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Synthesis and Evaluation of the *Bis-Nor*-Anachelin Chromophore as Potential Cyanobacterial Ligand

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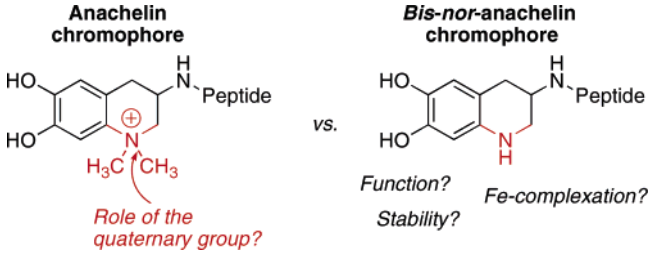

The cyanobacterial metabolite anachelin, postulated to serve as a biological ligand to Fe (siderophore), is composed of a fascinating blend of polyketide, peptide, and alkaloid building blocks. In particular, the latter consists of a *N*,*N*-dimethyltetrahydroquinolinium fragment, of which the biosynthesis is unknown. To investigate the role of this permanently positively charged fragment, we developed a synthesis of both the anachelin chromophore and its *bis-nor* derivative lacking the *N*,*N*-dimethyl groups starting from suitably protected nitro-DOPA in six and five steps, respectively, and in 50−64% overall yield. Both compounds were then compared for their chemical behavior toward oxidation. It was found that the *bis-nor*-anachelin chromophore is readily oxidized in solution in the presence of air, with a clear dependence of the rate of oxidation on the pH value. In addition, we could demonstrate that the enzyme tyrosinase, postulated to serve as key catechol oxidase in the biosynthesis of anachelin, also oxidized the *bis-nor*-hydroquinonamine derivative. Last, Fe(III) was shown to be an effective oxidant for the *bis-nor*-anachelin chromophore, resulting in all cases in the corresponding aminoquinone. In stark contrast, the anachelin chromophore resisted oxidation under various conditions surveyed (i.e., mediated by air, by tyrosinase, and by Fe(III)). In particular, Fe(III) was readily complexed by the anachelin chromophore, and the resulting complexes were characterized. In conclusion, these experiments demonstrate that the *bis-nor*-anachelin chromophore is unlikely to serve as cyanobacterial ligand, due to its instability toward oxidation. Moreover, the permanent quaternary ammonium group in anachelin renders the alkaloid chromophore much more stable against oxidation and thus results in its use as ligand for Fe(III).

Introduction

The complex secondary metabolite anachelin H (**1**) and related compounds were recently isolated from the cyanobacterium *Anabaena cylindrica* and postulated to serve as ligands for iron (siderophores) mediating iron uptake (Figure 1).[1,2] We have performed investigations on the solution structure of anachelin[3] and its biogenesis,[4]

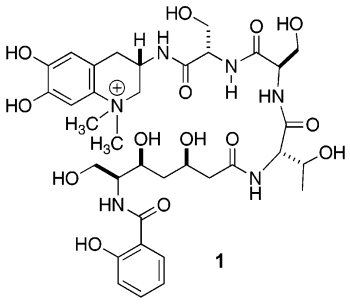

FIGURE 1. Anachelin H (**1**), a complex secondary metabolite isolated from *Anabaena cylindrica*.

and we also developed a biomimetic route to anachelin H,[5] which resulted in the total synthesis of this complex secondary metabolite.[6]

(1) (a) Beiderbeck, H.; Taraz, K.; Budzikiewicz, H.; Walsby, A. E. *Z. Naturforsch.* **2000**, *55c*, 681−687. (b) Beiderbeck, H. Ph.D. Dissertation, Universität zu Köln, Köln, Germany, 2000. (c) This compound was probably isolated more than 30 years ago, but its constitution could not be elucidated. See: Walsby, A. E. *Br. Phycol. J.* **1974**, *9*, 383−391.

(2) (a) Itou, Y.; Okada, S.; Murakami, M. *Tetrahedron* **2001**, *57*, 9093−9099. (b) Ito, Y.; Ishida, K.; Okada, S.; Murakami, M. *Tetrahedron* **2004**, *60*, 9075−9080.

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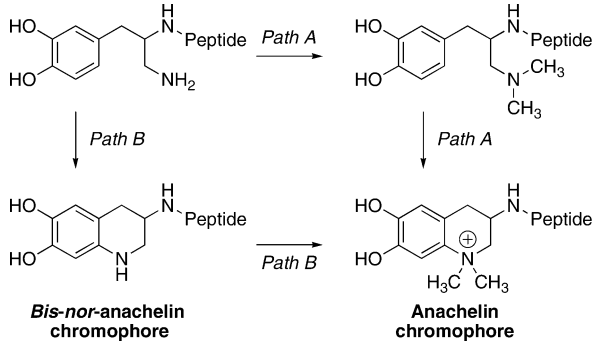
SCHEME 1. Possible Biosynthetic Pathways for the Formation of the Anachelin Chromophore

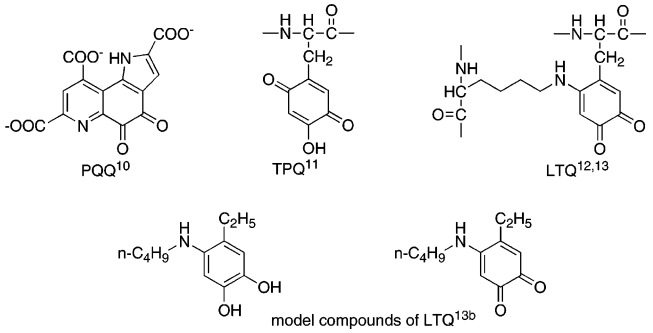


The structure of anachelin is characterized by a fascinating blend of polyketide, peptide, and alkaloid building blocks. In particular, the latter fragment containing the aminotetrahydroquinolinium ring system is unique among known natural products according to a recent literature search.[7] In general, tetrahydroquinoline rings are much less abundant compared to their isoquinoline counterparts, and also the aromatic quaternary ammonium group is highly uncommon. Therefore, this structural motif of the anachelin chromophore might point to an interesting biogenetic mechanism, which, however, remains to be elucidated.

