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Development of a Solid-Phase Approach to the Natural Product Class of Ahp-Containing Cyclodepsipeptides

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The 3-amino-6-hydroxy-2-piperidone (Ahp) containing cyclodepsipeptides are an interesting class of natural products that inhibit S1 (trypsin and chymotrypsin-like) serine protease in a reversible, noncovalent manner, turning them into potential chemical tools for protease research. Their systematic use in chemical biology is however hampered by their tedious solution-phase chemical synthesis. To overcome this limitation, we report a solid-phase approach to Ahp cyclodepsipeptides that is based on the use of a masked glutamic aldehyde moiety as a general Ahp precursor molecule. As a proof-of-concept, we therefore recently reported the solid-phase synthesis of Symplocamide A. Here, we want to give a full account on the development and application of the masked glutamic aldehyde moiety as well as the optimization of the solid-phase synthesis, which allowed the successful synthesis of the natural product Symplocamide A.

Introduction

Cyanobacteria of freshwater or marine origin have been recognized as proliferative producers of biologically active secondary metabolites.[1] From different strains of cyanobacteria such as *Microcystis*, *Anabaena*, *Oscillatoria*, *Nostoc*, *Lyngbya*, or *Symploca* spp, an interesting class of natural products known as 3-amino-6-hydroxy-2-piperidone (Ahp) containing cyclodepsipeptides have been isolated (Figure 1). Ahp cyclopeptides are characterized by unique chemical structures and potent bioactivities. In fact, Ahp cyclodepsipeptides show selective, reversible, and most intriguingly noncovalent inhibition of S1 (trypsin- and chymotrypsin-like) serine proteases.[2]

Their noncovalent inhibition mode is of particular interest for medicinal chemistry as well as chemical biology applications.[2h] Structural analysis of co-complexes of Ahp cyclodepsipeptides obtained from natural product isolation with S1 serine proteases have shed light on the underlying molecular basis of this favorable inhibition mode. A crystallographic study of a complex of the Ahp cyclodepsipeptide A90720A and the serine protease trypsin elucidated that Ahp cyclodepsipeptides bind in a substrate-like manner, raising the question on how Ahp cyclodepsipeptides prevent their own proteolytic processing. In one hypothesis, the overall fold of the Ahp cyclodepsipeptides that critically relies on the Ahp moiety results in an increased hydrolysis activation barrier.[3] In this model, Ahp cyclodepsipeptides

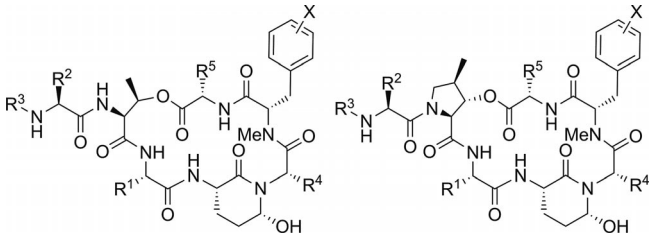

Figure 1. Conserved structural features of Ahp cyclodepsipeptides. Ahp cyclodepsipeptides are built up from a hexadepsipeptide macrocycle that includes the eponymous 3-amino-6-hydroxy-2-piperidone (Ahp) residue and one *N*-methylated phenylalanine derivative. In most known Ahp cyclodepsipeptides, a threonine moiety is involved in the formation of the ester linkage; however, also derivatives with a hydroxyproline residue on this position are known. In addition, an exocyclic side chain is attached to the threonine or hydroxyproline moiety.

mimic the mode of action of proteinaceous protease inhibitors such as canonical serine protease inhibitors.[4] The structural analysis of a complex of the Ahp cyclodepsipeptide Scyptolin A and the serine protease elastase however indicated additional factors that contribute to the potent inhibition: in this co-complex structure, the authors noted that the hydroxy moiety of the Ahp residue is accommodating the position of the "catalytic" water molecule of the hydrolysis reaction.[5] The authors suggest that this might lead to an impairment of the hydrolysis reaction. Besides, also steric hindrance of the movement of the catalytic triad of the protease during the hydrolysis reaction has been suggested as an important factor,[6] although the role of active site dynamics on catalysis is still under debate.[7] In order to gain deeper insights into the structural basis of inhibition as well as for exploring the medicinal potential of Ahp cyclodepsipeptides, the availability of structural analogs of

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Ahp cyclodepsipeptides for structure–activity studies would be highly desirable. So far, this approach is however hampered due to a lack of suitable synthetic methods for rapid access to derivatives for structure–activity studies.

To date, Ahp cyclodepsipeptides are usually obtained by natural product isolation, resulting however in only minute amounts of substance. In addition, total syntheses of the Ahp cyclodepsipeptides Somamide A and Micropeptin T-20 through extensive solution-phase total synthesis have been reported.[8] To gain a more convenient access to Ahp cyclodepsipeptides and to non-natural analogs for structure–activity studies, we therefore aimed to establish a solid-phase-based synthetic route to this natural product class. To illustrate the potential of the developed methodology, we investigated a solid-phase-based synthetic route to the Ahp cyclodepsipeptide natural product and potent chymotrypsin inhibitor Symplocamide A (Figure 2). The present work thereby represents a full account on our previously published work on its synthesis.[9]

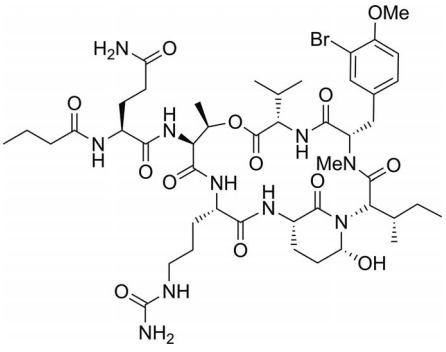
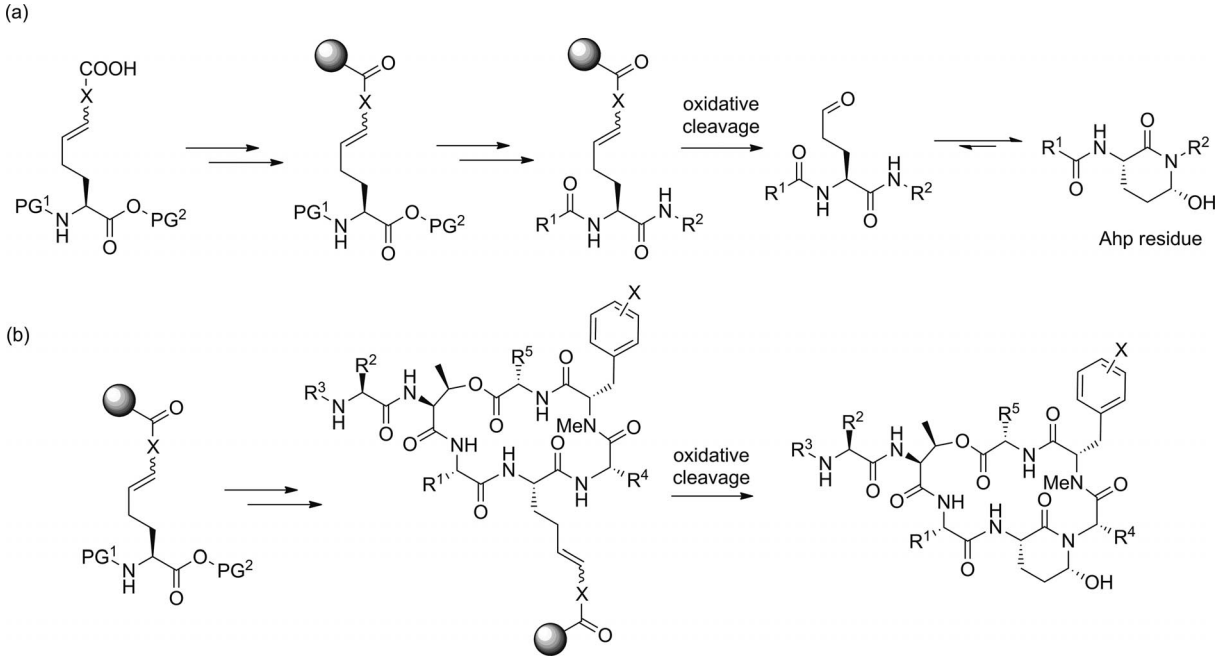


Figure 2. Chemical structure of the target Ahp cyclodepsipeptide Symplocamide A.

