

# Solid phase total synthesis of the 3-amino-6-hydroxy-2-piperidone (Ahp) cyclodepsipeptide and protease inhibitor Symplocamide A†‡

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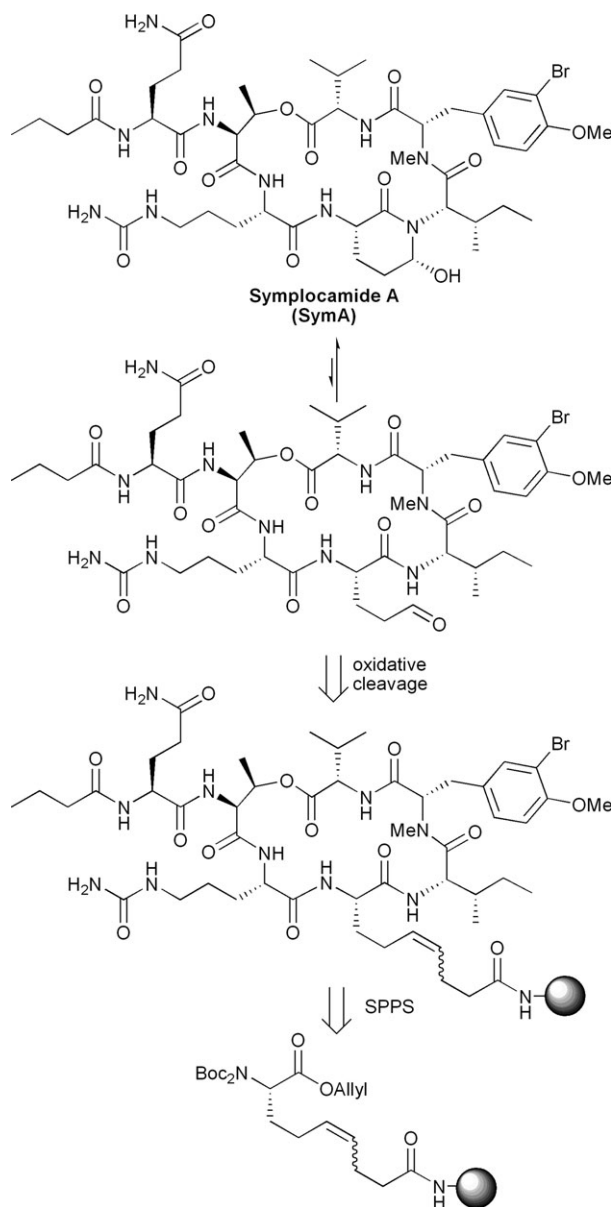
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The solid phase total synthesis of the marine cyanobacterial Ahp-cyclodepsipeptide Symplocamide A is reported as a model for a general route for the synthesis of tailor-made non-covalent serine protease inhibitors.

Freshwater and marine cyanobacteria produce a wide variety of secondary metabolites with diverse activities.<sup>1</sup> Among them, the class of 3-amino-6-hydroxy-2-piperidone (Ahp) containing cyclic depsipeptides is of particular interest due to their potent, selective and most intriguingly non-covalent inhibition of S1 (trypsin- and chymotrypsin-like) serine proteases.<sup>2,3</sup> These interesting biological activities suggest that this class of natural products might serve as a promising lead for serine protease inhibitor design.<sup>2</sup>

However, limited availability interferes with the wider application of Ahp cyclodepsipeptides as protease inhibitors in chemical biology research. Isolation usually yields very low amounts and chemical synthesis has been to date only reported for two Ahp cyclodepsipeptides *i.e.* Somamide A and Micropeptin T20, both generated after extensive multi-step solution phase chemistry.<sup>4</sup> To facilitate and speed up their synthesis and more importantly to gain access to custom-made Ahp cyclodepsipeptides, a versatile and straight-forward solid phase synthesis route would therefore be highly desirable. To establish such a methodology, we considered the Ahp cyclodepsipeptide natural product and strong chymotrypsin inhibitor Symplocamide A (SymA) as a suitable model target structure (Scheme 1).<sup>5</sup> To date, no synthesis of SymA has been reported and SymA furthermore introduces two interesting new features to the Ahp natural product class, *i.e.* a citrulline residue at the P1 site and the unusual and rarely found *N,O*-dimethyl-3-bromotyrosine residue in the macrocyclic part of the molecule.

