  Bull. Chem. Soc. Jpn., 61, 3653—3657 (1988)

Peptide Synthesis in Alcohol Solvents by the Mixed Anhydride Method Using Dimethylphosphinothioyl Chloride

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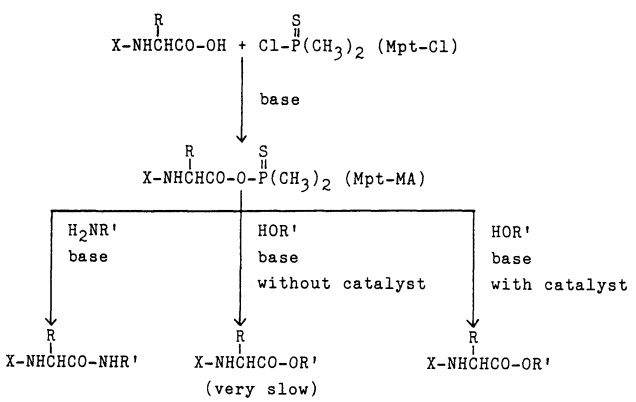
(Received July 1, 1988)

The formation and coupling of the dimethylphosphinothioic mixed anhydrides of protected amino acids can be performed in alcohol solvents. Sparingly soluble protected C-terminal segments of vasoactive intestinal peptide (VIP) up to the heptapeptide stage were synthesized by this technique and fully characterized.

In peptide synthesis low solubility of even small peptides can result in incomplete reaction giving unpurifiable materials with lack of purification method. Thus, solubility problems may prevent further elongation of the peptide chain. The solid phase method[1] is one solution to this problem. For solution syntheses various kinds of organic solvents having high solubilizing power such as *N,N*-dimethylformamide, dimethyl sulfoxide, and hexamethylphosphoric triamide, all of which are classified as dipolar aprotic solvents, have been utilized. Among polar protic solvents alcohols, especially methanol, have high solubilizing ability for peptides, although their use as solvents in peptide bond formation is not normally possible because of their high reactivity toward the activated amino acid derivatives.

Recently we reported that dimethylphosphinothioic mixed anhydrides (Mpt-MAs), formed from protected amino acids by reaction with dimethylphosphinothioyl chloride (Mpt-Cl)[2] in the presence of a base, were stable toward isolation and reacted very selectively toward amino functions.[3]

Mpt-MAs react with alcohols only in presence of basic catalysts such as 4-dimethylaminopyridine and imidazole.[4] Such high stability toward alcohols and selectivity toward the amino group can be attributed to the presence of the P=S bond. In this study we applied the Mpt-MA method to peptide synthesis in alcohol solvents in order to determine whether the selectivity was high enough for use in practical peptide syntheses. If this were the case it would represent an efficient solution to the often serious solubility problem.



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Results and Discussion

The possibility of using alcohol solvents in peptide synthesis was first investigated by examination of the synthesis of model peptides. The results are summarized in Table 1.

In these experiments the Mpt-MAs were generated in situ. Generally the formation of Mpt-MAs can be easily followed by thin layer chromatography (TLC) since these compounds can be visualized, without removing the amino protecting group, simply by spraying with ninhydrin reagent followed by warming. For reasons not yet clear a color reaction toward ninhydrin arises from the Mpt mixed anhydride group itself.

Methanol (Entries 3 and 6) and ethanol (Entry 7) could be used as well as chloroform (Entries 1, 2, 4, and 5) in the mixed anhydride formation. However, when 2,2,2-trifluoroethanol was used (Entry 8) formation of ester was observed even during the activation step. Methanol (Entries 3 and 6) and ethanol (Entry 7) could be used in both activation and coupling steps and yields of peptides obtained in this way were high. Since methanol is a more effective solvent than ethanol for the peptide segments, the former was used in the following experiments.

