Studies Related to a Convergent Fragment-Coupling Approach to Peptide Synthesis Using the Kaiser Oxime Resin

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In our efforts to synthesize the β-amyloid protein of Alzheimer's disease and other amyloid-forming peptides, we have used a convergent solid-phase fragment coupling strategy employing the *p*-nitrobenzophenone oxime resin developed by E. T. Kaiser.[1,2] The Kaiser oxime resin's lability to a variety of nucleophiles adds flexibility to the synthesis. We report improved procedures for the preparation and coupling of protected fragments on the Kaiser oxime resin.[3] Rearrangements which can occur during the synthesis, cleavage, and coupling of protected fragments have been characterized and steps have been taken to eliminate or minimize these side reactions. In addition, we report a procedure for the preparation of C-terminal primary amides using the Kaiser oxime resin.

Introduction

Stepwise solid-phase peptide synthesis has been applied successfully to the preparation of proteins such as the HIV-1 protease (99 residues).[4] The synthesis of long sequences is hampered, however, by the unavoidable accumulation of single amino acid deletion products which are difficult to separate from the target peptide. For example, the synthesis of a 50-mer in which all couplings proceed in 99.5% yield will produce the desired 50-mer and 49 different 49-mers. These single deletion impurities will make up ~20% of the final product but will be difficult to detect using the standard methods of peptide characterization. The mass spectrum of the crude product will have a signal for the target peptide which is 150 times more intense than the signals for each of the impurities, while amino acid analysis of the product mixture will indicate the correct composition because the mixture of deletion impurities contains the correct average composition.

The accumulation of significant amounts of deletion impurities in stepwise synthesis and the difficulties in separating them from the full length peptide are well documented. Clark-Lewis et al. synthesized interleukin-3 (140 residues) and reported that 59% of the product consisted of deletion impurities.[5] The resulting purification problem was not solved.[6] Even in short sequences, deletion impurities can pose a significant problem because the tendency of certain sequences of amino acids to aggregate can result in low-yield coupling steps.[7] Merrifield et al. reported an optimized synthesis of $Ala_{10}Val$ in which 35% of the crude product was the undesired decapeptide Ala_9Val resulting from single deletions.[8] Methods have been proposed to identify such "difficult sequences",[9] which are characterized by reproducible stretches of incomplete couplings. Although the physical basis for this phenomenon (aggregation of the resin-bound peptide via β-sheet formation) has been studied extensively,[10-18] little progress has been made in improving the synthesis of these sequences.[9]

In the fragment-coupling approach, protected peptide fragments are purified and fully characterized, eliminating impurities which lack a single amino acid. Because the target peptide is assembled by coupling purified protected fragments, a failed coupling results in an impurity that lacks an entire fragment and therefore is easier to identify and separate from the final product. In addition, side reactions which might go unnoticed in stepwise synthesis can be identified and avoided. Another advantage of the fragment coupling approach is that it provides a convergent synthetic route which is ideally suited to the preparation of analogs for structure–activity studies. Several approaches to fragment synthesis have been employed, including the preparation and coupling of fragments in solution,[19] the preparation of fragments by solid-phase methods followed by coupling of fragments in solution,[20-22] and the preparation and coupling of fragments on a solid support.[1,23] This last approach takes full advantage of the time-saving and labor-saving elements of solid-phase peptide synthesis.

Several strategies for the solid-phase synthesis of protected peptides have been employed, including peptide-resin linkers which are labile to dilute acid[22,24,25] and light.[26] The Kaiser oxime peptide-resin linkage can be cleaved by a variety of nucleophiles including amino acids,[28] alkylamines,[29] and *N*-hydroxypiperidine (HOPip), which, after reductive cleavage, yields the peptide free acid.[27] The

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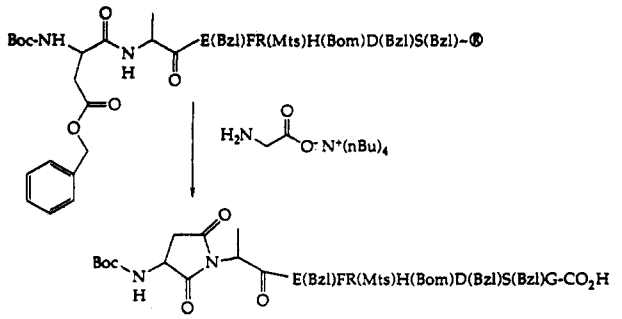

Figure 1.

Kaiser oxime resin has been employed in several published syntheses,[1,27,30-33] with mixed results. All fragment coupling strategies must overcome the same problems: solubilization and purification of protected peptides is difficult and activation of protected fragments to provide high yield coupling often results in significant epimerization at the C-terminal residue.[34] In addition, with the Kaiser resin, rearrangements may occur during the nucleophilic cleavage reaction. We report the optimization of fragment synthesis, cleavage, and fragment coupling using the Kaiser oxime resin. In addition, we report conditions for the minimization of two common side reactions, epimerization and aspartimide formation.

Results

Stepwise Synthesis of Protected Fragments. Boc-VFFAE(Bzl)D(Bzl)-OH[35] was synthesized using both symmetric anhydride activation[27] (with diisopropylcarbodiimide (DIC) as the activating agent) and (benzotriazol-1-yloxy)tris(dimethylamino)phosphonium hexafluorophosphate (BOP) activation.[36] The best results of the symmetric anhydride synthesis were achieved using double couplings at every step. The target peptide accounted for ~35% of the crude product and was obtained in 16% purified yield (based on resin-bound Asp). Amino acid analysis of the resin-bound intermediates indicated that the Phe to Phe coupling proceeded in very low yield (<35% for the first step of the double coupling). Synthesis of the same peptide using BOP activation with no double couplings resulted in a nearly homogeneous crude product and a purified yield of 66%.

Boc-VFFAE(Bzl)D(cHex)VG-OH was first synthesized in 15% purified yield (based on Gly-resin) using BOP activation with dimethylformamide (DMF) as the coupling solvent. A capping step with acetic anhydride followed each coupling. A mixture of five products was observed in the crude material. An otherwise identical synthesis was carried out using dimethyl sulfoxide (DMSO) in *N*-methylpyrrolidone (NMP) (15% v/v)[14] as the coupling solvent. This synthesis produced two products: one truncated peptide (acetyl-FFAE(Bzl)D(cHex)VG-OH), which accounted for ~10% of the crude material, and the target peptide, which was obtained in a purified yield of 45%.

