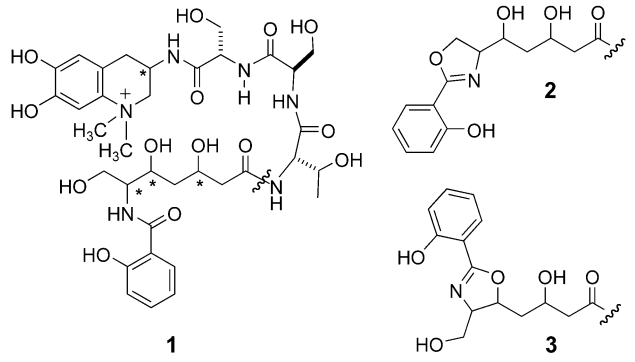
Biomimetic Synthesis

A Biomimetic Route to the Peptide Alkaloid Anachelin**

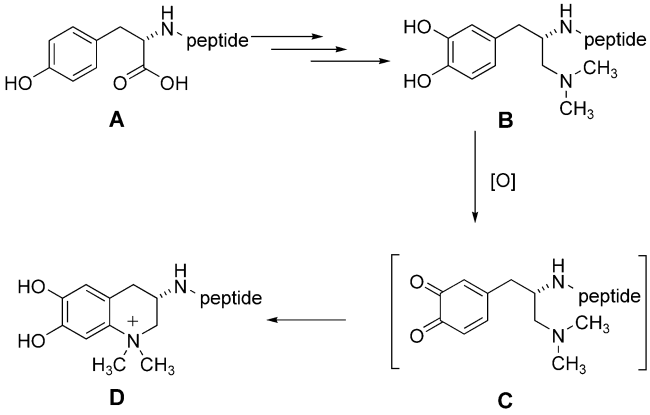
Karl Gademann and Yann Bethuel*

The acquisition and storage of iron is a central challenge for virtually all life forms due to its low bioavailability. Although cyanobacteria are one of the most successful life forms on earth, the molecular mechanism of iron acquisition by cyanobacteria remains largely unknown. Recently, the first complex secondary metabolites were isolated from *Anabaena cylindric*a.[1] These compounds were postulated to have biological activity as ligands for Fe (siderophores). While Budzikiewicz et al. isolated mixtures of anachelin H (**1**) and anachelin-1 (**2**),[1] Murakami et al. described the isolation and determination of the constitution of **2** and anachelin-2 (**3**).[2]



These compounds are composed of a fascinating blend of alkaloid, peptide, and polyketide building blocks. In particular, they contain structurally interesting fragments such as the rare tetrahydroquinolinium ring, which contains a quaternary N atom, as well as a polyhydroxylated ε-amino acid.[3] The biological activity and the mode of action of compounds **1**–**3** as siderophores are not yet established. In addition, the absolute and relative configuration of the four stereogenic centers was not determined. Since these questions can be addressed by total synthesis, we chose anachelin H as the subject for our synthetic studies.[4]

We propose the following hypothetical transformations for the biogenesis of the unique tetrahydroquinolinium chromophore (Scheme 1): It is reasonable to assume that peptide **A** containing a C-terminal tyrosine residue could serve as precursor. Oxidation to dihydroxyphenylalanine, subsequent reductive amination, and methylation would



Scheme 1. A biogenetic hypothesis for the formation of the anachelin chromophore **D**. The key intermediate **B** could be formed from the tyrosine peptide **A**. Subsequent oxidation to the *ortho*-quinone **C** and spontaneous intramolecular aza annulation would result in formation of the chromophore **D**.

result in the dopamine derivative **B**. This key precursor could then be transformed by enzymatic oxidation into the *ortho*-quinone **C**, which would react by spontaneous aza annulation to generate the anachelin chromophore **D**.[5]

In order to evaluate this biogenetic hypothesis in the chemical laboratory, we chose the transformation **B**→**D** as the key step of our biomimetic strategy.[6] The starting material L-DOPA (**4**) was Boc-protected using standard reagents (Scheme 2). Activation via the mixed anhydride and subsequent transformation with dimethylamine in THF gave Boc-L-DOPA-dimethylamide (**5**) as a crystalline solid.[7] A three-step sequence[8] (deprotection using trifluoroacetic acid, borane reduction, and Boc protection) resulted in the air-sensitive Boc-protected diamine **6**, the low yield of which is attributed to problems in the isolation and purification. The key intermediate **7** analogous to **B** in Scheme 1 was obtained after Boc removal and coupling with a serine derivative in good yield. After testing different oxidants, we found that the oxidative aza annulation of **7** using dianisyltellurium oxide[9] gave the desired cyclization product in 80% yield. Again, this compound is not stable on silica gel. Thus the hydroxy groups were protected as benzyl ethers, and the resulting quaternary ammonium salt **8** was isolated after flash chromatography. Cleavage of the N-terminal protecting group and coupling with the protected dipeptide derivative Boc-Thr-Ser-OH, which is readily available in four steps,[6] gave, after removal of the Boc group of **9**, fragment **10**.