Results and Discussion

Synthetic Strategy

In the total syntheses of the Ahp cyclodepsipeptides Micropeptin T-20 and Somamide A, Yokokawa and coworkers demonstrated that the Ahp hemiaminal forms spontaneously from a glutamic aldehyde macrolactam that was generated at a late stage of the synthesis.[8] Consequently, we envisioned that Ahp cyclodepsipeptides could be obtained upon solid-phase synthesis of a cyclodepsipeptide with a masked glutamic aldehyde residue that is unmasked at the end of the synthesis. Such an approach seemed feasible, as the Meldal group demonstrated that the oxidative cleavage of double bonds by a dihydroxylation–glycol cleavage protocol represents a powerful approach for the generation of aldehydes on solid phase.[10] As double bonds are stable to acidic as well as basic reaction conditions, such a masked aldehyde residue would be compatible with Fmoc/*t*Bu as well as Merrifield solid-phase peptide synthesis conditions. Furthermore, a substituted double bond would allow linkage of the masked glutamic aldehyde residue to solid support through its side chain, thereby enabling solid-phase manipulation of the N-terminal as well as the C-terminal part of the masked aldehyde residue and liberation of the glutamic aldehyde moiety upon resin cleavage (Scheme 1).



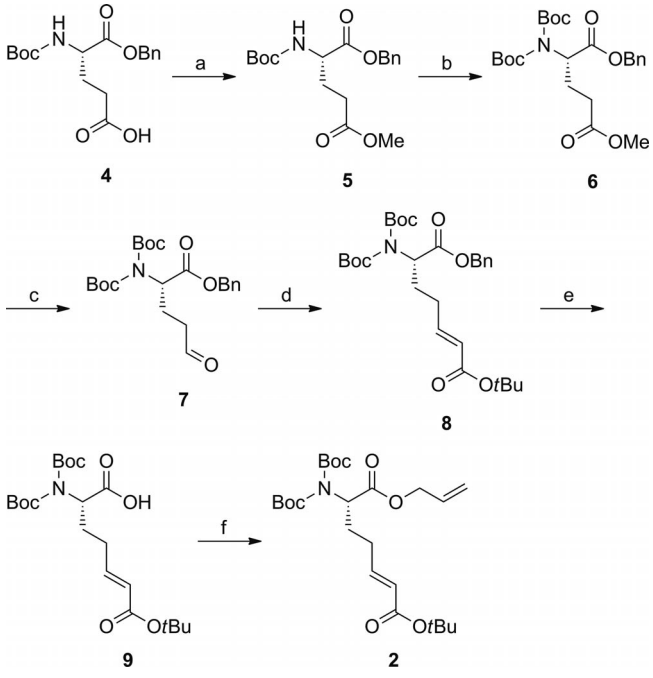
Scheme 1. (a) Proposition of a homoallylglycine derivative as a masked glutamic aldehyde residue for generation of the Ahp residue and (b) its potential application to the solid-phase synthesis of Ahp cyclodepsipeptides.

For linkage of the masked glutamic aldehyde residue to solid support, a building block with a terminal carboxylic acid moiety at its side chain for facile coupling to the resin was chosen. Moreover, in order to allow solid-phase peptide synthesis as well as an *on-resin* macrolactamization, suitable protecting groups for the N-terminal amino and C-terminal carboxyl group were required. We therefore started our studies on the solid-phase synthesis of Ahp cyclodepsipeptides with the development of a suitably masked glutamic aldehyde residue. In a second step, we then employed this building block for a solid-phase synthesis of an Ahp cyclodepsipeptide derivative.

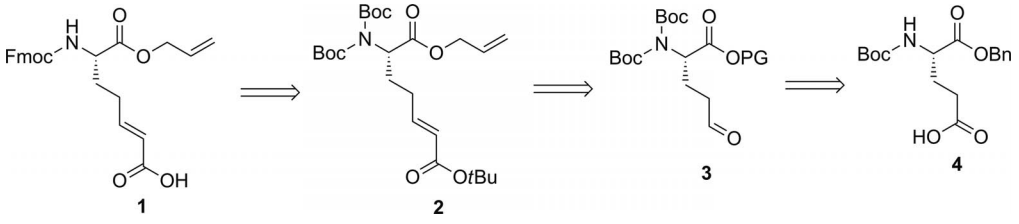
Synthesis of a Masked Glutamic Aldehyde Building Block

We first evaluated α,β-unsaturated carboxylic acid **1** with N-terminal Fmoc and C-terminal allyl ester protecting groups as a masked glutamic aldehyde building block for solid-phase synthesis (Scheme 2). We thought that this building block could be obtained from intermediate **2**, which could be derived from corresponding glutamic aldehyde **3** through a Wittig reaction. As glutamic aldehydes, however, tend to form hemiaminals, which significantly hampers the efficiency of Wittig reactions,[11] we decided to fully protect the amine functionality with two Boc protecting groups.[12] This would allow the use of the commercially available *N*-Boc glutamic acid benzyl ester **4** as a starting material for the synthesis.

With this plan in mind, we initiated the synthesis of the masked glutamic aldehyde building block, converting first *N*-Boc glutamic acid benzyl ester **4** into methyl ester **5** by methylation with methyl iodide (Scheme 3). Introduction of the second Boc protecting group on the N-terminal amino group was achieved by following Apella's protocol.[13] Consequently, **5** was treated with Boc_2O and DMAP as a catalyst, delivering intermediate **6** in 98% yield. Chemoselective reduction of the methyl ester without concurrent reduction of the benzyl ester was performed with DIBAL-H as the reducing agent in toluene at –78 °C. Despite these mild reaction conditions, desired aldehyde **7** was however isolated in only 27% yield; alongside, reduction of the benzyl ester as well as overreduction to the alcohol was observed, explaining the low yield in this reaction step. Subsequent Wittig olefination of aldehyde **7** led to α,β-unsaturated *tert*-butyl carboxylic acid ester **8** in 84% yield. At this stage, we went to replace the benzyl ester protecting group with an allyl ester moiety. We chose an allyl ester protecting group, as this moiety can be removed under mild and orthogonal conditions during solid-phase synthesis. Consequently, this protecting group change called for selective hydrogenation of the benzyl ester without concurrent reduction of the double bond of the α,β-unsaturated carboxylic acid ester. To achieve this, the hydrogenation conditions of Coleman et al. were employed.[14] These conditions use Et_3SiH as a hydrogen source and Pd^{II} acetate as the corresponding catalyst and delivered desired free acid **9** in 62% yield. In our hands, however, yields were dependent of the reaction batch size. In particular, large batch reactions led to lower yields. Despite this drawback, the free acid was subsequently transformed into allyl ester intermediate **2** by allylation of the corresponding cesium salt with allyl bromide as described by Kunz et al. in 76% yield.[15]



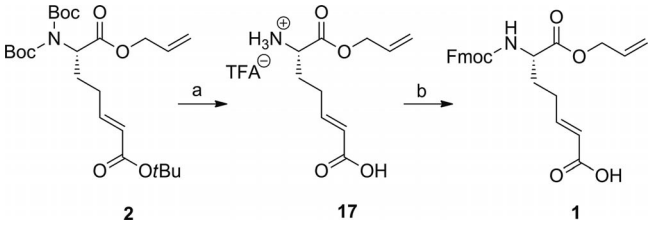
Scheme 3. Synthesis of the bis-Boc protected masked glutamic aldehyde intermediate **2**. Reagents and conditions: (a) MeI, K_2CO_3, acetone, reflux, 4 h (quant). (b) Boc_2O, DMAP, CH_3CN, r.t., 20 h (98%). (c) DIBAL-H, toluene, –78 °C, 2 h (27%). (d) 1. Ph_3P, $ClCH_2COOtBu$, 2 M NaOH/toluene (1:1), 1 h, r.t.; 2. **7**, ylide, CH_2Cl_2, r.t., overnight (84%). (e) $Pd(OAc)_2$, Et_3SiH, Et_3N, CH_2Cl_2, r.t., overnight (62%). (f) 1. Cs_2CO_3, MeOH; 2. allyl bromide, DMF, r.t., overnight (76%).



