Synthesis of Retrohydroxamate Analogues of the Microbial Iron-Transport Agent Ferrichrome

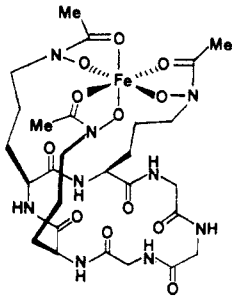
Richard K. Olsen* and K. Ramasamy

Department of Chemistry and Biochemistry, Utah State University, Logan, Utah 84322

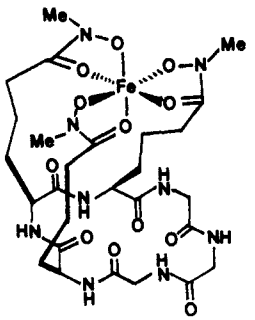
Received September 17, 1984

A retrohydroxamate analogue (2) of the peptidyl iron transport agent ferrichrome 1 has been prepared in which transposition of the hydroxamate functionalities in the respective amino acid side chains, as compared to ferrichrome, has been effected. The requisite benzyl *N*-methyl-N^{α}-Boc-L-α-aminoadipohydroxamate derivative was prepared and incorporated, via the corresponding mixed carbonic anhydride, into an appropriate linear hexapeptide containing three residues each of glycine and L-α-aminoadipic acid. Cyclization of the linear peptide was effected by application of the carbodiimide/*N*-hydroxybenzotriazole method in 51% yield, or in lower yield (19%), via the corresponding *p*-nitrophenyl ester. Removal of the *O*-benzyl group on the hydroxamate hydroxyl function provided the peptide ligand, which upon complexation with ferric ion furnished the desired retrohydroxamate ferrichrome analogue 2. A second retrohydroxamate analogue 22 also was prepared in which the *N*-methyl group on the hydroxamate nitrogen was replaced by hydrogen.

Ferrichrome 1 is a well-known microbial transport agent responsible for cellular uptake of iron.[1] Transport of ferric



1

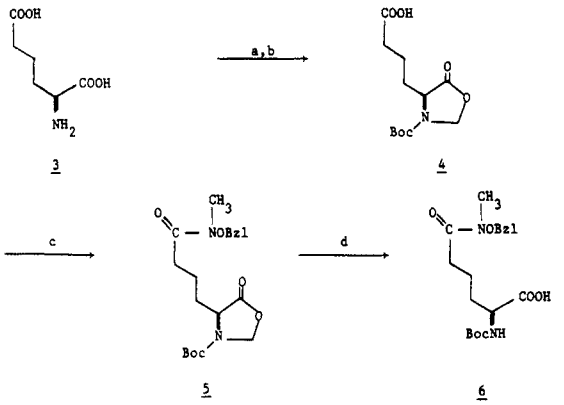


2

ion into the cell occurs via the hexacoordinate ferric ion complex formed by internal ligation with the hydroxamate functions in the side chains of the three functionalized ornithine residues. Reduction of the iron in ferrichrome within the cell to the ferrous state results in the release of the iron for use by the cell.[1] Total syntheses of ferrichrome[2] and of enantioferrichrome[3] have been reported, while the semisynthesis from ferrichrome of analogues in which the *N*-acetyl groups have been replaced with other acyl groups are known.[4] However, the preparation, by total synthesis, of structurally modified analogues of ferrichrome has not been developed. We wish to report the preparation of a retrohydroxamate analogue 2 of ferrichrome, in which the *N*-hydroxy and carbonyl functions are transposed relative to their positions in the ornithine side chains in ferrichrome.[5] While the effect of this structural modification on cellular transport was of interest, an additional rationale for preparation of the analogue was the anticipated synthetic advantage that may result from elimination of the need to introduce the N^{δ}-hydroxyornithine unit, the introduction of which had proven to be a major challenge in previous syntheses.[2,3]

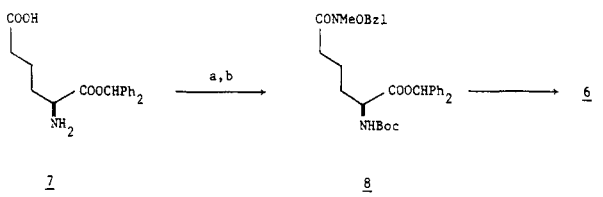
Preparation of retrohydroxamate ferrichrome 2 by transposition of the hydroxamate functions in ferrichrome requires replacement of the three N^{δ}-acetyl-N^{δ}-hydroxy-L-ornithine residues in ferrichrome by three *N*-methyl-L-α-aminoadipo-δ-hydroxamate units. The protected α-aminoadipic acid derivative utilized in our peptide synthesis was benzyl *N*-methyl-N^{α}-Boc-L-α-aminoadipo-δ-hydroxamate (6), which was prepared (Scheme I) from L-α-aminoadipic acid 3.[6] Transformation of 3 to the di-

Scheme I[a]



[a] (a) Di-*tert*-butyl dicarbonate, Et_3N, DMF, H_2O, 96%, mp 121-122 °C; (b) TsOH, paraformaldehyde, $ClCH_2CHCl_2$, 86%, oil; (c) isobutyl chloroformate, THF, -10 °C; $TFA^-CH_3NH_2OBzl^+$, *N*-methylmorpholine, 70%, oil; (d) 1 N NaOH, MeOH, then H^+, oil, 97%.

Scheme II[a]



[a] (a) Di-*tert*-butyl dicarbonate, DMF, H_2O; (b) isobutyl chloroformate, THF, *N*-methylmorpholine, -10 °C; BzlONHMe, 63% from 7; (c) 1 N NaOH, acetone, then H^+, 90%.

(1) Emery, T. *Am. Sci.* **1982**, *70*, 626-632. Emery, T. "Metals in Biological Systems"; Sigel, H., Ed.; Marcel Dekker; New York, 1978; Vol. 7, pp 77-126. Neilands, J. B. "Inorganic Biochemistry"; Eichorn, G. L., Ed.; Elsevier: New York, 1973; Vol. 1, pp 167-202. Isolation: Neilands, J. B. *J. Am. Chem. Soc.* **1952**, *74*, 4846-4847. Structure: Rogers, S.; Warren, R. A. J.; Neilands, J. B. *Nature (London)* **1963**, *200*, 167. Rogers, S., Neilands, J. B. *Biochemistry* **1964**, *3*, 1850-1855. Crystal Structure: van der Helm, D.; Baker, J. R.; Eng-Wilmont, D. L.; Hossain, M. B.; Loghry, R. A. *J. Am. Chem. Soc.* **1980**, *102*, 4224-4231.

(2) Keller-Schierlein, W.; Maurer, B. *Helv. Chim. Acta* **1969**, *52*, 603-610. Isowa, Y.; Ohmori, M.; Kurita, H. *Bull. Chem. Soc. Jpn.* **1974**, *49*, 215-220.

(3) Naegeli, H.-U.; Keller-Schierlein, W. *Helv. Chim. Acta* **1978**, *61*, 2088-2095.

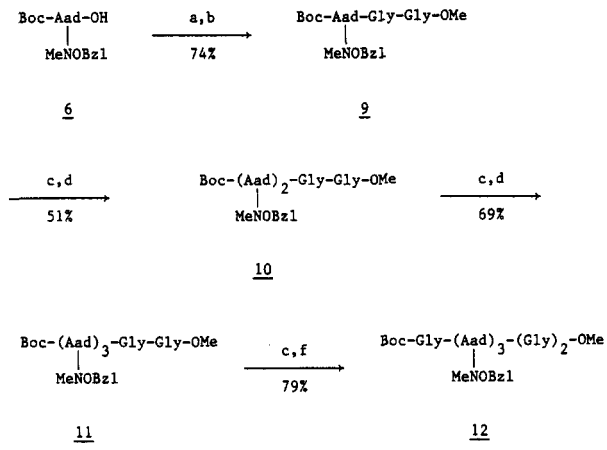
(4) Emery, T.; Emery, L. *Biochem. Biophys. Res. Commun.* **1973**, *50*, 670-675.

(5) Portions of this work have appeared in preliminary form: Ramasamy, K.; Olsen, R. K.; Emery, T. In "Proceedings of the Eighth American Peptide Symposium"; Hruby, V., Rich, D., Eds.; Pierce Chemical: Rockford, IL, 1983; p 187.

(6) Scott, A. I.; Wilkinson, T. J. *Synth. Commun.* **1980**, *10*, 127-131.

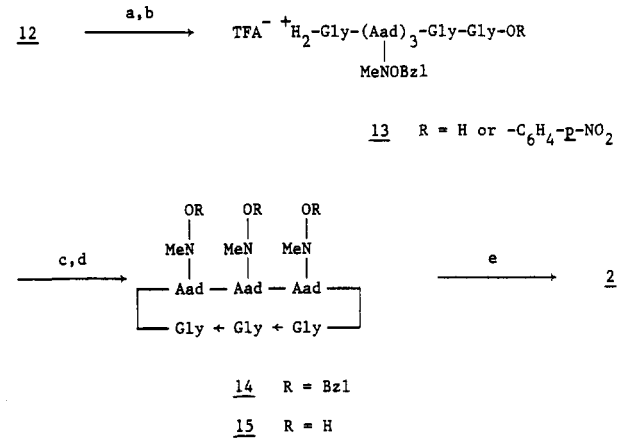
0022-3263/85/1950-2264$01.50/0

Scheme III[a]



[a] (a) Isobutyl chloroformate, R_3N, THF, −10 °C; (b) H-Gly-Gly-OMe, oil; (c) TFA, CH_2Cl_2, 25 °C; (d) isobutyl carbonic anhydride of **6**, R_3N, THF, −10 °C; (f) isobutyl carbonic anhydride of Boc-Gly-OH, R_3N, THF, −10 °C.

