Carbohydrate Protease Conjugates: Stabilized Proteases for Peptide Synthesis

Charles A. Wartchow,*,† Peng Wang,‡ Mark D. Bednarski,‡ and Matthew R. Callstrom†

Department of Chemistry, The Ohio State University, Columbus, Ohio 43210 and the Center for Advanced Materials, Lawrence Berkeley Laboratories, Berkeley, California 94720

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The synthesis of oligopeptides using stable carbohydrate protease conjugates (CPCs) was examined in acetonitrile solvent systems. CPC[α-chymotrypsin] was used for the preparation of peptides containing histidine, phenylalanine, tyrosine, and tryptophan in the P_1 position in 60–93% yield. The CPC[α-chymotrypsin]-catalyzed synthesis of octamer Z-Gly-Gly-Phe-Gly-Gly-Phe-Gly-Gly-OEt from Z-Gly-Gly-Phe-Gly-Gly-Phe-OMe was achieved in 71% yield demonstrating that synthesis was the dominant kinetic process relative to amide hydrolysis. CPC[papain] was used to synthesize peptides containing both hydrophilic and hydrophobic amino acids. The P_2 specificity of papain for aromatic residues was utilized for the 2 + 3 coupling of Z-Tyr-Gly-OMe to H_2N-Gly-Phe-Leu-OH to generate the leucine enkephalin derivative in 79% yield. Although papain is nonspecific for the hydrolysis of *N*-benzyloxycarbonyl amino acid methyl esters in aqueous solution, the rates of synthesis for these derivitives with nucleophile leucine *tert*-butyl ester differed by nearly 2 orders of magnitude. CPC[thermolysin] was used to prepare the aspartame precursor Z-Asp-Phe-OMe in 90% yield. The increased stability of CPCs prepared from periodate-modified poly(2-methacrylamido-2-deoxy-D-glucose), poly(2-methacrylamido-2-deoxy-D-galactcose), and poly(5-methacrylamido-5-deoxy-D-ribose), carbohydrate materials designed to increase the aldehyde concentration in aqueous solution, suggests that the stability of CPCs is directly related to the aldehyde concentration of the carbohydrate material. Periodate oxidation of poly(2-methacrylamido-2-deoxy-D-glucose) followed by covalent attachment to α-chymotrypsin gave a CPC with catalytic activity in potassium phosphate buffer at 90 °C for 2 h.

Introduction

The protease-catalyzed synthesis of peptides and proteins for structure/function studies or for the preparation of amino acid-based materials could prove to be a useful alternative to established methods such as site-directed mutagenesis,[1] unnatural amino acid mutagenesis,[2] and the expression of artificial genes.[3] Peptides and proteins are prepared by solid phase or solution phase methods[4,5] and by expression in a foreign host, such as bacteria or yeast. However, these methods can be difficult or impossible due to inefficient peptide or amino acid couplings during chemical synthesis, problems associated with the expression of soluble proteins in a foreign host, and difficulties in the purification of the target peptides and proteins. In contrast, methodology employing the enzymatic ligation of oligopeptides routinely prepared through solid or solution phase methods potentially eliminates or reduces these problems. The combined chemical and enzymatic synthesis of proteins has recently been demonstrated for the synthesis of RNase A,[6] and promising results have been obtained for the synthesis of oligopeptides.[7,8]

A representative protease-catalyzed synthesis of a tripeptide is shown in Scheme 1 for the α-chymotrypsin (CT)-catalyzed preparation of Z-Tyr-Gly-Gly-OEt (Z, benzyloxycarbonyl; Tyr, tyrosine; Gly, glycine; Et, ethyl). After the binding of the substrate to the protease (not shown), a covalent acyl-enzyme intermediate is formed between P_1 substrate[9] Z-Tyr-OMe and active site serine-195 resulting in the loss of methanol from the substrate. The peptide bond is formed when nucleophile glycylglycine ethyl ester (H_2N-Gly-Gly-OEt) adds to the acyl-enzyme intermediate to generate the tripeptide and regenerate the hydroxyl side chain of serine-195. Alternatively, water can add to the acyl-enzyme intermediate to generate unreactive *N*-(benzyloxycarbonyl)tyrosine.

Protease-catalyzed peptide synthesis has been investigated in both aqueous solutions and organic solvent systems for the thermodynamic and kinetic synthesis of peptides.[8,10,11] The advantages of protease-catalyzed peptide synthesis are established and include the ability

† Center for Advanced Materials, Lawrence Berkeley Laboratories, Berkeley, CA 94720.

‡ Department of Chemistry, The Ohio State University, Columbus, OH 43210.

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(1) Hedstrom, L.; Graf, L.; Stewart, C.-B.; Rutter, W. J.; Phillips, M. A. In *Methods in Enzymology*; Langone, J. J., Ed.; Academic Press, Inc.: San Diego, 1991; Vol. 202; pp 671-687.

(2) Ellman, J.; Mendel, D.; Anthony-Cahill, S.; Noren, C. J.; Schultz, P. G. In *Methods in Enzymology*; Langone, J. J., Ed.; Academic Press: San Diego, 1991; Vol. 202; pp 301–336.

(3) McGrath, K. P.; Fournier, M. J.; Mason, T. L.; Tirrell, D. A. *J. Am. Chem. Soc.* **1992**, *114*, 727–733.

(4) Jones, J. *The Chemical Synthesis of Peptides*; Clarendon Press: Oxford, 1991.

(5) Kaiser, E. T. *Acc. Chem. Res.* **1989**, *22*, 47–54.

(6) Jackson, D. Y.; Burnier, J.; Quan, C.; Stanley, M.; Tom, J.; Wells, J. A. *Science* **1994**, *266*, 243–247.

(7) Hwang, B. K.; Gu, Q.-M.; Sih, C. J. *J. Am. Chem. Soc.* **1993**, *115*, 7912–7913.

(8) Kullman, W. *Enzymatic Peptide Synthesis*; CRC Press: Boca Raton, 1987.

(9) The notation P_1, P_2, P_1', etc. refers to the location of a particlular amino acid residue occupying a subsite of a protease. The P_1 residue contains the carboxyl group of a cleaved peptide bond, and the P_2 residue is at the amino terminus of the P_1 residue. The P_1' residue contains the amino group generated during the hydrolysis of a peptide bond. In an analogous manner, this notation is also used in reference to peptide synthesis. See Schechter, I.; Berger, A. *Biochem. Biophys. Res. Commun.* **1967**, *27*, 157.

(10) Wong, C.-H.; Chen, S.-T.; Hennen, W. J.; Bibbs, J. A.; Wang, Y.-F.; Liu, J. L.-C.; Pantoliano, M. W.; Whitlow, M.; Bryan, P. N. *J. Am. Chem. Soc.* **1990**, *112*, 945–953.

(11) Schellenberger, V.; Jakubke, H.-D. *Angew. Chem. Int. Ed. Engl.* **1991**, *30*, 1437–1449.

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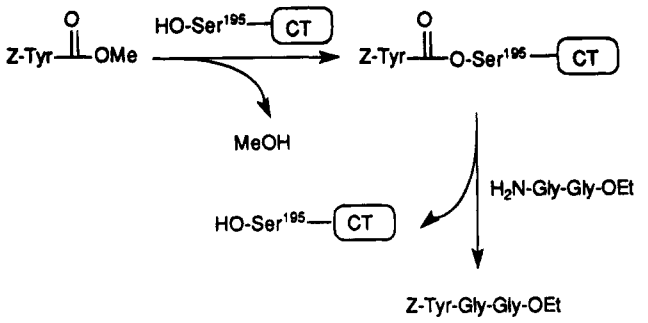
Scheme 1



of the protease to effect highly regio- and stereoselective transformations in the absence of side chain protecting groups.[8,12-14] However, general methodology for the enzymatic synthesis of oligopeptides in organic solvents has not been achieved.

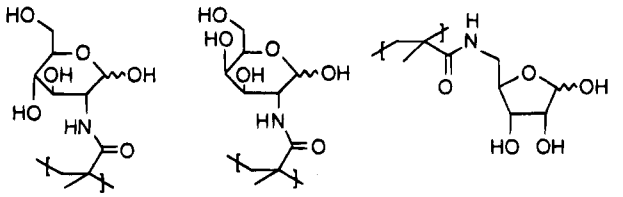
The use of organic solvents is often necessary for the reduction of the hydrolysis of peptide precursors in favor of peptide ligation[12,15,16] and also for the solubility of peptide precursors.[12] However, a limitation of the use of proteases for peptide synthesis is the instability of the enzymes in the presence of organic solvents.[12] In order to provide stability for proteases, we have developed a series of carbohydrate-based materials that can be covalently attached to the surface of proteins by reductive amination to generate carbohydrate protease conjugates (CPCs) which catalyze peptide synthesis in organic solvent systems (Scheme 2).[17,18] This mild, multisite attachment does not affect the activity of proteases, as determined by measuring the Michaelis–Menten parameters k_{cat} and K_M for chymotrypsin, subtilisin BPN', and trypsin in aqueous solution.[17] We have previously demonstrated that CPCs are efficient catalysts for the preparation of peptides in organic solvents and are remarkably stable in aqueous solution at elevated temperatures.[17]

Successful protease-catalyzed peptide synthesis requires protease stability and favorable kinetics for peptide ligation relative to amide hydrolysis and ester hydrolysis. Additionally, many proteases have specificity for certain amino acid side chains, and the direct application of the known specificity for substrate hydrolysis in aqueous solution to peptide synthesis in organic solvent systems is desirable. In order to address these important issues and to develop efficient strategies for the preparation of peptides and proteins through the enzymatic ligation of peptide oligomers, we report the development of a small library of stable CPC[proteases] and their use as catalysts for the synthesis of peptides in acetonitrile solvent systems. After determining the P_1 selectivity of the CPCs in acetonitrile, CPCs were used for the preparation of a leucine enkephalin derivative and a peptide octamer containing potential sites for amide and ester hydrolysis.

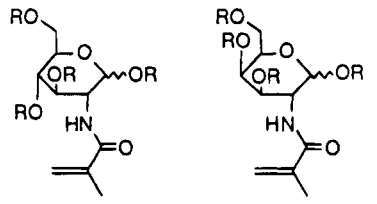
Results and Discussion

Preparation of CPCs. As part of our strategy to develop stabilized enzymes for peptide bond formation, we explored a series of materials that we expected to differ in the concentration of free aldehyde available for reaction with the ϵ-amine of lysine residues on the surface of enzymes. Previous studies have shown that the stability of trypsin attached to aldehyde-containing agarose was directly related to the aldehyde content of the agarose support.[19] In order to determine if the concentration of free aldehyde available from the carbohydrate based macromolecule could be a limiting factor for CPC stability, we prepared galactose (Gal)-based material **2** and ribose (Rib)-based material **3**. In addition, the glucose-based material was chemically modified by periodate oxidation to increase the aldehyde concentration of the material. The concentration of the open chain aldehyde form of D-ribose is approximately 25 times that of D-glucose and that of D-galactose is 10 times that of D-glucose.[20] This observation is consistent with the rates of tritium exchange at C2 of the carbohydrate, which must occur through the enol tautomer. D-Ribose exchanges tritium 28 times faster than D-glucose.[21]

Glucose-, galactose-, and ribose-based methacrylamide materials **1**–**3** were prepared from the corresponding methacrylamide-based monomers by reaction of the amino deoxy carbohydrates with methacryloyl chloride as illustrated for the preparation of poly(2-methacrylamido-2-deoxy-D-glucose) (**1**) in Scheme 3. Glucose-based monomer **4** and galactose-based monomer **6** were characterized as the trimethylsilyl derivatives **5** and **7**.



