A Novel Resin Linker for Solid-Phase Peptide Synthesis Which Can Be Cleaved Using Two Sequential Mild Reactions

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The interest in developing new linkers for solid-phase peptide and organic synthesis has increased tremendously as a result of the rapid development of combinatorial chemistry. Herein, we report the development of a new redox-sensitive linker for solid-phase peptide synthesis. This linker can be readily cleaved under mild conditions by using two sequential mild reactions, a reduction followed by a base ($Bu_4N^+F^-$)-catalyzed cyclic ether formation. By using this new linker, two short peptides, a tetrapeptide [Boc-Trp-Ala-Gly-Gly-OH] and a pentapeptide [Boc-Asn-Ala-Ser(OBn)-Gly-Glu(OBn)-OH)], were synthesized. Because the cleavage does not use acidic conditions, this resin linker provides an alternative when acidic conditions are not desirable. Furthermore, the cleavage conditions do not affect most of the side chain protecting group. Therefore, the peptides synthesized can be used for the segment synthesis of larger peptides without the need to reprotect the side chain functional groups.

Introduction

Since its invention by Merrifield, solid-phase synthesis has made an enormous impact not only in the peptide chemistry field[1−3] but also in the general field of organic synthesis.[4−8] With the rapid development of combinatorial chemistry, solid-phase synthesis is playing an even more important role in the synthesis and search for organic molecules of biological importance.[5−7] The recent rapid development of solid-phase synthesis also resulted in an increased demand for new resin linkers that can be cleaved under different conditions.[7,9−26] These new linkers should be stable and yet readily cleavable under mild reaction conditions when needed. We have recently reported the design and synthesis of a new redox-sensitive resin linker for the synthesis of C-terminal modified peptides.[27] Herein, we report a new linker which can be used for the synthesis of unmodified peptides and can be cleaved under mild conditions.

The design takes advantage of a "trimethyl lock"-facilitated cyclic ether formation (Scheme 1),[28−31] which is different from the design of the linker reported earlier from our group[27] in that the earlier linker utilized a facilitated lactone formation reaction. Consequently, the linker reported in this study allows for the preparation of peptides with free C-termini. In contrast, the linker reported earlier[27] only allows for the preparation of C-terminally modified peptides. In the current design, the first amino acid of the peptide is attached to the linker through ester formation. Peptide synthesis can then proceed from the C-terminus to the N-terminus. At the end of the synthesis, the peptide can be cleaved from the resin through a two-step process. First, the reduction

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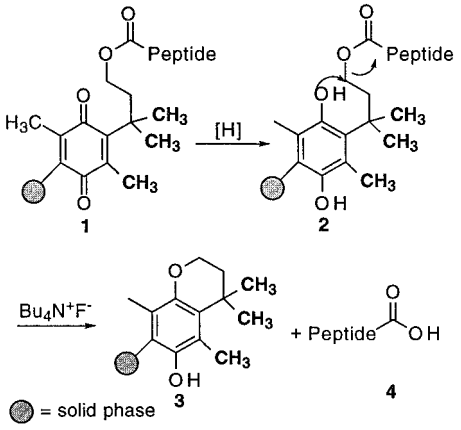
Scheme 1. Redox-Sensitive Resin Linker for Solid-Phase Peptide Synthesis



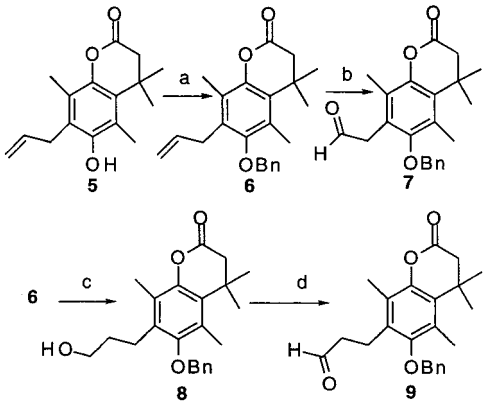
of the quinone moiety to the dihydroquinone **2** can be accomplished using a mild reducing agent. Then, treatment with tetrabutylammonium fluoride (TBAF) accomplishes the cyclization with the concomitant release of the peptide synthesized (Scheme 1).

Results and Discussion

The demonstration of the feasibility of the concept requires (1) the synthesis of the quinone-linker moiety with a pendent chain, (2) attachment of this linker moiety to a resin material, and (3) peptide synthesis using the resin linker prepared. We chose to use a polystyrene-based resin as the solid-phase material because it is most commonly used in the solid-phase synthesis of peptides and organic compounds. In designing the approach used for the attachment of the linker to the resin material, we considered several options, including ester, amide, and carbon−carbon bond formations. We chose a carbon−carbon bond formation method because of the expected stability of such an attachment, which will allow for the necessary chemical transformations after the initial attachment. We therefore chose to use a Wittig reaction for the attachment because similar reactions have long been used in solid-phase chemistry.[32]

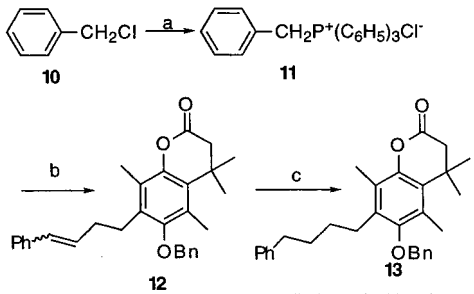
Preparation of Aldehydes 7 and 9. The design of the synthesis requires the preparation of the linker moiety with a pendent aldehyde functional group for the subsequent Wittig reaction. Therefore, the synthesis started with compound **5**, which has been prepared in a previous study.[27] The phenol hydroxyl group of **5** was first protected as a benzyl ether by using benzyl chloride in the presence of potassium carbonate and a catalytic amount sodium iodide in 90% yield (Scheme 2). There are two ways that the protected lactone **6** could be converted to an aldehyde. Ozonolysis of the side-chain double bond of **6** gave aldehyde **7** in 61% yield. Alternatively, hydroboration−oxidation of **6** followed by PCC (pyridinium chlorochromate) oxidation in DCM (dichloromethane) gave aldehyde **9** in 75% overall yield for two steps. Because the synthesis of the two aldehydes gave comparable yields, we chose to use the aldehyde (**9**) with a longer side chain for the attachment to a resin material to minimize the potential interference of the solid-phase material during the subsequent cleavage reaction. The

Scheme 2. Synthesis of Aldehydes 7 and 9



a. BnCl, K_2CO_3, NaI, acetone, reflux, 90%; b. i) O_3, DCM-MeOH, -78 °C, ii) CH_3SCH_3, 61%; c. i) BH_3, THF, ii) H_2O_2, NaOH, 87%; d. PCC, DCM, 86%.

Scheme 3. Model Wittig Reaction in Solution Phase



a. Ph_3P, PhH, reflux, 100%; b. n-BuLi, **9**, PhH, reflux, 85%; c. H_2 (55 psi), $[(C_6H_5)_3P]_3RhCl$, EtOH, PhH, CH_3COOH, 24 h, 100%.

preparation of **9** involves a total of 6 steps from a commercially available starting material, 2,5-dimethylbenzoquinone, with an overall yield of 38%.

