Novel Antibiotics for the Treatment of Gram-Positive Bacterial Infections

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The natural dipeptide antibiotic TAN 1057 A,B displays excellent antibacterial activity against staphylococci including methicillin resistant *Staphylococcus aureus*. However, the in vitro activity against additional Gram-positive strains, in particular pneumococci and *Enterococcus faecalis*, proved to be considerably lower. We report the synthesis and pharmacological evaluation of new derivatives of this natural product that displayed increased antibacterial potency against staphylococci and were also active against pneumococci. In particular, the analogues bearing modified β-homoarginine side chains with methylated guanidine moieties were shown to be significantly more potent than the natural product TAN 1057 A,B.

Introduction

The emergence and spread of bacterial resistance represent a severe global problem.[1] The escalating resistance has led to the appearance of multiresistant staphylococci, enterococci, and pneumococci in nosocomial- and community-acquired infections. Therefore, there is an urgent need for novel chemical entities that are particularly effective against Gram-positive pathogens including the multiresistant strains.

Natural products have always been a valuable source for the identification of new antibacterial entities.[2] A natural antibiotic that has received only moderate attention in the past years is the dipeptide TAN 1057 A,B (**1**) (Figure 1), which was first isolated and characterized by researchers at the Takeda company.[3] The natural product is an inhibitor of the bacterial translation,[3a] and it displays excellent minimal inhibitory concentrations (MIC) against staphylococci including methicillin resistant *Staphylococcus aureus* strains (MRSA).[3]

Moreover, a promising in vivo activity against *S. aureus* was observed in a murine sepsis model after ip or sc administration of the natural product.[3] However, it proves to be less active in vitro against *Enterococcus feacalis* and pneumococci.[3a] Our own results as depicted in Table 2 confirm these results for TAN 1057 A,B.[4] Additionally, TAN 1057 A,B suffers from toxic side effects (LD_{50} in the mouse: 50 mg/kg, ip and 100 mg/kg, iv),[3] which are in our view prohibitive for the therapeutic use in humans.

The promising antibiotic activity and the unique molecular architecture of TAN 1057 A,B prompted us to launch a chemical optimization program.[5] Our goal was to identify analogues that are better tolerated and display improved antibiotic activity toward a broader panel of Gram-positive pathogens including enterococci and pneumococci.

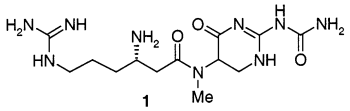

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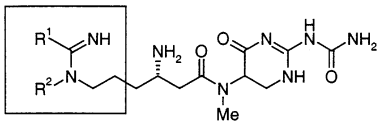
Figure 1. Natural dipeptide antibiotic TAN 1057 A,B.

Table 1. MIC Values against *S. aureus 133* and IC_{50} Values for Prokaryotic Translation for Compounds **4a**−**c**, **8a**−**b**, **14**, and **18**



compd	R^1	R^2	MIC, *S. aureus 133* (μg/mL)	IC_{50}, prokaryotic translation (μM)
4a	MeNH	H	0.8	0.5
4b	EtNH	H	6.3	12.5
4c	*i*-PrNH	H	12.5	12.5
8a	Me	H	0.2	0.1
8b	*i*-Pr	H	3.2	not determined
14	H_2N	Me	0.025	0.06
18	MeNH	Me	0.4	0.4

Chemistry

Two independent total syntheses of TAN 1057 A,B have been disclosed to date[6] setting the stage for a facile construction of analogues. Primarily, we focused our attention on alterations of the β-amino acid side chain with a special emphasis on modified guanidine derivatives. In this regard, a convergent synthesis strategy that allowed the introduction of a modified guanidine or amidine at a late stage in the process was elaborated. As illustrated in Scheme 1, the synthesis commenced with orthogonally protected β-lysine **2**,[7] which was coupled with the heterocyclic core **3**[8] using a cocktail of water soluble *N*-(3-dimethylaminopropyl)-*N*′-ethylcarbodiimide (EDC) and *N*-hydroxybenzotriazole (HOBt) as the coupling reagent. The coupling product from this reaction could conveniently be purified by crystallization. Subsequently, the BOC group was removed under acidic conditions to yield the amine hydrochloride salt **5** in quantitative yield.

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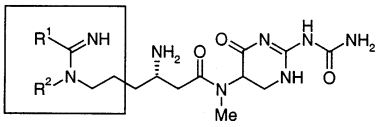
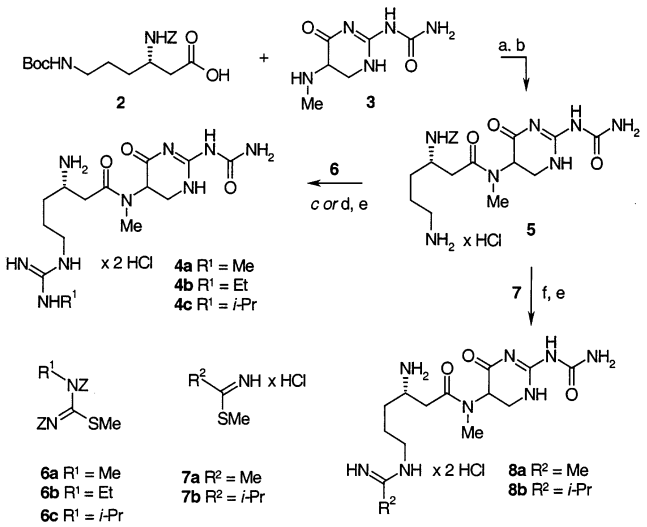
Table 2. MIC (μg/mL) for TAN 1057 A,B and Its Analogues **4a**, **8a**, **14**, and **18** against a Panel of Gram-Positive Pathogens



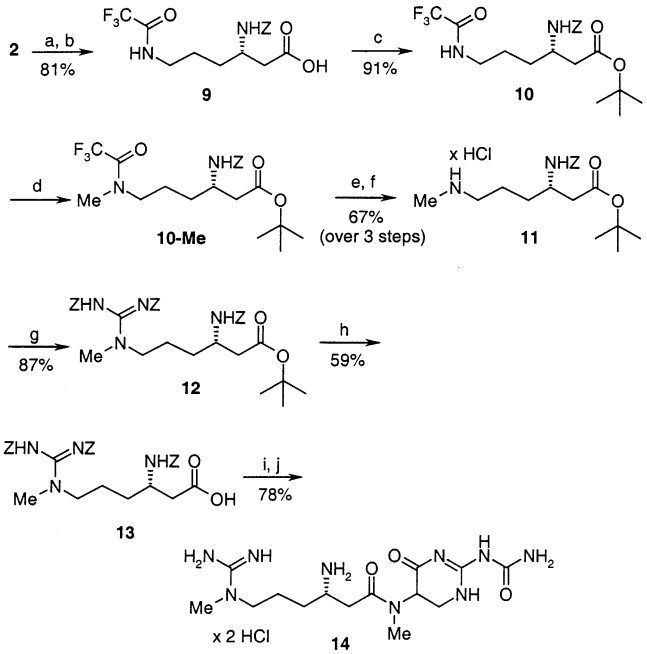
	compd									
	TAN-1057		**4a**		**8a**		**14**		**18**	
	R^1	R^2	R^1	R^2	R^1	R^2	R^1	R^2	R^1	R^2
bacterial strains	H	H	MeNH	H	Me	H	H_2N	Me	MeNH	Me
S. aureus 133	0.25		0.8		0.2		0.025		0.5	
S. epidermis 18570	0.25		0.13		0.2		0.12		0.5	
Str. Pyogenes Wacker	8		4		1.0		0.5		8	
E. faecium L4001	2		16		16		2		8	
E. faecalis 18531	>16		64		>64		64		>64	
S. pneumoniae G9a	16		16		16		4		32	

Scheme 1[a]



[a] Reagents and conditions: (a) EDC, HOBt, 60%. (b) 4 M HCl in dioxane, quant. (c) Compound **6a**, diisopropyl ethylamine (DIPEA), 50%. (d) Compound **6b** or **6c**, $HgCl_2$, Et_3N, DMF, 36−51%. (e) $PdCl_2$, H_2, MeOH, 82−100%. (f) Compound **7**, DIPEA, 44−57%.

Scheme 2[a]



[a] Reagents and conditions: (a) 4 M HCl in dioxane, 100%. (b) CF_3CO_2Me, MeOH, Et_3N, 81%. (c) *t*-BuOAc, 70% aqueous $HClO_4$. (d) MeI, Cs_2CO_3, DMF, 80 °C, 50 h or K_2CO_3, TBAB, acetone, reflux, 100 h, 97%. (e) 1 M LiOH, MeOH, 0 °C, 95%. (f) Ether, 4 M HCl in dioxane, 73%. (g) *N*,*N*′-Bis-*Z*-1-amidinopyrazole, DIPEA, CH_2Cl_2, reflux. (h) TFA, Et_3SiH, CH_2Cl_2, 0 °C. (i) Compound **3**, HATU, DIPEA, DMF, 82%. (j) $H_2/PdCl_2$, MeOH, 95%.