We proposed a possible pathway of the formation of this unique chromophore via an oxidative aza annulation of a dihydroxyphenylalanine (or tyrosine)-derived dimethylated precursor (Path A, Scheme 1).[4,5] This diaminocatechol would be oxidized to the *o*-quinone and subsequently cyclized via an intramolecular 1,4-addition. On the basis of this proposal, our biomimetic synthetic route to anachelin was executed featuring a tyrosinase-catalyzed[4,5] or Te-mediated[5,6] oxidative aza annulation reaction as key step. However, a similar pathway via the *bis-nor*-anachelin chromophore and subsequent methylation also appear mechanistically feasible (Path B, Scheme 1). A shortcoming of our biomimetic route is that the corresponding compounds lacking the quaternary N-atom are synthetically not accessible.[5,6]

With regard to further elucidating the biosynthesis using feeding experiments, a synthetic route to prepare the corresponding *N*,*N*-*bis-nor* compound lacking the two methyl groups on the N-atom of the chromophore would be desirable. In addition, the question arises why anachelin H (**1**) possesses a *quaternary* alkaloid fragment and not a "neutral" fragment such as the *bis-nor*-anachelin chromophore. This question is supported by the notion that aminohydroquinones related to the *bis-nor*-anachelin

SCHEME 2. Lysine Tyrosyl Quinone Cofactors and Some Model Compounds



chromophore are found in nature and used in so-called quinoproteins as cofactors.[8−11] For example, both pyrroloquinoline quinone (PQQ) and 2,4,5-trihydroxyphenylalanine quinone (TPQ) represent the two major classes of quinonoid cofactors, covalently or ionically bound to the protein (Scheme 2). In particular, lysine tyrosyl quinone (LTQ), structurally related to the *bis-nor*-anachelin chromophore, has been thoroughly characterized both in model systems and in proteins.[12,13]

For all these reasons, we wanted to develop a synthetic route to both the anachelin chromophore as well as its *bis-nor* derivative. This would allow for the comparison of the chemical properties of both target compounds, in particular with respect to their stability toward oxidation and to their iron-chelating properties.

Results and Discussion

Synthesis. Our synthesis first targeted the known[14] O-protected amino tetrahydroquinoline **7** as the advanced intermediate of which both target compounds could be prepared (Scheme 3). In contrast to the route by Blank and co-workers,[14] we followed a modified procedure of Miller and co-workers[15] and started from a suitably protected nitro-DOPA derivative **4**, which is readily available.[15] This compound was reduced using iron powder in acetic acid and gave the resulting lactam **5** in high yield on a 10-g scale.

Direct treatment of **5** with borane resulted in the undesired reduction of the carbamate group to the corresponding *N*-Me derivative. Therefore, the Alloc group was first removed using catalytic amounts of Pd(0) to give the amino lactam **6**.[16] The tetrahydroquinoline derivative **7** was then obtained after subsequent reduc-

(3) Gademann, K.; Budzikiewicz, H. *Chimia* **2004**, *58*, 212−214.
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(5) Gademann, K.; Bethuel, Y. *Angew. Chem.* **2004**, *116*, 3389−3391; *Angew. Chem., Int. Ed.* **2004**, *43*, 3327−3329.
(6) Gademann, K.; Bethuel, Y. *Org. Lett.* **2004**, *6*, 4707−4710.
(7) Structurally related are the pyoverdine, azobactin, and pseudobactin chromophores. For a review of peptide-derived siderophores, see: (a) Drechsel, H.; Jung, G. *J. Pept. Sci.* **1998**, *4*, 147−181. For examples of pyoverdine review: (b) Budzikiewicz, H. *Prog. Chem. Org. Nat. Prod.* **2004**, *87*, 81−237. (c) Pyoverdine isolation: Teintze, M.; Hossain, M. B.; Barnes, C. L.; Leong, J.; Van der Helm, D. *Biochemistry* **1981**, *20*, 6446−6457. (d) Partial synthesis: Kinzel, O.; Budzikiewicz, H. *J. Pept. Res.* **1999**, *6*, 618−625 and also ref 15. (e) Biogenetic investigations: Dorrestein, P. C.; Poole, K.; Begley, T. P. *Org. Lett.* **2003**, *5*, 2215−2217.
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(9) Mure, M. *Acc. Chem. Res.* **2004**, *37*, 131−139.
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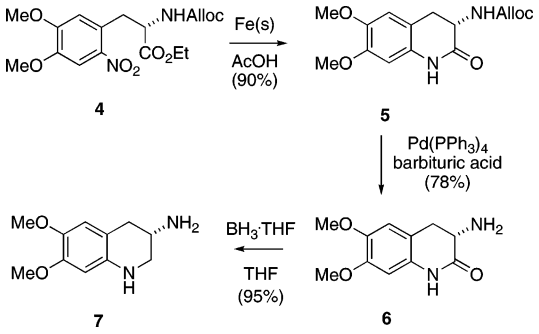
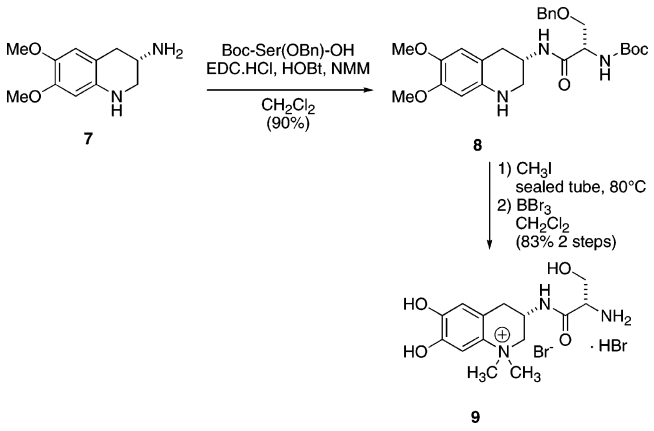
(12) Wang, S. X.; Mure, M.; Medzihradszky, K. F.; Burlingame, A. L.; Brown, D. E.; Dooley, D. M.; Smith, A. J.; Kagan, H. M.; Klinman, J. P. *Science* **1996**, *273*, 1078−1084.
(13) (a) Ling, K.-Q.; Kim, J.; Sayre, L. M. *J. Am. Chem. Soc.* **2001**, *123*, 9606−9611. (b) Mure, M.; Wang, S. X.; Klinman, J. P. *J. Am. Chem. Soc.* **2003**, *125*, 6113−6125. (c) Wang, S. X.; Nakamura, N.; Mure, M.; Klinman, J. P.; Sanders-Loehr, J. *J. Biol. Chem.* **1997**, *272*, 28841−28844.
(14) Lewis, R. J.; Francis, C. A.; Lehr, R. E.; Blank, C. L. *Tetrahedron* **2000**, *56*, 5345−5352.
(15) Kolasa, T.; Miller, M. J. *J. Org. Chem.* **1990**, *55*, 4246−4255.

