Protein Backbone Modification by Novel C^{α}–C Side-Chain Scission†

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Abstract: α-Ketoamide (-NH-CO-CO-) units in intact peptides are generated from Ser/Thr residues via Ru(VIII)-catalyzed C^{α}–C side-chain scission. Facets associated with this novel α-carbon modification have been probed with 75 peptides chosen to represent every possible peptide environment. The reactions were carried out at room temperature with *in situ* generated Ru(VIII) in biphasic (CH_3CN/CCl_4/pH 3 phosphate buffer, 1:1:2 v/v) medium. Whereas Ser/Thr residues placed at the C-terminal end in peptides undergo N–C bond scission leading to des-Ser/Thr peptide amides—thus acting as Gly equivalents in simulating the α-amidating action of pituitary enzymes—those located at the N-terminal or nonterminal or even at the C-terminal position (protected as amide) were found to undergo oxidative C–C bond scission (involving C^{α} and C side-chain bond), resulting in the generation of α-ketoamide (-NH-CO-CO-) units in the intact peptide backbone. The difference in the products arising from C^{α}–C side-chain scission of Ser/Thr esters and amides is rationalized on the basis of a common mechanism involving either oxaloesters [Pep-NH-CO-COX; X = OMe] or oxalamides [X = NH_2 or NH-Pep] arising from the oxidation of initially formed carbinolamide intermediates [Pep-NH-CH(OH)-COX], wherein, while the former are shown to undergo hydrolysis to terminal amides [Pep-NH_2], the oxalamides are found to be stable to hydrolysis. Ancillary noteworthy findings are those of peptide bond scission when contiguous Ser-Ser/Thr-Thr residues are present and the oxidative cleavage at C-terminal Tyr/Trp sites generating des amides. The oxidative methodology presented here is mild, simple, and practical and proceeds with chiral retention. The insensitivity of a large number of amino acid residues, such as Gly, Ala, Leu, Asn, Gln, Asp, Glu, Pro, Arg, Phe, Lys, Val, and Aib, and N-protecting groups, such as Boc, Z, and Bz, toward Ru(VIII) under the experimental conditions should make this methodology practical and useful. Sulfur-containing amino acids Cys and Met get oxidized to sulfones in the products.

Introduction

The capability to design and construct new proteins with tailor-made structural and functional properties is a goal that is currently being vigorously pursued.[1] The rapid developments that have taken place pertaining to protein synthesis[2] have not been matched with knowledge relating to the relationship between the primary sequence and its folding profile.[3] A promising approach toward protein design[4] would be to take advantage of detailed information pertaining to structure ensembles collected from protein crystallographic data bases.

Proteins with altered functions can be created from existing molecules by modification, either by genetic manipulations[5] involving addition or deletion of specific amino acid residues at the genomic level or, particularly in the case of relatively small peptides, by chemical methods,[6,7] through side-chain alteration, or by backbone modification. Although protein modification by gene manipulation finds ample illustration, there are only a few examples of proteins where new functional molecules have been created through side-chain alteration.[8] To the best of our knowledge, there has been no approach to protein design based on genetically or chemically mediated backbone modification.[9] We felt that this approach must be explored, particularly since the rigidity and flexibility of the protein molecule is manifested largely in its backbone and even marginal changes in the peptide backbone may lead to conformers with altered biological profiles. Indeed, in the past 10–15 years, design and synthesis of backbone-modified pseudopeptides or peptide isosteres[10] containing numerous surrogates of the amide carbonyl (CO), the amide nitrogen (NH), or both groups (CONH) has emerged as a popular endeavor in peptide chemistry and is fast becoming the most popular approach to overcome the poor stability, lack of oral absorption, and marginal ability to cross the blood–brain barrier in the use of peptides as therapeutic agents. The resulting isosteres have

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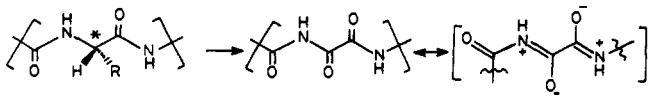

Figure 1.

shown, for instance, great biological interest as protease-resisting analogs and, in some cases, as potent protease inhibitors. Inherent in the concept of isosteric replacement of amide bonds in biologically active peptides is the postulate that it might be possible to modify one or more amide bonds in peptides such that the conformation and binding are maintained but enzymatic hydrolysis is prevented.[11]

The peptide backbone (-NH-CHR-CO)$_n$ comprises three repeating elements, NH, CHR and CO. Modification of the backbone would mean a change at any one of these elements or a combined (CONH) unit of the peptide. In our opinion, the most challenging task, synthetically, appears to be modification at the α-carbon in the peptide backbone. Unfortunately, so far this transformation has involved arduous synthetic endeavors. It is no wonder that there are only a few examples[12] of backbone modification at the α-carbon in peptides and there is only one example of backbone modification in proteins involving none other than the most common L → D isomer change.[13]

In this article, a novel strategy for backbone modification at the α-carbon of Ser and Thr residues in peptides is presented. The transformation of the α-carbon to a carbonyl (CO) function was considered a novel and exciting possibility to create peptide designs with altered conformation and modified electronic properties. This transformation, in essence, would mean the replacement of a chiral sp^3 carbon with a sp^2 hybridized center and would be expected to impart more rigidity and extended conjugation, arising from the resulting bis-dipeptide segment -CO-NH-CO-CO-NH-, as shown in Figure 1.

Logically, this transformation would arise from oxidative scission of the C$^\alpha$–C side chain of an amino acid. Our recent demonstration[14] of such a C$^\alpha$–C side-chain scission involving C-terminal Ser/Thr residues, leading to the successful chemical simulation of the terminal amidation[15] associated with the formation of pituitary hormones from their Gly extended precursors, suggested that the desired C$^\alpha$ → C=O modification might be feasible with the proper delineation of conditions that would promote the further oxidation of the carbinolamide intermediate (Scheme 1) to stable products. This objective has been realized from studies with 75 peptides, chosen to represent every possible environment.

Extensive work[16,17] from our laboratory on the side-chain engineering of amino acids in peptides by oxidative methods has shown that Ru(VIII), generated *in situ* from $RuCl_3$ and $NaIO_4$, oxidizes 13 of the 20 coded amino acids and is, in terms of the volt equivalent, a powerful oxidizing agent,[18] second only to ozone! The experimental protocol for Ru(VIII) oxidation of coded amino acids, developed by us, offers several advantages. The Ru(VIII) species here is generated in a cyclic operation promoted by added periodate, wherein the reagent, formed in water of the biphasic $CCl_4/CH_3CN/H_2O$ system, is partitioned preponderantly in favor of the organic phase. Thus, substrates and products remain largely in the organic phase, permitting easy isolation of the products. A high degree of selectivity can be obtained by permuting the time for the reaction, the pH of the aqueous phase, and the number of equivalents of periodate.[16]

Results and Discussion

The discovery of the novel C$^\alpha$–C side-chain fragmentation resulted when N,C-protected serine and threonine (entry **1**, Table 1) were subjected to oxidation, wherein the Ru(VIII) species was generated in a catalytic cycle from $RuCl_3$ (2 mol %) and $NaIO_4$ (18 equiv) in CH_3CN/CCl_4/pH 3 phosphate buffer, 1:1:2 v/v/v, at room temperature for 1.5 h. Surprisingly, the sole isolable product in both cases was benzamide (~80%). The rationalization of this change led to the delineation of a novel scission, which has the potential for the generation of terminal amides from Ser/Thr extended precursors.

Thus, entries **1–21** (Table 1) representing peptides, terminating in Ser and Thr, on treatment with Ru(VIII) under identical conditions afforded the expected C-terminal amides in good yields and with chiral retention.

This facile C–N bond rupture of a Ser/Thr residue can be rationalized on the basis of a retroaminal fragmentation of carbinolamide arising from addition of water to the initially formed acylimine, which, in turn, is produced by the oxidative scission of a Ser/Thr C$^\alpha$–C side-chain bond, involving either a cyclic or an open-chain ruthenium intermediate. Alternatively, the carbinolamides may undergo further oxidation to oxalamido esters and then to terminal amides by hydrolytic cleavage (Scheme 1). The overall process generates a C-terminal amide retaining the Ser/Thr nitrogen atom and releasing the C_2 unit either as glyoxylate or as oxalate.[19]

The oxidative scission of Ser/Thr-extended precursors to des-Ser/Thr peptide amides simulates[20] the natural process of terminal α-amidation in the post-translational processing of Gly-extended hormonal precursors by pituitary enzymes. It is important to note that both the natural and the chemical processes proceed with the intermediacy of a carbinolamide.

Table 1 presents a large variety of Ser and Thr peptides with Ser/Thr in combination with almost all the coded amino acids and placed at the C-terminal end of the peptide. These peptides were prepared by conventional coupling procedures using the solution-phase method. The oxidation profile of peptides **1–21** as presented in Table 1 shows that all peptides, with the exception **3**, **4**, and **9**, were transformed directly into terminal amides in good yields and with chiral retention via the pathway shown in Scheme 1.

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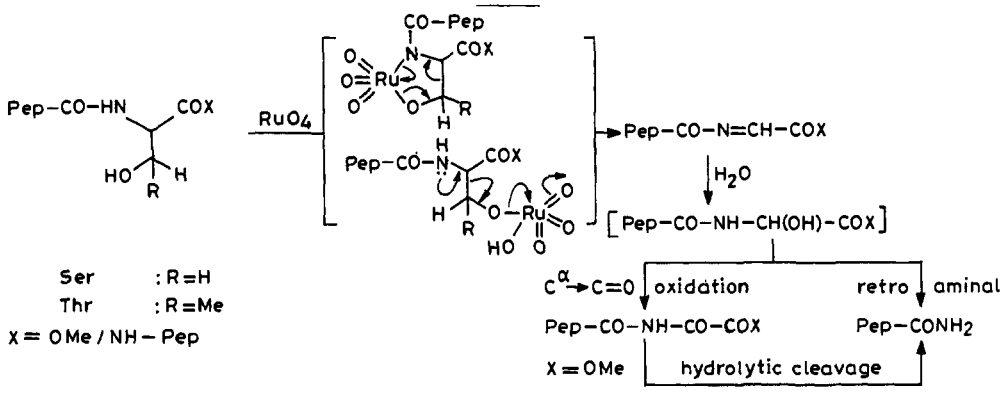
Scheme 1. Oxidative Scission of Ser/Thr Precursors with Ru(VIII)



The isolation of crystalline, chain intact, oxalamido esters in the controlled (0.5 h) oxidation of Z/Bz-Gly-Ser/Thr-OMe (**3**/**4**, Table 1) and their further transformation to terminal amides (8 h) indicated that C-terminal amides may arise by slow hydrolysis of oxalamido esters.

In Table 1, the insensitivity of Gly, Ala, Leu, Asn, Gln, Asp, Glu, Phe, Pro, Arg, and Val residues and N-protecting groups Boc (*tert*-butyloxycarbonyl), Z (benzyloxycarbonyl), or Bz (benzoyl) to Ru(VIII) oxidation is noteworthy. Methionine residue, however, was oxidized to sulfone (entry **14**, Table 1). An additional attractive feature of Table 1 is the smooth formation of α-amides, otherwise difficult to obtain from Asp and Glu bis-esters (entries **10** and **11**).

These results have enabled the delineation of a practical, *in vitro* model for the terminal α-amidation reaction using either a serine or a threonine residue in place of glycine.

A dramatic variant of Ru(VIII)-catalyzed terminal α-amidation involving Ser/Thr residues was discovered when the C-terminal protection was -$CONH_2$ in place of -COOMe. Thus, whereas Bz-Ser-OMe (**1**) resulted in N–C bond scission to afford Bz-NH_2 (74%), the corresponding terminal amides Bz-Ser-NH_2 and Z-Ser-NH_2 (**43** and **44**, Table 3) yielded the chain intact oxalamido derivatives in respectively 91% and 65% yields, wherein the C^{α} center was neatly transformed into a C=O function.

$$\text{Bz-NH-CH(CH}_2\text{OH)-COOMe} \xrightarrow{\text{i}} \text{Bz-NH}_2$$

$$\text{Bz/Z-NH-CH(CH}_2\text{OH)-CONH}_2 \xrightarrow{\text{i}} \text{Bz/Z-NH-CO-CO-NH}_2$$

i = *in situ* generated RuO_4

On the basis of the above experimental findings, it was rationalized that if the Ser/Thr residue were placed in a peptide environment either at the nonterminal or at the N-terminal location, the oxidation would lead to the incorporation of an α-ketoamide (-NH-CO-CO-) unit in place of a Ser/Thr residue in the peptide backbone (Figure 2).

With this objective, a variety of Ser/Thr peptides were prepared and subjected to Ru(VIII) oxidation.

The oxidation profile of N-terminal Ser/Thr peptides (**22**–**42**) is presented in Table 2. The peptides **22**–**42**, all containing Ser/Thr residues at the N-terminal position, underwent oxidation to afford backbone-modified products wherein the C^{α} center of the Ser/Thr unit was neatly transformed into a C=O function, generating an α-ketoamide (-NH-CO-CO-) unit in good yield. The products were easily characterized by the presence of a typical singlet (D_2O exchangeable) in ^1H NMR in the range of δ 9–11, assigned to the -CO-N*H*-CO-CO proton.

In the examples cited above, the crucial step is the oxidative scission of the C^{α}–C side chain of the Ser/Thr residues. In principle, any such C^{α}–C side-chain scission can lead to similar products. This aspect found illustration in an unexpected manner on reaction of Z-Ser-Tyr-OMe (**27**, Table 2) and Z-Ser-Trp-OMe (**28**, Table 2). These afforded as major product Z-NH-CO-CO-NH_2, clearly showing that here Tyr and Trp residues are acting as Ser/Thr equivalents! The fact that Z-Ser-Asp(β-OMe)-OMe (**30**, Table 2) gave the expected Z-NH-CO-CO-Asp(β-OMe)-OMe, coupled with the earlier findings[21] that Tyr and Trp side chains are oxidized readily with Ru(VIII) to -CH_2-CO_2H, suggested that in both cases this unit is responsible for the scission. This general notion was supported by treatment of Z-Ala-Trp-OMe under identical conditions to afford Z-Ala-NH_2 (38%).

The intermediacy of Z-Ala-Asp(β-OH)-OMe in the above reaction was confirmed by its preparation from Z-Ala-Trp-OMe,[21] followed by treatment with Ru(VIII) under the usual reaction conditions to afford Z-Ala-NH_2 in 80% yield. That the overall change involved oxidation was confirmed by the stability of Z-Ala-Asp(β-OH)-OMe in the absence of Ru(VIII).

These findings clearly support a mechanism wherein an Asp(β-OH) side chain undergoes oxidative C^{α}–C side-chain scission leading to products, similar to that encountered involving Ser/Thr residues. The transformation of Z-Ser-Tyr/Trp-OMe to Z-NH-CO-$CONH_2$ is rationalized in Scheme 2. Similar pathways would explain the observed Z-Ala-Trp-OMe → Z-Ala-NH_2 change (*vide supra*).

The fact that a Ser-Trp combination in peptides can efficiently lead to oxidative scission at that site was clearly brought out by oxidation of the 26-residue bee venom peptide, melittin, containing the Ser^{18}-Trp^{19} sequence in its chain Gly-Ile-Gly-Ala-Val-Leu-Lys-Val-Leu-Thr-Thr-Gly-Leu-Pro-Ala-Leu-Ile-Ser^{18}-Trp^{19}-Ile-Lys-Arg-Lys-Arg-Gln-Gln-NH_2, which under normal conditions of cleavage at pH 3 fragmented to Gly-Ile-Gly-Ala-Val-Leu-Lys-Val-Leu-Thr-Thr-Gly-Leu-Pro-Ala-Leu-Ile-NH_2 and Ile-Lys-Arg-Lys-Arg-Gln-Gln-NH_2 in ~30% yield. These fragments were isolated in pure form by HPLC, and their nature was ascertained by amino acid analysis.[22] Endeavors to further understand this novel cleavage are being planned.

A noteworthy feature of the present study is the oxidation profile of Z-Ser-Ser-OMe, Z-Ser-Aib-Ser-OMe, Z-Ser-Leu-Ser-OMe, Z-Ser-Gly-Ser-OMe, and Z-Ser-Pro-Ser-OMe (**31**, **39**, **40**, **41**, and **42**, respectively, Table 2), all having N- as well as C-terminal Ser residues, and Z-Gly-Ser-Gly-Ser-OMe (**55**, Table 4), having nonterminal and C-terminal Ser residues. Under the reaction conditions normally used, the oxidation of both of the Ser residues was observed, leading to the anticipated products. The transformation of Z-Ser-Met-OMe and Z-Thr-Cys(S-Bzl)-OMe (**32** and **36**, respectively, Table 2) to the corresponding oxalamido sulfone esters was as expected.

