N-Hydroxy Amides. X.[1] Synthesis of a Nonapeptide with an Ala-(HO)Gly-Ala[2] Sequence and Its Spectral and Iron(III) Holding Properties

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A nonapeptide with an Ala–(HO)Gly–Ala sequence has been synthesized via condensation of appropriately protected tripeptide units. ^{1}H NMR and CD spectral data indicate that the nonapeptide has a disordered structure in DMSO and water. The peptide forms a 1 : 3 complex of iron(III) with hydroxamate groups at neutral pH when mixed with an aqueous iron(III) solution. The complex shows λ_{max} at 410 nm with an ε of 2160 at pH 7. Alanine residues influence the hydroxamate groups to produce a chiral complex, the CD spectrum of which reveals bands at 355 nm ($\Delta\varepsilon$ +0.8) and 435 nm ($\Delta\varepsilon$ −1.8), showing a preference for the Δ-configuration around the iron.

Naturally occurring hydroxamic acids show unique biological activity, acting as tumour inhibitors, antibiotics, or growth factors.[3-8] The hydroxamate group is a powerful bidentate oxygen ligand for certain metal ions, including iron(III).[8,9] Thus, three hydroxamate groups of peptide desferrichromes[3] or oligoamide desferrioxamines[4] entrap iron(III) in a hexadentate octahedral configuration for cell-membrane transport.[3-8] In mimicking natural hydroxamate ligands, we prepared retrohydroxamate desferrioxamines with ω-amino acids derivatives by way of peptide synthesis.[10] In view of these natural and artificial analogs, we thought it useful to synthesize similar trihydroxamate ligands comprising solely α-amino acid residues, since they may show the properties of hydroxamic acids, while retaining other important peptide features.[11]

Our previous reports described the synthesis of a few poly(*N*-hydroxy-DL-α-amino acid)s[12] and a hexapeptide containing an *N*-hydroxy-DL-alanylglycyl sequence, together with their iron(III)-holding properties.[13] In those derivatives, close spacings (of one or four atoms) between hydroxamic acid groups produced no stable tris(hydroxamato) iron(III) complexes; also, the racemic *N*-hydroxy-α-amino acid residues gave no conformational and configurational information. Higher sequential analogs, however, are expected to have an increased tendency for the formation of the tris-(hydroxamato) iron(III) complex, because of flexibility due to their wider spacing. As the next higher homolog of the *N*-hydroxy-DL-alanylglycyl sequence, we synthesized a nonapeptide with an L-Ala–(HO)Gly–L-Ala tripeptide sequence. The purpose of this paper is to examine: i) if there is any conformational tendency for the *N*-hydroxypeptide, ii) whether a tripeptide sequence is suitable to make an intramolecular tris(hydroxamato) iron(III) complex or not, and iii) how the chiral units in the chain influence the configuration of the formed complex.

Results and Discussion

Synthesis. The *N*-hydroxyamino acid residue was introduced through a protected form, H–(BzlO)Gly–OH. The use of the Boc group in combination with an alkali-sensitive (BzlO)Gly unit required such a group as the phenacyl[14] for protecting a carboxyl group. Both the intermediates and final products prepared are shown in Fig. 1. Nonapeptide **3d** was derived from the appropriately protected tripeptide units via fragment condensation processes (Fig. 2); the condensation was carried out by a racemization-saving EDC–HOBt method.[15] Final catalytic hydrogenation, after acetylation of the *N*-terminal amino group, afforded the desired nonapeptide, **3d**. Good solubility of **3d** in methanol, ethanol and water was notable. The acetyltripeptide **1d** and -hexapeptide **2d** were derived from the protected derivatives, **1a** and **2a**. Although nonapeptide **3d** has only a 7-atom spacing between the hydroxamic acid groups, its close natural analogs could be linear desferrioxamines, having an 8- or 9-atom spacing.[4]

Spectral Data. In order to obtain structural information concerning *N*-hydroxy peptides, ^{1}H NMR and CD spectral data were collected. The ^{1}H NMR spectra were

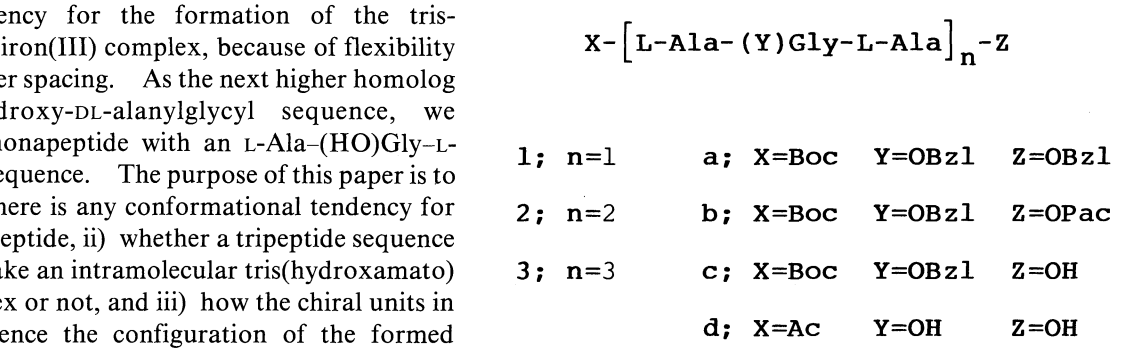

Fig. 1. Intermediates and final products in the synthesis of nonapeptide **3d**.

