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One-Step Synthesis of 3-(Fmoc-amino acid)-3,4-diaminobenzoic Acids

Min-Cheng Chien,[○] Yi Kai Lin,[○] Yong Liao, Szu-Hsuan Chen, Yen-Wei Chen, Chien-Yu Liang, Vijayasimha Molakaseema, Sodio C. N. Hsu, Chun-Cheng Lin, Hui-Ting Chen,* and Chai-Lin Kao*

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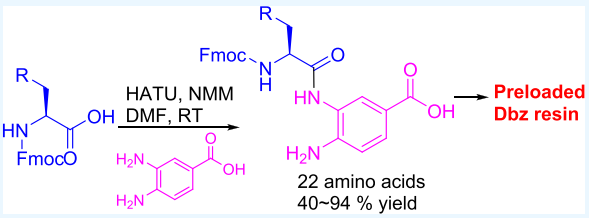
ABSTRACT: A one-step method for synthesizing 3-(Fmoc-amino acid)-3,4-diaminobenzoic acids was used to prepare preloaded diaminobenzoate resin. The coupling of free diaminobenzoic acid and Fmoc-amino acids gave pure products in 40−94% yield without any purification step in addition to precipitation except for histidine. For the proline residue, crude products were collected and used for solid-phase peptide synthesis to give a moderate yield of a pentapeptide. In addition, this method was used to prepare unusual amino acid derivatives, namely, (2-naphthyl) alanine and 6-aminohexanoic acid derivatives, in 50 and 65% yield, respectively.



INTRODUCTION

C-terminal functionalized peptides are in high demand for various purposes, such as a thioester as a precursor for native chemical ligation.[1] Despite the critical role of C-terminal functionalities in peptide chemistry, their preparation remains challenging due to the nature of C- to N-terminal preparation via conventional solid-phase peptide synthesis (SPPS).[2] The use of a safety-catch resin is a feasible strategy for this purpose; in this approach, a given moiety is introduced in the cleavage step to the C-terminus of the product.[3] Diaminobenzoate (Dbz) resin is an efficient safety-catch resin that is widely used to prepare thioesters.[4,5] Dbz resin was also used in the C-terminal functionalization of other peptides, including thiolactones,[6] acylbenzimidazolinones,[7] and cyclic peptides.[8] Dbz resin has been used to prepare polymer−peptide conjugates and to introduce carboxaldehydes at the C-terminus.[9]

Stepwise and reduced methods have been reported to synthesize the Dbz resin (Figure 1). In the stepwise method, 3-Fmoc-Dbz-OH was first introduced into resin.[10] After deprotection of the Fmoc group, the 3-amino group is coupled with the next amino acid. Due to the insensitivity of the Kaiser method to the aniline group of Dbz, it is challenging to follow the progress of the removal of the Fmoc group and the coupling of the next amino acid residue. Consequently, these two steps are the major impediments in this stepwise approach. In contrast, in the reduced method, 3-amino-4-nitrobenzoic acid is first incorporated into resin. SPPS then proceeds after the reduction of the 4-nitro group to the corresponding amine.[11] However, this method has the same issue in tracking the reaction progress as the stepwise method. This leads to the consumption of large amounts of valuable building blocks, which limits the applicability of these methods.

Recently, Molakaseema et al. reported a preloaded method that provides Dbz resin in higher yield than the reduced method. In this approach, Fmoc-amino acid-Dbz-OH (**1**) is individually prepared and incorporated into resin.[12] This method requires various Fmoc-amino acid-Dbz-OH. Unfortunately, only two analogues, Fmoc-Ala-Dbz-OH (**1a**) and Fmoc-Lys (Fmoc)-Dbz-OH (**1b**), have been reported (Figure 2). Meanwhile, the preparation of **1a** and **1b** requires chromatographic purification, which hinders the efficiency of preparation and impeded their application in SPPS. Herein, we report an ameliorated synthetic method for other Fmoc-amino acid-Dbz-OH derivatives with precipitation as the major purification method.

RESULTS AND DISCUSSION

The previously reported[12] method uses *O*-(benzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium hexafluorophosphate (HBTU) (1.1 equiv) and diisopropylethylamine (DIPEA) (3 equiv) to prepare **1a** and **1b** in 75 and 78% yields, respectively, after 8 h of reaction. However, the same approach afforded the phenylalanine derivative (**1c**) in only 15% yield in 12 h (entry 1, Table 1).

To improve the efficiency and yields, we first increased the concentration of Dbz to accelerate the reaction by adding free Dbz instead of Dbz in a solution of DMF. Dbz was added in four portions in 10 min intervals to prevent its self-coupling.

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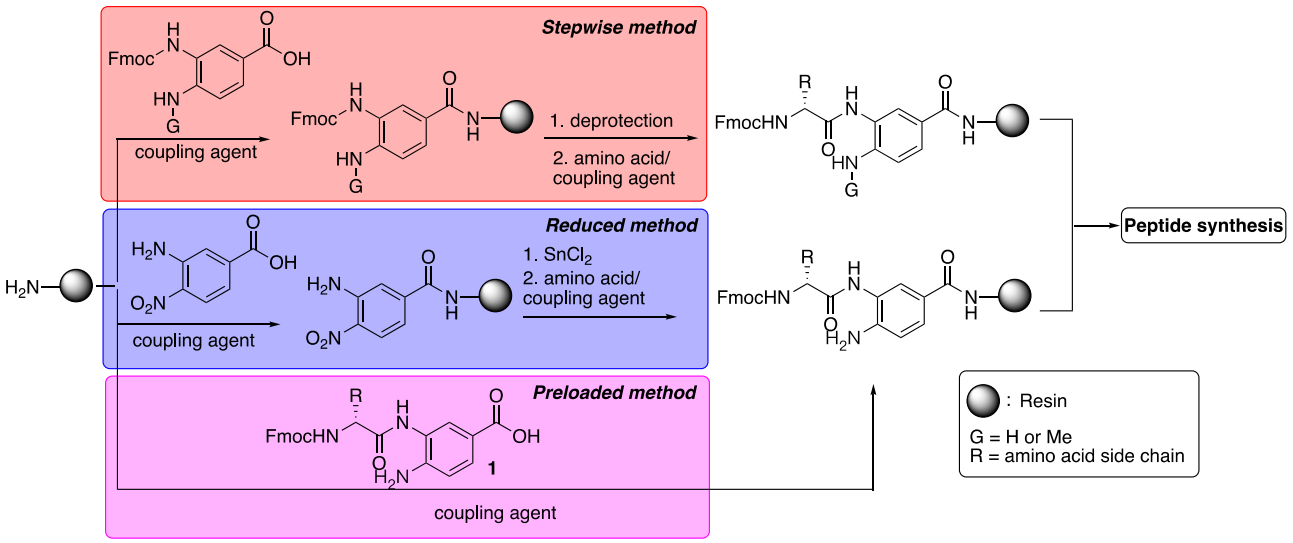
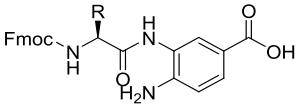


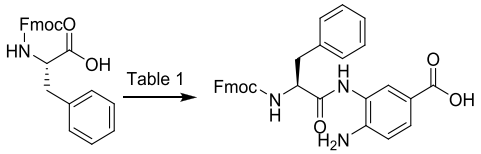
Figure 1. Schematic showing the three methods for preparing Dbz resin.



1a R= Me (75%)
1b R= $CH_2CHCH_2CH_2NHFmoc$ (78%)

Figure 2. Structure of compounds **1a** and **1b**.

Table 1. Reaction Conditions for the Preparation of 1c[a]



2 **1c**

entry	coupling agent (equiv)	incubation time (min)[b]	yield of **1c** (%)
1	HBTU (1.1)	15	15
2[b]	HBTU (1.2)	15	61
3	HATU (1.0)	15	68
3	HATU (1.0)	60	72
4[a]	HATU (1.2)	60	91
5	HATU (1.5)	60	60

[a]Reaction conditions: To the solution of phenylalanine (0.26 M), *N*-methylmorphorine (NMM) (2 equiv) was added with Dbz (1.3 equiv) in four portions every 10 min under N_2. The reaction was carried out at room temperature for 140 min under N_2. [b]The mixing time of **2** and coupling agents.

The reaction was completed in 140 min. When 1.2 equiv of HBTU was used, the yield of **1c** was 61% (entry 2, Table 1). For further improvement, HBTU was replaced with *N*-[(dimethylamino)-1*H*-1,2,3-triazolo[4,5-*b*]pyridin-1-ylmethylene]-*N*-methylmethanaminium hexafluorophosphate *N*-oxide (HATU, 1.0 equiv), increasing the yield of **1c** to 68% (entry 3, Table 1). Subsequently, the activation time of **2** with HATU was extended to 60 min to allow the formation of more active ester, further improving the yield to 72%. Increasing the content of HATU to 1.2 equiv afforded **1c** in 91% yield. However, using 1.5 equiv of HATU resulted in a lower yield of 60%. We analyzed the solution of this reaction and identified one unanticipated side product (**3**) as an ester of **1c** with 1-hydroxy-1*H*-1,2,3-triazolo[4,5-*b*]pyridine (Scheme 1, route a). Presumably, this side product **3** formed due to the presence of excess HATU. The attempt to recover **1c** by hydrolyzing **3** failed. After 2 h of reaction, no significant amount of **1c** was observed. In addition, two amines in Dbz and *meta*-amine are more reactive than the other.[4,12] Since the sole product was identified, 3-substituted Dbz was the product. Thereby, the presence of a rotamer at the amide was not observed.

We also investigated a convenient purification procedure. After the reaction, the reaction mixture was poured into ice water to remove excess Dbz. After filtration, the residue was added to dichloromethane, and the resulting mixture was stirred for an additional 1 h. The desired product **1c** was collected by filtration. This purification procedure required no chromatography and is suitable for large-scale production, which is important for SPPS. Benzimidazole derivatives are known side products of reactions using Dbz resin. To identify the presence of **4** in this preparation, pure **4** was prepared by treating **1c** with TFA. The high-performance liquid chromatograph of the obtained product **4** showed no clear signal of the crude product. However, when aqueous HCl (0.5 N) was used instead of ice water to remove excess Dbz, a small amount of benzimidazole derivatives (**4**) was identified in the HPLC chromatogram (Figure 3), and the yield of **1c** decreased (Scheme 1, route b).

