JOC Article

Biomimetic Total Synthesis and Antimicrobial Evaluation of Anachelin H

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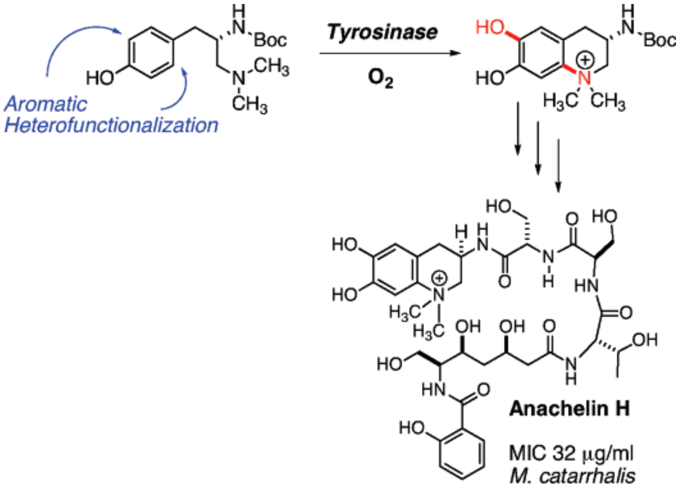

The first biomimetic total synthesis of the iron chelator anachelin H isolated from the cyanobacterium *Anabaena cylindrica* is reported. A first generation approach delivered one enantiomeric series of the polyketide fragment. Comparison of the ^{1}H NMR data suggested the relative configuration of this anachelin fragment. The relative and absolute configuration of anachelin H was then established by total synthesis. A second generation approach involved the enzymatic conversion of *N*,*N*-dimethyltyramine to the anachelin chromophore. It was demonstrated that the enzyme tyrosinase is activated by the product during this reaction, the anachelin chromophore can serve as a tyrosinase activator. Anachelin H was evaluated against a panel of eleven bacterial and fungal pathogens, and moderate antibiotic activity (32 μg/mL) against *Moraxella catarrhalis* was found.

Introduction

Cyanobacteria (or blue-green algae) are considered to be among the oldest life forms still present on earth, populating this planet since 3.5 billion years.[1] These species are thought to have modified the atmosphere of earth by performing oxygenic photosynthesis.[2] The release of oxygen caused a dramatic threat for all life forms present, and those unable to cope became extinct. In addition, the oxidative atmosphere resulted in the conversion of soluble Fe(II) ions to Fe(III) salts, of which the dominant iron oxide hydrates are insoluble at physiological pH (poor bioavailability).[3] Therefore, iron acquisition became crucial for every organism, and many sophisticated strategies were developed by evolution. It is interesting to note that although cyanobacteria probably caused this shortage of iron by performing oxygenic photosynthesis,[4] little was known

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[§] Actelion.

(1) Schopf, J. W. *Science* **1993**, *260*, 640−646.

(2) Review: (a) Alexander, M. *Annu. Rev. Microbiol.* **1971**, *25*, 361−392. (b) Howard, D. H. *Clin. Microbiol. Rev.* **1999**, *12*, 394−404.

(3) Bergeron, R. J.; McManis, J. S.; Phanstiel, O.; Vinson, J. R. *J. Org. Chem.* **1995**, *60*, 109−114.

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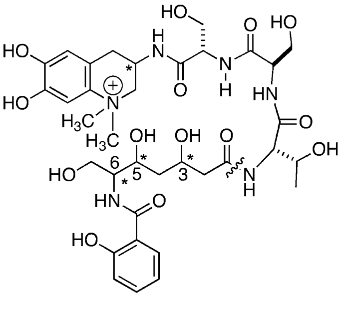
concerning their mechanism of iron uptake. In particular, no complex siderophores, that is, small molecules secreted for iron binding, transport, and uptake, have been isolated from cyanobacteria until recently. In 2000, the first complex metabolites postulated to serve as siderophores from the freshwater bacterium *Anabaena cylindrica* were isolated: Budzikiewicz, Walsby, and co-workers isolated mixtures of anachelin H (**1**) and anachelin-1 (**2**)[5] and determined the constitution of the former. Later, Murakami and co-workers reported the isolation and constitution of anachelin-1 and anachelin-2 (**3**) together with two related esters.[6] In these isolation reports, the absolute and relative configuration of four stereogenic centers could not be determined.[7] As this question could be addressed by total synthesis, we chose to initiate a research program on the synthesis of anachelin.



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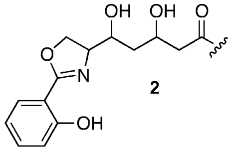

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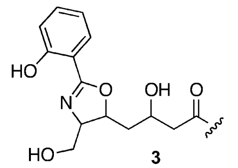

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Herein, we describe the biomimetic total synthesis of anachelin H (**1**) in full detail.[8] In addition, we present an optimized, second generation route, which utilizes an enzyme-mediated oxidative heterofunctionalization/cyclization cascade. The biological activity of anachelin H as antimicrobial agent was also evaluated and moderate antibiotic activity against *Moraxella catarrhalis* was determined.

Results and Discussion

Retrosynthetic analysis of anachelin is straightforward. As anachelin is composed of different modules of polyketide, peptide, and alkaloid biosynthetic origin, the preparation of each fragment precedes their coupling. Moreover, as the four stereogenic centers on the polyketide and alkaloid fragment were not described in the original isolation reports, a stereodivergent synthetic strategy allowing for the (possible) preparation of all sixteen diastereoisomers of anachelin was mandatory.

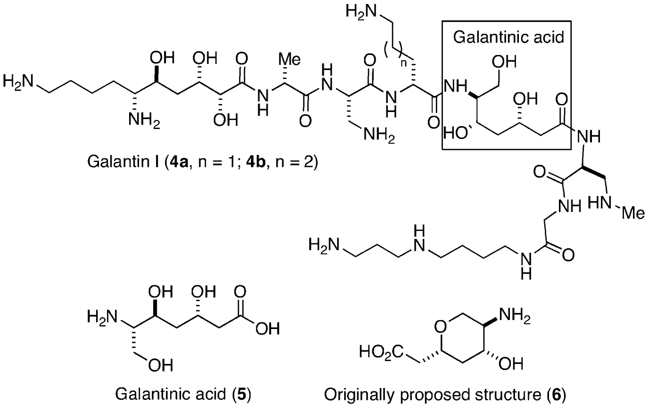

FIGURE 1. Galantin I (**4a**, **4b**), galantinic acid (**5**), and its originally proposed structure (**6**).

The polyketide fragment of anachelin resembles galantinic acid (**5**), constituent of the peptide antibiotic galantin I (**4**) isolated from the culture broth of *Bacillus pulvifaciens* (Figure 1).[9] The originally reported constitution of galantinic acid **6** (and of galantin) were later shown to be incorrect and revised by synthesis[10] to **5**. As galantinic acid features the same constitution as the polyketide fragment of anachelin, we compared the reported ^{1}H NMR chemical shifts of both fragments. However, the spectral data did not provide evidence that the relative configuration of the polyketide fragment in anachelin was (3*S**, 5*S**, 6*S**) as in galantinic acid (**5**). Therefore because of the unknown relative and absolute configuration of this fragment in anachelin, a stereodivergent synthetic strategy allowing for the access to all possible eight stereoisomers of the polyketide fragment was chosen.

The β-hydroxy-γ-amino ester **9** with the relative configuration 3,4-*unlike* (i.e., 3*R*, 4*S*) was targeted first, as this relative configuration occurs in several natural products (so-called statins) such as tandamarin, didemnin,[11-13] and others. Therefore, we followed well-established protocols used in the context of their synthesis for the preparation of the new compound **9** (Scheme 1). A biomimetic C_2-homologation according to Masamune[14] resulted in the clean formation of β-keto ester **8** on up to 40 g scale. Reduction of this compound by $NaBH_4$ resulted in the formation of a mixture of separable diastereoisomers **9** and **10**, of which both compounds are of use in our stereodivergent synthesis.[15] The major *unlike*-isomer **9** was O-silylated with more reactive TBSOTf (standard conditions using TBSCl gave poorer yields) and saponified. Another C_2-homologation then resulted in the aminohydroxyketoester **11** in good yield.

Another stereodivergent step was carried out next in producing both isomers **13** and **15**. The 3,5-*syn*-reduction of the hydroxyketoester **11** was addressed first (Scheme 2). After a literature survey, we realized that the manifold of different functional groups present in **11** could pose challenges to the

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(4) Hoehler, T. M.; Bebout, B. M.; Marais, D. J. *Nature* **2001**, *412*, 324−327.