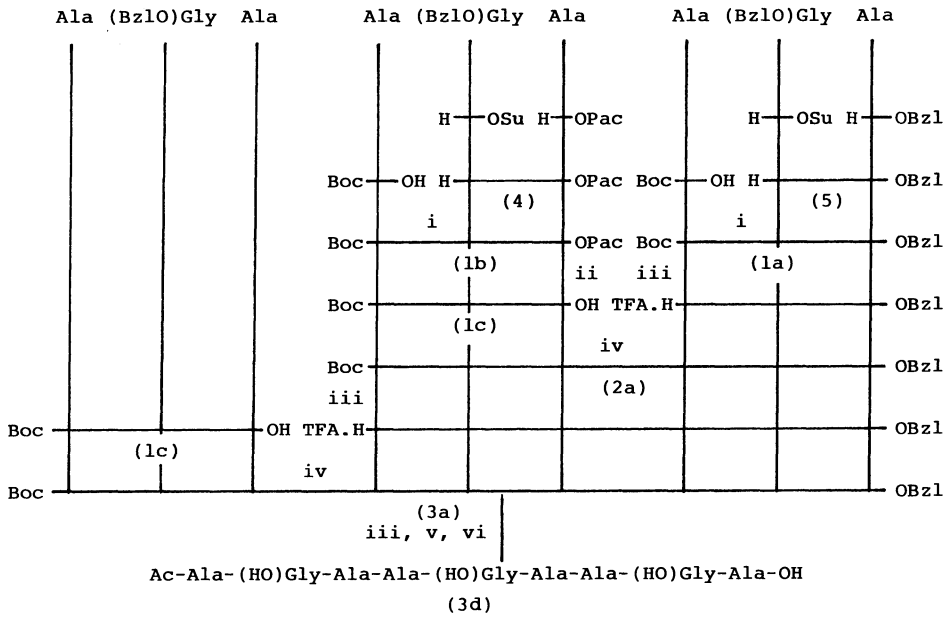

Fig. 2. Synthesis of nonapeptide **3d** from *N*-benzyloxytripeptide units. Reaction conditions: (i) *i*-BuOCOCl–Et_3N; (ii) Zn–AcOH; (iii) TFA in CH_2Cl_2; (iv) EDC–HOBt; (v) Ac_2O in THF; (vi) H_2–Pd/C in MeOH.

Table 1. ^1H NMR Data for *N*-Hydroxypeptides **1d**, **2d**, and **3d** in DMSO-d_6 at 80°C[a)]

Ac	CH_3[b)]		CH_2[c)]	C–H[b)]	NH[b)]
			Ac–X_1–(HO)X_2–X_3 (**1d**)		
1.83 s	X_1,	1.23 d (7.1)	4.09 and 4.21	X_1, 4.87 quin (7.1)	X_1, 7.72 d (7.1) [−5.0]
	X_3,	1.28 d (6.6)	ABq (17.7)	X_3, 4.25 quin (6.6)	X_3, 7.80 d (6.6) [−6.3]
			Ac–X_1–(HO)X_2–X_3–X_4–(HO)X_5–X_6–OH (**2d**)		
1.84 s	X_1,	1.24 d (7.1)	X_2 and X_5	X_1, 4.87 quin (7.1)	X_1, X_3, X_4, and X_6
	X_3,	1.27 d (7.1)	4.10 and 4.22	X_3, 4.37 quin (7.1)	7.75 br d
	X_4,	1.23 d (7.1)	ABq (17.3)	X_4, 4.87 quin (7.1)	[−5.8]
	X_6,	1.28 d (7.6)		X_6, 4.1—4.3 m	
			Ac–X_1–(HO)X_2–X_3–X_4–(HO)X_5–X_6–X_7–(HO)X_8–X_9–OH (**3d**)		
1.83 s	X_1,	1.24 d (7.1)	X_2, X_5, and X_8	X_1, X_4, and X_7	All NHs
	X_3, X_6,	1.26 d (7.1)	4.0—4.5 m	4.86 quin	7.8 br s
	X_4, X_7,	1.22 d (7.1)		X_3, X_6, and X_9	[−5.0]
	X_9,	1.27 d (7.1)		4.0—4.5 m	

a) *N*-Hydroxy protons, not observed; *J* values, in Hz in parentheses; temp coefficients×10^3 (ppm/deg), in [ ]. b) For Ala (X_1, X_3, X_4, X_6, X_7, and X_9). c) For Gly (X_2, X_5, and X_8).

