Efficient Peptide Thioester Synthesis from Hydrazides Using Near-Stoichiometric Thiol Quantities [1]

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Introduction

Peptide alkyl thioesters serve as versatile peptide reagents across various synthetic applications. Consequently, significant efforts have been directed over the past two decades towards accessing peptide thioesters through both solution and solid-phase methods. Notably, the generation of peptide thioesters from **peptide hydrazides** is particularly prevalent in the field, owing to the ease of access to the latter via Fmoc-SPPS.

However, a notable limitation of many of these synthetic approaches, including the hydrazide technique, is the requirement for **a substantial excess of thiol reagent**.[2] This limitation confines these synthetic strategies to the production of thioesters from relatively **simple and cheap alkyl thiol nucleophiles**.

We present here a novel method to access various types of alkyl thioesters using nearly **stoichiometric quantities** of the alkyl thiol nucleophile.

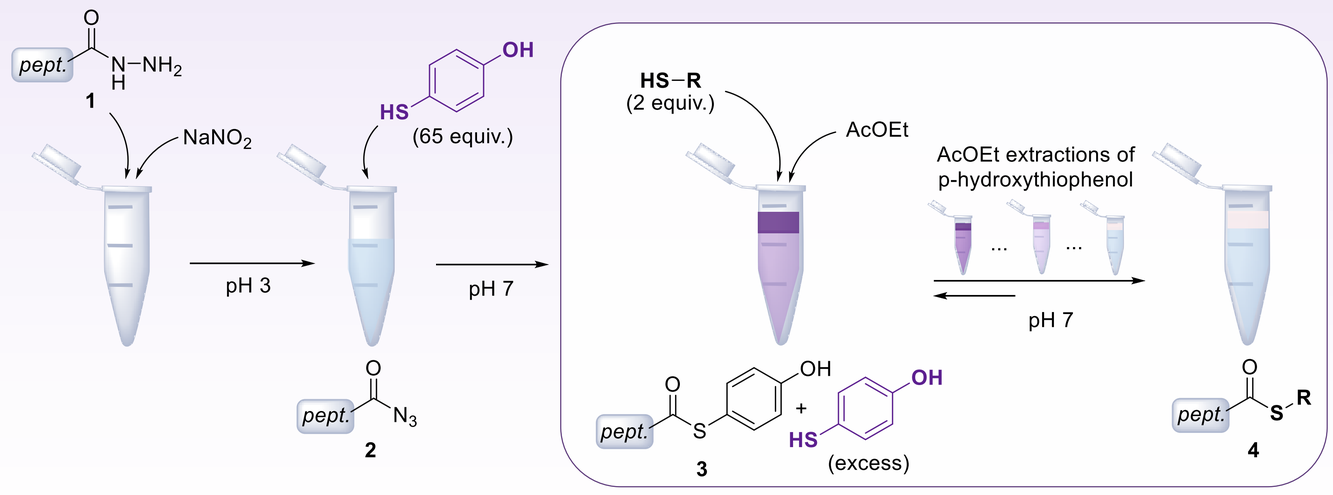


Fig. 1: One pot synthesis of peptide alkyl thioesters from peptide hydrazides is favoured by shifting thiol-thioester equilibrium through aryl thiol extraction.

Method

Initially, the hydrazide undergoes **activation** with sodium nitrite to yield a peptide azide (1→2, Fig.1). Subsequently, an excess of aryl thiol is added to the peptide azide solution under neutral pH conditions, resulting in the formation of an **intermediate aryl thioester** (2→3, Fig.1)**,** following a procedure reported by Liu and colleagues.[3]

In our method, an additional step involves a **thiol-thioester exchange** between the in situ formed aryl thioester and an alkyl thiol added in **quasi-stoichiometric amounts** to the reaction, under conditions enabling to concomitantly remove the excess of aryl thiol (3→4, Fig.1).

The excess of aryl thiol is essential to displace the azide and neutralize any remaining nitrous acid, but logically inhibits the formation of the alkyl thioester which proceeds through an **equilibrated thiol-thioester exchange** process.

