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SYNTHESIS OF LINEAR AND CYCLIC HEXAPEPTIDES WITH N^{δ}-(1,2-DIHYDRO-1-HYDROXY-2-OXOPYRIMIDIN-4-YL)-L-LYSYL-β-ALANYL AS THE REPEATING UNIT AND PROPERTIES OF THEIR IRON(III) COMPLEXES†

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Abstract — Linear and cyclic hexapeptides including N^{δ}-(1,2-dihydro-1-hydroxy-2-oxopyrimidin-4-yl)-L-lysyl-β-alanyl as the repeating unit have been synthesized. These hexapeptides formed 1:1 iron(III) complexes; λmax 466 nm and ε4700 for a linear hexapeptide (**8**), and λmax 458 nm and ε4300 for a cyclic hexapeptide (**9**). The relative stability constants of **8**- and **9**-iron(III) complexes were estimated to be 26.1 and 28.0, respectively. A linear hexapeptide (**8**)-iron(III) complex predominantly existed in Δ-configuration. From kinetic data on iron(III) removal from human transferrin, a linear hexapeptides (**8**) was found to efficiently remove three times as much iron(III) from transferrin as desferrioxamine B, only a chemotherapeutic agent for the iron overload disease.

The iron overload disease arises from a long term blood transfusionfor treatment of a certain anemia, particularly β-thalassemia. An excess iron deposit in liver and pancreas causes the damage of these organs or other serious diseases. Siderophores, naturally-occurring low-molecular-weight iron-sequestering compounds, have paid much attention for the treatment of iron overload. They are primarily classified into two general structural classes, catecholates and hydroxamates.[1-3] Three sets of these bidentate ligands scavenge iron(III) effectively and selectively by embedding it into an octahedral binding cavity. In the case of catecholate-type ligands, design and synthesis based on these ligands have been precluded, because several catecholate ligands strongly promote the growth of pathogenic microorganisms. On the other hand, in the case of hydroxamate-type ligand, naturally-occurring trihydroxamate, desferrioxamine B (Desferal by Ciba-Geigy) has been shown to be an effective chelating agent and has reigned supreme for over 30 years in the treatment of iron overload, in spite of its problems; oral inactiveness,[4] a short half-life in blood plasma,[5] and a number of side effects such as septicemia.[6] Many efforts,[6,7] therefore, have been devoted to design and synthesize novel artificial siderophores in place of DFB.

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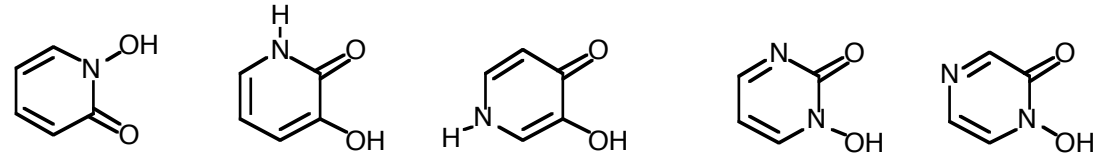
† Dedicated to Professor A. I. Meyers, Colorado State University, on the occasion of the 70th birthday.

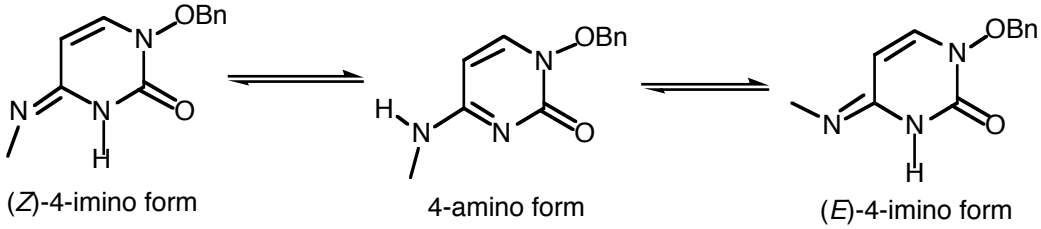
Since 1980 properties of iron(III) complexes of hydroxymonoazine-type heterocyclic bidentate ligands[7] such as 1-hydroxy-2(1*H*)-pyridinone (1,2-HOPO),[8] 3-hydroxy-2(1*H*)-pyridinone (3,2-HOPO),[8a,9] and 3-hydroxy-4(1*H*)-pyridinone (3,4-HOPO)[8a,10] have been investigated in detail. Among them, *N*-substituted 2-alkyl-3-hydroxy-4(1*H*)-pyridinones were the first compounds among hydroxymonoazine-type heterocycles to enter clinical trials, and proved their potency as new chemotherapeutic agents for the iron overload disease.[7,10c] Further, hexadentate derivatives may have certain advantages over simpler heterocyclic bidentate ligands, in particular a higher binding affinity by the chelate effect at low (clinical level) ligand concentration.

