Total Synthesis

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Total Synthesis of the Ribosomally Synthesized Linear Azole-Containing Peptide Plantazolicin A from *Bacillus amyloliquefaciens***

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Dedicated to the Bayer company on the occasion of its 150th anniversary

The lack of new lead structures is a major obstacle in developing drugs for the treatment of bacterial infections. A promising yet under-investigated source for such compounds is ribosomally synthesized and posttranslationally modified peptides (RiPPs), a growing group of peptides, mostly produced by microorganisms.[1] Besides the cyclic azole peptides of the cyanobactin type (patellamides, ulithiacyclamide)[2–4] and thiopeptides (thiostrepton, noshiheptide),[5–8] the linear azole-containing peptides (LAPs), which include prominent natural products such as microcin B17 and goadsporin,[9,10] are an important subgroup of the RiPPs. The distinguishing structural features of LAPs are oxazoles and thiazoles, heterocyclic amino acids derived from Ser/Thr and Cys[11] by enzymatic cyclodehydration and dehydrogenation reactions.[12] Recently, we extended the LAP family with plantazolicin A (**1a**) and B (**1b**; Figures 1 and 2). These are produced from the Gram-positive soil bacterium *Bacillus amyloliquefaciens* FZB42,[13,14] which is also used in the industrial production of α-amylase and serine proteases.[15] Interestingly, studies reported that plantazolicin A showed a selective growth inhibition against nine *Bacillus* species,[13,16] but not against *Staphylococcus* or *Enterococcus* species. Thus, plantazolicin can be considered as a new lead compound to fight *Bacillus anthracis* (anthrax) infections.

The structure of plantazolicin A was elucidated by ^{15}N-labeling studies facilitated by *B. amyloliquefaciens* cultures growing in ^{15}N-enriched media. This approach provided crucial signals from ^{15}N nuclei in 2D NMR spectra (^{1}H-^{15}N-HSQC, ^{1}H-^{15}N-HMBC) to solve the structure. From the structure elucidation of **1a**, information concerning the functions of enzymes of the corresponding biosynthesis gene cluster (*pzn* cluster) was deduced (Figure 1). Hence, the structural gene PznA codes for a ribosomally synthesized linear 41-mer precursor peptide with the 14-mer core peptide

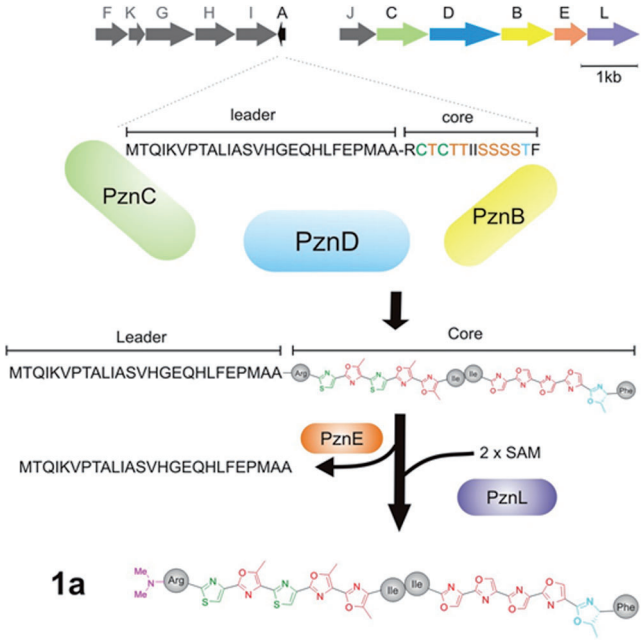

Figure 1. Structure of plantazolicin A (**1a**) and suggested course of the biosynthesis from a ribosomally synthesized prepeptide: multiple cyclodehydration (PznB/C/D), removal of the leader (PznE), and *N*,*N*-bismethylation (PznL).[12,13]

RCTCTTIISSSSTF. The trimeric protein complex PznBCD (cyclodehydratase, dehydrogenase, and docking/scaffolding protein) likely encodes posttranslational modifications, that is ten cyclodehydrations followed by nine dehydrogenations.[13] After protease PznE cleaves off the leader peptide to yield desmethylplantazolicin **1b**, a final *N*,*N*-bismethylation by methyltransferase PznL gives **1a**.

