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A silver-promoted solid-phase guanidylation process enables the first total synthesis of Stictamide A

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The first total synthesis of Stictamide A, a structurally unique peptide with a statine motif and a N-prenyl modified arginine in the side chain, is disclosed. The requisite statine was achieved via stereoselective hydrogenation of a functionalized ketone. The N-prenyl modified arginine was constructed by a novel silver-promoted solid-phase strategy for the first time. This synthetic method can be generally applied to the efficient synthesis of peptides containing statine and/or arginine N-alkylation groups.

A *N*-modified arginine has been proved to exist in many natural compounds and play a vital role for their biological activities.[1] Among them, Stictamide A (as shown in **Figure 1**), isolated from a new *Sticta* sp. of lichen, was shown to inhibit matrix metalloproteinase-12 (MMP12) at 2.3 μM (IC_{50} values) and significantly reduce cell invasion in the human glioma cell line U87MG. It is illustrated that the *N*-prenyl modified arginine in the side chain is necessary for the biological activities of Stictamide A.[2]

Actually, an increasing attention has been focused on the modification of guanidyl including the modification of arginine. An important example is that *N*-glycosylation of arginine in proteins.[3] Both the discovery of NleB with an arginine GlcNAc (*N*-acetylglucosamine) transferase activity and EarP with rhamnosyltransferase activity prove the importance of arginine glycosylation.[4] Another example is Martinellic acid, isolated from the species *Martinella iquitoensis*, also contains *N*-prenyl modified guanidyls (as shown in **Figure 1**). And these key motifs are imperative for potent activity for treating eye ailments. [5]

Meanwhile, Stictamide A harbors a kind of statine, which is naturally occurring nonribosomal amino acid widely present in many peptide natural products. As the inhibitors of some key proteases,many natural peptides containing statines have already been reported to display various biological activities[6], such as Grassystatin A[7], Tasiamide B[8], dolastatins[9], and hapalosin[10] (as shown in **Figure 1**). Therefore there is no denying that the statine groups are vitally important to the biological activities of these natural products.[11]

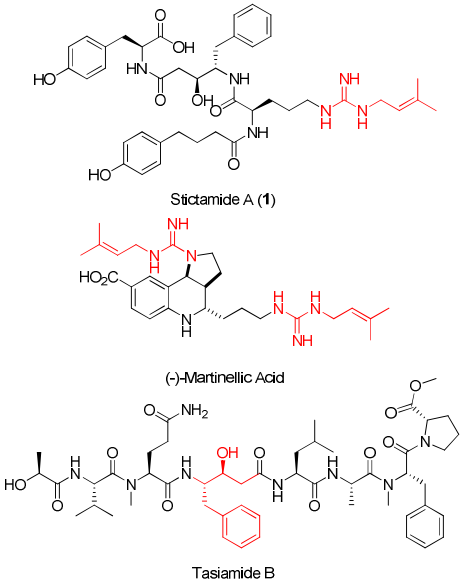

Figure 1. Representative structure of natural peptides constituted with *N*-prenyl modified arginine or statines

As a protease-inhibiting peptide, Stictamide A could be represented as a potent anticancer leading compound. However, d-etailed biological studies of Stictamide A are impeded by limited access to this compound due to its low abundance in natural sources. Herein, we report the first total synthesis of Stictamide A, which was facilitated by a novel silver-promoted solid-phase guanidylation pr ocess. To accomplish the total synthesis of Stictamide A, we chose a solid-phase synthetic route, which we expected would be more efficient than a solution-phase route. According to the retrosynthetic analysis (as shown in **Figure 2**), the synthetic target **1**

