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A NEW BASE-CATALYZED CLEAVAGE REACTION FOR THE PREPARATION OF PROTECTED PEPTIDES

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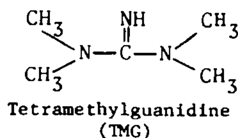
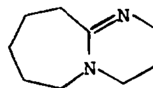
Abstract - A new base-catalyzed elimination reaction employing the hindered, non-nucleophilic bases tetramethylguanidine or 1,8-diazabicyclo-[5.4.0.]-undec-7-ene has been developed for the removal of protected peptides from a 2-[4-(hydroxymethyl)phenyl-acetoxy]propionyl-resin. The proposed reaction mechanism involved cleavage of the ester bond between the peptide and resin via a base-catalyzed elimination. The protected peptide-resin cleavage reaction is mild, rapid and proceeds in good yield with a very simple work-up procedure. Four protected peptide-resins varying in size from seven to sixteen residues were prepared using the 2-[4-(hydroxymethyl)phenyl-acetoxy]-propionyl-resin and then cleaved in the protected form to demonstrate the utility of the new cleavage technique. The protected peptide cleavage products can be used in the preparation of larger peptides by fragment condensation.

The preparation of peptides by solid phase fragment synthesis[1,2] is a viable alternative to the more ubiquitous stepwise approach,[3] particularly for peptides consisting of approximately twenty-five or more amino acid residues. A successful solid phase fragment synthesis is dependent on several factors, one of which is a rapid and efficient method of preparing the individual protected peptide fragments that serve as intermediates for the larger target synthesis.

A major obstacle to the successful preparation of these protected peptide substrates is encountered at the end of the synthesis when the peptide has to be removed from the solid support. The protected peptide must be cleaved at the bond that anchors the peptide to the resin while, at the same time, leaving the other protecting groups intact. Therefore, the method used must be rapid, highly selective and give reasonably good product yields. Methods that have been reported in the literature to date have included hydrogenolysis,[4] nucleophilic displacement of the peptide[5] and photolytic cleavage of the peptide-resin bond.[6] The method described here will demonstrate how protected peptide fragments that contain a free C-terminal amino acid can be synthesized with a minimum of side reactions and then be cleaved from the solid support by a new base-catalyzed elimination reaction employing the hindered, non-nucleophilic bases tetramethylguanidine or 1,8-diazabicyclo-[5.4.0.]-undec-7-ene.



Tetramethylguanidine
(TMG)



1,8-diazabicyclo-[5.4.0.]-undec-7-ene
(DBU)

We have recently described the synthesis of a thirteen residue protected peptide prepared on a multidetachable resin.[7] The multidetachable resins are a new class of solid phase supports that have been specifically designed to give maximum flexibility in the method used for removing the peptide from the resin. The protected tridecapeptide was removed from the resin by photolysis which gave the Boc-protected peptidyl-oxymethylphenylacetic acid (OMPA) derivative as the principal

product. The protected peptide was purified and reattached to 2-bromopropionyl-resin to regenerate the original peptide-resin which was then ready for extension of the sequence (Fig 1). The peptide-resin was extended to give a forty-two residue peptide by the coupling of a thirteen- and a sixteen-residue protected peptide, respectively. The forty-two residue peptide product corresponded to

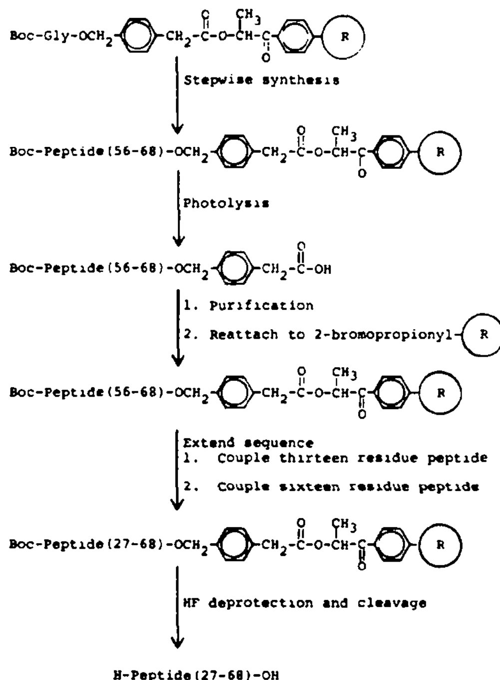


Figure 1. Strategy for the synthesis of peptides by fragment coupling on solid supports. The solid support used here is the multidetachable Pop-resin.

