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Synthesis of the Pyoverdin Chromophore by a Biomimetic Oxidative Cyclization

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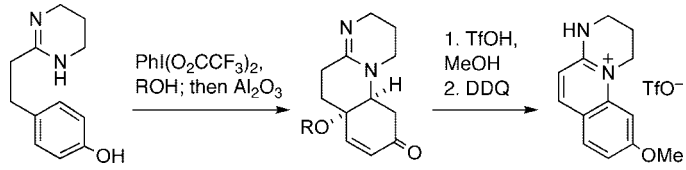
ABSTRACT



The fluorescent dihydropyrimido[1,2-*a*]quinoline chromophore of the pyoverdin siderophores has been synthesized by a biomimetic oxidative cyclization using an iodine(III) reagent, followed by elimination and dehydrogenation.

The pyoverdins are siderophores secreted by fluorescent *Pseudomonas* sp. including the pathogenic *P. aeruginosa* under conditions of iron starvation.[1] Structurally they comprise a species-specific peptide of 6−19 residues containing D-amino acids and hydroxamates and a characteristic 2,3-dihydro-1*H*-pyrimido[1,2-*a*]quinoline chromophore, as exemplified by pyoverdin Pf CCM2798 **1** from *P. fluorescens* (Figure 1).[2] This example also illustrates the occurrence of the tetrahydropyrimidine amino acid unit in some pyoverdins. Our interest in cyclic amidine amino acids as dipeptide mimics[3] led us to prepare some tetrahydropyrimidine pseudodipeptides **2** related to pyoverdins (Figure 2).[4] The chromophore is believed to be derived biogenetically by oxidative cyclization of tetrahydropyrimidine amino acid residue **3**, a proposal supported by incorporation studies with tyrosine and 2,4-diaminobutyric acid[5] and by the co-occurrence of

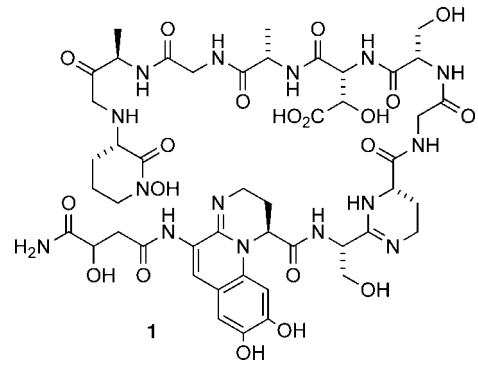


Figure 1. Structure of pyoverdin Pf CCM2798. In subsequent structures, substituents on the chromophore are represented as R groups.

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(1) (a) Fuchs, R.; Budziekewicz, H. *Curr. Org. Chem.* **2001**, *5*, 265–288. (b) Budziekewicz, H. *FEMS Microbiol. Rev.* **1993**, *104*, 209–228.
(2) Demange, P.; Bateman, A.; Macleod, J. K.; Dell, A.; Abdallah, M. A. *Tetrahedron Lett.* **1990**, *31*, 7611–7614.
(3) (a) Jones, R. C. F.; Dickson, J. *J. Peptide Sci* **2001**, *42*, 220–223, and references cited therein. (b) Jones, R. C. F.; Gilbert, I. H.; Rees, D. C.; Crockett, A. K. *Tetrahedron* **1995**, *51*, 6315–6336.
(4) Jones, R. C. F.; Crockett, A. K. *Tetrahedron Lett.* **1993**, *34*, 7459–7462.

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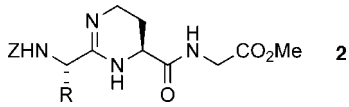


Figure 2. Tetrahydropyrimidine pseudodipeptides.

other related metabolites such as ferribactins,[6] which lack the chromophore but contain the tyrosine-based tetrahydropyrimidine **3**, isopyoverdins **4**,[7] and the corresponding 5,6-dihydro metabolites **5** and **6** (Figure 3).[8]

