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A traceless approach to amide and peptide construction from thioacids and dithiocarbamate-terminal amines†

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A novel and traceless strategy has been devised that allows a coupling of thioacids and dithiocarbamate-terminal amines. This strategy had been assumed to be dependent on the attachment of a functional equivalent of a cysteine side chain in earlier native chemical ligation approaches. This approach enables the traceless removal of CS_2 to directly generate the desired amide bond and is compatible with a range of unprotected side chains of amino acid. The ability to produce amide or peptides by a traceless removal of the auxiliary is a significant virtue of the method. Meanwhile, the application of this new peptide-bond-forming reaction to the synthesis of novel endomorphin (EM) derivatives with various binding potencies was realized.

Introduction

The amide bond is one of the most prevalent structural elements which appears in medicine and materials, especially proteins, conjugates and small molecule pharmaceuticals.[1] In recent years, many powerful methods of amide synthesis have appeared in the literature, such as conventional amide synthesis *via* coupling reagents,[2] Staudinger ligation,[3] the amidation of thioacids with azides,[4] hydrative amide synthesis through alkyne–azide coupling,[5] oxidative amidation of alcohols,[6] aldehydes,[7] or alkynes,[8] ketoacid–hydroxylamine ligation,[9] amide synthesis by isocyanate/isothiocyanate–thioacid coupling,[10] activated thioacid-mediated coupling,[11] amide formation by isonitrile intermediates[12a,b] and acyl disulfide-mediated ligation.[12c] One of the very best methods to demonstrate efficacy is peptide ligation,[13] that is polypeptide synthesis by segment coupling in buffered aqueous solutions of neutral pH without epimerization. While considerable progress has been made in this area over the past years, there is still certainly room for improvement.

In this article, we describe a traceless and convenient route to the preparation of amides and peptides. This amide synthesis uses thioacids as the acyl donor, but explores a new chemical template for the amine feedstock through the implementation of dithiocarbamates. This design is shown in Fig. 1. The dithiocarbamate-terminal amine or peptide (**1-B**) was obtained by reacting the desired amine or peptide (**1-A**) with CS_2. Thereafter, thioacid (**1-C**) reacts with dithiocarbamate (**1-B**) to afford an amide bond (**1-D**) by a traceless loss of a simple, volatile byproduct, carbon disulfide.[10a] The central feature of this strategy represents a novel, successful and traceless approach for amide and peptide synthesis. Additionally, we believe that this is the first example of the incorporation of the dithiocarbamate template into amide formation.

Results and discussion

This design was guided by the knowledge that thioacids are poorly reactive with amines to form amides.[14] We found that treatment of benzylamine in MeOH followed by addition of thiobenzoic acid gave the amide product (<10%) after 24 h in N_2 atmosphere at room temperature. Our study of the amide bond-forming reaction began with the model reaction of the thiobenzoic acid and potassium of *N*-benzyl dithiocarbamate[15] in MeOH. We are pleased to find that the conversion (>95%) and isolated yield (90%) were improved substantially after 8 h. This observation described above led to the hypothesis that dithiocarbamate might be the key one in the desired transformation.

A standard experimental protocol was developed in order to ascertain an initial scope for the reaction. These conditions included thioacid (2.0 equiv.) and dithiocarbamate salt (1.0 equiv.) in MeOH, operating at room temperature for a standard time. Encouraged by the initial result, we set out to explore the thioacid–dithiocarbamate coupling in greater details (Table 1). The thioacids were either commercially available or prepared using sodium sulfide and carbonyldiimidazole (CDI) and used without further purification.[16] Amines bearing

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‡ These authors contributed equally.

