Nucleo-*β*-Amino Acids: Synthesis and Oligomerization to *β*-Homoalanyl-PNA

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Dedicated to Professor *Dieter Seebach* on the occasion of his 65th birthday

The synthesis of thyminyl-, uracilyl-, cytosinyl-, and guaninyl-β^3-amino acids and the oligomerization of the cytosinyl- and guaninyl-β^3-amino acids to β-homoalanyl-PNA are presented. The pyrimidinyl nucleobases were connected to the γ-position of β-homoalanine by *Mitsunobu* reaction with a β-homoserine derivative or by nucleophilic substitution of methanesulfonates. For the preparation of the guaninyl-β^3-amino acid, a β-lactam route was established that might be of interest also for the synthesis of other β^3-amino acid derivatives. The cytosinyl and guaninyl building blocks were oligomerized to hexamers. They form quite stable self-pairing complexes in H_2O as indicated by temperature dependent UV and CD spectroscopy.

1. Introduction. – Peptide nucleic acids (PNAs) with a backbone composed of *N*-(2-aminoethyl)glycine units have attracted considerable interest because of their potential use as diagnostic tools and application in antigene or antisense therapy [1]. With alanyl-PNA, a new kind of PNA oligomer was introduced that is based on a regular peptide backbone: alanyl amino acids with alternating configuration carry nucleobases in the β-position of the side chain [2]. These oligomers are able to form linear double strands based on nucleobase recognition. Recently, oligomers have been reported that are composed of nucleo-β^3-amino acid building blocks[1]) in which adenine or 7-carbaadenine are connected to β-homoalanine at the γ-position [4]. These β-homoalanyl-PNAs are a special case of β-peptides with a β-sheet-like backbone conformation. They organize in higher ordered structures by nucleobase pairing.

While β-peptides with proteinogenic and cyclic nonproteinogenic side chains usually are known to fold into well-defined helical or sheet like secondary structures [5], nucleo-β^3-amino acids offer the potential to influence the β-peptide structure through base pairing and aromatic π-interactions (stacking).

Here, we describe the syntheses of new *N*-Boc-nucleo-β^3-amino acids with uracil, thymine, cytosine, and guanine in the side chains. The cytosine- and guanine-containing β-amino acids were oligomerized to β-homoalanyl-PNA by solid-phase techniques. Furthermore, initial studies of the pairing properties of these oligomers are presented.

2. Synthesis of Pyrimidinyl-β^3-Amino Acids. – Stereoselective syntheses of β-amino acids have already been reviewed extensively [5a][6]. Many methods known for the preparation of β-amino acids with proteinogenic side chains are not applicable to the

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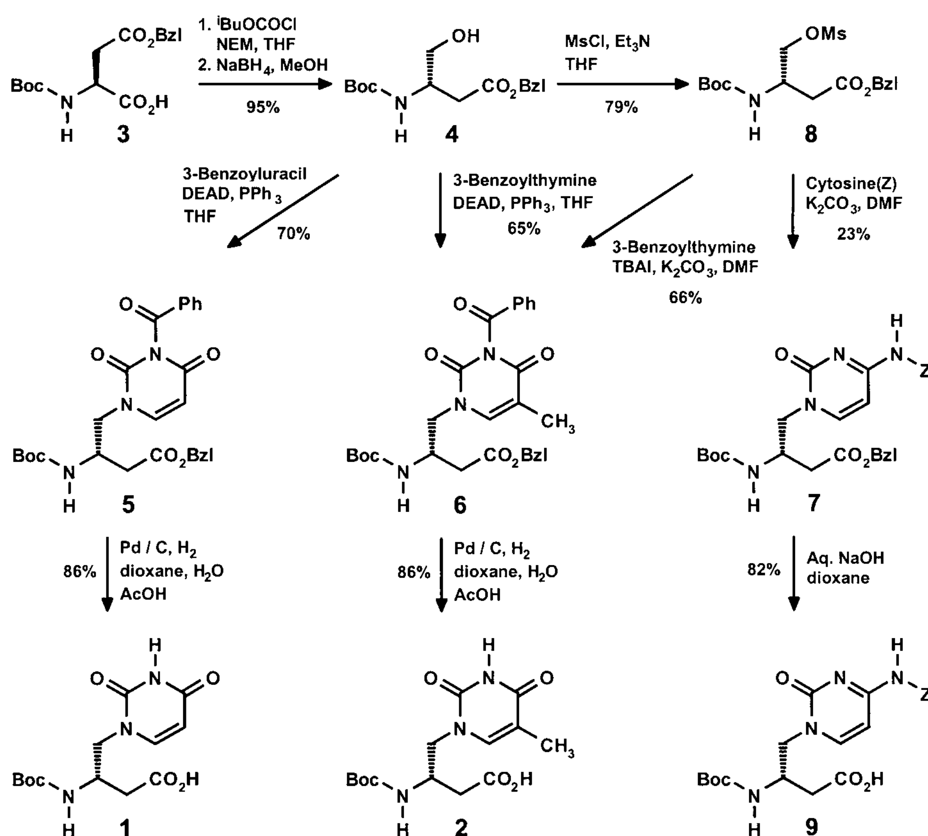
1) The designation of β^3-amino acids or β^3-peptides is proposed by *Seebach* to specify the position of the side chain [3a]. The preparation of a nucleo-β^2-amino acid has recently been described [3b].

synthesis of nucleo-β-amino acids. As a standard procedure, the *Arndt–Eistert* homologation of α-amino acids was established by *Seebach* and co-workers [5a][7]. However, since *Arndt–Eistert* homologation of aromatic α-amino acids is expected to proceed with racemization [7b], it should not be applied to the generation of nucleo-β^3-amino acids. Another obstacle is the poor solubility of nucleobases in most organic solvents. In case of Boc-β-HalA-OH and Boc-β-Hal^{7C}A-OH (H-β-HalA-OH = (*S*)-γ-(adenin-9-yl)-β-homoalanine, H-β-Hal^{7C}A-OH = (*S*)-γ-(7-carbaadenin-9-yl)-β-homoalanine) the *Mitsunobu* reaction was used to attach the nucleobase precursors 6-chloropurine and 6-chloro-7-carbapurine to the γ-position of a suitably protected β-homoalanyl derivative [4].

The *Mitsunobu* reaction was chosen also as the key step in the syntheses of the pyrimidinyl-nucleo-β-amino acids **1** and **2** (*Scheme 1*). Reduction of the commercially available Boc-L-Asp(Bzl)-OH (**3**) led to the required β-homoserine derivative **4** [8]. For the syntheses of the uracil and thymine monomers, the N^3-benzoyl-protected nucleobases [9] were used. The protecting group not only prevents N^3-alkylation but

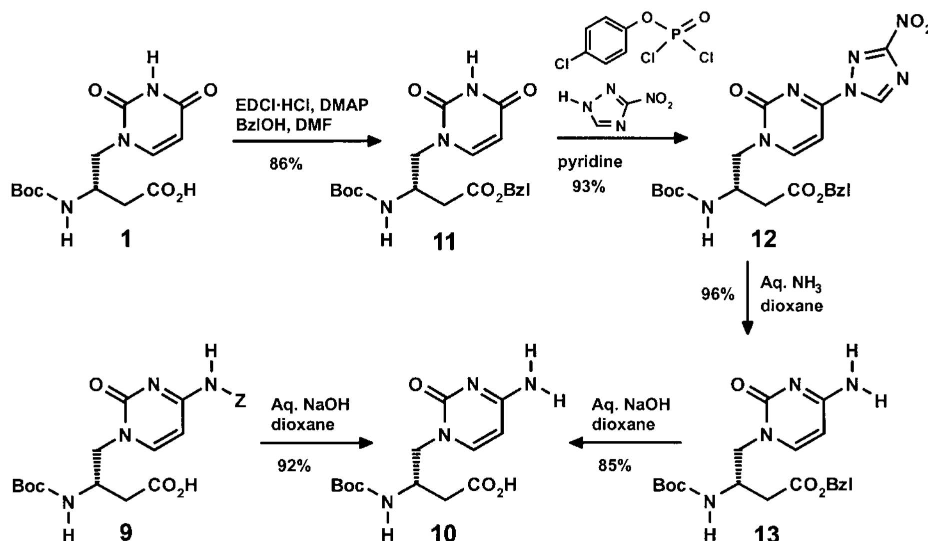
Scheme 1. *Synthesis of Pyrimidinyl-nucleo-β-amino Acids* **1**, **2**, *and* **9** *from β-Homoserine Derivative* **4** *as the Key Intermediate*



