Phosphinyl- and Phosphinothioylamino Acids and Peptides. IV. Further Examples of Use of the Diphenylphosphinothioyl Group for the Protection of Amino Acids

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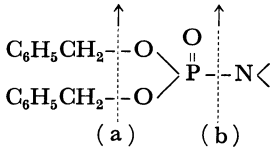
The preparation and purification of diphenylphosphinothioyl(Ppt)-amino acids were studied. The side-chain functional groups of tyrosine and cysteine were found to react also with diphenylphosphinothioyl chloride to form *O*-Ppt and *S*-Ppt derivatives. Acidic reagents for the removal of *N*-Ppt group were examined by using *N*,*O*-bis-Ppt-tyrosine ethyl ester (III) as the most difficult model, HCl in HCO_2H–CH_2Cl_2 being found to be most preferable. The *O*- and *S*-Ppt bonds were stable during the course of acidic treatments, but could be removed by alkaline hydrolysis. An application of Ppt-amino acids to the solid phase peptide synthesis is given.

Selection of the α-amino protecting group is important in working out peptide synthesis because an *N*-terminal elongation strategy is generally favored. Urethane groups such as benzyloxycarbonyl(Z)[1] and *t*-butoxycarbonyl(Boc)[2] have been used since they are superior in selectivity for the removal and prevention of racemization. However, complete prevention of side-reactions by carbonium ions formed during the course of deprotection seems difficult. We have investigated a new series of amino protecting groups not producing such an active species by cleavage.

The phosphazo method[3] is well-known as regards its utilization of the reactivity of trivalent phosphorus-nitrogen compounds with carboxylic acids. The acid lability of P–N bonds decreases in the oxidated state of phosphorus. In fact, the bis(benzyloxy)phosphinylidyne $(C_6H_5CH_2O)_2P(O)$– group has been used as an amino protecting group in the syntheses of amino sugars[4] and peptides.[5] The group can be removed, as in the case of the Z group, by catalytic hydrogenation or hydrogen bromide in acetic acid. In the latter case of deprotection, it is not clear which bond, C–O (a) or P–N (b), is cleaved initially. Recently



we have used monofunctional phosphinyl, RR'P(O)–, and phosphinothioyl, RR'P(S)–, groups as amino protecting groups and found that they can be removed not only by HBr in acetic acid but also by HCl reagents usually used for the removal of the Boc groups.[6,7] Among various kinds of phosphinyl and phosphinothioyl groups examined the diphenylphosphinothioyl (Ppt) group was thought to be the most useful since diphenylphosphinothioyl chloride can be easily prepared both in laboratories and on an industrial scale starting from benzene and thiophosphoryl chloride by the Friedel-Crafts reaction.[8] This paper gives further examples of the use of the Ppt group for the protection of amino acids.

$$2C_6H_6(\text{benzene}) + S{=}PCl_3 \xrightarrow{AlCl_3} (C_6H_5)_2P(S)\text{-}Cl \quad (\text{Ppt-Cl})$$

$$\text{Ppt-Cl} + H_2N\text{-CHR-}CO_2R' \xrightarrow{N(C_2H_5)_3} \text{Ppt-NH-CHR-}CO_2R'$$

$$\downarrow \text{1) } OH^- \text{ 2) } H^+$$

$$\text{Ppt-Cl} + H_2N\text{-CHR-}CO_2^- \xrightarrow[\text{2) } H^+]{\text{1) } OH^-} \text{Ppt-NH-CHR-}CO_2H$$

Ppt-amino acids were prepared by alkaline hydrolysis of Ppt-amino acid esters obtained from Ppt–Cl and amino acid esters.[6] Difficulties in the alkaline hydrolysis of *N*-diisopropoxyphosphinylidyne- and *N*-diphenoxyphosphinylidyneamino acid esters were reported.[9,10] However, no such steric hindrance was encountered in the case of Ppt derivatives. Importance of the Ppt group exists in the direct synthesis of Ppt-amino acids from amino acid betaines.

Phosphinothioyl chlorides are surprisingly resistant to hydrolysis as compared with phosphinyl chlorides.[11] The rate of hydrolysis was accelerated by the addition of a suitable solvent such as dioxane (Fig. 1). Addition of dioxane up to the same volume of water is admissible.