The unknown relative and absolute configuration of the ε-amino acid forced us to choose a flexible strategy for the construction of the aldol skeleton. Through biomimetic C_2-homologation by means of a crossed Claisen condensation and subsequent reduction of the β-keto ester, we can prepare all the possible isomers in good yields and few steps. Our

[*] Dr. K. Gademann, Y. Bethuel
Laboratorium für Organische Chemie
Eidgenössische Technische Hochschule Zürich
ETH Hönggerberg, 8093 Zürich (Switzerland)
Fax: (+41) 1-632-1328
E-mail: gademann@org.chem.ethz.ch

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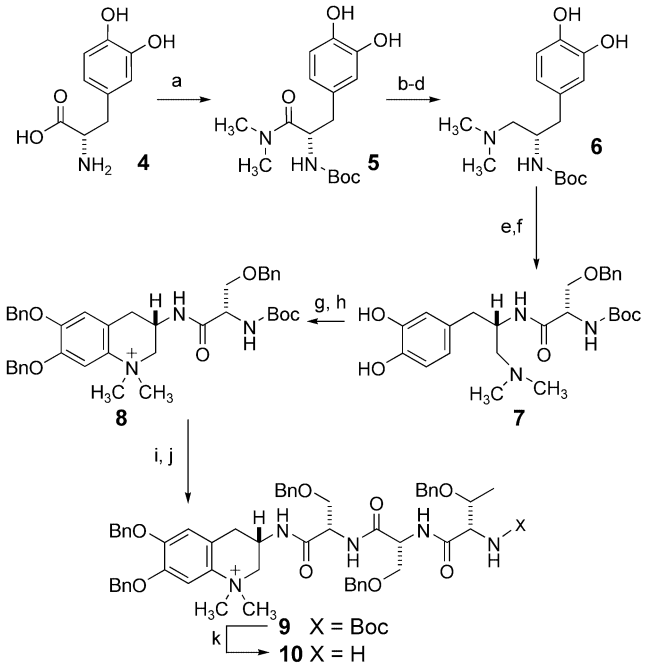
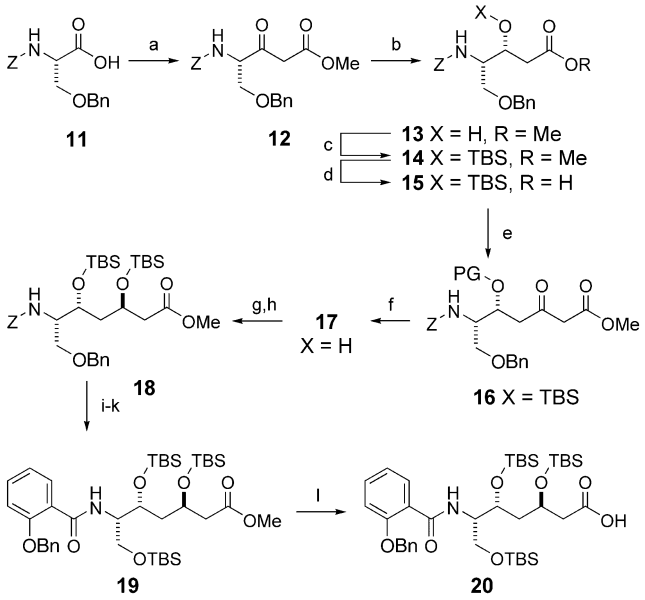


