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COMMUNICATION

Metal-free direct amidation of peptidyl thiol esters with α-amino acid esters†

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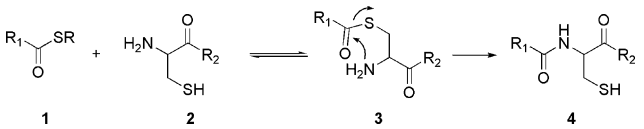
Metal-free direct amidation of peptidyl thiol esters with α-amino acid esters in the presence of bis(trimethylsilyl) acetamide (BSA) has been developed. This general method provides convenient access to *N*-protected peptides in good yields under mild conditions and demonstrates a high tolerance to functionality.

Amide bond linkage is an ubiquitous and important motif in a wide range of biological applications (*cf.* vaccines, antibodies, enzymes and factors), polymers, chemicals, as well as natural products.[1] Its importance in organic syntheses and medicinal chemistry has received enormous attention from organic chemists and encouraged the development of new synthetic pathways to construct a C–N bond. The most popular strategy for amide bond formation relies heavily upon the coupling reaction of an acid chloride or an activated carboxylic acid derivative with an amine.[2] However, due to some limitations including the liability of activated carboxylic acid derivatives and tedious procedures, alternative strategies toward the synthesis of amides have been explored for years. Transition-metal catalyzed amidation of alkenes,[3] aldoximes,[4] nitriles,[5] haloarenes,[6] alkynes,[7] alcohols using Ru-, Rh-, and Ag-based catalytic systems,[7a,8] and aldehydes using Cu, Pd, Rh, Ru, and lanthanide complexes[9] with amines, hydrative amide formation with alkynes,[10] and azides in the modified Staudinger reaction[11] have been employed for amide synthesis. However, most of these systems involve toxic solvents and expensive transition-metal catalysts. Thus, developing new procedures for the synthesis of amides is highly desirable. Peptide ligation methods have gained significant attention and have been shown to be particularly important for the synthesis of large polypeptides or mini-proteins.[12] In 1994, Kent and co-workers developed a major breakthrough method termed native chemical ligation (NCL) for the coupling of large peptidic fragments.[13] This method is outlined in Scheme 1, which allows for the use of completely unprotected peptide for the coupling. The coupling process included the intermolecular transthioesterification and intramolecular S→N acyl transfer. Nevertheless, this NCL methodology requires a cysteine residue or a cysteine-mimicking auxiliary of the C-terminal peptide segment and the rate-limiting step is S→S acyl transfer.[12d] To avoid the S→S acyl transfer, Danishefsky and co-workers reported the NCL reaction using C-terminal *ortho*-thiophenolic ester, direct oxo-ester ligation method,[14] and direct peptide synthesis from C-terminal thiol acids and N-terminal peptides in the presence of HOBT.[14i]



Scheme 1 Native chemical ligation.

The earlier research on metal aided direct conversion of thiol esters to peptides has been reported by Tam[15a] and Kurosu[15b] They have revealed that the thiol esters[15c] can be sufficiently activated by silver ion or dimethylaluminum to facilitate the peptide synthesis. It was also found that amide bonds can be formed by acyl fluorides with sterically hindered secondary amines in the presence of BSA in moderate yields.[15d,15e] Kammermeier and co-workers reported an example of the peptide synthesis through a mercaptobenzothiazole thiol ester with a primary amine in the presence of BSA in *N*-methylpyrrolidin-2-one (NMP) for the synthesis of Cefdaloxime.[15f] However, the information for metal-free direct conversion of thiol esters to peptides which is independent of the cysteine is very limited and the reaction has not been fully investigated. In this communication, we report the metal-free direct amidation of peptidyl thiol esters with α-amino acid esters in the presence of BSA in EtOH under mild conditions without any intermediacy of acyl transfer (Scheme 2).