also improves the solubility. The way of bringing the reactants together seems to play a crucial role to avoid the conversion of *N*-Boc-amino acids into *N*-Boc-aziridines under *Mitsunobu* conditions [10]. Consequently, diethyl azodicarboxylate (DEAD), Ph_3P, and the N^3-benzoyl-protected nucleobases were dissolved in THF at $-30°$ and added to a precooled solution of β-homoserine derivative **4**. After stirring for 4–5 d at low temperature, the coupling products **5** and **6** were isolated in yields of 70 and 65%, respectively. Saponification of the benzyl ester and deprotection of the N^3-atom were achieved by catalytic hydrogenation on Pd/C. The analogous *Mitsunobu* reaction of the N^4-protected cytosine derivatives N^4-(benzyloxycarbonyl)cytosine (cytosine(Z)), N^4-benzoylcytosine, and N^4-[4-(*tert*-butyl)benzoyl]cytosine failed. However, the preparation of **7** by alkylation of cytosine(Z) was achieved with methanesulfonate **8**. The latter compound was prepared according to the literature [11]. The low yield of 23% for the alkylation contrasts with the high yield obtained for the analogous reaction with 3-benzoylthymine in preliminary studies. Not only the extremely poor solubility of cytosine(Z) even in polar aprotic solvents accounts for the difficulties in the preparation of **7**. Methanesulfonate **8** showed a tendency to decompose under the reaction conditions. Nevertheless, the cytosinyl-β^3-amino acid **9** was prepared by saponification of the benzyl ester **7** on gram-scale.

HMBC-NMR Experiments were performed to establish the N^1-connectivity of **9**. However, no correlation between H–C(γ) and C(6), or H–C(6) and C(γ) in the spectra was detectable. Therefore, the unprotected cytosinyl-β^3-amino acid **10** was prepared from the uracilyl-β^3-amino acid **1** with known N^1-connectivity as depicted in *Scheme 2*. The manipulation of the *C*(4)-substituent in **1** was carried out by the 1,2,4-triazole method [12]. The β^3-amino acid **1** was first converted into the benzyl ester **11**. After activation of the C(4)-position with 4-chlorophenyl dichlorophosphate, the

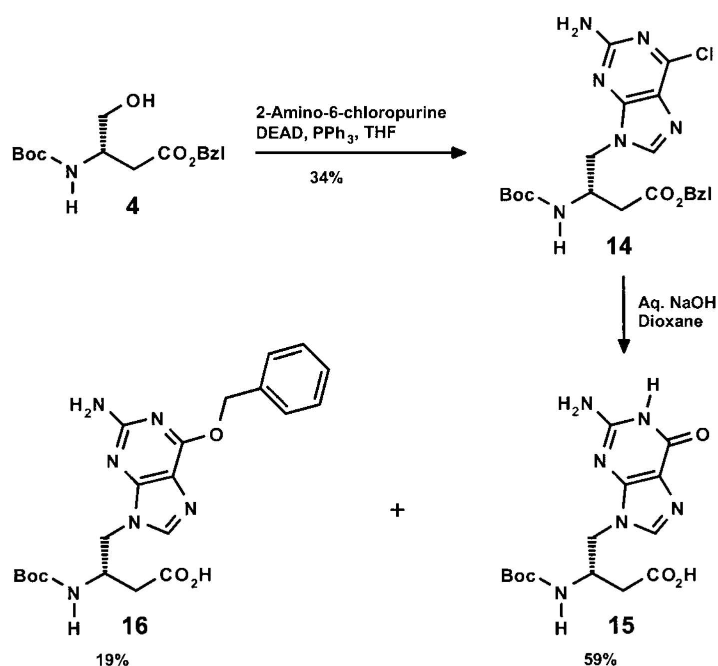
Scheme 2. *Conversion of the Uracilyl-β-amino Acid* **1** *into the Unprotected Cytosinyl-β-amino Acid* **10** *to Establish the Connectivity of the Protected Cytosinyl-β-amino Acid* **9**



substitution of the O-atom by 3-nitro-1,2,4-triazole proceeded smoothly. When the cytosine derivative **12** was treated with aqueous NH_3/dioxane the cytosinyl-β^3-amino acid benzyl ester **13** was obtained. Deprotection of the N^4-protected cytosinyl-β^3-amino acid **9**, as well as saponification of the unprotected cytosinyl-β^3-amino acid benzyl ester **13**, gave the same cytosinyl-β^3-amino acid **10** and hereby established the connectivity of compound **9**.

3. Synthesis of the Guaninyl-β^3-Amino Acid. – Guanine cannot be selectively alkylated at N^9. 2-Amino-6-chloropurine, however, gives almost exclusively the N^9-substituted product and is usually employed as a synthon for guanine [13]. Therefore, its reaction with β-homoserine derivative **4** under *Mitsunobu* conditions was investigated. Compound **14** was isolated in 34% yield after laborious chromatography (*Scheme 3*). Unfortunately, the treatment of **14** with aqueous NaOH/dioxane not only gave the desired guaninyl-β^3-amino acid **15** in 59% yield but also the O^6-benzylguaninyl-β^3-amino acid **16** in 19% yield.

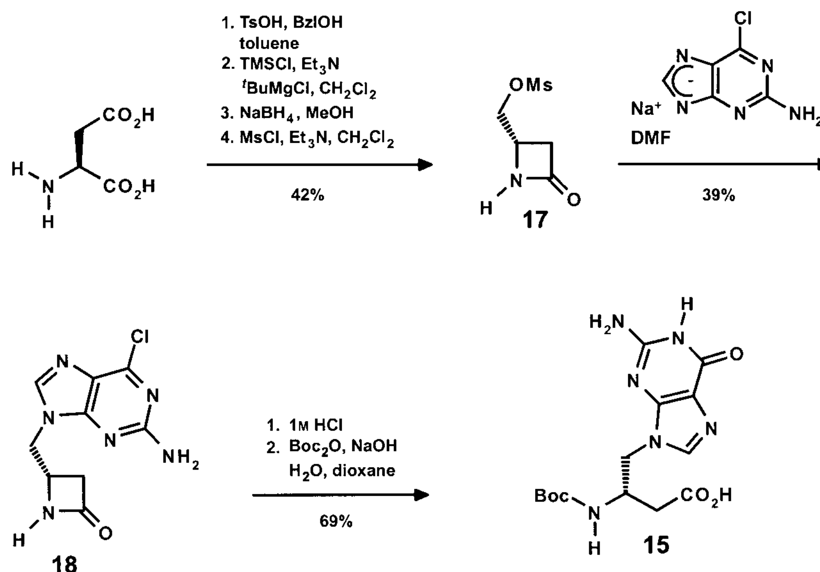
Scheme 3. *Synthesis of the Guaninyl-β-amino Acid* **15** *from the β-Homoserine Derivative* **4** *with the* Mitsunobu *Reaction as the Key Step*



In the light of these difficulties, preparation of **15** from a cheaper starting material that is unable to undergo aziridine formation, and that does not contain an ester functionality was desirable. Methanesulfonate **17** was the candidate of choice (*Scheme 4*). It can easily be prepared from L-aspartic acid by a procedure of *Salzmann et al.* [14]. The preformed Na salt of 2-amino-6-chloropurine was alkylated by this compound in 39% yield. The nucleo-β-lactam **18** was then converted to the guaninyl-β^3-

amino acid **15** by treatment with refluxing HCl, followed by protection with Boc_2O to give **15** in high yield and excellent optical purity (> 99% ee as determined by Boc deprotection, coupling with Boc-(*S*)-Ala-OSu and Boc-(*R*)-Ala-OSu resp., and HPLC analysis of the diastereoisomers).

