New, Easily Removable Poly(ethylene glycol) Supports for the Liquid-Phase Method of Peptide Synthesis

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Poly(ethylene glycol) (PEG) was derivatized with a number of acid-cleavable and photocleavable anchoring groups in order to test the applicability of these derivatives as supports in liquid-phase peptide synthesis. PEG was subjected to rapid and quantitative derivatization with aliphatic and aromatic isocyanates to give the corresponding urethanes. These PEG derivatives were tested for their stability under various conditions employed in peptide synthesis. Urethane derivatives, formed with 6-chlorohexylisocyanate and 1-isocyanato-4-(4-methyl-3-nitrophenoxy)butane, were found to be stable and hence applicable as supports in the liquid-phase method. Derivatization of PEG with 4-(bromomethyl)benzoyl chloride afforded a suitable support which permits easy attachment of the first amino acid residue and acid cleavage of the finished peptides. Replacement of the terminal hydroxyl groups of PEG with amino groups permitted a more facile introduction of the anchoring groups. Reaction of PEG, as well as its terminal amino analogue, with 4-(bromomethyl)-3-nitrobenzoic acid resulted in photolytically removable soluble polymeric supports for the stepwise synthesis of fully protected peptides. Furthermore, the use of 4-(aminomethyl)-3-nitrobenzoyl poly(ethylene glycol) derivatives permits a photolytic release of protected peptide amides under neutral conditions. The applicability of these PEG derivatives in the liquid-phase method is illustrated by the synthesis of a number of test peptides.

The liquid-phase method of peptide synthesis on poly(ethylene glycol) (PEG)[2] introduced in 1971[3,4] combines the strategical features of the classical and solid-phase methods. With the help of kinetic investigations it has been shown that the method essentially follows the classical homogeneous peptide synthesis in its coupling steps.[5,6] Since its introduction, the liquid-phase method has been successfully applied for the synthesis of a number of biologically active peptides and peptide fragments.[7] Originally, the attachment of the C-terminal amino acid to poly(ethylene glycol), which serves as a soluble polymeric protecting group, has been achieved through the formation of an aliphatic ester bond, which is finally cleaved under alkaline reaction conditions.[8] The danger of racemization during alkaline hydrolysis and the low yields of saponification in many cases have prompted us to try new modes of attachment of the C-terminal end of the peptide to the poly(ethylene glycol). Thus for the sake of higher strategic flexibility, the availability of covalent bonds between PEG and the peptide chain with different chemical stability is essential.

The introduction of the 3-nitro-4-(bromomethyl)benzoyl group, which can be removed photolytically and also by catalytic hydrogenolysis, has been observed to offer an attractive alternative method for the removal of peptides from the support.[9-11] By use of this approach, protected peptide acids and amides have been cleaved efficiently from PEG after liquid-phase synthesis.[12,13] Further attempts to derivatize poly(ethylene glycol) with acid-labile and photolabile anchoring groups and the investigation of the use of the resulting PEG derivatives as solubilizing macromolecular C-protecting groups are described in this paper.

Results and Discussion

Derivatization of PEG with Isocyanates Containing Acid-Labile Anchoring Groups. In order to incorporate a benzyl ester grouping between PEG (1) and peptide chain, the reaction of PEG with 4-(bromomethyl)phenyl isocyanate (**2b**) or its (*tert*-butyloxy)carbonyl amino acid ester (**2c**) was investigated. The formation of the urethane derivative **3** proceeded in good yield. However, this polymer was found to be unsuitable for stepwise synthesis of peptides because under the reaction conditions necessary to remove the Boc group (which is also incorporated through a urethane linkage), the urethane linkage to the anchoring group was significantly cleaved in contrast to the reported stability of the phenyl urethane linkage under similar conditions.[14]

The stability of aliphatic urethane derivatives of PEG was investigated in order to test whether the acid instability of such polymer-bound urethane linkages is due to the presence of the aromatic ring or is inherent of the

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(2) Abbreviations: PEG, poly(ethylene glycol); DCC, dicyclohexylcarbodiimide; DMF, dimethylformamide; TFA, trifluoroacetic acid. The abbreviations for amino acids and protecting groups follow the IUPAC-IUB Tentative Rules on Biochemical Nomenclature, *J. Biol. Chem.*, **247**, 977 (1972); *Int. J. Pept. Protein Res.*, **7**, 91 (1975).

(3) M. Mutter, H. Hagenmaier, and E. Bayer, *Angew. Chem.*, **83**, 883 (1971); *Angew. Chem., Int. Ed. Engl.*, **12**, 811 (1971).

(4) E. Bayer and M. Mutter, *Nature*, **237**, 512 (1972).

(5) E. Bayer, M. Mutter, R. Uhmann, H. Mauser, and J. Polster, *J. Am. Chem. Soc.*, **96**, 7333 (1974).

(6) E. Bayer, M. Mutter, J. Polster, and R. Uhmann in "Peptides 1974", Wiley, New York, 1975, p 129.

(7) For a review on the scope of the liquid-phase method of peptide synthesis, see M. Mutter and E. Bayer, "The Peptides: Analysis, Synthesis and Biology", E. Gross and J. Meienhofer, Eds., Vol. II, Academic Press, New York, 1980, p 236.

(8) M. Mutter, R. Uhmann, and E. Bayer, *Justus Liebigs Ann. Chem.*, 901 (1975).

(9) F.-S. Tjoeng, W. Staines, S. St. Pierre, and R. S. Hodges, *Biochim. Biophys. Acta.*, **490**, 489 (1977).

(10) F.-S. Tjoeng, E. K. Tong, and R. S. Hodges, *J. Org. Chem.*, **43**, 4190 (1978).

(11) F.-S. Tjoeng and R. S. Hodges, *Tetrahedron Lett.*, 1273 (1979).

(12) V. N. R. Pillai, M. Mutter, and E. Bayer, *Tetrahedron Lett.*, 3409 (1979).

(13) V. N. R. Pillai, *Synthesis*, 24 (1980).

(14) K. L. Agarwal and H. G. Khorana, *J. Am. Chem. Soc.*, **94**, 3578 (1972).

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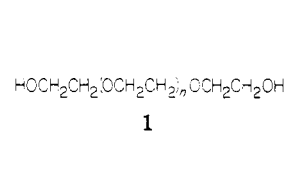

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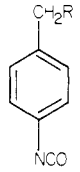
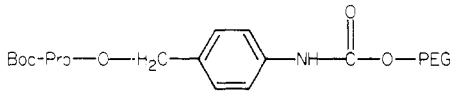
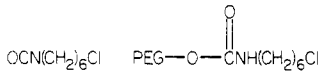


2a, R = H
b, R = Br
c, R = Boc-Pro—O



3[15]

urethane bond itself. To this end, 2-chloroethyl isocyanate was reacted with PEG in quantitative yield. Yet, the incorporation of the first amino acid into this PEG derivative proceeded to an extent of only about 5%; this low yield was due to a β-elimination of HCl from the PEG derivative. The urethane derivative **5** formed between 6-chlorohexyl isocyanate (**4**) and PEG was examined for



4 5

its acid stability to avoid any such side reaction. To this end **5** was treated with 1.2 N HCl in acetic acid for different periods at room temperature. The chlorine content of the polymer was found to be almost independent of the period of treatment. As expected from this result the esterification of Boc-Gly to the polymer **5** proceeded in excellent yield.