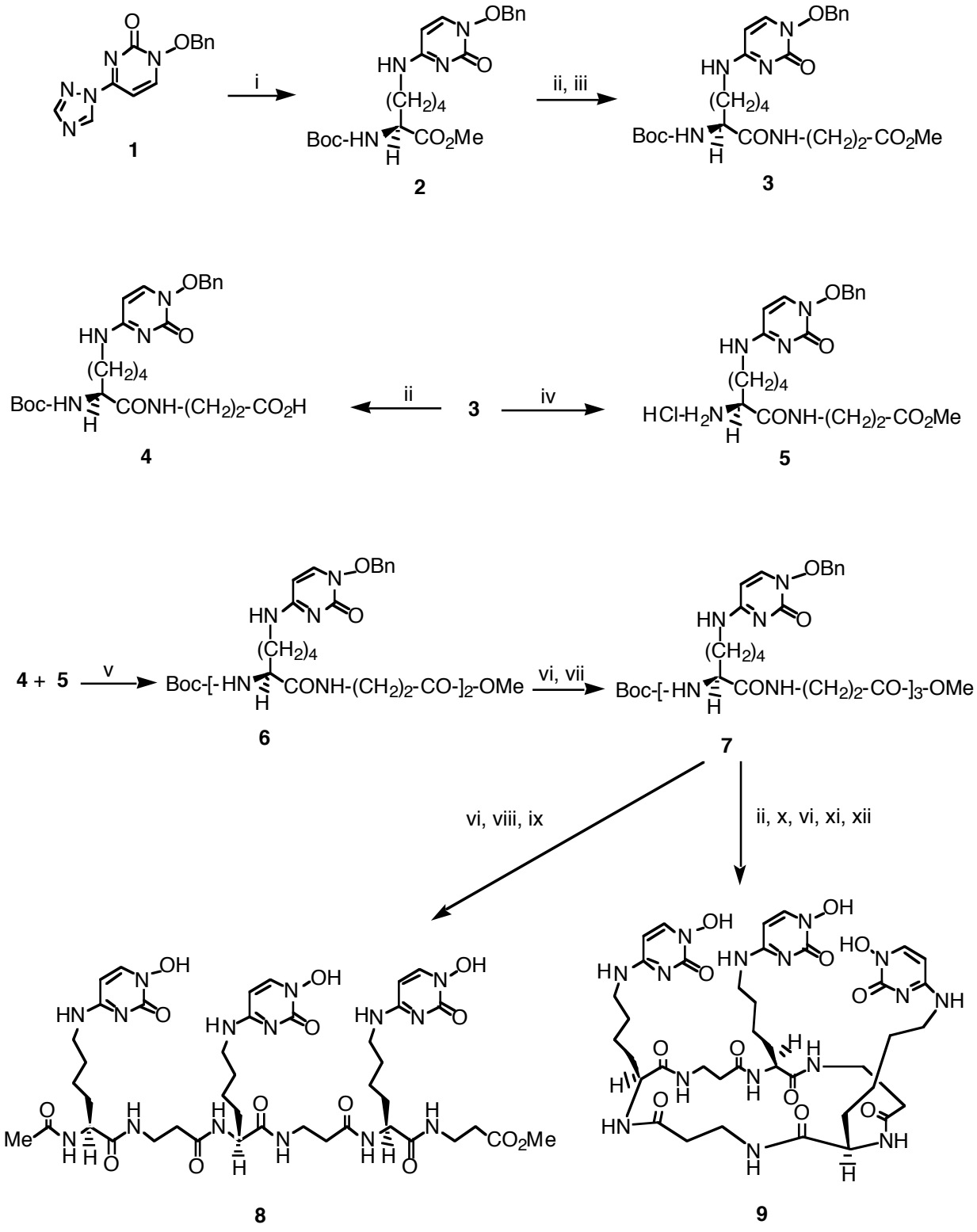


Over a decade, we have independently investigated synthesis of new hexadentate ligands containing *N*-hydroxydiazine-type heterocycles such as 1-hydroxy-2(1*H*)-pyrimidinone (HOPY)[11] and 1-hydroxy-2(1*H*)-pyrazinone (HOPR),[12] which are regarded as aza analogues of the monoazines, and functional evaluation of their iron(III) complexes. As extensive studies on the design of functional molecules and the development of chemotherapeutic agents based on hydroxyazine-type heterocycles,[13] we describe here synthesis of new linear and cyclic hexapeptides including N^{δ}-(1,2-dihydro-1-hydroxy-2-oxopyrimidin-4-yl)-L-lysyl-β-alanyl as the repeating unit and properties of thir iron(III) complexes.

RESULTS AND DISCUSSION

Synthesis of Hexapeptide Ligands: The synthetic procedure for linear and cyclic hexapeptide ligands is depicted in Scheme 1. 1-Benzyloxy-4-(1',2',4'-triazol-1'-yl)-2(1*H*)-pyrimidinone (**1**)[11b] was allowed to react with Boc-L-Lys-OMe in CH_2Cl_2 at 40 °C for 6 days to give N^{δ}-*tert*-butoxycarbonyl(Boc)-N^{δ}-(1,2-dihydro-1-hydroxy-2-oxopyrimidin-4-yl)-L-lysine methyl ester (**2**). The hydrolysis of **2** and subsequent coupling with H-β-Ala-OMe in DMF-CH_2Cl_2 mixture by using 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride (WSC·HCl) and 1-hydroxybenzotriazole (HOBt) afforded the dipeptide (**3**) as the repeating unit of hexapeptides. Dipeptide (**3**) was converted to the *C*-terminal free dipeptide (**4**) by treatment with 1M NaOH in MeOH. From ^{1}H-NMR spectral data, it is suggested that compound (**4**) exists in a tautomeric equilibrium between 4-amino and two isomers (*E* and *Z*) of 4-imino forms in $CDCl_3$ solution by virtue of internal conversion.[11c]