Previous synthetic studies have shown that the Ahp moiety forms spontaneously from a glutamic aldehyde generated within the correctly assembled precursor cyclodepsipeptide (Scheme 1).<sup>4</sup> A general synthetic route could therefore consist of a solid phase synthesis of the cyclodepsipeptide backbone, followed by the regio- and chemoselective generation of a glutamic aldehyde. Consequently, such a strategy would



**Scheme 1** A solid phase-based retrosynthesis of Symplocamide A (SymA), using an unsaturated building block as a masked glutamic aldehyde equivalent.

require a suitable masked aldehyde equivalent that should (a) be compatible with Fmoc- as well as Boc-protecting group strategies; (b) generate the glutamic aldehyde residue upon cleavage from the resin, thereby spontaneously forming also the hemiaminal of the Ahp unit in a convenient one-pot procedure; and (c) represent a suitable anchoring group for

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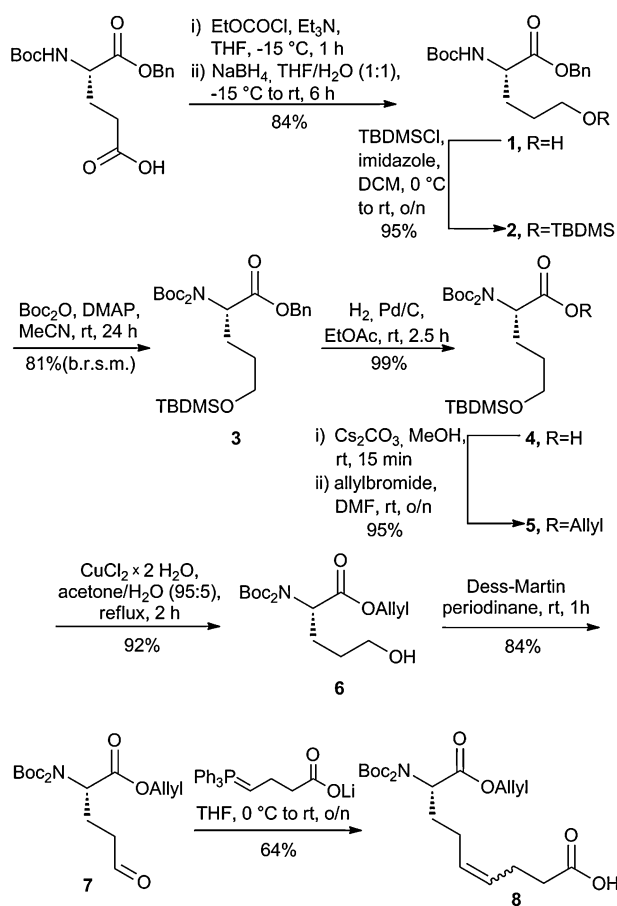
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the attachment to the solid support that still allows subsequent chemical modification of the N- and C-termini of the glutamic aldehyde equivalent. We envisioned that a novel amino acid consisting of a double bond as a masked aldehyde function and a free carboxylic acid for linking to the resin *via* its side chain could serve these requirements, as the Meldal group has previously shown that oxidative cleavage of double bonds on solid support efficiently generates aldehydes.<sup>6</sup>

Consequently, our synthetic investigations started with the solution phase synthesis of the Ahp precursor molecule **8** (Scheme 2). To this end, *N*-Boc protected glutamic acid benzyl ester was activated as a mixed anhydride and then reduced with sodium borohydride using the protocol by Shioiri *et al.*,<sup>4</sup> thereby yielding alcohol **1**. **1** was then protected as a silyl ether and the resulting intermediate **2** was converted to the di-Boc protected derivative **3**.<sup>7</sup> Removal of the benzyl ester protecting group by hydrogenation led to the acid **4**,<sup>8</sup> which was reprotected as an allyl ester using the well-established protocol by Kunz *et al.*,<sup>9</sup> thereby obtaining the fully protected intermediate **5**. The subsequent removal of the silyl ether to obtain alcohol **6** emerged to be demanding, as the standard cleavage conditions using fluoride or mild acidic conditions led to the loss of one of the two Boc protecting groups. This issue was solved with the very mild copper-mediated cleavage conditions described by Wang *et al.*<sup>10</sup> The following oxidation of **6** to

