A New Synthetic Route to *tert*-Butyloxycarbonylaminoacyl-4-(oxymethyl)phenylacetamidomethyl-resin, an Improved Support for Solid-Phase Peptide Synthesis[1]

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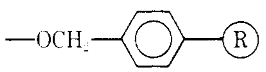
The preferred route to the aminoacylated 4-(oxymethyl)phenylacetamidomethyl-resin (-OCH$_2$-Pam-resin) involves the condensation of a Boc-aminoacyl-4-(oxymethyl)phenylacetic acid (Boc = *tert*-butyloxycarbonyl) with aminomethyl-resin. Aminomethyl-resin was synthesized by direct amidoalkylation of polystyrene resin to give the phthalimidomethyl-resin. The extent of reaction was monitored by IR, allowing the reaction to be stopped at any chosen level of substitution. Hydrazinolysis gave aminomethyl-resin. The Boc-amino acid was converted in solution to a substituted benzyl ester by reaction with 4-(bromomethyl)phenylacetic acid phenacyl ester. Zinc–acetic acid reduction removed the phenacyl group to give the Boc-aminoacyl-4-(oxymethyl)phenylacetic acid, which was coupled to aminomethyl-resin with DCC. The benzyl ester bond of the resulting aminoacyl-OCH$_2$-Pam-resins was approximately 100-fold more stable in refluxing trifluoroacetic acid than the aminoacyl-OCH$_2$-resin. Comparison with a solution analogue showed that this was due to the inductive effect of the *p*-acetamidomethyl group. Cleavage yields (HF–anisole, 9:1 v/v, 30 min, 0 °C) were 82–100% for the aminoacyl- and peptidyl-OCH$_2$-Pam-resins examined. The aminoacyl-OCH$_2$-Pam-resins showed resistance to primary amine nucleophiles similar to that of the aminoacyl-OCH$_2$-resin. No racemization (<0.1%) occurred in the synthesis of Boc-L-Val-OCH$_2$-Pam-resin, and this resin gave improved results in syntheses of the model peptides Leu-Ala-Gly-Val and ribonuclease A (111–124).

It is known that some of the peptide chains covalently bound to oxymethylpoly(styrene-*co*-divinylbenzene) resin (**1**), the support commonly used for solid-phase peptide synthesis,[3,4] are lost by acidolysis during the synthesis.[5–8] The resin 4-(oxymethyl)phenylacetamidomethylpoly(styrene-*co*-divinylbenzene) (**2**) was introduced to minimize this loss.



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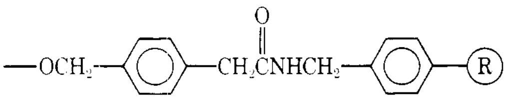

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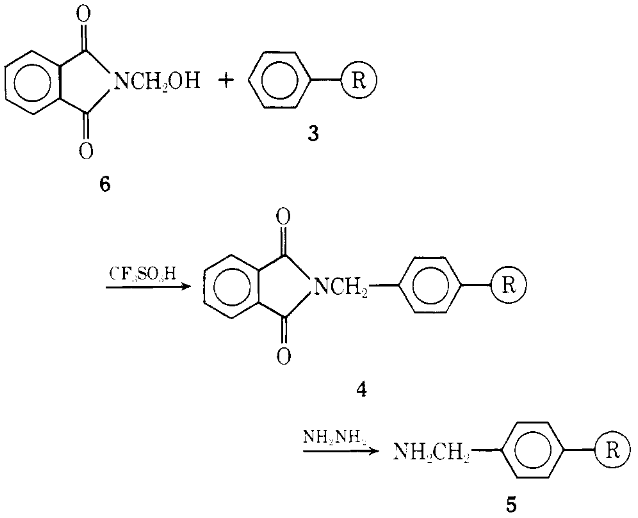
The presence of the electron-withdrawing phenylacetamidomethyl (Pam) bridge was shown to increase the stability of the peptide ester of **2** by 100-fold relative to the peptide ester of **1**, in 50% trifluoroacetic acid in dichloromethane.[9] Use of Pam-resin is expected to result in much higher yields of large peptides prepared by solid-phase peptide synthesis. It is also important that the aminoacyl-OCH$_2$-Pam-resin can be prepared by routes which avoid side reactions known to be possible in the preparation of aminoacyl-OCH$_2$-resin from chloromethyl-resin, and that this chemically well-defined resin exhibits improved results in peptide synthesis.

In this article we report our exploration of synthetic routes to aminoacyl-OCH$_2$-Pam-resins. We have devised a convenient general synthesis of Boc-aminoacyl-4-(oxymethyl)-phenylacetic acids, the key intermediates in the preparation of the aminoacyl-OCH$_2$-Pam-resins from aminomethyl-resin. Aminomethyl-resin has been prepared on a large scale directly from polystyrene resin without the intermediacy of chloromethyl-resin. In addition, further data are presented on the low trifluoroacetic acid labilities of several Boc-aminoacyl-OCH$_2$-Pam-resins, their high HF cleavage yields, and their resistance to amine nucleophiles.

Results and Discussion

A. Aminomethyl-resin (5). Aminomethyl-resin (**5**) previously used in the preparation of Pam-resin was synthesized via the chloromethyl-resin.[9–12] A preparation of **5** from unsubstituted polystyrene by direct amidoalkylation (the Tscherniac–Einhorn reaction[13]) was recently developed in this laboratory[14] (Scheme I). This avoids the use of the carcinogenic reagent chloromethyl methyl ether.[15] In addition, Scheme I requires one less step, the reactions are easy to perform, and the undesirable side reactions of the chloromethyl-resin are not possible.[4]

Scheme I. Preferred Route to Aminomethyl-resin



The preferred reagent for this synthesis is the readily available *N*-(hydroxymethyl)phthalimide (**6**), with trifluoromethanesulfonic acid as catalyst. Polystyrene–divinylbenzene copolymer beads were thoroughly washed before use to remove residual monomer, crosslinking agent, catalyst, and additives remaining from the polymerization, and noncrosslinked oligomer. The amidoalkylation proceeds smoothly in 50% (v/v) trifluoroacetic acid–dichloromethane as solvent at room temperature, and the extent of reaction can be readily controlled by IR monitoring of resin samples. The ratio of the intensity of the phthalimide carbonyl band at 1720 cm^{-1} to that of the polystyrene at 1601 cm^{-1} allows the degree of substitution to be approximately determined, and the reaction can be terminated at the desired level by filtration and washing. For the levels of substitution required for use in solid-phase peptide synthesis ($\leq$1 mmol/g), the reaction rapidly (<6 h) proceeds to completion if only the calculated

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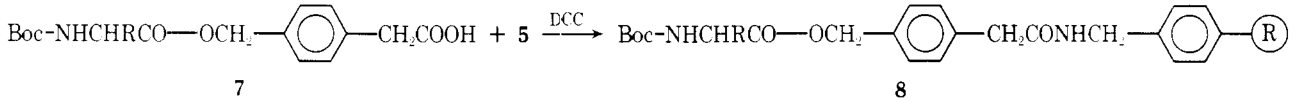
Scheme II. Preferred Route to Boc-aminoacyl-OCH_2-Pam-resin



amount of N-(hydroxymethyl)phthalimide is used. This is the simplest method of precisely obtaining a predetermined loading. In addition, we have varied the concentrations of reagent and catalyst and the reaction time to yield phthalimidomethyl-resin (4) having substitutions of 0.05–3.60 mmol/g. Hydrazinolysis in refluxing ethanol gives the aminomethyl-resin (5). Both of these steps have been conveniently carried out on 10-mg to 200-g amounts of resin.

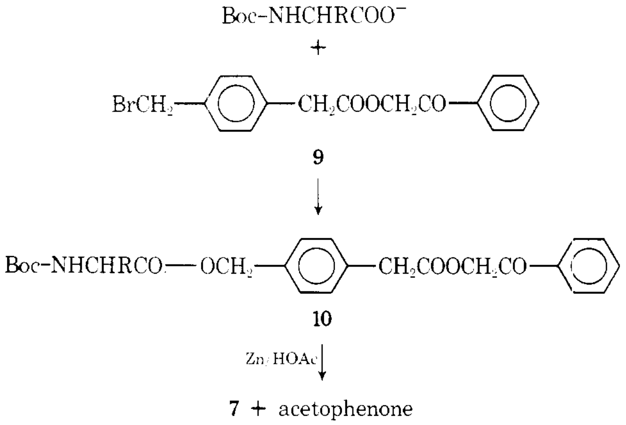
B. Synthesis of Boc-aminoacyl-4-(oxymethyl)-Pam-resin (8). There are two approaches to Boc-aminoacyl-OCH_2-Pam-resins. In the first of these, the Boc-amino acid is derivatized to form the Boc-aminoacyl-4-(oxymethyl)-phenylacetic acid which, after purification, is coupled to the aminomethyl-resin. This is the preferred route. In the second approach the aminomethyl-resin is derivatized with a substituted tolylacetic acid to give a functionalized Pam-resin onto which the C-terminal Boc-amino acid is then loaded. This approach is simpler, but is susceptible to all the side reactions known for the loading of normal resins.

