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Synthesis of the Hydroxamate Siderophore Nannochelin A

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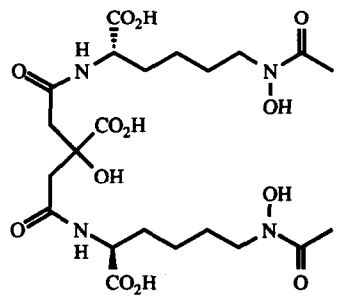
Abstract: A total synthesis of nannochelin A, a novel cinnamoyl-hydroxamate siderophore produced by *Nannocystis exedens*, is described.

Ferric iron plays an essential role in metabolism. Its ligand controlled redox properties are essential to the operation of for example ribonucleotide reductase and the cytochromes. The aquisition of iron by natural systems presents particular difficulties arising from the very low solubility of ferric salts at physiological pH, and microorganisms have evolved a mechanism for chelation of ambient ferric ions by low molecular weight metabolites known as siderophores.[1] The ferri-siderophores are recognised and transported into the cell by specialist surface receptors. Biosynthesis of both siderophore and receptor is activated in response to iron deficiency.[2]

Other natural transport mechanisms are well known, for example those for dipeptides and cytidine, and the exploitation of these channels to infiltrate bioactive molecules into cells has become a recognised goal.[3] Our interest in this area, and in the broader field of metal chelation by natural products,[4] has lead us to examine the receptor specificity towards a number of natural and synthetic siderophores with the objective of delineating the structural requirements for transport.

One of the groups of siderophores we selected for this purpose was the set including aerobactin **1**,[5] arthrobactin **2**,[6] and schizokinin **3**,[7] all of which are citrate based systems with two flexible N-acetyl hydroxamate arms attached to the peripheral carboxyl groups of citrate, to provide appropriate ligands. During our studies of synthetic relatives of these metabolites a related series, nannochelins A-C, **4-6**, were reported,[8] in which the *N*-acetyl moiety was replaced by the relatively large *N*-cinnamoyl unit, with retention of iron-transporting activity (to the appropriate organism, *Nannocystis exedens*). This suggests that other variations



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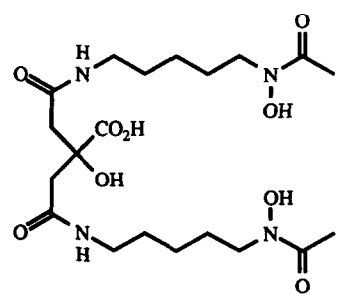

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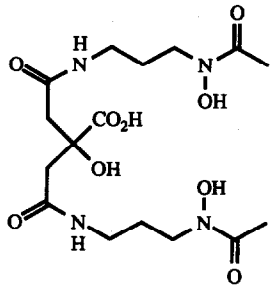

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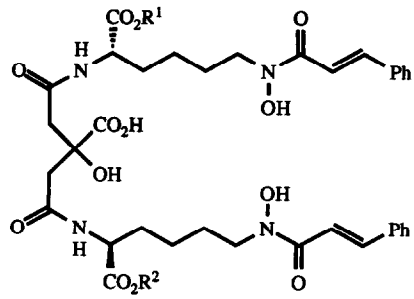
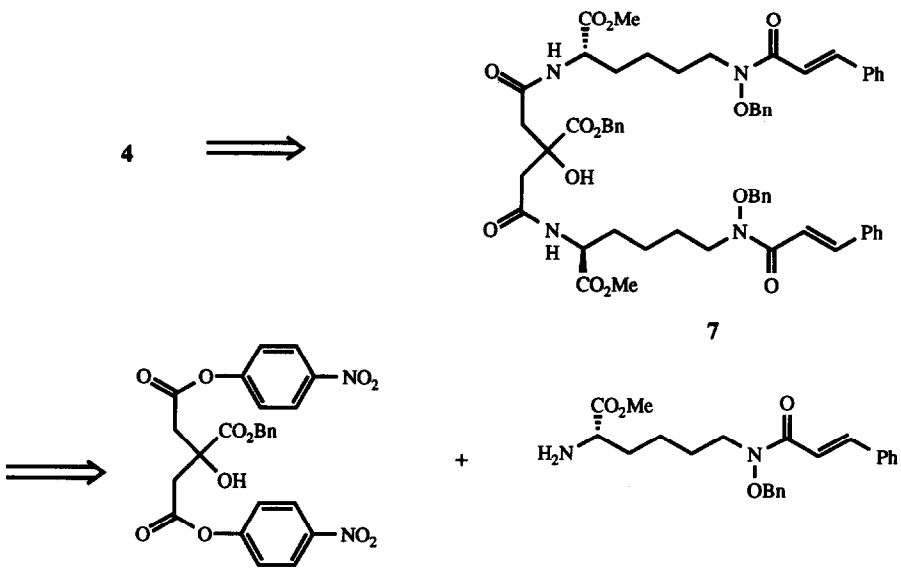


