pH's, which are given in Table V, showed structure dependence. Thus, whereas compound IIIg retains the fluorescence properties of the parent hydrazide IIg, compounds IIIc and IIId show emission characteristics (λ_{em} 550 nm) due to their much longer wavelength absorption bands. The fluorescence excitation maximum for IIIc was found at 400 nm (pH 6.2 or 10.7), corresponding within limit of error to the longest wavelength ultraviolet absorption maximum for this compound. Moreover, the observed smooth fluorescence emission of IIIc is a mirror image of the absorption band. The fluorescent cytidine derivatives are, in general, detectable down to concentrations of the order of 10^{-5} to 10^{-6} *M*, with a quantum yield lower than 0.01.

Compounds IIIc, IIId, and IIIh, especially in zwitterionic form, show a clear enhancement of fluorescence when adsorbed on silica gel (Table III). Since the emission maximum of IIIc is shifted in going from water (550 nm) to dimethyl sulfoxide (578 nm), corresponding to the bathochromic shift in the ultraviolet absorption, the influence of the environment on the fluorescence of this molecule is clear and may be of advantage. The incorporation of these fluorophores into single-stranded oligo- and polynucleotides and nucleic acids at original cytidine sites would result in changes in fluorescence properties depending upon the nature of the "solvent" in the region of the modified cytidine and upon interactions[34] with other portions of the larger matrix.

For application as converters of cytidine units to fluorescent modifications, compounds IIIc and IIId offer the best possibilities. Excitation is possible at 400–410 nm, well outside the range of absorption of proteins and nucleic acids. In addition, the absorption bands are in a favorable region for energy transfer studies with other dyes. Specifically, the fluorescent modification of adenine residues with chloroacetaldehyde[8-14] introduces fluorescence emission at about 410 nm and may be an excellent donor, since it has a sufficiently long fluorescence lifetime (~23 nsec) and the emission band of the 1,N^6-ethenoadenylate overlaps the absorption band of IIIc or IIId. Thus, if the spacing and relative orientation of the oscillators permitted strong interaction, the energy transfer should be efficient.[35]

The bifunctional reagent IIh reacts with two molecules of cytidine to give IIIh, which is fluorescent and emits at 460 nm at pH's above its pK_a' when excited at 335 nm. The appearance of fluorescence implies reaction with two cytidine moieties and may therefore signify intramolecular reaction when the cytidines have the proper spacing (bifunctional reagents with other distances between hydrazide functions can be engineered). Alternatively, it may be possible to use the reagent to bind together polynucleotide chains containing exposed cytidine units. In general, the fluorescence data for IIIc, IIId, and IIIh indicate that these derivatives of cytidine may provide advantages in further studies of tRNA structure and function by chemical modification.

Acknowledgment. We wish to thank Professor Gregorio Weber for valuable discussion and for providing the necessary facilities for fluorescence determinations. This work was supported by Research Grant GM-05829 from the National Institutes of Health, U. S. Public Health Service.

(34) A. J. Pesce, C. G. Rosèn, and T. L. Pasby, "Fluorescence Spectroscopy," Marcel Dekker, New York, N. Y., 1971.

(35) L. Stryer, *Radiat. Res. Suppl.*, **2**, 432 (1960).

p-Alkoxybenzyl Alcohol Resin and *p*-Alkoxybenzyloxycarbonylhydrazide Resin for Solid Phase Synthesis of Protected Peptide Fragments

Su-Sun Wang

Contribution from the Chemical Research Department, Hoffmann-La Roche Inc., Nutley, New Jersey 07110. *Received August 9, 1972*

Abstract: Two new resins—*p*-alkoxybenzyl alcohol resin ($HOCH_2C_6H_4OCH_2C_6H_4$ resin) and *p*-alkoxybenzyloxycarbonylhydrazide resin ($H_2NNHCOOCH_2C_6H_4OCH_2C_6H_4$ resin)—were prepared. The former resin is suitable for the synthesis of protected peptide fragments possessing a free carboxyl group while the latter is useful for the synthesis of protected peptide hydrazides. Applications of these resins in the syntheses of Z-Leu-Leu-Val-Phe, Z-Phe-Val-Ala-Leu-$HNNH_2$, Asp-Arg-Val-Tyr-Val-His-Pro-Phe, Z-Lys(Z)-Phe-Phe-Gly, and Z-Lys(Z)-Phe-Phe-Gly-Leu-Met-NH_2 are described.

Recent developments in solid phase peptide synthesis have been reviewed by Merrifield[1,2] and discussed by others.[3,4] The method has been widely and quite successfully utilized for the rapid and convenient synthesis of numerous polypeptides. However, the products obtained by this technique are, in general, rather difficult to purify. Although in certain instances, effective purification can be achieved by selective proteolysis[5] or affinity chromatography,[6]

(1) R. B. Merrifield, *Advan. Enzymol.*, **32**, 221 (1969).
(2) G. R. Marshall and R. B. Merrifield in "Biochemical Aspects of Reactions on Solid Supports," G. R. Stark, Ed., Academic Press, New York, N. Y., 1971, p 111.
(3) E. Wünsch, *Angew. Chem.*, **83**, 773 (1971).
(4) J. Meienhoffer, 163rd National Meeting of the American Chemical Society, Boston, Mass., April 1972, M15.

(5) B. Gutte and R. B. Merrifield, *J. Biol. Chem.*, **246**, 1922 (1971).
(6) H. Taniuchi and C. B. Anfinsen, *ibid.*, **244**, 3864 (1969).

those techniques are not universally applicable to all synthetic poypeptides. In view of obtaining the final peptides in greater purity, the use of modified resins suitable for solid phase synthesis of protected peptide fragments has been described by several authors.[7–14] In a previous communication[15] we have described the preparation of a *tert*-alkyloxycarbonylhydrazide resin that is useful for the synthesis of protected peptide hydrazides. In this paper, the preparation of the *p*-alkoxybenzyl alcohol resin (III) and the *p*-alkoxybenzyloxycarbonylhydrazide resin (V) and their application in the synthesis of a few protected peptide fragments are described.

