potassium hydroxide was refluxed for 1.5 h. Starting material was recovered quantitatively.

[2-(Pentafluorophenyl)tetrafluorophenyl]acetic Acid by Acid-Catalyzed Hydrolysis of 11. A mixture of 2.00 g (4.97 mmol) of 11 and 0.96 g (9.94 mmol) of methanesulfonic acid in 20 mL of 90% formic acid was refluxed with magnetic stirring for 23.5 h. The reaction mixture was then poured into 100 mL of water and the oil which formed solidified immediately. The crude white solid was collected by vacuum filtration, washed with water, and finally crystallized twice from ethanol–water to yield 1.31 g (70.4%) of a white microcrystalline acid: mp 151–152.5 °C; IR (CCl_4) 3300–2500 (br, carboxylic acid OH), 1722 (vs, C=O), 1644 (w, aromatic C=C), 1514, 1502, and 1487 (vs, aromatic ring), 1002 and 993 cm^{-1} (w, C–F); ^1H NMR (pyridine) δ 3.83 (d, 2, CH_2, J_{HF} = 1.8 Hz) and 10.12 (m, 1, COOH); neutralization equivalent calcd 374, found 379. Anal. Calcd for $C_{14}H_3O_2F_9$: C, 44.94; H, 0.81. Found: C, 44.50; H, 0.80.

Pentafluorophenylacetonitrile. This compound was prepared in 58% yield from hexafluorobenzene and ethyl cyanoacetate according to a previously described procedure.[27]

Bis(pentafluorophenyl)acetonitrile.[24] To a suspension of 2.2 g (0.092 mol) of sodium hydride in 40 mL of DMF and 21.0 g (0.113 mol) of hexafluorobenzene was added 4.8 g (0.023 mol) of (pentafluorophenyl)acetonitrile at room temperature during 50 min. The resulting dark solution was stirred for 4 h and 40 mL of ether was added, followed by 40 mL of 5% HCl. The organic layer was separated and the aqueous layer extracted three times with ether. The ethereal extracts were combined, washed with water, and dried over anhydrous sodium sulfate. After removal of the ether, the crude material was distilled to afford 2.14 g (25%) of product, bp 120–124 °C (2.8 torr), mp 65 °C (sublimation).

Ethyl Bis(pentafluorophenyl)acetate (13). Bis(pentafluorophenyl)acetonitrile (2.14 g, 0.057 mol) was heated under reflux for 10 h with 5 mL of absolute ethanol and 2 mL of concentrated H_2SO_4. After the solution was cooled, water was added and the product was extracted with ether. The aqueous layer was also extracted with ether. The combined ethereal layers were washed with water, 10% sodium bicarbonate, and again with water and then dried over anhydrous sodium sulfate. After removal of ether, the residue was distilled to give 0.8 g (27%) of ester, bp 108–110 °C (3.5 torr).

Alkaline hydrolysis of the ester with 5% potassium hydroxide gave bis(pentafluorophenyl)acetic acid, mp 108–109 °C (lit.[24] 112.0–112.5 °C); mass spectrum m/e 226 ($C_6F_5CHCO_2H$,$^+$ 181 ($C_6H_5CH_2$),$^+$ 162 (181 – F). A higher melting product (mp 148 °C) containing a phenolic function ($FeCl_3$ test) was also isolated.

Acidic hydrolysis of the ester with *p*-toluenesulfonic acid and 97% formic acid afforded, after 90 h of reflux, a 62% yield of bis(pentafluorophenyl)acetic acid, mp 106–108 °C (heptane).

Bis(pentafluorophenyl)acetic Acid via Decafluorobenzilic Acid. Methyl decafluorobenzilate and decafluorobenzilic acid were prepared according to previously described procedures.[5]

Bis(pentafluorophenyl)acetic Acid. Glacial acetic acid (5 mL), 0.3 g of purified red phosphorus, and 0.1 g of iodine crystals were placed in a round-bottom flask fitted with a reflux condenser. The mixture was stirred (magnetic stirrer) for 20 min and 2.0 g of decafluorobenzilic acid was added. The mixture was heated under reflux, with stirring, for 4 h. Hexane was added and 1.6 g of crystalline acid, mp 108–109 °C, was obtained on cooling the solution. This material did not depress the melting point on admixture with the acid obtained on hydrolysis of 13.

Reaction of Bis(pentafluorophenyl)acetate with Lithium Aluminum Hydride. Lithium aluminum hydride (0.1 g) in 30 mL of ether was placed in a two-necked flask and 1.1 g of ester in 30 mL of ether was added dropwise from a dropping funnel during 30 min. The mixture was heated under reflux for 30 min and excess hydride was decomposed by slowly adding 50 mL of ethyl acetate with magnetic stirring. Then, 20 mL of 3 N HCl was added. After the organic layer was separated, the aqueous layer was extracted with ether and the ethereal layers were combined. After removal of ether, the liquid residue revealed a strong hydroxyl absorption in the 3500–3400-cm^{-1} region.

