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Synthesis of a 7-Deazaguanine-Functionalized β-Amino Acid: Improved Specificity of β-Peptide Helix Organization

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Nucleobase-functionalized β-peptides are a suitable scaffold for the construction of well-defined tertiary structures organized by nucleobase pair recognition. Since guanine-rich oligomers are especially known to form higher aggregates, an enantiomerically pure 7-deazaguanine (^{Z}G)-functionalized nucleo-β^3-amino acid was synthesized from a β-lactam derivative as a key intermediate. Incorporated into β-peptide 14-helices it was possible to reduce aggregation phenomena and to increase recognition selectivity. Evidence for base-pair-mediated helix recognition was obtained by temperature-dependent UV and CD spectroscopy.

Introduction

In protein chemistry the folding and three-dimensional organization of structural elements like helices, sheets, and turns are essential for protein function. Typically, spatial organization and conformational reorganization of secondary structures are involved in the recognition of biomolecules, in catalysis, or in signalling processes. In recent years, the design, recognition, and organization of biomimetic molecules has received growing interest especially with respect to the formation and cooperativity of well-defined secondary structures.[1–5] In this regard β-peptides have turned out to be especially interesting since these oligomers composed of β-amino acids have been successfully developed as artificial mimics of secondary structures.[6] Stable β-peptide secondary structures can be predicted to exist in organic solvents but also under physiological conditions.[7] The secondary structure of β-peptides can be determined by the substitution pattern and the β-amino acid configuration at the 2- and 3-positions.[8] The 14-helix is one of the most prominent β-peptide secondary structures. It is derived from β^3-amino acids with lateral side-chain configuration. Only six β-amino acids are required to provide a 14-helix with high conformational stability.[9] Since three amino acids form one turn, all side-chains *i* and *i* + 3 are oriented alike to provide a helix with three faces which can have different physical properties depending on the side-chain functionalities. This design has already been used for the preparation and organization of amphiphilic β-peptide helices.[10]

Recently, we reported on nucleobase-functionalized β-peptide 14-helices and their reversible, antiparallel, and selective organization by nucleobase recognition (Figure 1).[11] Base-pairing provides an excellent driving force and selectivity for the organization of β-peptide helices similar to that in the known self-association of unnatural oligomers like peptidic nucleic acids[12,13] and α-helical peptides.[14] In the series of nucleobase-modified β-peptides the high helical content leads to a preorganization of the attached nucleobases with respect to double-strand formation. This entropy contribution provides pairing complexes with a much higher stability than those of oligonucleotides with an identical sequence. Only four nucleobases are required for β-peptide oligomers to form stable duplexes at room temperature. In addition to the high pairing stability of β-peptides derived from the helix topology, an extraordinary high stability was observed for guanine-rich sequences,[11a] reminiscent of complexes of guanine-rich DNA[15] and PNA oligomers.[16] Simultaneous base-pairing of guanine over the Watson–Crick and Hoogsteen sites leads to highly stabilized G quadruplexes or band-like aggregates. The carba-analogous nucleobase 7-deazaguanine (^{Z}G) can be used as a guanine analog that only forms base pairs over the Watson–Crick site.[17,18] Therefore, the incorporation of ^{Z}G instead of guanine into β-peptide 14-helices should suppress higher aggregation due to Hoogsteen pairing of guanine.[19] In addition, the incorporation of ^{Z}G as a guanine analog into double strands diminishes the double-strand stability if guanine recognition on the Hoogsteen site is required. A comparison of guanine- and ^{Z}G-containing oligomers might allow conclusions to be drawn on the preferred guanine-pairing mode in β-peptide double strands.

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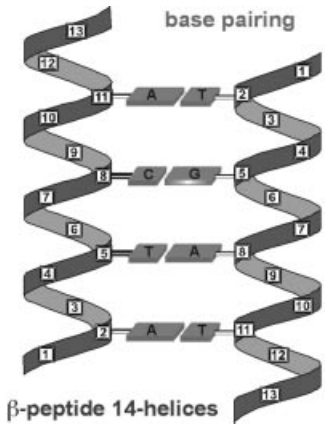


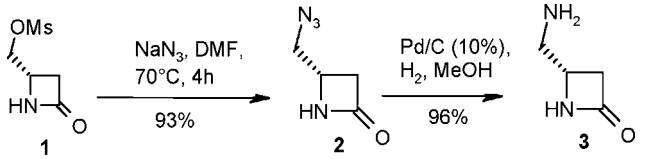
Figure 1. Double-strand formation of nucleobase-functionalized β-peptide 14-helices.

In this work 7-deazaguanine was incorporated into β-peptide 14-helices. It was introduced by solid-phase peptide synthesis as enantiomerically pure 7-deazaguaninyl-β^3-nucleo-amino acid. The corresponding β-amino acid synthesis via a β-lactam key intermediate is described. Furthermore, evidence for higher aggregation phenomena was obtained from the pairing stabilities of ^{Z}G-β-peptides relative to the corresponding guanine sequences.

