ORGANIC LETTERS

2001
Vol. 3, No. 9
1403−1405

Peptide Bond Formation Mediated by 4,5-Dimethoxy-2-mercaptobenzylamine after Periodate Oxidation of the N-Terminal Serine Residue

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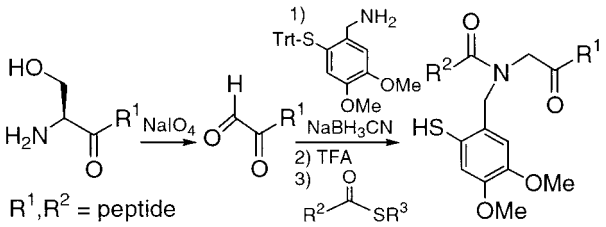
Received March 2, 2001

ABSTRACT



A thiol linker-attached peptide was prepared from a nonprotected peptide via an N^{α}-α-oxoacyl peptide. Selective oxidation of the N-terminal serine with sodium periodate gave the N^{α}-glyoxyloyl peptide, reductive amination of which with 4,5-dimethoxy-2-(triphenylmethylthio)benzylamine gave an N^{α}-4,5-dimethoxy-2-mercaptobenzyl glycyl peptide after removal of the trityl group. The N^{α}-4,5-dimethoxy-2-mercaptobenzyl peptide can be condensed with a peptide thioester, and the linker is removable. This strategy provides a useful method for the synthesis of peptides using recombinant proteins.

We wish to report herein a new strategy for the synthesis of polypeptides using recombinant proteins (nonprotected peptides) in combination with *S*-alkyl peptide thioesters (peptide thioesters)[1,2] for use as building blocks which do not contain protecting groups.

Semisynthesis using biologically prepared peptides has been investigated via chemical ligation methods.[3] For example, Gaertner et al. reported on hydrazone bond formation as the result of the reaction of peptide hydrazide and N^{α}-glyoxyloyl peptides, which were prepared from recombinant proteins.[4] Although these reactions were observed to proceed chemoselectively, they do not give rise to a native peptide bond. On the other hand, Dawson et al. reported on a chemoselective reaction of peptide thioesters and peptides, which have a cysteine residue at the N-terminus, which results in the formation of a native peptide bond.[5] This peptide bond formation via an S−N acyl shift during the reaction of the thioester and cysteine had been previously reported by Wieland et al.[6] Thiol-containing linkers, such as 2-mercaptoethoxy,[7] 2-mercaptobenzyl,[8] and 1-phenyl-2-

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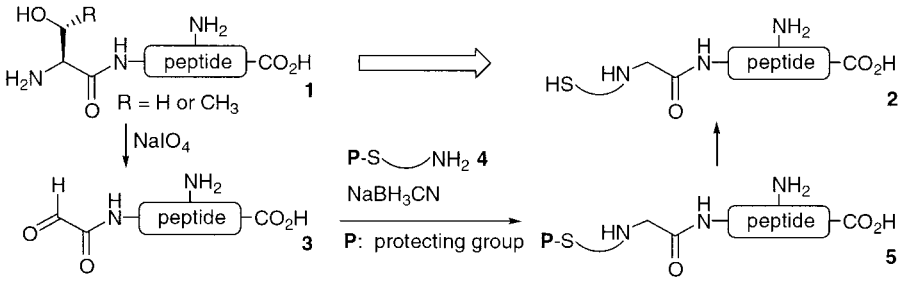
10.1021/ol0157813 CCC: $20.00
Published on Web 04/06/2001

Scheme 1. Strategy for the Preparation of Thiol Linker-Attached Peptides as C-Terminal Building Blocks from Free Peptides

