 9.5 Hz)	5.67 (dd, *J* = 8.5, 5.0 Hz)	3.31	2.46	3.84	1.13	1.69	0.84/ 0.87	-
	2d	5.27 (d, *J* = 9.5 Hz)	5.68 (broad signal)	3.31	2.45	3.85	1.12/ 1.26	1.60	0.85	-
	2e	5.21 (d, *J* = 9.4 Hz) 5.20 (d, *J* = 9.3 Hz) (*diastereomers)*	5.64 (m, overlapped with NHLeu$_4$u)	3.31	2.49	3.85	1.15	1.60	0.85	-
	2g	5.21 (d, *J* = 9.3 Hz)	5.63 (dd, *J* = 7.5, 4.8 Hz)	3.30	2.42	3.87	1.11	1.61	0.85	-
Ala2u	**2a**	5.46 (d, *J* = 8.6 Hz)	5.84 (*pseudo*-d, *J* = 7.0 Hz)	3.47	2.35	3.84	0.99	-	-	-
	2b	5.45 (d, *J* = 8.7 Hz)	5.82 (overlapped with NH Ala$_3$U)	3.49	2.33	3.86	0.99	-	-	-
	2c	5.47 (broad signal, overlapped with DCM)	5.84 (overlapped with NH Ala$_3$U)	3.49	2.36	3.86	0.99	-	-	-
	2d	5.46 (broad signal, overlapped with DCM)	5.85 (overlapped with NH Ala$_3$U)	3.48	2.32	3.86	0.99	-	-	-
	2e	5.41 (broad signal)	5.88 (overlapped with NH Ala$_3$U)	3.49	2.36	3.89	1.00	-	-	-
	2g	5.40 (d, *J* = 9.5 Hz)	5.81 (overlapped with NH Ala$_3$U)	3.48	2.31	3.91	0.99	-	-	-
Ala3u	**2a**	5.79 (d, *J* = 8.5 Hz)	5.93 (*pseudo*-d, *J* = 5.5 Hz)	3.43	2.58	3.74	0.95	-	-	-
	2b	5.79 (overlapped with N'H Ala$_2$U)	5.94 (pseudo-d, J = 6.2 Hz)	3.50	2.54	3.73	0.95	-	-	-
	2c	5.80 (overlapped with N'H Ala$_2$U)	5.94 (*pseudo*-d, *J* = 6.5 Hz)	3.46	2.56	3.74	0.97	-	-	-
	2d	5.79 (overlapped with N'H Ala$_2$U)	5.96 (*pseudo*-d, *J* = 5.9 Hz)	3.48	2.53	3.74	0.96	-	-	-
	2e	5.83 (overlapped with N'H Ala$_2$U)	5.97 (broad signal)	3.49	2.54	3.77	0.97	-	-	-

	2g	5.79 (overlapped with N'H Ala_2U)	5.99 (*pseudo*-d, *J* = 6.4 Hz)	3.49	2.54	3.73	0.95	-	-	-
Leu4u	**2a**	5.62 (d, *J* = 9.3 Hz)	-	3.32	-	4.04	1.17	1.68	0.86	-
	2b	5.61 (d, J = 9.3 Hz)	-	3.34	-	4.03	1.20	1.66	0.86	-
	2c	5.59 (d, *J* = 9.5 Hz) 5.63 (d, *J* = 9.3 Hz) *(diastereomers)*	-	3.32	-	4.03	1.19	1.66	0.83/ 0.88	-
	2d	5.63 (d, *J* = 8.9 Hz)	-	3.33	-	4.00	1.18	1.66	0.86	-
	2e	5.61 (m, overlapped with N'HLeu_1u)	-	3.34	-	4.03	1.21	1.68	0.85	-
	2g	5.48 (overlapped with NHtBu)	-	3.66	3.57	4.02	1.15	1.68	0.84	-

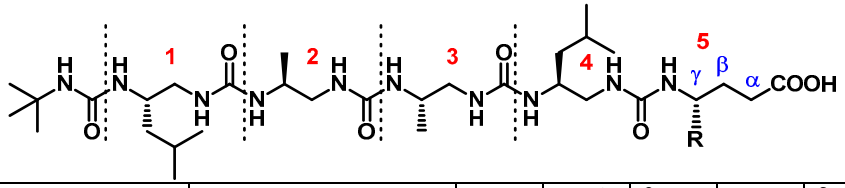
hydantoin dihydrouracil ring				
Res		**NH1**	**CH5**	**Substituents at C5**
Aib_{hyd}	**2a**	7.29 (s)	-	1.31/1.30 (CH3)
Gly_{hyd}	**2b**	7.11 (s)	3.80	-
Ala_{hyd}	**2c**	7.25 (s)	3.94 4.00 *(diastereomers)*	1.30/1.29 (CH_3, *diastereomers*)
Val_{hyd}	**2d**	7.31 7.29 *(doubled, diastereomers)*	3.83 3.88 *(diastereomers)*	2.08 (CH), 0.84 (CH_3)
Leu_{hyd}	**2e**	7.22 7.26 *(diastereomers)*	3.94 4.00 *(diastereomers)*	1.56/1.46 (CH_2), 1.80 (CH), 0.94 (CH_3)
$Ala_{dihydrouracil}$	**2g[@]**	6.73 (s)	3.58 (C4H) 2.27/2.68 (C5H)	1.16 (CH_3)

[*] 10% DMSO-d6 in CD_3CN

[$] CD_3CN

[@] different numbering of dihydrouracil (from *N*-foldamer) than the hydantoin ring

Table S 2. Signal assignments of oligoureas (**1b,c,f-j**) with amino acid residue at C-terminus based on 2D NMR spectra



Res	Cmpd	HN	HN'	$^{\alpha}CH^{1}$	$^{\alpha}CH^{2}$	$^{\beta}CH$	$^{\gamma}CH$	$^{\delta}CH$	$^{\varepsilon}CH$	CH_3 (tBu)
tBuNH	**1b***	-	5.62 (s)	-	-	-	-	-	-	1.27
	1c&	-	5.58 (s) 5.57 (s)	-	-	-	-	-	-	1.27
	1f	-	5.58 (s)	-	-	-	-	-	-	1.27
	1g	-	5.64 (s)	-	-	-	-	-	-	1.27
	1h	-	5.57 (s)	-	-	-	-	-	-	1.27
	1i	-	5.60 (s)	-	-	-	-	-	-	1.28
	1j	-	5.55 (s)	-	-	-	-	-	-	1.27
Leu1u	**1b**	5.37 (d, *J* = 10.0 Hz)	5.80 (dd, *J* = 7.6, 4.0 Hz)	3.40	2.31	3.89	1.16	1.65	0.89	-
	1c	5.29 (d, *J* = 9.5 Hz) 5.30 (d, *J* = 9.4 Hz)	5.76 5.78 (overlapped with $NHLeu_4U$)	3.40	2.31	3.90	1.15	1.64	0.87 0.91	-
	1f	5.30 (d, *J* = 9.6 Hz)	5.75 (dd, *J* = 8.2, 4.3 Hz)	3.40	2.29	3.89	1.16	1.64	0.87	-
	1g	5.38 (d, *J* = 9.6 Hz)	5.80 (dd, *J* = 8.1, 4.4 Hz)	3.39	2.32	3.88	1.17	1.64	0.88	-
	1h	5.30 (d, *J* = 9.6 Hz)	5.79 (dd, *J* = 8.1, 4.4 Hz)	3.39	2.31	3.88	1.17	1.64	0.88	-
	1i	5.33 (d, *J* = 9.5 Hz)	5.80 (overlapped with NH γ-Phe)	3.38	2.31	3.88	1.16	1.66	0.85	-
	1j	5.28 (d, *J* = 9.5 Hz)	5.79 (overlapped with $NHLeu_4U$)	3.40	2.29	3.88	1.15	1.64	0.89	-
Ala2u	**1b**	5.54 (d, *J* = 10.2 Hz)	6.31 (overlapped with N'H Ala_3U)	3.61	2.18	3.75	0.95	-	-	-
	1c	5.50 (d, *J* = 9.8 Hz) 5.54 (d, *J* = 10.5 Hz)	6.30/6.33 (overlapped with N'H Ala_3U)	3.67 3.54	2.16 2.20	3.73	0.94	-	-	-
	1f	5.50 (d, *J* = 10.0 Hz)	6.28 (overlapped with N'H Leu_4U)	3.51	2.18	3.72	0.92	-	-	-
	1g	5.56 (d, *J* = 10.1 Hz)	6.31 (dd, *J* = 10.0, 2.6 Hz)	3.55	2.20	3.73	0.93	-	-	-

	1h	5.54 (d, *J* = 10.1 Hz)	6.30 (dd, *J* = 10.0, 2.7 Hz)	3.51	2.20	3.75	0.93	-	-	-
	1i	5.56 (d, *J* = 10.1 Hz)	6.30 (overlapped with N'HLeu$_4$U)	3.52	2.20	3.71	0.95	-	-	-
	1j	5.54 (overlapped with NHtBu and NH γ-Leu)	6.29 (overlapped with N'HLeu$_4$U)	3.52	2.21	3.74	0.95	-	-	-
Ala3u	**1b**	6.00 (d, *J* = 10.5 Hz)	6.33 (overlapped with N'HAla$_2$U)	3.51	2.20	4.00	0.93	-	-	-
	1c	5.99 (d, *J* = 10.7 Hz) 6.01 (d, *J* = 10.8 Hz)	6.28 6.38 (overlapped with N'H Ala$_2$U and N'HLeu$_4$U)	3.52	2.18	3.99	0.95	-	-	-
	1f	5.95 (d, *J* = 10.5 Hz)	6.24 (dd, *J* = 10.0, 3.0 Hz)	3.51	2.18	3.97	0.92	-	-	-
	1g	5.99 (d, *J* = 10.5 Hz)	6.27 (dd, *J* = 9.8, 2.9 Hz)	3.52	2.20	3.98	0.93	-	-	-
	1h	5.99 (d, *J* = 10.5 Hz)	6.34 (dd, *J* = 9.7, 2.9 Hz)	3.51	2.20	4.00	0.93	-	-	-
	1i	6.00 (d, *J* = 10.5 Hz)	6.49 (dd, *J* = 9.1, 2.2 Hz)	3.51	2.22	4.01	0.92	-	-	-
	1j	6.00	6.50	3.51	2.22	4.00	0.92	-	-	-
Leu4u	**1b**	5.76 (d, *J* = 9.7 Hz)	6.52 (*pseudo-d, J* = 8.1 Hz)	3.56	2.39	3.86	1.14	1.62	0.89	-
	1c	5.71 (d, *J* = 9.8 Hz) 5.77 (overlapped with N'HLeu$_1$U)	6.39 6.42 (overlapped with N'HAla$_3$U and NH α-Ala)	3.51 3.55	2.38 2.41	3.86 3.87	1.13	1.61	0.86	-
	1f	5.70 (d, *J* = 9.7 Hz)	6.28 (overlapped with N'H Ala$_2$U)	3.55	2.40	3.82	1.13	1.62	0.88	-
	1g	5.72 (d, *J* = 9.8 Hz)	6.20 (*pseudo*-d, *J* = 8.1 Hz)	3.52	2.39	3.84	1.14	1.61	0.88	-
	1h	5.72 (d, *J* = 9.8 Hz)	6.25 (*pseudo*-d, *J* = 8.2 Hz)	3.57	2.36	3.83	1.14	1.61	0.87	-
	1i	5.76 (d, *J* = 10.3 Hz)	6.30 (overlapped with N'H Ala$_2$U)	3.57	2.19	3.80	1.11	1.60	0.88	-
	1j	5.78 (overlapped with N'HLeu$_1$U)	6.32 (overlapped with N'HAla$_2$U)	3.61	2.25	3.84	1.14	1.64	0.96	-

amino acid residue

Res	**Cmpd**	**NH**	$^{\alpha}$CH	$^{\beta}$CH	$^{\gamma}$CH	Side chain	OH
Gly	**1b**	6.41 (t, *J* = 5.6 Hz)	3.79	-	-	-	11.47 (broad signal)

α-Ala	**1c**	6.43 (d, *J* = 7.2 Hz, 1H) 6.50 (d, *J* = 6.8 Hz, 1H)	4.17	-	-	1.29 (CH_3)	12.06
β-hGly	**1f**	6.17 (t, *J* = 6.0 Hz)	2.38	3.29	-	-	11.81
β³hAla	**1g**	6.04 (d, *J* = 8.2 Hz, 1H)	2.28/2.51 (*diastereotopic)*	4.00	-	1.18 (CH_3)	11.85
GABA	**1h**	6.05 (t, *J* = 5.8 Hz)	2.28	1.62/1.70 (*diastereotopic)*	3.02/3.20 *(diastereotopic)*	-	in the backgr ound
γ-Phe	**1i**	5.79 (overlapped with N'HLeu$_1$U)	2.34/2.40 (*diastereotopic)*	1.44/1.83 (*diastereotopic)*	3.95	2.61/2.71 (CH_2, *diastereotopic*) 7.15-7.26 (Ph)	11.93
γ-Leu	**1j**	5.57 (overlapped with NHtBu and NHAla$_2$U)	2.37	1.34/1.80 (*diastereotopic)*	3.74	1.18 (CH_2) 1.63 (CH) 0.88 (CH_3)	11.99

