Angewandte Chemie
Eine Zeitschrift der Gesellschaft Deutscher Chemiker

Supporting Information

69451 Weinheim, Germany

Mapping the Landscape of Potentially Primordial Informational Oligomers: Oligo-Dipeptides Tagged with 2,4-Disubstituted 5-amino-pyrimidines as Recognition Elements**

Gopi Kumar Mittapalli, Yazmin M. Osornio, Miguel A. Guerrero, Kondreddi Ravinder Reddy, Ramanarayanan Krishnamurthy* and Albert Eschenmoser*

[*] Prof. Dr. A. Eschenmoser
Laboratorium für Organische Chemie
Eidgenössische Technische Hochschule
HCI Hönggerberg CH-8093 Zürich Switzerland
Fax: (+41)1-632-1043
E-mail: Eschenmoser@org.chem.ethz.ch

Prof. Dr. A. Eschenmoser, Prof. Dr. R. Krishnamurthy, Dr. G. K. Mittapalli, Dr. Y. M. Osornio, Dr. M. A. Guerrero, Dr. K. R. Reddy
Department of Chemistry and The Skaggs Institute for Chemical Biology
The Scripps Research Institute
10550 North Torrey Pines Road, La Jolla, CA 92037
Fax: (+1)858-784-9573
E-mail: rkrishna@scripps.edu

[**] Chemistry of α-aminonitriles Part 46. For part 45 see preceding paper [1]. This work was supported by the *Skaggs* Research Foundation. G. K. M., Y. M. O, M. A. G, and K. R. R. are *Skaggs* Postdoctoral Fellows.

Experimental Part

General. Solvents for extraction: ACS grade. Solvents for reaction: reagent grade. Reagents: unless otherwise noted, from *Acros*, *Fluka*, or *Aldrich*, highest quality available. TLC: silica gel *60 F_{254}* aluminum plates, (*Whatman*, type *Al Sil G/UV*, 250 µm layer); visualization by UV absorption) and/or by dipping in a soln. anisaldehyde/H_2SO_4/AcOH/EtOH 5:5:1:18) cerium(IV)sulfate (3 mM)/ammonium molybdate (250 mM) in aq. H_2SO_4 (10%); followed by heating. Flash column chromatography (CC) was performed on silica gel *60* (0.40 – 0.63 mm, 230 – 440 mesh, *EM Science*) at low pressure (max. 2 bar). Melting points (uncorrected) *MEL-TEMP II* (*Laboratory Devices Inc*., USA). NMR: ^{1}H: *d* values in ppm (TMS as internal standard); *J* [Hz], assignments of ^{1}H resonances were in some cases based on 2D experiments (^{1}H,^{1}H-COSY); ^{13}C: *d* values in ppm (TMS as internal standard); *J* [Hz]; assignments and multiplicities were based on 2D experiments (^{1}H,^{13}C-COSY). ESI-MS (mode): *m/z* (intensity in %); performed with Micromat-LCT. Matrix-assisted laser-desorption-ionization time-of-flight mass spectrometry (MALDI-TOF-MS) was performed on a *Voyager-Elite* mass spectrometer (*Perseptive Biosystems*) with delayed extraction with THAP or DHB as the matrix with ammonium citrate added to the sample. Oligo-dipeptides were synthesized on an *Expedite 8909 Nucleic Acid Synthesis* system (*Perseptive Biosystems*) in the PNA mode with the following modifications: 0.14M dipeptide monomer in NMP/DMSO 4:1; 0.13M of HATU in NMP; washing soln. A: NMP/DMSO 4:1; washing soln. B: NMP/DMSO 4:1; deblocking soln. 30% piperidine in NMP/DMSO 4:1; capping: 5% Ac_2O, 6% lutidine in NMP/DMSO 4:1; base-mixture: 0.14M DIPEA, 0.21M lutidine in NMP; coupling time of 30 min; in some cases double coupling step was used. Coupling efficiency was calculated by Fmoc-assay. For a majority of the synthesis, the average coupling efficiency was greater than 95%. HPLC purification of oligopeptides was achieved either by (a) ion-exchange: with MONO-Q HR 5/5 Pharmacia, 10 ⌀ 0.5 cm or Nucleogen-DEAE 60-7 *Machery Nagel*, 125 ⌀ 4, flow 1 ml / min; mobile phase: eluant A: 10 mM Na_2HPO_4, H_2O, pH 11.5 (unless otherwise specified); eluant B: 10 mM Na_2HPO_4, 1 M NaCl, H_2O, pH 11.5 (unless otherwise specified); or with Nucleogen-DEAE 60-7 *Machery Nagel*, 125 ⌀ 4, flow 1 ml / min, mobile phase: eluant A: 10 mM Na_2HPO_4, H_2O, pH 7.0; eluant B: 10 mM Na_2HPO_4, 1 M NaCl, H_2O, pH 7.0 or by (b) reverse-phase: Aquapore ODS 20 micron Brownlee, 250 ⌀ 10.0 mm, flow 4 ml / min. Mobile phase: eluant A: 0.1% TFA in H_2O; eluant B: 0.1% TFA in MeCN. UV Spectra were recorded on a *Cary 1 C*

spectrophotometer (*Varian*). Melting point (T_m) measurements of oligonucleotides were determined with *Cary 1 Bio* spectrophotometer (*Varian*). CD Spectrum was measured on an *AVIV 61 DS* CD spectropolarimeter. All measurements were made with the ′phosphate buffer′, 10 mM aq. NaH_2PO_4 buffer containing 0.1 mM Na_2EDTA, 150 mM (or 1M) NaCl at pH 7.0, with a total oligonucleotide concentration of *ca.* 10 μM, unless indicated otherwise, and the samples were thoroughly degassed, either by heating or by vacuum and ultrasonication. The following molar extinction coefficients were used for the heterocyclic bases at : ε_{260} (uracil) = 10,000, ε_{260} (thymine) = 10,000, ε_{260} (adenine) = 15,000, ε_{260} (2,4-diaminopurine) = 10,000, ε_{260}(2,4-diaminotriazine) = 4200, ε_{250} (2,4-dioxotriazine) = 7500, ε_{287} (2,4,5-triaminopyridine) = 5500, ε_{280} (2,4-dioxo-5-aminopyrimidine) = 6550, ε_{283} (2,5-diamino-4-oxoaminopyrimidine) = 6500, ε_{271} (4,5-diamino-2-oxoaminopyrimidine) = 5400. Abbreviations: Asp = aspartic acid; Bn = Benzyl; Boc = tert-butyloxy carbonyl; CBz = carboxybenzyl; DBU = diazabicyclo undecane; DIPEA: diisopropylethylamine; EDCI= 1-ethyl-3-(3’-dimethylaminopropyl)carbodiimide; Fmoc= 9-fluorenylmethyloxycarbonyl; Glu = glutamic acid; HATU = 2-(1H-7-azabenzotriazol-1-yl)-1,1,3,3-tetramethyl uronium hexafluorophosphate methanaminium; HOBt = 1-hydroxy benzotriazole; MBHA resin = 4-methylbenzhydrylamine resin; NMP = N-methyl-2-pyrrolidone.

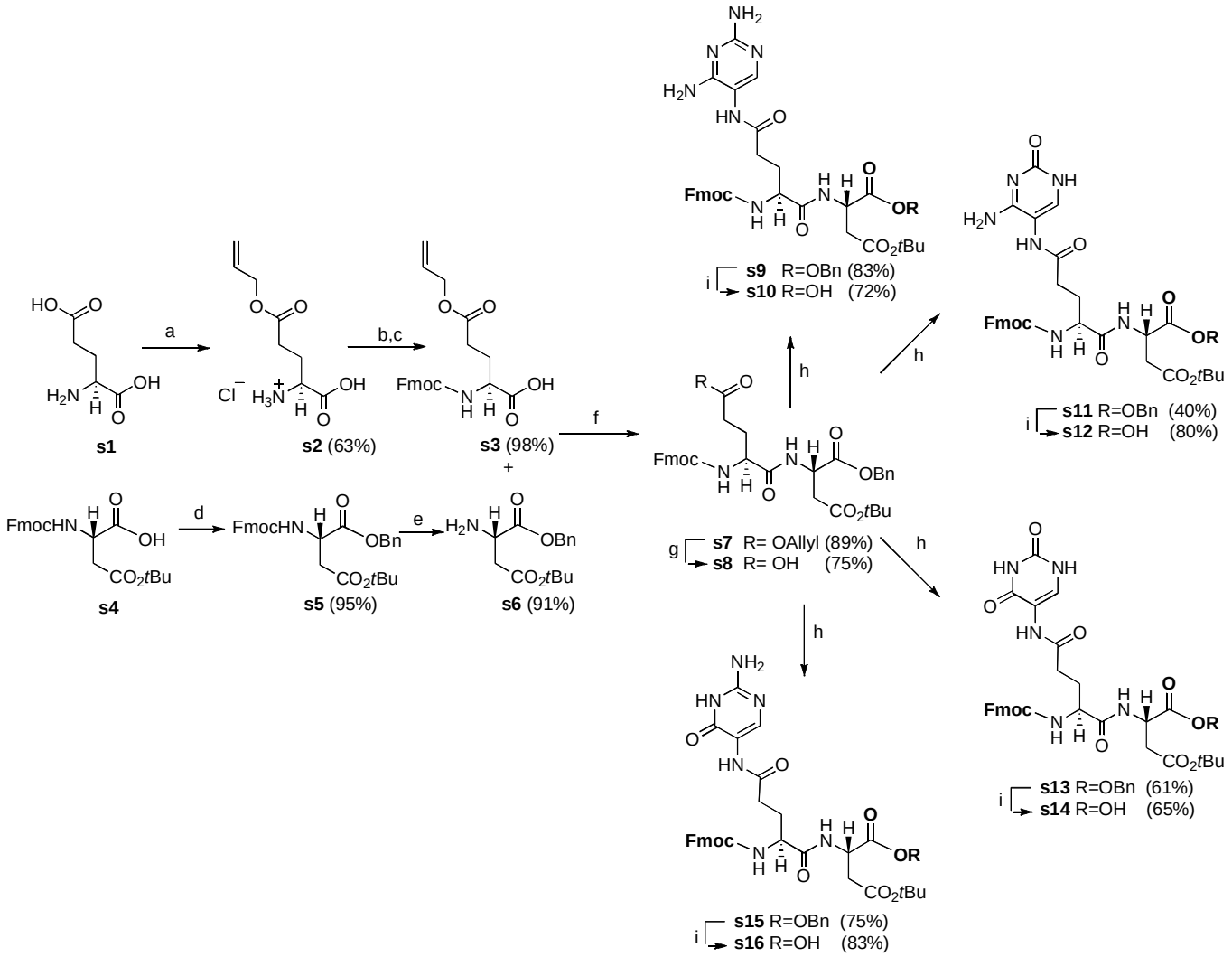

Figure 1. Synthesis of the four 5-amino-pyrimidine-tagged AspGlu-dipeptide building blocks used in the solid support synthesis of the oligomers. (a) 2.5 equiv (0.5M) Me_3SiCl, Allyl alcohol, 0°C, 24h. (b) 1.7 (0.24) K_2CO_3, H_2O, 0°C, 20 min. (c) 1.1 (0.07 M) FmocOSu, dioxane, RT, 8h. (d) 2.5 (0.49) $NaHCO_3$, 2.5 (0.58) $PhCH_2Br$, DMF, RT, 36 h. (e) 5.0 (2.0) 20 % Piperidine in DMF, RT, 1h. (f) 1.0 (0.5 M) EDCI, 1 (0.5) HOBt, DMF, RT, 4h. (g) 4.0 (0.12) $PhSiH_3$, 0.05 $(Ph_3P)_4Pd$, CH_2Cl_2, RT, 5h. (h) 1.5 (0.54) HBTU, 1 (0.36) HOBt, 2 (0.72) of corresponding 5-amino-pyrimidine, DMF, RT-35°C, 36-60 h. (i) 10% Pd/C, H_2 (1 atm), MeOH/DMF/HCO_2H (4.8:4.8:0.4), 0°C, 2-3.5h.

