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O-Aminoanilides in Protein Synthesis: *N*-Acylureas, Aryloxycarbonyl-*o*-Aminoanilides and Benzotriazoles

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Introduction

Peptide α-thioesters (peptide-COSR) play a key role in native chemical ligation (NCL). They react chemoselectively with peptides featuring *N*-terminal cysteine residues, forming a native amide bond [1,2]. The synthesis of peptide-COSR by Fmoc-solid phase peptide synthesis (Fmoc-SPPS) is a non-direct process because piperidine induces thioester aminolysis. Several methods have been described for the synthesis of these important intermediates, but the lack of reproducibility and cumbersome steps did not result in any general protocol. Thus, in 2008 the *N*-acyl-benzimidazolinone (Nbz) method was introduced, which relies on the 3,4-diaminobenzoic acid for the syntheses of peptide-Nbz-$CONH_2$ that are precursors of peptide-COSR (Figure 1) [3]. Herein we recapitulate the family of *o*-aminoanilide linkers used in protein synthesis.

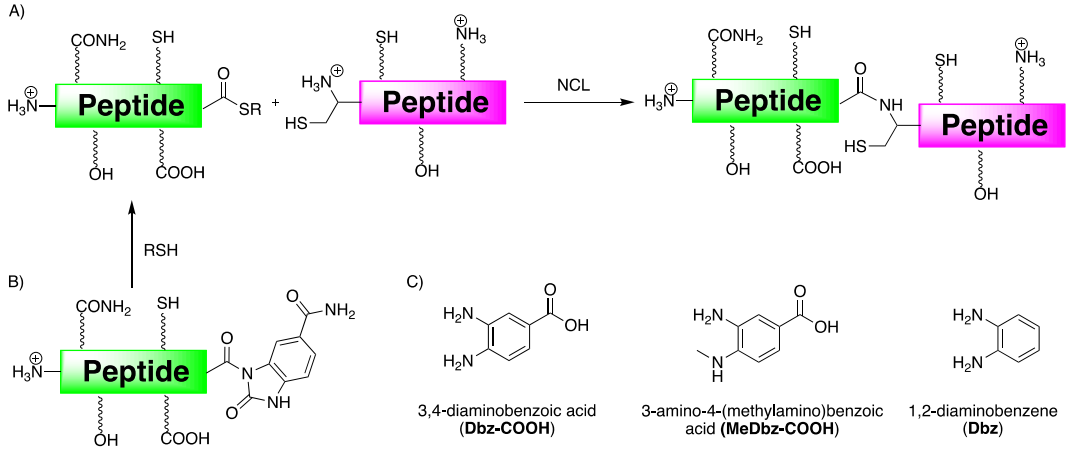

Fig. 1. A) The native chemical ligation (NCL) reaction. B) N-acyl-benzimidazolinone peptide (Peptide-Nbz-$CONH_2$) precursor of peptide-COSR. C) 3,4-diamonobenzoic acid, 3-amino-4-(methylamino)benzoic acid, and 1,2-diaminobenzene scaffolds.

Results and Discussion

Peptide-(*N*-acyl-benzimidazolinone) (peptide-Nbz-$CONH_2$) are prepared from 3,4-diaminobenzoic acid (Dbz-COOH) [3,4]. Starting from an aminomethylated resin functionalized with an acid labile linker (Rink, PAL, Sieber…), incorporation of Dbz-COOH followed by chemoselective monoacylation yields the Fmoc-Xaa_1-Dbz-CONH-resin (**1**, Xaa_1 = any amino acid, Figure 2). Next, stepwise chain elongation using Fmoc-SPPS leads to the target peptide sequence (peptide-Dbz-CONH-resin, **2**). The resulting *o*-aminoanilide peptide **2** is then acylated using *p*-nitrophenyl chloroformate, which under basic conditions undergoes intramolecular cyclization to give the peptide-Nbz-CONH-resin (**3**). TFA-mediated acidolytic cleavage of **3** releases the unprotected peptide-Nbz-$CONH_2$ (**4**), which under an excess of a thiol compound undergoes an intermolecular

N -to-*S* acyl exchange that forms the peptide-COSR (**5**). Alternatively, the presence of an *N*-terminal Cys-peptide yields the ligated product (**6**) in a one-pot NCL.

In addition, peptide **2** has a second reaction pathway via benzotriazole (Bt) derivatives [5,6]: activation with sodium nitrite at pH 3 and low temperature (~ -15ºC) leads to a peptide-Bt-$CONH_2$ (**7**) that, upon addition of a thiol compound, undertakes thioesterification followed by NCL (Figure 2).