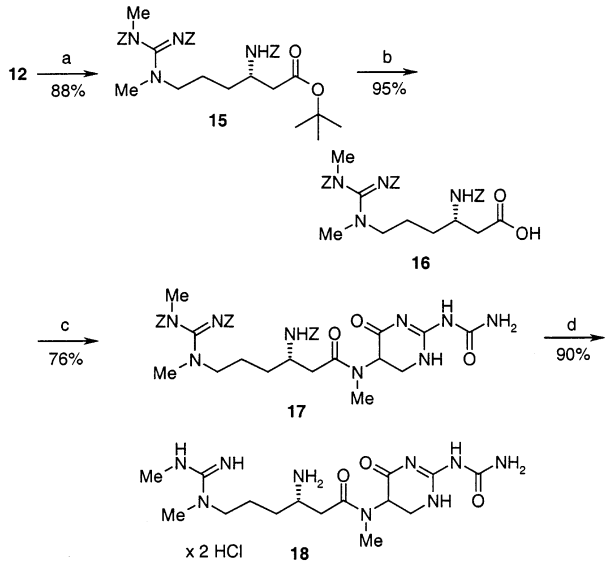
The methylguanidine moiety was installed by treatment of **5** with *N*-methylated bis-*Z*-*S*-methylisothiourea **6a** in the presence of an excess of diisopropylethylamine at 60 °C.[9] The yield for this transformation was moderate (50%) due to the inherent instability of the heterocycle toward basic reaction conditions at elevated temperatures. For the use of sterically more encumbered *S*-methylthioureas **6b**,**c**, the reaction even failed to furnish the corresponding guanidines. In these cases, the use of $HgCl_2$ as promoter for the guanylation proved to be beneficial, and the desired intermediates were obtained in acceptable yields (36% for **6b** and 51% for **6c**).[10] At the final step of the synthesis, all *Z* groups were removed by hydrogenation in MeOH using $PdCl_2$, and the target molecules **4a**−**c** were obtained as a 1:1 mixture of diastereomers in good yields (82−100%).[11]

In close analogy to the derivatives with substituted guanidine moieties, the corresponding amidine derivatives of TAN 1057 were prepared starting from **5** using *S*-methylthioamides as electrophiles. The amidine formation as depicted in Scheme 1 worked with reasonable yields, and the resulting intermediates were deprotected to provide the target molecules **8a**,**b** in 47 and 30% yield (for two steps), respectively.

In TAN 1057 A,B, the β-homoarginine side chain represents a key structural element. A related β-amino acid motif is found in the naturally occurring antibiotic Blasticidin S, which bears a N^{δ}-methyl-β-arginine side chain and acts as an inhibitor of protein synthesis.[12] In analogy, we considered decorating TAN 1057 A,B with an N^{ϵ}-methyl-β-homoarginine amino acid fragment exploiting the structural and functional resemblance with Blasticidin. For the synthesis of such a arginine analogue with a methyl group on the proximal guanidine nitrogen, the initial strategy had to be extended (Schemes 2 and 3).[13]

Starting from the same orthogonally protected β-lysine **2**,[7] the terminal *tert*-butoxycarbonyl protecting group was changed to a trifluoroacetyl residue,[14] and

Scheme 3[a]



[a] Reagents and conditions: (a) MeI, K_2CO_3, Bu_4NBr, acetone, 60 °C, 2 d. (b) TFA, Et_3SiH, CH_2Cl_2, 0 °C. (c) Compound **3**, HATU, DIPEA, DMF. (d) $H_2/PdCl_2$, MeOH.

subsequently, the carboxyl function in compound **9** was blocked as a *tert*-butyl ester. For the latter step, several methods were tried out, but reasonably good yields (81%) were achieved only upon treatment of the acid with *tert*-butyl acetate in the presence of 70% aqueous $HClO_4$.[15] Total conversion of the starting material **9** was achieved when this operation was performed twice, and the reisolated unreacted acid was recycled. The trifluoroacetylamino group in the protected β-amino acid ester **10** was selectively methylated by treatment with methyl iodide in the presence of either Cs_2CO_3 in dimethyl formamide (DMF) at 80 °C or K_2CO_3 in refluxing acetone.[16] Both procedures gave good yields of the crude product **10**-Me; however, an impurity containing a N^{β}-MeZ-group resulting from 2-fold methylation was sometimes detected (up to 7%). Because **10**-Me was obtained as an oil, an additional purification step was necessary. Subsequently, the trifluoroacetyl group was removed and the amino ester **11** was converted into its hydrochloride salt, which was purified by recrystallization. An impurity containing two methyl groups was easily removed by a single crystallization. Several guanidinylation agents were scrutinized to obtain a high yield of the protected guanidino ester **12**. In our hands, the best yield (87%) was achieved with *N*,*N*′-bis-*Z*-1-amidinopyrazole[17] in dichloromethane in the presence of $(i\text{-Pr})_2NEt$ under reflux. Subsequently, liberation of the carboxylic acid was performed under mild conditions employing trifluoroacetyl (TFA) and Et_3SiH. The latter reagent has been reported to facilitate the cleavage of *tert*-butyl esters.[18] Indeed, without addition of Et_3SiH, one of the two terminal *Z* groups was partially cleaved off by TFA. Finally, tris-*Z*-N^{ϵ}-methyl-β-(*S*)-arginine **13** was coupled with the heterocycle **3** of TAN-1057 A,B, and after it was deprotected, the dipeptide **14** was obtained. Its structure was confirmed by NMR spectroscopy. Unequivocal assignment of all signals, including those of the quaternary carbon atoms, was based on the regularities found in the two-dimensional (2D) ^{13}C-^{1}H correlation spectroscopy (COSY) and COLOC spectra.

Compound **12** was further used as a precursor for ester **15** with two methyl groups on the guanidine moiety (Scheme 3). A Mitsunobu protocol may be used for the methylation of the urethane-protected NH groups in guanidines.[19] It turned out, however, that the separation of the resulting ester **15** from the byproduct triphenylphosphine oxide by chromatography proved to be tedious, and it was difficult to obtain the ester **15** in a pure form. Therefore, the previously described approach for the methylation of trifluoroacetylamine **10**, namely, a 2.3-fold excess of MeI and 2 equiv of K_2CO_3 in the presence of catalytic amounts of TBAB in anhydrous acetone, was used. In this case, the methylation reaction proceeded faster than for compound **10**, and the reaction was complete in 2 days. The ester **15** was purified by chromatography and deprotected, and the acid **16** was coupled with heterocycle **3**. Finally, hydrogenolytic cleavage of the *Z* groups from the coupling product **17** delivered the double-methylated guanidine derivative **18** in 65% yield.

Results and Discussion

All synthesized TAN 1057 A,B analogues were tested for their antibacterial properties employing a prokaryotic translation assay (cf. Table 1). In this regard, we have used a Gram-negative transcription−translation assay based on *Escherichia coli* since a well-established Gram-positive in vitro translation assay is not available. Nevertheless, we consider our IC_{50} values in Table 1 as meaningful because the translation machineries of Gram-positive and Gram-negative bacteria have been highly conserved during evolution as indicated by the large number of available broad spectrum translation inhibitors.

Furthermore, we determined the MIC values of all TAN 1057 A,B analogues against *S. aureus 133*.

In general, the data presented in Table 1 show that a good activity in the translation assay translates into a good MIC for the given compound. In turn, less active inhibitors of the bacterial translation display a reduced efficacy in the whole cell MIC assay. From these observations, we conclude that antibacterial activity of our analogues refers to an inhibition of the bacterial translation confirming the results reported by the Takeda group for TAN 1057 A,B.[3a]

More important, a comprehensive structure−activity relationship (SAR) can be extracted from the data shown in Table 1. When a substituent is introduced at the terminal guanidine nitrogen, the antibacterial activity decreases rapidly with the size of the substituent. Merely the terminal methylation led to a highly active analogue **4a**, whereas for compounds bearing slightly larger groups such as ethyl (**4b**) or isopropyl (**4c**) a drastic loss in activity was observed.

Interestingly, the guanidine moiety can be replaced by an amidine without loss of the biological activity (compounds **8a**,**b**). Thus, a guanidine is not essential for activity and may be replaced by other basic surrogates. In parallel to the SAR of the guanidine derivatives, lower activities were found for substrates with sterically more demanding groups such as isopropyl in the amidine series (compound **8b**).

