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Amino Acid Pyridoxyl Esters in Peptide Synthesis

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Abstract—Pyridoxyl residue was suggested to be used as a multifunctional protective and modifying group in peptide synthesis. The modification was carried out by introducing the pyridoxyl residue in free or partially protected peptides or by the addition of amino acid pyridoxyl esters by the methods of conventional peptide synthesis without the removal of the pyridoxyl group at the terminal stages of the synthesis (the second approach is more convenient). Pyridoxyl residue was also used as a spacer in solid phase peptide synthesis. It was attached to the polymer by the alkylation of the hydroxyl groups or of the pyridine ring of the pyridoxyl derivatives with the chloromethylated styrene-divinylbenzene copolymer (the standard Merrifield resin). Potentials for the use of pyridoxyl derivatives in the synthesis of linear, multiplet, and cyclic peptides are discussed.

Key words: amino acid 5'-pyridoxyl esters, new carboxylic protective groups, ketals, peptide synthesis

INTRODUCTION

The creation of a peptide bond both by the conventional synthesis in solution and by solid phase synthesis on polymeric matrices is often hindered because of the low solubility (solvation) of the reaction components.[2] Unfortunately, during the synthesis of peptides, their solubility changes (and often unpredictably), forcing researchers to look for new protective [1] and activating groups that could facilitate the solution of this problem [2]. The success of the synthesis often depends on the physicochemical properties of the protected peptides [3, 4]. One of the approaches to solving the problem of solubility is the use of amino acid derivatives with multifunctional protective groups, as their properties can vary depending on the presence of one or another additional reactive radical in the permanent protective group [5]. Such additional groups can not only affect the solubility of the peptides to be synthesized but also have a number of other useful functions, e.g., to be markers, to bear a charge, or to dictate the biological properties of target compounds [6]. The physiological acceptability and the presence of several reactive groups in the pyridoxine derivatives make them promising for the synthesis of biologically active analogues of peptides, nucleotides, peptidomimetics, and enzyme inhibitors, especially taking into account the fact that heterocyclic residues can be complexones and unique acceptors of hydrogen bonds [7]. These properties provide their contacts with a receptor or an enzyme molecule. The possibility to impart lipophilic properties to a pyridoxine derivative makes them promising for use as medicines [8]. Taking all this into account, we developed methods for obtaining some intermediates (ketal, acyl, and aminoacyl derivatives of pyridoxine) [9] and showed that it is fundamentally possible to use them in peptide synthesis (for the preliminary communication at the European Peptide Symposium, see [10]).

RESULTS AND DISCUSSION

This work is devoted to various aspects of the use of pyridoxyl derivatives and to the special features of carrying out various stages of the synthesis of peptides with a pyridoxyl group. We have shown that the stage of peptide bond formation with these derivatives occurs without difficulty due to the high solubility of pyridoxyl esters of both the starting peptides with a removed N^{α}-protective group and the totally protected peptides generated during the synthesis. The presence of additional protective groups at the pyridoxyl residue allowed us to use usual solvents (e.g., ethyl acetate and chloroform) at the coupling stage in the synthesis of scarcely soluble peptides. We most often used compounds containing ketal groups in the pyridoxyl residue, since the preparative methods for their synthesis are most developed [9]. Moreover, the isopropylidene protective groups can be selectively removed or substituted by other additional groups. Compounds containing cyclohexylidene or palmitoyl protective groups more resistant to acidic hydrolysis were also easily soluble in the aforementioned solvents. Pyridoxyl esters of free peptides devoid of additional protective groups at the pyridoxyl residue are easily soluble in polar solvents such as DMF, alcohols, and water.

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[2] Abbreviations: $(Boc)_2O$, di-*tert*-butyl oxycarbonyl pyrocarbonate; ClZ, 4-chlorobenzyloxycarbonyl; cPyr5', 4',3-*O*-cyclohexylidene-5'-pyridoxyl; Fmoc, fluorenylmethyloxycarbonyl; For, formyl; iPyr5', 4',3-*O*-isopropylidene-5'-pyridoxyl; $(OPlm)_2Pyr5'$, 4',3-*O*-dipalmitoyl-5'pyridoxyl; and Pyr, pyridoxyl.