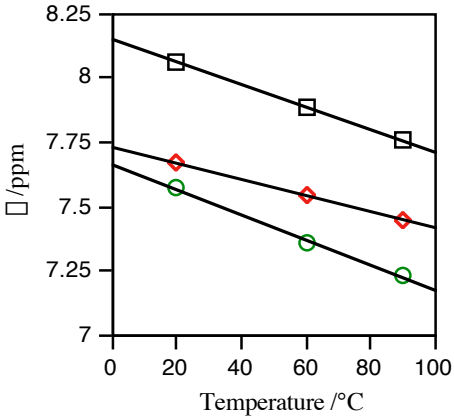




Scheme 1 *Reagents and conditions*: i) Boc-L-lys-OMe in CH_2Cl_2; ii) 1M NaOH in MeOH; iii) H-β-Ala-OMe·HCl, *N*-methylmorpholine, WSC·HCl-HOBt in DMF-CH_2Cl_2; iv) 4M HCl in dioxane; v) *N*-methylmorpholine, WSC·HCl-HOBt in DMF-CH_2Cl_2; vi) TFA in CH_2Cl_2; vii) **4**, *N*-methylmorpholine, WSC·HCl-HOBt in DMF-CH_2Cl_2; viii) Ac_2O, *N*-methylmorpholine in DMF; ix) H_2/10% Pd-C in MeOH; x) WSC·HCl-HOSu in DMF; xi) pyridine (a high dilution condition); xii) H_2/10%Pd-C in DMF-MeOH.

On the other hand, the deprotection of the Boc group of compounds (**3**) with 4M HCl in dioxane gave $N^{\square}$-terminal free dipeptide (**5**). Two components (**4** and **5**) were coupled by means of WSC·HCl-HOBt method to give the tetrapeptide (**6**). The Boc group of the tetrapeptide (**6**) was removed by treatment with TFA to give the corresponding TFA salts, and then this salts was coupled with the dipeptide (**4**) by means of WSC·HCl-HOBt method to give the hexapeptide (**7**). The Boc deprotection of the hexapeptide (**7**), *N*-acetylation with Ac_2O, and then the debenzylation by the catalytic hydrogenation gave the desired linear hexapeptide ligand (**8**). The hexapeptide (**7**) was treated with 1M NaOH to give the *C*-terminal free hexapeptide, and then it was converted into the corresponding *O*-succinimide ester. After removal of the Boc group of the *O*-succinimide ester and subsequent removal of the Boc group with TFA, the cyclization of the resulting TFA salt was carried out in pyridine at 60 ℃ for 3 days under high dilution conditions. Finally the deprotection of the *O*-benzyl group afforded the desired cyclic peptide (**9**).

Conformation in Solution: ^{1}H-NMR spectrum of cyclic hexapeptide (**9**) in DMSO-d_6 solution at 20 ℃ exhibited three sets of –NH- protons (□ 8.1-7.55), a doublet, a triplet, and a broad singlet, indicating that cyclic hexapeptide (9) possesses the pseudo-C_3-symmetry. The temperature dependence of the –NH-proton chemical shifts was measured in the range from 20 to 90 ℃, and plots gave straight lines as shown in Figure 1. The temperature coefficient of –3.15 x 10^{-3} ppm K^{-1} in Table 1 is comparable to the value (-3 x 10^{-3} ppm K^{-1}) for intramolecularly hydrogen-bonded amide-protons,[14] indicating the existence of a strong hydrogen bond between □-alanyl –NH- and –CO-. In a case of linear hexapeptide (**8**), no particular hydrogen bonds were observed in DMSO-d_6 solution.



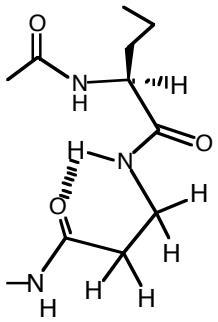


Table 1 Temperature coefficients (x 10^{-3} ppm K^{-1})

a	-4.58
b	-3.15
c	-4.89

Figure 1 The temperature dependence of the -NH- proton chemical shifts: (a, -□-); lysyl NH, (b, -◇-); □-alanyl NH, (c, -○-); lysyl $N^{\square}$-H

Iron(III) Complex Formation: In order to examine iron(III) complexation ability of hexapeptide ligands (**8** and **9**), UV-VIS spectra of a 1:1 molar mixture of hexapeptide and $Fe(NO_3)_3$ in aqueous solution under various pH conditions were measured. In the case of linear hexapeptide (**8**), the absorption maxima due to the characteristic LMCT (ligand-to-metal charge transfer) were observed at 465-473 nm over a wide pH range from 2.4 to 8.5. At pH 5.4 □max and □values were 456 nm and 4700, respectively, which are comparable to iron(III) complexes of 1-hydroxy-2(1*H*)-pyridinone-containing tripodal hexapeptate ligands,[11b,11c] indicating the formation of an intramolecular 1:1 complex of iron(III) to hexapeptide (**8**).

Cyclic hexapeptiide (**9**) also showed similar behaviors; □max=458 nm and □=4300 at pH 6.6.

The relative stability constant ($K_{Fe(L)}$) of linear and cyclic hexapeptide ligands (**8** and **9**) with iron(III) is defined by the following equation 1.

$$Fe^{3+} + L^{3-} \rightleftarrows Fe(L) \qquad K_{Fe(L)} = [Fe(L)]/[Fe^{3+}][L^{3-}] \qquad (1)$$

The competitive reaction between EDTA and these ligands was carried out in order to estimate the stability constants of iron(III) complexes.[10d,15] The relative stability constant (log K=28.0) of cyclic hexapeptide (**9**)-iron(III) complex was higher than that (log K=26.1) of linear hexapeptide (**8**)-iron(III) one. This result is consistent with that seen in naturally-occurring siderophores-iron(III) complexes; log K=30.5 for linear trihydroxamate-iron(III) complex, ferrioxamine B vs. log K=32.4 for cyclic trihydroxamate-iron(III) one, ferrioxamine E.[16]

