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Ynamides as Racemization-Free Coupling Reagents for Amide and Peptide Synthesis

Long Hu,[†] Silin Xu,[†] Zhenguang Zhao,[†] Yang Yang, Zhiyuan Peng, Ming Yang, Changliu Wang, and Junfeng Zhao*

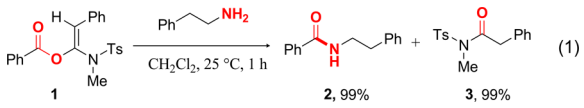
Key Laboratory of Chemical Biology of Jiangxi Province, College of Chemistry and Chemical Engineering, Jiangxi Normal University, Nanchang 330022, P. R. China

Supporting Information

ABSTRACT: A highly efficient, two-step, one-pot synthetic strategy for amides and peptides was developed by employing ynamides as novel coupling reagents under extremely mild reaction conditions. The ynamides not only are effective for simple amide and dipeptide synthesis but can also be used for peptide segment condensation. Importantly, no racemization was detected during the activation of chiral carboxylic acids. Excellent amidation selectivity toward amino groups in the presence of −OH, −SH, $-CONH_2$, $ArNH_2$, and the NH of indole was observed, making the protection of these functional groups unnecessary in amide and peptide synthesis.

The amide is a ubiquitous functional group in nature. It is not only the fundamental structural unit of proteins but also is widely found in pharmaceuticals, agrochemicals, polymers, materials, and other fine chemicals. A straightforward synthetic strategy for amide bond formation is the dehydration coupling of a carboxylic acid with an amine.[1] Numerous amide coupling reagents such as carbodiimides,[2] phosphoniums,[3] and uronium/aminium salts,[4] which are widely used for the activation of carboxylic acids, have been developed and commercialized. Meanwhile, synthetic methodologies employing surrogates of carboxylic acids or amines have also been developed for constructing amide bonds.[5] However, it is still far from ideal, particularly because large amounts of chemical wastes are often produced during amide bond formation in industry. "Amide formation avoiding poor atom economy reagents" was identified as one of the top challenges for organic chemistry.[6] In addition, growing needs for peptides in life science require manufacturing peptide products at reasonably low prices. Much more efficient and atom-economic protocols for amide bond formation are urgently demanded. In this regard, carboxylic acids and amines are still the ideal starting materials, as they are cheap and easily available. Consequently, the search for highly efficient coupling reagents with low molecular weights has become a great challenge.[7] We herein disclose for the first time that ynamides can be used as coupling reagents to facilitate amide and peptide bond formation under extremely mild reaction conditions.

During our efforts to develop new methodologies for amide bond formation,[8] we found that the amide **2** could be formed in quantitative yield along with the valuable *N*-acylsulfonamide **3** when *α*-acyloxyenamide **1** was treated with 1 equiv of 2-phenylethan-1-amine at room temperature (eq 1). Such



(1)

unprecedented reactivity made *α*-acyloxyenamide **1** an attractive active ester for amide bond formation because the aminolysis reaction proceeded smoothly in a "click" manner without the assistance of any additive or catalyst. We noticed that the *α*-acyloxyenamide could be prepared via the hydroacyloxylation of an ynamide with a carboxylic acid, albeit transition metal catalysis[9] or a high reaction temperature[10] was required. The hydroacyloxylation of ynamide and the subsequent aminolysis of *α*-acyloxyenamide together enabled the ynamide to act as a coupling reagent for amide bond formation. Decades ago, efforts to develop ynamine coupling reagents for amide bond formation proved to be unsuccessful because ynamines are thermally unstable, moisture-sensitive, and highly prone to causing serious racemization.[11] In contrast, ynamides with an electron-withdrawing group (EWG) on the nitrogen atom are stable, synthetically accessible, and easy to handle.[12] Importantly, ynamides are less basic or near neutral and will eliminate the risk of base-induced racemization. Thus, we envisioned that ynamides would be practical coupling reagents for amide and peptide synthesis if they could undergo highly efficient hydroacyloxylation under mild reaction conditions.

Extensive optimization of reaction conditions revealed that the solvent has a significant effect in ynamide hydroacyloxylation and that dichloromethane (DCM) was the best solvent (for details, see the Supporting Information (SI)). Further optimization regarding the ynamide structure illustrated that not only do the R^2 substituent and the EWG attached to the nitrogen atom have a remarkable effect on the reaction efficiency but R^1 on the other side of the C−C triple bond does as well (Scheme 1). The EWG is crucial for balancing the stability and reactivity of the ynamide while R^1 substituent has a notable impact on reaction time (**4c**, **4e**). The best results were obtained for terminal ynamides (R^1 = H) including *N*-methylynemethylsulfonamide (MYMsA) and *N*-methylynetoluenesulfonamide (MYTsA). Both MYMsA and MYTsA are easily accessible and stable for handling.[12c] No deterioration was detected after they had been exposed to open air at room temperature for 2 days or kept in a refridgerator for 2 months.

