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Tag-Assisted Liquid-Phase Peptide Synthesis Using Hydrophobic Benzyl Alcohols as Supports

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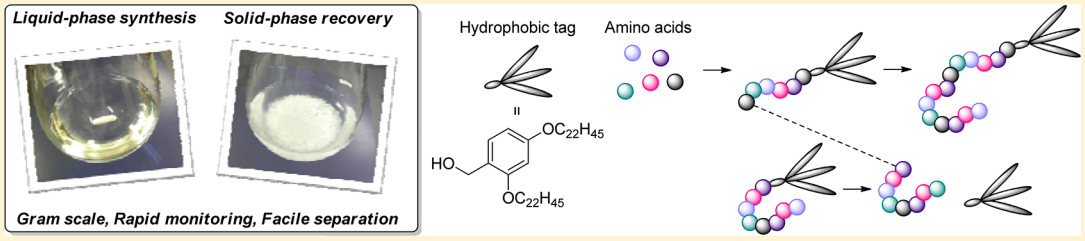
Supporting Information



ABSTRACT: A soluble tag-assisted liquid-phase peptide synthesis was successfully established based on simple hydrophobic benzyl alcohols, which can be easily prepared from naturally abundant materials. Excellent precipitation yields can be obtained at each step, combining the best properties of solid-phase and liquid-phase techniques. This approach can also be applied efficiently to fragment couplings, allowing chemical synthesis of several bioactive peptides.

INTRODUCTION

Since Novartis launched lypressin, a vasopressin analogue, peptides have attracted much attention as medicinal candidates. Naturally occurring peptides and their analogues offer extensive bioactivity with high specificity, which has resulted in an expansion in the market of therapeutic peptides. A particular advantage is that peptides have a fully established method of chemical synthesis based on solid-phase techniques in combination with progress in effective protection and deprotection methods. These techniques have combined well with automated and combinatorial strategies to comprise a substantial fraction of current peptide synthesis in both academic and industrial fields.

Alternative approaches consisting of soluble polymer-tagged liquid-phase reactions have also been developed to facilitate reaction workup and product isolation, with some success in preparing bioactive peptides.[1] In principle, liquid-phase methods have no scale limitations, and the reactions can be monitored directly using standard analytical techniques. For example, polystyrenes and polyethylene glycols have been employed as soluble supports for liquid-phase peptide synthesis. In both cases, reaction mixtures are simply diluted with poor solvents to induce precipitation of the polymer-tagged products, which can be recovered through filtration, while excess amino acids and coupling agents are rinsed away. For such strategies to be successful, the solubility and the precipitation yield of support-tagged products must be key components of the overall procedures and further improvement in these properties of the polymers would be a great aid for their practical uses.[2]

Hydrophobic supports are preferable, because both excess amino acids and coupling agents are generally polar and can be removed effectively by washing with hydrophilic solvents. In recent years, hydrophobic supports based simply on long alkyl chains have been proposed, especially in oligosaccharide synthesis, to improve reaction workup and product isolation.[3] These strategies have also been applied to peptide synthesis to enable practical application.[4]

In this context, we have developed liquid-phase techniques, assisted by hydrophobic supports, for oligosaccharide and peptide synthesis.[5] In particular, the discovery that excellent precipitation yields can be obtained when benzyl alcohols bearing long alkyl chains are used as hydrophobic supports has led to versatile preparation of bioactive peptides.[6] However, their application toward relatively long peptides remains challenging. Described herein are liquid-phase syntheses of several bioactive peptides enabled by hydrophobic tag-assisted fragment couplings.

RESULTS AND DISCUSSION

The present work began with the synthesis of a short bioactive peptide, leuprolide (**1**), which has two prominent features: (a) the C-terminal residue is proline, which induces diketopiper-

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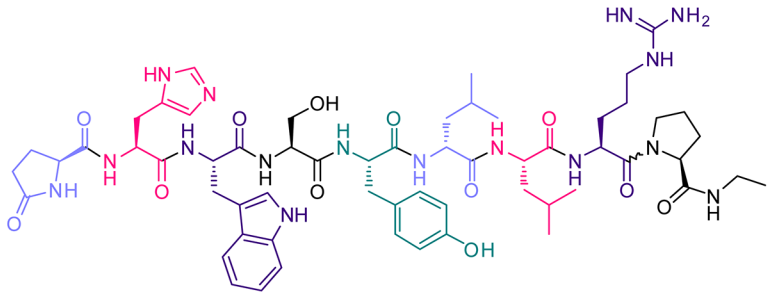
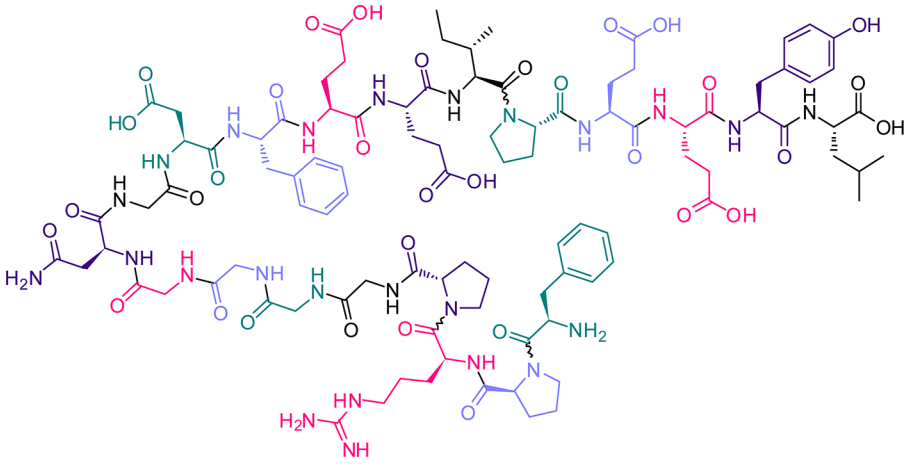
Scheme 1. Structures of Bioactive Peptides 1−3