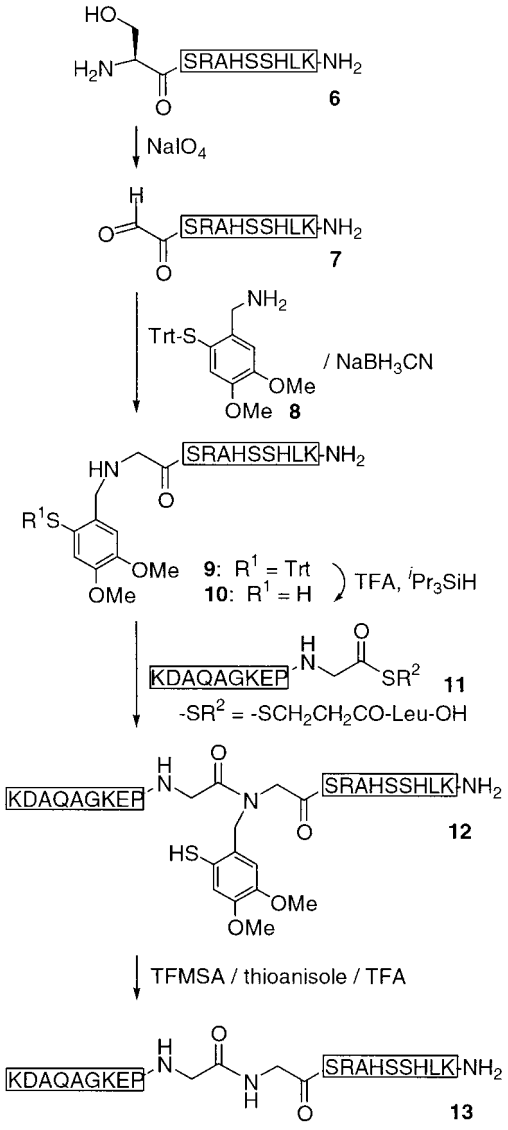


mercaptoethyl[9] groups on the terminal amino group, can be used to replace the cysteine residue. These methods are quite convenient for polypeptide synthesis, because protecting groups are not required and the reaction can be carried out in neutral aqueous solutions. Furthermore, biologically prepared peptides have been used in polypeptide synthesis using the native chemical ligation method.[10,11] An expressed peptide was also used as a C-terminal building block for polypeptide synthesis, in which a partially protected peptide segment was condensed with a peptide thioester in the presence of silver ions.[12]

Here we present an alternative method for the use of expressed peptides in the peptide bond formation at Xxx-Gly sequences. In our method, peptide building block **2** are prepared from recombinant proteins (nonprotected peptide **1**), which have a serine residue at the N-terminus, by the following steps (Scheme 1): first, the N-terminal serine or threonine residue of free peptide **1** is oxidized with periodate to give N^{α}-glyoxyloyl peptide **3**, then thiol-containing amine derivative **4** is introduced to give thiol-liker attached glycyl peptide **2** via peptide **5**. Peptide **2** would undergo condensation with a peptide thioester which contains no protecting groups. In this study, we chose the 4,5-dimethoxy-2-mercaptobenzyl (Dmmb) group as a novel linker, because this group can be readily removed under acidic conditions.

A model sequence, Gly-Ser-Arg-Ala-His-Ser-Ser-His-Leu-Lys, was examined as a C-terminal segment. The N^{α}-glyoxyloyl peptide, CHOCO-Ser-Arg-Ala-His-Ser-Ser-His-Leu-Lys-NH_2 (**7**),[13] was obtained by the periodate oxidation of a peptide, Ser-Ser-Arg-Ala-His-Ser-Ser-His-Leu-Lys-NH_2 (**6**), which is a convenient procedure for producing an N^{α}-glyoxyloyl moiety at the N-terminus (Scheme 2).[14,15] This peptide was treated with 4,5-dimethoxy-2-(triphenylmethylthio)benzylamine (**8**)[16] and sodium cyanoborohydride in DMF containing acetic acid (Figure 1A),

Scheme 2. 4,5-Dimethoxy-2-mercaptobenzylamine-Mediated Condensation of Peptide with Peptide Thioester



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(13) Sodium periodate (6.9 mg, 32 μmol) was added to a solution of peptide **6** (20 mg, 11 μmol) in 50 mM sodium phosphate buffer (pH 7.2, 1.0 mL). After stirring for 10 min, Ser (34 mg) was added, and product **7** was then purified by RP-HPLC [Cosmosil 5C18AR-II (4.6 × 250 mm), 0.1% TFA in aqueous acetonitrile] (11 mg, 7.1 μmol, 65%): MS (MALDI-TOF) found 1077.6 (MH^+), calcd 1077.6. Amino acid analysis: $Ser_{2.6}$-$Ala_{0.91}Leu_1Lys_{0.94}His_{1.8}Arg_{0.92}$.