1. First Approach. The most definitive route to a Boc-aminoacyl-OCH_2-Pam-resin (8) is illustrated in Scheme II. This approach allows the simultaneous attachment of the C-terminal Boc-amino acid and its benzyl ester protecting group[9] onto the polystyrene support. The acetamidomethyl group that is formed serves both as the covalent link between the benzyl ester and the resin and as the electron-withdrawing substituent that increases the acid stability of the peptide benzyl ester.

The compound 7 containing the amino acid benzyl ester linkage is formed in solution, purified, and characterized. It can then be used to acylate the resin 5 quantitatively under the same mild coupling conditions used in peptide bond formation. The reaction is applicable to all the protected amino acids normally used in peptide synthesis and is generally free from side reactions. This coupling can be readily incorporated into automated syntheses, allowing peptides to be made starting directly from the aminomethyl-resin support. Such a mild unambiguous loading method is a major advantage associated with the use of this route to aminoacyl-OCH_2-Pam-resins (8).

a. Preparation of Boc-aminoacyl-4-(oxymethyl)-phenylacetic Acids. A general route to the Boc-aminoacyl-4-(oxymethyl)phenylacetic acids (7) would involve the condensation of a Boc-amino acid salt with a carboxyl-protected halomethylphenylacetic acid. The carboxyl protecting group would have to be stable to the conditions of formation of the benzyl ester bond, and selectively removable without affecting the N^{α}-Boc group, the benzyl ester, and any side-chain protecting group present in the amino acid. One carboxyl-protecting group that satisfies the above requirements is the phenacyl ester.[16-18] The successful general route based on the use of this group is shown in Scheme III. The protected compound 9 is readily obtained from the reaction of a salt of 4-(bromomethyl)phenylacetic acid with bromoacetophenone. The use of 4-(bromomethyl)phenylacetic acid[12,19] is preferred over the 4-(chloromethyl)phenylacetic acid because the latter compound is obtained in low yield by the published procedure[20] and is less reactive. Reaction of 9 with a Boc-amino acid salt gives the phenacyl ester (10) of the desired product. Removal of excess Boc-amino acid by basic washes gives a product suitable for use without further purification. The phenacyl group can be removed by zinc/acetic acid reduction at room temperature, without cleaving the Boc or benzyl ester groups, to give the desired product 7. The reduction is readily monitored by proton NMR, which shows clean, rapid cleavage of the phenacyl ester. Provided the starting protected halomethylphenylacetic acid phenacyl ester (9) is pure, the final product is free of polycondensation products. Complete removal of excess Boc-amino acid from 10 ensures a final product free of the Boc-amino acid. Workup is by a simple extractive procedure, and residual acetic acid is removed by azeotroping with benzene. The Boc-amino-acyl-4-(oxymethyl)phenylacetic acid (7) is obtained from ether as the solid CHA or DCHA salt in good overall yield.

Scheme III. A General Route to Boc-aminoacyl-4-(oxymethyl)phenylacetic Acids



The route shown in Scheme III can be used for a variety of protected amino acids. Most of the commonly used protecting groups are stable to the reductive cleavage conditions.[21] We have prepared the 4-(oxymethyl)phenylacetic acid derivatives of the following amino acids, as CHA salts: Boc-L-Val, Boc-L-Lys(Z), Boc-L-Asp(OBzl), Boc-L-Ser(Bzl), and Boc-L-Met.

A simpler but less general route to 7 is the reaction of the Boc-amino acid salt with a 4-halomethylphenylacetic acid. However, this reaction can give rise to a multiplicity of products in addition to the desired product and unreacted starting material. For example, the halomethylphenylacetic acid can first dimerize before reacting with the Boc-amino acid salt. Further reaction of the desired product with halomethylphenylacetic acid would also give a similar spectrum of products. Although it could be achieved, the purification of the product 7 has been difficult.[9] Preparative thick-layer chromatography was necessary and, in addition to the desired product, the dimeric Boc-aminoacyl-[4′-(oxymethyl)phenylacetyl]-4-(oxymethyl)phenylacetic acid was isolated. Different Boc-amino acids required the development of new solvent systems.

We also explored the use of 4-(bromomethyl)phenylacetic acid N-hydroxysuccinimide ester. It was hoped that the N-hydroxysuccinimide ester would serve as a carboxyl protecting group during the formation of the benzyl ester bond, and then serve as an active ester to allow the acylation of aminomethyl-resin to give Boc-aminoacyl-OCH_2-Pam-resin (8). Unfortunately, the reaction of Boc-valine cesium salt[22] with 4-(bromomethyl)phenylacetic acid N-hydroxysuccinimide ester proceeded poorly as determined by thin-layer chromatography of the crude reaction mixture vs. a reference sample of the desired Boc-Val-4-(oxymethyl)phenylacetic acid N-hydroxysuccinimide ester. This was presumably due to the reaction of the carboxylate group with the active ester.[23]

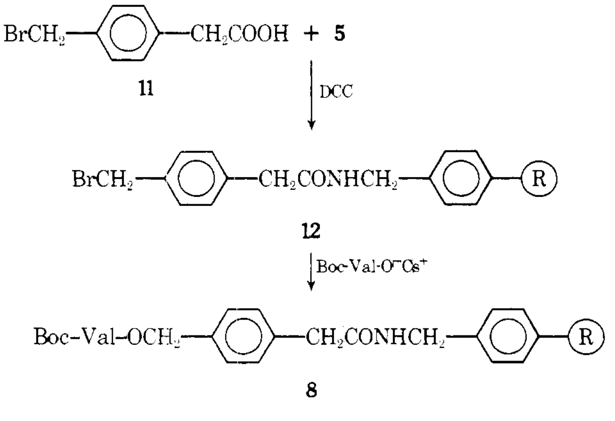
b. Physical Properties, Optical Purity, and Use in Synthesis. This first approach using the Boc-aminoacyl-4-(oxymethyl)phenylacetic acid (**7**) formed in solution to couple to the aminomethyl-resin (**5**), as shown in Scheme II, is the route of choice to aminoacyl-OCH$_2$-Pam-resins (**8**). Examination of the colorless loaded resin under the microscope showed unpitted translucent spheres identical in appearance with the unsubstituted resin, washed or unwashed. There was no evidence of broken or damaged beads. Measurement of the diameters of the aminomethyl-resin and the loaded Pam-resins showed that each swelled in methylene chloride to the same extent (4.4-fold), comparable to the unsubstituted resin (fivefold).[7] Sometimes the resin **8** showed an increased tendency to clump during some manipulations in the course of a synthesis. This had no effect on the excellent synthetic results obtained with the resin. In one instance[24] no clumping was observed in a prolonged stepwise synthesis using a silanized reaction vessel.[25] The loaded resins are optically pure and give good synthetic results, as shown by the following data.

Boc-L-Val-4-(oxymethyl)phenylacetic acid was purified and reacted with aminomethyl-resin (**5**) to give Boc-L-Val-OCH$_2$-Pam-resin. This was deprotected and coupled with Boc-L-Leu. The Boc-L-Leu-L-Val-OCH$_2$-Pam-resin was cleaved and the unpurified dipeptide was subjected to ion-exchange chromatography under conditions that allow the separation and quantitative determination of one part of L-Leu-D-Val in the presence of 1000 parts of L-Leu-L-Val.[26] The absence of L-Leu-D-Val (<0.1%) indicated that the synthesis of Boc-valyl-4-(oxymethyl)phenylacetic acid and its subsequent coupling to aminomethyl-resin proceeded without detectable racemization.

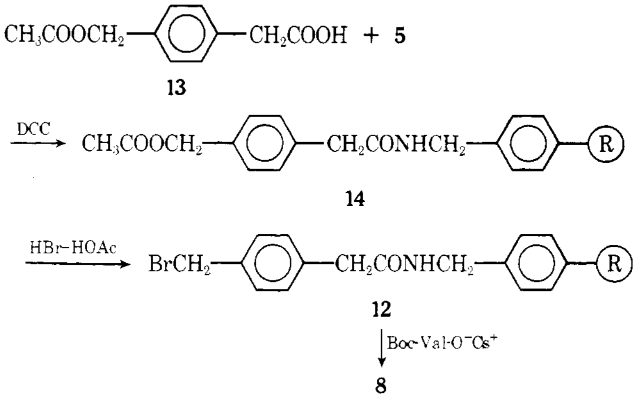
The Boc-Val-OCH$_2$-Pam-resin described above has been carried through three cycles of synthesis by standard procedures to give Boc-Leu-Ala-Gly-Val-OCH$_2$-Pam-resin. Treatment of this material with anhydrous HF has resulted in essentially quantitative cleavages, as indicated by recoveries of product peptide and by the levels of amino acids in acid hydrolyzates of the residual resin. Ion-exchange chromatography has routinely indicated the presence of over 99 mol % Leu-Ala-Gly-Val in the unpurified product. Levels of deletion peptides and other byproducts are substantially lower than in identical syntheses performed on normal Boc-Val-OCH$_2$-resin.