At the onset of the research, this idea was tested by mixing two readily available substrates, *N*-Cbz-L-phenylalanine thiol ethyl ester **8b** and glycine ethyl ester **6a** (1.0 equiv.) in the presence of BSA (1.0 equiv.) under a variety of reaction conditions. When

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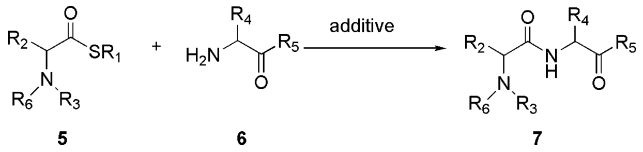
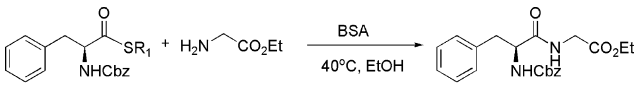
[b]*State Key Laboratory of Phytochemistry and Plant Resources in West China, Kunming Institute of Botany, Chinese Academy of Sciences, Kunming, 650204, P. R. China*
† Electronic supplementary information (ESI) available: See DOI: 10.1039/c1gc15401j



5 6 7

Scheme 2 Proposed approach for the peptide synthesis.

Table 1 Optimization and thiol esters screening for the formation of peptide **7a**[a]



8 6a 7a

Entry	R_1	Thiol ester **8**	Molar ratio (**8** : **6a**)	Equiv. of BSA	Yield (%)[b]
1	tBu	**8a**	1 : 1	1	Trace
2	Et	**8b**	1 : 1	1	35
3	*p*-Tolyl	**8c**	1 : 1	1	68
4	*p*-Tolyl	**8c**	1 : 2	1	90[c]
5	*p*-Tolyl	**8c**	1 : 2	0.5	37
6	*p*-Tolyl	**8c**	1 : 2	0	Trace

[a] All reactions performed at 0.4–0.6 mmol in EtOH (2 mL) at 40 °C for 18 h. [b] Isolated yield based on the thiol ester. [c] 22% isolated yield when the reaction was run in NMP at 40 °C for 96 h with (DL)-**8c**.

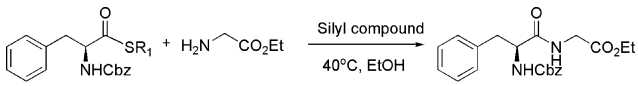
the reactions were carried out in acetonitrile, tetrahydrofuran, toluene, dichloromethane or methanol solvents at ambient temperature or 40 °C, the peptide **7a** was not observed at all. A trace amount of the product **7a** was formed in NMP solvent at 40 °C for 18 h. However, we were pleased to find that a solution of these two reactants in ethanol at 40 °C for 18 h produced the desired product **7a** in 35% yield (Table 1, entry 2).

Having concluded from the above results that the optimal reaction solvent system was ethanol, the effect of various thiol esters was further evaluated using similar reaction conditions (Table 1). Optimization studies revealed that *p*-toluene thiol ester provided the best results compared to that of ethyl thiol ester and *t*-butyl thiol ester (Table 1, entries 1–3). We found that the BSA loading is critical determinant of the reaction efficiency and increasing the BSA equiv (from 0 to 1 equiv.) can boost the yield significantly, up to 90% yield for *p*-toluene thiol ester **8c** (Table 1, entries 4–6). More importantly, the yield was improved as well with increased α-amino ester (Table 1, entry 3 *vs.* 4).

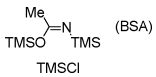
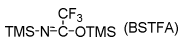
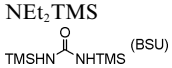
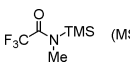
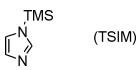
Various silyl compounds for the coupling were employed to probe the effect of the additives. The data are listed in Table 2. Pleasingly, all of the silyl compounds, regardless of TMSOTf, BSTFA, TMSCl, proceeded smoothly in moderate to good yields to afford the expected product **7a** (49–90% yield). It is worthy to note that BSA is the most promising coupling additive for *N*-Cbz-L-phenylalanine *p*-toluene thiol ester **8c** and glycine ethyl ester **6a** C–N bond formation.