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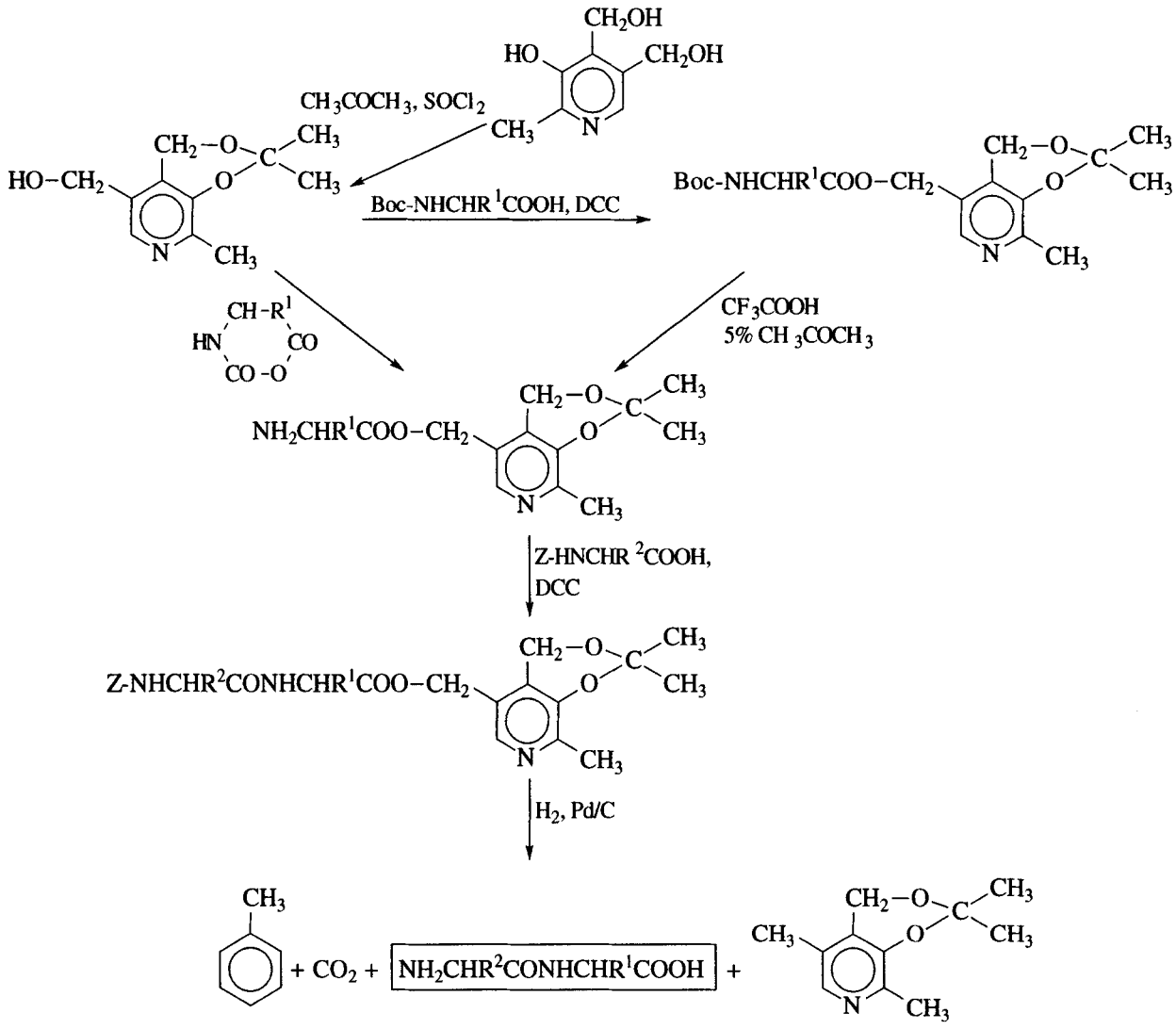
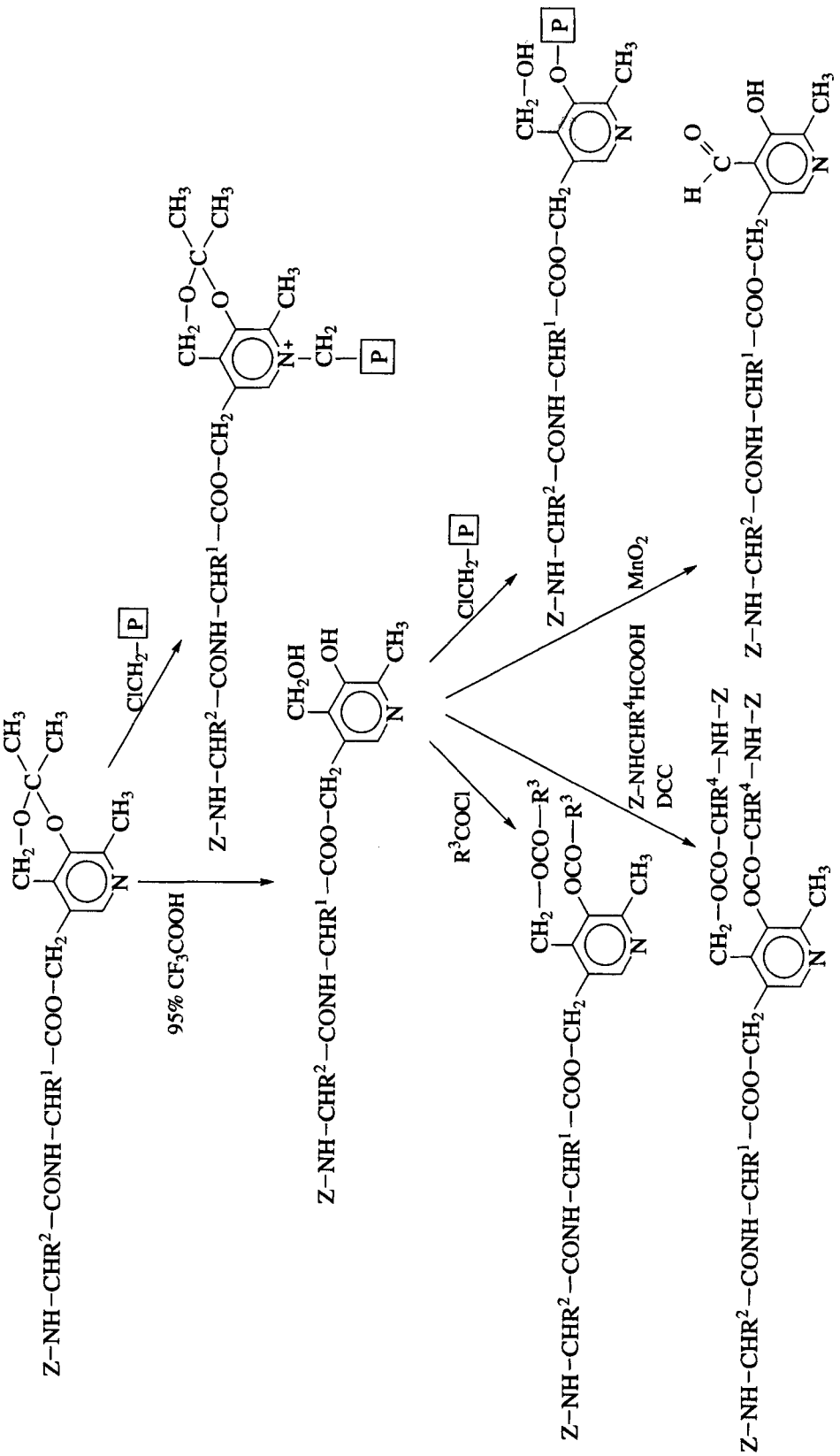


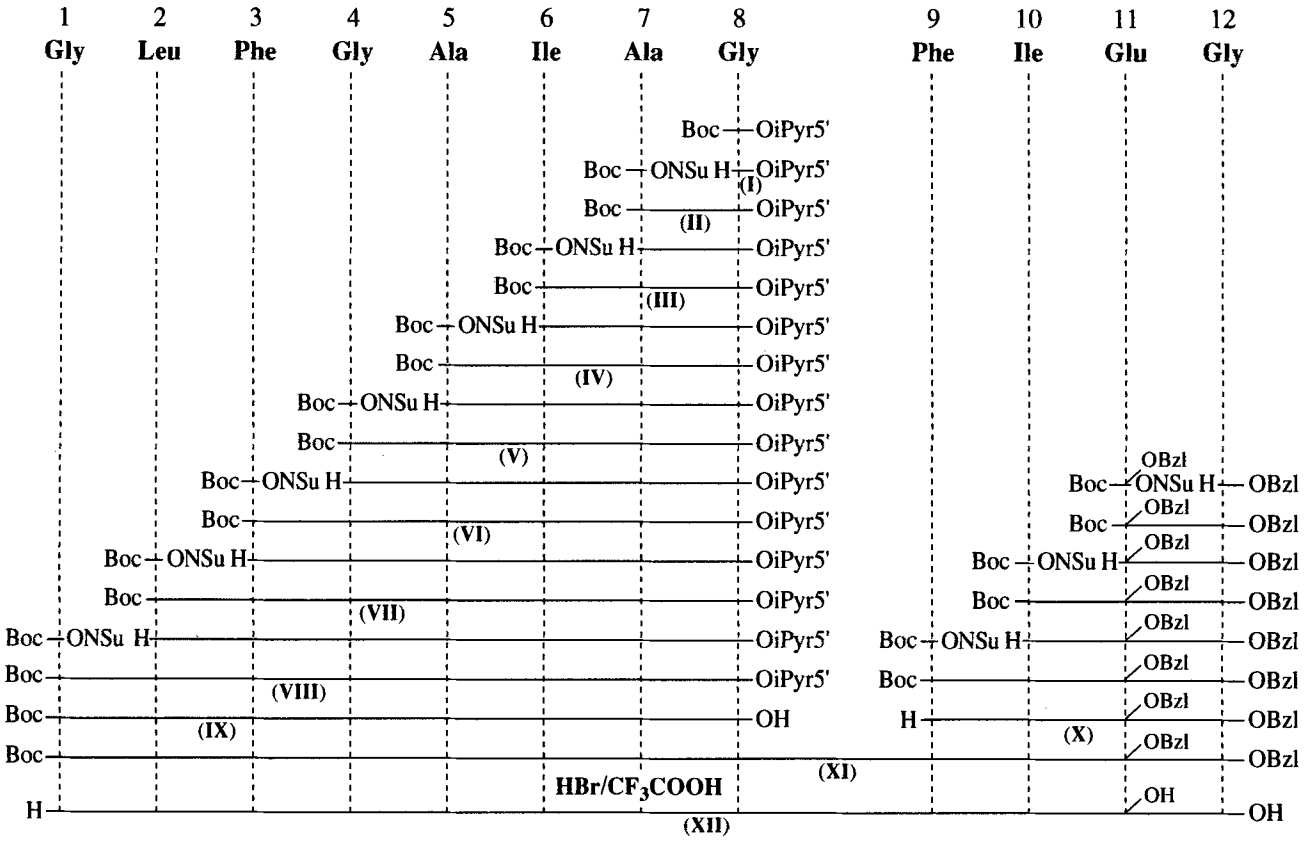
Scheme 1. The synthesis of amino acid -5'-pyridoxyl esters and their use in peptide synthesis.

