N-Hydroxy Amides. II[1]. *N*-Benzyloxy α-Amino Acid *N*-Hydroxysuccinimide Esters and Synthesis of a Hexapeptide Having an Alternating *N*-Hydroxy Amide-Amide Sequence

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Acylation of a series of *N*-benzyloxy α-amino acid derivatives with *N*,*N*-phthaloylglycine shows that the yield decreases with increasing bulkiness of the substituent even by the acyl chloride or mixed anhydride procedure, and that the use of an excess reagent or a double acylation technique is needed to attain a good yield. The low reactivity of the benzyloxyamino group enables us to utilize *N*-benzyloxy α-amino acid *N*-hydroxysuccinimide esters without *N*-protection in the acylation of the usual amino group. In an attempt to lengthen the chain of an *N*-hydroxy peptide, a hexapeptide anilide having an *N*-hydroxy-DL-alanylglycyl sequence was synthesized *via* *N*-benzyloxy peptides. The complex forming tendency of the hexapeptide with iron(III) is examined.

Considerable effort has been directed to the synthesis of peptides having *N*-hydroxy amide bonds in the chain.[1-4] In view of the unique biological activity of naturally occurring hydroxamic acids,[5-7] the synthetic *N*-hydroxy peptides are of interest as they may serve as models for intermediates in biogenetic transformation,[8] precursors of dehydropeptides[9] and ionophoric peptides[10] as well as physiologically active substances, such as hadacidin,[11] rhodotorulic acid and ferrichromes.[6] The biological activity of the natural hydroxamic acids centers on their unique ability to chelate iron(III) for cell-membrane transport.[6] Except for a few total syntheses of natural substances,[7,12] oligopeptides with two or more *N*-hydroxy amide groups in the chain have rarely been obtained.[13] There are few general procedures for introducing the *N*-hydroxy amide bond into a peptide sequence.[3,14] *N*-Benzylozy α-amino acids have been utilized under these circumstances.[15] Previously, we reported on the use of *N*-benzyloxy α-amino acid *N*-carboxy anhydrides (*N*-benzyloxy-NCAs) for a stepwise synthesis of an *N*-benzyloxy tetrapeptide.[16] Also it was reported that polymerization of these NCAs, followed by debenzylation, led to poly(*N*-hydroxy peptide)s.[1] In this paper we describe a procedure for obtaining sequential *N*-hydroxy peptides together with the related investigations, including an examination of iron(III) chelation.

Results and Discussion

By the use of benzyloxy-NCAs, introduction of an *N*-benzyloxy α-amino acid residue into a growing peptide having the usual amino terminus has become an easy process,[16] so that a key step in the synthesis of *N*-hydroxy peptides is subsequent acylation of the terminal benzyloxyamino group. Nucleophilic reactivity of the benzyloxyamino group decreases considerably due to the inductive effect of the oxygen and steric hindrance by the benzyloxy moiety. Kolasa and Chimiak commented briefly that acylation of the *N*-benzyloxy α-amino acids with a bulky side chain was difficult,[3] but the scope and limitations of the acylation reactions have not yet been fully investigated.

Careful examination of a coupling reaction between *N*,*N*-phthaloylglycine (Pht=GlyOH) and methyl *N*-benzyloxy-DL-alaninate (DL-H(PhCH$_2$O)AlaOMe) by using a few selected coupling methods[17] gave the following results (yield of the coupling after recrystallization); acyl chloride in DMF (69%), isobutyl chloroformate (65%), *N*-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline (EEDQ) (46%), pivaloyl chloride (40%), 1,1-carbonyldiimidazole (CDI) (37%), *N*-hydroxysuccinimide (0%): Care was taken to allow a sufficient time for each coupling procedure. These results confirm that strong activation of the acyl group is necessary to attain practical yields. Using the acyl chloride or the mixed anhydride of *N*,*N*-phthaloylglycine, acylation of a series of methyl esters of *N*-benzyloxy α-amino acids and *N*-benzyloxy dipeptides (Scheme 1) was carried out as shown in Fig. 1. The dipeptide