Vasoactive intestinal peptide (VIP), isolated in 1970 by Said and Mutt from the small intestine of the hog,[5] is a linear octacosapeptide amide.[6] Total synthesis of VIP has been achieved by many groups by solution[7,8] and solid phase[9-11] methods. In all previous solution syntheses much effort was required to overcome the solubility problem.[12] In a recent synthesis Schaaper and Beyerman temporarily substituted Asn[28] by the corresponding *β-t*-butyl aspartate ester which was subsequently converted to Asn.[13] Clearly for such sparingly soluble peptides the development of a generally applicable solvent would offer significant improvements over current techniques. As an example to demonstrate the efficiency of methanol as a solvent for peptide bond formation, stepwise synthesis of the relatively insoluble C-terminal segments of VIP up to the heptapeptide stage was investigated. Results obtained in the case of di-, tri-, and tetrapeptide amides are

Table 1. Peptide Synthesis in Alcohol Solvents[a)]

$$\text{Prot-AA}^1\text{-OH} + \text{Cl-Mpt} \xrightarrow[\text{Solvent}^1]{\text{TEA}} [\text{Prot-AA}^1\text{-OMpt}] \xrightarrow[\text{Solvent}^2(\text{R}^2\text{-OH})]{\text{H-AA}^2\text{-OR}^1 + \text{TEA}} \text{Prot-AA}^1\text{-AA}^2\text{-OR}^1 + \text{Prot-AA}^1\text{-OR}^2$$

Entry	Peptide	Solvent[1]	Solvent[2]	Yield/%	Mp θ_m/°C	Yield (%) of Prot-AA[1]-OR[2]
1	Boc-Pro-Phe-OBzl	$CHCl_3$	$CHCl_3$	86	(oil)	—
2		$CHCl_3$	CH_3OH	93	(oil)	0
3		CH_3OH	CH_3OH	95	(oil)	0
4	Boc-Val-Val-OMe	$CHCl_3$	$CHCl_3$	96	165.0—166.5	—
5		$CHCl_3$	CH_3OH	93	163.0—165.0	0
6		CH_3OH	CH_3OH	82	163.0—165.0	0
7		C_2H_5OH	C_2H_5OH	93	162.0—163.5	0
8		CF_3CH_2OH	CF_3CH_2OH	14	160.5—161.5	37

a) Unless otherwise noted the MA was generated at 0 °C for 1 h and coupled with an amino component at 0 °C overnight.

Table 2. Synthesis of VIP C-terminal Di-, Tri-, and Tetrapeptide Amide

Entry	Peptide	Method	Solvent		Yield/%
			Activation	Coupling	
1	Z-Leu-Asn-NH_2	Mpt-MA(direct)	$CHCl_3$	CH_3OH	88
2		Mpt-MA(isolated)		CH_3OH	95
3		Mpt-MA(isolated)		CH_3OH-H_2O	98
4		NP active ester		DMF	93[a)]
5	Z-Ile-Leu-Asn-NH_2	Mpt-MA(direct)	$CHCl_3$	CH_3OH	52
6		Mpt-MA(isolated)		CH_3OH	95
7		NP active ester		DMF	87[a)]
8	Z-Ser-Ile-Leu-Asn-NH_2	Mpt-MA(direct)	$CHCl_3$	CH_3OH	94
9		Mpt-MA(isolated)		CH_3OH	94

a) Ref. 12.

summarized in Table 2.

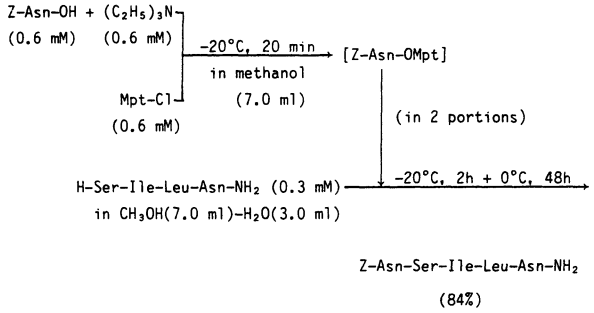
In these reactions the desired products precipitated during the reaction. Z-Leu-Asn-NH_2 was obtained in 88% yield when Z-Leu-OMpt, generated in situ in chloroform, was added to a solution of H-Asn-NH_2·HBr in methanol containing triethylamine (Entry 1). However, when synthesis of the related tripeptide was carried out by the same method (Entry 5) the yield of Z-Ile-Leu-Asn-NH_2 was only 52%. In this case precipitation of the product began too early thus causing coprecipitation of the free amino component which was difficult to remove from the tripeptide. In order to avoid this problem Z-Ile-OMpt was first freed from triethylamine hydrochloride by washing the solution with water and then coupled with free H-Leu-Asn-NH_2 in methanol (Entry 6). This technique gave Z-Ile-Leu-Asn-NH_2 in a yield of 95%. Similar result was also obtained in the case of dipeptide synthesis (Entry 2). These results compare favorably with those obtained by Bodanszky, et al., which involved coupling of the *p*-nitrophenyl active ester in *N,N*-dimethylformamide.[12)]