With these optimized conditions in hand, we explored the scope of the BSA-mediated direct coupling of a variety of *N*-protected thiol esters with α-amino acid esters (Table 3). Generally, the amidation reaction of α-unsubstituted amino acid esters such as Gly-OEt **6a**, proceeds very well to provide the desired *N*-Ac or *N*-Cbz peptides **7a–7b**, **7d–7f**, **7k** and **7n** in 79–97% yields (Table 3, entries 1, 3–5, 10–11 and 14). An

Table 2 Optimization of various silyl compounds for the formation of peptide **7a**[a]



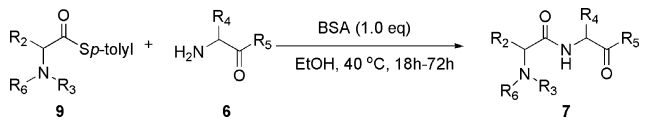
8 6a 7a

Entry	Silyl compounds	Yield (%)[b]
1	 (BSA)	90
	TMSCl	
2	TMSCl	Trace
3		Trace
4	 (BSTFA)	Trace
5	NEt_2TMS	49
6	 (BSU)	73
7	 (MSTFA)	80
8		79
9	 (TSIM)	72

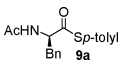
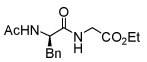
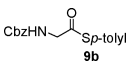
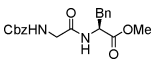
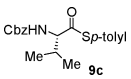
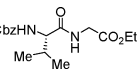
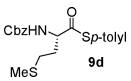
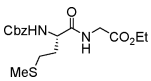
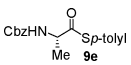
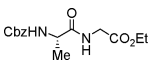
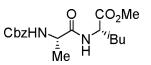
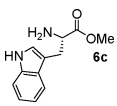
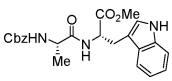
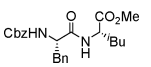
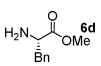
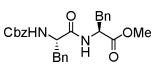
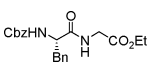
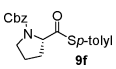
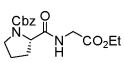
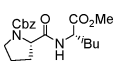
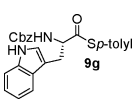
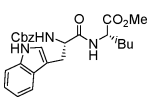
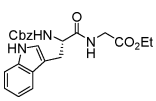
[a] All reactions performed at 0.4–0.6 mmol in EtOH (2 mL) at 40 °C for 18 h, **8c** : **6a** : silyl compound = 1 : 2 : 1 (mol/mol mol^{-1}). [b] Isolated yield based on the thiol ester.

extended reaction time (72 h) is usually required for a steric hindered *N*-Cbz protected thiol ester (Table 3, entry 3) and all the reactions can be completely converted to the desired products over times based on HPLC analysis. However, the coupling reactions of α-substituted amino acid esters are quite different and much slower compared to that of Gly-OEt **6a**. Most of them needed an extended reaction time (48–72 h) to push the reaction further, except when the coupling partner is α-unsubstituted thiol ester **9b** (78% yield, 24 h, Table 3, entry 2) and the yield was decreased due to the steric effect of the substituted group R_4. For example, the reactions of *N*-Cbz-L-alanine *p*-toluene thiol ester **9e** with L-leucine methyl ester **6b** and L-tryptophan methyl ester **6c** provided peptides **7g** and **7h** in 70% and 72% yields respectively (Table 3, entries 6–7) which are much lower than that of Gly-OEt **6a** (97% yield). When the other *N*-Cbz protected thiol ester such as **8c**, **9f–9g** was utilized as the coupling partner with the α-substituted amino acid ester, the desired peptide was obtained in a low yield, ranging from 53% to 60% (Table 3, entries 8–9, 12–13). In order to examine the extent of racemization, an enatiomeric mixture of **7a** or **7c** was synthesized in the same manner and HPLC data revealed that no detectable racemization occurred for the synthesis of L-**7a** or L-**7c** in the BSA-mediated coupling reaction. Significant racemization was observed (reduced from >99% ee to 54% ee) for the preparation of *N*-Ac protected thiol ester **9a** according to the previous DCC procedure. However, no further racemization occurred in the BSA-mediated coupling reaction in EtOH. The

