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SUPPORTING INFORMATION

Title: The Convenient Synthesis of Hydrogen-Bonded Ureidopyrimidinones
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General Remarks:
Unless stated otherwise, all reagents were obtained from commercial sources and used without further purification. Dry tetrahydrofuran (THF) was obtained by distillation from Na/K/benzophenone. Column chromatography was carried out on Merck silica gel 60 (70-230 mesh). ^{1}H-NMR, and ^{13}C-NMR spectra were recorded on a Bruker AM-400 or a Varian Gemini spectrometer at frequencies for ^{1}H at 400.1 or 300.1 MHz resp. and for ^{13}C 100.6 and 75.0 MHz respectively. Chemical shifts in ^{1}H-NMR are in ppm downfield from TMS. Elemental analyses were carried out using a Perkin Elmer 2400 series II CHNS/O Analyzer. Electrospray ionization mass spectrometry (ESI-MS) was carried out on a PE-Sciex API 300 LC/MS/MS System mass spectrometer with a mass range of 3000 g/mol. Matrix assisted laser desorption/ionization mass-time of flight (Maldi-TOF) spectra were obtained using a PerSeptive Biosystems Voyager-DE PRO spectrometer. Infrared spectra (IR) were recorded on a Perkin-Elmer Spectrum One FT-IR spectrometer equipped with an universal Attenuated Total Reflection (ATR) sampling accessory. Melting points were determined on a Jevenal polarization microscope with a Linkam THMS 600 hot stage, and are uncorrected. GC/MS measurements were performed on a Shimadzu GCMS-QP5000 using a Zebron ZB-5 column.

Isocytosines **1a** - **1d** were synthesized by a ring closure of the corresponding beta-ketoesters with guanidine carbonate in ethanol. [1, 2] In case of isocytosine **1b** the starting beta-keto ester was obtained from Fluka, for isocytosines **1a**, **1c** and **1d** [2] the beta-keto esters were synthesized as described before.

Imidazolide 2a.
6-(2-ethylpentyl) isocytosine **1a** (4 g, 19.14 mmol) and CDI (4.03 g, 24.88 mmol) were dissolved in 20 ml of $CHCl_3$ and this solution was stirred for three hours under nitrogen at room temperature. To the reaction mixture 50 ml of $CHCl_3$ was added and the organic layer was washed with 20 ml water followed by 20 ml brine and dried with Na_2SO_4. The organic layer was evaporated in vacuo resulting in a light yellow powder in a yield of 98%.
^{1}H-NMR ($CDCl_3$): δ = 8.85 (s, 1H, N-*CH*=N), 7.65 (s, 1H, (C=O)N-*CH*=CH), 7.07 (s, 1H, (C=O)N-CH=*CH*), 5.83 (s, 1H, (C=O)*CH*=C), 2.55 (m, 1H, $(CH_2)_2$*CH*C), 1.75 (m, 4H, CH_2), 1.32 (m, 4H, CH_2), 0.95 (t, 3H, CH_3), 0.92 (t, 3H, CH_3) ppm. ^{13}C-NMR ($CDCl_3$): δ = 162.3, 160.3, 157.3, 128.4, 117.3 103.7, 45.4, 32.7, 29.2, 26.5, 22.4, 13.8, 11.5 ppm. FTR-IR (ATR) ν = 3149, 2959, 2932, 2860, 2661, 1916, 1706, 1691, 1626, 1600, 1466, 1418, 1375, 1311, 1277, 1221, 1175, 1092, 1067, 1023, 1004, 989, 952, 912, 857, 833, 823, 790, 754 cm^{-1}. DIP(EI)-MS: found m/z 235, 210, 206 192, 179, 164 153, 138 g/mol.

