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Hydrophobic tag-assisted liquid-phase synthesis of a growth hormone-inhibiting peptide somatostatin

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ABSTRACT

Hydrophobic tag-assisted liquid-phase peptide synthesis technique and disulfide bond formation have been well-combined, leading to the efficient and practical preparation of a growth hormone-inhibiting peptide somatostatin. Intramolecular disulfide bond formation has successfully been carried out even under relatively high concentrations, enabling the effective peptide modifications in preparative scale.

Intramolecular cross-linking plays a significant role in both naturally occurring and artificial peptides to preorganize an amino acid sequence into a rigid conformation, to impart and/or enhance several biological activities, and to stabilize their structures. A vast number of cyclic peptides have been synthesized[1–17] to investigate their potential applications as nanomaterials,[18] imaging agents,[19] and therapeutics.[20,21] In particular, disulfide bonds formed between cysteine residues are essential for many peptides to induce a wide variety of biological processes. The development of solid-phase techniques have led to practical and facile peptide synthesis, into which effective disulfide bond formation can also be introduced.[22–31] Alternatively, disulfide bond-containing peptides have been prepared efficiently through liquid-phase methodologies under high dilution conditions.[32–36]

Soluble polymer-assisted liquid-phase synthesis has proven to be promising, especially for multi-step reactions, where the advantages of solid-phase separation and liquid-phase efficiency are both attained.[37–40] Previously, we developed a hydrophobic tag-assisted liquid-phase peptide synthesis method.[41–46] In this system, a simple hydrophobic tag, 3,4,5-trioctadecyloxybenzyl alcohol, which could be prepared from naturally abundant materials in one step (Scheme S1 in Supplementary data), served as an effective protecting group for the peptide C-terminus and as a phase-tag. The desired hydrophobically tagged peptides, which were soluble in less polar organic solvents to realize efficient homogeneous liquid-phase peptide elongation/deprotection, could be separated as precipitates from reaction solutions through the addition of polar organic solvents. Most recently, we demonstrated that several types of hydrophobic tags could be designed simply to incorporate both Boc- and Fmoc-chemistry, allowing the elaboration of desired peptide sequences (Scheme S2 in Supplementary data).[47] The combination of this technique with disulfide bond formation would be a great aid for furthering the potential of hydrophobic tag-assisted peptide synthesis.

The present work began with the evaluation of the stability of the hydrophobic tag against the conditions used for disulfide bond formation. To this extent, our previous studies also indicated that three octadecyloxy substituted groups (in total 54 carbons) of the hydrophobic tag could be replaced by two docosyloxy substituted groups (in total 44 carbons) with high precipitability that responded to the addition of polar organic solvents. Thus, we introduced *N*-Fmoc-Cys(Acm)-OH **1** to 1.00 mmol of the hydrophobic tag, 2,4-didocosyloxybenzyl alcohol, (HO-**TAG**) **2** using diisopropylcarbodiimide (DIC) in the presence of a catalytic amount of dimethylaminopyridine (DMAP), forming *N*-Fmoc-Cys(Acm)-O-**TAG 3** (Scheme 1). In order to integrate disulfide bond formation reactions into the hydrophobic tag-assisted peptide synthesis, we chose one of the most common conditions which involved simple iodine oxidation of cysteine residues, where side chains were protected with *N*-(acetyl)aminomethyl (Acm) groups, constructing desired disulfide bond in one step, including both deprotection and oxidative coupling.[48–52] *N*-Fmoc-Ala-OH **4** and *N*-Boc-Cys(Acm)-OH **5** were then introduced into **3** using *O*-benzotriazole-*N*,*N*,*N*′,*N*′-tetramethyluronium hexafluorophosphate (HBTU) and 1-hydroxy-1*H*-benzotria-