A test peptide, H-Ile-Ala-Val-Gly-OH, was synthesized stepwise on this poly(ethylene glycol) derivative, using established procedures.[7,8,16] The results of the amino acid analysis at the various stages of the synthesis (Table I) show that the polymer–urethane linkage remains absolutely stable to the treatment with 1.2 N HCl/HOAc, required for the removal of the Boc group. The tetrapeptide was cleaved from PEG under mild basic conditions and purified by chromatography. The absence of any failure sequences and the homogeneity of the peptide was established by ^{19}F NMR and high-pressure liquid chromatography of the dansylated peptide.

From the foregoing results, derivative **5** can be seen as a suitable polymeric protecting group for peptide synthesis when the danger of racemization of the C-terminal amino acid is low.

Derivatization of PEG with Isocyanates Containing *p*-(Bromomethyl)phenoxy Anchoring Groups. In an attempt to achieve a C-terminal attachment which permits a more selective and mild cleavage of the synthesized peptide, derivatization of PEG with isocyanates containing *p*-(bromomethyl)phenoxy anchoring groups was investigated. Thus PEG was reacted with 1-isocyanato-4-(4-(bromomethyl)phenoxy)butane (**6d**) and the resulting polymeric urethane derivative **7**, which was formed in quantitative yield, was tested for its use as a C-terminal soluble polymeric protecting support in peptide synthesis.

The isocyanate **6d** was prepared as depicted in Scheme I, starting from *p*-cresol.

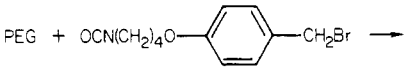
After esterification of **7** with Boc-Gly, the benzyl ester

(15) In the structural formulas used throughout this article, PEG is intended to mean the bivalent radical, $-CH_2CH_2(OCH_2CH_2)_nOCH_2CH_2-$. Thus for example, PEG–OX means $XO-CH_2CH_2-(OCH_2CH_2)_nOCH_2CH_2-OX$.

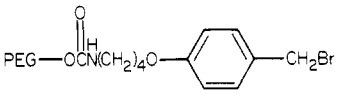
(16) M. Mutter and E. Bayer, *Angew. Chem.*, **86**, 101 (1974); *Angew. Chem., Int. Ed. Engl.*, 13, 88 (1974).

Table I. Amino Acid Analysis During the Synthesis of the Test Peptide H-Ile-Ala-Val-Gly-OH on Support 5 (mol wt 6000)

	capacity, mmol/g			
synthesis step	Gly	Val	Ala	Ile
esterification with Boc-Gly-OH	0.137			
monopeptide → Boc dipeptide	0.136	0.130		
tripeptide → Boc tetrapeptide	0.136	0.130	0.146	0.127



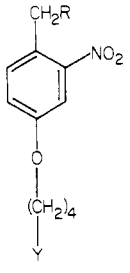
6d

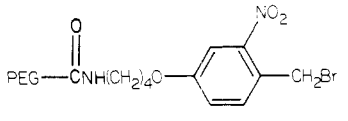


7

turned out to be unstable under the conditions of the Boc cleavage. This indicates an increased acid lability of the benzyl ester group in a position para to the ether function.[17]

A nitro group was introduced in a position ortho to the ester linkage[17] in order to increase the acid stability of the benzyl ester bond in the above-mentioned PEG derivative. Thus poly(ethylene glycol) was derivatized with 1-isocyanato-4-(4-(bromomethyl)-3-nitrophenoxy)butane (**8d**) to give the urethane **9**, containing the *o*-nitrobenzyl bromide grouping. Isocyanate **8d** was prepared analogously to **6d**, starting from 4-methyl-3-nitrophenol.





9

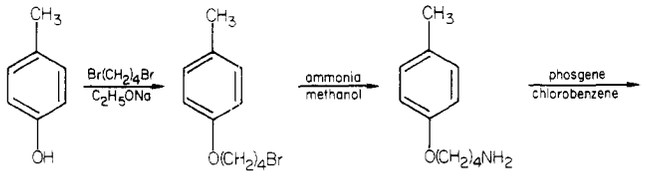
8a, R = H; Y = Br
b, R = H; Y = NH_2
c, R = H; Y = NCO
d, R = Br; Y = NCO

PEG derivative **9** was tested for its applicability as a soluble support for peptide synthesis. Boc-Gly was esterified to the polymer by the reaction of its silver salt. A capacity of 0.051 mmol of Gly per gram of polymer could be achieved in a reaction period of 24 h. The removal of the Boc group proceeded without any loss in the loading of the polymer, as evidenced by potentiometric titration and amino acid analysis. During the complete synthesis of the test peptide, the amino acid capacity of the support was found to remain constant (Table II), confirming that the nitro group induces an acid-stabilizing effect on the benzyl ester linkage.

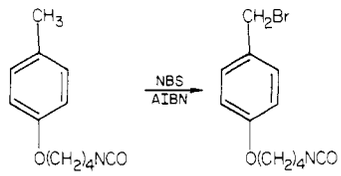
The synthesized peptide was cleaved quantitatively from the support by treatment with (i) HBr in acetic acid, (ii) HF, and (iii) by catalytic hydrogenolysis. It is important to note that the benzyl ester without the *o*-nitro group under the same conditions could not be cleaved by catalytic hydrogenolysis. These observations suggest that PEG derivative **9** is a suitable soluble support for the stepwise synthesis of peptides. The possibility of cleavage by em-

(17) R. B. Merrifield, *J. Am. Chem. Soc.*, **85**, 2149 (1963).

Scheme I



6a 6b



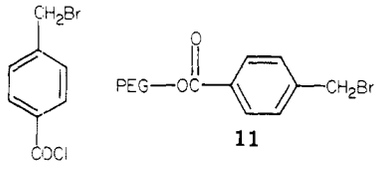
6c 6d

Table II. Synthesis of the Test Peptide H-Ile-Ala-Val-Gly-OH on the Support 9 (mol wt 6000)

reaction step	% conversion	capacity of Gly on the support, mmol/g
esterification with Boc-Gly-Ag	36	0.051
monopeptide → Boc dipeptide	96	0.050
dipeptide → Boc tripeptide	98	0.054
tripeptide → Boc tetrapeptide	98	0.053

ploying different acidic conditions or by catalytic hydrogenolysis is definitely an advantage over the originally employed aliphatic PEG–ester bond which undergoes cleavage only under alkaline reaction conditions.

