Supporting information for:

The Influence of Ethylene Glycol Chains on the Thermodynamics of Hydrogen-bonded Supramolecular Assemblies in Apolar Solvents.

Tom F. A. de Greef, Marko M. L. Nieuwenhuizen, Patrick J. M. Stals, Carel F. C. Fitié, Anja R. A. Palmans, Rint P. Sijbesma and E. W. Meijer*

Laboratory of Macromolecular and Organic Chemistry, Eindhoven University of Technology, P.O. Box 513,

5600 MB Eindhoven, The Netherlands

RECEIVED DATE (automatically inserted by publisher); e.w.meijer@tue.nl

Extended list of references

Due to space limitations we are unable to cite a number of important papers using oligo and poly ethylenegycol chains in supramolecular assemblies. Although the list reported here is far from complete it does give the reader a broader view on the subject.

General

1) Brunsveld, L.; Vekemans, J. A. J. M.; Hirschberg, J. H. K. K., Sijbesma, R. P.; Meijer, E. W. *Proc. Natl. Acad. Sci. U.S.A.***2002**, *99*, 4977-4982.

2) Brunsveld, L.; Lohmeijer, B. G. G.; Vekemans, J. A. J. M.; Meijer, E. W. *Chem. Commun.* **2000**, 2305-2306.

3) Sinkeldam, R. W.; van Houtem, M. H. C. J.; Pieterse, K.; Vekemans, J. A. J. M.; Meijer, E. W. *Chem. Eur. J.* **2006**, *12*, 6129-6137.

4) Mathews, J. R. ; Goldoni, F.; Schenning, A. P. H. J.; Meijer, E. W. *Chem. Commun.* **2005**, 5503-5505.

5) Kilbinger, A. F. M.; Schenning, A. P. H. J.; Goldoni, F.; Feast, W. J.; Meijer, E. W. *J. Am. Chem. Soc.* **2000**, *122*, 1820-1821.

6) Wolffs, M.; Hoeben, F. J. M.; Beckers, E. H. A.; Schenning, A. P. H. J.; Meijer, E. W. *J. Am. Chem. Soc.* **2005**, *127*, 13484-13485.

7) Harada, A.; Cammas,S.; Kataoka, K. *Macromolecules* **1998**, *29*, 6183-6188.

8) Scmuck, C.; Frey, P.; Heil, M. *ChemBioChem* **2005**, 6, 628-631.

9) Schmuck, C.; Rehm, T.; Gröhn, F.; Klein, K.; Reinhold, F. *J. Am. Chem. Soc.* **2006**, *128*, 1430-1431.

10) Inoue, Y.; Miyauchi, M.; Nakajima, H.; Takashima, Y.; Yamaguchi, H.; Harada, A. *Marcomolecules* **2007**, *40*, 3256-3262.

11) Kim, B.-S.; Hong, D.-J.; Bae, J.; Lee, M. *J. Am. Chem. Soc.* **2005**, *127*, 16333-16337.

12) Bae, J.; Choi, J.-H.; Yoo, Y.-S.; Oh, N.-K.; Kim, B.-S.; Lee, M. *J. Am. Chem. Soc.* **2005**, *127*, 9668-9669.

13) Hirschberg, J. H. K.. K.; Brunsveld, L.; Ramzi, A.; Vekemans, J. A. J. M.; Sijbesma, R. P.; Meijer, E. W. *Nature* **2000**, *402*, 167-170.

14) Arnaurd, A.; Belleney, J.; Boué, F.; Bouteiller, L.; Carrot, G.; Wintgens, V *Angew. Chem. Int. Ed.* **2004**, 43, 1718-1721.

15) Scmuck, C.; Wienand, W. *J. Am. Chem. Soc.* **2003**, *125*, 452-459.

16) Inoue, Y.; Kuad, P.; Okumura, Y.; Takashima, Y.; Yamaguchi, H.; Harada, A. *J. Am. Chem. Soc.* **2006**, *129*, 6396-6397.

17) Lee, M.; Lee, S.-J.; Jiang, L.-H. *J. Am. Chem. Soc.* **2004**, *126*, 12724-12725.

18) Stone, M. T.; Moore, J. S. *Org.* Lett.**2004**, *6*, 469-472.

Hydrogels

19) Friggeri, A.; van der Pol, C.; van Bommel, K. J. C.; Heeres, A.; Stuart, M. C. A.; Feringa, B. L.; van Esch, J. *Chem. Eur. J.* **2005**, *11*, 5353-5361.

Multivalent ligands

20) Bilgicer, B.; Moustakas, D. T.; Whitesides, G. M. *J. Am. Chem. Soc.* **2007**, *129*, 3722-3728.

21) Krishnamurthy, V. M.; Semetey, V.; Bracher, P. J.; Shen, N.; Whitesides, G. M. *J. Am. Chem. Soc.* **2007**, *129*, 1312-1320.

22) Crespo-Biel, O.; Lim, C. W.; Ravoo, B. J.; Reinhoudt, D. N.; Huskens, J. *J. Am. Chem. Soc.* **2006**, *128*, 17024-17032.

23) Mulder, A.; Auletta, T.; Sartori, A.; Del Ciotto, S.; Casnati, A.; Ungaro, R.; Huskens, J.; Reinhoudt, D. N. *J. Am. Chem. Soc.* **2004**, *126*, 6627-6636.

24) Kiessling, L.; Gestwicki, J. E.; Strong, L. E. *Curr. Opin. Chem. Biol.* **2004**, *4*, 696-703.

Experimental Details

General Considerations.

All chemicals were purchased from Aldrich or Acros and were used as received unless otherwise noted. Dichloromethane was distilled from P_2O_5. $CDCl_3$ was dried over 4Å molsieves and THF was distilled from 4Å molsieves. All moisture-sensitive reactions were performed under an atmosphere of dry argon. All reactions were followed by thin-layer chromatography (precoated 0.25 mm silica gel plates from Merck), and silica gel column chromatography was carried out with silica gel 60 (mesh 70-230). ^{1}H-NMR and ^{13}C-NMR spectra were recorded on a 400 MHz NMR (Varian Mercury, 400 MHz for ^{1}H-NMR and 100 MHz for ^{13}C-NMR), a 300 MHz NMR (Varian Gemini, 300 MHz for ^{1}H-NMR and 75 MHz for ^{13}C-NMR) or 500 MHZ NMR (Varian Unity Inova, 500 MHZ for ^{1}H-NMR and 125 MHZ for ^{13}C-NMR) . Proton chemical shifts are reported in ppm downfield from tetramethylsilane (TMS). The following splitting patterns are designated as s, singlet; d, doublet; t, triplet; q, quartet; b, broad; m, multiplet. Carbon chemical shifts are reported downfield from TMS using the resonance of the deuterated solvent as the internal standard. CD and UV measurements were recorded on a Jasco J-815 spectrometer at room temperature using spectroscopic grade solvents. Cells with an

optical path length of 1 cm (10^{-5} M solutions), 1 mm (10^{-4} M solutions), 0.1 mm (10^{-3} M solutions) and 0.01 mm (10^{-2} M solutions) were employed. The anisotropy value was calculated from $\Delta\varepsilon$ and ε: $g = \Delta\varepsilon/\varepsilon$ and $\Delta\varepsilon$ = CD-effect/(32980xcxl) where c is the concentration in mol/L and l = the optical path length in cm.

Matrix assisted laser desorption/ionization mass-time of flight (MALDI-TOF) were obtained using a PerSeptive Biosystems Voyager-DE PRO sprectrometer using an acid α-cyanohydroxycinnamic acid (CHCA) or a neutral 2-[(2E)-3-(4-*tert*-butylphenyl)-2-methylprop-2-enylidene]malononitrile (DCTB) matrix.