Scheme IV[a]



[a] (a) 1 N NaOH, acetone, then H^+, 68%, foam; (b) TFA, CH_2Cl_2, 25 °C; (c) 1-ethyl-3-[3-(dimethylamino)propyl]-carbodiimide·HCl, *N*-hydroxybenzotriazole, *N*-methylmorpholine, DMF/CH_2Cl_2, 51%, mp 175–180 °C; (d) H_2, Pd/C, 42%; or $NH_4^+HCOO^-$, Pd, 70%; (e) $FeCl_2$, O_2.

protected α-aminoadipic acid **4** was effected in good yield by introduction of the *tert*-(butyloxy)carbonyl (Boc) function, followed by treatment with paraformaldehyde and tosic acid to form the oxazolidinone **4**.[7] Coupling of **4**, as the isobutyl carbonic anhydride, with *N*-methyl-*O*-benzylhydroxylamine[8] gave the protected hydroxamate **5**. Liberation of the α-carboxyl group by saponification of **5** furnished the hydroxamate **6**.

A second route (Scheme II) to the protected α-aminoadipic acid **6** utilized the known α-benzhydryl ester **7**.[9] The α-amino group in **7** was protected with the Boc function, after which coupling with *N*-methyl-*O*-benzylhydroxylamine provided the fully protected α-aminoadipic acid **8**. Removal of the benzhydryl group by alkaline hydrolysis yielded **6**. In practice, the procedure outlined in Scheme I proved to be more convenient and was the method of choice for the preparation of **6**.

Our next objective was the preparation of the linear hexapeptide **12** (Scheme III). A glycine residue was placed at the C-terminus in **12** so as to eliminate the potential for racemization at the C-terminal unit in the subsequent cyclization reaction. Methodology for elaboration of the linear hexapeptide **12** utilized the mixed anhydride method[10] for the coupling reactions, while the Boc protecting groups were removed with 50% trifluoroacetic acid (TFA) in dichloromethane. Thus, coupling of the isobutyl carbonic anhydride of **6** with the dipeptide ester, H-Gly-Gly-OMe, gave **9**. A repetitive sequence of deprotection and coupling effected conversion of **9**, via **10** and **11**, to hexapeptide **12**. All of these peptide intermediates were oils or foams and were purified by medium-pressure liquid chromatography[11] on silica gel. The various peptides were characterized by analysis of their corresponding ^1H NMR (360 MHz) spectra.

Saponification of **12** to the corresponding acid, followed by deprotection of the amino terminus, provided hexapeptide **13** (R = H) (Scheme IV). Cyclization of **13** was effected by the carbodiimide/*N*-hydroxybenzotriazole method[12] at high dilution in dimethylformamide/dichloromethane Cyclic product **14** was obtained in a yield of 51%. Removal of the *O*-benzyl groups on the side-chain hydroxamate functions was accomplished by hydrogenolysis to yield the cyclic deferriretrohydroxamate analogue **15**. Complexation of **15** with ferric ion by standard procedures[13] gave retrohydroxamate ferrichrome **2**.

A second cyclization method was investigation for the preparation of cyclic peptide **14**. Hexapeptide **12** was converted to the *p*-nitrophenyl active ester[14] **13** (R = C_6H_4-*p*-NO_2) by a sequence involving saponification, esterification with bis (*p*-nitrophenyl)sulfite,[15] and removal of the Boc group. Cyclization of the active ester **13** in dimethylformamide/pyridine at 50–60 °C furnished cyclic peptide **14** in only 19% yield.

The hydrogenolysis of cyclic peptide **14** to the deferriretro analogue **15** proved to be somewhat problematic. The hydrogenolysis reaction, when carried out on a small scale (0.1 mmol) with 5% palladium/carbon in methanol, gave **15** in only moderate yield (42%). The above reaction, when performed on a larger scale (0.36 mmol), provided **15** in slightly reduced yield (38%). Hydrogenolysis of **14** (0.05 mmol) using palladium and ammonium formate as a source of hydrogen[16] gave deprotected hexapeptide **15** in a yield of 70%; this method appears to be the most suitable for deprotection of the *O*-benzyl hydroxamate protecting group in cyclic peptide **14**.

In this study, we also have prepared the *N*-demethylretrohydroxamate analogue **22**. This compound differs from **2** by replacement of the methyl groups on the three hydroxamate nitrogen atoms with a hydrogen; **22** would correspond to an *N*-formyl ferrichrome, a presently unknown siderophore.

The L-α-aminoadipic acid hydroxamate **16** was readily prepared from either **4** or **7** by application of the procedures used for the above described synthesis of **6**. Preparation of the linear hexapeptide **17** was effected by coupling **16** with H-Gly-Gly-OMe, followed by a sequence of deprotection (TFA in CH_2Cl_2) and coupling of the ap-

(7) Gut, V.; Rudinger, J. *Coll. Czech. Chem. Commun.* **1963**, *28*, 2953–2967.
(8) Ramasamy, K.; Olsen, R. K.; Emery, T. *J. Org. Chem.* **1981**, *46*, 5438–5441.
(9) Wolfe, S.; Jokiner, M. G. *Can. J. Chem.* **1979**, *57*, 1388.
(10) Anderson, A. W.; Zimmerman, J. E.; Callahan, F. M. *J. Am. Chem. Soc.* **1967**, *89*, 5012–5017.
(11) Meyers, A. I.; Slade, J.; Smith, R. K.; Mihelich, E. D.; Hershenson, F. M.; Liang, C. D. *J. Org. Chem.* **1979**, *44*, 2247–2249.
(12) Konig, W.; Geiger, R. *Chem. Ber.* **1970**, *103*, 788–798.
(13) Garibaldi, J. A.; Neilands, J. B. *J. Am. Chem. Soc.* **1975**, *77*, 2429–2430.
(14) Ludeschev, U.; Schwyzer, R. *Helv. Chim. Acta* **1972**, *55*, 2052–2060.
(15) Iselin, B.; Rittel, W.; Sieber, P.; Schwyzer, R. *Helv. Chim. Acta* **1957**, *40*, 373. Iselin, B.; Schwyzer, R. *Helv. Chim. Acta* **1960**, *43*, 1760.
(16) Anwer, M. K.; Spatola, A. F. *Synthesis* **1980**, 929.

Chart I

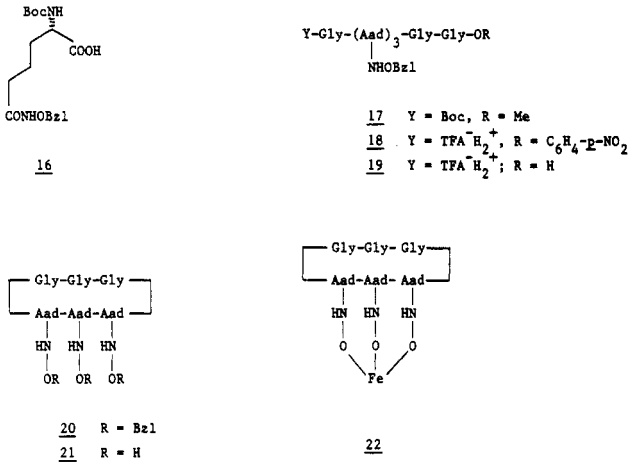
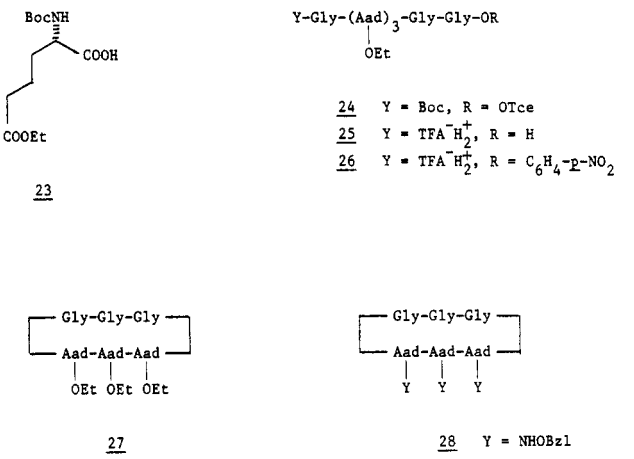


Chart II



propriate *N*-Boc-α-amino acid via the corresponding mixed carbonic anhydride. We observed in the purification of the tetra- and pentapeptides leading to **17**, as also with hexapeptide **17**, that these peptides were quite insoluble in organic solvents. This necessitated the use of a mixture of 1-butanol/ethyl acetate (1:2) as the organic phase in extraction procedures, otherwise, poor recovery of the peptide from the aqueous phase was encountered (Chart I).[17]

In contrast to the *N*-methyl retro analogue, cyclization to provide cyclic hexapeptide **20** was effected in the highest yield by use of the *p*-nitrophenyl ester method. Thus, hexapeptide **17** was converted to the *p*-nitrophenyl ester TFA salt **18** by standard procedures. Cyclization of **18** in dimethylformamide/pyridine at 60–65 °C furnished **20** in 54%. By comparison, cyclization of **19** using the carbodiimide/*N*-hydroxybenzotriazole procedure gave cyclic product **20** in a yield of 34%. Use of diphenylphosphoryl azide[18] as a reagent for cyclization of **19** also provided **20** in low yield (19%).

Simultaneously we have investigated another approach for synthesis of the cyclic deferri retro analogues that would involve introduction of the hydroxamate function into the α-aminoadipic acid side chains after the cyclic hexapeptide has been formed. We, therefore, prepared cyclic hexapeptide **27** having an ethyl ester as a side-chain functionality, which would be an appropriate functional group to allow transformation into the desired hydroxamate. The linear hexapeptide **24** was readily prepared from the 2,2,2-trichloroethyl ester (Tce) of glycylglycine by the stepwise coupling of three units of the α-aminoadipic acid δ-ethyl ester[19] **23** and one unit of Boc-Gly-OH, as their respective mixed carbonic anhydrides. The C-terminal glycine residue was protected with the Tce ester so as to allow selective deprotection at the C-terminus in presence of the side-chain ethyl ester groups by treatment with zinc in acetic acid.[20] Cyclization to provide **27** was effected from either **25** (carbodiimide/*N*-hydroxybenzotriazole, 59%) or active ester **26** (55%) (Chart II).

Attempts to transform the side-chain ethyl ester functions in **27** to the corresponding trihydroxamate **20** were unsuccessful. Attempted amination of **27** with *O*-benzylhydroxylamine in dimethylformamide at room temperature resulted in recovery of the reactant triester. Reaction of **27** with *O*-benzylhydroxylamine in sodium methoxide/methanol at reflux temperature was next attempted. Thin-layer chromatographic analysis showed a complex mixture of products, including some of the desired hydroxamate **20**, to have been formed. These results, however, indicated a lack of promise in this approach to the cyclic trihydroxamate compounds and studies along this line were not continued.