Imidazolide 2b.
6-*tert*-butylisocytosine **1b** (2.5 g, 14.97 mmol) and CDI (3.16 g, 19.46 mmol) were dissolved in 15 ml of dry THF and this solution was heated at reflux for three hours under nitrogen. The reaction mixture was concentrated in vacuo and to the concentrated

solution acetone was added. The resulting precipitate was washed thoroughly with acetone, filtered off and dried (yield 88 %).
^{1}H-NMR ($CDCl_3$): δ = 8.69 (s, 1H, N-*CH*=N), 7.62 (s, 1H, (C=O)N-*CH*=CH), 7.15 (s, 1H, (C=O)N-CH=*CH*), 5.96 (s, 1H, (C=O)*CH*=C), 1.41 (t, 9H, CH_3) ppm. ^{13}C-NMR ($CDCl_3$): δ =162.37, 161.92, 158.21, 156.77, 137.79, 129.26, 117.15, 102.43, 35.19, 28.11 ppm. FTR-IR (ATR): ν = 3463, 3348, 3148, 3077, 2957, 2719, 1979, 1699, 1660, 1615, 1555, 1519, 1473, 1416, 1367, 1328, 1305, 1280, 1223, 1170, 1095, 1067, 1002, 975, 953, 909, 859, 835, 796, 764 cm^{-1}. DIP(EI)-MS: found m/z 235, 220, 205 194, 178, 166 152, 125 g/mol.

Imidazolide 2c.
6-*n*-butylisocytosine **1c** (0.5 g, 2.99 mmol) and CDI (0.63 g, 3.89 mmol) were dissolved in 5 ml of $CHCl_3$ and this solution was heated at reflux for three hours under nitrogen. The reaction mixture was concentrated in vacuo and to the concentrated solution acetone was added. The resulting precipitate was washed thoroughly with acetone, filtered off and dried resulting in a white powder (yield 85%).
^{1}H-NMR ($CDCl_3$): δ = 8.83 (s, 1H, N-*CH*=N), 7.62 (s, 1H, (C=O)N-*CH*=CH), 7.00 (s, 1H, (C=O)N-CH=*CH*), 5.83 (s, 1H, (C=O)*CH*=C), 2.67 (t, 2H, CH=C-CH_2), 1.75 (q, 2H, CH_2), 1.52 (sextet, 4H, CH_2), 1.02 (t, 3H, CH_3) ppm. ^{13}C-NMR ($CDCl_3$): δ = 160.80, 157.05, 156.76, 156.66, 137.97, 127.61, 117.65 103.93, 32.53, 29.52, 22.28, 13.73 ppm. FTR-IR (ATR) ν = 3154, 3080, 2961, 2931, 2865, 2744, 1695, 1651, 1602, 1470, 1364, 1341, 1328, 1313, 1275, 1232, 1223, 1177 cm^{-1}, DIP(EI)-MS: found m/z 193, 178, 164, 151, 136 g/mol.

Imidazolide 2d.
6-tridecylisocytosine **1d** (2.5 g, 8.53 mmol) and CDI (1.79 g, 11.09 mmol) were dissolved in 10 ml of $CHCl_3$ and this solution was stirred for three hours under nitrogen at 50 ^{o}C. After cooling down to room temperature the reaction mixture was concentrated in vacuo and to the concentrated solution acetone was added. The resulting precipitate was washed thoroughly with acetone, filtered off and dried resulting in a white powder (yield 91%).
^{1}H-NMR ($CDCl_3$): δ = 8.86 (s, 1H, N-*CH*=N), 7.64 (s, 1H, (C=O)N-*CH*=CH), 7.00 (s, 1H, (C=O)N-CH=*CH*), 5.81 (s, 1H, (C=O)*CH*=C), 2.64 (t, 2H, CH=C-CH_2), 1.75 (q, 4H, CH=C-CH_2-CH_2), 1.43-1.19 (m, 10H, CH_2), 0.87 (t, 3H, CH_3) ppm. ^{13}C-NMR ($CDCl_3$): δ = 161.03, 157.32, 156.89, 156.77, 138.22, 127.90, 117.92, 104.21, 33.08, 31.14, 29.88, 29.87, 29.84, 29.79, 29.66, 29.58, 29.49, 29.33, 27.80, 22.92, 14.36 ppm. FTR-IR (ATR) ν = 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}. DIP(EI)-MS: found m/z 319, 293, 276, 264, 250, 234, 220, 206, 192, 178, 164 g/mol.

