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Amino Acids and Peptides. XXIII. Synthesis of N^{α}-Protected Amino Acid 6-Chloro-2-pyridyl Esters and Their Evaluation for Peptide Synthesis[1,2]

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6-Chloro-2-pyridyl esters (OPyCl) of N^{α}-benzyloxycarbonyl and *tert*-butyloxycarbonylamino acids were synthesized by the *N,N'*-dicyclohexylcarbodiimide (DCC) method from the acids and 6-chloro-2-hydroxypyridine in dimethylformamide (DMF). The reactivity of the 6-chloro-2-pyridyl ester with amino group is much higher than that of the corresponding 2-pyridyl ester (OPy) and *p*-nitrophenyl esters (ONp) in dioxane and DMF, and a peptide bond is formed without acylation at the side chain hydroxyl group of amino acids. Z–Asp(OBzl)–OPyCl reacted with amino acid methyl esters in dioxane to give the corresponding dipeptide without any detectable aspartimide formation.

Keywords N^{α}-protected amino acid 6-chloro-2-pyridyl ester; reactivity; amino group; hydroxyl group; solvent effect; active ester; synthesis

Previously, it was reported that 2-pyridyl esters reacted with the amino groups of amino acids and peptides much faster than the corresponding *p*-nitrophenyl esters in non-polar solvents, while *p*-nitrophenyl esters reacted with amino groups much faster than the corresponding 2-pyridyl esters in polar solvents.[3] In the above reactions, the reactivity of the *p*-nitrophenyl esters is dependent upon the electron-attracting power of the nitro-system in the leaving group and is therefore markedly increased in the change from a non-polar to a polar solvent. The activation of 2-pyridyl esters occurs by intramolecular general base catalysis and thus is favored in a non-polar solvent.[3] In spite of the excellent reactivity of 2-pyridyl esters in non-polar solvents and easy purification after peptide formation by the 2-pyridyl ester method, those advantages are counterbalanced by the problem of the limited solubility of the growing peptides in non-polar solvents.

This report deals with the synthesis of 6-chloro-2-pyridyl esters of *N*-protected amino acids and examination of their reactivity in non-polar and polar solvents with the objective of obtaining more reactive esters by utilizing both the electron-attracting property (inductive effect) of the chlorine atom in the leaving group and by making possible intramolecular base catalysis even in polar solvents.

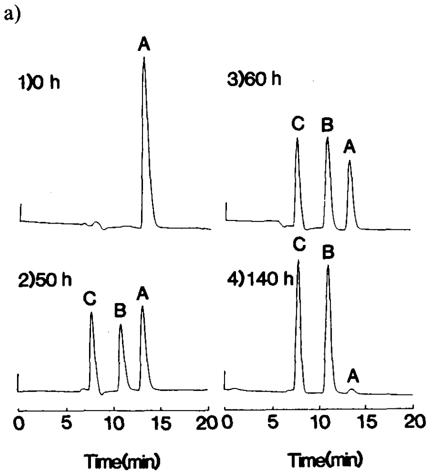
N^{α}-Benzyloxycarbonyl (Z) and *tert*-butyloxycarbonyl (Boc) amino acid 6-chloro-2-pyridyl esters were prepared by the DCC method[4] in DMF in good yields. The esters obtained were more crystallizable than the corresponding 2-pyridyl esters,[3] as summarized in Table I. The reactivity of the esters with various nucleophiles was examined using high performance liquid chromatography (HPLC).