*10% DMSO-d6 in CD_3CN

& doubling of signals resulting from epimerization of amino acid residue at C-terminus

7. Additional 1D and 2D NMR spectra

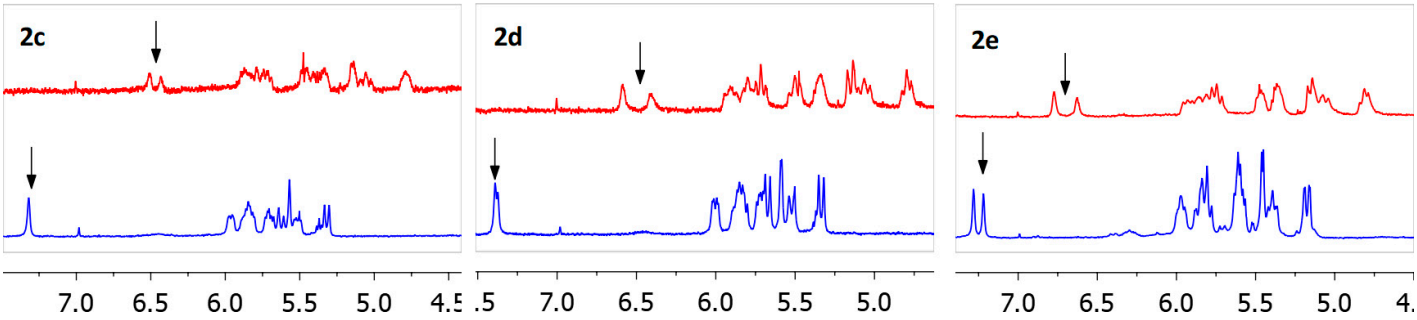


Figure S 47. Part of ^{1}H NMR spectra (300MHz) of oligourea-hydantoins **2c-2e** showing NH region. Red spectra were recorded in pure CD3CN, blue spectra were recorded in the mixture of DMSO-d_6 in CD_3CN. An arrow indicates hydantoin protons, doubled because of the epimerisation of the C-terminus amino acid residues

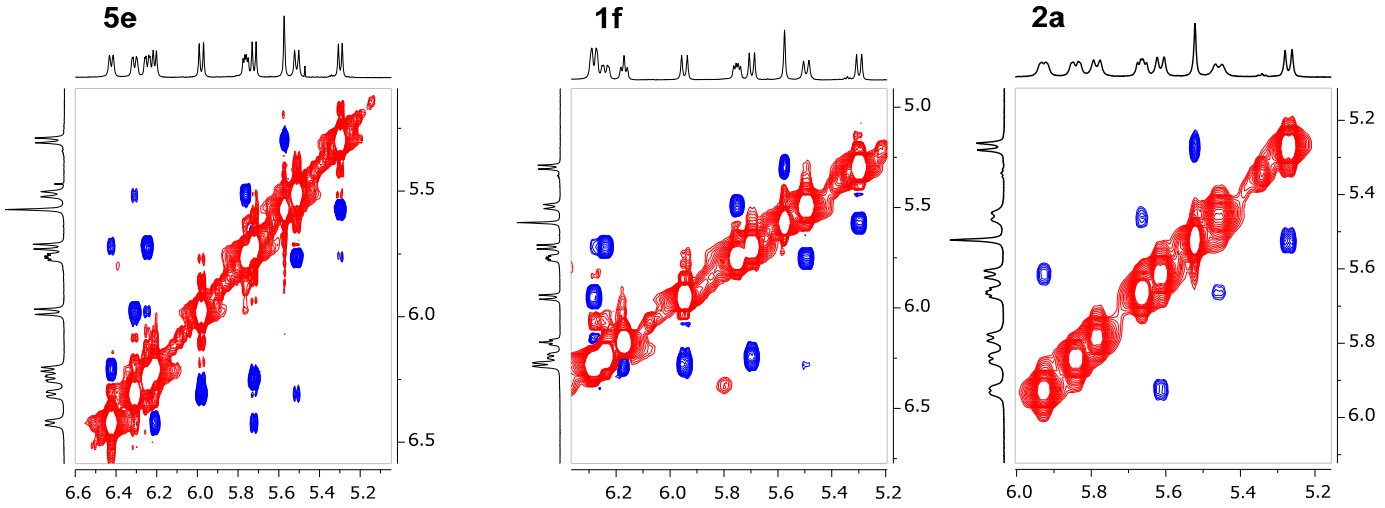


Figure S 48. Part of the NH/NH region of the ROESY spectra, showing NH/N'H cross peaks, characteristic for *trans/trans* conformation of urea groups in compounds **5e, 1f** and **2a**

8. RP-HPLC chromatograms of reaction mixtures of oligourea-esters 5 under alkaline conditions

Analytical RP-HPLC analyses were performed using a Jupiter 4u Proteo 90Å column (4.6 × 250 mm) at a flow rate of 1 mL × min^{-1}. The mobile phase was composed of 0.1% (v/v) TFA/H_2O (phase A) and 0.1% (v/v) TFA− CH_3CN (phase B). The detection was performed at 200 nm and a method 10% of B for 5 min. and a gradient 10-97% of B in 30 min was used.

a) reaction of 5a

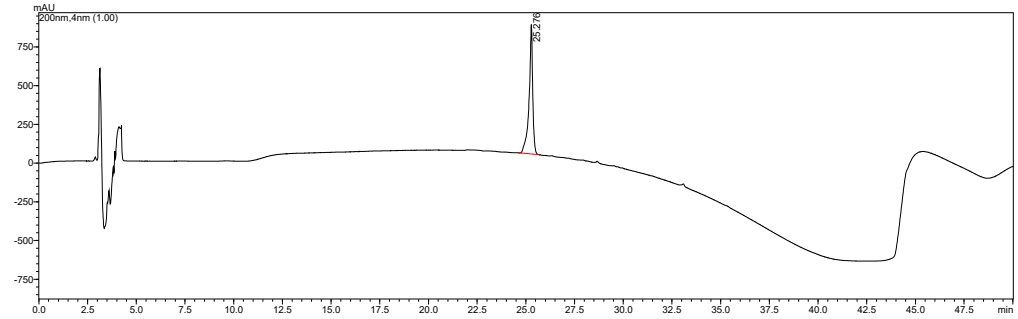


Figure S 49. RP-HPLC chromatogram of crude reaction mixture of **5a** under alkaline conditions. The product **2a** was formed

b) reaction of 5b

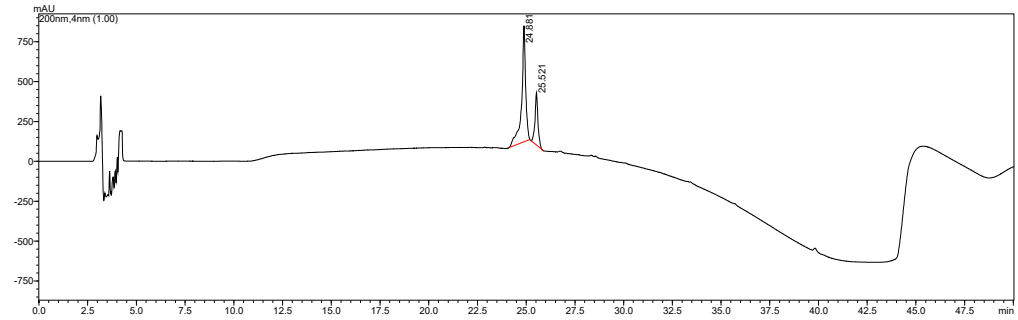


Figure S 50. RP-HPLC chromatogram of crude reaction mixture of **5b** under alkaline conditions. The products **1b**:**2b** were formed in 3:7 ratio.

c) reaction of 5c

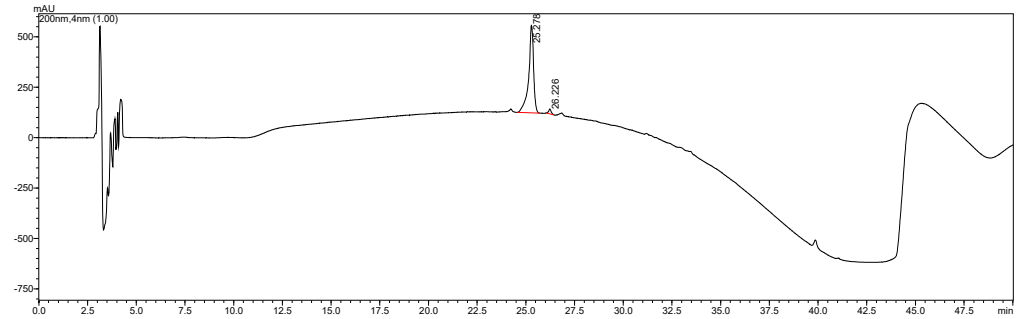


Figure S 51. RP-HPLC chromatogram of crude reaction mixture of **5c** under alkaline conditions. The products **1c**:**2c** were formed in 4:96 ratio.

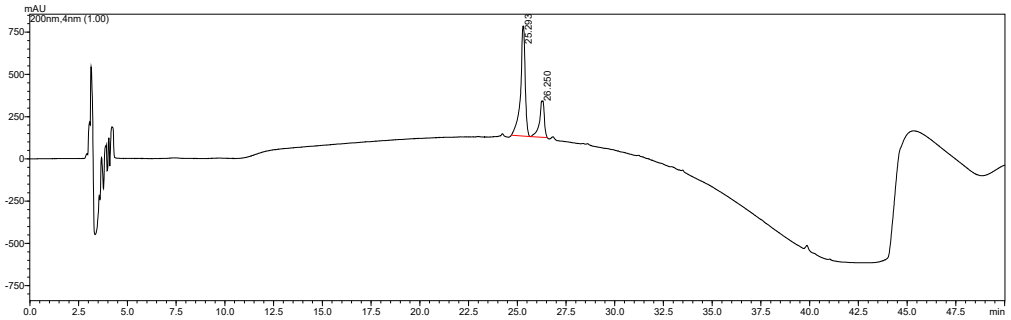


Figure S 52. RP-HPLC chromatogram of crude reaction mixture of **5c** under alkaline conditions after evaporation to dryness. The products **1c**:**2c** were formed in 3:7 ratio.

d) reaction of 5d

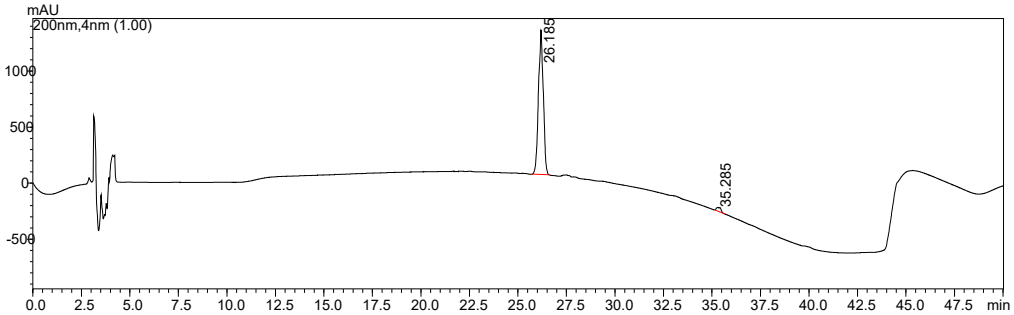


Figure S 53. RP-HPLC chromatogram of crude reaction mixture of **5d** under alkaline conditions. The product **2d** was formed

e) reaction of 5e

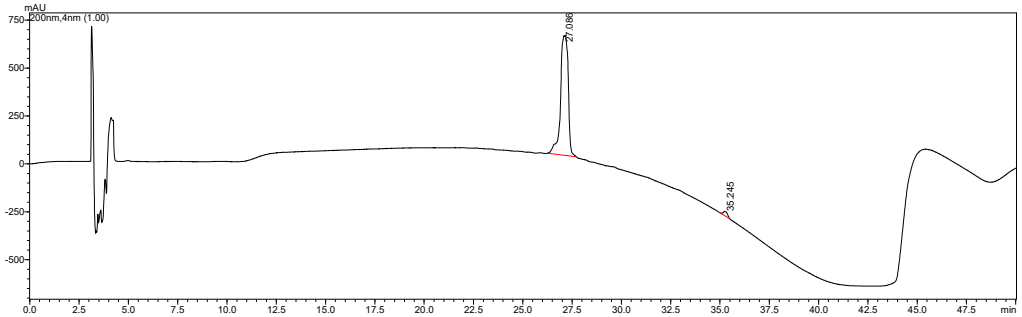


Figure S 54. RP-HPLC chromatogram of crude reaction mixture of **5e** under alkaline conditions. The product **2e** was formed