Attachment of the Linker Moiety to Resin Beads. Before the attachment of aldehyde **9** to resin beads using a Wittig reaction, we examined the corresponding solution-phase model reactions to understand the necessary conditions for the solid-phase reactions. Therefore, Wittig reagent **11** was prepared by following well-established literature procedures starting from benzyl chloride (Scheme 3).[33] Subsequent Wittig reaction with **9** using *n*-BuLi as the base gave the desired product **12** in 85% yield. The double bond was reduced through hydrogenation using Wilkinson's catalyst [tris(triphenylphosphine)-rhodium chloride] in quantitative yield. Wilkinson's catalyst was used because it is soluble in organic solvent. An insoluble catalyst, such as Pd/C, will most likely not work in the subsequent solid-phase synthesis because of the heterogeneous nature of the reaction and the involvement of two solid-phase materials, the resin beads and Pd/C.

With the successful model reaction, we started the procedure to attach the linker to polystyrene resin beads. First, triphenylphosphine chloride in anhydrous benzene was refluxed with a benzyl chloride resin **14** for 36 h to give the resin−Wittig reagent **15** (Scheme 4). Subsequent Wittig reactions with aldehyde **9** yielded **16** in about 60% yield (Scheme 4). IR was used for monitoring the reac-

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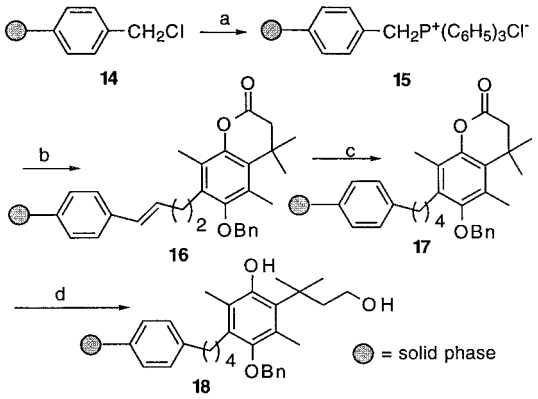
Scheme 4. Attachement of the Resin Linker Synthesized to Resin Beads Using a Wittig Reaction



a. Ph_3P, PhH, reflux; b. n-BuLi, **9**, PhH, reflux, 60%; c. H_2 (55 psi), $[(C_6H_5)_3P]_3RhCl$, EtOH, PhH, CH_3COOH; d. LAH/THF.

tions (Figure 1). The appearance of a peak at 1770 cm^{-1} (Figure 1b) after the Wittig reaction, corresponding to the lactone absorption of **16**, is indicative of a successful coupling. The coupling yield was calculated on the basis of the amount of triphenylphosphine oxide recovered from the reaction mixture. The double bond was then hydrogenated in the presence of Wilkinson's catalyst by using conditions similar to that of the solution-phase model reaction. However, it needs to be noted that the IR spectra before and after the hydrogenation did not show major differences that would allow us to unequivocally characterize the resin as the one with the double bond saturated. The lactone was then reduced with lithium aluminum hydride (LAH) to give the diol resin **18** (Scheme 4). The strong IR absorption at 1770 cm^{-1} disappeared (Figure 1c) after the reduction, indicating the complete conversion of the lactone **17** to the diol **18**.

Peptide Synthesis Using the Resin Linker Prepared. To study the feasibility of using this linker for peptide synthesis, we prepared two short peptides, a tetrapeptide [Boc-Trp-Ala-Gly-Gly-OH] and a pentapeptide [Boc-Asn-Ala-Ser(OBn)-Gly-Glu(OBn)-OH)].

The solid-phase peptide synthesis followed well-established procedures using Boc-protected amino acids and either DCC (dicyclohexylcarbodiimide) or DIC (diisopropylcarbodiimide) as the activating reagent in the presence of HOBt (1-hydroxybenzotriazole) and a catalytic amount of DMAP (4-*N*,*N*-(dimethylamino)pyridine) (Scheme 5).[1] After the coupling of the first amino acid, the dihydroquinone moiety of **19** was oxidized to a quinone moiety by using NBS (*N*-bromosuccinimide) to mask the phenol hydroxyl group.[27,34] It should be noted that methionine and cysteine are not compatible with the NBS oxidation to give **20**. In a separate solution-phase study, we noticed that NBS oxidation with sulfur-containing amino acids gave a complex mixture, presumably through oxidation of the sulfur atom. These solid-phase reactions were also followed by IR (Figure 1). The IR after coupling of the first amino acid (Figure 1d) showed new peaks at 1748 (ester) and 1719 (Boc) cm^{-1}, indicating the attachment of the first amino acid. Subsequent NBS oxidation resulted in a product **20** with new a peak 1646 (quinone C═O) cm^{-1} (Figure 1e), indicating

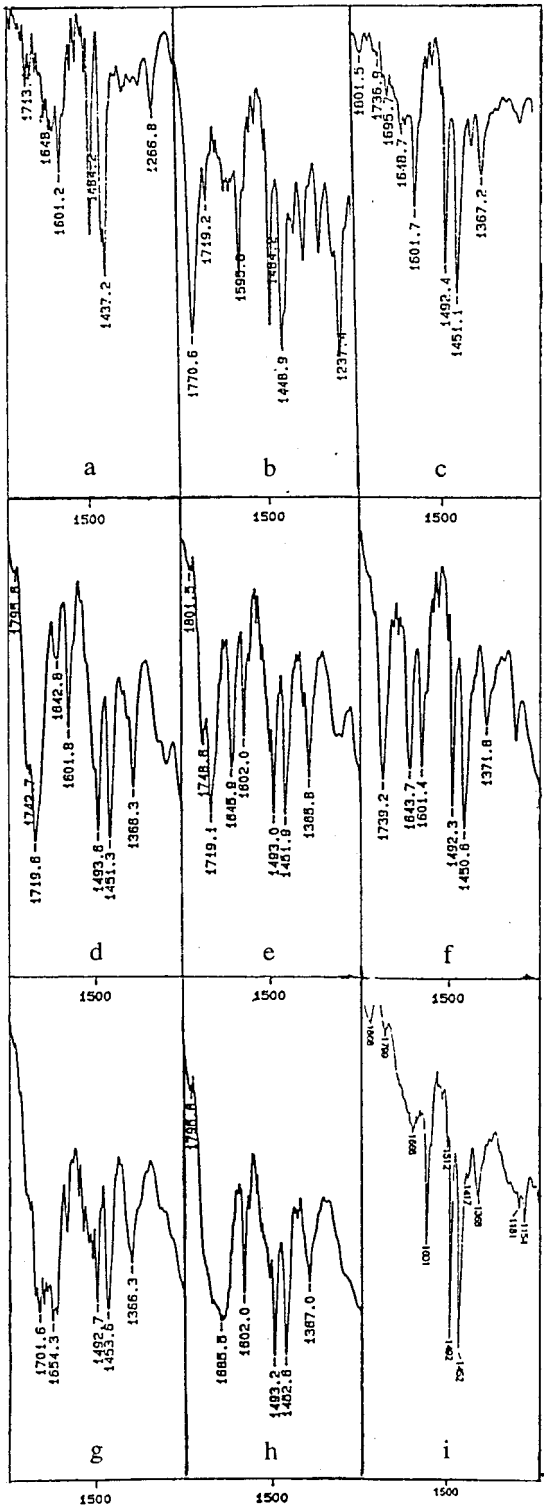


Figure 1. The IR spectra of resins **15** (a), **16** (b), **18** (c), **19** (d), **20** (e), **21** (f), **22** (g), **23** (h), and **24** (i).