Configuration of Iron(III) Complex: CD spectra of hexapeptide-iron(III) complexes were measured in aqueous solutions in order to examine the influence of the peptide backbone composed of a L-lysyl-□-alanyl sequence upon the abusolute configuration[17] of iron(III) complexes. Iron(III) complex of linear hexapeptide (**8**) showed a negative band at around 490 nm and a positive band at around 400 nm as shown in Figure 2, indicating that **8**-iron(III) complex predominantly exists in a □-configuration. It has already reported that iron(III) complex of 3-hydroxy-4(1*H*)-pyridinone-containing linear hexapeptide with a L-lysyl-□-alanyl sequence predominantly exists in a □-configuration.[10d] It is the fist example that the difference in heterocyclic bidentates, 1-hydroxy-2(1*H*)-pyrimidinone vs. 3-hydroxy-4(1*H*)-pyridinone, causes change in the absolute configuration of iron(III) complexes. On the other hand, CD spectrum of **9**-iron(III) complex showed no particular pattern.

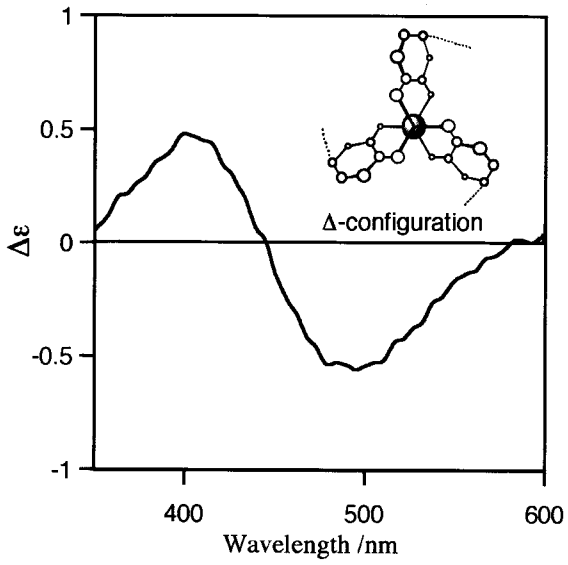


Figure 2 CD spectrum of **8**-iron(III) complex in aqueous solution

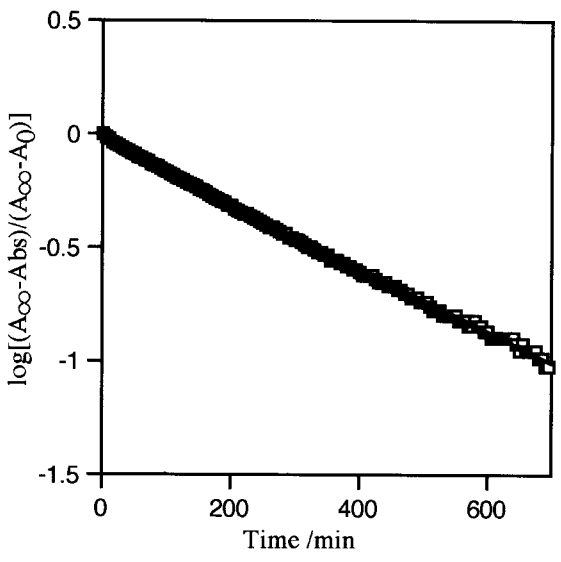


Figure 3 The plots of log[(A∞-Abs)/(A∞-A0)] vs. time on iron(III) removal of **8** from Fe2.00Tf

Iron(III) Removal from Transferrin: The iron(III) removal efficiency of synthetic hexapeptide ligands from human trannsferrin[18] *in vitro* is one of the important factors for elucidating the potential application to a

chemotherapeutic agent for the iron overload disease. After addition of a 20-fold excess of linear hexapeptide (**8**) to a solution of diiron(III) human transferrin ($Fe_{2.00}$TF) in 0.1 M Tris buffer (pH 7.4), the absorbance at 465 nm was measured at appropriate intervals.[10d] Plots of $\log[(A_\infty - A_{obs})/(A_\infty - A_0)]$ as a function of time gave a good linear relationship as shown in Figure 3, indicating that the reaction of iron removal from transferrin by linear hexapeptide (**8**) proceeded in the pseudo-first-order kinetics. From the slope of the line, k_{obs} value was caliculated to be 3.2 x 10^{-3} min^{-1}, this value being comparable to those of 1-hydroxy-2(1*H*)-pyrimidinone-containing tripodal hexadentate ligands.[11b, 11c] Further, linear hexapeptide (**8**) efficiently removed 3 times as much iron(III) from transferrin as DFB did, primarily for kinetic rather than thermodynamic reasons, even at a smaller concentration ratio at 30 min after the reaction was initiated. Unfortunately, kinetic data for cyclic hexapeptide (**9**) could not be obtained because of low water solubility under the employed conditions.

Table 2 Iron(III) removal of linear hexapeptide (**8**) from transferrin at pH 7.4

Ligand	[ligand]/[$Fe_{2.00}$Tf]	k_{obs} (x10^{-3} min^{-1})	% Fe removed (at a point 30 min)
8	20	3.2±0.1	15
DFB*	100	0.66	5

* Ref. 18a

EXPERIMENTAL

Melting points were recorded on a Mel-Temp apparatus in open capillaries and are uncorrected. IR spectra were recorded on JASCO FT/IR-230 infrared spectrophotometer. ^{1}H-NMR spectra were recorded on a JNM-LA400D spectrometers using Me_4Si as an internal standard. Optical rotations were measured on a JASCO DIP-370 digital polarimeter. UV-VIS spectra were recorded on a JASCO Ubest V-550 spectrophotometer. CD spectra were measured with a JASCO J-500 spectropolarimeter. Column chromatography was carried out with Merck Kieselgel 60 (230-400 mesh). Combustion analyses were performed on a Perkin Elmer 2400 II CHNS Analyzer.