recorded for a DMSO-d_6 solution at 80°C for spectral resolution. In Table 1 tripeptide **1d** is shown as having a diastereotopic glycyl CH_2 unit, as well as two alanyl CH_3 groups and two alanyl NH protons at slighly different positions. Hexapeptide **2d** reveals four different alanyl CH_3 groups as well as two glycyl CH_2 groups as one set of diastereotopic protons. Nonapeptide **3d** shows six alanyl CH_3 groups as four different signals, two of which represent four CH_3 groups in a consecutive Ala–Ala sequence. In going from tripeptide **1d** to nonapeptide **3d**, although the NMR spectra became complex, the peak positions remained almost unchanged. A singlet for the amide NH protons of hexa- **2d** and nona-peptides **3d** suggests that there is no particular conformation involving intramolecularly hydrogen bonded NH protons. The temperature dependence of the amide proton chemical shifts over the range 30—100°C gave larger negative values (−0.005 ppm deg^{-1}) for the tri-, hexa-, and nona-peptide than that expected for an intramolecularly hydrogen-bonded proton.[16] These oligopeptides have disordered structures in a DMSO solution.

The CD spectra for peptides **1d**, **2d**, and **3d** in water gave similar curves, but of different intensity. Nonapeptide **3d** showed a positive band at 220 nm (θ_M=5000) and a large negative extreme below 200 nm, indicative of a disordered structure. The 220 nm band was ascribed to the presence of the N–OH group; the maximum grew larger in alkaline solution. A similar CD pattern, which was ascribed to a disordered structure, was reported for a

water-soluble poly(Ala–Ala–Gly)$_n$ derivative.[17]

The UV spectrum of nonapeptide **3d** in water gave λ_{max} at 190 nm (ε=48000 dm^3 mol^{-1} cm^{-1}).

Iron(III) Complex Formation. A 1 : 1 molar mixture of iron(III) and nonapeptide **3d** in water produced a 1 : 3 complex of iron(III) with hydroxamate groups at neutral pH, where iron(III) might precipitate unless it is complexed. The absorption maximum was observed at 410 nm (ε=2160 dm^3 mol^{-1} cm^{-1}). The maximum is slightly small for the ε value, when compared with the ligand-to-metal charge-transfer band observed for such a 1 : 3 complex (λ_{max} 425—440 nm and ε=2500—3000 dm^3 mol^{-1} cm^{-1}).[3b,9] The visible spectra for the complex were obtained at different pH values in order to estimate its stability against H^+ or OH^- attack. The data are presented as a plot of pH vs. absorbance at 410 nm (Fig. 3). The narrow plateau span in a neutral pH region (pH 6.5—8.5) suggests that the 1 : 3 complex formed is not sufficently stable to resist attack of H^+ or OH^- ions of low concentration.

In the region of pH 5.4—6.6, the absorption spectra showed an isosbestic point at 464 nm, reflecting the following mono-protonation equilibrium reaction:

$$Fe\cdot L + H^+ \rightleftharpoons Fe\cdot HL^+, \quad (1)$$

where Fe·L and Fe·HL$^+$ represent the tris(hydroxamato) iron(III) complex and its protonated complex, respectively. Equation 1 is also expressed by Eq. 2, $K_{Fe\cdot HL}$ being the protonation equilibrium constant of the reaction:

$$K_{Fe\cdot HL} = [Fe\cdot HL^+]/[Fe\cdot L]\cdot[H^+]. \quad (2)$$

The spectral data were analyzed according to the Schwarzenbach equation, given as follows:[6,9]

$$A_{obs} = (A_0 - A_{obs})/([H^+]\cdot K_{Fe\cdot HL}) + \varepsilon_{Fe\cdot HL}\cdot C_t. \quad (3)$$

Here A_{obs} is the observed absorbance at each pH value, and A_0 is the plateau region absorbance at pH 7.4, both at 410 nm. The extinction coefficient at 410 nm for the protonated species is expressed by $\varepsilon_{Fe\cdot HL}$, and the total concentration of the ligand L by C_t. When A_{obs} was plotted against $(A_0-A_{obs})/[H^+]$, a straight line was obtained, as shown in Fig. 4. The values of $K_{Fe\cdot HL}$ and $\varepsilon_{Fe\cdot HL}$ were derived from the slope and intercept, respectively. The value of 1760 for $\varepsilon_{Fe\cdot HL}$ suggests that an average incremental value of 880 is assignable to the first and second hydroxamato ligands; hence, only a small contribution of 400 is estimated for the third ligand in the 1 : 3 complex. The obtained $K_{Fe\cdot HL}$ value of 2.24×10^{-6} corresponds to a half protonation pH of 6.35. These data imply that the third hydroxamato ligand of nonapeptide **3d** coordinates insufficiently to the iron(III) in forming the tris(hydroxamato) complex. Unlike ferrioxamine B, where three hydroxamato ligands are iocated at an optimal distance, the spacing of the nonapeptide ligands is not sufficiently long to produce a stable 1 : 3 complex. A gradual decrease in A_{obs} observed below pH 5.4 (Fig. 3) is ascribed to the presence of more than two species (mono- and di-protonated), as well as Fe·L.