First of all, the reactivity of Z–Phe–OPyCl with various alcohols was examined. As an example, the method used to

TABLE I. Yield, Melting Point, $[\alpha]_D$, Elemental Analysis and Crystallization Solvents of 6-Chloro-2-pyridyl Esters

Ester –OPyCl	Yield (%)	mp (°C)	$[\alpha]_D$	Formula	Elemental analysis Calcd (Found) C	H	N	Solvents for cryst.
Boc–Gly	56.1	120—123		$C_{12}H_{15}ClN_2O_4 \cdot 1/4\,H_2O$	51.6 (51.6	5.61 5.62	10.0 9.90)	Ether–pet.
Boc–Ala	60.2	79—80	−47.9° (c=1.0, DMF)	$C_{13}H_{17}ClN_2O_4$	51.9 (52.2	5.71 6.00	9.31 9.60)	Ether–pet.
Boc–Val	63.2	63—64	−41.1° (c=1.0, DMF)	$C_{15}H_{21}ClN_2O_4$	54.8 (54.7	6.39 6.45	8.52 8.58)	Ether–pet.
Boc–Leu	—	Oil	−25.2° (c=0.9, DMF)					
Boc–Ile	—	Oil	+0.93° (c=1.7, DMF)					
Boc–Gln	60.0	144—147	−40.0° (c=1.0, DMF)	$C_{15}H_{20}ClN_3O_5$	50.4 (50.6	5.63 5.75	11.7 11.7)	Ether
Z–Ala	78.3	73—74	−41.9° (c=1.0, DMF)	$C_{16}H_{15}ClN_2O_4 \cdot 1/2\,H_2O$	57.9 (58.0	4.87 5.02	8.45 8.20)	Ether–pet.
Z–Val	44.4	54—55	−26.4° (c=1.0, DMF)	$C_{18}H_{17}ClN_2O_4 \cdot 2H_2O$	60.2 (60.1	5.95 5.65	7.80 8.05)	Ether–pet.
Z–Phe	61.8	103—105	−37.3° (c=1.0, DMF)	$C_{22}H_{19}ClN_2O_4 \cdot 3/4\,H_2O$	64.1 (64.1	5.02 4.88	6.79 6.86)	Ether
Z–Gln	62.3	139—142	−16.0° (c=1.0, DMF)	$C_{18}H_{18}ClN_3O_5 \cdot 1/2\,H_2O$	55.6 (55.5	4.94 4.88	10.8 10.9)	Ether
Z–Asp(OBzl)	79.3	55—60	−23.4° (c=1.0, DMF)	$C_{24}H_{21}ClN_2O_6$	61.5 (61.8	4.51 4.71	6.00 6.21)	Ether–pet.

—: not determined. pet. = petroleum ether.



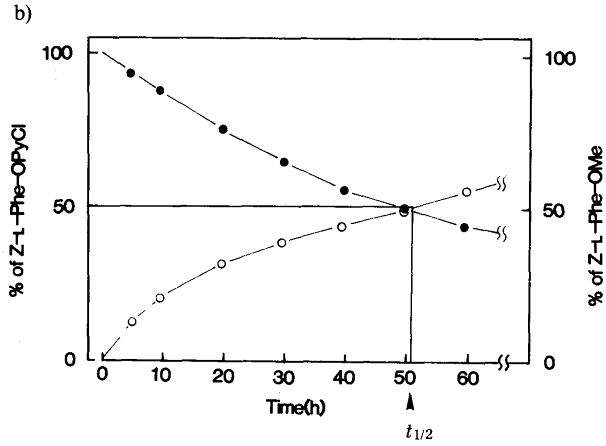


Fig. 1a. HPLC Pattern of the Reaction Mixture of Z–Phe–OPyCl and MeOH

Column, YMC R-ODS-5 (4.6×250 mm); solvent MeOH–H_2O (8:2) containing 0.1% TFA; flow rate, 0.5 ml/min; ditection, absorbance at 220 nm. Peak A, Z–Phe–OPyCl; peak B, Z–Phe–OMe; peak C, 6-chloro-2-hydroxypyridine.

Fig. 1b. Reactivity of Z–Phe–OPyCl with MeOH

—●—, Z–Phe–OPyCl; —○—, Z–Phe–OMe.