4-(Bromomethyl)benzoyl–Poly(ethylene glycol) as a Soluble Support. The successful application of the benzyl anchoring group for solid-phase peptide synthesis by Merrifield and co-workers[18] prompted an investigation of this group for the PEG-supported peptide synthesis. Thus, poly(ethylene glycols) of varying molecular weights were found to react with 4-(bromomethyl)benzoyl chloride (10) in quantitative yields to give 4-(bromomethyl)-benzoyl–poly(ethylene glycols) (11). Esterification of PEG with 4-(bromomethyl)benzoic acid has been reported by Künzi.[19]



10 11

N-Protected C-terminal amino acids were attached to the PEG derivative 11 by esterification in the presence of triethylamine, following the procedure described by Merrifield[18] or via the cesium salt.[20] The conditions and yields of the reaction in the case of a number of amino acid derivatives are presented in Table III.

During the stepwise elongation of peptide chains on this PEG support, no loss of peptide was observed, suggesting the support to be stable under the usual conditions employed in the peptide synthesis. Final cleavage of the finished peptide from the support was effected by treatment with HBr/TFA, HF, or by catalytic hydrogenolysis.

(18) R. B. Merrifield, *J. Am. Chem. Soc.*, **86**, 304 (1964).
(19) H. Künzi, Ger. Patent 24356422 (1976).
(20) B. F. Gisin, *Helv. Chim. Acta*, **56**, 1476 (1973).

Table III. Attachment of the First Amino Acid to the Support 11 (mol wt 6000)

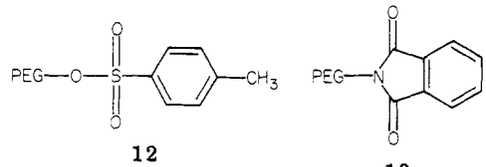
	cesium salt method		esterification with triethylamine	
amino acid derivative	period, h	loading, %	period, h	loading, %
Boc-Ala-OH	24	98	30	100
Boc-Gly-OH	24	105	24	98
Boc-Phe-OH	24	92	36	95
Boc-Val-OH	24	97	24	100
Boc-Asn(MbH)-OH	48	65		
Boc-Gln(MbH)-OH	48	85		
Boc-Thr(OBzl)-OH	48	75		

Table IV. Cleavage of H-Phe-Leu-Ala-Val-OH from the 4-(Bromomethyl)benzoyl Poly(ethylene glycol) Support 11 (mol wt 6000)

cleavage procedure	reaction period, h	% yield
HBr/TFA	1.5	75
HF	1.5	60
H_2/Pd	48	100
H_2/Pd, 50 bar	12	100
H_2/Pd, homogeneous	12	100

The conditions and yields of cleavage of the test peptide, H-Phe-Leu-Ala-Val-OH from PEG under different conditions are given in Table IV.

Conversion of Terminal Hydroxyl Groups of PEG to Amino Groups. Compared to the terminal hydroxyl groups of PEG, amino groups on PEG offered a more convenient attachment of the anchoring groups. "Amino PEG" (14) was prepared from PEG by a two-step polymer analogous reaction.[21] PEG on treatment with tosyl chloride in the presence of pyridine in CH_2Cl_2 afforded the tosylate 12. This was reacted with potassium phthalimide to give PEG–phthalimide (13) which on hydrolysis with hydrazine hydrate gave "amino PEG" (14). An overall conversion of 80% of the terminal hydroxyl groups of PEG could be achieved in this reaction. Alternative methods of introduction of terminal amino groups in PEG have recently been described.[22,23]



12 13

PEG-NH_2

14

In addition to providing a more facile attachment of anchoring groups, the access to "amino PEG" has also permitted a quantitative DCC-mediated coupling of C-terminal amino acids to PEG. The subsequent liquid-phase synthesis on these solubilizing amide supports permits the conformational analysis of a number of homooligopeptides.[24]

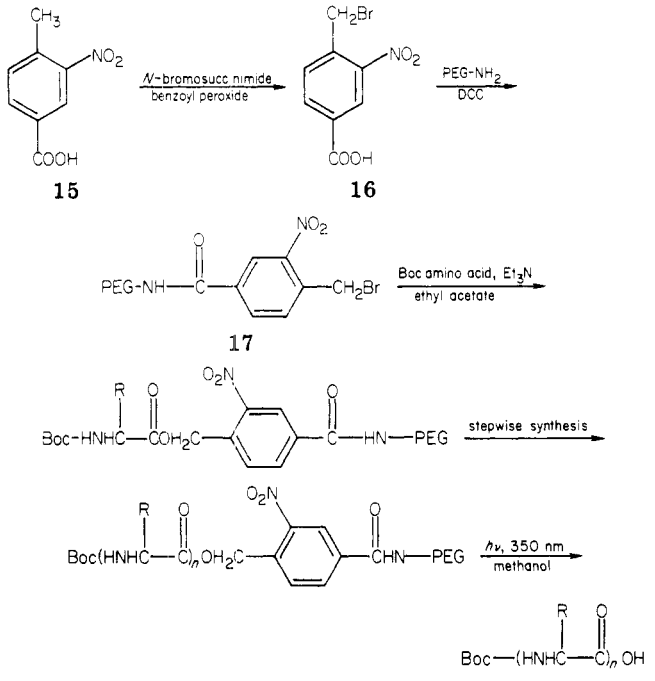
(21) M. Mutter, *Tetrahedron Lett.*, 2839 (1978).
(22) A. F. Bueckmann, M. Morr, and G. Johansson, *Makromol. Chem.*, in press.
(23) K. Geckeler, *Polym. Bull.* 1, 427 (1979).
(24) C. Toniolo, G. M. Bonora, and M. Mutter, *J. Am. Chem. Soc.*, **101**, 450 (1979); M. Mutter, *Macromolecules*, **10**, 1413 (1977); C. Toniolo, G. M. Bonora, and M. Mutter, *Int. J. Biol. Macromolecules*, **1**, 188 (1979); S. A. Rahman, H. Anzinger, and M. Mutter, *Biopolymers*, **19**, 173 (1980); C. Toniolo, G. M. Bonora, V. N. R. Pillai, and M. Mutter, *Macromolecules*, **13**, 772 (1980); C. Toniolo, G. M. Bonora, V. N. R. Pillai, and M. Mutter, *Gazz. Chim. Ital.*, in press; C. Toniolo, G. M. Bonora, M. Mutter, and V. N. R. Pillai, *Makromol. Chem.*, in press.