residues 27-68 of the M603 murine immunoglobulin V_H domain.[8,9] The thirteen- and sixteen-residue peptides used in the synthesis had to have a free C-terminal amino acid residue because they were being coupled to a peptide-resin (see Fig 1).

The protected peptides requiring a free C-terminal amino acid were prepared on a 2-bromopropionyl-resin and were cleaved from the resin by photolysis. The above procedure was subject to several drawbacks, two of which were a deleterious side reaction resulting in large amounts of chain loss[10] and the long reaction times required for the photolytic cleavage of the peptide-resin bond. The side reactions that we have observed during the synthesis of these protected peptide fragments prompted us to develop the alternative strategy described here.

RESULTS AND DISCUSSION

Synthesis of Protected Peptide Fragments

A series of peptide-resins were prepared that represent the peptide substrates for a solid phase fragment synthesis of the 1-68 residue sequence corresponding to the N-terminal half of the V_H domain of M603 murine myeloma immunoglubulin.[9] The target protected peptides all contain glycine as the C-terminal amino acid. The synthesis of all the peptides began with the preparation of Boc-glycyl-oxymethylphenylacetic acid from Boc-glycine followed by coupling of the Boc-glycine derivative to 2-bromopropionyl-resin as described by Tam et al.[11] with a slight modification found necessary when using glycine. Boc-glycyl-OMPA was added to a suspension of 2-bromopropionyl-resin in NMP. Finely ground anhydrous potassium fluoride was added and the mixture was stirred for eighteen hours at room temperature. The suspension was then stirred for an additional two hours at 40° C. The additional two hours at 40° C was required to drive the reaction to completion. The reaction proceeds smoothly to give the multidetachable Boc-glycyl-2-[4-(oxymethyl)phenyl-acetoxy]propionyl-resin (Boc-glycyl-OCH_2-Pop-resin) (see Fig 1). The yields for the coupling step were consistently better than 90% as determined by amino acid analysis of the aminoacyl-resin product.

The sequences of the peptides synthesized are shown below:

Boc-Glu(OBzl)-Val-Lys(2ClZ)-Leu-Val-Glu(OBzl)-Ser(Bzl)-Gly-OCH_2-Pop-(R) **1a**

Boc-Gly-Gly-Leu-Val-Gln-Pro-Gly-OCH_2-Pop-(R) **1b**

Boc-Trp(For)-Val-Arg(Tos)-Gln-Pro-Pro-Gly-OCH_2-Pop-(R) **2**

Boc-Phe-Thr(Bzl)-Phe-Ser(Bzl)-Asp(OcHex)-Phe-Tyr(Cl_2-Bzl)-Met(O)-Glu(OcHex)-Trp(For)-Val-Arg(Tos)-Gln-Pro-Pro-Gly-OCH_2-Pop-(R) **3**

Boc-Lys(2ClZ)-Arg(Tos)-Leu-Glu(OcHex)-Trp(For)-Ile-Ala-Ala-Ser(Bzl)-Arg(Tos)-Asn-Lys(2ClZ)-Gly-$OCH(CH_3)CO$-(R) **4**

Boc-Asn-Lys(2ClZ)-Tyr(Cl_2-Bzl)-Thr(Bzl)-Thr(Bzl)-Glu(OBzl)-Tyr(Cl_2-Bzl)-Ser(Bzl)-Ala-Ser(Bzl)-Val-Lys(2ClZ)-Gly-OCH_2-Pop-(R) **5**