Therefore, the 60 min reaction with 1.2 equiv of HATU was ideal for the preparation of Fmoc-amino acid-Dbz-OH (**1**) (Figure 4). With the exception of isoleucine derivatives (**1n**), aliphatic side-chain derivatives were collected in 83−87% yields. Glycine derivatives (**1e**) had to be washed with a solution of 2% MeOH in dichloromethane, and 90% of the product was collected. Methionine (**1h**), aspartic acid (**1i**), and lysine (**1j**) derivatives were obtained through the same procedure with yields of 86−94%. The attempt to prepare serine (**1k**) and glutamate (**1l**) derivatives afforded in-pure crude products. After several attempts, the crude products of **1k** and **1l** were redissolved in acetone for reprecipitation by adding H_2O. After filtration, the desired products **1k** and **1l** were collected in 78 and 82% yields, respectively. This method was also used to prepare two unnatural amino acid derivatives, Fmoc-(2-naphthyl)alanine (**1m**) and *N*-Fmoc-6-aminohexa-

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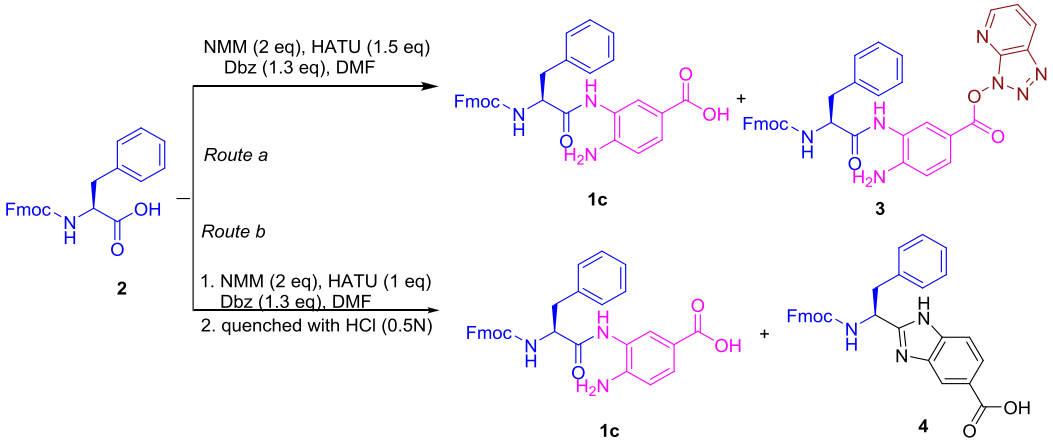
Scheme 1. Formation of Compound 3 (Route a) and Compound 4 (Route b)



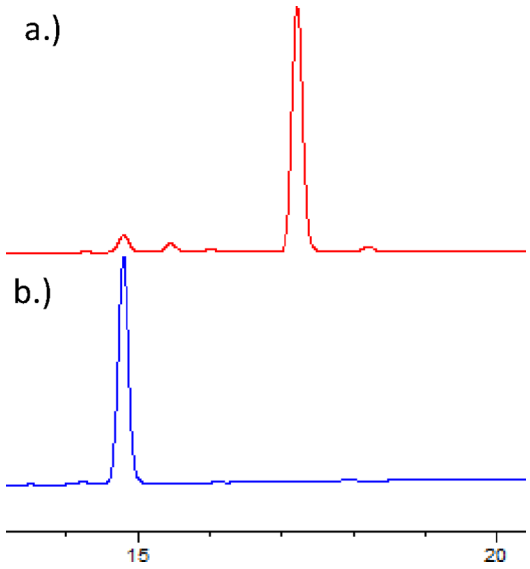


Figure 3. HPLC profile of (a) crude **1c** after the treatment of HCl and (b) **4** through the reaction condition shown in Scheme 1, route b. HPLC conditions were the same as those shown in Figure s51.

noic acid (*N*-Fmoc-AHX) (**1n**), in 50 and 65% yields, respectively. AHX is a popular linker used to prepare peptide derivatives for biomedical applications. These results demonstrate the flexibility of this synthetic approach and its potential for the construction of peptide derivatives.

The remaining derivatives were collected in low yields based on the same procedure. When each step was monitored, a significant proportion of the product was observed in the dichloromethane filtrate. Compared to the derivatives shown in Figure 4, most compounds in this group possess polar side chains (Figure 5). Therefore, the reaction residues were washed with a mixture of dichloromethane and hexane (1:1) instead of pure dichloromethane, allowing isoleucine (**1o**) and glutamic acid (**1p**) to be collected in 91 and 93% yields, respectively. The cystine (**1q**), threonine (**1r**), arginine (**1s**), tryptophane (**1t**), tyrosine (**1u**), and asparagine (**1v**) derivatives required additional acetone−H_2O precipitation as a procedure for **1k** and **1l**. The yields of **1q**−**1v** ranged from 60 to 83%. Histidine (**1w**) was prepared but required additional chromatographic separation to collect the final pure products in only a 40% yield.

The proline derivatives (**1x**) could be prepared through the same way as synthesizing **1c**, but a mixture of two products was

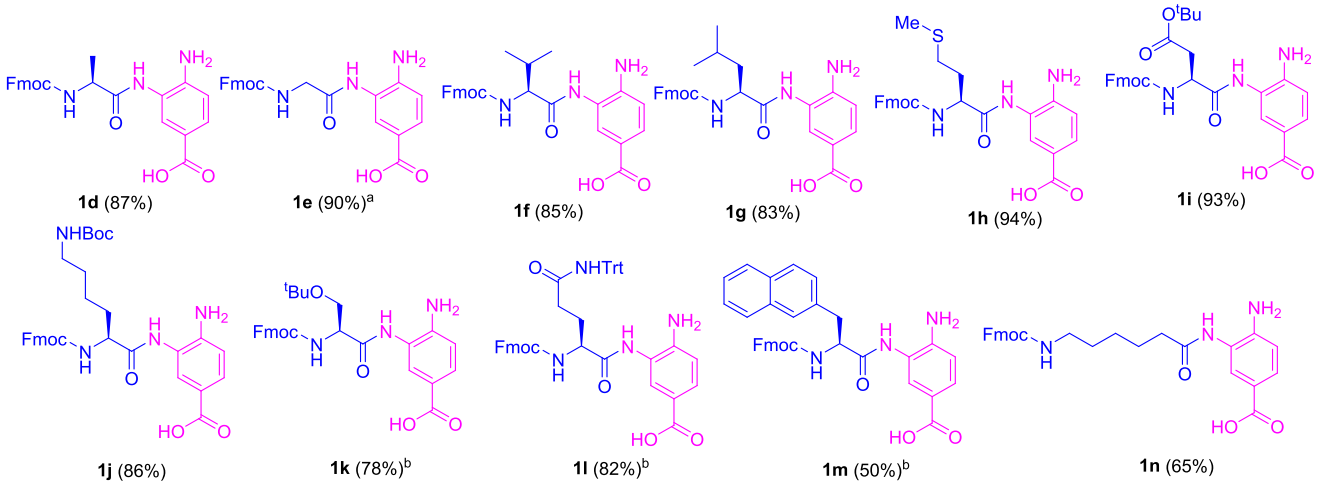


Figure 4. Results of the preparation of Fmoc-amino acid-Dbz derivatives **1d**−**1n**. The purification procedure was as follows. After the completed reaction, the crude product was poured into ice water and precipitated. The resulting mixture was filtrated, and the residue was stirred in dichloromethane for 1 h. The final product was collected by filtration. [a] Methanol (2%) in dichloromethane was used. [b] The collected product was dissolved in acetone and reprecipitated by adding H_2O.

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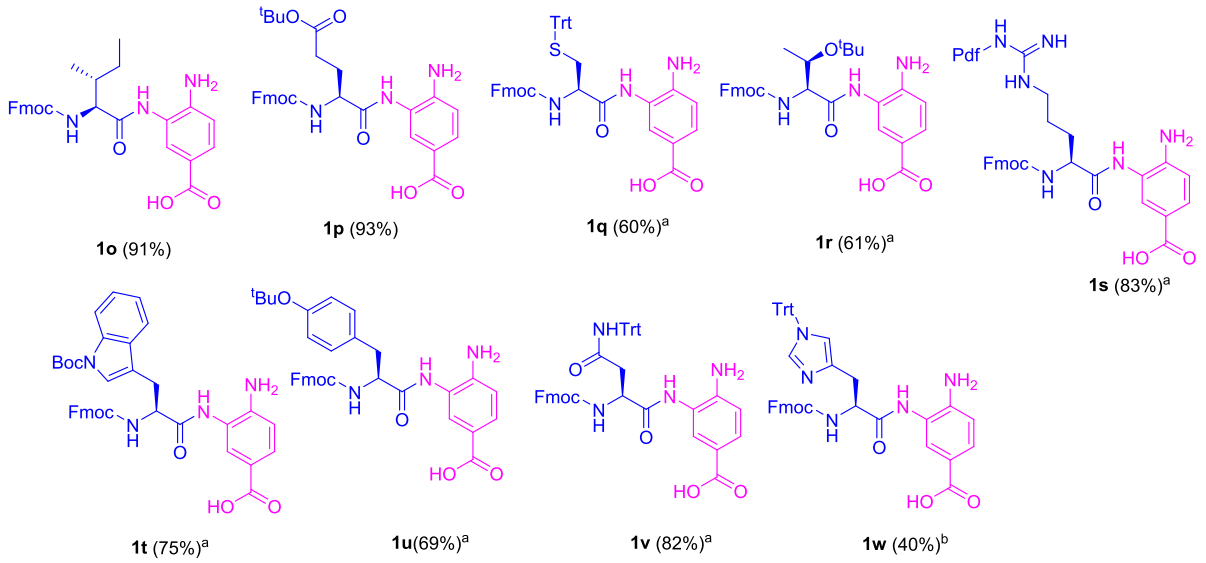
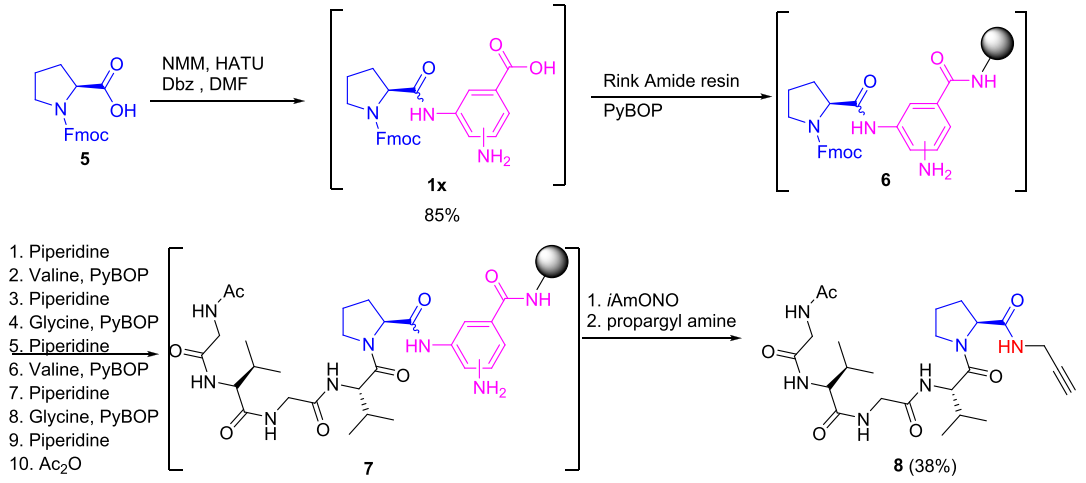