(S)-2-amino-(5-allyloxy)-5-oxo-pentanoic acid. HCl salt (**s2**):[2] To a stirred suspension of (2*S*)-(+)-glutamic acid **s1** (22.1 g, 150 mmol, *Aldrich*) in 700 ml of allyl alcohol under nitrogen was added dropwise chlorotrimethylsilane (40.8 g, 47.6 ml, 375 mmol) at 0 °C. The reaction mixture was allowed to warm to RT, and stirring was continued for 24 h. 2000 ml of cold diethyl ether was added to give a white precipitate, which was collected by filtration, washed with cold diethyl ether (2 x 200 ml) and dried under h.v. to afford 21.2 g (63 %) of **s2** as a white solid. TLC (CH_2Cl_2/MeOH, 75:25): R_f 0.47. ^{1}H-NMR (600 MHz, (D_6)DMSO): 13.77 (br. *s*, COOH), 8.59 (br. *s*, NH_3), 5.91 (appears as *ddd*, *J* = 16.2 10.8, 5.4, CH_2=C*H*), 5.29 (*d*, *J* = 16.2, 1H, CH_2=CH), 5.20 (*d*, *J* = 10.8, 1H, CH_2=CH), 4.55 (*d*, *J* = 5.4, CH_2O), 3.89 (*t*, *J* = 6.0, H-C(α)), 2.48 (*m*, H-C(γ)), 2.05-2.12 (*m*, H-C(β)). ^{13}C-NMR (150 MHz, (D_6)DMSO): 171.31 (COOH), 170.34 (CO), 132.51 (CH_2=CH), 117.75 (CH_2=CH), 64.51 (CH_2O), 51.06 (C(α)), 29.16 (C(γ)), 25.07 (C(β)). ESI-MS: 210 (10, [*M* + Na of free amine]$^+$), 188 (90, [*M* + H]$^+$), 130 (100, [*M*–(CH_2=CH-O)]$^{\cdot+}$).

(S)-2-[((9H-fluoren-9-yl)ethoxy)carbonylamino]-5-(allyloxy)-5-oxopentanoic acid (**s3**):[2b] To an ice-cold stirred suspension of **? 2** (20.12 g, 90 mmol) in 640 ml of water was added K_2CO_3 (21.14 g, 153 mmol). After 20 minutes, a solution of FmocOSu (33.39 g, 99 mmol) in 640 ml of dioxane was added and the resulting solution was allowed to warm to RT, and stirred for 8 h. The reaction mixture was poured into 500 ml of water and dioxane was removed under reduced pressure. The aqueous solution was washed with diethyl ether (2 x 400 ml), acidified to pH 2 with HCl (1 M solution) at 0 °C and extracted with dichloromethane (3 x 500 ml). The organic phase was dried (Na_2SO_4) and the solvent was removed under reduced pressure to give 36.3 g (98 %) of **3** as a white solid, which was used without further purification. TLC (CH_2Cl_2/MeOH, 90:10): R_f 0.48. ^{1}H-NMR (600 MHz, (D_6)DMSO): 12.62 (br. *s*, COOH), 7.88 (*d*, *J* = 7.2, 2 arom. H), 7.73 (*dd* appearing as *t*, *J* = 7.2, 2 arom. H), 7.66 (*d*, *J* = 8.4, NH(Fmoc)), 7.41 (*dd* appearing as *t*, *J* = 7.2, 2 arom. H), 7.32 (*dd* appearing as *t*, *J* = 7.2, 2 arom. H), 5.91 (appears as *ddd*, *J* = 16.2 10.8, 5.4, CH_2=C*H*), 5.29 (*d*, *J* = 16.2, 1H, CH_2=CH), 5.20 (*d*, *J* = 10.8, 1H, CH_2=CH), 4.55 (*d*, *J* = 5.4, CH_2O), 4.21-4.30 (*m*, 3H, HC(Fmoc), H_2CC(Fmoc)), 3.99-4.01 (*m*, H-C(α)), 2.40-2.45 (*m*, H-C(γ)), 1.99-2.06 (*m*, 1H, H-C(β)), 1.81-1.87 (*m*, 1H, H-C(β)). ^{13}C-NMR (150 MHz, (D_6)DMSO): 173.32 (COOH), 171.77 (CO), 156.05

(CO(Fmoc)), 143.75 (arom. C), 140.63 (arom. C), 132.58 (CH_2=$\underline{C}$H), 127.55 (arom. C), 126.97 (arom. C), 125.17 (arom. C), 120.01 (arom. C), 117.55 ($\underline{C}H_2$=CH), 65.55 (CH_2(Fmoc)), 64.32 ($\underline{C}H_2CH=CH_2$), 52.87 (C(α)), 46.59 (CH(Fmoc)), 29.96 (C(γ)), 25.98 (C(β)). ESI-MS: 432 (100, $[M + Na]^+$), 410 (10, $[M + H]^+$).

(S)-1-benzyl-4-tert-butyl 2-[((9H-fluoren-9-yl)methoxy)cabonylamino]succinate (**s5**):[3a] To a stirred solution of **s4** (20 g, 48.6 mmol, *Novabiochem*) in 250 ml of dry DMF was added $NaHCO_3$ (10.25 g, 122 mmol) and benzyl bromide (24.97 g, 17.36 ml, 146 mmol). The reaction mixture was stirred at RT, for 36 h and then diluted with 400 ml of H_2O and extracted with CH_2Cl_2 (3 x 300 ml). The organic phase was washed sequentially with H_2O (2 x 300 ml), 300 ml of sat. aq. $NaHCO_3$, 300 ml sat. aq. NaCl and evaporated in vacuo. To the resulting residue, 400 ml of cold hexane was added and the mixture stirred vigorously until precipitation of compound **s5** was complete. Filtration and washing with hexane (2 x 200 ml) gave 23.16 g (95 %) of **s5** as a white solid. TLC (Hexane/AcOEt, 80:20): R_f 0.48. ^{1}H-NMR (600 MHz, (D_6)DMSO): 7.87-7.89 (*m*, 2 arom. H, NH(Fmoc)), 7.68 (*dd* appearing as *t*, *J* = 7.2, 2 arom. H), 7.41 (*dd* appearing as *t*, *J* = 7.2, 2 arom. H), 7.29-7.32 (*m*, 7 arom. H), 5.12 (*s*, Ph$\underline{CH_2}$O), 4.46-4.49 (*m*, H-C(α)), 4.20-4.32 (*m*, 3H, HC(Fmoc), H_2CC(Fmoc)), 2.75 (*dd*, *J* = 16.2, 5.4, H-C(β)), 2.63 (*dd J* = 16.2, 7.8, H-C(β)),1.35 (*s*, $C(CH_3)_3$). ^{13}C-NMR (150 MHz, (D_6)DMSO): 170.68 (CO), 168.83 (CO), 155.72 (CO(Fmoc)), 143.62 (arom. C), 140.61 (arom. C), 135.65 (arom. C), 128.24 (arom. C), 127.89 (arom. C), 127.57 (arom. C), 127.51 (arom. C), 126.93 (arom. C), 125.02 (arom. C), 120.00 (arom. C), 80.36 ($\underline{C}(CH_3)_3$), 66.11 (CH_2(Fmoc)), 65.66 (Ph$\underline{C}H_2$O), 50.52 (C(α)), 46.47 (CH(Fmoc)), 36.87 (C(β)), 27.51 (C($\underline{C}H_3)_3$). ESI-MS: 302 (30, $[M$ – Fmoc + H + $Na]^+$), 280 (50, $[M$ – Fmoc + H + $H]^+$), 224 (100, [M – (Fmoc – ^{t}Bu + H + H) + H).

(S)-1-benzyl-4-tert-butyl 2-aminosuccinate (**s6**):[3b] To a stirred solution of **s5** (23 g, 45.8 mmol) in 90 ml of dry DMF under N_2 was added piperidine (19.5 g, 22.6 ml, 229 mmol). The reaction mixture was stirred at RT for 1 h, and DMF was removed under reduced pressure at RT The resulting residue was purified by CC (SiO_2 eluted with Hexane/AcOEt, 100:0 → 20:80) to afford 11.64 g (91 %) of **6** as a yellow oil. TLC (Hexane/AcOEt, 30:70): R_f 0.56. ^{1}H-NMR (600 MHz, (D_6)DMSO): 7.35-7.37 (*m*, 5 arom. H), 5.11 (*d*, *J* = 16.8, Ph$\underline{CH_2}$O), 3.65-3.67 (*m*, H-C(α)), 2.56 (*dd*, *J* = 16.2, 6.0, 1H-

C(β)), 2.51 (*dd*, *J* = 16.2, 7.8, 1H-C(β)), 1.93 (br. *s*, NH_2), 1.35 (*s*, $C(CH_3)_3$). ^{13}C-NMR (150 MHz, (D_6)DMSO): 174.06 (CO), 169.76 (CO), 135.97 (arom. C), 128.25 (arom. C), 127.83 (arom. C), 127.64 (arom. C), 79.95 ($\underline{C}(CH_3)_3$), 65.58 ($Ph\underline{C}H_2O$), 51.09 (C(α)), 40.03 (C(β)), 27.54 ($C(\underline{C}H_3)_3$). ESI-MS: 205 (30, $[M - {}^{t}Boc - H]^+$).