At the tetrapeptide stage a somewhat different situation emerged. Serine is found as the fourth amino acid from the C-terminal position. As previously reported[3)] incorporation of serine into a peptide chain is possible without protecting the side-chain hydroxyl group when using the Mpt-MA method. However the isolated yield of Z-Ser-OMpt is exceptionally low because of the lactone formation. Since yields using isolated and in-situ-generated Z-Ser-OMpt are nearly the same, the method involving in situ activation is more convenient.

At the pentapeptide stage another difficulty was encountered. Solubility of the free tetrapeptide amide, H-Ser-Ile-Leu-Asn-NH_2, in methanol is much lower than the free C-terminal mono-, di-, and tripeptide amides. This may limit the use of methanol as a sole solvent in the synthesis of peptide segments containing asparagine and glutamine. However, it is known that peptides containing these amino acids, although not soluble in a single organic solvent, often dissolve in aqueous organic solvents. Synthesis was therefore examined in aqueous methanol.

When a methanolic solution of Z-Leu-OMpt was added, in 2 portions with a 20 min interval between additions, to a methanol-water (2 : 3) solution of asparaginamide, the dipeptide Z-Leu-Asn-NH_2 was obtained in 98% yield. This result shows that aqueous methanol can be more effective solvent than methanol itself for the synthesis of longer peptides containing asparagine and glutamine.

At the 24-position of VIP is found asparagine whose activation via the Mpt technique required some caution. Mpt-MA formation is usually performed at room temperature except in the case of serine which requires cooling to 0 °C in order to avoid lactone formation. In the case of asparagine, complex reactions, possibly including nitrile formation, were observed even at 0 °C. Fortunately this side-reaction could be suppressed by carrying out the reaction at −20 °C in either methanol or chloroform. These conditions were applied to the synthesis of Z-VIP(24—28)-NH_2.



When a methanolic solution of Mpt-MA prepared from Z-Asn and Mpt-Cl at −20 °C for 20 min in the presence of triethylamine was added to a solution of H-Ser-Ile-Leu-Asn-NH_2 in methanol-water and the mixture stirred at the same temperature for 2 h and then at 0 °C for 48 h the pentapeptide Z-Asn-Ser-Ile-Leu-Asn-NH_2 was obtained in 84% yield in an analytically pure form.

The sixth amino acid, Leu, could be introduced without any problem using the salt-free, washed Mpt-MA along with free H-Asn-Ser-Ile-Leu-Asn-NH_2 dissolved in a methanol-water mixture. However, when Z-Tyr was coupled to H-Leu-Asn-Ser-Ile-Leu-Asn-NH_2 in a similar manner the crude product was contaminated with the free amino component and obtained in only 48% yield. Reprecipitation of the crude product from DMSO-H_2O or DMSO-methanol did not give pure material. Except for special amino acids such as Ser and Asn Mpt-MAs are sufficiently stable to be used for coupling reactions above room temperature thus avoiding premature precipitation. For example, when Z-Tyr-OMpt in methanol was added to a solution of H-Leu-Asn-Ser-Ile-Leu-Asn-NH_2 in a methanol-water mixture followed by stirring at 38—40 °C for 2 h and at room temperature for 46 h the desired product was obtained in 74% yield without contamination by the free amino component. In this case the unprotected tyrosine side-chain hydroxyl group caused no difficulty.

In the report of Bodanszky, et al.,[12] intermediates involved in the VIP synthesis could not be characterized except for the trifluoroacetates of *N*-deprotected peptide amides and for protected peptide amides longer than tripeptide only melting points and amino acid analyses were recorded. In the present study it was possible to characterize all of the protected and free peptide amides by amino acid analysis, elemental analysis and TLC. In addition melting points and optical rotation values could be recorded for the first time as shown in Tables 3 and 4.