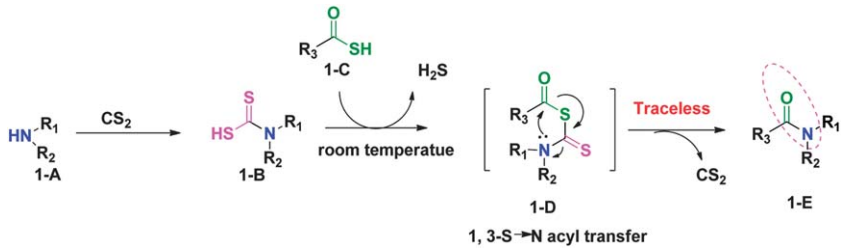
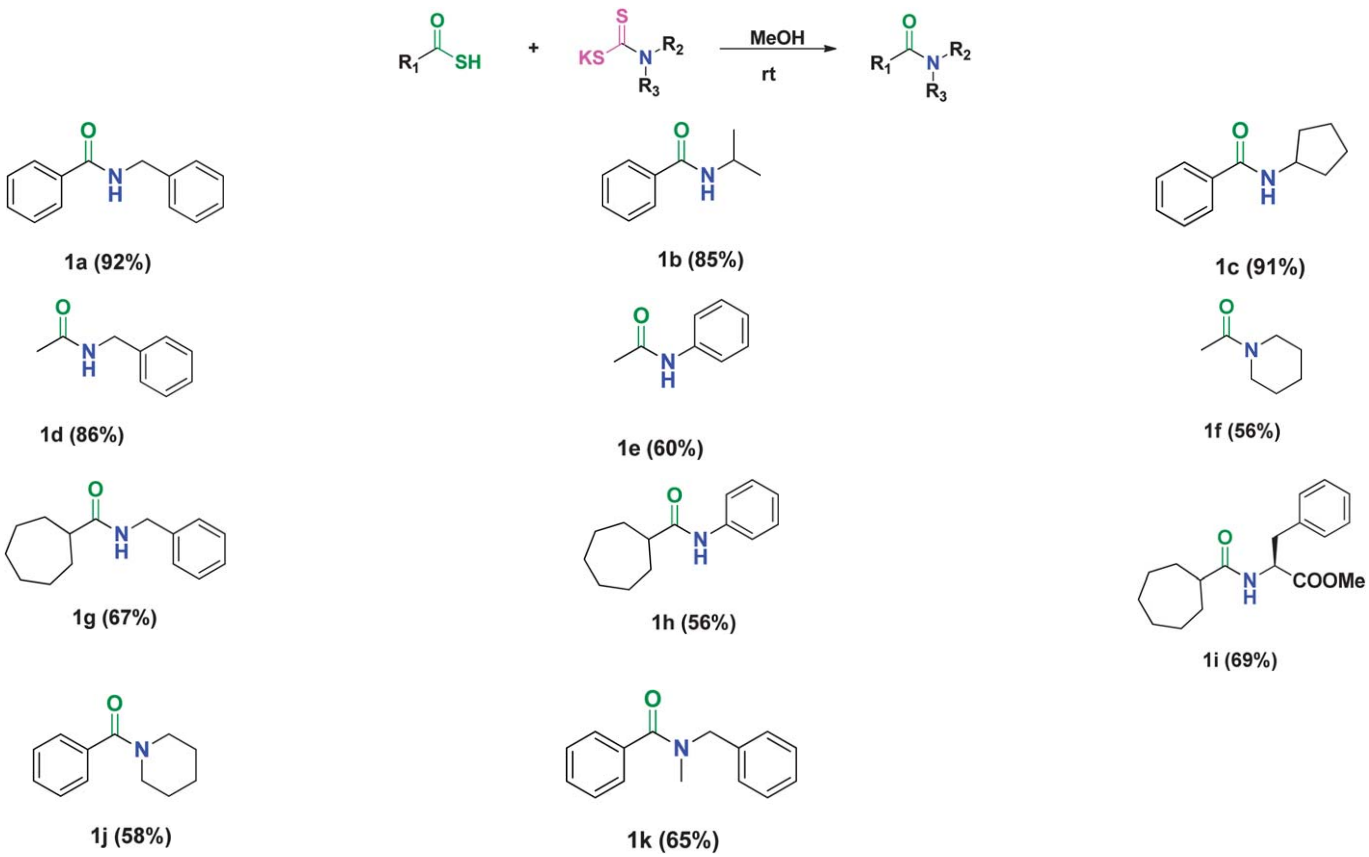


Fig. 1 Amide and peptide synthesis by thioacid–dithiocarbamate coupling.