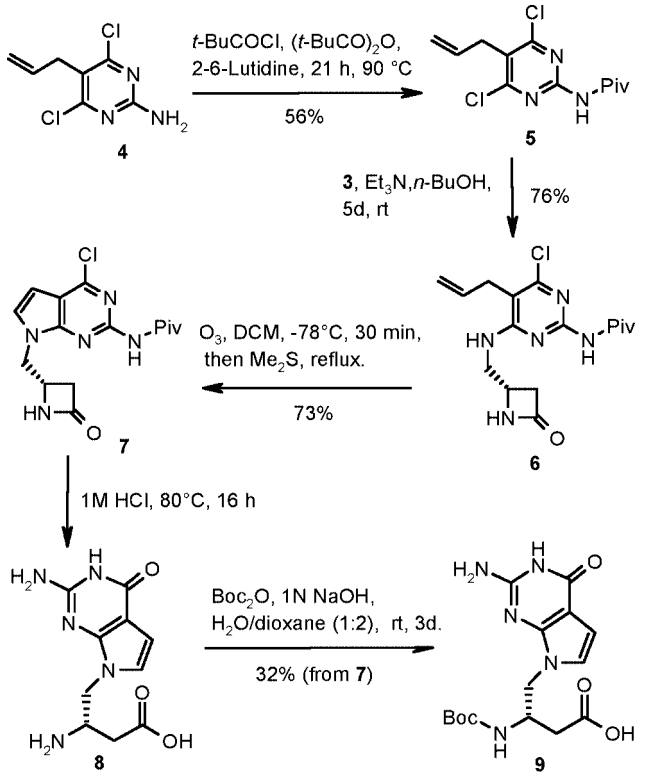
Results and Discussion

The synthetic approach to the ^{Z}G-modified nucleo-β^3-amino acid involves the direct substitution of a β-lactam with the nucleophilic nucleobase as the key step.[20] Therefore, the first attempts were based on the β-lactam mesylate **1** which is readily accessible from L-aspartic acid as reported by Salzmann et al.[21] Nevertheless, neither the β-lactam mesylate **1** nor the corresponding bromide was successfully substituted with 7-deazaguanine or 2-amino-6-chloro-7-deazapurine, the corresponding precursor, most likely due to a lack of nucleophilicity.

In a second approach, a pyrimidine derivative was attached to the β-lactam by nucleophilic aromatic substitution before the 7-deazaguanine was generated. The required β-lactam amine nucleophile **3** was obtained from the corresponding β-lactam mesylate **1** by conversion to the β-lactam azide **2** with NaN_3 in DMF followed by reduction with H_2 in the presence of Pd/C in almost quantitative yield (Scheme 1). The pyrimidine derivative was prepared starting from the readily accessible 5-allyl-4,6-dichloro-2-aminopyrimidine (**4**) which has already been reported as a ^{Z}G precursor by Legraverend et al. (Scheme 2).[22] The exocyclic amino group of heterocycle **4** was protected as the corresponding 2-pivaloylaminopyrimidine **5** by using *t*-BuCOCl/$(t\text{-BuCO})_2$O.[23] Nucleophilic aromatic substitution of pyrimidine **5** with the β-lactam amine **3** under mild conditions provided the ^{Z}G-β-amino acid precursor **6**. Oxidative cleavage of the terminal double bond by ozonolysis in DCM at –78 °C and subsequent cyclization furnished N^9-substituted 2-pivaloyl-6-chlorocarbapurine **7** in 73% yield. Finally, deprotection, conversion to the carboxylic functionality, and lactam ring-opening were performed in a one-pot transformation by first refluxing in 1 M HCl followed by basic β-lactam hydrolysis. After selective Boc protection of the aliphatic amine of amino acid **8** using Boc_2O, the desired nucleo-β-amino acid **9** was obtained in an overall yield of 32% from the lactam precursor **7**.



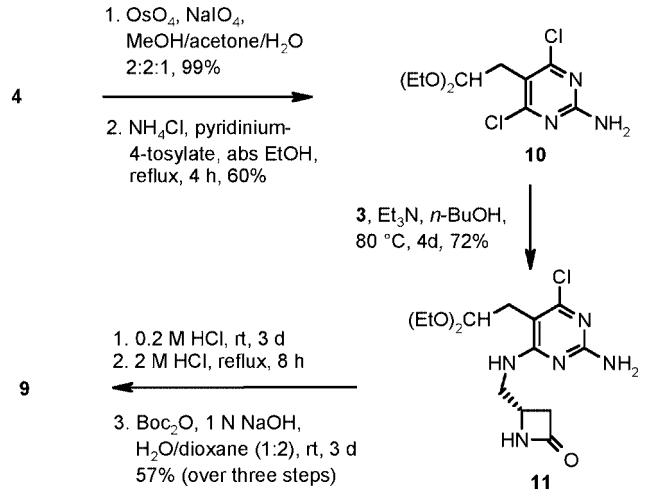
Scheme 1. Synthesis of β-lactam amine **3**.



Scheme 2. Synthesis of 7-deazaguaninyl-β-amino acid **9**.