The anchoring bonds employed are structurally similar to the *p*-methoxybenzyl ester[16] or *p*-methoxybenzyloxycarbonylhydrazide carboxyl protection of the classical peptide synthesis. These bonds can be selectively cleaved by trifluoroacetic acid under conditions where the side-chain protecting groups normally used are stable. When the very acid-labile 2-(*p*-biphenylyl)-2-propyloxycarbonyl[17,18] (Bpoc)[19] group is utilized for amino protection, resins III and V can be used satisfactorily for solid phase synthesis of protected peptide free acids and protected peptide hydrazides, respectively.

As outlined in Scheme I, Merrifield resin (I) (0.90 mmol of Cl/g) was allowed to react with methyl 4-hydroxybenzoate to form resin II. The product contained 0.87 mmol of OCH_3/g but no detectable amount of chlorine. An intense ester band at 1712 cm^{-1} and an aryl alkyl ether band at 1220 cm^{-1} appeared in the ir spectrum (Figure 1). Reduction of II with $LiAlH_4$ gave the desired *p*-alkoxybenzyl alcohol resin IIIa. In the ir spectrum, the ester band disappeared completely whereas the aryl alkyl ether band remained essentially unchanged. The same resin can also be conveniently prepared by reacting Merrifield resin with 4-hydroxybenzyl alcohol in the presence of $NaOCH_3$. The product obtained by this route (IIIb) is identical with IIIa as can be seen in Figure 1. Reaction of the alcohol resin III with phenyl chloroformate yielded IV which on hydrazinolysis gave *p*-alkoxybenzyloxycarbonylhydrazide resin V (see Figure 2). Nitrogen analysis indicated that there was 0.88 mmol/g of hydrazide on the resin. All of the reactions seem to have proceeded nearly quantitatively.

Bpoc-amino acids can be esterified to the alcohol

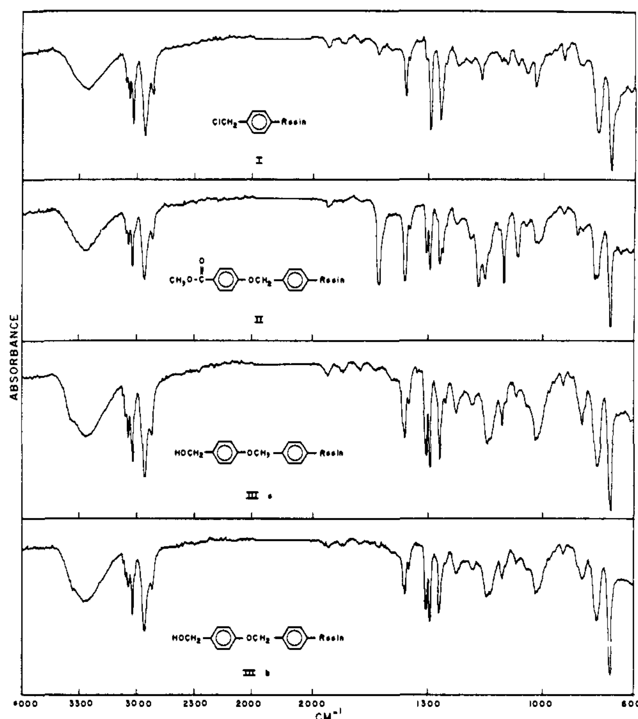
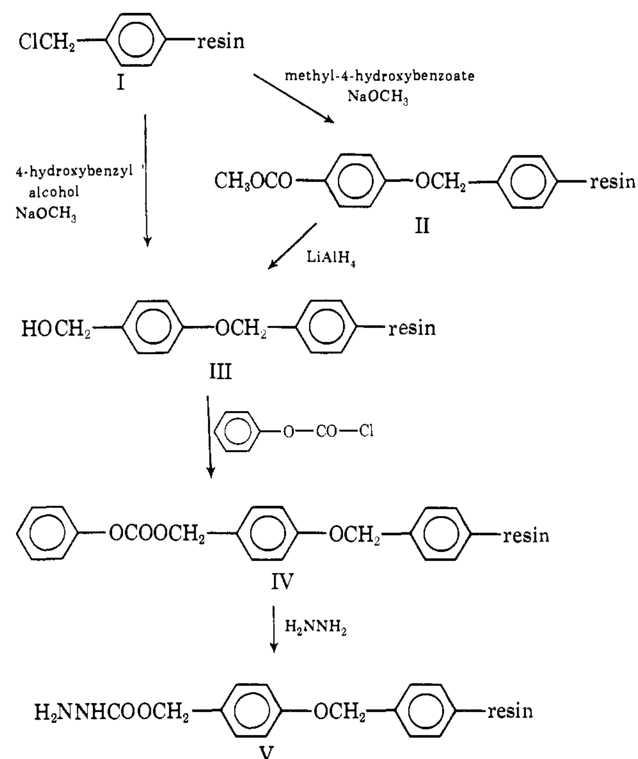


Figure 1. Infrared spectra of Merrifield resin (I); methyl *p*-alkoxybenzoate resin (II); *p*-alkoxybenzyl alcohol resin prepared from resin II by $LiAlH_4$ reduction (IIIa); *p*-alkoxybenzyl alcohol resin prepared directly from resin I and 4-hydroxybenzyl alcohol plus $NaOCH_3$ (IIIb).

Scheme I



resin III by DCC or active ester procedure. The extent of substitution was normally 0.2–0.6 mmol/g. To eliminate the unsubstituted free hydroxyl groups left on the resin, benzoylation or acetylation was found to be necessary. Attachment of Bpoc-amino acids to hydrazide resin V proceeded smoothly with the DCC method. To ascertain that no significant racemization