Registry No. 1, 27053-34-5; 2, 19925-96-3; 2 2,4-dinitrophenylhydrazone, 19925-97-4; 3, 5576-19-2; 4, 19925-95-2; 5, 16583-10-1; 6, 72844-06-5; 7, 1093-66-9; 8, 72844-07-6; 9, 72844-08-7; 10, 72866-24-1; 11, 27053-32-3; 12, 27053-33-4; 13, 42238-45-9; 1,2-dibromotetrafluorobenzene, 827-08-7; octafluoro-9-fluorenol, 72844-09-8; ethyl diazoacetate, 623-73-4; [2-(pentafluorophenyl)tetrafluorophenyl]-acetic acid, 72844-10-1; bis(pentafluorophenyl)acetonitrile, 42238-34-6; (pentafluorophenyl)acetonitrile, 653-30-5; (pentafluorophenyl)acetic acid, 653-21-4; decafluorobenzilic acid, 29688-34-4; methyl decafluorobenzilate, 38449-79-5.

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Polymer-Bound Oxime Esters as Supports for Solid-Phase Peptide Synthesis. Preparation of Protected Peptide Fragments

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A series of polystyrene-bound substituted benzophenone oximes (II) have been synthesized and tested as potential supports for the solid-phase preparation of protected peptide fragments. The polymer-bound *p*-nitrobenzophenone oxime (IID) has been found to be a suitable support for stepwise peptide synthesis. Protected peptides can be assembled on IID by coupling and deprotection steps similar to those employed in the usual Merrifield solid-phase procedures. Cleavage of peptides from IID can be accomplished with hydrazine and amino acid esters under mild conditions which do not affect benzyl ester side-chain protecting groups. The utility of IID has been illustrated in the synthesis of protected peptide hydrazides and esters, several of which have aspartic and glutamic acid side chains protected by benzyl groups.

Introduction

The solid-phase method of peptide synthesis[1] has had many notable successes. However, the preparation of peptides greater than 20 amino acids in length using the solid-phase technique often poses major problems in that very extensive purification of the final product is needed. In contrast to the solid-phase method, the classical solution methods[2] of peptide synthesis are likely to give more homogeneous products since intermediates are usually purified after each coupling step. A major problem though

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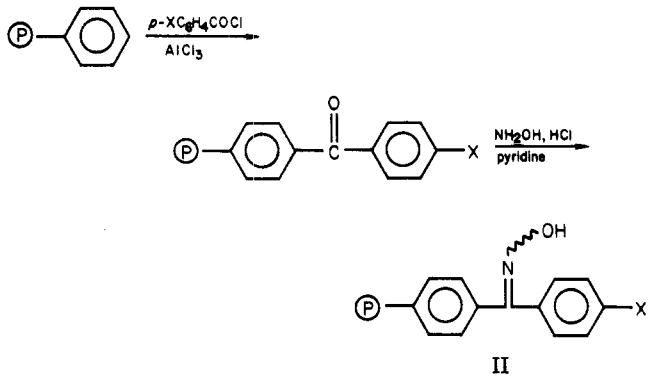
0022-3263/80/1945-1295$01.00/0

Scheme I



II

P = polystyrene; A, X = OMe; B, X = H; C, X = Cl; D, X = NO_2

with solution-phase synthesis of peptides is that it is very time consuming to prepare large peptides by this route. The synthesis of protected peptide fragments on solid supports, followed by the coupling of these fragments in solution or on the solid phase, could combine the advantages of the solid-phase and classical methods of peptide synthesis.

Existing methods for the preparation of protected fragments on the solid phase involve the use of anchoring bonds which are cleaved by acidolysis,[3-6] hydrazinolysis,[7-9] alcoholysis,[10-12] or photolysis.[13] However, each of these methods has certain disadvantages. The use of anchoring bonds of intermediate acid lability[3-6] requires the employment of amino acid derivatives with very acid labile α-amino protecting groups such as Bpoc or Ddz,[14] which are not as convenient to use as the readily available Boc amino acids. Alcoholysis or hydrazinolysis of benzyl ester type resins is of limited utility since β- or γ-carboxyl protected aspartyl and glutamyl residues are also transesterified or converted to the side-chain hydrazides. If photosensitive resins are used, sluggish cleavage of peptides from the polymer is encountered when the C-terminal residue is not glycine.

In addition, "safety-catch" type resins have been proposed for the synthesis of protected peptide fragments.[15,16] These resins take advantage of an anchoring bond which is stable to the conditions of peptide synthesis but may be activated by a chemical reaction after the peptide has been synthesized on the support. This allows facile release of the fully protected peptides. The major limitation of these types of resins is that the reaction which is used to labilize the resin either modifies certain amino acids[15] or severely limits the choice of α-amino protecting groups.[16] For example, the 4-alkylthiophenyl ester resin[15] is activated by converting it to the sulfone, a reaction which would also oxidize cysteine derivatives, methionine, and tryptophan.

It would be clearly desirable, thus, to develop a support from which a peptide might be quantitatively cleaved under very mild conditions by hydrazinolysis or by aminolysis using amino acid esters. To have wide applicability, the cleavage procedure should not cause aminolysis of benzyl ester side-chain protecting groups or racemization of the carboxyl-terminal amino acid. The linkage between the growing peptide chain and the support should also be stable to at least 25% trifluoroacetic acid in methylene chloride (25% TFA), if the Boc group is to be used for α-amino protection. The oxime resin IID (Scheme I), prepared from 1% divinylbenzene–polystyrene copolymer (Biogel SX1), meets these criteria, and we wish to report the synthesis of several protected peptides employing this resin.