The synthesis of Ppt-amino acids was studied by Schotten-Baumann type reactions in water or aqueous dioxane. An amino acid was dissolved in a 2 M (1 M=1 mol dm^{-3}) NaOH solution and Ppt–Cl was added in one portion. The mixture was stirred vigorously to initiate a rapid reaction within several minutes. Then, 2 M NaOH solution was added, the pH of the solution being kept in the desired range. An 8.5—9.5 pH range was recommended,[6] but yields from

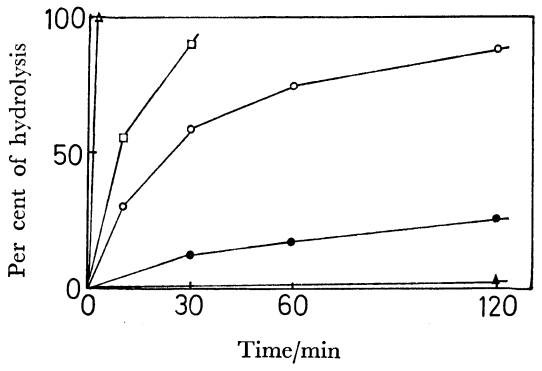


Fig. 1. Rate of hydrolysis of Ppt-Cl in dioxane–water (△: 2/1, □: 1/1, ○: 1/2, ●: 1/5) and in water (▲) at pH 9.5.

reactions in this range were later found to be sometimes poor. This could be attributed to the second reaction of the Ppt-amino acid salt with Ppt–Cl to give the Ppt-amino acid diphenylphosphinothioic anhydride,[12] since better yields were obtained by reactions at higher pH. This was confirmed by isolation of a small amount of dicyclohexylamine salt of Ppt–Gly–Gly–OH, Mp 187—192 °C, Found: C, 63.52; H, 7.46; N, 7.99; P, 5.45%. Calcd for $C_{28}H_{40}N_3O_3PS$: C, 63.53; H, 7.56; N, 7.93; P, 5.85%, from the mother liquor of recrystallization of Ppt–Gly–OH.

Most of the Ppt-amino acids were obtained as free acids which could be easily purified by recrystallization. Complete removal of contaminating diphenylphosphinothioic acid(Ppt–OH) was difficult in the case of oily substances. However, the presence of a small amount of Ppt–OH does not seem to hinder peptide synthesis by the dicyclohexylcarbodiimide (DCC) method since Ppt–OH reacts very rapidly with DCC to produce a stable 1 : 1 adduct.[13] Pure samples of Ppt-amino acids were obtained by utilizing the difference of acid strength of Ppt-amino acids and Ppt–OH. Ppt-amino acids, which are weaker as an acid and more lipophilic than Ppt–OH, can be extracted preferentially by organic solvents from slightly acidic aqueous solution. A considerable amount of dicyclohexylamine(DCHA) salt of Ppt–OH remained without being made free after the usual treatment to regenerate a free Ppt-amino acid from its DCHA salt by shaking with ethyl acetate and 5% aqueous citric acid solution. Ppt-glutamine could be purified by recrystallization from ethyl acetate or by column chromatography on silica gel. When a solution of crude Ppt–Gln–OH in ether saturated with water was passed through a silica gel column, Ppt–Gln–OH was absorbed completely, only Ppt–OH being eluted out. Pure Ppt–Gln–OH was then obtained by eluting with methanol.

In the case of γ-benzyl glutamate and ε-Z-lysine only hydrolysis of Ppt–Cl occurred under the conditions described above. Various conditions were tested; Ppt–Glu(OBzl)–OH can be prepared by using 50% aqueous dioxane as a solvent and triethylamine as a base and isolated in 32% yield as the *t*-butylamine salt.

