

N-Hydroxy Amides. X.¹⁾ Synthesis of a Nonapeptide with an Ala-(HO)Gly-Ala²⁾ 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. ¹H 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 (Δε +0.8) and 435 nm (Δε -1.8), showing a preference for the Δ-configuration around the iron.

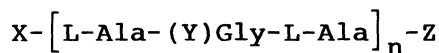
Naturally occurring hydroxamic acids show unique biological activity, acting as tumour inhibitors, antibiotics, or growth factors.^{3–8)} The hydroxamate group is a powerful bidentate oxygen ligand for certain metal ions, including iron(III).^{8,9)} Thus, three hydroxamate groups of peptide desferriochromes³⁾ or oligoamide desferrioxamines⁴⁾ 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 acids)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*-hydroxy peptide, 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 phenacyl¹⁴⁾ 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 acetyltri-peptide **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, ¹H NMR and CD spectral data were collected. The ¹H NMR spectra were



1; n=1	a; X=Boc	Y=OBzl	Z=OBzl
2; n=2	b; X=Boc	Y=OBzl	Z=OPac
3; n=3	c; X=Boc	Y=OBzl	Z=OH
	d; X=Ac	Y=OH	Z=OH

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

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 ($\theta_M=5000$) 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 $\lambda_{\max}$ at 190 nm ($\epsilon=48000 \text{ dm}^3 \text{ mol}^{-1} \text{ cm}^{-1}$).

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 ($\epsilon=2160 \text{ dm}^3 \text{ mol}^{-1} \text{ cm}^{-1}$). 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 ($\lambda_{\max}$ 425–440 nm and $\epsilon=2500\text{--}3000 \text{ dm}^3 \text{ mol}^{-1} \text{ cm}^{-1}$).^{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 sufficiently 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:



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

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

The spectral data were analyzed according to the Schwarzenbach equation, given as follows:^{6,9)}

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

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 $\epsilon_{\text{Fe}\cdot\text{HL}}$, and the total

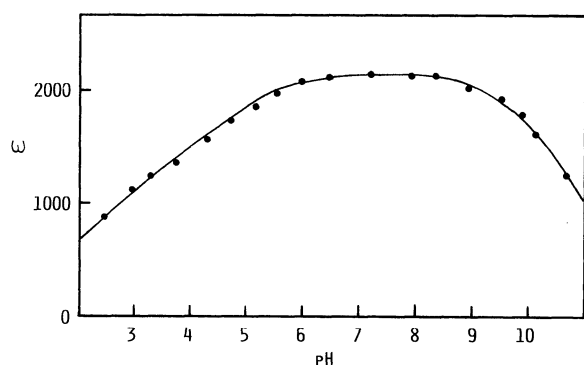


Fig. 3. Absorbance at 410 nm vs. pH for a 1 : 1 molar mixture of iron (III) and nonapeptide **3d** in water.

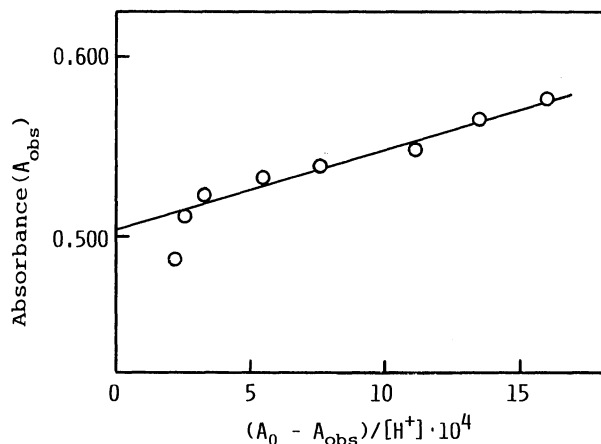
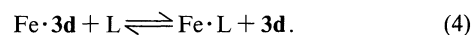