Infrared (IR) spectra were recorded on a Perkin Elmer Spectrum One FT-IR spectrometer with a Universal ATR sampling Accessory. Solutions of **1a** in $CDCl_3$ or **2a** in decaline were loaded between a pair of KBr windows using a 1 mm (for 10^{-3} M solution) or 5 mm (for 10^{-5}-10^{-4} M solutions) Teflon spacer contained in a demountable liquid cell. The absorbance due only to **1a** or **2a** was obtained by subtracting the spectra measured for pure $CDCl_3$ or decaline under otherwise identical conditions. For ureido-pyrimidinone **1a**, the resulting corrected spectra were flat in the region between 1800-1550 cm^{-1}. For each spectrum, a 128-scan interferogram was collected with 4-cm^{-1} resolution. Measurements were performed without active temperature control.

Elemental analysis was performed on a Perkin Elmer 2400 series II CHNS/O Analyzer. Melting points were determined on a Büchi Melting Point B-540 apparatus.

2-(2-(2-methoxyethoxy)ethoxy)ethyl amine and 2-(2-(2-methoxyethoxy)ethoxy)ethyl tosylate were prepared as described by Scherman et al.[1] *(R)*-3,7-Dimethyloctylamine was prepared as described by Fontana et al.[2]

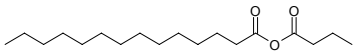
1) Scherman, O. A.; Ligthart, G. B. W. L.; Ohkawa, H.; Sijbesma, R. P.; Meijer, E. W. *Proc. Natl. Acad. Sci. U. S. A.* **2006**, *103*, 11850-11855.

2) Fontana, M; Chanzy, H; Caseri, W.R. ; Smith, P.; Schenning, A.P.H.J.; Meijer, E.W.;

Gröhn, F. *Chem. Mater.,* **2002**, *14*, 1730-1735.

Synthesis

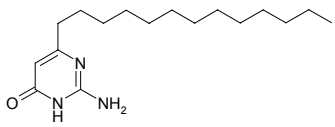
Ethyl 3-oxopalmitoate.



To a mixture of potassium ethyl malonate (24.4 g, 144 mmol) and 150 ml acetonitrile (DNA reagent grade) at 0°C, triethylamine (dried over KOH, 22.5 g, 223 mmol) was added and the mixture was stirred for 15 minutes. Subsequently, $MgCl_2$ (16.5 g, 173 mmol) was added together with 50 ml acetonitrile and the mixture was stirred at 10°C during 2 hours. The mixture was cooled to 0°C again, after which myristoic acid chloride (17.0 g, 69 mmol) was added dropwise and stirred overnight at room temperature. The acetonitrile was removed *in vacuo* and the resulting yellowish solid was transferred to a separation funnel with 150 ml 3M HCl and 200 ml Et_2O. After extraction, the organic layer was washed with 50 ml saturated $NaHCO_3$ solution, dried with $MgSO_4$ and evaporated *in vacuo*. The crude product, a yellowish oil, was purified by silica filtration using $CHCl_3$ as eluent, after which the beta-keto ester was obtained pure (18.4 g, 89% yield).

^{1}H-NMR: 12.11 (s, enol OH), 4.97 (s, enol CH), 4.19 (q, 2H), 3.24 (s, 2H), 2.53 (t, 2H), 1.59 (m, 2H), 1.34-1.15 (m, 20H), 0.88 (t, 3H) ppm. FTIR-ATR: 2923, 2854, 1743, 1717, 1646, 1466, 1411, 1368, 1311, 1232, 1150, 1096, 1033, 937, 800, 721 cm^{-1}.

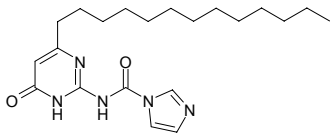
6-Tridecylisocytosine.



To a solution of ethyl 3-oxopalmitoate (18.0 g, 60.4 mmol) in 175 ml EtOH, guanidinium carbonate (9.25 g, 102.7 mmol) and potassium tert-butoxide (6.77 g, 60.4 mmol) were added and refluxed for 2 days. The reaction mixture was then cooled, filtered and the filtrate was evaporated *in vacuo*. To the resulting solid 300 ml mildly acidic water (pH 6) was added, resulting in a milk-like emulsion, which was subsequently filtered off over a glass filter, rinsed with acetone and Et_2O and dried *in vacuo* at 50°C. The pale yellowish solid was recrystallised from 2-propanol, resulting in the pure product (8.7 g, 49% yield)

^{1}H-NMR (DMSO-d_6): 10.57 (s, 1H), 6-7(br, 2H), 5.36 (s, 1H), 2.24 (t, 2H), 1.55 (t, 2H), 1.26 (m, 20H), 0.86 (t, 3H) ppm. ^{13}C-NMR(DMSO-d_6): 171.8, 162.7, 155.0, 99.2, 36.1, 30.4, 28.1 (multiple), 26.6, 21.1, 12.6 ppm. IR: 3365, 3146, 2920, 2850, 2713, 1662, 1634, 1553, 1468, 1400 cm^{-1}.

N-(6-oxo-4-tridecyl-1,6-dihydropyrimidin-2-yl)-1H-imidazole-1-carboxamide.

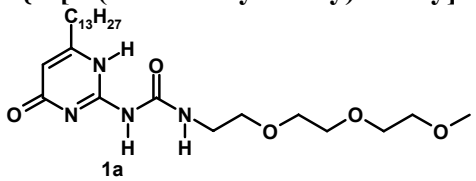


In a 500 ml flask, a mixture of 6-tridecylisocytosine (5.00 g, 17.0 mmol) and carbonyl diimidazole (3.32 g, 20,4 mmol) in 250 ml dry $CHCl_3$ was stirred for 4 hours at 50°C, until the mixture becomes clear. After evaporation of the solvent *in vacuo*, the residue was transferred to a glass filter with 3x50 ml acetone, filtered off, rinsed with 4x50 ml acetone and 50 ml Et_2O and dried *in vacuo* at 50°C. The product was obtained as a white powder (5.94 g, 90%).

^{1}H-NMR ($CDCl_3$): 8.86 (s, 1H), 7.64 (s, 1H), 7.00 (s, 1H), 5.81 (s, 1H), 2.64 (t, 2H), 1.75 (q, 2H,), 1.43-1.19 (m, 20H), 0.87 (t, 3H) ppm. ^{13}C-NMR ($CDCl_3$): 161.0, 157.3, 156.9,

156.8, 138.2, 127.9, 117.9, 104.2, 33.1, 31.1, 29.9, 29.8, 29.7, 29.6, 29.5, 29.3, 27.8, 22.9, 14.4 ppm. IR: 3172, 3080, 2951, 2922, 2853, 2664, 1979, 1693, 1649, 1603, 1470, 1409, 1371, 1342, 1322, 1278, 1232, 1224, 1180, 1092, 1068, 1027, 979, 914, 874, 858, 805, 751 cm^{-1}.

2{2-[2-(2-Methoxyethoxy)ethoxy]ethyl}ureido-6-(tridecyl)-4[1*H*] pyrimidinone (1a).



1a

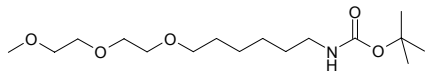
CDI activated isocytosine (1.04 g, 2.68 mmol) and 2-(2-(2-methoxyethoxy)ethoxy)ethyl amine (0.889 g, 5.45 mmol) were added to dry $CHCl_3$ (9 ml) and stirred at 60°C for 2 days. After cooling to room temperature, 25 ml $CHCl_3$ was added, and the mixture was extracted 3 times with 10 ml 0.1 M HCl, neutralized with 15 ml saturated $NaHCO_3$ and washed with 20 ml brine. After drying with $MgSO_4$ the solvent was removed by evaporation *in vacuo*, resulting in the crude ureidopyrimidinone (1.1 g, 2.3 mmol, 85% yield). Further purification by recrystallisation from 2-propanol, resulted in pure **1a** as a white powder (mp: 82.6-83°C).