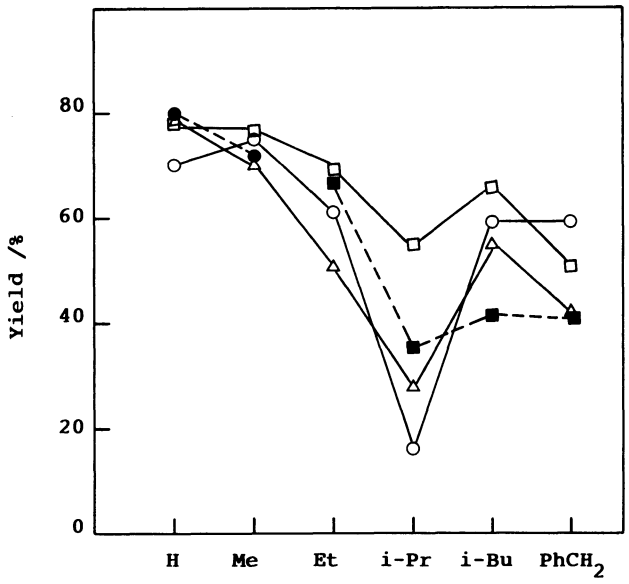
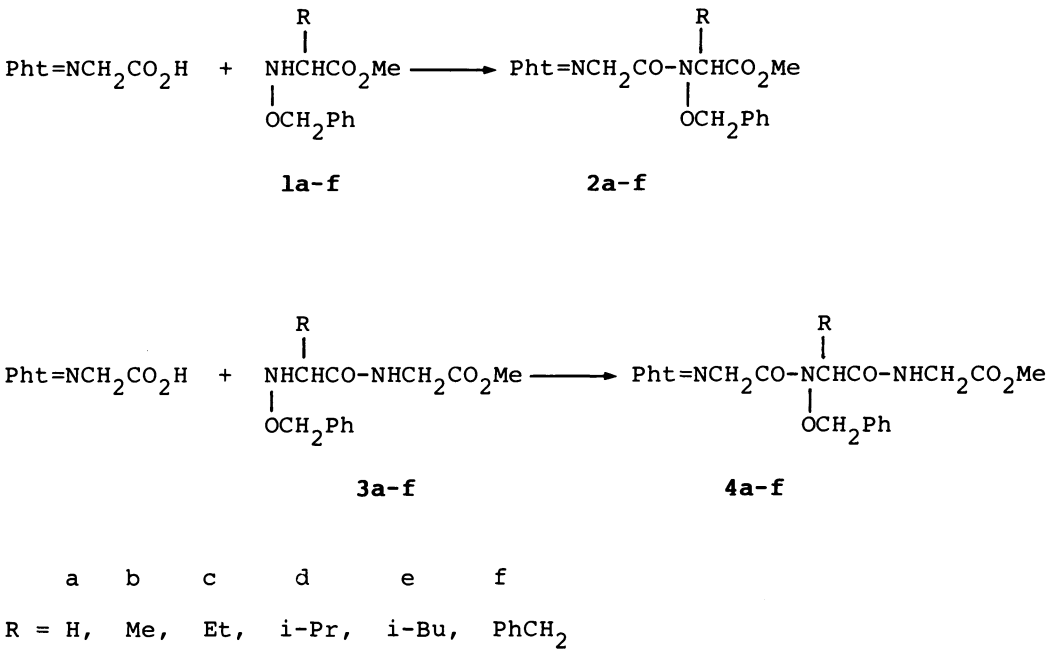


Fig. 1. Yield *vs.* α-substituent in the acylation of **1a—f** and **3a—f** with Pht=Gly-OH: —○— **1a—f** by acyl chloride under Schotten-Baumann conditions; —□— **1a—f** by acyl chloride in DMF; —△— **1a—f** by mixed anhydride with isobutyl chloroformate; —●— **3a—f** by acyl chloride under Schotten-Baumann conditions; —■— **3a—f** by acyl chloride in DMF. For a key, see Scheme 1.



Scheme 1.

Table 1. Products on acylation of *N*-benzyloxy amino acid derivatives

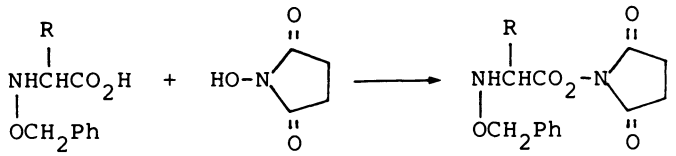
Product	Mp θ_m/°C	TLC[a] R_f^1	TLC[a] R_f^2	TLC[a] R_f^3	Found (Calcd)/% C	H	N
2a	117—118	0.85	0.81	0.62	61.96 (62.09	4.77 4.82	7.51 7.24)[b]
2b	136—137 (133—134)[c]	0.88	0.85	0.63			
2c	oil	0.88	0.85	0.63	64.08 (64.38	5.68 5.40	6.77 6.83)
2d	126—127	0.90	0.88	0.67	65.17 (65.08	5.64 5.70	6.59 6.60)
2e	oil	0.91	0.91	0.67	65.24 (65.08	6.21 5.99	6.53 6.33)[b]
2f	135—136	0.90	0.91	0.64	68.06 (67.98	5.05 5.03	5.88 5.87)[b]
4a	151—152	0.67	0.59	0.32	59.99 (60.13	4.82 4.82	9.55 9.56)
4b	132—133	0.79	0.66	0.52	60.50 (60.92	5.08 5.11	9.33 9.27)
4c	92— 94	0.84	0.69	0.57	61.67 (61.66	5.49 5.39	8.77 8.99)
4d	108—110	0.77	0.87	0.65	62.95 (62.36	6.06 5.65	8.38 8.73)
4e	136—137	0.88	0.74	0.66	62.83 (63.03	5.89 5.90	8.36 8.48)
4f	146—149	0.85	0.79	0.61	65.69 (65.77	5.14 5.14	8.09 7.94)

a) R_f^1, EtOAc; R_f^2, $CHCl_3$-MeOH-C_6H_6 (6:1:3); R_f^3, THF-hexane (1:1). b) $(1/4)H_2O$ is included in the calcd value. c) Ref. 3).

esters may serve as a model for growing *N*-benzloxy peptides. A decrease in the yield with increasing bulkiness of the α-substituents is clearly seen. This is ascribed to steric hindrance by the substituents. Only acyl chloride in DMF gave satisfactory results for most of the cases. The products in the acylation are summarized in Table 1. By a repetitive acylation or the use of excess reagents, these low yields may be improved (Table 2). This suggests a possibility of synthesizing longer peptides by the stepwise method.

