A Redox-Sensitive Resin Linker for the Solid Phase Synthesis of *C*-Terminal Modified Peptides

Ailian Zheng,† Daxian Shan,† and Binghe Wang*

Department of Chemistry, North Carolina State University, Raleigh, North Carolina 27695-8204

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With the rapid development of combinatorial chemistry using solid phase synthesis, there is a great deal of interest in developing new solid phase linkers, which are stable during the solid phase synthesis process and yet readily cleavable under mild conditions. By taking advantage of a "trimethyl lock"-facilitated lactonization reaction, we have developed a redox-sensitive resin linker for the synthesis of *C*-terminal-modified peptides. The cleavage only requires mild reducing agents such as sodium hydrosulfite, which is not expected to cause any problem with the commonly seen organic functional groups. Using this new linker, three short peptides were synthesized with high isolated yields (70−90%). Such a linker could potentially be used for the synthesis of modified peptide libraries, which allows for the ready cleavage of the linker under mild conditions.

Introduction

With the rapid development of combinatorial chemistry using solid-phase synthesis, it is becoming obvious that the "traditional" solid-phase linkers[1] cannot always meet the requirements of this new field. Therefore, there is a great deal of interest in developing new solid phase linkers, which are stable during the solid phase synthesis process and yet readily cleavable under mild conditions. Because of the wide variety of compounds that are being synthesized on solid phase and the large number of reactions involved, it is difficult, if possible at all, to design a "universal linker," which can be used in all different situations. Therefore, the focus in the field has been on designing solid phase linkers that address specific questions and concerns. Several new linkers have recently been developed, which can be cleaved under mild conditions. These new linkers include an allylic linker,[2] safety-catch and traceless linkers,[3−9] photolabile linkers,[10−14] silicon linkers,[15−17] several new linkers for the synthesis of peptide amides,[18−20] enzyme-labile linkers,[21−24] an oxidation-sensitive linker,[25] and other linkers for more specific applications.[26−34] There have also been efforts to find ways to cleave the Wang's linker using new methods.[35] Herein, we report our effort of developing a redox-sensitive linker, which can be used for the synthesis of *C*-terminal modified peptides in combinatorial chemistry or in single sequence synthesis (Scheme 1). The design takes advantage of a "trimethyl lock"-

* To whom correspondence should be addressed. Phone: (919) 515-2948. Fax: (919) 515−3757. E-mail: binghe_wang@ncsu.edu.

† Ailian Zheng and Daxian Shan made equal contributions to this manuscript.

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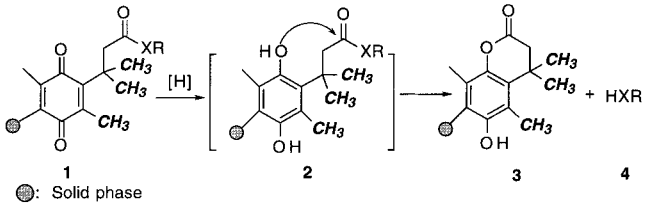
Scheme 1. A Redox-Sensitive Resin Linker for the Solid Phase Synthesis of *C*-Terminal-Modified Peptides



The methyl groups involved in the "trimethyl lock" are shown in bold-italic.

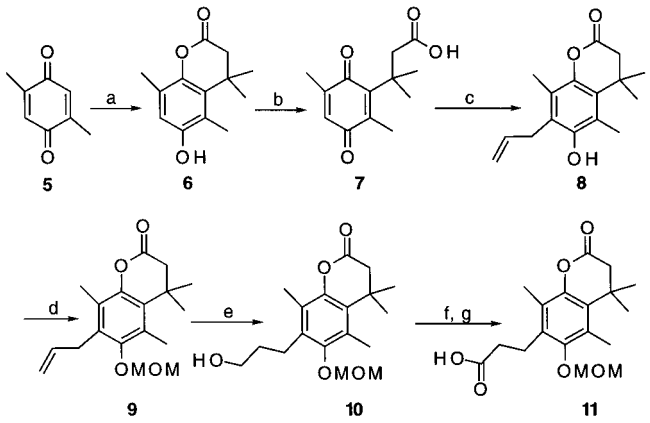
facilitated lactonization, which has previously been used for the development of redox-sensitive prodrugs and amine protecting groups.[36−38] As described in Scheme 1, when attached to a solid-phase material, this facile lactonization system can be used as a redox-sensitive linker for solid-phase synthesis of *C*-terminal modified peptide. Previous studies have demonstrated that the cyclization reaction has a very short ($t_{1/2}$ = 100 s in aqueous solution) half-life,[36,38] and the quinone moiety is stable under conditions required for the deprotection of the Boc group (TFA/DCM) and peptide coupling reactions.[37,39,40]

Results and Discussion

To study the feasibility of the concept, we first synthesized the linker with a pendent chain attached to the quinone moiety. This linker was then attached to a resin material, which was then used for the syntheses of three model *C*-terminal modified peptides.