Unfortunately, the chemoselectivity of the *o*-aminoanilide peptide **2** is compromised in Gly-rich sequences, resulting in the acylation of the free *o*-aminoanilide (Figure 2, **8**) [6]. Therefore, we developed the second generation of Dbz linkers to overcome this problem: 3-amino-4-methylamino-benzoic acid (MeDbz-COOH, Figure 3) [8]. The MeDbz-COOH is incorporated in the *C*-terminal position of peptides following a similar synthetic scheme as for Dbz-COOH. However, it behaves more robustly and can bear strong coupling microwave coupling conditions without noticeable

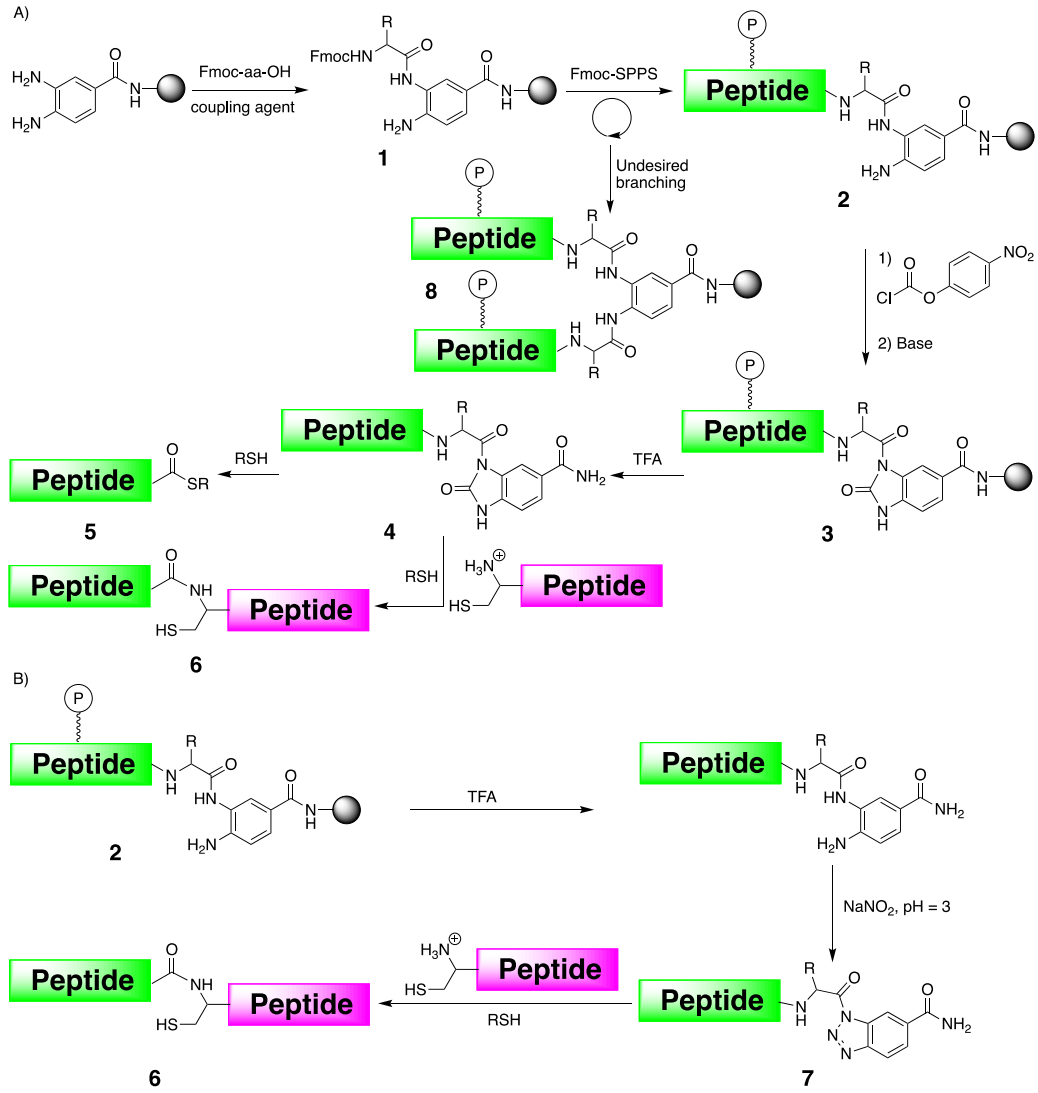

Fig. 2. A) Synthetic scheme for peptide-Nbz-$CONH_2$ and NCL. B) Synthetic route for peptide-Bt-$CONH_2$ and NCL. P = protecting group.

secondary acylations. Similarly to that peptide **2**, peptide-MeDbz-CONH-resin (**9**) can be converted into peptide-MeNbz-CONH-resin and then cleaved to give peptide-MeNbz-$CONH_2$ (**10**). Alternatively, acylation with *p*-cyanophenyl chloroformate followed by TFA-mediated cleavage affords the peptide-(*p*CN-Phoc)MeDbz-$CONH_2$ (**11**) [9]. Both peptides, **10** and **11**, undergo thiolysis in the presence of thiols, and NCL. In addition, the slower reactivity of **11** enables the use of (*p*CN-Phoc)MeDbz-$CONH_2$ peptides in sequential one-pot ligations under kinetic control [9,10].