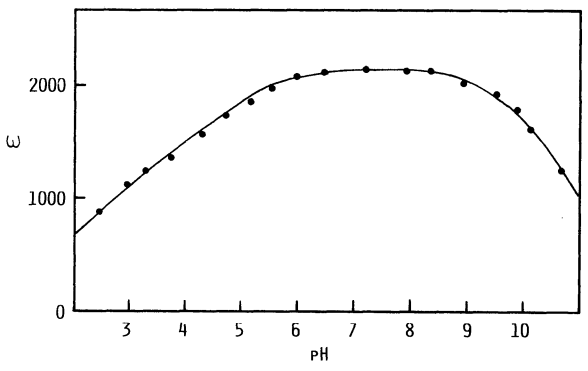

Fig. 3. Absorbance at 410 nm vs. pH for a 1 : 1 molar mixture of iron (III) and nonapeptide **3d** in water.

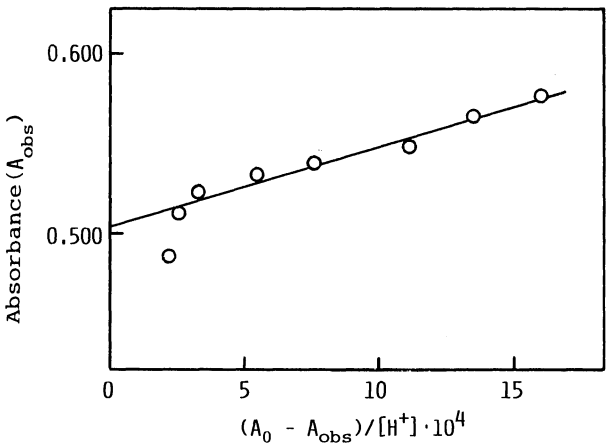

Fig. 4. Schwarzenbach plot of the absorbance vs. pH data from pH 5.39 to 6.60 in Fig. 3. A point at pH 5.19, which deviates from the line, is omitted from the calculation. The intercept gives absorbance at 410 nm due to Fe·HL$^+$ species and the value of $K_{Fe\cdot HL}$ is calculated from the slope.

In order to estimate the stability of the nonapeptide **3d** complex (Fe·**3d**) we carried out the following equilibrium ligand exchange reaction, using a Fe·**3d** : ligand=1.0 : 1.0 mixture:

$$Fe\cdot\mathbf{3d} + L \rightleftharpoons Fe\cdot L + \mathbf{3d}. \quad (4)$$

An apparent equilibrium constant (K_{ex}) for Eq. 4 can be expressed by

$$K_{ex} = [Fe\cdot L]\cdot[\mathbf{3d}]/[Fe\cdot\mathbf{3d}]\cdot[L]. \quad (5)$$

The ligands used were ethylenediaminetetraacetic acid (EDTA), *N*-hydroxyethylethylenediaminetriacetic acid (HEDT), nitrilotriacetic acid (NTA), and iminodiacetic acid (IDA).

The reaction was followed by measuring the decrease

Table 2. Equilibrium Data for the Ligand Exchange Reaction[a)]

Ligand (log K)[b)]	pH	Fe·**3d** unexchanged %	K_{ex}
EDTA (25.1)	7.1	10	81
HEDT (19.8)	7.2	17	24
NTA (15.9)	7.1	66	0.27
IDA (10.4)	7.1	83	0.042

a) Initial concentrations of Fe·**3d** and ligand, 1.5×10^{-4} mol dm^{-3}; in water at 25 °C and ionic strength 0.04 (KNO_3). b) Ref. 18.

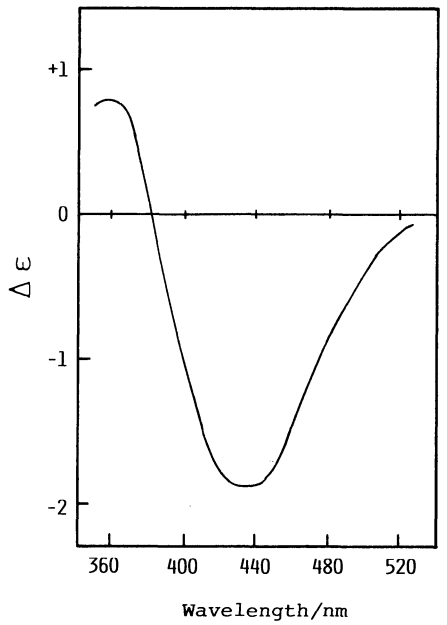

Fig. 5. CD spectrum for the iron (III) nonapeptide complex in water at pH 7.0.

in the optical density at 410 nm for Fe·**3d** in water at neutral pH and 25 °C. The reaction equilibrium was complete within hours. The results are summarized in Table 2, together with the iron(III) stability constant (log K) of the ligands.[18)] These data suggest that the stability constant of Fe·**3d** is between that of HEDT and NTA. Although we were unable to determine three protonation constants (pK_as) for **3d**, due to its small sample size, we could estimate log K for **3d** to be about 17 from the ligand protonation constants[18)] and K_{ex} for HEDT or NTA. We tentatively assumed pK_as (8.0) for **3d**. The estimated constant of 10^{17} for Fe·**3d** is far below that (10^{30})[9)] of ferrioxamine B.