As a result of the low overall yield for the preparation of β-nucleo-amino acid **9**, an alternative route to 7-deazaguanine without pivaloyl protection was investigated (Scheme 3). Dichloropyrimidine **4** was readily converted by oxidative double-bond cleavage with $OsO_4/NaIO_4$ to the corresponding aldehyde which was isolated as acetal **10**. Nucleophilic aromatic monosubstitution of the pyrimidine **10** with β-lactam amine **3** was carried out over 5 days at 80 °C to provide the β-lactam precursor **11** in 72% yield. Cyclization to the 7-deazaguanine was achieved under mild acidic conditions followed by carbonyl generation at the C-6 atom of the 7-deazaguanine under strong acidic conditions, base-mediated hydrolysis of the β-lactam ring, and Boc protection. The desired 7-deazaguaninyl-β-amino acid **9** was obtained in an overall yield of 57% over the last three steps. High

enantiomeric purity (*ee* > 99%) was indicated by HPLC analysis of the diastereoisomers obtained by coupling with Boc-L-Ala-OSu (see the Supporting Information).



Scheme 3. Synthesis of β-amino acid **9**: Alternative cyclization of the 7-deazaguanine.

Nucleo-β-amino acid **9** was used for the preparation of oligomers **13**, **15**, and **16** (Figure 2). Their sequential design was based on oligomers **14**, **17**, and **18**. Previously, exceptionally high stabilities were reported for these β-peptides indicating a high probability of aggregate formation.[11a] The constitutional design of the β-peptide oligomers was based on the 14-helical conformation with all recognition units oriented on one face of the helix. Therefore, the nucleo-β^3-amino acids were incorporated into every third position. The remaining two positions per turn were occupied by the helix-inducing *trans*-(1*R*,2*R*)-2-aminocyclohexanecarboxylic acid (ACHC) and β-homolysine for increased helix solubility under physiological conditions.[6b,9c]

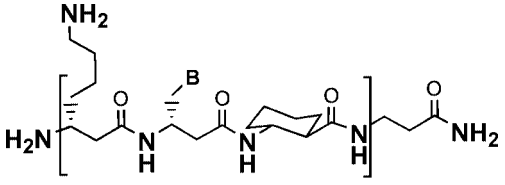


Figure 2. Nucleobase-modified β-peptide oligomers with 7-deazaguanine (^{Z}G) or guanine (G) incorporated.

Oligomers **13**, **15**, and **16** were prepared by solid-phase peptide synthesis on a 4-methylbenzhydrylamine-polystyrene (MBHA-PS) resin, coupling at 50 °C, and activation with 1-[bis(dimethylamino)methyliumyl]-1*H*-1,2,3-triazolo-[4,5-*b*]pyridine 3-oxide hexafluorophosphate (HATU), 1-hydroxy-7-azabenzotriazole (HOAt), and Hünig's base in DMF. Purification was performed by HPLC. The constitutional integrity of the oligomers was proven by high-resolution FT-ICR mass spectrometry.

Previously, it has been shown that β-peptide 14-helices modified with four nucleobases form highly stable double strands. The duplex formed between oligomer **14** (TGAT) and the complementary β-peptide ATCA (**12**) has a stability of T_m = 44 °C mainly due A-T and G-C base-pairing and to a high degree of preorganization of the helices with respect to the formation of the pairing complex.[11a] Surprisingly, the nucleobase-modified β-peptide **14** with the sequence TGAT also forms a stable self-aggregation (T_m = 32 °C) despite the self-complementarity of the sequence being missing (Figure 3).[11]

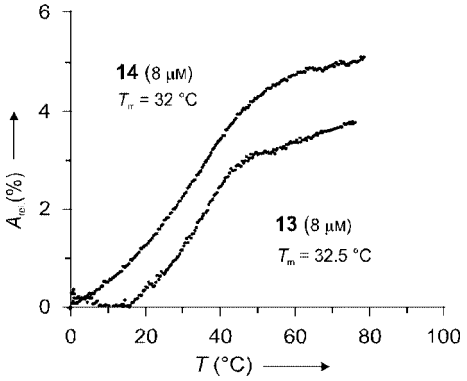


Figure 3. Temperature-dependent UV spectra of self-aggregating oligomers **13** (T^ZGAT) and **14** (TGAT).

The double-strand stabilities were determined from a correlation between the sigmoidal increase of absorption with rising temperature and the cooperative depairing of a nucleobase stack. Replacement of guanine by 7-deazaguanine was investigated in order to evaluate the possibility of greater aggregation over the Hoogsteen site and, furthermore, to characterize the G-C pairing mode. The double-strand formation of self-pairing β-peptide **13** with the 7-deazaguaninyl sequence T^ZGAT (T_m = 32.5 °C, Figure 3) was investigated as well as its interaction with the complementary oligomer **12** (T_m = 43 °C). Both double-strand stabilities were found to be quite similar to those obtained for the corresponding self-aggregation of 14-helix β-peptide **14** (Figure 3) and the equimolar complex formed between β-peptides **14** and **12** (see the Supporting Information). Therefore, we conclude that the Hoogsteen site of guanine is not involved in duplex formation in either case. This also implies that the Watson–Crick pairing mode is responsible for the G-C recognition in these nucleobase-modified β-peptide helices with linear backbone topology since the Hoogsteen mode, as the only alternative at neutral pH, would be suppressed in the ^{Z}G-C case.