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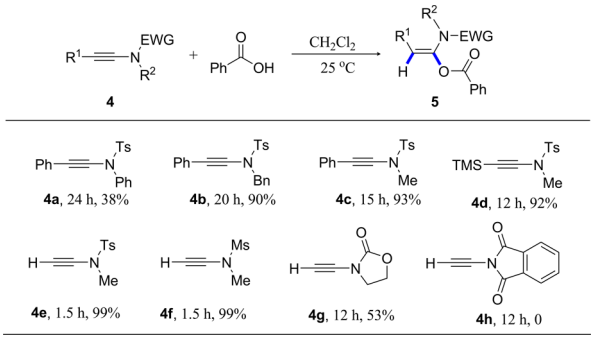
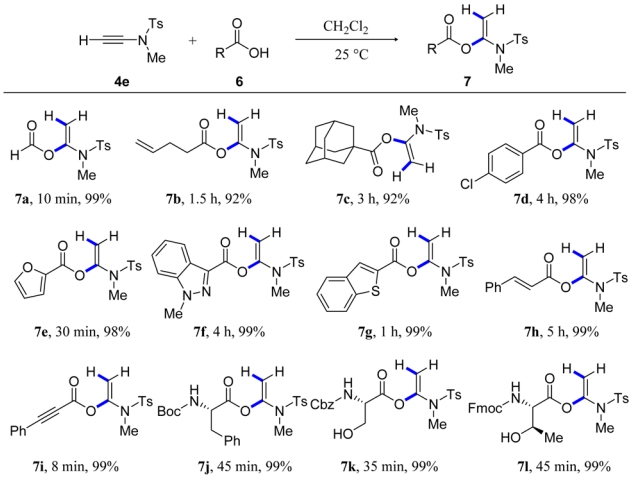
Scheme 1. Representative Results of the Optimization of Hydroacyloxylation of Ynamides[a]



[a]Reaction conditions: ynamide **4** (0.2 mmol), benzoic acid (0.2 mmol), isolated yield.

Scheme 2. Hydroacyloxylation of Ynamide MYTsA with Various Carboxylic Acids[a]

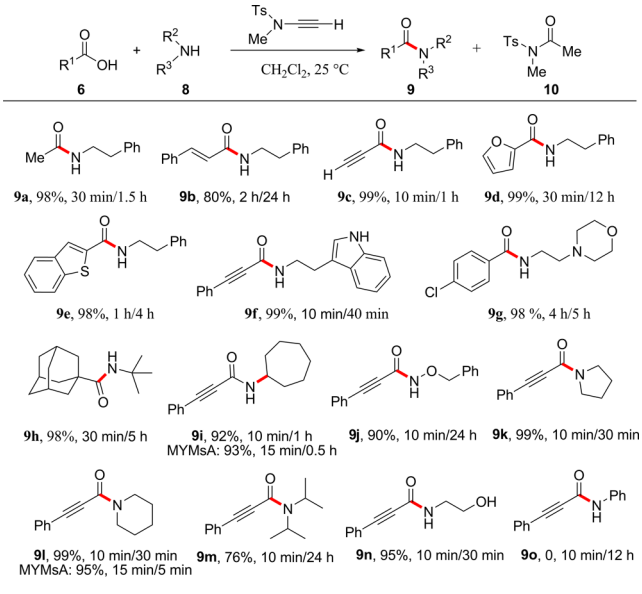


[a]Reaction conditions: MYTsA **4e** (0.2 mmol), carboxylic acid (0.2 mmol), isolated yield.

The scope of the hydroacyloxylation reaction was investigated with respect to the carboxylic acids. As shown in Scheme 2, all of the tested carboxylic acids including aliphatic, aryl, and α,β-unsaturated acids reacted smoothly at room temperature to afford the hydroacyloxylation products in excellent yields. Generally, stronger acids reacted faster than the weaker ones. For example, the reactions of formic acid and 3-phenylpropargylic acid were complete in only a few minutes (**7a**, **7i**). Heteroaryl carboxylic acids also worked well for this transformation (**7e**−**7g**). α-Amino acids reacted faster than common aliphatic acids (**7k**, **7l**). All of these α-acyloxyenamides were stable to air and moisture and could be stored at room temperature.