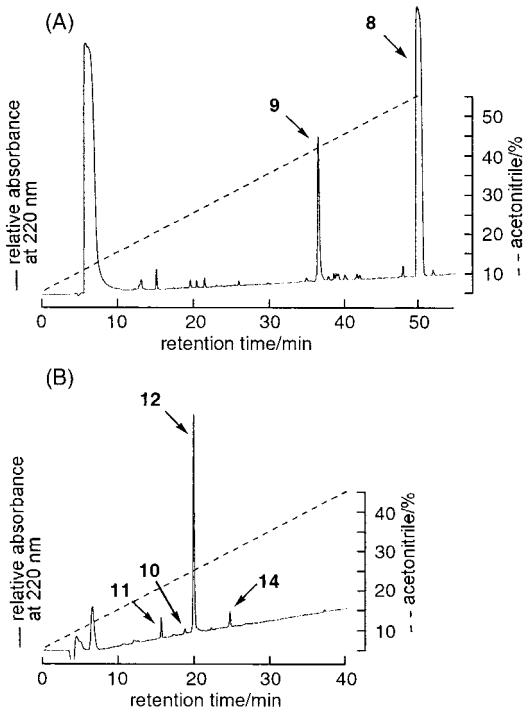

Figure 1. RP-HPLC elution profiles of the reaction mixtures. (A) Reductive amination product **9**. (B) Condensation product **12**. An arrow, **14**, indicates thiophenyl ester derived from peptide **11**. Column: Cosmosil 5C18AR-II (4.6 × 150 mm), eluent 0.1% TFA in aqueous acetonitrile, 1.0 mL/min.

followed by trifluoroacetic acid (TFA) containing triisopropylsilane to give the N^{α}-Dmmb peptide **10**.[17]

Peptide **10** was condensed with a peptide thioester, Lys-Asp-Ala-Gln-Ala-Gly-Lys-Glu-Pro-Gly-SCH_2CH_2CO-Leu-OH (**11**),[18] in neutral phosphate buffer (pH 7.2) containing 6 M guanidine hydrochloride and 0.20 M thiophenol[19] (Figure 1B). After stirring for 24 h, dithiothreitol was added to the reaction mixture, and the product, Lys-Asp-Ala-Gln-Ala-Gly-Lys-Glu-Pro-Gly-(Dmmb)Gly-Ser-Arg-Ala-His-Ser-Ser-His-Leu-Lys-NH_2 (**12**),[20] was purified by RP-HPLC. The Dmmb group was removed by treatment with trifluoromethanesufonic acid (TFMSA) in TFA to give Lys-Asp-Ala-Gln-Ala-Gly-Lys-Glu-Pro-Gly-Gly-Ser-Arg-Ala-His-Ser-Ser-His-Leu-Lys-NH_2 (**13**).[21]

The condensation of N^{α}-2-mercaptobenzyl peptides with peptide thioesters has been examined by Offer and Dawson.[8] The *N*-2-mercaptobenzyl group on the backbone of peptide is too stable at acidic conditions even under conditions of hydrogen fluoride treatment.[8] The introduction of two methoxy groups on the benzene ring permitted the Dmmb group to be removed by treatment with 1 M TFMSA in TFA.

In conclusion, we reported the successful preparation of a thiol linker-attached peptide, for condensation with the peptide thioesters, from a nonprotected peptide via periodate oxidation of the N-terminal serine residue, and reductive amination with the Dmmb amine derivative. Transamination reaction of N-terminal amino groups would also be used instead of the periodate oxidation of serine and threonine residues, in principle, though the stereochemistry, formed in the reductive amination, should be controlled. After the condensation with a peptide thioester, the thiol linker can be removed by treatment with an acid, such as TFMSA.

Acknowledgment. This research was supported, in part, by a Grant-in-Aid for Scientific Research (10179103 and 12780440) from the Ministry of Education, Science, Sports and Culture, Japan.