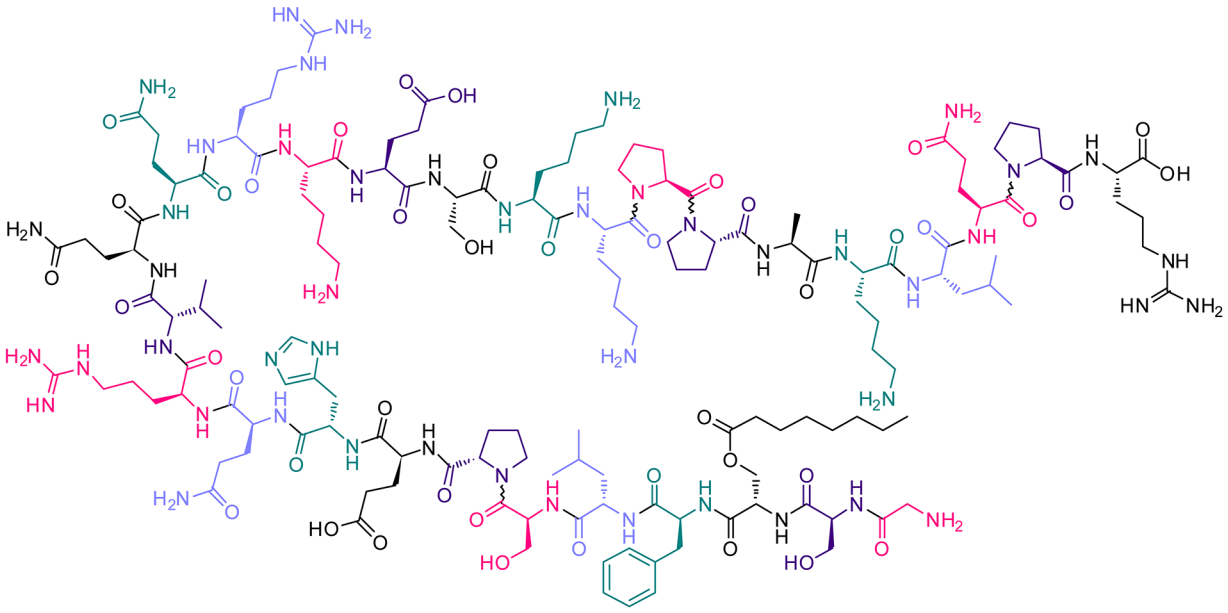
Pyr-His-Trp-Ser-Tyr-D-Leu-Leu-Arg-Pro-NHEt

1



H-D-Phe-Pro-Arg-Pro-Gly-Gly-Gly-Gly-Asn-Gly-Asp-Phe-Glu-Glu-Ile-Pro-Glu-Glu-Tyr-Leu-OH

2



H-Gly-Ser-Ser(*n*-Octanoyl)-Phe-Leu-Ser-Pro-Glu-His-Gln-Arg-Val-Gln-Gln-Arg-Lys-Glu-Ser-Lys-Lys-Pro-Pro-Ala-Lys-Leu-Gln-Pro-Arg-OH

3

azine formation during basic deprotection of the N-terminal Fmoc-group, and (b) the C-terminus is ethyl amidated (Scheme 1). Depending on the desired sequence, two different types of hydrophobic tag, acid-resistant (**4**) and readily cleavable (**5**), can be used; these are simply designed based on the substitution pattern and easily synthesized in two steps (Scheme 2).

We selected the "acid-resistant" hydrophobic tag **4** for leuprolide (**1**) synthesis to allow the use of Boc chemistry. Initially, Boc-Pro-OH was introduced into the tag, followed by removal of the Boc group under acidic conditions using a

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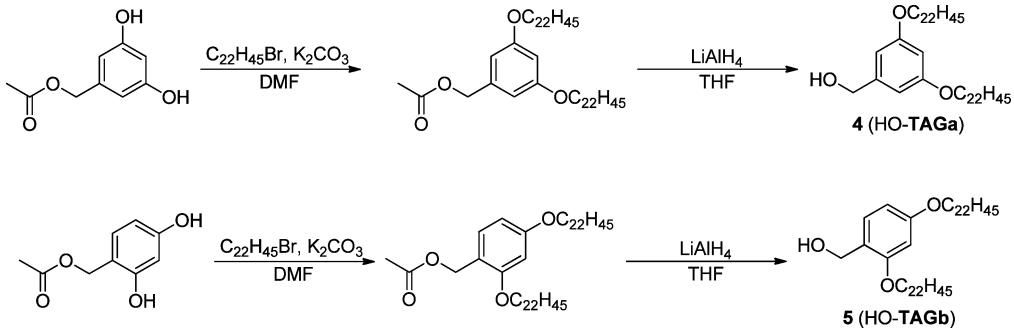
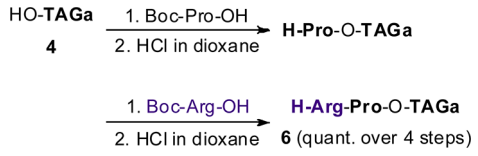
Scheme 2. Structures and Synthesis of Hydrophobic Tags 4 and 5 Used in This Work



dioxane solution of HCl. Both reactions could be carried out in homogeneous liquid phase in less polar solvents (typically CH_2Cl_2, THF, or toluene), and the products were obtained as precipitates through dilution with polar solvents (typically, MeOH or MeCN). Then, coupling of Boc-Arg-OH was carried out, followed by removal of the Boc group. Using this technique, excellent precipitation yields were obtained and excess reagent was effectively rinsed away, affording tagged dipeptide **6** quantitatively over four steps in excellent purity (Scheme 3). The reactions were directly monitored at each step