Scheme 2. Retrosynthesis of a masked glutamic aldehyde building block featuring a N-terminal Fmoc protecting group and an α,β-unsaturated carboxylic acid moiety for solid-phase synthesis.

Although we successfully obtained intermediate **2**, the overall yields were rather low, mainly due to inefficient reduction to aldehyde **7**. We therefore sought an alternative synthetic approach to **2** that circumvented this inefficient step. To this end, commercially available *N*-Boc glutamic acid benzyl ester **4** was first transformed into glutamic alcohol **10** by using Shiori's protocol (Scheme 4).[8b] Consequently, conversion of the acid functionality to a mixed anhydride, followed by reduction with sodium borohydride led to **10** in 84% yield. The hydroxy function of **10** was then protected as a *tert*-butyldimethylsilyl (TBDMS) ether; resulting intermediate **11** was again blocked completely by introduction of a second Boc group. The benzyl ester protecting group of bis-Boc-protected intermediate **12** was removed by hydrogenation to enable the required exchange of the protecting groups at the acid functionality. To achieve this, a modified hydrogenation protocol was used and, under the standard conditions involving the use of methanol or ethanol as solvent, resulted in partial cleavage of the silyl ether moiety. Use of ethyl acetate during hydrogenation however allowed efficient benzyl cleavage, thereby generating free acid **13** in quantitative yield.[16] The allyl protecting group was introduced again by using Kunz's conditions, yielding allyl ester **14** in 95% yield. Subsequent selective removal of the silyl ether moiety however proved to be more difficult than expected, as standard TBAF-mediated silyl cleavage resulted in the formation of an undesired lactone. An alternative method consisting of the use of phosphomolybdic acid immobilized on silica gel was more efficient but resulted in the cleavage of one of the Boc groups at the N-terminal amino group.[17] Desired alcohol **15** was finally obtained by a very mild, Cu^{II}-mediated cleavage reaction.[18] Dess–Martin oxidation by using standard conditions led to aldehyde intermediate **16**, which after a final Wittig reaction, led to desired intermediate **2** in 94% yield.

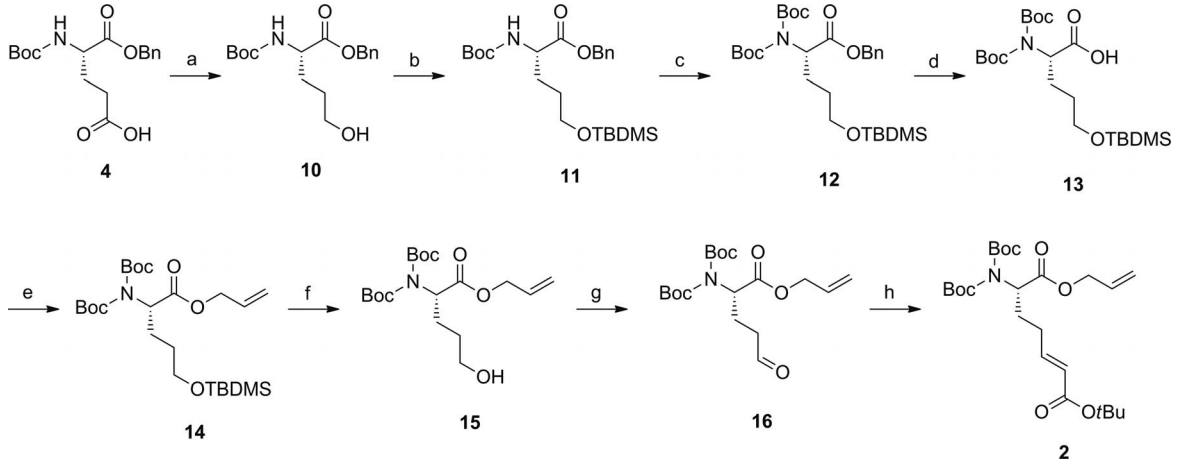
With **2** in hand, desired Fmoc-protected building block **1** for solid-phase synthesis should be available by an exchange of the N-terminal protecting group and cleavage of the *tert*-butyl ester (Scheme 5). Cleavage of the Boc and *tert*-butyl ester protecting groups with TFA in dichloromethane swiftly led to **17**. However, synthesis of **1** proved to be difficult and reaction yields were rather irreproducible, ranging from 49% in the best case to no product formation in the worst case.



Scheme 5. Attempted synthesis of Fmoc-protected masked glutamic aldehyde building block **1**. Reagents and conditions: (a) TFA/CH_2Cl_2 (1:1), r.t., 2 h (quant); (b) FmocCl, $NaHCO_3$ (sat.), pH > 8, H_2O/dioxane (1:1), 40 °C, overnight (max. 49%).

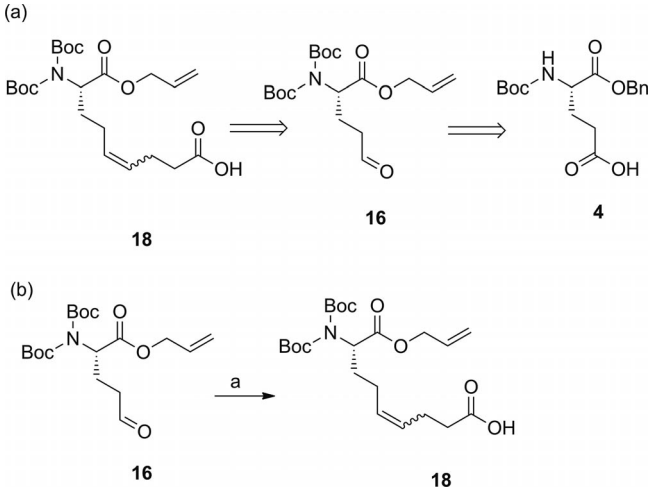
Even more devastating, we noticed that if this building block was coupled to the solid phase through its side chain carboxyl moiety, subsequent Fmoc cleavage led to undesired side products, preventing its use in solid-phase synthesis. We deduced that the low yield during installation of the Fmoc group on the amino group as well as its unexpected reactivity on solid phase is most probably a result of the α,β-unsaturated carboxyl moiety that renders this compound prone to nucleophilic attack, for example from the amino group.