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Table 1. Cleavage of C-Terminal Serine and Threonine Peptides to C-Terminal Amides with *in Situ* Generated RuO_4 at pH 3

$$\text{X-Pep-Ser/Thr-OMe}^{a} \xrightarrow{\text{i}} \text{X-Pep-NH}_2$$

peptide no.	X-Pep-Ser/Thr-OMe	X-Pep-NH_2 [yield, %; mp, °C; $[\alpha]^{30}_D$, deg (*c*, solvent)[b]]
1	Bz-Ser/Thr-OMe	Bz-NH_2 [74/84; 126]
2	*o*-NO_2Bz-Ser-OMe	*o*-NO_2Bz-NH_2 [83; 168–169]
3	Z-Gly-Ser/Thr-OMe	Z-Gly-NH-CO-CO_2Me [90/86; 92–93][c]
4	Bz-Gly-Ser/Thr-OMe	Bz-Gly-NH-CO-COOMe [89/90; 143][d]
5	Bz-Ala-Ser/Thr-OMe	Bz-Ala-NH_2 [49/65; 232–234; +21.1 (1.7, MeOH)]
6	Bz-Leu-Ser/Thr-OMe	Bz-Leu-NH_2 [68/72; 169–170; + 2.1 (1.6, $CHCl_3$)]
7	Z-Leu-Thr-OMe	Z-Leu-NH_2 [50; 117–118; –11.81 (0.66, MeOH)][e]
8	Bz-Phe-Ser/Thr-OMe	Bz-Phe-NH_2 [79/68; 183–184; –27.8 (2.8, MeOH)]
9	Bz-Asp(β-OMe)-Ser-OMe	Bz-Asp(β-OMe)-NH-CO-CO_2Me [92, 157; –26.87 (0.64, MeOH)]
10	Boc-Asp(β-OBzl)-Ser-OMe	Boc-Asp(β-OBzl)-NH_2 [96; 146; +33.83 (0.13, $CHCl_3$)]
11	Bz-Glu-(γ-OMe)-Ser-OMe	Bz-Glu(γ-OMe)-NH_2 [90; 136–137]
12	Z-Asn-Ser-OMe	Z-Asn-NH_2 [65; 220–222; –2.32 (0.56, MeOH)]
13	Z-Gln-Ser-OMe	Z-Gln-NH_2 [90; 138–139; +3.20 (0.25, MeOH)]
14	Z-Met-Ser-OMe	Z-Met(SO_2)-NH_2 [95; 111–112; +5.65 (0.56, MeOH)]
15	Bz-Val-Ser-OMe	Bz-Val-NH_2 [95; 216–217]
16	Bz-Pro-Ser-OMe	Bz-Pro-NH_2 [86; syrup; –56.85 (0.35, MeOH)]
17	Boc-Arg(N^GNO_2)-Ser-OMe	Boc-Arg(N^GNO_2)-NH_2 [91; syrup]
18	Bz-Pro-Phe-Ser-OMe	Bz-Pro-Phe-NH_2 [70; 188–190; –75.2 (2, MeOH)]
19	Boc-Ala-Ala-Ser/Thr-OMe	Boc-Ala-Ala-NH_2 [78/86; 145–148; –38.9 (1.7, MeOH)]
20	Bz-Val-Phe-Ser/Thr-OMe	Bz-Val-Phe-NH_2 [65/57; 238–239; –26.8 (0.9, MeOH)]
21	Bz-Gly-Phe-Thr-OMe	Bz-Gly-Phe-NH_2 [51; 177–178; +2.6 (2.6, MeOH)]

[a] X = Bz/Boc/Z; Pep = peptide unit; i, $NaIO_4$, $RuCl_3 \cdot 3H_2O$, CH_3CN/CCl_4/pH 3 phosphate buffer, 1:1:2 v/v/v, room temperature, 1.5 h. [b] ±0.05°. [c] Carried out at pH 3 with Z-Gly-Ser-OMe and Z-Gly-Thr-OMe for 30 min; with 8 h reaction time at pH 3, both yielded 92% of Z-Gly-NH_2, mp 131–132 °C. [d] 0.5 h reaction time; in 8 h reaction time, both afforded Bz-Gly-NH_2 [54/72; 170–171]. [e] 0.5 h reaction time; ~40% of starting material was recovered.

Table 2. Oxidation of N-Terminal Serine and Threonine Peptides and N,C-Terminal Bis-Ser Peptides with RuO_4 at pH 3: Isolation of Oxalamido Derivatives

$$\text{X-Ser/Thr-Pep-OMe}^{a} \xrightarrow{\text{i}} \text{X-NH-C*O-CO-Pep-OMe}$$

$$\text{X-Ser-Pep-Ser-OMe} \xrightarrow{\text{i}} \text{X-NH-C*O-CO-Pep-NH}_2$$

peptide no.	X-Ser/Thr-Pep-OMe or X-Ser-Pep-Ser-OMe	X-NH-C*O-CO-Pep-OMe/NH_2[e] [mp, °C; yield, %; $[\alpha]^{25}_D$, deg (*c*, solvent)[b]]
22	Z-Ser/Thr-Gly-OMe	Z-NH-CO-CO-Gly-OMe [132–133; 91/83]
23	Z-Ser/Thr-Ala-OMe	Z-NH-CO-CO-Ala-OMe [70–71; 66/93]
24	Z-Ser/Thr-Phe-OMe	Z-NH-CO-CO-Phe-OMe [82–83; 82/85; +32.00 (3.2, $CHCl_3$)]
25	Z-Ser/Thr-Leu-OMe	Z-NH-CO-CO-Leu-OMe [syrup; 85/86; –7.5 (13.5, $CHCl_3$)]
26	Z-Ser-methyl anthranilate	Z-NH-CO-CO-methyl anthranilate [125–126; 96]
27	Z-Ser-Tyr-OMe	Z-NH-CO-CO-Asp(β-OH)-OMe[d] + Z-NH-CO-CO-NH_2 [201–202; 38]
28	Z-Ser-Trp-OMe	Z-NH-CO-CO-Asp(β-OH)-OMe[e] + Z-NH-CO-CO-NH_2 [198–200; 85]
29	Z-Ser-Pro-OMe	Z-NH-CO-CO-Pro-OMe [syrup, 50]
30	Z-Ser-Asp[β-OMe)-OMe	Z-NH-CO-CO-Asp(β-OMe)-OMe [syrup; 85]
31	Z-Ser-Ser-OMe/Z-Thr-Thr-OMe	Z-NH-CO-CO-NH_2 [199–200; 32/40]
32	Z-Ser-Met-OMe	Z-NH-CO-CO-Met(SO_2)-OMe [180–181; 88; –14.3 (1.83, MeOH)]
33	Z-Thr-Lys(N^{ω}Z)-OMe	Z-NH-CO-CO-Lys(N^{ω}Z)-OMe [syrup; 60]
34	Boc-Thr-Ala-Ala-OMe	Boc-NH-CO-CO-Ala-Ala-OMe [syrup; 92]
35	Z-Thr-Ala-Ala-OMe	Z-NH-CO-CO-Ala-Ala-OMe [syrup; 76]
36	Z-Thr-Cys(S-Bzl)-OMe	Z-NH-CO-CO-Cys(SO_2-Bzl)-OMe [101–102; 79; +1.82 (1.7, $CHCl_3$)]
37	Z-Thr-Leu-Leu-OMe	Z-NH-CO-CO-Leu-Leu-OMe [syrup; 96; –36.22 (1.06, MeOH)]
38	Z-Thr-Leu-Leu-Leu-OMe	Z-NH-CO-CO-Leu-Leu-Leu-OMe [syrup; 36]
39	Z-Ser-Aib-Ser-OMe	Z-NH-CO-CO-Aib-NH_2 [syrup; 84]
40	Z-Ser-Leu-Ser-OMe	Z-NH-CO-CO-Leu-NH_2 [syrup; 50][f]
41	Z-Ser-Gly-Ser-OMe	Z-NH-CO-CO-Gly-NH_2 [204–205; 83][g]
42	Z-Ser-Pro-Ser-OMe[h]	Z-NH-CO-CO-Pro-NH_2 [gummy; 40]

[a] See note *a* of Table 1. [b] ±0.05°. [c] C* was originally C^{α} of serine or threonine. [d] Identified as ester (syrup; 16%). [e] Identified as ester (syrup; 6%). [f] Reaction at pH 6 yielded 30% of Z-NH-CO-CO-Leu-NH_2. [g] Same product was obtained at pH 6. [h] Oxidation was carried out at pH 6.

Table 2 illustrates two additional noteworthy features. First, the smooth transformation of Z-Thr-Lys(N^{ω}Z)-OMe (**33**, Table 2) to Z-NH-CO-CO-Lys(N^{ω}Z)-OMe should provide a methodology for peptide backbone modification when Lys residues are present, since it has been demonstrated[23] that N^{α}Z-Lys-OH in pH 3 phosphate buffer is recovered on treatment with Ru(VIII) under conditions described here even after 18 h! The results from melittin, described above, are also in agreement with the stability of the Lys side chain. Second, with the excellent stability shown by the Boc protecting group under the conditions of the reaction, the transformation of Boc-Thr-Ala-Ala-OMe (**34**, Table 2) to Boc-NH-CO-CO-Ala-Ala-OMe in 92% yield would thus provide a practical route to *N*-oxalamido peptides, a new class of N-protected peptides.

The foregoing account has clearly shown the dichotomy in the Ru(VIII)-mediated scission of Ser/Thr residues. Those having

(23) Reference 17. See also: Bhattacharyya, D. Ph.D. Thesis, IIT, Kanpur, India, 1988, p 191.

Table 3. Oxidation of C-Terminal Ser and Thr Peptide Amides to C-Terminal Oxalamido Peptide Amides with RuO_4 at pH 3

$$\text{X-Pep-Ser/Thr-NH}_2{}^{a} \xrightarrow{\text{I}} \text{X-Pep-NH-C*O-CONH}_2$$

peptide no.	X-Pep-Ser/Thr-NH_2	X-Pep-NH-C*O-$CONH_2$[c] [mp, °C; yield, %; $[\alpha]^{26}_D$, deg (*c*, solvent)[b]]
43	Bz-Ser-NH_2	Bz-NH-CO-$CONH_2$ [111–112; 91]
44	Z-Ser-NH_2	Z-NH-CO-$CONH_2$ [202; 65]
45	Z-Leu-Ser-NH_2	Z-Leu-NH-CO-$CONH_2$ [151–152; 88; –20.94 (0.85, MeOH)]
46	Bz-Gly-Ser-NH_2	Bz-Gly-NH-CO-$CONH_2$ [164–165; 90]
47	Z-Gly-Ser-NH_2	Z-Gly-NH-CO-$CONH_2$ [171–172; 85]
48	Z-Thr-NH_2	Z-NH-CO-CO-NH_2 [200–201; 61]
49	Z-Ser-Leu-NH_2	Z-NH-CO-CO-Leu-NH_2 [gummy; 80]
50	Z-Ser-Ser-NH_2	Z-NH-CO-CO-NH_2 [200–201]

[a] See note *a* of Table 1. [b] ±0.05°. [c] C* was originally Cα of Ser/Thr.

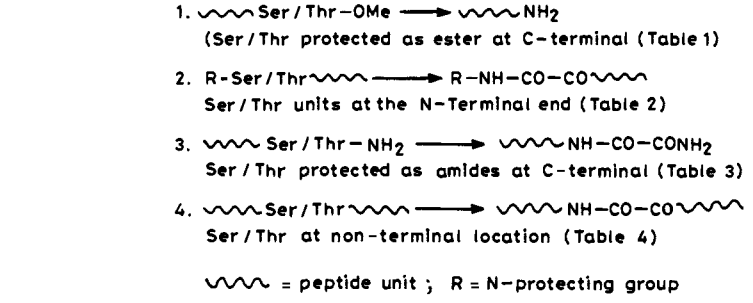

Figure 2. Cα–C side-chain scission profile of Ser/Thr residues in peptides.

the profile -CONH-Ser/Thr-CO-O- provide terminal amides (-$CONH_2$), and those of the type -CONH-Ser/Thr-CO-NH- are transformed to oxalamides (-CONH-CO-CO-NH-). Logically, then, the fate of the substrate is dictated by the C-terminal heteroatom; when this is O-, amidation results, and with NH-, backbone-intact oxalamides result. Fortunately, this aspect could be easily tested using C-terminal Ser/Thr amides in place of C-terminal esters as substrates. The expectation here, that C-terminal Ser/Thr amides would lead to chain-intact oxalamides, was indeed fully realized experimentally. Thus, a range of C-terminal Ser/Thr amides (Table 3, **43–50**) uniformally afforded in good to excellent yields the C-terminal amides. This aspect is highlighted below, comparing substrates that differ only at the C-terminal end.

substrate	product (yield, %)
Bz-Ser-OMe	Bz-NH_2 (74)
Bz-Gly-Ser-OMe	Bz-Gly-NH_2 (54)
Bz-Leu-Ser-OMe	Bz-Leu-NH_2 (68)
Bz-Thr-OMe	Bz-NH_2 (84)
Bz-Ser-NH_2	Bz-NH-CO-CO-NH_2 (91)
Bz-Gly-Ser-NH_2	Bz-Gly-NH-CO-CO-NH_2 (90)
Z-Leu-Ser-NH_2	Z-Leu-NH-CO-CO-NH_2 (88)
Z-Thr-NH_2	Z-NH-CO-CO-NH_2 (61)

A practical outcome of the above study is the prediction that nonterminal Ser/Thr residues in peptides on treatment with Ru(VIII) would give rise to backbone-modified peptides involving a -CO-NH-CH(CHROH)-CO-NH- → -CO-NH-CO-CO-NH- change. This expectation has been fully realized.

In Table 4 are presented peptides having nonterminal Ser/Thr residues (**51–59**). The reaction here affords exclusively the expected oxalamides. Barring factors that can add new dimensions during the oxidation, the methodology here provides a practical route to peptide backbone modification. This is particularly interesting in the case of the Bz-Pro-Ser/Thr-Pro-OMe → Bz-Pro-NH-CO-CO-Pro-OMe (**56**, Table 4) change, since the possibility for a γ-turn hydrogen bonding is likely to reduce the 1,2-dicarbonyl dihedral angle from 106° observed from X-ray crystallography for the related MeO-Pro-CO-CO-Pro-OMe.[24] The Z-Leu-Ser-His-OMe → Z-Leu-NH-CO-CO-NH-CH(CO_2Me)-CH_2-CO-NHCHO change (**53**, Table 4) is appealing in the sense that the same reaction brings about both peptide backbone modification and side-chain transformation and is in line with expectations based on the conversion of Z-His-OMe to Z-NH-CH(CO_2Me)-CH_2-CO-NHCHO—*via* the sequence 4.5 π bond oxidative scission, water addition, and oxidation—reported previously.[17]

A limitation of the methodology is highlighted in the oxidation of the amphipathic heptapeptide Z-Leu-Ser-Ser-Leu-Leu-Ser-Leu-OMe[25] (**59**, Table 4), a heptamer repeat of an ion-channel-forming, 21-residue peptide. Such problems have also been previously noted when contiguous Ser-Ser or Thr-Thr units are present in the chain (**31**, Table 2). The complication arises from the fact that the Ser-Ser or Thr-Thr units here would be transformed to -NH-C*O-CO-NH-C*O-CO-, forming an extended stretch of oxalamide units. All indications are that such an ensemble is susceptible to hydrolytic cleavage, resulting in the formation of a terminal amide on one hand and a HOOC-CO-NHR unit on the other. Although in the examples cited above, the latter eluded isolation, the pathways involved were clearly shown by using as the substrate the 12-residue hydrophobic segment[26] of the signal peptide pertaining to the release of *E. coli* protein, lipoprotein. Thus, peptide Boc-Gly-Ala-Val-Ile-Leu-Gly-Thr-Thr-Leu-Leu-Ala-Gly-OMe, on treatment with Ru(VIII) under usual conditions followed by HPLC separation of the reaction product, showed, besides recovered starting material, the presence of two peptides which were identified by amino acid analysis as Gly-Ala-Val-Ile-Leu-Gly and Leu-Leu-Ala-Gly segments resulting from cleavage at the Thr-Thr junction. We feel that the present method can be used with confidence for peptide scission at Ser-Ser or Thr-Thr sites. Of interest is the finding that cyclo(Ser-Ser)[28] afforded, in 30% yields, tetraketopiperazine, involving double Cα → C=O change on Ru(VIII) treatment.

Thus, the Cα–C side-chain scission of Ser/Thr residues, mediated by Ru(VIII) species and probed with 75 model substrates, has provided a wealth of experimental data, the most notable aspects of which are the formation of des amides from

(24) Karle, I.; Ranganathan, D.; Shah, K.; Vaish, N. K. *Int. J. Pept. Protein Res.* **1994**, *43*, 160.
(25) Lear, J. D.; Wasserman, Z. R.; DeGrado, W. F. *Science* **1988**, *240*, 1177. DeGrado, W. F.; Wasserman, Z. R.; Lear, J. D. *Science* **1989**, *243*, 622.
(26) This peptide was kindly supplied by Dr. R. Nagaraj, CCMB Hyderabad (Reddy, G. L.; Bikshapathy, E.; Nagaraj, R. *Tetrahedron Lett.* **1985**, 4257).
(27) Hydrolytic studies were carried out in pH 3 phosphate buffer:CH_3CN (2:1 v/v). Initial experiments showed that while Z-Gly-NH-CO-$CONH_2$ was unchanged after 24 h, Bz-Gly-NH-CO-CO_2Me and Z-Gly-NH-CO-CO_2Me were transformed to Bz-Gly-NH_2 and Z-Gly-NH_2, respectively. The rates of hydrolysis of the latter two substrates were determined by withdrawing aliquots at time intervals of 0.5, 2, 3.5, 8, and 24 h, followed by workup as described in a typical Ru(VIII) oxidation procedure. The extent of hydrolysis was monitored by ^{1}H NMR by the disappearance of the -COOMe peak against internal standards. Processing of the data in the usual manner afforded pseudo-first-order rate constants for Bz-Gly-NH-CO-CO_2Me and Z-Gly-NH-CO-CO_2Me respectively of 1.68×10^{-5} and 1.57×10^{-5} s^{-1}.
(28) Greenstein, J. P.; Winitz, M. *Chemistry of the Amino Acids*; John Wiley and Sons, Inc.: New York, 1961; Vol. 2, p 797.