Structurally, plantazolicin comprises of two unprecedented extended stretches of contiguous heterocycles. The configuration of the only non-oxidized methyloxazoline (5-MeOxH[13]) stereocenters were assigned as 4*S*,5*R* from the biosynthesis logic of it being derived from L-Thr.[12] Fermentation only yielded minute amounts of **1a**, thus hampering further studies on the molecule and its mode of action. Furthermore, the complex structure of **1a** also represents an attractive synthetic target for total synthesis. In this study, we present the first total synthesis of plantazolicin A (**1a**). Our synthetic route is designed in such a way that structural analogues for structure–activity relationship (SAR) studies

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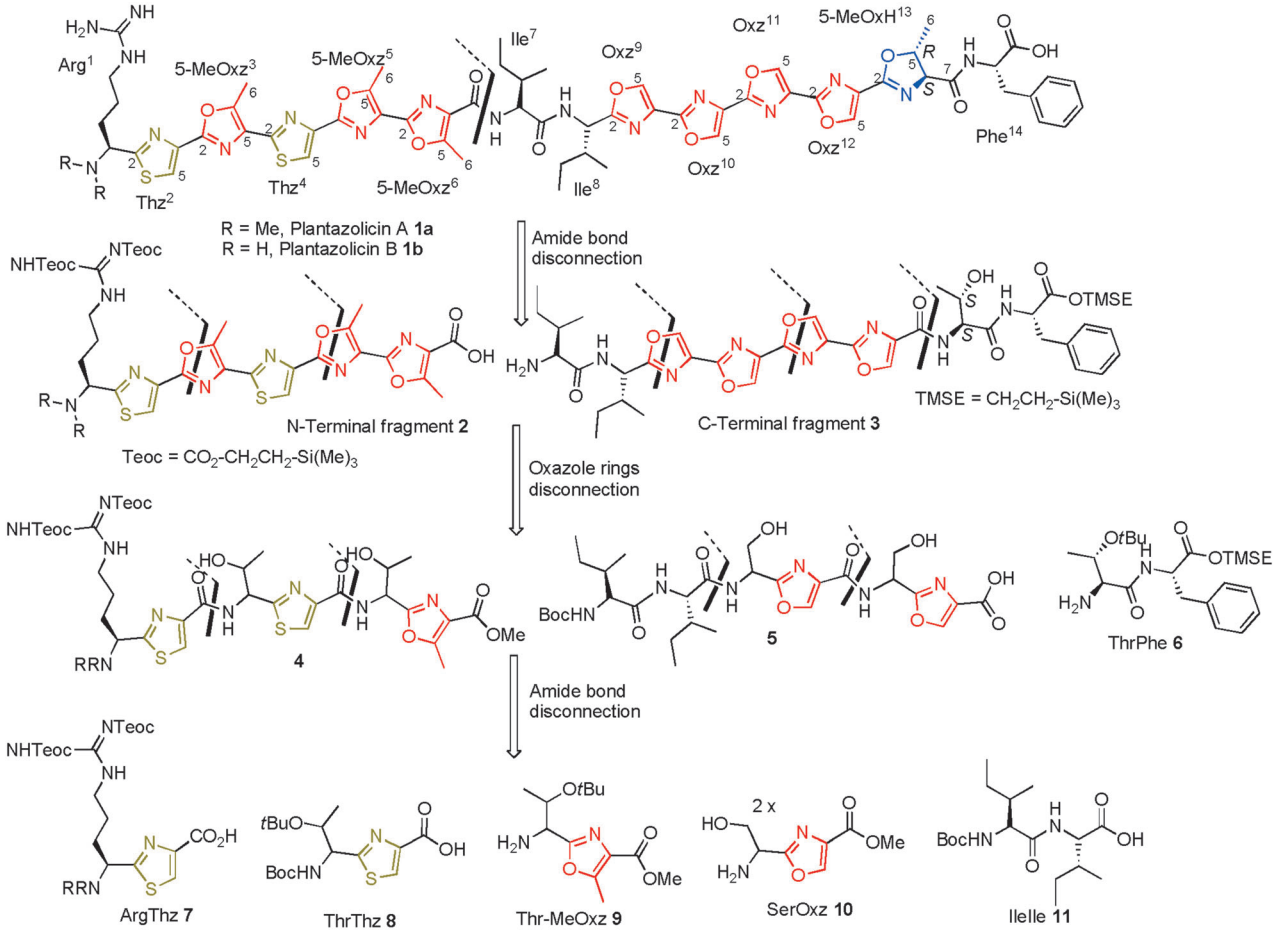


