Natural Product Synthesis

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Total Synthesis of the Antifungal Agent Echinocandin C**

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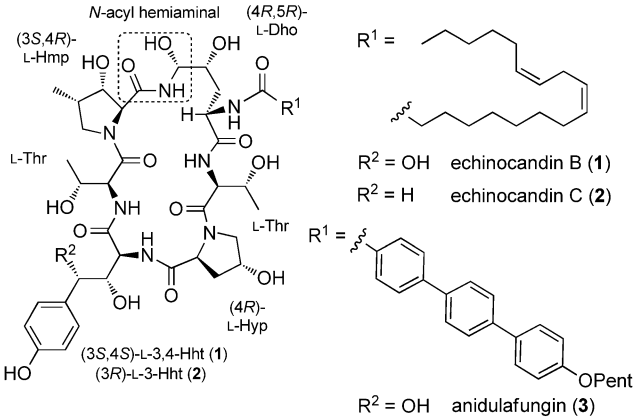
Invasive fungal infections, which are a major threat for immunocompromised patients, especially in a hospital setting, are difficult to treat because of the small number of effective antimycotic agents that are currently available.[1] Accordingly, the introduction of the echinocandins, a new class of antimycotic drugs that can be used to combat invasive candidiasis and aspergillosis, has been considered a major improvement for cases where other medication has failed or is not tolerated.[2] The three approved drugs, caspofungin, micafungin, and anidulafungin (**3**), are semisynthetic derivatives of naturally occurring cyclic lipopeptides produced by various fungi. All echinocandins share a similar structure within the cyclopeptide portion, which consists of six L-amino acids. As shown in Scheme 1 for the eponymous echinocandins B (**1**) and C (**2**),[3] and the echinocandin B-derived drug anidulafungin (**3**), a unique structural feature of most echinocandins and all three approved drugs is the *N*-acyl hemiaminal linkage between a highly substituted proline (Hmp) and a dihydroxyornithine unit (Dho).[4]



Scheme 1. Structure of echinocandin B (**1**), echinocandin C (**2**), and the drug anidulafungin (**3**), a semisynthetic derivative of **1**. The dashed box indicates the *N*-acyl hemiaminal unit. Hmp = 3-hydroxy-4-methylproline, Dho = 4,5-dihydroxyornithine, Hyp = 4-hydroxyproline, Hht = hydroxyhomotyrosine.

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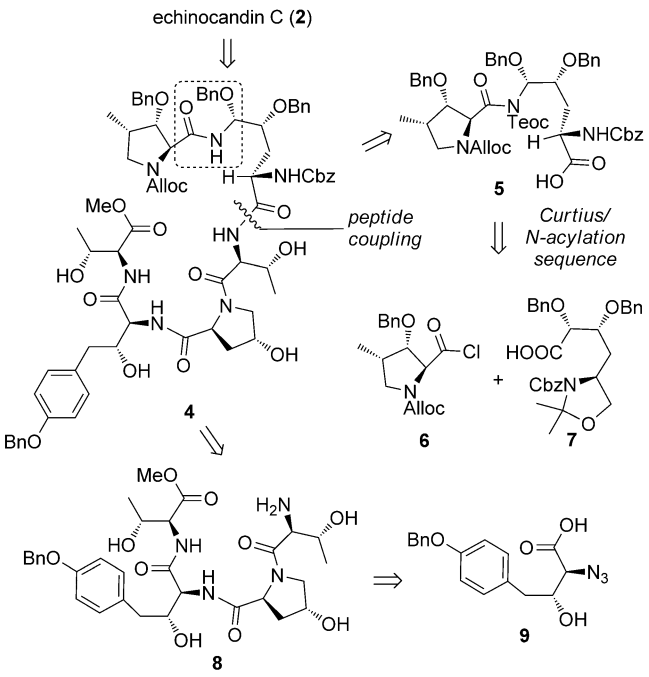
Despite their interesting structure and their clinical importance, no total synthesis of echinocandins with an *N*-acyl hemiaminal unit has been reported to date. Solely cyclopeptides with a more easily accessible amide bond at this position in which an L-ornithine unit replaces the hydroxylated derivative were prepared, for example, echinocandin D[5] and various further simplified analogues that were used for structure–activity relationship studies.[6] A possible biosynthetic approach to modified echinocandins with an *N*-acyl hemiaminal unit is also still in its infancy.[7] Accordingly, an efficient chemical access to cyclopeptides that contain this structural motif will not only allow more thorough activity studies with analogues that are closer to the parent structure but can also grant a flexible access to functionalized derivatives for biological studies. This is especially important because the detailed mechanism of action of the echinocandins, which are inhibitors of fungal cell-wall biosynthesis,[8] remains to be elucidated. We therefore embarked on the total synthesis of echinocandin C (**2**), which contains the more stable 3-hydroxyhomotyrosine unit instead of the 3,4-dihydroxy derivative found in **1**.[3]

