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Registry No. **1a**, 69649-19-0; **1b**, 85507-41-1; **3**, 85507-43-3; **4**, 87830-51-1; **5**, 2958-72-7; **6**, 117251-75-9; **7**, 117251-76-0; **8**, 117251-77-1; **9**, 117251-78-2; **10**, 117269-14-4; **11**, 117251-79-3; **12**, 117251-80-6; **13**, 117251-81-7; **14**, 117251-82-8; **15**, 75996-72-4; **16**, 117251-83-9; **17**, 69028-03-1.

N-Hydroxy Amides. 7.[1] Synthesis and Properties of Linear and Cyclic Hexapeptides Containing Three N^5-Acetyl-N^5-hydroxy-L-ornithine Residues as Models for Ferrichrome[2]

Masayasu Akiyama,* Akira Katoh, and Tsutomu Mutoh

Department of Applied Chemistry for Resources, Tokyo University of Agriculture and Technology, Koganei, Tokyo 184, Japan

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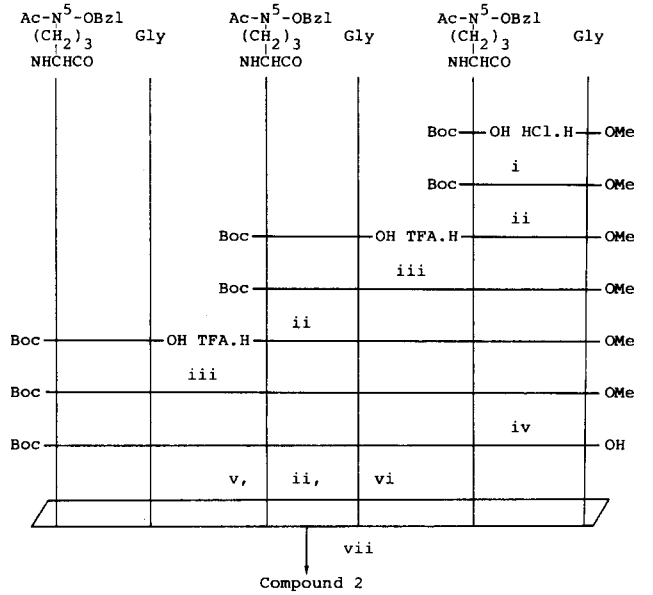
As sequence-modified analogues of desferriferrichrome (1), linear and cyclic peptides have been prepared. They are (OrnAH-Gly)$_3$ (**2**), Ac-Gly$_2$-OrnAH$_3$-Gly-OMe (**3**), Ac-(OrnAH-Gly)$_3$-OMe (**4**), and Ac-Gly-OrnAH-(OrnAH-Gly)$_2$-OMe (**5**), where OrnAH stands for an N^5-acetyl-N^5-hydroxy-L-ornithine residue. ^{1}H NMR study in DMSO-d_6 shows that most of the analogues have no particular intramolecular hydrogen bonds and that **2** assumes a C_3 symmetric conformation both in its free state and in a complex with Al(III). The 1:1 molar mixture of these analogues with iron(III) in water forms the 1:3 complex of the metal ion to the hydroxamate unit in a wide pH range. From absorption vs pH curves the stability of the iron complexes is estimated to fall in order of **1** > **4** > **2** > **3** > **5** against H^+ attack and **1** > **4** > **3** > **2** > **5** against OH^- attack. Iron transfer experiments to excess EDTA reveal a better binding of cyclic peptides with the following order of iron holding: **2** > **1** > **4** > **3** > **5**. CD spectra of iron complexes for all the analogues are virtually similar to that of ferrichrome, indicating a predominance of the Λ-cis configuration for the hexapeptides containing three OrnAH residues.

There is continuing interest in synthetic iron sequestering agents for use as drugs or models of naturally occurring compounds.[3,4] Microorganisms excrete metal binding compounds called siderophores to sequester Fe(III) by complexation.[4-7] Ferrichrome is the iron(III) complex of a representative hydroxamate siderophore, first isolated from the fungus *Ustilago sphaerogena* by Neilands.[5,8] It has an octahedrally coordinate Λ-cis configuration held by three ornithyl side chains.[8-10] The iron-free ligand, desferriferrichrome (1), is a cyclic hexapeptide composed of the three consecutive residues of glycine and of N^5-acetyl-N^5-hydroxy-L-ornithine. Considerable information has been gathered on microbial iron transport with ferrichrome related compounds.[4-7,11,12] Iron is transferred

Scheme I.[a]



[a] Reagents and conditions: (i) *N*-methylmorpholine/DCC-HOBt; (ii) TFA, 0 °C; (iii) *N*-methylmorpholine/DCC-HOSu; (iv) 1 N NaOH in MeOH at room temperature; (v) DEC·HCl (2 equiv)–HOSu (2 equiv) at −10 °C and then 0 °C; (vi) 3 mM in pyridine at 60 °C (48 h); (vii) 10% Pd-C in MeOH with H_2.

(1) Part 6: Akiyama, M.; Katoh, A.; Tsuchiya, Y. *J. Chem. Soc., Perkin Trans. 1*, in press.

(2) Abbreviations: OrnAH, N^5-acetyl-N^5-hydroxy-L-ornithine; DMSO, dimethyl sulfoxide; DCC, dicyclohexylcarbodiimide; HOBt, 1-hydroxybenzotriazole; TFA, trifluoroacetic acid; HOSu, *N*-hydroxysuccinimide; EDTA, ethylenediaminetetraacetic acid; DCU, dicyclohexylurea; DEC, 1-(3-(dimethylamino)propyl)-3-ethylcarbodiimide.

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(10) Total synthesis: (a) Keller-Schierlein, W.; Maurer, B. *Helv. Chim. Acta* **1969**, *52*, 603. (b) Isowa, Y.; Ohmori, M.; Kurita, H. *Bull. Chem. Soc. Jpn.* **1974**, *49*, 215.

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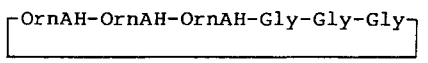
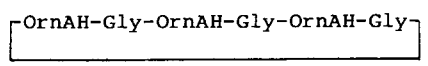
into the cell by a retro-hydroxamate ferrichrome analogue,[13] and to a variable degree by acyl portion modified ferrichromes;[14] markedly lower rates of uptake were observed for enantio-ferrichrome.[15] Thus it is now accepted that there is a specific site (receptor) on the cell that recognizes the particular iron carrier (siderophore iron complex) to allow its entrance into the cell.[4-7] The molecular shape of an iron complex seems to be crucial in microbial iron transport.[4,6,15] In this context, sequence-modified desferriferrichrome analogues are of interest for further understanding chemical properties of ligands and iron complexes in terms of the molecular shape.