The stability of the pyridoxyl esters under the conditions for creating peptide bonds, removing the temporary protective groups, attaching to the polymeric matrices, and chemical conversions of the pyridoxyl group itself dictate the choice of temporary N^{α}-protective groups (Schemes 1 and 2). The high stability of ketal groups at the pyridoxyl residue in alkaline medium [11, 12] makes the Fmoc protective group most acceptable [10, 13]. On the other hand, the high lability of the ketals in acidic media (especially isopropylidene groups) [12], requires special conditions during the use of the most widespread acid-labile N^{α}-protective groups. Thus, it is desirable to obtain dihydrochlorides or ditrifluoroacetates of pyridoxyl esters for the peptide synthesis immediately before the coupling stage, as these salts are hygroscopic during storage in the open air and can suffer from a partial splitting off of the isopropylidene group (splitting of the ketal groups is not observed in the desiccator). Moreover, the amino acid pyridoxyl esters contain additional pyridine nitrogen, which can be an acceptor of some acidic reagents (e.g., pentafluorophenol or *N*-hydroxysuccinimide) generated at the peptide formation stage during acylation by active esters. Therefore, it is desirable to add two equivalents of a tertiary amine at the coupling stage.

The peptide synthesis can be easily monitored by chromatography due to the high UV absorption (ε = 6000 M^{-1} cm^{-1} at 297 nm) and fluorescence of the target products as in the case of the synthesis of amino acid pyridoxyl esters [9]. The resulting peptides can be detected on TLC plates by the Gibbs reagent and also by iron salts [14]. The Gibbs test is very sensitive and informative. This reagent was first suggested for the visualization of phenol compounds [15] that acquire an intensive blue color. We have also observed it to stain



Scheme 2. The use of pyridoxyl residue as a protective group, a spacer, and a modifier.



Scheme 3. The synthesis of the peptide from influenza hemagglutinin sequence.

compounds containing a free sulfhydryl group (cysteine), imidazole (histidine), and indole (tryptophan), but the resulting color is yellow. This same reagent helps determine the degree of substitution of the pyridoxyl residue, as staining is observed only after the removal of additional groups (e.g., ketal or acyl groups) in the reaction solution or right on the TLC plate.

The pyridoxyl esters of amino acids and peptides can form peptide bonds with N^{α}-amino groups (in the presence of free hydroxyl groups on the pyridoxyl residue) in the presence of mild agents for the activation of their carboxyl groups (e.g., their *N*-succinimide or *p*-nitrophenyl esters are useful). The method of active esters is also useful in the case of a ketal protective group at the pyridoxyl residue, as it allows one to avoid the formation of by-products as a result of the additional acylation of the pyridoxyl residue, when, e.g., moisture or an alcohol accidentally falls into the reaction mixture [9]. After the coupling reaction, the target product(s) can easily be isolated by conversion into salts (e.g., hydrochloride) or into a complex with picric acid. Washing or recrystallization of the resulting precipitate helps separate by-products. These salts are dissolved rather easily when removing the N^{α}-protective groups at the next stage. This is likely to be due to the decrease in the aggregation of peptide chains because of the greater solvation of the pyridoxyl derivatives. In the solid phase synthesis, this aggregation is a serious cause for the decrease in the acylation and deprotection rates (so-called difficult sequences) [1].

The last stage of the synthesis, the removal of pyridoxyl protective groups, presents no problem, as the products of pyridoxyl ester cleavage, 5-methylpyridoxine (at reduction), pyridoxyl ketals, and pyridoxine itself (at hydrolysis, ammonolysis, or hydrazinolysis) can be separated from the free peptide due to their high solubility in organic solvents and their volatility in a vacuum. The cleavage of the pyridoxyl group results in the formation of products (see Scheme 1) that can give peculiar colors and fluoresce, which facilitates the monitoring of this stage of synthesis.

To illustrate the use of pyridoxyl esters (**I**)–(**VIII**) in combination with the Boc-protective group (Scheme 3), we report here the synthesis of dodecapeptide (**XII**) from the sequence of influenza virus protein (for the preliminary communication, see [16]).

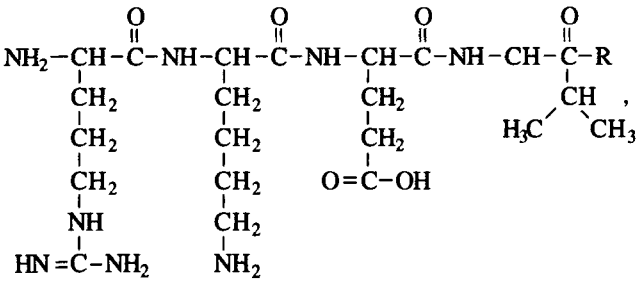
We have previously shown that the use of the benzyl ester of *C*-terminal glycine in the synthesis of peptide (**IX**) (fragment 1-8) results in the formation of intermediates insoluble even in hot DMF [17]. Therefore, Khaitov *et al.* [17] had to use *N*,*O*-bis(trimethylsilyl)acetamide to obtain temporary trimethylsilyl deriv-

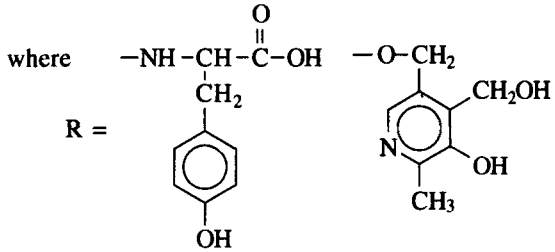
atives of the intermediate peptides in the synthesis of this fragment. However, the yield of the target product was low even in this case. We have no problems in the synthesis of this poorly soluble fragment when starting from Boc-glycine pyridoxyl ester and performing all coupling reactions by the method of *N*-succinimide active esters. The intermediate protected peptides (**II**)–(**VIII**) were dissolved in ethyl acetate or chloroform and the Boc-protective group was removed by treatment with trifluoroacetic acid containing 5 vol % of anhydrous acetone. Acetone was added immediately before the reaction to retain the isopropylidene group in the presence of traces of moisture, since isopropylidene derivatives are readily cleaved under conditions of acidic hydrolysis.