Scheme 3. Preparation of Tagged Dipeptide 6



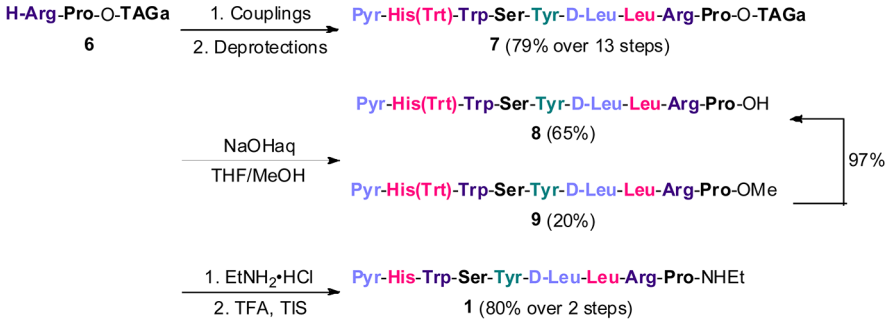
using standard analytical techniques, which showed that side reactions did not take place to a significant extent (Schemes S1 and S2, Supporting Information).

Through careful examination, it was found that several amino acid couplings had taken place even when their side chains were not protected, which improved the overall process with regard to atom economy. However, as it was observed that several byproducts were formed when Boc-His-OH was used, Fmoc-His(Trt)-OH was used instead. Thus, tagged dipeptide **6** was subjected to consecutive couplings and deprotections to give the desired sequence **7** in 79% yield over 13 steps (Scheme 4). Based on a method described in a patent application, saponification using NaOH(aq) in THF/MeOH was attempted to cleave the tag.[7] The saponification reaction took place to afford the desired peptide **8** in 65% yield, along with its methyl ester **9**. Although the mechanism of this transesterification was unclear, the methyl ester **9** could be transformed into the desired peptide **8** in 97% yield through further saponification in NaOH(aq)/diisopropyl ether (DIPE). The C-terminus was amidated with ethylamine hydrochloride, and the trityl group at the histidine side chain was removed using trifluoroacetic acid (TFA) to give leuprolide (**1**) in 80% yield over two steps (Scheme S3, Supporting Information).

For the synthesis of longer peptides, convergent synthetic schemes are preferable. We turned our attention to fragment couplings based on our tagging strategy. In this instance, bivalirudin (**2**) was chosen as a model bioactive peptide, and we selected a readily cleavable hydrophobic tag (**5**) with a view to preparing coupling fragments in which only the tag should be removed, with side-chain protecting groups maintained. The desired sequence was divided into the corresponding three fragments composed of six or seven amino acid residues, which were prepared using general Fmoc-chemistry (Scheme 5 and Scheme S4, Supporting Information). For N-terminal residues only, Boc-D-Phe-OH was used so as to be removed through final acidic deprotection. We then attempted to cleave the tags of the coupling fragments **10** and **12** selectively under mildly acidic conditions. To this end, 1% TFA in combination with 10% 2,2,2-trifluoroethanol (TFE) in CH_2Cl_2 was found to be effective. Under these conditions, the tag was rapidly cleaved with little deprotection of the side chains. Both peptides **11** and **13** could be isolated as precipitates through dilution of the reaction mixtures with DIPE.

These tagged peptides were found to exhibit a vivid red color after acidic treatment due to the formation of resorcinarene (Scheme S5, Supporting Information). Therefore, the readily cleavable hydrophobic tag **5** can also serve as a highly sensitive probe for ratiometric detection of peptides, facilitating monitoring of the reaction using basic analytical techniques such as TLC (Scheme 6).

Scheme 4. Synthesis of Leuprolide (1)



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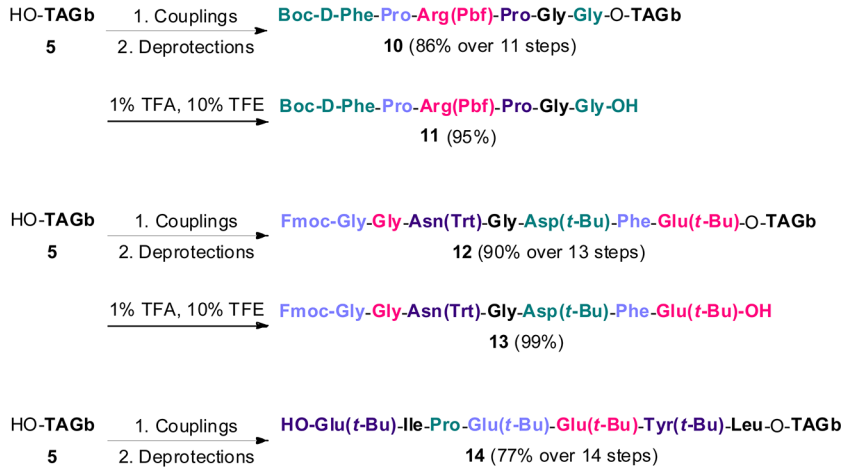
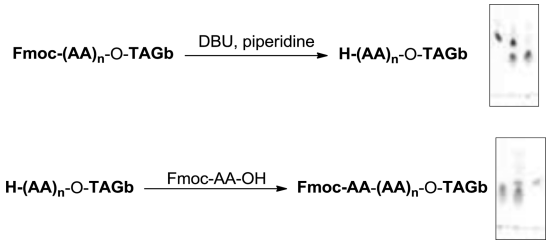
Scheme 5. Preparation of Coupling Fragments of Bivalirudin (2)