Table 1 Substrate scope of amide formation[a]



[a] The reaction conditions: dithiocarbamate salt (1.0 equiv.), thioacid (2.0 equiv.) in MeOH for 8–17 h at room temperature.

aliphatic and aromatic substituents performed well, delivering the desired amide in good to excellent yield (Table 1). Amines with increasing steric hindrance provided comparable levels of efficiency (Table 1, **1b** and **1c**). Piperidine and *N*-methyl benzylamine were evaluated as representative secondary amines, as these are often more difficult substrates for dehydrative amide synthesis. This protocol provided the desired tertiary amide **1f** (56%), **1j** (58%) and **1k** (65%). **1g**, **1h** and **1i** of Table 1 established the viability of the method and nicely illustrated the potential for application to a hindered thioacid. The formation of anilides **1e** and **1h**, notwithstanding the relatively weak reactivity of aniline nucleophiles, was encouraging. Amide formation using phenylalanine ethyl ester provided a promising indication that applications in peptide synthesis might be possible. (Table 1, **1i**) The ability to produce such products under mild conditions is a strong virtue of the method.

Attempts to extend this methodology to the construction of peptides were not initially encouraging (Fig. 2). It was found that dithiocarbamate-terminal peptide (**A**) formation underwent conversion to their corresponding 2-thioxoimidazolidin-4-ones (**C**) when KOH was utilized as the base during the preparation process. The corresponding 2-thioxoimidazolidin-4-ones (**C**) cannot undergo a coupling reaction with the peptide thioacids. Accordingly, we investigated the optimal conditions for the formation of dithiocarbamates (Table 2). An array of bases was assessed for their ability to prevent this side reaction. Importantly, pyridine was found to significantly favor the reaction and was superior to other bases,

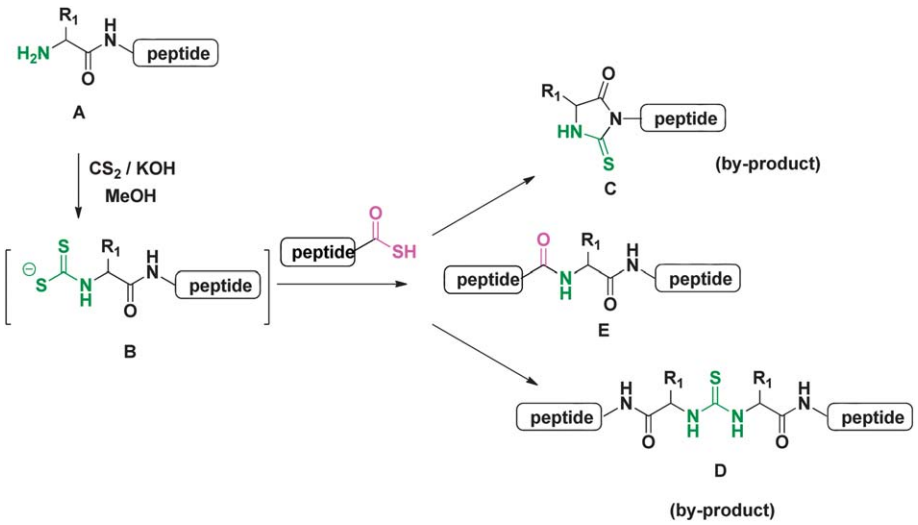
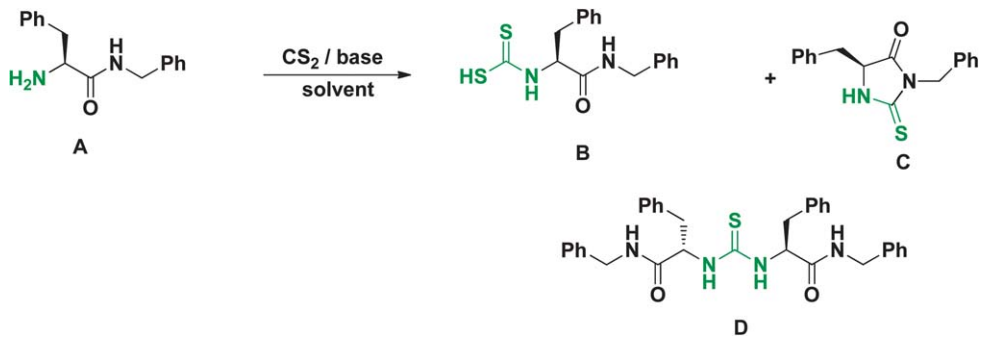


Fig. 2 Challenge: thioacid–dithiocarbamate coupling in peptide synthesis.