Both the hydroacyloxylation of ynamides and the aminolysis of active esters, the α-acyloxyenamides, proceeded in a "click" manner. By combining these two reactions, a two-step, one-pot strategy, in which the isolation of the α-acyloxyenamide was unnecessary, for amide bond formation with an ynamide as the coupling reagent was developed. A control experiment with Fmoc-glycine, 2-phenylethan-1-amine, and ynamide MYTsA as the carboxylic acid, amine, and coupling reagent, respectively, demonstrated that the two-step strategy and the two-step, one-pot strategy produced similar results (for details, see the SI),

Scheme 3. MYTsA-Mediated Amide Bond Formation between Carboxylic Acids and Amines[a]



[a]Reaction conditions: MYTsA **4e** (0.2 mmol), carboxylic acid (0.2 mmol), amine (0.22 mmol), reaction times for the first and second steps, respectively, isolated yield.

which unambiguously confirmed that ynamide MYTsA could be used as an efficient coupling reagent.

To explore the general applicability in amide synthesis, various carboxylic acids and amines were evaluated and the results are summarized in Scheme 3. Broad substrate scopes with respect to both coupling partners were observed. Aliphatic, aryl and α,β-unsaturated carboxylic acids reacted smoothly to produce the target amides in good to excellent yields. This transformation was also applicable to heteroaryl carboxylic acids (**9d**, **9e**). Propargylic amide, an attractive alkyne tag for "click chemistry", was formed quantitatively (**9c**). Moclobemid (**9g**), an antidepressant used in clinics, could also be obtained in quantitative yield from the corresponding carboxylic acid and amine. Interestingly, secondary amines reacted faster than primary ones in the aminolysis step owing to the increased nucleophilicity (**9k**, **9l**). Sterically demanding substrates were well tolerated with longer reaction times (**9h**, **9m**). Even a less nucleophilic *O*-benzylhydroxylamine could be used as a valid substrate (**9j**). Excellent selectivity was observed when polyfunctionalized amines such as 2-aminoethanol and tryptamine were employed as the amine (**9f**, **9n**). Arylamines such as aniline (**9o**) was inert in this reaction, which guaranteed selectivity between aryl and aliphatic amines. Comparable yields of amides were obtained when using MYTsA and MYMsA, with a faster aminolysis step for MYMsA (**9i**, **9l**).

A serious obstacle in the development of peptide coupling reagents is the epimerization/racemization during the activation of carboxylic acid and the subsequent coupling step. The potential loss of chiral integrity during peptide bond formation was investigated before we moved on to peptide synthesis. Owing to its high potential to racemization, Fmoc-L-Ser(O*t*Bu)-OH was chosen as the model carboxyl component for the epimerization/racemization study.[13] As shown in Table 1, significant racemization was observed with conventional coupling reagents such as *O*-(benzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HBTU), *O*-(7-azabenzo-

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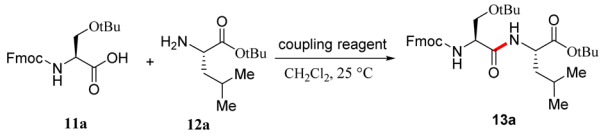
Table 1. Comparative Study of Epimerization/Racemization during Dipeptide Synthesis[a]



entry	coupling reagent	additive	time	yield[b] (%)	dr[c]
1	HBTU	DIEA	10 min	90	82:18
2	HATU	DIEA	10 min	70	87:13
3	PyBop	DIEA	10 min	91	88:12
4	DCC		10 min	98	91:9
5	DEPBT	DIEA	20 min	61	99:1
6	MYMsA		22 h	99	100:0
7	MYTsA		22 h	94	100:0

