Supplementary data

Side chain homologation of alanyl peptide nucleic acids: pairing selectivity and stacking

Ulf Diederichsen,*a Daniel Weicherdingab and Nicola Diezemanna

a Institut für Organische und Biomolekulare Chemie, Tammannstr. 2, D-37077 Göttingen, Germany. Fax: +49 551 392944; Tel: +49 551 393221; E-mail: udieder@gwdg.de

b Clondiag Chip Technologies GmbH, Löbstedter Str. 103-105, D-07749 Jena, Germany. E-mail: daniel@clondiag.com

Synthesis of Nucleo Amino Acids

Alanyl Nucleo Amino Acids

An enantioselective preparation of alanyl nucleo amino acids was first described for the thyminyl and adeninyl derivatives by Eschenmoser et al.16 Nucleo amino acids S1 and S2 were synthesized by nucleophilic ring opening of the butoxycarbonyl (Boc) protected serine lactone S317,18 with the respective nucleobase (Scheme 1).
Scheme S1. Synthesis of the alanyl nucleo amino acids

The benzyloxycarbonyl (Z) protected cytosine and the guanine substituted amino acids S4 and S5 were prepared in a similar manner. The exocyclic amine of cytosine had to be Z-protected, whereas 2-amino-6-chloropurine was used as a precursor for guanine. The guaninyl nucleo amino acid S5 was generated by treatment with TFA followed by Boc-reprotection.

(S)-N-tert-Butoxycarbonyl-β-(N4-benzyloxycarbonyl-1-cytosinyl)alanine S4

Under argon DBU (2.12 g, 13.9 mmol) was added to a suspension of N4-benzyloxycarbonylcytosine (4.56 g, 18.6 mmol) in anh. DMSO (15 mL). Within 15 min (S)-N-Boc-β-serine lactone\(^{17}\) (1.74 g, 9.30 mmol), dissolved in DMSO (10.5 mL) was added. After stirring the mixture for 3 h at room temperature the reaction was stopped by addition of AcOH (797 µL, 13.9 mmol). Purification by flash chromatography on silica with AcOEt/MeOH/AcOH 9:1: gradient 0-0.05 provided of nucleo amino acid S4 (2.41 g, 60%, > 98% e.e.) as a white solid (HPLC for H-(S)-Ala-(S)-AlaC(Z)-OH: \(t_R = 26.2\) min, gradient 10 - 50% B’ in 30 min). mp 188 °C; \(R_f\) [AcOEt/MeOH/H\(2\)O/AcOH 10:1:1:0.5, saturated NaCl] = 0.31; \([\alpha]^{20}_{D} = 86.3\) (c 0.7 in MeOH); \(\delta_{400}\) (400 MHz; [D\(_6\)]DMSO; Me\(_4\)Si) 1.26 (9 H, s, Boc), 3.54 (1 H, m, H-Į), 4.20 (1 H, m, H-ȕ), 4.42 (1 H, d, \(J_{H,H} = 10\) Hz, H-ȕ), 5.16 (2 H, s, Ph), 6.58 (0.7 H, br, NH/Boc), 6.89 (1 H, d, \(J_{H,H} = 6\) Hz, H-5), 7.37 (5 H, s, Ph), 7.86 (1 H, d, \(J_{H,H} = 6\) Hz, H-6), 10.69 (1 H, br, COOH); \(\delta_{100}\) (100 MHz; [D\(_6\)]DMSO; Me\(_4\)Si) 28.2 (Boc), 40.6 (C-ȕ), 52.0, 52.8 (C-ȫ), 66.5 (CH\(_2\)-Ph), 78.1 (Boc), 93.6 (C-5), 128.0 (Ph), 128.3 (Ph), 136.2 (CO-Z), 150.4 (C-6), 153.4 (C-4), 155.1 (C-2), 155.3 (CONH), 162.8 (COOH); \(\nu_{max}/\text{cm}^{-1}\) (KBr) 3400, 3240, 1750, 1700, 1655, 1625, 1370, 1215, 790, 700; \(\lambda_{max}/\text{nm}\) 250 (\(e/\text{dm}^3\,\text{mol}^{-1}\,\text{cm}^{-1}\) 13 800), 300 (550); ESI-MS m/z: 433.1 [M + H]\(^{+}\), 865.0 [2M + H]\(^{+}\).