Table 2 Screening the optimal condition for the formation of dithiocarbamates[a]



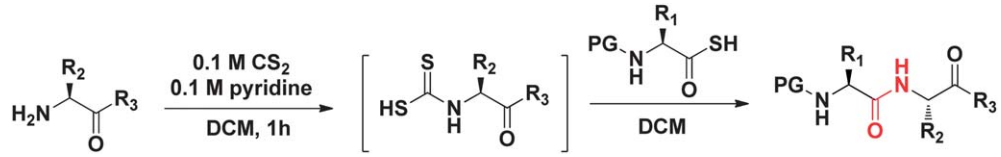
Entry	Base	B[b]%	C[b]%	D[b]%
1[c]	KOH	10	>85	Trace
2	Et_3N	10	>85	Trace
3	Imidazole	40	20	40
4	Pyridine	88	6	6

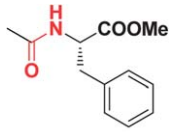
[a] **A** (1.0 mmol), 0.1 M CS_2 and 0.1 M base were stirred in DCM (2.0 ml) in ice-bath. [b] The conversion of **B**, **C** and **D** was monitored by HPLC. [c] Entry 1 was carried out in MeOH–H_2O (10 : 1).

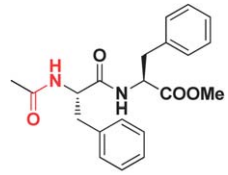
including KOH, Et_3N and imidazole. Our premise that the base would be an important factor was validated by the yield of the dithiocarbamates. The data (in Table 2) indicates that pyridine favors the formation of dithiocarbamates with limited side reactions.

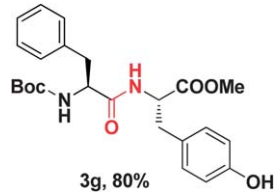
With these conditions in hand, a more detailed study of the peptide synthesis was undertaken. A series of thioesters was prepared by the coupling of suitably protected amino acids with 9-fluorenylmethanethiol,[17] or triphenylmethanethiol.[10a] The thioacids were released with piperidine or trifluoroacetic acid and triethylsilane, depending on which thioesters were employed. The corresponding dithiocarbamates were formed *in situ* from the amino ester in the presence of pyridine and CS_2.[18] The first coupling was investigated by using thioacetic acid with L-Phe–L-Phe–COOMe derived dithiocarbamate. The acetamide was obtained in excellent yield (88%, Table 3, **3d**). A series of substrates were then assessed in DCM (Table 3). As shown in Table 3, C-terminal Phe, Aib, Gln and Val residue derived thioacids were readily accommodated (Table 3, **3d–3p**). Importantly, even sterically hindered peptide-derived thioacids such as Val and Aib could be coupled effectively in good to excellent yields under these conditions. Moreover, coupling of a range of hindered amino acid derived dithiocarbamates, including Phe, Trp, Val, Ile, Tyr and His, were efficiently prepared (Table 3, **3a–3p**). The experimental results in Table 3 confirm the compatibility of this methodology with functionalities, such as phenolic, indole, and imidazole (Table 3, **3g**, **3h**, **3l** and **3n**). The question of N-terminal epimerization during the course of

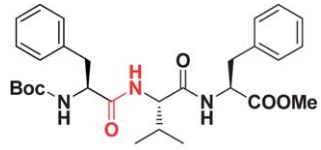
Table 3 Substrate scope of peptide synthesis[a]