To the best of our knowledge, Losse et al.[17] were the first workers to report the use of oxime esters in peptide synthesis. They observed that the reactions of Z-Gly esters of substituted acetophenone oximes with nucleophiles such as benzylamine were slow compared to those of the corresponding *p*-nitrophenyl esters. Fujino and Nishimura[18] and Romanovski et al.[19] found that in the presence of acetic acid or formic acid aminolysis of oxime esters is very much accelerated. Recently, Vlassa[20] has employed acetone oxime and cyclohexanone oxime esters of Z-Pro and Z-Pro-Leu in the synthesis of Pro-Leu-Gly-NH_2 (melanocyte) inhibiting factor. The oxime esters prepared from 4-amino-3-methyl-4-nitroso-1-phenylpyrazole and 2 equiv of an amino-protected amino acid are acylating agents that react rapidly at room temperature with amino acid esters without detectable racemization.[21] Although polystyrene-bound oxime esters have potential as polymeric active esters, the present study has concentrated on the use of oxime resin IID as an anchoring bond for stepwise peptide synthesis. When condensed with α-amino-protected amino acids, IID forms an ester which is active enough to be cleaved by aminolysis with the α-nucleophile hydrazine or with amino acid esters and yet is not active enough to cause racemization of the carboxyl-terminal amino acid during cleavage of the peptide from the support. Various peptide hydrazides and methyl, ethyl, and *tert*-butyl esters have been synthesized by using this support, including compounds with aspartic and glutamic acid side chains protected with benzyl esters, which would not have been accessible by other solid-phase methods.

Results

The benzophenone oxime IIB has been described by Ontjes et al.[22] as an intermediate in the synthesis of the benzhydrylamine polymer. Oxime polymers IIA, IIC, and IID have been prepared in the present work by similar

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(14) List of abbreviations: Boc, *tert*-butyloxycarbonyl; Bpoc, [[2-(*p*-biphenylyl)-2-propyl]oxy]carbonyl; Bz, benzoyl; Bzl, benzyl (ethers); DCC, dicyclohexylcarbodiimide; Ddz, α,α-dimethyl-3,5-dimethyloxybenzyloxycarbonyl; DMF, dimethylformamide; high-performance LC, high-performance liquid chromatography; OBzl, benzyl ester; O-*t*-Bu, *tert*-butyl ester; OEt, ethyl ester; OMe, methyl ester; TFA, trifluoroacetyl or trifluoroacetic acid; Z, benzyloxycarbonyl.
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Table I. Properties of Peptides Synthesized on Polymer IID

peptide	yield, %	exptl mp, °C	exptl $[\alpha]_D$, deg	lit. mp, °C	lit. $[\alpha]_D$, deg
IV	99	135-136	−17.4 (c 2, DMF)		
V	89	140-141	−31.3 (c 2, DMF)		
VI	57	248-249	−23.1 (c 2, DMF)	250[a]	−23 ± 1[a] (c 2, DMF)
VII	60	249-251	−19.4 (c 0.7, DMF)	252-254[b]	
VIII	40	228-229	−27.5 (c 2, 95% AcOH)	230[a]	−26.5[a] (c 2, 95% AcOH)
X	92	69-70	−12.5 (c 0.8, MeOH)		
XI	82	78-79	−22.8 (c 2, MeOH)		
XIII	68	104-104.5	−40.8 (c 1, MeOH)		
XIV	62	165-166	−31.4 (c 0.5, MeOH)	163-165[c]	−31.5[c] (c 0.5, MeOH)

[a] R. Sandrin and R. A. Boissonnas, *Helv. Chim. Acta*, 46, 1637 (1964). [b] Reference 4. [c] Reference 30.

procedures. As illustrated in Scheme I, oxime polymers IIA–D were synthesized via the ketone polymers IA,[23] IB,[22,23] IC,[23] and ID, which in turn were obtained by Friedel–Crafts acylation of polystyrene cross-linked with 1% divinylbenzene, using the appropriate acid chloride. The resulting benzophenone polymers had strong carbonyl absorbances in their IR spectra between 1653 and 1665 cm^{-1}, depending on the nature of the substituent X. The acylation of the polystyrene–divinylbenzene copolymer with the acid chlorides was nearly quantitative, thus providing an easy method for controlling the substitution level. The benzophenone polymers obtained from the acylation reaction were quantitatively converted to the corresponding oximes by reaction with hydroxylamine hydrochloride and pyridine in refluxing ethanol. The IR spectra of the oxime resins IIA–D showed no carbonyl absorptions and had strong absorptions at 3530 cm^{-1}. The configurations of the polymer-bound oximes were not determined, but it seemed probably that a combination of syn and anti isomers was formed. To test the stability of the respective oxime ester linkages, the polymer-bound oximes were condensed with BocLeu·H_2O by using DCC as the condensing agent. The oxime esters formed were tested for their stability toward 25% TFA. Only the ester linkage formed from oxime polymer IID was judged to be stable enough to exposure to 25% TFA for use with the Boc group for α-amino protection. With this concentration of TFA, less than 5% of the original Leu loaded on oxime polymer IID was cleaved in 4 h, which corresponds to the total amount of exposure to TFA encountered in the synthesis of a nonapeptide.