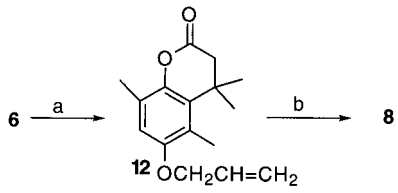
Preparation of the "Trimethyl Lock" Linker and Its Attachment to a Resin Material. For this project to work, one has to find a way to attach the linker to a solid phase material. We were interested in first studying the feasibility with polystyrene-based polymers because it has been widely used in solid-phase peptide synthesis.[1] As for the modification of the quinone moiety needed for the attachment, it is important that the attachment does not interfere with the facile lactonization reaction. With this in mind, we designed linker **1**, which uses a three-carbon chain to attach the linker to the para position of the pendant carboxylate side chain on the quinone moiety (Scheme 1). The synthesis of the linker started with commercially available 2,5-dimethylbenzoquinone (**5**) (Scheme 2). The lactone **6** was prepared in about 80% yield through reaction with 3,3-dimethylacrylic acid under acidic conditions.[36,37,41] For the attachment of an allyl group to give **8**, we first planned to use organotin chemistry as it was reported to give very high yields.[42] Therefore, the lactone **6** was oxidized to the quinone acid **7** with NBS (*N*-bromosuccinimide) following established procedures in 61% yield.[36,37] This low yield was unexpected because similar reactions[36,37] gave much higher yields. It was suspected that the bromination of the ortho-position of the phenol hydroxyl group was the problem.[43] However, no efforts were made to isolate and characterize the side product(s). Reaction of this quinone acid **7** with allytributyltin gave the desired allylated product **8** in 88% yield. However, because of the low yield of the NBS oxidation reaction, we were interested in finding another method that would give higher yield. Therefore, we studied the feasibility of using the Claisen rearrangement reaction (Scheme 3). The lactone **6** was then reacted with allyl bromide in the presence of K_2CO_3 to give the *O*-allylated product **12** in almost quantitative yield. Claisen rearrangement of the intermediate gave the desired allylated product **8** in quantitative yield. Therefore, further studies used the second approach via Claisen rearrangement because of the high yields and ready availability of the reagents.

Scheme 2. Synthesis of the Lactone 11



a. 3,3-dimethylacrylic acid, CH_3SO_3H, 81%; b.NBS/CH_3CN-H_2O, 59%; c. Allyltributyltin, BF_3-Et_2O, 88%; d. MOMCl, DIPEA, 87%; e. BH_3, 83%; f. PCC, 93%; g. $KMnO_4$, 64%.

Scheme 3. Introduction of the Allyl Group through Claisen Rearrangment



a. K_2CO_3, 99%; b. 200 °C, 100%.

The phenol hydroxyl group of **8** was first protected using MOM-Cl[44] and DIPEA (diisopropylethylamine) before the conversion of the allyl side chain to a carboxylate functional group. Hydroboration−oxidation of the MOM-protected intermediate **9** gave the alcohol **10**, which was oxidized in two steps with PCC and $KMnO_4$ to a carboxylic acid **11** in about 60% overall yield. The overall yield from 2,5-dimethylbenzoquinone (**5**) was about 35%. Then the latent linker **11** was attached to a benzylamine resin through DCC (dicyclohexylcarbodiimide)−HOBt (1-hydroxylbenzotriazole)-mediated coupling to give **14** (Scheme 4). The remaining free amino groups were capped by acetylation with acetic anhydride. The coupling reaction was followed with IR. Strong absorp-

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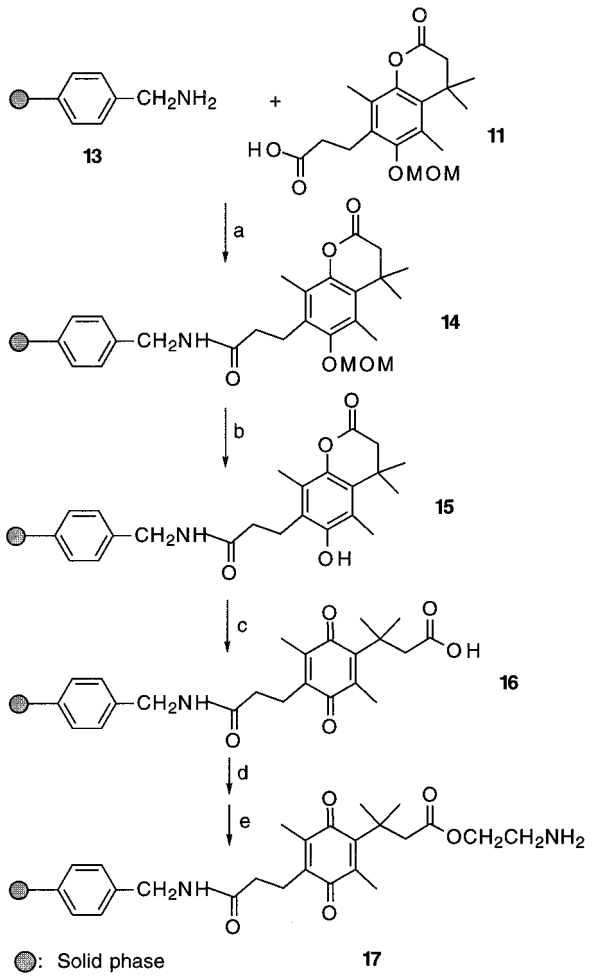
Scheme 4. Attachment of the Resin Linker to a Polystyrene-Based Resin with a Benzylamine Handle



a, DCC/HOBt, DCM; b, Me_3SiBr, -26 °C; c, NBS, THF/H_2O; d, $BocNHCH_2CH_2OH$, DIC/HOBt; e, 25%TFA