In summary, two retrohydroxamate analogues, **2** and **22**, of ferrichrome have been prepared. These compounds represent analogues in which transposition of the side-chain *N*-hydroxy and acyl functions have been effected relative to their respective positions in the natural iron-transport agent ferrichrome **1**. Evaluation of the retrohydroxamate ferrichrome **2** as an iron-transport agent in microbial systems established this compound to be indistinguishable from natural ferrichrome. In contrast, analogue **22**, which lacks the *N*-methyl group on the hydroxamate function, was found to show no activity as an iron-transport agent when compared to ferrichrome. Details on the biological evaluation of these analogues have been published.[21]

Experimental Section

The L-amino acids and coupling reagents used in this study were commercially available. Tetrahydrofuran was distilled prior to use from sodium benzophenone ketyl. Methylene chloride was distilled from phosphorus pentoxide and stored over Linde 3A molecular sieves. Dimethylformamide was distilled from calcium hydride.

Melting points were determined with a Thomas-Hoover capillary melting point apparatus and are uncorrected. Thin-layer chromatography was performed on commercial silica gel on glass plates (1 × 3 in.). Medium-pressure liquid chromatography (MPLC) was performed at 60–100 psi in glass columns packed with silica gel 60 (0.040–0.063 mm).

N^{α}-(*tert*-Butyloxycarbonyl)-L-α-aminoadipic Acid. To a stirred solution of L-α-aminoadipic acid (3.2 g, 20 mmol) and triethylamine (4.0 g, 40 mmol) in 50 mL of DMF/H_2O (30:20) was added di-*tert*-butyl dicarbonate (4.2 g, 20 mmol). The reaction mixture was stirred at room temperature for 2 h and the solvent

(17) These peptides were found to be insoluble in neat ethyl acetate so we used EtOAc/1-butanol mixed solvent system for extraction. For similar use of a mixed solvent system, see ref 3.

(18) Nutt, R. F.; Veber, D. F.; Saperstein, R. *J. Am. Chem. Soc.* **1980**, *102*, 6539–6545.

(19) Ester **23** was prepared by two different routes: (a) from the known[9] *N*-Boc-L-α-aminoadipic acid α-benzhydryl ester by esterification of the δ-carboxyl with ethanol/carbodiimide/DMAP, followed by hydrogenolysis of the benzhydryl ester and (b) from L-glutamic acid by the procedure of Ramasamy et al. (Ramasamy, K.; Olsen, R. K.; Emery, T. *Synthesis* **1982**, 42).

(20) Woodward, R. B.; Heusler, K.; Bosteli, J.; Naegeli, P.; Oppolzer, W.; Ramage, R.; Ranganthan, S.; Vorbruggen, H. *J. Am. Chem. Soc.* **1966**, *88*, 852. Ciardelli, T. L.; Chakravarty, P. K.; Olsen, R. K. *J. Am. Chem. Soc.* **1978**, *100*, 7684.

(21) Emery, T.; Emery, L.; Olsen, R. K. *Biochem. Biophys. Res. Commun.* **1984**, *119*, 1191–1197.

was removed in vacuo to give an oily residue. The oil was suspended in 100 mL of EtOAc and acidified with 1 N HCl to pH 2. The organic extract separated out and the aqueous layer was saturated with solid NaCl. The saturated aqueous solution was reextracted with EtOAc (50 mL). The combined EtOAc extract was washed with water (35 mL) and brine (25 mL) and dried over Na_2SO_4 (anhydrous) and the solvent removed to give an oily product. The oil was recrystallized from methylene chloride: yield, 5.0 g (96%); mp 121–122 °C; $[\alpha]^{25}_D$ +10.6° (*c* 1, $CHCl_3$).

(*S*)-4-[3-(*tert*-Butyloxycarbonyl)-5-oxo-4-oxazolidinyl]-butanoic Acid (4). *N*-(*tert*-Butyloxycarbonyl)-L-α-aminoadipic acid (7.8 g, 30 mmol), *p*-toluenesulfonic acid (400 mg), and paraformaldehyde (5 g, 55.6 mmol) were heated at reflux in 200 mL of 1,1,2-trichloroethane using a Dean–Stark apparatus. After 6 h, the reaction mixture was cooled and concentrated in vacuo to a residue. The residue was partitioned between EtOAc/saturated $NaHCO_3$ (100:50 mL) and extracted with saturated sodium bicarbonate. The aqueous extract was made acidic with 1 N HCl to pH 2 and extracted with ethyl acetate (3 × 75 mL). The organic extract was washed with water (25 mL) and brine (25 mL), dried over anhydrous Na_2SO_4, and evaporated to leave an oil with very little impurity: yield 7.0 g (86%); $[\alpha]^{25}_D$ +59.8° (*c* 2, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 1.43 (s, 9 H, Boc), 1.7–2.1 (m, 4 H), 2.25–2.67 (m, 2 H), 4.3 (m, 1 H, α-H), 5.21 (d, 1 H), 5.55 (d, 1 H), 10.2 (br s, 1 H, COOH).

Benzyl *N*-Methyl-4-[(*S*)-3-(*tert*-butyloxycarbonyl)-5-oxo-4-oxazolidinyl]butanohydroxamate (5). Butanoic acid 4 (15 g, 5.5 mmol) was dissolved in 50 mL of dry THF and cooled to −15 °C. *N*-Methylmorpholine (0.59 mL, 5.5 mmol) and isobutyl chloroformate (0.78 g, 5.7 mmol) were added and the mixture was stirred at −15 °C for 0.5 h. A solution of *N*-methyl-*O*-benzylhydroxylamine trifluoroacetate (1.6 g, 6.4 mmol)[8] and *N*-methylmorpholine (0.7 mL, 6.6 mmol) in 50 mL of dry THF was added, dropwise, during a 0.5-h period, after which the reaction mixture was stirred at −15 °C for 1 h and at room temperature overnight. The solvent was evaporated and the residue was suspended in water (20 mL) and extracted with EtOAc (2 × 75 mL). The organic extract was washed with 1 N HCl (2 × 30 mL), saturated $NaHCO_3$ solution (2 × 20 mL), H_2O (20 mL), and brine (20 mL) and dried over Na_2SO_4 (anhydrous) and the solvent removed. The crude product was purified on MPLC, with hexane/acetone (7:3) as the eluent, to give **5**, 1.5 g (80%), as an oil. The oil was dissolved in a minimum amount of dry ether and the resulting solution, upon refrigeration for a few days, gave the product as a white crystalline compound: $[\alpha]^{25}_D$ +73.6° (*c* 1, $CHCl_3$); mp 68–72 °C; ^1H NMR ($CDCl_3$) δ 1.5 (s, 9 H, Boc), 1.83 (m, 4 H); 2.40 (br t, 2 H), 3.23 (s, 3 H, *N*-CH_3), 4.31 (m, 1 H, α-H), 4.88 (s, 2 H, CH_2 Ph), 5.2 (d, 1 H), 5.53 (d, 1 H), 7.46 (s, 5 H, Ph).

Anal. Calcd for $C_{20}H_{28}N_2O_6$: C, 61.21; H, 7.19; N, 7.14. Found: C, 61.00; H, 7.19; N, 7.22.

Benzyl *N*-Methyl-*N*$^\alpha$-(*tert*-butyloxycarbonyl)-L-α-aminoadipo-δ-hydroxamate (6). The protected hydroxamate **5** (2.0 g, 5.1 mmol) was treated with methanol (20 mL) and 1 N NaOH (10 mL, 10 mmol) and the mixture was stirred at room temperature for 4 h. Methanol was removed under pressure and the alkaline solution was washed with ether (50 mL). The aqueous alkaline solution was made acidic with 1 N HCl and extracted with EtOAc (2 × 50 mL). The EtOAc extract was washed with water (20 mL) and brine (20 mL) and dried (anhydrous Na_2SO_4), and the solvent was evaporated to give an oil: 1.9 g (98%); $[\alpha]^{25}_D$ +8.5° (*c* 1, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 1.43 (s, 9 H, Boc), 1.73 (m, 4 H), 2.43 (br t, 2 H), 3.25 (s, 3 H, *N*-CH_3), 4.2 (m, 1 H, α-H), 4.88 (s, 2 H, CH_2Ph), 5.47 (d, 1 H, NH), 7.48 (s, 5 H, Ph), 10.33 (s, 1 H, COOH).

Anal. Calcd for $C_{19}H_{28}N_2O_6$: C, 59.98; H, 7.42; N, 7.36. Found: C, 59.89; H, 7.53; N, 7.37.

N$^\alpha$-(*tert*-Butyloxycarbonyl)-L-α-aminoadipic Acid α-Benzhydryl Ester. L-α-Aminoadipic acid α-benzhydryl ester[9] (3.2 g, 10 mmol) was suspended in 70 mL of DMF–H_2O (50:20) and treated with triethylamine (1.01 g, 10 mmol) to give a clear solution within 10 min. Di-*tert*-butyl dicarbonate (2.6 g, 12 mmol) was added and the reaction mixture was allowed to stir at room temperature for 2 h. DMF–H_2O was removed in vacuo and the residue was made acidic to pH 2 with aqueous $KHSO_4$ solution and extracted with ethyl acetate (2 × 75 mL). The organic extract was washed with 1 N HCl (25 mL), water (25 mL), and brine (20 mL). The mixture was dried (anhydrous Na_2SO_4) and the solvent removed to give an oil, which on crystallization from EtOAc/hexane gave a yellow powder: yield 4.0 g (97%); mp 140–142 °C.