Ribonuclease A (111–124) was also synthesized on Boc-Val-OCH$_2$-Pam-resin prepared according to Scheme II. A standard double-coupling synthesis, as described for the synthesis of Leu-Ala-Gly-Val, gave the protected tetradecapeptide-OCH$_2$-Pam-resin. After treatment with HF–anisole (9:1, v/v) for 1 h at 0 °C, the unpurified product was chromatographed on Aminex 50W-X4 in a pyridine–acetate gradient as previously described.[27] Chromatography at very high loading showed the desired tetradecapeptide as 94.8 mol % of the ninhydrin-positive products. None of the byproducts was present in more than 1.2 mol%. The peptide contained tritium label in Ala^{122}. Tritium monitoring of the column effluent showed the desired tetradecapeptide, but did not detect further byproducts. Previous syntheses on Boc-Val-OCH$_2$-resin have given higher levels of byproducts.[27]

Scheme IV



Scheme V



2. Second Approach. An example of the preparation of Boc-aminoacyl-OCH$_2$-Pam-resins (**8**) by derivatization of aminomethyl-resin (**5**) prior to the loading of the first Boc-amino acid is shown in Scheme IV. This route to **8** is less desirable, since precise analytical control of the chemistry performed on the functionalized resin **12** is not possible. A similar approach was investigated by Sparrow.[12] The reaction of **5** with DCC-activated **11** should give rise primarily to **12**, but the N-benzylation of some aminomethyl sites by **11** has not been ruled out as a competing side reaction. We have found that Boc-Val-OCH$_2$-Pam-resin (**8**) obtained via Scheme IV furnishes the model Leu-Ala-Gly-Val[3,28] containing higher levels of deletion peptides than normally observed in our syntheses from **8** obtained via Scheme II.

An alternative example of this second route which we have investigated is shown in Scheme V. Boc-Val-OCH$_2$-Pam-resin (**8**) prepared in this manner allowed the synthesis of Leu-Ala-Gly-Val in high purity.

This sequence avoids the possibility of the N-benzylation side reaction. However, both Schemes IV and V have the drawback that unreacted bromomethyl-Pam sites (**12**) may participate in undesirable side reactions later in a synthesis.[29]

C. Properties of Aminoacyl-4-(oxymethyl)-Pam-resins (8). 1. Acid Stability. The stability of three Boc-aminoacyl-OCH$_2$-Pam-resins to acidolytic conditions was determined. Boc-Gly-, Boc-Phe-, and Boc-Val-OCH$_2$-Pam-resins, Boc-Val-OCH$_2$-resin, and Boc-valyl-4-(oxymethyl)phenylacetamidomethylbenzene (**15**) were refluxed in anhydrous trifluoroacetic acid. The rate constants and the relative rates of cleavage (compared to Boc-Val-OCH$_2$-resin) of the various benzylic derivatives are given in Table I.

The aminoacyl-OCH$_2$-Pam-resins are 100- to 200-fold more stable than Boc-Val-OCH$_2$-resin in refluxing trifluoroacetic acid. The cleavage of Boc-Val-OCH$_2$-Pam-resin and **15** affords an interesting comparison. The latter soluble derivative was cleaved about fourfold faster than the resin bound analogue. This observation is consistent with the general observation that a reaction within a solid support proceeds somewhat slower than the same reaction in solution.[4] Therefore, it can be concluded that the increased acid stability of the acyl-OCH$_2$-Pam-resin is not due primarily to steric factors such as the polystyrene backbone, but to the acetamidomethyl group acting as an electron-withdrawing substituent.

The increased stability of acyl-OCH$_2$-Pam-resins in hot

Table I. Cleavage of Amino Acid Benzyl Ester Derivatives in Refluxing Trifluoroacetic Acid

Benzylic derivative	k,[a] 10^{-6} s^{-1}	% loss per min	k_{rel}
Boc-Val-OCH$_2$-resin	717	4.2	[100]
Boc-Gly-OCH$_2$-Pam-resin	7.4	0.044	1.0
Boc-Val-OCH$_2$-Pam-resin	5.1	0.031	0.7
Boc-Phe-OCH$_2$-Pam-resin	3.6	0.022	0.5
Boc-Val-OCH$_2$-Pam-benzene	20.4	0.12	2.8

[a] Apparent first-order rate constants were determined from plots of ln $[a/(a - x)]$ vs. time where a is the amino acid content of the starting material and x is the amount of acid released at a given time.

trifluoroacetic acid indicates a possible application of these supports in solid-phase peptide sequencing of resin-bound synthetic peptides.[30] The new preparation of aminomethyl-resin[14] may also be useful for sequencing of free peptides.

2. Cleavage Yields. It is important to note that the lability of the acyl-OCH$_2$-Pam-resin to anhydrous HF, a cleavage reagent commonly used in solid-phase peptide synthesis, is still adequate despite the increased stability of the acyl-OCH$_2$-Pam resin to trifluoroacetic acid. Thus, treatment of Leu-Ala-Gly-Val-OCH$_2$-Pam-resin and the aminoacyl-OCH$_2$-Pam-resins listed in Table I with 9:1 (v/v) HF–anisole for 30 min at 0 °C resulted in cleavages ranging from 82 (Boc-Phe-OCH$_2$-Pam-resin) to 100% (Boc-Gly-OCH$_2$-Pam-resin) as determined by the amount of product released and amino acid analysis of the cleaved resins. It is known that peptides with C-terminal phenylalanine are especially difficult to cleave with HF, and that those with C-terminal glycine are the most readily cleaved.[31] Even with the mild cleavage conditions tested here, phenylalanyl-OCH$_2$-Pam-resin gave a high cleavage yield.

3. Susceptibility to Amine Nucleophiles. The lability of the benzyl ester bond in acyl-OCH$_2$-Pam-resins toward attack by primary amines was compared with the lability of the standard acyl-OCH$_2$-resin under the same conditions (Table II). Runs 1 and 2 indicate that n-butylamine penetrates the polystyrene beads and converts all of the chloromethyl sites to butylaminomethyl sites at room temperature (18 h). Runs 3 and 4 show that Boc-aminoacyl-OCH$_2$-resin and Boc-aminoacyl-OCH$_2$-Pam-resin have significant lability in neat n-butylamine. As expected, reaction of these resins with 5–10% (v/v) amine in methylene chloride proceeded much less rapidly (runs 5–8). Both resins were cleaved at approximately the same rate.

These results show that the aminoacyl-OCH$_2$-Pam-resins are not significantly more susceptible to nucleophilic attack by primary amines than the aminoacyl-OCH$_2$-resin. Use of the aminoacyl-OCH$_2$-Pam-resin in prolonged stepwise synthesis has not resulted in any detectable loss of chains from the resin.[24] In the light of these data, it is not anticipated that the formation of diketopiperazines and concomitant loss of chains observed with the standard aminoacyl-OCH$_2$-resin will be any greater with the Pam-resin. Methods exist for overcoming this problem where it is observed.[4]

D. Other Applications. The aminomethyl derivative of the non-crosslinked KelF-g-styrene[2] resin[32] has been prepared and converted to Boc-aminoacyl-OCH$_2$-Pam-(KelF-g-styrene) according to Schemes I and II. A preliminary evaluation[24] of this resin showed properties comparable to those reported for the resin **8**.

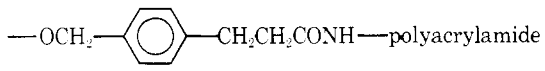
The chemistry of the Pam-resins that has been discussed in this paper and elsewhere[9] should find ready application in systems not utilizing polystyrene supports. For example, the soluble polyethylene glycol used in the liquid-phase method of peptide synthesis[33,34] could be modified to furnish an acid-resistant support that can be cleaved at the end of a synthesis with hydrogen bromide or hydrogen fluoride. The system in present use[35] requires a saponification step which not only releases the peptide in low yield, but may also give rise to racemization.

Table II. Reaction of Poly(styrene-*co*-1% divinylbenzene) Derivatives with Amines at 25 °C

run	derivative[a]	Reagent[b]	time, h	product[c]	% yield
1	Cl-CH$_2$-R	100% C$_4$H$_9$NH$_2$	1	C$_4$H$_9$NH-CH$_2$-resin	58
2	Cl-CH$_2$-R	100% C$_4$H$_9$NH$_2$	18	C$_4$H$_9$NH-CH$_2$-resin	100
3	Boc-Val-OCH$_2$-R	100% C$_4$H$_9$NH$_2$	16	Boc-Val-NHC$_4$H$_9$	7.2
4	Boc-Val-OCH$_2$-Pam-R	100% C$_4$H$_9$NH$_2$	16	Boc-Val-NHC$_4$H$_9$	6.7
5	Boc-Val-OCH$_2$-R	10% C$_4$H$_9$NH$_2$	29	Boc-Val-NHC$_4$H$_9$	0.03
6	Boc-Val-OCH$_2$-Pam-R	10% C$_4$H$_9$NH$_2$	29	Boc-Val-NH-C$_4$H$_9$	0.05
7	Boc-Gly-OCH$_2$-R	5% BzlNH$_2$	29	Boc-Gly-NHBzl	0.14
8	Boc-Gly-OCH$_2$-Pam-R	5% BzlNH$_2$	29	Boc-Gly-NHBzl	0.33

[a] R represents polystyrene resin. [b] In runs 5–8 the amine was diluted with methylene chloride. [c] The progress of runs 1–2 was followed by elemental analyses for nitrogen and chlorine, indicating the appearance of butylaminomethyl groups and disappearance of chloromethyl groups. Boc-Val-NHC$_4$H$_9$ and Boc-Gly-NHBzl were deprotected in CF$_3$CO$_2$H and detected on the ion-exchange column of a Beckman 120B Amino Acid Analyzer.

