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Amine Capture Strategy for Peptide Bond Formation by Means of Quinolinium Thioester Salts

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To go beyond the limits of the stepwise solid-phase peptide synthesis (SPPS),[1] various convergent methods have been successfully investigated allowing the preparation of large peptides.[2] Among them, "*native chemical ligation*" represents today one of the most promising approaches that has made the total chemical synthesis of proteins a practical reality.[3] The general principle of this approach relies on an efficient ligation reaction of two unprotected peptide fragments to bring the two coupling sites into close proximity. As a result of this entropic activation, the resultant "*ligated product*" undergoes acyl transfer to furnish the final coupled peptide containing a native peptide bond at the ligation junction. The "*Prior Amine Capture Strategy*", initially reported by Kemp in 1975, is the first general approach to ligation-based coupling method.[4] In this context, innovative templates for developing new amine capture strategies are highly desirable. Our approach reported herein capitalizes on conjugated additions to quinolinium salts-type **2**[5] to design a new "*electrophilic platform*" capable of acting as an amine capture device. The reversible nature of these conjugated additions would offer the advantage to realize in a single step the acyl transfer and release of the template, which represents a real benefit over existing ligation processes previously reported. A thioester link was selected to join the amino acid residue and the quinoline template. This functional group is expected to display a good balance between reactivity and stability, so that intramolecular acyl transfer occurs through the ligated product intermediate, while remaining nonreactive in the absence of this entropic activation.[6] As a consequence of the incapacity of quinolines **1** to form 1,4-adducts, this precursor may be considered as a "*latent capture amine site*" activated by quaternization. In this perspective, quinolinium thioester salts **2** are appealing candidates, fulfilling all the criteria of a promising template for developing a new amine capture strategy (Figure 1).

To validate this approach, Boc-Gly-S-quinolinium salt **2a** and Boc-D-Phe-S-quinolinium **2b** were selected as models and were involved in peptide bond formation with various amino esters. Table 1 summarizes the main results obtained after optimization of the reaction conditions. After screening several solvents, acetonitrile provided a good compromise between solubility and reactivity. Coupling reactions proceeded with excellent conversion rates (>90%) and, importantly, without detectable epimerization. Dipeptides **3a**−**f** could be isolated by flash chromatography with satisfactory yields ranging from 55 to 75%. It has been established that a reaction time of 5−6 h was generally required to drive the coupling reaction to completion (Table 1).

A control experiment revealed that the peptide coupling is totally ineffective in the absence of triethylamine, pointing out the crucial role of this base. Triethylamine probably takes part at some stage of the amine capture step for assisting the deprotonation of the amino ester. This observation strongly argues in favor of an amine capture prior to peptide bond formation. Finally, indirect confirmation was obtained for the proposed intermediate in a separated UV

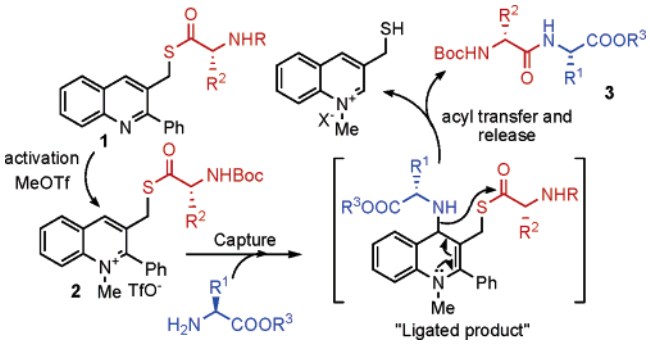


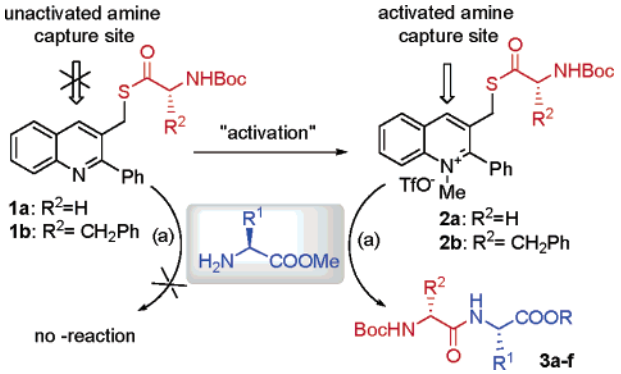
Figure 1. Amine capture strategy by means of a quinolinium thioester salt.

Table 1. Preparation of Dipeptides **3a**−**f** by Means of Quinolinium Thioester Salts **2a**,**b**[a]

entry	template	aminoester[b]	dipeptide **3a**–**f**	conv. %[c] (yield %)
1	**2a**	H−L-PheOMe	Boc-Gly-L-PheOMe **3a**	>90 (50)
2	**2a**	H−L-Pro-OMe	Boc-Gly-L-Pro-OMe **3b**	>90(65)
3	**2a**	H−L-Phg-OMe	Boc-Gly-L-Phg-OMe **3c**	>90(70)
4	**2b**	H−L-Phe-OMe	Boc-D-Phe-L-Phe-OMe **3d**	>90(55)
5	**2b**	H−L-Ala-OMe	Boc-D-Phe-L-Ala-OMe **3e**	>90(66)
6	**2b**	H−L-Pro-OMe	Boc-D-Phe-L-Pro-OMe **3f**	>90(75)

[a] Reagents and conditions: NEt_3 (2equiv), CD_3CN, room temperature, 6 h. [b] Hydrochloride salts are used. [c] Measured by ^1H NMR spectroscopy.