The most active analogues were those with a methyl group at the proximal guanidine nitrogen. Accordingly,

compounds **14** and **18** effectively inhibit the bacterial translation process and, moreover, they exhibit excellent MIC values against *S. aureus 133*. For further assessment, the most active TAN 1057 A,B derivatives were tested for their activity toward a broader panel of Gram-positive pathogens. In this regard, MIC values against a range of Gram-positive bacteria including *Enterococcus faecium*, *E. faecalis*, and *Streptococcus pneumoniae* were recorded (Table 2).

Derivatives **4a**, **8a**, and **18** showed similar antibiotic activity against Gram-positive bacteria as the natural product **1**. In contrast, analogue **14** bearing a methyl group at the proximal guanidine nitrogen displayed significantly improved in vitro potency. In comparison to the natural product, analogue **14** was superior in the overall antibiotic activity, and more important, a drastic increase in the in vitro potency against *S. pneumoniae* was observed. Finally, compound **14** was tested in vivo using a murine *S. aureus* sepsis model and an excellent ED_{100} of ≤0.5 mg/kg (route iv) was found underscoring the high antibacterial potency of derivative **14**.

Conclusion

A new class of antibiotics based on the natural dipeptide antibiotic TAN 1057 A,B have been made available by novel synthetic routes leading to analogues with alterations at the terminal guanidine moiety. In the course of this program, derivatives of the natural product that exhibit good to excellent in vitro and in vivo efficacy against a broad panel of Gram-positive pathogens were identified. In particular, compound **14** was found to be a promising new candidate for the treatment of bacterial infections caused by Gram-positive pathogens. Further in-depth pharmacological characterization of **14** is being conducted.

Experimental Section

Pharmacology. Prokaryotic Translation Assay. The inhibition of the bacterial translation was assayed by using an *E. coli* transcription−translation assay with a luciferase reporter as read out based on published and commercially available methods.[20]

MIC Determination. The MICs were determined by the microdilution method using AOAC broth (Difco) supplemented with 4% dextrose (staphylococci, enterococci, and *Streptococcus pyogenes*) or 1:10 diluted BHI broth (Oxoid) supplemented with 10% bovine serum (*S. pneumoniae*). Microtiter plates containing the test compounds (concentration range: 0.125−64 μg/mL) were inoculated with 1:50 (streptococci, enterococci) or 1:500 (staphylococci) dilutions of overnight cultures of the test bacteria. Plates were incubated in the presence of 8−10% CO_2 at 37 °C and read after approximately 20 h. The MIC was considered to be the first concentration with no visible growth.

Systemic Infection with *S. aureus 133*. Staphylococci from an overnight culture were grown to the logarithmic growth phase in BHI broth (Oxoid). The log-culture was precipitated and washed twice with phosphate-buffered saline. Bacteria were suspended in phosphate-buffered saline containing 5% mucin to a cell density of 4×10^6 cells/mL. Female CFW1-mice (20 g) were infected with 0.25 mL of the bacterial suspension. The test compounds were dosed iv at 30 min postinfection. The survival in each treatment group was monitored for 6 days.

Chemistry. General. Melting points (uncorrected) were determined in capillaries using an apparatus manufactured by Büchi. Routine NMR spectra were recorded using Bruker AM 250 (250 MHz), Varian UNITY 300 (300 MHz), Bruker DRX400, or Bruker AC500 instruments. All spectra were calibrated against tetramethylsilane as an internal standard ($\delta = 0$) or the signals of the residual protons of deuterated solvents: 7.26 for $CHCl_3$, 2.50 for [D_6]DMSO, and 3.30 for [D_4]-MeOH. Coupling constants (J) are given in Hz. Electron impact mass spectrometry (EI-MS) were recorded on Finnigan MAT 95 and Varian CH 5 spectrometers (70 eV). High-resolution MS (HRMS) were aquired on a Micromass LCT. High-performance liquid chromatography (HPLC)−MS spectra were recorded on a Hewlett-Packard 1100 (Micromass Platform LCZ); UV detection was at 210 nm. HPLC-MS parameters: column symmetry C18, 50 mm × 2.1 mm, 3.5 μm; eluent A, MeCN + 0.1% HCOOH; B, 0.3 g of 30% aqueous HCl in 1 L of H_2O; 0−4 min, from 10 to 90% A; 4−6 min, 90% A, 0.5 mL/min, 40 °C. HPLC, method A: Kromasil RP-18, 125 mm × 4 mm, eluent A: heptanesulfonic acid (WAT 084282 Fa. Waters) low UV, 4 bottles/L H_2O; B: MeCN; gradient, 0−1 min 90% A; 1−9 min from 90% A to 90% B; 9−13 min 90% B; 2 mL/min; room temperature. HPLC, method B: Kromasil RP-18, 50 mm × 2.1 mm, eluent A: MeCN + 0.1% HCOOH; B: H_2O + 0.1% HCOOH; gradient, 0−4 min from 90% B to 90% A; 4−6 min 90% A; 0.5 mL/min; 40 °C. HPLC, method C: Kromasil RP-18, 60 mm × 2.1 mm, eluent A: H_2O + 5 mL $HClO_4$/L; B: MeCN; gradient, 0−4.5 min from 98% A to 90% B; 4.5−6.5 min 90% B; 0.75 mL/min; 30 °C. Capillary electrophoresis (CE) was performed on an ALIGENT 3D CE device with 64.5 cm capillary (50 μ). HP-CE chromatograms were recorded with sodium citrate buffer (pH 4, 20 mM) + carboxymethyl-α-CD, 40 mM, CY-E-1006; 25 °C; voltage 20 kV; detection 214 nm.

Analytical thin-layer chromatography (TLC) was performed on Macherey−Nagel ready-to-use plates AluGram Sil G/UV_{254}. Detection was under a UV lamp at 254 nm, and development was with molybdatophosphoric acid solution (5% in EtOH) or ninhydrin solution in butanol/glacial acetic acid mixture. Elemental analyses were obtained at the Mikroanalytisches Laboratorium des Instituts für Organische Chemie in Göttingen (Georg-August Universität). Solvents were purified according to standard procedures. Organic solutions were dried over $MgSO_4$, unless otherwise stated.

(3′*S*,5*RS*)-5-[*N*-(3-Benzyloxycarbonylamino-6-*tert*-butoxycarbonylamino)hexanoyl-*N*-methylamino]-5,6-dihydro-2-ureidopyrimid-4(1*H*)-one (5-Boc). To a solution of 32.0 g (84.2 mmol) of N^{β}-*Z*-N^{ϵ}-BOC-β-lysine (**2**) in 800 mL of DMF was added 19.4 g (101 mmol) of EDC and 15.5 g (101 mmol) of HOBt (monohydrate), and the mixture was stirred at room temperature for 1 h. Subsequently, 17.1 g (92.4 mmol) of *rac*-3,4,5,6-tetrahydro-5-methylamino-2-ureidopyrimidin-4-one (**3**) and 13.1 g (101 mmol) of *N*,*N*-diisopropylethylamine were added, and the mixture was maintained at room temperature for 16 h. To the reaction mixture was added 5.6 L of water, and the aqueous layer was extracted three times with 2.1 L each of ethyl acetate. The combined organic layers were washed twice with 1.6 L each of water. During the washing process, the product precipitated. It was filtered off and washed with 100 mL of cold methanol. The organic layer was dried, and the solvent was removed in vacuo. The resulting oil was treated with 640 mL of methanol, and the precipitated product was filtered off and subsequently washed with 160 mL of methanol followed by washing with 160 mL of diethyl ether. From the filtrate, another crop of solids precipitated upon standing. It was filtered and washed as described before. In total, 28.0 g (60%) of the desired coupling product was isolated. HPLC (method B): R_t 3.05 min, peak area 100%. ^{1}H NMR (300 MHz, [D_6]DMSO): δ 1.36 (s, 9 H), 1.40 (m, 4 H), 2.79 (m, 2 H), 2.89 (m, 2H), 3.12 (m, 3 H), 3.36−3.90 (m, 5 H), 5.00 (m, 3 H), 6.76 (m, 3 H), 7.12 (m, 1 H), 7.34 (m, 5 H). ^{13}C NMR (125 MHz, $CDCl_3$/[D_4]MeOH, 1:1): δ 176.5, 172.2, 172.1, 159.7, 157.3, 156.3 (2 C), 156.2, 136.5, 136.4, 128.4, 128.3, 128.0, 127.9, 127.8, 127.7 (2 C), 127.6, 79.2, 77.2, 66.4, 66.3, 52.3, 51.6, 48.1, 47.7, 39.8, 38.5, 38.3, 37.7, 33.7, 33.1, 31.5, 31.2, 28.2 (3 C), 26.5. HRMS (ESI) calcd for $C_{25}H_{36}N_7O_7$ [$M^+ - H$], 546.2676; found, 546.2665.