The observation that the benzyloxyamino group did not react with the *N*-hydroxysuccinimide (HOSu) ester prompted us to utilize the benzyloxyamino acids in the

form of active esters without *N*-protection. Treatment of the *N*-benzyloxy α-amino acids with HOSu in the presence of DCC gave the corresponding active esters in good yields (Eq. 1). The esters were characterized as crystalline hydrochlorides except for that of *N*-benzyloxyglycine, which was converted into a more stable pentachlorophenyl ester (Table 3). These active esters reacted readily with methyl glycinate to afford the corresponding dipeptide methyl esters (Eq. 2 and Table 4), as did the *N*-benzyloxy-NCAs.[16]

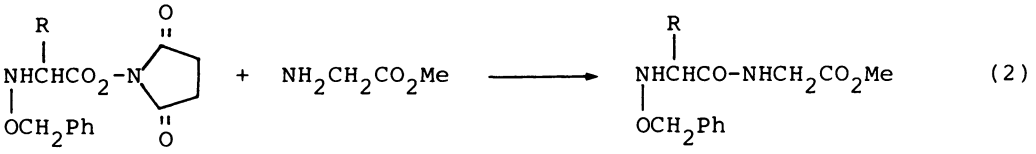


(1)

5a-f

a b c d e f

R = H, Me, Et, i-Pr, i-Bu, $PhcH_2$



(2)

3a-f

TABLE 2. EXCESS REAGENTS OR A REPETITIVE METHOD ON ACYLATION

Run	Acyl Component (mmol)	Amino Component (mmol)	DMF ml	Temp °C	Time h	Yield %
1	Pht=Gly-Cl (2.8)	H-(BzlO)Phe-OMe (1.1)	10	−5	24	92
2	Pht=Gly-Cl (4.25)	H-(BzlO)Val-Gly-OMe (1.7)	25	−5	24	86
3	Pht=Gly-Cl (6.0)	H-(BzlO)Phe-Gly-OMe (2.5)	20	−5	24	91
4	Pht=Gly-OH[a] (10.0)	H-(BzlO)Phe-Gly-OMe (2.0)	30	−15	30	85
5	Pht=Gly-OH[a,b] (2.2)	H-(BzlO)Phe-Gly-OMe (2.0)	40	−15	30	68

a) Mixed anhydride procedure. b) Coupling was repeated 3 times under these conditions.

TABLE 3. *N*-BENZYLOXY DL-α-AMINO ACID *N*-HYDROXYSUCCINIDE ESTERS (**5a—f**)

	Yield %	Mp θ_m/°C	TLC[a] R_f^1	TLC[a] R_f^4	Found (Calcd)[b]/(%) C	H	N
5a	98	113—114[c]	0.76	0.79	46.20 (49.61)	4.80 (4.80)	9.02 (8.90)
5a'[d]	79	92— 93			41.92 (41.95)	2.36 (2.35)	3.37 (3.26)
5b	94	134—136[c]	0.81	0.79	50.93 (51.15)	5.23 (5.21)	8.62 (8.52)
5c	85	129—130[c]	0.89	0.79	52.72 (52.56)	5.61 (5.59)	8.20 (8.17)
5d	87	139—140	0.84	0.96	54.07 (53.86)	6.05 (5.93)	7.77 (7.85)
5e	81	84— 85	0.87	0.96	60.85 (61.07)	6.65 (6.63)	8.42 (8.38)[e]
5f	88	135—136[c]	0.84	0.76	59.02 (59.34)	5.10 (5.23)	6.74 (6.92)

a) R_f^1, EtOAc; R_f^4, $CHCl_3$-MeOH (3:1). b) As the hydrochloride. c) With decomp. d) *N*-Benzyloxyglycine pentachlorophenyl ester. e) As the free base.

Removal of the protective group from *N*-benzyloxy peptides is also an important step for synthesis of *N*-hydroxy peptides. Using Pht=Gly-DL-(PhCH$_2$O)-Phe-Gly-OMe as a substrate, several debenzylation procedures were tested: These are HBr in AcOH, $(CF_3CO_2)_3B$ in CF_3CO_2H, and hydrogenation with Ni or a few types of Pd catalysts. The reaction was followed by HPLC. Hydrogenation with Pd catalysts[3,14] was found to be the most satisfactory.

In order to show the usefulness of the active esters and a facile peptide chain elongation, synthesis of a hexapeptide having an *N*-hydroxyalanylglycyl sequence was carried out. Thus, DL-H(PhCH$_2$O)Ala-OSu was coupled with glycinanilide to give a benzyloxy dipeptide anilide (**6**). A reaction cycle for lengthening the benzyloxy peptide chain was performed; *viz.* acylation of the terminal benzyloxyamino group with Boc-Gly-OH by the mixed anhydride method, followed by deprotection of the Boc-group and then subsequent coupling with H(PhCH$_2$O)Ala-OSu. The cycle was repeated and finally interrupted by coupling with Ac-(PhCH$_2$O)Ala-OSu. A critical step was acylation of the benzyloxyamino group by the mixed anhydride method. To secure a practical yield, a double acylation technique was performed for this step. The resulting acetyl hexapeptide (**10**) was debenzylated by catalytic hydrogenation to give an *N*-hydroxy hexapeptide (**11**). The outline of the synthesis is shown in Fig. 2 and the data for the intermediate peptides are summarized in Table 5.

