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Supplementary data

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

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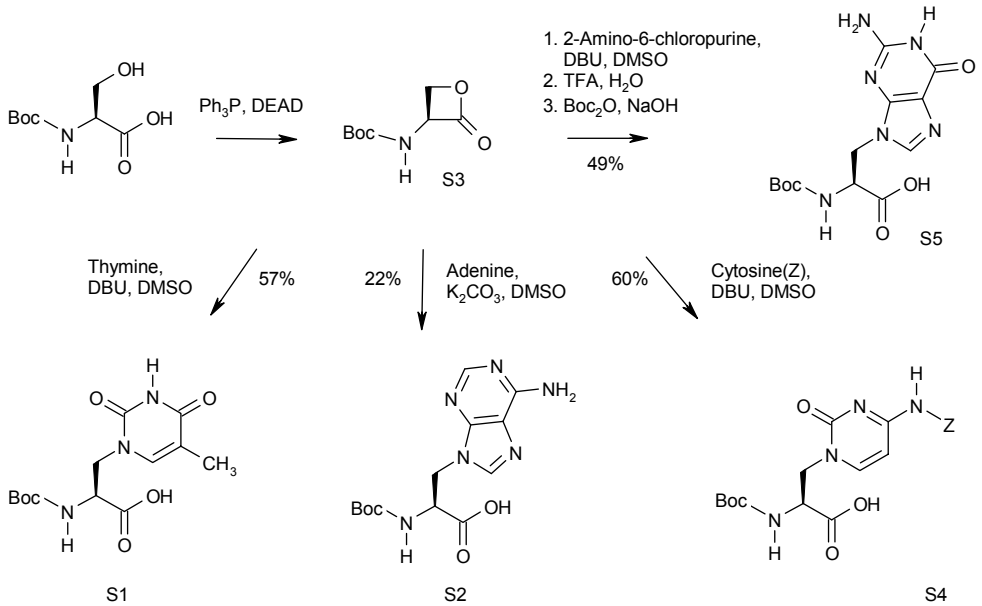
Synthesis of Nucleo Amino Acids

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 **S3**[17,18] with the respective nucleobase (Scheme 1).



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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*-(*N*4-benzyloxycarbonyl-1-cytosinyl)alanine S4

Under argon DBU (2.12 g, 13.9 mmol) was added to a suspension of *N*4-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_2O/AcOH 10:1:1:0.5, saturated NaCl] = 0.31; $[\alpha]^{20}_D$ –86.3 (*c* 0.7 in MeOH); δ_H (400 MHz; [D_6]DMSO; Me_4Si) 1.26 (9 H, s, Boc), 3.54 (1 H, m, H-*α*), 4.20 (1 H, m, H-*β*), 4.42 (1 H, d, ^3J (H,H) = 10 Hz, H-*β*), 5.16 (2 H, s, CH_2Ph), 6.58 (0.7 H, br, N*H*Boc), 6.89 (1 H, d, ^3J (H,H) = 6 Hz, H-5), 7.37 (5 H, s, Ph), 7.86 (1 H, d, ^3J (H,H) = 6 Hz, H-6), 10.69 (1 H, br, COO*H*); δ_C (100 MHz; [D_6]DMSO; Me_4Si) 28.2 (Boc), 40.6 (C-*β*), 52.0, 52.8 (C-*α*), 66.5 (CH_2-Ph), 78.1 (Boc), 93.6 (C-5), 128.0 (Ph), 128.3 (Ph), 128.6 (Ph), 136.2 (CO-Z), 150.4 (C-6), 153.4 (C-4), 155.1 (C-2), 155.3 (CONH), 162.8 (COOH); ν_{max}/cm^{-1} (KBr) 3400, 3240, 1750, 1700, 1655, 1625, 1370, 1215, 790, 700; λ_{max}(MeOH)/nm 250 (ε/dm^3 mol^{-1} cm^{-1} 13 800), 300 (550); ESI-MS m/z: 433.1 $[M + H]^+$, 865.0 $[2M + H]^+$.

(*R*)-*N*-*tert*-Butoxycarbonyl-*β*-(*N*4-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**[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_2O/AcOH 70:30:3:0.3] = 0.24; $[\alpha]^{20}_{;D}$ –86.3 (*c* 0.6 in MeOH); δ_H (400 MHz; [D_6]DMSO; Me_4Si) 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, N*H*Boc), 7.92 (1 H, s, H-8); δ_C (100 MHz; [D_6]DMSO; Me_4Si) 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 (*C*ONH), 159.9 (C-4), 171.4 (*C*OOH); ν_{max}/cm^{-1} (KBr) 3400, 3320, 3210, 1695, 1640, 1610, 1160; λ_{max}(MeOH)/nm 253 (ε/dm^3 mol^{-1} 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_2O 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_2O/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_2O/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_2O/ 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_R = 13.9 min, gradient 10 - 20% B' in 30 min; mp 235 °C; R_f [*i*-propanol/H_2O/AcOH 5:2:1, saturated NaCl] = 0.55; $[\alpha]^{20}_{;D}$ –