(5) (a) Beiderbeck, H.; Taraz, K.; Budzikiewicz, H.; Walsby, A. E. *Z. Naturforsch. C* **2000**, *55*, 681−687. (b) Beiderbeck, H. Dissertation, Universität Köln, Germany, 2000.

(6) Itou, Y.; Okada, S.; Murakami, M. *Tetrahedron* **2001**, *57*, 9093−9099.

(7) Three years after the initial report, the configuration was assigned by degradation experiments: Ito, Y.; Ishida, K.; Okada, S.; Murakami, M. *Tetrahedron* **2004**, *60*, 9075−9080.

(8) For preliminary communications, see (a) Gademann, K.; Bethuel, Y. *Angew. Chem., Int. Ed.* **2004**, *43*, 3327−3329. (b) Gademann, K.; Bethuel, Y. *Org. Lett.* **2004**, *6*, 4707−4710.

(9) Shoji, J.; Sakajaki, R.; Wakisaka, Y.; Koizumi, K.; Mayama, M.; Matsurra, S. J. *J. Antibiot.* **1975**, *28*, 122−125.

(10) (a) Sakai, N.; Ohfune, Y. *Tetrahedron Lett.* **1990**, 29, 4151−4154. (b) Sakai, N.; Ohfune, Y. *J. Am. Chem. Soc.* **1992**, *114*, 998−1010.

(11) Rinehart, K. L., Jr.; Gloer, J. B.; Cook, J. C.; Miszak, S. A.; Scahill, T. A. *J. Am. Chem. Soc.* **1981**, *103*, 1857−1859.

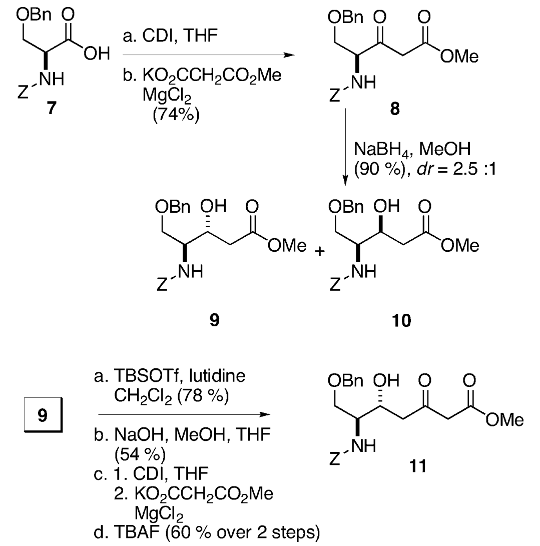
(12) Rinehart, K. L., Jr.; Kishore, V.; Bible, K. C.; Sakai, R.; Sullins, D. W.; Li, K.-M. *J. Nat. Prod.* **1988**, *51*, 1−21.

(13) Rinehart, K. L., Jr.; Kishore, V.; Nagarajan, S.; Lake, R. J.; Gloer, J. B.; Bozich, F. A.; Li, K.-M.; Maleczka, R. E., Jr.; Todsen, W. L.; Munro, M. H. G.; Sullins, D. W.; Sakai, R. *J. Am. Chem. Soc.* **1987**, *109*, 6846−6848.

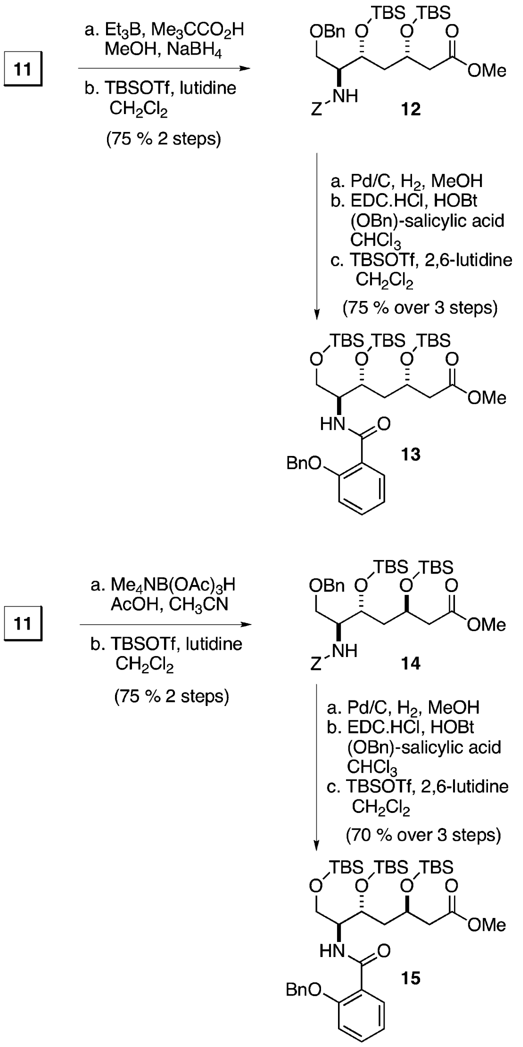
(14) Brooks, D. W.; Lu, L. D.-L.; Masamune, S. *Angew. Chem., Int. Ed.* **1979**, *18*, 72−74. Mansour, T. S.; Evans, C. A. *Synth. Commun.* **1990**, *20*, 773−781.

(15) The relative configuration of **9** and **10** was assigned after derivatization to the *N,O*-acetal using the method of Joullié: Harris, B. D.; Joullié, M. M. *Tetrahedron* **1988**, *110*, 3560−3578.

SCHEME 1. Preparation of 5,6-*unlike*-Hydroxyketone 11

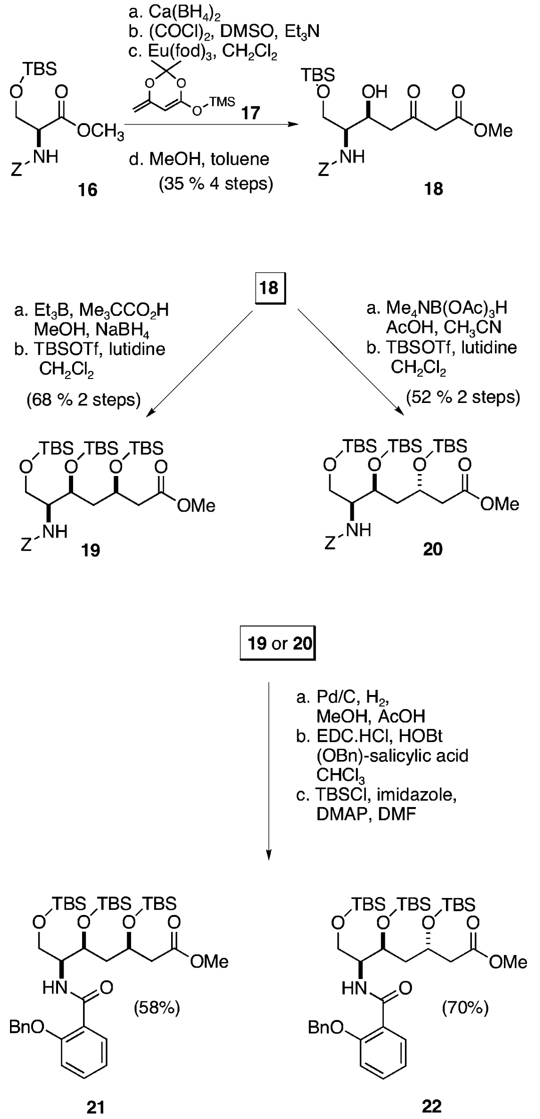


SCHEME 2. Stereodivergent Synthesis of Trihydroxyaminoesters 13 and 15



usual protocols of Narasaka et al.[16] or Prasad et al.,[17] as substrates containing this dense array of functional groups, in particular the carbamate protected amine, were found to be incompatible with the standard reaction conditions. In fact, the addition of pivalic acid in the course of the elaboration of the borane reagent was found to be crucial for a high-yielding and stereoselective reaction. Therefore, Et_3B was allowed to react with pivalic acid and MeOH before addition of the substrate,[18] which was subsequently, after chelation, reduced by $NaBH_4$. TBS protection of the secondary alcohols resulted in the preparation of fully protected **12**, which was isolated in 75% yield over two steps. Liberation of the 6,7-aminoalcohol by hydrogenolysis followed by acylation of the amino group by a protected salicylate[19] (EDC, HOBt) and reprotection of the primary alcohol (TBSOTf, 2,6-lutidine) gave the building block **13** in very good yield. The 3,5-*anti* reduction was carried out using the method of Evans and co-workers[20] utilizing triacetoxyborohydride, which resulted, after *O*-protection, in the

SCHEME 3. Preparation of Both Isomers with the Relative Configuration 5,6-*like*-21 and -22



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(16) Narasaka, K.; Pai, F.-C. *J. Org. Chem.* **1978**, *43*, 2923−2925.