Table V. Photolytic Release of Protected Peptide Acids from the Support 17 (mol wt 6000)

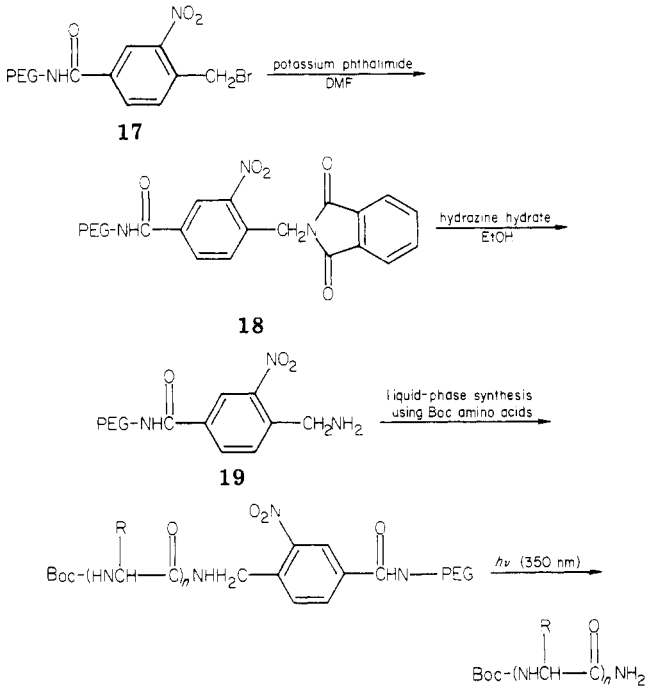
peptide	duration of photolysis for cleavage, h	cleavage yield,[a] %	amino acid analysis
Boc-Leu-Ala-Gly-Val-OH	14	95	Leu, 1.00; Ala, 0.95; Gly, 1.03; Val, 1.00
Boc-Leu-Arg(Tos)-Pro-Gly-OH	18	86	Leu, 1.00; Arg, 0.97; Pro, 1.00; Gly, 0.98
Boc-Lys(Z)-Gly-OH	12	85	Lys, 1.03; Gly, 0.96
Boc-Ser(Bzl)-Tyr(Bzl)-Gly-OH	12	90	Ser, 1.02; Tyr, 0.87; Gly, 1.04
Boc-Ala-Gly-Val-OH	18	95	Ala, 0.97; Gly, 1.05; Val, 1.00

[a] Based on amino acid analysis of the peptide remaining on the polymer after removal of the cleaved peptide.

Scheme II



Scheme III



[3-Nitro-4-(bromomethyl)benzoyl]amino-Poly(ethylene glycol) for the Synthesis of Fully Protected Peptide Acids. The photolabile 2-nitrobenzyl anchoring group has been successfully applied in solid-phase[25-27] and liquid-phase[9-11] methods for the preparation of fully protected peptide acids. In the liquid-phase method, the incorporation of this anchoring group has been achieved through the reaction of PEG with 3-nitro-4-(bromomethyl)benzoic acid[9-11]. A more convenient and quantitative derivatization was achieved by the reaction of "amino PEG" with 3-nitro-4-(bromomethyl)benzoic acid (**16**). 3-Nitro-4-(bromomethyl)benzoic acid was prepared in 77% yield by bromination of 3-nitro-4-methylbenzoic acid (**15**) in the presence of benzoyl peroxide. The photosensitive PEG derivative **17** formed was tested for its use in liquid-phase peptide synthesis. The C-terminal amino acid was attached to **17** in about 90% yield by direct esterification in presence of triethylamine in ethyl acetate. Subsequent synthetic steps followed the general procedure of the liquid-phase method. The anchoring group was found to be stable to all conditions of peptide synthesis and permitted the photolytic release of the protected peptide acids in almost quantitative yields under neutral conditions (Scheme II). The results of the photolyses are summarized in Table V.

For the photolysis, a methanol solution of the peptide–PEG support was irradiated at 350 nm under nitrogen atmosphere. A 40% copper sulfate solution was used to filter out wavelengths below 320 nm, which can be harmful to aromatic amino acids like Tyr and Trp. In addition to the Boc group, the *O*-benzyl group, the (benzyloxy)carbonyl group and the tosyl group were not destroyed by photolysis under these conditions.

Synthesis of Protected Peptide Amides. Conversion of the 4-bromomethyl group in the photosensitive PEG support **17** to a 4-aminomethyl group by reaction with potassium phthalimide followed by hydrolysis afforded [3-nitro-4-(aminomethyl)benzoyl]amino–poly(ethylene glycol) (**19**). After liquid-phase synthesis of several test peptides, fully protected amino acid or peptide amides were obtained by photolytic cleavage as outlined in Scheme III. The results of the irradiation of a number of PEG–peptide supports are given in Table VI.

In summary, these investigations provide several methodological improvements to liquid-phase synthesis of peptides on a poly(ethylene glycol) support. The methods of attachment of the first amino acid to the polymer and the final cleavage of the synthesized peptide have been significantly improved. The danger of racemization during the alkaline reaction conditions employed for the splitting of the peptide from the PEG–peptide support can be avoided by using photolabile or acid-labile anchoring groups between PEG and peptide. A new soluble polymeric support which permits synthesis and final photolytic removal of peptide amides under neutral conditions was developed. This method has potential applications in the synthesis of peptide hormones, particularly of those with hindered C-terminal amino acid residues.

(25) D. H. Rich and S. K. Gurwara, *J. Chem. Soc., Chem. Commun.*, 610 (1973).
(26) D. H. Rich and S. K. Gurwara, *J. Am. Chem. Soc.*, **97**, 1575 (1975).
(27) D. H. Rich and S. K. Gurwara, *Tetrahedron Lett.*, 301 (1975).

Table VI. Photolytic Cleavage of Peptide and Amino Acid Amides from the Support 19 (mol wt 6000)

amino acid or peptide amide	photolysis period for cleavage, h	% yield[a]	amino acid analysis
Boc-Val-NH_2	16	93	
Boc-Gly-NH_2	12	95	
Boc-Pro-NH_2	12	93	
Boc-Pro-Val-NH_2	18	97	Pro, 0.90; Val, 1.04
Boc-Lys(Z)-Gly-NH_2	12	90	Lys, 1.02; Gly, 0.97
Boc-Leu-Ala-Gly-Val-NH_2	16	95	Leu, 1.00; Ala, 0.95; Gly, 1.02; Val, 0.97

[a] Based on amino acid analysis of the residue remaining on the polymer after removal of the cleaved peptide.

Experimental Section

Melting points are uncorrected. Infrared spectra were taken on a Perkin-Elmer Model 221 spectrophotometer, using KBr pellets or a film between NaCl plates. Thin-layer chromatography was performed on precoated silica gel plates (Merck F 254). NMR spectra were run in $CDCl_3$ or CCl_4 on a Varian EM-360 spectrometer and on a Brucker HFX-9 spectrometer with Me_4Si as internal standard. Column chromatography was done on Kieselgel (pore size ≤ 0.063 mm) or on Sephadex LH-20. Amino acid analyses were obtained on a Biotronik amino acid analyser (chromatography system LC 6000 E) after hydrolysis of the samples with 6 N HCl in sealed evacuated tubes at 110 °C for 24 h for cleaved peptides and 24–36 h for PEG-bound peptides. The irradiations were carried out with a Hanovia 679 A-36 high-pressure mercury lamp housed in a water-cooled quartz immersion well.

