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Vanchrobactin: absolute configuration and total synthesis

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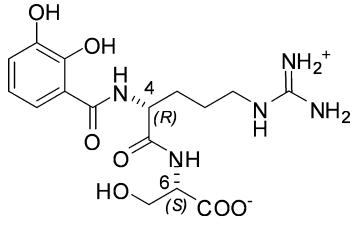
Abstract—The stereochemistry of vanchrobactin, a siderophore produced by the bacterial fish pathogen *Vibrio anguillarum* serotype O2, was elucidated by chiral capillary electrophoresis analysis and total synthesis as *N*-[*N*′-(2,3-dihydroxybenzoyl)-D-arginyl]-L-serine.

Iron is the fourth most abundant element in the earth's crust and is an essential nutrient for most microorganisms. However, the low solubility of Fe(III) in aqueous media (10^{-8} M at neutral pH) prompted many bacteria to develop specialised mechanisms for its acquisition. Iron uptake is generally mediated by siderophores, which are low molecular weight (300–2000 Da) high-affinity chelators produced by microorganisms to sequester Fe^{3+}. These molecules, which are generally excreted into the culture medium, are able to strongly chelate in a specific manner to solubilize and deliver Fe^{3+} into the cells.[1]

In the course of our studies into siderophores from marine microorganisms, vanchrobactin was isolated from iron-deficient cultures in the CM9 medium of the bacterial fish pathogen *V. anguillarum* serotype O2 strain RV22.[2] This pathogen is the causative agent of vibriosis, an extremely fatal hemorrhagic septicaemia that results in considerable economic losses in aquaculture farming worldwide.[3] It is known that the ability to scavenge iron through the utilization of siderophores is a key factor in the virulence of this fish pathogen.[4] The planar structure of vanchrobactin was established by extensive NMR studies on the natural sample. The identification and characterization of the genes and enzymes involved in the biosynthesis of vanchrobactin in strain RV22, confirmed not only that arginine and serine are the amino acids incorporated in vanchrobactin but also the assembly order of the three components (DHBA, arginine, and serine).[5] However, the stereochemistry of vanchrobactin could not be elucidated by spectroscopic analysis.

In order to confirm the proposed planar structure of vanchrobactin and to determine its absolute configuration, a synthetic sample was required. A practical method for the synthesis of vanchrobactin would allow the development of new strategies to interfere with the iron-acquisition mechanisms of *V. anguillarum* or approaches that could provide 'trojan horses'[6] for the development of new rationally designed antibacterial agents against vibriosis.[7]

Herein we report a short and efficient method for the synthesis of vanchrobactin and the elucidation of its absolute configuration by chiral capillary electrophoresis analysis. The configuration was unequivocally determined by comparison of the natural product with a synthetic sample.



Vanchrobactin

Keywords: Vanchrobactin; Marine siderophore; Vibriosis; *Vibrio anguillarum*; Iron uptake; Synthesis; Absolute configuration.
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Firstly, we planned the synthesis of two of the four possible stereoisomers,[8] compounds **1a** and **1b**, using dihydroxybenzoic acid, L-ornithine, D-serine, and L-serine as starting materials, and introducing the guanidine functionality in a later step.