The peptides were prepared by the standard solid phase method. The synthesis of **4** and **5** has been previously reported.[8] The t-butoxycarbonyl (Boc) group was used for α-amine protection. The Boc protecting group was removed by treatment with 50% TFA/CH_2Cl_2 followed by neutralization with 5% DIEA/CH_2Cl_2. The amino acids were coupled as symmetric anhydrides in CH_2Cl_2, followed by coupling with the 1-hydroxybenzotriazole (HOBt) ester derivatives of the amino acids in DMF. The symmetric anhydrides and HOBt esters were prepared by preactivation with dicyclohexylcarbodiimide (DCC) and all couplings were for two hours. A third coupling was performed when necessary and all couplings were essentially complete as measured by the quantitative ninhydrin test.[12] Glutamine was coupled as its HOBt ester for both couplings in DMF to reduce nitrile formation.[13] A continuous CH_2Cl_2 wash procedure was used between the deprotection and neutralization of the N-terminal glutamine residue during the synthesis of peptides **1b**, **2** and **3** to inhibit the formation of pyro-glutamic acid terminated sequences. Amino acid analyses on the peptide-resins were satisfactory and are shown in Table I.

Table I

Amino Acid Ratios From Hydrolysis of Peptide-Resins **1a**, **1b**, **3**, **4**, and **5**

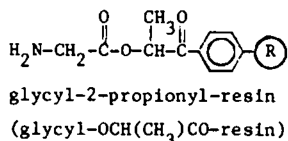
Amino Acid	1a	1b	3	4	5
Asp(Asn)			1.10	1.00	1.00
Thr			0.61[a]		1.68[a]
Ser	0.40[a]		0.34[a]	0.82[a]	1.29[a]
Glu(Gln)	2.04	1.02	1.90	0.85	1.00
Gly	1.05	2.94	0.99	1.00	1.03
Ala				1.96	0.98
Pro		1.02	—		
Val	1.92	0.99	1.10		0.98
Met			0.56[a]		
Leu	1.03	0.99		0.86	
Ile				0.86	
Tyr			0.46[a]		1.62[a]
Phe			2.90		
Lys	0.96			1.82	
Arg			0.96	1.71	
Trp			—		

a) Low recovery due to hydrolysis conditions

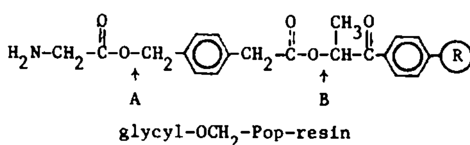
b) (—) not determined

Peptide-resins **1a**, **1b** and **3** represent residues 1-8, 9-15 and 27-42 in the M603 V_H, respectively. Peptide-resin **2** corresponds to the first seven residues in **3** and was prepared to see the effect of increased chain length on the resin cleavage reaction between **2** and **3**. The above peptides have all been synthesized previously on 2-bromopropionyl-resin and the current preparation on the multi-detachable Pop-resin shows a distinct improvement. For example, a 35% drop in substitution after two cycles was observed when peptide-resin **1a** was prepared on the 2-bromopropionyl-resin. The chain loss is believed to arise from Schiff base formation between the C-terminal glycine and the carbonyl group in the propionyl-resin. The OMPA spacer that gives the Pop-resin its multidetachable

properties also insulates the glycine from the carbonyl group on the propionyl-resin so that this side reaction does not occur. A similar effect was found in the preparation of peptide-resins



glycyl-2-propionyl-resin
(glycyl-$OCH(CH_3)CO$-resin)



glycyl-OCH_2-Pop-resin

1b, 2 and 3. Peptide-resin 2 and 3 contain the sequence Pro-Pro-Gly which has been shown to be especially susceptible to not only Schiff base but also diketopiperazine formation between Pro-Gly-propionyl-resin resulting in increased chain loss.[14] A prior synthesis of peptide-resin 3 employing the 2-bromopropionyl-resin as the solid support circumvented these side reactions by employing a more complex synthetic strategy using dipeptide fragment couplings at the beginning stages of the synthesis. The synthesis on the Pop-resin does not suffer from either of these side reactions so a standard stepwise synthesis was used in the preparation of 3. The use of the multidetachable resin for the preparation of these peptides demonstrated a distinct improvement in their synthesis.