This kind of alternating sequential peptides is of interest, since it has three *N*-hydroxy peptide units and presents possibilities of holding iron(III) intramolecularly and/or intermolecularly. Natural trihydroxamic acids such as desferrioxames form stable complexes

Table 4. Results of the reaction of **5a—f** with H-Gly-OMe

Product	Yield %	Mp (lit,[a]) Mp) θ_m/°C	TLC R_f^1	TLC R_f^2
3a	70	122—125 (124—126)	0.56	0.48
3b	86	164—165 (165—166)	0.63	0.53
3c	73	170—171 (172—173)	0.74	0.43
3d	79	177—179 (183—184)	0.79	0.49
3e	86	137—138 (138—139)	0.91	0.61
3f	85	168—169 (168—169)	0.90	0.80

a) Ref. 16).

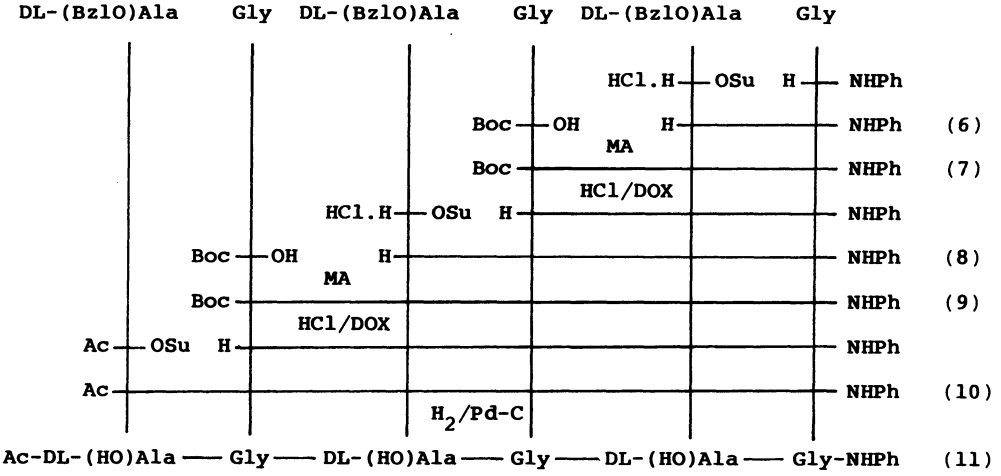


Fig. 2. Synthesis of an *N*-hydroxy hexapetide (**11**) *via* *N*-benzyloxy peptides (**6—10**): BzlO stands for PhCH$_2$O and DOX for dioxane. Acylation by the mixed anhydride procedure was repeated 3 times.

Table 5. Synthesis of *N*-hydroxy peptides (**6—11**)

Compound	Yield %	Mp θ_m/°C	TLC R_f^1	TLC R_f^2	TLC R_f^3	Found (Calcd)/% C	H	N
6	86	143—144	0.45	0.63	0.88	66.35 (66.03	6.79 6.47	12.89 12.84)
7	74	121—122	0.58	0.60	0.88	62.28 (61.96	6.84 6.66	11.71 11.56)
8	80	122—124	0.34	0.56	0.88	64.00 (64.16	6.21 6.28	12.59 12.47)
9	63	135—136	0.50	0.61	0.88	61.85 (61.82	6.47 6.45	11.58 11.69)
10	75	113—115	0.21	0.66	0.90	61.99 (61.69	6.15 6.19	11.31 11.45)
11	94	191—192 (decomp)			0.62	48.34 (48.67	5.86 5.86	16.96 17.28)

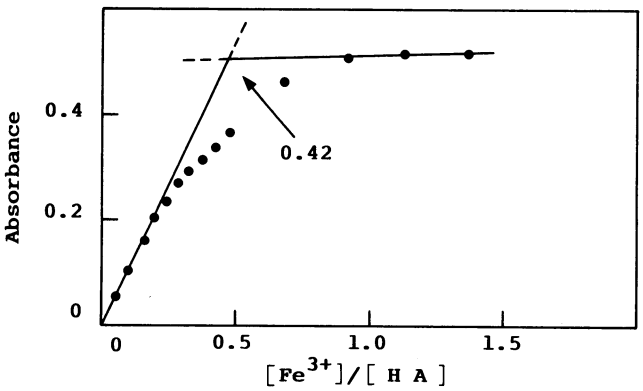


Fig. 3. Absorbance *vs.* the ratio of iron(III) to the hydroxamic acid (HA) units in 50 % aqueous DMF at pH=3 and 25 °C, ionic strength 0.1 (KNO_3). Absorbance at 463—487 nm.

with iron(III). On the other hand, little is known about the chelation tendency of synthetic *N*-hydroxy peptides.