(7) T. Mizoguchi, K. Shigezane, and Takamura, *Chem. Pharm. Bull.*, **18**, 1465 (1970).
(8) G. L. Southard, G. S. Brooke, and J. M. Pettee, *Tetrahedron*, **27**, 1701 (1970).
(9) T. Wieland, J. Lewalter, and C. Birr, *Justus Liebigs Ann. Chem.*, **740**, 31 (1970).
(10) D. L. Marshall and I. E. Liener, *J. Org. Chem.*, **35**, 867 (1970).
(11) E. Flanigan and G. R. Marshall, *Tetrahedron Lett.*, 2403 (1970).
(12) S. S. Wang and R. B. Merrifield, *Proc. 10th Eur. Peptide Symp., 1969*, 74 (1971).
(13) G. Losse and K. Neubert, *Tetrahedron Lett.*, 1267 (1970).
(14) G. W. Kenner, J. R. McDermott, and R. C. Sheppard, *Chem. Commun.*, 636 (1971).
(15) S. S. Wang and R. B. Merrifield, *J. Amer. Chem. Soc.*, **91**, 6488 (1969).
(16) F. Weygand and K. Hunger, *Chem. Ber.*, **95**, 1 (1962).
(17) P. Sieber and B. Iselin, *Helv. Chim. Acta*, **51**, 614, 622 (1968).
(18) S. S. Wang and R. B. Merrifield, *Int. J. Protein Res.*, **1**, 235 (1969).
(19) Abbreviations used are: Bpoc, 2-(*p*-biphenylyl)-2-propyloxycarbonyl; Boc, *tert*-butyloxycarbonyl; Z, benzyloxycarbonyl; Tos, *p*-toluenesulfonyl; Bzl, benzyl; But, *tert*-butyl; ONP, *p*-nitrophenyl ester; TFA, trifluoroacetic acid; NMM, *N*-methylmorpholine; DCC, dicyclohexylcarbodiimide; THF, tetrahydrofuran.

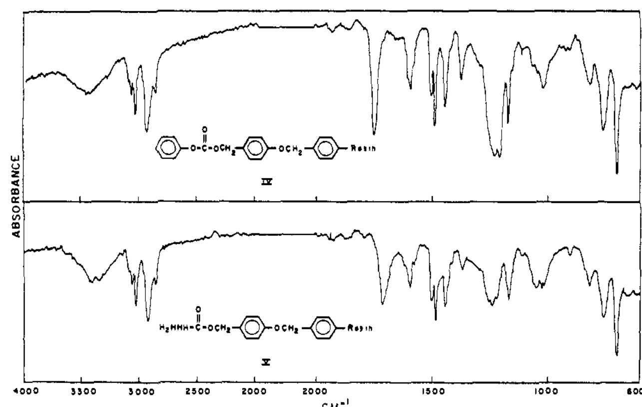


Figure 2. Infrared spectra of phenyl carbonate resin (IV); *p*-alkoxybenzyloxycarbonylhydrazide resin (V).

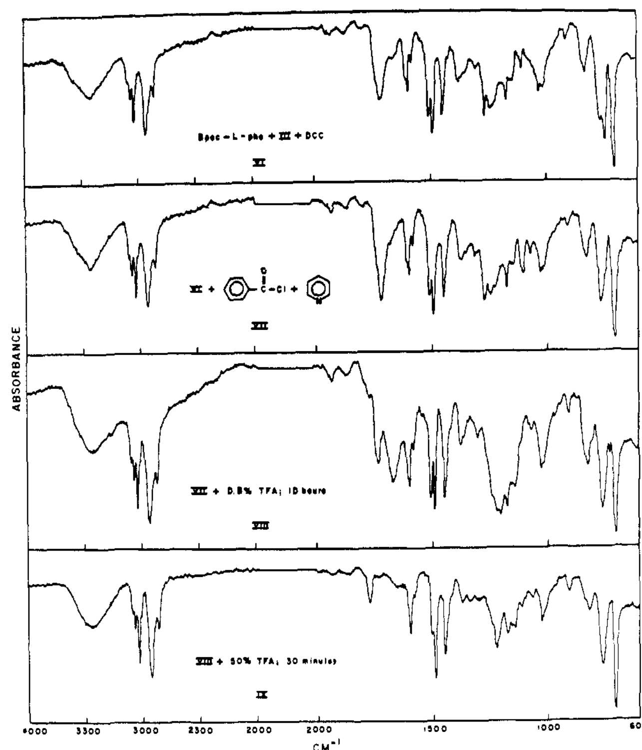


Figure 3. Infrared spectra of Bpoc-phenylalanyl resin (VI); resin VI after benzoylation (VII); resin VII that was treated with 0.5% TFA for 10 hr (VIII); resin VIII that was treated with 50% TFA for 30 min (IX).

took place during esterification of Bpoc-amino acids to resin III, Bpoc-Phe-resin (VII) was deblocked, neutralized, and coupled with Boc-L-Glu(But) to give Boc-L-Glu(But)-Phe-resin. The dipeptide was released from the resin with 50% TFA (30 min) and then examined with an amino acid analyzer by a procedure similar to that described by Manning and Moore.[20] The product chromatographed identically with L-Glu-L-Phe. Less than 0.1% of contaminant L-Glu-D-Phe was found. The optical purity of Bpoc-Leu-resin prepared from Bpoc-L-Leu, DCC, pyridine, and resin III was also examined. The free amino acid was cleaved from the resin by 50% TFA and allowed to react with L-Leu-*N*-carboxyanhydride at pH 10.2. The resultant dipeptide was chromatographed with an amino acid analyzer. In this case, too, very little (less than 0.1%) L-Leu-D-Leu was detected. The product chromatographed identically with L-Leu-L-Leu.