We therefore decided to replace the conjugated double bond of the α,β-unsaturated carboxyl moiety with a masked glutamic aldehyde building block in which the double bond



Scheme 4. Alternative synthesis of bis-Boc protected intermediate **2**. Reagents and conditions: (a) 1. EtOCOCl, Et_3N, THF, –15 °C, 1 h; 2. $NaBH_4$, THF/H_2O (1:1), –15 °C, 1 h, then r.t., 6 h (84%). (b) TBDMSCl, imidazole, CH_2Cl_2, 0 °C to r.t., overnight (95%). (c) Boc_2O, DMAP, CH_3CN, r.t., 24 h (81% based on recovered starting material). (d) 10% Pd/C, H_2, EtOAc, r.t., 2.5 h (99%). (e) 1. Cs_2CO_3, MeOH; 2. allyl bromide, DMF, r.t., overnight (95%). (f) $CuCl_2{\cdot}2H_2O$, acetone/H_2O (95:5), reflux, 2 h (92%). (g) Dess–Martin-periodinane, CH_2Cl_2, r.t., 1 h (84%). (h) 1. Ph_3P, $ClCH_2COOtBu$, 2 M NaOH/toluene (1:1), 1 h, r.t.; 2. **16**, ylide, CH_2Cl_2, r.t., overnight (94%).

was isolated. Moreover, we envisioned that the previously difficult exchange of the two N-terminal Boc groups for an Fmoc group could become obsolete, if a resin would be used that is stable both to the acidic and basic conditions. This would allow the use of Boc as well as Fmoc chemistry during solid-phase synthesis. Consequently, we changed our attention to the synthesis of masked glutamic aldehyde building block **18** that featured both a bis-Boc protected amino group as well as an isolated double bond in the side chain residue (Scheme 6a). Indeed, this compound should be obtainable by a Wittig reaction from aldehyde intermediate **16**, which we were able to obtain from the commercially available starting material *N*-Boc glutamic acid benzyl ester **4** (Scheme 4).



Scheme 6. (a) Retrosynthesis to the alternative masked glutamic aldehyde building block **18** that features an N-terminal bis-Boc protecting group and an isolated double bond. This building block should be available via aldehyde **16** for which a synthesis from the commercially available starting material *N*-Boc glutamic acid benzyl ester **4** has been established. (b) Synthesis of **18**. Reagents and conditions: (a) 1. $BrPh_3P(CH_2)_3CO_2H$, LHMDS, THF, r.t., 55 min; 2. **16**, THF, 0 °C to r.t., overnight (64%).

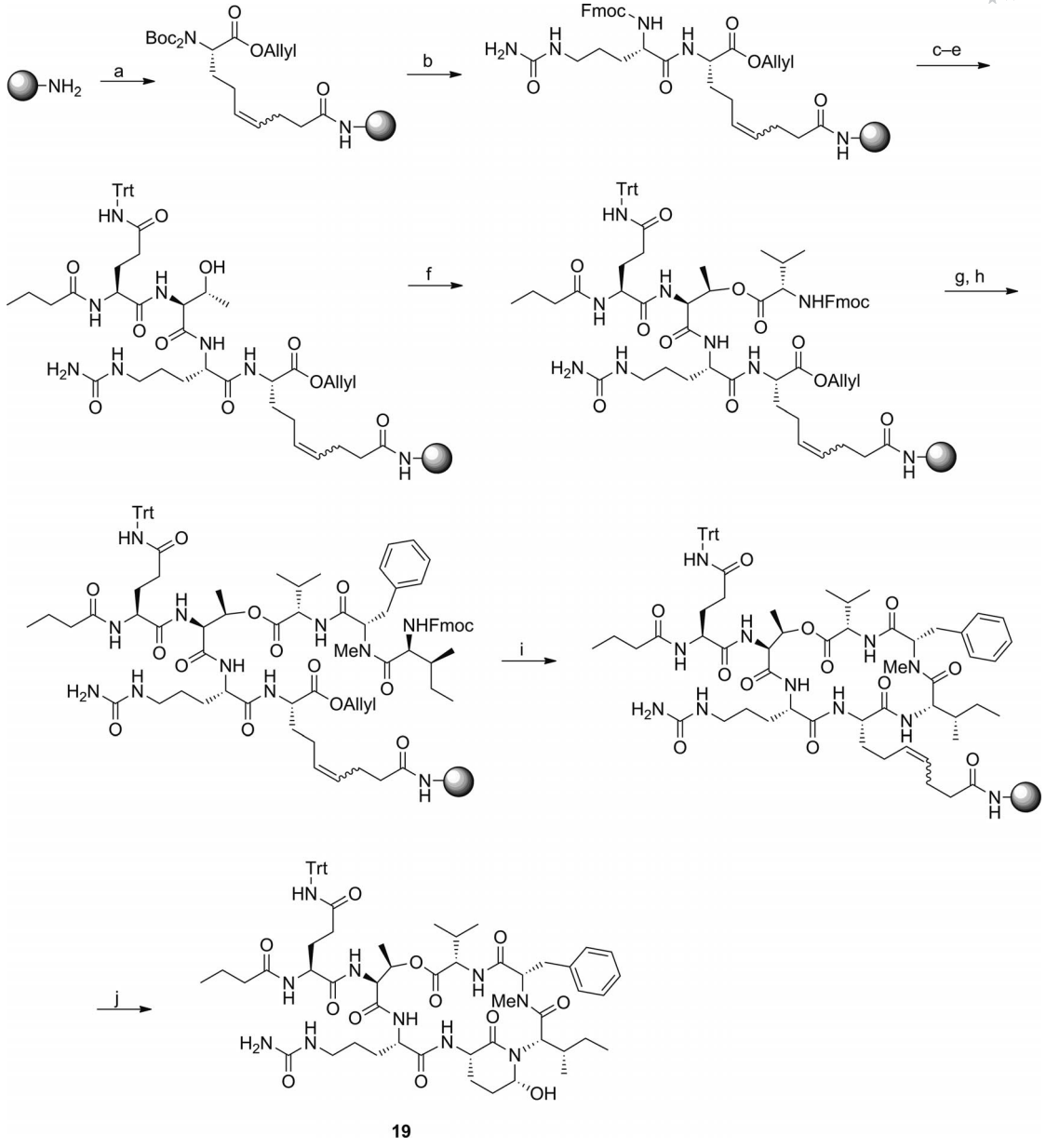
Consequently, Wittig reaction with triphenylphosphonium butyric acid and aldehyde intermediate **16** led to the desired masked glutamic aldehyde building block **18** (with a *Z*/*E* ratio of 9:1) in 64% yield.

Development of a Solid-Phase Synthesis for Ahp Cyclodepsipeptides

With **18** in hand, we commenced with the solid-phase-based approach to Ahp cyclodepsipeptides according to the envisioned strategy (Scheme 1). As the planned solid-phase synthesis features a rather unusual oxidative cleavage as a key step, we first investigated the oxidative cleavage reaction with different resin types. As reagents for cleavage, we used sodium periodate, osmium tetroxide, and DABCO to suppress the formation of hydroxymethyl ketones as established by the Meldal group.[10b] Thus, first "standard" aminomethyl polystyrene resin was acylated with acetic anhydride and subjected to the oxidative cleavage cocktail, and the resulting reaction products were analyzed, revealing insufficient stability of the resin to the cleavage conditions. To overcome these problems, two resins based on a PEG core (NovaPEG amino resin, aminoPEGA resin) instead of a polystyrene core were next tested. Both resins were also acylated and then subjected to the same cleavage conditions. This time, fewer impurities were observed with both resins, demonstrating that in principle both resins could be used for the subsequent solid-phase synthesis. For practical reasons, we therefore chose to use the NovaPEG resin for our future synthesis experiments.

We then started with a solid-phase synthesis of a Symplocamide A model compound (**19**, Scheme 7). This derivative contained a "standard" *N*-methylphenylalanine residue instead of the 3-bromo-4-methoxy-*N*-methyl tyrosine moiety of Symplocamide A. Moreover, the glutamine moiety was incorporated with a trityl protecting group on its side chain amide residue as we used the standard Fmoc-Gln(Trt)-OH amino acid during solid-phase synthesis. We aimed to perform a full deprotection of the Ahp cyclodepsipeptide after oxidative cleavage from the resin.