The extraordinary high stability (T_m = 47.5 °C) of an equimolar mixture of oligomers **17** (CGC) and **18** (GCG) clearly seems to reflect a higher aggregation presumably with participation of the guanine Hoogsteen site.[24] Usually, three base pairs are not sufficient for double-strand formation. Therefore, the formation of higher aggregates by simultaneous pairing over the Watson–Crick and Hoogs-

teen sites needs to be considered. Consequently, suppression of the recognition between β-peptide helices **15** (C^ZGC) and **16** ($^ZGC^ZG$), which lack the N^7 hydrogen acceptor, was expected. Indeed, neither homodimer nor heterodimer formation based on nucleobase recognition of β-peptides **15** and **16** was observed (see the Supporting Information and Figure 4). Hydrogen bonding over the Hoogsteen site of guanine seems essential for the aggregate formation of oligomers **17** and **18** as is already known, for example, for guanine tetrad formation.[15,16]

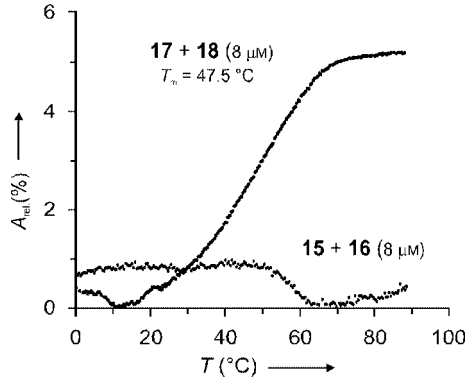


Figure 4. Temperature-dependent UV spectra of equimolar mixtures of oligomers **17** + **18** and **15** + **16**.

CD spectra of the 7-deazaguaninyl β-peptides **15** and **16** (see the Supporting Information) as well as of an equimolar mixture of β-peptides **15** and **16** (Figure 5) measured at various temperatures support the data obtained by temperature-dependent UV spectroscopy. There is a Cotton effect at around 260 nm indicating a preferred orientation of the nucleobases with respect to the helical backbone at lower temperatures. Nevertheless, the decrease in intensity with increasing temperature is continuous; there is no cooperativity as would be expected for the separation of a base stack. Furthermore, the helical content indicated by the Cotton effect at around 215 nm is of the same order as that known in comparable helices and diminishes parallel to the preferred nucleobase orientation with increasing temperature.

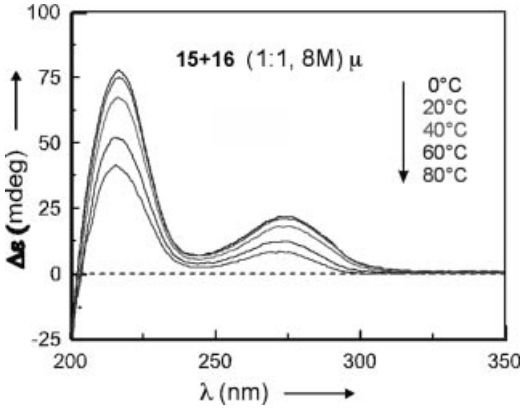


Figure 5. CD spectra of an equimolar mixture of oligomers **15** and **16** in Tris·HCl buffer (pH 7.5).

Conclusion

An efficient method for the preparation of an enantiomerically pure 7-deazaguaninyl-β^3-nucleo-amino acid has been presented based on β-lactam substitution of a suitable pyrimidine derivative followed by 7-deazaguaninyl ring-closure. This β-nucleo-amino acid was incorporated into β-peptide 14-helices in order to compare the recognition potential of the respective guaninyl-modified β-peptides. Two cases were determined in which the guanine Hoogsteen recognition site contributes to duplex stability. In double strands the overall topology seems to favor the Watson–Crick G-C pairing, whereas short sequences that are only stable as aggregates require the Hoogsteen site for stable complex formation. In future, the 7-deazaguaninyl-β-amino acid will allow the organization of β-peptide helices with increased base-pair specificity.

Experimental Section

General Remarks: All reagents were of analytical grade and used without further purification. Solvents were of the highest grade available. Dry solvents were purchased from Fluka and stored over molecular sieves (4 Å). (1*R*,2*R*)-Boc-ACHC-OH was prepared as described in the literature.[25] Boc-β-HLys(Z)-OH was obtained by Arndt–Eistert homologation of the corresponding α-amino acids.[9,26] Nucleo-β-amino acids Boc-β-HalA-OH, Boc-β-HalT-OH, Boc-β-HalG-OH, and Boc-β-HalC-OH were synthesized as described in the literature.[11,27] The 4-methylbenzhydrylamine-polystyrene (MBHA-PS) resin was obtained from Novabiochem. Analytical thin-layer chromatography (TLC) was performed on silica gel 60 F_{254} plates (Merck) and detection was carried out under UV light or by coloring with ninhydrin solution (3% in ethanol). Flash chromatography (FC) was performed using silica gel 60 (0.040–0.063 mm, Merck). Melting points were obtained with a Büchi-501 melting-point apparatus and are not corrected. Optical rotations were measured with a Perkin–Elmer 241 polarimeter. IR spectra were recorded with a Perkin–Elmer 1600 Series FT-IR spectrometer using either KBr pellets or as neat samples. NMR spectra were recorded with a Varian Unity 300, Varian Mercury 300, or Varian INOVA 600 instrument. Chemical shifts are referenced to the residual solvent peaks of [D_6]DMSO (^{1}H: δ = 2.49 ppm; ^{13}C: δ = 39.5 ppm) or $CDCl_3$ (^{1}H: δ = 7.26 ppm; ^{13}C: δ = 77.16 ppm). Mass spectra were recorded with a Finnigan LCQ spectrometer. High-resolution mass spectra were recorded with a Bruker APEX-IV FT-ICR mass spectrometer. HPLC analysis and purification of the oligomers was performed with a Pharmacia Äkta basic system (pump type P-900, variable wavelength detector of type UV-900) with a linear gradient of A (0.1% TFA in H_2O) to B [MeCN/H_2O (9:1) + 0.1% TFA]. Oligomers were purified using a YMC J'sphere column ODS-H80, RP-C18; 250 × 4.6 mm, 5 μm, 120 Å at a flow rate of 1 mL min^{-1} for analytical and 250 × 20 mm, 5 μm, 120 Å at a flow rate of 10 mL min^{-1} for preparative samples. CD spectra were recorded with a JASCO J-810 spectrometer equipped with a JASCO ETC-505S/PTC-423S temperature controller. All CD measurements were carried out in Tris·HCl buffer (pH 7.5) in a quartz cell of 1 cm path length. Spectra represent the average of five scans after baseline correction. Temperature-dependent UV spectra were measured with a JASCO V-550 UV/Vis spectrometer equipped with a JASCO ETC-505S/ETC-505T temperature controller. All measurements were carried out in Tris·HCl buffer at pH 7.5. The data