Fig. 4. Schwarzenbach plot of the absorbance vs. pH data from pH 5.39 to 6.60 in Fig. 3. A point at pH 5.19, which deviates from the line, is omitted from the calculation. The intercept gives absorbance at 410 nm due to $\text{Fe}\cdot\text{HL}^+$ species and the value of $K_{\text{Fe}\cdot\text{HL}}$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

concentration of the ligand L by C_{L} . When A_{obs} was plotted against $(A_0 - A_{\text{obs}})/[\text{H}^+]$, a straight line was obtained, as shown in Fig. 4. The values of $K_{\text{Fe}\cdot\text{HL}}$ and $\epsilon_{\text{Fe}\cdot\text{HL}}$ were derived from the slope and intercept, respectively. The value of 1760 for $\epsilon_{\text{Fe}\cdot\text{HL}}$ 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 $K_{\text{Fe}\cdot\text{HL}}$ 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 located 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 $\text{Fe}\cdot\text{L}$.

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



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

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

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 <i>K</i>) ^{b)}	pH	Fe·3d unexchanged	<i>K</i> _{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 (KNO₃).

b) Ref. 18.

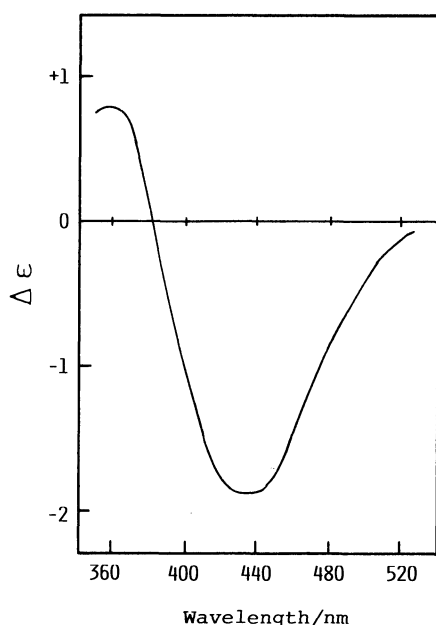


Fig. 5. CD spectrum for the iron (III) nonapeptide complex in water at pH 7.0.

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 (p*K*_{as}) for 3d, due to its small sample size, we could estimate log *K* for 3d to be about 17 from the ligand protonation constants¹⁸⁾ and *K*_{ex} for HEDT or NTA. We tentatively assumed p*K*_{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 ($\Delta\epsilon$ +0.8) and a negative band at 435 nm ($\Delta\epsilon$ -1.8). This pattern clearly shows a predominance of the Δ -configuration around the metal ion.¹⁹⁾ An examination of CPK models reveals a

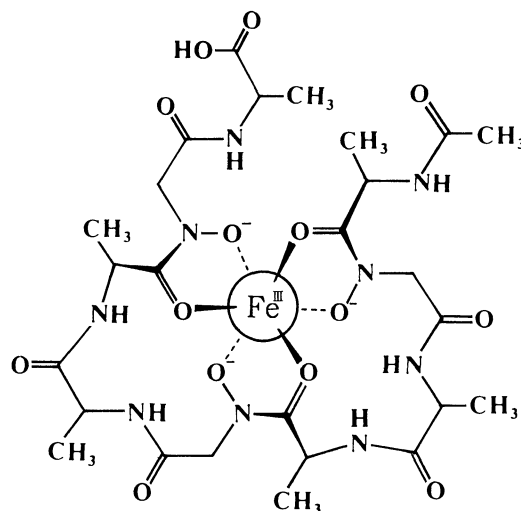


Fig. 6. Possible structure of the iron (III) nonapeptide complex shown in a Δ -*N*-cis, cis arrangement.

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 Ala⁴ and/or Ala⁷ 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 Twinkle apparatus using a column packed with Finepak SIL C₁₈. It was driven at a flow rate of 2 ml min⁻¹ with a solvent system: CH₃CN-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 F₂₅₄ plates; *R*_f (EtOAc-CHCl₃ 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-OSu¹³ was used without purification.