Scheme 6. Reaction Monitoring Using TLC Analysis (AA: amino acid)



Through the investigation of numerous coupling reagents, we found the use of *O*-(7-aza-1*H*-benzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium hexafluorophosphate (HATU) in combination with 1-hydroxy-7-azabenzotriazole (HOAt) was effective for fragment couplings. The coupling fragment **13** was coupled efficiently to tagged peptide **14**, even when only a slight excess of the fragment was used, followed by deprotection of its Fmoc-group under basic conditions to give tagged peptide **15** in 93% yield over two steps (Scheme 7). The coupling fragment **11** was then efficiently introduced into tagged peptide **15** using the same reaction conditions; this was treated with TFA under strongly acidic conditions to give bivalirudin (**2**) in 85% yield over two steps (Scheme S6, Supporting Information).

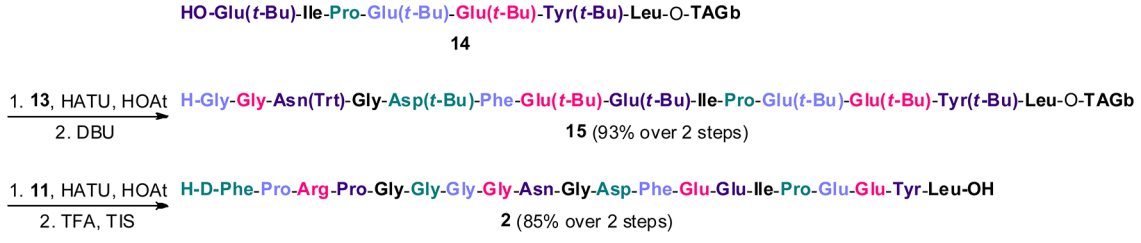
Finally, to achieve the synthesis of another long peptide, we chose h-ghrelin (**3**) as a model bioactive peptide. The desired sequence was divided into the corresponding four fragments composed of six or eight amino acid residues, which were prepared using general Fmoc-chemistry (Scheme 8 and Scheme S7, Supporting Information). In the preparation of the coupling fragment **16**, Fmoc-Ser-OH was used with its side chain unprotected; this was esterified using *n*-octanoyl acid before removal of the tag. However, the trityl group at the histidine residue was also partially cleaved through acidic cleavage of the tag, lowering the overall yield of the coupling fragment (**17**).

While the coupling fragment **18** could be coupled effectively to the tagged peptide **19** using HATU in the presence of HOAt, 4-(4,6-dimethoxy-1,3,5-triazin-2-yl)-4-methylmorpholinium chloride *n*-hydrate (DMT-MM) was found to be more efficient for subsequent fragment couplings. These facts were interpreted as indicating complexity of the fragment couplings; thus, the coupling conditions should be considered carefully depending on the desired sequences. Coupling of the remaining fragments **16** and **17** was carried out using DMT-MM to afford the tagged peptide, which was then treated with TFA under strongly acidic conditions to give h-ghrelin (**3**) (Scheme 9 and Scheme S8, Supporting Information).

CONCLUSION

We successfully established a procedure for soluble tag-assisted liquid-phase peptide synthesis using simple hydrophobic benzyl alcohols as supports. Excellent precipitation yields were obtained at each step, and the technique combined the best properties of solid-phase and liquid-phase synthesis methods. Two different types of hydrophobic tag, acid-resistant and readily cleavable, were easily prepared from naturally abundant materials and were used according to the desired sequence. Both deprotection and coupling of the tagged peptides took place effectively in homogeneous liquid phase, enabling rapid reaction monitoring using basic analytical techniques. When the reactions were complete, the desired tagged peptides were selectively isolated from the reaction mixtures as precipitates, similarly to the heterogeneous solid-phase method, requiring only filtration. The acid-resistant hydrophobic tag enabled the use of Boc chemistry, while the readily cleavable hydrophobic

Scheme 7. Synthesis of Bivalirudin (2)



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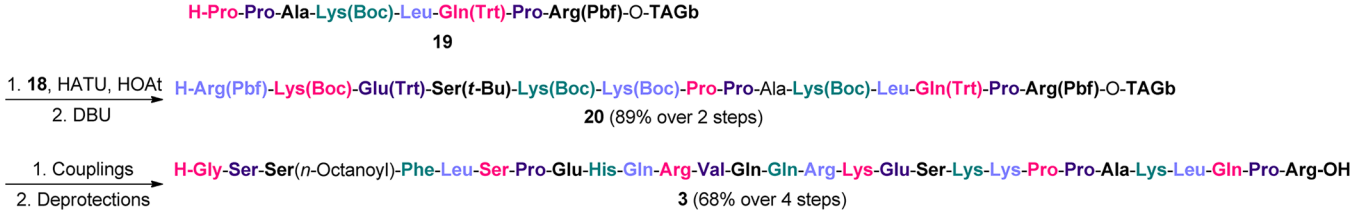
Scheme 8. Preparation of Coupling Fragments of h-Ghrelin (3)

Scheme 9. Synthesis of h-Ghrelin (3)



tag allowed effective fragment couplings. Using this technique, we demonstrated the production of several bioactive peptides on a gram scale.