Scheme 4. *Preparation of Guaninyl-nucleo-β-amino Acid* **15** *from Methanesulfonate* **17**



4. Guanine- and Cytosine-Containing β-Homoalanyl-PNAs. – The synthesis and structural characterization of β-HalA and β-Hal^{7C}A containing β-homoalanyl-PNAs has recently been reported [4]. The oligomers H-(β-HalA)$_n$-Lys-NH_2, where n = 5 or 6, form very stable higher aggregates in aqueous solution. This was explained by an extended backbone conformation enabling simultaneous pairing of the *Watson–Crick* and *Hoogsteen* pairing planes. As part of our ongoing studies of association processes in β-homoalanyl-PNAs, the following oligomers were synthesized: H-β-HalG-β-HalG-β-HalC-β-HalG-β-HalC-β-HalC-Lys-NH_2 (**19**, sequence GGCGCC, β-HalG = (*S*)-γ-(guanin-9-yl)-β-homoalanine, β-HalC = (*S*)-γ-(cytosin-9-yl)-β-homoalanine) and H-β-HalG-β-HalC-β-HalG-β-HalC-β-HalG-β-HalC-Lys-NH_2 (**20**, sequence GCGCGC). Oligomerization of Boc-β-HalG-OH (**15**) and Boc-β-HalC(Z)-OH (**9**) was carried out by solid-phase peptide synthesis on a MBHA-polystyrene (MBHA = 4-methylbenzhydrylamine) resin loaded with *N*-α-Boc-ω-Z-protected D-lysine. The lysine amide at the C-terminal of the PNAs was incorporated to increase the solubility of the oligomer. The amino acids were coupled in DMF with *O*-(7-aza-1*H*-benzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HATU), 1-hydroxy-7-azabenzotriazole (HOAt), and (ethyl)diisopropylamine (DIEA) for activation. To ensure chain elongation yields ≥ 97% each coupling step was performed twice – first with 5 and then 3 equiv. of nucleo-$β^3$-amino acid. After deprotection and cleavage from the solid

support with trifluoroacetic acid (TFA)/trifluoromethanesulfonic acid/dimethyl sulfide/*m*-cresol 5:1:3:1, the oligomers were precipitated with Et_2O and purified by HPLC on a *RP-C18* column. They were characterized with electrospray ionization mass spectrometry (ESI-MS).

The formation of pairing complexes and their stabilities in an aqueous solution were examined by temperature dependent UV spectroscopy. Water was chosen as the solvent, since the oligomers precipitated in phosphate buffer. Cooperative depairing of double strands or higher aggregates leads to destacking of the nucleobases, which can be recognized by the sigmoidal increase of the absorption (hyperchromicity, A_{rel}) with rising temperature [15]. The temperature T_m at the point of inflection characterizes the double strand stability. The self-pairing complex of oligomer **19** (sequence GGCGCC) showed a stability of $T_m = 46°$ (15 μM, 25% A_{rel}), whereas for oligomer **20** (sequence GCGCGC) a stability of $T_m = 51°$ (14 μM, 21% A_{rel}) was found (*Fig. 1*).

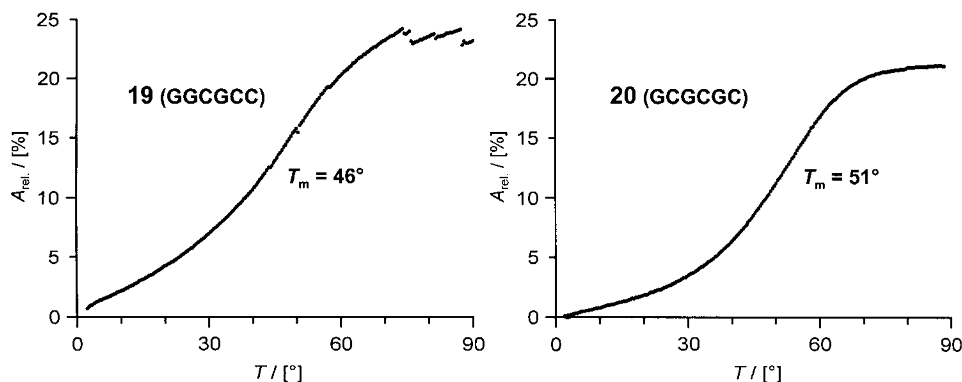


Fig. 1. *UV Melting curves* (270 nm) *of oligomers* **19** (left, 15 μM in H_2O) *and* **20** (right, 14 μM in H_2O)

Similar stabilities and hyperchromicities found for both oligomers indicate the formation of double strands with six G-C base pairs each. From simple model studies, the antiparallel double strand formation in the *Watson–Crick*-pairing mode is expected. In comparison to the alanyl-PNA hexamer with GGCGCC sequence ($T_m = 58°$, 6 μM, 14% A_{rel}) [2b], the β-homoalanyl-PNA double strands are slightly less stable. This is probably due to higher conformational freedom of the homologated oligomers. In case of the linear alanyl-PNA G-C hexamers, the GGCGCC sequence clearly defines the formation of an antiparallel double strand, whereas higher aggregation is preferred for the GCGCGC hexamer. In contrast, the β-homoalanyl-PNA oligomer **20** does not show higher aggregation. Since linear double strand formation is a prerequisite for band-like aggregates in alanyl-PNA, a deviation from the linearity is likely for β-homoalanyl-PNA. Helicalization would be in agreement with the need to lower the base pair distance of *ca.* 4.5 Å in β-homoalanyl-PNA double strands with linear topology.

With circular dichroism (CD) spectroscopy, it is possible to distinguish between the pairing complex in which the chromophore (nucleobase) is conformationally fixed in a chiral environment (backbone) and the statistical distribution in a single strand at higher temperatures. The CD spectra of oligomer **19** (sequence GGCGCC) and

oligomer **20** (sequence GCGCGC) distinctly show that the secondary structures at lower temperatures are very similar (*Fig. 2*). Both oligomers seem to form double strands with the same pairing mode and topology that differ from linearity because of the huge *Cotton* effect. Furthermore, the decrease of the *Cotton* effect between 40° and 60° is in agreement with the results obtained with the temperature dependent UV spectroscopy.

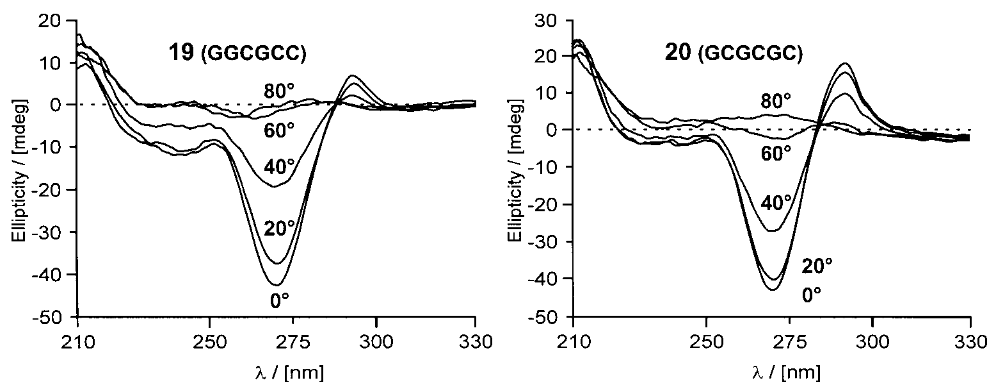


Fig. 2. *CD Spectra of oligomers* **19** (left, 10 μM in H_2O) *and* **20** (right, 12 μM in H_2O)

5. Conclusions. – As the syntheses of the nucleo-β^3-amino acids with adenine and 7-carbaadenine were described in previous articles [4], now the preparation of the remaining canonical nucleo-β^3-amino acids has been presented. Next to the *Mitsunobu* reaction for the attachment of the nucleobases to the γ-position of β-homoalanine, the nucleophilic substitution of a methanesulfonate was used in case of Z-protected cytosine. For the preparation of the guaninyl-β^3-amino acid, an efficient nucleo-β-lactam route has been established that might also be applied for the syntheses of the other nucleo-β^3-amino acids. Finally, β-homoalanyl-PNA G-C hexamers were shown to form quite stable *Watson–Crick*-pairing complexes with a double-strand topology that is most likely helical.