H-(BzlO)Gly-Ala-OPac (4). Boc-L-Ala-OPac (4.10 g, 13.3 mmol) in CH₂Cl₂ (10 ml) was treated with TFA (30 ml) for 0.5 h at 0°C and recrystallized from CH₂Cl₂–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 CHCl₃ (30 ml) was added at 0°C a CHCl₃ 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% NaHCO₃ and brine, and then dried (Na₂SO₄). 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, OCH₂COPh), 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 CHCl₃ (20 ml) with H-Ala-OBzl (15 mmol) in DMF (20 ml), followed by column chromatography with EtOAc–CHCl₃ (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–CHCl₃ (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; [α]_D²⁵ -44° (c 1.2 MeOH); ¹H NMR δ=1.29 (d, 3H, *J*=6.8 Hz), 1.40 (d, 3H, CH₃ 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_αH) 5.00 (s, 2H), 5.10 and 5.18 (ABq, 2H, OCH₂COPh), 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 C₂₇H₃₅N₃O₇: 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 CH₂Cl₂ (30 ml). After the usual workup, column chromatography (CHCl₃–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, CH₃ 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 C₂₈H₃₅N₃O₈: 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 C₂₀H₂₉N₃O₇·1/2H₂O: 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 CH₂Cl₂ (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 CH₂Cl₂ (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; [α]_D²⁵ -83° (c 0.9 MeOH); ¹H NMR δ=1.24 (d, 6H, *J*=7.2 Hz, two CH₃ for Ala¹ and Ala⁴), 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_αH), 4.85–5.00 (m, 1H, C_αH for Ala⁴), 4.97 and 5.02 (ABq, 4H, *J*=10 Hz), 5.09 and 5.18 (ABq, 2H, *J*=11.7 Hz, CO₂CH₂Ph), 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 C₄₂H₅₄N₆O₁₁·1/2H₂O: 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 CH₂Cl₂ (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 CH₂Cl₂ (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; [α]_D²⁵ -125° (c 1.2 MeOH); ¹H NMR (DMSO-*d*₆ at 80°C) δ=1.18 (d, 3H, *J*=6.7 Hz, CH₃ for Ala¹), 1.22 (d, 6H, *J*=7.2 Hz, CH₃ for Ala⁴ and Ala⁷), 1.23 (d, 6H, *J*=6.7 Hz, CH₃ for Ala³ and Ala⁶), 1.30 (d, 3H, *J*=7.2 Hz, CH₃ for Ala⁹), 1.38 (s, 9H), 4.1–4.6 (m, 10H, three CH₂ for Gly², Gly⁵, and Gly⁸, and four C_αH for Ala¹, Ala³, Ala⁶, and Ala⁹), 4.8–5.0 (m, 2H, two C_αH for Ala⁴ and Ala⁷), 4.97 (s, 6H), 5.12 (s, 2H, CO₂CH₂Ph), 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; N, 11.08%. Calcd for C₅₇H₇₃N₉O₁₅·H₂O: 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 CH₂Cl₂ (3 ml) was treated with TFA (1 ml) in CH₂Cl₂ (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 (MgSO₄). 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*_f 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]₃OH (**3d**) (67 mg, 91%) as a hygroscopic solid: IR 1726, 1642 cm⁻¹; [α]_D²³ -68° (*c* 1.0 in MeOH). Found: C, 41.92; H, 6.11; N, 16.70%. Calcd for C₂₆H₄₃N₉O₁₄·2H₂O: 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; [α]_D²² -52° (*c* 1.0 in MeOH) and Ac-Ala-(HO)Gly-Ala-OH (**1d**) as a glassy solid; IR 1726, 1648 cm⁻¹; [α]_D²² -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 HNO₃, 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 KNO₃ (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 KNO₃ 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.

References

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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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