

# Total Synthesis of Spiruchostatin A via Chemoselective Macrocyclization using an Accessible Enantiomerically Pure Latent Thioester

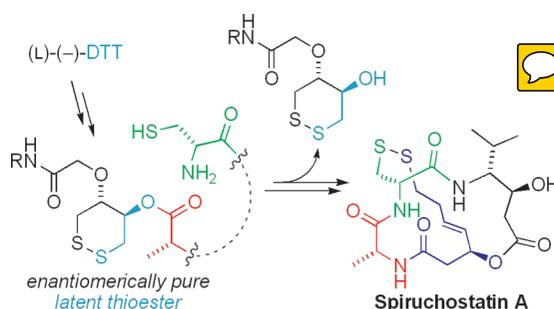
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## ABSTRACT



HDAC inhibitor Spiruchostatin A was synthesized via a route that differs significantly from previously reported routes. The key step involves a latent thioester that initiates a chemoselective transformation similar to native chemical ligation to form the macrocyclic alanine–cysteine amide bond. The easily prepared latent thioester—the first such moiety reported in enantiomerically pure form—is designed with a pendant carboxylic acid to serve as a solid-phase linker for the synthesis of cyclic, cysteine-containing, peptidic materials.

Spiruchostatin A (**1a**)<sup>1</sup> is a member of a class of cyclic, cysteine-containing, depsipeptidic natural products shown in Figure 1 that also includes FK228 (**2**)<sup>2</sup> and FR901,375 (**3**),<sup>3</sup> and can also include Largazole (**4**).<sup>4</sup> Due to their histone deacetylase (HDAC) inhibitory activity, tremendous effort has been thrust into the synthesis of these compounds and their analogs as potential chemotherapeutic targets.<sup>5–7</sup> Struc-

tural features shared by these molecules include an (*S,E*)-3-hydroxy-7-mercapto-4-heptenoate (Hmh) residue shown in blue, in most cases a D-Cys residue illustrated in green, and an Ala or Val residue drawn in red appended to the Hmh

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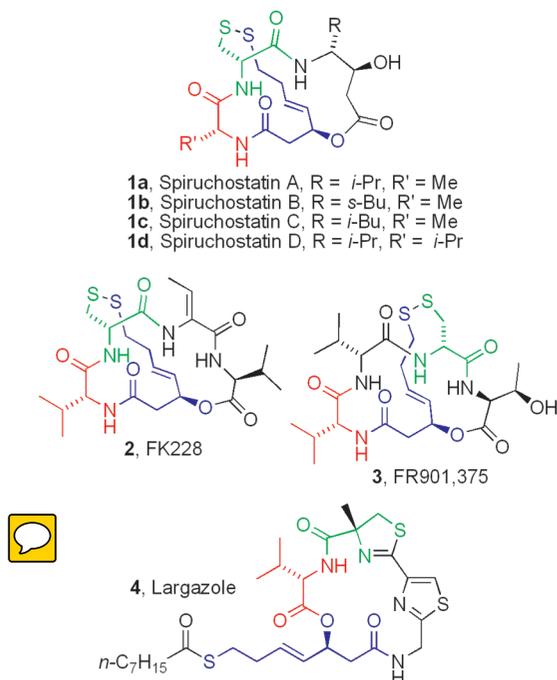
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moiety via an amide bond (except in Largazole). The Hmh residue has been shown to be involved in HDAC inhibitory activity.<sup>8</sup>