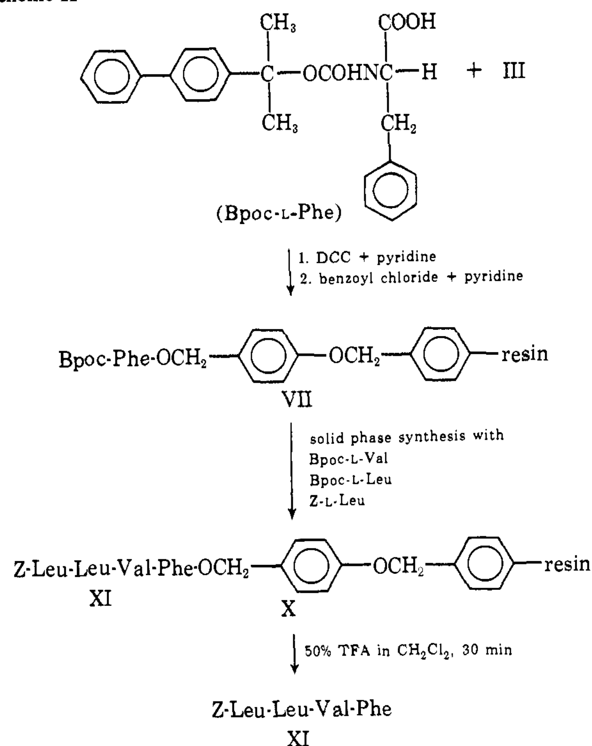
In agreement with our previous observations,[15,18] the Bpoc group on VII can be removed by 0.5% TFA CH_2Cl_2 within a few minutes, while the anchoring bond is stable even after VII was treated with 0.5% TFA for 10 hr, as ascertained by ir spectroscopy. No free phenylalanine was liberated into the solution during this period of time. The anchoring linkage on the other hand can be very efficiently cleaved by 50% TFA in 30 min. As illustrated in Figure 3, the carbonyl band in the ir spectrum disappeared completely after this treatment and more than 85% of free phenylalanine was recovered from the filtrate. The experiments with Bpoc-Leu-HNNH-resin (XIV) gave similar results.

The synthesis of the protected tetrapeptide XI is outlined in Scheme II. Esterification of Bpoc-L-Phe to resin III was achieved by the DCC method using an equivalent amount of pyridine as catalyst. After benzoylation, Bpoc-L-Val, Bpoc-L-Leu, and Z-L-Leu were successfully coupled to VII according to the general principle of solid phase peptide synthesis[1,2] with the necessary changes described before.[15] Treatment of X with 50% TFA gave XI as a pure crystalline compound in 68% overall yield calculated from the phenylalanine content of VII. Compound XI was further converted into its methyl ester XII and hydrogenated to give the tetrapeptide methyl ester XIII.

In Scheme III, the synthesis of the protected peptide hydrazide XVI is outlined. A crystalline, pure product was obtained in 42% overall yield.

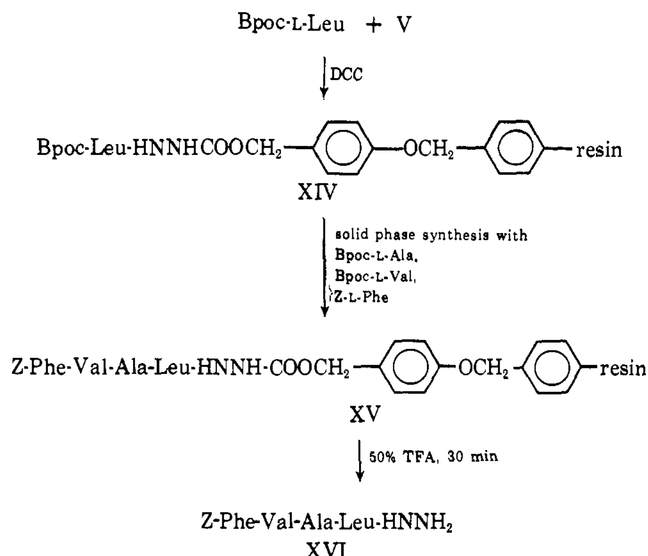
(20) J. Manning and S. Moore, *J. Biol. Chem.*, **243**, 5591 (1968).

Scheme II

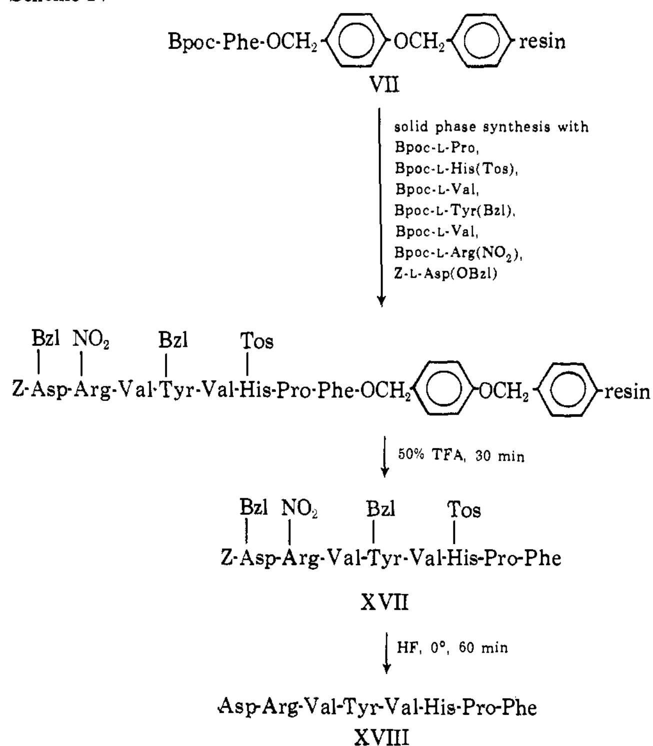


The alcohol resin III was utilized as the solid support for the synthesis of Val^5-angiotensin II (XVIII) (Scheme IV). In this synthesis, Bpoc-L-His(Tos) was used since partial racemization takes place when N^{Im}-benzyl-

