Synthesis of the Pyoverdin Chromophore by a Biomimetic Oxidative Cyclisation

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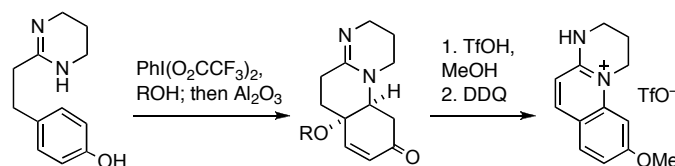
The fluorescent dihydropyrimido[1,2-a]quinoline chromophore of the pyoverdin siderophores has been synthesized by a biomimetic oxidative cyclisation using an iodine(III) reagent, followed by elimination and dehydrogenation.

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 cyclisation 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 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]
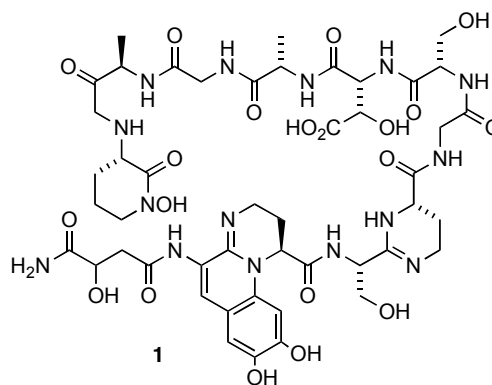

Figure 1. Structure of pyoverdin Pf CCM2798. In subsequent structures substituents on the chromophore are represented as R groups.
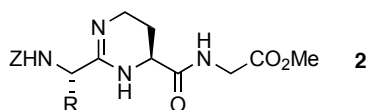
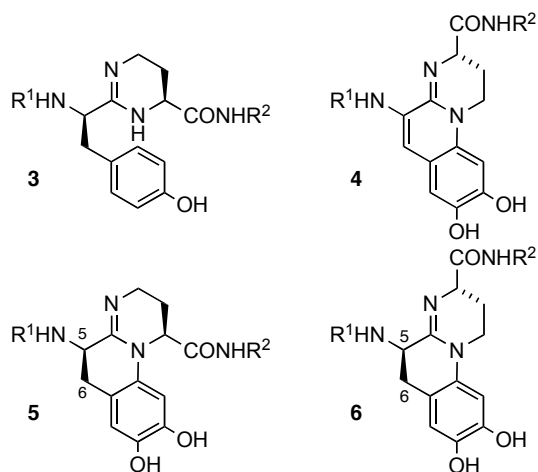**Figure 2.** Tetrahydropyrimidine pseudodipeptides

Figure 3. Co-metabolites 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$_2$CO$_3$, 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 trifluororomethanesulfonate (CH$_2$Cl$_2$, 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

**Scheme 1.** Synthesis of cyclic amidine oxidation substrates
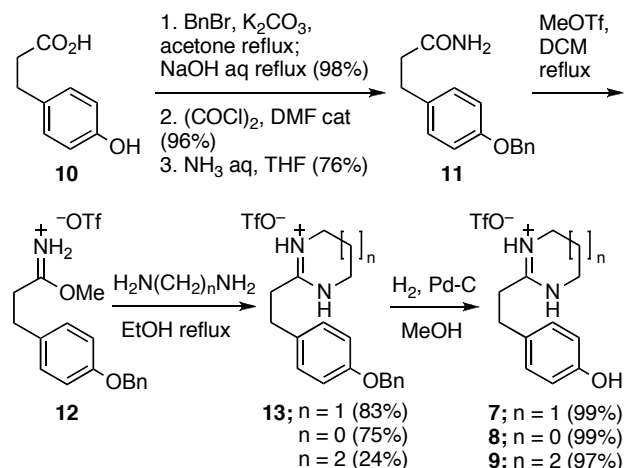
trifluoromethanesulfonate salt which was debenzylated by hydrogenolysis (Pd-C, 1 atm H$_2$, EtOH) to afford oxidation substrate **7** (99%). Likewise, reaction of **12** with 1,2-diaminoethane or 1,4-diaminobutane, and subsequent hydrogenolysis, led to the corresponding imidazoline **8** (75 and 99%)[12] and 1,3-diazepine **9** (24 and 97%), respectively, again as the trifluoromethanesulfonate salts.

Oxidative cyclisation 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 cyclise 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, dihydrooxazoles) although in the latter case the imidate then undergoes cleavage.[16] Since our initial attempts at direct oxidative cyclisation 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 ºC, 5 min) afforded a dienone intermediate **14a** (Table 1) that could not be fully characterised but when chromatographed on basic alumina afforded tetrahydropyrimidoquinolinone trifluoromethanesulfonate **15a** (46%) as product of the desired oxidative cyclisation 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 cyclisation, 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*-butanol and benzyl alcohol