tions for the amide and lactone groups were observed in the IR of the coupled product **14** at 1672 and 1766 cm^{-1}, respectively (Figure 1). In addition, the peak intensity around 3424 cm^{-1} for the amine resin **13** also decreased tremendously. Although peaks in this region are affected by moisture contents, the decreased intensity further substantiates the anticipated acylation of the amine group of the resin **13**. With the attachment of the linker, **14** also had new peaks at 1243, 1155, and 967 cm^{-1}. Deprotection of the MOM group using trimethylsilyl bromide at −26 °C, followed by NBS oxidation, gave the carboxylate linker **16**, which can be used for the attachment of compound to be modified on the solid phase. Upon deprotection of the MOM group of **14**, the peaks at 1155 and 967 cm^{-1} disappeared, indicating the completion of the reaction. The generation of the free hydroxyl group of **15** is also reflected in the IR with the appearance of a broad intense peak at around 3425 cm^{-1}. The oxidation of the lactone **15** intermediate to quinone linker **16** was accompanied by the appearance of a bright yellow color, characteristic of the quinone moiety. The IR of **16** also showed the disappearance of the peak at around 1766 cm^{-1}, characteristic of the lactone structural moiety, and the peak at 1243 cm^{-1}, characteristic of the lactone C−O stretching frequency. The resulting quinone acid resin **16** had an absorption in its IR region between 1702

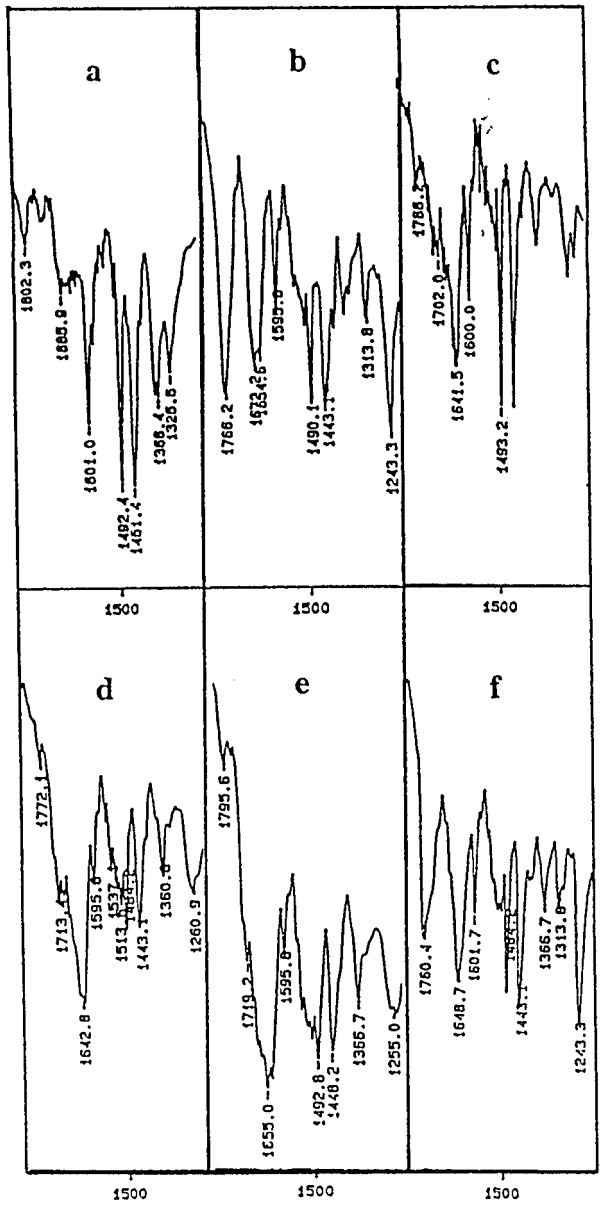


Figure 1. IR spectra of the resins: (a) aminomethyl resin; (b) resin **14**; (c) resin **16**; (d) resin **17**; (e) resin **17** with the tripeptide (Boc-D-Ala-Gly-Phe-$NHCH_2CH_2OH$) synthesized; (f) resin after the release of the tripeptide (Boc-D-Ala-Gly-Phe-$NHCH_2CH_2OH$).

and 1642 cm^{-1} (Figure 1), characteristic of the presence of a carboxylic acid and amide functional group.

Synthesis of Model-Protected Peptides Using the Linker Prepared. The feasibility of using this linker for solid phase synthesis was studied through the synthesis of three model peptides, a tripeptide, a tetrapeptide, and a pentapeptide. Because the linker has a carboxyl group as the handle, only hydroxyl groups or amino groups can be attached. Because peptides are normally synthesized from the *C*-terminal to the *N*-terminal for a variety of reasons,[1] a two-carbon spacer, ethanolamine, was used to introduce a functional group for the attachment of the carboxyl group of the first amino acid. Therefore, the resin was reacted with *N*-Boc-ethanolamine in the presence of DIC and catalytic amount DMAP (4-*N*,*N*-(dimethylamino)pyridine). The cleavage of the Boc group with 25% TFA (trifluoroacetic acid) in CH_2Cl_2 gave the intermediate **17** with a free

amino anchor, which allows for the attachment of the first amino acid of the peptide. Quantitative ninhydrin test[1] was performed to determine the loading degree of the resin at this stage. Typically, starting with a benzylamine linker with a loading degree of about 0.8 mmol/g, a 0.35 mmol/g loading could be obtained for linker **17**, whereas a quantitative derivatization of all the benzylamino group would give a loading degree of about 0.63 mmol/g.

