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Peptide Diversification through Addition Reaction of Free Carboxylic Acids to Ynamides

Zhefan Zhang, Lingchao Cai * and Liangliang Song *

Jiangsu Provincial Key Lab for the Chemistry and Utilization of Agro-Forest Biomass, Jiangsu Co-Innovation Center of Efficient Processing and Utilization of Forest Resources, Jiangsu Key Lab of Biomass-Based Green Fuels and Chemicals, International Innovation Center for Forest Chemicals and Materials, College of Chemical Engineering, Nanjing Forestry University, Nanjing 210037, China; zzf863766281@icloud.com
* Correspondence: cailingchao@njfu.edu.cn (L.C.); liangliangsong@njfu.edu.cn (L.S.)

Abstract: Peptide modification has emerged as an important topic in the academic community and pharmaceutical industry. However, they are primarily focused on the diversification of amines, thiols, and alcohols. Direct and chemoselective modification of acid residues in peptides is relatively underdeveloped. In this context, we report a novel and efficient method for the direct functionalization of acid residues in peptides. By using ynamides as reaction partners, the adducts are rapidly obtained in moderate to excellent yields at room temperature in water. This approach shows excellent chemoselectivity and a broad scope including dipeptides bearing unprotected Trp or Tyr residue and free Ser or Gln residue.

Keywords: peptide; ynamide; addition reaction

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1. Introduction

In contrast with small molecules, peptide therapeutics have recently obtained more interest from the pharmaceutical industry due to the distinct protein–protein interactions and superior specificity for their targets [1,2]. Post-translational modification of peptides has emerged as a vital issue in modulating activity under physiological conditions [3,4]. Considering unnatural peptides showing improved pharmacokinetics and bioactivity, the chemoselective modification of peptides has emerged as an important task because of its ability to fine-tune structural characteristics in helping regulate physicochemical and biological properties [5–7]. Furthermore, chemoselectively modified peptides could improve metabolic stability and membrane permeability and/or tune bioactive conformation [8,9]. Although many strategies for the diversification of OH-, SH-, and NH_2-groups in peptides have been developed [10,11], direct and chemoselective modification of COOH-group in peptides is relatively less common. In addition to classical esterification and amidation, carbodiimides have been used to directly modify the carboxylic acid residues of peptides, while the adducts are highly reactive and not stable (Figure 1a) [12–14]. Additionally, Raines developed an interesting esterification of carboxylic acids and diazo compounds [15,16], and Jørgensen reported a stereoselective oxidative bioconjugation between the carboxylic acid residues of peptides and aldehydes (Figure 1c) [17]. Alternatively, decarboxylative methods have been explored to generate decorated peptides [18–20], especially with the aid of photoredox catalysis (Figure 1b) [21–23]. Despite major advances, most of them have limited functional-group tolerance, requiring extra protections for certain special amino acids, such as Trp, Tyr, Ser, and Gln. In addition, they are primarily performed in organic solvents and are not compatible with water as a solvent, largely restricting the synthetic utility. Therefore, it is highly desirable to develop an efficient strategy for the direct peptide modifications starting from free carboxylic acids under mild reaction conditions using water as a solvent.

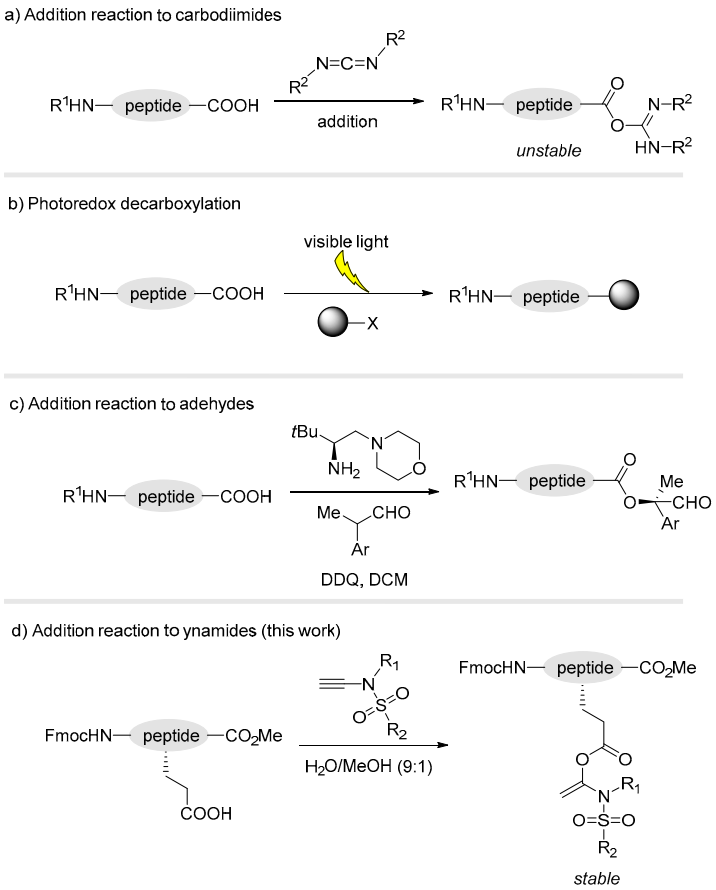

Figure 1. Modification of acid residues in peptides and this work.