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Experimental Part

General. All reagents were of anal. grade and used as supplied. Solvents were of the highest grade available. HPLC: *Pharmacia Äkta* basic with *YMC J'sphere ODS-H80, RP-C18*, 150 × 10 mm, 4 μm, 80 Å for preparation of the β-homoalanyl-PNAs, and 150 × 4.6 mm, 4 μm, 80 Å for anal. samples of the β-homoalanyl-PNAs, and *Pharmacia Biotech Sephasil Peptide C18 ST*, 250 × 4.6 mm, 5 μm, 100 Å for ee determination. The oligomer concentration was calculated by taking the extinction coefficient at 90° as being the sum of the extinction coefficients of the nucleo-β^3-amino acids. M.p.: Apparatus according to *Dr. Tottoli*, *Büchi 501*. Optical rotation: *Perkin-Elmer 241* polarimeter. UV Melting curves: *JASCO V-550 UV/VIS* spectrophotometer with *JASCO ETC-505S/ETC-505T* temp. controller. CD Spectra: *JASCO J-500A* spectropolarimeter with *JASCO* temp. controller. IR: *Perkin-Elmer 841*. NMR Spectra: *Bruker AC-250*, *Bruker AMX-500*, *Bruker DMX-500*, *Varian Unity-300*, or *Varian INOVA-500* spectrometer. ESI-MS: *LCQ Finnigan* spectrometer.

(S)-N-[(tert-Butoxy)carbonyl]-γ-(3-benzoyluracil-1-yl)-β-homoalanine Benzyl Ester (**5**). DEAD (1.64 ml, 10.4 mmol) was added to a suspension of 3-benzoyluracil (1.80 g, 8.33 mmol) and Ph_3P (2.73 g, 10.4 mmol) in dry THF (21 ml) at $-30°$ under Ar. The mixture was allowed to warm to $-15°$ and added dropwise to a soln. of *(S)-N-Boc-β-homoserine benzyl ester* (**4**, 1.25 g, 4.04 mmol) in dry THF, which had been precooled to $-15°$. After 4 d, the reaction was quenched with H_2O (570 µl), and the mixture was concentrated. Purification by repeated chromatography (silica gel; hexane/AcOEt 2:3 and $CHCl_3$/MeCN 9:1) yielded **5** (1.44 g, 70%). Colorless solid. M.p. 80–81°. R_f ($CHCl_3$/MeCN 9:1) 0.30. $[\alpha]_D^{20} = +86.0$ (c = 1.1, $CHCl_3$). IR: 3401, 3093, 3069, 3040, 2983, 2936, 1752, 1710, 1665, 1599, 1513, 1445, 1390, 1365, 1345, 1239, 1169, 1115, 1090, 1053, 1029, 982, 904, 860, 802, 784, 763, 699, 629. ^{1}H-NMR ($CDCl_3$): 1.41 (br. *s*, ^{t}Bu), 2.60–2.75 (*m*, 2 H–C(α)); 3.50–3.66 (*m*, H–C(γ)); 4.00–4.12 (*m*, H–C(γ)); 4.25–4.36 (*m*, H–C(β)); 5.11 (*s*, PhCH_2); 5.43 (*d*, ^3J = 8, BocN*H*); 5.78 (*d*, ^3J = 8, H–C(5)); 7.20 (*d*, ^3J = 8, H–C(6)); 7.25–7.40 (*m*, 5 arom. H); 7.46 (*m*, 2 arom. H); 7.61 (*m*, 1 arom. H); 8.08 (*m*, 2 arom. H). ^{13}C-NMR ($CDCl_3$): 28.4 (^{t}Bu), 36.0 (C(α)); 45.9 (C(β)); 52.4 (C(γ)); 66.9 (PhCH_2); 80.0 (^{t}Bu); 101.7 (C(5)); 128.3 (Ph); 128.5 (Ph); 128.7 (Ph); 129.0 (Ph); 130.8 (Ph); 131.6 (Ph); 134.9 (Ph); 135.2 (Ph); 144.4 (C(6)); 150.1 (C(2)); 155.5 (C=O (Boc)); 162.4 (C(4)); 168.8 (Ph*C*O); 171.0 (*C*OOBzl). ESI-MS: 1036.9 (89, [2 *M* + Na]$^+$), 1014.8 (100, [2 *M* + H]$^+$), 530.1 (89, [*M* + Na]$^+$).

(S)-N-[(tert-Butoxy)carbonyl]-γ-(uracil-1-yl)-β-homoalanine (**1**). Pd/C (751 mg, 30% Pd on charcoal containing 53.1% H_2O) was added to a soln. of **5** (753 mg, 1.48 mmol) in a mixture of dioxane (40 ml), H_2O (20 ml), and AcOH (2 ml). The mixture was stirred overnight under an atmosphere of H_2 (1 bar). The catalyst was filtered off and washed several times with MeOH. The filtrate and the washing solns. were pooled. After removal of the solvents, the crude product was recrystallized from AcOEt/MeOH 4:1 (10 ml) and as much H_2O as necessary to obtain a clear soln. under reflux. Additional material was obtained from the mother liquor. Compound **1** (399 mg, 86%) was isolated as a colorless solid. M.p. 203° (dec.). R_f (AcOEt/MeOH/H_2O 80:14:6, 2% AcOH) 0.48. $[\alpha]_D^{20} = +117.0$ (*c* = 1.0, DMSO). IR: 3466, 3449, 3194, 2984, 2609, 1721, 1694, 1651, 1493, 1474, 1410, 1395, 1369, 1350, 1298, 1284, 1248, 1212, 1168, 1117, 1060, 993, 890, 842, 815, 771, 717, 637. ^{1}H-NMR ((D_6)DMSO): 1.10–1.52 (br. *s*, ^{t}Bu); 2.19–2.45 (*m*, 2 H–C(α)); 3.11–3.50 (*m*, H–C(γ)); 3.78–4.00 (*m*, H–C(γ)); 4.01–4.22 (*m*, H–C(β)); 5.48 (*d*, ^3J = 7, H–C(5)); 6.39 (*d*, ^3J = 9, 0.13 H, BocN*H*); 6.72 (*d*, ^3J = 9, 0.87 H, BocN*H*); 7.32 (*d*, ^3J = 8, H–C(6)); 11.11 (*s*, 0.8 H, H–C(3)); 11.26 (*s*, 0.2 H, H–C(3)); 12.20 (br. *s*, COOH). ^{13}C-NMR ((D_6)DMSO): 27.7; 27.8; 28.1; 36.8; 37.2; 46.0; 51.5; 51.9; 77.9; 78.1; 100.2; 100.4; 145.8; 146.1; 151.0; 155.0; 163.8; 171.8. ESI-MS: 627.1 (100, [2 *M* + H]$^+$), 649.1 (87, [2 *M* + Na]$^+$), 314.0 (28, [*M* + H]$^+$). Anal. calc. for $C_{13}H_{19}N_3O_6$ (313.31): C 49.84, H 6.11, N 13.41; found: C 49.88, H 6.10, N 13.36.