Typical experimental procedure for synthesis of mono-functional ureido-pyrimidinones.
The procedure for reacting imidazolides with amines is exemplified by the synthesis of mono-functional ureidopyrimidinone **12**. Imidazolide **2a** (1.04 g, 3.44 mmol) and 2,5-dimethylaniline (0.5 g, 4.13 mmol) were dissolved in 10 ml of $CHCl_3$ and this solution

was stirred for three hours under nitrogen at 50 ^{o}C. To the reaction mixture 50 ml of $CHCl_3$ was added and the organic layer was washed with 20 ml 1N HCl, 20 ml saturated $NaHCO_3$ and, 20 ml brine. After drying with Na_2SO_4 the organic layer was reduced to about 5 ml by evaporation in vacuo. This concentrated solution was slowly added to 30 ml of MeOH under vigorous stirring, which resulted in a precipitate. The precipitate was filtered off, and washed thoroughly with MeOH. The resulting white powder was obtained in a 78 % yield.
^{1}H-NMR ($CDCl_3$): δ = 13.18 (s, 1H, *NH*-C=N), 12.47 (s, 1H, C-*NH* (C=O)NH-Ar), 11.81 (s, 1H, C-NH(C=O)*NH*-Ar), 7.17 (s+d, 2H, Ar-*H*), 7.15 (d, 1H, Ar-*H*) 5.88 (s, 1H, (C=O)*CH*=C), 2.36 (m, 7H, (Ar-CH_3 + $(CH_2)_2CH$C), 1.72-1.54 (m, 4H, CH_2), 1.34-1.23 (m, 4H, CH_2), 0.90 (m, 6H, CH_3) ppm. ^{13}C-NMR ($CDCl_3$): δ = 173.0, 155.6, 154.8, 135.9, 134.2, 131.3, 130.5, 127.6, 127.3, 106.3, 45.3, 32.7, 29.2, 26.4, 22.3, 20.8, 17.6, 13.8, 11.6 ppm. MALDI-TOF-MS: found ($M+H^+$ = 357.15, $M+Na^+$ = 379.14 g/mol) calc. (M^+ = 356 g/mol). Elemental analysis: $C_{20}H_{28}N_4O_2$ (356.47) calculated: C, 67.39 H, 7.92 N, 15.72 found: C, 67.29 H, 7.86 N, 15.75. Mp = 178 ^{o}C, FTR-IR: 3029, 2959, 2927, 2860, 2785, 2593, 1670, 1611, 1591, 1556, 1439, 1388, 1319, 1281, 1261, 1222, 1187, 1009, 992, 886, 845, 795, 743, 704 cm^{-1}.

Mono-functional ureidopyrimidinone 10.
Synthesis was performed according to general procedure for mono-functional ureidopyrimidinones. For further purification a silica column (eluens 1%MeOH in $CHCl_3$) was used. The product was obtained as a white powder in yield of 82%.
^{1}H-NMR ($CDCl_3$): δ = 13.31 (s, 1H, *NH*-C=N), 11.95 (s, 1H, ring-*NH* (C=O)NH), 10.23 (s, 1H, ring-NH(C=O)*NH*), 5.87 (s, 1H, (C=O)*CH*=C), 3.31 (m, 2H, (C=O)NHCH_2) 2.34 (m, 1H, $((CH_2)_2CH$C), 1.72-1.57 (m, 6H, CH_2), 1.34-1.23 (m, 7H, CH_2 + CH_3), 0.96 (m, 6H, CH_3) ppm. ^{13}C-NMR ($CDCl_3$): δ = 173.2, 156.7, 155.4, 154.8, 106.1, 45.2, 39.7, 32.8, 31.4, 29.2, 26.3, 22.3, 20.1, 13.7, 13.6, 11.6 ppm. MALDI-TOF-MS: found ($M+H^+$ = 309.17, $M+Na^+$ = 331.16 g/mol) calc. (M^+ = 308.22 g/mol). Elemental analysis: $C_{16}H_{28}N_4O_2$ (308.43) calculated: C, 62.31 H, 9.15 N, 18.17 found: C, 62.45 H, 9.22 N, 17.91. Mp = 178 ^{o}C, FTR-IR: ν = 3145, 2958, 2929, 2861, 1696, 1650, 1578, 1524, 1464, 1440, 1304, 1254, 1147, 1079, 999, 958, 920, 852, 794, 742 cm^{-1}.