^{1}H-NMR ($CDCl_3$): 13.08 (s, 1H), 11.93 (s, 1H), 10.28 (s, 1H), 5.80 (s, 1H), 3.64 (m, 8H), 3.52 (m, 4H), 3.47 (s, 3H), 2.45 (t, 2H), 1.63 (m, 2H), 1.28 (m, 20H), 0.87 (t, 3H) ppm.

^{13}C-NMR ($CDCl_3$): 173.0, 156.8, 154.6, 152.4, 105.8, 72.0, 70.5, 70.3, 69.4, 59.0, 39.5, 32.7, 31.9, 29.6-28.8, 27.0, 22.7, 14.1 ppm. FTIR (ATR, solid state): 3216, 3135, 3030, 2955, 2918, 2873, 2853, 2817, 1702, 1676, 1641, 1620, 1594, 1562, 1470, 1456, 1418, 1400, 1333, 1280, 1262, 1202, 1186, 1122, 1045, 1028, 997, 982, 949, 937, 925, 866, 854, 827, 799, 791, 780, 772, 767, 742, 720, 708, 694 cm^{-1}. Anal. Calcd for $C_{25}H_{46}N_4O_5$

(MW=482.66) C, 62.21; H, 9.61; N, 11,61. Found: C, 62.51; H, 9.69; N, 11.70. Maldi-TOF Calcd. (MH^+): 483.35. Observed: 483.37 (MH^+), 505.34 (MNa^+).

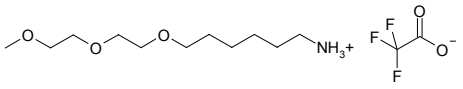
tert-butyl 6-(2-(2-methoxyethoxy)ethoxy)hexylcarbamate.



2-(2-(2-methoxyethoxy)ethoxy)ethyl tosylate (2.20 g, 8.0 mmol), N-Boc protected 6-aminohexanol (2.26 g, 10.4 mmol) and grinded KOH (0.772 g, 13.6 mmol) were refluxed in 10 ml distilled THF during 24 h. After subsequent cooling to room temperature 100 ml $CHCl_3$ was added, and the resulting solution was extracted with 100 ml 10% citric acid solution. The organic layer was then washed with 50 ml saturated $NaHCO_3$ solution and subsequently with 50 ml brine, dried over $MgSO_4$ and evaportated *in vacuo*. The crude product was purified using column chromatography using 1~4% MeOH in $CHCl_3$ as eluent, yielding the pure product as a colorless oil (0.26 g, 0.81 mmol, 10% yield).

^{1}H-NMR ($CDCl_3$): 3.66-3.53 (m, 8H), 3.45 (t, 2H), 3.38 (s, 3H), 3.09 (m, 2H), 1.59-1.30 (m, 17H) ppm. ^{13}C-NMR ($CDCl_3$): 156, 78.8, 71.8, 71.2, 70.5, 70.4, 69.9, 58.9, 40.4, 29.9, 29.4, 28.3, 26.5, 25.7 ppm. FTIR (ATR, solid state): 3356, 2931, 2862, 1712, 1699, 1521, 1455, 1391, 1365, 1268, 1248, 1171, 1105, 1040, 1028, 983, 927, 853, 781, 757, 730, 664 cm^{-1}.

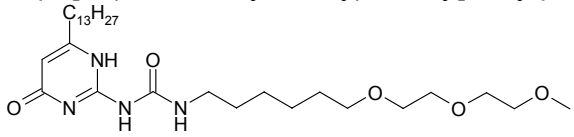
6-(2-(2-Methoxy-ethoxy)-ethoxy)-hexylammonium trifluoroacetate.



Tert-butyl 6-(2-(2-methoxyethoxy)ethoxy)hexylcarbamate (0.26 g, 0.81 mmol) was dissolved in 30 ml DCM. Subsequently, 1.8 ml trifluoroacetic acid was added and the solution was stirred for 3h at room temperature. After evaporation *in vacuo* and co-evaporation with toluene, the TFA salt was obtained as a yellowish oil (0.24 g, 0.76 mmol) in 94% yield.

^{1}H-NMR ($CDCl_3$): 7.90 (s, 3H), 3.66-3.55 (m, 8H), 3.50 (t, 2H), 3.39 (s, 3H), 2.96 (m, 2H), 1.72-1.34 (m, 8H) ppm. ^{13}C-NMR ($CDCl_3$): 71.6, 71.0, 70.1, 69.9, 69.7, 58.6, 39.6, 28.8, 26.9, 25.7, 25.0 ppm. FTIR (ATR, solid state): 3060, 2934, 2866, 1675, 1641, 1525, 1457, 1427, 1393, 1367, 1353, 1247, 1199, 1175, 1127, 1025, 983, 932, 870, 833, 798, 721, 706 cm^{-1}.

2-{6-[2-(2-Methoxyethoxy)ethoxy]hexyl}ureido-6-(tridecyl)-4[1*H*] pyrimidinone 1b.



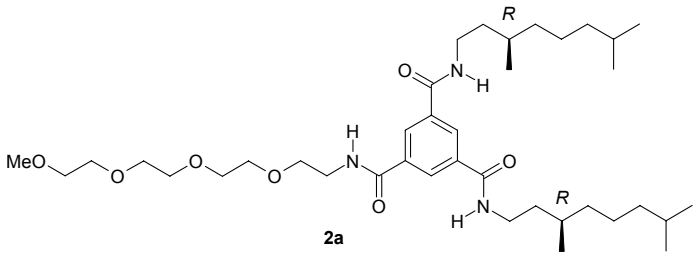
1b

CDI activated isocytosine (0.44 g, 1.13 mmol), 6-(2-(2-methoxyethoxy)ethoxy)hexyl ammonium trifluoroacetate (0.50 g; 1.58 mmol) and triethylamine (0.18 g; 1.80 mmol) were added to dry $CHCl_3$ (5 ml) and stirred at 55°C for 2 days. After cooling, 25 ml $CHCl_3$ was added, and the mixture was extracted 3 times with 10 ml 0.1 M HCl, neutralized with 15 ml saturated $NaHCO_3$ and washed with 20 ml brine. After drying with $MgSO_4$ the solvent was removed by evaporation *in vacuo*, resulting in the crude ureidopyrimidinone (0.53 g; 0.99 mmol, 88% yield). This was purified by

recrystallisation from 2-propanol, yielding the pure product as a white powder (mp:68.3-68.5°C)

^{1}H-NMR ($CDCl_3$): 13.17 (s, 1H), 11.88 (s, 1H), 10.18 (s, 1H), 5.81 (s, 1H), 3.66-3.63 (m, 4H), 3.59-3.54 (m, 4H), 3.44 (t, 2H), 3.38 (s, 3H), 3.24 (m, 2H), 1.66-1.54 (m, 6H), 1.38-1.26 (m, 24H), 0.88 (t, 3H) ppm. ^{13}C-NMR ($CDCl_3$): 173.2, 156.6, 154.7, 152.4, 105.8, 72.0, 71.4, 70.7, 70.5, 70.1, 59.0, 40.0, 32.7, 31.9, 29.6-28.9, 27.0, 26.8, 25.8, 22.7, 14.1 ppm. FTIR (ATR, solid state): 2922, 2850, 1698, 1662, 1579, 1526, 1466, 1438, 1307, 1259, 1203, 1124, 1005, 948, 885, 813, 771, 744 cm^{-1}. Anal. Calcd for $C_{29}H_{54}N_4O_5$ (MW=538.78): C, 64.65%; H, 10.10%; N, 10.40%. Found: C, 64.44%; H, 10.30%; N, 10.63%. Maldi-TOF Calcd. (MH^+): 539.41 Observed: 539.50 (MH^+), 561.48 (MNa^+), 577.45 (MK^+)

N-(2-{2-[2-(2-methoxyethoxy)-ethoxy]-ethoxy}-ethyl)-N',N"-di(*(R)*-3,7-dimethyl-octyl)benzene-1,3,5-tricarboxamide (2a).

