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An Improved Stereocontrolled Synthesis of Pyochelin, Siderophore of *Pseudomonas aeruginosa* and *Burkholderia cepacia*

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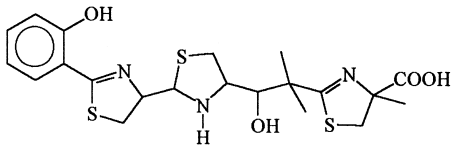
Abstract—A considerably improved stereocontrolled synthesis of pyochelin, a hydroxyphenylthiazolinylthiazolidine type of siderophore common to most strains of *Pseudomonas aeruginosa* and *Burkholderia cepacia* is described. 2′-(2-Hydroxyphenyl)-2′-thiazoline-4′-carboxaldehyde, a key molecule involved in this synthesis has been prepared by reduction of 2′-(2-hydroxyphenyl)-2′-thiazoline-4′-(*N*-methoxy,*N*-methyl) carboxamide with lithium aluminium hydride. The aldehyde was further coupled with (*R*)-*N*-methylcysteine to yield pyochelin. Under the conditions reported, epimerization at the C-4′ center was considerably diminished.

Introduction

Pyochelin **1a** is a hydroxyphenylthiazolinylthiazolidine type of siderophore which was isolated for the first time from iron-deficient cultures of *Pseudomonas aeruginosa* ATCC 15692 by Liu and Shokrani.[1] This siderophore was later shown to be produced by a great number of strains of *Pseudomonas aeruginosa* but also by many strains of *Burkholderia* (ex *Pseudomonas*) *cepacia*,[2] both species being involved in severe lung infections occurring in cystic fibrosis patients (Scheme 1).

The structure of pyochelin was established later by Cox et al.;[3] it possesses a hydroxyphenylthiazoline group as well as a carboxylic acid group, both involved in the chelation of iron(III) with a 2:1 stoichiometry. Although its association constant with iron(III) is very weak (estimated to be $5\times10^5\ M^{-1}$ l in methanol),[4] the pyochelin-mediated iron transport is very efficient.[5]

Natural pyochelin had initially been isolated as a mixture of two steroisomers **1a** and **1b** resulting from epimerization of the 2″ center.[6] The absolute configuration of the asymmetric centers was deduced from the 3D structure of 4-methylpyochelin determined by X-ray crystallography and by comparison of the ^{1}H- and ^{13}C NMR data of the same 4-methylpyochelin prepared by mutasynthesis. The absolute configurations of the asymmetric centers of pyochelin **1a** are, respectively, (4′*R*), (2″*R*) and (4″*R*) whereas for its diastereoisomer **1b**, they are (4′*R*), (2″*S*) and (4″*R*). These two diastereoisomeric forms were observed in synthetic pyochelins as well.[7]



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Epimerization of the C-2″ center has also been reported for yersiniabactin **2**[8] where it was assumed to occur as in thiazolidine derivatives via a Schiff base.[9–11]

The first synthesis of pyochelin had been reported by Ankenbauer et al.[6] It is based on the condensation of (*R*)-*N*-methylcysteine with aldehyde **4**. This latter was prepared after condensation of 2-hydroxybenzonitrile with (*R*)-cysteine followed by reduction of acid **3a** by thexylborane, but the yield of this last step was unfortunately particularly low (15%) due to the formation of a stable boron/thiazoline complex (Scheme 2).

This was followed by cyclization of aldehyde **4** with *N*-methylcysteine (prepared in two steps from (*R*)-cysteine) which gave a mixture of diastereoisomers: pyochelin **1a** (4′*R*,2″*R*,4″*R*), pyochelin **1b** (4′*R*,2″*S*,4″*R*) both corresponding to natural pyochelin (after epimerization of the C-2″ center), together with pyochelin **1d** (4′*S*,2″*R*,4″*R*) (no mention was made at that time of pyochelin **1c**).[7]

These results show how difficult it was to prepare pure pyochelin in large amounts and preparing synthetic

Keywords: pyochelin; siderophores; thiazolines; thiazolidines.

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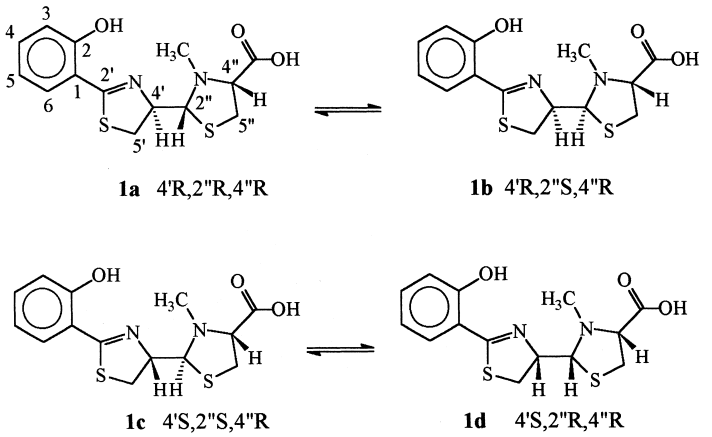
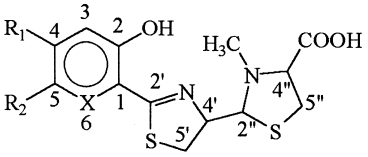


Scheme 1.