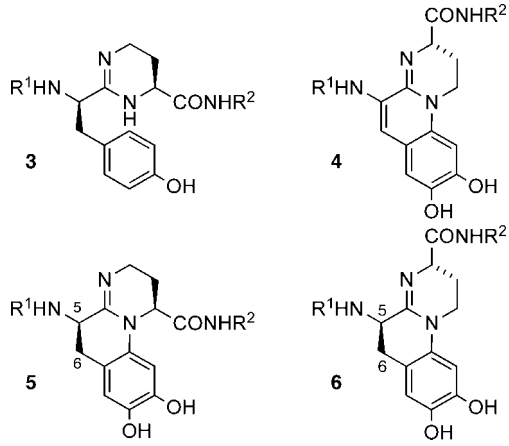
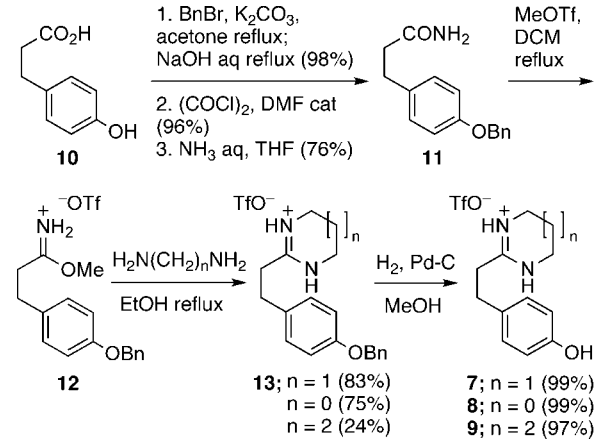


Figure 3. Cometabolites biogenetically related to pyoverdins.

The recent demonstration of enzymic oxidation of the simple analogue **7** of tetrahydropyrimidine **3** to form a model for the dihydropyrimido[1,2-*a*]quinoline chromophore, albeit in very low yield,[9,10] prompts us to report here our biomimetic chemical synthesis of a model for the pyoverdin chromophore and of homologues in the cyclic amidine ring using phenolic oxidation by hypervalent iodine.[11]

The required substrates for oxidation were the phenolic cyclic amidines **7**−**9**. These were all prepared from 2-(4-hydroxyphenyl)propanoic acid **10** by the sequence outlined in Scheme 1. Treatment with benzyl bromide (K_2CO_3, acetone reflux) and subsequent ester hydrolysis (NaOH, MeOH aq) afforded the benzyl ether (98%) which was converted into amide **11** via the acid chloride (oxalyl chloride, THF, DMF cat.; 96%) and ammonolysis (NH_3 aq, *d* 0.88, THF; 76%). After less reliable attempts at *O*-alkylation with Meerwein's salt, our preferred protocol for carboxamide activation was treatment with methyl trifluoromethanesulfonate (CH_2Cl_2, reflux) to afford the imidate salt **12** (we have also successfully employed *S*-alkylation of piperidine thioamides to achieve this carboxyl activation[4]). The crude imidate was treated directly with the appropriate diamine (EtOH, reflux) to form the required cyclic amidine. Thus, 1,3-diaminopropane led to the tetrahydropyrimidine **13** (n = 1) (83%)[12] as its trifluoromethanesulfonate salt which was debenzylated by hydrogenolysis (Pd−C, 1 atm H_2, EtOH) to afford oxidation substrate **7** (99%). Likewise, reaction of **12** with 1,2-diaminoethane or 1,4-diaminobutane, and subsequent hydrogenolysis, led to the corresponding imidazoline **8** (75 and 99%)[12] and 1,3-diazepine **9** (24 and 97%), respectively, again as the trifluoromethanesulfonate salts.

Scheme 1. Synthesis of Cyclic Amidine Oxidation Substrates



Oxidative cyclization of tetrahydropyrimidine **7** to the pyoverdin chromophore ring system requires closure via a nitrogen atom. Our own studies and the work of others have clearly demonstrated that primary amides such as **11** or the corresponding piperidine amides cyclize via the carbonyl oxygen atom on iodine(III) oxidation.[13] Ring closure via nitrogen has been demonstrated for acyl hydrazides,[14] sulfonamides,[15] or cyclic imidates (oxazines, dihydroox-

(5) (a) Thompson, B. N.; Gould, S. J. *Tetrahedron* **1994**, *50*, 9865–9872. (b) Böckmann, M.; Taraz, K.; Budziekewicz, K. *Z. Naturforsch. C: Biosci.* **1997**, *52*, 319–324.

(6) Hohlneicher, U.; Hartmann, R.; Taraz, K.; Budziekewicz, H. *Z. Naturforsch.* **1995**, *50C*, 337–344.