Scheme III



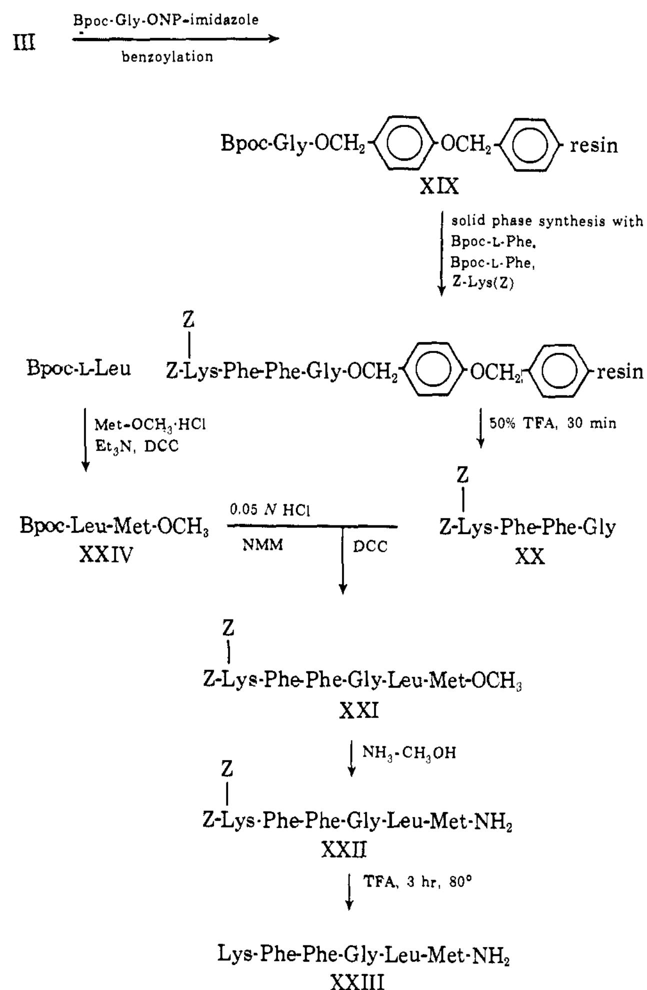
Scheme IV



histidine derivatives are used in solid phase synthesis.[21] The protected octapeptide intermediate XVII was released from the resin by 50% TFA. All of the protecting groups were then removed by anhydrous hydrogen fluoride[22] and the crude product was purified by countercurrent distribution followed by gel filtration. Analytically pure material was obtained in 15% overall yield.

Resin III was also used in the synthesis of the eledoisin analog Lys-Phe-Phe-Gly-Leu-Met-NH_2 (XXIII)[23] by a combination of solid phase method and fragment condensation procedures (see Scheme V). The crystalline protected tetrapeptide fragment XX was prepared by the solid phase technique (60% yield) and then condensed with the dipeptide methyl ester XXIV (prepared by the conventional method) to give the hexapeptide methyl ester XXI. Ammonolysis of this ester afforded the corresponding amide XXII in good yield. The carbobenzoxyl groups on XXII were then removed by hot TFA and the crude peptide was purified by countercurrent distribution followed by gel filtration. The product was shown to be homogeneous by thin layer chromatography and electrophoresis. It gave the expected amino acid analysis after acid hydrolysis.

Scheme V



Experimental Section

Melting points are uncorrected. Infrared spectra were taken on a Beckman IR-8 spectrophotometer with KBr pellets. Amino acid analyses were carried out on a Jeolco 5AH amino acid analyzer or Beckman Model 120B amino acid analyzer. A 500-tube Post countercurrent distribution apparatus was used for purification purposes. Paper electrophoresis was performed on a Camag high voltage electrophoresis apparatus and thin layer chromatography was carried out on the precoated silica gel plates (Merck, F-254).

The Merrifield resin (chloromethylated copolystyrene–1% divinylbenzene, 0.90 mequiv/g, 200–400 mesh) used in this work was purchased from Cyclo Chemical Co. Amino acid derivatives were obtained from Fox Chemical Co. or Schwarz/Mann. All the Bpoc-amino acids were prepared in this laboratory and were of L configuration unless otherwise stated.

Methyl *p*-Alkoxybenzoate Resin (II). Merrifield resin (I) (45 g, 40.5 mmol) in 250 ml of dimethylacetamide was allowed to react with 16 g of methyl 4-hydroxybenzoate (105 mmol) and 5.8 g of

(21) E. C. Jorgensen, G. C. Windridge, and T. C. Lee, *J. Med. Chem.*, 13, 352 (1970).

(22) S. Sakakibara in "Chemistry and Biochemistry of Amino Acids, Peptides and Proteins," Vol. 1, B. Weinstein, Ed., Marcel Dekker, New York, N. Y., 1971, p 51.

(23) L. Bernardi, G. Bosisio, F. Chillemi, G. de Caro, R. de Castriglione, V. Erspamer, A. Glaeser, and O. Goffredo, *Experientia*, **20**, 306 (1964).

$NaOCH_3$ (107 mmol) at 80° for 24 hr. The resin was collected and washed with DMF, dioxane, CH_2Cl_2, and methanol to give 49.5 g of II. The product absorbed strongly at 1712 and 1220 cm^{-1} (see Figure 1). It contained 2.69% (0.87 mmol/g) of OCH_3 but no detectable amount of Cl.

p-Alkoxybenzyl Alcohol Resin (III). Resin II (45 g) was treated with 4.6 g of $LiAlH_4$ in 600 ml of dry ether for 6 hr. The resin was then collected and washed carefully with ethyl acetate, methanol, CH_2Cl_2, and methanol to give a slightly grayish product. The color was removed by stirring in 2 l. of a 1:1 mixture of dioxane and 1 *N* H_2SO_4 for 45 hr. The snow white product (IIIa, 43 g) contained 0.3% OCH_3 (0.1 mmol/g). The ester band in the ir spectrum disappeared completely.

The same resin can also be prepared directly by treating Merrifield resin (5.1 g, 4.6 mmol) with 4-hydroxybenzyl alcohol (0.74 g, 5.9 mmol) and $NaOCH_3$ (0.32 g, 6.1 mmol) under similar conditions. The product obtained (IIIb, 5.2 g) gave an identical ir spectrum as that of IIIa. Microanalysis indicated that there was less than 0.07% (0.02 mmol/g) of chlorine.

p-Alkoxybenzyloxycarbonylhydrazide Resin (V). Resin III (8 g) in 70 ml of CH_2Cl_2 was treated with 7.8 ml of phenyl chloroformate and 5.5 ml of pyridine at 0° overnight. The reaction mixture was suction filtered and the resin washed consecutively with cold water, dioxane–water, DMF, CH_2Cl_2, and methanol to give 8.1 g of phenyl carbonate resin IV. It was immediately suspended in 70 ml of DMF and treated with anhydrous hydrazine (6.6 ml). After 6 hr of gentle stirring, the resin was collected and washed to give 6.8 g of the desired compound. Nitrogen analysis indicated that there was 0.88 mmol/g (2.45% N) of hydrazide. The ir spectra of IV and V are shown in Figure 2.