***N*□-*tert*-Butoxycarbonyl-*N*□-(1-benzyloxy-1,2-dihydro-2-oxopyrimidin-4-yl)-L-lysine methyl ester (2):** A mixture of Boc-L-Lys-OMe (360 mg, 1.38 mmol) and compound (**1**) (372 mg, 1.38 mmol) in CH_2Cl_2 (20 mL) was stirred for 6 days at 40 ℃. CH_2Cl_2 (100 mL) was added to the reaction mixture. The organic layer was successively washed with 10% citric acid (40 mL), 5% $NaHCO_3$ (40 mL), H_2O (40 mL), saturated NaCl (40 mL), and then dried over anhydrous Na_2SO_4. After evaporation of the solvent, the residual oil was chromatographed on silica gel with $CHCl_3$:acetone:EtOH (100:10:2) mixture to give the product (**2**) (526 mg, 83%) as an amorphous white solid: $[□]_D^{20}$ –3.4° (c=1 in MeOH); IR (KBr): 3283, 1741, 1705, 1632, 753 and 699 cm^{-1}; ^{1}H-NMR (□, $CDCl_3$, 400 MHz): 1.43 (9H, s), 1.36-1.81 (6H, m), 3.41-3.48 (2H, m), 3.74 (3H, s), 4.28 (1H, m), 5.14 (1H, br s), 5.21 (2H, s), 5.26 (1H, d, J=7.7 Hz), 6.88 (1H, d, J=7.7 Hz), and 7.36-7.41 ppm (5H, m). *Anal.* Calcd for $C_{23}H_{32}N_4O_6$: C, 59.98; H, 6.96; N, 12.17. Found: C, 59.69; H, 7.18; N, 12.26.

N^{α}-*tert*-Butoxycarbonyl-N^{ε}-(1-benzyloxy-1,2-dihydro-2-oxopyrimidin-4-yl)-L-lysyl-β-alanine methyl ester (3): To a solution of compound (**2**) ((340 mg, 0.74 mmol) in MeOH (5 mL) was slowly added 1M NaOH (7.5 mL, 0.75 mmol) at 0 ℃ and the reaction mixture was stirred for 3 h at rt. The solution was adjusted to pH 7 with 10% citric acid, and then the solvent was evaporated under reduced pressure. 10% Citric acid (30 mL) was added to the residue, and then the aqueous solution was extracted with $CHCl_3$ (100 mL x 2). The organic layer was washed with H_2O (50 mL), saturated NaCl (50 mL), and then dried over anhydrous Na_2SO_4. Removal of the solvent afforded the *C*-terminal-free carboxylic acid as an amorphous white solid, N^{α}-*tert*-butoxycarbonyl-N^{ε}-(1-benzyloxy-1,2-dihydro-2-oxopyrimidin-4-yl)-L-lysine (324 mg, 98%): IR (KBr): 3327, 1703, 1634, 752 and 700 cm^{-1}; ^{1}H-NMR (δ, $CDCl_3$, 400 MHz): 1.36 (9H, s), 1.47-1.89 (6H, m), 3.08-3.35 (2H, m), 4.12-4.37 (1H, m), 5.09 (s, 1H) and 5.07 and 5.10 (1H, ABq, J=10.9 and 13.9 Hz), 5.39 (d, 0.5H, J=7.8 Hz) and 5.51 (d, 0.5H, J=7.8 Hz), 5.43 (0.5H, J=7.4 Hz) and 5.47 (0.5H, d, J=7.4 Hz), 6.82 (0.5H, d, J=7.8 Hz) and 7.12 (0.5H, d, J=7.8 Hz), and 7.20-7.31 ppm (5H, m).

To a solution of the *C*-terminal-free carboxylic acid (537 mg, 1.2 mmol), H-β-Ala-OMe·HCl (182 mg, 1.3 mmol), *N*-methylmorpholine (131 mg, 1.3 mmol), HOBt·H_2O (200 mg, 1.3 mmol) in dry DMF (4 mL) was added a solution of WSC·HCl (249 mg, 1.3 mmol) in dry CH_2Cl_2 (6 mL) at 0 ℃, and then the reaction mixture was stirred for 2 h at 0 ℃ and then another 48 h at rt. After evaporation of the solvent, the residue was dissolved in H_2O (40 mL), and the aqueous solution was extracted with $CHCl_3$ (100 mL x 2). The organic layer was successively washed with 10% citric acid (50 mL x 3), 5% $NaHCO_3$ (50 mL x 3), H_2O (50 mL x 3), saturated NaCl (50 mL), and then dried over anhydrous Na_2SO_4. After evaporation of the solvent, the residue was chromatograped on silica gel with $CHCl_3$:acetone:EtOH (100:40:8) mixture to give the product (**3**) (551 mg, 87%) as a white amorphous white solid: $[\alpha]_D^{20}$ –5.3° (c=0.4 in MeOH); IR (KBr): 3286, 1745, 1700, 1650, 1633, 751 and 700 cm^{-1}; ^{1}H-NMR (δ, $CDCl_3$, 400 MHz): 1.33 (9H, s), 1.45-1.94 (6H, m), 2.52 (2H, t, J=6.7 Hz), 3.23-3.40 (0.8H, m), 3.45-3.66 (3.2H, m), 3.59 (3H, s), 4.08-4.17 (1H, m), 5.09 (2H, s), 5.36 (0.2H, d, J=7.6 Hz), 5.43 (0.8H, d, J=7.6 Hz), 5.52 (1H, d, J=8.0 Hz), 6.69 (1H, br s), 6.82 (0.8H, d, J=7.6 Hz), 7.04 (0.2H, d, J=7.6 Hz), 7.31-7.40 (5H, m), and 7.71 ppm (1H, br s). *Anal*. Calcd for $C_{26}H_{37}N_5O_7 \cdot H_2O$: C, 56.82; H, 7.15; N, 12.74. Found: C, 57.10; H, 7.08; N, 12.89.