(3′*S*,5*RS*)-5-[*N*-(6-Amino-3-benzyloxycarbonylamino-hexanoyl)-*N*-methylamino]-5,6-dihydro-2-ureidopyrimid-

4(1*H*)-one Hydrochloride (5). A solution of 31.0 g (56.7 mmol) of the coupling product in 566 mL of 4 M HCl in dioxane was stirred at room temperature for 2.5 h. All volatile components were evaporated in vacuo, and the resulting product **5** (28.4 g, quantitative) was used without further purification and characterization.

(3′*S*,5*RS*)-5-[*N*-[3-Benzyloxycarbonylamino-6-[2,3-di-(benzyloxycarbonyl)-2(3)-methylguanidin-1-yl]hexanoyl]-*N*-methylamino]-5,6-dihydro-2-ureidopyrimid-4(1*H*)-one (tris-*Z*-4a). A solution of 37.6 g (77.8 mnmol) of **5**, 34.7 g (93.2 mmol) of **6a**, and 20.1 g (26.6 mL, 155 mmol) of *N*,*N*-diisopropylethylamine in 710 mL of ethanol was heated to 60 °C for 16 h. The solvent was evaporated in vacuo, and the residue was purified by flash chromatography using a gradient of dichloromethane with increasing polarity to dichloromethane/methanol (95:5) to yield 30.0 g (50%) of the desired tris-*Z*-**4a**. HPLC (method A): R_t 6.08 min, peak area 92%. ^{1}H NMR (200 MHz, $CDCl_3$): 1.5 (m, 4 H), 2.60 (m, 2 H), 3.14 (s, 3 H), 3.39−4.05 (m, 5 H), 5.01 (m, 1 H), 5.06−5.18 (m, 6 H), 7.31 (m, 15 H), 9.25 (m, 1 H), 10.34 (m, 1 H). HRMS (ESI) calcd for $C_{38}H_{44}N_9O_9$ [M^+ − H], 770.3262; found, 770.3254.

(3′*S*,5*RS*)-5-[*N*-[3-Amino-6-[2(3)-methylguanidin-1-yl]-hexanoyl]-*N*-methyylamino]-5,6-dihydro-2-ureidopyrimid-4(1*H*)-one Dihydrochloride (4a). A solution of tris-*Z*-**4a** (13.8 g, 17.9 mmnol) and 6.34 g (35.8 mmol) of palladium(II) chloride in 700 mL of methanol (4 L flask) was stirred in a hydrogen atmosphere for 1 h. Because TLC (dichloromethane/methanol, 9:1) indicated complete conversion, the reaction mixture was filtered through a plug of Celite, and it was washed thoroughly with methanol. The filtrate was concentrated, and the crude product was treated with acetone. The solid was filtered off (D2-frit) and dried in vacuo to yield 8.3 g (quantitative) of **4a**. HPLC (method A): R_t 3.52 min, peak area 98%. HPLC (method C): R_t 0.70 min, peak area 97%. HP-CE: injection time 8 s; R_t 17.90 and 18.77 min (two diastereomers), peak areas 48.85 and 48.93%, respectively (98% in total). ^{1}H NMR (400 MHz, [D$_4$]MeOH): δ 1.70 (m, 4 H), 2.72−2.94 (m, 2 H), 2.84 (s, 3 H), 3.14 (s, 3 H), 3.23 (m, 2 H), 3.61 (m, 1 H), 3.90 (m, 1 H), 4.02 (dt, 1 H), 5.15 (m, 1 H). ^{13}C NMR (125 MHz, $CDCl_3$/[D$_4$]MeOH, 1:1): δ 173.2, 173.1, 167.2, 157.9, 157.0, 155.5, 153.9, 55.6, 55.4, 41.9, 40.2, 36.1, 35.9, 30.6, 28.5, 25.7. HRMS (ESI) calcd for $C_{14}H_{26}N_9O_3$ [M^+ − H], 368.2159; found, 368.2169.

(3′*S*,5*RS*)-5-[*N*-[3-Benzyloxycarbonylamino-6-[2,3-di-(benzyloxycarbonyl)-2(3)-ethylguanidin-1-yl]hexanoyl]-*N*-methylamino]-5,6-dihydro-2-ureidopyrimid-4(1*H*)-one (tris-*Z*-4b). A solution of 300 mg (0.780 mmol) of *S*-methylisothiourea **6b**, 376 mg (0.778 mmol) of **5**, 231 mg (0.85 mmol) of $HgCl_2$, and 471 mg (4.66 mmol) of triethylamine in 4 mL of DMF was stirred at room temperature for 72 h. The solvent was evaporated in vacuo, and the residue was redissolved in dichloromethane and filtered through a plug of Celite. The solvent was evaporated, and the crude product was purified by flash chromatography on silica gel (dichloromethane/methanol/concentrated aqueous NH_3, 90:10:1) to yield 225 mg (36%) of the desired tris-*Z*-**4b**. HPLC (method C): R_t 4.30 min, peak area 93%. ^{1}H NMR ($CDCl_3$, 500 MHz): δ 1.18 (m, 3 H), 1.55 (m, 4 H), 2.57 (m, 2 H), 2.92 (m, 3 H), 3.12 (m, 2 H), 3.22 (m, 2 H), 3.39−3.65 (m, 3 H), 3.71 (m, 2 H), 3.90 (m, 1 H), 5.04−5.26 (m, 7 H), 5.80 (m, 1H), 7.25−7.40 (m, 15 H), 9.19 (m, 1 H), 10.28 (m, 1 H). HRMS (ESI) calcd for $C_{39}H_{46}N_9O_9$ [M^+ − H], 784.3419; found, 784.3412.

(3′*S*,5*RS*)-5-[*N*-[3-Amino-6-[2(3)-ethylguanidin-1-yl]hexanoyl]-*N*-methylamino]-5,6-dihydro-2-ureidopyrimid-4(1*H*)-one Dihydrochloride (4b). According to the procedure described for **4a**, 109 mg (0.14 mmol) of tris-*Z*-**4b** was deprotected to yield 63 mg (99%) of the desired product **4b**. HPLC (method A): R_t 4.54 min, peak area 98%. HP-CE: injection time 8 s; R_t 21.24 and 22.21 min (two diastereomers), peak areas 48.65 and 51.35%, respectively (100% in total). ^{1}H NMR (500 MHz, D_2O): δ 1.19 (t, 3 H), 1.74 (m, 4 H), 2.85 (m, 1 H), 3.02 (m, 1 H), 3.14 (s, 3 H), 3.23 (m, 4 H), 3.68 (m, 1 H), 4.00 (m, 2 H), 5.11 (m, 1 H). ^{13}C NMR (125 MHz, $CDCl_3$/[D$_4$]MeOH, 1:1): δ 175.2, 169.7, 158.2, 157.4, 154.9, 57.0, 50.9, 43.1, 41.3, 39.0, 37.9, 37.5, 31.9, 26.9, 16.0. HRMS (ESI) calcd for $C_{15}H_{28}N_9O_3$ [M^+ − H], 382.2315; found, 382.2321.

(3′*S*,5*RS*)-5-[*N*-[3-Benzyloxycarbonylamino-6-[2,3-di-(benzyloxycarbonyl)-2(3)-isopropylguanidin-1-yl]hexanoyl]-*N*-methylamino]-5,6-dihydro-2-ureidopyrimid-4(1*H*)-one (tris-*Z*-4c). The synthesis was conducted in analogy to the synthesis of tris-*Z*-**4b** on a 207 mg (0.428 mmol) scale of **5** using **6c** (171 mg, 0.428 mmol) as an electrophilic component. The guanylation yielded 174 mg (51%) of tris-*Z*-**4c**. HPLC (method C): R_t 4.40 min, peak area 100%. ^{1}H NMR (500 MHz, [D$_4$]MeOH): δ 1.18−1.69 (m, 10 H), 2.50 (m, 2 H), 3.08 (m, 3 H), 3.25−3.40 (m, 2 H), 3.66 (m, 1 H), 3.80 (m, 1 H), 3.92 (m, 1 H), 3.90 (m, 1 H), 4.11 (m, 1 H), 4.96−5.24 (m, 7 H), 7.32 (m, 15 H). HRMS (ESI) calcd for $C_{40}H_{48}N_9O_9$ [M^+ − H], 798.3575; found, 798.3525.

