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Oxidative peptide bond formation of glycine–amino acid using 2-(aminomethyl)malononitrile as a glycine unit†

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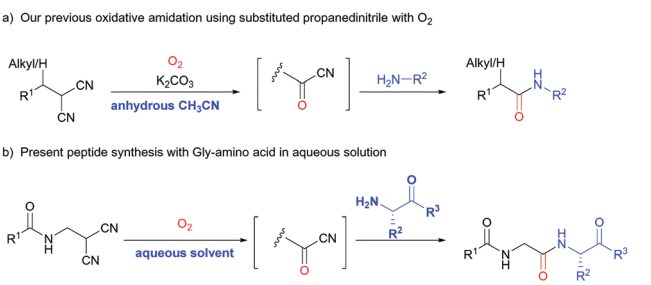
Amide linkage of glycine–amino acid was synthesized by coupling of substituted 2-(aminomethyl)malononitrile as a C-terminal glycine unit and N-terminal amine using CsOAc and O_2 in an aqueous solution. This is a coupling reagent-free and catalyst-free peptide synthesis *via* oxidative amide bond formation. Various tripeptides and tetrapeptides were synthesized efficiently and the sulfide moiety is inert even under an oxygen atmosphere.

Peptides and proteins are essential components in almost all living organisms and play various central functions.[1] Their abundant natural occurrence and unparalleled biological and medicinal significance have made them a vital research topic in both biological and chemical research fields.[2] The chemical synthesis represents the most reliable method to provide the desired peptides and proteins in both reasonable quantity and purity compared to the other two main pathways that are natural isolation and recombinant expressions in microorganisms.[3] There are many methods for amide bond synthesis.[4] The direct amide formation between C-terminal carboxylic acid and N-terminal amine amino acids and peptides is well utilized by employing coupling reagents,[5] and recently excellent catalytic methods have been reported.[6] Other methods such as native chemical ligation,[7] Staudinger ligation,[8] and ketoacid–hydroxylamine ligation[9] have been developed. The amide bond formation from aldehyde or alcohol with an amine in the presence of a catalyst is also reported.[10] Our group[11] and Johnston's group[12] have reported oxidative amide bond formation recently. An ideal synthetic method for peptides requires no coupling reagents, catalysts, and toxic metals, often in aqueous solution and a simple work-up, which has been a long-standing goal.[13] Therefore, a new simple coupling methodology for peptide synthesis is still in great demand.

We have previously developed a protocol for the oxidative synthesis of amide from α-substituted propanedinitrile and amine in the presence of O_2 and K_2CO_3 in anhydrous CH_3CN (Scheme 1a).[11c] This method accommodates a broad range of both steric hindered propanedinitrile and amine in moderate to excellent yield. The only reagents needed are K_2CO_3 and oxygen with the generation of KCN as a byproduct. An anhydrous condition was employed in order to suppress the undesired hydrolysis of acyl cyanide intermediate in the reaction of sterically hindered substrates. If protected amino acids and peptides can be employed as a nucleophile and a substituted 2-(aminomethyl)malononitrile can be employed as an electrophile, it would be a new method for the synthesis of an amide linkage between glycine and amino acid with the generation of the elongated peptides, without coupling reagent and with minimum waste generation (Scheme 1b). As amino acids and peptides are soluble in an aqueous solvent, the challenge in the present reaction is whether it can proceed efficiently in such an aqueous solvent. In this paper, we will describe the realization of this scenario.

We began our initial investigation by optimizing the reaction conditions using Boc-protected L-phenylalanine substituted propanedinitrile **1a** and L-phenylalanine methyl ester **2a**



Scheme 1 Previous amide bond formation in our group and present peptide synthesis.

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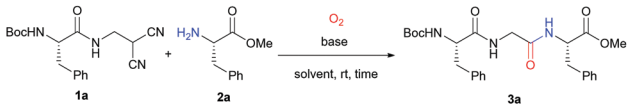
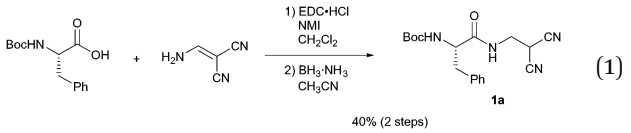
Table 1 The effect of base and solvent in the reaction of **1a** and **2a**[a]



Entry	Base	Solvent	Time [h]	Yield[b] [%]
1	K_2CO_3	DMF/H_2O = 9/1	17	<5
2	Cs_2CO_3	DMF/H_2O = 9/1	18	<5
3	KOAc	DMF/H_2O = 9/1	15	52
4	CsOAc	DMF/H_2O = 9/1	12	62
5	CsOAc	DMF/H_2O = 6/1	12	56
6	CsOAc	DMF/H_2O = 3/1	24	42
7[c]	CsOAc	DMF/H_2O = 6/1	4	47
8	CsOAc	CH_3CN/H_2O = 6/1	20	<5
9	CsOAc	DMSO/H_2O = 6/1	12	54