Figure 5. Results of the preparation of Fmoc-amino acid-Dbz derivatives **1o**−**1w**. The purification procedure was as follows. After the completed reaction, the crude product was poured into ice water and precipitated. The resulting mixture was filtered, and the residue was washed with dichloromethane and hexane (1:1) to collect the product. [a]Additional purification: the crude product was dissolved in acetone and reprecipitated by adding dichloromethane/hexane (1:1). [b]Chromatography was applied as an extra purification step.

Scheme 2. Synthesis of Pentapeptide 8 from Crude 3-(Fmoc-proline)-3,4-diaminobenzoic Acids 1x[a]



[a]The yield of **8** was calculated based on the amount of crude **1x**.

collected (Scheme 2). Although the HPLC chromatogram and the MS spectrum (Figures S52 and S53) indicated a sole signal, the ^{1}H NMR spectrum (Figure S43) suggested a pair of isomers. The signals at 9.47 and 9.37 ppm suggested the presence of a pair of rotamers in a ratio of 0.57:1 (Figure S43). The temperature-dependent ^{1}H NMR spectra supported this assumption (Figure S56). However, a pair of signals at 6.74 and 6.70 ppm was not altered by temperature. This observation indicated the presence of a constitutional isomer. The mixture was proposed to be 3- and 4-substituted analogues, which should be used in the peptide synthesis. Therefore, crude **1x** (4 equiv) was introduced to Rink amide resin. The following standard Fmoc chemistry was used to prepare peptides. After activation by ^{t}AmONO, the treatment with propargylamine gave a mixture including **8** and acid derivatives, which was a product from the hydrolysis of acylbenzotriazole to acid (Figure S55a). This hydrolysis was a common side product during the acyl nucleophilic cleavage of acylbenzotriazole resin.[12] The HPLC-MS chromatogram indicated that the ratio of **8** in the mixture was 81% (Figure S54). The desired product was isolated in 38% yield. This fact indicated that the mixture of **1x** could be used for SPPS without further purification.

CONCLUSIONS

A one-step method to prepare Fmoc-amino acid-Dbz (**1**) was reported. Various reaction conditions were studied, and 1.2 equiv of HATU with 1.3 equiv of Dbz gave the best result. The yields of products were excellent to moderate. Remarkably, plain diaminobenzoic acid was used in this approach. Meanwhile, precipitation is the only purification procedure except for the histidine derivatives. When using this preparation method, chromatographic purification was only required for histidine derivatives. This approach gave a mixture of proline derivatives. Fortunately, the crude **1x** could be used for SPPS without further purification and gave a moderate

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yield of the product. This approach was also applied to prepare two unnatural amino acids, Fmoc-(2-naphthyl)alanine (**1m**) and *N*-Fmoc-6-AHX (**1n**), in 50 and 65% yields, respectively. Therefore, this approach should facilitate the application of the Dbz resin and the C-terminal functionalization of peptides.

EXPERIMENTAL SECTION

General Information. All commercial materials were used without further purification. NMR spectra were obtained on a JEOL 400 MHz spectrometer. Molecular weight was calculated from the experimental mass/charge (m/z) ratios. Analytical HPLC was carried out in an Agilent model 1100 equipped and performed using a Pyramid C18 column (Nucleodex, 5.0 μm, Φ 4.6 mm × 250 mm) at a flow rate of 0.5 mL/min. Analytical injections were monitored at 220 nm.

Fmoc-Phe-Dbz-OH (1c). To a solution of Fmoc-Phe-OH (1.00 g, 2.6 mmol) and HATU (1.18 g, 3.1 mmol, 1.2 equiv) in DMF (10 mL) was added NMM (0.51 mL, 5.2 mmol, 2 equiv) under N_2. After the solution was stirred for 60 min under N_2, diaminobenzoic acid (0.51 g, 3.4 mmol, 1.3 equiv) was added to the resulting solution in four portions every 10 min at 0 °C. The resulting solution was stirred at room temperature for 140 min under N_2. Half of the DMF was removed under vacuum, and water (75 mL) was added to the resulting mixture. The resulting slurry was stirred for 60 min and filtrated. The residue was dried under vacuum and suspended in a mixture of DCM (75 mL). After stirring at room temperature for 60 min, filtration gave the desired product (1.23 g, 91% yield). mp = 174−176 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 12.14 (s, 1H; OH), 9.32 (s, 1H; NH), 7.88 (d, J = 7.6 Hz, 2H; CH), 7.81 (d, J = 7.6 Hz, 1H; CH), 7.68−7.66 (m, 3H; CH, NH), 7.52 (dd, J = 8.4, 2.0 Hz, 1H; CH), 7.41 (t, J = 7.2 Hz, 2H; CH), 7.36−7.27 (m, 4H; CH), 7.23 (t, J = 7.2 Hz, 2H; CH), 6.70 (d, J = 8.4 Hz, 1H; CH), 5.53 (s, 2H; NH_2), 4.42 (q, J = 8, 8 Hz, 1H; CH), 4.23−4.16 (m, 3H; CH, CH_2), 3.10 (dd, J = 9.2, 4 Hz, 1H; CH_2), 2.93 (dd, J = 13.6, 9.6 Hz, 1H; CH_2). ^{13}C{^{1}H} NMR (101 MHz, DMSO-d_6): δ 170.8, 167.2, 156.1, 147.1, 143.8, 143.7, 140.7, 137.9, 129.3, 128.4, 128.1, 127.7, 127.1, 126.4, 125.3, 121.3, 120.1, 117.4, 114.2, 65.7, 56.6, 46.6. MS (ESI$^+$) calcd for $[C_{31}H_{28}N_3O_5]^+$, $(M + H)^+$: 522. Found: 522; HRMS (ESI$^+$) calcd for $[C_{31}H_{28}N_3O_5]^+$, $(M + H)^+$: 522.2029. Found: 522.2024.

Fmoc-Ala-Dbz-OH (1d). The same procedure to produce **1c** was applied with Fmoc-Ala-OH (1.00 g, 3.2 mmol), HATU (1.47 g, 3.9 mmol, 1.2 equiv), and diaminobenzoic acid (0.64 g, 4.2 mmol, 1.3 equiv) to give **1d** (1.24 g, 87%). mp = 171−173 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 9.24 (s, 1H; CH), 7.89 (d, J = 7.6 Hz, 2H, CH), 7.76−7.69 (m, 4H), 7.53 (dd, J = 8.4 Hz, 2.0 Hz, 1H; CH), 7.42 (t, J = 7.2 Hz, 2H; CH), 7.33 (t, J = 7.6 Hz, 1H; CH), 6.71 (d, J = 8.4 Hz, 1H; NH), 5.62 (s, 2H; NH_2), 4.32−4.18 (m, 4H), 1.33 (d, J = 6.8 Hz, 3H; CH_3). ^{13}C{^{1}H} NMR (101 MHz, DMSO-d_6): δ 171.9, 167.3, 156.0, 147.2, 143.9, 143.8, 140.7, 128.3, 128.0, 127.7, 127.1, 125.3, 125.3, 121.4, 120.1, 117.4, 114.2, 65.7, 50.6, 46.7, 17.9. MS (ESI$^+$) calcd for $[C_{25}H_{24}N_3O_5]^+$, $(M + H)^+$: 446. Found: 446; HRMS (ESI$^+$) calcd for $[C_{25}H_{24}N_3O_5]^+$, $(M + H)^+$: 446.1716. Found: 446.1712.

Fmoc-Gly-Db-OH (1e). The same procedure to produce **1c** was applied with Fmoc-Gly-OH (1.00 g, 3.4 mmol), HATU (1.53 g, 4.0 mmol, 1.2 equiv), and diaminobenzoic acid (0.67 g, 4.4 mmol, 1.3 equiv). Half of the DMF was removed under vacuum, and H_2O (75 mL) was added. The resulting slurry was stirred for 60 min and filtrated. The residue was dried under vacuum and suspended in DCM (2% MeOH, 75 mL). After the mixture was stirred at rt for 60 min, filtration gave **1e** (1.32 g, 90%). mp = 162−164 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 12.17 (s, 1H; OH), 9.15 (s, 1H; NH), 7.90 (d, J = 7.6 Hz, 2H; CH), 7.76−7.71 (m, 3H), 7.62 (t, J = 6.0 Hz, 1H; CH), 7.52 (dd, J = 8.4, 2.0 Hz, 1H; CH), 7.42 (t, J = 7.2 Hz, 2H; CH), 7.33 (t, J = 7.2 Hz, 2H; CH), 6.70 (d, J = 8.4 Hz, 1H; CH), 5.68 (s, 2H; NH_2), 4.32 (d, J = 6.8 Hz, 2H; CH_2), 4.25 (t, J = 6.8 Hz, 1H; CH), 3.83 (d, J = 5.6 Hz, 2H; CH_2). ^{13}C{^{1}H} NMR (101 MHz, DMSO-d_6): δ 168.4, 167.3, 156.7, 147.1, 143.9, 140.8, 128.2, 128.0, 127.7, 127.1, 125.3, 121.4, 120.1, 117.3, 114.1, 65.8, 46.7, 43.8. MS (ESI$^+$) calcd for $[C_{24}H_{21}N_3NaO_5]^+$, $(M + Na)^+$: 454. Found: 454; HRMS (ESI$^+$) calcd for $[C_{24}H_{21}N_3NaO_5]^+$, $(M + Na)^+$: 454.1379. Found: 454.1373.