Mono-functional ureidopyrimidinone 11.
Synthesis was performed according to general procedure for mono-functional ureidopyrimidinones. The product was obtained as a white powder in yield of 66%.
^{1}H-NMR ($CDCl_3$): δ = 13.38 (s, 1H, *NH*-C=N), 11.76 (s, 1H, ring-*NH* (C=O)NH), 9.65 (s, 1H, ring-NH(C=O)*NH*), 5.85 (s, 1H, (C=O)*CH*=C), 2.33 (m, 1H, $(CH_2)_2CH$C), 1.76-1.57 (m, 4H, CH_2), 1.47 (s, 9H, CH_3), 1.38-1.28 (m, 4H, CH_2), 0.94 (m, 6H, CH_3) ppm. ^{13}C-NMR ($CDCl_3$): δ = 172.9, 155.8, 155.1, 105.9, 51.1, 45.2, 32.8, 29.2, 29.0, 28.7, 26.5, 22.4, 13.7, 11.6 ppm. MALDI-TOF-MS: found ($M+H^+$ = 309.17, $M+Na^+$ = 331.15 g/mol) calc. (M^+ = 308.22 g/mol). Elemental analysis: $C_{16}H_{28}N_4O_2$ (308.43) calculated: C, 62.31 H, 9.15 N, 18.17 found: C, 62.39 H, 9.25 N, 18.26. Mp = 190-191 ^{o}C, FTR-IR: ν = 3222, 3145, 2960, 2931, 2874, 2480, 1662, 1606, 1558, 1453, 1396, 1363, 1317, 1290, 1222, 1172, 1100, 929, 852, 799, 717, 690 cm^{-1}.

Mono-functional ureidopyrimidinone 13.

Synthesis was performed according to general procedure for mono-functional ureidopyrimidinones. For precipitation EtOH was used instead of MeOH. The product was obtained as a white powder in yield of 70%.
^{1}H-NMR ($CDCl_3$ + TFA): δ = 8.46 (d, 1H, NH-C-N-*CH*), 8.31 (t, 1H, NH-C-CH-*CH*), 7.79 (d, 1H, NH-C-*CH*), 7.50 (t, 1H, NH-C-CH-*CH*), 6.11 (s, 1H, (C=O)*CH*=C), 2.53 (m, 1H, $((CH_2)_2CH$C), 1.83-1.71 (m, 2H, CH_2), 1.69-1.58 (m, 2H, CH_2), 1.35-1.26 (m, 2H, CH_2), 0.96 (m, 6H, CH_3), 0.90 (m, 6H, CH_3) ppm. ^{13}C-NMR ($CDCl_3$): δ = 155.5, 154.9, 151.9, 148.3, 137.9, 119.1, 144.9, 106.6, 45.4, 32.8, 29.2, 26.5, 22.4, 13.8, 11.6 ppm. MALDI-TOF-MS: found ($M+H^+$ = 330.09, $M+Na^+$ = 352.07 g/mol) calc. (M^+ = 329.19 g/mol). Elemental analysis: $C_{17}H_{23}N_5O_2$ (329.41) calculated: C, 61.99 H, 7.04 N, 21.26 found: C, 61.97 H, 7.09 N, 21.19. Mp = 160-161^{o}C, FTR-IR: ν = 3228, 3177, 3011, 2958, 2930, 2448, 1696, 1681, 1641, 1606, 1585, 1541, 1461, 1428, 1329, 1292, 1265, 1223, 1174, 1146, 1100, 1057, 1007, 861, 834, 805, 777, 767, 732 cm^{-1}.