The reactions of Ppt–Cl with side-chain functional groups were investigated. Alcoholic hydroxyl groups of serine and threonine were not affected by Ppt–Cl since Ppt–Ser–OH and Ppt–Thr–OH were obtained as DCHA salts in 66 and 47% yields, respectively, by the Schotten-Baumann type reactions. Ppt–Ser–OH can be benzylated with benzyl bromide and sodium hydride[14] to give Ppt-*O*-benzyl-serine in 61% yield as the DCHA salt. When tyrosine was treated with 2 molar equiv. of Ppt–Cl under the conditions described above, a mixture of two phosphinothioylated tyrosines was obtained. The mixture was separated by silica gel column chromatography to give *N*-Ppt-tyrosine (I), which was isolated as the DCHA salt, and *N,O*-bis-Ppt-tyrosine (II), Mp 117—124 °C, in 6 and 30% yields, respectively. Ethyl tyrosinate also reacted with Ppt–Cl in the presence of triethylamine to give *N,O*-bis-Ppt-tyrosine ethyl ester (III) contaminated with *N*-Ppt-tyrosine ethyl ester. When III was hydrolyzed by 1 M NaOH at room temperature for 48 h, both carboxylate ester and phosphinothioate ester bonds were cleaved to afford I in 80% yield. The *O*-Ppt group could also be cleaved slowly by hydrazinolysis.

$$\text{H-Tyr-OH} \xrightarrow[\text{OH}^-]{\text{2 Ppt-Cl}} \underset{\text{(I)}}{\text{Ppt-Tyr-OH}} + \underset{\text{(II)}}{\text{Ppt-Tyr(Ppt)-OH}} \quad (\xrightarrow{\text{OH}^-})$$

$$\text{H-Tyr-OEt} \xrightarrow[\text{N}(C_2H_5)_3]{\text{2 Ppt-Cl}} \underset{\text{(III)}}{\text{Ppt-Tyr-OEt}} + \text{Ppt-Tyr(Ppt)-OEt}$$

The *N*-Ppt group of compound III was found to be unexpectedly slow in cleavage by acidic reagents. Hydrogen bromide in acetic acid could remove the *N*-Ppt group within 5 min, but it took 4 days for complete removal by trifluoroacetic acid–dichloromethane (1/1). Hydrogen chloride in dioxane or acetic acid and triphenylphosphine dihydrochloride solution in dichloromethane[15] were also so impotent that it took more than 2—3 h for complete deprotection. The most preferable reagent for removal of the *N*-Ppt group was HCl in formic acid. When compound III was dissolved in dichloromethane and the same volume of 0.6 M HCl in formic acid was added, complete deprotection took place within 20 min to give *O*-Ppt-tyrosine ethyl ester hydrochloride (IV). These conditions are generally applicable to the removal of *N*-Ppt groups. The *O*-Ppt group was quite stable during the course of acidic treatment since no Pauly positive spot[16] was detected by TLC. The results suggest the possible use of the *O*-Ppt group for the protection of the OH function of tyrosine. This was exemplified in the following synthesis. IV was coupled in the presence of triethylamine with Z–Ser(Bu^t)–OH by DCC to give Z–Ser(Bu^t)–Tyr(Ppt)–OEt(V) in 91% yield. Alternative cleavage of the two *O*-protecting groups was accomplished by alkaline hydrolysis for the *O*-Ppt group and by treatment with trifluoroacetic acid for the *O*-Bu^t group.

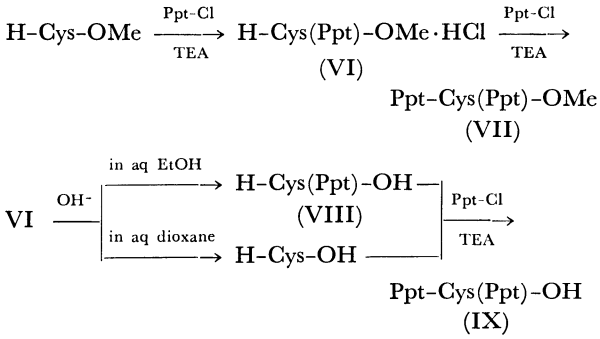
$$\text{III} \xrightarrow{\text{0.3M HCl/HCO}_2\text{H-CH}_2\text{Cl}_2\text{(1/1)}} \underset{\text{(IV)}}{\text{H-Tyr(Ppt)-OEt}\cdot\text{HCl}}$$

$$\text{Z-Ser(Bu}^t\text{)-OH} + \text{IV} \xrightarrow{\text{DCC}} \underset{\text{(V)}}{\text{Z-Ser(Bu}^t\text{)-Tyr(Ppt)-OEt}}$$

$$\text{V} \begin{cases} \xrightarrow{\text{OH}^-} \text{Z-Ser(Bu}^t\text{)-Tyr-OH} \\ \xrightarrow{\text{F}_3\text{CCO}_2\text{H}} \text{Z-Ser-Tyr(Ppt)-OEt} \end{cases}$$