Results and Discussion

Synthesis. Linear and cyclic sequence-modified analogues (**2**–**5**) of desferriferrichrome (**1**) were prepared by the standard peptide synthesis. Scheme I illustrates a synthetic route for analogue **2** as example. The DCC-



1



2

Ac-Gly-Gly-OrnAH-OrnAH-OrnAH-Gly-OMe

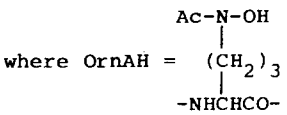
3

Ac-OrnAH-Gly-OrnAH-Gly-OrnAH-Gly-OMe

4

Ac-Gly-OrnAH-OrnAH-Gly-OrnAH-Gly-OMe

5



HOSu method[16] was used for most of the coupling reactions. Intramolecular cyclization was effected under high-dilution conditions in pyridine. Debenzylation followed by gel chromatographic purification afforded the desired compound **2**. Linear hexapeptides (**3**–**5**) were prepared by the similar procedure. The linear peptides were blocked at the amino and carboxy terminuses with acetyl and methyl ester groups, respectively.

There are four sequence isomers for cyclic peptides comprising the three residues of Gly and OrnAH. Compound **1** has the most one-sided sequence while compound **2** is symmetrical, the other two being intermediate between the two. Several possibilities exist for modification of a linear sequence. Analogues **3** and **4** correspond to linear versions of **1** and **2**, respectively. Analogue **5** situates in between **3** and **4**.

Table I. Temperature Dependence (TD) of Amide Proton Chemical Shifts in DMSO-d_6

compd	TD ($\times 10^3$ ppm K^{-1})	residue
2	−3.7	3Gly, 3OrnAH
3	−5.5, −4.7, −4.4	3Gly
	−4.6, −4.3, −4.0	3OrnAH
4	−4.8	Gly
	−4.4	2Gly, OrnAH
	−4.8, −3.2[a]	2OrnAH
5	−5.2, −4.4	2Gly
	−4.6	Gly, 3OrnAH

[a] A possibility of intramolecular hydrogen bonding.

Table II. Iron(III) Exchange Reactions with EDTA[a]

ligand (L)	k_{tr}, s^{-1}	relative rate
1	6.1×10^{-4}	1.0
2	2.0×10^{-4}	0.3
3	1.2×10^{-2}	20
4	4.6×10^{-3}	7.5
5	1.9×10^{-2}	30

[a] Equation i, where $k_{tr} \doteq k_f$, under conditions: in AcOH–AcONa buffer at 25 °C, pH 5.4, and ionic strength 0.1, with an initial concentration ratio, $[Fe{\cdot}L]_0/[EDTA]_0 = (3.8 \times 10^{-4}\ M)/(7.6 \times 10^{-3}\ M)$.

$$Fe{\cdot}L + EDTA \underset{k_r}{\overset{k_f}{\rightleftharpoons}} L + Fe{\cdot}EDTA \qquad (i)$$

Conformation of Peptides. ^1H NMR spectra in DMSO-d_6 at 50 °C exhibit characteristic proton signals (δ): *N*-OH (9.5), amide NH (8.8–7.7), ornithyl C^2H (4.5–4.1), glycyl C^2H (4.0), ornithyl C^5H (3.5), N^5-acetyl (2.0), and ornithyl C^3H and C^4H (1.8–1.4). Signals sometimes appear overlapped, although their positions are comparable with those[17] observed for **1**. The temperature dependence of amide proton chemical shifts was determined in the range of 25–100 °C (Table I). Conformational changes are gradual as expressed by good linear plots. Cyclic peptide **2** reveals six amide NH as a broad singlet, three ornithyl C^2H as a quartet, and six glycyl C^2H as one set of diastereomeric protons, indicating that the molecule stays in a rather rigid conformation with C_3 symmetry. No particular intramolecular amide hydrogen bonds are noted in the NMR time scale, while compound **1** reveals two intramolecular hydrogen bonds in the same solvent.[17] For linear peptides **3**–**5**, signals of amide NH, ornithyl C^2H, and glycyl C^2H appear at slightly different positions since there is no symmetry. Compound **4** may possess one ornithyl NH hydrogen bond, but the other ones (**3** and **5**) none.

CD spectra in water gave curves of different patterns, none of which are considered to be of ordered nature.

Iron Complex Formation. Visible spectra for the 1:1 molar mixture of iron(III) and compounds **1**–**5** were determined in water at different pH values. The data are represented by the plots of absorbance at 425 nm vs pH (Figure 1). A nearly constant absorption coefficient, that is, a wide plateau region, is noted for every plot. The wavelength (425 nm) and ϵ in this region are comparable to those of ferrichrome,[8,9,13] characteristic of the 1:3 complex of iron(III) to the hydroxamic acid unit.[5] Off the plateau region, λ_{max} shifts to a longer wavelength and absorbance at 425 nm decreases, in response to transformation of the 1:3 complex into 1:2 and further 1:1 complexes by attack of H^+ or OH^-. A plateau region expanse used as a measure for the stability of the 1:3 complex indicates

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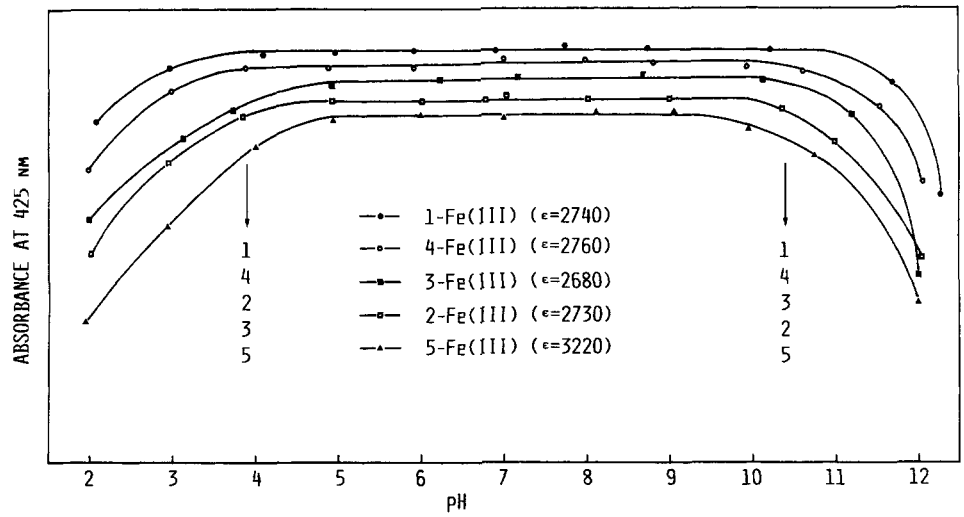

Figure 1. Absorbance vs pH for the 1:1 molar mixture of Fe(III) and hexapeptides (1–5) in water. The scale in the ordinate is arbitrary to indicate the onset of curvature. Decreasing order of stability for the peptides is given under the arrow. The ϵ value for each complex is shown in parentheses.