Bi-functional ureidopyrimidinone 14.
Imidazolide **2a** (6.0 g, 15.5 mmol) and 1.6-hexamethylenediamine (0.78 g, 6.74 mmol) were dissolved in 10 ml of $CHCl_3$ and this solution was stirred for three hours under nitrogen. To the reaction mixture 50 ml of $CHCl_3$ was added and the organic layer was washed with 20 ml 1N HCl, 20 ml sat. $NaHCO_3$, and 20 ml brine. The organic layer was dried with Na_2SO_4 and was reduced to about 5 ml by evaporation in vacuo. To this concentrated solution 30 ml of acetone was added to under vigorous stirring, which resulted in a precipitate. The precipitate was filtered off, and washed thoroughly with MeOH. The resulting white powder was obtained in a yield of 95 %.
^{1}H-NMR ($CDCl_3$): δ = 13.25 (s, 2H, *NH*-C=N), 11.90 (s, 2H, C-*NH* (C=O)NH-Ar), 10.19 (s, 2H, C-NH(C=O)*NH*-Ar), 5.82 (s, 2H, (C=O)*CH*=C), 3.24 (m, 4H, NH-(C=O)-NH-CH_2), 2.30 (m, 2H, $(CH_2)_2CH$C), 1.72-1.48 (m, 12H, CH_2), 1.40 (m, 4H, CH_2), 1.35-1.18 (m, 8H, CH_2) 0.93 (m, 12H, CH_3) ppm. ^{13}C-NMR ($CDCl_3$): δ = 173.1, 156.6, 155.3, 154.7, 106.1, 45.2, 39.9, 32.7, 29.3, 29.2, 26.6, 26.5, 22.3, 13.7, 11.6 ppm. MALDI-TOF-MS: found ($M+H^+$ = 587.39, $M+Na^+$ = 609.37 g/mol) calc. (M^+ = 586.40 g/mol)). Elemental analysis: $C_{30}H_{50}N_8O_4$ (586.71) calculated: C, 61.41 H, 8.59 N, 19.10 found: C, 61.35 H, 8.57 N, 18.86. Mp = 131-133 ^{o}C, FTR-IR (ATR): ν = 2929, 2859, 1690, 1644, 1568, 1520, 1458, 1381, 1335, 1299, 1250, 1223, 1129, 1067, 985, 949, 843, 802, 723 cm^{-1}.

Bi-functional ureidopyrimidinone 15.
Imidazolide **2a** (5.62 g, 18.56 mmol) and 3, 5-diamino-4- methyl benzoic acid ethyl ester (1.51 g, 7.73 mmol) were dissolved in 20 ml of $CHCl_3$ and this solution was stirred for three hours under nitrogen at 50 ^{o}C. To the reaction mixture 50 ml of $CHCl_3$ was added and the organic layer was washed with 20 ml 1N HCl followed by 20 ml brine and dried with Na_2SO_4. The organic layer was reduced to about 10 ml by evaporation in vacuo. This concentrated solution was slowly added to 50 ml of MeOH under vigorous stirring, which resulted in a precipitate. The precipitate was filtered off, and washed thoroughly with MeOH. The resulting white powder was obtained in an 88 % yield.
^{1}H-NMR ($CDCl_3$): δ = 13.11 (s, 2H, *NH*-C=N), 12.55 (s, 2H, C-*NH* (C=O)NH-Ar), 12.00 (s, 2H, C-NH(C=O)*NH*-Ar), 8.02 (s, 2H, Ar-*H*), 5.89 (s, 2H, (C=O)*CH*=C), 4.41 (q, 2H, (C=O)-O-CH_2), 2.47 (s, 3H, (Ar-CH_3), 2.35 (m, 2H, $(CH_2)_2CH$C), 1.7-1.6 (m, 8H, CH_2),