f) reaction of 5f

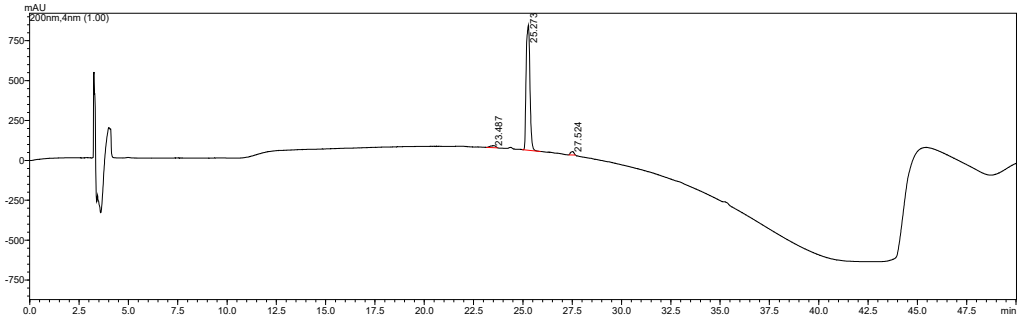


Figure S 55. RP-HPLC chromatogram of crude reaction mixture of **5f** under alkaline conditions. The product **1f** was formed

g) reaction of 5g

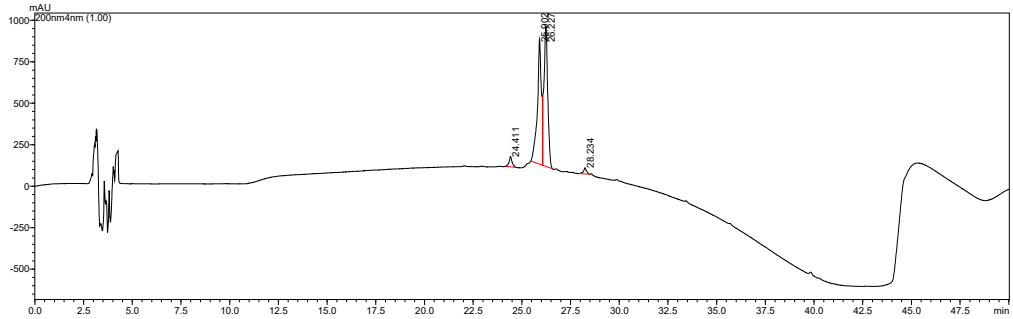


Figure S 56. RP-HPLC chromatogram of crude reaction mixture of **5g** under alkaline conditions. The products **1g**:**2g** were formed in 1:1 ratio.

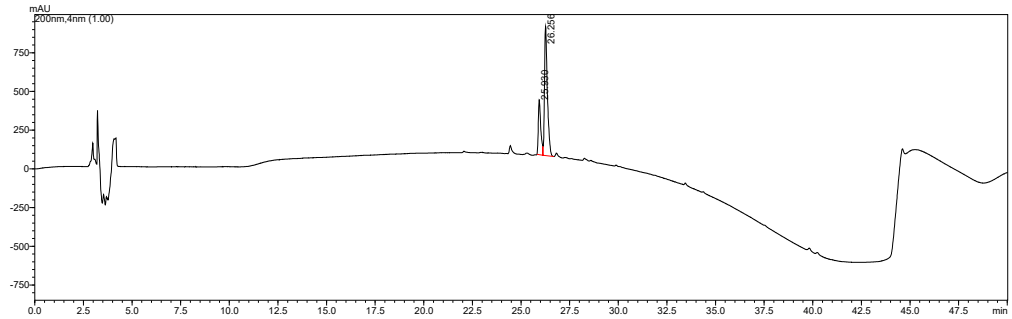


Figure S 57. RP-HPLC chromatogram of crude reaction mixture of **5g** under alkaline conditions after evaporation to dryness. The products **1g**:**2g** were formed in 7:3 ratio.

h) reaction of 5h

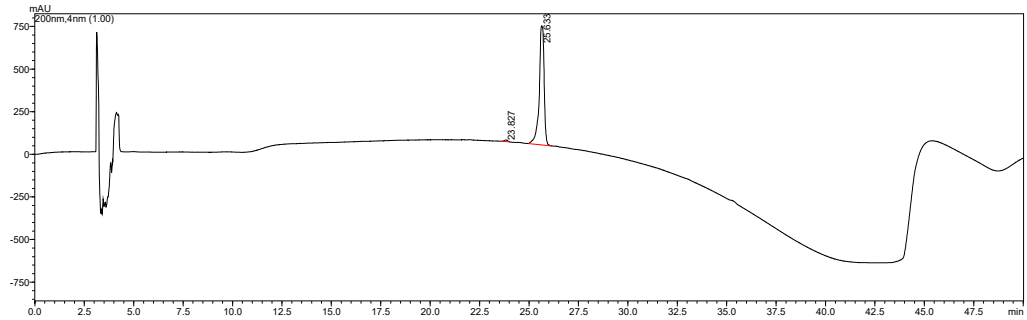


Figure S 58. RP-HPLC chromatogram of crude reaction mixture of **5h** under alkaline conditions. The product **1h** was formed.

i) reaction of 5i

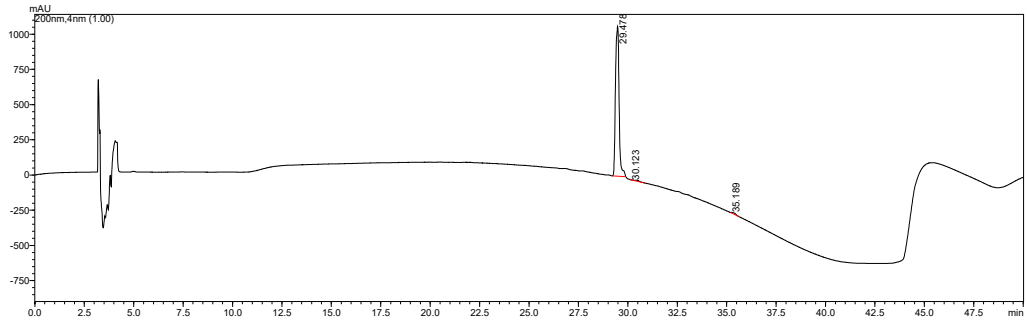


Figure S 59. RP-HPLC chromatogram of crude reaction mixture of **5i** under alkaline conditions. The product **1i** was formed.

j) reaction of 5j

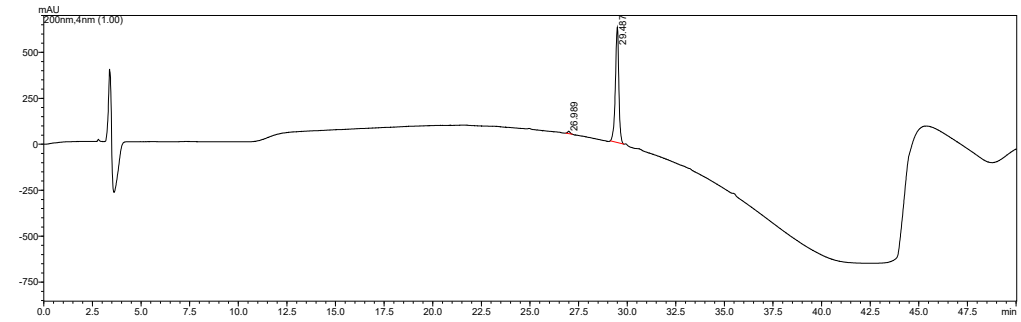


Figure S 60. RP-HPLC chromatogram of crude reaction mixture of **5j** under alkaline conditions. The product **1j** was formed.