Table II. Half-Lives ($t_{1/2}$) of Reactions of N^α-Benzyloxycarbonylphenylalanine 6-Chloro-2-pyridyl, 2-Pyridyl and *p*-Nitrophenyl Esters with Alcohols

Alcohol	$t_{1/2}$ (h)		
	6-Chloro-2-pyridyl	2-Pyridyl	*p*-Nitrophenyl
Methanol	51	12	63
Ethanol	19% (150 h)	22	ND
Isopropanol	—	31	ND
tert-Butanol	—	43% (50 h)	ND

—: no reaction. ND: not determined.

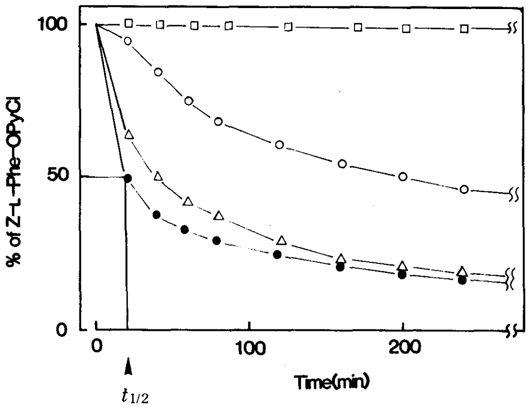


Fig. 2. Reactivity of Z–Phe–OPyCl with H–Ala–Val–Gly–OMe

—●—, in dioxane; —△—, in DMF; —○—, in $CHCl_3$; —□—, absence of amino component in dioxane, DMF and $CHCl_3$.

Table III. Half-Lives ($t_{1/2}$) of Reactions of N^α-Benzyloxycarbonylphenylalanine 6-Chloro-2-pyridyl, 2-Pyridyl and *p*-Nitrophenyl Esters with Amino Component

Amino component	Solvent	$t_{1/2}$ (min)		
		6-Chloro-2-pyridyl	2-Pyridyl	*p*-Nitrophenyl
H–Ala–Val–Gly–OMe	Dioxane	20	67	1050
	DMF	41	150	72
	Chloroform	210	149	—

—: not reaction.

Table IV. Half-Lives ($t_{1/2}$) of Reactions of N^α-Benzyloxycarbonylphenylalanine 6-Chloro-2-pyridyl, 2-Pyridyl and *p*-Nitrophenyl Esters with Hydroxyl Components

Hydroxyl component	Solvent	$t_{1/2}$ (h)		
		6-Chloro-2-pyridyl	2-Pyridyl	*p*-Nitrophenyl
Boc–Ser–OMe	Dioxane	—	ND	ND
	DMF	ND	22% (46 h)	—
	Chloroform	—	21	ND
Z–Tyr–OMe	Dioxane	—	ND	ND
	DMF	ND	ND	36% (60 h)
	Chloroform	—	42	ND

—: no reaction. ND: not determined.

determine the reaction rate between Z–Phe–OPyCl and MeOH will be described.

As shown in Fig. 1a, the peak of Z–Phe–OPyCl (A) decreased and peaks of Z–Phe–OMe[5] (B) and liberated 6-chloro-2-hydroxypyridine (C) increased with time. The peak areas were plotted as a function of time as shown in Fig. 1b, and the half-life ($t_{1/2}$) was determined. The half-lives in the reactions of Z–Phe–OPyCl with various alcohols are summarized in Table II in comparison with those of Z–Phe–OPy and Z–Phe–ONp.[6] The reactivity of Z–Phe–OPyCl with alcohols was decreased compared with that of Z–Phe–OPy, presumably due to the decrease of basicity of the 6-chloropyridine ring compared with that of the pyridine ring owing to the inductive effect of the chlorine atom (pK_a of pyridine, 5.8; pK_a of 2-chloropyridine, 0.72).[7]