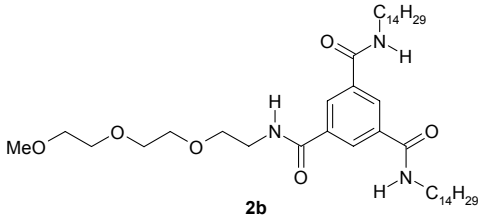


2a

A 100 mL two-necked round bottom flask was charged with a solution of 2-{2-[2-(2-methoxyethoxy)-ethoxy]-ethoxy}-ethyl amine (2.505 g, 12.09 mmol), triethyl amine (2.523 g, 24.93 mmol) and *(R)*-3,7-dimethyloctylamine (1.915 g, 12.17 mmol) in 25 mL of chloroform (amylene stabilised), and stirred under inert atmosphere. To this mixture, a solution of 1,3,5-benzenetricarboxylic acid chloride (1.924 g, 7.247 mmol) in 10 mL of

chloroform (amylene stabilized), was added dropwise. After this addition was completed, the reaction mixture was stirred overnight at room temperature and under inert atmosphere. To the reaction mixture, 20 mL of chloroform was added, and the solution was washed with 25 mL 1 M HCl (check for acidity). The organic layer was collected and evaporated to yield 5.50 g of the crude product as a yellowish/orange oil, which also contained solid product. Column chromatography was performed using 65 g silica in a column of 35 cm in length and a diameter of 4 cm. The first eluent (ethylene glycol dimethyl ether/*n*-heptane, 3/7) eluted the tri-3,7-dimethyloctyl derivative (checked with TLC). Then, the eluent was changed to ethylene glycol dimethyl ether/ *n*-heptane (7/3) to obtain the desired second product fraction (checked with TLC). The fractions containing the desired product were collected and evaporation *in vacuo* resulted in a white, sticky compound (1.4 g).^{1}H-NMR ($CDCl_3$): δ = 8.40 (d, 3H, Ar-*H*), 7.65 (t, 1H, N-*H*), 6.67 (t, 2H, N-*H*), 3.68 -3.58 (m, 14 H, O-CH_2), 3.48 (m, 6H, NH-CH_2), 3.21 (s, 3H, O-CH_3), 1.67-1.13 (m, 24H, C*H*, CH_2), 0.94 (d, 6H, CH_3), 0.86 (d, 12H, CH_3). IR ν (cm^{-1}) 3238 (NH stretch), 1637 (C=O), 1556 (amide II). Maldi-TOF Calcd. [M + Na^+] = 700.50 Da; Obs. [M + Na^+] = 700.47 Da. Anal. Calcd for $C_{38}H_{67}N_3O_7$ (MW = 677.97 g/mol) C, 67.32; H, 9.96; N, 6.20. Found: C, 67.34; H, 10.18; N, 6.16.

N-{2-[2-(2-methoxyethoxy)-ethoxy]-ethyl}-N',N"-di(tetradecyl)benzene-1,3,5-tricarboxamide compound (2b).



2b

Synthesis and isolation of compound **2b** was identical to that of compound **2a** with the exception that a mixture of *n*-tetradecylamine and 2-[(2-methoxyethoxy)-ethoxy]-ethyl amine was used. ^{1}H-NMR ($CDCl_3$): δ = 8.40 (d, 3H, Ar-*H*), 7.27 (t, 1H, N-*H*), 6.60 (t, 2H, N-*H*), 3.68 (m, 10H, O-C*H*$_2$), 3.58 (dd, 2H, NH-C*H*$_2$), 3.48 (qua, 4H, NH-C*H*$_2$), 3.21 (s, 3H, OC*H*$_3$), 1.62 (m, 4H, CH_2C*H*$_2$), 1.30 (bs, 44H, C*H*$_2$), 0.90 (d, 6H, C*H*$_3$).Maldi-TOF Calcd. [M + Na^+] = 768.60 Da; Obs. [M + Na^+] = 768.52 Da. Anal. Calcd for $C_{44}H_{79}N_3O_6$ (MW = 700.50 g/mol) C, 70.83; H, 10.67; N, 5.63. Found: C, 70.85; H, 10.88; N, 5.46.

NMR measurements

^{1}H-NMR dilution experiments on **1a** and **1b** in $CDCl_3$ were performed on a Varian Unity Inova, 500 MHZ equipped with a 5mm 1H/X Inverse Detection probe equipped with gradient capabilities at 298K. Ureido-pyrimidinones **1a** and **1b** were dried over P_2O_5 under dynamic vacuum for at least a period of 12 hours to remove any trace amounts of water. $CDCl_3$ used for the ^{1}H-NMR dilution and DOSY studies was distilled over P_2O_5 and collected under a constant stream of argon to avoid any contact with water. The NMR tubes used for the studies were dried over P_2O_5 under high vacuum.

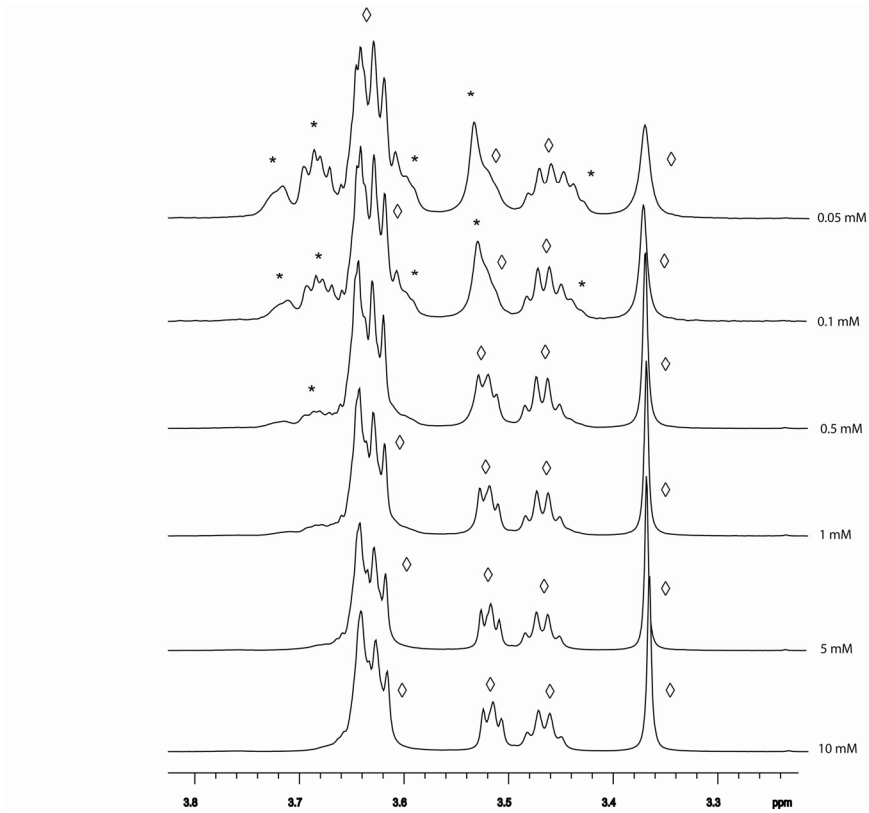


Figure S1: Partial ^{1}H-NMR spectrum of **1a** in $CDCl_3$ at different concentrations showing the changes in the region were the $NHC\underline{H}_2C\underline{H}_2OC\underline{H}_2C\underline{H}_2OC\underline{H}_2C\underline{H}_2OC\underline{H}_3$ protons resonate. The symbols denote signals belonging to dimeric (◊) and monomeric (*) species. The spectra were normalized with respect to the peak at 3.36 ppm.