9. HRMS of building blocks, oligourea esters and final compounds

a) building blocks BB1-4

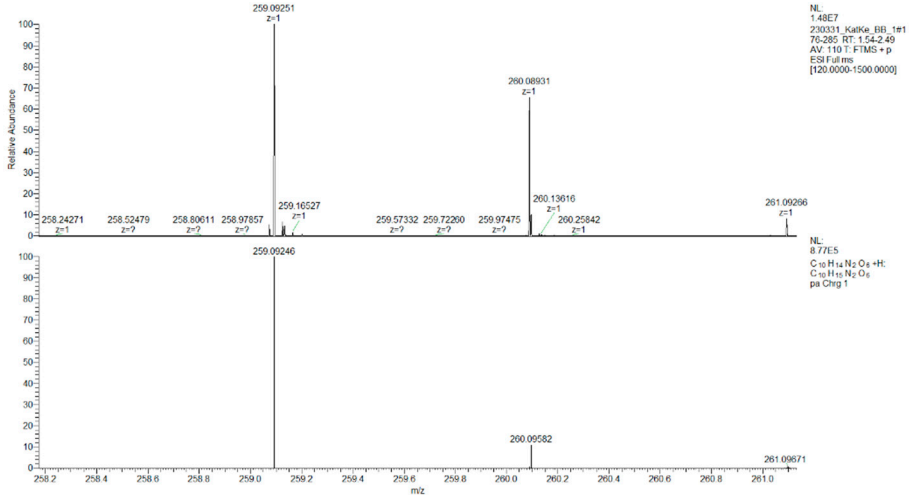


Figure S 61. HRMS spectra of building block **BB1**

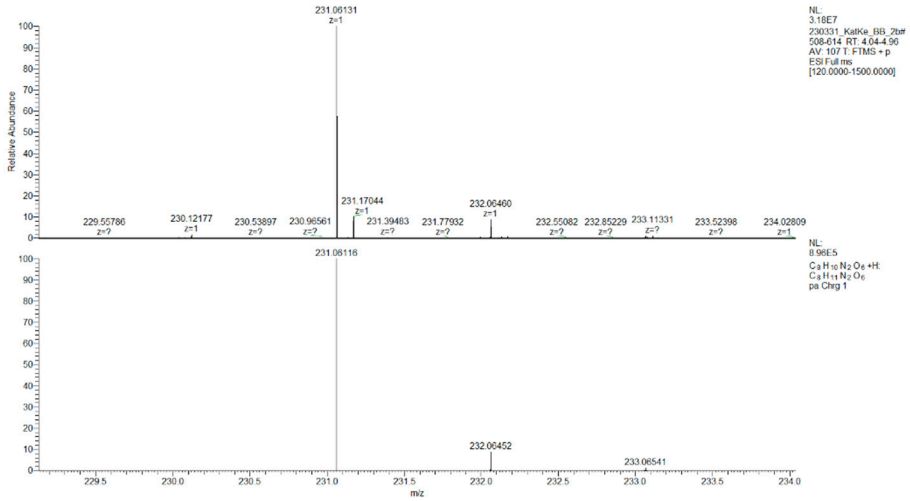


Figure S 62. HRMS spectra of building block **BB2b**

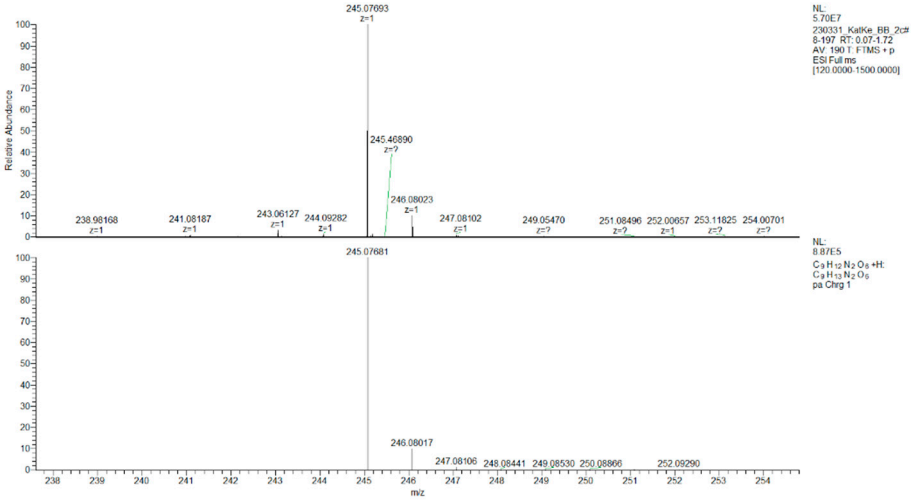


Figure S 63. HRMS spectra of building block **BB2c**

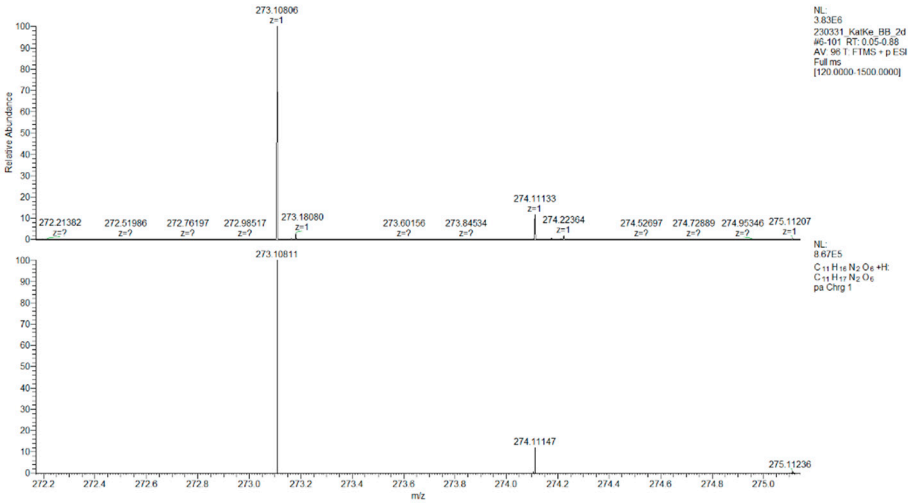


Figure S 64. HRMS spectra of building block **BB2d**

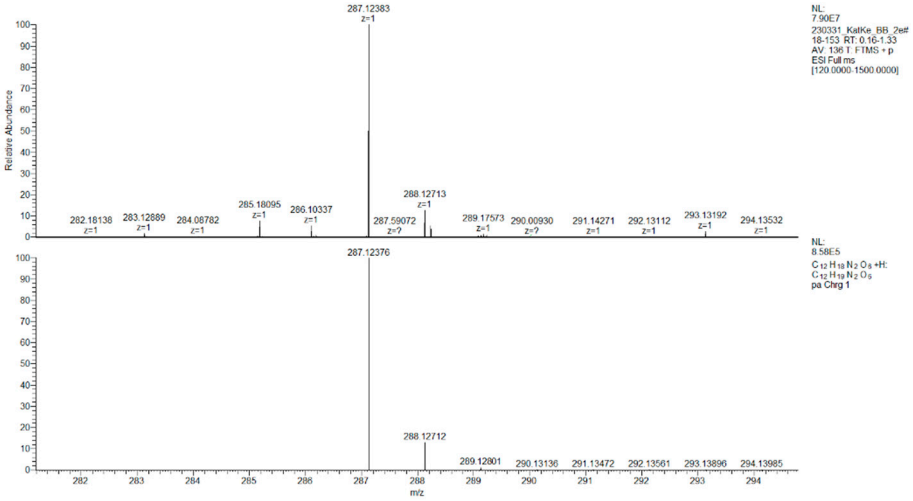


Figure S 65. HRMS spectra of building block **BB2e**

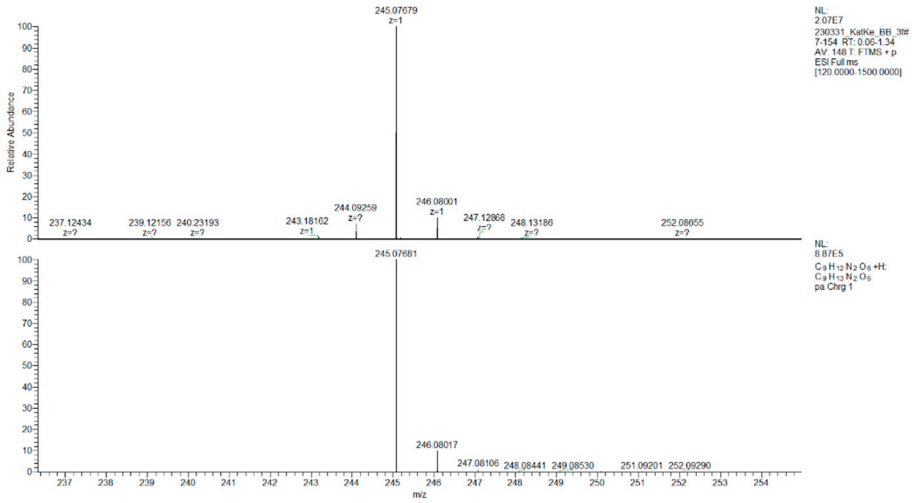


Figure S 66. HRMS spectra of building block **BB3f**

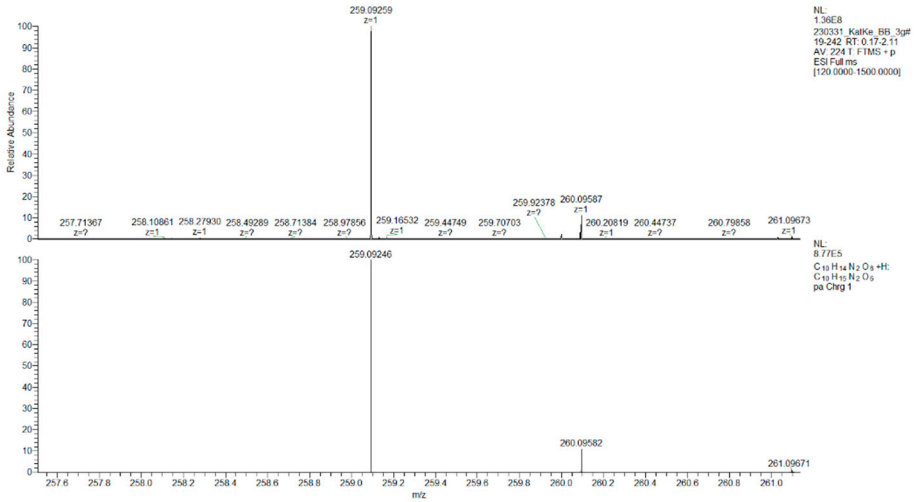


Figure S 67. HRMS spectra of building block **BB3g**

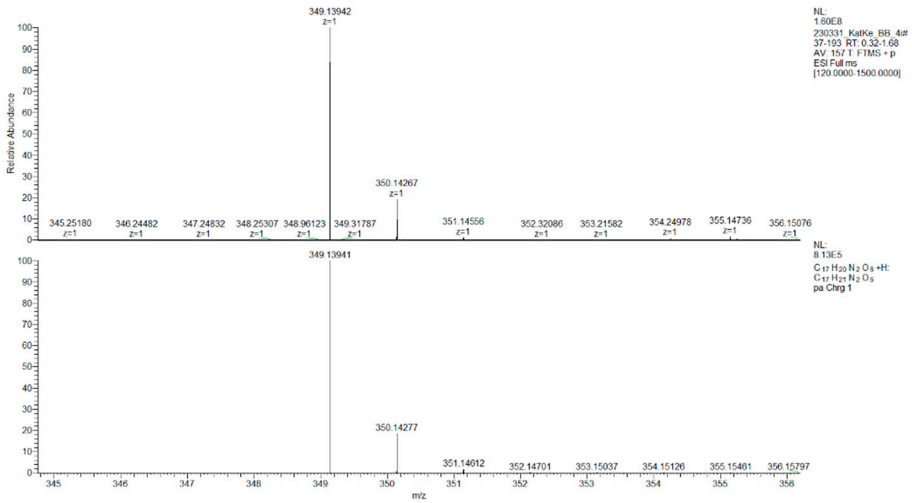


Figure S 68. HRMS spectra of building block **BB4i**

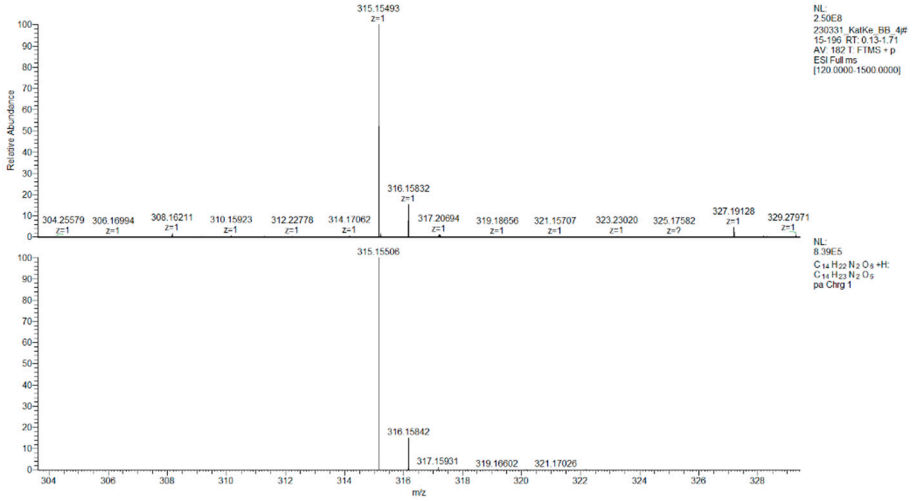


Figure S 69. HRMS spectra of building block **BB4j**

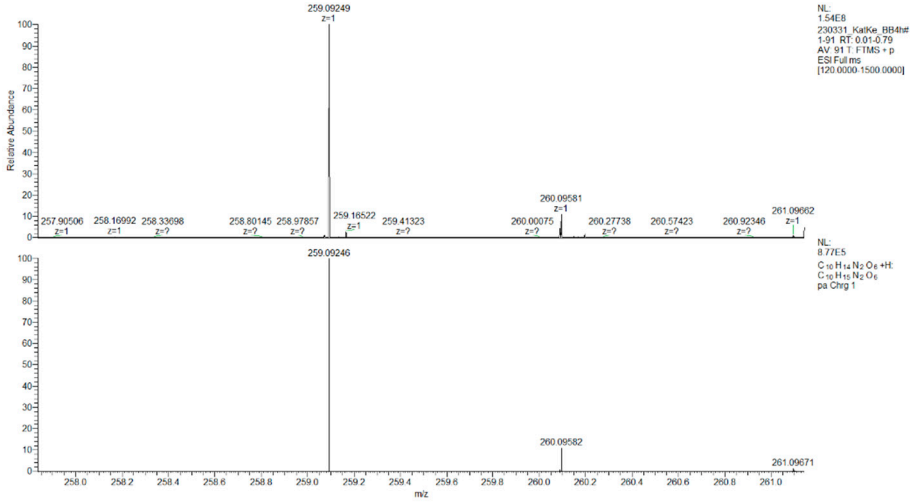


Figure S 70. HRMS spectra of building block **BB4h**