Next, the reactivity of Z–Phe–OPyCl with H–Ala–Val–Gly–OMe in $CHCl_3$, dioxane and DMF (order of polarity, DMF > dioxane > $CHCl_3$) was examined. As described above, the decrease of peak area of Z–Phe–OPyCl in the reaction mixtures was plotted as a function of time (Fig. 2) and the half-lives of the reactions of Z–Phe–OPyCl with H–Ala–Val–Gly–OMe are summarized in Table III in comparison with those of Z–Phe–OPy and Z–Phe–ONp. The newly developed OPyCl ester reacted with amino com-

TABLE V. Comparison between 6-Chloro-2-pyridyl Ester Method and Other Activation Methods

Compound	Method	Yield (%)	mp (°C)	[α]D	Rf^1	Rf^4
Z–Ala–Phe–OMe	OPyCl	87.8	97—99	+1.0° (c=1.0, DMF)	0.70	0.27
	ONSu[8]	86	99—100			
	ONp[9]	89	98—99			
	DCC[10]	64	99—101	+1.1° (DMF)		
Z–Phe–Ala–OMe	OPyCl	95.0	128—129	−16.5° (c=1.0, DMF)	0.70	0.31
	DCC[11]	97	132—133	−20° (DMF)		
Z–Ala–Gly–OBzl	OPyCl	82.3	108—109	−26.5° (c=1.0, MeOH)	0.75	0.31
	DCC[12]	92	109—110			
Boc–Val–Ala–OMe	OPyCl	68.4	112—116	−60.9° (c=1.0, AcOH)	0.53	0.30
	Mix[13]	70	125—126	−62° (AcOH)		
Z–Gln–Gly–OMe	OPyCl	37.9	169—172	−15.6° (c=1.0, AcOEt)	0.20	0.69[a]
	MCA[14]	60	173—175	−16° (AcOEt)		
Z–Asp(OBzl)–Phe–OMe	OPyCl	92.5	110—111	−13.7° (c=1.0, DMF)	0.73	0.82
	ONp[15]	72.4	114—116	−15° (DMF)		
Z–Asp(OBzl)–Ser–OMe	OPyCl	79.1	114—115	0.0° (c=1.0, MeOH)	0.69	0.23
	DCC[16]	73	125—126	0.0° (c=1.0, MeOH)		
Boc–Val–Thr–OMe	OPyCl	65.4	78—81	−37.6° (c=1.0, MeOH)	0.70	0.13

[a] Rf^2.

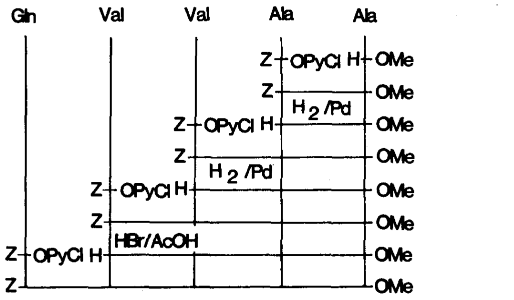


Fig. 3. Synthetic Scheme for Z–Gln–Val–Val–Ala–Ala–OMe by the OPyCl Method

TABLE VI. Comparison between 6-Chloro-2-pyridyl Ester Method and Other Methods for Synthesis of Z–Gln–Val–Val–Ala–Ala–OMe

Compound	Method	Yield (%)	mp (°C)	[α]D	Rf^1	Rf^2
Z–Ala–Ala–OMe	OPyCl	42.9	88—93	−51.4° (c=1.0, MeOH)	0.68	0.75
	Mix	43.2	92—93	−50.9° (c=1.0, MeOH)	0.70	0.75
Z–Val–Ala–Ala–OMe	OPyCl	63.8	186—187	−15.6° (c=1.0, DMF)	0.54	0.51
	Azid	72.2	183—185	−15.1° (c=1.0, DMF)	0.54	0.51
Z–Val–Val–Ala–Ala–OMe	OPyCl	79.0	249—252	−14.6° (c=1.0, DMF)	0.78	0.51
	Mix	94.5	230—233	−16.2° (c=1.0, DMF)	0.77	0.51
Z–Gln–Val–Ala–Ala–Ala–OMe	OPyCl	79.0	281—283 (dec.)	−35.6° (c=0.5, DMSO)	0.42	0.33
	ONp	53.2	274 (dec.)	−35.3° (c=0.5, DMSO)	0.42	0.34

ponents much faster than the corresponding OPy and ONp esters in dioxane and even in DMF. Z–Phe–ONp favored DMF as a solvent and Z–Phe–OPy favored dioxane.