Table 1. Oxidative cyclisation of pyrimidine **7**
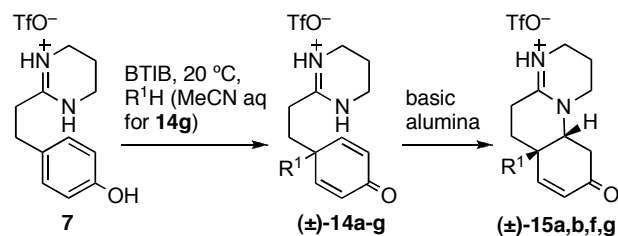
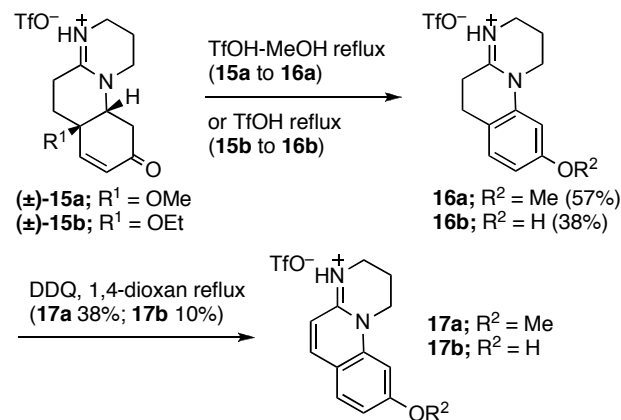| Entry	R[1]	(±)-Dienone salt 14[a]	(±)-Pyrimidoquinolinone salt 15 (Yield %)
1	OMe	**14a**	**15a** (46%)
2	OEt	**14b**	**15b** (34%)
3	OCHMe$_2$	**14c**	–
4	OCMe$_3$	**14d**	–
5	OCH$_2$Ph	**14e**	–
6	NHCOMe	**14f**	**15f** (41%)
7	OH	**14g**	**15g** (4%)

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

afforded dienones **14c-e** that were not completely characterized. From an oxidation in moist acetonitrile, the cyclisation product **15f** was isolated (41%) via acetamide adduct **14f**, and the hydroxyl adduct **15g** was observed in low yield (approx. 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]

Aromatisation 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.

Scheme 2. Completion of the tetrahydropyrimidoquinolines
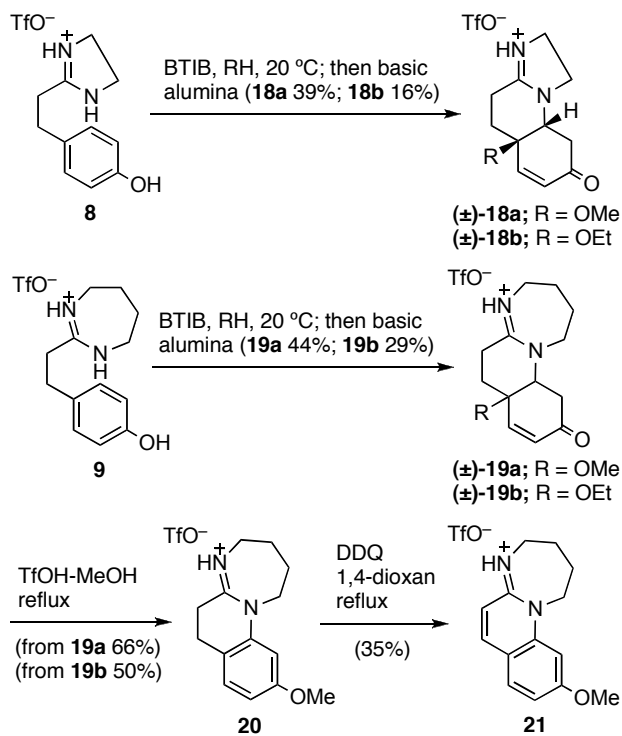

Treatment of **15a** with trifluoromethanesulfonic acid afforded a mixture of the methoxy- and hydroxy-tetrahydropyrimidoquinoline salts **16a** and **16b**. We propose that *O*-alkylation arises by formation of the methylating agent methyl trifluoromethanesulfonate from the eliminated methanol in the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

Scheme 3. Oxidative cyclisations of imidazoline **8**, diazepine **9**

BTIB, RH, 20 ºC; then basic alumina (**18a** 39%; **18b** 16%)

8

(±)-**18a**; R = OMe
(±)-**18b**; R = OEt

BTIB, RH, 20 ºC; then basic alumina (**19a** 44%; **19b** 29%)

9

(±)-**19a**; R = OMe
(±)-**19b**; R = OEt

TfOH-MeOH reflux

(from **19a** 66%)
(from **19b** 50%)

20

DDQ 1,4-dioxan reflux

(35%)

21

18a and **18b** from imidazoline **8** (39% and 16%, respectively). 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 (approx. 25% of that of **17a**). The UV spectra of chromophores **17** and **21** compared acceptably with those of the natural pyoverdins,[22] recognising 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 cyclisation. Efforts continue towards synthesis of the pyoverdins.

Supporting Information Available: Procedures and spectral data for the synthesis of **7-9** and oxidative cyclisations to **17a**, **16b**, **18a**, **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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12. Some recovered amide **11** was isolated along with tetrahydropyrimidine **13** (n = 1) and imidazoline **13** (n = 0) (15 and 4%, respectively).

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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.

21. **17a**: fluorescence em (MeOH-H$_2$O 1:1 v/v, pH 6.5, ex 338, 353, λ_{max}) 372 (ε 5.09 x 10^6) and 382 (4.80 x 10^6), respectively; UV-vis (MeOH-H$_2$O 1:1 v/v, pH 6.5, λ) 219 (ε 6.3 x 10^4), 338 (2.3 x 10^4) and 353 (2.0 x 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 *Pseudomonas aeruginosa* ATCC 15692: UV-vis (pH 4.2, λ) 365 nm (ε l.4 x l0^4) and 380 (1.4x10^4).