4, $R^1 = R^2 = Me$
5, $R^1 = H, R^2 = Me$
6, $R^1 = R^2 = H$

are possible at this site, and therefore we undertook the synthesis of nannochelin A **4**, for comparative biological studies. Prompted by the recent disclosure by Bergeron and Phanstiel[9] of their route to nannochelin A, we now describe our approach to this target.

The strategy we designed for a concise synthesis of nannochelin A **4** was based on an amide coupling reaction between the differentially protected citrate **8** and the L-lysine ester **9** (Scheme 1).

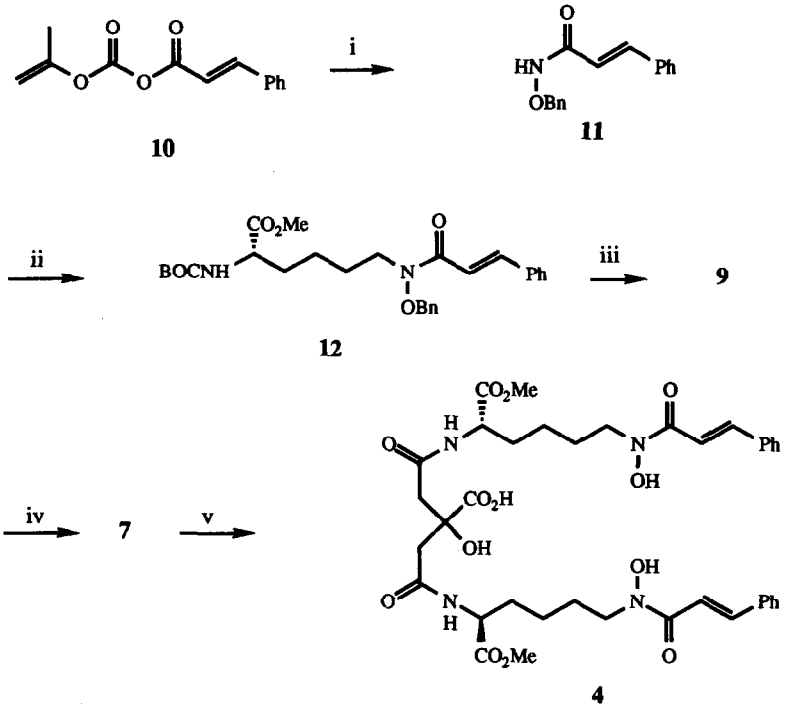


7

8 **9**

Scheme 1

Thus, the *O*-benzyl-*N*-cinnamoyl hydroxylamine **11** was first prepared starting from cinnamic acid following reaction with isopropenyl chloroformate to form the mixed anhydride **10** and condensation of the latter with *O*-benzylhydroxylamine. *N*-Alkylation of the protected amide **11** with L-*N*-BOC-ε-bromonorleucine,[10] next gave the double *N*-protected nannochelin sidearm **12** which on treatment with trifluoroacetic acid led to the L-lysine ester **9**, ready for coupling to the citrate unit **8** (Scheme 2).



Scheme 2

Reagents: *i*; $BnONH_2$, Et_3N, CH_2Cl_2, RT, 30 min; *ii*; L-*N*-BOC-ε-bromonorleucine, KI, K_2CO_3, Me_2CO, reflux, 24h; *iii*; TFA, 0°, 10min; *iv*; **8**, Et_3N, CH_3CN, RT, 4.5h ; *v*; BF_3-Et_2O, EtSH, CH_2Cl_2, RT, 10h