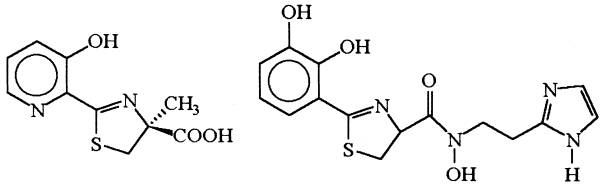
pyochelin analogs has always been found to be a rather difficult challenge, the only access to analogs being mutasynthesis. Using this technique Ankenbauer et al. have prepared three analogs of pyochelin: 5-fluoropyochelin **5**, 4-methylpyochelin **6** and 6-azapyochelin **7**. After incubation of 13 analogs of salicylic acid with a mutant of *Pseudomonas aeruginosa* Sal$^-$ IA602, only three of them (5-fluorosalicylic acid, 4-methylsalicylic acid and 3-hydroxypicolinic acid) gave the corresponding analog of pyochelin. These analogs present biological activities quite comparable to pyochelin in relation to the pyochelin-mediated iron-transport in *Pseudomonas aeruginosa*.[12]



5	R_1 = H	R_2 = F	X = CH
6	R_1 = CH_3	R_2 = H	X = CH
7	R_1 = H	R_2 = H	X = N

Other analogs of pyochelin such as desferriferrithiocine **8** (from S*treptomyces antibioticus*),[13] anguibactin **9** (produced by *Vibrio anguillarum*)[14] and yersiniabactin (or yersiniophore) **2** excreted by *Yersinia enterocolitica* were also reported.[8,15]

At present the pyochelin-dependent iron-transport system of these bacteria is not very clearly understood. A few reports have shown the existence of receptor proteins on the outer membrane and also a direct relationship between the rate of iron transport and the amount of these receptor proteins.[16] In addition, any study concerning these receptors implies handling large amounts of pyochelin and/or some specially designed analogs of pyochelin.



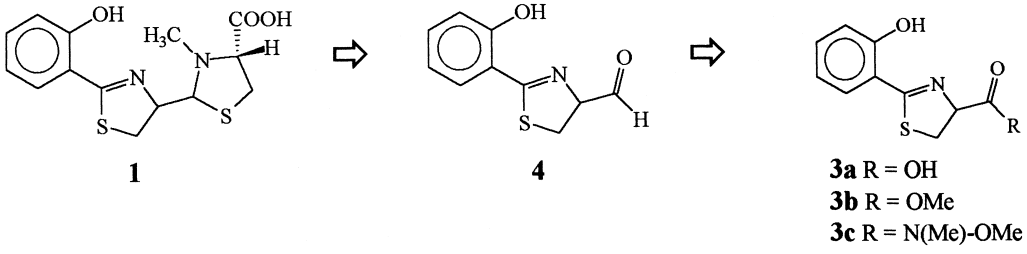
8 **9**

Therefore, after having carefully examined the previous conditions reported for the synthesis of pyochelin, we introduced several modifications leading to a considerably improved synthesis of pyochelin which we describe in this paper, and discuss the conditions leading to a complete stereocontrolled synthesis.

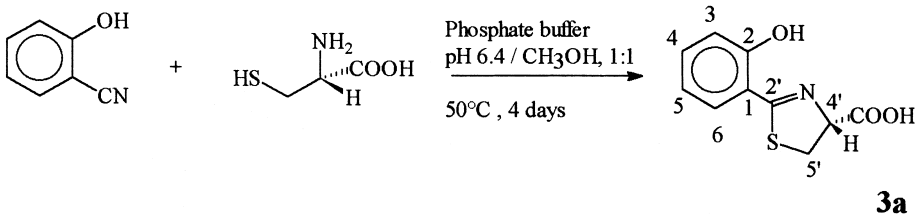
Results and Discussion

The key molecule for the synthesis of pyochelin **1** is aldehyde **4** which is then readily condensed with *N*-methylcysteine. The aldehyde has already been obtained either by reduction of the carboxylic acid group of thiazoline **3a** with thexylborane,[17] or by reduction of ester **3b** with diisobutylaluminum hydride.[7]

The first synthesis of thiazoline **3a** has been described by Mathur et al.[18] condensing (*R*)-cysteine hydrochloride with

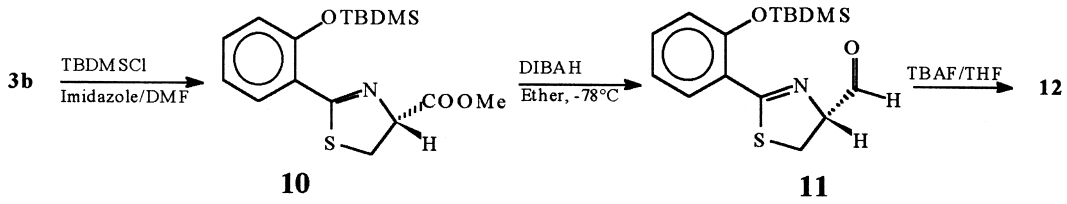


Scheme 2.



3a

Scheme 3.



10 **11**

Scheme 4.

2-hydroxybenzonitrile in the presence of a base (piperidine at pH ca. 9.0). Ankenbauer et al.[6] then Rinehart et al.[7] used the same method but reported that the corresponding methyl ester **3b** had no optical activity.

With a view to understanding the reasons for this epimerization we have prepared thiazoline **3a** as described by Mathur et al.[18] and found a slight optical activity with an $[\alpha]_D=-6.5°$ ($c=0.9$; MeOH/HCl 6N 2%). We performed the same reaction according to Bergeron et al.[19] in a 1:1 mixture of phosphate buffer 0.1M, pH 6.4 and methanol at 50°C for 4 days, and obtained thiazoline **3a** with a yield as high as 84% but with a very different optical rotation: $[\alpha]_D=-190°$ ($c=0.9$; MeOH/HCl 6N 2%) (Scheme 3). We assigned this large difference to the epimerization of the C-4′ asymmetric center caused by the very basic pH conditions caused by piperidine (pH ca. 9.0).

Esterification of thiazoline **3a** with methanol/HCl at 50°C under anhydrous conditions yielded ester **3b** in 93% yield. This was reduced at −78°C with DIBAH (2 equiv.) to yield aldehyde **4** (61%). It was however accompanied by the starting material in an 8:2 ratio (determined from TLC), and during its purification by column chromatography on silica gel epimerization of the carbon atom C-4′ in the position α to the aldehyde function occurred.[20] Therefore it was used without further purification.