Ynamides, a special kind of electron-rich heteroatom-substituted alkynes, have emerged as important building blocks in synthetic chemistry during the past decade [24–26]. The electron-withdrawing groups on the nitrogen atom offer enhanced stability. Notably, the nitrogen atom could impose an electronic bias, leading to highly regioselective nucleophilic α-addition via keteniminium intermediates [27,28]. Based on this, ynamides have been successfully used in the preparation of novel N-containing molecules and versatile N-heterocycles [29,30]. Within our program on the efficient peptide modification [5,7,31–33], we herein disclose a direct peptide modification protocol in an aqueous solution at room temperature through the addition reaction of free carboxylic acids to ynamides (Figure 1d).

2. Materials and Methods

2.1. General Information

All reagents were used as received from commercial sources. Reactions were monitored through thin-layer chromatography (TLC) on 0.25-mm silica gel plates and visualized under UV light. Flash column chromatography (FCC) was performed using Flash silica gel (90-Å pore size, 200–300 µm). NMR spectra were recorded on a Bruker Avance-400 or -600 instrument, calibrated to $CD(H)Cl_3$ as the internal reference (7.26 and 77.0 ppm for ^1H and ^{13}C NMR spectra, respectively) and $CD(H)_3OD(H)$ as the internal reference (3.31 and 49.0 ppm for ^1H and ^{13}C NMR spectra, respectively). ^1H NMR spectral data were reported in terms of the chemical shift (δ, ppm), multiplicity, coupling constant (Hz), and integration. ^{13}C NMR spectral data were reported in terms of the chemical shift (δ, ppm). The following abbreviations indicated multiplicities: s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet; and br, broad. High-resolution mass spectra were recorded using a SCIEX X500R LC-Q-TOF, ESI ion Source.

2.2. Synthesis of Product 3

In a microcentrifuge tube, **1** (0.05 mmol, 1 equiv), **2** (0.075 mmol, 1.5 equiv), H_2O (450 μL), and MeOH (50 μL) were added. The reaction mixture was put in a constant-temperature oscillating metal bath and stirred at r.t. for 24 h. The solvent was removed in *vacuo* and the remaining residue was purified by silica gel column chromatography (petroleum ether/EtOAc or methanol/dichloromethane) to afford the products **3a**–**3m**.

Following the general procedure, **1a** (22.0 mg, 0.05 mmol, 1 equiv) and **2a** (15.7 mg, 0.075 mmol, 1.5 equiv) were used to give **3a** (18.5 mg, 57%). white solid. R_f = 0.5 (Petroleum ether/EtOAc, 1:4). **^{1}H NMR** (600 MHz, $CDCl_3$) δ 7.78 (d, *J* = 7.5 Hz, 2H), 7.73 (d, *J* = 8.0 Hz, 2H), 7.62 (d, *J* = 7.5 Hz, 2H), 7.42 (t, *J* = 7.4 Hz, 2H), 7.36–7.30 (m, 4H), 6.76 (d, *J* = 7.9 Hz, 1H), 5.57 (s, 1H), 4.81 (d, *J* = 2.7 Hz, 1H), 4.69 (td, *J* = 8.1, 5.1 Hz, 1H), 4.51–4.46 (m, 1H), 4.43 (d, *J* = 7.2 Hz, 2H), 4.25 (t, *J* = 7.1 Hz, 1H), 3.96 (d, *J* = 5.7 Hz, 2H), 3.78 (s, 3H), 3.01 (s, 3H), 2.58–2.46 (m, 2H), 2.45 (s, 3H), 2.36–2.21 (m, 1H), 2.13–2.05 (m, 1H). **^{13}C NMR** (101 MHz, $CDCl_3$) δ 171.7, 170.3, 169.1, 156.5, 147.3, 144.3, 143.8, 141.3, 133.1, 129.6, 128.0, 127.7, 127.1, 125.1, 120.0, 99.6, 67.3, 52.7, 51.6, 47.1, 44.4, 37.7, 30.1, 26.6, 21.6. **HRMS** (ESI, *m*/*z*) calcd for $C_{33}H_{36}N_3O_9S$ $[M + H]^+$: 650.2167, found: 650.2167.