b) oligourea-esters 5

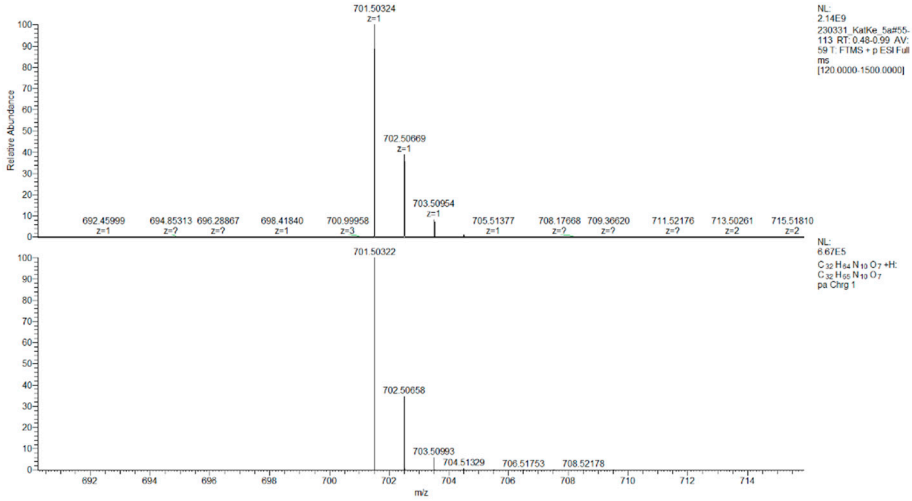


Figure S 71. HRMS spectra of compound **5a**

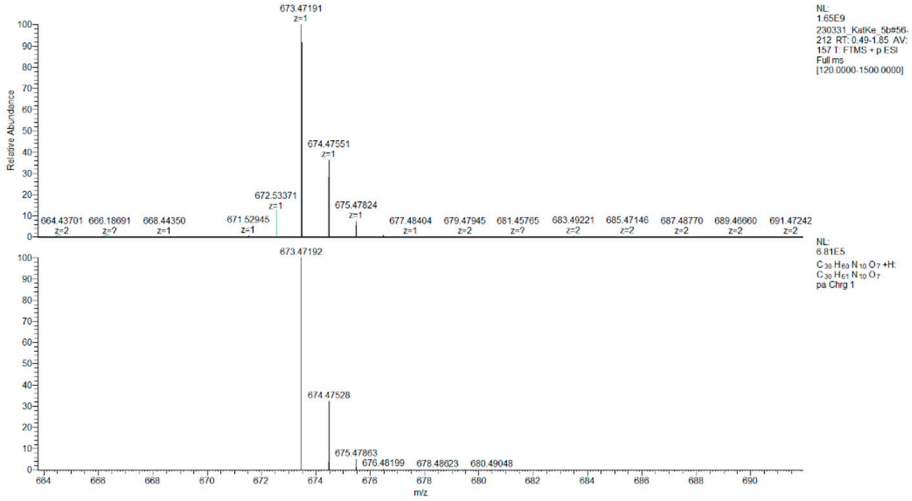


Figure S 72. HRMS spectra of compound **5b**

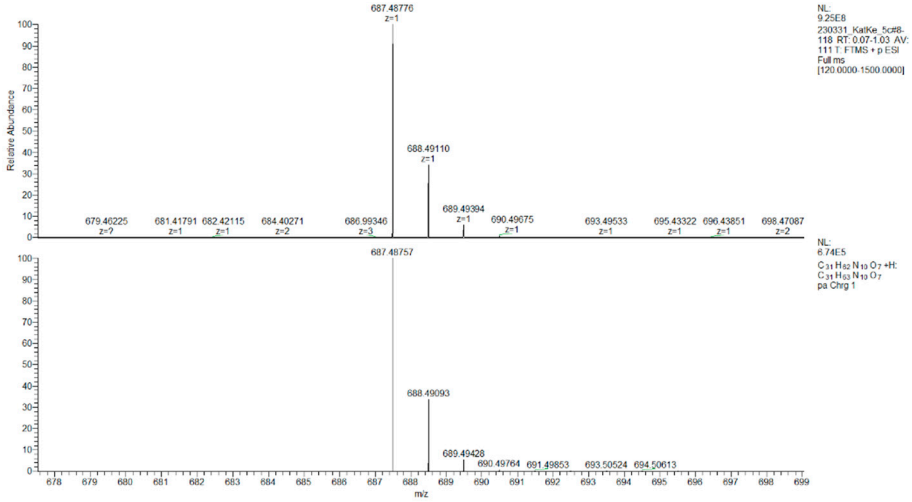


Figure S 73. HRMS spectra of compound **5c**

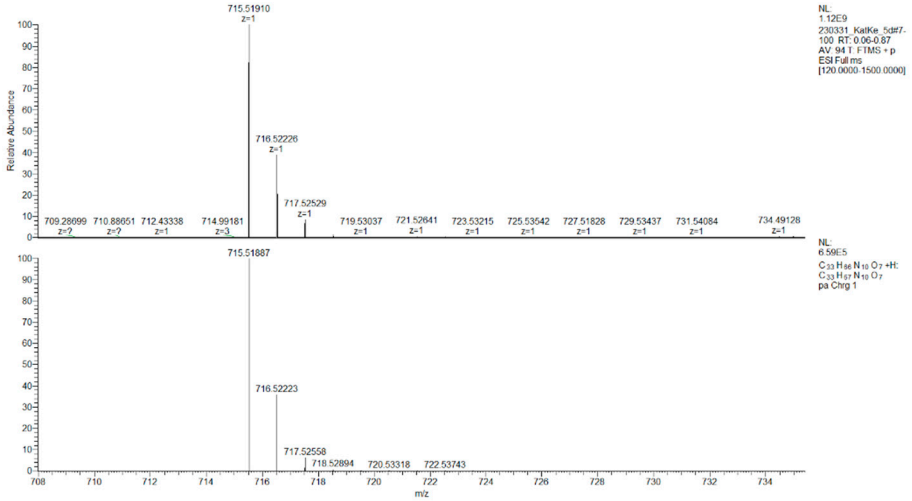


Figure S 74. HRMS spectra of compound **5d**

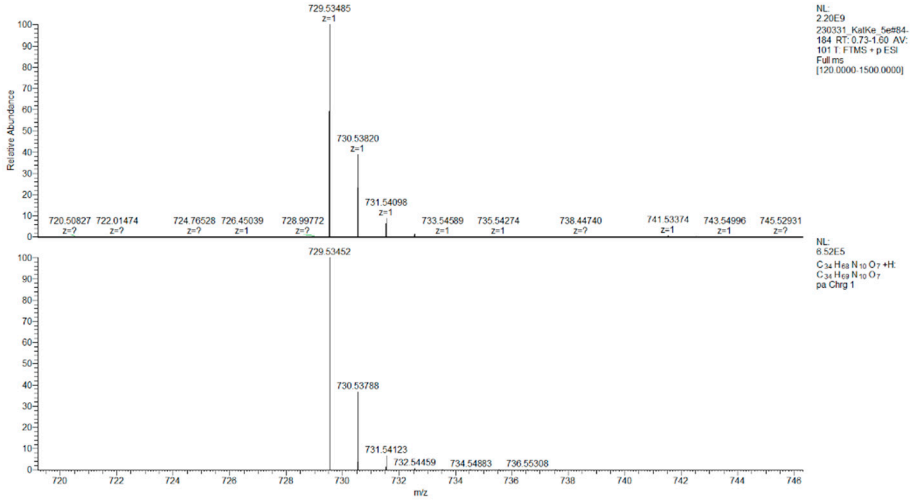


Figure S 75. HRMS spectra of compound **5e**

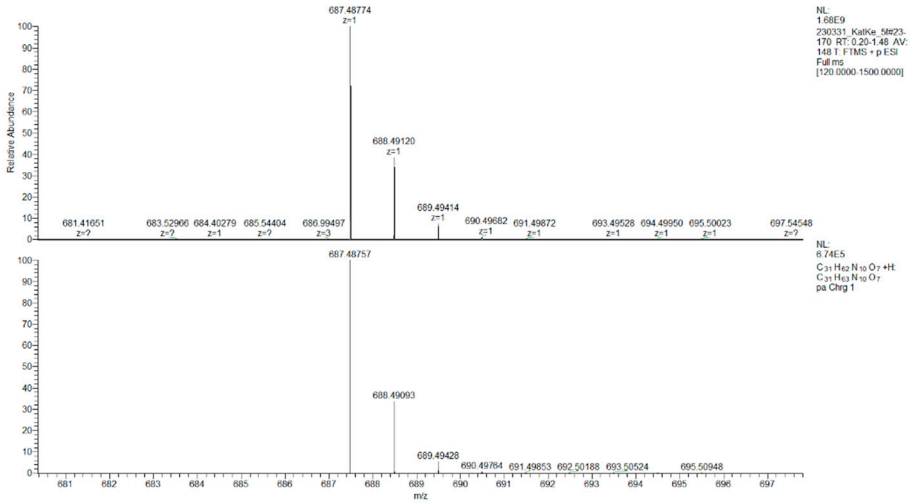


Figure S 76. HRMS spectra of compound **5f**

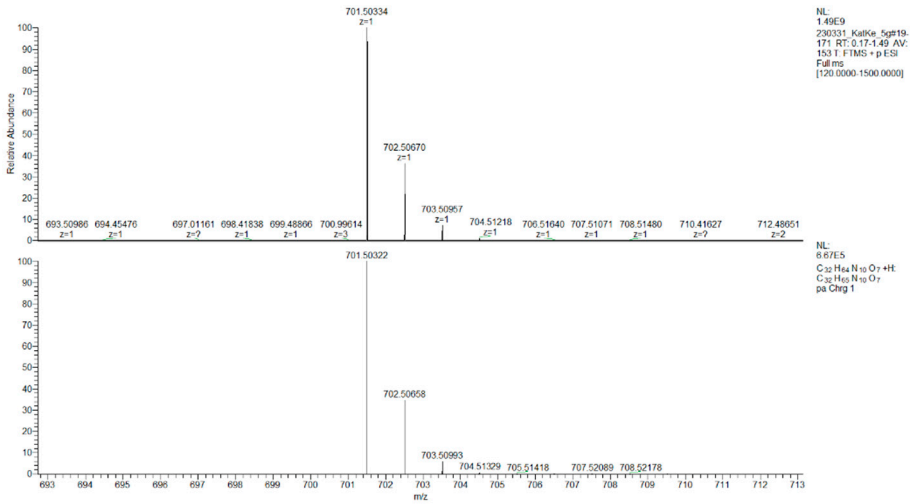


Figure S 77. HRMS spectra of compound **5g**

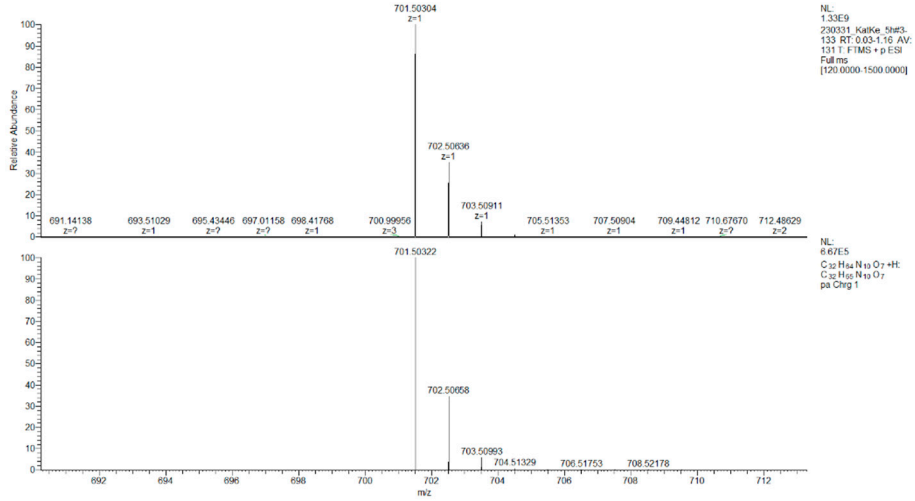


Figure S 78. HRMS spectra of compound **5h**

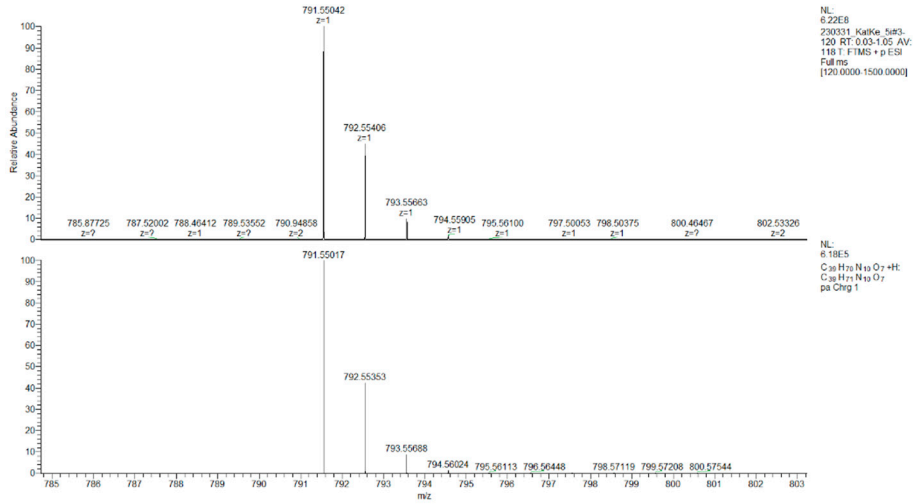


Figure S 79. HRMS spectra of compound **5i**

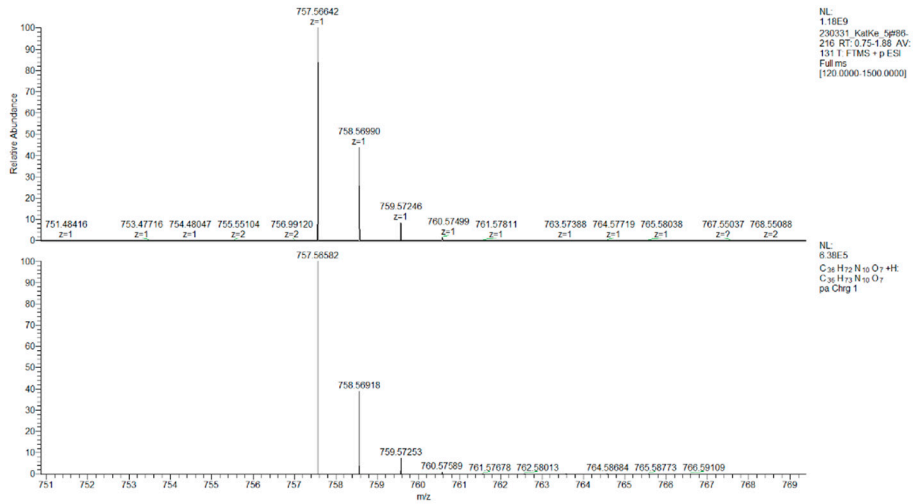


Figure S 80. HRMS spectra of compound **5j**

c) oligourea-acids 1

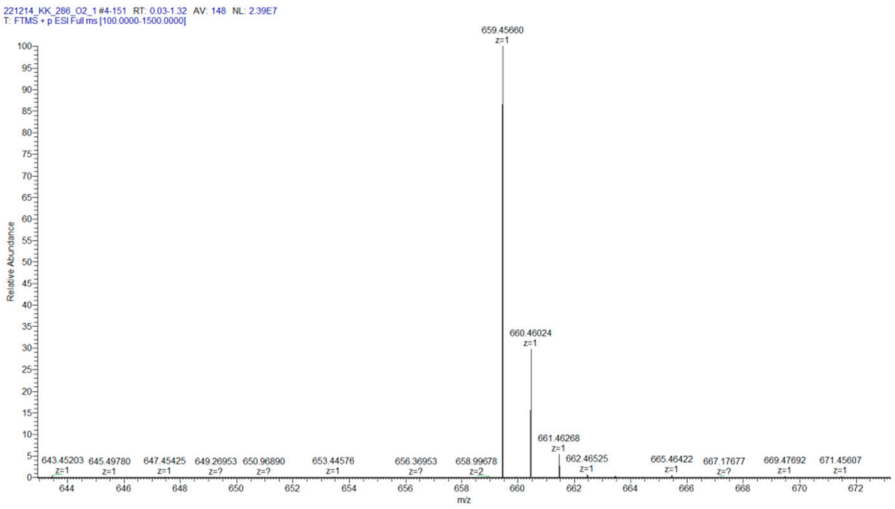


Figure S 81. HRMS spectra of compound **1b**.