1-Benzyl-4-tert-butyl 2-[2-{((9H-fluoren-9-yl)-methoxy)carbonylamino}-5-(allyloxy)-5-oxopentanamido]succinate (**s7**): To a stirred solution of the acid **s3** (17.1 g, 41.76 mmol) in 73 ml of dry DMF was added EDCI (8.0 g, 41.76 mmol) and HOBt (5.76 g, 41.76 mmol) followed by the addition of **s6** (11.65 g, 41.76 mmol) in 10 ml of DMF. The reaction mixture was stirred for 4h at RT, quenched with 400 ml of sat. aq. $NaHCO_3$ solution and the aqueous phase was extracted with AcOEt (3 x 200 ml). The combined organic extracts were dried (Na_2SO_4) and concentrated in vacuo. The residue was purified by CC (SiO_2, Hexane/EtOAc, 100:0 → 70:30) to afford 25.3 g (89 %) of **s7** as a white solid. TLC (Hexane/EtOAc, 70:30): R_f 0.45. ^{1}H-NMR (600 MHz, (D_6)DMSO): 8.43 (*d*, *J* = 7.8, NHCO), 7.88 (*d*, *J* = 7.2, 2 arom. H), 7.73 (*dd* appearing as *t*, *J* = 7.2, 2 arom. H), 7.59 (*d*, *J* = 8.4, NH(Fmoc)), 7.41 (*dd* appearing as *t*, *J* = 7.2, 2 arom. H), 7.32-7.34 (*m*, 7 arom. H), 5.90 (appears as *ddd*, *J* = 16.8, 10.8, 5.4, CH_2=C*H*), 5.11 (*d*, *J* = 19.8, $Ph\underline{CH_2}O$), 4.68-4.71 (*m*, H-C(α-Asp)), 4.54 (*d*, *J* = 5.4, CH_2=$CH\underline{CH_2}O$), 4.19-4.30 (*m*, 3H, HC(Fmoc), H_2CC(Fmoc)), 4.07-4.10 (*m*, H-C(α-Glu)), 2.74 (*dd*, *J* = 16.8, 5.7, 1H-C(β-Asp)), 2.63 (*dd*, *J* = 16.8, 7.0, 1H-C(β-Asp)), 2.36-2.39 (*m*, 2H-C(γ-Glu)), 1.91-1.96 (*m*, 1H-C(β-Glu)), 1.77-1.84 (*m*, 1H-C(β-Glu)), 1.33 (*s*, $C(CH_3)_3$). ^{13}C-NMR (150 MHz, (D_6)DMSO): 171.80 (CO), 171.22 (CO), 170.29 (CO), 168.83 (CO), 155.74 (CO(Fmoc)), 143.75 (arom. C), 140.59 (arom. C), 135.58 (arom. C), 132.57 (CH_2=$\underline{C}$H), 128.23 (arom. C), 127.88 (arom. C), 127.59 (arom. C), 127.51 (arom. C), 126.93 (arom. C), 125.18 (arom. C), 119.97 (arom. C), 117.53 ($\underline{C}H_2$=CH), 80.49 ($\underline{C}(CH_3)_3$), 66.15 (CH_2(Fmoc)), 65.58 ($Ph\underline{C}H_2O$), 64.27 ($\underline{C}H_2CH$=CH_2), 53.46 (C(α-? ? ?)), 48.55 (C(α-Glu)), 46.53 (CH(Fmoc)), 36.72 (C(β-? ? ?)), 29.85 (C(γ-Glu)), 27.46 ($C(\underline{C}H_3)_3$, 27.18 (C(β-Glu)). ESI-MS: 693 (100, $[M + Na]^+$), 671 (40, $[M + H]^+$).

4-[{((9H-fluoren-9-yl)methoxy)carbonylamin}-5-(1-benzyloxy)-4-tert-butoxy-1,4-dioxobutan-2-ylamino]-5-oxopentanoic acid (**s8**): To a stirred solution of the dipeptide **s7** (24 g, 35.7 mmol) and phenylsilane (15.5g, 17.6 ml, 143.1 mmol) in 1200 ml of dry

CH_2Cl_2 under N_2 was added tetrakis(triphenylphosphine)Pd(0) (280 mg, 1.8 mmol) at 0 ^{O}C. The reaction mixture was stirred for 5 h at RT (till no more starting material was detected by TLC). The solvent was removed under reduced pressure at RT, and the residue was purified by CC (SiO_2, CH_2Cl_2/MeOH, 90:10) to afford 16.9 g (75 %) of **s8** as a white solid. TLC (CH_2Cl_2/MeOH, 94:6): R_f 0.47. ^{1}H-NMR (600 MHz, (D_6)DMSO): 12.14 (br. *s*, COOH), 8.41 (*d*, *J* = 7.8, NHCO), 7.89 (*d*, *J* = 7.2, 2 arom. H), 7.72 (*dd* appearing as *t*, *J* = 7.2, 2 arom. H), 7.54-7.63 (*m*, NH(Fmoc), 2 arom. H), 7.41 (*dd* appearing as *t*, *J* = 7.2, 2 arom. H), 7.31-7.34 (*m*, 5 arom. H), 5.11 (*d*, *J* = 19.8, Ph$\underline{CH_2}$O), 4.69-4.72 (*m*, H-C(α-Asp)), 4.21-4.30 (*m*, 3H, HC(Fmoc), H_2CC(Fmoc)), 4.07-4.10 (*m*, H-C(α-Glu)), 2.74 (*dd*, *J* = 16.2, 6.0, 1H-C(β-Asp)), 2.63 (*dd*, *J* = 16.2, 6.6, 1H-C(β-Asp)), 2.27-2.30 (*m*, 2H-C(γ-Glu)), 1.91-1.95 (*m*, 1H-C(β-Glu)), 1.74-1.80 (*m*, 1H-C(β-Glu)), 1.33 (*s*, C(CH_3)$_3$). ^{13}C-NMR (150 MHz, (D_6)DMSO): 173.80 (COOH), 171.40 (CO), 170.32 (CO), 168.84 (CO), 155.77 (CO(Fmoc)), 143.76 (arom. C), 140.59 (arom. C), 135.6 (arom. C), 128.25 (arom. C), 127.88 (arom. C), 127.62 (arom. C), 127.52 (arom. C), 126.94 (arom. C), 125.21 (arom. C), 119.98 (arom. C), 80.49 ($\underline{C}$(CH_3)$_3$), 66.15 (CH_2(Fmoc)), 65.61 (Ph$\underline{C}H_2$O), 53.61 (C(α-Asp)), 48.54 (C(α-Glu)), 46.53 (CH(Fmoc)), 36.76 (C(β-Asp)), 30.08 (C(γ-Glu)), 27.46 (C($\underline{C}H_3$)$_3$, 27.26 (C(β-Glu)). ESI-MS: 653 (10, [*M* + Na]$^+$), 631 (4, [*M* + H]$^+$), 279 (100, [*M* – Glu]$^+$).

1-benzyl-4-tert-butyl-2-{2-[((9H-fluoren-9-yl)methoxy)carbonylamino)-5-(2,4-dioxo-1,2,3,4-tetrahydropyrimidin-5-ylamino)-5-oxopentanamido}succinate (**s9**): To a stirred solution of the acid **s8** (2.75 g, 4.36 mmol) in 12 ml of dry DMF was added HBTU (2.48 g, 6.54 mmol), HOBt (588 mg, 4.36 mmol) and 2,4,5-triaminopyrimidine (1.1 g, 8.72 mmol). The stirring was stirred at 35 ^{o}C for 36 h, diluted with 100 ml of H_2O, extracted with AcOEt (3 x 80 ml). The organic phase was washed with 150 ml of sat. aq. $NaHCO_3$ solution, dried (Na_2SO_4) and concentrated in vacuo. The residue was purified by CC (SiO_2, eluted with CH_2Cl_2/MeOH, 98:2 to 90:10) to afford 2.67 g (83 %) of **9** as a white solid. TLC (CH_2Cl_2/MeOH, 9:1): R_f 0.55. ^{1}H-NMR (600 MHz, (D_6)DMSO): 8.87 (br. *s*, NHCO), 8.42 (*d*, *J* = 7.8, NHCO), 7.89 (*d*, *J* = 7.2, 2 arom. H), 7.71 (*d*, *J* = 7.2, 2 arom. H), 7.61 (*s*, H-(C6)), 7.59 (*d*, *J* = 8.4 Hz, *NH*Fmoc), 7.41 (*dd* appearing as *t*, *J* = 7.2, 2 arom. H), 7.29-7.34 (*m*, 7 arom. H), 6.36 (br. *s*, NH_2), 6.06 (br. *s*, NH_2), 5.11 (*d*, *J* = 18.8, Ph$\underline{CH_2}$O), 4.69-4.72 (*m*, H-C(α-Asp)), 4.20-4.26 (*m*, 3H, HC(Fmoc),

H_2CC(Fmoc)), 4.07-4.11 (*m*, H-C(α-Glu)), 2.75 (*dd*, *J* = 16.8, 6.0, 1H-C(β-Asp)), 2.63 (*dd*, *J* = 16.8, 6.6, 1H-C(β-Asp)), 2.33-2.37 (*m*, 2H-C(γ-Glu)), 1.99-2.01 (*m*, 1H-C(β-Glu)), 1.80-1.82 (*m*, 1H-C(β-Glu)), 1.33 (*s*, C(CH_3)$_3$). ^{13}C-NMR (150 MHz, (D_6)DMSO): 171.59 (*C*O), 171.36 (*CO*), 170.34 (CO), 168.89 (CO), 160.32 (C(4)), 159.86 (C(2)), 155.86 (NH*CO*Fmoc), 151.12 (C(6)), 143.70 (arom. C), 140.59 (arom. C), 135.62 (arom. C), 128.26 (arom. C), 127.89 (arom. C), 127.61 (arom. C), 127.54 (arom. C), 126.98 (arom. C), 125.24 (arom. C), 120.00 (arom. C), 107.29 (C(5)), 80.49 (<u>C</u>(CH_3)$_3$), 66.14 (CH_2(Fmoc)), 65.72 (Ph<u>C</u>H_2O), 53.95 (C(α-Asp)), 48.54 (C(α-Glu)), 46.52 (CH(Fmoc)), 36.80 (C(β-Asp)), 31.71 (C(γ-Glu)), 27.67 (C(β-Glu)), 27.47 (C(<u>C</u>H_3)$_3$). ESI-MS: 760 (10, [*M* + Na]$^+$), 738 (100, [*M* + H]$^+$).

