# Stereochemical Assignment of the Pyochelins[1]

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The synthesis of pyochelin has been much improved but gives four stereoisomers. The stereochemistry of the two naturally occurring pyochelins I and II has been assigned, based on (1) the similarity of the NMR spectra of pyochelin I methyl ester to those for 4-methylpyochelin I methyl ester, whose X-ray structure has been determined and relative stereochemistry assigned; (2) comparison of the rotation of the natural material to that of pyochelin I methyl ester synthesized from *N*-methyl-L-cysteine methyl ester, which is not expected to epimerize during the synthesis and thus assigns pyochelin I methyl ester as 4′*R*,2″*R*,4″*R*; and (3) the known facile epimerization at C-2″ of 2″-substituted thiazolidine-4-carboxylic acids, which assigns the other (unfavored) naturally occurring isomer, pyochelin II, as 4′*R*,2″*S*,4″*R*. The remaining two synthetic products, neopyochelin I methyl ester and neopyochelin II methyl ester, were assigned the stereochemistry 4′*S*,2″*S*,4″*R* and 4′*S*,2″*R*,4″*R*, respectively, based on (1) the use of *N*-methyl-L-cysteine methyl ester in their synthesis, which establishes C-4″ as *R*; (2) the known instability at C-2″, which favors neopyochelin II (2″*R*) over neopyochelin I (2″*S*); and (3) the requirement of nonidentity with pyochelins I and II, which requires the *S* configuration at C-4′.

Pyochelin (**1a**, Scheme 1) is a unique iron-chelating siderophore isolated from *Pseudomonas aeruginosa*.[2,3] As produced in nature pyochelin exists as a mixture of two interconvertible isomers, pyochelins I and II, whose absolute and relative configurations at the three chiral centers have not previously been assigned. The proposed structure of pyochelin has been corroborated by total synthesis[4] and further spectroscopic studies,[5] and three mutasynthetic analogues were recently isolated from a salicylic acid idiotrophic mutant (Sal$^-$ phenotype).[6,7] We have found that the free carboxylic acids cannot be isolated in pure form due to their rapid interconversion, but the methyl esters (**1b**) are more stable configurationally and can be easily purified. We report here the assignment of the absolute stereochemistry of the natural and synthetic isomers as well as the mutasynthetic analogues of pyochelin, based on spectral data and an X-ray crystal structure. We also report a significant improvement in the synthetic approach to the compounds, one that provides four of the eight possible stereoisomers with the ring system of pyochelin.[1]

## Results

**Synthesis of Pyochelin.** The previous synthetic approach to the pyochelins (Scheme 1) proceeded in 8% overall yield from salicylonitrile (**2**), with the critical step being the selective thexylborane reduction of the acid **3a**[8] to provide the very unstable aldehyde **4** in 15% yield. Condensation of **4** with *N*-methylcysteine[9] was reported to provide three isomers of pyochelin (pyochelins I and II and neopyochelin). A significant improvement in the yield of **4** (to 75%) was obtained in the present study through a diisobutylaluminum hydride (DIBAL-H) reduction of the methyl ester **3b**, followed by quenching with saturated ammonium chloride.[10] The aldehyde proved too unstable to provide acceptable analytical data, although a $^1$H NMR spectrum on the partially purified material clearly showed the presence of the aldehyde proton and the H-4′ proton. The aldehyde was immediately used for the condensation with *N*-methylcysteine, as before, for an overall 65% yield of *four* carboxylic acids (pyochelins I and II and neopyochelins I and II) from **2**. Pyochelins I and II were identical to the natural product isomers by TLC and $^1$H NMR spectroscopy; neopyochelin II was identical to the third isomer isolated from the previously reported synthesis,[4] while neopyochelin I was previously unreported (although the compound has been detected in earlier samples of neopyochelin II). The carboxylic acids resisted all attempts at preparative separation by chromatographic means.

**Isolation and Spectral Characterization of Pyochelin Methyl Esters.** The carboxylic acids **1a** were converted to the methyl esters **1b** with ethereal diazomethane,[4] and the esters could be successfully separated and purified by repeated silica gel chromatography using carbon tetrachloride–ethyl acetate and hexane–isopropyl alcohol solvent mixtures. Although the $^1$H and $^{13}$C NMR spectra (summarized in Tables 1 and 2, respectively) were