SCHEME 3. Preparation of 3-Amino-tetrahydroquinoline 7



SCHEME 4. Preparation of the Anachelin Chromophore 9

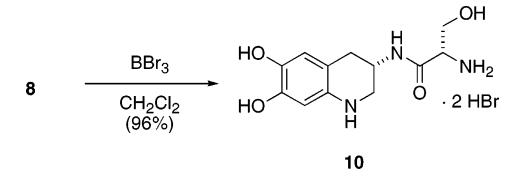


tion with borane tetrahydrofuran complex at elevated temperatures.

Differentiation of the amino groups in **7** proved to be the key sequence of the synthesis of the anachelin chromophore derivative **9** (Scheme 4). While we first tried to protect the primary amine as Schiff base followed by quarternization, these efforts were rendered fruitless as such functionalities were found to be incompatible with the conditions of N-methylation. Next, we tried protection of the exocylic amine with the standard carbamate-protecting groups followed by N-methylation, but witnessed only decomposition. Last, we devised to protect the primary amine as amide by using an amino acid. Therefore, the amino tetrahydroquinoline **7** was coupled to *tert*-butoxycarbonyl (Boc)-Ser(OBn)-OH (ethyl-(*N*, *N*-dimethylaminopropyl)carbodiimide, EDC, 1-hydroxybenzotriazole, HOBt) to give the tetrahydroquinoline-serine derivative **8** (Scheme 4). To carry out the desired quarternization on the tetrahydroquinoline N-atom, several conditions were evaluated. The optimal conditions were found to be heating compound **8** in neat CH_3I in a sealed tube at 80 °C overnight. The addition of cosolvents and/or bases all resulted in lower yields; other methylating agents were also found to be ineffective.

It is interesting to note that under these conditions (neat CH_3I, 80 °C) partial cleavage of the Boc group was observed, without any methylation of the primary amine.[17]

SCHEME 5. Preparation of *Bis-Nor*-Anachelin Chromophore 10



We decided to deprotect the OMe groups using standard BBr_3 solution, which proceeded smoothly to the deprotected anachelin chromophore **9** as the dihydrobromide salt in 83% yield.

The route described herein also provides a rapid synthetic access to the *bis-nor*-anachelin chromophore. Therefore, compound **8** was smoothly deprotected using BBr_3 to give the *N*,*N*-*bis-nor* derivative **10** as dihydrobromide salt in very good yield (Scheme 5).

Evaluation of the Chemical Stability toward Oxidation. Having both the *bis-nor* compound **10** and the anachelin chromophore **9** at hand, we first examined the chemical stability of these compounds toward oxidation. This is of particular interest, as the hydroquinonamine **10** resembles eukaryotic cofactors such as the LTQ of lysyl oxidase, which mediates redox processes.[13] With respect to the oxidation potential of **9** and **10**, the main structural difference resides in the electron density of the aromatic ring. Whereas ammonium **9** is substituted with an electron-withdrawing group, the donor substituent (the secondary amine) renders **10** more electron-rich and thus, in principle, easier to oxidize. Consequently, we decided to examine the rate of oxidation of compound **10** at different pH values using UV−vis spectroscopy.

The rate of air oxidation of the hydroquinonamine **10** in 50 mM phosphate buffer solution under physiologically relevant conditions (pH = 6.5) is shown in Figure 2. Before addition of air, the spectrum displayed a single absorption band with a λ_{max} at 288 nm. In the course of the oxidation reaction, a broad absorption band with a λ_{max} at 520 nm and a sharp band with a λ_{max} at 275 nm appeared. After 15 min, complete oxidation was observed, and the resulting line shape of the *o*-quinone was in very good agreement with those observed for lysine tyrosyl quinone cofactors and related model systems.[13] Moreover, the presence of signals in the NMR spectrum of the lyophilized reaction mixture supports these UV spectroscopic observations. Even though the NMR spectrum showed a mixture of more than one product, which can be explained by tautomerization of the quinone (Scheme 6) and further decomposition, the characteristic signals of the amino quinone could be observed in the ^1H NMR spectrum. In particular, the peaks at 6.36 and 6.68 ppm are distinctive for aromatic *o*-quinone protons and are in good agreement to values reported in the literature for similar compounds.[13] The other signals may be related to polymerization as described for compounds such as actinomycin[18] or melanin.[19] Finally, mass spectroscopy

(16) (a) Racemic: Bowman, R. E.; Islip, P. J.; Lockhart, I. M.; Richards, K. E.; Wright, M. *J. Chem. Soc.* **1965**, 1080−1087. (b) Enantiomerically pure: Santangelo, F.; Casagrande, C.; Miragoli, G.; Vecchietti, V. *Eur. J. Med. Chem.* **1994**, *29*, 877−882.

(17) Upon searching the Beilstein and SciFinder Scholar databases, we did not find any example of the use of CH_3I to cleave Boc groups. However, it is well-known that a Si counterpart, iodotrimethylsilane, is able to cleave Boc groups under mild conditions. Review: Olah, G. A.; Narang, S. C. *Tetrahedron* **1982**, *38*, 2225−2277.

(18) (a) Meienhofer, J. *J. Am. Chem. Soc*. **1970**, *92*, 3771−3777. (b) Mauger, A. B.; Gallagher, K. S.; Silverton, J. V.; Ferretti, J. A. *Biopolymers* **1989**, *28*, 1771−1780.