Derivatization of PEG with 6-Chlorohexyl Isocyanate. Preparation of Urethane 5. 6-Chlorohexyl isocyanate (2.0 g, 12.4 mmol) was dissolved in minimum amount of methylene chloride. This was added to a solution of PEG (mol wt 6000; 12 g, 2 mmol) in minimum methylene chloride. The reaction mixture was stirred overnight at room temperature and the product was precipitated by the dropwise addition of ether with stirring. The precipitate was filtered and washed several times with ether, recrystallized from ethanol by the slow addition of ether with rapid stirring, filtered, washed with ether, and dried under vacuum to yield 11.5 g (91%) of **5**; Cl, 1.12%.

Liquid-Phase Synthesis of H-Ile-Ala-Val-Gly-OH on the PEG Derivative 5. A mixture of **5** (6.0 g, 0.95 mmol), Boc-Gly (1.75 g, 10 mmol), a 40% solution of benzyltrimethylammonium hydroxide (4 mL, 9.5 mmol), and absolute ethanol (70 mL) in presence of a little sodium iodide (as catalyst) was heated under reflux for 50 h. The solvent was then removed under vacuum and the residue recrystallized from ethanol by the addition of ether. The recrystallization was repeated twice till the product was found to be absolutely free from any low-molecular-weight impurities; yield, 5.8 g (94%). Hydrolysis of the polymer and amino acid analysis indicated a loading of 0.137 mmol of Gly/g of polymer. Deprotection of the amino group and further stepwise coupling of the amino acids as their symmetrical anhydrides were performed, following the established procedures for the liquid-phase method of peptide synthesis.[8] Each coupling step was controlled by the ninhydrin test and amino acid analysis. Yield of the polymer-bound protected tetrapeptide Boc-Ile-Ala-Val-Gly-O$(CH_2)_6$HNCOO-PEG was 4.5 g. Amino acid analysis gave Gly:Val:Ala:Ile = 1.00:0.97:1.08:0.94. The peptide was cleaved from the polymer by basic hydrolysis. A solution of 1 g of PEG-bound peptide in 10 mL of water and 0.5 mL of 1 N NaOH was stirred for 3 h at room temperature. This was then neutralized with 1 N HCl and evaporated in vacuo to dryness. The residue was taken up in water (10 mL) and extracted with $CHCl_3/CH_2Cl_2$ (1:1) (3 × 20 mL). The crude peptide which was obtained by lyophilization of the aqueous phase was purified by chromatography on a Sephadex G 10 column (40 × 3 cm). The ninhydrin-positive fractions were collected and freeze-dried to yield 34 mg of H-Ile-Ala-Val-Gly-OH: mp 243–244 °C dec; R_f 0.46 (*n*-butanol–acetic acid–water, 3:1:1), 0.77 (chloroform–methanol–acetic acid–water, 8:4:2:1); amino acid analysis, Gly:Val:Ala:Ile = 1.00:1.06:0.99:1.01).

1-Bromo-4-(4-methylphenoxy)butane (6a). To a solution of *p*-cresol (7.0 g, 63 mmol) and 1,4-dibromobutane (65 g, 304 mmol) in 30 mL of absolute ethanol was added 4.3 g (64 mmol) of C_2H_5ONa and the mixture was heated under reflux for 10 h. The solvent was then removed in vacuo. The residue was extracted with C_2H_5OAc and the extract was distilled in vacuo to remove the excess 1,4-dibromobutane. The remaining brown oil was then purified by distillation to give **6a** (9.5 g, 62%) as a colorless oil: bp 102 °C (0.4 atm); IR (film) 1615 (aromatic), 1100 (ether) cm^{-1}; NMR ($CDCl_3$) δ 7.00 (A_2B_2, 4 H, aromatic), 3.97 (t, J = 6 Hz, 2 H, OCH_2), 3.50 (t, J = 6 Hz, 2 H, CH_2Br), 2.35 (s, 3 H, CH_3), 2.00 (m, 4 H, CH_2CH_2). Anal. Calcd for $C_{11}H_{15}OBr$: C, 54.34; H, 6.22; Br, 32.86. Found: C, 54.14; H, 5.83; Br, 30.84.

1-Amino-4-(4-methylphenoxy)butane (6b). Compound **6a** (7.0 g, 28.8 mmol) was dissolved in methanolic ammonia (300 mL) and kept for 3 days at room temperature. The residual oil obtained after the removal of the solvent was taken up in C_2H_5OAc and treated with acid. After neutralization and extraction with C_2H_5OAc, the extract was distilled to remove the solvent to obtain **6b** (4.7 g, 91%) as a bright yellow oil: R_f 0.56 (*n*-butanol–acetic acid–water, 3:1:1); IR (film) 3400 (NH), 3250 (NH), 1615 (aromatic) cm^{-1}.

1-Isocyanato-4-(4-methylphenoxy)butane (6c). A solution of compound **6b** (4.7 g, 26.2 mmol) in a minimum amount of dry benzene was added dropwise to a saturated solution of phosgene in chlorobenzene at −5 °C. The reaction mixture was then heated under reflux for 4 h. After cooling, the yellow solution was filtered to remove the insoluble materials and the filtrate was distilled in vacuo to remove the solvent. The residual yellow oil was purified by distillation to give **6c** (3.5 g, 65%) as a colorless viscous oil, bp 103–107 °C (0.45 atm). On prolonged keeping at −20 °C the substance was found to crystallize as fine needles: mp 29.5–30.5 °C; IR (film) 2250 (NCO), 1605 (aromatic), 1100 (ether), 815 (*p*-substituted aromatic) cm^{-1}; NMR (CCl_4) δ 6.92 (A_2B_2, 4 H, aromatic), 3.95 (t, J = 6 Hz, 2 H, OCH_2), 3.45 (t, J = 6 Hz, 2 H, CH_2NCO), 2.47 (s, 3 H, CH_3), 1.95 (m, 4 H, CH_2CH_2). Anal. Calcd for $C_{12}H_{15}NO_2$: C, 70.22; H, 7.37; N, 6.82. Found: C, 70.24; H, 7.36; N, 6.61. A urea derivative of **6c** was obtained by reaction with water, mp 152–152.5 °C.

1-Isocyanato-4-(4-(bromomethyl)phenoxy)butane (6d). A solution of **6c** (410 mg, 2 mmol), powdered *N*-bromosuccinimide (360 mg, 2 mmol), and a pinch of azobis(isobutyronitrile) in 50 mL of dry carbon tetrachloride was heated under reflux for 90 min. The precipitated succinimide was removed by filtration. Solvent was distilled off from the filtrate to give **6d**; NMR (CCl_4) δ 6.95 (A_2B_2, 4 H, aromatic), 4.40 (s, 2 H, CH_2Br), 3.95 (t, J = 6 Hz, 2 H, OCH_2), 3.38 (t, J = 6 Hz, 2 H, CH_2NCO), 1.83 (m, 4 H, CH_2CH_2).