Peptide-resin cleavage

The multidetachable Pop-resin has two anchoring sites that are susceptible to cleavage. The phenacyl ester linkage between the resin and the OMPA handle (site B) is labile to photolysis and to a variety of nucleophiles. The bond between the peptides and the OMPA handle (site A) is essentially a benzyl ester and is labile to acidolysis, saponification and hydrogenolysis. Cleavage at bond B gives the peptide OMPA derivative which is unsuitable as a substrate in a solid phase fragment synthesis. The desired cleavage at bond A can be effected by the above mentioned methods (hydrogenolysis, etc.) but these procedures lack the desired selectivity to make them generally useful.

We have recently found that hindered, non-nucleophilic amidine type bases such as tetramethylguanidine (TMG) and 1,8-diazabicyclo-[5.4.0.]-undec-7-ene (DBU) cleave Boc-aminoacyl-OCH_2-Pop-resins in high yield to give the Boc-amino acid as the principal product.[11] Tetramethylguanidine and 1,8-diazabicyclo-[5.4.0.]-undec-7-ene have been used in peptide synthesis and organic synthesis as reagents for base catalyzed reactions (e.g. esterification) due to their low nucleophilicity and high basicity (pKa $\sim$12.5).[15,16] The unexpected result from the cleavage of the Boc-aminoacyl-resins was that the Boc-amino acid was the sole product. The cleavage reactions for the solution equivalent of the Boc-aminoacyl-resins were quite rapid. A sample of Boc-Val-oxymethylphenylacetic acid phenacyl ester was cleaved by both DBU and TMG in NMP to 99% ($\sim$6-7 half-lives) conversion in less than five minutes.[16]

The peptide-resins 1a, 1b, 2 and 3 were all treated with TMG or DBU to determine if the Boc-peptidyl-OCH_2-Pop-resins were also susceptible to cleavage. Additionally, peptide-resins 4 and 5 which have been previously synthesized, were tested for comparison with the others. The results are shown in Table II.

Table II

Yields for the Cleavage of Protected Peptides[a,b]

Peptide-Resin	Resin Type	Base	Solvent	Time (h)	Yield (%)
1a	Pop	TMG	NMP	1	76
1b	Pop	TMG	NMP	1	73
2	Pop	TMG	NMP	1.5	68
3	Pop	TMG	NMP	2.5	74
4	2-propionyl	TMG	NMP	1.0	0[b]
4	2-propionyl	DBU	NMP	2.0	0[b]
5	Pop	DBU	NMP	1	65

a) Yields determined by quantitative ninhydrin test and/or amino acid analysis.

b) Peptide-resin 4 was used as a control.

All of the peptide-resins studied, except for 4 gave high yields of protected peptide product when treated with the hindered bases. The cleavage yields were comparable to the corresponding photolytic cleavage yields. The Boc-peptide-resins were treated with 2.3 equivalents of TMG or

DBU in NMP for one to two hours. The peptide-resin suspension immediately changed color on addition of the base, followed by color loss within five minutes. The most dramatic color change was observed with peptide 1b. The reactions were quenched using dilute aqueous hydrochloric acid. The resin suspensions were filtered, the resin collected, and the cleavage yields were determined by the quantitative ninhydrin reaction and/or amino acid analysis of peptide remaining on the resin. A drawback of the ninhydrin reaction is that the efficiency of the reaction between the free amino group on the resin and the ninhydrin to give Ruhemann's purple is not 100%. The efficiency is dependent on the nature of the N-terminal amino acid, the solid support and the sequence of the peptide. Therefore, to assure a quantitative result, the free amine content of both the base-treated and original untreated peptide-resin was determined by the ninhydrin test under similar conditions. The results were converted to mmoles of peptide per gram of polystyrene and the drop in substitution for the base-treated peptide-resin was used to determine the yield of the cleavage reaction. Amino acid analysis was used as a check for some of the base-treated peptide-resins and the yield values were in good agreement with those obtained by the ninhydrin analysis. The yield of the cleavage reaction did not vary to a great extent as a function of the size of the peptides cleaved. Peptide-resin 2 represents the first seven residues of peptide-resin 3 and the yields for the cleavage of the two are quite similar even though 3 is more than double the size of 2. The finding that the size of the peptide seems irrelevant to the cleavage yield is fortunate, because we eventually intend to use this method to cleave peptide-resins of up to seventy residues. The reactions were conducted in NMP or DMF because these solvents gave the most rapid reactions when cleaving the Boc-Val-oxymethylphenylacetic acid phenacyl ester. The choice of solvent is especially critical when cleaving peptide-resins because of the well known insolubility of protected peptides in most common solvents. We have found through experience that NMP not only provides excellent solvation of these protected peptides but also has good resin-swelling properties.