Configuration of the Iron Complex. Reflecting the chiral influence of L-alanine residues, the 1 : 1 complex of iron(III) and nonapeptide **3d** in water gave its CD spectrum (Fig. 5), which showed a characteristic spectral pattern with a positive band at 355 nm ($\Delta\varepsilon$ +0.8) and a negative band at 435 nm ($\Delta\varepsilon$ −1.8). This pattern clearly shows a predominance of the *Δ*-configuration around the metal ion.[19)] An examination of CPK models reveals a

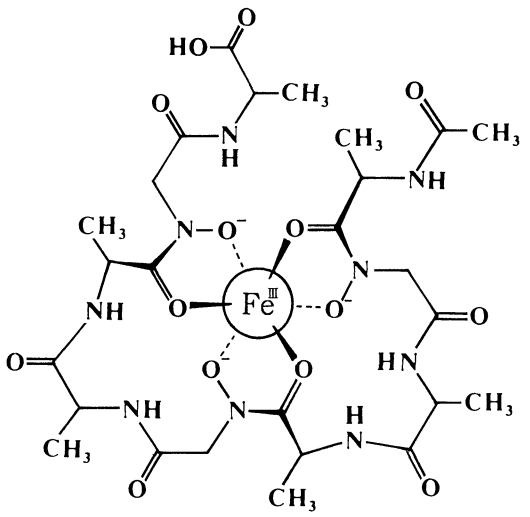

Fig. 6. Possible structure of the iron (III) nonapeptide complex shown in a *Δ-N-cis, cis* arrangement.

number of possibilities for configurational isomers, i.e, geometrical (*cis* and *trans*) and optical (*Δ* and *Λ*) isomers. Regarding the *Δ*-configuration of the nonapeptide complex, possibilities are open for five configurations out of eight theoretical varieties. They are designated *N-cis, cis*; *N-trans, cis*; *N-cis, trans*; *C-trans, trans*; and *C-trans, cis*, according to the nomenclature presented by Raymond.[20)] Except for the *N-cis, cis*, the other four configurations require a sharp turn at the Ala[4] and/or Ala[7] residue. These four are thus virtually eliminated from the possibility. Figure 6 depicts a possible configuration (*Δ-N-cis, cis*) for the complex.

The formation of the present chiral 1 : 3 complex is due primarily to the intramolecular nature of the three ligating hydroxamate functions. A mixture of iron(III) and three equivalent tripeptide **1d** showed no evidence for the formation of a tris(hydroxamato) iron(III) complex; only a small λ_{max} was noted at 440 nm, and no characteristic CD bands were observed.

In summary, the present nonapeptide carrying the Ala–(HO)Gly–Ala sequence is capable of making a chiral 1 : 3 iron(III) complex. This complex formation is the first example among linear peptide hydroxamic acids, even though the stability of the complex is low.

Experimental

The melting points are uncorrected. CD spectra were taken with a JASCO J-40 spectrometer. The other spectrometers used were mentioned in a previous paper.[13)] HPLC was carried out with a JASCO model Twincle apparatus using a column packed with Finepak SIL C_{18}. It was driven at a flow rate of 2 ml min^{-1} with a solvent system: CH_3CN–water (3 : 1 v/v) containing 0.1% phosphoric acid. The retention time (R_t) is expressed in min. TLC was carried out using Merck precoated silica gel 60 F_{254} plates; R_f (EtOAc–$CHCl_3$ 3 : 8 v/v). Gel chromatography was performed using Sephadex LH-20 or

Toyopearl HW-40 with MeOH as the eluant. Wakogel C-300 was used for column chromatography.

Boc-Ala-OPac was prepared by a method mentioned in the literature,[21] and recrystallized from EtOAc-light petroleum (66%); mp 96—98°C; IR (KBr) 1765, 1695 cm^{-1}. H-(BzlO)Gly-OSu[13] was used without purification.