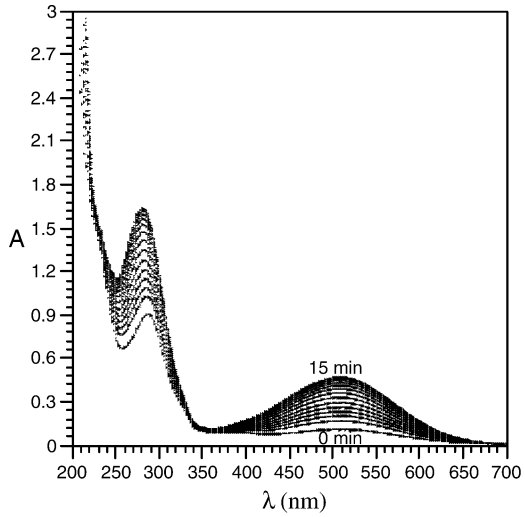
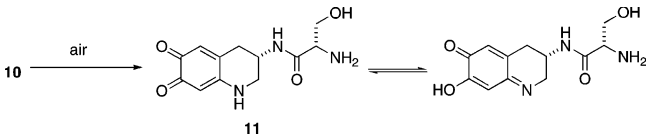


FIGURE 2. Air oxidation of compound **10** (0.15 mM) at pH = 6.5 in degassed phosphate buffer (50 mM). UV−vis spectra were measured every 30 s, and air was added between each measurement.

SCHEME 6. Possible Pathways for the Oxidation of the Hydroquinonamine 10 in the Presence of Air to the Aminoquinone 11 and a Possible Tautomer



(ESI, positive mode) confirmed the presence of the oxidized compound **11** in the reaction mixture.

This oxidation process of the hydroquinonamine **10** is accelerated in basic medium (pH = 9.2), as the reaction is complete within 5 min as judged by UV−vis spectroscopy (data in Supporting Information). This behavior is in accordance to the well-known instability of catechols and dopamines in basic medium toward oxidation.[20]

Complementary to these observations, the oxidation at pH = 4 (acetate buffer) by air is much slower, and complete conversion was only observed after 120 min (Figure 3). This could be explained by protonation of the endocyclic amino group (p*K*a = 3.75 of the corresponding acid) at such acidic pH values and, consequently, a decreased oxidation potential of the aromatic ring.

There is a clear dependence of the rate of oxidation on the pH value: as the reaction is accelerated the more basic the aqueous medium becomes. It is also important to note that the band at 500 nm displays no spectral differences over the range of pH. This implies that the same oxidized *o*-quinone product was formed at every pH value examined (data in Supporting Information). Moreover, the red shift observed with increasing pH for the band at 280 nm might be explained by the different population of tautomers with varying pH values. For example, as the secondary amine (pK_a = 3.75) is protonated at low pH values, the deprotonated catecholate is probably the stable species at high pH values, and therefore, the pH-dependent red shift observed can be correlated to the protonation state of the molecule. All these observations support the existence of an air oxidation process of the fragment **10** as shown in Scheme 6.

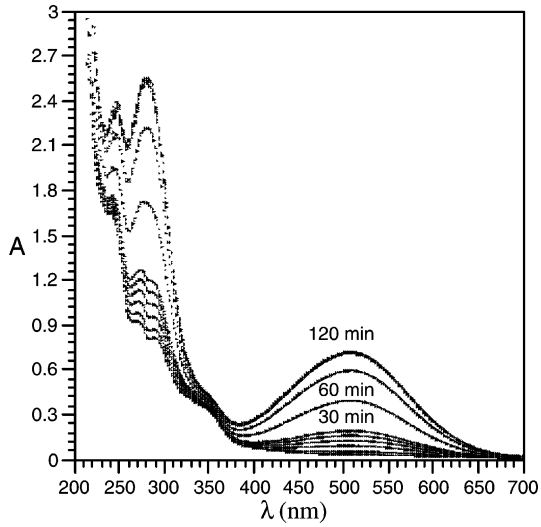


FIGURE 3. Air oxidation of compound **10** (0.15 mM) in degassed acetate buffer (40 mM, pH = 4). UV−vis spectra were taken every 30 s until 15 min, then at 30, 60, and 120 min. Air (2 mL) was bubbled through the solution between each measurement via syringe.

In addition to chemical oxidation by air, we evaluated whether the *bis-nor*-anachelin chromophore could also be oxidized by an enzyme. Catechol oxidases (such as tyrosinases) are known to oxidize catechols to quinones,[21] and this class of enzymes has been shown to catalyze the Path A shown in Scheme 1.[4,5] We found that the *bis-nor* compound **10** was readily oxidized by a catechol oxidase at pH = 6.5 accelerated by 1 order of magnitude related to the background rate (data in Supporting Information).

It is well-known that Fe(III) salts are able to oxidize catechols to the corresponding quinones.[22] Therefore, we thought about introducing Fe(III) (0.14 mM $Fe(NO_3)_3$) into the buffer medium at pH = 4 to examine its influence on the rate of oxidation. As expected, the reaction was much faster, and already within the first minute complete conversion was observed. The UV−vis spectrum obtained was superimposable to the one that resulted from the iron-free oxidation reaction. To distinguish between a hypothetical Fe catechol complex and the quinone product, which could in principle display similar spectral characteristics, EDTA was added to the Fe-containing reaction mixture. No change in either the color or the UV spectrum was observed, which supported the notion that indeed complete oxidation of the catechol to the quinone mediated by Fe(III) was observed. The oxidation by iron was possible because of the similarity of the reduction potentials of Fe^{3+}/Fe^{2+} and quinone/catechol, 0.749 and 0.792 V, respectively.[23] According to

(19) Katritzky, A. R.; Akhmedov, N. G.; Denisenko, S. N.; Denisko, O. V. *Pigm. Cell Res*. **2002**, *15*, 93−97.
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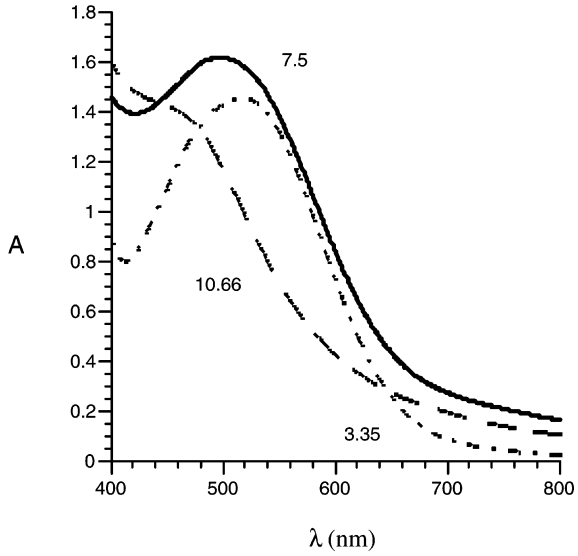

FIGURE 4. UV spectra of oxidized **10** at different pH values in the presence of $Fe(NO_3)_3$.