The peptide ester of the polyacrylamide support (**16**) developed by Sheppard and co-workers[36] for peptide synthesis



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is reported to have the same lability to acid as the peptide ester of the polystyrene support (**1**) most commonly used for solid-phase peptide synthesis.[3,4] Acylation of the polyacrylamide support with a Boc-aminoacyl-4-(oxymethyl)phenylacetic acid (**7**), rather than a Boc-aminoacyl-4-(oxymethyl)-phenylpropionic acid, should provide a peptide ester of the polyacrylamide support having the 100-fold greater acid stability displayed by the Boc-aminoacyl-4-(oxymethyl)-Pam-resins.

Conclusions

The preparation of aminomethyl-resin from unsubstituted styrene polymers allows precise control of the extent of substitution and is free from the undesirable side reactions of chloromethyl-resin. The chemically well-defined, general route to the Boc-aminoacyl-OCH$_2$-Pam-resins reported here represents a significant improvement over the previous, less-defined syntheses of Boc-aminoacyl-OCH$_2$-resins. The resulting Pam-resins show lower levels of byproducts in model peptide syntheses. The problem of the relative acidolytic labilities of the N$^\alpha$-Boc group and the peptide–resin linkage has been solved by the 100-fold increase in the acid stability of the peptidyl-OCH$_2$-Pam-resin linkage, without sacrificing HF cleavage yields and without significantly increasing the susceptibility to nucleophilic side reactions.

Experimental Section

Infrared spectra were taken with a Perkin-Elmer Model 237B grating infrared spectrophotometer. Melting points were taken on a Thomas-Hoover capillary melting point apparatus and are uncorrected. Nuclear magnetic resonance spectra were recorded on a Varian Model T-60 spectrometer. Elemental analyses were performed by Mr. S. T. Bella of the Microanalytical Laboratory, The Rockefeller University. The solvents used for thin-layer chromatography (TLC) (precoated 0.25-mm silica gel GF plates, Analtech) were: I, petroleum ether (bp 30–60 °C)–acetic acid, 9:1; II, petroleum ether–acetic acid (8:2); III, chloroform–acetic acid (99:1); IV, chloroform–acetic acid (95:5); V, chloroform–methanol–acetic acid (85:10:5). Spots were visualized with ultraviolet light (254 nm) followed by spraying with 0.2% ninhydrin in 1-butanol and heating. Preparative layer chromatography (PLC) was performed using 30 × 30 × 0.5 cm or 40 × 40 × 0.5 cm plates[37] prepared with silica gel PF-254 containing $CaSO_4$ (Brinkman Instruments). All solvents and bulk chemicals were reagent grade. DMF was MCB-Spectroquality and was stored over 4 Å molecular sieves. Boc-amino acids were obtained from Beckman Instruments or Chemical Dynamics. *p*-Tolylacetic acid, *N*-(hydroxymethyl)phthalimide, and bromoacetophenone were obtained from Aldrich.

Poly(styrene-*co*-1% divinylbenzene) beads (200–400 mesh) were purchased from Bio-Rad Laboratories. Chloromethylpoly(styrene-*co*-1% divinylbenzene) resin was obtained from Bio-Rad, Pierce, or Lab Systems. The materials and methods for solid phase synthesis were similar to those described elsewhere,[3,4,7] but modified as indicated.

Ion-exchange chromatography was performed using a Beckman amino acid analyzer (Model 120B or 121). The buffers were prepared from Beckman buffer concentrates. Borate buffer (pH 10) was prepared by dissolving boric acid (12 g), sodium hydroxide (8 g), and sodium chloride (35 g) in 4 L of distilled water; boric acid was added to bring the solution to pH 10.

Phthalimidomethyl-resin (4). Copoly(styrene-1% divinylbenzene) resin (200 g) was thoroughly washed[38] according to the following protocol to remove non-covalently-bound material:[39] the resin was placed in a 4-L round-bottom flask, fitted with an overhead stirrer and reflux condenser, in a water bath at 70 °C. The resin was stirred slowly with benzene (2 L) for 30 min and the solvent was removed by aspiration through a coarse sintered glass filter. This was repeated once with benzene, then twice each with 2 L of methanol, DMF, dioxane–2 N aqueous NaOH (1:1, v/v), dioxane–H_2O (1:1, v/v), dioxane–2 N aqueous HCl (1:1, v/v), and dioxane–H_2O (1:1, v/v). The resin was then rinsed with 4 L of hot methanol, 4 L of benzene, 4 L of methanol, and 4 L of CH_2Cl_2, filtered, and dried under vacuum. The washed resin and *N*-(hydroxymethyl)phthalimide (90 mol % pure by NMR[46]) (8.14g, 41 mmol) were placed in a three-neck round-bottom flask (5 L) equipped with an overhead stirrer. CF_3COOH–CH_2Cl_2 (2 L) (1:1, v/v) was added. The resin was suspended by rapid stirring and trifluoromethanesulfonic acid (18 mL, 0.20 mol) was slowly added. Stirring was continued at room temperature. The amidoalkylation reaction was followed by IR of KBr pellets of washed resin samples (~10 mg). The substitution of the resin is given approximately by: ([intensity 1720 cm^{-1}]/[intensity 1601 cm^{-1}]) × 0.17 = mmol/g. The reaction was allowed to proceed to completion as indicated by no further change in the IR spectrum (less than 6 h), and the resin was filtered and washed with: CF_3COOH–CH_2Cl_2 (1:1, v/v) (4 L), CH_2Cl_2 (8 L), and ethanol (8 L). The resin was dried under vacuum overnight to give **4**. Anal.: N, 0.28% (0.20 mmol N/g).

Aminomethyl-resin (5). Resin 4 (180 g) was refluxed without stirring for 16 h in ethanol (2 L) containing 5% hydrazine (Eastman 95 + %). The resin was filtered hot and washed (with stirring 5–10 min each wash) with boiling ethanol (4 × 2 L) and methanol (4 × 2 L). The product was dried under vacuum to give **5**, which contained 0.26% N (0.19 mmol N/g) by elemental analysis, 0.22 mmol of NH_2/g by picric acid titration,[40] and no carbonyl groups by IR. Examination of the CH_2Cl_2-swollen resin under the microscope showed the beads to be identical in appearance with the starting polystyrene resin.

4-(Bromomethyl)phenylacetic Acid (11). Prepared by photobromination.[19] *p*-Tolylacetic acid (30 g, 0.20 mol) was dissolved in CCl_4 (400 mL) and brought to reflux with magnetic stirring in a two-neck round-bottom flask (2 L) fitted with a reflux condenser and a 250-mL addition funnel. Bromine (14.5 mL, 0.56 mol) in CCl_4 (150 mL) was added slowly over a 1–2 h period to the refluxing solution, while the reaction was illuminated with a 150-W tungsten lamp placed 6 in. from the flask. The rate of reaction can be estimated from the white fuming HBr evolved (*Caution:* these fumes should be led directly to a hood vent), and controlled by the degree of illumination. The ambient light level may be sufficient to sustain the reaction. When HBr evolution had ceased (typically, overnight) the reaction mixture was cooled to room temperature. The insoluble product was collected by filtration and washed with CCl_4 (6 × 200 mL). The off-white solid was recrystallized from hot benzene (2 L) by addition of hexane (about 200 mL) to turbidity to give **11** (23.4 g, 51% yield): mp 177–178 °C; NMR (Me_2SO-d_6) 3.55 (s, 2 H, CH_2CO), 4.67 (s, 2 H, $BrCH_2$), and 7.30 ppm (m, 4 H, *p*-C_6H_4). Anal. Calcd. for $C_9H_9O_2Br$: C, 47.18; H, 3.96; Br, 34.89. Found: C, 47.26; H, 4.01; Br, 34.59.