(S)-N-[(tert-Butoxy)carbonyl]-γ-(3-benzoylthymin-1-yl)-β-homoalanine Benzyl Ester (**6**) *from* **4**. 3-Benzoylthymine (1.92 g, 8.33 mmol), Ph_3P (2.73 g, 10.4 mmol), and DEAD (1.64 ml, 10.4 mmol) were added to dry THF (20 ml) at $-30°$ under Ar. After 10 min, the now clear soln. was added dropwise to a soln. of **4** (1.25 g, 4.04 mmol) in dry THF (10 ml) at $-30°$ within 10 min. The mixture was stirred for 5 d at $-10°$. After removal of the solvent, purification was achieved by repeated chromatography (silica gel; $CHCl_3$/MeOH 9:1 and hexane/acetone 7:3) to give **6** (1.37 g, 65%). Colorless solid. M.p. 73–75°. R_f ($CHCl_3$/MeCN 9:1) 0.45. $[\alpha]_D^{20} = +77.0$ (*c* = 0.9, $CHCl_3$). IR: 3365, 3071, 3038, 2982, 2938, 2343, 1749, 1706, 1658, 1599, 1513, 1439, 1391, 1367, 1349, 1245, 1169, 1063, 1031, 984, 903, 874, 850, 820, 781, 764, 699, 685, 631. ^{1}H-NMR ($CDCl_3$): 1.39 (*s*, ^{t}Bu); 1.89 (*s*, Me); 2.55–2.75 (*m*, 2 H–C(α)), 3.46–3.70 (*m*, H–C(γ)), 3.96–4.13 (*m*, H–C(γ)); 4.18–4.40 (*m*, H–C(β)); 5.11 (*s*, PhCH_2); 5.45 (*d*, ^3J = 9, BocN*H*); 7.04 (*d*, *J* = 1, H–C(6)); 7.26–7.39 (*m*, 5 arom. H); 7.40–7.55 (*m*, 2 arom. H); 7.56–7.69 (*m*, 1 arom. H); 7.98–8.13 (*m*, 2 arom. H). ^{13}C-NMR ($CDCl_3$): 12.3; 28.3; 30.9; 36.0; 46.0; 51.9; 66.9; 80.0; 110.2; 128.3; 128.5; 128.6; 129.0; 130.7; 131.6; 134.8; 135.2; 140.5; 150.1; 155.3; 163.1; 169.1; 171.0. ESI-MS: 544.0 (100, [*M* + Na]$^+$).

Benzyl Ester **6** *from (S)-N-[(tert-Butoxy)carbonyl]-γ-(methanesulfonyl)-β-homoserine Benzyl Ester* (**8**). A soln. of **8** (500 mg, 1.29 mmol), Bu_4NI (143 mg, 387 µmol), K_2CO_3 (267 mg, 1.93 mmol), and 3-benzoylthymine (327 mg, 1.42 mmol) in dry DMF (50 ml) was stirred under Ar at r.t. for 3 d. After quenching the reaction with AcOH (221 µl, 3.86 mmol), the solvent was removed and the residue was subjected to chromatography (silica gel; $CHCl_3$/MeCN 9:1). Compound **6** (442 mg, 66%) was isolated as a colorless solid. The anal. data correspond to those obtained from **6** prepared *via* the *Mitsunobu* reaction.

(S)-N-[(tert-Butoxy)carbonyl]-γ-(thymin-1-yl)-β-homoalanine (**2**). Pd/C (1.80 g, 30% Pd/C containing 53.1% H_2O) was added to a soln. of **6** (1.18 g, 2.27 mmol) in a mixture of dioxane (60 ml), H_2O (15 ml), and AcOH (1.5 ml). The mixture was stirred overnight under an atmosphere of H_2 (1 bar). The catalyst was filtered off and washed several times with MeOH. The filtrate and the washing solns. were pooled. After removal of the solvents and repeated co-evaporation with toluene, the crude product was purified by recrystallization from AcOEt/MeOH 4:1 and as much H_2O as necessary to obtain a clear soln. under reflux. Compound **2** (638 mg, 86%) was isolated as a colorless solid. M.p. 212° (dec.). R_f (AcOEt/MeOH/H_2O 80:14:6, 2% AcOH) 0.54.

$[\alpha]_D^{20} = +97.0$ ($c = 1.1$, DMSO). IR: 3442, 3186, 3068, 3000, 2953, 1723, 1709, 1682, 1660, 1504, 1465, 1445, 1416, 1401, 1373, 1348, 1272, 1225, 1173, 1133, 1063, 1052, 1035, 924, 894, 853, 818, 788, 779, 761, 716, 618. ^{1}H-NMR ((D_6)DMSO): 1.05–1.39 (br. *s*, ^{t}Bu); 1.70 (*s*, Me); 2.20–2.50 (*m*, 2 H–C(α)); 3.10–3.51 (*m*, H–C(γ)); 3.73–3.97 (*m*, H–C(γ)); 3.99–4.21 (*m*, H–C(β)); 6.32 (*d*, $^3J = 9$, 0.15 H, BocN*H*); 6.74 (*d*, $^3J = 9$, 0.85 H, BocN*H*); 7.23 (*s*, H–C(6)); 11.11 (*s*, 0.87 H, H–C(3)); 11.25 (*s*, 0.13 H, H–C(3)); 12.00–12.55 (br. *s*, COOH). ^{13}C-NMR ((D_6)DMSO): 12.0; 27.8; 28.0; 36.7; 37.1; 46.0; 51.3; 77.8; 107.6; 141.9; 150.9; 155.0; 164.3; 171.9. ESI-MS: 350.0 (100, $[M + Na]^+$), 676.9 (48, $[2M + Na]^+$).

(S)-N-[(tert-Butoxy)carbonyl]-γ-[N⁴-(benzyloxycarbonyl)cytosin-1-yl]-β-homoalanine Benzyl Ester (**7**). A suspension of **8** (5.91 g, 15.3 mmol), N^4-(benzyloxycarbonyl)cytosine (4.88 g, 19.9 mmol), and K_2CO_3 (3.39 g, 24.5 mmol) in dry DMF (100 ml) was stirred at r.t. for 14 d under N_2. The solvent was removed, and the residue was extracted with AcOEt and acetone. After evaporation of the solvents and purification by chromatography (silica gel: AcOEt/hexane 3:1 to AcOEt), **7** (1.85 g, 23%) was isolated as a colorless solid. M.p. 162°. R_f (AcOEt/hexane 3:1) 0.29. $[\alpha]_D^{20} = +74.4$ ($c = 0.25$, MeOH). IR: 3367, 2984, 2361, 1743, 1729, 1684, 1627, 1558, 1516, 1369, 1344, 1223, 1167, 1055, 787. ^{1}H-NMR ($CDCl_3$): 1.36 (*s*, ^{t}Bu), 2.58 (br. *m*, H–C(α)); 2.86 (br. *m*, H–C(α)); 4.07 (br. *m*, 2 H–C(γ)); 4.23 (br. *m*, H–C(β)); 5.13 (*s*, PhC*H*$_2$); 5.21 (*s*, PhC*H*$_2$); 5.51 (br. *m*, H–C(5)); 7.25–7.45 (*m*, 10 arom. H), 7.49–7.62 (br. *m*, H–C(6)). ^{13}C-NMR ($CDCl_3$): 28.2; 36.2; 47.3; 52.4; 66.7; 67.7; 80.5; 95.0; 127.9; 128.1; 128.3; 128.4; 128.5; 128.6; 135.0; 149.2; 152.5; 155.3; 162.6; 170.9. ESI-MS: 559.5 (75, $[M + Na]^+$), 1095.4 (100, $[2M + Na]^+$).