The three peptides synthesized using this new linker were Boc-D-Ala-Gly-Phe-$NHCH_2CH_2OH$, Boc-Leu-D-Ala-Gly-Phe-$NHCH_2CH_2OH$, and Boc-Trp(For)-Leu-D-Ala-Gly-Phe-$NHCH_2CH_2OH$. The synthesis of these protected peptides was carried out manually using diisopropylcarbodiimide (DIC) and HOBt as the coupling reagents using normal solid-phase peptide synthesis protocols.[1] The deprotection of Boc group was achieved using 25% TFA in methylene chloride and indole[1] for about 30 min.

The key to the design of this linker was that the peptide synthesized could be released readily under mild conditions. As it has been demonstrated before, the quinone moiety could be easily reduced using mild reducing agents such as sodium hydrosulfite ($Na_2S_2O_4$), which are not expected to cause any problems for the commonly seen organic functional groups.[36,37] As expected, the peptides could be readily cleaved from the solid phase upon reduction with sodium hydrosulfite. Because the polystyrene-based resin used is very hydrophobic and the reducing agent, sodium hydrosulfite, is only soluble in aqueous solutions, the reduction reaction was conducted in a mixture of THF and water using excess amount of the reducing agent. Therefore, after being washed with CH_2Cl_2, MeOH, and THF, the resin (about 1 g) was treated with sodium hydrosulfite (850 mg, 85% pure) in H_2O (5 mL) and THF (8 mL) for 2.5 h at room temperature. The disappearance of the yellow color of the resin serves as a good indicator of the progress of the reduction reaction. After release, the crude peptides were purified using silica gel columns to give 89% of the tripeptide (Boc-D-Ala-Gly-Phe-$NHCH_2CH_2OH$), 83% of the tetrapeptide (Boc-Leu-D-Ala-Gly-Phe-$NHCH_2CH_2$-OH), and 70% of the pentapeptide (Boc-Trp (For)-Leu-D-Ala-Gly-Phe-$NHCH_2CH_2OH$).

Reversed-phase HPLC was used for the analysis of the reaction mixture. Analyses of the crude reaction mixtures indicated that even before purification, the peptides cleaved from the resin were of high purity. Figure 2 shows the HPLC profiles of crude mixtures as compared with the purified products and the standards prepared through solution phase synthesis.

It should be noted that the mild cleavage conditions are not expected to cause any problems for the commonly seen amino acid side chain protecting groups either.[1] However, if *C*-terminal peptides with side chain functional groups are to be synthesized, an additional deprotection step using standard methods[1] will be needed to generate the free peptides.