(17) Chen, K.-M.; Hardtmann, G. E.; Prasad, G. E.; Repic, O.; Shapiro, M. J. *Tetrahedron Lett.* **1987**, *28*, 155−158. See also: Sletzinger, M.; Verhoeven, T. R.; Volante, R. P.; McNamara, J. M.; Corley, E. G.; Liu, T. M. H. *Tetrahedron Lett.* **1985**, *26*, 2951−2954.

(18) Lee, H. T.; Woo, P. W. K. *J. Labelled Compd Radiopharm.* **1999**, *42*, 129−133.

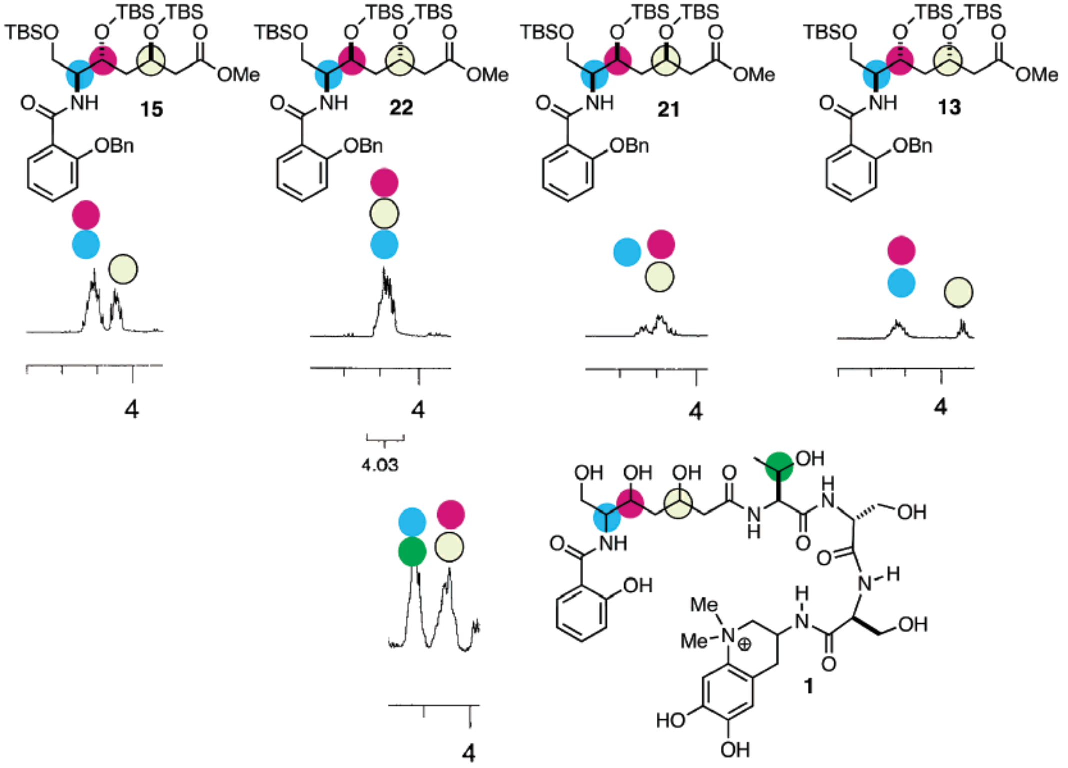
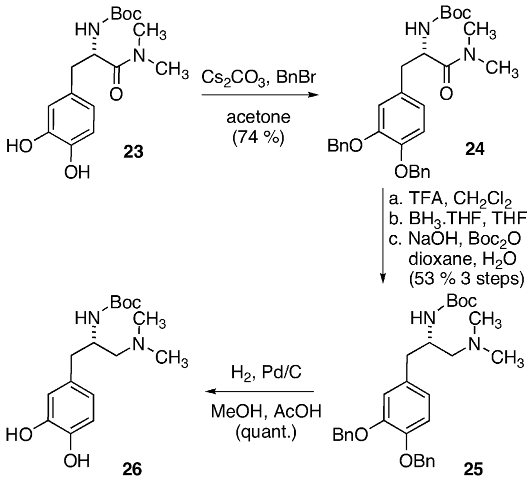


FIGURE 2. Proton chemical shifts in natural anachelin H (**1**, 300 MHz, D_2O, rt) and in the polyketide fragments (**13**, **15**, **21**, and **22**, 300 MHz, $CDCl_3$, rt).

SCHEME 4. Preparation of the *N*,*N*-Dimethylaminomethyldopamine 26



intermediate **14**. As for its diastereoisomer, elaboration of this fragment was carried out by hydrogenation, amide coupling and reprotection to give the target fragment **15**.

The building blocks carrying the 5,6-*like* configuration were accessed by performing the vinylogous aldol reaction catalyzed by Eu(III) developed by Campagne and co-workers (Scheme 3).[21] In contrast to their approach, we chose to transform the intermediate dioxanone by heating in the presence of MeOH in toluene thus affording directly the Me ester **18**.[22] This aminohydroxyketoester was then reduced following chelate-controlled via either the modified Sandoz conditions (→ **19**) or Evans' conditions (→ **20**). Elaboration of both intermediates (*Z* deprotection and coupling to the protected salicylic acid) yielded both diastereoisomers **21** and **22**. By all these synthetic operations, all possible diastereoisomers **13**, **15**, **21**, and **22** of one enantiomeric series (i.e., from L-serine) have been prepared. By following the same chemical transformations starting from D-Ser, *ent*-**21** and *ent*-**22** were also prepared.

Comparison of the chemical shifts of these protected fragments in the ^1H NMR spectrum in $CDCl_3$ to those of the natural product **1** in D_2O revealed an interesting pattern (Figure 2). Of all isomers, the shape of the peaks and in particular the chemical shifts of the relevant protons of **21** matched best to those of **1**. In the spectrum of isomer **21**, the signals of C(3)-H and C(5)-H both appear at around 4.2 ppm, whereas C(6)-H resonates at 4.27 ppm. This pattern (superposition of C(3)-H and C(5)-H, separation of C(6)-H) appears slightly shifted also in the natural product. Other isomers such as **15** display the C(3)-H and C(5)-H resonances separated in their chemical shifts. In addition, the anachelin H isomer derived from **15** has been previously prepared by us[8a] and has been shown to display clear differences in the ^1H NMR spectrum when compared to the natural product. Therefore, we decided to advance the fragment having relative configuration shown in **21**, namely, (3*R**, 5*S**, 6*S**).

Preparation of the Peptide Alkaloid Fragment. The tetrahydroquinolinium fragment of anachelin, which serves as a fluorescent chromophore, is unprecedented in other natural

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(19) Protection of the salicylic acid was carried out following a procedure by Farkas and coworkers: Farkas, L.; Vermer, B.; Nògràdi, M. *Tetrahedron* **1967**, *23*, 741−744.

(20) Evans, D. A.; Chapman, K. T.; Carreira, E. M. *J. Am. Chem. Soc.* **1988**, *110*, 3560−3578.