Following the general procedure, **1b** (23.5 mg, 0.05 mmol, 1 equiv), **2a** (15.7 mg, 0.075 mmol, 1.5 equiv) were used to give **3b** (18.7 mg, 55%). white solid. R_f = 0.6 (Petroleum ether/EtOAc, 1:4). **^{1}H NMR** (400 MHz, $CDCl_3$) δ 7.80–7.77 (m, 2H), 7.75–7.68 (m, 2H), 7.62 (s, 2H), 7.41 (d, *J* = 8.1 Hz, 2H), 7.36–7.30 (m, 4H), 7.20 (s, 1H), 5.94 (s, 1H), 4.80 (d, *J* = 3.3 Hz, 1H), 4.68–4.63 (m, 1H), 4.46–4.41 (m, 2H), 4.34 (s, 1H), 4.24 (s, 1H), 4.11 (s, 1H), 3.99 (s, 1H), 3.78 (s, 3H), 3.66 (s, 1H), 3.29 (d, *J* = 10.5 Hz, 1H), 3.00 (s, 3H), 2.54 (d, *J* = 6.1 Hz, 1H), 2.48 (d, *J* = 5.0 Hz, 1H), 2.44 (s, 3H), 2.31 (s, 2H). Mixture of rotamers. **^{13}C NMR** (101 MHz, $CDCl_3$) δ 171.9, 171.1, 170.4, 147.4, 141.3, 130.0, 129.6, 128.0, 127.8, 127.3, 127.1, 125.2, 120.0, 99.5, 67.4, 63.0, 55.7, 52.8, 51.8, 47.1, 37.8, 30.2, 26.2, 21.6. **HRMS** (ESI, *m*/*z*) calcd for $C_{34}H_{38}N_3O_{10}S$ $[M + H]^+$: 680.2273, found: 680.2274.

Following the general procedure, **1c** (28.5 mg, 0.05 mmol, 1 equiv), **2a** (15.7 mg, 0.075 mmol, 1.5 equiv) were used to give **3c** (13.2 mg, 34%). white solid. R_f = 0.4 (Petroleum ether/EtOAc, 1:4). **^{1}H NMR** (400 MHz, $CDCl_3$) δ 8.82 (s, 1H), 7.80 (d, *J* = 7.7 Hz, 2H), 7.76 (d, *J* = 8.1 Hz, 2H), 7.67 (d, *J* = 7.6 Hz, 1H), 7.61 (t, *J* = 8.6 Hz, 2H), 7.47–7.41 (m, 3H), 7.37–7.32 (m, 4H), 7.22 (d, *J* = 7.6 Hz, 1H), 7.16 (d, *J* = 7.6 Hz, 2H), 6.54 (d, *J* = 7.7 Hz, 1H), 5.55 (s, 1H), 4.85 (s, 1H), 4.65 (s, 1H), 4.57 (s, 1H), 4.46 (s, 2H), 4.44–4.36 (m, 1H), 4.27–4.19 (m, 1H), 3.69 (s, 3H), 3.53 (d, *J* = 15.0 Hz, 1H), 3.13–3.23 (m, 1H), 3.03 (s, 3H), 2.46 (s, 3H), 2.30 (s, 1H), 2.18 (d, *J* = 11.4 Hz, 2H), 1.87 (q, *J* = 10.7 Hz, 1H). **^{13}C NMR** (101 MHz, $CDCl_3$) δ 174.6, 171.5, 171.4, 170.3, 156.0, 147.5, 144.5, 143.7, 141.3, 136.4, 132.8, 130.0, 129.7, 128.1, 127.8, 127.6, 127.3, 127.1, 125.2, 123.8, 122.2, 120.0, 118.3, 111.8, 100.6, 67.3, 60.4, 52.6, 51.4, 47.1, 38.0, 28.0, 26.9, 21.6, 20.4. **HRMS** (ESI, *m*/*z*) calcd for $C_{42}H_{43}N_4O_9S$ $[M + H]^+$: 779.2745, found: 779.2732.