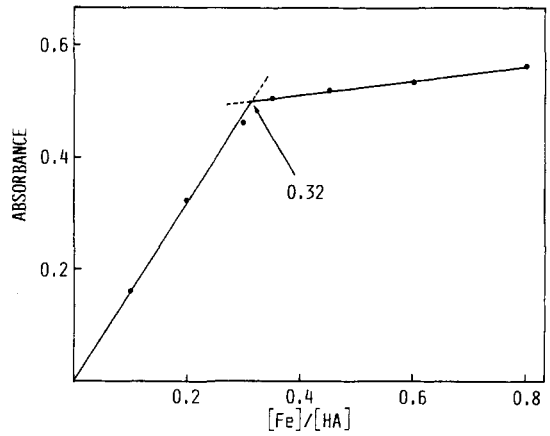

Figure 2. The mole ratio plot for compound **2**; in water at pH 5.0 with [2] = 1.94×10^{-4} M, wavelength at 425 nm.

that the iron complexes are stable in the following order: **1** > **4** > **2** > **3** > **5** against H^+ and **1** > **4** > **3** > **2** > **5** against OH^- attack, ferrichrome being the most stable. Neither a cyclic structure nor a consecutive ornithyl sequence is a single factor for stabilization of the complexes. It appears that ferrichrome cyclic structure is favorably guarded by hydrogen bondings and hence advantageous to survive in the natural environment.

Formation of the 1:3 complex of iron to the hydroxamate group was confirmed by the mole ratio plot, shown for compound **2** as example (Figure 2). Inflection points appear at 0.32–0.34 with λ_{max} 425 nm, as expected for the 1:3 complex. A value of 0.26 observed for **5** may suggest formation of a slightly distorted complex, although CD data (later section) indicate that it is rather the 1:3 complex.

Iron(III) Exchange Reactions. Iron of the complexes was transferred to EDTA present in excess at pH 5.4 and 25 °C (Table II with the reaction equation). The transfer reaction is a reversible reaction,[18] but initial rates for the reaction give a relative order of iron holding ability for the ferrichrome analogues. The order seen in Table II is different from those observed for the pH stability. Cyclic peptides hold iron more firmly than linear ones, **2** being a better holder than **1**. Tufano and Raymond studied kinetics of iron exchange of ferrioxamine B with ferrichrome A or EDTA,[19] and Monzyk and Crumbliss reported stepwise dissociation of iron(III) from ferrioxamine B in aqueous acid.[20] In the iron transfer process an incoming ligand competes for the metal with the existing ligand. Iron is in a stable complex at pH 5.4, although H^+ acts to loosen the complex.[19] The transfer is considered to take place via proton-aided dissociation of one of three hydroxamate groups from the inner coordination shell,[20] followed by the formation of a ternary complex between the leaving and incoming ligands.[19] Steric hindrance is produced upon attack by incoming ligand EDTA. Ferrichrome holds iron at one-sided site on the ring and leaves an open space. Compound **2** keeps the metal on the center of the ring with its three symmetrically extending side chains, being less favorable in formation of a ternary complex. Relative to cyclic compounds linear ones are flexible and vulnerable to attack.

The one-sided structure of ferrichrome could be more convenient than **2** for further iron exchange in the cell, if such a process exists as it is sometimes implied.[4,5,21]

Stability Constant of Iron Complex of 2. In order to evaluate the stability constant, the proton dissociation constants of the three hydroxamic acid groups of **2** were obtained at 25 °C: $pK_1 = 8.42$, $pK_2 = 9.13$, $pK_3 = 9.76$. These are similar to those of **1**.[18] The iron stability constant ($\log K = 31.8$) of **2** was obtained from the acid dissociation constants and the stability constant of iron(III)-EDTA complex,[18] after determination of an equilibrium point for the iron exchange reaction of **2** with EDTA (at 25 °C and ionic strength 0.1). The value is larger than that ($\log K = 29.1$)[18] for ferrichrome.

Aluminum Complex of 2. The formation of alumichrome has been studied by NMR spectroscopy.[22] Formation of an Al(III) complex for analogues **2–5** was attempted by mixing them with $Al(OH)_3$ in DMSO-d_6. The NMR spectrum of such solution (100 °C) for compound **2** exhibited distinct spectral changes; disappearance of NOH protons, separation of a singlet for six amide protons (7.96) into glycyl (7.99) and ornithyl (7.80) protons, and

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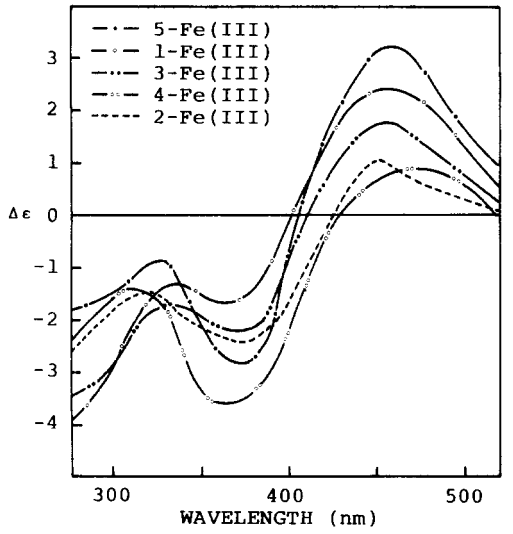

Figure 3. CD spectra for the complexes of Fe(III) with hexapeptides (1–5) in water at pH 7.0.

small shifts of ornithyl C^3H and C^4H (from 1.4–1.9 to 1.3–1.7) and glycyl CH_2 protons (from 3.58 and 3.96 to 3.62 and 3.89). These spectral changes are in accord with Al complex formation. Since chemical shifts of like protons of the OrnAH-Gly unit are the same, the metal complex assumes a C_3 symmetric structure. No intramolecular hydrogen bonds are formed, as the temperature dependences of amide protons are large and negative: −4.35 for 3Gly, −4.09 for 3OrnAH. The structure of this complex is clearly different from that of alumichrome[22] or ferrichrome.[9] The C_3 symmetric structure of the ligand and complex indicates an easy complex formation by preorganization of the ligand.