In conclusion, peptide synthesis by the Mpt-MA method using methanol or methanol-water mixtures as solvents provides a convenient and efficient solution to the solubility problem in peptide synthesis.

Experimental

Amino acids and their derivatives used here are all of the L configuration. Abbreviations given by the IUPAC-IUB Commission (*J. Biol. Chem.*, **247**, 977 (1972)) are used throughout. Additional abbreviations: Z, benzyloxycarbonyl; Boc, *t*-butyloxycarbonyl; DCHA, dicyclohexylamine; TEA, triethylamine; AcOH, acetic acid; DMF, *N,N*-dimethylformamide; DMSO, dimethyl sulfoxide. DMF (Kokusan Chemical Works, special grade) was distilled at reduced pressure and stored over molecular sieves. Amino acid analyses were performed on the Atto Model MLC 203 Amino Acid Analyzer. Peptides were hydrolyzed in 6 mol dm^{-3} HCl in sealed, evacuated tubes at 110 °C for 24 h. TLC was carried out on precoated silica gel 60 (F-254) plates (Merck) in the following solvent systems: (A) 1-butanol-acetic acid-water (4 : 1 : 1); (B) chloroform-methanol-water (8 : 3 : 1).

Salt-Free, Washed Z-Amino Acid-Dimethylphosphinothioic Mixed Anhydrides (General Procedure): To a solution of Z-amino acid (2.0 mmol) and triethylamine (2.0 mmol) in chloroform (3.0 ml) was added Mpt-Cl[2] (2.2 mmol) in chloroform (1.0 ml) and the mixture stirred at room temperature for 20 min. Chloroform was removed under reduced pressure and the residue divided between ethyl acetate and water. The organic layer was successively washed with 5% aqueous citric acid solution (3 times), water (3 times), saturated NaCl solution, 5% aqueous sodium hydrogencarbonate solution (3 times), water (3 times), and saturated NaCl solution, and dried over anhydrous sodium sulfate. Removal of the solvent gave the Mpt-MAs as oils.

In the case of tyrosine 1.0 equiv of the Mpt-Cl was used in the presence of DCHA in order to avoid undesirable formation of *O*-dimethylphosphinothioylated product. The washing procedures were carried out without changing the solvent. After removal of the solvent Z-Tyr-OMpt was obtained as amorphous solid.

Thus obtained Mpt-MAs were used in the following experiments without further purification.

Preparation of Z-Ser-OMpt was already described in the previous communication.[3]

Preparation of Z-Leu-Asn-NH_2 (1): (A) To a suspension of Z-Asn-NH_2[12] (530.4 mg, 2.0 mmol) in AcOH (2.0 ml) was added 25% HBr/AcOH (8.3 ml). After 1 h the solution was diluted with ether to give a precipitate, which was dissolved in water and dehydrobrominated by stirring for 5 min with Dowex 1 resin. The resin was filtered and washed with water. The filtrate and washings were collected and evaporated under reduced pressure. The residue was dissolved in methanol and purified by gel filtration on Sephadex LH-20 to give the free asparaginamide as a white powder; 229.1 mg (87%), mp 138—139 °C, $[\alpha]_D^{27}$ +4.7° (*c* 2.0, H_2O), R_f(A) 0.12, R_f(B) 0.01.

To an ice-cooled solution of H-Asn-NH_2 (131.1 mg, 1.0 mmol) in methanol (6.5 ml) and TEA (209 μl, 1.5 mmol) was added Z-Leu-OMpt (536.1 mg, 1.5 mmol) in methanol (1.2 ml) and the mixture stirred at 0 °C for 24 h. The precipitate was collected by filtration and washed with chloroform, water and ether, and dried to give **1** as a white powder. After concentration of the filtrate and washings a second crop was obtained giving a total yield of 359.4 mg (95%). Physical properties and analytical data are given in Tables 3 and 4.

(B) H-Asn-NH_2 (19.7 mg, 0.15 mmol) and TEA (21 μl, 0.15 mmol) were dissolved in methanol (0.2 ml) and water (0.3 ml). To this solution was added at 0 °C a solution of Z-Leu-OMpt (53.6 mg, 0.15 mmol) in methanol (0.7 ml). After stirring for 20 min the same amounts of TEA and Z-Leu-OMpt were again added. After the mixture was stirred at 0 °C for 24 h the precipitate was collected as described above. Yield 55.5 mg (98%), mp 235—236 °C (decomp), $[\alpha]_D^{23}$ −7.6° (*c* 1, DMF).