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**Scheme 1**

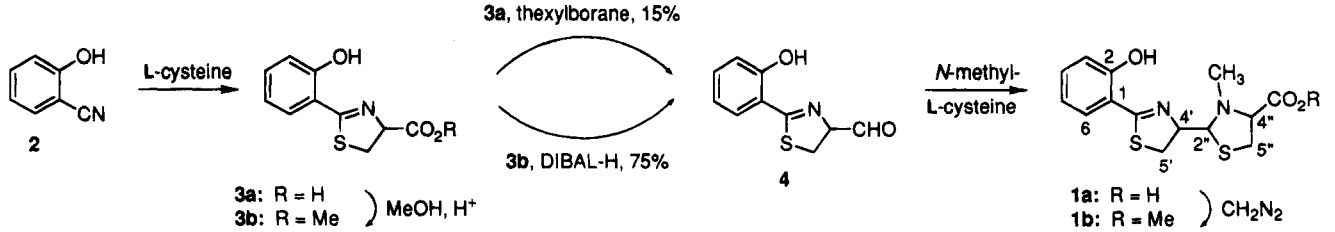



**Table 1. $^1$H NMR Spectral Data for Two Mutasynthetic and Four Synthetic Pyochelin Methyl Ester Isomers**

| | δ, m (J in Hz)[a] | | | | | |
|---|---|---|---|---|---|---|
| proton, H- | 4-methylpyochelin I | 4-methylpyochelin II | pyochelin I | pyochelin II | neopyochelin I | neopyochelin II |
| 3 | 6.73, s | 6.73, s | 6.99, d (7.5) | 6.99, d (7.5) | 6.99, d (7.5) | 6.99, d (7.5) |
| 4 | | | 7.35, t (7.9) | 7.35, t (7.9) | 7.35, t (7.9) | 7.35, t (7.9) |
| 5 | 6.61, dd (8.0, 1.0) | 6.61, dd (7.9, 0.9) | 6.87, t (7.7) | 6.87, t (7.7) | 6.87, t (7.7) | 6.87, t (7.7) |
| 6 | 7.20, d (8.0) | 7.19, d (7.9) | 7.41, d (7.4) | 7.41, d (7.4) | 7.41, d (7.4) | 7.41, d (7.4) |
| 4′ | 5.08, td (8.8, 5.2) | 4.97, q (7.7) | 5.08, td (8.8, 5.0) | 4.92, q (8.2) | 5.12, td (9.1, 4.6) | 4.79, q (8.0) |
| 5′a | 3.38, dd (11.2, 8.5) | 3.43, dd (11.2, 8.6) | 3.48, dd (11.3, 8.6) | 3.52, dd (11.8, 8.2) | 3.42, dd (10.9, 8.2) | 3.51, dd (11.3, 6.9) |
| 5′b | 3.33, dd (11.2, 8.9) | 3.30, dd (11.2, 8.2) | 3.41, dd (11.3, 9.3) | 3.40, dd (11.2, 8.2) | 3.37, dd (10.9, 9.5) | 3.49, dd (11.3, 7.1) |
| 2″ | 4.44, d (5.1) | 4.48, d (6.9) | 4.53, d (5.2) | 4.56, d (6.9) | 5.04, d (4.6) | 4.24, d (7.7) |
| 4″ | 3.57, dd (9.1, 6.3) | 4.01, t (6.0) | 3.65, dd (9.1, 6.3) | 4.09, t (6.0) | 4.13, dd (6.4, 2.1) | 3.83, t (6.8) |
| 5″a | 3.10, dd (10.5, 9.2) | 3.17, dd (10.7, 6.5) | 3.13, dd (10.8, 9.1) | 3.25, dd (10.7, 6.5) | 3.29, dd (10.7, 6.2) | 3.43, dd (11.2, 6.6) |
| 5″b | 3.03, dd (10.6, 6.2) | 3.10, dd (10.7, 5.6) | 3.05, dd (10.8, 6.3) | 3.18, dd (10.5, 5.7) | 3.02, dd (10.7, 2.0) | 3.24, dd (11.2, 6.8) |
| $NCH_3$ | 2.51, s | 2.41, s | 2.56, s | 2.59, s | 2.56, s | 2.59, s |
| $OCH_3$ | 3.68, s | 3.70, s | 3.76, s | 3.78, s | 3.77, s | 3.77, s |
| $CH_3$ | 2.29, s | 2.26, s | | | | |

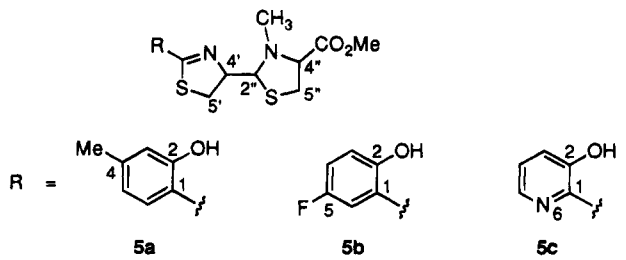
[a] Chemical shift (δ) in ppm; multiplicity, m: s = singlet, d = doublet, t = triplet, q = quartet.

**Table 2. $^{13}$C NMR Spectral Data[a] and Optical Rotations[b] for Two Mutasynthetic and Four Synthetic Pyochelin Methyl Ester Isomers**

| carbon, C- | 4-methylpyochelin I | 4-methylpyochelin II | pyochelin I | pyochelin II | neopyochelin I | neopyochelin II |
|---|---|---|---|---|---|---|
| 1 | 113.8[c] | 113.8[c] | 116.1 | 116.2 | 116.2 | 116.1 |
| 2 | 158.9 | 158.9 | 159.0 | 159.1 | 159.1 | 159.0 |
| 3 | 117.2[c] | 117.3[c] | 117.0 | 117.1 | 117.0 | 117.0 |
| 4 | 144.1 | 144.1 | 133.0 | 133.1 | 133.0 | 132.9 |
| 5 | 120.0 | 120.0 | 118.8 | 118.7 | 118.7 | 118.6 |
| 6 | 130.2 | 130.2 | 130.6 | 130.6 | 130.6 | 130.3 |
| 2′ | 170.6 | 170.6 | 171.3 | 170.6 | | 171.9 |
| 4′ | 79.1 | 79.7 | 79.4 | 79.9 | 78.4 | 81.9 |
| 5′ | 32.8 | 34.0 | 32.9 | 34.7 | 32.0 | 33.7 |
| 2″ | 76.0 | 75.7 | 76.1 | 75.5 | 72.7 | 77.6 |
| 4″ | 72.3 | 70.4 | 72.4 | 70.4 | 70.0 | 73.0 |
| 4″a | 172.5 | 172.5 | 172.8 | 172.7 | 170.2 | 172.4 |
| 5″ | 32.2 | 31.9 | 32.3 | 32.0 | 31.8 | 33.4 |
| $NCH_3$ | 41.2 | 37.8 | 41.2 | 37.7 | 36.3 | 44.2 |
| $OCH_3$ | 52.4 | 52.3 | 52.3 | 52.2 | 51.8 | 52.4 |
| Ar-$CH_3$ | 21.7 | 21.7 | | | | |
| $[\alpha]^{20}_D$ (c, $CHCl_3$) | +36.2° (0.2)[d] | −46.6° (0.1)[d] | +54.8° (0.4) | −51.2° (1.2) | −140° (0.3) | −0.9° (5.0) |

[a] In δ, ppm downfield of TMS in $CDCl_3$ solution. [b] In $CHCl_3$ solution. [c] Resonances may be interchanged. [d] $[\alpha]^{25}_D$.

**Scheme 2**




characteristically different for each of the four isomers, there were some similarities in the chemical shifts and coupling patterns for specific pairs of the isomers (e.g., pyochelin I and neopyochelin I; pyochelin II and neopyochelin II). However, the stereochemistry of the individual isomers could not be unambiguously assigned a priori from the spectra. Attempts to obtain nuclear Overhauser enhancements were unsuccessful, possibly due to nonrigid conformations of the contiguous rings.