α-Benzhydryl *O*-Benzyl *N*-Methyl-*N*$^\alpha$-(*tert*-butyloxycarbonyl)-L-α-aminoadipo-δ-hydroxamate (8). *N*$^\alpha$-(*tert*-butyloxycarbonyl)-L-α-aminoadipic acid α-benzhydryl ester (1.0 g, 2.4 mmol) was dissolved in 50 mL of dry THF and cooled to −15 °C. To this cold, stirred solution, *N*-methylmorpholine (0.26 mL, 2.4 mmol) and isobutyl chloroformate (0.33 g 2.4 mmol) were added and the mixture was stirred at −15 °C for 0.5 h. *N*-Methyl-*O*-benzylhydroxylamine trifluoroacetate (0.6 g, 2.4 mmol) in 50 mL of dry THF was neutralized with *N*-methylmorpholine (0.27 mL, 2.5 mmol) and the resulting solution was added dropwise during a 0.5-h period. The reaction mixture was stirred at −15 °C for 1 h and at room temperature overnight. The solvent was evaporated; the oily residue was partitioned between EtOAc (75 mL)/water (20 mL), and brine (20 mL), dried over anhydrous Na_2SO_4, and evaporated to dryness. The crude product was purified by using MPLC on elution with hexane/acetone (7:3). The pure product was crystallized from ether–petroleum ether (30–60 °C): yield 0.8 g (63%); $[\alpha]^{25}_D$ −6.4° (*c* 1, $CHCl_3$); mp 83–85 °C; ^1H NMR ($CDCl_3$) δ 1.42 (s, 9 H, Boc), 1.67 (m, 4 H), 2.33 (br t, 2 H), 3.18 (s, 3 H, *N*-CH_3), 4.4 (m, 1 H, α-H), 4.8 (s, 2 H, CH_2Ph), 5.3 (d, 1 H, NH), 6.96 (s, 1 H, $CHPh_2$), 7.3 (d, 15 H, Ph).

Anal. Calcd for $C_{32}H_{38}N_2O_6$: C, 70.3; H, 7.01; N, 5.12. Found: C, 70.36; H, 6.90; N, 5.12.

N-(*tert*-Butyloxycarbonyl)glycylglycine Methyl Ester. *N*-(*tert*-Butyloxycarbonyl)glycine (5.25 g, 30 mmol) and glycine methyl ester hydrochloride (3.78 g, 30 mmol) in 150 mL of dry CH_2Cl_2 were cooled with stirring to 0 °C. Triethylamine was added and the stirring was continued for 0.5 h. Dicyclohexylcarbodiimide (DCC; 6.8 g, 33 mmol) was added and the reaction mixture was stirred at 0 °C for 2 h and at room temperature overnight. The precipitated solid was filtered and the filtrate evaporated to dryness to give an oil. The oil was dissolved in EtOAc (120 mL), washed with 10% citric acid (40 mL), 1 N $NaHCO_3$ (20 mL), H_2O (20 mL), and brine (20 mL), dried over anhydrous Na_2SO_4, and concentrated in vacuo. The crude product was purified by using MPLC with elution by hexane/acetone: yield 4 g (54%); ^1H NMR ($CDCl_3$) δ 1.43 (s, 9 H, Boc), 3.7–4.3 (m, 7 H), 5.83 (br t, 1 H, NHBoc), 7.33 (br t, 1 H, NH).

Glycylglycine Methyl Ester Trifluoroacetate Salt. *N*-(*tert*-Butyloxycarbonyl)glycylglycine methyl ester (1.8 g, 7.3 mmol) was dissolved in 5 mL of dry CH_2Cl_2 and treated with trifluoroacetic acid (5 mL). The reaction mixture was stirred at room temperature for 0.5 h and evaporated to dryness in vacuo. The residue was dissolved in dry CH_3OH and the methanol was removed under pressure. This was repeated 3 times to remove traces of TFA. The residue was dried inside a vacuum desiccator for a day over KOH pellets and used as such for the next reaction.

N-[N$^\alpha$-Boc-N$^\epsilon$-(benzyloxy)-N$^\epsilon$-methyl-α-amino-δ-L-adipamidyl]glycylglycine Methyl Ester (9). A General Procedure for the Mixed Anhydride Method. *O*-Benzyl *N*-methyl-*N*$^\alpha$-(*tert*-butyloxycarbonyl)-L-α-aminoadipo-δ-hydroxamate (**6**) (3.65 g, 9.6 mmol) was dissolved in dry THF (75 mL) and cooled to −15 °C and *N*-methylmorpholine (1.1 mL, 10 mmol) was added. To this cold stirred solution, isobutyl chloroformate (1.37 g, 10 mmol) was added and the stirring was continued for 20 min at −15 °C. A solution of glycylglycine methyl ester trifluoroacetate (2.6 g, 10 mmol), in 75 mL of dry THF, and neutralized with *N*-methylmorpholine (1.1 mL, 10 mmol), was added dropwise during a 0.5-h period. After the addition, the reaction mixture was stirred at −15 °C for 1 h and at room temperature overnight. The solvent was removed in vacuo, and the residue was partitioned between EtOAc (75 mL)/water (25 mL). The EtOAc extract was washed with 1 N HCl (30 mL), 1 N $NaHCO_3$ (30 mL), water (25 mL), and brine (20 mL), dried over Na_2SO_4 (anhydrous), and evaporated under reduced pressure. The crude product was purified by MPLC with $CHCl_3$/acetone as eluant: yield 3.69 (74%); $[\alpha]^{25}D$ +2.0° (*c* 0.5, MeOH); ^1H NMR ($CDCl_3$) δ 1.41 (s, 9 H, Boc), 1.87 (m, 4 H), 2.4 (br t, 2 H), 3.18 (s, 3 H, NCH_3), 3.68 (s, 3 H, $COOCH_3$), 3.96–4.03 (m, 5 H), 4.82 (s, 2 H, CH_2Ph), 5.8 (d, 1 H, NH), 7.4 (s, 5 H, Ph), 7.58 (br t, 2 H, NH).

Anal. Calcd for $C_{24}H_{36}N_4O_8 \cdot H_2O$: C, 54.74; H, 7.27; N, 10.63. Found: C, 54.68; H, 7.23; N, 10.55.

TFA⁻ $^{+}H_2$-Aad(NMeOBzl)-Gly-Gly-OMe: A General Procedure for Preparing TFA Salt. Tripeptide **9** (3.5 g, 6.9 mmol) was dissolved in 10 mL of dry CH_2Cl_2 and treated with trifluoroacetic acid (10 mL). The reaction mixture was stirred at room temperature for 0.5 h and evaporated to dryness in vacuo. The residue was dissolved in dry CH_3OH (15 mL) and evaporated to dryness under reduced pressure. This procedure was repeated 3 times to remove traces of TFA. The residue was dried inside a vacuum desiccator for 12 h over KOH pellets. The dried TFA salt was used as such for further reaction without characterization: yield 3.5 g (97%).

Boc-Aad(NMeOBzl)-Aad(NMeOBzl)-Gly-Gly-OMe (10). Compound **10** was prepared by the same procedure employed for the preparation of **9**. The following materials were used: hydroxamate **6** (2.7 g, 7 mmol), *N*-methylmorpholine (1.67 mL, 14.6 mmol), isobutyl chloroformate (0.98 g, 7 mmol), TFA salt of **9** (4 g, 7.6 mmol), and dry THF (150 mL). Yield, 2.8 g (51%); foam; $[\alpha]^{25}_D$ −4.8° (*c* 0.5, MeOH); ^{1}H NMR ($CDCl_3$) δ 1.42 (s, 9 H, Boc), 1.73 (m, 8 H), 2.46 (m, 4 H), 3.19 (d, 6 H, NCH_3), 3.69 (s, 3 H, $COOCH_3$), 3.8–4.4 (m, 6 H), 4.82 (s, 4 H, CH_2Ph), 5.73 (d, 1 H, NH), 7.37 (s, 10 H, Ph), 7.85 (d, 3 H, NH).

Anal. Calcd for $C_{38}H_{54}N_6O_{11}\cdot1^1/_2H_2O$: C, 57.20; H, 7.20; N, 10.52. Found: C, 57.15; H, 7.16; N, 10.44.

TFA⁻ $^{+}H_2$-Aad(NMeOBzl)-Aad(NMeOBzl)-Gly-Gly-OMe (10·TFA). This was prepared by the same procedure employed for the preparation of **9·TFA**. Materials used were the following: tetrapeptide **10** (1.5 g, 3.3 mmol), dry CH_2Cl_2 (5 mL), and trifluoroacetic acid (5 mL), yield 2.5 g (98%).

Boc-[Aad(NMeOBzl)]$_3$-(Gly)$_2$-OMe (11). Compound **11** was prepared by the same procedure employed for the preparation of **9**. The following materials were used: hydroxamate **6** (1.33 g, 3.5 mmol), *N*-methylmorpholine (0.81 mL, 7.1 mmol), isobutyl chloroformate (0.49 g, 3.5 mmol), TFA salt of **10** (2.8 g, 3.6 mmol), and THF (150 mL). Yield 2.5 g (69%); foam; $[\alpha]^{25}_D$ −10.4° (*c* 1, MeOH); ^{1}H NMR ($CDCl_3$) δ 1.4 (s, 9 H, Boc), 1.76 (m, 12 H), 2.46 (m, 6 H), 3.19 (t, 9 H, NCH_3), 3.68 (s, 3 H, $COOCH_3$), 3.9–4.5 (m, 7 H), 4.81 (s, 6 H, CH_2Ph), 5.87 (d, 1 H, NH), 7.38 (s, 15 H, Ph), 7.55–7.85 (m, 3 H, NH), 8.35 (m, 1 H, NH).

Anal. Calcd for $C_{52}H_{72}N_8O_{14}\cdot1^1/_2H_2O$: C, 58.90; H, 7.13; N, 10.56. Found: C, 58.54; H, 7.03, N, 10.53.

TFA⁻ $^{+}H_2$-[Aad(NMeOBzl)]$_3$-(Gly)$_2$-OMe (11·TFA). This was prepared by the same procedure employed for the preparation of **9·TFA**. Materials used were the following: pentapeptide **11** (2.4 g), CH_2Cl_2 (5 mL), and TFA (5 mL), yield 2.4 g (98%).