The SH function of cysteine was more reactive than the amino function with Ppt–Cl. When cysteine methyl ester was treated with equimolar amounts of Ppt–Cl in the presence of triethylamine, *S*-Ppt-cysteine methyl ester hydrochloride (VI) was obtained as the main product. When 2 molar equiv. of the reagent was used, the α-amino group of VI was also phosphinothioylated to give *N,S*-bis-Ppt-cysteine methyl ester (VII) in 79% yield. When VI was hydrolyzed by 1 M NaOH in ethanol, selective removal of the methyl ester occurred to give *S*-Ppt-cysteine (VIII) in 61% yield. Stability of the *S*-Ppt group changed

with solvent. When aqueous dioxane was used as a solvent the phosphinothioate ester bond also underwent hydrolysis. However, *N*,*S*-bis-Ppt-cysteine (IX) could be obtained in 12% yield when VIII or cysteine itself was reacted for a short time with Ppt–Cl in aqueous dioxane in the presence of triethylamine. Since the *S*-Ppt group was also stable under the acidic conditions, IX would be useful for the synthesis of cysteine containing peptides.



TEA: triethylamine

Arginine reacted very rapidly with Ppt–Cl, but no useful Ppt derivative was obtained probably because of the lactam formation.[17] Since Ppt–Cl is unreactive toward the indole moiety of tryptophan, tryptophan reacted with Ppt–Cl to give Ppt-tryptophan as a sole product. Pure acid was obtained as crystals containing 1 mol of ethanol by recrystallization from ethanol.

The physical properties of Ppt-amino acids prepared in the present experiments are summarized in Table 1.

Coupling of the Ppt-amino acids was successful[6] with use of DCC, mixed anhydride and the oxidation-reduction condensation[18] methods. No steric hindrance was observed in the coupling of the Ppt-amino acids contrary to the case of *N*-trityl-amino acids.[19]

Ppt-amino acids can be used for the solid phase synthesis of peptides.[20] Ppt-glycine was esterified *via* caesium salt[21] to a chloromethylated resin. Deprotection was performed by treatment with 1 M HCl in HCO_2H–CH_2Cl_2(2/1) for 30 min, carried out twice. Couplings were mediated with oxidation-reduction condensation. After incorporation of Ppt–Leu followed by Ppt–Pro, the protected tripeptide

Table 1. Diphenylphosphinothioylamino acids and their salts

Ppt deriv. of	Yield, %[a] (Method)[b]	Mp, (°C)	$[\alpha]_D$[c] (deg.)	TLC R_f[d] A	TLC R_f[d] B	Found (Calcd), % C	H	N	P
Gly	66 (*A*)	118—119		0.53	0.87	57.65 (57.75)	4.53 (4.81)	4.86 (4.81)	10.44 (10.64)
L-Ala	58 (*A*)	120	−13.7	0.55	0.87	58.86 (59.03)	5.31 (5.24)	4.69 (4.57)	10.25 (10.14)
L-Ala·DCHA	e)	177—178	−3.7			67.78 (67.75)	8.06 (8.09)	5.87 (5.75)	6.36 (6.36)
L-Val	62 (*A*)	112—114	−17.5	0.53	0.93	61.12 (61.27)	6.11 (6.00)	4.37 (4.20)	9.33 (9.29)
L-Val·DCHA	e)	149—151	−10.0			67.63 (67.72)	8.33 (8.36)	5.39 (5.44)	5.60 (6.02)
L-Leu	40 (*C*)	60— 70[g]	−17.5	0.52	0.91	59.12 (59.19)[k]	6.41 (6.57)[k]	3.72 (3.83)[k]	8.66 (8.48)[k]
L-Leu·DCHA	e)	137—138	−15.0			67.92 (68.14)	8.78 (8.59)	5.15 (5.29)	6.19 (5.85)
L-Ile·CHA	52 (*E*)	181—183	−7.5	0.54	0.90	64.39 (64.54)	7.89 (7.91)	6.14 (6.27)	6.89 (6.93)
L-Phe·DCHA	75 (*D*)	190—191	+8.7	0.54	0.89	70.72 (70.42)	7.71 (7.71)	5.09 (4.97)	5.30 (5.50)
L-Pro	62 (*A*)	128—130	−15.0	0.52	0.90	61.47 (61.64)	5.45 (5.43)	4.14 (4.22)	9.03 (9.35)
L-Pro·DCHA	e)	194—195	−40.0			67.72 (67.93)	7.87 (8.08)	5.50 (5.46)	5.79 (6.04)
L-Met·DCHA	30 (*D*)	145—146	−1.2	0.52	0.88	63.88 (63.74)	7.84 (7.86)	5.14 (5.12)	5.83 (5.66)
L-Cys(Bzl)·DCHA	30 (*D*)	170—171	+22.5[h]	0.52	0.92	66.96 (67.06)	7.48 (7.46)	4.65 (4.60)	5.00 (5.09)
L-Cys(Ppt)·CHA	12 (*F*)	141—144	+7.5	0.53	0.94	60.36 (60.74)	5.71 (5.82)	4.22 (4.29)	9.18 (9.49)
L-Asp(OBzl)·DCHA	50 (*D*)	156—157	−13.7	0.47	0.96	67.57 (67.75)	7.47 (7.25)	4.51 (4.51)	5.15 (4.99)
L-Glu(OBzl)·TBA	32 (*F*)	94— 98	+10.0	0.53	0.88	63.85 (63.89)	6.93 (6.65)	5.38 (5.32)	5.85 (5.88)
L-Asn	56 (*B*)	163—164 (dec)	−5.0[h]	0.31	0.77	55.19 (55.16)	4.86 (4.93)	7.93 (8.04)	8.79 (8.89)
L-Gln	50 (*B*)	77— 79	+22.5	0.31	0.77	56.04 (56.36)	5.29 (5.24)	7.76 (7.73)	8.38 (8.55)