4-(Bromomethyl)phenylacetic Acid Phenacyl Ester (9). Triethylamine (8.49 mL, 60.6 mmol) and bromoacetophenone (12.05 g, 60.6 mmol) were dissolved in ethyl acetate (450 mL). **11** (13.89 g, 60.6 mmol) was added in seven equal portions over a 3-h period to the stirred solution at 40–50 °C. Stirring was continued for a further 2 h at the same temperature. Precipitated $Et_3N{\cdot}HBr$ was removed by filtration, and the ethyl acetate solution was washed with aqueous solutions (4 × 50 mL each) of 10% citric acid, saturated sodium chloride, saturated sodium bicarbonate, and saturated sodium chloride. The organic phase was dried over anhydrous magnesium sulfate and freed of solvent by rotary evaporation under reduced pressure. The residue was crystallized from CH_2Cl_2–petroleum ether (bp 30–60 °C) (1:3, v/v) to give **9** (8.07 g, 40% yield) as fine white crystals: mp 85–87 °C; TLC, pure (100 μg loading, solvent II); NMR ($CDCl_3$) 3.83 (s, 2 H, CH_2COO), 4.50 (s, 2 H, $BrCH_2$), 5.37 (s, 2 H, OCH_2CO), 7.38 (apparent s, 4 H, *p*-C_6H_4), and 7.7 ppm (m, 5 H, C_6H_5). The presence of dimer, 4′-($BrCH_2$)$PhCH_2CO$-4-(OCH_2)$PhCH_2COOCH_2COPh$, would be shown by NMR peaks at 3.67(s) and 5.17 ppm (s) with a detection level of <1 mol%. Anal. Calcd, for $C_{17}H_{15}BrO_3$: C, 58.80; H, 4.35; Br, 23.02. Found: C, 58.32; H, 4.26; Br, 23.26.

Boc-valyl-4-(oxymethyl)phenylacetic Acid Phenacyl Ester (10a). The valine compound is typical. Boc-L-Val (3.10 g, 14.3 mmol), DCHA (2.82 mL, 14.4 mmol), and **9** (2.50 g, 7.3 mmol) were reacted in 60 mL of DMF for 4 h at 50 °C and overnight at room temperature. Precipitated DCHA·HBr was removed by filtration, and the filtrate was freed of solvent by rotary evaporation under high vacuum. The yellow residue was dissolved by stirring for 2 h in EtOAc (450 mL), and insoluble DCHA·HBr was removed by filtration. The ethyl acetate solution was thoroughly extracted with 10% aqueous citric acid (3 × 75 mL) to remove residual DCHA, water (3 × 75 mL), pH 9.5 buffer (one part 0.5 M K_2CO_3 plus two parts 0.5 M $NaHCO_3$) (10 × 75 mL) to remove excess Boc-Val, and water (3 × 75 mL). Removal of all traces of excess Boc-amino acid is crucial and can be monitored by TLC. After drying over $MgSO_4$, the EtOAc was removed by rotary evaporation to give a white solid which was dried under vacuum: weight 3.02 g (theoretical for 7.3 mmol, 3.52 g); TLC (benzene–HOAc, 95:5, v/v) showed **10a**, R_f 0.45, and several minor (<1%) UV-active components of lower R_f. No **9**, R_f 0.9, and no free Boc-Val, R_f 0.4, were detected. This product was suitable for reduction to **7a** as described below. Products of comparable purity containing Lys(Z), Asp(OBzl), Ser(Bzl), and Met were similarly prepared in near-quantitative yields.

An analytical sample of the valine compound was purified by PLC (solvents I, and III) yielding hard, amorphous solid **10a**: $[\alpha]^{24}{}_D$ −18.5° (*c* 2, CH_3OH); NMR ($CDCl_3$) 0.97 (m, 6 H, $(CH_3)_2$), 1.53 (s, 9 H, *t*-Bu), 2.18 (m, 1 H, $C_\beta H$), 3.87 (s, 2 H, CH_2COO), 4.28 (m, 1 H, α-CH), 5.05 (br d, *J* = 8 Hz, 1 H, NH), 5.21 (s, 2 H, OCH_2), 5.40 (s, 2 H, OCH_2CO), 7.38 (apparent s, 4 H, *p*-C_6H_4), and 7.72 ppm (m, 5 H, C_6H_5). Anal. Calcd for $C_{27}H_{33}NO_7$: C, 67.06; H, 6.88; N, 2.90. Found: C, 67.09; H, 6.90; N, 2.78.

Boc-valyl-4-(oxymethyl)phenylacetic Acid (7a). Crude **10a** (3.02 g, 6.25 mmol), purified as described above, was dissolved in 90 mL of HOAc–H_2O (85:15, v/v), and the NMR spectrum in the 2.4–6-ppm region was recorded. Zinc dust (9.64 g, 147 mmol) was added and the suspension was stirred vigorously at room temperature. [Zinc dust (40 g) had previously been acid washed, as follows: 1 N aqueous HCl (6 × 150 mL; 2 min each), H_2O (6 × 150 mL; 1 min), EtOH (6 × 150 mL; 1 min), Et_2O (6 × 150 mL; 1 min). After 5 min aspiration, it was stored in a screw-capped brown bottle. The activity did not change significantly after more than 6 months storage at room temperature.] The reduction was conveniently monitored by NMR of aliquots of the suspension, which were subsequently returned to the reaction vessel. The phenacyl ester CH_2 singlet at 5.4 ppm gradually disappeared with concomitant formation of acetophenone at 2.85 ppm (s, 3 H, CH_3). Similarly, the phenylacetic acid ester singlet at 3.85 ppm disappeared and was replaced by the singlet due to the free acid, at 3.65 ppm. The reduction was always complete within 6 h. No cleavage (<5%) of the benzyl ester bond occurred in 72 h under these conditions. Only ~15% of the N^α-Boc group was removed after 72 h. Therefore, after 6 h only 1–2% of product would be deprotected and removed in the workup. After 6 h the zinc was removed by filtration and washed with 15 mL of 85% HOAc in H_2O. The filtrate, 105 mL, was placed in a separatory funnel with 200 mL of Et_2O, and then 170 mL of water was added, forming a biphasic system. The aqueous phase was titrated in the presence of the Et_2O with 6 N HCl to pH 1–1.5

Table III

Boc-L-Lys(Z)-$OCH_2PhCH_2COOH\cdot CHA$	(**7b**)	83–93 °C dec	Calcd for $C_{34}H_{49}N_3O_8$:	C, 65.05; H, 7.87; N, 6.69
			Found:	C, 65.29; H, 8.00; N, 6.47
Boc-L-Asp(OBzl)-$OCH_2PhCH_2COOH\cdot CHA$	(**7c**)	136–138 °C	Calcd for $C_{31}H_{42}N_2O_8$:	C, 65.24; H, 7.42; N, 4.91
			Found:	C, 65.32; H, 7.47; N, 5.01
Boc-L-Ser(Bzl)-$OCH_2PhCH_2COOH\cdot CHA\cdot 1.5H_2O$	(**7d**)	125–128 °C	Calcd for $C_{30}H_{44}N_2O_8$:	C, 63.30; H, 7.96; N, 4.92
			Found:	C, 63.04; H, 7.69; N, 5.11
Boc-L-Met-$OCH_2PhCH_2COOH\cdot CHA$	(**7e**)	hard oil	Calcd for $C_{25}H_{40}N_2O_6S$:	C, 60.45; H, 8.12; N, 5.64
			Found:	C, 60.31; H, 8.02; N, 5.27

(narrow range paper). After vigorous shaking, the Et_2O (product-containing) layer was separated and the aqueous phase was extracted a second time with 200 mL of Et_2O. The combined ether layers were backwashed with five 200-mL portions of water to remove the bulk of the acetic acid.

TLC showed that more than 99% of the product was in the combined Et_2O layers, together with acetophenone. Ether was removed by rotary evaporation under reduced pressure. The acetic acid remaining was removed by rotary evaporation, at 40 °C, under high vacuum. Residual traces of acetic acid were removed as an azeotrope by the evaporation of six 20-mL portions of benzene. The residue was pumped for 16 h over KOH pellets. Removal of all acetic acid is critical to avoid contamination of the final product with this terminating impurity. The absence of acetic acid (to <1 mol %) can be determined by NMR at this stage. The residue was dissolved in 100 mL of Et_2O and filtered to remove a small (~100 mg) amount of insoluble material. The salt was formed by titration of the Et_2O solution with CHA (or DCHA) to a pH 8 end point (moist narrow range paper). After 72 h at 4 °C white crystals of the CHA salt of **7a** were recovered and washed with Et_2O–petroleum ether: weight 1.85 g (4.0 mmol); yield 55% based on **9**; mp 148–152 °C (lit.[9] 153–154 °C). A second crop was obtained: 0.20 g; mp 138–143°C. Recrystallization of the combined crops gave: 1.80 g (52%); mp 150–152 °C; NMR ($CDCl_3$) 0.90 (m, 6 H, $(CH_3)_2$), 0.8–1.8 (m, 10 H, CHA methylenes), 1.48 (s, 9 H, *t*-Bu), 2.15 (m, 1 H, β-CH), 2.53 (m, 1 H, CHA methine), 3.47 (s, 2 H, CH_2CO), 4.20 (m, 1 H, α-CH), 5.03 (br d, J = 8 Hz, 1 H, NH), 5.13 (s, 2 H, OCH_2), 7.0 (br s, 3 H, NH_3), and 7.27 ppm (apparent s, 4 H, *p*-C_6H_4); TLC (petroleum ether (bp 30–60 °C)–HOAc, 96:4, v/v, five passes, 100 μg) showed: the desired **7a**, apparent R_f 0.4; CHA, R_f 0, no (<0.1%) Boc-Val-OH, apparent R_f 0.9; no acetophenone (high R_f on initial pass). Anal. Calcd for $C_{25}H_{40}N_2O_6$: C, 64.63; H, 8.68; N, 6.03. Found: C, 64.66; H, 8.49; N, 5.84.