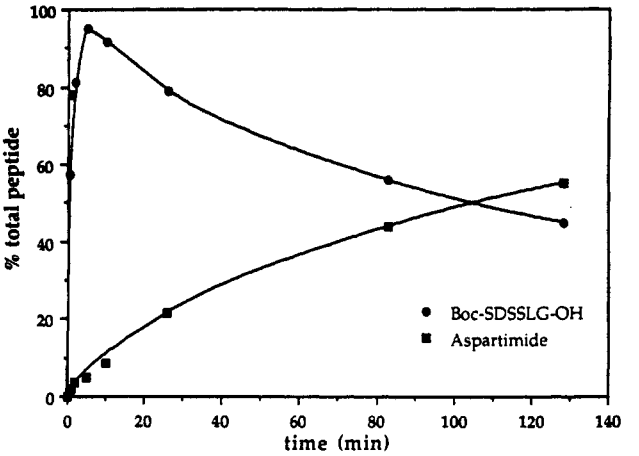

Figure 2. Formation of Boc-S(Bzl)D(aspartimide)S(Bzl)S-(Bzl)LG-OH and Boc-S(Bzl)D(cHex)S(Bzl)S(Bzl)LG-OH during the salt cleavage (3 equiv of Gly salt)[28] of Boc-S(Bzl)D(cHex)S-(Bzl)S(Bzl)L-resin. The two peptides were separated by reverse-phase HPLC. The integrated area of each HPLC peak is expressed as a percentage of the total peptide, which is defined as the sum of the two peaks after 128 min.

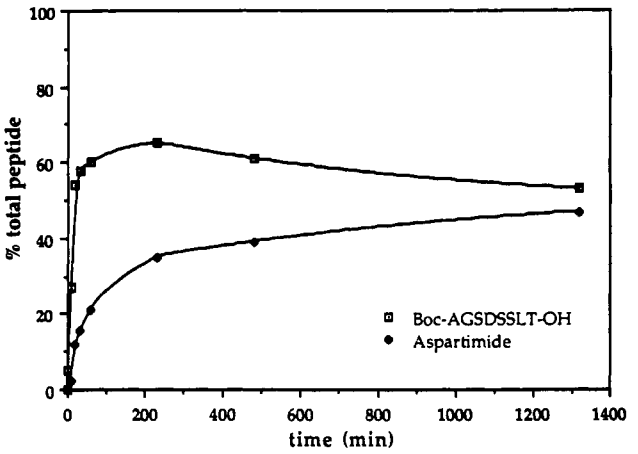

Figure 3. Formation of Boc-AGS(Bzl)D(aspartimide)S(Bzl)S-(Bzl)LT(Bzl)-OH and Boc-AGS(Bzl)D(cHex)S(Bzl)S(Bzl)LT-(Bzl)-OH during the salt cleavage (3 equiv of Thr(Bzl) salt)[28] of Boc-AGS(Bzl)D(cHex)S(Bzl)S(Bzl)L-resin. The two peptides were separated by reverse-phase HPLC. The integrated area of each HPLC peak is expressed as a percentage of the total peptide, which is defined as the sum of the two peaks after 1320 min.

Nucleophilic Cleavage of Protected Fragments. Synthesis of Boc-D(Bzl)AE(Bzl)FR(NO_2)H(Bom)D(Bzl)-S(Bzl)-resin followed by cleavage from the resin with glycine tetra-*n*-butylammonium salt[28] resulted in a peptide which contained an aspartimide as position 1 (Figure 1). None of the target protected peptide was detected in the crude product (MS). The aspartimide was identified by FAB mass spectrometry; its position in the sequence was confirmed by tandem FAB-collision mass spectrometry.[37] Substitution of a *tert*-butyl protecting group for the benzyl group at position 1 completely eliminated aspartimide formation.[38,39]

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For determination of whether aspartimide formation occurs during the salt cleavage procedure, three model peptides having the sequence Boc-S(Bzl)D(*)S(Bzl)S-(Bzl)L-OH were synthesized with three different aspartyl protecting groups (*): benzyl, cyclohexyl,[45] and 2-adamantyl.[40] Each peptide was dissolved in DMF in the presence of glycine tetra-*n*-butylammonium salt (3 equiv). Conversion to the aspartimide and concomitant loss of the protecting group was monitored by reverse-phase HPLC. The cyclohexyl ($t_{1/2}$ = 10 min) and 2-adamantyl ($t_{1/2}$ = 45 min) esters were converted to aspartimide much more slowly than the benzyl ester ($t_{1/2}$ < 1 min.).

Boc-S(Bzl)D(cHex)S(Bzl)S(Bzl)L-resin was suspended in DMF containing glycine tetra-*n*-butylammonium salt in order to compare the rates of salt cleavage and aspartimide formation. Aliquots of the supernatant were removed periodically and analyzed by HPLC for the presence of the fully protected peptide and the apartimide-containing peptide. A second experiment was performed using the related peptide Boc-AGS(Bzl)D(cHex)S(Bzl)S-(Bzl)L-resin and threonine(benzyl) salt. The results are shown in Figures 2 and 3. In the case of the Gly salt, the cleavage reaction was complete in 15 min and provided a 9:1 ratio of protected peptide to aspartimide. After 15 min, no further cleavage was observed but conversion to the apartimide continued (50% conversion at 110 min). In the Thr salt cleavage, both the cleavage rate and the rate of aspartimide formation were slower. After 1.5 h, ca. 80% of the peptide was cleaved from the resin and a 3:1 ratio of protected peptide to aspartimide was obtained. The peptide was completely cleaved from the resin after 3.5 h, but at this time the ratio of protected peptide to aspartimide was 2:1.

An HPLC assay was used to measure epimerization in both the amino acid salt and the C-terminal residue of the resin-bound peptide during the salt cleavage. Boc-S-(Bzl)T(Bzl)QT(Bzl)A-resin was cleaved with Leu salt.[28] The product was analyzed by HPLC and compared with three synthetic standards: Boc-S(Bzl)T(Bzl)QT(Bzl)AL-OH, Boc-S(Bzl)T(Bzl)QT(Bzl)DAL-OH, and Boc-S(Bzl)-T(Bzl)QT(Bzl)ADL-OH. Integration of the HPLC peaks indicated <1% epimerization at both A and L.

Cleavage of the oxime resin with primary alkyl amines to yield the peptide C-terminal alkyl amide has been reported previously.[29] We observed extensive aspartimide formation in the cleavage of several Asp-containing sequences. The cleavage of Boc-D(Bzl)GGG-resin with CH_3NH_2·HCl (5 equiv) in DMF with DIEA (2 equiv) was carried out at 22 °C and at −2 °C; both reactions were monitored by HPLC. In both cases, the relatively fast cleavage reaction was followed by the slower cyclization to form aspartimide. The cleavage was >90% complete after 3 h at both temperatures. The low-temperature conditions reduced aspartimide formation. At 22 °C, the crude product was 35% aspartimide after 3 h and 75% aspartimide after 24 h. At −2 °C, the crude product was 15% aspartimide after 3 h and 40% aspartimide after 24 h.