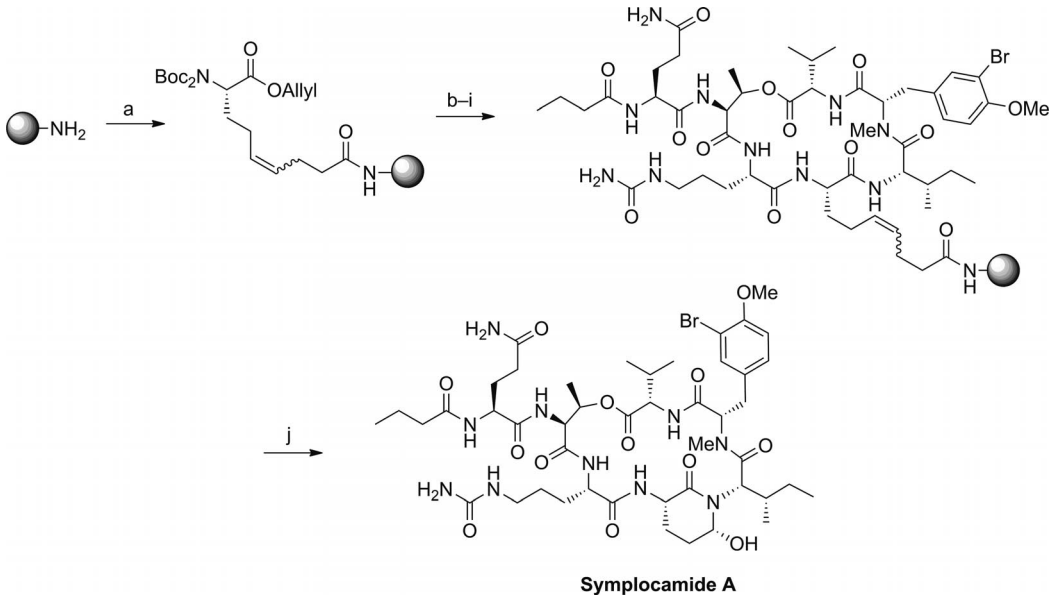
Thus, the synthesis started with coupling of building block **18** to a NovaPEG amino resin using DIC/HOBt activation. This was followed by a capping step. The Boc groups were removed using 50% TFA in DCM followed by neutralization with 10% triethylamine in DCM. Then, a standard solid-phase peptide synthesis protocol consisting of HBTU/HOBt activation was used to couple Fmoc-protected citrulline. Removal of the Fmoc protecting group with 20% piperidine in DMF was followed by coupling of Fmoc-protected threonine by using again HBTU/HOBt activation. Of note, the hydroxy group of the threonine side chain was incorporated without a protecting group. Again, the Fmoc protecting group was cleaved and the next amino acid, Fmoc-Gln(Trt)-OH, was coupled. Fmoc cleavage and subsequent coupling of butyric acid with HBTU/HOBt activation finished the elongation of the N-terminal end of the Ahp cyclodepsipeptide. Next, the esterification of the hydroxy group of the threonine moiety with Fmoc-Val-OH was performed. In order to achieve an efficient reaction, quadrupole couplings with Fmoc-Val-OH and diisopropylcarbodiimide as the coupling agent and DMAP as a catalyst were performed for 2 h (per coupling) to drive the reaction to completion. After subsequent Fmoc cleavage, again with 20% piperidine in DMF, the Fmoc-protected *N*-methyl amino acid phenylalanine was coupled after HBTU/HOBt activation to the resin. Fmoc cleavage then set the stage for the coupling of Fmoc-Ile-OH. In order to assure efficient coupling to the *N*-methyl group, the strong activating reagent PyBrOP was used instead of the HBTU/HOBt reagent pair. Cleavage of the Fmoc group, followed by cleavage of the allyl ester by a protocol established by Vaz et al. was used to generate a linear precursor molecule ready for subsequent *on-resin* macrolactamization.[19] The cyclization reaction was then performed with PyBOP and HOBt as coupling reagents and a long reaction time of 24 h. Oxi-



Scheme 7. Solid-phase peptide synthesis of Symplocamide A model Ahp cyclodepsipeptide **19**. Reagents and conditions: (a) 1. AminoNovaPEG resin (0.66 mmol/g), **18** (2.4 equiv.), HOBt, DIC, CH_2Cl_2/DMF (9:1), r.t., 24 h; 2. CH_2Cl_2/DIEA/Ac_2O (3:1:1), r.t., 3 h. (b) 1. TFA/CH_2Cl_2 (1:1), r.t., 1 h; 2. Et_3N/CH_2Cl_2 (1:9), r.t., 2 × 10 min; 3. Fmoc-Cit-OH, HOBt, HBTU, DIEA, DMF, r.t., 5 h (30% by Fmoc determination, 2 steps). (c) 1. Piperidine/DMF (1:4), r.t., 2 × 15 min; 2. Fmoc-Thr-OH, HOBt, HBTU, DIEA, DMF, r.t., 2 h. (d) 1. Piperidine/DMF (1:4), r.t., 2 × 15 min; 2. Fmoc-Gln(Trt)-OH, HOBt, HBTU, DIEA, DMF, r.t., 2 h (88% by Fmoc determination, 2 steps). (e) 1. Piperidine/DMF (1:4), r.t., 2 × 15 min; 2. butyric acid, HOBt, HBTU, DIEA, DMF, r.t., 2 h. (f) Fmoc-Val-OH, DIC, DMAP, CH_2Cl_2/DMF (9:1), r.t., 4 × 2 h (79% by Fmoc determination, 2 steps). (g) 1. Piperidine/DMF (1:4), r.t., 2 × 15 min; 2. Fmoc-*N*Me-Phe-OH, HOBt, HBTU, DIEA, DMF, r.t., 2 h. (h) 1. Piperidine/DMF (1:4), r.t., 2 × 15 min; 2. Fmoc-Ile-OH, PyBrOP, DIEA, DMF, r.t., 24 h (60% by Fmoc determination, 2 steps). (i) 1. Piperidine/DMF (1:4), r.t., 2 × 15 min; 2. $Pd(PPh_3)_4$, morpholine, CH_2Cl_2, r.t., 30 min; 3. PyBOP, HOBt, DIEA, DMF, r.t., 24 h. (j) $NaIO_4$, OsO_4 (0.1 M in *t*BuOH), DABCO, H_2O/THF (1:1), r.t., 20 h (1.2 overall yield%).

dative cleavage was then achieved with sodium periodate, osmium tetroxide, and DABCO, following essentially the protocol established by the Meldal group, which after HPLC purification delivered desired Ahp cyclodepsipeptide **19** in 1.2% overall yield.

With this Ahp cyclodepsipeptide in hand, we next tested the cleavage of the trityl group from the glutamine residue. In general, the reaction conditions for removing an amide trityl group are very harsh and require the use of 95% TFA. When we submitted peptide **19** to these conditions, we observed rapid and complete decomposition of the Ahp cyclodepsipeptide, most probably due to side reactions on the Ahp moiety. These findings indicate that Ahp cyclodepsipeptides are not stable to strong acidic conditions, calling