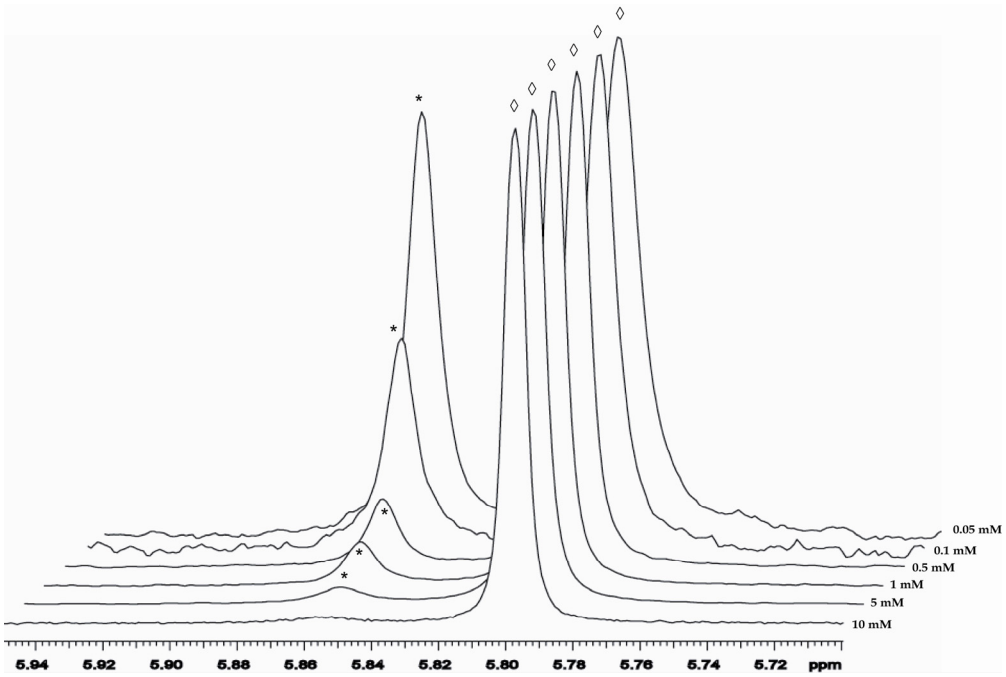


Figure S2: Partial ^{1}H-NMR spectrum of **1** in $CDCl_3$ at different concentrations showing the changes in the region were the C=CH proton on the ureido-pyrimidinone ring resonates. The symbols denote signals belonging to dimeric (◊) and monomeric (*) species. The spectra were normalized with respect to the peak at 5.82 ppm.

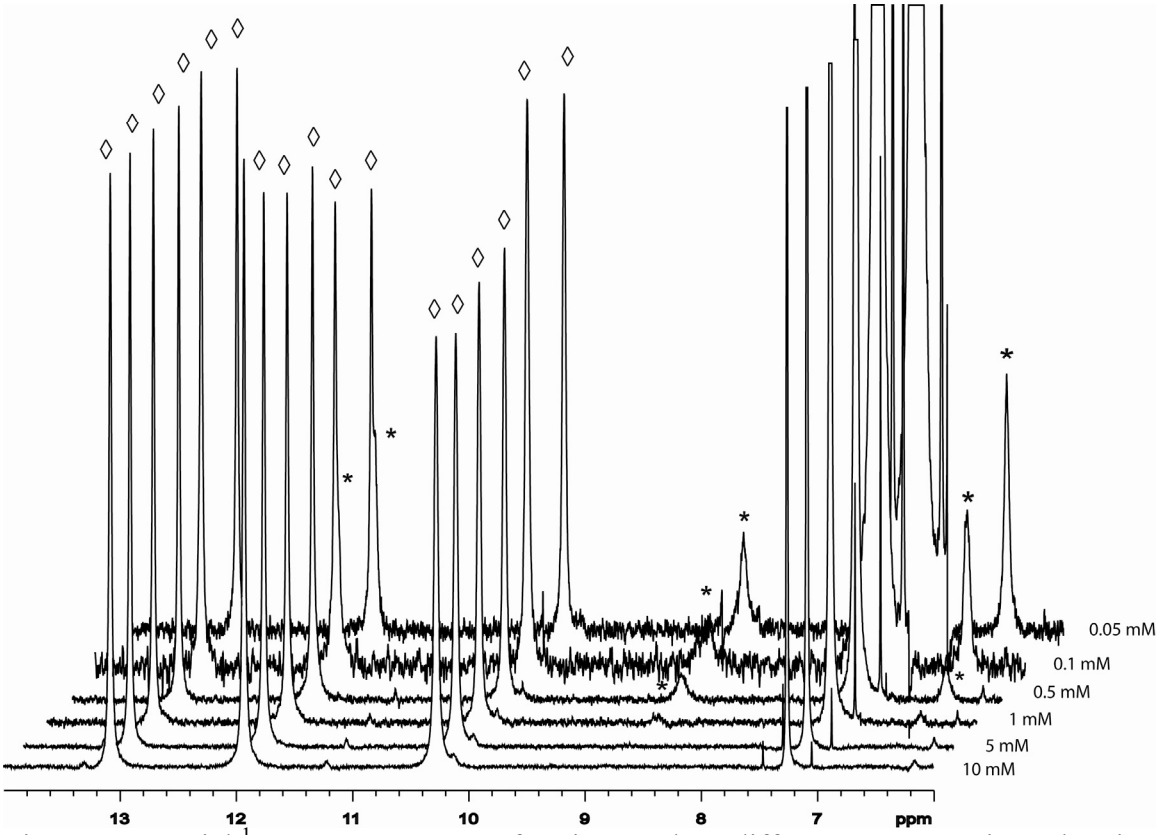


Figure S3: Partial ^{1}H-NMR spectrum of **1a** in $CDCl_3$ at different concentrations showing the changes in the region were the NH protons resonate. The symbols denote signals belonging to dimeric (◊) and monomeric (*) species. The spectra were normalized with respect to the peak at 13.1 ppm.

To establish the influence of the hydrophobic hexyl spacer, a ^{1}H-NMR dilution experiment was also performed on Upy **1b** in $CDCl_3$. In contrast to **1a**, no additional signals arising from monomeric ureido-pyrimidinone could be detected nor did any significant shift occur up to a concentration of 0.05 mM (Figure S4, S5 and S6). Under the assumption that at least 10% dissociation is required to be observable at this concentration, a lower limit on the dimerization constant can be placed: $K_{dim} > 1 \times 10^{6}$ M^{-1}.

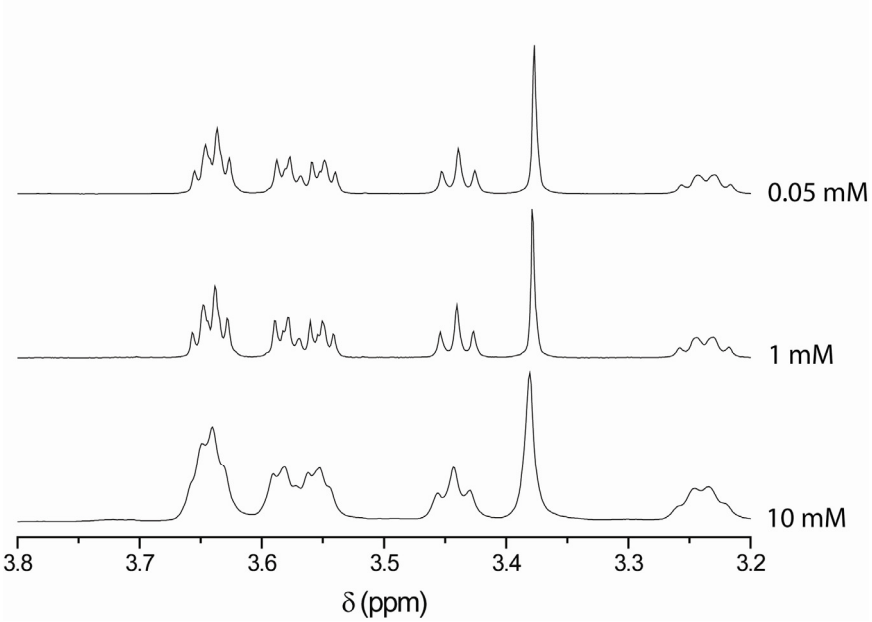


Figure S4: Partial ^{1}H-NMR spectrum of **1b** in $CDCl_3$ at different concentrations showing the changes in the region were the NHC$\underline{H_2}$C$\underline{H_2}$OC$\underline{H_2}$C$\underline{H_2}$OC$\underline{H_2}$C$\underline{H_2}$OC$\underline{H_3}$ protons resonate.