The hydrogenolysis of protected peptide (**VIII**) was monitored by TLC, the Gibbs reagent being more sensitive for detection of the unprotected compounds than ninhydrin. The time of hydrogenolysis of (**XI**) (6 h) was comparable with that necessary for the deprotection of benzyl ester (**XI**) (10 h) [17]. The hydrogenolysis was accompanied by the formation of 5-methylpyridoxine (2,5-dimethyl-3-hydroxy-4'-hydroxymethylpyridine) ketal (see Scheme 1), whose TLC-spots turned bright blue with the Gibbs reagent. The subsequent condensation by the carbodiimide method with benzyl ester (**X**) synthesized in a convergent scheme proceeded with no complications.

Obtaining hydrophobic peptide (**VIIIa**) (table) bearing palmitoyl residues on the pyridoxyl moiety also presented no difficulties. In this case, the synthesis was also started from the 4',3-isopropylidenepyridoxyl ester of Boc-glycine. The removal of the Boc-protective and the isopropylidene group from peptide (**III**) was carried out by treatment with 95% trifluoroacetic acid. The subsequent attachment of Boc-Ala was performed by the method of *N*-succinimide active esters in chloroform with subsequent palmitoylation of the resulting (**IVa**). Further elongation of the peptide chain was carried out similarly to that in the synthesis of octapeptide (**VIII**). However, the usual treatment with trifluoroacetic acid for the removal of the Boc-protective group was less successful because of the noticeable solubility of the peptide trifluoroacetates in diethyl ether, which was used to separate the by-products and the traces of trifluoroacetic acid. Therefore, the Boc-protective group in palmitoylated derivatives was removed with 20% HCl in dioxane. The moiety bearing the palmitoyl residues was not removed from peptide (**VIIIa**). The palmitoyl residue attached to the pyridoxyl moiety, which was used as a spacer group, made it possible to obtain the lipophilic form of peptide (**VIII**) with a higher immunogenicity, which can be compared with that of some high-molecular conjugates difficult for preparation [16].

The pyridoxyl residue was also not removed during the synthesis of a biologically active analogue of splenopentine [table, (**XIII**)] (see preliminary communication [18]).

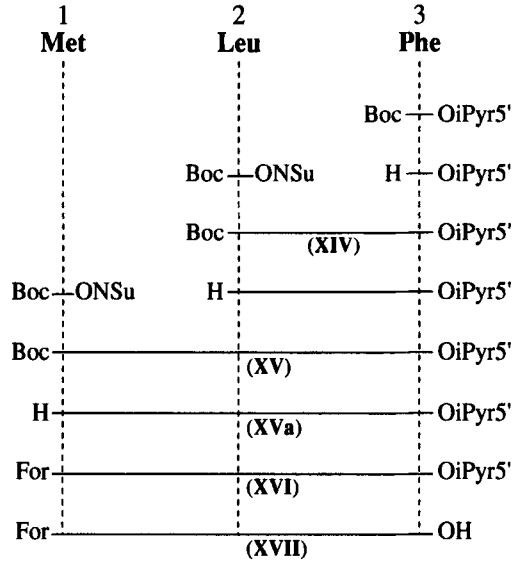




Splenopentine Splenopentine analogue (**XIII**)

In this case, the pyridoxyl residue appeared to mimic Tyr residue (an aromatic amino acid): the absence of Tyr in the molecule of splenopentine or its substitution by an aliphatic amino acid residue is known to result in the loss of its specific activity [19].

The same approach was used in the synthesis of the pyridoxyl ester of chemotactic peptide (**XVII**). The presence of the *C*-terminal pyridoxyl ester allowed formylation (Scheme 4) by the excess of acetoformic anhydride without racemization. The pyridoxyl ester of chemotactic peptide (**XVI**) exhibited a higher biological activity than chemotactic peptide (**XVII**) itself [16].



Scheme 4. The synthesis of a chemotactic peptide (for preliminary communication, see [20]).