1 **2** **3**



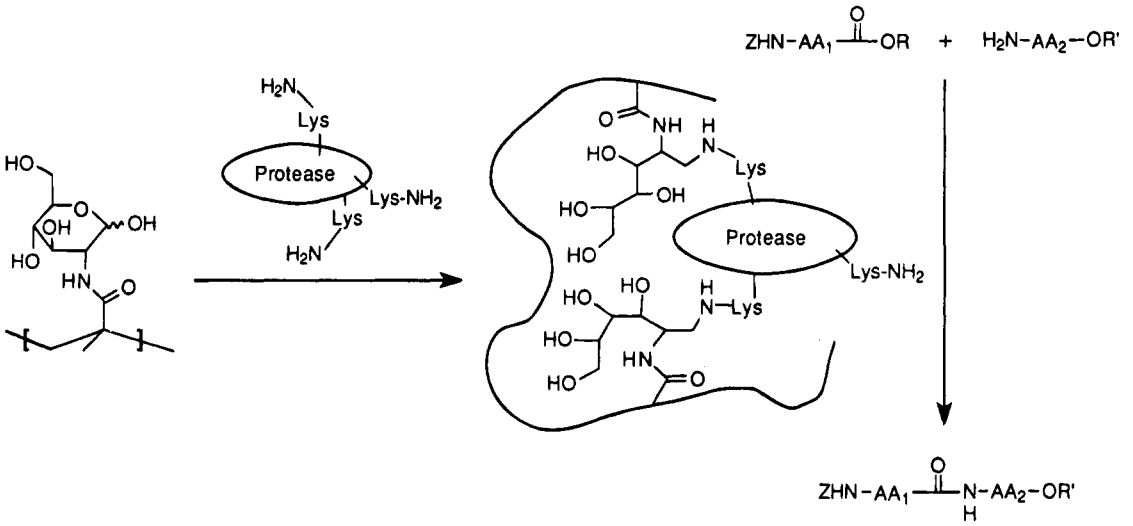
4 R = H
5 R = TMS

6 R = H
7 R = TMS

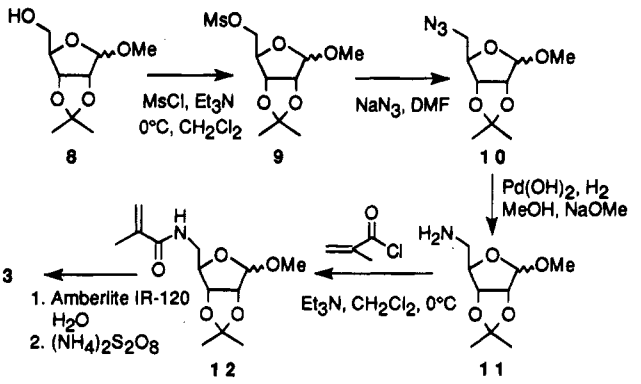
The ribose-based material **3** was prepared from D-ribose according to Scheme 3. Methyl 2,3-*O*-isopropylidene-D-ribofuranoside (**8**) was converted to mesylate **9**, followed by displacement with azide to give **10**. The azide

(12) Wong, C.-H.; Wang, K.-T. *Experientia* **1991**, *47*, 1123–1129.
(13) Fruton, J. S. *Adv. Enzymol. Relat. Areas Mol. Biol.* **1982**, *53*, 239–306.
(14) Bielka, H.; Scheler, W. *Biomed. Biochim. Acta* **1991**, *50*, S4-S216.
(15) Barbas, C. F.; Matos, J. R.; West, J. B.; Wong, C.-H. *J. Am. Chem. Soc.* **1988**, *110*, 5162–5166.
(16) Bibbs, J. A.; Zhong, Z.; Wong, C.-H. In *Materials Research Society Symposium Proceedings*; Rieke, P. C., Calvert, P. D., Alper, M., Eds.; Materials Research Society: Pittsburg, PA, 1990; Vol. 174; pp 223–228.
(17) Wang, P.; Hill, T. G.; Wartchow, C. W.; Huston, M. E.; Oehler, L. M.; Smith, M. B.; Bednarski, M. D.; Callstrom, M. R. *J. Am. Chem. Soc.* **1992**, *114*, 378–382.
(18) Hill, T. G.; Wang, P.; Huston, M. E.; Wartchow, C. A.; Oehler, L. M.; Smith, M. B.; Bednarski, M. D.; Callstrom, M. R. *Tetrahedron Lett.* **1991**, *32*, 6823.
(19) Blanco, R. M.; Calvette, J. J.; Guisán, J. M. *Enzyme Microb. Technol.* **1989**, *11*, 353–366.
(20) Hayward, L. D.; Angyal, S. *J. Carbohydrate Res.* **1977**, *53*, 13–20.
(21) Isbell, H. S.; Frush, H. L.; Wade, C. W. R.; Hunter, C. E. *Carbohydrate Res.* **1969**, *9*, 163–175.

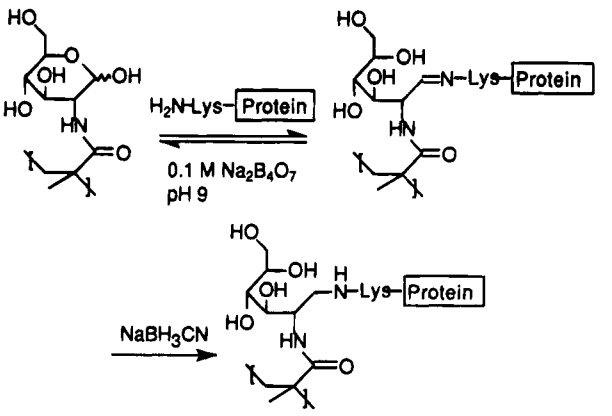
Scheme 2



Scheme 3



Scheme 4



was reduced to the amine **11** by hydrogenation and the amine was subsequently treated with methacryloyl chloride to give the protected monomer **12**. Removal of the isopropylidene and the methyl glycoside protecting groups with acidic resin afforded the highly reactive monomer 5-deoxy-5-methacrylamido-D-ribofuranose, which was not stable as a concentrated solution at room temperature. The deprotected monomer was polymerized in aqueous solution using ammonium persulfate as the initiator, resulting in the formation of poly(5-deoxy-5-methacrylamido-D-ribofuranose) (**3**) with a molecular weight of approximately 10^6 daltons.

The free radical polymerization of the carbohydrate monomers in aqueous solution at room temperature using initiator ammonium persulfate resulted in the generation of water-soluble carbohydrate based materials **1**–**3** with molecular weights of 10^5–10^6 daltons as determined by light scattering measurements. These materials were used directly for the attachment of proteins to form carbohydrate protein conjugates (CPC) or were chemically modified with sodium periodate in order to increase the aldehyde density of the material.[22]

The covalent attachment of the carbohydrate materials to proteases was achieved by reductive amination of the imine that is formed between the ϵ-amino group of a surface lysine residue and the open-chain aldehyde form of the carbohydrates[23,24] (Scheme 4). This method can be performed in aqueous solution under mild conditions of temperature and pH and results in the multisite attachment of the carbohydrate-based materials to the protein.[17]

Stability of CPC[proteases]. The stability of CPC[CT] was evaluated by assay in aqueous solution with the expectation that the stability of CPCs observed in aqueous solution would correlate with the stability observed during the enzymatic synthesis of peptides in organic solvents. CPC*[CT], a CPC prepared from sodium periodate oxidized glucose-based macromolecule **1**, was active at 90 °C for 1–2 h (Figure 1A) while native chymotrypsin lost all activity at 50 °C within 10 min. Approximately 90% of the original activity remained for CPC*[CT] at 50 °C after 3 h. CPC[CT] materials Rib-CPC[CT], Glc-CPC[CT], and Gal-CPC[CT] were prepared under identical reaction conditions, and the stability of the conjugates was evaluated in pH 7 phosphate buffered solution at 50 °C. Rib-CPC[CT] was the most stable conjugate with 45% activity remaining after 20 h (Figure 1B). Glc-CPC[CT] retained 33% activity, and Gal-CPC[CT] retained 23% activity for the same period of time. The greater stability of CPC*[CT] and Rib-CPC[CT] supports the hypothesis that the stability of CPCs is directly related to the available aldehyde content of the carbohydrate material from which the CPC is prepared.

We found that the increased stability of CPCs in aqueous solution correlated with the activity of CPCs in

(22) Wong, S. S. *Chemistry of Protein Conjugation and Cross-Linking*; CRC Press, Inc.: Boca Raton, 1991.
(23) Gray, G. R.; Schwartz, B. A.; Kamicker, B. J. *Prog. Clin. Biol. Res.* **1978**, *23*, 583–594.
(24) Schwartz, B. A.; Gray, G. R. *Arch. Biochem. Biophys.* **1977**, *181*, 542–549.

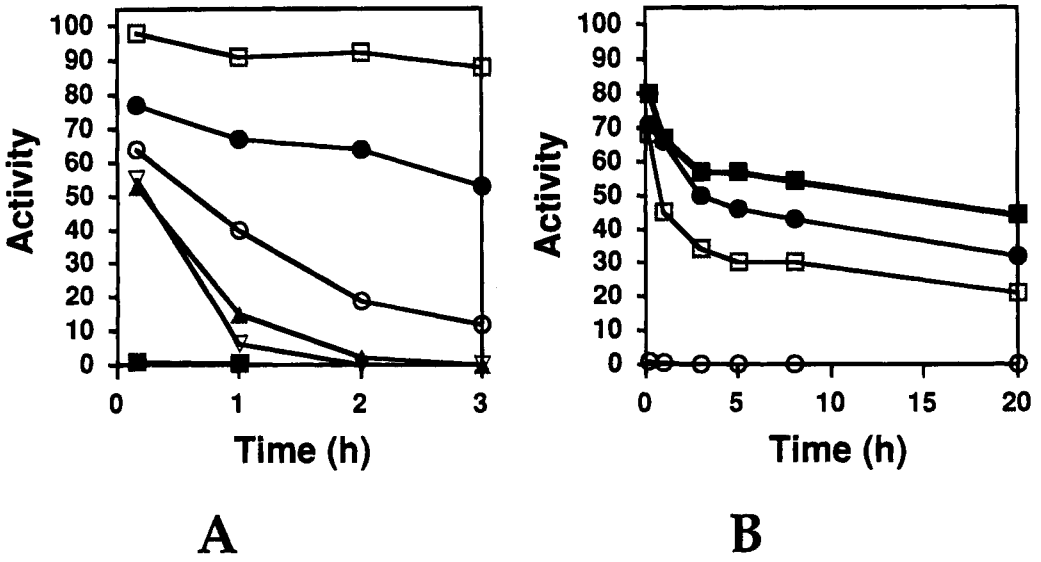


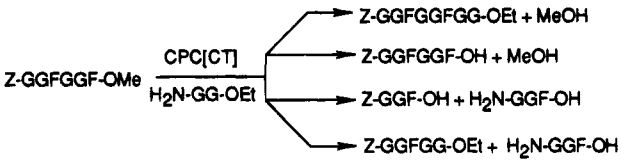
Figure 1. Stability of chymotrypsin conjugates in 0.1 M potassium phosphate at pH 7. (A) Stability of CPC*[CT] at 50 °C (□), 60 °C (●), 70 °C (○), 80 °C (▲), and 90 °C (▽) and nativee chymotrypsin at 50 °C (■). (B) Comparison of thermal stability of Rib-CPC[CT] (B), Glc-CPC[CT] (●), Gal-CPC[CT] (□), and native chymotrypsin (○) in 0.1 M potassium phosphate at pH 7 at 50 °C.

acetonitrile solvent systems. CPC[chymotrypsin] and CPC[papain] efficiently catalyzed the synthesis of peptides while the native proteases were inefficient catalysts under the conditions of this study.

CPC[protease]-Catalyzed Peptide Synthesis. CPC[chymotrypsin] (CPC[CT]), CPC[papain] (CPC[P]), CPC[proteinase K] (CPC[pK]), CPC[subtilisin BPN'] (CPC[BPN']), and CPC[thermolysin] (CPC[Th]) were prepared by the attachment of glucose-based macromolecule **1**, which was chosen because it could be easily prepared in sufficient quantities, to the native protease. The resulting conjugates were sufficiently stable as catalysts for peptide bond formation at 35–40 °C in acetonitrile solvent systems containing 10–20% water and 5–10% triethylamine.

The successful ligation of peptide fragments by a protease requires that the rate of synthesis of the desired peptide or protein be approximately 1 order of magnitude faster than the amide hydrolysis of the peptide precursors or products, transpeptidation, or ester hydrolysis. In aqueous solution, the kinetics for the latter processes are sometimes favorable, and undesirable side products have been observed.[8] In order to qualitatively evaluate the relative kinetics of these undesirable side-reactions during protease catalyzed oligopeptide synthesis in acetonitrile, the octamer Z-GGFGGFGG-OEt (Z, benzyloxycarbonyl; G, glycine; F, phenylalanine; Et, ethyl) was prepared by the CPC[CT] catalyzed 6 + 2 coupling of hexamer Z-GGFGGF-OMe (**13**) to glycylglycine ethyl ester. This hexamer, which was prepared using standard solution phase methodology,[25] contains only two possible sites for activation by α-chymotrypsin, the Phe-Gly amide and the C-terminus methyl ester. The possible reactions of hexamer **13** during the synthesis of the octamer Z-GGFGGFGG-OEt (**14**) are shown in Scheme 5. These reactions are synthesis, ester hydrolysis, amide hydrolysis, and transpeptidation. The CPC[CT]-catalyzed synthesis of the desired octamer **14** was achieved in 71%

Scheme 5



yield (unoptimized) demonstrating that synthesis was the dominant kinetic process under the conditions of this study. Tripeptide and pentamer products, formed from the activation of the C-terminal carboxyl group of the internal phenylalanine residue followed by the addition of water or nucleophile glycylglycine ethyl ester, were not observed at a detection limit of 1–2%. Clearly amide hydrolysis and transpeptidation were not kinetically favorable processes for this peptide under the conditions of this study.

The P_1 selectivity of CPC[CT] for the aromatic side chains is consistent with that previously determined for native α-chymotrypsin-catalyzed peptide synthesis.[26] *N*-benzyloxycarbonyl methyl esters of phenylalanine, tyrosine, and tryptophan were efficiently coupled to nucleophile glycylglycine ethyl ester during the CPC[CT]-catalyzed synthesis of tripeptides Z-Phe-Gly-Gly-OEt (**15**), Z-Tyr-Gly-Gly-OEt (**16**), and Z-Trp-Gly-Gly-OEt (**18**) in 60–93% yield (Table 1). In addition to the aromatic substrates, we also observed that *N*-benzyloxycarbonyl histidine methyl ester was efficiently coupled to glycylglycine ethyl ester to give the desired product Z-His-Gly-Gly-OEt (**17**) in 83% yield. However, *N*-benzyloxycarbonyl leucine methyl ester was a poor substrate under the same conditions. The other product isolated for these reactions was the corresponding *N*-benzyloxycarbonyl amino acid which was generated by the CPC-catalyzed hydrolysis of the ester substrates. Although α-chymotrypsin has P_2 specificity for aromatic residues,[8] products resulting from activation of the Gly-Gly amide bond of

(25) Slomczynska, U.; Beusen, D. D.; Zabrocki, J.; Kociolek, K.; Redlinski, A.; Reusser, F.; Hutton, W. C.; Leplawy, M. T.; Marshall, G. R. *J. Am. Chem. Soc.* **1992**, *114*, 4095–4106.