Following the general procedure, **1d** (27.3 mg, 0.05 mmol, 1 equiv), **2a** (15.7 mg, 0.075 mmol, 1.5 equiv) were used to give **3d** (11.3 mg, 30%). white solid. R_f = 0.6 (Petroleum ether/EtOAc, 1:2). **^{1}H NMR** (400 MHz, $CDCl_3$) δ 7.80 (d, *J* = 7.5 Hz, 2H), 7.73 (d, *J* = 8.3 Hz, 2H), 7.59 (t, *J* = 6.9 Hz, 2H), 7.43 (t, *J* = 7.4 Hz, 2H), 7.37–7.31 (m, 4H), 7.01 (d, *J* = 8.0 Hz, 2H), 6.83 (d, *J* = 8.5 Hz, 2H), 6.58 (s, 1H), 6.09 (s, 1H), 5.29 (d, *J* = 8.6 Hz, 1H), 4.86 (d, *J* = 2.7 Hz, 1H), 4.60 (td, *J* = 7.9, 4.4 Hz, 1H), 4.51 (d, *J* = 8.0 Hz, 2H), 4.46 (d, *J* = 2.8 Hz, 1H), 4.39 (s, 1H), 4.23 (t, *J* = 6.8 Hz, 1H), 3.73 (s, 3H), 3.25 (d, *J* = 14.0 Hz, 1H), 3.02 (s, 3H), 2.90–2.81 (m, 1H), 2.45 (s, 3H), 2.38 (t, *J* = 7.4 Hz, 1H), 2.32 (t, *J* = 7.0 Hz, 1H), 2.28–2.21 (m, 1H), 1.94 (d, *J* = 8.2 Hz, 1H). **^{13}C NMR** (101 MHz, $CDCl_3$) δ 171.5, 171.0, 170.3, 147.4, 144.6, 143.9, 143.6, 141.3, 132.7, 130.6, 129.7, 128.1, 127.8, 127.2, 125.1, 120.0, 115.9, 100.8, 67.1, 56.0, 52.7, 51.5, 47.2, 38.0, 37.2, 29.8, 26.8, 21.6. **HRMS** (ESI, m/z) calcd for $C_{40}H_{42}N_3O_{10}S$ $[M + H]^+$: 756.2586, found: 756.2581.

Following the general procedure, **1e** (27.3 mg, 0.05 mmol, 1 equiv), **2a** (15.7 mg, 0.075 mmol, 1.5 equiv) were used to give **3e** (24.8 mg, 69%). white solid. R_f = 0.5 (Methanol/Dichloromethane, 1:20). **^{1}H NMR** (400 MHz, $CDCl_3$) δ 7.78 (d, *J* = 7.4 Hz, 2H), 7.72 (d, *J* = 7.9 Hz, 2H), 7.62 (d, *J* = 6.3 Hz, 2H), 7.42 (d, *J* = 7.5 Hz, 2H), 7.36–7.29 (m,

4H), 6.24 (s, 1H), 6.11 (d, *J* = 7.4 Hz, 1H), 5.76 (s, 1H), 4.81 (s, 1H), 4.65 (s, 1H), 4.49 (s, 1H), 4.38 (d, *J* = 7.2 Hz, 2H), 4.23 (t, *J* = 7.4 Hz, 1H), 3.76 (s, 3H), 3.30 (s, 1H), 3.00 (s, 3H), 2.96 (s, 1H), 2.52 (s, 1H), 2.47 (s, 1H), 2.44 (s, 3H), 2.42 (s, 1H), 2.32–2.23 (m, 1H), 2.15 (s, 1H), 2.12–2.05 (m, 2H). Mixture of rotamers. **^{13}C NMR** (101 MHz, $CDCl_3$) δ 172.1, 171.7, 170.2, 156.4, 147.2, 145.0, 144.4, 143.9, 141.3, 141.3, 133.2, 130.0, 129.6, 128.0, 127.7, 127.3, 127.1, 125.2, 125.2, 120.0, 99.9, 67.2, 52.7, 51.6, 47.1, 43.5, 37.7, 33.1, 30.2, 26.4, 25.0, 21.6. **HRMS** (ESI, *m*/*z*) calcd for $C_{36}H_{41}N_4O_{10}S$ $[M + H]^+$: 721.2538, found: 721.2542.

Following the general procedure, **1a** (22.0 mg, 0.05 mmol, 1 equiv), **2b** (21.4 mg, 0.075 mmol, 1.5 equiv) were used to give **3f** (27.2 mg, 75%). white solid. R_f = 0.5 (Petroleum ether/EtOAc, 1:1). **^{1}H NMR** (400 MHz, $CDCl_3$) δ 7.81–7.74 (m, 4H), 7.61 (d, *J* = 6.7 Hz, 2H), 7.42 (t, *J* = 7.5 Hz, 2H), 7.36–7.29 (m, 9H), 6.66 (d, *J* = 7.9 Hz, 1H), 5.49 (s, 1H), 4.92 (d, *J* = 2.6 Hz, 1H), 4.67–4.59 (m, 2H), 4.52 (s, 1H), 4.47 (d, *J* = 3.3 Hz, 1H), 4.43 (d, *J* = 7.1 Hz, 2H), 4.25 (t, *J* = 7.1 Hz, 1H), 3.93 (d, *J* = 5.6 Hz, 2H), 3.77 (s, 3H), 2.45 (s, 3H), 2.37–2.30 (m, 2H), 2.23–2.14 (m, 1H), 2.02–1.91 (m, 1H). Mixture of rotamers. **^{13}C NMR** (101 MHz, $CDCl_3$) δ 171.7, 169.7, 144.2, 143.8, 141.3, 135.4, 129.7, 128.6, 128.5, 128.0, 127.9, 127.8, 127.1, 125.1, 120.0, 104.1, 67.3, 53.0, 52.7, 51.6, 47.1, 38.6, 30.2, 26.7, 21.6. **HRMS** (ESI, *m*/*z*) calcd for $C_{39}H_{40}N_3O_9S$ $[M + H]^+$: 726.2480, found: 726.2480.