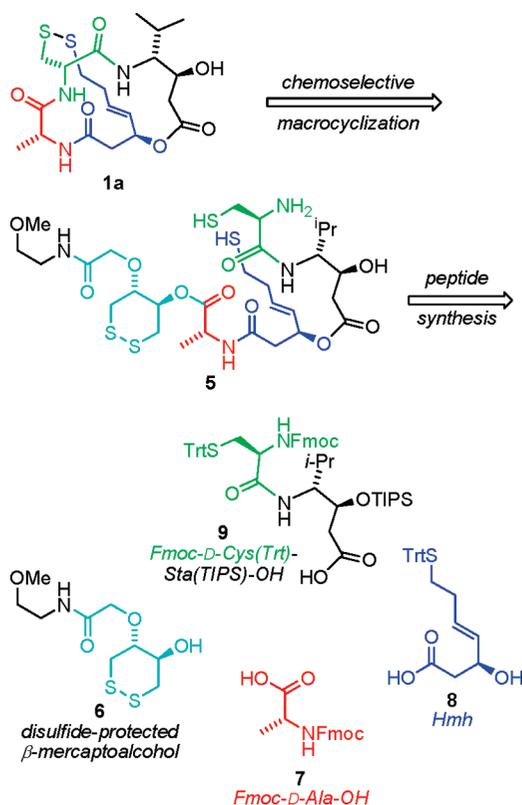
**Figure 1.** Various macrocyclic depsipeptides, including Spiruchostatin A (**1a**), containing Hmh (blue), cysteine (green), and Ala or Val residues (red).

Several syntheses of **1a** have been published, with each reported synthesis employing a key **macrolactonization** to form the depsipeptidic linkage between the D-Ala and Hmh residues.<sup>5,9</sup> Such synthetic routes have been shown to be effective in generating both the parent compound and even analogs of **1a**.<sup>6</sup> However, similar macro-lactonizations en route to FK228 (**2**) have been reported to be **difficult due to the sensitivity of the reacting allylic alcohol moieties or steric concerns**.<sup>7k,9a,10</sup> Issues regarding the reaction conditions for these macrolactonizations apparently render such routes ill-suited to the more demanding environment of solid-phase synthesis, given the lack of reported solid-phase syntheses for any of these compounds. Peptidic isosteres of FK228 were synthesized on the solid phase, but only when the lactone functionality was replaced with a lactam.<sup>11</sup> A solid-phase synthetic route toward **1a**, and by extension **2–4**, would facilitate the generation of their analogs for **SAR**.

Ganesan, et al. have noted the shortcomings of the macrolactonization routes<sup>10b</sup> and have recently developed a different route toward FK228 (**2**) that involves **macrolactamization via standard peptide coupling methods** and the concomitant required protecting group manipulations.

We envisioned instead a route, illustrated in Scheme 1, that **employs the D-Cys residue in a key macrolactamization involving the N-terminal D-Cys residue and the C-terminal D-Ala latent thioester of 5**. Macrocyclizations such as this

**Scheme 1.** Retrosynthesis of Spiruchostatin A (**1a**)<sup>a</sup>



<sup>a</sup> Abbreviations: NCL = native chemical ligation; Sta = statine.

one that are similar to **native chemical ligation (NCL)**<sup>12</sup> have been demonstrated using C-terminal thioesters<sup>13</sup> and even a latent aryl thioester.<sup>14</sup> Moreover, since latent thioesters have been shown previously to function as solid-phase linkers,<sup>15</sup> if the latent thioester here were designed to serve as a solid-phase linker, then the synthetic route would have the clear advantage over previous routes of making the transition from solution phase to the solid phase manageable. The remainder

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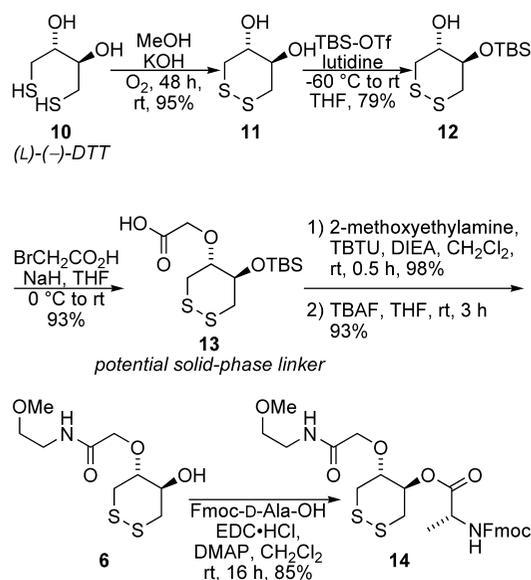
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of the strategy involves peptide synthesis using commercially available amino acids (**7**) or materials that can be accessed through previously described routes (**8** and **9**).<sup>3,5,16</sup>