The probable mechanism for this reaction is shown in Figure 2.[11]

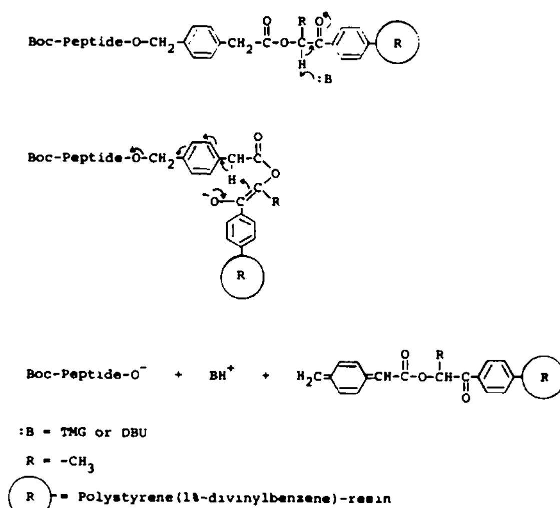


Figure 2. The probable mechanism for the cleavage of protected peptide-resins using either TMG or DBU.

The base (TMG or DBU) removes the acidic proton from the carbon α to the keto group, generating an enolate anion. The anion then abstracts the proton α to the carboxy group in a five membered cyclic transition followed by expulsion of the peptide. The last step can be envisioned as an internal base-catalyzed elimination reaction across an aromatic ring, generating a highly conjugated resin-bound spacer and the peptide product. The presence of the OMPA spacer is critical for this reaction to occur. The benzyl ester bond of several amino acid derivatives was resistant to cleavage by this method and peptide-resin 4, which contains only the phenacyl ester bond between peptide and solid support, gave no detectable cleavage products (see Tables II and III).

The useful feature of the new peptide-resin cleavage reaction is that the product peptide contains a free C-terminal amino acid. The OMPA spacer apparently remains bound to the resin.

The product identity was determined by several methods. The amino acid analysis of the products verified that they all contained the proper amino acids but amino acid analysis did not show whether the OMPA group was present or not. Previous data obtained from cleavage of the Boc-aminoacyl-resins have shown that the only product of the reaction was the Boc-amino acid.[11] The mechanism for the reaction is presumed to be similar for the peptide-resins so by extrapolation the products should be the Boc-peptides without the OMPA group. Secondly, when peptide-resin **4**, which contains only the phenacyl ester function of the Pop-resin was treated with TMG or DBU, no cleavage occurred. The absence of a cleavage reaction demonstrates the stability of the phenacyl ester bond to the hindered base when the OMPA spacer is not present. This result is not surprising because the hindered bases are non-nucleophilic. It is therefore highly unlikely that the peptide-resin would cleave at the phenacyl ester bond and give the Boc-peptide-OMPA product. Evidence of a more direct and conclusive nature was obtained by subjecting the peptide product from the TMG cleavage of peptide-resin **1a** to molecular weight analysis by Californium-252 fission fragment ionization mass spectrometry.[17,18] The molecular weights of the peptide products would be 1547.20 for the peptide with the OMPA group and 1399.05 for the peptide with a free α carboxyl group. The results showed two peaks of mass 1421.94 $(M + Na)^+$ and 1444.11 $(M + 2\ Na-H)^+$ for the peptide from **1a**, which correspond to an average molecular weight of 1399.05 and verify the presence of a free C-terminal amino acid in the peptide. No ion corresponding to protected peptide was found. The mass spectrometry data provide direct evidence in support of the proposed mechanism and product composition for this reaction.