The interesting properties of Dbz-$CONH_2$ and MeDbz-$CONH_2$ peptides as surrogates of peptide-COSR via peptide-Nbz/MeNbz-$CONH_2$ or peptide-Bt-$CONH_2$ motivated the search for an *o* -aminoanilide surrogate that would combine the advantages of both and keep the robustness of MeDbz-$CONH_2$ peptides. Thus, we found that 1,2-diaminobenzene linked to a PAL-resin can

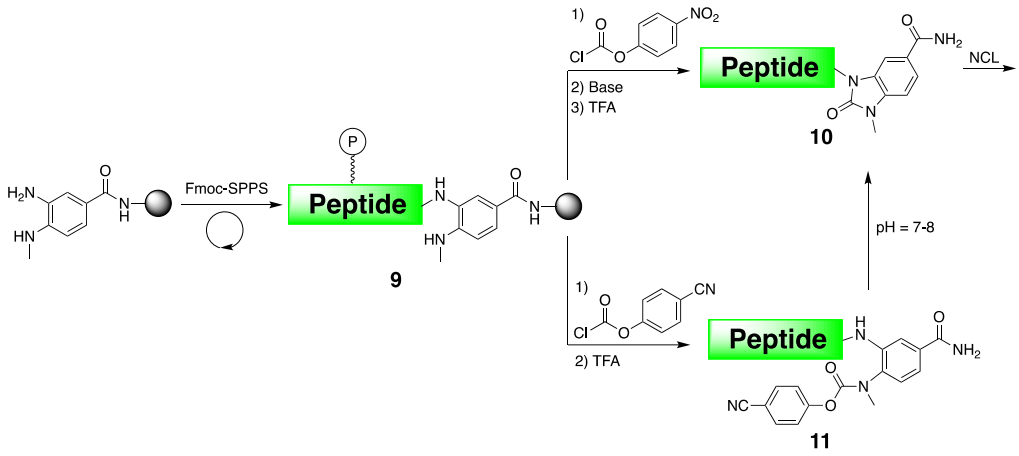

Fig. 3. Synthesis of peptide-MeNbz-$CONH_2$ and peptide-(pCN-Phoc)MeDbz-$CONH_2$ surrogates for peptide-COSR.

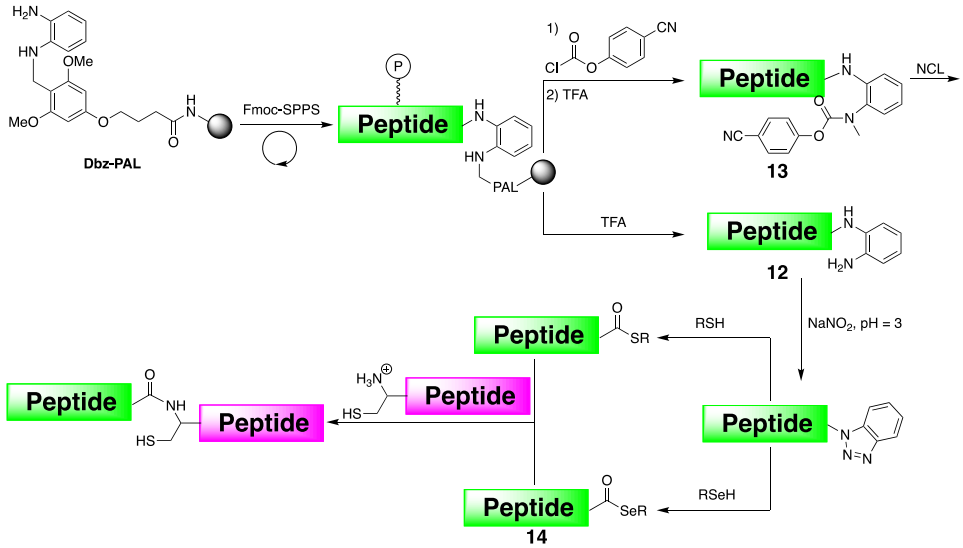

Fig. 4. Synthesis of peptide-Dbz-PAL-resin and pathways for the preparation of peptide-(pCN-Phoc)Dbz, peptide-COSR, and peptide-SeR.

generate a peptide-Dbz (**12**) after TFA cleavage, or a peptide-(*p*CN-Phoc)Dbz (**13**) precursor of peptide-Nbz (Figure 4) [11]. The Dbz-PAL-resin is chemoselective, but it does not impair the acylation in the presence of strong electrophiles such as chloroformates. In addition, we have found that peptide-Bt is labile enough to undergo transselenoesterication in the presence of benzeneselenol. C-terminal peptide-selenoesters (peptide-COSeR, **14**) react outstandingly fast with *N*-terminal cysteine and selenocysteine-peptides. Here, we have developed an efficient strategy for the synthesis of peptide-COSeR that hold a great deal of promise for chemical protein synthesis.

Conclusions

The synthesis of peptide-COSR and peptide-COSeR for NCL can be efficiently accomplished by the different moieties derived from *o*-aminoanilides. Activation via *N*-acylurea or *N*-acylbenzotriazole leads to precursors that undergo transthioesterification or transselenoesterification in the presence of the corresponding thiols or selenols.

Acknowledgments

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