Scheme 2. Role of Tyr/Trp as Ser/Thr Equivalents in Chemical Model of PAM Action

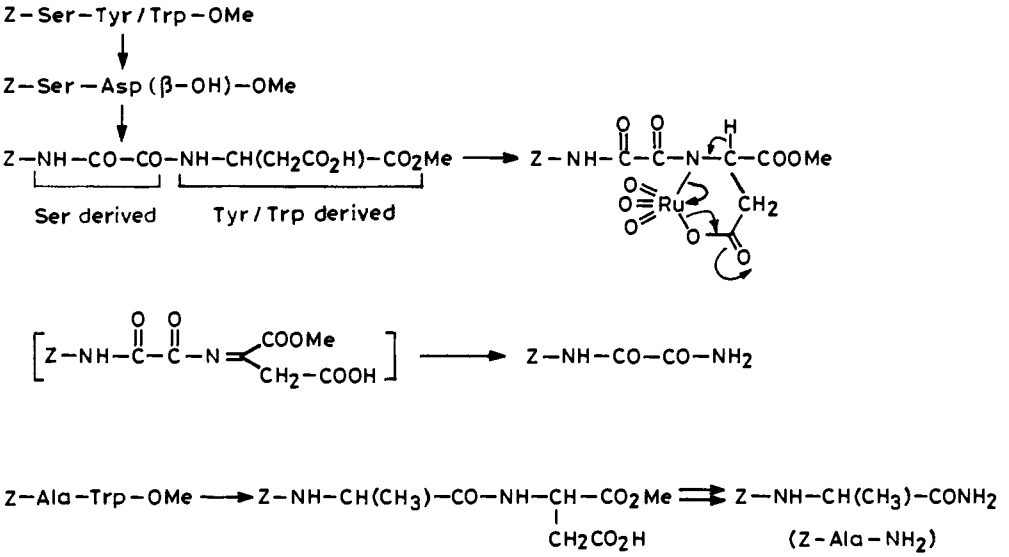

Table 4. Oxidation of Nonterminal Serine and Threonine Peptides with RuO_4 at pH 3: Isolation of Oxalamido Peptides

$$\text{X-Pep-Ser/Thr-Pep'-OMe}^{a} \xrightarrow{\text{i}} \text{X-Pep-NH-C*O-CO-Pep'-OMe}$$

peptide no.	X-Pep-Ser/Thr-Pep'-OMe	X-Pep-NH-C*O-CO-Pep'-OMe[c] [mp, °C; yield, %; $[\alpha]^{28}_D$, deg (*c*, solvent)[b]]
51	Bz-Leu-Ser-Leu-OMe	Bz-Leu-NH-CO-CO-Leu-OMe [75–77; 55]
52	Bz-Ala-Ser/Thr-Ala-OMe	Bz-Ala-NH-CO-CO-Ala-OMe [140–141; 89/80]
53	Z-Leu-Ser-His-OMe	Z-Leu-NH-CO-CO-NH-CH(CO_2Me)-CH_2-CONHCHO [79–85; 73][d]
54	Z-Gly-Ser-Gly-OMe	Z-Gly-NH-CO-CO-Gly-OMe [115–116; 50]
55	Z-Gly-Ser-Gly-Ser-OMe	Z-Gly-NH-CO-CO-Gly-NH_2 [semisolid; 39]
56	Bz-Pro-Ser/Thr-Pro-OMe	Bz-Pro-NH-CO-CO-Pro-OMe [syrup; 40/69; –38.18 (0.27, $CHCl_3$)]
57	Bz-Aib-Ser-Aib-OMe	Bz-Aib-NH-CO-CO-Aib-OMe [173–174; 85]
58	Bz-Ala-Ala-Thr-Ala-Ala-OMe	Bz-Ala-Ala-NH-CO-CO-Ala-Ala-OMe [gummy; 50[e]; –18.97 (1.66, MeOH)]
59	Z-Leu-Ser-Ser-Leu-Leu-Ser-Leu-OMe	Z-Leu-NH_2[f] [118, 11]

[a] See note *a* of Table 1. [b] ±0.05°. [c] C* was originally C^{α} of serine or threonine. [d] Attempted purification on silica gel gave the cleaved product Z-Leu-NH_2 in >90% yield. [e] Yield based on recovered starting material (30%). [f] This was the sole product which could be isolated from the reaction mixture.

C-terminal Ser/Thr extended terminal esters and the formation of backbone-intact oxalamides either from C-terminal Ser/Thr amides or from N-terminal and nonterminal Ser/Thr peptides.

Studies with representative model systems have enabled the delineation of likely pathways involved in the formation of the above products on the basis of an integrated mechanistic scheme.

Hydrolytic studies[27] using pH 3 phosphate buffer with Z-Gly-NH-CO-$CONH_2$ (**47**, Table 3) have shown that it does not undergo any perceptible cleavage. In sharp contrast, Z-Gly-NH-CO-CO_2Me and Bz-Gly-NH-CO-CO_2Me (**3** and **4**, Table 1) are hydrolyzed to Z-Gly-NH_2 and Bz-Gly-NH_2, the pseudo-first-order rate constants being respectively 1.57×10^{-5} and 1.68×10^{-5} s^{-1}.

Thus, products arising from C^{α}–C side-chain scission of Ser/Thr residues can be accounted for on the basis of a common mechanism involving either oxalamides or oxaloesters arising from the oxidation of initially formed carbinolamide intermediates, wherein, while the oxalamides are stable to hydrolysis, the oxaloesters are hydrolyzed to terminal amides. Thus, electronic factors play a major role pertaining to the nature of the oxidation products formed in the reaction.

Conclusion

The comprehensive study outlined above has enabled, *inter alia*, the delineation of pathways involved in the C^{α}–C side-chain scission of peptides having Ser/Thr residues. Regardless of the finer aspects of the reaction mechanisms, the major experimental findings are the conversion of peptides having C-terminal Ser/Thr esters to des Ser/Thr amides, the conversion of Ser/Thr terminal amides to oxalamides, and the transformation of N-terminal and nonterminal Ser/Thr residues to peptides having oxalamide units in their backbones. The demonstrated stability of, among the 20 coded amino acids, Phe, Leu (therefore Ile), Val, Pro, Ala, Asn, Gln, Lys, Arg, and Gly to the scission and knowledge pertaining to the behavior of the remaining residues, namely, Ser, Thr, Cys, Met, Tyr, His, Asp, Glu, and Trp, coupled with the lack of any procedure to incorporate an unsymmetrical oxalamide unit[29] in the peptide backbone, should make the methodology delineated here useful for the preparation of a variety of analogs of biologically important peptides. Detailed biological studies of the compounds reported here are in progress, and the results will be reported elsewhere. In selected cases, the illustrations cited here would enable changes in protein secondary structures, particularly proximate to Pro residues. Ancillary noteworthy findings are peptide bond scission when contiguous Ser-Ser/Thr-Thr are present and the oxidative cleavage at C-terminal Tyr/Trp sites generating des amides.

In a broader perspective, these endeavors pertain to the development of methods for conducting selective chemistry on assembled peptides and proteins, a worthwhile goal which will likely see more contributions in future.

(29) To the best of our knowledge, no methodology exists for preparation of *N*-acyloxalamides either from general substrates or from peptides. Although tangential to the present work, endeavors were made to prepare simple examples of such systems, particularly *N*-benzoyloxalamido methyl ester, from azlactone precursors without success.

Experimental Section

Melting points are uncorrected. Optical rotations were measured with an automatic JASCO polarimeter; concentrations are given in grams/100 mL. Infrared spectra were recorded with a Perkin-Elmer 580/1600 FT spectrometers either as neat liquids or as KBr pellets, and prominent peaks are expressed in cm^{-1}. ^1H NMR spectra were determined on Bruker WM 400, WP 80, and Hitachi R 600 spectrometers. The chemical shifts are recorded in δ, with TMS at 0.00 as internal reference. FAB masses were obtained on a JEOL SX-120/DA-6000 instrument using *m*-nitrobenzyl alcohol as the matrix. Elemental analyses were carried out with an automatic C, H, N analyzer. Silica gel G (Merck) was used for TLC, and column chromatography was done on silica gel (Acme, 100–200 mesh) columns, which were generally made from a slurry in hexane or benzene. Reactions were monitored wherever possible by TLC.

Synthesis of Peptides. General. All amino acids used were of L-configuration. Dipeptides were prepared by coupling N-protected (Bz,[30] or Boc,[32]) amino acids with C-protected [as methyl ester, prepared by using either dry HCl[33] gas (e.g. Ser, Gly, Ala, Leu, Pro, Asp, His, etc.) or $SOCl_2$[34] (Phe, Tyr, Aib, Met, Thr, Trp, etc.) in dry MeOH] partner using any one of the general procedures A, B, or C in solution phase.[35]

For the preparation of tripeptides, the deprotected dipeptide either at the N-terminal (de-Boc,[36] de-Z[37]) or at the C-terminal (ester hydrolysis[38])was coupled with the appropriate N- or C-protected partner by using any of the above procedures. Higher peptides were synthesized using blockwise condensation of smaller units.

α-Amino Acid Coupling. General Procedures for the Synthesis of Peptides. All peptides were synthesized by the solution-phase method[35] using either DCC/HOBt-mediated coupling (method A) or azide coupling via hydrazide (method B) or diphenyl phosphorazidate (method C). The crude peptides were, in most cases, directly crystallized from EtOAc/hexane mixture or purified on a short column of silica gel using benzene/EtOAc as eluents.

(A) Preparation of C-Terminal Ser Peptides Listed in Table 1. The method used for the synthesis is given with each entry.

Bz-Ser-OMe. Prepared from Ser-OMe hydrochloride by using the Schotten–Baumann procedure in bicarbonate medium: yield 83%; mp 85–86 °C; IR (KBr) 3430, 3300, 1740, 1620, 1530 cm^{-1}; ^1H NMR (60 MHz, $CDCl_3$) δ 3.15 (1H, br), 3.71 (3H, s), 3.95 (2H, dd), 4.80 (1H, m), 7.11–8.05 (6H, m).

(*o*-NO_2Bz)-Ser-OMe. Prepared by treating Ser-OMe hydrochloride (1 mmol) with *o*-NO_2BzCl (1 mmol) and triethylamine (3 mmol) at 0 °C in dry CH_2Cl_2 (20 mL) for 1 h. The reaction mixture was washed with cold 2 N H_2SO_4 (~5 mL), water (10 mL), and saturated bicarbonate solution (10 mL), dried, evaporated *in vacuo*, and residue crystallized from ethyl acetate–hexane as pale yellow needles: yield 50%; mp 92–93 °C; IR (KBr) 3568, 3283, 2950, 1743, 1634, 1611, 1573, 1522, 1356 cm^{-1}; ^1H NMR (80 MHz, $CDCl_3$) δ 3.84 (3H, s), 4.09 (2H, m), 4.81 (1H, m), 6.79 (1H, br), 7.34–8.28 (4H, m).

Z-Gly-Ser-OMe[39] (method A): yield 94%; mp 85–86 °C; $[\alpha]^{30}_D$ = +24.72 (*c*, 0.55, $CHCl_3$); IR (KBr) 3395, 3310, 1733, 1718, 1688, 1657, 1540, 1513 cm^{-1}; ^1H NMR (80 MHz, $CDCl_3$) 3.73 (5H, s + m), 3.89 (2H, d, *J* = 5.0 Hz), 4.60 (1H, m), 5.09 (2H, s), 6.00 (1H, t), 7.32 (6H, s). Anal. Calcd for $C_{14}H_{18}N_2O_6$: C, 54.19; H, 5.81; N, 9.03. Found: C, 54.33; H, 5.65; N, 8.87.

Bz-Gly-Ser-OMe (method A): yield 70%; mp 82–84 °C; $[\alpha]^{30}_D$ = –2.3 (*c*, 3.3, MeOH); IR (KBr) 3360, 3285, 3085, 2955, 1730, 1655, 1630, 1555, 1490 cm^{-1}; ^1H NMR (80 MHz, $CDCl_3$ + $(CD_3)_2SO$) δ 3.80 (3H, s), 3.91 (2H, m), 4.14 (2H, d, *J* = 5.0 Hz), 4.60 (1H, m), 7.20–8.25 (7H, m); MS (*m/z*) 281 $(MH)^+$. Anal. Calcd for $C_{13}H_{16}N_2O_5$: C, 55.71; H, 5.71; N, 10.00. Found: C, 55.44; H, 5.98; N, 10.09.

Bz-Ala-Ser-OMe (method A): yield 65%; mp 135–136 °C; $[\alpha]^{30}_D$ = +10.8 (*c*, 0.4, MeOH); IR (KBr) 3455, 3325, 2935, 2860, 1740, 1655, 1625, 1600, 1570, 1530, 1490 cm^{-1}; ^1H NMR (80 MHz, $CDCl_3$ + $(CD_3)_2SO$) δ 1.47 (3H, d, *J* = 7.5 Hz), 3.75 (3H, s), 3.87 (2H, m), 4.39–4.95 (2H, m), 7.20–8.00 (7H, m). Anal. Calcd for $C_{14}H_{18}N_2O_5$: C, 57.14; H, 6.12; N, 9.52. Found: C, 57.42; H, 6.38; N, 9.72.

Bz-Leu-Ser-OMe (method A): yield 70%; mp 95–97 °C; $[\alpha]^{30}_D$ = +24.1 (*c*, 3.3, MeOH); IR (KBr) 3284, 3068, 2955, 1750, 1638, 1544 cm^{-1}; Anal. Calcd for $C_{17}H_{24}N_2O_5$: C, 60.71; H, 7.14; N, 8.33. Found: C, 60.36; H, 7.42; N, 8.42.

Bz-Phe-Ser-OMe (method A): yield 63%; mp 105–106 °C; $[\alpha]^{30}_D$ = +2.1 (*c*, 3.3, MeOH); IR (KBr) 3330, 3280, 3070, 3040, 2940, 2860, 1740, 1725, 1635, 1575, 1545, 1535, 1490 cm^{-1}; MS (*m/z*) 370 $(M)^+$. Anal. Calcd for $C_{20}H_{22}N_2O_5$: C, 64.86; H, 5.94; N, 7.57. Found: C, 65.07; H, 6.23; N, 7.73.

Bz-Asp(β-OMe)-Ser-OMe (method A): yield 78%; mp 135–136 °C; IR (KBr) 3428, 3286, 2928, 1747, 1718, 1652, 1562, 1542 cm^{-1}; ^1H NMR (80 MHz, $CDCl_3$) δ 2.94 (2H, dd, *J* = 5.5 Hz, 1 Hz), 3.72, 3.75 (3H, 3H, s, s), 3.91 (2H, d, *J* = 3.0 Hz), 4.56 (1H, m), 5.00 (1H, m), 7.28–7.84 (7H, m); MS (*m/z*) 353 $(MH)^+$. Anal. Calcd for $C_{16}H_{20}N_2O_7$: C, 54.54; H, 5.68; N, 7.95. Found: C, 54.86; H, 5.90; N, 8.15.

Boc-Asp(β-OBzl)-Ser-OMe (method A): yield 58%; syrup; $[\alpha]^{30}_D$ = +25.53 (*c*, 0.32, $CHCl_3$); IR (KBr) 3370, 3010, 1740, 1670, 1530 cm^{-1}; ^1H NMR (60 MHz, $CDCl_3$) δ 1.43 (9H, s), 2.83 (2H, d, *J* = 5.5 Hz), 3.70 (3H, s), 3.80 (2H, br), 4.53 (2H, m), 5.06 (2H, s), 6.03 (1H, d, *J* = 8.7 Hz), 7.23 (5H, s), 7.46 (1H, d, *J* = 7.25 Hz). Anal. Calcd for $C_{20}H_{28}N_2O_8$: C, 56.60; H, 6.60; N, 6.60. Found: C, 56.47; H, 6.38; N, 6.87.

Bz-Glu(γ-OMe)-Ser-OMe (method A): yield 65%; mp 134–136 °C; IR (KBr) 3273, 1734, 1707, 1655, 1626, 1576, 1532 cm^{-1}; ^1H NMR (80 MHz, $CDCl_3$) δ 2.09–2.71 (4H, m), 3.68, 3.76 (3H, 3H, s, s), 4.00 (2H, m), 4.68 (2H, m), 7.34–8.00 (7H, m). Anal. Calcd for $C_{17}H_{22}N_2O_7$: C, 55.74; H, 6.01; N, 7.65. Found: C, 55.64; H, 6.36; N, 7.33.

Z-Asn-Ser-OMe[40] (method C): yield 65%; mp 194–195 °C; IR (KBr) 3421, 3298, 2927, 1731, 1686, 1653, 1609, 1539 cm^{-1}; ^1H NMR (80 MHz, $CDCl_3$ + $(CD_3)_2SO$) δ 2.50 (2H, d, *J* = 5.0 Hz), 3.68 (5H, s + m), 4.45 (2H, m), 5.09 (2H, s), 6.90 (1H, br), 7.34 (7H, br s), 8.09 (1H, d, *J* = 8.75 Hz). Anal. Calcd for $C_{16}H_{21}N_3O_7$: C, 52.32; H, 5.72; N, 11.44. Found: C, 52.43; H, 5.63; N, 11.62.

Z-Gln-Ser-OMe[41] (method C): yield 63%; mp 156–160 °C; IR (KBr) 3403, 3312, 2954, 1747, 1642, 1535 cm^{-1}; ^1H NMR (80 MHz, $CDCl_3$ + $(CD_3)_2SO$) δ 1.68–2.40 (4H, br m), 3.68 (5H, br s), 4.00–4.59 (2H, m), 5.06 (2H, s), 6.50 (1H, br), 6.84–7.56 (7H, s + br), 8.03 (1H, br). Anal. Calcd for $C_{17}H_{23}N_3O_7$: C, 53.54; H, 6.04; N, 11.02. Found: C, 53.93; H, 6.18; N, 11.44.

Z-Met-Ser-OMe (method A): yield 90%; mp 143–144 °C; $[\alpha]^{30}_D$ = +20.94 (*c*, 0.42, $CHCl_3$); IR (KBr) 3535, 3300, 2960, 2930, 1725, 1682, 1645, 1555, 1540 cm^{-1}; ^1H NMR (80 MHz, $CDCl_3$) δ 2.09 (5H, s + m), 2.56 (2H, t), 3.75 (3H, s), 3.87 (2H, br d), 4.15–4.75 (2H, m), 5.06 (2H, s), 5.62 (1H, d, *J* = 7.5 Hz), 6.90–7.53 (6H, s + m). Anal. Calcd for $C_{17}H_{24}N_2O_6S$: C, 53.12; H, 6.25; N, 7.29. Found: C, 53.40; H, 6.38; N, 7.26.

Bz-Val-Ser-OMe (method A): yield 78%; mp 169–170 °C; $[\alpha]^{30}_D$ = +15.44 (*c*, 1.58, $CHCl_3$); IR (KBr) 3340, 3290, 2940, 2860, 1750, 1623, 1570 cm^{-1}; ^1H NMR (80 MHz, $CDCl_3$) δ 1.06 (6H, d, *J* = 5.0 Hz), 2.18 (1H, m), 3.65–4.10 (5H, s + m), 4.62 (2H, m), 6.93–8.00 (7H, m). Anal. Calcd for $C_{16}H_{22}N_2O_5$: C, 59.63; H, 6.83; N, 8.70. Found: C, 59.86; H, 6.47; N, 8.58.