Boc-Gly-[Aad(NMeOBzl)]$_3$-(Gly)$_2$-OMe (12). This compound was prepared by the same procedure employed for the preparation of **9**; the product was purified by MPLC with $CHCl_3$/MeOH (95:5) as eluant. The following materials were used: *N*-(*tert*-butyloxycarbonyl)glycine (438 mg, 2.5 mmol), *N*-methylmorpholine (0.56 mL, 4.8 mmol), isobutyl chloroformate (0.35 g, 2.5 mmol), TFA salt of **11** (2.4 g, 2.3 mmol), and THF (200 mL). Yield 1.9 g (79%); foam; $\alpha]^{25}_D$ −14.4° (*c* 0.5, MeOH); ^{1}H NMR ($CDCl_3$) δ 1.41 (s, 9 H, Boc), 1.45–2.00 (m, 12 H), 2.25–2.45 (m, 6 H), 3.22 (t, 9 H, NCH_3), 3.65 (s, 3 H, $COOCH_3$), 3.7–4.22 (m, 8 H), 4.4 (br s, 1 H, α-H), 4.82 (dd, 6 H, CH_2Ph), 6.21 (br s, 1 H, NH), 7.4 (m, 15 H, Ph), 7.44–7.62 (m, 3 H, NH), 8.22 (br s, 2 H, NH).

Anal. Calcd for $C_{54}H_{75}O_9O_{15}\cdot1^1/_2H_2O$: C, 58.05; H, 7.03; N, 11.28. Found: C, 57.94; H, 6.99; N, 11.36.

Boc-Gly-[Aad(NMeOBzl)]$_3$-(Gly)$_2$-OH. Hexapeptide **12** (450 mg, 0.42 mmol) was dissolved in acetone (15 mL) and treated with 1 N NaOH solution (1.0 mL, 1 mmol). The reaction mixture was stirred at room temperature for 4 h and acetone was removed under reduced pressure. The alkaline solution was made acidic with 1 N HCl and extracted with EtOAc/1-butanol (2 × 30:10 mL). The organic extract was washed with H_2O (20 mL) and brine, dried over Na_2SO_4 (anhydrous), and evaporated to dryness to give a foam (380 mg, 85%). This material was found to be sufficiently pure and used as such for the next reaction without characterization.

TFA⁻ $^{+}$H-Gly-[Aad(NMeOBzl)]$_3$-(Gly)$_2$-OH (13a). The above acid (280 mg) was dissolved in a mixture of TFA/CH_2Cl_2 (3:3 mL) and the solution was stirred at room temperature for 0.5 h. The reaction mixture was concentrated to dryness in vacuo and the residue was dissolved in 10 mL of methanol and evaporated to dryness. This process was repeated 3 times and the residue was triturated well with dry ether, filtered, and dried inside a vacuum desiccator for 12 h over KOH pellets. The resulting foam was used as such for the cyclization reaction without characterization, yield 270 mg (95%).

TFA⁻ $^{+}H_2$-Gly-[Aad(NMeOBzl)]$_3$-(Gly)$_2$-ONp (13b). Boc-Gly-[Aad(NMeOBzl)]$_3$-(Gly)$_2$-OH (350 mg, 0.33 mmol) was dissolved in a mixture of DMF (20 mL) and pyridine (10 mL). To this stirred solution, *p*-nitrophenol (60 mg, 0.4 mmol) and 1-ethyl-3-[3-(dimethylamino)propyl]carbodiimide hydrochloride (200 mg, 1 mmol) were added and the mixture was stirred at room temperature for 36 h. The reaction mixture was evaporated to dryness, and the residue was partitioned between EtOAc (60 mL)/1-butanol (20 mL)/H_2O (10 mL). The organic extract was washed with 25% citric acid (20 mL), H_2O (20 mL), and brine (20 mL) and dried (Na_2SO_4) and the solvent removed in vacuo to give a foam. This material was used as such for the next reaction without characterization.

The above foam (100 mg) was dissolved in TFA (5 mL) and dry CH_2Cl_2 (5 mL) and the mixture was stirred at room temperature for 0.5 h. The reaction mixture was concentrated in vacuo and the residue was dissolved in dry CH_3OH (10 mL) and evaporated to dryness. The above process was repeated 3 times and the residue was dried in a desiccator for 12 h over KOH pellets. This dried TFA salt was used as such for the cyclization reaction.

***cyclo*-[Triglycyltris(*N'*-(benzyloxy)-*N'*-methyl-L-α-amino-δ-adipamidyl] (14). Method A.** 1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (382 mg, 2.0 mmol) and 1-hydroxybenzotriazole (268 mg, 2.0 mmol) in 100 mL of dry DMF was diluted with 150 mL of dry CH_2Cl_2 and cooled to 0 °C. To this cold solution, *N*-methylmorpholine (0.035 mL, 0.3 mmol) was added and the mixture was stirred for 15 min. TFA salt **13a** (270 mg, 0.25 mmol), dissolved in dry DMF (100 mL) and diluted with dry CH_2Cl_2 (100 mL), was added during a 5-h period at 0–5 °C. After the addition, the reaction mixture was stirred at 0 °C for 1 h and then at room temperature for 4 days. The reaction mixture was evaporated to dryness, the residue partitioned between EtOAc/*n*-BuOH/H_2O (60/20/20 mL) and extracted in EtOAc/*n*-BuOH (2 × 60:20). The organic extract was washed with 1 N HCl (30 mL), 1 N $NaHCO_3$ (30 mL), H_2O (30 mL), and brine (25 mL), dried (Na_2SO_4), and evaporated to dryness. The crude product was purified by MPLC with elution by $CHCl_3$/MeOH (92:8). The fractions having R_f 0.43 [$CHCl_3$/MeOH (9:2)] were collected and evaporated to give a white solid. This material was crystallized from methanol/ether: yield 125 mg (51%); mp 175–180 °C; $[\alpha]^{25}_D$ +8.6° (*c* 0.5, MeOH); ^{1}H NMR ($CDCl_3$ δ 1.57–1.8 (m, 12 H), 2.3–2.5 (m, 6 H), 3.18 (d, 9 H, NCH_3), 3.55–3.95 (m, 4 H), 4.1–4.2 (m, 4 H), 4.6–4.7 (br d, 1 H, α-H), 4.8 (d, 6 H, CH_2Ph), 7.4 (d, 15 H, Ph), 7.5–7.9 (m, 4 H, NH), 8.08–8.2 (m, 2 H, NH).

Anal. Calcd for $C_{48}H_{63}N_9O_{13}\cdot CH_3OH$: C, 58.50; H, 6.71; N, 12.52. Found: C, 58.44; H, 6.76; N, 12.66.

Method B. TFA salt **13b** (100 mg, 0.08 mmol) in dry DMF (30 mL) was neutralized with *N*-methylmorpholine (0.06 mL, 0.5 mmol). The above solution was added to a stirred solution of hot pyridine (60–65 °C) during a 4-h period. After the addition, the reaction mixture was stirred at 60–65 °C for 24 h and concentrated to dryness. The residue, upon workup as described in method A, gave 15 mg (19%) of pure product.

***cyclo*-[Triglycyltris(*N*$^{\epsilon}$-hydroxy-*N*$^{\epsilon}$-methyl-L-δ-amino-δ-adipamidyl)] (15). Method A.** Cyclic hexapeptide **14** (100 mg) and 50 mg of 5% palladium–carbon in 20 mL of methanol was shaken under hydrogen (10 psi) for 26 h. The catalyst was filtered and the filtrate evaporated to give an oily product. The residue was purified on a gravity column of silica gel (22 cm × 1.5 cm, 230–400 mesh) with $CHCl_3$/methanol (7:3; R_f 0.24) as eluant. Pure compound was triturated well with ether, filtered, and dried: yield 30 mg (42%); mp 190 °C dec; $[\alpha]^{25}_D$ +15.5° (*c* 0.5, DMF); ^{1}H NMR ($CDCl_3$) δ 1.4–1.8 (m, 12 H), 2.3–2.4 (m, 6 H), 3.1 (s, 9 H, NCH_3), 3.5–4.25 (m, 9 H), 8.0–8.5 (m, 6 H, NH).

In one experiment, the above hydrogenolysis was carried out in a mixture of MeOH and $CHCl_3$, in which $CHCl_3$ was added to facilitate solubility of the cyclic peptide **14**. However, the product formed under these conditions lacked the hydroxamate functionality as evidenced by lack of complexation with ferric ion. Hydrogenolysis in $CHCl_3$ likely generated hydrogen chloride;

under the acidic conditions formed reduction of the N–O bonds of the hydroxamate groups must have occurred.

Method B. A solution of cyclic hexapeptide **14** (50 mg, 0.05 mmol), ammonium formate (0.11 g, 1.8 mmol), 10% palladium–carbon (50 mg), in 10 mL of methanol was stirred at room temperature for 3 h. After filtration through a Celite column the filtrate was evaporated to dryness to give a yellowish sticky residue. This material was purified on a gravity column of silica gel (1.5 × 25 cm, 230–400 mesh) using $CHCl_3$/methanol as eluant. The fractions having R_f 0.24 [$CHCl_3$/MeOH (7:3)] were pooled and evaporated to dryness. The residue was triturated well with dry ether to give an orange powder, which was filtered and dried: yield 25 mg (70%); mp 190 °C dec.

Retrohydroxamate Ferrichrome (2). The chelate **2** was prepared[13] following the reported procedure for chelation of natural ferrichromes. An aqueous solution of the ligand **15** was treated with an excess of ferrous sulfate and air was passed into the solution for about 45 min. The solution was saturated with ammonium sulfate and the complex extracted with two portions of benzyl alcohol. The combined alcohol extracts were diluted with a 3-fold volume of ethyl ether and one-tenth volume of water. The organic layer was extracted with several small volumes of water, and the aqueous extracts were combined and washed once with ethyl ether. Removal of the water in vacuo yielded the product **2** as an orange-red solid: R_f 0.06, ferrichrome, 0.21 (*n*-BuOH/AcOH/H_2O/pyridine, 4:1:5:1); R_f 0.19, ferrichrome, 0.60 (ethyl acetate/pyridine/AcOH/H_2O, 5:5:1:3).