(3′*S*,5*RS*)-5-[*N*-[3-Amino-6-[2(3)-isopropylguanidin-1-yl]hexanoyl]-*N*-methylamino]-5,6-dihydro-2-ureidopyrimnid-4(1*H*)-one Dihydrochloride (4c). The removal of the *Z* groups was conducted as described for **4a** on a 50 mg (0.07 mmol) scale of tris-*Z*-**4c**. The hydrogenation yielded 32 mg (97%) of **4c**. HPLC (method A): R_t 4.64 min, peak area 100%. HP-CE: injection time 20 s; R_t 24.91 and 26.19 min (two diastereomers), peak areas 51.66 and 48.34%, respectively (100% in total). ^{1}H NMR (400 MHz, [D$_4$]MeOH): δ 1.23 (d, 6 H), 1.75 (m, 4 H), 2.76 (dd, 1 H), 2.97 (dt, 1 H), 3.13 (s, 3 H), 3.26 (m, 2 H), 3.60 (mn, 1 H), 3.72 (m, 1 H), 3.88 (m, 1 H), 4.01 (dt, 1 H), 5.19 (m, 1 H). ^{13}C NMR (125 MHz, D_2O): δ 172.8, 167.6, 155.1, 152.8, 54.6, 49.2, 48.5, 44.2, 40.8, 38.9, 35.4, 35.1, 29.5, 24.5, 21.8 (2C). HRMS (ESI) calcd for $C_{16}H_{30}N_9O_3$ [M^+ − H], 396.2472; found, 396.2508.

(3′*S*,5*RS*)-5-[*N*-[6-Acetimidoylamino-3-(benzyloxycarbonylamino)hexanoyl]-*N*-methylamino]-5,6-dihydro-2-ureidopyrimid-4(1*H*)-one (*Z*-8a). A solution of 500 mg (1.03 mmol) of **5**, 175 mg (1.39 mmol) of **7a**, and 400 mg (3.10 mmol) of *N*,*N*-diisopropylethylamine in 10 mL of ethanol was stirred at 40 °C for 16 h. The solvent was evaporated in vacuo, and the crude product was purified by flash chromatography on silica gel using dichloromethane/methanol/concentrated aqueous NH_3 (85:15:3) to yield 287 mg (57%) of the desired product *Z*-**8a**. HPLC (method C): R_t 3.4 min, peak area 94%. ^{1}H NMR (500 MHz, [D$_4$]MeOH): δ 1.51−1.73 (m, 4 H), 2.20 (m, 3 H), 2.64 (m, 2 H), 3.02 (m, 3 H), 3.27 (m, 2 H), 3.49 (m, 1 H), 3.68 (m, 2 H), 4.02 (m, 1 H), 5.06 (m, 3 H), 7.31 (m, 5 H), 8.45 (s, 1 H). HRMS (ESI) calcd for $C_{22}H_{31}N_8O_5$ [M^+ − H], 487.2417; found, 487.2431.

(3′*S*,5*RS*)-5-[*N*-(6-Acetimidoylamino-3-aminohexanoyl)-*N*-methylamino]-5,6-dihydro-2-ureidopyrimid-4(1*H*)-one Dihydrochloride (8a). The removal of the *Z* group was conducted as described for **4a** on a 66 mg (0.14 mmol) scale of *Z*-**8a**. The hydrogenation yielded 47 mg (82%) of **8a**. HPLC (method A): R_t 4.36 min, peak area 94%. HP-CE: injection time 8 s; R_t 16.45 and 17.21 min (two diastereomers), peak areas 55.39 and 44.61%, respectively (100% in total). ^{1}H NMR (400 MHz, [D$_4$]MeOH): δ 1.81 (m, 4 H), 2.26 (s, 3 H), 2.80 (dd, 1 H), 2.99 (m, 1 H), 3.17 (s, 3 H), 3.32 (m, 2 H), 3.62 (m, 2 H), 3.90 (m, 1 H), 4.04 (m, 1 H), 5.18 (m, 1 H). ^{13}C NMR (125 MHz, D_2O): δ 175.2, 169.8, 167.6, 157.4, 155.0, 57.1, 50.8, 44.2, 41.3, 37.9, 37.5, 32.0, 25.6, 21.1. HRMS (ESI) calcd for $C_{14}H_{25}N_8O_3$ [M^+ − H], 353.2050; found, 353.2020.

(3′*S*,5*R*,*S*)-5-[*N*-[3-Benzyloxycarbonylamino-6-(isobutyrimidoylamino)hexanoyl]-*N*-methylamino]-5,6-dihydro-2-ureidopyrimid-4(1*H*)-one (*Z*-8b). The synthesis was conducted in analogy to the synthesis of *Z*-**8a** with 400 mg (0.83 mmol) of **5** using **7b** as an electrophilic component. The reaction yielded 190 mg (44%) of *Z*-**8b**. HPLC (method C): R_t 3.57 min, peak area 79%. ^{1}H NMR (500 MHz, [D$_4$]MeOH): δ 1.25 (m, 6 H), 1.68 (m, 4 H), 2.56−2.82 (m, 2 H), 3.02 (m, 3 H), 3.26 (m, 2 H), 3.49 (m, 1 H), 3.69 (m, 2 H), 4.02 (m, 1 H), 4.91−5.11 (m, 3 H), 7.31 (m, 5H). HRMS (ESI) calcd for $C_{24}H_{35}N_8O_5$ [M^+ − H], 515.2730; found, 515.2643.

(3′*S*,5*R*,*S*)-5-[*N*-(3-Amino-6-isobutyrimidoylaminohexanoyl)-*N*-methylamino]-5,6-dihydro-2-ureidopyrimiid-4(1*H*)-one Dihydrochloride (8b). The removal of the *Z* group was conducted as described for **4a** on a 130 mg (0.25

mmol) scale of *Z*-**8b**. The hydrogenation yielded 95 mg (83%) of **8b**. HPLC (method A): R_t 4.52 min, peak area 100%. HP-CE: injection time 20 s; R_t 20.01 and 21.01 min (two diastereomers), peak areas 47.32 and 45.87%, respectively (93% in total). ^{1}H NMR (400 MHz, D_2O): δ 1.24 (d, 6 H), 1.76 (m, 4 H), 2.76 (m, 3 H), 2.92 (m, 1 H), 3.05 (m, 3 H), 3.31 (m, 3 H), 3.62 (m, 1 H), 3.74 (m, 2 H), 4.87 (m, 1 H). HRMS (ESI) calcd for $C_{16}H_{29}N_8O_3$ [M^+ − H], 381.2363; found, 381.2396.

***N*$^\beta$-Benzyloxycarbonyl-*N*$^\epsilon$-trifluoroacetyl-(*S*)-β-lysine (9).** To a suspension of *N*$^\beta$-*Z*-*N*$^\epsilon$-Boc-(*S*)-β-lysine (**2**) (30.4 g, 80 mmol) in 10 mL of anhydrous dioxane was added dropwise with stirring 90 mL of 4 M HCl in dioxane at 20−25 °C. The solution was kept at +5 °C overnight, the dioxane was evaporated in vacuo, and the crystalline mass was washed twice with anhydrous ether. The hydrochloride of *N*$^\beta$-*Z*-(*S*)-β-lysine was dried in vacuo and dissolved in 40 mL of anhydrous MeOH, and triethylamine (33.2 mL, 24.2 g, 0.24 mol) was added dropwise with stirring while the reaction flask was cooled with ice water. The thick precipitate, which appeared soon was gradually dissolved by adding methyl trifluoroacetate (9.6 mL, 12.3 g, 96 mmol) at +5 °C. The reaction mixture was warmed to room temperature and was stirred overnight. All volatile materials were removed in vacuo, the semisolid residue was dissolved in water (250 mL), and 20% aqueous HCl was added to it with stirring and cooling until the pH value was 1−2. The precipitated product was extracted with ethyl acetate (4 × 150 mL). The combined organic solutions were washed with water (2 × 40 mL) and brine (100 mL), and then dried and concentrated to yield a solid, which was recrystallized from ethyl acetate/hexane mixture in two crops. The first crop (21.9 g) with mp 122−124 °C was used in the next step; $[\alpha]_D^{20} = -6.4°$ (c = 1.05, EtOAc). Total yield, 24.5 g (81%). IR (KBr): 3307, 3108, 2955, 1700, 1542, 1456, 1213, 1183, 1069 cm^{-1}. ^{1}H NMR (250 MHz, [D$_4$]MeOH): δ 1.55−1.61 (m, 4 H), 2.46 (d, J = 7.5, 2 H), 3.28 (m, 2 H), 3.95 (br. m, 1 H), 5.06 (s, 2 H), 7.28−7.33 (m, 5 H). ^{13}C NMR (62.9 MHz, [D$_4$]MeOH): δ 173.0, 158.7, 156.5 (q, J = 35), 138.7, 129.7, 129.2, 129.0, 116.3 (q, J = 286), 67.6, 49.5, 41.0, 40.7, 33.2, 26.8. HRMS (EI) calcd for $C_{16}H_{19}F_3N_2O_5$, 376.1247; found, 376.1246.