Physicochemical characteristics of the compounds synthesized

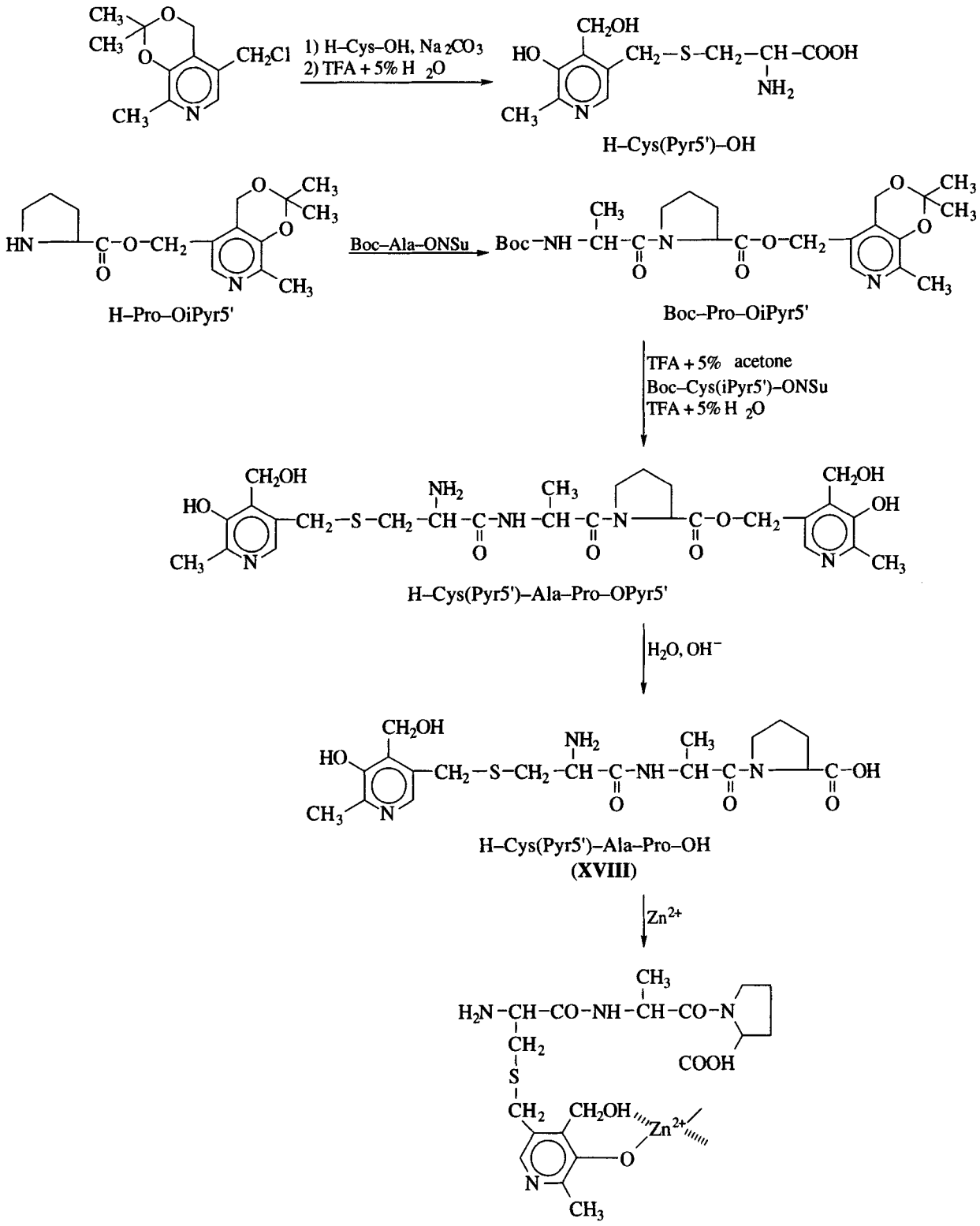
Number	Compound	Mp, °C	$[\alpha]_D$, 1% MeOH	R_f	Yield at the last stage, %
(I)	H-Gly-OiPyr5' (trifluoroacetate)	53–55		0.55 (E)	94.7
(Ia)	H-Gly-OiPyr5' (picrate)	105–107		0.55 (E)	98
(Ib)	H-Gly-OcPyr5'(hydrochloride)	134–136		0.64 (E)	74
(II)	Boc-Ala-Gly-OiPyr5' *	71–73	–8.9	0.41 (A); 0.35 (G)	93.5
(IIa)	Boc-Ala-Gly-OcPyr5'*	96–98	–6.1	0.50 (A); 0.39 (G)	81
(III)	Boc-Ile-Ala-Gly-éiPyr5' *	82–84	–20.8	0.38 (A); 0.71 (B)	95
(IV)	Boc-Ala-Ile-Ala-Gly-éiPyr5' *	129–131	–49.2	0.23 (A); 0.64 (B)	96
(IVa)	Boc-Ala-Ile-Ala-Gly-$(OPlm)_2$Pyr5'	110–112	–36.5	0.61 (A); 0.83 (B)	52
(V)	Boc-Gly-Ala-Ile-Ala-Gly-OiPyr5' *	141–145	–40.8	0.29 (A); 0.58 (B)	93
(Va)	Boc-Ala-Ile-Ala-Gly-OPyr***	166–169	–37	0.2 (A); 0.43 (B)	47
(VI)	Boc-Phe-Gly-Ala-Ile-Ala-Gly-OiPyr5' *	138–141	–24	0.1 (A); 0.52 (B)	95.5
(VII)	Boc-Leu-Phe-Gly-Ala-Ile-Ala-Gly-OiPyr5' **	151–153	–41.8	0.09 (A); 0.68 (B)	93
(VIII)	Boc-Gly-Leu-Phe-Gly-Ala-Ile-Ala-Gly-OiPyr5' **	148–150	–40.1	0.41 (B); 0.73 (C)	93.5
(VIIIa)	Boc-Gly-Leu-Phe-Gly-Ala-Ile-Ala-Gly-$(OPlm)_2$Pyr5'	131–133	–36	0.58 (A); 0.74 (B)	48
(IX)	Boc-Gly-Leu-Phe-Gly-Ala-Ile-Ala-Gly-OH	243–247 (decomp.)	–38.3	0.64 (E); 0.38 (F)	90.9
(XI)	Boc-Gly-Leu-Phe-Gly-Ala-Ile-Ala-Gly- Phe-Ile-Glu(OBzl)-Gly-OBzl	267–269	–23		82
(XII)	H-Gly-Leu-Phe-Ala-Ile-Ala-Gly-Phe-Ile-Glu-Gly-OH	289–291 (decomp.)	–30	0.6 (E); 0.35 (F)	98
(XIII)	H-Arg-Lys-Glu-Val-OPyr5'***	188–190	–29	0.11 (A); 0.2 (E)	79
(XIV)	Boc-Leu-Phe-OiPyr5'*	92.5–94	–7.5	0.91 (E); 0.41 (G)	96
(XV)	Boc-Met-Leu-Phe-OiPyr5' *	120–123	–8.8	0.8 (E); 0.38 (G)	95
(XVa)	H-Met-Leu-Phe-OiPyr5'	207–210	–8.1	0.28 (A); 0.61 (E)	90
(XVI)	For-Met-Leu-Phe-OiPyr5'	146–148	–15.8	0.5 (A); 0.77 (A)	95
(XVII)	For-Met-Leu-Phe-OH	212–214			95
(XVIII)	H-Cys(Pyr5')-Ala-Pro-OH***	176–180	–12.8	0.1 (C); 0.26 (E)	84
(XIX)	Z-Cys(Pyr5')-Pro-OPyr5'(hydrochloride)	142–145	–11.4	0.55 (A); 0.72 (C)	80

* Recrystallized from 1 : 1 ethyl acetate-petroleum ether.
** Recrystallized from 1 : 1 chloroform-petroleum ether.
*** Recrystallized from 1 : 1 isopropanol-hexane.

Furthermore, it was more soluble in water, which allowed us to eliminate DMSO from the cellular medium and, therefore, to ensure a more objective determination of its phagocytic activity in clinical immunology [20]. Pyridoxyl ester **(XVI)** was smoothly hydrolyzed under the conditions developed previously [9]. The solid phase synthesis of this peptide is described below.

We also successfully used a Boc-amino acid pyridoxyl ester, H-Lys(Z(Cl))-OiPyr5', in the synthesis of peptides of cell contact interaction and their fluorescent analogues [16]. Our attempts to synthesize them starting from free H-Lys(Z(Cl))-OBzl resulted in the formation of hardly separable mixtures because of the poor ability of intermediates to crystallize.