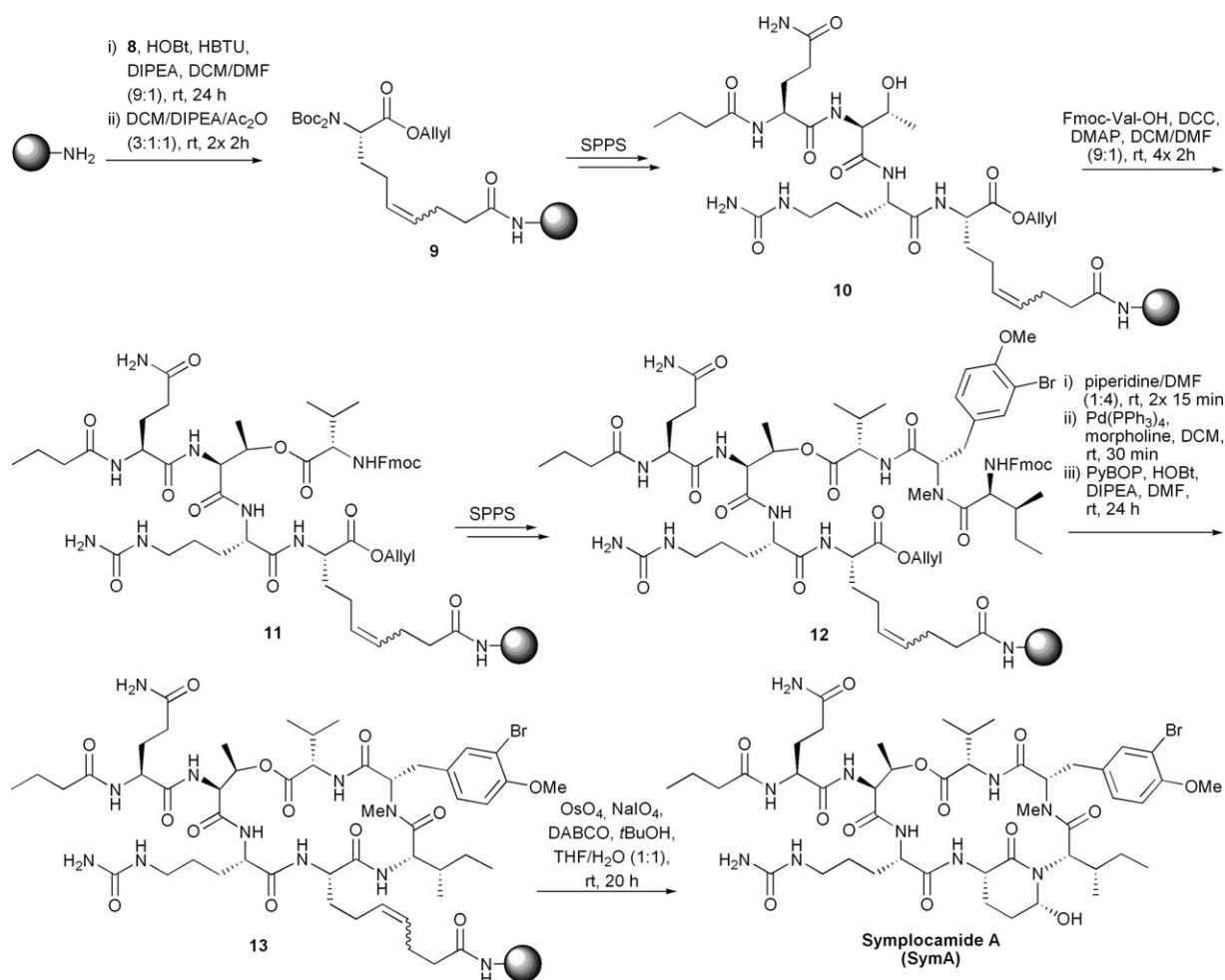


**Scheme 2** Solution synthesis of building block **8**, the general precursor for the solid phase synthesis of Ahp containing cyclodepsipeptides.

aldehyde **7** under Dess–Martin conditions proceeded smoothly in 84% yield. **7** was then immediately converted by a Wittig reaction to obtain building block **8** for the subsequent solid phase synthesis.