The reactivity of Z–Phe–OPyCl with side chain hydroxyl groups of amino acids was then examined, and the results are summarized in Table IV in comparison with those for Z–Phe–OPy and Z–Phe–ONp. Z–Phe–OPyCl did not react with hydroxyl groups of Ser and Tyr in dioxane, which is the most favorable solvent for Z–Phe–OPyCl, during 3 d. However, Z–Phe–OPy reacted with them in $CHCl_3$ with half-lives of 21 and 42 h, respectively. Z–Phe–ONp did not react with the hydroxyl group of Ser in DMF but reacted with the hydroxyl group of Tyr to the extent of 35% during 60 h. From these results, it can be concluded that the protection of hydroxyl group of amino acid is not required when OPyCl active esters are employed for peptide synthesis.

Next, several dipeptides were prepared using OPyCl active esters for evaluation of the usefulness of the esters in peptide synthesis. Yields, melting points and [α]D values are summarized in Table V in comparison with those of the corresponding peptides prepared by other methods. The yields are similar to or higher than those of dipeptides prepared by other methods except for the case of Z–Gln–Gly–OMe. The melting points and [α]D values are identical with those of dipeptides prepared by other methods. Z–Asp(OBzl)–Ser–OMe and Boc–Val–Thr–OMe were obtained in good yields without acylation of the hydroxyl groups of Ser and Thr. Interestingly, reaction of Z–Asp(OBzl)–OPyCl with H–Phe–OMe or H–Ser–OMe in dioxane gave Z–Asp(OBzl)–Phe–OMe or Z–Asp(OBzl)–Ser–OMe, respectively without any detectable aspartimide formation so far as examined by thin-layer chromatography (TLC). However, in the reaction of Z–Asp(OBzl)–ONp or Z–Asp(OBzl)–OPyCl with H–Ser–OMe in DMF, aspartimide formation was observed on TLC. Dioxane is a good solvent for the synthesis of Asp-containing peptides.

Finally, a thiol proteinase inhibitor, Z–Gln–Val–Val–Ala–Ala–OMe,[17] which is fairly insoluble in organic solvents and contains the sterically bulky Val–Val sequence, was prepared in a stepwise manner starting from H–Ala–OMe and using Z–Ala–OPyCl, Z–Val–OPyCl and Z–Gln–OPyCl in DMF as illustrated in Fig. 3. The yields, melting points and [α]D values are summarized in Table VI in comparison with those of the corresponding peptides prepared by other methods. Yields were fairly good and melting points and [α]D values are identical with those of the corresponding peptides prepared by other methods, as summarized in Table VI.

The newly developed OPyCl active esters are concluded to be very effective for peptide synthesis in dioxane and DMF.

Experimental

The melting points are uncorrected. Optical rotations were measured with an automatic polarimeter, model DIP-360 (Japan Spectroscopic Co., Ltd.). Amino acid compositions of acid hydrolysates (110 °C, 18 h, 6 N HCl, for peptides containing a Val–Val bond, 6 N HCl, 110 °C, 72 h) were determined with an amino acid analyzer, K-101 AS (Kyowa Seimitsu Co., Ltd.). On TLC (Kieselgel G, Merck), Rf^1, Rf^2, Rf^3 and Rf^4 values refer to the systems of $CHCl_3$, MeOH and AcOH (90 : 8 : 2), $CHCl_3$, MeOH and H_2O (89 : 10 : 1), $CHCl_3$, MeOH and H_2O (8 : 3 : 1, lower phase) and $CHCl_3$ and ether (4 : 1), respectively.