(7) Jacques, Ph.; Ongena, M.; Gwose, I.; Seinsche, D.; Schröder, H.; Delfosse, Ph.; Thonart, Ph.; Taraz, K.; Budziekewicz, H. *Z. Naturforsch.* **1995**, *50C*, 622–629.

(8) (a) Gwose, I.; Taraz, K. *Z. Naturforsch.* **1992**, *47C*, 487–502. (b) Michalke, R.; Taraz, K.; Budziekewicz, H.; Thonart, Ph.; Jacques, Ph. *Z. Naturforsch.* **1995**, *50C*, 855–857.

(9) Dorrestein, P. C.; Poole, K.; Begley, T. P. *Org. Lett.* **2003**, *5*, 2215–2217.

(10) (a) Routes to cyclic amidine [1,2-*a*]-fused quinolines have also been reported by closure of the amidine ring as the final step using conventional or electrochemical protocols. See, for example: Jones, R. C. F.; Smallridge, M. J.; Chapleo, C. B. *J. Chem. Soc., Perkin Trans. 1* **1990**, *38*, 5–391. (b) Okimoto, M.; Yoshida, T.; Hoshi, M.; Hattori, K.; Komata, M.; Numata, K.; Tomozawa, K. *Aust. J. Chem.* **2007**, *60*, 236–242.

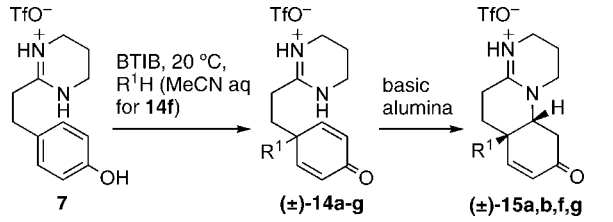
(11) (a) Wirth, T. *Angew. Chem., Int. Ed.* **2005**, *44*, 3656–3665. (b) Wirth, T. *Hypervalent Iodine Chemistry: Modern Developments in Organic Synthesis*; Wirth, T., Ed.; Springer: Berlin, 2003; Vol. 224, pp 185−208. (c) Tohma, H.; Kita, Y. *Hypervalent Iodine Chemistry: Modern Developments in Organic Synthesis*; Wirth, T., Ed.; Springer: Berlin, 2003; Vol. 224, pp 209−248.

(12) Some recovered amide **11** was isolated along with tetrahydropyrimidine **13** (n = 1) and imidazoline **13** (n = 0) (15 and 4%, respectively).

(13) (a) Smith, J. E. M.Phil. Thesis, The Open University, 2004. (b) Kita, Y.; Tohma, H.; Kikuchi, K.; Inagaki, M.; Yakura, T. *J. Org. Chem.* **1991**, *56*, 435–438.

azoles) although in the latter case the imidate then undergoes cleavage.[16] Since our initial attempts at direct oxidative cyclization of the cyclic amidines **7**−**9** were unpromising, we elected to oxidize in the presence of nucleophilic alcohol solvents.[17] Thus, treatment of tetrahydropyrimidine **7** with bis(trifluoroacetoxy)iodobenzene (BTIB) in MeOH (20 °C, 5 min) afforded a dienone intermediate **14a** (Table 1) that

Table 1. Oxidative Cyclization of Pyrimidine **7**



7 **(±)-14a-g** **(±)-15a,b,f,g**

entry	R^1	(±)-dienone salt **14**[a]	(±)-pyrimidoquinolinone salt **15** (yield, %)
1	OMe	**14a**	**15a** (46)
2	OEt	**14b**	**15b** (34)
3	$OCHMe_2$	**14c**	
4	$OCMe_3$	**14d**	
5	OCH_2Ph	**14e**	
6	NHCOMe	**14f**	**15f** (41)
7	OH	**14g**	**15g** (4)

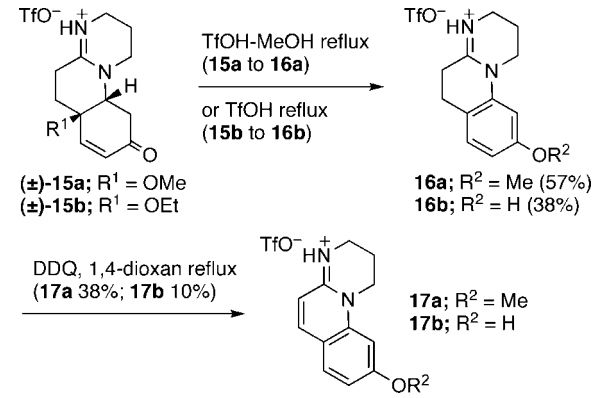
[a] Dienones **14** were not fully characterized.