(S)-N-[(tert-Butoxy)carbonyl]-γ-[N⁴-(benzyloxycarbonyl)cytosin-1-yl]-β-homoalanine (**9**). A soln. of **7** (1.68 g, 3.13 mmol) in a mixture of dioxane (15 ml), H_2O (10 ml), and 1M aq. NaOH (5 ml) was stirred at r.t. for 9 h. The mixture was then neutralized with 1M aq. HCl, concentrated, and freeze-dried. The residue was subjected to chromatography (*RP-C18* silica gel; H_2O to H_2O/MeOH 4:6) to yield **9** (1.15 g, 82%). Colorless solid. M.p. 172–182° (dec.). R_f (AcOEt/MeOH/H_2O 80:14:6, 3% AcOH) 0.55. $[\alpha]_D^{20} = +94.0$ ($c = 0.25$, MeOH). IR: 3410, 2977, 1750, 1653, 1574, 1504, 1370, 1213, 1053, 789, 746, 697. ^{1}H-NMR ((D_6)DMSO): 1.25 (*s*, ^{t}Bu); 1.97–2.13 (*m*, 2 H–C(α)); 3.56–3.66 (*m*, H–C(γ)); 3.85–4.00 (*m*, 2 H–C(β), H–C(γ)); 5.16 (*s*, PhC*H*$_2$); 6.86 (*d*, $^3J = 7$, H–C(5)); 7.26–7.40 (*m*, 5 arom. H); 7.81 (*d*, $^3J = 7$, H–C(6)); 10.57 (br. *s*, COOH). ^{13}C-NMR ((D_6)DMSO): 28.1 (^{t}Bu); C(α signal probably overlaps with the solvent signal; 46.5 (C(β)); 53.6 (C(γ)); 66.3 (Ph*C*H$_2$); 77.5 (^{t}Bu); 93.7 (C(5)); 127.8–128.4 (Ph); 136.0 (Ph); 150.0 (C(6)); 153.3; 154.8; 162.8; 174.5 (COOH). ESI-MS: 469.6 (100, $[M + Na]^+$), 915.4 (56, $[2M + Na]^+$).

(S)-N-[(tert-Butoxy)carbonyl]-γ-(uracil-1-yl)-β-homoalanine Benzyl Ester (**11**). Compound **1** (1.00 g, 3.19 mmol) was dissolved in a mixture of DMF (5 ml) and BzlOH (5 ml). DMAP (195 mg, 1.60 mmol) and *N*-[3-(dimethylamino)propyl]-*N'*-ethylcarbodiimide hydrochloride (EDCI · HCl, 672 mg, 3.51 mmol) were added at 0°, and the mixture was stirred at r.t. for 16 h. After removal of the solvents, the residue was purified by repeated chromatography (silica gel; $CHCl_3$/EtOH 95:5) to give **11** (1.11 g, 86%). Colorless solid. M.p. 148–149°. R_f ($CHCl_3$/EtOH 95:5) 0.24. $[\alpha]_D^{20} = +113.0$ ($c = 0.9$, DMSO). IR: 3370, 3058, 2375, 2001, 1679, 1522, 1453, 1418, 1390, 1368, 1345, 1275, 1244, 1167, 1112, 1056, 982, 848, 804, 765, 697, 622. ^{1}H-NMR ($CDCl_3$): 1.36 (br. *s*, ^{t}Bu); 2.55–2.82 (*m*, 2 H–C(α)); 3.70–3.87 (*m*, H–C(γ)); 3.88–4.00 (*m*, H–C(γ)); 4.13–4.30 (*m*, H–C(β)); 5.12 (*s*, PhC*H*$_2$); 5.38 (*d*, $^3J = 8$, BocN*H*); 5.61 (*d*, $^3J = 8$, H–C(5)); 7.11 (*d*, $^3J = 8$, H–C(6)): 7.27–7.41 (*m*, 5 arom. H); 9.16 (br. *s*, H–C(3)). ^{13}C-NMR ($CDCl_3$): 28.2 (^{t}Bu), 36.1 (C(α)); 46.9 (C(β)); 51.0 (C(γ)); 66.8 (Ph*C*H$_2$); 80.1 (^{t}Bu); 102.1 (C(5)); 128.3 (Ph); 128.5 (Ph); 128.6 (Ph); 135.4 (Ph); 144.7 (C(6)); 151.3 (C(2)); 155.3 (Boc*C*O); 163.5 (C(4)); 170.9 (*C*OOBzl). ESI-MS: 426.1 (100, $[M + Na]^+$).

(S)-N-[(tert-Butoxy)carbonyl]-γ-[4-(3'-nitro-1',2',4'-triazol-1'-yl)-2-oxo-1H-pyrimidin-1-yl]-β-homoalanine Benzyl Ester (**12**). A soln. of **11** (894 mg, 2.22 mmol) in dry pyridine (14 ml) was cooled to 0°. 4-Chlorophenyl dichlorophosphate (434 µl, 2.66 mmol) was added dropwise, followed by a soln. of 3-nitro-1,2,4-1*H*-triazole (760 mg, 5.84 mmol) in dry pyridine (11 ml). After stirring for 16 h at r.t., the solvent was removed by co-evaporation with toluene, and the residue was purified by chromatography (silica gel; $CHCl_3$/EtOH 95:5) to give **12** (1.03 g, 93%). Yellow solid. M.p. 176–177°. R_f ($CHCl_3$/EtOH 95:5) 0.40. $[\alpha]_D^{20} = +163.0$ ($c = 1.0$, DMSO). IR: 3567, 3374, 3096, 2986, 1736, 1680, 1634, 1561, 1514, 1470, 1426, 1403, 1367, 1348, 1302, 1246, 1163, 1116, 1059, 1001, 939, 835, 786, 751, 697, 655. ^{1}H-NMR ($CDCl_3$): 1.31 (br. *s*, ^{t}Bu), 2.70 (*dd*, $^1J = 17$, $^3J = 5$, H–C(α)); 2.84 (*dd*, $^1J = 17$, $^3J = 5$, H–C(α)); 3.91–4.10 (*m*, H–C(γ)); 4.22–4.42 (*m*, H–C(β), H–C(γ)); 5.14 (*s*, PhC*H*$_2$); 5.40 (*d*, $^3J = 8$, BocN*H*); 6.99 (*d*, $^3J = 7$, H–C(5)); 7.26–7.42 (*m*, 5 arom. H); 7.93 (*d*, $^3J = 7$, H–C(6)); 9.29 (*s*, H–C(5')). ^{13}C-NMR ($CDCl_3$): 28.1; 36.2; 46.6; 54.0; 67.0; 80.4; 93.9; 95.8; 128.4; 128.6; 128.7; 135.2; 144.6; 152.6; 154.7; 155.3; 158.5; 170.8. ESI-MS: 998.7 (100, $[2M + H]^+$), 522.0 (21, $[M + Na]^+$), 499.7 (16, $[M + H]^+$).

(S)-N-[(tert-Butoxy)carbonyl]-γ-(cytosin-1-yl)-β-homoalanine Benzyl Ester (**13**). Aq. NH_3 (39 ml) was added to a soln. of **12** (984 mg, 1.96 mmol) in dioxane (78 ml). After stirring for 1 h at r.t., the mixture was

concentrated, and the residue was purified by repeated chromatography (silica gel; $CHCl_3$/MeOH 4:1). Compound **13** (761 mg, 96%) was isolated as a colorless solid. M.p. 94–97°. R_f ($CHCl_3$/MeOH 4:1) 0.88. $[\alpha]_D^{20} = +95.0$ ($c = 0.9$, DMSO). IR: 3346, 2982, 2001, 1699, 1649, 1522, 1494, 1455, 1438, 1392, 1367, 1282, 1170, 1053, 1027, 979, 912, 855, 789, 751, 697, 676, 619. ^{1}H-NMR ($CDCl_3$): 1.36 (*s*, ^{t}Bu), 2.48–2.60 (*m*, H–C(α)); 2.78–2.92 (*m*, H–C(α)); 3.89–4.07 (*m*, 2 H–C(γ)); 4.12–4.27 (*m*, H–C(β)); 5.11 (*s*, PhC*H*$_2$); 5.30–6.60 (br. *s*, NH_2); 5.63 (*d*, $^3J = 7$, H–C(5)); 5.67 (*d*, $^3J = 7$, BocN*H*); 7.21 (*d*, $^3J = 7$, H–C(6)), 7.28–7.41 (*m*, 5 arom. H). ^{13}C-NMR ($CDCl_3$): 28.3; 36.4; 48.3; 51.8; 66.7; 79.7; 94.0; 105.3; 128.4; 128.6; 135.6; 146.4; 155.5; 157.0; 165.6; 171.0. ESI-MS: 805.0 (100, $[2M + H]^+$), 403.0 (37, $[M + H]^+$).