2-{2-[((9H-fluoren-9-yl)methoxy)carbonylamino)-5-(2,4-diaminopyrimidin-5-ylamino)-5-oxopentanamido}-4-tert-butoxy-4-oxobutanoic acid (**s10**): To a stirred suspension of 10 % Pd-C (150 mg) in 15 ml of DMF/MeOH 1:3 was added **s9** (500 mg, 0.67 mmol) in 5 ml of DMF, followed by formic acid (800 µl) at 0 °C. The reaction mixture was stirred under H_2 atmosphere (balloon) for 3.5 h at 0-5 °C, filtered through celite and washed with hot MeOH (2 x 30 ml). The washings were combined with the filtrate and concentrated in vacuo. The residue was purified on reverse phase CC (C18-silica gel; H_2O/DMF 100:0→20:80) to afford 312 mg (72 %) of **s10** as a brown solid. (AcOEt/MeOH/H_2O/CH_3COOH 75:15:6:2): R_f 0.54. ^{1}H-NMR (600 MHz, (D_6)DMSO): 12.78 (br. *s*, COOH), 8.99 (br. *s*, NHCO), 8.12 (*d*, *J* = 7.8, NHCO), 7.89 (*d*, *J* = 7.2, 2 arom. H), 7.73 (*dd* appearing as *t*, *J* = 7.2, 2 arom. H), 7.65 (*d*, *J* = 6.0, H-(C6)), 7.56 (*d*, *J* = 8.4 Hz, *NH*Fmoc), 7.41 (*dd* appearing as *t*, *J* = 7.2, 2 arom. H), 7.33 (*dd* appearing as *t*, *J* = 7.2, 2 arom. H), 6.48 (br. *s*, NH_2), 6.22 (br. *s*, NH_2), 4.51-4.54 (*m*, H-C(α-Asp)), 4.21-4.25 (*m*, 3H, HC(Fmoc), H_2CC(Fmoc)), 4.05-4.08 (*m*, H-C(α-Glu)), 2.67 (*dd*, *J* = 16.2, 6.0, 1H-C(β-Asp)), 2.54 (*dd*, *J* = 16.2, 7.2, 1H-C(β-Asp)), 2.33-2.37 (*m*, 2H-C(γ-Glu)), 1.99-2.01 (*m*, 1H-C(β-Glu)), 1.81-1.83 (*m*, 1H-C(β-Glu)), 1.36 (*s*, C(CH_3)$_3$). ^{13}C-NMR (150 MHz, (D_6)DMSO): 173.00 (COOH), 171.50 (*C*O), 171.01 (*CO*), 169.49 (CO), 160.06 (C(4)), 159.90 (C(2)), 155.77 (NH*CO*Fmoc), 148.37 (C(6)), 143.75 (arom. C), 140.59 (arom. C), 127.77 (arom. C), 126.99 (arom. C), 125.26 (arom. C), 119.98 (arom. C), 107.66 (C(5)), 79.92 (<u>C</u>(CH_3)$_3$), 65.71 (CH_2(Fmoc)), 54.02 (C(α-Asp)), 49.37 (C(α-

Glu)), 46.55 (CH(Fmoc)), 37.62 (C(β? ? ?)), 31.75 (C(γ?Glu)), 27.85 (C(β?Glu)), 27.55 (C(CH_3)$_3$). ESI-MS: 670 (10, [*M* + Na]$^+$), 648 (100, [*M* + H]$^+$).

1-benzyl-4-tert-butyl-2-{2-[((9H-fluoren-9-yl)methoxy)carbonylamino)-5-(4-amino-2-oxo-1,2-dihydropyrimidin-5-ylamino)-5-oxopentanamido}succinate (**s11**): To a stirred solution of the acid **s8** (2.75 g, 4.36 mmol) in 12 ml of dry DMF was added HBTU (2.48 g, 6.54 mmol), HOBt (588 mg, 4.36 mmol) and 5-aminocytosine (1.09 g, 8.72 mmol). The reaction mixture was stirred a RT, for 36 h, diluted with 100 ml of H_2O and extracted with AcOEt (3 x 80 ml). The organic phase was washed with 150 ml of sat. aq. $NaHCO_3$ solution, dried (Na_2SO_4) and concentrated in vacuo. The residue was purified by CC (SiO_2, eluted with CH_2Cl_2/MeOH, 100:0 to 85:15) to afford 1.28 g (40 %) of **s11** as a white solid. TLC (CH_2Cl_2/MeOH, 9:1): R_f 0.46. ^{1}H-NMR (600 MHz, (D_6)DMSO): 10.41 (br. *s*, NH), 8.77 (*s*, NHCO), 8.43 (*d*, *J* = 7.8, NHCO), 7.89 (*d*, *J* = 7.2, 2 arom. H), 7.72 (*d*, *J* = 7.2, 2 arom. H), 7.59 (*d*, *J* = 8.4 Hz, *NH*Fmoc), 7.41 (*dd* appearing as *t*, *J* = 7.2, 2 arom. H), 7.29-7.36 (*m*, 7 arom. H, H-C(6)), 6.73 (br. *s*, NH_2), 5.11 (*d*, *J* = 19.8, PhC*H*$_2$O), 4.69-4.72 (*m*, H-C(α-Asp)), 4.21-4.25 (*m*, 3H, HC(Fmoc), H_2CC(Fmoc)), 4.06-4.09 (*m*, H-C(α?Glu)), 2.76 (*dd*, *J* = 16.8, 6.6, 1H-C(β?Asp)), 2.63 (*dd*, *J* = 16.8, 7.2, 1H-C(β?Asp)), 2.32-2.38 (*m*, 2H-C(γ?Glu)), 1.99-2.01 (*m*, 1H-C(β?Glu)), 1.78-1.81 (*m*, 1H-C(β?Glu)), 1.33 (*s*, C(CH_3)$_3$). ^{13}C-NMR (150 MHz, (D_6)DMSO): 171.65 (*C*O), 171.55 (*CO*), 170.32 (CO), 168.88 (CO), 163.38 (C(4)), 155.85 (NH*CO*Fmoc), 155.60 (C(2)), 143.70 (arom. C), 140.59 (arom. C), 139.05 (C(6)), 135.61 (arom. C), 128.26 (arom. C), 127.82 (arom. C), 127.67 (arom. C), 127.60 (arom. C), 126.98 (arom. C), 125.24 (arom. C), 120.00 (arom. C), 104.3 (C(5)), 80.50 (C(CH_3)$_3$), 66.15 (CH_2(Fmoc)), 65.73 (PhCH_2O), 53.88 (C(α? ? ?)), 48.55 (C(α-Glu)), 46.53 (CH(Fmoc)), 36.80 (C(β? ? ?)), 31.67 (C(γ?Glu)), 27.48 (C(CH_3)$_3$), 25.13 (C(β?Glu)). ESI-MS: 761 (5, [*M* + Na]$^+$), 739 (50, [*M* + H]$^+$), 775 (50), 553 (100).

2-{2-[((9H-fluoren-9-yl)methoxy)carbonylamino)-5-(4-amino-2-oxo-1,2-dihydropyrimidin-5-ylamino)-5-oxopentanamido}-4-tert-butoxy-4-oxobutanoic acid (**s12**): To a stirred suspension of 10 % Pd-C (150 mg) in 15 ml of DMF/MeOH 1:3 was added the compound **s11** (500 mg, 0.67 mmol) in 5 ml of DMF, followed by formic acid (800 µl) at 0 °C. The reaction mixture was stirred under H_2 atmosphere (balloon) for 2 h at 0-5 °C, filtered through celite and washed with hot MeOH (2 x 30 ml). The washings

were combined with the filtrate and concentrated in vacuo. The residue was purified on reverse phase CC (C18-silica gel; H_2O/MeOH 100:0→40:60) to afford 351 mg (80 %) of **s12** as a white solid. TLC (AcOEt/MeOH/H_2O/CH_3COOH 75:15:6:2): R_f 0.47. ^{1}H-NMR (600 MHz, (D_6)DMSO): 12.79 (br. s, COOH), 10.41 (br. *s*, NH), 8.81 (*s*, NHCO), 8.18 (*d*, *J* = 7.8, NHCO), 7.89 (*d*, *J* = 7.2, 2 arom. H), 7.72 (*dd* appearing as *t*, *J* = 7.2, 2 arom. H), 7.57 (*d*, *J* = 8.4 Hz, NHFmoc), 7.39-7.42 (*m*, 2 arom. H, H-C(6)), 7.33 (*dd* appearing as *t*, *J* = 7.2 Hz, 2 arom. H), 6.75 (br. *s*, NH_2), 4.51-4.58 (*m*, H-C(α-Asp)), 4.20-4.25 (*m*, 3H, HC(Fmoc), H_2CC(Fmoc)), 4.06-4.07 (*m*, H-C(α-Glu)), 2.68 (*dd*, *J* = 16.2, 6.0, 1H-C(β-Asp)), 2.56 (*dd*, *J* = 16.2, 7.2, 1H-C(β-Asp)), 2.32-2.34 (*m*, 2H-C(γ-Glu)), 1.97-1.99 (*m*, 1H-C(β-Glu)), 1.77-1.80 (*m*, 1H-C(β-Glu)), 1.37 (*s*, $C(CH_3)_3$). ^{13}C-NMR (150 MHz, (D_6)DMSO): 172.12 (COOH), 171.72 (CO), 171.24 (CO), 169.19 (CO), 163.31 (C4), 155.82 (NHCOFmoc), 155.50 (C2), 143.74 (arom. C), 140.59 (arom. C), 139.05 (C-6), 127.55 (arom. C), 127.00 (arom. C), 125.27 (arom. C), 120.00 (arom. C), 104.38 (C5), 80.22 ($\underline{C}(CH_3)_3$), 65.73 (CH_2(Fmoc)), 53.93 (C(α-? ? ?)), 48.68 (C(α-Glu)), 46.54 (CH(Fmoc)), 37.17 (C(β-? ? ?)), 31.66 (C(γ-Glu)), 27.54 ($C(\underline{C}H_3)_3$, C(β-Glu)). ESI-MS: 671 (10, $[M + Na]^+$), 649 (100, $[M + H]^+$).