Detailed studies by the group of Ohfune[5c] directed at the synthesis of **2** showed that a cyclization of a linear hexapeptide with amide and aldehyde termini is not feasible for the generation of the *N*-acyl hemiaminal. In contrast, our own retrosynthetic analysis focused on the introduction of this linkage at a much earlier stage of the synthesis (Scheme 2), which led to the *N*-acyl hemiaminal-containing linear hexapeptide **4** as a precursor for echinocandin C (**2**). In the forward direction, C- and N-terminal deprotection of **4**, cyclization between Thr and Hmp, global deprotection of the Bn- and Cbz-groups by hydrogenation, and a final α-NH-acylation of the Dho unit would lead to **2** or other lipidated echinocandin derivatives.

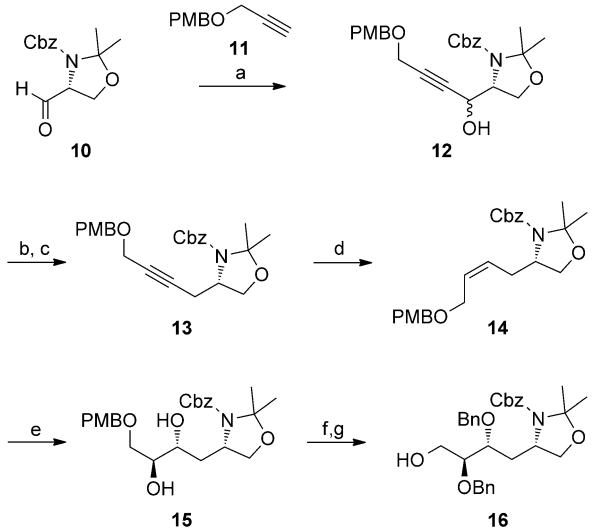
This strategy is more flexible compared to late-stage hemiaminal formation. At the beginning of our endeavor, however, it was not clear if the hemiaminal would be stable throughout the synthesis. Accordingly, we tried to limit the number of remaining transformations following *N*-acyl hemiaminal formation and disconnected hexapeptide **4** into the Hmp-Dho dipeptide **5** and Thr-(3-Hht)-Hyp-Thr tetrapeptide **8**, which required the synthesis of the nonproteinogenic 3-hydroxyhomotyrosine derivative **9**. Based on a number of model studies, we were confident that the crucial *N*-acyl hemiaminal building block **5** could be assembled stereoselectively by a reaction sequence that features a Curtius rearrangement of the acyl azide derived from carboxylic acid **7** and a subsequent acylation of the so obtained Teoc-protected hemiaminal with acid chloride **6**,[9] followed by oxidation of the C-terminus to the corresponding carboxylic acid.

The stereoselective synthesis of primary alcohol **16**, which is the direct precursor of carboxylic acid **7**, the substrate for



Scheme 2. Retrosynthesis of echinocandin C (**2**). Alloc = allyloxycarbonyl, Teoc = 2-(trimethylsilyl)ethoxycarbonyl, Cbz = benzyloxycarbonyl.

the Curtius rearrangement, is shown in Scheme 3. Starting from the known (*R*)-Cbz-Garner's aldehyde **10**,[10] the reaction with the lithium acetylide obtained from alkine **11**[11] first afforded alcohol **12** (72%, ca. 7:1 mixture of diastereomers). The removal of the epimeric hydroxy groups was achieved in