The hexapeptide showed an intense violet color when mixed with $FeCl_3$ solution. The complex forming tendency was examined by determing the molar ratio of iron(III) to the *N*-hydroxy amide unit in 50% aq DMF solution.[1] The absorbance *vs.* the molar ratio plot (Fig. 3) reveals a curvature around an intersection of the two extrapolated straight lines. The intersection corresponds to the molar ratio of iron(III) to the *N*-hydroxy amide unit; the value being 0.33 for a 1:3 complex. A value of 0.42 was obtained for the present case, which is interpreted as a formation of an unstable complex rather than a stable 1:3 complex. This may be supported by a shift of λ_{max} to a longer wavelength (470 nm) coupled with a decrease in absorbance. It is known that a typical 1:3 complex, ferrioxamine B, shows its maximum at 420 nm while a 1:1 complex, which is usually observed for a monohydroxamic acid with iron(III) at low pH, has the maximum at 520 nm.[5] A 1:2 complex formation has been suggested for a polmyer system with λ_{max} at 466 nm[18] and also for an intermediate stage during transformation of a 1:3 complex to a 1:1 complex by an increasing acidity of the solution.[19-20] It appears that an alternating sequence of the *N*-hydroxy amide units is too dense to form a stable 1:3 complex either intramolecularly or intermolecularly.

Experimental

All the melting points are uncorrected. IR spectra were obtained with a JASCO model 403G spectrophotometer and ^1H NMR spectra were recorded on a JEOL FX200 spectrometer with TMS as internal standard. HPLC was carried out with a JASCO twincle apparatus by using a column packed with a Finepak SIL C_{18}. Microanalysis was performed in the Analytical Section, Institute of Physical and Chemical Research, Saitama. TLC was carried out by using Merck precoated silica gel 60 F_{254} plates: R_f^1, EtOAc; R_f^2, $CHCl_3$–MeOH–C_6H_6 (6:1:3); R_f^3, THF-hexane (1:1); R_f^4, $CHCl_3$–MeOH (3:1). The iron (III) complex molar ratio was determined as previously.[1] *N*-Benzyloxy-DL-amino acid methyl ester hydrochlorides ($PhCH_2ONHCH(R)CO_2Me \cdot HCl$) were obtained by treating the corresponding acid with HCL-saturated methanol: R, mp (lit,[21] mp); H, 125—126 °C (125—126 °C); Me, 130—131 °C (131—132 °C); Et. 114—116 °C (115—117 °C); *i*-Pr, 127—128 °C (127—130 °C); *i*-Bu, 115—117 °C (120—123 °C); $PhCH_2$, 119—120 °C (118—119 °C).

Coupling Methods. The following coupling procedures (a—f) were applied to the reaction of Pht=GlyOH with DL-$H(PhCH_2O)$AlaOMe; (a) acyl chloride in DMF, 3 h at −5 °C and 24 h at 25 °C; (b) mixed anhydride with $ClCO_2Bu^i$ in THF, 24 h at −15 °C and 48 h at 25 °C; (c) EEDQ in THF, 48 h at 30 °C; (d) mixed anhydride with pivaloyl chloride in THF, 48 h at 25 °C; (e) CDI in THF, 24 h at 0 °C and 24 h at 25 °C; (f) HOSu ester in THF, 72 h at 25 °C. In each case, a solution of an acyl active derivative of Pht=GlyOH was prepared either by simply dissolving the derivative or by adding an appropriate reagent to the solution of Pht=GlyOH (0.84 g, 4 mmol). To this solution was added $H(PhCH_2O)$Ala-OMe·HCl (0.982 g, 4 mmol) and Et_3N (0.404 g, 4 mmol, or 0.808 g in the case of acyl chloride). The resulting mixture was subjected to the above procedure (a—f). After evaporation of the solvent, the residue was extracted with AcOEt (100 mL) and the extract washed with 0.1 M (1 M=1 mol dm^{-3}) HCl, 5% $NaHCO_3$, and water, and then dried (Na_2SO_4). Evaporation of the solvent gave a crude product, which was recrystallized from AcOEt, mp 136—137 °C (lit,[3] mp 133—134 °C). Mp was depressed when the yield was less than 50%.

General Procedure for Acylation of N-*Benzyloxy α-Amino Acid Derivatives. (A) Acyl Chloride under Schotten-Baumann Conditions*: As a typical example, the preparation of Pht=Gly-DL-$(PhCH_2O)$Ala-Gly-OMe is described. To a mixture of DL-$H(PhCH_2O)$Ala-Gly-OMe·HCl (0.605 g, 2 mmol) in AcOEt (20 mL) and 5% $NaHCO_3$ (7 mL) was added Pht=Gly-Cl (0.447 g, 2 mmol) in AcOEt (20 mL) at 0—5 °C with vigorous stirring. The stirring was continued for 1 h at 5 °C and 48 h at room temperature, and AcOEt (60 mL) was added to extract the reaction products. The organic layer was washed with 0.1 M HCl, 5% Na_2CO_3, and water, and then dried over Na_2SO_4. Evaporation of the solvent gave the crude product; yield, 0.65 g (72%); mp 127—129 °C. The product was recrystallized from AcOEt–hexane to give a pure sample.