1.40 (t, 3H, O-CH_2-*CH_3*), 1.36-1.27 (m, 8H, *CH_2*), 0.93 (m, 12H, *CH_3*) ppm. ^{13}C-NMR ($CDCl_3$): δ = 168.9, 161.5, 152.9, 152.3, 134.8, 132.3, 132.0, 122.4, 105.4, 45.8, 40.9, 32.6, 30.9, 29.1, 26.4, 22.3, 19.9, 13.6, 13.5, 12.4, 11.2 ppm. ESI-MS: found $M+H^+$ = 665.31 g/mol calc. M^+ = 664.37 g/mol. Elemental analysis: $C_{34}H_{48}N_8O_6$ (664.37) calculated: C, 61.43 H, 7.22 N, 16.86 found: C, 61.08 H, 7.18 N, 16.62. Mp = 186 ^oC, FTR-IR (ATR): ν = 2958, 2931, 2872, 1721, 1700, 1644, 1563, 1555, 1520, 1482, 1367, 1327, 1304, 1247, 1215, 1106, 1014, 957, 849, 764 cm^{-1}.

Tri-functional ureidopyrimidinone 16.

Imidazolide **2d** (1.08 g, 2.79 mmol) and 4-aminomethyl-octane-1,8-diamine (0.14 g, 0.81 mmol) were dissolved in 20 ml of $CHCl_3$ and this solution was stirred for three hours under nitrogen at room temperature. To the reaction mixture 50 ml of $CHCl_3$ was added and the organic layer was washed with 20 ml 1N HCl followed by 20 ml brine and dried with Na_2SO_4. The organic layer was reduced to about 10 ml by evaporation in vacuo. The reaction mixture was slowly added to 100 ml of MeOH under vigorous stirring, which resulted in a precipitate. The precipitate was filtered off, and washed thoroughly with MeOH. The resulting white powder was obtained in an 84 % yield.

^{1}H-NMR ($CDCl_3$ + CF_3CO_2H): δ = 6.16 (s, 3H, (C=O)*CH*=C)), 3.30 (m, 6H, *CH_2*-NH(C=O)-), 2.62 (t, 6H, C=C-*CH_2*, J=7Hz), 1.66 (t, 6H, C=C-CH_2*CH_2*, J=7Hz), 1.58 (m, 7H, *CH_2*CH_2NH(C=O)-), 1.4-1.2 (m, 26H, CH_2-CH_2-*CH_2*), 0.88 (t, 9H, *CH_3*, J=7Hz).

^{1}H-NMR ($CDCl_3$): δ = 13.17 (br.s,3H, *NH*-C=N), 11.85 (br.s., 3H, CH_2-*NH*-(C=O)NH), 10.19 + 9.99-9.75 (br.s. 3H, CH_2-NH-(C=O)*NH*), 5.82 + 5.64 (br.s., 3H, C=CH), 3.24 (br.s., 6H, *CH_2*-NH(C=O)-), 2.45 (br.s., 6H, C=C-*CH_2*), 1.62 (br.s., 6H, C=C-CH_2*CH_2*), 1.4-1.2 (m, 33H, CH_2-CH_2-*CH_2*), 0.87 (br.s., 9H, *CH_3*).

^{13}C-NMR ($CDCl_3$): δ = 173.24, 172.75, 156.72, 154.83, 152.45, 152.00, 105.91, 105.06, 40.14, 32.80, 31.99, 29.72, 29.42, 29.28, 29.05, 27.75, 14.18. MALDI-TOF-MS: found ($M+H^+$ = 1131.69, $M+Na^+$ = 1153.68 g/mol) calc. (M^+ = 1130.87 g/mol). Elemental analysis: $C_{63}H_{110}N_{12}O_6$ (1130.87) calculated: C, 66.87 H, 9.80 N, 14.85 found: C, 66.48 H, 9.64 N, 14.87, Mp = 204-209 ^oC, FTR-IR (ATR): ν = 2922, 2852, 1660, 1561, 1524, 1440, 1377, 1323, 1253, 1188, 1147, 1077, 953, 830, 766, 710 cm^{-1}.

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[2] F. H. Beijer, H. Kooijman, A. L. Spek, R. P. Sijbesma, E. W. Meijer, *Angew. Chem., Int. Ed.* **1998**, *37*, 75.