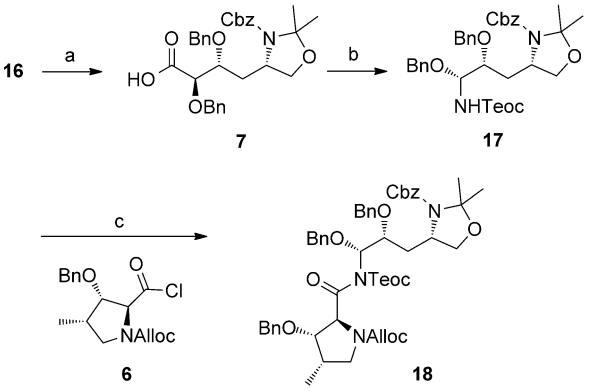


Scheme 3. Synthesis of *Z*-olefin **14**. a) **11**, *n*BuLi, THF, −78 °C, 72%; b) MsCl, Et_3N, CH_2Cl_2, quant.; c) NH_4HCO_2, $[Pd_2(dba)_3]$, PBu_3, benzene, 80%; d) Lindlar's catalyst, quinoline, H_2 (1 bar), EtOAc, 81%; e) AD-mix-α, $CH_3SO_2NH_2$, *t*BuOH/H_2O, 0 °C, 73%; f) BnBr, Ag_2O, DMF, 76%; g) DDQ, CH_2Cl_2/H_2O, 74%. PMB = *p*-methoxybenzyl, Dba = dibenzylideneacetone, DDQ = 2,3-dichloro-5,6-dicyano-1,4-benzoquinone.

good yields (80%) under conditions developed for the deoxygenation of propargylic alcohols[12] by conversion of the alcohol into the mesylate and its reduction using a combination of ammonium formate and a Pd^0 catalyst. A *Z*-selective hydrogenation of alkine **13** then afforded olefin **14** in multigram quantities (81%).

The *Z*-configuration of olefin **14** is necessary to secure the relative configuration of the two hydroxy groups of diol **15** that were introduced by means of a Sharpless asymmetric dihydroxylation (SAD) reaction. *Z*-olefins are notoriously difficult substrates for the SAD reaction,[13] and the reaction outcome is often difficult to predict even for simple substrates. By using the regular AD-mix-α reagent, we observed the opposite stereoselectivity that would be predicted according to the mnemonic devised by Sharpless[14] and obtained the required stereoisomer of **15** as the major product (3.2:1 dr, 73% yield of isolated **15**).[15] This was confirmed by the independent synthesis of **15** from a precursor in which the configuration of the two newly introduced stereocenters was unambiguously proven by an X-ray structure.[16] Benzylation of the two hydroxy groups and oxidative removal of the PMB ether then afforded alcohol **16** (56%, 2 steps).

For the crucial Curtius rearrangement (Scheme 4), alcohol **16** was then oxidized in one step to carboxylic acid **7**

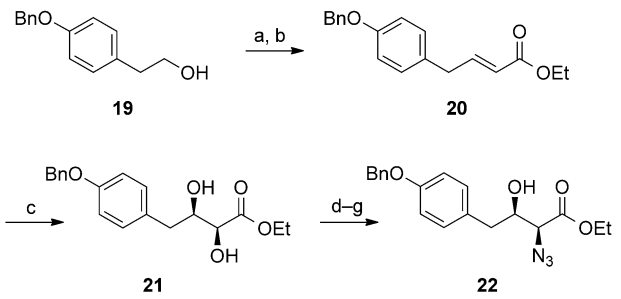


Scheme 4. Synthesis of the *N*-acyl hemiaminal-linked "dipeptide" **18** by a Curtius rearrangement/N-acylation sequence. a) TEMPO, NaOCl, $NaClO_2$, CH_3CN/pH 6.7 buffer, 40 °C; b) *i*BuOC(O)Cl, DIPEA, THF, 0 °C; aq. NaN_3; $TMSCH_2CH_2OH$, toluene, 80 °C, 71% (3 steps); c) LiHMDS, DMAP, THF, −78 °C; then **6**, 70% plus 7% recovered **17**. DMAP = 4-*N*,*N*-dimethylaminopyridine, DIPEA = *N*,*N*-diisopropylethylamine, LiHMDS = lithium hexamethyldisilazide, TEMPO = 2,2,6,6-tetramethylpiperidine-1-oxyl.

(TEMPO, NaOCl, $NaClO_2$).[17] Treatment of the crude **7** with isobutyl chloroformate and subsequent reaction with aqueous NaN_3 then produced the corresponding acyl azide, which was heated in the presence of 2-(trimethylsilyl)ethanol to afford the Teoc-protected *N*-acyl hemiaminal **17** in good overall yield (71%).

With **17** in hand, we then explored the N-acylation of the hemiaminal. To our delight, N-deprotonation with LiHMDS and subsequent acylation with acid chloride **6**[18] cleanly afforded the *N*-acyl hemiaminal containing dipeptide **18** in 70% yield.