EXPERIMENTAL SECTION

Leuprolide (1). *Preparation of the Tagged Peptide 6.* The "acid-resistant" hydrophobic tag **4** (1.52 g, 2.00 mmol) was dissolved in CH_2Cl_2 (20 mL). Boc-Pro-OH (646 mg, 3.00 mmol), *N*,*N*′-diisopropylcarbodiimide (DIC) (378 mg, 3.00 mmol), and *N*,*N*-dimethyl-4-aminopyridine (DMAP) (48.8 mg, 0.400 mmol) were then added to the solution. The reaction mixture was stirred at room temperature until the reaction was completed (30 min). After completion, MeCN was added to the reaction mixture to give the product quantitatively as a precipitate. The precipitate was filtered and washed with MeCN. The product was then dissolved in a 3:2 (v/v) mixture of 4 M HCl/dioxane and toluene (30 mL). The reaction mixture was stirred at room temperature until the reaction was completed (30 min). After completion, MeCN was added to give the product quantitatively as a precipitate. The precipitate was filtered and washed with MeCN. The product was then dissolved in a 4:1 (v/v) mixture of THF and DMF (40 mL). Boc-Arg-OH (658 mg, 2.40 mmol), DMT-MM (664 mg, 2.40 mmol), and *N*-methylmorpholine (NMM) (405 mg, 4.00 mmol) were then added to the solution. The reaction mixture was stirred at room temperature until the reaction was completed (30 min). After completion, MeCN was added to the reaction mixture to give the product quantitatively as a precipitate. The precipitate was filtered and washed with MeCN. Finally, the product was dissolved in a 3:2 (v/v) mixture of 4 M HCl/dioxane and toluene (30 mL). The reaction mixture was stirred at room temperature until the reaction was completed (30 min). After completion, MeCN was added to the tagged peptide **6** quantitatively as a precipitate. The precipitate was filtered and washed with MeCN.

General Method for the Couplings. Tagged peptide was dissolved in a 4:1 (v/v) mixture of THF and DMF (40 mL). Boc-AA-OH (1.20 mol equiv), except for the N-terminal pyroglutamic acid (Pyr-OH) and histidine next to the N-terminus (Fmoc-His(Trt)-OH), DMT-MM (1.20 mol equiv), and NMM (2.00 mol equiv) were then added to the solution. The reaction mixture was stirred at room temperature until the reaction was completed. After completion, MeCN was added to the reaction mixture to give the product as a precipitate. The precipitate was filtered and washed with MeCN.

General Method for Boc Group Deprotections. Tagged peptide was dissolved in a 3:2 (v/v) mixture of 4 M HCl/dioxane and toluene (30 mL). The reaction mixture was stirred at room temperature until the reaction was completed. After completion, MeCN was added to give the product as a precipitate. The precipitate was filtered and washed with MeCN.

Basic Deprotection of the C-Terminal Hydrophobic Tag of the Tagged Peptide 7. The tagged peptide **7** (4.00 g, 1.85 mmol) was dissolved in a 1:1 (v/v) mixture of THF and MeOH (92 mL). Aqueous 1.0 M NaOH (6.47 mL) was added to the solution at 0 °C. The reaction mixture was stirred at 0 °C until the reaction was completed (20 h). After completion, the solution was filtered and then washed with MeOH. DIPE was added to the filtrate to give the products **8** in 65% yield as a precipitate with the formation of its methyl ester **9** in 20% yield. The methyl ester was then dissolved in a 1:1 (v/v) mixture of DIPE and H_2O (17 mL). Aqueous 1.0 M NaOH (2.14 mL) was added to the solution at 0 °C to hydrolyze the C-terminus (20 h), affording the product **8** in 97% yield (in total 84% yield).

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C-Terminal Amidation and Acidic Deprotection of the Product 9. The product **8** (2.84 g, 1.95 mmol) was dissolved in DMF (20 mL). Monoethylamine hydrochloride (5.00 mol equiv), DMT-MM (1.20 mol equiv), and NMM (2.00 mol equiv) were then added to the solution. The reaction mixture was stirred at room temperature until the reaction was completed (2 h). After completion, the solution was filtered and then purified with gel filtration (GE Healthcare Sephadex LH-20) to give the products in 80% yield as a precipitate, which was dissolved in 2.5% triisopropylsilane (TIS) and 2.5% H_2O in TFA (74 mL). The reaction mixture was stirred at room temperature until the reaction was completed (2 h). After completion, the solution was evaporated and then washed with DIPE to give leuprolide (**1**) quantitatively as a precipitate, which was finally purified by RP-HPLC using H_2O−MeCN, including 0.1% TFA.

Bivalirudin (2). *Preparation of the Fragment 11.* The "readily-cleavable" hydrophobic tag **5** (2.27 g, 3.00 mmol) was dissolved in CH_2Cl_2 (60 mL). Fmoc-Gly-OH (1.34 g, 4.50 mmol), DIC (567 mg, 4.50 mmol), and DMAP (73.2 mg, 0.600 mmol) were then added to the solution. The reaction mixture was stirred at room temperature until the reaction was completed (30 min). After completion, MeCN was added to the reaction mixture to give the product as a precipitate. The precipitate was filtered and washed with MeCN. The product was then dissolved in 1% (v/v) 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) in the presence of piperidine (384 mg, 4.50 mmol) in THF (60 mL). The reaction mixture was stirred at room temperature until the reaction was completed (5 min). After completion, 6 M HCl was added to the solution to neutralize (pH 7.0), and then MeCN was added to give the product in 98% over two steps as a precipitate. The precipitate was filtered and washed with MeCN. The product was then subjected to the consecutive couplings and deprotections to give the tagged peptide **10** in 88% yield over nine steps, which was then dissolved in a 100:10:1 (v/v) mixture of CH_2Cl_2, TFE, and TFA (100 mL). The reaction mixture was stirred at room temperature until the reaction was completed (30 min). After completion, the solution was filtered by PTFE filter, and then DIPE was added to the filtrate to give the fragment **11** in 95% yield as a precipitate.