1-benzyl-4-tert-butyl-2-{2-[((9H-fluoren-9-yl)methoxy)carbonylamino)-5-(2,4-dioxo-1,2,3,4-tetrahydropyrimidin-5-ylamino)-5-oxopentanamido}succinate (**s13**): To a stirred solution of the acid **s8** (2.75 g, 4.36 mmol) in 12 ml of dry DMF was added HBTU (2.48 g, 6.54 mmol), HOBt (588 mg, 4.36 mmol) and 5-aminouracil (1.1 g, 8.72 mmol). The reaction mixture was stirred a RT, for 60 h. At the end of the reaction the remaining 5-aminouracil was filtered and the solid was washed with 15 ml of DMF. The filtrate was combined with the washings and diluted with 100 ml of H_2O, extracted with AcOEt (3 x 80 ml). The organic phase was washed with 150 ml of sat. aq. $NaHCO_3$ solution, dried (Na_2SO_4) and concentrated in vacuo. The residue was purified by CC (SiO_2, eluted with CH_2Cl_2/MeOH, 98:2 to 90:10) to afford 1.96 g (61 %) of **s13** as a white solid. TLC (CH_2Cl_2/MeOH, 92:8): R_f 0.56. ^{1}H-NMR (600 MHz, (D_6)DMSO): 11.40 (br. *s*, NHCO), 10.63 (br. *s*, NHCO), 8.98 (br. *s*, NHCO), 8.38 (*d*, *J* = 7.8, NHCO), 8.05 (*s*, H-(C6)), 7.89 (*d*, *J* = 7.2, 2 arom. H), 7.71 (*dd* appearing as *t*, *J* = 7.2, 2 arom. H), 7.51 (*d*, *J* = 8.4 Hz, *NH*Fmoc), 7.41 (*dd* appearing as *t*, *J* = 7.2, 2 arom. H), 7.27-7.34 (*m*, 7 arom. H), 5.11 (*d*, *J* = 18, Ph$\underline{C}H_2$O), 4.67-4.71 (*m*, H-C(α-Asp)), 4.19-4.29 (*m*, 3H, HC(Fmoc),

H_2CC(Fmoc)), 4.02-4.06 (*m*, H-C(α-Glu)), 2.73 (*dd*, *J* = 16.8, 6.6, 1H-C(β-Asp)), 2.62 (*dd*, *J* = 16.8, 7.2, 1H-C(β-Asp)), 2.31-2.45 (*m*, 2H-C(γ-Glu)), 1.91-1.95 (*m*, 1H-C(β-Glu)), 1.74-1.81 (*m*, 1H-C(β-Glu)), 1.33 (*s*, C(CH_3)$_3$). ^{13}C-NMR (150 MHz, (D_6)DMSO): 171.53 (*C*O), 170.92 (*CO*), 170.36 (CO), 168.87 (CO), 160.58 (C(4)), 155.76 (NH*CO*Fmoc), 149.52 (C(2)), 143.66 (arom. C), 140.60 (arom. C), 135.60 (arom. C), 129.19 (C(6)), 128.23 (arom. C), 127.85 (arom. C), 127.65 (arom. C), 127.54 (arom. C), 126.97 (arom. C), 125.10 (arom. C), 119.99 (arom. C), 113.15 (C(5)), 80.50 (C(CH_3)$_3$), 66.17 (CH_2(Fmoc)), 65.61 (PhCH_2O), 53.92 (C(α-Asp)), 48.55 (C(α-Glu)), 46.55 (CH(Fmoc)), 36.79 (C(β-Asp)), 32.28 (C(γ-Glu)), 28.00 (C(β-Glu)), 27.48 (C(CH_3)$_3$). ESI-MS: 762 (5, [*M* + Na]$^+$), 740 (100, [*M* + H]$^+$).

2-{2-[((9H-fluoren-9-yl)methoxy)carbonylamino)-5-(2,4-dioxo-1,2,3,4-tetrahydropyrimi din-5-ylamino)-5-oxopentanamido}-4-tert-butoxy-4-oxobutanoic acid (**s14**): To a stirred suspension of 10 % Pd-C (150 mg) in 15 ml of DMF/MeOH 1:3 was added the compound **s13** (500 mg, 0.67 mmol) in 5 ml of DMF, followed by formic acid (800 µl) at 0 °C. The reaction mixture was stirred under H_2 atmosphere (balloon) for 3.5 h at 0-5 °C, filtered through celite and washed with hot MeOH (2 x 30 ml). The washings were combined with the filtrate and concentrated in vacuo. The residue was purified on reverse phase CC (C18-silica gel; H_2O/MeOH 100:0→20:80) to afford 283 mg (65 %) of **s14** as a white solid. TLC (CH_2Cl_2/MeOH, 4:6): R_f 0.51. ^{1}H-NMR (600 MHz, (D_6)DMSO): 12.78 (br. *s*, COOH), 11.41 (br. *s*, NHCO), 10.61 (*d*, *J* = 6.0 Hz, NHCO), 8.99 (*s*, NHCO), 8.15 (*d*, *J* = 7.8, NHCO), 8.03 (*d*, *J* = 6.0, H-(C6)), 7.89 (*d*, *J* = 7.2, 2 arom. H), 7.71 (*dd* appearing as *t*, *J* = 7.2, 2 arom. H), 7.51 (*d*, *J* = 8.4 Hz, *NH*Fmoc), 7.41 (*dd* appearing as *t*, *J* = 7.2, 2 arom. H), 7.33 (dd appearing as *t*, *J* = 7.2, 2 arom. H), 4.52-4.56 (*m*, H-C(α-Asp)), 4.20-4.25 (*m*, 3H, HC(Fmoc), H_2CC(Fmoc)), 4.00-4.04 (*m*, H-C(α-Glu)), 2.66 (*dd*, *J* = 16.2, 6.0, 1H-C(β-Asp)), 2.56 (*dd*, *J* = 16.2, 7.2, 1H-C(β-Asp)), 2.32-2.45 (*m*, 2H-C(γ-Glu)), 1.90-1.96 (*m*, 1H-C(β-Glu)), 1.74-1.80 (*m*, 1H-C(β-Glu)), 1.36 (*s*, C(CH_3)$_3$). ^{13}C-NMR (150 MHz, (D_6)DMSO): 172.16 (COOH), 171.38 (*C*O), 171.09 (*CO*), 169.21 (CO), 160.69 (C4), 155.83 (NH*CO*Fmoc), 149.62 (C2), 143.82 (arom. C), 140.66 (arom. C), 129.48 (C-6), 127.61 (arom. C), 127.06 (arom. C), 125.28 (arom. C), 120.26 (arom. C), 113.16 (C5), 80.32 (C(CH_3)$_3$), 65.66 (CH_2(Fmoc)), 54.03 (C(α-Asp)),

48.64 (C(α-Glu)), 46.61 (CH(Fmoc)), 37.13 (C(β? ? ?)), 32.31 (C(γ?Glu)), 28.05 (C(β?Glu)), 27.57 (C(<u>C</u>H_3)$_3$). ESI-MS: 672 (30, [*M* + Na]$^+$), 650 (100, [*M* + H]$^+$).

1-benzyl-4-tert-butyl-2-{2-[((9H-fluoren-9-yl)methoxy)carbonylamino)-5-(2-amino-6-oxo-1,6-dihydropyrimidin-5-ylamino)-5-oxopentanamido}succinate (**s15**): To a stirred solution of the acid **s8** (2.75 g, 4.36 mmol) in 12 ml of dry DMF was added HBTU (2.48 g, 6.54 mmol), HOBt (588 mg, 4.36 mmol) and 5-aminoisocytosine (1.1 g, 8.72 mmol). The stirring was stirred at 35 ^{o}C for 36 h, diluted with 100 ml of H_2O and extracted with AcOEt (3 x 80 ml). The organic phase was washed with 150 ml of sat. aq. $NaHCO_3$ solution, dried (Na_2SO_4) and concentrated in vacuo. The residue was purified by CC (SiO_2, eluted with CH_2Cl_2/MeOH, 98:2 to 88:12) to afford 2.41 g (75 %) of **s15** as a white solid. TLC (CH_2Cl_2/MeOH, 92:8): R_f 0.53. ^{1}H-NMR (600 MHz, (D_6)DMSO): 11.39 (br. *s*, NH), 8.74 (br. *s*, NHCO), 8.39 (*d*, *J* = 7.8, NHCO), 8.15 (*s*, H-(C6)), 7.88 (*d*, *J* = 7.2, 2 arom. H), 7.71 (*dd* appearing as *t*, *J* = 7.2, 2 arom. H), 7.53 (*d*, *J* = 8.4 Hz, *NH*Fmoc), 7.41 (*dd* appearing as *t*, *J* = 7.2, 2 arom. H), 7.28-7.33 (*m*, 7 arom. H), 6.49 (br. *s*, NH_2), 5.11 (*d*, *J* = 17.4, Ph<u>C*H*$_2$</u>O), 4.67-4.73 (*m*, H-C(α-Asp)), 4.21-4.30 (*m*, 3H, HC(Fmoc), H_2CC(Fmoc)), 4.03-4.06 (*m*, H-C(α?Glu)), 2.73 (*dd*, *J* = 16.8, 6.0, 1H-C(β?Asp)), 2.62 (*dd*, *J* = 16.8, 6.6, 1H-C(β?Asp)), 2.29-2.40 (*m*, 2H-C(γ?Glu)), 1.93-1.95 (*m*, 1H-C(β?Glu)), 1.78-1.80 (*m*, 1H-C(β?Glu)), 1.33 (*s*, C(CH_3)$_3$). ^{13}C-NMR (150 MHz, (D_6)DMSO): 171.55 (*C*O), 170.59 (*CO*), 170.37 (CO, C(4)), 168.86 (CO), 155.75 (NH*CO*Fmoc, C(2)), 152.75 (C(6)), 143.66 (arom. C), 140.59 (arom. C), 135.59 (arom. C), 128.23 (arom. C), 127.85 (arom. C), 127.66 (arom. C), 127.52 (arom. C), 126.97 (arom. C), 125.20 (arom. C), 119.98 (arom. C), 115.91 (C(5)), 80.49 (<u>C</u>(CH_3)$_3$), 66.16 (CH_2(Fmoc)), 65.60 (Ph<u>C</u>H_2O), 53.96 (C(α? ? ?)), 48.53 (C(α-Glu)), 46.54 (CH(Fmoc)), 36.79 (C(β? ? ?)), 32.43 (C(γ?Glu)), 28.10 (C(β?Glu)), 27.47 (C(<u>C</u>H_3)$_3$). ESI-MS: 761 (28, [*M* + Na]$^+$), 739 (35, [*M* + H]$^+$).