the progress of the reactions as designed. Unreacted free hydroxyl groups were capped by reacting with acetic anhydride in the presence of triethylamine (TEA) in DCM. Cleavage of the Boc protecting group was achieved by treatment with 25% TFA (trifluoroacetic acid) in DCM. The ninhydrin test[1] was used for the determination of the degree of substitution of the resin, usually 0.3−0.5 mmol/g after coupling of the first amino acid. Therefore, the overall yields from **14** and **16** were 37−63% and 73−100%, respectively. The remaining amino acids were

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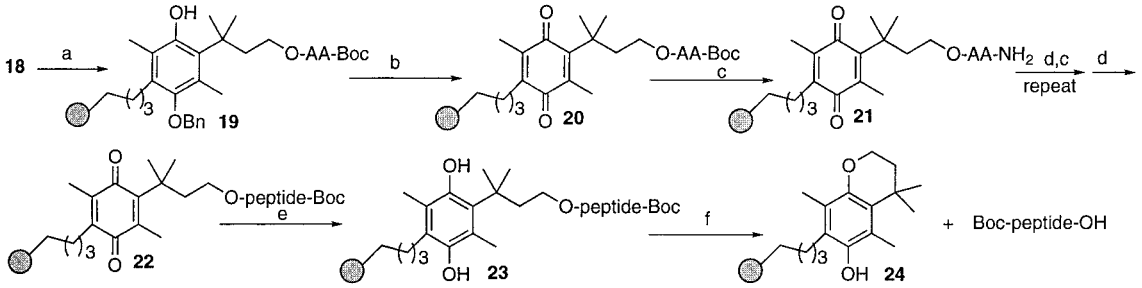
Scheme 5. Solid-Phase Peptide Synthesis Using the Resin Linker Prepared



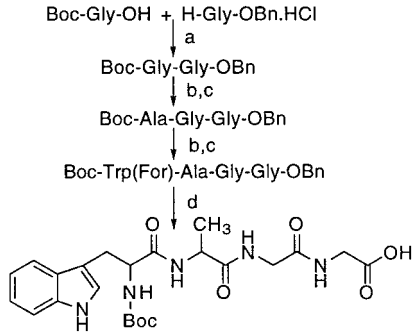
a. Boc-AA-OH, DCC, DMAP; b. NBS; c. i) Ac_2O/TEA; ii) 25% TFA/DCM; d. Boc-AA-OH, DIC, HOBt, DMAP; e. $NaBH_4$; f. TBAF/THF

assembled by using standard solid-phase synthesis procedure as described in the Experimental Section. Figure 1f shows the IR of **21**, which lacks the peak corresponding to the Boc group (1719 cm^{-1}) indicating the successful Boc deprotection. Figure 1g shows the IR of **22** with multiple peaks around 1654 cm^{-1} indicating the presence of amide bonds.

As designed (Schemes 1 and 5), the cleavage of the peptide synthesized requires first the reduction of the quinone moiety to dihydroquinone followed by base-catalyzed cyclization. It is known that the reduction of such a quinone moiety could be accomplished using aqueous $Na_2S_2O_4$.[27,34] However, in this study it was difficult to achieve complete reduction with $Na_2S_2O_4$ to give a satisfactory yield for the subsequent cleavage. Therefore, the reduction was carried out using $NaBH_4$. For the based-catalyzed cyclization reaction, we studied several methods including tetramethylguanidine (TMG) in DMF,[35] CsF, and tetrabutylammonium fluoride (TBAF). The TBAF-catalyzed reactions gave the best results. Therefore, after the reduction, the resin was treated with 1 M anhydrous TBAF in THF to achieve the desired cleavage. The IR spectrum (Figure 1i) of the resin **24** after the cleavage clearly indicated the disappearance of the peaks corresponding to the peptide bonds. The crude peptide was separated by using a mixed bed of Amberlyst 15 calcium sulfate resin and Amberlyst 15 sulfonic acid resin[36] followed by chromatography or recrystallization to give the purified product in about 35% yield. It should be noted that after the cleavage, most of the protecting groups are left intact. Among the side chain protecting groups encountered in this study, the formyl protecting group of tryptophan was not stable under the cleavage conditions. The side chain benzyl ester (glutamic acid) and benzyl ether (serine) and the terminal *N*-Boc protecting groups were unaffected during the cleavage when anhydrous TBAF was used. This makes it possible for these peptides to be used for the segment synthesis of larger peptides.

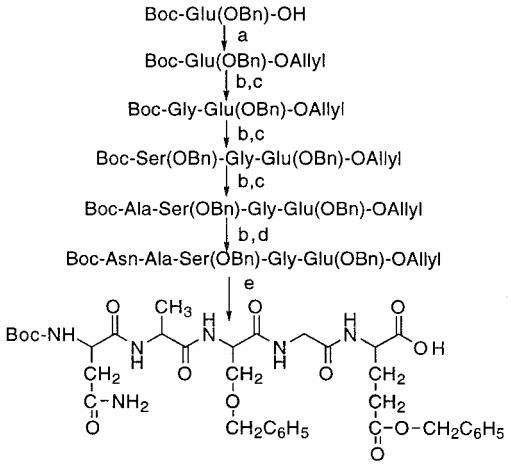
For comparison purposes, both the standard tetrapeptide and the standard pentapeptide were synthesized by using standard solution-phase peptide synthesis methods with Boc-protected amino acids as described in Schemes 6 and 7.[1] EDC [1-(3-dimethylaminopropyl)-3-ethylcarbodiimide] was used as the activating reagent in the solution-phase approach because the urea side product was water soluble and, therefore, the subsequent purification was easier. The synthesis of the protected tetrapeptide (Boc-Trp-Ala-Gly-Gly-OH) started with H-Gly-OBn. The C-terminal benzyl protection was cleaved at the end by catalytic hydrogenation (Scheme 6). For the synthesis of the standard protected pentapeptide (Boc-Asn-Ala-Ser(OBn)-Gly-Glu(OBn)-OH), the C-terminal protection cannot be a benzyl group because of the presence of side chain benzyl protection of the desired peptide. Therefore, a C-terminal allyl protection was used, which was cleaved at the end by using tetrakis(triphenylphosphin)palladium(0) in the presence of morpholine (Scheme 7). The coupling of the last amino acid in the synthesis of the pentapeptide gave a poor yield when EDC was used as the activating reagent. Therefore, PyBOP (benzotria-

Scheme 6. Preparation of the Standard Tetrapeptide



a. EDC, HOBt, TEA, 87%; b. 25% TFA/DCM; c. Boc-AA-OH, EDC, HOBt, d. i) H_2, 10% Pd/C, 97% ii) 20% piperidine/DMF.