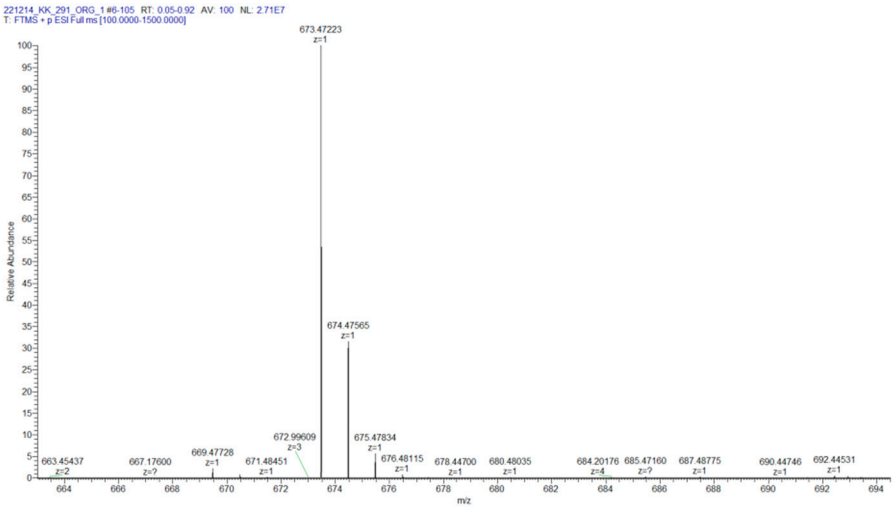


Figure S 82. HRMS spectra of compound **1c**

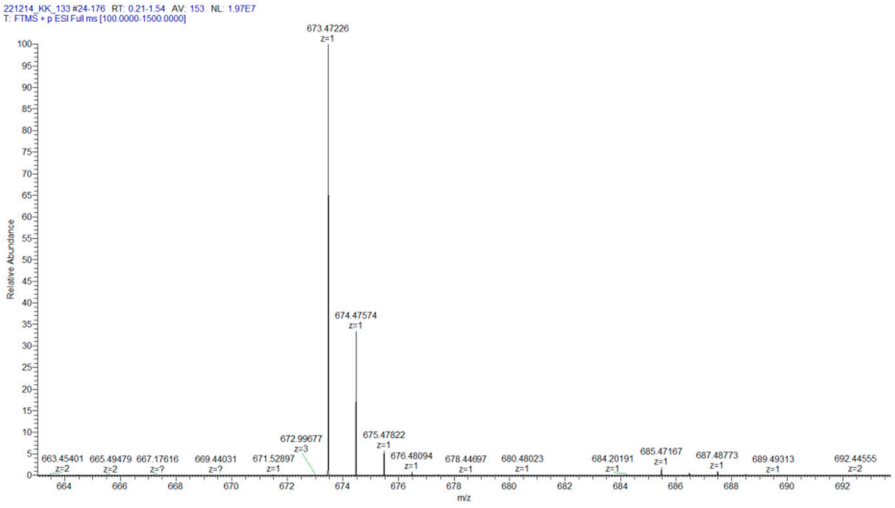


Figure S 83. HRMS spectra of compound **1f**

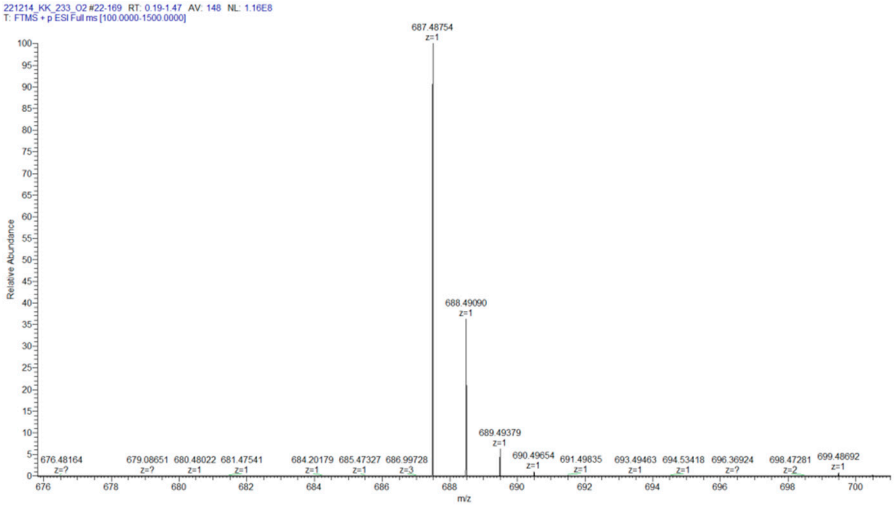


Figure S 84. HRMS spectra of compound **1g**

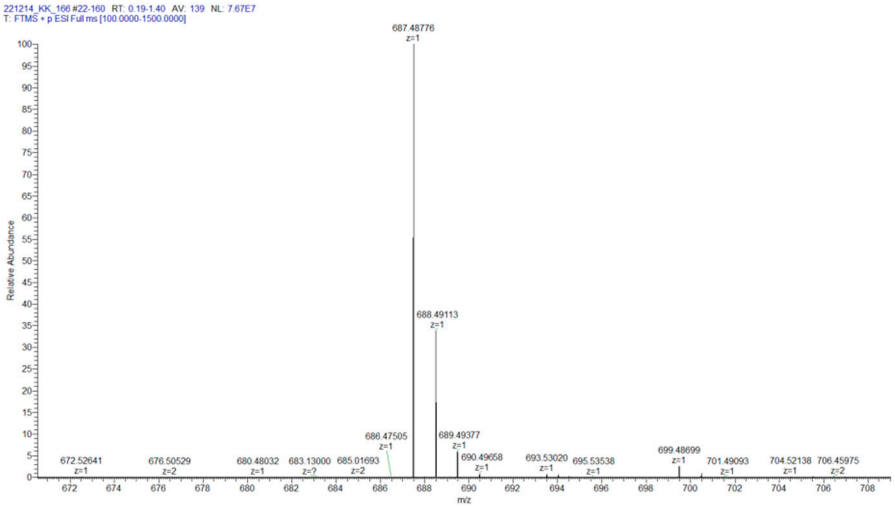


Figure S 85. HRMS spectra of compound **1h**

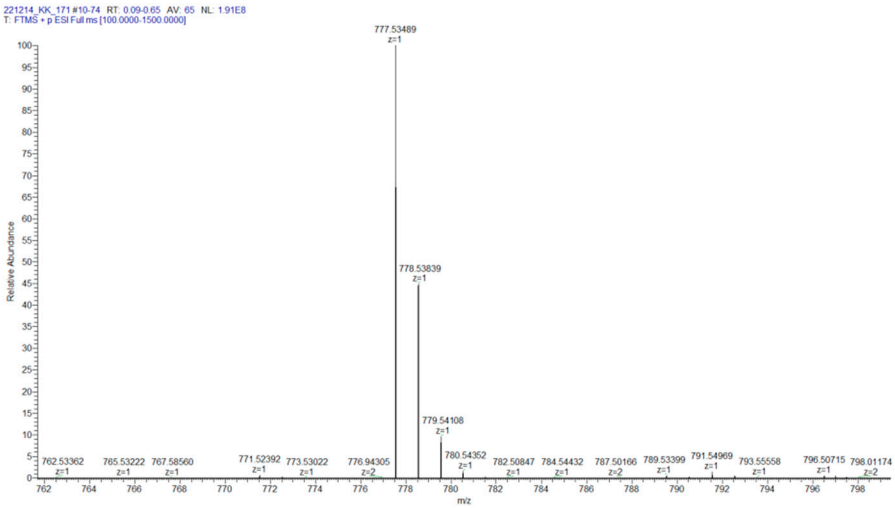


Figure S 86. HRMS spectra of compound **1i**

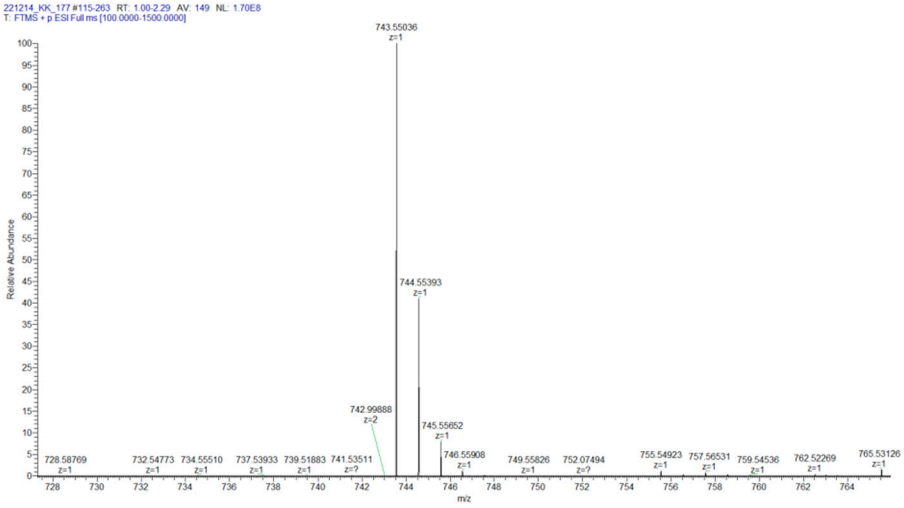


Figure S 87. HRMS spectra of compound **1j**

d) oligourea-hydantoin/dihydrouracil 2

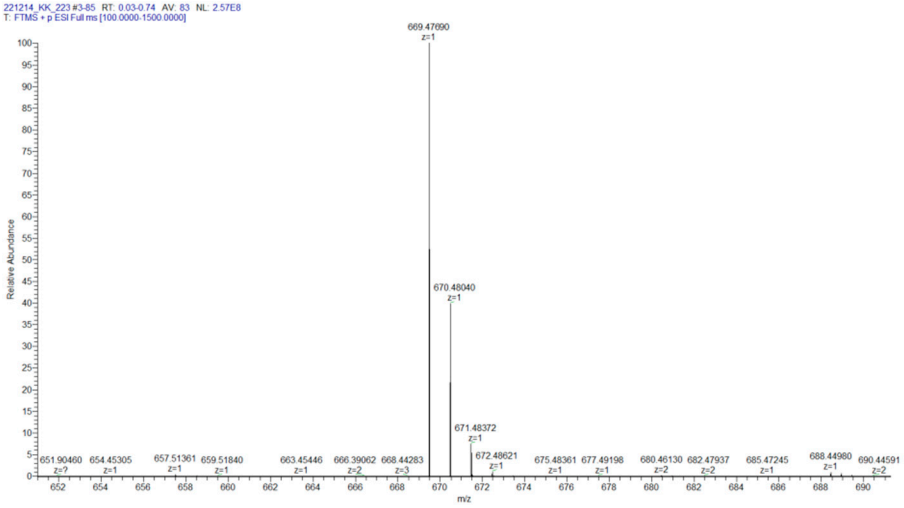


Figure S 88. HRMS spectra of compound **2a**

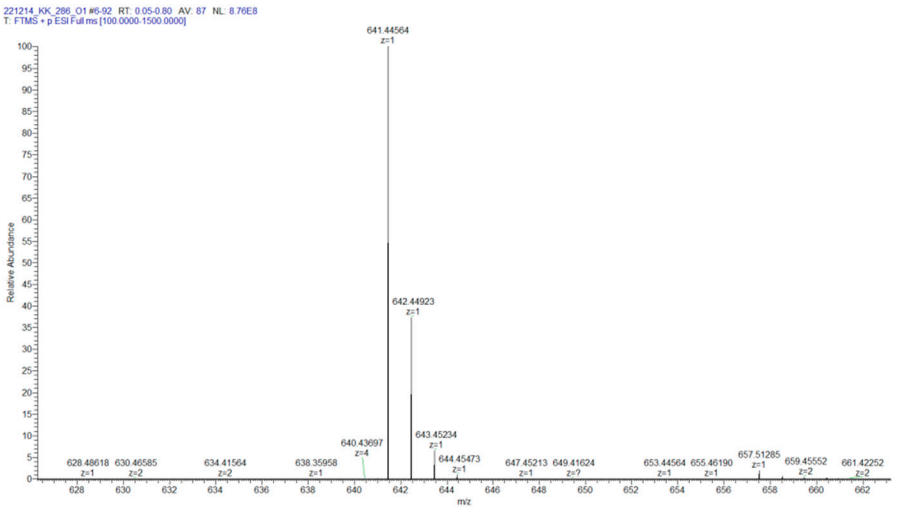


Figure S 89. HRMS spectra of compound **2b**

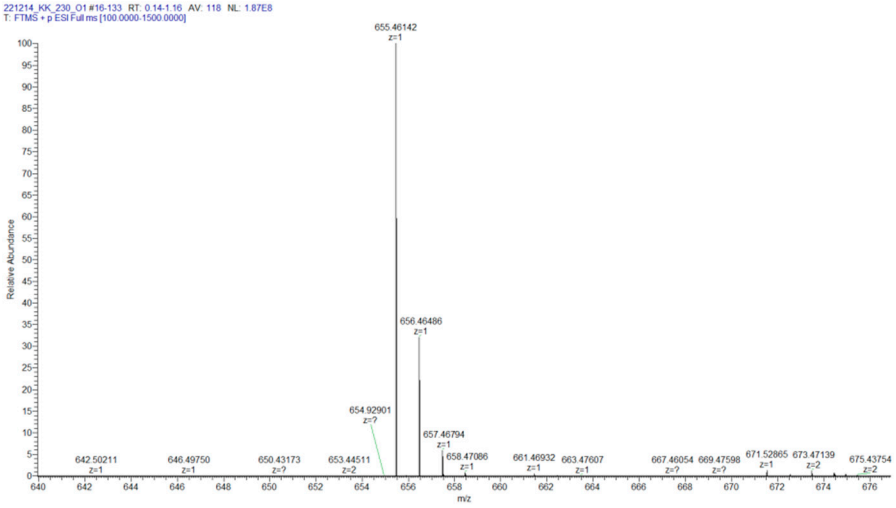


Figure S 90. HRMS spectra of compound **2c**

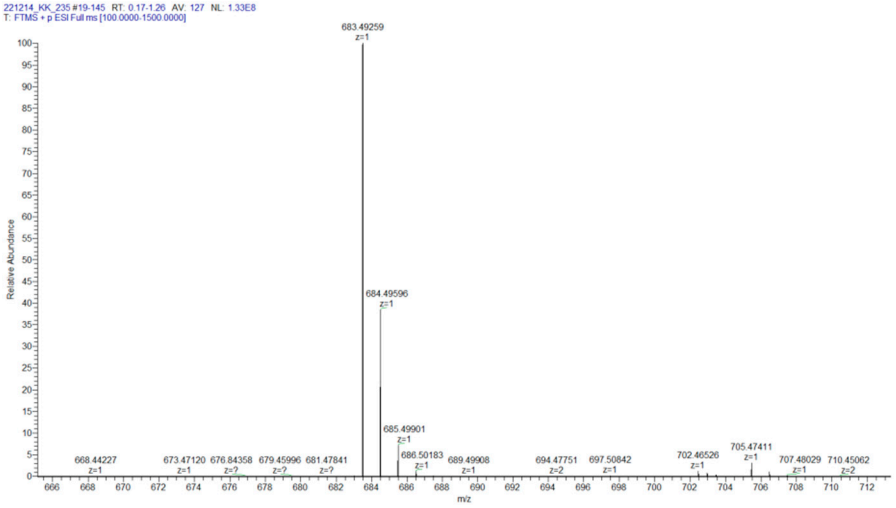


Figure S 91. HRMS spectra of compound **2d**

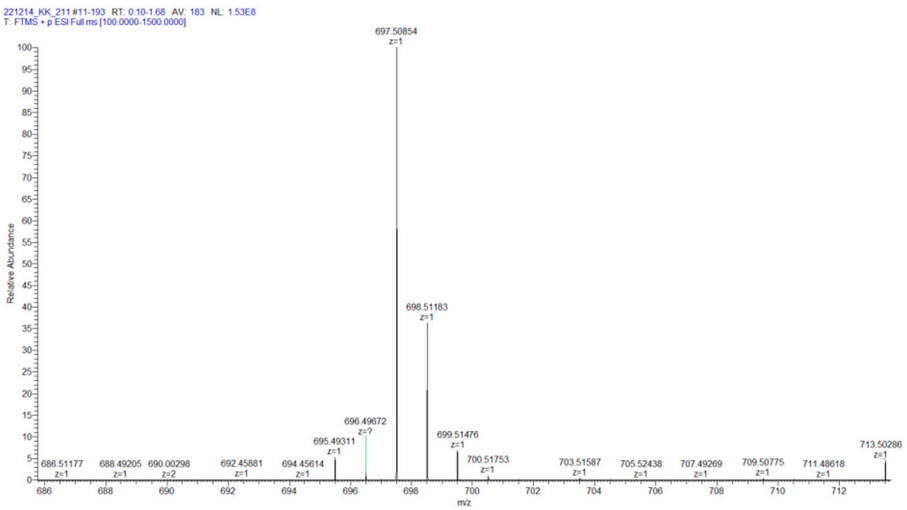


Figure S 92. HRMS spectra of compound **2e**

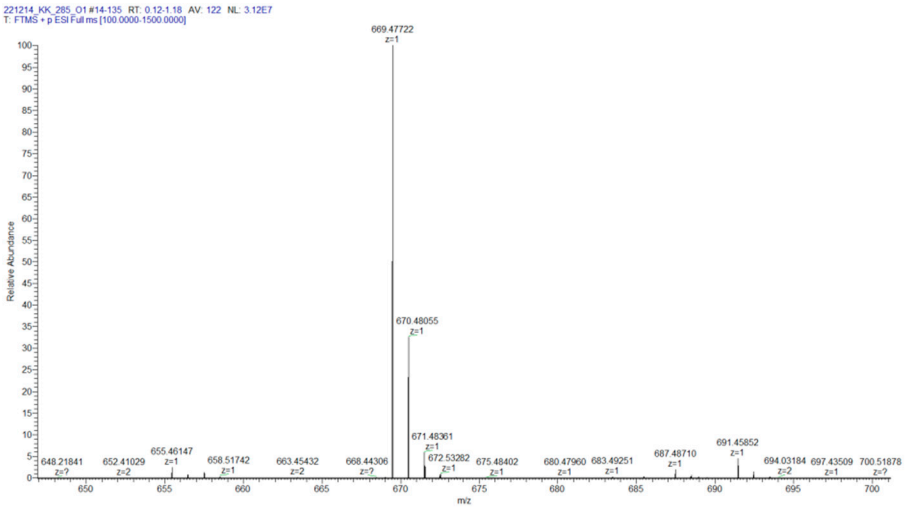


Figure S 93. HRMS spectra of compound **2g**

10. CD spectra of selected oligourea-esters 5, oligourea-acids 1 and oligourea-hydantoin/dihydrouracil 2

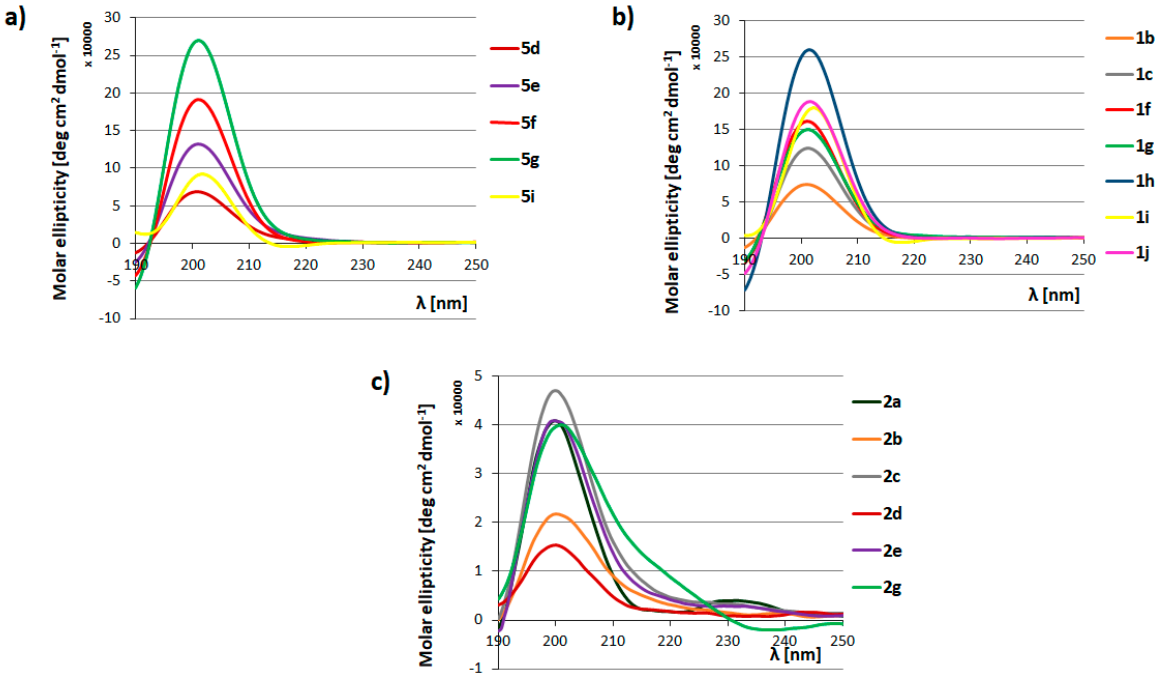


Figure S 94. ECD spectra measured at c= 0.2 mM in TFE, at 20°C: a) oligourea-esters; b) oligourea-acids; c) oligourea-hydantoins/dihydrouracil

11. Additional HPLC chromatograms