[a] Unless otherwise shown, the reaction was performed by employing **1a** (0.2 mmol), **2a** (0.4 mmol), base (0.4 mmol), and solvent (total volume = 2 mL) under O_2 atmosphere at room temperature at the indicated time. [b] Isolated yield. [c] The reaction temperature is 40 °C.

en-route to a Boc-Phe-Gly-Phe-OMe tripeptide **3a** (Table 1). The starting substituted propanedinitrile **1a** was easily prepared from Boc-protected L-phenylalanine and aminomethylene propanedinitrile[14] by the coupling reaction, followed by the reduction with $BH_3{\cdot}NH_3$ (eqn (1)).



(1)

Previous oxidative amide bond formation was conducted under anhydrous conditions, and in some cases in the presence of MS4Å to remove water completely.[11c] However, as the solubility of protected amino acids and peptides toward an organic solvent is generally poor, the aqueous solvent has to be employed. We chose a solvent system of DMF/H_2O = 9/1 in the initial investigation. Although K_2CO_3 and Cs_2CO_3 are suitable bases under previous anhydrous conditions, a complex mixture was obtained (entries 1 and 2). We were happy to find that the reaction proceeded even under aqueous conditions to afford product **3a** in 52% yield when KOAc was employed as a base (entry 3). The yield was further improved to 62% in the presence of CsOAc (entry 4). Next, we screened the amount of water using CsOAc as a base. A mixture of DMF and H_2O (6/1) also gave an acceptable yield, but the yield decreased when the amount of water increased such as DMF/H_2O (3/1) (entry 5 and 6). DMSO gave a similar result with DMF (entry 9), but CH_3CN was found to be a poor solvent although it was the best solvent under anhydrous conditions (*cf.* entry 8 and ref. 11*c*). Therefore, the optimal reaction conditions were found to use CsOAc as a base in a solvent of a mixture of DMF and H_2O at room temperature. We selected the ratio (6/1) of DMF and H_2O considering the better solubility of other amino acids.

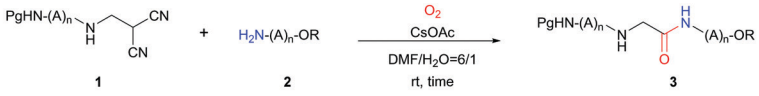
With the optimal conditions in hand, the generality of the reaction was investigated (Table 2). A wide range of tripeptides was synthesized by applying various N-terminal free amines of amino acid esters with acceptable yields (Table 2a). Not only Boc-protected L-phenylalanine substituted propanedinitrile **1a** but also Cbz-protected leucine substituted and Boc-protected serine benzyl ether substituted propanedinitriles are suitable C-terminal glycine units. Suitable nucleophilic amino acids are Phe-OMe, Phe-O*t*-Bu (Bu = butyl), Tyr-OMe, Leu-OMe, Ile-OMe, Met-OMe, Ser(O*t*-Bu)-O*t*-Bu, Thr(O*t*-Bu)-O*t*-Bu, Glu(OMe)-OMe. Trp-OMe, Cys(STr)-OMe (Tr = trityl), Lys(NHBoc)-OMe, His(NTr)-OMe and Arg(Pbf)-OMe.[15] Thus, the tripeptides such as Boc-Phe-Gly-Phe-OMe (**3a**), Boc-Phe-Gly-Phe-O*t*-Bu (**3b**), Boc-Phe-Gly-Tyr-OMe (**3c**), Boc-Phe-Gly-Leu-OMe (**3d**), Boc-Phe-Gly-Ile-OMe (**3e**), Boc-Phe-Gly-Met-OMe (**3f**), Boc-Phe-Gly-Ser(O*t*-Bu)-O*t*-Bu (**3g**), Boc-Phe-Gly-Thr(O*t*-Bu)-O*t*-Bu (**3h**), Boc-Phe-Gly-Glu(OMe)-OMe (**3i**), Boc-Phe-Gly-Trp-OMe (**3j**), Boc-Phe-Gly-Cys(STr)-OMe (**3k**), Boc-Phe-Gly-Lys(NHBoc)-OMe (**3l**), Boc-Phe-Gly-His(NTr)-OMe (**3m**), Boc-Phe-Gly-Arg(Pbf)-OMe (**3n**), Cbz-Leu-Gly-Phe-OMe (**3o**), Cbz-Leu-Gly-Met-OMe (**3p**) and Cbz-Leu-Gly-Trp-OMe (**3q**) and Boc-Ser(OBn)-Gly-Lys(NHBoc)-OMe (**3r**) were synthesized in good yield, in which Gly–amino acid bonds were constructed oxidatively. Moreover, tetrapeptides such as Boc-Phe-Gly-Leu-Phe-O*t*-Bu (**3s**), Cbz-Leu-Gly-Leu-Phe-O*t*-Bu (**3t**), Boc-Phe-Leu-Gly-Phe-OMe (**3u**), Boc-Phe-Leu-Gly-Ile-OMe (**3v**), and Boc-Phe-Leu-Gly-Tyr-OMe (**3w**) were also synthesized in a moderate yield, in which Gly–amino acid bonds were prepared (Table 2b).