With **8** in hand, the assembly of the cyclodepsipeptide on solid support could be initiated (Scheme 3). To prevent unwanted side reactions during the on resin macrolactamization, the reaction conditions were optimized towards a low loading (0.24 mmol g<sup>−1</sup> resin) of **8** to a NovaPEG amino resin, resulting in **9**. Deprotection of the Boc-protecting groups with 50% TFA in DCM sets the stage for the subsequent amino acid couplings. Couplings were performed with HBTU (3.5 eq.) and HOBT (4 eq.) as the coupling reagents, DIPEA (3 eq.) as the base and the corresponding Fmoc- or Boc-protected amino acids (4 eq.). First, Fmoc-citrulline was coupled, followed by Fmoc-Thr-OH, Boc-Glu-OH and finally butyric acid, leading to intermediate **10**. For cleavage of the Fmoc- or Boc-protecting groups, either 20% piperidine in DMF or 50% TFA in DCM were used. The adjacent esterification of the threonine hydroxyl group side chain with Fmoc-Val-OH (10 eq.), thereby yielding **11**, was achieved with DMAP (1 eq.) and di-isopropyl carbodiimide (10 eq.) as the activating reagents. Fmoc-deprotection followed by coupling with *N*-Boc-*N,O*-dimethyl-3-bromotyrosine using standard coupling conditions and subsequent Boc-deprotection yielded an *N*-methyl intermediate, to which Fmoc-isoleucine (5 eq.) was coupled, using PyBOP (4.9 eq.) as the coupling reagent and DIPEA (10 eq.) as the base and a reaction time of 24 h. This intermediate **12** was deprotected at the N-terminus by Fmoc-cleavage and the C-terminus by removal of the allyl protection group by the protocol of Vaz and Brunsfeld<sup>11</sup> led to the cyclization precursor. Macrolactamization was obtained by PyBOP (4 eq.)/HOBT (8 eq.)/DIPEA (12 eq.) activation and a reaction time of 24 h, thereby yielding **13**. The final conversion of the double bond of **13** into the aldehyde moiety for establishing the Ahp residue in SymA and for simultaneous cleavage from the resin was achieved with OsO<sub>4</sub> and NaIO<sub>4</sub> as oxidation reagents and DABCO to prevent the formation of hydroxymethyl ketones.<sup>6</sup> To the best of our knowledge, this is the first time that these reagents are used for cleaving aldehydes from a resin. Subsequent chromatographic purification of the crude product yielded the desired natural product Symplocamide A (SymA). The resulting <sup>1</sup>H and <sup>13</sup>C NMR spectra of the synthetic compound corresponded well to the data published for the natural product.<sup>5</sup> Moreover, the synthetic SymA demonstrated potent chymotrypsin inhibition, displaying a *K<sub>i</sub>* value of 0.32 ± 0.09 μM, in accordance with the literature report (lit.,<sup>5</sup> IC<sub>50</sub> = 0.38 ± 0.08 μM).

We have established the first solid phase synthesis of an Ahp cyclodepsipeptide by synthesizing the natural product Symplocamide A. Essential for the overall synthesis strategy was the development of the masked aldehyde precursor **8** that proved to be a useful building block to install the Ahp moiety at the last stage of the synthesis. The established synthetic route holds promise for the synthesis of custom-made Ahp depsipeptides, thereby opening the possibility to generate non-covalent S1 serine protease inhibitors as chemical biology tools in a fast and efficient manner for protease research.



**Scheme 3** The solid phase synthesis of Symplocamide A as a model synthesis for establishing a general route towards the synthesis of tailor-made Ahp containing cyclodepsipeptides.

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