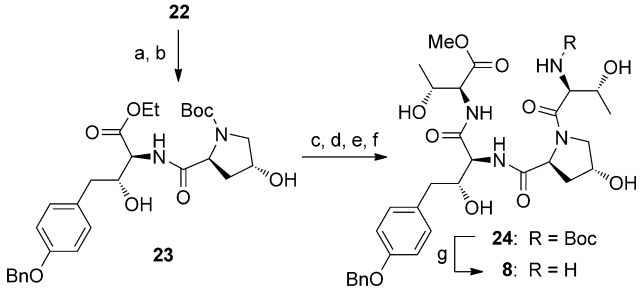
The synthesis of the second peptide building block, tetrapeptide **8**, commenced with the synthesis of a suitable (*R*)-3-hydroxy-L-homotyrosine derivative.[5,19] Toward this end, the commercially available primary alcohol **19** was first oxidized to the corresponding aldehyde and then converted to the known *E*-olefinic ester **20**[20] by a Wittig olefination (81%; Scheme 5). A SAD reaction with AD-mix-β proceeded



Scheme 5. Synthesis of the protected (*R*)-3-hydroxyhomotyrosine derivative **22**. a) DMP, CH_2Cl_2; b) $Ph_3P{=}CHCO_2Et$, THF, reflux, 81% (2 steps); c) AD-mix-β, $CH_3SO_2NH_2$, *t*BuOH/H_2O, 0°C, 92%; d) $SOCl_2$, Et_3N, CH_2Cl_2, 0°C; e) $NaIO_4$, $RuCl_3$ (cat.), CH_3CN/H_2O; f) LiBr, THF, then H_2SO_4 (20%), Et_2O; g) NaN_3, DMSO, 65% (4 steps). DMP = Dess–Martin periodinane.

efficiently to afford 2,3-diol **21** (92%), which was then transformed to the corresponding 2-azido derivative **22**. To achieve this goal under retention of the C2 configuration, a double inversion strategy was employed, which features the opening of the corresponding cyclic sulfate with LiBr and a second substitution of the bromide with NaN_3.[21] This sequence conveniently afforded azide **22** in good overall yield (65%) and in multigram quantities.

Following Staudinger reduction of azide **22** (97%, Scheme 6), the amine thus obtained was coupled with Boc-L-Hyp by activation with DEPBT[22] to afford dipeptide **23** (87%). Next, the fully protected tetrapeptide **24** was generated by coupling with appropriate L-Thr derivatives with DEPBT activation following the removal of the N- and C-terminal protecting groups, respectively (75%, 4 steps).



Scheme 6. Synthesis of tetrapeptide **8**. a) PPh_3, THF/H_2O, reflux, 97%; b) Boc-L-Hyp, DEPBT, DIPEA, THF, 87%; c) TFA/CH_2Cl_2, 0°C; d) Boc-L-Thr, DEPBT, DIPEA, THF, 92% (2 steps); e) aq. LiOH, THF, 0°C; f) L-Thr-OMe, DEPBT, DIPEA, THF, 81% (2 steps); g) TFA/CH_2Cl_2, 0°C, quant. DEPBT = 3-(diethoxyphosphoryloxy)-1,2,3- benzotriazin-4(3*H*)-one, TFA = trifluoroacetic acid.

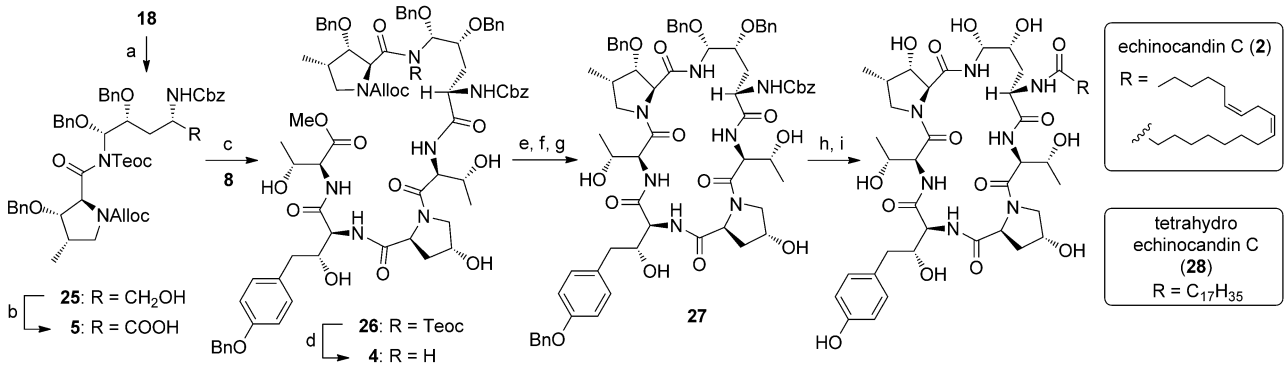
Finally, the N-terminal Boc-group was removed under acidic conditions to provide the tetrapeptide building block **8**.