It should be noted that there is no necessity to protect the phenol moiety of tyrosine (**3c**, **3w**), and the reaction is compatible with methyl sulfide of methionine (**3f**, **3p**) even though the reaction was conducted under an oxygen atmosphere. Indole of tryptophan (**3j**, **3q**) and ester moiety of glutamic methyl ester (**3i**) did not disturb the reaction. Protected functionalized amino acids such as cysteine (**3k**), lysine (**3l**, **3r**), histidine (**3m**), and arginine (**3n**) can be successfully employed. As all the substrates are soluble in an aqueous solvent and aqueous condition did not affect the reaction, the reaction proceeded efficiently to afford elongated peptides in a good yield. More important, unprotected leucine (**3y**), phenylalanine (**3x**, **3aa**) and proline (**3z**) were successfully coupled demonstrating the orthogonality of the current method with condensative peptide couplings (Table 2c).

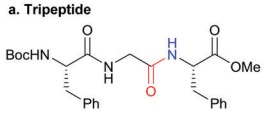
Although the amide bond formation using 2-(aminomethyl)-malononitrile as a glycine unit was successful, we do not have a good result in the reaction using 2-substituted 2-(aminomethyl)malononitrile as the other amino acid surrogate because of the racemization.

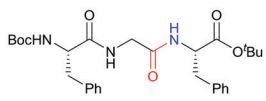
In conclusion, an oxidative synthetic method of the peptide bond of glycine–amino acid has been developed under an O_2 atmosphere in the presence of CsOAc in an aqueous solution without coupling reagents and catalyst. Substituted 2-(aminomethyl)malononitrile acts as a glycine unit to react with a wide variety of amino acids to afford tripeptides and tetrapeptides under mild reaction conditions. Although the present method is limited to the generation of an amide linkage between glycine and other amino acids, it offers an

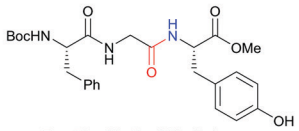
Table 2 Oxidative peptide synthesis[a]

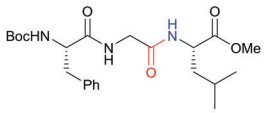


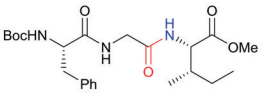
a. Tripeptide

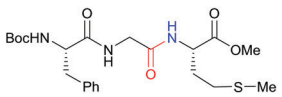

Boc-Phe-Gly-Phe-OMe (3a)
58%, 13.5 h[b]

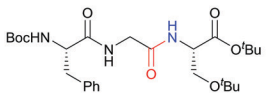

Boc-Phe-Gly-Phe-O*t*-Bu (3b)
50%, 20 h

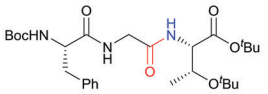

Boc-Phe-Gly-Tyr-OMe (3c)
52%, 16 h

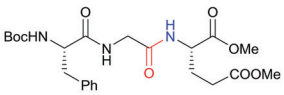

Boc-Phe-Gly-Leu-OMe (3d)
65%, 13 h

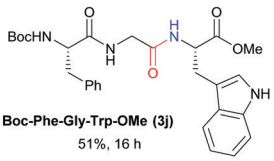

Boc-Phe-Gly-Ile-OMe (3e)
60%, 13 h

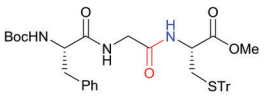

Boc-Phe-Gly-Met-OMe (3f)
47%, 12 h

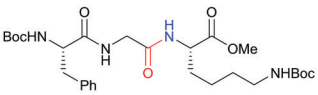

Boc-Phe-Gly-Ser(O*t*-Bu)-O*t*-Bu (3g)
56%, 12 h[c]