We have developed a method for the preparation of primary amides using the oxime resin. The model peptide Ac-(β)D(Bzl)VG-resin was cleaved with NH_4OAc (10–20 equiv) in the presence of DIEA with DMF as solvent. Amino acid analysis of the resin indicated >97% cleavage, and the pure protected peptide amide was obtained in 68% yield. A previously reported 16-residue peptide[41] was cleaved with NH_4OAc (4 equiv) in 1.5 M LiBr in THF with a cleavage yield of 99%. The NH_4OAc cleavage proceeds with minimal epimerization; in the cleavage of Boc-S-(Bzl)T(Bzl)QT(Bzl)A-resin, the amount of the D-Ala epimer detected was <1%. Aspartimide formation did not occur during the NH_4OAc cleavage of Ac-(β)D(Bzl)VG-resin or Boc-S(Bzl)D(cHex)S(Bzl)S(Bzl)L-resin, but significant aspartimide formation occurred during the cleavage of two peptides containing benzyl-protected aspartate (Table I).

Table I. Aspartimide Formation in NH_4OAc Cleavage[a]

peptide	NH_4OAc, equiv	DIEA, equiv	temp, °C	time, h	% aspartimide
Ac-YDLTS	20	10	22	18.5	17
Ac-YDLTS	10	3	4	15.5	<10
Ac-DGF	20	3	22	12	97
Ac-DGF[b]	20	1	4	16	58

[a] Protecting groups: Y(Cl_2Bzl), D(Bzl), T(Bzl), S(Bzl). Cleavage solvent: DMF. [b] Cleavage solvent: methylene chloride.

We previously reported the use of 2 M LiBr in THF as a solvent for the cleavage of aggregated resin-bound peptides.[12] Subsequent experiments have shown that LiBr/THF itself can cleave peptides from the oxime resin. Treatment of a test resin-bound peptide (Boc-VFAE-(Bzl)D(Bzl)-resin) with 1 M LiBr/THF for 15 h at 22 °C resulted in 25% cleavage from the resin. The product obtained after standard workup was identified as the peptide carboxylic acid.

Coupling of Protected Fragments on the Kaiser Oxime Resin. An HPLC assay was used to monitor epimerization during the coupling of Boc-H(Bom)QK(Cl-Z)L-OH to resin-bound Val (1.5 equiv of BOP, 1 equiv of hydroxybenzotriazole (HOBt), 2 equiv of DIEA, 0–4 °C). Boc-H(Bom)QK(Cl-Z)DLV-OH was synthesized as a standard. After cleavage with HOPip, the amount of the D-Leu epimer detected was <2.5% of the product. Similar results were obtained in a previously reported BOP-mediated fragment coupling.[41]

Solvation of the resin-bound peptide is a critical factor in both single amino acid couplings and fragment couplings. Four solvent systems were tested on a fragment coupling in which the resin-bound fragment is known to aggregate.[12] Boc-S(Bzl)NK(Cl-Z)GAIIG-OH was coupled to H_2N-LMVGGVVIA-resin[42] (0.3–0.4 mmol/g) using standard BOP activation (1.5 equiv of BOP, 1 equiv of HOBt, 3 equiv of DIEA, 12 h). Coupling yields were measured by amino acid analysis of the resin. The coupling yield in DMF was 40–45%. The yield in 0.4 M $NaClO_4$/DMF[43] was 30–35%, while in 0.4 M KSCN/DMF[43] the yield was 20–25%. The best results were obtained in 15% DMSO/NMP.[14] The coupling yield in this solvent was 55–65%.

Discussion

Stepwise Synthesis of Protected Fragments: BOP and DMSO/NMP Improve Yields for "Difficult" Sequences. While symmetric anhydride activation has been used successfully in the synthesis of protected fragments,[1,23] the BOP reagent is reported to be better for "difficult" couplings (couplings which routinely proceed

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in low yield).[36] Our results support this assertion. The peptide Boc-VFFAE(Bzl)D(Bzl)-OH was repeatedly synthesized in poor yield (0–16%) using symmetric anhydride activation, with the Phe to Phe coupling proving especially problematic. Synthesis of the same peptide using BOP activation required no double couplings and produced a homogeneous product in 66% yield.

Many sequences are difficult to synthesize due to the tendency of the resin-bound peptide to aggregate.[7] The yields for these sequences can be improved by using a coupling solvent which disrupts aggregation. The solvent 15% DMSO/NMP is a good coupling solvent for aggregation-prone sequences;[14] its use resulted in a 3-fold increase in yield for the synthesis of Boc-VFFAE(Bzl)D-(cHex)VG-OH.

Nucleophilic Cleavage of Protected Fragments from the Oxime Resin: Rearrangements Can Result. Nucleophilic cleavage of the peptide-resin linkage is the most important feature of the Kaiser oxime resin. The cleavage reaction provides great flexibility; a single batch of peptide-resin can be used to prepare a series of peptides with different C-terminal amino acids (salt cleavage)[28] and/or a series of peptides with different C-terminal functional groups: carboxylic acid (cleavage with HOPip),[27] primary amide (ammonium acetate), secondary amide (alkyl amines),[29] and ester (amino acid esters).[44] However, we observed aspartimide formation in the salt, ammonium acetate, and alkylamine cleavages. The rate of aspartimide formation is dependent on the sequence of the peptide,[38] the aspartate protecting group,[45] and the cleavage reaction conditions.

Extensive aspartimide formation was observed in the peptide Boc-S(Bzl)D(*)S(Bzl)S(Bzl)L-OH under standard salt cleavage conditions. This sequence forms aspartimide very rapidly, but the aspartate protecting group (*) had a large effect on the rate of the reaction; the cyclohexyl[45] ($t_{1/2}$ = 10 min) and 2-adamantyl[40] ($t_{1/2}$ = 45 min) esters were converted to aspartimide much more slowly than the benzyl ester ($t_{1/2}$ < 1 min). The *tert*-butyl ester provided complete protection against aspartimide formation for the peptide Boc-D(tBu)AE(Bzl)FR(Mts)H(Bom)D(cHex)S-(Bzl)-resin (2 equiv of Gly salt, 24 h, 22 °C) but is not generally useful in Boc-based strategies because of its acid lability. It can, however, be used at the N-terminus of a peptide.[3] For sequences prone to aspartimide formation (Asp-Gly, Asp-Ser, and Asp-Ala are especially at risk),[38] a sterically hindered ester (cyclohexyl, 2-adamantyl, or *tert*-butyl) is a better aspartate protecting group than the benzyl ester.

When the salt cleavage is to be used with an aspartimide-prone sequence, a small scale test cleavage can assist in the choice of appropriate reaction time and temperature. The products of the cleavage reaction can be monitored by reverse-phase HPLC and the cleavage stopped after most of the peptide has been removed from the resin but before the slower conversion to the aspartimide has occured. This approach was demonstrated in the cleavage of Boc-S(Bzl)D(cHex)S(Bzl)S(Bzl)L-resin with Gly salt and in the cleavage of Boc-AGS(Bzl)D(cHex)S(Bzl)S-(Bzl)L-resin with Thr(Bzl) salt. In the first experiment, 15 min was the optimal reaction time at room temperature, while for the second cleavage, 1.5 h proved to be the appropriate reaction time (see Figures 2 and 3).