2-{2-[((9H-fluoren-9-yl)methoxy)carbonylamino)-5-(2-amino-6-oxo-1,6-dihydropyrimi din-5-ylamino)-5-oxopentanamido}-4-tert-butoxy-4-oxobutanoic acid (**s16**): To a stirred suspension of 10 % Pd-C (150 mg) in 15 ml of DMF/MeOH 1:3 was added the compound **s15** (500 mg, 0.67 mmol) in 5 ml of DMF, followed by formic acid (800 µl) at 0 ^{o}C. The reaction mixture was stirred under H_2 atmosphere (balloon) for 3.5 h at 0-5 ^{o}C, filtered through celite and washed with hot MeOH (2 x 30 ml). The washings

were combined with the filtrate and concentrated in vacuo. The residue was purified on reverse phase CC (C18-silica gel; H_2O/MeOH 100:0→30:70) to afford 364 mg (83 %) of **s16** as a white solid. TLC (AcOEt/MeOH/H_2O/CH_3COOH 75:15:6:2): R_f 0.54. ^{1}H-NMR (600 MHz, (D_6)DMSO): 12.77 (br. *s*, COOH), 11.31 (br. *s*, NHCO), 8.75 (*s*, NHCO), 8.17 (*d*, *J* = 7.8, NHCO), 8.11 (*s*, H-(C6)), 7.88 (*d*, *J* = 7.2, 2 arom. H), 7.72 (*dd* appearing as *t*, *J* = 7.2, 2 arom. H), 7.52 (*d*, *J* = 8.4 Hz, *NH*Fmoc), 7.41 (*dd* appearing as *t*, *J* = 7.2, 2 arom. H), 7.33 (*dd* appearing as *t*, *J* = 7.2, 2 arom. H), 6.45 (br. *s*, NH_2), 4.52-4.56 (*m*, H-C(α-Asp)), 4.20-4.28 (*m*, 3H, HC(Fmoc), H_2CC(Fmoc)), 4.00-4.04 (*m*, H-C(α-Glu)), 2.66 (*dd*, *J* = 16.2, 6.0, 1H-C(β-Asp)), 2.56 (*dd J* = 16.2, 7.2, 1H-C(β-Asp)), 2.32-2.40 (*m*, 2H-C(γ-Glu)), 1.92-1.97 (*m*, 1H-C(β-Glu)), 1.75-1.82 (*m*, 1H-C(β-Glu)), 1.36 (*s*, C(CH_3)$_3$). ^{13}C-NMR (150 MHz, (D_6)DMSO): 172.19 (COOH), 171.31 (*C*O), 170.67 (*CO*, C(4)), 169.12 (CO), 155.74 (NH*CO*Fmoc, C(2)), 152.89 (C(6)), 143.77 (arom. C), 140.59 (arom. C), 127.53 (arom. C), 126.98 (arom. C), 125.20 (arom. C), 119.99 (arom. C), 115.90 (C5), 80.24 (<u>C</u>(CH_3)$_3$), 65.61 (CH_2(Fmoc)), 54.03 (C(α-? ? ?)), 48.62 (C(α-Glu)), 46.56 (CH(Fmoc)), 37.11 (C(β-? ? ?)), 32.39 (C(γ-Glu)), 28.10 (C(β-Glu)), 27.53 (C(<u>C</u>H_3)$_3$). ESI-MS: 649 (100, $[M + H]^+$).

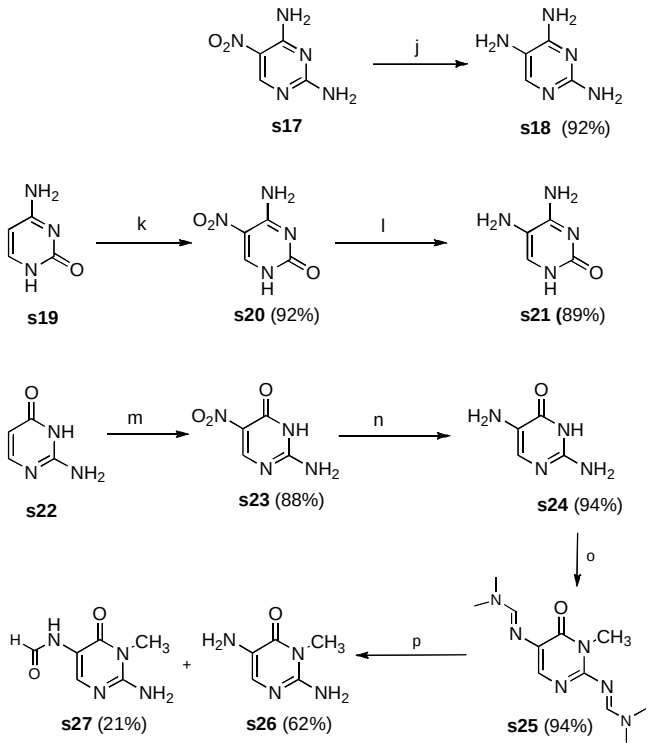

Figure 2. Synthesis of the three members of the 5-amino-pyrimidine family. (j) Raney® 2800 nickel, H_2 (ballon), DMF, RT, 24h.(k) HNO_3/H_2SO_4 (5:1), 80°C, 18h. (l) H_2, (7%wt) Pd/C(10%), H_2O/EtOH (2:1), rt, 3h. (m) HNO_3/H_2SO_4 (1:1), 90°C, 1h. (n) H_2,(10%wt) Pd/C(10%), H_2O/EtOH (9:1), rt, 5h. (o) 0.5 M of **s24**, 15.18 N,N-dimethylformamide dimethylacetal, 105 °C**.** (p) 0.05M of **s25**, NH_4OH, RT, 60h.

2,4,5-triaminopyrimidine (**s18**):[4] To a suspension of Raney® 2800 nickel (4 g wet, *Aldrich*) in 100 ml of DMF was added 5-nitro-2,4-diaminopyrimidine **s17** (5 g, 32.2 mmol, *TRC Biomedical Research Chemicals*) and stirred under H_2 atmosphere (Balloon) for 24 h at RT The suspension was filtered over celite and washed with DMF (2 x 50 ml) and MeOH (2 x 40 ml). The washings were combined with the filtrate and removed in vacuo to afford 3.7 g (92 %) of **s18** as a purple solid. TLC (CH_2Cl_2/CH_3OH 7:3): R_f 0.48. ^{1}H-NMR (600 MHz, (D_6)DMSO): 7.23 (*s*, H-(C6)), 5.96 (br. *s*, NH_2), 5.14 (br. *s*, NH_2), 3.75 (br. *s*, NH_2). ^{13}C-NMR (150 MHz, (D_6)DMSO): 157.12 (C(4)), 155.84 (C(2)), 140.12 (C(6)), 118.09 (C(5)). ESI-MS: 148 (5, $[M + Na]^+$), 126 (100, $[M + H]^+$). UV (1.25 mmol NaH_2PO_4, 12.5 µM Na_2EDTA, pH 7): λ_{max} =226 (ε = 8200), λ_{max} =295 (ε = 4170), λ_{min} =267 (ε = 2650).

4-amino-5-nitropyrimidin-2(1H)-one (**s20**):[5] *4-aminopyrimidin-2(1H)-one* **s19** (20 g, 180 mmol, *Acros*) was added over 30 min to a stirred mixture of anhydrous nitric acid (125 ml) and conc. sulfuric acid (25 ml). The resulting mixture was heated at 80 ^{o}C for 18 h. The mixture was poured onto ice (1 kg), neutralized with sat. aq. NaOH. When the mixture reached pH 7 a white solid start to precipitate. The solid was filtered off and washed with water (2 x 300 ml). The crude solid was dissolved in 700 ml of hot water and the pH adjusted to 12 with sat. aq. NaOH, followed by neutralization with conc. acetic acid. The mixture was cooled on ice bath for 3 h, the resulting solid filtered, washed with ice-cold water (2 x 400 ml) and dried in h.v. to give 25.85 g (92 %) of 4-amino-5-nitropyrimidin-2(1*H*)-one **s20** as a pale yellow solid. TLC (CH_2Cl_2/CH_3OH 8:2): R_f 0.55. ^{1}H-NMR (300 MHz, (D_6)DMSO): 8.88 (*s*, H-C(6)), 7.95 (br. *s*, NH_2). ^{13}C-NMR (75 MHz, (D_6)DMSO): 163.40 (C(2)), 158.59 (C(4)), 157.47 (C(6)), 116.78 (C(5)).

4,5-diaminopyrimidin-2(1H)-one (**s21**):[5] A suspension of **s20** (10 g, 64 mmol) and 10% Pd-C (700 mg) in 105 ml of H_2O/EtOH (2:1) was shaken under H_2 atmosphere (55 psi.) in a Parr apparatus until no more hydrogen was absorbed (3 h at RT). The suspension was filtered over celite and washed with hot water (2 x 200 ml), the washings were combined with the filtrate and concentrated in vacuo. The residue was dissolved in 60 ml of hot water in an attempt to crystallize, but instead gave 7.2 g (89 %) of **s21** as an amorphous yellow solid. TLC (CH_2Cl_2/CH_3OH 7:3): R_f 0.41. ^{1}H-NMR (600 MHz, (D_6)DMSO): 6.79 (*s*, H-(C6)), 3.84 (br. *s*, NH_2), 3.36 (br. *s*, NH_2). ^{13}C-NMR (150 MHz, (D_6)DMSO): 161.62 (C(4)), 155.64 (C(2)), 123.79 (C(6)), 114.94 (C(5)). ESI-MS: 149 (5, [M + Na^+]), 127 (100, [M + H^+]). UV (c= 68.2 x 10^{-6} M in (1.25 mmol NaH_2PO_4, 12.5 µM Na_2EDTA, pH 7): λ_{max}=290 nm (ε= 4500); λ_{max}=220 nm (ε= 11900); λ_{min}=260 nm (ε= 2700). Spectra data were identical with those reported before.[4]

2-amino-5-nitropyrimidin-4(3H)-one (**s23**):[5] *2-aminopyrimidin-4(3H)-one* **s22** (6 g, 54 mmol, *TRC Biomedical Research Chemicals*) was added over 30 min to a stirred mixture of anhydrous nitric acid (18 ml) and conc. sulfuric acid (18 ml). The resulting mixture was heated at 80 ^{o}C for 1.5 h and then was poured onto 100 ml of ice-water. The clear yellow solution was made slightly alkaline with aq. 5 M NaOH soln. The mixture was cooled on ice bath for 3 h, the resulting solid filtered, washed with ice-cold water (2 x 100 ml) and dried in hv to afford 7.41 g (88 %) of 2-amino-5-nitropyrimidin-4(3*H*)-one

s23 as a pale yellow solid. TLC (CH_2Cl_2/CH_3OH 8:2): R_f 0.50. ^{1}H-NMR (600 MHz, (D_6)DMSO): 8.64 (*s*, H-(C6)), 6.35 (br. *s*, NH_2). ^{13}C-NMR (150 MHz, (D_6)DMSO): 165.06 (C(4)), 164.62(C(2)), 158.07 (C(6)), 125.47 (C(5)). ESI-MS: 179 [100, [M + Na^+]), 157 (60, [M + H^+]).