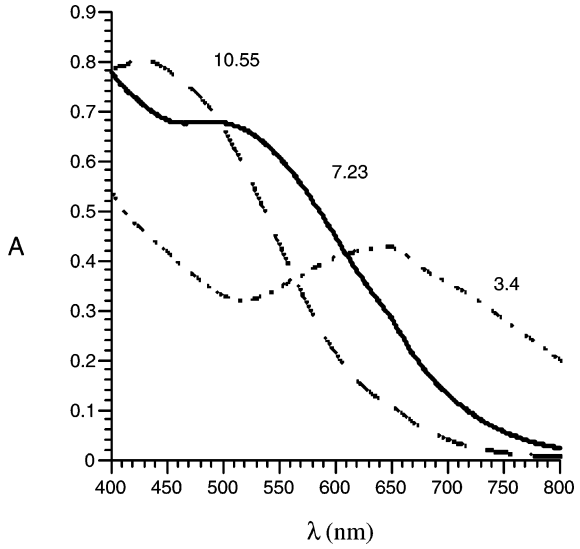

FIGURE 5. UV−vis spectra of the anachelin chromophore **9** at different pH values in the presence of $Fe(NO_3)_3$.

literature precedents, the iron catechol complex is rapidly formed which initiates the intramolecular reduction/oxidation process, and the resulting monodentate complex underwent internal electron transfer.[24]

To evaluate the oxidation behavior of the *bis-nor*-anachelin chromophore at different pH values, we consequently titrated compound **10** in the presence of $Fe(NO_3)_3$ at different pH values (Figure 4). We observed only a small difference between the two spectra at pH 3.35 and 7.5, which is in agreement with the broad oxidation band previously observed and with the literature data.[25] In the whole pH range studied, the absorbance monitored remained identical, supporting the presence of quinones that are not able to bind to iron. At pH 10.66, the maximum is more shifted to lower wavelength (λ_{max} = 450 nm), which may indicate further decomposition into melanin-type polymers.[26]

From all these experiments, it could be demonstrated that the *bis-nor*-anachelin chromophore **10** is not stable in the presence of various oxidizing agents. Even air quickly oxidized the aminohydroquinone **10** in aqueous solution with complete conversion observed ranging from 5 to 120 min. Enzymes such as catechol oxidases also oxidized compound **10** within minutes. Last, it could be shown that the *bis-nor* chromophore **10** is not a ligand to Fe(III), but is again oxidized instantaneously in the presence of this metal.

The anachelin chromophore derivative **9** was studied next. This compound exhibited completely different properties compared to the *bis-nor* chromophore **10**. It was fascinating to see that under all conditions evaluated no oxidation of the quarternary hydroquinone took place. The compound is stable exposed to air and light in both the solid state and solution over several weeks. Moreover, it could be shown that the anachelin chromophore **9** is not a substrate for enzymes such as catechol oxidases and thus resists enzymatic oxidation. In addition, under strong ionizing conditions as in the course of mass spectroscopic measurements, the quaternary compound **9** resisted oxidation. Last, addition of Fe(III) (0.12 mM $Fe(NO_3)_3$) to a solution of the anachelin chromophore resulted in complex formation and not in oxidation, as different complexes were reversibly formed upon change of pH. Titration of Fe(III)·**9** reveals a similar trend as was observed for other catechol-derived Fe complexes[27] (Figure 5). At low pH, a ligand−metal charge transfer (LMCT) band at 650 nm is observed, which results from a mixture of 1:1 and 1:2 Fe/ligand complexes and in a strong dark blue color. The lower absorbance can be explained by the smaller molar extinction of the 1:1 complex, as was shown for related complexes in the literature.[28] Upon increasing the pH value to physiologically relevant conditions, the 1:3 Fe/ligand complex is observed as evidenced by an LMCT band at 500 nm. Last, at pH > 10, hydrolysis of the FeL_3 complex occurs.

It is interesting to note that the introduction of a quaternary ammonium group completely changes the chemical behavior, whereas *bis-nor* derivative **10** is unstable and oxidized under a variety of conditions, and the anachelin chromophore derivative **9** is stable toward oxidation and can thus serve as a ligand for iron.

Conclusion

We have developed a rapid and high-yielding synthetic route to the *bis-nor*-anachelin derivative **10** (five steps, 64% overall) and the anachelin chromophore derivative **9** (six steps, 50% overall). Characterization of these compounds allowed for the evaluation whether the *bis-nor*-anachelin chromophore **10** constitutes a viable al-

(24) Jameson, G. N. L.; Linert, W. *J. Chem. Soc., Perkin Trans. 2* **2001**, 569−575.
(25) Lee, B. P.; Dalsin, J. L.; Messersmith, P. B. *Polym. Prepr. (Am. Chem. Soc., Div. Polym. Chem.)* **2001**, *42*, 151.
(26) Di, J.; Bi, S. *Anal. Sci.* **2004**, *20*, 629−634.
(27) For example, for (a) Fe/catechol, see: Avdeef, A.; Sofen, S. R.; Bregante, T. L.; Raymond, K. N. *J. Am. Chem. Soc.* **1978**, *100*, 5362−5370. (b) Fe/β-DOPA, see: Gerard, C.; Chehhal, H.; Hugel, R. P. *Polyhedron* **1994**, *13*, 591−597. (c) Fe/DOPA, see: von Nussbaum, F.; Spiteller, P.; Rüth, M.; Steglich, W.; Wanner, G.; Gamblin, B.; Stievano, L.; Wagner, F. E. *Angew. Chem., Int. Ed.* **1998**, *37*, 3293−3295.
(28) Kipton, H.; Powell, J.; Taylor, M. C. *Aust. J. Chem.* **1982**, *35*, 739−756.

ternative to the parent, quaternized structure **9**. It is fascinating to see how sensitive the *bis-nor*-anachelin chromophore **10** is toward oxidation. Simple oxidation of **10** by air is pH-dependent and a fast process ranging from 5 min at pH = 9 to 15 min at pH = 6.5 until 120 min at pH = 4. In addition, the *bis-nor* derivative **10** is instantaneously oxidized by Fe(III) in solution as well as enzymatically with a catechol oxidase under physiologically relevant conditions. In stark contrast, the anachelin chromophore **9** is resistant to oxidation in the presence of air, Fe(III) salts, as well as a catechol oxidase. In addition and again in contrast to *bis-nor* derivative **10**, the anachelin chromophore **9** can serve as ligand to iron and different complexes could be obtained and spectroscopically characterized.