Scheme 7. Preparation of the Standard Pentapeptide



a. i) Cs_2CO_3, CH_3OH, ii) CH_2=$CHCH_2Br$, DMF, 94%; b. 25% TFA/DCM; c Boc-AA-OH, EDC, HOBt, DMAP, about 85%; d. Boc-AA-OH, PyBop, DIPEA, 67%; e. $(Ph_3P)_4Pd$, morpholine, THF, 80%.

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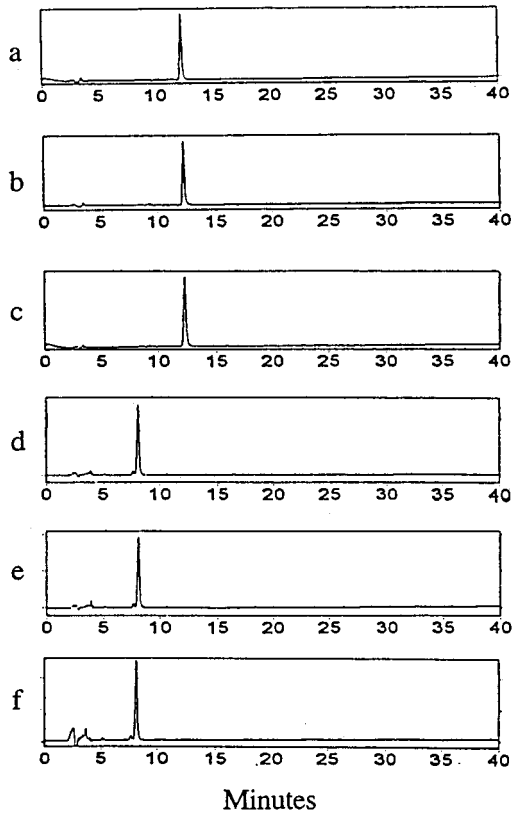

Figure 2. HPLC profiles of the peptides synthesized: (a) the standard tetrapeptide [Boc-Trp-Ala-Gly-Gly-OH] prepared through solution-phase synthesis, (b) the tetrapeptide prepared through solid-phase synthesis, (c) co-injection of the standard and the sample (tetrapeptide), (d) the standard pentapeptide [Boc-Asn-Ala-Ser(OBn)-Gly-Glu(OBn)-OH] prepared through solution-phase synthesis, (e) the pentapeptide prepared through solid-phase synthesis, and (f) co-injection of the standard and the sample (pentapeptide).

zolyoxy-tris(pyrrolidino)-phosphonium hexafluorophosphate)[37] was used for this step as the activating reagent, and DIPEA (diisopropylethylamine) was used as the base. The peptides synthesized by using the solid-phase methods were identical to the standards from the solution-phase methods by ^{1}H NMR and HPLC (Figure 2).

Conclusions

We have developed a new linker for the solid-phase synthesis of peptides. This linker can be cleaved under mild conditions by using two sequential reactions. Because the cleavage does not require acidic conditions, which are commonly used for the cleavage of the conventional Merrifield resin and Wang's resin, this resin provides an alternative when acidic conditions are not desirable. Furthermore, the cleavage conditions do not affect most of the side chain protecting groups. Therefore, the peptides synthesized can be used for the segment synthesis of larger peptides without the need to reprotect the side chain functional groups. Although the feasibility was only studied for polystyrene-based resin, it should be feasible to attach this linker to other resin materials for solid-phase synthesis. Furthermore, attachment of the resin linker to a more hydrophilic resin could make it easier to reduce quinone resin **22** to the dihydroquinone linker **23** with aqueous $Na_2S_2O_4$, which is a much milder reducing reagent and is not expected to cause any problem for most of the functional groups commonly encountered in peptides and organic compounds.[27,34]

Experimental Section

General. Mass spectra were obtained at the Mass Spectrometry Laboratory for Biotechnology at North Carolina State University. HPLC studies were carried out by using a dual pump system with a UV−vis detector (detection wavelength: 220, 245 nm; solvent: acetonitrile and water with 0.1% TFA). For the tetrapeptide (Boc-Trp-Ala-Gly-Gly-OH) the mobile-phase gradient was 70−50% H_2O/CH_3CN. For the pentapeptide (Boc-Asn-Ala-Ser(OBn)-Gly-Glu(OBn)-OH) the mobile-phase gradient was 55−50% H_2O/CH_3CN. The column was a C_{18} reversed-phase analytical column from YMC (4.6 mm × 250 mm, ODS-A, S-5 micron 120A). Unless stated otherwise, commercial reagents were from Aldrich and used without further purification. Merrifield resin (1.0 mequiv Cl/g, 1% cross linked) and protected amino acids were from Bachem Bioscience. Acetonitrile and THF were of HPLC grade from Fisher Scientific Inc. DCM was distilled from CaH_2; THF, benzene, and ethanol were distilled from sodium under N_2. TBAF was dried with P_2O_5 before the preparation of the solution. TFA was of peptide synthesis grade.

7-Allyl-6-benzyloxy-4,4,5,8-tetramethylhydrocoumarin 6. A mixture of compound **5** (635 mg, 2.44 mmol), benzyl chloride (618 mg, 4.88 mmol), K_2CO_3 (674 mg, 4.88 mmol), and sodium iodide (36 mg, 0.244 mmol) in 8 mL of acetone was refluxed for 20 h under N_2 with stirring. To the reaction mixture was added 20 mL of water, and then acetone was removed under reduced pressure. The aqueous solution was extracted with EtOAc and dried over $MgSO_4$. Filtration, evaporation, and purification by a silica gel column afforded a white solid (772 mg, 90%). ^{1}H NMR ($CDCl_3$): δ 7.46−7.30 (5H, m), 5.96−5.93 (1H, m), 5.06−4.90 (2H, m), 4.74 (2H, s), 3.50 (2H, d, J = 5.6 Hz), 2.59 (2H, s), 2.43 (3H, s), 2.23 (3H, s), 1.47 (6H, s). ^{13}C NMR ($CDCl_3$): δ 168.4, 152.6, 146.3, 137.5, 136.2, 131.3, 129.3, 128.6, 128.1, 127.6, 126.8, 124.6, 115.6, 75.3, 46.0, 35.8, 31.5, 27.7, 15.2, 12.4. IR (film): 1768, 1245, 1120, 735, 696 cm^{-1}. MS (FAB) m/z: 351 (M + H). Anal. Calcd for $C_{23}H_{26}O_3$: C, 78.80; H, 7.48. Found: C, 78.64; H, 7.39.