Bpoc-Phenylalanyl p-Alkoxybenzyl Alcohol Resin (VII). p-Alkoxybenzyl alcohol resin IIIa (5 g, 4.4 mmol) was washed several times with CH_2Cl_2 and then allowed to react with 2.5 g of Bpoc-L-Phe (6.3 mmol) and 1.3 g of DCC in the presence of 0.51 ml of pyridine for 150 min. After washings, 5.8 g of Bpoc-Phe-resin (VI) was obtained. It was then treated with 1.65 ml of pyridine and 1.95 ml of benzoyl chloride in 58 ml of CH_2Cl_2 at 0° for 15 min (see next section) to give 6.1 g of VII. The ir spectra of these resins are shown in Figure 3. There was 0.410 mmol of phenylalanine/g of resin according to amino acid analysis. Microanalysis indicated that there was 0.60% N (0.428 mmol/g) in this resin.

When resin IIIb was esterified with Bpoc-Phe in the same manner as above, the Bpoc-Phe-resin (VII) obtained was found to have 0.406 mmol/g of phenylalanine. The ir spectrum was identical with the corresponding aminoacyl resin described above.

Elimination of the Unreacted Hydroxyl Groups on the Resin. It was found necessary to eliminate the excess unreacted hydroxyl groups left on the resin after the first Bpoc-amino acid was attached. The rate of acylation of resin III by benzoyl chloride or acetic anhydride was therefore studied. A sample of resin III (0.3 g) was suspended in 3 ml of CH_2Cl_2 that contained 0.1 ml of pyridine. Benzoyl chloride (0.11 ml) was added at 0° while the mixture was kept stirred magnetically. Aliquots (0.1 ml) were withdrawn at different intervals and washed immediately with CH_2Cl_2 and methanol. The rate of increase in the ratio of the ester band at 1720 cm^{-1} to the polystyrene band at 1600 cm^{-1} in the ir spectra of these samples was taken as the rate of benzoylation. The reaction proceeded quite rapidly to completion with a half-life time of 40 sec. When similar experiments were carried out with tenfold excess of acetic anhydride with triethylamine as base, the reaction was found to proceed only to 70% completion after 60 min at 25°. Addition of a catalytic amount (0.05 equiv) of 4-dimethylaminopyridine[24] to the reaction mixture did bring the reaction to completion within 1 hr. Thus, acetylation with acetic anhydride in the presence of the catalyst appears to be satisfactory also.

Racemization Tests for Bpoc-Amino Acid Resins. Amino acid resin VII (0.5 g) was deprotected with 0.5% TFA (10 min), neutralized, and then coupled with 0.25 g of Boc-L-Glu(But) in the presence of 0.16 g of DCC to form Boc-L-Glu(But)-Phe-resin. The dipeptide was cleaved from the resin with 50% TFA (30 min) and the filtrate chromatographed on an amino acid analyzer in a system similar to that reported by Manning and Moore.[20] The product chromatographed identically with L-Glu-L-Phe. Only a trace amount of L-Glu-D-Phe (less than 0.1%) was detected.

In another experiment, 66 mg of Bpoc-Leu-$OCH_2C_6H_4OCH_2$-C_6H_4-resin was treated with 2 ml of 50% TFA (30 min) and the liberated free leucine was allowed to react with 4 mg of L-Leu-*N*-carboxyanhydride at pH 10.2, 0°. The product (L-Leu-L-Leu) contained very little (less than 0.1%) L-Leu-D-Leu as revealed by the chromatographic analysis.

Bpoc-Glycyl p-Alkoxybenzyl Alcohol Resin (XIX). The alcohol resin III (6.1 g) was washed a few times with dioxane and stirred with 3.3 g of Bpoc-Gly-ONP (7.5 mmol) plus 5.1 g of imidazole (75 mmol) in 50 ml of dioxane for 18 hr. The esterified resin was benzoylated as above to give 7.0 g of XIX. Amino acid analysis indicated that there was 0.36 mmol/g of glycine.

Z-Leu-Leu-Val-Phe (XI). Phenylalanyl resin VII (3.2 g, 1.3 mmol) was placed in the peptide synthesis flask[25] and the solid phase synthesis carried out with 65-ml portions of solvents according to the procedures described previously[15] using a threefold excess (4.2 mmol) of amino acid derivative and DCC in each cycle. Thus, Bpoc-L-Val (1.5 g), Bpoc-L-Leu (1.55 g), and Z-L-Leu (1.1 g) were sequentially coupled to the resin to give 3.8 g of protected tetrapeptide resin X (see Scheme II). According to amino acid analysis, X contained 0.38 mmol/g of peptide and had an amino acid composition of $Val_{0.98}Leu_{2.00}Phe_{1.21}$. To liberate the protected peptide from the resin, 3.47 g of X was stirred in 70 ml of 50% TFA for 30 min. After removal of the resin particles by filtration and the solvents by evaporation, an oily residue obtained was triturated with petroleum ether. The white powder was taken up in 200 ml of ethyl acetate–ether mixture and left standing overnight at 4°. Crystalline white solid formed slowly during this time: yield, 0.54 g (68.3%); mp 216–219°; nmr spectrum consistent with the structure. It had the amino acid composition of $Val_{0.95}Leu_{1.94}$-$Phe_{1.08}$.

Anal. Calcd for $C_{34}H_{48}N_4O_7$ (624.8): C, 65.36; H, 7.74; N, 8.97. Found: C, 65.32; H, 7.59; N, 8.77.

Z-Leu-Leu-Val-Phe-OCH_3 (XII). Compound XI (0.42 g) was dissolved in 300 ml of 0.2 *N* methanolic HCl and kept overnight at 4°. Evaporation of the solvent left an oil which was crystallized from ethyl acetate with petroleum ether: yield, 0.35 g (82%); mp 204–206°.

Anal. Calcd for $C_{35}H_{50}N_4O_7$ (638.8): C, 65.80; H, 7.89; N, 8.77. Found: C, 65.22; H, 7.80; N, 8.78.

Leu-Leu-Val-Phe-OCH_3 (XIII). Hydrogenation of the above compound (XII, 0.57 g) in 20 ml of solvent mixture containing MeOH-THF-acetic acid (10:10:1) at 48 psi for 20 hr in the presence of 0.2 g of catalyst (5% Pd on $BaSO_4$) gave the tetrapeptide methyl ester free amine in a moderate yield (0.19 g, 43%), mp 157–159°.