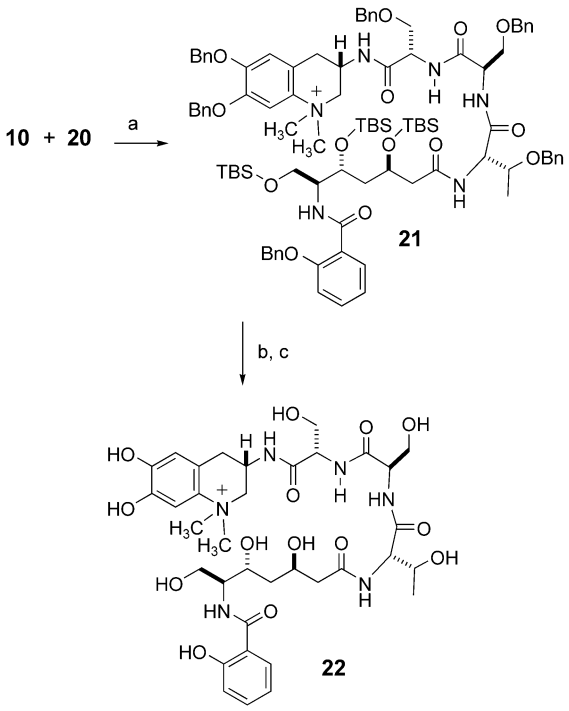
Scheme 2. a) 1. Boc_2O, dioxane, aq. NaOH, 2. *i*BuOCOCl, THF, 3. $HN(CH_3)_2$, 61%; b) TFA, CH_2Cl_2; c) BH_3·THF; d) Boc_2O, dioxane, aq. NaOH, (16% over three steps starting from **5**); e) TFA, CH_2Cl_2, quant.; f) Boc-L-Ser(OBn)-OH, EDC, HOBt, 58%; g) dianisyltellurium oxide, CH_2Cl_2, 80%; h) BnBr, Cs_2CO_3, acetone, 85%; i) TFA, CH_2Cl_2, quant.; j) Boc-L-Thr(OBn)-D-Ser(OBn)-OH, EDC, HOBt, 73%; k) TFA, CH_2Cl_2, quant. Abbreviations: Boc = butoxycarbonyl, DOPA = 3,4-dihydroxyphenylalanine, EDC = *N*-ethyl-*N*′-(dimethylaminopropyl)carbodiimide hydrochloride, HOBt = 1-hydroxybenztriazole, TFA = CF_3CO_2H.



Scheme 3. a) 1. CDI, THF, 2. $KO_2CCH_2CO_2Me$, $MgCl_2$, 71%; b) $NaBH_4$, MeOH, Et_2O, −60°C, 90%, then separation of the diastereoisomers, 48%; c) TBSOTf, 2,6-lutidine, CH_2Cl_2, 78%; d) NaOH, MeOH, THF, 54%; e) 1. CDI, THF, 2. $KO_2CCH_2CO_2Me$, $MgCl_2$; f) TBAF (1 M in THF), 60% (over two steps starting from **15**); g) $Me_4NB(OAc)_3H$, AcOH, CH_3CN (d.r. > 95:5); h) TBSOTf, 2,6-lutidine, CH_2Cl_2, 75% (over two steps from **17**); i) Pd/C, H_2, MeOH; j) EDC, HOBt, 2-BnO-salicylic acid, $CHCl_3$; k) TBSCl, imidazole, DMAP, DMF, 70% (over three steps from **18**); l) NaOH, MeOH, THF, quant. Abbreviations: CDI = carbonyldiimidazole, DMAP = 4-*N*,*N*-dimethylaminopyridine, PG = protecting group, TBAF = tetrabutylammonium fluoride, TBS = *tert*-butyldimethylsilyl, Tf = triflate.

synthesis started with the protected serine derivative **11**, which, after activation to the imidazolide, was allowed to react using the procedure of Masamune et al. with monomethylpotassium malonate in the presence of $MgCl_2$[10] (Scheme 3). The resulting β-keto ester **12** was deliberately reduced with low selectivity using $NaBH_4$ in order to produce both isomers.[11] The secondary hydroxy group in **13** was protected (→**14**) and the methyl ester saponified. This reaction called for careful optimization of the reaction conditions, as major amounts of the corresponding γ-lactam were isolated as a side product. The carboxylic acid **15** was again homologated to **16** using a Claisen condensation, and the silyl group was cleaved. A stereoselective 1,3-*anti* reduction of the hydroxyketoester following the Evans protocol[12] and subsequent TBS protection gave **18** in 75% yield. Hydrogenolytic cleavage of the benzyl protecting groups, coupling to the salicylic acid derivative, and TBS protection resulted in **19** (70% yield over three steps); the methyl ester was easily hydrolyzed at this time to give **20**.

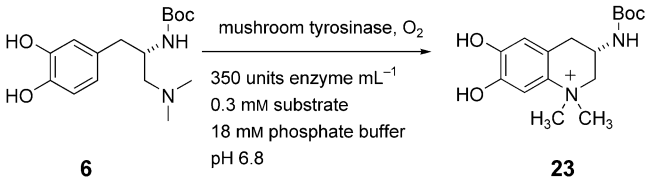
Fragments **10** and **20** were then coupled using EDC to the protected anachelin H derivative **21** (Scheme 4) The benzyl groups were cleaved hydrogenolytically in acidic medium (MeOH, AcOH). Since the use of TBAF resulted only in partial deprotection of the TBS-protected secondary hydroxy groups, the reagent of choice proved to be 1% HCl in MeOH, and the completely deprotected peptide alkaloid **22** was isolated.