(26) Kise, H.; Tomiuchi, Y.; Nagashima, T. *Biomed. Biochim. Acta* **1991**, *50*, S127-S130.

Table 1. CPC[protease]-Catalyzed Peptide Synthesis

entry	CPC	acyl donor	nucleophile	product	% yield[a]
1	CPC[CT]	Z-Phe-OMe	H_2N-Gly-Gly-OEt	Z-Phe-Gly-Gly-OEt (**15**)	93
2	CPC[CT]	Z-Tyr-OMe	H_2N-Gly-Gly-OEt	Z-Tyr-Gly-Gly-OEt (**16**)	90
3	CPC[CT]	Z-His-OMe	H_2N-Gly-Gly-OEt	Z-His-Gly-Gly-OEt (**17**)	83
4	CPC[CT]	Z-Trp-OMe	H_2N-Gly-Gly-OEt	Z-Trp-Gly-Gly-OEt (**18**)	60
5	CPC[CT]	Z-Tyr-OMe	H_2N-Gly-OMe	Z-Tyr-Gly-OMe (**19**)	41
6	CPC[P]	Z-Phe-Gly-OMe	H_2N-Leu-OtBu	Z-Phe-Gly-Leu-OtBu (**20**)	85
7	CPC[P]	Z-Phe-Gly-OMe	H_2N-Leu-OMe	Z-Phe-Gly-Leu-OMe (**21**)	77
8	CPC[P]	Z-Gly-OMe	H_2N-Leu-OtBu	Z-Gly-Leu-OtBu (**22**)	74
9	CPC[P]	Z-Ala-OMe	H_2N-Leu-OtBu	Z-Ala-Leu-OtBu (**23**)	72
10	CPC[P]	Z-Phe-Gly-OMe	H_2N-Phe-OtBu	Z-Phe-Gly-Phe-OtBu (**24**)	70
11	CPC[P]	Z-Ser-OMe	H_2N-Leu-OtBu	Z-Ser-Leu-OtBu (**25**)	50
12	CPC[P]	Z-Gln-OMe	H_2N-Leu-OtBu	Z-Gln-Leu-OtBu (**26**)	44
13	CPC[P]	Z-Leu-OMe	H_2N-Leu-OtBu	Z-Leu-Leu-OtBu (**27**)	41
14	CPC[P]	Z-Met-OMe	H_2N-Leu-OtBu	Z-Met-Leu-OtBu (**28**)	35
15	CPC[P]	Z-Gly-Gly-OMe	H_2N-Leu-OtBu	Z-Gly-Gly-Leu-OtBu (**29**)	31
16	CPC[P]	Z-Gln-OMe	H_2N-Phe-OtBu	Z-Gln-Phe-OtBu (**30**)	23
17	CPC[P]	Z-Phe-OMe	H_2N-Leu-OtBu	Z-Phe-Leu-OtBu (**31**)	12
18	CPC[P]	Z-Tyr-Gly-OMe	H_2N-Gly-Phe-Leu-OH	Z-Tyr-Gly-Gly-Phe-Leu-OH (**32**)	79
19	CPC[pK]	Z-Tyr-Gly-Gly-OEt	H_2N-Phe-Leu-OH	Z-Tyr-Gly-Gly-Phe-Leu-OH (**32**)	32
20	CPC[BPN']	Z-Tyr-Gly-Gly-OEt	H_2N-Phe-Leu-OH	Z-Tyr-Gly-Gly-Phe-Leu-OH (**32**)	28
21	CPC[Th]	Z-Asp-OH	H_2N-Phe-OMe	Z-Asp-Phe-OMe (**33**)	90
22	CPC[BPN']	Z-Ala-OMe	H_2N-Phe-Leu-OH	Z-Ala-Phe-Leu-OH (**34**)	39

[a] All reactions were performed as described in the Experimental Section. Yields were determined by reverse phase HPLC analysis. Abbreviations: CT, chymotrypsin; P, papain; BPN', subtilisin BPN', pK, proteinase K, Th, thermolysin; Ala, alanine; tBu, *tert*-butyl; Gly, glycine; Et, ethyl; His, histidine; Leu, leucine; Me, methyl; Phe, phenylalanine; Trp, tryptophan; Tyr, tyrosine; Z, benzyloxycarbonyl.

the products were not observed under the conditions of this study. Control reactions using an equivalent amount of native chymotrypsin as found in CPC[CT] resulted in yields of 8−38% for the desired product. The other product isolated during the reactions catalyzed by the native protease was the unreacted methyl ester. When the D isomer of Z-Tyr-OMe or Z-Phe-OMe was used, no product was observed under the conditions of this study.

Use of CPC[papain] for peptide synthesis in acetonitrile in catalytic quantities has resulted in the preparation of the leucine enkephalin derivative *N*-Z-Tyr-Gly-Gly-Phe-Leu-OH, described in further detail below, and the preparation of dipeptides and tripeptides in yields ranging from 85−12% (Table 1, entries 6−18). Peptides prepared in high yield (70−85%) were Z-Phe-Gly-Leu-OtBu (**20**), Z-Phe-Gly-Leu-OMe (**21**), Z-Gly-Leu-OtBu (**22**), Z-Ala-Leu-OtBu (**23**), and Z-Phe-Gly-Phe-OtBu (**24**). Peptides prepared in modest yield (44−23%) were Z-Ser-Leu-OtBu (**25**), Z-Gln-Leu-OtBu (**26**), Z-Leu-Leu-OtBu (**27**), Z-Met-Leu-OtBu (**28**), Z-Gly-Gly-Leu-OtBu (**29**), and Z-Gln-Phe-OtBu (**30**). The preparation of Z-Phe-Leu-OtBu (**31**) was achieved in poor yield (12%). The other product generated during these syntheses was the corresponding *N*-benzyloxycarbonyl amino acid, except for syntheses with Z-Phe-OMe and Z-Leu-OMe which resulted in the recovery of the unreacted esters in 51 and 32% yields after 72 h. The synthesis of Z-Phe-Gly-Leu-OtBu using native papain lyophilized from 0.05 M sodium phosphate at pH 6.0 resulted in a yield of 3%.[27] Additionally, the rate of amide hydrolysis for tripeptide Z-Phe-Gly-Leu-OtBu to Z-Phe-Gly-OH was determined in acetonitrile and found to be 1 order of magnitude less than the rate of synthesis.

Although we determined that papain is a relatively nonspecific protease for the hydrolysis of *N*-benzyloxycarbonyl amino acid methyl esters in aqueous solution, the initial rates of synthesis using CPC[P] in acetonitrile differed by approximately 2 orders of magnitude. The initial rates of ester hydrolysis in aqueous solution were 0.7−2.4 μmol min^{-1} mg papain^{-1} while the rates of synthesis with nucleophile leucine *tert*-butyl ester varied from 1.4 μmol min^{-1} mg papain^{-1} to 19 nmol min^{-1} mg papain^{-1}. The highest rates of synthesis were observed for *N*-benzyloxycarbonyl amino acid methyl esters of alanine, glycine, methionine, and glutamine with rates of synthesis of 0.57−1.4 μmol min^{-1} mg papain^{-1}. For synthesis with the leucine and phenylalanine derivatives, the rates were 0.17 and 0.019 μmol min^{-1} mg papain^{-1}. These results suggest that the specificity of a protease in aqueous solution may not be directly applied to rates of the synthesis of peptides under the conditions of this study.

Comparison of the initial velocities of synthesis to hydrolysis during the CPC[papain]-catalyzed synthesis of peptides with nucleophile leucine *tert*-butyl ester showed that for the synthesis of Z-Phe-Gly-Leu-OtBu, the rate of synthesis is 48 times faster than that of hydrolysis. For derivatives of glycine, alanine, leucine, and methionine, the ratio is 7.3−2.9, for serine and glutamine, the ratio is nearly equal, and for phenylalanine, hydrolysis is the favored by a factor of 3.

The specificity of CPC[proteases] in acetonitrile solvent systems was applied to the convergent synthesis of the *N*-benzyloxycarbonyl leucine enkephalin Z-Tyr-Gly-Gly-Phe-Leu-OH (**32**). This synthesis was achieved utilizing CPC[CT] to prepare the fragment Z-Tyr-Gly-OMe (**19**) in 41% yield (unoptomized) and CPC[P] to couple this fragment to H_2N-Gly-Phe-Leu-OH in 79% yield (32% for two steps). Attempts to use CPC[BPN'] and CPC[pK] for the 3 + 2 coupling of Z-Tyr-Gly-Gly-OEt to H_2N-Phe-Leu-OH resulted in poor yields of 28−32%.

Other CPC[protease]-catalyzed syntheses include the preparation of the aspartame precursor Z-Asp-Phe-OMe (**33**) in 90% yield using CPC[thermolysin]. Native thermolysin has P_1' specificity for hydrophobic amino acids such as phenylalanine, leucine, valine, and isoleucine.[28] We also prepared Z-Ala-Phe-Leu-OH (**34**, 39%) from Z-Ala-OMe and the unprotected dipeptide phenylalanylleucine using CPC[BPN']. However, CPC[BPN'] was

(27) Zaks, A.; Klibanov, A. M. *Proc. Natl. Acad. Sci. U.S.A.* **1985**, *82*, 3192−3196.

(28) Morihara, K.; Tsuzuki, H. *Eur. J. Biochem.* **1970**, *15*, 374−380.

not a good catalyst for the synthesis of peptides from acyl components Z-Gly-OMe, Z-Gly-Gly-OMe, Z-Phe-OMe, Z-Tyr-OMe, and Z-Ala-OMe with nucleophiles H_2N-Gly-Gly-OEt or H_2N-Leu-OtBu due to the hydrolysis of the P_1 ester to the unreactive acid. Although CPC[BPN'] was not an efficient catalyst with single amino acid substrates, we have previously demonstrated that CPC[BPN'] is an efficient catalyst for the synthesis of oligopeptides Z-Leu-Leu-Phe-Leu-OtBu (95%) and Z-Val-Leu-Phe-Leu-OtBu (90%) from Z-Leu-Leu-OMe and Z-Val-Leu-OMe.[17] This suggests that for CPC[BPN'], synthesis is favorable for dipeptide acyl components but not single amino acid acyl components.

Conclusion

In summary, we have investigated the use of a small library of stabilized CPC[proteases] for the catalytic formation of peptides containing 2–8 amino acids in acetonitrile solvent systems. The selectivity of CPC[α-chymotrypsin] for peptide synthesis was consistent with the specificity known for the hydrolysis of aromatic amino acid containing substrates in aqueous solution. The histidine-containing peptide Z-His-Gly-Gly-OEt was also prepared using CPC[α-chymotrypsin]. The known specificity of CPC[papain] for aromatic amino acids in the P_2 position was observed during peptide synthesis, and P_1 selectivity was observed for single amino acid substrates during peptide synthesis in acetonitrile, although no selectivity was observed for the papain-catalyzed hydrolysis of *N*-benzyloxycarbonyl methyl esters in aqueous solution. The aspartame precursor Z-Asp-Phe-OMe was prepared using CPC[thermolysin]. CPC[BPN'] was a useful catalyst for the preparation of tetramers from dipeptide acyl components, but was not an efficient catalyst for the preparation of tri- and dipeptides from single amino acid acyl components. The selectivities of CPCs observed in acetonitrile was applied to the convergent synthesis of a leucine enkephalin derivative Z-YGGFL-OH and the octamer Z-GGFGGFGG-OEt. The increased stability of CPCs prepared from carbohydrate materials designed to increase the aldehyde concentration in aqueous solution suggests that the stability of CPCs is directly related to aldehyde concentration.

Experimental Section

General Experimental. Sources. 2-Amino-2-deoxy-D-glucopyranose (D-glucosamine hydrochloride) and 2-amino-2-deoxy-D-galactopyranose (D-galactosamine hydrochloride) was purchased from the Sigma Chemical Co. Amino acid derivatives and peptides were purchased from the Sigma Chemical Co. or Bachem California. Enzymes were obtained from the Sigma Chemical Co. *N*-benzyloxycarbonyl (Z) amino acid and peptide methyl esters were prepared using acetyl chloride (0.5% v/v) in methanol, except CBZ-His-OMe and CBZ-Met-OMe which were prepared using methanol acidified with HCl gas. All water used was purified using a Nanopure water purification system. Methacryloyl chloride, ammonium persulfate, sodium azide, palladium hydroxide on carbon, and sodium periodate were obtained from the Aldrich Chemical Co. Thin layer chromatography was performed using EM Science 0.2 mm precoated silica gel 60 plates.