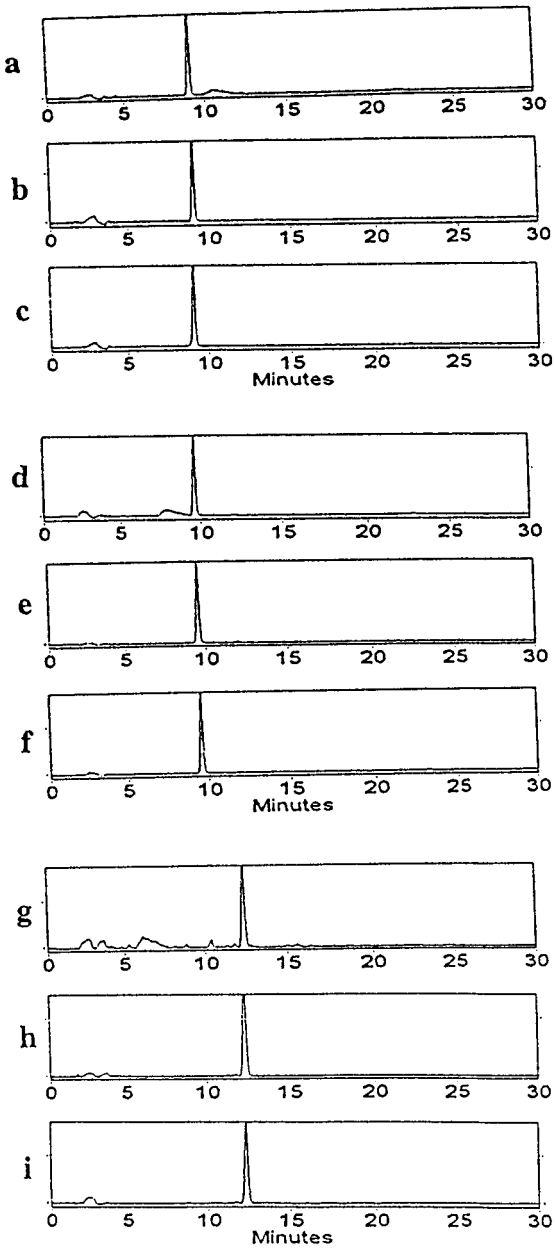


Figure 2. HPLC profiles of the peptides synthesized: (a) the tripeptide (Boc-D-Ala-Gly-Phe-$NHCH_2CH_2OH$) after being released from the resin; (b) the tripeptide after purification; (c) the standard tripeptide prepared through solution phase peptide synthesis; (d) the tetrapeptide (Boc-Leu-D-Ala-Gly-Phe-$NHCH_2CH_2OH$) after being released from the resin; (e) the tetrapeptide after purification; (f) the standard tetrapeptide prepared through solution phase peptide synthesis; (g) the pentapeptide (Boc-Trp(for)-Leu-D-Ala-Gly-Phe-$NHCH_2CH_2OH$) after being released from the resin; (h) the pentapeptide after purification; (i) the standard pentapeptide prepared through solution phase peptide synthesis. The HPLC mobile phase was a mixture of water and acetonitrile in a ratio 7:3 for the analysis of the tripeptide (Boc-D-Ala-Gly-Phe-$NHCH_2CH_2OH$) (a−c), 6:4 for the analysis of the tetrapeptide (Boc-Leu-D-Ala-Gly-Phe-$NHCH_2CH_2OH$) (d−f), and 55:45 for the analysis of the pentapeptide (Boc-Trp(for)-Leu-D-Ala-Gly-Phe-$NHCH_2CH_2$-OH) (g−i).

Conclusions

We have developed a redox-sensitive linker for solid-phase synthesis of *C*-terminally modified peptides. This linker can be cleaved using mild reduction conditions. Although the feasibility was only studied using polystyrene-based resin, it should be feasible to attach this linker to other resin materials for solid-phase synthesis. For example, attachment of this linker to a more hydrophilic resin material would allow for the cleavage of the peptides in an aqueous solution, which could allow for in situ bioassays of a peptide library.

Experimental Section

^{1}H NMR spectra were recorded at 300 MHz. IR spectra were recorded on a FTIR spectrophotometer with KBr pellets. Mass spectra were obtained at the Mass Spectrometry Laboratory for Biotechnology at North Carolina State University. HPLC analyses were carried out using a dual pump system with a UV−vis detector (detection wavelength: 220 nm, solvent: acetonitrile and water). The column was a C_{18} reversed phase analytical column from YMC (4.6 × 250 mm, ODS-A, S-5 μm 120A). Unless stated otherwise commercial reagents were from Aldrich and used without further purification. The aminomethyl resin (0.8 meq/g, 1% cross linking, 100−200 mesh) and protected amino acids were from Bachem Inc. Acetonitrile and THF were HPLC grade from Fisher Scientific Inc. TFA was peptide synthesis grade. The standard tripeptide, tetrapeptide, and pentapeptide were prepared using standard solution phase peptide synthesis procedures.[1]

6-Hydroxy-4,4,5,8-tetramethylhydrocoumarin (6). 2,5-Dimethylbenzoquinone (**5**, 10 g, 73.5 mmol) was dissolved in 300 mL of ether, which was mixed with an aqueous solution of sodium hydrosulfite (178 g in 150 mL of water, 85% pure, 870 mmol). The mixture was shaken until the ether layer became near colorless. The ether layer was separated. The aqueous layer was extracted with ethyl ether (3 × 200 mL). The combined ether layers were washed with brine (2 × 150 mL) and dried over $MgSO_4$. Filtration and solvent evaporation gave 10.03 g (72.6 mmol) of the dihydroquinone as a white solid.

The dihydroquinone was mixed with 3,3-dimethylacrylic acid (8.00 g, 80 mmol) and methanesulfonic acid (111 mL). The mixture was stirred at 85 °C under nitrogen atmosphere for 3 h and then cooled to room temperature. To the mixture was added 300 g of ice with stirring. The gray-white precipitate was extracted with ethyl acetate (4 × 100 mL). The combined organic layers were washed with saturated sodium bicarbonate solution (2 × 100 mL) and water (3 × 100 mL) and dried over $MgSO_4$. After filtration and evaporation, a residue was obtained, which was recrystallized in ethyl acetate and hexanes (1:1, v/v) to give 13.02 g (81%) of the desired product. ^{1}H NMR ($CDCl_3$) δ: 6.57 (s, 1H), 4.85 (s, 1H), 2.56 (s, 2H), 2.33 (s, 3H), 2.22 (s, 3H), 1.46 (s, 6H). MS *m*/*z* 220 (M^+). Anal. Calcd for $C_{13}H_{16}O_3$: C, 70.89; H, 7.32. Found: C, 70.73; H, 7.41.

3-(3′,6′-Dioxo-2′,5′-dimethylcyclohexa-1′,4′-diene)-3,3-dimethylpropionic Acid (7). To a solution of lactone **6** (300 mg, 1.364 mmol) in a mixture of acetonitrile (9 mL), acetone (1 mL), and water (9 mL) was added NBS (243 mg, 1.364 mmol) in portions with stirring at room temperature. After being stirred at room temperature for 30 min, the reaction solution turned yellow. Then the organic solvents were evaporated under reduced pressure, and the remaining solution was extracted with ether (1 × 30 mL). The ethereal solution was then extracted with sat. $NaHCO_3$ (3 × 10 mL), and the combined aqueous phase was washed with ether (3 × 10 mL). After acidification of the aqueous solution with concd HCl to pH 2−3, the aqueous solution was extracted with ether (2 × 20 mL). After drying over $MgSO_4$, ether was evaporated to give a yellow oily product (197 mg, 61%). ^{1}H NMR ($CDCl_3$) δ 6.44 (s, 1H), 3.01 (s, 2H), 2.13 (s, 3H), 1.97 (s, 3H), 1.45 (s, 6H).

