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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-1H-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 mimics3 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 47 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.
The recent demonstration of enzymic oxidation of the simple analogue 7 of tetrahydropyrimidine 3 to form a model for the dihydropyrimido[1,2-α]quinoline chromophore, albeit in very low yield, 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. 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 (K₂CO₃, 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 (NH₃ 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 (CH₃Cl₂, reflux) to afford the imidate salt 12 (we have also successfully employed S-alkylation of piperidine thioamides to achieve this carboxyl activation⁴). 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%) as its trifluoromethanesulfonate salt which was debenzylated by hydrogenolysis (Pd-C, 1 atm H₂, 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%) 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. Ring closure via nitrogen has been demonstrated for acyl hydrazides, sulfonamides, or cyclic imidates (oxazines, dihydrooxazoles) although in the latter case the imidate then undergoes cleavage. Since our initial attempts at direct oxidative cyclisation of the cyclic amidines 7-9 were unpromiseing, we elected to oxidize in the presence of nucleophilic alcohol solvents. Thus treatment of tetrahydropyrimidine 7 with bis(trifluoroacetoxy)iodobenzene (BTIB) in MeOH (20 °C, 5 min) afforded a dieneone 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. It is apparent that the intramolecular conjugate addition is alumina-mediated; indeed, stirring the dieneone 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

**Scheme 1. Synthesis of cyclic amidine oxidation substrates**

**Figure 2. Tetrahydropyrimidine pseudodipeptides**

**Figure 3. Co-metabolites biogenetically related to pyoverdins**
tricyclic salt 15b was formed (34%) via dienone 14b. Likewise, 2-propanol, tert-butanol and benzyl alcohol afforded dienones 14c-e that were not completely characterized. From an oxidation in moist acetonitrile, the cyclisation product 15f was isolated (41%) via acetonamide 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.

Table 1. Oxidative cyclisation of pyrimidine 7

<table>
<thead>
<tr>
<th>Entry</th>
<th>R’</th>
<th>(±)-Dienone</th>
<th>(±)-Pyrimidoquinoline salt 15 (Yield %)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>OMe</td>
<td>14a</td>
<td>15a (46%)</td>
</tr>
<tr>
<td>2</td>
<td>OEt</td>
<td>14b</td>
<td>15b (34%)</td>
</tr>
<tr>
<td>3</td>
<td>OCHMe2</td>
<td>14c</td>
<td>–</td>
</tr>
<tr>
<td>4</td>
<td>OCHMe</td>
<td>14d</td>
<td>–</td>
</tr>
<tr>
<td>5</td>
<td>OCHPh</td>
<td>14e</td>
<td>–</td>
</tr>
<tr>
<td>6</td>
<td>NHCOMe</td>
<td>14f</td>
<td>15f (41%)</td>
</tr>
<tr>
<td>7</td>
<td>OH</td>
<td>14g</td>
<td>15g (4%)</td>
</tr>
</tbody>
</table>

*Dienones 14 were not fully characterized.

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 dihydrohydropyrimidoquinoline 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 trifluoromethanesulfonates.

Scheme 3. Oxidative cyclisations of imidazoline 8, diazepine 9
18a and 18b from imidazoline 8 (39% and 16%, respectively). Diazepine 9 similarly afforded the reduced alkoxyazepinoquinolinoine 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 methoxyhexahydroazepinoquinolinoine salt 20 (66% and 50%, respectively). DDQ dehydrogenation of 20 as previously led to the homologue 21 of the pyoverdin chromophore model (35%).

The dihydroxypyrindioquinoline 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 dihydroprymido[1,2-α]quinoline chromophore of the pyoverdin siderophores via a biomimetic route involving oxidative cyclisation. Efforts continue towards synthesis of the pyoverdins.

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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.