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Facile and Mild Synthesis of Linear and Cyclic Peptides via Thioesters

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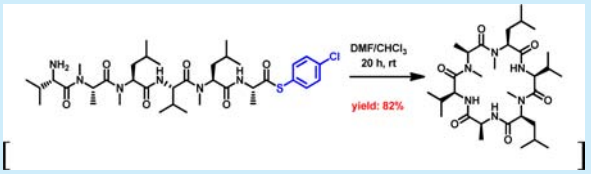
Supporting Information

ABSTRACT: Thioester-mediated peptide bond formation has recently garnered a lot of attention, most notably in its relevance to condensation of large peptide fragments. Herein, a simple and general ligation method for the preparation of linear and cyclic peptides, starting from peptide thioester, mainly *p*-chlorophenyl, precursors is reported. The inherent advantages of this method are the low epimerization, reduced dimerization, use of mild reaction conditions, and elimination of superfluous coupling reagents.



Natural cyclopeptides play an important role in pharmaceutical research.[1] Owing to their enhanced resistance to proteases[2] and their reduced conformational flexibility compared to their open-chain counterparts, cyclopeptides are meeting the stability, potency and selectivity criteria of drugs.[3] Many natural cyclopeptides such as Vancomycin, Cyclosporin A and Romidepsin, to mention only a few, have been developed as drugs. Indeed, natural cyclopeptides have presented themselves as formidable starting points in drug discovery and have helped inspire generations of medicinal chemists. It is within this area of small cyclic peptides, which is seen by many as a natural progression from traditional small molecule entities, that there is a need for enlarging the tool arsenal for their preparation.

Most common synthetic methods of preparing head-to-tail cyclopeptides rely on lactamization of the fully protected amino acid prepared by SPPS.[4] The use of the classical carboxylic acid activation requires the presence of all side-chain protecting groups, which can jeopardize the cyclization step due to the intrinsic low solubility of these peptides. Since the pioneering work of Kemp, chemists have tried to develop amide bond formation methods which are compatible with most of the functional groups present in amino acids.[5] This type of amide formation has been called ligation.[6] Arguably, the most important extension of this method into peptide synthesis is the native chemical ligation (NCL).[7] In NCL, the thiolate/thiol of an N-terminal cysteine is reacted reversibly with a thioester, leading to an intermediate cysteine thioester. In a second step, this intermediate rearranges via an intramolecular 5-membered *S*,*N*-acyl shift which then gives rise to a native cysteine amide.[8] In practice, the addition of an excess of thiol catalyzes the transthioesterification. Since the scope of the NCL is restricted to the preparation of cysteine amides, methods have been developed to overcome this restriction. In all of these methods, the role of the cysteine side chain is mimicked by a thiol-containing auxiliary.[9]

Thioesters can be considered as activated acids. Thus, Jakubke[10] reported the peptide bond formation, with near-quantitative yields, of the Cbz-Gly-Phe-8-thioquinolyl ester with glycine ethyl ester by simply stirring the components at room temperature. More recently, this method was extended by Tam.[11] He found that poorly reactive alkyl thioesters, activated by silver salts, react selectively in buffered aqueous solutions with amines. Finally, Houghten[12] reported that thioesters react with amines in the presence of imidazole. The authors propose a two-step mechanism. The thioester is reacted reversibly with imidazole to give the putative acyl imidazole. This intermediate is then trapped immediately by the amine to give the observed amide. In theory, this process appears to be catalytic with respect to the imidazole. In practice, however, concentrations of 1.5 M are essential in order to drive the reactions to completion. As a consequence of using these latter methods, the products tend to be contaminated by traces of silver or imidazole, which could lead to artifacts in biological assays. In this regard, we were, naturally, very keen to develop a method which avoided the use of such catalysts.