7-Allyl-6-hydroxy-4,4,5,8-tetramethylhydrocoumarin (8). Method A. To a solution of quinone **7** (393 mg, 1.66 mmol) in anhydrous methylene chloride (4 mL) was added BF_3·OEt_2 (610 mL, 4.98 mmol) at −78 °C with constant stirring under N_2. A solution of allyltributyltin (1.275 g, 3.30 mmol) in anhydrous methylene chloride (5 mL) was slowly added from a syringe, and then the reaction was warmed to room temperature. After stirring at room temperature for 1 h, the reaction mixture was quenched with 10 mL of 10% KF aqueous solution and stirred for 2 h. Then the white solid was filtered off, and the organic phase was separated and washed with sat. $NaHCO_3$ solution and water. After drying over $MgSO_4$, the solvent was evaporated to give a residue, which was purified on a silica gel column (CH_2Cl_2 as a eluent) to give 407 mg (88%) of a solid product. ^{1}H NMR ($CDCl_3$) δ: 6.01−5.88 (m, 1H), 5.12 (m, 2H), 4.80 (bs, 1 H), 3.44 (d, J = 5.7 Hz, 2H), 2.56 (s, 2H), 2.35 (s, 3H), 2.23 (s, 3H), 1.46 (s, 6H). MS *m*/*z* 261 (M + 1). Anal. Calcd for $C_{16}H_{20}O_3$: C, 73.80; H, 7.75. Found: C, 73.58; H, 7.79.

Method B. Compound **12** (8.26 g, 31.65 mmol) was heated at 200 °C under N_2 for 7 h to give 8.26 g (100%) of the rearranged product **8**.

7-Allyl-6-(methoxymethoxy)-4,4,5,8-tetramethylhydrocoumarin (9). Compound **8** (8.26 g, 31.65 mmol) and DIPEA (57.2 mL, 317.7 mmol) were dissolved in 160 mL of methylene chloride. The mixture was cooled in an ice−water bath and stirred under N_2. Methoxymethyl chloride (12.2 mL, 159 mmol) was added dropwise. After the addition, the mixture was stirred at room temperature under N_2 overnight. The mixture was diluted with 140 mL of methylene chloride and washed with 10% HCl solution (2 × 50 mL) and water (3 × 50 mL) and dried over $MgSO_4$. Filtration and solvent evaporation gave a yellow residue, which was purified on a silica gel column (hexanes and ethyl acetate, 3/1, v/v) to give 8.61 g (87%) of a solid product. ^{1}H NMR ($CDCl_3$) δ: 5.96 (m, 1H), 5.07−4.95 (m, 2H), 4.90 (s, 2H), 3.63 (s, 3H), 3.47 (d, J = 5.7 Hz, 2H), 2.58 (s, 2H), 2.40 (s, 3H), 2.22 (s, 3H), 1.47 (s, 6H). MS *m*/*z* 305 (M + 1). Anal. Calcd for $C_{18}H_{24}O_4$: C, 71.03; H, 7.95. Found: C, 70.98; H, 7.87.

7-(3-Hydroxy-1-propyl)-6-(methoxymethoxy)-4,4,5,8-tetramethylhydrocoumarin (10). Compound **9** (4.09 g, 13.46 mmol) was dissolved in 7 mL of freshly distilled THF. Argon was passed through the mixture for 20 min. The solution was cooled in an ice−water bath. BH_3−THF solution (1.0 M, 5.05 mL, 5.05 mmol) was added dropwise with stirring. After the addition, the mixture was stirred at 0 °C for 2 h. Water (about 1 mL) was added dropwise to destroy the excessive borane hydride until hydrogen evolution ceased. NaOH solution (2 N, 3.4 mL) and 30% H_2O_2 solution (1.7 mL, 16.2 mmol) were added. The mixture was stirred for 30 min and neutralized with 5% HCl solution. The organic compounds were extracted with EtOAc (3 × 50 mL), washed with water (3 × 30 mL), and dried over $MgSO_4$. The solvents were removed in a vacuum. The residue was purified on a silica gel column (EtOAc/hexanes = 1/3, v/v) to give 3.19 g (70%) of the product. ^{1}H NMR ($CDCl_3$) δ: 4.91 (s, 2H), 3.63 (s, 3H), 3.60 (t, J = 6.0 Hz, 2H), 2.81 (t, J = 7.4 Hz, 2H), 2.56 (s, 2H), 2.38 (s, 3H), 2.24 (s, 3H), 1.77 (m, 2H), 1.45 (s, 6H). MS *m*/*z* 323 (M + 1). Anal. Calcd for $C_{18}H_{26}O_5$: C, 67.06; H, 8.13. Found: C, 66.98; H, 8.09.