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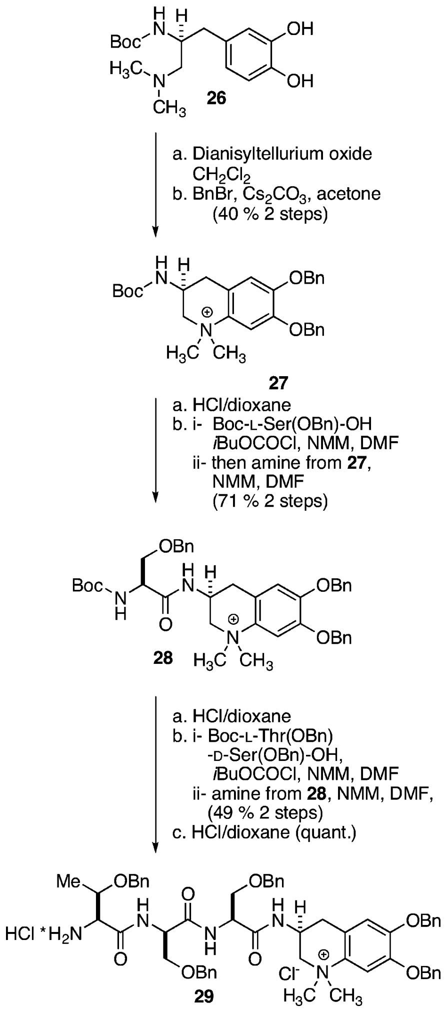
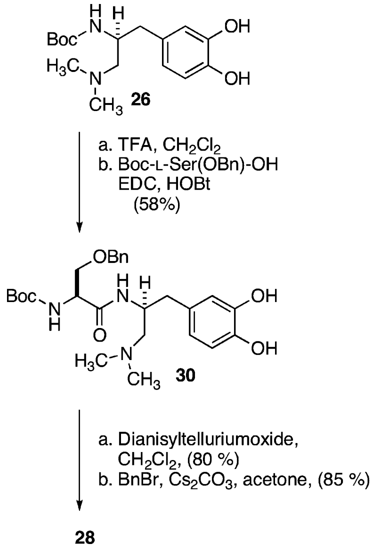
(22) Sato, M.; Sunami, S.; Sugita, Y.; Kaneko, C. *Chem. Pharm. Bull.* **1994**, *42*, 839−845. See also Snider, B. B.; Song, F. *Org. Lett.* **2001**, *3*, 1817−1820.

SCHEME 5. Preparation of the Anachelin Chromophore Tripeptide Fragment 29

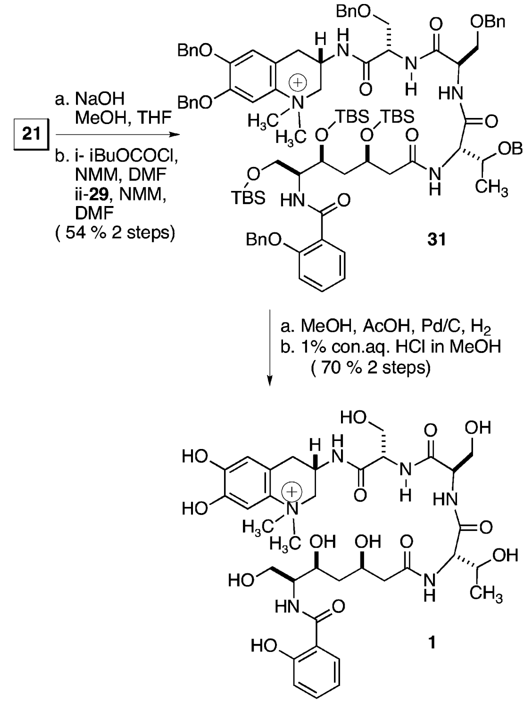


SCHEME 6. Alternative Route to the Tripeptide Anachelin Chromophore Fragment 28



products. We postulated a biogenetic hypothesis for this fragment featuring an oxidative aza annulation reaction.[23] Key

SCHEME 7. Completion of the Synthesis



to this strategy is the cyclization of protected diamine **26**, of which we developed a synthesis earlier. However, this synthesis suffered from poor yields (10% over five steps from L-DOPA),[23] which prompted us to develop the following, higher-yielding route.

Boc-L-DOPA-dimethylamide (**23**) was OBn protected, the Boc group removed and the amide reduced (Scheme 4). The removal of the Boc group proved to be critical to prevent side reactions such as carbamate reduction to the N−CH_3 taking place. Introduction of the Boc group and hydrogenolytic cleavage of the Bn ethers delivered target diamine **26** in 40% overall yield over five steps.

The cyclization to the anachelin chromophore was investigated next. Departing from the diamine **26**, two routes were examined. The first route (Scheme 5) consisted in the direct oxidative aza annulation of **26** using dianisyltellurium oxide,[24] resulting in the formation of the tetrahydroquinolinium ring. Protection of the catecholic OH groups allowed for the purification of **27** on silical gel. This heterocyclic structure could then be coupled, after removal of the Boc group, to a protected serine derivative. The intermediate **28** was thus prepared from diamine **26** in 29% yield over four steps.

An improved route (Scheme 6) was found, by first attaching the protected Ser acid to the diamine resulting in **30** (58% yield). Analogous cyclization of compound **30** furnished the intermediate **28** in an improved yield (39% over four steps). Elaboration of this compound by attaching the dipeptide Boc-L-Thr(OBn)-D-Ser(OBn)-OH to **28** (49% yield over two steps) followed by Boc-deprotection gave target fragment **29** (Scheme 5).

Merging of the peptide alkaloid part **29** with the polyketide fragment was carried out by the mixed anhydride method (Scheme 7), which proved superior to alternative coupling agents

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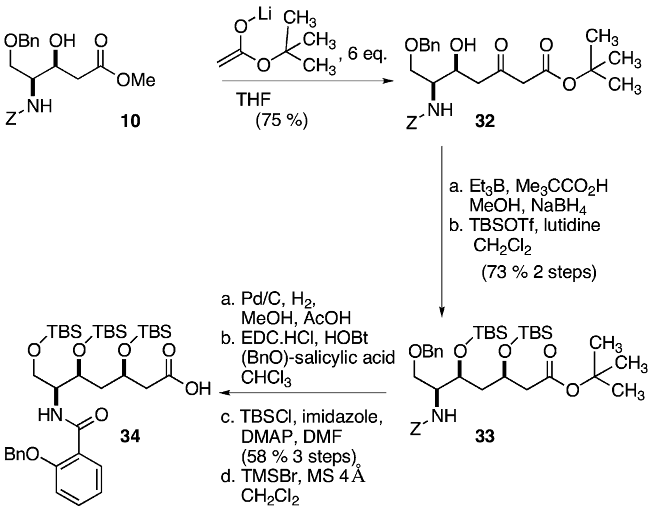
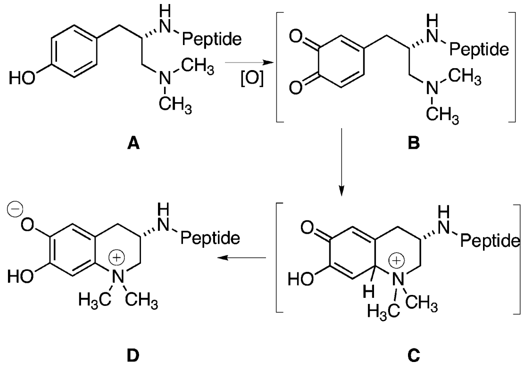
[23] Gademann, K. *Chem. Bio. Chem.* **2005**, *6*, 913−919.

[24] Ley, S. V.; Meerholz, C. A.; Barton, D. H. R. *Tetrahedron* **1981**, *37*, 213−233. For a related application, see: Clews, J.; Cooksey, C. J.; Garratt, P. J.; Land, E. J.; Ramsden, C. A.; Riley, P. A. *J. Chem. Soc., Pekin Trans.* **2000**, *1*, 4306−4315.

SCHEME 8. Second-Generation Route to the Target Diketide 34



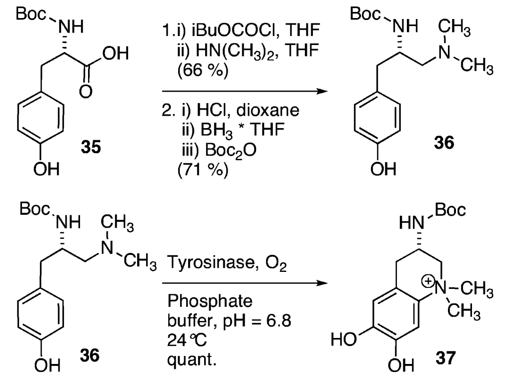
SCHEME 9. Biogenetic Hypothesis for the Generation of the Anachelin Chromophore



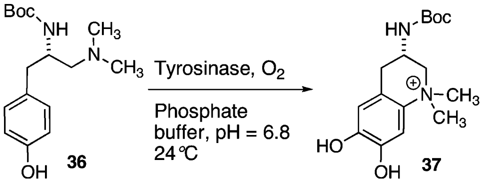
such as BOP–Cl, HBTU or EDC with respect to yield and ease of purification. No epimerization was observed in the ^{1}H NMR spectrum after the coupling reaction. Thus, the acid resulting from saponification of the ester **21** was activated with ^{i}BuOCOCl in the presence of tertiary amine base and the resulting anhydride coupled to fragment **29** resulting in protected anachelin H **31** (54% yield over two steps). Deprotection of the Bn groups was carried out by hydrogenolysis in acidic medium catalyzed by Pd/C. The removal of the Si-protecting groups by standard F-based reagents proved cumbersome, as slow and only partial deprotection was observed. We were successful by using acidic MeOH (0.5 or 1% aq HCl in MeOH) in deprotecting the TBS groups. These reactions resulted in synthetic anachelin H (**1**), which was found to be identical to the natural product as judged by the ^{1}H NMR and MS data.[25] The NMR spectra of this cationic peptide alkaloid **1** are dependent on concentration and other parameters. Therefore the final proof of identity was delivered by mixing samples of synthetic and natural anachelin H and observing their identity in the NMR spectrum of the mixture and, independently, by HPLC co-injection of natural and synthetic samples. Therefore, the absolute and relative configuration of anachelin H (**1**) was confirmed to be as shown in Scheme 7 by total synthesis.