Anal. Calcd for $C_{27}H_{44}N_4O_5$ (504.7): C, 64.20; H, 8.77; N, 11.10; OCH_3, 6.19. Found: C, 63.95; H, 8.72; N, 10.96; OCH_3, 6.09.

Z-Phe-Val-Ala-Leu-$HNNH_2$ (XVI). Bpoc-L-Leu (0.69 g, 1.8 mmol) was acylated to resin V (1.1 g, 0.96 mmol) by stirring in 15 ml of CH_2Cl_2 for 90 min in the presence of 0.37 g of DCC. The product (XIV, 1.2 g) was found to have 0.86 mmol/g of leucine according to amino acid analysis. A small sample was treated with 50% TFA and the filtrate examined on thin layer chromatography. Only Leu-$HNNH_2$ was found. There was no free leucine present. These results indicated that the conversion of resin III into resin V had proceeded quite completely. All of the hydroxyl groups on resin III appeared to have been transferred to hydrazide function.

Solid phase synthesis[1,15] was carried out on 0.6 g (0.52 mmol) of XIV using 12-ml portions of solvents with a threefold excess of amino acid derivatives and DCC in each cycle. As outlined in Scheme III, Bpoc-L-Ala (0.52 g), Bpoc-L-Val (0.57 g), and Z-L-Phe (0.47 g) were sequentially coupled to the resin. The protected tetrapeptide hydrazide resin XV weighed 0.63 g after drying. Part of the material (0.5 g) was stirred in 10 ml of 50% TFA for 30 min and the peptide released was crystallized from methanol with ether: yield, 0.11 g (42%); mp 252–254° (lit.[15] mp 255–257°).

Anal. Calcd for $C_{31}H_{44}N_6O_6$ (596.7): C, 62.39; H, 7.43; N, 14.09. Found: C, 62.29; H, 7.33; N, 13.73.

Asp-Arg-Val-Tyr-Val-His-Pro-Phe (XVIII). Bpoc-Phe-resin VII (1.0 g, 0.41 mmol) was placed in the peptide synthesis flask and the solid phase synthesis[15] carried out with 18-ml portions of solvents. In each cycle, fourfold excesses of amino acid derivative and DCC were used. Thus, Bpoc-L-Pro (0.56 g), Bpoc-L-His(Tos) (1.08 g), Bpoc-L-Val (0.57 g), Bpoc-L-Tyr(Bzl) (0.82 g), Bpoc-L-Val (0.57 g), Bpoc-L-Arg(NO_2), and Z-L-Asp(OBzl) (0.57 g) were successively coupled to the resin. The resultant octapeptide resin (1.36 g) was stirred in 26 ml of 50% TFA for 30 min. Protected octapeptide (0.42 g) was isolated as amorphous white solid. It had an amino

(24) W. Steglich and G. Hofle, *Angew. Chem.*, **81**, 1001 (1969).

(25) R. B. Merrifield and M. A. Corigliano, *Biochem. Prep.*, **12**, 98 (1968).

acid composition of $Asp_{0.96}Pro_{0.99}Val_{2.34}Tyr_{0.25}Phe_{1.14}His_{0.80}Arg_{1.00}$. Part of the sample (0.35 g) was then dissolved in 2 ml of TFA. Anisole (0.35 ml) was added and the mixture treated with 8 ml of anhydrous HF for 60 min at 0°. Removal of the excess acids left an oily residue which was taken up in 60 ml of water, washed several times with ether, and lyophilized to give 0.17 g of crude peptide. It was then purified by countercurrent distribution in a solvent system made up from *n*-BuOH–HOAc–H_2O (4:1:5) for 200 transfers (K = 0.27) followed by gel filtration on a Sephadex G-10 column (2.5 × 85 cm) using 0.2 *M* acetic acid as eluent. The material in the major peak was collected and lyophilized to give 56 mg of pure product. It was shown to be homogeneous on thin layer chromatography and paper electrophoresis. On acid hydrolysis, the compound gave the correct amino acid analysis: $Asp_{1.06}Pro_{1.03}Val_{1.93}Tyr_{1.02}Phe_{1.01}His_{0.93}Arg_{1.00}$.

Anal. Calcd for $C_{51}H_{83}N_{13}O_{19}$ (1182.3): C, 51.81; H, 7.08; N, 15.40. Found: C, 51.84; H, 7.06; N, 15.47.

Bpoc-Leu-Met-OCH_3 (XXIV). Bpoc-L-Leu (6.9 g, 18.6 mmol) was allowed to react with 3.73 g of Met-OCH_3·HCl (18.7 mmol) and 3.9 g of DCC in 80 ml of CH_2Cl_2 containing 2.6 ml of triethylamine at 0° for 2 hr. The insoluble by-product formed was filtered off and the filtrate washed a few times with water. The solution was dried over Na_2SO_4 and then concentrated to an oil. It was dissolved in a small volume of CH_2Cl_2 and treated with petroleum ether. Upon cooling, the product started to crystallized slowly: yield, 7.5 g (78%); mp 80–82°; $[\alpha]^{25}D$ −38.09° (*c* 1, MeOH).

Anal. Calcd for $C_{28}H_{38}N_2O_5S$ (514.7): C, 65.34; H, 7.44; N, 5.44. Found: C, 65.73; H, 7.83; N, 5.38.

Bpoc-Leu-Met-NH_2. Ammonolysis of the above compound (XXIV, 3 g) in 80 ml of methanol that had been saturated with dry ammonia resulted in the formation of the corresponding peptide amide (1.96 g, 67%): mp 99–102°; $[\alpha]^{25}D$ −37.46° (*c* 1, MeOH).

Anal. Calcd for $C_{17}H_{37}N_3O_4S$ (499.7): C, 64.90; H, 7.46; N, 8.41. Found: C, 64.90; H, 7.78; N, 8.27.