Derivatization of PEG with 1-Isocyanato-4-(4-(bromomethyl)phenoxy)butane. Preparation of Urethane 7. The isocyanate **6d** from the above step was allowed to react with PEG (mol wt 6000; 5 g, 0.83 mmol). Workup of the reaction mixture as in the preparation of **5** afforded **7** (4.9 g, 98%): IR (KBr) 3350 (NH), 1710 (urethane C=O), 1600 (aromatic); Br, 2.63%.

Coupling of Boc-Gly with Urethane 7. A mixture of urethane **7** (4.9 g, 0.75 mmol), Boc-Gly (3.5 g, 20 mmol), a 40% solution of benzyltrimethylammonium hydroxide (4 mL, 9.5 mmol), and a pinch of NaI was heated under reflux for 50 h. The reaction mixture was worked up as in the case of the coupling of urethane **5** to yield 4.6 g. Amino acid analysis after total hydrolysis of the polymer indicated a capacity of 0.04 mmol of Gly/g of polymer.

1-Bromo-4-(4-methyl-3-nitrophenoxy)butane (8a). To a solution of 4-methyl-3-nitrophenol[28] (19.8 g, 129 mmol) and 1,4-dibromobutane (138 g, 650 mmol) in 150 mL of absolute ethanol was added 10 g (147 mmol) of C_2H_5ONa and the mixture was heated under reflux for 10 h. Workup of the reaction mixture as in the case of **6a** afforded **8a** (30.5 g, 82%) as a bright yellow oil: R_f 0.75 (benzene); n^{23}_D 1.5445; IR 1625 (aromatic), 1520 (NO_2),

(28) M. Copisarow, *J. Chem. Soc.*, 251 (1929).

1350 (NO_2), 1040 (ether) cm^{-1}; NMR ($CDCl_3$) δ 7.39–6.80 (m, ABC, 3 H, aromatic), 3.93 (t, J = 6 Hz, 2 H, OCH_2), 3.39 (t, J = 6 Hz, 2 H, CH_2Br), 2.49 (s, 3 H, CH_3). Anal. Calcd for $C_{11}H_{14}NO_3Br$: C, 45.85; H, 4.90; N, 4.86; Br, 27.73. Found: C, 45.94; H, 4.99; N, 5.13; Br, 27.83.

1-Amino-4-(4-methyl-3-nitrophenoxy)butane (8b). Compound **8a** (7.0 g, 24 mmol) was reacted with methanolic ammonia as in the case of the reaction of **6a**. Workup in the same manner afforded **8b** (5.03 g, 93%) as a bright yellow oil: R_f 0.5 (*n*-butanol–acetic acid–water, 3:1:1); IR 3450 (NH), 3300 (NH), 1620 (aromatic), 1520 (NO_2), 1350 (NO_2) cm^{-1}.

1-Isocyanato-4-(4-methyl-3-nitrophenoxy)butane (8c). Compound **8b** (5.0 g, 22.3 mmol) was allowed to react with phosgene as in the case of **6b**. Similar workup afforded **8c** (3.1 g, 55%) as a viscous bright yellow oil: bp 139–143 °C (0.15 atm); IR 2260 (NCO), 1625 (aromatic), 1530 (NO_2), 1350 (NO_2); NMR ($CDCl_3$) δ 7.46–6.96 (m, 3 H, aromatic), 4.01 (t, J = 6 Hz, 2 H, CH_2O), 3.42 (t, J = 6 Hz, 2 H, CH_2NCO), 2.47 (s, 3 H, CH_3), 1.83 (m, 4 H, CH_2CH_2). Anal. Calcd for $C_{12}H_{14}N_2O_4$: C, 57.57; H, 5.64; N, 11.20. Found: C, 57.53; H, 5.78; N, 11.26. The symmetrical urea derivative of **8c** was obtained by reaction with water, mp 126–127 °C.

1-Isocyanato-4-(4-(bromomethyl)-3-nitrophenoxy)butane (8d). Compound **8c** (500 mg, 2 mmol) was allowed to react with *N*-bromosuccinimide as in the case of **6c**. Similar workup afforded **8d**: IR 2260 (NCO); NMR (CCl_4), CH_2Br/CH_3 protons = 27:12.

Derivatization of PEG with 1-Isocyanato-4-(4-(bromomethyl)-3-nitrophenoxy)butane. Preparation of Urethane 9. The isocyanate **8d** from the above step was allowed to react with PEG (mol wt 6000; 5 g, 0.83 mmol). Workup as in the preparation of **6a** afforded **9** (4.8 g, 96%): IR (KBr) 3440 (NH), 1720 (urethane C=O), 1620 (aromatic), 1530 (NO_2), 1345 (NO_2) cm^{-1}; Br, 1.14%.

Synthesis of the Peptide Boc-Ile-Ala-Val-Gly-OH on the PEG Derivative 9. PEG derivative **9** (4.5 g, 0.68 mmol) and Boc-Gly-Ag (3.7 g, 6.3 mmol) were suspended in dry benzene (70 mL) and the suspension was heated under reflux in the dark for 24 h. The precipitated AgBr and excess Boc-Gly-Ag were filtered off. The solvent from the filtrate was then removed under vacuum and the residue was recrystallized twice from ethanol by the addition of ether to yield 4.5 g (98%); amino acid analysis, 0.051 mmol of Gly/g of polymer. Boc-Val, Boc-Ala, and Boc-Ile were then stepwise coupled to this, following the established procedures of the liquid-phase method. The results of analysis during each step are given in Table II.

Splitting of the Peptide as H-Ile-Ala-Val-Gly-OH. (i) With HBr/HOAc. The poly(ethylene glycol)–peptide support (60 mg) from the above step was dissolved in 10 mL of a 40% solution of HBr in acetic acid. The solution was stirred at room temperature for 90 min, and then the solvent was removed under vacuum. The residual oil was treated with 50 mL of absolute ether. The precipitated peptide together with the polymer was filtered and dried. TLC indicated a homogeneous product, which was ninhydrin and chlorine–tolidine positive and was identical with an authentic test peptide; R_f 0.46 (*n*-butanol–acetic acid–water, 3:1:1), 0.77 (chloroform–methanol–acetic acid–water, 60:45:6:14), 0.54 (pyridine–ethanol–benzene–water, 8:4:2:1).

(ii) By Catalytic Hydrogenation. Polymer–tetrapeptide (40 mg) was dissolved in methanol (30 mL) and the pH adjusted to 5 by the addition of a few drops of acetic acid. This was shaken overnight in a hydrogen atmosphere in the presence of Pd/C. After filtration and removal of the solvent a residue was obtained, which on TLC indicated two ninhydrin- and chlorine–tolidine-positive spots, suggesting an incomplete (~60%) splitting; R_f 0.00 and 0.46 (*n*-butanol–acetic acid–water, 3:1:1).

(iii) With HF. Polymer–tetrapeptide (150 mg) was allowed to remain in liquid HF (7 mL) at room temperature for 75 min. The excess HF was blown off through a NaOH solution with dry nitrogen. The residue was dried in vacuo. TLC of this residue indicated a homogeneous product; R_f 0.46 (*n*-butanol–acetic acid–water, 3:1:1), 0.76 (chloroform–methanol–acetic acid–water, 60:45:6:14), 0.54 (pyridine–ethanol–benzene–water, 8:4:2:1).