In contrast, the removal of the aryl thiol during the thiol-thioester exchange reaction accelerates and promotes the formation of the alkyl thioester. This was conveniently achieved by using ***p*-hydroxythiophenol**, a thiol extractable under neutral pH conditions. (*Fig. 1*)

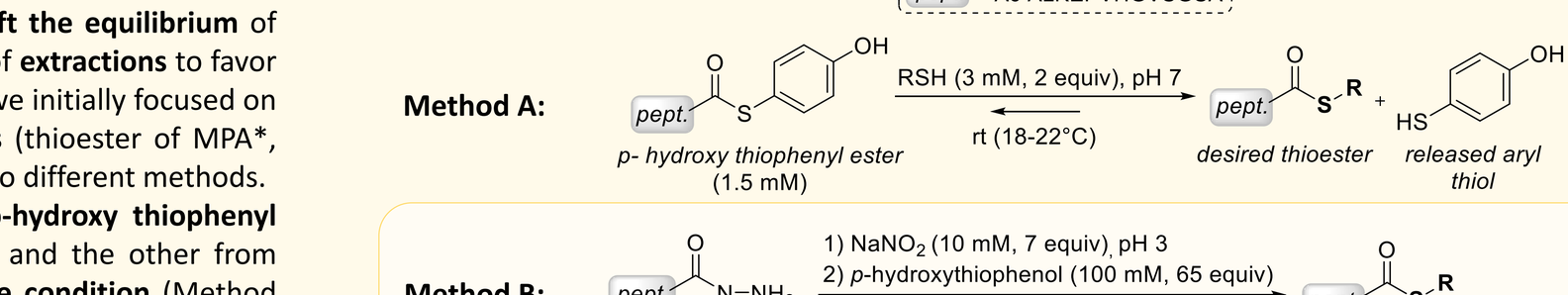
Results and discussion

As the aim of our method is to **shift the equilibrium** of the thiol thioester exchange by means of **extractions** to favor the formation of the desired thioester, we initially focused on this exchange to form **three thioesters** (thioester of MPA*, MPA-R_6L-NH_2 and coenzyme A) using two different methods.

The first method starts from the **p-hydroxy thiophenyl ester without extractions** (Method A) and the other from the **peptide hydrazide under extractive condition** (Method B). (*Fig. 2*)

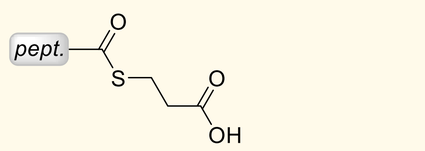
Then, we compared these exchanges thanks to the **kinetics data** we obtained during the synthesis of our thioesters using both methods. (*Fig. 3*)

* MPA = 3-Mercaptopropionic acid

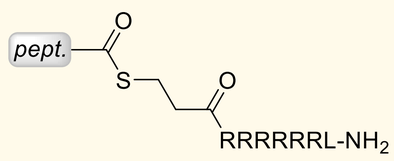


Desired thioester	Isolated yield (%)
4a	40
4b	31
4c	52

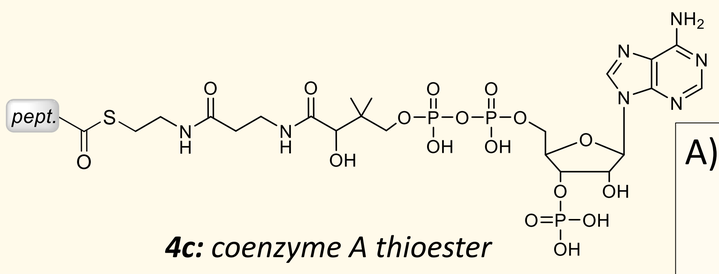
Fig. 2: Methods A and B are performed under the **same conditions for the exchange step** (1.5 mM of starting material, 2 equiv of alkyl thiol, pH 7) but Method B allows the extractions of *p*-hydroxythiophenol.