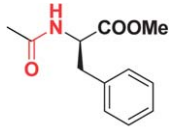


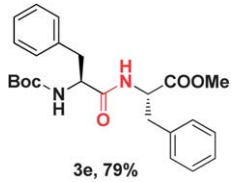

3a, 93%

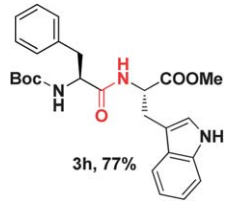

3d, 88%

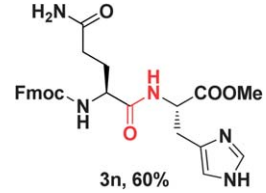

3g, 80%

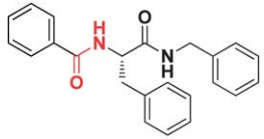

3j, 71%

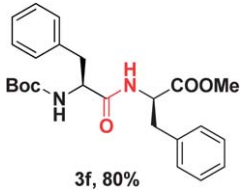

3b, 94%

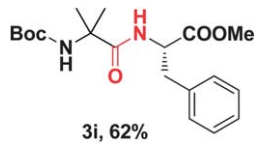

3e, 79%

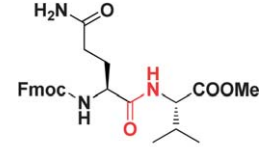

3h, 77%

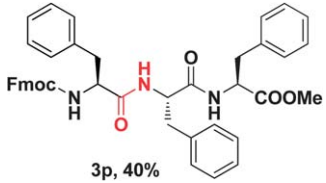

3n, 60%

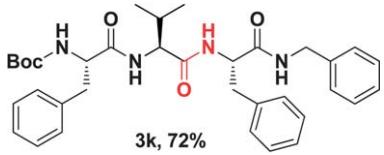

3c, 92%

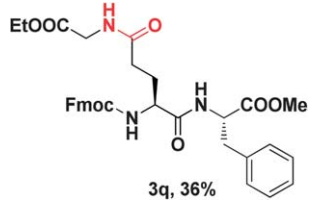

3f, 80%

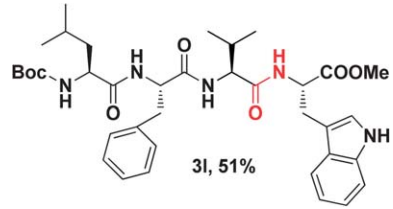

3i, 62%

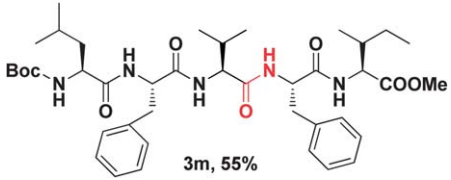

30, 35%

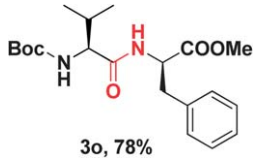

3p, 40%

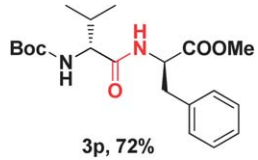

3k, 72%


3q, 36%


3l, 51%

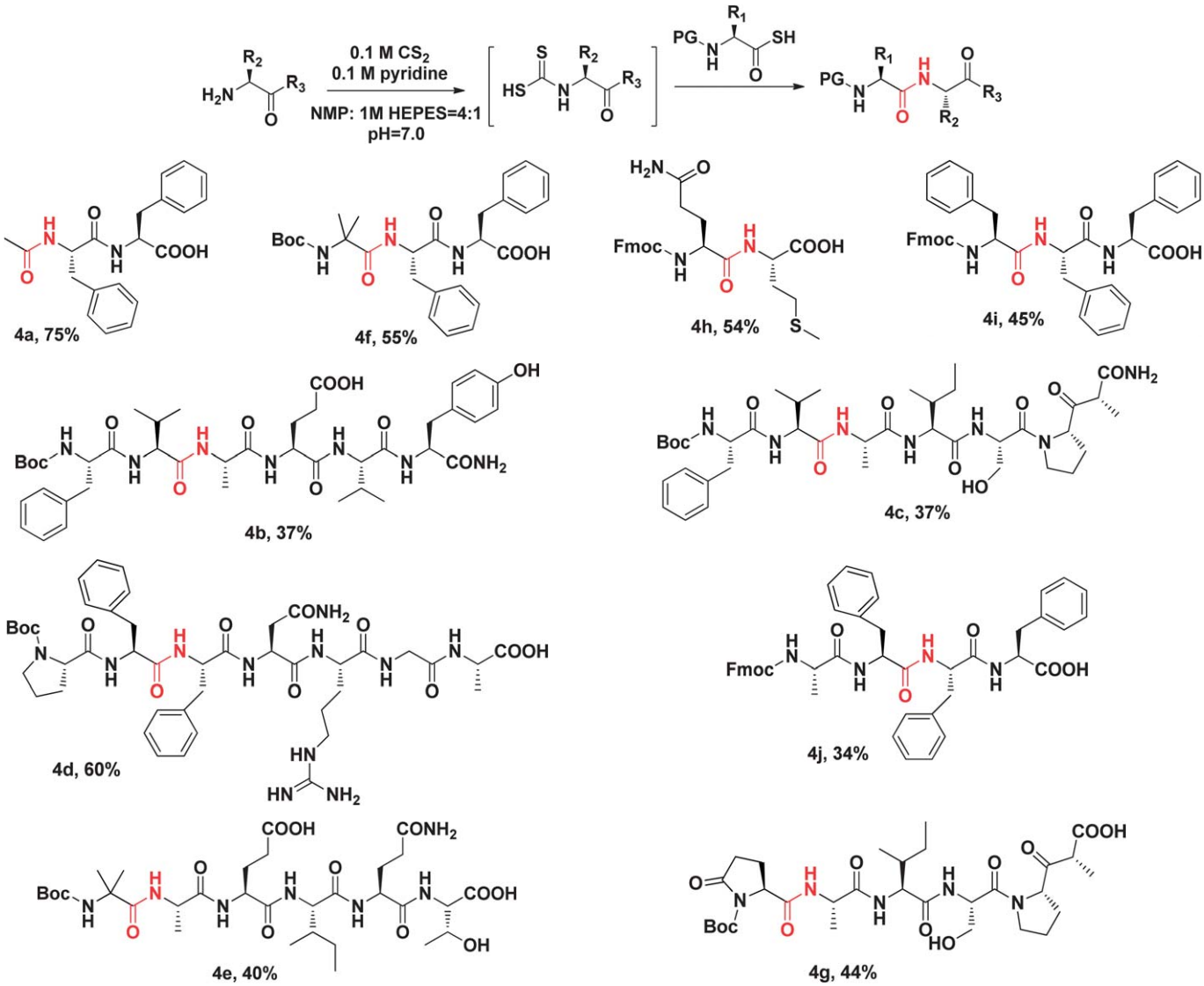

3m, 55%


3o, 78%


3p, 72%

[a] General procedure: 1.0 equiv. of amino ester, 0.1 M CS_2 and 0.1 M pyridine were stirred at room temperature in DCM for 1 h and then 2.0 equiv. of thioacid was added. The reaction mixture was stirred for 24 h to 48 h.

Table 4 Substrate scope of peptide synthesis in aqueous media[a]



[a] General procedure: 1.0 equiv. of amino acid, 0.1 M CS_2 and 0.1 M pyridine were stirred at room temperature in NMP:1 M HEPES buffer (V/V = 4 : 1, pH = 7.0) and then 2.0 equiv. of thioacid was added. The reaction mixture was stirred for 48 h.