The final assembly of the echinocandin C cyclopeptide started with the C-terminal oxidation of dipeptide **18** (Scheme 7). Acidic removal of the *N*,*O*-acetal (TsOH/MeOH) did not affect the hemiaminal and afforded primary alcohol **25** in good yield (87%). Direct oxidation (TEMPO, NaOCl, $NaClO_2$) to the corresponding crude carboxylic acid **5** and coupling with amine **8** then provided the linear hexapeptide **26** (87%, 2 steps). To achieve good cyclization yields, it was imperative to remove the Teoc group from the *N*-acyl hemiaminal (TBAF, 98%) to afford hexapeptide **4**. Liberation of the N-terminus by removal of the Alloc group (Pd^0, thiosalicylic acid, 90%) and C-terminal ester hydrolysis with LiOH in THF then set the stage for the cyclization. Here, activation of the carboxylic acid with DEPBT in DMF and addition of solid $NaHCO_3$ as a base were found to provide optimal cyclization conditions, which afforded cyclopeptide **27** in excellent yield (90%, 2 steps). This first total synthesis of an echinocandin-type cyclopeptide with *N*-acyl hemiaminal unit (longest linear sequence: 21 steps starting from D-serine, 4.2% overall yield) easily provides **27** in quantities of more than 200 mg, which can be used for the synthesis of echinocandin C (**2**) and various lipidated analogues.

Toward this end, the removal of the Bn and Cbz groups was initially attempted under standard conditions for hydrogenation (H_2 (1 bar, 10 bar or 48 bar), Pd/C, MeOH or Raney-Nickel, EtOH) and transfer hydrogenation (NH_4HCO_2, Pd/C, 40–60°C, MeOH). However, the global deprotection proceeded very slowly, which ultimately led to partial degradation of the cyclopeptide. A solution to this problem was eventually found by using cyclohexene as the hydrogen source in the transfer hydrogenation (EtOH, 50°C), which accelerated the deprotection substantially and provided the deprotected cyclopeptide after 24 h as the major product (as judged by analytical HPLC). The final acylation at the α-amino group of the Dho unit was achieved employing known conditions for the lipidation of unprotected echinocandin cyclopeptides.[23] In the event, reaction of the deprotected cyclopeptide with the HOBt ester of linoleic acid and subsequent purification by silica gel chromatography provided echinocandin C (**2**) as a colorless solid with a melting point and an optical rotation that corresponds well to published data.[3b] Furthermore, the ^1H and ^{13}C NMR spectra in combination with extensive 2D NMR experiments proved the correct structure of synthetic echinocandin C (**2**).

The small scale lipidation of unprotected echinocandins with this type of reagent and subsequent chromatographic purification is known to proceed with moderate yields,[23] but affords various lipid derivatives in a highly convergent fashion. Accordingly, we easily prepared a second echinocandin derivative, namely the known tetrahydro derivative **28** of echinocandin C, by deprotection of **27** and subsequent acylation with stearic acid HOBt ester (Scheme 7). The ^{13}C spectral data of this compound matched the reported data completely.[3b]

In conclusion, we have developed an efficient synthesis of echinocandin C (**2**), which features an early introduction of the *N*-acyl hemiaminal by a Curtius rearrangement. The



Scheme 7. Synthesis of echinocandin C (**2**) and tetrahydroechinocandin C (**28**). a) TsOH, MeOH, 87%; b) TEMPO, NaOCl, $NaClO_2$, CH_3CN/pH 6.7 buffer, 40°C; c) **8**, DEPBT, DIPEA, THF, 87% (2 steps); d) TBAF, THF, 98%; e) $[Pd(PPh_3)_4]$, thiosalicylic acid, THF, 90%; f) aq. LiOH, THF, 0°C; g) DEPBT, $NaHCO_3$, DMF, 0°C→8°C→RT, 6 d, 90% (2 steps); h) Pd/C, cyclohexene, EtOH, 50°C; i) linoleic acid HOBt ester (for **2**) or stearic acid HOBt ester (for **28**), KH_2PO_4, acetone/H_2O, 50°C, **2**: 28% (2 steps); **28**: 26% (2 steps). TBAF = tetrabutylammonium fluoride, HOBt = 1-hydroxybenzotriazole.

synthesis is highly convergent and should provide an easy access to structurally modified echinocandins with intact *N*-acyl hemiaminal unit. The synthesis of such analogues and their antimycotic activity will be reported in due course.

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