Second-Generation Synthesis. The preparation of anachelin H (**1**) as presented above delivered synthetic material in 33 steps (17 steps longest linear sequence) and 4.9% overall yield. To streamline the synthesis, we optimized the routes for both polyketide and alkaloid fragments.

SCHEME 10. Preparation of Anachelin Chromophore 37 Starting from Boc-L-Tyr-OH (35)



SCHEME 11. The Tyrosinase-Mediated Oxidative Cascade for the Preparation of 37



Entry	Enzyme units	Conversion (%)
1	100	15
2	200	18
3	50	15
4	2 x 100	60
5	3 x 100	> 95 %

The key fragment for the polyketide part is the hydroxy keto ester **18**, which was prepared via the vinylogous Mukaiyama aldol (Scheme 3) reaction in seven steps overall from commercially available *Z*-Ser(OH)-OH.[26] We found that the corresponding *t*-Bu ester **32** can be prepared in only one step from **10** employing a direct Claisen condensation according to Heathcock[27] using an excess of *tert*-butyl lithium enolate (6 equiv) in 75% (Scheme 8).[28] This reduces the number of overall steps for this key fragment from seven to three from commercially available starting materials, in particular as **10** can be obtained as major isomer from the ketone **8** by using different reagents (see ref 15 for a detailed discussion). Elaboration of this fragment by similar methods as outlined above resulted in the preparation of **34**. Noteworthy is the fast, clean, and high-yielding cleavage of the *t*-Bu ester by TMSBr to **34**.

Optimizing the synthetic route for the alkaloid fragment was addressed next. We have postulated earlier[23] that the biosynthesis

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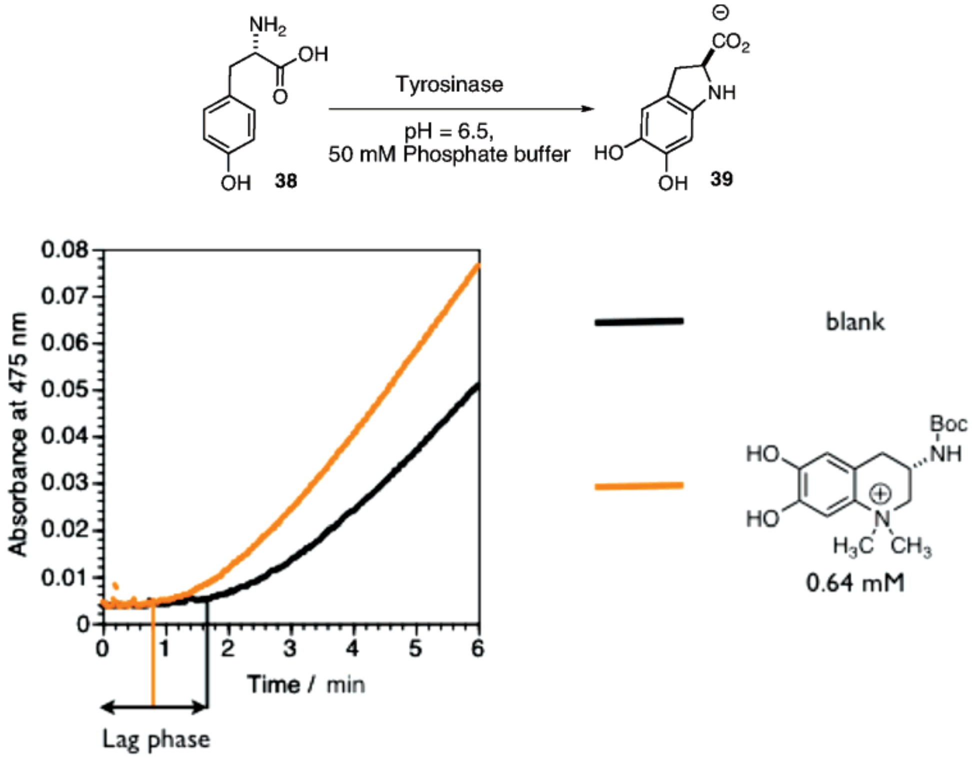
(25) The HPLC traces and ^{1}H NMR and MS spectra of the synthetic anachelin H can be found in the Supporting Information.

(26) The aldehyde precursor according to: Kang, M.; Park, J.; Konfradi, A. W.; Pedersen, S. F. *J. Org. Chem.* **1996**, *61*, 5528–5531.

(27) Hubbs, J. L.; Heathcock, C. H. *J. Am. Chem. Soc.* **2003**, *125*, 12836–12843.

(28) We employed this reaction in the stereoselective total synthesis of galantinic acid, see: Bethuel, Y.; Gademann, K. *Synlett* **2006**, 1580–1582.

SCHEME 12[a]



[a] The anachelin chromophore **37** shortens the lag phase and induces greater initial velocity in the conversion of L-Tyr (**38**) to *cyclo*-DOPA (**39**). Thus, the anachelin chromophore **37** serves as a *tyrosinase activator*.

of the anachelin chromophore originates from a dihydroxyphenylalanine derivative, which is cyclized after reductive amination. One of us (K.G.) demonstrated that this hypothesis can be chemically realized in the laboratory by the enzyme tyrosinase, and mechanistic studies have provided insight into these cyclizations.[23] Herein, we would like to expand this biogenetic hypothesis and corroborate it by enzyme-mediated biomimetic synthesis of the anachelin chromophore.

It can be postulated that a *mono-hydroxylated diamine* derived from tyrosine such as **A** can serve as precursor for the enzyme-mediated aza annulation (Scheme 9). Oxidation (either first by hydroxylation and then quinone oxidation or direct quinone oxidation) would give quinone **B** which allows for an intramolecular 1,4-addition providing either spiro-five-membered compound or directly the six-ring annulated product **C**. Tautomerization would then furnish the anachelin chromophore **D**. Tyrosinase (E.C. 1.14.18.1) is well-known to serve as dual function of both polyphenol oxidase and catechol oxidase, therefore, we set out to test this hypothesis on a preparative scale.

The precursor was prepared from Boc-tyrosine in four steps as shown in Scheme 10. Thus, activation and amidation gave Boc-Tyr(OH)-NMe$_2$, which was reduced via a three-step sequence to the diamine **36** (Scheme 10). The substrate **36** was subjected to oxidation by molecular oxygen in the presence of commercial mushroom tyrosinase (phosphate buffer, pH = 6.8 at 24 °C). The reaction was monitored by taking aliquots, which were characterized both by NMR and UV spectroscopy, and therefore the product formation of **37** was measured. Initial experiments were encouraging: Up to 15% conversion was observed using 100 units[29] of tyrosinase (Scheme 11, entry 1).[30] However, when the initial enzyme concentration was doubled, only a small increase in conversion to 18% could be observed (entry 2).[31] In general, varying enzyme concentration had only a very small effect on conversion, as the use of 50 units resulted in roughly equal conversion after 2 h. The breakthrough was achieved when the initial amount of enzyme was split, and the second portion added after the reaction stopped (entry 4). Surprisingly, conversion around 50−60% was measured. Finally, splitting the enzyme in three portions added after 0, 2, and 4 h led to full conversion noted by the disappearance of starting material (entry 5). An explanation for this unusual kinetic behavior could be that the product **37** of the transformation in Scheme 11 is *activating* the tyrosinase.