coupling was also addressed here. **3a** and **3b**, **3e** and **3f** of Table 3 verified the absence of epimerization.[19] We next addressed the question of C-terminal epimerization during the course of ligation. The preparation of the L,D and D,D isomer of Boc–Val–Phe–COOMe (**3o** and **3p** in Table 3) verified the absence of racemization, as also determined by LC-MS.[19] Furthermore, this methodology was also applicable for the coupling of short peptide fragments (Table 3, **3k**, **3l** and **3m**). We further explored the efficiency of our reaction in the presence of an internal glutamic thioacid (Table 3, **3q**) as well as an unprotected glutamine-derived thioacid (Table 3, **3n and 3o**). As expected, the reaction proceeded smoothly to give the desired products in moderate yields.

To further extend the scope of this reaction, we have also applied it in buffered aqueous media for the synthesis of peptides with a carboxylic acid at the C-terminal (Table 4).[18] A series of peptides were smoothly prepared in a mixed organic and buffer system with acceptable yields (Table 4, **4a–4j**). As the same in Table 3, the influence of functionalities and steric effects are shown. Similarly, the hindered thioacids (Table 4, **4b**, **4c**, **4e–4g**) coupled with dithiocarbamate-terminal peptides under mild conditions. Meanwhile, we also demonstrated the application of this approach to peptide fragment synthesis, such as tripeptides (Table 4, **4f** and **4i**), tetrapeptides (Table 4, **4j**), hexapeptides (Table 4, **4b**, **4e** and **4g**), and heptapeptides (Table 4, **4c** and **4d**). In fact, some of the fragments are terminated by the incorporation of a further acid, which enable the "left to right" peptide synthesis strategy.