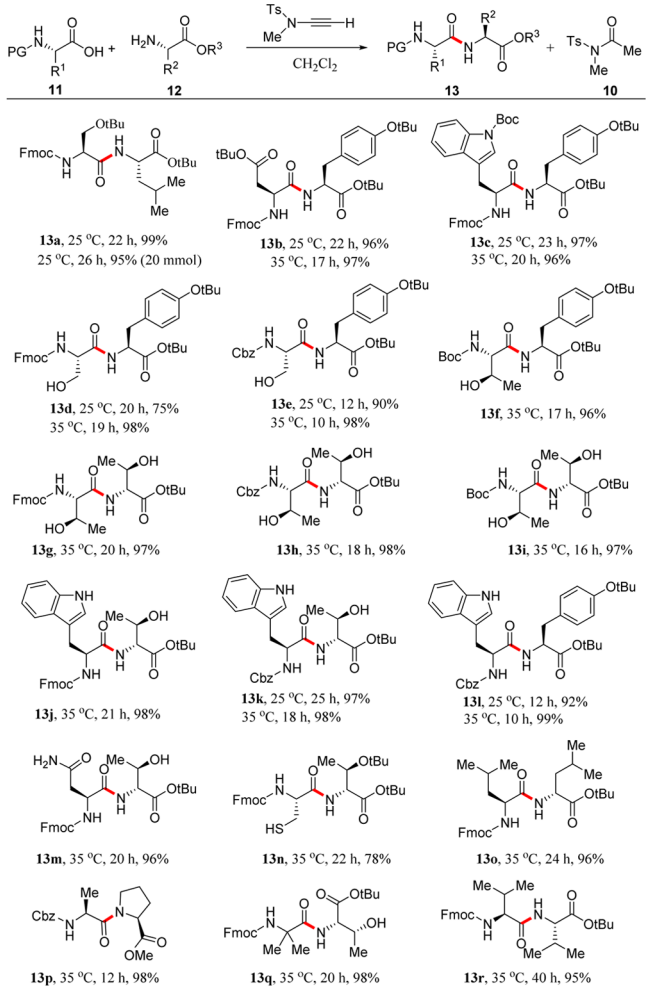
[a]Reaction conditions: **11a** (0.2 mmol), **12a** (0.22 mmol), coupling reagent (0.22 mmol), diisopropylethylamine (DIEA) (0.4 mmol). [b]Isolated yield. [c]Determined by HPLC analysis.

triazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HATU), benzotriazol-1-yloxytri(pyrrolidino)phosphonium hexafluorophosphate (PyBop), and *N*,*N*′-dicyclohexylcarbodiimide (DCC) during the coupling of Fmoc-L-Ser(O*t*Bu)-OH and H-L-Leu-O*t*Bu (Table 1, entries 1−4). In contrast, no epimerization/racemization was detected when either MYTsA or MYMsA was used as the coupling reagent (Table 1, entries 6 and 7). Although excellent chirality retention was observed for 3-(diethoxyphosphoryloxy)-1,2,3,-benzotriazin-4(3*H*)-one (DEPBT), the reaction efficiency was low (Table 1, entry 5).

The generality of the two-step, one-pot amide bond formation strategy was further evaluated in dipeptide synthesis.[14] Almost all of the dipeptides were obtained in excellent yields without any detectable epimerization/racemization at 25 °C. Longer reaction times compared to those for common amines were attributed to the decreased nucleophilicity of α-amino esters. Increasing the temperature to 35 °C could shorten the reaction time while higher temperatures had detrimental effects on the reaction efficiency. Unlike conventional coupling reagents that require a base as a co-reagent, the ynamides could be used alone to mediate peptide bond formation. Both common amine protecting groups such as Boc, Cbz and the base-sensitive Fmoc could be tolerated. Fmoc deprotection, which generally occurs under basic reaction conditions, was not detected when using this protocol. Different protecting groups had no noticeable influence on reaction efficiency. Excellent amidation selectivity toward amino groups in the presence of −OH, −SH, $-CONH_2$, and the NH of indole was observed, making the protection of these functional groups unnecessary in amide and peptide synthesis. However, the side-chain functional groups of Lys, His, Arg, Tyr, Asp, and Glu are not compatible. Interestingly, L-proline ester reacted faster than other α-amino esters (**13p**, Scheme 4). Sterically hindered coupling partners such as the esters of 2-aminoisobutyric acid (Aib) (**13q**) and Val (**13r**), which are difficult substrates for peptide coupling, also reacted smoothly to furnish the dipeptides in excellent yields, albeit longer reaction times were required. This strategy is not limited to the small scale (0.2 mmol), as a larger-scale reaction of 20 mmol to produce 11 g of dipeptide **13a** could be conveniently performed with the excellent efficiency retained, which paves the way for practical application.