Other compounds prepared in the same way in good yield are given in Table III. Satisfactory analytical data (±0.4% for C, H, N; TLC purity; expected NMR) were obtained for all the compounds listed. For **7d**, the 1.5 mol of H_2O was seen by ^1H NMR in $CDCl_3$.

Boc-aminoacyl-4-(oxymethyl)-Pam-resin (8). The CHA or DCHA salt of **7** was first converted to the free acid as follows. The CHA salt of **7** (4.4 mmol) was suspended in 150 mL of water and 150 mL of Et_2O. The calculated amount of 3 N HCl was added with vigorous shaking. The aqueous layer was titrated to pH 1–2 (narrow range paper) by the addition of further small amounts of 3 N HCl. The Et_2O layer was separated, and the aqueous layer was extracted with 2 × 150 mL of Et_2O. The combined Et_2O layers were backwashed with 100 mL of water. TLC of the Et_2O and aqueous solutions showed quantitative extraction of **7** into the Et_2O, while all CHA remained in the aqueous phase. After drying over $MgSO_4$, the Et_2O was evaporated and the free **7** was taken up in 100 mL of CH_2Cl_2 and added to aminomethyl-resin (**5**) (10 g, 2.2 mmol). After 5 min of shaking DCC (0.91 g, 4.4 mmol) in 100 mL of CH_2Cl_2 was added and the mixture was shaken for 16 h at room temperature. The resin was filtered and washed with 6 × 200 mL of CH_2Cl_2. The extent of coupling was determined by picric acid titration[40] of free amino groups. If necessary, residual amino groups were acetylated with 200 mL of acetic anhydride–pyridine (1:1, v/v) for 2 h. The resin was filtered and washed with CH_2Cl_2, CH_2Cl_2–HOAc (1:1), HOAc, 2-propanol, and CH_2Cl_2, and vacuum dried to furnish **8** with a loading of 0.21 mmol/g (amino acid analysis,[41] picrate after deprotection).

Alternative Preparation of 7a. 7a was prepared by direct reaction of Boc-L-Val DCHA salt with 4-$(BrCH_2)PhCH_2COOH$, as previously described.[9] After PLC, valine-containing **7a** was obtained as the CHA salt (3.08 g, 53% yield): mp 149–150 °C; $[\alpha]^{24}{}_D$ −23.0° (*c* 2, CH_3OH). Anal. Calcd for $C_{25}H_{40}N_2O_6$: C, 64.63; H, 8.68; N, 6.03. Found: C, 64.72; H, 8.70; N, 5.99. The dimeric Boc-L-Val-4-(OCH_2) $PhCH_2CO$-4-$(OCH_2)PhCH_2COOH$ was also isolated: mp 89–91 °C; NMR ($CDCl_3$) 0.95 (m, 6 H, $(CH_3)_2$), 1.48 (s, 9 H, *t*-Bu), 2.18 (m, 1 H, β-CH), 3.66 and 3.68 (apparent (d, 4 H, $(CH_2CO)_2$), 4.25 (m, 1 H, $C_\alpha H$), 5.15 and 5.18 (apparent d, 4 H, $(OCH_2)_2$), and 6.98 ppm (apparent s, 8 H, (*p*-$C_6H_4)_2$). Anal. Calcd for $C_{28}H_{35}NO_8$: C, 65.48; H, 6.87; N, 2.73. Found: C, 65.36; H, 6.13; N, 2.67.

4-(Bromomethyl)phenylacetic Acid *N*-Hydroxysuccinimide Ester (17): from **11**, *N*-hydroxysuccinimide, and DCC by the method of Anderson et al.[42] The crude product was crystallized from 2-propanol to give white needles of **17**: yield 68%; mp 148–149.5 °C; NMR ($CDCl_3$) 2.87 (s, 4 H, CH_2CH_2), 3.97 (s, 2 H, CH_2COO), 4.51 (s, 2 H, $BrCH_2$), and 7.40 ppm (apparent s, 4 H, *p*-C_6H_4). Anal. Calcd for $C_{13}H_{12}BrNO_4$: C, 47.87; H, 3.70: N, 4.29; Br, 24.53. Found: C, 47.79; H, 3.72; N, 4.63; Br, 24.40.

Boc-valyl-4-(oxymethyl)phenylacetic Acid *N*-Hydroxysuccinimide Ester (18). An authentic sample was prepared from the reaction of **7a**, *N*-hydroxysuccinimide, and DCC by the general method of Anderson et al:[42] yield 48%; mp 101–102 °C; $[\alpha]^{24}{}_D$ −17.9° (*c* 2, CH_3OH); NMR ($CDCl_3$) 0.95 (m, 6 H, $(CH_3)_2$), 1.50 (s, 9 H, *t*-Bu), 2.13 (m, 1 H, $C_\beta H$), 2.85 (s, 4 H, CH_2CH_2), 3.95 (s, 2 H, CH_2COO), 4.23 (m, 1 H, $C_\alpha H$), 5.00 (br d, J = 10 Hz, 1 H, NH), 5.15 (s, 2 H, OCH_2), and 7.35 ppm (apparent s, 4 H, *p*-C_6H_4). Anal. Calcd for $C_{23}H_{30}N_2O_8$: C, 59.73; H, 6.54; N, 6.06. Found: C, 59.66; H, 6.54; N, 6.02.

The reaction of Boc-L-ValOCs[22] and **17** in DMF gave a multiplicity of products (TLC, system I). The presence of **18** was detected, but preliminary attempts to separate the pure compound were unsuccessful and the preparation was abandoned.

4-(Acetoxymethyl)phenylacetic Acid (13). Sodium acetate and **11** were reacted as previously described using the 4-$(ClCH_2)$-$PhCH_2COOH$.[9] Recrystallization from hot water gave **13**: yield 77%; mp 85–86 °C (lit.[9] mp 84–86 °C); TLC pure (R_f 0.35, twice developed in II); NMR ($CDCl_3$) 2.23 (s, 3 H, CH_3CO), 3.75 (s, 2 H, CH_2CO), 5.20 (s, 2 H, OCH_2), 7.40 (apparent s, 4 H, *p*-C_6H_4), and 10.67 ppm (br s, 1 H, COOH).

4-(Acetoxymethyl)-Pam-resin (14). A solution of **13** (3.64 g, 17.5 mmol) in 250 mL of CH_2Cl_2 was shaken with **5** (25.0 g, 8.94 mmol NH_2) for 5 min. DCC (3.60 g, 17.5 mmol) was added in 50 mL of CH_2Cl_2 and the suspension was shaken at room temperature for 3 h. The resin **14** was filtered and washed with 4 L of CH_2Cl_2. Picric acid titration[40] gave 0.0006 mmol of NH_2/g. Strong carbonyl absorptions were observed in the IR spectrum at 1740 (ester) and 1680 cm^{-1} (amide).

4-(Bromomethyl)-Pam-resin (12). A. From HBr Cleavage of 14. A saturated solution of HBr in acetic acid was prepared by bubbling HBr through a trap containing anisole–acetic acid–CH_2Cl_2 and then into 10:1 acetic acid–anisole (55 mL) for several hours. The cleavage solution was added to **14** (5.00 g, 1.59 mmol) and the suspension was shaken for 2 h. The suspension was filtered and the resin was washed with acetic acid (3 × 50 mL), methanol (3 × 50 mL), and dichloromethane (10 × 50 mL) and dried under vacuum to give **12**. The infrared spectrum of the resin showed a weak residual carbonyl absorption at 1740 cm^{-1} (ester) and a strong band at 1680 cm^{-1} (amide).

B. From 4-(Bromomethyl)phenylacetic acid (11) and Aminomethyl-resin (5). 4-(Bromomethyl)phenylacetic acid (**11**; 2.29 g, 10.0 mmol) and DCC (1.03 g, 5.00 mmol) were allowed to react in 25 mL of tetrahydrofuran at 5 °C for 1 h. The suspension was filtered and the filtrate was added to **5** (5.00 g, 1.10 mmol). The reaction mixture was shaken at room temperature for 1 h. The suspension was filtered and the resin was washed with tetrahydrofuran, CH_2Cl_2, CH_2Cl_2–HOAc (1:1, v/v), HOAc, ethanol, and methanol. The resin **12** was dried under vacuum. Anal.: N, 0.25% (0.18 mmol N/g); Br, 1.62% (0.20 mmol Br/g).

