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A photoremovable ligation auxiliary for use in polypeptide synthesis

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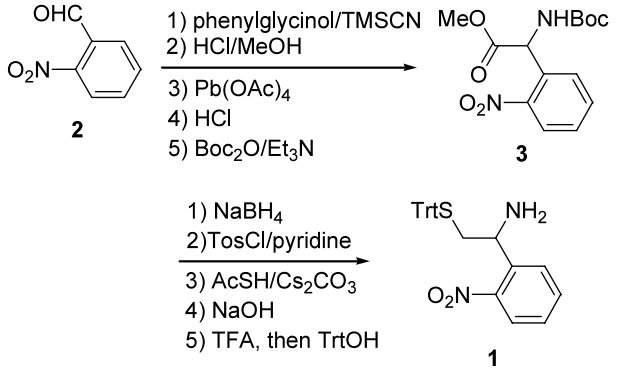
Abstract—A photoremovable auxiliary for use in peptide synthesis via the native chemical ligation method is described. A 2-mercapto-1-(2-nitrophenyl)ethylamine (Mnpe-amine) moiety was attached to the N-terminus of a peptide via the periodate oxidation of a seryl peptide. The resulting peptide was then ligated to a peptide thioester, and UV (365 nm) irradiation resulted in the removal of the auxiliary from the peptide.

Peptide thioesters are versatile synthetic intermediates that are useful in polypeptide synthesis.[1] For example, native chemical ligation using peptide thioesters as building blocks is a very useful and convenient method for polypeptide synthesis.[2] This can be performed under neutral conditions in aqueous solutions without the need for protecting groups, although a cysteine residue is required at the condensation site. Recently several groups have introduced auxiliaries for the ligation of peptides that permit the condensation without the need for a cysteine residue at the condensation site. Three scaffolds of removable auxiliaries have been reported to date: 2-mercaptoethoxy group,[3] methoxy groups-attached 2-mercaptobenzyl[4] and 1-phenyl-2-mercaptoethyl[5] groups. Since the conditions required for their removal frequently cause the adsorption of the peptides to zinc powder or their degradation under the harsh acidic conditions used, we initiated an investigation of a new auxiliary, that can be removed under mild conditions.

We previously reported the 4,5-dimethoxy-2-mercaptobenzyl group, an auxiliary that can be removed by acid treatment.[4a] An *o*-nitro group on benzyl group confers photolability,[6] but its introduction on thiophenol scaffolds decreases its nucleophilicity, thus reducing thioester exchange in the first step of the chemical ligation reaction. Herein, we describe the use of the 2-mercapto-1-phenylethyl group as a scaffold.

1-(2-Nitrophenyl)-2-(triphenylmethylthio)ethylamine (**1**) was prepared as shown in Scheme 1. Methyl *t*-butoxycarbonyloxy-2′-nitrophenylglycinate (**3**) was prepared from 2-nitrobenzaldehyde (**2**) via a Strecker type amino acid synthesis,[7] and a transformation similar to a reported literature procedure[8] gave the amine **1**.[9]

Scheme 2 shows a model peptide synthesis using the N^{α}-2-mercapto-1-(2-nitrophenyl)ethyl (Mnpe) auxiliary. The N^{α}-glyoxyloyl peptide, CHOCO-Ser-Arg-Ala-His-Ser-Ser-His-Leu-Lys-NH_2 (**5**), was prepared by the periodate oxidation of a peptide, Ser-Ser-Arg-Ala-His-Ser-Ser-His-Leu-Lys-NH_2 (**4**).[4a] This peptide was treated with amine **1** and sodium cyanoborohydride in DMF containing acetic acid (Fig. 1A), followed by treatment with trifluoroacetic acid (TFA) containing triisopropylsilane to give the N^{α}-Mnpe peptide **7**.[10]



Scheme 1. Synthesis of auxiliary molecule **1**.