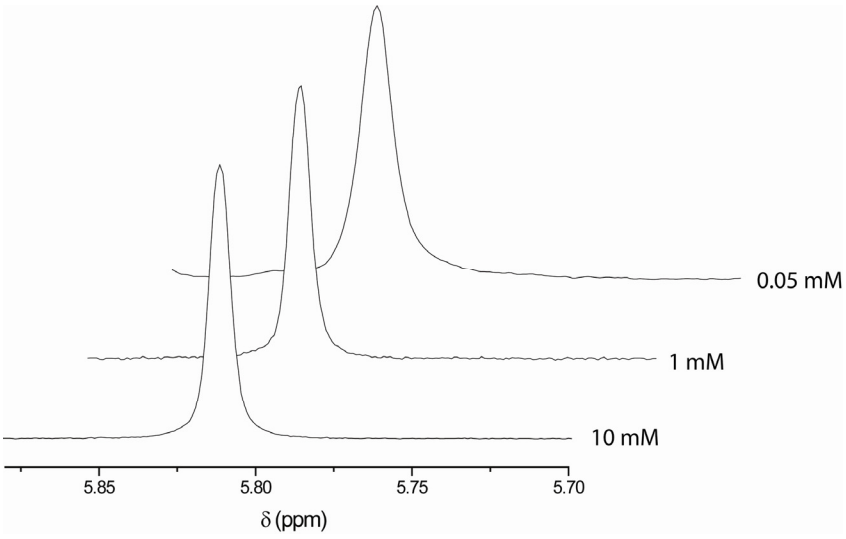


Figure S5: Partial ^{1}H-NMR spectrum of **1b** in $CDCl_3$ at different concentrations showing the changes in the region were the C=CH proton on the ureido-pyrimidinone ring resonates.

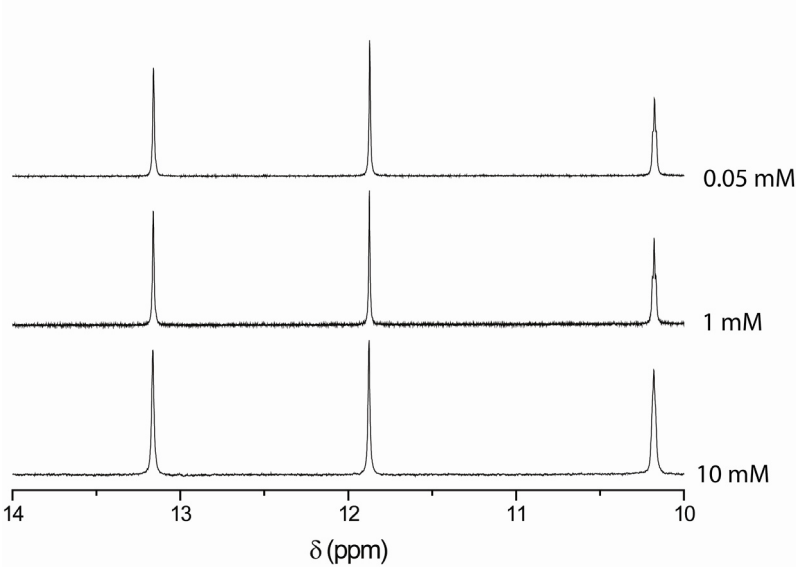


Figure S6: Partial ^{1}H-NMR spectrum of **1b** in $CDCl_3$ at different concentrations showing the changes in the region were the NH protons resonate.

2D-DOSY experiments on **1a** in $CDCl_3$ (0.5 mM concentration) were performed on a Varian Unity Inova, 500 MHZ equipped with a 5mm 1H/X Inverse Detection probe equipped with gradient capabilities (Performa II/III, maximum gradient strength of 70 gauss/cm) at 295 and 298 K. Ureido-pyrimidinone **1a** was dried over P_2O_5 under dynamic vacuum for at least a period of 12 hours to remove any trace amounts of water. $CDCl_3$ used for the ^{1}H-NMR dilution and DOSY studies was distilled over P_2O_5 and collected under a constant stream of argon to avoid any contact with water. The NMR tubes (5 mm) used for the studies were dried over P_2O_5 under high vacuum.

The DOSY bipolar pulse pair stimulated echo (Dbppste) sequence[1] was used for the determination of the self-diffusion of the different components. For the DOSY measurements performed on $CDCl_3$ solutions, active temperature control was switched off in order to remove any artifacts arising from convective flows. Temperature

calibration was achieved by observing the temperature dependent chemical-shift separation between the OH and CH_3 resonance in methanol. In all experiments the 90^o pulse widths were determined. Firstly, the strength of the B0 field gradient was calibrated by measuring the self-diffusion coefficient of the residual HDO signal in a 1% D_2O sample, at 298 K (D(H2O) = 19 x 10^{-10} m^2/s).[2] The experimental diffusion data can be fitted into the following equation:

$$I(G) = I(0)\exp(-D\,\gamma^2\delta^2(G)^2(\Delta - \delta/3 - \tau/2)) \qquad (1)$$

In which $I(G_{zi})$ represents the experimental signal intensity, I(0) the initial signal intensity, γ_h is the magnetogyric ratio for ^1H, τ the time interval between the bipolar pulse pair, δ the length of the pulsed field gradient and Δ the diffusion period. Using this equation we can determine D from a plot of ln(I(G)/I(0)) *vs* G^2.

Using the calibrated field gradients we first measured the diffusion constant of $CHCl_3$ in $CDCl_3$ (99.99%) resulting in a value of 23.72 x 10^{-10} m^2/s at 295 K. 2D DOSY measurements on **1** in $CDCl_3$ (0.5 mM) were performed using a diffusion period (Δ) of 70 ms a pulsed field gradient length of 2 ms (δ) and a gradient stabilisation delay of 3 ms. A total of 1024 transients were collected for each of the 30 steps while the gradient strength was changed from 1.085 G/cm^{-1} to 32.55 G/cm^{-1}. At the highest gradient strength approximately 80% signal reduction was observed for the signals of interest. Fitting of the intensities as a function of gradient strength resulted in the following apparent diffusion constants at 295 K:

D_{dimer} = 6.93 x 10^{-10} m^2/s

$D_{monomer}$ = 8.52 x 10^{-10} m^2/s

D_{CHCl_3} = 28.52 x 10^{-10} m^2/s

The value for the diffusion constant of residual $CHCl_3$ in a solution of **1** in $CDCl_3$ deviates from the diffusion constant measured in 'pure' $CDCl_3$. We therefore corrected the measured values according to the measured value of the diffusion constant of $CHCl_3$ in $CDCl_3$ (99.99%) (23.72×10^{-10} m^2/s at 295 K):