***4a**: MPA thioester*



***4b:** MPA-R_6L-NH_2 thioester*



***4c:** coenzyme A thioester*

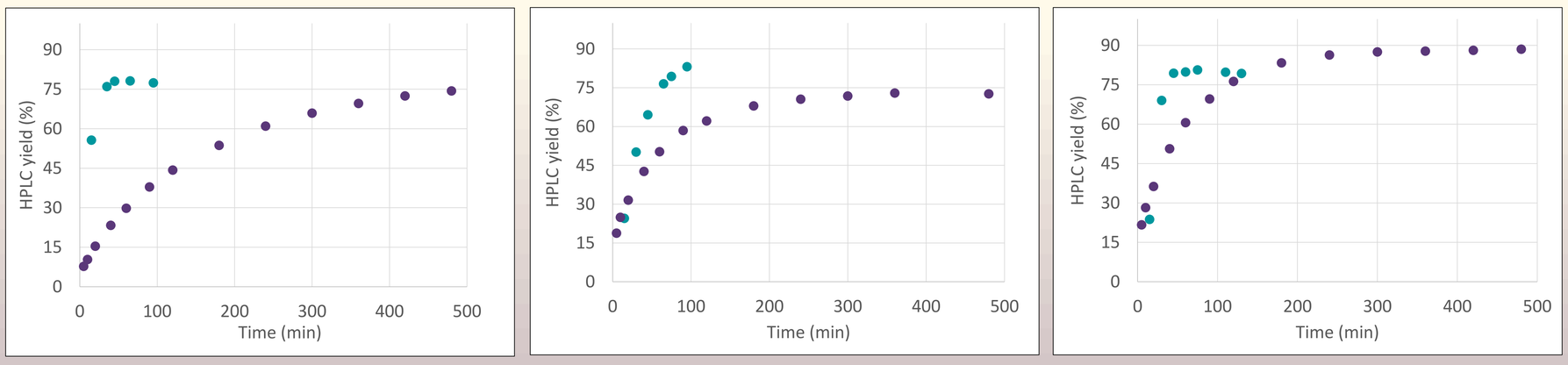


Fig. 3: Comparison of the kinetics data obtained for various thioesters synthesis using Methods A (●) and B (●).

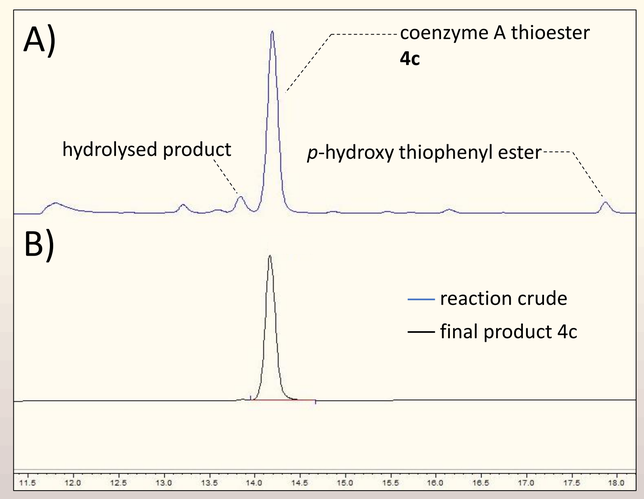


Fig. 4: Synthesis of thioester **4c** using Method B. A) HPLC chromatograms obtained after 2 h of reaction. B) Purified peptide thioester **4c**.

Figure 3 highlights that by shifting the equilibrium of the thiol-thioester exchange using Method B, the desired maximum thioester yield can be achieved **more quickly**. In addition, for some thioester the **HPLC yield** using method B are higher.

The improvement in alkyl thioester synthesis obtained using Method B is due to the fact that both the initial excess of aryl thiol and the released aryl thiol are removed by extraction, while in Method A the released *p*-hydroxythiophenol remains in solution and favours the backward reaction.

Conclusion

Our method has proven **highly effective** in accessing peptide thioesters from a variety of alkyl thiols. Indeed, the extraction of the initial aryl thiol and aryl thiol released during the exchange **shifts the equilibrium** of the reaction, favouring the formation of the desired alkyl thioester using **near-stoichiometric conditions**.

The method is thus applicable to the use of **complex or expensive thiols** such as coenzyme A.

References:

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[2] Agouridas, V.; El Mahdi, O.; Diemer, V.; Cargoët, M.; Monbaliu, J.-C. M.; Melnyk, O. *Chem. Rev.* **2019**, *119* (12), 7328–7443.

[3] Fang, G.-M.; Li, Y.-M.; Shen, F.; Huang, Y.-C.; Li, J.-B.; Lin, Y.; Cui, H.-K.; Liu, L. *Angew. Chem. Int. Ed.* **2011**, *50* (33), 7645–7649.