Preparation of the Fragment 13. The "readily-cleavable" hydrophobic tag **5** (2.27 g, 3.00 mmol) was dissolved in CH_2Cl_2 (60 mL). Fmoc-Glu(*t*-Bu)-OH (1.91 g, 4.50 mmol), DIC (567 mg, 4.50 mmol), and DMAP (73.2 mg, 0.600 mmol) were then added to the solution. The reaction mixture was stirred at room temperature until the reaction was completed (30 min). After completion, MeCN was added to the reaction mixture to give the product as a precipitate. The precipitate was filtered and washed with MeCN. The product was then dissolved in 1% (v/v) DBU in the presence of piperidine (384 mg, 4.50 mmol) in THF (60 mL). The reaction mixture was stirred at room temperature until the reaction was completed (5 min). After completion, 6 M HCl was added to the solution to neutralize (pH 7.0), and then MeCN was added to give the product quantitatively over two steps as a precipitate. The precipitate was filtered and washed with MeCN. The product was then subjected to the consecutive couplings and deprotections to give the tagged peptide (**13**) in 90% yield over 11 steps, which was then dissolved in a 100:10:1 (v/v) mixture of CH_2Cl_2, TFE, and TFA (100 mL). The reaction mixture was stirred at room temperature until the reaction was completed (30 min). After completion, the solution was filtered by PTFE filter, and then DIPE was added to the filtrate to give the fragment **13** in 99% yield as a precipitate.

Preparation of the Tagged Peptide 14. The "readily-cleavable" hydrophobic tag **5** (2.27 g, 3.00 mmol) was dissolved in CH_2Cl_2 (60 mL). Fmoc-Leu-OH (1.59 g, 4.50 mmol), DIC (567 mg, 4.50 mmol), and DMAP (73.2 mg, 0.600 mmol) were then added to the solution. The reaction mixture was stirred at room temperature until the reaction was completed (30 min). After completion, MeCN was added to the reaction mixture to give the product as a precipitate. The precipitate was filtered and washed with MeCN. The product was then dissolved in 1% (v/v) DBU in the presence of piperidine (384 mg, 4.50 mmol) in THF (60 mL). The reaction mixture was stirred at room temperature until the reaction was completed (5 min). After completion, 6 M HCl was added to the solution to neutralize (pH 7.0), and then MeCN was added to give the product quantitatively over two steps as a precipitate. The precipitate was filtered and washed with MeCN. The product was then subjected to the consecutive couplings and deprotections to give the fragment **14** in 77% yield over 12 steps as a precipitate. The precipitate was filtered and washed with MeCN.

General Method for the Couplings. Tagged peptide was dissolved in a 9:1 (v/v) mixture of THF and DMF (60 mL). Fmoc-AA-OH (1.20 mol equiv), except for the N-terminal D-phenylalanine (Boc-D-Phe-OH) of the fragment **11**, *O*-(1*H*-benzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium hexafluorophosphate (HBTU) (1.20 mol equiv), 1-hydroxy-1*H*-benzotriazole (HOBt) (1.20 mol equiv), and *N*,*N*-diisopropylethylamine (DIPEA) (5.00 mol equiv) were then added to the solution. The reaction mixture was stirred at room temperature until the reaction was completed. After completion, MeCN was added to the reaction mixture to give the product as a precipitate. The precipitate was filtered and washed with MeCN.

General Method for N-Fmoc Group Deprotections. Tagged peptide was dissolved in 1% (v/v) DBU and in the presence of piperidine (1.50 mol equiv) in THF (60 mL). The reaction mixture was stirred at room temperature until the reaction was completed. After completion, 6 M HCl was added to the solution to neutralize (pH 7.0), and then MeCN was added to give the product as a precipitate. The precipitate was filtered and washed with MeCN.

Synthesis of Bivalirudin (2). The tagged peptide **14** (2.00 mmol) was dissolved in a 9:1 (v/v) mixture of THF and DMF (80 mL). The fragment **13** (1.05 mol equiv), HATU (1.05 mol equiv), HOAt (1.05 mol equiv), DIPEA (5.00 mol equiv), and DIC (1.00 mol equiv) were then added to the solution. The reaction mixture was stirred at room temperature until the reaction was completed (2 h). After completion, MeCN was added to the reaction mixture to give the product as a precipitate. The precipitate was filtered and washed with MeCN. The product was then dissolved in THF (200 mL) in the presence of piperidine (12.0 mol equiv) and DBU (3.00 mol equiv). The reaction mixture was stirred at room temperature until the reaction was completed (5 min). After completion, 6 M HCl was added to the solution to neutralize (pH 7.0), and then MeCN was added to give the product **15** in 93% yield over two steps as a precipitate. The precipitate was filtered and washed with MeCN. The product **15** (2.00 mmol) was dissolved in a 9:1 (v/v) mixture of THF and DMF (80 mL). The fragment **11** (1.05 mol equiv), HATU (1.05 mol equiv), HOAt (1.05 mol equiv), DIPEA (5.00 mol equiv), and DIC (1.00 mol equiv) were then added to the solution. The reaction mixture was stirred at room temperature until the reaction was completed (2 h). After completion, MeCN was added to the reaction mixture to give the product in 85% yield as a precipitate. The precipitate was filtered and washed with MeCN. The product was then dissolved in 2.5% TIS and 2.5% H_2O in TFA (200 mL). The reaction mixture was stirred at room temperature until the reaction was completed (3 h). After completion, the solution was filtered by PTFE filter, and then DIPE was added to the filtrate to give bivalirudin (**2**) quantitatively as a precipitate, which was finally purified by RP-HPLC using H_2O-MeCN.