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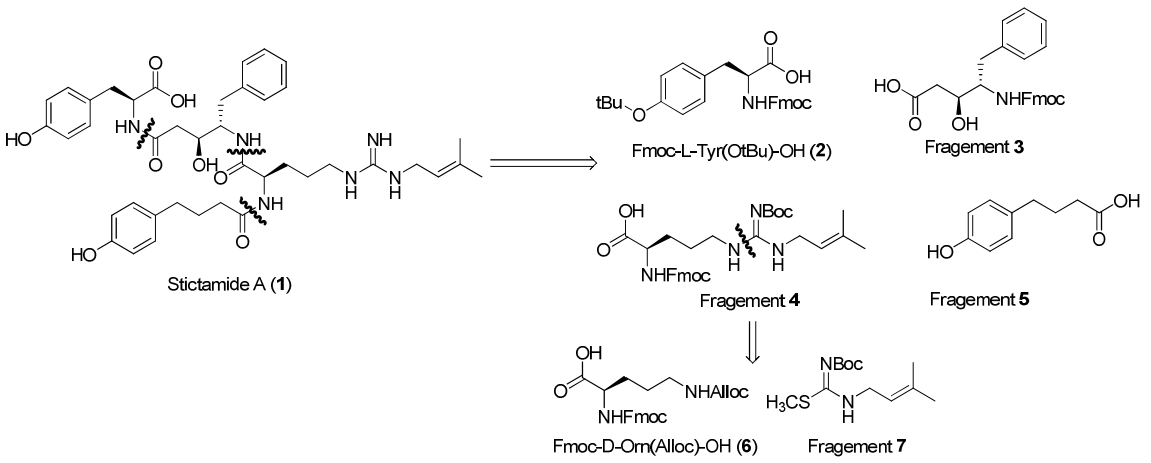


Figure 2[a]. Retrosynthetic analysis of Stictamide A.
[a]Abbreviations: Fmoc = 9-Fluorenylmethoxycarbonyl; Alloc = allyloxycarbonyl; tBu = tertiary butyl; Boc = tertiary butyloxy carbonyl.

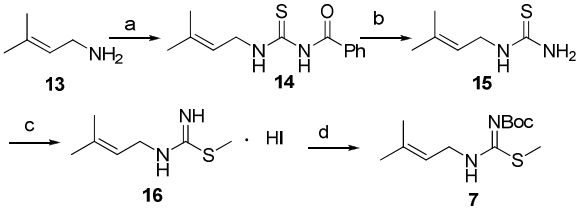
would be accessed from two α-amino acids (**2** and **4**), one *γ*-amino-*δ*-hydroxy acid (**3**) and one butanoic acid (**5**), in which the fragment **2** and **5** are commercial available. Once the preparation of these fragments is completed, fragment **2** could be installed to the resin and **3**, **4** and **5** could be sequentially introduced using standard Fmoc solid-phase peptide synthesis (SPPS) to afford the title compound.

However, the synthesis of Stictamide A is still challenging due to the following problems: 1) The building block **4** cannot be obtained easily with either solution-phase or solid-phase approaches;[12] and 2) the preparation of statine-like amino acid **3** requires stereoselective chemistry. The traditional approach to preparing *N*-prenyl modified arginine derivatives is *via* a solution-phase route. [13] Therefore, we pursued a solution-phase synthesis of modified arginine block **4** for subsequent use in the SPPS construction of stictamide A. Unfortunately, after extensive investigation we found that the preparation of **4** was inefficient due to the requirement for multiple protection and deprotection steps, plus it was very difficult to purify. Therefore, we turned to another strategy featuring direct guanidylation of the amino acid side chain while on solid support, which has been successfully used to prepare glycopeptides with Arginine *N*-glycosylation in previous study[14] including our work[14a].

To accomplish this strategy, we were interested in the silver-promoted guanidylation reaction between an *S*-alkyl-isothiourea and an amine, which is an effective approach for the construction of guanidine moieties *via* a solution-phase route in natural product synthesis. For example, this method has been successfully applied for the synthesis of Martinellic acid, which contained the same *N*-prenyl-modified arginine as the title compound.[15]