Bz-Pro-Ser-OMe (method A): yield 40%; mp 71–72 °C; $[\alpha]^{30}_D$ = –26.50 (*c*, 0.8, $CHCl_3$); IR (KBr) 3460, 3390, 3320, 2960, 2890, 1743, 1642, 1613, 1570, 1555 cm^{-1}; ^1H NMR (80 MHz, $CDCl_3$) δ 2.18 (4H, m), 3.46–4.12 (7H, s + m), 4.62 (2H, m), 7.03–7.81 (6H, m). Anal. Calcd for $C_{16}H_{20}N_2O_5$: C, 60.00; H, 6.25; N, 8.75. Found: C, 59.68; H, 6.52; N, 8.57.

Boc-Arg(N^GNO_2)-Ser-OMe (method A): yield 52%; mp 74 °C; IR (KBr) 3318, 2976, 1744, 1661, 1600, 1532 cm^{-1}; ^1H NMR (80 MHz, $CDCl_3$ + $(CD_3)_2SO$) δ 1.44 (9H, s), 1.72 (4H, m), 3.34 (2H, m), 3.72–4.00 (5H, s + m), 4.12 (1H, m), 4.52 (1H, m), 6.31 (1H, d, *J* = 7.5 Hz),

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7.56–8.18 (4H, m); MS (m/z) 421 $(MH)^+$. Anal. Calcd for $C_{15}H_{28}N_6O_8$: C, 42.85; H, 6.66; N, 20.00. Found: C, 43.25; H, 6.80; N, 19.64.

Bz-Pro-Phe-Ser-OMe. Prepared by coupling Bz-Pro-Phe-OH (mp 188–190 °C) with Ser-OMe by method A: yield 68%; mp 182–184 °C; $[\alpha]^{30}_D = -112.3$ (c, 3.4, $CHCl_3$); IR (KBr) 3400, 3340, 2945, 1742, 1660, 1600, 1570, 1535 cm^{-1}; ^1H NMR (80 MHz, $CDCl_3$) δ 1.96 (4H, m), 3.15–3.93 (9H, s + m), 4.50 (3H, m), 6.90 (1H, d, J = 7.5 Hz), 7.15–7.59 (11H, s + m). Anal. Calcd for $C_{25}H_{29}N_3O_6$: C, 64.24; H, 6.21; N, 8.99. Found: C, 64.21; H, 6.32; N, 8.72.

Boc-Ala-Ala-Ser-OMe. Prepared by coupling Boc-Ala-Ala-OH (mp 88–89 °C) with Ser-OMe using method A: yield 68%; mp 156–158 °C; $[\alpha]^{30}_D = -24.00$ (c, 0.5, $CHCl_3$); ^1H NMR (80 MHz, $CDCl_3$) δ 1.43 (15H, s + m), 3.81 (3H, s), 3.96 (2H, m), 4.18 (1H, m), 4.62 (2H, m), 5.28 (1H, d, J = 7.5 Hz), 7.06 (1H, d, J = 7.5 Hz), 7.46 (1H, d, J = 7.5 Hz). Anal. Calcd for $C_{15}H_{27}N_3O_7$: C, 49.86; H, 7.48; N, 11.63. Found: C, 49.58; H, 7.33; N, 11.52.

Bz-Val-Phe-Ser-OMe. Prepared by coupling Bz-Val-Phe-OH (mp 185–187 °C) with Ser-OMe using method A: yield 87%; mp 165–167 °C; $[\alpha]^{30}_D = -13.9$ (c, 3.3, MeOH); IR (KBr) 3338, 3298, 2943, 2868, 1743, 1630, 1580, 1538 cm^{-1}. Anal. Calcd for $C_{25}H_{31}N_3O_6$: C, 63.96; H, 6.61; N, 8.95. Found: C, 64.34; H, 6.76; N, 8.79.

(B) Preparation of C-Terminal Thr Peptides Listed in Table 1. Bz-Thr-OMe.[42] Prepared by esterification (ethereal CH_2N_2) of Bz-Thr-OH (mp 143–144 °C): yield 79%; mp 91–92 °C; IR (KBr) 3410, 3345, 1730, 1630, 1510 cm^{-1}.

Z-Gly-Thr-OMe (method A): yield 92%; mp 112–113 °C; IR (KBr) 3464, 3313, 1711, 1692, 1653, 1543 cm^{-1}; ^1H NMR (60 MHz, $CDCl_3$) δ 1.16 (3H, d, J = 6.5 Hz), 3.70 (3H, s), 3.90 (2H, d, J = 5.0 Hz), 4.13–4.73 (2H, br), 5.10 (2H, s), 5.83 (1H, br), 6.96–7.43 (6H, s + br).

Bz-Gly-Thr-OMe (method A): yield 90%; mp 138–140 °C; $[\alpha]^{30}_D = -6.21$ (c, 3.3, MeOH); IR (KBr) 3485, 3370, 3315, 2940, 1723, 1671, 1647, 1580, 1549 cm^{-1}; ^1H NMR (80 MHz, $CDCl_3$ + $(CD_3)_2SO$) δ 1.15 (3H, d, J = 6.5 Hz), 3.71 (3H, s), 4.12 (2H, d, J = 5.0 Hz), 4.25 (1H, m), 4.46 (1H, dd, J = 8.75 Hz, 2.5 Hz), 7.28–8.06 (6H, m), 8.37 (1H, m). Anal. Calcd for $C_{14}H_{18}N_2O_5$: C, 57.14; H, 6.12; N, 9.52. Found: C, 57.40; H, 6.26; N, 9.83.

Bz-Ala-Thr-OMe (method A): yield 64%; mp 68–70 °C; $[\alpha]^{30}_D = +6.1$ (c, 3.3, $CHCl_3$); IR (KBr) 3320, 3075, 3035, 2995, 2955, 1740, 1660, 1530, 1490 cm^{-1}; ^1H NMR (80 MHz, $CDCl_3$ + $(CD_3)_2SO$) δ 1.15 (3H, d, J = 6.5 Hz), 1.53 (3H, d, J = 6.5 Hz), 3.78 (3H, s), 4.28 (1H, m), 4.56 (1H, dd, J = 8.75 Hz, 2.5 Hz), 4.88 (1H, m), 7.19–8.00 (7H, m); MS (m/z) 309 $(MH)^+$. Anal. Calcd for $C_{15}H_{20}N_2O_5$: C, 58.44; H, 6.49; N, 9.09. Found: C, 57.73; H, 6.82; N, 8.49.

Bz-Leu-Thr-OMe (method A): yield 65%; mp 113–114 °C; $[\alpha]^{30}_D = -5.4$ (c, 3.3, MeOH); IR (KBr) 3300, 2975, 1747, 1670, 1639, 1537 cm^{-1}; ^1H NMR (80 MHz, $CDCl_3$) δ 0.94 (6H, br s), 1.20 (3H, d, J = 6.5 Hz), 1.73 (3H, br), 3.76 (3H, s), 4.00 (1H, br), 4.31 (1H, br), 4.56 (1H, m), 4.85 (1H, m), 7.05–8.14 (7H, m); MS (m/z) 351 $(MH)^+$. Anal. Calcd for $C_{18}H_{26}N_2O_5$: C, 61.71; H, 7.43; N, 8.00. Found: C, 61.23; H, 7.18; N, 8.22.

Z-Leu-Thr-OMe (method A): yield 85%; mp 93–94 °C; IR (KBr) 3478, 3292, 1714, 1693, 1655, 1542 cm^{-1}; ^1H NMR (80 MHz, $CDCl_3$) δ 0.96 (6H, d, J = 5.0 Hz), 1.18 (3H, d, J = 6.5 Hz), 1.65 (3H, m), 3.81 (3H, s), 4.15–4.50 (2H, br), 4.65 (1H, m), 5.15 (2H, s), 5.56 (1H, d, J = 7.5 Hz), 7.06 (1H, d, J = 7.5 Hz), 7.37 (5H, s).

Bz-Phe-Thr-OMe (method A): yield 63%; mp 145–146 °C; IR (KBr) 3475, 3310, 3270, 1720, 1671, 1641, 1541 cm^{-1}; MS (m/z) 384 $(M)^+$. Anal. Calcd for $C_{21}H_{24}N_2O_5$: C, 65.62; H, 6.25; N, 7.29. Found: C, 65.69; H, 6.37; N, 7.18.

Bz-Gly-Phe-Thr-OMe. Prepared by condensing Bz-Gly-Phe-OH (gummy foam) with Thr-OMe by method A: yield 79%; mp 157–159 °C; IR (KBr) 3320, 1750, 1660, 1555, 1530 cm^{-1}; ^1H NMR (80 MHz, $CDCl_3$ + $(CD_3)_2SO$) δ 1.14 (3H, d, J = 6.5 Hz), 3.12 (2H, m), 3.70 (3H, s), 3.96 (2H, dd, J = 5.0 Hz, 1.0 Hz), 4.19–4.60 (2H, m), 4.75 (1H, m), 7.00–8.00 (12H, m), 8.15 (1H, t); MS (m/z) 441 $(M)^+$. Anal. Calcd for $C_{23}H_{27}N_3O_6$: C, 62.58; H, 6.12; N, 9.52. Found: C, 62.38; H, 6.29; N, 9.17.

Bz-Val-Phe-Thr-OMe. Prepared by coupling Bz-Val-Phe-OH with Thr-OMe by method A: yield 52%; mp 205–207 °C; $[\alpha]^{30}_D = -20.9$ (c, 2.3, MeOH); IR (KBr) 3495, 3340, 3320, 3280, 2950, 2875, 1722, 1630, 1580, 1540 cm^{-1}; ^1H NMR (80 MHz, $CDCl_3$ + $(CD_3)_2SO$) δ 0.75–1.34 (9H, m), 2.14 (1H, m), 3.15 (2H, t), 3.75 (3H, s), 4.18–5.15 (4H, m), 7.09–8.00 (13H, m). Anal. Calcd for $C_{26}H_{33}N_3O_6$: C, 64.60; H, 6.83; N, 8.69. Found: C, 64.65; H, 6.69; N, 8.56.

Boc-Ala-Ala-Thr-OMe. Made by coupling Boc-Ala-Ala-OH with Thr-OMe by method A: yield 53%; mp 155–156 °C; $[\alpha]^{30}_D = -53.9$ (c, 3.3, MeOH); IR (KBr) 3390, 3310, 3000, 2970, 2875, 1740, 1695, 1638, 1530 cm^{-1}; ^1H NMR (80 MHz, $CDCl_3$ + $(CD_3)_2SO$) δ 1.18 (3H, d, J = 6.5 Hz), 1.34 (6H, d, J = 6.5 Hz), 1.49 (9H, s), 3.81 (3H, s), 4.06–4.81 (4H, m), 5.65 (1H, d, J = 7.5 Hz), 7.46 (2H, m). Anal. Calcd for $C_{16}H_{29}N_3O_7$: C, 51.20; H, 7.73; N, 11.20. Found: C, 50.87; H, 7.48; N, 11.06.

(C) Preparation of N-Terminal Ser Peptides and N,C-Terminal Bis-Ser Peptides Listed in Table 2. Z-Ser-Gly-OMe[43] (method A): yield 78%; mp 80 °C; $[\alpha]^{25}_D = -8.1$ (c, 3.3, $CHCl_3$); IR (KBr) 3310, 2955, 1753, 1682, 1648, 1529 cm^{-1}; ^1H NMR (80 MHz, $CDCl_3$) δ 3.71 (5H, s + m), 4.00 (2H, d, J = 6.25 Hz), 4.26 (1H, m), 5.12 (2H, s), 5.96 (1H, d, J = 7.5 Hz), 6.96–7.46 (6H, s + m). Anal. Calcd for $C_{14}H_{18}N_2O_6$: C, 54.19; H, 5.81; N, 9.03. Found: C, 54.23; H, 5.55; N, 9.25.

Z-Ser-Ala-OMe[44] (method A): yield 78%; mp 106 °C; $[\alpha]^{25}_D = -7.8$ (c, 3.7, $CHCl_3$); IR (KBr) 3314, 1762, 1694, 1657, 1539 cm^{-1}; ^1H NMR (80 MHz, $CDCl_3$) δ 1.33 (3H, d, J = 7.5 Hz), 3.71 (5H, s + m), 4.15–4.75 (2H, m), 5.09 (2H, s), 6.03 (1H, d, J = 7.5 Hz), 7.36 (6H, s + m); MS (m/z) 325 $(MH)^+$. Anal. Calcd for $C_{15}H_{20}N_2O_6$: C, 55.55; H, 6.17; N, 8.64. Found: C, 55.43; H, 6.37; N, 8.59.

Z-Ser-Phe-OMe (method A): yield 50%; mp 104 °C; $[\alpha]^{25}_D = -2.7$ (c, 3.3, MeOH); IR (KBr) 3300, 2950, 1732, 1688, 1650, 1528, 1450 cm^{-1}; ^1H NMR (80 MHz, $CDCl_3$) δ 3.02 (2H, br d), 3.61 (5H, s + m), 4.22 (1H, m), 4.79 (1H, m), 5.03 (2H, s), 6.05 (1H, d, J = 7.5 Hz), 6.87–7.43 (11H, s + m). Anal. Calcd for $C_{21}H_{24}N_2O_6$: C, 63.00; H, 6.00; N, 7.00. Found: C, 62.74; H, 6.11; N, 7.26.

Z-Ser-Leu-OMe[45] (method A): yield 84%; mp 77–78 °C; $[\alpha]^{25}_D = -32.65$ (c, 1.66, MeOH); IR (KBr) 3400, 3310, 2960, 2930, 1745, 1695, 1660, 1645, 1550 cm^{-1}; ^1H NMR (80 MHz, $CDCl_3$) δ 0.84 (6H, d, J = 5.0 Hz), 1.53 (3H, m), 3.71 (5H, s + m), 4.00–4.62 (2H, m), 5.10 (2H, s), 5.90 (1H, d, J = 7.5 Hz), 7.03 (1H, d, J = 7.5 Hz), 7.37 (5H, s). Anal. Calcd for $C_{18}H_{26}N_2O_6$: C, 59.02; H, 7.10; N, 7.65. Found: C, 59.33; H, 7.18; N, 7.43.

Z-Ser-Methyl Anthranilate (method A): yield 43%; mp 100–101 °C; IR (KBr) 3459, 3391, 3293, 2949, 1708, 1680, 1626, 1606, 1588, 1519 cm^{-1}; ^1H NMR (80 MHz, $CDCl_3$) δ 3.90 (3H, s), 4.16 (2H, m), 4.47 (1H, m), 5.20 (2H, s), 5.84 (1H, br d), 7.03–7.66 (8H, m), 8.06, 8.69 (1H, 1H, dd, dd, J = 7.5 Hz, 1.25 Hz). Anal. Calcd for $C_{19}H_{20}N_2O_6$: C, 61.29; H, 5.38; N, 7.53. Found: C, 60.82; H, 4.86; N, 7.78.

Z-Ser-Tyr-OMe[46] (method A): yield 60%; mp 115–116 °C; $[\alpha]^{25}_D = +3.66$ (c, 3.33, MeOH); IR (KBr) 3397, 3315, 1750, 1708, 1649, 1570, 1515, 1453 cm^{-1}; ^1H NMR (80 MHz, $CDCl_3$) δ 3.00 (2H, m), 3.71 (5H, s + m), 4.20 (1H, m), 4.60 (1H, m), 5.06 (2H, s), 5.84 (1H, d, J = 7.5 Hz), 6.50 (1H, br d), 6.66–7.12 (4H, dd), 7.31 (5H, s). Anal. Calcd for $C_{21}H_{24}N_2O_7$: C, 60.58; H, 5.77; N, 6.73. Found: C, 60.36; H, 5.68; N, 6.48.

Z-Ser-Trp-OMe[47] (method A): yield 71%; mp 99–100 °C; $[\alpha]^{25}_D = +9.38$ (c, 0.81, MeOH); IR (KBr) 3350, 2935, 1720, 1655, 1508, 1450 cm^{-1}; ^1H NMR (80 MHz, $CDCl_3$) δ 3.26 (2H, d, J = 5.0 Hz), 3.71 (5H, s + m), 4.25 (1H, m), 4.81–5.25 (3H, s + m), 6.03 (1H, d, J = 7.5 Hz), 6.89–7.57 (11H, s + m), 8.64 (1H, br s); MS (m/z) 439 $(M)^+$. Anal. Calcd for $C_{23}H_{25}N_3O_6$: C, 62.87; H, 5.69; N, 9.57. Found: C, 62.86; H, 5.34; N, 9.65.

Z-Ser-Pro-OMe[48] (method A): yield 40%; mp 113–115 °C; $[\alpha]^{25}_D = -79.15$ (c, 1.66, MeOH); IR (KBr) 3396, 3280, 2956, 1735, 1713, 1617, 1558, 1531 cm^{-1}; ^1H NMR (80 MHz, $CDCl_3$) δ 2.09 (4H, m), 3.50–4.03 (7H, s + m), 4.66 (2H, m), 5.19 (2H, s), 5.78 (1H, br d), 7.42 (5H, s). Anal. Calcd for $C_{17}H_{22}N_2O_6$: C, 58.28; H, 6.28; N, 8.00. Found: C, 58.34; H, 6.16; N, 8.34.

Z-Ser-Asp(β-OMe)-OMe (method A): yield 58%; mp 97–98 °C; $[\alpha]^{25}_D = -14.77$ (c, 3.33, MeOH); IR (KBr) 3314, 2994, 1727, 1685, 1649, 1549, 1526 cm^{-1}; ^1H NMR (60 MHz, $CDCl_3$) δ 2.90 (2H, d, J = 5.5 Hz), 3.63, 3.73 (3H, 3H, s, s), 3.90 (2H, m), 4.16 (1H, m), 4.76 (1H, m), 5.10 (2H, s), 5.83 (1H, br), 7.26 (6H, s + m). Anal. Calcd for $C_{17}H_{22}N_2O_8$: C, 53.40; H, 5.76; N, 7.33. Found: C, 53.78; H, 5.57; N, 7.23.