4(Bromomethyl)benzoyl Chloride (10). 4-(Bromomethyl)benzoic acid[26] (8.6 g, 40 mmol) and thionyl chloride (11.8 g, 100 mmol) were heated under reflux for 5 h. The excess thionyl chloride was then distilled off. The residue was recrystallized from petroleum ether (bp 60–90 °C): yield 8.2 g (87%); mp 56 °C (lit.[29] mp 56 °C).

Derivatization of PEG with 4-(Bromomethyl)benzoyl Chloride. Preparation of 4-(Bromomethyl)benzoyl–Poly(ethylene glycol) (11). PEG (mol wt 6000; 15 g, 5 mmol) and 4-(bromomethyl)benzoyl chloride (4.7 g, 20 mmol) were dissolved in dry toluene (120 mL) and the solution was heated at reflux for 8 h under a continuous slow stream of nitrogen to drive off the HCl formed. After cooling, the solution was concentrated by rotary evaporation and the PEG derivative precipitated by slow addition of ether with stirring. The precipitate was filtered, washed several times with ether, reprecipitated from EtOH by the addition of ether, filtered, washed, and dried in vacuo: yield 14.3 g; Br, 2.4%.

Attachment of the First Amino Acid to 4-(Bromomethyl)benzoyl–Poly(ethylene glycol) (11). Method A. Direct Esterification. The following procedure is typical of the method.[18] Compound 11 (6.0 g, 2 mmol) was dissolved in 50 mL of ethyl acetate. Boc-Ala (0.38 g, 2 mmol) and triethylamine (0.18 g, 1.8 mmol) were added successively to the solution. This was then heated gently under reflux for 48 h. After the solution was cooled to room temperature, ether (600 mL) was slowly added with stirring until precipitation was complete. The stirring was continued for another 0.5 h. The precipitate was then filtered and washed with ether until no traces of Boc-Ala were detected by TLC. The precipitate was dried in vacuo to give 5.9 g of Boc-Ala esterified PEG derivative. The yields and the conditions of the reaction with a number of other Boc amino acids are presented in Table III.

Method B. Cesium Salt Method.[20] The Boc amino acid (10 mmol) was suspended in about 50 mL of water. To this was added cesium carbonate (5 mmol). After the evolution of CO_2 had finished, the solution was frozen and lyophilized. The resulting solid mass was then dried over P_2O_5 in vacuo. A fourfold molar excess of the cesium salt of the Boc amino acid prepared in this manner was dissolved in a minimum quantity of DMF. This solution was then added to the bromobenzyl–poly(ethylene glycol) derivative dissolved in a little DMF. The mixture was then stirred while kept at 60 °C by means of a thermostated water bath for varying periods in the case of the different amino acids (Table III). The solution was then cooled and ether was added to precipitate the PEG derivative. The precipitate was dissolved in methylene chloride and the solution was centrifuged to remove the insoluble CsBr and the excess cesium salt of the amino acid. The clear solution was concentrated and again precipitated by slow addition of ether. The precipitate was filtered and recrystallized twice from methylene chloride by the addition of ether until it was homogeneous to TLC. The C-terminal amino acid capacities of the PEG derivatives were then determined by amino acid analysis after hydrolysis (Table III).

Peptide Synthesis on the Bromobenzyl–PEG Derivative. To the bromobenzyl–PEG derivative to which the C-terminal amino acid of the desired peptide had been attached, the next amino acid derivatives were coupled stepwise, following the established methodology for the liquid-phase method. All couplings were made via the in situ symmetrical anhydride method, using the N-protected amino acid and DCC in a 2:1 molar ratio. The amino acid derivative and DCC were separately dissolved in CH_2Cl_2 and cooled to 0 °C. Both solutions were then combined and allowed to stand at this temperature for 30 min. The solution containing the symmetrical anhydride of the Boc amino acid was filtered directly into the flask containing the deprotected peptide support dissolved in a suitable solvent. Then the mixture was neutralized with *N*-methylmorpholine and stirred at room temperature for 1 h. The completion of coupling was tested by the fluram reagent. After the coupling, the solution was concentrated by evaporation in vacuo and the peptide–PEG support was precipitated by the addition of ether, filtered, washed with ether, and dried in vacuo. Precipitation was repeated until the support was free from any Boc amino acid as evidenced by TLC.

Splitting of the Finished Peptides from the Bromobenzyl–PEG Support. General Procedure. A. With HBr. Peptide–PEG support (1 g) was dissolved in 10 mL of TFA and this solution was treated with 1 mL of anisole. Gaseous HBr was

(29) J. Titley, *J. Chem. Soc.*, 2581 (1928).

passed through this solution with constant stirring for 30 min. Nitrogen gas was then passed through the solution for another 30 min. The resulting solution was then evaporated to dryness in vacuo. The residue was treated with methanol and again evaporated. The residue was then purified by gel filtration of the methanol solution through a Sephadex LH 20 column.

B. Splitting with HF. To 1 g of the polymer–peptide and 1 mL of anisole in a polypropylene flask, a flow of HF was led through an apparatus made of polypropylene or Teflon. This reaction mixture was stirred for 90 min at 0 °C. Then the HF flow was stopped and at room temperature the remaining HF in the reactions mixture was removed through a stream of nitrogen. The residue was then dried in vacuo and the peptide was isolated and purified by chromatography of a methanol solution on a Sephadex LH 20 column.

C. By Heterogeneous Catalytic Hydrogenation. To a solution of 1 g of the peptide–PEG support, 200 mg of palladized charcoal (9.7%) was added. The hydrogenation apparatus was flushed twice with hydrogen and the reaction mixture was then shaken in the hydrogen atmosphere for 48 h. The catalyst was then filtered off and the solvent was removed from the filtrate by rotary evaporation. The peptide was separated and purified from the residue by chromatography as usual.

D. By Homogeneous Catalytic Hydrogenation. To 15 mL of a 2% solution of polyvinylpyrrolidone in isopropanol was added 1 mL of a 1% aqueous solution of $PdCl_2$. This was then diluted with 15 mL of water and treated with 4% sodium bicarbonate solution. To this catalyst solution was added 0.5 g of the peptide–PEG support and the reaction mixture was stirred for 30 min in hydrogen atmosphere. The reaction mixture was worked up as in the above case.

Replacement of the Terminal Groups of PEG with Amino Groups. Synthesis of "Amino PEG". (i) PEG–Tosylate (12). Poly(ethylene glycol) (mol wt 6000; 20 g), which had been previously dried in vacuo at 80 °C for 4 h, was dissolved in methylene chloride (100 mL). *p*-Toluenesulfonyl chloride (36 g) and pyridine (6 mL) were added to this solution and the mixture was stirred in a nitrogen atmosphere overnight. The reaction mixture was concentrated to about 30% of its original volume and stirred for another 2 h at room temperature. The polymer was precipitated from this solution by dropwise addition of ether (300 mL) with rapid stirring. Stirring was continued for another 15 min, keeping the reaction mixture in an ice bath. The precipitate was filtered and washed several times with ether. The product was crystallized from ethanol filtered, washed, and dried under vacuum to yield 18 g, R_f 0.00.