(B) Acyl Chloride in DMF: The preparation of Pht=Gly-DL-$(PhCH_2O)$Phe-Gly-OMe is given as an example. To a mixture of DL-$H(PhCH_2O)$Phe-Gly-OMe·HCl (1.13 g, 3 mmol) and Et_3N (0.61 g, 6 mmol) in DMF (30 mL) was added Pht=Gly-Cl (0.67 g, 3 mmol) in DMF (10 mL) at −10—−5 °C. After the mixture was stirred for 3 h at −5 °C and 48 h at room temperature, the solvent was removed under vacuum. The residue was dissolved in AcOEt (100 mL), and the organic layer was washed with 0.1 M HCl, 5% Na_2CO_3 and water, and dried over Na_2CO_3. Evaporation of the solvent gave a crude product, which was purified by column chromatography using Wakogel C-200; yield, 0.67 g (42%); mp 146—149 °C.

(C) Mixed Anhydride Procedure: The preparation of Pht=Gly-DL-$(PhCH_2O)$Abu-OMe is given as an example. A mixed anhydride was prepared from Pht=GlyOH (1.02 g, 5 mmol), Et_3N (0.505 g, 5 mmol) and isobutyl chloroformate (0.68 g, 5 mmol) in THF (30 mL) at −10 °C. To this was added DL-$H(PhCH_2O)$Abu-OMe·HCl (1.30 g, 5 mmol) and Et_3N (0.505 g, 5 mmol) in THF (10 mL) at −15 °C, and the mixture was stirred for 24 h at −20 °C and at 5 °C for 2 d. After evaporation of the solvent, the workup was carried out as above, to give an oily product.

N-*Benzyloxy* DL-*α-Amino Acid* N-*Hydroxysuccinimide Ester Hydrochlorides. General Procedure*: To a solution of *N*-benzyloxy α-amino acid (5 mmol) and *N*-hydroxysuccinimide (0.58 g, 5 mmol) in THF (10 mL) was added DCC (1.03 g, 5 mmol) in THF (10 mL) at 0 °C with stirring. The solution was kept for 1 h at 0 °C and for 20 h in the refri-

gerator. *N,N'*-Dicyclohexylurea (DCU) which separated was removed by filtration and the filtrate was evaporated to give a residue. It was diluted with AcOEt (20 mL) to give additional DCU. After separation of DCU, the filtrate was concentrated to give an oil or solid. This was treated with 1 M HCl in THF (20 mL) for 1 h at room temperature. Evaporation of the solvent gave the crude product. The product could be used for further coupling without purification. For an analytical sample, it was recrystallized from AcOEt-hexane.

Reactions of N-*Benzyloxy-α-amino Acid* N-*Hydroxysuccinimide Esters with H-Gly-OMe. General Procedure*: To a solution of H-Gly-OMe·HCl (0.25 g, 2 mmol) and Et_3N (0.202 g, 2 mmol) in $CHCl_3$ (10 mL), was added H($PhCH_2O$)-NHCHRCOOSu·HCl (2 mmol) and Et_3N (0.20 g, 2 mmol) in $CHCl_3$ (10 mL) at 0°C under stirring. The solution was kept for 2 h at 0°C and 1 d at room temperature. After evaporation of the solvent, the residue was diluted with AcOEt (50 mL), and the AcOEt solution was washed with 5% $NaHCO_3$ and water, and dried (Na_2SO_4). After the solvent was evaporated, the residue was treated with 2 M HCl in dioxane (3 mL) for 1 h at room temperature. Evaporation of the solvent gave the crude dipeptide hydrochloride, which was recrystallized from MeOH-ether.

*Pht=Gly-*DL*-(HO)Phe-Gly-OMe.* Pht=Gly-DL-($PhCH_2O$)Phe-Gly-OMe (2.65 g, 5 mmol), 10% Pd on carbon (0.030 g) and MeOH (10 mL) were placed in a hydrogenation apparatus. The mixture was shaken with H_2 under the atmospheric pressure for 24 h at 30 °C. The catalyst was filtered off, and the filtrate was evaporated to give the crude product. It was recrystallized from $CHCl_3$-hexane; yield, 1.93 g (88%); mp 183—184 °C (decomp). Found: C, 59.51; H, 4.76; N, 9.43. Calcd for $C_{22}H_{21}N_3O_7\cdot(1/4)H_2O$: C, 59.65; H, 4.66; N, 9.49%.

When the sample was mixed with a $FeCl_3$ solution, it gave an intense voilet color.

DL-*H($PhCH_2O$)Ala-Gly-NHPh (6).* To a solution of DL-H($PhCH_2O$)Ala-OSu·HCl (4.92 g, 15 mmol) and Et_3N (1.52 g, 15 mmol) in $CHCl_3$ (25 mL) was added H-Gly-NHPh·HCl (2.80 g, 15 mmol) in $CHCl_3$ (15 mL) containing Et_3N (1.52 g), and the mixture was stirred at room temperature. After 30 h, the mixture was washed with 5% $NaHCO_3$ and water, and dried (Na_2SO_4). Evaporation of the solvent gave the crude product, which was recrystallized from AcOEt-hexane; yield, 4.1 g (86%).