N^{α}-*tert*-Butoxycarbonyl-N^{ε}-(1-benzyloxy-1,2-dihydro-2-oxopyrimidin-4-yl)-L-lysyl-β-alanyl-N^{ε}-(1-benzyloxy-1,2-dihydro-2-oxopyrimidin-4-yl)-L-lysyl-β-alanine methyl ester (6): The hydrolysis of compound (**3**) (170 mg, 0.32 mmol) with 1M NaOH (4 mL, 4 mmol) in MeOH (10 mL) was carried out in the same manner as described above to give N^{α}-*tert*-butoxycarbonyl-N^{ε}-(1-benzyloxy-1,2-dihydro-2-oxopyrimidin-4-yl)-L-lysyl-β-alanine (**4**) (141 mg, 85 %) as an amorphous with solid: IR(KBr): 3286, 3080 (br), 1707, 1634, 755 and 700 cm^{-1}; ^{1}H-NMR (δ, CD_3OD, 400 MHz): 1.43 (9H, s), 1.44-1.74 (6H, m), 2.51 (2H, t, J=6.6 Hz), 3.31-3.52 (2H, m), 3.45-3.66 (2H, m), 4.02 (1H, m), 5.13 (2H, s), 5.56 (1H, d, J=7.6 Hz), 7.31 (1H, d, J=7.6 Hz), and 7.37-7.42 ppm (5H, m).

To a solution of compound (**3**)(170 mg, 0.32 mmol) in dry dioxane (1 mL) was carefully added 4M HCl in dioxane (2.4 mL), and then the reaction mixture was stirred for 30 min on an ice-bath. After evaporation of

the solvent under reduced pressure, the residue was dissolved in benzene (10 mL) and then evaporated off. This work-up was repeated 3 times to give N^{α}-(1-benzyloxy-1,2-dihydro-2-oxopyrimidin-4-yl)-L-lysyl-β-alanine methyl ester HCl salt (**5**) (149 mg, 100%), which was used for the next reaction without further purification.

To a solution of compound (**4**) (141 mg, 0.27 mmol), compound (**5**) (149 mg, 0.32 mmol), *N*-methylmorpholine (92 mg, 0.91 mmol), HOBt·H_2O (49 mg, 0.32 mmol) in dry DMF (2 mL) was added a solution of WSC·HCl (61 mg, 0.32 mmol) in dry CH_2Cl_2 (3 mL) at –10 ℃, and then the reaction mixture was stirred for 1 h at 0 ℃ and for another 48 h at rt. After evaporation of the solvent, $CHCl_3$ (150 mL) was added to the residue. The organic layer was successively washed with 10% citric acid (30 mL x 3), 5% $NaHCO_3$ (30 mL x 3), H_2O (30 mL x 3), saturated NaCl (30 mL), and then dried over anhydrous Na_2SO_4. Removal of the solvent, and sebsequent colum chromatography on silica gel with $CHCl_3$:MeOH (12:1) mixture afforded the tetrapeptide (**6**) (183 mg, 73%) as an amorphous white solid: $[\alpha]_D^{28}$ –4.5° (c=0.3 in MeOH); IR (KBr): 3283, 1734, 1708, 1655, 1635, 748 and 699 cm^{-1}; ^{1}H-NMR (δ, CD_3OD, 400 MHz): 1.42 (9H, s), 1.23-1.77 (12H, m), 2.43 (2H, t, J=6.6 Hz), 2.55 (2H, t, J=6.6 Hz), 3.30-3.49 (8H, m), 3.66 (3H, s), 3.97 (1H, m), 4.22 (1H, m), 5.13 (4H, s), 5.56 (2H, d, J=7.6 Hz), 7.32 (2H, d, J=7.6 Hz), 7.37-7.39 (5H, m), and 7.43-7.46 ppm (5H, m). *Anal.* Calcd for $C_{46}H_{62}N_{10}O_{11}\cdot 3H_2O$: C, 56.07; H, 6.96; N, 14.21. Found: C, 55.74; H, 6.72; N, 13.88.

N^{α}-*tert*-Butoxycarbonyl-N^{α}-(1-benzyloxy-1,2-dihydro-2-oxopyrimidin-4-yl)-L-lysyl-β-alanyl-N^{α}-(1-benzyloxy-1,2-dihydro-2-oxopyrimidin-4-yl)-L-lysyl-β-alanyl-N^{α}-(1-benzyloxy-1,2-dihydro-2-oxopyrimidin-4-yl)-L-lysyl-β-alanine methyl ester (7):

To a solution of compound (**6**) (160 mg, 0.17 mmol) in dry CH_2Cl_2 (2 mL) was added dropwise CF_3CO_2H (2 mL) on an ice-water bath, and then the reaction mixture was stirred for 30 min at rt. After evaporation of the solvent under reduced pressure, the residue was dissolved in EtOH (10 mL) and then evaporated off. This work-up was repeated 4 times to give the TFA salt of the corresponding tetrapeptide (162 mg, 100%), which was used for the next reaction without further purification.