Following the general procedure, **1a** (22.0 mg, 0.05 mmol, 1 equiv) and **2c** (19.9 mg, 0.075 mmol, 1.5 equiv) were used to give **3g** (34.9 mg, 99%). White solid. R_f = 0.6 (Petroleum ether/EtOAc, 1:1). **^{1}H NMR** (400 MHz, $CDCl_3$) δ 7.82–7.72 (m, 4H), 7.62 (d, *J* = 7.4 Hz, 2H), 7.42 (t, *J* = 7.6 Hz, 2H), 7.37–7.29 (m, 4H), 6.69 (d, *J* = 7.8 Hz, 1H), 5.52 (s, 1H), 5.05 (s, 1H), 4.77 (s, 1H), 4.67 (q, *J* = 7.5 Hz, 1H), 4.44 (d, *J* = 7.2 Hz, 2H), 4.26 (d, *J* = 6.9 Hz, 1H), 3.95 (s, 2H), 3.78 (s, 3H), 3.30 (t, *J* = 7.7 Hz, 2H), 2.48 (d, *J* = 8.5 Hz, 1H), 2.44 (s, 3H), 2.34 (s, 1H), 2.29–2.20 (m, 1H), 2.14–1.93 (m, 2H), 1.51 (t, *J* = 7.6 Hz, 2H), 0.90 (d, *J* = 6.7 Hz, 6H). **^{13}C NMR** (101 MHz, $CDCl_3$) δ 171.7, 169.8, 169.0, 144.0, 143.8, 141.3, 130.2, 129.5, 127.9, 127.8, 127.5, 127.1, 125.1, 120.0, 103.5, 67.4, 52.7, 51.6, 47.7, 47.1, 36.7, 30.3, 26.7, 25.4, 22.3, 21.6. **HRMS** (ESI, *m*/*z*) calcd for $C_{37}H_{44}N_3O_9S$ $[M + H]^+$: 706.2793, found: 706.2794.

Following the general procedure, **1a** (22.0 mg, 0.05 mmol, 1 equiv) and **2d** (20.3 mg, 0.075 mmol, 1.5 equiv) were used to give **3h** (17.1 mg, 48%). White solid. R_f = 0.4 (Petroleum ether/EtOAc, 1:1). **^{1}H NMR** (400 MHz, $CDCl_3$) δ 7.79 (d, *J* = 7.8 Hz, 2H), 7.71–7.60 (m, 4H), 7.47–7.39 (m, 3H), 7.39–7.29 (m, 7H), 7.26 (s, 1H), 6.66 (d, *J* = 8.3 Hz, 1H), 5.51 (s, 1H), 4.95 (s, 1H), 4.88 (s, 1H), 4.71–4.61 (m, 1H), 4.45 (t, *J* = 8.2 Hz, 2H), 4.31–4.21 (m, 1H), 3.93 (d, *J* = 6.0 Hz, 2H), 3.77 (s, 3H), 2.83 (s, 3H), 2.45 (s, 2H), 2.25–2.19 (m, 1H), 2.06–2.00 (m, 1H). Mixture of rotamers. **^{13}C NMR** (101 MHz, $CDCl_3$) δ 169.8, 143.8, 141.3, 129.9, 129.6, 129.4, 128.8, 128.0, 127.8, 127.1, 125.1, 120.0, 101.2, 67.3, 52.8, 51.6, 47.1, 38.6, 30.3, 26.6, 21.7. **HRMS** (ESI, *m*/*z*) calcd for $C_{38}H_{38}N_3O_9S$ $[M + H]^+$: 712.2324, found: 712.2312.

