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Copper(II) mediated facile and ultra fast peptide synthesis in methanol†

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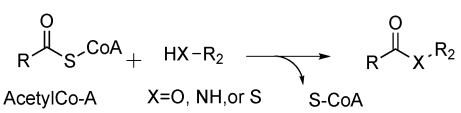
A novel, ultrafast, mild and scalable amide bond formation strategy in methanol using simple thioacids and amines is described. The mechanism suggests that the coupling reactions are initially mediated by $CuSO_4{\cdot}5H_2O$ and subsequently catalyzed by *in situ* generated copper sulfide. The pure peptides were isolated in satisfactory yields in less than 5 minutes.

Amide bond formation is one of the most important reactions in contemporary organic chemistry. Numerous strategies and reagents have been developed for the synthesis of amide–peptide bonds.[1,2] Recently, the thioacid mediated amide bond coupling reactions have been gaining momentum due to their unique reactivity and selectivity compared to those of carboxylic acids.[3] The reactivity of thioacids with azides,[4] isonitriles,[5] sulphonamides,[6] nitroso derivatives,[7] thioisocyanates,[8] isocyanates,[8] dinitrofluorobenzene[9] and aziridines[10] has been effectively utilized in the direct peptide bond formation or native chemical ligation. Herein we disclose a novel, ultrafast, mild and scalable copper sulfate mediated and subsequently copper sulfide catalyzed amide bond formation involving thioacids and amines in methanol.

Inspired by the thioester strategies in the acylation reactions of coenzyme, acetyl-CoA (Scheme 1), and in non-ribosomal peptide synthesis,[11] we anticipate that the coenzyme in the thioester is replaced with sulfur loving metal (M) which would readily react with amines to form highly stable metal sulfides (Scheme 2).

We began our search for a suitable metal salt or metal complex to mediate the amide bond synthesis using thioacid Boc-Ala-SH and benzylamine in DMF. Various metal salts and complexes including Ag(I), Au(III), Fe(II), Fe(III), Cu(I), Cu(II), Mo(IV) and Zn(II) have been screened and better yields



Scheme 1 Acetyl transfer reactions by acetyl coenzyme A through thioesters.

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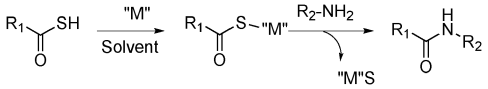
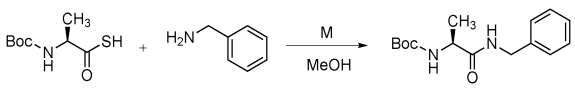


Scheme 2 Schematic representation of the metal mediated amide bond formation.

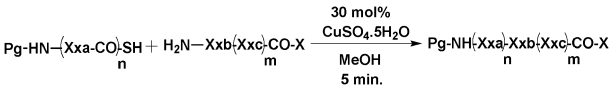
were observed in Cu(I) and Cu(II) compounds. In comparison, Cu(II) complexes $CuSO_4{\cdot}5H_2O$ and $Cu(OAc)_2{\cdot}H_2O$ gave better yields than Cu(I)I. We continued our work with $CuSO_4{\cdot}5H_2O$ as it is cheap and commonly available in any undergraduate laboratory. The slow reactivity and the longer time duration of $CuSO_4{\cdot}5H_2O$ [also $Cu(OAc)_2{\cdot}H_2O$] in both DMF and DMSO, as compared to the standard coupling reagents such as HBTU and HATU, led us to search for a solvent in which both reactants and the metal complex are soluble. As $CuSO_4{\cdot}5H_2O$ has better solubility in methanol, we carried out the same coupling reaction in methanol with a suspicion, since methanol has never been used as a common solvent in any acylation reactions. Surprisingly, the amide bond formation was observed in good yield within 5 min and no other byproduct was observed except the insoluble metal sulfide. As the metal complex is insoluble in EtOH, EtOAc and THF, we did not further pursue the amide bond synthesis in these solvents.



Scheme 3 Amide bond synthesis using thioacids. A comparison of various metal salts and complexes in the acylation reaction. Time is given in minutes.

Entry	M	Time (min)	%Yield
a	$Cu(OAc)_2$	5	57
b	$CuSO_4{\cdot}5H_2O$	5	71
c	CuI	30	41
d	$ZnCl_2$	30	14
e	AgOAc	5	38
f	$FeCl_2$	420	14
g	$FeCl_3$	420	14
h	$AuCl_3$	30	20

Encouraging results of copper sulfate mediated coupling reactions in methanol promoted us to probe the amide bond synthesis using other metal salts and complexes (Scheme 3).



Scheme 4 Synthesis of peptides using 30 mol% of $CuSO_4{\cdot}5H_2O$ in MeOH.