Scheme 8. Solid-phase peptide synthesis of Symplocamide A. Reagents and conditions: (a) 1. AminoNovaPEG resin (0.66 mmol/g), **16** (2.5 equiv.), HOBt, HBTU, DIEA, CH_2Cl_2/DMF (9:1), r.t., 24 h; 2. CH_2Cl_2/DIEA/Ac_2O (3:1:1), r.t., 2 × 2 h. (b) 1. TFA/CH_2Cl_2 (1:1), r.t., 1 h; 2. Et_3N/CH_2Cl_2 (1:9), r.t., 2 × 10 min; 3. Fmoc-Cit-OH, HOBt, HBTU, DIEA, DMF, r.t., 4 h (36%, by Fmoc determination, 2 steps). (c) 1. Piperidine/DMF (1:4), r.t., 2 × 15 min; 2. Fmoc-Thr-OH, HOBT, HBTU, DIEA, DMF, r.t., 5 h. (d) 1. Piperidine/DMF (1:4), r.t., 2 × 15 min; 2. Boc-Gln-OH, HOBT, HBTU, DIEA, DMF, 4 h. (e) 1. TFA/CH_2Cl_2 (1:1), r.t., 1 h; 2. Et_3N/CH_2Cl_2 (1:9), r.t., 2 × 10 min; 3. butyric acid, HOBT, HBTU, DIEA, DMF, r.t., 4 h. (f) Fmoc-Val-OH, DIC, DMAP, CH_2Cl_2/DMF (9:1), 4 × 2 h, (92% by Fmoc determination, 4 couplings). (g) 1. Piperidine/DMF (1:4), r.t., 2 × 15 min; 2. Boc-mTyr(3-Br, 4-OMe)-OH, HOBT, HBTU, DIEA, DMF, 4.5 h. (h) 1. TFA/CH_2Cl_2 (1:1), r.t., 1 h; 2. Et_3N/CH_2Cl_2 (1:9), r.t., 2 × 10 min; 3. Fmoc-Ile-OH, PyBrOP, DIEA, DMF, 24 h (73%, by Fmoc determination, 2 couplings). (i) 1. Piperidine/DMF (1:4), r.t., 2 × 15 min; 2. $Pd(PPh_3)_4$, morpholine, CH_2Cl_2, r.t., 30 min; 3. PyBOP, HOBt, DIEA, DMF, r.t., 24 h. (j) $NaIO_4$, OsO_4 (0.1 M in *t*BuOH), DABCO, H_2O/THF (1:1), r.t., 20 h (3.0% overall yield).

for a synthesis route in which such protecting groups are avoided or are removed before formation of the Ahp moiety.

After showing that the masked glutamic aldehyde approach enables the generation of Ahp cyclodepsipeptides, we continued our investigations with the synthesis of the natural product Symplocamide A (Scheme 8). In order to prevent the cleavage of the trityl protecting group, we used Boc-Gln-OH instead of Fmoc-Gln(Trt)-OH as an amino acid building block. Moreover, in this case, the Symplocamide A-specific amino acid Boc-*N*-methyl 3-bromo-4-methoxyphenylalanine was used instead of Fmoc-*N*-methylphenylalanine. This amino acid derivative was prepared by solution-phase chemistry by using standard methods.

For the solid-phase synthesis of Symplocamide A, some reaction conditions were slightly changed (Scheme 8). For example, for loading the resin with the first amino acid derivative, that is, masked glutamic aldehyde building block **18**, HBTU and HOBt were used this time, resulting in a loading efficiency of 36%. However, most of the reaction conditions were kept as previously established and oxidative cleavage from the resin delivered the crude natural product in fair purity (see the Supporting Information, Figure 1, for the corresponding HPLC chromatogram). Subsequent purification by HPLC led to the desired natural product symplocamide A in 3% overall yield. The spectroscopic data obtained for the synthetic compound corresponded well to the data published for the natural product.[2g,9]

Inhibition Properties of Symplocamide-Based Ahp Cyclodepsipeptides

Ahp cyclodepsipeptides are known inhibitors of S1 serine proteases. For Symplocamide A, a potent inhibition of the serine protease chymotrypsin was reported, as expected from the large, hydrophobic citrulline moiety that – according to the binding mode that has been elucidated with other analogs of the Ahp cyclodepsipeptides – should bind to the S1 pocket. We therefore tested the inhibition potency of our synthetically prepared Symplocamide A analog – and as a further proof for its successful synthesis – we observed a potent K_i value of 0.32 ± 0.09 μM, which is in good accordance with that reported (ref.[2g] IC_{50} = 0.38 ± 0.08 μM).

In addition, it was suggested that Symplocamide A might also act as an inhibitor of the proteasomal β5 subsite that displays chymotrypsin-like proteolytic activity.[2g] Consequently, we performed a biochemical inhibition assay by using standard conditions but unfortunately could not detect any significant inhibition.[9] We furthermore tested the inhibitory potential of Ahp cyclodepsipeptide **19**, which also should represent a potent chymotrypsin inhibitor. Indeed, **19** displayed potent chymotrypsin inhibition with a

K_i value of 1.09 ± 0.11 μM, ranking this compound almost equipotent to the natural product.

Conclusions

In summary, we have developed a first solid-phase approach towards the synthesis of natural products from the Ahp cyclodepsipeptide class and exemplified this approach by the synthesis of the natural product Symplocamide A.

Towards this end, the use of a masked glutamic aldehyde building block as a precursor for the Ahp moiety represented a key element in the synthetic strategy. The oxidative cleavage conditions reported herein are – to the best of our knowledge – used for the first time to generate an aldehyde residue and simultaneously achieve cleavage from the solid support. The synthesis of the Ahp precursor was achieved from commercially available *N*-Boc glutamic acid benzyl ester and in high overall yields. The solid-phase synthesis contains some unusual transformations that required the use of nonstandard reaction conditions, in particular the solid-phase esterification of the threonine residue as well as an *on-resin* macrolactamization. We anticipate that the developed synthetic approach should present a general strategy for the synthesis of Ahp cyclodepsipeptides, thereby paving the way for the synthesis of tailor-made Ahp cyclodepsipeptides. This should enable more facile use of these inhibitors as chemical biology tools in protease research.

Experimental Section

Chemical Synthesis of Selected Compounds

(*S*)-Benzyl-2-(*tert*-butoxycarbonylamino)-5-hydroxypentanoate (10): Boc-(*S*)-glutamic acid benzyl ester (**4**, 5.00 g, 14.8 mmol) was dissolved in anhydrous THF (60 mL) under an argon atmosphere and cooled to –15 °C in an ice-acetone cooling bath. A white precipitate formed upon successive addition of triethylamine (6.2 mL, 44.5 mmol, 3 equiv.) and ethyl chloroformate (4.2 mL, 44.5 mmol, 3 equiv.). The reaction mixture was maintained at –15 °C for 1 h. After this time, sodium borohydride (2.24 g, 59.3 mmol, 4 equiv.) dissolved in water (60 mL) was slowly added at –15 °C resulting in the dissolution of the precipitate. After stirring for another 1 h at –15 °C, the reaction mixture was warmed to room temperature. A TLC check after 5 h indicated the complete consumption of the starting material. The reaction mixture was then quenched with 1 M aq. $KHSO_4$ solution (100 mL), and the aqueous phase was extracted with ethyl acetate (3 × 100 mL). The combined organic extracts were washed with brine (150 mL) and dried with Na_2SO_4. After evaporation to dryness, the crude material was purified by flash column chromatography (50% ethyl acetate in cyclohexane) to afford **10** (4.03 g, 12.5 mmol, 84%) as a pale yellow oil. TLC (50% ethyl acetate in cyclohexane): R_f = 0.36. $[\alpha]_D^{20}$ = –2.3 (*c* = 0.98, $CHCl_3$) {ref.[8b] $[\alpha]_D^{24}$ = –3.9 (*c* = 1.0, $CHCl_3$)}. ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.32–7.34 (m, 5 H), 5.10–5.19 (m, 2 H), 4.29–4.34 (m, 1 H), 3.58 (t, *J* = 6.20 Hz, 2 H), 1.93–2.00 (m, 1 H), 1.84–1.91 (m, 1 H), 1.66–1.76 (m, 1 H), 1.53–1.60 (m, 2 H), 1.41 (s, 9 H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 172.6, 141.0, 135.3, 128.5, 128.4, 128.2, 79.8, 65.0, 61.8, 53.2, 29.2, 28.2, 25.4 ppm. LC–MS: t_R = 8.90 min (C_{18}), *m*/*z* (%) = 346 (30) [M + Na]$^+$, 224 (100) [M – Boc]$^+$. HRMS (EI): calcd. for $C_{17}H_{25}O_5N^+$ [M + H]$^+$ 324.1805; found 324.1810.