Following the general procedure, **1a** (22.0 mg, 0.05 mmol, 1 equiv) and **2e** (21.4 mg, 0.075 mmol, 1.5 equiv) were used to give **3i** (16.7 mg, 46%). White solid. R_f = 0.5 (Petroleum ether/EtOAc, 1:1). **^{1}H NMR** (400 MHz, $CDCl_3$) δ 8.00–7.91 (m, 1H), 7.79 (d, *J* = 7.6 Hz, 2H), 7.71–7.58 (m, 4H), 7.43 (d, *J* = 6.7 Hz, 2H), 7.38–7.30 (m, 4H), 7.19–7.10 (m, 3H), 6.68 (d, *J* = 7.8 Hz, 1H), 5.53 (s, 1H), 4.91 (s, 1H), 4.84 (s, 1H), 4.65 (d, *J* = 7.5 Hz, 1H), 4.50–4.40 (m, 2H), 4.26 (d, *J* = 7.2 Hz, 1H), 3.94 (s, 2H), 3.77 (s, 3H), 2.48 (s, 1H), 2.45 (s, 3H), 2.35 (s, 3H), 2.23 (d, *J* = 9.4 Hz, 1H), 2.11–1.95 (m, 2H). Mixture of rotamers. **^{13}C NMR** (101 MHz, $CDCl_3$) δ 171.7, 171.4, 169.9, 144.2, 143.8, 141.3, 138.9, 136.3, 135.8, 130.9, 130.6, 130.0, 129.6, 129.4, 129.2, 128.7, 128.0, 127.8, 127.1, 125.2, 125.0, 120.0, 100.7, 67.4, 52.8, 51.6, 47.1, 43.5, 30.3, 26.6, 21.7, 21.2. **HRMS** (ESI, *m*/*z*) calcd for $C_{39}H_{40}N_3O_9S$ $[M + H]^+$: 726.2480, found: 726.2472.

Following the general procedure, **1a** (22.0 mg, 0.05 mmol, 1 equiv) and **2f** (21.1 mg, 0.075 mmol, 1.5 equiv) were used to give **3j** (22.4 mg, 62%). White solid. R_f = 0.4 (Petroleum ether/EtOAc, 1:2). **^{1}H NMR** (400 MHz, $CDCl_3$) δ 7.87–7.76 (m, 4H), 7.62 (d, *J* = 7.4 Hz, 2H), 7.41 (d, *J* = 7.8 Hz, 2H), 7.36–7.30 (m, 2H), 7.04–6.95 (m, 2H), 6.69 (d, *J* = 7.9 Hz, 1H), 5.54 (s, 1H), 5.05 (s, 1H), 4.80 (s, 1H), 4.66 (d, *J* = 6.8 Hz, 1H), 4.44 (d, *J* = 6.7 Hz, 2H), 4.26 (d, *J* = 7.2 Hz, 1H), 3.95 (s, 2H), 3.87 (s, 3H), 3.78 (s, 3H), 3.34–3.24 (m, 2H), 2.48–2.37 (m, 2H), 2.28–2.20 (m, 1H), 2.08–1.96 (m, 2H), 1.50 (d, *J* = 7.6 Hz, 2H), 0.90 (d, *J* = 5.4 Hz, 6H). **^{13}C**

NMR (101 MHz, $CDCl_3$) δ 171.7, 169.8, 169.1, 163.2, 144.1, 143.8, 141.3, 130.1, 127.8, 127.1, 125.1, 120.0, 114.1, 103.7, 67.3, 55.7, 52.7, 51.5, 47.5, 47.1, 36.7, 30.3, 26.8, 25.4, 22.3. **HRMS** (ESI, *m*/*z*) calcd for $C_{37}H_{44}N_3O_{10}S$ $[M + H]^+$: 722.2742, found: 722.2735.

Following the general procedure, **1a** (22.0 mg, 0.05 mmol, 1 equiv) and **2g** (23.9 mg, 0.075 mmol, 1.5 equiv) were used to give **3k** (20.5 mg, 54%). White solid. R_f = 0.4 (Petroleum ether/EtOAc, 1:1). **^{1}H NMR** (600 MHz, $CDCl_3$) δ 8.10–7.98 (m, 2H), 7.89–7.77 (m, 4H), 7.62 (d, *J* = 7.4 Hz, 2H), 7.42 (t, *J* = 7.7 Hz, 2H), 7.33 (t, *J* = 7.2 Hz, 2H), 6.64 (d, *J* = 7.4 Hz, 1H), 5.47 (s, 1H), 5.09 (s, 1H), 4.85 (s, 1H), 4.66 (d, *J* = 7.7 Hz, 1H), 4.45 (d, *J* = 7.2 Hz, 2H), 4.27 (d, *J* = 8.1 Hz, 1H), 3.93 (s, 2H), 3.78 (s, 3H), 3.34 (t, *J* = 7.5 Hz, 2H), 2.41 (q, *J* = 7.6 Hz, 2H), 2.29–2.17 (m, 1H), 2.10–1.91 (m, 2H), 1.52 (d, *J* = 7.4 Hz, 2H), 0.92 (d, *J* = 5.9 Hz, 6H). Mixture of rotamers. **^{13}C NMR** (101 MHz, $CDCl_3$) δ 171.7, 169.7, 169.0, 143.7, 141.3, 128.4, 127.8, 127.1, 126.1, 125.1, 120.0, 104.5, 67.3, 52.8, 51.4, 51.0, 47.7, 47.1, 36.7, 30.1, 26.9, 25.4, 22.3. **HRMS** (ESI, *m*/*z*) calcd for $C_{39}H_{41}F_3N_3O_9S$ $[M + H]^+$: 760.2510, found: 760.2502.