Fmoc-Val-Dbz-OH (1f). The same procedure to produce **1c** was applied with Fmoc-Val-OH (1.00 g, 2.9 mmol), HATU (1.34 g, 3.5 mmol, 1.2 equiv), and diaminobenzoic acid (0.58 g, 3.8 mmol, 1.3 equiv) to give **1f** (1.17 g, 85%). mp = 198−199 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 12.19 (s, 1H; OH), 9.32 (s, 1H; NH), 7.89 (d, J = 7.6 Hz, 2H; CH), 7.76 (t, J = 6.4 Hz, 3H; CH, NH), 7.66 (d, J = 8 Hz, 1H; CH), 7.53 (dd, J = 8.4, 2.0 Hz, 1H; CH), 7.41 (t, J = 7.2 Hz, 2H; CH), 7.33 (t, J = 7.2 Hz, 2H; CH), 6.71 (d, J = 8.4 Hz, 1H; CH), 5.61 (s, 2H; NH_2), 4.30−4.24 (m, 3H), 3.99 (t, J = 7.6 Hz, 1H; CH), 2.07 (dquent, J = 6.8, 6.8 Hz, 1H; CH), 0.97 (d, J = 6.8 Hz, 6H; CH_3). ^{13}C{^{1}H} NMR (101 MHz, DMSO-d_6): δ 170.8, 167.3, 156.5, 147.0, 143.9, 143.8, 140.7, 128.3, 127.7, 127.1, 125.4, 121.5, 120.1, 117.5, 114.3, 65.8, 61.0, 46.7, 29.9, 19.3, 18.8. MS (ESI$^+$) calcd for $[C_{27}H_{28}N_3O_5]^+$, $(M + H)^+$: 474. Found: 474; HRMS (ESI$^+$) calcd for $[C_{27}H_{28}N_3O_5]^+$, $(M + H)^+$: 474.2029. Found: 474.2024.

Fmoc-Leu-Dbz-OH (1g). The same procedure to produce **1c** was applied with Fmoc-Leu-OH (1.00 g, 2.8 mmol), HATU (1.29 g, 3.4 mmol, 1.2 equiv), and diaminobenzoic acid (0.56 g, 3.7 mmol, 1.3 equiv) to give **1g** (1.13 g, 83%). mp = 204−206 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 9.31 (s, 1H; NH), 7.89 (d, J = 7.6 Hz, 2H; CH), 7.75−7.70 (m, 3H), 7.67 (d, J = 7.2 Hz, 1H; CH), 7.52 (dd, J = 8.4, 2.0 Hz, 1H; CH), 7.41 (t, J = 7.6 Hz, 2H; CH), 7.32 (t, J = 7.6 Hz, 2H; CH), 6.70 (d, J = 8.4 Hz, 1H; CH), 5.59 (s, 2H; NH_2), 4.31−4.16 (m, 4H), 1.71−1.54 (m, 3H), 0.92 (dd, J = 12.4, 6.4 Hz, 6H; CH_3). ^{13}C{^{1}H} NMR (101 MHz, DMSO-d_6): δ 171.7, 167.2, 156.2, 147.2, 143.9, 143.7, 140.7, 128.3, 128.0, 127.7, 127.1, 125.3, 121.5, 120.1, 117.4, 114.2, 65.7, 53.5, 46.7, 24.3, 23.0, 21.7. MS (ESI$^+$) calcd for $[C_{28}H_{30}N_3O_5]^+$, $(M + H)^+$: 488. Found: 488; HRMS (ESI$^+$) calcd for $[C_{28}H_{30}N_3O_5]^+$, $(M + H)^+$: 488.2185. Found: 488.2181.

Fmoc-Met-Dbz-OH (1h). The same procedure to produce **1c** was applied with Fmoc-Met-OH (1.00 g, 2.7 mmol), HATU (1.23 g, 3.2 mmol, 1.2 equiv), and diaminobenzoic acid (0.53 g, 3.5 mmol, 1.3 equiv) to give **1h** (1.28 g, 94%). mp = 177−179 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 12.15 (s, 1H; OH), 9.30 (s, 1H; NH), 7.89 (d, J = 7.6 Hz, 2H; CH), 7.76−7.73 (m, 4H), 7.53 (dd, J = 8.4, 2.0 Hz, 1H; CH), 7.42 (t, J = 7.6 Hz, 2H; CH), 7.33 (t, J = 7.6 Hz, 2H; CH), 6.71 (d, J = 8.4 Hz, 1H; CH), 5.61 (s, 2H; NH_2), 4.33−4.22 (m, 4H), 2.61−2.51 (m, 2H; CH_2), 2.08 (s, 3H; CH_3), 2.04−1.89 (m, 2H; CH_2). ^{13}C{^{1}H} NMR (101 MHz, DMSO-d_6): δ 170.9, 167.2, 156.3, 147.2, 143.9, 143.7, 140.7, 128.4, 128.1, 127.7, 127.1, 125.3, 121.4, 120.1, 117.4, 114.2, 65.7, 54.4, 46.7, 31.3, 29.8, 14.7. MS (ESI$^+$) calcd for $[C_{27}H_{28}N_3O_5S]^+$, $(M + H)^+$:

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506. Found: 506; HRMS (ESI$^+$) calcd for $[C_{27}H_{28}N_3O_5S]^+$, $(M + H)^+$: 506.1750. Found: 506.1741.

Fmoc-Asp(OtBu)-OH (1i). The same procedure to produce **1c** was applied with Fmoc-Asp(OtBu)-OH (1.00 g, 2.4 mmol), HATU (1.11 g, 2.9 mmol, 1.2 equiv), and diaminobenzoic acid (0.48 g, 3.2 mmol, 1.3 equiv) to give **1i** (1.22 g, 93%). mp = 150−152 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 12.14 (s, 1H; OH), 9.29 (s, 1H; NH), 7.89 (d, J = 7.6 Hz, 2H; CH), 7.79 (d, J = 7.6 Hz, 1H; CH), 7.74−7.70 (m, 3H), 7.53 (dd, J = 8.4, 2.0 Hz, 1H; CH), 7.41 (t, J = 7.6 Hz, 2H; CH), 7.32 (t, J = 7.6 Hz, 2H; CH), 6.70 (d, J = 8.4 Hz, 1H; CH), 5.65 (s, 2H; NH_2), 4.52 (q, J = 6.4 Hz, 1H; CH), 4.34−4.22 (m, 3H; CH, CH_2), 2.68 (dd, J = 14.8, 6.0 Hz, 1H; CH_2), 2.57 (dd, J = 16.0, 8.0 Hz, 1H; CH_2), 1.40 (s, 9H; CH_3). ^{13}C{^{1}H} NMR (101 MHz, DMSO-d_6): δ 169.7, 169.5, 167.2, 155.9, 151.1, 147.4, 143.8, 143.7, 140.7, 128.5, 128.4, 127.7, 127.1, 125.3, 121.2, 120.1, 117.3, 114.1, 80.3, 65.8, 63.7, 52.8, 51.8, 46.6, 37.5, 27.7. MS (ESI$^+$) calcd for $[C_{30}H_{31}N_3NaO_7]^+$, $(M + Na)^+$: 568. Found: 568; HRMS (ESI$^+$) calcd for $[C_{30}H_{31}N_3NaO_7]^+$, $(M + Na)^+$: 568.2060. Found: 568.2057.

Fmoc-Lys(Boc)-Dbz-OH (1j). The same procedure to produce **1c** was applied with Fmoc-Lys(Boc)-OH (1.00 g, 2.1 mmol, 1 equiv), HATU (0.97 g, 2.6 mmol, 1.2 equiv), and diaminobenzoic acid (0.42 g, 2.8 mmol, 1.3 equiv) to give **1i** (1.09 g, 86%). mp = 181−183 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 12.16 (s, 1H; OH), 9.26 (s, 1H; NH), 7.89 (d, J = 7.2 Hz, 2H; CH), 7.75−7.72 (m, 3H), 7.65 (d, J = 7.2 Hz, 1H; CH), 7.52 (dd, J = 8.4, 2.0 Hz, 1H; CH), 7.41 (t, J = 7.2 Hz, 2H; CH), 7.33 (t, J = 7.2 Hz, 2H; CH), 6.79 (t, J = 5.6 Hz, 1H; NH), 6.70 (d, J = 8.4 Hz, 1H; CH), 5.60 (s, 2H; NH_2), 4.30−4.22 (m, 3H; CH, CH_2), 4.11 (q, J = 6.4 Hz, 1H; CH), 2.92 (q, J = 5.6 Hz, 2H; CH_2), 1.78−1.61 (m, 2H; CH_2), 1.46−1.25 (m, 2H, CH_2), 1.36 (s, 9H, CH_3), ^{13}C{^{1}H} NMR (101 MHz, DMSO-d_6): δ 171.4, 167.2, 156.3, 155.6, 147.0, 143.9, 143.8, 140.7, 128.3, 127.9, 127.7, 127.1, 125.3, 121.5, 120.1, 117.4, 114.2, 77.4, 65.7, 55.2, 46.7, 31.3, 29.3, 28.3, 23.0. MS (ESI$^+$) calcd for $[C_{33}H_{39}N_4O_7]^+$, $(M + H)^+$: 603. Found: 603; HRMS (ESI$^+$) calcd for $[C_{33}H_{39}N_4O_7]^+$, $(M + H)^+$: 603.2819. Found: 603.2811.