Figure 2. Structure of plantazolicin A (**1a**) and its retrosynthetic analysis by dissection into two main peptide fragments and breakdown into single heterocyclic amino acid building blocks.

can be readily prepared by iterative couplings of precursor fragment peptides.

Our retrosynthesis strategy (Figure 2) was inspired by enzymatic cyclodehydrations, which represent key steps in the biosynthesis of **1**. To begin with, we identified the two extended stretches of contiguous heterocycles including one acid-sensitive oxazoline as well as the selective *N*,*N*-bismethylation of the arginyl residue as major synthetic challenges. For the synthesis of the heterocycles we selected dimethylaminosulfur trifluoride (DAST) as the cyclodehydration agent to synthesize oxazolines from the respective aminoacyl-Ser or aminoacyl-Thr precursor peptides.[17] As an alternative, cyclodehydrations with catalytic amounts of molybdenum oxide (MoO_2) under azeotropic removal of water were considered.[18]

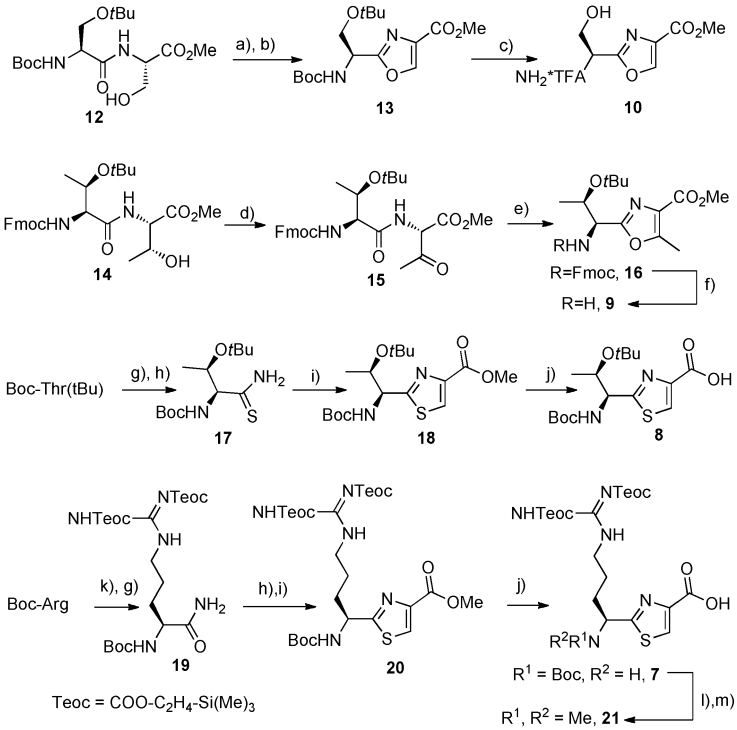
The assembly of acid-sensitive 5-MeOxH^{13} (methyloxazoline) was planned using DAST in the penultimate step to removal of protecting groups from the 14-mer heterocyclic precursor of **1**. For protection of the C terminus and of the guanidine side chain (Arg^1), we chose concomitantly removable, fluoride-labile 2-(trimethylsilyl)ethyl ester (TMSE),[19] and 2-(trimethylsilyl)ethyl carbamate (Teoc),[20] respectively, both being orthogonal to acid- and base-labile protecting groups. Protection as an TMSE ester was considered advantageous to suppress diketopiperazine formation during coupling reactions of dipeptides (e.g. **6** with **5**).[21] The *N*,*N*-bismethylation was planned to be established on a late-stage fragment because the purification of Boc-protected peptides is easier.