**X-ray Structure of 4-Methylpyochelin I.** As noted above, we have recently described the isolation and structures of a series of mutasynthetic pyochelin analogues (**5a-c**, Scheme 2) derived from a Sal$^-$ phenotype of *Ps. aeruginosa*.[6,7] The methyl ester (**5a**) of the major mutasynthetic product derived from 4-methylsalicylic acid crystallized from MeOH in a form suitable for X-ray analysis. Of 1805 unique intensities only the 467 "observed" data were used for refinement of the proposed model. The structure was solved by direct methods. The final agreement factors were $R = 0.093$ and $R_w = 0.082$. Due to paucity of data the absolute configuration could not be assigned by anomalous dispersion. An ORTEP plot of the structure is shown in Figure 1. Of the features shown in the crystal structure, relative stereochemistry and the spatial arrangement of the three rings are of the most interest. The phenolic ring and the thiazoline ring are nearly coplanar, with a C-2, C-1, C-2′, N-3′ torsional angle of −3°, while the thiazoline and thiazolidine rings

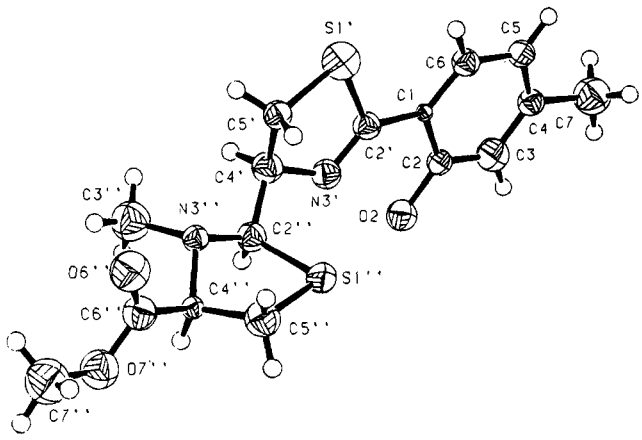



**Figure 1.** ORTEP view of 4-methylpyochelin I methyl ester.

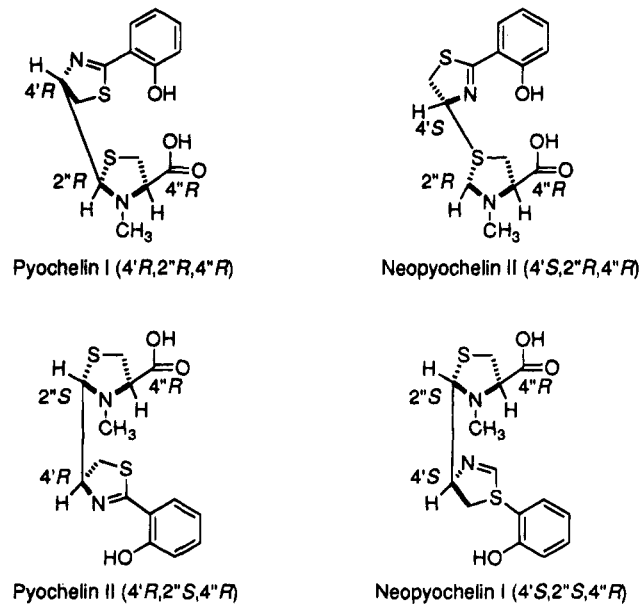
are considerably skewed relative to each other with an N-3′, C-4′, C-2″, S-1″ torsional angle of −67° and an N-3′, C-4′, C-2″, N-3″ torsional angle of −55°. The thiazolidine ring is the thermodynamically favored 2′,4′-cis disubstituted isomer. The crystal structure clearly defines the stereochemistry to be 4′*R*,2″*R*,4″*R* or, far less likely (cf. below), 4′*S*,2″*S*,4″*S*.[11]

The spectral parameters (chemical shift, coupling constants) of the heterocyclic ring protons (H-4′, H-2″, and H-4″) for the two pyochelin isomers (I, II) of synthetic **1b** corresponded very closely to the parameters for the two corresponding mutasynthetic 4-methylpyochelins I and II (**5a**).[1,7] In fact, a similarity of spectral data and optical rotations (sign and magnitude) for the corresponding isomers of all three pairs of mutasynthetic pyochelins has been noted.[7] This result is to be expected because incorporation of the mutasynthetic precursors (4-methylsalicylic acid, 5-fluorosalicylic acid, and 3-hydroxypicolinic acid) changes only the aromatic chromophore of the derived mutasynthetic pyochelins **5a-c** and should not affect the stereochemical integrity of the remainder of the product. This leads to the reasonable conclusion that, given the similarity of spectral data and optical rotations between synthetic pyochelin I (and II) and "natural"[12] 4-methylpyochelin I (and II), pyochelin I has the absolute configuration 4′*R*,2″*R*,4″*R* or, less likely, 4′*S*,2″*S*,4″*S*. Further spectral and chemical analysis of the remaining synthetic pyochelin isomers, discussed below, leads to the stereochemical assignments of 4′*R*,2″*S*,4″*R* for pyochelin II, 4′*S*,2″*S*,4″*R* for neopyochelin I, and 4′*S*,2″*R*,4″*R* for neopyochelin II. The stereostructures of all four isomers are shown in Scheme 3.