6-Hydroxy-7-(3-propionic acid)-4,4,5,8-tetramethylhydrocoumarin (11). Compound **10** (2.11 g, 6.55 mmol) was dissolved in 50 mL of methylene chloride. PCC (2.127 g, 9.89 mmol) was added in portions with stirring at room temperature. The total reaction time was 3.5 h. The reaction mixture was filtered through a short silica gel column. The silica gel was washed with a solution of ethyl acetate and hexane (1:1, v/v). The filtrates were evaporated to give the aldehyde (1.97 g) which was dissolved in 8 mL of acetone and 2 mL of water, and then $KMnO_4$ (1.00 g) was added. The mixture was stirred for 1 h and then acidified with 5% HCl solution. The white precipitates were extracted with ethyl acetate (3 × 30 mL), washed with water (3 × 15 mL), and dried over $MgSO_4$. Filtration and solvent evaporation gave 1.27 g (58%) of the product. ^{1}H NMR (CD_3OD) δ: 4.91 (s, 2H), 3.58 (s, 3H), 3.02 (t, J = 8.6 Hz, 2H), 2.59 (s, 2H), 2.46 (t, J = 8.6 Hz, 2H), 2.38 (s, 3H), 2.23 (s, 3H), 1.43 (s, 6H). MS *m*/*z* 337 (M + 1). Anal. Calcd for $C_{18}H_{24}O_6$: C, 64.27; H, 7.19. Found: C, 64.17; H, 7.14.

6-(Allyloxy)-4,4,5,8-tetramethylhydrocoumarin (12). The lactone **6** (7.08 g, 32.17 mmol) and allyl bromide (7.79 g, 64.35 mmol) were dissolved in 50 mL of acetone. K_2CO_3 (8.88 g, 64.35 mmol) and NaI (0.2 g) were added into the solution. The mixture was stirred at 60 °C for 80 h and then evaporated. The residue was partitioned between water and methylene chloride. The organic layer was separated. The aqueous layer was further extracted with methylene chloride (4 × 50 mL), and the combined CH_2Cl_2 layers were washed with water (3 × 50 mL) and dried over $MgSO_4$. Solvent evaporation gave 8.26 g (99%) of a white solid (**12**). ^{1}H NMR ($CDCl_3$) δ: 6.63 (s, 1H), 6.13−6.01 (m, 1H), 5.46−5.26 (m, 2H), 4.50 (d, J = 5.1

Hz, 2H), 2.56 (s, 2H), 2.35 (s, 3H), 2.27 (s, 3H), 1.46 (s, 6H). MS *m*/*z* 261 (M + 1). Anal. Calcd for $C_{16}H_{20}O_3$: C, 73.80; H, 7.75. Found: C, 73.79; H, 7.82.

Protected Lactone−Resin Conjugate 14. Aminomethyl resin (0.8 mmol/g, 4.737 g, 3.76 mmol) was washed with methylene chloride (30 mL) and 10% TEA (triethylamine)/methylene chloride solution (2 × 30 mL). The lactone acid **11** (1.52 g, 4.51 mmol) was dissolved in 30 mL of CH_2Cl_2. To this solution were added DCC (929 mg, 4.51 mmol) and HOBt (609 mg, 4.51 mmol). The mixture was stirred for 1 h at room temperature. The activated lactone acid (**11**) mixture was transferred into the funnel charged with aminomethyl resin. CH_2Cl_2 (30 mL) and DMAP (110 mg, 0.90 mmol) were added. The mixture was shaken at room temperature for 22 h. The resin was filtered, washed with MeOH (5 × 30 mL), a mixture of MeOH and CH_2Cl_2 (5 × 30 mL), and CH_2Cl_2 (5 × 30 mL), and dried on an oil pump for 20 h to give resin **14** (5.59 g in 71.5% yield). IR (KBr) cm^{-1}: 1766 (lactone CO), 1654 (amide CO).

The resin was treated with 2 mL of acetic anhydride and 1.0 mL of TEA in 40 mL of methylene chloride for 2 h to cap the unreacted amino groups. The resin was then washed with CH_2Cl_2 (5 × 30 mL) and MeOH (5 × 30 mL) and dried on an oil pump.

Resin−Linker Conjugate 16. Resin **14** (5.59 g) was washed with methylene chloride (3 × 30 mL) and swelled in 35 mL of methylene chloride. The mixture was cooled in a freezer (−21 °C). Bromotrimethylsilane (Me_3SiBr) (1.07 mL) was added. The mixture was stored at −26 °C for 50 min and shaken occasionally. Then methanol (13 mL) was added. The mixture was shaken at room temperature for 30 min. The resin was washed with MeOH and CH_2Cl_2 alternatively.

Then the resin was washed with THF (3 × 30 mL) and swelled in 30 mL of THF. Water (15 mL) and NBS (1.14 g) were added. The mixture was shaken at room temperature for 40 min. The resin was washed with MeOH and CH_2Cl_2 several times and dried in a vacuum for 24 h to give 5.51 g of resin **16**. IR (KBr): 1702 cm^{-1} (COOH), 1642 cm^{-1} (amide and quinone CO).

Resin−Linker Conjugate 17. The resin **16** (5.50 g) was washed with methylene chloride (3 × 35 mL) and swelled in 40 mL of CH_2Cl_2. *N*-Boc-ethanolamine (1.41 g, 8.73 mmol), DIC (998 mg, 1.24 mL, 7.92 mmol), and DMAP (68 mg, 0.56 mmol) were added into the funnel charged with the resin (**16**). The reaction mixture was shaken at room temperature for 18 h, washed with CH_2Cl_2, and dried with an oil pump for 24 h to get the resin (5.83 g). IR (KBr): 1719 cm^{-1} (ester CO), 1654 cm^{-1} (quinone and amide CO).