Herein, we describe a set of conditions which allowed the uncatalyzed reaction of simple phenyl thioesters with amino acids. This reaction covers a broad scope and allows the buildup of linear and cyclic peptides from readily available and shelf-stable thioesters which, in turn, are obtained by simply mixing the components together at room temperature.

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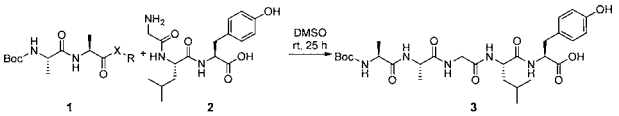
Compared to the in situ activation of carboxylic acids, this method is more selective with a simpler workup and the yields tend to be higher.

First, the influence of the leaving group was investigated. Thus, different aryl and alkyl thioesters were tested in a model ligation test system, consisting of peptide thioester Boc-Ala-Ala-COSR and the N-terminal unprotected tripeptide Gly-Leu-Tyr. The peptide thioalkyl and thioaryl esters have been synthesized from the carboxylic acids[13] with 61−91% overall yield (see the Supporting Information). The amino thioesters have been proven to be shelf-stable. In fact, when these compounds were stored without efforts to exclude moisture, we were surprised to learn that they were stable for many months without detectable decomposition. Thioesters can also be replaced by oxoesters. However, because of their lower reactivity, ligations tend to be incomplete, even with prolonged reaction times (Table 1, entries 12−14). From the leaving groups tested, *p*-nitrophenyl and, in some sense surprisingly, *p*-chloro thioesters (entries 1 and 4) gave the best yields. In the case of the alkyl thioesters, there was no conversion at all (entries 9 and 10). We also found oxoesters to be far less reactive.[14] In general, the results observed could be explained by the reactivity of the leaving group. Interestingly, a relatively high reactivity of the *p*-chloro thioester has been shown.

Table 1. Screening of Different Esters with Gly-Leu-Tyr[a]



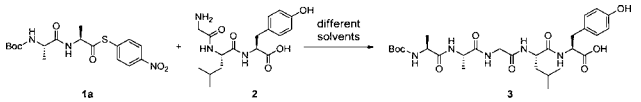
entry	peptide	XR	yield[b] (%)
1	**1a**	SC_6H_4-4NO_2	81
2	**1b**	SPh	84
3	**1c**	SC_6H_4-4OCH_3	50
4	**1d**	SC_6H_4-4Cl	95
5	**1e**	SC_6H_4-2Cl	64
6	**1f**	SC_6H_4-3Cl	74
7	**1g**	SC_6H_4-4F	78
8	**1h**	SBzl	no conversion
9	**1i**	SC_6H_{11}	no conversion
10	**1j**	SEt	no conversion
11	**1k**	OPh	no conversion
12	**1l**	OC_6H_4-4Cl	81[c]
13	**1m**	OC_6H_4-4NO_2	54[d]
14	**1n**	OC_6H_4-4OCH_3	no conversion
15	**1o**	OC_6F_5	46

[a]Reaction conditions: thioester (1.2 equiv), amine (1 equiv), DMSO (60 mM), 25 h, rt. All the reactions were stopped after complete consumption of starting materials shown by LCMS. [b]Isolated yields. [c]Reaction was stopped after 8 days. [d]Reaction was stopped after 4 days.

Next, different solvents were screened for the same reaction (Table 2). As expected, polar aprotic solvents were found to be the best suited. In the case of less polar solvents such as THF, $CHCl_3$, and MeCN, solubility of the reagents was problematic leading to lower conversions.

As described in the literature, the addition of *N*-OH additives could accelerate the ligation reaction.[15] Thus, it was reported that peptide bond formation via 1-hydroxy-1*H*-benzotriazole (HOBt) esters resulted in high yields.[16] Accordingly, different additives were investigated (Table 3). However, with our model, no enhancement of the yield was found using these additives. Even in the case of *p*-nitrophenyl thioester, yields were higher in the absence of HOBt or other polar additives, typically from 70 to 81% yield. This fact alone favors the development of a simpler ligation method whereby polar additives are completely eliminated, facilitating the recovery and purification processes.