Following the general procedure, **1a** (22.0 mg, 0.05 mmol, 1 equiv) and **2h** (22.2 mg, 0.075 mmol, 1.5 equiv) were used to give **3l** (19.5 mg, 53%). White solid. R_f = 0.4 (Petroleum ether/EtOAc, 1:1). **^{1}H NMR** (400 MHz, $CDCl_3$) δ 8.37 (d, *J* = 8.4 Hz, 2H), 8.05 (d, *J* = 8.4 Hz, 2H), 7.79 (d, *J* = 7.6 Hz, 2H), 7.62 (d, *J* = 7.5 Hz, 2H), 7.42 (t, *J* = 7.5 Hz, 2H), 7.33 (t, *J* = 7.5 Hz, 2H), 6.65 (d, *J* = 8.0 Hz, 1H), 5.49 (d, *J* = 6.0 Hz, 1H), 5.11 (s, 1H), 4.92 (s, 1H), 4.68–4.59 (m, 1H), 4.45 (d, *J* = 7.2 Hz, 2H), 4.26 (t, *J* = 7.2 Hz, 1H), 3.92 (d, *J* = 5.5 Hz, 2H), 3.78 (s, 3H), 3.36 (t, *J* = 7.5 Hz, 2H), 2.40 (q, *J* = 8.2 Hz, 2H), 2.25–2.18 (m, 1H), 1.95–1.88 (m, 1H), 1.61 (d, *J* = 7.0 Hz, 1H), 1.51 (q, *J* = 7.2 Hz, 2H), 0.92 (d, *J* = 6.3 Hz, 6H). **^{13}C NMR** (101 MHz, $CDCl_3$) δ 171.7, 169.6, 169.0, 150.3, 143.7, 142.8, 141.3, 129.1, 127.8, 127.1, 125.1, 124.2, 120.1, 105.0, 67.4, 52.9, 51.3, 47.5, 47.1, 44.5, 36.7, 30.1, 27.1, 25.4, 22.3. **HRMS** (ESI, *m*/*z*) calcd for $C_{36}H_{41}N_4O_{11}S$ $[M + H]^+$: 737.2487, found: 737.2503.

Following the general procedure, **1a** (22.0 mg, 0.05 mmol, 1 equiv) and **2i** (10.0 mg, 0.075 mmol, 1.5 equiv) were used to give **3m** (13.5 mg, 47%). White solid. R_f = 0.5 (Petroleum ether/EtOAc, 1:4). **^{1}H NMR** (400 MHz, $CDCl_3$) δ 7.78 (d, *J* = 7.6 Hz, 2H), 7.62 (d, *J* = 7.5 Hz, 2H), 7.42 (t, *J* = 7.6 Hz, 2H), 7.33 (t, *J* = 7.6 Hz, 2H), 6.72 (d, *J* = 8.0 Hz, 1H), 5.52 (s, 1H), 4.94 (s, 1H), 4.85 (s, 1H), 4.71 (d, *J* = 6.4 Hz, 1H), 4.44 (d, *J* = 7.2 Hz, 2H), 4.26 (t, *J* = 7.3 Hz, 1H), 3.93 (s, 2H), 3.77 (s, 3H), 3.11 (s, 3H), 2.99 (s, 3H), 2.82 (s, 1H), 2.56 (d, *J* = 8.3 Hz, 1H), 2.37–2.27 (m, 1H), 2.03 (d, *J* = 15.8 Hz, 1H). **^{13}C NMR** (101 MHz, $CDCl_3$) δ 171.8, 170.2, 169.1, 143.7, 141.3, 127.7, 127.1, 125.0, 120.0, 99.0, 67.3, 52.8, 51.3, 47.0, 44.4, 37.3, 36.3, 29.9, 26.8. **HRMS** (ESI, *m*/*z*) calcd for $C_{38}H_{46}N_3O_6$ $[M + H]^+$: 574.1854, found: 574.1858.

3. Results

Our investigation started with the addition reaction of dipeptide **1a** bearing a Gly residue and ynamide **2a** using H_2O/MeOH (9:1) (Figure 2). Fortunately, product **3a** was afforded in 57% yield. Next, diverse dipeptides with functionalized amino acids were examined. Dipeptides bearing free Ser or Gln residue were tolerated, giving the corresponding products **3b** (55%) and **3e** (69%), respectively. Dipeptides bearing unprotected Trp or Tyr residue worked well, delivering products **3c** (34%) and **3d** (30%), respectively. For these cases, only the addition reaction of free carboxylic acids to ynamides was observed, proving the excellent chemoselectivity of this method. Moreover, various ynamides were also explored. Diverse aliphatic and aromatic groups on the nitrogen atom were tolerated, resulting in products **3f**–**3i** in 46–99% yields. The results suggested that aliphatic groups on the nitrogen atom are favorable to delivering high yields. The electron-donating and electron-withdrawing groups on the phenylsulfonyl moiety were compatible with the reaction conditions, without a significant impact on this transformation, affording products **3j**–**3l** in 53–62% yields. Interestingly, methylsulfonyl-based ynamide was applicable to form products **3m** in 47% yield.