The differentially protected citrate **8** was prepared from citric acid as described previously by Maurer and Miller.[10] When a solution of the amino ester **9** and the doubly activated citrate ester **8** in acetonitrile was stirred at room temperature in the presence of triethylamine, work up and chromatography gave *bis*-benzylnannochelin **7** as a semi-solid in 31% yield. The problem of debenzylation of **7** to give the free carboxylic acid *bis*-hydroxylamine **4**, without simultaneously affecting the cinnamoyl double bonds in **7** was overcome by the use of a boron trifluoride etherate-ethanethiol reagent.[11] Using this deprotection protocol nannochelin A **4** was produced as a colourless glass, which displayed an ^1H n.m.r. spectrum closely superimposable on that of naturally derived nannochelin A. Its ^{13}C n.m.r. data were also closely similar to those reported for the natural product,[8] and in mass spectrometry it displayed a molecular ion (electron impact, negative ion spectrum) and M + 23 (Na), M + 39 (K) (electrospray methods). The optical rotation was slightly lower than the literature values (see Experimental) suggesting that minor racemisation/epimerisation has

occurred, although the meso form could not be detected in the n.m.r. spectra. The synthetic nannochelin A was shown to act as a chelator for ferric ion, and its biological activity together with that of some of its analogues will be discussed elsewhere.

Experimental

General Details: Optical rotations were measured on a JASCO DIPA-370 polarimeter. Ultraviolet spectra were recorded on a Philips PU 8700 spectrophotometer as solutions in spectroscopic grade ethanol. Infrared spectra were obtained using a Perkin-Elmer 1720-X or Perkin-Elmer 1600 series FT-IR instrument as either liquid films or potassium bromide discs. Proton N.M.R. spectra were recorded on Bruker WP 80 SY (80MHz), Bruker WM 250 (250MHz), Bruker AM 400 (400MHz) or Jeol EX-270 (270MHz) spectrometer. The chemical shifts are recorded relative to an internal tetramethylsilane standard and the multiplicity of a signal is designated by one of the following abbreviations: s, singlet; d, doublet; t, triplet; q, quartet; brd, broad; m, multiplet. All observed couplings, *J*, are reported in Hertz. Carbon-13 n.m.r.. spectra were recorded on Bruker WM 250 (62.9MHz), Bruker AM 400 (100.6MHz) or Jeol EX-270 (67.8MHz) instruments. The spectra were recorded as dilute solutions in deuterio-solvents with chemical shifts reported relative to internal tetramethylsilane or chloroform standard on a broad band decoupled mode, and the number of attached hydrogen revealed using a DEPT sequence. Mass spectra were recorded on AE1 MS-902, MM-701CF or a Finnigan TSQ 700 spectrometers using electron ionisation (EI), fast atom bombardment (FAB) or electrospray (ES) techniques.

O-Benzyl-N-Cinnamoyl Hydroxylamine (**11**). Isopropenyl chloroformate (387 μL, 3.37 mmol) was added over 5 min to a stirred solution of cinnamic acid (0.5 g, 3.37 mmol) and triethylamine (470 μL, 3.37 mmol) in dry dichloromethane (20 μL) at 0°C. The solution was allowed to warm to ambient temperature, and then stirred for 30 min under an atmosphere of nitrogen. This solution was added *via* cannula to a solution of O-benzyl hydroxylamine hydrochloride (0.415 g, 3.37 mmol) and triethylamine (470 μL, 3.37 mmol) in dry dichloromethane (20 mL), and the solution was then allowed to stir overnight at ambient temperature under an atmosphere of nitrogen. The mixture was washed with water (30 mL), and the separated organic extract was then dried over anhydrous magnesium sulphate, filtered and evaporated *in vacuo* to leave a solid residue. Chromatography on silica gel eluting with 3:2 hexane:ethyl acetate gave a white solid which was recrystallised from ethyl acetate:hexane to produce the *hydroxylamine* (0.35 g, 49%) as white crystals. m.p. 100-101°C; ν_{max} 3157, 2964, 1650, 1613, 1508, 1028, 745 and 695 cm^{-1}; δ_H (400 MHz; $CDCl_3$) 8.66 (1H, broad s, N*H*), 7.66 (1H, d, *J* 15.5 Hz, C*H*), 7.33-7.47 (10H, m, 2 x Ph), 6.49 (1H, d, *J* 15.4Hz, C*H*), 4.98 (2H, s, CH_2); δ_C (100 MHz; $CDCl_3$) 177.3 (CO), 142.5 (CH), 135.5 (C), 135.1 (C), 130.1 (CH), 129.3 (CH), 128.9 (CH), 128.7 (CH), 128.0 (CH), 116.6 (CH), 78.5 (CH_2); m/z (EI) Found 253.105; $C_{16}H_{15}NO_2$ requires 253.110.