Poly(2-methacrylamido-2-deoxy-D-glucose) (1).[17] Solutions of 2-methacrylamido-2-deoxy-D-glucose (5 g) in water (50 mL) and ammonium persulfate (1 g, 4 mmol in 2 mL of water) were purged with argon for 30 min. The ammonium persulfate solution was added to the stirred monomer solution via gastight syringe at rt, and the mixture was stirred for 20 h. The solution was dialyzed against water (50k MWCO Spectra Por CE membrane, 3 × 2 L water, 24 h) and lyophilized to give 3 g (75% monomer conversion) of a water soluble (100 mg/mL) white powder: ^{1}H NMR (500 MHz, D_2O) δ 0.50–2.40 (bm), 3.25 (bs), 3.57 (bm), 4.80–5.1 (bm); ^{13}C NMR (125 MHz, D_2O) δ 14.04, 46.93, 56.76, 59.34, 63.00, 72.69, 73.90, 76.05, 78.21, 92.55, 97.54, 181.35.

Poly(2-methacrylamido-2-deoxy-D-galactose) (2). To a solution of D-galactosamine hydrochloride (5 g, 23 mmol) in water (50 mL) was added sodium methoxide solution (25% w/w in methanol, 3.7 mL, 23 mmol) to give a pH of 8.8 as determined by pH meter. Methacryloyl chloride (2.4 g, 23.2 mmol, 2.2 mL) and sodium methoxide solution (3.7 mL, 23 mmol) were added in alternating portions over 3 h to give a final pH of 7.2. The pH was adjusted to 4, and the solution was extracted with ethyl acetate (3 × 50 mL). The pH was adjusted to 7 with sodium bicarbonate, and the solution was freeze thaw degassed. To this solution was added a freeze thaw degassed solution of ammonium persulfate (0.52 g, 2.3 mmol in 3 mL of water), and the solution was stirred at room temperature for 24 h. The solution was added to methanol (300 mL), and the resulting white precipitate was collected by vacuum filtration, dissolved in water (100 mL), dialyzed (Spectra Por 12-14k MWCO, 3 × 2 L water, 48 h), and lyophilized to give 2.5 g (63% monomer conversion) of white powder: ^{1}H NMR (500 MHz, D_2O) δ 0.40–2.30 (bm), 3.2–4.2 (bm), 4.8–5.2 (bm); ^{13}C NMR (125 MHz, D_2O) δ 19.51, 47.70, 53.35, 56.74, 63.67, 65.06, 69.36, 71.16, 72.82, 77.40, 92.74, 97.76, 181.81.

Poly(5-methacrylamido-5-deoxy-D-ribose) (3). To a solution of methyl 2,3-*O*-isopropylidene-5-methacrylamido-5-deoxy-D-ribofuranoside (**12**) (2.83 g, 10.4 mmol) in water, (50 mL) was added 1.3 g of acidic ion exchange resin (Amberlite IR-120), and the solution was stirred at 50 °C for 30 h. The heterogeneous solution was filtered, and the solution was concentrated to 15 mL under reduced pressure and freeze thaw degassed, and a solution of freeze thaw degassed ammonium persulfate (154 mg, 0.66 mmol in 1.5 mL of water) was added. The solution was stirred at room temperature for 18 h, dialyzed against water (Spectra Por 1000 MWCO membrane), and lyophilized to give 0.373 g (16%) of a white solid: ^{1}H NMR (200 MHz, D_2O) δ 0.7 (bs, 3 H), 1.7 (bm, 2 H), 3.2 (bs, 2 H), 5.2 (bm, 3 H), 7.5 (bs, 1 H); ^{13}C NMR (125 MHz, D_2O) δ 18.50, 43.43, 47.41, 56.44, 70.59, 71.77, 74.18, 74.90, 94.75, 98.86, 181.69.

2-Methacrylamido-2-deoxy-D-glucose (4).[17] To a stirred slurry of 2-deoxy-D-glucosamine hydrochloride (25 g, 0.12 mol) in anhydrous methanol (100 mL) at 2 °C was added 1.5 M sodium methoxide in methanol (80 mL, 0.12 mol, prepared within 24 h of use from sodium metal) to give a final pH of 8. Distilled methacryloyl chloride was added in 1 mL portions (14 mL total, 13.1 g, 0.13 mol) over 2 h. After each addition, the pH was adjusted to 8–9 with 1.5 M sodium methoxide in methanol. After the addition of methacryloyl chloride was complete, TLC analysis (silica, methanol eluent) showed 2-methacrylamido-2-deoxy-D-glucose (R_f 0.8) and 2-deoxy-D-glucosamine (R_f 0.4). An additional 5.5 mL of methacryloyl chloride (5.1 g, 0.05 mol) was added in 1 mL portions. The pH was adjusted to 8–9 after each addition with 1.5 M sodium methoxide in methanol. After the final 0.5 mL of methacryloyl chloride was added, TLC analysis showed no remaining 2-deoxy-D-glucosamine and the pH was adjusted to 7. The methanol was removed by rotary evaporation to give 35 g (100%) of 2-methacrylamido-2-deoxy-D-glucose as a mixture of α and β anomers containing sodium chloride which was used without further purification: ^{1}H NMR (200 MHz, D_2O) δ 1.79 (s, 3 H), 3.30–3.85 (m, 6 H), 5.08 (bs, α), 5.10 (s, 1 H), 5.56 (s, 1 H).

2-Deoxy-2-methacrylamidotetra-*O*-(trimethylsilyl)-D-glucopyranose (5). This derivative was prepared according to Sweeley and co-workers.[29] To a solution of 2-methacrylamido-2-deoxy-D-glucose (**4**, 50 mg, 0.2 mmol) in pyridine (5 mL) was added 1,1,1,3,3,3-hexamethyldisilazane (1 mL, 4.7 mmol) followed by trimethylsilyl chloride (0.5 mL, 4.0 mmol), and the

(29) Sweeley, C. C.; Bentley, R.; Makita, M.; Wells, W. W. *J. Am. Chem. Soc.* **1963**, *85*, 2497.

solution was shaken for 30 s and stored at rt for 1 min. The solvent was removed by rotary evaporation and the residue was purified by silica gel chromatography using ethyl acetate/hexane (10/1) to give a white solid: mp 105–107 °C; ^{1}H NMR (250 MHz, $CDCl_3$) δ 0.10 (s, 18 H), 0.14 (s, 9 H), 0.17 (s, 9 H), 1.97 (s, 3 H), 3.60–3.74 (m, 5 H), 4.10 (dt, 1 H, J = 9.8, 3.5 Hz), 5.06 (d, 1 H, J = 3.5 Hz), 5.37 (t, 1 H, J = 1.3 Hz), 5.69 (s, 1 H), 5.78 (d, 1 H, J = 9.9 Hz); ^{13}C NMR (125 MHz, $CDCl_3$) δ −0.37 (q), −0.19 (q), 0.01 (q), 0.27 (q), 0.55 (q), 0.74 (q), 1.01 (q), 1.30 (q), 18.52 (q), 18.57 (q), 54.54 (d), 59.48 (d), 61.71 (d), 62.25 (t), 71.80 (d), 72.01 (d), 72.65 (d), 74.06 (d), 76.50 (d), 76.91 (d), 92.61 (d), 94.05 (d), 119.44 (t), 119.59 (t), 140.08 (s), 140.43 (s), 167.65 (s), 168.18 (s); IR (CH_2CL_2) 3930, 3742, 3680, 3439, 3041, 2978, 2950, 2920, 2895, 2830, 1665, 1620, 1544, 1503, 1415, 1375, 1260, 1140, 1100, 1055, 970, 865, 730 cm^{-1}; HRMS calcd for $C_{22}H_{49}NO_6Si_4$ 535.2638, m/z found 535.2669. Anal. Calcd for $C_{22}H_{49}NO_6Si_4$: C, 49.30; H, 9.21. Found: C, 49.20; H, 9.16.

2-Methacrylamido-2-deoxy-D-galactose (6). This compound was prepared as described for 2-methacrylamido-2-deoxy-D-glucose (**4**). To a heterogeneous solution of D-galactosamine hydrochloride (1.0 g, 4.7 mmol) in methanol (200 mL) was added 1 equiv of 1.3 M sodium methoxide in methanol (4 mL) at 5–10 °C to give a pH of 9 as determined by pH paper wetted with water. Methacryloyl chloride (0.49 g, 4.7 mmol) and a second equivalent of sodium methoxide solution were added in alternating portions over 1 h. The methanol was removed by rotary evaporation to give 1.4 g (100%) of white powder: ^{1}H NMR (200 MHz, D_2O) δ 1.82 (s, 3 H), 3.5–4.1 (m, 6 H), 5.34 (s, 1 H), 5.59 (s, 1 H).

2-Deoxy-2-methacrylamidotetra-*O*-(trimethylsilyl)-D-galactopyranose (7). This derivative was prepared as described for **5**. To a room temperature solution of 2-methacrylamido-2-deoxy-D-galactose (0.50 g, 1.6 mmol) in pyridine (20 mL) was added 1,1,1,3,3,3-hexamethyldisilazane (7.65 g, 10 mL, 48 mmol) followed by trimethylsilyl chloride (6.9 g, 64 mmol, 8 mL), and the solution was stirred for 1 h. The solvent was removed by rotary evaporation, and the residue was purified by silica gel chromatography (10:1 hexanes/ethyl acetate) to give a white solid: mp 135–140 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 0.07 (s, 9 H), 0.08 (s, 9 H), 0.13 (s, 9 H), 0.27 (s, 9 H), 0.46 (s, 3 H), 3.80 (dd, 1 H, J = 9.5, 5.6 Hz), 3.92–3.97 (m, 2 H), 4.09 (dd, 1 H, J = 13.4, 6.4 Hz), 4.15 (d, 1 H, J = 2.0 Hz), 4.99–5.05 (m, 2 H), 5.32 (d, 1 H, J = 3.4 Hz), 5.72–5.82 (m, 2 H); ^{13}C NMR (125 MHz, DMSO-d_6) δ −0.53 (q), −0.27 (q), 0.37 (q), 0.87 (q), 18.83 (q), 50.75 (d), 61.77 (t), 71.13 (d), 71.67 (d), 72.26 (d), 94.06 (d), 118.43 (t), 141.53 (s), 166.96 (s). IR (KBr) 3374, 2959, 2922, 1656, 1617, 1533, 1458, 1373, 1251, 1175, 1134, 1119, 1095, 1059, 982, 910, 839, 750 cm^{-1}. Anal. Calcd for $C_{22}H_{49}NO_6Si_4$: C, 49.30; H, 9.21; N, 2.61. Found: C, 49.58; H, 9.22; N, 2.61.

Methyl 2,3-*O*-isopropylidene-D-ribofuranoside (8).[30] To a suspension of D-ribose (10 g, 67 mmol) in 2,2-dimethoxypropane (20 mL, 0.16 mol) and acetone (80 mL) at 5 °C was added 60% perchloric acid (4.7 mL) dropwise over 5 min. The solution was stirred at room temperature for 2 h, methanol (14 mL) was added, and the solution was stirred for an additional 2 h. The solution was cooled to 5 °C and neutralized with sodium carbonate (3.2 g in 10 mL of water). The solution was filtered and the solvent was removed by rotary evaporation to give a yellow solid that was dissolved in diethyl ether (200 mL), extracted with water (100 mL) and saturated sodium chloride solution (100 mL), dried (Na_2SO_4), and concentrated by rotary evaporation to give a colorless oil. The compound was purified by distillation at reduced pressure (85–89 °C, 0.25 mm of mercury) to give 5.38 g (40%) of a colorless oil: ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.28 (s, 3 H), 1.43 (s, 3 H), 3.27 (m, 1 H), 3.39 (s, 3 H), 3.59 (m, 2 H), 4.38 (t, 1 H, J = 2.6 Hz), 4.54 (d, 1 H, J = 6.3 Hz), 4.80 (d, 1 H, J = 6.3 Hz), 4.92 (s, 1 H).

Methyl 2,3-*O*-Isopropylidene-5-*O*-(methylsulfonyl)-D-ribofuranoside (9). This compound was prepared using a modified procedure.[31] To a stirred solution of **8** (1.0 g, 4.9 mmol) in dichloromethane (12 mL) containing triethylamine (1.6 mL, 12 mmol) at 0 °C was added methanesulfonyl chloride (0.92 mL, 12 mmol) dropwise over 5 min, and the solution was stirred for 5 h. The solution was extracted with saturated sodium bicarbonate (2 × 7 mL) and water (2 × 7 mL), and the organic layer was dried (Na_2SO_4). The solvent was removed by rotary evaporation to give 1.18 g (86%) of a yellow solid that was used without further purification: mp 66–73 °C (lit. mp 73–74 °C);[31] ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.31 (s, 3 H), 1.49 (s, 3 H), 3.09 (s, 3 H), 3.37 (s, 3 H), 4.20 (d, 2 H, J = 7.1 Hz), 4.40 (t, 1 H, J = 7.1 Hz), 4.60 (d, 1 H, J = 5.6 Hz), 4.70 (d, 1 H, J = 5.6 Hz), 5.00 (s, 1 H).