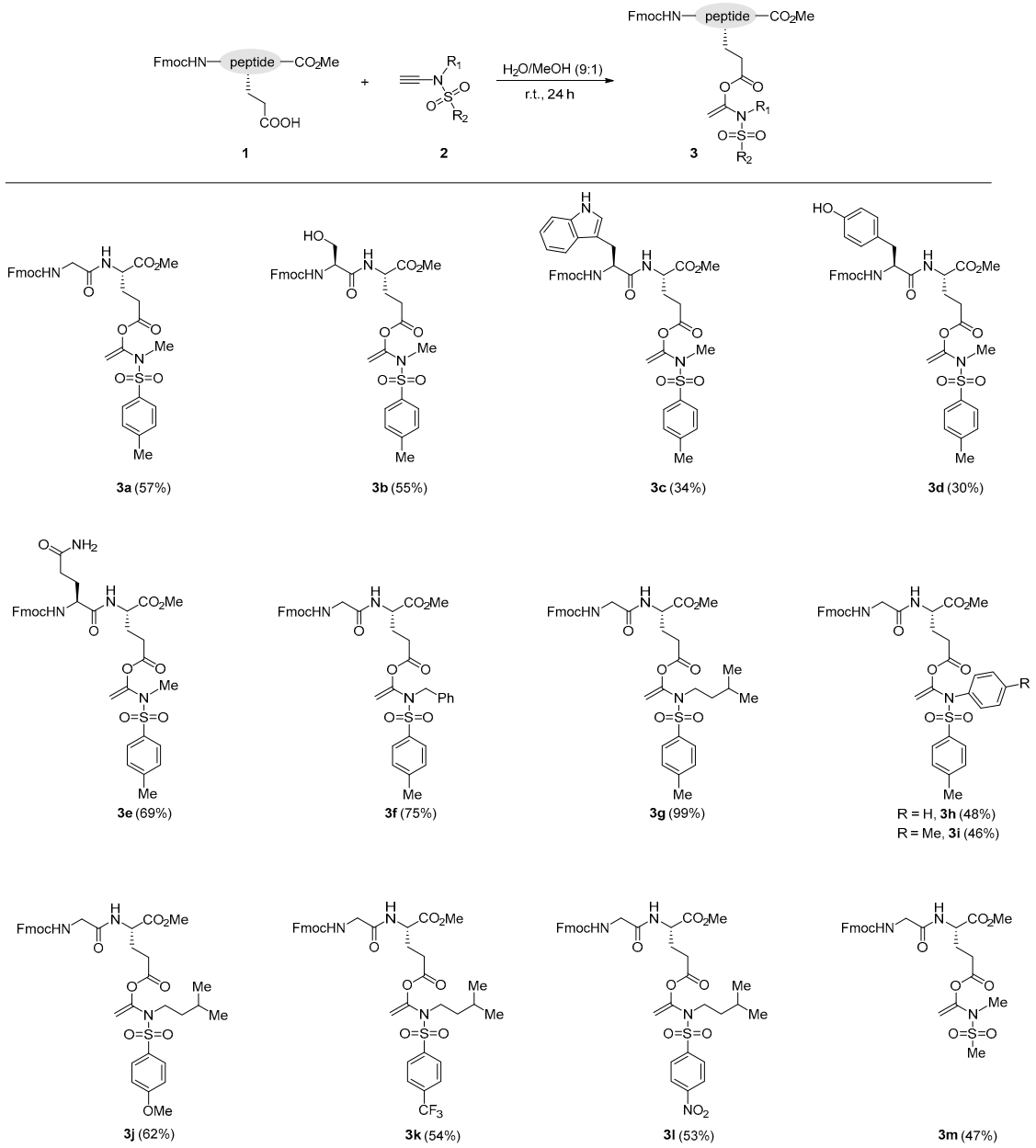

Figure 2. Scope for the addition reaction of free carboxylic acids to ynamides. Condition: **1** (0.05 mmol), **2** (0.075 mmol), H_2O (450 µL), MeOH (50 µL), isolated yield.

4. Conclusions

In conclusion, an efficient and novel reaction between carboxylic acid residues of peptides and ynamides has been developed. By performing the reaction in water, the adducts are delivered in moderate to excellent yields at room temperature in a green and rapid manner. This method features excellent chemoselectivity and a broad scope including dipeptides bearing unprotected Trp or Tyr residue and free Ser or Gln residue.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/pr11082262/s1. Refs. [34–36] are cited in the Supplementary Materials.

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