In the retrosynthetic analysis, the structure of **1** was dissected at the amide bond between Ile^7 and 5-MeOxz^6 to obtain N-terminal fragment **2** and C-terminal fragment **3**, as activation of 5-MeOxz^6 does not lead to racemization. The fragments **2** and **3** were further dissected at (methyl)oxazole rings 5-MeOxz^3, 5-MeOxz^5, Oxz^9, and Oxz^{11} to obtain the corresponding heterocyclic Thr/Ser-containing precursor peptides **4** and **5**. Subsequent amide-bond disconnections led to dipeptide building blocks Thr-Phe **6**, Ile-Ile **11**, and heterocyclic amino acids **7**, **8**, **9**, and **10**. The total synthesis commenced with the synthesis of dipeptide building blocks from protected Ser/Thr by using TBTU/DIPEA (see the Supporting Information).[22] The subsequent cyclodehydration was achieved with DAST, as this reagent is known to mediate cyclodehydrations of various Ser- and Thr-containing dipeptides with inversion of the configuration at β-C.[17] Indeed the reaction of Boc-Ser(*t*Bu)-Ser-OMe (**12**) with DAST at −78 °C in CH_2Cl_2 gave the corresponding oxazoline in >95% yield. Subsequent dehydrogenation with $CBrCl_3$/DBU provided the protected oxazole **13** in an overall yield of

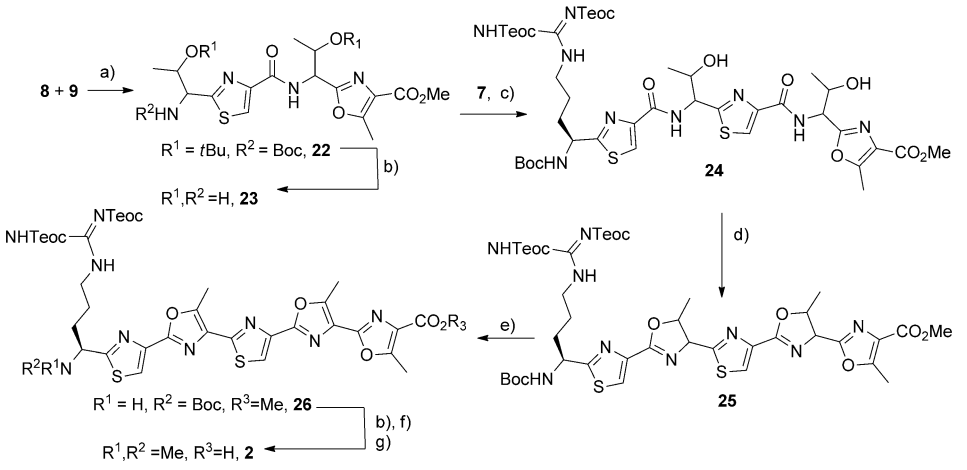
70% (Scheme 1).[23] Analogously Boc-Thr(*t*Bu)-Thr-OMe (**14**) was converted into its corresponding 5-methyloxazoline in quantitative yield, but the dehydrogenation to **16** was not successful with the above-mentioned reagents ($CBrCl_3$/DBU) or under various other conditions (CCl_4/DBU/pyridine,[24] MnO_2,[25] CuBr/Cu(OAc)$_2$/*tert*-butyl peroxybenzoate,[26] and DDQ). This prompted us to apply Wipf's modified Gabriel–Robinson oxazole synthesis.[27] Accordingly, the oxidation of **14** to ketone **15** with Dess–Martin periodinane and subsequent treatment with $PMe_3/I_2/NEt_3$ provided 5-methyloxazole **16** in 68%. Thiazoles **7** and **8** were prepared by using a modified Hantzsch thiazole procedure.[28,29] Thionation of the primary carboxamides of Boc-Thr(O*t*Bu) or **19** with Lawesson's reagent followed by the $KHCO_3$-mediated addition of methyl bromopyruvate in 1,2-DME and further treatment with TFAA/2,6-lutidine provided thiazoles **18** and **20**, both in an overall yield of 60%. Initially, the *N*,*N*-bismethylation of **7** by reductive amination seemed to be trivial; however, considerable optimization was required. Finally, selective cleavage of the Boc group in **7** with HCl followed by imine formation with 37% aq HCHO in the presence of NaOAc at 0°C and reduction with $NaCNBH_3$ at 0°C gave **21** in quantitative yield.[30]