Stereochemistry of Iron Complexes. The Λ-cis configuration of hydroxamate ligands around the central iron has been proved for ferrichrome from X-ray analysis and CD spectra in the solid state and in solution.[9,23] From molecular model examination of the metal complexes, it is evident that the trans geometrical isomer is difficult and that there are two equally possible, Δ and Λ, configurations for the cis isomer.[4,6,9] Figure 3 shows CD spectra for iron complexes of hexapeptides 1–5 in water. All the CD spectra have a similar pattern, a negative band at 350, a crossover point at about 400, and a positive band at 450 nm. This is a characteristic of the Λ-cis configuration.[9,23] Therefore these iron(III) complexes stay predominantly in the Λ-cis configuration in water. It is tempting to speculate that there is controlling force at the chiral α-carbon in the ornithine residue to guide its side-chain terminal group to form only one configuration out of two possibilities.

It will be interesting to study whether these complexes are taken up into microorganisms after recognition in the cell membrane.

Experimental Section

All the melting points are uncorrected. IR spectra were recorded on JASCO Model A-302 and FT/IR-5M infrared spectrometers. ^1H NMR spectra were obtained on a JEOL JNM-FX 200 spectrometer with Me_4Si as the internal standard. Optical rotations were determined on a JASCO ORD/UV spectrometer. CD spectra were taken with a JASCO J-40 AS spectropolarimeter equipped with a DP-600 data processor. HPLC was carried out with a JASCO Model Twincle apparatus using a column packed with Finepak SIL C_{18}. TLC was performed with Merck Kieselgel 60 F_{254} and R_f was obtained in chloroform–methanol (7:1). N^2-(*tert*-Butoxycarbonyl)-N^5-acetyl-N^5-(benzyloxy)-L-ornithine (**6**) was prepared according to the literature.[24]

N^2-(*tert*-Butoxycarbonyl)-N^5-acetyl-N^5-(benzyloxy)-L-ornithylglycine Methyl Ester (7). DCC (0.76 g, 3.7 mmol) was added to a mixture of compound **6** (1.28 g, 3.36 mmol), H-Gly-OMe·HCl (0.42 g, 3.35 mmol), *N*-methylmorpholine (0.39 g, 3.8 mmol), and HOBt (0.91 g, 6.7 mmol) in DMF (20 mL) at −10 °C. The mixture was stirred for 48 h at room temperature before the removal of DCU by filtration. The filtrate was evaporated under vacuum, and the residue was dissolved in EtOAc. DCU, which separated, was filtered. The organic layer was washed with water, 5% $NaHCO_3$ (×3), 5% citric acid (×3), and brine and dried (Na_2SO_4). Evaporation of the solvent, followed by purification with gel chromatography (Sephadex LH-20; MeOH) gave the product as an oil, 1.28 g (84%): $[\alpha]^{25}_D$ −4.3° (*c* 1.0, MeOH); R_f 0.86; IR (neat) 1751, 1710, 1677 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.40 (s, 9 H), 1.70 (m, 4 H), 2.10 (s, 3 H), 3.68 (s, 3 H), 3.80 (AB q, 2 H), 4.02 (AB q, 2 H), 4.32 (m, 1 H), 4.82 (s, 2 H), 5.27 (d, 1 H, HN), 6.98 (s, 1 H, HN), 7.35 (s, 5 H). Anal. Calcd for $C_{22}H_{33}N_3O_7 \cdot {}^1/_2H_2O$: C, 57.38; H, 7.44; N, 9.12. Found: C, 57.73; H, 7.49; N, 9.12.

N^2-(*tert*-Butoxycarbonyl)-N^5-acetyl-N^5-(benzyloxy)-L-ornithylglycine (8). Compound **7** (0.75 g, 1.66 mmol) in acetone (14 mL) was mixed with 1 N NaOH (3.3 mL, 3.3 mmol) at 0 °C. The disappearance of **7** was followed with TLC. After 15 min, the mixture was neutralized with 1 N citric acid and evaporated. The residue was dissolved in 1 N citric acid and extracted with EtOAc. The organic layer was washed with water and brine and dried ($MgSO_4$). Evaporation of the solvent gave the product as an oil, 0.69 g (95%): $[\alpha]^{25}_D$ −4.3° (*c* 1.0, MeOH); R_f 0.51; IR (neat) 1710, 1670 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.40 (s, 9 H), 1.70 (m, 4 H), 2.06 (s, 3 H), 3.71 (AB pattern, 2 H), 4.00 (m, 2 H), 4.32 (br s, 1 H), 4.80 (s, 2 H), 5.67 (d, 1 H, HN), 7.19 (br s, 1 H, HN), 7.35 (s, 5 H).

TFA Salt of N^5-Acetyl-N^5-(benzyloxy)-L-ornithylglycine Methyl Ester (9). Compound **7** (0.55 g, 1.21 mmol) was dissolved in TFA (13.9 g, 0.122 mol) at 0 °C. Disappearance of **7** was monitored by TLC. The mixture was evaporated to remove TFA. MeOH was added to the residue and evaporated. Addition and evaporation of MeOH were repeated three times before the product was obtained (ca. 100%).