α-Benzhydryl *O*-Benzyl-N^{α}-(*tert*-butyloxycarbonyl)-L-α-aminoadipio-δ-hydroxamate. To a cold stirred solution of *O*-benzylhydroxylamine (1.4 g, 12 mmol) and N^{α}-(*tert*-butyloxycarbonyl)-L-α-aminoadipic acid α-benhydryl ester (5.5 g, 12 mmol) in 50 mL of THF/H_2O (1:4), was added 2.2 g (14 mmol) of 1-ethyl-3-[3-(dimethylamino)propyl]carbodiimide hydrochloride (EDCI). The reaction mixture was stirred at 0 °C for 3 h and at room temperature overnight. The solvent was removed and the residue was extracted with ethyl acetate (2 × 70 mL). The EtOAc extract was washed with 1 N HCl (30 mL), 1 N $NaHCO_3$ (25 mL), H_2O (20 mL), and brine (20 mL), dried (Na_2SO_4), and evaporated to dryness. The crude product was purified by using MPLC with elution by hexane/acetone (7:3): yield 5.05 g (72%); mp 98–100 °C (hexane); $[\alpha]^{25}_D$ −1.5° (*c* 1, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 1.43 (s, 9 H, Boc), 1.56–1.83 (m, 4 H), 2.2–2.36 (m, 2 H), 4.4 (m, 1 H, α-H), 4.9 (s, 2 H, CH_2Ph), 5.53 (d, 1 H, NH), 6.97 (s, 1 H, $CHPh_2$), 7.4 (s, 15 H, phenyl).

Anal. Calcd for $C_{31}H_{36}N_2O_6$: C, 69.85; H, 6.81; N, 5.25. Found: C, 70.06; H, 6.84; N, 5.27.

Benzyl 4-[3-(*tert*-Butyloxycarbonyl)-5-oxo-4(*S*)-oxazolidinyl]butanohydroxamate. A mixture of the oxazolidinone **4** (2.1 g, 7.7 mmol), *O*-benzylhydroxylamine hydrochloride (1.3 g, 8.0 mmol), triethylamine (0.81 g, 8 mmol) and 1-ethyl-3-[3-(dimethylamino)propyl]carbodiimide hydrochloride (1.9 g, 10 mmol) in 75 mL of dry CH_2Cl_2 was stirred at 0 °C for 2 h and at room temperature overnight. The solvent was removed in vacuo to give an oil. The oil was dissolved in 125 mL of EtOAc/H_2O (100:25). The organic extract was washed with 1 N HCl (25 mL), 1 N $NaHCO_3$ (20 mL), H_2O (20 mL), and brine (20 mL), dried (Na_2SO_4), and concentrated in vacuo. The crude product was purified by MPLC with hexane/acetone (7:3) as eluant to give 1.8 g (62%): $[\alpha]^{25}_D$ +73.2° (*c* 1, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 1.43 (s, 9 H, Boc), 1.7–2.1 (m, 4 H), 2.25–2.67 (m, 2 H), 4.3 (m, 1 H, α-H), 4.93 (s, 2 H, CH_2Ph), 5.21 (d, 1 H), 5.55 (d, 1 H), 7.43 (s, 5 H, Ph), 9.73 (br s, 1 H, NH).

Anal. Calcd for $C_{19}H_{26}N_2O_6 \cdot {}^1/_2H_2O$: C, 59.06; H, 7.04; N, 7.25. Found: C, 59.10; H, 6.96; N, 7.28.

Benzyl N^{α}-(*tert*-Butyloxycarbonyl)-L-α-aminoadipo-δ-hydroxamate (16). Method A. A solution of α-benzhydryl *O*-benzyl-N^{α}-*tert*-butyloxycarbonyl-L-α-aminoadipo-δ-hydroxamate (2.65 g, 5 mmol) and 1 N NaOH (10 mL, 10 mmol) in 20 mL of acetone was stirred at room temperature for 1 h. Acetone was removed and the aqueous portion was washed with ether (25 mL). The aqueous alkaline solution was adjusted to pH 2–3 with 10% citric acid and extracted with ethyl acetate (2 × 50 mL). The organic extract was washed with water (20 mL) and brine (20 mL), dried (Na_2SO_4), and concentrated under reduced pressure to give an oil. The oil was dissolved in dry CH_2Cl_2 and left at room temperature to provide the white crystalline compound **16**: 1.62 g (88%); mp 128–130 °C; $[\alpha]^{25}D$ −2.4° (*c* 1.5, CH_3OH); ^1H NMR ($CDCl_3$) δ 1.43 (s, 9 H, Boc), 1.56–1.83 (m, 4 H), 2.2–2.36 (m, 2 H), 4.4 (m, 1 H, α-H), 4.9 (s, 2 H, CH_2Ph), 5.53 (d, 1 H, NH), 7.4 (s, 5 H, Ph), 10.17 (br s, 1 H, COOH).

Anal. Calcd for $C_{18}H_{26}N_2O_6$: C, 59.00; H, 7.15; N, 7.64. Found: C, 59.21; H, 7.27; N, 7.58.

Method B. A solution of α-benzhydryl *O*-benzyl-N^{α}-(*tert*-butyloxycarbonyl)-L-α-aminoadipo-δ-hydroxamate (2.6 g, 4.9 mmol) in 15 mL of trifluoroacetic acid was stirred at room temperature for 1 h. The mixture was concentrated in vacuo and the residue dissolved in EtOAc (100 mL) and extracted with water (2 × 50 mL). The combined aqueous extract was evaporated in vacuo to give about 1 mL of clear colorless oil. The oil was diluted with 5 mL of H_2O and the pH of the solution was adjusted to 5–6 by the addition of pyridine. Absolute ethanol (12 mL) was added and the precipitated acid was collected after chilling at 5 °C overnight, yield 0.8 g (61%), mp 155–156 °C.

The above solid (0.46 g, 2 mmol), triethylamine (0.2 g, 2 mmol), and di-*tert*-butyl dicarbonate (0.5 g, 2.5 mmol) were stirred at room temperature in 10 mL of dimethylformamide for 0.5 h. The solvent was removed in vacuo, and the residue was acidified to pH 2 with 1 N $KHSO_4$ solution and extracted with EtOAc (2 × 25 mL). The EtOAc extract was washed with H_2O (10 mL) and brine (10 mL) and dried over Na_2SO_4 (anhydrous) and the solvent removed in vacuo. The oil obtained was dissolved in CH_2Cl_2 and left as such at room temperature to give a crystalline white solid: yield 0.52 g (82%); mp 128–130 °C; $[\alpha]^{25}_D$ −2.4° (*c* 1.5, CH_3OH).

Method C. A solution of benzyl 4-[3-(tert-butyloxycarbonyl)-5-oxo-4(*S*)-oxazolidinyl]butanohydroxamate (6 g, 15.9 mmol) in methanol (15 mL) was stirred with 1 N NaOH (35 mL, 35 mmol) at room temperature for 4 h. Methanol was removed under reduced pressure and the alkaline solution was washed with ether (50 mL), acidified with 1 N HCl to pH 2, and extracted with EtOAc (2 × 75 mL). The organic extract was washed with H_2O (20 mL) and brine (20 mL) and dried and the solvent removed to give an oil. Crystalline product was deposited from CH_2Cl_2: yield 5.0 g (86%); mp 128–130 °C; $[\alpha]^{25}_D$ −2.6° (*c* 1, CH_3OH).

Boc-Aad(NHOBzl)-Gly-Gly-OMe. Method A. To a stirred solution of benzyl *N*-(*tert*-butyloxycarbonyl)-L-α-aminoadipo-δ-hydroxamate (**16**) (0.36 g, 1 mmol), *N*-hydroxysuccinimide (0.23 g, 2 mmol), and *N*-methylmorpholine (0.12 mL, 1.1 mmol) in 60 mL of dry DMF/CH_2Cl_2 (15:45) was added 1-ethyl-3-[3-(dimethylamino)propyl]carbodiimide hydrochloride (0.26 g, 1.4 mmol) at −10 °C. The reaction mixture was stirred at room temperature for 48 h and evaporated to dryness in vacuo. The oily residue was dissolved in EtOAc (120 mL) and washed with 2 N HCl (30 mL), 1 N $NaHCO_3$ (30 mL), H_2O (25 mL), and brine (20 mL). The organic extract was dried over anhydrous Na_2SO_4 and evaporated to dryness to give an oil, which was purified by MPLC using $CHCl_3$/MeOH (95:5) as eluant. The pure product was crystallized from acetone/hexane: yield 0.30 g (61%); mp 110–112 °C; $[\alpha]^{25}_D$ +1.9° (*c*, 1, acetone); ^1H NMR ($CDCl_3$ + Me_2SO) δ 1.43 (s, 9 H, Boc), 1.5–1.74 (m, 4 H), 2.03 (br s, 2 H), 3.7 (s, 3 H, $COOCH_3$), 3.71–4.02 (m, 5 H), 4.84 (s, 2 H, CH_2Ph), 6.74 (d, 1 H, NHBoc), 7.3–7.4 (m, 5 H, Ph), 8.12 (t, 1 H, NH), 8.17 (t, 1 H, NH), 10.89 (s, 1 H, $NHOCH_2Ph$).

Anal. Calcd for $C_{23}H_{34}N_4O_8$: C, 55.86; H, 6.93; N, 11.32. Found: C, 55.59; H, 6.91, N, 11.07.

Method B. A solution of hydroxamate **16** (1.8 g, 5 mmol) and N-methylmorpholine (0.55 mL, 5 mmol) in 30 mL of dry THF was cooled to −15 °C. Isobutyl chloroformate (0.7 g, 5 mmol) was added and the mixture was stirred at −15 °C for 0.5 h. A solution of glycylglycine methyl ester trifluoroacetate salt (1.3 g, 5.5 mmol) in 20 mL of dry THF containing *N*-methylmorpholine (0.61 g, 5.5 mmol) was added over a 0.5-h period, after which the reaction mixture was stirred at −15 °C for 1 h and at room temperature overnight. Workup of the reaction mixture as described in method A furnished the tripeptide in 82% yield.

TFA^- $^+H_2$-Aad(NHOBzl)-Gly-Gly-OMe. This was prepared by the general procedure employed for the preparation of **9·TFA**. Materials used were the following: tripeptide (8.6 g, 17.5 mmol), dry CH_2Cl_2 (15 mL), TFA (15 mL); yield 8.6 g (98%).