(C) To a solution of Z-Leu-OH·DCHA (147.4 mg, 0.33 mmol) in chloroform (1.0 ml) was added Mpt-Cl (42.4 mg, 0.33 mmol) in chloroform (1.0 ml) and the mixture stirred at room temperature for 45 min. The solution was cooled to 0 °C and treated with a solution of asparaginamide hydrobromide (71.1 mg, 0.33 mmol) in methanol (2.5 ml) containing TEA (92.0 μl, 0.66 mmol) and the mixture stirred at the same temperature for 24 h. The precipitate was collected as described above to give 109.2 mg (88%) of the amide, mp 234—235 °C (decomp), $[\alpha]_D^{22}$ −7.6° (*c* 1.0, DMF).

Preparation of H-Leu-Asn-NH_2 (2): To a suspension of **1** (473.5 mg, 1.25 mmol) in AcOH (2.5 ml) was added 25% HBr/AcOH (5.2 ml) and the mixture kept standing at room temperature for 1 h and diluted with ether. The precipitate was collected, dehydrobrominated and purified as described for H-Asn-NH_2. Yield 257.0 mg (84%). Physical properties and analytical data are given in Tables 3 and 4.

Preparation of Z-Ile-Leu-Asn-NH_2 (3): (A) To an ice-cooled solution of **2** (244.3 mg, 1.0 mmol) and TEA (209 μl, 1.5 mmol) in methanol (6.7 ml) was added a solution of Z-Ile-OMpt (536.1 mg, 1.5 mmol) in methanol (1.1 ml), and the mixture stirred at 0 °C for 24 h. The precipitate was collected by filtration, washed as described for compound **1** and dried. Yield 468.3 mg (95%). Physical properties and analytical data are given in Tables 3 and 4.

(B) To a solution of Z-Ile-OH·DCHA (223.3 mg, 0.5 mmol) in chloroform (1.0 ml) was added a solution of Mpt-Cl (64.3 mg, 0.5 mmol) in chloroform (0.5 ml) and the mixture stirred for 1 h. The solution was cooled to 0 °C and treated with a solution of **2** hydrobromide (162.5 mg, 0.5 mmol) and TEA (140 μl, 1.0 mmol) in methanol (2.0 ml). The mixture was stirred at 0 °C for 24 h and worked up as described in (A). Yield 128.3 mg (52%), mp 262—263 °C (decomp).

Preparation of H-Ile-Leu-Asn-NH_2 (4): To a suspension of **3** (453.6mg, 0.92 mmol) in AcOH (5.0 ml) was added 25% HBr/AcOH (5.0 ml) and the mixture kept standing at room temperature for 1 h and diluted with ether. The precipitate was collected, dehydrobrominated and purified as described for H-Asn-NH_2. Yield 270.7 mg (82%). Physical properties and analytical data are given in Tables 3 and 4.

Preparation of Z-Ser-Ile-Leu-Asn-NH_2 (5): (A) To an ice-cooled solution of Z-Ser-OH (71.8 mg, 0.3 mmol) and TEA (41.8 μl, 0.3 mmol) in chloroform (1.0 ml) was added a solution of Mpt-Cl (38.5 mg, 0.3 mmol) in chloroform (0.5 ml). After stirring for 20 min the resulting solution was added to an ice-cooled solution of **4** (71.5 mg, 0.2 mmol) and TEA (41.8 μl, 0.3 mmol) in methanol (4.4 ml) and the mixture stirred at 0 °C for 24 h. The precipitate was collected by filtration and washed with chloroform, water and ether, and dried to give **5** as a white powder. After concentration of the filtrate and washings a second crop was obtained giving a total amount of 112.2 mg (94%). Physical properties and analytical data are given in Tables 3 and 4.

(B) To an ice-cooled solution of **4** (89.4 mg, 0.25 mmol) and TEA (52.2 μl, 0.375 mmol) in methanol (4.8 ml) was added a solution of Z-Ser-OMpt (124.3 mg, 0.375 mmol) in methanol (1.2 ml) and the mixture stirred at 0 °C for 24 h. Work up as described above gave **5**; 136.3 mg (94%), mp 257—258 °C (decomp).