We propose that this difference in stability toward oxidation is the reason why the complex secondary metabolite anachelin H (**1**) contains a quarternary tetrahydroquinolinium chromophore. In addition, the experiments suggest that a pathway in the biosynthesis of anachelin is more likely to furnish directly the anachelin chromophore (Path A in Scheme 1), thus avoiding an unstable aminoquinone of type **10**. Nonetheless, however, it might be possible that such aminoquinones could be biosynthetically available to cyanobacteria, and it is tempting to speculate whether these prokaryotes are able to use related intermediates of **10** as cofactors in amine oxidase enzymes.

Experimental Section

General. The preparation of nitro-DOPA derivative **4** following a modified procedure of Kolosa and Miller[15] is reported in the Supporting Information. Ether refers to diethyl ether.

Reactions were run under an atmosphere of Ar in dry glassware (at least 24 h in an oven at 140 °C, followed by heating with a heat gun under high vacuum). Analytical thin layer chromatograpy (TLC) was performed on silica gel plates (0.25-mm thickness) precoated with a fluorescent indicator. The developed plates were examined under UV light and stained with ceric ammonium molybdate (CAM) stain or $KMnO_4$ stain followed by heating. Flash chromatography (FC) was performed using silica gel 60 (230−400 mesh). All ^1H and ^{13}C NMR spectra were recorded at ambient temperature, chemical shifts δ are given in ppm, and coupling constants J are in Hz. IR spectra were recorded as $CHCl_3$ solution, and absorptions are given in cm^{-1}. Optical rotations were measured using a 1-mL cell with a 1-dm path length, and the concentration c is given in g/100 mL. In mass spectroscopy, fragment ions are given in m/z with relative intensities (%) in parentheses. Nomenclature is according to Autonom 2.0, Beilstein Information Systeme GmbH.

(3*S*)-*N*-(6,7-Dimethoxy-2-oxo-1,2,3,4-tetrahydro-quinolin-3-yl)-carbamic Acid Allyl Ester (5). To a solution of **4** (3.86 g, 10 mmol) in AcOH (50 mL) at 80 °C was added iron powder (10 g, 0.179 mol). After 30 min of manual stirring the reaction was completed. The reaction mixture was filtered over Celite and poured into water. $NaHCO_3$ was added until pH = 12, and the aqueous layer was extracted 3× with AcOEt. The combined organic layers were dried over Na_2SO_4, filtered, and evaporated under reduced pressure. FC (CH_2Cl_2/MeOH 95:5) gave **5** (2.85 g, 9 mmol, 90%). White solid. mp = 138−140 °C. R_f = 0.26 (CH_2Cl_2/MeOH 96:4). $[\alpha]_D$ −20.3 (c 0.54, $CHCl_3$, T = 26 °C). ^1H NMR ($CDCl_3$, 300 MHz) 2.8 (t, 1H, J = 14.3), 3.6 (dd, 1H, J_1 = 6.2, J_2 = 14.9), 3.85 (s, 6H), 4.3−4.4 (m, 1H), 4.6 (d, 2H, J = 5.3), 5.23 (d, 1H, J = 10.34), 5.33 (d, 1H, J = 17), 5.86−6 (m, 2H), 6.3 (s, 1H), 6.7 (s, 1H), 8.2 (bs, 1H). ^{13}C NMR ($CDCl_3$, 75 MHz) 32.2, 50.5, 56.2, 56.3, 66.8, 100.3, 111.9, 114.0, 117.8, 129.1, 132.4, 145.1, 148.6, 155.8, 168.9. IR 3408*w*, 3156*w*, 2359*w*, 2253*s*, 1692m, 1464*m*, 1383*m*, 1213*s*. MS 329.1 (100, $[M + Na]^+$), 307.1 (37, $[M + H]^+$). HRMS calcd. for $C_{15}H_{18}N_2O_5Na$ $(M + Na)^+$: 329.1108, found: 329.1108.

(3*S*)-(6,7-Dimethoxy-1,2,3,4-tetrahydro-quinolyn-3-yl)-amine (6). To a solution of **5** (0.400 g, 1.31 mmol) in dry CH_2Cl_2 (100 mL) with *N*,*N*′-dimethylbarbituric acid (0.654 g, 4.19 mmol) was added $Pd(PPh_3)_4$, freshly prepared from $(Pd_2dba)_3{\cdot}CHCl_3$ (0.040 g, 0.0392 mmol, 6 mol % Pd) and PPh_3 (0.062 mg, 0.235 mmol, 18 mol %). It was stirred overnight at room temperature (RT) and then washed 3× with saturated Na_2CO_3. The organic phase was dried over Na_2SO_4, filtered, and evaporated under reduced pressure. FC (CH_2Cl_2/MeOH 95:5) gave the desired product **6** (225 mg, 1.01 mmol, 78%). R_f = 0.22 (CH_2Cl_2/MeOH 97:3). Reddish foam. $[\alpha]_D$ −102.7 (c 0.31, $CHCl_3$, T = 27 °C). ^1H NMR ($CDCl_3$, 300 MHz) 1.89 (bs, 2H), 2.81 (t, 1H, J = 15.25), 3 (dd, 1H, J_1 = 6.53, J_2 = 15.25), 3.6 (dd, 1H, J_1 = 6.53, J_2 = 13.07), 3.83 (s, 6H), 6.4 (s, 1H), 6.6 (s, 1H), 9.1 (bs, 1H). ^{13}C NMR ($CDCl_3$, 75 MHz) 34.1, 50.5, 56.2, 56.4, 100.3, 111.8, 114.0, 129.9, 144.7, 148.4, 173.4. IR 3405*m*, 3216*w*, 3024*m*, 3009*m*, 2964*m*, 2843*w*, 1682*s*, 1624m, 1522*s*. MS 245.1 (33, $[M + Na]^+$), 223.1 (100, $[M + H]^+$), 206.1 (25, $[M - NH_2]^+$). HRMS calcd. for $C_{11}H_{14}N_2O_3Na$ $(M + H)^+$: 223.1077, found: 223.1075.