2-[7-(6-Benzyloxy-4,4,5,8-tetramethylhydro)coumarinyl]-ethanal 7. Compound **6** (2.0 g, 5.71 mmol) was dissolved in a mixture of 20 mL of DCM and 20 mL of methanol. Then, ozone was passed through the reaction mixture at −78 °C for 5 h. The reaction was stopped by flushing with N_2 for 2 h followed by the addition of dimethyl sulfide (1 mL). The reaction mixture was then stirred for 10 h. Evaporation of the solvents gave a pale yellow oil. Ether (70 mL) was added, and the solution was washed with water and dried over $MgSO_4$. Filtration, evaporation, and purification by a silica gel column (hexanes/EtOAc = 2/1) gave product **7** (1.226 g, 61%) as white solid. ^{1}H NMR ($CDCl_3$): δ 9.67 (1H, s), 7.38 (5H, s), 4.69 (2H, s), 3.81 (2H, s), 2.60 (2H, s), 2.44 (3H, s), 2.16 (3H, s), 1.48 (6H, s). ^{13}C NMR ($CDCl_3$): δ 199.1, 168.1, 153.0, 146.4, 136.8, 130.8, 128.8, 128.4, 127.9, 127.1, 125.2, 124.8, 75.3, 45.8, 42.9, 35.9, 27.6, 15.3, 13.1. IR (film): 2719, 1768, 1719, 1244, 1121, 736, 696 cm^{-1}. MS (FAB) m/z: 353 (M + H). Anal. Calcd for $C_{22}H_{24}O_4$: C, 74.98; H, 6.86. Found: C, 74.69; H, 6.79.

7-(3-Hydroxy-1-propyl)-6-benzyloxy-4,4,5,8-tetramethylhydrocoumarin 8. To a cooled solution of compound **6** (8.07 g, 23.06 mmol) in freshly distilled THF (95 mL) was added dropwise a borane solution (1.0 M in THF) (30.0 mL, 30.0 mmol) via a syringe under N_2 at 0 °C with stirring. After addition, stirring was continued for 2 h at 0 °C. The reaction was stopped by addition of 10 mL of water in an ice bath. Then, to the reaction mixture was added dropwise 3 N NaOH until the pH of the solution reached 7−8. Hydrogen peroxide (3.5 mL, 30−35%) was added at 0 °C. Stirring was continued for an additional 20 min. The reaction mixture was acidified with 1 N HCl to pH 6−7. Ether (200 mL) was added into the

(37) Coste, J.; Le-Nguyen, D.; Castro, B. *Tetrahedron Lett.* **1990**, *31*, 205−208.

mixture, the organic phase was separated, and the aqueous phase was extracted with ether. The combined organic layer was washed with brine and dried over $MgSO_4$. Filtration and evaporation gave an oil, which was purified by silica gel column (EtOAc/hexanes = 1/3−1/1 as eluent) to give a white solid (7.413 g, 87%). ^{1}H NMR ($CDCl_3$): δ 7.50−7.37 (5H, m), 4.75 (2H, s), 3.50 (2H, q, J = 6.0 Hz), 2.81 (2H, t, J = 7.2 Hz), 2.58 (2H, s), 2.45 (3H, s), 2.26 (3H, s), 1.77 (2H, m), 1.47 (6H, s). ^{13}C NMR ($CDCl_3$): δ 168.4, 152.6, 146.6, 137.0, 133.2, 129.1, 128.8, 128.4, 127.9, 126.5, 124.0, 75.7, 61.59, 46.0, 35.8, 32.4, 27.7, 23.3, 15.3, 12.3. IR (film): 3442, 1766, 1246, 735, 697. MS (FAB) *m*/*z*: 369 (M + H). Anal. Calcd for $C_{23}H_{28}O_4$: C, 74.96; H, 7.66. Found; C, 74.94; H, 7.77.

3-[7-(6-Benzyloxy-4,4,5,8-tetramethylhydro)coumarinyl]-propanal 9. A solution of compound **8** (3.04 g, 8.152 mmol) in anhydrous DCM (15 mL) was added dropwise into a suspension of PCC (3.315 g, 16.304 mmol) in anhydrous DCM (45 mL) with stirring under N_2 at room temperature. The flask was washed with 10 mL of anhydrous DCM, and the washing was added into the reaction flask. After addition, this mixture was stirred for 2 h. The black material was filtered off through a funnel with silica gel (70−230 mesh) and washed with DCM. Evaporation of the solvent gave a brown solid, which was purified on a silica gel column (hexanes/EtOAc = 1/1) to afford a white solid (2.57 g, 86%). ^{1}H NMR ($CDCl_3$): δ 9.75 (1H, s), 7.49−7.34 (5H, m), 4.75 (2H, s), 2.98 (2H, t, J = 7.5 Hz), 2.62 (4H, m), 2.43 (3H, s), 2.24 (3H, s), 1.47 (6H, s). ^{13}C NMR ($CDCl_3$): δ 201.6, 168.3, 152.7, 146.4, 137.2, 132.3, 129.6, 128.8, 128.3, 127.7, 127.0, 123.8, 75.5, 46.0, 43.9, 35.9, 27.7, 20.2, 15.3, 12.4. IR (film): 2719, 1765, 1720, 1244, 737, 696 cm^{-1}. MS (FAB) *m*/*z*: 366 (M^+). Anal. Calcd for $C_{23}H_{26}O_4$: C, 75.58; H, 7.15. Found: C, 75.29; H, 7.16.

Benzyltriphenylphosphonium Chloride 11.[33] A mixture of triphenylphosphine (4.039 g, 15.42 mmol) and benzyl chloride (2.3 mL, 20.04 mmol) was heated without solvent under N_2 in an oil bath with stirring until it became a solution. Heating was continued for an additional 15 min, and the reaction was allowed to cool to room temperature. The resulting solid was recrystallized in EtOH to give a white crystalline solid (5.98 g, 100%). ^{1}H NMR (CD_3OD): δ 7.91−6.98 (20H, m), 3.31 (2H, s).