Synthesis of Protected Peptide Hydrazides Using Oxime Polymer IID. The use of oxime polymer IID for the synthesis of protected peptide hydrazides is illustrated by the preparation of BocAsp(OBzl)-LeuNHNH$_2$ (IV) (Scheme II). Resin IID was acylated with BocLeu·H_2O by using DCC to give III with a substitution level of 0.27 mmol of amino acid/g of resin. The remaining oxime groups were blocked by acetylation with acetic anhydride and diisopropylethylamine (DIEA). The Boc protecting group was then removed by treatment with 25% TFA for 30 min. After the appropriate washing steps, BocAsp-(OBzl) was added as the symmetric anhydride[24,25] (BocAsp(OBzl)SA) in 3-fold excess in the presence of a 2.2-fold excess of DIEA, and the coupling reaction was allowed to proceed for 2 h. The resulting dipeptide resin was treated for 10 min with 0.5 M anhydrous hydrazine in CH_2Cl_2/MeOH (2:1). The IR spectrum of the polymer after cleavage was identical with that of IID, indicating

Scheme II

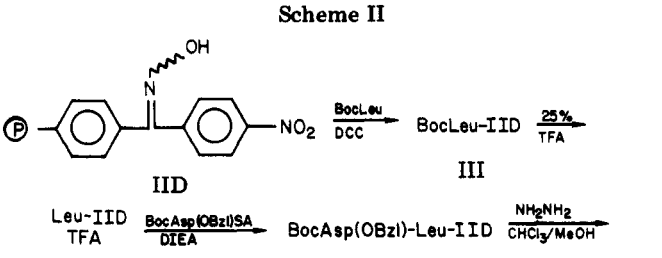

Table II. Elemental Analyses of Peptides

peptide		C, %	H, %	N, %
IV	calcd	59.46	7.81	12.07
	found	59.29	7.93	11.96
V	calcd	58.63	7.61	12.44
	found	58.72	7.66	12.26
VI	calcd	59.59	8.05	15.09
	found	59.45	8.13	15.00
VII	calcd	62.39	7.43	14.09
	found	62.55	7.79	13.97
VIII	calcd	56.98	7.41	16.61
	found	56.85	7.41	16.47
X	calcd	59.71	7.15	6.63
	found	59.69	7.20	6.65
XI	calcd	60.11	7.78	8.76
	found	60.32	7.84	8.71
XIII	calcd	64.44	8.89	7.27
	found	64.43	8.94	7.29
XIV	calcd	53.47	8.13	11.69
	found	53.37	8.24	11.59

that cleavage was complete and that recycling of the polymer should be possible. After workup, the dipeptide product IV was crystallized in 89% yield.

The properties of the peptide hydrazides BocGlu-(OBzl)-LeuNHNH$_2$ (IV), BocAla-Phe-IleNHNH$_2$ (VI), ZPhe-Val-Ala-LeuNHNH$_2$ (VII), and BocAla-Phe-AlaNHNH$_2$ (VIII), synthesized through the use of oxime polymer IID, and their elemental analyses are illustrated in Tables I and II, respectively.

Solid-phase syntheses of the tetrapeptide hydrazide ZPhe-Val-Ala-LeuNHNH$_2$ (VII) using Wang's "hydrazide resins" and giving 76%[3] and 42%[4] yields have been described in the literature. Our yield (60%) compares well with these yields without requiring the use of the very acid-labile Bpoc amino acids.

To determine the extent of racemization during the synthesis of peptide hydrazides using oxime polymer IID, the method of Manning and Moore[26,27] was employed.

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Table III. Results of a Study of Racemization in the Syntheses of BocGly-Ile-GlyOMe

procedure[a]	mp, °C crude	mp, °C recrystallized	% yield crude	% yield recrystallized[d]	$[\alpha]_D$, deg	% alle, crude
A	155-158	166-167	81	64	-30.6	1.0
B	149-153	166.5-167.5	71	50	-31.0	0.8
C	159-161.5	165-166	70	62	-31.4	0.6
lit.[b]		163-165		68[c]	-31.5	

[a] See Experimental Section for description of reaction conditions for procedures A, B, and C. [b] Reference 30. [c] Based on the amount of dipeptide used in the coupling of BocGly with Ile-GlyOMe by the mixed anhydride method. [d] Corrected to allow for the amount of crude product removed for amino acid analysis and thin layer chromatography.

The peptide BocAla-AlaNHNH$_2$ was prepared by using IID, and the crude product was hydrolyzed with 6 N HCl at 110 °C for 24 h. The amount of D-Ala in the hydrolysate was 0.53%. Since some racemization occurs during hydrolysis,[27] it is reasonable to conclude that both the coupling of the first amino acid to the resin and the hydrazinolysis step proceeded without significant racemization.

Cleavage of Protected Peptides from Oxime Polymer IID with Amino Acid Esters. Cleavage of peptide–IID linkages with glycine methyl and ethyl esters gave protected peptide esters. For instance, when BocGlu(OBzl)–IID polymer was treated with 4 equiv of glycine ethyl ester for 18 h, the absorptions due to the oxime ester and the Boc group in the IR spectrum completely disappeared, and BocGlu(OBzl)GlyOEt (X) was recovered in 92% yield. The tripeptide ester BocSer(Bzl)-Leu-GlyOMe (XI) has similarly been synthesized in 82% yield. The extent of racemization occurring when a peptide was cleaved from IID with glycine ethyl ester was estimated by the use of the Young test.[28] Aminolysis of benzoyl-leucine polymer–IID with 4 equiv of glycine ethyl ester hydrochloride and 4 equiv of DIEA for 16 h yielded BzLeu-GlyOEt with 98% optical purity.