Fmoc-Ser(tBu)-Dbz-OH (1k). To a solution of Fmoc-Ser(tBu)-OH (1.00 g, 2.6 mmol) and HATU (1.19 g, 3.1 mmol, 1.2 equiv) in DMF (10 mL) was added NMM (0.57 mL, 5.2 mmol, 2 equiv) under N_2. After the solution was stirred for 60 min under N_2, diaminobenzoic acid (0.52 g, 3.4 mmol, 1.3 equiv) was added to the resulting solution in four portions every 10 min at 0 °C. The resulting solution was stirred at room temperature for 140 min under N_2. Half of the DMF was removed under vacuum, and water (75 mL) was added to the resulting mixture. The resulting slurry was stirred for 60 min and filtrated. The residue was dried under vacuum and suspended in DCM (1:1, 75 mL). After stirring at room temperature for 60 min, filtration gave the crude product dissolved in acetone (c.a. 300 mL). Water (300 mL) was then added to the solution at 0 °C. The resulting mixture was centrifuged (6000 rpm), and filtration gave the desired product (1.08 g, 78% yield). mp = 150−152 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 12.17 (s, 1H; OH), 9.33 (s, 1H; NH), 7.89 (d, J = 7.6 Hz, 2H; CH), 7.75 (dd, J = 7.2, 4.0 Hz, 2H; CH), 7.68 (d, J = 2.0 Hz, 1H; CH), 7.55 (dd, J = 8.4, 2.0 Hz, 1H; NH), 7.52 (d, J = 8.4 Hz, 1H; CH), 7.42 (t, J = 7.6 Hz, 2H; CH), 7.33 (t, J = 7.6 Hz, 2H; CH), 6.70 (d, J = 8.8 Hz, 1H; CH), 5.62 (s, 2H; NH_2), 4.32−4.22 (m, 5H), 3.60−2.53 (m, 3H), 1.16 (s, 9H; CH_3). ^{13}C{^{1}H} NMR (101 MHz, DMSO-d_6): δ 169.4, 167.2, 156.0, 151.1, 147.5, 143.9, 143.8, 140.7, 128.9, 128.7, 128.4, 127.7, 127.1, 125.4, 121.3, 120.1, 117.3, 114.0, 73.1, 65.9, 63.5, 61.9, 55.5, 52.6, 46.7, 27.2. MS (ESI$^+$) calcd for $[C_{29}H_{32}N_3O_6]^+$, $(M + H)^+$: 518. Found: 518; HRMS (ESI$^+$) calcd for $[C_{29}H_{32}N_3O_6]^+$, $(M + H)^+$: 518.2291. Found: 518.2289.

Fmoc-Gln(Trt)-Dbz-OH (1l). The same procedure to produce **1k** was applied with Fmoc-Gln(Trt)-OH (1.00 g, 1.6 mmol), HATU (0.75 g, 2.0 mmol, 1.2 equiv), and diaminobenzoic acid (0.32 g, 2.1 mmol, 1.3 equiv) to give **1l** (0.98 g, 82%). mp = 178−179 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 12.17 (s, 1H; OH), 9.22 (s, 1H; NH), 8.64 (s, 1H; NH),7.89 (d, J = 7.6 Hz, 2H; CH), 7.76−7.73 (m, 3H), 7.63 (d, J = 7.2 Hz, 1H; CH), 7.52 (d, J = 8.4 Hz, 1H; CH), 7.41 (t, J = 7.2 Hz, 2H; CH), 7.34−7.17(m, 17H), 6.70 (d, J = 8.4 Hz, 1H; CH), 5.60 (s, 2H; NH_2), 4.32 (d, J = 6.8 Hz, 2H; CH_2), 4.24 (t, J = 6.0 Hz, H; CH), 4.11 (q, J = 6.4 Hz, 1H; CH), 2.45−4.32 (m, 2H; CH_2), 2.00−1.79 (m, 2H; CH_2). ^{13}C{^{1}H} NMR (101 MHz, DMSO-d_6): δ 171.4, 170.9, 167.2, 156.2, 151.1, 147.0, 144.9, 143.8, 143.8, 140.7, 128.5, 128.0, 127.7, 127.5, 127.1, 126.3, 125.3, 121.4, 120.1, 117.4, 114.2, 112.8, 69.2, 65.8, 63.5, 55.0, 52.6, 46.7, 32.8, 27.7. MS (ESI$^+$) calcd for $[C_{46}H_{41}N_4O_6]^+$, $(M + H)^+$: 745. Found: 745; HRMS (ESI$^+$) calcd for $[C_{46}H_{41}N_4O_6]^+$, $(M + H)^+$: 745.3026. Found: 745.3022.

Fmoc-Nap-Dbz-OH (1m). The same procedure to produce **1k** was applied with Fmoc-Nap-OH (1.00 g, 1.8 mmol), HATU (0.80 g, 2.1 mmol, 1.2 equiv), and diaminobenzoic acid (0.35 g, 2.3 mmol, 1.3 equiv) to give **1m** (0.52 g, 50%). mp = 175−177 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 12.13 (s, 1H; OH), 9.32 (s, 1H; NH), 8.27 (d, J = 8.4 Hz, 1H; CH), 7.94−7.81 (m, 5H), 7.69−7.49 (m, 7H), 7.42−7.27 (m, 6H), 6.69 (d, J = 8.4 Hz, 1H; CH), 5.53 (s, 2H; NH_2), 4.56 (s, 1H, CH_2), 4.22−4.13 (m, 3H), 3.62 (d, J = 13.6 Hz, 1H, CH_2). ^{13}C{^{1}H} NMR (101 MHz, DMSO-d_6): δ 188.5, 170.1, 162.7, 159.6, 158.9, 141.8, 132.8, 130.5, 126.4, 124.1, 120.1, 115.9, 108.7, 99.9, 67.1, 65.0, 25.2. MS (ESI$^+$) calcd for $[C_{35}H_{30}N_3O_5]^+$, $(M + H)^+$: 572. Found: 572; HRMS (ESI$^+$) calcd for $[C_{35}H_{30}N_3O_5]^+$, $(M + H)^+$: 572.2186. Found: 572.2186.

Fmoc-AHX-Dbz-OH (1n). The same procedure to produce **1c** was applied with Fmoc-AHX-OH (1.00 g, 2.1 mmol), HATU (0.96 g, 2.5 mmol, 1.2 equiv), and diaminobenzoic acid (0.42 g, 2.8 mmol, 1.3 equiv) to give **1n** (0.67 g, 65%). mp = 174−175 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 12.15 (s, 1H; OH), 9.05 (s, 1H; NH), 7.88 (d, J = 7.6 Hz, 2H; CH), 7.81 (d, J = 1.6 Hz, 1H; CH), 7.68 (d, J = 7.2 Hz, 2H; CH), 7.49 (dd, J = 8.8, 2.0 Hz, 1H; CH), 7.41 (t, J = 7.2 Hz, 2H; CH), 7.35−7.27 (m, 3H), 6.70 (d, J = 7.6 Hz, 1H; CH), 5.64 (s, 2H; NH_2), 4.29 (d, J = 6.8 Hz, 2H; CH_2), 4.21 (t, J = 6.4 Hz, 1H; CH), 2.99 (q, J = 6.4 Hz, 2H; CH_2), 2.32 (t, J = 7.2 Hz, 2H; CH_2), 1.59 (p, J = 7.2 Hz, 2H; CH_2), 1.44 (p, J = 7.2 Hz, 2H; CH_2), 1.30 (p, J = 7.2 Hz, 2H; CH_2). ^{13}C{^{1}H} NMR (101 MHz, DMSO-d_6): δ 167.3, 146.5, 144.0, 140.7, 127.8, 127.6, 127.4, 127.1, 125.2. 122.1, 120.1, 117.4, 114.3, 65.2, 46.8, 35.7, 29.2, 26.0, 24.9. MS (ESI$^+$) calcd for $[C_{28}H_{29}N_3NaO_5]^+$, $(M + H)^+$: 510. Found: 510; HRMS (ESI$^+$) calcd for $[C_{28}H_{29}N_3NaO_5]^+$, $(M + H)^+$: 510.1999. Found: 510.1998.

Fmoc-Ile-Dbz-OH (1o). The same procedure to produce **1c** was applied with Fmoc-Ile-OH (1.00 g, 2.8 mmol), HATU (1.29 g, 3.4 mmol, 1.2 equiv), and diaminobenzoic acid (0.56

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g, 3.7 mmol, 1.3 equiv). After the reaction completed, half of the DMF was removed under vacuum, and H_2O was added (75 mL). The resulting slurry was stirred for 60 min and filtrated. The residue was dried under vacuum and suspended in a mixture of DCM and hexane (1:1, 75 mL). After the mixture was stirred at rt for 60 min, filtration gave **1o** (1.23 g, 91%). mp = 176−178 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 12.19 (s, 1H; OH), 9.33 (s, 1H; NH), 7.89 (d, J = 4.8 Hz, 2H; CH), 7.76−7.73 (m, 3H), 7.68 (d, J = 8.0 Hz, 1H; CH), 7.53 (dd, J = 8.4, 2.0 Hz, 1H; CH), 7.41 (t, J = 7.2 Hz, 2H; CH), 7.32 (q, J = 7.2 Hz, 2H; CH), 6.71 (d, J = 8.4 Hz, 1H; CH), 5.60 (s, 2H; NH_2), 4.33−4.22 (m, 3H; CH, CH_2), 4.02 (t, J = 8.4 Hz, 1H, CH), 1.89−1.82 (m, 1H), 1.58−1.53 (m, 1H), 1.26−1.18 (m, 1H), 0.94 (d, J = 6.8 Hz, 3H; CH_3), 0.88 (t, J = 7.6 Hz, 3H; CH_3). ^{13}C{^{1}H} NMR (101 MHz, DMSO-d_6): δ 170.9, 167.3, 156.4, 147.0, 143.9, 143.8, 140.7, 128.3, 127.7, 127.1, 125.4, 125.4, 121.5, 120.1, 117.5, 114.3, 65.8, 59.7, 46.7, 35.8, 24.8, 15.4, 10.8. MS (ESI$^+$) calcd for $[C_{28}H_{30}N_3O_5]^+$, $(M + H)^+$: 488. Found: 488; HRMS (ESI$^+$) calcd for $[C_{28}H_{30}N_3O_5]^+$, $(M + H)^+$: 488.2180. Found: 488.2178.