Preparation of H-Ser-Ile-Leu-Asn-NH_2 (6): Compound **5** (597.2 mg, 1.03 mmol) was hydrogenolyzed for 4 h in the presence of 10% Pd-C in 80% AcOH (45 ml). The catalyst was filtered and washed with the same solvent. The filtrate and washings were combined and evaporated under reduced pressure. The residue was dissolved in water by addition of the minimum amount of methanol and the solution treated for 5 min with Dowex 1 resin. The resin was removed and washed with water, and the combined filtrate and washings were evaporated under reduced pressure. The residual mass was washed with ether to give **6**; 424.9 mg (93%). Physical properties and analytical data are given in Tables 3 and 4.

Preparation of Z-Asn-Ser-Ile-Leu-Asn-NH_2 (7): A solution of Z-Asn-OH (79.8 mg, 0.3 mmol) and TEA (41.8 μl, 0.3 mmol) in methanol (2.6 ml) was cooled to −20 °C, and treated with a solution of Mpt-Cl (38.5 mg, 0.3 mmol) in methanol (0.9 ml), and the mixture stirred for 20 min. The solution was added to a solution of **6** (133.3 mg, 0.3 mmol) and TEA (41.8 μl, 0.3 mmol) in methanol (7.0 ml) and water (3.0 ml) at −20 °C. After the mixture had been stirred for 40 min the Z-Asn-OMpt solution and TEA (0.3 mmol each) were added and the stirring continued at −20 °C for 2 h and at 0 °C for 48 h. The precipitate was filtered and washed with chloroform, ethanol, water, and ether, and dried to give **7**; 173.7 mg (84%). Physical properties and analytical data are given in Tables 3 and 4.

Preparation of H-Asn-Ser-Ile-Leu-Asn-NH_2 (8): Compound **7** (291.0 mg, 0.42 mmol) was hydrogenolyzed for 24 h in the presence of 10% Pd-C in 80% AcOH (45 ml). The same work up described for compound **6** gave **8**; 197.5 mg (82%). Physical properties and analytical data are given in Tables 3 and 4.

Preparation of Z-Leu-Asn-Ser-Ile-Leu-Asn-NH_2 (9): Compound **8** (55.9 mg, 0.1 mmol) and TEA (14.0 μl, 0.1 mmol) were dissolved in methanol (3.0 ml) and water (1.0 ml) and the solution cooled to 0 °C. To the solution was added Z-Leu-OMpt (35.8 mg, 0.1 mmol) in methanol (0.9 ml) and the mixture stirred at 0 °C for 20 min. After the same amounts of the Z-Leu-OMpt solution and TEA were again added the mixture was stirred at 0 °C for 48 h. Filtration, washing and drying as described for the compound **7** gave **9**; 64.5 mg (80%). Physical properties and analytical data are given in Tables 3 and 4.

Preparation of H-Leu-Asn-Ser-Ile-Leu-Asn-NH_2 (10): Compound **9** (133.5 mg, 0.165 mmol) was hydrogenolyzed for 8 h in the presence of 10% Pd-C in 80% AcOH (17 ml). The same work up described for compound **6** gave **10**; 97.0 mg (83%). Physical properties and analytical data are given