General Procedure for Preparation of N^α-Protected Amino Acid 6-Chloro-2-Pyridyl Esters An N^α-protected amino acid (8.5 mmol) and 6-chloro-2-hydroxypyridine (1.10 g, 8.5 mmol) were dissolved in DMF (30 ml) and cooled with ice-salt. DCC (2.10 g, 10.2 mmol) was added to the solution. The reaction mixture was stirred at 4 °C overnight. After removal of dicyclohexylurea and the solvent, the residue was extracted with AcOEt. The extract was washed with 5% $NaHCO_3$ and water, dried over Na_2SO_4 and evaporated down *in vacuo*. The residue was crystallized from the appropriate solvent shown in Table I. Yield, melting point, $[\alpha]_D$ value, elemental analysis data and Rf values are summarized in Table I.

General Procedure for Examination of Reactivity of Z–Phe–OPyCl (Z–Phe–OPy and Z–Phe–ONp) with Alcohol Z–Phe–OPyCl (4 mg, 0.01 mmol) was dissolved in MeOH (EtOH, iso-PrOH or *tert*-BuOH, 4 ml) and the solution was stirred at room temperature. The reaction mixture (5 μl) was injected into an HPLC apparatus [column, YMC-Pack R-ODS-5 (4.6 × 250 mm); solvent, 80% MeOH containing 0.1% TFA; flow rate, 0.5 ml/min; detection, 220 nm] and the decrease of the peak area of Z–Phe–OPyCl was plotted as a function of time (Fig. 1b).

General Procedure for Examination of Reactivity of Z–Phe–OPyCl (Z–Phe–OPy and Z–Phe–ONp) with Amino Component 1) Synthesis of Standard Sample: Z–Ala–Val–Gly–OMe: The title compound was prepared from a mixed anhydride (prepared from 0.58 g of Z–Ala–OH, 0.3 ml of ethyl chloroformate and 0.4 ml of Et_3N) and H–Val–Gly–OMe (prepared from 0.70 g of Z–Val–Gly–OMe[18]) by catalytic hydrogenation). The crude product was recrystallized from MeOH, yield 0.47 g (55.2%), mp 203—207 °C, $[\alpha]_D^{27}$ $-52.2°$ ($c=1.0$, MeOH), Rf^1 0.40, Rf^3 0.62. *Anal.* Calcd for $C_{19}H_{27}N_3O_6 \cdot 1/4H_2O$: C, 57.3; H, 6.98; N, 10.6. Found: C, 57.3; H, 6.98; N, 10.8.

H–Ala–Val–Gly–OMe: The title compound was prepared from Z–Ala–Val–Gly–OMe (220 mg) by catalytic hydrogenation. After removal of Pd and the solvent, water was added to the residue and the mixture was lyophilized to give an amorphous powder, yield 140 mg (97.0%), $[\alpha]_D^{25}$ $-32.1°$ ($c=0.2$, MeOH), Rf^3 0.59. *Anal.* Calcd for $C_{11}H_{21}N_3O_4 \cdot H_2O$: C, 47.6; H, 8.37; N, 15.1. Found: C, 47.6; H, 8.15; N, 14.8.

Z–Phe–Ala–Val–Gly–OMe: The title compound was prepared from Z–Phe–ONp (136 mg) and H–Ala–Val–Gly–OMe (70 mg). The crude product was recrystallized from MeOH, yield 63.8 mg (43.7%), mp 229—232 °C, $[\alpha]_D^{25}$ $-8.8°$ ($c=1.0$, DMSO), Rf^1 0.47, Rf^3 0.78. *Anal.* Calcd for $C_{29}H_{38}N_4O_7$: C, 64.4; H, 7.10; N, 10.4. Found: C, 64.5; H, 7.18; N, 10.2.