(R)-N-tert-Butoxycarbonyl-β-(N4-benzyloxycarbonyl-1-cytosinyl)alanine ent-S4

The synthesis followed the procedure for enantiomer S4. The analytical data of ent-S4 and S4 are identical except for \([\alpha]^{20}_{D} = 95.4\) (c 0.5 in MeOH) and HPLC for H-(S)-Ala-(R)-AlaC(Z)-OH: \(t_R = 27.0\) min, gradient 10 - 50% B’ in 30 min.
(S)-N-tert-Butoxycarbonyl-β-(2-amino-6-chloro-9-purinyl)alanine

DBU (1.56 g, 10.2 mmol) was added over 15 min to a suspension of 2-amino-6-chloropurine (2.05 g, 12.1 mmol) in DMSO (5 mL). After 10 min N-Boc-β-serine lactone S3\textsuperscript{17} (1.74 g, 9.30 mol), dissolved in DMSO (5 mL), was added drop wise and the mixture was stirred for additional 3 h. The reaction was stopped by adding AcOH (585 µL, 10.22 mmol) and the solvent was removed. Purification of the crude product was done by flash chromatography using silica and AcOEt/MeOH/AcOH 8:2: gradient 0-0.05. After coevaporation with toluene the product was obtained as a white solid (2.68 g, 81%). mp 207-212 °C; \( R_f \) [CHCl\(_3\)/MeOH/H\(_2\)O/AcOH 70:30:3:0.3] = 0.24; \([\alpha]\)\textsubscript{D}\(_{20}\) –86.3 (c 0.6 in MeOH); \( \delta_t \) (400 MHz; [D\(_6\)]DMSO; Me\(_4\)Si) 1.05 (1.5 H, s, Boc), 1.26 (7.5 H, s, Boc), 4.15 (1 H, dd, \( ^3J \) (H,H) = 10 Hz, \( ^2J \) (H,H) = 14 Hz, H-β), 4.30 (1 H, m, H-α), 4.46 (1 H, dd, \( ^2J \) (H,H) = 14 Hz, \( ^3J \) (H,H) = 4 Hz, H-β), 6.90 (2 H, s, NH\(_2\)), 7.00 (1 H, d, \( ^3J \) (H,H) = 8 Hz, NH/Boc), 7.92 (1 H, s, H-8); \( \delta_c \) (100 MHz; [D\(_6\)]DMSO; Me\(_4\)Si) 27.6 (Boc), 44.2 (C-β), 53.2 (C-α), 78.5 (Boc), 123.4 (C-5), 143.6 (C-8), 149.3 (C-6), 154.4 (C-2), 155.3 (CONH), 159.9 (C-4), 171.4 (COOH); \( \lambda_{max/cm}^{-1} \) (KBr) 3400, 3320, 3210, 1695, 1640, 1610, 1160; \( \lambda_{max}(\text{MeOH})/nm \) 253 (\( \epsilon/\text{dm}^3 \text{mol}^{-1} \text{cm}^{-1} \) 7 000), 315 (5 700); ESI-MS m/z: 357.0 [M + H]+, 712.9 [2M + H]+.