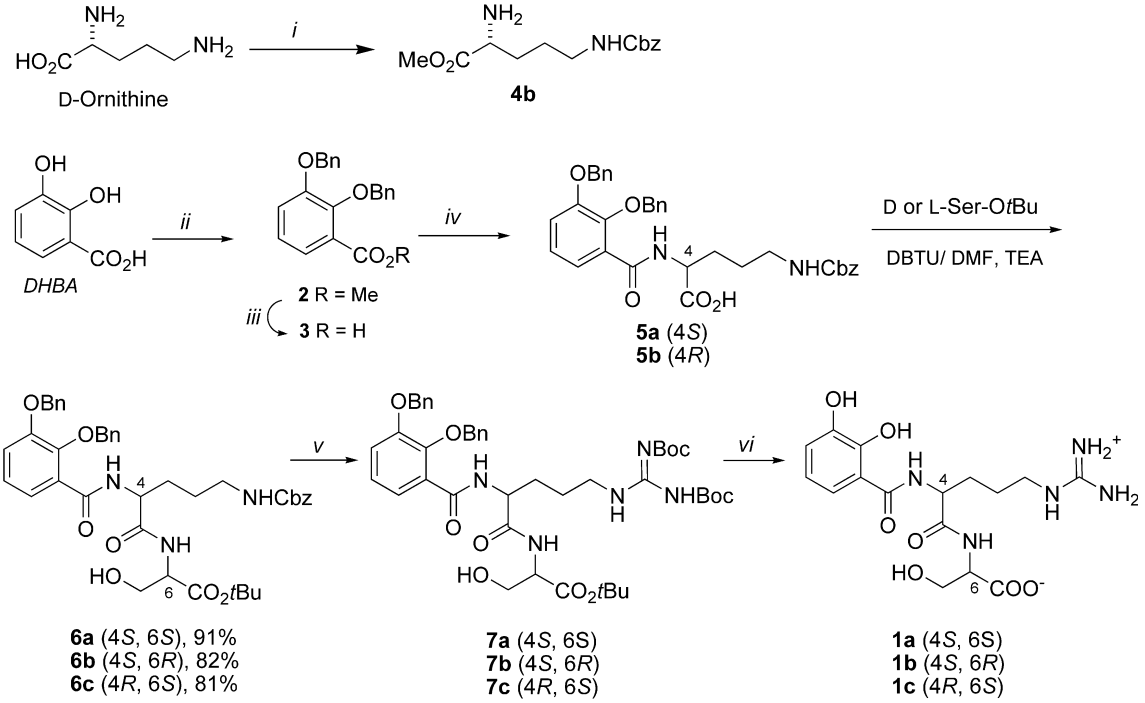
The synthesis started from the commercially available 2,3-dihydroxybenzoic acid (DHBA) which, after conversion into the methyl ester with MeOH and $SOCl_2$, was protected with benzyl bromide in the presence of potassium carbonate under reflux to give **2**. Subsequent saponification with barium hydroxide in THF/H_2O (2:1) gave 2,3-dibenzyloxybenzoic acid (**3**) in quantitative yield.[9] On the other hand, N^{δ}-Cbz-L-ornithine-OMe (**4a**) was prepared by esterification of the commercially available N^{δ}-Cbz-L-ornithine.[10] The coupling of DHBA derivative **3** and N^{δ}-Cbz-L-ornithine-OMe (**4a**) using TBTU as the coupling agent,[11] followed by hydrolysis with barium hydroxide in THF/H_2O (1:1), gave acid **5a** in very good yield (Scheme 1).

L-Serine and D-serine were converted into the corresponding *tert*-butyl esters with AcO^tBu in the presence of a catalytic amount of $HClO_4$.[12] These compounds were then coupled to acid **5a** with TBTU to afford compounds **6a** and **6b** in 91% and 82% yield, respectively.

Derivatives **6a** and **6b** were submitted to catalytic hydrogenation to remove the benzyl protecting groups, and the guanidine function was introduced by reaction with 1,3-di-Boc-2-(trifluoromethanesulfonyl)guanidine[13] to give **7a** and **7b** in 69% and 63% yield, respectively. Finally, acidic hydrolysis of the *tert*-butoxy protecting group afforded compounds **1a** and **1b**, respectively, in quantitative yield as amorphous solids.

HPLC analysis of these stereoisomers using a reversed-phase achiral column (Discovery® HS F5) showed that compound **1b** had the same retention time as the natural product (see Fig. 1). However, chiral capillary electrophoresis analysis of compounds **1a** and **1b** on a highly sulfated gamma cyclodextrin chiral selector, in comparison to the natural product, indicated that they did not correspond to the same diastereoisomer. The two compounds gave different migration times (see Fig. 2), allowing us to postulate that vanchrobactin must be the enantiomer of **1b**. In order to confirm this hypothesis, we carried out the synthesis of the stereoisomer bearing D-arginine and L-serine amino acid residues according to the synthetic strategy described previously for compounds **1a** and **1b**.

In this case, N^{δ}-Cbz-D-ornithine-OMe (**4b**) was prepared from the Cbz derivative of D-ornithine copper complex, elimination of the metal cation with EDTA and esterification of the free acid group.[14] Coupling of **4b** with DHBA derivative **3** gave compound **5b** which, after saponification, was coupled to L-serine *tert*-butyl ester to afford **6c**.[15] Deprotection of the benzyl groups in **6c**, introduction of the guanidine functionality to give **7c** and acidic hydrolysis yielded **1c**. Chiral capillary electrophoresis analysis showed that **1c** has the same migration time as the natural product and these compounds were coeluted when a mixture was injected (see Fig. 2).