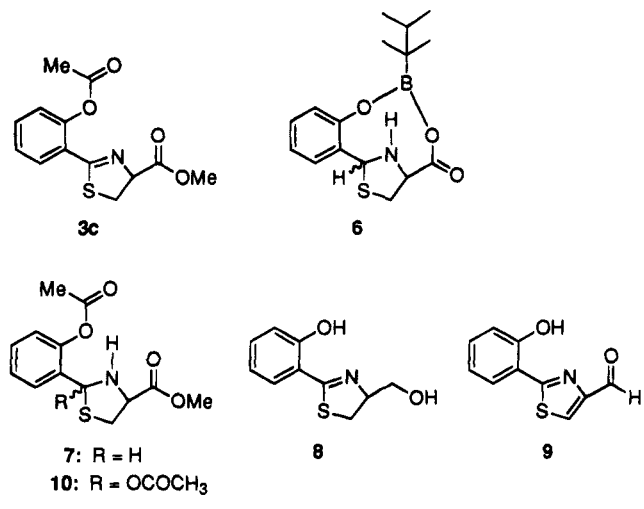
## Discussion

**Synthetic Approach.** During development of the synthetic sequence, the reduction of **3a** to the critical aldehyde **4** was reinvestigated. The low yield from the previously reported thexylborane reduction of **3a** was explained by the isolation in the present study of the intramolecularly chelated alkylboronate **6** (Scheme 4), whose formation blocks reduction of the carboxyl group. The structure of **6** was assigned from its molecular formula, $C_{16}H_{22}BNO_3S$, in accord with HRFABMS data, a carboxyl IR band at 1730 $cm^{-1}$, the intact thexyl (two

**Scheme 3**




**Scheme 4**




methyl singlets, two methyl doublets), *o*-phenylene units in the $^1H$ NMR spectrum (cf. Experimental Section), and a thiazolidine unit with $^1H$ NMR signals at 7.82 ppm (br s, H-3), 5.92 (d, H-2), 4.85 (td, H-4), 3.52 (dd, H-5a), and 3.26 (dd, H-5b). This compound, which can be isolated in 77% yield from the thexylborane reaction mixture, is extremely stable to acidic and basic hydrolytic conditions. Its structure also suggested that the reduction of the thiazoline C=N double bond was particularly facile.

The complexation of boron with the free phenolic hydroxyl suggested protection of the phenol as its acetate. However, the free carboxylic acid proved difficult to handle, so the methyl ester of the acetate (**3c**) was prepared and was treated with DIBAL-H at −78 °C. In this case, reduction of the thiazoline C=N double bond again proved more facile than reduction of the carbomethoxy group, as evidenced by the isolation of **7** in 67% yield. The structure assigned (**7**) derives from its molecular formula ($C_{13}H_{15}NO_4S$, microanalyses, LRE-

(11) Although the ORTEP plot indicates an (*R*,*R*,*R*) configuration, (*S*,*S*,*S*) could not be ruled out here because the absolute stereochemistry could not be determined from the X-ray data.

(12) Although the mutasynthetic pyochelins are not normal metabolic products of *Ps. aeruginosa*, they can be considered "natural" in the sense that they were derived from this microbial source, as opposed to a synthetic source.

IMS) and the $^1$H NMR spectrum (Experimental Section), which showed an intact *o*-phenylene acetate, carbomethoxy, and five thiazolidine hydrogens ($SCH_2CHN$, ABX; NCHS, br s; NH, br s, exch) similar to those noted above for **6**.

Alternative approaches to the production of **4** that involved the reoxidation of alcohol **8**[13] (which can be obtained as a byproduct of the thexylborane reaction or produced by a sodium borohydride reduction of methyl ester **3b**) proved fruitless. Several variations of the Swern-type DMSO-based oxidations[14] provided the known aeruginaldehyde[5] **9** as the only isolable product. This implies that oxidation and aromatization of the thiazoline ring is more facile than oxidation of the alcohol to an acid, but whether aromatization precedes or follows oxidation to the aldehyde has not been determined.

**Assignment of Stereochemistry.** The following arguments were employed:

First, bacterial pyochelin I was shown[4] to be identical with synthetic pyochelin I and the stereochemistry of the latter is the same as that of 4-methylpyochelin I, since their methyl esters have similar rotations and $^1$H and $^{13}$C NMR signals (Tables 1 and 2). Thus, their *relative* stereochemistry is assigned as 4′*R*,2″*R*,4″*R* (or 4′*S*,2″*S*,4″*S*) by the X-ray study of the 4-methyl analog.

Second, the present synthesis gives only four stereoisomers instead of the possible eight allowed by the three chiral centers. The fixed center is assigned as C-4″ since there is no record of isomerization at this center during thiazolidine syntheses under the present conditions.[15,16] Since C-4″ is derived from *N*-methyl L-cysteine, it is assigned as *R*. Taken with the X-ray relative stereochemistry this completes the stereochemical assignment of pyochelin I, and by extension 4-methylpyochelin I, as 4′*R*,2″*R*,4″*R*. With a fixed C-4″ the observed four isomers must be due to isomerism at C-4′ and C-2″.

Third, there is ample precedent for isomerization at C-2 of 2-substituted thiazolidine-4-carboxylic acids,[17] hence at C-2″ of pyochelin. In fact, pyochelin I (natural or synthetic) isomerizes spontaneously to a mixture with pyochelin II and vice versa,[4] with pyochelin I favored by *ca.* 3:1. Similarly, in the present study the methyl esters of pyochelins I and II equilibrate with each other as do the methyl esters of neopyochelins I and II (*ca.* 1:3 ratio). These ratios are typical of *cis,trans* isomerization of 2,4-disubstituted thiazolidines. These observations assign pyochelin II the 4′*R*,2″*S*,4″*R* configuration and indicate that neopyochelins I and II differ at C-2″.

Fourth, since C-4″ is fixed as *R* in both neopyochelins I and II and neopyochelin II is favored in the equilibrium it must be *R* at C-2″ with C-2″ being *S* in neopyochelin I. By default, both neopyochelins must then be *S* at C-4′. Significantly, the aldehyde **4** employed in the present synthesis had no detectable rotation, arguing for a racemate in **4**,[18] which would yield a mixture of stereoisomers at C-4′, leading to the pyochelin–neopyochelin pairs and the four stereoisomers shown in Scheme 3. The drawings in Scheme 3 illustrate a point of interest: pyochelin I and neopyochelin II both contain *cis*-2,4-disubstituted thiazolidine rings, which leads to equilibria (pyochelin I ⇌ pyochelin II; neopyochelin I ⇌ neopyochelin II) favoring the *cis* isomers as noted above. Moreover, the presence of the phenol and carboxyl groups on the same side of the thiazolidine ring promotes chelation, and this is manifest by partitioning of the four isomers between chloroform and aqueous zinc chloride.[5] The two *cis* isomers (pyochelin I and neopyochelin II) cleanly partition into the aqueous layer, while the *trans* isomers (pyochelin II and neopyochelin I) remain in the chloroform layer.