were collected at 260 nm at a heating rate of 0.5 °C min^{-1} in a quartz cell of 1 cm path length. The oligomer concentrations were determined based on the absorption at 260 nm measured at 80 °C assuming all nucleobases to be completely destacked at this temperature. The extinction coefficient of each oligomer was assumed to be the sum of the extinction coefficients of the contained nucleobases.[28]

General Procedure for Solid-Phase β-Peptide Synthesis: β-Peptide oligomers were synthesized by manual solid-phase peptide synthesis in a small fritted glass column (ø = 1.5 cm). 4-Methylbenzhydrylamine-polystyrene (MBHA-PS) resin was used preloaded with Boc-β-HGly-OH. Oligomers were synthesized on a 16.75 μmol scale using *N*-Boc-β-HGly-MBHA-PS resin (25.0 mg, 0.67 mmol g^{-1} loading); peptide coupling was performed at 50 °C. For each coupling reaction an excess of 5 equiv. amino acid (83.75 μmol) was used, pre-activated with 1-[bis(dimethylamino)-methyliumyl]-1*H*-1,2,3-triazolo[4,5-*b*]pyridine 3-oxide hexafluorophosphate (HATU) (4.5 equiv.), 1-hydroxy-7-azabenzotriazole (HOAt) (5 equiv. of a 0.5 M solution in DMF), and DIEA (14 equiv.) in dry DMF (400 μL). After swelling the loaded resin for 2 h in CH_2Cl_2 (2 mL), the following procedure was applied for each coupling step: 1) deprotection twice for 3 min with TFA/*m*-cresol (95:5, 2 mL); 2) washing five times with CH_2Cl_2/DMF (1:1, 2 mL), then five times with pyridine (2 mL); 3) coupling steps, 2 h gentle movement at 50 °C; 4) washing with CH_2Cl_2/DMF (1:1, 3 × 2 mL), DMF/piperidine (95:5, 3 × 2 mL), and then CH_2Cl_2/DMF (1:1, 3 × 2 mL); 5) capping twice for 3 min with DMF/Ac_2O/DIEA (8:1:1, 2 mL). After the final coupling step the resin was washed with TFA (3 × 2 mL) and CH_2Cl_2 (5 × 2 mL), and dried overnight in vacuo. The resin was transferred into a small glass vessel and suspended in *m*-cresol/thioanisole/ethanedithiol (2:2:1, 500 μL). After stirring for 30 min at room temperature, TFA (2 mL) was added and the mixture cooled to –20 °C. Trifluoromethanesulfonic acid (TFMSA) (200 μL) was added dropwise with vigorous stirring. The mixture was warmed to room temperature over 1.5 h and stirring continued for another 2 h. The mixture was filtered through a fritted glass funnel and TFA was removed under reduced pressure. The crude oligomer was isolated by precipitation from cold diethyl ether (–15 °C) and dried in vacuo. The crude peptide was dissolved in water/CH_3CN and purified by preparative HPLC.

(*S*)-4-Azidomethylazetidin-2-one (2): NaN_3 (2.17 g, 33.48 mmol) was added to a solution of β-lactam mesylate **1** (2.00 g, 11.16 mmol) in dry DMF (50 mL) under argon. The reaction mixture was stirred at 70 °C for 6 h. DMF was removed under reduced pressure and the crude product was purified by flash column chromatography (EtOAc/*n*-hexane, 8:2); the desired product **2** (1.31 g, 93%) was obtained as a colorless oil. R_f = 0.48 (EtOAc). $[\alpha]_D^{20}$ = +51.3 (*c* = 0.3, MeOH). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 2.75 (ddd, $^3J_{H,H}$ = 15, 2, 1 Hz, 1 H, α-CH_2), 3.09 (ddd, $^3J_{H,H}$ = 15, 2, 1 Hz, 1 H, α-CH_2), 3.42 (dd, $^3J_{H,H}$ = 13, 7 Hz, 1 H, γ-CH_2), 3.60 (dd, $^3J_{H,H}$ = 13, 7 Hz, 1 H, γ-CH_2), 3.79–3.85 (m, 1 H, β-CH), 6.74 (br. s, 1 H, NH) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 46.0, 48.5, 53.8, 167.3 ppm. IR (film): $\tilde{\nu}$ = 3259.7 (br), 2945.6 (w), 2106.0 (s), 1749.1 (s), 1285.4 cm^{-1}. MS (DCI): *m*/*z* = 144.1 [M + NH_4]$^+$, 270.1 [2M + NH_4]$^+$. HRMS: calcd. for $C_4H_6N_4O$: 126.0542; found 126.0542.