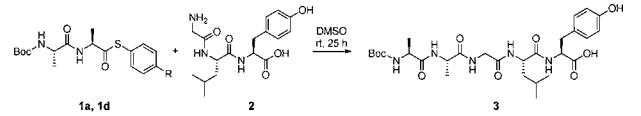
Table 2. Screening of Different Solvents[a]



		conversion[b] (%)			
entry	solvent	1.5 h	8 h	24 h	48 h
1	DMSO	49	100	100	100
2	THF	8	41	89	100
3	ACN	traces	traces	traces	traces
4	DMF	81	100	100	100
5[c]	DMF/$CHCl_3$	53	99	99	100
6	$CHCl_3$	traces	traces	traces	traces
7	NMP	53	84	99	100
8	MeOH	traces	18	49	60
9	H_2O	traces	traces	traces	traces

[a]Reaction conditions: peptide thioester Boc-Ala-Ala-COSC_6H_4-4NO_2 (1.2 equiv), Gly-Leu-Tyr (1 equiv), solvent (60 mM), rt. [b]All the conversions were determined by HPLC of the crude reaction mixture and checked for 96 h. [c]DMF/$CHCl_3$ (50:50).

Table 3. Screening of Different Additives[a]



entry	R	additive	yield[b] (%)
1	SPh	HOBt	84
2	SPh		73
3[c]	SPh	HOOBt	66
4	SPh	HOAt	67
5	SC_6H_4-4NO_2	HOBt	70
6	SC_6H_4-4NO_2		81
7	SC_6H_4-4NO_2	HOOBt	81
8	SC_6H_4-4NO_2	HOAt	59
9	SC_6H_4-4NO_2	PS-HOOBt	51

[a]Reaction conditions: peptide 1: thioester Boc-Ala-Ala-COSR (1.2 equiv), Gly-Leu-Tyr (1 equiv), additive (2 equiv) DMSO (60 mM), 25 h, rt. [b]Isolated yield.

In order to investigate the scope of the method, Boc-Phe-Phe-Gly-SC_6H_4-*p*-NO_2 was ligated with a representative set of amino acid esters having reactive side chains (Table 4). In all cases, including the *N*-methylamino acid sarcosine (entry 11), products were obtained in medium/moderate to high yields.[17] Even the coupling with the His-OEt derivative (entry 10) gave a good yield. In the case of the lysine, the presence of side-chain acylation product in the reaction mixture accounts for the low yield (entry 9).

In this context, a number of tri- and pentapeptides were acylated by different *p*-nitrophenyl thioesters (Table 5). In all cases, fair to good coupling yields could be obtained by varying the reaction times between 1 and 4 days.

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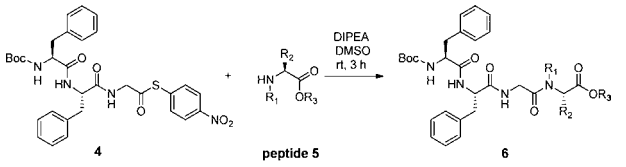
Table 4. Screening of Amino Esters[a]

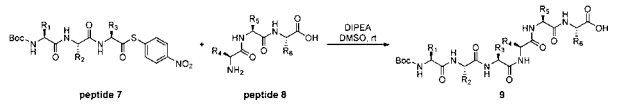


4 peptide 5 6

entry	product	peptide **5**	yield[b] (%)
1	**6a**	Gln-O-*t*-Bu	70
2	**6b**	Tyr-OBz	71
3	**6c**	Asp-OBz	60
4	**6d**	Ser-OBz	72
5	**6e**	Arg-OBz	89
6	**6f**	Trp-OMe	83
7	**6g**	Asn-OMe	53
8	**6h**	Thr-OMe	51
9[c]	**6i**	Lys-OMe	50
10	**6j**	His-OEt	75
11[d]	**6k**	Sar-OBz	70

[a]Reaction conditions: peptide 1: thioester (1.2 equiv), amine (1 equiv), DMSO (10 mM), DIPEA (5 equiv), 25 h, rt. [b]Isolated yields. [c]Isolated yield as a 1:1 mixture of regioisomers. [d]Reaction time 96 h.