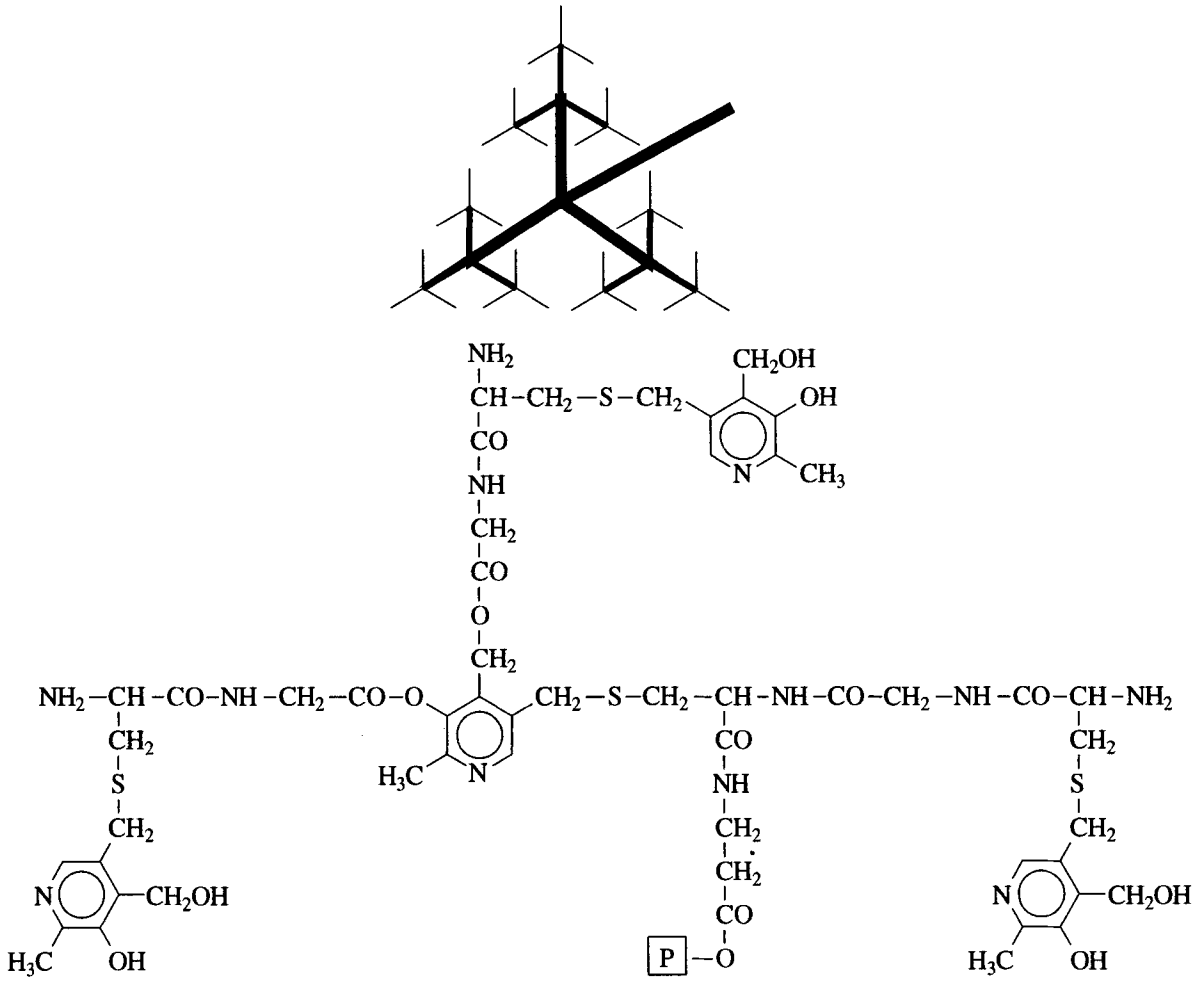
The removal of the Boc-group by treatment with formic or trifluoroacetic acid or saturated hydrogen chloride in dioxane with the addition of 5% water resulted in the simultaneous removal of the isopropylidene protective group [9] and the formation of compounds with complexing properties. We used these properties of the pyridoxyl compounds in order to obtain inhibitors of metalloenzymes, including the inhibitor of the angiotensin-converting enzyme



Scheme 5. The synthesis of *S*-5'-pyridoxylcysteinylalanylproline, a peptide inhibitor of the angiotensin-converting enzyme. One of the possible structures of the Zn(II)-complex is shown [29].

(Scheme 5) [21]. Moreover, the presence of the adjacent hydroxyl groups in the pyridoxyl derivatives of peptides can be the basis for the synthesis of diol inhibitors of HIV proteases as demonstrated in [22].

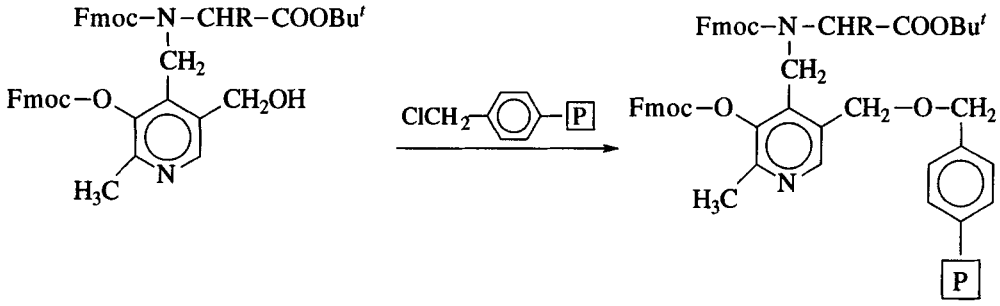
Thus, a Boc-group can be used as a temporary N^{α}-protective group in combination with the permanent pyridoxyl protective group bearing additional acid-labile (ketal) groups. The removal of a Boc-protective



Scheme 6. A fragment of the branched core of the multiplet peptide on the basis of *S*-5'-pyridoxylcysteine. The graphic image (three branching levels) of the free multiplet is given.

group requires certain conditions, such as the absence of moisture in trifluoroacetic acid and, in some cases, the use of hydrogen chloride. For example, the use of hydrogen chloride should be given preference over trifluoroacetic acid for removing the Boc-protective group in the synthesis of hydrophobic peptides or when using cyclohexylidene derivatives of pyridoxine, which are more hydrophobic than the isopropylidene derivatives. Furthermore, it was noted [23] that prolonged heating (at 60–80°C) at evaporation of trifluoroacetic acid containing ~1% water (in the case of a low vacuum or a large load) results not only in the splitting off of the ketal protective group but also in the polycondensation of pyridoxyl residues due to the high reactivity of the hydroxymethyl group in position 4 of the pyridoxine molecule. On the other hand, the polycondensation process can easily be controlled [24] and can be used for practical synthetic purposes, e.g., for the transition from the monomolecular protective group to the polymeric one. Multiplet peptides and conjugates could probably be obtained as a result of this process [25].

As expected, the use of pyridoxyl derivatives in combination with Boc-amino acids in the solid phase peptide synthesis caused no problems. The high swelling ability of peptidylpolymers in the synthesis of the determinants of HIV-proteins for Peptoscreen diagnosticum was described [26]. The pyridoxyl residue can play the role of a spacer in solid phase peptide synthesis due to the easy alkylation of the polymeric matrix by the chloromethyl groups or the reaction of the pyridoxal aldehyde group with the amino acid attached to the polymeric matrix [27, 28] (Scheme 2). The resistance of the spacer to the action of HF, HBr, and other acidic reagents helps finally synthesize unprotected peptides attached to matrices (e.g., immunosorbents, immunogens, and cyclopeptides). If necessary, free peptides can be split off under mild conditions by hydrolysis, ammonolysis, hydrazinolysis, hydrogenolysis, or photolysis; see, e.g., the synthesis of (**XVII**).