(*S*)-Benzyl-2-(*tert*-butoxycarbonylamino)-5-(*tert*-butyldimethylsilyloxy)pentanoate (11): Compound **10** (3.92 g, 12.1 mmol) was dissolved in DCM (80 mL) and cooled to 0 °C. Imidazole (2.00 g, 30.3 mmol, 1.2 equiv.) and *tert*-butyldimethylsilyl chloride (2.19 g, 14.5 mmol, 2.5 equiv.) were added, and the reaction mixture was stirred at 0 °C for 1 h and warmed to room temperature overnight. Water was added to the reaction mixture, the phases were separated, and the aqueous phase was extracted with ethyl acetate (3 × 80 mL). The combined organic extracts were washed with 1 M aq. $KHSO_4$ solution (150 mL) and aq. saturated $NaHCO_3$ solution (150 mL) and dried with Na_2SO_4. After evaporation of the solvent to dryness, the crude product was purified by flash column chromatography (10% ethyl acetate in cyclohexane) to afford **11** (5.05 g, 11.5 mmol, 95%) as a yellow oil. TLC (10% ethyl acetate in cyclohexane): R_f = 0.35. $[\alpha]_D^{20}$ = –5.8 (*c* = 1.13, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.32–7.35 (m, 5 H), 5.13–5.19 (m, 2 H), 4.31–4.36 (m, 1 H), 3.58–3.61 (t, *J* = 6.05 Hz, 2 H), 1.94–1.95 (m, 1 H), 1.76–1.85 (m, 1 H), 1.51–1.56 (m, 2 H), 1.43 (s, 9 H), 0.88 (s, 9 H), 0.03 (s, 6 H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 172.6, 155.4, 135.5, 128.5, 128.3, 128.1, 79.6, 66.8, 62.2, 53.3, 29.0, 28.3, 25.9, 18.3, –5.4 ppm. LC–MS: t_R = 13.31 min (C_{18}), *m*/*z* (%) = 460 (40) [M + Na]$^+$, 338 (100) [M – Boc]$^+$. HRMS (EI): calcd. for $C_{23}H_{40}O_5NSi^+$ [M + H]$^+$ 438.2670; found 438.2670.

(*S*)-Benzyl-2-[bis(*tert*-butoxycarbonyl)amino]-5-(*tert*-butyldimethylsilyloxy)-pentanoate (12): Compound **11** (4.94 g, 11.3 mmol) was dissolved in acetonitrile (80 mL). After the addition of 4-(dimethylamino)pyridine (2.07 g, 16.9 mmol, 1.5 equiv.) at room temperature, the mixture was stirred for 10 min. Di-*tert*-butyl dicarbonate (13.0 mL, 56.5 mmol, 5 equiv.) was added, and the reaction mixture was stirred overnight at room temperature, thereby turning from a pale yellow color to bright red. The reaction was quenched by the addition of water (80 mL), and the aqueous phase was extracted with ethyl acetate (3 × 80 mL). To accelerate the sluggish phase separation, brine was added in small portions. The combined organic extracts were washed with 1 M aq. HCl (150 mL) and brine (4 × 150 mL). After evaporation of the solvents to dryness, the crude mixture of product and unreacted starting material was purified by flash column chromatography (5% ethyl acetate in cyclohexane) to yield **12** (3.88 g (7.2 mmol, 64%, 81% based on recovered starting material) as a colorless oil along with unreacted starting material **11**. TLC (5% ethyl acetate in cyclohexane): R_f = 0.26. $[\alpha]_D^{20}$ = –20.2 (*c* = 1.26, $CHCl_3$). ^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.31–7.33 (m, 5 H), 5.11–5.18 (m, 2 H), 4.88–4.92 (m, 1 H), 3.61–3.65 (dt, *J* = 6.40, 1.38 Hz, 2 H), 2.12–2.14 (m, 1 H), 1.91–2.01 (m, 1 H), 1.54–1.61 (m, 2 H), 1.44 (s, 18 H), 0.88 (s, 9 H), 0.03 (s, 6 H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 170.7, 152.2, 135.7, 128.4, 128.0, 127.9, 82.9, 66.7, 62.6, 58.1, 29.6, 27.9, 26.0, 25.9, 18.3, –5.3 ppm. LC–MS: t_R = 11.29 min (C_4), *m*/*z* (%) = 1097 (100) [2M + Na]$^+$, 438 (35) [M – Boc]$^+$. HRMS (EI): calcd. for $C_{56}H_{94}O_{14}N_2NaSi_2^+$ [2M + Na]$^+$ 1097.6136; found 1097.6141.

(*S*)-2-[Bis(*tert*-butoxycarbonyl)amino]-5-(*tert*-butyldimethylsilyloxy)-pentanoic acid (13): Compound **12** (5.85 g, 10.9 mmol) was dissolved in ethyl acetate (80 mL) and transferred to a two-necked round-bottomed flask equipped with a rubber septum and a stopcock under an argon atmosphere. Pd/C (10%) (2.93 g, 5 wt.-%) was added at room temperature in small portions through a funnel, after each portion the funnel was flushed with ethyl acetate (70 mL in total). After the complete addition, the flask was equipped with a hydrogenation balloon and carefully flushed by opening the stopcock from time to time. The hydrogenation was carried out for

2.5 h at room temperature, then the reaction mixture was filtered through a pad of Celite, which was rinsed thoroughly with ethyl acetate (4 × 100 mL). Evaporation to dryness yielded **13** (4.84 g, 10.8 mmol, 99%) as a white solid. $[\alpha]_D^{20}$ = –19.8 (*c* = 0.91, $CHCl_3$). ^1H NMR (400 MHz, $CDCl_3$): δ = 4.91–4.95 (m, 1 H), 3.62–3.65 (dt, *J* = 6.30, 1.74 Hz, 2 H), 2.11–2.20 (m, 1 H), 1.91–2.00 (m, 1 H), 1.54–1.59 (m, 2 H), 1.50 (s, 18 H), 0.89 (s, 9 H), 0.04 (s, 6 H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 177.1, 152.0, 83.2, 62.5, 57.8, 29.5, 27.9, 26.1, 25.9, 18.3, –5.3 ppm. LC–MS: t_R = 11.31 min (C_{18}), *m*/*z* (%) = 917 (100) $[2M + Na]^+$, 347 (20) $[M – Boc]^+$. HRMS (EI): calcd. for $C_{42}H_{82}O_{14}N_2NaSi_2^+$ $[2M + Na]^+$ 917.5197; found 917.5191.

(*S*)-Allyl-2-[bis(*tert*-butoxycarbonyl)amino]-5-(*tert*-butyldimethylsilyloxy)-pentanoate (14): Compound **13** (4.61 g, 10.3 mmol) and cesium carbonate (3.36 g, 10.3 mmol, 1 equiv.) were dissolved in anhydrous methanol (40 mL). The solvent was evaporated, and the remaining solid was coevaporated twice with toluene (40 mL) to obtain the cesium salt of **13**. The cesium salt was suspended in DMF (80 mL) and allyl bromide (17.4 mL, 206 mmol, 20 equiv.) was added slowly at room temperature. The reaction mixture was stirred overnight at room temperature, the solvent was evaporated, and the residue was coevaporated twice with toluene (40 mL) to remove remaining DMF. The crude material was then purified by flash chromatography (10% ethyl acetate in cyclohexane) to afford **14** (4.75 g, 9.7 mmol, 95%) as a pale yellow oil. TLC (5% ethyl acetate in cyclohexane): R_f = 0.13. $[\alpha]_D^{20}$ = –20.1 (*c* = 1.21, $CHCl_3$). ^1H NMR (400 MHz, $CDCl_3$): δ = 5.81–5.93 (m, 1 H), 5.28–5.32 (dd, *J* = 17.20, 1.55 Hz, 1 H), 5.18–5.21 (dd, *J* = 10.50, 1.45 Hz, 1 H), 4.86–4.88 (m, 1 H), 4.58–4.60 (m, 2 H), 3.60–3.64 (dt, *J* = 6.40, 1.80 Hz, 2 H), 2.12–2.21 (m, 1 H), 1.89–1.96 (m, 1 H), 1.54–1.60 (m, 2 H), 1.48 (s, 9 H), 0.87 (s, 9 H), 0.03 (s, 6 H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 170.5, 152.1, 131.9, 117.9, 82.9, 65.5, 62.6, 58.1, 29.6, 27.9, 26.2, 25.9, 18.3, –5.3 ppm. LC–MS: t_R = 14.37 min, *m*/*z* (%) = 510 (100) $[M + Na]^+$, 410 (80) $[M – Boc + Na]^+$. HRMS (EI): calcd. for $C_{24}H_{45}O_7NNaSi^+$ $[M + Na]^+$ 510.2858; found 510.2860.