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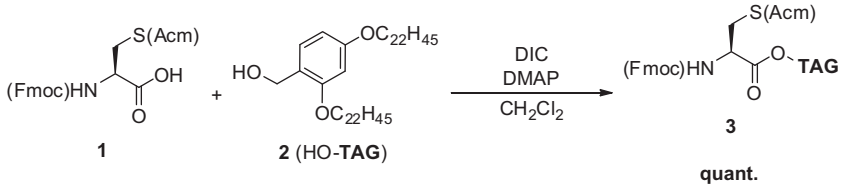
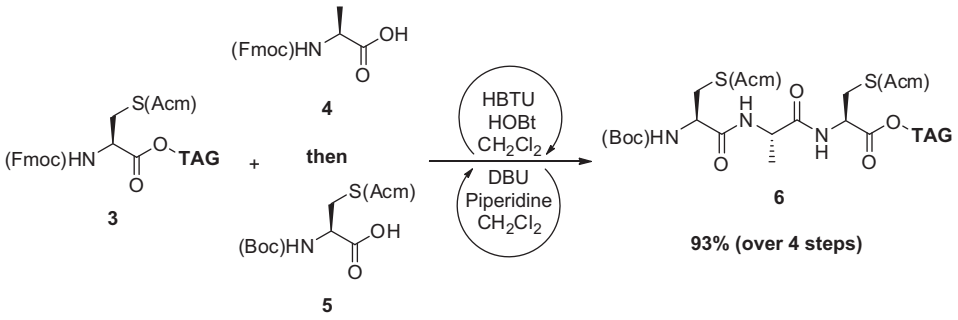
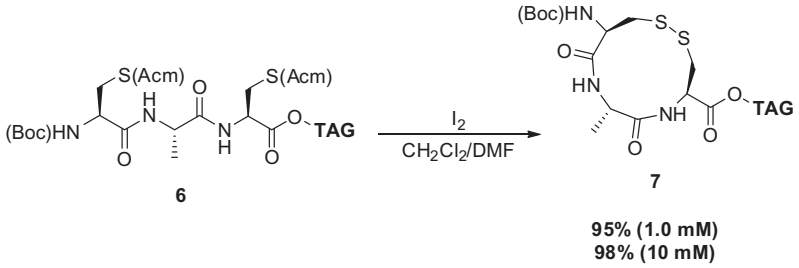


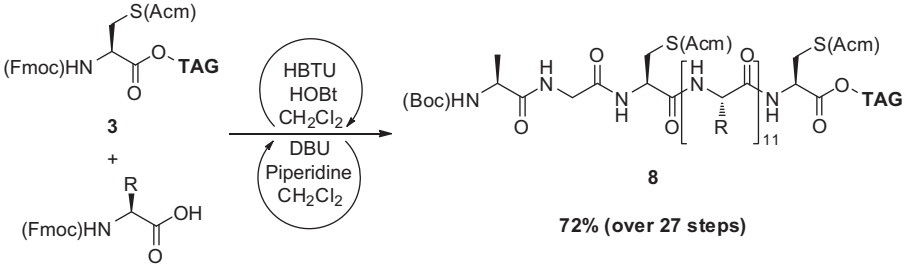
Scheme 1. Introduction of *N*-Fmoc-Cys(Acm)-OH **1** to the hydrophobic tag, 2,4-didocosyloxybenzyl alcohol, (HO-**TAG**) **2**.



Scheme 2. Construction of the model peptide for the evaluation of the stability of the hydrophobic tag against the conditions used for disulfide bond formation.



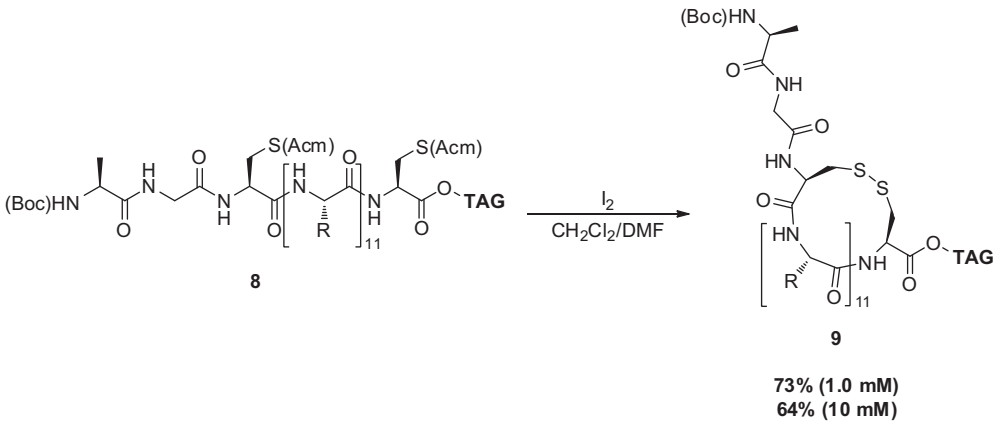
Scheme 3. Iodine oxidation of *N*-Boc-Cys(Acm)-Ala-Cys(Acm)-O-**TAG 6**.