(*S*)-4-(Aminomethyl)azetidin-2-one (3): 10% Pd/C (800 mg) was added to a suspension of compound **2** (1.6 g, 12.6 mmol) in MeOH (50 mL) and the mixture stirred overnight under H_2. After completion of the reaction (monitored by TLC) the reaction mixture was filtered through a pad of Celite and concentrated in vacuo. The desired amine **3** (1.21 g, 96%) was obtained as a colorless oil and used without further purification. $[\alpha]_D^{20}$ = +6.0 (*c* = 0.1, MeOH). ^{1}H NMR (300 MHz, D_2O): δ = 2.68–2.76 (m, 1 H, α-CH_2), 2.81–2.94 (m, 2 H, γ-CH_2), 3.05–3.12 (m, 1 H, α-CH_2), 3.78–3.84 (m, 1 H) ppm. ^{13}C NMR (75 MHz, D_2O): δ = 40.5 (C-α or C-γ), 45.0 (C-α or C-γ), 51.8 (C-β), 173.0 (C=O) ppm. IR (neat): $\tilde{\nu}$ = 3357.6, 2949.1, 1734.4 (s), 1654.0, 1576.2, 1474.9, 1192.4 cm^{-1}. HRMS: calcd. for $C_4H_8N_2O$: 100.0637; found 100.0637.

***N*-(5-Allyl-4,6-dichloropyrimidin-2-yl)-2,2-dimethylpropionamide (5):** Pivaloyl anhydride (27 mL) was added to a mixture of pyrimidine **4** (1.0 g, 4.90 mmol), pivaloyl chloride (2.36 g, 19.6 mmol), and 2,6-lutidine (2.10 g, 19.6 mmol) under argon. The mixture was heated at 90 °C for 21 h. The solvent was evaporated under reduced pressure and the residue was purified by silica gel column chromatography (DCM) to give **5** (789 mg, 56%) as a colorless solid. R_f = 0.39 (DCM). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 1.28 (s, 9 H, *t*Bu), 3.52–3.58 (m, 2 H, CH_2CH-*CH*$_2$R), 5.00–5.14 (m, 2 H, *CH*$_2$CHCH_2R), 5.73–5.88 (m, 1 H, CH), 8.07 (br. s, 1 H, NH) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 27.2 (*t*Bu), 33.5 (CH_2), 40.3 (*t*Bu), 117.5, 124.8, 131.2 (CH), 154.5, 162.3, 175.3 (C=O) ppm. MS (ESI): *m*/*z* = 310.2 [M + Na]$^+$, 598.9 [2M + Na]$^+$.

***N*-{5-Allyl-4-chloro-6-[(S)-4-oxoazetidin-2-ylmethylamino]pyrimidin-2-yl}-2,2-dimethylpropionamide (6):** β-Lactam amine **3** (189 mg, 1.89 mmol) and dry NEt_3 (205 μL, 1.47 mmol) were added to a solution of compound **5** (212 mg, 0.736 mmol) in dry DMF (10 mL). The mixture was stirred for 5 days at room temperature under argon. The reaction mixture was concentrated in vacuo and the residue was purified by flash column chromatography (DCM/MeOH, 96:4) to give the pure product **6** (196 mg, 76%) as a colorless solid. R_f = 0.29 (DCM/MeOH, 95:5). ^{1}H NMR (300 MHz, [D_6]DMSO): δ = 1.19 (s, 9 H, *t*Bu), 2.62–2.74 (m, 1 H, α-CH_2), 2.82 (dd, $^3J_{H,H}$ = 15, 4 Hz, 1 H, α-CH_2), 3.22–3.40 (m, 3 H, β-CH, CH_2CH-*CH*$_2$R), 3.68–3.83 (m, 2 H, γ-CH_2), 4.93–5.06 (m, 2 H, *CH*$_2$CHCH_2R), 5.72–5.88 (m, 1 H, CH_2*CH*CH_2R), 7.31 (m, 1 H, NH), 7.87 (s, 1 H, NH), 9.74 (s, 1 H, NH) ppm. ^{13}C NMR (75 MHz, [D_6]DMSO): δ = 26.7 (*t*Bu), 29.4 (CH_2CH*CH*$_2$R), 40.2, 43.3, 46.0 (C-β), 106.9, 115.5, 132.9 (CH_2*CH*CH_2R), 154.9, 156.9, 162.3, 166.5, 176.4 (C=O) ppm. HRMS: calcd. for $C_{16}H_{22}ClN_5O_2$: 351.1462; found 351.1462.