Aspartimides can also form during the ammonium acetate and alkylamine cleavages. Amino acid sequence, aspartate protecting group, amount of nucleophile and base present, and the reaction time and temperature all affect the rate of aspartimide formation. For example, in the methylamine cleavage of Boc-D(Bzl)GGG-resin, cleavage was >90% complete within 3 h, and extended reaction times led to continued aspartimide formation. In that cleavage, lowering the temperature significantly lowered the rate of aspartimide formation relative to the rate of cleavage. In the ammonium acetate cleavage, lower reaction temperatures were used together with less DIEA and, in one case, less ammonium acetate, to dramatically lower the amount of aspartimide formed (Table I). The results in Table I also serve to underscore the varying propensities of different sequences to form aspartimide.

Cleavage reactions can be run in many solvents; the most important requirement is that the solvent provide good solvation of the resin-bound peptide as evidenced by the swelling of the resin.[12] We have previously reported the use of anhydrous LiBr in THF as a cleavage solvent.[12] While improved yields were observed for both HOPip and salt cleavages of strongly aggregating peptides, further studies have shown that LiBr/THF slowly cleaves peptide from the oxime resin in the absence of any additional nucleophiles. The product obtained after standard workup is the peptide carboxylic acid. Therefore, while LiBr/THF can be used for HOPip cleavages, its use in prolonged salt cleavages will result in an impurity which lacks the additional C-terminal amino acid. It should not be used in cases where such an impurity is not easily separable from the desired product.

Coupling Protected Fragments on the Oxime Resin: Epimerization Can Be Minimized and Solvation Is Critical. Solid-phase fragment coupling yields vary considerably depending on the size and sequence of both the resin-bound fragment and the acylating fragment. However, our work on the Alzheimer's amyloid protein[3] and several other peptides has revealed some general strategies for increasing fragment-coupling yields. Solvation of the resin-bound peptide is a critical factor in enhancing coupling yield. In the case of resin-bound peptides which tend to aggregate, the degree of swelling is a good indicator of the ability of the solvent to disaggregate the resin-bound peptide.[12,46] Unfortunately, not all solvents are amenable to the coupling reaction.[47,48] The coupling of Boc-S-(Bzl)NK(Cl-Z)GAIIG-OH to H_2N-LMVGGVVIA-resin was run in several different solvent systems, all of which have been reported as superior to methylene chloride in the *stepwise* synthesis of peptides with a tendency to aggregate.[14,43] The best results for this fragment coupling were obtained with DMSO (15%) in *N*-methylpyrrolidone.[14] Solutions of chaotropic salts (KSCN, $NaClO_4$) in DMF[43] resulted in lower fragment coupling yields than DMF alone.

We reported previously that the BOP reagent is superior to DIC/HOBt activation for fragment couplings.[41] Concerns have been raised about the levels of epimerization in BOP-mediated fragment couplings; the addition of HOBt to the reaction mixture has been reported to reduce epimerization.[34] Monitoring epimerization via separation of diastereomers after fragment coupling reveals that when reaction temperature and amount of tertiary base are minimized, epimerization levels are low. Couplings in which the C-terminal residue of the acylating fragment is

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not glycine are run at 0–4 °C with 1.5 equiv of BOP, 1 equiv of HOBt, and 2 equiv of DIEA. Under these conditions, observed epimerization levels for two different fragment couplings run in DMF were <2.5%.[41] Slightly higher epimerization levels may be obtained when 15% DMSO in NMP is used as the coupling solvent; while the polarities of the two solvents are similar,[14] the reported epimerization level for one BOP-mediated fragment coupling was 25% higher when NMP was the coupling solvent than when DMF was used.[34] If the C-terminal residue is glycine, coupling yields can be improved by running the reaction at room temperature, omitting HOBt, and using 5 equiv of DIEA.

Conclusions

Solid-phase fragment coupling provides an alternative to stepwise synthesis for the preparation of "difficult" peptides or peptides for which a convergent synthetic route is desired. Sequences which have a tendency to aggregate are difficult to synthesize because they are prone to low yield coupling steps which result in the contamination of the final product with single deletion impurities. Synthesis of these sequences by fragment coupling can facilitate the purification of the final product. Impurities which are normally carried through to the end of a stepwise synthesis can be removed during the purification of the intermediates. In addition, the ability to detect synthetic impurities which result from side reactions allows reaction conditions to be adjusted in order to minimize these reactions.

The formation of aspartimides under the basic conditions used for coupling amino acids and fragments has been widely reported.[38,39,49,50] Aspartimides can be hydrolyzed during acidolytic deprotection and workup to yield two isomers: α-aspartyl and β-aspartyl,[45] which are indistinguishable by the commonly used techniques of mass spectrometry and amino acid analysis. In contrast, the formation of an aspartimide in a protected fragment is easily detected by mass spectrometry.

Solid-phase synthesis using the Kaiser oxime resin is one approach to the preparation of protected peptides for fragment coupling. The key advantage of the Kaiser oxime resin is the nucleophilic cleavage procedure, which allows the preparation of many related peptides from one batch of peptide resin. The variability in the cleavage adds further flexibility and convergence to the synthetic strategy.

There are still some difficulties associated with fragment synthesis using the Kaiser oxime resin. The formation of aspartimides during some of the cleavage procedures has hindered the preparation of Asp-containing peptides. This problem can be solved, however, by judicious choice of aspartate protection and reaction conditions and by careful monitoring of the cleavage reaction. Better activation methods and solvent systems have improved fragment coupling yields, but some couplings, especially those involving fragments of ≥15 amino acids, remain difficult.[33]

The potential advantages of an oxime-based fragment coupling approach are many. Structural studies of hydrophobic peptides such as transmembrane sequences and amyloid-forming peptides[51] require the preparation of pure peptide, labeled analogs, and sequence analogs. A convergent synthetic strategy utilizing the Kaiser oxime resin provides a means to do this. Further improvements in activation methods and solvent systems will serve to increase the utility of the Kaiser oxime resin. The modifications presented in this paper demonstrate some progress toward this goal. The viability of the reported methodology is demonstrated in the accompanying paper, which describes the preparation of a 25 amino acid protected fragment of the β-amyloid protein of Alzheimer's disease.[3]

Experimental Section

Equipment, Materials, and Methods. DIEA was distilled from ninhydrin under reduced pressure. Polystyrene oxime resin,[2,44,52] HOPip,[53] and amino acid tetra-*n*-butylammonium salts[28] were prepared according to published procedures. Hydrolyses of peptides were performed in HCl/propionic acid (1:1, v/v) at 130 °C for 3 h. Analytical reverse-phase HPLC was carried out using Waters Deltapak C_4 100-Å and 300-Å columns (3.9 mm × 30 cm). Semipreparative reverse-phase HPLC was carried out using Waters Deltapak C_4 300-Å columns (19 mm × 30 cm), and preparative reverse-phase HPLC was carried out using a Waters C_4 Prep-pak radial compression column (57 mm × 30 cm). Proton NMR were measured in DMSO-d_6. All coupling constants are reported in hertz.