Table III

Stability of Amino Acid Derivatives Towards Tetramethylguanidine[a]

Amino Acid	Stable	Unstable
Boc-Glu(OBzl)-Gly-OEt	x	
Boc-Ala-Glu(OBzl)-Gly-OEt	x	
Boc-Asp(OBzl)-OH	x	
Boc-Glu(OcHex)-OBzl	x	
Boc-Met(O)-OH	x	
Boc-His(DNP)-OH		x
Boc-His(Bzl)-OH	x	
Boc-Tyr(2BrZ)-OH		x
Boc-Tyr(Cl_2-Bzl)-OH	x	
Boc-Ser(Bzl)-OH	x	
Boc-Lys(2ClZ)-O·TBA	x	
Boc-Trp(For)-OH	x	

a) Reaction conditions were 2.3 eq TMG in 1 ml NMP and reaction times $\geq$ 2.5 h.

The hindered base reagents used in this study appear to be quite selective with respect to the protecting groups commonly used in peptide synthesis. The t-butoxycarbonyl group used for α-amine protection is stable to the conditions used in the cleavage reaction. A number of Boc-amino acid derivatives were tested for their stability towards tetramethylguanidine under the same conditions used where peptide-resin cleavage is observed. The data are shown in Table III. Boc-Tyr(2BrZ) and Boc-His(DNP) were the only amino acid derivatives decomposed by the reaction conditions. The Boc-Tyr(Cl_2-Bzl) and Boc-His(Bzl) derivatives however, were quite stable to the tetramethylguanidine reagent and therefore could be used in any synthesis that employs the hindered base reagent for final peptide cleavage from the resin. The di- and tripeptides Boc-Glu(OBzl)-Gly-OEt and Boc-Ala-Glu(OBzl)-Gly-OEt were also stable under the reaction conditions employed for cleavage. The pyro-glutamic or aspartimide forming side reactions however, may occur with particularly susceptible peptide sequences (Asp-Gly, etc.) due to the basicity of the tetramethylguanidine and 1,8-diazabicyclo-[5.4.0.]-undec-7-ene reagents. The use of the cyclohexyl ester group for protection of glutamic and aspartic acid residues is recommended as a precautionary

measure. The steric bulk of the cyclohexyl group retards the ring formation that leads to the above mentioned side reactions.[19]

An attractive feature of this method is the short reaction time required for the peptide-resin cleavage and the rapid product isolation as compared with other methods such as photolysis. The following example is provided to exemplify this statement. Peptide-resin **1a** was added to 1 ml of NMP and stirred. Ten microliters of tetramethylguanidine (neat) was added and the suspension was stirred for one hour. The reaction was quenched with aqueous acid, filtered and then the filtrate was chromatographed using a Sephadex LH-20 column in DMF. The peptide fractions were pooled (the tetramethylguanidine and NMP elute in the column volume) and DMF solvent was removed under vacuum. The product oil was taken up in a minimal amount of DMF and the peptide was precipitated by addition of water. The peptide was then collected and lyophilized. The thin layer chromatogram of the product showed a single spot using both starch/iodine and $AgNO_3$/toluidine spray reagents for the detection of peptide, and the amino acid analysis showed good correlation with the theoretical values.

CONCLUSION

Protected peptide fragments for use in solid phase fragment synthesis can be prepared in good yield and purity employing the multidetachable Pop-resin as the solid support. The protected peptide can be cleaved from the resin via a new elimination reaction catalyzed by tetramethylguanidine or 1,8-diazabicyclo-[5.4.0.]-undec-7-ene. The cleavage reaction conditions are mild, selective, proceed in good yield and the workup is simple.