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(16) Amine **8** was prepared by the reaction of 4,5-dimethoxy-2-mercaptobenzylamine and triphenylmethanol in TFA: 55% yield; ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.36 (s, 3H), 3.62 (s, 2H), 3.85 (s, 3H), 6.70 (s, 1H), 6.91 (s, 1H), 7.19−7.31 (m, 15H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 41.18, 55.58, 55.98, 71.37, 113.22, 118.77, 124.13, 127.33, 127.96, 130.03, 131.29, 143.66, 149.09, 150.71; MS (FAB) 442 (MH^+), calcd 442.

(17) A solution of sodium cyanoborohydride (1.6 mg, 25 μmol) in DMF (0.10 mL) was added to a solution of peptide **7** (7.2 mg, 5.0 μmol) and amine **8** (22 mg, 50 μmol) in DMF (0.90 mL) containing acetic acid (8.0 μL). After stirring for 24 h, product **9** was purified by RP-HPLC [Cosmosil 5C18AR-II (4.6 × 250 mm), 0.1% TFA in aq acetonitrile] (3.7 mg, 1.9 μmol, 38%): MS (MALDI-TOF) found 1260.4 [$(M - Trt + H)^+$], calcd 1260.6. Amino acid analysis: $Ser_{2.5}Gly_{nd}Ala_{1.1}Leu_1Lys_{0.89}His_{1.8}Arg_{0.87}$. Peptide **9** was treated with TFA in the presence of triisopropylsilane (5%) for 0.5 h and then washed with ether and dried in vacuo. The residue was used for condensation with peptide **10**: MS (MALDI-TOF) found 1260.9 (MH^+), calcd 1260.6.

(18) Starting from Boc-Gly-SCH_2CH_2CO-Leu-OCH_2-Pam resin,[2] the peptide chain was elongated by means of a peptide synthesizer 430A (Applied Biosystems) using Boc chemistry to give Boc-Lys(Cl-Z)-Asp(O*c*Hex)-Ala-Gln-Ala-Gly-Lys(Cl-Z)-Glu(OBzl)-Pro-Gly-SCH_2CH_2CO-Leu-OCH_2-Pam resin. This resin was treated with hydrogen fluoride containing anisole (10%) on an ice bath for 1.5 h. After evaporation of the hydrogen fluoride, the mixture was washed with ether and freeze-dried. The peptide, **11**, was purified by RP-HPLC [Cosmosil 5C18AR-II (10 × 250 mm), 0.1% TFA in aqueous acetonitrile]: MS (MALDI-TOF) found 1160.0 (MH^+), calcd 1159.5. Amino acid analysis: $Asp_{0.99}Glu_{2.0}Pro_{0.78}Gly_{2.1}Ala_3Lys_{2.0}$.

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(20) Peptides **10** (3.0 mg, 1.9 μmol) and **11** (4.2 mg, 2.8 μmol) were dissolved in 50 mM sodium phosphate buffer (pH 7.2, 0.95 mL) containing 6 M guanidine hydrochloride in the presence of thiophenol (20 μL). After stirring for 25 h, dithiothreitol (30 mg) was added, the mixture was stirred for an additional 1 h, and product **12** was purified by RP-HPLC [Cosmosil 5C18AR-II (4.6 × 250 mm), 0.1% TFA in aqueous acetonitrile] (5.2 mg, 1.6 μmol, 85%): MS (MALDI-TOF) found 2243.7 (MH^+), calcd 2243.5 (average). Amino acid analysis: $Asp_{1.1}Ser_{2.5}Glu_{2.2}Pro_{0.98}Gly_{2.3}Ala_{3.2}Leu_1Lys_{3.3}His_{1.8}Arg_{0.94}$.

(21) Peptide **12** (5.2 mg, 1.6 μmol) was treated with 1 M TFMSA and 1 M thioanisole in TFA (0.20 mL) on an ice bath for 1 h, and the mixture was then washed with ether and freeze-dried. The product, **13**, was purified by RP-HPLC [Cosmosil 5C18AR-II (4.6 × 250 mm), 0.1% TFA in aqueous acetonitrile] (1.6 mg, 0.73 μmol, 45%): MS (MALDI-TOF) found 2061.1 (MH^+), calcd 2061.3 (average). Amino acid analysis: $Asp_{0.99}Ser_{2.7}Glu_{2.1}Pro_{1.2}Gly_{2.9}Ala_{2.9}Leu_1Lys_{2.9}His_{1.9}Arg_{0.99}$.