General Procedure for the Synthesis of Protected Peptides. Synthesis of Protected Peptides. The resin-bound Boc-amino acid[1] (ca. 0.4 mmol/g) was placed in a reaction vessel and swollen with methylene chloride (15 mL/g of resin). Acetic anhydride (10 equiv) and diisopropylethylamine (DIEA, 5 equiv) were added. The mixture was shaken for 6–12 h at 23 °C.

The following procedure was used for the addition of each amino acid: (1) wash resin with CH_2Cl_2 (15 mL/g of resin, 2 × 1 min), (2) wash with 25% trifluoroacetic acid (TFA) in CH_2Cl_2 (20 mL/g, 1 min), (3) shake resin with 25% TFA in CH_2Cl_2 (20 mL/g, 30 min), (4) wash with CH_2Cl_2 (15 mL/g, 2 × 1 min), (5) wash with 2-propanol (15 mL/g, 2 × 1 min), (6) wash with CH_2Cl_2 (15 mL/g, 2 × 1 min), (7) wash with 2-propanol (15 mL/g, 2 × 1 min), (8) wash with CH_2Cl_2 (15 mL/g, 4 × 1 min), (9) remove a small amount of resin and perform the Kaiser test for free amine[54] (the test should be positive (blue)), (10) wash with DMF (15 mL/g, 2 × 1 min), (11) add Boc-amino acid (3 equiv, 0.1 M in DMF) and BOP reagent (3 equiv, 0.1 M in DMF), shake resin for 30 s, (12) add DIEA (5.3 equiv), shake mixture for 1 h at 23 °C, (13) wash with DMF (15 mL/g, 2 × 1 min), (14) wash with CH_2Cl_2 (15 mL/g, 3 × 1 min), (15) wash with 2-propanol (15 mL/g, 2 × 1 min), (16) wash with CH_2Cl_2 (15 mL/g, 3 × 1 min), (17) remove a small amount of resin and perform the Kaiser test for free amine:[54] if positive (blue) then repeat steps 10–17 until a negative (yellow) result is obtained.

Boc-D(aspartimide)AE(Bzl)FR(NO_2)H(Bom)D(Bzl)S(Bzl)G-OH. Protected Boc-D(Bzl)AE(Bzl)FR(NO_2)H(Bom)D(Bzl)S(Bzl)-resin was synthesized according to the standard procedure and cleaved with the tetra-*n*-butylammonium salt of glycine according to our previously published procedure.[28] The combined filtrate from the cleavage was evaporated to a solid residue and dissolved in DMF. The solution was purified by HPLC [semiprep: 57/43 H_2O/2:1 2-propanol:CH_3CN, (0.5% acetic acid), R_v = 255 mL]. FABMS: 1550 (calcd 1657.7, 1550 corresponds to M – ($CH_2C_6H_5$ + H_2O)). Tandem FAB-collision MS sequencing[37] indicated an aspartimide at position 1.

Boc-D(tBu)AE(Bzl)FR(Mts)H(Bom)D(cHex)S(Bzl)G-OH. Boc-D(tBu)AE(Bzl)FR(Mts)H(Bom)D(cHex)S(Bzl)-resin was synthesized according to a modified standard procedure (5 equiv of BOP, 5 equiv of aa, and 8.9 equiv of DIEA used for each coupling). An acetylation step (10 equiv of acetic anhydride, 5 equiv of DIEA) was performed after each coupling. The peptide was cleaved with the tetra-*n*-butylammonium salt of glycine.[28] The combined filtrate from the cleavage was evaporated to a solid residue and dissolved in DMF. The solution was purified by HPLC [prep: 37/63 H_2O/CH_3CN, (0.1% TFA), R_v = 1040 mL].

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FABMS: 1753.1 $(M + H)^+$ (calcd 1752.8). Amino acid analysis: D 2.0 (2), A 1.0 (1), E 1.0 (1), F 1.2 (1), R 1.1 (1), H 1.2 (1), S 0.5 (1), G 2.1 (1). Yield: 45% based on Gly-resin.

Boc-H(Bom)QK(Cl-Z)L-OH. Boc-H(Bom)QK(Cl-Z)L-resin was synthesized according to the standard procedure, cleaved with HOPip, and reduced to the free acid.[27] The peptide was purified by HPLC [prep: 37/63 H_2O/CH_3CN, (0.1% TFA), R_v = 618 mL]. FABMS: 913.6 $(M + H)^+$ (calcd 912.4). Amino acid analysis: H 1.2 (1), Q 1.9 (1), K 0.9 (1), L 1.0 (1). Sequence was confirmed by tandem FAB-collision MS.[37] Yield: 63% based on Leu-resin.

Boc-VFFAE(Bzl)D(Bzl)-OH. Boc-VFFAE(Bzl)D(Bzl)-resin was synthesized using symmetric anhydride activation.[27] Double couplings were employed at every step. The peptide was cleaved with HOPip, reduced to the free acid,[27] and purified by HPLC [semiprep: 43/57 H_2O/CH_3CN (5% trifluoroethanol), (0.1% TFA), R_v = 375 mL]. FABMS: 1030 $(M + Na)^+$, 1042 $(M + 2Na - H)^+$ (calcd 1006.4). Amino acid analysis: V 1.0 (1), F 1.5 (2), A 1.1 (1), E 2.1 (1), D 2.4 (1). Sequence was confirmed by tandem FAB-collision MS.[37] Yield: 16% based on Asp-resin.

The same peptide was synthesized using the standard (BOP activation) procedure. The peptide was cleaved with HOPip, reduced to the free acid,[27] and purified by HPLC. FABMS: 1007 $(M + H)^+$, 1029 $(M + Na)^+$ (calcd 1006.4). Amino acid analysis: V 0.9 (1), F 2.0 (2), A 1.2 (1), E 1.0 (1), D 0.9 (1). Sequence was confirmed by tandem FAB-collision MS.[37] Yield: 66% based on Asp-resin.

Boc-VFFAE(Bzl)D(cHex)VG-OH. Boc-VFFAE(Bzl)D(cHex)VG-resin was synthesized using the standard procedure. An acetylation step (10 equiv of acetic anhydride, 5 equiv of DIEA, 30 min) was added after each coupling. The peptide was cleaved with HOPip, reduced to the free acid,[27] and purified by HPLC [semiprep: 15 mL/min 60/40 H_2O/CH_3CN, (0.1% TFA), 3 min isocratic followed by linear gradient to 30/70 (17 min)]. FABMS 1155 $(M + H)^+$ (calcd 1155). Yield: 15% based on Gly-resin.

The same peptide was synthesized using a modified standard procedure in which DMSO (15% v/v) in *N*-methylpyrrolidone was used as the coupling solvent. The peptide was cleaved with HOPip, reduced to the free acid,[27] and purified by HPLC. Yield: 45% based on Gly-resin. A truncated impurity was detected in the crude product: FABMS 998 (acetyl-FFAE(Bzl)D(cHex)VG-OH, calcd 997).