h-Ghrelin (3). *Preparation of the Fragment 16.* The "readily-cleavable" hydrophobic tag **5** (7.57 g, 10.0 mmol) was dissolved in CH_2Cl_2 (200 mL). Fmoc-Ser(*t*-Bu)-OH (5.75 g, 15.0 mmol), DIC (1.89 g, 15.0 mmol), and DMAP (244 mg, 2.00 mmol) were then added to the solution. The reaction mixture was stirred at room temperature until the reaction was completed (30 min). After completion, MeCN was added to the reaction mixture to give the product as a precipitate. The precipitate was filtered and washed with MeCN. The product was then dissolved in 1% (v/v) DBU in the presence of piperidine (1.28 g, 15.0 mmol) in THF (200 mL). The reaction mixture was stirred at room temperature until the reaction was completed (5 min). After completion, 6 M HCl was added to the solution to neutralize (pH 7.0), and then MeCN was added to give the product quantitatively over two steps as a precipitate. The precipitate was filtered and washed with MeCN. The product was then subjected to the consecutive couplings and deprotections to give the desired tagged peptide in 66% yield over nine steps. *n*-Octanoic acid (1.50 mol equiv), DIC (1.50 mol equiv), and DMAP (0.20 mol equiv) were then

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added to the solution. The reaction mixture was stirred at room temperature until the reaction was completed (2 h). After completion, MeCN was added to the reaction mixture to give the product as a precipitate, which was then dissolved in a 100:10:1 (v/v) mixture of CH_2Cl_2, TFE, and TFA (500 mL). The reaction mixture was stirred at room temperature until the reaction was completed (30 min). After completion, the solution was filtered by PTFE filter, and then DIPE was added to the filtrate to give the fragment (**21**) in 94% yield over two steps as a precipitate.

*Preparation of the Fragments **17** and **18**.* The "readily-cleavable" hydrophobic tag **5** (7.57 g, 10.0 mmol) was dissolved in CH_2Cl_2 (200 mL). Fmoc-Gln(Trt)-OH (9.16 g, 15.0 mmol) or Fmoc-Lys(Boc)-OH (7.03 g, 15.0 mmol), DIC (1.89 g, 15.0 mmol), and DMAP (244 mg, 2.00 mmol) were then added to the solution. The reaction mixture was stirred at room temperature until the reaction was completed (30 min). After completion, MeCN was added to the reaction mixture to give the product as a precipitate. The precipitate was filtered and washed with MeCN. The product was then dissolved in 1% (v/v) DBU in the presence of piperidine (1.28 g, 15.0 mmol) in THF (200 mL). The reaction mixture was stirred at room temperature until the reaction was completed (5 min). After completion, 6 M HCl was added to the solution to neutralize (pH 7.0), and then MeCN was added to give the product quantitatively over two steps as a precipitate, respectively. The precipitate was filtered and washed with MeCN. The product was then subjected to the consecutive couplings and deprotections to give the tagged peptides in 52% yield over 13 steps or 95% yield over 9 steps, respectively, which was then dissolved in a 100:10:1 (v/v) mixture of CH_2Cl_2, TFE, and TFA (500 mL). The reaction mixture was stirred at room temperature until the reaction was completed (30 min). After completion, the solution was filtered by PTFE filter, and then DIPE was added to the filtrate to give the fragments **17** and **18** in 72% yield or quantitatively as a precipitate, respectively.

*Preparation of the Tagged Peptide **19**.* The "readily-cleavable" hydrophobic tag **5** (7.57 g, 10.0 mmol) was dissolved in CH_2Cl_2 (200 mL). Fmoc-Arg(Pbf)-OH (9.73 g, 15.0 mmol), DIC (1.89 g, 15.0 mmol), and DMAP (244 mg, 2.00 mmol) were then added to the solution. The reaction mixture was stirred at room temperature until the reaction was completed (30 min). After completion, MeCN was added to the reaction mixture to give the product as a precipitate. The precipitate was filtered and washed with MeCN. The product was then dissolved in 1% (v/v) DBU in the presence of piperidine (1.28 g, 15.0 mmol) in THF (200 mL). The reaction mixture was stirred at room temperature until the reaction was completed (5 min). After completion, 6 M HCl was added to the solution to neutralize (pH 7.0), and then MeCN was added to give the product quantitatively over two steps as a precipitate. The precipitate was filtered and washed with MeCN. The product was then subjected to the consecutive couplings and deprotections to give the fragment **19** in 64% yield over 14 steps as a precipitate. The precipitate was filtered and washed with MeCN.

General Method for the Couplings. Tagged peptide was dissolved in a 9:1 (v/v) mixture of THF and DMF (200 mL). N-Fmoc-AA-OH (1.20 mol equiv), except for the N-terminal glycine (Boc-Gly-OH) of the fragment **16**, HBTU (1.20 mol equiv), HOBt (1.20 mol equiv), and DIPEA (5.00 mol equiv) were then added to the solution. The reaction mixture was stirred at room temperature until the reaction was completed. After completion, MeCN was added to the reaction mixture to give the product as a precipitate. The precipitate was filtered and washed with MeCN.