To further demonstrate the potential applicability of ynamide coupling reagents in peptide synthesis, a convergent [2+3]

Scheme 4. MYTsA-Mediated Peptide Bond Formation[a]



[a]Reaction conditions: acid partner **11** (0.2 mmol), amine partner **12** (0.22 mmol), MYTsA (0.2 mmol), 25 or 35 °C, total reaction times for the two steps (the reaction time of the first step was less than 1 h), isolated yield.

segment condensation strategy was employed for the synthesis of protected Leu-enkephalin **20** (Scheme 5). Dipeptides Fmoc-L-Tyr(Bzl)-Gly-O*t*Bu **14** and Fmoc-L-Phe-L-Leu-O*t*Bu **16** and tripeptide Fmoc-Gly-L-Phe-L-Leu-O*t*Bu **18** could be prepared quantitatively by employing the two-step, one-pot strategy using MYMsA as the coupling reagent. The last step, the MYMsA-mediated [2+3] segment condensation of dipeptide acid **15** and tripeptide amine **19**, proceeded smoothly to release target protected Leu-enkephalin **20** in good yield. It is notable that Leu-enkephalin **20** was obtained in 58% total yield over seven steps, four of which were MYMsA-mediated peptide bond formation. No detectable evidence of racemization was observed in the entire synthesis. The synthesis of Leu-enkephalin **20** clearly demonstrated that the ynamide not only can be used as a coupling reagent for amides and dipeptides but also is effective for peptide segment condensation.

In conclusion, we have successfully developed a two-step, one-pot strategy for amide and peptide bond formation by employing ynamides as novel coupling reagents. It was established based on an extremely efficient hydroacyloxylation of ynamides with carboxylic acids and an unprecedented aminolysis of α-acyloxyenamides. Both the hydroacyloxylation and the ami-

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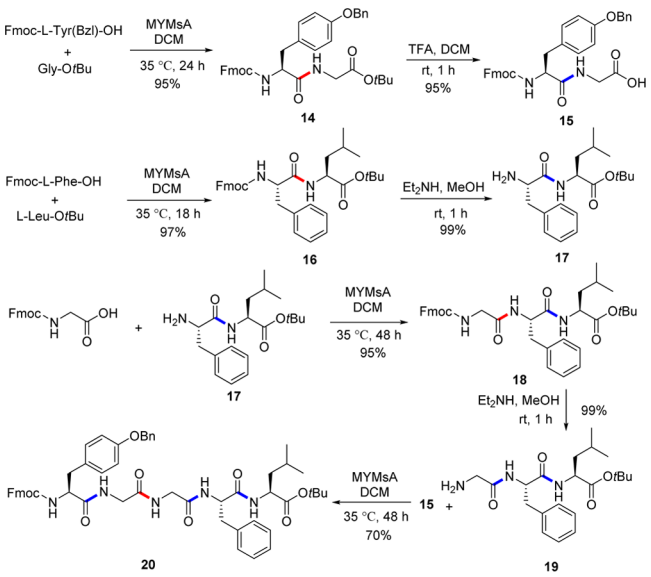
Scheme 5. Synthesis of Protected Leu-Enkephalin with MYMsA as the Coupling Reagent



nolysis proceeded under very mild reaction conditions in a "click" manner, making ynamides as efficient coupling reagents. The ynamide coupling reagents have several advantages: (1) ynamides MYTsA and MYMsA can be prepared easily from readily available chemicals and are stable to air and moisture; (2) the ynamide coupling reagents can be used alone without the assistance of any additive or catalyst; (3) MYMsA (mol wt = 133.02) is the smallest racemization-free peptide coupling reagent, highlighting its atom-economic advantage; (4) excellent amidation selectivity toward amino group in the presence of −OH, −SH, $-CONH_2$, $ArNH_2$, and the NH of indole renders the protection of these functional groups unnecessary in amide and peptide synthesis; and (5) ynamide coupling reagents can also be used for peptide segment condensation as well as larger-scale reactions. All of these features make ynamides practical coupling reagents for amide and peptide synthesis in both academia and industry. Further studies toward more effective ynamides, more efficient coupling reaction systems, and their application in solid-phase peptide synthesis are underway in our laboratory.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/jacs.6b07230.

Experimental procedures and characterization data (PDF)

■ AUTHOR INFORMATION

Corresponding Author

*zhaojf@jxnu.edu.cn

Author Contributions

†L.H., S.X., and Z.Z. contributed equally.

Notes

The authors declare no competing financial interest.

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(13) A comparative study of epimerization/racemization of a non-natural amino acid Fmoc-L-phenylglycine, which is extremely prone to epimerization/ racemization, was also performed (see the SI).

(14) Although the first step had to be performed in DCM, the subsequent aminolysis of α-acyloxyenamide could also be performed in other solvents such as DMF with a significantly shorter reaction time (see the SI).

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