To evaluate whether the anachelin chromophore is indeed a tyrosinase activator, we examined the action of tyrosinase on its natural substrate L-Tyr resulting in *cyclo*-DOPA (Scheme 12).[32] The control experiment (black line in Scheme 12) displays the usual kinetics of this transformation featuring a lag phase, where the inactive *met*-tyrosinase is converted into the active

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(29) Unit definition: One unit will cause an increase in A_{280nm} of 0.001 per minute at pH 6.5 at 25° in a 3 mL reaction mix containing L-tyrosine. Enzyme activity as expressed in units was measured according to the Sigma Quality Control Test Procedure, SPTYR001.001, T7755, Rev. 02/22/94. Sigma Biochemicals, 3050 Spruce Street, Saint Louis, Missouri 53103.

(30) Tyrosinase is slowly decomposing in the course of the experiments at 24 °C. Generally, there was no enzyme activity observed after 2 h anymore.

(31) This experimental evidence can be correlated to the literature, where it was shown that tyrosinase in its resting native form (*met*-Tyrosinase) is catalytically inactive. See ref 32 for reviews.

(32) Reviews: (a) Duckworth, H. W.; Coleman, J. E. *J. Biol. Chem.* **1970**, *245*, 1613−1625. (b) Sanchez-Ferrer, A.; Rodriguez-Lopez, J. N.; Garcia-Canovas, F.; Garcia-Carmona, F. *Biochim. Biophys. Acta* **1995**, *1247*, 1−11. (c) Land, E. J.; Ramsden, C. A.; Riley, P. A. *Acc. Chem. Res.* **2003**, *36*, 300−308.

form.[32] The addition of a 0.64 mM solution of the anachelin chromophore derivative **37** (orange line in Scheme 12) led to the observation of both a shortened lag phase as well as higher initial rates. Therefore, one can conclude that the anachelin chromophore **37** displays biological activity as a tyrosinase activator ($AC_{50} \approx 1$ mM).

These results support the biosynthetic proposal outlined in Scheme 9 that a tyrosine precursor could be involved in the biogenesis of anachelin. The feature of autoactivation is mechanistically interesting, where the product of this enzymatic transformation is activating the enzyme of its own formation. In addition, the transformation from **36** to **37** demonstrates the power of enzymatic reactions in total synthesis further featuring a formal aminohydroxylation/cyclization cascade.

Antimicrobial Evaluation. Many iron chelators display antimicrobial activity by sequestering free Fe ions and thus preventing bacterial iron uptake. Such so-called sideromycins are very effective antibiotics with the drawback that resistance quickly develops. We therefore wanted to profile the antimicrobial activity of anachelin H (**1**). The antibacterial activities of anachelin H (**1**) and its chromophore (**37**) were tested against a panel of well-characterized bacterial strains (*Staphylococcus aureus* ATCC 29213, *Staphylococcus aureus* A-798, *Enterococcus faecalis* ATCC 29212, *Enterococcus faecium* A-949, *Streptococcus pneumoniae* ATCC 49619, *Streptococcus pneumoniae* A-70, *Haemophilus influenzae* A-921, *Moraxella catarrhalis* A-894, *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, and *Saccharomyces cerevisiae* A-136). Anachelin H (**1**) and the anachelin chromophore (**37**) displayed moderate activity (MIC, 32 μg/mL) against *Moraxella catarrhalis*, a respiratory tract pathogen, but were not active against the other strains at the highest concentration tested (64 μg/mL). To make the assay more sensitive to iron chelators,[33,34] we also used iron limited medium (M9 medium with and without iron supplement) for determination of MICs for *E. coli* and *P. aeruginosa*. However, also under these conditions no antibacterial activity was observed for these strains. Whether anachelin H (**1**) and its chromophore fragment **37** act upon *Moraxella* via a mechanism of iron deprivation is currently not known. Another possible explanation concerning the mode of action of **1** and **37** would reside in the presence of a quaternary ammonium group, a fragment known for antibacterial activity.

Conclusion

We have described the total synthesis of the cyanobacterial polyketide alkaloid anachelin H **1**. This synthesis delivered the structural proof for anachelin H by mixing samples of synthetic and natural anachelin H and detecting identity by NMR and HPLC. The first generation synthesis was characterized by a stereodivergent approach resulting in the identification of the relative configuration. The second generation approach is based on a biomimetic oxidative heterofunctionalization/cyclization cascade that shortened the synthesis and added further support to the biogenetic proposal of anachelin. The anachelin chromophore was shown to *activate* tyrosinase thus facilitating its own formation via this enzyme-mediated process. Finally, biological evaluation of anachelin H (**1**) and its chromophore **37** demonstrated moderate antibacterial activity against *Moraxella catarrhalis*, a respiratory tract pathogen.

Experimental Section

General. Reagents, abbreviations, instruments, and standard methods are reported in the Supporting Information.

(6*S*)-6-Benzyloxycarbonylamino-7-(*tert*-butyl-dimethyl-silanyloxy)-(5*S*)-5-hydroxy-3-oxo-heptanoic Acid Methyl Ester (18). To a solution of [1-(*tert*-butyl-dimethyl-silanyloxymethyl)-3-(2,2-dimethyl-6-oxo-6*H*-[1,3]dioxin-4-yl)-((3*S*)-2-hydroxy-propyl]-(2*S*)-carbamic acid benzyl ester (175 mg, 0.36 mmol) in toluene (3 mL) was added MeOH (58 μL, 1.44 mmol, 4 equiv), and the reaction mixture was refluxed for 4 h. Then it was cooled to rt, and the solvents were removed under reduced pressure. FC (EtOAc/hexane 2.5:7.5) gave the title compound **18** (141 mg, 0.31 mmol, 85%). $R_f = 0.5$ (EtOAc/hexane 4:6). $[\alpha]_D = +0.65$ (c 2.00, $CHCl_3$, $T = 27.2$ °C). ^1H NMR ($CDCl_3$, 300 MHz): δ 0.051 (s, 3H), 0.06 (s, 3H), 0.87 (s, 9H), 2.65 (dd, 1H, $J_1 = 4.35$, $J_2 = 17.44$), 2.79 (dd, 1H, $J_1 = 8.4$, $J_2 = 17.43$), 3.34 (s, 1H), 3.47 (s, 2H), 3.62−3.63 (m, 1H), 3.72 (s, 3H), 3.8 (d, 2H, $J = 4.35$), 4.44−4.49 (m, 1H), 5.1 (s, 2H), 5.32 (br. d, $J = 9.34$), 7.31−7.37 (m, 5H). ^{13}C NMR ($CDCl_3$, 75 MHz): −5.4, 18.4, 26.0, 47.0, 49.8, 52.6, 54.5, 65.0, 67.1, 68.1, 128.4, 128.4, 128.8, 136.6, 156.7, 167.5, 202.5. IR: 3647−3170*m*, 1744*m*, 1716*s*. MS 476.2 (100, $[M+Na]^+$), 444.2 (79, $[M-MeOH+Na]^+$. HRMS calcd for $C_{22}H_{35}NO_7SiNa$ $(M+Na)^+$, 476.2075; found, 476.2069.

(6*S*)-6-Benzyloxycarbonylamino-(3*R*,5*S*)-3,5,7-tris-(*tert*-butyl-dimethyl-silanyloxy)-heptanoic Acid Methyl Ester (19). A solution of (6*S*)-6-benzyloxycarbonylamino-7-(*tert*-butyl-dimethyl-silanyloxy)-(3*R*,5*S*)-4,5-hydroxy-3-oxo-heptanoic acid methyl ester (19.0 mg, 41.7 μmol) in dry CH_2Cl_2 was cooled to −20 °C, and 2,6-lutidine (19.4 μL, 0.17 mmol, 4 equiv) was added dropwise followed by the addition of TBSOTf (29 μL, 0.125 mmol, 3 equiv). The reaction mixture was stirred at −20 °C for 1 h 15 min. Then CH_2Cl_2 was added and the organic phase was washed 3× with citric acid (10%) and 2× with H_2O, dried over Na_2SO_4, filtered, and evaporated under reduced pressure. FC (EtOAc/hexane 1:9) gave **19** (21.0 mg, 30.7 μmol, 75%). $R_f = 0.68$ (EtOAc/hexane 2:8). $[\alpha]_D = -1.3$ (c 1.05, $CHCl_3$, $T = 25.5$ °C). ^1H NMR ($CDCl_3$, 300 MHz): δ 0.056 (s, 6H), 0.062 (s, 3H), 0.07, 0.083(s, 6H), 0.098 (s, 3H), 0.86(s, 9H), 0.88 (s, 9H), 0.89 (s, 9H), 1.63−1.84 (m, 2H), 2.43 (d, 2H, $J = 6.22$), 3.52 (dd, 1H, $J_1 = 7.79$, $J_2 = 9.03$), 3.62 (s, 3H), 3.66 (d, 1H, $J = 5$), 3.69 (s, 1H), 3.73 (m, 1H), 4.99 (br.d, 1H, $J = 8.7$), 5.1 (s, 2H), 7.3−7.39 (m, 5H). ^{13}C NMR ($CDCl_3$, 75 MHz): −5.1, −4.5, −4.0, 18.0, 18.2, 18.6, 26.0, 26.1, 26.2, 42.1, 43.1, 51.6, 55.1, 62.0, 66.7, 66.9, 67.4, 128.3, 128.4, 128.7, 156.4, 171.6. IR: 1727*m*. MS 706.3 (100). HRMS calcd for $C_{34}H_{65}NO_7Si_3Na$ $(M+Na)^+$, 706.3961; found, 706.3960.