As a more accurate and direct test of racemization, BocGly-Ile-GlyOMe was synthesized by aminolysis of Boc-Gly-Ile-IID with glycine methyl ester under various conditions (Table III). The resulting products, BocGly-Ile-GlyOMe (XIV), were then hydrolyzed, and the amount of alloisoleucine (aIle) was determined by ion-exchange chromatography on an amino acid analyzer.[29] When 3 equiv of GlyOMe·HCl and 3 equiv of DIEA in CH_2Cl_2 were employed (procedure A), the reaction was very slow, achieving completion only after 5 days, as judged by IR. The crude product contained 1.0% aIle after hydrolysis. When THF was used as the solvent (procedure B) the reaction was still slower, reaching only 85% completion after 5 days, and 0.8% aIle was recovered in the hydrolysate of the crude product. However, when 3 equiv of GlyOMe·HCl, 3 equiv of DIEA, and 1.5 equiv of acetic acid (AcOH as catalyst) in CH_2Cl_2 were used (procedure C), the reaction was complete within 1 day, and the crude product contained 0.6% aIle after hydrolysis. The optical rotation of the latter product after one recrystallization was also in very good agreement with that described in the literature for BocGly-Ile-GlyOMe synthesized by a classical solution-phase method.[30] The slow cleavage of the peptide from the polymer in the uncatalyzed reactions (e.g., in the absence of HOAc) may be due to the steric bulk of the C-terminal isoleucine residue. Acetic acid appears to be a very potent catalyst for this aminolysis reaction, and it also reduces the degree of racemization. Since a small amount of racemization of isoleucine always accompanies acid hydrolysis of peptides,[31] we believe that negligible racemization was due to the cleavage with glycine methyl ester in the presence of HOAc.

Preliminary investigations have shown that oxime esters of IID may be cleaved with amino acid *tert*-butyl esters to give protected peptide *tert*-butyl esters. For instance, when BocSer(Bzl)-Leu–IID was treated with 3 equiv of LeuO-*t*-Bu·HCl and 3 equiv of DIEA in CH_2Cl_2 for 12 h, crude BocSer(Bzl)-Leu-LeuO-*t*-Bu was obtained in quantitative yield. Recrystallization gave the analytically pure tripeptide in 68% yield.

The physical properties and analytical data for some of the peptide esters synthesized are listed in Tables I and II, respectively. As in the cases of the peptide hydrazides, the esters were obtained in satisfactory yield. The 270-MHz ^{1}H NMR spectra of the peptides prepared agreed with their structures. All peptides described in this work were homogeneous by TLC with at least two solvent systems and by reverse and/or normal phase high-performance LC.

Discussion

The use of resin IID should make a valuable contribution to the methods available for peptide synthesis. The high degree of carboxyl activation allows facile cleavage by aminolysis under very mild conditions. Initially, it was feared that such a high level of activation might lead to unwanted inter- and intrasite aminolysis reactions. For instance, the apparent first-order rate constant for diketopiperazine formation from the dipeptide resin Glu(OBzl)-Ser(Bzl)–IID at ambient temperature was 2.6×10^{-2} min^{-1} ($t_{1/2}$ = 27 min, as determined by the method of Gisin and Merrifield[32]) when the dipeptide resin was prepared in situ from the corresponding TFA salt with 3 equiv of DIEA in CH_2Cl_2. Additionally, when TFA–Leu–IID resin was treated with 5% DIEA in CH_2Cl_2 for 2 min, filtered, and suspended in CH_2Cl_2, half of the leucine was lost from the resin within 1 h (as determined by amino acid analysis of a hydrolysate of the resin), presumably by a mechanism very recently discussed by Rebek and Trend.[33] However, we anticipated that the rate of coupling would be much faster than these side reactions if the neutralization step normally used in solid-phase peptide synthesis was omitted and coupling was accomplished with 3 equiv of the symmetric anhydride and a slight excess of DIEA. Indeed, we found that under these conditions, coupling was usually complete, as judged by the ninhydrin test,[34] and that after cleavage from the resin, protected peptides could be obtained in homogeneous form in good yields by simple

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crystallization. Fragments terminating in glycine or proline, where direct attachment of these amino acids to the resin might cause excessively low yields due to diketopiperazine formation, can be acessible via aminolysis of peptide esters of oxime polymer IID using glycine or proline esters.

The oxime IID has several advantages over other resins thus far proposed as supports for the synthesis of protected peptide fragments. Existing resins have been limited either to the preparation of peptides lacking certain amino acids or to the use of very acid-labile α-protected amino acids. The anchoring linkage of the oxime resin IID suffers from neither of these limitations, since it is compatible with the use of the Boc group for α-amino protection and since it can be cleaved by aminolysis under conditions which do not affect benzyl ester protecting groups. The synthesis of several peptides using IID has been shown to be essentially free from racemization. A further advantage is that fragments containing C-terminal *tert*-butyl ester functions can be quite easily synthesized with IID. Such fragments were previously inaccessible via solid-phase methods. Finally, acetic acid has been reported to be a potent catalyst for the aminolysis of oxime esters,[18] and our experience with polymer-bound oxime esters derived from IID has confirmed this observation. We also found that acetic acid reduced racemization in the case of the aminolysis of BocGly-Ile–IID with glycine methyl ester. Thus, acetic acid catalyzed aminolysis has the potential to provide a racemization-free route for the cleavage of peptides from IID employing amino components of nucleophilicity lower than those used in the present study, including N-deprotected peptides and sterically hindered amino acid esters, for instance. Work is being continued in our laboratory along these lines.