To serve ultimately as a solid-phase linker for a synthesis that will precisely mimic the solution-phase version described here, the latent thioester requires an attachment point to the solid phase, and therefore a stereocenter, somewhere along its  $\beta$ -mercaptoalcohol functionality.<sup>17</sup> The enantiomeric purity of this functional group is critical for the spectroscopic analysis required during the solution-phase synthesis of **1a** and would also facilitate similar analysis of cleaved intermediates that still contain the linker during the envisioned solid-phase synthesis. Disulfide protection of the thiol is valuable because this protecting group is generally stable, but is readily removed under the reducing conditions of NCL. To fulfill the structural requirements of an enantiomerically pure, disulfide protected  $\beta$ -mercaptoalcohol, we developed the short, efficient route displayed in Scheme 2 toward

**Scheme 2.** Synthesis of an Enantiopure Latent Thioester



internally disulfide protected  $\beta$ -mercaptoalcohol **13**, which is a potential solid-phase linker due to its pendant carboxylic acid. The starting material for this linker is (L)-(-)-1,4-dithiothreitol (**10**), which is a commercially available enantiomerically pure compound with much of the essential functionality. The linker synthesis requires only three high-yielding steps: oxidation to form disulfide **11**,<sup>18</sup> selective protection of one of the two C<sub>2</sub>-symmetrical secondary

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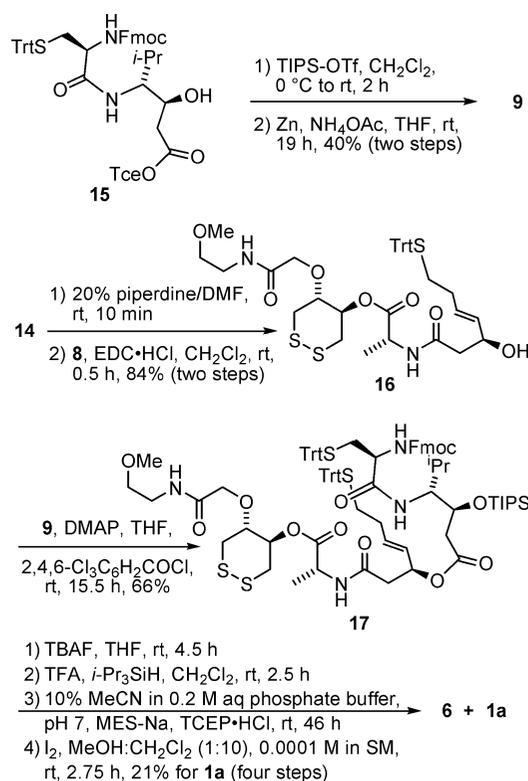
(17) Although alkyl  $\beta$ -mercaptoalcohol linkers with all of the appropriate functionality can be designed without a stereocenter, these would generally require a tertiary thiol or alcohol, which would confer unwanted reactivity or steric hindrance. Disulfide-protected aryl  $\beta$ -mercaptoalcohols (*o*-mercaptophenols) are not suitable as Fmoc solid-phase linkers because their esters are cleaved by the secondary amines used for Fmoc deprotection.

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alcohols to give TBS ether **12**, and etherification of the remaining alcohol with bromoacetic acid to give **13** in 70% combined yield for the three steps. To occupy the position designed for a solid-phase resin, acid **13** was coupled with 2-methoxyethylamine (to simulate a PEG-resin), which upon TBAF removal of the silyl ether afforded alcohol **6**. In a step equivalent to resin loading, Fmoc-D-Ala-OH was coupled under standard esterification conditions to give **14**.

Further elaboration of the depsipeptide required that two intermediates, **8** and **9**, be synthesized. Trityl-protected hydroxymercaptoheptenoate **8** was synthesized as described, and exhibited spectroscopic properties identical to those reported.<sup>3</sup> Compound **9** was prepared from known **15** (Scheme 3), which also displayed spectroscopic properties

**Scheme 3.** Completing the Synthesis of Spiruchostatin A (**1a**)<sup>a</sup>



<sup>a</sup> Abbreviations: MES-Na = sodium 2-mercaptoethanesulfonate; TCEP = tris(carboxyethyl)phosphine; Tce = trichloroethyl.

identical to those reported.<sup>5</sup> Conversion of **15** into **9** involved protection of the secondary alcohol as a silyl ether followed by removal of the Tce protecting group, as shown in Scheme 3.