could not be fully characterized but when chromatographed on basic alumina afforded tetrahydropyrimidoquinolinone trifluoromethanesulfonate **15a** (46%) as product of the desired oxidative cyclization through a nitrogen atom.[18] It is apparent that the intramolecular conjugate addition is alumina-mediated; indeed, stirring the dienone in solution with alumina also led to cyclization, but chromatographic conversion was the more efficient protocol. When the BTIB oxidation and alumina treatment sequence was repeated with EtOH as oxidation solvent, the corresponding tricyclic salt **15b** was formed (34%) via dienone **14b**. Likewise, 2-propanol, *tert*-butyl alcohol, and benzyl alcohol afforded dienones **14c**−**e** that were not completely characterized. From an oxidation in moist acetonitrile, the cyclization product **15f** was isolated (41%) via acetamide adduct **14f**, and the hydroxyl adduct **15g** was observed in low yield (approximately 4%) from a reaction in water, via **14g**. The structures of the methoxy derivative **15a** and ethoxy derivative **15b** were confirmed by X-ray crystallographic analysis, which also revealed *cis*-6,6-ring fusion in the crystalline material.[19,20]

Aromatization of the carbocyclic ring by elimination of alcohol from **15a**,**b** under acidic conditions was more difficult than originally anticipated, presumably because the quinolinones were already protonated at the amidine function, hindering alkoxy-group protonation.

Treatment of **15a** with trifluoromethanesulfonic acid afforded a mixture of the methoxy- and hydroxytetrahydropyrimidoquinoline salts **16a** and **16b**. We propose that *O*-alkylation arises by formation of the methylating agent methyl trifluoromethanesulfonate from the eliminated methanol in the reaction medium. In support of this, addition of MeOH to the acidic reaction medium afforded methyl ether **16a** as the major product (57%) (Scheme 2). In contrast,

Scheme 2. Completion of the Tetrahydropyrimidoquinolines



elimination from ethoxy compound **15b** afforded solely the phenolic dihydropyrimidinoquinoline **16b** (38%), as presumably any ethyl trifluoromethanesulfonate formed is either less effective as an alkylating agent, or undergoes elimination. The structure of methyl ether **16a** was confirmed by an X-ray crystal structure.[19] Completion of the pyoverdin chromophore model was achieved by dehydrogenation of **16a** using DDQ (1,4-dioxane, reflux) to afford tricyclic salt **17a** (38%), purified by reverse-phase HPLC; the structure of **17a** was also confirmed by a crystal structure determination. Dehydrogenation of **16b** could also be achieved in low yield (10%) to give a highly polar product **17b** that was not fully characterized. These dehydrogenations could also be achieved on silica by microwave irradiation but gave less pure products.

The oxidative cyclization sequence was also performed on the imidazoline **8** and 1,3-diazepine **9** (Scheme 3). Thus, BTIB oxidation using MeOH or EtOH as solvent, and subsequent alumina chromatography, afforded reduced imidazoquinolinone trifluoromethanesufonates **18a** and **18b** from imidazoline **8** (39% and 16%, respectively). Diazepine **9**

(14) Clemente, D.-T. V.; Lobo, A. M.; Prabhakar, S. *Tetrahedron Lett.* **1994**, *35*, 2043–2046.

(15) Ciufolini, M. A.; Canesi, S.; Ousmer, M.; Braun, N. A. *Tetrahedron* **2006**, *62*, 5318–5337.

(16) Braun, N. A.; Bray, J. D.; Ousmer, M.; Peters, K.; Peters, E. M.; Bouchou, D.; Ciufolini, M. A. *J. Org. Chem.* **2000**, *65*, 4397–4408.