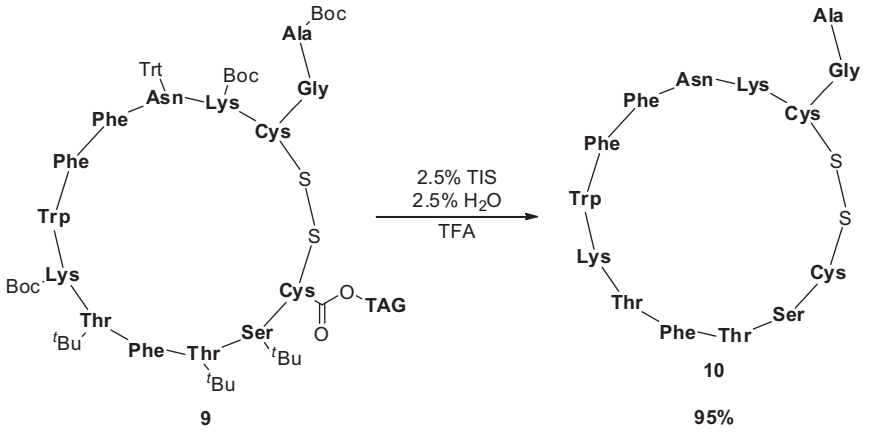
Scheme 4. Synthesis of hydrophobically tagged peptide **8**.

zole (HOBt) in the presence of *N*,*N*-diisopropylethylamine (DIPEA) to afford *N*-Boc-Cys(Acm)-Ala-Cys(Acm)-O-**TAG 6** in 93% isolated yield over four steps (Scheme 2). All reaction steps, including both elongation and deprotection, were efficiently carried out homogeneously in the liquid-phase, realizing both high reaction rate and rapid reaction monitoring. The desired hydrophobically tagged peptides could be separated as precipitates from the reaction solutions after the completion of the reactions. Iodine oxidation was then attempted with **6** at 1.0 mM concentration to give the desired disulfide bond-containing peptide **7** in 95% isolated yield (Scheme 3). Notably, the iodine oxidation of **6** also gave **7** in 98% isolated yield even at a 10 mM concentration of **6**. These results suggested that disulfide bond formation was effectively combined with the hydrophobic tag-assisted peptide synthesis, which was successfully carried out even under relatively high concentrations.

With these results in hand, we next turned our attention to the synthesis of a growth hormone-inhibiting peptide, somatostatin, as a model of bioactive disulfide bond-containing peptides. The receptor subtypes of somatostatin are well-established targets for several therapeutic studies. To date, several synthetic strategies that address the disulfide bond formation have been proposed to elaborate somatostatin based on both solid-phase and liquid-phase techniques.[53–55] In order to remove all protective groups under acidic conditions at the final step of the synthesis, we chose the



Scheme 5. Iodine oxidation of hydrophobically tagged peptide **8**.



Scheme 6. Deprotection of hydrophobically tagged disulfide bond-containing peptide **9**.

Boc and *t*-Bu groups for the protection of both the *N*-terminus and side chains. Several *N*-Fmoc amino acids (AAs) (except the last, *N*-Boc-Ala-OH) were repeatedly introduced to **3** using HBTU and HOBt in the presence of DIPEA to afford the desired hydrophobically tagged peptide **8** in 72% isolated yield over 27 steps (Scheme 4). When **8** at a concentration of 1.0 mM was oxidized using iodine, the desired disulfide bond-containing peptide **9** was formed in 73% isolated yield (Scheme 5). Furthermore, even at a concentration of 10 mM, **8** could give **9** in 64% isolated yield through iodine oxidation. Finally, deprotection of **9**, including the N-terminal Boc group, side chain protective groups, and C-terminal **TAG**, was achieved in a single step under acidic conditions to give deprotected compound, somatostatin, **10** in 95% isolated yield (Scheme 6).

In conclusion, disulfide bond formation has effectively been combined with hydrophobic tag-assisted peptide synthesis, leading to the efficient and practical preparation of a growth hormone-inhibiting peptide somatostatin. Disulfide bond formation has successfully been carried out even under high concentrations. This approach should find applications not only for the synthesis of naturally occurring disulfide bond-containing peptides, but also for the creation of artificial cyclic peptides.

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Supplementary data

Supplementary data (general information, experimental details, characterization data, and copies of ^{1}H NMR and ^{13}C NMR spectra of new compound) associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2011.06.004.

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