Methyl 2,3-*O*-Isopropylidene-5-azido-D-ribofuranoside (10). This compound was prepared as described previously except the reaction mixture was heated at reflux.[32] To a stirred solution of **9** (1.0 g, 3.6 mmol) in dimethylformamide (21 mL) was added sodium azide (0.94 g, 14.5 mmol), and the solution was heated at reflux for 1 h to give a heterogeneous brown mixture. The mixture was cooled, 20 mL of water was added, and the mixture was extracted with diethyl ether (3 × 20 mL). The ether layer was washed with water (40 mL) and dried ($MgSO_4$). The solvent was removed by rotary evaporation to give 0.56 g (68%) of a light yellow liquid that was used without further purification: ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.30 (s, 3 H), 1.40 (s, 3 H), 3.22 (m, 1 H), 3.36 (s, 3 H), 3.42 (m, 1 H), 4.27 (t, 1 H, J = 7.6 Hz), 4.59 (s, 2 H), 4.98 (s, 1 H).

Methyl 2,3-*O*-Isopropylidene-5-amino-5-deoxy-D-ribofuranoside (11). This compound was prepared using a modified procedure.[33] To a solution of **10** (3.50 g, 15.3 mmol) in methanol (29 mL) containing 3.7 mL of a 25% w/w solution of sodium methoxide in methanol was added 424 mg of palladium hydroxide on carbon. The flask was purged with hydrogen and stirred for 20 h at room temperature under hydrogen stored in a balloon. The reaction mixture was filtered over Celite followed by silica gel, and the solvents were removed by rotary evaporation to give 2.0 g (64%) of a yellow liquid which was purified by flash chromatography (1:1 ethyl acetate/hexanes, then methanol) as a colorless liquid: ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.20 (s, 3 H), 1.37 (s, 3 H), 1.59 (s, 2 H), 2.68 (d, 2 H, J = 7.3 Hz), 3.25 (s, 3 H), 4.05 (m, 1 H), 4.48 (m, 2 H), 4.87 (s, 1 H); ^{13}C NMR (63 MHz, $CDCl_3$) δ 24.71, 26.24, 45.25, 54.84, 81.94, 85.22, 88.58, 109.36, 112.03; IR (neat) 3382, 3314, 2988, 2938, 2834, 1598, 1457, 1373, 1220, 1160, 1090 cm^{-1}.

Methyl 2,3-*O*-Isopropylidene-5-methacrylamido-5-deoxy-D-ribofuranoside (12). To a solution of **11** (18.22 g, 90 mmol) in dichloromethane (237 mL) under argon was added freshly distilled triethylamine (58 mL) at 0 °C followed by the dropwise addition of a solution of freshly distilled methacryloyl chloride (10.7 mL, 110 mmol) in dichloromethane (150 mL) over 2 h to give a cloudy, yellow solution that was stirred at room temperature for 9 h. Removal of the solvents by rotary evaporation gave a brown, viscous liquid that was purified by flash chromatography using 2:1 hexanes/ethyl acetate to give 13.5 g (56%) of a white solid: mp 67–69 °C; ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.29 (s, 3 H), 1.53 (s, 3 H), 1.96 (m, 3 H), 3.28–3.39 (m, 1 H), 3.41 (s, 3 H), 3.66–3.80 (m, 1 H), 4.43 (t, 1 H, J = 4.7 Hz), 4.56 (m, 2 H), 4.99 (s, 1 H), 5.34 (m, 1 H), 5.74 (m, 1 H), 6.65 (bs, 1 H); ^{13}C NMR (63 MHz, $CDCl_3$) δ 18.35, 24.69, 26.24, 42.08, 55.10, 81.89, 85.44, 85.94, 109.98, 112.21, 119.71, 139.52, 168.12; IR (KBr) 3342, 2986, 2931, 2835, 1654, 1614, 1534, 1445, 1215, 1107, 1048, 869 cm^{-1}. Anal. Calcd for $C_{13}H_{21}NO_5$: C, 57.61; H, 7.81; N, 5.17. Found: C, 57.61; H, 7.86; N, 5.10.

General Method for the Preparation of Carbohydrate Protease Conjugates (CPCs). CPCs were prepared according to the following example for CPC[α-chymotrypsin]. To a solution of poly(2-methacrylamido-2-deoxy-D-glucose) (**23**, 2 g) in 0.1 M sodium tetraborate at pH 9.2 (40 mL) was added

(30) Ali, S. M.; Ramesh, K.; Borchardt, R. T. *Tetrahedron Lett.* **1990**, *31*, 1509–1512.

(31) Kissman, H. M.; Weiss, M. J. *J. Am. Chem. Soc.* **1958**, *80*, 5559–5564.

(32) Ohrui, H. *J. Org. Chem.* **1985**, *50*, 3007–3009.

(33) Secrist, J. A., III; Logue, M. W. *J. Org. Chem.* **1972**, *37*, 335–336.

α-chymotrypsin (Sigma type II, 0.40 g, 16 μmol) followed by sodium cyanoborohydride (0.40 g, 6 mmol) which was purified from its dioxane complex.[34] The solution was shaken at 35 °C for 30 h at 60 osc/min in a linear shaking water bath. The solution was dialyzed (Spectra Por 50k MWCO, alternating 0.1 M calcium chloride and water, 4 × 2 L, 24 h) to give 1.5 g of a white powder after lyophilization. The yield was determined by assay after isolation of the CPC by gel filtration chromatography (Pharmacia HR 16/50 with Sephacryl S-200 gel, 0.05 M sodium tetraborate (pH 8) eluent at 1.5 mL/min, 254 nm). CPC eluted from 26 to 40 min and α-chymotrypsin eluted from 40 to 55 min. The yield was determined by assay for α-chymotrypsin as described below. Comparison of the activity in the CPC fraction with that in the initial solution before the addition of **23** and sodium cyanoborohydride resulted in a calculated 30% yield.

α-Chymotrypsin Assay.[35] This assay was performed using a modified procedure. α-Chymotrypsin (10 μg/mL) was added to 1 mL of 5×10^{-4} M *N*-succinyl-Ala-Ala-Pro-Phe-*p*-nitroaniline in 0.05 M sodium tetraborate pH 8 containing 10% methanol, and the absorbance was monitored at 410 nm and 25 °C. A unit of activity for chymotrypsin is defined as the amount of enzyme or CPC required to hydrolyze 1 μmol of *N*-succinyl-Ala-Ala-Pro-Phe-*p*-nitroaniline in 1 min. The hydrolysis is quantified by relating the slope observed at V_{max} to the extinction coefficient of *p*-nitroaniline (8800)[36] at 410 nm.

Subtilisin BPN' assay.[37] Subtilisin BPN' and CPC[BPN'] were assayed as described for α-chymotrypsin.

Papain Assay. This assay was performed using a modified procedure.[38] To a solution of Z-Phe-Gly-OMe (1.0 mL, 3.0×10^{-3} M) in 0.05 M sodium phosphate at pH 6 containing 25% dimethylformamide or 20% acetonitrile at 35 °C was added papain (0.3 nmol) in 0.05 M sodium phosphate pH 6. The mixture was separated by reverse phase HPLC (Alltech Econosil C_{18} column, 1 × 25 cm; 260 nm; acetonitrile/water containing 0.1% trifluoroacetic acid, 30/70 to 90/10 over 18 min). The amount of Z-Phe-Gly-OH that was formed was measured at 5−15% hydrolysis of Z-Phe-Gly-OMe. One unit of activity is the amount of enzyme or CPC required to hydrolyze 1 μmol of Z-Phe-Gly-OMe in 1 min.

Determination of the Stability of CPC[CT] and Native α-Chymotrypsin in Aqueous Solution. The activity of CPC[CT] or the native protease in potassium phosphate buffer (0.1 M, pH 7.0) was monitored at various temperatures by assay as described above.

Periodate Oxidation of Poly(2-methacrylamido-2-deoxy-D-glucose). Periodate oxidation was performed using a modified procedure.[39] To a solution of poly(2-methacrylamido-2-deoxy-D-glucose) (1.2 g, 6 mmol of carbohydrate residues) in water (120 mL) was added sodium periodate (0.33 g, 1.5 mmol) to give a pH of 4. The flask was wrapped in aluminum foil and the solution was stirred for 4 h. The solution was dialyzed (Spectra Por 12−14K MWCO, 3 × 2 L water, 24 h) and lyophilized to give 1 g (0.83%) of a white powder.

Z-Gly-Gly-Phe-Gly-Gly-Phe-OMe (13). The solution phase methodology used to synthesize hexamer **13** was identical to that reported for the synthesis of the emerimicins.[25] To a solution of Z-Gly-Gly-Phe-OH (0.75 g, 1.8 mmol), dicyclohexylcarbodiimide (0.371 g, 1.8 mmol), and 1-hydroxybenzotriazole (0.243 g, 1.8 mmol) in DMF (10 mL) was added a solution of HCl·H_2N-Gly-Gly-Phe-OMe (0.597 g, 1.8 mmol) and 4-methylmorpholine (0.183 g, 1.8 mmol) in DMF (5 mL), and the solution was stirred for 6 h. The solution was filtered through a fritted funnel and concentrated by rotary evaporation at 50 °C and 0.25 mm of mercury to give a an off white semisolid. Addition of 1:1 ethyl acetate/diethyl ether (100 mL) resulted in the formation of a precipitate that was collected by filtration through a fritted funnel to give 0.65 g of a white solid. An additional 0.12 mg of solid was isolated from the filtrate (total yield 62%). The peptide was purified using reverse phase HPLC on an Alltech Econosil 1 × 25 cm C_{18} column using gradient elution with acetonitrile/water containing 0.1% trifluoroacetic acid eluent (30/70 to 90/10, 18 min, 3 mL/min, 260 nm detection) to give a white solid after removal of the solvent at 40 °C and 0.25 mm of mercury: mp 138−142 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 2.79 (dd, 1 H, J = 13.7, 9.7 Hz), 2.91 (dd, 1 H, J = 13.7, 8.8 Hz), 3.00−3.07 (m, 2 H), 3.58 (s, 3 H), 3.59−3.77 (m, 8 H), 4.45−4.52 (m, 2 H), 5.02 (s, 2 H), 7.14−7.35 (m, 15 H), 7.46 (t, 1 H, J = 5.9 Hz), 7.92 (t, 1 H, J = 5.7 Hz), 7.97 (s, 1 H), 8.10 (d, 1 H, J = 8.1 Hz), 8.29 (m, 2 H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 36.73 (t), 37.38 (t), 41.47 (t), 41.79 (t), 42.01 (t), 43.48 (t), 51.78 (q), 53.56 (d), 53.99 (d), 65.48 (t), 126.20 (d), 126.54 (d), 127.65 (d), 127.72 (d), 128.01 (d), 128.22 (d), 128.27 (d), 129.01 (d), 129.01 (d), 136.97 (s), 137.79 (s), 156.47 (s), 168.60 (s), 168.68 (s), 168.78 (s), 169.31 (s), 171.32 (s), 171.75 (s); HRMS calcd for $C_{35}H_{41}N_6O_9$ (M + H) 689.2935, m/z found 689.2976.

Z-Gly-Gly-Phe-Gly-Gly-Phe-Gly-Gly-OEt (14). Hexamer **13** (68.8 mg, 0.1 mmol) and glycylglycine ethyl ester hydrochloride (196.6 mg, 1 mmol) were dissolved in triethylamine (0.2 mL), water (0.15 mL), and acetonitrile (0.5 mL). CPC[α-chymotrypsin] (25 mg, 35 units) was added to give a heterogeneous solution that was shaken in an orbital shaking water bath at 300 osc/min and 40 °C for 19 h. Acetonitrile (2 mL) was added, and the solution was filtered through a fritted funnel. Acetic acid (0.5 mL) was added, and the octamer **14** was isolated by reverse phase HPLC as described for **13** to give 14.9 mg (71% HPLC yield, 18% isolated) of a white solid: ^{1}H NMR (500 MHz, DMSO-d_6) δ 1.18 (t, 3 H, J = 7.1 Hz), 2.79 (m, 2 H), 3.05 (dd, 2 H, J = 13.9, 4.1 Hz), 3.59 (d, 1 H, J = 5.4 Hz), 3.62 (d, 3 H, J = 5.9 Hz), 3.69−3.78 (m, 6 H), 3.83 (d, 2 H, J = 6.0 Hz), 4.09 (q, 2 H, J = 7.1 Hz), 4.52 (m, 2 H), 5.02 (s, 2 H), 7.15−7.31 (m, 15 H), 7.45 (bs, 1 H), 7.89 (t, 1 H, J = 5.6 Hz), 7.97 (bs, 1 H), 8.09−8.13 (m, 3 H), 8.27−8.31 (m, 2 H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 13.98 (q), 37.43 (t), 40.60 (t), 41.76 (t), 42.02 (t), 43.47 (t), 53.99 (d), 60.38 (t), 65.49 (t), 126.24 (d), 127.69 (d), 127.76 (d), 128.05 (d), 128.32 (d), 129.14 (d), 136.99 (s), 137.82 (s), 156.52 (s), 168.62 (s), 168.90 (s), 169.18 (s), 169.37 (s), 169.69 (s), 171.32 (s), 171.40 (s); HRMS calcd for $C_{40}H_{49}N_8O_{11}$ (M + H) 817.3521, m/z found 817.3504.