Having the heterocyclic amino acids in hand, their iterative coupling was performed (Scheme 2). For the synthesis of N-terminal fragment **4**, a HATU/DIPEA-mediated coupling of **8** and **9** yielded **22** in 60%, which was followed by cleavage of the Boc and *t*Bu groups with HCl to give the bisheterocyclic compound **23**. Further coupling of dipeptide **7** in the presence of HATU/DIPEA furnished heterocyclic peptide **24** in 45% yield.

DAST-mediated cyclodehydration of peptide **24** gave **25** in 86% yield. However, the dehydrogenation of 5-methyloxazolines with $CBrCl_3$/DBU proved difficult. Complete dehydrogenation of one of the newly formed methyloxazolines was observed after 6 h at 0°C (**26**-$\mathbf{H_2}$, LC-MS analysis), but only trace amounts of the twofold dehydrogenated compound **26** were observed. Finally we found that an excess of reagents in a reaction over three days was needed to effect dehydrogenation of the second oxazoline, although it did not drive the reaction to completion. Fortunately, the mono- and bisoxidized products could be separated by flash chromatography (yields: 15% **26**-$\mathbf{H_2}$ and 50% **26**). *N*,*N*-



Scheme 1. Synthesis of heterocyclic amino acids (**7**, **8**, **9**, and **10**): Reagents and conditions: a) DAST, CH_2Cl_2, −78°C, 2 h, 95%. b) DBU, $CBrCl_3$, CH_2Cl_2, −10°C, 1 h to 25°C, 2 h, 79%. c) TFA, Et_3SiH, CH_2Cl_2, 25°C, 40 h, 99%. d) Dess–Martin periodinane, CH_2Cl_2, 25°C, 0.5 h, 95%. e) $PMe_3/I_2/NEt_3$, CH_2Cl_2, −40°C, 2 h, 68%. f) Piperidine, EtOAc (1:5, v/v), 25°C, 0.5 h, 99%. g) $ClCO_2Et$, NEt_3, THF, 0°C, 0.5 h, then 30% aq NH_3, 25°C, 3 h, 99%. h) Lawesson's reagent, CH_2Cl_2, 40°C, 3 h, 72%. i) $BrCH_2COCO_2Me$, $KHCO_3$, DME, −40°C, 0.5 h to −17°C, 14 h; then TFAA, 2,6-lutidine, 0°C, 14 h, 86%. j) LiOH, THF, MeOH, H_2O (1:1:1), 25°C, 3 h, 99%. k) $SiMe_3Cl$, NEt_3, CH_2Cl_2, 0°C, 0.5 h, 40°C, 0.5 h, then $(CH_3)_3Si$-C_2H_4-OCO-Suc (Teoc-OSu), NEt_3, 25°C, 3 days, 99%. l) 4 M HCl in dioxane, 25°C, 0.5 h. m) 37% HCHO (in H_2O), THF, H_2O (2:1), NaOAc 0°C, 10 min, then $NaCNBH_3$, 0°C, 1 h, 99%. DBU = 1,8-diazabicyclo[5.4.0]undec-7-ene, TFA = trifluoroacetic acid, TFAA = trifluoroacetic anhydride.