Boc-S(Bzl)D(*)S(Bzl)S(Bzl)L-OH. Three protected peptide-resins, Boc-S(Bzl)D(*)S(Bzl)S(Bzl)L-resin (* = benzyl, cyclohexyl, 2-adamantyl), were synthesized using the standard procedure, cleaved with HOPip, and reduced to the free acid.[27] The peptides were purified by HPLC [prep: 40/60 H_2O/CH_3CN, (0.1% TFA), R_v(Bzl) = 880 mL, R_v(cHex) = 1100 mL, R_v(Ada) = 1480 mL]. Boc-S(Bzl)D(Bzl)S(Bzl)S(Bzl)L-OH: amino acid analysis D 1.0 (1), S 1.9 (3), L 1.0 (1), FABMS 968.7 $(M + H)^+$ (calcd 968.5). ^1H NMR: 0.77 (d, 3 H, J = 8, LeuCH_3), 0.82 (d, 3 H, J = 8, LeuCH_3), 1.38 (s, 9 H, Boc), 1.45 (m, 2 H, LeuβH), 1.57 (m, 1 H, LeuγH), 2.63 (dd, 1 H, J = 17, 9, AspβH), 2.84 (dd, 1 H, J = 17, 9, AspβH), 3.6 (m, 6 H, SerβH), 4.25 (m, 2 H), 4.45 (m, 6 H, Ser(Bzl)), 4.58 (m, 2 H), 4.77 (m, 1 H), 5.05 (s, 2 H, Asp(Bzl)), 6.90 (d, 1 H, J = 9, Ser NH), 7.2–7.4 (m, 20 H, Bzl), 8.06 (m, 2 H, NH), 8.18 (d, 1 H, J = 8, NH), 8.36 (d, 1 H, J = 8, NH), 12.6 (br s, 1 H, CO_2H). Boc-S(Bzl)D(cHex)S(Bzl)S(Bzl)L-OH: amino acid analysis D 1.6 (1), S 1.6 (3), L 1.0 (1), FABMS 960.9 $(M + H)^+$ (calcd 960.5). ^1H NMR: 0.77 (d, 3 H, J = 6, LeuCH_3), 0.82 (d, 3 H, J = 6, LeuCH_3), 1.1–1.8 (br m, 10 H, cyclohexyl + LeuγH), 1.38 (s, 9 H, Boc), 1.45 (m, 3 H, LeuβH), 2.57 (m, 1 H, AspβH), 2.78 (m, 1 H, DβH), 3.6 (m, 6 H, SerβH), 4.2 (m, 2 H), 4.40 (m, 6 H, Ser(Bzl)), 4.60 (m, 2 H), 4.70 (m, 2 H), 6.88 (d, 1 H, J = 8, SerNH), 7.3 (m, 15 H, Ser(Bzl)), 8.04 (m, 2 H, NH), 8.18 (d, 1 H, J = 8, NH), 8.30 (d, 1 H, J = 8, NH). Boc-S(Bzl)D(Ada)S(Bzl)S(Bzl)L-OH: FABMS 1012.8 $(M + H)^+$ (calcd 1012.6).

Aspartimide Formation in the Presence of Glycine Salt. Each of the Boc-S(Bzl)D(*)S(Bzl)L-OH peptides (5 μmol) was dissolved in DMF (200 μL). Glycine salt (3 equiv) in DMF (200 μL) was added and the solution was stirred. An aliquot (10 μL) of the reaction mixture was removed at 0, 2, 5, 10, 25, and 60 min and analyzed by HPLC [analytical: 2 mL/min 40/60 H_2O/CH_3CN, (0.1% TFA), 2 min isocratic followed by linear gradient to 30/70 (5 min), R_v(aspartimide) = 11.0 mL, R_v(Bzl) = 14.0 mL, R_v(cHex) = 20.5 mL, R_v(Ada) = 28.5 mL], and the peak areas were integrated. FABMS of aspartimide: 860.1 (calcd 859.5).

Aspartimide Formation during Cleavage of Boc-S(Bzl)-D(cHex)S(Bzl)S(Bzl)L-resin with Glycine Salt. Boc-S(Bzl)D(cHex)S(Bzl)S(Bzl)L-resin (100 mg, 3 μmol) was stirred with glycine salt (3 equiv) in DMF (1 mL). An aliquot (30 μL) of the reaction mixture was removed at 0, 1, 2, 5, 10, 26, 83, and 128 min and quenched in acetic acid (20 μL). Resin was removed and 10 μL of the supernatant was analyzed by HPLC [analytical: 2 mL/min 60/40 H_2O/CH_3CN, (0.1% TFA), linear gradient to 10/90 (15 min), R_v(aspartimide) = 20 mL, R_v(cHex) = 25 mL]. Peak areas were integrated and plotted as a percentage of the total peak area at 128 min (see Figure 2). Amino acid analysis of the cleaved resin after 20 min indicated >99% cleavage. Products were characterized by FABMS: cHex, 1017.0 (calcd 1016.5); aspartimide, 917.1 (calcd 916.5).

Aspartimide Formation during Cleavage of Boc-AGS-(Bzl)D(cHex)S(Bzl)S(Bzl)L-resin with Threonine(*O*-benzyl) Salt. Boc-AGS(Bzl)D(cHex)S(Bzl)S(Bzl)L-resin (200 mg, 22 μmol) was stirred with threonine(Bzl) salt (3 equiv) in DMF (2 mL). An aliquot (30 μL) of the reaction mixture was removed at 0, 2, 5, 10, 20, 30, 60, 230, 480, and 1320 min and quenched in acetic acid (20 μL). Resin was removed and 10 μL of the supernatant was analyzed by HPLC [analytical: 2 mL/min 60/40 H_2O/CH_3CN, (0.1% TFA), linear gradient to 10/90 (15 min), R_v(aspartimide) = 21 mL, R_v(cHex) = 26.4 mL]. Peak areas were integrated and plotted as a percentage of the total peak area at 1320 min (see Figure 3). Amino acid analysis of the cleaved resin after 120 min indicated 80–85% cleavage. Products were characterized by FABMS: cHex 1280.1 (calcd 1279.6), aspartimide 1180.0 (calcd 1179.5).