(3*S*)-6,7-Dimethoxy-1,2,3,4-tetrahydro-quinolin-3-yl-amine (7). To a solution of **6** (0.270 g, 1.2 mmol) in tetrahydrofuran (THF) (5 mL) was added BH_3/THF (1M) (12 mL). It was stirred at RT for 1 h and then neutralized with HCl (6 N) until no hydrogen evolved from the solution. The solution was stirred for 30 min, and subsequently, NaOH solution (1 M) was added until pH = 14. The reaction mixture was extracted 3× with chloroform. The combined organic phases were dried over Na_2SO_4, filtered, and evaporated under reduced pressure. FC (CH_2Cl_2/MeOH 90:10) gave **7** (240 mg, 1.15 mmol, 95%). White solid. mp = 118−120 °C. R_f = 0.22 (CH_2Cl_2/MeOH 90:10). $[\alpha]_D$ +20.7 (c 0.155, $CHCl_3$, T = 25 °C). ^1H NMR ($CDCl_3$, 300 MHz) 2.5 (dd, 1H, J_1 = 6.2, J_2 = 16.2), 2.94 (dd, 1H, J_1 = 4.7, J_2 = 15.5), 3.3 (ddd, 1H, J_1 = 1.24, J_2 = 6.53, J_3 = 7.78), 3.25−3.27 (m, 2H), 3.78 (s, 6H), 6.1 (s, 1H), 6.5 (s, 1H). ^{13}C NMR ($CDCl_3$, 75 MHz) 35.8, 44.7, 48.9, 55.8, 56.6, 99.3, 110.2, 114.1, 137.4, 141.5, 148.2. IR 3690*w*, 3028*w*, 2956*w*, 2838*w*, 2360*s*, 1602*w*, 1518*s*, 1485*m*, 1464*m*, 1228*s*. MS 208.1 (100, $[M + H]^+$), 192.1 (68, $[M - NH_2]^+$). HRMS calcd. for $C_{11}H_{16}N_2O_2$ $(M + H)^+$: 208.1206, found: 208.1212. Anal. Calcd. for $C_{11}H_{16}N_2O_2$ (208.12): C 59.45, H 6.35, N 12.6; found: C 59.64, H 6.38, N 12.41.

(1*S*)-[2-Benzyloxy-1-(3*S*)-(−6,7-dimethoxy-1,2,3,4-tetrahydro-quinolin-3-ylcarbamoyl)-ethyl]-carbamic Acid *tert*-Butyl Ester (8). To a solution of Boc-Ser(OBn)-OH (0.135 g, 0.65 mmol) in dry CH_2Cl_2 was added EDC * HCl (0.137 g, 0.71 mmol), HOBt (0.097 g, 0.71 mmol), and *N*-methylmorpholine (0.197 g, 1.9 mmol). Then compound **7** (0.210 g, 0.71 mmol) was added, and the mixture was stirred at RT overnight. The solvent was evaporated, and the residue was purified by FC (CH_2Cl_2/MeOH 99:1) to give **8** (379 mg, 0.78 mmol, 90%). White solid. mp = 58−60 °C. R_f = 0.7 (CH_2Cl_2/MeOH 90:10). $[\alpha]_D$ −4.73 (c 0.945, $CHCl_3$, T = 29 °C). ^1H NMR ($CDCl_3$, 300 MHz) 1.39 (s, 9H), 2.6 (d, 1H, J = 16.5), 2.98 (dd, 1H, J_1 = 4.9, J_2 = 16.5), 3.1 (ddd, 1H, J_1 = 1.87, J_2 = 4.36, J_3 = 11.52), 3.23 (d, 1H, J = 11.21), 3.56 (dd, 1H, J_1 = 6.5, J_2 = 9.3), 3.75 (s, 3H), 3.77 (s, 3H), 3.84−3.88 (m, 1H), 4.19 (bs, 1H), 4.39−4.52 (m, 3H), 5.4 (bs, 1H), 6.04 (s, 1H), 6.45 (s, 1H), 6.83 (d, 1H, J = 8.1), 7.2−7.34 (m, 5H). ^{13}C NMR ($CDCl_3$, 125 MHz) 28.3, 32.1, 42.4, 45.4, 53.9, 55.8, 56.5, 70.1, 73.4, 80.0, 99.4, 109.5, 113.9, 127.5, 127.7, 128.2, 137.1, 137.4, 141.8, 148.2, 155.2, 169.6. IR 3419*m*, 3021*m*, 2936*m*, 2848*w*, 1710*m*, 1669*m*, 1517*s*, 1486*s*. MS 508 (76, $[M + Na]^+$), 485 (25, $[M]^+$), 430.2 (57, $[M - Boc + H]^+$), 408 (68, $[M - Boc + Na]^+$), 386

(29) Still, W. C.; Kahn, M.; Mitra, A. *J. Org. Chem.* **1978**, *43*, 2923−2925.

(100, $[M - Boc]^+$). HRMS calcd. for $C_{26}H_{35}N_3O_6$ $(M + Na)^+$: 508.2418, found: 508.2407.