H-(BzlO)Gly-Ala-OPac (4). Boc-L-Ala-OPac (4.10 g, 13.3 mmol) in CH_2Cl_2 (10 ml) was treated with TFA (30 ml) for 0.5 h at 0°C and recrystallized from CH_2Cl_2-hexane to give TFA·H-Ala-OPac (3.84 g, 90%); mp 124—125°C. To a solution of triethylamine (1.17 g, 11.6 mmol) and TFA·H-Ala-OPac (3.72 g, 11.6 mmol) in $CHCl_3$ (30 ml) was added at 0°C a $CHCl_3$ solution (20 ml) of H-(BzlO)Gly-OSu derived from H-(BzlO)Gly-OH (3.00 g, 16.6 mmol) and HOSu (1.91 g, 16.6 mmol) by the use of DCC. The mixture was stirred for 2 h at 0°C and 24 h at room temperature and then filtered. After evaporation, the residue was dissolved in EtOAc, and *N,N'*-dicyclohexylurea (DCU) was removed. The organic layer was washed with 5% $NaHCO_3$ and brine, and then dried (Na_2SO_4). Evaporation of the solvent, followed by chromatography with EtOAc-hexane (1 : 1 v/v), yielded a product (2.45 g, 58%) as an oil: TLC R_f 0.26; IR (neat) 1750, 1690, 1665 cm^{-1}; ^1H NMR δ=1.58 (d, 3h, J=6.2 Hz), 3.55 (s, 2H), 4.71 (s, 2H), 4.82 (quin, 1H, J=6.2 Hz), 5.25 and 5.48 (ABq, 2H, J=16.3 Hz, OCH_2COPh), 7.2—7.7 (m, 8H), and 7.8—8.0 (m, 2H).

H-(BzlO)Gly-Ala-OBzl (5). Similarly, a reaction of H-(BzlO)-Gly-OSu (22 mol) in $CHCl_3$ (20 ml) with H-Ala-OBzl (15 mmol) in DMF (20 ml), followed by column chromatography with EtOAc-$CHCl_3$ (1 : 9 v/v), gave a product (1.2 g, 24%) as an oil: TLC R_f 0.35; IR (neat) 1741, 1655 cm^{-1}.

Boc-Ala-(BzlO)Gly-Ala-OBzl (1a). To a solution of Boc-Ala-OH (2.00 g, 10.6 mmol) and triethylamine (1.07 g, 10.6 mmol) in THF (35 ml) was added dropwise a solution of isobutyl chloroformate (1.46 g, 10.6 mmol) in THF (15 ml) at −18°C. To the mixture, after 15 min, was added dropwise a solution of compound **5** (2.90 g, 8.46 mmol) in THF (40 ml) at −18°C. The mixture was stirred for 3 h at −18°C and kept in a refrigerator for 48 h. After the usual workup, column chromatography of a residual oil with EtOAc-$CHCl_3$ (3 : 8 v/v) gave a crude product, which was recrystallized from EtOAc-light petroleum (3.04 g, 71%); mp 110—111°C; TLC R_f 0.34; HPLC R_t 5.14 (flow rate 1 ml min^{-1}); IR (KBr) 1739, 1700, 1653 cm^{-1}; $[\alpha]_D^{22}$ −44° (c 1.2 MeOH); ^1H NMR δ=1.29 (d, 3H, J=6.8 Hz), 1.40 (d, 3H, CH_3 for Ala-OBzl), 1.43 (s, 9H), 4.26 and 4.37 (ABq, 2H, J=16.6 Hz), 4.55—4.78 (m, 2H, two $C_\alpha H$) 5.00 (s, 2H), 5.10 and 5.18 (ABq, 2H, OCH_2COPh), 5.27 (br s, 1H, Boc-NH), 6.97 (br s, 1H, NH), 7.3—7.4 (m, 10H). Found: C, 63.02; H, 6.88; N, 8.14%. Calcd for $C_{27}H_{35}N_3O_7$: C, 63.14; H, 6.87; N, 8.18%.

Boc-Ala-(BzlO)Gly-Ala-OPac (1b). Similarly, Boc-L-Ala-OH (0.686 g, 3.63 mmol), triethylamine (0.367 g, 3.63 mmol) in THF (20 ml), and isobutyl chloroformate (0.495 g, 3.62 mmol) in THF (20 ml) produced a mixed anhydride, which was allowed to react with compound **4** (1.12 g, 3.02 mmol) in CH_2Cl_2 (30 ml). After the usual workup, column chromatography ($CHCl_3$-EtOAc, 9 : 1 v/v) gave a residue, which was recrystallized from EtOAc-hexane (1.13 g, 69%); mp 139—140°C; TLC R_f 0.36; IR (KBr) 1754, 1690, 1667 cm^{-1}; ^1H NMR δ=1.32 (d, 3H, J=7.2 Hz), 1.44 (s, 9H), 1.58 (d, 3H, J=6.7 Hz, CH_3 for Ala-OPac), 4.29 and 4.39 (ABq, 2H, J=16.7 Hz), 4.75 (quin, 2H, J=7.8 Hz), 5.05 (s, 2H), 5.20 (br s, 1H, NH), 5.25 and 5.49 (ABq, 2H, J=16.6 Hz), 6.93 (br s, 1H, NH), 7.2—7.6 (m, 8H) and 7.7—7.9 (m, 2H). Found: C, 62.19; H, 6.44; N, 7.78%. Calcd for $C_{28}H_{35}N_3O_8$: C, 62.10; H, 6.51; N, 7.76%.