EXPERIMENTAL

Materials

The Boc-amino acids were purchased from Peninsula Laboratories and were checked for purity by TLC before use. Sephadex LH-20 for gel filtration chromatography was from Pharmacia. Silica gel plates for thin layer chromatography were from Analtech. Gold label N-methylpyrrolidinone was from Aldrich and dimethylformamide (omni-solve, glass distilled) was from Matheson, Coleman and Bell. Trifluoroacetic acid was from Halocarbon Products Inc. Dichloromethane was distilled over sodium carbonate before use. 1-hydroxybenzotriazole was purchased from Aldrich and dicyclohexylcarbodiimide was from the Pierce Chemical Company. Bio Beads S-XI (1% crosslinked divinylbenzene-polystyrene resin) was from Bio-Rad Laboratories. Boc-aspartic acid α-benzyl ester, Boc-glutamic acid α-benzyl ester, tetramethylguanidine and water soluble carbodiimide (1-ethyl-3-(3-dimethylaminopropyl)carbodiimide) were from Sigma. 1,8-diazabicyclo-[5.4.0.]-undec-7-ene was from Aldrich.

Analytical

Amino acid analyses were done on a Durrum D-500 or Beckman 121 amino acid analyzer. The peptide resins were hydrolyzed in 12 N HCl/propionic acid (1:1) at 130° C for 6 h in screw cap test tubes. The peptides were hydrolyzed in 6 N HCl at 110° for 24 h in evacuated tubes. The quantitative ninhydrin test was performed as described by Sarin et al.[12] Molecular weight determinations were obtained using Californium-252 fission fragment ionization mass spectrometry at the Rockefeller Laboratory. Elemental analyses were performed at the Microanalytical Laboratory, Rockefeller University.

Synthesis of Boc-Asp(OcHex)-OH and Boc-Glu(OcHex)-OH

Boc-Asp(OcHex)-OH and Boc-Glu(OcHex)-OH were prepared as previously described[19] except for a reduction in the hydrogenation time from 18 h to 6.5 h.

Synthesis of Boc-Ala-Glu(OBzl)-Gly-OEt

The purity of Boc-Glu(OBzl)-Gly-OEt was determined by amino acid analysis and elemental analysis Glu (1.03), Gly (0.97). Anal. Calc. for $C_{21}H_{30}N_2O_7$: C, 59.70%; H, 7.17%; N, 6.63%. Found: C, 59.88%; H, 7.06%; N, 6.63%. The dipeptide (243 mg, 0.575 mmol) was treated with 4.0 ml of 50% TFA/CH_2Cl_2 for 25 minutes with stirring. The residual TFA was removed by flushing with N_2. The residue was neutralized with 0.7 ml of 10% triethylamine in CH_2Cl_2. The Boc-Ala-OH was activated to its HOBt ester by adding one ml of 0.587 M DCC/CH_2Cl_2 to a solution of Boc-Ala-OH (111.5 mg, 0.59 mmol) and HOBt (81 mg, 0.59 mmol) in CH_2Cl_2. The reaction was stirred for ten minutes at 0° C, filtered and the CH_2Cl_2 was removed by flushing with N_2. The residue was taken up in 3 ml DMF and was then added to the deprotected, neutralized dipeptide residue. The coupling reaction was continued for 20 h. The DMF solvent was removed under vacuum. The residue was taken up in 50 ml ethyl acetate and the residual dicyclohexylurea was removed by filtration. The ethyl acetate fraction was extracted by the following protocol: 3 x 50 ml 1 M aqueous HCl; 2 x 50 ml water; 3 x 50 ml Na_2CO_3 pH 9.4; 2 x 50 ml water. TLC on silica gel plates using a chloroform: acetic acid solvent (95:5) gave one spot for the product (RF, 0.29). The peptide residue was dissolved in hot ethyl acetate and recrystallized by adding petroleum ether. The tripeptide product (105 mg) crystallized as white needles in 37% yield. The yield was not optimized. Amino acid analysis for the tripeptide gave Glu (1.03), Gly (1.00), Ala (0.97). Anal. calc. for C, 24; H, 35; O, 8; N, 3: C, 58.40%; H, 7.14%, N, 8.51. Found: C, 58.28%, H, 7.03; N, 8.51%.