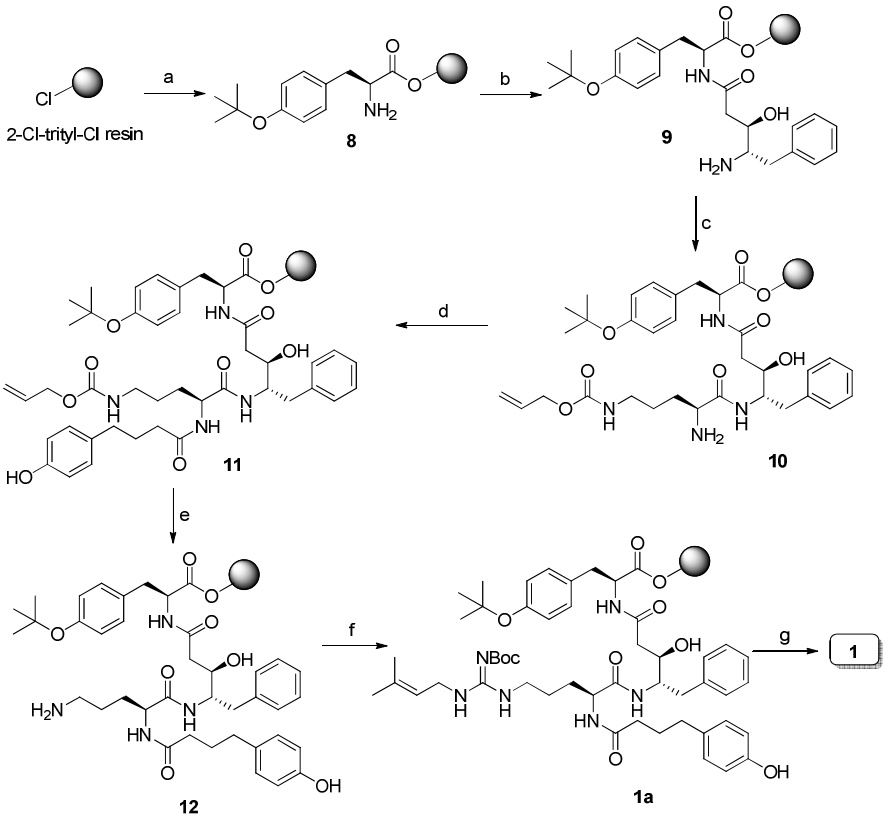
In this study, we report the first the formation of an *N*-prenyl-modified arginine by a silver-promoted solid-phase route. Specifically, the building block **4** can be obtained from fragments **6** and **7**. For the key building block **7**, i.e. *N*-prenyl-*S*-alkyl-isothiourea, the synthetic route is shown in **Scheme 1**. Treatment of prenylamine (**13**) with phenylcarbonyl isothiocyanate in acetonitrile at room temperature provided the compound **14**[16], which was deprotected with K_2CO_3 in methanol to give the thiourea **15**, which was methylated with CH_3I to afford methyl isothiourea hydroiodide salt **16**. Finally, the treatment of **16** with Boc_2O assisted by Et_3N and DMAP gave *N*-(tert-butoxycarbonyl)-*N*'-(3-methyl-2-butenyl)-*S*-methylisothiourea **7**.[17]

Another key building block **3**, a statine-like amino acid derivative, which might play an important role for the biological activity of stictamide A, is 4-amino-3-hydroxy-5-phenylpentanoic acid. In our study, steric hindrance caused by the *N,N*-dibenzyl protected group was exploited to acquire the product with correct stereochemistry. (as shown in **Scheme 2**). First, naturally occurring *L*-amino acid (**17**) was protected at nitrogen to provide tribenzylated esters and then the benzyl ester was converted to the *N,N*-dibenzylamino acid **18**[18]. Second, **18** was converted to the corresponding *α*-keto ester **19**



Scheme 1 Synthesis of building block 7[a]

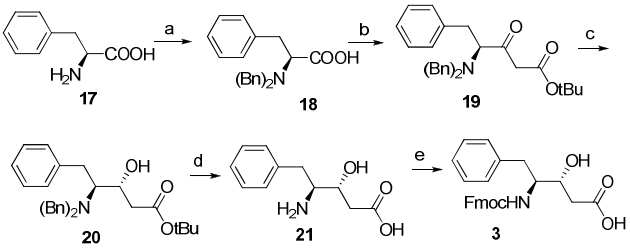
[a]Reagents and conditions: (a) benzoyl chloride, KSCN, acetone, acetonitrile, 2h, 68 %; (b) K_2CO_3, methanol, 1h, 61%; (c) CH_3I, DMF, 12h, 82%; (d) Boc_2O, Et_3N, DMAP, DCM, 84%. Abbreviations: KSCN = potassium thiocyanate; K_2CO_3 = potassium carbonate; DMF = *N,N*-dimethylformamide; DCM = dichloromethane; CH_3I = potassium iodide; Boc_2O = di-tert-butyl dicarbonate; Et_3N = triethylamine; DMAP = 4-dimethylaminopyridine; Ph = phenyl.