N^2-(*tert*-Butoxycarbonyl)-N^5-acetyl-N^5-(benzyloxy)-L-ornithylglycyl-N^5-acetyl-N^5-(benzyloxy)-L-ornithylglycine Methyl Ester (10). A procedure described for **7** was followed, with use of compound **8** (0.53 g, 1.2 mmol), compound **9** obtained above (1.2 mmol), *N*-methylmorpholine (0.14 g, 1.4 mmol), HOSu (0.28 g, 1.8 mmol), DCC (0.30 g, 1.4 mmol), and DMF (10 mL) to give the product, 0.75 g (80%): R_f 0.68; $[\alpha]^{25}_D$ −6.7° (*c* 1.0, MeOH); IR (neat) 1740, 1710, 1670 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.40 (s, 9 H), 1.70 (m, 8 H), 2.08 (s, 6 H), 3.68 (s, 3 H), 3.71 (AB pattern, 4 H), 3.90 (m, 4 H), 4.23 (m, 1 H), 4.61 (m, 1 H), 4.80 (s, 4 H), 5.65 (d, 1 H, HN), 7.30 (br s, 3 H, HN), 7.35 (s, 10 H). Anal. Calcd for $C_{38}H_{54}N_6O_{11} \cdot H_2O$: C, 57.93; H, 7.04; N, 10.67. Found: C, 58.18; H, 7.13; N, 10.68.

TFA Salt of N^5-Acetyl-N^5-(benzyloxy)-L-ornithylglycyl-N^5-(benzyloxy)-L-ornithylglycine Methyl Ester (11). A procedure for compound **9** was followed, with use of compound **10** (0.70 g, 0.91 mmol) and TFA (10 g, 90 mmol) to give the product (ca. 100%).

N^2-(*tert*-Butoxycarbonyl)-N^5-acetyl-N^5-(benzyloxy)-L-ornithyldi(glycyl-N^5-acetyl-N^5-(benzyloxy)-L-ornithyl)-glycine Methyl Ester (12). With use of compound **8** (0.424 g, 0.97 mmol), **11** (0.97 mmol), HOSu (0.223 g, 1.94 mmol), *N*-methylmorpholine (0.126 g, 1.25 mmol), DCC (0.22 g, 1.07 mmol), and DMF (20 mL), a coupling procedure for **7** was followed to give the product, 0.89 g (84%): mp 95–98 °C; R_f 0.61; $[\alpha]^{25}_D$ +4.2° (*c* 1.0, MeOH); IR (KBr) 1754, 1723, 1659 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.40 (s, 9 H), 1.70 (br s, 12 H), 2.10 (br s, 9 H), 3.68 (s, 3 H), 3.80 (AB q, 6 H), 3.90 (m, 6 H), 4.25 (br s, 1 H), 4.49 (m, 2 H), 4.82 (s, 6 H), 5.61 (br s, 1 H, NH), 7.35 (s, 15 H), 7.40 (m, 2 H, NH), 7.68 (m, 1 H, NH), 7.95 (m, 2 H, NH). Anal. Calcd for

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$C_{54}H_{75}N_9O_{15}\cdot H_2O$: C, 58.52; H, 7.00; N, 11.37. Found: C, 58.77; H, 6.90; N, 11.41.

N^2,N^5-Diacetyl-N^5-(benzyloxy)-L-ornithyldi(glycyl-N^5-acetyl-N^5-(benzyloxy)-L-ornithyl)glycine Methyl Ester (13). Deprotection of the Boc group in compound **12** (0.30 g, 0.27 mmol) was effected in TFA (3.1 g, 27 mmol) as described for **8** to give a residue, which was dissolved in THF. Et_3N (0.03 g, 0.3 mmol) was added. Acetic anhydride (0.034 g, 0.32 mmol) was added to this with stirring at 0 °C. A resulting gel was dissolved in $CHCl_3$, and the organic layer was washed with water and brine and dried (Na_2SO_4). The product was purified by gel chromatography (Sephadex LH-20; MeOH), 0.274 g (96%): mp 170–171 °C; R_f 0.51; IR (KBr) 1750, 1640 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.70 (m, 12 H), 1.96 (s, 3 H), 2.10 (s, 9 H), 3.64 (s, 3 H), 3.80 (AB q, 6 H), 3.90 (m, 6 H), 4.40 (br s, 1 H), 4.70 (br s, 2 H), 7.35 (s, 15 H), 7.40 (m, 3 H, NH), 7.75 (m, 1 H, NH), 8.00 (m, 2 H, NH).

N^2,N^5-Diacetyl-N^5-hydroxy-L-ornithyldi(glycyl-N^5-acetyl-N^5-hydroxy-L-ornithyl)glycine Methyl Ester (4). 10% Pd–C (210 mg) suspended in anhydrous methanol (20 mL) was prehydrogenated with H_2 for 0.5 h. Compound **13** (210 mg, 0.2 mmol) in anhydrous methanol (10 mL) was added to this suspension. After hydrogenation with H_2 under the atmospheric pressure at room temperature for 8 h, the catalyst was removed by filtration. The filtrate was evaporated to give a residue, which was purified by gel chromatography (Sephadex LH-20; MeOH) to afford the product, 100 mg (68%): IR (KBr) 1660 cm^{-1}; $[\alpha]^{25}_D$ −4.4° (*c* 1.0, MeOH); ^1H NMR (DMSO-d_6 at 50 °C) δ 1.55 and 1.65 (m, 12 H), 1.86 (s, 3 H), 1.97 (s, 9 H), 3.48 (br s, 6 H), 3.62 (s, 3 H), 3.74 (m, 4 H), 3.82 (d, 2 H), 4.15, 4.30, and 4.40 (br s, 3 H), 7.83 (d, 2 H, NH), 8.03 (m, 3 H, NH), 8.21 (t, 1 H, NH), 9.57 (br s, 3 H, HO). Anal. Calcd for $C_{30}H_{51}N_9O_{14}\cdot{}^3/_2H_2O$: C, 45.68; H, 6.89; N, 15.98. Found: C, 45.75; H, 6.77; N, 16.12.

N-(*tert*-Butoxycarbonyl)di(N^5-acetyl-N^5-(benzyloxy)-L-ornithyl)glycyl-N^5-acetyl-N^5-(benzyloxy)-L-ornithylglycine Methyl Ester (14). With use of compound **6** (0.30 g, 0.79 mmol), **11** (0.79 mmol), *N*-methylmorpholine (80 mg, 0.79 mmol), HOSu (0.18 g, 1.6 mmol), DCC (0.18 g, 0.87 mmol), and DMF (10 mL), coupling was carried out by a similar procedure described for **7** to give the product, 0.50 g (61%): $[\alpha]^{25}_D$ +7.0° (*c* 1.0, MeOH); IR (neat) 1751, 1710, 1640 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.40 (s, 9 H), 1.70 (m, 12 H), 2.10 (s, 9 H), 3.68 (s, 3 H), 3.72 (AB q, 6 H), 3.90 (m, 4 H), 4.15, 4.28, and 4.55 (br s, 3 H), 4.80 (s, 6 H), 5.75 (d, 1 H, NH), 7.30 (m, 2 H, NH), 7.35 (s, 15 H), 7.48 (m, 1 H, NH), 7.62 (m, 1 H, NH).