Compound 12. To a solution of benzyltriphenylphosphonium chloride **11** (194 mg, 0.5 mmol) in anhydrous benzene (4 mL) was added dropwise 2.5 M BuLi solution in hexanes (200 μL, 0.5 mmol) via a syringe under N_2 at room temperature. The resulting deep-red solution was stirred for 20 min at room temperature. A solution of aldehyde **9** (201 mg, 0.55 mmol) in anhydrous DCM (2 mL) was added with stirring under N_2. The red color turned to an orange color. After stirring for 2 h at room temperature, the reaction mixture was refluxed for 3 h. After evaporation of the solvent, the residue was dissolved in ether (80 mL), washed with water, and dried over $MgSO_4$. Filtration and evaporation gave a yellow oil, which was separated with silica gel column (hexanes/EtOAc = 4/1) to give compound **12** as a colorless oil (187 mg, 85%). ^{1}H NMR ($CDCl_3$): δ 7.50−7.17 (10H, m), 6.46−5.68 (2H, m), 4.78, 4.72 (2H, ss), 2.84 (2H, m), 2.59 (2H, s), 2.42 (5H, m), 2.29, 2.20 (3H, ss), 1.46 (6H, S); IR (film): 1768, 1598, 1244, 1167, 736, 695 cm^{-1}. ^{13}C NMR ($CDCl_3$): δ 168.6, 152.8, 146.5, 137.9, 137.6, 133.7, 132.0, 130.5, 130.3, 129.7, 129.1, 128.8, 128.7, 128.3, 128.2, 127.6, 127.2, 126.9, 126.8, 126.2, 124.0, 75.4, 46.1, 35.9, 33.6, 29.2, 27.8, 15.4, 12.6. MS (FAB) *m*/*z*: 440 (M^+). Anal. Calcd for $C_{30}H_{32}O_3$: C, 81.78; H, 7.32. Found: C, 81.66; H, 7.37.

Compound 13. To a medium-pressure hydrogenation flask was added compound **12** (17 mg, 0.039 mmol), 4 mL of anhydrous and degassed benzene, 1 mL of ethanol, and 5 drops of glacial acetic acid. Then, tris(triphenylphosphine)rhodium chloride (6 mg, 0.0065 mmol) was added. The flask was flushed with hydrogen gas five times. Then, hydrogenation was carried out under 55 psi of hydrogen pressure for 24 h at room temperature. After evaporation of solvent, ether (5 mL) was added to the residue. The black solid was filtered off, and the ether phase was washed with saturated $NaHCO_3$ and water and dried over $MgSO_4$. After filtration and evaporation, the crude product was purified by using chromatotron (hexanes/EtOAc = 5/1) to give compound **13** as a colorless oil (19 mg, quantitative). ^{1}H NMR ($CDCl_3$): δ 7.43−7.12 (10H, m), 4.73 (2H, s), 2.66 (4H, m), 2.57 (2H, s), 2.42 (3H, s), 2.22 (3H, s), 1.69 (2H, m), 1.54 (2H, m), 1.45 (6H, s). ^{13}C NMR ($CDCl_3$): δ 168.7, 152.7, 146.5, 142.7, 137.8, 134.6, 128.8, 128.6, 128.5, 128.4, 127.6, 126.8, 125.9, 124.0, 76.5, 46.2, 35.9, 32.0, 30.0, 27.9, 15.4, 12.5, 0.2. IR (film): 1768, 1244, 732, 697 cm^{-1}. MS (FAB) *m*/*z*: 442 (M^+). Anal. Calcd for $C_{30}H_{34}O_3$: C, 81.41; H, 7.74. Found: C, 81.33; H, 7.80.

Resin 15. A suspension of Merrifield resin (1 mequiv Cl/g) (3.047 g, 3.047 mmol) and triphenylphosphine (2.358 g, 9.00 mmol) in 20 mL of anhydrous benzene was refluxed for 36 h under N_2. The mixture was allowed to cool to room temperature. The solid was filtered, washed with benzene and ether, and dried in vacuo. The resin **15** (3.75 g, 99%) was obtained.

Resin 16. To a slurry of resin **15** (1.00 g, 1.00 mmol) in anhydrous benzene (8 mL) was added dropwise BuLi in hexanes (2.5 M) (400 μL, 1.00 mmol) under N_2 via a syringe. A red coloration developed immediately. After the mixture stirred for 20 min at room temperature, a solution of aldehyde **9** (403 mg, 1.10 mmol) in anhydrous DCM (5 mL) was added with stirring under N_2. The red color of the resin vanished immediately, and the reaction mixture was stirred for 2 h at room temperature. Then, the reaction mixture was refluxed for 5 h in an oil bath (bath temperature 80−85 °C). After cooling to room temperature, the resin was filtered, washed with ethanol, benzene, ether, and DCM, and dried in vacuo to give a cream-colored resin (1.017 g). The filtrate was concentrated under reduced pressure to give a residue, which was dissolved in 30 mL of benzene, washed with water, and dried over $MgSO_4$. After filtration, evaporation, and purification (by silica gel column, DCM/MeOH = 10/1) triphenylphosphine oxide (172 mg, 0.62 mmol) was obtained. The yield of the Wittig reaction was calculated (62%) on the basis of the amount of triphenylphosphine oxide recovered. The substitution degree was determined to be 0.6 mequiv/g. IR (KBr): 1770, 1595, 1490, 1448, 1243 cm^{-1}.

Resin 17. To a slurry of resin **16** (5.844 mg, 3.506 mmol) in anhydrous and degassed benzene (70 mL), ethanol (15 mL), and glacial acetic acid (1 mL) was added tris(triphenylphosphine)rhenium chloride (540 mg, 0.584 mmol). This mixture was flushed three times with hydrogen. Then, hydrogenation was carried out under a hydrogen pressure of 55 psi for 24 h at room temperature. The resin was filtered and washed with ethanol, benzene, and DCM. Then, the resin was dried on an oil pump overnight to give resin **17** (5.899 g).

Resin 18. To a slurry of resin **17** (5.655 g, 3.393 mmol) in anhydrous THF (40 mL) was added lithium aluminum hydride (1.445 g, 10.179 mmol) in three portions with stirring under N_2 at 0 °C. Stirring was continued for 1 h at 0 °C and overnight at room temperature. Hydrogen chloride (3 N HCl aqueous solution, 30 mL) was slowly added into the mixture at 0 °C. The resin was filtered, washed with 3 N HCl aqueous solution, water, ethanol, and DCM, and dried on an oil pump overnight to give the resin **18** (0.94 g). IR (KBr): 3400, 1601, 1492, 1451, 757, 690, 540 cm^{-1}.

General Procedure for the Attachment of the First Amino Acid to Resin 18. The resin **18** (600 mg, 0.36 mmol) was washed twice with DCM and swelled with DCM (7 mL). Protected amino acid (1.08 mmol), DCC (222 mg, 1.08 mmol), and DMAP (9 mg, 0.07 mmol) were added. The mixture was shaken for 6 h at room temperature. The resin was filtered and washed with DCM (7 × 7 mL), methanol and DCM (5 × 7 mL), and DCM (3 × 7 mL). The resin was dried on an oil pump. IR (KBr): 1748, 1719, 1161 cm^{-1}.

To a suspension of the resin in THF (8 mL) and H_2O (4 mL) was added NBS (158 mg, 0.89 mmol). This mixture was shaken for 2.5 h. The resin was filtered, washed with H_2O and THF (3 × 7 mL), THF (3 × 7 mL), and DCM (3 × 7 mL), and dried in vacuo. The free hydroxyl groups on the resin were capped with Ac_2O (132 mg, 1.29 mmol) and Et_3N (131 mg, 1.294 mmol) in 7 mL of anhydrous DCM for 2 h. The yellow resin was filtered and washed with DCM and dried in vacuo. IR (KBr): 1748, 1719, 1645, 1163 cm^{-1}.