Scheme 3. The synthetic route of Stictamide A[a]

[a]Reagents and conditions: (a) i. **2**, DIEA, DCM/DMF, ii. 20% piperidine/DMF; (b) i. **3**, HCTU, DIEA/DMF, ii. 20% piperidine/DMF; (c) i. **6**, HCTU, DIEA/DMF, ii. 20% piperidine/DMF; (d) i. **5**, HCTU, DIEA/DMF, ii. 20% piperidine/DMF; (e) $Pd[P(Ph)_3]_4$, Phenylsilane/DCM; (f) **7**, $AgNO_3$, Et_3N/DMF; (g) TFA/water. Abbreviations: HCTU = *O*-(6-chloro-1-hydrocibenzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphat; $Pd[P(Ph)_3]_4$ = tetrakis (triphenylphosphine)palladium(0); $AgNO_3$ = silver nitrate.

by the CDI-mediated coupling of the amino acid with lithiotert-butyl acetate in excellent yield. Third, borohydride reduction of



Scheme 2 Synthesis of building block 3[a]

[a]Reagents and conditions: (a) K_2CO_3, benzoyl bromide, ethanol, reflux, 5h; then NaOH, H_2O, dioxane, 82%; (b) *n*-BuLi, DIEA, butyl acetate, CDI, THF, 2h, 61%; (c) $NaBH_4$, methanol, 12h, 82%; (d) TFA/DCM; then Pd/C, H_2, methanol, 86%; (e) Fmoc-Cl, DMAP, DCM, 75%. Abbreviations: Bn = benzoyl; NaOH = sodium hydroxide; *n*-BuLi = normal-butyllithium; DIEA = diisopropylamine; CDI = carbonyldiimidazole; THF = tetrahydrofuran; $NaBH_4$ = sodium borohydride; TFA = trifluoroacetic acid; Pd/C = 10% palladium on charcoal; Fmoc-Cl = 9-fluorenylmethyl chloroformate.

19 gave statine **20** with the correct relative and absolute stereochemistry owing to the steric effect (dr = 19: 1)[19]. Fourth, in presence of TFA and Pd/C, the tBu and dibenzyl groups of **20** were removed sequentially to afford **21**. Finally, the amino group of **21** was protected by an Fmoc group to give the key building block **3**[20], which was ready for use in standard Fmoc SPPS.

With **3** and **7** in hand, we then carried out the synthesis of **1** using the on-resin guanidylation strategy (as shown in Scheme **3**). The linear peptide was first prepared using standard Fmoc SPPS procedures with 2-chlorotrityl resin as the solid support. The building block **2**, **3**, **6** and **5** were successively assembled onto the resin, where Fmoc-D-Orn(Alloc)-OH was used as the precursor for the *N*-prenyl-modified arginine residue. After the peptide assembly was completed to give **11**, the Alloc group was removed using $Pd[P(Ph)_3]_4$ to yield compound **12** on resin[21]. The free amino side chain of **12** was then treated with $AgNO_3$ and **7** to afford protected stictamide A (**1a**) on resin[22]. The resin was then treated with 5% water in TFA to release **1**, which was purified by preparative reverse-phase HPLC. The overall isolated yield of **1** was 28% as calculated from the resin loading, indicated the good efficiency of the on-resin

guanidylation process. All the key intermediates were monitored by analytical HPLC and successfully characterized by ESI-MS. The NMR and MS data of the synthetic product are identical to those of natural **1**.

In summary, the first total synthesis of stictamide A employing a silver-promoted solid-phase guanidylation process has been achieved. This work represents the first report of forming *N*-prenyl-modified arginine with a silver-promoted solid-phase route. This strategy allows for the preparation of sufficient quantities of the natural product for detailed biological studies. Meanwhile, our synthetic method can be generally applied to the efficient synthesis of peptides containing statine and/or arginine *N*-alkylation groups.

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