Table 5. Scope of Thioester Reaction in Ligation Test[a]



peptide 7 peptide 8 9

entry	product	peptide **7**, peptide **8**	time (h)	yield[b] (%)
1	**9a**	Boc-Ala-Ala- $COSC_6H_4$-4NO_2 Ala-Tyr-Tyr-Ala-Gly-OH	96	67
2	**9b**	Boc-Ala-Ala- $COSC_6H_4$-4NO_2 Val-Gly-Phe-Thr-Ser-OH	48	75
3	**9c**	Boc-Ala-Ala- $COSC_6H_4$-4NO_2 Glu-Gly-Phe-Thr-Ser-OH	48	70
4	**9d**	Boc-Ala-Ala- $COSC_6H_4$-4NO_2 Gly-Arg(NO_2)-Gln-Phe-Thr-Ser-OH	25	72
5	**9e**	Boc-Gly-Val-Pro- $COSC_6H_4$-4NO_2 Val-Trp-Ala-OH	48	59
6	**9f**	Boc-Val-Ala-Gly- $COSC_6H_4$-4NO_2 Val-Pro-Val-OH	48	76

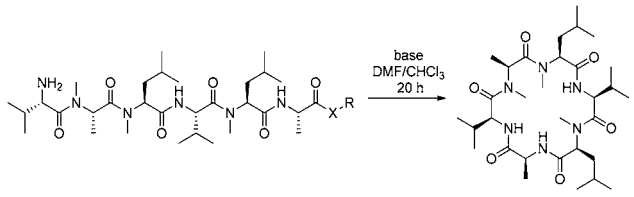
[a]Reaction conditions: thioester (1.2 equiv), amine (1 equiv), DMSO (60 mM), 25 h, rt. [b]Isolated yields.

Next, the applicability of this method for the preparation of cyclopeptides was explored using the hexapeptide H-Val-MeAla-MeLeu-MeVal-MeLeu-Ala-OH as a core sequence.

First of all, different phenyl (thio)esters were investigated. The cyclizations were performed at 5 mM concentration (Table 6).

The best results were obtained for the *p*-chloro thioester (entry 1), clearly superior to the *p*-nitro thioester in terms of yield (82% vs 47%) and less dimer formation (<1% vs 4%). Furthermore, it was also superior to the classical coupling reaction conditions using HATU, which is considered the reagent of choice (46%) (entry 7). In addition, we examined the analogous oxo-ester (entry 5) that gave a lower yield compared to the thioesters derivatives. This result is in accordance with the previous observation for the ligation tests (Table 2, entries 11−14).

Table 6. Cyclization Condition Screening[a]



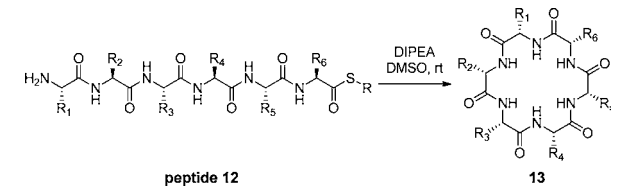
peptide 10 11

entry	peptide **10**	XR[b]	base	temp (°C)	yield[c] (%)	dimer[d] (%)
1	**10a**	SC_6H_4-4Cl	DIPEA	25	82	<1
2	**10a**	SC_6H_4-4Cl	$NaHCO_3$	25	65	16
3	**10a**	SC_6H_4-4Cl	$NaHCO_3$	60	58	16
4	**10b**	SC_6H_4-4NO_2	DIPEA	25	47	4
5	**10c**	OC_6H_4-4NO_2	DIPEA	25	37	11
6	**10d**	SC_6H_4-4OCH_3	DIPEA	25	40	7
7[e]	**10e**	OH	DIPEA	25	46	15