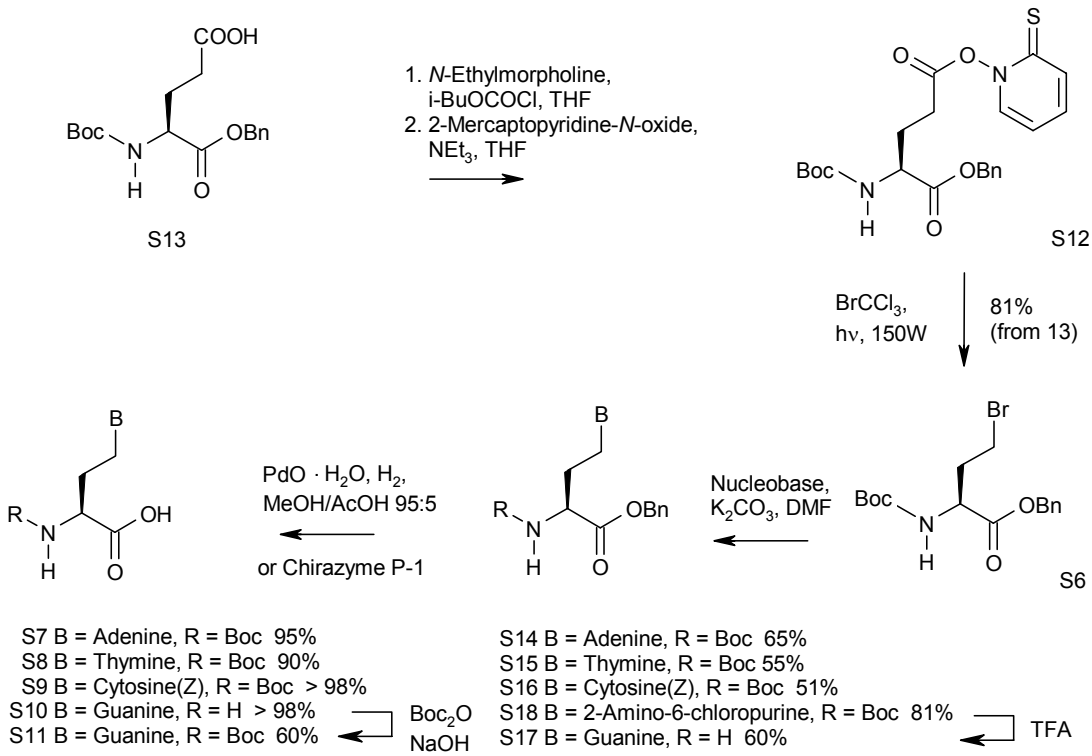
20.7 (*c* 0.8 in MeOH); δ_H (400 MHz; [D_6]DMSO; Me_4Si) 1.10 (2 H, s, Boc), 1.27 (7 H, s, Boc), 3.93 (2 H, m, H-*β*, H-*α*), 4.42 (1 H, d, ^3J (H,H) = 10 Hz, H-*β*), 6.17 (1 H , d, ^3J (H,H) = 7 Hz, N*H*Boc), 6.93 (2 H, s, NH_2), 7.47 (1 H, s, H-8), 11.57 (1 H, br s, COO*H*); δ_C (100 MHz; [D_6]DMSO; Me_4Si) 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 (*C*ONH), 157.6 (C-4), 172.4 (*C*OOH); ν_{max}/cm^{-1}(KBr) 3360, 3120, 1690, 1610, 1370, 1170; λ_{max}(MeOH)/nm 259 (ε/dm^3 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 $[\alpha]^{20}_D$ +14.3 (*c* 1.0 in MeOH) and HPLC for H-(*S*)-Ala-(*R*)-AlaG-OH: t_R = 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 *N*7 (purine) and *N*3 (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-*γ*-(9-adeninyl)homoalanine S7