Boc-[Aad(NHOBzl)]$_2$-Gly$_2$-OMe. The hydroxamate **16** (6.2 g, 17 mmol) in 100 mL of dry THF was cooled to −15 °C. To this cold stirred solution, *N*-methylmorpholine (2.04 mL, 17 mmol) and isobutyl chloroformate (2.33 g, 17 mmol) were added and the

mixture was stirred for 0.5 h at −15 °C. A solution of TFA⁻ $^{+}H_2$-Aad(NHOBzl)-Gly-Gly-OMe (8.6 g, 17 mmol) in 100 mL of dry THF containing *N*-methylmorpholine (2.04 mL, 17 mmol) was added over a 0.5-h period. The reaction mixture was stirred at −15 °C for an additional 1 h and at room temperature overnight. The THF was removed under reduced pressure and the residue was dissolved in 200 mL of *n*-BuOH/EtOAc (1:2). The organic extract was washed with 1 N HCl (30 mL), 1 N $NaHCO_3$ (30 mL), H_2O (25 mL), and brine (20 mL), dried (Na_2SO_4), and concentrated to leave an oil. The oil was dissolved in acetone (75 mL) and left at room temperature for 1 h with occasional shaking to give a white gel. The gel was filtered, washed with minimum amount of water, and dried. The white solid was crystallized from CH_2Cl_2 as an amorphous powder: yield 9.3 g (74%); mp 161–163 °C; $[\alpha]^{25}_D$ −13.5° (*c* 1, CH_3OH); ^1H NMR (Me_2SO) δ 1.4 (s, 9 H, Boc), 1.43–1.70 (m, 8), 1.94 (br s, 4 H), 3.64 (s, 3 H, $COOCH_3$), 3.67–3.99 (m, 5 H), 4.2–4.31 (m, 1 H, α-H), 4.8 (s, 4 H, CH_2Ph), 6.9 (d, 1 H, NHBoc), 7.39 (s, 10 H, Ph), 7.9 (d, 1 H, NH), 8.26 (t, 1 H, NH), 8.3 (t, 1 H, NH), 10.99 (s, 2 H, NH).

Anal. Calcd for $C_{36}H_{50}N_6O_{11}$: C, 57.82; H, 6.74; N, 11.23. Found: C, 57.58; H, 6.88; N, 11.07.

TFA⁻ $^{+}H_2$-[Aad(NHOBzl)]$_2$-(Gly)$_2$-OMe. This was prepared by the general procedure employed for the preparation of **9·TFA**. Materials used were the following: tetrapeptide (2.2 g, 3 mmol), dry CH_2Cl_2 (5 mL), TFA (5 mL); yield 2.2 g (96%).

Boc-[Aad(NHOBzl)]$_3$-(Gly)$_2$-OMe. Benzyl N$^{\alpha}$-(*tert*-butyloxycarbonyl)-L-α-aminoadipo-δ-hydroxamate (**16**) (0.9 g, 2.5 mmol) in dry THF (50 mL) was cooled to −15 °C. To this cold solution, *N*-methylmorpholine (0.3 mL, 2.5 mmol) and isobutyl chloroformate (0.35 g, 2.5 mmol) were added and the mixture was stirred for 0.5 h at −15 °C. A solution of TFA⁻ $^{+}H_2$-[Aad(NHOBzl)]$_2$-(Gly)$_2$-OMe (1.9 g, 2.5 mmol) in dry THF (50 mL) containing *N*-methylmorpholine (0.3 mL, 2.5 mmol) was added during a 0.5-h period at −15 °C, and the reaction mixture was stirred at −15 °C for 1 h and at room temperature overnight. The THF was removed under reduced pressure and the residue was dissolved in 200 mL of *n*-BuOH/EtOAc (1:2). The organic phase was washed with 1 N HCl (30 mL), 1 N $NaHCO_3$ (30 mL), H_2O (25 mL), and brine (20 mL), dried over Na_2SO_4 (anhydrous), and evaporated to dryness. The reisue was treated with acetone to give a white solid, which was filtered and washed with water (20 mL). The white solid was crystallized from methanol/ether as an amorphous powder: yield 1.6 g (66%); mp 168–171 °C; $[\alpha]^{25}_D$ −7.9° (*c* 1, DMF); ^1H NMR (Me_2SO) δ 1.37 (s, 9 H, Boc), 1.43–1.7 (m, 12 H), 1.96 (br s, 6 H) 3.6 (s, 3 H, $COOCH_3$), 3.7–3.97 (m, 5 H), 4.2–4.37 (m, 2 H, α-H), 4.8 (s, 6 H, CH_2Ph), 6.96 (d, 1 H, NHBoc), 7.39 (s, 15 H, Ph), 7.84 (d, 1 H, NH), 8.12 (d, 1 H, NH), 8.26 (d, 2 H, NH), 10.99 (br s, 3 H, $NHOCH_2Ph$).

Anal. Calcd for $C_{49}H_{66}N_8O_{14}$: C, 59.38; H, 6.71; N, 11.3. Found: C, 59.03; H, 6.53; N, 11.21.

TFA⁻ $^{+}H_2$-[Aad(NHOBzl)]$_3$-(Gly)$_2$-OMe. This was prepared by the general procedure employed for the preparation of **9·TFA**. Materials used were the following: pentapeptide (2.0 g, 2.02 mmol), dry CH_2Cl_2 (4 mL), TFA (4 mL); yield 2.0 g (95%).

Boc-Gly-[Aad(NHOBzl)]$_3$-(Gly)$_2$-OMe. A solution of N-(*tert*-butyloxycarbonyl)glycine (0.35 g, 2 mmol) and *N*-methylmorpholine (0.24 mL, 2 mmol) in dry THF (50 mL) was cooled to −15 °C. To this cold solution, isobutyl chloroformate (0.24 g, 2 mmol) was added and the mixture stirred at −15 °C for 0.5 h. TFA⁻ $^{+}H_2$-[Aad(NHOBzl)]$_3$-(Gly)$_2$-OMe (1.9 g, 1.8 mmol) in dry DMF (50 mL) containing *N*-methylmorpholine (0.24 mL, 2 mmol) was added during a 0.5-h period and the reaction mixture was stirred at −15 °C for 1 h and at room temperature overnight. The solvent was removed in vacuo and the residue dissolved in 200 mL of *n*-BuOH/EtOAc (1:2). The organic extract was washed with 1 N HCl (30 mL), 1 N $NaHCO_3$ (30 mL), H_2O (25 mL), and brine (20 mL), dried (Na_2SO_4), and evaporated to dryness. The crude product was purified by MPLC using $CHCl_3$/MeOH (9:1) as eluant. The pure product was crystallized from hot methanol: yield 1.6 g (80%); mp 180–182 °C; $[\alpha]^{25}_D$ −8.6° (*c* 1, DMF); ^1H NMR (Me_2SO) δ 1.37 (s, 9 H, Boc), 1.43–1.70 (m, 12 H), 1.96 (br s, 6 H), 3.59 (m, 2 H), 3.62 (s, 3 H, $COOCH_3$), 3.76 (m, 2 H), 3.87 (m, 2 H), 4.2–4.38 (m, 3 H, α-H), 4.81 (s, 6 H, CH_2Ph), 6.98 (t, 1 H, NHBoc), 7.5 (s, 15 H, phenyl), 7.9 (d, 1 H, NH), 7.99 (d, 1 H, NH), 8.1 (d, 1 H, NH), 8.26 (d, 2 H, NH), 10.99 (d, 3 H, $NHOCH_2Ph$).

Anal. Calcd for $C_{51}H_{69}N_9O_{15}$: C, 58.44; H, 6.63; N, 11.92. Found: C, 58.17; H, 6.66; N, 11.92.

TFA⁻ $^{+}H_2$-Gly-[Aad(NHOBzl)]$_3$-(Gly)$_2$-ONp (18). Method A. To a stirred solution of Boc-Gly[Aad(NHOBzl)]$_3$-Gly-Gly-OH (0.51 g, 0.5 mmol) and *p*-nitrophenol (70 mg, 0.5 mmol) in 30 mL of dry DMF/pyridine (2:1) was added to 1-ethyl-3-[3-(dimethylamino)propyl]carbodiimide hydrochloride (200 mg, 1 mmol). The reaction mixture was stirred at room temperature for 36 h and evaporated to dryness in vacuo. The residue was dissolved in 150 mL of *n*-BuOH/EtOAc (1:2) and washed with 25% citric acid (2 × 75 mL), H_2O (50 mL), and brine (30 mL). After drying (Na_2SO_4), the solution was evaporated in vacuo to give a yellow solid, which was dissolved in 20 mL of hot methanol and diluted to a volume of 200 mL with chloroform. The entire 200 mL of solution was passed through a MPLC column and eluted with $CHCl_3$/methanol (9:1). The fractions having R_f 0.32 [$CHCl_3$/MeOH (9:1)] were pooled and evaporated to a solid, which was crystallized from hot methanol/ether: yield 400 mg (70%); mp >170 °C(dec; $[\alpha]^{25}_D$ −4.4° (*c* 1, DMF).

Method B. To a stirred solution of Boc-Gly-[Aad(NHOBzl)]$_3$-Gly-Gly-OH (520 mg, 0.5 mmol) in 24 mL of pyridine/EtOAc (1:1) at 40 °C was added freshly prepared bis(*p*-nitrophenyl) sulfite (648 mg, 2 mmol) in small portions. After being stirred at 40 °C for 3 h, the reaction mixture was concentrated in vacuo to a yellow residue. The residue was dissolved in 150 mL of *n*-BuOH/EtOAc (1:2) and washed with 2.5% citric acid (2 × 75 mL), water (40 mL), and brine (20 mL). The organic layer was dried over anhydrous Na_2SO_4 and evaporated to dryness. The crude product was crystallized from methanol/ether, filtered, washed well with ether, and dried. This solid 350 mg (60%) was used as such for the next reaction without characterization.

The above *p*-nitrophenyl ester (250 mg, 0.22 mmol) was dissolved in 6 mL of dry CH_2Cl_2/TFA (3:3) and stirred at room temperature for 0.5 h. The reaction mixture was evaporated to dryness in vacuo. The residue was dissolved in dry CH_3OH (10 mL) and evaporated to dryness under reduced pressure. This was repeated 3 times, and the residue, upon trituration with dry ether, gave a solid that was filtered, washed with ether, and dried inside a vacuum desiccator for 12 h over KOH pellets, yield 220 mg (87%).