(S)-N-tert-Butoxycarbonyl-β-(9-guaninyl)alanine S5

(S)-N-Boc-β-(2-Amino-6-chloro-9-purinyl)alanine (2.86 g, 7.51 mmol) was dissolved in a mixture of TFA/H\(_2\)O 3:1 (33 mL) and stirred for 48 h. After completion of the reaction toluene was added and the solvent was evaporated. Exchange of the counterion was done by solvation in 1 N HCl followed by solvent evaporation providing (S)-β-(9-guaninyl)alanine (\( R_f \) [i-propanol/H\(_2\)O/AcOH 5:2:1, saturated NaCl] = 0.10). The crude reaction mixture was used for Boc-protection. A suspension of (S)-β-(9-guaninyl)-alanine in H\(_2\)O/1N NaOH/dioxane 1:1:2 (48 mL) was cooled to 0 °C and di-tert-butyldicarbonate (1.80 g, 8.25 mmol) was added. After stirring for 45 min at 0°C the ice bath was removed. 1N NaOH was added to keep the pH value at 9.0-9.5. After 60 h the solution was acidified to pH 6.5 by adding 1 N HCl. After evaporation of the solvent purification was done by flash chromatography with RP silica (H\(_2\)O/ gradient 10% MeOH each 500 mL) and product S5 was obtained as white solid (1.53 g, 60%, >99% e.e.). HPLC for H-(S)-Ala-(S)-AlaG-OH: \( t_k \) = 13.9 min, gradient 10 - 20% B’ in 30 min; mp 235 °C; \( R_f \) [i-propanol/H\(_2\)O/AcOH 5:2:1, saturated NaCl] = 0.55; \([\alpha]\)\textsubscript{D}\(_{20}\) –
20.7 (c 0.8 in MeOH); δH (400 MHz; [D6]DMSO; Me4Si) 1.10 (2 H, s, Boc), 1.27 (7 H, s, Boc), 3.93 (2 H, m, H-β, H-α), 4.42 (1 H, d, 3J(H,H) = 10 Hz, H-β), 6.17 (1 H , d, 3J(H,H) = 7 Hz, NHBoc), 6.93 (2 H, s, NH2), 7.47 (1 H, s, H-8), 11.57 (1 H, br s, COOH); δC (100 MHz; [D6]DMSO; Me4Si) 28.3 (Boc), 45.6 (C-β), 55.6 (C-α), 77.7 (Boc), 116.3 (C-5), 137.7 (H-8), 151.7 (C-6), 154.1 (C-2), 155.1 (CONH), 157.6 (C-4), 172.4 (COOH); νmax/cm⁻¹(KBr) 3360, 3120, 1690, 1610, 1370, 1170; λmax(MeOH)/nm 259 (ε/dm³ mol⁻¹ cm⁻¹ 11 700); ESI-MS m/z: 339.0 [M + H]+, 677.0 [2M + H]+.

(R)-N-tert-Butoxycarbonyl-β-(9-guaninyl)alanine ent-S5

The synthesis followed the procedure for enantiomer S5. The analytical data of ent-S5 and S5 are identical except for [α]D 20 +14.3 (c 1.0 in MeOH) and HPLC for H-(S)-Ala-(R)-AlaG-OH: tR = 16.6 min, gradient 10 - 20% B' in 30 min.

Homoalanyl Nucleo Amino Acids

Homoalanyl nucleo amino acids were prepared by substitution of primary bromide S6 with the nucleobases. Therefore, homoalanyl nucleo amino acids S7-S11 were chiefly prepared following procedures of Taddei (Scheme S2).19-22 The γ-bromo amino acid S6 was generated by photochemical degradation of the Barton-ester S12 which was prepared from glutamic acid S13. Finally, the primary bromide S6 was substituted by the nucleobases. After separation from the undesired N7 (purine) and N3 (pyrimidine) regioisomers by chromatography, the C-terminal deprotection of the benzyl protected nucleo amino acids S14-S17 to the desired nucleo amino acids S7-S11 was obtained either under hydrogenolytic conditions or using the endopeptidase Chirazyme® P-1 (Roche Diagnostics GmbH, Penzberg, Germany). The guanine derivative S17 was generated form the 2-amino-6-chloropurinyl amino acid S18 by treatment with TFA. The Boc-group lost during this conversion was attached in the final step to yield amino acid S11.
Scheme S2. Synthesis of the homoalanyl nucleo amino acids$^{19-22}$

(S)-N-tert-Butoxycarbonyl-$\gamma$-(9-adeninyl) homoalanine S7

(S)-N-Boc-$\gamma$-(9-adeninyl) homoalanine benzyl ester S14$^{22}$ (2.00 g, 4.70 mmol) was dissolved in a mixture of MeOH (20 mL) and H$_2$O (1 mL) and reduced by PdO-H$_2$O (250 mg) within 3 d. The palladium oxide was separated by filtration over celite and washed with MeOH. By extraction with CHCl$_3$/H$_2$O starting material (1.00 g, 2.35 mmol) was recovered in the organic phase. Evaporation of H$_2$O followed by coevaporation with toluene yielded 790 mg (50%, >98% e.e.) amino acid S7 as a white solid (HPLC for H-(S)-Ala-(S)-HalA-OH: $t_R$ = 24.3 min, gradient 10 - 15% B in 30 min). mp 227 °C; $R_f$ [AcOEt/MeOH/H$_2$O/AcOH 10:1:1:0.5, saturated NaCl] = 0.43; $\delta_h$ (400 MHz; [D$_6$]DMSO; Me$_4$Si) 1.29–1.40 (9 H, s, Boc), 1.95–2.10 (1 H, m, H-$\gamma$), 2.20–2.35 (1 H, m, H-$\beta$), 3.80 (1 H, m, H-$\alpha$), 4.19 (2 H, t, $^3J$(H,H) = 7 Hz, H-$\gamma$), 7.17 (2 H, s, NH$_2$), 7.25 (1 H, d, $^2J$(H,H) = 8 Hz, BocNH), 8.02 (1 H, s, H-2), 8.12 (1 H, s, H-8), 12.60 (0.7 H, br s, COOH); $\delta_c$ (100 MHz; [D$_6$]DMSO; Me$_3$Si) 28.5, 31.1, 51.3, 78.4, 118.9, 140.9, 152.5, 155.7, 156.1, 173.6; $\nu_{\max}$/cm$^{-1}$ (KBr) 3450, 3280, 3120, 1730, 1690, 1350; $\lambda_{\max}$(MeOH)/nm 266 ($\varepsilon$/dm$^3$ mol$^{-1}$ cm$^{-1}$ 14 800); ESI-MS m/z: 337.1 [M + H]$^+$; C$_{14}$H$_{20}$N$_6$O$_4$ (336.4) calcd. C 49.99, H 5.99, N 24.99; found C 49.75, H 6.01, N 24.33.
(R)-N-tert-Butoxycarbonyl-γ-(9-adeninyl)homoalanine ent-S7