Z-Phe-Gly-Gly-OEt (15). Z-Phe-OMe (313 mg, 1 mmol) and glycyl-glycine ethyl ester hydrochloride (589 mg, 3 mmol) was dissolved in a mixture of triethylamine (0.39 mL), water (0.3 mL), and acetonitrile (1.0 mL). CPC[CT] (50 mg, 250 units) was added to give a heterogeneous solution that was shaken in an orbital shaking water bath at 300 osc/min and 40 °C for 72 h. Acetonitrile (2 mL) and acetic acid (1 mL) were added, and the solution was filtered through a fritted glass funnel. Reverse phase HPLC analysis was performed by linear gradient elution with acetonitrile/water containing 0.1% trifluoroacetic acid eluent (30/70 to 90/10, 18 min, 3 mL/min, 260 nm). Z-Phe-Gly-Gly-OEt eluted at 12.3 min, and the yield was determined to be 93% by peak integration (% isolated). Z-Phe-OH (7%) eluted at 12.84 min. Z-Phe-Gly-Gly-OEt was isolated as a white solid after removal of the solvent by rotary evaporation at 0.25 mm of mercury and 40 °C. The compound was dried over P_2O_5 at 0.05 mm of mercury for 24 h at 80 °C: mp 90−92 °C; ϵ_{260} (CH_3CN) 301 M^{-1} cm^{-1}; ^{1}H NMR (500 MHz, DMSO-d_6) δ 1.18 (t, 3 H, J = 7.10 Hz), 2.77 (dd, 1 H, J = 13.6, 10.8 Hz), 3.06 (dd, 1 H, J = 13.8, 3.9 Hz), 3.78 (d, 2 H, J = 5.7 Hz), 3.85 (dd, 2 H, J = 5.8, 2.4 Hz), 4.09 (q, 2 H, J = 7.1 Hz), 4.31 (m, 1 H), 4.92 (d, 1 H, J = 12.8 Hz), 4.96 (d, 1 H, J = 12.8 Hz), 7.19 (m, 10 H), 7.52 (d, 1 H, J = 8.5 Hz), 8.18 (t, 1 H, J = 5.7 Hz), 8.32 (t, 1 H, J = 5.6 Hz); ^{13}C NMR (125 MHz, DMSO-d_6) δ 14.00 (q), 37.33 (t), 40.61 (t), 41.79 (t), 56.12 (d), 60.39 (t), 65.21 (t), 126.17 (d), 126.77 (s), 127.37 (d), 127.61 (d), 127.98 (d), 128.22 (d), 129.15 (d), 136.94 (s), 138.10 (s), 155.89 (s), 169.18 (s), 169.63 (s), 171.78 (s); HRMS calcd for

(34) Borch, R. F.; Bernstein, M. D.; Durst, H. D. *J. Am. Chem. Soc.* **1971**, *93*, 2897−2904.
(35) DelMar, E. G.; Largman, C.; Brodrick, J. W.; Geokas, M. C. *Anal. Biochem.* **1979**, *99*, 316−320.
(36) Erlanger, B. F.; Kokowsky, N.; Cohen, W. *Arch. Biochem. Biophys.* **1961**, *95*, 271−278.
(37) Abrahmsén, L.; Tom, J.; Burnier, J.; Butcher, K. A.; Kossiakoff, A.; Wells, J. A. *Biochemistry* **1991**, *30*, 4151−4159.
(38) Lowe, G.; Yuthavong, Y. *Biochem. J.* **1971**, *124*, 107.
(39) Guthrie, R. D. In *Methods in Carbohydrate Chemistry*; Whistler, R. L., Wolfrom, M. L., Eds.; Academic Press: New York, 1962; Vol. 1, pp 432−441.

$C_{23}H_{27}N_3O_6$ 441.1900, *m/z* found (EI) 441.1874. Anal. Calcd for $C_{23}H_{27}N_3O_6$: C, 62.57; H, 6.16; N, 9.52. Found: C, 62.45; H, 6.17; N, 9.44.

Z-Tyr-Gly-Gly-OEt (16). To a solution of Z-Tyr-OMe (758 mg, 2.3 mmol) and glycylglycine ethyl ester hydrochloride (1080 mg, 5.5 mmol) in acetonitrile containing 10% water and 10% triethylamine (10 mL) was added CPC[CT] (60 mg, 420 units), and the solution was stirred for 20 h at 40 °C. TLC analysis (2:1 hexanes/ethyl acetate) showed no Z-Tyr-OMe (R_f 0.4). The solution was filtered through a fritted glass funnel, and water (100 mL) was added. This peptide was isolated by precipitation, but could be purified by reverse phase HPLC as described for Z-Phe-Gly-Gly-OEt (**15**). After 1 h, a white precipitate formed that was filtered and dried over P_2O_5 at 0.05 mm of mercury for 24 h at 80 °C to give 817 mg (90% HPLC yield, 78% isolated) of a white powder: mp 160–163 °C; ϵ_{260} (CH_3CN) 664 M^{-1} cm^{-1}; ^1H NMR (500 MHz, DMSO-d_6) δ 1.18 (t, 3 H, J = 7.1 Hz), 2.64 (dd, 1 H, J = 13.8, 10.5 Hz), 2.92 (dd, 1 H, J = 13.8, 4.1 Hz), 3.76 (d, 2 H, J = 5.8 Hz), 3.84 (dd, 2 H, J = 5.8, 2.8 Hz), 4.09 (q, 2 H, J = 7.1 Hz), 4.20 (m, 1 H), 4.92 (d, 1 H, 12.7 Hz), 4.97 (d, 1 H, 12.8 Hz), 6.64 (d, 2 H, J = 8.4 Hz), 7.05 (d, 2 H, J = 8.3 Hz), 7.22–7.34 (m, 5 H), 7.43 (d, 1 H, J = 8.4 Hz), 8.13 (t, 1 H, J = 5.8 Hz), 8.26 (t, 1 H, J = 5.7 Hz), 9.14 (s, 1 H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 14.00 (q), 36.58 (t), 40.60 (t), 41.76 (t), 56.45 (d), 60.39 (t), 65.16 (t), 114.82 (d), 127.33 (d), 127.59 (d), 128.06 (s), 128.22 (d), 130.06 (d), 136.98 (s), 155.72 (s), 155.88 (s), 169.19 (s), 169.61 (s), 171.90 (s). Anal. Calcd for $C_{23}H_{27}N_3O_7$: C, 60.39; H, 5.95; N, 9.19. Found: C, 60.32; H, 5.91; N, 8.97.

Z-His-Gly-Gly-OEt (17). To a solution of Z-His-OMe·HCl (302 mg, 1 mmol) and glycylglycine ethyl ester hydrochloride (589 mg, 3 mmol) in acetonitrile (1 mL) containing triethylamine (0.4 mL) and water (0.3 mL) was added CPC[CT] (50 mg, 70 units), and the solution was shaken in an orbital shaking water bath at 300 osc/min and 40 °C for 72 h. The solution was filtered and Z-His-Gly-Gly-OEt was isolated by reverse phase HPLC using isocratic elution with acetonitrile/0.05 M ammonium bicarbonate (15 min) and acetonitrile (5 min) at a flow rate of 3 mL/min or gradient elution with 0.05 M ammonium bicarbonate/acetonitrile 30/70 to 0/100 over 18 min. Removal of the solvent by rotary evaporation at 0.25 mm of mercury and 40 °C gave 100 mg (83% HPLC yield, 18% isolated) of a white solid that was dried over P_2O_5 at 0.05 mm of mercury for 24 h at 80 °C: mp 150–156 °C dec; ^1H NMR (500 MHz, DMSO-d_6) δ 1.18 (t, 3 H, J = 7.1 Hz), 2.95 (dd, 1 H, J = 15.2, 8.7 Hz), 3.12 (dd, 1 H, J = 15.2, 5.2 Hz), 3.73–3.86 (m, 4 H), 4.09 (q, 2 H, J = 7.2 Hz), 4.38 (m, 1 H), 4.98 (d, 1 H, J = 12.6 Hz), 5.02 (d, 1 H, J = 12.6 Hz), 7.28–7.37 (m, 5 H), 7.60 (d, 1 H, J = 8.3 Hz), 8.28 (t, 1 H, J = 5.6 Hz), 8.32 (t, 1 H, J = 5.6 Hz), 8.88 (s, 1 H), 14.18 (bs, 2 H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 14.00 (q), 27.06 (t), 40.59 (t), 41.82 (t), 53.67 (d), 60.40 (t), 65.59 (t), 116.96 (d), 127.51 (d), 127.76 (d), 128.29 (d), 129.57 (s), 133.81 (d), 136.73 (s), 155.85 (s), 169.25 (s), 169.59 (s), 170.43 (s); HRMS calcd for $C_{20}H_{26}N_5O_6$ (M + H) 432.1883, *m/z* found 432.1898.

Z-Trp-Gly-Gly-OEt (18). This peptide was prepared exactly as descried for Z-His-Gly-Gly-OEt (**5**) except Z-Trp-Gly-Gly-OEt (352 mg, 1 mmol) was used, and the product was isolated as described for Z-Phe-Gly-Gly-OEt (**15**) to give 290 mg (56% HPLC yield, 60% isolated) of a white solid: mp 131–135 °C; ^1H NMR (500 MHz, DMSO-d_6) δ 1.19 (t, 3 H, J = 7.0 Hz), 2.94 (dd, 1 H, J = 14.6, 4.6 Hz), 3.15 (dd, 1 H, J = 14.6, 4.6 Hz), 3.76 (dd, 2 H, J = 5.5, 3.3 Hz), 3.83 (dd, 2 H, J = 5.7, 3.7 Hz), 4.09 (q, 2 H, J = 7.1 Hz), 4.32 (m, 1 H), 4.95 (s, 2 H), 6.96 (t, 1 H, J = 7.4 Hz), 7.06 (t, 1 H, J = 7.2 Hz), 7.16 (d, 1 H, J = 1.5 Hz), 7.24–7.33 (m, 6 H), 7.41 (d, 1 H, J = 8.0 Hz), 7.61 (d, 1 H, J = 7.9 Hz), 8.12 (t, 1 H, J = 5.6 Hz), 8.33 (t, 1 H, J = 5.6 Hz), 10.79 (s, 1 H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 14.01 (q), 27.56 (t), 40.60 (t), 41.87 (t), 55.51 (d), 60.39 (t), 65.28 (t), 110.07 (s), 111.24 (d), 118.15 (d), 118.41 (d), 120.77 (d), 123.78 (d), 127.25 (s), 127.43 (d), 127.62 (d), 128.24 (d), 136.04 (s), 136.90 (s), 155.90 (s), 169.23 (s), 169.61 (s), 172.11 (s). Anal. Calcd for $C_{25}H_{28}N_4O_6$: C, 62.49; H, 5.87; N, 11.66. Found: C, 62.50; H, 5.80; N, 11.49.

Z-Tyr-Gly-OMe (19). To a solution of Z-Tyr-OMe (758 mg, 2 mmol) and glycine methyl ester hydrochloride (1.25 g, 10 mmol) in acetonitrile (2 mL) containing triethylamine (1 mL) and water (0.5 mL) was added CPC[CT] (100 mg, 140 units), and the solution was shaken at 300 osc/min at 40 °C for 72 h. The solution was filtered and acetic acid (1 mL) was added. Z-Tyr-Gly-OMe was isolated as described for Z-Phe-Gly-Gly-OEt (**3**) to give 90 mg (41% HPLC yield, 12% isolated) of a white powder: mp 137–140 °C (lit.[40] 141–143 °C); ^1H NMR (200 MHz, DMSO-d_6) δ 2.61 (m, 1 H), 2.88 (dd, 1 H, J = 14.0, 3.69 Hz), 3.41 (s, 3 H), 3.86 (m, 2 H), 4.14 (m, 1 H), 4.89 (d, 1 H, J = 12.9 Hz), 4.97 (d, 1 H, J = 12.9 Hz), 6.66 (d, 2 H, J = 8.2 Hz), 7.06 (d, 2 H, J = 8.2 Hz), 7.08–7.38 (m, 5 H), 7.47 (d, 1 H, J = 8.7 Hz), 8.46 (t, 1 H, J = 5.5 Hz), 9.19 (s, 1 H); HRMS calcd for $C_{20}H_{23}N_2O_6$ (M + H) 387.1556, *m/z* found 387.1542.