***N*$^\beta$-Benzyloxycarbonyl-*N*$^\epsilon$-trifluoroacetyl-(*S*)-β-lysine *tert*-Butyl Ester (10).** Compound **9** (21.9 g, 58.2 mmol) was suspended in *t*-BuOAc (830 mL), and 4.5 mL of 70% aqueous $HClO_4$ was added. Stirring in the closed reaction flask was continued for 2 days at ambient temperature. The flask was opened carefully, the solution was diluted with ether (1 L), and the mixture was carefully washed with many portions of saturated aqueous $KHCO_3$, until no gas evolution was observed, and TLC of the organic layer (CH_2Cl_2/MeOH/AcOH, 100:5:1) displayed no spot of **9** (R_f 0.15) any more. The combined aqueous solutions were extracted once with ethyl acetate (200 mL) and carefully acidified with 20% aqueous HCl with cooling. Unreacted **9** was extracted with ethyl acetate (3 × 150 mL), and the combined organic layers were washed with brine (100 mL) and dried. Evaporation of the solvent in vacuo left 8.4 g of the crude acid **9**. The organic solution that contained ether was washed with brine and dried. Evaporation of the solvents in vacuo yielded a two phase liquid residue. The upper oily layer (diisobutylene) was decanted, and the lower layer was triturated with pentane (150 mL), seeded with **10**, and stirred. More pentane was added, when the crystallization proceeded. The mixture was kept for 2 h at +5 °C, and the colorless powder was collected on a filter, washed with cold pentane, and dried to yield 14.1 g of **10** with mp 44−47 °C. The yield was 91% based on the converted acid **9**. A second run was conducted with the recovered starting material (8.4 g), and a second crop of **9** was obtained in the previous experiment (2.6 g). *t*-BuOAc (700 mL) and 2.8 mL of 70% aqueous $HClO_4$ were used, and 2.8 g of unreacted acid **9** was isolated; the yield of **10** was found to be 6.9 g (73% based on the reacted acid **9**); $[\alpha]_D^{20} = -8.0°$ (c = 1.03, EtOAc). IR (KBr): 3336, 2944, 1701, 1681, 1534, 1165 cm^{-1}. ^{1}H NMR (250 MHz, $CDCl_3$): δ 1.42 (s, 9 H), 1.52−1.64 (m, 4 H), 2.46 (t, J = 5.8, 2 H), 3.37 (m, 2 H), 3.96 (br. m, 1 H), 5.08 (s, 2 H), 5.37 (d, J = 9, 1 H), 7.10 (br. m, 1 H), 7.26−7.35 (m, 5 H). ^{13}C NMR (62.9 MHz, $CDCl_3$): δ 170.8, 155.3 (q, J = 37), 156.2, 136.3, 128.5, 128.2, 128.1, 116.3 (q, J = 286), 81.5, 66.8, 47.4, 40.2, 39.4, 32.0, 27.3, 24.9. EI-MS m/z (rel intensity, %): 376 (31), 359 (10), 269 (11), 241 (9), 178 (14), 108 (39), 91 (100). DCI (NH_3)-MS m/z (rel intensity, %): 450 ($M + NH_4^+$, 31), 299 (100). Anal. ($C_{20}H_{27}F_3N_2O_5$) C, H, N.

***N*$^\beta$-*Z*-*N*$^\epsilon$-methyl-*N*$^\epsilon$-trifluoroacetyl-(*S*)-β-lysine *tert*-Butyl Ester (10-Me).** A mixture of the compound **10** (2.64 g, 6.11 mmol), Cs_2CO_3 (3.98 g, 12.2 mmol), and MeI (1.91 mL, 4.33 g, 30.5 mmol) in 30 mL of DMF was heated at 80 °C with rapid stirring in a closed reactor for 50 h. (A round-bottom cylindrical glass reactor with thick walls was used, volume 200 mL.)

After it was cooled to ambient temperature, the reaction mixture was filtered, and the filter cake was washed with ether (200 mL). The combined liquids were filtered once more to remove the precipitated salts, and the solution was concentrated in vacuo, first at about 10 Torr and then at 1 Torr with a cold trap (−78 °C) and a warm water bath (<40 °C). The residual oil was taken up in ether (250 mL), and the solution was washed with water (3 × 30 mL) and brine (30 mL) and dried. The TLC of the crude title compound displayed a single spot with R_f 0.70 (EtOAc/hexane, 1:1), yield 2.7 g (100%). IR (neat): 3338, 2978, 2941, 1694, 1530, 1454, 1368, 1247, 1146 cm^{-1}. ^{1}H NMR (250 MHz, $CDCl_3$): δ 1.42 (s, 9 H), 1.50−1.63 (m, 4 H), 2.33−2.46 (m, 2 H), 2.94 and 3.05 (2s, total 3 H), 3.39−3.47 (m, 2 H), 3.96 (m, 1 H), 5.08 (s, 2 H), 5.38 (m, 1 H), 7.33−7.42 (m, 5 H). ^{13}C NMR (62.9 MHz, $CDCl_3$): δ (signals of the major isomer are marked*) 170.6, 156.6 (q, J = 35), 155.9, 136.5, 128.4, 128.1, 128.0, 116.4 (q, J = 286), 81.4/81.3*, 66.64/66.56*, 49.0/48.9*, 47.8*/47.6, 40.3/40.2*, 34.6* (q, J = 3.8)/34.2, 31.5*/31.3, 27.9, 24.75, 23.1. The progress of the methylation reaction was monitored by mass spectroscopy. The product of the selective *N*$^\epsilon$-methylation has two characteristic peaks in the EI-MS: a more intensive one with m/z = 390 ($M^+ - C_4H_8$) and 373 ($M^+ - C_4H_8 - OH$). For the starting material **10**, they are found at m/z = 376 and 359 and at for the "overmethylated" product at 404 and 387. As a rule, when the conversion is complete, the reaction mixture contains about 7% of the impurity with an NMeZ group.

***N*$^\beta$-Benzyloxycarbonyl-*N*$^\epsilon$-methyl-(*S*)-β-lysine *tert*-Butyl Ester Hydrochloride (11).** The crude methylation product **10**-Me (19.7 g, 44 mmol) was dissolved in MeOH (100 mnL), and a solution of $LiOH \cdot H_2O$ (2.1 g, 50 mmol) in MeOH (150 mL) was added carefully at about +5 °C. The mixture was allowed to warm to ambient temperature, and after 3 h, it was evaporated in vacuo. The residue was dissolved in ether (500 mL), washed with water (3 × 30 mL) and brine (50 mL), and dried. Evaporation of the solvent provided 14.6 g of the free amine (95% crude yield). ^{1}H NMR (250 MHz, $CDCl_3$): δ 1.41 (s, 9 H), 1.53 (m, 4 H), 2.03 (br. s, 1 H), 2.39 (s, 3 H), 2.42 (d, J = 5.8, 2 H), 2.56 (br. t, 2 H), 3.97 (br. s, 1 H), 5.06 (s, 2 H), 5.58 (br. s, 1 H), 7.32 (m, 5 H). ^{13}C NMR (62.9 MHz, $CDCl_3$): δ 170.8, 155.8, 136.6, 128.4, 128.1, 127.9, 81.0, 66.4, 51.4, 48.1, 40.2, 36.1, 32.1, 28.0, 25.9. ESI-MS m/z, positive ions, 351 ($M^+ + H$, 100): 295 (38). The oil was dissolved in anhydrous ether (200 mL), and 11 mL of 4 M HCl in dioxane was slowly added at +5 °C with vigorous stirring. The suspension was kept at +5 °C for 2 h, and the solid was filtered off, washed with anhydrous ether, and dissolved in a minimal amount of boiling 2-propanol. Anhydrous ether was added until a precipitate began to appear, and then, the solution was left overnight at room temperature. The salt **11** was collected on a filter, washed with anhydrous ether (200 mL), and dried to yield 11.8 g (73%) of hydrochloride **11** with mp 143 °C; $[\alpha]_D^{20} = -10.6°$ (c = 1.15, MeOH). IR (KBr): 3343, 2948, 2867, 2793, 1719, 1694, 1539, 1267 cm^{-1}. ^{1}H NMR (250 MHz, [D$_4$]MeOH): δ 1.40 (s, 9 H), 1.53−1.79 (m, 4 H), 2.29−2.42 (m, 2 H), 2.64 (s, 3 H), 3.00 (m, 2 H), 3.96 (m, 1 H), 5.05/5.07 (AB-q, J = 12.5, 2 H), 7.27−7.34 (m, 5 H). ^{13}C NMR (62.9 MIHz, [D$_4$]-MeOH): δ 172.4, 158.6, 138.6, 129.8, 129.3, 129.1, 82.3, 67.6, 50.2, 49.5, 42.5, 33.8, 33.2, 28.6, 24.0. Anal. ($C_{19}H_{31}N_2O_4Cl$) C, H, N.