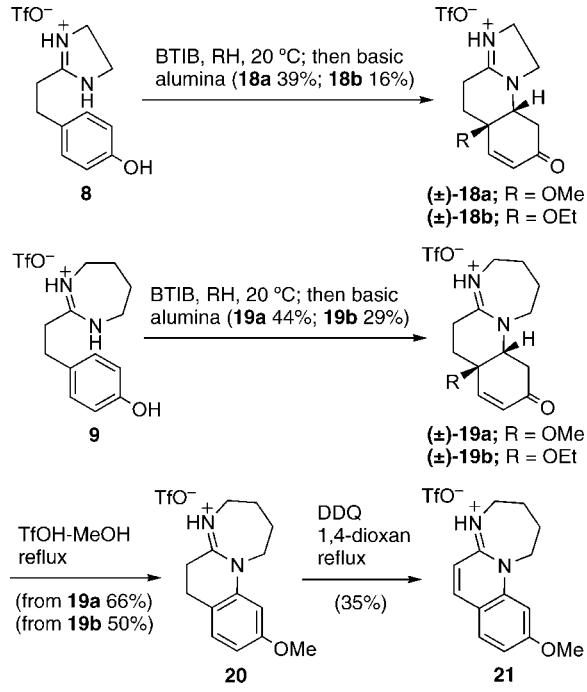
(17) Tohma, H.; Morioka, H.; Takizawa, S.; Yarisawa, M.; Kita, Y. *Tetrahedron* **2001**, *57*, 345–352, and references cited therein.

(18) (a) Pouysegu, L.; Avella, A.-V.; Quideau, S. *J. Org. Chem.* **2002**, *61*, 3425–3436, and references cited therein. (b) Wipf, P.; Methot, J.-L. *Org. Lett.* **2000**, *2*, 4213.

(19) Crystal structures of **15a**, **15b**, **16a**, and **17a** were refined to convergence with *R* factors of 0.061, 0.054, 0.042 and 0.097, respectively. Full details are presented in the Supporting Information.

(20) A cis-ring junction is expected from attack of the amidine onto the dienone from the face opposite the alkoxy group.

Scheme 3. Oxidative Cyclizsations of Imidazoline **8** and Diazepine **9**



similarly afforded the reduced alkoxyazepinoquinolinone salts **19a** and **19b** (44% and 29%, respectively). Intermediates **18** have not been taken further, but treatment of both **19a** and **19b** in trifluoromethanesulfonic acid-MeOH gave the methoxyhexahydroazepinoquinoline salt **20** (66% and 50%, respectively). DDQ dehydrogenation of **20** as previously led to the homologue **21** of the pyoverdin chromophore model (35%).

The dihydropyrimidoquinoline **17a** showed strong fluorescence,[21] as do the natural pyoverdins; the seven-ring analogue **21** showed a much weaker fluorescence (approximately 25% of that of **17a**). The UV spectra of chromophores **17** and **21** compared acceptably with those of the natural pyoverdins,[22] recognizing that they have an incomplete substituent set.

We have thus chemically generated models for the dihydropyrimido[1,2-*a*]quinoline chromophore of the pyoverdin siderophores via a biomimetic route involving oxidative cyclization. Efforts continue toward synthesis of the pyoverdins.

Acknowledgment. We acknowledge the financial support of Loughborough University (S.C.Y.), the Open University (J.D.), and EPSRC (J.E.S.), and the EPSRC National Crystallography Service at Southampton for the structure determination of **15a**.

Supporting Information Available: Procedures and spectral data for the synthesis of **7**−**9** and oxidative cyclizations to **17a**, **16b**, **18a**, and **21**; X-ray crystal data including CIF files for **15a**, **15b**, **16a**, and **17a**. This material is available free of charge via the Internet at http://pubs.acs.org.

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(21) **17a**: fluorescence em (MeOH−H_2O 1:1 v/v, pH 6.5, ex 338, 353, λ_{max}) 372 (ε 5.09 × 10^6), and 382 (4.80 × 10^6), respectively; UV−vis (MeOH-H_2O 1:1 v/v, pH 6.5, λ) 219 (ε 6.3 × 10^4), 338 (2.3 × 10^4), and 353 (2.0 × 10^4). No significant change over pH range 6.5−9.

(22) For example: Demange, P.; Wendenbaum, S.; Linget, C.; Mertz, C.; Cung, M. T.; Dell, A.; Abdallah, M. A. *Biol. Met.* **1990**, *3*, 155–170. For pyoverdin Pa from *P. aeruginosa* ATCC 15692: UV−vis (pH 4.2, λ) 365 nm (ε l.4 × l04) and 380 (1.4 × 104).