TFA⁻ $^{+}H_2$-Gly-[Aad(NHOBzl]$_3$-Gly-Gly-OH (19). Hexapeptide methyl ester **17** (570 mg, 0.54 mmol) was dissolved in 15 mL of methanol/H_2O (10:5) and stirred with 1 N NaOH (2 mL, 2 mmol) at room temperature. After 4 h, the methanol was removed under reduced pressure and the aqueous alkaline solution was neutralized with 1 N HCl to pH 2. The aqueous phase was extracted with 2 × 100 mL of *n*-BuOH/EtOAc (1:1) and washed with H_2O (25 mL) and brine (20 mL). The extract was dried (Na_2SO_4) and evaporated to dryness to leave a white solid, which was crystallized from methanol/ether to give a white amorphous powder: yield, 480 mg (85%); mp 189–190 °C; $[\alpha]^{25}_D$ −8.3° (*c* 1, DMF).

The above solid (600 mg, 0.58 mmol) was dissolved in 10 mL of dry CH_2Cl_2/TFA (5:5) and stirred at room temperature for 0.5 h. The reaction mixture was concentrated to a residue. The residue was dissolved in 10 mL of dry CH_3OH and evaporated to dryness. This process was repeated 3 times. The residue was treated with dry ether to give a solid, which was filtered, washed with ether, and dried in vacuo over KOH pellets, yield 0.6 g (98%).

***cyclo*-[Triglycyltris(*N*$^{\epsilon}$-(benzyloxy)-L-α-amino-δ-adipamidyl)] (20). Method A.** A solution of the TFA salt **18** (200 mg, 0.17 mmol) and *N*-methylmorpholine (0.1 mL, 0.9 mmol) in 20 mL of dry DMF was added dropwise over 2 h to a stirred solution of hot pyridine (150 mL) at 60–65 °C. After the addition, the reaction mixture was stirred at 60–65 °C for 10 h and evaporated to dryness in vacuo. The residue was dissolved in 150 mL of *n*-BuOH/EtOAc (1:2) and washed with 1 N HCl (25 mL), 1 N $NaHCO_3$ (25 mL), H_2O (20 mL), and brine (20 mL). After drying over anhydrous Na_2SO_4, the solvent was evaporated to dryness in vacuo. The crude product was dissolved in 10 mL of hot methanol and diluted to 100 mL with $CHCl_3$. The resulting solution was passed through a MPLC column followed by elution with $CHCl_3$ (200 mL). The column was eluted with 300 mL of $CHCl_3$/MeOH (8:2), and the fractions containing the product, Rf 0.5, were pooled and evaporated to give a solid. The solid upon crystallization from hot methanol gave **20**, 85 mg (54%): mp

187–189 °C; ^{1}H NMR (Me_2SO) δ 1.45–1.69 (m, 12 H), 1.95 (br s, 6 H), 3.77 (d, 2 H), 3.81 (t, 2 H), 3.87 (m, 2 H), 4.18–4.36 (m, 3 H, α-H), 4.81 (s, 6 H, CH_2Ph), 7.41 (s, 15 H, phenyl), 7.99 (d, 1 H, NH), 8.1 (d, 1 H, NH), 8.15 (d, 1 H, NH), 8.25 (t, 1 H, NH), 11.08 (d, 3 H, $NHOCH_2Ph$).

Anal. Calcd for $C_{45}H_{57}N_9O_{12}\cdot CH_3OH\cdot{}^1/_2H_2O$: C, 57.74; H, 6.53; N, 13.16. Found: C, 57.43; H, 6.51; N, 13.18.

Method B. TFA salt **19** (180 mg, 0.17 mmol) and triethylamine (0.03 mL, 0.2 mmol) in dry DMF (150 mL) were cooled to 0 °C. To this cold solution diphenylphosphoryl azide (DPPA) (0.08 mL, 0.4 mmol) was added and the mixture was stirred at 0 °C for 5 h and at room temperature for 4 days, during which time the pH of 7.5 was maintained by the addition of triethylamine. The reaction mixture was concentrated to a small volume (5 mL) and partitioned between *n*-BuOH/EtOAc/0.5 M aqueous citric acid (50:100:50 mL). The organic extract was washed with 0.5 M citric acid (25 mL), 1 N HCl (25 mL), 1 N $NaHCO_3$ (25 mL), H_2O (20 mL), and brine, dried (Na_2SO_4), and evaporated to dryness in vacuo. The crude product was purified as described in method A to give **20**, 30 mg (19%).

Method C. TFA salt **19** (0.52 g, 0.5 mmol) and 1-hydroxybenzotriazole (220 mg, 1.6 mmol) in dry DMF (100 mL) was cooled to 0 °C and diluted with dry CH_2Cl_2 (100 mL). *N*-Methylmorpholine (0.06 mL. 0.5 mmol) in dry DMF (25 mL) was added and the mixture was stirred for 1 h at 0 °C. 1-Ethyl-3-[3-(dimethylamino)propyl]carbodiimide hydrochloride (0.28 g, 1.5 mmol) in 150 mL of dry DMF/CH_2Cl_2 (50:100) was added at 0 °C over a period of 2 h. The reaction mixture was stirred at 0 °C for 1 h and at room temperature for 4 days. The solvent was removed in vacuo and the residue was taken up in 200 mL of *n*-BuOH/EtOAc (4:6). The organic phase was washed with 1 N HCl (30 mL), 1 N $NaHCO_3$ (30 mL), water (50 mL), and brine (20 mL). After drying (Na_2SO_4), the solution was evaporated to dryness, and the crude product was purified as described in method A, yield 152 mg (33%).

***cyclo*-[Triglycyltris(*N*$^\epsilon$-hydroxy-L-α-amino-δ-adipamidyl)] (21).** The cyclic hexapeptide **20** (40 mg) in DMF (25 mL) was shaken under hydrogen (10 psi) with 5% palladium–carbon (20 mg) for 6 h at room temperature. The catalyst was filtered and the filtrate evaporated to dryness. The residue was dissolved in a minimum amount of methanol and diluted with ether, which on cooling gave crystalline compound. The product was filtered, washed with ether, and dried in vacuo over P_2O_5: yield 20 mg (71%); mp >150 °C dec; ^{1}H NMR (Me_2SO) δ 1.57 (m, 12 H), 1.94 (br s, 6 H), 3.6–3.83 (m, 6 H), 4.21 (m, 3 H, α-H), 8.08–8.68 (m, 6 H, NH), 10.36 (br s, 3 H, NH).

***N*-Demethylretrohydroxamate Ferrichrome (22).** This compound was prepared by the same procedure as used for retrohydroxamate ferrichrome (**2**).

Acknowledgment. Appreciation is expressed to the National Institutes of Health (Grant GM 26711) for support of the work and to the Colorado State University Regional NMR Center (funded by National Science Foundation Grant No. CHE-8208821) for providing 360-MHz NMR data. We acknowledge the contribution of Professor T. Emery of this department for the genesis of the rationale for attempting the synthesis of a retrohydroxamate analogue of ferrichrome.

Supplementary Material Available: Additional experimental information for other compounds in this paper (7 pages). Ordering information is given on any current masthead page.

Reductive Alkylation of β-Alkanolamines with Carbonyl Compounds and Sodium Borohydride†[1]

Joseph E. Saavedra

LBI-Basic Research Program, Laboratory of Chemical and Physical Carcinogenesis, NCI—Frederick Cancer Research Facility, Frederick, Maryland 21701

Received July 27, 1984

A synthesis of secondary alkylalkanolamines from primary alkanolamines in a rapid process in which overalkylation is virtually suppressed is described. The procedure combines the ease of formation of oxazolidines from alkanolamines with aldehydes or ketones in absolute ethanol and the lability of the newly formed C–O bond toward sodium borohydride. The entire process is carried out in 15–35 min depending on the carbonyl substrate.

In reactivity patterns, alkylalkanolamines combine the characteristics of the amine and hydroxyl groups. This combination of functionalities makes them versatile intermediates for countless industrial applications; they are of particular interest to the textile, pharmaceutical and household products industries.[2] Secondary alkylalkanolamines are also precursors for β-hydroxylated nitrosamines. These important compounds are used in the study of nitrosamine exposure in the workplace and metabolic and carcinogenesis studies.[3]

The title compounds are generally prepared by the ring opening of an epoxide with an alkylamine.[4] The addition of imidoosmium reagents to alkenes[5] and methods for the alkylation of primary amines with 2-bromo alcohols[6] are also well-established procedures for the preparation of *N*-alkyl-1,2-alkanolamines. A major limitation of some of these methods is the possibility of further alkylation to tertiary amines and quaternary ammonium compounds.

†Research sponsored by the National Cancer Institute, DHHS, under Contract N01-CO-23909 with Litton Bionetics, Inc. The contents of this publication do not necessarily reflect the views or policies of the Department of Health and Human Services, nor does mention of trade names, commercial products, or organizations imply endorsement by the U.S. Government.

(1) Presented at 188th National Meeting of American Chemical Society, Philadelphia, PA, Aug 1984; American Chemical Society: Washington, DC, 1984; Abstract No. ORGN 245.

(2) The Alkanolamines Handbook 1981, The Dow Chemical Co.

(3) Lijinsky, W. "Genotoxicology of N-Nitroso Compounds"; Rao, T. K., Lijinsky, W., Epler, J. L. Plenum Press: New York, 1984; Chapter 10 and references therein.

(4) (a) Knorr, L.; Schmidt, W. *Ber. Deutsch. Chem. Ges.* **1898**, *31*, 1072. (b) Berlini A. Ya.; Sycheva, T. P. *Zh. Obsch. Khim.* **1950**, *20*, 640. (c) Burfield, D. R.; Gan, S.-N.; Smithers, R. H. *J. Chem. Soc., Perkin Trans. 1*, **1977**, 666.

(5) Sharpless, K. B.; Patrick, D. W.; Truesdale, L. K.; Biller, S. A. *J. Am. Chem. Soc.* **1975**, *97*, 2305.

(6) Goldberg, US Patent 2 139 818; English Patent 482 886.

0022-3263/85/1950-2271$01.50/0