Experimental Section

Materials and Methods. Melting points were taken on a Thomas-Hoover apparatus and are uncorrected. IR spectra were recorded on a Perkin-Elmer 283 infrared spectrophotometer by using KBr pellets. Optical rotations were measured with a Perkin-Elmer 141 polarimeter. The syntheses were carried out with a Beckman Model 990 peptide synthesizer, or manually. Amino acid analyses were performed with a Beckman Model 121 amino acid analyzer. Elemental analyses were by Galbraith Laboratories, Knoxville, TN. ^{1}H NMR spectra were recorded on a Bruker HS-270 spectrometer equipped with a Nicolet data processor system.

Precoated silica gel TLC plates, Merck F-254, were purchased through Scientific Products. The following solvent systems were used: A, chloroform/methanol (9:1); B, chloroform/methanol (5:1); C, chloroform/methanol/acetic acid (85:10:5); D, chloroform/acetic acid (95:5); E, 1-butanol/acetic acid/water (4:1:1); F, 1-butanol/acetic acid/water/pyridine (15:3:12:10). All amino acids were purchased from Bachem and were homogeneous in solvent systems C and D. Acid chlorides from Aldrich were used without further purification.

Preparation of Oxime Resins IIA–D. In a typical synthesis of the *p*-nitrobenzoyl oxime polymer IID, 4.34 g of Biogel SX1 was swelled in 70 mL of 1,2-dichloroethane. *p*-Nitrobenzoyl chloride (5.0 mmol) was added, followed by slow addition of 7.5 mmol of $AlCl_3$ with vigorous stirring. Stirring was continued, and the mixture was heated at reflux for 10 h. The polymer was collected by filtration and washed several times with dioxane/4 N HCl (3:1), dioxane/water (3:1), dioxane, MeOH, and CH_2Cl_2, yielding 4.64 g of ID. The IR spectrum of ID had strong absorbances at 1665, 1525, and 1310 cm^{-1}, and elemental analysis indicated that the resin contained 1.30% nitrogen (0.93 mmol/g or 86% conversion). The *p*-nitrobenzoylated resin (4.38 g) was suspended in 50 mL of ethanol; 6 mL of pyridine and 6 g of NH_2OH·HCl were added, and the mixture was heated at reflux with stirring for 22 h. The resin was washed with ethanol, 50% ethanol, ethanol, acetone, and CH_2Cl_2 to yield 4.37 g. The IR spectrum showed strong absorbances at 3530, 1525, and 1310 cm^{-1}, and the absorption at 1665 cm^{-1} had completely disappeared. Elemental analysis showed the presence of 2.66% nitrogen which corresponds to a substitution level of 0.95 mmol/g and is consistent with complete conversion of the ketone to the oxime. The other resins were synthesized by similar procedures. While the Friedel–Craft's acylation with *p*-chlorobenzoyl chloride was carried out at reflux in 1,2-dichloroethane, the other acylations were carried out at room temperature in nitrobenzene.

BocGlu(OBzl)-LeuNHNH$_2$ (IV). To 2.00 g of IID was added 25 mL of CH_2Cl_2, 1.20 mmol of BocLeu·H_2O, and 1.20 mmol of DCC. The mixture was shaken for 15 h to yield 2.14 g of BocLeu–IID (III) after washing with CH_2Cl_2, DMF, *i*-PrOH, and CH_2Cl_2 and drying in vacuo. The substitution level was determined to be 0.270 mmol/g by amino acid analysis.[35] The rest of the synthesis was carried out automatically by the synthesizer programmed to execute the following steps: (1) wash, CH_2Cl_2 (3×); (2) wash, DMF (2×); (3) acetylate with 3 mL of acetic anhydride, 1 mL of DIEA, and 12 mL of DMF (1 × 30 min); (4) wash, DMF (2×); (5) wash, *i*-PrOH/CH_2Cl_2 (1:3) (3×); (6) wash, CH_2Cl_2 (6×); (7) prewash, 25% TFA (1×); (8) deprotect, 25% TFA (1 × 30 min); (9) wash, CH_2Cl_2 (4×); (10) wash, *i*-PrOH (1×); (11) wash, CH_2Cl_2 (2×); (12) wash, *i*-PrOH (1×); (13) wash, CH_2Cl_2 (4×); (14) couple, 3 equiv of symmetric anhydride, 2.2 equiv of DIEA in CH_2Cl_2 (1 × 2 h); (15) wash, CH_2Cl_2 (2×); (16) wash, DMF (1×); (17) wash, *i*-PrOH/CH_2Cl_2 (1:3) (2×); (18) wash, CH_2Cl_2 (2×). The time for all wash steps was 1 min. To the reaction vessel was addded 0.95 g of III, and the mixture was acetylated and then coupled with BocGlu(OBzl) by the above program to yield 1.03 g of resin. The dipeptide resin (0.98 g) was then shaken with 0.5 M anhydrous hydrazine in $CHCl_3$/MeOH (2:1) for 10 min, filtered, and washed twice with 10 mL portions of $CHCl_3$ and once with 10 mL of MeOH. To the combined washes was added 20 mL of water and the organic layer was separated and evaporated in vacuo to give an oil. After the residue was dissolved in MeOH and reevaporated several times, a glassy solid resulted which was crystallized from 3 mL of ethanol by addition of about 4 mL of water to yield 114 mg of peptide IV (99%): mp 135–136 °C; R_f(B) 0.58; R_f(E) 0.70; R_f(F) 0.75; $Glu_{1.00}Leu_{1.04}$.