Protected Ala fragment **14** was extended by removal of Fmoc using piperidine followed by standard carbodiimide peptide coupling with carboxylic acid **8** to give alcohol **16**. Yamaguchi-type esterification<sup>19</sup> of carboxylic acid **9** with alcohol **16** afforded protected depsipeptide **17**, which contains

(19) Du, Y.; Chen, Q.; Linhardt, R. J. *J. Org. Chem.* **2006**, *71*, 8446.

all of the functionality of **1a** but is absent in both macrocycles. In elaborating **16**, the statine and D-Cys residues were first joined into a single unit to prevent the expected<sup>16</sup> unproductive cyclization of the statine  $\gamma$ -amine onto the newly formed allylic ester. Although a Boc-protected statine derivative could in principle circumvent this issue through in situ neutralization,<sup>20</sup> removal of the Boc group in the presence of the trityl thioether proved difficult in this case, and such a route was not pursued further.

Because it involves only coupling and deprotection reactions, the linear route from **13** to **17** will likely benefit from adaptation of this synthesis to the solid phase, where excess reagents and their byproducts are easily removed. For example, optimizing the Yamaguchi reaction affording **17** was challenging because **17** and excess **9** were difficult to separate chromatographically. Because no products from elimination of the allylic alcohol (or ester, after the fact) were observed, and **17** was stable for weeks, the other issues with this coupling may largely stem from side reactions of **9** (e.g., premature cyclization of the activated acid), and such issues are readily addressed during a solid-phase synthesis using excess **9**.

Conclusion of the synthesis from **17** required global deprotection followed by the key chemoselective macrocyclization and further oxidative macrocyclization.<sup>5</sup> TBAF-mediated removal of both the silyl ether and the Fmoc protecting group,<sup>10b</sup> followed immediately by trityl deprotection with TFA, yielded a compound consistent<sup>21</sup> with **5** (Scheme 1). Purification of this relatively unstable (oxidation) and likely water- and organic-soluble material was not attempted; the intermediate was instead subjected immediately to the aqueous conditions required for NCL.<sup>15b</sup> The products of this reaction were not isolated, thus preventing intermolecular disulfide formation of the still readily oxidized unprotected thiols. The crude product mixture was treated immediately under dilute conditions with iodine,<sup>5</sup> affording the desired **1a** and also disulfide **6**, as

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(21) During TLC, the color change and rate of oxidation (little heat required) with ceric ammonium molybdate stain were consistent with the presence of free thiol, and ninhydrin stain indicated a free amine.

expected. All of the spectroscopic and analytical properties of our synthetic **1a** were identical to those reported previously for Spiruchostatin A,<sup>1</sup> thus confirming a successful synthesis.

We have clearly demonstrated an effective new strategy for generating Spiruchostatin A. Furthermore, the isolation of both **1a** and **6**, along with the stability of the alaninyl ester of **6** throughout the synthesis, indicates that the ester of **6** functioned as expected for a latent thioester. This proof of principle not only provides a new method for generating the Spiruchostatins and their analogs; it also opens the door to new syntheses of FK228 and FR901,375 and their analogs, as well. Moreover, carboxylic acid **13** is designed to be loaded onto suitable peptide synthesis resins using standard coupling conditions and, following removal of the silyl ether protection, used as would any other peptide ester resin. This work will hopefully thus facilitate the solid-phase synthesis of analog libraries for SAR and drug development of these cysteine-containing, cyclic depsipeptides. Finally, the easily synthesized **13** is a single enantiomer, which enabled the analysis of the resulting diastereomerically homogeneous latent thioester intermediates described here, and will by extension do the same for intermediates containing **13** that are cleaved for analysis during solid-phase synthesis using **13** as a linker. Results from our solid-phase synthetic efforts will be reported in due course.

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**Supporting Information Available:** Experimental procedures along with spectroscopic and analytical data for all new compounds. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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