2,5-diaminopyrimidin-4(3H)-one (**s24**)**:**[6] A suspension of **s23** (6 g, 38.4 mmol) and 10% Pd-C (600 mg) in 200 ml of H_2O/EtOH (9:1) was shaken under H_2 atmosphere (55 psi.) in a Parr apparatus until no more hydrogen was absorbed (5 h at RT). The suspension was filtered over celite and washed with hot water (2 x 100 ml), the washings were combined with the filtrate. The solvents were removed in vacuo to afford 4.55 g (94 %) of **s24** as a yellow solid. TLC (CH_2Cl_2/CH_3OH 7:3): R_f 0.63. ^{1}H-NMR (600 MHz, (D_6)DMSO): 6.88 (*s*, H-(C6)), 4.96 (br. *s*, NH_2), 3.40 (br. *s*, NH, NH_2). ^{13}C-NMR (150 MHz, (D_6)DMSO): 168.78 (C(4)), 157.66 (C(2)), 134.17 (C(6)), 123.71 (C(5)). ESI-MS: 149 (10, [*M* + $Na]^+$), 127 (100, [*M* + $H]^+$). UV (1.25 mmol NaH_2PO_4, 12.5 µM Na_2EDTA, pH 7; Figure 4): λ_{max}=287 nm (ε= 4000); λ_{max}=239 nm (ε= 6100); λ_{max}=217 nm (ε= 10,280); λ_{min}=266 nm (ε= 3400).

(1E,1'E)-N',N''-(1-methyl-6-oxo-1,6-dihydropyrimidine-2,5-diyl)bis(N,N-dimethylformimidamide) (**s25**): A stirred suspension of 5-aminoisocytosine (200mg, 1.58mmol, 0.5 M) in N,N-dimethylformamide dimethylacetal (3.2 ml, 24.0 mmol) was heated to boiling for 30 h (after 4 h a clear solution resulted) under nitrogen atmosphere. The N,N-dimethylformamide dimethylacetal was removed in vacuo to give 383 mg (97% yield) of **s25** as a yellow solid. ^{1}H-NMR (600 MHz, (D_6)DMSO): 8.52 (*s*, HC=N), 8.44 (*s*, HC=N), 7.03 (*s*, H-C(6)), 3.43 (*s*, 3H, N-CH_3), 3.14 (*s*, 3H, =C-N-CH_3), 3.03 (*s*, 3H, =C-N-CH_3), 2.88 (br. *s*, 6H, =C-N-CH_3). ^{13}C-NMR (150 MHz, (D_6)DMSO): 160.31(C(4)), 156.86 (C(2)), 153.88 (=C- of side chain), 153.62 (=C- of side chain), 141.06 (C(6)), 129.04 (C(5)), 40.36 (CH_3 at N-1), 34.45 (CH_3 of side chain), 29.16 (CH_3 of side chain). ESI-MS: 273 (33[*M* + $Na]^+$), 251 (100 [*M* + $H]^+$).

A mixture of the **s25** (200 mg, 0.8mmol, 0.05M) and concentrated ammonium hydroxide solution (16 ml) was stirred at room temperature for 50 h. The reaction mixture was concentrated in vacuo. The resulting residue was purified by CC (SiO_2, eluted with CH_2Cl_2/MeOH, 95:05 to 70:30) to afford 69.5 mg (0.49 mmol, 62 %) of **s26** as a pale yellow solid and 28.22 mg (0.16mmol, 21%) of **s27** as a yellow solid.

2,5-diamino-3-methylpyrimidin-4(3H)-one (**s26**): TLC (CH_2Cl_2/CH_3OH 8:2): R_f 0.36. ^{1}H-NMR (600 MHz, (D_6)DMSO): 7.02 (*s*, H-C(6)), 6.13 (br. *s*, 2H, NH_2-C(2)), 3.89 (br. *s*, 2H, NH_2-C(5)), 3.28 (*s*, 3H, N-CH_3). ^{13}C-NMR (150 MHz, (D_6)DMSO): 158.66 (C(4)), 149.24 (C(2)), 131.22 (C(6)), 124.39 (C(5)). ESI-MS: 141 (100 $[M + H]^+$). UV (c= 97.1 x 10^{-6} M in 1.25 mmol NaH_2PO_4, 12.5 µM Na_2EDTA, pH 7; Figure 4): λ_{max} =308 (ε=7300) and λ_{max} =242 (ε=6900).

N-(2-amino-1-methyl-6-oxo-1,6-dihydropyrimidin-5-yl)formamide (**s27**): TLC (DCM/Methanol 8:2): R_f 0.50. ^{1}H-NMR (600 MHz, (D_6)DMSO): 9.33 (br. *s*, 1H, NH), 8.30 (*s*, O=C-H), 8.16 (H-C(6)), 7.02 (br. *s*, 2H, NH_2), 3.30 (*s*, N-CH_3). ^{13}C-NMR (150 MHz, (D_6)DMSO): 160.09 (H-C=O), 157.79 (C(4)), 153.03 (C(2)), 143.02 (C(6)), 114.24(C(5)), 28.87 (N-CH_3). ESI-MS: 169 (100 $[M + H]^+$). UV (c= 94.5 x 10^{-6} M in 1.25 mmol NaH_2PO_4, 12.5 µM Na_2EDTA, pH 7; Figure 4): λ_{max} =295 (ε=8800) and λ_{max} =235 (ε=6900).

(E)-N-(2-((dimethylamino)methyleneamino)-1-methyl-6-oxo-1,6-dihydropyrimidin-5-yl)formamide (**s28**): A suspension of **s25** (20mg, 0.08mmol) in 300µl of DMF-dimethylacetal was heated in an open vial for 30 seconds at 120°C, until a clear solution was obtained. The vial was covered with a small portion of cotton and left at room temperature for 4 days until no more DMF-dimethylacetal was observed (slow evaporation of DMF dimethylacetal was observed, b.p.=102°C), to give two clearly differentiable solids (mixed with each other): pale yellow crystals (**s28**) and a yellow powder (that looks very much like the starting material **s25**). A pale yellow crystal was carefully separated and given for x-ray analysis. Very small quantities of pale yellow crystals were separated and submitted for mass spectroscopy.

The remaining mixture of solids (**s25** and **s28**) was characterized by ^{1}H-NMR and ^{13}C-NMR spectroscopy; by elimination of the signals corresponding to the known compound **s25**, signals corresponding to **s28** were assigned. **s28**: ^{1}H-NMR (600 MHz, (D_6)DMSO): 9.52 (s, HN), 8.58 (s, HC=O), 8.54 (*s*, HC=N), 8.24 (s, H-C(6)), 3.47 (s, 3H, N-CH_3), 3.17 (s, 3H, =C-N-CH_3), 3.05 (s, 3H, =C-N-CH_3). ^{13}C-NMR (150 MHz, (D_6)DMSO): 159.76 (CH=0), 158.13 (C(4)), 157.60 (C(2)), 154.5 (CH=N of side chain), 139.02 (C(6)), 118.51 (C(5)), 40.57 (CH_3 at N-1), 34.63 (CH_3 of side chain), 29.40 (CH_3 of side chain). ESI-MS: 246 (5$[M + Na]^+$), 224 (100 $[M + H]^+$).

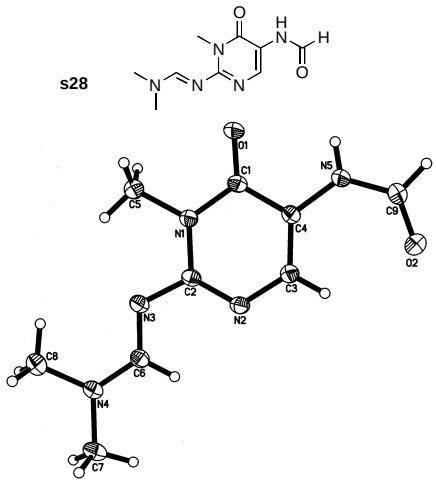

Figure 3: X-ray structure of **s28**.[7]

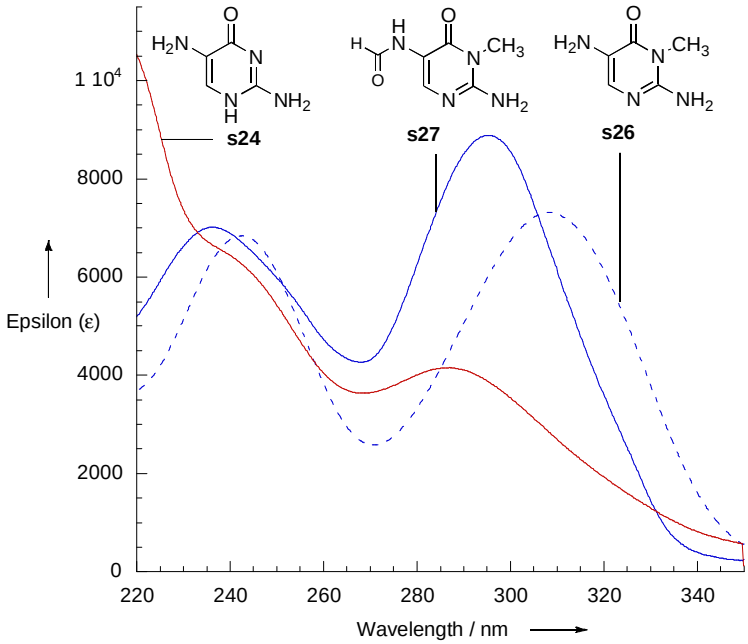

Figure 4. UV-spectra of 5-amino-isocytosine and its derivatives. Measurements were made in 1.25 mmol aq. NaH_2PO_4, 12.5 µM Na_2EDTA, pH 7.