[a]Reaction conditions: thioester (1 equiv), base (2 equiv) DMF/$CHCl_3$(5 mM), 20 h. [b]The thioester was prepared from the corresponding Boc derivative, which was removed before the cyclization. [c]Isolated yields. [d]Isolated yields. [e]HATU, DIPEA, DMF (1 mM), 30 min, rt. Reaction was stopped after complete consumption of starting material as shown by LCMS.

Finally, the efficiency of this approach was further demonstrated by cyclization tests with different sequences (Table 7). All peptides **12** were obtained without any optimization efforts with high yields and, for the case of *p*-chloro thioesters, with no (**12c**,**e**) or very low epimerization (**12a**,**d**). In the case of *p*-nitro thioester (**12b**), the result was not satisfactory. Importantly, all final cyclic peptides were

Table 7. Study of Cyclization of Thioester for Different Sequences[a]



peptide 12 13

entry	peptide **12** SR	product	time (h)	yield[b] (%)	dimer[c] (%)	dr[d]
1	**12a**, Ala-Ala-Gly-Leu-Tyr SC_6H_4-4Cl	**13a**	20	43	9	95:5
2	**12b**, Ala-Ala-Gly-Leu-Tyr SC_6H_4-4NO_2	**13a**	18	49	22	99:1
3	**12c**, Gly-Ile-Thr-Pro-Val-Ile-Phe SC_6H_4-4Cl	**13b**	24	51	<1	99:1
4	**12d**, Gly-Gly-Tyr-Pro-Ile-Leu-Ile SC_6H_4-4Cl	**13c**	24	45	<1	99:1
5[e]	**12e**, Ala-Ile-Pro-Phe-Asn-Ser-Leu SC_6H_4-4Cl	**13d**	24	75	2	98:2

[a]Reaction conditions: thioester (1 equiv), DIPEA (2 equiv) DMSO (5 mM). [b]Isolated yields. [c]Isolated cyclized dimer. [d]dr determinated by RP-UPLC-MS or chiral-HPLC analysis. [e]Solvent DMF/$CHCl_3$.

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obtained with high diastereomeric ratio, as monitored by chiral HPLC analysis (Table 7, entries 1−5).

Again, the *p*-Cl-thioester gave excellent and better results than the *p*-NO_2 in terms of dimerization (Table 7).

Heterophyllin A, a natural cyclopeptide isolated from the *roots of Pseudostellaria heterophylla*[18] (entry 3) was synthesized for the first time using our methodology based on *p*-Cl thioester with 51% of isolated yield. In addition pseudostellarin D (entry 4), a natural heptapeptide, was synthesized in 45% isolated yield.[19] This clearly demonstrated the convenience and robustness (very low epimerization and dimer formation) of our methodology based on the *p*-Cl-thioester for the synthesis of cyclic peptides such as pseudostellarin D.

In conclusion, we have demonstrated that the uncatalyzed reaction of aryl thioesters, preferably *p*-Cl derivatives, with peptides provides a far superior ligation method than many currently existing procedures. Interestingly, the *p*-Cl gave superior results compared to the *p*-NO_2 in terms of epimerization during the preparation of the linear thioester and cyclization and reduced dimerization. By utilization of this method, peptide synthesis is reduced to simple mixing of two shelf-stable components. The mild reaction conditions and stability of the activated acid is compatible with a broad range of amino acids. Because of the high selectivity and simple workup, coupling yields are good to excellent and exceed reported yields in several cases. We believe that this method can be particularly interesting for the preparation of cyclic peptides as it has been demonstrated for heterophyllin A and pseudostellarin D.

■ ASSOCIATED CONTENT

Supporting Information

Complete experimental procedures and compound characterization. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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