Z-Lys(Z)-Phe-Phe-Gly (XX). Bpoc-Gly-resin XIX (7.0 g, 2.52 mmol) was placed in the peptide synthesis flask and the solid phase synthesis[15] carried out with 150-ml portions of solvents using a 2.4-fold excess of amino acid derivative and DCC in each cycle. As outlined in Scheme V, Bpoc-L-Phe (2.42 g), Bpoc-L-Phe (2.42 g), and Z-L-Lys(Z) (2.43 g) were sequentially coupled to the resin to give 7.5 g of the protected tetrapeptide resin. The peptide was then released from the resin by stirring in 150 ml of 50% TFA for 30 min. After removing the resin particles and the solvents, the oily residue left was treated with 50 ml of ethyl acetate. The solid obtained was dissolved in THF and crystallized by addition of water: yield, 1.02 g (60%); mp 220–222°; $[\alpha]^{25}D$ −25.55° (*c* 1, DMF); nmr spectrum consistent with the structure.

Anal. Calcd for $C_{42}H_{47}N_5O_9$ (765.9): C, 65.87; H, 6.19; N, 9.14. Found: C, 65.81; H, 6.19; N, 9.14.

Z-Lys(Z)-Phe-Phe-Gly-Leu-Met-OCH_3 (XXI). Bpoc-Leu-Met-OCH_3 (XXIV) (0.52 g, 1.0 mmol) was dissolved in a mixture of 1 ml of 2.4 *N* HCl in ethyl acetate and 47.5 ml of CH_2Cl_2. After 10-min standing, the solvents were removed at 25° under reduced pressure and the oily residue of the dipeptide hydrochloride was taken up in 20 ml of DMF–CH_2Cl_2 mixture. The solution was cooled to 0° while 0.77 g of Z-Lys(Z)-Phe-Phe-Gly (1 mmol) was added followed immediately by 0.3 ml of *N*-methylmorpholine and 0.23 g of DCC. The mixture was stirred at 0° overnight. The insoluble material formed was filtered off and the filtrate washed a few times with water, dried over Na_2SO_4, and then evaporated to an oil. It was dissolved in DMF–CH_2Cl_2 mixture and precipitated with ether. The product was crystallized from THF by slow addition of water: yield, 0.85 g (83%), mp 180–184°.

Anal. Calcd for $C_{54}H_{69}N_7O_{11}S$ (1024.3): C, 63.32; H, 6.79; N, 9.57. Found: C, 63.87; H, 6.74; N, 9.54.

Z-Lys(Z)-Phe-Phe-Gly-Leu-Met-NH_2 (XXII). The above compound XXI (0.75 g, 0.73 mmol) was suspended in 100 ml of dry methanol and bubbled with dry ammonia gas for 2 hr at 0°. The compound became soluble in the solution but started to crystallize out slowly during overnight standing at room temperature. The product was collected and washed with ether to give 0.58 g of the desired compound: mp 238–242°; $[\alpha]^{25}D$ −39.28° (*c* 1, DMF).

Anal. Calcd for $C_{53}H_{68}N_8O_{10}S$ (1009.3): C, 63.08; H, 6.70; N, 11.10; S, 3.18. Found: C, 62.79; H, 6.70; N, 11.25; S, 2.90.

Lys-Phe-Phe-Gly-Leu-Met-NH_2 (XXIII). Compound XXII (0.15 g) was dissolved in 10 ml of TFA containing 0.5 ml of mercaptoethanol as well as 1 ml of anisole. The mixture was warmed at 80° for 3 hr during which time some white insoluble material came out of the solution. It was fitered off and the filtrate was treated with a large volume of ether to precipitate the product. The crude peptide was then purified by countercurrent distribution in a solvent system of *n*-BuOH–HOAc–pyridine–H_2O (8:2:2:9) for 300 transfers (K = 2.8) followed by gel filtration on a Sephadex G-10 column (2.5 × 85 cm) using 0.2 *M* acetic acid as eluent. The material in the main fraction was collected and lyophilized to give 33 mg of pure product. It gave correct amino acid analyses upon acid hydrolysis: $Gly_{1.00}Met_{0.93}Leu_{1.05}Phe_{2.11}Lys_{0.92}$. The product was shown to be homogeneous on thin layer chromatography and paper electrophoresis.

Anal. Calcd for $C_{37}H_{56}N_8O_6S \cdot 2CH_3COOH$ (861.1): C, 57.19; H, 7.49; N, 13.01. Found: C, 57.38; H, 7.61; N, 13.00.

Acknowledgment. The author wishes to thank Dr. R. B. Merrifield for helpful discussions and Drs. G. Saucy and A. M. Felix for valuable suggestions.

Communications to the Editor

The Reverse Anomeric Effect and the Synthesis of α-Glycosides

Sir:

One of the classical problems of carbohydrate chemistry has been the preparation of α-glycosides—especially those of cis-1,2 configuration. The problem has been difficult since the usual substituents used as leaving groups on C-1 are electronegative and have a preferred axial or α configuration because dipolar interactions predominate over the usual steric factors. Therefore, when nonparticipating groups are present on C-2 and replacement by alkoxyl occurs with inversion, β-glycosides are usually the preferred product. When participating groups are present on C-2, the configuration of the resulting glycoside is predominantly determined by participation of the C-2 substituent and the product is largely trans 1,2. The picture is further complicated by the partial carbonium ion character of the intermediate, partial inversion of the reagent by negative ion before glycoside formation, steric hindrance, the probable participation of groups on other sites, and the possibility in some instances of ortho ester formation.

Variable yields of α-linked glycosides have been prepared in select cases by controlling one or all of the above-mentioned factors that influence the stereoselectivity of the reaction employed. One of the more promising recent approaches to α-glycoside syntheses was originated by Ishikawa and Fletcher[1] and is being extended by others.[2,3]

In these and other examples, α-glycosides have been prepared by controlling the possible participation of groups on sites other than C-2,[2–4] the C-1 configura-

(1) T. Ishikawa and H. G. Fletcher, *J. Org. Chem.*, **34**, 563 (1969).
(2) M. Dejter-Juszynski and H. M. Flowers, *Carbohyd. Res.*, **23**, 41 (1972).
(3) J. M. Frechet and C. Schuerch, *J. Amer. Chem. Soc.*, **94**, 604 (1972).
(4) P. F. Lloyd, B. Evans, and R. J. Fielder, *Carbohyd. Res.*, **22**, 111 (1972).