On biogenetic considerations, one would expect the configuration in the natural products (pyochelins I and II) to be 4′*S*,2″*R*,4″*R* and 4′*S*,2″*S*,4″*R*, respectively, if both centers are derived from L-cysteine.[19] However, the configurations at C-4′ for natural pyochelins I and II are found to be 4′*R*. This implies either that the natural product is biosynthesized from one molecule each of L- and D-cysteine or that the configuration at C-4′ is epimerized at a late stage in the biosynthetic pathway. D-Amino acids are well known in bacteria, so the incorporation of D-cysteine into pyochelin would not be surprising.

## Experimental Section

**General.** $^1$H NMR spectra were obtained on an NT-360 spectrometer operating at 360 MHz, and chemical shifts ($\delta$) are reported as downfield from TMS (internal). IR spectra were obtained on a 30-S FTIR spectrophotometer. Fast atom bombardment (FAB) mass spectra were obtained in a dithioerythritol–dithiothreitol matrix ("magic bullet")[20] on a ZAB SE instrument operating in the positive ion mode with an Ion Tech fast atom gun and Xe atoms (8 keV). Electron ionization (EI) mass spectra were obtained on a CH5 instrument operating at 70 eV accelerating potential, and field desorption (FD) mass spectra were obtained on a 731 mass spectrometer. Optical rotations were obtained in dilute solution on a DIP-360 digital polarimeter.

**Methyl 2-(2-Hydroxyphenyl)-2-thiazoline-4-carboxylate (3b).** Acid **3a**[4,8] (10.2 g, 45.7 mmol) was dissolved in MeOH (500 mL), and concd $H_2SO_4$ (20 mL) was added. The mixture refluxed under $N_2$ overnight and then was cooled, concd, and diluted with $H_2O$. The aqueous suspension was extracted with ether (3 × 100 mL) and worked up to yield **3b**, obtained analytically pure as a light-yellow syrup (9.98 g, 42.1 mmol, 92% yield) that slowly solidified on standing in a freezer for 4 days (mp 42–44 °C): $^1$H NMR ($CDCl_3$) $\delta$ 7.65 (1 H, dt, $J$ = 7.8, 1.4 Hz), 7.61 (1 H, dd, $J$ = 7.9, 1.2 Hz), 7.28 (1 H, d, $J$ = 8.1 Hz), 7.13 (1 H, t, $J$ = 7.2 Hz), 5.58 (1 H, dd, $J$ = 9.5, 9.4 Hz), 4.06 (3 H, s), 3.90 (1 H, dd, $J$ = 11.3, 9.4 Hz), 3.81 (1 H, dd, $J$ = 11.3, 9.5 Hz); $^{13}$C NMR ($CDCl_3$) $\delta$ 174.3, 170.6, 159.1, 133.5, 130.7, 118.9, 117.2, 116.1, 76.7, 52.8, 33.6; IR (neat) 1744, 1622, 1593, 1489, 1437, 1402, 1296, 1221, 1155, 1120, 1061, 1035, 953, 822, 754 cm$^{-1}$; UV (MeOH) $\lambda_{max}$ (log $\epsilon$) 313 (3.66), 256 (4.04), 250 (4.04), 212 (4.34); EIMS *m/z* 237, 178, 150, 121, 119, 59. The compound was optically inactive.

(13) Alcohol **8** has recently been reported as a natural product: Zunnundzhanov, A.; Bessanova, I. A.; Abdullaev, N. D.; Ogai, D. K. *Chem. Nat. Compd. (Engl. Transl.)* **1987**, *23*, 461–465.

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Anal. Calcd for $C_{11}H_{11}NO_3S$: C, 55.68; H, 4.67; N, 5.90; S, 13.51. Found: C, 55.62; H, 4.62; N, 5.88; S, 13.51.

**Methyl 2-(2-Acetoxyphenyl)-2-thiazoline-4-carboxylate (3c).** Ester **3b** (10.2 g, 43.0 mmol) was dissolved in $CH_2Cl_2$ (250 mL), and $Ac_2O$ (8 mL, 78 mmol) was added dropwise with stirring at 0 °C. $Et_3N$ (20 mL) and (*N,N*-dimethylamino)-pyridine (10 mg) were then added, and the mixture was stirred overnight at 25 °C. Quenching with MeOH at 0 °C and workup provided 16.4 g of a 1:1 mixture of two compounds as a sticky oil. The compounds were separated by gradient silica gel chromatography ($CHCl_3$ to $CHCl_3$–MeOH, 9:1) to provide pure **3c** (6.32 g, 22.6 mmol, 53%) as a light-brown oil: $^1H$ NMR ($CDCl_3$) $\delta$ 7.85 (1 H, dd, $J$ = 7.8, 1.6 Hz), 7.48 (1 H, dt, $J$ = 7.8, 1.6 Hz), 7.29 (1 H, dt, $J$ = 7.6, 1.0 Hz), 7.13 (1 H, dd, $J$ = 8.0, 1.0 Hz), 5.24 (1 H, t, $J$ = 9.4 Hz), 3.83 (3 H, s), 3.65 (1 H, dd, $J$ = 11.2, 9.4 Hz), 3.58 (1 H, dd, $J$ = 11.2, 9.5 Hz), 2.35 (3 H, s); $^{13}C$ NMR ($CDCl_3$) $\delta$ 170.8, 169.3, 166.5, 148.4, 132.0, 131.0, 125.9, 125.4, 123.5, 78.1, 52.5, 34.8, 21.1; IR (neat) 1767, 1740, 1602, 1597, 1485, 1441, 1367, 1290, 1220, 1188, 1105, 1045, 1012, 947, 908, 868, 819, 762, 656, 617 $cm^{-1}$; EIMS *m/z* 279, 237, 220, 178, 150, 121, 119, 59, 43.

Anal. Calcd for $C_{13}H_{13}NO_4S$: C, 55.90; H, 4.69; N, 5.02. Found: C, 55.85; H, 4.45; N, 4.93.