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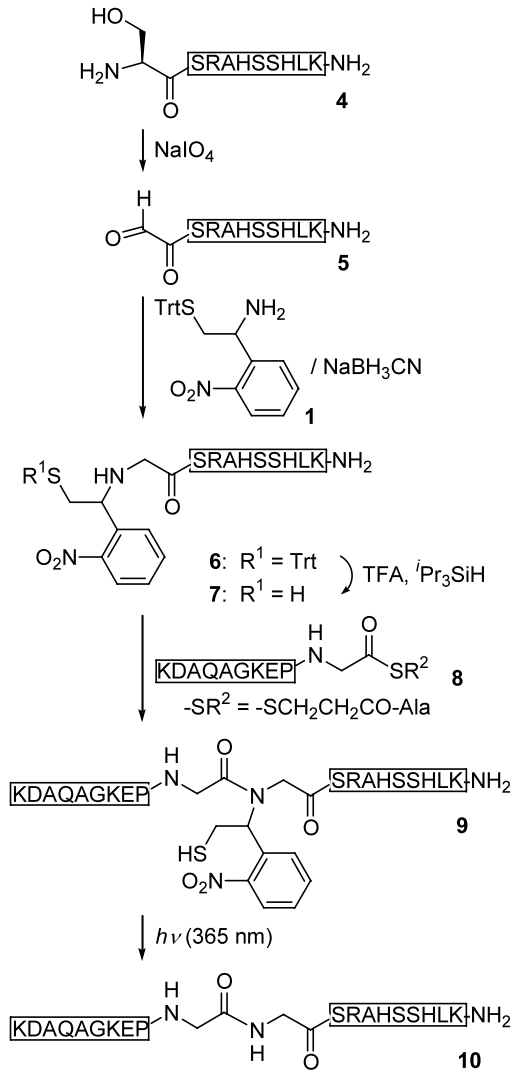

Scheme 2. 2-Mercapto-1-(2-nitrophenyl)ethylamine (Mnpe-amine)-assisted ligation of a peptide with the peptide thioester.