Table 1 List of peptides synthesized using 30 mol% of $CuSO_4{\cdot}5H_2O$

Entry	Xxa	Xxb	Xxa–Xxb	% Yield
Pg = Boc, *n* = 1, *m* = 0, X = OMe				
1	Ala	Leu	Ala-Leu	68
2	^{D}Ala	Leu	^{D}Ala-Leu	62
3	Val	Ala	Val-Ala	76
4	Ser(OBu^t)	Leu	Ser(OBu^t)-Leu	72
5	Trp	Val	Trp-Val	63
Pg = Fmoc, X = OMe				
6	Val	Leu	Val-Lue	77
7	Aib	Phe	Aib-Phe	69
8	Ile	Val	Ile-Val	70
9	Pro	Val	Pro-Val	65
Pg = Cbz, X = OMe				
10	Leu	Trp	Leu-Trp	60
n = 1, *m* = 1, Pg = Boc, X = OMe				
	Xxa	Xxb–Xxc	Xxa–Xxb–Xxc	
11	Ser(OBu^t)	Ala-Leu	Ser(OBu^t)-Ala-Leu	64
12	Ser(OBu^t)	Ala-Val	Ser(OBu^t)-Ala-Val	61
Pg = Cbz, X = OMe				
13	Leu	Val-Val	Leu-Val-Val	64
n = 2, *m* = 1, Pg = Boc, X = OMe				
14	Ala-Val	Leu-Leu	Ala-Val-Leu-Leu	74
15	Val-Leu	Val-Val	Val-Leu-Val-Val	72
Pg = Boc, X = OEt				
16	Aib-Ala	dgL-dgL	Aib-Ala-dgL-dgL	69

(dgL = α,β-unsaturated γ-leucine).

As shown in Scheme 3, $CuSO_4{\cdot}5H_2O$ gave better yield (71%) followed by $Cu(OAc)_2{\cdot}H_2O$ (57%) and AgOAc gave only 38% in 5 min. Other metal complexes gave low yields with longer time durations. In their pioneering work, Blake and Li reported the Ag(I) mediated fragment coupling reactions of peptide thioacids in 50% aqueous DMF.[12] The results observed for the Ag(I) mediated coupling reaction in methanol are in accordance with the reported results. In the optimization of the concentration of copper sulfate from 1 to 100 mol%, we found that reaction with 30 mol% of copper sulfate was comparable with that with the 100 mol%, however even 10 mol% also mediated the amide bond formation with longer time duration (3 h). In a control reaction without the metal complex, 5% of the product was isolated after 24 h. We speculate that the oxidative dimerization of thioacids in solution may result in the formation of the product.[3,13] Further, we continued peptide coupling reactions using 30 mol% of $CuSO_4{\cdot}5H_2O$ (Scheme 4). The pH measurement studies suggest that the reaction mixture was almost neutral (pH 7.76) during the amide bond coupling. The elemental analysis of the black material obtained as a byproduct in the reaction gave 31.5% of sulfur, which is nearly equivalent to the CuS. However, the MALDI-TOF analysis suggests the complex mixture of copper and sulfur (see ESI†). It is also evident from the literature that the copper sulfide may exist in the complex of $[Cu(I)–Cu(II)]_nS_x$.[14] As solubility experiments suggest that the metal sulfide is insoluble in all solvents including methanol (except con. HNO_3), we subjected it to the powder XRD analysis. Results from the X-ray analysis reveal the formation of CuS (covellite) as a byproduct in the amide bond coupling reaction. The powder XRD data of the byproduct along with the standard CuS (covellite) are shown in Fig. 1a and b, respectively.[14] Further, the UV absorption measurements of Boc-Ala-SH and H-Leu-OMe with and without copper sulfate in time course experiments suggest the complete disappearance of thiol absorption within 1 min, indicating the formation of an amide bond within a minute. In addition, to investigate the racemization during the amide bond synthesis, the two diastereomeric dipeptides **1** and **2** (Table 1) were synthesized using Boc-Ala-SH, and Boc-^{D}Ala-SH along with the racemic mixture of Boc-(±)-Ala-SH. The chiral HPLC results indicate that no racemization occurred during the amide bond formation (see ESI†). Additionally, it has also been reported that the presence of Cu(II) complexes reduces the racemization during the peptide coupling reactions.[15] Further, to understand the compatibility of this protocol with other *N*-terminal protecting groups, a series of *N*-protected thioacids were synthesized and subjected to coupling reactions in methanol using 30 mol% of copper sulfate. The list of dipeptides (**1–10**) synthesized from this protocol is given in Table 1. All *N*-protected thioacids were synthesized using the reported procedure.[16] Instructively, no methyl esters or free carboxylic acids of the corresponding thioacids were observed in the acylation reactions. Out of all the thioacids, we were able to obtain single crystals of Boc-Leu-SH and its X-ray structure is shown in the ESI.† All dipeptides were isolated in satisfactory yields including sterically hindered amino acids, Val(**3**, **6**), Aib(**7**) and Ile(**8**), in less than 5 min. Though the reaction proceeds with the coupling efficiencies more than 90% (HPLC analysis), the isolated yields after the column purification are given in Table 1. Inspired by the clean, efficient and fast amide bond coupling reaction, we further extended this strategy to synthesize tri and tetrapeptides. The dipeptide amine **1** was coupled to the Boc-Ser(OBu^t)-SH, as anticipated the tripeptide **11** was isolated in good yield in less than 5 min. Similarly, other tripeptides **12** and **13** were isolated with satisfactory yields. The tetrapeptides (**14–16**) were synthesized using a 2 + 2 convergent strategy. The corresponding dipeptide thioacids were synthesized similar to the *N*-protected thioacids and subjected to the coupling reactions with dipeptide amines. Peptide **15** with highly β-branched valine residues was isolated in satisfactory yield within 5 min. To ensure the compatibility of the thioacid coupling reactions in the presence of electron