BocAsp(OBzl)-LeuNHNH$_2$ (V). To the reaction vessel was added 0.95 g of III, and the syntheses using BocAsp(OBzl) and workup were conducted as for IV: yield 89%; mp 140–141 °C; R_f(B) 0.63; R_f(E) 0.66; R_f(F) 0.75; $Asp_{1.05}Leu_{1.00}$.

BocAla-Phe-IleNHNH$_2$ (VI). BocIle–IID resin (0.88 g, 0.260 mmol) was added to the reaction vessel and BocPhe and BocAla were coupled sequentially. BocPhe was recoupled due to a positive ninhydrin test.[34] For addition of BocAla, steps 1–5 of the program were omitted. After hydrazinolysis and workup as for IV, peptide VI was recrystallized from 10 mL of MeOH by slow addition of 70 mL of ether: yield 69.4 mg (57%); mp 248–249 °C; R_f(B) 0.50; R_f(E) 0.70; R_f(C) 0.50; $Ala_{1.00}Ile_{0.95}Phe_{1.00}$. The peptide was homogeneous by high-performance LC with a Waters Associates C_{18} column with MeOH/H_2O (6:4) as eluting buffer.

ZPhe-Val-Ala-LeuNHNH$_2$ (VII). Boc-Leu–IID resin (0.81 g, 0.45 mmol) was added to the vessel of the peptide synthesizer and BocAla, BocVal, and ZPhe were coupled sequentially by skipping steps 1–5 after addition of BocAla. No recouplings were necessary; yield 0.90 g. Hydrazinolysis of 0.61 g of this resin and workup as for IV yielded a powder which was crystallized from MeOH by addition of ether: yield 110 mg (60%); mp 249–251 °C; R_f(A) 0.58; R_f(E) 0.75; R_f(F) 0.79; $Ala_{1.00}Val_{1.03}Leu_{0.99}Phe_{0.97}$. The purity was greater than 99% as determined by high-performance LC with eluting buffer MeOH/H_2O (7:3) and by employing UV detection at 230 nm.

BocAla-Phe-AlaNHNH$_2$ (VIII). BocAla–IID resin (0.92 g, 0.352 mmol) was added to the reaction vessel and BocPhe and BocAla were coupled sequentially. After hydrazinolysis and workup the tripeptide was crystallized twice from MeOH by slow addition of ether: yield 59.4 mg (40%); mp 228–229 °C; R_f(A) 0.27; R_f(E) 0.52; R_f(F) 0.73.

Manning and Moore Test,[26,27] BocAla-AlaNHNH$_2$ (IX). BocAla–IID resin (1.02 g, 0.45 mmol) was acetylated and coupled with BocAla. BocAla–IID hydrazinolysis with 0.5 M hydrazine

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in $CHCl_3$/MeOH (2:1) for 15 min the resin was collected by filtration and washed. Evaporation of the filtrate in vacuo resulted in a white solid (149 mg). Without further purification a portion of the product was hydrolyzed at 110 °C with 6 N HCl. Analysis of this hydrolysate by the method of Manning and Moore[27] gave 0.52% D-Ala.

BocGlu(OBzl)-GlyOEt (X). To 0.65 g of BocGlu(OBzl)–IID polymer (0.257 mmol) was added 1.1 mmol of GlyOEt in 7 mL of CH_2Cl_2. The progress of the reaction was followed by watching the disappearance of the Boc and oxime ester absorbances in the IR spectra of aliquots of the resin. After 18 h the reaction was judged to be complete. The resin was collected by filtration and washed with CH_2Cl_2, and the filtrate was reduced to an oil. The oil was taken up in ethyl acetate and the resultant solution washed with 0.1 N HCl, saturated sodium bicarbonate solution, water, and brine, dried over $MgSO_4$, and evaporated in vacuo to give an oil. The oil was crystallized from ethyl acetate/petroleum ether: yield 100 mg (92%); mp 69–70 °C; R_f(B) 0.73; R_f(E) 0.82.

BocSer(Bzl)-Leu-GlyOMe (XI). To the reaction vessel of the synthesizer was added 5.52 g of BocLeu–IID resin (2.65 mmol). The resin was acetylated and coupled with 6.00 mmol of Boc-Ser(Bzl) symmetric anhydride. After a positive ninhydrin test[34] recoupling was performed by using 2.00 mmol of the anhydride. The resin was then treated with 5.00 mmol of GlyOMe·HCl and 5.0 mmol of DIEA in 60 mL of CH_2Cl_2 for 5.5 h. Subsequently, the resin was collected by filtration and washed with CH_2Cl_2 and MeOH and then treated again with 5.00 mmol of GlyOMe·HCl and DIEA in CH_2Cl_2 for 7.5 h. The resin was then isolated by filtration and washed as before. The filtrates from both treatments were evaporated in vacuo, taken up in ethyl acetate, washed with 0.1 N HCl, water, and brine, dried over $MgSO_4$, and evaporated to give an oil. The oil was dissolved in ether and it slowly crystallized: yield 1.035 g (82%); mp 78–79 °C; R_f(C) 0.75; R_f(E) 0.80; $Ser_{0.89}Gly_{1.00}Leu_{0.99}$; homogeneous by high-performance LC by using a Waters Associates μ-Porasil column and $CHCl_3$ as eluting solvent.