***N*$^\beta$,*N*$^\omega$,*N*$^{\omega'}$-Tris(benzyloxycarbonyl)-*N*$^\epsilon$-methyl-(*S*)-[β-homoarginine *tert*-Butyl Ester (12).** A mixture of compound

11 (1.48 g, 3.83 mmol) and *N,N′*-bis-*Z*-1-guanidinylpyrazole[17] (1.51 g, 3.98 mmol) in 10 mL of CH_2Cl_2 and 3 mL of Et_3N was heated overnight under reflux. The product was isolated by chromatography on basic aluminum oxide (200 g, activity grade I) eluting with CH_2Cl_2/MeOH/40% NH_3 in MeOH (100:1:0.5); R_f 0.75; yield 2.21 g (87%) of a glasslike foam. IR (neat): 3330, 3033, 2941, 1723, 1615, 1498, 1455, 1404, 1368, 1214, 1065 cm^{-1}. ^1H NMR (250 MHz, $CDCl_3$): δ 1.41 (s, 9 H), 1.48 (m, 2 H), 1.67 (m, 2 H), 2.41 (t, J = 6.5, 2 H), 2.98 (s, 3 H), 3.52 (m, 2 H), 3.98 (m, 1 H), 4.72 (s, 1 H), 5.06 (s, 2 H), 5.15 (s, 4 H), 5.41 (m, 1 H), 7.23−7.38 (m, 15 H). ^{13}C NMR (62.9 MHz, $CDCl_3$): δ 170.7, 156.3, 156.0, 136.6, 128.5, 128.2, 128.0, 127.9, 127.4, 126.8, 81.0, 67.6 (br), 67.2, 66.5, 51.0 (br), 48.0, 40.5, 37.3 (br), 31.3, 28.0, 23.5. ESI-MS *m/z*: positive ions, 683 (M + Na, 100), 661 (M + H, 92); negative ions, 659 (M − H, 100).

***N*$^{\beta}$,*N*$^{\omega}$,*N*$^{\omega'}$-Tris(benzyloxycarbonyl)-*N*$^{\epsilon}$-methyl-(*S*)-*β*-homoarginine (13).** To a solution of the ester **12** (2.35 g, 3.56 mmol) and Et_3SiH (1.03 g, 8.86 mmol) in 6 mL of CH_2Cl_2 was added TFA (3.5 mL) carefully with stirring at + 5 °C. The reaction mixture was left overnight at the same temperature and concentrated in vacuo. The oily residue was washed with anhydrous ether (3 × 30 mL with sonification followed by decantation) and coevaporated with CH_2Cl_2 (3 × 30 mL). After it was dried at 0.1 Torr overnight, 2.1 g of the crude title product was obtained; R_f 0.20 (hexane/EtOAc/AcOH, 4:2:1). The purity was found to be 80% (HPLC-MS) corresponding to a yield of 78%. All attempts to obtain the crystalline dicyclohexyl-ammonium salt failed. Purification by chromatography on silica gel (100 g) eluting with a hexane/EtOAc/AcOH mixture (1:1:1) was accompanied by losses: 1.27 g (59%) were recovered. ^1H NMR (300 MHz, 50 °C, $C_2D_2Cl_4$): δ 1.43 (m, 2 H), 1.62 (m, 2 H), 2.43 (m, 2 H), 2.95 (s, 3 H), 3.44 (m, 2 H), 3.96 (m, 1 H), 5.05/5.12 (s, 6 H), 5.47 (m, 1 H), 7.25−7.38 (m, 15 H), 8.8 (br. s, 2 H). ^{13}C NMR (75.5 MHz, 35 °C, $C_2D_2Cl_4$): δ 175.2, 157.7, 156.0, 155.7, 136.3, 135.6, 128.4, 128.3, 128.2, 128.1, 127.9, 127.6, 67.4, 66.4, 50.2, 47.8, 39.6, 36.9, 30.8, 23.3. ESI-MS *m/z*: positive ions, 1231 (2M + Na, 14), 649 (M − H + 2Na, 26), 627 (M + Na, 100), 605 (M + H, 12), 519 (M − $PhCH_2OH$ + Na, 12); negative ions, 1207 (2M − H, 80), 625 (M − 2H + Na, 50), 603 (M − H, 50), 495 (M − $PhCH_2OH$ − H, 100).

(3′*S*,5*RS*)-5-[*N*-[3-Benzyloxycarbonylamino-6-[2,3-di-(benzyloxycarbonyl)-1-methylguanidin-1-yl]hexanoyl]-*N*-methylamino]-5,6-dihydro-2-ureidopyrimid-4(1*H*)-one (tris-*Z*-14). Under an argon atmosphere, the acid **13** (1.20 g, 1.98 mmol) was dissolved in DMF (20 mL), and the heterocycle **3** (368 mg, 1.98 mmol) was added followed by HATU (1.13 g, 2.98 mmol) and DIPEA (385 mg, 0.510 mL, 2.98 mmol), which was added dropwise with stirring at room temperature. The mixture was stirred overnight at room temperature, DMF was evaporated in vacuo (1 Torr), and the residue was dissolved in dichloromethane (200 mL). This solution was washed with water (20 mL), 0.5 aqueous H_2SO_4 (10 mL), water (20 mL), saturated aqueous $NaHCO_3$ (10 mL), and brine (10 mL). After the solvent was dried and evaporated, the coupling product was isolated by repeated chromatography on silica gel eluting with CH_2Cl_2/MeOH (9:1, R_f = 0.27), EtOAc/EtOH/AcOH (90:10:5), and CH_2Cl_2/MeOH (100:7); yield 1.26 g (82%) tris-*Z*-**14** as a colorless foam. HPLC (method C): R_t 4.3 min, peak area 98%. IR (KBr): 3410, 2945, 1714, 1616, 1576, 1456, 1378, 1273, 1225 cm^{-1}. ^1H NMR (200 MHz, $CDCl_3$): δ 1.60 (m, 4 H), 2.45−2.70 (m, 2 H), 2.87 and 2.92 (2 s, total 3 H), 2.99 (s, 3 H), 3.35−3.52 (m, 4 H), 3.97 (m, 1 H), 4.88 (m, 1 H), 5.04 (s, 2 H), 5.11 (s, 4 H), 5.68−5.90 (m, 1 H), 7.38 (m, 15 H), 9.28 (br. s, 1 H), 10.26 (br. s, 1H), 10.41 (br. s, 1H), 11.43 (br. s, 1H). HPLC-MS: R_t 2.31 min. ESI-MS *m/z*: positive ions, 772 (M + H, 100), 407 (69), 387 [(M + 2H)/2, 78]; negative ions, 770 (M − H, 100).

(3′*S*,5*R*,*S*)-5-[*N*-[3-Amino-6-(1-methylguanidin-1-yl)hexanoyl]-*N*-methylamino]-5,6-dihydro-2-ureidopyrimid-4(1*H*)-one Dihydrochloride (14). Tris-*Z*-**14** (110 mg, 0.143 mmol) was dissolved in MeOH/CH_2Cl_2 (1:1, 5 mL), $PdCl_2$ (38 mg, 0.21 mmol) was added, the solution was flushed with nitrogen and then with H_2. Hydrogenation under normal pressure was continued for 1.5 h, while the reaction mixture was stirred vigorously, and a slow stream of hydrogen was passed above the solution. The catalyst (coagulated Pd-black) was removed by filtering the solution through Celite. The filter cake was washed with anhydrous methanol (15 mL), and the combined solutions were evaporated to dryness. The residue was dissolved in MeOH (1.5 mL), anhydrous acetone (20 mL) was added carefully with stirring, and the precipitated salt was filtered off and washed with anhydrous ether to yield 60 mg of a hygroscopic colorless solid. HPLC-MS: R_t 0.29 min, peak area 98%. HPLC (method A): R_t 3.52 min, peak area 97%. HP-CE: injection time 8 s; R_t 24.52 and 25.79 min (two diastereomers), peak areas 51.46 and 46.88%, respectively (98% in total). ESI-MS *m/z*: positive ions, 370 (M + H, 100); yield was calculated to be 95% (by 98% purity). ^1H NMR (400 MHz, [D$_4$]MeOH): δ 1.62 (br. s, 4 H, 4′/5′−H), 2.76 (dd, J = 8.8 and 17.5, 1 H, 2′−H), 2.97 (dt, J = 2.7 and 17, 1 H, 2′−H), 3.04 (s, 3 H, 6′-NMe), 3.12 (s, 3 H, CONMe), 3.38 (br. s, 2 H, 6′−H), 3.60 (br.s, 1 H, 3′−H), 3.87 (m, 1 H, 6-H), 4.02 (m, 1 H, 6-H), 5.14 (m, 1 H, 5-H). ^{13}C NMR (400 MHz, [D$_4$]MeOH, two rotamers, ratio ca. 1:1): δ 173.3 (C−1′), 173.4 (C−1′), 168.0 (C-2), 158.5 (C-guanidino), 155.2 (C-ureido), 154.2 (C-4), 55.5 (C-5), 51.0 (C-6′), 49.0 (C-3′), 40.2 (C-6), 36.8 (C-6′−N-Me), 36.1 (C-2′), 35.6 (C-2′), 35.4 (CONMe), 30.6 (C-5′), 30.5 (C-5′), 24.0 (C− 4′).