*(*S*)-*N*-[(*tert*-Butoxy)carbonyl]-γ-(cytosin-1-yl)-β-homoalanine* (**10**) *from* **13**. A soln. of NaOH (100 mg, 2.50 mmol) in H_2O (5.5 ml) was added to a soln. of **13** (586 mg, 1.46 mmol) in dioxane (5 ml). After being stirred overnight at 30°, the mixture was acidified with 1M aq. HCl (pH 2–3), and the precipitating crystalline **10** was separated. The mother liquor was concentrated, and additional product was isolated after chromatography (*RP-C18* silica gel, first H_2O then H_2O/MeOH with increasing amounts of MeOH). A total of 385 mg of **10** (85%) was obtained as a colorless solid. M.p. 211–213° (dec.). R_f (AcOEt/MeOH/H_2O 80:14:6, 3% AcOH) 0.39. ee 96% (determined by HPLC of the dimer prepared with Boc-(*S*)-Leu-OSu on *RP-C18*; 10–30% *B* (*B* = MeCN + 0.1% TFA) in 30 min, t_R(*like*) 22.8 min; t_R(*unlike*) 21.4 min). $[\alpha]_D^{20} = +120.3$ ($c = 0.8$, DMSO). IR: 3414, 3079, 2986, 2924, 2749, 1959, 1692, 1650, 1559, 1486, 1447, 1419, 1397, 1352, 1308, 1286, 1274, 1220, 1168, 1108, 1059, 1018, 1002, 900, 850, 806, 768, 715, 611. ^{1}H-NMR ((D_6)DMSO): 1.19–1.40 (br. *s*, ^{t}Bu); 2.23–2.41 (*m*, 2 H–C(α)); 3.35–3.51 (*m*, H–C(γ)); 3.82–3.95 (*m*, H–C(γ)); 3.98–4.15 (*m*, H–C(β)); 5.60 (*d*, $^3J = 7$, H–C(5)); 6.12–6.31 (br. *s*, 0.2 H, BocN*H*); 6.65 (*d*, $^3J = 7$, 0.8 H, BocN*H*); 6.72–7.12 (br. *s*, NH_2); 7.33 (*d*, $^3J = 7$, H–C(6)). ^{13}C-NMR ((D_6)DMSO): 28.1; 37.0; 46.8; 51.7; 77.7; 92.8; 146.0; 155.7; 165.8; 171.8. ESI-MS: 313.0 (98, $[M + H]^+$), 625.0 (100, $[2M + H]^+$).

Compound **10** *from* **9**. A soln. of NaOH (145 mg, 3.63 mmol) in H_2O (4 ml) was added to a soln. of **9** (50.0 mg, 112 μmol) in dioxane (3 ml). After being stirred for 2 d at 50°, the mixture was acidified with 1M aq. HCl (pH 2–3) and concentrated. The product **10** (32.2 mg, 92%) was isolated after chromatography (*RP-C18* silica gel; first H_2O then H_2O/MeOH with increasing amounts of MeOH) as a colorless solid. The anal. data correspond to those of **10** obtained from **13** as described above.

*(*S*)-*N*-[(*tert*-Butoxy)carbonyl]-γ-(2-amino-6-chloropurin-9-yl)-β-homoalanine Benzyl Ester* (**14**). Ph_3P (2.11 g, 8.04 mmol) was dissolved in dry MeCN (30 ml) under N_2, and the soln. was cooled to −45°. DEAD (1.47 ml, 9.34 mmol) was added within 20 min, and the soln. was allowed to warm to −30°. A −20° cold suspension of **4** (1.38 g, 4.46 mmol) and 2-amino-6-chloropurine (1.14 g, 6.72 mmol) in a mixture of dry THF (30 ml) and dry MeCN (10 ml) was added within 30 min. After stirring for 2 h at −20°, the soln. was warmed to 0°, and stirring was continued for 11 d. The solvents were removed, and the residue was taken up in $CHCl_3$ and centrifuged. After concentration of the supernatent suspension and precipitation of Ph_3PO in cold Et_2O, purification by repeated chromatography (silica gel; $CHCl_3$/MeOH 95:5 and $CHCl_3$/MeCN 3:1) afforded **14** (707 mg, 34%). Yellowish solid. M.p. 79–80°. R_f ($CHCl_3$/EtOH 95:5) 0.32. $[\alpha]_D^{20} = +23.0$ ($c = 1.0$, $CHCl_3$). IR: 3392, 2983, 1716, 1616, 1565, 1518, 1464, 1409, 1394, 1368, 1311, 1283, 1253, 1163, 1055, 1001, 913, 786, 752, 698, 642. ^{1}H-NMR ($CDCl_3$): 1.18–1.55 (br. *s*, ^{t}Bu); 2.58 (*dd*, $J = 17, 6$, H–C(α)); 2.67 (*dd*, $J = 15, 4$, H–C(α)); 4.10–4.42 (br. *m*, H–C(β), H–C(γ)); 4.97–5.10 (br. *s*, NH_2); 5.10–5.16 (*m*, PhC*H*$_2$); 5.34–5.51 (*m*, BocN*H*); 7.30–7.41 (*m*, 5 arom. H); 7.65 (*s*, H–C(8)). ^{13}C-NMR ($CDCl_3$): 28.2; 36.1; 46.0; 47.7; 66.9; 80.2; 125.2; 128.4; 128.6; 128.7; 135.3; 142.8; 151.5; 154.1; 155.0; 159.1; 170.7. ESI-MS: 461.0 (100, $[M + H]^+$).

*(*S*)-*N*-[(*tert*-Butoxy)carbonyl]-γ-(guanin-9-yl)-β-homoalanine* (**15**) *from* **14**. A soln. of **14** (1.00 g, 2.17 mmol) in a mixture of dioxane (11 ml) and 1M aq. NaOH (22 ml) was stirred at 50° for 20 h. The mixture was then neutralized with 1M aq. HCl and concentrated. The residue was subjected to chromatography (silica gel; AcOEt/MeOH/H_2O 80:14:6, 3% AcOH; after removal of *(*S*)-*N*-[*tert*-butoxy)carbonyl]-γ-(*O*^{6}-benzylguanin-9-yl)-β-homoalanine* (**16**): THF/MeOH/H_2O 80:14:6, 3% AcOH). Desalting was achieved by chromatography (*RP-C18* silica gel; first H_2O then H_2O/MeOH 9:1). Compounds **16** (184 mg, 19%) and **15** (453 mg, 59%) were isolated as colorless solids.

Data of **16**: M.p. 187–190°. R_f (AcOEt/MeOH/H_2O 80:14:6, 3% AcOH) 0.70. $[\alpha]_D^{20} = +54.0$ ($c = 0.8$, DMSO). IR: 3368, 2985, 2001, 1687, 1620, 1588, 1525, 1455, 1414, 1361, 1336, 1251, 1162, 1070, 1028, 911, 848, 789, 753, 700, 639. ^{1}H-NMR ((D_6)DMSO): 0.90–1.10 (br. *s*, 1.4 H, ^{t}Bu); 1.12–1.55 (br. *s*, 7.6 H, ^{t}Bu); 2.26–2.45 (*m*, 2 H–C(α)); 3.90–4.31 (*m*, H–C(β), 2 H–C(γ)); 5.39–5.62 (*m*, PhC*H*$_2$); 6.40–6.52 (br. *s*, NH_2); 6.87 (*d*, $^3J = 8$, BocN*H*); 7.28–7.43 (*m*, 3 arom. H); 7.44–7.58 (*m*, 2 arom. H); 7.63 (*s*, H–C(8)). ^{13}C-NMR ((D_6)DMSO): 27.5; 28.0; 37.0; 46.0; 47.3; 66.7; 77.8; 90.6; 113.6; 128.0; 128.3; 136.7; 140.0; 154.6; 154.7; 159.6; 159.9; 171.9. ESI-MS: 443.1 (85, $[M + H]^+$).