Scheme 7. The attachment of the starting *N*-pyridoxyl amino acids to a matrix (a hypothetical scheme).

Thus, it is possible to use the combination of the temporary Boc-protective group and the permanent pyridoxyl protective group in both the conventional and solid phase peptide synthesis. The isopropylidene group can be removed simultaneously with the Boc-protective group by treatment with 95% trifluoroacetic acid. The use of another, more stable acid-labile group (benzyloxycarbonyl) together with the pyridoxyl protective groups has its own peculiarities: both groups can be removed simultaneously by hydrogenolysis (Scheme 1), or the ketal groups at the pyridoxyl residue can be removed selectively under mild acidic hydrolysis as, e.g., in the synthesis of (**XIX**). The procedure for the selective removal of the ketal groups is necessary for introduction into the pyridoxyl residue of other substituents, including the amino acid residues [9].

The pyridoxyl derivatives can be used in the synthesis of libraries of multiplet peptides, since they enable the synthesis of the core with three levels of branching (Scheme 6) [28, 29]. This imparts to the core the ability for selective cleavage. Thus, 3-*O*-, 4'-*O*-, and 5'-*O*-pyridoxyl esters can be saponified, whereas the 3-*O*-pyridoxyl group can be cleaved by treatment with HF [9]. The resulting linear peptides can be used for the analysis of the multiplet synthesized. This possibility was specifically discussed in [30]. For example, three amino groups of Cys residues and six hydroxyl groups of pyridoxyl residues served as the centers of attachment and subsequent solid phase synthesis of a multiplet core on the basis of 5'-*S*-pyridoxylcysteine (Scheme 6). β-Alanine and glycine were introduced to decrease the steric hindrances. They also served as internal standards in the amino acid analysis of the multiplet.

Casella and Gullotti [31] proposed *N*-pyridoxylamino acids easily obtainable from pyridoxal. They can be starting compounds in the synthesis of amino acid derivatives that should contain two different protective substituents at the α-amino group. The advantage of N^{α}-pyridoxyl derivatives over the compounds suggested previously [1] is the feasibility of their attachment to a polymeric matrix using the additional functions of pyridoxyl residue, as in the case of pyridoxyl esters (Scheme 2). This makes possible the subsequent synthesis of a linear chain and cyclization on the same matrix. Polyfunctional amino acids are usually used for this purpose [29–35], which limits the feasibilities of obtaining the cyclic peptides. We have suggested a hypothetical scheme (Scheme 7) for the synthesis of a polymeric matrix suitable for obtaining cyclic peptides.

Taking into account the complexing properties and the easy transformation of carbinol functions into haloalkyl derivatives or aldehydes (Scheme 2) [10, 13], the pyridoxyl derivatives appear to be most promising for their use as synthons in the synthesis of peptidomimetics [29], e.g., inhibitors, which requires some reactive groups (Scheme 5).

EXPERIMENTAL

Melting points (uncorrected) were determined on a Boetius instrument. The precoated Kieselgel GF 254 (Merck) plates and seven chromatographic systems [(A) 10 : 1 : 0.1 ethyl acetate-methanol-acetic acid, (B) 3 : 1 chloroform-methanol, (C) 3 : 1 : 0.3 chloroform-methanol-acetic acid, (D) 9 : 9 : 2 acetone-dioxane-25% ammonia, (E) 3 : 1 : 1 *n*-butanol-acetic acid-water, (F) 3 : 7 2-propanol-25% ammonia, and (G) ethyl acetate] were used for TLC. The optical rotation angles were measured on a Perkin-Elmer Model 141 polarimeter. The structure of all compounds synthesized was confirmed by the data of amino acid analysis carried out on a Biotronic LC 5001 amino acid analyzer after hydrolysis with 6 N HCl at 110°C for 18–24 h.

1. The general procedure for the synthesis of the salts of 4',3-*O*-isopropylidene-, cyclohexylidene-, or dipalmitoyl-5'-pyridoxyl esters of amino acids and peptides. Trifluoroacetic acid (10 ml), a mixture of 20% HCl in dioxane (20 ml) and anhydrous acetone (0.5 ml), or 20% HCl in dioxane (20 ml) [in the synthesis of (**IVa**) and (**VIIIa**)] was added to a solution, cooled to 0°C, of 4',3-*O*-isopropylidene-5'-pyridoxyl ester of a Boc-amino acid (0.184 mmol, obtained as described in [9]) or a peptide in chloroform (10 ml). The reaction mixture was stirred for 30 min at room temperature and evaporated. The residue was washed with diethyl ether, and the crystalline precipitate was dried.

For example, 0.89 g of **H-Gly-OiPyr5' · 2 TFA (I)** was obtained. The treatment of this product with 1 equiv of picric acid resulted in a quantitative yield of crystalline picrate (**Ia**).

Cyclohexylidene derivative **H-Gly-OcPyr · 2HCl (Ib)** (0.56 g) was similarly obtained. In this case, cyclohexanone (0.5 ml) was added to HCl in dioxane.

1a. The general procedure for the synthesis of salts of -5'-pyridoxyl esters of amino acids and peptides. Distilled water (0.5 ml) was added to a solution, cooled to 0°C, of 4',3-*O*-isopropylidene-5'-pyridoxyl ester of an amino acid [9] or a peptide (1 mmol) in 20% solution of HCl in dioxane (10 ml) or in TFA (10 ml). The reaction mixture was stirred for 1 h at room temperature and evaporated in a vacuum. The residue was washed with diethyl ether and dried to yield, e.g., 0.57 g of **Z-Cys(Pyr5')-Ala-Pro-OH · HCl (XIX)**.

2. The general procedure for creating the peptide bond using 4',3-*O*-isopropylidene-, cyclohexylidene-, or dipalmitoyl-5'-pyridoxyl esters of amino acids and peptides (II)–(VIII), (IIa), (IVa), (VIIIa), (XIV), (XV), (XVa), and (XVI). Triethylamine (1.39 ml, 10 mmol) in CH_2Cl_2 or DMF (30 ml) and *N*-succinimide ester of a Boc-amino acid (5 mmol) were added to an amino ester (5 mmol) obtained by method 1. The mixture was stirred at room temperature for 18 h and evaporated in a vacuum to dryness. The residue was dissolved in ethyl acetate (40 ml). The resulting solution was washed with 5% $NaHCO_3$, water, 3% citric acid (30 ml × 3), 5% $NaHCO_3$, and water. The organic layer was dried over Na_2SO_4 and evaporated in a vacuum. The residue was crystallized from a suitable solvent (table).

2a. The general procedure for creating the peptide bond using 5'-pyridoxyl esters of amino acids and peptides (Va). Triethylamine (1.39 ml, 10 mmol) and *N*-succinimide ester of a Boc-amino acid (5 mmol) were added to an amino ester (5 mmol) obtained by method 1a. The mixture was stirred at room temperature for 18 h and evaporated to dryness in a vacuum. The residue was dissolved in *n*-butanol (40 ml) and washed with the saturated NaCl solution containing 3% acetic acid and a saturated $NaHCO_3$ solution. *n*-Butanol was evaporated, and the residue was crystallized from 1 : 1 2-propanol-hexane.