Boc-aminoacyl-4-(oxymethyl)-Pam-resin (8) from Resin 12. The cesium salts[22] (2 equiv) of Boc-Gly, Boc-L-Phe, and Boc-L-Val were allowed to react with resin **12** (0.20 mmol of Br/g), prepared from **11** and **5**, in DMF for 36 h at room temperature (Scheme IV). The Boc-aminoacyl-OCH_2-Pam-resins so produced had loadings of 0.160 mmol of Gly/g, 0.184 mmol of Phe/g, and 0.175 mmol of Val/g as determined by acid hydrolysis for 6 h (130 °C) in HCL–propionic acid (1:1, v/v)[41] and subsequent amino acid analysis.

In a similar manner, Boc-Val-OCs was allowed to react with resin

12 (~0.32 mmol of Br/g), prepared from **14**, yielding Boc-Val-OCH_2-Pam-resin (Scheme V) having a loading of 0.26 mmol of Val/g by amino acid analysis.

Test for Racemization. Synthesis of Boc-Leu-Val-OCH_2-Pam-resin. Boc-Val-OCH_2-Pam-resin (**8a**; 0.200 g, 0.0432 mmol) was placed in a 6-mL reaction vessel. The resin was suspended in trifluoroacetic acid–CH_2Cl_2 (1:1, v/v) and shaken for 1 h. The resin was filtered, washed with CH_2Cl_2, neutralized with 5% ethyldiisopropylamine in CH_2Cl_2, washed with CH_2Cl_2, and coupled for 30 min with 4 equiv of Boc-Leu and 4 equiv of DCC in 4 mL of CH_2Cl_2. The resin was filtered, washed with CH_2Cl_2, CH_2Cl_2–HOAc (1:1, v/v), HOAc, ethanol, and methanol, and dried under vacuum.

A sample (51 mg) of the Boc-Leu-Val-OCH_2-Pam-resin was shaken in 2 mL of trifluoroacetic acid–CH_2Cl_2 (1:1, v/v) containing 20 equiv of trifluoromethanesulfonic acid[43] for 30 min. The cleavage solution was filtered and the resin was washed with (3 × 2 mL) trifluoroacetic acid–CH_2Cl_2 (1:1 v/v). The pooled filtrates were evaporated in vacuo and the residue was dissolved in 10 mL of pH 4.25 sodium citrate buffer (0.2 N). A 1-mL sample of this solution was injected into the long column (0.9 × 58 cm; AA-15 sulfonated polystyrene) of a Beckman 120B amino acid analyzer and eluted with pH 4.25 citrate buffer (61 mL/h; 57 °C). A large peak corresponding to L-Leu-L-Val (153 min) was seen, whereas no detectable peak (<0.1%) was observed at or near the elution position of L-Leu-D-Val (136 min).[26]

Synthesis of Leu-Ala-Gly-Val. The following protocol was used for the syntheses of the model tetrapeptide. Boc-L-Val-OCH_2-Pam-resin (**8a**; 1 g) was placed in a reaction vessel on a shaker and treated as follows for the incorporation of each residue: (1) washed with 20 mL of CH_2Cl_2 (3 × 1 min); (2) shaken with 20 mL of trifluoroacetic acid–CH_2Cl_2 (1:1, v/v) for 30 min; (3) washed with 20 mL of CH_2Cl_2 (6 × 1 min); (4) shaken with 20 mL of 5% ethyldiisopropylamine in CH_2Cl_2 for 5 min: (5) washed with 20 mL of CH_2Cl_2 (3 × 1 min); (6) repeat step 4; (7) repeat step 5; (8) shaken with Boc-Gly (4 equiv) in 15 mL of CH_2Cl_2 for 5 min; (9) without filtration, DCC (4 equiv) in 5 mL of CH_2Cl_2 was added and shaken for 30 min; (10) washed with 20 mL of CH_2Cl_2 (3 × 1 min). The cycle was repeated with Boc-L-Ala, then with Boc-L-Leu. In a double-coupling synthesis, steps 6–10 were repeated in each cycle. The Boc-Leu-Ala-Gly-Val-OCH_2-Pam-resin was washed with CH_2Cl_2-HOAc (1:1 v/v), HOAc, 2-propanol, and CH_2Cl_2, and vacuum dried. The peptide was cleaved from the resin with HF-anisole (9:1, v/v) at 0 °C for 30 min. The cleaved material was taken up in 5% HOAc, filtered, evaporated to dryness, and dissolved in water for analysis. The sample was injected onto the 0.9 × 58 cm column (AA-15 cation exchange resin) of a Beckman 120B amino acid analyzer and eluted (61 mL/h; 57 °C) with pH 3.49 citrate buffer (0.2 N in sodium). The sample was intentionally overloaded (about 4 μmol of peptides) so that less than one part per 1000 of ninhydrin-positive components could be detected.[28]

The following resins were used for syntheses of Leu-Ala-Gly-Val.

I. 8a Obtained from 5 and 7a (Scheme II). Analysis showed the desired tetrapeptide as 98.0 mol % of the unpurified peptide product, together with 0.10–0.22 mol % of each single-deletion peptide. A double coupling synthesis gave the tetrapeptide as 99.2 mol % and reduced to <0.06 mol% each of the deletion peptides.

II. 8a Obtained from Boc-Val-OCs and 12 (Scheme IV). Analysis showed the tetrapeptide as 97.3 mol % of the unpurified peptide product, together with 0.32–0.57 mol % of each single-deletion peptide.

III. 8a Obtained from 14 (Scheme V). A double-coupling synthesis was performed. Analysis showed the tetrapeptide as 99.2 mol % of the unpurified peptide product, together with 0.06–0.10 mol % of each single-deletion peptide.

Boc-valyl-4-(oxymethyl)-Pam-benzene (15). Compound 18 (0.93 g, 2.00 mmol) and benzylamine (0.24 mL, 2.2 mmol) were reacted in ethyl acetate (15 mL) for 16 h at room temperature. The product was worked up in the usual manner and crystallized from ethyl acetate–petroleum ether (bp 30–60 °C) to give 0.56 g (62% yield) of **15:** mp 101.5–103 °C; $[\alpha]^{24}{}_D$ −21.6° (*c* 2, CH_3OH); NMR ($CDCl_3$) 0.95 (m, 6 H, $(CH_3)_2$), 1.50 (s, 9 H, *t*-Bu), 2.15 (m, 1 H, β-CH), 3.65 (s, 2 H, CH_2CO), 4.27 (m, 1 H, α-CH), 4.20 (d, J = 6 Hz, 2 H, N-CH_2), 5.02 (br d, 1 H, urethane NH), 5.18 (s, 2 H, OCH_2), 5.82 (br s, 1 H, benzylamide NH), and 7.32 ppm (m, 9 H, aryl). Anal. Calcd for $C_{26}H_{34}N_2O_5$: C, 68.69; H, 7.54; N, 6.16. Found, C, 68.65; H, 7.41; N, 6.08.

Stability of Boc-amino acid-resins and Boc-valyl-4-(oxymethyl)-Pam-benzene (15) in Refluxing Trifluoroacetic Acid. Boc-amino acid-resin (50 mg) was placed in a 25-mL round-bottom flask equipped with a water condenser and drying tube. Anhydrous trifluoroacetic acid (10 mL) was added and the suspension was refluxed. At a given time the resin was filtered and washed with trifluoroacetic acid. The combined filtrates were evaporated in vacuo and the residue was dissolved in water for amino acid analysis. The extent of cleavage was measured for the Boc-aminoacyl-OCH_2-Pam-resins and compound **15** at 2, 4, and 6 h. Cleavage of Boc-Val-OCH_2-resin was measured at 15, 30, and 45 min. The results are summarized in Table I.

HF Cleavage Yields. Boc-Gly-OCH_2-Pam-resin (50.5 mg, 0.0081 mmol), Boc-L-Phe-OCH_2-Pam-resin (59.1 mg, 0.0096 mmol), Boc-L-Val-OCH_2-Pam-resin (54.7 mg, 0.0096 mmol), and Boc-Leu-Ala-Gly-Val-OCH_2-Pam-resin (106.9 mg, 0.0208 mmol) were cleaved with 10 mL of HF plus 1 mL of anisole for 32 min at 0 °C. After evaporation of the HF, residual anisole was removed by two 25-mL Et_2O rinses. The products were taken up by rinsing with 20% HOAc (2 × 25 mL) and HOAc (2 × 25 mL) After filtration, the solvent was evaporated and the residue was taken up in H_2O for analysis. The resin remaining after the HF cleavage was hydrolyzed for 6 h (130 °C) in HCl–propionic acid[41] to determine the residual amino acid content. Observed recoveries from HF cleavage were (residual amino acid shown in parentheses): Gly, 106% (4%); Phe, 82% (9%); Val, 88% (6%); Leu-Ala-Gly-Val, 94%.