To a solution of compound (**4**) (98 mg, 0.19 mmol), the TFA salt (162 mg, 0.17 mmol), *N*-methylmorpholine (124 mg, 1.23 mmol), HOBt·H_2O (29 mg, 0.19 mmol) in dry DMF (1 mL) was added a solution of WSC·HCl (36 mg, 0.19 mmol) in dry CH_2Cl_2 (2 mL) at –10 ℃, and then the reaction mixture was stirred for 1 h at 0 ℃ and for another 72 h at rt. After evaporation of the solvent, the residue was extracted with hot $CHCl_3$ (100 mL x 7). The organic layer was successively washed with 10% citric acid (200 mL), 5% $NaHCO_3$ (200 mL), H_2O (200 mL), saturated NaCl (200 mL), dried over anhydrous Na_2SO_4, and then the solvent was evaporated off under reduced pressure. The crude product was purified by colum chromatography on silica gel with $CHCl_3$:MeOH (6:1) mixture and subsequent gel chromatography on Sephadex LH-20 with MeOH as an eluent to give the hexapeptide (**7**) (186 mg, 82%) as an amorphous white solid: IR (KBr): 3294, 1735, 1702, 1650, 1635, 761 and 700 cm^{-1}; ^{1}H-NMR(δ, CD_3OD, 400 MHz): 1.42 (9H, s), 1.53-1.60 (18H, m), 2.43-2.46 (4H, m), 2.52-2.56 (2H, m), 3.30-3.51 (12H, m), 3.65 (3H, s), 4.98 (1H, m), 4.19-4.24 (2H, m), 5.13 (6H, s), 5.57 (3H, d, J=7.6 Hz), 7.32 (3H, d, J=7.6 Hz), and 7.37-7.46 ppm (15H, m). *Anal.* Calcd for $C_{66}H_{87}N_{15}O_{15}\cdot H_2O$: C, 58.78; H, 6.65; N, 15.58. Found: C, 58.92; H, 6.63; N, 15.30.

N^{α}-Acetyl-N^{α}-(1,2-dihydro-1-hydroxy-2-oxopyrimidin-4-yl)-L-lysyl-β-alanyl-N^{α}-(1,2-dihydro-1-hydroxy-2-oxopyrimidin-4-yl)-L-lysyl-β-alanyl-N^{α}-(1,2-dihydro-1-hydroxy-2-oxopyrimidin-4-yl)-L-lysyl-β-alanine methyl ester (8):

The treatment of compound (**7**) (165 mg, 0.12 mmol) in dry CH_2Cl_2 (1 mL) with CF_3CO_2H (2.5 mL) was carried out in the same manner as described above to give the TFA salt of the hexapeptide (167 mg, 100%), which was used for the next reaction without further purification.

To a solution of the TFA salt (167 mg, 0.12 mmol) and *N*-methylmorpholine (104 mg, 1 mmol) in dry DMF (1.5 mL) was added dropwise a solution of Ac_2O (38 mg, 0.37 mmol) in dry DMF (1.5 mL) at rt, and then the reaction mixture was stirred for 2 h at rt. After removal of the solvent under reduced pressure, the residue was extracted with $CHCl_3$ (100 mL x 8), and then dried over anhydrous Na_2SO_4. After evaporation of the solvent, the residue was purified by gel chromatography on Sephadex LH-20 with MeOH as an eluent to give *N*-acetylated hexapeptide (61 mg, 39%) as an amorphous white solid: ^{1}H-NMR (δ, CD_3OD, 400 MHz): 1.29-1.78 (18H, m), 2.00 (3H, s), 2.41-2.56 (6H, m), 3.30-3.50 (12H, m), 3.64 (3H, s), 4.22-4.34 (3H, m), 5.13 (6H, s), 5.57 (3H, d, J=7.6 Hz), 7.25 (3H, d, J=7.6 Hz), and 7.31-7.45 ppm (15H, m).

A suspension of 10% Pd-C (6 mg) in MeOH (10 mL) was pre-hydrogenated with H_2 for 30 min. To the suspension was added a solution of the *N*-acetylated hexapeptide (61 mg, 0.048 mmol) in MeOH (5 mL). After hydrogenation under H_2 atmospheric pressure for 1 h, the catalyst was removed by filtration. The filtrate was evaporated off, and then the residue was purified by gel chromatography on Sephadex LH-20 with MeOH to give the desired linear hexapeptide (**8**) (39 mg, 81%) as an amorphous white solid: hydroxamic acid test (+); $[\alpha]_D^{23}$ –33.2° (c=0.1 in H_2O); IR (KBr): 3281, 3160 (br), 1730, 1722, 1702, 1672, and 1637 cm^{-1}; ^{1}H-NMR (δ, CD_3OD, 400 MHz):1.27-1.79 (18H, m), 2.00 (3H, s), 2.45-2.59 (6H, m), 3.30-3.52 (12H, m), 3.66 (3H, s), 4.24 (3H, m), 5.73 (3H, d, J=7.6 Hz), and 7.61 ppm (3H, d, J=7.6 Hz). Anal. Calcd for $C_{42}H_{63}N_{15}O_{14}\cdot H_2O$: C, 49.45; H, 6.42; N, 20.59. Found: C, 49.25; H, 6.46; N, 20.30.