Epimerization during Cleavage of Boc-S(Bzl)T(Bzl)QT-(Bzl)A-resin with Leucine Salt. Boc-S(Bzl)T(Bzl)QT(Bzl)A-resin was synthesized using the standard procedure. Epimerized standards were prepared in the following manner: Boc-S(Bzl)-T(Bzl)QT(Bzl)(D/L)A-resin was synthesized using racemic alanine. Boc-S(Bzl)T(Bzl)QT(Bzl)A-resin was stirred with D-leucine salt and Boc-S(Bzl)T(Bzl)QT(Bzl)(D/L)A-resin was stirred with L-leucine salt. After 12 h the resin was removed and 20 μL of the supernatant analyzed by HPLC. Boc-S(Bzl)T(Bzl)QT(Bzl)A-resin (500 mg, 150 μmol) was stirred with leucine tetra-*n*-butylammonium salt (2 equiv) in DMF (1 mL) at rt. After 12 h the resin was removed and 20 μL of the supernatant analyzed by HPLC [YMC-ODSAQ column (4.7 × 150 mm), 1 mL/min, 50/50 H_2O/CH_3CN, (0.1% TFA), R_v(S(Bzl)T(Bzl)QT(Bzl)AL) = 19 mL, R_v(S(Bzl)T(Bzl)QT(Bzl)DAL) = 20 mL, R_v(S(Bzl)T(Bzl)QT-(Bzl)ADL) = 20.5 mL]. The amount of epimerized peptide was measured by integrating peak areas: Boc-STQTDAL (<1%), Boc-STQTADL (<1%).

Preparation of Peptide C-Terminal Amides. Methylamine Cleavage of Resin-Bound Boc-D(Bzl)GGG. Boc-D(Bzl)-GGG-resin was synthesized using the standard procedure. The resin (75 mg, 30 μmol) was suspended in DMF (600 μL) and treated with CH_3NH_2·HCl (10.2 mg, 150 μmol) and DIEA (10 μL, 60 μmol). In addition, naphthalene was added to the reaction as an HPLC standard. This reaction was carried out at 22 and at −2 °C. In both cases, aliquots (20 μL) were removed at various time points and quenched into 15 μL of acetic acid. The aliquots were analyzed by HPLC [analytical: 90/10 H_2O/CH_3CN (0.1% AcOH) linear gradient to 15/85 over 14 min]. Two peaks were observed. The first (R_v = 8.0 mL) was identified as Boc-D(aspartimide)GGG-$NHCH_3$ (FABMS 400 $(M + H)^+$ (calcd 399.2)); the second (R_v = 16.8 mL) was identified as Boc-D(Bzl)GGG-$NHCH_3$ (FABMS 508 $(M + H)^+$ (calcd 507.3)). For determination of the amounts of the two compounds, peak heights at 225 nm were measured and both peaks were scaled to the naphthalene standard. In addition, the early peak (Boc-D(aspartimide)-GGG-$NHCH_3$) was multiplied by 2 as a width factor (this corresponds to the ratio of the two peaks' widths at half-height), and the second peak (Boc-D(Bzl)GGG-$NHCH_3$) was multiplied by 0.91 to compensate for the additional absorbance at 225 nm of the benzyl group. In this way, the amounts (adjusted peak heights) of the aspartimide and the fully protected peptide were obtained at each time point. The cleavage was assumed to be complete when the sum of these values no longer changed.

Ac-(β)D(Bzl)VG-NH_2. Ac-(β)D(Bzl)VG-resin was synthesized using the standard procedure incorporating *N*-Boc-aspartic acid

α-benzyl ester followed by deprotection (25% TFA, 30 min) and acetylation (acetic anhydride (10 equiv) and DIEA (5 equiv)). The peptide-resin (500 mg, 135 μmol) was stirred with ammonium acetate (10 equiv) and DIEA (10 equiv) in DMF (5 mL) for 15 h. The ammonium acetate never completely dissolves, and a white solid remains at the end of the cleavage. Amino acid analysis of the resin indicated >97% cleavage. The peptide was purified by HPLC [semiprep: 75/25 H_2O/CH_3CN, (0.1% TFA), R_v = 195 mL] to give 92 μmol of pure peptide (68% yield). Amino acid analysis: D 1.1 (1), V 0.8 (1), G 1.1 (1). ^1H NMR: 0.81 (d, 3 H, J = 6, ValCH_3), 0.82 (d, 3 H, J = 6, ValCH_3), 1.82 (s, 3 H, acetyl), 1.97 (m, 1 H, ValβH), 2.65 (d, 2 H, J = 7, AspβH), 3.58 (dd, 1 H, J = 18, 6, GlyαH), 3.66 (dd, 1 H, J = 18, 6, GlyαH), 4.11 (dd, 1 H, J = 8, 7, ValαH), 4.59 (dt, 1 H, J = 7, 7, AspαH), 5.09 (s, 2 H, Asp(Bzl)), 7.02 (s, 1 H, amide NH), 7.18 (s, 1 H, amide NH), 7.35 (m, 5 H, Asp(Bzl)), 8.01 (d, 1 H, J = 9), 8.11 (t, 1 H, J = 6, GlyNH), 8.22 (d, 1 H, J = 8).

Boc-S(Bzl)T(Bzl)QT(Bzl)A-NH_2. Boc-S(Bzl)T(Bzl)QT-(Bzl)A-resin was synthesized using the standard procedure. The peptide-resin (500 mg, 150 μmol) was stirred with ammonium acetate (10 equiv) and DIEA (10 equiv) in DMF (1 mL). The peptide-resin Boc-S(Bzl)T(Bzl)QT(Bzl)(D/L)A-resin (from above) was treated in the same manner. After 12 h, resin was removed and the supernatant were analyzed by HPLC [YMC-ODSAQ column (4.7 × 150 mm), 50/50 H_2O/CH_3CN, (0.1% TFA), R_v-(Boc-S(Bzl)T(Bzl)QT(Bzl)A-NH_2) = 15.0 mL, R_v(Boc-S(Bzl)T-(Bzl)QT(Bzl)DA-NH_2) = 15.9 mL]. The amount of Boc-S(Bzl)-T(Bzl)QT(Bzl)DA-NH_2, determined by integration of peak areas, was <1%. Boc-S(Bzl)T(Bzl)QT(Bzl)A-NH_2 was purified by HPLC [semiprep: 50/50 H_2O/CH_3CN, (0.1% TFA), R_v = 212 mL] to give 83 μmol of pure peptide (55% yield). Amino acid analysis: Q 0.4 (1), S 1.1 (1), T 2.0 (2), A 1.4 (1). ^1H NMR: 1.01 (d, 3 H, J = 6.5, ThrCH_3), 1.05 (d, 3 H, J = 6.5, ThrCH_3), 1.22 (m, 3 H, J = 8, AlaCH_3), 1.36 (s, 9 H, Boc), 1.7–1.9 (m, 2 H, GlnβH), 2.15 (m, 2 H, GlnγH), 3.5–3.7 (m, 2 H), 3.9 (m, 2 H), 4.25 (m, 2 H), 4.3–4.6 (m, 8 H, Ser(Bzl), Thr(Bzl) and αH's), 6.77 (s, 1 H), 7.09 (s, 1 H), 7.13 (d, 1 H, J = 9, SerNH), 7.2–7.4 (m, 17 H), 7.75 (d, 1 H, J = 8), 7.81 (d, 1 H, J = 7.5), 8.06 (d, 1 H, J = 9), 8.12 (d, 1 H, J = 8).