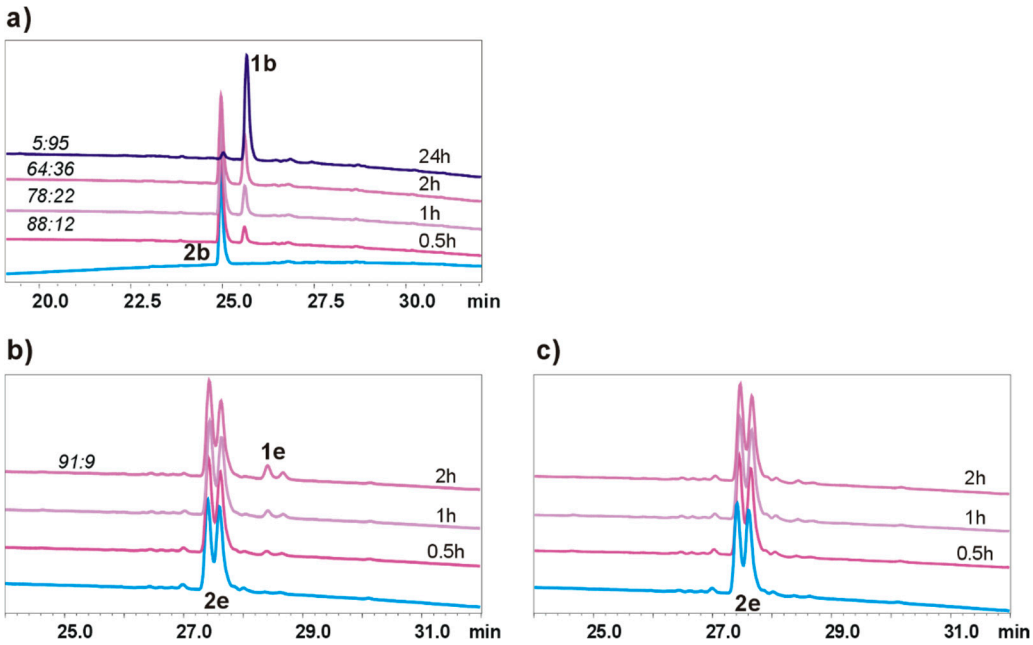


Figure S 95. Stability studies of oligourea-hydantoin under alkaline conditions: a) stability studies of compound **2b** at RT; b) stability studies of compound **2e** at 45°C; c) stability studies of compound **2e** at RT

12. Crystallography

The X-ray measurement of **2a** was performed at 130.0(5) K on a Bruker D8 Venture PhotonII diffractometer equipped with a INCOATEC IμS micro-focus source (CuKα, λ = 1.54178 Å) and a mirror monochromator. A total of 3586 frames were collected with Bruker APEX3 program.[1] The frames were integrated with the Bruker SAINT software package[2] using a narrow-frame algorithm. The integration of the data using a monoclinic unit cell yielded a total of 71438 reflections to a maximum ϑ angle of 59.09° (0.90 Å resolution), of which 22715 were independent (average redundancy 3.145, completeness = 99.4%, R_{int} = 4.85%, R_{sig} = 5.04%) and 16483 (72.56%) were greater than $2\sigma(F^2)$. The final cell constants of a = 14.9921(4) Å, b = 35.4478(9) Å, c = 15.0011(4) Å, β = 90.7754(18)°, V = 7971.4(4) Å^3, are based upon the refinement of the XYZ-centroids of 9904 reflections above 20 $\sigma(I)$ with 5.895° < 2ϑ < 117.8°. Data were corrected for absorption effects using the Multi-Scan method (SADABS).[3] The ratio of minimum to maximum apparent transmission was 0.863. The calculated minimum and maximum transmission coefficients (based on crystal size) are 0.784 and 0.964.