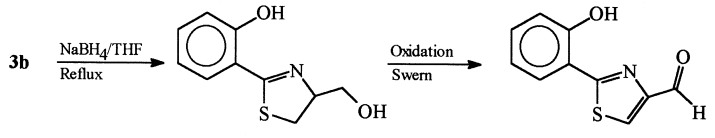
Attempts were made to improve the yield by modification of the solvent, the amount of DIBAH and the reaction temperature: raising the temperature above 50°C or using more than 2 equiv. of DIBAH considerably increased the amount of alcohol **10**.

The protection of the phenol group as its *t*-butyldimethylsilyl ether gave the protected aldehyde **11** with very high yields (87%) (Scheme 4). Unfortunately the deprotection of the TBDMS group at this stage with tetrabutylammonium fluoride (TBAF) afforded a mixture of colored compounds which after purification by column chromatography on silica gel yielded the thiazole aldehyde **12**. Performing this deprotection after the final step of the synthesis of pyochelin afforded a mixture of colored compounds which were even more difficult to purify.

The structure of thiazole aldehyde **12** was confirmed by proton NMR and by mass spectrometry. The signals of proton H-4′ at 5.33 ppm and of the two protons H-5′ at 3.39 and 3.69 ppm present in aldehyde **4** had completely disappeared. Instead a signal at 8.1 ppm corresponding to a single proton appears (H-5′), whereas the aldehydic proton shifts from 9.86 to 10.06 ppm. The FAB mass spectrum shows a molecular peak at $m/z=206$ $(M+H)^+$ corresponding to the aldehyde **12**.

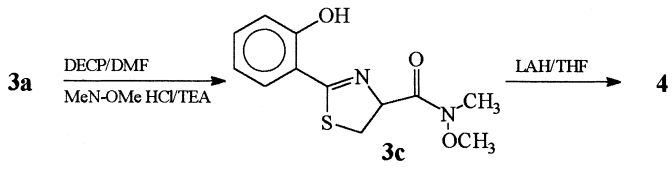
We further attempted to prepare aldehyde **4** by oxidation of alcohol **13** (itself obtained by reduction of ester **3b** with sodium borohydride in 60% yield). However this oxidation step performed by different methods according to Swern, Moffat or Collins always yielded aldehyde **12** (Scheme 5), showing that aromatization of alcohol **13** is faster than its oxidation into aldehyde **4**.[7,21]

For these reasons we used a method reported in 1983 by Fehrentz and Castro[22] for the preparation of aldehydes derived from protected amino acids based on the reduction

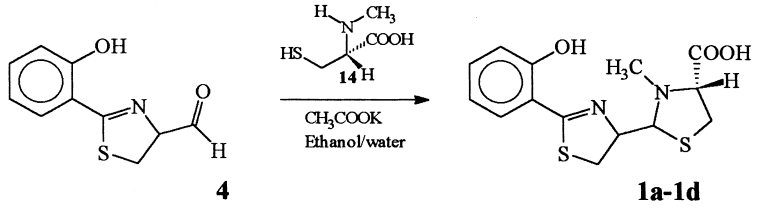


13 **12**

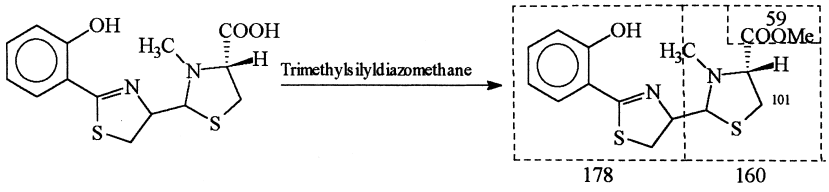
Scheme 5.



Scheme 6.



Scheme 7.



Scheme 8.

of the corresponding *N*-methoxy-*N*-methyl hydroxamates using excess lithium aluminum hydride (5 equiv.) at 0°C. These aldehydes were obtained with excellent yields without any epimerization.

We have adapted their reaction conditions to prepare aldehyde **4**, and also modified the conditions of preparation of hydroxamate **3c** and the temperature of reduction of this latter.

Diethyl cyanophosphonate (DECP) was used as a coupling agent for acid **3a** and *N*,*O*-dimethylhydroxylamine,[23] and afforded hydroxamate **3c** in excellent yield (94%).

In order to prevent the secondary reaction yielding alcohol **13**, the reduction of hydoxamate **3c** was performed with 3 equiv. of lithium aluminum hydride (instead of 5 equiv.) and at −20°C (instead of 0°C). Under these conditions the reduction was complete after 20 min, yielding aldehyde **4** with no trace of starting material and no trace of alcohol **13** (Scheme 6).

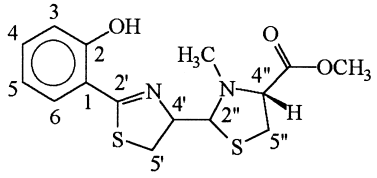
Aldehyde **4** was condensed with (*R*)-*N*-methylcysteine hydrochloride[24] in a 4:1 mixture of ethanol and water in the presence of potassium acetate. The yield of this step is 70%, and pyochelin was obtained as a mixture of four diastereoisomers **1a**–**1d** (Scheme 7).

The structure of pyochelins **1a**–**1d** and the absolute configuration of the asymmetric centers were determined by comparison of the proton NMR spectra and the mass spectra of their methyl esters, with the corresponding reference spectra described by Rinehart et al.[7] In this respect we have quantitatively derivatized the acids **1a**–**1d** as their methyl esters **15a**–**15d** using trimethylsilyl diazomethane,[27] these esters being easier to handle and purify than the corresponding acids **1a**–**1d**[7] (Scheme 8).