The second compound isolated from the mixture (5.98 g, 17.6 mmol, 41%, mp 100–102 °C) was the product of AcOH addition across the thiazoline ring double bond, methyl 2-(2-acetoxyphenyl)-2-acetoxy-2-thiazolidine-4-carboxylate (**10**): $^1H$ NMR ($CDCl_3$) $\delta$ 7.90 (1 H, dd, $J$ = 7.8, 1.4 Hz), 7.56 (1 H, dt, $J$ = 7.8, 1.4 Hz), 7.32 (1 H, t, $J$ = 7.6 Hz), 7.12 (1 H, d, $J$ = 8.0 Hz), 6.39 (1 H, $D_2O$ exch, d, $J$ = 7.2 Hz), 4.89 (1 H, dt, $J$ = 7.5, 5.6 Hz), 3.76 (3 H, s), 3.56 (1 H, dd, $J$ = 14.3, 4.8 Hz), 3.49 (1 H, dd, $J$ = 14.3, 6.0 Hz), 2.33 (3 H, s), 2.01 (3 H, s); $^{13}C$ NMR ($CDCl_3$) $\delta$ 189.2, 170.5, 170.0, 169.2, 147.7, 133.9, 129.6, 129.4, 126.1, 123.8, 52.6, 51.8, 30.7, 22.9, 21.1; IR (Nujol) 3310, 1764, 1734, 1672, 1645, 1603, 1551, 1483, 1437, 1367, 1346, 1252, 1205, 1116, 1035, 1010, 925, 902, 871, 814, 792, 777, 684, 655 $cm^{-1}$; UV (MeOH) $\lambda_{max}$ (log $\epsilon$) 262 (3.86), 235 (4.01), 204 (4.40); EIMS *m/z* 339, 297, 280, 238, 163 (100).

Anal. Calcd for $C_{15}H_{17}NO_6S$: C, 53.08; H, 5.05; N, 4.13; S, 9.45. Found: C, 53.11; H, 5.05; N, 4.13; S, 9.51.

**Pyochelins.** Methyl ester **3b** (2.52 g, 10.6 mmol) was dissolved in dry distilled $Et_2O$ (75 mL) at −78 °C under $N_2$. DIBAL-H (20 mmol, 20 mL of a 1.0 M solution in $C_6H_{14}$) was added by syringe over 30 min, and the mixture was stirred at −78 °C for 1 h and then quenched with MeOH at −78 °C, followed by saturated aqueous $NH_4Cl$ (50 mL). The mixture was warmed to 0 °C and shaken with $H_2O$ (100 mL), the organic layer was removed, and the aqueous layer was reextracted with $Et_2O$ (20 mL). The combined ether layers were worked up to leave crude 2-(2-hydroxyphenyl)-2-thiazoline-4-carboxaldehyde (**4**) as a green-yellow foam (1.89 g, 9.13 mmol, 86%), optically inactive: $^1H$ NMR ($CDCl_3$) $\delta$ 12.2 (1 H, br s), 9.80 (1 H, s), 7.50–7.62 (2 H, m), 6.98 (1 H, t, $J$ = 7.8 Hz), 6.92 (1 H, d, $J$ = 7.9 Hz), 5.22 (1 H, dd, $J$ = 9.1, 7.8 Hz), 3.68 (1 H, dd, $J$ = 11.3, 7.8 Hz), 3.37 (1 H, t, $J$ = 10.1 Hz); $^{13}C$ NMR ($CDCl_3$) $\delta$ 159.1, 133.8, 130.5, 118.2, 117.0, 83.1, 30.8. The crude aldehyde was condensed immediately with *N*-methyl-L-cysteine.

Potassium acetate (5.04 g, 63 mmol) and *N*-methyl-L-cysteine hydrochloride (2.5 g, 14 mmol)[8] were added to a solution of **4** (1.89 g, 9.13 mmol) in EtOH (200 mL) and $H_2O$ (50 mL). The mixture was stirred at 25 °C overnight and then diluted with $H_2O$, adjusted to pH 4.5 with 6 N HCl, extracted with EtOAc, and reextracted into $NaHCO_3$ and then back into EtOAc after readjusting the aqueous layer to pH 4.5. The organic layers were worked up to yield 2.39 g of crude pyochelin (7.4 mmol, 81% yield from **4**), a mixture of four isomers in about a 4:1:1:4 ratio after analysis by TLC (upper layer of 2:2:1:1 BuOH–$H_2O$–MeOH–$C_6H_{14}$), $R_f$ 0.42, 0.35, 0.26, and 0.16 (pyochelins I and II, neopyochelins I and II, respectively). The first and last isomers showed a strong, bright-blue fluorescence under long-wavelength UV light, while the two other isomers showed a darker, less fluorescent spot. All isomers were positive to $I_2$ vapor and to 0.1 M $FeCl_3$ spray reagent.

**Neopyochelin II.** Synthetic pyochelins (3.2 g) were dissolved in $CHCl_3$ (5 mL) and applied to a silica gel column (37.5 g, 22 mm × 280 mm, equilibrated with $CHCl_3$–MeOH–AcOH, 98:2:0.1). The acids were eluted with the same solvent (400 mL), followed by $CHCl_3$–MeOH–AcOH (150 mL, 95:5:1). TLC analysis of the column fractions (as above) indicated partial separation (total 1.38 g, 43%): fraction 1, 239 mg, pyochelin I ($R_f$ 0.42) and pyochelin II ($R_f$ 0.35), 3:1; fraction 2, 856 mg, mainly pyochelin II and neopyochelin I ($R_f$ 0.26), 1:1; fraction 3, 289 mg, neopyochelin II only ($R_f$ 0.16).

**Neopyochelin II methyl ester** was produced by treatment of the purified carboxylic acid with ethereal $CH_2N_2$: $^1H$ NMR ($CDCl_3$), see Table 1; $^{13}C$ NMR ($CDCl_3$), see Table 2; $[\alpha]^{25}_D$ −0.9° (*c* 5.0, $CHCl_3$).