Table 1. (Continued)

Ppt deriv. of	Yield, %[a] (Method)[b]	Mp, (°C)	$[\alpha]_D$[c] (deg.)	TLC R_f[d] A	TLC R_f[d] B	Found (Calcd), % C	H	N	P
L-Gln·DCHA	e)	172—174	+8.7			63.37 (63.05)[l]	7.70 (7.60)[l]	7.74 (7.60)[l]	5.76 (5.60)[l]
L-Ser·DCHA	66 (*D*)	157—158	−5.0	0.29	0.83	64.37 (64.50)	7.80 (7.83)	5.42 (5.57)	6.17 (6.16)
L-Ser(Bzl)·DCHA	39[f]	138—139	+5.0	0.52	0.97	68.74 (68.93)	7.62 (7.59)	4.57 (4.77)	5.25 (5.22)
L-Thr·DCHA	47 (*D*)	147—149	−10.0	0.31	0.84	64.91 (65.08)	8.10 (8.01)	5.39 (5.42)	5.78 (5.99)
L-Tyr·DCHA	6 (*B*)	192—193	−1.2[h]	0.31	0.84	66.18 (66.46)[m]	7.62 (7.54)[m]	4.50 (4.69)[m]	5.12 (5.25)[m]
L-Tyr·(Ppt)	33 (*B*)	117—124	−17.5	0.57	0.93	63.56 (63.68)[n]	4.85 (4.66)[n]	2.01 (2.25)[n]	9.41 (9.95)[n]
L-Tyr(Bzl)·DCHA	43 (*D*)	182—184	+15.0[h]	0.57	0.94	71.89 (71.87)	7.36 (7.33)	4.14 (4.19)	4.62 (4.63)
L-Trp	e)	76— 82	−8.7[i]	0.54	0.92	64.58 (64.39)[o]	5.71 (5.79)[o]	6.05 (6.00)[o]	6.71 (6.64)[o]
L-Trp·DCHA	84 (*E*)	190—191	+7.5			69.74 (69.87)	7.43 (7.31)	6.87 (6.98)	5.33 (5.14)
L-Arg(Tos)	30 (*A*)	199—201	+4.9[j]	0.20	0.84	54.06 (54.26)[p]	5.35 (5.42)[p]	10.29 (10.12)[p]	5.63 (5.60)[p]
L-Arg(NO_2)	61 (*C*)	129—132	+2.5[h]	0.17	0.86	48.23 (48.34)[p]	5.42 (5.22)[q]	15.50 (15.65)[q]	6.86 (6.93)[q]
L-Arg(NO_2)·DCHA	e)	159—161	+5.0[h]			58.08 (58.45)	7.13 (7.30)	13.37 (13.62)	4.94 (5.02)