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Z-Ser-Ser-OMe[49] (method A): yield 60%; mp 136 °C; $[\alpha]^{25}_D$ = –4.2 (*c*, 3.3, MeOH); IR (KBr) 3445, 3305, 3280, 3080, 2950, 1738, 1663, 1636, 1547 cm^{-1}; ^{1}H NMR (80 MHz, $CDCl_3$ + $(CD_3)_2SO$) δ 3.68–3.93 (7H, s + br), 4.44 (2H, m), 5.04 (2H, s), 6.44 (1H, d, *J* = 7.5 Hz), 7.25 (5H, s), 7.61 (1H, d, *J* = 7.5 Hz). Anal. Calcd for $C_{15}H_{20}N_2O_7$: C, 52.94; H, 5.88; N, 8.23. Found: C, 53.08; H, 5.96; N, 8.41.

Z-Ser-Met-OMe[50] (method A): yield 63%; mp 98–99 °C; $[\alpha]^{25}_D$ = –25.42 (*c*, 1.66, MeOH); IR (KBr) 3305, 2946, 1756, 1695, 1656, 1545 cm^{-1}; ^{1}H NMR (60 MHz, $CDCl_3$) δ 2.06 (5H, s + m), 2.46 (2H, m), 3.73 (3H, s), 3.86 (2H, m), 4.20 (1H, m), 4.63 (1H, m), 5.01 (2H, s), 5.86 (1H, br d), 7.43 (6H, s + m). Anal. Calcd for $C_{17}H_{24}N_2O_6S$: C, 53.12; H, 6.25; N, 7.29. Found: C, 53.52; H, 6.27; N, 7.63.

Z-Ser-Aib-Ser-OMe. Prepared by coupling Z-Ser-Aib-azide—generated from Z-Ser-Aib-hydrazide (mp 128–130 °C)—with Ser-OMe using method B: yield 47%; mp 160–168 °C; IR (KBr) 3441, 3306, 3275, 2927, 1749, 1671, 1648, 1627, 1560 cm^{-1}; ^{1}H NMR (80 MHz, $CDCl_3$) δ 1.53 (6H, d, *J* = 5.0 Hz), 3.84 (3H, s), 4.03 (4H, m), 4.20 (1H, m), 4.64 (1H, m), 5.22 (2H, s), 6.03 (1H, d, *J* = 7.5 Hz), 7.00 (1H, s), 7.15 (1H, m), 7.46 (5H, s). Anal. Calcd for $C_{19}H_{27}N_3O_8$: C, 53.65; H, 6.35; N, 9.88. Found: C, 53.27; H, 6.53; N, 9.64.

Z-Ser-Leu-Ser-OMe. Prepared by coupling Z-Ser-Leu-azide—generated from Z-Ser-Leu-hydrazide (mp 178–179 °C, yield 99% from Z-Ser-Leu-OMe)—with Ser-OMe by method B: yield 60%; mp 182–183 °C; $[\alpha]^{25}_D$ = –39.25 (*c*, 0.21, MeOH); IR (KBr) 3440, 3280, 2960, 1735, 1683, 1638, 1615, 1535 cm^{-1}; ^{1}H NMR (80 MHz, $CDCl_3$ + $(CD_3)_2$-SO) δ 0.87 (6H, d, *J* = 5.0 Hz), 1.62 (3H, m), 3.71 (7H, s + m), 4.12–4.89 (3H, m), 5.09 (2H, s), 6.71 (1H, d, *J* = 7.5 Hz), 7.39 (5H, s), 7.85 (2H, m); MS (*m*/*z*) 454 (MH)$^+$. Anal. Calcd for $C_{21}H_{31}N_3O_8$: C, 55.63; H, 6.84; N, 9.27. Found: C, 55.37; H, 6.48; N, 9.38.

Z-Ser-Gly-Ser-OMe.[51] Prepared by coupling Z-Ser-Gly-azide—generated from Z-Ser-Gly-hydrazide (mp 173–174 °C, yield 85% from Z-Ser-Gly-OMe)—with Ser-OMe by method B: yield 60%; crystals from MeOH; mp 171–172 °C; $[\alpha]^{25}_D$ = –11.20 (*c*, 0.5, MeOH); IR (KBr) 3470, 3390, 3315, 2945, 2885, 1732, 1680, 1650, 1548, 1512 cm^{-1}; ^{1}H NMR (80 MHz, $CDCl_3$ + $(CD_3)_2SO$) δ 3.56–4.03 (9H, s + m), 4.37–4.87 (2H, m), 5.06 (2H, s), 6.78 (1H, br), 7.31 (5H, s), 7.75 (1H, d, *J* = 7.5 Hz), 8.09 (1H, t). Anal. Calcd for $C_{17}H_{23}N_3O_8$: C, 51.38; H, 5.79; N, 10.58. Found: C, 51.37; H, 5.43; N, 10.36.

Z-Ser-Pro-Ser-OMe. Prepared by coupling Z-Ser-Pro-azide—generated from Z-Ser-Pro-hydrazide (mp 130–131 °C; yield 76% from Z-Ser-Pro-OMe)—with Ser-OMe by method B: yield 30%; syrup; $[\alpha]^{25}_D$ = –65.53 (*c*, 3.16, MeOH); IR (KBr) 3381, 2953, 1742, 1719, 1639, 1533, 1452 cm^{-1}; ^{1}H NMR (80 MHz, $CDCl_3$) δ 2.00 (4H, br), 3.07–4.00 (9H, s + m), 4.53 (3H, m), 5.03 (2H, s), 6.29 (1H, d, *J* = 7.5 Hz), 7.28 (5H, s), 7.68 (1H, d, *J* = 7.5 Hz). Anal. Calcd for $C_{20}H_{27}N_3O_8$: C, 54.92; H, 6.18; N, 9.61. Found: C, 55.19; H, 6.33; N, 9.45.

(D) Preparation of N-Terminal Thr Peptides Listed in Table 2. Z-Thr-Gly-OMe[52] (method A): yield 78%; mp 97 °C; $[\alpha]^{25}_D$ = –12.3 (*c*, 2.2, MeOH); IR (KBr) 3288, 1731, 1689, 1649, 1556 cm^{-1}; ^{1}H NMR (80 MHz, $CDCl_3$) δ 1.15 (3H, d, *J* = 6.5 Hz), 3.71 (3H, s), 3.92 (2H, d, *J* = 5.0 Hz), 4.21 (2H, m), 5.09 (2H, s), 6.06 (1H, d, *J* = 7.5 Hz), 7.34 (6H, s + m). Anal. Calcd for $C_{15}H_{20}N_2O_6$: C, 55.55; H, 6.17; N, 8.64. Found: C, 55.09; H, 6.42; N, 8.36.

Z-Thr-Ala-OMe[53] (method A): yield 60%; mp 128 °C; $[\alpha]^{25}_D$ = –28.3 (*c*, 3.3, MeOH); IR (KBr) 3404, 3300, 3065, 2924, 1756, 1692, 1648, 1542 cm^{-1}; ^{1}H NMR (80 MHz, $CDCl_3$) δ 1.14 (3H, d, *J* = 6.5 Hz), 1.34 (3H, d, *J* = 7.0 Hz), 3.75 (3H, s), 4.22 (2H, m), 4.53 (1H, m), 5.12 (2H, s), 6.00 (1H, d, *J* = 7.5 Hz), 7.35 (6H, s + m). Anal. Calcd for $C_{16}H_{22}N_2O_6$: C, 56.80; H, 6.51; N, 8.28. Found: C, 57.09; H, 6.43; N, 8.38.

Z-Thr-Phe-OMe[54] (method A): yield 63%; mp 99 °C; $[\alpha]^{25}_D$ = –2.8 (*c*, 3.3, MeOH); IR (KBr) 3299, 3063, 2929, 1743, 1697, 1648, 1540 cm^{-1}; ^{1}H NMR (80 MHz, $CDCl_3$) δ 1.09 (3H, d, *J* = 6.5 Hz), 3.06 (2H, m), 3.68 (3H, s), 4.15 (2H, m), 4.81 (1H, m), 5.06 (2H, s), 5.62 (1H, d, *J* = 7.5 Hz), 6.75–7.56 (11H, s + m). Anal. Calcd for $C_{22}H_{26}N_2O_6$: C, 63.77; H, 6.28; N, 6.76. Found: C, 63.38; H, 6.59; N, 6.95.

Z-Thr-Leu-OMe[55] (method A): yield 75%; syrup; IR (KBr) 3415, 3322, 3066, 2959, 1739, 1692, 1650, 1542 cm^{-1}; ^{1}H NMR (80 MHz, $CDCl_3$) δ 0.90 (6H, d, *J* = 5.0 Hz), 1.15 (3H, d, *J* = 6.5 Hz), 1.59 (3H, m), 3.72 (3H, s), 4.22 (2H, m), 4.50 (1H, m), 5.10 (2H, s), 5.88 (1H, d, *J* = 7.5 Hz), 7.00 (1H, br d, *J* = 7.5 Hz), 7.31 (5H, s). Anal. Calcd for $C_{19}H_{28}N_2O_6$: C, 60.00; H, 7.37; N, 7.37. Found: C, 60.19; H, 7.46; N, 7.24.

Z-Thr-Thr-OMe (method A): yield 59%; mp 99 °C; $[\alpha]^{25}_D$ = –10.7 (*c*, 3.6, MeOH); IR (KBr) 3472, 3408, 3312, 3073, 2989, 2931, 1738, 1703, 1664, 1549 cm^{-1}; ^{1}H NMR (80 MHz, $CDCl_3$) δ 1.18 (6H, d, *J* = 6.5 Hz), 3.74 (3H, s), 4.28 (2H, m), 4.53 (2H, dd, *J* = 8.7 Hz, 2.5 Hz), 5.12 (2H, s), 6.09 (1H, d, *J* = 7.5 Hz), 7.37 (6H, s + m). Anal. Calcd for $C_{17}H_{24}N_2O_7$: C, 55.43; H, 6.52; N, 7.61. Found: C, 55.64; H, 6.46; N, 7.46.

Z-Thr-Lys(N$^\omega$Z)-OMe[56] (method A): yield 59%; mp 98–99 °C; $[\alpha]^{25}_D$ = –12.29 (*c*, 1.66, MeOH); IR (KBr) 3320, 3088, 2928, 2862, 1742, 1686, 1649, 1541 cm^{-1}; ^{1}H NMR (80 MHz, $CDCl_3$) δ 1.12 (3H, d, *J* = 6.5 Hz), 1.40 (4H, m), 1.68 (2H, m), 3.12 (2H, m), 3.71 (3H, s), 4.04–4.60 (3H, m), 5.04, 5.11 (2H, 2H, s, s), 5.78 (1H, d, *J* = 7.5 Hz), 7.00 (1H, d, *J* = 7.5 Hz), 7.34 (11H, s + m); MS (*m*/*z*) 530 (MH)$^+$. Anal. Calcd for $C_{27}H_{35}N_3O_8$: C, 61.25; H, 6.62; N, 7.94. Found: C, 61.44; H, 6.23; N, 7.88.

Boc-Thr-Ala-Ala-OMe. Prepared by condensing Boc-Thr-Ala-OH with Ala-OMe using method A: yield 45%; mp 127–128 °C; IR (KBr) 3325, 2980, 2933, 1737, 1702, 1676, 1635, 1543 cm^{-1}; ^{1}H NMR (80 MHz, $CDCl_3$) δ 1.15 (3H, d, *J* = 6.5 Hz), 1.37 (6H, d, *J* = 7.5 Hz), 1.43 (9H, s), 3.73 (3H, s), 4.00–4.72 (4H, m), 5.53 (1H, d, *J* = 7.5 Hz), 7.00 (2H, m). Anal. Calcd for $C_{16}H_{29}N_3O_7$: C, 51.20; H, 7.73; N, 11.20. Found: C, 50.89; H, 7.67; N, 11.28.

Z-Thr-Ala-Ala-OMe. Prepared by coupling Z-Thr-Ala-azide—made from Z-Thr-Ala-hydrazide (mp 195–196 °C)—with Ala-OMe using method B: yield 42%; mp 165–166 °C; $[\alpha]^{25}_D$ = –51.60 (*c*, 0.56, MeOH); IR (KBr) 3296, 3075, 2959, 1738, 1697, 1636, 1551 cm^{-1}; ^{1}H NMR (80 MHz, $CDCl_3$) δ 1.15 (3H, d, *J* = 6.5 Hz), 1.34 (6H, d, *J* = 7.5 Hz), 3.75 (3H, s), 4.09–4.68 (4H, m), 5.12 (2H, s), 5.78 (1H, d, *J* = 7.5 Hz), 7.34 (5H, s), 7.84 (2H, m). Anal. Calcd for $C_{19}H_{27}N_3O_7$: C, 55.74; H, 6.60; N, 10.27. Found: C, 55.92; H, 6.72; N, 10.38.

Z-Thr-Cys(*S*-Bzl)-OMe (method A): yield 90%; mp 140–141 °C; $[\alpha]^{25}_D$ = –26.14 (*c*, 0.83, MeOH); IR (KBr) 3306, 2928, 1744, 1694, 1644, 1530 cm^{-1}; ^{1}H NMR (80 MHz, $CDCl_3$) δ 1.12 (3H, d, *J* = 6.5 Hz), 2.81 (2H, m), 3.71 (3H, s), 3.73 (2H, s), 3.93–4.50 (2H, m), 4.71 (1H, m), 5.12 (2H, s), 5.68 (1H, d, *J* = 7.5 Hz), 7.12–7.56 (11H, s + s + m); MS (*m*/*z*) 461 (MH)$^+$. Anal. Calcd for $C_{23}H_{28}N_2O_6S$: C, 60.00; H, 6.09; N, 6.09. Found: C, 59.87; H, 5.83; N, 6.38.

Z-Thr-Leu-Leu-OMe. Prepared by coupling Z-Thr-Leu-azide with Leu-OMe using method B: yield 59%; mp 136–137 °C; $[\alpha]^{25}_D$ = –45.84 (*c*, 1.66, MeOH); IR (KBr) 3290, 2957, 2927, 1746, 1699, 1640, 1542 cm^{-1}; ^{1}H NMR (60 MHz, $CDCl_3$) δ 0.90 (12H, br s), 1.13 (3H, d, *J* = 6.5 Hz), 1.59 (6H, m), 3.66 (3H, s), 4.03–4.76 (4H, m), 5.06 (2H, s), 5.96 (1H, br d), 6.73–7.46 (7H, s + m); MS (*m*/*z*) 494 (MH)$^+$. Anal. Calcd for $C_{25}H_{39}N_3O_7$: C, 60.85; H, 7.91; N, 8.52. Found: C, 61.38; H, 7.84; N, 8.09.

Z-Thr-Leu-Leu-Leu-OMe. Prepared by coupling Z-Thr-Leu-azide—generated from Z-Thr-Leu-hydrazide—with Leu-Leu-OMe (freshly prepared by deprotecting Boc-Leu-Leu-OMe (mp 128–129 °C) with TFA in CH_2Cl_2 (25%, 2 h, 0 °C)) using method B: yield 52%; mp 197–198 °C; $[\alpha]^{25}_D$ = –61.5 (*c*, 1.66, MeOH); IR (KBr) 3281, 2956, 1750, 1699, 1637, 1543 cm^{-1}; ^{1}H NMR (80 MHz, $CDCl_3$) δ 0.89 (18H, br s), 1.11 (3H, d, *J* = 6.5 Hz), 1.66 (9H, m), 3.69 (3H, s), 3.93–4.84 (5H, m), 5.09 (2H, s), 6.03 (1H, d, *J* = 7.5 Hz), 7.34 (8H, s + m); MS (*m*/*z*) 607 (MH)$^+$. Anal. Calcd for $C_{31}H_{50}N_4O_8$: C, 61.39; H, 8.25; N, 9.24. Found: C, 61.44; H, 8.08; N, 9.43.

(E) Preparation of C-Terminal Ser and Thr Peptides Amides Listed in Table 3. The amides were made from the corresponding esters by treatment of their methanolic solution with dry ammonia gas at 0 °C.[57]

Bz-Ser-NH$_2$: yield 75%; mp 162–163 °C; IR (KBr) 3378, 3292, 3188, 1634, 1578, 1525 cm^{-1}.

Z-Ser-NH$_2$: yield 82%; mp 130–131 °C; $[\alpha]^{26}_D$ = +7.95 (*c*, 1.66, MeOH); IR (KBr) 3376, 3318, 3204, 2947, 1686, 1650, 1532, 1465 cm^{-1}; ^{1}H NMR (80 MHz, $CDCl_3$ + $(CD_3)_2SO$) δ 3.65 (2H, m), 4.06 (1H, m), 5.06 (2H, s), 6.56–7.46 (8H, s + m). Anal. Calcd for $C_{11}H_{14}N_2O_4$: C, 55.46; H, 5.88; N, 11.76. Found: C, 55.65; H, 5.96; N, 11.89.

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Z-Leu-Ser-NH$_2$: yield 74%; mp 148–149 °C; IR (KBr) 3299, 2956, 1689, 1646, 1540 cm^{-1}; ^{1}H NMR (80 MHz, $CDCl_3$ + $(CD_3)_2SO$) δ 0.87 (6H, d, J = 5.0 Hz), 1.56 (3H, m), 3.68 (2H, m), 4.15 (1H, m), 4.80 (1H, m), 5.06 (2H, s), 6.78–7.37 (8H, s + m), 7.65 (1H, d, J = 7.5 Hz). Anal. Calcd for $C_{17}H_{25}N_3O_5$: C, 58.12; H, 7.12; N, 11.97. Found: C, 58.24; H, 7.34; N, 11.80.

Bz-Gly-Ser-NH$_2$: yield 65%; mp 110–111 °C; IR (KBr) 3382, 3283, 1661, 1552 cm^{-1}; ^{1}H NMR (80 MHz, $CDCl_3$ + $(CD_3)_2SO$) δ 3.78 (2H, m), 3.96 (2H, d, J = 5.0 Hz), 4.39 (1H, m), 4.80 (1H, br), 6.84 (1H, br), 7.09–8.25 (7H, m), 8.60 (1H, br). Anal. Calcd for $C_{12}H_{15}N_3O_4$: C, 54.34; H, 5.66; N, 15.85. Found: C, 54.52; H, 5.72; N, 15.89.