The resin was washed with 25% TFA in DCM−indole (1 mg/mL) (7 mL) and shaken for 30 min with 7 mL of a 25% TFA solution. The resin was then washed with DCM, 10% TEA (2 × 7 mL), and DCM and dried on an oil pump for 2 h. Then a quantitative ninhydrin test was performed to give a substitution level of approximately 0.31 mmol/g.

General Peptide Coupling Procedure. To a suspension of the resin (571 mg, 0.177 mmol) in 6.5 mL of anhydrous DCM was added protected amino acid (0.531 mmol), DIC (67 mg, 0.531 mmol), HOBt (72 mg, 0.531 mmol), and DMAP (4.3 mg, 0.035 mmol). This mixture was shaken for 2 h at room temperature. The resin was filtered and washed with DCM (6 × 8 mL), and the ninhydrin test was conducted. When positive ninhydrin test was obtained, the coupling reaction was repeated.

General Procedure for the Cleavage of the Peptides from the Resin. Reduction with $NaBH_4$. The quinone resin **22** (250 mg, 0.36 mmol/g, 0.09 mmol) was swelled in 5 mL of THF. Sodium borohydride (35 mg, 0.9 mmol) and methanol (0.5 mL) were added. The mixture was stirred for 0.5 h at room temperature. The hydroquinone resin was filtered and washed with methanol (3 × 10 mL), THF (3 × 10 mL), and anhydrous THF (5 × 5 mL). The hydroquinone resin **23** was swelled in 3 mL of anhydrous THF. Anhydrous TBAF/THF (3 mL, 0.76 M) was added, and the mixture was stirred for 20 h under N_2. IR showed that most of peptide had been cleaved. The resin was filtered and washed with THF (3 × 5 mL), DMF (3 × 5 mL), MeOH (3 × 5 mL), and THF (3 × 5 mL). The filtrate was combined and solvent was removed at 30 °C and dried on oil pump for 20 h.

Typical Procedure for the Purification of the Peptides Cleaved from the Resin. The dried resin mixture from 250 mg of resin **22** was swelled in 20 mL of anhydrous THF, Amberlyst-15 calcium sulfonate resin (8.5 g, 29.75 mmol, approximately 1.76 mequiv/g of Ca^{2+}) and Amberlyst-15 sulfonic acid resin (300 mg, 14.1 mmol, 4.7 mequiv/g of H^+) were added, and the mixture was stirred for 4 h at room temperature.[36] The Amberlyst-15 resin was filtered and washed with DMF (10 × 10 mL), and the solvent was removed at 30 °C. The residue was recrystallized in a suitable solvent to afford a pure peptide. The filtrate was further purified on a silica gel column to afford another part of the pure peptide.

Boc-Trp-Ala-Gly-Gly-OH. To the residue obtained following the general procedures described above from 0.25 g of resin was added 1 mL of ethyl acetate. The solution was stored at about −12 °C for 6 h. The white precipitates were filtered and washed with ethyl acetate to afford 4 mg of the product. TLC showed a single spot. The filtrate was further purified with a silica gel column (DCM/CH_3OH = 10/1, 0.1% HOAc) to afford 12 mg of a white solid. The total yield was 16 mg (37%). ^{1}H NMR and HPLC studies indicated that this peptide was identical to the standard prepared by the solution-phase method.

Boc-Asn-Ala-Ser(OBn)-Gly-Glu(OBn)-OH. The residue from 50 mg of resin was recrystallized using methanol to afford a white solid (3 mg, 34%). ^{1}H NMR and HPLC studies indicated that this peptide was identical to the standard prepared using solution phase method.

Synthesis of the Standard Tetrapeptide [Boc-Trp-Ala-Gly-Gly-OH] and Pentapeptide [Boc-Asn-Ala-Ser(OBn)-Gly-Glu(OBn)-OH] by the Solution-Phase Method. Typical Coupling Procedure Using Boc-Gly-Gly-OBn as an Example. To a cooled solution of Boc-Gly-OH (525 mg, 3.00 mmol) in 25 mL of anhydrous DCM was added EDC (634 mg, 3.30 mmol), HOBt (446 mg, 3.30 mmol), H-Gly-OBn·HCl (495 mg, 3.00 mmol), DMAP (73 mg, 0.60 mmol), and TEA (636 mg, 6.20 mmol). This mixture was stirred under N_2 for 2 h at 0 °C and 3 h at room temperature. Evaporation of the solvent under reduced pressure gave a residue, which was dissolved in EtOAc (70 mL), washed with 10% citric acid, saturated $NaHCO_3$, and brine, and dried over $MgSO_4$. Filtration, evaporation, and drying in vacuo gave a white solid (835 mg, 86%).^{1}H NMR ($CDCl_3$): δ 7.35 (s, 5H), (5.17 (s, 2H), 4.09 (d, J = 5.3 Hz, 2H), 3.84 (d, J = 5.3 Hz, 2H), 1.45 (s, 9H).

Typical Boc Deprotection Procedure Using Boc-Gly-Gly-OBn as an Example. Boc-Gly-Gly-OBn (835 mg, 2.593 mmol) was treated with 4 mL of 25% TFA in DCM for 1 h at room temperature. After evaporation of the solvent, the residue was dried in vacuo. The resulting $CF_3CO_2^-\cdot{}^+NH_3$-Gly-Gly-OBn was used for the next coupling reaction step without purification.

Boc-Ala-Gly-Gly-OBn. ^{1}H NMR ($CDCl_3$): δ 7.34 (s, 5H), 5.15 (s, 2H), 4.20−3.90 (m, 5H), 1.39 (s, 9H), 1.35 (d, J = 7.0 Hz).

Boc-Trp(For)-Ala-Gly-Gly-OBn. ^{1}H NMR (DMSO): δ 9.60−7.00 (m, 6H), 5.12 (s, 2H), 4.31 (m, 2H), 3.93 (d, J = 5.7 Hz, 2H), 3.76 (d, J = 4.5 Hz, 2H), 3.13 (m, 1H), 2.85 (m, 1H), 1.24 (s, 9H), 1.17 (m, 3H). HRMS (FAB) for $C_{31}H_{37}N_5O_8$: calcd 608.2720, found 608.2753.

Boc-Trp(For)-Ala-Gly-Gly-OH. A suspension of Boc-Trp-(For)-Ala-Gly-Gly-OBn (503 mg, 0.828 mmol) in 30 mL of methanol was hydrogenated via a balloon in the presence of 10% Pd/C (75 mg) for 1 h at room temperature. The catalyst was removed by filtration, and the reaction mixture was evaporated to dryness. After purification using a silica gel column (DCM/MeOH = 10/1, 1% acetic acid), a white solid product (385 mg, 90%) was obtained. ^{1}H NMR (CD_3OD): δ 9.50−7.09 (m, 6H), 4.46−4.25 (m, 2H), 3.98−3.78 (m, 4H), 3.28 (m, 1H), 3.02 (m, 1H), 1.36 (s, 9H), 1.23 (m, 3H). HRMS (FAB) for $C_{24}H_{31}N_5O_8$: calcd 518.2251, found 518.2280.