Boc-Ala-(BzlO)Gly-Ala-OH (1c). A mixture of compound **1b** (2.30 g, 4.25 mmol) and zinc powder (13.9 g; pretreated with 2% HCl solution) in AcOH (80 ml) was stirred for 2.5 h at room temperature and filtered. After evaporation, the residue was dissolved in 5% citric acid and extracted with EtOAc. After evaporation, a crude oil was washed with hexane to afford the product (1.38 g, 77%); mp 145—147°C; TLC R_f 0.1 (EtOAc); IR (neat) 1720, 1665 cm^{-1}. Found: C, 55.62; H, 6.70; N, 9.52%. Calcd for $C_{20}H_{29}N_3O_7 \cdot 1/2H_2O$: C, 55.55; H, 6.99; N, 9.71%.

Boc-[Ala-(BzlO)Gly-Ala]$_2$-OBzl (2a). Compound **1a** (2.00 g, 3.89 mmol) in CH_2Cl_2 (10 ml) was treated with TFA (30 ml) for 30 min at 0°C, and then evaporated to give a residue. To a solution of the residue (3.89 mmol), compound **1c** (1.30 g, 3.07 mmol), triethylamine (0.53 g, 5.2 mmol), and HOBt (0.54 g, 4.00 mmol) in DMF (25 ml) was added HCl·EDC (0.77 g, 4.02 mmol) in CH_2Cl_2 (10 ml) at −15°C. The mixture was stirred for 3 h at 0°C and 24 h at room temperature. Evaporation of the solvent gave a residue. Column chromatography (EtOAc-MeOH, 30 : 1 v/v) afforded the product (2.28 g, 70%); mp 75—78°C; HPLC R_t 5.02 (flow rate 1 ml min^{-1}); IR (KBr) 1740, 1700, 1690, 1650 cm^{-1}; $[\alpha]_D^{22}$ −83° (c 0.9 MeOH); ^1H NMR δ=1.24 (d, 6H, J=7.2 Hz, two CH_3 for Ala^1 and Ala^4), 1.39 (d, 6H, J=6.7 Hz), 1.43 (s, 9H), 4.27 and 4.38 (ABq, 4H, J=17.2 Hz), 4.5—4.8 (m, 3H, 3×$C_\alpha H$), 4.85—5.00 (m, 1H, $C_\alpha H$ for Ala^4), 4.97 and 5.02 (ABq, 4H, J=10 Hz), 5.09 and 5.18 (ABq, 2H, J=11.7 Hz, CO_2CH_2Ph), 5.22 (s, 1H, NH), 7.07 (br s, 3H, NH), 7.25—7.45 (m, 15H). Found: C, 61.08; H, 6.68; N, 10.12%. Calcd for $C_{42}H_{54}N_6O_{11} \cdot 1/2H_2O$; C, 60.93; H, 6.70; N, 10.15%.

Boc-[Ala-(BzlO)Gly-Ala]$_3$-OBzl (3a). Compound **2a** (1.00 g, 1.22 mmol) in CH_2Cl_2 (5 ml) was treated with TFA (9.2 ml). To this were added a solution of compound **1c** (0.61 g, 1.22 mmol), triethylamine (0.28 g, 2.8 mmol) and HOBt (0.198 g, 1.22 mmol) in DMF (15 ml) and a solution of HCl·EDC (0.28 g, 1.46 mmol) in CH_2Cl_2 (10 ml); the mixture was stirred for 2 h at −10°C and for 24 h at room temperature. The usual workup and gel chromatography (Sephadex LH-20 and Toyopearl HW-40) afforded the product (0.98 g, 71%); mp 89—90°C; HPLC R_t 2.54 and 4.98 (flow rate 1 ml min^{-1}); IR (KBr) 1735, 1700, 1650 cm^{-1}; $[\alpha]_D^{22}$ −125° (c 1.2 MeOH); ^1H NMR (DMSO-d_6 at 80°C) δ=1.18 (d, 3H, J=6.7 Hz, CH_3 for Ala^1), 1.22 (d, 6H, J=7.2 Hz, CH_3 for Ala^4 and Ala^7), 1.23 (d, 6H, J=6.7 Hz, CH_3 for Ala^3 and Ala^6), 1.30 (d, 3H, J=7.2 Hz, CH_3 for Ala^9), 1.38 (s, 9H), 4.1—4.6 (m, 10H, three CH_2 for Gly^2, Gly^5, and Gly^8, and four $C_\alpha H$ for Ala^1, Ala^3, Ala^6, and Ala^9), 4.8—5.0 (m, 2H, two $C_\alpha H$ for Ala^4 and Ala^7), 4.97 (s, 6H), 5.12 (s, 2H, CO_2CH_2Ph), 6.61 (br s, 1H, NH), 7.25—7.45 (m, 20H), 7.85 (d, 2H, J=7.2 Hz, two NH), 7.95 (d, 2H, J=6.7 Hz, two NH), 8.17 (d, 1H, J=6.1 Hz, NH). Found: C, 59.95; H, 6.53; 11.08%. Calcd for $C_{57}H_{73}N_9O_{15} \cdot H_2O$: C, 59.94; H, 6.62; N, 11.04%.