The yellow resin (3.29 g) was treated with 25% TFA in CH_2Cl_2, and washed with 10% TEA in CH_2Cl_2 and CH_2Cl_2 to give the resin **17**. This resin was dried on an oil pump for 3 h. Then a quantitative ninhydrin test was performed to give a substitution level of 0.348 mmol/g.

General Peptide Coupling Procedures. The resin **17** (992 mg, 0.348 mmol/g, 0.345 mmol) was washed twice with CH_2Cl_2 and then swelled with 10 mL of CH_2Cl_2. Boc-protected amino acid (1.143 mmol), DIC (144 mg, 1.143 mmol), HOBt (154 mg, 1.143 mmol), and DMAP (5.0 mg, 0.04 mmol) were added. The mixture was shaken for 2 h at room temperature. After coupling, the resin was washed with CH_2Cl_2 (7 × 10 mL), and the ninhydrin test was conducted. If positive ninhydrin test was obtained, the coupling reaction was repeated.

General Boc Cleavage Procedures. The resin (about 1 g) was washed with 25% TFA in CH_2Cl_2−indole (1 mg/mL) (1 × 10 mL) and shaken for 30 min with 10 mL of 25% TFA solution. The resin was then washed with CH_2Cl_2, 10% TEA/CH_2Cl_2, and CH_2Cl_2.

General Procedures for the Cleavage of the Peptides from the Resin. After washing with CH_2Cl_2, MeOH, and THF, the resin (about 1 g) was treated with sodium hydrosulfite (850 mg, 85% pure) in H_2O (5 mL) and THF (8 mL) for 2.5 h at room temperature. The progress of the reaction was monitored by IR. The yellow color of the resin disappeared with the progression of the reaction. After filtration and washing with THF, MeOH, and CH_2Cl_2, the resin was recovered. The combined solvents were evaporated. Ethyl acetate (40 mL) and water (5 mL) were added into the residue, and this mixture was stirred for 20 min. The organic layer was separated, and the aqueous layer was extracted with ethyl acetate. The combined organic layers was dried over $MgSO_4$. After filtration and evaporation, the crude product was obtained.

Boc-D-Ala-Gly-Phe-NHCH$_2$CH$_2$OH. The preparation started with 920 mg of the resin **17**. The peptide was prepared following the general procedures described above. The crude product (158 mg) was purified on a silica gel column using a gradient of 100% ethyl acetate to an ethyl acetate/methanol mixture (4/1, v/v) as the eluent to give a white solid 135 mg (89%). TLC analysis showed a single spot (silica gel plate, CH_2Cl_2/MeOH = 9/1 as the eluent, phosphomolybdic acid in EtOH as the staining agent). HPLC analysis showed that this product is identical to the standard prepared using the solution phase method. ^{1}H NMR (CD_3OD) δ: 7.27−7.19 (m, 5H), 4.56 (m, 1H), 4.02 (q, J = 7.05 Hz, 1H), 3.77 (m, 2H), 3.56 (m, 2H), 3.27 (m, 3H), 2.98 (m, 1H), 1.45 (s, 9H), 1.29 (d, J = 7.05 Hz, 3H). HRMS (M + H): Calcd for $C_{21}H_{32}N_4O_6$: 437.2400; Found: 437.2396.

Boc-Leu-D-Ala-Gly-Phe-NHCH$_2$CH$_2$OH. The synthesis started with 954 mg (0.332 mmol) of resin **17**. The peptide was prepared following the general procedures described above. The crude product after extraction (179 mg) was purified by column chromatography (silica gel) using a gradient of methylene chloride/methanol (20/1−9/1) to afford a white solid (151 mg, 83%). ^{1}H NMR (CD_3OD) δ: 7.27−7.19 (m, 5H), 4.58 (m, 1H), 4.26 (m, 1H), 4.09 (m, 1H), 3.78 (m, 2H), 3.55 (m, 2H), 3.30−3.15 (m, 5H), 3.03 (m, 1H), 1.67 (m, 1H), 1.56 (m, 2H), 1.43 (s, 9H), 1.38 (d, J = 7.09 Hz, 3H), 0.97 (m, 6H). HRMS (M + H): Calcd for $C_{27}H_{43}N_5O_7$: 550.3241; Found: 550.3243.

Boc-Trp(For)-Leu-D-Ala-Gly-Phe-NHCH$_2$CH$_2$OH. The synthesis started with 786 mg (0.274 mmol) of resin **17**. The peptide was prepared following the general procedures described above. The crude product after extraction (179 mg) was purified by column chromatography (silica gel) using a gradient of methylene chloride/methanol (20/1−9/1) to afford a white solid (147 mg, 70%). ^{1}H NMR (CD_3OD) δ: 9.13 (s, 1H), 8.30 (s, 1H), 7.69−7.17 (m, 9H), 4.55 (m, 1H), 4.48 (m, 1H), 4.37 (m, 1H), 4.26 (m, 1H), 3.79−3.59 (m, 2H), 3.48 (m, 2H), 3.30−3.09 (m, 6H), 1.59 (m, 3H), 1.36 (m, 12H), 0.92 (m, 6H). HRMS (M + H): Calcd for $C_{39}H_{53}N_7O_9$: 764.3983; Found: 764.4002.

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