N-(*tert*-Butoxycarbonyl)glycyldi(N^5-acetyl-N^5-(benzyloxy)-L-ornithyl)glycyl-N^5-acetyl-N^5-(benzyloxy)-L-ornithylglycine Methyl Ester (15). Deprotection of the Boc group of compound **14** (0.45 g, 0.436 mmol) and simultaneous transformation into the TFA salt was carried out by the above-described procedure. Boc-Gly-OH (90 mg, 0.51 mmol) was coupled with this TFA salt (0.436 mmol) by a procedure described for **7**, with *N*-methylmorpholine (44 mg, 0.43 mmol), HOSu (0.1 g, 0.87 mmol), DCC (0.10 g, 0.48 mmol), and DMF (8 mL). The product was obtained, 0.33 g (68%): R_f 0.59; IR (neat) 1750, 1710, 1650 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.41 (s, 9 H), 1.75 (m, 12 H), 2.10 (s, 9 H), 3.68 (s, 3 H), 3.75 (m, 12 H), 4.15, 4.40, and 4.52 (br s, 3 H), 4.80 (s, 6 H), 5.73 (m, 1 H, NH), 7.35 (s, 15 H), 7.40 (m, 2 H, NH), 7.68 (m, 1 H, NH), 7.80 (m, 2 H, NH).

N^2-Acetylglycyldi(N^5-acetyl-N^5-(benzyloxy)-L-ornithyl)glycyl-N^5-acetyl-N^5-(benzyloxy)-L-ornithylglycine Methyl Ester (16). A similar procedure described for **13** gave the product **16** by the use of compound **15** (0.323 g, 0.297 mmol), TFA (3.38 g, 29.6 mmol), Et_3N (0.032 g, 0.31 mmol), Ac_2O (36 mg, 0.35 mmol), and THF (5 mL), 0.23 g (74%): mp 134–137 °C; R_f 0.47; $[\alpha]^{25}_D$ −15.4° (*c* 1.0, MeOH); IR (KBr) 1750, 1660 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.70 (m, 12 H), 1.97 (s, 3 H), 2.07 (s, 9 H), 3.68 (s, 3 H), 3.80 (m, 6 H), 3.90 (m, 6 H), 4.25, 4.40 and 4.55 (br s, 3 H), 4.80 (s, 6 H), 7.30 (m, 4 H, NH), 7.35 (s, 15 H), 7.70 (m, 2 H, NH). Anal. Calcd for $C_{51}H_{69}N_9O_{14}\cdot H_2O$: C, 58.33; H, 6.81; N, 12.00. Found: C, 58.53; H, 6.76; N, 12.11.

N-Acetylglycyldi(N^5-acetyl-N^5-hydroxy-L-ornithyl)glycyl-N^5-acetyl-N^5-hydroxy-L-ornithylglycine Methyl Ester (5). Compound **16** (0.2 g, 0.19 mmol) was hydrogenated in MeOH (12 mL) with 10% Pd–C (0.20 g) by the procedure described for **4**, affording the product, 0.13 g (86%): $[\alpha]^{25}_D$ −11.0° (*c*, 1.0, MeOH); IR (KBr) 1659 cm^{-1}; ^1H NMR (DMSO-d_6 at 50 °C) δ 1.4–1.7 (m, 12 H), 1.86 (s, 3 H), 1.97 (s, 9 H), 3.47 (s, 6 H), 3.62 (s, 3 H), 3.74 (m, 6 H), 4.30 (br s, 3 H), 7.88 (br s, 4 H, NH), 8.01 (t, 1 H, NH), 8.22 (t, 1 H, NH), 9.57 (br s, 3 H, HO). Anal. Calcd for $C_{30}H_{51}N_9O_{14}\cdot H_2O$: C, 46.21; H, 6.85; N, 16.03. Found: C, 46.52; H, 6.81; N, 16.16.

N^2-(*tert*-Butoxycarbonyl)di(N^5-acetyl-N^5-(benzyloxy)-L-ornithyl)glycine Methyl Ester (17). Compound **9** (0.89 mmol) was coupled with compound **6** (0.34 g, 0.89 mmol) by use of HOSu (0.2 g, 1.7 mmol), *N*-methylmorpholine (91 mg, 0.90 mmol), DCC (0.21 g, 1.0 mmol), and DMF (5 mL) according to the procedure described for **7** to give the product, 0.42 g (66%): R_f 0.69; $[\alpha]^{25}_D$ −15.9° (*c* 1.1, MeOH); IR (neat) 1760, 1710, 1650 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.42 (s, 9 H), 1.70 (m, 8 H), 2.10 (s, 6 H), 3.68 (s, 3 H), 3.71 (AB q, 4 H), 3.94 (AB q, 2 H), 4.27 (t, 1 H), 4.60 (t, 1 H), 4.80 (s, 4 H), 5.40 (d, 1 H, NH), 7.15 (t, 2 H, NH), 7.35 (s, 10 H).

N-(*tert*-Butoxycarbonyl)tri(N^5-acetyl-N^5-(benzyloxy)-L-ornithyl)glycine Methyl Ester (18). Compound **17** (0.60 g, 0.84 mmol) was converted to a deprotected TFA salt with TFA (9.6 g, 0.84 mol) by the procedure for **9**. The TFA salt was coupled with compound **6** (0.32 g, 0.84 mmol) by the use of HOSu (0.19 g, 1.7 mmol), *N*-methylmorpholine (90 mg, 0.89 mmol), DCC (0.21 g, 1.0 mmol), and DMF (7 mL) to give the product, 0.53 g (64%): R_f 0.61; $[\alpha]^{25}_D$ −18.1° (*c* 1.0, MeOH); IR (neat) 1750, 1710, 1650 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.42 (s, 9 H), 1.70 (m, 12 H), 2.10 (s, 9 H), 3.68 (s, 3 H), 3.75 (AB q, 6 H), 3.94 (m, 2 H), 4.26 (m, 1 H), 4.40 (m, 2 H), 4.80 (s, 6 H), 5.40 (d, 1 H, NH), 7.12 (t, 1 H, NH), 7.30 (m, 2 H, NH), 7.35 (s, 15 H).