(6*S*)-6-(2-Benzyloxy-benzoylamino)-(3*R*,5*S*)-3,5,7-tris-(*tert*-butyl-dimethyl-silanyloxy)-heptanoic Acid Methyl Ester (21). To a solution of (6*S*)-6-benzyloxycarbonylamino-(3*R*,5*S*)-3,5,7-tris-(*tert*-butyl-dimethyl-silanyloxy)-heptanoic acid methyl ester (**19**) (70 mg, 102 μmol) in MeOH (1 mL) previously 3× flushed with argon was added Pd/C. Then the flask was flushed with H_2, and the reaction mixture was stirred at rt for 4 h. Pd/C was filtered over celite, and the solvent was removed under reduced pressure. This residue was added to a solution of *O*-Bn-salicylic acid (27.9 mg, 122 μmol, 1.2 equiv) dissolved in dry $CHCl_3$ (1.5 mL) with EDC.HCl (23.4 mg, 122 μmol, 1.2 equiv), HOBt (18.9 mg, 122 μmol, 1.2 equiv), and NMM (34 μL, 306 μmol, 3 equiv). The reaction mixture was stirred at rt for 20 h. The solvent was then

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[33] For articles on the role of iron in infectious diseases see: (a) Weinberg, E. D.; Weinberg, G. A. *Curr. Opin. Infect. Diseases* **1995**, *8*, 164−169. (b) Weinberg, E. D. *Eur. J. Cancer Prev.* **1996**, *5*, 19−36. (c) Weinberg, E. D. *Emerging Infect. Dis.* **1999**, *5*, 346−352.

[34] For articles on the effect of iron deprivation see: (a) Archibald, F. S.; DeVoe, I. W. *J. Bacteriol.* **1978**, *136*, 35−48. (b) Al-Younes, H. M.; Rudel, T.; Brinkmann, V.; Szczepek, A. J.; Meyer, T. F. *Cell. Microbiol.* **2001**, *3*, 427−437.

removed under reduced pressure, and the residue was taken up with EtOAc. It was washed 3× with citric acid (10%) and 3× with saturated $NaHCO_3$. The organic phase was dried over $MgSO_4$, filtered, and evaporated under reduced pressure. FC (EtOAc/hexane 1:9) gave **21** (45 mg, 59 μmol, 58%). $R_f = 0.43$ (EtOAc/hexane 2:8). $[\alpha]_D = -4.2$ (*c* 2.235, $CHCl_3$, $T = 26.5$ °C). ^1H NMR ($CDCl_3$, 300 MHz): δ 0.061 (s, 6H), 0.071 (s, 3H), 0.091 (s, 3H), 0.094 (s, 3H), 0.105 (s, 3H), 0.84 (s, 9H), 0.86 (s, 9H), 0.89 (s, 9H), 1.67−1.78 (m, 2H), 2.35−2.48 (m, 2H), 3.49 (s, 3H), 3.6 (dd, 1H, $J_1 = 8.4$, $J_2 = 9.33$), 3.71 (dd, 1H, $J_1 = 5.6$, $J_2 = 9.6$), 4.1−4.21 (m, 2H), 4.23−4.28 (m, 1H), 5.29 (s, 2H), 6.91 (dd, 1H, $J_1 = 0.6$, $J_2 = 8.4$), 7.0−7.1 (m, 1H), 7.28−7.37 (m, 6H), 8.0 (d, 1H, $J = 8.4$), 8.16 (dd, 1H, $J_1 = 1.87$, $J_2 = 7.8$). ^{13}C NMR ($CDCl_3$, 75 MHz): −5.1, −4.5, −4.2, −4.0, 17.9, 18.1, 18.5, 25.9, 26.0, 26.1, 42.1, 43.2, 51.4, 53.8, 61.2, 66.5, 67.1, 70.7, 113.2, 121.3, 122.3, 126.7, 128.1, 128.7, 132.2, 132.3, 135.9, 156.3, 164.9, 171.3. IR: 2953*m*, 2928*m*, 2856*m*, 1740*m*, 1654*m*. MS 760 (100, $[M+H]^+$). HRMS calcd for $C_{40}H_{69}NO_7Si_3Na$ $(M+Na)^+$, 782.4274; found, 782.4285.

(*S*,*S*)-{2-Benzyloxy-1-[1-(3,4-dihydroxy-benzyl)-2-dimethylamino-ethylcarbamoy]-ethyl}-carbamic Acid *tert*-Butyl Ester (30). The Boc-diamine (**26**) (108 mg, 0.348 mmol) was dissolved in CH_2Cl_2 (1 mL) and cooled to 0 °C under an Ar atmosphere. Trifluoroacetic acid (1 mL) was added dropwise, and the resulting solution was stirred for 1 h at 0 °C and for 1 h at rt. The solvent was removed under reduced pressure. The residue was twice dissolved in toluene, and the solvent again was removed under reduced pressure. The residue was dried under high vacuum and used without further purification.

Boc-L-Ser(OBn)-OH (102.9 mg, 0.348 mmol) was dissolved in THF (1 mL) under Ar and cooled to −20 °C (bath temp.). To this solution, Et_3N (48.5 mL, 0.348 mmol) and ^{i}BuOCOCl (45.3 mL, 0.348 mmol) were added dropwise, and a precipitate began to form after 5 min. The reaction mixture was stirred for 15 min at −20 °C. In the meantime, the diamine trifluoroacetate prepared above was dissolved in THF (1 mL) and Et_3N (0.97 mL, 0.696 mmol) was added. This solution of the free base was slowly added to the above mixture of the mixed anhydride at −20 °C. The reaction mixture was allowed to warm to 10 °C in three h. The solvent was evaporated under reduced pressure, and the residue taken up in EtOAc. The organic layer was twice washed with 10% citric acid solution, twice washed with brine, dried ($MgSO_4$), and filtered, and the solvent was evaporated under reduced pressure. FC (CH_2Cl_2/MeOH 10:1) gave the title compound (**30**) (98.0 mg, 0.20 mmol, 58%). Colorless oil. $R_f = 0.12$ (CH_2Cl_2/MeOH 10:1). $[\alpha]_D = -12.3$ (*c* 1.00, MeOH). ^1H NMR (CD_3OD, 300 MHz): 1.45 (s, 9H), 2.63−2.76 (m, 2H), 2.82 (br. s, 6H), 3.14−3.25 (m, 2H), 3.57 (d, $J = 5.6$, 2 H), 4.15 (t, $J = 5.3$, 1 H), 4.36−4.40 (m, 1H), 4.47 (s, 2H), 6.53 (dd, $J_1 = 8.1$, $J_2 = 2.0$, 1H), 6.66 (d. $J = 2.0$, 1H), 6.70 (d, $J = 8.1$, 1H), 7.24−7.35 (m, 5H). ^{13}C NMR (CD_3OD, 75 MHz): 27.5, 37.6, 43.2, 55.6, 60.6, 69.4, 73.1, 80.3, 115.4, 116.3, 120.6, 127.7, 127.9, 128.3, 137.9, 144.3, 145.3, 157.3, 172.4. IR: (thin film): 3313s, 2947*m*, 1674*s*, 1529*m*. MS 488.3 (100, $[M+H]^+$), 432.2 (31, $[M\text{-}tBu+H]^+$), 388.2 (17, $[M\text{-}Boc]^+$). HRMS calcd for $C_{26}H_{38}N_3O_6$ $(M+H)^+$, 488.2755; found, 488.2750.