Scheme 1. Reagents and conditions: (i) (1) $CuSO_4$, H_2O, NaOH, (2) NaOH, dioxane, ClCbz, (3) EDTA, 1 M HCl, (4) $SOCl_2$/MeOH (48% four steps); (ii) (1) $SOCl_2$/MeOH (89%), (2) $BnBr/K_2CO_3$ (99%); (iii) $Ba(OH)_2$, THF, H_2O, 50 °C, 12 h, quant.; (iv) (1) N^{δ}-Cbz-L-ornithine-OMe (**4a**) or N^{δ}-Cbz-D-ornithine-OMe (**4b**), TBTU, Et_3N, DMF, rt, 16 h (78% and 79%, respectively); (2) $Ba(OH)_2$, THF, H_2O, 50 °C, 12 h, quant; (v) (1) H_2, Pd-C, MeOH, rt, 3 h, quant.; (2) $(NHBoc)_2NTf$, Et_3N, $CHCl_3$, rt, 1 h (69% for **7a**, 63% for **7b**, 61% for **7c**); (vi) TFA/DCM 3:7, rt, 16 h, quant.

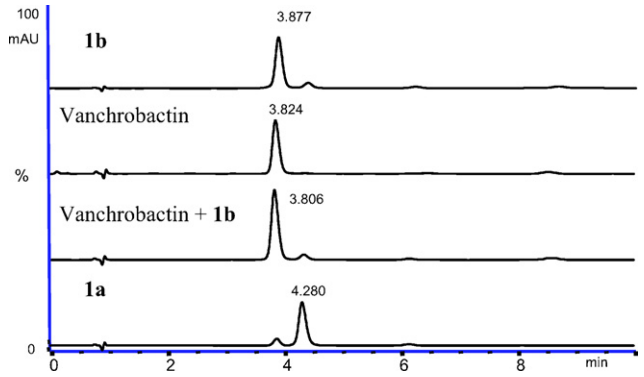


Figure 1. UV_{214nm} chromatograms (from top to bottom) of **1b**, vanchrobactin, vanchrobactin + **1b** (1 to 1 mixture), and **1a**. Column 4.6 × 50 mm, 3 μm; mobile phase, H_2O 0.05% TFA and MeCN 0.05% TFA (90/10); flow rate, 1.0 ml/min.

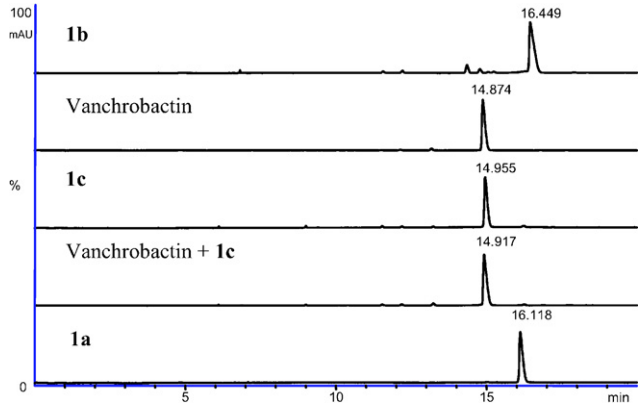


Figure 2. UV_{214nm} electropherograms (from top to bottom) of **1b**, vanchrobactin, **1c**, vanchrobactin + **1c** (1 to 1 mixture), and **1a**. Capillary dimension, 50 μm i.d., 363 μm o.d.; total capillary length, 38.5 cm; length to detector, 30 cm; running buffer, 5% gamma-HS-CD, 25 mM phosphate buffer pH 2.5; hydrodynamic injection, 60 mbars.

The NMR data for the synthetic compound **1c** are practically identical to those of vanchrobactin.[16] The slight chemical shift differences observed are due to the strong pH dependence on the chemical shifts of amino acids in D_2O solution.[17]

The $[\alpha]_D$ value of −16.4 (*c* 0.7, MeOH) of the synthetic sample was similar to that of vanchrobactin {$[\alpha]_D$ −13.6 (*c* 25×10^{-3}, MeOH)}.