Nε-Cinnamoyl-Nε-Benzyloxy-Nα-Boc-L-Lysine Methyl Ester (**12**). A mixture of the amide (**11**) (1.23 g, 4.86 mmol), L-*N*-BOC-ε-bromonorleucine[8] (1.31 g, 4.05 mmol), potassium iodide (0.134 g, 0.807 mmol) and anhydrous potassium carbonate (1.39 g, 0.01 mol) in dry acetone (35 mL) was stirred and heated at reflux for 24h under an atmosphere of nitrogen. The solution was cooled, filtered and evaporated to dryness in vacuo and the resulting slurry was then taken into ether (50 mL) and washed with 0.5 M sodium hydroxide (30 mL). The organic phase was dried over anhydrous magnesium sulphate, filtered, and concentrated *in vacuo* to leave a yellow residue. Chromatography on silica gel eluting with 3:1 hexane:ethyl acetate gave the substituted *amide* (0.76 g, 32%) as a yellowish oil; $[\alpha]_D$ +2.56° (c 1.47, CH_2Cl_2, 24°C); ν_{max} 3321, 2950, 1713, 1652, 1616, 1394, 1366, 1168, 982, 762 and 701 cm^{-1}; δ_H (400 MHz; $CDCl_3$) 7.58 (1H, d, *J* 15.8Hz, C*H*), 7.18-7.39 (10H, m, 2 x Ph), 6.90 (1H, d, *J* 16Hz, C*H*), 5.07 (1H, d, *J* N*H*), 4.78 (2H, s, CH_2), 4.18 (1H, m, C*H*), 3.63-3.68 (2H,

m, CH_2), 3.61 (3H, s, OCH_3), 1.17-1.70 (6H, m, 3 x CH_2) 1.34 (9H, s, 3 x CH_3); δ_C (100 MHz; $CDCl_3$) 172.6 (CO), 166.6 (CO), 154.8 (CO), 142.6 (CH), 134.5 (C), 133.7 (C), 129.1 (CH), 128.6 (CH), 128.4 (CH), 128.1 (CH), 127.3 (CH), 115.7 (CH), 79.1 (C), 76.6 (CH_2), 52.6 (CH), 51.5 (CH_3), 44.7 (CH_2), 31.5 (CH_2), 27.7 (CH_3), 25.9 (CH_2), 21.8 (CH_2); m/z (EI) Found 396.203 (M^+ - BOC); $C_{23}H_{27}N_2O_4$ requires 396.205.

Nε-Cinnamoyl-Nε-Benzyloxy-L-Lysine Methyl Ester (**9**). The protected amine (**12**) (0.23 g, 0.0463 mmol) was stirred with trifluoroacetic acid (1.5 mL) for 10 mins at 0°C and then the excess trifluoroacetic acid was removed *in vacuo*. The residue was taken into chloroform (10 mL) and the solution was washed with 1M sodium bicarbonate (10 mL), then dried over anhydrous magnesium sulphate, filtered, and concentrated *in vacuo* to leave the free amine (0.163 g, 88%) as a yellow oil. The amine was not purified further and was used directly in the next reaction.