The structure was solved and refined using SHELXTL Software Package[4,5] using the space group $P2_1$, with Z = 1 for the formula unit, $C_{248}H_{474}N_{80}O_{53}$ and Flack parameter[6] yielding 0.01(8). The final anisotropic full-matrix least-squares refinement on F^2 with 1802 variables converged at $R1$ = 7.78%, for the observed data and $wR2$ = 23.16% for all data. The goodness-of-fit was 1.036. The largest peak in the final difference electron density synthesis was 0.460 e$^-$/Å^3 and the largest hole was -0.317 e$^-$/Å^3 with an RMS deviation of 0.060 e$^-$/Å^3. On the basis of the final model, the calculated density was 1.130 g/cm^3 and F(000), 2946 e$^-$. Crystal data and refinement parameters for **2a** are collected in **Table S 3**. The crystal contains in the asymmetric part four oligourea molecules and 2½ water moieties (hydrogen atoms not assigned resulting in three Alerts B in the checkCIF report). The scattering power of the crystal is limited giving another Alert B. In one of the oligourea the hydantoin part is disordered over two positions with refined occupancy ratio yielding 0.69(2):0.31(2) what is associated with two alternative positions of one of the water molecules and occupancy ratio equal 0.31(2):0.69(3). The crystal is probably composed of two domains rotated along Y axis. This is probably related to pseudotetragonal symmetry of the lattice. The structure was refined with TWIN instruction 0 0 -1 0 1 0 1 0 0 (4-fourfold rotation along Y) giving BASF parameter equal 0.062(2). Introduction of the TWIN refinement slightly improved final R parameters.

During structure refinement a number of restraints (distances, angles, ADPs) was used. All major component (>0.5) disordered moieties were refined anisotropically. All hydrogen atoms were placed in calculated positions and refined within the riding model. The temperature factors of hydrogen atoms were not refined and were set to be either 1.2 or 1.5 times larger than U_{eq} of the corresponding heavy atom. The atomic scattering factors were taken from the International Tables.[7] Molecular graphics was prepared using program Mercury 2022.3.0.[8] Atomic displacement parameters of **2a** are presented in **Figure S 96**. Packing of the molecules are presented in **Figure S 97**. The structure in the form of ribbon representation generated in CCP4mg package[9] is presented in **Figure S 98**.

Table S 3. Data collection and structure refinement parameters for **2a**

Formula	$C_{248}H_{474}N_{80}O_{53}$
M_x/ g·mol^{-1}	5425.04
T/ K	130.0(5)
λ/ Å	1.54178
Crystal size	0.056 × 0.172 × 0.389
Space group	$P2_1$
Unit cell dimensions	a = 14.9921(4) Å b = 35.4478(9) Å β = 90.7754(18)° c = 15.0011(4) Å
V/ Å^3, Z	7971.4(4), 1
D_x/ g·cm^{-3}	1.130
μ/ mm^{-1}	0.659
F(000)	2946
ϑ_{min}, ϑ_{max}	2.49°, 59.09°
Index ranges	-15 ≤ h ≤ 16 -39 ≤ k ≤ 39 -16 ≤ l ≤ 16
Reflections collected/ independent	71438 / 22715 R_{int} = 0.0485
Completeness	99.4%
Absorption correction	Multi-Scan
T_{max}, T_{min}	0.964, 0.784
Refinement method	Full-matrix LSQ on F^2
Data / restraints / parameters	22715 / 118 / 1802
Goodness-of-fit on F^2	1.036
Final R indices	16483 data; I>2σ(I) R1 = 0.0778, wR2 = 0.2094

	all data *R*1 = 0.1005, *wR*2 = 0.2316
Absolute structure parameter	0.01(8)
$\Delta\rho_{max}$, $\Delta\rho_{min}$	0.460, -0.317 eÅ^{-3}

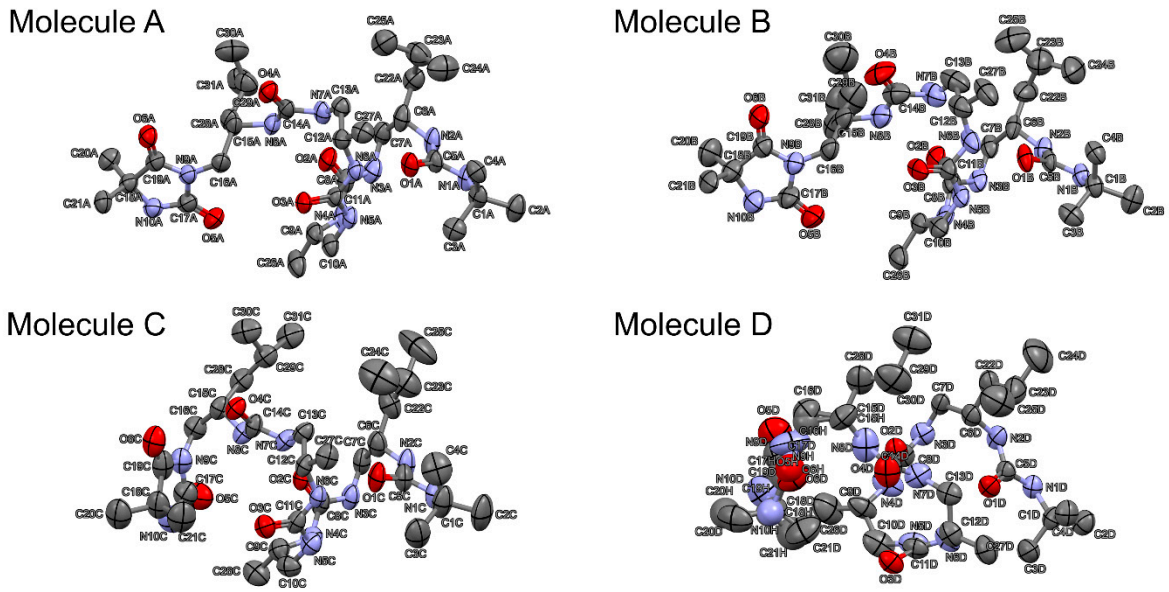


Figure S 96. Atomic displacement parameters of **2a** at 50% probability level presented for each molecule separately. Solvent moieties and hydrogen atoms omitted for clarity.

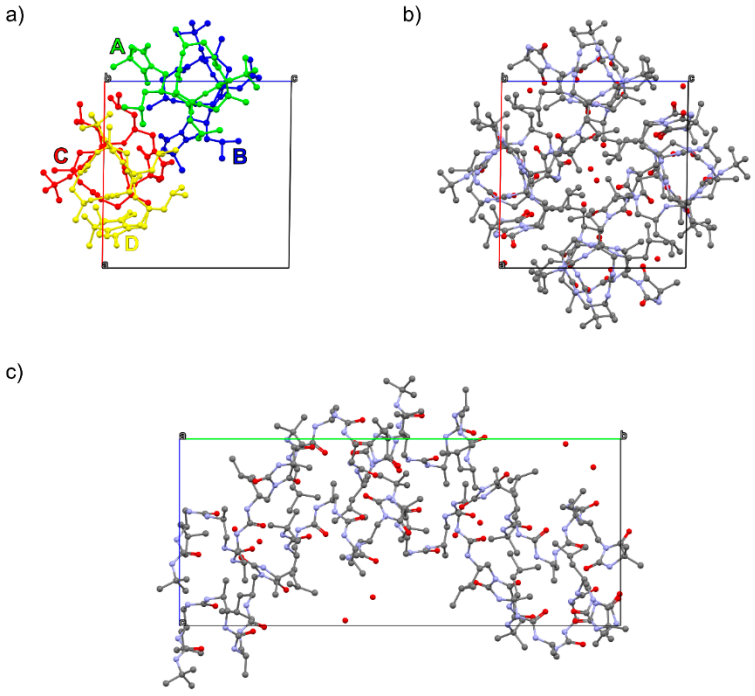


Figure S 97. Asymmetric part of the structure with differently coloured molecules a) and packing diagrams of **2a** - view along [010] b) and [100] c). H atoms omitted for clarity.

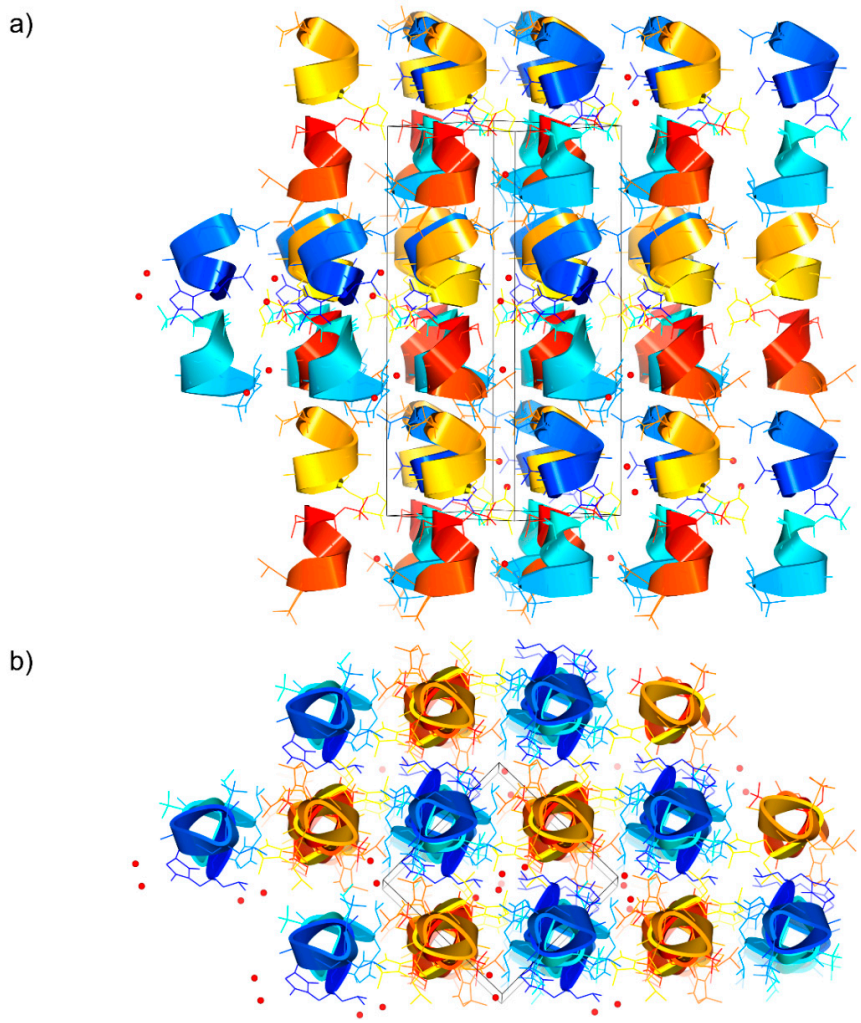


Figure S 98. Structure view using ribbon representation for each peptide molecule, view parallel to [010] a), view along [010] b).

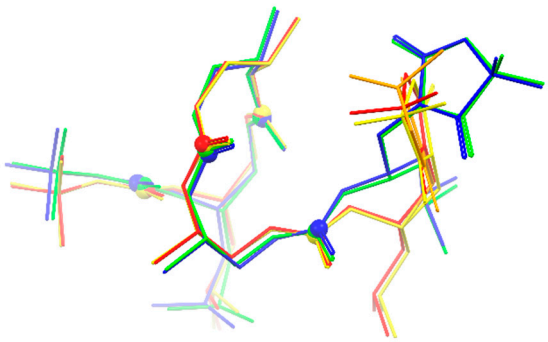
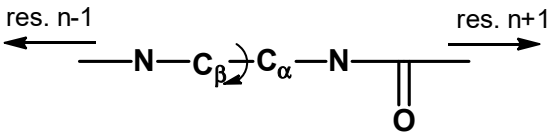


Figure S 99. Overlay of molecules A-D; matching for all urea carbons (indicated as balls)

Table S 4. The backbone torsion angles in **2a** (taken from the crystal structure)



Residue	A	B	C	D
Leu1u	50(1)	54(1)	54(1)	54(1)
Ala2u	52.8(9)	57.4(9)	54(1)	55(1)
Ala3u	53.8(9)	47(1)	52.5(9)	55(1)
Leu4(hyd)	-159.3(7)	-163.6(8)	41(1)	43(2)

13. References

1. APEX3 V2019, Bruker Nano, Inc., **2019**.
2. SAINT V8.40A, Bruker Nano, Inc., **2019**.
3. SADABS V2016/2, Bruker Nano, Inc., **2019**.
4. *G. M. Sheldrick*, *Acta Cryst.*, **2015**, *A71*, 3-8.
5. G. M. Sheldrick, *Acta Cryst*., **2015**, *C71*, 3–8.
6. S. Parsons, H. D. Flack, T. Wagner, *Acta Cryst.*, **2013**, *B69*, 249–259.
7. *International Tables for Crystallography*, Ed. A. J. C. Wilson, Kluwer: Dordrecht, **1992**, Vol.C.
8. C. F. Macrae, I. Sovago, S. J. Cottrell, P. T. A. Galek, P. McCabe, E. Pidcock, M. Platings, G. P. Shields, J. S. Stevens, M. Towler and P. A. Wood, "Mercury 4.0: from visualization to analysis, design and prediction", *J. Appl. Cryst.*, **2020**, *53*, 226-235.
9. S. McNicholas, E. Potterton, K. S. Wilson, M. E. M. Noble, Presenting your structures: the CCP4mg molecular-graphics software, *Acta Cryst.*, **2011**, *D67*, 386-394.