$D_{dimer} = 5.78 \times 10^{-10}$ m^2/s

$D_{monomer} = 7.10 \times 10^{-10}$ m^2/s

For perfectly spherical molecules it has been claimed[3] that the ratio of the diffusion coefficients for two different molecular species is inversely proportional to the cubic root of the ratio of their molecular weights:

$$\sqrt[3]{\frac{M_1}{M_2}} = \frac{D_2}{D_1} \qquad (2)$$

From this we can calculate a molecular weight ratio of 1.85 which is in close agreement with the expected value of 2 for a monomer-dimer equilibrium. Although the monomer and dimer are in slow exchange on the ^{1}H-NMR timescale, care must be taken in using the calculated values of the diffusion constants for $D_{monomer}$ and D_{dimer} as true diffusion constants as they can still be under fast exchange on the DOSY timescale. Cabrita and co workers have previously shown[4] that under the condition $\Delta/\tau_{ab} < 0.1$ (with $\tau_{ab} = 1/k_{ab}$ and k_{ab} rate constant of the exchange process) the true diffusion constants are obtained (slow exchange on the DOSY time scale) while in the fast exchange limit ($\Delta/\tau_{ab} > 10$) a single diffusion constant is obtained. Because we currently do not have any information regarding the lifetime of the 6[1*H*] ureido-pyrimidinone monomer of **1a** in $CDCl_3$ it is difficult to justify whether the obtained diffusion constants are true or averaged diffusion constants. However, given the close agreement between the true and calculated molecular

weight ratio it is most likely that the two species are in slow exchange on the DOSY timescale.

1) Jerschow, A.; Müller, N. *J. Magn. Reson.* **1997**, *125*, 372.

2) Longsworth, L. G.; *J. Phys. Chem.* **1960**, *64*, 1914.

3) Waldeck, A. R.; Kuchel, P. W.; Lennon, A. J.; Capman, B. E. *Prog. Nucl. Magn. Reson. Spectrosc.*, **1997**, *30*, 39.

4) Cabritta, E. J.; Berger, S.; Bräuer, P; Kärger, J. *J. Magn. Res.* **2002**, *157*, 124.

Concentration dependent FTIR measurements of 1a and 1c in $CDCl_3$.

Figure S7 shows the FTIR spectra of the amide I and amide II region of solutions of **1a** in $CDCl_3$, (between 0.025 mM and 1.0 mM) normalized for the concentration. The most obvious changes can be seen in the bands at 1585 and 1600 cm^{-1}. However, the latter must be assigned to residual H_2O. A less pronounced change can be seen in the bands around 1550 cm^{-1} and 1650 cm^{-1}.

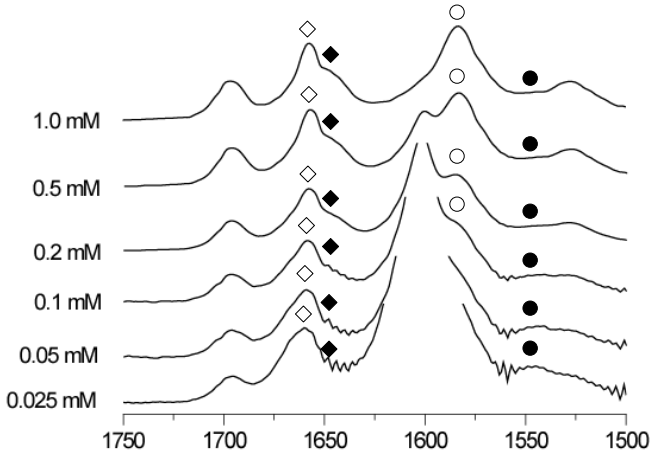


Figure S7: Amide I and amide II region of the FTIR spectrum at various concentrations of **1a** in $CDCl_3$.

FTIR spectra in $CDCl_3$ were also collected of a derivative of **1a** (**1c**), carrying the same ethylene glycol chain but with a methyl group rather than a tridecyl chain at the 6-position of the pyrimidinone ring. This compound has a lower dimerization constant ($1.2*10^4$ M^{-1} obtained by ^{1}H-NMR in dry $CDCl_3$) compared to **1a**. Indeed, changes in the spectrum are more pronounced allowing a quantitative analysis of the binding process (Figure S8).

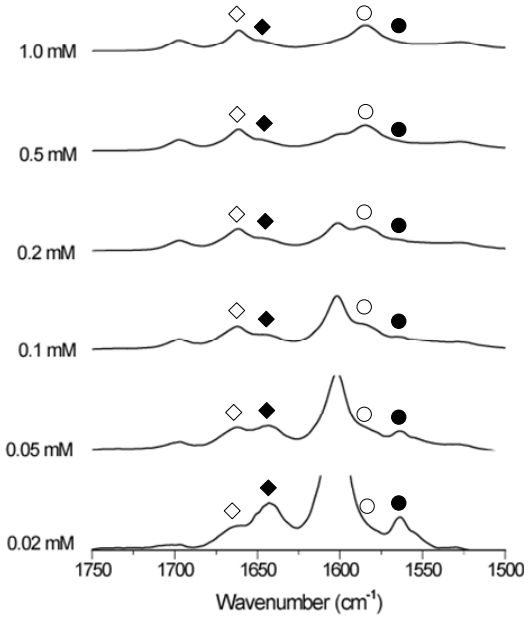


Figure S8: Amide I and amide II region of the FTIR spectrum at various concentrations of **1c** in $CDCl_3$.

To allow for a quantative analysis, the FTIR spectrum between 1500 and 1710 cm^{-1} at each concentration was deconvoluted into 7 peaks using a Lorentzian[1] lineshape (Figure S9).

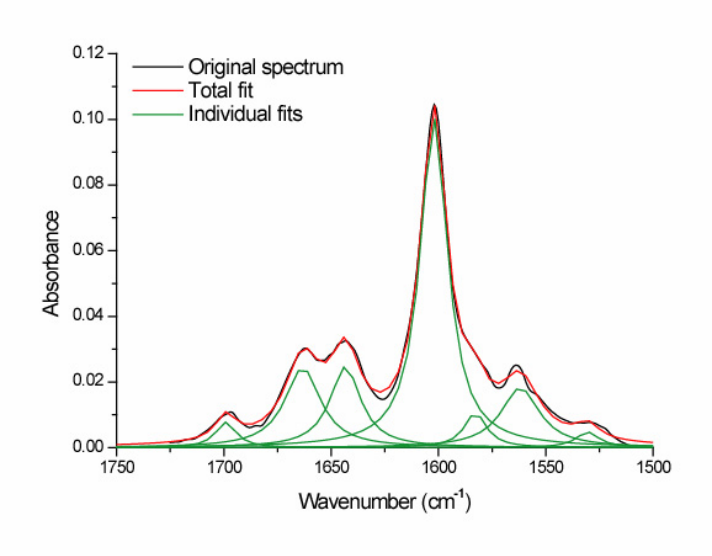


Figure S9: Lorentz fit of the amide region of compound **1c** at 0.05 mM.

A plot of the relative area of the band at 1560 cm^{-1} (corrected for the concentration) against the calculated monomer concentration reveals that this band originates from monomeric ureido-pyrimidinone.

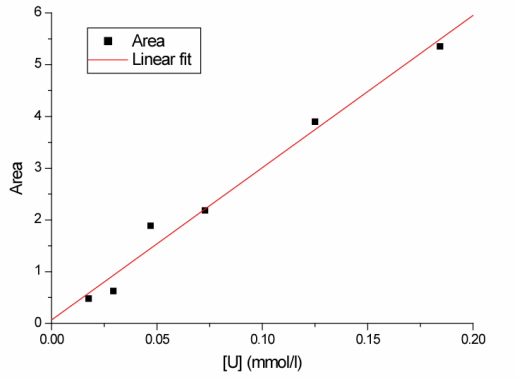


Figure S10: Relative areas of the IR band at 1560 cm^{-1} of **1c** plotted against the calculated monomer concentration (U).