General Procedure for Conversion of Boc-[Ala-(BzlO)Gly-Ala]$_n$-OBzl(1a, 2a, and 3a) into Ac-[Ala-(HO)Gly-Ala]$_n$-OH (1d, 2d, and 3d). As an example, compound **3a** (150 mg, 0.133 mmol) in CH_2Cl_2 (3 ml) was treated with TFA (1 ml) in CH_2Cl_2 (2 ml) at 0°C for 40 min. Evaporation of the solvent gave a residue (HPLC R_t 6.28). To a solution of the residue (0.13 mmol) and triethylamine (51 mg, 0.5 mmol) in THF

(10 ml) was added acetic anhydride (16 mg, 0.16 mmol) in THF (5 ml). After 2 h at room temperature, the mixture was evaporated to give a residue, which was transferred into EtOAc. The organic layer was washed with water and dried ($MgSO_4$). Evaporation of the solvent and gel chromatography (Sephadex LH-20) afforded the Ac-nonapeptide benzyl ester (116 mg, 81%) as a glassy solid: HPLC R_t 2.00; IR (KBr) 1742, 1652 cm^{-1}.

A mixture of the ester (105 mg, 0.098 mmol) and 10% Pd-C (20 mg) was added in MeOH (10 ml) under a nitrogen atmosphere; hydrogen was then introduced. After being stirred for 3 h at room temperature, the catalyst was filtered off. Evaporation of the solvent gave a residue, which was chromatographed (Sephadex LH-20) to afford a product, Ac-[Ala-(HO)Gly-Ala-]$_3$OH (**3d**) (67 mg, 91%) as a hygroscopic solid: IR 1726, 1642 cm^{-1}; $[\alpha]_D^{23}$ −68° (*c* 1.0 in MeOH). Found: C, 41.92; H, 6.11; N, 16.70%. Calcd for $C_{26}H_{43}N_9O_{14}\cdot 2H_2O$: C, 42.10; H, 6.38; N, 16.99%.

Similarly, Ac-[Ala-(HO)Gly-Ala]$_2$-OH (**2d**) was obtained as a glassy solid: IR 1724, 1650 cm^{-1}; $[\alpha]_D^{22}$ −52° (*c* 1.0 in MeOH) and Ac-Ala-(HO)Gly-Ala-OH (**1d**) as a glassy solid; IR 1726, 1648 cm^{-1}; $[\alpha]_D^{22}$ −42° (*c* 0.8 in MeOH).

Iron(III) Complex Formation. Visible spectra of a 1:1 molar mixture of compound **3d** and iron(III) in water were obtained at different pH values. An iron(III) nitrate solution of 3.32×10^{-3} mol dm^{-3} was prepared (the concentration was determined by the bismuth method with EDTA). Compound **3d** (1.764 mg, 2.38×10^{-6} mol) and the iron(III) nitrate solution (0.79 ml, 2.38×10^{-6} mol) were mixed, diluted to 10.0 ml with distilled water, and kept for 1 h. The pH of the solution was adjusted by the addition of either aqueous 0.05 mol dm^{-3} HNO_3, or 0.1 or 0.05 mol dm^{-3} KOH solution.

Ligand Exchange Reactions. A solution (pH 6.9) of 10.0 ml of Fe·**3d** (3.0×10^{-4} mol dm^{-3}) containing 0.04 mol dm^{-3} of KNO_3 (ionic strength of 0.04) was prepared in a manner similar to that mentioned above. A ligand solution was prepared, for example, by the dilution of 1.0 ml of an aqueous EDTA·2Na stock solution (1.5×10^{-3} mol dm^{-3}) and 0.5 ml of an aqueous KNO_3 solution (0.2 mol dm^{-3}) to 5.0 ml with distilled water. The pH of the solution was then adjusted to 6.9. Other ligand solutions were also prepared as disodium salts with aqueous NaOH. The reaction was initiated by putting 1.5 ml of the ligand solution into 1.5 ml of the Fe·**3d** solution in a 10 mm quartz cell in a thermostated cell compartment of a Hitachi 430 spectrometer. After 24 h the equilibrium concentration was determined and the solution pH was measured.

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2) Abbreviations: H-(HO)Gly-OH and H-(BzlO)Gly-OH, *N*-hydroxy- and *N*-(benzyloxy)glycine; Boc, *t*-butoxycarbonyl; Pac, phenacyl; DMSO, dimethyl sulfoxide; EDC, 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide; HOBt, 1-hydroxybenzotriazole; DMF, *N*,*N*-dimethylformamide; DCC, dicyclohexylcarbodiimide; TFA, trifluoroacetic acid; EtOAc, ethyl acetate; HOSu, *N*-hydroxysuccinimide; THF, tetrahydrofuran.

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