The siderophore activity and biological function of the synthetic compound was confirmed by testing the restoration of growth of the producer cells in CM9 medium containing the iron chelator 2,2′-dipyridyl at inhibiting concentrations. Furthermore, stereoisomers **1a** and **1b** showed also growth promotion under the same conditions. On the basis of these data, the absolute structure of vanchrobactin was thus established as *N*-[*N*′-(2,3-dihydroxybenzoyl)-D-arginyl]-L-serine.

Chrysobactin is a siderophore isolated from the phytopathogenic bacterium *Erwinia chrysanthemi* and is structurally related to vanchrobactin but bears a lysine residue instead of arginine.[18] Interestingly, this compound also has the L configuration for the serine residue, whereas the lysine residue has a D configuration.[19]

In conclusion, the elucidation of the absolute stereochemistry of vanchrobactin and its total synthesis have been achieved. This has important implications for the mechanism of vanchrobactin biosynthesis and for the development of new rationally designed antibacterial compounds against vibriosis. Studies on the structure-activity relationships of vanchrobactin are currently under way.

Acknowledgments

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15. *Selected data for* **6c**: $[\alpha]_D^{20}$ +13.9 (*c* 1.8, $CHCl_3$); ^1H NMR (300 MHz, $CDCl_3$): δ 1.45 (s, 9H, $C(CH_3)_3$), 1.79–1.90 (m, 4H, H-2 and H-3), 3.03–3.11 (m, 1H, H-1a), 3.17–3.25 (m, 1H, H-1b), 3.50 (dd, $J = 8.7$, 2.9 Hz, 1H, H-7a), 3.79 (dd, $J = 8.7$, 2.9 Hz, 1H, H-7b), 4.59–4.70 (m, 2H, H-4 and H-6), 5.07–5.27 (m, 6H, $3 \times OCH_2Ph$), 6.90 (d, $J = 8.4$ Hz, 1H, NH), 7.13–7.46 (m, 17H, aromatics), 7.72 (dd, $J = 6.5$, 2.3 Hz, 1H, aromatic), 8.51 (d, $J = 7.1$ Hz, 1H, NH); ^{13}C NMR (75 MHz, $CDCl_3$): δ 25.72 (C-2), 27.97

($C(CH_3)_3$), 29.39 (C-3), 40.06 (C-1), 52.73, 53.09 (C-4 and C-6), 62.06 (C-7), 71.32, 73.08, 76.08 ($3\times$–OCH_2Ph), 81.76 ($C(CH_3)_3$), 117.32, 123.28, 124.21, 127.72, 127.90, 128.03, 128.21, 128.38, 128.49, 128.62, 128.91, 128.82, 136.20, 136.26, 146.91, 151.67, 156.43 (*C*ONH), 165.04 (*C*ONH), 169.26 (*C*ONH) 171.11 ($CO_2C(CH_3)_3$); HR-ESIMS: *m*/*z* 726.3352 $[M+H]^+$ (calcd for $C_{41}H_{48}N_3O_9$, 726.3385).

16. *Selected data for* **1c**: $[\alpha]_D^{24}$ −16.4 (*c* 0.7, MeOH); ^{1}H NMR (500 MHz, D_2O): δ 1.74–2.00 (m, 4H, H-2 and H-3), 3.25 (t, $J = 6.7$ Hz, 2H, H-1), 3.91 (dd, $J = 4.0$, 11.5 Hz, 1H, H-7a), 4.00 (dd, $J = 5.0$, 11.5 Hz, 1H, H-7b), 4.59 (t, $J = 4.5$ Hz, 1H, H-6), 4.67 (dd, $J = 5.5$, 8.5 Hz, 1H, H-4), 6.90 (t, $J = 8.0$ Hz, 1H, H-6 DHBA), 7.11 (dd, $J = 1.5$, 8.0 Hz, H-5 DHBA), 7.31 (dd, $J = 1.5$, 8.0 Hz, H-7 DHBA); ^{13}C NMR (125 MHz, D_2O): δ 24.82 (C-2), 28.91 (C-3), 41.00 (C-1), 53.98 (C-4), 55.32 (C-6), 61.46 (C-7), 117.08, 119.90, 120.15, 120.21, 145.00, 147.17, 157.16 (*C*=N), 170.36 (*C*ONH), 173.54 (*C*ONH), 174.26 (CO_2H). HRESIMS: *m*/*z* 398.1674 $[M+H]^+$ (calcd for $C_{16}H_{24}N_5O_7$ 398.1670).
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