2) Z–Phe–OPyCl (4 mg, 0.01 mmol) and H–Ala–Val–Gly–OMe (2.6 mg, 0.01 mmol) were dissolved in dioxane (4 ml), DMF (4 ml) or chloroform (4 ml). The reaction mixture was stirred at room temperature. An aliquot (10 μl) was taken periodically and diluted with MeOH (200 μl), and 20 μl was injected into an HPLC apparatus [solvent, 78% MeOH containing 0.1% TFA; detection, 265 nm]. The decrease of peak area of Z–Phe–OPyCl was plotted as a function of time (Fig. 2).

General Procedure for Examination of Reactivity of Z–Phe–OPyCl (Z–Phe–OPy and Z–Phe–ONp) with Hydroxyl Group of Ser and Tyr Z–Phe–OPyCl (4 mg, 0.01 mmol) and Z–Ser–OMe (2.6 mg, 0.01 mmol) or Z–Tyr–OMe (2.6 mg, 0.008 mmol) were dissolved in dioxane (4 ml), DMF (4 ml) or $CHCl_3$ (4 ml). The reaction mixture was stirred at room temperature. An aliquot (10 μl) was taken periodically, and diluted with MeOH (200 μl), and 20 μl was injected into an HPLC apparatus [solvent, 78% MeOH containing 0.1% TFA; detection, 265 nm]. The decrease of peak area of Z–Phe–OPyCl was plotted as a function of time and the half-life of Z–Phe–OPyCl was estimated.

General Procedure for Preparation of Dipeptide Using 6-Chloro-2-Pyridyl Esters and Amino Acid Methyl Esters The active ester (1.2 mmol) and an amino acid methyl ester were dissolved in dioxane (30 ml). The reaction mixture was stirred at room temperature overnight. After removal of the solvent, the residue was dissolved in AcOEt. The solvent was washed with 1 N HCl or 10% citric acid, 5% $NaHCO_3$ and water, dried over Na_2SO_4 and evaporated down. The residue was crystallized from the appropriate solvent. The yield, melting point, $[\alpha]_D$ values, Rf values and solvent for recrystallization are summarized in Table V.

Z–Ala–Ala–OMe Z–Ala–OPyCl (2.3 g) and H–Ala–OMe·HCl (1.0 g) were dissolved in DMF (10 ml) containing Et_3N (1.0 ml). The reaction mixture was stirred at room temperature overnight. After removal of the solvent, the residue was extracted with AcOEt. The extract was washed with 1 N HCl, 5% Na_2CO_3 and water, dried over Na_2SO_4 and evaporated. Ether was added to the residue to give crystals, which were collected by filtration and recrystallized from AcOEt and ether. Yield, melting point, $[\alpha]_D$ value and Rf values are summarized in Table VI.

Z–Val–Ala–Ala–OMe Z–Val–OPyCl (1.1 g) and H–Ala–Ala–OMe·HCl (prepared from 0.9 g of Z–Ala–Ala–OMe by catalytic hydrogenation) were dissolved in DMF (10 ml) containing Et_3N (0.5 ml). The reaction mixture was stirred at room temperature overnight. After removal of the solvent, AcOEt and H_2O were added to the residue to give crystals, which were collected by filtration and recrystallized from MeOH.

Z–Val–Val–Ala–Ala–OMe The title compound was prepared from Z–Val–OPyCl (0.7 g) and H–Val–Ala–Ala–OMe (prepared from 0.7 g of Z–Val–Ala–Ala–OMe by catalytic hydrogenation) in the same manner as described for the synthesis of Z–Val–Ala–Ala–OMe.

Z–Gln–Val–Val–Ala–Ala–OMe The title compound was prepared from Z–Gln–OPyCl (0.7 g) and H–Val–Val–Ala–Ala–OMe (prepared from 0.6 g of Z–Val–Val–Ala–Ala–OMe and 0.3 ml of 25% HBr/AcOH) in DMF in the same manner as described above.

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References and Notes

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