***N*-{4-Chloro-7-[(*S*)-4-oxoazetidin-2-ylmethyl]-7*H*-pyrrolo[2,3-*d*]pyrimidin-2-yl}-2,2-dimethylpropionamide (7):** Compound **6** (40.0 mg, 0.114 mmol) was suspended in dry DCM (20 mL) and cooled to –78 °C under argon. O_3 was slowly passed through the solution for 30 min. The reaction mixture was diluted in DCM (10 mL) and then Me_2S (1 mL) was added. The mixture was refluxed for 1 h. The solvent was removed in vacuo and the residue was purified by silica gel column chromatography (EtOAc/MeOH, 98:2) to afford the 7-deazapurinyl product **7** (28.1 mg, 73%) as a colorless solid. R_f = 0.21 (EtOAc/MeOH, 98:2). ^{1}H NMR (300 MHz, [D_6]DMSO): δ = 1.24 (s, 9 H, *t*Bu), 2.73 (m, 1 H, α-CH_2), 2.96 (m, 1 H, α-CH_2), 3.88–3.96 (m, 1 H, β-CH), 4.39 (m, 2 H, γ-CH_2), 6.57 (d, $^3J_{H,H}$ = 4 Hz, 1 H, 7-H), 7.60 (d, $^3J_{H,H}$ = 4 Hz, 1 H, 8-H), 8.20 (s, 1 H, NH), 10.2 (s, 1 H, NH) ppm. ^{13}C NMR (75 MHz, [D_6]DMSO): δ = 26.8 (*t*Bu), 41.1 (C-α), 46.1 (C-β), 48.9 (C-γ), 98.6 (C-7), 113.3 (C-5), 130.8 (C-8), 150.5, 151.3, 151.7, 166.3 (C=O), 176.1 (C=O) ppm. MS (ESI): *m*/*z* = 358.3 [M + Na]$^+$, 693.0 [2M + Na]$^+$. HRMS: calcd. for $C_{15}H_{18}ClN_5O_2$: 335.1149; found 335.1149.

(*S*)-4-[2-Amino-6-chloro-5-(2,2-diethoxyethyl)pyrimidin-4-ylaminomethyl]azetidin-2-one (11): β-Lactam amine **3** (185 mg, 1.85 mmol) and NEt_3 (1.5 mL) were added to a solution of pyrimidine **10** (316 mg, 1.22 mmol) in *n*BuOH (40 mL) under argon. The reaction

mixture was heated to 80 °C and stirred for 4 days under an inert atmosphere. The reaction mixture was then cooled and the solvent removed under reduced pressure. The residue was purified by silica gel flash chromatography (EtOAc/*n*-hexane, 9:1) to yield the desired product **11** (302 mg, 72%) as a pale yellow oil. R_f = 0.18 (EtOAc). $[\alpha]_D^{20}$ = +17.3 (*c* = 0.075, MeOH). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 1.14 (t, $^3J_{H,H}$ = 7 Hz, 6 H, OEt), 2.66 (ddd, $^3J_{H,H}$ = 15, 2, 1 Hz, 1 H, α-CH_2), 2.74 [d, $^3J_{H,H}$ = 5.4 Hz, 2 H, CH_2–CH(OEt)$_2$], 2.99 (ddd, $^3J_{H,H}$ = 15, 2, 1.8 Hz, 1 H, α-CH_2), 3.42–3.52 (m, 4 H, OEt), 3.64–3.71 (m, 2 H, γ-CH_2), 3.74–3.80 (m, 1 H, β-CH_2), 4.44 [t, $^3J_{H,H}$ = 5.4 Hz, 1 H, C*H*(OEt)$_2$], 5.09 (s, 2 H, NH_2), 6.23 (t, $^3J_{H,H}$ = 5.8 Hz, 1 H, NH), 6.65 (s, 1 H, NH) ppm. ^{13}C NMR (75 MHz, $CDCl_3$): δ = 15.2 (OEt), 32.9 (CH_2), 41.5 (C-γ), 44.8 (C-α), 47.2 (C-β), 63.5 (OEt), 63.5 (OEt), 100.8, 103.6 (CH), 158.6, 160.5, 164.1, 167.9 (C=O) ppm. IR (neat): $\tilde{\nu}$ = 3404.2 (s), 2973.6 (w), 1746.0 (s), 1568.1 (s), 1480.2, 1368.3, 1111.2, 1057.8 cm^{-1}. MS (ESI): *m*/*z* = 344.2 [M + H]$^+$, 708.9 [2M + Na]$^+$. HRMS (ESI): calcd. for $C_{14}H_{23}ClN_5O_3$: 344.1484; found 344.1483 [M + H]$^+$.

(*S*)-4-(2-Amino-4-oxo-3,4-dihydropyrrolo[2,3-*d*]pyrimidin-7-yl)-3-*tert*-butoxycarbonylaminobutyric Acid (9). Method A: A solution of β-lactam **7** (18.0 mg, 53.6 mmol) in 1 M HCl (5 mL) was heated for 16 h at 80 °C. The solvent was removed in vacuo and the residue was neutralized with 1 M NaOH and lyophilized. After addition of dioxane/H_2O/1 M NaOH (2:1:1, 5 mL) and Boc_2O (23.4 mg, 107 mmol), stirring was continued overnight. An additional portion of Boc_2O (23.4 mg, 107 mmol) was added followed by a small portion of 1 M NaOH to adjust the pH value to 10. Stirring was continued for another 2 days. The reaction mixture was neutralized with 1 M HCl and lyophilized. The residue was applied to a RP silica gel column and eluted first with water and then with water/methanol (9:1). After freeze-drying the desired product **9** (6.10 mg, 32%) was obtained as a white solid.