a) Purified yields. b) Described in Experimental. c) *c* 1 in EtOH unless otherwise stated. d) Solvent systems: A; chloroform: methanol : acetic acid = 95 : 5 : 3. B; 1-butanol : acetic acid : water = 4 : 1 : 1. e) Pure sample prepared for analytical purpose. f) Yield for the *O*-benzylation. g) Dehydration temperature. h) *c* 1 in MeOH. i) The value we reported previously[23] is corrected. j) *c* 1 in *N*,*N*-dimethylformamide. k) Calcd for $C_{18}H_{22}NO_2PS \cdot H_2O$. l) Calcd for $C_{29}H_{42}N_3O_3PS \cdot 1/2H_2O$. m) Calcd for $C_{33}H_{43}N_2O_3PS \cdot H_2O$. n) Calcd for $C_{33}H_{29}NO_3P_2S_2 \cdot 1/2H_2O$. o) Calcd for $C_{23}H_{21}N_2O_2PS \cdot C_2H_5OH$. p) Calcd for $C_{25}H_{29}N_4O_4PS_2 \cdot 1/2H_2O$. g) Calcd for $C_{18}H_{22}N_5O_4PS \cdot 1/2H_2O$.

was removed from the resin by transesterification[22] to give Ppt–Pro–Leu–Gly–OMe in 95% yield. No undesirable product, Ppt–Leu–Gly–OMe, was detected by TLC.

Experimental

Diphenylphosphinothioyl chloride (Wako Pure Chemical Ind. Ltd.) was distilled before use.

N-Diphenylphosphinothioylamino acids were prepared by the reaction of diphenylphosphinothioyl chloride and α-amino acids (or α-amino acids bearing a protected side-chain functional group) in aqueous alkaline solution or in aqueous dioxane containing triethylamine. The diphenylphosphinothioylamino acids thus prepared were isolated either as free acids (procedures *A*, *B*, and *C*) or in the form of dicyclohexylamine(DCHA), cyclohexylamine(CHA) or *t*-butylamine(TBA) salt (procedures *D*, *E*, and *F*). They are listed in Table 1.

A. The amino acid (0.85 mol) was dissolved in 400 ml of 2 M NaOH solution. Diphenylphosphinothioyl chloride (214 g, 0.85 mol) was added in one portion and the mixture was stirred vigorously to initiate a rapid reaction within several minutes. 2 M NaOH solution was added at a rate to keep the pH of the solution in the range 9.5—10.0. After the pH change had ended the reaction mixture was diluted with 1 l of water and the remaining chloride was removed by extraction with ethyl acetate. The aqueous layer was acidified to pH 6 with solid citric acid, saturated with NaCl and extracted 3 times with ethyl acetate. Combined ethyl acetate extracts were washed successively with saturated $NaHCO_3$ solution, 5% citric acid solution and saturated NaCl solution, dried over anhydrous Na_2SO_4 and evaporated to give a pasty mass. This was crystallized by trituration with petroleum ether. The crude products were recrystallized from ethyl acetate, ethyl acetate–hexane or benzene–hexane.

B. The amino acid was treated with diphenylphosphinothioyl chloride as described in procedure *A*. The solution was acidified with 5% citric acid solution, ether being added. The resulting precipitate was collected by filtration and washed thoroughly with water and then with ether and dried. In the case of tyrosine, a mixture of *N*,*O*-bis-Ppt-L-tyrosine (II) and *N*-Ppt-L-tyrosine (I) was obtained. The mixture was dissolved in dichloromethane and applied to a silica gel column. II was obtained by elution with the same solvent. Following elution with ether gave I.

C. After synthesis and extraction as described in procedure *A*, dicyclohexylamine was added to the ethyl acetate extracts, the corresponding salt separating out. Sometimes, addition of the same volume of ether was necessary. The product was filtered off and washed with ethyl acetate or ether. The crude salt was shaken vigorously in a separatory funnel with a mixture of ethyl acetate and 5% citric acid solution. After removal of insoluble substance, mainly DCHA salt of diphenylphosphinothioic acid, by filtration, the aqueous layer was separated and extracted twice with ethyl acetate. The combined ethyl acetate extracts were washed with 5% citric acid solution and satu-

rated NaCl solution, dried and evaporated to dryness. Ppt-L-leucine was crystallized as a monohydrate by addition of water and recrystallized from methanol–water.