Scheme 4. a) EDC, HOBt, $CHCl_3$, 63%; b) Pd/C, H_2, MeOH, AcOH; c) 1% conc. aq. HCl in MeOH (45% over two steps).

Both the exact mass of compound **22** as well as the fragmentation pattern in the mass spectrum are identical to those of an authentic sample of anachelin H (**1**);[13] however, the ^{1}H NMR spectra of **1** and **22** display minor differences. This indicates that compound **22** could be a diastereoisomer of anachelin H (**1**). The synthetic route shown here allows for the preparation of all possible fifteen diastereoisomers of **22** and thus should lead the determination of the absolute and relative configuration of anachelin H (**1**).

In addition, our synthesis described herein delivers intermediates that serve in the further evaluation of our biogenetic hypothesis. For example, the postulated cascade **B**→**D** in Scheme 1 can be corroborated by biochemical experiments. While one can assume that in this transformation **C** spontaneously reacts to give **D**, we postulate that an enzyme in the class of catechol oxidases catalyzes the oxidation of **B** to **C**. Therefore, we decided to test this hypothesis using an intermediate of our synthesis. We chose diamine **6** as model substrate, which was transformed in 18 mM phosphate buffer at pH 6.8 with commercially available tyrosinase (catechol oxidase, EC 1.14.18.1) isolated from mushrooms (Scheme 5). Interestingly, the formation of the



Scheme 5. Catalytic oxidation of diamine **6** with O_2 and catechol oxidase.

cyclization product **23** could be observed by UV spectroscopy at 305 nm![14] This biochemical experiment corroborates our postulated biogenesis and indeed suggests that a catechol oxidase is likely involved in the biosynthesis of the anachelin chromophore.

We present in this communication a biomimetic strategy for the preparation of the peptide alkaloid anachelin featuring a Te-mediated oxidative aza annulation as well as Claisen condensations under mild conditions as key steps. This route also delivers substrates, by which our biogenetic hypothesis can be corroborated. For example, we were able to show that the enzyme tyrosinase catalyses the cyclization of a key model substrate of the biogenetic hypothesis. This experiment suggests that a catechol oxidase could be involved in the biosynthesis of anachelin. Additional experiments concerning the biosynthesis, the configuration of anachelin, and the mechanism of iron acquisition of *A. cylindrica* are being pursued in our laboratory and will be reported in due course.

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[2] Y. Itou, S. Okada, M. Murakami, *Tetrahedron* **2001**, *57*, 9093–9099. This group did not isolate anachelin H (**1**), but instead two esters isomeric to **1** of the salicylic acid with C(5)-O and C(7)-O of the ε-amino acid of anachelin.
[3] This amino acid is only to be found in the peptide antibiotic galantin. Isolation: J. Shoji, R. Sakazaki, Y. Wakisaka, K. Koizumi, M. Mayama, S. Matsuura, *J. Antibiot.* **1975**, *28*, 122–125; First synthesis: N. Sakai, Y. Ohfune, *J. Am. Chem. Soc.* **1992**, *114*, 998–1010.
[4] Compound **2** can be transformed by hydrolysis of the oxazoline ring into **1** (see [1]). Therefore, we assume that anachelin H is the more stable compound in the extracellular aquatic environment of *A. cylindri*ca.
[5] The transformation **B**→**C** is analogous to the biosynthesis of *cyclo*-DOPA starting from L-DOPA, important intermediates in the biogenesis of betanidin and the melanins.
[6] Experimental procedures and analytical data of all new compounds are reported in the Supporting Information and can be obtained from http://www.angewandte.org or from the corresponding author.
[7] We did not observe acylation of the aromatic hydroxy groups, as no corresponding by-products could be isolated.
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[11] The diastereoisomers were separated by crystallization. The relative configuration of **13** was assigned after derivatization to the N,O-acetal using the method of Joullié: B. D. Harris, M. M. Joullié, *Tetrahedron* **1988**, *44*, 3489–3500.
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[13] We thank Prof. Dr. H. Budzikiewicz for an authentic sample of natural anachelin H.
[14] A reference sample of **23** was prepared by chemical oxidation of **6** using dianisyltellurium oxide and characterized: (UV: λ_{max} = 284 nm, $\varepsilon = 3960\,M^{-1}s^{-1}$, shoulder at $\lambda = 305$ nm, $\varepsilon = 850\,M^{-1}s^{-1}$).