***N*$^{\beta}$,*N*$^{\omega}$,*N*$^{\omega'}$-Tris(benzyloxycarbonyl)-*N*$^{\epsilon}$,*N*$^{\omega}$-dimethyl-(*S*)-*β*-homoarginine *tert*-Butyl Ester (15).** Dry, finely powdered K_2CO_3 (362 mg, 2.62 mmol) was added to a solution of the ester **12** (866 mg, 1.31 mmol), MeI (428 mg, 3.02 mmol), and TBAB (21 mg, 66 μmol) in anhydrous acetone (10 mL). The suspension was vigorously stirred in a closed screw-cap Pyrex bottle at 60 °C for 2 days. After the bottle was cooled, the inorganic salts were filtered off and washed with anhydrous acetone, and the filtrate was evaporated. The residue was dissolved in AcOEt (50 mL) and washed with 5% aqueous $KHSO_4$, water, saturated aqueous $NaHCO_3$, water, and brine and dried. The product was isolated by chromatography on silica gel (hexane/EtOAc, 1:1, R_f 0.31), yielding 781 mg (88%) of a colorless oil. HPLC (method C): R_t 5.14 min, peak area 99%. IR (neat): 3054, 2977, 1724, 1591, 1508, 1422, 1265, 1156 cm^{-1}. ^1H NMR-(300 MHz, 60 °C, $C_2D_2Cl_4$): δ 1.43 (s, 9 H), 1.40−1.68 (m, 4 H), 2.36 (m, 2 H), 2.91 (br. s, 3 H), 2.97 (s, 3 H), 3.20−3.52 (m, 2 H), 3.83−3.96 (m, 1 H), 5.06 (s, 6 H), 5.15−5.40 (m, 1 H), 7.24−7.42 (m, 15 H). ^{13}C NMR (75.5 MHz, 60 °C, $C_2D_2Cl_4$): δ 170.2, 159.9, 156.3, 155.5, 153.4, 136.55, 136.50, 135.7, 128.30, 128.25, 128.2, 128.1, 127.9, 127.8, 127.7, 127.6, 80.9, 67.6 (br), 67.7, 67.1, 66.3, 49.9 (br), 47.8, 40.3, 35.6 (br), 34.4, 31.4, 27.9, 22.9 (br). DCI (NH_3)-MS *m/z*: 675 (M + H, 62), 183 (100). HR LC-MS (EI) calcd for $C_{37}H_{47}N_4O_8$ [M^+ + H], 675.3394; found, 675.3370.

***N*$^{\beta}$,*N*$^{\omega}$,*N*$^{\omega'}$-Tris(benzyloxycarbonyl)-*N*$^{\epsilon}$,*N*$^{\omega}$-dimethyl-(*S*)-*β*-homoarginine (16).** TFA (0.857 mL) was added dropwise with stirring to a solution of the compound **15** (577 mg, 0.856 mmol) and Et_3SiH (0.344 mL, 2.14 mmol) in CH_2Cl_2 (2 mL) at +5 °C. The reaction mixture was allowed to warm to room temperature, and after 6 h, volatiles were removed in vacuo, and the oily residue was dissolved in EtOAc (40 mL), washed with water (3 × 10 mL) and brine, and dried. Evaporation in vacuo gave 500 mg (95%) of the title compound as an off-white foam. IR (KBr): 3330, 3034, 2953, 1792, 1723, 1588, 1521, 1327, 1174 cm^{-1}. ^1H NMR (300 MHz, 50 °C, [D$_6$]DMSO): δ 1.39 (m, 2 H), 1.48 (m, 2 H), 2.23−2.42 (m, 2 H), 2.87 (s, 6 H), 3.33 (m, 2 H), 3.81 (m, 1 H), 4.98 (s, 2 H), 5.05 (s, 4 H), 7.08 (d, 1 H), 7.24−7.38 (m, 15 H). ^{13}C NMR (75.5 MHz, 50 °C, [D$_6$]-DMSO): δ 172.0, 158.9, 155.7, 155.4, 152.8, 137.1, 136.7, 135.9, 128.11, 128.08, 128.05, 127.75, 127.54, 127.45, 127.3, 67.0, 66.2, 65.0, 49.6, 47.5 (br), 40.3, 35.1 (br), 31.2, 22.9 (br). DCI (NH_3)-MS *m/z* (rel intensity, %): 619 (M + H^+, 38), 183 (64), 143 (60), 126 (100).

(3′*S*,5*RS*)-5-[*N*-[3-Benzyloxycarbonylamino-6-[2,3-di-(benzyloxycarbonyl)-2,3-dimethylguanidin-1-yl)hexanoyl]-*N*-methylamino]-5,6-dihydro-2-ureido-4(1*H*)-pyrimidone (17). HATU (494 mg, 1.30 mmol) was added to the

solution of the heterocycle **3** (118 mg, 0.637 mmol) and the acid **16** (360 mg, 0.582 mmol) in DMF (3 mL). Then, DIPEA (0.225 mL, 1.30 mmol) was added dropwise, and the reaction mixture was stirred at room temperature overnight. After the workup (similar to tris-*Z*-**14**), the title compound was isolated by chromatography on silica gel (50 mL), eluting with CH_2Cl_2/MeOH (10:1), R_f 0.50; yield 0.350 g (76%) of a glasslike foam. HPLC (method C): R_t 4.42 min, peak area 94%. IR (KBr): 3399, 3336, 3151, 2942, 1717, 1577, 1497, 1456, 1375, 1338, 1273, 1143, 1055 cm^{-1}. ^1H NMR (500 MHz, CD_3OD/$CDCl_3$, 1:1): δ 1.58 (m, 4 H), 2.52 (m, 2 H) 2.79−3.00 (m, 9 H), 3.26−4.01 (m, 5 H), 4.98 (m, 7 H), 7.29 (m, 15 H). ESI-MS *m*/*z*: positive ions, 808 (M + Na, 100); negative ions, 784 (M − H, 100). HR LC-MS (EI) calcd for $C_{39}H_{48}N_9O_9$ [M + H], 786.3575; found, 786.3575.

(3′*S*,5*RS*)-5-[*N*-[3-Amino-6-(1,2-dimethylguanidin-1-yl)-hexanoyl]-*N*-methylamino]-5,6-dihydro-2-ureidopyrimid-4(1*H*)-one Dihydrochloride (18). A suspension of **17** (306 mg, 0.389 mmol) in MeOH (15 mL) with $PdCl_2$ (69.1 mg, 0.390 mmol) was hydrogenated and worked-up as described for compound **14**. After the methanol evaporated, the residue was triturated with several portions of anhydrous ether (ca. 10 mL each) to yield 160 mg (90%) of white powder. HPLC (method C): R_t 1.15 min, peak area 93%. HPLC (method A): R_t 4.44 min, peak height 94%. IR (KBr): 3375, 3194, 2941, 1716, 1622, 1569, 1275 cm^{-1}. ^1H NMR (500 MHz, [D$_4$]MeOH): δ 1.73 (br s, 4 H, 4′/5′-H), 2.79 (dd, J = 9 and 17, 1 H, 2′-H), 2.82 (s, NMe), 2.89 (s, NMe), 2.96 (dt, J = 4 and 17, 1 H, 2′-H), 3.05 (s, NMe), 3.08 (s, total intensity of all N-Me singlets is 12 H), 3.40 (br s, 2 H, 6′-H), 3.62 (m, 1 H, 3′-H), 3.73 (dd, J = 8 and 13, 1 H, 6-H), 3.86 (t, J = 13, 1 H, 6-H), 5.21 (dd, J = 8 and 13, 1 H, 5-H). ^{13}C NMR (250 MHz, [D$_4$]MeOH): δ 173.4 (C−1′), 158.9, 158.3, 158.0, 54.6 (C-5), 51.4 (C-6′), 50.1 (C-3′), 40.3 (C− 6), 39.2 (NMe), 37.2 (NMe), 36.5 (C-2′), 34.5 (NMe), 30.8 (C-5′), 29.6 (NMe), 24.5 (C-4′). ESI-MS *m*/*z*: positive ions, 384 (M + H, 100); negative ions, 382 (M − H, 100). HR LC-MS (EI) calcd for $C_{15}H_{28}N_9O_3$ [M − H], 382.2315; found, 382.2325.

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