(3*S*)-(3-(2*S*)-(2-Amino-3-hydroxy-propionylamino)-6,7-dihydroxy-1,1-dimethyl-1,2,3,4-tetrahydro-quinolinium Bromide (9). Compound **8** (136 mg, 0.28 mmol) was dissolved in MeI (342 mg, 2.4 mmol), and the solution was heated in a sealed tube overnight at 80 °C. Then MeI was removed with a flow of argon, and it was suspended in dry CH_2Cl_2. BBr_3/CH_2Cl_2 (1 M, 2.8 mmol) was added, and the reaction mixture was stirred at RT for 3 h. The excess of BBr_3 was hydrolyzed with MeOH (10 mL), and the solution was poured into Et_2O (100 mL). The precipitate was filtered to give **9** (105 mg, 0.23 mmol, 83%). Off-white foam. $R_f = 0.03$ ($CHCl_3$/MeOH/H_2O 10:6:1). ^1H NMR (CD_3OD, 300 MHz) 3.0 (dd, 1H, $J_1 = 10.4$, $J_2 = 15.8$), 3.1 (dd, 1H, $J_1 = 4.1$, $J_2 = 16.1$), 3.3 (s, 3H), 3.61 (s, 3H), 3.65 (s, 3H), 3.66−3.73 (m, 1H), 3.87−4 (m, 3H), 4.05 (m, 1H), 4.6−4.75 (m, 1H), 6.7 (s, 1H), 7.2 (s, 1H). MS 296.2 (100, $[M]^+$). HRMS calcd. for $C_{14}H_{22}N_3O_4$ $(M)^+$: 296.1605, found: 296.1604. UV [$\epsilon = 684$ ($\lambda = 395$ nm, H_2O, pH = 1.3)].

(2*S*)-2-Amino-*N*-[(3S)-6,7-dihydroxy-1,2,3,4-tetrahydro-quinolin-3-yl]-3-hydroxypropionamide (10). Compound **8** (19 mg, 39.1 μmol) was dissolved in dry CH_2Cl_2 (0.3 mL), and BBr_3 (1 M in CH_2Cl_2) (3.91 mL) was added dropwise. After 1.5 h at RT, the excess BBr_3 was hydrolyzed with MeOH (0.5 mL), and a large excess of Et_2O (4 mL) was added so that the expected product precipitated. The suspension was stirred for 20 min and filtered. The residue was washed with Et_2O and dried under high vacuum to give **10** (13 mg, 37.5 μmol, 96%). Off-white foam. $R_f = 0.15$ ($CHCl_3$/MeOH/H_2O 10:6:1). $[\alpha]_D$ −1.53 (*c* 0.125, MeOH, $T = 25$ °C). ^1H NMR (CD_3OD, 300 MHz) 2.85 (dd, 1H, $J_1 = 9.03$, $J_2 = 16.5$), 3.08 (dd, $J_1 = 5.92$, $J_2 = 16.5$), 3.37 (dd, 1H, $J_1 = 9.3$, $J_2 = 11.83$), 3.62 (dd, 1H, $J_1 = 2.49$, $J_2 = 11.83$), 3.90 (dd, 1H, $J_1 = 5.92$, $J_2 = 11.52$), 3.94 (d, 1H, $J = 4.05$), 4.01 (m, 1H), 4.42 (m, 1H), 6.70 (s, 1H), 6.72 (s, 1H). IR 3600−2200 br·*m*, 1677*m*, 1531*s*, 1456*s*. MS 268.2 $(M + H)^+$. UV ([$\epsilon = 1032$ ($\lambda = 520$ nm, H_2O, pH = 1.3)].

Oxidation of the *Bis-Nor*-Anachelin Chromophore with Air. Three buffer solutions were prepared: 40 mM acetate buffer at pH = 4, 50 mM phosphate buffer at pH = 6.5, and 100 mM carbonate buffer at pH = 9.2. All buffers were degassed during 2 h with Ar prior to the experiments. The *bis-nor*-anachelin chromophore **10** (0.2 mg, ca. 0.45 μM) was dissolved in each buffer solution (3 mL). UV−vis spectra of the resulting solutions (0.15 mM) were taken every 30 s until complete oxidation persisted. Air was bubbled between each measurement through the solution. The initially light purple solution turned dark purple over time. The resulting graphs are shown in Figures 1, 2, and 5 (Supporting Information).

Oxidation of the *Bis-Nor*-Anachelin Chromophore with Fe(III). The acetate buffer solution (40 mM, pH = 4) was previously degassed with Ar. Then a solution of $Fe(NO_3)_3$ (0.14 mM) was prepared, and the *bis-nor*-anachelin chromophore **10** (0.2 mg, ca. 0.45 μM) was dissolved. UV−vis spectra of the solution (0.15 mM) were taken every 30 s until oxidation was complete. The data are shown in the Supporting Information. Air was bubbled between each measurement through the solution. The solution turned almost immediately dark purple. Once the oxidation completed, EDTA was added and the reaction was followed by UV−vis spectroscopy. No change in the spectrum was observed. In a similar experiment, to an aqueous solution of the *bis-nor*-anachelin chromophore (7 mL, 0.7mM) was added KNO_3 (36 mM) as an electrolyte and $Fe(NO_3)_3{\cdot}9H_2O$ (0.18 mM in Fe). This solution was first acidified with HNO_3 (6 M) until a pH value of ca. 1.5 was obtained and then titrated with a solution of NaOH (0.05 M). The resulting UV−vis spectra are shown in Figure 3.

Generation of [Fe(Anachelin Chromophore)] (*n* = 1−3) Complexes. To an aqueous solution of the anachelin chromophore **9** (7 mL, 0.5 mM) was added KNO_3 (36 mM) as an electrolyte and $Fe(NO_3)_3{\cdot}9\ H_2O$ (0.12 mM in Fe). This solution was first acidified with HNO_3 (6 M) until a pH around 1.5 was obtained and then titrated with a solution of NaOH (0.05 M). The color of the solution turned from light green (pH = 1.29), to dark green (pH = 3.39), brown (pH = 7.26), and finally orange (pH = 11−12). At the same time, a spectrophotometric titration was carried out by measuring UV spectra at different pH values with the same solutions. The resulting data is shown in Figure 4.

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Supporting Information Available: General methods, general experimental details for compound **4**, ^1H spectra of compounds **4**−**10**, MS spectrum of compound **10**, UV−vis spectra of the air oxidation of compound **10** at pH = 9.2, UV−vis spectra of the oxidized form of **10** at pH = 4, 6.5, 9.2, kinetic data of the mushroom tyrosinase oxidation of **10**, and kinetic data of the oxidation of **10** by $Fe(NO_3)_3$. This material is available free of charge via the Internet at http://pubs.acs.org.

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