The chemical ionization mass spectrum of the mixture **15a**–**15d** showed fragmentations similar to those observed by Cox et al.[3]

The proton NMR spectrum of the mixture of esters presented great similarities with the spectrum reported by Rinehart et al. (Table 1).[7] In our hands a mixture of four

Table 1. Chemical shifts of the protons H-4′, H-2″ and H-4″ of the diastereoisomers of pyochelin methyl esters **15a**–**15d**. The values in brackets represent the coupling constants expressed in hertz



	H-4′	H-2″	H-4″
15a	5.08 t,d (8.7; 5.2)	4.51 d (5.2)	3.65 d,d (8.7; 6.6)
	5.08 t,d (8.7; 5.2)[a]	4.53 d (5.2)[a]	3.65 d,d (9.1; 6.3)[a]
15b	4.82 q (8.1)	4.56 d (6.9)	4.09 t (6.2)
	4.92 q (8.2)[a]	4.56 d (6.9)[a]	4.09 t (6.0)[a]
15c	5.13 t,d (9.3; 4.7)	5.02 d (4.7)	4.14 d,d (4.8; 2.2)
	5.12 t,d (9.1; 4.6)[a]	5.04 d (4.6)[a]	4.13 d.d (6.4; 2.1)[a]
15d	4.80 q (8.1)	4.23 d (7.7)	3.83 t (6.8)
	4.79 q (8.0)[a]	4.24 d (7.7)[a]	3.83 t (6.8)[a]

[a] Data reported by Rinehart et al.[7]

diastereoisomers was obtained: **15a** (4′*R*,2″*R*,4″*R*), **15b** (4′*R*,2″*S*,4″*R*), **15c** (4′*S*,2″*S*,4″*R*) and **15d** (4′S,2″*R*,4″*R*), but in a ratio of 2:1:2:5, respectively (calculated from the signals of proton H-4′, H-2″ and H-4″). Rinehart et al.[7] had indicated a ratio of 4:1:1:4 for the corresponding acids (determined by TLC). It is very likely that the starting compound they used for the condensation reaction was already epimerized at C-4′ before the DIBAH reduction affording aldehyde **4**.

From these results, we have drawn the following conclusions:

1. There is an epimerization at the C-4′ (30%), occurring probably during the condensation reaction of aldehyde **4** with *N*-methylcysteine in the presence of potassium acetate.
2. The diastereoisomers possessing the (4′*S*) configuration (**15c**+**15d**) represent 70% of the mixture, which is not surprising since the starting product is (*R*)-cysteine and that all care was taken to prevent epimerization of this asymmetric center (Rinehart et al.[7] had a total epimerization of this center).
3. The diastereoisomers possessing the (2″*R*) configuration (**15a**+**15d**) also represent 70% of the mixture and not 50% as would be expected since the C-2″ asymmetric center is formed *after* addition of *N*-methylcysteine on a planar aldehydic carbonyl group. This result indicates that this last step is performed with asymmetric induction created by the C-4′ center, which is not finally so surprising since all the molecules participating in the reaction are chiral.
4. The esterification reaction having no effect on the chiral centers, pyochelins **1a**–**1d** are obtained in the same proportions as their corresponding methyl esters.

Experimental

General indications

The melting points (mps) were measured in a capillary tube using a Büchi SMP-20 instrument and are not corrected. The proton NMR spectra were measured on Bruker AC-200 (200 MHz), AM-400 (400 MHz), ARX-500 (500 MHz), AMX 500 (500 MHz) or with gradient instruments. The mass spectra (MS) were determined using either an LKB 9000S or a Thomson THN 208 spectrometer. The FAB (fast atom bombardment) spectra were performed using a ZAB-HF instrument (VG Analytical, Manchester, UK). The relative intensities of the signals are stated in parentheses after the mass *m*/*z* of the fragment considered. Optical rotations were determined with a Perkin–Elmer 241 MC instrument. The elemental analyses (microanalyses) were performed in Strasbourg by the Service de Microanalyse of the Institut Charles Sadron. The column chromatographies were performed using Merck 9385 silica gel (Darmstadt, Germany) 40–63 μm mesh. Thin layer chromatographies (TLCs) were performed using silica gel analytical plates Merck 5715 (F_{254}) of 0.25 mm thickness. The detection on TLC plates was performed by UV light at 254 or 365 nm or using a spray and heating the plates. The sprays used were:

—a 0.3% solution of ninhydrin in a mixture of butanol/acetic acid (97:3, v/v).

—a 0.4% solution of 2,4-dinitrophenylhydrazine in 2M hydrochloric acid.

—a 10% solution of phosphomolybdic acid in ethanol.

The anhydrous solvents were obtained by distillation from an appropriate drying agent: phosphoric anhydride for methylene chloride and dimethylformamide; calcium hydride for toluene, ether and acetonitrile; sodium for methanol. The oxygen- or humidity-sensitive reactions were performed under argon.

2′-(2-Hydroxyphenyl)-2′-thiazoline-4′-carboxylic acid 3a

According to Mathur et al.[18] 2-Hydroxy-2-benzonitrile (6.0 g, 50 mmol), (*R*)-cysteine hydrochloride (8.0 g, 50 mmol) and sodium bicarbonate (4.2 g, 50 mmol) were dissolved in absolute ethanol (50 ml) and heated under reflux for 30 min. The pH was adjusted to 9.0 by addition of piperidine and the suspension stirred ovenight. Hot water (10 ml) was added and the pH was carefully adjusted to 4.0 by addition of acetic acid. After filtration, the precipitate was washed with cold water, then with ethanol, dried under reduced pressure to yield a clear yellow powder (9.48 g, 42.5 mmol, 85%).

Mp: 268–270°C (lit. 269°C[18]).