Z-Gly-Ser-NH$_2$: yield 85%; mp 123–124 °C; IR (KBr) 3356, 3294, 3201, 1705, 1661, 1518 cm^{-1}; ^{1}H NMR (80 MHz, $CDCl_3$ + $(CD_3)_2SO$) δ 3.56–4.00 (4H, m), 4.37 (1H, m), 5.12 (2H, s), 6.62–7.50 (8H, s + m), 7.71 (1H, d, J = 7.5 Hz).

Z-Thr-NH$_2$: yield 60%; mp syrup; IR (KBr) 3280, 1670, 1638, 1600, 1525 cm^{-1}.

Z-Ser-Leu-NH$_2$: yield 90%; mp 110–115 °C; IR (KBr) 3444, 3303, 2955, 1644, 1541 cm^{-1}. Anal. Calcd for $C_{17}H_{25}N_3O_5$: C, 58.12; H, 7.12; N, 11.97. Found: C, 58.30; H, 7.29; N, 11.72.

Z-Ser-Ser-NH$_2$: yield 76%; mp 221–222 °C; IR (KBr) 3401, 3297, 3268, 2926, 1685, 1647, 1548 cm^{-1}; ^{1}H NMR (80 MHz, $CDCl_3$ + $(CD_3)_2SO$) δ 3.68 (4H, m), 4.20 (2H, m), 5.04 (2H, s), 6.82 (2H, br), 7.10 (1H, br), 7.32 (5H, s), 7.79 (1H, br); MS (m/z) 326 $(MH)^+$.

(F) Preparation of Nonterminal Ser Peptides Listed in Table 4. Bz-Leu-Ser-Leu-OMe. Prepared by coupling Bz-Leu-Ser-azide—generated from Bz-Leu-Ser-hydrazide (mp 172–173 °C)—with Leu-OMe using method B: yield 74%; mp 87–88 °C; $[\alpha]^{26}_D$ = –25.9 (c, 3.3, MeOH); IR (KBr) 3286, 3066, 2957, 1747, 1636, 1532 cm^{-1}; ^{1}H NMR (80 MHz, $CDCl_3$) δ 0.93 (12H, m), 1.62 (6H, m), 3.72 (3H, s), 3.87 (2H, br), 4.37–5.00 (3H, m), 7.06–7.96 (8H, m). Anal. Calcd for $C_{23}H_{35}N_3O_6$: C, 61.47; H, 7.79; N, 9.35. Found: C, 61.16; H, 7.83; N, 9.48.

Bz-Ala-Ser-Ala-OMe. Prepared by coupling Bz-Ala-Ser-azide—generated from Bz-Ala-Ser-hydrazide (mp 208–209 °C)—with Ala-OMe using method B: yield 62%; mp 195–196 °C; $[\alpha]^{26}_D$ = –30.96 (c, 1.76, MeOH); IR (KBr) 3270, 3070, 2980, 2930, 1743, 1688, 1625, 1533 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$ + $(CD_3)_2SO$) δ 1.28, 1.36 (3H, 3H, d, d, J = 7.2 Hz, 7.2 Hz), 3.40 (2H, m), 3.60 (3H, s), 4.28 (2H, m), 4.48 (1H, m), 7.50 (3H, m), 7.90 (3H, m), 8.16 (1H, d, J = 7.5 Hz), 8.54 (1H, d, J = 7.5 Hz); MS (m/z) 365 $(M)^+$. Anal. Calcd for $C_{17}H_{23}N_3O_6$: C, 55.89; H, 6.30; N, 11.51. Found: C, 55.54; H, 6.27; N, 11.59.

Z-Leu-Ser-His-OMe. Prepared by coupling Z-Leu-Ser-azide—generated from Z-Leu-Ser-hydrazide (mp 148–149 °C)—with His-OMe using method B: yield 68%; mp 126–127 °C; $[\alpha]^{26}_D$ = –15.66 (c, 1.66, MeOH); IR (KBr) 3298, 2953, 1732, 1688, 1640, 1537 cm^{-1}; ^{1}H NMR (80 MHz, $CDCl_3$ + $(CD_3)_2SO$) δ 0.90 (6H, d, J = 5.0 Hz), 1.62 (3H, m), 3.06 (2H, m), 3.53–4.00 (5H, s + m), 4.37 (1H, m), 4.53–5.37 (4H, s + m), 6.50 (1H, d, J = 7.5 Hz), 6.81 (1H, s), 7.18–8.15 (8H, s + m); MS (m/z) 504 $(MH)^+$. Anal. Calcd for $C_{24}H_{33}N_5O_7$: C, 57.27; H, 6.56; N, 13.92. Found: C, 57.43; H, 6.67; N, 13.49.

Z-Gly-Ser-Gly-OMe. Prepared by coupling Z-Gly-Ser-azide—generated from Z-Gly-Ser-hydrazide (mp 205 °C)—with Gly-OMe using method B: yield 50%; mp 154–155 °C; $[\alpha]^{26}_D$ = –16.6 (c, 1.0, MeOH); IR (KBr) 3297, 3066, 2947, 1760, 1704, 1646, 1555 cm^{-1}; ^{1}H NMR (80 MHz, $CDCl_3$ + $(CD_3)_2SO$) δ 3.50–4.03 (9H, s + m), 4.53 (1H, m), 5.12 (2H, s), 7.00 (1H, br), 7.37 (5H, s), 7.69 (1H, m), 8.00 (1H, m). Anal. Calcd for $C_{16}H_{21}N_3O_7$: C, 52.32; H, 5.72; N, 11.44. Found: C, 52.49; H, 5.81; N, 11.38.

Z-Gly-Ser-Gly-Ser-OMe. Prepared by coupling Z-Gly-Ser-Gly-azide—generated from Z-Gly-Ser-Gly-hydrazide (mp 158–159 °C; IR (KBr) 3295, 2927, 2850, 1737, 1647, 1627, 1602, 1571, 1537 cm^{-1})—with Ser-OMe using method B: yield 35%; sticky solid; IR (KBr) 3477, 3325, 2927, 2850, 1739, 1705, 1626, 1575, 1533 cm^{-1}.

Bz-Pro-Ser-Pro-OMe. Prepared by coupling Bz-Pro-Ser-azide—generated from Bz-Pro-Ser-hydrazide (sticky solid)—with Pro-OMe using method B: yield 44%; mp 70–71 °C; $[\alpha]^{26}_D$ = –42.52 (c, 1.74, $CHCl_3$); IR (KBr) 3340, 2960, 1735, 1625, 1570, 1530, 1440 cm^{-1}; ^{1}H NMR (80 MHz, $CDCl_3$) δ 2.06 (8H, m), 3.40–4.03 (9H, s + m), 4.25–5.21 (3H, m), 7.18–8.06 (6H, m). Anal. Calcd for $C_{21}H_{27}N_3O_6$: C, 60.43; H, 6.47; N, 10.07. Found: C, 60.28; H, 6.35; N, 9.87.

Bz-Aib-Ser-Aib-OMe. Prepared by coupling Bz-Aib-Ser-azide—generated from Bz-Aib-Ser-hydrazide (mp 57–58 °C)—with Aib-OMe using method B: yield 75%; mp 73–74 °C; IR (KBr) 3290, 2988, 1737, 1649, 1537 cm^{-1}; ^{1}H NMR (80 MHz, $CDCl_3$) δ 1.66 (12H, m), 3.69 (3H, s), 3.87–4.56 (3H, m), 7.10–8.00 (8H, m); MS (m/z) 394 $(MH)^+$. Anal. Calcd for $C_{19}H_{27}N_3O_6$: C, 58.01; H, 6.87; N, 10.69. Found: C, 57.91; H, 6.56; N, 10.59.

Z-Leu-Ser-Ser-Leu-Leu-Ser-Leu-OMe. Prepared by coupling Z-Leu-Ser-Ser-Leu-Leu-azide—generated from Z-Leu-Ser-Ser-Leu-Leu-hydrazide—with Ser-Leu-OMe using method B. Z-Leu-Ser-Ser-Leu-Leu-hydrazide [mp 172–173 °C; IR (KBr) 3293, 2957, 2872, 1653, 1541 cm^{-1}; ^{1}H NMR (400 MHz, $(CD_3)_2SO$) δ 0.82 (18H, m), 1.34–1.68 (9H, m), 3.44–3.70 (4H, m), 4.02–4.38 (5H, m), 5.02 (2H, s), 5.16 (1H, br), 7.02 (2H, m), 7.34 (5H, s), 7.48 (1H, d, J = 7.5 Hz), 7.60 (1H, d, J = 7.5 Hz), 8.00 (3H, m)] was obtained in 63% yield from Z-Leu-Ser-Ser-Leu-Leu-OMe (sticky solid) which, in turn, was prepared by condensing Z-Leu-Ser-Ser-azide—generated from Z-Leu-Ser-Ser-hydrazide (mp 209 °C)—with Leu-Leu-OMe using method B. Heptapeptide was obtained in 41% yield: mp 145–146 °C; IR (KBr) 3291, 3064, 2957, 1748, 1694, 1648, 1534 cm^{-1}; ^{1}H NMR (400 MHz, $(CD_3)_2SO$) δ 0.93 (24H, br d), 1.66 (12H, m), 3.73 (3H, br s), 4.00 (2H, m), 4.20 (4H, m), 4.46 (4H, m), 5.15 (5H, s + m), 5.66 (1H, br), 6.26 (1H, br), 7.08–7.84 (9H, s + m), 8.35 (1H, br). Anal. Calcd for $C_{42}H_{69}N_7O_{13}$: C, 57.34; H, 7.85; N, 11.15. Found: C, 57.44; H, 7.53; N, 11.36.

(G) Preparation of Nonterminal Thr Peptides Listed in Table 4. Bz-Ala-Thr-Ala-OMe. Prepared by coupling Bz-Ala-Thr-OH (mp 154–155 °C) with Ala-OMe using method A: yield 65%; also prepared by condensing Bz-Ala-Thr-azide—prepared from Bz-Ala-Thr-hydrazide (mp 164–165 °C)—with Ala-OMe using method B: yield 45%; mp 206–207 °C; $[\alpha]^{26}_D$ = –30.9 (c, 3.3, MeOH); IR (KBr) 3326, 3268, 2928, 2850, 1738, 1683, 1623, 1577, 1532 cm^{-1}; ^{1}H NMR (80 MHz, $CDCl_3$) δ 1.12 (3H, d, J = 6.5 Hz), 1.40 (3H, d, J = 7.5 Hz), 1.53 (3H, d, J = 7.5 Hz), 3.78 (3H, s), 4.15–4.96 (4H, m), 7.31–8.22 (8H, m). Anal. Calcd for $C_{18}H_{25}N_3O_6$: C, 56.99; H, 6.60; N, 11.08. Found: C, 57.14; H, 6.26; N, 10.87.

Bz-Pro-Thr-Pro-OMe. Prepared by coupling Bz-Pro-Thr-OH (mp 128–129 °C) with Pro-OMe using method A: yield 83%; syrup; IR (KBr) 3326, 2928, 2850, 1745, 1626, 1574, 1533, 1435 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.26 (3H, d, J = 6.5 Hz), 1.76–2.38 (8H, m), 3.40–3.94 (7H, s + m), 4.18 (1H, m), 4.52 (1H, m), 4.72 (2H, m), 7.26–7.64 (6H, m); MS (m/z) 432 $(MH)^+$. Anal. Calcd $C_{22}H_{29}N_3O_6$: C, 61.25; H, 6.73; N, 9.74. Found: C, 61.67; H, 7.03; N, 9.93.

Bz-Ala-Ala-Thr-Ala-Ala-OMe. Prepared by coupling Bz-Ala-Ala-Thr-azide—generated from Bz-Ala-Ala-Thr-hydrazide (mp 196–197 °C)—with Ala-Ala-OMe [prepared by deprotection (Pd/C, 10%/H_2) of Z-Ala-Ala-OMe (mp 104–105 °C)]: yield 43%; mp 253 °C; $[\alpha]^{26}_D$ = –43.13 (c, 1.66, MeOH); IR (KBr) 3271, 3071, 2928, 2850, 1753, 1690, 1627, 1531 cm^{-1}; ^{1}H NMR (80 MHz, $CDCl_3$ + $(CD_3)_2SO$) δ 0.90–1.56 (15H, m), 3.71 (3H, s), 4.09–4.87 (6H, m), 7.28–8.47 (10H, m); MS (m/z) 522 $(MH)^+$. Anal. Calcd for $C_{24}H_{35}N_5O_8$: C, 55.28; H, 6.72; N, 13.44. Found: C, 55.11; H, 6.49; N, 13.74.

Cyclo(Ser-Ser):[28] yield 56%; mp 247 °C dec; IR (KBr) 3273, 1676, 1465, 1325 cm^{-1}; ^{1}H NMR (80 MHz, $(CD_3)_2SO$) δ 3.78 (6H, br), 4.92 (2H, br s, exchangeable), 8.00 (2H, br s, exchangeable).

Ru(VIII) Oxidation of Ser/Thr Peptides: Cleavage of C-Terminal Ser/Thr Peptides Listed in Table 1 to Des-Ser/Thr C-Terminal Amides. A typical procedure for the Ru(VIII) mediated cleavage of Ser/Thr peptides is as follows. A mixture of the Ser/Thr peptide (1 mmol), $NaIO_4$ (18 mmol), $RuCl_3 \cdot 3H_2O$ (2.2 mol%) and MeCN/CCl_4/pH 3 phosphate buffer (or distilled water when pH 6 was required), 4 mL/4 mL/8 mL, was mechanically shaken in a sealed flask at room temperature for 1.5 h and then cooled. The flask was cautiously opened and the mixture filtered. The residue was washed with CH_3CN (2 × 5 mL). The combined filtrates were evaporated *in vacuo* without heating, and the residue was stirred with saturated aqueous $NaHCO_3$ (15 mL), extracted with EtOAc (3 × 20 mL), and dried ($MgSO_4$). The solvents were removed to yield the crude product amide in the case of C-terminal Ser/Thr peptides and uncleaved product in the case of N-terminal or nonterminal Ser/Thr peptides. The products were either crystallized from hot EtOAc or MeOH or purified on a column of silica gel (100–200 mesh) using benzene/EtOAc as eluents. In cases where acidic product was expected, the bicarbonate extract was cooled, acidified with 2 N H_2SO_4 (pH ~ 3), saturated with solid NaCl, and extracted with EtOAc (2 × 30 mL), dried ($MgSO_4$), evaporated and crystallized or directly esterified with diazomethane in ether.

The structures of the product C-terminal amides were confirmed by direct comparison of their spectra and melting points with those of the authentic samples (prepared[57] from peptide methyl esters and dry NH_3 gas).

The same experimental protocol was used for Ser/Thr peptides listed in Tables 1–4.

Cleavage reactions were carried out at pH 3 for 1.5 h unless otherwise stated. Product yields and melting points are reported in Tables 1–4 against their respective entries.

Cleavage of Bz-Ser-OMe and Bz-Thr-OMe yielded Bz-NH_2.

Cleavage of o-NO_2Bz-Ser-OMe afforded o-NO_2Bz-NH_2, mp 173–174 °C (lit.[58] mp 174–178 °C).

Cleavage of Z-Gly-Ser-OMe and Z-Gly-Thr-OMe for 0.5 h at pH 3 gave Z-Gly-NH-CO-CO_2Me: IR (KBr) 3370, 3260, 3190, 1742, 1678, 1525, 1490 cm^{-1}; ^1H NMR (80 MHz, $CDCl_3$ + $(CD_3)_2SO$) δ 3.88 (3H, s), 4.09 (2H, d, J = 6.5 Hz), 5.13 (2H, s), 7.06 (1H, br, exchangeable with D_2O), 7.38 (5H, s), 11.25 (1H, s, exchangeable); MS (m/z) 295 $(MH)^+$. Anal. Calcd for $C_{13}H_{14}N_2O_6$: C, 53.06; H, 4.76; N, 9.52. Found: C, 53.21; H, 4.47; N, 9.39.

Cleavage of Z-Gly-Ser-OMe and Z-Gly-Thr-OMe for 8 h at pH 3 yielded Z-Gly-NH_2 in 92% yield: mp 131–132 °C; IR (KBr) 3382, 3327, 3184, 1680, 1642, 1525 cm^{-1}; ^1H NMR (80 MHz, $CDCl_3$ + $(CD_3)_2SO$) δ 3.75 (2H, d, J = 6.5 Hz), 5.09 (2H, s), 6.34–7.16 (3H, br), 7.38 (5H, s).

Cleavage of Bz-Gly-Ser-OMe and Bz-Gly-Thr-OMe for 8 h yielded Bz-Gly-NH_2: IR (KBr) 3270, 3150, 3075, 2940, 1695, 1675, 1633, 1603, 1575, 1550 cm^{-1}; ^1H NMR (80 MHz, $CDCl_3$ + $(CD_3)_2SO$) δ 3.91 (2H, d, J = 6.5 Hz), 6.97 (1H, br), 7.19–8.09 (6H, m), 8.59 (1H, t).

In 0.5 h reaction time, both peptides yielded terminal oxaloester Bz-Gly-NH-CO-CO_2Me: IR (KBr) 3329, 1753, 1663, 1635, 1545 cm^{-1}; ^1H NMR [80 MHz, $CDCl_3$ + $(CD_3)_2SO$] δ 3.93 (3H, s), 4.37 (2H, d, J = 5.0 Hz), 7.31–8.03 (5H, m), 8.21 (1H, br), 11.18 (1H, br s).

Cleavage of Bz-Ala-Ser-OMe and Bz-Ala-Thr-OMe gave Bz-Ala-NH_2; IR (KBr) 3300, 3165, 1690, 1635, 1603, 1577, 1548 cm^{-1}.

Cleavage of Bz-Leu-Ser-OMe and Bz-Leu-Thr-OMe gave Bz-Leu-NH_2: IR (KBr) 3390, 3325, 3195, 2980, 1635, 1612, 1588, 1562, 1532 cm^{-1}; ^1H NMR (80 MHz, $CDCl_3$ + $(CD_3)_2SO$) δ 0.91 (6H, d, J = 5.0 Hz), 1.69 (3H, m), 4.75 (1H, m), 5.75 (1H, br), 6.70 (1H, br), 6.96 (1H, d, J = 7.5 Hz), 7.34–8.00 (5H, m).