Ammonium Acetate Cleavage: Aspartimide Formation. Ac-Y(Cl_2Bzl)D(Bzl)LT(Bzl)S(Bzl)-resin and Ac-D(Bzl)GF-resin were synthesized using the standard procedure. Results of the next four experiments are summarized in table I.

YDLTS (high aspartimide conditions). Ac-Y(Cl_2Bzl)D-(Bzl)LT(Bzl)S(Bzl)-resin (2.0 g, 0.8 mmol) was suspended in DMF (30 mL), treated with NH_4OAc (1.23 g, 16 mmol) and DIEA (1.4 mL, 8 mmol), and stirred for 18.5 h at 22 °C. The suspension was filtered, the resin was washed with DMF, CH_2Cl_2, and MeOH, and the washes were concentrated. The product was purified by HPLC [semiprep: 43/57 H_2O/CH_3CN (0.1% AcOH)], yielding 17% Ac-Y(Cl_2Bzl)D(aspartimide)LT(Bzl)S(Bzl)-NH_2 (R_v = 90 mL; FABMS 959 $(M + H)^+$ (calcd 958)) and 83% Ac-Y-(Cl_2Bzl)D(Bzl)LT(Bzl)S(Bzl)-NH_2 (R_v = 150 mL; FABMS 1067.4 $(M + H)^+$ (calcd 1066.4)).

YDLTS (low aspartimide conditions). Ac-Y(Cl_2Bzl)D-(Bzl)LT(Bzl)S(Bzl)-resin (3.0 g, 1.2 mmol) was suspended in DMF (50 mL), treated with NH_4OAc (925 mg, 12 mmol) and DIEA (612 μL, 3.6 mmol), and stirred for 15.5 h at 4 °C. The suspension was filtered, the resin was washed, and the washes were concentrated. This material was then hydrogenated at ~55 PSI, using a 10% Pd/C catalyst, to remove the protecting groups. The product was purified by HPLC [semiprep: 87/13 H_2O/CH_3CN (0.1% AcOH)], yielding Ac-YDLTS-NH_2 (R_v = 132 mL; FABMS 639.0 $(M + H)^+$ (calcd 638.3)). A later peak (R_v = 177 mL) constituted <10% of the crude product; no other significant peaks were observed. Cleavage yield measured by amino acid analysis of the resin before and after cleavage was 88–95%.

DGF (high aspartimide conditions). Ac-D(Bzl)GF-resin (~0.25 mmol) was suspended in DMF (7.5 mL), treated with NH_4OAc (385 mg, 5 mmol) and DIEA (128 μL, 0.75 mmol), and stirred for 12 h at 22 °C. The suspension was filtered, the resin was washed, and the washes were concentrated. This material was then hydrogenated at ~45 PSI, using a 10% Pd/C catalyst, to remove the protecting groups. The product was purified by HPLC [semiprep: 87/13 H_2O/CH_3CN (0.1% AcOH)], yielding Ac-DGF-NH_2 (R_v = 48 mL; FABMS 379.1 $(M + H)^+$ (calcd 378.2); 3%) and Ac-D(aspartimide)GF-NH_2 (R_v = 108 mL; FABMS 361.0 $(M + H)^+$ (calcd 360.1); 97%).

DGF (low aspartimide conditions). Ac-D(Bzl)GF-resin (~0.25 mmol) was suspended in CH_2Cl_2 (7 mL), treated with NH_4OAc (385 mg, 5 mmol) and DIEA (42 μL, 0.25 mmol), and stirred for 16 h at 5 °C. The reaction was filtered, the resin was washed, and the washes were concentrated. This material was then hydrogenated at ~45 PSI, using a 10% Pd/C catalyst, to remove the protecting groups. A portion of this material was purified as above to give 42% of Ac-D(Bzl)GF-NH_2 and 58% of Ac-D(aspartimide)GF-NH_2.

Cleavage of Peptide from Resin in LiBr/THF. Boc-VFAE(Bzl)D(Bzl)-resin (0.48 g) was suspended in LiBr (1 M) in THF and stirred for 15 h. The suspension was filtered to remove resin and the resin was washed with LiBr (0.1 M) in THF, THF, DMF, 2-propanol, and CH_2Cl_2. A control sample of resin was suspended in DMF for 15 h and washed with DMF, 2-propanol, and CH_2Cl_2. The resins were hydrolyzed for amino acid analysis. Control: V 0.37 mmol/g, F 0.28, A 0.43, E 0.32, D 0.23. LiBr/THF: V 0.31, F 0.25, A 0.31, E 0.21, D 0.14. The combined filtrate from the LiBr/THF sample was analyzed by HPLC. The product coeluted with Boc-VFAE(Bzl)D(Bzl)-OH.

Test for Epimerization during BOP-Mediated Fragment Coupling. V-resin (0.5 g, 0.2 mmol) was deprotected with TFA (25% in CH_2Cl_2, 30 min), neutralized (5% DIEA in CH_2Cl_2, 3 × 1 min), and coupled with Boc-H(Bom)QK(Cl-Z)L-OH (20 mg, 0.02 mmol) using BOP (0.03 mmol), HOBt (0.02 mmol), and DIEA (0.04 mmol) in DMF (2 mL) for 14 h. The resin was washed with DMF and 2-propanol. A portion of the resin (50 mg) was placed in a test tube with HOPip (20 mg) and DMF (1 mL) and was stirred at room temperature for 2 h. Boc-H(Bom)QK(Cl-Z)-DLV-resin was synthesized by standard methods. A portion of the resin (50 mg) was placed in a test tube with HOPip (20 mg) and DMF (1 mL) and was stirred at room temperature for 2 h. Aliquots (20–50 μL) of the supernatants were removed and analyzed by HPLC [analytical: 60/40 H_2O/CH_3CN, (0.1% TFA), R_v(Boc-HQKLV-OPip) = 26 mL; R_v(Boc-HQKDLV-OPip) = 32 mL]. Integration of the HQKDLV-OPip peak in the HPLC trace indicates 2% epimerization during coupling.

Comparison of Fragment Coupling Solvents. Boc-LMVGGVVIA-resin[42] was deprotected with TFA (25% in CH_2Cl_2, 30 min), neutralized (5% DIEA in CH_2Cl_2, 3 × 1 min), and suspended in four different solvents: DMF, $NaClO_4$ (0.4M) in DMF, KSCN (0.4M) in DMF, and DMSO (15% v/v) in NMP. Boc-S(Bzl)NK(Cl-Z)GAIIG-OH[42] (1 equiv), BOP (2 equiv), HOBt (1.4 equiv), and DIEA (5 equiv) were added to each sample. After 24 h, the resins were filtered, washed, dried, and hydrolyzed for amino acid analysis. Coupling yields are derived from the ratios of Asn and Lys to Leu and Met. The yields were 40–45% for DMF, 30–35% for $NaClO_4$/DMF, 20–25% for KSCN/DMF, and 55–65% for DMSO/NMP.

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