Table 1. HPLC and MS Data of 5-aminopyrimidine Tagged Oligo-dipeptide Sequences.[a]

No.	Sequences[a]	Deprotection[b] *Method*	Analytical HPLC[c]		MALDI-TOF-M[e]	
			0→100%B, t_R [min.]	pH	$[M + H]^+$ (obs.)	$[M + H]^+$ (calc.)
1	^{HOOC}**AspGlu**$(^{AP}OO)_{12}$	A	30.39	11.0	4297	4296
2	^{HOOC}**AspGlu**$(^{AP}OO)_{16}$	A	16.24[d]	11.0	5671	5664
3	^{HOOC}**AspGlu**$(^{AP}NN)_{12}$	A	13.60[d]	8.0	4233	4232
4	^{HOOC}**AspGlu[**$(^{AP}NN)(^{AP}OO)]_6$	A	26.81	11.0	4244	4242
5	^{HOOC}**AspGlu**$(^{AP}NN)_6(^{AP}OO)_6$	A	25.92	8.5	4248	4242
6	^{HOOC}**AspGlu**$(^{AP}OO)_4(^{AP}ON)_4(^{AP}OO)_4$	A	14.90[d]	11.0	4249	4251
7	^{HOOC}**AspGlu**$(^{AP}OO)_4(^{AP}NO)_4(^{AP}OO)_4$	A	29.09	11.0	4256	4251
8	^{HOOC}**AspGlu**$[(^{AP}ON)(^{AP}NO)]_6$	A	12.83[d]	11.0	4239	4243
9	^{HOOC}**AspGlu**$(^{AP}NO)_6(^{AP}ON)_6^{NHAc}$	A	23.17	7.8	4285	4285

[a] All sequences refer to 5-aminopyrimidine tagged oligo-dipeptide backbone sequences (Figure 1 in main paper). N,N = 2,4,5-triaminopyrimidin-5-yl; O,O = 2,4-dioxo-5-aminopyrimidin-5-yl; N,O = 4-oxo-2,5-diaminopyrimidin-5-yl; O,N = 2-oxo-4,5-diaminopyrimidin-5-yl;AcNH = end NH_2 group was acetylated after final FMOC-deprotection. [b] Method A: TFA/m-cresol (95:5); [c] MONO-Q HR 5/5 Pharmacia, 10 µ 0.5 cm or Nucleogen-DEAE 60-7 *Machery Nagel*, 125 µ 4, flow 1 ml / min. Mobile phase: eluant A: 10 mM Na_2HPO_4, H_2O; eluant B: 10 mM Na_2HPO_4, 1 M NaCl, H_2O. [d] Nucleogen-DEAE 60-7 *Machery Nagel*, 125 µ 4, flow 1 ml / min. Mobile phase: eluant A: 10 mM Na_2HPO_4, H_2O, pH 7.0; eluant B: 10 mM Na_2HPO_4, 1 M NaCl, H_2O, pH 7.0. [e] Matrix assisted laser-desorption ionization time-of-flight mass spectroscopy; matrix: 3-hydropicolinic acid or α-cyanohdroxycinnamic acid or 2,4,6-trihydroxyacetophenone and ammonium citrate buffer.

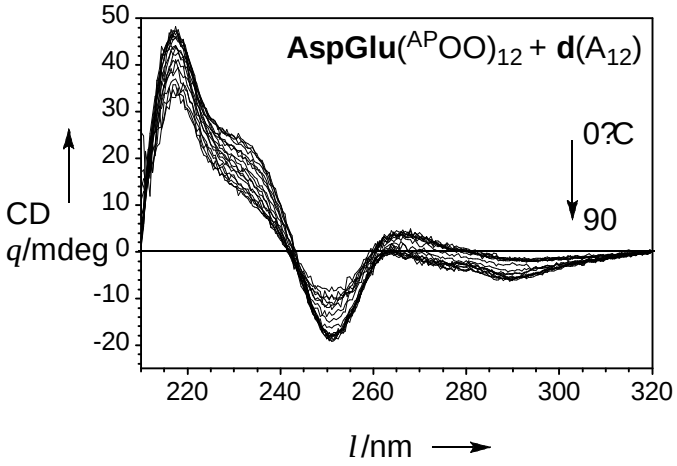

Figure 5. Temperature dependent CD-spectrum of the duplexes formed by **AspGlu**$(^{AP}OO)_{12}$ with DNA (**d**A_{12}). Measurements were made with c ~ 10 µM (1:1) in 1M NaCl, 10 mM aq. NaH_2PO_4, 0.1 mM Na_2EDTA, pH 7.0. CD-Temperature increments in 5°C steps.

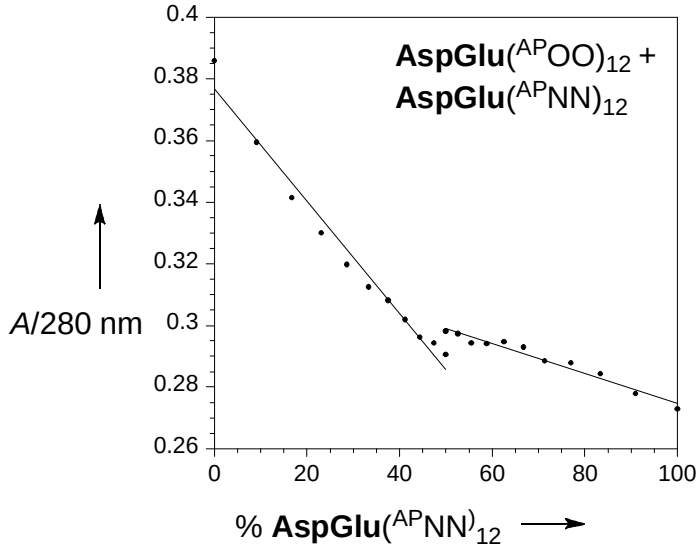

Figure 6. Job-plot of showing the 1:1 ratio of the pairing partners in the homo-duplex formed at 0° (Table 1, entry 17 of main manuscript). Measurements were made with a total c ˜ 10 µM (1:1) in 1M NaCl, 10 mM aq. NaH_2PO_4, 0.1 mM Na_2EDTA, pH 7.0.

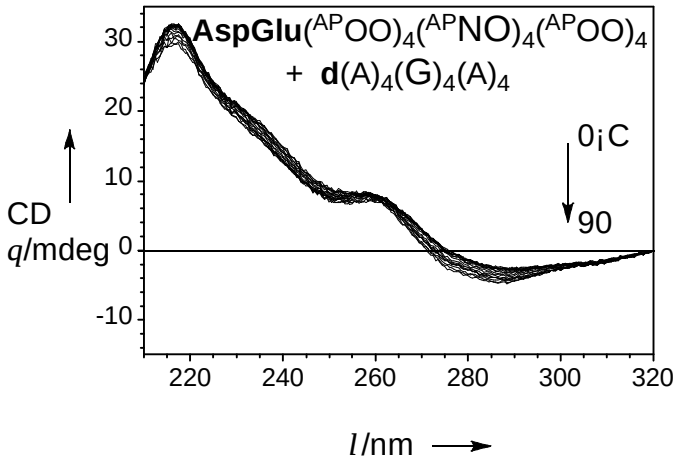

Figure 7. Temperature dependent CD-spectra documenting the ambiguous behavior of (^{AP}NO) with guanine (G). Measurements were made with a total c ˜ 10µM (1:1) in 1M NaCl, 10 mM aq. NaH_2PO_4, 0.1 mM Na_2EDTA, pH 7.0. CD-Temperature increments in 5°C steps.

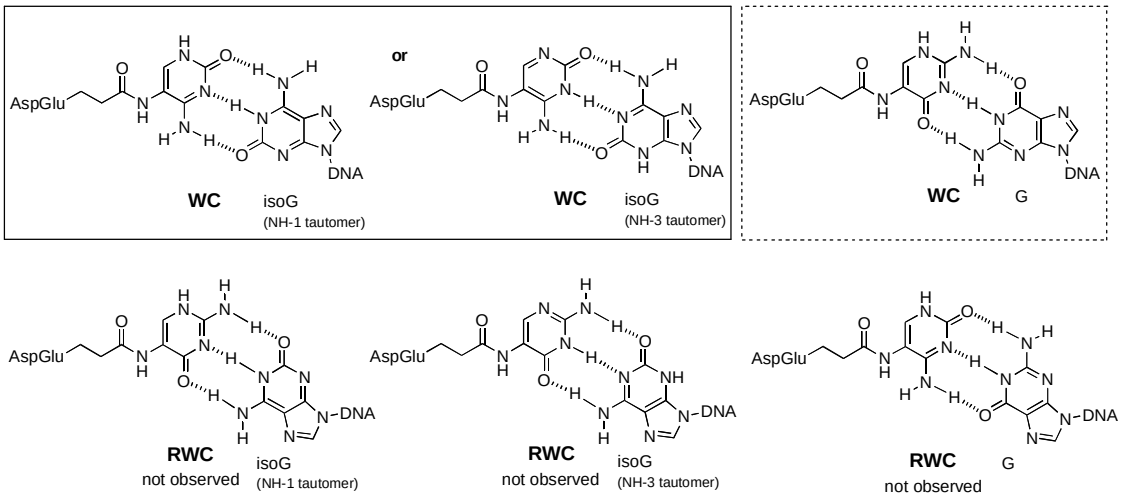

Figure 8. The possible modes of Watson-Crick (WC) and Reverse-Watson-Crick (RWC) base-pairing available for the two isomeric oxo-amino-members when pairing with with guanine and isoguanine.

References:

[1] G. K. Mittapalli, K. R. Reddy, H. Xiong, O. Munoz, B. Han, F. De Riccardis, R. Krishnamurthy, A. Eschenmoser, *Angew. Chem. Int. Ed*. **2006**, *xx*, xxxx.

[2] (a) P. Wipf, A. Cunningham, R. L. Rice, J. S. Lazo. *Bioorg. Med. Chem.* **1997**, *5*, 165. (b) Procedure used in the preparation for the (D)-enantiomer was used: K. L. Webster, A. B. Maude, M. E. O'Donnell, A. P. Mehorotra, D. Gani. *J. Chem. Soc., Perkin Trans*. **2001**, *1*, 1673

[3] Procedure used in the preparation of Fmoc-Asp(O^{t}Bu)-OBzl was used: S. Nakabayashi, C. D. Warren, R. W. Jeanloz. *Carbohydrate Research*. **1988**, *174*, 279.

[4] P. Ratsep, R. Pless. *J. Org. Chem.* **1998**, *53*, 3241.

[5] G. Andresen, L. L. Gundersen, M. Lundmark, F. Rise, S. Sundell, *Tetrahedron*. **1995**, *51*, 3655. (b) A. Holy, *Coll. Czech. Chem. Commun*. **1979**, *44*, 2846.

[6] T. B. Johnson, C. O. Johns, *Am. Chem. Soc*. **1905**, *34*, 554.

[7] X-ray analysis was carried out by Dr. Raj Chada, TSRI. Crystallographic data for the structure has been deposited with the Cambridge Crystallographic Data Centre as deposition No. CCDC 613878. Copies of the data can be obtained, free of charge, on application to the CCDC, 12 Union Road, Cambridge CB12 1EZ UK (fax: + 44(1233)336 0333; e-mail: deposit@ccdc.cam.ac.uk).