Cleavage of Z-Leu-Thr-OMe at pH 3 for 0.5 h afforded Z-Leu-NH_2: IR (KBr) 3391, 3321, 3191, 2955, 1657, 1543 cm^{-1}; ^1H NMR (80 MHz, $CDCl_3$) δ 0.90 (6H, d, J = 5.0 Hz), 1.62 (3H, m), 4.18 (1H, m), 5.09 (2H, s), 5.31 (1H, d, J = 7.5 Hz, exchangeable), 5.90 (2H, br, exchangeable), 7.34 (5H, s). Anal. Calcd for $C_{14}H_{20}N_2O_3$: C, 63.64; H, 7.58; N, 10.61. Found: C, 63.43; H, 7.29; N, 10.43.

Cleavage of Bz-Phe-Ser-OMe and Bz-Phe-Thr-OMe yielded Bz-Phe-NH_2: IR (KBr) 3410, 3335, 3200, 1662, 1635, 1608, 1583, 1525 cm^{-1}.

Cleavage of Bz-Asp(β-OMe)-Ser-OMe gave Bz-Asp(β-OMe)-NH-CO-CO_2Me: IR (KBr) 3271, 2923, 2852, 1782, 1734, 1633, 1578, 1531, 1500 cm^{-1}; ^1H NMR (80 MHz, $CDCl_3$ + $(CD_3)_2SO$) δ 2.93 (2H, m), 3.68, 3.87 (3H, 3H, s, s), 5.03 (1H, m), 7.25–8.06 (5H, m), 8.65 (1H, d, J = 7.5 Hz), 11.40 (1H, s); MS (m/z) 337 $(MH)^+$. Anal. Calcd for $C_{15}H_{16}N_2O_7$: C, 53.57; H, 4.76; N, 8.33. Found: C, 53.73; H, 4.48; N, 8.26.

Cleavage of Boc-Asp(β-OBzl)-Ser-OMe yielded Boc-Asp(β-OBzl)-NH_2: IR (KBr) 3405, 3350, 3210, 2990, 2970, 1725, 1665, 1635, 1510 cm^{-1}; ^1H NMR (80 MHz, $CDCl_3$) δ 1.43 (9H, s), 2.87 (2H, m), 4.53 (1H, m), 5.12 (2H, s), 5.65 (2H, br, exchangeable), 6.40 (1H, br, exchangeable), 7.37 (5H, s); MS (m/z) 323 $(MH)^+$. Anal. Calcd for $C_{16}H_{22}N_2O_5$: C, 59.63; H, 6.83; N, 8.70. Found: C, 59.36; H, 6.63; N, 8.53.

Cleavage of Bz-Glu(γ-OMe)-Ser-OMe yielded Bz-Glu(γ-OMe)-NH_2: IR (KBr) 3396, 3306, 3185, 1730, 1659, 1633, 1577, 1523 cm^{-1}; ^1H NMR (80 MHz, $CDCl_3$ + $(CD_3)_2SO$) δ 1.87–2.56 (4H, m), 3.69 (3H, s), 4.59 (1H, m), 6.46 (1H, br), 7.06–8.03 (7H, m); MS (m/z) 264 $(M)^+$. Anal. Calcd for $C_{13}H_{16}N_2O_4$: C, 59.09; H, 6.06; N, 10.61. Found: C, 58.69; H, 5.76; N, 10.64.

Cleavage of Z-Asn-Ser-OMe gave Z-Asn-NH_2: IR (KBr) 3383, 3321, 3185, 1696, 1655, 1533 cm^{-1}; ^1H NMR (80 MHz, $CDCl_3$ + $(CD_3)_2SO$) δ 2.50 (2H, d, J = 6.5 Hz), 4.34 (1H, m), 5.07 (2H, s), 6.73–7.31 (10H, s + m); MS (m/z) 266 $(MH)^+$. Anal. Calcd for $C_{12}H_{15}N_3O_4$: C, 54.34; H, 5.66; N, 15.85. Found: C, 54.25; H, 5.36; N, 15.75.

Cleavage of Z-Gln-Ser-OMe gave Z-Gln-NH_2: IR (KBr) 3390, 3315, 3199, 2925, 1654, 1539 cm^{-1}; ^1H NMR (80 MHz, $CDCl_3$ + $(CD_3)_2SO$) δ 1.50–2.31 (4H, m), 3.96 (1H, m), 5.09 (2H, s), 6.64–7.50 (10H, s + m); MS (m/z) 280 $(MH)^+$. Anal. Calcd for $C_{13}H_{17}N_3O_4$: C, 55.91; H, 6.09; N, 15.05. Found: C, 56.22; H, 6.04; N, 14.83.

Cleavage of Z-Met-Ser-OMe gave Z-Met(SO)$_2$-NH_2: IR (KBr) 3425, 3380, 3190, 1650, 1525, 1415 cm^{-1}; ^1H NMR (80 MHz, $CDCl_3$ + $(CD_3)_2$-SO) δ 2.00–3.34 (7H, s + m), 4.37 (1H, m), 5.12 (2H, s), 6.33 (1H, br), 6.62 (1H, br d), 7.40 (6H, s + m); MS (m/z) 315 $(MH)^+$. Anal. Calcd for $C_{13}H_{18}N_2O_5S$: C, 49.68; H, 5.73; N, 8.92. Found: C, 49.43; H, 5.94; N, 8.63.

Cleavage of Bz-Val-Ser-OMe afforded Bz-Val-NH_2: IR (KBr) 3400, 3320, 3210, 2980, 1660, 1632, 1602, 1578, 1520 cm^{-1}; ^1H NMR (80 MHz, $CDCl_3$ + $(CD_3)_2SO$) δ 1.00 (6H, dd, J = 5.0 Hz, 2.5 Hz), 2.15 (1H, m), 4.50 (1H, m), 6.31 (1H, br), 7.12–7.96 (7H, m); MS (m/z) 221 $(MH)^+$.

Cleavage of Bz-Pro-Ser-OMe gave Bz-Pro-NH_2: IR (KBr) 3370, 3180, 2960, 1660, 1602, 1562 cm^{-1}; ^1H NMR (80 MHz, $CDCl_3$ + $(CD_3)_2SO$) δ 2.12 (4H, m), 3.59 (2H, m), 4.71 (1H, m), 6.31 (1H, br), 7.46 (6H, m); MS (m/z) 219 $(MH)^+$. Anal. Calcd for $C_{12}H_{14}N_2O_2$: C, 66.06; H, 6.42; N, 12.84. Found: C, 66.18; H, 6.52; N, 12.93.

Cleavage of Boc-Arg(N^GNO_2)-Ser-OMe afforded Boc-Arg(N^GNO_2)-NH_2: IR (KBr) 3324 (br), 2972, 2851, 1675, 1625, 1595, 1527, 1367 cm^{-1}; ^1H NMR (80 MHz, $CDCl_3$ + $(CD_3)_2SO$) δ 1.40 (9H, s), 2.00 (4H, m), 3.34 (2H, m), 4.06 (1H, m), 5.53 (1H, br), 7.00–7.56 (5H, br); MS (m/z) 319 $(MH)^+$. Anal. Calcd for $C_{11}H_{22}N_6O_5$: C, 41.51; H, 6.92; N, 26.41. Found: C, 41.09; H, 7.23; N, 27.11.

Cleavage of Bz-Pro-Phe-Ser-OMe gave Bz-Pro-Phe-NH_2: IR (KBr) 3310, 3160, 1675, 1655, 1615, 1532, 1440 cm^{-1}. Anal. Calcd for $C_{21}H_{23}N_3O_3$: C, 69.04; H, 6.30; N, 11.51. Found: C, 69.22; H, 6.27; N, 11.64.

Cleavage of Boc-Ala-Ala-Ser-OMe and Boc-Ala-Ala-Thr-OMe gave Boc-Ala-Ala-NH_2: IR (KBr) 3390, 3350, 3315, 3205, 3005, 1685, 1645, 1540 cm^{-1}; ^1H NMR (80 MHz, $CDCl_3$ + $(CD_3)_2SO$) δ 1.26 (3H, d, J = 6.5 Hz), 1.37 (3H, d, J = 6.5 Hz), 1.43 (9H, s), 3.87–4.59 (2H, m), 6.31 (2H, m), 7.06 (1H, br), 7.53 (1H, d, J = 7.5 Hz). Anal. Calcd for $C_{11}H_{21}N_3O_4$: C, 50.96; H, 8.11; N, 16.22. Found: C, 50.84; H, 7.93; N, 16.64.

Cleavage of Bz-Val-Phe-Ser-OMe and Bz-Val-Phe-Thr-OMe yielded Bz-Val-Phe-NH_2: IR (KBr) 3423, 3318, 3275, 3215, 2980, 2940, 1675, 1655, 1632, 1618, 1580, 1540 cm^{-1}. Anal. Calcd for $C_{21}H_{25}N_3O_3$: C, 68.66; H, 6.81; N, 11.44. Found: C, 68.38; H, 7.16; N, 11.29.

Cleavage of Bz-Gly-Phe-Thr-OMe gave Bz-Gly-Phe-NH_2: IR (KBr) 3435, 3380, 3285, 3210, 2935, 2860, 1682, 1670, 1640, 1607, 1580, 1540, 1492 cm^{-1}; ^1H NMR (80 MHz, $CDCl_3$ + $(CD_3)_2SO$) δ 3.03 (2H, m), 3.87 (2H, m), 4.60 (1H, m), 6.80 (1H, br s), 7.00–8.03 (12H, s + m), 8.50 (1H, t). Anal. Calcd for $C_{18}H_{19}N_3O_3$: C, 66.46; H, 5.85; N, 12.92. Found: C, 66.11; H, 5.75; N, 12.74.

Oxidation of N-Terminal Ser/Thr Peptides Listed in Table 2 to Oxalamido Peptides. Oxidation of Z-Ser-Gly-OMe and Z-Thr-Gly-OMe yielded oxalamido peptide Z-NH-CO-CO-Gly-OMe: IR (KBr) 3250, 3215, 3185, 3050, 2980, 2958, 1778, 1755, 1745, 1698, 1680, 1500 cm^{-1}; ^1H NMR (80 MHz, $CDCl_3$) δ 3.81 (3H, s), 4.11 (2H, d, J = 6.5 Hz), 5.25 (2H, s), 7.40 (5H, s), 7.81 (1H, br, exchangeable), 9.37 (1H, br s, exchangeable); ^{13}C NMR (100 MHz, $CDCl_3$) δ 41.4, 52.6, 68.4, 128.5, 128.7, 134.5, 149.7, 157.0, 158.3, 168.5; MS (m/z) 294 $(M)^+$. Anal. Calcd for $C_{13}H_{14}N_2O_6$: C, 53.06; H, 4.76; N, 9.52. Found: C, 52.78; H, 4.63; N, 9.26.

Oxidation of Z-Ser-Ala-OMe and Z-Thr-Ala-OMe afforded Z-NH-CO-CO-Ala-OMe: IR (KBr) 3332, 3254, 1792, 1749, 1691, 1478 cm^{-1}; ^1H NMR (80 MHz, $CDCl_3$) δ 1.46 (3H, d, J = 6.5 Hz), 3.75 (3H, s), 4.53 (1H, m), 5.22 (2H, s), 7.34 (5H, s), 7.87 (1H, br d, exchangeable), 9.43 (1H, br s, exchangeable); ^{13}C NMR (100 MHz, $CDCl_3$) δ 17.8, 48.7, 52.7, 68.3, 128.5, 128.7, 134.6, 149.7, 157.1, 157.5, 171.5; MS (m/z) 309 $(MH)^+$. Anal. Calcd for $C_{14}H_{16}N_2O_6$: C, 54.54; H, 5.19; N, 9.09. Found: C, 54.74; H, 5.46; N, 8.78.

Oxidation of Z-Ser-Phe-OMe and Z-Thr-Phe-OMe gave Z-NH-CO-CO-Phe-OMe: IR (KBr) 3318, 1779, 1740, 1718, 1677, 1474 cm^{-1}; ^1H NMR (60 MHz, $CDCl_3$) δ 3.15 (2H, d, J = 6.0 Hz), 3.70 (3H, s), 4.76 (1H, m), 5.18 (2H, s), 6.09–7.50 (10H, s + m), 7.73 (1H, br d, exchangeable), 9.26 (1H, br s, exchangeable). Anal. Calcd for $C_{20}H_{20}N_2O_6$: C, 62.50; H, 5.21; N, 7.29. Found: C, 62.17; H, 5.05; N, 6.93.

Oxidation of Z-Ser-Leu-OMe and Z-Thr-Leu-OMe afforded Z-NH-CO-CO-Leu-OMe: IR (neat) 3321 (br), 2957, 1793, 1743, 1690 (br), 1487 (br) cm^{-1}; ^1H NMR (80 MHz, $CDCl_3$) δ 0.87 (6H, d, J = 5.0 Hz), 1.62 (3H, m), 3.71 (3H, s), 4.53 (1H, m), 5.20 (2H, s), 7.34 (5H, s), 7.75 (1H, d, J = 7.5 Hz), 9.43 (1H, br s); MS (m/z) 350 $(M)^+$. Anal. Calcd for $C_{17}H_{22}N_2O_6$: C, 58.29; H, 6.29; N, 8.00. Found: C, 58.73; H, 6.48; N, 8.36.

Oxidation of Z-Ser-Methyl anthranilate yielded Z-NH-CO-CO-methyl anthranilate: IR (KBr) 3302, 3185, 1758, 1735, 1702, 1687, 1603, 1588, 1541, 1491 cm^{-1}; ^1H NMR (60 MHz, $CDCl_3$) δ 3.93 (3H, s), 5.26 (2H,

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s), 6.93–7.76 (7H, s + m), 8.00 (2H, m), 8.56 (1H, d, J = 9.0 Hz), 9.41 (1H, s, exchangeable); MS (m/z) 356 $(M)^+$. Anal. Calcd for $C_{18}H_{16}N_2O_6$: C, 60.67; H, 4.49; N, 7.86. Found: C, 60.89; H, 4.34; N, 7.64.

Oxidation of Z-Ser-Tyr-OMe gave Z-NH-CO-CO-NH_2 as the neutral component [IR (KBr) 3375, 3171, 1802, 1776, 1684, 1515, 1404 cm^{-1}; ^1H NMR (80 MHz, $CDCl_3$ + $(CD_3)_2SO$) δ 5.25 (2H, s), 7.40 (5H, s), 8.00 (2H, br, exchangeable), 10.31 (1H, s, exchangeable); MS (m/z) 223 $(MH)^+$] and Z-NH-CO-CO-Asp(β-OH)-OMe as the acidic part [obtained from the bicarbonate triturated portion by acidification (2N H_2SO_4), saturation (solid NaCl), extraction (EtOAc, 2 × 30 mL), drying ($MgSO_4$), and evaporation *in vacuo* and directly transformed to the methyl ester, Z-NH-CO-CO-Asp(β-OMe)-OMe, by esterification with ethereal diazomethane: IR (neat) 3333, 3033, 2955, 1788, 1729, 1687, 1485 cm^{-1}; ^1H NMR (60 MHz, $CDCl_3$) 2.96 (2H, m), 3.68, 3.76 (3H, 3H, s, s), 4.80 (1H, m), 5.23 (2H, s), 7.30 (5H, s), 8.06 (1H, br d, exchangeable), 9.30 (1H, s, exchangeable); MS (m/z) 367 $(MH)^+$. Anal. Calcd for $C_{16}H_{18}N_2O_8$: C, 52.46; H, 4.92; N, 7.65. Found: C, 52.43; H, 4.67; N, 7.56].

Oxidation of Z-Ser-Trp-OMe afforded Z-NH-CO-CO-NH_2 as neutral part and Z-NH-CO-CO-Asp(β-OH)-OMe (characterized as the methyl ester) as the acidic portion.

Oxidation of Z-Ser-Pro-OMe afforded Z-NH-CO-CO-Pro-OMe: IR (neat) 3360, 3278, 2955, 1790, 1734, 1652, 1560, 1436 cm^{-1}; ^1H NMR (80 MHz, $CDCl_3$) δ 2.09 (4H, m), 3.81 (3H, s), 4.03 (2H, m), 4.60 (1H, m), 5.26 (2H, s), 7.47 (5H, s), 9.75 (1H, br s); MS (m/z) 335 $(MH)^+$. Anal. Calcd for $C_{16}H_{18}N_2O_6$: C, 57.48; H, 5.39; N, 8.38. Found: C, 57.76; H, 5.43; N, 8.44.

Oxidation of Z-Ser-Asp(β-OMe)-OMe yielded Z-NH-CO-CO-Asp-(β-OMe)-OMe as the sole isolable product.

Oxidation of Z-Ser-Ser-OMe and Z-Thr-Thr-OMe afforded a mixture of Z-NH-CO-$CONH_2$ and trace amounts of Z-NH-CO-CO-NH-CO-CO_2Me: IR (KBr) 3375, 3172, 2924, 1776, 1686, 1513 cm^{-1}; ^1H NMR (80 MHz, $CDCl_3$ + $(CD_3)_2SO$) δ 3.96 (3H, s), 5.23 (2H, s), 7.46 (5H, s), 10.46 (1H, br, exchangeable), 11.25 (1H, br, exchangeable); MS (m/z) 308 $(M)^+$.

Oxidation of Z-Ser-Met-OMe yielded Z-NH-CO-CO-Met-(SO_2)-OMe: IR (KBr) 3300, 3025, 2931, 1785, 1750, 1670, 1497, 1375, 1298, 1269, 1220, 1186, 1122 cm^{-1}; ^1H NMR (80 MHz, $CDCl_3$) δ 2.44 (2H, m), 2.92 (3H, s), 3.04 (2H, m), 3.81 (3H, s), 4.66 (1H, m), 5.22 (2H, s), 7.35 (5H, s), 8.19 (1H, d, J = 7.5 Hz, exchangeable), 9.38 (1H, s, exchangeable); MS (m/z) 401 $(MH)^+$.