Tris-Benzyl Nannochelin A (**7**). A solution of the free amine (**9**) (71 mg, 0.179 mmol) in dry acetonitrile (2 mL) was added in one portion to a stirred solution of the ester (**8**) (47 mg, 0.089 mmol) and triethylamine (24.9 mL, 0.179 mmol) in dry acetonitrile (8 mL) and the mixture was then stirred at ambient temperature for 4.5h under an atmosphere of nitrogen. The solution was evaporated to dryness *in vacuo* and the residue was then taken into dichloromethane (15 mL). The organic solution was washed with water (2 x 15 mL), then dried over anhydrous magnesium sulphate, filtered, and evaporated *in vacuo* to afford a yellow residue. Chromatography on silica gel eluting with ethyl acetate gave *tris-benzyl nannochelin* (27 mg, 30.6%) as a pale brown semi-solid; $[\alpha]_D$ -5.37° (c 2.05, CH_2Cl_2, 23°C); ν_{max} 3317, 2949, 1742, 1715, 1649, 1537, 980, 754 and 700 cm^{-1}; δ_H (400 MHz; $CDCl_3$) 7.65 (2H, d, *J* 15.8 Hz, 2 x C*H*), 7.27-7.57 (25H, m, 5 x Ph), 7.00 (2H, d, *J* 15.7 Hz, 2 x C*H*), 5.19 (2H, s, CH_2), 4.86 (4H, s, 2 x CH_2), 4.45-4.53 (2H, m, 2 x C*H*), 3.68-3.82 (10H, m, 2 x OCH_3, 2 x CH_2), 2.61-2.90 (4H, m, 2 x CH_2), 1.60-1.88 (8H, m, 4 x CH_2), 1.35-1.50 (4H, m, 2 x CH_2); δ_C (100 MHz, $CDCl_3$) 174.1 (CO), 173.8 (CO), 171.9 (CO), 171.6 (CO), 169.50 (CO), 167.4 (CO), 167.3 (CO), 143.6 (CH), 143.4 (CH), 135.6 (C), 135.1 (C), 135.0 (C), 134.4 (C), 134.3 (C), 129.9 (CH), 129.9 (CH), 129.3 (CH), 129.1 (CH), 128.8 (CH), 128.5 (CH), 128.3 (CH), 128.2 (CH), 128.2 (CH), 128.0 (CH), 116.2 (CH), 116.1 (CH), 77.4 (CH_2), 74.5 (C), 67.5 (CH_2), 52.7 (CH_3), 52.6 (CH_3), 52.3 (CH), 52.1 (CH), 45.3 (CH_2), 45.2 (CH_2), 44.2 (CH_2), 31.0 (CH_2), 30.8 (CH_2), 26.5 (CH_2), 26.4 (CH_2), 22.8 (CH_2), 22.7 (CH_2); m/z (FAB) Found; 1039 (MH^+,10%), 1061 (MNa^+, 2%); $C_{59}H_{48}N_4O_{13}$ requires M 1038.

Nannochelin A (**4**). A solution of the benzyl ester (**7**) (59 mg, 0.0568 mmol), boron trifluoride-etherate (164 µL, 1.36 mmol) and ethanethiol (0.85 mL) in dry dichloromethane (2 mL) was stirred at ambient temperature for 10h under an atmosphere of nitrogen. The mixture was poured onto water (5 mL), and the product was then extracted into ethyl acetate (20 mL). The ether solution was washed with sat. brine (10 mL) and water (10 mL), then dried over anhydrous sodium sulphate, filtered, and concentrated *in vacuo* to leave a brown solid. Chromatography on silica gel eluting with 9:1 dichloromethane:methanol gave nannochelin A (13.1 mg, 30%) as an almost colourless glass; $[\alpha]_D$ -9.56° (c 0.79, MeOH, 33°C) (lit.[8] $[\alpha]_D$ -13° (c 0.9, MeOH, 25°C) (lit.[9] $[\alpha]_D$ -12°C (c.0.05, MeOH, 26°C)); λ_{max} 278.4nm (lit [8]. 280nm); ν_{max} 3356, 2924, 1738, 1644, 1580, 1453 and 736 cm^{-1}; δ_H (400 MHz; CD_3OD) 7.62 (6H, m, 2 x C*H* arom., 2 x C*H* olefin), 7.41 (8H, m, 6 x C*H* arom., 2 x C*H* olefin.), 4.45 (2H, m, 2 x C*H*), 3.74-3.81 (10H, m, 2 x CH_2, 2 x OCH_3), 2.83 (4H, m, 2 x CH_2), 1.89, 1.76, 1.48 (12H, m, 6 x CH_2); δ_C (100 MHz; CD_3OD) 177.0 (CO), 174.4 (CO), 172.6 (CO), 172.3 (CO), 168.7 (CO), 144.1 (CH), 136.8 (C), 131.3 (CH), 130.3 (CH), 129.3 (CH), 117.9 (CH), 75.4 (C), 53.8 (CH), 53.1 (CH_3), 44.9 (CH_2), 32.3 (CH_2), 31.0 (CH_2), 27.5 (CH_2), 24.0 (CH_2); m/z (ESMS) Found 768.6; 767.1 (M^+-1), 768.6 (M^+), 791.3 (MNa^+), 807.4 (MK^+); $C_{38}H_{48}N_4O_{13}$ requires M 768.9.

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