(ii) PEG–Phthalimide (13). PEG–tosylate (**12**, 18 g) and potassium phthalimide (8 g) in DMF (50 mL) were heated under reflux in a nitrogen atmosphere for 4 h. The precipitate was then filtered off and to the clear filtrate was added ether slowly with stirring. Stirring was continued for another 30 min in an ice bath after the precipitation was complete. The precipitate was filtered, washed with ether, and then digested with 50 mL of CH_2Cl_2. The insoluble impurities were filtered off and the filtrate was concentrated. The PEG–phthalimide was then precipitated from the CH_2Cl_2 solution by addition of ether. The precipitate was filtered, washed with ether, and dried in vacuo to give **13**: yield 16 g; R_f 0.00.

(iii) "Amino PEG" (14). PEG–phthalimide (**13**, 16 g) and hydrazine hydrate (8 mL) in absolute ethanol (60 mL) were heated under reflux for 12 h. After cooling to room temperature the product was precipitated by the dropwise addition of ether to the solution. The precipitate was filtered and redissolved in CH_2Cl_2 (30 mL), and the insoluble impurities were removed by filtration. The filtrate was concentrated and ether added slowly to the filtrate. The precipitated "amino PEG" was filtered, washed, and dried in vacuo to yield 15 g of **14**, R_f 0.00. Microtitration with HCl indicated the amino group capacity of the polymer to be 0.28 mequiv/g corresponding to about 85% conversion of the terminal hydroxyl groups.

3-Nitro-4-(bromomethyl)benzoic Acid (16). A mixture of 3-nitro-4-methylbenzoic acid (Aldrich) (**15**, 9.1 g, 50 mmol), *N*-bromosuccinimide (8.9 g, 50 mmol), and benzoyl peroxide (0.2 g) in dry benzene (100 mL) was heated under reflux for 36 h. The solvent was then removed under vacuum and the residue was washed thoroughly with hot water. The precipitate was filtered, dried, and recrystallized from CH_2Cl_2–petroleum ether to give **16** (9.8 g, 77%) as light yellow needles: mp 132 °C; IR (KBr) 2780–2300, 1680, 1600 (COOH), 1615 (aromatic), 1520, 1350 cm^{-1} (NO_2); NMR ($CDCl_3$, Me_2SO-d_6) δ 4.7 (s, 2 H), 7.9 (d, 1 H), 8.2 (dd, 1 H), 8.6 (d, 1 H), 10.8 (s, 1 H). Anal. Calcd for $C_8H_6NBrO_4$: C, 36.95; H, 2.3; N, 5.38; Br, 30.73. Found: C, 35.40; H, 2.1; N, 5.30; Br, 30.21.

[3-Nitro-4-(bromomethyl)benzoyl]amino–PEG (17). To a solution of 3-nitro-4-(bromomethyl)benzoic acid (**16**, 5.2 g, 20 mmol) and amino PEG (**14**, 15 g, 4 mmol of NH_2 groups) in methylene chloride (50 mL) was added a solution of DCC (4.2 g, 20 mmol) in CH_2Cl_2 (400 mL) and the reaction mixture stirred at room temperature for 48 h. The precipitated dicyclohexylurea was removed by filtration, the filtrate was concentrated in vacuo, and the product was precipitated by the addition of ether. The precipitate was filtered, washed thoroughly with ether, recrystallized from ethanol by the slow addition of ether with rapid stirring, filtered, washed with ether, and dried under vacuum: yield 14 g; Br, 2.4% (0.30 mequiv/g).

Synthesis of Peptides on the PEG Derivative 17. The conditions for the stepwise synthesis of peptides on this support were essentially the same as those described in previous section, except that care was taken to protect these derivatives from prolonged exposure to light.

Photolytic Cleavage of the Finished Protected Peptide Acids from the Support 17. General Procedure. A solution of the peptide–PEG support in methanol or ethanol (1.5 to 2.5 g in 150 mL) was irradiated with a 450-W lamp under a nitrogen atmosphere in a Hanovia photochemical reactor with a double-walled quartz immersion well. A 40% copper sulfate solution was used to filter out wavelengths below 320 nm. The irradiation periods for the different PEG–peptide supports ranged from 12 to 18 h. After the photolysis, the solvent was removed under vacuum and the residue containing the cleaved peptide, the PEG derivative, and any unchanged peptide–PEG support was worked up and purified as usual to get the protected peptide acids (Table V).

[3-Nitro-4-(phthalimidomethyl)benzoyl]amino–PEG (18). [3-Nitro-4-(bromomethyl)benzoyl]amino–PEG (**17**, 10 g, 3 mequiv of Br) and potassium phthalimide (4 g) in DMF (60 mL) were heated at 90 °C under a nitrogen atmosphere for 6 h. The precipitate was then filtered off and ether was added to the clear filtrate slowly with stirring. The precipitate was filtered, washed several times with ether, and then digested with 100 mL of CH_2Cl_2. The insoluble impurities were filtered off and the filtrate was concentrated. Ether was then added to the filtrate to precipitate the product. The precipitate was then filtered, washed with ether, and dried in vacuo to yield **18**: 10 g; unchanged Br, 0.2% (92% conversion); R_f 0.00.

[3-Nitro-4-(aminomethyl)benzoyl]amino–PEG (19). Compound **18** (10 g) and hydrazine hydrate (6 mL) in absolute ethanol (60 mL) were heated under reflux for 12 h. After the solution cooled to room temperature, the product was precipitated by the dropwise addition of ether. The precipitate was filtered and purified by repeated crystallization as in the above case to yield 9.5 g. Microtitration with HCl indicated the amino group capacity of the polymer to be 0.24 mequiv/g.

Attachment of N-Protected Amino Acids to the PEG Derivative 19. The following procedure is typical for the method. To a solution of Boc-Val (1.3 g, 6 mmol) in CH_2Cl_2 (15 mL) kept at 0 °C was added an ice-cold solution of DCC (0.63 g, 3 mmol) in 15 mL of CH_2Cl_2. The mixture was stirred at 0 °C for 1 h. This solution was filtered directly into a flask containing a solution of the PEG derivative **19** (5 g, 1.2 mequiv of NH_2) in 20 mL of CH_2Cl_2, and the reaction mixture stirred at room temperature overnight. The solution was concentrated to about 10 mL and ether was added dropwise with rapid stirring. The precipitate was filtered and washed several times with ether. The product was twice crystallized from CH_2Cl_2 solution by the addition of ether to give 5 g of pure Boc-Val derivative of the aminomethyl polymer. Stepwise synthesis of peptides on this derivative and photolytic cleavage of N-protected C-terminal amino acid amides (Table VI) were carried out as described in the previous cases.

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