(*S*)-Allyl-2-[bis(*tert*-butoxycarbonyl)amino]-5-hydroxypentanoate (15): Compound **14** (4.63 g, 9.5 mmol) was dissolved in a solution of 5% water in acetone (100 mL) at room temperature. Copper(II) chloride dihydrate (81 mg, 0.5 mmol, 0.05 equiv.) was added, and the reaction mixture was heated to reflux. A TLC check after 1 h of refluxing indicated the complete conversion of the starting material. The solvent was evaporated to dryness, and the crude product was purified by flash column chromatography (33% ethyl acetate in cyclohexane) to afford **15** (3.27 g, 8.7 mmol, 92%) as a colorless oil. TLC (33% ethyl acetate in cyclohexane): R_f = 0.26. $[\alpha]_D^{20}$ = –30.2 (*c* = 1.01, $CHCl_3$). ^1H NMR (400 MHz, $CDCl_3$): δ = 5.82–5.91 (m, 1 H), 5.26–5.31 (dd, *J* = 17.20, 1.50 Hz, 1 H), 5.17–5.20 (dd, *J* = 10.50, 1.40 Hz, 1 H), 4.85–4.89 (m, 1 H), 4.57–4.59 (m, 2 H), 3.62–3.65 (t, *J* = 6.40 Hz, 2 H), 2.17–2.26 (m, 1 H), 1.87–1.97 (m, 1 H), 1.57–1.64 (m, 2 H), 1.46 (s, 18 H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 170.5, 152.1, 131.7, 118.0, 83.1, 65.6, 62.1, 57.9, 29.3, 27.9, 26.1 ppm. LC–MS: t_R = 9.90 min (C_{18}), *m*/*z* (%) = 396 (100) $[M + Na]^+$, 295 (80) $[M – Boc + Na]^+$, 173 (70) $[M – 2Boc]^+$. HRMS (EI): calcd. for $C_{18}H_{31}O_7NNa^+$ $[M + Na]^+$ 396.1990; found 396.1993.

(*S*)-Allyl-2-[bis(*tert*-butoxycarbonyl)amino]-5-oxopentanoate (16): Compound **15** (1.60 g, 4.3 mmol) was dissolved in anhydrous DCM under an argon atmosphere. Dess–Martin periodinane (1.91 g, 4.5 mmol, 1.05 equiv.) was added at room temperature, and the reaction mixture was stirred at room temperature for 1.5 h, when a TLC check indicated the complete conversion of the starting material. The reaction mixture was quenched by the addition of aq. saturated K_2CO_3 solution (60 mL), the phases were allowed to separate, and the aqueous layer was extracted with ethyl acetate (3 × 60 mL). The combined organic extracts were washed with brine (150 mL) and dried with Na_2SO_4. After evaporation to dryness, the crude product was purified by flash column chromatography (17% ethyl acetate in cyclohexane) to afford **16** (1.34 g, 3.6 mmol, 84%) as a colorless oil, which was directly used for the next step. TLC (17% ethyl acetate in cyclohexane): R_f = 0.23. $[\alpha]_D^{20}$ = –26.2 (*c* = 1.26, $CHCl_3$). ^1H NMR (400 MHz, $CDCl_3$): δ = 9.75 (s, 1 H), 5.83–5.92 (m, 1 H), 5.27–5.32 (dd, *J* = 17.20, 1.50 Hz, 1 H), 5.18–5.21 (dd, *J* = 10.50, 1.40 Hz, 1 H), 4.86–4.90 (m, 1 H), 4.58–4.60 (m, 2 H), 2.48–2.59 (m, 3 H), 2.13–2.20 (m, 1 H), 1.47 (s, 18 H) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 200.8, 169.9, 152.0, 131.7, 118.2, 83.4, 65.7, 57.4, 40.4, 27.9, 22.3 ppm. LC–MS: t_R = 11.11 min (C_{18}), *m*/*z* (%) = 394 (80) $[M + Na]^+$, 294 (90) $[M – Boc + Na]^+$. HRMS (EI): calcd. for $C_{18}H_{30}O_7N^+$ $[M + H]^+$ 372.2017; found 372.2018.

(*S*)-9-(Allyloxy)-8-[bis(*tert*-butoxycarbonyl)amino]-9-oxonon-4-enoic Acid (18): 3-(Carboxypropyl)triphenylphosphonium bromide (1.92 g, 4.5 mmol, 1.5 equiv.) was suspended in anhydrous THF (20 mL) under an argon atmosphere. A solution of lithium bis(trimethylsilyl) amide (1 M in THF, 9.7 mL, 9.7 mmol, 3.25 equiv.) was added within 40 min to the suspension at room temperature, which was stirred for additional 15 min at room temperature. The resulting dark red solution was cooled to 0 °C and **16** (1.11 g, 3.0 mmol) dissolved in THF (20 mL) was added over 40 min. The reaction mixture was warmed slowly to room temperature, and after 4 h, a TLC check indicated the completion of the reaction. 2 M HCl was added to the reaction mixture until an acidic pH was obtained. The aqueous phase was extracted with ethyl acetate (3 × 40 mL), and the combined extracts were dried with Na_2SO_4. After evaporation of the solvents to dryness, the crude mixture was purified by flash column chromatography (50% ethyl acetate in cyclohexane) to afford **18** (824 mg, 1.9 mmol, 64%) in a 9:1 *Z*/*E* ratio as a yellow oil. TLC (50% ethyl acetate in cyclohexane): R_f = 0.35. ^1H NMR (500 MHz, $CDCl_3$): δ = 5.83–5.91 (m, 1 H), 5.43–5.44 (t, *J* = 5.20 Hz 1 H), 5.37–5.42 (dt, *J* = 11.10, 5.90 Hz, 1 H), 5.27–5.31 (d, *J* = 16.20 Hz, 1 H), 5.17–5.19 (d, *J* = 10.50 Hz, 1 H), 4.83–4.86 (m, 1 H), 4.57–4.58 (d, *J* = 5.35 Hz, 2 H), 2.28–2.40 (m, 4 H), 2.11–2.19 (m, 2 H), 2.02–2.06 (m, 1 H), 1.89–1.95 (m, 1 H), 1.47 (s, 18 H) ppm. ^{13}C NMR (126 MHz, $CDCl_3$): δ = 178.9, 170.5, 152.1, 131.8, 130.3, 130.0, 128.9, 128.4, 117.9, 83.0, 65.6, 57.8, 33.9, 29.7, 29.5, 29.1, 27.9 ppm. LC–MS: t_R = 10.73 min (C_{18}), *m*/*z* (%) = 486 (45) $[M + 2Na]^+$, 464 (45) $[M + Na]^+$, 242 (100) $[M – 2Boc]^+$. HRMS (EI): calcd. for $C_{22}H_{36}O_8N^+$ $[M + H]^+$ 442.2435; found 442.2436.

Inhibition assays: The biochemical inhibition assays have been performed following the protocols reported in ref.[20]

Supporting Information (see footnote on the first page of this article): General information on the synthesis conditions and experimental protocols for the solution-phase synthesis of compounds **1**–**2**, **5**–**9**, and **17**; HPLC chromatogram of the crude resin cleavage product of Symplocamide A.

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