Boc-valine-butylamide. Boc-Val (1.08 g, 5.00 mmol) and *p*-nitrophenyl trifluoroacetate[44] (1.41 g, 6.00 mmol) were reacted in dry pyridine (4 mL) for 1.5 h at room temperature. Water (0.018 mL, 1.00 mmol) was then added to destroy the excess *p*-nitrophenyl trifluoroacetate. After 5 min, *n*-butylamine was added (0.99 mL, 10 mmol) and the solution was allowed to stand overnight. The solvent was removed in vacuo and the resulting oil was worked up in the usual manner. A crystallization of the title compound from acetone–H_2O gave white needles (0.399 g, 29% yield): mp 113–116 °C; TLC (Solvent V) R_f 0.82; $[\alpha]^{25}{}_D$ − 22.7° (*c* 2.2, CH_3OH); NMR ($CDCl_3$) 1.00 (m, 9 H, Val γ-$(CH_3)_2$ and *n*-BuCH_3), near 1.4 (m, 4 H, *n*-Bu β,γ-CH_2CH_2), 1.50 (s, 9 H, *t*-Bu), 2.10 (m, 1 H, $C_\beta H$), 3.30 (q, J = 6 Hz, 2 H, NCH_2), 3.87 (m, 1 H, $C_\alpha H$), 5.17 (br d, J = 8 Hz, 1 H, urethane NH), and 6.17 ppm (br s, 1 H, CONHBu). Anal. Calcd for $C_{14}H_{28}N_2O_3$: C, 61.73; H, 10.36; N, 10.29. Found: C, 61.65; H, 10.24; N, 10.19.

Treatment of Chloromethyl-resin and Boc-amino acid-resins with Amines. A. Reaction with *n*-Butylamine. Chloromethylpoly(styrene-*co*-1% divinylbenzene) resin (Pierce, 0.69 mmol of Cl/g of resin) was suspended in *n*-butylamine (25 mL/g of resin) and either shaken at 25 °C (1, 18 h) or refluxed (1 h). The suspension was filtered and the resin was washed with dimethylformamide, dichloromethane, 2-propanol, and ethanol, and vacuum dried. The resin treated with *n*-butylamine for 1 h (25 °C) contained 0.40 mmol of N/g of resin and 0.30 mmol of Cl/g of resin. The 18-h sample (25 °C) contained 0.71 mmol of N/g of resin and no Cl. Similarly, a resin refluxed in *n*-butylamine (1 h) contained 0 72 mmol of N/g of resin and no Cl. See Table II (runs 1–2).

Boc-Val-resin (0.100 g) was suspended in 4 mL of 100% *n*-$C_4H_9NH_2$ (Table II, runs 3 and 4) or 10% (v/v) *n*-$C_4H_9NH_2$–CH_2Cl_2 (Table II, runs 5 and 6) and shaken at 25 °C. At a given time the suspension was filtered and the resin was washed with dichloromethane. The pooled filtrates were evaporated in vacuo and the residue was treated with trifluoroacetic acid (25 °C) for 30 min. The trifluoroacetic acid was evaporated in vacuo and the residue was dissolved in water (5 mL) for injection into the long column (0.9 × 58 cm AA-15 cation-exchange resin) of the Beckman 120B amino acid analyzer. The column was eluted with borate (pH 10) buffer at 57 °C (61 mL/h). A standard was prepared by treating Boc-Val-NHC_4H_9 with trifluoroacetic acid and removing trifluoroacetic acid in vacuo. The resulting valine *n*-butylamide eluted at 49 min using the ion-exchange column just described.

B. Reaction with Benzylamine. Boc-Gly-resin (0.100 g) was suspended in 5% (v/v) benzylamine–dichloromethane (Table II, runs 7 and 8) and treated as described for Boc-Val-resin. Glycine benzylamide[45] eluted at 86 min under the conditions of ion-exchange chromatography described for valine *n*-butylamide. The results obtained from treatment of the polystyrene derivatives with amines are summarized in Table II.

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Registry No.—*N*-(Hydroxymethyl)phthalimide, 118-29-6; copoly(styrene–divinylbenzene, 9003-70-7; *p*-tolylacetic acid, 622-47-9; 4-(bromomethyl)phenylacetic acid, 13737-36-5; bromoacetophenone, 70-11-1; 4-(bromomethyl)phenylacetic acid phenacyl ester, 66270-97-1; Boc-L-Val, 13734-41-3; Boc-Valyl-4-(oxymethyl)phenylacetic acid phenacyl ester, 66402-58-2; Boc-Valyl-4-(oxymethyl)phenylacetic acid CHA salt, 66270-98-2; Boc-L-Lys(Z)-OCH_2PhCH_2COOH CHA salt, 66271-00-9; BOC-L-Asp(OBzl)OCH_2PhCH_2COOH CHA salt, 66271-02-1; Boc-L-Ser(Bzl)-$\mathrm{OCH_2PhCH_2COOH}$ CHA salt, 66271-04-3; Boc-L-Met-$\mathrm{OCH_2PhCH_2COOH}$ CHA salt, 66271-06-5; Boc-L-Lys(Z)-$\mathrm{OCH_2C_6H_4}$-*p*-$\mathrm{CH_2COOH_2COPh}$, 66271-07-6; Boc-L-Asp(OBzl)-$\mathrm{OCH_2C_6H_4}$-*p*-$\mathrm{CH_2COOCH_2COPh}$, 66271-08-7; Boc-L-Ser(Bzl)-$\mathrm{OCH_2C_6H_4}$-*p*-$\mathrm{CH_2COOCH_2COPh}$, 66271-09-8; Boc-L-Met-$\mathrm{OCH_2C_6H_4}$-*p*-$\mathrm{CH_2COOCH_2COPh}$, 66271-10-1; Boc-Val-$\mathrm{OCH_2C_6H_4}$-*p*-$\mathrm{CH_2CONHCH_2Ph}$, 66271-11-2; Boc-L-Val DCHA salt, 16944-17-5; Boc-L-Val-4-($\mathrm{OCH_2}$)$\mathrm{PhCH_2CO}$-4-($\mathrm{OCH_2}$)$\mathrm{PhCH_2COOH}$, 66271-12-3; *N*-hydroxysuccinimide, 6066-82-6; 4-(bromomethyl)phenylacetic, acid *N*-hydroxysuccinimide ester, 66271-13-4; Boc-Valyl-4-(oxymethyl)phenylacetic acid *N*-hydroxysuccinimide ester, 66271-14-5; 4-(acetoxymethyl)phenylacetic acid, 61165-81-9; Boc-Gly Cs salt, 42538-64-7; Boc-L-Phe Cs salt, 42538-61-4; Boc-L-Val Cs salt, 42538-62-5; Boc-Leu, 13139-15-6; L-Leu-L-Val, 13588-95-9; Boc-Gly, 4530-20-5; Boc-L-Ala, 15761-38-3; Leu-Ala-Gly-Val, 17195-26-5; benzylamine, 100-46-9; *p*-nitrophenyltrifluoroacetate, 658-78-6; butylamine, 109-73-9; Boc-Valine butylamide, 66271-15-6; Boc-Gly-NHBzl, 19811-52-0.

References and Notes

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(2) Abbreviations used: Boc, *tert*-butyloxycarbonyl; CHA, cyclohexylamine; DCC, dicyclohexylcarbodiimide; DCHA, dicyclohexylamine; DMF, *N,N*-dimethylformamide; KelF-g-styrene, radiation-induced graft polymer of styrene on solid poly(trifluorochloroethylene); NMR, nuclear magnetic resonance; Pam, phenylacetamidomethyl; PLC, preparative layer chromatography; R, resin; TLC, thin-layer chromatography. Other nomenclature and symbols follow the Tentative Rules of the IUPAC–IUB Commission on Biochemical Nomenclature, *J. Biol. Chem.*, **241,** 2491 (1966); **242,** 555 (1967); **247,** 977 (1972).

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Synthesis of Oxysanguinarine

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Base-catalyzed condensation of the homophthalate ester **14** with the imine **15** supplied the lactam amide **16.** This compound was saponified to the acid **17** which was homologated by an Arndt–Eistert sequence to the ester **19.** Hydrolysis and acid-catalyzed cyclization provided the keto lactam **21.** Acid dehydration of the lactam alcohol **22,** derived from reduction of **21,** was accompanied by air oxidation to provide the desired alkaloid oxysanguinarine (**23**).

A number of aromatic benzophenanthridine alkaloids possess interesting biological activity. Nitidine (**1**) and fagaronine (**2**) have shown anticancer activity,[1] while sanguinarine (**3**), chelerythrine (**4**), and chelirubine (bocconine) (**5**) are nematocides.[2]

The aim of the present study was to synthesize a naturally occurring aromatic benzophenanthridine, namely oxysanguinarine (**23**),[3] through a route based on the previously reported finding that base-catalyzed condensation of diethyl glutaconate with *N*-benzylidenemethylamine yields lactam **6.**[4] The first hurdle was to prepare the homophthalic ester **14,** which was to be condensed with piperonylidenemethylamine (**15**) to afford such lactams as **16, 17,** or **18.** Homologation of the acid **17** to the acid **20,** followed by intramolecular Friedel–Crafts acylation, would then afford keto lactam **21,** which would be readily convertible into oxysanguinarine (**23**).

An eight-step sequence to the homophthalic ester **14** was developed which parallels to some extent, but is superior to, that recorded by Haworth and co-workers for the construction of the corresponding homophthalic acid **13.**[5] Doebner con-

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