Fmoc-Glu(tBu)-Dbz-OH (1p). The same procedure to produce **1o** was applied with Fmoc-Glu(tBu)-OH (1.00 g, 2.4 mmol), HATU (1.07 g, 2.8 mmol, 1.2 equiv), and diaminobenzoic acid (0.47 g, 3.1 mmol, 1.3 equiv) to give **1p** (1.25 g, 93%). mp = 171−172 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 12.17 (s, 1H; OH), 9.28 (s, 1H; NH), 7.89 (d, J = 7.6 Hz, 2H; CH), 7.76−7.70 (m, 4H), 7.53 (dd, J = 8.4, 2.0 Hz, 1H; CH), 7.42 (t, J = 7.6 Hz, 2H; CH), 7.33 (t, J = 7.6 Hz, 2H; CH), 6.71 (d, J = 8.8 Hz, 1H; CH), 5.62 (s, 2H; NH_2), 4.35−4.22 (m, 3H; CH, CH_2), 4.16 (q, J = 5.6 Hz, 1H; CH), 2.38−2.24 (m, 2H; CH_2), 2.04−1.83 (m, 2H; CH_2), 1.41 (s, 9H; CH_3). ^{13}C{^{1}H} NMR (101 MHz, DMSO-d_6): δ 171.6, 170.7, 167.2, 156.2, 147.2, 143.9, 143.7, 140.7, 128.4, 128.1, 127.7, 127.1, 125.3, 121.3, 120.1, 117.4, 114.2, 79.8, 65.8, 54.4, 46.7, 31.4, 27.8, 26.9. MS (ESI$^+$) calcd for $[C_{31}H_{33}N_3NaO_7]^+$, $(M + Na)^+$: 582. Found: 582; HRMS (ESI$^+$) calcd for $[C_{31}H_{33}N_3NaO_7]^+$, $(M + Na)^+$: 582.2211. Found: 582.2209.

Fmoc-Cys(Trt)-Dbz-OH (1q). The same procedure to produce **1o** was applied with Fmoc-Cys(Trt)-OH (1.00 g, 1.7 mmol), HATU (0.78 g, 2.1 mmol, 1.2 equiv), and diaminobenzoic acid (0.34 g, 2.2 mmol, 1.3 equiv). After the first filtration, the residue was dissolved in acetone (c.a. 300 mL). To the solution was added water (300 mL) at 0 °C. The resulting mixture was centrifuged (6000 rpm), and decantation gave **1q** (0.74 g, 60%). mp = 170−172 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 12.19 (s, 1H; OH), 9.33 (s, 1H; NH), 7.88 (d, J = 7.6 Hz, 2H; CH), 7.82 (d, J = 7.6 Hz, 1H; CH), 7.75−7.70 (m, 3H), 7.53 (d, J = 8.4 Hz, 1H; CH), 7.40 (t, J = 7.6 Hz, 2H; CH), 7.36−7.23 (m, 19H), 6.69 (d, J = 8.4 Hz, 1H; CH), 5.60 (s, 2H; NH_2), 4.33−4.21 (m, 4H). ^{13}C{^{1}H} NMR (101 MHz, DMSO-d_6): δ 169.1, 167.2, 155.8, 151.0, 147.2, 144.2, 143.8, 143.7, 140.7, 129.1, 128.8, 128.6, 128.2, 128.1, 127.6, 127.1, 126.8, 125.3, 121.0, 120.7, 120.1, 117.3, 114.1, 66.0, 65.9, 63.7, 54.0, 52.9, 46.6, 42.9, 33.7. MS (ESI$^+$) calcd for $[C_{44}H_{38}N_3O_5S]^+$, $(M + H)^+$: 720. Found: 720; HRMS (ESI$^+$) calcd for $[C_{44}H_{38}N_3O_5S]^+$, $(M + H)^+$: 720.2527. Found: 720.2524.

Fmoc-Thr(tBu)-Dbz-OH (1r). The same procedure to produce **1q** was applied with Fmoc-Thr(tBu)-OH (1.00 g, 2.5 mmol), HATU (1.15 g, 3.0 mmol, 1.2 equiv), and diaminobenzoic acid (0.50 g, 3.3 mmol, 1.3 equiv) to give **1r** (0.81 g, 61%). mp = 154−155 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 12.19 (s, 1H; OH), 8.95 (s, 1H; NH), 7.89 (d, J = 7.2 Hz, 2H; CH), 7.81−7.72 (m, 3H), 7.54 (d, J = 8.4 Hz, 1H; CH), 7.42 (t, J = 7.6 Hz, 2H; CH), 7.33 (t, J = 7.6 Hz, 2H; CH), 7.18 (d, J = 7.2 Hz, 1H; CH), 6.72 (d, J = 8.4 Hz, 1H; CH), 5.69 (s, 2H; NH_2), 4.37−4.22 (m, 4H), 4.00 (t, J = 5.2 Hz, 1H; CH), 1.18 (s, 9H; CH_3), 1.10 (d, J = 6.0 Hz, 3H; CH_3). ^{13}C{^{1}H} NMR (101 MHz, DMSO-d_6): δ 168.6, 167.2, 156.0, 151.1, 146.9, 143.9, 143.7, 140.7, 128.9, 128.4, 127.7, 127.1, 125.4, 121.3, 120.7, 120.1, 117.5, 114.1, 74.2, 67.3, 65.9, 63.5, 59.8, 52.6, 46.7, 42.5, 27.9, 19.0. MS (ESI$^+$) calcd for $[C_{30}H_{34}N_3O_6]^+$, $(M + H)^+$: 532. Found: 532; HRMS (ESI$^+$) calcd for $[C_{30}H_{34}N_3O_6]^+$, $(M + H)^+$: 532.2442. Found: 532.2441.

Fmoc-Arg(Pbf)-Dbz-OH (1s). The same procedure to produce **1q** was applied with Fmoc-Arg(Pbf)-OH (1.00 g, 1.5 mmol), HATU (0.70 g, 1.9 mmol, 1.2 equiv), and diaminobenzoic acid (0.31 g, 2.0 mmol, 1.3 equiv) to give **1s** (1.00 g, 83%). mp = 169−171 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 12.20 (s, 1H; OH), 9.29 (s, 1H; NH), 7.89 (d, J = 7.6 Hz, 2H; CH), 7.75−7.68 (m, 4H), 7.52 (d, J = 8.4, 2 Hz, 1H; CH), 7.41 (t, J = 7.6 Hz, 2H; CH), 7.32 (t, J = 7.6 Hz, 2H; CH), 6.71 (d, J = 8.4 Hz, 1H; CH), 6.40 (s, 2H; NH), 5.61 (s, 2H; NH_2), 4.31−4.21 (m, 3H; CH, CH_2), 4.13 (q, J = 5.6 Hz, 1H; CH), 3.15−3.03 (m, 3H; CH_3), 2.92 (s, 3H; CH_3), 2.42 (s, 3H; CH_3), 1.98 (s, 3H; CH_3), 1.82−1.42 (m, 6H; CH_2), 1.38 (s, 6H; CH_3). ^{13}C{^{1}H} NMR (101 MHz, DMSO-d_6): δ 171.1, 167.2, 157.5, 156.2, 156.1, 151.2, 147.1, 143.9, 143.7, 140.7, 137.3, 131.5, 128.9, 128.4, 127.9, 127.7, 127.1, 125.3, 124.3, 121.4, 120.8, 120.1, 117.4, 116.3, 114.2, 86.3, 65.7, 63.5, 54.8, 52.6, 46.7, 42.4, 28.3, 19.0, 17.6, 12.3. MS (ESI$^+$) calcd for $[C_{41}H_{47}N_6O_8S]^+$, $(M + H)^+$: 783. Found: 783; HRMS (ESI$^+$) calcd for $[C_{41}H_{47}N_6O_8S]^+$, $(M + H)^+$: 783.3171. Found: 783.3173.

Fmoc-Trp(Boc)-Dbz-OH (1t). The same procedure to produce **1q** was applied with Fmoc-Trp(Boc)-OH (1.00 g, 1.9 mmol), HATU (0.87 g, 2.3 mmol, 1.2 equiv), and diaminobenzoic acid (0.38 g, 2.5 mmol, 1.3 equiv) to give **1t** (0.94 g, 75%). mp = 182−183 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 12.16 (s, 1H; OH), 9.44 (s, 1H; NH), 8.04 (d, J = 8.0 Hz, 1H; CH), 7.90−7.86 (m, 3H), 7.80 (d, J = 8.0 Hz, 1H; CH), 7.71 (s, 1H; CH), 7.65−7.64 (m, 3H), 7.53 (d, J = 8.8 Hz, 1H; CH), 7.41−7.22 (m, 6H), 6.71 (d, J = 8.0 Hz, 1H; CH), 5.58 (s, 2H; NH_2), 4.54 (q, J = 5.6 Hz, 1H; CH), 4.25−4.18 (m, 3H), 3.26−3.04 (m, 2H), 1.57 (s, 9H; CH_3). ^{13}C{^{1}H} NMR (101 MHz, DMSO-d_6): δ 170.7, 167.3, 156.1, 149.1, 147.2, 143.8, 143.7, 140.7, 134.7, 130.3, 128.9, 128.5, 128.2, 127.6, 127.0, 125.3, 124.4, 124.1, 122.5, 121.3, 120.7, 120.1, 119.6, 117.4, 116.7, 114.7, 114.2, 83.5, 65.9, 63.7, 54.9, 52.8, 46.6, 42.8, 27.7. MS (ESI$^+$) calcd for $[C_{38}H_{37}N_4O_7]^+$, $(M + H)^+$: 661. Found: 661; HRMS (ESI$^+$) calcd for $[C_{38}H_{37}N_4O_7]^+$, $(M + H)^+$: 661.2657. Found: 661.2659.