Cyclo[N^{α}-(1,2-dihydro-1-hydroxy-2-oxopyrimidin-4-yl)-L-lysyl-β-alanyl-N^{α}-(1,2-dihydro-1-hydroxy-2-oxopyrimidin-4-yl)-L-lysyl-β-alanyl-N^{α}-(1,2-dihydro-1-hydroxy-2-oxopyrimidin-4-yl)-L-lysyl-β-alanyl] (9):

To a solution of compound (**7**) (398 mg, 0.30 mmol) in MeOH (10 mL) was added 1M NaOH (3 mL, 3 mmol), and then the reaction mixture was stirred for 2 h at rt. The solution was adjusted to pH 7 with 5% citric acid, and then the solvent was removed under reduced pressure. The solution of the residue in H_2O (50 mL) was adjusted to pH 2 with 6M HCl on an ice-bath, and then extracted with $CHCl_3$ (100 mL x 2). The organic layer was washed with H_2O (50 mL) and dried over anhydrous Na_2SO_4. Removal of the solvent afforded the corresponding *C*-terminal free hexapeptide (376 mg 95%) as an amorphous solid: ^{1}H-NMR (δ, CD_3OD, 400 MHz): 1.42 (9H, s), 1.53-1.60 (18H, m), 2.39-2.56 (6H, m), 3.30-3.49 (12H, m), 3.64 (3H, s), 3.98 (1H, m), 4.18-4.24 (2H, m), 5.11 (6H, s), 5.57 (3H, d, J=7.6 Hz), 6.89 (3H, d, J=7.6 Hz), and 7.31-7.40 ppm (15H, m). *Anal.* Calcd for $C_{65}H_{85}N_{15}O_{15}\cdot H_2O$: C, 57.72; H, 6.63; N, 15.53. Found: C, 57.43; H, 6.38; N, 15.26.

To a solution of the *C*-terminal free hexapeptide (356 mg, 0.27 mmol) in dry DMF (5 mL) was added HOSu (62 mg, 0.54 mmol) at -10 ℃. A solution of WSC·HCl (103 mg, 0.54 mmol) in dry CH_2Cl_2 (5 mL) was added to the reaction mixture at -10 ℃, and then the reaction mixture was stirred for 1 h at 0 ℃ and another for 24 h at rt. After removal of the solvent under reduced pressure, the residue was dissolved in $CHCl_3$ (200 mL). The organic layer was washed with H_2O (50 mL x 2) and dried over anhydrous Na_2SO_4. Removal of the solvent under reduced pressure afforded the corresponding *O*-succinimide ester (359 mg, 94%): IR(neat): 1814, 1780, and 1738 cm^{-1}. This product was used for the next reaction without further purification.

To a solution of the *O*-succinimide ester (359 mg, 0.25 mmol) in dry CH_2Cl_2 (2 mL) was added CF_3CO_2H (3 mL), and then the reaction mixture was stirred for 1 h on an ice-water bath. Removal of the solvent under reduced pressure afforded the corresponding TFA salt (363 mg, 100%) as an amorphous white solid. This salt was used for the next reaction without further purification.

A solution of the TFA salt (363 mg, 0.25 mmol) in dry DMF (5 mL) was added dropwise to pyridine (100 mL), and then the reaction mixture was stirred for 3 days at 60 ℃. After removal of the solvent, the crude product was purified by gel chromatography on Sephadex LH-20 with DMF as an eluent to give the cyclic hexapeptide (88 mg, 29%) as an amorphous pale brown solid: ninhydrin test (-); IR(KBr): 3278, 1639, 761, and 700 cm^{-1}; ^{1}H-NMR (□, DMSO-d_6, 400 MHz): 1.46-1.81 (18H, m), 2.27 (3H, m) and 2.60 (3H, m), 3.30-3.52 (12H, m), 4.01-4.06 (3H, m), 5.18 (6H, s), 5.65 (3H, d, J=7.6 Hz), 7.41-7.53 (15H, m), 7.68 (3H, d, J=7.6 Hz), 7.80 (3H, t, J=5.1 Hz), 7.88 (3H, t, J=5.8 Hz), and 8.24 ppm (3H, d, J=5.8 Hz). *Anal.* Calcd for $C_{60}H_{75}N_{15}O_{12}\cdot 3H_2O$: C, 57.54; H, 6.51; N, 16.77. Found: C, 57.22; H, 6.37; N, 16.75.

A suspension of 10% Pd-C (10 mg) in MeOH (2 mL) was pre-hydrogenated with H_2 for 0.5 h. To the suspension was added a solution of the cyclic hexapeptide (72 mg, 0.06 mmol) in DMF (5 mL). After hydrogenation under H_2 atmospheric pressure for 8 h, the catalyst was removed by filtration. The filtrate was evaporated, and the residue was purified by gel chromatography on Sephadex LH-20 with DMF as an eluent to give the desired cyclic hexapeptide (**9**) (20 mg, 32%) as an amorphous pale brown solid: hydroxamic acid test (+); $[□]_D^{28}$ +3.3° (c=0.1 in DMF); ^{1}H-NMR (□, DMSO-d_6, 400 MHz): 1.23-1.65 (18H, m), 2.27 (3H, m) and 2.60 (3H, m), 3.30-3.52 (12H, m), 4.01-4.06 (3H, m), 5.18 (6H, s), 5.55 (3H, d, J=7.6 Hz), 7.57 (3H, t, J=5.8 Hz), 7.65 (3H, d, J=7.6 Hz), 7.66 (3H, t, J=5.1 Hz), 8.07 (3H, d, J=5.8 Hz), and 10.9 ppm (3H, br s). *Anal.* Calcd for $C_{60}H_{75}N_{15}O_{12}\cdot 3H_2O$: C, 57.54; H, 6.51; N, 16.77. Found: C, 57.22; H, 6.37; N, 16.75.

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