The synthesis followed the procedure for enantiomer S7. The analytical data of ent-S7 and S7 are identical except HPLC for H-(S)-Ala-(R)-HalA-OH: $t_R = 26.5$ min, gradient 10 - 15% B’ in 30 min.

(S)-N-tert-Butoxycarbonyl-γ-((N4-benzoxy carbonyl-1-cytosinyl)homoalanine S9

(S)-N-Boc-γ-(N4-Benzoxycarbonyl-1-cytosinyl)homoalanine benzyl ester S16$^{20}$ (1.83 g, 3.41 mmol) was dissolved in acetone (50 mL) and was added within 2 days dropwise to a solution of Chirazyme P-1 (1 g) in phosphate-buffer (50 mM, pH 7.0). After 20 h the solution was evaporated and amino acid S9 was isolated as a white solid (1.52 g) by a reversed phase flash chromatography in quantitative yield (>99% e.e.; HPLC for H-(S)-Ala-(S)-HalC(Z)-OH: $t_R = 29.5$ min, 0 - 30% B’). mp 180 °C; $R_f$ [AcOEt/MeOH/H2O/AcOH 10:1:1:0.5, saturated NaCl] = 0.43; $[\alpha]^2_0 = -8.5$ (c 0.4 in MeOH); $\delta_1$ (400 MHz; [D$_6$]DMSO; Me$_4$Si) 1.34 (9 H, s, Boc), 1.78–2.03 (2 H, m, H-β), 3.52 (1 H, m, H-α), 3.79 (2 H, t, $^3J$ (H,H) = 7 Hz, H-γ), 5.17 (2 H, s, CH$_2$Ph), 6.03 (1 H, d, $^3J$ (H,H) = 6 Hz, BocNH), 6.92 (1 H, d, $^3J$ (H,H) = 7 Hz, H-5), 7.30–7.45 (5 H, m, Ph), 8.9 (1 H, d, $^3J$ (H,H) = 7 Hz, H-6), 10.6 (0.7 H, br, COO$^-$); $\delta_c$ (100 MHz; [D$_6$]DMSO; Me$_4$Si) 28.3 (Boc), 32.4 (C-β), 47.6 (C-γ), 53.2 (C-α), 66.5 (CH$_2$Ph), 77.6 (Boc), 93.8 (C-5), 128.0 (Ph), 128.3 (Ph), 128.6 (Ph), 136.2 (CO-Z), 150.3 (C-6), 153.4 (C-4), 155.0 (C-2), 162.7 (CONH), 173.3 (COOH); $\nu_{max}$/cm$^{-1}$ (KBr) 3400, 1750, 1700, 1655, 1625, 1370, 790, 700; $\lambda_{max}$(MeOH)/nm 245 ($\varepsilon$/dm$^3$ mol$^{-1}$ cm$^{-1}$ 15 700), 300 (6 000); ESI-MS m/z: 469.2 [M + Na]$^+$, 915.2 [2M + Na]$^+$.

(R)-N-tert-Butoxycarbonyl-γ-(N4-benzoxy carbonyl-1-cytosinyl)homoalanine ent-S9

The synthesis followed the procedure for enantiomer S9. The analytical data of ent-S9 and S9 are identical except for $[\alpha]^2_0 = +4.2$ (c 0.6 in MeOH) and HPLC for H-(S)-Ala-(R)-HalC(Z)-OH: $t_R = 28.4$ min, 0 - 30% B’.