N-(*tert*-Butoxycarbonyl)diglycyltri(N^5-acetyl-N^5-(benzyloxy)-L-ornithyl)glycine Methyl Ester (19). Compound **18** (0.50 g, 0.513 mmol) was transformed with TFA (6.0 g, 53 mmol) into a deprotected TFA salt.

The TFA salt (0.513 mmol) was coupled with Boc-Gly-Gly-OH (0.12 g, 0.52 mmol) in DMF (7 mL) in the presence of HOSu (0.12 g, 1 mmol), *N*-methylmorpholine (55 mg, 0.54 mmol), and DCC (160 mg, 0.77 mmol) according to the procedure for **7** to give the product, 0.33 g (58%): mp 128–131 °C; R_f 0.54; $[\alpha]^{25}_D$ −13.8° (*c* 0.87, MeOH); IR (KBr) 1750, 1710, 1660 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.40 (s, 9 H), 1.75 (m, 12 H), 2.10 (s, 9 H), 3.68 (s, 3 H), 3.75 (m, 12 H), 4.28 (m, 2 H), 4.42 (m, 1 H), 4.81 (s, 6 H), 6.02 (s, 1 H, NH), 7.30 (m, 3 H, NH), 7.35 (s, 15 H), 7.60 (br s, 2 H NH). Anal. Calcd for $C_{54}H_{75}N_9O_{15}\cdot H_2O$: C, 58.52; H, 7.00; N, 11.37. Found: C, 58.17; H, 6.89; N, 11.39.

N-Acetyldiglycyltri(N^5-acetyl-N^5-(benzyloxy)-L-ornithyl)glycine Methyl Ester (20). Compound **19** (175 mg, 0.158 mmol), with TFA (1.83 g, 16 mmol), Et_3N (17 mg, 0.16 mmol), and Ac_2O (21 mg, 0.2 mmol), was transformed into the product: 0.10 g (60%): mp 158–160 °C; R_f 0.26 ($CHCl_3$/MeOH, 10); IR (KBr) 1740, 1640 cm^{-1}.

N-Acetyldiglycyltri(N^5-acetyl-N^5-hydroxy-L-ornithyl)glycine Methyl Ester (3). Compound **20** (0.1 g, 0.095 mmol) was hydrogenated in MeOH/H_2O (13 mL/5 mL) with 10% Pd–C (0.10 g) by the procedure for **4**. The product was obtained by gel chromatography (Sephadex G-10; H_2O), 63 mg (85%): $[\alpha]^{25}_D$ −35.0° (*c* 1.0, MeOH); IR (KBr) 1659 cm^{-1}; ^1H NMR (DMSO-d_6 at 50 °C) δ 1.60 (m, 12 H), 1.86 (s, 3 H), 1.97 (s, 9 H), 3.49 (br s, 6 H), 3.62 (s, 3 H), 3.78 (m, 6 H), 4.25 (m, 3 H), 7.76 (d, 1 H, NH), 7.84 (d, 1 H, NH), 7.87 (d, 1 H, NH), 8.02 (t, 1 H, NH), 8.07 (t, 1 H, NH), 8.14 (t, 1 H, NH), 9.59 (br s, 3 H, HO). Anal. Calcd for $C_{30}H_{51}N_9O_{14}\cdot{}^5/_2H_2O$: C, 44.66; H, 6.99; N, 15.61. Found: C, 44.60; H, 6.51; N, 15.18.

Cyclotri(N^5-acetyl-N^5-(benzyloxy)-L-ornithylglycyl) (21). The C-terminal methyl ester of compound **12** (0.55 g, 0.50 mmol) was hydrolyzed with 1 N NaOH (0.99 mL) in MeOH (6 mL) by a similar procedure for **8** to give the hydrolyzed product, 0.50 g (90%): mp 90–92 °C; IR (KBr) 1660 cm^{-1}.

DEC·HCl (88 mg, 0.46 mmol) in CH_2Cl_2 (5 mL) was added to a DMF solution (3 mL) of the carboxylic acid obtained above (0.24 g, 0.22 mmol) and HOSu (0.051 g, 0.44 mmol) at −10 °C. After 15 h of stirring at 0 °C, ethyl acetate (50 mL) was added to the mixture. The organic layer was washed with water (×3) and dried (Na_2SO_4). Evaporation of the solvent gave the C-terminal *N*-hydroxysuccinimide ester, which was used directly for the next reaction.

The crude ester (0.22 g, 0.18 mmol) in CH_2Cl_2 was dissolved in TFA (3.1 g, 27 mmol) at 0 °C. The reaction was monitored with TLC. The solvent was evaporated when the starting material

was consumed. The residue, dissolved in DMF (4 mL), was added to pyridine (62 mL) heated at 60 °C. The pyridine solution, after being stirred for 48 h at 60 °C, was evaporated under reduced pressure. The residue was extracted with $CHCl_3$, washed with water, dried (Na_2SO_4), and evaporated. The product was obtained by gel chromatography (Sephadex LH-20; MeOH), 83 mg (47%): mp 164-166 °C; $[\alpha]^{25}_D$ -19.2° (c 0.83, MeOH); ^1H NMR ($CDCl_3$) δ 1.75 (m, 12 H), 2.07 (s, 9 H), 3.63 (m, 9 H), 4.08 (part of AB q, 3 H), 4.35 (br s, 3 H), 4.82 (s, 6 H), 7.35 (s, 15 H), 7.63 (d, 3 H, NH), 7.87 (t, 3 H, NH).

Cyclotri(N^5-acetyl-N^5-hydroxy-L-ornithylglycyl) (2). Compound **21** (0.18 g, 0.19 mmol) was hydrogenated with 10% Pd-C (0.18 g) in MeOH/H_2O (20 mL/10 mL), as described for **3**, to afford the product, 0.12 g (84%): $[\alpha]^{25}_D$ -14.9° (c 1.0, MeOH); IR (KBr) 1650 cm^{-1}; ^1H NMR (DMSO-d_6 at 50 °C) δ 1.50 (m, 6 H), 1.70 (m, 6 H), 1.96 (s, 9 H), 3.45 (t, 6 H), 3.78 (AB q, 6 H), 4.22 (q, 3 H), 8.15 (br s, 6 H), 9.54 (br s, 3 H). Anal. Calcd for $C_{27}H_{45}N_9O_{12}{}^3/_2H_2O$: C, 45.37; H, 6.77; N, 17.64. Found: C, 45.58; H, 6.78; N, 17.31.