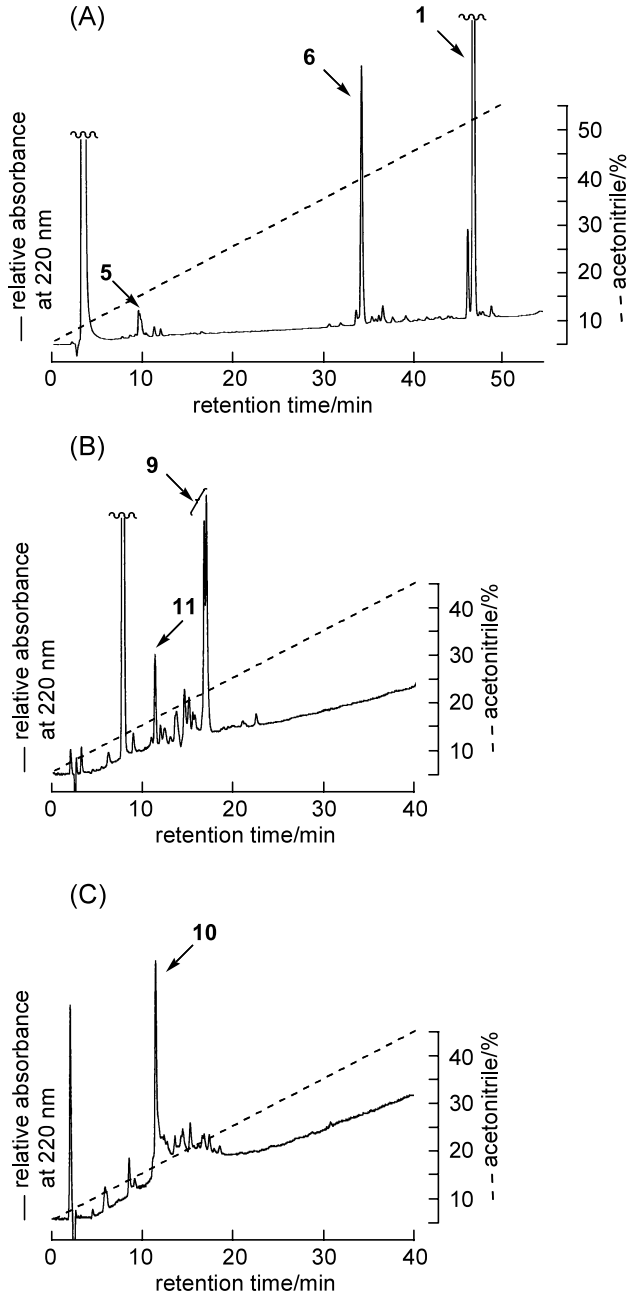

Figure 1. RP-HPLC elution profiles of the reaction mixtures. (A) Reductive amination product **6** (24 h). (B) Ligation product **9** (24 h-reaction followed by the addition of DTT). Since the Mnpe moiety has chirality, the diastereomers **9** were separated as split peaks by HPLC. An arrow, **11**, indicates a hydrolysis product of peptide thioester **8** and oxidized DTT. (C) Removal of Mnpe group (6 h). Column: YMC-Pack Pro C18 (4.6×150 mm), eluent: 0.1% TFA in aq. acetonitrile, 1.0 mL/min.

Peptide **7** was then ligated to a peptide thioester, Lys-Asp-Ala-Gln-Ala-Gly-Lys-Glu-Pro-Gly-SCH_2CH_2CO-Ala (**8**), in sodium phosphate buffer (0.10 M, pH 7.4) containing 50 mM thiophenol.[11] After stirring for 24 h, dithiothreitol (DTT) was added to the reaction mixture, and the ligation product, Lys-Asp-Ala-Gln-Ala-Gly-Lys-Glu-Pro-Gly-(Mnpe)Gly-Ser-Arg-Ala-His-Ser-Ser-His-Leu-Lys-NH_2 (**9**),[12] was purified by RP-HPLC (Fig. 1B). The Mnpe group was removed by UV irradiation at 365 nm in sodium phosphate buffer (pH 7.4) for 6 h to give Lys-Asp-Ala-Gln-Ala-Gly-Lys-Glu-Pro-Gly-Gly-Ser-Arg-Ala-His-Ser-Ser-His-Leu-Lys-NH_2 (**10**) (Fig. 1C).[4a,13]

In conclusion, the Mnpe group can be successfully used as a chemical ligation auxiliary for peptide synthesis. The N^{α}-Mnpe-attached peptide, for condensation with the peptide thioesters, can be prepared from a non-protected peptide via periodate oxidation of the N-terminal serine residue, followed by reductive amination with the Mnpe amine derivative **1**. After ligation with a peptide thioester the Mnpe group can be removed under mild conditions by UV irradiation to give a native peptide bond.

Acknowledgements

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9. **1** (as a TFA salt): ^{1}H NMR (400 MHz, $CDCl_3$) δ 3.01 (dd, $J=8.0$, 14.0 Hz, 1H), 3.15 (dd, $J=7.3$, 14.0 Hz, 1H), 3.62 (t, $J=7.5$ Hz, 1H), 7.18–7.28 (m, 9H), 7.34–7.39 (m, 6H), 7.46–7.50 (m, 2H), 7.56–7.61 (m, 1H), 7.84–7.88 (m, 1H); ^{13}C NMR (100 MHz, $CDCl_3$) δ 32.8, 49.7, 67.9, 125.3, 127.2, 128.3, 128.9, 129.3, 129.6, 130.6, 134.2, 143.7, 149.3.
10. **6** (50% yield in the 10 μmol-scale reaction, in which 5 equiv. of amine **1** and 6 equiv. of sodium cyanoborohydride were used, after RP-HPLC purification): MS (MALDI-TOF) found 1502.3, calcd 1501.7 (MH^+). **7**: MS (MALDI-TOF) found 1260.1, calcd 1259.6 (MH^+).
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12. **9** (45% yield in the 1.3 μmol-scale reaction, in which 1.5 equiv. of peptide thioester **8** was used, after RP-HPLC purification): MS (MALDI-TOF) found 2242.4, calcd 2242.5 (MH^+) (average).
13. **10** (37% yield after RP-HPLC purification): MS (MALDI-TOF) found 2062.1, calcd 2061.3 (MH^+) (average).