Z-Phe-Gly-Leu-OtBu (20). To a solution of Z-Phe-Gly-OMe (185 mg, 0.5 mmol) and leucine *tert*-butyl ester hydrochloride (223 mg, 1 mmol) in acetonitrile (1.05 mL) containing triethylamine (0.2 mL), water (0.15 mL), and 2-mercaptoethanol (0.1 mL) was added CPC[papain] (CPC[P], 40 mg, 15–30 units), and the heterogeneous solution was shaken at 40 °C and 300 osc/min for 72 h. Z-Phe-Gly-Leu-OtBu was isolated as described for Z-Phe-Gly-Gly-OEt (**15**) to give 125 mg (81% HPLC yield, 48% isolated) of a white solid: mp 89–95 °C; ϵ_{260} (CH_3CN) 280 $M^{-1}cm^{-1}$; ^1H NMR (500 MHz, DMSO-d_6) δ 0.84 (d, 3 H, J = 6.6 Hz), 0.89 (d, 3 H, J = 6.6 Hz), 1.39 (s, 9 H), 1.49 (m, 2 H), 1.62 (m, 1 H), 2.74 (dd, 1 H, J = 13.7, 10.7 Hz), 3.02 (dd, 1 H, J = 13.8, 3.9 Hz), 3.75 (d, 2 H, J = 5.7 Hz), 4.18 (ddd, 1 H, J = 8.1, 8.1, 8.1 Hz), 4.26 (m, 1 H), 4.92 (d, 1 H, J = 12.7 Hz), 4.95 (d, 1 H, J = 12.7 Hz), 7.17–7.33 (m, 10 H), 7.52 (d, 1 H, J = 8.3 Hz), 7.96 (d, 1 H, J = 7.8 Hz), 8.27 (t, 1 H, J = 5.6 Hz); ^{13}C NMR (125 MHz, DMSO-d_6) δ 21.43 (q), 22.59 (q), 24.17 (d), 27.56 (q), 37.26 (t), 40.06 (t), 41.70 (t), 50.87 (d), 56.15 (d), 65.17 (t), 80.47 (q), 126.16 (d), 127.36 (d), 127.61 (d), 127.97 (d), 128.21 (d), 129.11 (d), 136.88 (s), 138.08 (s), 155.86 (s), 168.56 (s), 171.52 (s), 171.71 (s); HRMS calcd for $C_{32}H_{40}N_3O_6$ (M + H) 526.2917, *m/z* found 526.2922. Anal. Calcd for $C_{29}H_{39}N_3O_6$: C, 66.27; H, 7.48; N, 7.99. Found: C, 66.15; H, 7.39; N, 7.94.

Z-Phe-Gly-Leu-OMe (21). This peptide was prepared exactly as described for Z-Phe-Gly-Leu-OtBu (**20**) except leucine methyl ester hydrochloride (181 mg, 1 mmol) was used to give 181 mg (77% HPLC yield, 37% isolated) of a white solid: mp 91–94 °C; ^1H NMR (500 MHz, DMSO-d_6) δ 0.84 (d, 3 H, J = 6.5 Hz), 0.88, (d, 3 H, J = 6.5 Hz), 1.47–1.64 (m, 3 H), 2.74 (dd, 1 H, J = 13.7, 10.8 Hz), 3.02 (dd, 1 H, J = 13.8, 4.0 Hz), 3.62 (s, 3 H), 3.76 (d, 2 H, J = 5.7 Hz), 4.26 (m, 1 H), 4.32 (m, 1 H), 4.92 (d, 1 H, J = 12.7 Hz), 4.95 (d, 1 H, J = 12.7 Hz), 7.18–7.33 (m, 10 H), 7.52 (d, 1 H, J = 8.3 Hz), 8.10 (d, 1 H, J = 7.7 Hz), 8.28 (t, 1 H, J = 5.6 Hz); ^{13}C NMR (125 MHz, DMSO-d_6) δ 21.31 (q), 22.61 (q), 24.08 (d), 37.24 (t), 39.83 (t), 39.87 (t), 41.66 (t), 50.15 (d), 51.79 (q), 56.13 (d), 65.19 (t), 126.16 (d), 127.37 (d), 127.61 (d), 127.97 (d), 128.21 (d), 129.11 (d), 136.88 (s), 138.07 (s), 155.88 (s), 168.69 (s), 171.72 (s), 172.77 (s). Anal. Calcd for $C_{26}H_{33}N_3O_6$: C, 64.58; H, 6.88; N, 8.69. Found: C, 64.13; H, 6.85; N, 8.59.

Z-Gly-Leu-OtBu (22). To a solution of Z-Gly-OMe (116 mg, 0.5 mmol) and leucine *tert*-butyl ester hydrochloride (335 mg, 1.5 mmol) in acetonitrile (1.0 mL) containing triethylamine (0.2 mL), water (0.2 mL), and 2-mercaptoethanol (0.1 mL) was added CPC[P] (50 mg, 20 units), and the solution was shaken at 40 °C and 300 osc/min for 72 h. The solution was filtered through a fritted funnel, and Z-Gly-Leu-OtBu was isolated by reverse phase HPLC as described for Z-Phe-Gly-Gly-OEt (**15**) to give 136.4 mg (74% HPLC yield, 72% isolated) of a semisolid: ^1H NMR (500 MHz, DMSO-d_6) δ 0.83 (d, 3 H, J = 6.5 Hz), 0.88 (d, 3 H, J = 6.5 Hz), 1.38 (s, 9 H), 1.42–1.57 (m, 2 H), 1.61 (m, 1 H), 3.61 (dd, 1 H, J = 16.8, 6.2 Hz), 3.67 (dd, 1 H, J = 16.8, 6.2 Hz), 4.15 (dd, 1 H, J = 14.3, 8.0 Hz), 5.02 (s, 2 H), 7.30–7.39 (m, 6 H), 8.03 (d, 1 H, J = 7.8 Hz); ^{13}C NMR (125 MHz, DMSO-d_6) δ 21.41 (q), 22.61 (q), 24.20 (d), 27.56 (q), 40.04 (t), 43.08 (t), 50.89 (d), 65.35 (t), 80.42 (s), 127.60 (d), 127.70 (d), 128.26 (d), 137.02 (s), 156.37 (s), 169.01 (s), 171.60 (s); HRMS calcd for $C_{20}H_{31}N_2O_5$ (M + H) 379.2233, *m/z* found 379.2232.

(40) Beacham, J.; Bentley, P. H.; Kenner, G. W.; MacLeod, J. K.; Mendive, J. J.; Sheppard, R. C. *J. Chem. Soc.* **1967**, 2520.

Z-Ala-Leu-OtBu (23). This peptide was prepared exactly as described for Z-Gly-Leu-OtBu (**22**) except Z-Ala-OMe (120 mg, 0.5 mmol) was used to give 133 mg (72% HPLC yield, 68% isolated) of a white solid: mp 90–92 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 0.83 (d, 3 H, J = 6.5 Hz), 0.89 (d, 3 H, J = 6.6 Hz), 1.21 (d, 3 H, J = 7.15 Hz), 1.40 (s, 9 H), 1.43–1.50 (m, 2 H), 1.53–1.67 (m, 1 H), 4.05–4.14 (m, 2 H), 5.00 (s, 2 H), 7.30–7.41 (m, 6 H), 8.00 (d, 1 H, J = 7.6 Hz). ^{13}C NMR (125 MHz, DMSO-d_6) δ 21.42 (q), 22.67 (q), 24.22 (d), 27.57 (q), 37.42 (t), 51.15 (d), 55.80 (d), 65.11 (t), 80.42 (s), 126.24 (d), 127.41 (d), 127.65 (d), 128.01 (d), 128.26 (d), 129.20 (d), 137.06 (s), 138.11 (s), 155.82 (s), 171.57 (s), 171.68 (s). HRMS calcd for $C_{21}H_{33}N_2O_5$ (M + H) 393.2389, m/z found 393.2379.

Z-Phe-Gly-Phe-OtBu (24). This peptide was prepared exactly as described for Z-Phe-Gly-Leu-OtBu (**20**) except phenylalanine *tert*-butyl ester hydrochloride (258 mg, 1 mmol) was used to give 149 mg (72% HPLC yield, 27% isolated) of a white solid: mp 60–66 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 1.30 (s, 9 H), 2.73 (dd, 1 H, J = 13.7, 10.9 Hz), 2.88–3.03 (m, 3 H), 3.74 (t, 2 H, J = 5.2 Hz), 4.27 (m, 1 H), 4.38 (m, 1 H), 4.91 (d, 1 H, J = 12.8 Hz), 4.94 (d, 1 H, J = 12.8 Hz), 7.18–7.32 (m, 15 H), 7.50 (d, 1 H, J = 8.4 Hz), 8.13 (d, 1 H, J = 7.6 Hz), 8.25 (t, 1 H, J = 5.6 Hz); ^{13}C NMR (125 MHz, DMSO-d_6) δ 27.46 (s), 37.07 (t), 37.32 (t), 39.00 (s), 41.67 (t), 54.04 (d), 56.12 (d), 65.16 (t), 80.71 (s), 126.15 (d), 126.46 (d), 127.35 (d), 127.59 (d), 127.97 (d), 128.12 (d), 128.20 (d), 129.13 (d), 136.90 (s), 136.99 (s), 138.09 (s), 155.85 (s), 168.53 (s), 170.36 (s), 171.72 (s); HRMS calcd for $C_{32}H_{38}N_3O_6$ (M + H) 560.2761, m/z found 560.2795. Anal. Calcd for $C_{32}H_{37}N_3O_6$: C, 68.68; H, 6.66; N, 7.51. Found: C, 68.25; H, 6.59; N, 7.42.

Z-Ser-Leu-OtBu (25). This peptide was prepared exactly as described for Z-Gly-Leu-OtBu (**22**) except Z-Ser-OMe (120 mg, 0.5 mmol) was used to give 102.3 mg (38% HPLC yield, 38% isolated) of an oil: ^{1}H NMR (500 MHz, DMSO-d_6) δ 0.83 (d, 3 H, J = 6.5 Hz), 0.88 (d, 3 H, J = 6.5 Hz), 1.37 (s, 9 H), 1.43–1.51 (m, 2 H), 1.62 (bs, 1 H), 3.50 (dd, 1 H, J = 10.9, 7.2 Hz), 3.61 (dd, 1 H, J = 11.0, 4.6 Hz), 4.09–4.17 (m, 2 H), 4.75 (bs, 1 H), 5.02 (s, 2 H), 7.16 (d, 1 H, J = 8.32 Hz), 7.29–7.36 (m, 5 H), 8.00 (d, 1 H, J = 7.7 Hz); ^{13}C NMR (125 MHz, DMSO-d_6) δ 21.46 (q), 22.64 (q), 24.14 (d), 27.51 (q), 39.91 (t), 51.02 (d), 57.13 (d), 61.79 (t), 65.38 (t), 80.39 (s), 127.64 (d), 127.71 (d), 128.25 (d), 136.94 (s), 155.82 (s), 169.95 (s), 171.40 (s); HRMS calcd for $C_{21}H_{33}N_2O_6$ (M + H) 409.2339, m/z found 409.2325.

Z-Gln-Leu-OtBu (26). This peptide was prepared exactly as described for Z-Gly-Leu-OtBu (**22**) except Z-Gln-OMe (147 mg, 0.5 mmol) was used to give 57.2 mg (44% HPLC yield, 25% isolated) of a white solid: mp 122–125 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 0.83 (d, 3 H, J = 6.5 Hz), 0.89 (d, 3 H, J = 6.5 Hz), 1.37 (s, 9 H), 1.40–1.53 (m, 2 H), 1.60–1.72 (m, 2 H), 1.90 (m, 1 H), 2.06–2.19 (m, 2 H), 4.00 (ddd, 1 H, J = 8.9, 8.7, 4.9 Hz), 4.12 (ddd, 1 H, 9.2, 7.8, 5.8 Hz), 5.01 (s, 2 H), 6.72 (s, 1 H), 7.21 (s, 1 H), 7.30–7.37 (m, 6 H), 8.06 (d, 1 H, 7.6 Hz); ^{13}C NMR (125 MHz, DMSO-d_6) δ 21.41 (q), 22.66 (q), 24.19 (d), 27.56 (q), 27.86 (t), 31.55 (t), 39.81 (t), 51.05 (d), 54.09 (d), 65.33 (t), 80.42 (s), 127.66 (d), 127.77 (d), 128.33 (d), 137.04 (s), 155.87 (s), 171.57 (s), 171.69 (s), 173.77 (s); HRMS calcd for $C_{23}H_{36}N_3O_6$ (M + H) 450.2604, m/z found 450.2633.

Z-Leu-Leu-OtBu (27). This peptide was prepared exactly as described for Z-Gly-Leu-OtBu (**22**) except Z-Leu-OMe (139 mg, 0.5 mmol) was used to give 27.5 mg (41% HPLC yield, 13% isolated) of a white solid: mp 126–127 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 0.82–0.89 (m, 12 H), 1.36 (s, 9 H), 1.40–1.53 (m, 4 H), 1.53–1.64 (m, 2 H), 4.06 (dd, 1 H, J = 15.4, 8.2 Hz), 4.13 (ddd, 1 H, J = 9.4, 7.7, 5.6 Hz), 4.99 (d, 1 H, J = 13.0 Hz), 5.02 (d, 1 H, J = 12.9 Hz), 7.29–7.36 (m, 6 H), 8.03 (d, 1 H, 7.7 Hz); ^{13}C NMR (125 MHz, DMSO-d_6) δ 21.38 (q), 21.46 (q), 22.67 (q), 22.97 (q), 24.10 (d), 24.16 (d), 27.54 (q), 39.75 (t), 40.78 (t), 51.00 (d), 52.79 (d), 65.24 (t), 80.27 (s), 127.60 (d), 127.73 (d), 128.30 (d), 137.13 (s), 155.85 (s), 171.53 (s), 172.36; HRMS calcd for $C_{24}H_{39}N_2O_5$ (M + H) 435.2859, m/z found 435.2889.