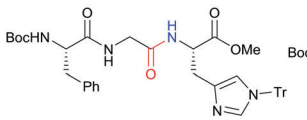

Boc-Phe-Gly-Thr(O*t*-Bu)-O*t*-Bu (3h)
51%, 17 h

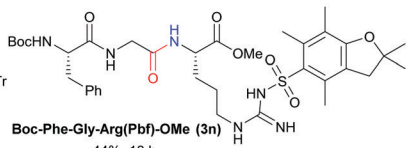

Boc-Phe-Gly-Glu(OMe)-OMe (3i)
32%, 12 h

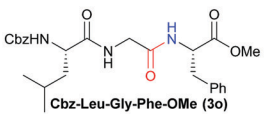

Boc-Phe-Gly-Trp-OMe (3j)
51%, 16 h

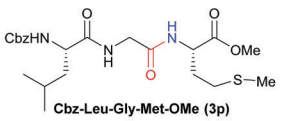

Boc-Phe-Gly-Cys(STr)-OMe (3k)
43%, 19 h


Boc-Phe-Gly-Lys(NHBoc)-OMe (3l)
53%, 16 h

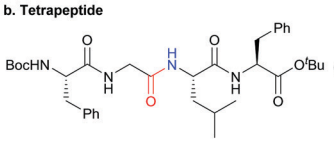

Boc-Phe-Gly-His(NTr)-OMe (3m)
49%, 20 h

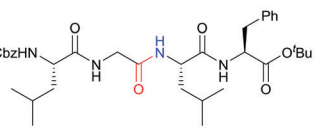

Boc-Phe-Gly-Arg(Pbf)-OMe (3n)
44%, 18 h

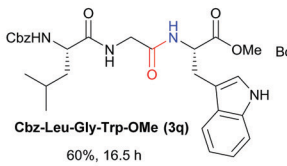

Cbz-Leu-Gly-Phe-OMe (3o)
57%, 16 h

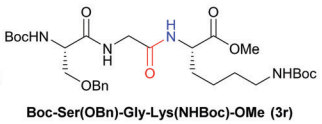

Cbz-Leu-Gly-Met-OMe (3p)
43%, 15.5 h

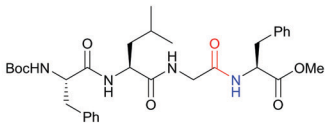
b. Tetrapeptide

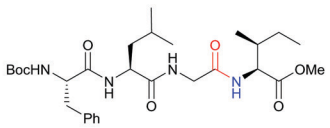

Boc-Phe-Gly-Leu-Phe-O*t*-Bu (3s)
60%, 18.5 h

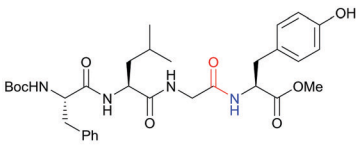

Cbz-Leu-Gly-Leu-Phe-O*t*-Bu (3t)
47%, 16 h


Cbz-Leu-Gly-Trp-OMe (3q)
60%, 16.5 h

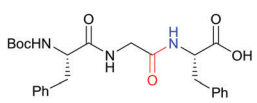

Boc-Ser(OBn)-Gly-Lys(NHBoc)-OMe (3r)
45%, 19.5 h

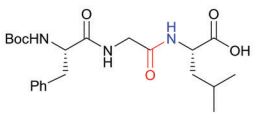

Boc-Phe-Leu-Gly-Phe-OMe (3u)
50%, 15 h

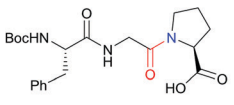

Boc-Phe-Leu-Gly-Ile-OMe (3v)
53%, 17.5 h

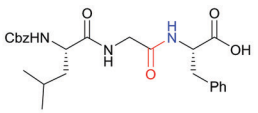

Boc-Phe-Leu-Gly-Tyr-OMe (3w)
51%, 18 h

C. Tripeptide by using unprotected amino acid[d]


Boc-Phe-Gly-Phe-OH (3x)
67%, 22 h


Boc-Phe-Gly-Leu-OH (3y)
64%, 22 h


Boc-Phe-Gly-Pro-OH (3z)
66%, 20 h


Cbz-Leu-Gly-Phe-OH (3aa)
55%, 24 h

[a] Unless otherwise shown, the reaction was performed by employing **1** (0.2 mmol), **2** (0.4 mmol), CsOAc (0.4 mmol), DMF/H_2O = 6/1 (total volume: 2 mL) under an O_2 atmosphere at room temperature at the indicated time. The yield is an isolated yield. [b] The reaction was conducted with 0.5 mmol of **1**. [c] DMF/H_2O = 9/1 was used. [d] The reaction was performed by using amino acid lithium salt in DMF/H_2O = 7/1. See the ESI for more detail.

alternative method for the chemical synthesis of an amide linkage in the peptides.

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Conflicts of interest

There are no conflicts to declare.

Notes and references

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