(S)-N-tert-Butoxycarbonyl-γ-(9-guaninyl)homoalanine S11

PdO·H$_2$O (600 mg) was added to a solution of (S)-γ-(9-guaninyl)homoalanine benzyl ester S17$^{22}$ in MeOH (70 mL) and AcOH (3.7 mL) under argon. After saturation with hydrogen, the reaction mixture was activated with ultra sound and stirred for 2 h. The mixture was centrifuged and the catalyst washed several times with MeOH.
The combined solutions were coevaporated with toluene and the desired amino acid S10 was isolated as a white solid (1.08 g, 93%); \( R_f [i\text{-propanol}\/H_2O\/AcOH 5:2:1, \text{saturated NaCl}] = 0.16; \) \( \delta_1 \) (250 MHz; \([D_6]DMSO; Me_4Si) 2.15–2.40 (2 H, m, H-\( \gamma \)), 3.77 (1 H, t, \(^3J(H,H) = 7 \text{ Hz}, \text{H-}\alpha \)), 4.15 (2 H, t, \(^3J(H,H) = 7 \text{ Hz}, \text{H-}\gamma \)), 6.60 (2 H, br s, NH\(_2\)), 7.89 (1 H, s, H-8), 8.40 (2 H, br s, NH\(_2\)). A suspension of the crude (S)-\( \gamma \)-(9-guaninyl)-homoalanine S10 in H\(_2\)O/1N NaOH/dioxane 1:1:2 (30 mL) was cooled to 0°C and di-tert-butyldicarbonate (1.08 g, 4.95 mmol) was added. After stirring 45 min at 0°C the ice bath was removed and the pH was kept at 9.0-9.5 with 1N NaOH. After 1.5 h the solution was acidified to pH 6.5 by adding 1 N HCl. After evaporation of the solvent the purification was done by flash chromatography with RP silica and the desired nucleo amino acid S11 was obtained as a white solid (0.95 g, 60%, >96% e.e.); HPLC for H-(S)-Ala-(S)-HalG-OH: \( t_R = 25.3 \text{ min, gradient 10 - 15% B' in 30 min}. \) mp 257 °C; \( R_f [i\text{-propanol}\/H_2O\/AcOH 5:2:1, \text{saturated NaCl}] = 0.55; \) \([\alpha]^{20}_D +9.7 \text{ (c } 0.3 \text{ in MeOH)}; \) \( \delta_1 \) (500 MHz; \([D_6]DMSO; Me_4Si) 1.35 (9 H, s, Boc), 1.85–2.15 (2 H, m, H-\( \gamma \)), 3.63 (1 H, m, H-\( \alpha \)), 3.94 (2 H, m, H-\( \gamma \)), 6.15 (1 H, d, \(^3J(H,H) = 7 \text{ Hz, BocNH})\), 6.98 (2 H, s, NH\(_2\)), 7.64 (1 H, s, H-8), 11.67 (1 H, br s, COOH); \( \delta_C \) (125 MHz; \([D_6]DMSO; Me_4Si) 28.4 (Boc), 34.1 (C-\( \beta \)), 40.7 (C-\( \gamma \)), 53.3 (C-\( \alpha \)), 77.8 (Boc), 116.7 (C-5), 137.4 (C-8), 151.3 (C-6), 154.4 (C-2), 155.5 (CONH), 157.6 (C-4), 174.3 (COOH); \( \nu_{max}/\text{cm}^{-1} \) (KBr) 3400, 3320, 3180, 1690, 1605, 1170; \( \lambda_{max}(\text{MeOH})/\text{nm} \) 259 (\( \epsilon/\text{dm}^3 \text{ mol}^{-1} \text{ cm}^{-1} \) 12 500); ESI-MS m/z: 353.2 [M + H]\(^+\), 705.1 [2M + H]\(^+\), 727.1 [2M + Na]\(^+\).

(R)-N-tert-Butoxycarbonyl-\( \gamma \)-(9-guaninyl)homoalanine ent-S11

The synthesis followed the procedure for enantiomer S11. The analytical data of ent-S11 and S11 are identical except for \([\alpha]^{20}_D +7.0 \text{ (c } 0.8 \text{ in MeOH)}\) and HPLC for H-(S)-Ala-(R)-HalG-OH: \( t_R = 28.0 \text{ min, gradient 10 - 15% B' in 30 min}. \)