Table 3 Investigation of BSA-promoted peptide bond formation[a]



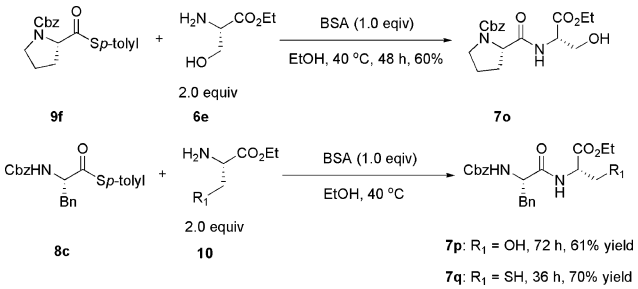
9 6 7

Entry	Thiol ester (**9**)	α-Amino Acid Ester (**6**)	Peptide (**7**)		Reaction Time (h)	Yield (%)[b]
1	 **9a**	**6a**		**7b**	24	91[c]
2	 **9b**	**6d**		**7c**	24	78[c]
3	 **9c**	**6a**		**7d**	72	79
4	 **9d**	**6a**		**7e**	72	80
5	 **9e**	**6a**		**7f**	36	97
6	**9e**	 **6b**		**7g**	48	70
7	**9e**	 **6c**		**7h**	72	72
8	**8c**	**6b**		**7i**	48	53
9	**8c**	 **6d**		**7j**	72	60
10	**8c**	**6a**		**7a**	18	90[d]
11	 **9f**	**6a**		**7k**	36	85
12	**9f**	**6b**		**7l**	48	55
13	 **9g**	**6b**		**7m**	72	57
14	**9g**	**6a**		**7n**	36	92

[a] All reactions performed at 0.4–0.6 mmol in EtOH (2 mL) at 40 °C. [b] Isolated yield based on the thiol ester. [c] **9a** (54% ee); **7b** (54% ee); **7c** (>99% ee); HPLC conditions: Lux 3 μm, Cellulose-2, 4.6 mm × 250 mm, *n*-hexane/2-propanol = 80 : 20, 206 nm, flow rate = 1.0 mL min^{-1}. [d] **7a** (>99% ee), Chiral Pak AD-H, 5 μm, 4.6 mm × 250 mm, *n*-hexane/2-propanol = 70 : 30, 230 nm, flow rate = 0.65 mL min^{-1}.

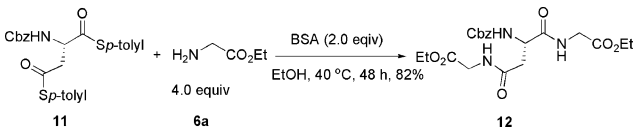
enantiomeric excess of the corresponding peptide **7b** is identical to that of the thiol ester **9a**.

Remarkably, the further screening revealed that our direct peptide synthesis procedure tolerates a wide range of functional groups. The amidation was compatible with a variety of amines containing hydroxyl group or thiol group. The reactions proceeded smoothly with or without a cysteine –SH residue and provided the desired products **7o–7q** in 60–70% yields (Scheme 3). Interestingly, when the *N*-Cbz-L-aspartic bis-*p*-toluene thiol ester **11** was applied to the amidation reaction, the



Scheme 3 Direct peptide synthesis from thiol esters with or without a cysteine –SH residue.

di-amidation reaction occurred smoothly (**11** : **6a** : BSA = 1 : 4 : 2 mol : mol : mol) in high yield (Scheme 4). Though the mechanism of the reaction is clear yet, we speculate that BSA could react with amines to form *N*-silylamine intermediates which would facilitate amide bond formation.[15d,15e]



Scheme 4 Di-amidation reaction.

In summary, we have developed a new, convenient route for the synthesis of optically pure *N*-protected peptides using a metal-free direct amidation of peptidyl thiol esters with α-amino acid esters mediated by BSA under mild conditions. This considerable promising method will be valuable for building a range of challenging peptide bond which is independent of the cysteine based NCL strategies. Further investigations regarding the mechanistic study, reaction scope and their application in pharmaceutical level discovery research will be reported in due course.

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