$[\alpha]_D = -6.5°$ (*c* 0.9 methanol/HCl 6N 2%).

According to Bergeron et al.[19] 2-Hydroxy-2-benzonitrile (5.33 g, 44.8 mmol) and (*R*)-cysteine (10.83 g, 89.5 mmol) were dissolved in a 1:1 (v/v) mixture of methanol and phosphate buffer pH 6.4 (200 ml). The mixture was stirred for 4 days at 50°C, filtered and concentrated under reduced pressure. Water (50 ml) was added and the solution extracted with methylene chloride (3×25 ml). The organic phase was washed with water (2×10 ml), then with brine (10 ml) and dried over sodium sulfate. After evaporation of the solvent under reduced pressure a yellow solid was obtained (8.4 g, 37.6 mmol, 84%).

Mp: 116–117°C.

$[\alpha]_D = -190°$ (*c* 0.9 methanol/HCl 6N 2%).

MS (EI): *m*/*z*=223 (55); 178 (100); 119 (17.2); 59 (38.04).

^{1}H NMR (200 MHz; $CDCl_3$): 3.69 (m; 2-H, H-5′); 5.42 (t; *J*=8.12 Hz, H-4′); 6.89 (t; *J*=7.5 Hz, 1-H, H-3); 7.03 (d; *J*=8.5 Hz, 1-H, H-4); 7.43 (m; 5-H, H-5 and H-6).

^{13}C NMR (100 MHz; $CDCl_3$): 33.6 (C-5′); 76.3 (C-4′); 115.84 (C-1); 117.38 (C-3); 119.09 (C-5); 130.8 (C-6); 133.8 (C-4); 159.12 (C-2′); 174.7 (C-2); 175.14 (COOH).

Calculated for $C_{10}H_9NO_3S$	C% 53.81	H% 4.04	N% 6.28	O% 21.52
Found	C% 53.84	H% 3.98	N% 6.23	O% 21.34

2′-(2-Hydroxyphenyl)-2′-thiazoline-4′-carboxaldehyde 4

Methyl-2′-(2-hydroxyphenyl)-2-thiazoline-4′-carboxylate 3b. To a solution of anhydrous hydrochloric acid in

methanol (prepared from 20 ml methanol and 5 ml acetyl chloride at 0°C), thiazoline **3a** (2.95 g, 13.2 mmol) was added and the mixture heated at 50°C for 24 h. After evaporation of the solvent, 50 ml ethyl acetate were added, and the organic phase washed with water (2×10 ml), then with brine, dried over sodium sulfate, filtered and evaporated under reduced pressure to yield a yellow oil (2.91 g, 12.28 mmol, 93%).

R_f 0.51 (methylene chloride–acetone 75:25, v/v).

^{1}H NMR (200 MHz; $CDCl_3$): 3.58 (m; 2-H, H-5′); 3.81 (s; 1-H, OMe); 5.12 (t; J=9.28 Hz, H-4′); 6.87 (t; J=8.3 Hz, 1-H, H-3); 6.97 (d; J=7.4 Hz, 1-H, H-5); 7.28 (dd; J=1.3 and 7.6 Hz, 1-H, H-4); 7.78 (d,t; J=1.3 and 7.7 Hz, 1-H, H-6).

^{13}C NMR (100 MHz; $CDCl_3$): 33.6 (C-5′); 52.8 (C–OMe); 76.6 (C-4′); 116.2 (C-1); 118.9 (C-3); 119.09 (C-5); 130.6 (C-6); 133.5 (C-4); 159.2 (C-2′); 174.3 (C-2); 170.6 (COOH).

Reduction of ester 3b with DIBAH into aldehyde 4

To a solution of ester **3b** (864 mg, 3.64 mmol) in anhydrous ether (20 ml) at −78°C under argon, a 1.5M solution of DIBAH 1.5M in toluene (4.5 ml, 6.75 mmol) was added dropwise during 8 min with a syringe. After 2 h, methanol (3 ml) then a saturated solution of ammonium chloride (17 ml) were added. The temperature was raised to 0°C and water (30 ml) was added. The organic phase was conserved and the aqueous phase was extracted with ether (3×15 ml). All the organic phases were pooled and washed with brine and dried over sodium sulfate. After evaporation of the solvents, a yellow resin was obtained (459 mg, 2.21 mmol, 61% as a 8:2 mixture of aldehyde **4** and ester **3b** according to NMR).

For the characteristics of aldehyde **4** see the following.

Attempts to synthesize aldehyde 4 after protection of the phenol group as a *t*-butyldiphenylsilyl ether

Methyl-2′-(2-*t*-butyldiphenylsilyloxyphenyl)-2′-thiazoline-4′-carboxylate 10. Imidazole (782 mg, 11.5 mmol) and *t*-butyldiphenylsilyl chloride (832 mg, 5.52 mmol) were added to a solution of ester **3b** (1.098 g, 4.6 mmol) in dimethylformamide (5 ml). The mixture was stirred at room temperature for 24 h. Water (25 ml) was added and the mixture extracted with ethyl acetate (3×10 ml). The organic phases were pooled, washed with brine (3×10 ml), dried over sodium sulfate and evaporated under reduced pressure. The crude compound (1.8 g) was purified by column chromatography on silica gel (50 g), and eluted with a 9/1 mixture of hexane–acetone 9/1 (v/v). After evaporation, a colorless oil was obtained (1.4 g, 4 mmol, 87%).

R_f 0.66 (hexane–ethyl acetate 7:3, v/v).

^{1}H NMR (200 MHz; $CDCl_3$): 0.26 (s; 6-H, $Si(Me)_2$); 0.97 (s; 9-H, Si-*t*-butyl); 3.57 (m; 2-H, H-5′); 3.81 (s; 1-H, OMe); 5.12 (t; J=9.36 Hz, H-4′); 6.85 (d,d; J=1 and 8.23 Hz, 1-H, H-3); 6.94 (t; J=9.2, 1-H, H-5); 7.28 (m; 1-H, H-4); 7.75 (dd; J=1.8 and 7.8 Hz, 1-H, H-6).