Scheme 1. Experimental Data Supporting an Amine Capture Strategy Prior to Peptide Bond Formation[a]



[a] Reagents and conditions: (a) NEt_3 (2eq)/CH_3CN/r.t./6h.

spectroscopy experiment performed with quinolinium salt **7**. Whereas UV spectra of quinolinium salt **7** remain unchanged by addition of benzylamine, sequential additions of NEt_3 led to the formation of the adduct product **8** which was further isolated and fully characterized by ^1H and ^{13}C NMR (Figure 2).

The incapability of quinolines **1a**,**b** to react with amino esters under the same conditions than those employed with quinolinium

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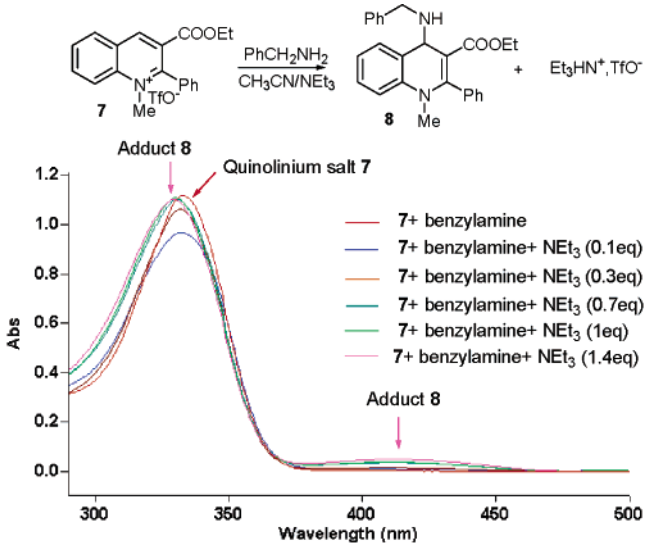
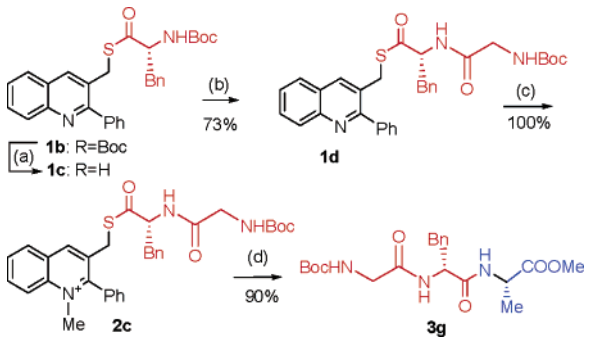


Figure 2. Participation of NEt_3 in conjugated additions of amines to the quinolinium salt **7** evidenced by means of UV spectroscopy.

Scheme 2. Preparation of a Tripeptide **3g** Using a Safety-Catch Approach[a]



[a] Reagents and conditions: (a) HCl (3 M) in AcOEt, 1 h, 0 °C; (b) Boc-glycine/EDCI/HOBt/DIEA/CH_2Cl_2, 20 °C, 12 h; (c) TfOMe/Na_2CO_3, 20 °C, 1 h; (d) L-alanine methyl ester hydrochloride (1 equiv)/NEt_3 (2 equiv)/CH_3CN, room temperature, 6 h.

salts **2a**,**b** provided additional evidence for the proposed sequential mechanism. With these experimental observations in mind, we then speculated that quinolines **1a**,**b** may be conceptually considered as a "*masked amine capture site*", which may possibly be used in the design of a new "*safety-catch*" linker[7] and exploitable in Boc peptide synthesis (Scheme 1).

To validate the whole sequence of this safety-catch approach, quinoline **1b** was subjected to Boc deprotection conditions (HCl/AcOEt) and subsequently coupled with Boc-glycine under standard activation conditions (EDCI/HOBt/DIEA/CH_2Cl_2), providing the dipeptide Boc-Gly-D-Phe-S-quinoline **1d** in 73% overall yield (Scheme 2). At this stage, no residual reactivity of the thioester was detected, demonstrating that both deprotection and activation conditions are compatible with a safety-catch approach. After activation of the amine capture site by quaternization of **1d**, the resulting quinolinium salt **2c** was reacted with L-alanine methyl ester, under the optimized conditions established above, to supply the desired tripeptide Boc-Gly-D-Phe-L-Ala-OMe **3g** in a satisfactory yield (Scheme 2).

In summary, we have demonstrated that quinolinium thioester salts-type **2** display an attractive potential in peptide synthesis. Interestingly, a number of experimental observations lend to the belief that a sequential mechanism related to a prior amine capture strategy is involved. The "*latent reactivity*" of the nonquaternized quinoline **1** renders this precursor an appealing synthetic tool in view of developing a new safety-catch linker. These preliminary results laid down the basis of future developments in SPPS.

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Supporting Information Available: Experimental procedures and characterization data for all new compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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