Table 3. Physical Properties of Synthetic Intermediates of VIP

Compound	Yield/%[a]	Mp (θ_m/°C)	$[\alpha]_D$ (solv., temp)	R_f(A)	R_f(B)
Z-VIP (27—28)-NH_2 (**1**)	95	235—236(decomp)	−7.5°(*c* 1 DMF, 22 °C)[b]	0.70	0.40
H-VIP(27—28)-NH_2 (**2**)	84	188—190(decomp)	−5.5°(*c* 1 H_2O, 26.5 °C)	0.33	0.06
Z-VIP (26—28)-NH_2 (**3**)	95	268—270(decomp)	−24.8°(*c* 0.5 DMSO, 24 °C)	0.78	0.64
H-VIP(26—28)-NH_2 (**4**)	82	218—220(decomp)	−20.3°(*c* 1 DMF, 19 °C)	0.51	0.21
Z-VIP (25—28)-NH_2 (**5**)	94[c]	261—262(decomp)	+6.0°(*c* 0.5 DMF, 25 °C)	0.73	0.43
H-VIP(25—28)-NH_2 (**6**)	93	216—220(decomp)	−29.1°(*c* 1 AcOH, 20.5 °C)	0.54	0.03
Z-VIP (24—28)-NH_2 (**7**)	84[c]	270—271(decomp)	−15.1°(*c* 0.5 DMSO, 20.5 °C)	0.72	0.11
H-VIP(24—28)-NH_2 (**8**)	82	230—233(decomp)	−33.5°(*c* 1 AcOH, 20.5 °C)	0.45	0.02
Z-VIP (23—28)-NH_2 (**9**)	80	259—261(decomp)	−20.7°(*c* 0.5 DMSO, 20.5 °C)	0.73	0.16
H-VIP(23—28)-NH_2 (**10**)	83	215—218(decomp)	−18.5°(*c* 1 AcOH, 27.5 °C)	0.35	0.04
Z-VIP (22—28)-NH_2 (**11**)	74	261—265(decomp)	−14.6°(*c* 0.5 DMSO, 27.0 °C)	0.66	0.30

a) Unless otherwise noted yields are given for reactions involving isolated Mpt-MAs. b) Lit,[12] −7.5°(*c* 1 DMF, 25 °C). c) In-situ-generated Mpt-MA was used.

Table 4. Analytical Data for Isolated Intermediates in the Synthesis of VIP

Compound	Amino Acid Ratios (6 mol dm^{-3}, 110 °C, 24 h)						Elemental Analysis
	Tyr	Ser	Ile	Leu	Asp	NH_3	
1				1.00(1)	1.00(1)	1.80(2)	
2				1.00(1)	1.00(1)	1.85(2)	($C_{10}H_{20}N_4O_3$)C,H,N
3			0.96(1)	1.00(1)	1.03(1)	1.85(2)	($C_{24}H_{37}N_5O_6$)C,H,N
4			0.94(1)	1.00(1)	1.00(1)	1.85(2)	($C_{16}H_{31}H_5O_4$)C,H,N
5		0.86(1)	0.96(1)	1.00(1)	1.00(1)	1.86(2)	($C_{27}H_{42}N_6O_8 \cdot H_2O$)C,H,N
6		0.88(1)	0.96(1)	1.02(1)	1.00(1)	1.80(2)	($C_{19}H_{36}N_6O_6$)C,H,N
7		0.92(1)	1.01(1)	1.05(1)	2.00(2)	2.91(3)	($C_{31}H_{48}N_8O_{10}$)C,H,N
8		0.97(1)	0.98(1)	1.03(1)	2.00(2)	2.69(3)	($C_{23}H_{42}N_8O_8$)C,H,N
9		0.87(1)	0.96(1)	2.00(2)	2.00(2)	2.88(3)	($C_{37}H_{59}N_9O_{11}$)C,H,N
10		0.93(1)	1.00(1)	2.05(2)	2.00(2)	3.12(3)	($C_{29}H_{53}N_9O_9 \cdot 2H_2O$)C,H,N
11	0.94(1)	0.97(1)	1.05(1)	2.17(2)	2.00(2)	3.22(3)	($C_{46}H_{68}N_{10}O_{13} \cdot 2.5H_2O$)C,H,N

in Tables 3 and 4.

Preparation of Z-Tyr-Leu-Asn-Ser-Ile-Leu-Asn-NH_2 (11): To a solution of **10** (26.9 mg, 0.04 mmol) and TEA (5.6 μl, 0.04 mmol) in methanol (1.5 ml) and water (0.5 ml) was added a solution of Z-Tyr-OMpt (16.3 mg, 0.04 mmol) in methanol (0.9 ml) and the mixture stirred at 38—40 °C for 20 min. After the same amounts of the Z-Tyr-OMpt solution and TEA were again added the mixture was stirred at 38—40 °C for 2 h and at room temperature for 46 h. The precipitate was filtered, washed successively with chloroform, ethanol, water and ether, and dried to give **11**. 30.0 mg (74%). Physical properties and analytical data are given in Tables 3 and 4.

The authers wish to express their thanks to Professor Louis A. Carpino, University of Massachusetts at Amherst, for stylistic revision of the manuscript.

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