General Method for N-Fmoc Group Deprotections. Tagged peptide was dissolved in 1% (v/v) DBU and in the presence of piperidine (1.50 mol equiv) in THF (200 mL). The reaction mixture was stirred at room temperature until the reaction was completed. After completion, 6 M HCl was added to the solution to neutralize (pH 7.0), and then MeCN was added to give the product as a precipitate. The precipitate was filtered and washed with MeCN.

Synthesis of h-Ghrelin (3). Tagged peptide **19** (3.00 mmol) was dissolved in a 9:1 (v/v) mixture of THF and DMF (120 mL). The fragment **18** (1.50 mol equiv), HATU (1.50 mol equiv), HOAt (1.50 mol equiv), and DIPEA (5.00 mol equiv) were then added to the solution. The reaction mixture was stirred at room temperature until the reaction was completed (1 h). After completion, MeCN was added to the reaction mixture to give the product as a precipitate. The precipitate was filtered and washed with MeCN. The product was then dissolved in THF (300 mL) in the presence of piperidine (12.0 mol equiv) and DBU (3.00 mol equiv). The reaction mixture was stirred at room temperature until the reaction was completed (5 min). After completion, 6 M HCl was added to the solution to neutralize (pH 7.0), and then MeCN was added to give the product **20** in 89% yield over two steps as a precipitate. The precipitate was filtered and washed with MeCN. The tagged peptide (**20**) (3.00 mmol) was dissolved in a 9:1 (v/v) mixture of THF and DMF (120 mL). The fragment **17** (1.50 mol equiv), DMT-MM (1.50 mol equiv), and DIPEA (5.00 mol equiv) were then added to the solution. The reaction mixture was stirred at room temperature until the reaction was completed (1 h). After completion, MeCN was added to the reaction mixture to give the product as a precipitate. The precipitate was filtered and washed with MeCN. The product was then dissolved in THF (300 mL) in the presence of piperidine (12.0 mol equiv) and DBU (3.00 mol equiv). The reaction mixture was stirred at room temperature until the reaction was completed (5 min). After completion, 6 M HCl was added to the solution to neutralize (pH 7.0), and then MeCN was added to give the product in 75% yield over two steps as a precipitate. The precipitate was filtered and washed with MeCN. The product (3.00 mmol) was dissolved in a 9:1 (v/v) mixture of THF and DMF (120 mL). The fragment **16** (1.50 mol equiv), DMT-MM (1.50 mol equiv), and DIPEA (5.00 mol equiv) were then added to the solution. The reaction mixture was stirred at room temperature until the reaction was completed (1 h). After completion, MeCN was added to the reaction mixture to give the product in 91% yield as a precipitate. The precipitate was filtered and washed with MeCN. The product was then dissolved in 2.5% TIS and 2.5% H_2O in TFA (300 mL). The reaction mixture was stirred at room temperature until the reaction was completed (3 h). After completion, the solution was filtered by PTFE filter, and then DIPE was added to the filtrate to give h-Ghrelin (**3**) quantitatively as a precipitate, which was finally purified by RP-HPLC using H_2O-MeCN.

*Leuprolide (**1**).* HRMS $[M + H]^+$ calcd for $C_{59}H_{85}N_{16}O_{12}$ 1209.6533, found 1209.6526.

*Coupling Fragment **11**.* HRMS $[M + H]^+$ calcd for $C_{47}H_{67}N_9O_{12}S$ 983.4708, found 983.4705.

*Coupling Fragment **13**.* HRMS $[M + Na]^+$ calcd for $C_{70}H_{78}N_8O_{15}Na$ 1293.5485, found 1293.5484.

*Tagged Peptide **14**.* HRMS M^+ calcd for $C_{108}H_{186}N_7O_{17}$ 1854.3983, found 1854.3983.

*Tagged Peptide **15**.* HRMS $[M + 2Na]^{2+}$ calcd for $C_{163}H_{253}N_{15}O_{29}Na_2$ 1465.4290, found 1465.4284.

*Bivalirudin (**2**).* HRMS $[M + H]^+$ calcd for $C_{98}H_{139}N_{24}O_{35}$ 2179.9936, found 2179.9887.

*Coupling Fragment **16**.* HRMS $[M + Na]^+$ calcd for $C_{47}H_{78}N_6O_{13}Na$ 957.5525, found 957.5520.

*Coupling Fragment **17**.* HRMS $[M + Na]^+$ calcd for $C_{150}H_{159}N_{16}O_{19}S$ 2520.1688, found 2520.1701.

*Coupling Fragment **18**.* HRMS $[M + Na]^+$ calcd for $C_{83}H_{128}N_{12}O_{21}SNa$ 1661.9116, found 1661.9090.

*Tagged Peptide **19**.* HRMS $[M + Na]^+$ calcd for $C_{129}H_{203}N_{13}O_{17}S$ 2239.5219, found 2239.5259.

*Tagged Peptide **20**.* HRMS $[M + 2Na]^{2+}$ calcd for $C_{197}H_{319}N_{25}O_{35}S_2Na_2$ 1852.6594, found 1852.6540.

*h-Ghrelin (**3**).* HRMS $[M + 2H]^{2+}$ calcd for $C_{149}H_{251}N_{47}O_{42}$ 1685.4475, found 1685.4486.

■ ASSOCIATED CONTENT

Supporting Information

Additional schemes and general information. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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