Fmoc-Tyr(tBu)-Dbz-OH (1u). The same procedure to produce **1q** was applied with Fmoc-Tyr(tBu)-OH (1.00 g, 2.2 mmol), HATU (1.00 g, 2.6 mmol, 1.2 equiv), and diaminobenzoic acid (0.43 g, 2.8 mmol, 1.3 equiv) to give **1u** (0.90 g, 69%). mp = 170−172 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 12.17 (s, 1H; OH), 9.30 (s, 1H; NH), 7.88 (d, J = 7.6 Hz, 2H; CH), 7.80 (d, J = 6.8 Hz, 1H; CH), 7.70−7.68 (m, 3H), 7.52 (d, J = 8.4 Hz, 1H; CH), 7.41 (t, J = 7.6 Hz, 2H; CH), 7.33 (t, J = 7.2 Hz, 1H; CH), 7.31 (t, J = 7.2 Hz, 1H; CH), 7.24 (d, J = 7.6 Hz, 2H; CH), 6.85 (d, J = 7.6 Hz, 2H; CH), 6.70 (d, J = 8.4 Hz, 1H; CH), 5.55 (s, 2H; NH_2), 4.39 (q, J = 8.0 Hz, 1H, CH), 4.24−4.15 (m, 3H), 3.07−2.85 (m, 2H; CH_2), 1.22 (s, 9H; CH_3). ^{13}C{^{1}H} NMR (101 MHz,

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DMSO-d_6): δ 170.8, 167.2, 156.0, 153.5, 147.1, 143.8, 143.7, 140.7, 132.4, 129.8, 128.4, 128.0, 127.6, 127.1, 125.3, 123.4, 121.3, 120.1, 117.4, 114.1, 77.6, 65.8, 63.5, 56.6, 52.6, 46.6, 36.8, 28.5. MS (ESI$^+$) calcd for $[C_{35}H_{36}N_3O_6]^+$, $(M + H)^+$: 594. Found: 594; HRMS (ESI$^+$) calcd for $[C_{35}H_{36}N_3O_6]^+$, $(M + H)^+$: 594.2599. Found: 594.2598.

Fmoc-Asn(Trt)-Dbz-OH (1v). The same procedure to produce **1q** was applied with Fmoc-Asn(Trt)-OH (1.00 g, 1.7 mmol), HATU (0.76 g, 2.0 mmol, 1.2 equiv), and diaminobenzoic acid (0.33 g, 2.2 mmol, 1.3 equiv) to give **1v** (1.02 g, 82%). mp = 167−169 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 12.18 (s, 1H; OH), 9.27 (s, 1H; NH), 8.65 (s, 1H; NH), 7.90 (d, J = 7.6 Hz, 2H; CH), 7.78−7.72 (m, 3H), 7.52 (dd, J = 8.4, 2.0 Hz, 1H; CH), 7.42 (t, J = 7.2 Hz, 1H; CH), 7.41 (t, J = 7.2 Hz, 1H; CH), 7.34−7.12 (m, 17H), 6.70 (d, J = 8.4 Hz, 1H; CH), 5.61 (s, 2H; NH_2), 4.45 (q, J = 6.8 Hz, 1H; CH), 4.40−4.23 (m, 3H), 2.74 (d, J = 7.6 Hz, 2H; CH_2). ^{13}C{^{1}H} NMR (101 MHz, DMSO-d_6): δ: 170.5, 168. 9, 167.3, 155.9, 150.8, 147.1, 144.7, 143.8, 143.8, 140.7, 128.7, 128.6, 128.3, 128.0, 127.7, 127.5, 127.1, 126.4, 125.3, 125.2, 121.4, 120.6, 120.1, 117.3, 114.2, 69.5, 65.8, 64.1, 53.2, 52.4, 46.7, 45.8, 8.7. MS (ESI$^+$) calcd for $[C_{45}H_{39}N_{34}O_6]^+$, $(M + H)^+$: 731. Found: 731; HRMS (ESI$^+$) calcd for $[C_{45}H_{39}N_{34}O_6]^+$, $(M + H)^+$: 731.2864. Found: 731.2863.

Fmoc-His(Trt)-Dbz-OH (1w). To a solution of Fmoc-His(Trt)-OH (1.00 g, 1.6 mmol) and HATU (0.74 g, 1.9 mmol, 1.2 equiv) in DMF (10 mL) was added NMM (0.35 mL, 3.2 mmol, 2 equiv) under N_2. After the mixture was stirred for 60 min under N_2, diaminobenzoic acid (0.32 g, 2.1 mmol, 1.3 equiv) was added to the resulting solution in four portions every 10 min at 0 °C. The resulting solution was stirred at room temperature for 140 min under N_2. Half of the DMF was removed under vacuum, and water (75 mL) was added to the resulting mixture. The resulting slurry was stirred for 60 min and filtrated. The residue was dried under vacuum and suspended in a mixture of DCM and hexane (1:1, 75 mL). After stirring at room temperature for 60 min, filtration gave the crude product dissolved in acetone (c.a. 300 mL). Water (300 mL) was then added to the solution at 0 °C. The resulting mixture was centrifuged (6000 rpm), and the supernatant was decanted. The resulting residue was chromatographically purified (ϕ 2.1 cm × 13 cm, eluted by DCM/MeOH = 95/5) to give **1w** (0.48 g, 40%, R_f = 0.3, DCM/MeOH = 24). mp = 142−145 °C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 9.28 (s, 1H; NH), 7.89 (d, J = 7.6 Hz, 2H; CH), 7.72−7.68 (m, 3H), 7.62 (d, J = 7.2 Hz, 1H; CH), 7.53 (dd, J = 8.0, 2.0 Hz, 1H; CH), 7.42−7.25 (m, 16H), 7.07−7.05 (m, 6H), 6.84 (s, 1H), 6.70 (d, J = 8.4 Hz, 1H; CH), 4.45 (q, J = 7.2 Hz, 1H; CH), 4.30−4.17 (m, 3H; CH, CH_2), 2.95 (ddd, 2H; CH_2). ^{13}C{^{1}H} NMR (101 MHz, DMSO-d_6): δ 170.0, 167.3, 155.9, 151.1, 147.3, 143.7, 141.5, 140.7, 139.6, 137.3, 129.2, 128.9, 128.3, 128.2. 127.7, 127.1, 125.3, 121.2, 120.7, 120.1, 117.2, 114.1, 65.9, 63.5, 54.6, 52.6, 46.7, 31.0, 22.1, 14.0. MS (ESI$^+$) calcd for $[C_{47}H_{40}N_5O_5]^+$, $(M + H)^+$: 754. Found: 754; HRMS (ESI$^+$) calcd for $[C_{47}H_{40}N_5O_5]^+$, $(M + H)^+$: 754.3024. Found: 754.3025.

Fmoc-Pro-Dbz (1x). The same procedure to produce **1c** was applied with Fmoc-Pro-OH (1.00 g, 2.96 mmol), HATU (1.35 g, 3.55 mmol, 1.2 equiv), and diaminobenzoic acid (0.59 g, 3.85 mmol, 1.3 equiv) to give **1x** (1.19g, 85%). ^{1}H NMR (400 MHz, DMSO-d_6): δ 12.15 (s, 1.72H; OH), 9.47 (s, 0.57H; NH), 9.37 (s, 1H; NH), 7.91 (s, 0.55H; CH), 7.89 (dd, J = 4 Hz, 3H; CH), 7.70 (t, J = 4, 8 Hz, 4H; CH), 7.54 (d, J = 8 Hz 0.5H; CH), 7.42 (m, 1.5H, CH), 7.42 (m, 7H, CH), 7.35 (m, 1.5H, CH), 6.75 (m, 1.5H, CH), 6.75 (s, 3H, NH), 4.32 (m, 0.5H, CH), 4.26 (m, 4H, CH), 4.19 (m, 0.5H, CH), 4.14 (m, 0.5H, CH), 3.53 (m, 2H, CH), 2.4 (m, 0.5H, CH), 2.3 (m, 1H, CH), 2.25 (m, 1H, CH), 2.20 (m, 5H, CH). ^{13}C NMR (101 MHz, DMSO-d_6): δ 171.2, 171.1, 167.2, 154.3, 153.9, 147.5, 143.9, 143.7, 140.7, 140.7, 128.5, 128.3, 127.8, 127.7, 127.1, 127.0, 125.3, 125.2, 121.8, 120.1, 117.7, 117.2, 114.6, 114.0, 67.2, 66.7, 60.3, 59.8, 47.3, 46.6, 30.7, 30.1, 24.1, 23.0. HRMS (ESI$^+$) calcd for $[C_{27}H_{25}N_3O_5]^+$, $(M + H)^+$: 472. Found: 472.

Fmoc-Phe-Dbz-7-azabenzotriazole (3). The same procedure for preparing **1c** with diaminobenzoic acid (1.5 equiv) was applied. The filtrate of DCM was concentrated *in vacuo* to give a residue, which was subjected to chromatography purification (silica gel, ϕ 2.5 mm × 8 cm, eluted by hexane/EtOAc = 1/2) to give **3** (50.4 mg yield = 4.67%, R_f = 0.25, hexane/EtOAc = 15). ^{1}H NMR (400 MHz, DMSO-d_6): δ 9.44 (s, 1H; NH), 8.82 (m, 1H; CH), 8.73 (dd, J = 8.4, 1.2 Hz, 1H; CH), 7.97 (d, J = 1.2 Hz, 1H; CH), 7.85 (m, 4H; CH), 7.66 (m, 3H; CH), 7.38−7.35 (m, 10H; CH), 7.20 (t, J = 7.3 Hz, 1H; CH), 6.90 (d, J = 8.6 Hz, 1H; CH), 6.92 (s, 2H; NH), 4.46 (dd, J = 14.7, 7.8 Hz, 1H; CH), 4.25 (d, J = 7.2 Hz, 2H; CH), 4.19 (t, J = 7.0 Hz, 1H; CH), 3.13 (dd, J = 13.6, 5.6 Hz, 1H; CH), 2.97 (dd, J = 13.5, 9.3 Hz, 1H; CH). ^{13}C{^{1}H} NMR (101 MHz, DMSO-d_6): δ 170.9, 162.0, 156.0, 152.4, 143.7, 143.6, 140.6, 140.1, 137.7, 134.43, 130.0, 129.7, 129.2, 128.9, 128.0, 127.5, 126.9, 126.3, 125.22, 120.2, 114.6, 108.0, 65.6, 56.5, 46.5, 37.1. MS (MALDI+) calcd for $[C_{36}H_{29}N_7O_5Na]^+$, $(M + H)^+$: 662.2. Found: 662.2; HRMS (MALDI+) calcd for $[C_{36}H_{29}N_7O_5Na]^+$, $(M + H)^+$: 662.2121. Found: 662.2122.