Cyclo(triglycyltri(N^5-acetyl-N^5-(benzyloxy)-L-ornithyl)) (22). Compound **19** (0.30 g, 0.27 mmol) was hydrolyzed with 1 N NaOH (1.0 mL, 1.0 mmol) in MeOH (4 mL) to give a crude product, 0.227 g (77%). The product was esterified with HOSu (0.50 g, 0.43 mmol) with DEC·HCl (81 mg, 0.42 mmol) in DMF (3 mL)/CH_2Cl_2 (5 mL) to give 0.185 g (74%). The OSu ester (0.156 mmol) in CH_2Cl_2 (2 mL) was treated with TFA (2.68 g, 23 mmol) and then dissolved in DMF (4 mL). Addition of the DMF solution into pyridine (52 mL) heated at 60 °C gave the cyclic product, 74 mg (49%): mp 176-179 °C; R_f 0.37; IR (KBr) 1660 cm^{-1}.

Compound **22** (74 mg, 0.076 mmol) was hydrogenated with 10% Pd-C (74 mg) in MeOH/H_2O (15 mL/5 mL) and purified with Sephadex G-10 to give compound **1**, 45 mg (83%): IR (KBr) 1660 cm^{-1}; ^1H NMR (DMSO-d_6 at 50 °C) gave signals virtually at the same positions as those of **1**. Anal. Calcd for $C_{27}H_{45}N_9O_{12}{}^3/_2H_2O$: C, 45.37; H, 6.77; N, 17.63. Found: C, 45.33; H, 6.59; N, 17.16.

Iron(III) Binding Ratio. A sample (14-15 mg) of each hexapeptide was dissolved in water (10.0 mL); 1.0 mL of the sample solution and 1.0 mL of 0.2 N aqueous KNO_3 solution were mixed. An appropriate amount of a standardized aqueous ferric nitrate solution (3.07×10^{-3} M) was added to this. The pH of the mixture was adjusted to 4.0 or 5.0 with 0.01 or 0.1 N KOH and diluted to 10.0 mL before spectral determination.

Spectral Determination of the 1:1 Mixture. An accurately weighed sample (1.2 mg) of an analogue was mixed with an equimolar amount of ferric nitrate solution (3.07×10^{-3} M) and diluted to 10.0 mL. The pH of a 3.0-mL solution was adjusted to an appropriate value with 0.1 or 0.01 N KOH or 0.01 N HNO_3, and after 1 h, the visible spectrum was measured.

Iron(III) Exchange Reactions. Iron complex solutions of hexapeptides were prepared by dissolving an accurately weighed sample (6-8 mg) in an equivalent amount of ferric nitrate solution (3.07×10^{-3} M) and an equal volume of 0.2 M aqueous KNO_3 solution. The solution was made pH 5.0 with aqueous KOH before dilution to 5.0 mL with 0.1 M KNO_3 solution.

EDTA in buffer solution was made by dissolving EDTA·$2Na^+$·$2H_2O$ in acetate buffer solution (ionic strength 0.1, pH 5.42) to give a concentration of 9.27×10^{-3} M.

Iron(III) exchange reaction was followed by observing the decrease of an absorbance at 425 nm by repeat scanning with a Hitachi 320 S spectrophotometer. Each run was carried out in a cuvette with 3.0 mL of solution containing an iron complex and EDTA with a ratio of 1/20. A constant temperature (25 °C) was maintained. The first-order rate constant (k_{obsd}) was obtained from the plot of ln $[(OD_0 - OD_\infty)/(OD_t - OD_\infty)]$ vs time.

Proton Dissociation and Iron Stability Constants for Compound 2. Compound **2** (25.5 mg) was dissolved in degassed and deionized water (25 mL), and 20 mL of the solution was titrated with 0.01 N NaOH (carbonate free) under an atmosphere of argon at 25 °C and ionic strength 0.10 ($NaClO_4$). The proton dissociation constants (pK_1, pK_2, and pK_3) were obtained by computer calculation with aid of Gran's plot.

The iron complex stability constant was obtained by using the iron stability constant of EDTA[18] and the pK's of the hydroxamic acid groups, after determination of an apparent equilibrium point in a mixture of the iron complex of **2** and EDTA at pH 5.3, ionic strength 0.1, and 25 °C.

Aluminum Complex Formation. A solution of compound **2**-Al(III) complex was prepared by dissolving **2** (10 mg) in DMSO-d_6 (0.5 mL), followed by the addition of aluminum hydroxide (10 equiv). The mixture was sonicated with slight heating and filtered to remove excess of the hydroxide. The precipitate was washed with DMSO-d_6 (0.3 mL), and the DMSO-d_6 solution was combined for NMR spectral measurement.

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Hydrotitanation-Protonation of Vitamin D_2 and Its Analogues: An Efficient Method for the Preparation of 10,19-Dihydrovitamins D_2 Including Dihydrotachysterol$_2$†

J. G. Cota, M. C. Meilán, A. Mouriño,* and L. Castedo*

Departamento de Química Orgánica, Facultad de Química y Sección de Alcaloides del CSIC, Santiago de Compostela, Spain

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In this study we describe an easy and efficient method for the preparation of the known 10,19-dihydrovitamins D_2 **2b** (DHV_2-II), **2c** (DHV_2-IV), **3c** (dihydrotachysterol$_2$, DHT_2), and the new dihydrovitamins D_2 **2f** and **2g**. This method is based on the regioselective hydrometalation reaction of vitamin D_2 and its derivatives with the system Cp_2TiCl_2-$LiAlH_4$ or Cp_2TiCl_2-Red-Al (Aldrich). Under optimal conditions, the reaction with the former of these hydrometalating systems takes place with a high degree of stereoselectivity and allows labeling at C-19.

Introduction

Dihydrovitamins D are a class of compounds derived by reduction of the natural vitamin D_3 (**1a**), the unnatural vitamin D_2 (**2a**), and their 5E isomers (5,6-trans derivatives) (Chart I). Among them, dihydrotachysterol$_2$ (DHT_2, **3c**), first isolated by von Werder,[1] is considered an interesting analogue of 1α,25-dihydroxyvitamin D_3 (**1b**), the hormonal form of vitamin D_3, because the former's 3β-OH

† This work was taken in part from the Ph.D. Thesis of J.G.C. and was presented as a communication in the Sixth Workshop on Vitamin D, Merano, Italy, 1985.

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