(2*S*, 3*S*)-6,7-Bis-benzyloxy-3-(3-benzyloxy-2-*tert*-butoxycarbonylamino-propionylamino)-6,7-dihydroxy-1,1-dimethyl-1,2,3,4-tetrahydro-quinolinium (28). (2*S*, 3*S*)-3-(3-benzyloxy-2-*tert*-butoxycarbonylamino-propionylamino)-6,7-dihydroxy-1,1-dimethyl-1,2,3,4-tetrahydro-quinolinium (149.2 mg, 0.3 mmol) was dissolved under Ar in dry acetone (3 mL). To this solution, Cs_2CO_3 (300 mg, 0.92 mmol) and BnBr (110 mL, 0.93 mmol) were added, and the resulting reddish solution was heated to reflux for 4 h. The color changed to white, and the solvent was removed under reduced pressure. The residue was taken up in EtOAc, and the organic layer was washed three times with 1 N HCl solution and twice with brine. The organic layer was dried ($MgSO_4$) and filtered, and the solvent was removed under reduced pressure. FC (5 g SiO_2, CH_2Cl_2/MeOH 10:1) gave the title compound (**28**) (169.0 mg, 0.253 mmol, 85%). Colorless oil. $R_f = 0.11$ (CH_2Cl_2/MeOH 10:1). ^1H NMR ($CDCl_3$, 300 MHz): 1.41 (s, 9H), 2.94 (dd, $J_1 = 16.5$, $J_2 = 5.3$, 1H), 3.24 (br. dd, $J_1 = 16.5$, $J_2 = 10.6$, 1H), 3.61 (s, 3H), 3.67 (s, 3H), 3.69−3.83 (m, 2H), 3.96−4.01 (m, 1H), 4.40−4.53 (m, 1H), 4.53 (d, $J = 3.7$, 2H), 5.11 (s, 2H), 5.37 (d, $J = 3.4$, 2H), 5.58 (d, $J = 7.5$, 1H), 6.58 (s, 1H), 7.22−7.35 (m, 16H), 7.37 (d, $J = 4$, 1H), 7.39 (br. s, 1H), 8.30 (br. s, 1H). MS 666.3 (43, M^+), 610.3 (100, $[M\text{-}tBu+H]^+$), 566.3 (28, $[M\text{-}Boc]^+$). HRMS calcd for $C_{40}H_{48}N_3O_6$ (M^+), 666.3538; found, 666.3531.

(3*R*, 5*S*, 6*S*)-6-(Benzyloxy-benzoylamino)-3,5,7-tris-(*tert*-butyl-dimethyl-silanoxy)-heptanoic acid-L-Thr(OBn)-D-Ser(OBn)-L-Ser(OBn)-(3*S*)-THQ(OBn) (31). Boc-L-Thr(OBn)-D-Ser(OBn)-L-Ser(OBn)-(3*S*)-THQ(OBn) (36 mg, 33.6 μmol) was dissolved in dioxane (0.6 mL) and HCl in dioxane (4 M, 100 μL, 400 μmol) was added at 0 °C. The reaction mixture was stirred 1 h at this temperature and 3 h at rt. The solvent was then removed under reduced pressure, and the product **29** was dried under high vacuum 2 h. The resulting residue was used without further purification.

To a solution of (3*R*,5*S*,6*S*)-6-(2-benzyloxy-benzoylamino)-3,5,7-tris-(*tert*-butyl-dimethyl-silanyloxy)-heptanoic acid **(21)** (30 mg, 40.3 μmol, 1.2 equiv) in DMF (0.5 mL) at −18 °C was added ^{i}-BuOCOCl (5.3 μL, 40.3 μmol, 1.2 equiv) and NMM (4.4 μL, 40.3 μmol, 2 equiv). The reaction mixture was stirred at this temperature for 20 min. Then L-Thr(OBn)-D-Ser(OBn)-L-Ser(OBn)-(3*S*)-THQ-(OBn) (33.8 mg, 33.6 μmol, 1 equiv) dissolved in DMF (0.5 mL) and NMM (4.4 μL, 40.3 μmol, 2 equiv) was added dropwise at −18 °C, and the reaction mixture was allowed to warm over 4 h. The solvent was evaporated under reduced pressure, and the residue taken up in EtOAc. The organic layer was washed 3× with H_2O, and the resulting water phase extracted 3× with EtOAc. The combined organic phases were dried over Na_2SO_4, filtered, and evaporated under reduced pressure. FC (CH_2Cl_2/MeOH 10:1) gave the title compound **31** (31 mg, 18.3 mmol, 54%). Colorless oil. $R_f = 0.18$ (CH_2Cl_2/MeOH 10:1). $[\alpha]_D = +7.9$ (*c* 0.55, $CHCl_3$, $T = 26$ °C). ^1H NMR ($CDCl_3$, 300 MHz): 0.013 (s, 3H), 0.032 (s, 6H), 0.061 (s, 6H), 0.079 (s, 3H), 0.80 (s, 9H), 0.83 (s, 9H), 0.86 (s, 9H), 1.15 (d, 3H, $J = 6.54$), 1.74−1.9 (m, 8H), 2.24 (dd, 1H, $J_1 = 6.54$, $J_2 = 14.6$), 2.35 (dd, 1H, $J_1 = 3.1$, $J_2 = 14.6$), 2.86 (dd, 1H, $J_1 = 5.0$, $J_2 = 16.2$), 3.18 (dd, 1H, $J_1 = 11.5$, $J_2 = 16.2$), 3.41 (s, 3H), 3.44 (s, 3H), 3.56−3.74 (m, 6H), 3.86−3.94 (m, 2H), 4.0−4.08 (m, 1H), 4.1−4.18 (m, 1H), 4.18−4.28 (m, 2H), 4.41 (s, 2H), 4.42 (s, 2H), 4.48 (s, 2H), 4.52−4.62 (m, 2H), 4.64−4.74 (m, 1H), 5.13 (d, 2H, $J = 2.5$), 5.21 (d, 2H, $J = 1.9$), 5.26 (s, 2H), 6.6 (s, 1H), 6.86−6.94 (m, 3H), 7.4−7.46 (m, 36H), 7.84 (d, 1H, $J = 7.5$), 7.97 (d, $J = 8.7$), 8.07 (dd, $J_1 = 1.9$, $J_2 = 7.5$), 8.38 (m, 2H). IR: 1653*m*, 1520*m*, 1216*s*. MS 1662.8 (100, $[M+H]^+$). HRMS calcd for $C_{95}H_{129}N_6O_{14}Si_3$ $(M+H)^+$, 1661.8869; found, 1661.8897.

Anachelin H (1). (3*R*,5*S*,6*S*)-6-(Benzyloxy-benzoylamino)-3,5,7-tris-(*tert*-butyl-dimethyl-silanoxy)-heptanoic acid-L-Thr(OBn)-D-Ser(OBn)-L-Ser(OBn)-(3*S*)-THQ(OBn) (**31**) (10 mg, 5.89 μmol) was dissolved in CH_3OH (400 μL), and glacial acetic acid (30 drops) was added. The reaction flask was flushed three times with Ar, and then Pd/C (10%, 8 mg) was added. The reaction flask was three times flushed with H_2, and the reaction mixture was stirred 2.5 h at rt. The reaction mixture was then filtered over celite, and the solvent was removed under reduced pressure. The residue was dissolved in cold MeOH (HCl 1%) (1 mL), and the solution stirred for 4 h at 0 °C. Then the solvent was evaporated under reduced pressure, and the residual acid was coevaporated three times with chloroform. The residue was dissolved in water and lyophilized to give anachelin H (**1**) (3.3 mg, 70%). Light yellow fluffy powder. ^1H NMR (D_2O, 300 MHz): 1.18 (d, 3H, $J = 6.2$), 1.6−1.64 (m, 1H), 1.65−1.74 (m, 1H), 1.99−2.08 (m, 1H), 2.27−2.35 (m, 1H), 2.89−3.04 (m, 1H), 3.05−3.15 (m, 1H), 3.36−3.44 (m, 1H), 3.53 (s, 6H), 3.66−4.1 (m, 7H), 4.1−4.17 (m, 2H), 4.2−4.33 (m, 2H), 4.38−4.45 (m, 3H), 4.53−4.7(m, 1H), 6.75 (s, 1H), 6.96−7.07 (m, 3H), 7.42−7.53 (m, 1H), 7.79 (d, 1H, *J* = 7.5). MS 779 (100, $[M]^+$). HRMS calcd for $C_{35}H_{51}N_6O_{14}$ $(M)^+$, 779.3463; found, 779.3471.

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Supporting Information Available: Experimental procedures, characterization data, and copies of spectra are provided as Supporting Information. This material is available free of charge via the Internet at http://pubs.acs.org.

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