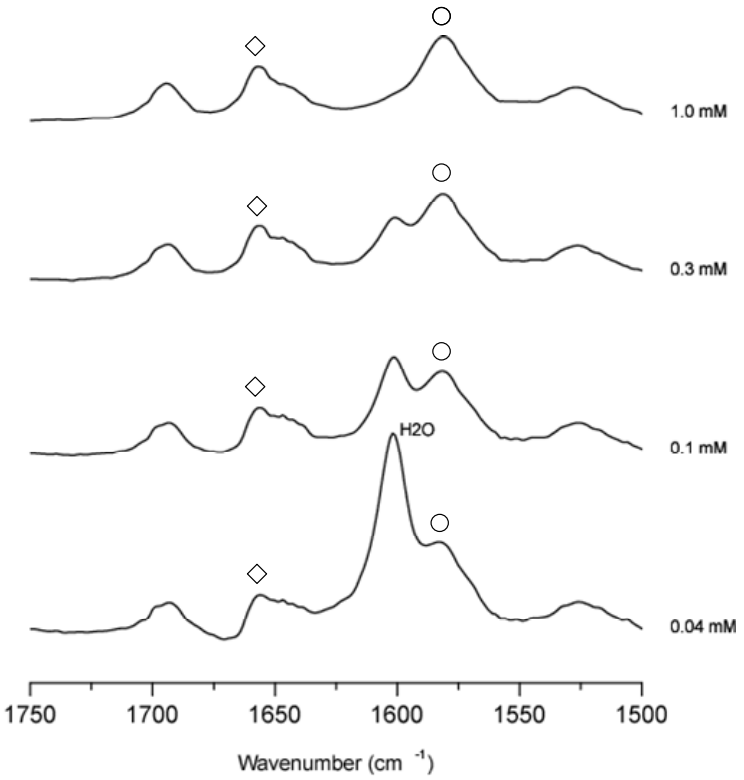


Figure S1: Amide I and amide II region of the FTIR spectrum at various concentrations of **1b** in $CDCl_3$.

1) Meier, R. J. *Vibrational Spectroscopy* **2005**, *39*, 266-269.

The sergeants-and-soldiers experiment.

The solutions of achiral **2b** and chiral **2a** were prepared with identical concentrations of 48 mM in decaline. The mixtures were prepared by mixing the 2 solutions in different ratios, heating the (rather viscous) solutions to around 70°C and then cooling slowly back to room temperature to ensure complete mixing. UV and CD spectra were measured with a 0.01 mm cell at room temperature. The anisotropy value *g* at 215 nm was calculated as a function of the amount of chiral **2a** added. The data were fitted with the Havinga model.[1] The optimal fit of the data points with the predicted g/g_0 values was obtained by

taking K_{ass} = 21 M^{-1} and S = 1. A linear relation between g/g_0 and mol% of chiral added is expected if no amplification of chirality would be present. Details of the Havinga model are given in reference 1.

1) Palmans, A. R. A.;Vekemans, J. A. J. M.; Havinga, E. E.; Meijer, E. W. *Angew. Chem., Int. Ed.* **1997**, *36*, 2648.

FT-IR measurements of 2a in decaline, c = 48 mM

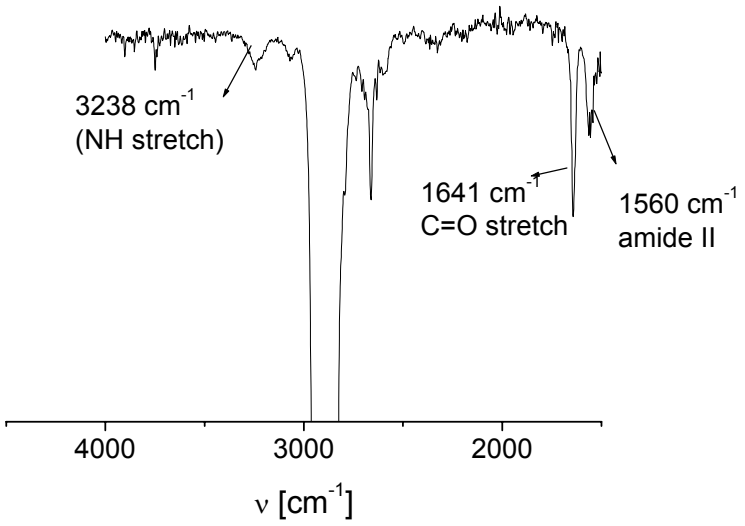


Figure S12: IR spectrum of compound **2a** in decaline, c = 48 mM

UV and CD spectra of 2a in decaline

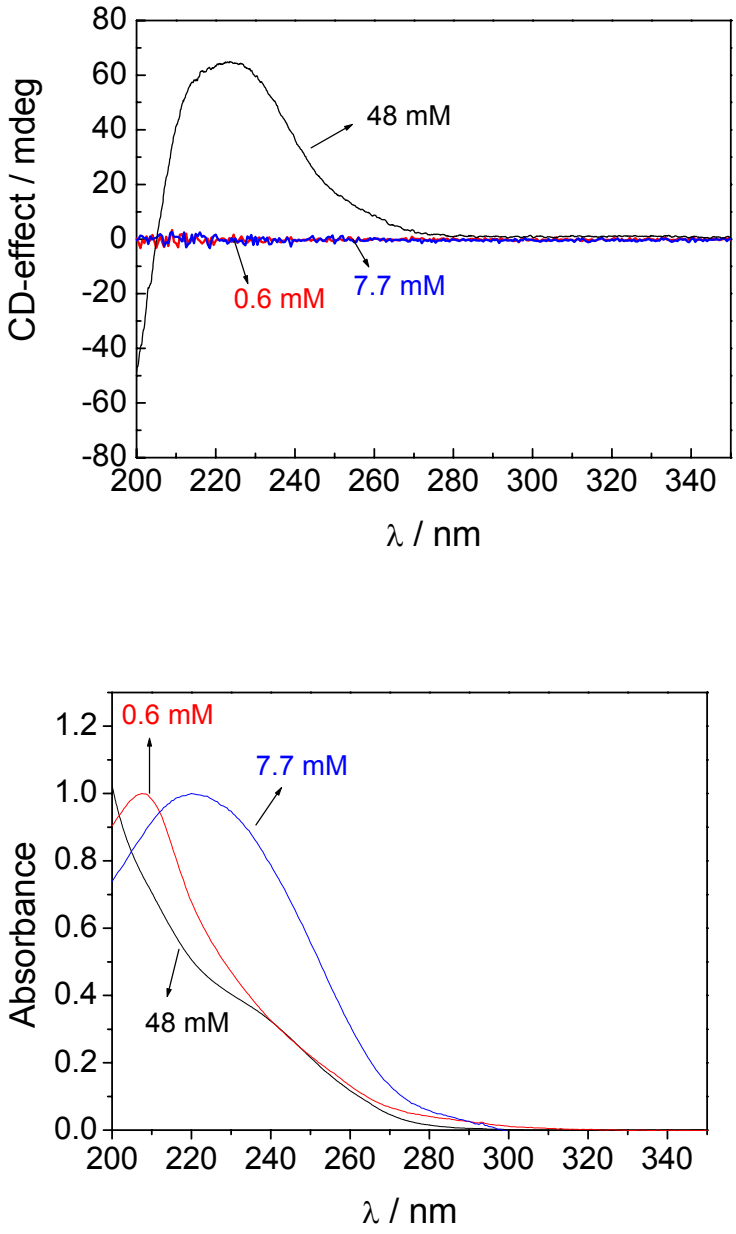


Figure S13: UV and CD spectra of compound **2a** in decaline at c = 48 mM, c = 7.7 mM and c = 0.6 mM.