*Boc-Gly-*DL*-(PhCH$_2$O)Ala-Gly-NHPh (7).* Boc-Gly-OH(0.29 g, 1.65 mmol) in THF (10 mL) containing Et_3N (0.167 g, 1.65 mmol) was converted into the mixed anhydride by adding isobutyl chloroformate (0.205 g, 1.5 mmol) in THF (5 mL) at −15 °C. To this mixture was added the dipeptide anilide (**6**) (0.491 g, 1.5 mmol) in DMF (10 mL), and the resulting mixture was stirred for 3 h at −15 °C and 12 h at 0 °C. After evaporation of the solvent, the residue was diluted with ethyl acetate (11 mL). The organic layer was washed with 5% $NaHCO_3$ and water, and dried over Na_2SO_4. Evaporation of the solvent gave the crude product (0.72 g). To ensure coupling, the crude product was subjected to the above acylation procedure two further times. The final product was chromatographed using Wakogel C-100 with EtOAc as eluant. The product obtained was 0.59 g (74% yield).

DL-*H($PhCH_2O$)Ala-Gly-*DL*-(PhCH$_2$O)Ala-Gly-NHPh(8).* Boc-tripeptide anilide (**7**) (0.82 g, 1.69 mmol) was dissolved in dioxane (6 mL). To this was added 3.6 M HCl in dioxane (5 mL). The reaction mixture was stirred for 2 h at 5 °C and evaporated. The residue was triturated with ether and filtered. The product was recrystallized from MeOH-ether to give 0.60 g (85%) of the tripeptide anilide hydrochloride, mp 175—176 °C (decomp).

The tripeptide anilide hydrochloride (0.69 g, 1.65 mmol) and triethylamine (0.168 g, 1.65 mmol) were dissolved in $CHCl_3$ (15 mL). To the solution was added a solution containing H($PhCH_2O$)Ala-OSu (0.54 g, 1.65 mmol) and Et_3N (0.168 g, 1.65 mmol) in $CHCl_3$(10 mL) at 0 °C. The solution was stirred for 1 h at 0 °C and then for 24 h at room temperature. The $CHCl_3$ layer was washed with 5% $NaHCO_3$ and water, and dried (Na_2SO_4). Evaporation of the solvent gave a product (0.80 g), which was recrystallized from EtOAc-hexane; yield, 0.74 g (80%).

*Boc-Gly-*DL*-(PhCH$_2$O)Ala-Gly-*DL*-(PhCH$_2$O)Ala-Gly-NHPh (9).* Boc-GlyOH (0.193 g, 1.1 mmol) and Et_3N (0.113 g, 1.1 mmol) were placed in THF (10 mL) and cooled. To this was added $ClCO_2Bu^i$ (0.137 g, 1 mmol) in THF (5 mL) at −16°C. After 10 min, the tetrapeptide anilide (**8**) (0.56 g, 1 mmol) in DMF (10 mL) was added slowly to the mixed anhydride solution at −16 °C. The reaction mixture was stirred for 24 h at −16 °C, evaporated and diluted with EtOAc (40 mL). The organic layer was washed with 5% $NaHCO_3$ and water, and dried over Na_2SO_4. Evaporation of the solvent gave a residue. This residue was found to contain the starting tetrapeptide anilide; it was subjected two more times to a similar Boc-Gly mixed anhydride acylation. The crude residue thus obtained amounted to 0.84 g. It was purified by chromatography on a column of Wakogel C-100 with EtOAc as eluant by the flash technique; yield, 0.45 g (63%).

*Ac-*DL*-(PhCH$_2$O)Ala-Gly-*DL*-(PhCH$_2$O)Ala-Gly-*DL*-(PhCH$_2$O)Ala-Gly-NHPh (10). Ac-*DL*-(PhCH$_2$O)Ala-OSu:* To a solution of Ac-DL-($PhCH_2O$)Ala-OH (0.68 g, 2,9 mmol) and HOSu (0.33 g, 2.9 mmol) in THF (10 mL) was added DCC (0.59 g, 2.9 mmol) in THF (5 mL) at 0 °C. The solution was stirred for 1 h at 0 °C and kept in the refrigerator for 24 h. The crude product, which was obtained by the usual workup performed for a DCC reaction, was recrystallized from EtOAc-hexane; yield, 0.65 g (67%); mp 100—100.5 °C. Found: C, 57.11; H, 5.45; N, 8.35. Calcd for $C_{16}H_{18}N_2O_6$: C, 57.48; H, 5.43; N, 8.38 (%).

The pentapeptide anilide (**9**) (0.44 g, 0.61 mmol) was dissolved in dioxane (5 mL), to remove the Boc group. After the addition of 3.6 M HCl in dioxane (20 mL), the resulting solution was stirred for 2 h at room temperature and evaporated. The residue was triturated with ether, and the precipitate was filtered to give the pentapeptide anilide hydrochloride; yield, 0.346 g (86%).

The pentapeptide anilide hydrochloride (0.451 g, 0.52 mmol) and Et_3N (0.52 g, 0.52 mmol) were stirred in THF (10 mL) at 0 °C for 1 h. To this mixture was added Ac-DL-($PhCH_2O$)Ala-OSu (0.174 g, 0.52 mmol) in THF (10 mL). The reaction mixture was stirred for 48 h at 25 °C, evaporated and extracted with EtOAc (50 mL). The organic layer was washed successively with 5% $NaHCO_3$, 5% citric acid and water, and dried over Na_2SO_4. Evaporation of the solvent gave the crude product, 0.431 g (99%). The product was chromatographed on Wakogel C-200, to give a pure sample; yield, 0.325 g (75%).