Scheme 2. Synthesis of N-terminal fragment **2**: Reagents and conditions: a) HATU, DIPEA, CH_2Cl_2, 0°C to 25°C, 14 h, then MeOH, 90% (60% of **22**, 30% of **18**). b) 4 M HCl in dioxane, 25°C, 6 h, 99%. c) HATU, DIPEA, DMF 25°C, 14 h, then MeOH, 56% (45% of **24**, 11% of **20**). d) DAST, CH_2Cl_2, −78°C, 14 h, 86%. e) DBU, $CBrCl_3$, CH_2Cl_2, 0°C to 25°C, 3 days, 60% (45% of **26**, 15% of **26**-$\mathbf{H_2}$). f) 37% aq HCHO, THF/H_2O (2:1), NaOAc, 0°C, 10 min, then $NaCNBH_3$, 0°C, 1 h. g) Me_3SnOH, 1,2-DCE, 85°C, 18 h, 97% (3 steps). HATU = *O*-(7-azabenzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium hexafluorophosphate, DIPEA = *N*,*N*-diisopropylethylamine, DCE = 1,2-dichloroethane.

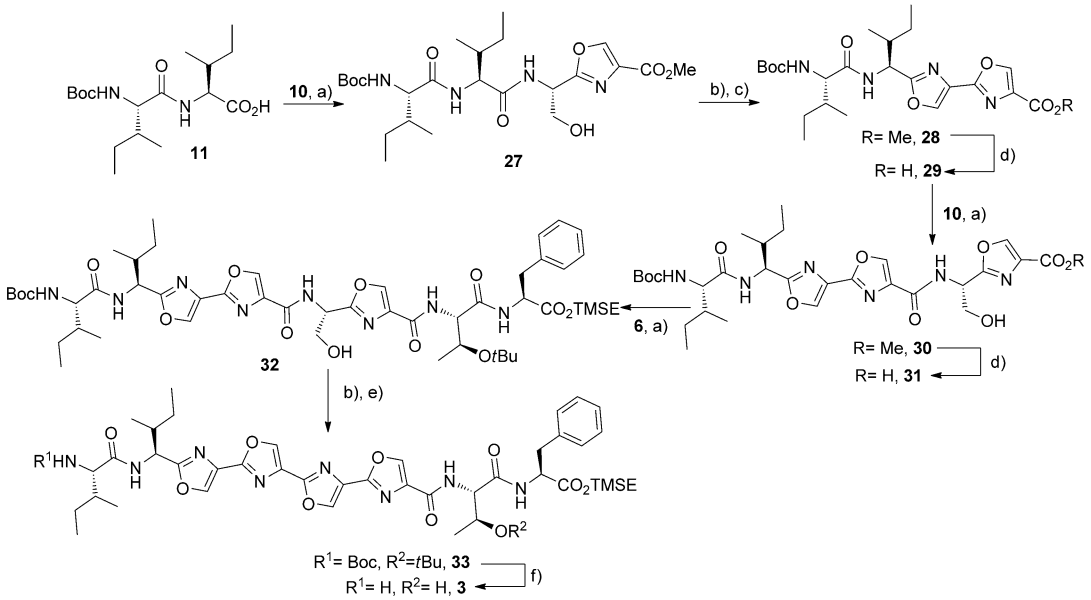
Bismethylation was carried out on fragment **26** by reductive amination under the conditions optimized for **21**. Final hydrolysis of the methyl ester with Me_3SnOH provided the N-terminal fragment **2** in 97% yield.