Boc-Trp-Ala-Gly-Gly-OH. Boc-Trp(For)-Ala-Gly-Gly-OH (52 mg, 0.10 mmol) was treated with 20% piperidine in DMF (2 mL) for 3 h at 0 °C and 19 h at room temperature under N_2. After evaporation of the solvent, the residue was dissolved in 10 mL of EtOAc. This solution was washed with 10% citric acid (2 × 5 mL) and brine (2 × 5 mL) and dried over $MgSO_4$. Filtration and evaporation gave an oil, which was purified by radial preparative TLC (DCM/MeOH = 10/1, 1% CH_3CO_2H). A white solid was obtained (18 mg, 36%). ^{1}H NMR (CD_3OD): δ 8.20−6.99 (5H, m), 4.36−4.24 (2H, m), 3.89−3.60 (4H, m), 3.23−3.05 (2H, m), 138 (9H, s), 1.23 (3H, d, J = 6.42 Hz). HRMS (FAB) for $C_{23}H_{31}N_5O_7$: calcd 489.2223, found 489.2235.

Boc-Glu(OBn)-$OCH_2CH{=}CH_2$. A solution of Boc-Glu-(OBn)-OH (709 mg, 2.1 mmol) in 4 mL of methanol was added to a solution of cesium carbonate (330 mg, 1.01 mmol) in 2 mL of water with stirring at room temperature. This solution was stirred for 30 min, and then the solvent was evaporated. Benzene (3 × 5 mL) was added to the mixture and then removed by evaporation to give a white solid. To the cesium salt was added DMF (6 mL) and allyl bromide (1 mL). After reaction for 1 h, the white solid was filtered off and washed with ethyl acetate several times. Evaporation of the filtrate under reduced pressure and purification of the residue using a silica gel column (hexanes/EtOAc = 2/1−1/1) gave a white solid (740 mg, 94%).^{1}H NMR (CD_3OD): δ 7.35 (s, 5H), 5.91−5.23 (m, 3H), 5.12 (s, 2H), 4.62 (d, J = 5.8 Hz, 2H), 4.38 (m, 1H), 2.50−2.46 (m, 2H), 2.24 (m, 1H), 1.96 (m, 1H), 1.43 (s, 9H).

Boc-Gly-Glu(OBn)-$OCH_2CH{=}CH_2$. ^{1}H NMR (CD_3OD): δ 7.34 (s, 5H), 5.94−5.12 (m, 3H), 5.11 (s, 2H), 4.61 (d, J = 5.6 Hz, 2H), 4.53 (m, 1H), 3.72 (brs, 2H), 2.48 (t, J = 7.5 Hz, 2H), 2.24 (m, 1H), 2.00 (m, 1H), 1.43 (s, 9H).

Boc-Ser(OBn)-Gly-Glu(OBn)-$OCH_2CH{=}CH_2$. ^{1}H NMR (CD_3OD): δ 7.34 (m, 10H), 5.93−5.15 (m, 3H), 5.10 (s, 2H), 4.60 (d, J = 5.4 Hz, 2H), 4.51−4.47 (m, 3H), 4.28−4.22 (m, 1H), 3.88 (m, 2H), 3.70 (m, 2H), 2.49−2.42 (m, 2H), 2.22−2.13 (m, 1H), 1.99−1.90 (m, 1H), 1.43 (s, 9H).

Boc-Ala-Ser(OBn)-Gly-Glu(OBn)-$OCH_2CH{=}CH_2$. ^{1}H NMR (CD_3OD): δ 7.34 (m, 10H), 5.93−5.19 (m, 3H), 5.10 (s, 2H), 4.60 (d, J = 5.4 Hz, 2H), 4.52 (s, 2H), 4.48 (m, 2H), 4.03−3.72 (m, 5H), 2.47 (t, J = 7.5 Hz, 2H), 2.23−2.17 (m, 1H), 2.02−1.95 (m, 1H), 1.39 (s, 9H), 1.27 (d, J = 7.1 Hz, 3H).

Boc-Asn-Ala-Ser(OBn)-Gly-Glu(OBn)-$OCH_2CH{=}CH_2$. $CF_3CO^-\cdot{}^+NH_3$-Ala-Ser(Bn)-Gly-Glu(OBn)-$OCH_2CH{=}CH_2$ was coupled with Boc-Asn-OH (121 mg, 0.523 mmol) in 5 mL of anhydrous DCM and 2 mL of DMF in the presence of the PyBOP (272 mg, 0.523 mmol) and DIPEA (202.8 mg, 1.569 mmol). This mixture was stirred for 30 min at 0 °C and overnight at room temperature under N_2. After workup and

purification by a silica gel column (DCM/MeOH = 15/1−10/1), the protected peptide was obtained as a white solid (278 mg, 67%). ^{1}H NMR (CD_3OD): δ 7.31 (m, 10H), 5.90−5.15 (m, 3H), 5.07 (s, 2H), 4.58 (d, J = 5.4 Hz, 2H), 4.48 (s, 2H), 4.46−4.23 (m, 4H), 3.87−3.71 (m, 4H), 2.68−2.57 (m, 2H), 2.46−2.40 (m, 2H), 2.20−2.09 (m, 1H), 2.00−1.89 (m, 1H), 1.38 (s, 9H), 1.31 (d, J = 6.9 Hz, 3H). HRMS (FAB) for $C_{39}H_{52}N_6O_{12}$: calcd 797.3721, found 797.3740.

Boc-Asn-Ala-Ser(OBn)-Gly-Glu(OBn)-OH. Under argon, tetrakis(triphenylphosphine) palladium(0) (44 mg, 0.0384 mmol) was added to a solution of Boc-Asn-Ala-Ser(OBn)-Gly-Glu(OBn)-$OCH_2CH{=}CH_2$ (306 mg, 0.384 mmol) in anhydrous THF (26 mL) and DMSO (2.6 mL), followed by the addition of morpholine (335 mg, 3.84 mmol). This pale yellow solution was stirred for 4.5 h under argon at room temperature. After evaporation of the solvent, the crude product was washed with 2 N HCl, chloroform, and ethyl acetate and dried in vacuo. A white solid was obtained (233 mg, 80%). ^{1}H NMR (DMSO-d_6): δ 7.34 (m, 10H), 5.08 (s, 2H), 4.48 (s, 2H), 4.40−4.22 (m, 4H), 3.76 (d, J = 5.3 Hz, 2H), 3.62 (d, J = 5.6 Hz, 2H), 2.41 (m, 4H), 2.00 (m, 1H), 1.82 (m, 1H), 1.37 (s, 9H), 1.19 (d, J = 6.8 Hz, 3H). HRMS (FAB) for $C_{36}H_{48}N_6O_{12}$: calcd 757.3408, found 757.3402.

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