2′-(2-*t*-Butyldiphenylsilyloxyphenyl)-2′-thiazoline-4′-carboxaldehyde 11

To a solution of ester **3b** (459 mg, 1.3 mmol) in anhydrous toluene (5 ml) at −78°C under argon, a 1.5M solution of DIBAH in toluene (1.3 ml, 1.95 mmol) was added dropwise during 8 min with a syringe. After 1 h, 2 ml methanol then a saturated solution of aqueous ammonium chloride (10 ml) was added. The temperature was raised to 0°C and water (20 ml) added. The organic phase was conserved and the aqueous phase was extracted with ether (3×15 ml). All the organic phases were pooled and washed with brine and dried over sodium sulfate. After evaporation of the solvents a colorless oil was obtained (362 mg, 1.13 mmol, 87%).

R_f 0.45 (hexane–ethyl acetate 7:3, v/v; detection either with the phosphomolybdic acid or with the 2,4-dinitrophenylhydrazine sprays).

^{1}H NMR (200 MHz; $CDCl_3$): 0.13 (s; 6-H, $Si(Me)_2$); 0.89 (s; 9-H, *t*-butyl); 3.57–3.80 (m; 2-H, H-5′); 5.25 (dd; J=6.15 and 9.8 Hz, 1-H, H-4′); 6.85–7.03 (m; 2-H, H-3 and H-5); 7.13–7.4 (m; 2-H, H-4, H-6).

Deprotection of the TBDMS function of aldehyde 11

To a solution of aldehyde **11** (233 mg, 0.73 mmol) in tetrahydrofuran (3 ml) at 0°C, 1.4 ml of a 1M solution of tetrabutylammonium fluoride in tetrahydrofuran (1.4 mmol), was added dropwise. After 30 min, the solvents were evaporated and the dark brown residue was purified by column chromatography on silica gel (3 g) and eluted with methylene chloride to yield 2′-(2-hydroxyphenyl)-2′-thiazole-4′-carboxaldehyde **12** (59 mg, 0.29 mmol, 40%).

Mp: 134–136°C (lit. 135–135.5°C)[21].

R_f 0.75 (methanol–methylene chloride 9:1, v/v).

FAB-MS: 206.1 $(M+H)^{+\cdot}$

^{1}H NMR (200 MHz; $CDCl_3$): 6.88–7.08 (m; 2-H, 3-H, H-4); 7.3–7.6 (m; 2-H, H-5 and H-6); 8.1 (s; 1-H, H-5′); 10.02 (s; 1-H, aldehydic proton); 11.5 (s; 1-H, –O*H*).

Attempts to synthesize aldehyde 4 by oxidation of alcohol 13

2′-(2-Hydroxyphenyl)-2″-thiazoline-4′-methanol 13. A mixture of ester **3b** (894 mg, 3.77 mmol), sodium borohydride (371 mg, 9.77 mol) and tetrahydrofuran (12 ml) was heated under reflux, and methanol (3 ml) was added within 15 min. The mixture was cooled down to 15°C, water (1 ml) was added dropwise, the stirring was continued for 10 min and after another addition of water (15 ml), it was extracted with ether (3×15 ml). The organic phase was washed with brine and dried over sodium sulfate. After filtration and evaporation of the solvents under reduced pressure an oil was obtained (690 mg). It was purified by column chromatography on silica gel (20 g), and afforded after elution with methylene chloride–methanol 9:1 (v/v) and evaporation, a yellowish oil (474 mg, 2.27 mmol, 60%).

R_f 0.43 (methylene chloride–methanol 9:1, v/v).

^{1}H NMR (200 MHz; CD_3OD): 3.32–3.52 (m; 2-H, H-5′); 4.82 (m; 1-H, H-4′); 6.85–6.94 (m; 2-H, H-3 and H-5); 7.13–7.4 (m; 2-H, H-4, H-6).

Oxidation of alcohol 13. To a solution of oxalyl chloride (0.24 ml, 2.6 mmol) and DMSO (0.34 ml, 4.4 mmol) in methylene chloride (30 ml) at −78°C, a solution of alcohol **13** (244 mg, 1.17 mmol) in methylene chloride (30 ml) was added dropwise in 20 min. Triethylamine (2 ml, 14 mmol) was then added, the temperature raised to 25°C before addition of water (20 ml). The organic phase was washed with brine, dried over sodium sulfate, evaporated to yield 240 mg of a yellow solid product. It was purified by column chromatography on silica gel (5 g) and afforded after elution with a mixture of methylene chloride and ether 99:1 (v/v), 2′-(2-hydroxyphenyl)-2′-thiazole-4′-carboxaldehyde **12** (96 mg, 0.46 mmol, 39%).

For the characteristics of aldehyde **12** see above.

Synthesis of aldehyde 4 by reduction of hydroxamate 3c

2′-(2-Hydroxyphenyl)-2′-thiazoline-4′-(*N*-methoxy,*N*-methyl) carboxamide 3c. To a solution of thiazoline **3a** (2.23 g, 10 mmol), *N*,*O*-dimethylhydroxylamine hydrochloride (1.07 g, 11 mmol) and diethylcyanophosphonate (2.0 g, 11 mmol) in DMF (15 ml) at 0°C, diisopropylethylamine (3.65 ml, 21 mmol) was added. The mixture was stirred for 30 min at 0°C, then another 30 min at room temperature. A 3% solution of citric acid (90 ml) was added and the mixture filtered. The precipitate was conserved and the filtrate extracted twice with a mixture of toluene and ethyl acetate (1:1, v/v). The organic phase was washed with water, then with brine, dried over sodium sulfate, filtered and evaporated. The residue and the former precipitate were pooled and dried under reduced pressure to yield a yellow powder (2.33 g, 8.9 mmol, 89%) which was crystallized from methylene chloride.