**Pyochelin Methyl Esters (1b).** The carboxylic acids **1a** were converted to their methyl esters (**1b**) in quantitative yield by treatment in MeOH with ethereal $CH_2N_2$ at 0 °C, as previously reported.[4] The isomeric esters were isolated by repeated silica gel chromatography using $CCl_4$–EtOAc mixtures (15:1–1:1, step gradients) and $C_6H_{14}$–IPA mixtures (95:5).

**Pyochelin II Methyl Ester.** The mixed methyl esters (129 mg) were dissolved in IPA (0.5 mL), applied to a silica gel column (14 mm × 340 mm, equilibrated with $C_6H_{14}$–IPA, 95:5), and eluted with the same solvent (250 mL). Fractions were pooled after TLC and $^1H$ NMR analysis to provide (total 39.9 mg, 31%): fraction 1, 10.7 mg, pyochelin I and neopyochelin I methyl esters, 3:1; fraction 2, 19.1 mg, neopyochelins I and II methyl esters, 1:4; fraction 3, 10.1 mg, pure pyochelin II methyl ester: $^1H$ NMR ($CDCl_3$), see Table 1; $^{13}C$ NMR ($CDCl_3$), see Table 2; $[\alpha]^{25}_D$ −51.2° (*c* 1.2, $CHCl_3$).

**Neopyochelin I Methyl Ester.** A mixture of pyochelin methyl esters (256 mg) was dissolved in EtOAc (0.5 mL), applied to a silica gel column (24 mm × 300 mm, equilibrated with $C_6H_{14}$–EtOAc, 4:1), and gradient-eluted ($C_6H_{14}$–EtOAc, 4:1, 50 mL; $C_6H_{14}$–EtOAc–IPA, 4:1:0.01, 250 mL; $C_6H_{14}$–EtOAc–IPA, 2:1:0.01, 200 mL). A few fractions were shown by TLC to contain only one isomer; all others contained at least three isomers. Pure neopyochelin I methyl ester (11.7 mg) crystallized into long, thin white needles, but the crystals were not deemed suitable for X-ray analysis: $^1H$ NMR ($CDCl_3$), see Table 1; $^{13}C$ NMR ($CDCl_3$), see Table 2; $[\alpha]^{25}_D$ −140° (*c* 0.3, $CHCl_3$).

The remaining fractions were combined to provide 137 mg of mixed pyochelin methyl ester isomers (total recovery 149 mg, 57%).

**Pyochelin I Methyl Ester.** A sample of esters enriched in pyochelin I and neopyochelin I methyl esters (45 mg) was separated by semipreparative HPLC on silica gel ($C_6H_{14}$–EtOAc, 4:1; 300-$\mu$L injection volume, flow rate 4.0 mL/min). Pyochelin I methyl ester (7.9 mg) eluted between 14.0 and 16.0 min: $^1H$ NMR ($CDCl_3$), see Table 1; $^{13}C$ NMR ($CDCl_3$), see Table 2; $[\alpha]^{25}_D$ +54.8° (*c* 0.4, $CHCl_3$).

Neopyochelin I methyl ester (6.8 mg) eluted between 16.2 and 18.2 min. Total recovery was 14.7 mg, 33%.

**4-(1,1,2-Trimethylpropyl)-3,5-dioxa-4-bora-9-thia-11-aza-2-oxobicyclo[6.2.1]benz[*f*]undecane (6).** Acid **3a**[4,8] (2.334 g, 10.5 mmol) was dissolved in dry THF (10 mL) at 25 °C, thexylborane (20 mmol, 40 mL of a 0.5 M solution in THF) was added slowly in 5-mL portions over 30 min, the mixture was stirred overnight under $N_2$, and more thexylborane solution (5 mL) was added. The mixture refluxed for 45 min and then was cooled in an ice bath, and $H_2O$ (200 mL) was added, followed by 5% aqueous HCl (10 mL). The resulting suspension was filtered, and the filter cake was washed with EtOAc (20 mL). The filtrate was separated, the aqueous layer was extracted with EtOAc, and the combined organic layers were worked up to give 7.72 g of a mixture, which after flash chromatography (silica gel, $C_6H_{14}$–EtOAc, 1:1) provided 2.32 g of **6** (8.1 mmol, 77%): mp 109 °C; $^1H$ NMR (acetone-$d_6$) $\delta$ 7.82 (1 H, br s, $D_2O$ exch), 7.42 (1 H, d, $J$ = 7.5 Hz), 7.26 (1 H, td, $J$ = 7.8, 1.6 Hz), 6.87 (1 H, td, $J$ = 7.6, 0.9 Hz), 6.84 (1 H, d, $J$ = 8.0 Hz), 5.92 (1 H, d, $J$ = 5.7 Hz), 4.85 (1 H, td, $J$ = 8.1, 4.8 Hz), 3.52 (1 H, dd, $J$ = 12.2, 8.0 Hz), 3.26 (1 H, dd, $J$ = 12.2, 4.8 Hz), 1.75 (1 H, septet, $J$ = 6.8 Hz), 0.92 (3 H, d, $J$ = 6.9 Hz), 0.91 (3 H, d, $J$ = 6.8 Hz), 0.74 (3 H, s), 0.73 (3 H, s);

$^{13}$C NMR (acetone-$d_6$) $\delta$ 171.1, 156.7, 131.8, 128.1, 120.9, 120.2, 118.1, 68.6, 67.7, 34.9, 34.8, 21.5, 20.6, 19.4, 19.0; IR (Nujol) 1730, 1603, 1579, 1483, 1456, 1390, 1317, 1277, 1238, 1107, 1080, 943, 908, 812, 770 cm$^{-1}$; UV $\lambda_{max}$ (log $\epsilon$) 276 (3.48), 228 (sh, 3.90), 204 (4.34); EIMS *m/z* 234 (100%, M − $C_6H_{13}$), 206, 200, 148; FABMS *m/z* 320 (M + H), 234, 216. The compound was optically inactive.

Anal. Calcd for $C_{16}H_{22}BNO_3S$: C, 60.20; H, 6.95; N, 4.34; S, 10.04; $M_r$ 320.1492 (M + H). Anal. Calcd for $C_{16}H_{22}BNO_3S{\cdot}H_2O$: C, 56.99; H, 7.17; N, 4.15; S, 9.50. Found: C, 56.50; H, 7.08; N, 4.07; S, 9.76; $M_r$ 320.1492 (M + H, HRFABMS).