Boc-Ala-Ile-Ala-Gly-4',3-$(OPlm)_2$Pyr5' (IVa). Palmitoyl chloride (0.9 ml, 3.2 mmol) in chloroform (5 ml) was added for 1 h to a solution of Boc-Ile-Ala-Gly-OPyr5' (prepared by method 2a) in chloroform (10 ml) containing 2 ml of pyridine at 0°C. The mixture was kept for 18 h at room temperature and evaporated in a vacuum. The residue was dissolved in ethyl acetate and washed with 3% citric acid, water, and 5% $NaHCO_3$. The organic layer was separated, dried over Na_2SO_4, and evaporated in a vacuum. The residue was crystallized from 1 : 1 2-propanol-hexane to yield 0.78 g of (**IVa**).

Boc-Gly-Leu-Phe-Gly-Ala-Ile-Ala-Gly-OH (IX). A solution of Boc-Gly-Leu-Phe-Gly-Ala-Ile-Ala-Gly-OiPyr5' (**VIII**) (1.09 g, 1.1 mmol) in methanol (30 ml) was hydrogenated over Pd/C for 6 h. The catalyst was filtered off, the filtrate was evaporated in a vacuum, and the residue was crystallized from 2-propanol. Yield: 0.80 g.

H-Gly-Leu-Phe-Ala-Ile-Ala-Gly-Phe-Ile-Glu-Gly-OH (XII). A solution of octapeptide (**IX**) (0.8 g, 1 mmol) in DMF (30 ml) was mixed at 0°C with a solution of pentafluorophenol (0.683 g, 3.6 mmol) and DCC (0.258 g, 1.2 mmol) in DMF (10 ml). Tetrapeptide (**X**) (0.645 g, 1.0 mmol, previously obtained [17]) was added, and the mixture was stirred for 4 h. The precipitate was filtered and washed with methanol and diethyl ether on the filter to yield 1.18 g (82%) of dodecapeptide (**XI**). This was treated with saturated HBr in TFA and precipitated with anhydrous diethyl ether. The precipitate was filtered, washed on the filter with anhydrous ethyl acetate, and dried in a vacuum. Yield: 0.98 g (98% from (**XI**)).

For-Met-Leu-Phe-OiPyr5' (XVI). Acetic anhydride (9.43 g, 100 mmol) was added to a mixture of sodium formate (7.28 g, 80 mmol) and formic acid (7.55 ml, 200 mmol) at 0°C. Met-Leu-Phe-OiPyr5' · 2HCl (**XVa**) (3.09 g, 4.6 mmol) obtained by method 1 was added to the resulting solution. The mixture was stirred for 20 min at 0°C and for 1 h at room temperature and, then, diluted with diethyl ether (100 ml). The precipitate was separated; washed with distilled water (5 ml × 5), diethyl ether, and hexane; dried in a vacuum; and crystallized from 2-propanol to yield 2.75 g of (**XVI**).

For-Met-Leu-Phe-OH (XVII). For-Met-Leu-Phe-OiPyr5' (2.75 g, 4.37 mmol) was dissolved in a mixture of methanol (50 ml) and 2 N NaOH (10 ml) at room temperature. The mixture was stirred for 20 min, neutralized with glacial acetic acid, and evaporated to dryness. The residue was dissolved in ethyl acetate and washed with water, filtered, washed with anhydrous diethyl ether, dried, and crystallized from 2-propanol-hexane to yield 1.72 g of (**XVII**).

The solid phase synthesis of For-Met-Leu-Phe-OH (XVII).

(a) The attachment of Boc-Phe-OiPyr5' to polymeric matrix (Scheme 2). Boc-Phe-OiPyr5' (0.228 g, 0.50 mmol), KI (50 mg), $NaHCO_3$ (50 mg), and a chloromethylated copolymer of styrene with 1% divinylbenzene (0.8 g, 1.4 mmol/g of chlorine) were suspended in DMF (2 ml). The mixture was kept for 24 h at 80°C (a reddish brown staining of the polymer was observed); filtered; washed with DMF (3 × 1 ml), 50% methanol, and diethyl ether; and dried in a vacuum. The content of Boc-Phe-OiPyr5' determined by quantitative amino acid analysis was 0.24 mmol/g of polymer.

(b) For-Met-Leu-Phe-OiPyr5'-polymer. A 1 : 1 TFA-CH_2Cl_2 mixture (6 ml) and acetone (0.6 ml) were added to 0.5 g of the polymer containing 0.12 mmol of

Boc-Phe-OiPyr5', and the mixture was kept for 30 min. The polymer was filtered and washed with CH_2Cl_2 (6 ml × 3) and diethyl ether. Trifluoroacetic acid was neutralized with 3% triethylamine in chloroform (3 ml, 5 min), and the polymer was washed with chloroform (3 ml × 2) and diethyl ether.

Anhydrous Boc-Leu-OH (0.84 g, 0.36 mmol) in CH_2Cl_2 (3 ml) and a solution of DCC (0.8 g, 0.38 mmol) in CH_2Cl_2 (3 ml) cooled to 0°C were added to the resulting polymer. The mixture was stirred for 2 h at room temperature. The precipitate of dicyclohexylurea was washed with CH_2Cl_2. The neutralization and acylation stages were repeated. Boc-Met-OH was attached in a similar manner. Formylation was performed on the polymer (after neutralization) at 0°C by the mixed anhydride of formic and acetic acids prepared *in situ* from 0.75 ml of formic acid as described for (**XVI**). The anhydride was preliminarily dissolved in DMF (3 ml) at 0°C. The peptidylpolymer was kept for 1 h at 20°C; filtered; washed with DMF, 50% methanol, and diethyl ether; and dried in a vacuum. According to quantitative amino acid analysis, the content of peptide (**XVII**) was 0.11 mmol/g.

(c) The photolysis of For-Met-Leu-Phe-OiPyr5'-polymer. A suspension of the peptidylpolymer (0.45 g) containing 0.049 mmol of peptide (**XVII**) and NH_4Cl (15 mg) in 50% methanol (1.5 ml) was irradiated by a SYLVAVIA Blacklight Blue BW-8W lamp in a 2-ml quartz cuvette for 18 h [36]. The polymer was filtered, washed with 50% methanol (3 ml × 3), and dried. The quantitative amino acid analysis of an aliquot of the polymer (peptide content was 0.079 mmol/g) showed that 28% of the peptide had been split off. The filtrate was evaporated in a vacuum. The residue was dissolved in methanol (0.5 ml), applied onto a silica gel column (0.5 × 10 cm), and eluted with a chloroform-methanol mixture. The fractions containing the peptide were collected and evaporated to give 5.1 mg (0.011 mmol) of formylpeptide with characteristics identical to those of peptide (**XVII**) obtained by the conventional synthesis in solution (table).

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