R_f 0.68 (methylene chloride–methanol 9:1, v/v).

Mp: 46–48°C.

MS (CI, NH_3^+): $(M+H)^+$=267.2 (28); 237.2 (5); 236.2 (12); 178 (100).

^{1}H NMR (200 MHz; $CDCl_3$): 3.3 (s; 3-H, CH_3); 3.48 (dd; *J*=9.2 and 11 Hz, 2-H, H-5′); 3.81 (s; 1-H, OMe); 5.70 (t; *J*=8.8 Hz, 1-H, H-4′); 6.88 (t; *J*=7.7 Hz, 1-H, H-3); 6.98 (d; *J*=8.3 Hz, 1-H, H-5); 7.34 (dd; *J*=1.4 and 7.3 Hz, 1-H, H-4); 7.42 (d,t; *J*=1.6 and 7.8 Hz, 1-H, H-6).

^{13}C NMR (100 MHz; $CDCl_3$): 32.64 (C-5′); 32.92 (N-CH_3); 61.87 (O-CH_3); 74.7 (C-4′); 116.2 (C-1); 117.2 (C-3); 119.02 (C-5); 130.6 (C-6); 133.5 (C-4); 159.2 (C-2′); 174.3 (C-2).

Calculated for $C_{12}H_{14}N_2O_3S$	C% 54.12	H% 5.30	N% 10.52	O% 18.02
Found	C% 54.10	H% 5.33	N% 10.52	O% 18.13

Reduction of hydroxamate 3c with lithium aluminum hydride. Hydroxamate **3c** (231 mg, 0.87 mmol) was dissolved in ether (10 ml) and the solution cooled down to −20°C. Lithium aluminum hydride (94 mg, 2.46 mmol) was added. After 20 min at −20°C (until the starting material had disappeared) methanol (1 ml) then a saturated aqueous solution of ammonium chloride (10 ml) and finally a 5% (v/v) solution of sulfuric acid (5 ml) were successively added. The organic phase was conserved and the aqueous phase extracted with ethyl acetate (3×10 ml). The organic phases were pooled, washed with brine, dried over sodium sulfate and evaporated under reduced pressure. 2′-(2-Hydroxyphenyl)-2′-thiazoline-4′-carboxaldehyde **4** (163 mg, 0.79 mmol, 90%) was obtained as a yellow foam.

R_f 0.45 (methylene chloride–methanol 9:1, v/v; detection either with the phosphomolybdic acid or with the 2,4-dinitrophenylhydrazine sprays).

^{1}H NMR (200 MHz; $CDCl_3$): 3.39 (t; *J*=10 Hz, 2-H, H-5′); 3.69 (dd; *J*=7.6 and 11.4 Hz, H-5′); 5.33 (dd; *J*=7.8 and 9.1 Hz, 1-H, H-4′); 7.50–7.62 (m; 3-H, H-4, H-5 and H-6); 9.84 (s; 1-H, aldehydic proton); 12.2 (s; 1-H, −O*H*).

(*R*)-*N*-Methylcysteine **14** was prepared according to the method of Blondeau et al.[25] by reduction of (*R*)-4-thiazolidinyl carboxylic acid using sodium in liquid ammonia at −78°C (68%). This latter acid was synthesized by the method of Ratner et al.[26] by condensation of cysteine hydrochloride with formaldehyde in 73% yield.

Synthesis of pyochelin 1

2′-(2-Hydroxyphenyl)-2′-thiazoline-4′-carboxaldehyde **4** (154 mg, 0.74 mmol) was dissolved in a mixture of ethanol (15 ml) and water (4 ml) and potassium acetate (0.4 g, 5.1 mmol) and (*R*)-*N*-methylcysteine (0.2 g, 1.12 mmol)[24] were added. After 24 h, water (50 ml) was added and the mixture extracted with hexane (3×10 ml). The aqueous phase was acidified to pH 5.0, extracted with ethyl acetate (3×10 ml), washed with brine and dried over sodium sulfate. After filtration and removal of the solvents a yellow foam was obtained (168 mg, 0.52 mmol, 70%).

	I	II	III	IV	
R_f	0.42	0.35	0.26	0.16	(Butanol:water:methanol:hexane 2:2:1:1)

Detection by UV light (254 *and* 366 *nm*): Compounds I and IV gave a bright blue fluorescence whereas II and III were less intensely fluorescent. The four compounds gave a brown color after spraying with a 0.1M solution of ferric chloride in methanol.

Preparation of the methyl esters of pyochelin 15

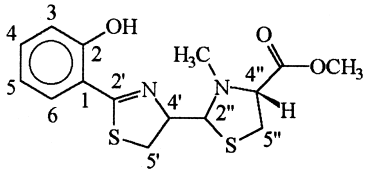
Pyochelin (123 mg, 0.38 mmol) was dissolved in 5 ml of a 4:1 mixture of benzene and methanol (v/v), and trimethylsilyldiazomethane was added (0.25 ml, 0.5 mmol). After 15 min, the solvents were evaporated under reduced pressure and the residue was purified by column chromatography on silica gel (4 g). The product was eluted with

^{1}H-NMR (200 MHz; $CDCl_3$).