Z-Met-Leu-OtBu (28). This peptide was prepared exactly as described for Z-Gly-Leu-OtBu (**22**) except Z-Met-OMe (149 mg, 0.5 mmol) was used to give 66.7 mg (35% HPLC yield, 30% isolated) of a white solid: mp 82–85 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 0.80 (d, 3 H, J = 6.4 Hz), 0.86 (d, 3 H, J = 6.5 Hz), 1.35 (s, 9 H), 1.38–1.54 (m, 3 H), 1.61 (m, 1 H), 1.77 (m, 1 H), 1.85 (m, 1 H), 2.00 (s, 3 H), 2.46 (m, 1 H), 4.09 (m, 2 H), 4.98 (s, 2 H), 7.27–7.35 (m, 5 H), 7.39 (d, 1 H, J = 8.2 Hz), 8.08 (d, 1 H, J = 7.6 Hz); ^{13}C NMR (125 MHz, DMSO-d_6) δ 14.55 (q), 21.38 (q), 22.63 (q), 24.18 (d), 27.53 (q), 29.56 (t), 31.85 (t), 39.70 (t), 51.07 (d), 53.59 (d), 65.31 (t), 80.33 (s), 127.60 (d), 127.70 (d), 128.24 (d), 136.97 (s), 155.82 (s), 171.43 (s), 171.46 (s); HRMS calcd for $C_{23}H_{37}N_2O_5S$ (M + H) 453.2423, m/z found 453.2455.

Z-Gly-Gly-Leu-OtBu (29). This peptide was prepared exactly as described for Z-Gly-Leu-OtBu (**22**) except Z-Gly-Gly-OMe (140 mg, 0.5 mmol) was used to give 67 mg (31% isolated) of an oil: ^{1}H NMR (500 MHz, DMSO-d_6) δ 0.83 (d, 3 H, J = 6.6 Hz), 0.88 (d, 3 H, J = 6.6 Hz), 1.38 (s, 9 H), 1.49 (m, 2 H), 1.62 (m, 1 H), 3.64 (d, 2 H, J = 5.9 Hz), 3.72 (dd, 1 H, J = 16.7, 5.7 Hz), 3.77 (dd, 1 H, J = 16.7, 5.8 Hz), 4.17 (m, 1 H), 5.03 (s, 2 H), 7.29–7.36 (m, 5 H), 7.48 (t, 1 H, J = 5.9 Hz), 7.99 (d, 1 H, J = 7.8 Hz), 8.06 (t, 1 H, J = 5.5 Hz); ^{13}C NMR (125 MHz, DMSO-d_6) δ 21.40 (q), 22.62 (q), 24.19 (d), 27.57 (q), 40.00 (t), 41.56 (t), 43.57 (t), 50.88 (d), 65.47 (t), 80.43 (s), 127.65 (d), 127.73 (d), 128.28 (d), 136.95 (s), 156.50 (s), 168.66 (s), 169.26 (s), 171.54 (s); HRMS calcd for $C_{22}H_{34}N_3O_6$ (M + H) 436.2448, m/z found 436.2448.

Z-Gln-Phe-OtBu (30). This peptide was prepared exactly as described for Z-Gly-Leu-OtBu (**22**) except Z-Gln-OMe (147 mg, 0.5 mmol) and phenylalanine *tert*-butyl ester hydrochloride (387 mg, 1.5 mmol) were used to give 54.9 mg (23% isolated) of a white solid: mp 122–125 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 1.30 (s, 9 H), 1.67 (m, 1 H), 1.86 (m, 1 H), 2.10 (m, 2 H), 2.94 (m, 2 H), 4.00 (ddd, 1 H, J = 8.7, 8.4, 5.1 Hz) 4.34 (dd, 1 H, J = 14.5, 7.3 Hz), 5.00 (s, 2 H), 6.72 (s, 1 H), 7.18–8.07 (m, 12 H), 8.15 (d, 1 H, J = 7.5 Hz); ^{13}C NMR (125 MHz, DMSO-d_6) δ 27.45 (q), 27.80 (t), 31.47 (t), 36.78 (t), 54.08 (d), 65.34 (t), 80.64 (s), 126.41 (d), 127.62 (d), 127.71 (d), 128.07 (d), 128.27 (d), 129.16 (d), 136.94 (s), 137.04 (s), 155.77 (s), 170.28 (s), 171.54 (s), 173.65 (s); HRMS calcd for $C_{26}H_{34}N_3O_6$ (M + H) 484.2448 m/z found 484.2471.

Z-Phe-Leu-OtBu (31). This peptide was prepared exactly as described for Z-Gly-Leu-OtBu (**22**) except Z-Phe-OMe (156 mg, 0.5 mmol) was used to give 16.6 mg (12% HPLC yield, 7% isolated) of a white solid: mp 89–92 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 0.85 (d, 3 H, J = 6.5 Hz), 0.91 (d, 3 H, J = 6.6 Hz), 1.38 (s, 9 H), 1.51 (m, 2 H), 1.65 (m, 1 H), 2.73 (dd, 1 H, J = 13.6, 11.0 Hz), 3.00 (dd, 1 H, J = 13.9, 3.5 Hz), 4.16 (m, 1 H), 4.29 (m, 1 H), 4.92 (s, 2 H), 7.16–7.33 (m, 10 H), 7.41 (d, 1 H, J = 8.8 Hz), 8.23 (d, 1 H, J = 7.7 Hz); ^{13}C NMR (125 MHz, DMSO-d_6) δ 21.41 (q), 22.65 (q), 24.21 (d), 27.55 (q), 37.41 (t), 39.84 (t), 51.12 (d), 55.77 (d), 65.07 (t), 80.38 (s), 126.18 (d), 126.79 (d), 127.34 (d), 127.59 (d), 127.95 (d), 128.19 (d), 129.13 (d), 136.99 (s), 138.04 (s), 155.74 (s), 171.48 (s), 171.59 (s); HRMS calcd for $C_{27}H_{37}N_2O_5$ (M + H) 468.2702, m/z found 468.2698.

Z-Tyr-Gly-Gly-Phe-Leu-OH (32). To a solution of Z-Tyr-Gly-OMe (10 mg, 26 μmol) and H_2N-Gly-Phe-Leu-OH (34.5 mg, 103 μmol) in acetonitrile (100 μL) containing triethylamine (30 μL), water (80 μL), and 2-mercaptoethanol (5 μL) was added CPC[P] (10 mg, 3 units), and the solution was shaken at 35 °C and 150 osc/min for 8 h. HPLC analysis was performed as described as for Z-Phe-Gly-Gly-OEt (**15**). Z-Tyr-Gly-OH was formed in 21% yield and Z-Tyr-Gly-Gly-Phe-Leu-OH was formed in 79% yield based on peak integration. ^{1}H NMR was identical to that determined for the same compound prepared by a CPC[proteinase K]-catalyzed synthesis. To a solution of Z-Tyr-Gly-Gly-OEt (100 mg, 0.22 mmol) and phenylalanylglycine (305 mg, 1.1 mmol, 5 eq) in acetonitrile (2.18 mL) containing triethylamine (0.4 mL) and water (0.02 mL) was added CPC[proteinase K] (CPC[pK], 25 mg), and the solution was shaken at 40 °C and 300 osc/min. The reaction was monitored by reverse phase HPLC. At 4 and 7 days, 25 additional mg of CPC[pK] was added, and the product was isolated by HPLC as described for Z-Phe-Gly-Gly-OEt (**15**) to give 48.7 mg (32%) of a white solid: mp 120–128 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 0.83 (d, 3 H, J = 6.5 Hz), 0.89 (d, 3 H, J = 6.5 Hz), 1.48–1.66 (m, 3 H), 2.62 (dd, 1 H, J = 13.7, 10.7 Hz), 2.75 (dd, 1 H, J = 13.9, 9.8 Hz), 2.91 (dd, 1 H, J = 13.9,

3.9 Hz), 3.03 (dd, 1 H, J = 14.0, 4.1 Hz), 3.61 (dd, 1 H, J = 16.8, 5.6 Hz), 3.69–3.73 (m, 3 H), 4.16–4.24 (m, 2 H), 4.56 (ddd, 1 H, J = 4.1, 4.0, 3.6 Hz), 4.90 (d, 1 H, J = 12.6 Hz), 4.96 (d, 1 H, J = 12.8 Hz), 6.63 (d, 2 H, J = 8.3 Hz), 7.04 (d, 2 H, J = 8.3 Hz), 7.14–7.34 (m, 10 H), 7.41 (d, 1 H, J = 8.5 Hz), 7.93 (t, 1 H, J = 5.5 Hz), 8.00 (d, 1 H, J = 8.5 Hz), 8.19–8.23 (m, 2 H), 9.13 (s, 1 H), 12.49 (bs, 1 H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 21.32 (q), 22.77 (q), 24.21 (d), 36.57 (t), 37.56 (t), 39.83 (t), 41.68 (t), 42.01 (t), 50.26 (d), 53.48 (d), 56.44 (d), 65.14 (t), 114.79 (d), 126.17 (d), 127.33 (d), 127.56 (d), 127.94 (d), 128.10 (d), 128.20 (d), 129.17 (d), 130.03 (d), 136.97 (s), 137.70 (s), 155.69 (s), 155.83 (s), 168.23 (s), 168.82 (s), 171.01 (s), 171.92 (s), 173.80 (s); HRMS calcd for $C_{36}H_{44}N_5O_9$ (M + H) 690.3139, m/z found 690.3171.

Z-Asp-Phe-OMe (33). To a solution of Z-Asp-OH (68 mg, 0.25 mmol) and phenylalanine methyl ester hydrochloride (430 mg, 2 mmol) in acetonitrile (1.6 mL) containing triethylamine (0.2 mL) and water (0.2 mL) was added CPC[thermolysin] (30 mg), and the solution was shaken at 40 °C and 300 osc/min for 72 h. Acetic acid (1 mL) was added, and the solution was filtered through a fritted funnel. HPLC was performed as described for Z-Phe-Gly-Gly-OEt (**15**) to give 69.2 mg (90% HPLC yield, 66% isolated) of a white powder: mp 131-133 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 2.44 (dd, 1 H, J = 16.6, 9.3 Hz), 2.59 (dd, 1 H, J = 16.6, 4.5 Hz), 2.94 (dd, 1 H, J = 13.8, 8.5 Hz), 3.01 (dd, 1 H, J = 13.8, 5.7 Hz), 3.57 (s, 3 H), 4.38 (ddd, 1 H, J = 8.7, 8.7, 4.4 Hz), 4.45 (ddd, 1 H, J = 7.0, 7.0, 7.0 Hz), 5.00 (d, 1 H, J = 12.7 Hz), 5.02 (d, 1 H, J = 13.8 Hz), 7.18–7.36 (m, 10 H), 7.48 (d, 1 H, J = 8.2 Hz), 8.21 (d, 1 H, J = 7.5 Hz), 12.30 (bs, 1 H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 36.18 (t), 36.44 (t), 51.11 (d), 51.79 (q), 53.63 (d), 65.44 (t), 126.51 (d), 126.80 (s), 127.63 (d), 127.74 (d), 128.18 (d), 128.28 (d), 129.03 (d), 136.86 (s), 136.94 (s), 155.70 (s), 171.01 (s), 171.53 (s), 171.53 (s), 171.64 (s). Anal. Calcd for $C_{22}H_{24}N_2O_7$: C, 61.68; H, 5.65; N, 6.54. Found: C, 61.42; H, 5.44; N, 6.45.

Z-Ala-Phe-Leu-OH (34). To a solution of Z-Ala-OMe (360 mg, 1.5 mmol) and phenylalanylleucine (834 mg, 3 mmol) in acetonitrile (4.5 mL) containing triethylamine (1.2 mL) and water (0.8 mL) was added CPC[subtilisin BPN'] (100 mg) and the solution was shaken at 40 °C and 300 osc/min for 20 h. Z-Ala-Phe-Leu-OH was isolated as described for Z-Phe-Gly-Gly-OEt (**15**) to give 113.5 mg (39% HPLC yield, 16% isolated) of a white powder: mp 142-146 °C; ^{1}H NMR (500 MHz, DMSO-d_6) δ 0.83 (d, 3 H, J = 6.4 Hz), 0.88 (d, 3 H, J = 6.5 Hz), 1.10 (d, 3 H, J = 7.1 Hz), 1.53 (m, 2 H), 1.63 (m, 1 H), 2.81 (dd, 1 H, J = 13.7, 9.2 Hz), 3.04 (dd, 1 H, J = 13.8, 4.0 Hz), 3.99 (dd, 1 H, J = 7.2, 7.2 Hz), 4.24 (dd, 1 H, J = 14.5, 8.3 Hz), 4.54 (bs, 1 H), 5.00 (d, 1 H, J = 12.5 Hz), 5.02 (d, 1 H, J = 12.7 Hz), 7.16–7.39 (m, 11 H), 7.84 (d, 1 H, J = 8.3 Hz), 8.12 (d, 1 H, J = 7.8 Hz), 12.54 (bs, 1 H); ^{13}C NMR (125 MHz, DMSO-d_6) δ 18.00 (q), 21.31 (q), 22.75 (q), 24.15 (d), 37.29 (t), 39.97 (t), 50.20 (d), 53.27 (d), 65.34 (t), 126.11 (d), 127.63 (d), 127.71 (d), 127.86 (d), 128.27 (d), 129.24 (d), 136.89 (s), 137.53 (s), 155.55 (s), 170.79 (s), 172.05 (s), 173.76 (s); HRMS calcd for $C_{26}H_{34}N_3O_6$ (M + H) 484.2448, m/z found 484.2452.

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