D. The Ppt-amino acid obtained as in procedure *C* was converted again into the corresponding DCHA salt.

E. After synthesis and extraction as in procedure *A*, cyclohexylamine or dicyclohexylamine was added to the ethyl acetate extracts, the corresponding salt separating out. The product was filtered off, washed with ethyl acetate and recrystallized from methanol or ethanol.

F. The amino acid (0.30 mol) was dissolved in a mixture of 425 ml of water and 425 ml of dioxane by addition of 56 ml (0.4 mol) of triethylamine with vigorous stirring under ice cooling. 90.7 g(0.36 mol) of diphenylphosphinothioyl chloride and 36.4 ml (0.26 mol) of triethylamine in 150 ml of 50% aqueous dioxane were added separately in one portion. Within 5 min a clear solution resulted and was extracted twice with ether. The ether extracts were back extracted with 5% $NaHCO_3$ solution. The combined aqueous solution was acidified to pH 4—5 with 5% citric acid solution and extracted 3 times with ethyl acetate. The ethyl acetate extracts were washed successively with 5% $NaHCO_3$ solution, two portions of 30 ml of 1% $NaHCO_3$ solution, three portions of 150 ml of 5% citric acid solution, two portions of 300 ml of water and saturated NaCl solution. The ethyl acetate layer was dried and concentrated to dryness. The oily residue was dissolved in dichloromethane, applied to a silica gel column (3×40 cm) and eluted with the same solvent (about 1.5 l). After evaporation of the eluate the product was dissolved in ether, the amine shown in Table 1 being added to give the corresponding salt.

Ppt-L-Tyr(Ppt)-OEt (III) and Ppt-L-Tyr-OEt. Ppt-Cl(5.05 g, 20 mmol) was added to a suspension of L-Tyr-OEt·HCl(2.48 g, 10 mmol) in 20 ml of chloroform and 4.2 ml (30 mmol) of triethylamine. After being stirred at room temperature for a day the solution was successively washed with water, 5% citric acid, water, 5% $NaHCO_3$, and water. The chloroform layer was dried and concentrated to dryness. The oily residue was dissolved in benzene, applied to a silica gel column (1.5×30 cm) and eluted with 150 ml of benzene to give III as amorphous white powder; 3.93 g (61%). It was homogeneous chromatographically. $[\alpha]_D$ −10.0° (*c* 1, EtOH); Found: C, 65.81; H, 5.04; N, 2.28%. Calcd for $C_{35}H_{33}NO_3P_2S_2$: C, 65.54; H, 5.14; N, 2.18%. Further elution with CH_2Cl_2 gave Ppt-L-Tyr-OEt; 0.86 g (20%). Mp 93—98 °C; $[\alpha]_D$ −32.5° (*c* 1, EtOH); Found: C, 64.92; H, 5.75; N, 2.90%. Calcd for $C_{23}H_{24}NO_3PS$: C, 64.96; H, 5.64; N, 3.29%.

H-L-Tyr(Ppt)-OEt·HCl (IV). III(0.64 g, 1 mmol) was dissolved in 10 ml of 0.3 M HCl/HCO_2H–CH_2Cl_2(2/1). After being left to stand at room temperature for 30 min the solution was evaporated *in vacuo*, the residue being distributed between water and ether. The layers were separated and the ether layer was extracted with 1 M hydrochloric acid (×5). The combined aqueous extracts were concentrated and dried under reduced pressure over NaOH pellets. The product was triturated with ether to give IV as white crystals; 0.42 g (93%). Mp 137—141 °C; $[\alpha]_D$ +22.5° (*c* 1, EtOH); Found: C, 58.37; H, 5.48; N, 3.07%. Calcd for $C_{23}H_{25}NO_3PSCl\cdot 1/2H_2O$: C, 58.68; H, 5.25; N, 2.97%.