Method B: β-Lactam **11** (302 mg, 0.88 mmol) was suspended in 0.2 M HCl (15 mL) and EtOH (15 mL). The mixture was stirred at room temperature for 3 days unless all starting material had disappeared (monitored by TLC). After removal of the solvents, the residue was suspended in 2 M HCl (20 mL) and refluxed for 8 h. The reaction mixture was neutralized with ammonium hydroxide and then lyophilized. The solid residue was suspended in dioxane/H_2O (2:1, 20 mL). Then 1 M NaOH (5 mL) was added. The solution was stirred for 2 days at room temperature and then Boc_2O (600 mg, 2.75 mmol) was added. The mixture was stirred for another 3 days at room temperature maintaining the solution at pH 9 by the addition of 1 M NaOH. The reaction mixture was neutralized by 1 M HCl and lyophilized. The residue was first purified by silica gel flash chromatography (EtOAc/MeOH, 4:1, 1% AcOH) and then by RP column chromatography (15% MeOH in H_2O) to yield the desired amino acid **9** (178 mg, 57%) as a white solid. R_f = 0.73 (EtOAc/MeOH/H_2O, 80:14:6, 3% AcOH); m.p. 263–265 °C. $[\alpha]_D^{20}$ = +24.35 (*c* = 0.115, MeOH). ^{1}H NMR (600 MHz, [D$_6$]DMSO): δ = 1.11 (s, 1.5 H, *t*Bu rotamer), 1.28 (s, 7.5 H, *t*Bu), 2.05–2.11 (m, 2 H, α-CH_2), 3.94–4.00 (m, 3 H, β-CH and γ-CH_2), 6.14 (d, $^3J_{H,H}$ = 1.8 Hz, 1 H, 7-H), 6.33 (s, 2 H, NH_2), 6.58–6.60 (m, 1 H, 8-H), 6.87 (s, 1 H, NH), 10.67 (br. s, 1 H, COOH or aromatic NH) ppm. ^{13}C NMR (150 MHz, [D$_6$]DMSO): δ = 28.1 (*t*Bu), 40.0 (C-α), 47.3 (C-γ), 48.2 (C-β), 77.4 (*t*Bu), 99.8, 100.6 (C-7), 120.6 (C-8), 150.4, 152.4, 154.6, 158.8 ppm. IR (KBr): $\tilde{\nu}$ = 3426.3 (s), 2934.3, 1667.7 (s), 1404.4, 1169.2 cm^{-1}. MS (ESI): *m*/*z* = 352.2 [M + H]$^+$, 374.1 [M + Na]$^+$. HRMS (ESI): calcd. for $C_{15}H_{22}N_5O_5$: 352.1616; found 352.1613 [M + H]$^+$.

H_2N-(β-HLys-β-HalT-ACHC-β-HLys-β-Hal^{z}G-ACHC-β-HLys-β-HalA-ACHC-β-HLys-β-HalT-ACHC-β-HGly)-$CONH_2$ (13): Analytical RP-HPLC: t_R = 24.17 min (gradient: 22–45% B in 30 min). MS (ESI): *m*/*z* (%) = 1014.5 (100) [M + 2H]$^{2+}$. HRMS (ESI): calcd. for $C_{96}H_{153}N_{31}O_{18}$: 1014.0999; found 1014.0999 [M + 2H]$^{2+}$.

H_2N-(β-HLys-β-HalC-ACHC-β-HLys-β-Hal^{z}G-ACHC-β-HLys-β-HalC-ACHC-β-HGly)-$CONH_2$ (15): Analytical RP-HPLC: t_R = 17.31 min (gradient: 18–42% B in 30 min). MS (ESI): *m*/*z* (%) = 756.8 (100) [M + 2H]$^{2+}$. HRMS (ESI): calcd. for $C_{71}H_{116}N_{24}O_{13}$: 756.4571; found 756.4568 [M + 2H]$^{2+}$.

H_2N-(β-HLys-β-Hal^{z}G-ACHC-β-HLys-β-HalC-ACHC-β-HLys-β-Hal^{z}G-ACHC-β-HGly)-$CONH_2$ (16): Analytical RP-HPLC: t_R = 20.88 min (gradient: 18–37% B in 30 min). MS (ESI): *m*/*z* (%) = 776.2 (100) [M + 2H]$^{2+}$. HRMS (ESI): calcd. for $C_{73}H_{117}N_{25}O_{13}$: 775.9626; found 775.9625 [M + 2H]$^{2+}$.

Supporting Information (for details see the footnote on the first page of this article): Temperature-dependent UV and CD spectra of oligomers **12**–**16** in their respective pairing complexes. ^{1}H NMR spectra of compounds **6**, **7**, **9**, and **11** and the HPLC profile of the enantiomeric excess determination of nucleo-amino acid **9**.

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