For the construction of the C-terminal fragment (Scheme 3), Boc-Ile-Ile-OH (**11**) was coupled with oxazole **10** in the presence of HATU/DIPEA to obtain peptide **27** in 64% yield. Cyclodehydration with DAST proceeded quantitatively; however, dehydrogenation with MnO_2 in hot toluene gave **28** in 26% yield only. Here, dehydrogenation with $CBrCl_3$/DBU was not successful, even after prolonged reaction times, presumably for steric reasons. After hydrolysis of the methyl ester with LiOH, a second coupling of **10** in the presence of HATU/DIPEA gave **30** in 57% yield. Saponification of the methyl ester with LiOH set the stage for the third coupling of dipeptide **6**. To our delight, HATU-mediated coupling proceeded smoothly to give the 8-mer fragment **32** in 81% yield. The DAST-mediated oxazoline formation and $CBrCl_3$/DBU dehydrogenation readily gave the contiguous tetraoxazole product **33** in 75% yield. Concurrent removal of the Boc and *t*Bu protecting groups with HCl gave the C-terminal fragment **3** ready for final coupling with N-terminal fragment **2**. This optimized fragment coupling route enables the synthesis of plantazolicin analogues, i.e. the introduction of labels via fragment **6**.

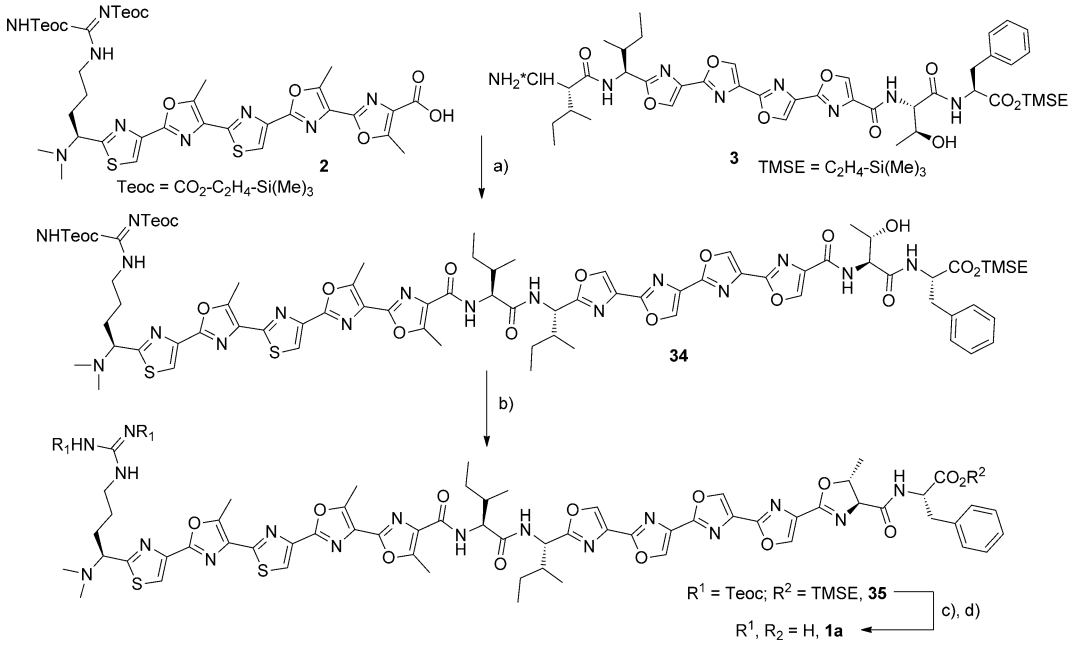
At this point we consider it necessary to discuss other attempts and problems we encountered during the synthesis of plantazolicin, which were mostly related to heterocyclic ring formation and dehydrogenation reactions. In our initial approaches, a "zipper"-like multiple cyclodehydration was pursued on protected linear peptides, synthesized by Fmoc-based solid-phase synthesis. Reaction of N-terminal fragment Boc-Arg(Z)$_2$-Cys-Thr(Bn)-Cys-Thr(Bn)-Thr(Bn)-OMe with MoO_2 gave two thiazolines along with multiple eliminations of benzyl groups to yield dehydroalanine-type products.[31] Likewise the use of Tf_2O/$OPPh_3$ gave an intractable mixture of by-products.[32] Similarly, the reaction of C-terminal fragment Boc-Ile-Ile-Ser-Ser-Ser-Ser-Thr(*t*Bu)-Phe-OMe with DAST resulted in four cyclodehydrations, but the oxidation was incomplete when either $CBrCl_3$/DBU or MnO_2 in hot toluene was used, as well as a variety of other methods mentioned above during the synthesis of **16**. During the stepwise synthesis of the contiguous tetraoxazole peptide Boc-Ile-Ile-Oxz^9-Oxz^{10}-Oxz^{11}-Oxz^{12}-OMe, the cyclodehydration of Boc-Ile-Ile-Oxz^9-Oxz^{10}-Ser^{11}-Oxz^{12}-OMe with DAST followed by $CBrCl_3$/DBU gave the desired tetraoxazole product, but it turned out that its solubility in a range of organic solvents was too low to continue along this route. The solubility of the azoles also caused difficulties in later stages of the total synthesis, especially during chromatographic purifications, which could be attributed to low yields in later steps.