*Ac-*DL*-(HO)Ala-Gly-*DL*-(HO)Ala-Gly-*DL*-(HO)Ala-Gly-NHPh (11).* The acetyl hexapeptide anilide (**10**) (0.240 g, 0.27 mmol), 10% Pd/C (0.040 g) and MeOH (20 mL) were placed in a hydrogenation apparatus, and the mixture shaken with H_2 for 72 h at 30 °C under atmospheric pressure. The catalyst was filtered off and the filtrate evaporated to give the product; yield, 0.154 g (95%). After recrystallization from MeOH, the product showed mp 191—192 °C (decomp). IR $\nu_{C=O}$, 1620—1680 cm^{-1} (-CONH- and -CON(OH)-); ^1H NMR (DMSO-d_6) δ=1.30 (9H, m, CH_3), 2.04 (3H, s, CH_3CO), 4.0 (6H, m, $-CH_2-$), 4.90 (3H, m, -CH-), 7.0—7.8 (8H, broad, -CONH- and $-C_6H_5$), 7.95 (1H, -HNPh), and 9.70 (3H, s, N-OH).

The sample gave a characteristic color when mixed with $FeCl_3$ solution.

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References

1) Part I: K. Shimizu, M. Hasegawa, and M. Akiyama, *Bull. Chem. Soc. Jpn.*, **57**, 495 (1984).
2) J. D. M. Herscheid, R. J. F. Nivard, M. W. Tijhuis, H. P. H. Scholten, and H. C. J. Ottenheijm, *J. Org. Chem.*, **45**, 1880 (1980).
3) T. Kolasa and A. Chimiak, *Tetrahedron*, **33**, 3285 (1977).
4) N. Shinmon, M. P. Cava, and R. F. C. Brown, *J. Chem. Soc., Chem. Commun.*, **1980**, 1020.
5) J. B. Neilands, *Science*, **156**, 1443 (1967).
6) J. B. Neilands, "Inorganic Biochemistry," ed by G. Eichorn, Elsevier, New York (1973), p.167; T. Emery, *Adv. Enzymol. Relat. Areas Mol. Biol.*, **33**, 135 (1971); "Microbial Iron Metabolism," ed by J. B. Neilands, Academic Press, New York (1974), Chap. 5.
7) H. Maehr, *Pure Appl. Chem.*, **28**, 603 (1971).
8) U. Schmidt, J. Häusler, E. Ohler, and H. Poisel, *Fortschr. Chem. Org. Naturst.*, **37**, 251 (1979); H. C. J. Ottenheijm, R. Plate, J. H. Noordik, and J. D. M. Herscheid, *J. Org. Chem.*, **47**, 2147 (1982).
9) C. Shin, K. Nanjo, E. Ando, and J. Yoshimura, *Bull. Chem. Soc. Jpn.*, **47**, 3109 (1974).
10) Yu. A. Ovichinnikov and V. T. Ivanov, *Tetrahedron*, **31**, 2177 (1975).
11) E. A. Kaczka, C. O. Gitterman, E. L. Dulaney, and K. Folkers, *Biochemistry*, **1**, 340 (1962).
12) W. Keller-Schierlein and B. Maurer, *Helv. Chim. Acta*, **52**, 603 (1969); Y. Isowa, M. Ohmori, and H. Kurita, *Bull. Chem. Soc. Jpn.*, **47**, 215 (1974). See also: W. Wierenga, "The Total Synthesis of Natural Products," ed by J. Apsimmon, Wiley, New York (1981), Vol. 4, Chap. 3, p.274.
13) The compounds which have the *N*-hydroxy amide units in the main chain appaer to be only cyclic dipeptide derivatives.[14]
14) A. H. Cook and C. A. Slater, *J. Chem. Soc.*, **1956**, 4130; C. Shin, K. Nanjo, M. Kato, and J. Yoshimura, *Bull. Chem. Soc. Jpn.*, **48**, 2584 (1975); J. D. M. Hersheid, J. H. Colstee, and H. C. J. Ottenheijm, *J. Org. Chem.*, **46**, 3346 (1981).
15) T. Kolasa and A. Chimiak, *Tetrahedron*, **30**, 3591 (1974).
16) M. Akiyama, M. Hasegawa, H. Takeuchi, and K. Shimizu, *Tetrahedron Lett.*, **1979**, 2599.
17) M. Bodanszky, Y. S. Klausner, M. A. Ondetti, "Peptide Synthesis," 2nd. ed. Wiley, New York (1976).
18) A. Winston and E. T. Mazza, *J. Polym. Sci., Polym. Chem. Ed.*, **13**, 2019 (1975); A. Winston and Kirchner, *Macromolecules*, **11**, 597 (1978).
19) R. V. Christian, I. D. Leffler, and J. S. Dahler, *Anal. Chem.*, **26**, 1666 (1954).
20) G. Schwarzenbach and K. Schwarzenbach, *Helv. Chim. Acta*, **46**, 1390 (1963).
21) T. Kolasa, A. Chimiak, and A. Kitowska, *J. Pract. Chem.*, **317**, 252 (1975).