Z-L-Ser(Bu^t)-L-Tyr(Ppt)-OEt (V). IV(0.23 g, 0.5 mmol), Z-L-Ser(Bu^t)-OH(0.15 g, 0.5 mmol) and triethylamine(0.07 ml, 0.5 mmol) were dissolved in 1 ml of chloroform. Dicyclohexylcarbodiimide (0.11 g, 0.5 mmol) was added under stirring at 0 °C. Stirring was continued at 0 °C for 30 min and at room temperature for 12 h. After the usual work-up the product was separated and purified by silica gel preparative layer chromatography to give an oil; 0.33 g (91%). $[\alpha]_D$ +30.0° (*c* 1, EtOH); Found: C, 64.91; H, 6.31; N, 3.86%. Calcd for $C_{38}H_{43}N_2O_7PS$: C, 64.97; H, 6.12; N, 3.99%.

Z-L-Ser-L-Tyr(Ppt)-OEt. V(0.18 g, 0.25 mmol) was treated with anhydrous trifluoroacetic acid(0.25 ml) at 0 °C for 20 min and at room temperature for 2 h. After the removal of trifluoroacetic acid by evaporation *in vacuo* the product was separated and purified by silica gel preparative layer chromatography to give an oil; 0.15 g (91%). $[\alpha]_D$ −5.0° (*c* 1, EtOH); Found: C, 62.71; H, 5.46; N, 4.13%. Calcd for $C_{34}H_{35}N_2O_7PS$: C, 63.17; H, 5.41; N, 4.33%.

Z-L-Ser(Bu^t)-L-Tyr-OH. A solution of 0.14 g (0.2 mmol) of V in 3 ml of ethanol and 0.66 ml of 1 M sodium hydroxide was stirred at room temperature for 3 h. After removal of ethanol *in vacuo* the aqueous solution was extracted with ethyl acetate and acidified to pH 4 with 5% citric acid. The acidified solution was extracted with ethyl acetate. The ethyl acetate extracts were washed with water, dried and concentrated. The oily residue was dissolved in methanol and purified by gel filtration on Sephadex LH-20 (1.7×17 cm) to give a chromatographically homogeneous oil; 0.073 g (80%). $[\alpha]_D$ +40.0° (*c* 1, EtOH); Found: C, 62.04; H, 6.74; N, 6.16%. Calcd for $C_{24}H_{30}N_2O_7\cdot 1/2H_2O$: C, 61.69; H, 6.63; N, 5.99%.

Solid Phase Synthesis of Ppt-L-Pro-L-Leu-Gly-OMe. Chloromethyl resin, prepared by chloromethylating the Bio-Beads S-X1 (Bio-Rad Laboratories), was esterified with Ppt-glycine by means of the caesium salt method(Gly content 0.43 mmol/g). One gram of the ester resin was placed in the reaction vessel of the Beckman model 990 peptide synthesizer. The machine was programmed to perform the following steps automatically: (1) washing three times with CH_2Cl_2, (2) pre-washing with 1 M HCl in HCO_2H–CH_2Cl_2 (2/1), (3) deprotection twice with 1 M HCl in HCO_2H–CH_2Cl_2 (2/1) for 30 min, (4) washing three times each with CH_2Cl_2, EtOH, and again CH_2Cl_2, (5) neutralization three times with 10% triethylamine in CH_2Cl_2 for 5 min, (6) washing three times with CH_2Cl_2, (7) successive addition of 1.29 mmol each of Ppt-amino acid, tris(*p*-methoxyphenyl)-phosphine and 2,2′-dithiodipyridine in CH_2Cl_2, (8) coupling for 60 min, (9) washing three times with CH_2Cl_2, (10) repetition of steps 7, 8, and 9, (11) washing twice each with *N*,*N*-dimethylformamide and EtOH. After the completion of coupling the protected peptide was removed from the resin by transesterification with use of 1 M triethylamine in MeOH for 12 h four times. The combined filtrates and washings were evaporated *in vacuo* and the residue was dissolved in ethyl acetate. The solution was washed with water, dried and concentrated to give a pasty mass. This was crystallized slowly by triturating with ether; 0.21 g (95%). Mp 143—144 °C; $[\alpha]_D$ −70.0° (*c* 0.5, EtOH); Found: C, 60.38; H, 6.61; N, 7.89; P, 5.98%. Calcd for $C_{23}H_{34}N_3O_4PS$: C, 60.59; H, 6.59; N, 8.15; P, 6.01%.

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