Having heterocylic fragments **2** and **3** in hand, fragment condensation with HATU/DIPEA provided the 14-mer heterocyclic peptide **34** in 30% yield (Scheme 4). As planned, DAST-mediated final cyclodehydration went smoothly to afford 5-methyloxazoline **35** in 81% yield. Deprotection with TBAF trihydrate in DMF did remove the TMSE, but only one of the guanidine Teoc protecting groups. Excess reagent and prolonged reaction times did not yield the desired product. The use of TFA, TFA/NEt_3, or NEt_3·3HF cleaved all the protecting groups, but also reopened the acid-labile 5-MeOxH[13]. Finally, the mild reagent hexafluoroisopropanol (HFIP) was able to remove the remaining Teoc group and provided the title compound, plantazolicin **1a** in 91% yield.

In conclusion we achieved the first total synthesis of plantazolicin A (**1a**). In our synthesis strategy, iterative coupling of dipeptide and heterocylic building blocks were



Scheme 3. Synthesis of C-terminal fragment **3**: Reagents and conditions: a) HATU, DIPEA, DMF, −30°C to 25°C, 20 h, 64%. b) DAST, CH_2Cl_2, −78°C to −20°C, 4 h. c) MnO_2, toluene, 70°C, 3 days, 26%. d) LiOH, THF, H_2O, 25°C, 2 h. e) DBU, $CBrCl_3$, CH_2Cl_2, −20°C to 25°C, 3 days, 75%. f) 4 M HCl, dioxane, 25°C, 5 h.



Scheme 4. Final stages of the total synthesis of plantazolicin A (**1a**): coupling of N-terminal and C-terminal peptides, oxazoline formation, and deprotection. Reagents and conditions: a) HATU, DIPEA, DMF, 25 °C, 14 h, 30%. b) DAST, CH_2Cl_2, −78 °C, 12–16 h, 81%. c) TBAF, DMF, 25 °C, 1.5 h. d) $(CF_3)_2CHOH$, 25 °C, 48 h, 91% (2 steps). TBAF = tetra-*n*-butylammonium fluoride.

used, which are amenable to the synthesis of analogues. A distinguishing feature of our route is the broad applicability of DAST in cyclodehydration reactions, which even proved successful with bulky substrates such as the 14-mer peptide. Further studies towards the investigation of the plantazolicin biosynthesis and target identification are currently ongoing and will be reported in due course.

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