2-(1-*N*-Fmoc-1-amino-2-phenyl-1*s*-ethyl)-benzimidazole-5-carboxylic Acid (4). Fmoc-Phe-Dbz-OH (**3**) (20 mg, 0.04 mmol) was added to TFA (3 mL) and stirred at 65 °C for 6 h. The solution was removed under vacuum, and the following residue was dissolved in TFA (1 mL) and ether (30 mL). The resulting mixture was left to stand at 0 °C for 2 h and centrifuged (6000 rpm × 15 min), and filtration gave the desired product (11 mg, 57%). ^{1}H NMR (400 MHz, DMSO-d_6): δ 8.24 (d, J = 7.6 Hz, 1H; CH), 8.19 (s, 1H; CH), 7.92 (d, J = 7.6 Hz, 1H; CH), 7.86 (d, J = 7.6 Hz, 2H; CH), 7.70 (d, J = 8.4 Hz, 1H; CH), 7.62 (d, J = 7.2 Hz, 2H; CH), 7.38 (t, J = 7.2 Hz, 2H; CH), 7.26−7.17 (m, 8H), 5.13 (q, J = 5.6 Hz, 1H; CH), 4.29−4.13 (m, 3H), 3.41 (dd, J = 13.6, 4.8 Hz, 1H; CH_2), 3.41 (t, J = 10.0 Hz, 1H; CH2). ^{13}C{^{1}H} NMR (101 MHz, DMSO-d_6): δ 167.8, 157.6, 156.3, 144.1, 141.1, 137.6, 129.6, 128.7, 128.1, 127.5, 127.1, 126.2, 125.7, 125.6, 124.9, 120.5, 117.0, 114.8, 66.2, 56.3, 51.6, 47.0, 30.0. MS (ESI+) calcd for $[C_{31}H_{26}N_3O_4]^+$, $(M + H)^+$: 504.2. Found: 504.2; HRMS (ESI+) calcd for $[C_{31}H_{26}N_3O_4]$+, $(M + H)^+$: 504.1923. Found: 504.1916.

Ac-GVGVP-Propargyl (8). Without further purification, crude **1x** was subjected to peptide synthesis: Rink amide resin (0.17 g, 0.05 mmol) was added into a PD-10 tube and swelled in DMF (2 mL) over 1 h. For the removal of Fmoc, the resin was treated with 2 mL of a piperidine solution (20% piperidine in DMF) and mechanical shaking (5 min × 2) followed by connectively washing with DMF (3 mL) and DCM (3 mL) three times. Crude **1x** (4 equiv), PyBOP (208.1 mg, 0.4 mmol, 4 equiv), and 5% NMM/DMF (0.5 mL) were sequentially added to the reaction vessel and reacted for 1 h. Fmoc was removed and washed as explained above for the coupling of the next amino acid residue through the same amount of residue,

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reagents, and the same procedure. After removal of Fmoc of the final glycine residue, 5% NMM (2 mL) with acetic anhydride (20 mL) was added into the reaction vessel and mechanically shaken for 30 min. The resin was added into a 20 mL vial and swelled in DMF (10.8 mL) over 30 min. To the resulting mixture was added the premixing solution of isoamyl nitrile (1.2 mL, 8.93 mmol, 180 equiv) and acetic acid (15.3 mL, 0.27 mmol, 5.4 equiv) and was shaken for 15 min. After removal of the solution, the resin was added with DMF (4 mL), propargyl amine (13 mL, 0.2 mmol, 4 equiv), and DIPEA (34 mL, 0.2 mmol, 4 equiv) and shaken for 1h. After filtration, the filtrate and the solvent were removed *in vacuo* to give a mixture. To the mixture was added 2 mL of TFA/TIS/H_2O (95:2.5:2.5 v/v/v), and the mixture was reacted for 1.5 h. After filtration, to the filtrate was added cold ether to afford the crude, which was subjected to chromatography (ϕ 2.1 cm × 13.3 cm, eluent: solvent A: water (0.1% TFA); solvent B: acetonitrile (0.1% TFA); from 5 to 100% solvent B over 30 min, flow rate: 15 mL min^{-1}) to give the final product (**8**) (9.6 mg, 38%). ^{1}H NMR (400 MHz, DMSO-d_6): δ 4.40 (d, J = 8 Hz, 1H; CH), 4.29 (t, J = 8 Hz, 1H; CH), 4.09 (d, J = 8 Hz, 1H; CH), 3.87 (m, 4H; CH), 3.66 (m, 1H; CH), 2.04 (m, 1H; CH), 2.01 (m, 5H; CH), 1.97 (m, 5H; CH), 0.92 (m, 9H; CH). ^{13}C{^{1}H} NMR (101 MHz, D_2O): δ 174.8, 174.2, 174.0, 172.0, 171.8, 171.0, 71.9, 60.9, 59.7, 57.0, 48.3., 42.4, 29.9, 29.3, 28.8, 24.7, 21.7, 18.4, 17.5, 17.4; MS (ESI$^+$) calcd for $[C_{24}H_{38}N_6O_6]^+$, $(M + H)^+$: 507.0. Found: 507.0.

Bulky Synthesis of Fmoc-AHX-Dbz-OH (1n). The same procedure to produce **1c** was applied with Fmoc-AHX-OH (5 g, mmol), HATU (6.46 g, mmol, 1.2 equiv) in DMF (100 mL), NMM (3.11 mL, mmol, 2 equiv), and diaminobenzoic acid (2.80 g, mmol, 1.3 equiv) to give **1n** (6.54 g, 95%). ^{1}H NMR (400 MHz, DMSO-d_6): δ 12.11 (s, 1H; OH), 9.05 (s, 1H; NH), 7.89 (d, J = 7.5 Hz, 2H; CH), 7.80 (d, J = 1.5 Hz, 1H; CH), 7.68 (d, J = 7.3 Hz, 2H; CH), 7.49 (dd, J = 8.4, 1.8 Hz, 1H; CH), 7.41 (t, J = 7.4 Hz, 2H; CH), 7.35−7.26 (m, 3H), 6.70 (d, J = 8.4 Hz, 1H; CH), 5.61 (s, 2H; NH_2), 4.29 (d, J = 6.9 Hz, 2H; CH_2), 4.20 (t, J = 6.7 Hz, 1H; CH), 2.99 (dd, J = 12.6, 6.4 Hz, 2H; CH_2), 2.32 (t, J = 7.3 Hz, 2H; CH_2), 1.67−1.54 (m, J = 14.7, 7.3 Hz, 2H; CH_2), 1.48−1.39 (m, J = 14.1, 7.0 Hz, 2H; CH_2), 1.37−1.24 (m, J = 7.0 Hz, 2H; CH_2).

■ ASSOCIATED CONTENT

Data Availability Statement

The data underlying this study are available in the published article and its Supporting Information.

Supporting Information

FAIR Data is available as Supporting Information for Publication and includes the primary NMR FID files The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsomega.3c06640.

^{1}H and ^{13}C{^{1}H} NMR spectra, HPLC chromatograms, and MS data for all the compounds (PDF)

■ AUTHOR INFORMATION

Corresponding Authors

Hui-Ting Chen − *Department of Pharmacy, National Yang Ming Chiao Tung University, Taipei 112, Taiwan*; Email: htchen1969@nycu.edu.tw

Chai-Lin Kao − *Department of Medicinal and Applied Chemistry and Drug Development and Value Creation Research Center, Kaohsiung Medical University, Kaohsiung 807, Taiwan; Department of Medical Research, Kaohsiung Medical University Hospital, Kaohsiung 807, Taiwan; Department of Chemistry, National Sun Yat-sen University, Kaohsiung 80424, Taiwan; College of Professional Studies, National Pingtung University of Science and Technology, Pingtung 912, Taiwan;* orcid.org/0000-0003-3991-9092; Email: clkao@kmu.edu.tw

Authors

Min-Cheng Chien − *Department of Medicinal and Applied Chemistry, Kaohsiung Medical University, Kaohsiung 807, Taiwan*

Yi Kai Lin − *Department of Medicinal and Applied Chemistry, Kaohsiung Medical University, Kaohsiung 807, Taiwan*

Yong Liao − *Department of Pharmacy, National Yang Ming Chiao Tung University, Taipei 112, Taiwan*

Szu-Hsuan Chen − *Department of Medicinal and Applied Chemistry, Kaohsiung Medical University, Kaohsiung 807, Taiwan*

Yen-Wei Chen − *Department of Medicinal and Applied Chemistry, Kaohsiung Medical University, Kaohsiung 807, Taiwan*

Chien-Yu Liang − *Department of Medicinal and Applied Chemistry, Kaohsiung Medical University, Kaohsiung 807, Taiwan*

Vijayasimha Molakaseema − *Department of Medicinal and Applied Chemistry, Kaohsiung Medical University, Kaohsiung 807, Taiwan*

Sodio C. N. Hsu − *Department of Medicinal and Applied Chemistry, Kaohsiung Medical University, Kaohsiung 807, Taiwan;* orcid.org/0000-0002-2576-7289

Chun-Cheng Lin − *Department of Medicinal and Applied Chemistry, Kaohsiung Medical University, Kaohsiung 807, Taiwan; Department of Chemistry, National Tsing Hua University, Hsinchu 30013, Taiwan;* orcid.org/0000-0002-2323-0920

Complete contact information is available at: https://pubs.acs.org/10.1021/acsomega.3c06640

Author Contributions

○M.-C.C. and Y.K.L. contributed equally to this manuscript.

Notes

The authors declare no competing financial interest.

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