Finally, we applied this new peptide-bond-forming reaction to the synthesis of a novel endomorphin (EM) derivative (**5a**)

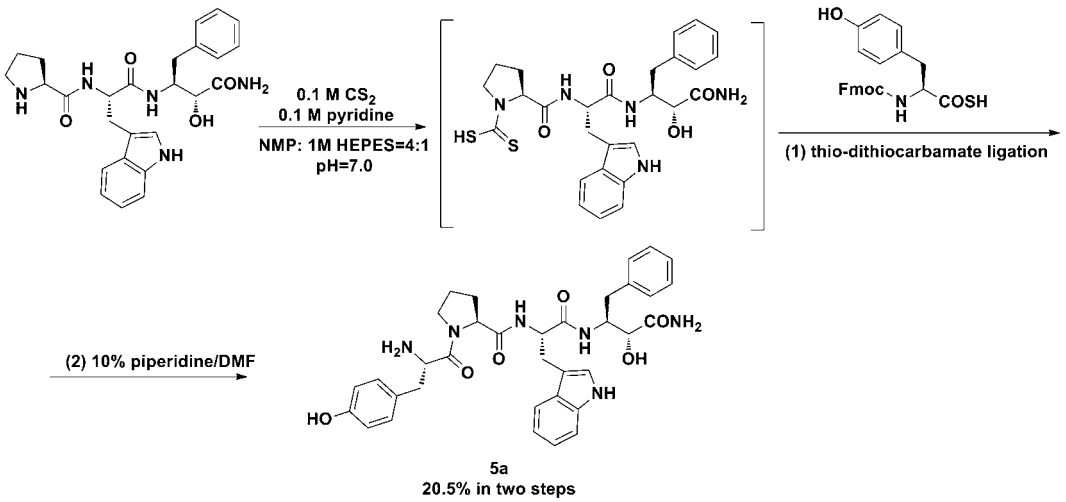


Fig. 3 Synthesis of endomorphin derivative **5a**.

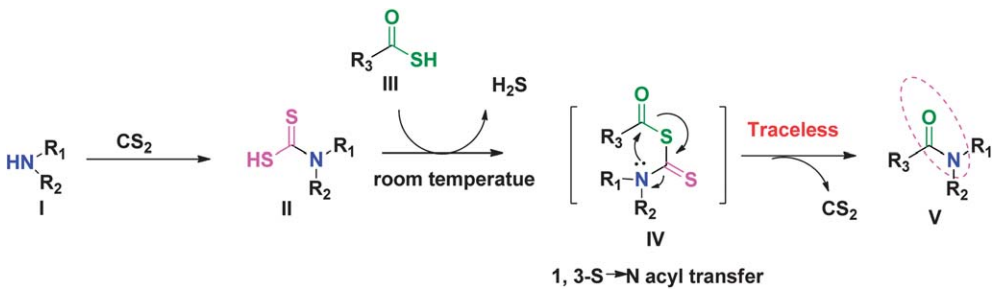


Fig. 4 Proposed mechanism.

(Fig. 3). This tetrapeptide, containing an α-hydroxyl-β-phenylalanine in the sequence, with the binding affinities for the μ and κ opioid receptor are 10.37 nM and 2087 nM, respectively (unpublished work). The unprotected residues of the both coupling fragments can be tolerated to this reaction condition.

The proposed mechanism of the reaction is shown in Fig. 4. The amine (**I**) first reacts with CS_2 to form the corresponding dithiocarbamate (**II**). Then the dithiocarbamate (**II**) is reacted with thioacid (**III**) to give the intermediate (**IV**).[20] Next, this intermediate undergoes a spontaneous intramolecular rearrangement (1,3-S → *N*-acyl transfer) with loss of CS_2 to form a desired amide bond. The target product (**V**) is obtained in the desired final form without further manipulation.

In summary, we have demonstrated a novel and straightforward amide and peptide synthesis approach. The success of this strategy had been assumed to be dependent on the attachment of a functional equivalent of a cysteine side chain in earlier native chemical ligation approaches. The ability to produce amides or peptides by a traceless removal of the auxiliary is a significant virtue of our described method. Simple and complex amide products can be obtained in moderate to excellent yields with this methodology. Furthermore, this method is tolerant to a range of naturally occurring amino acid side chains, except lysine and cysteine, which must be protected.[21] This methodology holds considerable promise for a supplement to current methods for peptide synthesis.

Acknowledgements

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19 NMR spectra and chiral HPLC spectra of **3a** and **3b** of Table 3 are listed in ESI.† LC-MS spectra of **3e** and **3f**, **3o** and **3p** in Table 3 are listed in ESI†.

20 Caution: H_2S is highly toxic and the reaction must be performed in a well-ventilated fume hood.

21 From a consideration of the reactivity of amine nucleophiles toward CS_2, the lysine and cysteine residues in a sequence must be protected when the ligation reaction is performed.