Data of **15**: M.p. 234° (dec.). R_f (AcOEt/MeOH/H_2O 80:14:6, 3% AcOH) 0.32. $[\alpha]_D^{20} = +45.0$ ($c = 1.2$, DMSO). IR: 3422, 3129, 2980, 1688, 1575, 1536, 1484, 1410, 1366, 1251, 1165, 1054, 1027, 850, 784, 691, 638. ^{1}H-NMR ((D_6)DMSO): 0.99-1.15 (br. *s*, 1.5 H, ^{t}Bu); 1.16–1.32 (br. *s*, 7.5 H, ^{t}Bu); 2.12–2.32 (*m*, 2 H−C(α)); 3.89–4.10 (*m*, H−C(β), 2 H−C(γ)); 6.50 (*s*, 0.15 H, BocN*H*); 6.65–6.79 (br. *s*, NH_2); 6.80–6.90 (*m*, 0.85 H, BocN*H*); 7.50 (*s*, H−C(8)). ^{13}C-NMR ((D_6)DMSO): 27.5; 28.0; 46.1; 47.8; 77.5; 116.3; 137.6; 151.4; 153.7; 154.6; 157.1; 175.6. ESI-MS: 353.1 (100, $[M+H]^+$), 705.0 (39, $[2M+H]^+$).

*(*S*)-4-[(2′-Amino-6′-chloropurin-9′-yl)methyl]azetidin-2-one* (**18**). NaH (1.74 g suspension in mineral oil, 43.5 mmol) was added to a suspension of 2-amino-6-chloropurine (6.82 g, 40.2 mmol) in dry DMF (100 ml) under Ar. After H_2 evolution had ceased, the mixture was heated to 50°, and a soln. of **17** (6.00 g, 33.5 mmol) in dry DMF (50 ml) was added. After stirring the suspension at 85° for 4 h, the solvent was removed, and the residue was thoroughly washed with THF/MeOH 9:1 (filtration through *Celite*). The soluble material was absorbed on silica gel and subjected to chromatography (silica gel; THF/CH_2Cl_2 4:1). Evaporation of the solvents yielded **18** (3.31 g, 39%). Cream-colored solid. R_f (THF/CH_2Cl_2 4:1) 0.24. $[\alpha]_D^{20} = -6.1$ ($c = 0.25$, DMSO). IR: 3319, 3200, 1752, 1650, 1615, 1560, 1525, 1477, 1386, 1346, 1310, 1166, 997, 907, 782, 733. ^{1}H-NMR ((D_6)DMSO): 2.69 (*m*, H−C(3)); 2.97 (*m*, H−C(3)); 3.93 (*m*, H−C(4)); 4.22 (*m*, CH_2); 6.92 (*s*, NH_2); 8.07 (*s*, NH); 8.11 (*s*, H−C(8′)). ^{13}C-NMR ((D_6)DMSO): 41.4 (C(3)); 45.4 (C(4)); 47.3 (CH_2); 123.4 (C(5′)); 143.4 (C(8′)); 149.6 (C(6′)); 154.4 (C(4′)); 160.0 (C(2′)); 166.6 (C=O). ESI-MS: 294 (100, $[M+H+MeCN]^+$), 253 (38, $[M+H]^+$).

Compound **15** *from* **18**. Compound **18** (3.10 g, 12.3 mmol) was dissolved in 1M aq. HCl (600 ml). The soln. was refluxed for 9 h and then evaporated to dryness. A stirred suspension of the residue in H_2O/1M aq. NaOH/dioxane 1:1:2 (80 ml) was treated portionwise with Boc_2O (7.12 g, 32.6 mmol) within 5 d. pH 9 was maintained by repeated addition of 1M aq. NaOH. The resultant mixture was washed with Et_2O (2 × 50 ml) and concentrated *in vacuo*. Purification of the residue by chromatography (*RP-C18* silica gel, first H_2O then H_2O/MeOH 9:1) provided, after freeze drying, **15** (3.00 g, 69%) as a colorless solid. The anal. data correspond to the those of **15** obtained from **14** as described above except for $[\alpha]_D^{20} = +54.9$ ($c = 0.25$, DMSO). ee > 99% (determined by HPLC of the dimers prepared with Boc-(*S*)-Ala-OSu and Boc-(*R*)-Ala-OSu on *RP-C18*; 7.8–14.4% *B* (*B* = MeCN/H_2O 9:1 + 0.1% TFA) in 30 min, t_R(*like*) 21.8 min; t_R(*unlike*) 19.2 min).

General Procedure for SPPS of β-Homoalanyl-PNA. Oligomerization was performed as a manual solid-phase peptide synthesis on a 4-methylbenzhydrylamine(MBHA)-polystyrene resin loaded with D-lysine(Z)-OH (39.4 mg, 22.4 μmol lysine amide). For the first step of the double coupling, an excess of 5 equiv. *N*-Boc-protected nucleo-β^3-amino acid (112 μmol) was used and activated by *O*-(7-azabenzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HATU, 38.4 mg, 101 μmol), 1-hydroxy-7-aza-1*H*-benzotriazole (HOAt, 15.2 mg, 112 μmol), and (ethyl)diisopropylamine (DIEA, 39.1 μl, 224 μmol) in DMF (500 μl). For the second step of the double coupling, an excess of 3 equiv. *N*-Boc-protected nucleo-β^3-amino acid (67.2 μmol) was used and activated by HATU (23.0 mg, 60.5 μmol), HOAt (9.2 mg, 68 μmol), and DIEA (23.5 μl, 134 μmol) in DMF (500 μl). After swelling the loaded resin for 1 h in 2 ml CH_2Cl_2, the following procedure was repeated for every nucleo-β^3-amino acid unit: *1*) deprotection twice, for 3 min with TFA/*m*-cresol 95:5 (2 ml); *2*) washing first 5× with CH_2Cl_2/DMF 1:1 (2 ml) and then 5× with pyridine (2 ml); *3*) double coupling steps, each 1 h gentle moving in a small column; *4*) washing with CH_2Cl_2/DMF 1:1 (3 × 2 ml), DMF/piperidine 95:5 (3 × 2 ml), and CH_2Cl_2/DMF 1:1 (3 × 2 ml). The resin was washed with TFA (3 × 2 ml) and CH_2Cl_2 (5 × 2 ml), dried overnight *in vacuo*, suspended in dimethyl sulfide (600 μl)/*m*-cresol (200 μl), and cooled to −20°. TFA (1 ml) was added, followed by trifluoromethanesulfonic acid (200 μl) after 10 min. The mixture was warmed to r.t. within 1.5 h, and stirring was continued for 1.5 h. The filtrate was concentrated by freeze drying, and the β-homoalanyl-PNA precipitated with Et_2O (30 ml at −10°) as a white solid and was purified by HPLC (*RP-C18*). The yield of each coupling step was estimated from HPLC to be higher than 97%.

H-β-HalG-β-HalG-β-HalC-β-HalG-β-HalC-β-HalC-Lys-NH$_2$ (**19**): Anal. HPLC: 15.1 min, *RP-C18*, gradient: 4–12% *B* (B = MeCN/H_2O 9:1 + 0.1% TFA) in 16 min. ESI-MS: 716.4 (92%, $[M+2H]^{2+}$), 728.2 (100%, $[M+H+Na]^{2+}$), 738.9 (86%, $[M+2Na]^{2+}$), 1430.7 (16%, $[M+H]^+$), 1452.7 (20%, $[M+Na]^+$).

H-β-HalG-β-HalC-β-HalG-β-HalC-β-HalG-β-HalC-Lys-NH$_2$ (**20**): Anal. HPLC: 18.6 min, *RP-C18*, gradient: 4–15% *B* (*B* = MeCN/H_2O 9:1 + 0.1% TFA) in 33 min. ESI-MS: 716.2 (100%, $[M+2H]^{2+}$), 727.1 (31%, $[M+H+Na]^{2+}$), 738.2 (20%, $[M+2Na]^{2+}$), 1430.5 (6%, $[M+H]^+$).

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