BzLeu-GlyOEt (Young Test)[28] (XII). BocLeu–IID resin (1.00 g, 0.530 mmol) was automatically deprotected by using steps 6–13. The deprotected resin was then benzoylated with 1.0 mmol of benzoyl chloride and 1.50 mmol of DIEA in CH_2Cl_2 for 10 min. After being washed with CH_2Cl_2, the resin was treated for 16 h with 2.00 mmol of GlyOEt·HCl and 2.00 mmol of DIEA in 10 mL of CH_2Cl_2. After the resin was filtered and washed with CH_2Cl_2, the peptide was worked up[28] to give 106 mg (64%): mp 155–157 °C; $[\alpha]_D$ −33.3° (c 2.5, EtOH) [lit.[28] mp 156–157 °C, $[\alpha]_D$ −34.0° (c 3.1, EtOH)].

Anal. Calcd for $C_{17}H_{24}N_2O_4$: C, 63.73; H, 7.55; N, 8.74. Found: C, 63.84; H, 7.63; N, 8.70.

BocSer(Bzl)-Leu-LeuO-*t*-Bu (XIII). BocLeu–IID resin (1.00 g, 0.31 mmol) was added to the reaction vessel and Boc-Ser(Bzl) was coupled as the symmetric anhydride (1.00 mmol) with 0.63 mmol of DIEA in the usual manner. LeuO-*t*-Bu·HCl (1.00 mmol) and DIEA (1.00 mmol) in 10 mL of CH_2Cl_2 were then added to the resin and the mixture was shaken for 12 h. The tripeptide was worked up as for XI to give an oil which was crystallized from ether/hexane: crude yield 179 mg (100%); mp 100–104 °C; R_f(A) 0.68, R_f(D) 0.52. An analytical sample, crystallized from ether/hexane, had mp 104–104.5 °C; yield 68%.

BocGly-Ile-GlyOMe (XIV). BocIle–IID resin (3.00 g, 1.35 mmol) was added to the reaction vessel of a manual synthesizer,[36] and Boc-Gly was coupled as the symmetric anhydride by using the same steps as for the automatic synthesis; yield 3.04 g of BocGly-Ile–IID. The dipeptide resin (1.00 g) was then treated with GlyOMe·HCl (1.50 mmol) and DIEA (1.50 mmol) in 10 mL of CH_2Cl_2 (procedure A). To a second portion of the resin (1.00 g) was added GlyOMe·HCl (1.50 mmol) and DIEA (1.50 mmol) in 10 mL of THF (procedure B). A third portion of BocGly-Ile–IID (0.90 g) was treated with GlyOMe·HCl, DIEA (1.50 mmol each), and AcOH (0.8 mmol) in 10 mL of CH_2Cl_2 (procedure C). The above reaction mixtures were mechanically shaken and the progress of the reaction was monitored by taking the IR spectra of aliquots of the resins after 1, 2, 3, and 5 days. The acetic acid catalyzed reaction (procedure C) was judged to be complete after 1 day and was worked up at that time. The uncatalyzed aminolysis reactions (procedures A and B) were much slower. The reaction with CH_2Cl_2 as solvent (procedure A) required 5 days for completion, while B was only about 85% complete at this time (as judged by IR). After 5 days, the reaction mixtures from procedures A and B were worked up in the same manner as for procedure C. The resins were collected by filtration and washed with MeOH. The filtrate was evaporated in vacuo and the residue was dissolved in ethyl acetate and washed with 0.1 N HCl (3×), water (2×), and brine. After drying the organic layer over $MgSO_4$, the solvent was removed in vacuo and the residue dried overnight under high vacuum. Without further purification, small amounts of the crude products from procedures A, B, and C were hydrolyzed with 5 N HCl at 110 °C for 36 h. The remaining portions were crystallized from ethyl acetate/petroleum ether; yields, melting points of the crystallized and crude products, and rotations of the crystallized products are given in Table I. The amount of aIle in the hydrolysate of the crude product was measured by amino acid analysis.[29]

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Registry No. IV, 72917-79-4; V, 72917-80-7; VI, 3081-44-5; VII, 24604-93-1; VIII, 7652-95-1; IX, 41863-52-9; X, 72917-81-8; XI, 72917-82-9; XII, 2418-77-1; XIII, 72917-83-0; XIV, 13734-50-4; BocLeu, 13139-15-6; BocGlu(OBzl)SA, 51499-95-7; BocAsp(OBzl), 7536-58-5; BocIle, 13139-16-7; BocPhe, 13734-34-4; BocAla, 15761-38-3; BocVal, 13734-41-3; ZPhe, 1161-13-3; D-Ala, 338-69-2; GlyOEt, 459-73-4; BocSer(Bzl), 23680-31-1; GlyOMe·HCl, 5680-79-5; GlyOEt·HCl, 623-33-6; LeuOzBu·HCl, 2748-02-9; BocGly, 4530-20-5.

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