Oxidation of Z-Ser-Aib-Ser-OMe afforded the C-terminal Ser cleaved product as Z-NH-CO-CO-Aib-NH_2: IR (KBr) 3486, 3348, 2923, 1785, 1687, 1497, 1456 cm^{-1}; ^1H NMR (80 MHz, $CDCl_3$) δ 1.62 (6H, br s), 5.18 (2H, s), 5.96 (2H, br), 7.33 (5H, s), 8.03 (1H, s), 9.40 (1H, s); MS (m/z) 308 $(MH)^+$. Anal. Calcd for $C_{14}H_{17}N_3O_5$: C, 54.72; H, 5.54; N, 13.68. Found: C, 54.63; H, 5.88; N, 13.43.

Oxidation of Z-Ser-Leu-Ser-OMe yielded the N-terminal Ser oxidized and C-terminal Ser cleaved Z-NH-CO-CO-Leu-NH_2 as the main product: IR (neat) 3392, 3294, 3194, 2958, 1783, 1659, 1492 cm^{-1}; ^1H NMR (60 MHz, $CDCl_3$) δ 0.91 (6H, br d), 1.63 (3H, m), 4.43 (1H, m), 5.18 (2H, s), 6.30 (2H, br, exchangeable), 7.31 (5H, s), 8.05 (1H, d, J = 7.5 Hz, exchangeable), 9.50 (1H, s, exchangeable); MS (m/z) 335 $(M)^+$. Anal. Calcd for $C_{16}H_{21}N_3O_5$: C, 57.31; H, 6.27; N, 12.54. Found: C, 57.43; H, 6.48; N, 12.17.

Z-Ser-Leu-Ser-OMe when reacted at pH 6, afforded a mixture of Z-NH-CO-CO-Leu-NH_2 (30%) and Z-Ser-Leu-NH_2 (50%).

Oxidation of Z-Ser-Gly-Ser-OMe gave the N-terminal Ser oxidized and C-terminal Ser cleaved product Z-NH-CO-CO-Gly-NH_2: IR (KBr) 3402, 3208, 1772, 1686, 1654, 1493 cm^{-1}; ^1H NMR (80 MHz, $CDCl_3$ + $(CD_3)_2SO$) δ 3.80 (2H, d, J = 6.5 Hz), 5.22 (2H, s), 7.09 (1H, br s, exchangeable), 7.42 (6H, s), 9.00 (1H, t, exchangeable), 10.75 (1H, s, exchangeable); MS (m/z) 279 $(M)^+$. Anal. Calcd for $C_{12}H_{13}N_3O_5$: C, 51.61; H, 4.66; N, 15.05. Found: C, 51.29; H, 4.38; N, 15.35. (Oxidation at pH 6 yielded the same product.)

Oxidation of Z-Ser-Pro-Ser-OMe at pH 6 yielded a mixture of two products which were identified as Z-NH-CO-CO-Pro-NH_2 [IR (KBr) 3348, 2955, 1785, 1679, 1497, 1453 cm^{-1}; ^1H NMR (80 MHz, $CDCl_3$) δ 2.10 (4H, m), 3.80 (2H, m), 4.50 (1H, m), 5.20 (2H, s), 7.33 (7H, br s), 9.80 (1H, s); MS (m/z) 320 $(MH)^+$] and Z-Ser-Pro-NH_2 [IR (KBr) 3220, 2959, 2883, 1672, 1448 cm^{-1}; ^1H NMR (80 MHz, $CDCl_3$) δ 2.00 (4H, br), 3.31–4.03 (4H, m), 5.09 (4H, s + br), 6.15 (1H, br, exchangeable), 6.93–7.53 (7H, s + br)].

Oxidation of Z-Thr-Lys(N^{ω}Z)-OMe afforded Z-NH-CO-CO-Lys-(N^{ω}Z)-OMe: IR (KBr) 3329, 2932, 1791, 1700 (br), 1494, 1455 cm^{-1}; ^1H NMR (80 MHz, $CDCl_3$) δ 1.03–1.93 (6H, m), 3.12 (2H, m), 3.68 (3H, s), 4.46 (1H, m), 5.09 (4H, s), 7.34 (11H, br s + m), 7.90 (1H, d, J = 7.5 Hz, exchangeable), 9.43 (1H, br s, exchangeable). Anal. Calcd for $C_{25}H_{29}N_3O_8$: C, 60.12; H, 5.81; N, 8.42. Found: C, 59.87; H, 5.82; N, 8.37.

Oxidation of Boc-Thr-Ala-Ala-OMe gave Boc-NH-CO-CO-Ala-Ala-OMe: IR (KBr) 3394, 3302, 2955, 1786, 1746, 1660, 1542 cm^{-1}; ^1H NMR (80 MHz, $CDCl_3$) δ 1.00–1.62 (15H, m), 3.79 (3H, s), 4.54 (2H, m), 6.73 (1H, d, J = 7.5 Hz), 8.06 (1H, d, J = 7.5 Hz), 9.28 (1H, s). Anal. Calcd for $C_{14}H_{23}N_3O_7$: C, 48.70; H, 6.67; N, 12.17. Found: C, 48.53; H, 6.44; N, 12.27.

Oxidation of Z-Thr-Ala-Ala-OMe yielded Z-NH-CO-CO-Ala-Ala-OMe: IR (KBr) 3328, 2929, 1793, 1742, 1689, 1487 cm^{-1}; ^1H NMR (80 MHz, $CDCl_3$) δ 1.46 (6H, d, J = 6.5 Hz), 3.78 (3H, s), 4.56 (2H, m), 5.25 (2H, s), 7.37 (6H, s), 7.93 (1H, br d), 9.46 (1H, br s). Anal. Calcd for $C_{17}H_{21}N_3O_7$: C, 53.83; H, 5.54; N, 11.08. Found: C, 53.47; H, 5.18; N, 11.33.

Oxidation of Z-Thr-Cys(S-Bzl)-OMe gave Z-NH-CO-CO-Cys-(SO_2-Bzl)-OMe: IR (KBr) 3302, 2949, 1783, 1743, 1673, 1483, 1311, 1173, 1134 cm^{-1}; ^1H NMR (80 MHz, $CDCl_3$) δ 3.40 (2H, d, J = 5.0 Hz), 3.78 (3H, s), 4.28 (2H, s), 4.96 (1H, m), 5.25 (2H, s), 7.43 (10H, s), 8.40 (1H, d, J = 7.5 Hz, exchangeable), 9.37 (1H, s, exchangeable); MS (m/z) 463 $(MH)^+$. Anal. Calcd for $C_{21}H_{22}N_2O_8S$: C, 54.54; H, 4.76; N, 6.06. Found: C, 54.18; H, 4.68; N, 5.83.

Oxidation of Z-Thr-Leu-Leu-OMe yielded Z-NH-CO-CO-Leu-Leu-OMe; IR (neat) 3308, 2956, 2870, 1788, 1744, 1665, 1535, 1484 cm^{-1}; ^1H NMR (80 MHz, $CDCl_3$) δ 0.93 (12H, br s), 1.62 (6H, m), 3.72 (3H, s), 4.56 (2H, m), 5.22 (2H, s), 6.78 (1H, d, J = 7.5 Hz, exchangeable), 7.37 (5H, s), 8.00 (1H, d, J = 7.5 Hz, exchangeable), 9.62 (1H, s, exchangeable); MS (m/z) 464 $(MH)^+$. Anal. Calcd for $C_{23}H_{33}N_3O_7$: C, 59.61; H, 7.13; N, 9.07. Found: C, 59.44; H, 7.23; N, 9.27.

Oxidation of Z-Thr-Leu-Leu-Leu-OMe afforded Z-NH-CO-CO-Leu-Leu-Leu-OMe: IR (neat) 3289, 2956, 2871, 1784, 1742, 1651, 1484 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 0.9 (18H, br s), 1.66 (9H, m), 3.72 (3H, s), 4.34–4.64 (3H, m), 5.24 (2H, s), 6.40 (2H, m, exchangeable), 7.36 (5H, s), 7.80 (1H, d, J = 7.5 Hz, exchangeable), 9.40 (1H, s, exchangeable); MS (m/z) 577 $(MH)^+$. Anal. Calcd for $C_{29}H_{44}N_4O_8$: C, 60.42; H, 7.64; N, 9.72. Found: C, 60.09; H, 7.73; N, 10.04.

Oxidation of C-Terminal Ser and Thr Peptide Amides Listed in Table 3. Oxidation of Bz-Ser-NH_2 yielded Bz-NH-CO-$CONH_2$: IR (KBr) 3428, 3376, 3324, 3283, 3172, 1763, 1702, 1678, 1646, 1597, 1578, 1500 cm^{-1}; ^1H NMR (80 MHz, $CDCl_3$) δ 6.36 (2H, br d, exchangeable), 7.15–8.12 (5H, m), 10.56 (1H, s, exchangeable); MS (m/z) 193 $(MH)^+$. Anal. Calcd for $C_9H_8N_2O_3$: C, 56.25; H, 4.17; N, 14.58. Found: C, 56.08; H, 4.26; N, 14.96.

Oxidation of Z-Ser-NH_2 gave Z-NH-CO-$CONH_2$.

Oxidation of Z-Leu-Ser-NH_2 afforded Z-Leu-NH-CO-$CONH_2$: IR (KBr) 3396, 3304, 2952, 1764, 1704, 1678, 1518, 1479 cm^{-1}; ^1H NMR (80 MHz, $CDCl_3$ + $(CD_3)_2SO$) δ 0.93 (6H, d, J = 5.0 Hz), 1.62 (3H, m), 4.65 (1H, m), 5.18 (2H, s), 6.71 (1H, d, J = 7.5 Hz, exchangeable), 7.12–7.75 (7H, s + br), 10.50 (1H, s, exchangeable); MS (m/z) 336 $(MH)^+$. Anal. Calcd for $C_{16}H_{21}N_3O_5$: C, 57.31; H, 6.27; N, 12.54. Found: C, 57.13; H, 6.48; N, 12.67.

Oxidation of Bz-Gly-Ser-NH_2 afforded Bz-Gly-NH-CO-$CONH_2$: IR (KBr) 3397, 3288, 3160, 1780, 1687, 1637, 1557, 1490 cm^{-1}; ^1H NMR (80 MHz, $CDCl_3$ + $(CD_3)_2SO$) δ 4.40 (2H, d, J = 5.0 Hz), 6.71 (1H, br, exchangeable), 7.00–8.46 (7H, m), 10.65 (1H, s, exchangeable); MS (m/z) 250 $(MH)^+$. Anal. Calcd for $C_{11}H_{11}N_3O_4$: C, 53.01; H, 4.42; N, 16.87. Found: C, 52.81; H, 4.46; N, 17.16.

Oxidation of Z-Gly-Ser-NH_2 gave Z-Gly-NH-CO-CO-NH_2: IR (KBr) 3396, 3365, 3292, 3252, 1705, 1685, 1538, 1476 cm^{-1}; ^1H NMR [80 MHz, $CDCl_3$ + $(CD_3)_2SO$] δ 4.15 (2H, d, J = 5.0 Hz), 5.09 (2H, s), 7.37 (6H, s + br), 8.03 (2H, br), 10.62 (1H, s).

Oxidation of Z-Thr-NH_2 gave Z-NH-CO-CO-NH_2.

Oxidation of Z-Ser-Leu-NH_2 yielded Z-NH-CO-CO-Leu-NH_2, identical in all respects with the major product obtained from the oxidation of Z-Ser-Leu-Ser-OMe at pH 6.

Oxidation of Z-Ser-Ser-NH_2 yielded the cleaved product Z-NH-CO-$CONH_2$, identical in all respects with the product obtained from the oxidation of Z-Ser-NH_2.

Oxidation of Nonterminal Ser/Thr Peptides Listed in Table 4. Oxidation of Bz-Leu-Ser-Leu-OMe yielded Bz-Leu-NH-CO-CO-Leu-OMe: IR (KBr) 3330, 2960, 2938, 2875, 1770, 1745, 1690, 1645, 1605, 1578, 1530, 1470 cm^{-1}; ^1H NMR (80 MHz, $CDCl_3$) δ 0.93 (12H, d, J = 5.0 Hz), 1.68 (6H, m), 3.75 (3H, s), 4.59 (1H, m), 5.34 (1H, m), 7.04 (1H, d, J = 7.5 Hz, exchangeable), 7.25–8.03 (6H, m), 10.23 (1H, s,

exchangeable). Anal. Calcd for $C_{22}H_{31}N_3O_6$: C, 60.97; H, 7.16; N, 9.70. Found: C, 61.28; H, 7.18; N, 9.36.

Oxidation of Bz-Ala-Ser-Ala-OMe and Bz-Ala-Thr-Ala-OMe gave Bz-Ala-NH-CO-CO-Ala-OMe: IR (KBr) 3277, 2936, 1762, 1740, 1674, 1630, 1534, 1488 cm^{-1}; ^1H NMR (80 MHz, $CDCl_3$) δ 1.46, 1.51 (3H, 3H, d, d, J = 6.5 Hz, 6.5 Hz), 3.78 (3H, s), 4.53 (1H, m), 5.31 (1H, m), 6.81 (1H, d, J = 7.5 Hz, exchangeable), 7.28–8.00 (6H, m), 10.09 (1H, s, exchangeable); MS (m/z) 349 $(M)^+$. Anal. Calcd for $C_{16}H_{19}N_3O_6$: C, 55.01; H, 5.44; N, 12.03. Found: C, 55.43; H, 5.08; N, 11.87.

Oxidation of Z-Leu-Ser-His-OMe yielded Z-Leu-NH-CO-CO-NH-$CH(CO_2Me)$-CH_2-CO-NH-CHO as the major product, wherein the serine residue was oxidized to the α-ketoamide (-NH-CO-CO-) unit and the histidine side chain was transformed to that of *N*-formylasparagine (-CH_2-CO-NH-CHO); pale yellow crystals from EtOAc/hexane; IR (KBr) 3396, 3319, 3209, 2955, 1775, 1732, 1660, 1543 cm^{-1}; ^1H NMR (80 MHz, $CDCl_3$) δ 0.90 (6H, br), 1.57 (3H, m), 3.14 (2H, m), 3.78 (3H, s), 4.87 (2H, m), 5.10 (2H, s), 5.50 (1H, br, exchangeable), 7.34 (5H, s), 8.40 (1H, d, J = 7.5 Hz, exchangeable), 9.12 (1H, m, nonexchangeable), 10.18 (1H, s, exchangeable), 10.43 (1H, d, J = 7.5 Hz, exchangeable). Attempted purification of this sample on silica gel by preparative TLC yielded the cleaved product, Z-Leu-NH_2.

Oxidation of Z-Gly-Ser-Gly-OMe afforded Z-Gly-NH-CO-CO-Gly-OMe: IR (KBr) 3383, 3325, 3272, 3210, 3169, 1760, 1720, 1690, 1651, 1531, 1512, 1493 cm^{-1}; ^1H NMR (80 MHz, $CDCl_3$ + $(CD_3)_2SO$) δ 3.75 (3H, s), 4.06, 4.34 (2H, 2H, d, d, J = 5.0 Hz, 5.0 Hz), 5.12 (2H, s), 6.46 (1H, br, exchangeable), 7.34 (5H, s), 8.75 (1H, br m, exchangeable), 10.25 (1H, s, exchangeable); MS (m/z) 351 $(M)^+$. Anal. Calcd for $C_{15}H_{17}N_3O_7$: C, 51.28; H, 4.84; N, 11.97. Found: C, 50.87; H, 4.64; N, 11.79.

Oxidation of Z-Gly-Ser-Gly-Ser-OMe yielded Z-Gly-NH-CO-CO-Gly-NH_2: IR (KBr) 3316, 2929, 2851, 1694, 1627, 1575, 1536 cm^{-1}; ^1H NMR (80 MHz, $CDCl_3$) δ 3.93, 4.09 (2H, 2H, d, d, J = 5.0 Hz, 5.0 Hz), 5.15 (2H, s), 5.71 (1H, br), 7.34 (7H, s + br), 9.03 (1H, br).

Oxidation of Bz-Pro-Ser-Pro-OMe and Bz-Pro-Thr-Pro-OMe yielded Bz-Pro-NH-CO-CO-Pro-OMe: IR (KBr) 3320, 2920, 1725, 1640, 1600, 1560 cm^{-1}; ^1H NMR (80 MHz, $CDCl_3$) δ 1.75–2.31 (8H, m), 3.59–3.93 (7H, s + m), 4.25–4.93 (2H, m), 6.18 (1H, br, exchangeable), 7.28–8.00 (5H, m).

Oxidation of Bz-Aib-Ser-Aib-OMe yielded Bz-Aib-NH-CO-CO-Aib-OMe: IR (KBr) 3377, 3326, 2997, 2948, 1770, 1738, 1702, 1680, 1657, 1519 cm^{-1}; ^1H NMR (80 MHz, $CDCl_3$) δ 1.66 (12H, d, J = 6.5 Hz), 3.71 (3H, s), 6.78 (1H, s), 7.25–8.00 (6H, m), 10.68 (1H, s); MS (m/z) 378 $(MH)^+$. Anal. Calcd for $C_{18}H_{23}N_3O_6$: C, 57.29; H, 6.10; N, 11.14. Found: C, 57.46; H, 6.27; N, 11.26.

Oxidation of Z-Leu-Ser-Ser-Leu-Leu-Ser-Leu-OMe yielded a mixture of products, from which only Z-Leu-NH_2 could be isolated and identified.

Oxidation of Bz-Ala-Ala-Thr-Ala-Ala-OMe gave the chain intact, Bz-Ala-Ala-NH-CO-CO-Ala-Ala-OMe: IR (KBr) 3295, 2929, 1756 (br), 1637, 1548 cm^{-1}; ^1H NMR (80 MHz, $CDCl_3$) δ 1.40 (12H, d, J = 6.5 Hz), 3.78 (3H, s), 4.59 (4H, m), 6.43–8.28 (10H, m); MS (m/z) 492 $(MH)^+$.

Oxidation of Cyclo(Ser-Ser) yielded as the sole isolable product tetraketopiperazine: yield (30%); IR (KBr) 3199, 1731 (br), 1419, 1361, 1329, 1290 cm^{-1}; ^1H NMR (80 MHz, $(CD_3)_2SO$) δ 12.06 (br s, exchangeable); MS (m/z) 142 $(M)^+$.

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