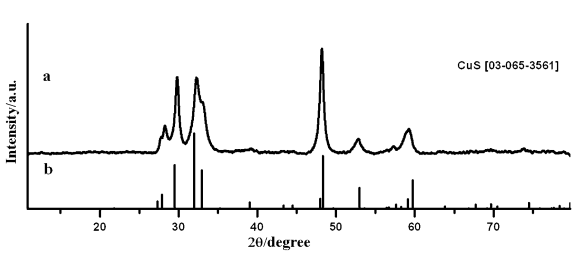
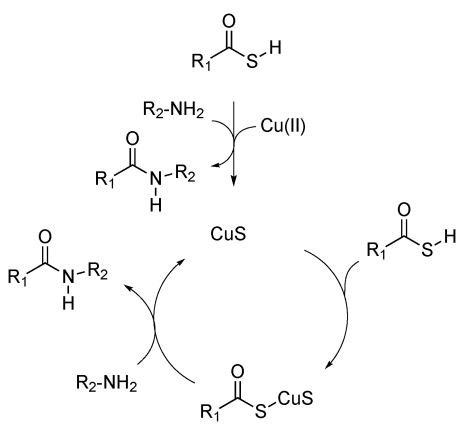


Fig. 1 Powder XRD pattern of the byproduct CuS (covellite).

deficient unsaturated amides and esters,[17] which are subjected to Michael addition reaction,[18] the tetrapeptide **16** was synthesized. Instructively, the tetrapeptide was isolated without any side products. All tetrapeptides were isolated with satisfactory yields within 5 min and are given in Table 1.

In hindsight, the comparable results of 30 mol% of copper sulfate with that of 100 mol% provoked us to investigate the role of insoluble byproduct CuS in the coupling reactions. To understand whether or not CuS is involved in the amide bond formation, a control reaction with Boc-Ala-SH and benzylamine was carried out in the presence of 30 mol% of CuS. Surprisingly, the formation of an amide bond was observed with the same rate as that of copper sulfate. To verify its compatibility, we further synthesized dipeptides **2**, **3**, **10**, tripeptide **13** and the tetrapeptide **14** (Table 1). We found no difference between the insoluble CuS and the soluble $CuSO_4$. Further, we carried out the amide bond formation using Boc-Ala-SH and H-Leu-OMe in neat methanol after filtering the CuS to verify whether or not the CuS treated methanol will accelerate reaction. Results suggest that there was no acceleration of amide bond formation even after 24 h, indicating the need of CuS to accelerate the reaction. Albeit it is contradicting to our initial assumption regarding the solubility of copper sulfate, the serendipity provides insight into the role of metal sulfides as catalysts in the peptide bond formation using thioacids.[19] In contrast to the model experiments on the fixation of the carbon monoxide and the activation of thioacetic acid on metal sulfides under primordial conditions,[19] we observed only amide bond formation in the CuS catalyzed reaction. Based on these observations, we propose the possible mechanism for the amide bond synthesis (Scheme 5). We anticipate that the initial step proceeds with direct involvement of the copper complex ($CuSO_4{\cdot}5H_2O$) in the reaction leading to the formation of CuS and the amide bond. Subsequently, the *in situ* generated CuS acts as a catalyst to activate the thioacid through coordination. The reaction between the activated intermediate and the free amine leads to the formation of an amide bond along with the regeneration of CuS (Scheme 5). Though the LC-MS analysis suggests the presence of a mixture of elemental sulphur (S_7, S_{18} *etc.*) in the reaction mixture, the detailed mechanism of the liberation of sulfur in the reaction needs to be investigated.



Scheme 5 Proposed mechanism for the $CuSO_4{\cdot}5H_2O$ mediated and CuS catalyzed amide bond synthesis.

In conclusion, we have successfully demonstrated a novel, ultrafast, scalable amide bond synthesis in methanol with a range of amides and peptides. The coupling products of sterically hindered amino acids, tri and tetrapeptides were isolated in less than few minutes. This protocol can be utilized for the synthesis of synthetically challenging membrane peptides and peptides containing sterically hindered amino acids.

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