(*S*)-*N*-Boc-*γ*-(9-adeninyl)homoalanine benzyl ester **S14**[22] (2.00 g, 4.70 mmol) was dissolved in a mixture of MeOH (20 mL) and H_2O (1 mL) and reduced by $PdO{\cdot}H_2O$ (250 mg) within 3 d. The palladium oxide was separated by filtration over celite and washed with MeOH. By extraction with $CHCl_3/H_2O$ starting material (1.00 g, 2.35 mmol) was recovered in the organic phase. Evaporation of H_2O 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_2O/AcOH 10:1:1:0.5, saturated NaCl] = 0.43; δ_H (400 MHz; [D_6]DMSO; Me_4Si) 1.29–1.40 (9 H, s, Boc), 1.95–2.10 (1 H, m, H-*β*), 2.20–2.35 (1 H, m, H-*β*), 3.80 (1 H, m, H-*α*), 4.19 (2 H, t, ^3J (H,H) = 7 Hz, H-*γ*), 7.17 (2 H, s, NH_2), 7.25 (1 H, d, ^3J (H,H) = 8 Hz, BocN*H*), 8.02 (1 H, s, H-2), 8.12 (1 H, s, H-8), 12.60 (0.7 H, br s, COOH); δ_C (100 MHz; [D_6]DMSO; Me_4Si) 28.5, 31.1, 51.3, 78.4, 118.9, 140.9, 152.5, 155.7, 156.1, 173.6; ν_{max}/cm^{-1} (KBr) 3450, 3280, 3120, 1730, 1690, 1350; λ_{max}(MeOH)/nm 266 (ε/dm^3 mol^{-1} cm^{-1} 14 800); ESI-MS m/z: 337.1 $[M + H]^+$; $C_{14}H_{20}N_6O_4$ (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*-(*N*4-benzoxycarbonyl-1-cytosinyl)homoalanine S9

(*S*)-*N*-Boc-*γ*-(*N*4-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/H_2O/AcOH 10:1:1:0.5, saturated NaCl] = 0.43; $[\alpha]^{20}_D$ –8.5 (*c* 0.4 in MeOH); δ_H (400 MHz; [D_6]DMSO; Me_4Si) 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_2Ph), 6.03 (1 H, d, ^3J (H,H) = 6 Hz, BocN*H*), 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*H*); δ_C (100 MHz; [D_6]DMSO; Me_4Si) 28.3 (Boc), 32.4 (C-*β*), 47.6 (C-*γ*), 53.2 (C-*α*), 66.5 (*C*H_2Ph), 77.6 (Boc), 93.8 (C-5), 128.0 (Ph), 128.3 (Ph), 128.6 (Ph), 136.2 (*C*O-Z), 150.3 (C-6), 153.4 (C-4), 155.0 (C-2), 162.7 (*C*ONH), 173.3 (*C*OOH); ν_{max}/cm^{-1} (KBr) 3400, 1750, 1700, 1655, 1625, 1370, 790, 700; λ_{max}(MeOH)/nm 245 (ε/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*-(*N*4-benzoxycarbonyl-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 $[\alpha]^{20}_D$ +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_2O (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*-propanol/H_2O/AcOH 5:2:1, saturated NaCl] = 0.16; δ_H (250 MHz; [D_6]DMSO; Me_4Si) 2.15–2.40 (2 H, m, H-*β*), 3.77 (1 H, t, ^3J (H,H) = 7 Hz, H-*α*), 4.15 (2 H, t, ^3J (H,H) = 7 Hz, H-*γ*), 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*)-*γ*-(9-guaninyl)-homoalanine **S10** in H_2O/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 min, gradient 10 - 15% B' in 30 min). mp 257 °C; R_f [*i*-propanol/H_2O/AcOH 5:2:1, saturated NaCl] = 0.55; $[\alpha]^{20}_{;D}$ +9.7 (*c* 0.3 in MeOH); δ_H (500 MHz; [D_6]DMSO; Me_4Si) 1.35 (9 H, s, Boc), 1.85–2.15 (2 H, m, H-*β*), 3.63 (1 H, m, H-*α*), 3.94 (2 H, m, H-*γ*), 6.15 (1 H, d, ^3J (H,H) = 7 Hz, BocN*H*), 6.98 (2 H, s, NH_2), 7.64 (1 H, s, H-8), 11.67 (1 H, br s, COO*H*); δ_C (125 MHz; [D_6]DMSO; Me_4Si) 28.4 (Boc), 34.1 (C-*β*), 40.7 (C-*γ*), 53.3 (C-*α*), 77.8 (Boc), 116.7 (C-5), 137.4 (C-8), 151.3 (C-6), 154.4 (C-2), 155.5 (*C*ONH), 157.6 (C-4), 174.3 (*C*OOH); ν_{max}/cm^{-1} (KBr) 3400, 3320, 3180, 1690, 1605, 1170; λ_{max}(MeOH)/nm 259 (ε/dm^3 mol^{-1} cm^{-1} 12 500); ESI-MS m/z: 353.2 $[M + H]^+$, 705.1 $[2M + H]^+$, 727.1 $[2M + Na]^+$.

(*R*)-*N*-*tert*-Butoxycarbonyl-*γ*-(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 (*c* 0.8 in MeOH) and HPLC for H-(*S*)-Ala-(*R*)-HalG-OH: t_R = 28.0 min, gradient 10 - 15% B' in 30 min.