**Methyl 2-(2-Acetoxyphenyl)thiazolidine-4-carboxylate (7).** DIBAL-H (35 mmol, 35 mL of a 1.0 M solution in $C_6H_{14}$) was added during 30 min to a solution of **3c** (6.32 g, 22.6 mmol) in dry $Et_2O$ (150 mL) at −78 °C under $N_2$, and the mixture was stirred for 30 min more and then was quenched by MeOH (50 mL) at −78 °C. Filtration of the product and washing with cold MeOH provided 3.91 g (15.2 mmol, 67%) of **7** as an off-white powder: mp 234−235 °C; $^1$H NMR (DMSO-$d_6$) $\delta$ 9.4 (1 H, br s, $D_2O$ exch), 7.86 (1 H, d, $J$ = 7.7 Hz), 7.10 (1 H, t, $J$ = 7.7 Hz), 6.82 (1 H, d, $J$ = 8.1 Hz), 6.80 (1 H, t, $J$ = 7.7 Hz), 6.31 (1 H, br s, $D_2O$ exch, H-2′), 4.66 (1 H, dd, $J$ = 9.2, 6.3 Hz, H-4′), 3.70 (3 H, s), 3.37 (1 H, dd, $J$ = 12.0, 6.4 Hz, H-5′), 2.97 (1 H, dd, $J$ = 11.9, 9.4 Hz), 1.78 (3 H, s); $^{13}$C NMR (DMSO-$d_6$) $\delta$ 170.6, 169.2, 153.8, 128.8, 127.9, 125.9, 119.0, 115.0, 64.3, 60.5, 52.3, 30.8, 22.0; IR (thin film) 1745, 1620, 1585, 1288, 1238, 1170, 1095, 765 cm$^{-1}$; UV (MeOH) $\lambda_{max}$ (log $\epsilon$) 279 (3.59), 204 (4.47); EIMS *m/z* 281, 238, 222, 195, 180, 153, 137 (100). The compound was optically inactive.

Anal. Calcd for $C_{13}H_{15}NO_4S$: C, 55.50; H, 5.37; N, 4.98; S, 11.39. Found: C, 55.10; H, 5.17; N, 5.00; S, 11.16.

**2-(2-Hydroxyphenyl)-2-thiazoline-4-methanol (8).** The method of Soai et al. was employed.[21] A mixture of methyl ester **3b** (1.472 g, 6.21 mmol), $NaBH_4$ (612 mg, 16.1 mmol, 2.6 equiv), and THF (20 mL) refluxed while MeOH (5 mL) was added dropwise during 30 min. The reaction was cooled to 15 °C, $H_2O$ was added, the mixture was stirred for 15 min, more $H_2O$ was added, the mixture was extracted with $Et_2O$, and the organic layer was worked up to provide **8** (541 mg, 2.58 mmol, 41%) as a chromatographically pure, light-yellow oil: $^1$H NMR ($CDCl_3$) $\delta$ 12.7 (1 H, br s, $D_2O$ exch), 7.37 (1 H, dd, $J$ = 8.2, 1.5 Hz), 7.34 (1 H, dd, $J$ = 8.4, 1.6 Hz), 6.97 (1 H, d, $J$ = 8.4 Hz), 6.85 (1 H, dd, $J$ = 8.6, 7.1 Hz), 4.81 (1 H, septet, $J$ = 5.0 Hz), 3.92 (1 H, dd, $J$ = 11.3, 5.2 Hz), 3.79 (1 H, dd, $J$ = 11.3, 4.8 Hz), 3.37 (1 H, dd, $J$ = 10.8, 8.9 Hz), 3.29 (1 H, dd, $J$ = 10.9, 8.2 Hz); $^{13}$C NMR ($CDCl_3$) $\delta$ 173.0, 158.8, 133.2, 130.6, 119.0, 117.0, 116.2, 77.7, 63.8, 32.8; IR (neat) 3400 (br), 1593, 1491, 1254, 1221, 1155, 1039, 956, 819, 752 cm$^{-1}$; UV (MeOH) $\lambda_{max}$ (log $\epsilon$) 310 (3.78), 254 (4.10), 250 (4.10), 212 (4.44); EIMS *m/z* 209, 178, 150, 146, 120, 59. The optically inactive compound was also isolated as a byproduct (7−20% yields) from the thexylborane and DIBAL-H reductions described above.

Anal. Calcd for $C_{10}H_{11}NO_2S$: C, 57.39; H, 5.30; N, 6.69; S, 15.33. Found: C, 57.41; H, 5.20; N, 6.65; S, 15.23.

**2-(2-Hydroxyphenyl)thiazole-4-carboxaldehyde (9).** A solution of alcohol **8** (122 mg, 0.584 mmol) in $CH_2Cl_2$ (10 mL) was added dropwise with stirring during 15 min to a solution of oxalyl chloride (0.12 mL, 1.3 mmol) and DMSO (0.17 mL, 2.2 mmol) in $CH_2Cl_2$ (25 mL) at −78 °C. $Et_3N$ (1.0 mL, 7 mmol) was added at once, the mixture was warmed to 25 °C, $H_2O$ was added, and the organic layer was worked up to give a crude mixture which was flash chromatographed (silica gel; $CH_2Cl_2$−$Et_2O$, 99:1) to provide **9** (54 mg, 0.263 mmol, 45%) as a yellow powder: mp 130−133 °C (lit.[5] mp 135−135.5 °C); $^1$H and $^{13}$C NMR, IR, and UV as reported.[5]

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**Supplementary Material Available:** Copies of $^1$H NMR spectra of methyl esters of 4-methylpyochelins I and II and the four pyochelin isomers (partial) (3 pages). This material is contained in libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information. The author has deposited atomic coordinates for 4-methylpyochelin I with the Cambridge Crystallographic Data Centre. The coordinates can be obtained, on request, from the Director, Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge, CB2 1EZ, UK.

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