Protons	I	II	III	IV
3	7.00 (d; 10 Hz)	7.00 (d; 10 Hz)	7.00 (d; 10 Hz)	7.00 (d; 10 Hz)
4	7.32 (m)	7.32 (m)	7.32 (m)	7.32 (m)
5	6.83 (t; 7.56 Hz)	6.83 (t; 7.56 Hz)	6.83 (t; 7.56 Hz)	6.83 (t; 7.56 Hz)
6	7.40 (m)	7.40 (m)	7.40 (m)	7.40 (m)
4′	5.08 (d,t, 5.2, and 8.7)	4.82 q (8.1)	5.13 (d,t; 4.7, and 9.3)	4.80 q (8.1)
5′	3.51 (m)	3.42–3.46 (m)	3.41–3.47 (m)	3.51 (m)
2″	4.51 (d; 5.2)	4.56 (d; 6.9)	5.02 (d;4.7)	4.23 (d;7.7)
4″	3.65 (dd; 6.6 and 8.7)	4.09 (t; 6.2)	4.14 (dd; 2.2 and 4.8)	3.83 (t; 6.8)
5″	3.01–3.1 (m)	3.18–3.24 (m)	3.28 (m)	3.24–3.36 (m)
N-CH_3	2.58 (s)	2.58(s)	2.58(s)	2.58(s)
O-CH_3	3.78 (s)	3.78 (s)	3.78 (s)	3,78 (s)

a mixture of hexane–ethyl acetate, and after evaporation of the solvents, a yellow foam (127 mg, 0.375 mmol, 98%) as a mixture of four diastereoisomers: I, II, III, IV, was obtained.

MS (CI, NH_3^+): *m/z* 339.1 $[M+H]^+$ (100); 279.1 $[M-COOMe]$ (4); 178.1 $[C_9H_8NOS]^+$ (16); 160.1 $[(C_6H_{10}NO_2S]^+$ (34); 101.1 $[C_4H_6NS]^+$ (4).



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References

1. Liu, P. V.; Shokrani, F. *Infect. Immun.* **1978**, *22*, 878–890.
2. Sokol, P. A. *J. Clin. Microbiol.* **1986**, *23*, 560–562.
3. Cox, C. D.; Rinehart, K. L.; Moore, M. L.; Cook, J. C. *Proc. Natl Acad. Sci. USA* **1981**, *78*, 4256–4260.
4. Cox, C. D.; Graham, R. *J. Bacteriol.* **1979**, *137*, 357–364.
5. Cox, C. D. *J. Bacteriol.* **1980**, *142*, 581–587.
6. Ankenbauer, R. G.; Toyokuni, T.; Staley, A. L.; Rinehart, K. L. *J. Bacteriol.* **1988**, *170*, 5344–5351.
7. Rinehart, K. L.; Staley, A. L.; Wilson, S. R.; Ankenbauer, R. G.; Cox, C. D. *J. Org. Chem.* **1995**, *60*, 2786–2791.
8. Drechsel, H.; Stephan, H.; Lotz, R.; Haag, H.; Zähner, H.; Hantke, K.; Jung, G. *Liebigs Ann. Chem.* **1995**, 1727–1733.
9. Poncticelli, F.; Marinello, E.; Missale, M. C. *Org. Magn. Res.* **1982**, *20*, 138–140.
10. Somogy, L. *Liebigs Ann. Chem.* **1985**, 657–876.
11. Chiba, T.; Sakaki, J.; Kobayashi, S.; Furuya, T.; Inukai, N.; Kaneko, C. *Chem. Pharm. Bull.* **1989**, *37*, 877–882.
12. Ankenbauer, R. G.; Staley, A. L.; Rinehart, K. L.; Cox, C. D. *Proc. Natl Acad. Sci. USA* **1991**, *88*, 1878–1882.
13. Naegeli, H. U.; Zähner, H. *Helv. Chim. Acta* **1980**, *63*, 1400–1406.
14. Jalal, M. A. F.; Hossain, M. B.; van der Helm, D.; Sanders-Loehr, J.; Actis, L. A.; Crosa, J. H. *J. Am. Chem. Soc.* **1989**, *111*, 292–296.
15. Chambers, C. E.; McIntyre, D. D.; Mouck, M.; Sokol, P. A. *BioMetals* **1996**, *9*, 157–167.
16. Heinrichs, D. A.; Young, L.; Poole, K. *Infect. Immun.* **1991**, *59*, 3680–3684.
17. Brown, C. B.; Heim, P.; Yoon, N. M. *J. Org. Chem.* **1972**, *37*, 2942–2950.
18. Mathur, K. B.; Iyer, R. N.; Dhar, M. L. *J. Sci. Ind. Res.* **1962**, *21B*, 34–37.
19. Bergeron, R. J.; Wiegand, J.; Dionis, J. B.; Egli-Karmakka, M.; Frei, J.; Huxley-Tencer, A.; Peter, H. *J. Med. Chem.* **1991**, *34*, 2072–2078.
20. Jurczak, J.; Golebiowski, A. *Chem. Rev.* **1989**, *89*, 149–164.
21. Cuppels, D. A.; Stipanovic, R. D.; Stoessl, A.; Stothers, J. B. *Can. J. Chem.* **1987**, *65*, 2126–2130.
22. Fehrentz, J. A.; Castro, B. *Synthesis* **1983**, 676–678.
23. Takuma, S.; Hamada, Y.; Shioiri, T. *Chem. Pharm. Bull.* **1982**, *30*, 3147–3153.
24. (*R*)-*N*-Methylcysteine **14** was prepared according to the method of Blondeau et al.[25] by reduction of (*R*)-4-thiazolidinyl carboxylic acid using sodium in liquid ammonia at −78°C (68%). This latter acid was synthesized by the method of Ratner et al.[26] by condensation of cysteine hydrochloride with formaldehyde in 73% yield.
25. Blondeau, P.; Berse, C.; Gravel, D. *Can. J. Chem.* **1967**, *45*, 49–52.
26. Ratner, S.; Clarke, H. T. *J. Am. Chem. Soc.* **1937**, *59*, 200–206.
27. Hashimoto, N.; Aoyama, T.; Shiori, T. *Chem. Pharm. Bull.* **1981**, *29*, 1475–1478.