Synthesis of Boc-glycyl-2-[4-(oxymethyl)phenylacetoxy]propionyl-resin

Boc-glycyl-oxymethylphenylacetic acid was prepared as described before.[11] The Boc-glycine derivative (561 mg, 1.73 mmol) was added to a suspension of 1.8 g of 2-bromopropionyl-resin (0.73 mmol/g, 1.3 mmol Br) in 20.6 ml Nmp. Potassium flouride (372 mg, 6.4 mmol), anhydrous and freshly ground, was added with stirring. The resulting suspension was stirred for two hours at room temperature. Potassium bicarbonate (174 mg, 1.74 mmol) was added and the mixture was stirred for 15 h. The temperature of the reaction was raised to 40° C and stirring was continued for an additional two hours. Methylene chloride (30 ml) was added to separate the resin from the inorganic protocol: DMF, 2 x 1 min; DMF/H_2O (1:1) 4 x 2 min; acetic acid/dioxane (1:1), 5 x 2 min; DMF/H_2O (1:1), 2 x 2 min; DMF, 4 x 1 min; CH_2Cl_2, 4 x 1 min. The resin was then dried under vacuum. A sample of the product Boc-aminoacyl-resin was hydrolyzed and analyzed for amino acids. The substitution was 0.70 mmol/g. Two other preparations of the Boc-glycyl-2-[4-(oxymethyl)phenyl-acetoxy]-propionyl-resin gave substitutions of 0.67 and 0.68 mmol/g.

Synthesis of peptide-resins

The following protocol was used in the synthesis of the peptide-resins **1a**, **1b**, **2** and **3**: 50% TFA/CH_2Cl_2, 1 x 2 min; 50% TFA/CH_2Cl_2, 1 x 25 min; CH_2Cl_2, 4 x 1 min; 5% DIEA/CH_2Cl_2, 2 x 2 min; CH_2Cl_2, 5 x 1 min; 2.5 eq symmetric anhydride/CH_2Cl_2, 1 x 120 min; CH_2Cl_2, 3 x 1 min; 5% DIEA/CH_2Cl_2, 1 x 1 min; CH_2Cl_2, 5 x 1 min; 2.5 eq HOBt ester/DMF, 1 x 120 min; CH_2Cl_2, 3 x 1 min; 5% DIEA/CH_2Cl_2; 1 x 1 min; CH_2Cl_2, 5 x 1 min.

The syntheses generally began with 1.0 to 2.5 grams of starting Boc-glycyl-2-[4-(oxymethyl)-phenylacetoxy]propionyl-resin. The amino acid derivatives used in the coupling steps were all preactivated with DCC and filtered before use. All couplings were checked for completion by the quantitative ninhydrin test[12] and a third coupling was performed when necessary. All coupling yields were ≧99.6%.

Peptide-Resin Cleavage

Typically a 100-mg sample of peptide-resin was added to one ml of NMP and stirred. Tetramethylguanidine (10 µl, 80 µmol, 2.3 eq) was added and the suspension was stirred for one hour. The suspension was then filtered to collect the resin. The resin product was then washed with an additional one ml NMP and all the NMP fractions were collected. Hydrochloric acid (1 M, 340 µl) was added to the pooled NMP fractions to quench the reaction. The filtrate was then chromatographed on an LH-20 (0.95 x 100 cm) column using DMF as the eluant. The peptide fractions were pooled and the peptide was isolated by precipitation from the DMF with water. The base treated resin and a sample of untreated resin (∿30-50 mg) were deprotected with 50% TFA/CH_2Cl_2 and neutralized with 5% DIEA/CH_2Cl_2. The quantity of free amine on the resin was determined for both by the quantitative ninhydrin test[12] and the yield for the cleavage was determined by a comparison of the two results. In several cases the results were checked by amino acid analysis of the base treated resin and the results in all cases agreed to within ±5%.

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